Berberrubine phosphate: A selective Fluorescent Probe for Quadruplex DNA

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Supporting Information

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1. Absorption and emission spectra

Solutions were prepared for each measurement from stock solutions of the derivative **2** in MeOH (*c* = 1.0 mM). Aliquots of the stock solution were thoroughly evaporated under a stream of nitrogen, and the residue was redissolved in the respective solvent or solvent mixture.

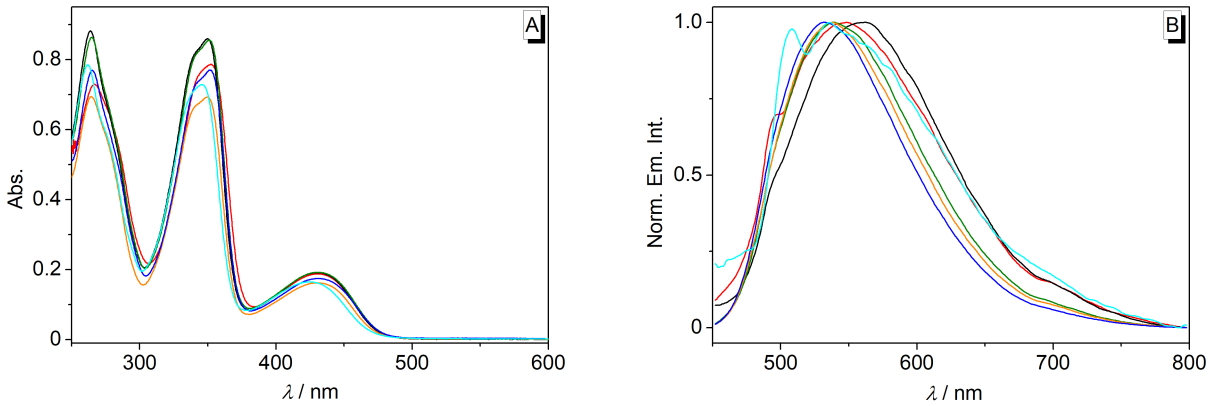

Figure S1. Absorption (A) and emission spectra (B) of **2** (c = 20 µM) in different solvents (red: DMSO, black: MeOH, cyan: aq. buffer, orange: MeCN, green: EtOH, blue: 1-PrOH; buffer: 16 mM BPE buffer solution at pH 7, λ_{ex} = 430 nm).

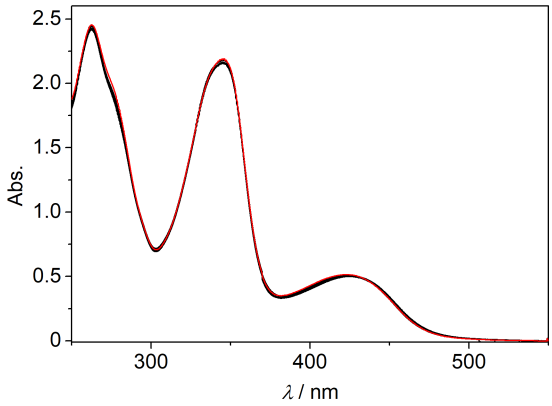

Figure S2. Absorption spectra of **2** (c = 20 µM) in BPE buffer at different temperature (black: 20–70 °C, red: 80°C.

2. Determination of fluorescence quantum yields

Solutions were prepared for each measurement as described above from stock solutions of **2** in MeOH (c = 1.0 mM). For the detection of fluorescence spectra, the excitation and emission slits were adjusted to 5 nm, and the excitation wavelengths were fixed to 340 nm. The relative fluorescence quantum yields, $\Phi_{fl,}$ of **2** were determined under identical conditions (detection wavelength, excitation wavelength, detector voltage, slit bandwidths, collection rate). The quantum yield, Φ_{fl}, was determined according to equation 1.

$$\Phi_{\mathrm{fl,\,X}} = \frac{F_X A_S}{F_S A_X} \cdot \frac{n_X^2}{n_S^2} \cdot \Phi_{\mathrm{fl,\,S}} \qquad \text{(eq. 1)}$$

where, the indices X and S indicate the analyte (X) and standard (S) solution.

Φ_{fl} = Fluorescence quantum yield.

F = Integral of the emission band.

A = Absorbance at the excitation wavelength.

n = Refraction index of the solution.

Measurements were performed with coumarin 153 in ethanol as standard [Φ = 0.544] 1.

The estimated error is ca. 10% of the given values.

4. Photometric and fluorimetric DNA titrations

Spectrophotometric and spectrofluorimetric titrations with ct DNA and quadruplex-forming oligonucleotides were performed according to published procedure [2]. All titrations were performed with 5% v/v DMSO to ensure a sufficient solubility of the ligand.

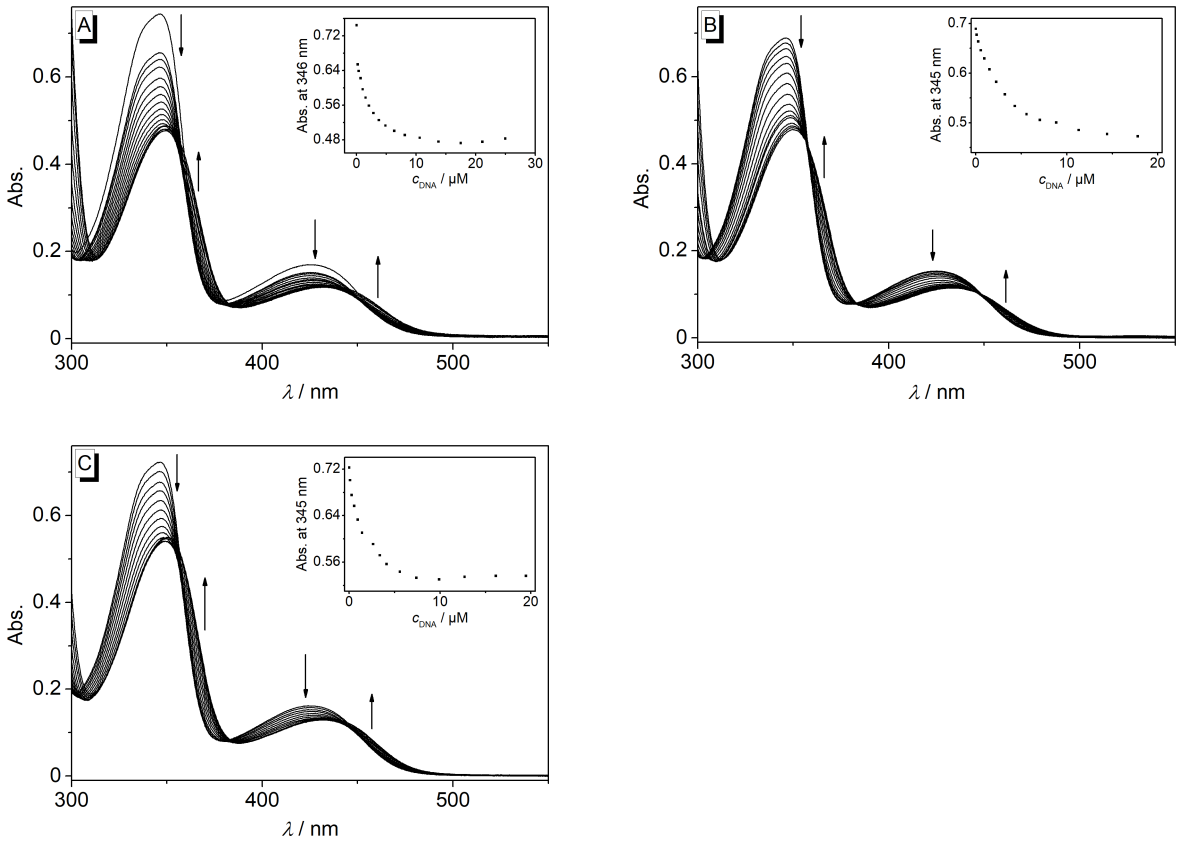

Figure S3. Photometric titration of **2** (*c* = 20 µM) with **22AG** (A), **c-kit** (B) and **c-myc** (C) in K-phosphate buffer (c_{K+} = 110 mM, pH 7.0, with 5% v/v DMSO). The arrows indicate the changes of the absorption bands upon addition of DNA. Inset: Plot of absorption at 345 nm *versus* DNA concentration.

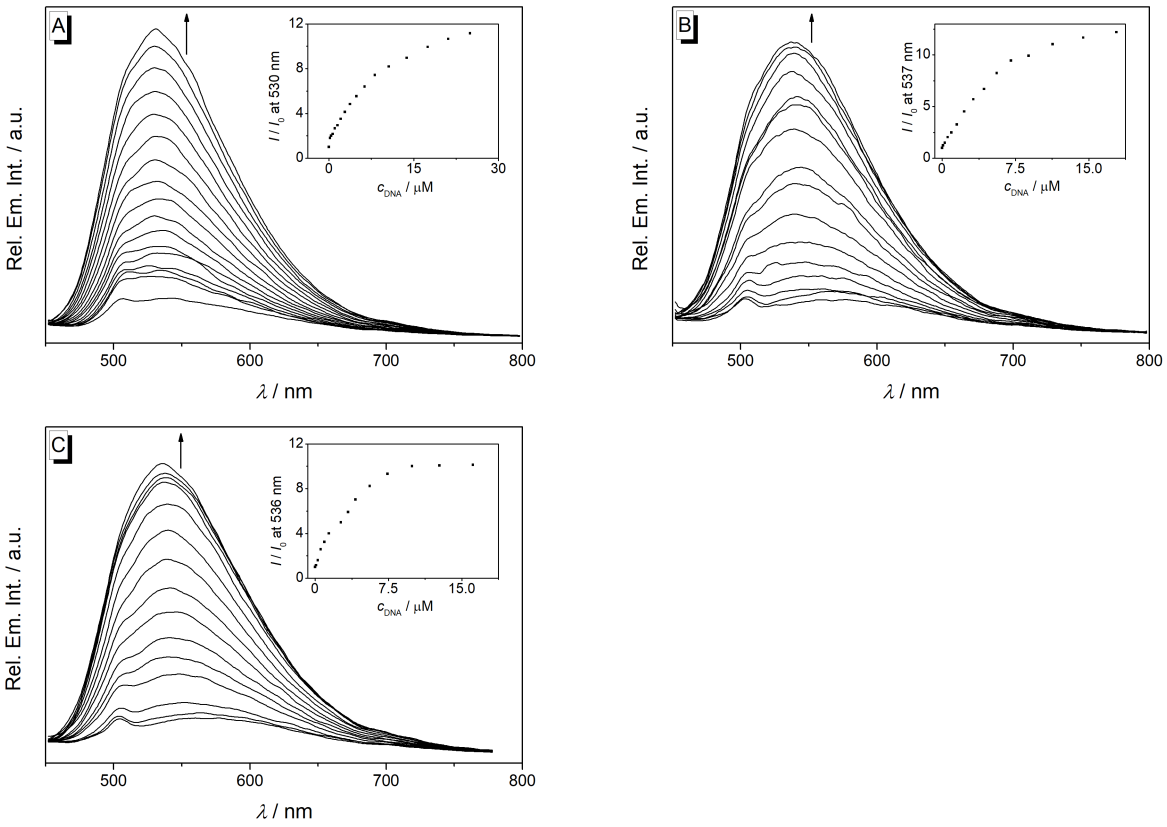

Figure S4. Fluorimetric titration of **2** (c = 20 μM) with **22AG** (A), **c-kit** (B) and **c-myc** (C) in K-phosphate buffer (c_{K+} = 110 mM, pH 7.0, with 5% v/v DMSO); λ_{ex} = 430 nm. The arrows indicate the changes of the emission bands upon addition of DNA. Inset: Plot of the relative fluorescence intensity *versus* c_{DNA}.

The binding constants, K_b, were determined from binding isotherms resulting from the photometric titration (Figure S6) and fitting of the experimental data to the theoretical model according to equation 2 [3].

$$\frac{I}{I_0} = 1 + \frac{Q-1}{2}\left(A + xn + 1 - \sqrt{(Q + xn + 1)^2 - 4xn}\right) \qquad \text{(eq. 2)}$$

$Q = I / I_0$ = Minimal absorbance in the presence of excess ligand

n = Number of independent binding sites per DNA

$A = 1 / (K_b \times c_{Ligand})$

$x = c_{DNA} / c_{Ligand}$

Standard derivations (SD) of K_b values were calculated according to equation 4.

$$\text{SD}\ (K_b) = \{(\text{SD of}\ A\ /\ A)\ /\ A\}\ /\ c_{\text{Ligand}} \qquad \text{(eq. 4)}$$

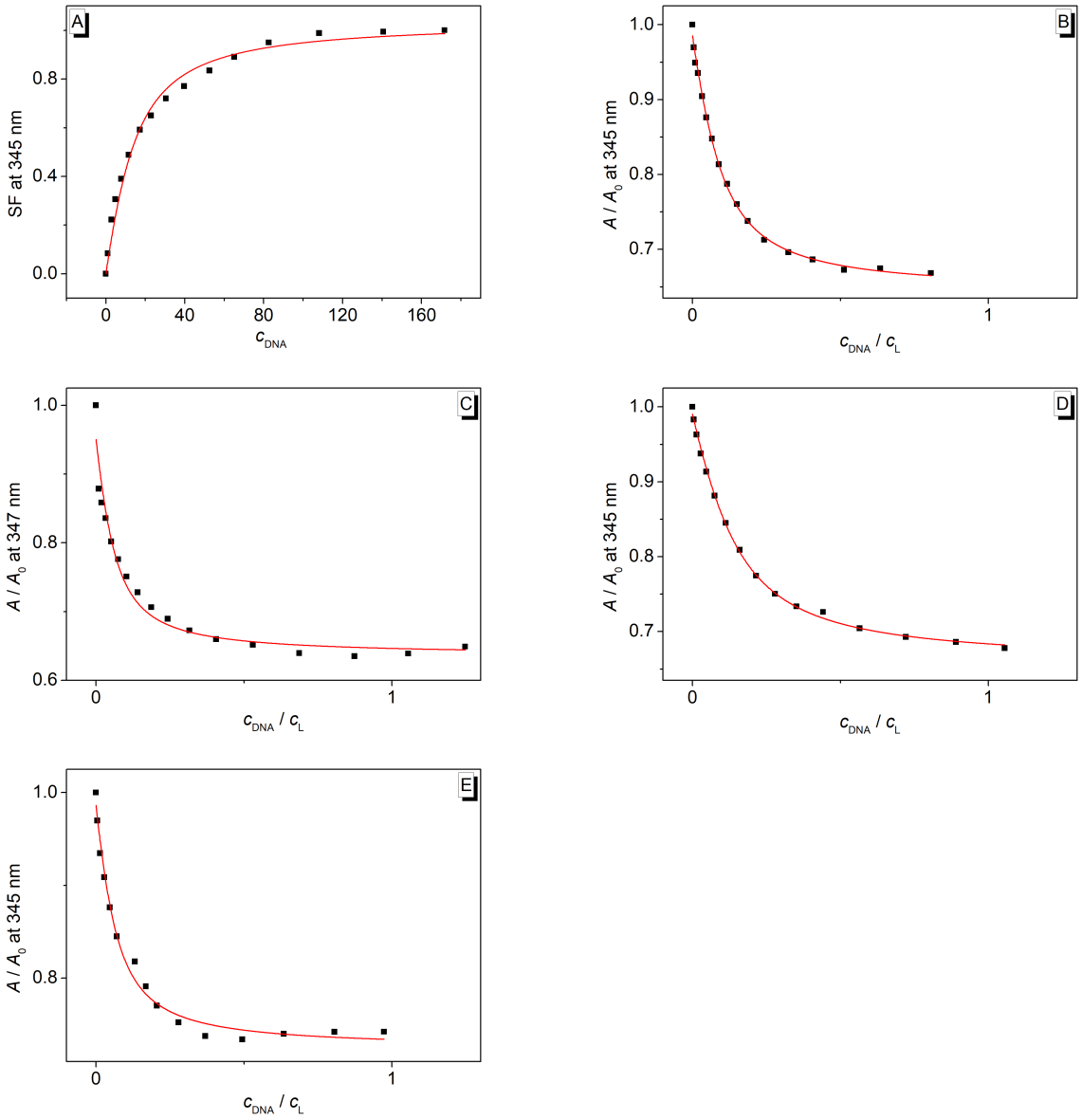

Figure S5. Fitting curves of binding isotherms from photometric titrations of **2** with ct DNA (A), **a2** (B), **22AG** (C), **c-kit** (D) and **c-myc** (E) for the determination of binding constants (K_b). Red lines represent the best fits of the experimental data to the theoretical model.

5. Thermal DNA-denaturation experiments

Thermal DNA-denaturation experiments with the dye-labeled quadruplex-forming oligonucleotides **F21T**, **Fa2T**, **FmycT**, and **FkitT** were performed according to published procedure [4]. ^{FRET}S values were calculated according to equation 3 [5].

$$^{FRET}S = \Delta T_{m,\ G4\text{-}DNA+ds26} / \Delta T_{m,\ G4\text{-}DNA} \qquad \text{(eq. 3)}$$

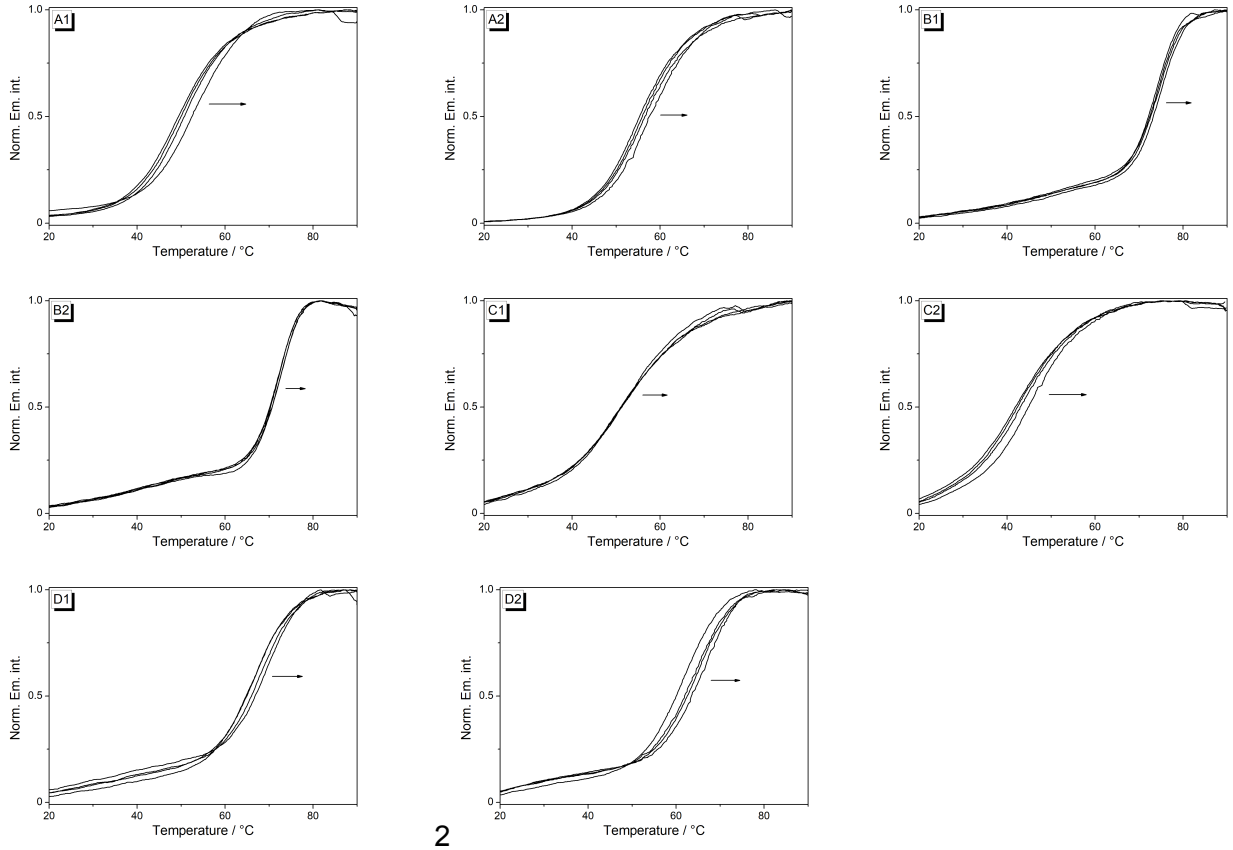

Figure S6. Normalized changes of emission intensity of the dye-labeled oligonucleotides **F21T** (A), **Fa2T** (B), **FkitT** (C), **FmycT** (D) (0.2 μM, λ_{ex} = 470 nm) at 515 nm between 20 °C and 90 °C on addition of ligand **2** in the absence (1) and presence (2) of **ds26**. Thermal denaturation experiments were performed in aqueous KCl-LiCl-Na-cacolydate buffer (c_{K+} = 10 mM, c_{Na+} = 10 mM, c_{K+} = 90 mM, pH 7.2) at different ligand-DNA ratios (0, 1.3, 2.5, 5.0).

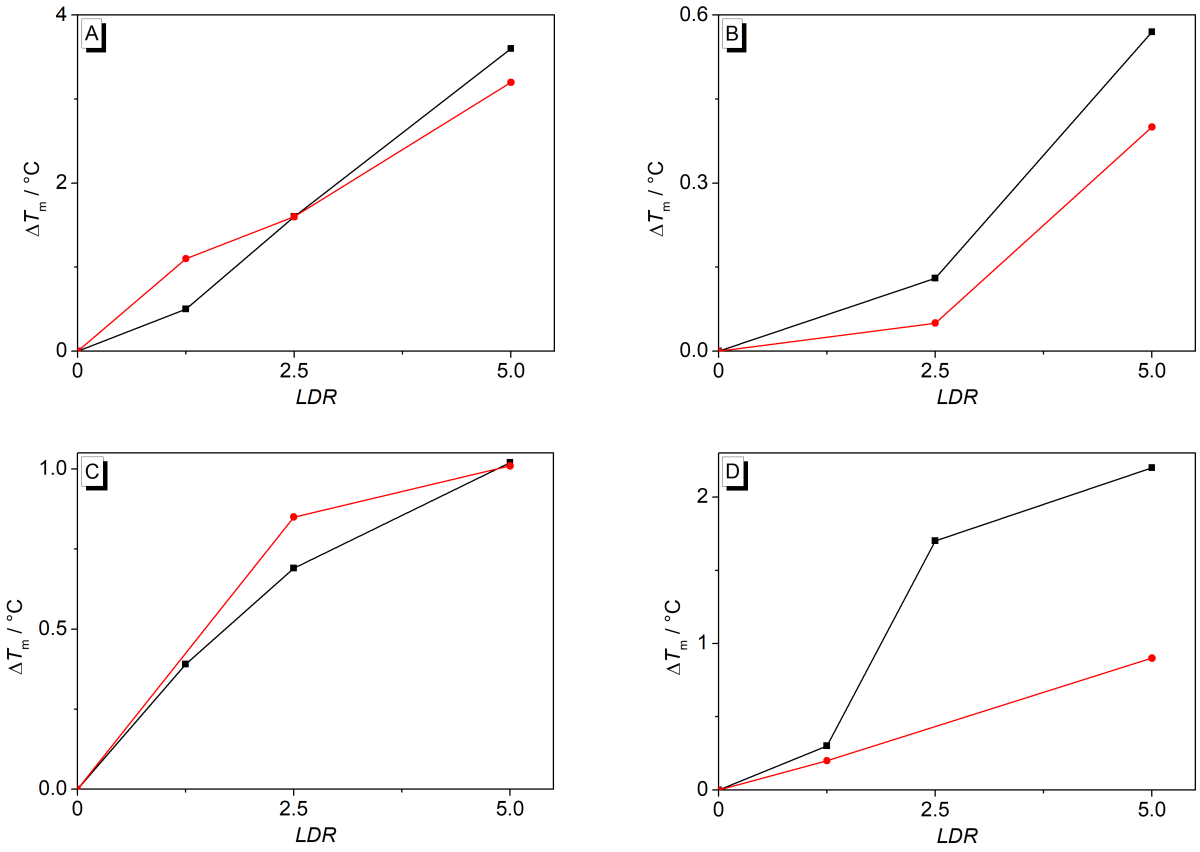

Figure S7. Induced shift of the melting temperature, ΔT_m, of **F21T** (A), **Fa2T** (B), **FkitT** (C) and **FmycT** (D) (c = 50 µM) upon addition of ligand **2** in the absence (black) and presence (red) of **ds26**. Thermal DNA-denaturation experiments were performed in aqueous KCl-LiCl-Na-cacolydate buffer (c_{K+} = 10 mM, c_{Na+} = 10 mM, c_{K+} = 90 mM, pH 7.2) at different ligand-DNA ratios (0, 1.3, 2.5, 5.0).

6. CD- and LD-spectroscopic analysis

CD and LD spectra were recorded in BPE buffer solution with ct DNA (c_{DNA} = 20.0 µM, with 5% v/v DMSO) and in K-phosphate buffer solution with **22AG, a2**, **c-kit**, **c-myc** (c_{DNA} = 20.0 µM, with 5% v/v DMSO) at different ligand-DNA ratios (*LDR*). The CD and LD measurements with ct DNA were performed at *LDR* = 0, 0.1, 0.2, 0.5, 1.0. CD signals were recorded with band width of 1 nm, recording speed of 1 nm/s and time per data point of 0.5 seconds. All samples were measured after an equilibration time of 1 h.

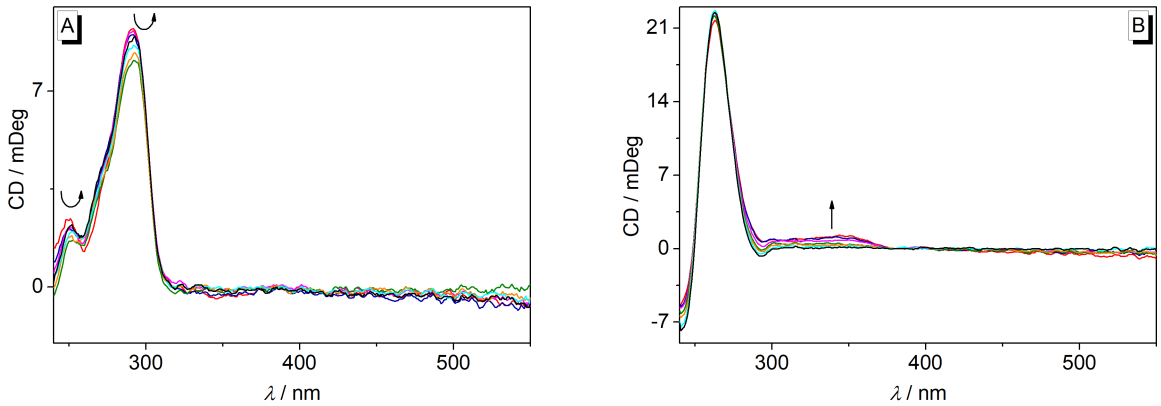

Figure S8. CD spectra of oligonucleotides **22AG** (A) and **c-kit** (B) (c_{DNA} = 20 μM) in the absence and presence of **2** [LDR = 0 (black), 0.05 (cyan), 0.2 (orange), 0.5 (green), 1.0 (magenta), 1.5 (blue), 2.0 (red)] in K-phosphate buffer (c_{K+} = 110 mM, pH 7.0, with 5% v/v DMSO). The arrows indicate the changes of CD bands upon addition of DNA.

7. NMR spectra

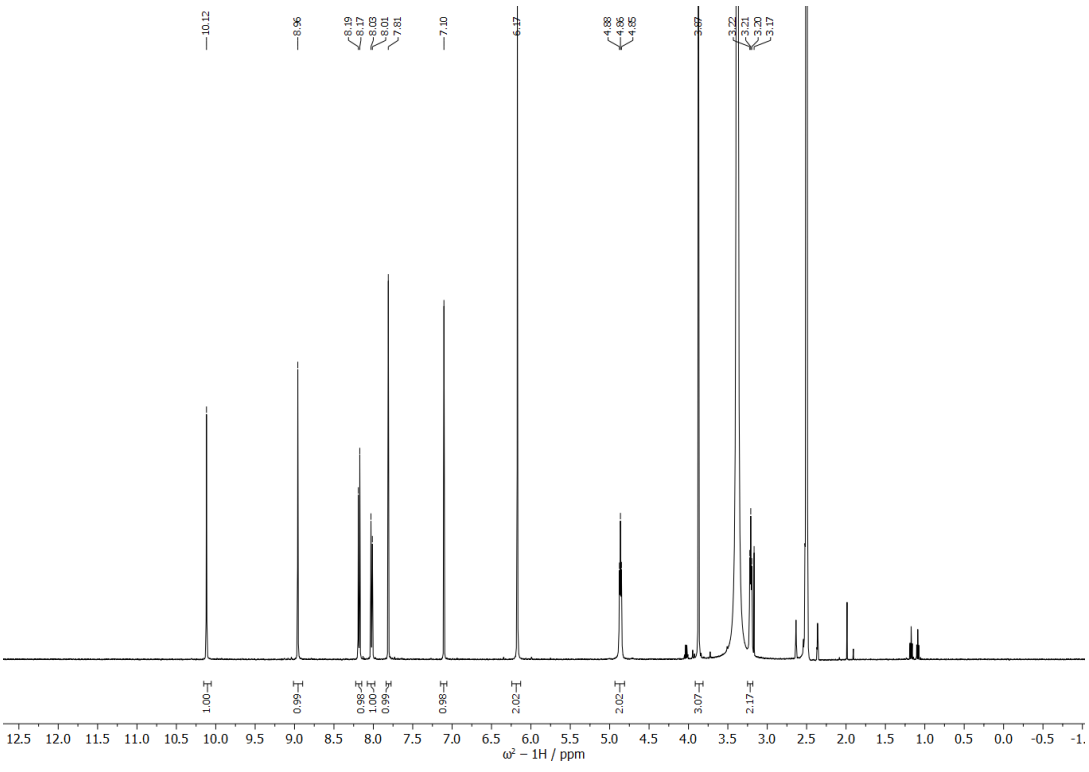

Figure S9. ^{1}H-NMR spectrum (500 MHz) of **2** in DMSO-d_6.

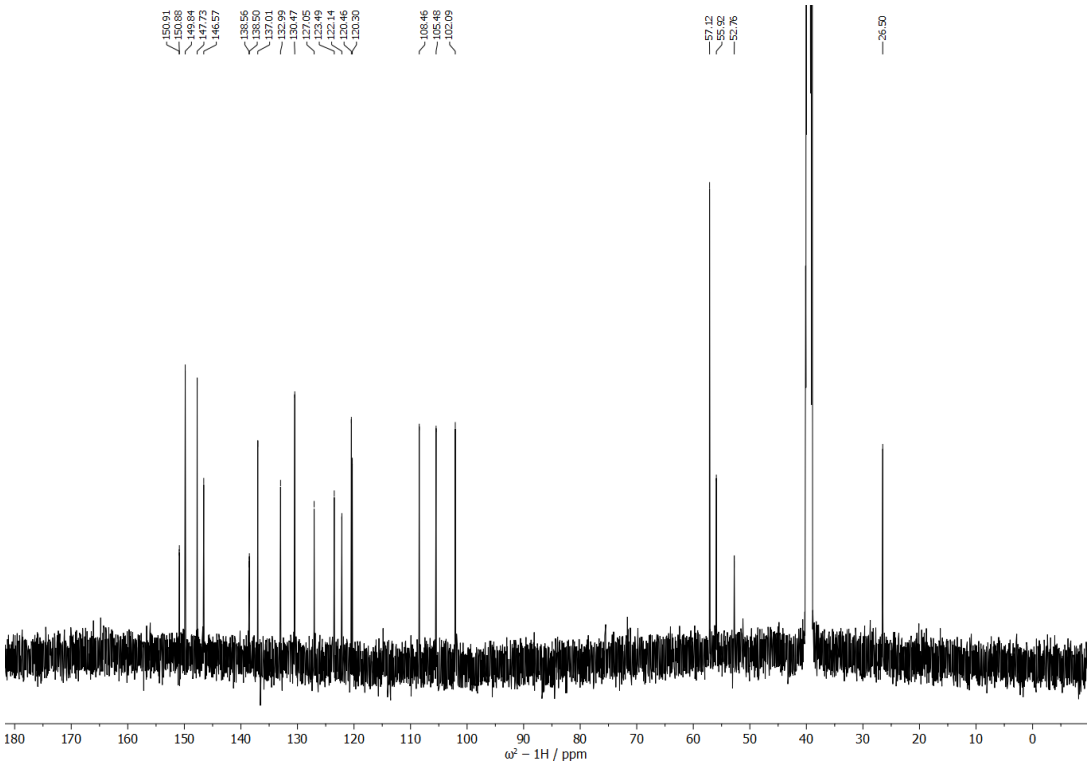

Figure S10. ^{13}C-NMR spectrum (125 MHz) of **2** in DMSO-d_6.

8. References

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