

## Electronic Supplementary Information

### Interaction of Spermine-Alanine Functionalized Perylene Bisimide Dye Aggregates with ds-DNA/RNA Secondary Structure

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### 1) General Information

Solvent and reagents were purchased from commercial sources, unless otherwise stated, and purified and dried according to standard procedures.<sup>[S1]</sup> Reactions were monitored by TLC on silica gel plates (Merck TLC Silica gel 60 F<sub>254</sub> aluminium sheets). All perylene derivatives are colored, so no additional visualization of the spots was necessary. Amines were visualized by spraying the TLC plate with a ninhydrine solution.<sup>[S2]</sup> Column chromatography was performed on silica gel (Merck Silica 60, particle size 0.04-0.063 mm). Semi-preparative HPLC was performed on a Jasco system (PU 2080 PLUS) with a UV/Vis detector (UV 2077 PLUS) using a semi-preparative NUCLEOSIL<sup>®</sup> C8 column (Macherey& Nagel). NMR experiments were conducted on a BrukerAvance 400 with TMS or residual undeuterated solvent as internal standard. The chemical shifts are reported in ppm from TMS ( $\delta$  scale). The apparent coupling constants  $J$  are given in Hertz (Hz). The description of the signal fine structure means: s = singlet, br. s = broad signal, d = doublet, t = triplet, q = quartet, dt = doublet of triplet, tt = triplet of triplet, m = multiplet. All melting points were measured with a Linkam TP 94 heating stage and are uncorrected. Mass spectra were performed on Bruker MALDI-TOF (autoflex II) and Bruker ESI-TOF (microTOF focus) spectrometers.

### 2) UV/Vis Absorption and Fluorescence Spectroscopy for General PBI Characterization

All absorption spectra were measured on a Perkin-Elmer Lambda 950 spectrometer equipped with a Peltier system as temperature controller in conventional quartz cells of appropriate path length. The spectral bandwidth and the scan rate were 2 nm and 140 nm/min, respectively. Either spectroscopically pure chloroform (Uvasol<sup>®</sup>) or MilliQ (deionized) water was used for measurements. The stock solutions for the concentration-dependent measurements of PBIs *L-5* and *D-5* were prepared by dissolving the accurately weighed compound in an appropriate volume of water. Dilutions of these stock solutions were used for absorption measurements over the indicated concentration or temperature range.

All emission spectra were recorded on a PTI QM4-2003 fluorescence spectrometer and were corrected against photomultiplier and lamp intensity. A long wavelength range emission corrected Hamamatsu photomultiplier R928 was used. Quartz glass cuvettes with a thickness of 10 mm were used for the measurements. Fluorescence quantum yields  $\Phi_{\text{fl}}$  were determined in MilliQ water vs. fluorescein ( $\Phi_{\text{fl}} = 0.92$  in 0.1 N NaOH) as reference.<sup>[S3]</sup> The given quantum yields are averaged from values measured at three different excitation wavelengths with OD 0.02 – 0.05 in the absorption maximum (standard deviation  $\sigma = 1\% - 3\%$ ).

### 3) Fluorescence, CD Spectroscopy and Viscometry Studies for the Characterization of PBI-Polynucleotide Interactions

CD spectra were recorded on a JASCO 815 and fluorescence spectra were recorded on a Varian Eclipse instrument. All experiments were performed in 1 cm path quartz cuvettes and by proper temperature control with Peltier elements or thermostats.

Viscometry measurements were conducted with an Ubbelohde viscometer system AVS 370 (Schott). The temperature was maintained at 25  $\pm$  0.1  $^{\circ}\text{C}$ . Aliquots of compound stock solutions were added to 3.0 mL of  $5 \times 10^{-4}$  mol dm<sup>-3</sup> *ct*-DNA solution in sodium cacodylate buffer,  $I = 0.05$  M, pH=7, with a compound to DNA phosphate ratio  $r < 0.1$ . Dilution never exceeded 1% and was corrected for in the calculations. The flow times were measured with a

digital stopwatch, while each sample was measured at least five times optically by light barrier with a deviation of  $\pm 0.1$  s. The viscosity index  $\alpha$  was obtained from the flow times at varying  $r$  according to the following equation:

$$L/L_0 = [ (t_r - t_0) / (t_{\text{DNA}} - t_0) ]^{1/3} = 1 + \alpha \cdot r$$

$t_0$ ,  $t_{\text{DNA}}$  and  $t_r$  denote the flow times of buffer, free DNA and DNA complex at compound / phosphate ratio  $r$ , respectively;  $L/L_0$  is the relative DNA lengthening. The  $L/L_0$  vs  $r$ -plot was fitted to a straight line that gave slope  $\alpha$ . The error in  $\alpha$  is  $< 0.1$ .

#### 4) Synthesis and Characterization of PBIs

**Synthesis of threefold 'Boc-protected spermine4:**<sup>[S4]</sup> A solution of spermine (1.00 g, 4.96 mmol) in methanol (70 mL) was cooled to  $-78$  °C under argon atmosphere. Trifluoroacetic acid ethylester (0.59 mL, 0.70 g, 4.96 mmol) was added dropwise to the solution within 30 min. The colorless suspension was stirred for 60 min at  $-78$  °C and subsequently warmed up to  $0$  °C within 3 h. A solution of di-*t*-butyldicarbonate (4.33 g, 19.5 mmol) in methanol (10 mL) was added dropwise within five minutes. The reaction solution was stirred for additional 20 h under argon atmosphere at room temperature. After this time period, the pH value of the solution was adjusted to 11 with concentrated aqueous ammonia solution (30 mL). The solution was stirred again for 24 h at room temperature. After removal of the solvent in vacuo ( $45$  °C, 11 mbar) the highly viscous oil was purified by column chromatography ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2$  : MeOH : conc. aq.  $\text{NH}_3$  = 70 : 10 : 1 v/v/v) to give 0.75 g (1.49 mmol, 30%) of **4** as a colorless oil. MW ( $\text{C}_{25}\text{H}_{50}\text{N}_4\text{O}_6$ ) = 502.69 g/mol;  $R_f$  = 0.5 ( $\text{CH}_2\text{Cl}_2$  : MeOH : conc. aqua.  $\text{NH}_3$  = 50:10:1 v/v/v);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  = 3.36-3.04 (m, 10H), 2.71 (t,  $J$  = 6.6 Hz, 2H), 1.74-1.55 (m, 4H), 1.52-1.35 (m, 33H); HRMS (ESI pos.):  $m/z$  = 503.3808; calcd. for  $\text{C}_{25}\text{H}_{50}\text{N}_4\text{O}_6 + \text{H}^+$  : 503.3808.

**General procedure for the synthesis of PBI dicarboxylic acids *L*- and *D*-3:**<sup>[S5]</sup> Perylene tetracarboxylic bisanhydride **1** (2.5 mmol, 1 equiv.), the appropriate  $\alpha$ -amino acid (*L*- or *D*-**2**; 2.1 eq) and imidazole (40 equiv.) were mixed in a flask which was flooded with argon gas for 15 minutes. The mixture was heated to  $120$  °C for 30 minutes and then cooled to  $85$  °C. The pH value of the reaction mixture was adjusted to 3 – 4 with 1 N HCl. The precipitate was collected by suction filtration, washed with water and dried in high vacuum ( $2 \times 10^{-3}$  mbar,  $60$  °C).

***L*-3:** Yield: 1.32 g (2.47 mmol, 97 %) of a red solid; MW ( $\text{C}_{30}\text{H}_{18}\text{N}_2\text{O}_8$ ) = 534.47 g/mol; m.p.:  $> 400$  °C;  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ ):  $\delta$  = 8.23 (d,  $J$  = 8.0, 4H), 8.18 (d,  $J$  = 8.0, 4H), 5.59 (q,  $J$  = 7.0 Hz, 2H), 1.68 (d,  $J$  = 7.1, 6H); MS (MALDI, matrix: DCTB):  $m/z$  = 534.101; calcd for  $\text{C}_{30}\text{H}_{18}\text{N}_2\text{O}_8 + \text{e}^-$  : 534.106.

***D*-3:** Yield: 1.32 g (2.47 mmol, 97 %) of a dark red solid; MW ( $\text{C}_{30}\text{H}_{18}\text{N}_2\text{O}_8$ ) = 534.47 g/mol; m.p.:  $> 400$  °C;  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ ):  $\delta$  = 8.23 (d,  $J$  = 7.3, 4H), 8.18 (d,  $J$  = 8.0, 4H), 5.59

(q,  $J = 7.0$ , 2H), 1.68 (d,  $J = 7.1$ , 6H); MS (MALDI, matrix: DCTB):  $m/z = 534.101$ ; calcd for  $C_{30}H_{18}N_2O_8 + e^-$ : 534.106.

**General procedure for the synthesis of the fully protected PBIs *L*- and *D*-5:** A solution of the respective PBI *L*- and *D*-3 (0.28 mmol, 1 equiv.), threefold<sup>t</sup>Boc-protected spermine<sup>4</sup> (2.1 equiv.), DCC (3 equiv.), HOBt (0.8 equiv.) and Hünig's base (0.1 mL) were stirred in DMF (5 mL) at 80 °C for 4 h. After the mixture was cooled down to room temperature, water (50 mL) and  $CH_2Cl_2$  (50 mL) were added. The phases were separated and the aqueous phase was extracted with  $CH_2Cl_2$  ( $2 \times 50$  mL). The combined organic phases were washed with water (200 mL) and concentrated under reduced pressure. The crude product was purified by column chromatography ( $SiO_2$ ,  $CH_2Cl_2$  : MeOH = 99 : 1 to 97 : 3) and subsequent HPLC (Nucleosil<sup>®</sup>,  $CH_2Cl_2$  : MeOH = 97 : 3).

**Sixfold<sup>t</sup>Boc-protected *L*-5:** Yield: 187 mg (0.12 mmol, 44 %) of a red solid; MW ( $C_{80}H_{114}N_{10}O_{18}$ ) = 1503.82 g/mol;  $R_f = 0.22$  ( $SiO_2$ ,  $CH_2Cl_2$  : MeOH = 97 : 3); m.p.: 100 – 102 °C;  $^1H$  NMR ( $CDCl_3$ ):  $\delta = 8.69$  (d,  $J = 7.9$ , 4H), 8.63 (d,  $J = 8.0$ , 4H), 7.62 (br.s, 2H), 5.78 (q,  $J = 6.72$ , 2H), 3.26 – 3.02 (m, 24H), 1.79 (d,  $J = 7.2$ , 6H), 1.62 (br. s, 8H), 1.42 (s, 46H), 1.01 (s, 16H); the amide and carbamate NH signals are broadened due to the fast exchange with the deuterated solvent; HRMS (APCI pos.):  $m/z = 1537.7994$ ; calcd for  $C_{80}H_{114}N_{10}O_{18} + Cl^-$ : 1537.8007; UV/Vis ( $CH_2Cl_2$ ,  $c = 5 \times 10^{-5}$  M):  $\lambda_{max}$  (nm) [ $\epsilon_{max}$  ( $M^{-1}cm^{-1}$ )] = 458.5 [18500], 489.5 [51200], 526.5 [85000].

**Sixfold<sup>t</sup>Boc-protected *D*-5:** Yield: 327 mg (0.21 mmol, 61 %) of a red solid; MW ( $C_{80}H_{114}N_{10}O_{18}$ ) = 1503.82 g/mol;  $R_f = 0.22$  ( $SiO_2$ ,  $CH_2Cl_2$  : MeOH = 97 : 3); m.p.: 99 – 102 °C;  $^1H$  NMR ( $CDCl_3$ ):  $\delta = 8.69$  (d,  $J = 7.6$ , 4H), 8.63 (d,  $J = 7.9$ , 4H), 7.62 (br.s, 2H), 5.79 (q,  $J = 6.8$ , 2H), 3.27 – 3.02 (m, 24H), 1.79 (d,  $J = 7.2$ , 6H), 1.62 (br. s, 8H), 1.42 (s, 46H), 1.01 (s, 16H); the amide and carbamate NH signals are broadened due to the fast exchange with the deuterated solvent; HRMS (ESI pos.):  $m/z = 1503.8371$ ; calcd for  $C_{80}H_{114}N_{10}O_{18} + H^+$ : 1503.8389; UV/Vis ( $CH_2Cl_2$ ,  $c = 5 \times 10^{-5}$  M):  $\lambda_{max}$  (nm) [ $\epsilon_{max}$  ( $M^{-1}cm^{-1}$ )] = 458.5 [18500], 489.5 [51100], 526.5 [85000].

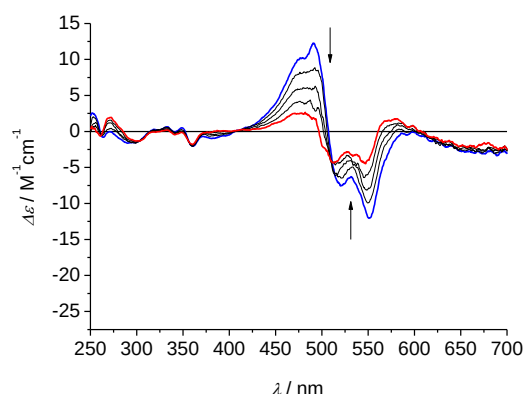
**General procedure for the synthesis of the target compounds PBIs *L*- and *D*-5:** A solution of the respective <sup>t</sup>Boc-protected PBI *L*- or *D*-5 ( $8 \times 10^{-6}$  mol, 1 equiv.) in trifluoroacetic acid (3 mL) was stirred for 2 h at room temperature and concentrated *in vacuo* afterwards. MilliQ(deionized) water (5 mL) was added to the residue, yielding a dark red solution, which was lyophilized. Yield: 100 % for each, as a red fluffy solid.

***L*-5:** MW ( $C_{62}H_{72}F_{18}N_{10}O_{18}$ ) = 1587.26 g/mol; m.p.: > 400 °C;  $^1H$  NMR ( $[D_6]DMSO$ ):  $\delta = 9.02$  (d,  $J = 8.0$ , 4H), 8.72 (br. s, 4H), 8.62 (d,  $J = 7.8$ , 4H), 8.52 (br. s, 4H), 8.13 (t,  $J = 5.9$ , 2H), 7.88 (br. s, 6H), 5.56 (q,  $J = 6.9$ , 2H), 3.15 (dt,  $J = 7.8$ ,  $J = 5.76$ ), 2.99 – 2.86 (m, 20H), 1.89 (tt,  $J = 7.8$ ,  $J = 7.4$ , 4H), 1.72 (tt,  $J = 7.6$ ,  $J = 6.0$ , 4H), 1.64 (br. s, 8H), 1.58 (d,  $J = 7.0$ , 6H); HRMS (ESI pos.):  $m/z = 903.5239$ ; calcd for  $C_{50}H_{66}N_{10}O_6 + H^+$ : 903.5240; UV/Vis ( $H_2O$ ,  $c = 5 \times 10^{-7}$  M):  $\lambda_{max}$  (nm) [ $\epsilon_{max}$  ( $M^{-1}cm^{-1}$ )] = 466 [18000], 497 [48500], 534 [77200]; fluorescence ( $H_2O$ ,  $\lambda_{ex} = 475$  nm):  $\lambda_{max}$  (nm) = 547;  $\Phi_{fl} = 0.72$ .

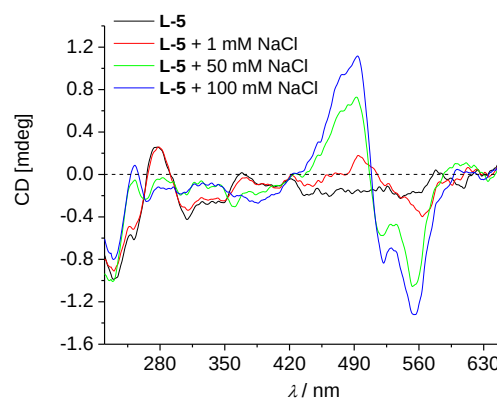
**D-5:** MW ( $C_{62}H_{72}F_{18}N_{10}O_{18}$ ) = 1587.26 g/mol; m.p.: > 400 °C;  $^1H$  NMR ( $[D_6]DMSO$ ):  $\delta$  = 9.01 (d,  $J$  = 8.56, 4H), 8.74 (br. s, 4H), 8.62 (d,  $J$  = 7.4, 4H), 8.53 (br. s, 4H), 8.13 (t,  $J$  = 6.4, 2H), 7.89 (br. s, 6H), 5.56 (q,  $J$  = 6.9, 2H), 3.15 (dt,  $J$  = 7.5,  $J$  = 8.0), 3.01 – 2.84 (m, 20H), 1.89 (tt,  $J$  = 7.4,  $J$  = 7.5, 4H), 1.76 – 1.68 (m, 4H), 1.65 (br. s, 8H), 1.58 (d,  $J$  = 6.9, 6H); HRMS (ESI pos.):  $m/z$  = 903.5239; calcd for  $C_{50}H_{66}N_{10}O_6 + H^+$ : 903.5240.

## 5) CD Spectroscopy

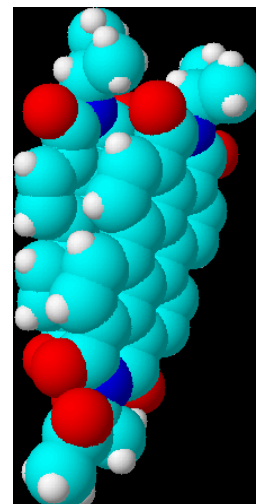
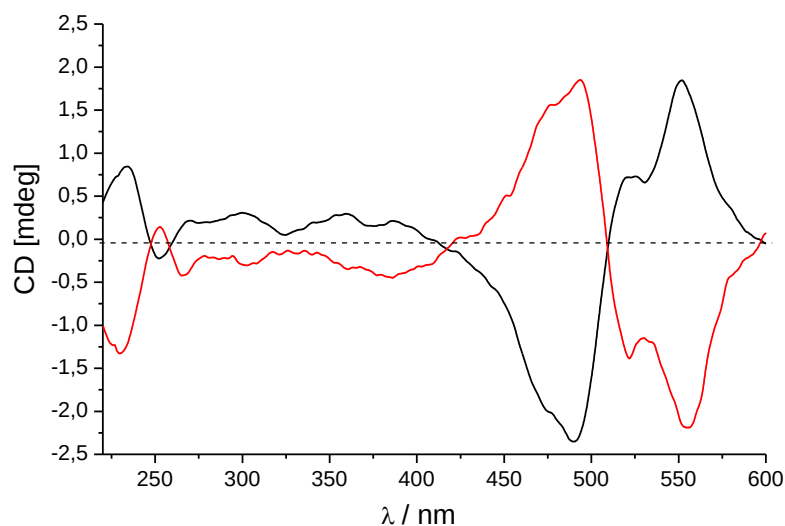
a)



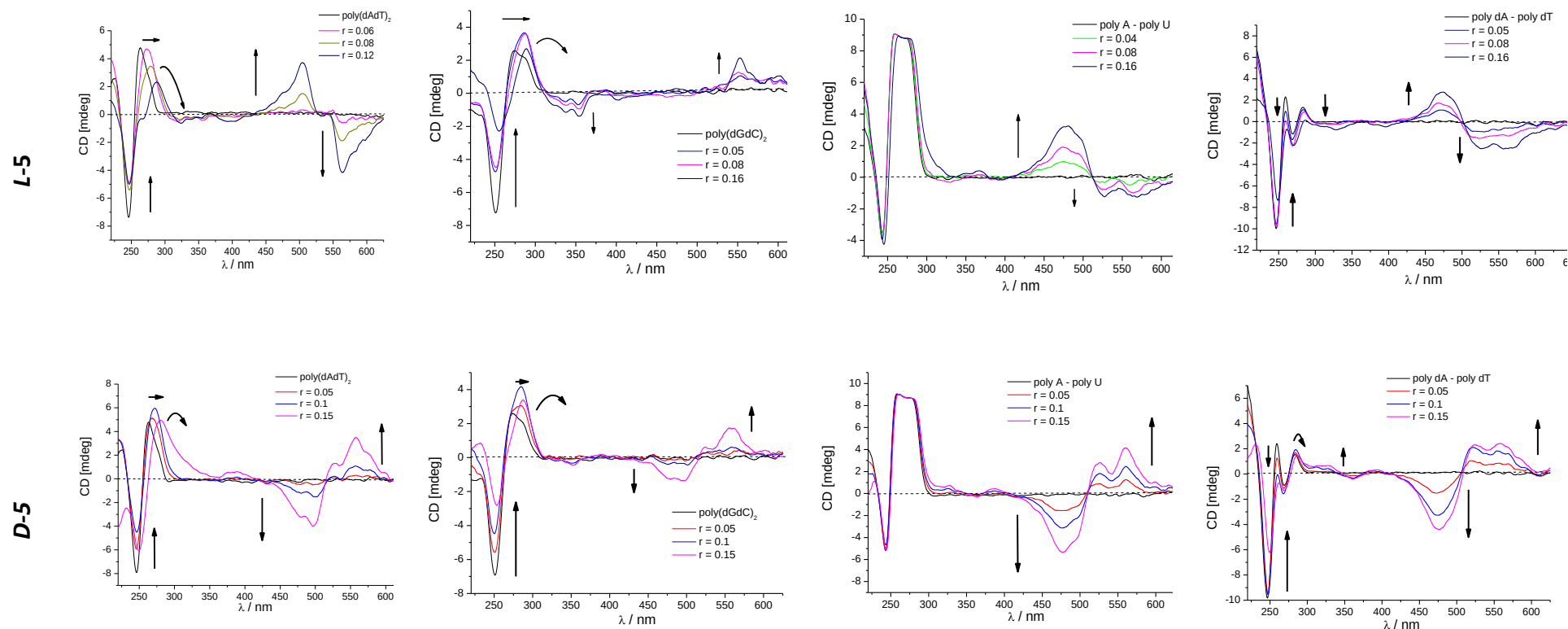
b)



**Figure S1.** a) Temperature-dependent CD spectra for *L-5* ( $c = 5 \times 10^{-3}$  M in water) from +10 °C to +50 °C; b) changes in the CD spectrum of *L-5* ( $c = 5 \times 10^{-6}$  M) upon successive addition of aliquots of 1M NaCl.



**Figure S2.** CD spectra of *L-5* (red line) and *D-5* (black line) aggregates ( $c = 1.0 \times 10^{-5}$  M) at 100 mM NaCl and model for the helical stacking arrangement between two enantiomers.

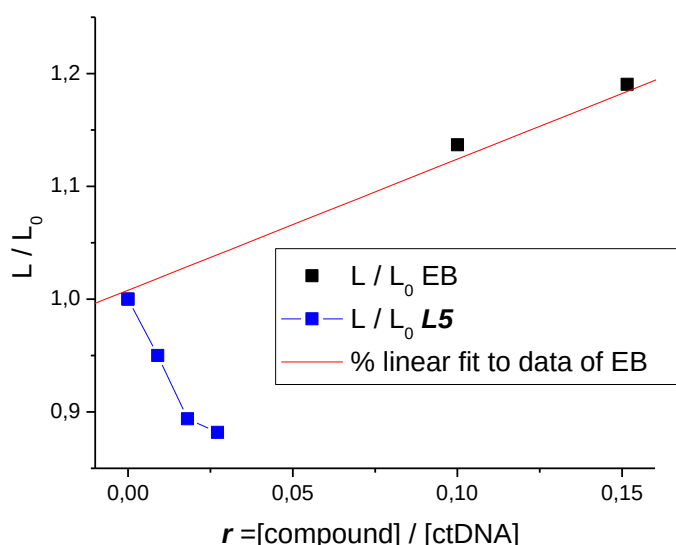


**Figure S3.** CD spectra of various DNA/RNA samples ( $c = 4 \times 10^{-5}$  M) in the presence of *L*-5 (top row) or *D*-5 (bottom row) at pH 7.0 in sodium cacodylate buffer ( $I = 0.05$  M).

## 6) Viscometry measurements

Upon intercalation of aromatic moieties in ds-DNA/RNA an increase of contour length is typically observed which is conveniently monitored by measuring the viscosity of sonicated rod like fragments of ds-DNA/RNA as a function of ligand binding ratio,  $r[\text{compound}] / [\text{polynucleotide}]$ .

Classical monointercalators like ethidium bromide (**EB**), proflavine and 9-aminoacridine have values of helix extension parameter (viscosity index) $\alpha$  of about 0.8-0.9<sup>[S10]</sup>. Complexes and ligands that bind exclusively or at least partially in the DNA grooves typically cause less pronounced or no changes in DNA/RNA solution viscosity.



**Figure S4.** Viscometric analysis of ct-DNA with **L-5** and **EB** as a referent intercalator. Aliquots of compound stock solutions were added to ct-DNA solution ( $c = 5 \times 10^{-4} \text{ mol dm}^{-3}$ ) in sodium cacodylate buffer,  $I = 0.05 \text{ mol dm}^{-3}$ , pH=7.

Viscometric analysis of ct-DNA with **L-5** revealed pronounced viscosity decrease already for the ratio  $r = 0.02 - 0.05$ , excluding intercalation of **L-5** into ds-DNA as the dominant binding mode<sup>[S8]</sup>. Similar viscosity decrease was observed for other DNA binders<sup>[S9]</sup> and attributed to groove binding, which, in contrast to intercalation, can shorten the DNA helix by the substantial neutralization of DNA backbone negative charge. Here presented PBIs can obviously cause such collapse of the double helical rod-like structure of ds-DNA by the positively charged spermine side chains.

## 7) Properties of the secondary structure of the most common ds-DNA/RNA.

**Table S1:** Minor groove widths for selected nucleic acid conformations.<sup>[S6,S7]</sup>

| Structure type                       | Minor groove width [Å] |
|--------------------------------------|------------------------|
| A-DNA                                | 11.0                   |
| rA <sub>n</sub> rU <sub>n</sub>      | 10.9                   |
| (dGdC) <sub>n</sub>                  | 9.5                    |
| (dAdT) <sub>n</sub>                  | 6.3                    |
| C-DNA                                | 4.8                    |
| d(A) <sub>n</sub> -d(T) <sub>n</sub> | 3.3                    |

## 8) Notes and References

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