Electronic Supporting Information

Short, rigid linker between pyrene and guanidinocarbonyl-pyrrole induced new set of spectroscopic responses to ds-DNA secondary structure

Marijana Radić Stojković,a Patrycium Piotrowski,b Carsten Schmuck,b,* Ivo Piantanidaa*

Experimental
Materials and methods
Synthetic details and characterisation of new compounds are given in ESI†. The UV/vis spectra were recorded on a Varian Cary 100 Bio spectrophotometer and the CD spectra on a JASCO J815 spectrophotometer at 25°C using 1 cm path quartz cuvettes. To study the interactions with DNA and RNA, aqueous solutions of the respective compounds buffered to pH 7.0 or pH 5.0 (buffer sodium cacodylate, I=0.05 M) were used.
Polynucleotides were purchased as noted: poly A-poly U, poly dAdT-poly dAdT and poly dGdC – poly dGdC (Sigma) and calf thymus (ct)-DNA (Aldrich). Polynucleotides were dissolved in sodium cacodylate buffer, I = 0.05 M, pH = 7.0. Calf thymus (ct)-DNA was additionally sonicated and filtered through a 0.45 µm filter.1,2 Polynucleotide concentration was determined spectroscopically2 and is given as the concentration of phosphate groups. Spectrophotometric titrations were performed and pH 7.0 or pH 5.0 (I=0.05 M, buffer sodium cacodylate) by adding portions of polynucleotide solutions into the solution of the compounds for UV/vis. For CD experiments aliquots of the compound stock solution were added into the solution of the polynucleotide. Titration data were processed using the Scatchard equation.3 Values for $K_s$ and $n$ given in Table 1 all have satisfactory correlation coefficients (>0.999).
Thermal melting curves for DNA, RNA and their complexes with the compounds studied were determined as previously described4 by following the absorption change at 260 nm as a function of temperature. Absorbance of the ligands was subtracted from every curve and the absorbance scale was normalized. The $T_m$ values are the midpoints of the transition curves determined from the maximum of the first derivative and checked graphically by the tangent method.2 The $\Delta T_m$ values were calculated subtracting $T_m$ of the free nucleic acid from $T_m$ of the complex. Every $\Delta T_m$ value here reported was the average of at least two measurements. The error in $\Delta T_m$ is ±0.5°C.
Scheme 1. Synthesis of pyrene-functionalized amino acid 12 and cationic guanidinio compound 5 (Boc = butoxycarbonyl, PIDA = (diacetoxyiodo)benzene, Cbz = carbobenzyloxy, MeOH = methanol, PyBOP = (benzotriazol-1-yl oxy)tripyrrolidinophosphonium hexafluorophosphate, TFA = trifluoroacetic acid, NMM = N-methylmorpholine, DCM = dichloromethane, NEt3 = Triethylamine).

Compound 5 was synthesized according to Scheme 1. The synthesis of the pyrene-functionalized artificial amino acid 12 started with a Hoffmann-type amide degradation of Boc-L-asparagine-OH 6 to the zwitter-ion 7 with PIDA in a mixture of ethyl acetate, acetonitrile and water (2:2:1) with a yield of 90 %. Cbz protection of the amine function with Cbz-Cl in toluene (8, 92 %), followed by methylation of the carboxylic acid group with methyl iodide and potassium carbonate gave the orthogonally protected amino acid 9 in 87 % yield. Hydrogenolytic Cbz deprotection to the amine 10 proceeded with 96 % yield and coupling to 1-pyrenecarboxylic acid 11 with PyBOP and NMM in a DMF/DCM mixture (1:1) gave the orthogonally protected, pyrene-functionalized amino acid 12 in 78 % yield. Boc-deprotection of 12 to the amine 13 (99 %) was achieved with TFA in DCM. Coupling of 13 to the GCP binding motive 14 with PyBOP and NMM in a DMF/DCM mixture (1:1) gave the
methyl ester 15 in 80% yield. Final acidic Boc-deprotection and treatment with aqueous hydrochloric acid provided the cationic compound 5 with 97% yield.

N$_{\alpha}$-Boc-$\beta$-amino-$L$-alanine 7
A suspension of 6 (5.0 g, 21.5 mmol, 1.0 eq) and PIDA (8.3 g, 25.7 mmol, 1.2 eq) in a mixture of ethyl acetate, acetonitrile (both 30 mL) and water (15 mL) was stirred at 10 °C overnight. The solvent mixture was evaporated and the colorless residue was sonicated with 20 mL ethyl acetate. The precipitate was filtered off, washed with diethyl ether and dried in vacuo to yield 7 as a colorless solid (4.0 g, 19.5 mmol, 90%). M.p. 206 °C (decomp); $^1$H NMR (300 MHz, [D$_6$]DMSO, 25 °C): $\delta$ = 1.39 (s, 9H, Boc-CH$_3$), 2.71 (m, 1H, CH$_2$), 3.01 (m, 1H, CH$_2$), 3.60 (m, 1H, CH), 6.16 (m, 1H, Boc-NH), 8.10 (br.s, 3H, NH$_3^+$); $^{13}$C NMR (75 MHz, [D$_6$]DMSO, 25 °C): $\delta$ = 28.1 (Boc-CH$_3$), 40.6 (CH$_2$), 50.9 (CH), 78.1 (Boc-Cq), 155.0 (Cq), 170.9 (Cq); IR (film, [cm$^{-1}$]) = 3057 (w), 3341 (s), 2969 (m), 2718 (w), 2653 (w), 1683 (s), 1657 (w), 1524 (s), 1456 (w), 1403 (w), 1364 (m), 1318 (w), 1282 (m), 1251 (w), 1168 (m), 1115 (w), 1056 (w), 1014 (w), 943 (w), 895 (w), 845 (w); HRMS (ESI): $m/z$ = calc. for C$_8$H$_{16}$N$_2$O$_4$: 205.1183 [M+H]$^+$; found 205.1180 [M+H]$^+$.

Cbz-protection of 7 to acid 8
To an ice cold suspension of 7 (4.0 g, 19.5 mmol, 1.0 eq) in water (30 mL) sodium bicarbonate (4.1 g, 48.8 mmol, 2.5 eq) was added until 7 was dissolved. A solution of Cbz-Cl (4.0 g, 23.4 mmol, 1.2 eq) in toluene (5 mL) was added and the mixture was stirred at 0 °C overnight. The solvent was evaporated and the residue was dissolved in water and extracted with 200 mL diethyl ether twice. After phase separation a concentrated citric acid solution was added to the water phase until a colorless precipitate formed, which was extracted with 200 mL diethyl ether twice. After washing the organic phase with water thoroughly, it was dried with magnesium sulfate, the solvent was evaporated and the colorless solid 8 was dried in vacuo (6.1 g, 18.0 mmol, 92%). M.p. 75 °C; $^1$H NMR (300 MHz, [D$_6$]DMSO, 25 °C): $\delta$ = 1.37 (s, 9H, Boc-CH$_3$), 3.15-3.46 (m, 2H, CH$_2$), 3.99-4.12 (m, 1H, CH), 5.02 (s, 2H, Cbz-CH$_2$), 6.95 (d, 1H, Boc-NH), 7.15-7.47 (m, 1H + 5H, Cbz-NH + Aryl-CH), 12.63 (br.s, 1H, COOH); $^{13}$C NMR (75 MHz, [D$_6$]DMSO, 25 °C): $\delta$ = 28.1 (Boc-CH$_3$), 41.6 (CH$_2$), 53.6 (CH), 65.3 (Cbz-CH$_2$), 78.1 (Boc-Cq), 127.6 (aryl-CH), 127.7 (aryl-CH), 128.3 (aryl-CH), 137.0 (aryl-Cq), 155.2 (Cq), 156.3 (Cq), 172.1 (Cq); IR (film, [cm$^{-1}$]) = 3334 (m), 2976 (m), 2643 (w), 1688 (s), 1598 (m), 1520 (m), 1455 (m), 1404 (m), 1365 (m), 1248 (m), 1164 (m), 1055 (w), 854 (w), 735 (w), 696 (w); LRMS (ESI): $m/z$ = calc. for C$_{16}$H$_{22}$N$_2$O$_6$: 361.14 [M+Na]$^+$; found 361.13 [M+Na]$^+$.

Protection of 8 to the methyl ester 9
A suspension of 8 (3.0 g, 8.8 mmol, 1.0 eq) and potassium carbonate (12.2 g, 88.2 mmol, 10.0 eq) in dry DMF (40 mL) was stirred at 10 °C. Methyl iodide (25.2 g, 177.5 mmol, 20.1 eq) was added slowly and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was dissolved in water and extracted with ethyl acetate (300 mL). The organic phase was dried with magnesium sulfate, the solvent was evaporated and the residue was purified by column chromatography (ethyl
acetate/cyclohexane = 6/4) to yield 9 as a colorless oil (2.7 g, 7.7 mmol, 87 %). \( R_f = 0.74 \) (SiO\(_2\), ethyl acetate/cyclohexane = 6/4); \(^1\)H NMR (300 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 1.38 \) (s, 9H, Boc-CH\(_3\)), 3.27-3.41 (m, 2H, CH\(_2\)), 3.59 (s, 3H, CH\(_3\)), 4.06-4.18 (m, 1H, CH), 5.03 (s, 2H, Cbz-CH\(_2\)), 7.11 (d, 1H, Boc-NH), 7.21-7.45 (m, 5H + 1H, aryl-CH + Cbz-NH); \(^1^3\)C NMR (75 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 28.1 \) (Boc-CH\(_3\)), 41.5 (CH\(_2\)), 51.8 (CH\(_3\)), 53.6 (CH), 65.4 (Cbz-CH\(_2\)), 78.5 (Boc-Cq), 127.6 (aryl-CH), 127.7 (aryl-CH), 128.3 (aryl-CH), 136.9 (aryl-Cq), 155.2 (Cq), 156.2 (Cq), 171.3 (Cq); HRMS (ESI): \( m/z \) = calc. for C\(_{17}\)H\(_{24}\)N\(_2\)O\(_6\): 375.1527 [M+Na]\(^+\); found 357.1560 [M+Na]\(^+\).

Cbz-deprotection of 9 to amine 10
A suspension of 9 (3.0 g, 8.5 mmol) and Pd/C (300 mg, 10%) was stirred in methanol (100 mL) under a hydrogen atmosphere at room temperature until reaction control (tlc, ethyl acetate/cyclohexane = 6/4) showed no more starting material. After filtration through a celite pad to remove the catalyst and washing with 50 mL methanol twice the solvent was evaporated and the residue was dried in vacuo to yield 10 as a colorless oil (1.8 g, 8.2 mmol, 96 %). \(^1\)H NMR (300 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 1.39 \) (s, 9H, Boc-CH\(_3\)), 2.39 (br.s, 2H, NH\(_2\)), 2.62-3.44 (m, 2H, CH\(_2\)), 3.61 (s, 3H, CH\(_3\)), 3.87-4.19 (m, 1H, CH), 7.14 (d, 1H, Boc-NH); \(^1^3\)C NMR (75 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 28.1 \) (Boc-CH\(_3\)), 42.9 (CH\(_2\)), 51.6 (CH\(_3\)), 56.8 (CH), 78.2 (Boc-Cq), 155.5 (Cq), 172.0 (Cq); IR (film, [cm\(^{-1}\)]) = 3317 (m), 2977 (m), 1697 (s), 1514 (m), 1454 (w), 1366 (m), 1275 (m), 1248 (m), 1161 (s), 1050 (w), 1026 (w), 857 (w), 780 (w); HRMS (ESI): \( m/z \) = calc. for C\(_9\)H\(_{18}\)N\(_2\)O\(_4\): 219.1339 [M+H]\(^+\); found 219.1336 [M+H]\(^+\).

Coupling of 10 with 1-pyrenecarboxylic acid 11 to 12
A solution of 11 (1.0 g, 4.0 mmol, 1.0 eq), PyBOP (3.2 g, 6.1 mmol, 1.5 eq), NMM (1.5 mL, 12 mmol, 3.0 eq) and a catalytic amount of DMAP was stirred in a 1:1 mixture of DMF and DCM (100 mL) for 30 minutes at room temperature. To this solution the amine 10 (0.88 g, 4.0 mmol, 1.0 eq), dissolved in DCM (5 mL) and NMM (0.5 mL), was added slowly and the mixture was stirred at room temperature overnight. The solvent mixture was evaporated; the residue was dissolved in 50 mL methanol and dropped into 1 L of stirred water. The precipitate was extracted with 200 mL ethyl acetate three times and the organic phase was dried with magnesium sulfate. The solvent was evaporated and the residue was purified by column chromatography (ethyl acetate/cyclohexane/dichloromethane = 6/3/1 + 1% triethyl amine) yielding 12 as a slightly yellow solid (1.4 g, 3.1 mmol, 78 %). \( R_f = 0.65 \) (SiO\(_2\), ethyl acetate/cyclohexane/dichloromethane = 6/3/1 + 1% triethyl amine), m.p. 85 °C; \(^1\)H NMR (300 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 1.40 \) (s, 9H, Boc-CH\(_3\)), 3.27-3.41 (m, 2H, CH\(_2\)), 3.64-3.88 (m, 2H, CH\(_2\)), 3.72 (s, 3H, CH\(_3\)), 4.32-4.51 (m, 1H, CH), 7.34 (d, 1H, Boc-NH), 8.05-8.17 (m, 2H, aryl-CH), 8.18-8.42 (m, 6H, aryl-CH), 8.50 (d, 1H, aryl-CH), 8.79 (t, 1H, NH); \(^1^3\)C NMR (75 MHz, \([D_6]DMSO\), 25 °C): \( \delta = 28.1 \) (Boc-CH\(_3\)), 52.1 (CH\(_3\)), 53.4 (CH), 78.5 (Boc-Cq), 123.6 (aryl-Cq), 123.8 (aryl-Cq), 124.3 (aryl-CH), 124.6 (aryl-CH), 125.2 (aryl-CH), 125.6 (aryl-CH), 125.8 (aryl-CH), 126.6 (aryl-CH), 127.2 (aryl-CH), 127.8 (aryl-Cq), 128.1 (aryl-CH), 128.4 (aryl-CH), 130.2 (aryl-Cq), 130.7 (aryl-Cq), 131.4 (aryl-Cq), 131.7 (aryl-Cq), 155.3 (Cq), 169.2 (Cq), 171.5 (Cq); IR (film, [cm\(^{-1}\)]) = 3325 (m), 2976 (m), 2360 (w), 1711 (s), 1640 (m), 1519 (s), 1435 (m), 1366 (m), 1274 (m), 1247 (m), 1208 (m), 1160 (s), 1063
Boc-deprotection of 12 to cation 13
A solution of 12 (2.0 g, 4.5 mmol) in DCM (50 mL) and TFA (20 mL) was stirred at room temperature until reaction control (tlc, ethyl acetate/cyclohexane/dichloromethane = 6/3/1 + 1% triethyl amine) showed no more starting material. After evaporation of the solvent mixture the colorless solid was lyophilized and dried in vacuo to give 13 (2.0 g, 4.4 mmol, 99 %). M.p. 223 °C; \(^1\)H NMR (300 MHz, \([\text{D}_6]\)DMSO, 25 °C): \(\delta = 3.83\) (s, 3H, CH\(_3\)), 3.87-3.95 (m, 2H, CH\(_2\)), 4.18-4.48 (m, 1H, CH), 8.10-8.42 (m, 8H, aryl-CH), 8.50-8.67 (m, 3H + 1H, NH\(_3^+\) + aryl-CH), 8.97 (t, 1H, NH); \(^{13}\)C NMR (75 MHz, \([\text{D}_6]\)DMSO, 25 °C): \(\delta = 39.5\) (CH\(_2\)), 52.1 (CH\(_3\)), 53.1 (CH), 123.5 (aryl-Cq), 123.8 (aryl-Cq), 124.3 (aryl-CH), 124.7 (aryl-CH), 125.7 (aryl-CH), 125.9 (aryl-CH), 126.6 (aryl-CH), 127.1 (aryl-CH), 128.0 (aryl-Cq), 128.2 (aryl-CH), 128.6 (aryl-CH), 130.1 (aryl-Cq), 130.5 (aryl-Cq), 130.7 (aryl-Cq), 131.9 (aryl-Cq), 168.6 (Cq), 169.8 (Cq); IR (film, \([\text{cm}^{-1}]\)) = 3371 (w), 3305 (w), 3044 (m), 2963 (m), 2635 (w), 2361 (w), 1746 (s), 1634 (s), 1601 (m), 1515 (s), 1447 (m), 1327 (w), 1283 (w), 1178 (s), 1132 (s), 1075 (m), 937 (w), 849 (m), 804 (w), 695 (w); HRMS (ESI): \(m/z\) = calc. for C\(_{26}\)H\(_{26}\)N\(_2\)O\(_5\): 469.1734 [M+Na\(^+\)]; found 469.1768 [M+Na\(^+\)].

Coupling of 13 with 14 to 15
A solution of 14 (0.50 g, 1.3 mmol, 1.0 eq), PyBOP (1.0 g, 1.9 mmol, 1.5 eq), NMM (0.5 mL, 3.9 mmol, 3.0 eq) and a catalytic amount of DMAP was stirred in a 1:1 mixture of DMF and DCM (50 mL) for 30 minutes at room temperature. To this solution a solution of the amine 13 (0.59 g, 1.3 mmol, 1.0 eq) in DMF (5 mL) and NMM (0.2 mL) was added slowly and the mixture was stirred at room temperature overnight. The solvent mixture was evaporated, the residue was dissolved in 50 mL methanol and dropped into 300 mL of stirred water. The precipitate was extracted with 100 mL ethyl acetate three times and the organic phase was dried with magnesium sulfate. The solvent was evaporated and the residue was purified by column chromatography (ethyl acetate) yielding the methyl ester 15 as a colorless solid (0.63 g, 1.0 mmol, 80 %). \(R_f = 0.60\) (SiO\(_2\), ethyl acetate), m.p. 204 °C; \(^1\)H NMR (300 MHz, \([\text{D}_6]\)DMSO, 25 °C): \(\delta = 1.46\) (s, 9H, Boc-CH\(_3\)), 3.74 (s, 3H, CH\(_3\)), 3.79-3.88 (m, 1H, CH\(_2\)), 3.90-3.98 (m, 1H, CH\(_2\)), 4.89 (m, 1H, CH), 6.81-6.93 (m, 2H, pyrrole-CH), (d, 1H, Boc-NH), 8.09-8.38 (m, 8H, aryl-CH), 8.48 (d, 1H, aryl-CH), 8.59 (br.s, 1H, guanidino-NH), 8.88 (d, 1H, NH), 8.94 (t, 1H, NH), 9.35 (br.s, 1H, guanidino-NH), 10.92 (br.s, 1H, guanidino-NH), 11.56 (br.s, 1H, pyrrole-NH); \(^{13}\)C NMR (75 MHz, \([\text{D}_6]\)DMSO, 25 °C): \(\delta = 27.8\) (Boc-CH\(_3\)), 40.4 (CH\(_2\)), 52.1 (CH\(_3\)), 52.2 (CH), 76.3 (Boc-Cq), 112.6 (pyrrole-CH), 113.7 (pyrrole-CH), 123.6 (aryl-Cq), 123.7 (aryl-Cq), 124.4 (aryl-CH), 124.7 (aryl-CH), 125.2 (aryl-CH), 125.6 (aryl-CH), 125.8 (aryl-CH), 126.6 (aryl-CH), 127.2 (aryl-CH), 127.8 (aryl-Cq), 128.0 (aryl-CH), 128.4 (aryl-CH), 128.7 (aryl-Cq), 130.2 (aryl-Cq), 130.7 (aryl-Cq), 131.5 (aryl-Cq), 131.7 (aryl-Cq), 158.4 (Cq), 159.8 (Cq), 169.4 (Cq), 170.4 (Cq), 171.0 (Cq); IR (film, \([\text{cm}^{-1}]\)) = 3380 (m), 3259 (m), 2978 (m), 2655 (w), 2360 (w), 1725 (m), 1632 (s), 1525 (s), 1469 (m), 1434 (s), 1369 (m), 1332 (m), 1296 (m), 1237 (s), 1145 (s), 1046 (m), 983 (w), 843 (m), 755 (m); HRMS (ESI): \(m/z\) = calc. for C\(_{33}\)H\(_{32}\)N\(_6\)O\(_7\): 625.2405 [M+H\(^+\)]; found 625.2444 [M+H\(^+\)].
**Boc-deprotection of 15 to cation 5**

A solution of 15 (0.10 g, 0.16 mmol) in DCM (20 mL) and TFA (5 mL) was stirred at room temperature until reaction control (tlc) showed no more starting material. After evaporation of the solvent mixture the residue was treated with aqueous hydrochloric acid (5%). The colorless solid was lyophilized and dried \textit{in vacuo} to give 5 (0.087 g, 0.155 mmol, 97%).

\[5^+\text{-TFA}^-\] \textit{H NMR} (500 MHz, [D$_6$]DMSO, 25 °C): \(\delta = 3.75\) (s, 3H, CH$_3$), 3.81-3.88 (m, 1H, CH$_2$), 3.90-3.98 (m, 1H, CH$_2$), 4.90 (m, 1H, CH), 6.96-6.99 (m, 1H, pyrrole-CH), 7.12-7.16 (m, 1H, pyrrole-CH), 8.09-8.28 (m, 5H, aryl-CH), 8.29-8.63 (m, 4H + 4H, aryl-CH + guanidinio-NH$_2^+$), 8.96 (t, 1H, NH), 8.99 (d, 1H, NH), 11.26 (s, 1H, guanidinio-NH), 12.60 (s, 1H, pyrrole-NH); \textit{13C NMR} (100 MHz, [D$_6$]DMSO, 25 °C): \(\delta = 40.4\) (CH$_2$), 52.2 (CH), 52.3 (CH$_3$), 113.2 (pyrrole-CH), 114.9 (pyrrole-Cq), 115.3 (pyrrole-CH), 117.3 (pyrrole-Cq), 123.6 (aryl-Cq), 123.7 (aryl-Cq), 124.4 (aryl-Cq), 124.6 (aryl-CH), 125.2 (aryl-CH), 125.6 (aryl-CH), 125.8 (aryl-CH), 126.6 (aryl-CH), 127.2 (aryl-CH), 127.8 (aryl-CH), 128.0 (aryl-CH), 128.4 (aryl-CH), 130.2 (aryl-Cq), 130.7 (aryl-Cq), 131.4 (aryl-Cq), 131.7 (aryl-Cq), 155.0 (Cq), 159.3 (Cq), 159.7 (Cq), 169.4 (Cq), 170.8 (Cq); HRMS (ESI): \(m/z = \text{calc. for C}_{28}\text{H}_{24}\text{N}_{6}\text{O}_{5}: 525.1881 \,[M+H]^+\); found 525.1908 [M+H]$^+$. 

\[5^+\text{-Cl}^-\] \textit{H NMR} (500 MHz, [D$_6$]DMSO, 25 °C): \(\delta = 3.75\) (s, 3H, CH$_3$), 3.81-4.01 (m, 2H, CH$_2$), 4.79-4.99 (m, 1H, CH), 6.87-7.11 (m, 1H, pyrrole-CH), 7.26-7.56 (m, 1H, pyrrole-CH), 7.86-8.76 (m, 5H + 9H, guanidinio-NH + aryl-CH), 8.80-9.20 (m, 2H, NH), 12.60 (s, 1H, pyrrole-NH); \textit{13C NMR} (100 MHz, [D$_6$]DMSO, 25 °C): \(\delta = 40.3\) (CH$_2$), 52.2 (CH), 52.4 (CH$_3$), 113.2 (pyrrole-CH), 115.4 (pyrrole-Cq), 123.5 (aryl-Cq), 123.7 (aryl-Cq), 124.3 (aryl-CH), 124.6 (aryl-CH), 125.2 (aryl-CH), 125.6 (aryl-CH), 125.8 (aryl-CH), 126.6 (aryl-CH), 127.1 (aryl-CH), 127.8 (aryl-Cq), 128.0 (aryl-CH), 128.3 (aryl-CH), 130.1 (aryl-Cq), 130.6 (aryl-Cq), 131.3 (aryl-Cq), 131.6 (aryl-Cq), 156.1 (Cq), 159.4 (Cq), 160.6 (Cq), 169.4 (Cq), 170.8 (Cq).
**Spectroscopic properties of 5**

**Table S1.** Electronic absorption maxima and corresponding molar extinction coefficients of 5 in aqueous medium compared with previously studied analogues 1-4.

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_{\text{max}}$ / nm ($\varepsilon \times 10^3 / \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)</th>
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<tbody>
<tr>
<td>1a</td>
<td>276 (38.1); 303 (28.1); 342 (20.4)</td>
</tr>
<tr>
<td>2a</td>
<td>276 (33.7); 307 (28.6); 344 (18.2); 377 (1.8)</td>
</tr>
<tr>
<td>3a</td>
<td>295 (29.9); 355 (22.5)</td>
</tr>
<tr>
<td>4a</td>
<td>295 (21); 355 (17.8)</td>
</tr>
<tr>
<td>5b</td>
<td>276 (30.7); 302 (25.2); 342 (18.1)</td>
</tr>
</tbody>
</table>

a Previous results.7,8,9,10; b Determined at pH 5 (Na cacodylate buffer, $I = 0.05 \text{ mol dm}^{-3}$), similar values at pH 7.
Study of interactions of 5 with DNA and RNA in aqueous medium

Hypochromic effects of 5-polynucleotide complexes at pH=7 and pH=5

The changes in the UV/Vis spectra of 5 upon the addition of all studied polynucleotides were rather small in the region of λ > 300 nm, thus preventing the use of UV/Vis titrations for further studies. Nevertheless, the addition of polynucleotides to 5 buffered solutions at ratio, r=0.1 (λ_{max}=342 nm) enabled the calculation of hypochromic effect.

Table S2. Spectroscopic changes of the UV/Vis spectra of 5 observed in titrations with ds-polynucleotides at pH = 7.0 and pH 5.0 (buffer sodium cacodylate, I = 0.05 mol dm^{-3}).

<table>
<thead>
<tr>
<th></th>
<th>poly(dAdT)₂</th>
<th>poly(dGdC)₂</th>
<th>poly A – poly U</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H / %⁻ᵃ</td>
<td>bΔλ / nm</td>
<td>H / %ᵃ</td>
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<tr>
<td>pH 7</td>
<td>38.6</td>
<td>6</td>
<td>28.9</td>
</tr>
<tr>
<td>pH 5</td>
<td>34.7</td>
<td>9</td>
<td>32.9</td>
</tr>
</tbody>
</table>

⁻ᵃΔλ = λ(5) - λ(complex); Absorbance maximum λ (5_{342 nm})⁻ᵇHypochromic effect for 5 calculated according to the equation; H=(Abs(5) - Abs(complex)) / Abs(5) × 100.
Fluorimetric titrations

Figure S1. a) Changes in fluorescence spectrum of 5 ($c = 4.0 \times 10^{-6}$ mol dm$^{-3}$) upon titration with ctDNA ($c = 2 \times 10^{-6} - 1.64 \times 10^{-4}$ mol dm$^{-3}$); b) Dependence of 5 absorbance at $\lambda_{\text{max}} = 402$ nm on c(ctDNA), at pH 7.0, sodium cacodylate buffer, $I = 0.05$ mol dm$^{-3}$.

Figure S2. a) Changes in fluorescence spectrum of 5 ($c = 4.7 \times 10^{-6}$ mol dm$^{-3}$) upon titration with poly(dG-dC)$_2$ ($c = 1.0 \times 10^{-6} - 1.11 \times 10^{-4}$ mol dm$^{-3}$); b) Dependence of 5 absorbance at $\lambda_{\text{max}} = 402$ nm on c(poly(dG-dC)$_2$), at pH 7.0, sodium cacodylate buffer, $I = 0.05$ mol dm$^{-3}$.
Figure S3. a) Changes in fluorescence spectrum of 5 (c = 1.0 \times 10^{-7} \text{ mol dm}^{-3}) upon titration with poly(dA·dT) (c = 4.75 \times 10^{-8} – 6.73 \times 10^{-6} \text{ mol dm}^{-3}); b) Dependence of 5 absorbance at \( \lambda_{\text{max}} = 402 \text{ nm} \) on c(poly(dA·dT)), at pH 7.0, sodium cacodylate buffer, \( I = 0.05 \text{ mol dm}^{-3} \).

Figure S4. a) Changes in fluorescence spectrum of 5 (c = 4.0 \times 10^{-6} \text{ mol dm}^{-3}) upon titration with poly A – poly U (c = 8.0 \times 10^{-7} – 8.21 \times 10^{-5} \text{ mol dm}^{-3}); b) Dependence of 5 absorbance at \( \lambda_{\text{max}} = 402 \text{ nm} \) on c(poly A – poly U), at pH 7.0, sodium cacodylate buffer, \( I = 0.05 \text{ mol dm}^{-3} \).
Figure S5. LEFT) Changes in fluorescence spectrum of 5 \((c = 1.0 \times 10^{-7} \text{ mol dm}^{-3})\) upon titration with poly(dA-dT)\(_2\) \((c = 5.8 \times 10^{-8} -1.3 \times 10^{-4} \text{ mol dm}^{-3})\); RIGHT) Changes in fluorescence spectrum of 5 \((c = 1.0 \times 10^{-7} \text{ mol dm}^{-3})\) upon titration with poly(dG-dC)\(_2\) \((c = 5 \times 10^{-7} - 6.3 \times 10^{-5} \text{ mol dm}^{-3})\). Done at pH 5.0, sodium cacodylate buffer, \(I = 0.05 \text{ mol dm}^{-3}\).

Figure S6. a) Changes in fluorescence spectrum of 5 \((c = 1.0 \times 10^{-7} \text{ mol dm}^{-3})\) upon titration with poly A – poly U \((c = 1 \times 10^{-6} - 1.5 \times 10^{-4} \text{ mol dm}^{-3})\); b) Dependence of 5 absorbance at \(\lambda_{\text{max}} = 402 \text{ nm}\) on c(poly A – poly U), at pH 5.0, sodium cacodylate buffer, \(I = 0.05 \text{ mol dm}^{-3}\).
Circular dichroism (CD) experiments

Figure S7. CD spectrum of $c = 3 \times 10^{-5}$ mol dm$^{-3}$ in sodium cacodylate buffer, pH 7.0.
Figure S8. CD titration of poly (dA – dT)$_2$, poly (dG - poly dC)$_2$ and poly A – poly U with 5 at molar ratios $r = [\text{compound}] / [\text{polynucleotide}]$ (pH 7.0, $c(\text{polynucleotide}) = 3.0 \times 10^{-5}$)
mol dm$^{-3}$ and pH 5.0, $c$ (polynucleotide) = $1.0 \times 10^{-5}$ mol dm$^{-3}$, buffer sodium cacodylate, $l=0.05$ mol dm$^{-3}$).

**Docking of 5 into AT-DNA**

Docking was performed by Pymol programme using earlier analysed complexes$^{3,5}$ with poly dAdT-poly dAdT as templates. Original molecules 1$^3$ and 4$^5$ already positioned within the AT-DNA were modified to get 5 and conformational space of small molecule within binding site was checked that VdW radii of DNA and ligand do not overlap. For the intercalative complex (Figure S9, LEFT) limited binding of the linker hampered positioning of GCP moiety deep in the minor groove, whereas minor groove binding model (Figure S9, RIGHT) could optimally accommodate to binding interactions.

Figure S9. Schematic presentation of 5 bound to poly dAdT – poly dAdT in two different binding modes. LEFT: pyrene intercalated into DNA by modification of 1$^3$ to 5; RIGHT: whole 5 in the minor groove by modification of 4$^5$.

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5 The zwitterion 7 forms in DMSO after sonification a strong and transparent gel
9 K. Gröger, D. Baretić, I. Piantanida, M. Marjanović, M. Kralj, M. Grabar, S. Tomić, C.