

Recognition of ATT triplex and DNA:RNA hybrid structures by benzothiazole ligands

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1. Spectroscopic characterization of benzothiazoles 1-9 in aqueous solutions.

1.1. Solubility

All studied benzothiazoles were dissolved in redistilled water ($c=3-5 \times 10^{-3} \text{ mol dm}^{-3}$) or aqueous buffer (sodium cacodylate/HCl buffer, $I = 0.05 \text{ mol dm}^{-3}$).

1.2. UV/Vis spectra, stability

Buffered solutions of studied compounds were stable for days at 4-8°C (refrigerator). The absorbances of buffered solutions of studied benzothiazole compounds were proportional to their concentrations up to $c = 2 \times 10^{-5} \text{ mol dm}^{-3}$ (Figures S1–S10).

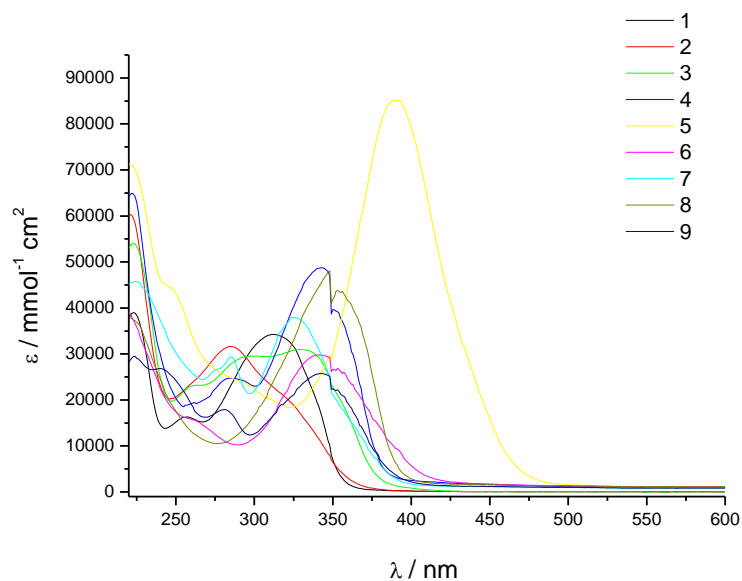


Figure S1. UV/Vis spectra of **1-9** at $c = 1.6 \times 10^{-5} - 2 \times 10^{-5} \text{ mol dm}^{-3}$; pH=7, sodium cacodylate/HCl buffer, $I = 0.05 \text{ mol dm}^{-3}$.

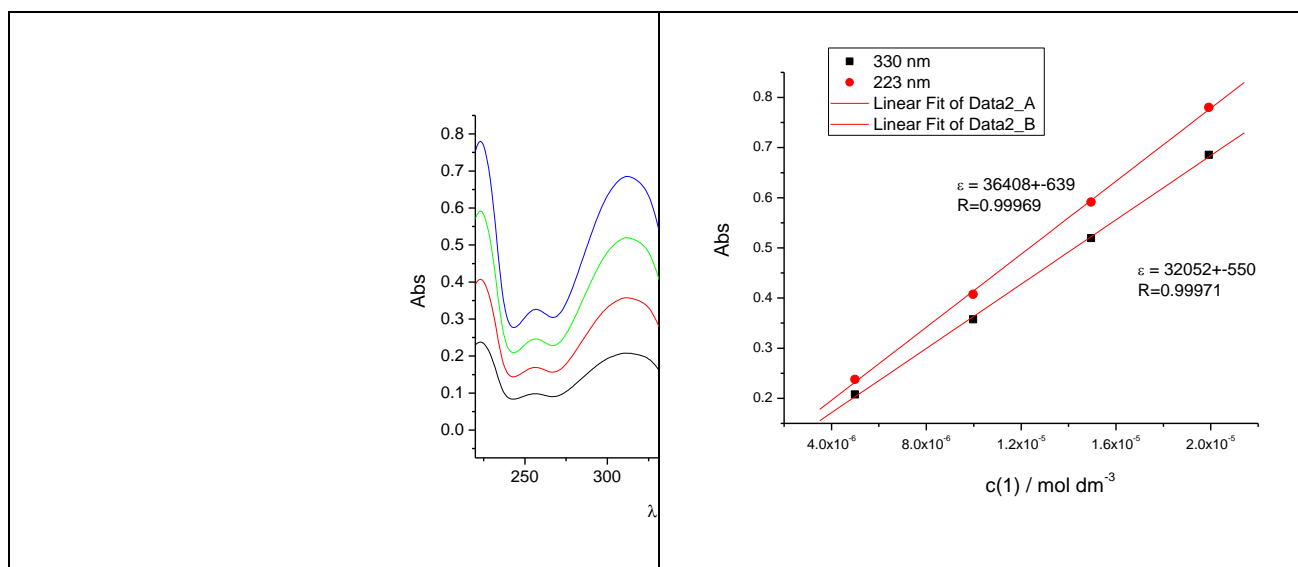


Figure S2. UV/Vis spectra changes of **1** at different concentrations (concentration range from $5 \times 10^{-6} - 2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 223 and 330 nm (■) on the **1** concentration (right); (pH=7, sodium cacodylate buffer, $I=0.05 \text{ M}$).

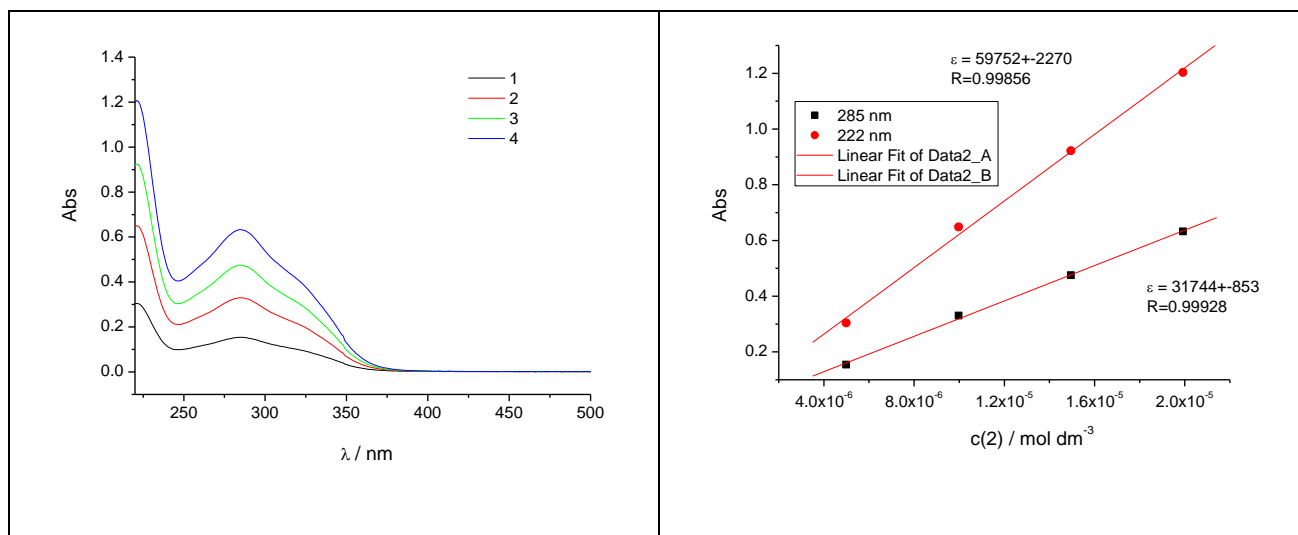


Figure S3. UV/Vis spectra changes of **2** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 222 and 385 nm (■) on the **2** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

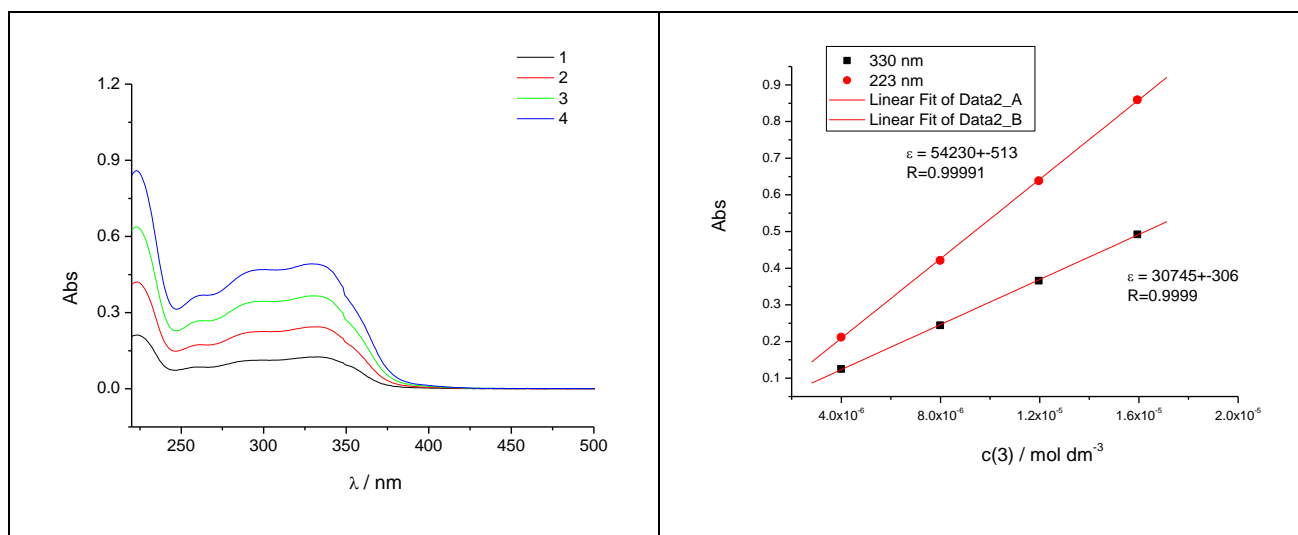


Figure S4. UV/Vis spectra changes of **3** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 223 and 330 nm (■) on the **3** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

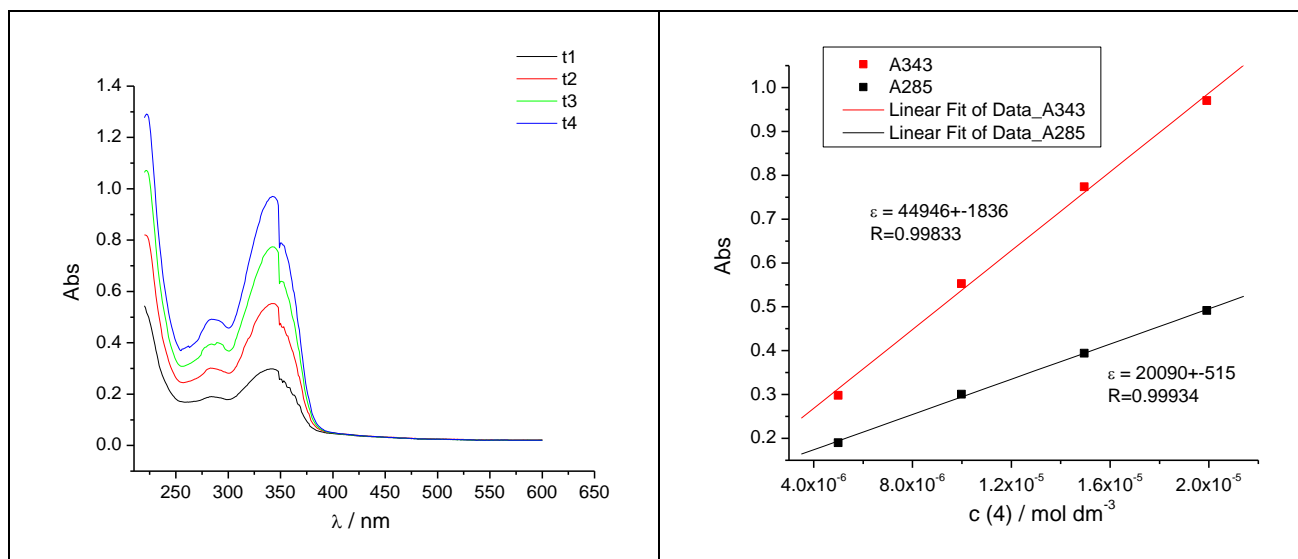


Figure S5. UV/Vis spectra changes of **4** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 285 and 343 nm (■) on the **4** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

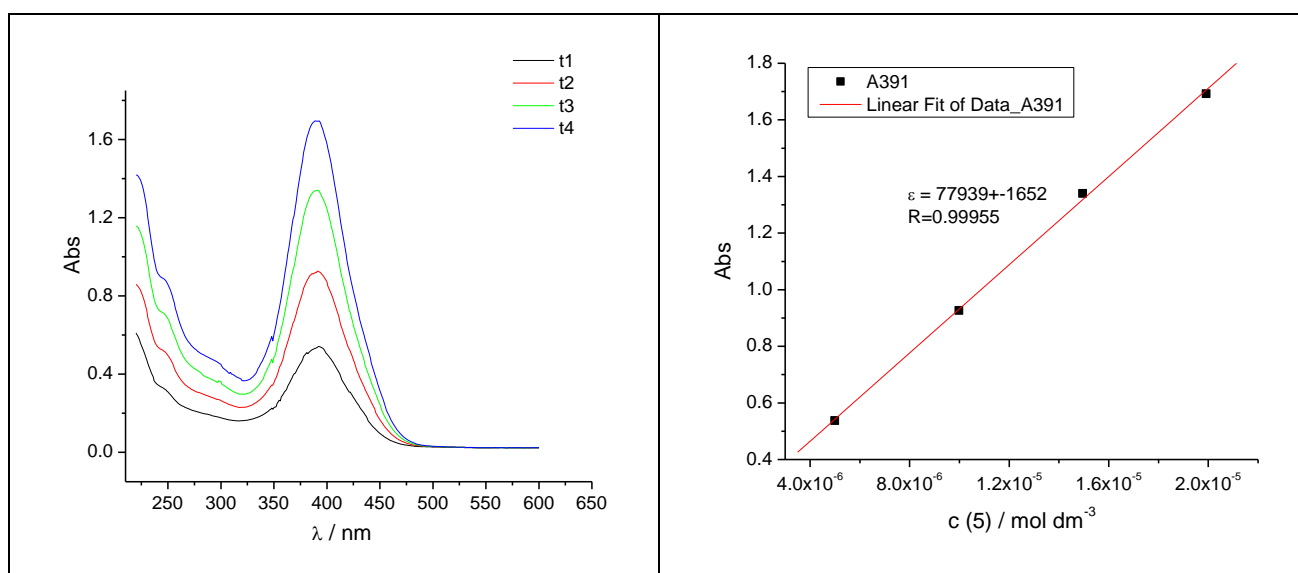


Figure S6. UV/Vis spectra changes of **5** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 391 nm (■) on the **5** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

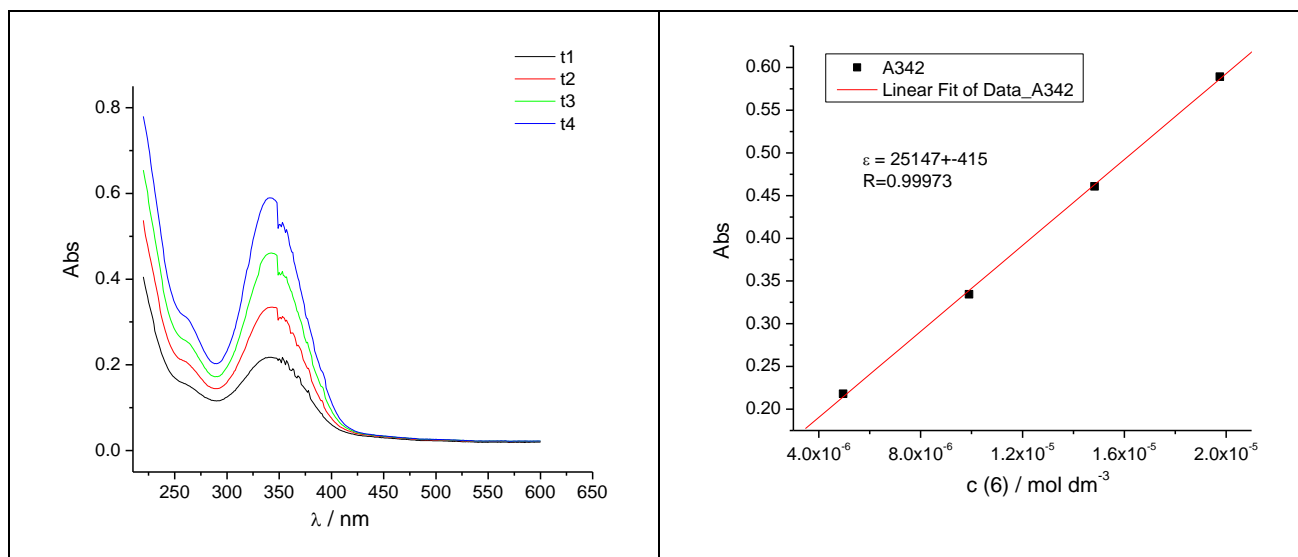


Figure S7. UV/Vis spectra changes of **6** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 342 nm (■) on the **6** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

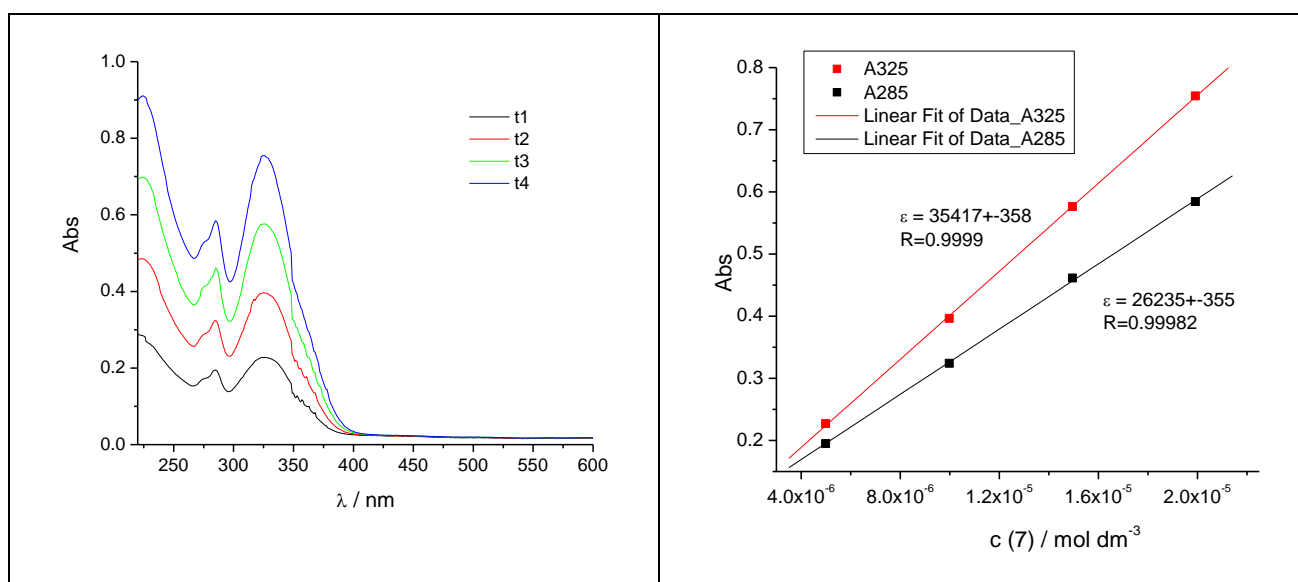


Figure S8. UV/Vis spectra changes of **7** at different concentrations (concentration range from 5×10^{-6} – $2 \times 10^{-5} \text{ mol dm}^{-3}$) (left); linear dependence (—) of the absorbance at 285 and 325 nm (■) on the **7** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05 \text{ M}$).

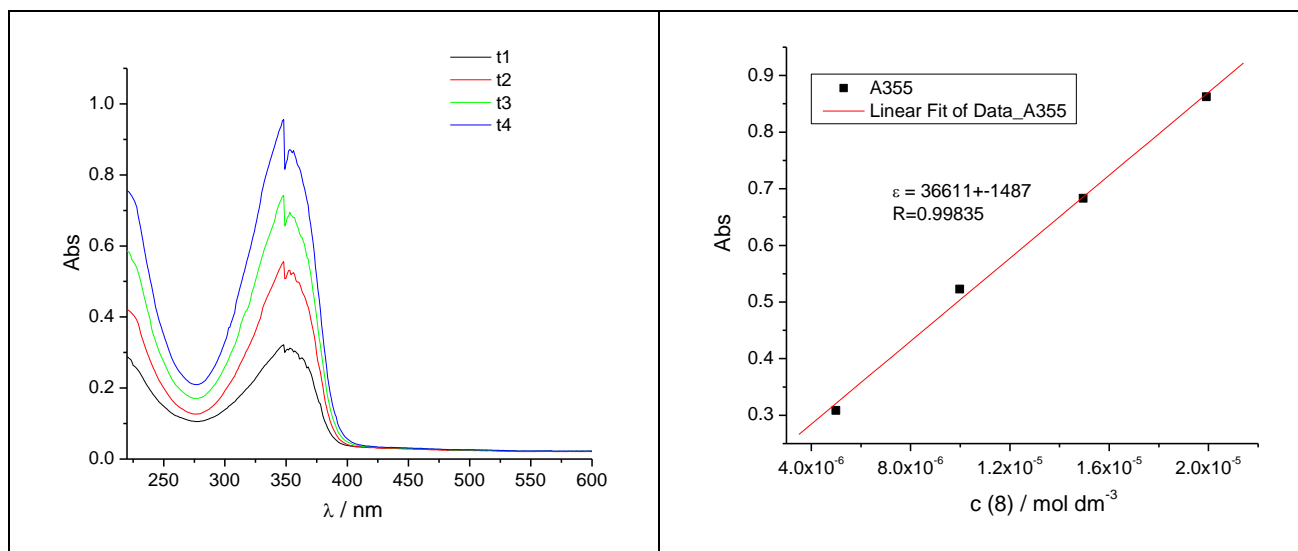


Figure S9. UV/Vis spectra changes of **8** at different concentrations (concentration range from 5×10^{-6} - 2×10^{-5} mol dm⁻³) (left); linear dependence (—) of the absorbance at 355 nm (■) on the **8** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05$ M).

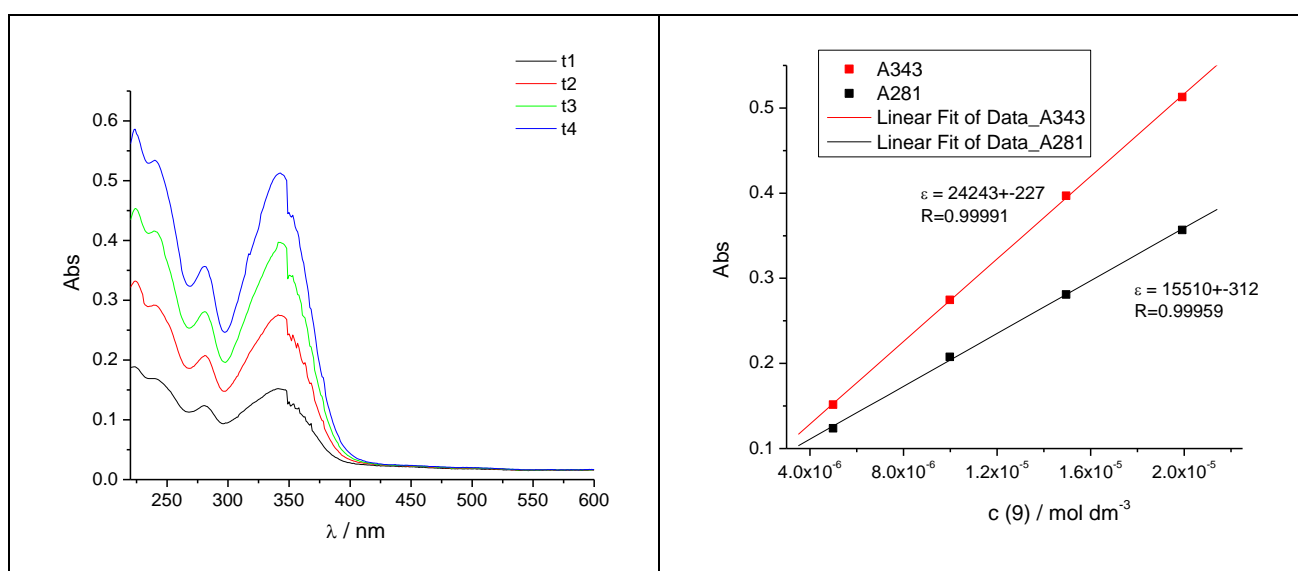


Figure S10. UV/Vis spectra changes of **9** at different concentrations (concentration range from 5×10^{-6} - 2×10^{-5} mol dm⁻³) (left); linear dependence (—) of the absorbance at 281 and 343 nm (■) on the **9** concentration (right); (pH=7, sodium cacodylate buffer, $I=0,05$ M).

1.3. Fluorescence spectra

The emission intensities of buffered aqueous solutions (sodium cacodylate buffer, $I = 0.05 \text{ mol dm}^{-3}$, $\text{pH} = 7$) of studied compounds are proportional to their concentrations up to $c = 2 \times 10^{-6} \text{ mol dm}^{-3}$ (Figures S11-S20).

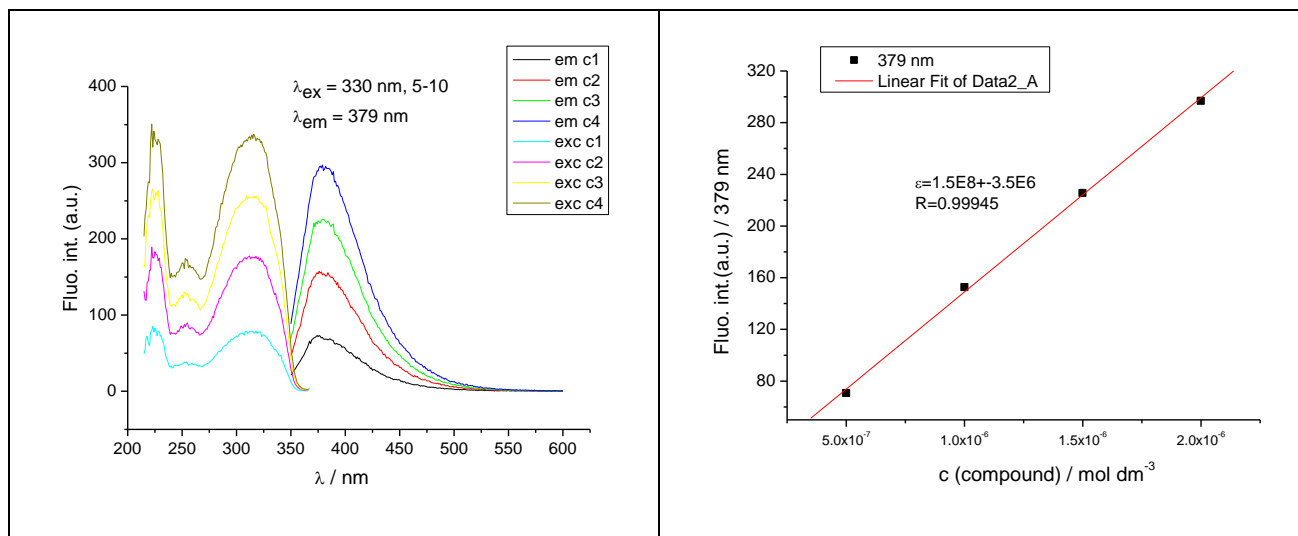


Figure S11. Emission and excitation spectra changes of **1** at different concentrations at $\lambda_{\text{exc}}=330 \text{ nm}$ (concentration range from 5×10^{-7} - $2 \times 10^{-6} \text{ mol dm}^{-3}$) at $\text{pH}=7.0$, sodium cacodylate buffer, $I=0.05 \text{ mol dm}^{-3}$.

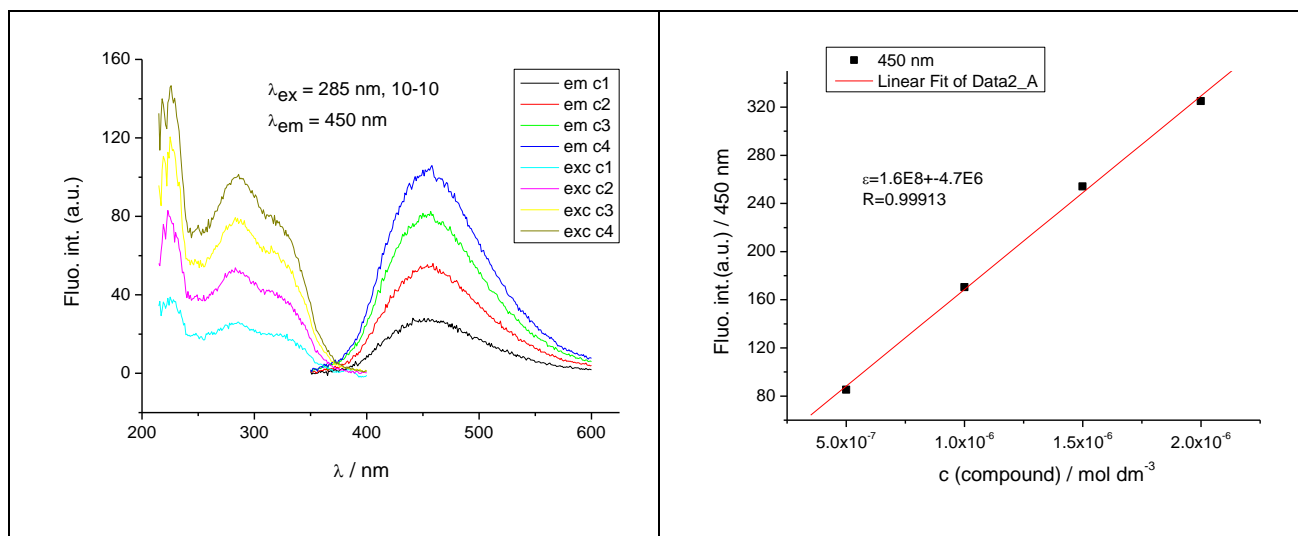


Figure S12. Emission and excitation spectra changes of **2** at different concentrations at $\lambda_{\text{exc}}=285 \text{ nm}$ (concentration range from 5×10^{-7} - $2 \times 10^{-6} \text{ mol dm}^{-3}$) at $\text{pH}=7.0$, sodium cacodylate buffer, $I=0.05 \text{ mol dm}^{-3}$.

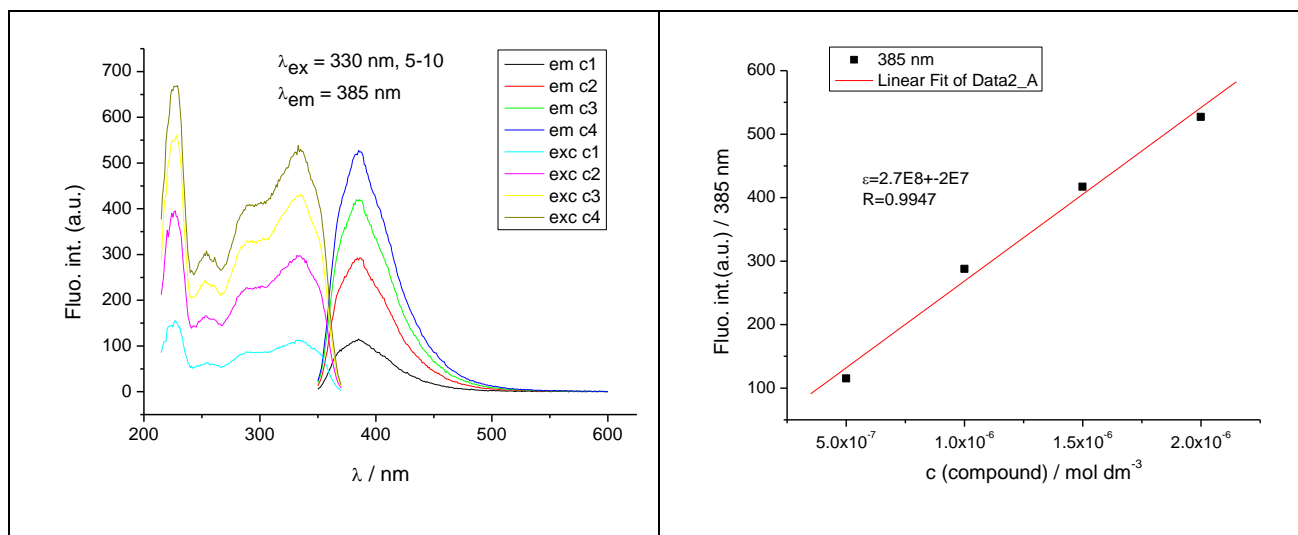


Figure S13. Emission and excitation spectra changes of **3** at different concentrations at $\lambda_{\text{exc}}=330$ nm (concentration range from 5×10^{-7} - 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I=0.05$ mol dm⁻³.

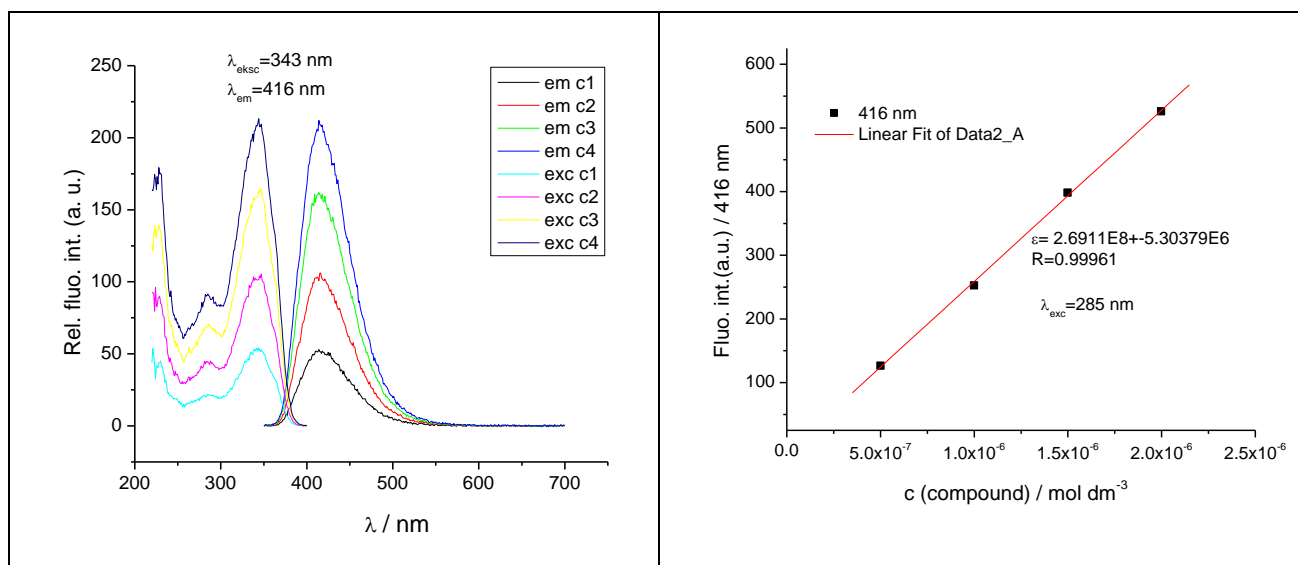


Figure S14. Emission and excitation spectra changes of **4** at different concentrations at $\lambda_{\text{exc}}=343$ nm (concentration range from 5×10^{-7} - 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I=0.05$ mol dm⁻³.

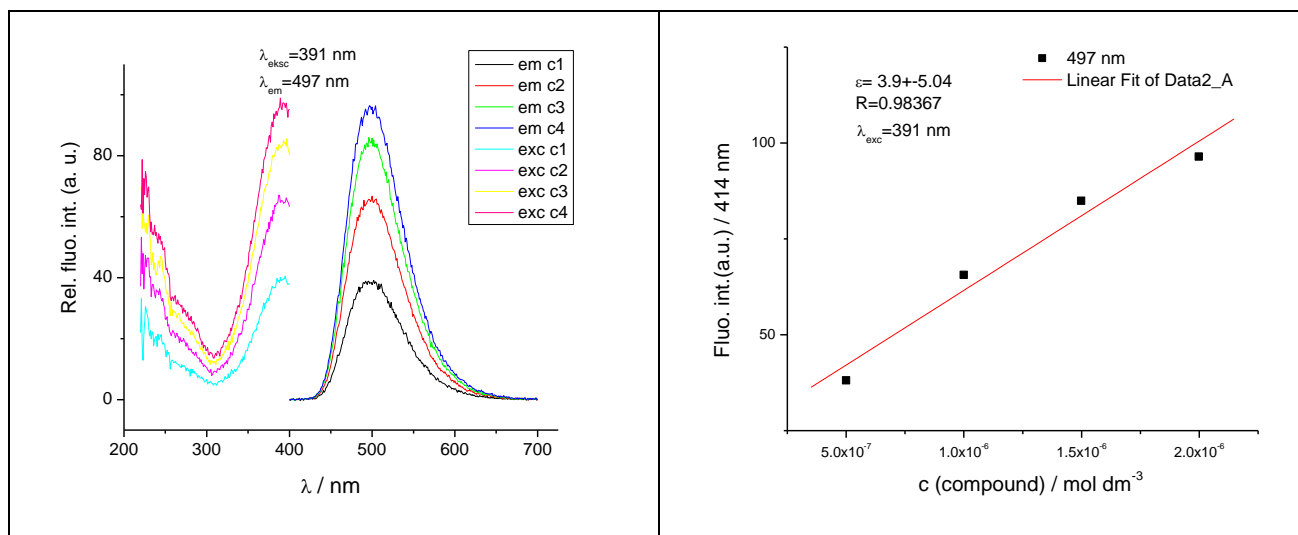


Figure S15. Emission and excitation spectra changes of **5** at different concentrations at $\lambda_{exc} = 391$ nm (concentration range from 5×10^{-7} – 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I = 0.05$ mol dm⁻³.

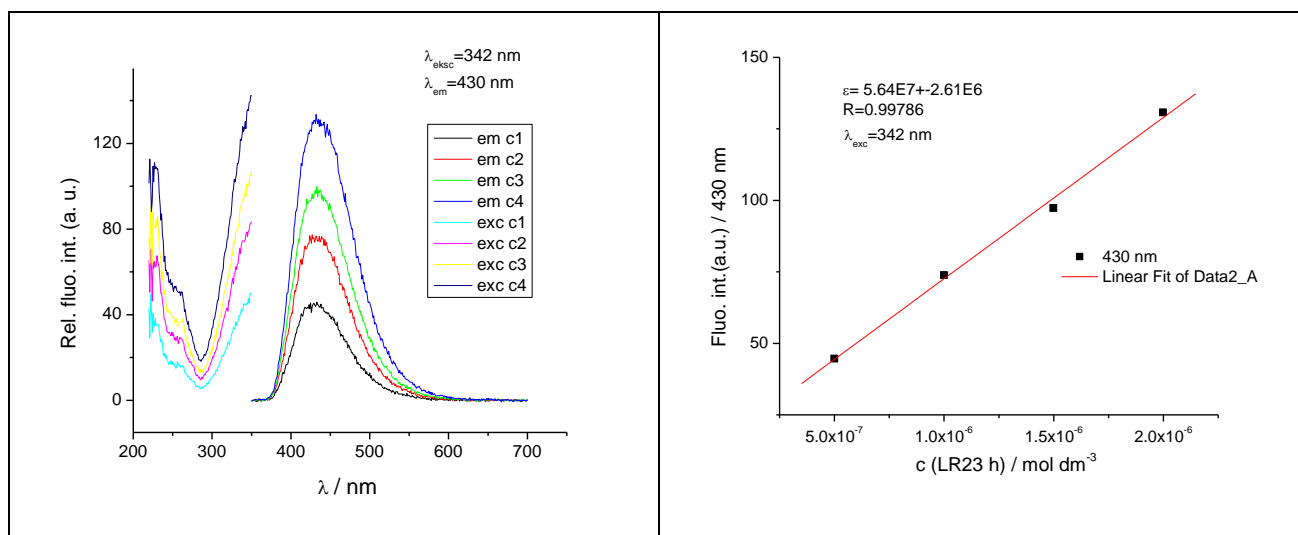


Figure S16. Emission and excitation spectra changes of **6** at different concentrations at $\lambda_{exc} = 342$ nm (concentration range from 5×10^{-7} – 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I = 0.05$ mol dm⁻³.

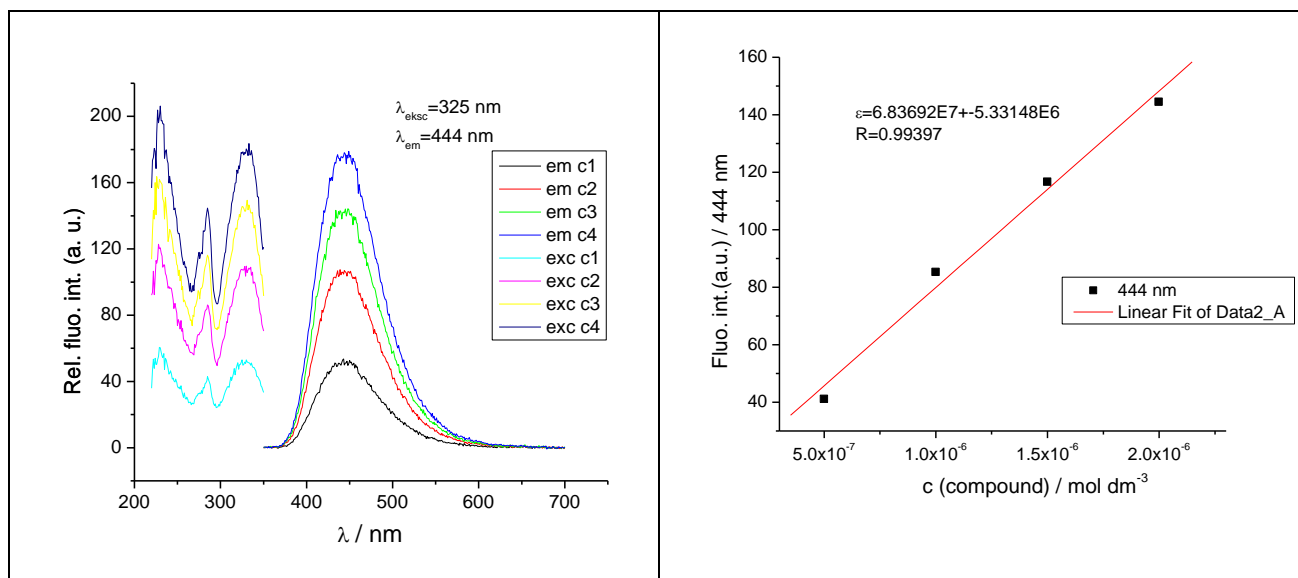


Figure S17. Emission and excitation spectra changes of **7** at different concentrations at $\lambda_{exc}=325$ nm (concentration range from 5×10^{-7} - 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I=0.05$ mol dm⁻³.

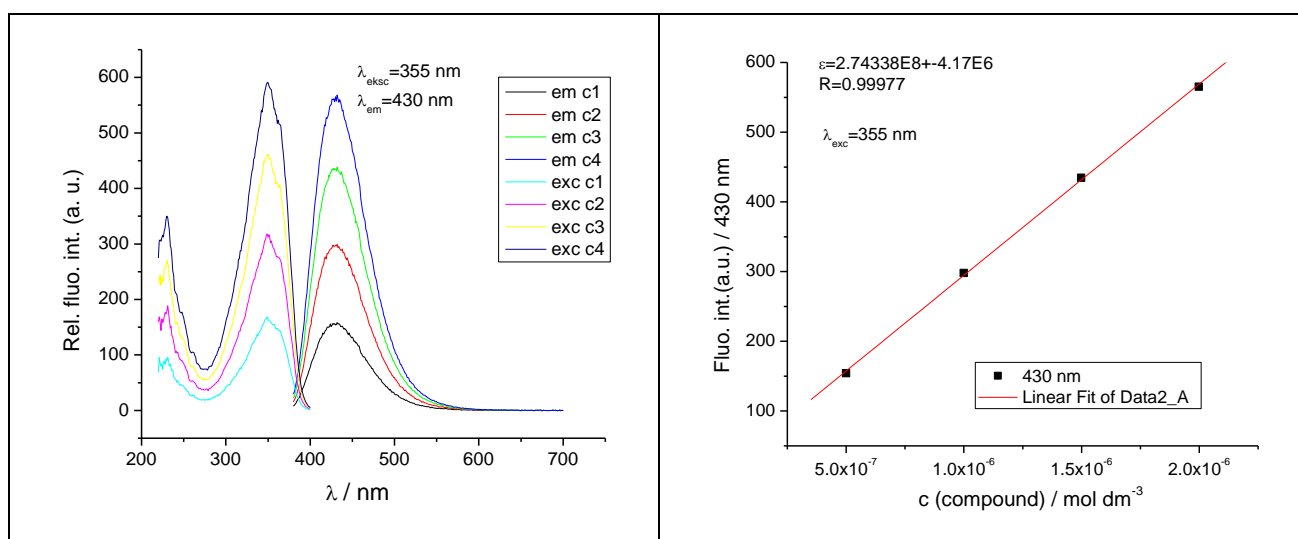


Figure S18. Emission and excitation spectra changes of **8** at different concentrations at $\lambda_{exc}=355$ nm (concentration range from 5×10^{-7} - 2×10^{-6} mol dm⁻³) at pH=7.0, sodium cacodylate buffer, $I=0.05$ mol dm⁻³.

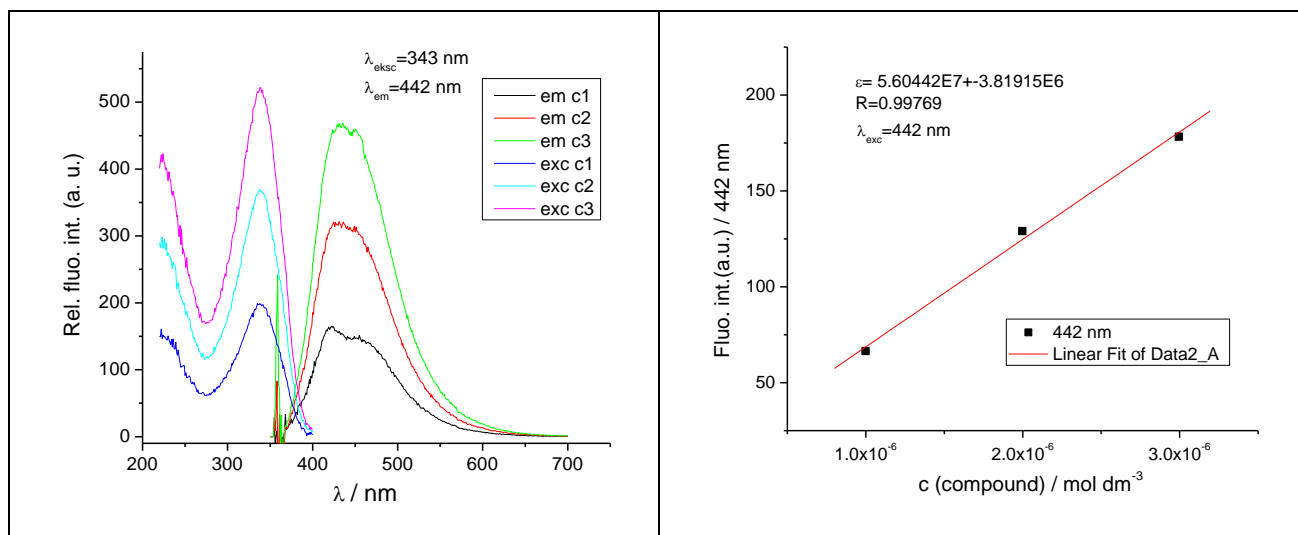


Figure S19. Emission and excitation spectra changes of **9** at different concentrations at $\lambda_{\text{exc}}=343$ nm (concentration range from 5×10^{-7} - 2×10^{-6} mol dm^{-3}) at pH=7.0, sodium cacodylate buffer, $I=0.05$ mol dm^{-3} .

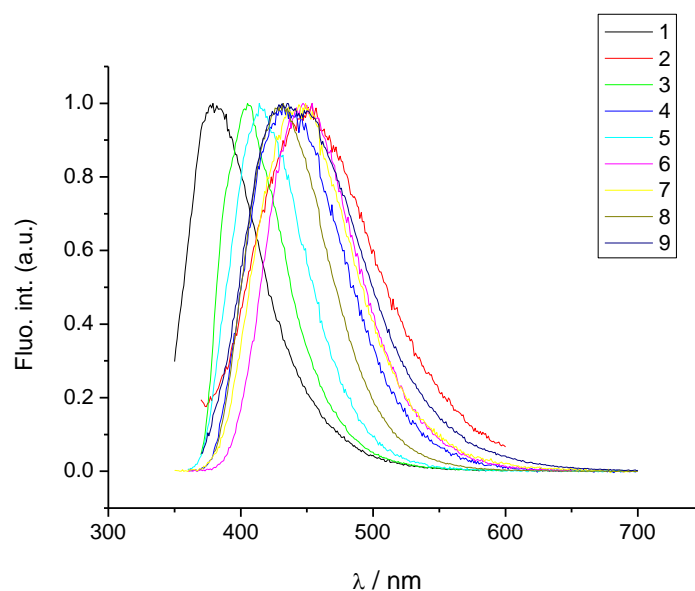


Figure S20. Normalized emission spectra of benzothiazoles **1-9**, $c=2 \times 10^{-6}$ mol dm^{-3} at pH=7, Na cacodylate buffer, $I=0.05$ mol dm^{-3} .

2. Interactions of benzothiazoles with polynucleotides in neutral medium (pH=7.0)

2.1. Competition dialysis

Specificity sum, SS, is a metric that identifies compounds with high binding selectivity.

$$SS = \sum_i \frac{c_{b,i}}{c_{max}}$$

where $c_{b,i}$ is the amount of ligand bound and c_{max} is the maximum amount bound to any species.

The index i ranges from 1 to 13 in the current version of the assay, corresponding to the 13 different nucleic acid structures used. The SS value 1 pointed absolute selectivity for compound that binds only one polynucleotide, while SS value 13 pointed equal binding to all polynucleotides without any selectivity.

Metric c_{max}/SS includes information both on selectivity and binding affinity.

2.2. Fluorimetric titrations

Stability constants were determined by nonlinear Scatchard equation:

$$I = I_0 + ((I_{lim} - I_0) / (2 \times c)) \times (c + n \times c_s + 1 / K_s - ((c + n \times c_s + 1 / K_s)^2 - 4 \times c \times n \times c_s)^{1/2})$$

where I is fluorescence intensity, while I_0 and I_{lim} denote fluorescence intensities of free and fully complexed ligand; c is concentration of free ligand; c_s is concentration of polynucleotide; n is ratio[bound ligand]/[polynucleotide]; K_s is complex stability constant.

In Scatchard equation values of stability constant (K_s) and ratio (n =[bound compound] / [polynucleotide]) are highly mutually dependent and similar quality of fitting calculated to experimental data is obtained for $\pm 20\%$ variation for K_s and n ; this variation can be considered as an estimation of the errors for the given binding constants. Given estimation was added to Footnote of Table 2.

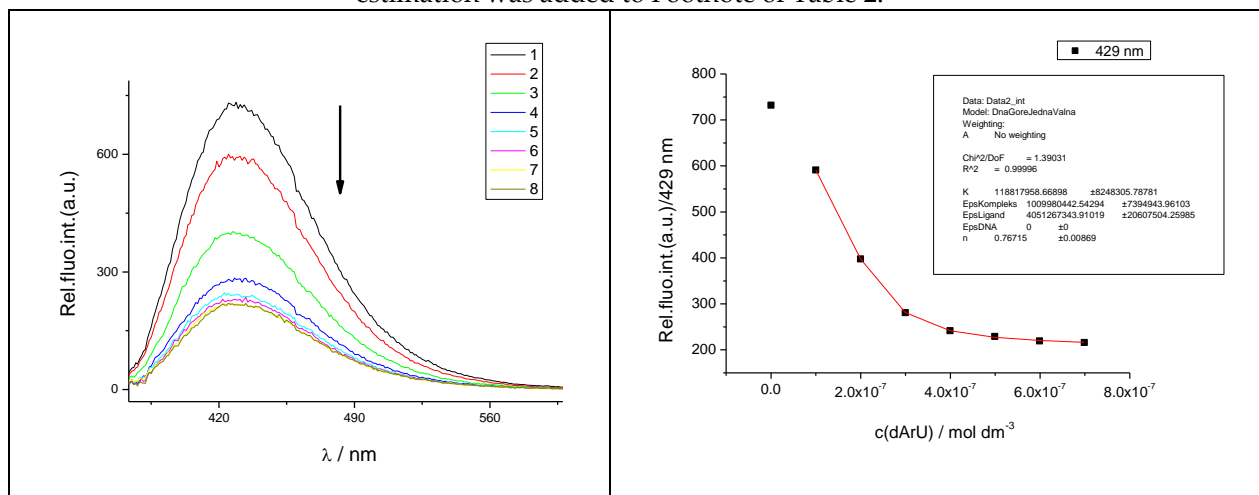


Figure S21. Left: Changes in fluorescence spectrum of **6** ($c = 2 \times 10^{-7}$ mol dm⁻³, $\lambda_{exc} = 342$ nm) upon titration with poly dA-poly rU ($c = 9.9 \times 10^{-8} - 7 \times 10^{-7}$ mol dm⁻³); Right: Experimental (●) and calculated (—) (by Scatchard eq.) fluorescence intensities of **6** at $\lambda_{em} = 429$ nm upon addition of poly dA-poly rU (pH = 7.0, Na cacodylate buffer, $I = 0.2$ mol dm⁻³ + 1mM EDTA).

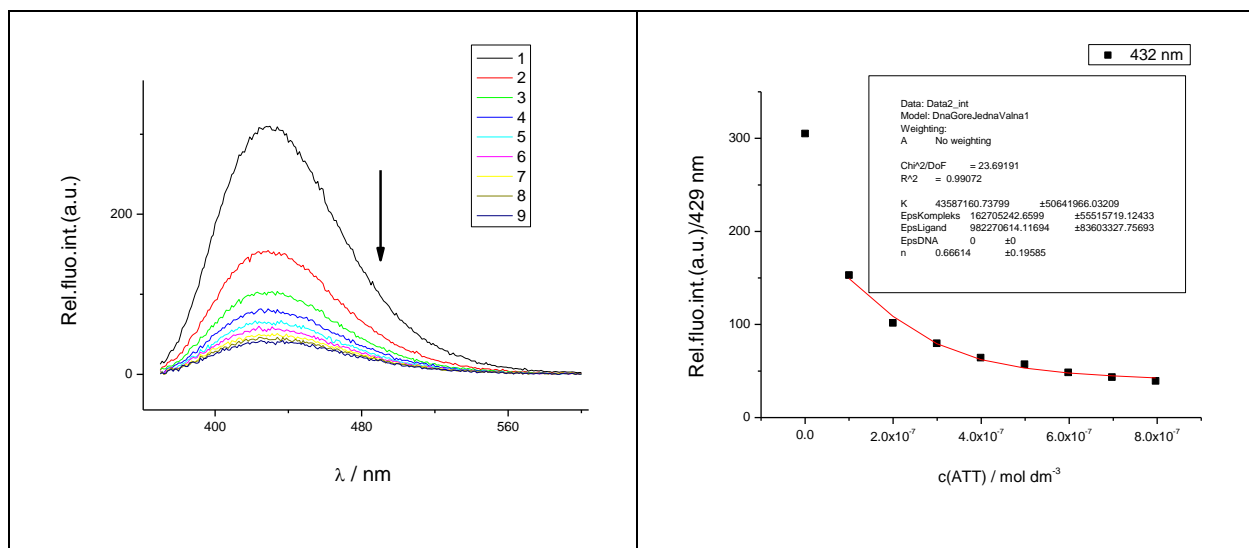


Figure S22. Left: Changes in fluorescence spectrum of **6** ($c = 2 \times 10^{-7} \text{ mol dm}^{-3}$, $\lambda_{\text{exc}} = 342 \text{ nm}$) upon titration with ATT triplex ($c = 9.9 \times 10^{-8} - 9.9 \times 10^{-7} \text{ mol dm}^{-3}$); Right: Experimental (●) and calculated (–) (by Scatchard eq.) fluorescence intensities of **6** at $\lambda_{\text{em}} = 432 \text{ nm}$ upon addition of ATT triplex (pH = 7.0, Na cacodylate buffer, $I = 0.05 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$).

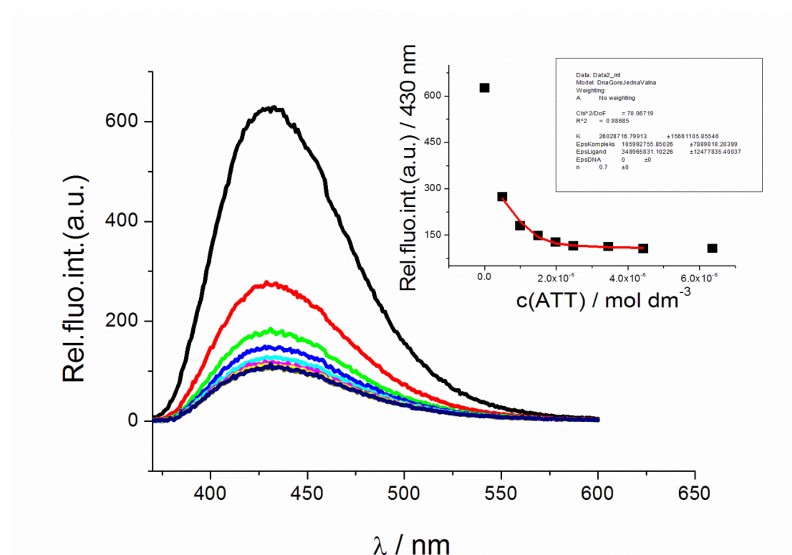


Figure S23. Changes in fluorescence spectrum of **6** ($c = 1 \times 10^{-6} \text{ mol dm}^{-3}$, $\lambda_{\text{exc}} = 342 \text{ nm}$) upon titration with 26mer ATT triplex ($c = 5 \times 10^{-7} - 6.36 \times 10^{-6} \text{ mol dm}^{-3}$); Insert: Experimental (●) and calculated (–) (by Scatchard eq.) fluorescence intensities of **6** at $\lambda_{\text{em}} = 430 \text{ nm}$ upon addition of 26mer ATT triplex (pH = 7.0, Na cacodylate buffer, $I = 0.05 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$).

2.3. Isothermal titration calorimetry

Titration of ATT triplex and poly rA–poly dT hybrid with the compound **1** were performed using an isothermal titration microcalorimeter Microcal VP-ITC. One aliquot of 2 μL , twelve aliquots of 10 μL and eight aliquots of 20 μL of the compound **1** ($c = 1.0 \times 10^{-4} \text{ mol dm}^{-3}$) were injected from rotating syringe (220 rpm) into the isothermal cell, equilibrated at 25.0 $^{\circ}\text{C}$, containing 1.4406 mL of ATT or poly rA–poly dT ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$).

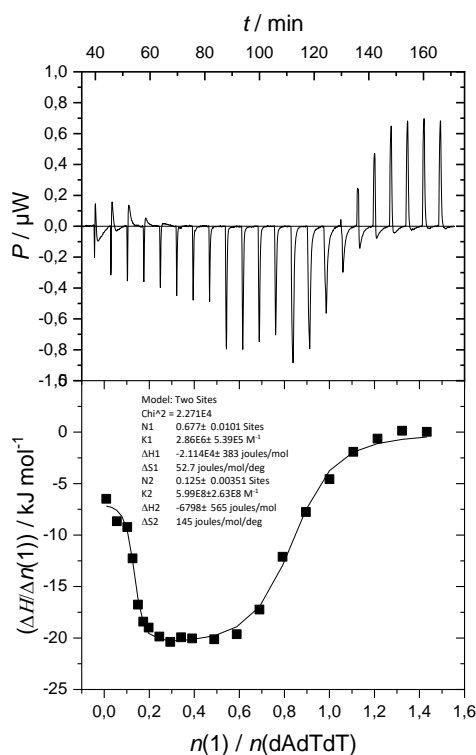


Figure S24. ITC experiment of ATT triplex titrated with **1**; experimental data (■) and calculated fit for model two sets of sites (—). Inset: raw titration data from the single injection of **1** into a solution of ATT triplex; $[\text{ATT}] = 3.0 \times 10^{-5} \text{ M}$; $\text{pH}=7.0$, Na-cacodylate buffer, $I=0.05 \text{ mol dm}^{-3} + 1\text{mM EDTA}$.

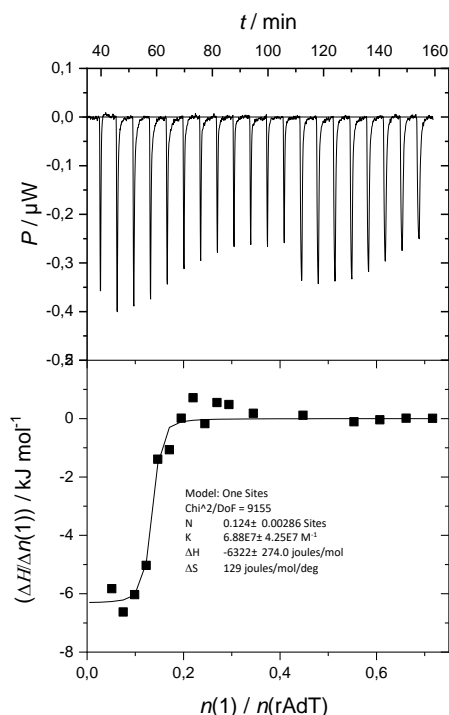


Figure S25. ITC experiment of poly rA–poly dT titrated with **1**; experimental data (■) and calculated fit for model two sets of sites (–). Inset: raw titration data from the single injection of **1** into a solution of poly rA–poly dT hybrid; [poly rA–poly dT] = 3.0×10^{-5} M; pH=7.0, Na-cacodylate buffer, $I=0.05 \text{ mol dm}^{-3} + 1\text{mM EDTA}$.

2.4. Thermal denaturation experiments

$$\Delta T_m = T_m (\text{complex polynucleotide-dye}) - T_m (\text{polynucleotide})$$

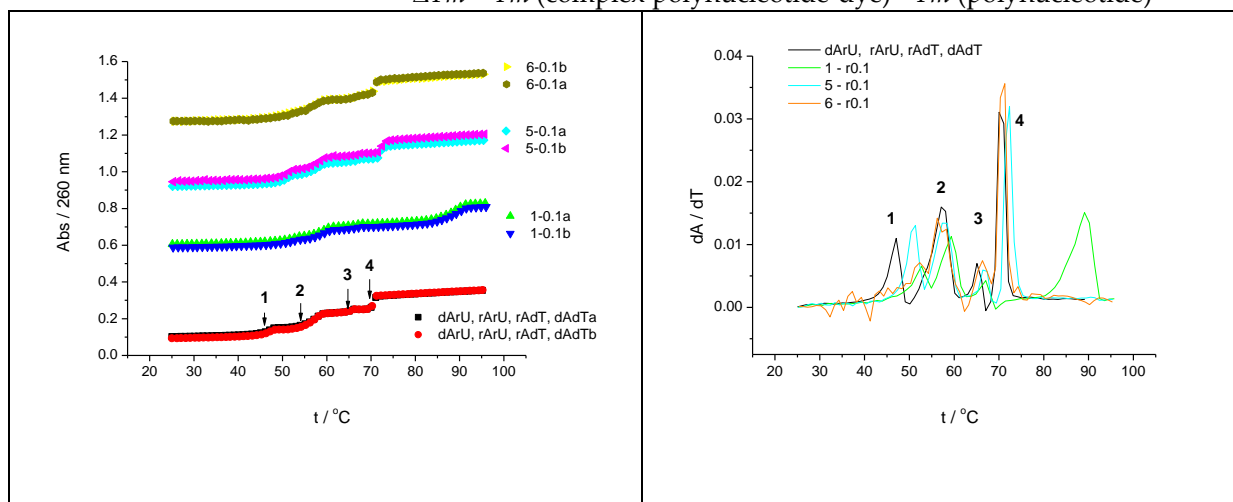


Figure S26. Left: Melting curves of polynucleotide mixtures (50mM sodium cacodylate buffer, 50mM NaCl, 1 mM EDTA): a DNA:RNA hybrid [poly dA–poly rU; peak 1], RNA [poly rA–poly rU; peak 2], an RNA:DNA hybrid [poly rA–poly dT; peak 3] and DNA [poly dA–poly dT; peak 4]. The concentration of each polynucleotide structure was 20 μM (bp); total polynucleotide concentration is 80 μM (bp). Effect of addition of ligand **1**, **5** and **6** (2 μM) at ratio, r ([compound/ [polynucleotide)]=0.025 to polynucleotide mixture was shown. Right: First derivative of absorbance at 260 nm in dependence of temperature.

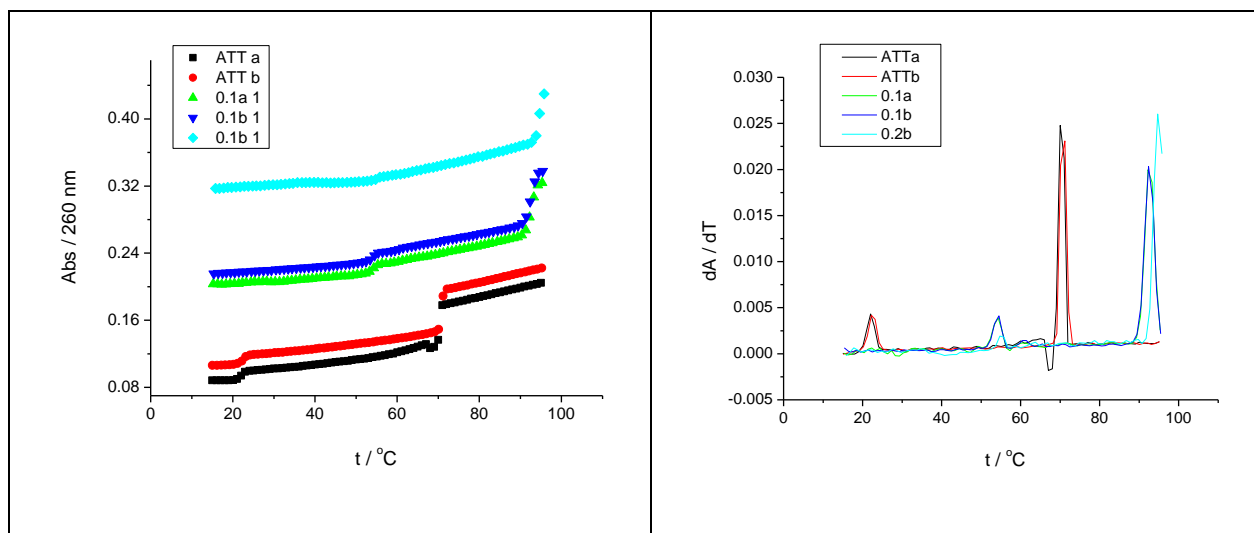


Figure S27. Left: Melting curve of ATT triplex upon addition of ratio, r ([compound]/ [polynucleotide]) = 0.1 of **1** at pH = 7.0 (sodium cacodylate buffer with NaCl, $I = 0.1 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$); Right: First derivative of absorbance at 260 nm in dependence of temperature.

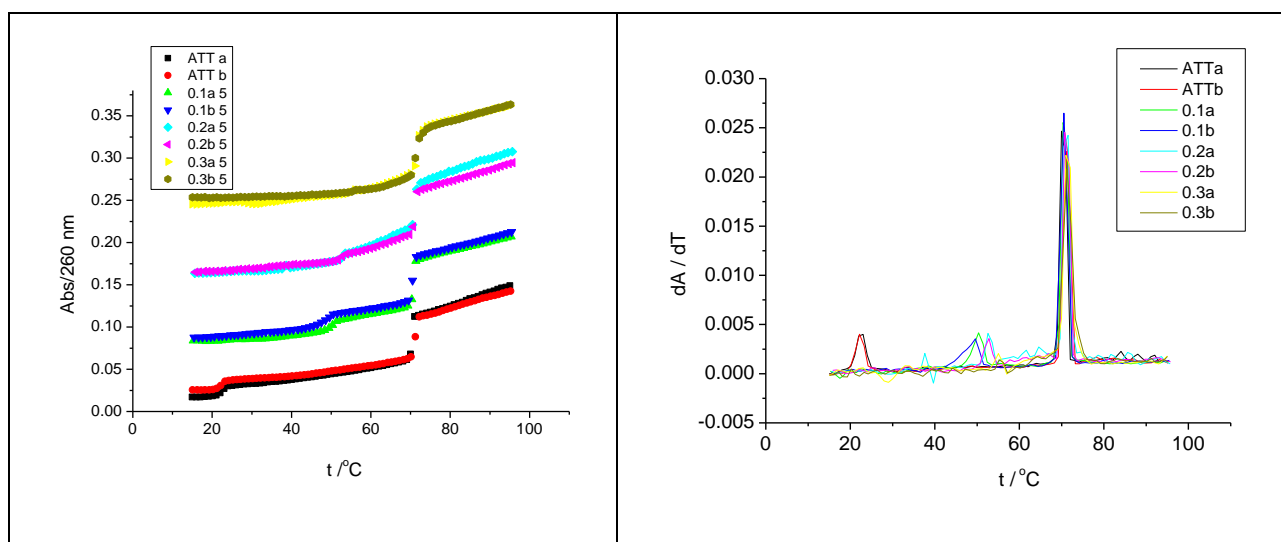


Figure S28. Left: Melting curve of ATT triplex upon addition of ratio, r ([compound]/ [polynucleotide]) = 0.1 of **5** at pH = 7.0 (sodium cacodylate buffer with NaCl, $I = 0.1 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$); Right: First derivative of absorbance at 260 nm in dependence of temperature.

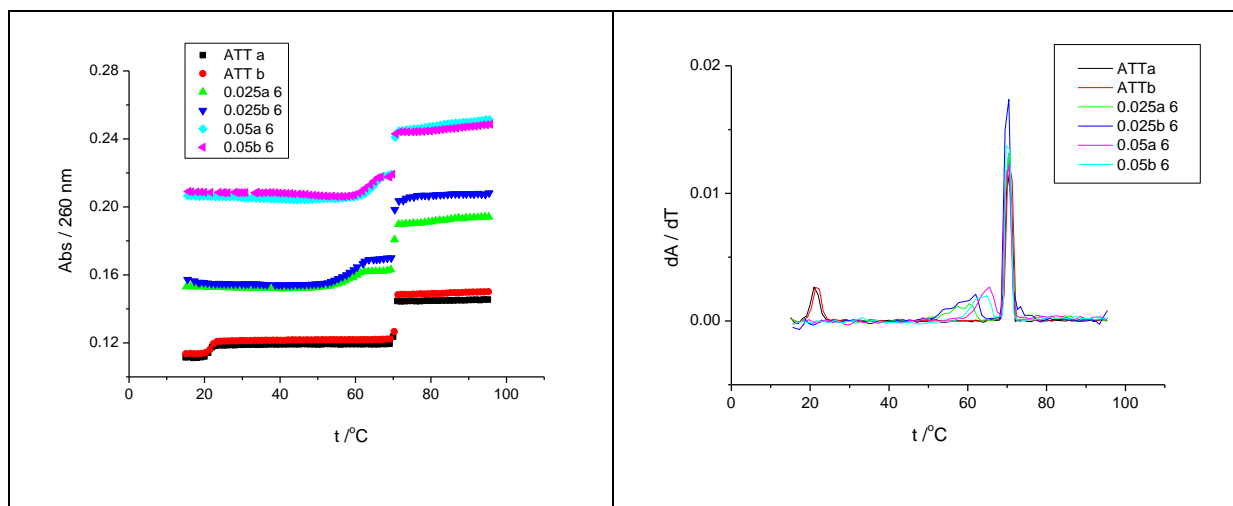


Figure S29. Left: Melting curve of ATT triplex upon addition of ratio, r ([compound]/[polynucleotide])=0.1 of **6** at pH=7.0 (sodium cacodylate buffer with NaCl, $I = 0.1 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$); Right: First derivative of absorbance at 260 nm in dependence of temperature.

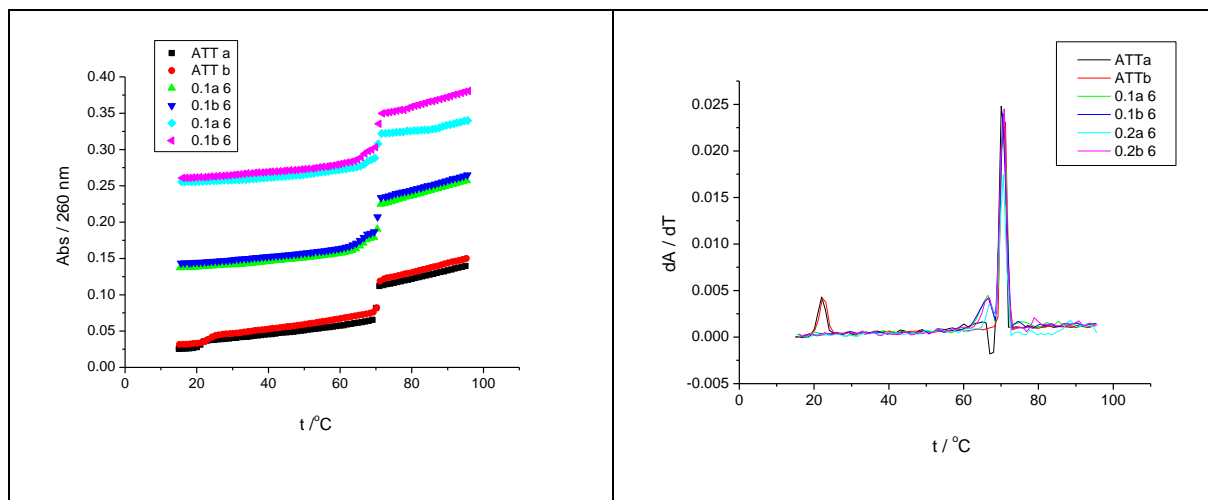


Figure S30. Left: Melting curve of ATT triplex upon addition of ratio, r ([compound]/[polynucleotide])=0.1 of **6** at pH=7.0 (sodium cacodylate buffer with NaCl, $I = 0.1 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$); Right: First derivative of absorbance at 260 nm in dependence of temperature.

2.5. Circular dichroism (CD) experiments

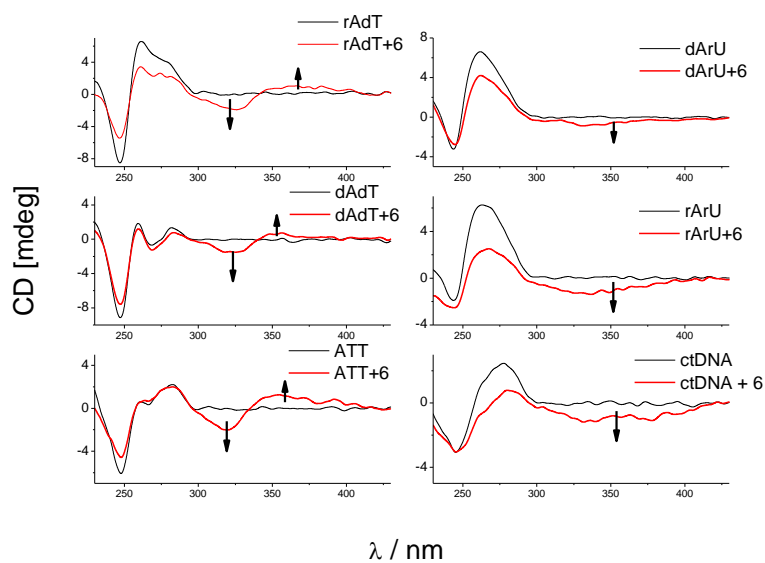


Figure S31. CD titrations of ATT triplex, poly rA-poly dT, poly rA-poly rU and poly dA-poly rU ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) with **6** at molar ratios $r = [\text{compound}] / [\text{polynucleotide}] = 0.3$ (pH = 7.0, buffer sodium cacodylate, $I = 0.05 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$ for all titrations except poly dA-poly rU ($I = 0.2 \text{ mol dm}^{-3} + 1 \text{ mM EDTA}$)).

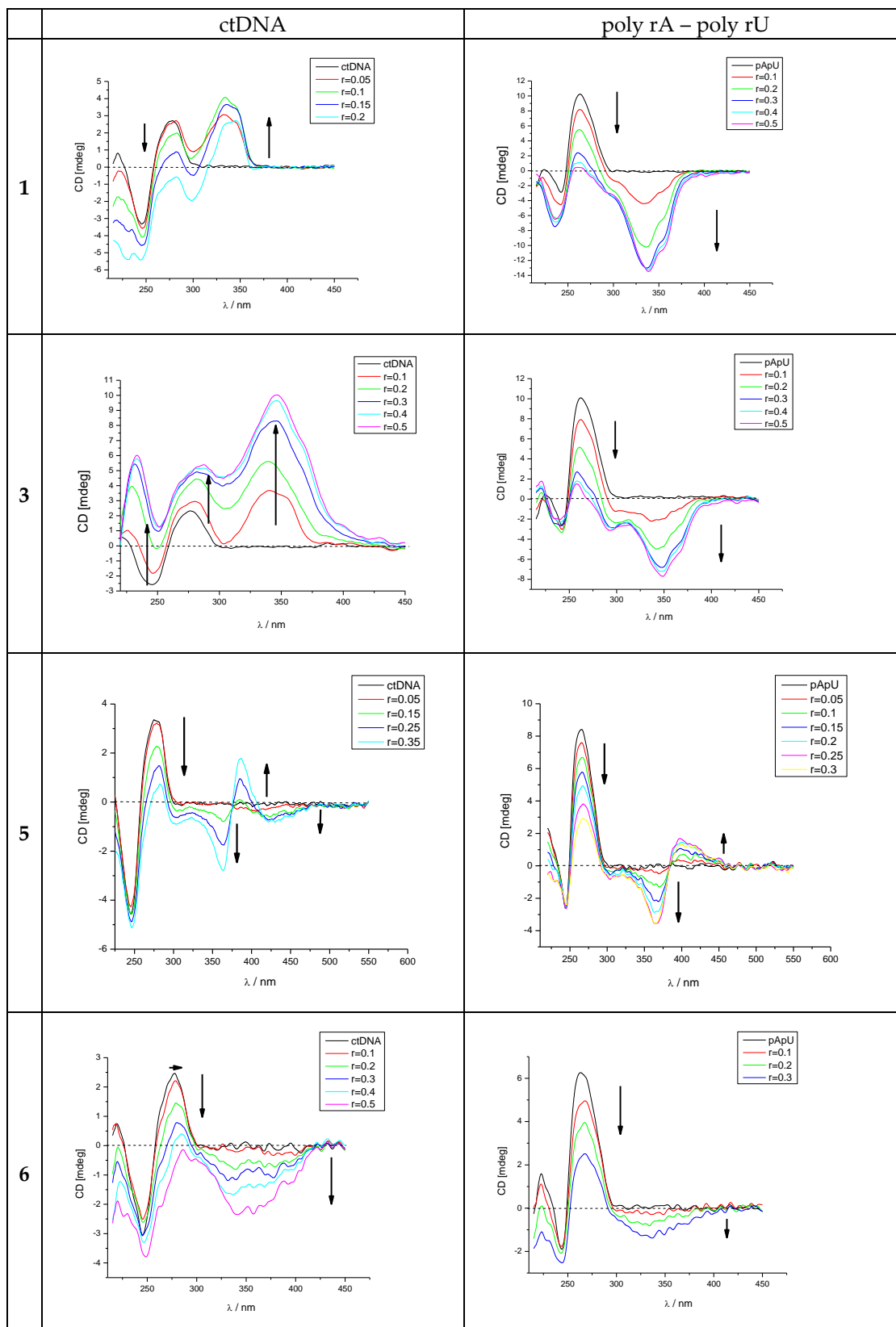


Figure S32. CD titrations of ctDNA ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) and poly rA-poly rU ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) with **1**, **3**, **5** and **6** at molar ratios $r = [\text{compound}] / [\text{polynucleotide}]$ (pH = 7.0, buffer sodium cacodylate, $I = 0.05 \text{ mol dm}^{-3}$).

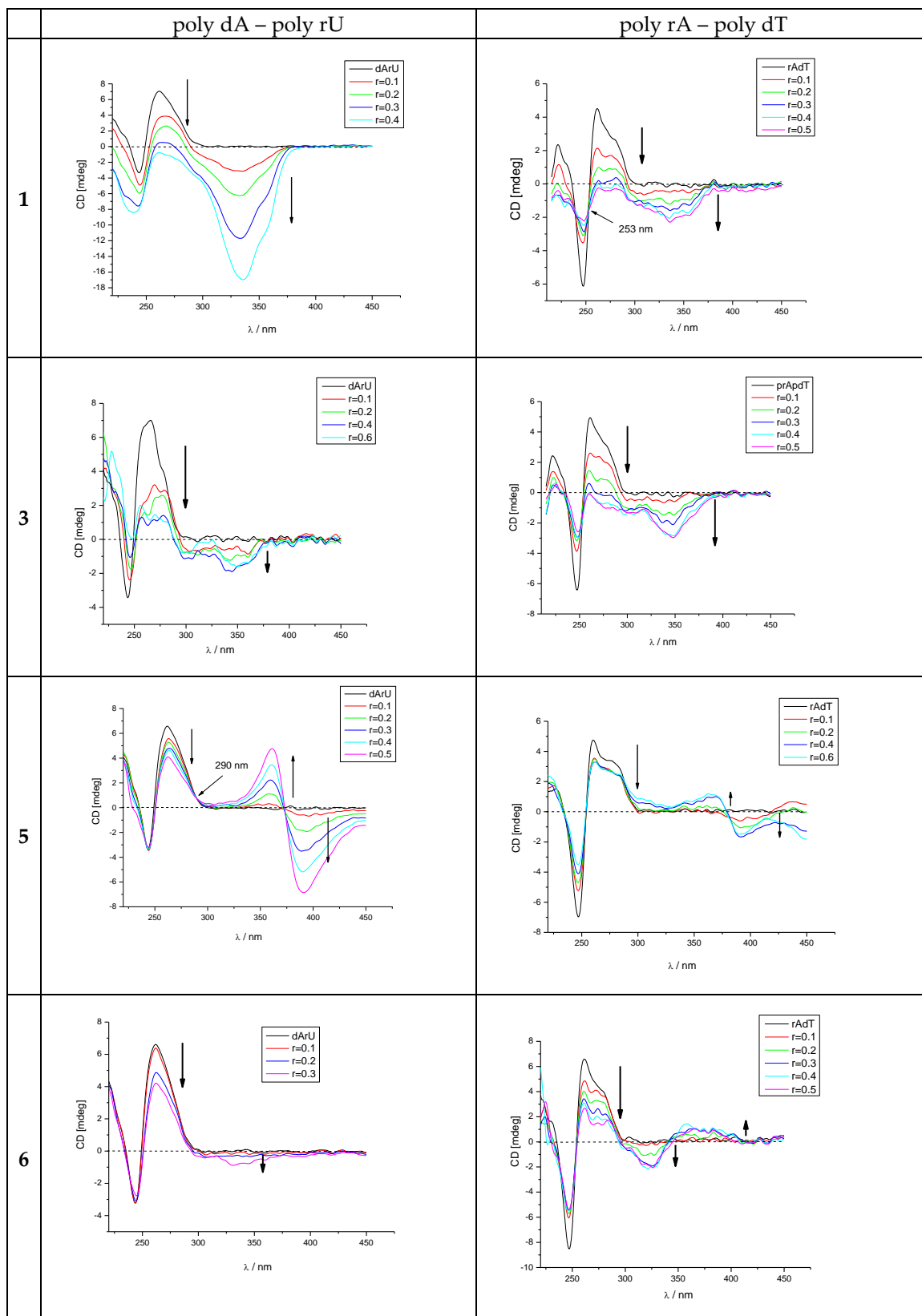


Figure S33. CD titrations of poly dA – poly rU ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) and poly rA – poly dT ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) with **1**, **3**, **5** and **6** at molar ratios $r = [\text{compound}] / [\text{polynucleotide}]$ (pH = 7.0, buffer sodium cacodylate, $I = 0.05 \text{ mol dm}^{-3}$).

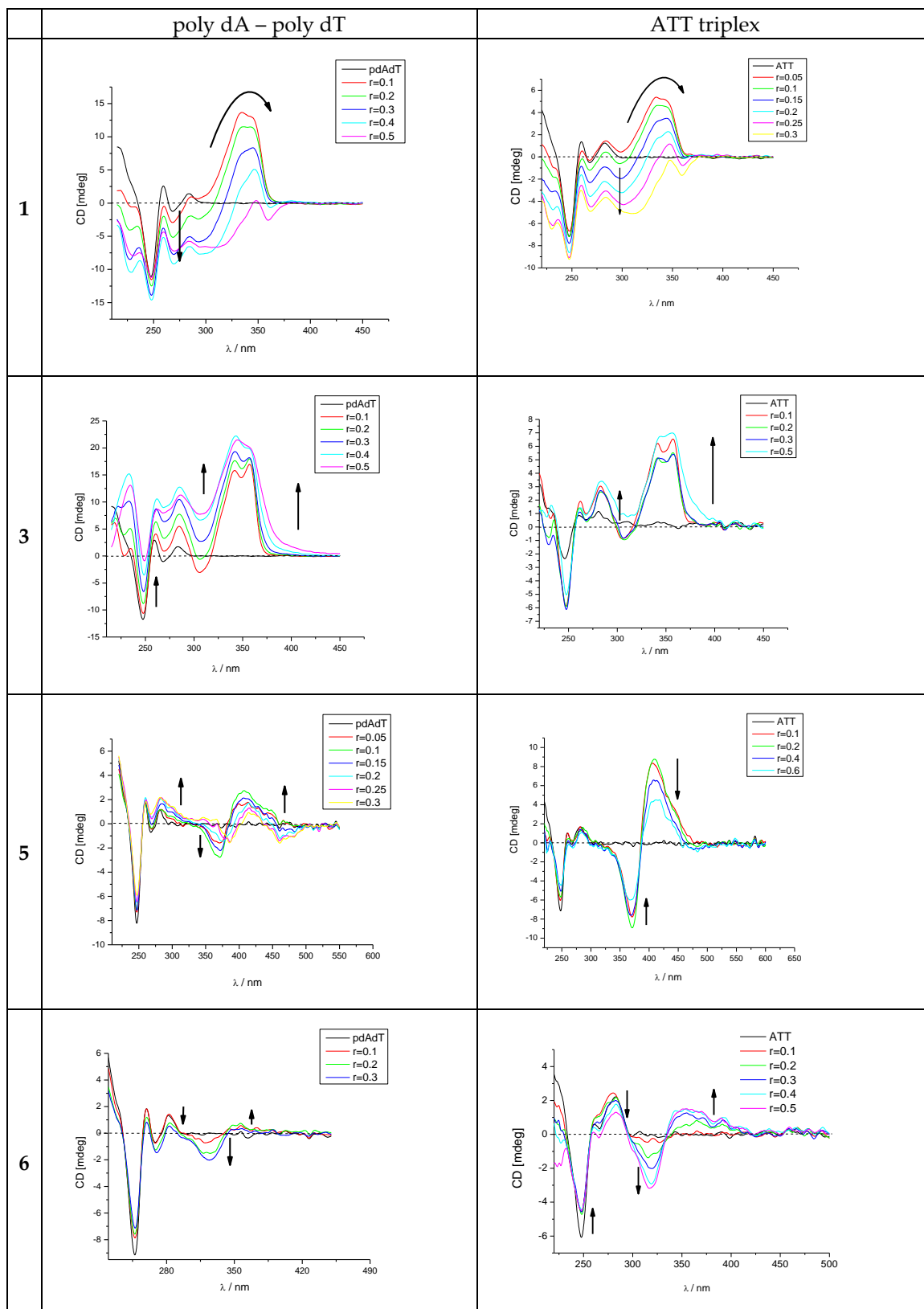


Figure S34. CD titrations of poly dA – poly dT ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) and ATT triplex ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) with **1**, **3**, **5** and **6** at molar ratios $r = [\text{compound}] / [\text{polynucleotide}]$ (pH = 7.0, buffer sodium cacodylate, $I = 0.05 \text{ mol dm}^{-3}$).

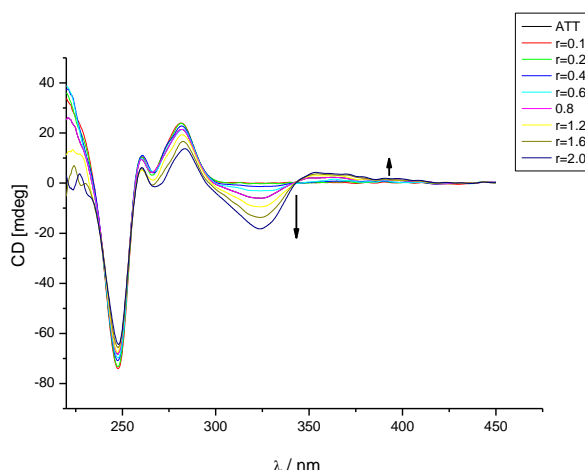


Figure S35. CD titration of ATT 26mer triplex ($c = 3.0 \times 10^{-5} \text{ mol dm}^{-3}$) and with **6** at molar ratios $r = [\text{compound}] / [\text{polynucleotide}]$ (pH = 7.0, buffer sodium cacodylate, $I = 0.05 \text{ mol dm}^{-3}$).

3. Characterization of 1-9 compounds (NMR, elemental analysis, ESI)

Compound 1. C. ^1H NMR (300 MHz, DMSO- d_6) (δ ppm): 10.70 (br s, 4H, H-Amd), 9.54 (s, 2H, H-Py), 9.13 (s, 1H, H-Py), 8.91 (s, 2H, H-Bt), 8.44 (d, 2H, $J = 8.3 \text{ Hz}$, H-Bt), 8.15 (d, 2H, $J = 8.4 \text{ Hz}$, H-Bt), 4.04 (s, 8H, H-CH $_2$). ^{13}C NMR (75 MHz, D $_2$ O, 70°C) (δ ppm): 167.9, 164.3, 155.7, 149.5, 135.0, 131.8, 127.8, 126.0, 123.7, 123.0, 118.7, 44.7. LC-MS (ESI) m/z : 482.4 [(M + H $^+$) calcd for free base C $_{25}$ H $_{19}$ N $_7$ S $_2$, 481.11]. Analysis calcd for C $_{25}$ H $_{21}$ C $_{12}$ N $_7$ S $_2$ \times 3H $_2$ O (608.56): C, 49.34; H, 4.47; N, 16.11, Cl, 11.65. Found C, 49.51; H, 4.54; N, 16.01; Cl, 11.83%.

Compound 2. ^1H NMR (300 MHz, DMSO- d_6) (δ ppm): 10.89 (br s, 4H, H-Amd), 9.02 (d, 1H, $J = 4.3 \text{ Hz}$, H-Py), 8.96 (s, 1H, H-Bt), 8.87 (s, 1H, H-Bt), 8.39 (d, 1H, $J = 7.2 \text{ Hz}$, H-Py), 8.33 (d, 1H, $J = 8.4 \text{ Hz}$, H-Bt), 8.18 (d, 1H, $J = 8.6 \text{ Hz}$, H-Bt), 8.00 (d, 1H, $J = 8.2 \text{ Hz}$, H-Bt), 7.88 (dd, 1H, $J = 4.6 \text{ Hz}$, $J = 7.5 \text{ Hz}$, H-Py), 7.65 (d, 1H, $J = 8.2 \text{ Hz}$, H-Bt), 4.07 (s, 4H, H-CH $_2$), 4.02 (s, 4H, H-CH $_2$). LC-MS (ESI) m/z : 482.4 [(M + H $^+$) calcd for free base C $_{25}$ H $_{19}$ N $_7$ S $_2$, 481.11]. Analysis calcd for C $_{25}$ H $_{21}$ C $_{12}$ N $_7$ S $_2$ \times 3H $_2$ O (608.56): C, 49.34; H, 4.47; N, 16.11, Cl, 11.65. Found C, 49.66; H, 4.35; N, 16.27; Cl, 11.53%.

Compound 3. ^1H NMR (300 MHz, DMSO- d_6) (δ ppm): 8.89 (d, 2H, $J = 1.3 \text{ Hz}$, H-Bt), 8.57 (d, 2H, $J = 7.8 \text{ Hz}$, H-Py), 8.39 (d, 2H, $J = 8.6 \text{ Hz}$, H-Bt), 8.36 (d, 1H, $J = 7.8 \text{ Hz}$, H-Py), 8.11 (dd, 2H, $J = 1.6 \text{ Hz}$, $J = 8.6 \text{ Hz}$, H-Bt), 4.04 (s, 8H, H-CH $_2$). ^{13}C NMR (75 MHz, D $_2$ O/DMSO- d_6 , 80°C) (δ ppm): 170.9, 162.2, 154.3, 146.3, 137.9, 134.1, 123.7, 122.0, 121.3, 121.1, 116.2, 43.0. LC-MS (ESI) m/z : 482.4 [(M + H $^+$) calcd for free base

C $_{25}$ H $_{19}$ N $_7$ S $_2$, 481.11]. Analysis calcd for C $_{25}$ H $_{21}$ C $_{12}$ N $_7$ S $_2$ \times 2H $_2$ O (590.55): C, 50.85; H, 4.27; N, 16.60, Cl, 12.01. Found C, 51.12; H, 4.23; N, 16.81; Cl, 11.92%.

Compound 4. ^1H NMR (300 MHz, DMSO- d_6) (δ ppm): 10.57 (s, 2H, -C(NH-) $_2^+$), 8.74 (d, 1H, $J = 1.6 \text{ Hz}$, Ar-H), 8.27 (d, 1H, $J = 8.6 \text{ Hz}$, Ar-H), 8.11-8.00 (m, 2H, Thioph.-H), 8.02 (dd, 1H, $J = 1.7 \text{ Hz}$, $J = 8.6 \text{ Hz}$, Ar-H), 4.06 (s, 4H, -CH $_2$ CH $_2$ -), 2.31 (s, 3H, CH $_3$ SO $_3$). ^{13}C NMR (150 MHz, DMSO- d_6) (δ ppm): 164.7 (s), 164.1 (s), 156.2 (s), 136.8 (s), 135.1 (s), 132.7 (d), 129.7 (d), 126.6 (d), 123.7 (d), 123.1 (d), 119.0 (s), 110.5 (s), 44.6 (t, 2C), 39.7 (q). LC-MS (ESI) m/z : 364.5 [(M+H $^+$) calcd for free base C $_{14}$ H $_{10}$ BrN $_3$ S $_2$, 362.95]. Analysis calcd for C $_{15}$ H $_{14}$ BrN $_3$ O $_3$ S $_3$ (460.39): C, 39.13; H, 3.07; N, 9.13. Found C, 38.98; H, 3.01; N, 9.08 %.

Compound 5. ^1H NMR (300 MHz, DMSO- d_6) (δ ppm): 10.50 (s, 2H, -C(NH-) $_2^+$), 8.69 (d, 1H, $J = 1.4 \text{ Hz}$, Ar-H), 8.20 (d, 1H, $J = 8.6 \text{ Hz}$, Ar-H), 8.01 (dd, 1H, $J = 1.7 \text{ Hz}$, $J = 8.6 \text{ Hz}$, Ar-H), 7.94 (d, 1H, $J = 4.0 \text{ Hz}$, Thioph.-H), 7.66 (d, 1H, $J = 5.0 \text{ Hz}$, Thioph.-H), 7.54 (d, 1H, $J = 3.4 \text{ Hz}$, Thioph.-H), 7.47 (d, 1H, $J = 4.0 \text{ Hz}$, Thioph.-H), 7.17 (dd, 1H, $J = 3.7 \text{ Hz}$, $J = 4.9 \text{ Hz}$, Thioph.-H), 4.01 (s, 4H, -CH $_2$ CH $_2$ -), 2.33 (s, 3H, CH $_3$ SO $_3$). ^{13}C NMR (150 MHz, DMSO- d_6) (δ ppm): 165.1 (s), 164.8 (s), 156.8 (s), 142.3 (s), 135.3 (s), 135.0 (s), 133.8 (s), 132.0 (d), 128.6 (d), 127.3 (d), 126.7 (d), 126.0 (d), 125.3 (d), 123.4 (d), 122.7 (d), 118.8 (s), 44.7 (t, 2C), 39.7

(q). LC-MS (ESI) m/z : 368.5 [(M+H⁺) calcd for free base C₁₈H₁₃N₃S₃, 367.03]. Analysis calcd for C₁₉H₁₇N₃O₃S₄ (463.62): C, 49.22; H, 3.70; N, 9.06. Found C, 49.10; H, 3.75; N, 9.22 %.

Compound 6. ¹H NMR (300 MHz, DMSO-d₆, 50°C) (δ ppm): 10.57 (s, 2H, -C(NH-)₂⁺), 8.81 (s, 1H, Ar-H), 8.37 (d, 1H, J = 8.6 Hz, Ar-H), 8.16 (d, 1H, J = 8.2 Hz, Ar-H), 8.07 (d, 1H, J = 8.8 Hz, Ar-H), 8.00 (d,

1H, J = 8.2 Hz, Ar-H), 7.69-7.61 (m, 2H, Ar-H), 4.08 (s, 4H, -CH₂CH₂-), 2.33 (s, 3H, CH₃SO₃). ¹³C NMR (75 MHz, DMSO-d₆, 50°C) (δ ppm): 165.5, 162.7, 155.2, 138.2, 136.9, 135.7, 130.9, 129.0, 127.3, 126.8, 124.2, 123.9, 123.8, 123.2, 122.9, 119.9, 45.2 (2C). LC-MS (ESI) m/z : 370.5 [(M+H⁺) calcd for free base

C₁₈H₁₂ClN₃S₂, 369.02]. Analysis calcd for C₁₉H₁₆ClN₃O₃S₃ (466.00): C, 46.97; H, 3.46; N, 9.02. Found C, 47.01; H, 3.33; N, 9.27 %.

Compound 7. ¹H NMR (300 MHz, DMSO-d₆) (δ ppm): 10.57 (s, 2H, -C(NH-)₂⁺), 8.80 (d, 2H, J = 2.0 Hz, Ar-H), 8.34 (d, 1H, J = 8.6 Hz, Ar-H), 8.26 (dd, 1H, J = 8.6, 1.8 Hz, Ar-H), 8.20 (m, 1H, Ar-H), 8.14 (d, 1H, J = 8.7 Hz, Ar-H), 8.09-8.01 (m, 2H, Ar-H), 7.70-7.64 (m, 2H, Ar-H), 4.07 (s, 4H, -CH₂-), 2.33 (s, 3H, CH₃SO₃⁻). ¹³C NMR (150 MHz, DMSO-d₆) (δ ppm): 172.2 (s), 164.7 (s), 157.0 (s), 135.1 (s), 134.6 (s),

132.7 (s), 129.5 (s), 129.2 (d), 129.1 (d), 128.4 (d), 128.3 (d), 127.9 (d), 127.4 (d), 126.5 (d), 123.9 (d), 123.7 (d), 123.4 (d), 118.9 (s), 44.6 (t, 2C). LC-MS (ESI) m/z : 330.1 [(M + H⁺) calcd for free base C₁₄H₁₁N₃OS, 329.10]. Analysis calcd for C₂₁H₁₉N₃O₃S₂ (425.52): C, 59.27; H, 4.50; N, 9.87. Found C, 59.31; H, 4.55; N, 10.01%.

Compound 8. ¹H NMR (600 MHz, DMSO-d₆) (δ ppm): 10.61 (s, 2H, -C(NH-)₂⁺), 8.82 (d, 1H, J = 1.4 Hz, Ar-H), 8.35 (d, 1H, J = 8.6 Hz, Ar-H), 8.06 (dd, 1H, J = 1.6 Hz, J = 8.6 Hz, Ar-H), 7.98 (s, 1H, Ar-H), 7.85 (d, 1H, J = 7.7 Hz, Ar-H), 7.79 (d, 1H, J = 8.3 Hz, Ar-H), 7.54 (m, 1H, Ar-H), 7.41 (t, 1H, J = 7.6 Hz, Ar-H), 4.07 (s, 4H, -CH₂-), 2.33 (s, 3H, CH₃SO₃⁻). ¹³C NMR (75 MHz, DMSO-d₆, 70°C) (δ ppm): 165.5 (s), 161.8 (s), 157.3 (s), 155.8 (s), 149.1 (s), 135.3 (s), 128.2 (s), 127.9 (d), 127.2 (d), 124.7 (d), 124.4 (d), 124.0 (d), 123.3 (d), 119.7 (s), 112.2 (d), 110.0 (d), 45.2 (t, 2C), 40.3 (q). LC-MS (ESI) m/z : 320.1 [(M + H⁺) calcd for free base C₁₈H₁₃N₃OS, 319.08]. Analysis calcd for C₁₉H₁₇N₃O₄S₂ (415.49): C, 54.92; H, 4.12; N, 10.11. Found

C, 54.98; H, 4.28; N, 10.07%.

Compound 9. ¹H NMR (300 MHz, DMSO-d₆) (δ ppm): 11.57 (s, 1H, Ind-H), 10.54 (s, 2H, -C(NH-)₂⁺), 8.70 (s, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.01 (dd, 1H, J = 1.2 Hz, J = 8.5 Hz, Ar-H), 7.93 (dd, 1H, J = 1.2 Hz, J = 8.5 Hz, Ar-H), 7.59 (d, 1H, J = 8.5 Hz, Ar-H), 7.52 (m, 1H, Ar-H), 6.65 (s, 1H, Ar-H), 4.06 (s, 4H, -CH₂-), 2.32 (s, 3H, CH₃SO₃⁻). ¹³C NMR (150 MHz, DMSO-d₆) (δ ppm): 174.0 (s), 164.7 (s), 157.3 (s), 138.2 (s), 134.7 (s), 128.0 (s), 127.6 (d), 126.3 (d), 123.4 (s), 123.2 (d), 122.6 (d), 122.8 (d), 122.6 (d), 117.9 (s), 112.4 (d), 102.6 (d), 44.5 (t, 2C), 39.7 (q). LC-MS (ESI) m/z : 319.1 [(M + H⁺) calcd for free base C₁₈H₁₄N₄S, 318.09]. Analysis calcd for C₁₉H₁₈N₄O₃S₂ (414.50): C, 55.05; H, 4.38; N, 13.52. Found C, 55.11; H, 4.51; N, 13.44%.