Supporting Information

Distinctive Nucleic Acid Recognition by Lysine-Embedded Phenanthridine Peptides

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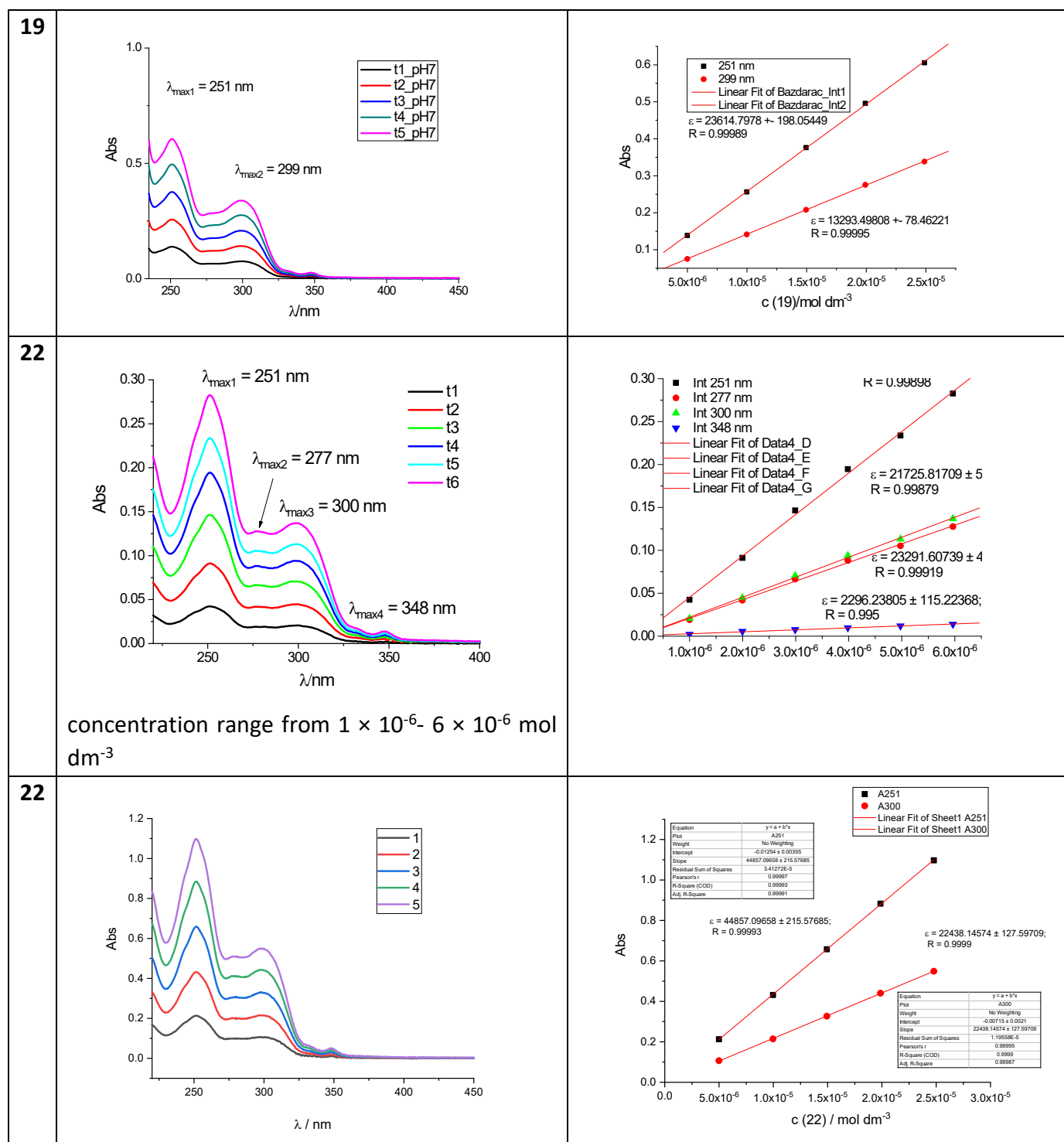
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†   Deceased 1 August 2019.

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1. Spectroscopic characterization of phenanthridine peptides in aqueous solutions

1.1. UV/Vis spectra at pH 7.0

19		
22	concentration range from 1×10^{-6}- 6×10^{-6} mol dm^{-3}	
22		

23

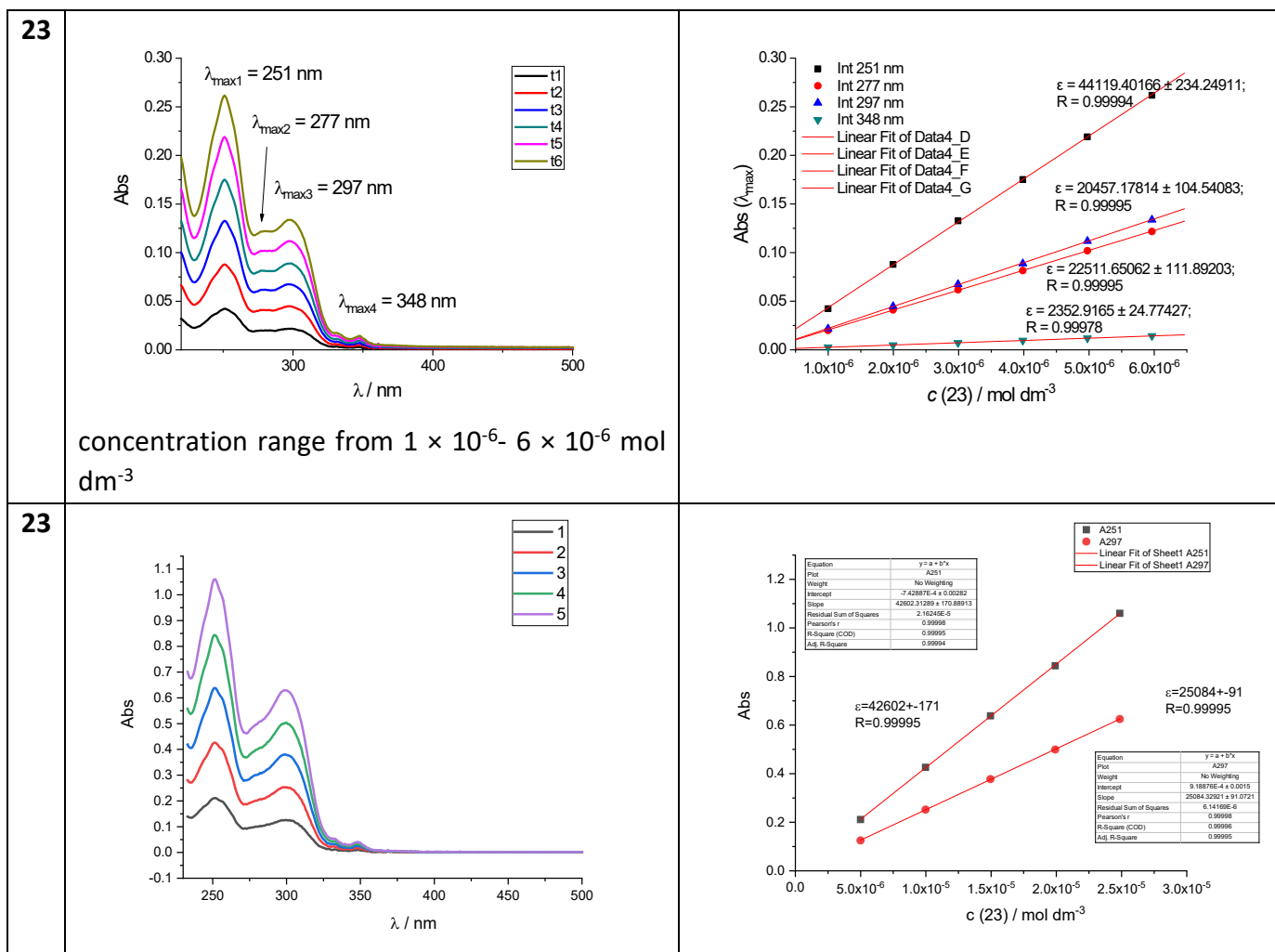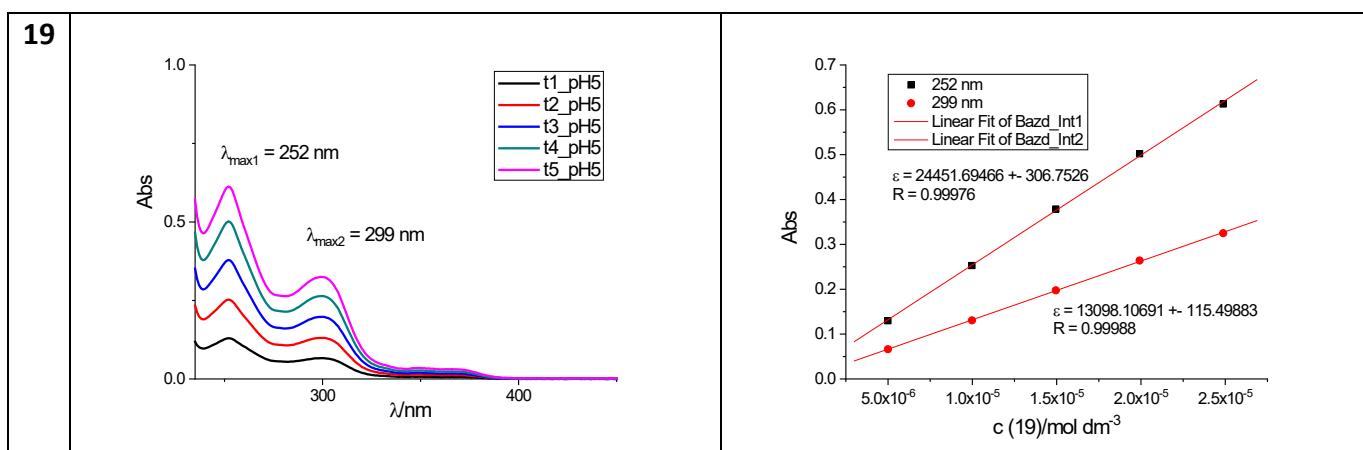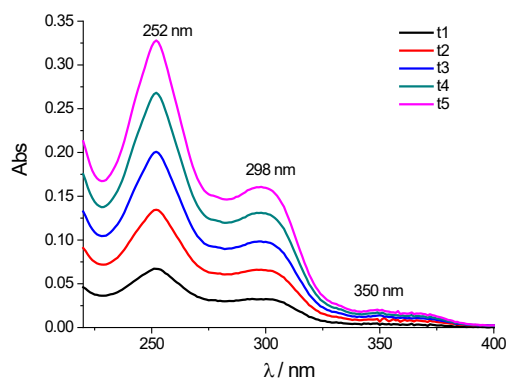concentration range from 1×10^{-6}- 6×10^{-6} mol dm^{-3}

23

Figure S1. UV/Vis spectra changes of **19, 22, 23** at different concentrations (concentration range from 5×10^{-6}- 2.5×10^{-5} mol dm^{-3}) at pH 7.0, sodium cacodylate buffer, *I*=0,05 M.
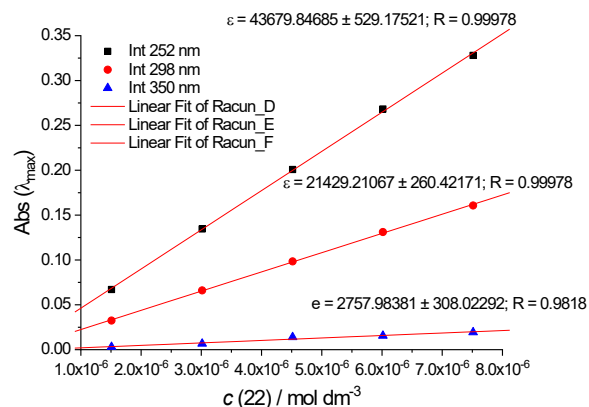
1.2. UV/Vis spectra at pH 5.0

19
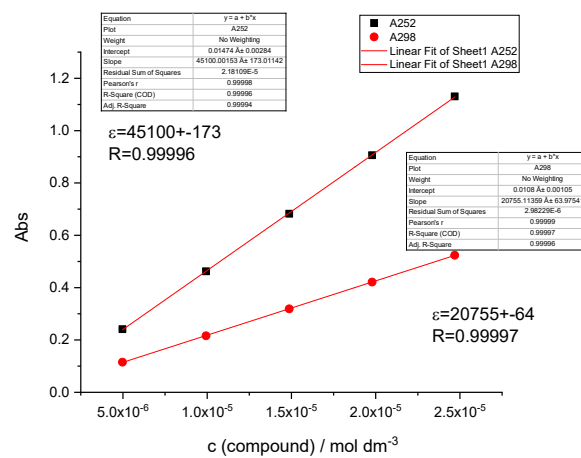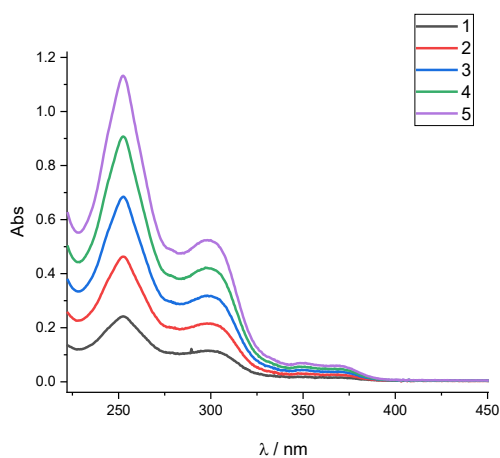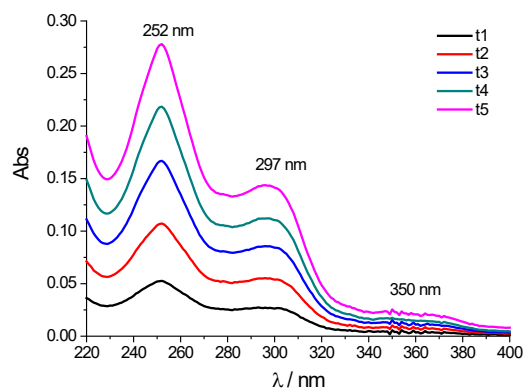
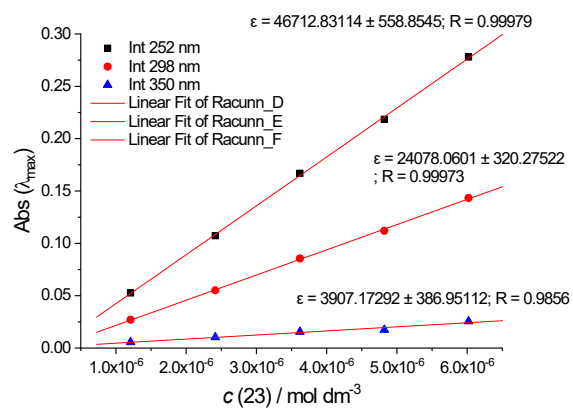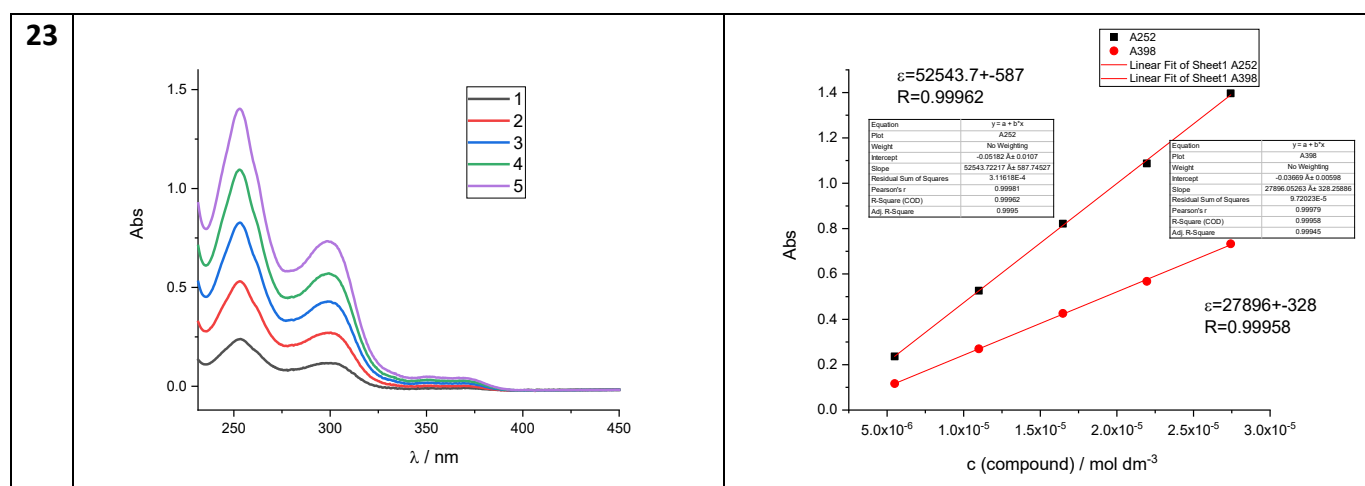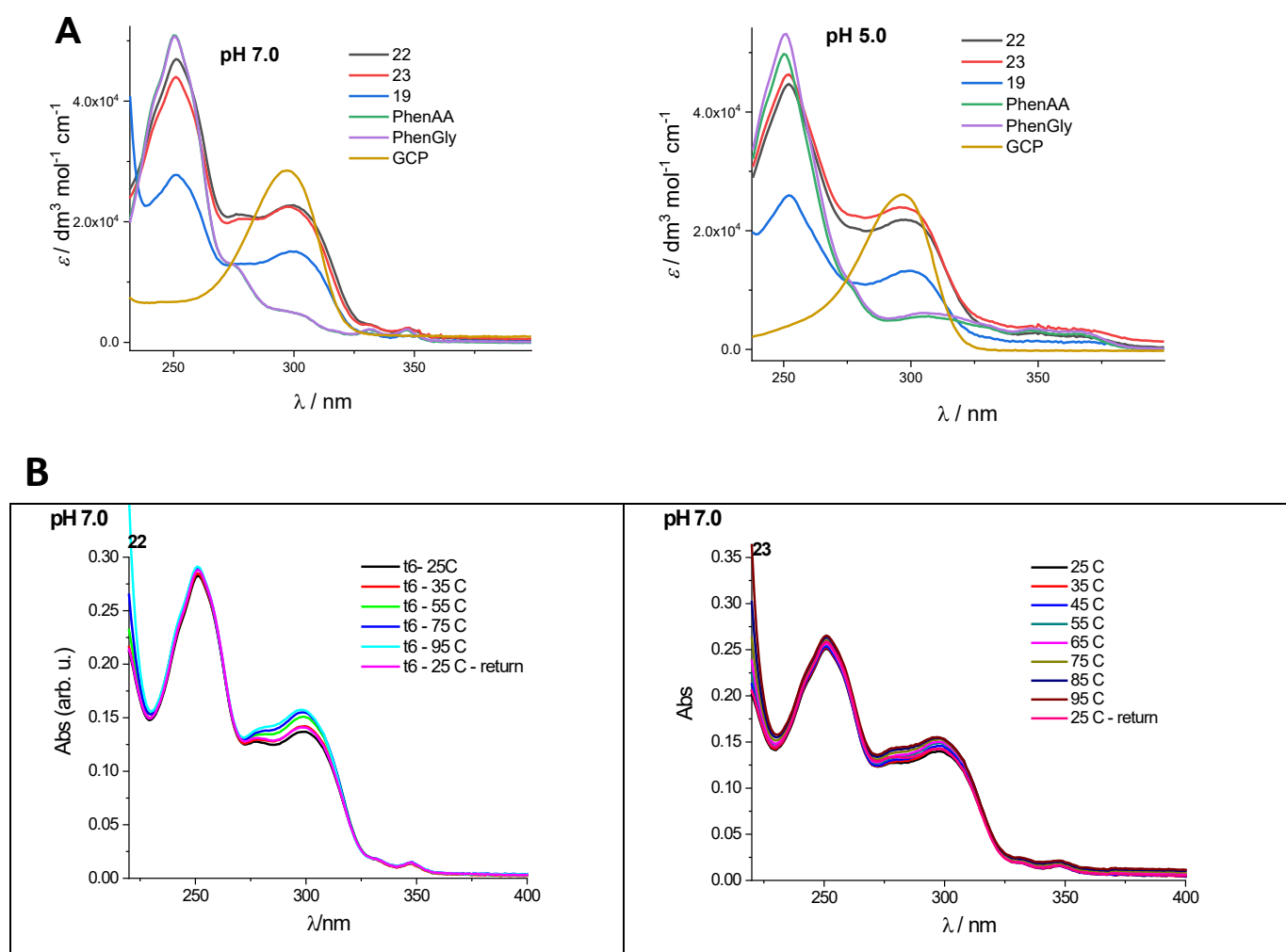| 22	
concentration range from 1×10^{-6} - 6×10^{-6} mol dm^{-3}	$\varepsilon = 43679.84685 \pm 529.17521$; R = 0.99978 $\varepsilon = 21429.21067 \pm 260.42171$; R = 0.99978 e = 2757.98381 ± 308.02292; R = 0.9818 Int 252 nm / Int 298 nm / Int 350 nm / Linear Fit of Racun_D / Linear Fit of Racun_E / Linear Fit of Racun_F
22	A252 / A298 / Linear Fit of Sheet1 A252 / Linear Fit of Sheet1 A298 $\varepsilon = 45100 \pm 173$ R=0.99996 $\varepsilon = 20755 \pm 64$ R=0.99997
23	$\varepsilon = 46712.83114 \pm 558.8545$; R = 0.99979 $\varepsilon = 24078.0601 \pm 320.27522$; R = 0.99973 $\varepsilon = 3907.17292 \pm 386.95112$; R = 0.9856 Int 252 nm / Int 298 nm / Int 350 nm / Linear Fit of Racunn_D / Linear Fit of Racunn_E / Linear Fit of Racunn_F
concentration range from 1×10^{-6} - 6×10^{-6} mol dm^{-3}	

Figure S2. UV/Vis spectra changes of **19,22,23** at different concentrations (concentration range from 5×10^{-6}- 2.5×10^{-5} mol dm^{-3}) at pH 5.0, sodium cacodylate buffer, I=0,05 M.
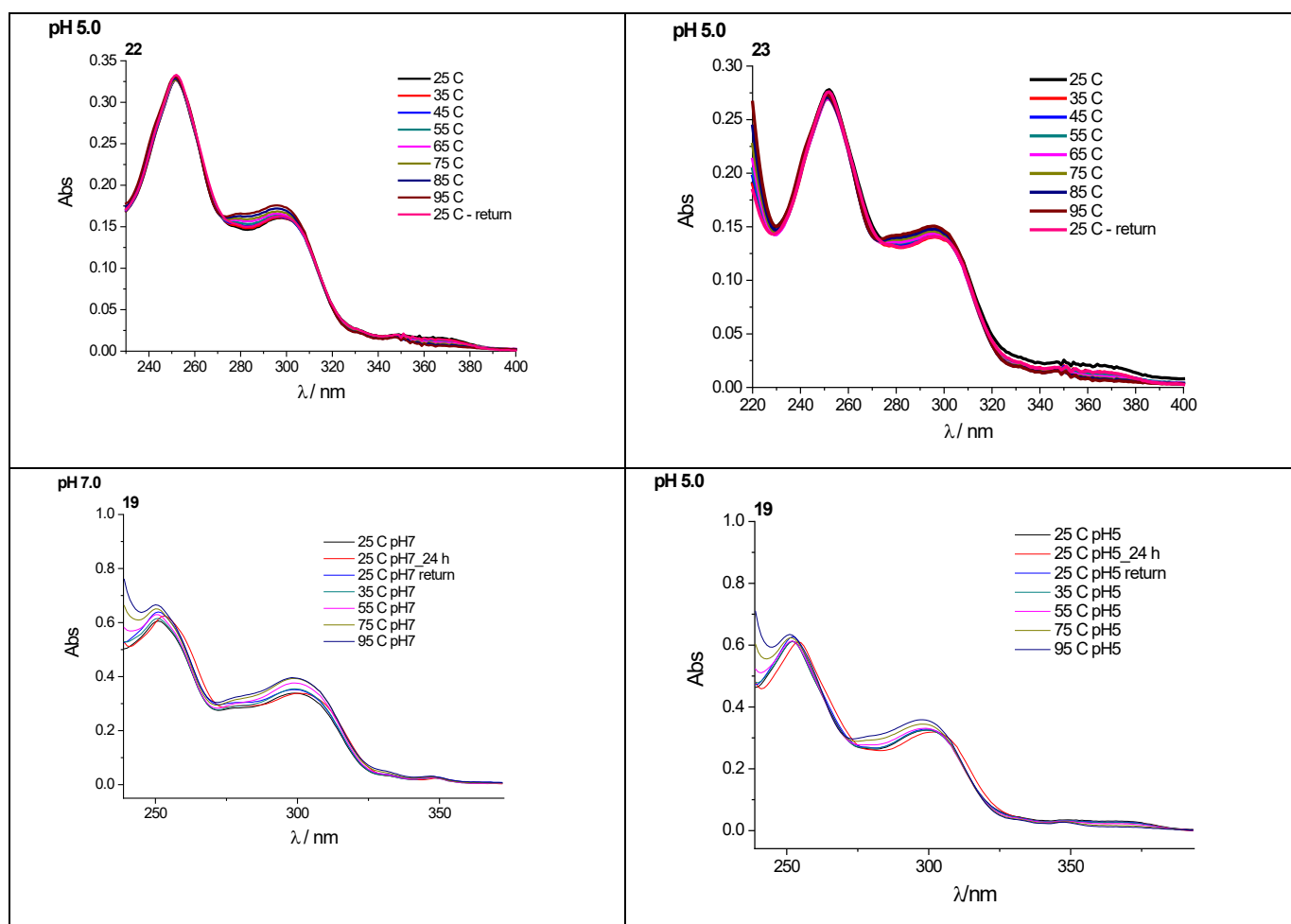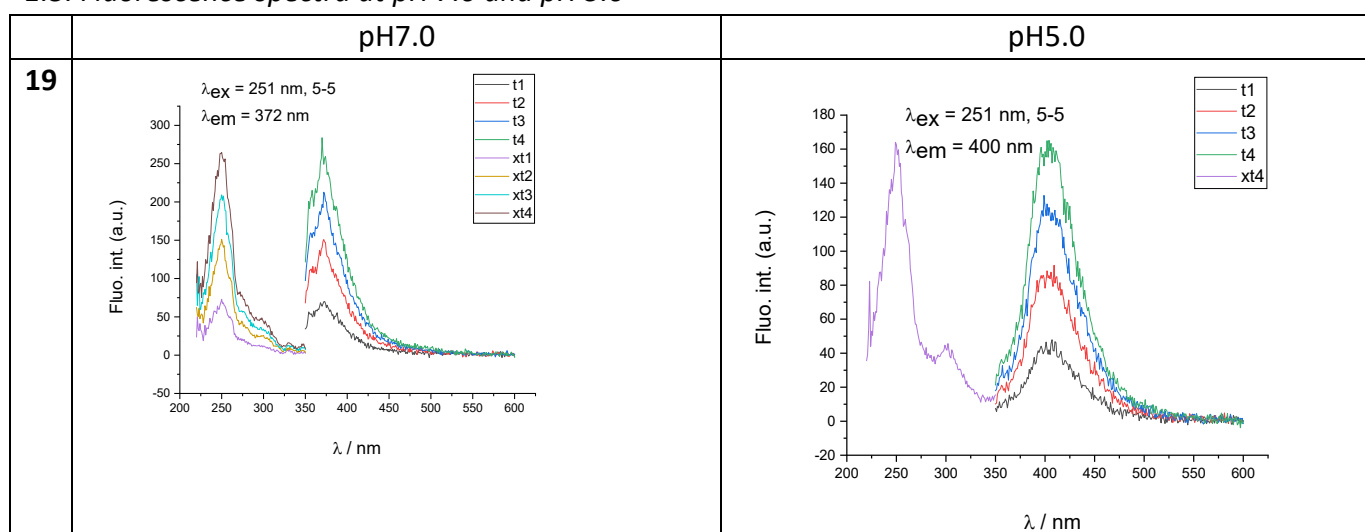
Figure S3. A) Overlapped UV spectra of **22, 23** and **19** with the reference compounds **Phen-AA**, **Phen-Gly** and **GCP**; showing hypochromicity of all chromophores; B) changes of UV spectra of **22, 23** and **19** upon temperature increase.
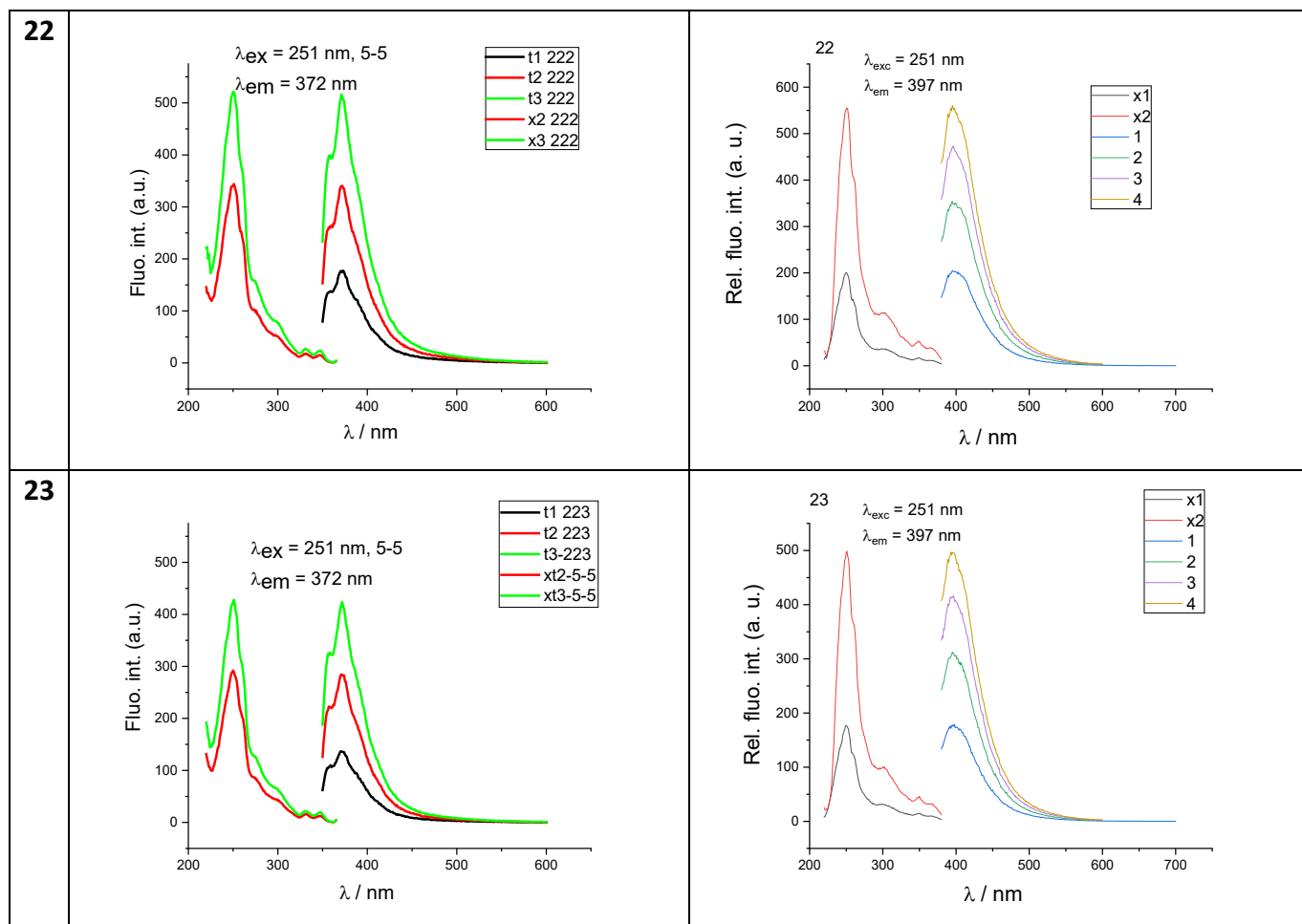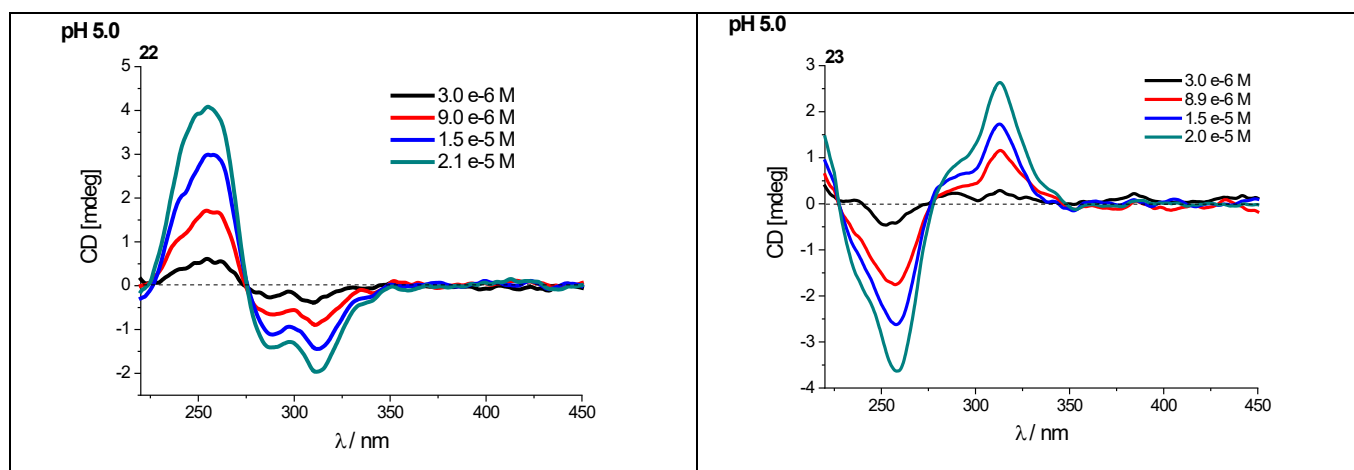
1.3. Fluorescence spectra at pH 7.0 and pH 5.0

Figure S4. Emission and excitation spectra changes of **19, 22, 23** at different concentrations at λ_{exc}=251 nm (concentration range from 5×10^{-7}- 2×10^{-6} mol dm^{-3}) at pH 7.0 and pH 5.0, Na cacodylate buffer, I=0.05 mol dm^{-3}.

*1.4. CD spectra of **19, 22** and **23***
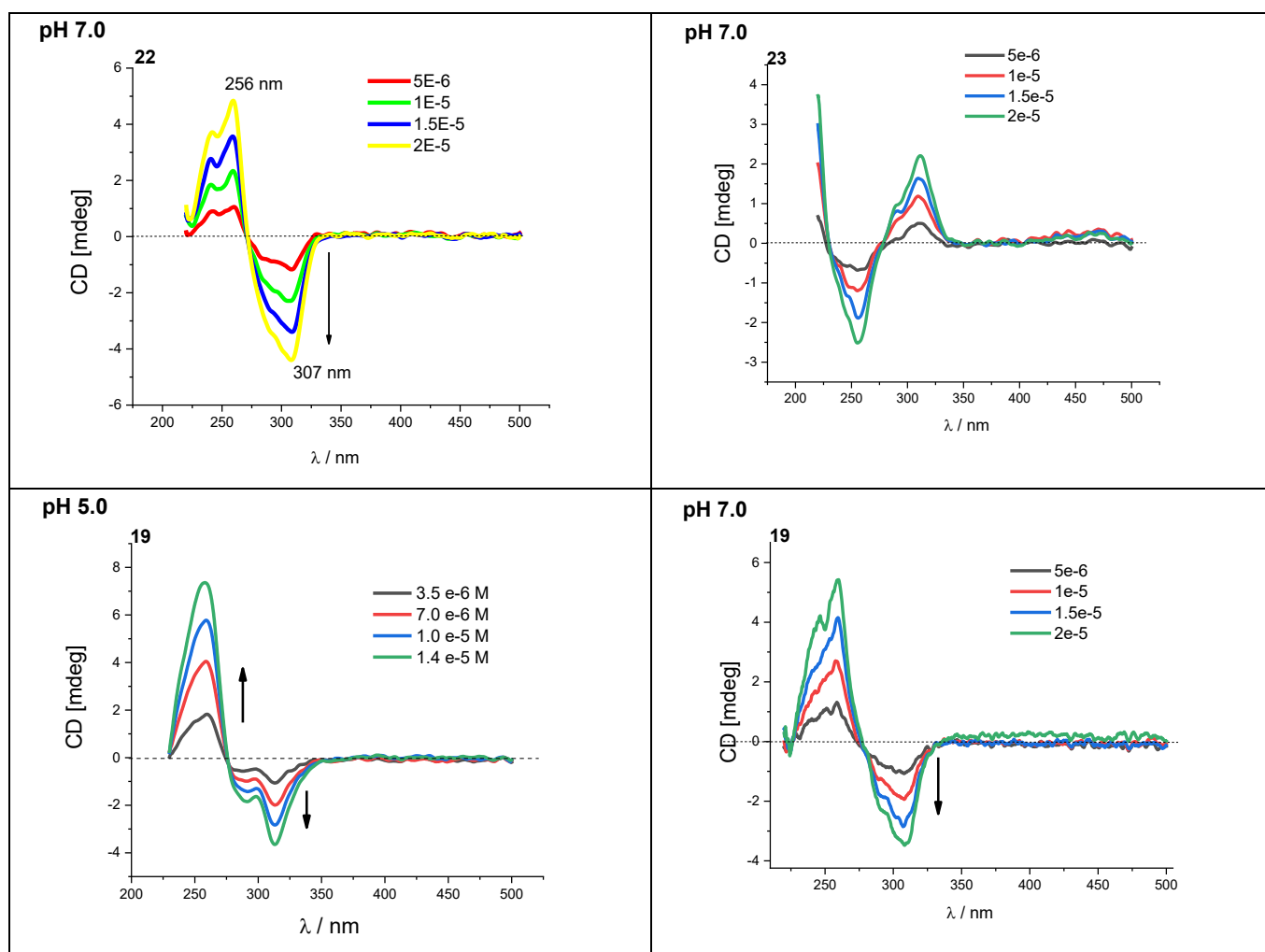
Figure S5. CD spectra changes of **19, 22** and **23** at different concentrations (concentration range from 3.0×10^{-6}- 6×10^{-5} mol dm^{-3}) at pH = 7.0 and 5.0 (buffer sodium cacodylate, I = 0.05 mol dm^{-3}).

2. Interactions of phenanthridine peptides with polynucleotides in neutral and weakly acid medium (pH 7.0 and pH 5.0)

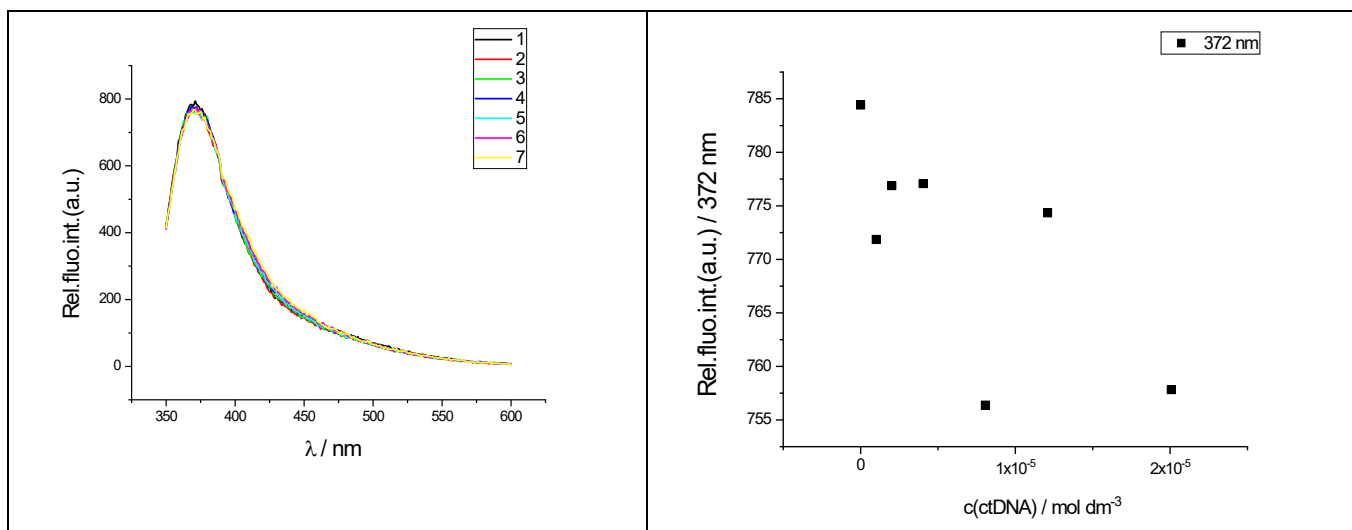2.1. Fluorimetric titrations

2.1.1. Fluorimetric titrations at pH 7.0

Figure S6. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA (c= 1.0 × 10^{-6} - 2 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 372 nm on c(ctDNA), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
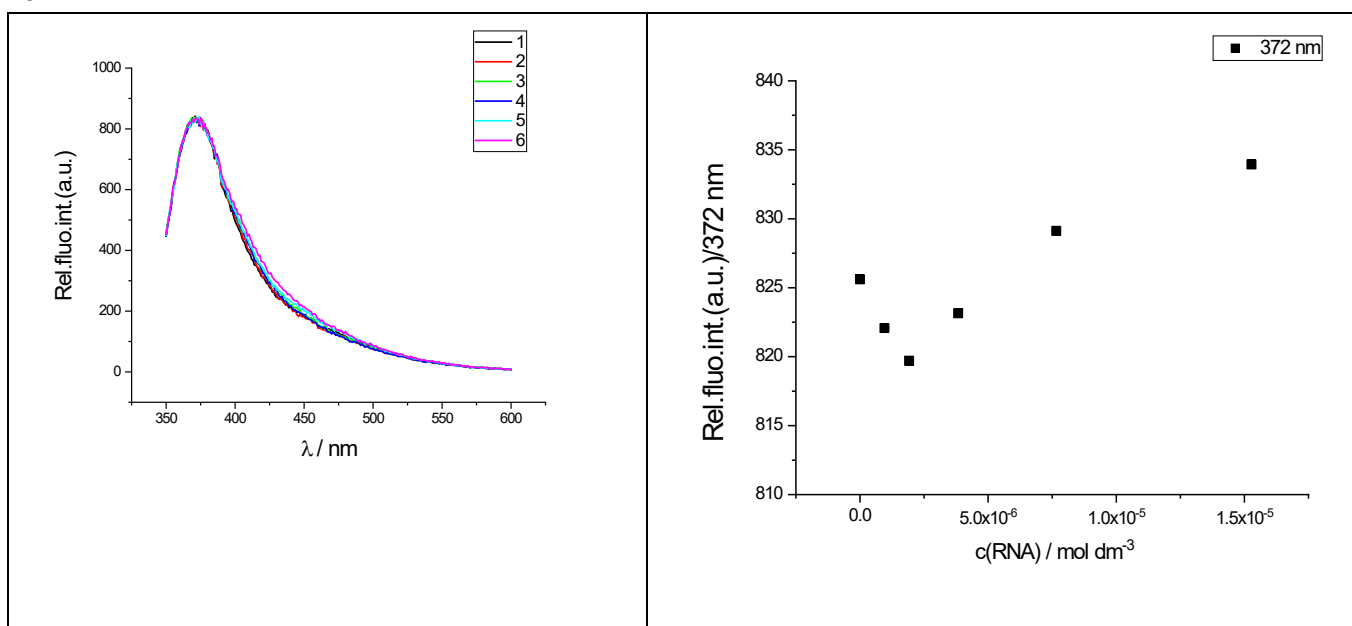

Figure S7. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U (c= 1.0 × 10^{-6} – 1.9 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 372 nm on c(poly A-poly U), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
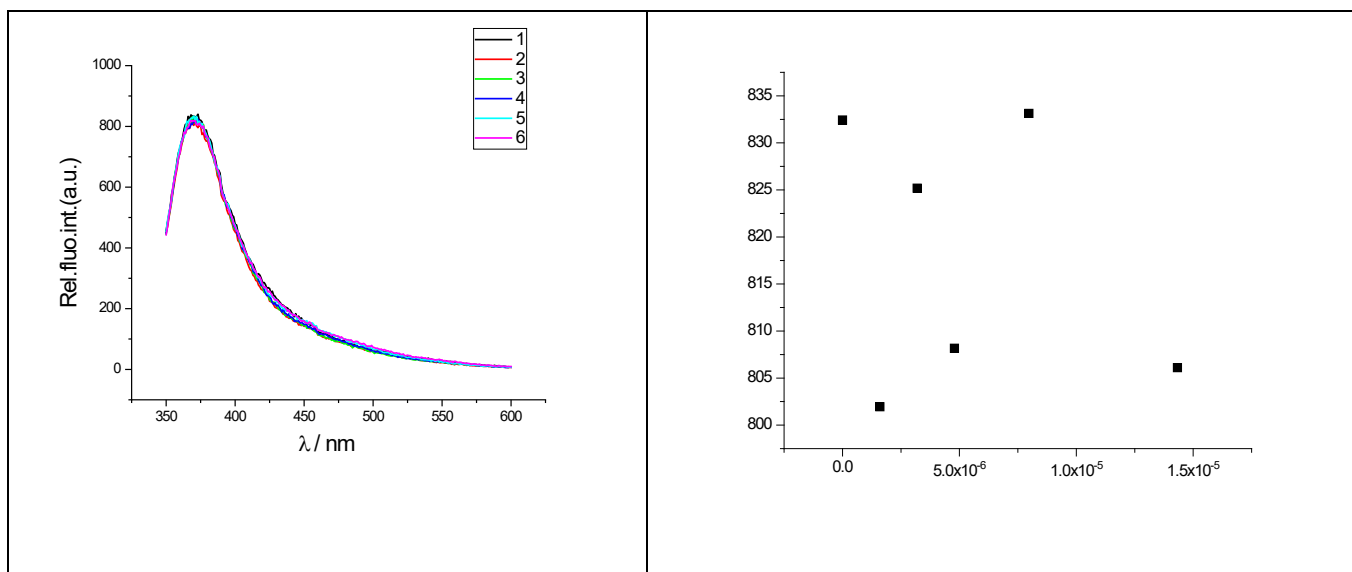
Figure S8. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ (c= 1.6 × 10^{-6} − 1.4 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 372 nm on c(poly(dGdC)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S9. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ (c= 1.0 × 10^{-6} − 1.9 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 372 nm on c(poly(dAdT)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S10. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT (c= 1.0 × 10^{-6} – 2.0 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 372 nm on c(poly dA-poly dT), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S11. a) Changes in fluorescence spectrum of **22** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA (c= 9.0 × 10^{-7} - 9 × 10^{-6} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 372 nm on c(ctDNA), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
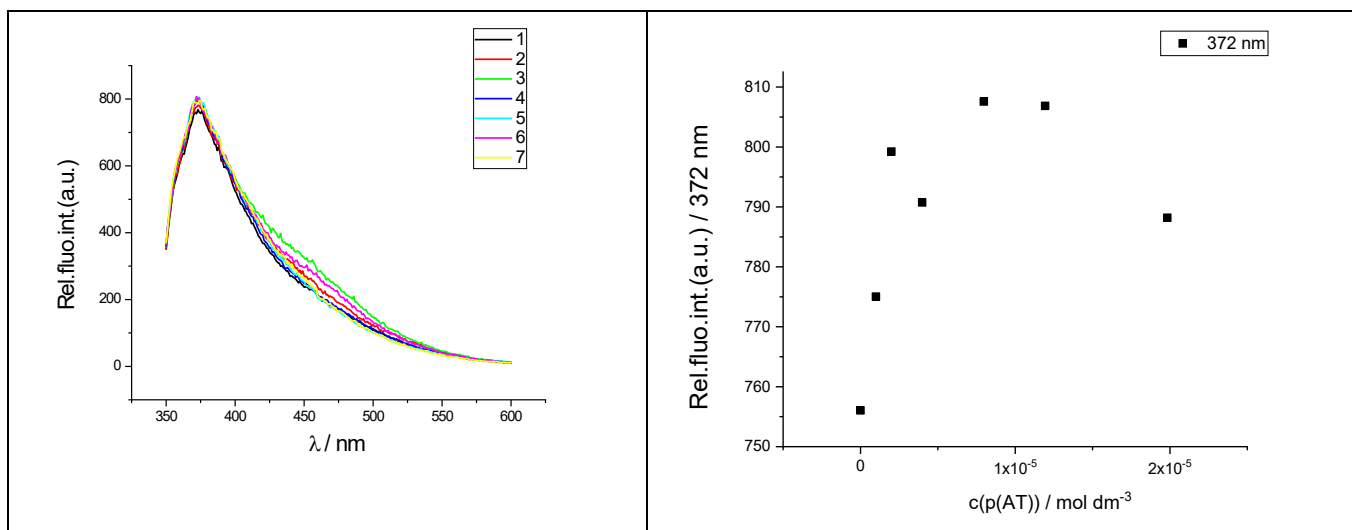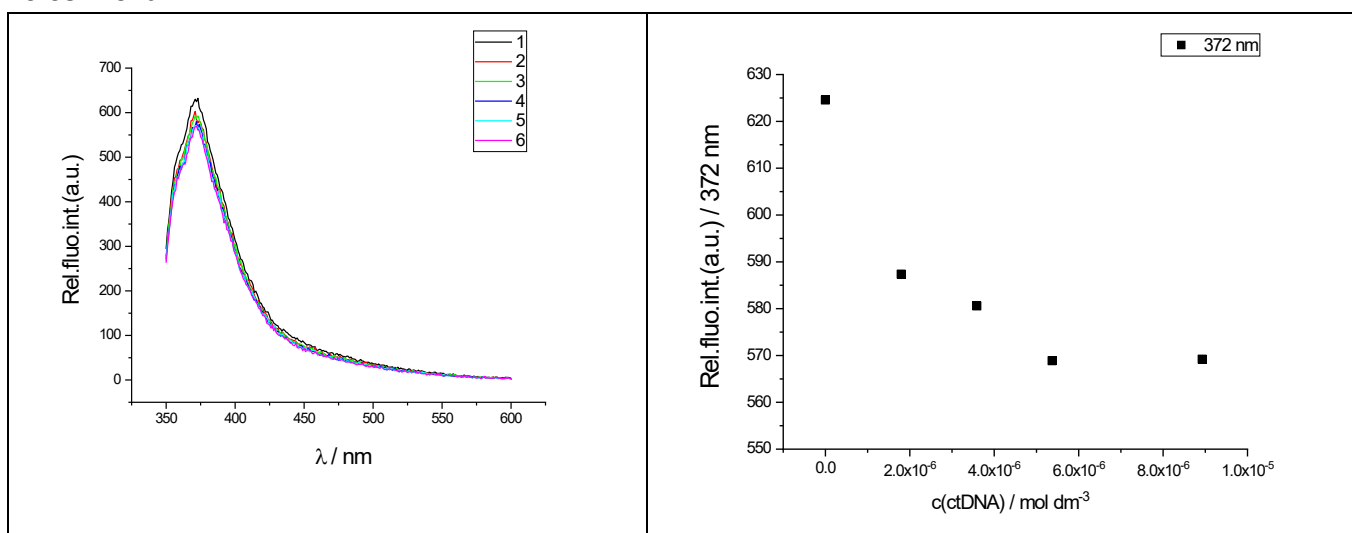
Figure S12. a) Changes in fluorescence spectrum of **22** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U (c= 1.0×10^{-6} – 1.5×10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 374 nm on c(poly A-poly U), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
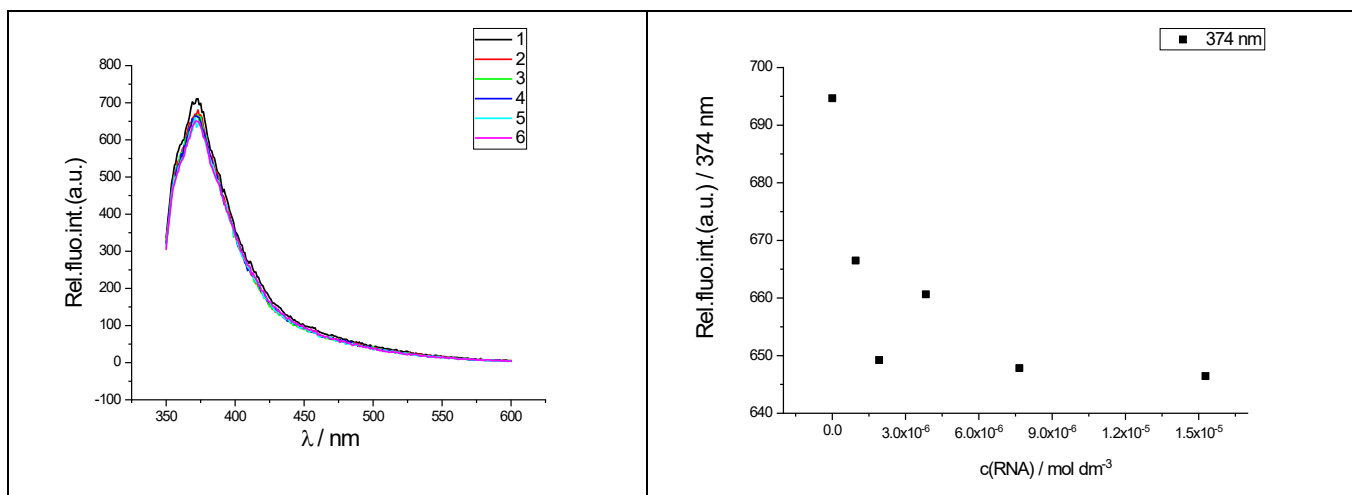

Figure S13. a) Changes in fluorescence spectrum of **22** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ (c= 1.0×10^{-6} – 1.2×10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 373 nm on c(poly(dGdC)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S14. a) Changes in fluorescence spectrum of **22** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ (c= 1.0×10^{-6} – 1.6×10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 372 nm on c(poly(dAdT)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
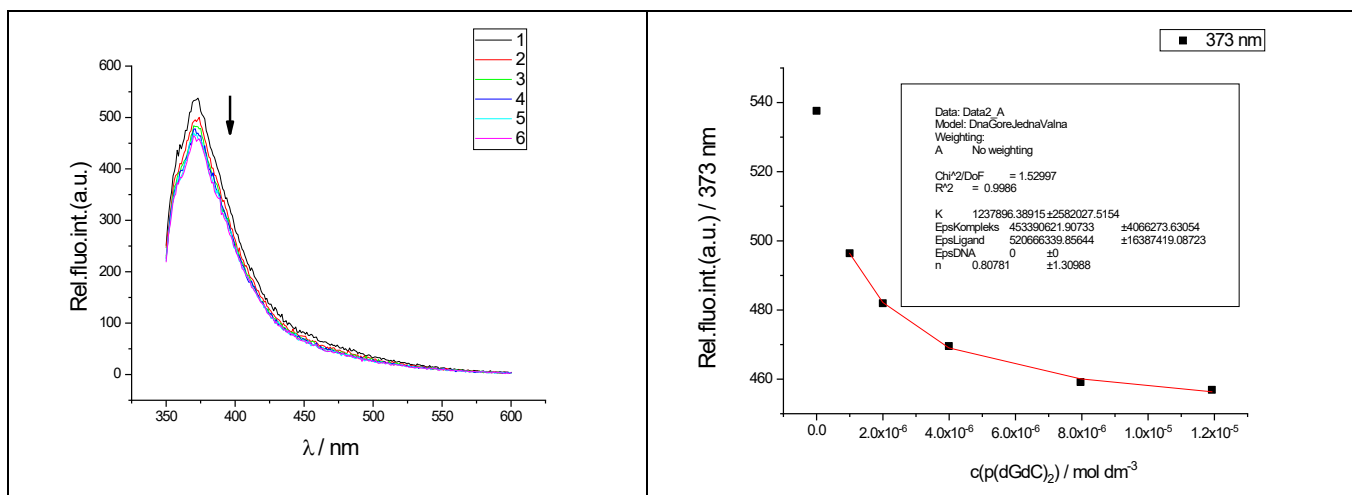

Figure S15. a) Changes in fluorescence spectrum of **22** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT (c= 1.0×10^{-6} – 1.8×10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 372 nm on c(poly dA-poly dT), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
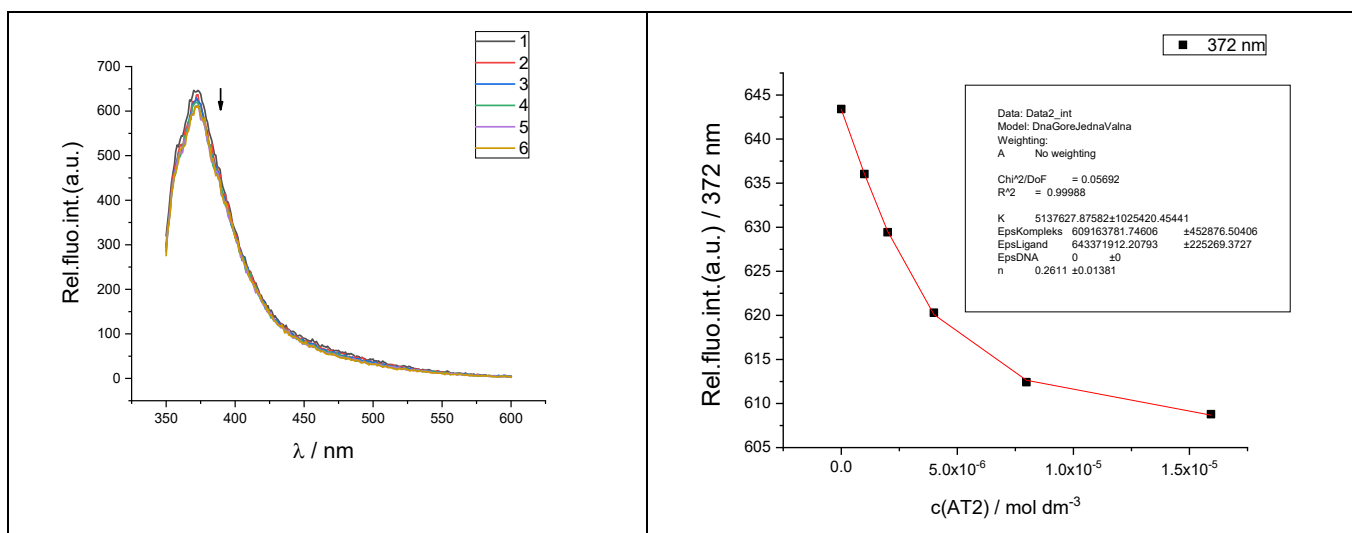

Figure S16. a) Changes in fluorescence spectrum of **23** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA (c= 1.0×10^{-6} – 9.82×10^{-6} mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 377 nm on c(ctDNA), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
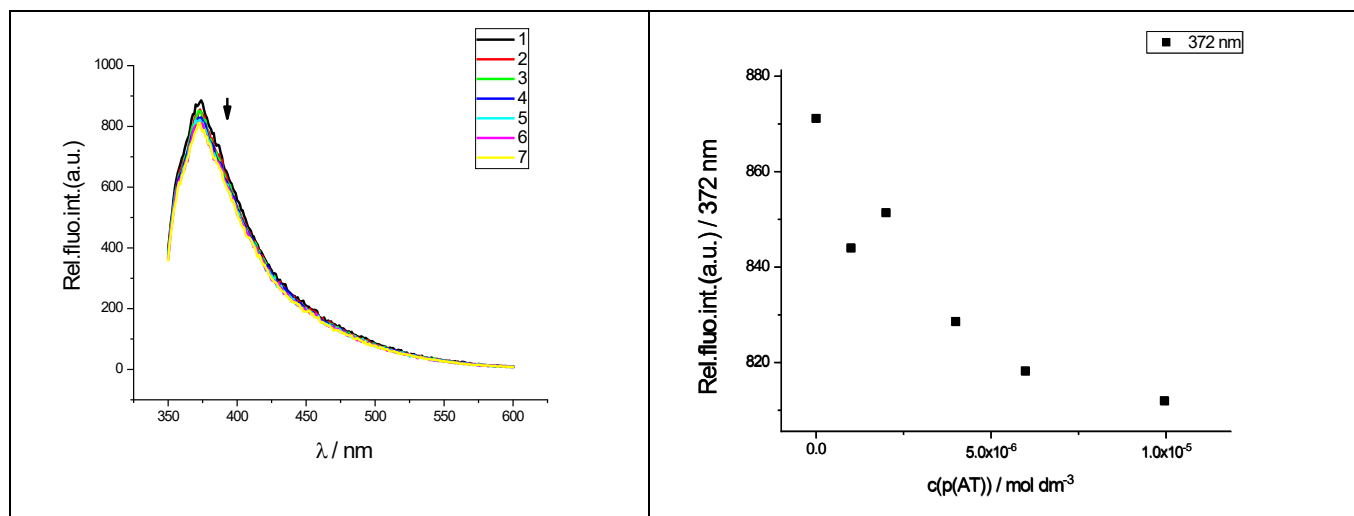
Figure S17. a) Changes in fluorescence spectrum of **23** ($c = 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U ($c = 1.0 \times 10^{-6} - 1.9 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 373 nm on c(poly A-poly U), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
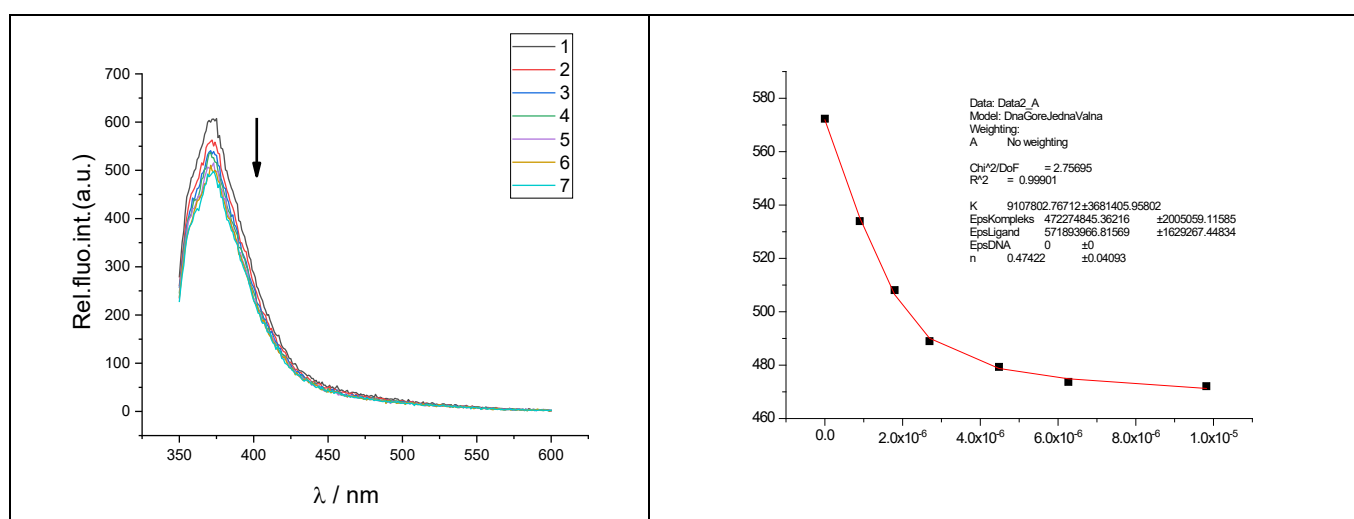

Figure S18. a) Changes in fluorescence spectrum of **23** ($c = 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ ($c = 1.0 \times 10^{-6} - 3.1 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 372 nm on c(poly(dGdC)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
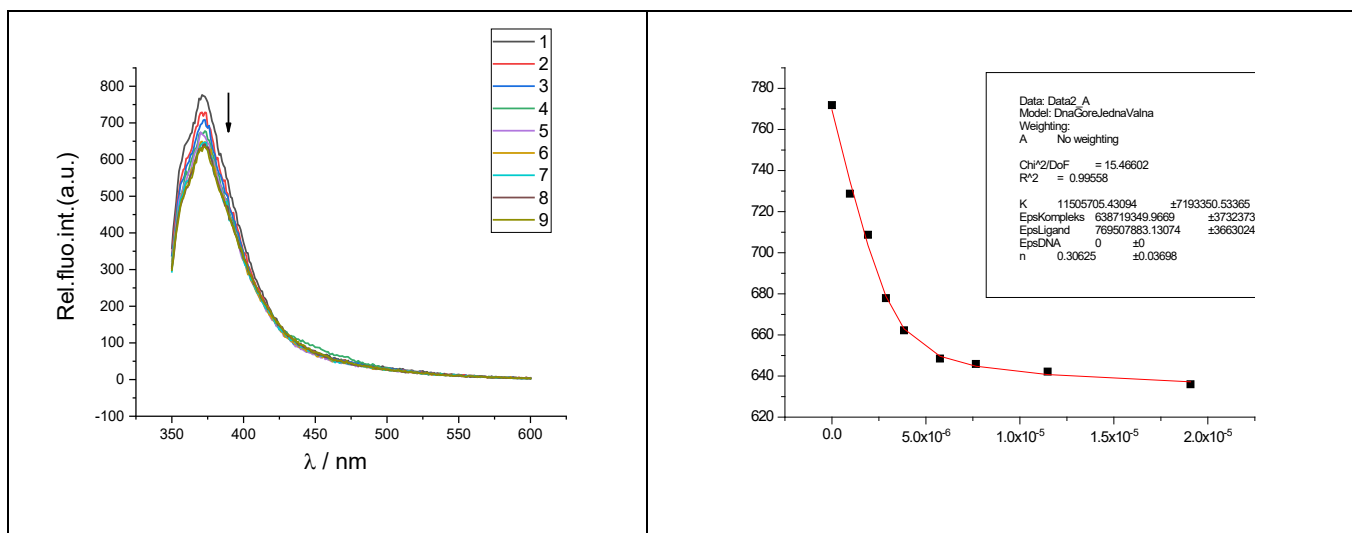
Figure S19. a) Changes in fluorescence spectrum of **23** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ (c= 1.0×10^{-6} – 4.1×10^{-5} mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 375 nm on c(poly(dAdT)$_2$), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S20. a) Changes in fluorescence spectrum of **23** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT (c= 1.0×10^{-6} – 1.6×10^{-5} mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 375 nm on c(poly dA-poly dT), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

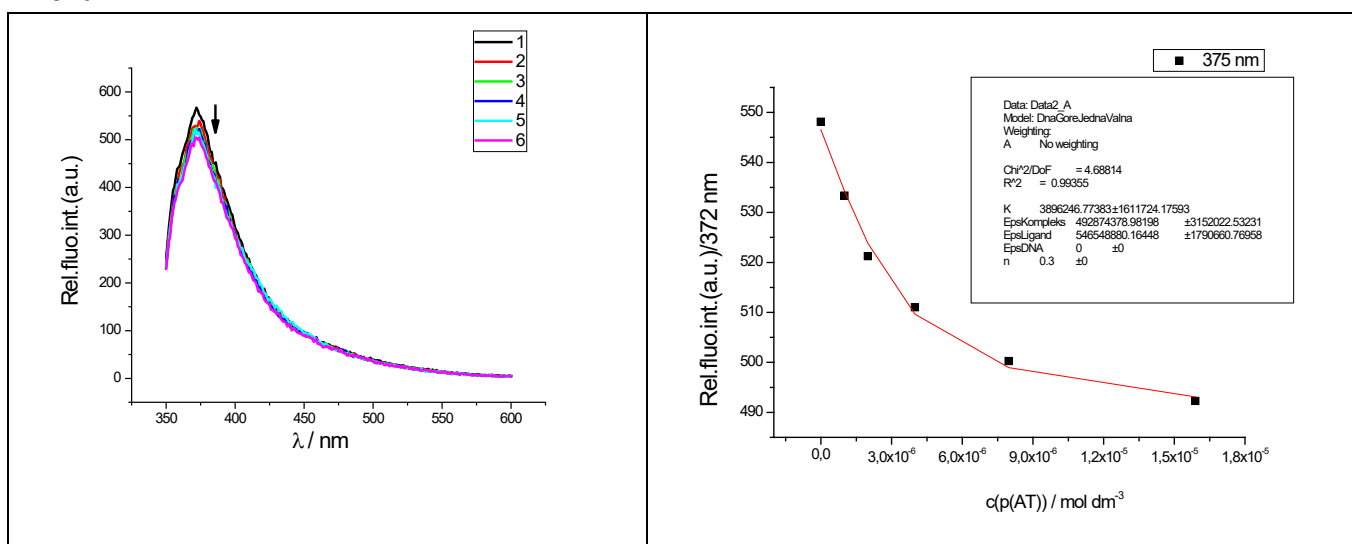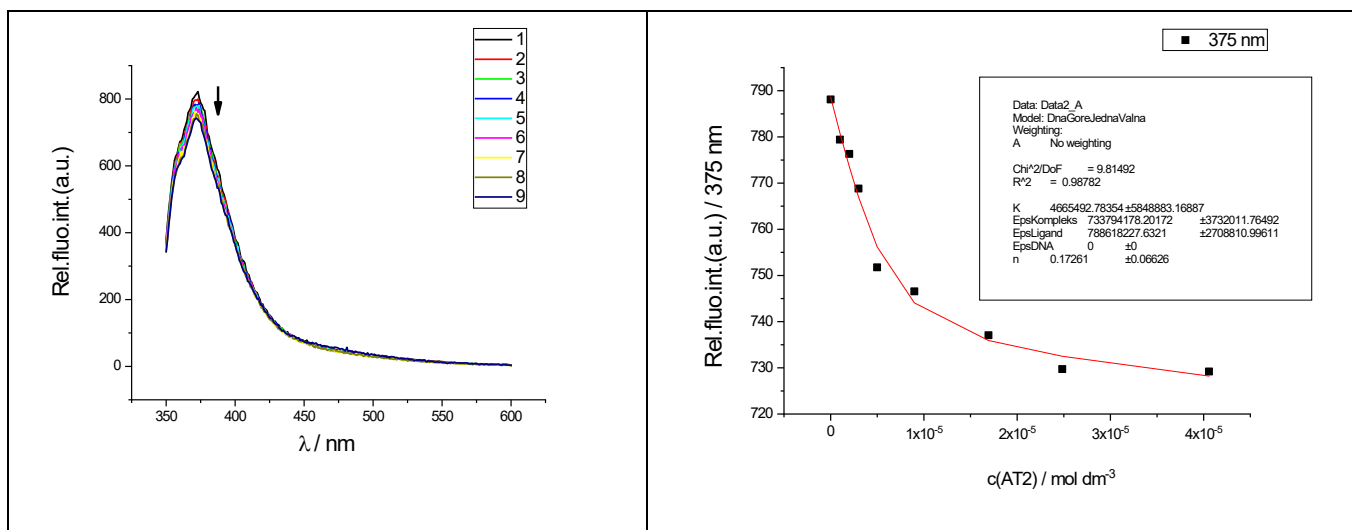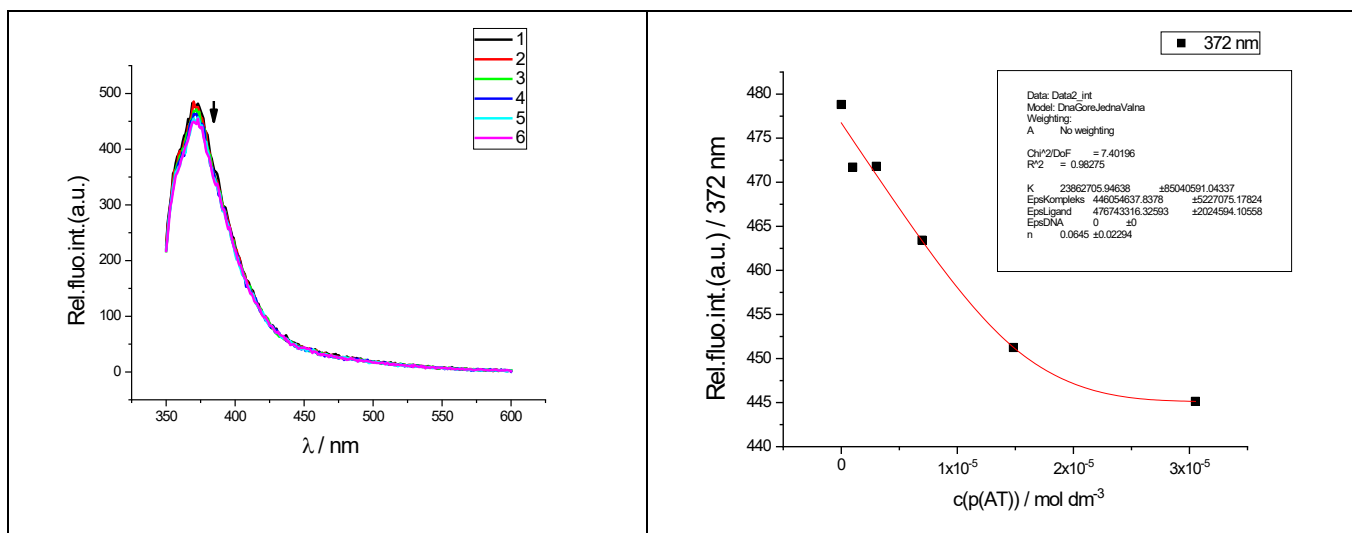Figure S21. a) Changes in fluorescence spectrum of **23** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ATT (c= 1.0×10^{-6} – 1.6×10^{-5} mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 372 nm on c(ATT), at pH 7.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
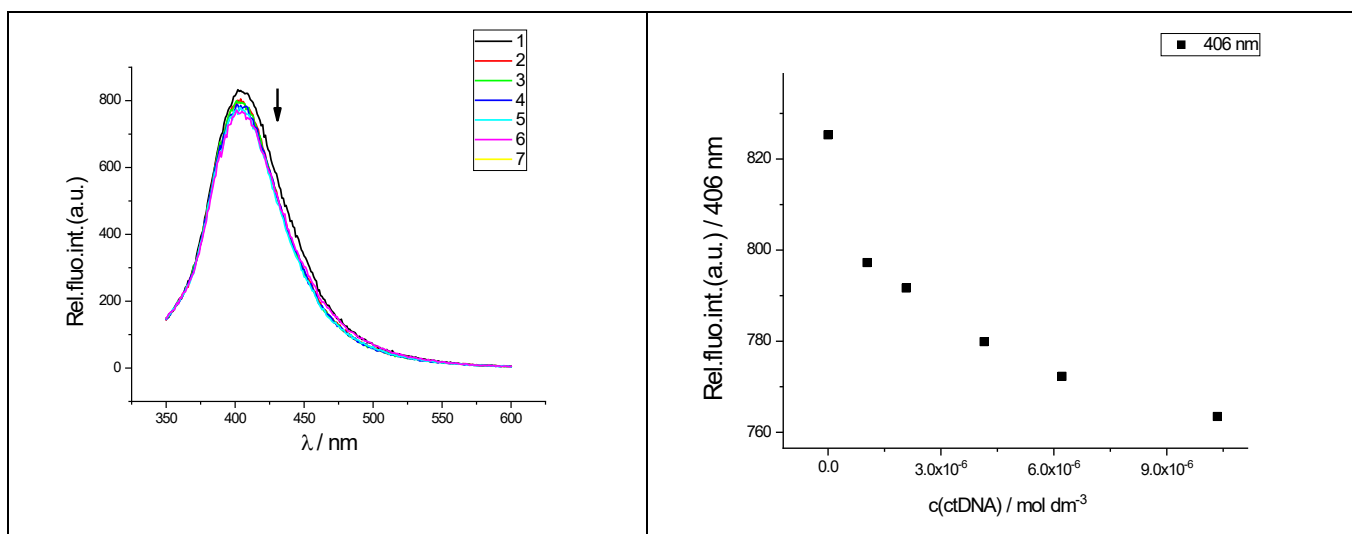
2.1.2. Fluorimetric titrations at pH 5.0

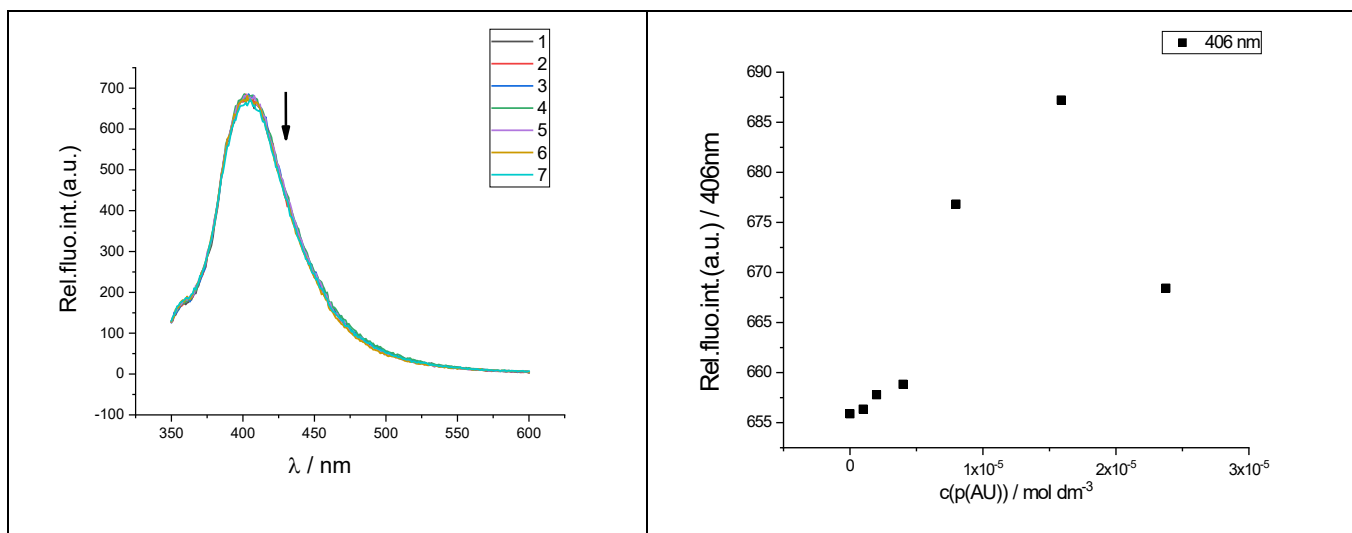Figure S22. a) Changes in fluorescence spectrum of **19** (c= 1.0×10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA (c= 1.0×10^{-6} - 1×10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 406 nm on c(ctDNA), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S23. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U (c= 1.0 × 10^{-6} − 1.8 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 406 nm on c(poly A-poly U), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
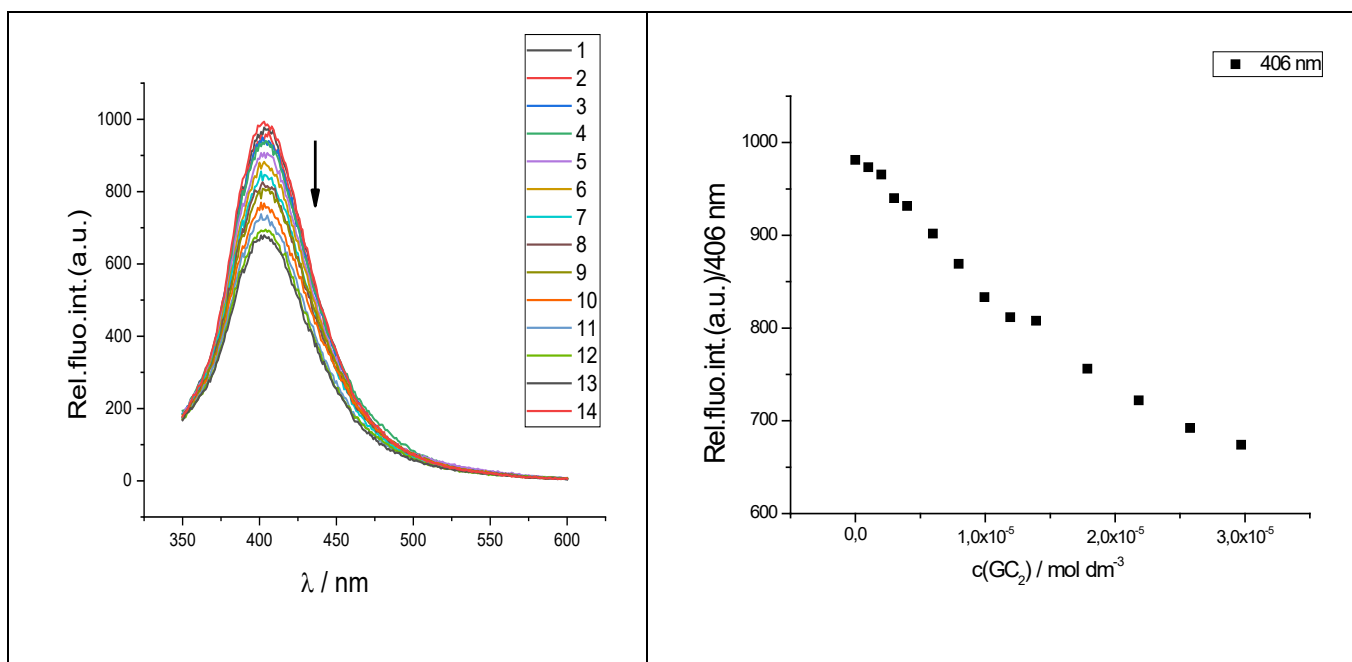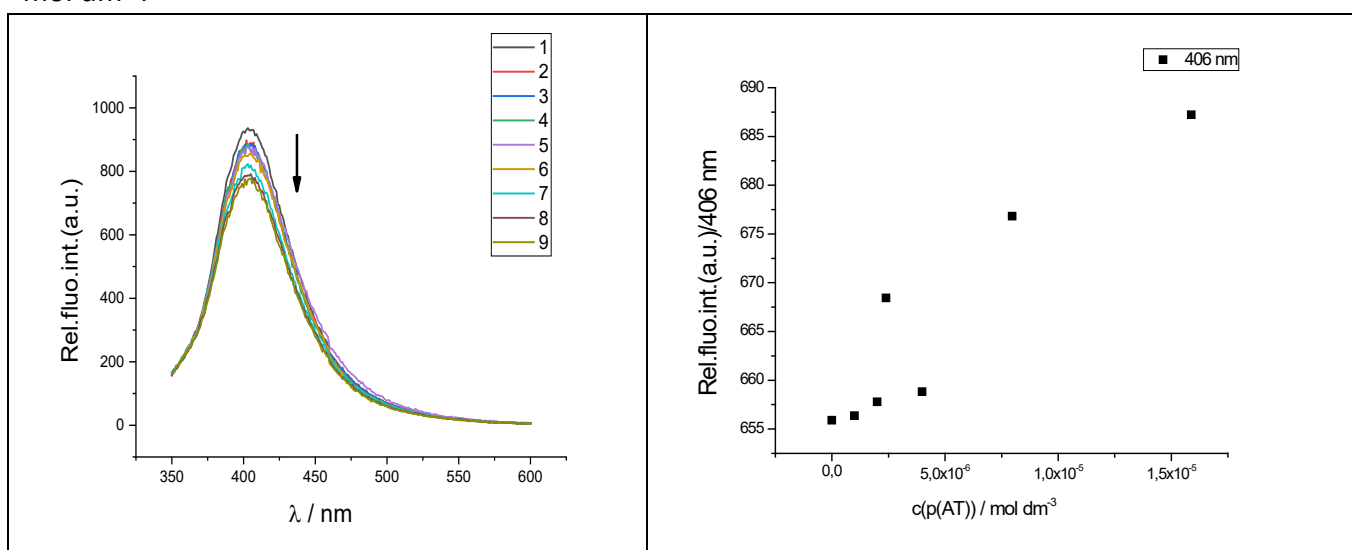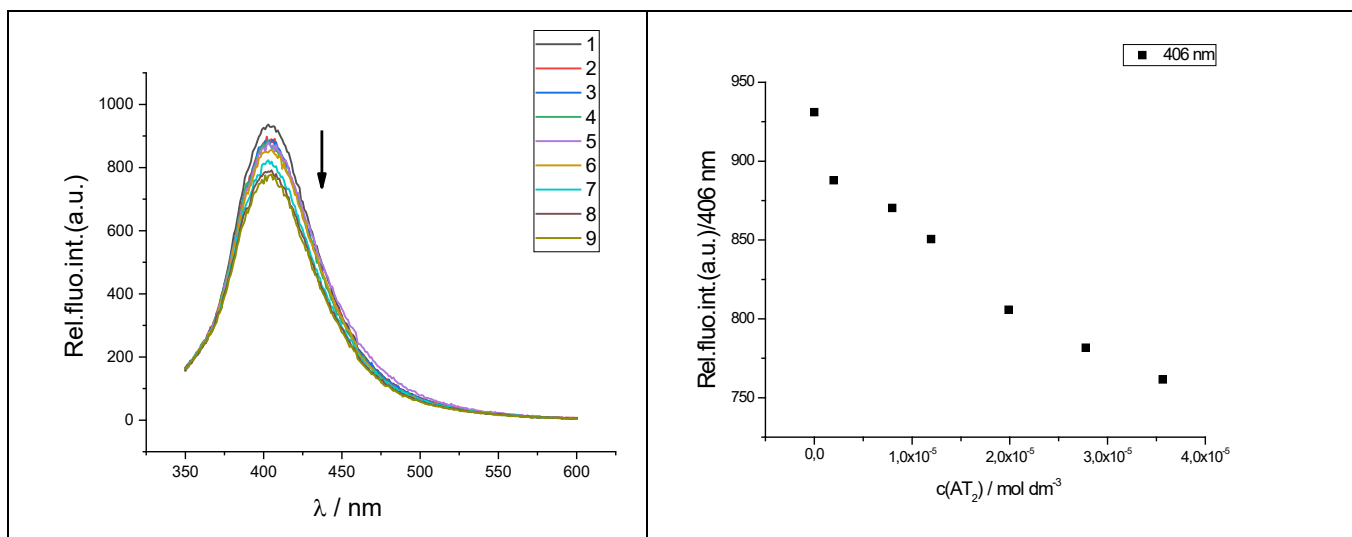

Figure S24. a) Changes in fluorescence spectrum of **19** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ (c= 1.0 × 10^{-6} − 2.9 × 10^{-5} mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 406 nm on c(poly(dGdC)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
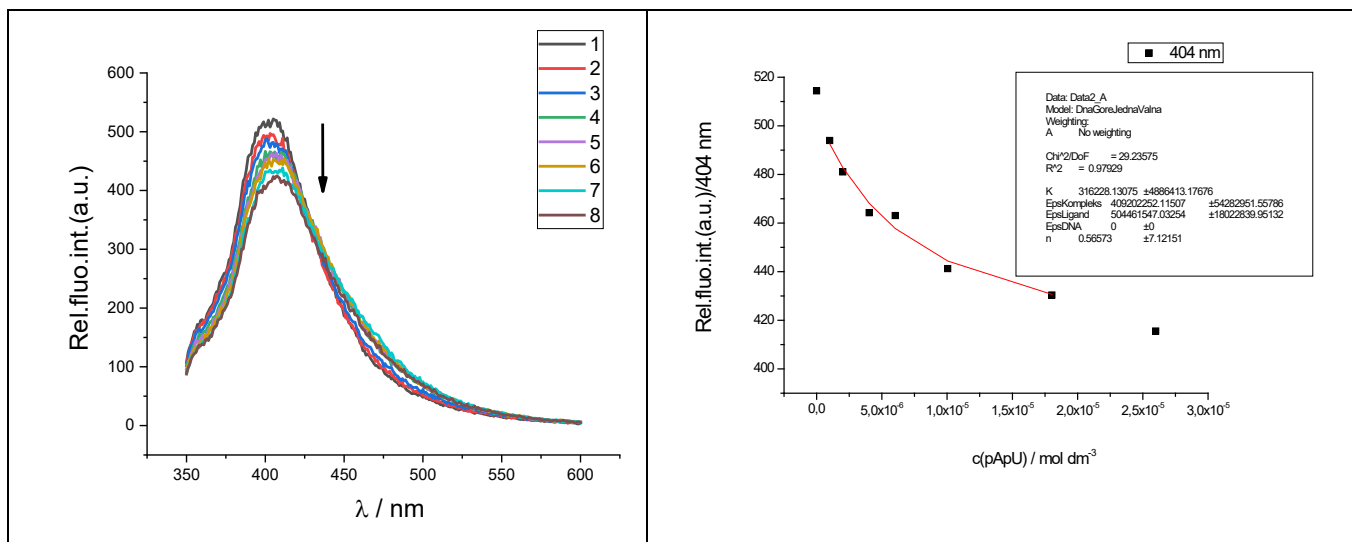
Figure S25. a) Changes in fluorescence spectrum of **19** ($c = 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ ($c = 1.0 \times 10^{-6} - 3.6 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 406 nm on c(poly(dAdT)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
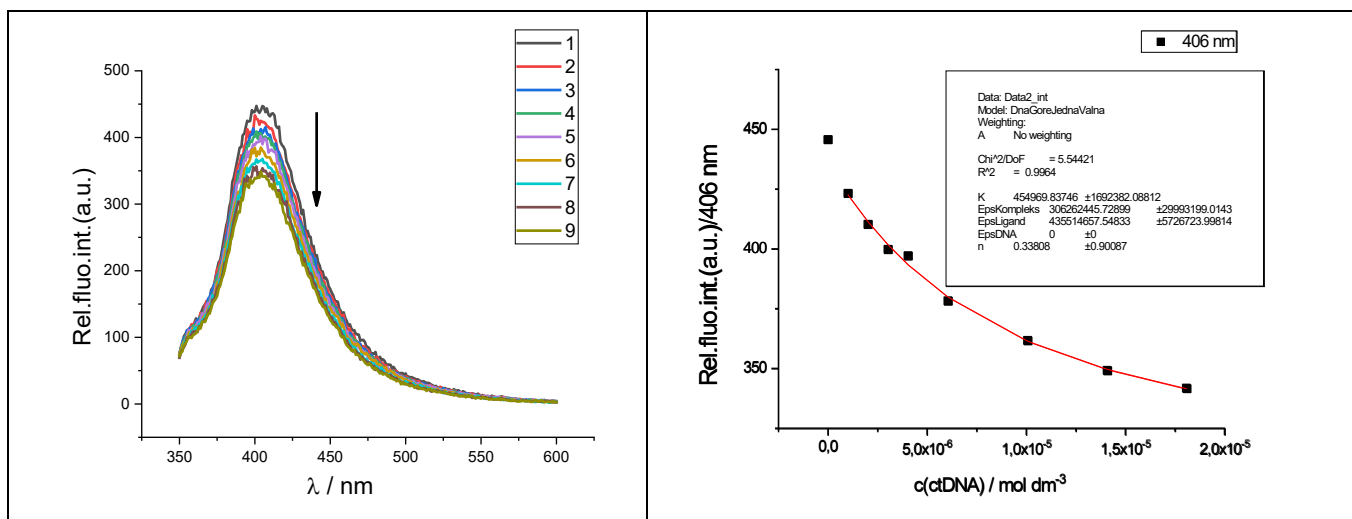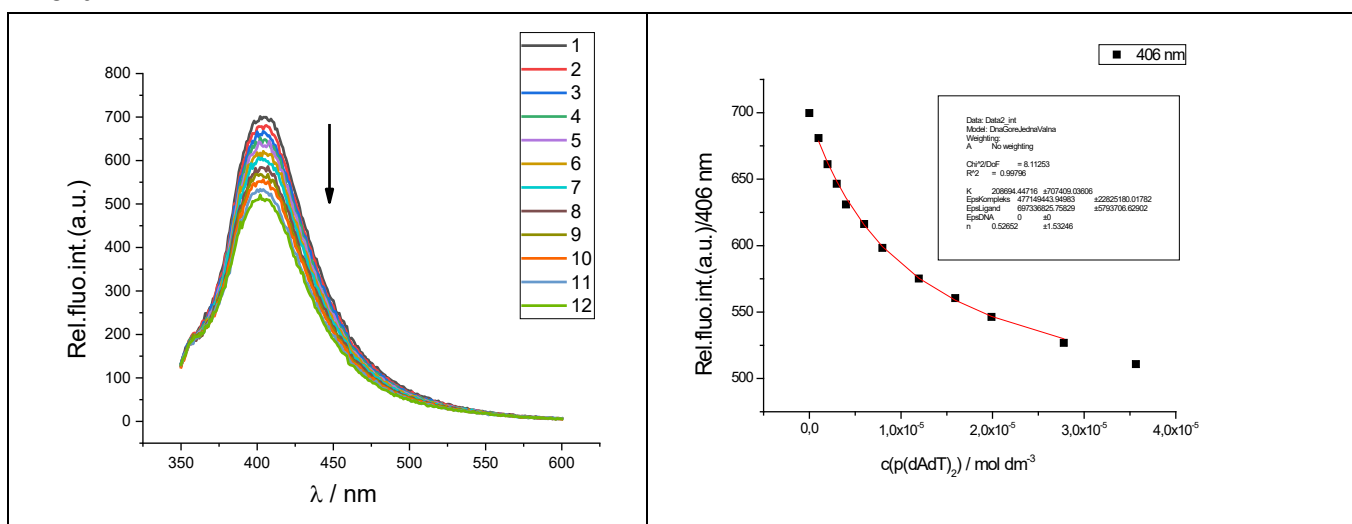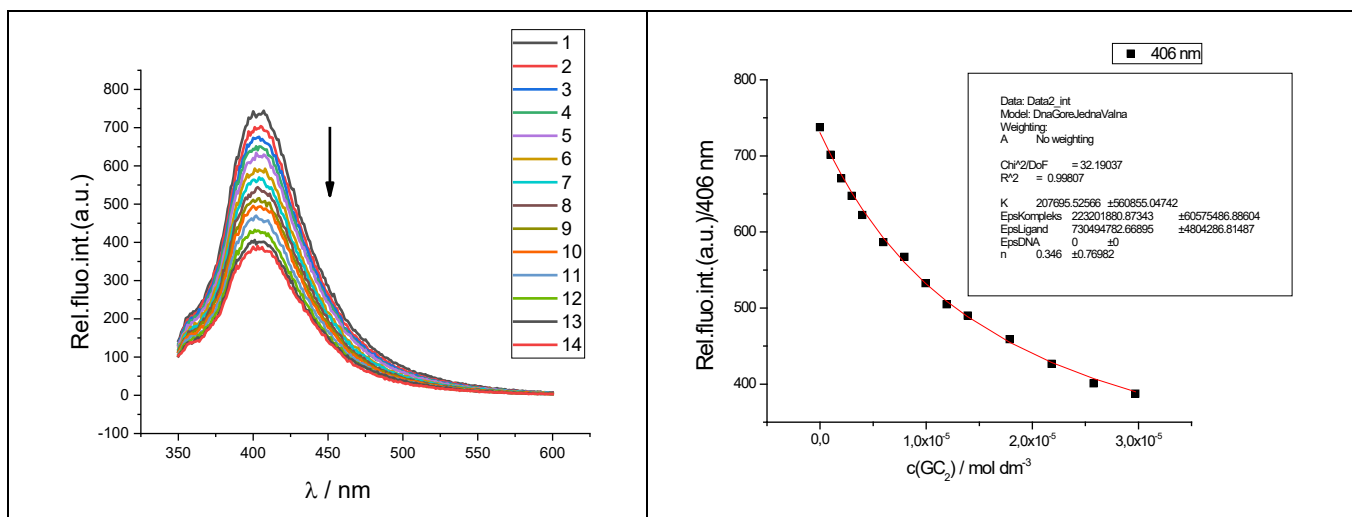

Figure S26. a) Changes in fluorescence spectrum of **19** ($c = 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT ($c = 1.0 \times 10^{-6} - 3.6 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **19** absorbance at λ_{max} = 406 nm on c(poly dA-poly dT), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S27. a) Changes in fluorescence spectrum of **22** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA (c= 1.0 × 10^{-6} − 1.8 × 10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 406 nm on c(ctDNA), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S28. a) Changes in fluorescence spectrum of **22** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U (c= 1.0 × 10^{-6} − 2.6 × 10^{-5} mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 473 nm on c(poly A-poly U), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S29. a) Changes in fluorescence spectrum of **22** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ ($c= 9.9 \times 10^{-7} - 2.9 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 406 nm on c(poly(dGdC)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
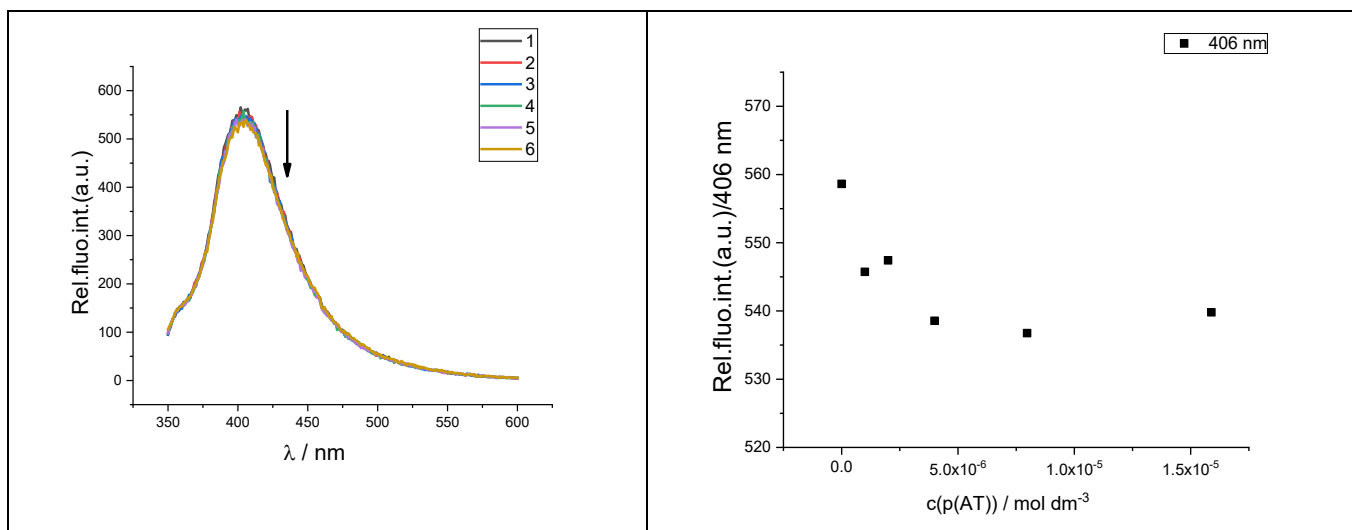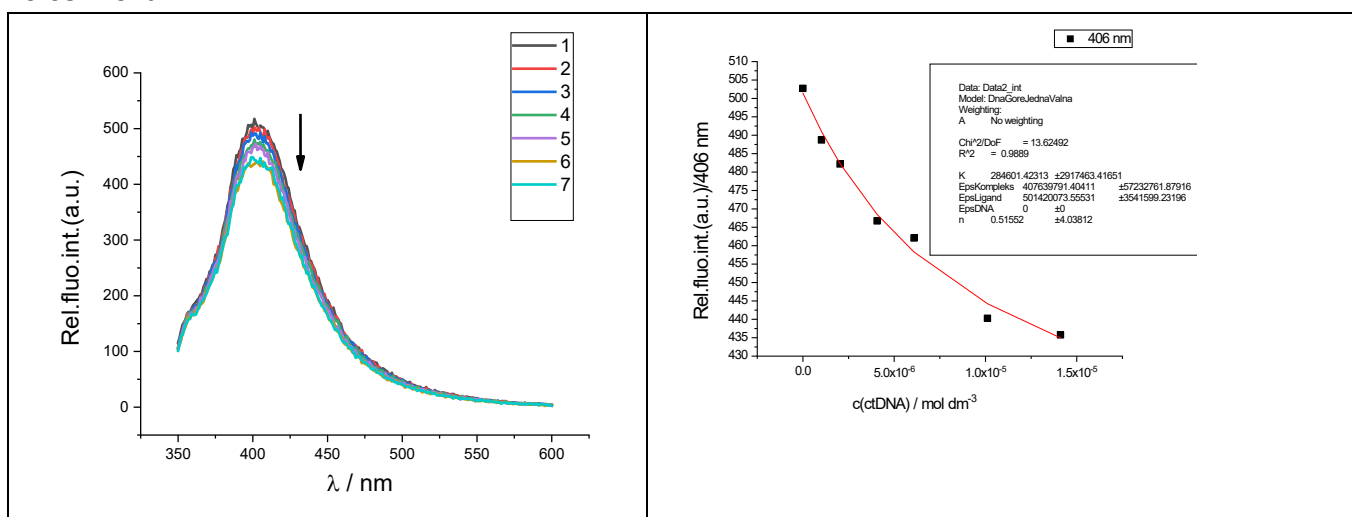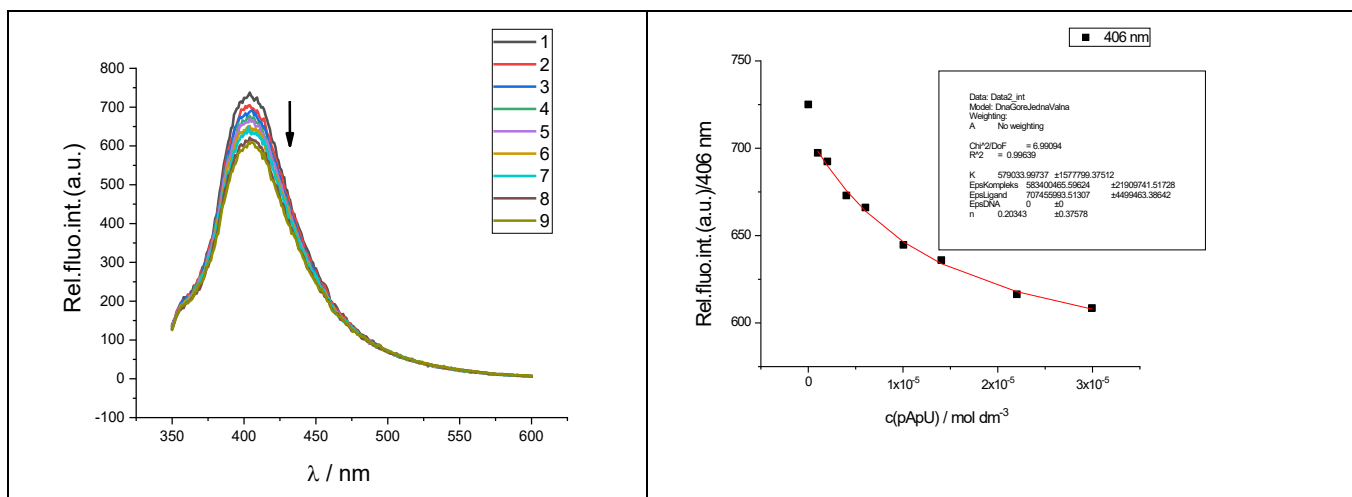

Figure S30. a) Changes in fluorescence spectrum of **22** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ ($c= 1.0 \times 10^{-6} - 3.6 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **22** absorbance at λ_{max} = 406 nm on c(poly(dAdT)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
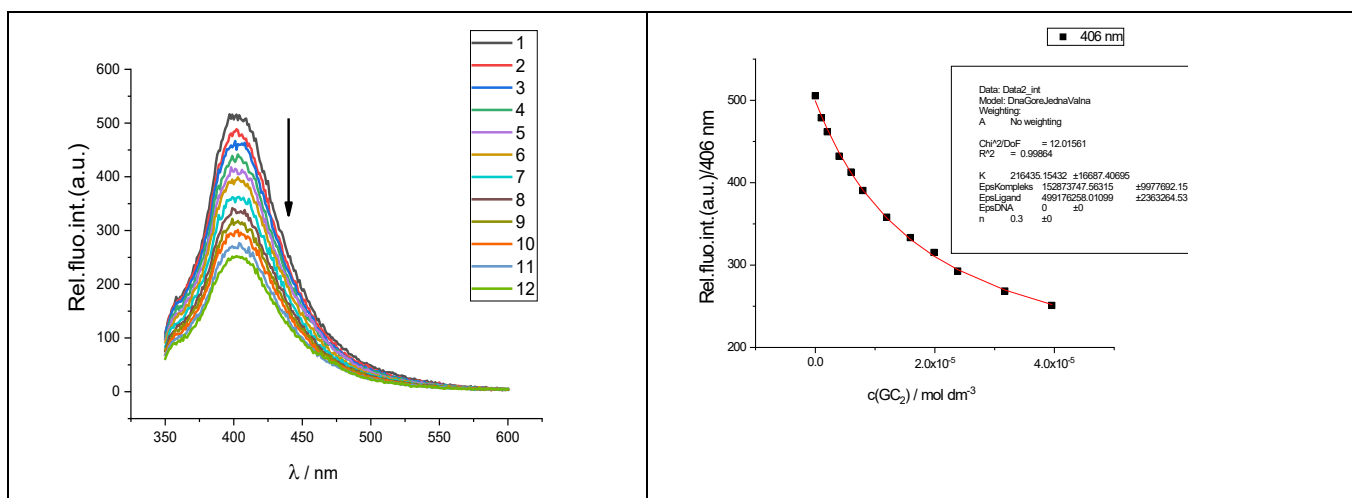
Figure S31. a) Changes in fluorescence spectrum of **22** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT ($c= 1.0 \times 10^{-6} - 1.6 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **22** absorbance at $\lambda_{max} = 406$ nm on c(poly dA-poly dT), at pH 5.0, sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}.
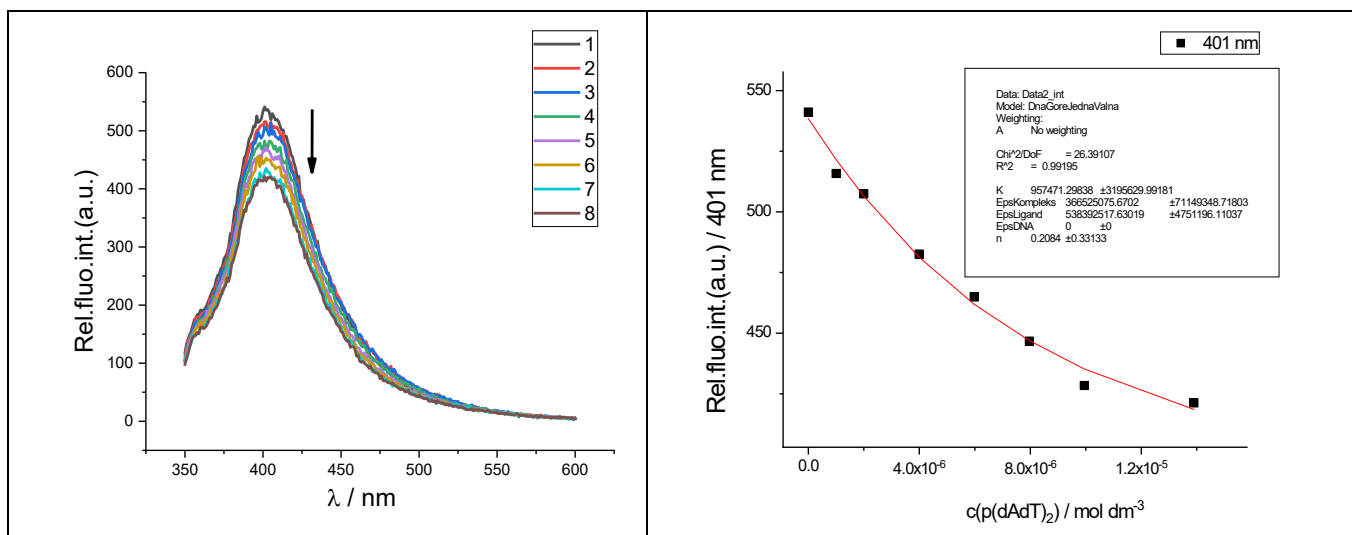

Figure S32. a) Changes in fluorescence spectrum of **23** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with ctDNA ($c= 1.0 \times 10^{-6} - 1.4 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at $\lambda_{max} = 406$ nm on c(ctDNA), at pH 5.0, sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}.
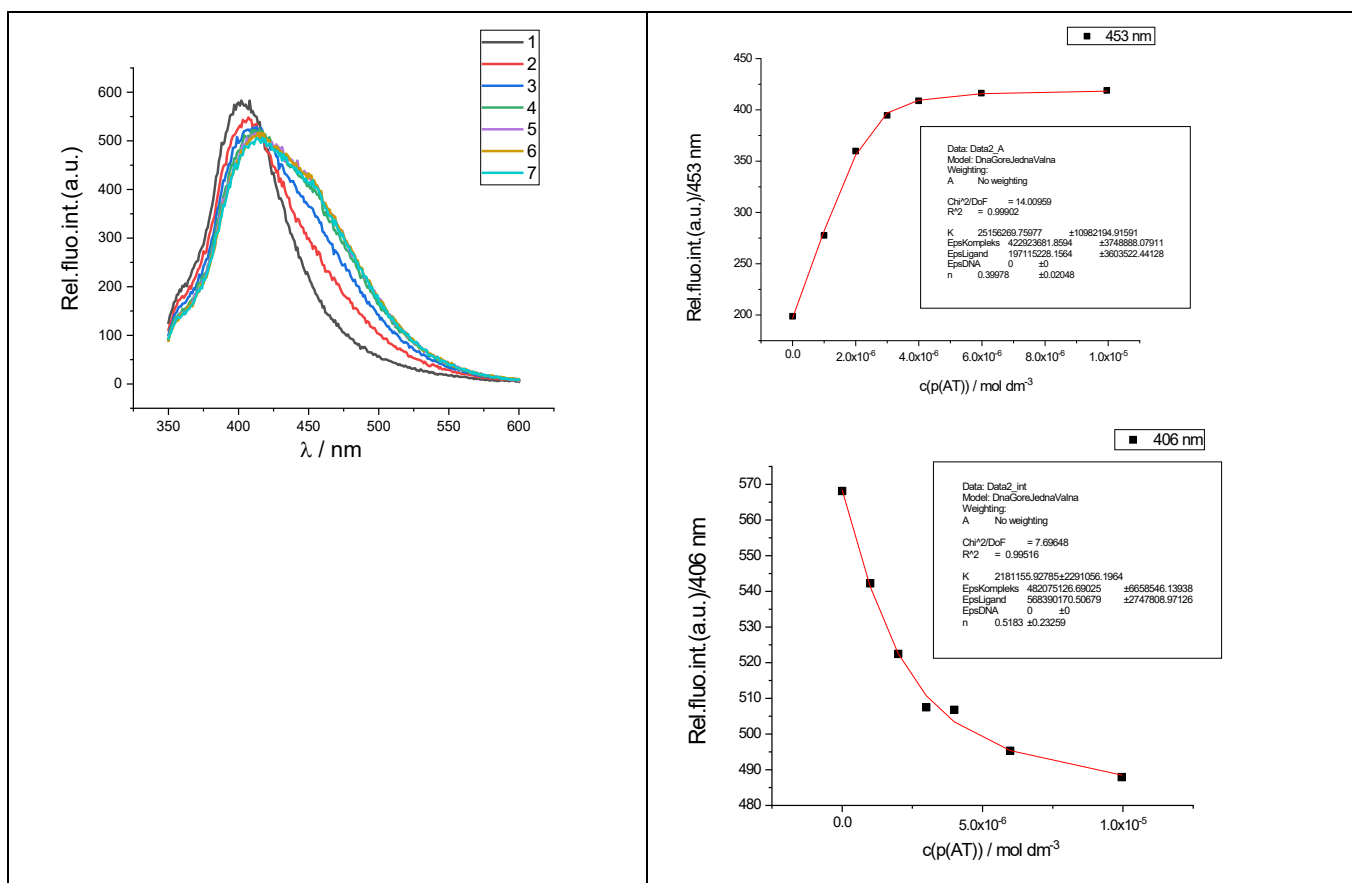
Figure S33. a) Changes in fluorescence spectrum of **23** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly A-poly U ($c= 1.0 \times 10^{-6} - 2.99 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 406 nm on c(poly A-poly U), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S34. a) Changes in fluorescence spectrum of **23** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dGdC)$_2$ ($c= 1.0 \times 10^{-6} - 3.96 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 406 nm on c(poly(dGdC)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
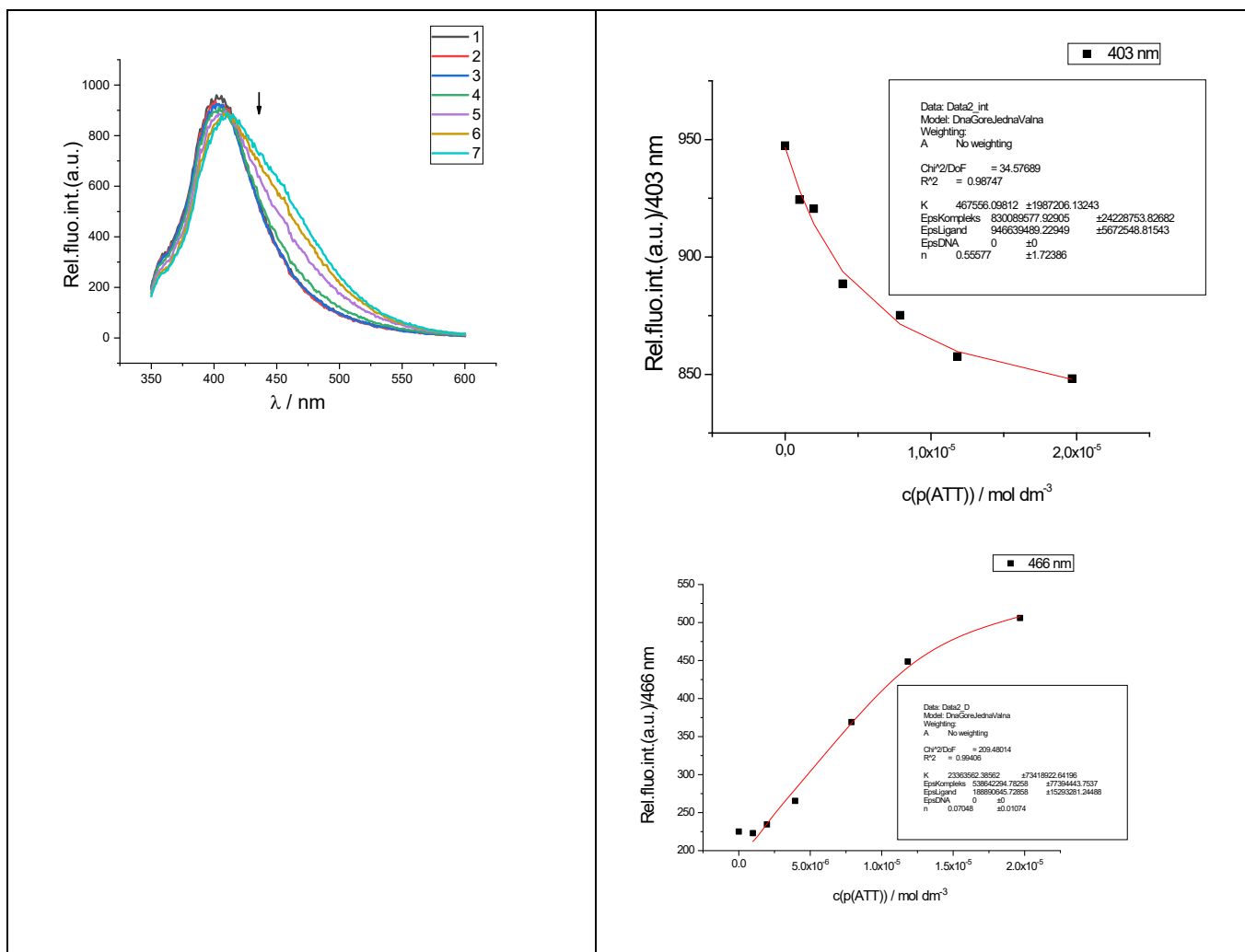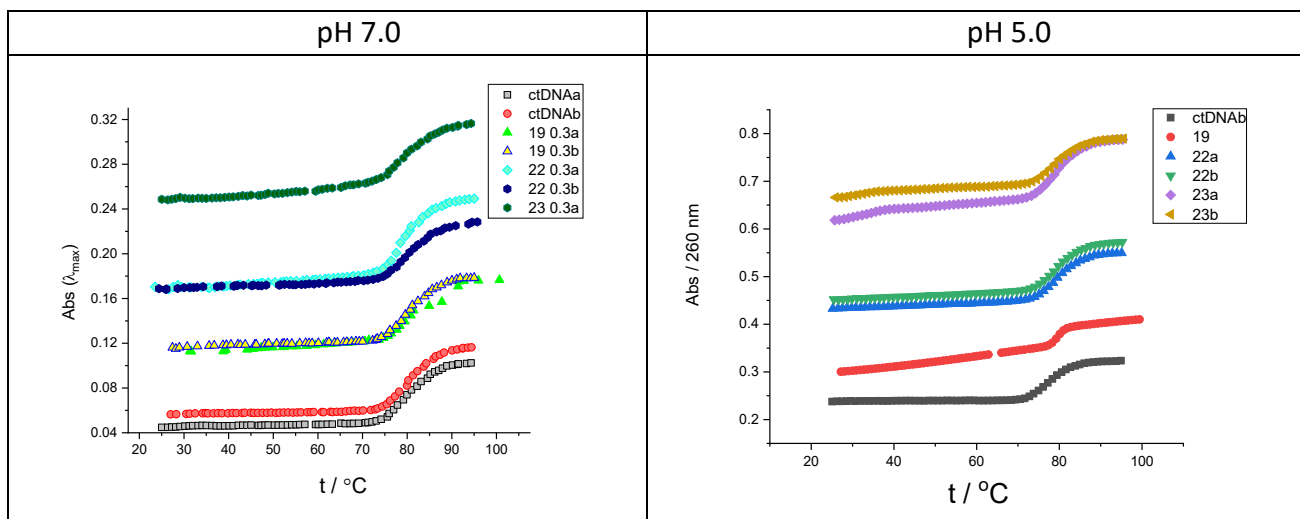
Figure S35. a) Changes in fluorescence spectrum of **23** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly(dAdT)$_2$ ($c= 1.0 \times 10^{-6} - 1.39 \times 10^{-5}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 401 nm on c(poly(dAdT)$_2$), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S36. a) Changes in fluorescence spectrum of **23** ($c= 1.0 \times 10^{-6}$ mol dm^{-3}, λ_{exc}=300 nm) upon titration with poly dA-poly dT ($c= 1.0 \times 10^{-6} - 9.96 \times 10^{-6}$ mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 406 nm and 453 nm on c(poly dA-poly dT), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure S37. a) Changes in fluorescence spectrum of **23** (c= 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}=300 nm) upon titration with ATT (c= 1.0 × 10^{-6} − 1.97 × 10^{-5} mol dm^{-3}); b) Dependence of **23** absorbance at λ_{max} = 403 nm and 466 nm on c(ATT), at pH 5.0, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.
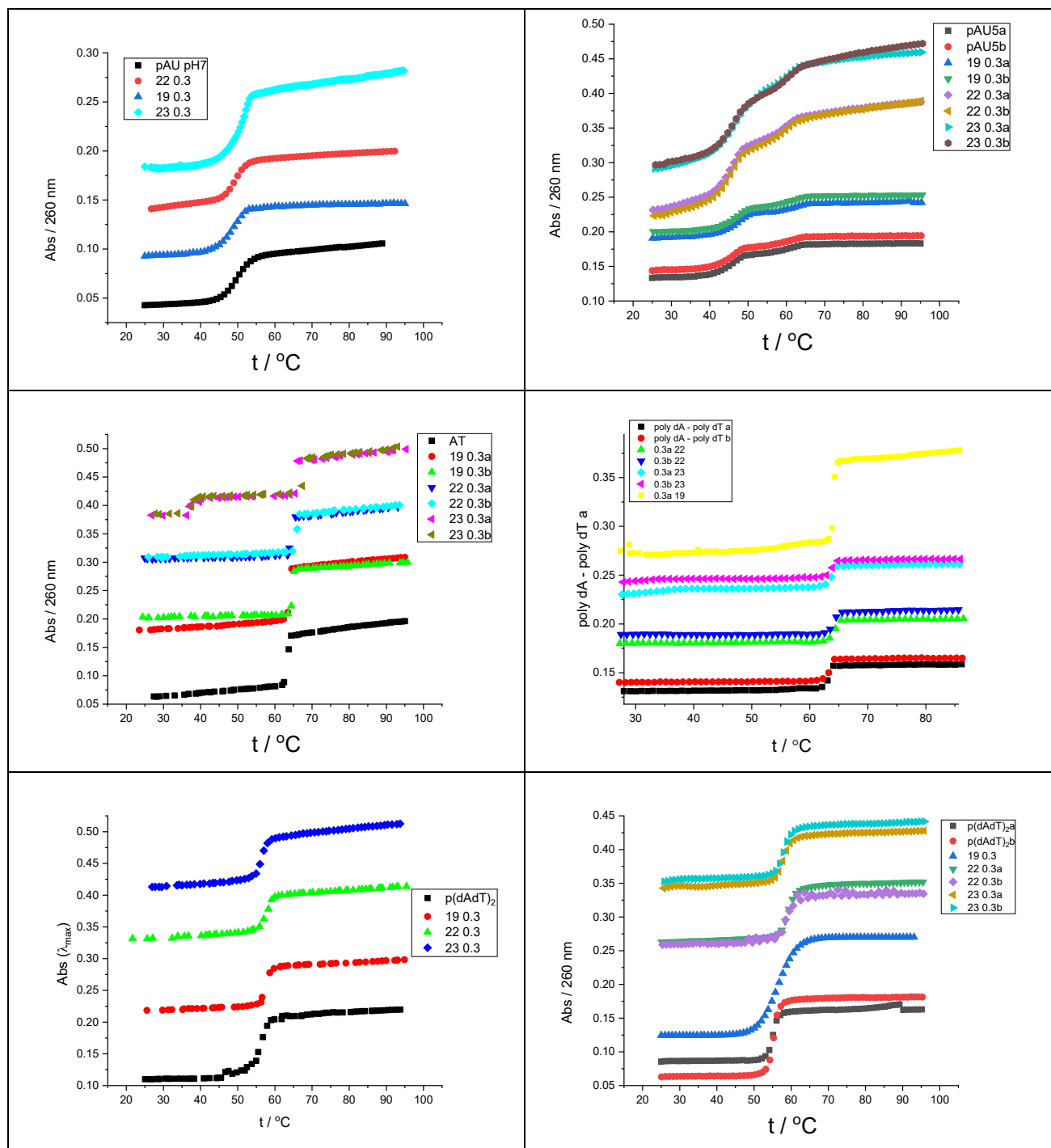
2.2. Thermal melting experiments

Figure S38. Melting curve of **ctDNA, poly A-poly U, poly dA-poly dT**, **poly (dA-dT)₂** upon addition of ratio, *r* ([compound/ [polynucleotide])=0.3 of **19**, **22** and **23** at pH = 7.0 and pH = 5.0 (buffer sodium cacodylate, *I* = 0.05 mol dm⁻³).
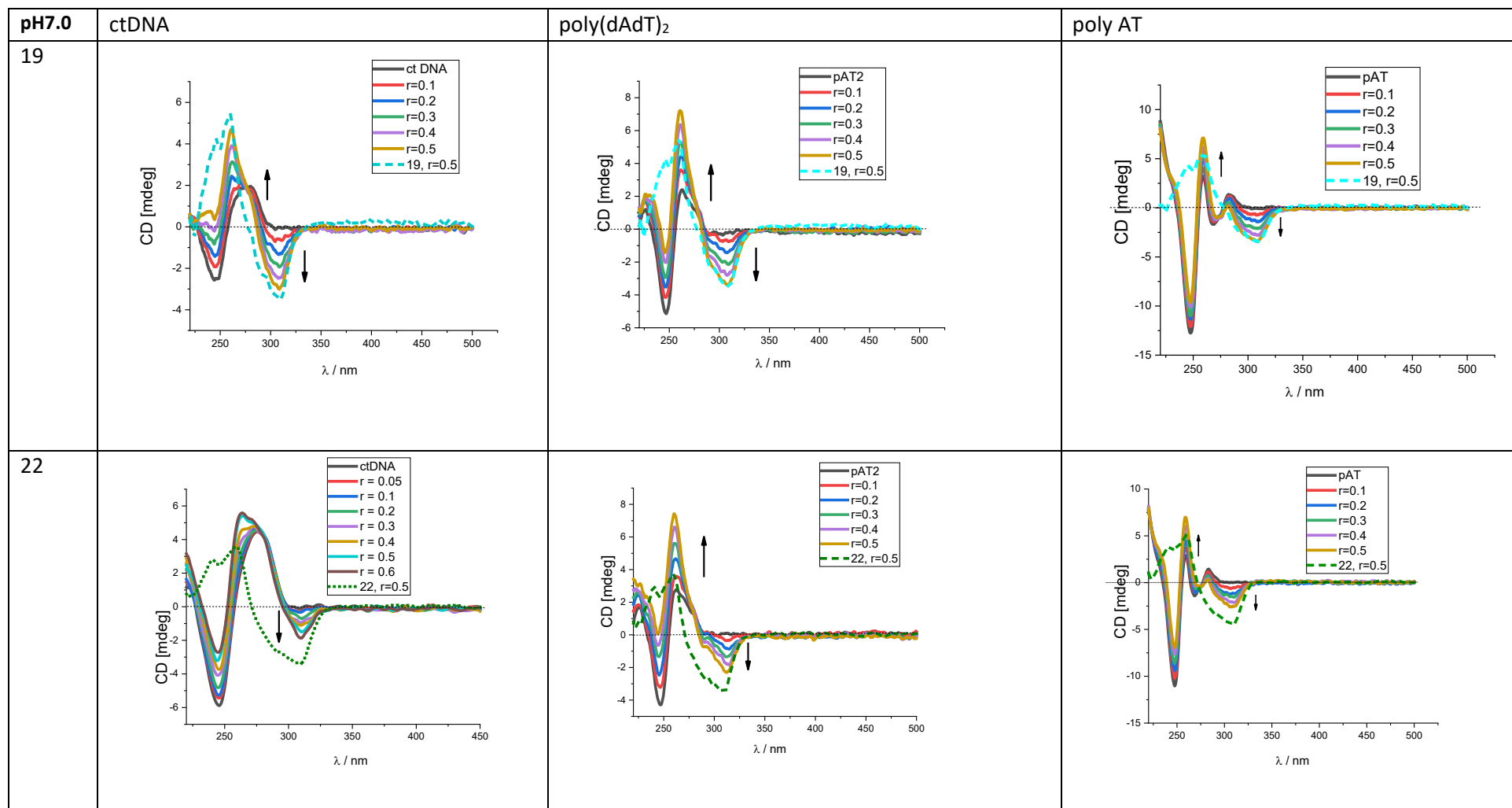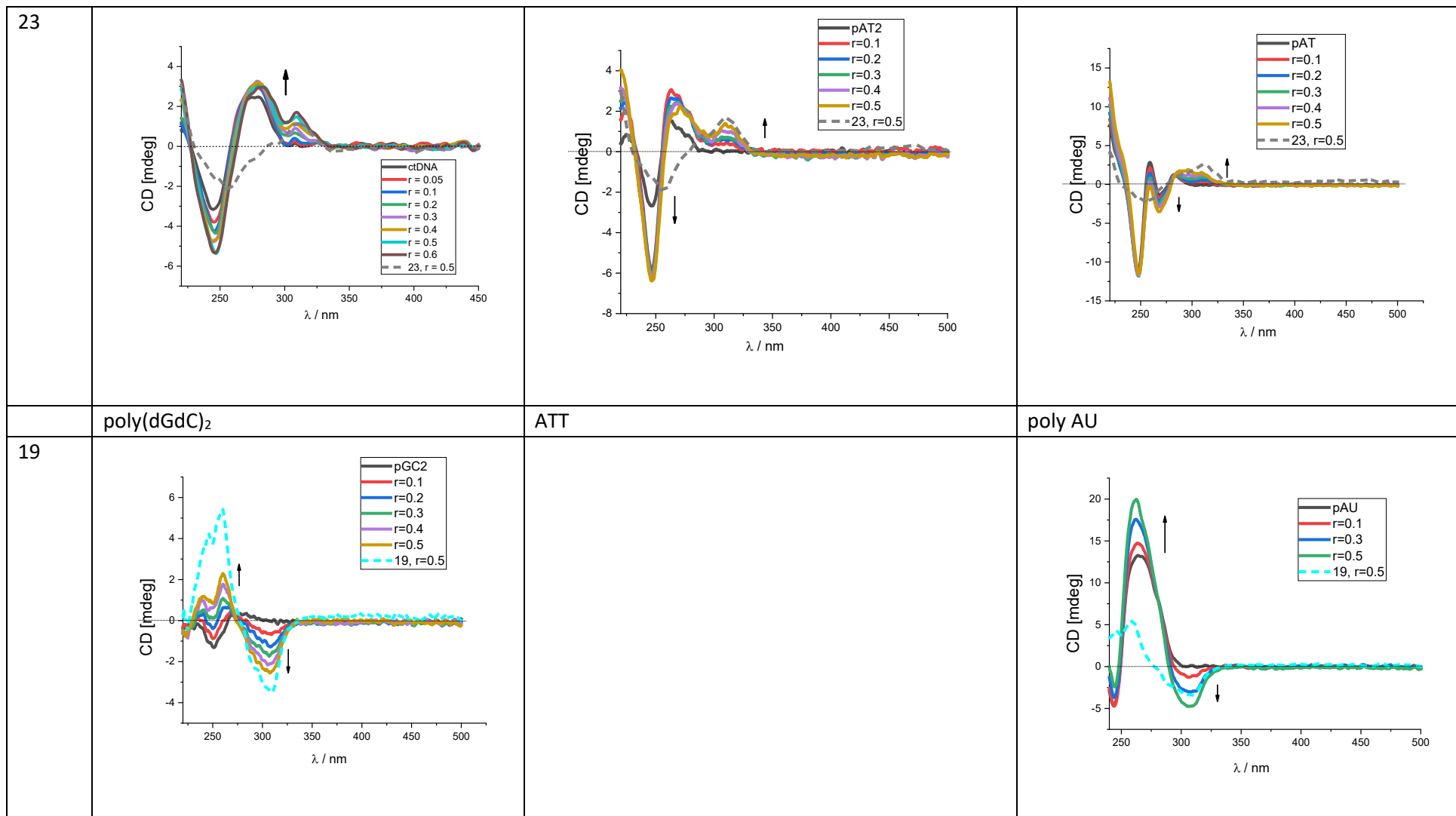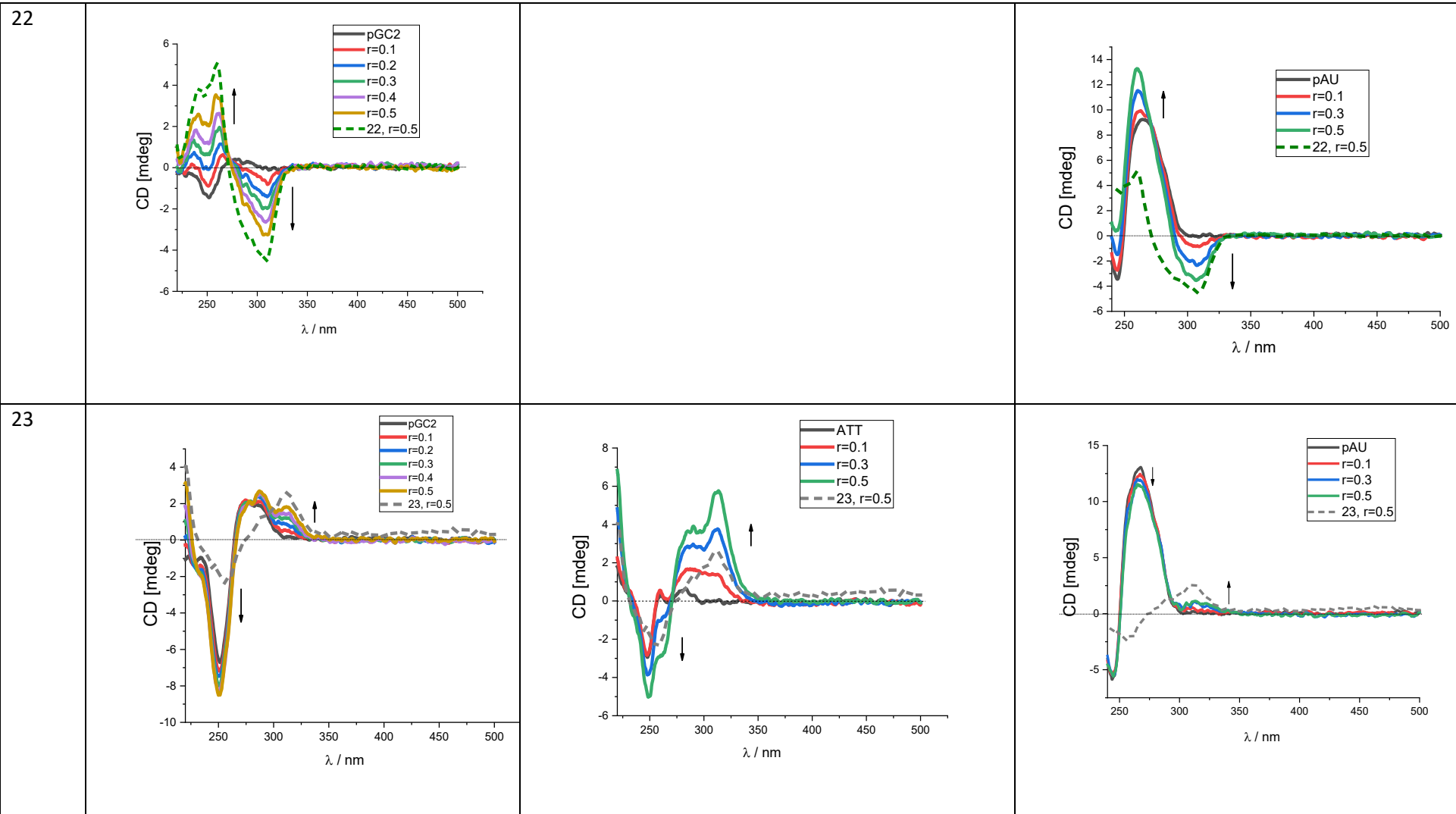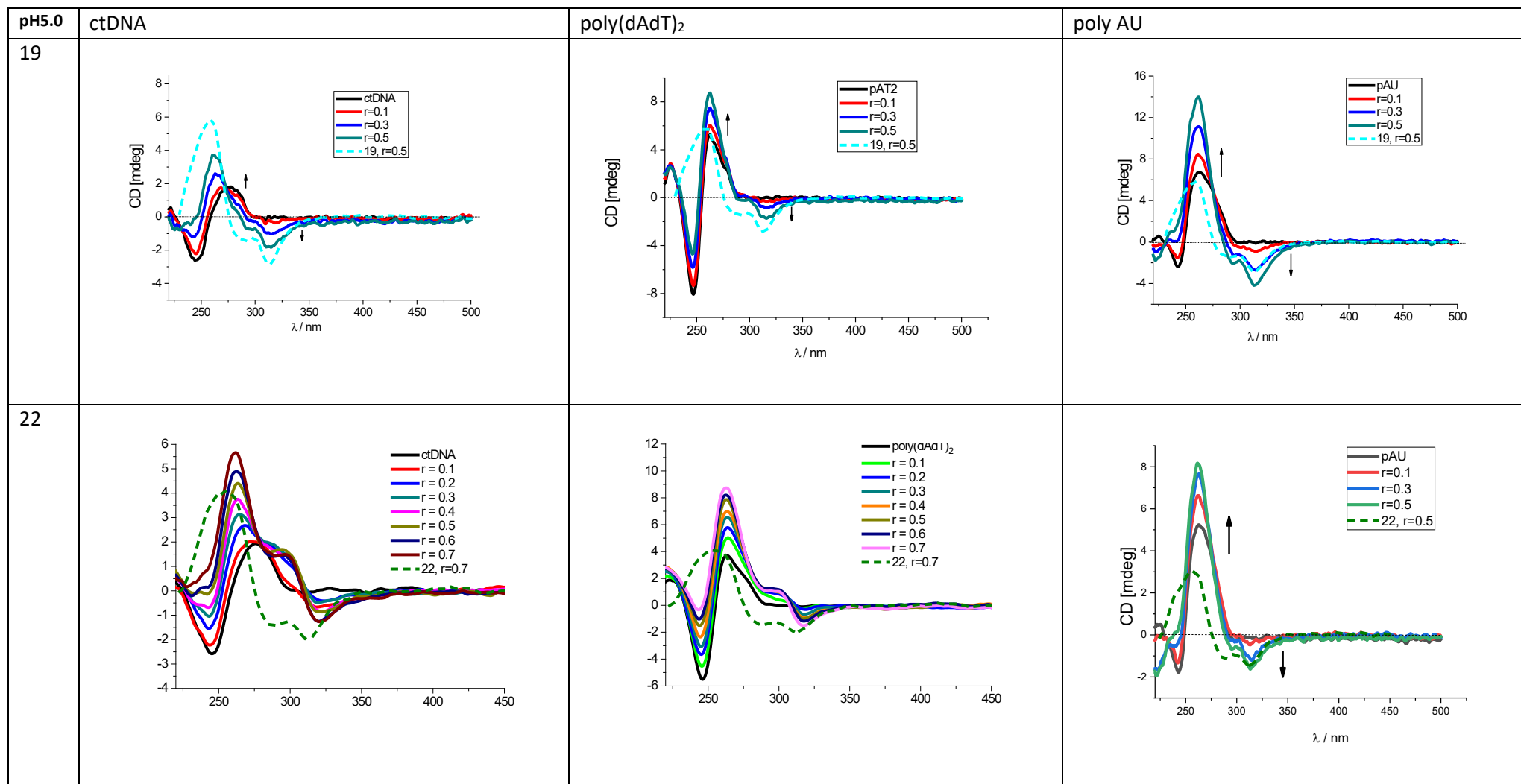
Figure S39. Melting curve of **poly dA-poly dT** and **dAdTdT** upon addition of ratio, *r* ([compound/ [polynucleotide])=0.3 of **23** at pH = 7.0 and pH = 5.0 (buffer sodium cacodylate, *I* = **0.1** mol dm^{-3})
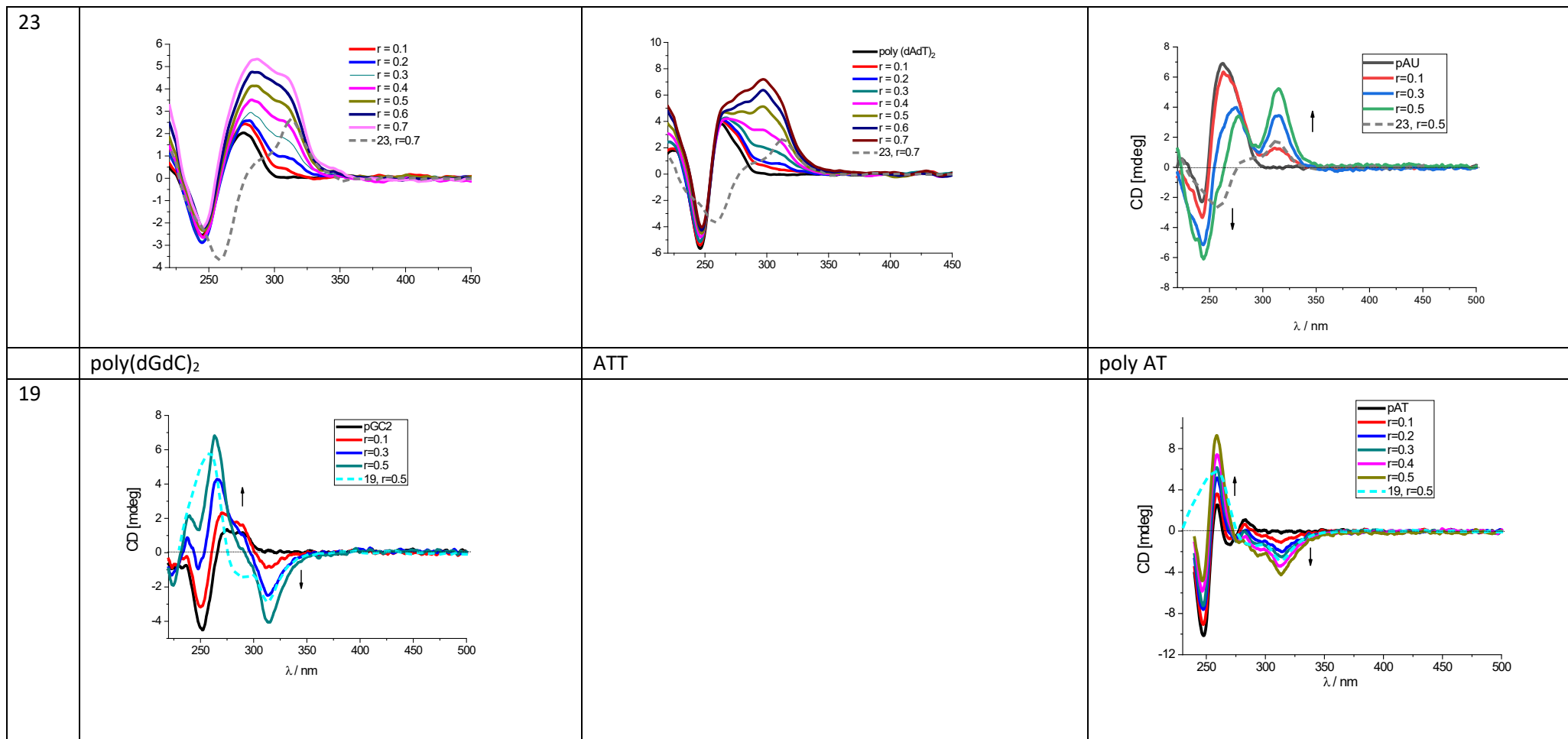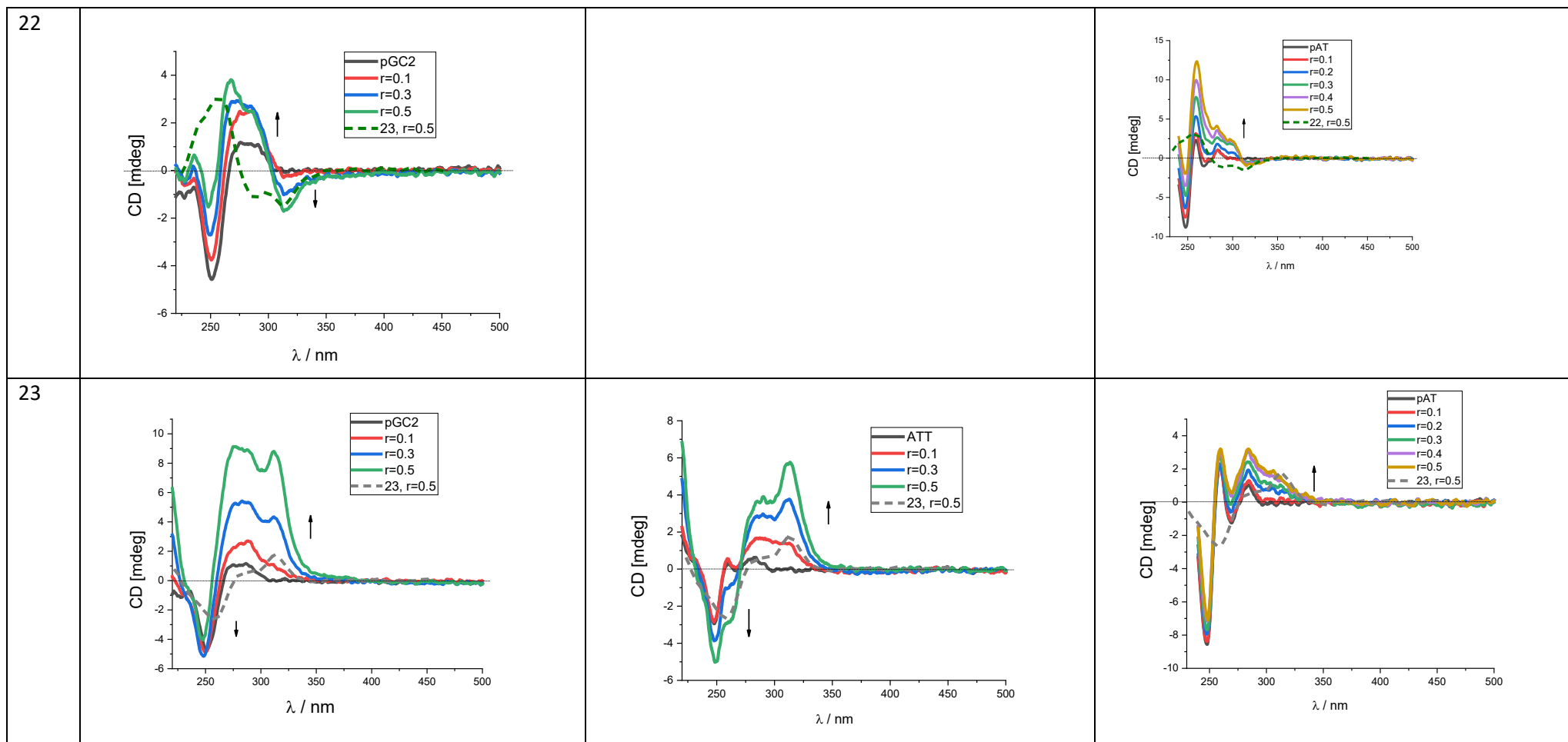
2.3.   Circular dichroism (CD) experiments

pH7.0	ctDNA	poly(dAdT)₂	poly AT
19			
22			

23			
	poly(dGdC)$_2$	ATT	poly AU
19			

22		

23		

pH5.0	ctDNA	poly(dAdT)$_2$	poly AU
19			
22			

23			
	poly(dGdC)$_2$	ATT	poly AT
19			

Figure S40. CD titration of polynucleotides (c = 3.0 × 10^{-5} mol dm^{-3}) with **19, 22** and **23** at molar ratios r = [compound] / [polynucleotide] (pH = 7.0 and 5.0, buffer sodium cacodylate, I = 0.05 mol dm^{-3}).

2.4. Mass spectra

2.4. Mass spectra

Figure S41 **a.** Snapshot scan of compound **19** (signal at *m/z 587* assigned as [**19**+H]$^+$ ion) dissolved in H_2O or D_2O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the single charged ion of compound **19**.

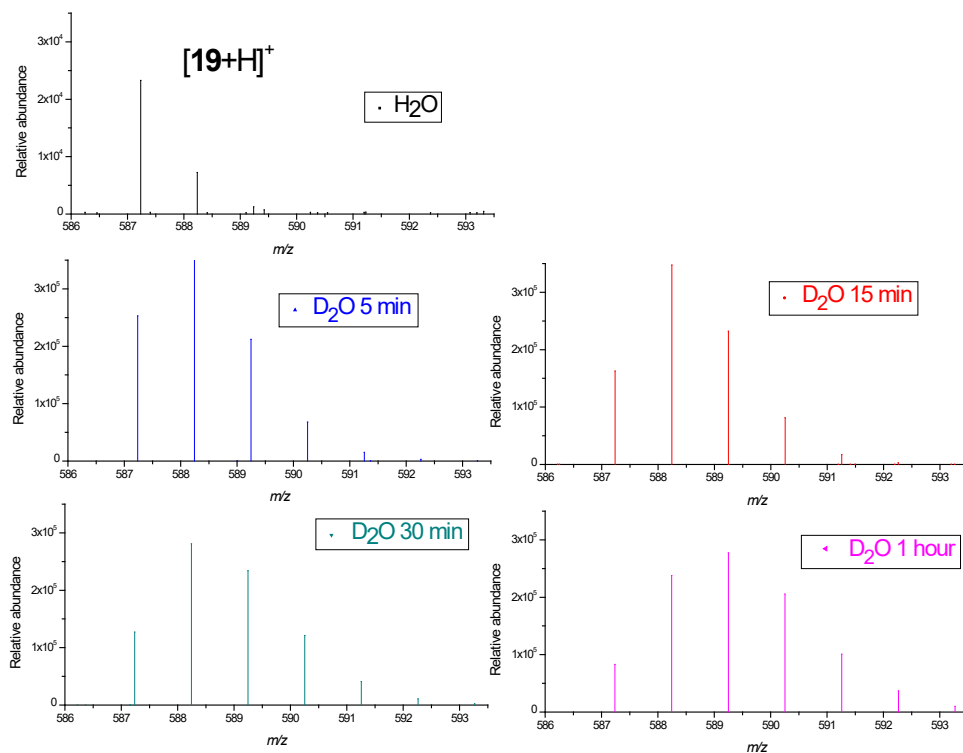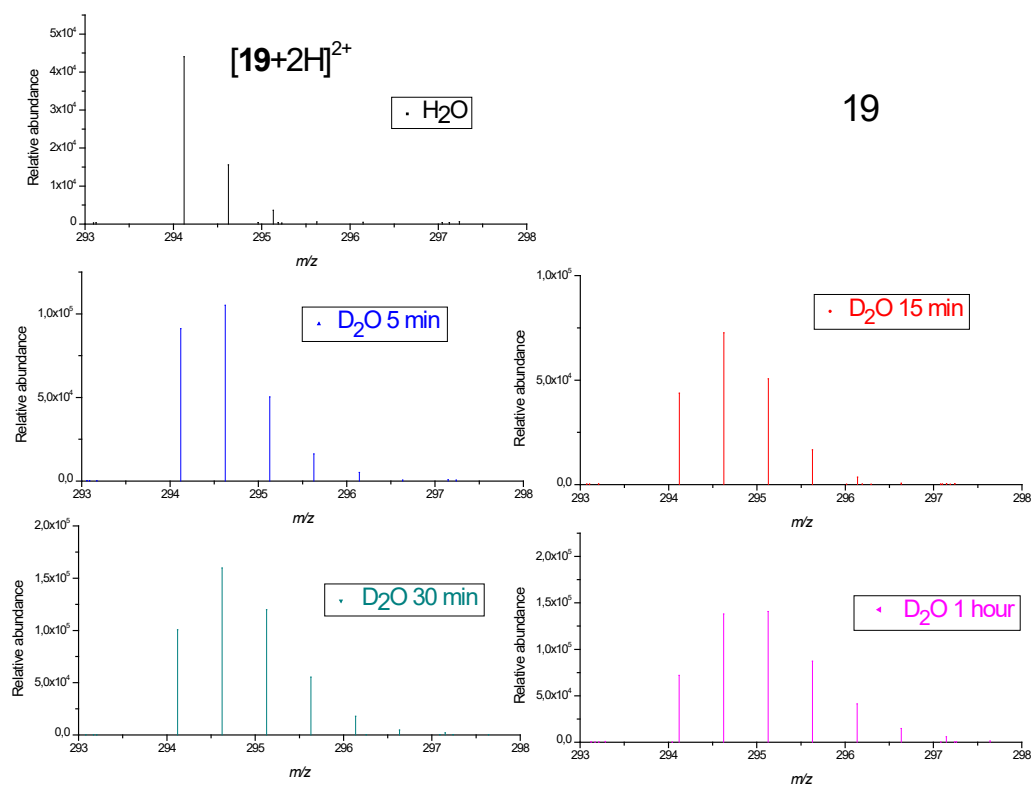Figure S41 **b.** Snapshot scan of compound **19** (signal at *m/z 294* assigned as [**19**+2H]$^{2+}$ ion) dissolved in H_2O or D_2O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the double charged ion of compound **19.**

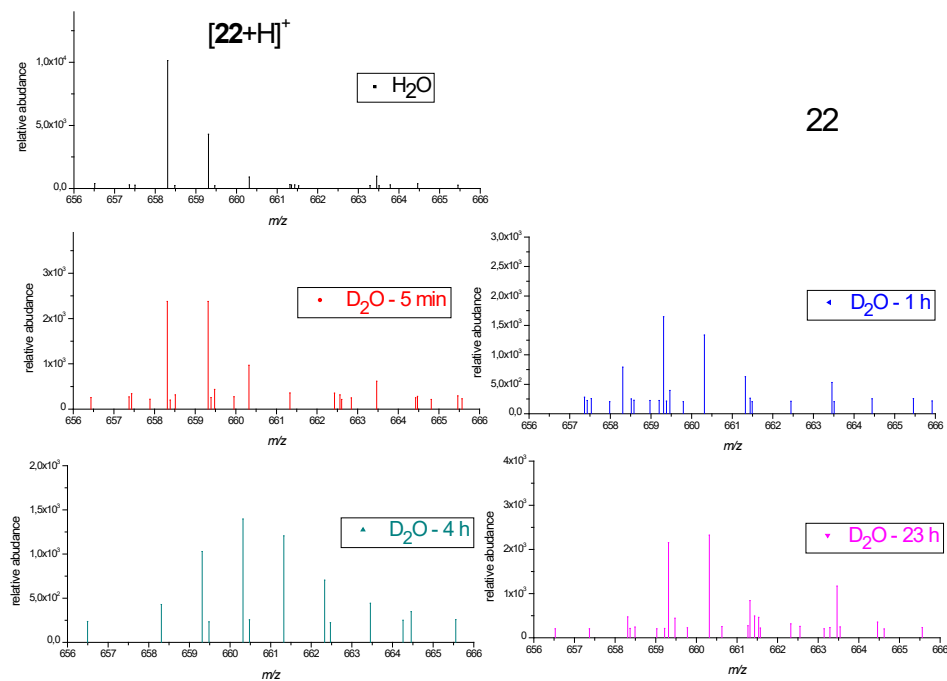Figure S41 **c.** Snapshot scan of compound **22** (signal at *m/z 658* assigned as [**22**+H]$^+$ ion) dissolved in H_2O or D_2O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the single charged ion of compound **22**.
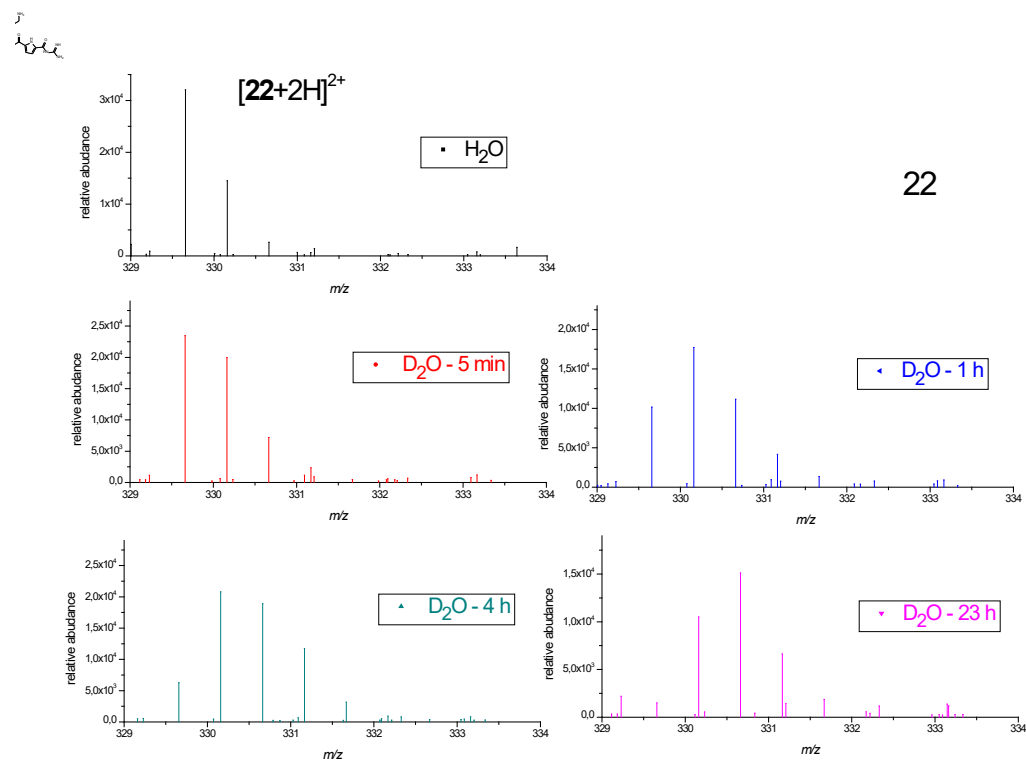
Figure S41 **d.** Snapshot scan of compound **22** (signal at *m/z 329* assigned as [**22**+2H]$^{2+}$ ion) dissolved in H$_2$O or D$_2$O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the double charged ion of compound **22.**

Figure S41 **e.** Snapshot scan of compound **23** (signal at *m/z 658* assigned as [**23**+H]$^+$ ion) dissolved in H$_2$O or D$_2$O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the single charged ion of compound **23**.

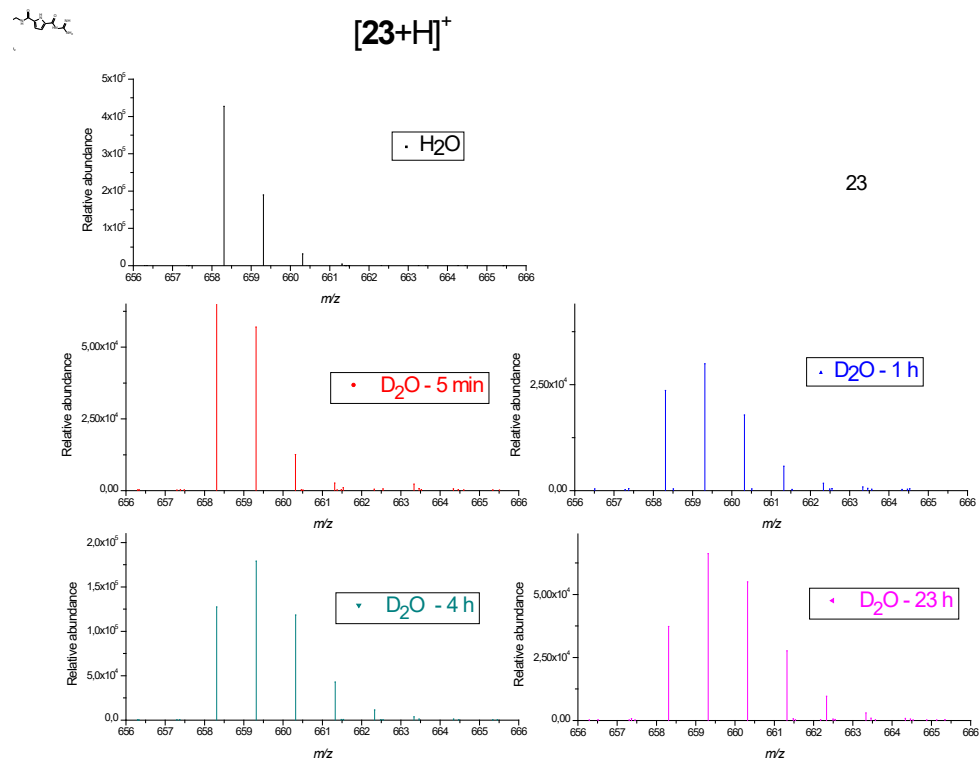Figure S41 **f.** Snapshot scan of compound **23** (signal at *m/z 329* assigned as [**23**+2H]$^{2+}$ ion) dissolved in H_2O or D_2O about 10^{-5}M. The observed signals correspond to the H/D exchange results observed for the double-charged ion of compound **23.**

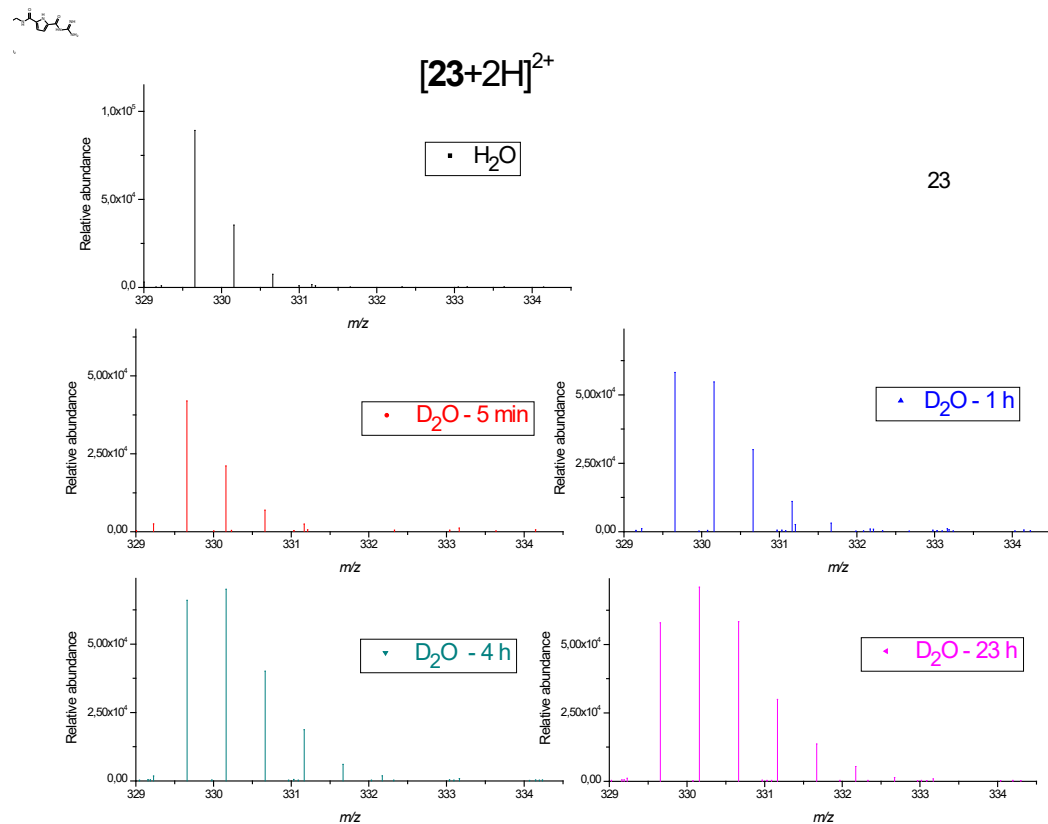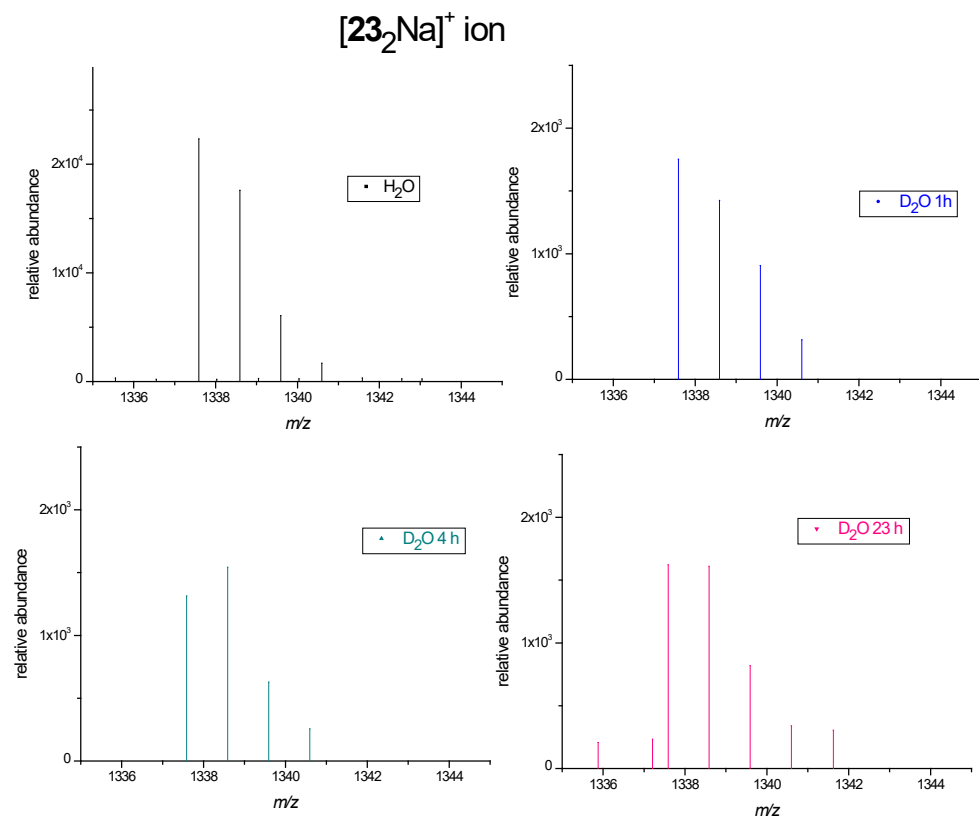Figure S41 **g**. Snapshot scan of compound **23** (signal at *m/z 1337* assigned as [**23₂**+Na]⁺ ion) dissolved in H_2O or D_2O about $10^{-5}M$. The observed signals correspond to the H/D exchange results observed for the single charged dimer ion (sodium adduct) of compound **23**.

3. Computational analysis of 19, 22 and 23

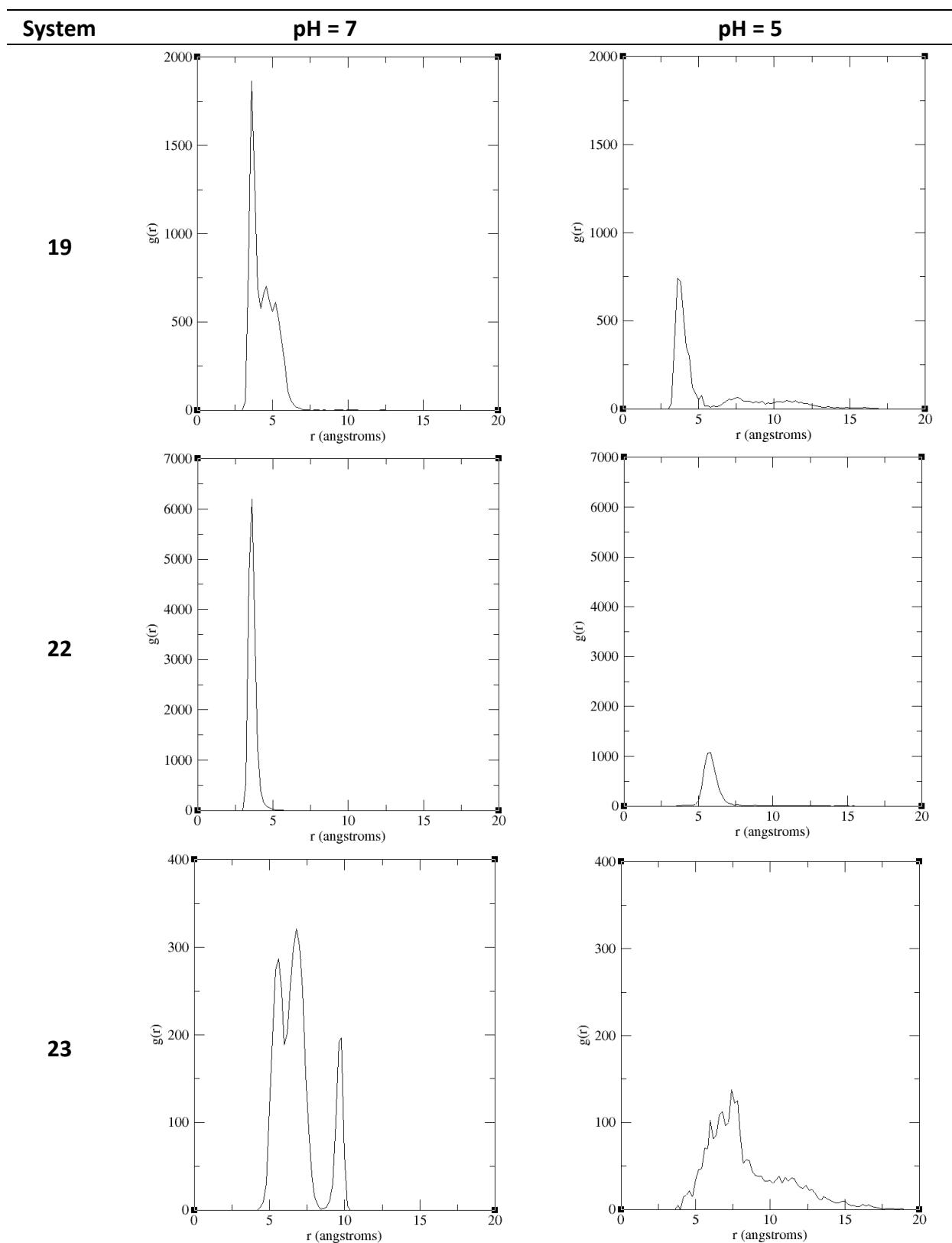| System	pH = 7	pH = 5
19		
22		
23		

Figure S42. RDF graphs describing the distance between centres of mass of phenanthridine and pyrrole rings during the last 30 ns of the matching molecular dynamics simulations.
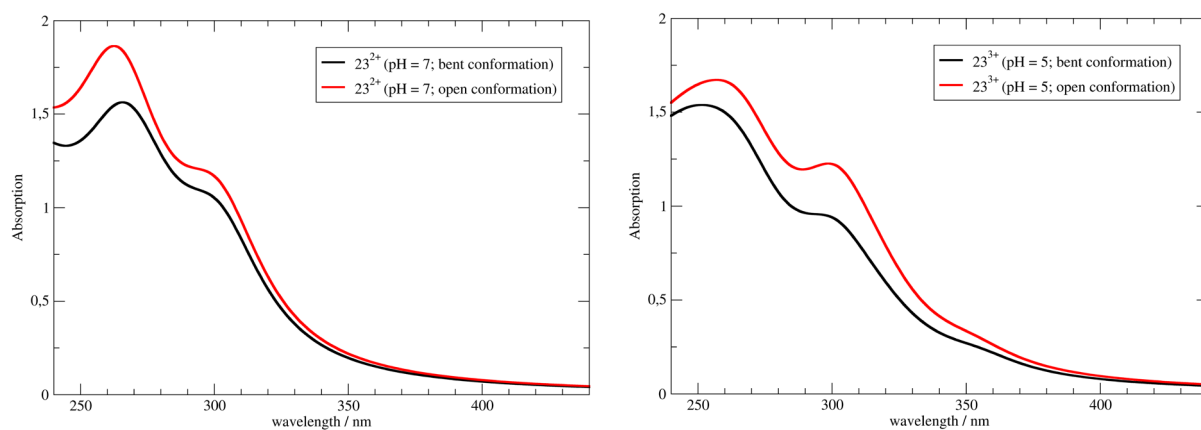
Figure S43. Calculated UV/Vis spectra for the representative structures of both bent and open conformations of systems **23** under normal (pH = 7, left) and acidic conditions (pH = 5, right) obtained with the TD-DFT approach and the (SMD)/B3LYP/6–31+G(d) model in water.