Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Guanidino-aryl derivatives: protonation and structure tuning for spectrophotometric recognition of ds-DNA and ds-RNA

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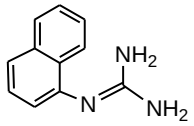
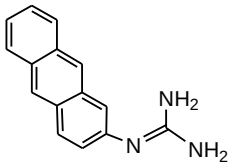
I. Structures of studied aryl-guanidines



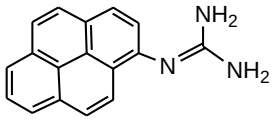
Molecular weight: 185.2251

***N*-(naphthalene-1-yl)guanidine (NGU)**



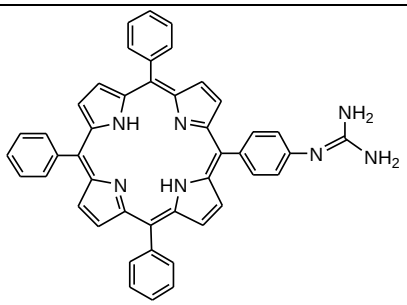
Molecular weight: 235.2838

***N*-(anthracene-2-yl)guanidine (AGU)**



Molecular weight: 259.3052

***N*-(pyrene-1-yl)guanidine (PyGU)**



Molecular weight: 671.7904

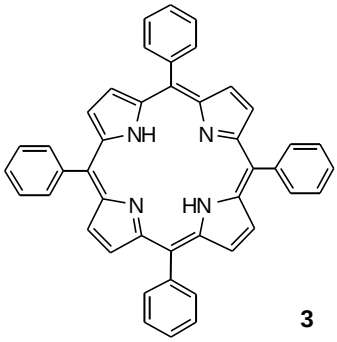
5-(*p*-guanidinophenyl)-10,15,20-triphenylporphyrin (PoGU)

Chart S1. Structures of studied aryl-guanidines

II. Synthesis and Analytical Data of compounds

***N*-(naphthalene-1-yl)guanidine (NGU), *N*-(anthracene-2-yl)guanidine (AGU), *N*-(pyrene-1-yl)guanidine (PyGU)** were synthesized as described before.[1]

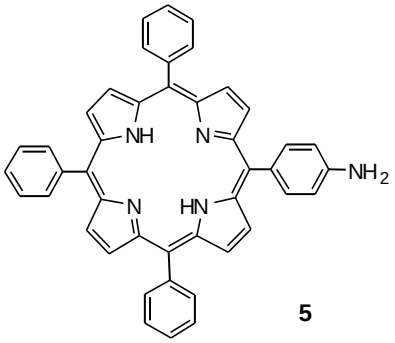
1.1. 5,10,15,20-tetraphenylporphyrin (3)



3

Porphyrin **3** was prepared by literature procedure.[2] To a hot propionic acid (600 mL), benzaldehyde (16.0 mL, 0.16 mol), and pyrrole (11.2 mL, 0.16 mol) were added consecutively. Reaction mixture was refluxed for 30 min. After colling, the precipitate was filtered off and washed with methanol to give purple crystals. After drying in the air, product **3** was obtained (4.9 g, 20 %). ^{1}H NMR spectrum is identical to literature.
^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm: 8.85 (s, 8H; H-2, H-3, H-7, H-8, H-12, H-13, H-17, H-18), 8.28 - 8.16 (m, 8H; H-2'), 7.82 - 7.68 (m, 12H; H-3', H-4'), -2.75 (brs, 2H; NH).

1.2. 5-(*p*-aminophenyl)-10,15,20-triphenylporphyrin (5)



5

Porphyrin **5** was synthesized from 5,10,15,20-tetraphenylporphyrin **3** in 19-62% yield (depending on the reaction scale), by modification of the literature procedure.[3]

To a solution of 5,10,15,20-tetraphenylporphyrin **3** in TFA, sodium nitrite (0.8 eq) was added. After 5 minutes of stirring at 0 °C, the reaction mixture was poured into distilled water and extracted with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and water. After evaporation of the solvent, the residue was dissolved in concentrated hydrochloric acid and, while stirring, tin(II) chloride (10 eq) was carefully added. The final mixture was heated to 65 °C for 2 hours, before being poured into cold water. The aqueous solution was neutralized with sodium hydroxide until pH = 8. The

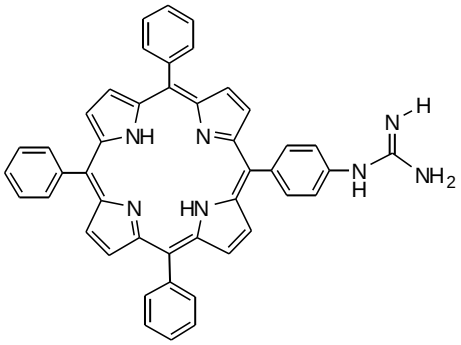
aqueous solution was extracted with dichloromethane. The organic layer was then concentrated under vacuum and the residue was purified on the column of silicagel employing a 1:1 mixture of petroleum ether/dichloromethane as the eluent, to give amine **5** as purple solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm: 8.94 (d, J = 4.8 Hz, 2H; H-3, H-7), 8.83 (s, 6H; H-2, H-8, H-12, H-13, H-17, H-18), 8.29-8.16 (m, 6H; H-6'), 8.00 (d, J = 8.3 Hz, 2H; H-2'), 7.83-7.67 (m, 9H; H-7', H-8'), 7.07 (d, J = 8.4 Hz, 2H; H-3'), 4.03 (brs, 2H; NH_2), -2.75 (brs, 2H; NH).

1.3. ***N*,*N*'-di-Boc-*N*''-trifluoromethanesulfonyl-guanidine** was synthesized in two steps from guanidine hydrochloride in 33% yield, following the literature procedure.[4]

^{1}H NMR (600 MHz, d_6-DMSO) δ/ppm: 11.03 (brs, 2H), 1.46 (s, 18H).

1.3. 5-(*p*-guanidinophenyl)-10,15,20-triphenylporphyrin (PoGU) (6)



PoGu

To a solution of *N*,*N*'-di-Boc-*N*''-trifluoromethanesulfonyl-guanidine (198.9 mg, 0.5082 mmol) and trimethylamine (75.0 μL, 0.5336 mmol) in dichloromethane (3 mL), 5-(*p*-aminophenyl)-10,15,20-triphenylporphyrin **5** (160.0 mg, 0.2541 mmol) was added and the reaction mixture was stirred at r.t. for 15 days, until the complete consumption of amine (followed by TLC). After the evaporation of solvent, reaction mixture was subjected to chromatography on the column of silicagel employing a 1:1 mixture of petroleum ether/dichloromethane as the eluent. The obtained solid (246.0 mg), containing 5-[(N^1,N^2-bis(*tert*-butyloxycarbonyl)-*p*-guanidinophenyl]-10,15,20-triphenylporphyrin **6** as desired product, contaminated with the excess of *N*,*N*'-di-Boc-*N*''-trifluoromethanesulfonyl-guanidine, was hydrolysed in the next step without further purification. 50.0 mg (0.0573 mmol) of the above mentioned solid, composed mainly of *N*,*N*'-di-Boc-protected guanidine, was suspended in methanol and excess of hydrochloric acid was added. The reaction mixture was stirred at r.t. for several hours, until the full consumption of substrate (monitored by TLC). Reaction mixture was basified with saturated aqueous solution of potassium hydroxide and stirred at r.t. for several hours. Methanol was removed under reduced pressure and residue was extracted with ethyl acetate. Organic extracts were collected, dried

over $MgSO_4$ and solvent was removed under reduced pressure to afford neutral **PoGU** as a purple solid (30.1 mg, 78% yield).

FTIR-ATR $\tilde{\nu}$/cm^{-1}: 3463, 3318, 3023, 1657, 1590, 1471, 1440, 1349, 1174, 1071, 1001, 965, 800, 734, 701.
^{1}H NMR (300 MHz, d_6-DMSO) δ/ppm: 9.04 (d, J = 4.8 Hz, 2H; H-3, H-7), 8.87-8.76 (m, 6H; H-2, H-8, H-12, H-13, H-17, H-18), 8.27-8.18 (m, 6H; H-6'), 8.00 (d, J = 8.2 Hz, 2H; H-2'), 7.89-7.78 (m, 9H; H-7', H-8'), 7.19 (d, J = 6.6 Hz, 2H; H-3'), 5.39 (brs, 4H; 2 NH_2), -2.87 (s, 2H; 2 $NH_{pyrrole}$).
^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm: 151,1 (C=N); 148,7; 142,2; 136,1; 135,8; 134,5; 127,6; 126,6; 121,3; 120,4; 119,9; 119,8.
HRMS-MALDI found: 672.2891; calc. for $C_{45}H_{33}N_7$ $[M+H]^+$: 672.2876.

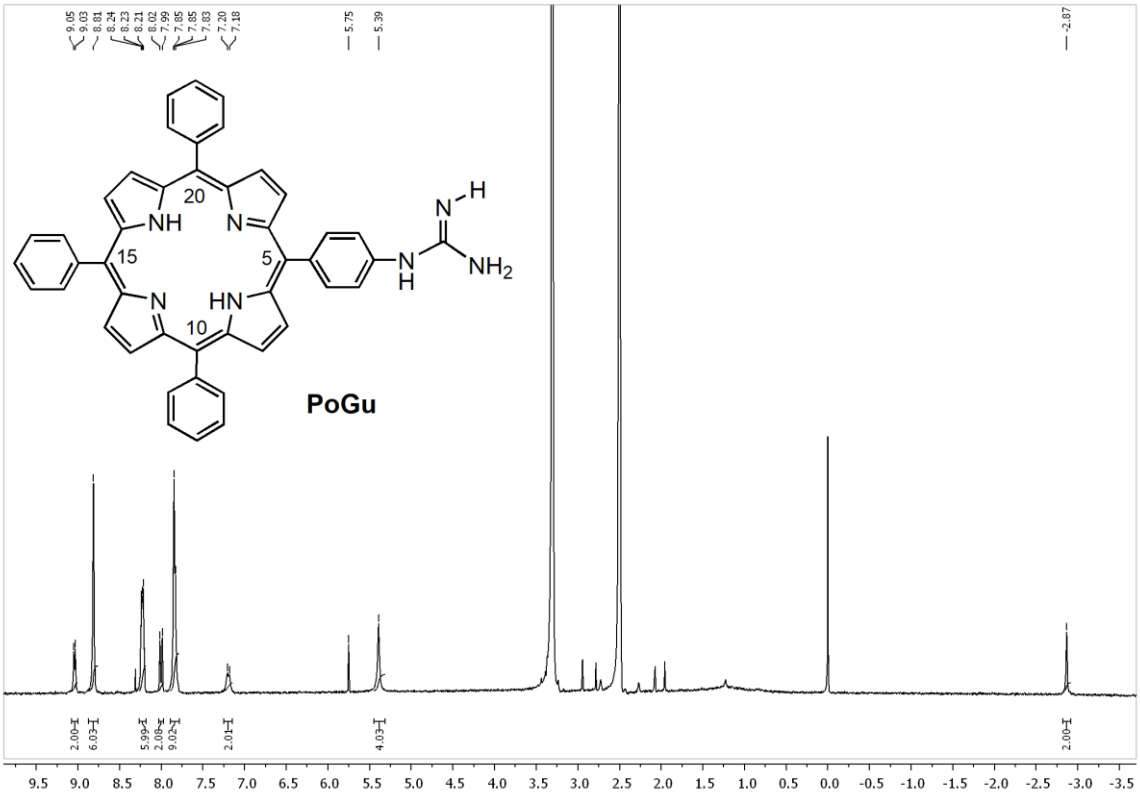


Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **PoGU**

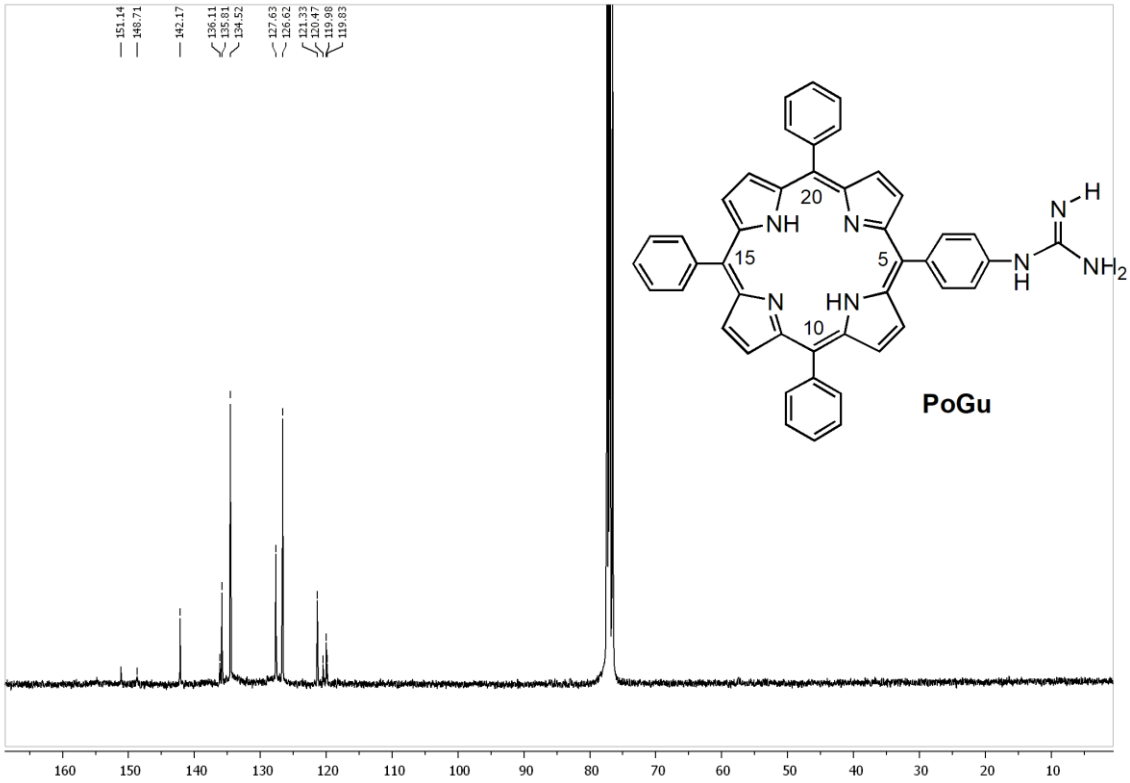


Figure S2. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **PoGU**

III. Physico-chemical properties of buffered solution (sodium cacodylate pH=7)

Solubility

NGU, **AGU**, **PyGU** and **PoGU** are soluble in dimethyl sulfoxide (c = 2×10^{-3} mol dm^{-3}). Stock solutions were diluted with buffer sodium cacodylate (pH = 7.0, I = 0.05 mol dm^{-3}).

UV/Vis spectra, stability

Buffered solutions of studied compounds were stable for more (1 or 4) days. The absorbancies of buffered solutions of studied compounds are proportional to their concentrations up to c = 6×10^{-6} mol dm^{-3}.

No significant changes of the UV/Vis spectra on the temperature increase up to 90 ^{o}C were observed, and reproducibility of UV/Vis spectra upon cooling back to 20 ^{o}C was excellent.

All mentioned is indicating that the studied compounds do not aggregate by intermolecular stacking at experimental conditions used. Exception is **PoGU,** which indicate some aggregation upon heating to 90 ^{o}C and cooling back to 20 ^{o}C (see Figure S3.).

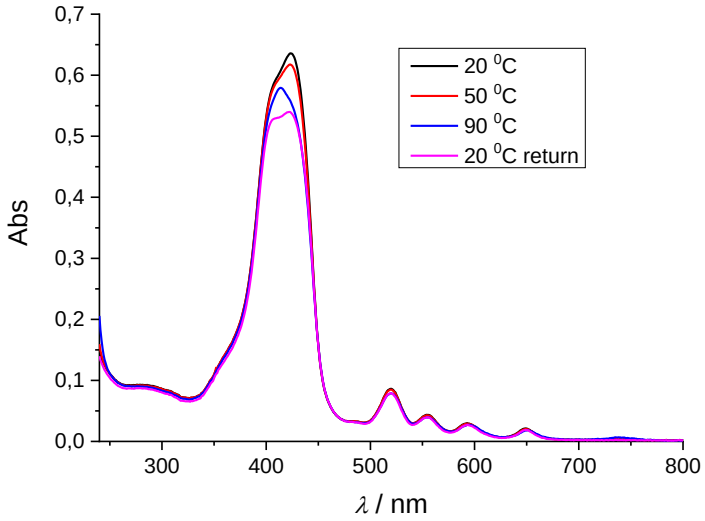
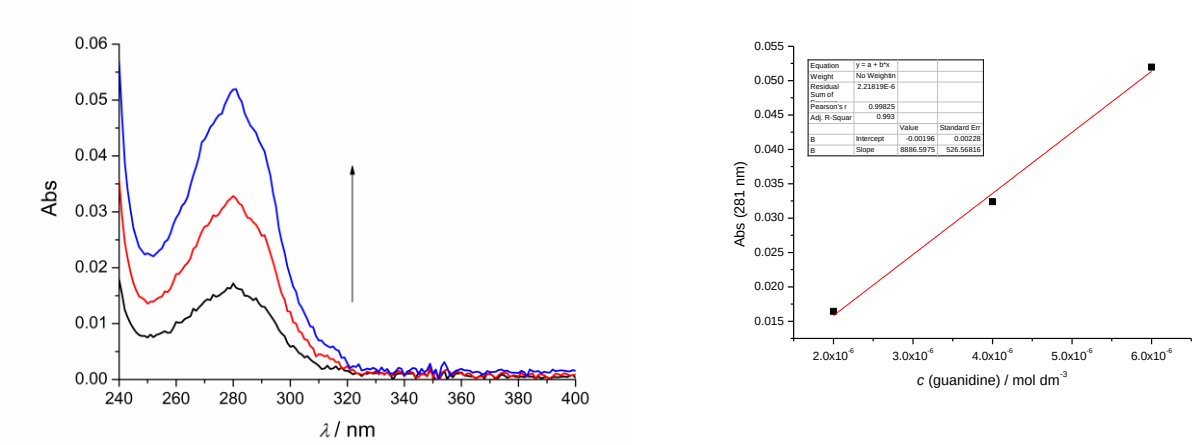
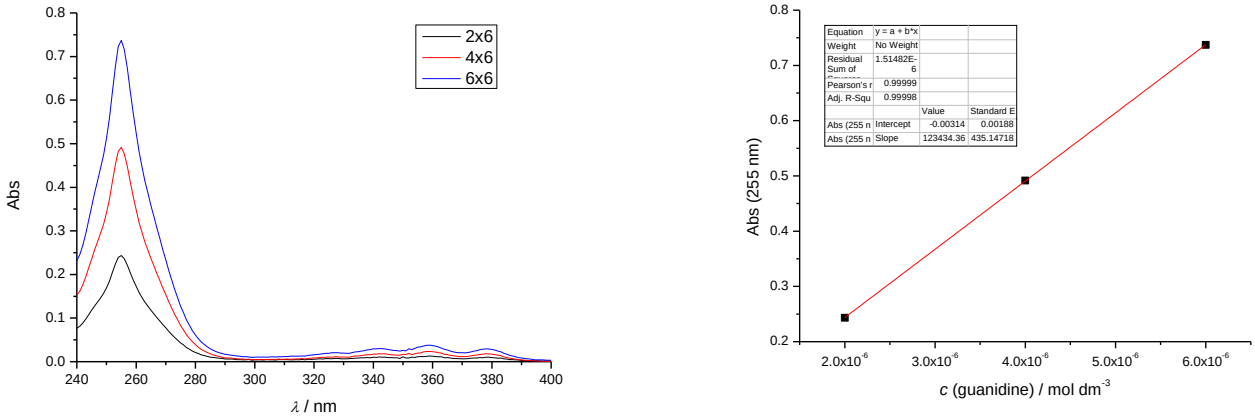


Figure S3. UV/Vis spectra changes of **PoGU (***c* = 6×10^{-6} mol dm^{-3}) in buffered solution (pH = 7.0, *I* = 0.05 mol dm^{-3}) at different temperatures.

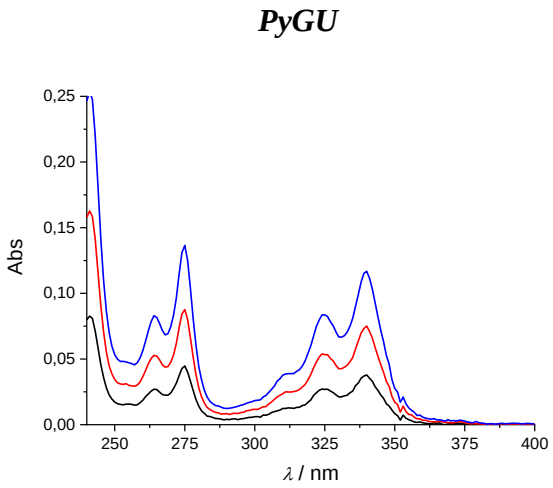
Absorption maxima and corresponding molar extinction coefficients (ε) are given in Table 1.

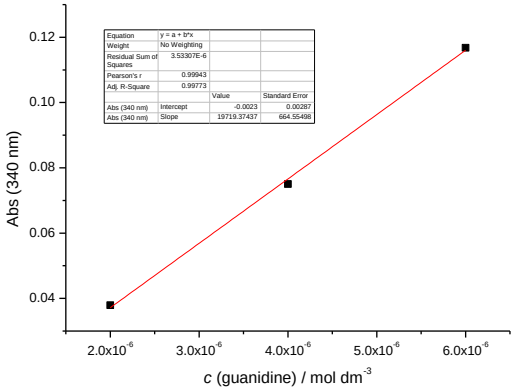


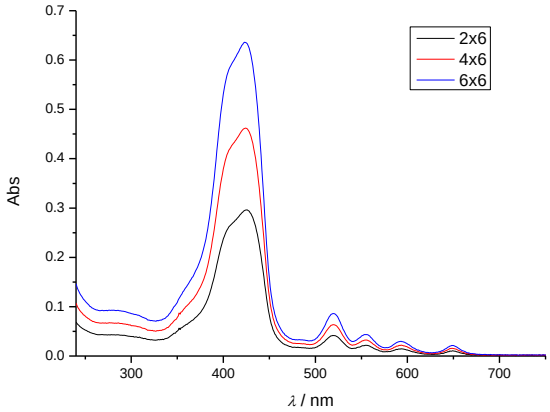
NGU

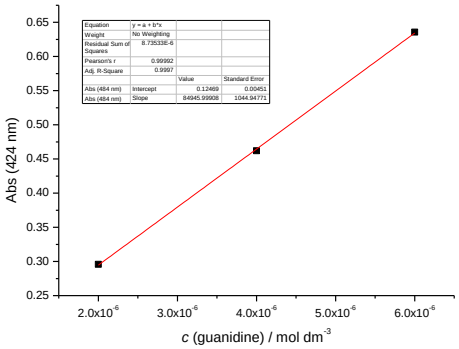


AGU





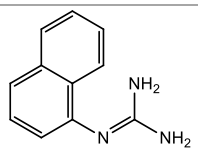
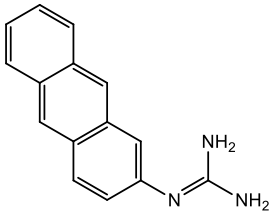
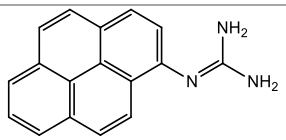
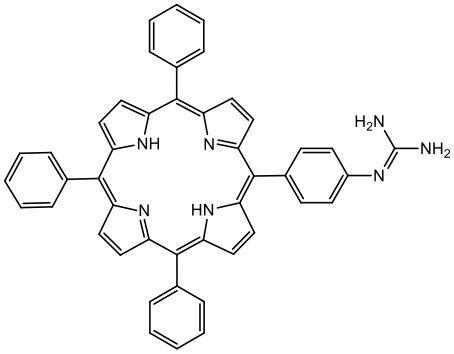




PoGU

Figure S4. UV/Vis spectra dependence of **NGU, AGU, PyGU** and **PoGU** on compound concentrations (range 2×10^{-6}– 6×10^{-6} mol dm^{-3}) in buffered solution (Na cacodylate, pH = 7.0, I = 0.05 mol dm^{-3}).

Table S1. Electronic absorption data of **NGU, AGU, PyGU** and **PoGU.**

	λ_{max}/nm	$\varepsilon \times 10^3$/mmol^{-1} cm^2
 Molecular Weight: 185.2251 ***N*-(naphthalene-1-yl)guanidine**	281	8.9 ± 0.5
 Molecular Weight: 235.2838 ***N*-(anthracene-2-yl)guanidine**	255	123.4 ± 0.4
 Molecular Weight: 259.3052 ***N*-(pyrene-1-yl)guanidine**	340	19.7 ± 0.6
 Molecular Weight: 671.7904 **5-(*p*-guanidinophenyl)-10,15,20-triphenylporphyrin**	424	84.9 ± 1.0

Stock solutions of **NGU, AGU, PyGU** and **PoGU** compounds were prepared in dimethyl sulfoxide (c = 2×10^{-3} mol dm^{-3}).

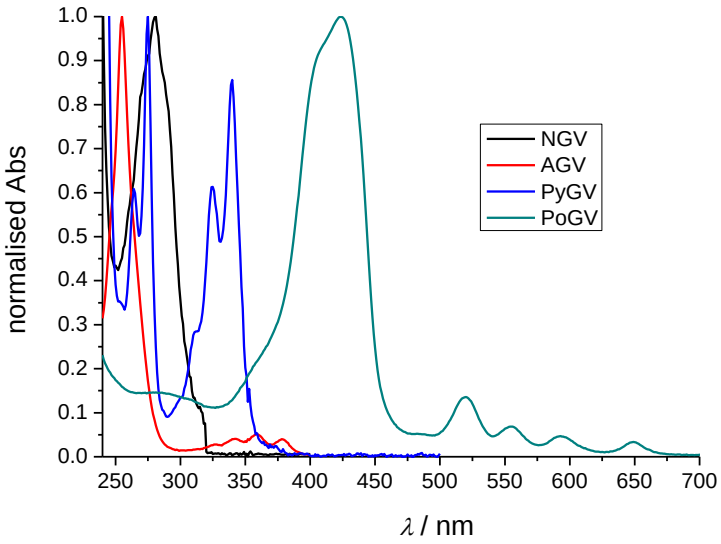
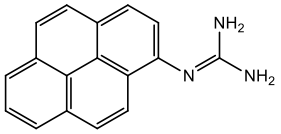


Figure S5. The UV/Vis spectra of studied compounds normalised on λ_{max} for each compound, c = 6×10^{-6} mol dm^{-3} in buffered solution (Na cacodylate, pH = 7.0, I = 0.05 mol dm^{-3}).

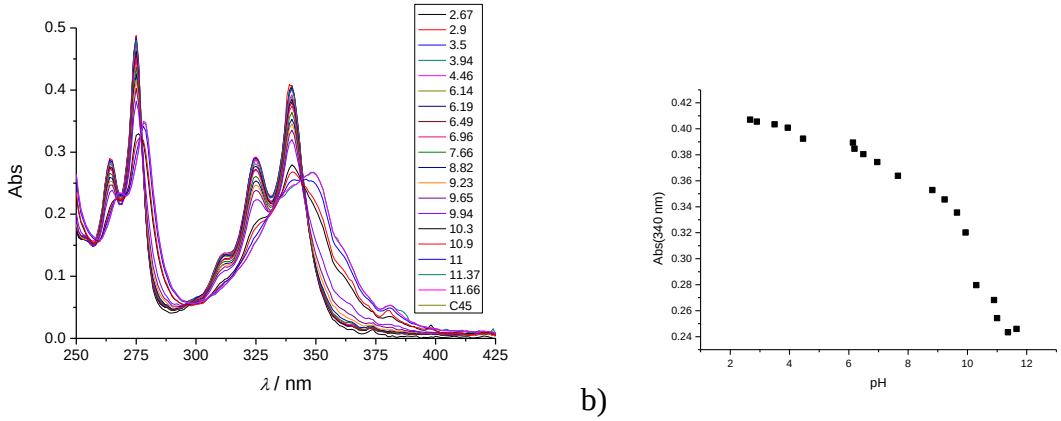
pK determination

_N_-(pyrene-1-yl)guanidine



Molecular Weight: 259.3052 as a model compound for all, due to convenient chromophore

Stock solution of **PyGU** was prepared in dimethyl sulfoxide (c = 2×10^{-3} mol dm^{-3}).



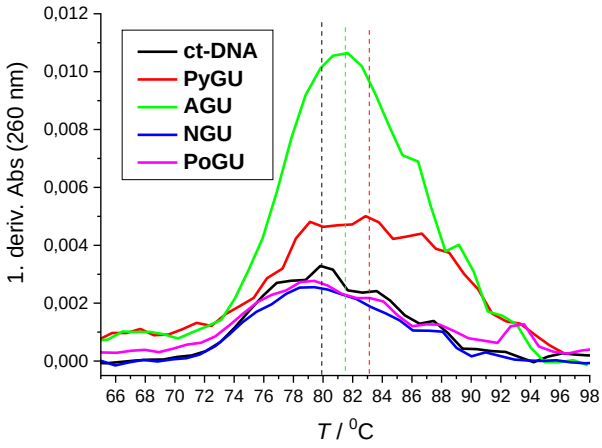
a) b)

Figure S6. a) UV/Vis titration of water solution of **PyGU** (c = 5×10^{-6} mol dm^{-3}), acidified with HCl (0.1 mol dm^{-3}), with NaOH (0.2 mol dm^{-3}); b) dependence of absorbance at λ_{max} = 340 nm on pH.

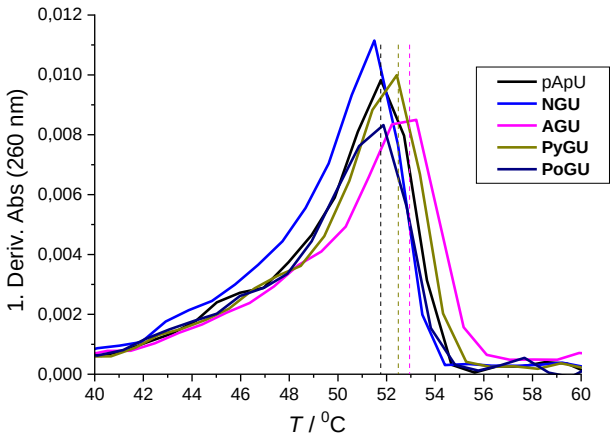
IV. Study of interactions of NGU, AGU, PyGU and **PoGU with DNA and RNA in buffered solution (sodium cacodylate pH = 7)**

Thermal melting experiments

It is well known that upon heating ds-helices of polynucleotides at well-defined temperature (T_m value) dissociate into two single stranded polynucleotides. Non-covalent binding of small molecules to ds-polynucleotides usually has certain effect on the thermal stability of helices thus giving different T_m values. Difference between T_m value of free polynucleotide and complex with small molecule (ΔT_m value) is important factor in characterisation of small molecule / ds-polynucleotide interactions.



a)



b)

Figure S7. a) Melting curves of ct-DNA upon addition of **NGU, AGU, PyGU** and **PoGU** (r = 0.2 [compound] / [polynucleotide]) at pH = 7.0 (buffer sodium cacodylate, I = 0.05 mol dm^{-3}) b) Melting curves of polyA-polyU upon addition of **NGU, AGU, PyGU** and **PoGU** (r = 0.2 [compound] / [polynucleotide]) at pH = 7.0 (buffer sodium cacodylate, I = 0.05 mol dm^{-3}).

Fluorimetric titrations with DNA/RNA

_N_-(anthracene-2-yl)guanidine (AGU)

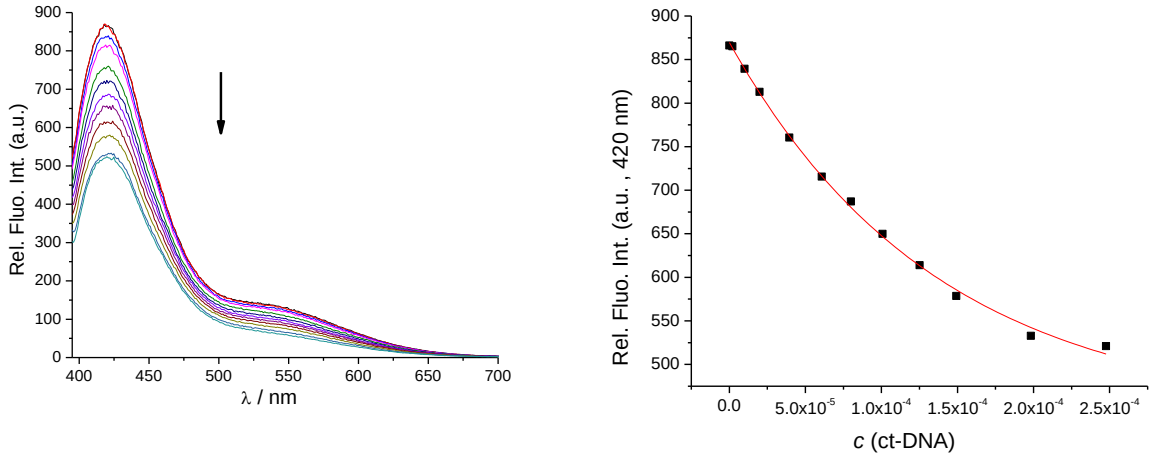


Figure S8. Fluorimetric titration of **AGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 380 nm) with **ct-DNA**. RIGHT: dependence of fluorescence at λ_{max} = 420 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

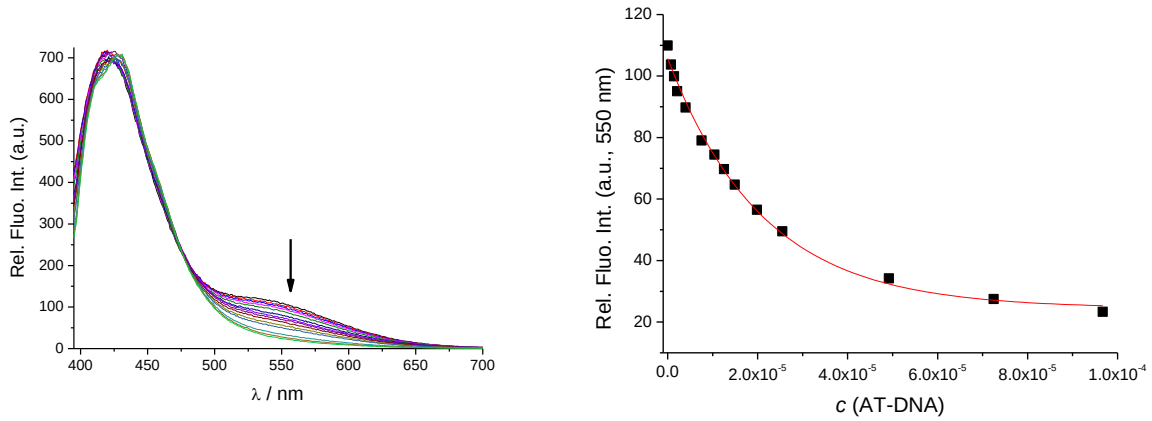


Figure S9. Fluorimetric titration of **AGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 380 nm) with **p(dAdT)$_2$**. RIGHT: dependence of fluorescence at λ_{max} = 550 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

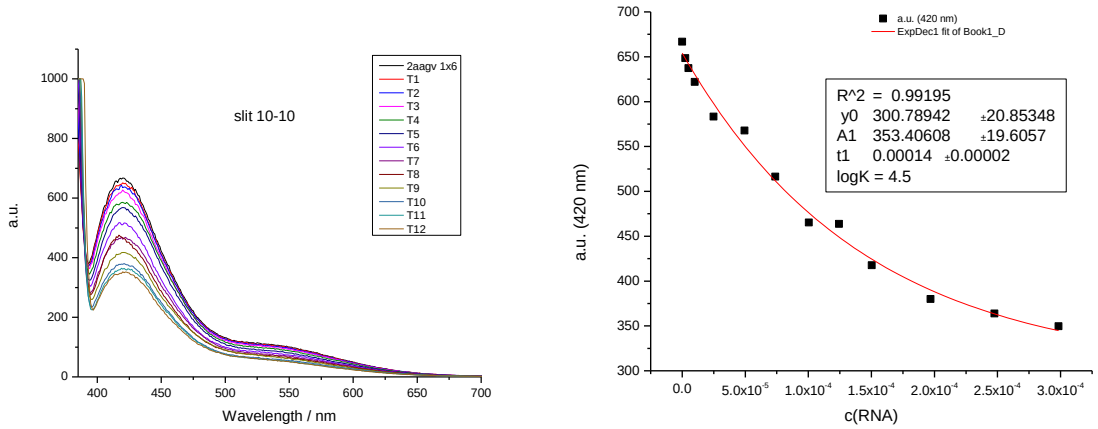


Figure S10. Fluorimetric titration of **AGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 380 nm) with **poly A - poly U**. RIGHT: dependence of fluorescence at λ_{max} = 420 nm on c(RNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

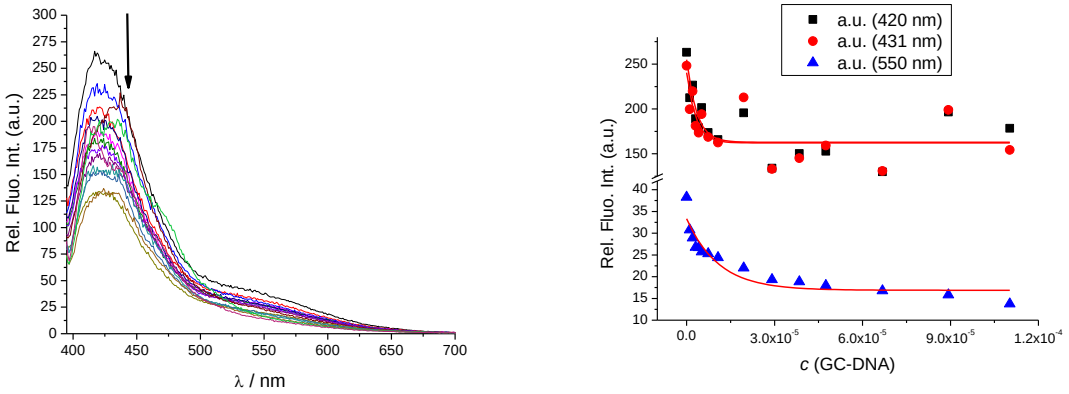


Figure S11. Fluorimetric titration of **AGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 380 nm) with GC-**DNA**. RIGHT: dependence of fluorescence at λ_{max} = 420 nm on c(DNA). **Too small** changes did not allow accumulation of enough data points for accurate analysis by Scatchard eq. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

***N*-(pyrene-1-yl)guanidine (PyGU)**

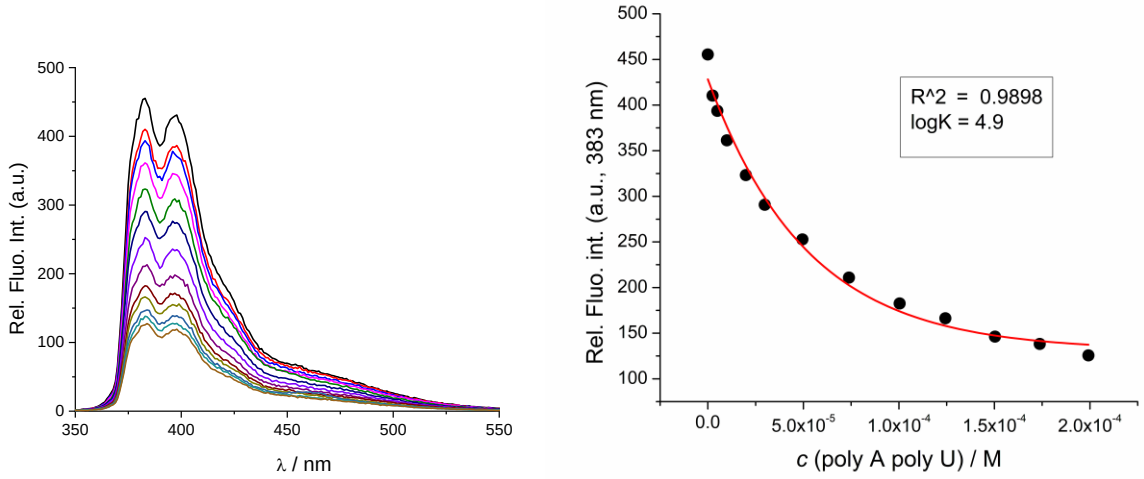


Figure S12. Fluorimetric titration of **PyGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 340 nm) with **poly A - poly U**. RIGHT: dependence of fluorescence at λ_{max} = 383 nm on c(RNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

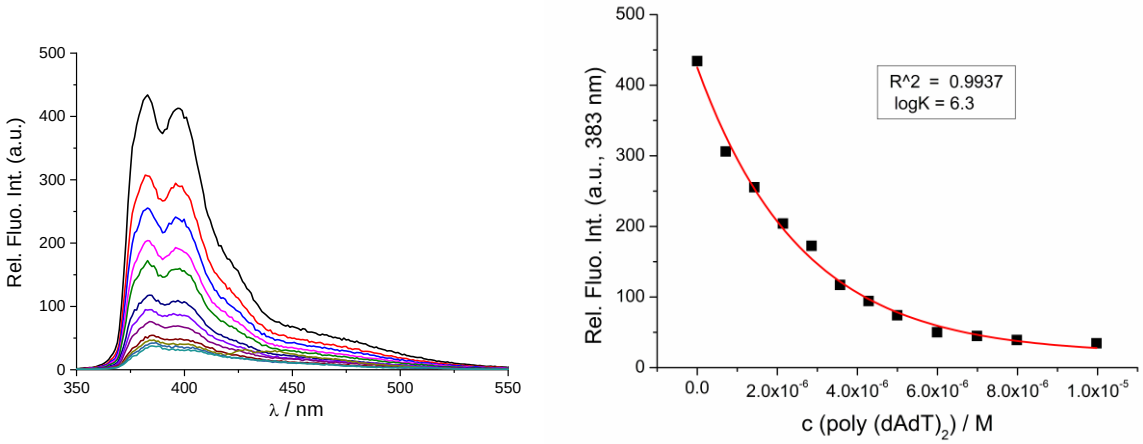


Figure S13. Fluorimetric titration of **PyGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 340 nm) with poly (dAdT)$_2$. RIGHT: dependence of fluorescence at λ_{max} = 383 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

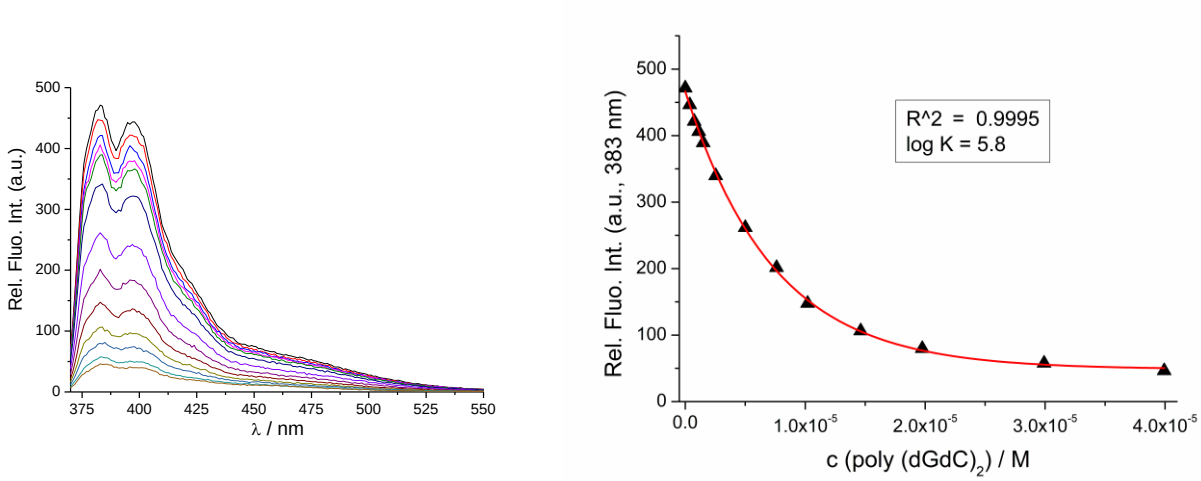


Figure S14. Fluorimetric titration of **PyGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 340 nm) with poly (dGdC)$_2$. RIGHT: dependence of fluorescence at λ_{max} = 383 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

5-(*p*-guanidinophenyl)-10,15,20-triphenylporphyrin (PoGU)

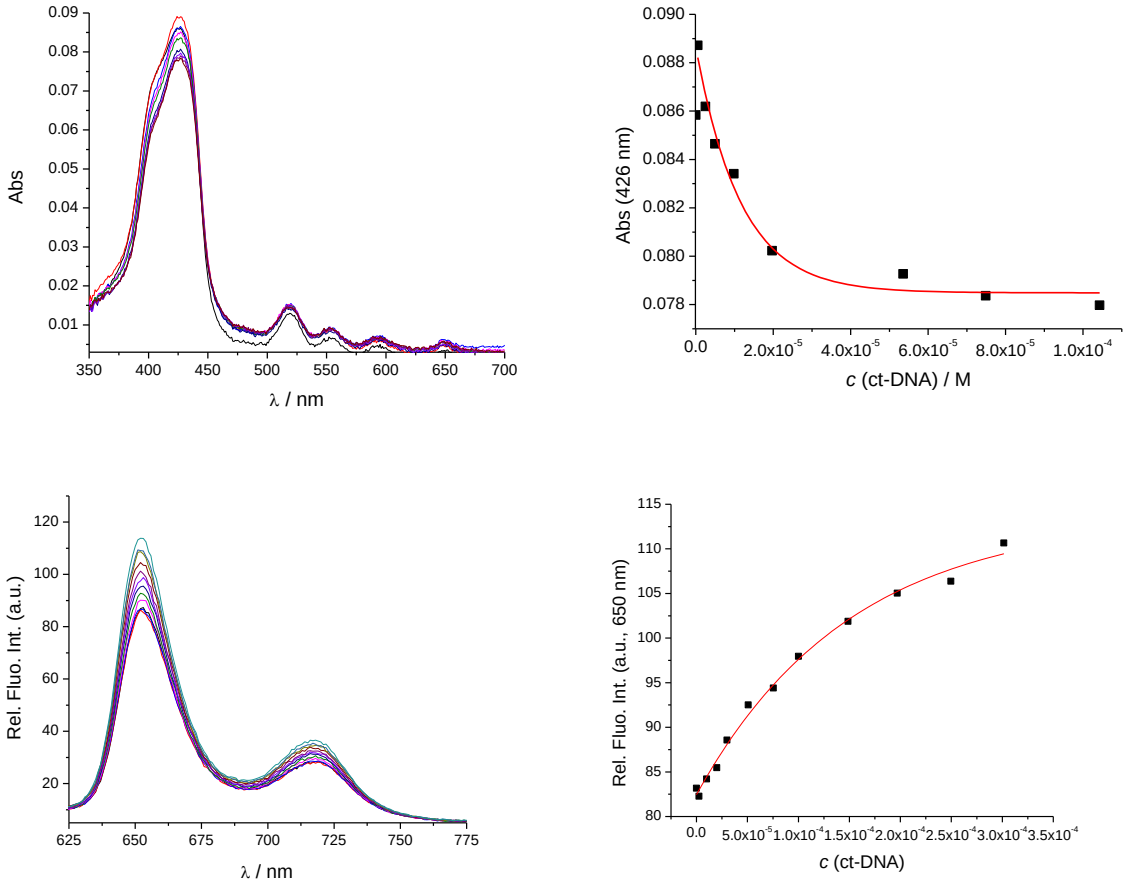


Figure S15. UV/vis and fluorimetric titration of **PoGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 420 nm) with ct-DNA. RIGHT: dependence of fluorescence at λ_{max} = 650 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

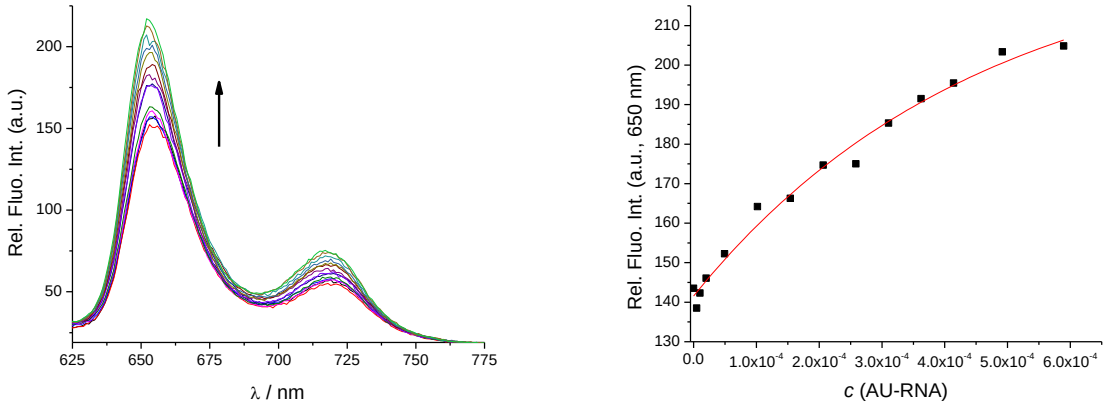


Figure S16. Fluorimetric titration of **PoGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 420 nm) with poly A - poly U. RIGHT: dependence of fluorescence at λ_{max} = 650 nm on c(RNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

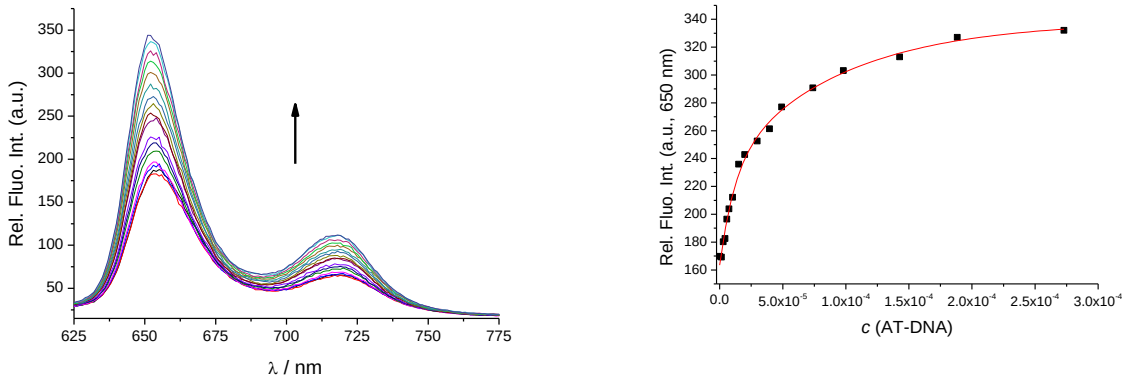


Figure S17. Fluorimetric titration of **PoGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 420 nm) with p(dAdT)$_2$. RIGHT: dependence of fluorescence at λ_{max} = 650 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

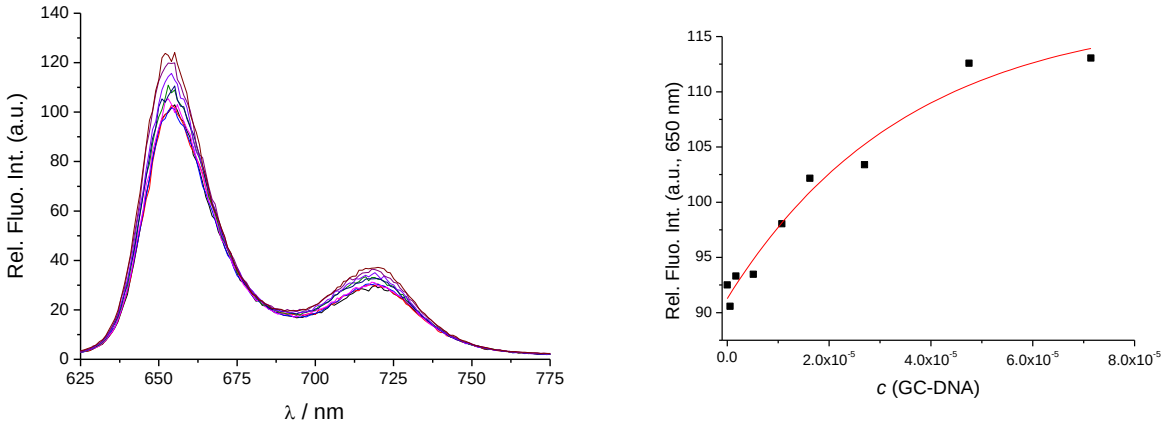


Figure S18. Fluorimetric titration of **PoGU** (c = 1×10^{-6} mol dm^{-3}; λ_{exc}= 420 nm) with **GC-DNA**. RIGHT: dependence of fluorescence at λ_{max} = 650 nm on c(DNA), red line is non-linear least square fitting of Scatchard eq. (McGhee, vonHippel formalism) on experimental data. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

Circular dichroism (CD) experiments

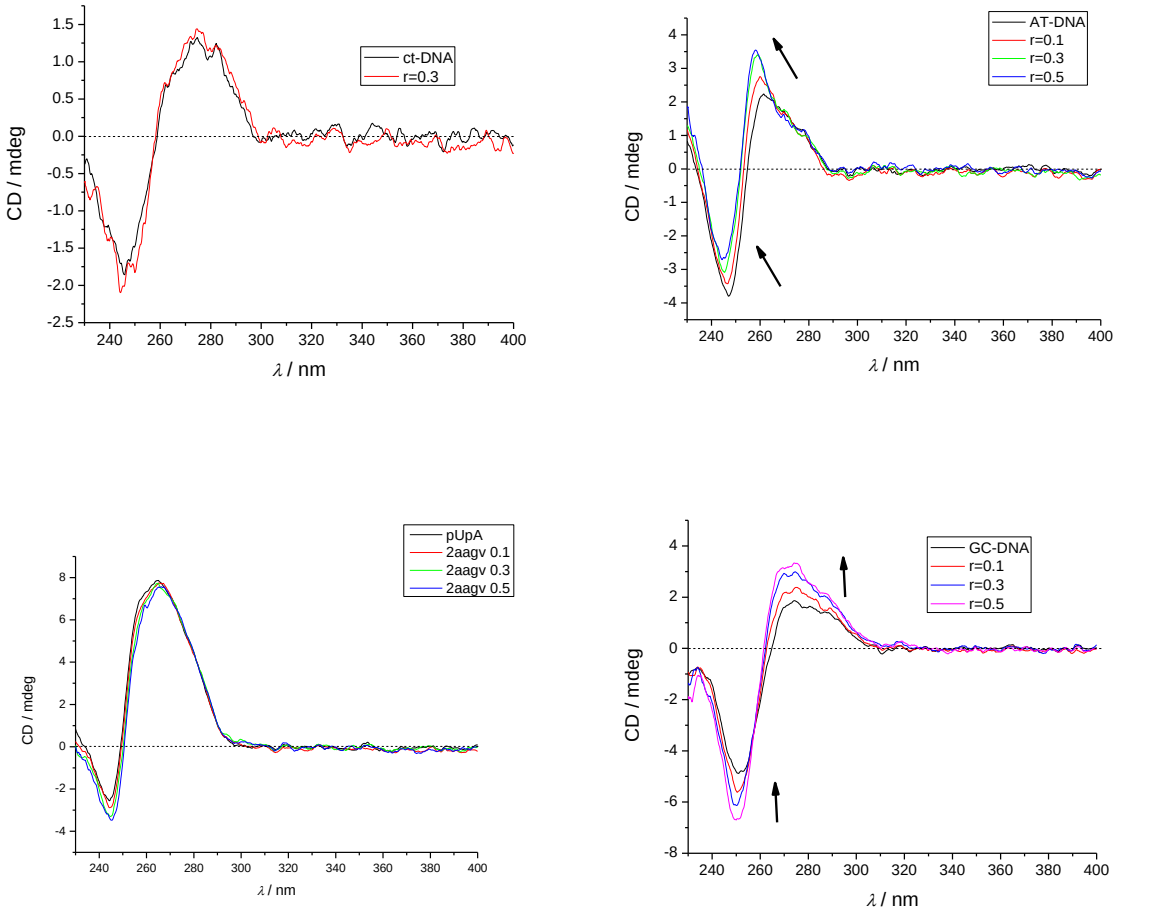
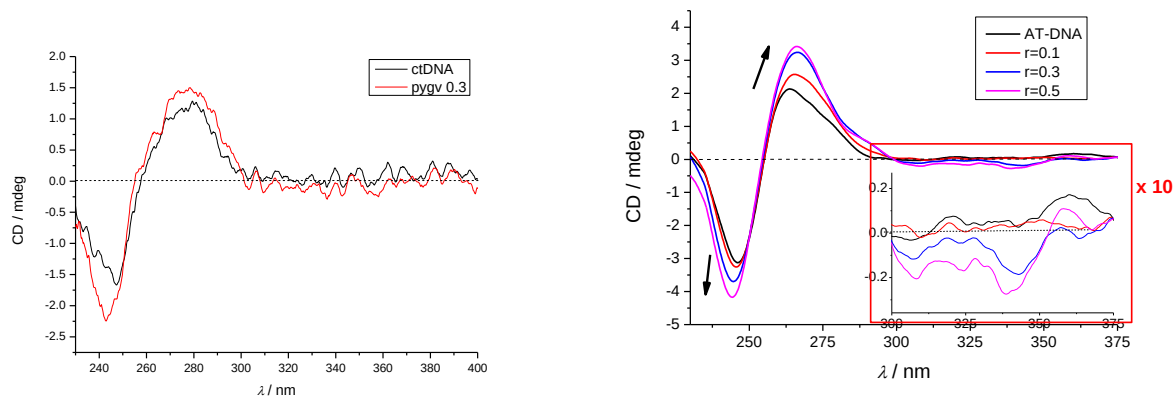


Figure S19. CD titration of ct-DNA, p(dAdT)$_2$, poly A - poly U and GC-DNA (all DNA and RNA c = 2×10^{-5} mol dm^{-3}) with **AGU** at molar ratios $\boldsymbol{r}$ [**AGU**] / [polynucleotide] = 0.1 - 0.5. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.



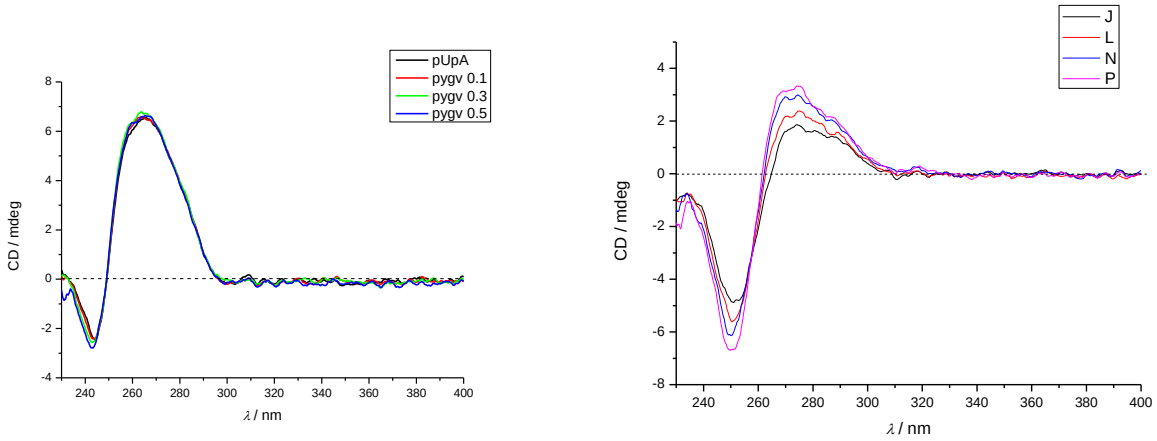


Figure S20. CD titration of ct-DNA, p(dAdT)$_2$, poly A - poly U and GC-DNA (all DNA and RNA c = 2×10^{-5} mol dm^{-3}) with **PyGU** at molar ratios $\boldsymbol{r}$ $_{[\boldsymbol{PyGU}]\ /\ [\text{polynucleotide}]}$ = 0.1 - 0.5. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

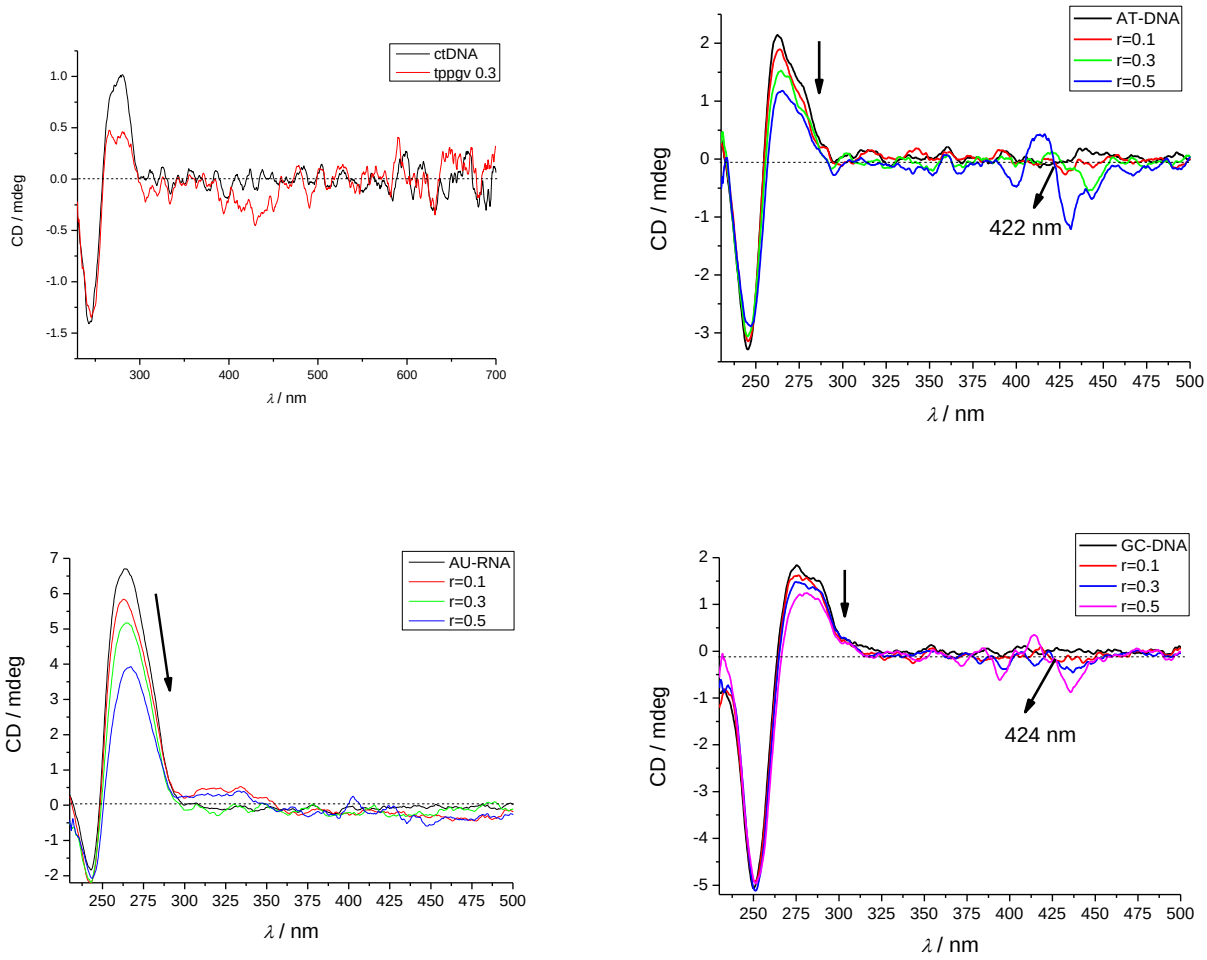


Figure S21. CD titration of ct-DNA, $p(dAdT)_2$, poly A - poly U and GC-DNA (all DNA and RNA c = 2×10^{-5} mol dm^{-3}) with **PoGU** at molar ratios $\boldsymbol{r}$ $_{[\mathbf{PoGU}]\ /\ [\text{polynucleotide}]}$ = 0.1 - 0.5. Done at pH = 7, sodium cacodylate buffer, I = 0.05 mol dm^{-3}.

V. Additional information

Table S2: Groove widths and depths for selected nucleic acid conformations. Note (marked red) the drastic differences in minor groove dimensions between alternating ($(dAdT)_n$, $(dGdC)_n$) and homo-DNA ($d(A)_nd(T)_n$), as well as major groove of ds-RNA.[5]

Structure type	Groove width [Å]		Groove depth [Å]	
	major	minor	major	minor
RNA A_nU_n	**3.8**	10.9	13.5	2.8
[a]B-DNA	11.7	**5.7**	8.5	7.5
$(dGdC)_n$	13.5	**9.5**	10.0	7.2
$(dAdT)_n$	11.2	**6.3**	-	-
$d(A)_nd(T)_n$	11.4	**3.3**	-	-

[a]For instance ct-DNA

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