Guanidiniocarbonyl-pyrrole-aryl derivatives: structure tuning for spectrophotometric recognition of specific DNA and RNA sequences and antiproliferative activity.

Laura Hernandez-Folgado,[a,b] Domagoj Baretić,[c] Ivo Piantanida,*[c] Marko Marjanović,[d] Marijeta Kralj,[d] Thomas Rehm[a] and Carsten Schmuck*[a]

Dedication ((optional))

Abstract: We present here a systematic study of different guanidiniocarbonyl-pyrrole-aryl derivatives designed to interact with DNA or RNA both by intercalation of an aromatic moiety into the base stack of the nucleotide as well as groove binding of a guanidinio-carbonyl pyrrole cation. We varied 1.) the size of the aromatic ring (benzene, naphthalene, pyrene and acridine), 2.) the length and flexibility of the linker connecting the two binding groups, as well as 3.) the total number of positive charges present at different pH values. The compounds and their interaction with DNA and RNA were studied by UV/Vis-, fluorescence and CD spectroscopy. Also the antiproliferative activity against human tumour cell lines was determined. Our studies show that efficient interaction with e.g. DNA requires a significantly large aromatic ring (pyrene) connected via a flexible linker to the pyrrole moiety. However, a positive charge as in **12** is also needed. Compound **12** allows for a base pair selective recognition of ds-DNA at physiological pH. The antiproliferative activity correlates with the binding affinity of these compounds towards DNA suggesting that the biological effect is most likely due to DNA binding.

Keywords: DNA/RNA binding • intercalation • molecular recognition • antiproliferation

Introduction

The search for molecules that specifically interact with DNA and RNA is of current interest for the development of sensors and new drug candidates.[1] A variety of biologically active compounds acts by interacting with DNA/RNA; e.g. netropsin, a potent antiviral and antitumor agent and DAPI, a fluorescent indicator with pronounced antitrypanosomal activity. Such compounds often owe their biological activity to the binding into the DNA minor groove by multiple interactions of cationic functional groups (e.g. guanidinium or amidinium groups) with DNA basepairs, and/or phosphate backbone. However, a strong DNA selectivity of minor groove binders is disadvantageous in cases of RNA targeting. Another common binding mode is intercalation (e.g. echinomycin, anthracyclines), which is the insertion of large aromatic moieties into the base stack of the nucleic acid, characterized by similar affinity toward DNA and RNA. Some compounds actually exhibit a binding mode switch (e.g. intercalation into G-C sequences and groove binding into A-T sequences).[2] The combination of two aromatic units of different but specific spectroscopic properties allows achieving specific spectroscopic responses upon interaction with DNA and/or RNA.[3] However, it was noticed that in many cases very strong binding to DNA (e.g. intercalation of large fused aromatic systems or *bis*-intercalators) severely limits the extravascular distributive properties of such compounds hampering their potential use as drugs, most likely due to the limited solubility of large aromatic groups in aqueous solvents. Therefore, one aim of research in this area is the development of minimal DNA intercalators.[4] Another option is to combine intercalation with groove binding, one area of research that we are currently interested in. Moreover, a recent report on a bis-guanidinium derivative of ethidium which is highly selective for AT-Rich DNA regions demonstrates the potential of hybrid compounds containing both an intercalator and a positively charged group like a guanidinium cation.[5] In addition one such compound was shown to have distinct TAR RNA of HIV-1 antiviral activity.[6]

We recently reported a first example of a guanidiniocarbonyl pyrrole-pyrene hybrid molecule **1**, which contains both an intercalator (the pyrene unit) and a cationic group, capable of groove binding (the guanidiniocarbonyl pyrrole cation).[7] Pyrene is a well known polarity-sensitive probe and its fluorescence is extensively employed to characterize microheterogeneous systems.[8] A long lifetime of the excited state and the possibility of easy excimer formation[9] are distinctive features of the pyrene fluorophore that allow its application for detection of nucleic acid interactions both as a single label[10] and in excimer-forming pairs or as multipyrene probes.[11] Moreover, the flat aromatic structure of the pyrene residue facilitates its stacking with nucleobases.[12] The guanidiniocarbonyl pyrrole cation was expected to undergo multiple non-covalent

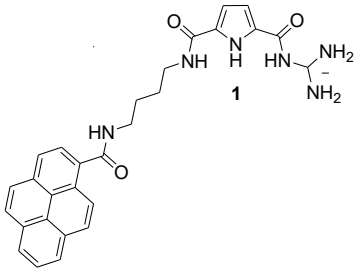
[a] Dr. Laura Hernandez-Folgado, Dr. Thomas Rehm, Prof. Dr. C. Schmuck
Institute for Organic Chemistry
University of Duisburg-Essen
Universitässtrasse 7, 45141 Essen, Germany
Fax: (+) 201 183 4259
E-mail: carsten.schmuck@uni-due.de

[b] Current address: Instituto de Quimica Medica, CSIC. Juan de la Cierva 3, 28006 Madrid, Spain

[c] Domagoj Baretić, Dr. Ivo Piantanida
Division of Organic Chemistry and Biochemistry, "Ruđer Bošković" Institute, P. O. Box 180, HR-10002 Zagreb, Croatia
E-mail: Ivo.Piantanida@irb.hr

[d] Marko Marjanović, Marijeta Kralj
Division of Molecular Medicine, "Ruđer Bošković" Institute, Zagreb, Croatia

interactions (hydrogen bonds and ion pairs) with DNA and RNA. In addition, electrostatic interactions are tuneable by external stimuli since protonation of the acyl guanidinium (pK_a ca. 6-7) is directly correlated to the pH of the aqueous solution.

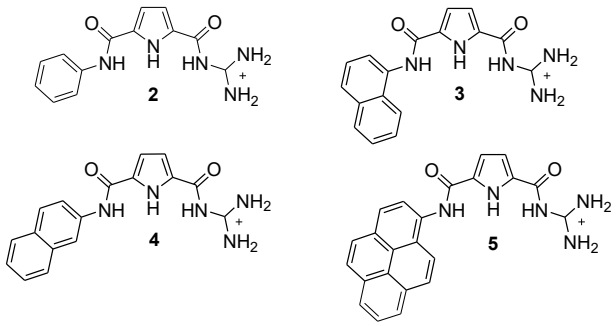


Most interestingly, compound **1** exhibited a very unique and distinctly different spectroscopic interaction with either DNA or RNA. With ds-DNA a strong induced CD signal at about λ = 300 nm was observed, while under the same conditions a new fluorescence maximum at λ = 480 nm appeared exclusively upon the addition of RNA. This different behaviour could be explained by a switch in binding mode. Whereas **1** intercalates its pyrene moiety into ds-DNA while the guanidiniocarbonyl pyrrole cation is binding into the minor groove (giving rise to the ICD signal), with ds-RNA two or more molecules of **1** form a π-stacked excimer most likely binding into the major groove of ds-RNA which gives rise to the strong excimer fluorescence at λ = 480 nm.

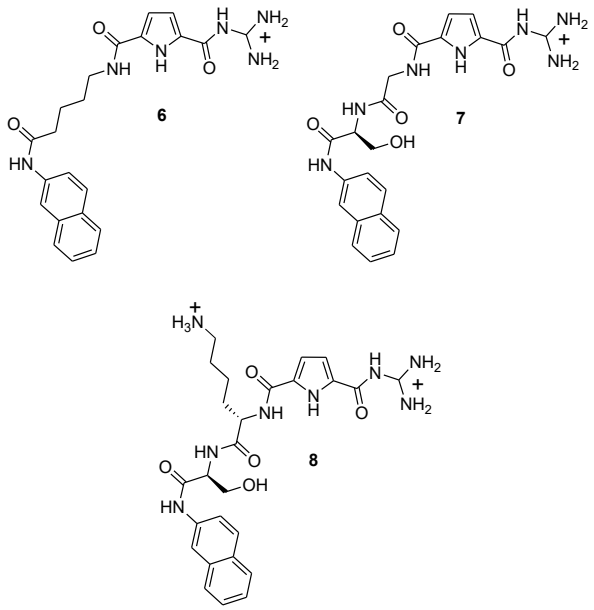
We now report on a systematic study of a series of related hybrid molecules, in which we varied the aromatic unit, the length and rigidity of the linker in between the aromatic and cationic groups or the number of charges present at different pH values. The driving force for the DNA/RNA binding of these compounds in analogy to the results obtained for **1** is assumed to consist of two parts: (1) hydrophobic/dispersive interactions associated with intercalative stacking of the aromatic ring system with the base pairs and (2) the interaction of the guanidiniocarbonyl pyrrole cation within one of the DNA/RNA grooves. While the former part is of predominantly attractive nature, the latter involves both attractive electrostatic interactions, including hydrogen bonding, as well as steric repulsion. In total we examined 13 compounds. We describe here their syntheses and study of interactions with DNA and RNA determined by UV/Vis-, fluorescence and CD spectroscopy as well as some first results on their antiproliferative activities against tumour cell lines. The experimental data clearly indicate a possibility of the fine tuning of small molecule-DNA or RNA interactions and indicate a correlation between the affinity of these small molecules toward DNA and the observed antiproliferative activity.

Results and Discussion

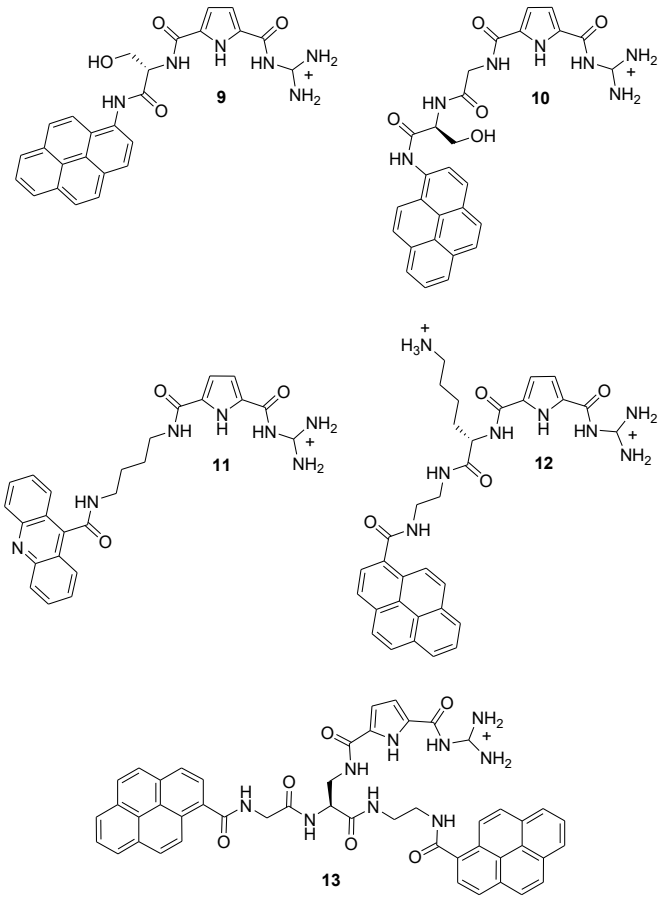
Design of the compounds: First, within the series **2** - **5** we directly connected an aromatic unit of increasing size to the guanidiniocarbonyl pyrrole cation.



Compounds **6**, **7** and **8** all contain a naphthalene unit similar to **4** but with an additional linker in-between the naphthyl-moiety and the guanidiniocarbonyl pyrrole cation to ease structural accommodation of the molecule within the double stranded helix of the polynucleotide. We varied the rigidity and polarity of the linker (flexible and non-polar in **6** and more rigid and polar in **7** and **8**) and the number of positive charges (**8** has one positive charge more than the other two compounds). The linkers in **7** and **8** are dipeptides, Gly-Ser for **7** and Lys-Ser for **8**, respectively. The amino acids were chosen to provide additional sites for potential interactions with the nucleic acids (by H-bonds to the amide groups e.g.) as well as to increase the solubility (serine, lysine) of the compounds.



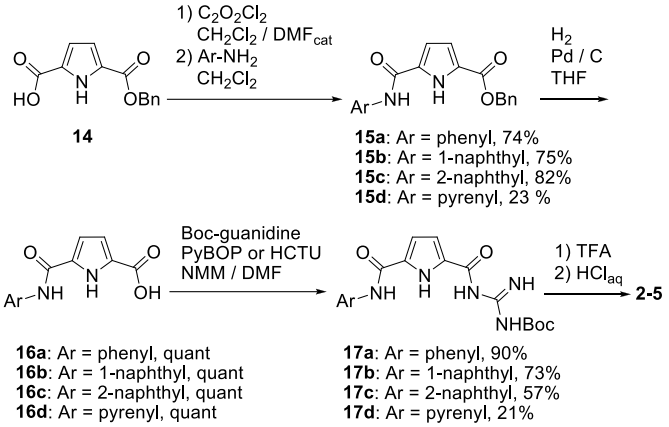
Furthermore, five compounds with either a pyrene or acridine moiety were synthesized in order to increase their affinity toward DNA/RNA (relative to the naphthalene based compounds) and to also take advantage of their superior fluorescent properties, whereby a pyrene moiety was chosen due to its strong fluorescence which could allow a selective and highly sensitive recognition of specific DNA/RNA structures.



9 and **10** are derived from amino pyrene and as a linker contain either serine or the dipeptide Gly-Ser. **11** is the acridine analogue of **1**, whereas **12** is again a pyrene derivative with a semi flexible linker and one additional positive charge (due to the lysine). **13** was designed as potential bis-intercalator.

Synthesis of the compounds: The synthesis of **1**[7] and **8**[13] were previously reported. Compounds **2-5** were synthesized according to scheme 1. Due to their low nucleophilicity the aryl amines used were coupled via the acid chloride to the starting compound **14**. After hydrogenolysis of the benzyl ester in **15a-d** with palladium on charcoal the free acid was activated with either PyBOP [(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate] (**16a-c**) or HCTU [*O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate] (**16d**) and reacted with *N*-Boc-protected guanidine yielding the protected compounds **17a-d**. Finally, trifluoroacetic acid (TFA) was used for the deprotection of the acylguanidines. After lyophilization from methanol and hydrochloric acid the compounds **2-5** could be obtained as chloride salts.

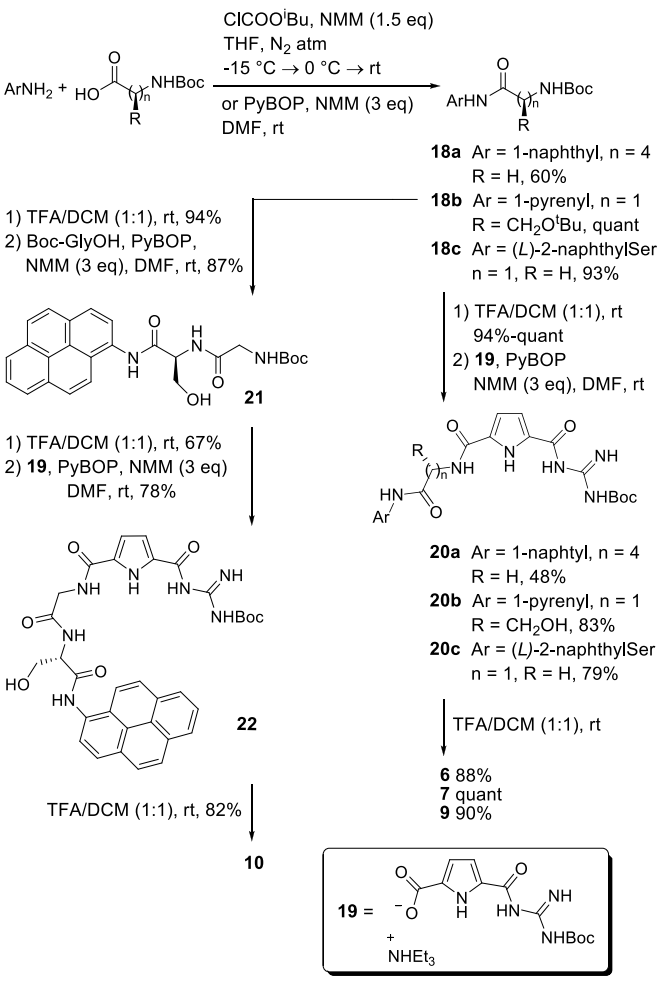
The syntheses of the new guanidiniocarbonyl-pyrrole-aryl hybrid compounds **6** - **12** were achieved by two different procedures described in Schemes 2 and 3. The procedure starting from aryl amines (considering (*L*)-*N*-2-naphthyl-serinamide also as an aryl amine) is shown in scheme 2: 1-naphthylamine and 1-pyrenyl-amine were coupled to 5-[(*tert*-butoxycarbonyl)amino]-pentanoic acid[14] and (*L*)-*N*-(*tert*-butoxycarbonyl)serine respectively, using isobutyl chloroformiate as acid activator. **18c** was prepared from (*L*)-*N*-2-naphthylserinamide, commercially available, with PyBOP as coupling reagent. Then, the Boc-protecting groups in **18a-c** were removed quantitatively with TFA. The resulting free amines were reacted without further purification with *N*-Boc-5-guanidinocarbonylpyrrole-2-carboxylate (**19**), prepared according to literature procedures.[15] Final Boc-deprotection of **20a-c** was carried out again with TFA, providing the guanidiniocarbonyl-pyrrole-aryl derivatives **6**, **7** and **9** in high yields as trifluoroacetate salts. The resulting free amine from **18b** was also coupled first to *N*-(*tert*-butoxycarbonyl)glycine (87% yield) and then to **19** (78% yield). Afterwards, the Boc-deprotection of **22** under acidic conditions led to the guanidiniocarbonyl-pyrrole-pyrenyl derivative **10**.



Scheme 1. Synthesis of the 1st intercalator generation **2-5**.

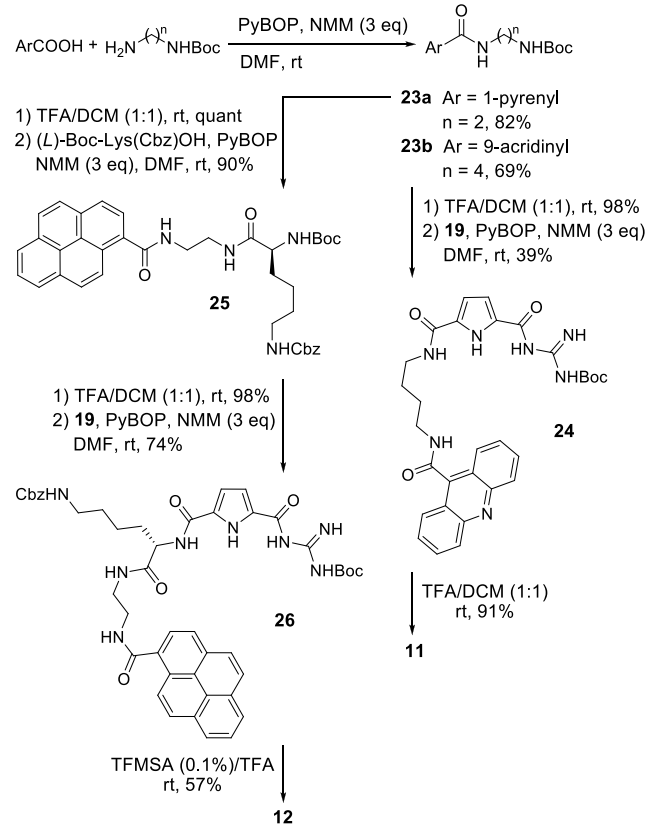
Preparation of compounds **11** and **12** is outlined in scheme 3. In this case starting materials were aryl acids (1-pyrenecarboxylic acid and 9-acridinecarboxylic acid), which were coupled to Boc-monoprotected alkyl diamines[16] by PyBOP activation. Afterwards **23a-b** were the subject of amine deprotection and consecutive amide-bond formation using PyBOP standard coupling conditions in a similar way as it was described above. Intermediates **24-26** were thus obtained in good yields. **24** was easily deprotected, affording **11** with high yield. However, deprotection of **26** was not so trivial. A mixture of 0.1% of trifluoromethanesulfonic acid (TFMSA) in TFA was used for the deprotection of both the Cbz-protected amine and the Boc-protected guanidine simultaneously, following our own previously reported results.[7] This procedure gave **12** in moderate yields of 57%, requiring RP18-chromatography to purify it from decomposition byproducts.

Synthesis of guanidiniocarbonyl-pyrrole-diaryl derivative **13** was achieved by a convergent procedure according to scheme 4. Preparation of intermediate **29** was performed in moderate yield by PyBOP activation couplings and acid Boc-deprotection. 1-pyrenecarboxylic acid, *N*-(*tert*-butoxycarbonyl)glycine and the arginine analogue **28**[17] were used as starting materials. Afterwards the methyl ester in **29** was deprotected quantitatively by basic hydrolysis. Then, the free acid was coupled to the free amine of **23a**, affording the diaryl Boc-protected intermediate **30** in moderate yield. Final deprotection led to **13** in 43% yield after reverse phase chromatographic purification.

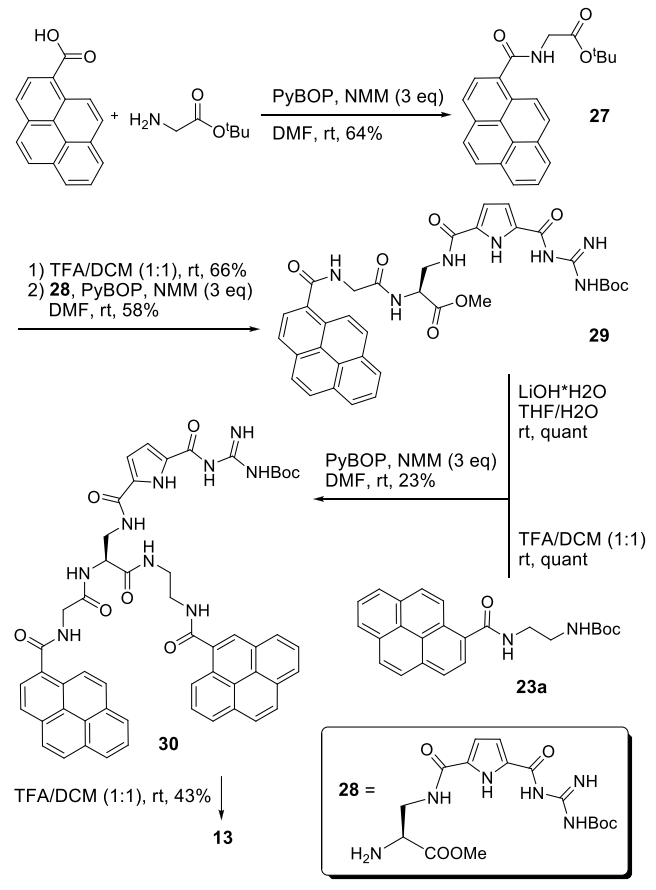


Scheme 2. Synthesis of **6**,**7**, **9** and **10**

Physico-chemical properties of the compounds in aqueous solution: Compounds **2** - **5** have only a limited solubility in water, namely **2** - **4** could be dissolved at $c \approx 3\text{-}4 \times 10^{-4}$ mol dm^{-3}, and a stock solution of **5** was prepared in DMSO (c = 0.01 mol dm^{-3}) and then diluted in water or buffer up to c(**5**)$\approx 1 \times 10^{-5}$ mol dm^{-3}. Introduction of a spacer significantly improved the water solubility of pyrene and acridine derivatives **1**, **6**, **7**, **11** and **13** ($\approx 10^{-4}$ mol dm^{-3} range), **8** and **12** (up to c=1.0 $\times 10^{-3}$ mol dm^{-3}). Compounds **4**, **9** and **10** decomposed after few hours in aqueous solution at room temperature or upon heating at 90 ^{0}C for several minutes as indicated by changes in the UV spectra, which excluded them from further studies. Aqueous solutions of all other compounds were stable, not showing any sign for decomposition upon standing for several days at room temperature or upon heating to 90 °C for at least 1 hour. The insufficient stability of aqueous solutions of **9** and **10** compared e.g. to **12** or **13** is most likely due to the fact that amino pyrene is a rather good leaving group in nucleophilic displacement reactions. Furthermore, the nearby serine OH group can intramolecularly assist in the cleavage via the intermediate formation of a β-lactone. In **12** or **13**, derived from pyrene carboxylic acid, the direction of the amide bond is reversed and thus the compound is much more stable.



Scheme 3. Synthesis of **11** and **12**



Scheme 4. Synthesis of bis-pyrene derivative **13**.

UV-Spectroscopy: Absorbencies of aqueous solutions of all compounds are proportional to their concentrations up to $c = 5 \times 10^{-5}$ mol dm^{-3} (1×10^{-5} mol dm^{-3} for **5** due to its limited solubility). Hence, in the concentration range needed for the following spectroscopic studies no significant intermolecular aggregation of the compounds occurred, which should give rise to hypochromicity effects. Absorption maxima and the corresponding molar extinction coefficients (ε) are given in Table 1.

Table 1. Electronic absorption maxima and corresponding molar extinction coefficients in aqueous medum,[a] fluorescence emission maxima and corresponding relative quantum yields (Q).[b]

	UV/Vis	Fluorescence emission		
	λ_{max} / nm ($\varepsilon \times 10^3$ /dm^3 mol^{-1} cm^{-1})	λ_{max} / nm	[b]Q pH 5	pH 7
2	308 (20.6)		0	0
3	301 (30.2)		0	0
5	231 (20.1); 284 (18.4); 343 (10.0)		>0.01	>0.01
6	294 (21.0)		>0.01	>0.01
7	243 (30.6); 295 (24.4)		>0.01	>0.01
8	243 (27.4); 295 (22.7)		>0.01	>0.01
1	242 (24.2); 276 (38.1); 303 (28.1); 342 (20.4)	382	0.03	0.04
11	250 (88.3); 300 (25); 360 (8.4)	425	0.06	0.04
12	231 (58.17); 242 (48.2); 276 (33.7); 307 (28.6); 344 (18.2); 377 (1.8)	387	0.01	0.02
13[c]	278 (26.2); 345 (19.3)	401	0.04	0.07

[a] Buffer pH = 7 (sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}). [b]Relative quantum yield (Q) was determined with respect to standard N-acetyl-L-tryptophanamide (NATA) Q = 0.14, excitation wavelength was underlined in the UV/Vis data.; [c]Determined at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}).

The guanidiniocarbonyl pyrrole moiety in all compounds absorbs at λ = 284 – 308 nm. It is interesting to note that the absorption maxima of the aryl-moieties (benzene, naphthalene, pyrene, acridine) and the absorption maximum of the pyrrole are overlapping for **2**, **3**, **6** and **5** but are well separated for **1**, **7**, **8**, **11** and **12**, most likely due to the pronounced conjugation of both groups in the former compounds, which is prevented by the additional linker in latter structures. Interestingly, for **13** the absorption maxima of the pyrene and the guanidiniocarbonyl pyrrole moieties are also overlapping despite the long linker. Furthermore, although **13** contains two pyrene subunits the ε value at the pyrene maximum (λ = 345 nm) is similar to the values of **1** and **12** which contain only one pyrene. Both observations strongly suggest an intramolecular aromatic stacking interaction between the two pyrene units of **13**.

For all compounds except **11** the UV/Vis spectra in buffer at pH 7 and pH 5 were the same as in pure water. This was unexpected since the protonation state of the guanidiniocarbonyl pyrrole moiety was expected to be different at pH 5 and pH 7. However, the pyrrole unit shows only a very weak absorbance, the main absorbance is due to the naphthalene or pyrene units, which are not affected by the protonation of the guanidine. Furthermore, weak pH dependent changes in the UV/Vis spectrum of **11** within the range attributed to the acridine moiety allowed estimation of a pK_a < 6 for protonation of the acridine.

Fluorescence spectra: The naphthalene derivatives (**6**, **7**, **8**) exhibited only very weak fluorescence in aqueous media. In contrast, pyrene and acridine derivatives, **1**, **11**, **12** and **13** showed strong fluorescence emission, linearly dependent on the concentration of the compound in water up to $c = 5.0 \times 10^{-6}$ mol dm^{-3} (**5** was not studied due to its low solubility). At higher concentrations the increase of fluorescence emission became non-proportional due to inner filter effects. For the bis-pyrene compound **13** the fluorescence emission is significantly stronger in comparison to the emission intensity of the mono-pyrene derivative **12** multiplied by two (Figure 1), which again confirms an intramolecular aromatic stacking interaction between the two pyrene units as was already suggested based on the differences in the UV/Vis spectra.

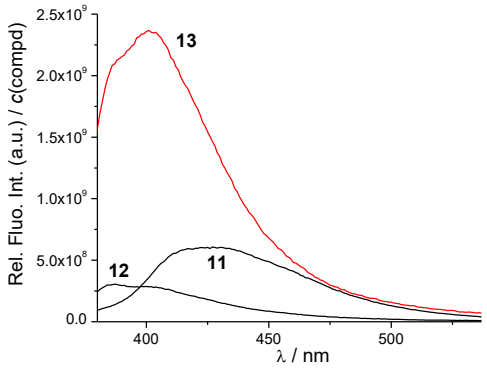


Figure 1. Fluorescence emission spectra of **11** (λ_{exc}=360 nm), **12** (λ_{exc}=344 nm), **13** (λ_{exc}=345 nm) collected under the same instrument setup, at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}).

Protonation state: The fluorescence of **11**, **12** and **13** was found to be weakly pH dependent in the range pH = 5-8, which was attributed to protonation of the guanidine group, and allowed an estimation of the p$Ka \approx 5.5$ - 6. The fluorescence of acridine of **11** cnage considerably between pH=3-5 (pKa = 4.1), thus **11** is neutral at pH 5. Hence, at pH 7 the guanidine moiety is not yet protonated so that all compounds except **8** and **12** are present in their neutral form whereas they are expected to be positively charged at pH 5. Compounds **8** and **12** possess an additional amino group in the side chain with p$Ka \approx 8$, and therefore at pH 7 have already one positive charge.

Interactions with polynucleotides in aqueous medium

Thermal denaturation of ds-DNA and ds-RNA: The experiments were performed at pH 7 and pH 5 since it was expected that the different protonation state of the compounds could have a significant impact on their interactions with DNA and RNA. At pH 7 (buffer Na cacodylate, $I = 0.05$ mol dm^{-3}) most of the compounds at ratio $r_{[compound] / [polynucleotide]} = 0.3$ or even higher ($r_{2 \text{ or } 3} = 1$) did not show any influence on the T_m value of the ct-DNA (calf thymus DNA). The only exception is a weak stabilisation ($\Delta T_m = 0.8$ °C at r = 0.3) of ct-DNA by **12**, which is the only compound positively charged also at pH 7 and possessing a large aromatic moiety (pyrene).

However, at pH 5 all compounds are positively charged due to protonation of the guanidine moiety and consequently the results

were significantly different (Figure 2). Compounds with small aromatic moieties (benzene, naphthalene) connected via short and rigid linkers to the guanidiniocarbonyl pyrrole (**2, 3**) did not stabilize ds-DNA and ds-RNA at all. Due to the larger aromatic moiety (pyrene) **5** weakly stabilized ct-DNA (ΔT_m = 1.0 °C at r = 0.3), while the introduction of a longer and more flexible linker between the guanidiniocarbonyl pyrrole and the pyrene (**1**, **12**) additionally increased stabilization of ds-DNA (Table 2). The highest ΔT_m value was obtained for **12** (ΔT_m = 9.7 °C), which showed that the additional positive charge of **12** (which **1** does not have) increased the affinity for ds-DNA. Most surprisingly, the *bis*-pyrene compound **13** also did not stabilize any of the polynucleotides studied, most likely because the intramolecular stacking of the two pyrene rings prevented the intercalation of **13** into the polynucleotide. The acridine derivative (**11**) stabilized ct-DNA less than the analogous pyrene compound **1** in agreement with the smaller aromatic surface of the former compound. Interestingly, even the naphthalene derivative **8** stabilized ct-DNA more efficiently than **11**, but this again most likely reflects the one additional positive charge present only in **8**. A more detailed analysis of those compounds which show significant stabilisation of ds-DNA revealed a strongly non-linear relation between ΔT_m values and ratio ***r*** = [compound] / [ds-DNA], pointing toward saturation of binding sites at about r = 0.2-0.3. The stabilisation effect of **11** and **12** on poly dA-poly dT was even more pronounced than for ct-DNA, most likely due to stronger interactions of the compounds within the narrower and deeper minor groove of the former polynucleotide.[19] However, the effect of the compounds studied on ds-RNA (poly A – poly U) was much weaker. Only **8** led to stabilization whereas **7** and **1** actually weakly destabilized RNA. This observation suggests that most of the compounds do not intercalate into ds-RNA.

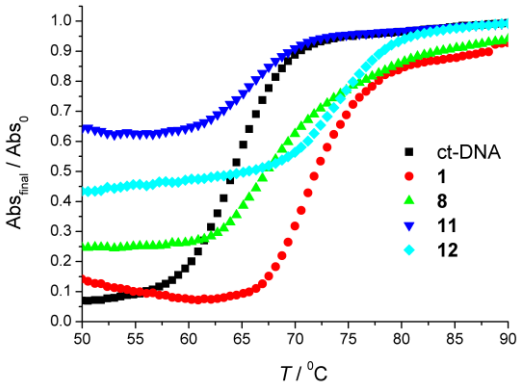


Figure 2. Thermal denaturation curves of ct-DNA (c=2×10^{-5} mol dm^{-3}) at pH 5 (sodium citrate buffer, I=0.03 M) upon addition of **1**, **8**, **11**, **12**. For measuring conditions, see Table 2 and the Experimental Section.

Table 2. ΔTm values[a] (°C) of various ds- polynucleotides upon addition of studied compounds at ratio [b]r = 0.3, pH = 5.0 (sodium citrate buffer, I = 0.03 mol dm^{-3}).

	7	[c]**1**	**8**	**11**	**12**	**13**
ct-DNA	0	+7.2	+3.4	+2.2	+9.7	0
poly dA-poly dT	[d]	[d]	[d]	+4.0	+11.7	0
[c] poly A- poly U	-1.0	-1.5	+3.9	<1.0	<1.0	0

[a] Error in ΔTm : ± 0.5°C; [b] ***r*** = [compound] / [polynucleotide]. [c]Biphasic transitions: the first transition at Tm = 30.3 °C is attributed to denaturation of poly A-poly U and the second transition at Tm = 85.8 °C is attributed to denaturation of poly AH$^+$-poly AH$^+$ since poly A at pH = 5.0 is mostly protonated and forms ds-polynucleotide.[18,19] For all compounds second transition stabilisation was 0. [d]Not determined. [e] Previous results.[7]

Fluorimetric titrations: At this point we have focused for all further studies on those compounds (**8**, **11**, **12**) which showed measurable thermal stabilisation effect (Table 2), or at least acceptable solubility and promising structure in respect to single stranded polynucleotides (**13**). Although all compounds possess UV/Vis bands at λ > 300 nm, UV/Vis titrations were not applicable to study their interactions with ds-polynucleotides as for example the addition of ct-DNA yielded only very small changes in their UV/Vis spectra, hampering accurate quantitative analysis. Except for **8** all other compounds showed strong fluorescence which allowed titration studies at low concentrations. The fluorescence changes were remarkably dependent on the type of polynucleotide added as well as on the pH of the solution (Table 3).

Table 3. The spectroscopic properties[a] of complexes of studied compounds with ds-polynucleotides observed in fluorimetric titrations at pH = 5 (sodium citrate buffer, I = 0.03 mol dm^{-3}) and pH = 7 (sodium cacodylate buffer, I = 0.05 mol dm^{-3}).

	ct-DNA		poly dAdT-poly dAdT		poly dGdC-poly dGdC		poly dA – poly dT		poly A – poly U	
	pH5	pH7	pH5	pH7	pH5	pH7	pH5	pH7	pH5	pH7
11	[b]0.5	[b]0.6	[b]0.5	[c]0.8	[b]0.5	[c]0.7	[b]0.7	[c]0.7	[b]0.8	[b]0.6
12	[b]0.7	[b]0.7	[b]8.9	[b]6.3	[b]0.3	[b]0.5	[b]8.8	[c]0.8	[b]3.4	[c]0.8
13	[c]0.9	-	[b]0.7	-	[b]0.7	-	1	-	[c]0.9	-

[a]Emission change; I = I(complex) / I(compd).; [b] I(complex) obtained from Scatchard analysis of titration data for correlation coeff. r<0.999 (error of I value <1%); [c] I(complex) estimated from titration data since Scatchard analysis was not possible due to small changes, changes in opposite directions or linear change abruptly ends at defined intensity; consequently error of I value 5-10%.

At pH 7, only **11** and **12** did not precipitate upon addition of DNA and/or RNA. Whereas the fluorescence of the acridine derivative **11** was quenched by the addition of any of ds-DNA and ds-RNA, the emission of **12** was strongly quenched by ds-DNAs containing G-C base pairs, while addition of alternating poly dAdT - poly dAdT resulted in a strong fluorescence increase. Most intriguingly, addition of the homo-polynucleotide poly dA- poly dT as well as a RNA analogue (poly A – poly U) induced weak fluorescence quenching of **12**.

At pH 5 results of the fluorimetric titrations were significantly different from those at pH 7. For compound **1** we previously found[7] that at pH 5 first agglomeration along the DNA takes place (r > 0.14) which leads to quenching of fluorescence, followed by intercalation of the pyrene into ds-DNA (r < 0.1) accompanied by strong fluorescence increase. However, upon addition of ds-RNA compound **1** revealed a new, specific emission maximum at 480 nm (Figure 3 top), attributed to pyrene excimer formation within the major groove of the RNA.[7] Compound **11**, which is the acridine analogue of **1**, showed only a strong, non-selective quenching upon addition of any ds-DNA or ds-RNA (Figure 3 bottom), confirming that the electronic properties of the pyrene moiety are responsible for the specific dual fluorimetric response of **1** towards ds-DNA and ds-RNA.

The specific fluorimetric response of **1** upon addition to ds-RNA (new maximum at 480 nm)[7] was not observed for its analogue **12**. However, compound **12** came up with a new feature (fluorimetric differentiation between basepair composition of polynucleotides), which was not observed for **1**. Namely, at pH 5 the fluorescence of **12** was strongly quenched by any G-C base pair containing polynucleotide, while polynucleotides with only A-T or A-U base

pairs induced a strong fluorescence increase (Table 3, Figure 4). Such a fluorimetric sensing was previously reported for proflavine[20] and some 4,9-diazapyrenium cations[21] and it was attributed to the guanine-induced fluorescence quenching, because guanine is more easily oxidised than any other nucleobase and can thus efficiently quench the fluorescence of an electron-accepting fluorophore. This quenching can occur either by direct aromatic stacking interactions with guanine or by remote G sites via electron-transfer through the π-stacked DNA helix.[22] Both quenching modes require that the fluorophore is efficiently stacked within the DNA double helix. Since the pyrene moiety of **1** and **12** is intercalated into DNA it seems that basepair differentiation by **12** but not **1** is the consequence of different orientation of the pyrene within the DNA double helix most likely due to a steric influence of the bulky linker with its positively charged side arm in **12**.

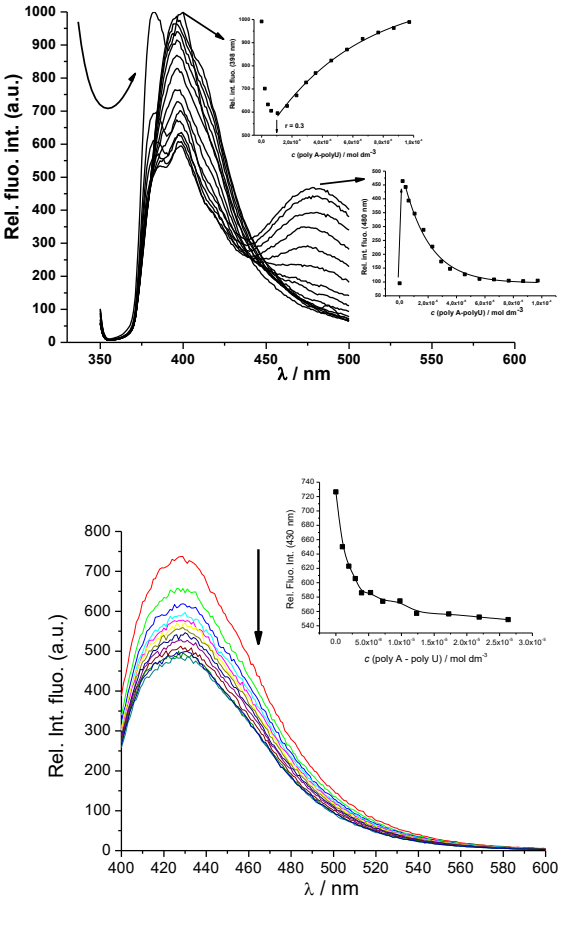


Figure 3. Fluorimetric titration with poly A-poly U at pH 5 (sodium citrate buffer, I=0.03 M): Top) **1** (c=3.3×10^{-6} mol dm^{-3}, λ_{exc}= 320 nm); Bottom) **11** c=2.0×10^{-6} mol dm^{-3}, λ_{exc}= 320 nm).

The fluorescence of the bis-pyrenyl-derivative **13** was also quenched by any G-C base pair containing polynucleotide, and increased by alternating poly dAdT - poly dAdT. However, negligible fluorescence changes were observed upon addition of homo-polynucleotides (poly dA – poly dT or poly A – poly U). Such a sensitivity of fluorescence response can be attributed to the specific properties of polynucleotide secondary structure. Namely, alternating polynucleotides adopt a B-helical structure[19] and most likely bulky compound **13** fits tightly within the minor groove. However, poly dA – poly dT is characterized by a peculiar twisted structure possessing a very narrow minor groove,[23] while poly A – poly U forms a A-form double helix, characterized by a shallow and broad minor groove[19] – in both cases it seems that binding of **13** was not supported.

All titrations with fluorescence changes above ≈ 10% were processed by means of the Scatchard equation[24] to obtain the binding constants and ratio $n_{[bound\ compound]\ /\ [polynucleotide]}$ (Table 4). At pH 7 due to small changes or precipitation only few titration experiments were applicable for processing, thus no comparison of the results for the various compounds was possible. However, at pH 5 for most of the titrations binding constants could be calculated and the obtained values of logK_s=5 – 6 reveal similar affinity of all compounds toward all studied polynucleotides. However, in some cases (e.g. **13**/ poly dGdC- poly dGdC complex) the high value of ratio n>0.5 strongly supports agglomeration of molecules along DNA or RNA double helix, thus the corresponding logK_s values should be considered as a cumulative affinity resulting from more than one binding mode.

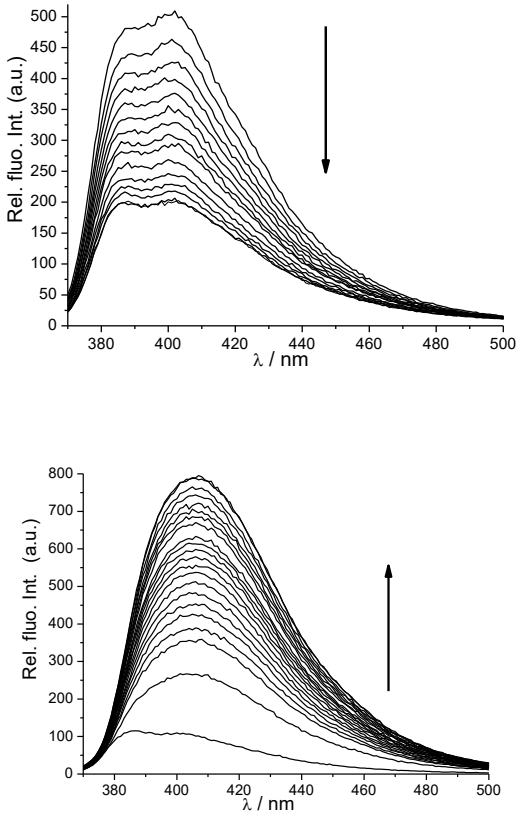


Figure 4. Fluorimetric titration of **12** at pH 5 (sodium citrate buffer, I=0.03 M): Top) poly dGdC-poly dGdC (c(**12**)=5.0×10^{-6} mol dm^{-3}, λ_{exc}= 350 nm); Bottom) poly dAdT - poly dAdT (c(**12**)=1.0×10^{-6} mol dm^{-3}, λ_{exc}= 350 nm).

Since the UV/Vis and fluorimetric titrations were not applicable to study the interaction of **8** with polynucleotides, as an alternative method for estimation of affinity we have performed an ethidium bromide (EB) displacement assays. This allows at least comparing the ability of **8** to compete for binding with a classical intercalator already bound to DNA. It should be taken into account that the applied ratios $r_{[8]/[polynucleotide]}$ and the concentration range of **8** and polynucleotides used in this displacement assay are comparable with those of the thermal denaturation experiments in which **8** showed a

distinct stabilization of both ds-DNA and ds-RNA (Table 2). We have also performed experiments with poly dA – poly dT and poly G – poly C but partial precipitation in the course of titration hampered accurate processing of the results. However, the obtained IC_{50} values show that a significantly higher concentration of **8** compared to *c*(EB) was needed to displace 50% of EB from both ct-DNA and poly A – poly U. From these results an estimate for the affinity of **8** could be derived using equation 1, by using the logK_s (**EB**) value determined previously under the same experimental conditions.[25]

$$\log Ks\ (\mathbf{8}) = \log K_s\ (\mathbf{EB}) \times IC_{50}\ \text{value} \qquad \text{Eq. 1}$$

Thus, binding constants of **8** toward ct-DNA and poly A - poly U are estimated to be about log$K_s \approx 5$.

Table 4. Binding constants (logK_s), (in parentheses ratios $\boldsymbol{n}$[bound compound]/[polynucleotide]) of studied compounds with ds-polynucleotides calculated from fluorimetric titrations at pH = 5 (sodium citrate buffer, I = 0.03 mol dm^{-3}) and pH = 7 (sodium cacodylate buffer, I = 0.05 mol dm^{-3}).[a]

	logKs (***n***)									
	ctDNA		poly dAdT-poly dAdT		poly dGdC-poly dGdC		poly dA - poly dT		poly A – poly U	
	pH5	pH7	pH5	pH7	pH5	pH7	pH5	pH7	pH5	pH7
1[7]	[d]5.9 (0.1)	[e] 6.0 (0.5)	-	-	-	-	[d]6.8 (0.1)	[e] 5.1 (0.8)	[d]6.3 (0.1)	[e] 5.1 (3.6)
11	6.2 (0.2)	4.4 (0.2)	>6[b]	c	5.9 (0.2)	>6[b]	5.9 (0.2)	>6[b]	[e]6.5 (0.5)	[e] 6.5 (1)
12	[e] 6.1 (0.5)	[e] 6.8 (1.7)	6.5 (0.3)	6.6 (0.2)	6.3 (0.2)	5.7 (0.2)	c	c	5.4 (0.2)	c
13	c	-	6.5 (0.2)	-	[e]5.3 (0.5)	-	c	-	c	-

[a] Titration data were processed using Scatchard equation,[24] accuracy of obtained $\boldsymbol{n}$ ± 10 - 30 %, consequently log K_s values vary in the same order of magnitude; [b] Linear change abruptly ends at r = 0.3 - 0.1, suggesting logKs>6; [c] Too small changes or accurate calculation; [d] Data calculated by Scatchard equation from the second part of titration experiment with polynucleotide in which fluorescence of primarily formed complex **1**/polynucleotide was enhanced by formation of secondary complex.; [e] Too high n value suggests agglomeration.

Fluorimetric titrations of 11, 12 and 13 with single stranded (ss-) polynucleotides: The fluorescence emissions of **12** and **13** were highly sensitive to the base pair composition of the ds-polynucleotide (Table 3). To study in more detail the role of each nucleobase on the fluorescence of these two compounds (and of the acridine derivative **11** as a reference), we performed a series of titrations with single stranded homo-polynucleotides. The fluorescence of **13** was not changed significantly by any studied ss-polynucleotide, while **11** and **12** revealed quite specific fluorimetric responses to some polynucleotides. At pH 5, most intriguingly, only addition of poly A yielded a strong increase of fluorescence of **12** (Figure 5), while other polynucleotides either completely (poly G) or partially (poly U, poly C) quenched the emission. However, at pH 7 no fluorescence change was observed for **12** upon addition of any polynucleotide. It should be stressed that only at pH 5 (not at pH 7) poly A is protonated and readily forms a double stranded helix of poly AH$^+$ - poly AH$^+$,[19] thus, the observed fluorescence specificity is actually related to interaction of **12** with a protonated adenine – adenine double strand. Since **12** did not stabilize poly AH$^+$ - poly AH$^+$ in thermal denaturation experiments (second transition of poly A – poly U in Table 2), intercalation of the pyrene subunit most likely can be excluded. Therefore, the observed fluorescence specificity could be attributed to the specific orientation of **12** within poly AH$^+$ - poly AH$^+$ grooves (hence the different binding compared to poly G, poly U, poly C), which allowed non-covalent contacts of **12** with the polynucleotide and/or interactions between the pyrene subunits of two or more molecules of **12**.

Furthermore, the fluorescence of the acridine derivative **11** was quenched by addition of any ss-polynucleotide studied. This absence of selectivity again stresses the importance of pyrene as a polarity sensitive fluorescence probe. Due to the small emission changes only a few fluorimetric titrations of **11** and **12** resulted in emission changes suitable for processing by means of the Scatchard equation[24] and obtained logK_s values (for pH 5 logK_s = 5 - 6, for pH 7 logK_s = 4 - 6). Hence, the affinity of these compounds to towards ss-polynucleotides is rather high.

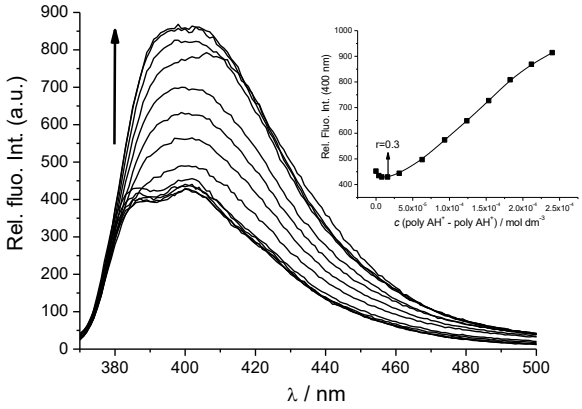


Figure 5. Fluorimetric titration of **12** with poly AH$^+$-poly AH$^+$ at pH 5[19] (c(**12**)=5.0 × 10^{-6} mol dm^{-3}, λ_{exc}= 350 nm, sodium citrate buffer, I=0.03 M).

CD spectroscopy: To obtain some more information on the binding mode and the structure of the complexes formed, we have chosen CD spectroscopy as a highly sensitive method to assess conformational changes in the secondary structure of polynucleotides.[26] In addition, achiral small molecules can show induced CD signals (ICD) upon binding to polynucleotides, which gives useful information about the modes of interaction.[7,26,27] For example, sign and magnitude of the ICD band can depend on the binding geometry: ligand-ligand stacking is expected to give strong bisignate exciton CD, minor groove binding to ds-DNA orientates the ligand approximately at 45° in respect to the DNA chiral axis thus giving a strong positive ICD band, while intercalation brings the aromatic moiety of the ligand in a co-planar arrangement with the base pairs giving only a weak ICD band (not always but in most cases of negative sign due to parallel orientation of the transition vector of the ligand and the longer axis of the surrounding base pairs).[28,29]

It should be noted that **1**, **11** and **13** do not exhibit any significant intrinsic CD spectrum on their own under the experimental conditions used, while **8** and **12** posses CD spectra (Figure 6). The intrinsic CD spectrum of **8** revealed two strong negative maxima, whereby according to the maxima in the UV/Vis spectrum (Table 1) the band at λ = 243 nm can be attributed to the naphthyl-moiety, while the band λ = 298 nm corresponds to the guanidiniocarbonyl pyrrole group. Intriguingly, CD bands of **12** at λ

= 243 nm and at λ = 290-305 nm are of opposite (strongly positive) sign in respect to corresponding band of **8**. The additional, negative band of **12** at λ=330-343 nm can be attributed to the pyrene.

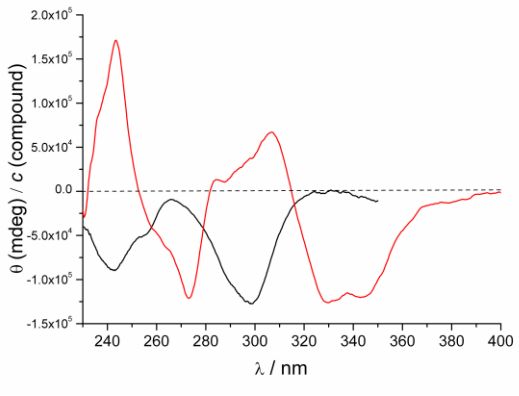
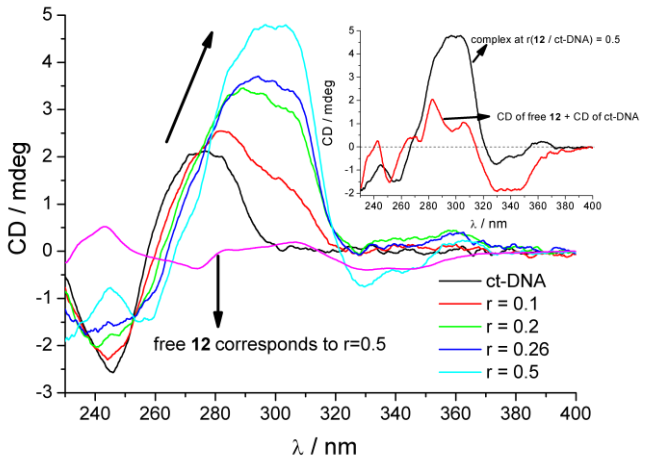


Figure 6. CD spectra of **8** (—) and **12** (—) at pH 5 (sodium citrate buffer, I = 0.03 mol dm^{-3}).



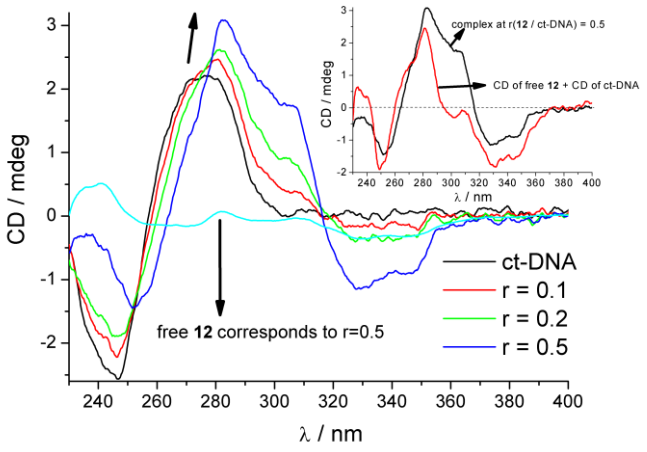
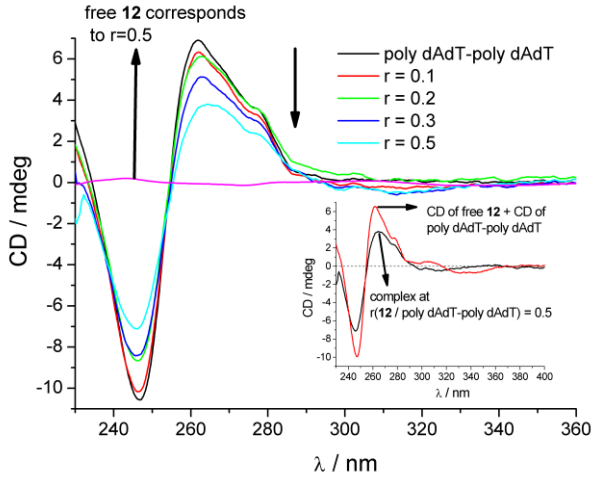


Figure 7. CD titration of ct-DNA (c =3.0 × 10^{-5} mol dm^{-3}) with **12** at: Top) pH 5, r$_{[12]\,/\,[ct\text{-}DNA]}$ = 0; 0.1; 0.2; 0.26; 0.50, (sodium citrate buffer, I = 0.03 mol dm^{-3}); Bottom) pH 7, r$_{[12]\,/\,[ct\text{-}DNA]}$ =0; 0.1; 0.2; 0.5; (sodium cacodylate buffer, I = 0.05 mol dm^{-3}).

Interactions with ds-DNA: The previously reported specific recognition of ds-DNA by **1** (ICD band at 310 nm), which was not observed for ds-RNA, was attributed to the positioning of the guanidiniocarbonyl pyrrole moiety exclusively in the minor groove.[7] However, **1** showed that specificity only at weakly acidic conditions (pH 5), at which the guanidine was protonated. Compound **11** (the acridine analogue of **1**) gave rise to similar ICD band again (λ=300 nm corresponds nicely to the electronic absorption maximum given in Table 1), but again only at pH 5 (results not shown), confirming that interactions of the protonated guanidiniocarbonyl pyrrole moiety within the DNA minor groove are essential for that ICD band. Upon mixing with ct-DNA at pH 5, also **12** revealed a similar ICD band at λ = 305-315 nm (Figure 7, Top), again in good accordance with the corresponding electronic absorption maximum given in Table 1.



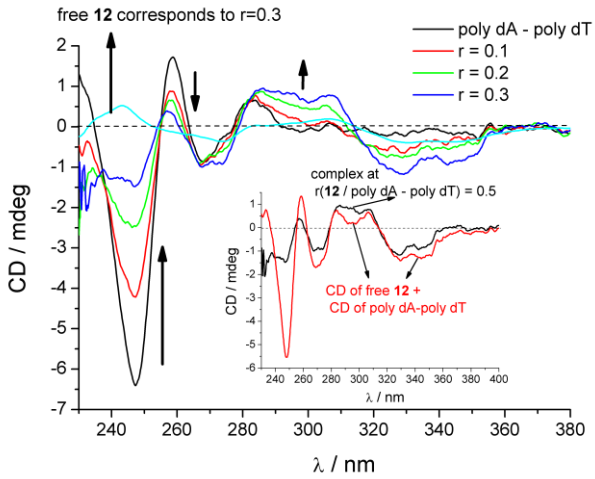


Figure 8. Titrations of alternating (top) and homo-(bottom) dAdT polynucleotides with **12** at pH 5 (sodium cacodylate buffer, I = 0.05 mol dm^{-3}), r= [**12**] / [polynucleotide].

However, in contrast to **1** or **11**, the ICD band of **12** was observed also at pH 7 (Figure 7, Bottom). A strong intensity decrease of the intrinsic negative CD band of **12** at λ = 330 – 343 nm upon mixing with ct-DNA (Figure 7, insets) suggests also intercalation of the pyrene moiety into the DNA double helix. More detailed studies revealed highly selective changes of the CD

spectrum of **12** in respect to a) pH of the solution and b) different base pair composition of ds-DNA (Figure 8). By far the strongest positive ICD band at λ = 310 nm was obtained for poly dGdC- poly dGdC, but only at pH 5, which underlines again the essential role of the protonated guanidiniocarbonyl pyrrole cation on the positioning of **12** in the minor groove of poly dGdC - poly dGdC. The neutral guanidine at pH 7 interacts only weakly with ds-DNA. It probably flanks out of the DNA minor groove resulting in the absence of a ICD band at λ = 310 nm. Furthermore, addition of **12** to A-T containing polynucleotides induced significantly smaller changes in the CD spectra compared to G-C containing polynucleotides, whereby the absence of a ICD band at λ = 310 nm A-T containing polynucleotides is the most prominent difference (Figure 8). It seems that specific substituents of the G-C base pair (like e.g. the amino group of guanidine which is exposed in the DNA minor groove) are responsible for the uniform orientation of the guanidiniocarbonyl pyrrole moiety of **12** in the minor groove. Hence, **12** shows a base pair selective recognition of ds-DNA at pH 5.

Addition of **8** (naphthyl-analogue of **12**) caused a strong increase of both, negative and positive CD bands of the ds-DNA polynucleotides (Figure 9). A distinct deviation from the isoelliptic points is pointing to the presence of several different **8**/DNA complexes. The increase of the positive DNA band (λ = 280 nm) can only be the consequence of changes in the secondary structure of the DNA double helix as **8** does not have a CD signal in this region (Figure 6). Also, the strong negative CD band (290 – 320 nm) in the CD spectrum of the **8**/DNA complexes (Fig 9a inset) is red shifted in respect to free **8** and free DNA, which can be attributed to the interactions of the chromophore of **8** with the ds-helix of polynucleotides. Most interestingly, in contrast to **1**, **11**, **12**, no positive ICD band at λ = 310 nm was observed for **8**/DNA complexes, probably due to the significantly smaller aromatic surface of naphthalene (**8**) in respect to pyrene or acridine, which does not lead to intercalation. Consequently, the guanidiniocarbonyl pyrrole part of **8** is not uniformly oriented within the DNA minor groove.

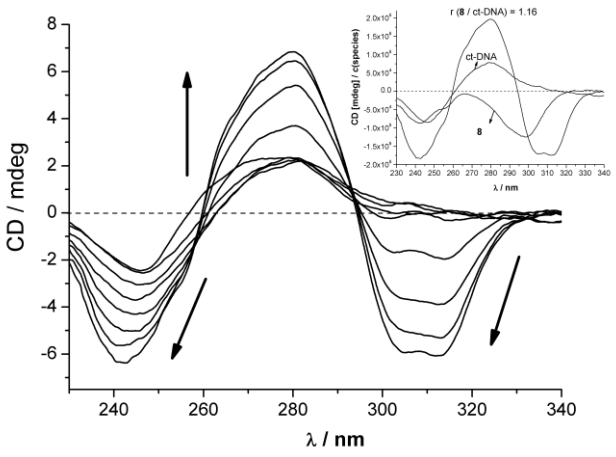


Figure 9. CD titration of ct-DNA (c = 3.0 × 10^{-5} mol dm^{-3}) with **8** at pH 5 (sodium citrate buffer, I = 0.03 mol dm^{-3}); $r_{[8]\,/\,[ct\text{-}DNA]}$=0; 0.17; 0.33; 0.50; 0.66; 0.83; 0.99; 1.16. Inset: comparison of the spectra of complex with the spectra of free **8** and free ct-DNA.

Addition of **13** did not yield any significant changes in the CD spectra of most ds-DNAs studied. The only exceptions are two weak but negligible positive ICD bands at λ = 295 nm and λ = 354 nm obtained for poly dAdT - poly dAdT and a moderate increase of the CD band at λ = 282 nm for poly dGdC- poly dGdC. Such minor changes point toward negligible structural changes of the polynucleotides upon mixing with **13**, due to agglomeration of **13** along the polynucleotide surface rather than specific binding.

Interactions with ds-RNA and ss-RNA: Opposite to ds-DNA, the addition of any compound led to a decrease of the positive CD bands of ds-RNA polynucleotides. For ds-RNA polynucleotides it is characteristic that changes in the CD spectra are almost proportional to the ratio $r_{[compd]\,/\,[RNA]}$, thus showing no saturation of binding sites even at excess of ligand over ds-RNA, which clearly shows that the compounds studied here interact significantly different with ds-DNA and ds-RNA. Accordingly, the CD spectra of ss-RNAs were only slightly changed upon the addition of any compound studied here, suggesting that upon binding the secondary structure of the polynucleotides was mainly preserved.

Discussion of the spectroscopic results

Our structure tuning of guanidiniocarbonyl-pyrrole-aryl hybrid probes aiming toward spectrophotometric recognition of specific DNA and RNA sequences started with benzene and naphthalene moieties attached to a guanidiniocarbonyl-pyrrole moiety by short and rigid linkers (**2** – **3**). These compounds did not show significant interactions with DNA/RNA. Enlarging the aromatic moiety by attaching pyrene instead of naphthalene (**5**), again by a short and rigid linker, resulted in a minimal stabilisation of ds-DNA (as seen in the thermal denaturation studies; ΔT_m = 1.0 °C at r = 0.3) but only at pH 5 when the guanidine was protonated. However, by introducing more flexibility in the linker between the pyrene and the guanidiniocarbonyl-pyrrole moiety and in combination with an additional positive charge (as in **1** at pH 5 or in **12** also at pH 7) the affinity was large enough to cause a measurable stabilization of the polynucleotides. Moreover, combining two positive charges and a longer and flexible linker in **8** allowed also a naphthyl-moiety to interact significantly with ds-DNA (logK_s ≈ 5, thermal stabilisation, CD spectrum change). However, the comparison of the CD titrations of ds-DNA with either **8** (no positive ICD band at λ >300 nm) or **1, 11, 12** (strong positive ICD band at λ = 310 nm) revealed the importance of the intercalative unit (pyrene in **12**) for the uniform orientation of guanidiniocarbonyl-pyrrole moiety within the DNA minor groove.

A pyrene connected by a flexible aliphatic linker to a positively charged guanidiniocarbonyl-pyrrole yielded compound **1** which, as recently published by us,[7] interacts with both ds-DNA and ds-RNA but differently giving rise to distinct and unique features – emission of a specific fluorescence signal for ds-RNA and a specific induced CD band for ds-DNA. However, the need for a positive charge restricted the use of **1** as a polynucleotide specific spectroscopic probe to pH 5. The newly prepared acridine analogue **11** stabilized ds-DNA to a lesser extent than **1**, underlining the importance of the size of aromatic surface for efficient intercalation into the DNA double helix. Furthermore, **11** yielded strong positive ICD band at λ = 310 nm upon binding to ds-DNA similar to **1**, but in contrast to **1** gave no specific fluorescence signal for any ds-DNA or ds-RNA studied, although acridine and pyrene in general exhibit similar fluorescence spectra. That observation stresses the sensitivity of the pyrene fluorescence on the microenvironment. Moreover, the affinity of acridine derivative **11** toward DNA/RNA (Table 4) is

comparable to affinities of some most intensively studied simple acridines as e.g. AMSA ((N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulphonanilide) and DACA (acridine-4-carboxamide); however the affinity and thermal stabilisation effect were significantly lower in respect to 9-amino-acridine derivatives with unsubstituted amino group (Chapter 18 in ref [4]).

Compound **13**, the bis-pyrene analogue of **1**, was prepared with the expectation that the two pyrenes will form an intramolecular excimer (characterised by specific fluorescence response) which can then specifically interact with some polynucleotides depending on their secondary structure (e.g. only with ds-RNA but not ds-DNA). Indeed, fluorimetric and UV/Vis properties of free **13** point toward intramolecular aromatic stacking interactions. However, **13** was rather weakly soluble in water and did not show any thermal stabilisation of any ds-DNA or ds-RNA. Moreover, additional spectroscopic studies suggested only weak interaction of **13** with DNA or RNA, most likely based on the agglomeration of molecules along the polynucleotide surface. Thus, intramolecular stacking interactions of **13** are obviously not disturbed by DNA or RNA and therefore **13** does not show any significant interaction with polynucleotides.

In order to keep the polynucleotide specific spectroscopic features of **1** but shift its applicability to physiological conditions (pH 7), compound **12** was prepared, characterised by an additional positive charge even at pH 7. Indeed, the thermal denaturation effect of **12** on ds-DNA increased in comparison with **1**, and in addition, **12** gave a strong positive ICD band at λ = 310 nm upon binding to ct-DNA even at pH 7, which was not observed for **1**. However, **12** did not show any fluorimetric response specific for ds-RNA (as emission of **1** at 500 nm)[7], most likely because steric hindrance and/or charge repulsion prevented dimer formation of **12** within the ds-RNA major groove. On the other hand, in contrast to **1** the fluorescence of **12** proved to be highly sensitive to the base pair composition of ds-DNA, especially at pH 5. Namely, at pH 5 addition of any A-T base pair polynucleotide to **12** resulted in a strong increase of its fluorescence, while G-C containing polynucleotides (even mixed base pair ct-DNA) strongly quenched its fluorescence. Similar specificity for the base pair composition was also seen in the CD spectra, pointing toward specific interaction of the positively charged guanidiniocarbonyl-pyrrole moiety with G-C base pairs within the DNA minor groove.

To study the role of each nucleobase on the fluorescence of **12** in more detail, we performed series of titrations of **12** with single stranded homo-polynucleotides. At pH 7 no significant interactions were observed at biologically relevant conditions, most likely due to a) the low affinity of the intercalative unit alone (pyrene, acridine) toward ss-sequences, b) the absence of any well defined groove necessary for accommodation of the guanidiniocarbonyl-pyrrole moiety as well as c) the presence of only one positive charge in **12**. However, at pH 5 the guanidiniocarbonyl-pyrrole moiety is also protonated, and in addition poly A and poly C are protonated as well and readily form double stranded helices with more or less well defined grooves.[19] Most intriguingly, at pH 5 only the addition of poly AH^+ - poly AH^+ yielded a strong increase of **12** fluorescence, while other polynucleotides either completely (poly G) or partially (poly U, poly CH^+ - poly CH^+) quenched the emission of **12**. Fluorescence quenching by poly G is in line with previous observations for proflavine and diazapyrenes, because guanine is much more easily oxidised than any other nucleobase and can thus efficiently quench the fluorescence of an electron-accepting fluorophore.[20,21,22] Fluorescence increase can be correlated to the more efficient aromatic stacking (intercalation) of pyrene in poly AH^+ - poly AH^+, which is characterized by significantly larger aromatic basepair surface in respect to poly U and poly CH^+ - poly CH^+.

Biological Results and Discussion

Compounds **1**, **7**, **8**, **11**, **12** and **13** were screened for their potential antiproliferative effects on a panel of 5 human cell lines, which were derived from different cancer types including HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), and H460 (lung carcinoma) (Table 6).

Table 6. *In vitro* inhibition of compounds **7**, **8**, **1**, **11**−**13** on the growth of tumour cells.

Compd	IC_{50} (μM)[a]				
	HeLa	MiaPaCa-2	SW 620	MCF-7	H 460
7	30 ± 8	53 ± 8	63 ± 37	21 ± 7	>100
8	30 ± 0.2	>100	>100	>100	>100
1	9 ± 5	4 ± 1.4	10 ± 0.5	8 ± 9	16 ± 1
11	47 ± 18	50 ± 6	57 ± 30	≥ 100	> 100
12	15 ± 2	14 ± 0.02	92 ± 1	25 ± 1	52 ± 47
13	>10[b]	>10	> 10	> 10	> 10

a IC_{50}; concentration that causes a 50% reduction of cell growth.
b The maximal tested concentration was c = 10 μM.

Compounds **7**, **8**, **11**, and **13** exhibited only moderate antiproliferative activity (IC_{50} values in the upper to middle μM range), while **1** and **12** showed activity in the lower μM range but with some interesting exceptions depending on the cell line. The high DNA affinity of **8**, **1**, **11** and **12** strongly suggests that cellular DNA is the main target, whereby intercalation of the pyrene seems to have the most pronounced effect. This can be seen from the significantly stronger biological activity of the two pyrene derivatives **1** and **12** in comparison to their naphthalene (**8**) and acridine (**11**) analogues. Bis-pyrene derivative **13**, in which the two pyrene subunits strongly interact intramolecularly and do not intercalate into isolated DNA in vitro and accordingly cannot interact significantly with cellular DNA, has only a negligible antiproliferative activity compared to **1**. Thus the biological results nicely correlate with the results from the spectroscopic studies. However, although **1** revealed the highest but a non-selective biological activity, its close analogue **12** (which is nearly as active as **1**) is also characterized by pronounced selectivity toward HeLa, MCF-7 and MiaPaCa-2 cell lines with only a very weak antiproliferative effect on SW 620 and H 460 cells. Such an intriguing impact of only one additional positive charge present in **12** but not in **1** cannot be explained within the presented experimental data but however strongly supports additional biological studies.

Conclusion

Systematic structure-activity relationship (SAR) study of interactions of guanidiniocarbonyl-pyrrole-aryl derivatives with various DNA and RNA polynucleotides revealed several critical factors, which control affinity and spectroscopic sensing of particular secondary structure or basepair composition of the polynucleotide. To start with, for efficient binding the linker between the aryl moiety and the guanidiniocarbonyl-pyrrole should be flexible enough to allow efficient accommodation within the groove of the polynucleotide. Furthermore, the acridine analogue (**11**) showed to be the minimal aromatic surface able to intercalate into DNA/RNA. Neutral molecules (**1**, **13**, **5** at pH 7) showed significantly stronger interactions with DNA/RNA when they are protonated at the guanidine (pH 5), which is in accord with previous studies.[4] Intercalation of the aryl subunit within DNA invariably led to the uniform positioning of the protonated guanidiniocarbonyl-pyrrole within the DNA minor groove, yielding a characteristic ICD signal. Furthermore, pyrene analogues revealed specific fluorescence changes either due to the different binding mode (compound **1**, intercalation in ds-DNA, dimer formation in ds-RNA) or signalling base pair composition (guanine quenched and adenine increased fluorescence). To some extent the affinity of the guanidinio-carbonyl-pyrrole-aryl conjugate toward DNA/RNA could be increased by addition of another positive charge. The number of positive charges and the size of the aromatic surface also seem to control the antiproliferative activity of these compounds. It should be stressed that most tumour cells in solid tumours consistently have lower extracellular pH levels than normal tissues because of the inefficient clearance of metabolic acids from chronically hypoxic cells.[30] Tumours of the bladder, kidney and gastrointestinal system in particular are exposed to extremely low pH values. Therefore, an uptake of weakly ionizing drugs by tumours is greatly influenced by the interstitial and intracellular pH, and the ionization properties of the compound. Consequently strategies for enhancing and exploiting pH gradients to drive the uptake of molecules into tumours are under investigation.[31] In this respect the compounds we studied here are interesting model systems for such studies in living cells due to the aforementioned specific fluorimetric properties.

Experimental Section

General remarks. Reaction solvents were dried and distilled under argon before use. All other reagents were used as obtained from BAChem, Aldrich, Acros, Novabiochem, GL Biochem and Lancaster. Flash column chromatographies were run on *ICN Silica* (0.032-0.063 nm) from *Biomedicals GmbH* or on medium pressure flash system (MPLC, CombiFlash®, Companion™, Isco Inc.) with prepacked silica gel cartridge (RP-18 Reverse Phase 4.3 g from *RediSep*). Melting points were measured in open end glass capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 MHz spectrometer. The chemical shifts are reported relative to the deuterated solvents. Peaks assignment is based on DEPT studies and comparison with literature data. ESI- and HR-mass spectra were recorded on a micrOTOF from *Bruker Daltonik*. Analytical HPLC was run on a Supelcosil LC18 (Supelco) 5 µm, (25 cm x 4.6 mm) column. Gua = guanidiniocarbonylpyrrole.

General procedure for the coupling with oxalyl chloride:

A solution of the free acid **14** (1 eq) was dissolved in dry DCM (15 ml per eq) and catalytic amounts of dry DMF. After addition of oxalyl chloride (3 eq) the solution was refluxed for two hours. Subsequently to the removal of the solvent and residual oxalyl chloride the resulting brown solid was redissolved in dry DCM (20 ml) and cooled to 0 °C. After addition of the appropriate arylamine (3 eq) the solution was stirred for one hour at 0 °C and one more hour at room temperature. The reaction solution was washed then with hydrochloric acid (5%, 3 x 50 ml). After phase separation the organic phase was dried over magnesium sulphate and the solvent was removed *in vacuo*. The resulting solid was purified by column chromatography.

15a was prepared from free acid **14** (500 mg, 2.04 mmol), oxalyl chloride (524 µl, 6.12 mmol) and aniline (559 µl, 6.12 mmol), obtaining a brown solid (480 mg, 74%); R_f = 0.54 (SiO_2, cyclohexane/ethyl acetate 6/4 + 1 vol% NEt_3); mp: 170 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.43 (s, 1H, pyrrole-NH), 10.06 (s, 1H, CONH), 7.73-7.70 (m, 2H, Ph-CH), 7.49-7.46 (m, 2H, Ph-CH), 7.43-7.34 (m, 5H, Ph-CH), 7.12-7.08 (m, 1H, Ph-CH), 7.01-6.99 (m, 1H, pyrrole-CH), 6.91-6.90 (m, 1H, pyrrole-CH), 5.33 (s, 2H, O-CH_2-Ph); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.9 (Cq), 157.8 (Cq), 138.7 (Cq), 136.2 (Cq), 131.1 (Cq), 128.7 (CH), 128.5 (CH), 128.1 (Cq), 127.9 (CH), 119.6 (Cq), 115.5 (pyrrole-CH), 113.5 (pyrrole-CH), 65.6 (CH_2); HR-MS (ESI$^+$) m/z = 321.1234 (calculated for $C_{19}H_{16}N_2O_3$ + H^+: 312.1234); m/z = 343.1053 (calculated for $C_{19}H_{16}N_2O_3$ + Na^+: 343.1053); m/z = 663.2265 (calculated for 2M + Na^+: 663.2214).

15b was prepared from free acid **14** (500 mg, 2.04 mmol), oxalyl chloride (524 µl, 6.12 mmol) and 1-naphthylamine (876 mg, 6.12 mmol) obtaining a brown solid (550 mg, 75%); R_f = 0.46 (SiO_2, cyclohexane/ethyl acetate 6/4 + 1 vol% NEt_3); mp: 155 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.52 (s, 1H, pyrrole-NH), 10.26 (s, 1H, CONH), 8.02-7.97 (m, 2H, naphthyl-CH), 7.86-7.84 (m, 1H, naphthyl-CH), 7.65-7.63 (m, 1H, naphthyl-CH), 7.58-7.53 (m, 3H, naphthyl-CH), 7.49-7.48 (m, 2H, Ph-CH), 7.43-7.35 (m, 3H, Ph-CH), 7.10-7.09 (m, 1H, pyrrole-CH), 6.96-6.95 (m, 1H, pyrrole-CH), 5.35 (s, 2H, O-CH_2-Ph); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.9 (Cq), 158.7 (Cq), 136.2 (Cq), 133.8 (Cq), 133.0 (Cq), 131.0 (Cq), 128.7 (Cq), 128.5 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 126.1 (CH), 126.0 (CH), 125.5 (CH), 124.8 (Cq), 123.4 (CH), 123.1 (CH), 115.6 (pyrrole-CH), 113.6 (pyrrole-CH), 65.5 (CH_2); HR-MS (ESI$^+$) m/z = 371.1390 (calculated for $C_{23}H_{18}N_2O_3$ + H^+: 371.1390); m/z = 393.1210 (calculated for $C_{23}H_{18}N_2O_3$ + Na^+: 393.1210); m/z = 763.2569 (calculated for 2M + Na^+: 763.2533).

15c was prepared from free acid **14** (143 mg, 0.58 mmol), oxalyl chloride (150 µl, 1.75 mmol) and 2-naphthylamine (250 mg, 1.75 mmol), obtaining a slightly brown solid (170 mg, 82%); R_f = 0.58 (SiO_2, cyclohexane/ethyl acetate 6/4 + 1 vol% NEt_3); mp: 185 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.49 (s, 1H, pyrrole-NH), 10.27 (s, 1H, CONH) , 7.38-7.37 (m, 1H, naphthyl-CH), 7.92-7.90 (m, 1H, naphthyl-CH), 7.87-7.84 (m, 2H, naphthyl-CH), 7.79-7.76 (m, 1H, naphthyl-CH), 7.52-7.33 (m, 5H, Ph-CH; m, 2H, naphtyl-CH), 6.94-6.93 (m, 1H, pyrrole-CH), 7.16-7.05 (m, 1H, pyrrole-CH), 5.35 (s, 2H, O-CH_2-Ph); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.9 (Cq), 158.0 (Cq), 136.4 (Cq), 136.2 (Cq), 133.1 (Cq), 129.9

(Cq), 128.5 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.5 (CH), 127.3 (CH), 126.4 (CH), 124.9 (Cq), 124.8 (CH), 120.5 (CH), 116.1 (CH), 115.5 (pyrrole-CH), 113.6 (pyrrole-CH), 65.6 (CH_2); HR-MS (ESI^+) m/z = 371.1390 (calculated for $C_{23}H_{18}N_2O_3 + H^+$: 371.1390); m/z = 393.1210 (calculated for $C_{23}H_{18}N_2O_3 + Na^+$: 393.1210); m/z = 763.2554 (calculated for 2M + Na^+: 763.2533).

15d was prepared from free acid **14** (250 mg, 1.02 mmol), oxalyl chloride (259 µl, 3.06 mmol) and 1-aminopyrene (651 mg, 3.06 mmol), obtaining a slightly brown solid (105 mg, 23%); R_f = 0.89 (SiO_2, cyclohexane/ethyl acetate/isopropanol 4/4/1 + 1 vol% NEt_3); mp: 228 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.61 (s, 1H, pyrrole-NH), 10.64 (s, 1H, CONH), 8.35-8.08 (m, 9H, pyrenyl-CH), 7.51-7.34 (m, 5H, Ph-CH), 7.18-7.17 (m, 1H, pyrrole-CH), 6.99-7.00 (m, 1H, pyrrole-CH), 5.37 (s, 2H, O-CH_2-Ph); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.9 (Cq), 159.9 (Cq), 158.8 (Cq), 136.2 (Cq), 131.1 (Cq), 130.0 (Cq), 130.5 (Cq), 128.8 (Cq), 128.5 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.3 (CH), 127.2 (CH), 126.9 (CH), 124.5 (CH), 125.4 (CH), 125.1 (CH), 124.9 (CH), 124.6 (CH), 124.4 (Cq), 123.8 (Cq), 122.7 (CH), 115.6 (pyrrole-CH), 111.1 (pyrrole-CH), 65.6 (CH_2); HR-MS (ESI^+) m/z = 445.1547 (calculated for $C_{29}H_{20}N_2O_3 + H^+$: 445.1547); m/z = 467.1366 (calculated for $C_{29}H_{20}N_2O_3 + Na^+$: 467.1366).

General procedure for the hydrogenolysis of the benzyl ester

A suspension of the appropriate benzyl ester and palladium on charcoal (10%) was stirred in MeOH under hydrogen atmosphere at 40 °C until tlc indicates full conversion of the starting material. The reaction solution was filtrated over a celite pad which was washed several times with ethyl acetate. The resulting solution was dried over magnesium sulphate and the solvent evaporated *in vacuo*.

16a was prepared from **15a** (370 mg, 1.15 mmol) and Pd/C (74 mg) in MeOH (50 ml), obtaining a colorless solid (265 mg, quant.); mp: 225 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.82 (br.s, 1H, COOH), 12.15 (s, 1H, pyrrole-NH), 10.04 (s, 1H, CONH), 7.72-7.70 (m, 2H, Ph-CH), 7.37-7.33 (m, 2H, Ph-CH), 7.11-7.07 (m, 1H, aryl-CH), 6.96-6.94 (m, 1H, pyrrole-CH), 6.80-6.79 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 161.6 (Cq), 157.8 (Cq), 138.8 (Cq), 130.1 (Cq), 128.7 (CH), 123.5 (CH), 119.8 (CH), 114.7 (pyrrole-CH), 113.7 (pyrrole-CH); HR-MS (ESI^+) m/z = 231.0764 (calculated for $C_{12}H_{10}N_2O_3 + H^+$: 231.0764); m/z = 253.0584 (calculated for $C_{12}H_{10}N_2O_3 + Na^+$: 253.0584); m/z = 483.1300 (calculated for 2M + Na^+: 483.1275); m/z = 713.1994 (calculated for 3M + Na^+: 713.1966); m/z = 943.2685 (calculated for 4M + Na^+: 943.2658).

16b was prepared from **15b** (530 mg, 1.48 mmol) and Pd/C (106 mg) in MeOH (50 ml), obtaining a slightly greenish solid (415 mg, quant.); mp: 173 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.25 (s, 1H, pyrrole-NH), 10.22 (s, 1H, CONH), 8.06-8.03 (m, 1H, naphthyl-CH), 7.99-7.96 (m, 1H, naphthyl-CH), 7.85-7.83 (m, 1H, naphthyl-CH), 7.66-7.64 (m, 1H, naphthyl-CH), 7.60-7.53 (m, 3H, naphthyl-CH), 7.03-7.02 (m, 1H, pyrrole-CH), 6.83-6.82 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 161.7 (Cq), 158.7 (Cq), 133.8 (Cq), 133.1 (Cq), 128.7 (Cq), 128.1 (CH), 126.1 (Cq), 125.9 (CH), 125.5 (CH), 123.2 (CH), 123.1 (CH), 114.7 (pyrrole-CH), 113.7 (pyrrole-CH); HR-MS (ESI^+) m/z = 281.0921 (calculated for $C_{16}H_{12}N_2O_3 + H^+$: 281.0921); m/z = 303.074 (calculated for $C_{16}H_{12}N_2O_3 + Na^+$: 303.074); m/z = 583.1606 (calculated for 2M + Na^+: 583.1588).

16c was prepared from **15c** (170 mg, 0.47 mmol) and Pd/C (34 mg) in MeOH (40 ml), obtaining a grey solid (132 mg, quant.); mp: 233 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.22 (s, 1H, pyrrole-NH), 10.28 (s, 1H, CONH), 8.40-8.39 (m, 1H, naphthyl-CH), 7.92-7.78 (m, 4H, naphthyl-CH), 7.51-7.40 (m, 2H, naphthyl-CH), 7.01-7.00 (m, 1H, pyrrole-CH), 6.81-6.80 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 161.7 (Cq), 158.0 (Cq), 150.9 (Cq), 138.2 (Cq), 136.5 (Cq), 133.4 (Cq), 129.9 (Cq), 128.3 (CH), 127.5 (CH), 127.3 (CH), 126.4 (CH), 124.7 (CH), 120.4 (CH), 116.0 (pyrrole-CH), 113.8 (pyrrole-CH); HR-MS (ESI^+) m/z = 281.0921 (calculated for $C_{16}H_{12}N_2O_3 + H^+$: 281.0921); m/z = 303.074 (calculated for $C_{16}H_{12}N_2O_3 + Na^+$: 303.074).

16d was prepared from **15d** (105 mg, 0.24 mmol) and Pd/C (11 mg) in MeOH (25 ml), obtaining a slightly yellow solid (84 mg, quant.); mp: 248 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.31 (s, 1H, pyrrole-NH), 10.63 (s, 1H, CONH), 8.34-8.07 (m, 9H, pyrenyl-CH), 7.10-7.09 (m, 1H, pyrrole-CH), 6.84-6.83 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 161.8 (Cq), 158.9 (Cq), 131.2 (Cq), 130.8 (Cq), 130.5 (Cq), 129.6 (Cq), 128.7 (Cq), 127.2 (CH), 127.2 (Cq), 126.8 (CH), 126.4 (Cq), 125.3 (CH), 125.0 (CH), 124.9 (Cq), 124.9 (CH), 124.5 (CH), 124.4 (Cq), 123.8 (Cq), 122.7 (Cq), 114.4 (pyrrole-CH), 114.0 (pyrrole-CH); HR-MS (ESI^+) m/z = 355.1077 (calculated for $C_{22}H_{14}N_2O_3 + H^+$: 355.1077); m/z = 377.0897 (calculated for $C_{22}H_{14}N_2O_3 + Na^+$: 377.0897).

General procedure for the synthesis of the Boc-protected intercalators 17a-d:

A solution of the appropriate free acid (1 eq) was dissolved in dry DMF. The coupling reagent (1.1 eq) and NMM was added to the solution which was stirred for 30 minutes at room temperature. After addition of Boc-guanidine (1.1 eq) the solution was stirred for additional 24 hours at rt. The reaction solution was diluted into vigorously stirred water (100 ml) and extracted with ethyl acetate. After phase separation the organic phase was dried over magnesium sulphate and the solvent was evaporated *in vacuo*. The resulting oil was purified by column chromatography.

17a was prepared from free acid **16a** (260 mg, 1.13 mmol) in DMF (20 ml), NMM (2 ml), PyBOP (646 mg, 1.24 mmol) and Boc-guanidine (198 mg, 1.24 mmol), obtaining a slightly yellow solid (380 mg, 90%); R_f = 0.40 (SiO_2, cyclohexane/ethyl acetate/isopropanol 6/2/1 + 1 vol% NEt_3); mp: 147 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.32 (br.s, 1H, pyrrole-NH), 10.81 (br.s, 1H, NH), 10.07 (s, 1H, CONH), 9.37 (br.s, 1H, NH), 8.56 (br.s, 1H, NH), 7.73-7.71 (m, 2H, Ph-CH), 7.37-7.33 (m, 2H, Ph-CH), 7.11-7.07 (m, 1H, Ph-CH), 7.01 (br.s, 1H, pyrrole-CH), 6.85 (br.s, 1H, pyrrole-CH), 1.47 (s, 9H, ^{t}Bu-CH_3); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 158.7 (Cq), 138.9 (Cq), 128.7 (CH), 123.5 (CH), 120.0 (CH), 113.2 (pyrrole-CH), 27.8 (^{t}Bu-CH_3); HR-MS (ESI^+) m/z = 372.1666 (calculated for $C_{18}H_{21}N_5O_4 + H^+$: 372.1666); m/z = 394.1486 (calculated for $C_{18}H_{21}N_5O_4 + Na^+$: 394.1486).

17b was prepared from free acid **16b** (410 mg, 1.46 mmol) in DMF (30 ml), NMM (3 ml), PyBOP (837 mg, 1.61 mmol) and Boc-guanidine (256 mg, 1.61 mmol), obtaining a colorless solid (450 mg, 73%); R_f = 0.62 (SiO_2, ethyl acetate/cyclohexane 6/4 + 1 vol%

NEt_3); mp: 189 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.54 (br.s, 1H, pyrrole-NH), 10.86 (br.s, 1H, NH), 10.28 (s, 1H, CONH), 9.37 (br.s, 1H, NH), 8.59 (br.s, 1H, NH), 8.02-7.96 (m, 2H, naphthyl-CH), 7.86-7.84 (m, 1H, naphthyl-CH), 7.63-7.62 (m, 1H, naphthyl-CH), 7.59-7.53 (m, 3H, naphthyl-CH), 7.59 (br.s, 1H, pyrrole-CH), 6.84 (br.s, 1H, pyrrole-CH), 1.47 (s, 9H, ^{t}Bu-CH$_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.0 (Cq), 158.5 (Cq), 133.8 (Cq), 133.1 (Cq), 128.9 (Cq), 128.1 (CH), 126.1 (CH), 125.7 (CH), 125.6 (CH), 123.5 (CH), 123.2 (CH), 113.8 (pyrrole-CH), 113.2 (pyrrole-CH), 81.0 (^{t}Bu-Cq), 27.8 (^{t}Bu-CH$_3$); HR-MS (ESI$^+$) m/z = 322.1307 (calculated for $C_{17}H_{15}N_5O_2$ + H$^+$: 322.1299); m/z = 344.1123 (calculated for $C_{17}H_{15}N_5O_2$ + Na$^+$: 344.1118); m/z = 422.1823 (calculated for $C_{22}H_{23}N_5O_4$ + H$^+$: 422.1823); m/z = 444.1642 (calculated for $C_{22}H_{23}N_5O_4$ + Na$^+$: 444.1642); m/z = 843.3590 (calculated for 2M + H$^+$: 843.3573).

17c was prepared from free acid **16c** (120 mg, 0.43 mmol) in DMF (15 ml), NMM (1.5 ml), PyBOP (245 mg, 0.47 mmol) and Boc-guanidine (75 mg, 0.47 mmol), obtaining a slightly brown solid (102 mg, 57%); R_f = 0.62 (SiO_2, ethyl acetate/cyclohexane 6/4 + 1 vol% NEt_3); mp: 149 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.44 (br.s, 1H, pyrrole-NH), 10.84 (br.s, 1H, NH), 10.28 (s, 1H, CONH), 9.39 (br.s, 1H, NH), 8.59 (br.s, 1H, NH), 8.37 (s, 1H, naphthyl-CH), 7.92-7.78 (m, 4H, naphthyl-CH), 7.51-7.41 (m, 2H, naphthyl-CH), 7.07 (br.s, 1H, pyrrole-CH), 6.88 (br.s, 1H, pyrrole-CH), 1.48 (s, 9H, ^{t}Bu-CH$_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 158.3 (Cq), 136.5 (Cq), 133.4 (Cq), 129.9 (Cq), 128.5 (CH), 127.5 (CH), 127.3 (CH), 126.4 (CH), 124.8 (CH), 128.5 (CH), 116.1 (pyrrole-CH), 113.3 (pyrrole-CH), 27.8 (^{t}Bu-CH$_3$); HR-MS (ESI$^+$) m/z = 422.1823 (calculated for $C_{22}H_{23}N_5O_4$ + H$^+$: 422.1823); m/z = 444.1642 (calculated for $C_{22}H_{23}N_5O_4$ + Na$^+$: 444.1642); m/z = 843.3600 (calculated for 2M + H$^+$: 843.3573).

17d was prepared from free acid **16d** (80 mg, 0.23 mmol) in DMF (20 ml), NMM (2 ml), HCTU (102 mg, 0.25 mmol) and Boc-guanidine (40 mg, 0.25 mmol), obtaining a slightly brown solid (40 mg, 36%); R_f = 0.49 (SiO_2, ethyl acetate/cyclohexane 1/1 + 1 vol% NEt_3); mp: 224 °C (decomp.); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.40 (br.s, 1H, pyrrole-NH), 10.80 (br.s, 1H, NH), 10.65 (s, 1H, CONH), 9.44 (br.s, 1H, NH), 8.55 (br.s, 1H, NH), 8.54-8.35 (m, 8H, pyrenyl-CH), 8.11-8.08 (m, 1H, pyrenyl-CH), 7.20 (br.s, 1H, pyrrole-CH), 6.89 (br.s, 1H, pyrrole-CH), 1.47 (s, 9H, ^{t}Bu-CH$_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.1 (Cq), 131.2 (Cq), 130.8 (Cq), 130.5 (Cq), 128.2 (Cq), 127.2 (CH), 126.9 (CH), 126.5 (CH), 125.4 (CH), 125.2 (Cq), 125.1 (CH), 124.9 (CH), 124.8 (CH), 124.4 (Cq), 123.8 (Cq), 122.8 (Cq), 113.4 (pyrrole-CH), 27.8 (^{t}Bu-CH$_3$); HR-MS (ESI$^+$) m/z = 496.1979 (calculated for $C_{28}H_{25}N_5O_4$ + H$^+$: 496.1979); m/z = 518.1799 (calculated for $C_{28}H_{25}N_5O_4$ + Na$^+$: 518.1799).

General procedure for the synthesis of the chloride salts of the intercalators 2-5:

The appropriate Boc-protected compound was dissolved in mixture of DCM and TFA. The solution was stirred at rt until tlc control indicated no more starting material. The solvent and the TFA were evaporated *in vacuo*, the resulting brown oil was dissolved in MeOH (2-5 ml) and hydrochloric acid (5%, 5 ml) was added. Subsequently the resulting suspension was lyophilized obtaining the chloride salts of the deprotected intercalators.

Chloride salt of 2-(*N*-Phenylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole

Chloride salt **2** was prepared from **17a** (380 mg, 1.02 mmol) in DCM (10 ml) and TFA (6 ml), obtaining a slightly grey solid (228 mg, 73%); mp: 290 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.61 (s, 1H, pyrrole-NH), 11.89 (s, 1H, NH), 10.25 (s, 1H, CONH), 8.48 (br.s, 4H, NH), 7.75-7.73 (m, 2H, Ph-CH), 7.50-7.49 (m, 1H, Ph-CH), 7.39-7.35 (m, 2H, Ph-CH), 7.13-7.07 (m, 2H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.6 (Cq), 157.6 (Cq), 155.3 (Cq), 138.6 (Cq), 132.5 (Cq), 128.8 (CH), 126.1 (Cq), 123.8 (CH), 120.0 (CH), 115.8 (pyrrole-CH), 113.2 (pyrrole-CH); HR-MS (ESI$^+$) m/z = 272.1142 (calculated for $C_{13}H_{13}N_5O_2$ + H$^+$: 272.1142); m/z = 543.2224 (calculated for 2M + H$^+$: 543.2211).

Chloride salt of 2-(*N*-Naphthalene-1-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole

Chloride salt **3** was prepared from **17b** (400 mg, 0.95 mmol) in DCM (15 ml) and TFA (8 ml), obtaining a slightly green solid (190 mg, 56%); mp: 229 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.69 (s, 1H, pyrrole-NH), 11.90 (s, 1H, NH), 10.42 (s, 1H, CONH), 8.52 (br.s, 4H, NH), 8.05-7.96 (m, 2H, naphthyl-CH), 7.88-7.86 (m, 1H, naphthyl-CH), 7.65-7.63 (m, 1H, naphthyl-CH), 7.59-7.52 (m, 1H, pyrrole-CH; m, 3H, naphthyl-CH), 7.18-7.17 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.6 (Cq), 158.5 (Cq), 155.4 (Cq), 133.8 (Cq), 132.9 (Cq), 132.4 (Cq), 128.8 (Cq), 128.1 (CH), 126.3 (CH), 126.2 (CH), 126.0 (CH), 125.6 (CH), 123.5 (CH), 123.1 (CH), 115.9 (pyrrole-CH), 113.6 (pyrrole-CH); HR-MS (ESI$^+$) m/z = 322.1299 (calculated for $C_{17}H_{15}N_5O_2$ + H$^+$: 322.1299); m/z = 643.2545 (calculated for 2M + H$^+$: 643.2524).

Chloride salt of 2-(*N*-Naphthalene-2-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole

Chloride salt **4** was prepared from **17c** (100 mg, 0.24 mmol) in DCM (10 ml) and TFA (5 ml), obtaining a slightly brown solid (75 mg, 88%); mp: 290 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.67 (s, 1H, pyrrole-NH), 11.91 (s, 1H, NH), 10.46 (s, 1H, CONH), 8.48 (br.s, 4H, NH), 8.39 (s, 1H, naphthyl-CH), 7.93-7.79 (m, 4H, naphthyl-CH), 7.52-7.22 (m, 1H, pyrrole-CH; m, 2H, naphthyl-CH), 7.15-7.13 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.6 (Cq), 157.8 (Cq), 155.3 (Cq), 136.2 (Cq), 133.3 (Cq), 132.5 (Cq), 130.0 (Cq), 128.4 (CH), 127.5 (CH), 127.4 (CH), 126.5 (CH), 126.2 (Cq), 124.9 (CH), 120.5 (CH), 116.3 (pyrrole-CH), 113.7 (pyrrole-CH); HR-MS (ESI$^+$) m/z = 322.1299 (calculated for $C_{17}H_{15}N_5O_2$ + H$^+$: 322.1299); m/z = 643.2549 (calculated for 2M + H$^+$: 643.2524).

Chloride salt of 2-(*N*-Pyrene-1-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole

Chloride salt **5** was prepared from **17d** (20 mg, 0.04 mmol) in DCM (8 ml) and TFA (4 ml), obtaining a yellow solid (17 mg, quant.); mp: 281 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.77 (s, 1H, pyrrole-NH), 11.70 (s, 1H, NH), 10.78 (s, 1H, CONH), 8.43 (br.s, 4H, NH), 8.36-8.09 (m, 9H, pyrenyl-CH), 7.52-7.42 (m, 1H, pyrrole-CH), 7.48-7.47 (m, 1H, pyrrole-CH), 7.28-7.26 (m, 1H, pyrrole-CH); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 159.7 (Cq), 158.7 (Cq), 155.4 (Cq), 132.5 (Cq), 130.9 (Cq), 130.8 (Cq), 130.5 (Cq), 128.9 (Cq), 127.3 (CH), 127.2 (CH), 127.0 (CH), 126.5 (CH), 123.2 (Cq), 125.4 (CH), 125.2 (Cq), 125.2 (CH), 124.9 (CH), 124.7 (CH), 123.8 (Cq), 122.7 (CH), 116.1 (pyrrole-CH), 113.8 (pyrrole-CH); HR-MS (ESI$^+$) m/z = 396.1455 (calculated for $C_{23}H_{17}N_5O_2$ + H$^+$: 396.1455); m/z = 791.2888 (calculated for 2M + H$^+$: 791.2837).

General procedure for the coupling with isobutyl chloroformiate

A solution of free acid (1.5 eq) in dry THF (6 mL) was kept under N_2 atm and cooled at -15 °C. Then *N*-methylmorpholine (NMM) (1.5 eq) and isobutyl chloroformiate (1 eq) were added. The white suspension was stirred at -15 °C for 20 min. Afterwards a solution of arylamine (1 eq) in dry DMF or dry THF (1-2 mL) was added. The mixture was stirred 10 min at -15°C and at 0 °C overnight (allowing to warming to rt). The white solid was filtered and solvent was removed from the liquid layer. The residue was dissolved in EtOAc (25-50 mL), washed with H_2O (2 x 50 mL), 5% HCl aq (6 x 50 mL) and brine (2 x 50 mL). The organic layer was dried over $MgSO_4$ and solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, eluent: EtOAc/*n*-hexane 1:1 for **18a** and EtOAc/*n*-hexane 1:2 for **18b**).

5-[(*tert*-Butoxycarbonyl)amino-*N*-2-naphthylpentanamide 18a was prepared from 5-[(*tert*-butoxycarbonyl)amino]pentanoic acid (300 mg, 1.38 mmol), isobutyl chloroformiate (0.12 mL, 0.92 mmol), NMM (0.15 mL, 1.38 mmol) and 1-naphthylamine (132 mg, 0.92 mmol), obtaining a white solid (189 mg, 60%); mp =129-130 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.83 (s, 1H, naphN*H*CO); 8.06-8.04 (m, 1H, naphthyl H$_8$); 7.94-7.92 (m, 1H, naphthyl H$_5$); 7.75 (d, 1H, naphthyl H$_4$, *J* = 8.2 Hz); 7.67 (d, 1H, naphthyl H$_2$, *J* = 8.2 Hz); 7.57-7.51 (m, 2H, naphthyl H$_{6,7}$); 7.47 (t, 1H, naphthyl H$_3$, *J* = 7.8 Hz); 6.82 (br s, 1H naphNHCOCH$_2$CH$_2$-CH$_2$CH$_2$N*H*Boc); 2.97 (q, 2H, naphNHCOCH$_2$CH$_2$CH$_2$C*H*$_2$, *J* = 6.4 Hz); 2.47 (m, 2H, naphNHCOC*H*$_2$CH$_2$CH$_2$CH$_2$); 1.53-1.46 (m, 2H, CH$_2$); 1.68-1.61 (m, 2H, CH$_2$); 1.53-1.46 (m, 2H, CH$_2$); 1.38 (s, 9H, C(CH$_3$)$_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 171.3 (naphNH*C*O); 155.1 (*C*OOC(CH$_3$)$_3$); 133.2 (naphthyl Cq); 127.5 (naphthyl CH); 127.3 (naphthyl Cq); 125.4 (naphthyl CH); 125.2 (naphthyl CH); 125.0 (naphthyl CH and Cq); 124.5 (naphthyl CH); 122.2 (naphthyl CH); 121.1 (naphthyl CH); 76.8 (*C*(CH$_3$)$_3$); 40.0 (naphNHCOCH$_2$CH$_2$CH$_2$*C*H$_2$); 35.1 (naphNHCO*C*H$_2$CH$_2$CH$_2$-CH$_2$); 28.7 (naphNHCOCH$_2$-CH$_2$*C*H$_2$CH$_2$); 27.7 (C(*C*H$_3$)$_3$); 22.3 (naphNHCOCH$_2$*C*H$_2$CH$_2$CH$_2$); HR-MS (ESI$^+$) m/z = 365.183 ±0.005 (calculated for $C_{20}H_{26}N_2O_3$+Na$^+$: 365.183).

(*L*)-1-PyrenylSer(O^{t}Bu)NHBoc 18b was prepared from (*L*)-BocSer(O^{t}Bu)OH.DCHA (300 mg, 0.68 mmol), isobutyl chloroformiate (58 µL, 0.45 mmol), NMM (74 µL, 0.68 mmol), and 1-aminopyrene (98 mg, 0.45 mmol), obtaining a brown solid (207 mg, quant); mp = 160-164°C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 10.31 (s, 1H, PyreneNH); 8.34-8.28 (m, 4H, Pyrene H); 8.19-8.06 (m, 5H, Pyrene H); 6.86 (d, 1H, Ser NH, *J* = 4.9 Hz); 4.47-4.46 (m, 1H, CH); 3.73-3.66 (m, 2H, CH$_2$); 1.45 (s, 9H, C(CH$_3$)$_3$); 1.23 (s, 9H, C(CH$_3$)$_3$); HR-MS (ESI$^+$) m/z = 483.227±0.005 (calculated for $C_{28}H_{32}N_2O_4$+Na$^+$: 483.225).

General procedure for the deprotection with TFA

Boc-protected guanidine (1 eq), was dissolved in a mixture 1:1 TFA/dry DCM (0.5-2 mL TFA, 0.5-2 mL dry DCM) and stirred at rt for 1-2 h. Solvent and excess of TFA were removed *in vacuo*, and the oily residue was lyophilized.

Trifluoroacetate of *N*-2-[(5-(naphthalen-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidinium 6 was prepared from **20a** (70 mg, 0.13 mmol), obtaining a white solid (63 mg, 88%); mp = 205 ºC (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.33 (s, 1H, pyrrole NH); 11.31 (s, 1H, guanidinium NH); 9.87 (s, 1H, naphNHCO); 8.47 (t, 1H, naphNHCOCH$_2$CH$_2$CH$_2$CH$_2$N*H*, *J* = 5.2 Hz); 8.37 (br s, 4H, guanidinium (NH$_2$)$_2$); 8.06-8.04 (m, 1H, naphthyl H$_8$); 7.95-7.91 (m, 1H, naphthyl H$_5$); 7.75 (d, 1H, naphthyl H$_4$, *J* = 8.1 Hz); 7.67 (d, 1H, naphthyl H$_2$, *J* = 7.1 Hz); 7.54-7.52 (m, 2H, naphthyl H$_{6,7}$); 7.47 (t, 1H, naphthyl H$_3$, *J* = 7.7 Hz); 7.17 (m, 1H, pyrrole CH); 6.87 (m, 1H, pyrrole CH); 3.35-3.32 (m, 2H, CH$_2$); 2.54-2.52 (m, 2H, CH$_2$); 1.74-1.71 (m, 2H, CH$_2$); 1.64-1.61 (m, 2H, CH$_2$); ^{13}C-NMR (CD$_3$OD, 100 MHz) δ = 174.7 (naphthylCONH); 161.3 (CONH or C(NH$_2$)$_2$); 161.0 (CONH or C(NH$_2$)$_2$); 156.7 (CONH or C(NH$_2$)$_2$); 135.1 (naphthyl Cq); 133.7 (pyrrole Cq); 133.3 (naphthyl Cq); 129.5 (naphthyl Cq); 128.7 (naphthyl CH); 126.9 (naphthyl CH); 126.7 (naphthyl CH); 126.5 (naphthyl CH); 126.3 (pyrrole Cq); 125.9 (naphthyl CH); 123.5 (naphthyl CH); 122.8 (naphthyl CH); 115.6 (pyrrole CH); 112.3 (pyrrole CH); 40.2 (naphNHCOCH$_2$CH$_2$CH$_2$*C*H$_2$); 36.3 (naphNHCO*C*H$_2$CH$_2$CH$_2$-CH$_2$); 29.5 (naphNHCOCH$_2$CH$_2$*C*H$_2$CH$_2$); 23.8 (naphNHCOCH$_2$-*C*H$_2$CH$_2$CH$_2$); HR-MS (ESI$^+$) m/z = 421.198±0.005 (calculated for $C_{22}H_{25}N_6O_3^+$: 421.198); HPLC t_R = 4.77 min (96%); eluent: 80% MeOH + 0.1% TFA and 20% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

(*L*)-1-PyrenylSerGuaNH.CF$_3$COOH 9 was prepared from **20b** (20 mg, 3.4 x 10^{-5} mol), obtaining a brownish solid (18 mg, 90%); mp = 229 °C (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.71 (s, 1H, pyrrole NH); 11.47 (s, 1H, N*H*C(NH$_2$)$_2$); 10.51 (s, 1H, pyreneNH); 8.82 (d, 1H, Ser NH, *J* = 7.4 Hz); 8.36 (br s, 4H, (NH$_2$)$_2$); 8.33-8.28 (m, 4H, pyrene H); 8.22-8.16 (m, 4H, pyrene H); 8.08 (t, 1H, pyrene H, *J* = 7.7 Hz); 7.30 (br s, 1H, pyrrole CH); 7.01 (br s, 1H, pyrrole CH); 5.32 (br s, 1H, OH); 4.95 (q, 1H, CH, *J* = 6.5 Hz); 3.96 (br s, 2H, CH$_2$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 169.9 (pyreneNHCO); 159.9 (pyrrole CONH); 159.3 (pyrrole CONH); 155.3 (C(NH$_2$)$_2$); 132.5 (pyrrole Cq); 131.5 (pyrene Cq); 130.8 (pyrene Cq); 130.5 (pyrene Cq); 127.2 (pyrene CH); 127.1 (pyrene CH); 126.7 (pyrene CH); 126.4 (pyrene CH); 125.7 (pyrrole Cq); 125.3 (pyrene CH); 125.0 (pyrene CH); 124.9 (pyrene CH); 124.6 (pyrene Cq); 124.3 (pyrene Cq); 123.9 (pyrene Cq); 123.8 (pyrene CH); 122.7 (pyrene CH); 115.1 (pyrrole CH); 113.7 (pyrrole CH); 61.3 (CH$_2$); 55.7 (CH); HR-MS (ESI$^+$) m/z = 483.178±0.005 (calculated for $C_{26}H_{23}N_6O_4^+$: 483.177); HPLC t_R = 8.44 min (94%); eluent: 50% MeOH + 0.1% TFA and 50% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

(*L*)-2-naphthylSerGlyGuaNH.CF$_3$COOH 7 was prepared from **20c** (30 mg, 5.3 x 10^{-5} mol), obtaining a white solid (31 mg, quant); mp > 200 ºC; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.48 (br s, 1H, pyrrole NH); 11.13 (br s, 1H, guanidinium NH); 10.12 (s, 1H, naphthylNHCO); 8.80 (t, 1H, Gly NH, *J* = 5.8 Hz); 8.31-8.23 (m, 6H, naphthyl H$_1$, Ser NH and guanidinium (NH$_2$)$_2$); 7.90-7.63 (m, 3H, naphthyl H$_{4,5,8}$); 7.51-7.38 (m, 1H, naphthyl H$_6$ or H$_7$); 7.09 (br s, 1H, pyrrole CH); 6.93-6.92 (m, 1H, pyrrole CH); 4.54 (dd, 1H, Ser CH, *J* = 13.1 Hz, *J* = 7.6 Hz); 4.03 (d, 2H, Gly CH$_2$, *J* = 5.8 Hz); 3.72 (d, 2H, Ser CH$_2$, *J* = 5.3 Hz); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 169.4 (CONH or C(NH$_2$)$_2$); 169.2 (CONH or C(NH$_2$)$_2$); 160.3 (CONH or C(NH$_2$)$_2$); 135.9 (naphthyl Cq); 133.3 (pyrrole Cq and naphthyl Cq); 130.1 ((naphthyl Cq); 128.6 (naphthyl CH); 127.6 (naphthyl CH); 127.4 (naphthyl CH); 126.8 (pyrrole Cq and naphthyl CH); 125.1 (naphthyl CH); 120.2 (naphthyl CH); 115.9 (pyrrole CH and naphthyl CH); 112.9 (pyrrole CH); 61.5 (Ser CH$_2$);

56.0 (Ser CH); 42.2 (Gly CH_2); HR-MS (ESI^+) m/z = 466.184±0.005 (calculated for $C_{22}H_{24}N_7O_5^+$: 466.183); HPLC t_R = 4.80 min (94%); eluent: 80% MeOH + 0.1% TFA and 20% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

(*L*)-1-PyrenylSerGlyGuaNH.CF_3COOH 10 was prepared from **22** (17 mg, 2.6 x 10^{-5} mol), obtaining a brownish solid (14 mg, 82%); mp = 215 ºC (decomposition); ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 12.47 (br s, 1H, pyrrole NH); 11.11 (s, 1H, NH); 10.33 (s, 1H, pyreneNH); 8.83 (br t, 1H, Gly NH, *J* = 5.5 Hz); 8.34-8.27 (m, 9H, pyrene H, $(NH_2)_2$ and Ser NH); 8.19-8.16 (m, 4H, pyrene H); 8.08 (t, 1H, pyrene H); 7.08 (br s, 1H, pyrrole H); 6.93 (br s, 1H, pyrrole H); 5.25 (br s, 1H, OH); 4.77 (m, 1H, CH); 4.10-4.07 (m, 2H, Gly CH_2); 3.90 (br s, 1H, Ser CH_2); 3.84 (br s, 1H, Ser CH_2); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 169.9 (CONH); 169.1 (CONH); 159.5 (pyrroleCONH); 155.1 ($C(NH_2)_2$); 132.3 (pyrrole Cq); 131.4 (pyrene Cq); 130.8 (pyrene Cq); 130.5 (pyrene Cq); 128.5 (pyrene Cq); 127.2 (pyrene CH); 127.1 (pyrene CH); 126.7 (pyrene CH); 126.4 (pyrene CH); 125.2 (pyrene CH); 125.0 (pyrene CH); 124.9 (pyrene CH); 124.4 (pyrene Cq); 123.7 (pyrene CH); 122.6 (pyrene CH); 115.3 (pyrrole CH); 112.8 (pyrrole CH); 61.9 (Ser CH_2); 55.8 (CH); 42.1 (Gly CH_2); HR-MS (ESI^+) m/z = 540.199±0.005 (calculated for $C_{28}H_{26}N_7O_5^+$: 540.199); HPLC t_R = 8.46 min (97%); eluent: 50% MeOH + 0.1% TFA and 50% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

Trifluoroacetate of *N*-2-[(5-(acridin-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidinium 11 was prepared from **24** (30 mg, 5.25 x 10^{-5} mol), obtaining a yellow solid (28 mg, 91%); mp > 230 ºC; ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 12.37 (s, 1H, pyrrole NH); 11.40 (s, 1H, NH); 9.07 (t, 1H, acridineCON*H*, *J* = 5.5 Hz); 8.52 (t, 1H, acridineCONHCH$_2$CH$_2$-CH$_2$CH$_2$N*H*, *J* = 5.5 Hz); 8.37 (br s, 4H, $(NH_2)_2$); 8.21 (d, 2H, $H_{1,8}$, *J* = 8.7 Hz); 8.00 (d, 2H, $H_{4,5}$, *J* = 8.7 Hz); 7.93-7.7.89 (m, 2H, $H_{3,6}$); 7.70-7.66 (m, 2H, $H_{2,7}$); 7.23-7.22 (m, 1H, pyrrole CH); 6.90-6.89 (m, 1H, pyrrole H); 3.57-3.52 (m, 2H, acridineCONHC*H*$_2$CH$_2$-CH$_2$CH$_2$NH); 3.38-3.34 (m, 2H, acridineCONHCH$_2$CH$_2$CH$_2$C*H*$_2$-NH); 1.72-1.69 (m, 4H, acridineCONHCH$_2$C*H*$_2$C*H*$_2$CH$_2$NH); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 165.1 (acridine*C*ONH); 159.1 (CONH or $C(NH_2)_2$); 158.5 (CONH or C(=NH)NH); 146.8 ($C_{4a,10a}$); 143.0 (C_9); 132.4 (pyrrole Cq); 130.8 ($CH_{3,6}$); 127.8 ($CH_{4,5}$); 126.4 ($CH_{2,7}$); 125.2 ($CH_{1,8}$); 124.8 (pyrrole Cq); 121.8 ($C_{8a,9a}$); 115.1 (pyrrole CH); 111.8 (pyrrole CH); 38.2 (acridineCONH*C*H$_2$CH$_2$-CH$_2$*C*H$_2$NH); 38.0 (acridineCONH*C*H$_2$CH$_2$-CH$_2$*C*H$_2$NH); 26.3 (acridineCONHCH$_2$*C*H$_2$*C*H$_2$CH$_2$NH); 26.1 (acridineCONHCH$_2$-*C*H$_2$*C*H$_2$CH$_2$NH); HR-MS (ESI^+) m/z = 472.210±0.005 (calculated for $C_{25}H_{26}N_7O_3$+: 472.210). HPLC: t_R = 5.57 min (95%); eluent: 50% MeOH + 0.1% TFA and 50% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

(*L*)-*N*-1-PyrenylGly-(1-pyrenoyldiaminoethane) Arg Analogue NH.CF_3COOH 13 was prepared from **30** (21 mg, 2.20 x 10^{-5} mol). The residue was purified by MPLC (RP18, eluent: 20% MeOH + 0.1% TFA in H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA) obtaining a pale brown solid (9 mg, 43%); ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 12.35 (s, 1H, pyrrole NH); 11.06 (s, 1H, pyrroleCON*H*); 8.95 (t, 1H, pyreneCONH, *J* = 5.7 Hz); 8.68 (t, 1H, pyreneCONH, *J* = 5.5 Hz); 8.61-8.56 (m, 2H, pyrene H and CON*H*CH$_2$CH$_2$); 8.48 (d, 1H, pyrene H, *J* = 9.2 Hz); 8.38 (d, 1H, Gly NH, *J* = 7.8 Hz); 8.34-8.07 (m, 21H, pyrene H, $(NH_2)_2$ and NH); 6.98 (br s, 1H, pyrrole H); 6.86-6.84 (m, 1H, pyrrole H); 4.59 (q, 1H, CH, *J* = 6.8 Hz); 4.18-4.07 (m, 2H, Gly CH_2); 3.77-3.42 (m, 6H, 3CH_2); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 172.2 (CONH or $C(NH_2)_2$); 169.4 (CONH or $C(NH_2)_2$); 169.1 (CONH or $C(NH_2)_2$); 168.7 (CONH or $C(NH_2)_2$); 168.6 (CONH or $C(NH_2)_2$); 159.3 (CONH or $C(NH_2)_2$); 157.8 (q, 1C, CF_3COO, $J_{C\text{-}F}$ = 31 Hz); 154.5 (CONH or $C(NH_2)_2$); 131.9 (pyrrole Cq); 131.2 (2pyrene Cq); 131.1 (pyrene Cq); 130.7 (pyrene Cq); 130.2 (2pyrene Cq); 129.7 (2pyrene Cq); 127.9 (pyrene CH); 127.8 (pyrene CH); 127.6 (pyrene CH); 127.5 (pyrene CH); 127.4 (pyrene Cq); 127.3 (pyrene Cq); 126.7 (pyrene CH); 126.6 (pyrene CH); 126.1 (2pyrene CH); 125.3 (2pyrene CH); 125.1 (2pyrene CH); 125.0 (pyrrole Cq); 124.8 (2pyrene CH); 124.3 (pyrene CH); 124.2 (pyrene CH); 123.8 (2pyrene CH); 123.2 (2pyrene Cq); 123.1 (2pyrene Cq); 116.6 (q, 1C, *C*F_3COO, $J_{C\text{-}F}$ = 299 Hz); 114.7 (pyrrole CH); 112.3 (pyrrole CH); 52.7 (CH); 42.6 (Gly CH_2); 40.3 (CH_2); 38.4 (2CH_2); HR-MS (ESI^+) m/z = 838.310±0.005 (calculated for $C_{48}H_{40}N_9O_6^+$: 838.309); HPLC: t_R = 22.58 min (99%); eluent: 40% MeOH + 0.1% TFA and 60% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

General procedure for coupling with PyBOP

A) A solution of acid (1 eq), PyBOP (1 eq) and NMM (3 eq) in dry DMF (3-7 mL) was stirred for 20 min at rt. Afterwards, amine (1 eq) was added and the solution was stirred at rt overnight. Then, it was poured onto water and the suspension was stirred at 0ºC for 2 h. The precipitate was filtered off, washed several times with water and lyophilized. The residue was used in the next step without further purification (**18c**) or was purified by flash chromatography (SiO_2) using the corresponding eluent (**23a-b** and **27**).

(*L*)-2-naphtylSerGlyNHBoc 18c was prepared from *N*-(*tert*-butoxycarbonyl)glycine (152 mg, 0.87 mmol), PyBOP (452 mg, 0.87 mmol), NMM (0.29 mL, 2.60 mmol) and (*L*)-*N*-2-naphthylserinamide (200 mg, 0.87 mmol), obtaining a white solid (314 mg, 93%); mp = 98-100 ºC; ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 10.04 (s, 1H, naphthylNHCO); 8.29 (d, 1H, naphthyl H_1); 7.96 (d, 1H, Ser NH, *J* = 7.7 Hz); 7.87-7.79 (m, 3H, naphthyl $H_{4,5,8}$); 7.67 (d, 1H, naphthyl H_3, *J* = 8.8 Hz); 7.47 (m, 1H, naphthyl $H_{6,7}$); 7.40 (m, 1H, naphthyl $H_{6,7}$); 7.06 (t, 1H, Gly NH, *J* = 5.4 Hz); 5.07 (t, 1H, OH, *J* = 5.4 Hz); 4.50 (q, 1H, Ser CH, *J* = 7.1 Hz); 3.74-3.63 (m, 4H, Ser CH_2 and Gly CH_2); 1.39 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 170.0 (CONH); 169.2 (CONH); 156.3 (NHCOO); 135.9 (naphthyl Cq); 133.3 (naphthyl Cq); 130.0 (naphthyl Cq); 128.5 (naphthyl CH); 127.6 (naphthyl CH); 127.3 (naphthyl CH); 126.7 (naphthyl CH); 125.0 (naphthyl CH); 120.1 (naphthyl CH); 115.9 (naphthyl CH); 79.0 (*C*$(CH_3)_3$); 61.4 (Ser CH_2); 55.7 (Ser CH); 43.3 (Gly CH_2); 28.2 (C(*C*$H_3)_3$); HR-MS (ESI^+) m/z = 410.169±0.005 (calculated for $C_{20}H_{25}N_3O_5$+Na^+: 410.169).

5-[(*tert*-Butoxycarbonyl)amino-*N*-2-pyrenylpropanamide 23a was prepared from 1-pyrenecarboxilic acid (200 mg, 0.81 mmol), PyBOP (423 mg, 0.81 mmol), NMM (0.27 mL, 2.44 mmol) and *tert*-butyl (2-aminoethyl)carbamate (130 mg, 0.81 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 2:1 → 9:1. Yield 260 mg (82%) of a white solid; mp = 196-198ºC; ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 8.66 (br t, 1H, pyreneCON*H*, *J* = 5.2 Hz); 8.52 (d, 1H, pyrene H, *J* = 9.3 Hz); 8.36-8.33 (m, 3H, pyrene H); 8.31-8.21 (m, 3H, pyrene H); 8.18-8.10 (m, 2H, pyrene H); 6.97 (br t, 1H, pyreneCONHCH$_2$CH$_2$N*H*, *J* = 5.4 Hz); 3.48-3.43 (m, 2H, pyreneCONHC*H*$_2$CH$_2$NH); 3.27-3.23 (m, 2H, pyreneCONH-

CH_2CH_2NH); 1.41 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 169.0 (pyrene*C*ONH); 155.8 (*C*OO^{t}Bu); 131.9 (pyrene Cq); 131.6 (pyrene Cq); 130.7 (pyrene Cq); 130.2 (pyrene Cq); 128.2 (pyrene CH); 128.0 (pyrene CH); 127.8 (pyrene Cq); 127.2 (pyrene CH); 126.5 (pyrene CH); 125.7 (pyrene CH); 125.6 (pyrene CH); 125.3 (pyrene CH); 124.8 (pyrene CH); 124.3 (pyrene CH); 123.8 (pyrene Cq); 123.6 (pyrene Cq); 77.7 ($C(CH_3)_3$); 39.2 (pyreneCONHCH_2CH_2NH); 28.3 ($C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 411.167±0.005 (calculated for $C_{24}H_{24}N_2O_3$+Na$^+$: 411.167).

5-[(*tert*-Butoxycarbonyl)amino-*N*-2-acridinylpentanamide 23b was prepared from 9-acridinecarboxylic acid (200 mg, 0.89 mmol), PyBOP (466 mg, 0.89 mmol) and NMM (0.29 mL, 0.92 mmol), and *tert*-butyl (4-aminobutyl)carbamate (169 mg, 0.89 mmol)[5]. Eluent for flash chromatography: EtOAc/*n*-hexane 7:1. Yield 242 mg (69%) of a yellow solid; mp = 153-155ºC; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 9.01 (t, 1H, acridineCON*H*, *J* = 5.5 Hz); 8.19 (d, 2H, $H_{1,8}$, *J* = 8.70 Hz); 7.98 (d, 2H, $H_{4,5}$, *J* = 8.7 Hz); 7.90-7.86 (m, 2H, $H_{3,6}$); 7.69-7.65 (m, 2H, $H_{2,7}$); 6.86 (br s, 1H, BocN*H*); 3.49 (q, 2H, BocNHCH$_2$CH$_2$CH$_2$C*H*$_2$, *J* = 6.4 Hz); 3.01 (q, 2H, BocNHC*H*$_2$CH$_2$CH$_2$CH$_2$, *J* = 6.4 Hz); 1.66-1.61 (m, 2H, BocNHCH$_2$C*H*$_2$C*H*$_2$CH$_2$); 1.57-1.51 (m, 2H, BocNHCH$_2$C*H*$_2$C*H*$_2$-CH$_2$); 1.39 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 165.9 (acridine*C*ONH); 155.7 (*C*OO^{t}Bu); 148.2 ($C_{4a,10a}$); 142.5 (C_9); 130.6 ($CH_{3,6}$); 129.3 ($CH_{4,5}$); 126.7 ($CH_{2,7}$); 125.6 ($CH_{1,8}$); 121.8 ($C_{8a,9a}$); 77.4 ($C(CH_3)_3$); 39.3 (BocNH*C*H$_2$CH$_2$CH$_2$*C*H$_2$); 38.8 (BocNH*C*H$_2$CH$_2$CH$_2$*C*H$_2$); 28.3 ($C(CH_3)_3$); 27.3 (BocNH*C*H$_2$*C*H$_2$-CH$_2$CH$_2$); 26.5 (BocNH*C*H$_2$*C*H$_2$CH$_2$CH$_2$); HR-MS (ESI$^+$) m/z = 394.212±0.005 (calculated for $C_{23}H_{28}N_3O_3^+$: 394.212).

***tert*-Butyl *N*-1-pyrenoylglycinate 27** was prepared from 1-pyrenecarboxilic acid (300 mg, 1.22 mmol), PyBOP (634 mg, 1.22 mmol), NMM (0.40 mL, 3.65 mmol) and *tert*-butyl glycinate (204 mg, 1.22 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 1:2. Yield 281 mg (64%) of a brownish solid; mp = 129-132 ºC; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 9.05 (t, 1H, NH, *J* = 5.9 Hz); 8.62 (d, 1H, pyrene H, *J* = 9.3 Hz); 8.38-8.34 (m, 3H, pyrene H); 8.29-8.22 (m, 3H, pyrene H); 8.16-8.11 (m, 2H, pyrene H); 4.05 (d, 2H, CH_2, *J* = 5.9 Hz); 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 168.8 (CONH or COO^{t}Bu); 168.5 (CONH or COO^{t}Bu); 131.2 (pyrene Cq); 130.9 (pyrene Cq); 130.2 (pyrene Cq); 129.7 (pyrene Cq); 127.8 (pyrene CH); 127.6 (pyrene CH); 127.3 (pyrene Cq); 126.6 (pyrene CH); 126.0 (pyrene CH); 125.3 (pyrene CH); 125.1 (pyrene CH); 124.6 (pyrene CH); 124.2 (pyrene CH); 123.9 (pyrene CH); 123.2 (pyrene Cq); 123.1 (pyrene Cq); 80.3 ($C(CH_3)_3$); 41.7 (CH_2); 27.3 ($C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 382.141±0.005 (calculated for $C_{23}H_{21}N_1O_3$+Na$^+$: 382.141).

B) Boc-protected amine (1 eq) was dissolved in a mixture 1:1 TFA/dry DCM (1-6 mL TFA, 1-6 mL dry DCM) and stirred at rt for 30 min. Solvent and excess of TFA were removed *in vacuo*, and the oily residue was lyophilized. Free amine was used in the next step without further purification. A solution of acid (1 eq), PyBOP (1 eq) and NMM (3 eq) in dry DMF (2-6 mL) was stirred for 20 min at rt. Afterwards, the free amine (1 eq) was added and the solution was stirred at RT overnight. Then, it was poured onto water and the suspension was stirred at 0ºC for 2 h. The precipitate was filtered off, washed several times with water and lyophilized. The residue was purified by flash chromatography (SiO_2) using the corresponding eluent, except **25**, which was used in the next step without further purification.

***N*-5-Boc *N*-2-[(5-(naphthalen-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidino 20a** was prepared from triethylammonium *N*-Boc 5-guanidinocarbonylpyrrole-2-carboxylate **19** (178 mg, 0.45 mmol), PyBOP (234 mg, 0.45 mmol), NMM (0.15 mL, 1.35 mmol) and free amine (160 mg, 0.45 mmol, from Boc-deprotection of **18a**, yield quant). Eluent for flash chromatography: EtOAc/*n*-hexane 4:1 → 9:1. Yield 112 mg (48%) of a white solid; mp = 127 ºC (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 10.85 (br s, 1H, Guanidino NH); 9.86 (s, 1H, naphN*H*CO); 9.31 (br s, 1H, Guanidino NH); 8.56 (br s, 1H, Guanidino NH); 8.36 (br s, 1H, naphNHCOCH$_2$CH$_2$CH$_2$CH$_2$N*H*); 8.06-8.04 (m, 1H, naphthyl H_8); 7.94-7.91 (m, 1H, naphthyl H_5); 7.75 (d, 1H, naphthyl H_4, *J* = 8.1 Hz); 7.68 (d, 1H, naphthyl H_2, *J* = 7.3 Hz); 7.54-7.52 (m, 2H, naphthyl $H_{6,7}$); 7.48 (t, 1H, naphthyl H_3, *J* = 7.8 Hz); 6.80 (br s, 2H, pyrrole CH); 3.32-3.30 (m, 2H, naphNHCOCH$_2$CH$_2$CH$_2$C*H*$_2$); 2.53-2.49 (m, 2H, naphNHCOC*H*$_2$-CH$_2$CH$_2$CH$_2$); 1.74-1.69 (m, 2H, CH_2); 1.63-1.60 (m, 2H, CH_2); 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 171.8 (CONH); 170.3 (CONH); 159.6 (CONH); 158.4 (CONH); 133.7 (naphthyl Cq and pyrrole Cq); 129.2 (pyrrole Cq); 129.1 (naphthyl CH); 127.8 (naphthyl Cq); 125.9 (naphthyl CH); 125.7 (naphthyl CH); 125.5 (naphthyl CH and Cq); 125.1 (naphthyl CH); 122.7 (naphthyl CH); 121.7 (naphthyl CH); 113.8 (pyrrole CH); 111.7 (pyrrole CH); 38.5 (naphNHCOCH$_2$CH$_2$CH$_2$*C*H$_2$); 35.6 (naphNHCO*C*H$_2$CH$_2$CH$_2$CH$_2$); 28.9 (naphNHCOCH$_2$CH$_2$*C*H$_2$-CH$_2$); 27.8 ($C(CH_3)_3$); 23.0 (naphNHCOCH$_2$*C*H$_2$CH$_2$CH$_2$); HR-MS (ESI$^+$) m/z = 521.251±0.005 (calculated for $C_{27}H_{32}N_6O_5$+H$^+$: 521.250).

(*L*)-1-PyrenylSerGuaNHBoc 20b was prepared from *N*-Boc 5-guanidinocarbonylpy-rrole-2-carboxylic acid **19** (71 mg, 0.24 mmol), PyBOP (124 mg, 0.24 mmol), NMM (79 µL, 0.71 mmol) and free amine (100 mg, 0.24 mmol, from Boc-deprotection of **18b**, yield 94%). Eluent for flash chromatography: EtOAc/*n*-hexane 5:1. Yield 115 mg (83%) of a yellowish solid; mp > 210 °C; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.64 (br s, 1H, pyrrole NH); 10.85 (br s, 1H, NH); 10.47 (s, 1H, pyrene NH); 9.31 (br s, 1H, NH); 8.66 (br s, 1H, Ser NH); 8.52 (br s, 1H, NH); 8.33-8.28 (m, 4H, pyrene H); 8.22-8.16 (m, 4H, pyrene H); 8.07 (t, 1H, pyrene H, *J* = 7.6 Hz); 6.90 (br s, 1H, pyrrole H); 6.87 (br s, 1H, pyrrole H); 5.27 (br s, 1H, OH); 4.92 (m, 1H, CH); 3.95 (br s, 2H, CH_2); 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 169.5 (CONH); 131.1 (pyrene Cq); 130.3 (pyrene Cq); 130.0 (pyrene Cq); 128.0 (pyrene Cq); 126.7 (pyrene CH); 126.6 (pyrene CH); 126.2 (pyrene CH); 125.9 (pyrene CH); 124.7 (pyrene CH); 124.5 (pyrene CH); 124.4 (pyrene CH); 124.0 (pyrene Cq); 123.8 (pyrene Cq); 123.3 (pyrene CH); 122.1 (pyrene CH); 112.5 (pyrrole CH); 61.3 (CH_2); 55.6 (CH); 27.2 ($C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 605.212±0.005 (calculated for $C_{31}H_{30}N_6O_6$+Na$^+$: 605.212).

(*L*)-2-naphtylSerGlyGuaNHBoc 20c was prepared from triethylammonium *N*-Boc 5-guanidinocarbonylpyrrole-2-carboxylate **19** (178 mg, 0.45 mmol), PyBOP (234 mg, 0.45 mmol), NMM (0.15 mL, 1.35 mmol) and free amine (180 mg, 0.45 mmol, from Boc-deprotection of **18c**, yield 94%), Eluent for flash chromatography: EtOAc/MeOH 98:2. Yield 200 mg (79%) of a brownish solid; mp = 178 ºC (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 10.81 (br s, 1H, Guanidino NH); 10.09 (s, 1H, naphthylNHCO); 9.33 (br s, 1H, Guanidino NH); 8.74 (t, 1H, Gly NH, *J* = 5.7 Hz); 8.55 (br s, 1H, Guanidino NH); 8.32 (d, 1H, naphthyl H_1, *J* = 1.6 Hz); 8.20 (d, 1H, Ser NH, *J* = 7.7 Hz); 7.89-7.81 (m, 3H, naphthyl $H_{4,5,8}$); 7.68 (dd, 1H, naphthyl H_3, *J* = 8.8 Hz,

J = 2.0 Hz); 7.47 (td, 1H, naphthyl H_6 or H_7, J = 6.8 Hz, J = 1.3 Hz); 7.41 (td, 1H, naphthyl H_6 or H_7, J = 6.8 Hz, J = 1.3 Hz); 6.84 (m, 2H, pyrrole CH); 5.08 (t, 1H, OH, J = 5.4 Hz); 4.55 (dd, 1H, Ser CH, J = 13.3 Hz, J = 5.4 Hz); 3.99 (d, 3H, Gly CH_2, J = 5.9 Hz); 3.75-3.72 (m, 2H, Ser CH_2); 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 168.6 (CONH or C=NH); 168.5 (CONH or C=NH); 159.6 (CONH or C=NH); 157.9 (CONH or C=NH); 135.8 (naphthyl Cq); 132.8 (pyrrole Cq and naphthyl Cq); 129.3 (naphthyl Cq); 127.7 (naphthyl CH); 126.9 (naphthyl CH); 126.7 (naphthyl CH); 125.8 (pyrrole Cq and naphthyl CH); 124.1 (naphthyl CH); 119.6 (naphthyl CH); 114.9 (pyrrole CH and naphthyl CH); 111.8 (pyrrole CH); 61.1 (Ser CH_2); 55.4 (CH); 41.6 (Gly CH_2); 27.2 ($C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 588.217±0.005 (calculated for $C_{27}H_{31}N_7O_7$+Na^+: 588.217).

(*L*)-1-PyrenylSerGlyNHBoc 21 was prepared from *N*-(*tert*-butoxycarbonyl)glycine (50 mg, 0.28 mmol), PyBOP (149 mg, 0.28 mmol), NMM (95 µL, 0.86 mmol) and free amine (120 mg, 0.28 mmol, from Boc-deprotection of **18b**, yield 94%). Eluent for flash chromatography: EtOAc/*n*-hexane 4:1. Yield 115 mg (87%) of a yellowish solid; mp = 182-185 °C; ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 10.28 (s, 1H, pyreneNH); 8.30-8.21 (m, 4H, pyrene H); 8.19-8.15 (m, 4H, pyrene H and Ser NH); 8.08 (t, 2H, pyrene H, J = 7.7 Hz); 7.08 (t, 2H, Gly NH); 5.24 (br t, 1H, OH, J = 5.3 Hz); 4.72-4.69 (m, 1H, CH); 3.91-3.87 (m, 1H, Ser CH_2); 3.82-3.78 (m, 1H, Ser CH_2); 3.72-3.69 (m, 2H, Gly CH_2); 1.36 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 169.9 (CONH); 169.7 (CONH); 155.9 (COO^{t}Bu); 131.5 (pyrene Cq); 130.8 (pyrene Cq); 130.5 (pyrene Cq); 128.5 (pyrene Cq); 127.2 (pyrene CH); 127.1 (pyrene CH); 126.7 (pyrene CH); 126.4 (pyrene CH); 125.2 (pyrene CH); 125.0 (pyrene CH); 124.5 (pyrene Cq); 124.3 (pyrene CH); 123.8 (pyrene CH); 122.6 (pyrene CH); 78.1 ($C(CH_3)_3$); 61.9 (Ser CH_2); 55.6 (CH); 43.4 (Gly CH_2); 30.4 ($C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 484.184±0.005 (calculated for $C_{26}H_{27}N_3O_5$+Na^+: 484.184).

(*L*)-1-PyrenylSerGlyGuaNHBoc 22 was prepared from **19** *N*-Boc 5-guanidinocarbonylpyrrole-2-carboxylic acid (30 mg, 9.9 x 10^{-5} mol), PyBOP (51 mg, 9.9 x 10^{-5} mol), NMM (33 µL, 29.6 x 10^{-5} mol) and free amine (47 mg, 9.9 x 10^{-5} mol, from Boc-deprotection of **21**, yield 67%). Eluent for flash chromatography: THF/*n*-hexane 3:1. Yield 49 mg (78%) of a yellowish solid; mp = 219 ºC (decomposition); ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 11.10 (br s, 1H, pyrrole NH); 10.73 (br s, 1H, NH); 10.31 (s, 1H, pyreneNH); 9.35 (br s, 1H, NH); 8.75 (br t, 1H, Gly NH, J = 5.7 Hz); 8.53 (br s, 1H, NH); 8.29-8.26 (m, 5H, pyrene H and Ser NH); 8.19-8.14 (m, 4H, pyrene H); 8.07 (t, 1H, pyrene H, J = 7.6 Hz); 6.87 (br s, 1H, pyrrole CH); 6.62 (br s, 1H, pyrrole CH); 4.10-4.00 (m, 2H, Gly CH_2); 3.93-3.88 (m, 1H, Ser CH_2); 3.86-3.81 (m, 1H, Ser CH_2); 1.46 (s, 9H, $C(CH_3)_3$); HR-MS (ESI$^+$) m/z = 662.233±0.005 (calculated for $C_{33}H_{33}N_7O_7$+Na^+: 662.233).

***N*-5-Boc *N*-2-[(5-(acridin-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidino 24** was prepared from *N*-Boc 5-guanidinocarbonylpyrrole-2-carboxylic acid **19** (40 mg, 0.13 mmol), PyBOP (70 mg, 0.13 mmol), NMM (44 µL, 0.41 mmol) and free amine (55 mg, 0.13 mmol, from Boc-deprotection of **23b**, yield 98%). Eluent for flash chromatography: EtOAc/*n*-hexane 10:1 → 14:1. Yield 30 mg (39%) of a yellow solid; mp = 198 ºC (decomposition); ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 11.81 (br s, 1H, NH); 9.61 (br s, 1H, NH); 9.06 (t, 1H, acridineCONH, J = 5.6 Hz); 8.80 (br s, 1H, NH); 8.47 (t, 1H, acridineCONHCH$_2$CH$_2$CH$_2$-CH$_2$NH, J = 5.4 Hz); 8.20 (d, 2H, $H_{1,8}$, J = 8.7 Hz); 8.00 (d, 2H, $H_{4,5}$, J = 8.7 Hz); 7.92-7.88 (m, 2H, $H_{3,6}$); 7.70-7.65 (m, 2H, acridine $H_{2,7}$); 6.97 (br s, 1H, pyrrole CH); 6.84 (s, 1H, pyrrole H); 3.56-3.52 (m, 2H, acridineCONHCH_2CH$_2$CH$_2$-CH$_2$NH); 3.48 (br s, 2H, acridineCONHCH$_2$CH$_2$CH$_2$CH_2NH); 1.72-1.69 (m, 4H, acridineCONHCH$_2$CH_2CH_2CH$_2$NH); 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 165.2 (acridine*C*ONH); 158.8 (acridineCONHCH$_2$CH$_2$CH$_2$CH$_2$NH*C*O); 147.1 ($C_{4a,10a}$); 142.6 (C_9); 130.5 ($CH_{3,6}$); 128.2 ($CH_{4,5}$); 126.3 ($CH_{2,7}$); 125.2 ($CH_{1,8}$); 121.3 ($C_{8a,9a}$); 114.5 (pyrrole CH); 111.6 (pyrrole CH); 82.1 ($C(CH_3)_3$); 38.2 (acridineCONH*C*H$_2$CH$_2$CH$_2$-CH$_2$NH); 37.7 (acridineCONHCH$_2$CH$_2$CH$_2$*C*H$_2$NH); 26.8 ($C(CH_3)_3$); 26.0 (acridineCONHCH$_2$*C*H$_2$*C*H$_2$CH$_2$NH); 25.9 (acridineCONHCH$_2$-*C*H$_2$*C*H$_2$CH$_2$NH); HR-MS (ESI$^+$) m/z = 572.261±0.005 (calculated for $C_{30}H_{34}N_7O_5^+$: 572.261).

(*L*)-1-PyrenoyldiaminoethaneLys(Cbz)NHBoc 25 was prepared from (*L*)-BocLys(Z)OH (69 mg, 0.18 mmol), PyBOP (94 mg, 0.18 mmol), NMM (0.06 mL, 0.54 mmol) and free amine (73 mg, 0.18 mmol, from Boc-deprotection of **23a**, yield quant), obtaining a brownish solid (106 mg, 90%); mp = 190 ºC (decomposition); ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 8.63-8.60 (m, 1H, pyreneCON*H*); 8.50 (d, 1H, pyrene H, J = 9.3 Hz); 8.34-8.31 (m, 3H, pyrene H); 8.27-8.23 (m, 3H, pyrene H); 8.20-8.09 (m, 2H, pyrene H); 8.03 (br t, 1H, pyreneCONHCH$_2$CH$_2$N*H*, J = 5.6 Hz); 7.36-7.28 (m, 5H, Ph); 7.19 (br t, 1H, CbzN*H*, J = 5.5 Hz); 6.79 (d, 1H, LysN*H*, J = 7.7 Hz); 4.99 (s, 2H, PhCH$_2$); 3.88-3.85 (m, 1H, Lys CH); 3.50-3.42 (m, 4H, pyreneCONHCH_2CH_2NH); 2.97-2.92 (m, 2H, CbzNHCH_2); 1.70-1.58 (m, 1H, CbzNHCH$_2$CH$_2$CH$_2$CH_2); 1.57-1.44 (m, 1H, CbzNHCH$_2$CH$_2$CH$_2$CH_2); 1.34-1.20 (br s, 13H, CbzNHCH$_2$CH_2CH_2CH$_2$ and $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 172.0 (pyrene*C*ONH); 168.5 (Lys CONH); 155.6 (COOBn or COO^{t}Bu); 154.9 (COOBn or COO^{t}Bu); 136.7 (C_1 Ph); 131.3 (pyrene Cq); 131.1 (pyrene Cq); 130.2 (pyrene Cq); 129.7 (pyrene Cq); 127.8 (C_2 Ph, C_3 Ph and pyrene CH); 127.6 (pyrene CH); 127.3 (pyrene Cq); 127.2 (C_4 Ph); 126.7 (pyrene CH); 126.1 (pyrene CH); 125.3 (pyrene CH); 125.1 (pyrene CH); 124.8 (pyrene CH); 124.3 (pyrene CH); 123.8 (pyrene CH); 123.3 (pyrene Cq); 123.1 (pyrene Cq); 77.5 ($C(CH_3)_3$); 64.6 (PhCH_2); 53.9 (CH); 39.6 (pyreneCONH*C*H$_2$*C*H$_2$); 37.9 (Lys CH_2); 31.2 (Lys CH_2); 28.6 (Lys CH_2); 27.6 ($C(CH_3)_3$); 22.3 (Lys CH_2); HR-MS (ESI$^+$) m/z = 673.299±0.005 (calculated for $C_{38}H_{42}N_4O_6$+Na^+: 673.299).

(*L*)-1-PyrenoyldiaminoethaneLys(Cbz)GuaNHBoc 26 was prepared from *N*-Boc 5-guanidinocarbonylpyrrole-2-carboxylic acid **19** (34 mg, 0.11 mmol), PyBOP (60 mg, 0.11 mmol), NMM (0.04 mL, 0.34 mmol) and free amine (76 mg, 0.11 mmol, from Boc-deprotection of **LH324**, yield 98%) Eluent for flash chromatography: EtOAc/MeOH 95:5 → 9:1. Yield 70 mg (74%) of a brownish solid; mp = 136-139 ºC; ^{1}H-NMR ([D_6]DMSO, 400 MHz) δ = 11.51 (br s, 1H, pyrrole NH); 10.87 (br s, 1H, NH); 9.31 (br s, 1H, NH); 8.61 (t, 1H, pyreneCON*H*, J = 5.4 Hz); 8.51 (d, 1H, pyrene H, J = 9.3 Hz); 8.47 (d, 1H, Lys NH, J = 7.6 Hz); 8.46 (br s, 1H, NH); 8.33 (t, 2H, pyrene H, J = 8.4 Hz); 8.26-8.08 (m, 7H, pyrene H and NH); 7.35-7.27 (m, 5H, Ph); 7.21 (t, 1H, N*H*Cbz, J = 5.4 Hz); 6.82 (br s, 2H pyrrole H); 4.98 (s, 2H, PhCH$_2$); 4.41 (m, 1H, Lys CH); 3.50-3.36 (m, 4H, pyrene CONHCH$_2$CH$_2$NH); 2.96 (q, 2H, CbzNHCH_2, J = 6.4 Hz); 1.79-1.76 (m, 1H, CbzNHCH$_2$CH$_2$-CH$_2$CH_2); 1.69-1.66 (m, 1H, CbzNHCH$_2$CH$_2$CH$_2$-CH_2); 1.46-1.39 (m, 13H, CbzNHCH$_2$CH_2CH_2CH$_2$ and $C(CH_3)_3$); ^{13}C-NMR ([D_6]DMSO, 100 MHz) δ = 171.6 (pyrene*C*ONH); 168.5 (Lys CONH); 159.0 (pyrroleCONH); 157.9 (pyrroleCONH); 155.5 (COOBn and COO^{t}Bu); 136.7 (C_1 Ph); 131.3 (pyrene Cq); 131.0

(pyrene Cq); 130.2 (pyrene Cq); 129.7 (pyrene Cq); 127.8 (C_2 Ph, C_3 Ph and pyrene CH); 127.7 (pyrene CH); 127.5 (pyrene CH); 127.3 (pyrene Cq); 127.2 (C_4 Ph); 126.6 (pyrene CH); 126.0 (pyrene CH); 125.2 (pyrene CH); 125.0 (pyrene CH); 124.8 (pyrene CH); 124.3 (pyrene CH); 123.8 (pyrene CH); 123.3 (pyrene Cq); 123.1 (pyrene Cq); 113.1 (pyrrole CH); 112.4 (pyrrole CH); 69.3 ($C(CH_3)_3$); 64.6 (PhCH_2); 52.5 (CH); 39.4 (pyreneCONHCH_2CH_2); 38.6 (pyreneCONHCH_2CH_2); 37.6 (Lys CH_2); 31.2 (Lys CH_2); 28.6 (Lys CH_2); 27.2 (C(CH_3)$_3$); 22.5 (Lys CH_2); HR-MS (ESI^+) m/z = 829.368±0.005 (calculated for $C_{45}H_{49}N_8O_8^+$: 829.366).

(*L*)-1-PyrenylGlyArgAnalogue(OMe)NHBoc 29 was prepared from free acid (92 mg, 0.30 mmol, from ^{t}Bu-protected acid **27**, yield 66%), PyBOP (157 mg, 0.30 mmol), NMM (0.10 mL, 0.91 mmol) and ArgAnalogue **28** (120 mg, 0.30 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 10:1 → EtOAc → EtOAc/MeOH 9:1. Yield 120 mg (58%) of a brownish solid; mp > 230 ºC; ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 10.82 (br s, 2H, pyrrole NH and NH); 9.33 (br s, 1H, NH); 8.91 (t, 1H, NH, *J* = 5.9 Hz); 8.65 (m, 1H, pyrene H); 8.54-8.52 (m, 3H, NH); 8.36-8.33 (m, 3H, pyrene H); 8.28-8.20 (m, 4H, pyrene H); 8.14-8.10 (m, 1H, pyrene H); 6.79 (br s, 2H, pyrrole H); 4.60 (q, 1H, CH, *J* = 6.7 Hz); 4.11 (d, 2H, Gly CH_2, *J* = 5.9 Hz); 3.77-3.67 (m, 1H, NHCH_2CH); 3.67 (s, 3H, CH_3); 3.62-3.51 (m, 1H, NHCH_2CH); 1.46 (s, 9H, C(CH_3)$_3$); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 170.4 (pyreneCONH); 168.8 (Gly CONH and *C*OOCH$_3$); 159.7 (pyrroleCONH); 157.9 (pyrroleCONH); 131.2 (pyrene Cq); 130.9 (pyrene Cq); 130.2 (pyrene Cq); 129.7 (pyrene Cq); 127.8 (pyrene CH); 127.5 (pyrene CH); 127.4 (pyrene Cq); 126.7 (pyrene CH); 126.0 (pyrene CH); 125.3 (pyrene CH); 125.1 (pyrene CH); 124.8 (pyrene CH); 124.4 (pyrene CH); 123.9 (pyrene CH); 123.2 (pyrene Cq); 123.1 (pyrene Cq); 113.2 (pyrrole CH); 111.7 (pyrrole CH); 66.5 ($C(CH_3)_3$); 51.7 (CH and COOCH_3); 42.0 (Gly CH_2); 39.5 (CH_2); 27.2 (C(CH_3)$_3$); HR-MS (ESI^+) m/z = 704.243±0.005 (calculated for $C_{35}H_{35}N_7O_8$+Na^+: 704.244).

(*L*)-1-Pyrenoyldiaminoethane-Lys(Cbz)Gua-NH.CF$_3$COOH 12

26 (70 mg, 8.44 x 10^{-5} mol, 1 eq) was dissolved in 1.5 mL of TFA and 1.5 µL (0.1%) of TFMSA were added. The solution was stirred at rt for 24h. Then, TFA and TFMSA were removed under reduced pressure (oil pump). The oil obtained was dried and lyophilized. The white solid residue was purified by MPLC (RP18 column, flow 40-20 mL/min, eluent: 100% H_2O + 0.1% TFA → 100 % MeOH + 0.1% TFA), obtaining 40 mg (57%) of **12** as a white solid; mp = 170 ºC (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 12.51 (s, 1H, pyrrole NH); 11.79 (s, 1H, NH); 8.67-8.64 (m, 2H, NH); 8.51 (br s, 4H, $(NH_2)_2$); 8.50 (d, 1H, pyrene H, *J* = 9.3 Hz); 8.43-8.10 (m, 9H, pyrene H and NH); 7.72 (br s, 3H, NH_3); 7.39 (br s, 1H pyrrole H); 6.90 (m, 1H, pyrrole H); 4.50-4.44 (m, 1H, Lys CH); 3.51-3.39 (m, 4H, pyreneCONHCH_2CH_2); 2.82-2.69 (m, 2H, NH_3CH_2); 1.91-1.78 (m, 1H, $NH_3CH_2CH_2CH_2CH_2$); 1.73-1.64 (m, 1H, $NH_3CH_2CH_2CH_2CH_2$); 1.57-1.52 (m, 2H, $NH_3CH_2CH_2CH_2CH_2$); 1.47-1.36 (m, 2H, $NH_3CH_2CH_2CH_2CH_2$); ^{13}C-NMR ([D$_6$]DMSO 100 MHz) δ = 171.2 (pyrene*C*ONH); 168.5 (Lys CONH); 158.4 (pyrroleCONH); 157.5 (pyrroleCONH); 131.2 (pyrene Cq); 131.1 (pyrene Cq); 130.2 (pyrene Cq); 129.6 (pyrene Cq); 129.4 (pyrrole Cq); 127.7 (pyrene CH); 127.5 (pyrene CH); 127.3 (pyrene Cq); 126.7 (pyrene CH); 126.0 (pyrene CH); 125.3 (pyrene CH); 125.1 (pyrene CH and pyrrole Cq); 124.8 (pyrene CH); 124.2 (pyrene CH); 123.8 (pyrene CH); 123.2 (pyrene Cq); 123.1 (pyrene Cq); 114.9 (pyrrole CH); 113.1 (pyrrole CH); 52.3 (Lys CH); 38.5 (pyreneCONHCH_2CH_2); 37.9 (pyreneCONHCH_2-CH_2); 37.7 (Lys CH_2); 31.0 (Lys CH_2); 26.2 (Lys CH_2); 22.0 (Lys CH_2); HR-MS (ESI^+) m/z = 595.277±0.005 (calculated for $C_{32}H_{35}N_8O_4^+$: 595.277); HPLC: t_R = 5.89 min (99%); eluent: 50% MeOH + 0.1% TFA and 50% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA, flow 1 mL/min, λ = 300 nm.

(*L*)-*N*-1-PyrenylGly-(1-pyrenoyldiaminoethane) Arg Analogue NHBoc 30

29 (40 mg, 4.5 x 10^{-5} mol, 1 eq) was dissolved in a 4:1 mixture of THF/H_2O (3 mL THF, 0.75 mL H_2O) and LiOH.H_2O (3 mg, 6.6 x 10^{-5} mol, 1.5 eq), was added. The solution was stirred at rt for 2h, afterwards neutralized until pH 6 with 5% HCl aq and lyophilized. The acid obtained was used in the next step without further purification. A solution of the acid (50 mg, 7.5 x 10^{-5} mol, 1 eq), PyBOP (39 mg, 7.5 x 10^{-5} mol, 1 eq) and NMM (25 µL, 0.91 mmol, 3 eq) in dry DMF (3 mL) was stirred for 20 min at rt. Afterwards, free amine from **23a** (30 mg, 7.5 x 10^{-5} mol, 1 eq) was added and the solution was stirred at rt overnight. Then, it was poured onto water and the suspension was stirred at 0 ºC for 2 h. The precipitate was filtered off, washed several times with water and lyophilized. The residue was purified by flash chromatography (SiO_2, eluent: EtOAc/MeOH 9:1 → EtOAc/MeOH 8:2), yielding 16 mg (23%) of **30** as a brownish solid; mp = 250 ºC (decomposition); ^{1}H-NMR ([D$_6$]DMSO, 400 MHz) δ = 11.54-11.11 (br s, 1H, pyrrole NH); 11.11-10.65 (br s, 1H NH); 9.29 (br s, 1H, NH); 8.95 (t, 1H, pyreneCON*H*, *J* = 5.7 Hz); 8.68 (t, 1H, NH, *J* = 5.6 Hz); 8.60 (d, 1H, pyrene H, *J* = 9.2 Hz); 8.54 (br s, 1H, NH); 8.48 (d, 1H, pyrene H, *J* = 9.3 Hz); 8.39 (d, 1H, Gly NH, *J* = 7.5 Hz); 8.34-8.06 (m, 18H, pyrene H and 2NH); 6.77 (br s, 2H, pyrrole H); 4.55 (q, 1H, CH, *J* = 6.6 Hz); 4.58-4.00 (m, 2H, Gly CH_2); 3.73-3.39 (m, 6H, 3CH_2); 1.44 (s, 9H, C(CH_3)); ^{13}C-NMR ([D$_6$]DMSO, 100 MHz) δ = 169.4 (CONH); 169.1 (CONH); 168.7 (CONH); 168.6 (CONH); 157.9 (CONH); 131.2 (2pyrene Cq); 131.0 (pyrene Cq); 130.7 (pyrene Cq); 130.1 (2pyrene Cq); 129.6 (2pyrene Cq); 127.8 (pyrene CH); 127.7 (pyrene CH); 127.5 (2pyrene CH); 127.4 (pyrene Cq); 127.2 (pyrene Cq); 126.6 (2pyrene CH); 126.0 (2pyrene CH); 125.2 (2pyrene CH); 125.0 (2pyrene CH); 124.8 (2pyrene CH); 124.3 (pyrene CH); 124.2 (pyrene CH); 123.8 (2pyrene CH); 123.2 (2pyrene Cq); 123.1 (pyrene Cq); 123.0 (pyrene Cq); 113.1 (pyrrole CH); 111.7 (pyrrole CH); 62.3 ($C(CH_3)_3$); 53.0 (CH); 42.5 (Gly CH_2); 40.2 (CH_2); 38.7 (2CH_2); 27.3 (C(CH_3)$_3$); HR-MS (ESI^+) m/z = 960.344±0.005 (calculated for $C_{53}H_{47}N_9O_8$+Na^+: 960.344).

Spectroscopic studies:

Polynucleotides were purchased as noted: poly dA- poly dT, poly dAdT- poly dAdT, poly dGdC – poly dGdC, polyA-polyU, poly A, poly G, poly C, poly U (Sigma), calf thymus (ct)-DNA (Aldrich). Polynucleotides were dissolved Na-cacodylate buffer, I = 0.05 mol dm^{-3}, pH=7. Calf thymus ct-DNA was additionally sonicated and filtered through a 0.45 µm filter. Polynucleotide concentration was determined spectroscopicaly as concentration of phosphates.

The electronic absorption spectra were obtained on Varian Cary 100 Bio spectrometer, CD spectra were collected on the Jasco J-810 spectrometer and fluorescence spectra were recorded on a Varian Cary Eclipse fluorimeter; all in quartz cuvettes (1 cm). The measurements were performed in aqueous buffer solution (pH=7 -

Na-cacodylate buffer, I = 0.05 mol dm^{-3}, pH = 5 - buffer citric acid/NaOH, I=0.03 M). Under the experimental conditions used the absorbance and fluorescence intensities of studied compounds were proportional to their concentration. Relative fluorescence quantum yields (Q) were determined according to the standard procedure.[32] All samples were purged with argon to displace oxygen, emission spectra were recorded from 350 – 600 nm and corrected for the effects of time- and wavelength-dependent light-source fluctuations using a standard rhodamine 101, a diffuser and the software provided with the instrument. The sample concentration in fluorescence measurements had an optical absorbance below 0.05 at the excitation wavelength. As the standard we used N-acetyl-L-tryptophanamide (NATA, Fluka, Buchs, Switzerland) with published fluorescence quantum yield Q=0.14.[33] In fluorimetric titrations excitation wavelength of λ_{exc} > 320 nm was used to avoid inner filter effects caused by absorption of excitation light by added polynucleotide. The binding constant (*Ks*) and [bound compound] / [polynucleotide phosphate] ratio (*n*) were calculated according to the Scatchard equation by non-linear least-square fitting, giving excellent correlation coefficients (>0.999) for obtained values for *Ks* and *n*. Thermal melting curves for ds-polynucleotides and their complexes with studied compounds were determined as previously described by following the absorption change at 260 nm as a function of temperature.[25] The absorbance of studied compound was subtracted from every curve, and the absorbance scale was normalized. Obtained Tm values are the midpoints of the transition curves, determined from the maximum of the first derivative or graphically by a tangent method. Given ΔTm values were calculated subtracting *Tm* of the free nucleic acid from *Tm* of complex. Every ΔTm value here reported was the average of at least two measurements, the error in ΔTm is ± 0.5 °C.

Ethidium bromide (**EB**) displacement assay: to polynucleotide solution ($c = 2 \times 10^{-5}$ mol dm^{-3}) ethidium bromide ($c = 5 \times 10^{-6}$ mol dm^{-3}) was added (***r*** ([**EB**]/ [polynucleotide] = 0.4), and quenching of the **EB**/ polynucleotide complex fluorescence emission (λ_{ex} = 520 nm, λ_{em} = 601 nm) was monitored as function of c(**EB**)/c(compound). The given IC_{50} values present the ratio c(**EB**)/c(compound) = [Int(**EB**/ polynucleotide) – Int(**EB**$_{free}$)] / 2, where Int(**EB**/ polynucleotide) is fluorescence intensity of **EB**/ polynucleotide complex and Int(**EB**$_{free}$) is fluorescence intensity of the free ethidium bromide before polynucleotide is added.

Proliferation assays:

The growth inhibition activity was assessed as described previously,[34] according to the slightly modified procedure of the National Cancer Institute, Developmental Therapeutics Program.[35] The cells were inoculated onto standard 96-well microtiter plates on day 0. Test agents were then added in five consecutive 10-fold dilutions (10^{-8} to 10^{-4} mol/l; 10^{-5} for **13**) and incubated for further 72 hours. Working dilutions were freshly prepared on the day of testing. The solvent (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations (maximal concentration of DMSO was 0.25%). After 72 hours of incubation, the cell growth rate was evaluated by performing the MTT assay which detects dehydrogenase activity in viable cells. The absorbency (OD, optical density) was measured on a microplate reader at 570 nm. Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (*i.e.* 50%). Each result is a mean value from three separate experiments.

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[1] R. B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, Elsevier Academic Press, New York, **2004**.

[2] W.D. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski, D. Boykin, *Biochemistry,* **1993**, *32*, 4098-4104.

[3] N. Stevens, N. O'Connor, H. Vishwasrao, D. Samaroo, E. R. Kandel, D. L. Akins, C. M. Drain, N. J. Turro, *J. Am. Chem Soc*. **2008**, *130*, 7182-7183.

[4] M. Demeunynck, C. Bailly, W. D. Wilson, *DNA and RNA binders*, Wiley-VCH, Weinheim, **2002**.

[5] C. Bailly, R. K. Arafa, F. A. Tanious, W. Laine, C. le Tardy, A. Lansiaux, P. Colson, D. W. Boykin, W. D. Wilson, *Biochemistry* **2005**, *44*, 1941-1952.

[6] V. Peytou, R. Condom, N. Patino, R. Guedj, A.-M. Aubertin, N. Gelus, C. Bailly, R. Terreux, D. Cabrol-Bass, *J. Med. Chem.* **1999**, *42*, 4042-4053

[7] L. Hernandez-Folgado, C. Schmuck, S. Tomić, I. Piantanida, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2977-2981.

[8] a) F.M. Winnik, *Chem. Rev*. **1993**, *93*, 587-614; b) K. Kalyanasundaram, J. K. Thomas, *J. Am. Chem. Soc.* **1977**, *99*, 2039-2044; c) D. C. Dong, M. A. Winnik, *Photochem. Photobiol.* **1982**, *35*, 17.

[9] a) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum, New York, **1999**; b) F. M. Winnik, *Chem. Rev.* **1993**, *93*, 587-614; c) S. S. Lehrer, *Meth. Enzymol.* **1997**, *287*, 286-295.

[10] a) P. C. Bevilacqua, R. Kierzek, K. A. Johnson, D. H. Turner, *Science* **1992**, *258*, 1355-1358; b) K. Yamana, T. Gokota, H. Ozaki, H. Nakano, O. Sangen, T. Shimidzu, *Nucleosides Nucleotides* **1992**, *11*, 383-390; c) R. Kierzek, Y. Li, D. H. Turner, P. C. Bevilacqua, *J. Am. Chem. Soc.* **1993**, *115*, 4985-4992; d) Y. Li, P. C. Bevilacqua, D. Mathews, D. H. Turner, *Biochemistry* **1995**, *34*, 14394-14399; e) M. Manoharan, K. L. Tivel, M. Zhao, K. Nafisi, T. L. Netzel, *J. Phys. Chem.* **1995**, *99*, 17461-17472; f) J. Yguerabide, E. Talavera, J. M. Alvarez, M. Afkir, *Anal. Biochem.* **1996**, *241*, 238-247; g) J. Dapprich, N. G. Walter, F. Salingue, H. Staerk, *J. Fluoresc.* **1997**, *7*, 87S-89S; h) R. Preu, J. Dapprich, N. G. Walter, *J. Mol. Biol.* **1997**, *273*, 600-613; i) K. Yamana, R. Iwase, S. Furutani, H. Tsuchida, H. Zako, T. Yamaoka, A. Murakami, *Nucleic Acids Res.* **1999**, *27*, 2387-2392; j) S. K. Silverman, T. R. Cech, *Biochemistry* **1999**, *38*, 14224-14237; k) K. Yamana, H. Zako, K. Asazuma, R. Iwase, H. Nakano, A. Murakami, *Angew. Chem.* **2001**, *113*, 1138-1140; *Angew. Chem. Int. Ed.* **2001**, 40, 1104-1106; m) V. A. Korshun, D. A. Stetsenko, M. J. Gait, *J. Chem. Soc., Perkin Trans. 1* **2002**, 1092-1104.

[11] a) H. Fritzsche, A. Akhebat, E. Taillandier, K. Rippe, T. M. Jovin, *Nucleic Acids Res.* **1993**, *21*, 5085-5091; b) K. Ebata, M. Masuko, H. Ohtani, M. Kashiwasake-Jibu, *Photochem. Photobiol.* **1995**, *62*, 836-839; c) G. Tong, J. M. Lawlor, G.W. Tregear, J. Haralambidis, *J. Am. Chem. Soc.* **1995**, *117*, 12151-12158; d) F. D. Lewis, Y. Zhang, R. L. Letsinger, *J. Am. Chem. Soc.* **1997**, *119*, 5451-5452; e) K. V. Balakin, V. A. Korshun, I. A. Prokhorenko, G. V. Maleev, I. A. Kudelina, S. V. Gontarev, Y. A. Berlin, *Bioorg. Khim.* **1997**, *23*, 33-41; f) P. L. Paris, J. M. Langenhan, E. T. Kool, *Nucleic Acids Res.* **1998**, *26*, 3789-3793; g) K. V. Balakin, V. A. Korshun, I. I. Mikhalev, G. V. Maleev, A. D. Malakhov, I. A. Prokhorenko, Y. A. Berlin, *Biosens. Bioelectron.* **1998**, *13*, 771-778; h) M. Masuko, H. Ohtani, K. Ebata, A. Shimadzu, *Nucleic Acids Res.* **1998**, *26*, 5409-5416; i) V. A. Korshun, K. V. Balakin, T. A. Proskurina, I. I. Mikhalev, A. D. Malakhov, Y. A. Berlin, *Nucleosides Nucleotides.* **1999**, *18*, 2662-2676; j) E. Kostenko, M. Dobrikov, D. Pyshnyi, V. Petyuk, N. Komarova, V. Vlassov, M. Zenkova, *Nucleic Acids Res.* **2001**, *29*, 3611-3620; k) A. Mahara, R. Iwase, T. Sakamoto, K. Yamana, T. Yamaoka, A. Murakami, *Angew. Chem.* **2002**, *114*, 3800-3802; *Angew. Chem. Int. Ed.* **2002**, *41*, 3648-3650.

[12] a) K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J., Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182-8183; b) B. R. Babu, A. K. Prasad, S. Trikha, N. Thorup, V. S. Parmar, J. Wengel, *J. Chem. Soc., Perkin Trans. 1* **2002**, *22*, 2509-2519; c) U. B. Christensen, E. B. Pedersen, *Nucleic Acids Res.* **2002**, *30*, 4918-4925; d) V. V. Filichev, E. B. Pedersen, *Org. Biomol. Chem.* **2003**, *1*, 100-103; e) J. Michel, K. Bathany, J.-M. Schmitter, J.-P. Monti, S. Moreau, *Tetrahedron* **2002**, *58*, 7975-7982.

[13] C. Schmuck, L. Hernandez-Folgado, *Org. Biomol. Chem.* **2007**, *5*, 2390-2394.

[14] R. Houssin, J.-L. Bernier, J.-P. Hénichart, *Synthesis* **1988**, 259-261.

[15] C. Schmuck, V. Bickert, M. Merschky, L. Geiger, D. Rupprecht, J. Dudaczek, P. Wich, Th. Rehm, U. Machon, *Eur. J. Org. Chem*. **2008**, 324-329.

[16] J.-F. Pons, J.-L. Fauchère, F. Lamaty, A. Molla, R. Lazaro, *Eur. J. Org. Chem.* **1998**, *5*, 853-859.

[17] C. Schmuck, L. Geiger, *Chem. Commun*. **2005**, 772-774.

[18] G. Malojčić, I. Piantanida, M. Marinić, M. Žinić, M. Marjanović, M. Kralj, K. Pavelić, H.-J. Schneider, *Org. Biomol. Chem*. **2005**, *3*, 4373-4381.

[19] C.R. Cantor, P.R. Schimmel, *Biophysical Chemistry*, Vol. 3. WH Freeman and Co., San Francisco, **1980**.

[20] J. Ramstein, M. Leng, *Biochim. Biophys. Acta* **1972**, *281*, 18; b) M. G. Badea, S. Georghiou, *Photochem. and Photobiol*., **1976**, *24*, 417; c) S. Georghiou, *Photochem. and Photobiol*. **1977**, *26*, 59.

[21] I. Piantanida, B. S. Palm, M. Žinić, H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1808-1816.

[22] a) S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375-381; b) S. A. E. Marras, F. R. Kramer, S. Tyagi, *Nucleic Acids Res*. **2002**, *30*, 122.

[23] W. D. Wilson, Y.-H. Wang, C. R. Krishnamoorthy, J. C. Smith, *Biochemistry* **1985**, *24*, 3991-3999.

[24] G. Scatchard, *Ann. N.Y. Acad. Sci*. **1949**, *51*, 660-672; J.D. McGhee, P.H. von Hippel, *J. Mol. Biol*. **1976**, *103*, 679-684.

[25] B. S. Palm I. Piantanida, M. Žinić, H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2* **2000**, 385-392.

[26] A. Rodger, B. Norden, *Circular Dichroism and Linear Dichroism*, Oxford University Press, New York, **1997**.

[27] N. Berova, K. Nakanishi, R. W. Woody, *Circular dichroism Principles and Applications*, 2nd Edn, Wiley-VCH, New York, **2000**.

[28] M. Eriksson, B. Norden, *Methods in Enzymology* **2001**, *340*, 68-98, Table I.

[29] a) B. Norden, F. Tjerneld, *Biopolymers* **1982**, *21*, 1713-1734; b) R. Lyng, A. Rodger, B. Norden, Biopolymers **1991**, *31*, 1709-1820; c) P. E. Schipper, B. Norden, F. Tjerneld, *Chem. Phys. Lett.* **1980**, *70*, 17-21.

[30] a) I. F. Tannock, D. Rotin, *Cancer Res.* **1989** *49*, 4373-4384; b) P. Wong, C. Lee, I. F. Tannock, *Clin. Cancer Res.* **2005**, 11, 3553-2557; c) R. J. Gillies, I. Robey, R. A. Gatenby, *J. Nucl. Med.* **2008**, *49*, 24S-42S.

[31] N. Raghunand, R. J. Gillies, *Drug Resistance Updates* **2000**, *3*, 39-47.

[32] J.N.Miller, In *Standards for Fluorescence Spectrometry*, Chapman and Hall, London, **1981**.

[33] M.R. Eftink, Y. Jia, D. Hu, C.A. Ghiron, *J. Phys. Chem*., **1995**, *99*, 5713-5723.

[34] M. Marjanović, M. Kralj, F. Supek, L. Frkanec, I. Piantanida, T. Šmuc, L. Tušek-Božić, *J. Med. Chem.* **2007**, *50*, 1007-1018.

[35] M.R. Boyd, D.P. Kenneth, *Drug Dev. Res.* **1995**, *34*, 91-109.

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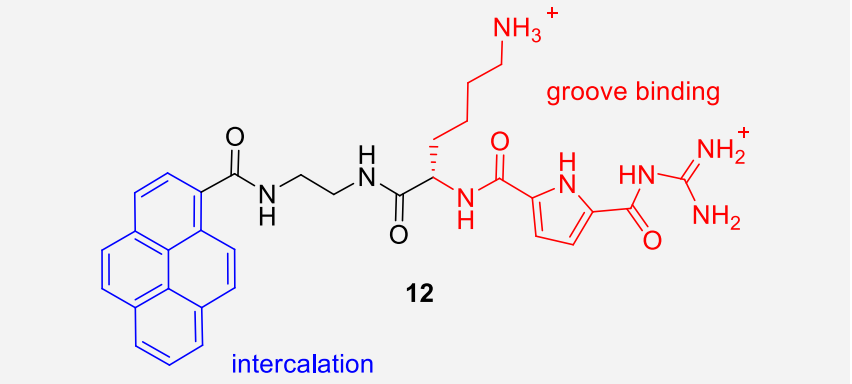
Entry for the Table of Contents (Please choose one layout only)

Layout 2:

DNA sensors with antiproliferative activity

Laura Hernandez-Folgado, Domagoj Baretić, Ivo Piantanida, Marko Marjanović, Marijeta Kralj, Thomas Rehm and Carsten Schmuck**…….…. Page – Page

Guanidiniocarbonyl-pyrrole-aryl derivatives: structure tuning for spectrophotometric recognition of specific DNA and RNA sequences and antiproliferative activity



A systematic study of guanidiniocarbonyl-pyrrole-aryl derivatives designed to interact with DNA or RNA both by intercalation as well as groove binding is presented. Distinctively different interactions with either DNA or RNA are seen by UV/Vis-, fluorescence and CD spectroscopy. For example, **12** shows base pair selective recognition of ds-DNA. The binding affinity to ds-DNA correlates with the antiproliferative activity of these compounds against several human tumour cell lines.

References:

[1] R. B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, Elsevier Academic Press, New York, **2004**.

[2] W.D. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski, D. Boykin, *Biochemistry,* **1993**, *32*, 4098-4104.

[3] N. Stevens, N. O'Connor, H. Vishwasrao, D. Samaroo, E. R. Kandel, D. L. Akins, C. M. Drain, N. J. Turro, *J. Am. Chem Soc*. **2008**, *130*, 7182-7183.

[4] M. Demeunynck, C. Bailly, W. D. Wilson, *DNA and RNA binders*, Wiley-VCH, Weinheim, **2002**, for **"**Minimal intercalator concept" see Chapter 18.

[5] C. Bailly, R. K. Arafa, F. A. Tanious, W. Laine, C. le Tardy, A. Lansiaux, P. Colson, D. W. Boykin, W. D. Wilson, *Biochemistry* **2005**, *44*, 1941-1952.

[6] V. Peytou, R. Condom, N. Patino, R. Guedj, A.-M. Aubertin, N. Gelus, C. Bailly, R. Terreux, D. Cabrol-Bass, *J. Med. Chem.* **1999**, *42*, 4042-4053

[7] L. Hernandez-Folgado, C. Schmuck, S. Tomić, I. Piantanida, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2977-2981.

[8] a) F.M. Winnik, *Chem. Rev*. **1993**, *93*, 587-614; b) K. Kalyanasundaram, J. K. Thomas, *J. Am. Chem. Soc.* **1977**, *99*, 2039-2044; c) D. C. Dong, M. A. Winnik, *Photochem. Photobiol.* **1982**, *35*, 17.

[9] a) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum, New York, **1999**; b) F. M. Winnik, *Chem. Rev.* **1993**, *93*, 587-614; c) S. S. Lehrer, *Meth. Enzymol.* **1997**, *287*, 286-295.

[10] a) P. C. Bevilacqua, R. Kierzek, K. A. Johnson, D. H. Turner, *Science* **1992**, *258*, 1355-1358; b) K. Yamana, T. Gokota, H. Ozaki, H. Nakano, O. Sangen, T. Shimidzu, *Nucleosides Nucleotides* **1992**, *11*, 383-390; c) R. Kierzek, Y. Li, D. H. Turner, P. C. Bevilacqua, *J. Am. Chem. Soc.* **1993**, *115*, 4985-4992; d) Y. Li, P. C. Bevilacqua, D. Mathews, D. H. Turner, *Biochemistry* **1995**, *34*, 14394-14399; e) M. Manoharan, K. L. Tivel, M. Zhao, K. Nafisi, T. L. Netzel, *J. Phys. Chem.* **1995**, *99*, 17461-17472; f) J. Yguerabide, E. Talavera, J. M. Alvarez, M. Afkir, *Anal. Biochem.* **1996**, *241*, 238-247; g) J. Dapprich, N. G. Walter, F. Salingue, H. Staerk, *J. Fluoresc.* **1997**, *7*, 87S-89S; h) R. Preu, J. Dapprich, N. G. Walter, *J. Mol. Biol.* **1997**, *273*, 600-613; i) K. Yamana, R. Iwase, S. Furutani, H. Tsuchida, H. Zako, T. Yamaoka, A. Murakami, *Nucleic Acids Res.* **1999**, *27*, 2387-2392; j) S. K. Silverman, T. R. Cech, *Biochemistry* **1999**, *38*, 14224-14237; k) K. Yamana, H. Zako, K. Asazuma, R. Iwase, H. Nakano, A. Murakami, *Angew. Chem.* **2001**, *113*, 1138-1140; *Angew. Chem. Int. Ed.* **2001**, 40, 1104-1106; m) V. A. Korshun, D. A. Stetsenko, M. J. Gait, *J. Chem. Soc., Perkin Trans. 1* **2002**, 1092-1104.

[11] a) H. Fritzsche, A. Akhebat, E. Taillandier, K. Rippe, T. M. Jovin, *Nucleic Acids Res.* **1993**, *21*, 5085-5091; b) K. Ebata, M. Masuko, H. Ohtani, M. Kashiwasake-Jibu, *Photochem. Photobiol.* **1995**, *62*, 836-839; c) G. Tong, J. M. Lawlor, G.W. Tregear, J. Haralambidis, *J. Am. Chem. Soc.* **1995**, *117*, 12151-12158; d) F. D. Lewis, Y. Zhang, R. L. Letsinger, *J. Am. Chem. Soc.* **1997**, *119*, 5451-5452; e) K. V. Balakin, V. A. Korshun, I. A. Prokhorenko, G. V. Maleev, I. A. Kudelina, S. V. Gontarev, Y. A. Berlin, *Bioorg. Khim.* **1997**, *23*, 33-41; f) P. L. Paris, J. M. Langenhan, E. T. Kool, *Nucleic Acids Res.* **1998**, *26*, 3789-3793; g) K. V. Balakin, V. A. Korshun, I. I. Mikhalev, G. V. Maleev, A. D. Malakhov, I. A. Prokhorenko, Y. A. Berlin, *Biosens. Bioelectron.* **1998**, *13*, 771-778; h) M. Masuko, H. Ohtani, K. Ebata, A. Shimadzu, *Nucleic Acids Res.* **1998**, *26*, 5409-5416; i) V. A. Korshun, K. V. Balakin, T. A. Proskurina, I. I. Mikhalev, A. D. Malakhov, Y. A. Berlin, *Nucleosides Nucleotides.* **1999**, *18*, 2662-2676; j) E. Kostenko, M. Dobrikov, D. Pyshnyi, V. Petyuk, N. Komarova, V. Vlassov, M. Zenkova, *Nucleic Acids Res.* **2001**, *29*, 3611-3620; k) A. Mahara, R. Iwase, T. Sakamoto, K. Yamana, T. Yamaoka, A. Murakami, *Angew. Chem.* **2002**, *114*, 3800-3802; *Angew. Chem. Int. Ed.* **2002**, *41*, 3648-3650.

[12] a) K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J., Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182-8183; b) B. R. Babu, A. K. Prasad, S. Trikha, N. Thorup, V. S. Parmar, J. Wengel, *J. Chem. Soc., Perkin Trans. 1* **2002**, *22*, 2509-2519; c) U. B. Christensen, E. B. Pedersen, *Nucleic Acids Res.* **2002**, *30*, 4918-4925; d) V. V. Filichev, E. B. Pedersen, *Org. Biomol. Chem.* **2003**, *1*, 100-103; e) J. Michel, K. Bathany, J.-M. Schmitter, J.-P. Monti, S. Moreau, *Tetrahedron* **2002**, *58*, 7975-7982.

[13] C. Schmuck, L. Hernandez-Folgado, *Org. Biomol. Chem.* **2007**, *5*, 2390-2394.

[14] R. Houssin, J.-L. Bernier, J.-P. Hénichart, *Synthesis* **1988**, 259-261.

[15] C. Schmuck, V. Bickert, M. Merschky, L. Geiger, D. Rupprecht, J. Dudaczek, P. Wich, Th. Rehm, U. Machon, *Eur. J. Org. Chem*. **2008**, 324-329.

[16] J.-F. Pons, J.-L. Fauchère, F. Lamaty, A. Molla, R. Lazaro, *Eur. J. Org. Chem.* **1998**, *5*, 853-859.

[17] C. Schmuck, L. Geiger, *Chem. Commun*. **2005**, 772-774.

[18] G. Malojčić, I. Piantanida, M. Marinić, M. Žinić, M. Marjanović, M. Kralj, K. Pavelić, H.-J. Schneider, *Org. Biomol. Chem*. **2005**, *3*, 4373-4381.

[19] C.R. Cantor, P.R. Schimmel, *Biophysical Chemistry*, Vol. 3. WH Freeman and Co., San Francisco, **1980**.

[20] J. Ramstein, M. Leng, *Biochim. Biophys. Acta* **1972**, *281*, 18; b) M. G. Badea, S. Georghiou, *Photochem. and Photobiol*., **1976**, *24*, 417; c) S. Georghiou, *Photochem. and Photobiol*. **1977**, *26*, 59.

[21] I. Piantanida, B. S. Palm, M. Žinić, H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1808-1816.

[22] a) S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375-381; b) S. A. E. Marras, F. R. Kramer, S. Tyagi, *Nucleic Acids Res*. **2002**, *30*, 122.

[23] W. D. Wilson, Y.-H. Wang, C. R. Krishnamoorthy, J. C. Smith, *Biochemistry* **1985**, *24*, 3991-3999.

[24] G. Scatchard, *Ann. N.Y. Acad. Sci*. **1949**, *51*, 660-672; J.D. McGhee, P.H. von Hippel, *J. Mol. Biol*. **1976**, *103*, 679-684.

[25] B. S. Palm I. Piantanida, M. Žinić, H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2* **2000**, 385-392.

[26] A. Rodger, B. Norden, *Circular Dichroism and Linear Dichroism*, Oxford University Press, New York, **1997**.

[27] N. Berova, K. Nakanishi, R. W. Woody, *Circular dichroism Principles and Applications*, 2nd Edn, Wiley-VCH, New York, **2000**.

[28] M. Eriksson, B. Norden, *Methods in Enzymology* **2001**, *340*, 68-98, Table I.

[29] a) B. Norden, F. Tjerneld, *Biopolymers* **1982**, *21*, 1713-1734; b) R. Lyng, A. Rodger, B. Norden, Biopolymers **1991**, *31*, 1709-1820; c) P. E. Schipper, B. Norden, F. Tjerneld, *Chem. Phys. Lett.* **1980**, *70*, 17-21.

[30] a) I. F. Tannock, D. Rotin, *Cancer Res.* **1989** *49*, 4373-4384; b) P. Wong, C. Lee, I. F. Tannock, *Clin. Cancer Res.* **2005**, 11, 3553-2557; c) R. J. Gillies, I. Robey, R. A. Gatenby, *J. Nucl. Med.* **2008**, *49*, 24S-42S.

[31] N. Raghunand, R. J. Gillies, *Drug Resistance Updates* **2000**, *3*, 39-47.

[32] J.N.Miller, In *Standards for Fluorescence Spectrometry*, Chapman and Hall, London, 1981.

[33] M.R. Eftink, Y. Jia, D. Hu, C.A. Ghiron, *J. Phys. Chem*., **1995**, *99*, 5713-5723.

[34] M. Marjanović, M. Kralj, F. Supek, L. Frkanec, I. Piantanida, T. Šmuc, L. Tušek-Božić, *J. Med. Chem.* **2007**, *50*, 1007-1018.

[35] M.R. Boyd, D.P. Kenneth, *Drug Dev. Res.* **1995**, *34*, 91-109.