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Guanidiniocarbonylpyrrole–Aryl Derivatives: Structure Tuning for Spectrophotometric Recognition of Specific DNA and RNA Sequences and for Antiproliferative Activity

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Abstract: We present a systematic study of different guanidiniocarbonylpyrrole-aryl derivatives designed to interact with DNA or RNA both through intercalation of an aromatic moiety into the base stack of the nucleotide and through groove binding of a guanidiniocarbonylpyrrole 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, and 3) the total number of positive charges present at different pH values. The compounds and their interactions with DNA and RNA were studied by UV/Vis, fluorescence and CD spectroscopy. Antiproliferative activities against human tumour cell lines were also determined. Our studies show that efficient interaction with, for example, DNA requires a significantly large aromatic ring (pyrene) connected through a flexible linker to the pyrrole moiety. However, a positive charge, as in **12**, is also needed. Compound **12** allows for base-pair-selective recognition of ds-DNA at physiological pH values. The antiproliferative activities of these compounds correlate with their binding affinities towards DNA, suggesting that their biological effects are most probably due to DNA binding.

Keywords: antiproliferation · DNA recognition · intercalations · structure–activity relationships · RNA recognition

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—such as netropsin, a potent antiviral and antitumor agent, and DAPI, a fluorescent indicator with pronounced antitrypanosomal activity—act by interacting with DNA/RNA. Such compounds often owe their biological activities to binding into the DNA minor groove through multiple interactions between cationic functional groups (e.g., guanidinium or amidinium groups) and the DNA base pairs and/or phosphate backbone. However, the strong DNA selectivity of minor groove binders is disadvantageous for the case of RNA targeting. Another common binding mode is intercalation (e.g., echinomycin, anthracyclines)—the insertion of a large aromatic moiety into the base stack of a nucleic acid—which is characterised by similar affinities toward DNA and RNA. Some compounds even exhibit a binding mode switch (e.g., intercalation into G-C sequences and groove binding into A-T sequences).[2] Combination of two aromatic units with different but specific spectroscopic properties allows specific spectroscopic responses to be achieved upon interaction both/either with DNA and/or RNA.[3] However, it has been 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 proba-

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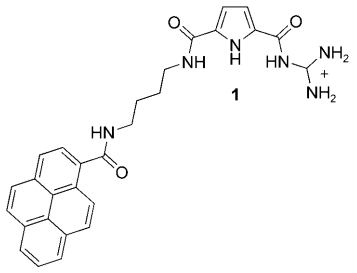
bly as a result of the limited solubilities of large aromatic groups in aqueous solvents. Therefore, one aim of research in this area is the development of minimal DNA intercalators.[4] Another possible option is to combine intercalation with groove binding, one area of research in which we are currently interested. Moreover, a recent report on a bis-guanidinium derivative of ethidium that is highly selective for AT-rich DNA regions demonstrates the potential of hybrid compounds containing both an intercalator and a positively charged group such as a guanidinium cation.[5] In addition, one such compound has been shown to have distinct antiviral activity towards HIV-1, based on TAR RNA.[6]

We recently reported a first example of a guanidiniocarbonylpyrrole-pyrene hybrid molecule in the form of compound **1**, containing both an intercalator unit (the pyrene)



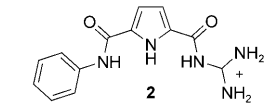
and a cationic group capable of groove binding (the guanidiniocarbonylpyrrole cation).[7] Pyrene is a well known polarity-sensitive probe and its fluorescence has been extensively employed for characterisation of microheterogeneous systems.[8] A long lifetime of the excited state and the possibility 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 guanidiniocarbonylpyrrole cation was expected to enter into multiple noncovalent interactions (hydrogen bonds and ion pairs) with DNA and RNA. In addition, electrostatic interactions are tuneable through external stimuli, because protonation of the acylguanidinium component (pK_a ca. 6–7) is directly correlated to the pH of the aqueous solution.

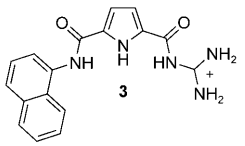
Most interestingly, compound **1** exhibited unique and distinctly different patterns of spectroscopic interaction behaviour with DNA and with RNA. With ds-DNA a strong induced CD signal was observed at about $\lambda = 300$ nm, whereas under the same conditions a new fluorescence maximum at $\lambda = 480$ nm appeared exclusively upon the addition of RNA. These differences in behaviour could be explained in terms of a switch in binding mode. With ds-DNA, compound **1** intercalates its pyrene moiety into the ds-DNA and at the same time the guanidiniocarbonylpyrrole cation binds into the minor groove (giving rise to the ICD signal), whereas with ds-RNA two or more molecules of **1** form a π-stacked excimer, most probably binding into the major groove of ds-RNA, which gives rise to the strong excimer fluorescence at $\lambda = 480$ nm.

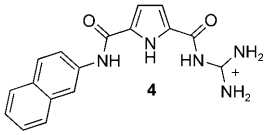
We now report on a systematic study of a series of related hybrid molecules, in which we varied 1) the aromatic unit, 2) the length and rigidity of the linker between the aromatic and the cationic groups, and 3) the number of charges present at different pH values. The driving force for the DNA/RNA binding 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 guanidiniocarbonylpyrrole cation within one of the DNA/RNA grooves. Whereas the former is predominantly attractive in nature, the latter involves both attractive electrostatic interactions—including hydrogen bonding—and steric repulsion. In total we examined 13 compounds. Here we describe their syntheses and a study of their 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 potential for fine-tuning of small-molecule–DNA or –RNA interactions and indicate a correlation between the affinities of these small molecules toward DNA and their observed antiproliferative activities.

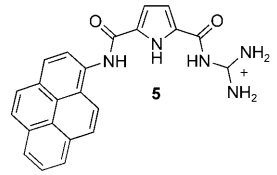
Results and Discussion

Design of the compounds: Firstly, in the series **2**–**5** we directly connected aromatic units of increasing size to the guanidiniocarbonylpyrrole cation.

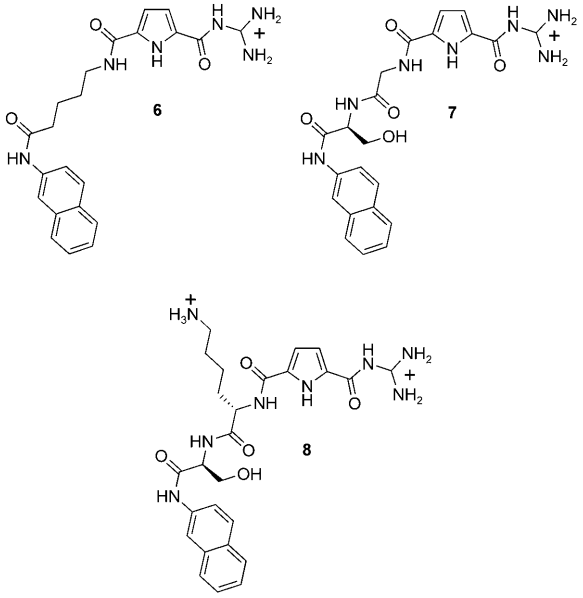








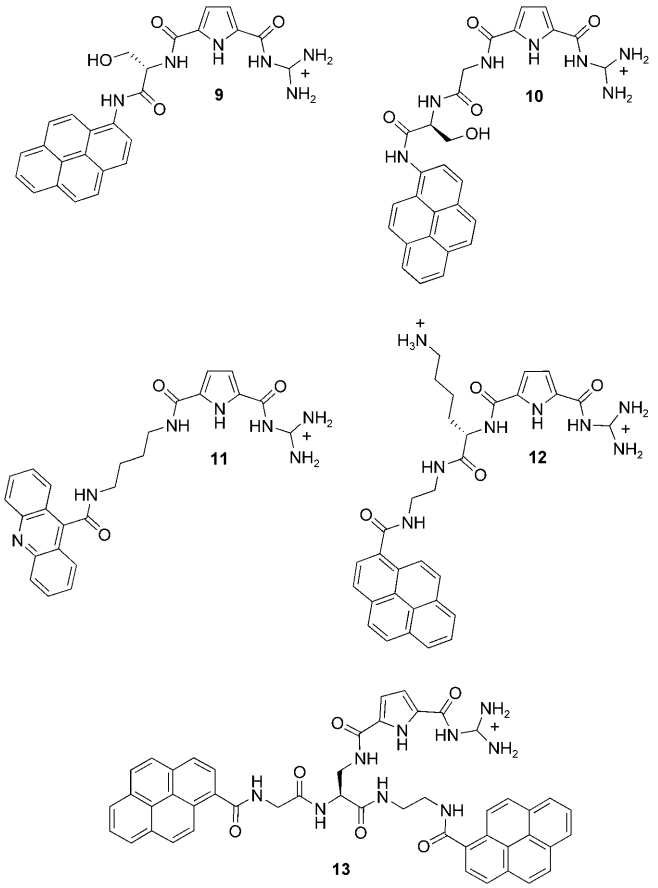
Like compound **4**, compounds **6**, **7** and **8** each contain a naphthalene unit but with an additional linker between the naphthyl moiety and the guanidiniocarbonylpyrrole cation, to ease structural accommodation of the molecule within the double-stranded helix of the polynucleotide. We varied the rigidity and polarity of the linker (flexible and nonpolar 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 in **7** and Lys-Ser in **8**. The amino acids were chosen both to provide additional sites for potential interac-



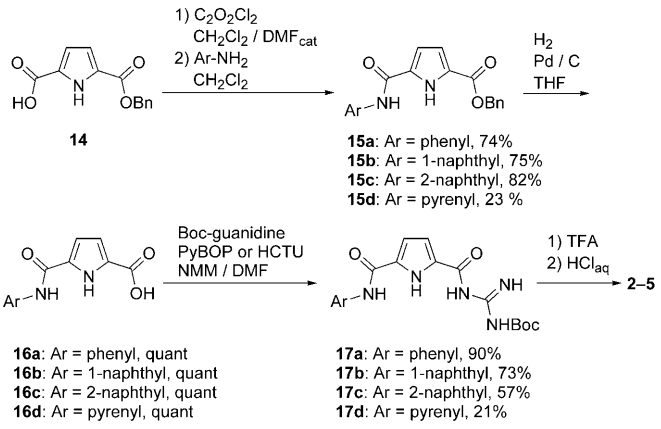
tions with the nucleic acids (by H-bonds to the amide groups, for example) and to increase the solubilities (serine, lysine) of the compounds.

Furthermore, five compounds each containing either a pyrene or an acridine moiety were synthesised, both to increase their affinities toward DNA/RNA (relative to the naphthalene-based compounds) and also to take advantage of their superior fluorescent properties, the pyrene moiety being chosen for its strong fluorescence, which should allow selective and highly sensitive recognition of specific DNA/RNA structures.

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

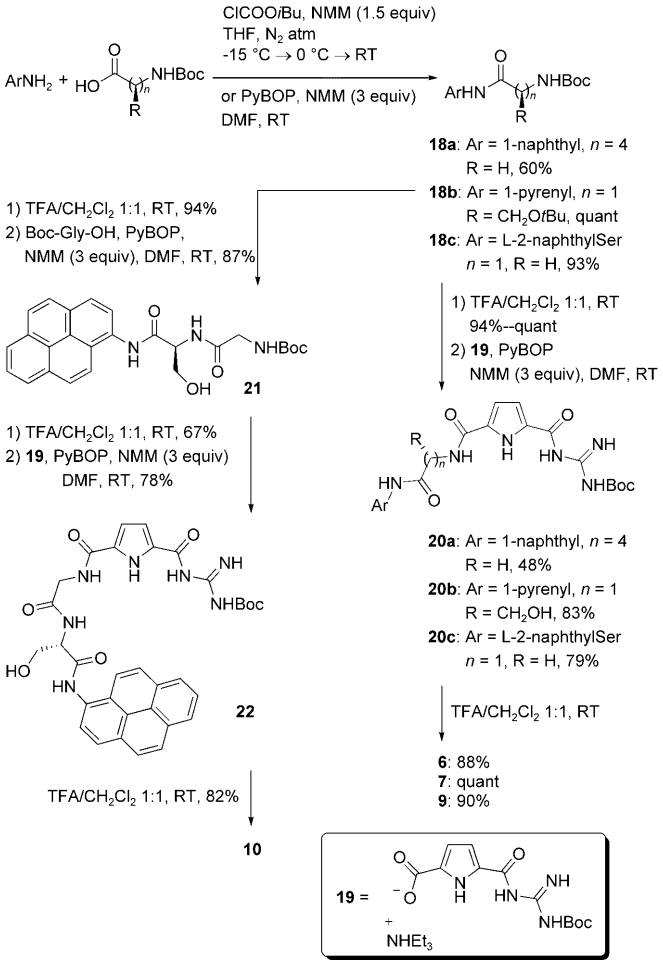


Synthesis of the compounds: The syntheses of **1**[7] and **8**[13] had been reported previously. Compounds **2–5** were synthesised as shown in Scheme 1. Because of their low nucleophilicities the aryl amines used were coupled to the starting compound **14** via its acid chloride. After hydrogenolysis of the benzyl ester moieties in **15a–d** (palladium on charcoal) the liberated free acids was activated either with PyBOP [(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate] (**16a–c**) or with HCTU [*O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate] (**16d**) and were then treated with *N*-Boc-protected guanidine, yielding the protected compounds **17a–d**. Finally, trifluoroacetic acid (TFA) was used for the deprotection of the acylguanidines. After lyophilisation from methanol and hydrochloric acid the compounds **2–5** could be obtained as chloride salts.

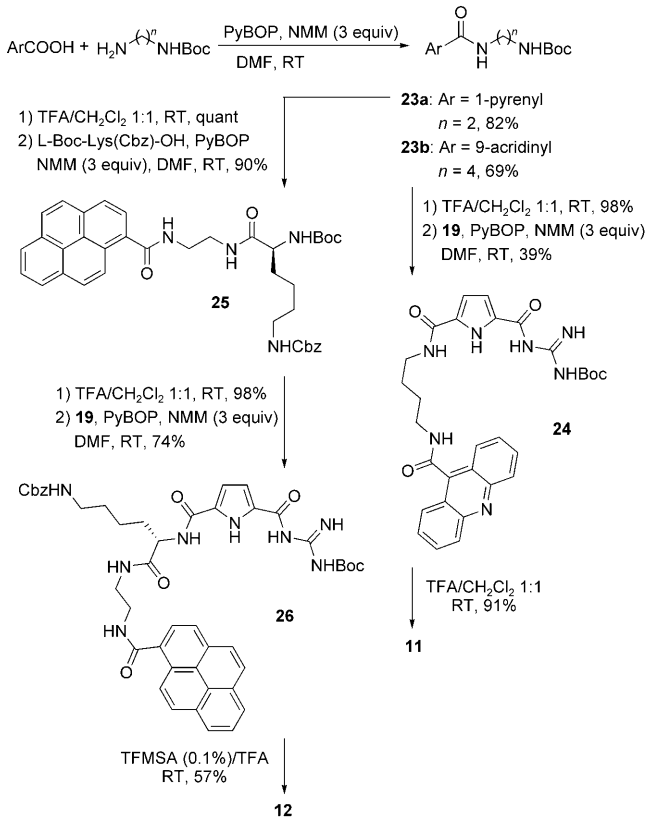


Scheme 1. Synthesis of the first intercalator generation **2–5**.

The syntheses of the new guanidiniocarbonylpyrrole-aryl hybrid compounds **6–12** were achieved by two different procedures as shown in Schemes 2 and 3 (see below). The procedure starting from aryl amines (also considering L-*N*-2-naphthyl-serinamide as an aryl amine) is shown in Scheme 2: 1-naphthylamine and pyren-1-ylamine were coupled to 5-[(*tert*-butoxycarbonyl)amino]pentanoic acid[14] and L-*N*-(*tert*-butoxycarbonyl)serine, respectively, with use of isobutyl chloroformate as acid activator. Compound **18c** was prepared from the commercially available L-*N*-2-naphthyl-



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



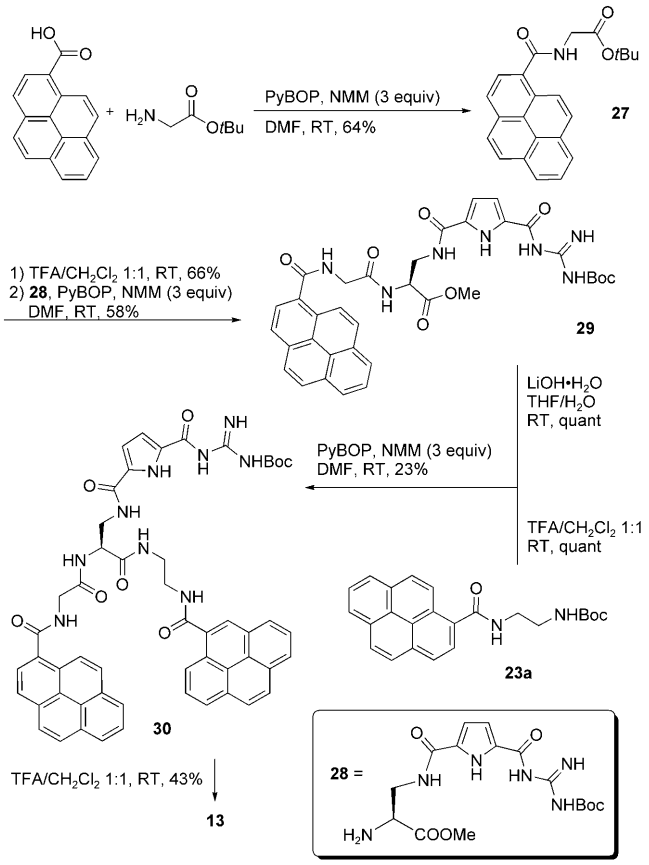
Scheme 3. Synthesis of **11** and **12**.

serinamide, with use of PyBOP as coupling reagent. The Boc protecting groups in **18a–c** were then removed quantitatively with TFA. The resulting free amines were treated without further purification with *N*-Boc-5-guanidinocarbonylpyrrole-2-carboxylate (**19**), prepared by literature procedures.[15] Final Boc removal from **20a–c** was again carried out with TFA, providing the guanidiniocarbonylpyrrole-aryl derivatives **6**, **7** and **9** in high yields as their 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, Boc-removal from **22** under acidic conditions led to the guanidiniocarbonylpyrrole-pyrenyl derivative **10**.

The preparation of compounds **11** and **12** is outlined in Scheme 3. In this case the starting materials were aryl acids (pyrene-1-carboxylic acid and acridine-9-carboxylic acid), which were coupled to Boc-monoprotected alkyl diamines[16] by PyBOP activation. Afterwards, **23a** and **23b** were subjected to amine deprotection and subsequent amide bond formation under standard PyBOP coupling conditions in a way similar to that described above. Intermediates **24**–**26** were thus obtained in good yields. Compound **24** was easily deprotected, affording **11** in high yield. However, deprotection of **26** was not so trivial. Trifluoromethanesulfonic acid (TFMSA) in TFA (0.1%) was used for the simultaneous deprotection both of the Cbz-protected amine and of the Boc-protected guanidine, 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 the guanidiniocarbonylpyrrole-diaryl derivative **13** was achieved by a convergent procedure as shown in Scheme 4. The preparation of intermediate **29** in moderate yield was achieved by PyBOP activation couplings and acidic *tert*-butyl ester removal. Pyrene-1-carboxylic acid, *N*-(*tert*-butoxycarbonyl)glycine, and the arginine analogue **28**[17] were used as starting materials, and the methyl ester in **29** was then removed quantitatively by basic hydrolysis. The free acid was then 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 reversed-phase chromatographic purification.

Physicochemical properties of the compounds in aqueous solution: Compounds **2–5** have only limited solubilities in water: **2–4** could be dissolved at $c \approx 3\text{–}4\times10^{-4}$ 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 $c(\mathbf{5}) \approx 1\times10^{-5}$ mol dm^{-3}. The introduction of spacers signifi-



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

cantly improved the water solubilities of the pyrene and acridine derivatives **1**, **6**, **7**, **11** and **13** ($\approx 10^{-4}$ mol dm^{-3} range) and of **8** and **12** (up to $c = 1.0 \times 10^{-3}$ mol dm^{-3}). Compounds **4**, **9** and **10** decomposed after a few hours in aqueous solution at room temperature or upon heating at 90 °C for several minutes, as indicated by changes in their UV spectra, which excluded them from further studies. Aqueous solutions of all other compounds were stable, not showing any signs of decomposition upon standing for several days at room temperature or upon heating to 90 °C for at least 1 hour. The relative instabilities of aqueous solutions of **9** and **10**—in comparison with, for example, **12** or **13**—are most probably due to the fact that aminopyrene is a rather good leaving group in nucleophilic displacement reactions. Furthermore, the nearby serine OH group can intramolecularly assist in the cleavage through the intermediate formation of a β-lactone. In compounds **12** or **13**, derived from pyrenecarboxylic acid, the direction of the amide bond is reversed and the compounds are thus much more stable.

UV spectroscopy: The 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} in the case of **5**, due to its limited solubility). Hence, no significant intermolecular aggregation of the compounds, which would be expected to give rise to hypochromicity effects, occurred in the concentration range needed for the following spectroscopic studies. 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 medium,[a] together with fluorescence emission maxima and corresponding relative quantum yields (Q).[b]

	UV/Vis	Fluorescence emission		
			Q[b]	
	λ_{max} [nm] ($\varepsilon \times 10^3$ [dm^3 mol^{-1} cm^{-1}])	λ_{max} [nm]	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 L-*N*-acetyltryptophanamide (NATA) standard; $Q = 0.14$, applied excitation wavelength marked in italic in the UV/Vis data. [c] Determined at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}).

The guanidiniocarbonylpyrrole moiety in all compounds absorbs at $\lambda = 284$–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 overlap in **2**, **3**, **6** and **5** but are well separated for **1**, **7**, **8**, **11** and **12**, most probably due to the pronounced conjugation of both groups in the former compounds, which is prevented by the additional linkers in the latter structures. Interestingly, in **13** the absorption maxima of the pyrene and the guanidiniocarbonylpyrrole moieties also overlap despite the long linker. Furthermore, although **13** contains two pyrene subunits the ε value at the pyrene maximum ($\lambda =$ 345 nm) is similar to the values of **1** and **12**, each containing 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 because the protonation states of the guanidiniocarbonylpyrrole moieties were expected to be different at pH 5 and pH 7. However, the pyrrole units show only very weak absorbances, the main absorbances being 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 value of <6 for protonation of the acridine.

Fluorescence spectra: The naphthalene derivatives (**6**, **7**, **8**) exhibited only very weak fluorescence in aqueous media. In

contrast, the pyrene and acridine derivatives **1**, **11**, **12** and **13** showed strong fluorescence emissions, linearly dependent on the concentrations of the compounds in water up to $c = 5.0 \times 10^{-6}$ mol dm^{-3} (**5** was not studied, because of its low solubility). At higher concentrations the increases in fluorescence emission became non-proportional, due to inner filter effects. In the case of the bis-pyrene compound **13** the fluorescence emission is significantly more than twice the emission intensity of the monopyrene derivative **12** (Figure 1), which again confirms an intramolecular aromatic stacking interaction between the two pyrene units as already suggested on the basis of the differences in the UV/Vis spectra.

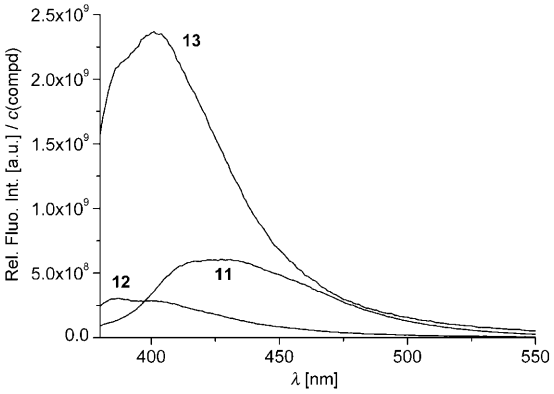


Figure 1. Fluorescence emission spectra of **11** ($\lambda_{exc} = 360$ nm), **12** ($\lambda_{exc} = 344$ nm) and **13** ($\lambda_{exc} = 345$ nm), all collected with the same instrument setup at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}).

Protonation states: The fluorescence of **11**, **12** and **13** was found to be weakly pH-dependent in the pH 5–8 range, which was attributed to protonation of the guanidine group, and allowed an estimation of the pK_a values as 5.5–6 (based on pH titrations; data not shown). The fluorescence of the acridine component in **11** changed considerably between pH 3–5 (p$K_a = 4.1$), so **11** is mostly neutral at pH 5. At pH 7 the guanidine moiety is therefore not yet protonated so all compounds except **8** and **12** are present in their neutral forms, whereas at pH 5 they would be expected to be positively charged. Compounds **8** and **12** each possess an additional amino group with p$K_a \approx 8$ in their side chains, and so already have one positive charge at pH 7.

Interactions with polynucleotides in aqueous medium

Thermal denaturation of ds-DNA and ds-RNA: The experiments were performed at pH 7 and pH 5 because it was expected that different protonation states 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 a ratio ($r_{[compound]/[polynucleotide]}$) of 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 also the only compound positively charged at pH 7 and possessing a large aromatic moiety (pyrene).

At pH 5, however, all compounds are positively charged as a result of protonation of their guanidine moieties and consequently the results were significantly different (Figure 2). Compounds with small aromatic moieties (benzene, naphthalene) connected to the guanidiniocarbonylpyrrole through short and rigid linkers (**2**, **3**) did not stabilise ds-DNA and ds-RNA at all. Because of its larger aromatic moiety (pyrene), **5** weakly stabilised ct-DNA ($\Delta T_m = 1.0$ °C at $r = 0.3$), whereas the introduction of a longer and more flexible linker between the guanidiniocarbonylpyrrole and the pyrene (**1**, **12**) additionally increased stabilisation of ds-DNA (Table 2). The highest ΔT_m value was obtained for **12** ($\Delta T_m = 9.7$ °C), which showed that the additional positive charge in **12** (which **1** does not possess) increased the affinity for ds-DNA. Most surprisingly, the bis-pyrene compound **13** also did not stabilise any of the polynucleotides studied, most probably because the intramolecular stacking of the two pyrene rings prevented the intercalation of **13** into the polynucleotide. The acridine derivative (**11**) stabilised ct-DNA less efficiently than the analogous pyrene compound **1**, consistently with the smaller aromatic surface of the former compound. Interestingly, even the naphthalene de-

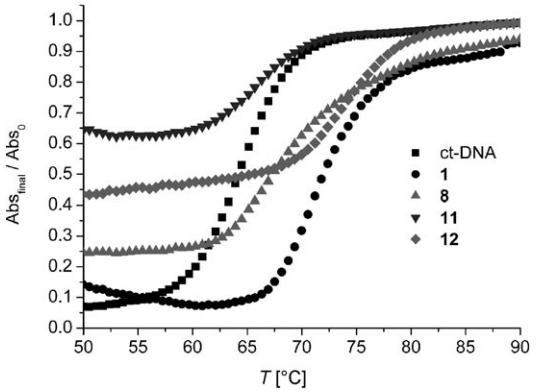


Figure 2. Thermal denaturation curves of ct-DNA ($c = 2 \times 10^{-5}$ mol dm^{-3}) at pH 5 (sodium citrate buffer, $I = 0.03$ M) upon addition of **1**, **8**, **11** and **12**. For measuring conditions, see Table 2 and the Experimental Section.

Table 2. ΔT_m 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	**1**[e]	**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
poly A–poly U[c]	−1.0	−1.5	+3.9	<1.0	<1.0	0

[a] Error in ΔT_m: ±0.5 °C. [b] r = [compound]/[polynucleotide]. [c] Biphasic transitions: the first transition at $T_m = 30.3$ °C is attributed to denaturation of poly A–poly U and the second transition at $T_m = 85.8$ °C is attributed to denaturation of poly AH$^+$–poly AH$^+$, because 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]

rivative **8** stabilised ct-DNA more efficiently than **11**, but this again most probably reflects the one additional positive charge present only in **8**. A more detailed analysis of those compounds that displayed significant stabilisation of ds-DNA revealed a strongly nonlinear relationship between ΔT_m values and r ratio ([compound]/[ds-DNA]), pointing to saturation of binding sites at about $r = 0.2$–0.3. The stabilisation effects of **11** and **12** on poly dA–poly dT were even more pronounced than for ct-DNA, most probably due to stronger interactions of the compounds in the narrower and deeper minor groove of the former polynucleotide.[19] However, the effects of the studied compounds on ds-RNA (poly A–poly U) were much weaker. Only **8** led to stabilisation, whereas **7** and **1** actually weakly destabilised RNA. This observation suggests that most of these compounds do not intercalate into ds-RNA.

Fluorimetric titrations: At this point we focused all further studies on those compounds (**8**, **11**, **12**) that showed measurable thermal stabilisation effects (Table 2), or at least acceptable solubility and promising structure with respect to single-stranded polynucleotides (**13**). Although all the compounds display UV/Vis bands at $\lambda > 300$ nm, UV/Vis titrations were not applicable for study of their interactions with ds-polynucleotides because, 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 highly 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	
	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7
11	0.5[b]	0.6[b]	0.5[b]	0.8[c]	0.5[b]	0.7[c]	0.7[b]	0.7[c]	0.8[b]	0.6[b]
12	0.7[b]	0.7[b]	8.9[b]	6.3[b]	0.3[b]	0.5[b]	8.8[b]	0.8[c]	3.4[b]	0.8[c]
13	0.9[c]	–	0.7[b]	–	0.7[b]	–	1	–	0.9[c]	–

[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 because 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 ds-DNA or ds-RNA, the emission of **12** was strongly quenched by ds-DNAs containing G-C base pairs, whereas 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 that of a RNA analogue (poly A–poly U), induced weak fluorescence quenching of **12**.

The results of the fluorimetric titrations at pH 5 were significantly different from those at pH 7. For compound **1** we had previously found[7] that at pH 5 agglomeration along the DNA, leading to quenching of fluorescence, takes place first ($r > 0.14$), followed by intercalation of the pyrene into ds-DNA ($r < 0.1$) accompanied by a strong fluorescence increase. Upon addition of ds-RNA, however, 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 in the case of its analogue **12**. However, compound **12** displayed a new feature—fluorimetric differentiation between polynucleotide base pair composition—that was not observed for **1**. At pH 5 the fluorescence of **12** was strongly quenched by any polynucleotide containing G–C base pairs, whereas polynucleotides with only A-T or A-U base pairs induced a strong fluorescence increase (Table 3, Figure 4). Such fluorimetric sensing had previously been reported for proflavine[20] and some 4,9-diazapyrenium cations[21] and it was attributed to 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 through direct aromatic stacking interactions with guanine or through remote G sites by electron-transfer through the π-stacked DNA helix.[22] Both quenching modes require that the fluorophore be efficiently stacked within the DNA double helix. Because the pyrene moieties in **1** and **12** are intercalated into DNA it seems that the base pair differentiation by **12** but not by **1** is the consequence of a different orientation of the pyrene within the DNA double helix, most probably due to a steric influence of the bulky linker with its positively charged side arm in **12**.

The fluorescence of the bis-pyrenyl derivative **13** was also quenched by any polynucleotide containing G–C base pairs 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). Sensitivity of fluorescence response of this kind can be attributed to the specific properties of polynucleotide secondary structure. Namely, alternating polynucleotides

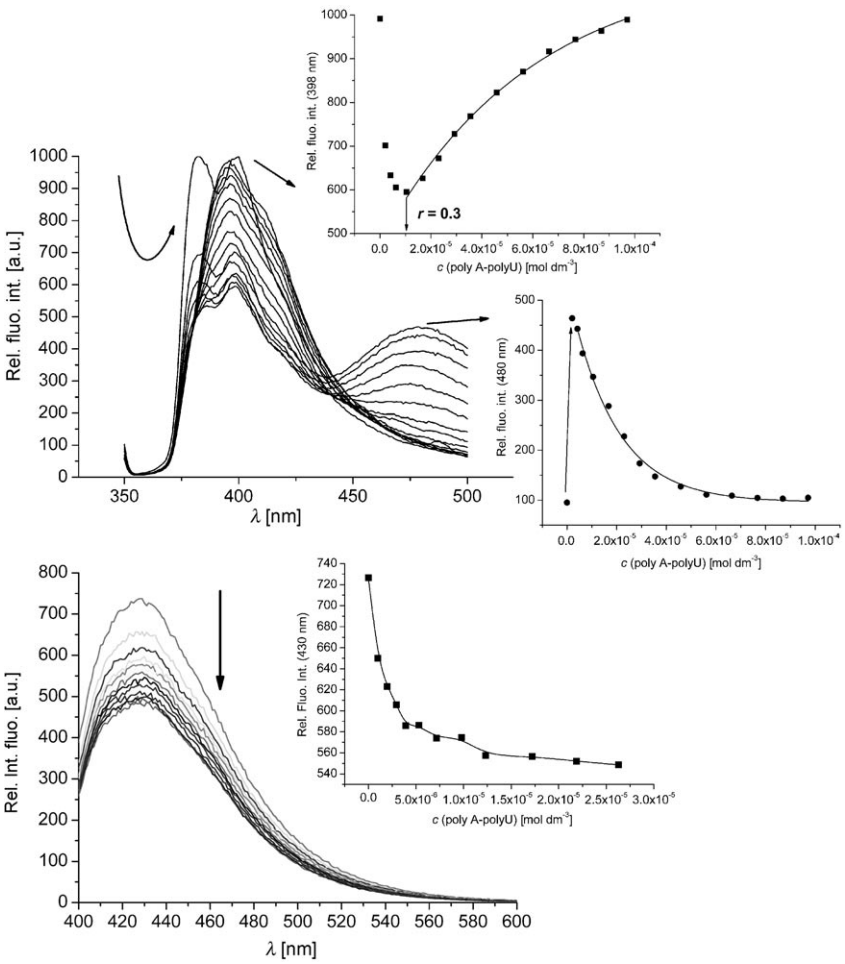


Figure 3. Fluorimetric titration with poly A–poly U at pH 5 (sodium citrate buffer, $I = 0.03$ M). Top three figures: **1** ($c = 3.3 \times 10^{-6}$ mol dm^{-3}, $\lambda_{exc} = 320$ nm). Bottom two figures: **11** $c = 2.0 \times 10^{-6}$ mol dm^{-3}, $\lambda_{exc} = 320$ nm).

adopt a B-helical structure[19] and most probably bulky compound **13** fits tightly within the minor groove. Poly dA–poly dT, however, is characterised by a peculiar twisted structure possessing a very narrow minor groove,[23] whereas poly A–poly U forms an A-form double helix, characterised by a shallow and broad minor groove.[19] In both cases it seems that binding of **13** was not supported.

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

Because the UV/Vis and fluorimetric titrations were not applicable for study of the interactions between **8** and polynucleotides, we performed ethidium bromide (EB) displacement assays as an alternative method for estimation of affinity. This at least allows comparison of 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 $r_{[\mathbf{8}]/[\text{polynucleotide}]}$ ratios 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 stabilisation of both ds-DNA and ds-RNA (Table 2). We 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** relative to c(EB) was needed to displace 50% of EB both from ct-DNA and from poly A–poly U. From these results an estimate for the affinity of **8** could be derived with the aid of Equation (1), by use of the $\log K_s$ (EB) value determined previously under the same experimental conditions.[25]

$$\log K_s\,(\mathbf{8}) = \log K_s\,(\text{EB}) \times \text{IC}_{50}\ \text{value} \qquad (1)$$

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

*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 compositions of the ds-polynucleotides (Table 3). To study the role of each nucleobase on the fluorescence of these two compounds in more detail (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, but **11** and **12** revealed quite specific fluorimetric responses to some polynucleotides. At pH 5, most intriguingly, only addi-

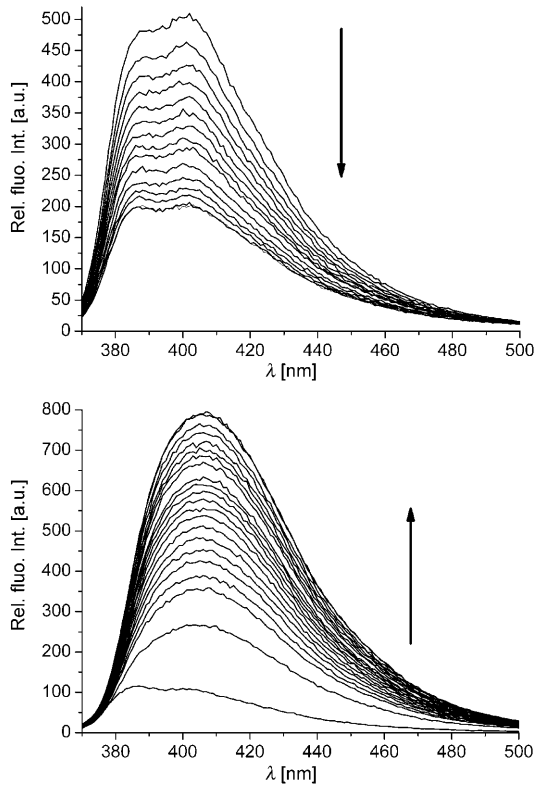


Figure 4. Fluorimetric titration of **12** at pH 5 (sodium citrate buffer, $I=0.03$ M). Top: poly dGdC–poly dGdC [$c(\mathbf{12})=5.0\times10^{-6}$ mol dm^{-3}, $\lambda_{exc}=350$ nm]. Bottom: poly dAdT–poly dAdT [$c(\mathbf{12})=1.0\times10^{-6}$ mol dm^{-3}, $\lambda_{exc}=350$ nm].

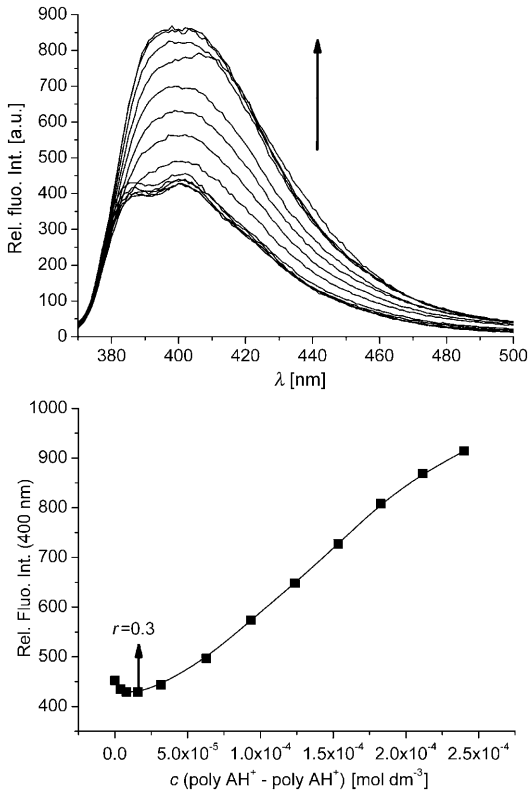


Figure 5. Fluorimetric titration of **12** with poly AH$^+$–poly AH$^+$ at pH 5[19] [c(**12**)$=5.0\times10^{-6}$ mol dm^{-3}, $\lambda_{exc}=350$ nm, sodium citrate buffer, $I=0.03$ M].

tion of poly A yielded a strong increase in the fluorescence of **12** (Figure 5), whereas other polynucleotides either completely (poly G) or partially (poly U, poly C) quenched the emission. At pH 7, however, no fluorescence change was observed for **12** upon addition of any polynucleotide. It should be stressed that poly A is protonated only at pH 5 (not at pH 7) and readily forms a double-stranded helix of poly AH$^+$–poly AH$^+$,[19] so the observed fluorescence specificity is actually related to interaction of **12** with a protonated adenine–adenine double strand. Because **12** had not stabilised poly AH$^+$–poly AH$^+$ in thermal denaturation experiments (second transition of poly A–poly U in Table 2), intercalation of the pyrene subunit can most probably be excluded. Therefore, the observed fluorescence specificity could be attributed to the specific orientation of **12** within the poly AH$^+$–poly AH$^+$ grooves (hence the different binding in relation to poly G, poly U, or poly C), allowing noncovalent 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. Because of the small emission changes, only a few fluorimetric titra-

Table 4. Binding constants (logK_s, in parentheses ratios $n_{[\text{bound compound}]/[\text{polynucleotide}]}$) 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]

	logK_s (n)									
	ctDNA		poly dAdT–poly dAdT		poly dGdC–poly dGdC		poly dA–poly dT		poly A–poly U	
	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7
1[7]	5.9[d] (0.1)	6.0[e] (0.5)	–	–	–	–	6.8[d] (0.1)	5.1[e] (0.8)	6.3[d] (0.1)	5.1[e] (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]	6.5[e] (0.5)	6.5[e] (1)
12	6.1[e] (0.5)	6.8[e] (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 with the aid of the Scatchard equation,[24] accuracy of obtained n values ±10–30%; consequently logK_s values vary with the same order of magnitude. [b] Linear change abruptly ends at $r=0.3$–0.1, suggesting log$K_s>6$. [c] Too small changes for accurate calculation. [d] Data calculated with the aid of the Scatchard equation from the second part of a titration experiment with a polynucleotide in which fluorescence of primarily formed complex **1**/polynucleotide was enhanced by formation of secondary complex. [e] Too high n value suggests agglomeration.

tions of **11** and **12** resulted in emission changes suitable for processing with the aid of the Scatchard equation[24] and obtained $\log K_s$ values (for pH 5 $\log K_s = 5$—6; for pH 7 $\log K_s =$ 4–6). Hence, the affinities of these compounds towards ss-polynucleotides are rather high.

CD spectroscopy: To obtain some more information on the binding modes and the structures of the complexes formed, we chose CD spectroscopy as a highly sensitive method to assess conformational changes in the secondary structures of polynucleotides.[26] In addition, achiral small molecules can show induced CD (ICD) signals upon binding to polynucleotides, which gives useful information about the modes of interaction.[7,26,27] The sign and magnitude of the ICD band can depend on, for example, the binding geometry: ligand–ligand stacking is expected to give strong bisignate exciton CD, minor groove binding to ds-DNA orientates the ligand at approximately 45° with respect to the DNA chiral axis thus giving a strong positive ICD band, whereas intercalation brings the aromatic moiety of the ligand into a coplanar 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 spectra on their own under the experimental conditions used, whereas **8** and **12** do display CD spectra (Figure 6). The intrinsic CD spectrum of **8** revealed

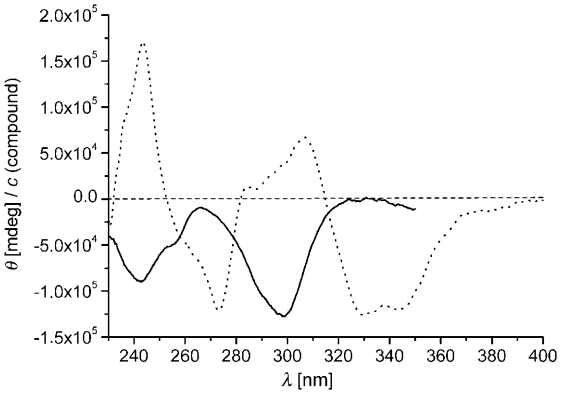


Figure 6. CD spectra of **8** (——) and **12** (·····) at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}).

two strong negative maxima: from the maxima in the UV/Vis spectrum (Table 1) the band at $\lambda = 243$ nm can be attributed to the naphthyl moiety, and the band at $\lambda = 298$ nm corresponds to the guanidiniocarbonylpyrrole group. Intriguingly, the CD bands of **12** at $\lambda = 243$ nm and at $\lambda = 290$–305 nm are of opposite (strongly positive) sign with respect to the corresponding bands in **8**. The additional negative band at $\lambda = 330$–343 nm for **12** can be attributed to the pyrene.

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 guanidiniocarbonylpyrrole moiety exclusively in the minor groove.[7] However, **1** showed that specificity only under weakly acidic conditions (pH 5), under which the guanidine was protonated. Compound **11** (the acridine analogue of **1**) also gave rise to a similar ICD band ($\lambda = 300$ nm, corresponding 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 guanidiniocarbonylpyrrole moiety within the DNA minor groove are essential for that ICD band. Compound **12** also revealed a similar ICD band at $\lambda = 305$–315 nm upon mixing with ct-DNA at pH 5 (Figure 7, top), again in good accordance with the corresponding electronic absorption maximum given in Table 1.

The ICD band of **12**, however, unlike those of **1** or **11**, was also observed at pH 7 (Figure 7, bottom). A strong decrease in the intensity of the intrinsic negative CD band of

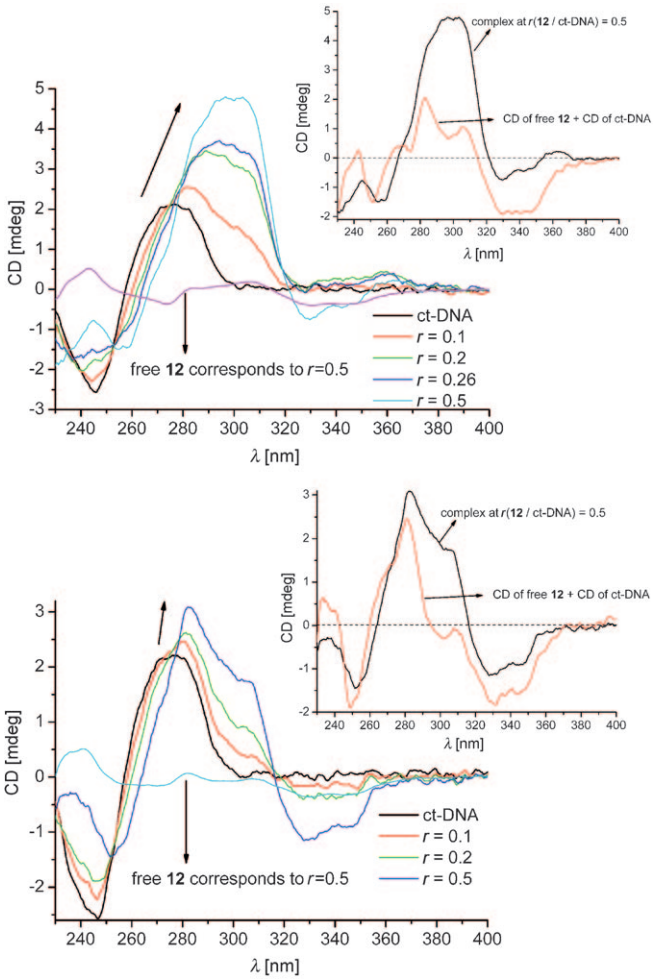


Figure 7. CD titration of ct-DNA ($c = 3.0 \times 10^{-5}$ mol dm^{-3}) with **12**. Top two figures: pH 5, $r_{[12]/[\text{ct-DNA}]} = 0$, 0.1, 0.2, 0.26, 0.50 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}). Bottom two figures: pH 7, $r_{[12]/[\text{ct-DNA}]} = 0$, 0.1, 0.2, 0.5 (sodium cacodylate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

12 at $\lambda = 330$–343 nm upon mixing with ct-DNA (Figure 7, insets) also suggests intercalation of the pyrene moiety into the DNA double helix. More detailed studies revealed highly selective changes of the CD spectrum of **12** with respect to a) the pH of the solution and b) different ds-DNA base pair compositions (Figure 8). By far the strongest positive ICD band at $\lambda = 310$ nm was obtained for poly dGdC–poly dGdC, but only at pH 5, which again underlines the essential role of the protonated guanidiniocarbonylpyrrole cation on the positioning of **12** in the minor groove of poly dGdC–poly dGdC. At pH 7 the neutral guanidine interacts only weakly with ds-DNA. It is probably situated outside of the DNA minor groove, resulting in the absence of a ICD band at $\lambda = 310$ nm. Furthermore, addition of **12** to polynucleotides containing A-T induced significantly smaller changes in the CD spectra than in the case of G-C-containing polynucleotides, with the absence of a ICD band at $\lambda = 310$ nm in the A-T-containing polynucleotides being the most prominent difference (Figure 8). It seems that specific substituents of the G-C base pair (such as the guanosine amino group, which is exposed in the DNA minor groove) are responsible for the uniform orientation of the guanidiniocarbonylpyrrole moiety of **12** in the minor groove. Compound **12** hence shows base-pair-selective recognition of ds-DNA at pH 5.

Addition of **8** (naphthyl analogue of **12**) caused strong increases in both negative and positive CD bands of the ds-DNA polynucleotides (Figure 9). A distinct deviation from the isoelliptic points to the presence of several different **8**/DNA complexes. The increase in the positive DNA band ($\lambda = 280$ nm) can only be the consequence of changes in the secondary structure of the DNA double helix, because **8** does not have a CD signal in this region (Figure 6). Moreover, the strong negative CD band (290–320 nm) in the CD spectrum of the **8**/DNA complexes (Figure 9 bottom) is red-shifted with respect to free **8** and free DNA, which can be attributed to the interactions of the chromophore of **8** with the polynucleotide ds-helix. Most interestingly, in contrast with **1**, **11** and **12**, no positive ICD band at $\lambda = 310$ nm was

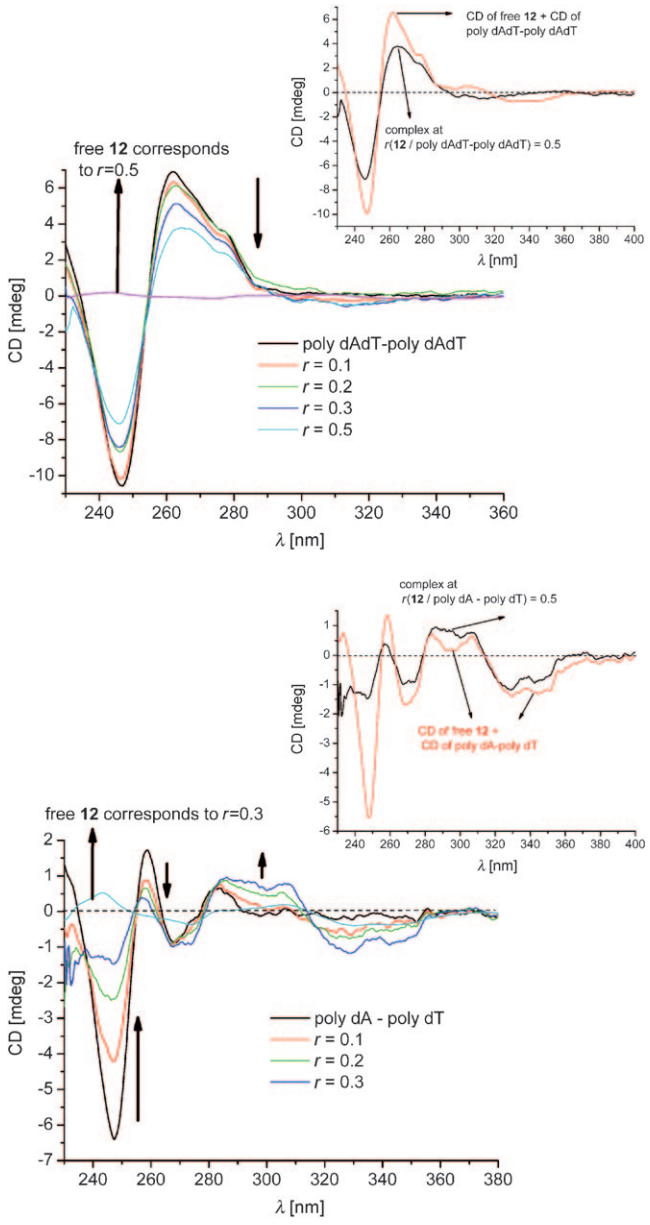


Figure 8. Titrations of alternating (top two figures) and homo-dAdT (bottom two figures) polynucleotides with **12** at pH 5 (sodium cacodylate buffer, $I = 0.05$ mol dm^{-3}), $r = [\mathbf{12}]/[\text{polynucleotide}]$.

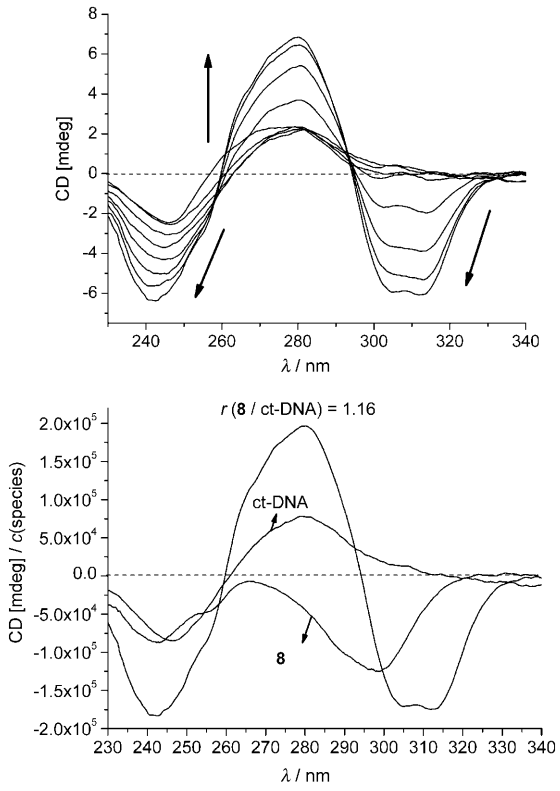


Figure 9. Top: CD titration of ct-DNA ($c = 3.0 \times 10^{-5}$ mol dm^{-3}) with **8** at pH 5 (sodium citrate buffer, $I = 0.03$ mol dm^{-3}); $r_{[\mathbf{8}]/[\text{ct-DNA}]} = 0$, 0.17, 0.33, 0.50, 0.66, 0.83, 0.99, 1.16. Bottom: comparison of the spectra of the complex with the spectra of free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

observed for **8**/DNA complexes, probably due to the significantly smaller aromatic surface of naphthalene (in **8**) with respect to pyrene or acridine, which does not lead to intercalation. Consequently, the guanidiniocarbonylpyrrole part of **8** is not uniformly oriented within the DNA minor groove.

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 $\lambda =$ 295 nm and $\lambda = 354$ nm obtained for poly dAdT–poly dAdT and a moderate increase in the CD band at $\lambda = 282$ nm for poly dGdC–poly dGdC. Such minor changes point to 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: Unlike in the case of ds-DNA, the addition of any compound led to decreases in 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 $r_{[compd]/[RNA]}$ ratio, thus showing no saturation of binding sites even in the presence of an excess of ligand over ds-RNA, which clearly shows that the compounds studied here interact significantly differently with ds-DNA and with ds-RNA. Accordingly, the CD spectra of the ss-RNAs were only slightly changed upon the addition of any compound studied here, suggesting that the secondary structures of the polynucleotides were mainly preserved upon binding.

Discussion of the spectroscopic results: Our structure tuning of guanidiniocarbonylpyrrole-aryl hybrid probes for the goal of spectrophotometric recognition of specific DNA and RNA sequences started with benzene and naphthalene moieties attached to a guanidiniocarbonylpyrrole moiety through short and rigid linkers (**2**, **3**). These compounds did not show significant interactions with DNA/RNA. Enlargement of the aromatic moiety by attachment of pyrene instead of naphthalene (**5**), again through a short and rigid linker, resulted in a minimal stabilisation of ds-DNA (as seen in the thermal denaturation studies; $\Delta T_m = 1.0\,^{\circ}C$ at $r =$ 0.3) but only at pH 5 when the guanidine was protonated. However, through the introduction of more flexibility in the linker between the pyrene and the guanidiniocarbonylpyrrole moiety, together with the presence of an additional positive charge (as in **1** at pH 5 or in **12** also at pH 7), the affinity was large enough to produce measurable stabilisation of the polynucleotides. Moreover, the combination of two positive charges and a longer and flexible linker in **8** also allowed a naphthyl moiety to interact significantly with ds-DNA ($\log K_s \approx 5$, thermal stabilisation, CD spectrum change). However, comparison of the CD titrations of ds-DNA with **8** (no positive ICD band at $\lambda > 300$ nm) or with **1**, **11** or **12** (strong positive ICD band at $\lambda = 310$ nm) revealed the importance of the intercalative unit (pyrene in **12**) for the uniform orientation of the guanidiniocarbonylpyrrole moiety within the DNA minor groove.

Connection of a pyrene through a flexible aliphatic linker to a positively charged guanidiniocarbonylpyrrole yielded compound **1**, which, as we have recently reported,[7] interacts both with ds-DNA and with ds-RNA but in different ways, 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** stabilised 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**, like **1**, yielded a strong positive ICD band at $\lambda =$ 310 nm upon binding to ds-DNA, but unlike **1** gave no specific fluorescence signal for any ds-DNA or ds-RNA studied, although acridine and pyrene in general exhibit similar fluorescence spectra. This observation stresses the sensitivity of the pyrene fluorescence in the microenvironment. Moreover, the affinity of acridine derivative **11** toward DNA/RNA (Table 4) is comparable to the affinities of some most intensively studied simple acridines such as AMSA {N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonanilide} and DACA (acridine-4-carboxamide); however, the affinity and thermal stabilisation effect were significantly lower relative to 9-amino-acridine derivatives with unsubstituted amino groups (Chapter 18 in ref. [4]).

Compound **13**, the bis-pyrene analogue of **1**, was prepared in the expectation that the two pyrenes would form an intramolecular excimer (characterised by specific fluorescence response) that would then be able to interact specifically with some polynucleotides, depending on their secondary structures (e.g., only with ds-RNA but not ds-DNA). Indeed, the 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 probably based on the agglomeration of molecules along the polynucleotide surface. Intramolecular stacking interactions of **13** are therefore obviously not disturbed by DNA or RNA, so **13** does not show any significant interaction with polynucleotides.

In order to keep the polynucleotide-specific spectroscopic features of **1**, but to shift its applicability to physiological conditions (pH 7), compound **12**, characterised by an additional positive charge even at pH 7, was prepared. Indeed, the thermal denaturation effect of **12** on ds-DNA was greater than that of **1**, and in addition, **12** gave a strong positive ICD band at $\lambda = 310$ 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 (such as the emission of **1** at 500 nm),[7] most probably because steric hindrance and/or charge repulsion prevented dimer formation of **12** within the ds-RNA major groove. On the other hand, the fluorescence of **12**, unlike that of **1**, proved to be highly sensitive to the base pair composition of ds-DNA, es-

pecially at pH 5. Namely, at pH 5 addition of any A-T base pair polynucleotide to **12** resulted in a strong increase in its fluorescence, whereas 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 to specific interaction of the positively charged guanidiniocarbonylpyrrole 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 probably due to: a) the low affinity of the intercalative unit alone (pyrene, acridine) toward ss-sequences and b) the absence of any well-defined groove necessary for accommodation of the guanidiniocarbonylpyrrole moiety, as well as c) the presence of only one positive charge in **12**. At pH 5, however, the guanidiniocarbonylpyrrole 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 in the fluorescence of **12**, whereas 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.[20–22] Fluorescence increase can be correlated with more efficient aromatic stacking (intercalation) of pyrene in poly AH^+–poly AH^+, which is characterised by a significantly larger aromatic base pair surface than 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 five human cell lines derived from different cancer types: HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma) and H460 (lung carcinoma) (Table 5).

Compounds **7**, **8**, **11** and **13** exhibited only moderate antiproliferative activity (IC_{50} values in the upper to middle μM range), whereas **1** and **12** showed activity in the lower μM range but with some interesting exceptions depending on the cell line. The high DNA affinities of **8**, **1**, **11** and **12** strongly suggest that cellular DNA is the main target, with intercalation of the pyrene seeming 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 relation to their naphthalene (**8**) and acridine (**11**) analogues. The 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 relative to **1**. The biological results thus correlate nicely with the results from the spectroscopic studies. However, although **1** revealed the highest—but non-selective—biological activity, its close analogue **12** (which is nearly as active as **1**) is also characterised by pronounced selectivity towards the 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 in terms of the presented experimental data, but does, however, strongly support additional biological studies.

Table 5. In vitro inhibition of the growth of tumour cells by compounds **7**, **8**, **1** and **11**–**13**.

	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 in cell growth. [b] The maximum concentration tested was $c = 10$ μM.

Conclusion

A systematic structure–activity relationship (SAR) study of interactions of guanidiniocarbonylpyrrole-aryl derivatives with various DNA and RNA polynucleotides has revealed several critical factors that govern their affinities towards the polynucleotides and spectroscopic sensing of particular secondary structures or base pair compositions. To start with, for efficient binding the linker between the aryl and the guanidiniocarbonylpyrrole moieties should be flexible enough to allow efficient accommodation within the groove of the polynucleotide. Furthermore, the acridine analogue (**11**) proved to represent the minimal aromatic surface able to intercalate into DNA/RNA. Molecules that were neutral at pH 7 (**1**, **13**, **5**) showed significantly stronger interactions with DNA/RNA when protonated at 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 guanidiniocarbonylpyrrole component within the DNA minor groove, yielding a characteristic ICD signal. Furthermore, pyrene analogues displayed specific fluorescence changes due either to the different binding mode (compound **1**, intercalation in ds-DNA, dimer formation in ds-RNA) or to the signalling base pair composition (guanine quenched fluorescence whereas adenine increased it). To some extent the affinities of the guanidiniocarbonylpyrrole-aryl conjugates 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 govern the antiproliferative activities 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 inefficient clearance of metabolic acids from chronically hypoxic cells.[30] Tumours of the bladder, kidney and gastrointestinal system in particular display extremely low pH values. Therefore, uptake of weakly ionizing drugs by tumours is greatly influenced by the interstitial and intracellular pH and the ionisation 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 have studied here are interesting model systems for such studies in living cells, due to the specific fluorimetric properties discussed.

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 chromatographic separations were run on ICN silica (0.032–0.063 nm) from Biomedicals, GmbH or on a medium-pressure flash system (MPLC, CombiFlash®, Companion™, Isco, Inc.) with prepacked silica gel cartridges (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 with a Bruker Avance 400 MHz spectrometer. The chemical shifts are reported relative to the deuterated solvents. Peaks assignments are based on DEPT studies and comparison with literature data. ESI and HR mass spectra were recorded with a micrOTOF instrument from Bruker Daltonik. Analytical HPLC was run on a Supelcosil LC18 (Supelco) 5 μm (25 cm × 4.6 mm) column. Gua = guanidiniocarbonylpyrrole.

General procedure for the coupling with oxalyl chloride: A solution of the free acid **14** (1 equiv) was dissolved in dry DCM (15 mL per equiv) and catalytic amounts of dry DMF. After addition of oxalyl chloride (3 equiv) the solution was heated at reflux for two hours. After 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 equiv) the solution was stirred for one hour at 0 °C and for one more hour at room temperature. The reaction solution was then washed with hydrochloric acid (5%, 3 × 50 mL). After phase separation the organic phase was dried over magnesium sulfate and the solvent was removed in vacuo. The resulting solid was purified by column chromatography.

Compound 15a: Compound **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) as a brown solid (480 mg, 74%); R_f = 0.54 (SiO_2, cyclohexane/ethyl acetate 6:4 + 1 vol% NEt_3); m.p. 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 ppm (s, 2H; OCH_2Ph); ^{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 ppm (CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{19}H_{16}N_2O_3+H^+$: 321.1234; found: 321.1234 [M+H$^+$], 343.1053 [M+Na$^+$], 663.2265 [2M+Na$^+$].

Compound 15b: Compound **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) as a brown solid (550 mg, 75%); R_f = 0.46 (SiO_2, cyclohexane/ethyl acetate 6:4 + 1 vol% NEt_3); m.p. 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 ppm (s, 2H; OCH_2Ph); ^{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 ppm (CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{23}H_{18}N_2O_3+H^+$: 371.1390; found: 371.1390 [M+H$^+$], 393.1210 [M+Na$^+$], 763.2569 [2M+Na$^+$].

Compound 15c: Compound **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) as a slightly brown solid (170 mg, 82%); R_f = 0.58 (SiO_2, cyclohexane/ethyl acetate 6:4 + 1 vol% NEt_3); m.p. 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; naphthyl CH), 6.94–6.93 (m, 1H; pyrrole CH), 7.16–7.05 (m, 1H; pyrrole CH), 5.35 ppm (s, 2H; OCH_2Ph); ^{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 ppm (CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{23}H_{18}N_2O_3+H^+$: 371.1390; found: 371.1390 [M+H$^+$], 393.1210 [M+Na$^+$], 763.2554 [2M+Na$^+$].

Compound 15d: Compound **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) as a slightly brown solid (105 mg, 23%); R_f = 0.89 (SiO_2, cyclohexane/ethyl acetate/isopropanol 4:4:1 + 1 vol% NEt_3); m.p. 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 ppm (s, 2H; OCH_2Ph); ^{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 ppm (CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{29}H_{20}N_2O_3+H^+$: 445.1547; found: 445.1547 [M+H$^+$], found: 467.1366 [M+Na$^+$].

General procedure for benzyl ester hydrogenolysis: A suspension of the appropriate benzyl ester and palladium on charcoal (10%) was stirred under hydrogen in MeOH at 40 °C until tlc indicated full conversion of the starting material. The reaction solution was filtered through a celite pad, which was washed several times with ethyl acetate. The resulting solution was dried over magnesium sulfate and the solvent was evaporated in vacuo.

Compound 16a: Compound **16a** was prepared from **15a** (370 mg, 1.15 mmol) by treatment under hydrogen with Pd/C (74 mg) in MeOH (50 mL) as a colourless solid (265 mg, quant.); m.p. 225 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 12.82 (brs, 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 ppm (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 ppm (pyrrole CH); HRMS (ESI$^+$): m/z: calcd for $C_{12}H_{10}N_2O_3+H^+$: 231.0764; found: 231.0764 [M+H$^+$], 253.0584 [M+Na$^+$], 483.1300 [2M+Na$^+$], 713.1994 [3M+Na$^+$], 943.2685 [4M+Na$^+$].

Compound 16b: Compound **16b** was prepared from **15b** (530 mg, 1.48 mmol) by treatment under hydrogen with Pd/C (106 mg) in MeOH (50 mL) as a slightly greenish solid (415 mg, quant.); m.p. 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 ppm (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 ppm (pyrrole CH); HRMS (ESI$^+$): *m/z*: calcd for $C_{16}H_{12}N_2O_3+H^+$: 281.0921; found: 281.0921 [$M+H^+$], 303.074 [$M+Na^+$], 583.1606 [$2M+Na^+$].

Compound 16c: Compound **16c** was prepared from **15c** (170 mg, 0.47 mmol) by treatment under hydrogen with Pd/C (34 mg) in MeOH (40 mL) as a grey solid (132 mg, quant.); m.p. 233 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=12.22$ (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 ppm (m, 1H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=161.7$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): *m/z*: calcd for $C_{16}H_{12}N_2O_3+H^+$: 281.0921; found: 281.0921 [$M+H^+$], 303.074 [$M+Na^+$].

Compound 16d: Compound **16d** was prepared from **15d** (105 mg, 0.24 mmol) by treatment under hydrogen with Pd/C (11 mg) in MeOH (25 mL) as a slightly yellow solid (84 mg, quant.); m.p. 248 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=12.31$ (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 ppm (m, 1H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=161.8$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): *m/z*: calcd for $C_{22}H_{14}N_2O_3+H^+$: 355.1077; found: 355.1077 [$M+H^+$], 377.0897 [$M+Na^+$].

General procedure for the synthesis of the Boc-protected intercalators 17a–d: A solution of the appropriate free acid (1 equiv) was dissolved in dry DMF. The coupling reagent (1.1 equiv) and NMM were added to the solution, which was stirred for 30 min at room temperature. After addition of Boc-guanidine (1.1 equiv) the solution was stirred for an additional 24 h at RT. The reaction solution was diluted in vigorously stirred water (100 mL) and extracted with ethyl acetate. After phase separation the organic phase was dried over magnesium sulfate and the solvent was evaporated in vacuo. The resulting oil was purified by column chromatography.

Compound 17a: Compound **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) as a slightly yellow solid (380 mg, 90%); $R_f=0.40$ (SiO_2, cyclohexane/ethyl acetate/isopropanol 6:2:1 + 1 vol% NEt_3); m.p. 147 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.32$ (brs, 1H; pyrrole NH), 10.81 (brs, 1H; NH), 10.07 (s, 1H; CONH), 9.37 (brs, 1H; NH), 8.56 (brs, 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 (brs, 1H; pyrrole CH), 6.85 (brs, 1H; pyrrole CH), 1.47 ppm (s, 9H; *t*Bu-CH$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=158.7$ (Cq), 138.9 (Cq), 128.7 (CH), 123.5 (CH), 120.0 (CH), 113.2 (pyrrole CH), 27.8 ppm (*t*Bu-CH$_3$); HRMS (ESI$^+$): *m/z*: calcd for $C_{18}H_{21}N_5O_4+H^+$: 372.1666; found: 372.1666 [$M+H^+$], 394.1486 [$M+Na^+$].

Compound 17b: Compound **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) as a colourless solid (450 mg, 73%); $R_f=0.62$ (SiO_2, ethyl acetate/cyclohexane 6:4 + 1 vol% NEt_3); m.p. 189 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.54$ (brs, 1H; pyrrole NH), 10.86 (brs, 1H; NH), 10.28 (s, 1H; CONH), 9.37 (brs, 1H; NH), 8.59 (brs, 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 (brs, 1H; pyrrole CH), 6.84 (brs, 1H; pyrrole CH), 1.47 ppm (s, 9H; *t*Bu-CH$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=159.0$ (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 ppm (*t*Bu-CH$_3$); HRMS (ESI$^+$): *m/z*: calcd for $C_{17}H_{15}N_5O_2+H^+$: 322.1299; found: 322.1307; *m/z*: calcd for $C_{17}H_{15}N_5O_2+Na^+$: 344.1118; found: 344.1123; *m/z*: calcd for $C_{22}H_{23}N_5O_4+H^+$: 422.1823; found: 422.1823 [$M+H^+$], 444.1642 [$M+Na^+$], found: 843.3590 [$2M+H^+$].

Compound 17c: Compound **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) as a slightly brown solid (102 mg, 57%); $R_f=0.62$ (SiO_2, ethyl acetate/cyclohexane 6:4 + 1 vol% NEt_3); m.p. 149 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.44$ (brs, 1H; pyrrole NH), 10.84 (brs, 1H; NH), 10.28 (s, 1H; CONH), 9.39 (brs, 1H; NH), 8.59 (brs, 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 (brs, 1H; pyrrole CH), 6.88 (brs, 1H; pyrrole CH), 1.48 ppm (s, 9H; *t*Bu-CH$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=158.3$ (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 ppm (*t*Bu-CH$_3$); HRMS (ESI$^+$): *m/z*: calcd for $C_{22}H_{23}N_5O_4+H^+$: 422.1823; found: 422.1823 [$M+H^+$], 444.1642 [$M+Na^+$], 843.3600 [$2M+H^+$].

Compound 17d: Compound **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) as a slightly brown solid (40 mg, 36%); $R_f=0.49$ (SiO_2, ethyl acetate/cyclohexane 1:1 + 1 vol% NEt_3); m.p. 224 °C (decomp.); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.40$ (brs, 1H; pyrrole NH), 10.80 (brs, 1H; NH), 10.65 (s, 1H; CONH), 9.44 (brs, 1H; NH), 8.55 (brs, 1H; NH), 8.54–8.35 (m, 8H; pyrenyl CH), 8.11–8.08 (m, 1H; pyrenyl CH), 7.20 (brs, 1H; pyrrole CH), 6.89 (brs, 1H; pyrrole CH), 1.47 ppm (s, 9H; *t*Bu-CH$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=159.1$ (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 ppm (*t*Bu-CH$_3$); HRMS (ESI$^+$): *m/z*: calcd for $C_{28}H_{25}N_5O_4+H^+$: 496.1979; found: 496.1979 [$M+H^+$], 518.1799 [$M+Na^+$].

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 monitoring 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 to afford the chloride salts of the deprotected intercalators.

Chloride salt of 2-(*N*-phenylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole: The chloride salt of **2** was prepared from **17a** (380 mg, 1.02 mmol) in DCM (10 mL) and TFA (6 mL) as a slightly grey solid (228 mg, 73%); m.p. 290 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=12.61$ (s, 1H; pyrrole NH), 11.89 (s, 1H; NH), 10.25 (s, 1H; CONH), 8.48 (brs, 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 ppm (m, 2H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=159.6$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): *m/z*: calcd for $C_{13}H_{13}N_5O_2+H^+$: 272.1142; found: 272.1142 [$M+H^+$], 543.2224 [$2M+H^+$].

Chloride salt of 2-(*N*-naphthalene-1-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole: The chloride salt of **3** was prepared from **17b** (400 mg, 0.95 mmol) in DCM (15 mL) and TFA (8 mL) as a slightly green solid (190 mg, 56%); m.p. 229 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=12.69$ (s, 1H; pyrrole NH), 11.90 (s, 1H; NH), 10.42 (s, 1H; CONH), 8.52 (brs, 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 ppm (m, 1H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=159.6$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): *m/z*: calcd for $C_{17}H_{15}N_5O_2+H^+$: 322.1299; found: 322.1299 [$M+H^+$], 643.2545 [$2M+H^+$].

Chloride salt of 2-(*N*-naphthalene-2-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole: The chloride salt of **4** was prepared from **17c** (100 mg, 0.24 mmol) in DCM (10 mL) and TFA (5 mL) as a slightly brown solid (75 mg, 88%); m.p. 290 °C; ^{1}H NMR ([D$_6$]DMSO,

400 MHz): $\delta = 12.67$ (s, 1H; pyrrole NH), 11.91 (s, 1H; NH), 10.46 (s, 1H; CONH), 8.48 (brs, 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 ppm (m, 1H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta = 159.6$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): m/z: calcd for $C_{17}H_{15}N_5O_2+H^+$: 322.1299; found: 322.1299 [M+H$^+$]; m/z: calcd for [2M+H$^+$]: 643.2524; found: 643.2549.

Chloride salt of 2-(*N*-pyrene-1-ylcarboxamide)-5-(guanidiniocarbonyl)-1*H*-pyrrole: The chloride salt of **5** was prepared from **17d** (20 mg, 0.04 mmol) in DCM (8 mL) and TFA (4 mL) as a yellow solid (17 mg, quant.); m.p. 281 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 12.77$ (s, 1H; pyrrole NH), 11.70 (s, 1H; NH), 10.78 (s, 1H; CONH), 8.43 (brs, 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 ppm (m, 1H; pyrrole CH); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta = 159.7$ (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 ppm (pyrrole CH); HRMS (ESI$^+$): m/z: calcd for $C_{23}H_{17}N_5O_2+H^+$: 396.1455; found: 396.1455 [M+H$^+$], 791.2888 [2M+H$^+$].

General procedure for the coupling with isobutyl chloroformate: A solution of free acid (1.5 equiv) in dry THF (6 mL) was kept under N_2 and cooled at −15 °C. *N*-Methylmorpholine (NMM, 1.5 equiv) and isobutyl chloroformate (1 equiv) were then added. The white suspension was stirred at −15 °C for 20 min. Afterwards a solution of an arylamine (1 equiv) in dry DMF or dry THF (1–2 mL) was added. The mixture was stirred for 10 min at −15 °C and at 0 °C overnight (allowing to warm to RT). The white solid was filtered and solvent was removed from the liquid layer. The residue was dissolved in EtOAc (25–50 mL) and washed with H_2O (2×50 mL), aq HCl (5%, 6×50 mL) and brine (2×50 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): This compound was prepared from 5-[(*tert*-butoxycarbonyl)amino]pentanoic acid (300 mg, 1.38 mmol), isobutyl chloroformate (0.12 mL, 0.92 mmol), NMM (0.15 mL, 1.38 mmol) and 1-naphthylamine (132 mg, 0.92 mmol) as a white solid (189 mg, 60%); m.p. 129–130 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 9.83$ (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, $J = 8.2$ Hz, 1H; naphthyl H-4), 7.67 (d, $J = 8.2$ Hz, 1H; naphthyl H-2), 7.57–7.51 (m, 2H; naphthyl H-6, H-7), 7.47 (t, $J = 7.8$ Hz, 1H; naphthyl H-3), 6.82 (brs, 1H naphNHCOCH$_2$CH$_2$CH$_2$CH$_2$N*H*Boc), 2.97 (q, $J = 6.4$ Hz, 2H; naphNHCOCH$_2$CH$_2$CH$_2$C*H*$_2$), 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 ppm (s, 9H; C(CH$_3$)$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta =$ 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 ppm (naphNHCOCH$_2$*C*H$_2$CH$_2$CH$_2$); HRMS (ESI$^+$): m/z: calcd for $C_{20}H_{26}N_2O_3+$ Na$^+$: 365.183; found: 365.183±0.005 [M+Na$^+$].

L-Pyren-1-ylSer(O*t*Bu)NHBoc (18b): This compound was prepared from L-BocSer(O*t*Bu)OH·DCHA (300 mg, 0.68 mmol), isobutyl chloroformate (58 μL, 0.45 mmol), NMM (74 μL, 0.68 mmol) and 1-aminopyrene (98 mg, 0.45 mmol) as a brown solid (207 mg, quant); m.p. 160–164 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 10.31$ (s, 1H; pyrene NH), 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 ppm (s, 9H; C(CH$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for $C_{28}H_{32}N_2O_4+$Na$^+$: 483.225; found: 483.227±0.005 [M+Na$^+$].

General procedure for the deprotection with TFA: Boc-protected guanidine (1 equiv), was dissolved in a TFA/dry DCM mixture (1:1, 0.5–2 mL TFA, 0.5–2 mL dry DCM) and stirred at RT for 1–2 h. Solvent and excess TFA were removed in vacuo, and the oily residue was lyophilised.

***N*-2-[(5-(Naphthalen-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidinium trifluoroacetate (6)**: This compound was prepared from **20a** (70 mg, 0.13 mmol) as a white solid (63 mg, 88%); m.p. 205 °C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 12.33$ (s, 1H; pyrrole NH), 11.31 (s, 1H; guanidinium NH), 9.87 (s, 1H; naphNHCO), 8.47 (t, $J = 5.2$ Hz, 1H; naphNHCOCH$_2$CH$_2$CH$_2$CH$_2$N*H*), 8.37 (brs, 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, $J = 8.1$ Hz, 1H; naphthyl H-4), 7.67 (d, $J = 7.1$ Hz, 1H; naphthyl H-2), 7.54–7.52 (m, 2H; naphthyl H-6, H-7), 7.47 (t, $J =$ 7.7 Hz, 1H; naphthyl H-3), 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 ppm (m, 2H; CH$_2$); ^{13}C NMR (CD$_3$OD, 100 MHz): $\delta = 174.7$ (naphthyl CONH), 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 ppm (naphNHCOCH$_2$*C*H$_2$CH$_2$CH$_2$); HRMS (ESI$^+$): m/z: calcd for $C_{22}H_{25}N_6O_3^+$: 421.198; found: 421.198± 0.005 [M^+]; 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^{-1}, $\lambda = 300$ nm.

L-Pyren-1-ylSerGuaNH·CF$_3$COOH (9): This compound was prepared from **20b** (20 mg, 3.4×10^{-5} mol) as a brownish solid (18 mg, 90%); m.p. 229 °C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 12.71$ (s, 1H; pyrrole NH), 11.47 (s, 1H; N*H*C(NH$_2$)$_2$), 10.51 (s, 1H; pyrene NH), 8.82 (d, $J = 7.4$ Hz, 1H; Ser NH), 8.36 (brs, 4H; (NH$_2$)$_2$), 8.33–8.28 (m, 4H; pyrene H), 8.22–8.16 (m, 4H; pyrene H), 8.08 (t, $J = 7.7$ Hz, 1H; pyrene H), 7.30 (brs, 1H; pyrrole CH), 7.01 (brs, 1H; pyrrole CH), 5.32 (brs, 1H; OH), 4.95 (q, $J = 6.5$ Hz, 1H; CH), 3.96 ppm (brs, 2H; CH$_2$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta = 169.9$ (pyrene NHCO), 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 ppm (CH); HRMS (ESI$^+$): m/z: calcd for $C_{26}H_{23}N_6O_4^+$: 483.177; found: 483.178±0.005 [M^+]; 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^{-1}, $\lambda = 300$ nm.

L-2-NaphthylSerGlyGuaNH·CF$_3$COOH (7): This compound was prepared from **20c** (30 mg, 5.3×10^{-5} mol) as a white solid (31 mg, quant); m.p. >200 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 12.48$ (brs, 1H; pyrrole NH), 11.13 (brs, 1H; guanidinium NH), 10.12 (s, 1H; naphthylNHCO), 8.80 (t, $J = 5.8$ Hz, 1H; Gly NH), 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, H-5, H-8), 7.51–7.38 (m, 1H; naphthyl H-6 or H-7), 7.09 (brs, 1H; pyrrole CH), 6.93–6.92 (m, 1H; pyrrole CH), 4.54 (dd, $J = 13.1$ Hz, $J = 7.6$ Hz, 1H; Ser CH), 4.03 (d, $J = 5.8$ Hz, 2H; Gly CH$_2$), 3.72 ppm (d, $J = 5.3$ Hz, 2H; Ser CH$_2$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta = 169.4$ (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 ppm (Gly CH$_2$); HRMS (ESI$^+$): m/z: calcd for $C_{22}H_{24}N_7O_5^+$: 466.183; found: 466.184±0.005 [M^+]; 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^{-1}, $\lambda = 300$ nm.

L-Pyren-1-ylSerGlyGuaNH·CF$_3$COOH (10): This compound was prepared from **22** (17 mg, 2.6×10^{-5} mol) as a brownish solid (14 mg, 82%);

m.p. 215 °C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 12.47 (brs, 1H; pyrrole NH), 11.11 (s, 1H; NH), 10.33 (s, 1H; pyrene NH), 8.83 (brt, J = 5.5 Hz, 1H; Gly NH), 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 (brs, 1H; pyrrole H), 6.93 (brs, 1H; pyrrole H), 5.25 (brs, 1H; OH), 4.77 (m, 1H; CH), 4.10–4.07 (m, 2H; Gly CH_2), 3.90 (brs, 1H; Ser CH_2), 3.84 ppm (brs, 1H; Ser CH_2); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 169.9 (CONH), 169.1 (CONH), 159.5 (pyrrole CONH), 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 ppm (Gly CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{28}H_{26}N_7O_5^+$: 540.199; found: 540.199 ± 0.005 [M^+]; 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^{-1}, λ = 300 nm.

***N*-2-[(5-(Acridin-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidinium (11) trifluoroacetate**: This compound was prepared from **24** (30 mg, 5.25×10^{-5} mol) as a yellow solid (28 mg, 91%); m.p. >230 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 12.37 (s, 1H; pyrrole NH), 11.40 (s, 1H; NH), 9.07 (t, J = 5.5 Hz, 1H; acridineCON*H*), 8.52 (t, J = 5.5 Hz, 1H; acridineCONHCH$_2$CH$_2$CH$_2$CH$_2$N*H*), 8.37 (brs, 4H; $(NH_2)_2$), 8.21 (d, J = 8.7 Hz, 2H; H-1, H-8), 8.00 (d, J = 8.7 Hz, 2H; H-4, H-5), 7.93–7.7.89 (m, 2H; H-3, H-6), 7.70–7.66 (m, 2H; H-2, H-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 ppm (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 (4a-C, 10a-C), 143.0 (9-C), 132.4 (pyrrole Cq), 130.8 (3-CH, 6-CH), 127.8 (4-CH, 5-CH), 126.4 (2-CH, 7-CH), 125.2 (1-CH, 8-CH), 124.8 (pyrrole Cq), 121.8 (8a-C, 9a-C), 115.1 (pyrrole CH), 111.8 (pyrrole CH), 38.2 (acridineCONH*C*H$_2$CH$_2$CH$_2$*C*H$_2$NH), 38.0 (acridineCONHCH$_2$CH$_2$CH$_2$*C*H$_2$NH), 26.3 ppm (acridineCONHCH$_2$*C*H$_2$-*C*H$_2$CH$_2$NH), 26.1 (acridineCONHCH$_2$*C*H$_2$CH$_2$CH$_2$NH); HRMS (ESI$^+$): m/z: calcd for $C_{25}H_{26}N_7O_3^+$: 472.210; found: 472.210 ± 0.005 [M^+]. 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^{-1}, λ = 300 nm.

L-*N*-Pyren-1-ylGly-(1-pyrenoyldiaminoethane)arginine analogue NH·CF$_3$COOH 13: This compound was prepared from **30** (21 mg, 2.20×10^{-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) to afford 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; pyrrole CONH), 8.95 (t, J = 5.7 Hz, 1H; pyreneCONH), 8.68 (t, J = 5.5 Hz, 1H; pyreneCONH), 8.61–8.56 (m, 2H; pyrene H and CON*H*CH$_2$CH$_2$), 8.48 (d, J = 9.2 Hz, 1H; pyrene H), 8.38 (d, J = 7.8 Hz, 1H; Gly NH), 8.34–8.07 (m, 21H; pyrene H, $(NH_2)_2$ and NH), 6.98 (brs, 1H; pyrrole H), 6.86–6.84 (m, 1H; pyrrole H), 4.59 (q, J = 6.8 Hz, 1H; CH), 4.18–4.07 (m, 2H; Gly CH_2), 3.77–3.42 ppm (m, 6H; 3×CH_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, $J_{C,F}$ = 31 Hz, 1C; CF_3COO), 154.5 (CONH or $C(NH_2)_2$), 131.9 (pyrrole Cq), 131.2 (2× pyrene Cq), 131.1 (pyrene Cq), 130.7 (pyrene Cq), 130.2 (2×pyrene Cq), 129.7 (2×pyrene 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 (2×pyrene CH), 125.3 (2× pyrene CH), 125.1 (2×pyrene CH), 125.0 (pyrrole Cq), 124.8 (2×pyrene CH), 124.3 (pyrene CH), 124.2 (pyrene CH), 123.8 (2×pyrene CH), 123.2 (2×pyrene Cq), 123.1 (2×pyrene Cq), 116.6 (q, $J_{C,F}$ = 299 Hz, 1C; *C*F$_3$COO), 114.7 (pyrrole CH), 112.3 (pyrrole CH), 52.7 (CH), 42.6 (Gly CH_2), 40.3 (CH_2), 38.4 ppm (2×CH_2); HRMS (ESI$^+$): m/z: calcd for $C_{48}H_{40}N_9O_6^+$: 838.309; found: 838.310 ± 0.005 [M]; 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^{-1}, λ = 300 nm.

General procedure for coupling with PyBOP—Procedure A: A solution of acid (1 equiv), PyBOP (1 equiv) and NMM (3 equiv) in dry DMF (3–7 mL) was stirred for 20 min at RT. Afterwards, amine (1 equiv) was added and the solution was stirred at RT overnight. It was then poured into water and the suspension was stirred at 0 °C for 2 h. The precipitate was filtered off, washed several times with water and lyophilised. The residue was used in the next step without further purification (**18c**) or was purified by flash chromatography (SiO_2) with the corresponding eluent (**23a**, **23b** and **27**).

L-2-NaphthylSerGlyNHBoc (18c): This compound 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) as a white solid (314 mg, 93%); m.p. 98–100 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.04 (s, 1H; naphthylNHCO), 8.29 (d, 1H; naphthyl H-1), 7.96 (d, J = 7.7 Hz, 1H; Ser NH), 7.87–7.79 (m, 3H; naphthyl H-4, H-5, H-8), 7.67 (d, J = 8.8 Hz, 1H; naphthyl H-3), 7.47 (m, 1H; naphthyl H-6, H-7), 7.40 (m, 1H; naphthyl H-6, H-7), 7.06 (t, J = 5.4 Hz, 1H; Gly NH), 5.07 (t, J = 5.4 Hz, 1H; OH), 4.50 (q, J = 7.1 Hz, 1H; Ser CH), 3.74–3.63 (m, 4H; Ser CH_2 and Gly CH_2), 1.39 ppm (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 ppm ($C(CH_3)_3$); HRMS (ESI$^+$): m/z: calcd for $C_{20}H_{25}N_3O_5$+Na$^+$: 410.169; found: 410.169 ± 0.005 [M+Na$^+$].

5-[(*tert*-Butoxycarbonyl)amino-*N*-2-pyrenylpropanamide (23a): This compound was prepared from pyrene-1-carboxylic 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; m.p. 196–198 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 8.66 (brt, J = 5.2 Hz, 1H; pyreneCON*H*), 8.52 (d, J = 9.3 Hz, 1H; pyrene H), 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 (brt, J = 5.4 Hz, 1H; pyreneCONHCH$_2$CH$_2$N*H*), 3.48–3.43 (m, 2H; pyreneCONHC*H*$_2$CH$_2$NH), 3.27–3.23 (m, 2H; pyreneCONHCH$_2$C*H*$_2$NH), 1.41 ppm (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 (pyreneCONH*C*H$_2$CH$_2$NH), 28.3 ppm ($C(CH_3)_3$); HRMS (ESI$^+$): m/z: calcd for $C_{24}H_{24}N_2O_3$+Na$^+$: 411.167; found: 411.167 ± 0.005 [M+Na$^+$].

5-[(*tert*-Butoxycarbonyl)amino-*N*-2-acridinylpentanamide (23b): This compound was prepared from acridine-9-carboxylic acid (200 mg, 0.89 mmol), PyBOP (466 mg, 0.89 mmol), 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; m.p. 153–155 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.01 (t, J = 5.5 Hz, 1H; acridineCON*H*), 8.19 (d, J = 8.70 Hz, 2H; H-1, H-8), 7.98 (d, J = 8.7 Hz, 2H; H-4, H-5), 7.90–7.86 (m, 2H; H-3, H-6), 7.69–7.65 (m, 2H; H-2, H-7), 6.86 (brs, 1H; BocN*H*), 3.49 (q, J = 6.4 Hz, 2H; BocNHCH$_2$CH$_2$CH$_2$C*H*$_2$), 3.01 (q, J = 6.4 Hz, 2H; BocNHC*H*$_2$CH$_2$-CH$_2$CH$_2$), 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 ppm (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 (4a-C, 10a-C), 142.5 (9-C), 130.6 (3-CH, 6-CH), 129.3 (4-CH, 5-CH), 126.7 (2-CH, 7-CH), 125.6 (1-CH, 8-CH), 121.8 (8a-C, 9a-C), 77.4 ($C(CH_3)_3$), 39.3 (BocNH*C*H$_2$CH$_2$CH$_2$CH$_2$), 38.8 (BocNH*C*H$_2$CH$_2$-CH$_2$*C*H$_2$), 28.3 ($C(CH_3)_3$), 27.3 (BocNHCH$_2$*C*H$_2$CH$_2$CH$_2$), 26.5 ppm (BocNHCH$_2$*C*H$_2$CH$_2$CH$_2$); HRMS (ESI$^+$): m/z: calcd for $C_{23}H_{28}N_3O_3^+$: 394.212; found: 394.212 ± 0.005 [M^+].

***tert*-Butyl *N*-pyren-1-oylglycinate (27)**: This compound was prepared from pyrene-1-carboxylic 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; m.p. 129–132°C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.05 (t, J = 5.9 Hz, 1H; NH), 8.62 (d, J = 9.3 Hz, 1H; pyrene H), 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, J = 5.9 Hz, 2H; CH$_2$), 1.52 ppm (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 ppm (C(*C*H$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for C$_{23}$H$_{21}$N$_1$O$_3$+Na$^+$: 382.141; found: 382.141±0.005 [M+Na$^+$].

General procedure for coupling with PyBOP—Procedure B: A Boc-protected amine (1 equiv) was dissolved in a TFA/dry DCM mixture (1:1, 1–6 mL TFA, 1–6 mL dry DCM) and the system was stirred at RT for 30 min. Solvent and excess TFA were removed in vacuo, and the oily residue was lyophilised. The free amine was used in the next step without further purification. A solution of an acid (1 equiv), PyBOP (1 equiv) and NMM (3 equiv) in dry DMF (2–6 mL) was stirred for 20 min at RT. Afterwards, the free amine (1 equiv) was added and the solution was stirred at RT overnight. Then it was poured onto water and the suspension was stirred at 0°C for 2 h. The precipitate was filtered off, washed several times with water and lyophilised. The residue was purified by flash chromatography (SiO$_2$) with the corresponding eluent, except in the case of **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)**: This compound 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 the free amine from Boc-deprotection of **18a** (yield quant, 160 mg, 0.45 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 4:1 → 9:1. Yield 112 mg (48%) of a white solid; m.p. 127°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.85 (brs, 1H; guanidino NH), 9.86 (s, 1H; naphN*H*CO), 9.31 (brs, 1H; guanidino NH), 8.56 (brs, 1H; guanidino NH), 8.36 (brs, 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, J = 8.1 Hz, 1H; naphthyl H-4), 7.68 (d, J = 7.3 Hz, 1H; naphthyl H-2), 7.54–7.52 (m, 2H; naphthyl H-6, H-7), 7.48 (t, J = 7.8 Hz, 1H; naphthyl H-3), 6.80 (brs, 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 ppm (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(*C*H$_3$)$_3$), 23.0 ppm (naphNHCOCH$_2$*C*H$_2$CH$_2$CH$_2$); HRMS (ESI$^+$): m/z: calcd for C$_{27}$H$_{32}$N$_6$O$_5$+H$^+$: 521.250; found: 521.251±0.005 [M+H$^+$].

L-Pyren-1-ylSerGuaNHBoc (20b): This compound was prepared from *N*-Boc-5-guanidinocarbonylpyrrole-2-carboxylic acid (**19**, 71 mg, 0.24 mmol), PyBOP (124 mg, 0.24 mmol), NMM (79 μL, 0.71 mmol) and the free amine from Boc-deprotection of **18b** (yield 94%, 100 mg, 0.24 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 5:1. Yield 115 mg (83%) of a yellowish solid; m.p. >210°C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 11.64 (brs, 1H; pyrrole NH), 10.85 (brs, 1H; NH), 10.47 (s, 1H; pyrene NH), 9.31 (brs, 1H; NH), 8.66 (brs, 1H; Ser NH), 8.52 (brs, 1H; NH), 8.33–8.28 (m, 4H; pyrene H), 8.22–8.16 (m, 4H; pyrene H), 8.07 (t, J = 7.6 Hz, 1H; pyrene H), 6.90 (brs, 1H; pyrrole H), 6.87 (brs, 1H; pyrrole H), 5.27 (brs, 1H; OH), 4.92 (m, 1H; CH), 3.95 (brs, 2H; CH$_2$), 1.46 ppm (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 ppm (C(CH$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for C$_{31}$H$_{30}$N$_6$O$_6$+Na$^+$: 605.212; found: 605.212±0.005 [M+Na$^+$].

L-2-naphthylSerGlyGuaNHBoc (20c): This compound 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 the free amine from Boc-deprotection of **18c** (yield 94%, 180 mg, 0.45 mmol). Eluent for flash chromatography: EtOAc/MeOH 98:2. Yield 200 mg (79%) of a brownish solid; m.p. 178°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.81 (brs, 1H; guanidino NH), 10.09 (s, 1H; naphthylNHCO), 9.33 (brs, 1H; guanidino NH), 8.74 (t, J = 5.7 Hz, 1H; Gly NH), 8.55 (brs, 1H; guanidino NH), 8.32 (d, J = 1.6 Hz, 1H; naphthyl H-1), 8.20 (d, J = 7.7 Hz, 1H; Ser NH), 7.89–7.81 (m, 3H; naphthyl H-4, H-5, H-8), 7.68 (dd, J = 8.8 Hz, 2.0 Hz, 1H; naphthyl H-3), 7.47 (td, J = 6.8 Hz, 1.3 Hz, 1H; naphthyl H-6 or H-7), 7.41 (td, J = 6.8 Hz, 1.3 Hz, 1H; naphthyl H-6 or H-7), 6.84 (m, 2H; pyrrole CH), 5.08 (t, J = 5.4 Hz, 1H; OH), 4.55 (dd, J = 13.3 Hz, 5.4 Hz, 1H; Ser CH), 3.99 (d, J = 5.9 Hz, 3H; Gly CH$_2$), 3.75–3.72 (m, 2H; Ser CH$_2$), 1.46 ppm (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 ppm (C(*C*H$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for C$_{27}$H$_{31}$N$_7$O$_7$+Na$^+$: 588.217; found: 588.217±0.005 [M+Na$^+$].

L-Pyren-1-ylSerGlyNHBoc (21): This compound 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 the free amine from Boc-deprotection of **18b** (yield 94%, 120 mg, 0.28 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 4:1. Yield 115 mg (87%) of a yellowish solid; m.p. 182–185°C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.28 (s, 1H; pyrene NH), 8.30–8.21 (m, 4H; pyrene H), 8.19–8.15 (m, 4H; pyrene H and Ser NH), 8.08 (t, J = 7.7 Hz, 2H; pyrene H), 7.08 (t, 2H; Gly NH), 5.24 (brt, J = 5.3 Hz, 1H; OH), 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 ppm (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 ppm (C(*C*H$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for C$_{26}$H$_{27}$N$_3$O$_5$+Na$^+$: 484.184; found: 484.184±0.005 [M+Na$^+$].

L-Pyren-1-ylSerGlyGuaNHBoc (22): This compound was prepared from *N*-Boc-5-guanidinocarbonylpyrrole-2-carboxylic acid (**19**, 30 mg, 9.9×10^{-5} mol), PyBOP (51 mg, 9.9×10^{-5} mol), NMM (33 μL, 29.6×10^{-5} mol) and the free amine from Boc-deprotection of **21** (yield 67%, 47 mg, 9.9×10^{-5} mol). Eluent for flash chromatography: THF/*n*-hexane 3:1. Yield 49 mg (78%) of a yellowish solid; m.p. 219°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 11.10 (brs, 1H; pyrrole NH), 10.73 (brs, 1H; NH), 10.31 (s, 1H; pyrene NH), 9.35 (brs, 1H; NH), 8.75 (brt, J = 5.7 Hz, 1H; Gly NH), 8.53 (brs, 1H; NH), 8.29–8.26 (m, 5H; pyrene H and Ser NH), 8.19–8.14 (m, 4H; pyrene H), 8.07 (t, J = 7.6 Hz, 1H; pyrene H), 6.87 (brs, 1H; pyrrole CH), 6.62 (brs, 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 ppm (s, 9H; C(CH$_3$)$_3$); HRMS (ESI$^+$): m/z: calcd for C$_{33}$H$_{33}$N$_7$O$_7$+Na$^+$: 662.233; found: 662.233±0.005 [M+Na$^+$].

***N*-5-Boc-*N*-2-[(5-(acridin-2-ylamino)-5-oxopentyl)carbamoyl]-1*H*-pyrrole-5-carbonylguanidino (24)**: This compound 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 the free amine from Boc-deprotection of **23b** (yield 98%, 55 mg, 0.13 mmol). Eluent for flash chromatography: EtOAc/*n*-hexane 10:1 → 14:1. Yield 30 mg (39%) of a yellow solid; m.p. 198°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 11.81 (brs, 1H; NH), 9.61 (brs, 1H; NH), 9.06 (t, J = 5.6 Hz, 1H; acridineCON*H*), 8.80 (brs, 1H; NH), 8.47 (t, J = 5.4 Hz, 1H; acridineCONHCH$_2$CH$_2$CH$_2$CH$_2$N*H*), 8.20 (d, J =

8.7 Hz, 2H; H-1, H-8), 8.00 (d, $J=8.7$ Hz, 2H; H-4, H-5), 7.92–7.88 (m, 2H; H-3, H-6), 7.70–7.65 (m, 2H; acridine 2-H, 7-H), 6.97 (brs, 1H; pyrrole CH), 6.84 (s, 1H; pyrrole H), 3.56–3.52 (m, 2H; acridine-CONHCH_2CH$_2$CH$_2$CH$_2$NH), 3.48 (brs, 2H; acridine-CONHCH$_2$CH$_2$CH$_2$CH_2NH), 1.72–1.69 (m, 4H; acridine-CONHCH$_2$CH_2CH_2CH$_2$NH), 1.48 ppm (s, 9H; C(CH$_3$)$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=165.2$ (acridine*C*ONH), 158.8 (acridine-CONHCH$_2$CH$_2$CH$_2$CH$_2$NH*C*O), 147.1 (4a-C, 10a-C), 142.6 (9-C), 130.5 (3-CH, 6-CH), 128.2 (4-CH, 5-CH), 126.3 (2-CH, 7-CH), 125.2 (1-CH, 8-CH), 121.3 (8a-C, 9a-C), 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 (acridine-CONHCH$_2$CH$_2$CH$_2$CH$_2$NH), 26.8 (C(*C*H$_3$)$_3$), 26.0 (acridine-CONHCH$_2$*C*H$_2$CH$_2$CH$_2$NH), 25.9 ppm (acridineCONHCH$_2$*C*H$_2$CH$_2$ CH$_2$NH); HRMS (ESI$^+$): *m*/*z*: calcd for $C_{30}H_{34}N_7O_5^+$: 572.261; found: 572.261 ± 0.005 [M^+].

L-Pyren-1-oyldiaminoethaneLys(Cbz)NHBoc (25): This compound 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 the free amine from Boc-deprotection of **23a** (yield quant, 73 mg, 0.18 mmol) as a brownish solid (106 mg, 90%); m.p. 190°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=8.63$–8.60 (m, 1H; pyreneCON*H*), 8.50 (d, $J=9.3$ Hz, 1H; pyrene H), 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 (brt, $J=5.6$ Hz, 1H; pyrene-CONHCH$_2$CH$_2$N*H*), 7.36–7.28 (m, 5H; Ph), 7.19 (brt, $J=5.5$ Hz, 1H; CbzN*H*), 6.79 (d, $J=7.7$ Hz, 1H; LysN*H*), 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 ppm (brs, 13H; CbzNHCH$_2$CH_2CH_2CH$_2$ and C(CH$_3$)$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=172.0$ (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 (Ph C-2, Ph C-3 and pyrene CH), 127.6 (pyrene CH), 127.3 (pyrene Cq), 127.2 (Ph C-4), 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 (Ph*C*H$_2$), 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(*C*H$_3$)$_3$), 22.3 ppm (Lys CH$_2$); HRMS (ESI$^+$): *m*/*z*: calcd for $C_{38}H_{42}N_4O_6+Na^+$: 673.299; found: 673.299 ± 0.005 [M+Na$^+$].

L-1-PyrenoyldiaminoethaneLys(Cbz)GuaNHBoc (26): This compound 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 the free amine from Boc-deprotection of **25** (yield 98%, 76 mg, 0.11 mmol). Eluent for flash chromatography: EtOAc/MeOH 95:5 → 9:1. Yield 70 mg (74%) of a brownish solid; m.p. 136–139°C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.51$ (brs, 1H; pyrrole NH), 10.87 (brs, 1H; NH), 9.31 (brs, 1H; NH), 8.61 (t, $J=5.4$ Hz, 1H; pyrene-CON*H*), 8.51 (d, $J=9.3$ Hz, 1H; pyrene H), 8.47 (d, $J=7.6$ Hz, 1H; Lys NH), 8.46 (brs, 1H; NH), 8.33 (t, $J=8.4$ Hz, 2H; pyrene H), 8.26–8.08 (m, 7H; pyrene H and NH), 7.35–7.27 (m, 5H; Ph), 7.21 (t, $J=5.4$ Hz, 1H; N*H*Cbz), 6.82 (brs, 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, $J=$ 6.4 Hz, 2H; CbzNHCH_2), 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 ppm (m, 13H; CbzNHCH$_2$CH_2CH_2CH$_2$ and C(CH$_3$)$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=171.6$ (pyrene*C*ONH), 168.5 (Lys CONH), 159.0 (pyrrole CONH), 157.9 (pyrrole CONH), 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 (Ph C-2, Ph C-3 and pyrene CH), 127.7 (pyrene CH), 127.5 (pyrene CH), 127.3 (pyrene Cq), 127.2 (Ph C-4), 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 (Ph*C*H$_2$), 52.5 (CH), 39.4 (pyreneCONH*C*H$_2$*C*H$_2$), 38.6 (pyreneCONH*C*H$_2$*C*H$_2$), 37.6 (Lys CH$_2$), 31.2 (Lys CH$_2$), 28.6 (Lys CH$_2$), 27.2 (C(*C*H$_3$)$_3$), 22.5 ppm (Lys CH$_2$); HRMS (ESI$^+$): *m*/*z*: calcd for $C_{45}H_{49}N_8O_8^+$: 829.366; found: 829.368 ± 0.005 [M^+].

L-Pyren-1-ylGlyArgAnalogue(OMe)NHBoc (29): This compound was prepared from the free acid from *t*Bu-protected acid **27** (yield 66%, 92 mg, 0.30 mmol), PyBOP (157 mg, 0.30 mmol), NMM (0.10 mL, 0.91 mmol) and Arg analogue **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; m.p. >230°C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=10.82$ (brs, 2H; pyrrole NH and NH), 9.33 (brs, 1H; NH), 8.91 (t, $J=5.9$ Hz, 1H; NH), 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 (brs, 2H; pyrrole H), 4.60 (q, $J=6.7$ Hz, 1H; CH), 4.11 (d, $J=5.9$ Hz, 2H; Gly CH$_2$), 3.77–3.67 (m, 1H; NHCH_2CH), 3.67 (s, 3H; CH$_3$), 3.62–3.51 (m, 1H; NHCH_2CH), 1.46 ppm (s, 9H; C(CH$_3$)$_3$); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta=170.4$ (pyreneCONH), 168.8 (Gly CONH and *C*OOCH$_3$), 159.7 (pyrrole CONH), 157.9 (pyrrole CONH), 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 COO*C*H$_3$), 42.0 (Gly CH$_2$), 39.5 (CH$_2$), 27.2 ppm (C(*C*H$_3$)$_3$); HRMS (ESI$^+$): *m*/*z*: calcd for $C_{35}H_{35}N_7O_8+Na^+$: 704.244; found: 704.243 ± 0.005 [M+Na$^+$].

L-Pyren-1-oyldiaminoethane-Lys(Cbz)Gua-NH·CF$_3$COOH (12): Compound **26** (70 mg, 8.44×10^{-5} mol, 1 equiv) was dissolved in TFA (1.5 mL) and TFMSA (0.1%, 1.5 μL) was added. The solution was stirred at RT for 24 h. The TFA and TFMSA were then removed under reduced pressure (oil pump). The oil obtained was dried and lyophilised. The white solid residue was purified by MPLC (RP18 column, flow 40–20 mL min^{-1}, eluent: 100% H_2O + 0.1% TFA → 100% MeOH + 0.1% TFA) to afford **12** (40 mg, 57%) as a white solid; m.p. 170°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=12.51$ (s, 1H; pyrrole NH), 11.79 (s, 1H; NH), 8.67–8.64 (m, 2H; NH), 8.51 (brs, 4H; (NH$_2$)$_2$), 8.50 (d, $J=$ 9.3 Hz, 1H; pyrene H), 8.43–8.10 (m, 9H; pyrene H and NH), 7.72 (brs, 3H; NH$_3$), 7.39 (brs, 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$_3$CH_2), 1.91–1.78 (m, 1H; NH$_3$CH$_2$CH$_2$CH$_2$CH_2), 1.73–1.64 (m, 1H; NH$_3$CH$_2$CH$_2$CH$_2$CH_2), 1.57–1.52 (m, 2H; NH$_3$CH$_2$CH_2CH_2CH$_2$), 1.47–1.36 ppm (m, 2H; NH$_3$CH$_2$CH_2CH_2CH$_2$); ^{13}C NMR ([D$_6$]DMSO 100 MHz): $\delta=171.2$ (pyrene*C*ONH), 168.5 (Lys CONH), 158.4 (pyrrole CONH), 157.5 (pyrrole CONH), 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 (pyreneCONH*C*H$_2$*C*H$_2$), 37.9 (pyreneCONH*C*H$_2$*C*H$_2$), 37.7 (Lys CH$_2$), 31.0 (Lys CH$_2$), 26.2 (Lys CH$_2$), 22.0 ppm (Lys CH$_2$); HRMS (ESI$^+$): *m*/*z*: calcd for $C_{32}H_{35}N_8O_4^+$: 595.277; found: 595.277 ± 0.005 [M^+]; 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^{-1}, $\lambda=300$ nm.

L-*N*-Pyren-1-ylGly-(pyren-1-oyldiaminoethane) Arg analogue NHBoc 30: Compound **29** (40 mg, 4.5×10^{-5} mol, 1 equiv) was dissolved in a THF/H_2O mixture (4:1, 3 mL THF, 0.75 mL H_2O) and LiOH·H_2O (3 mg, 6.6×10^{-5} mol, 1.5 equiv) was added. The solution was stirred at RT for 2 h and was then adjusted to pH 6 with aq HCl (5%) and lyophilised. The acid obtained was used in the next step without further purification. A solution of the acid (50 mg, 7.5×10^{-5} mol, 1 equiv), PyBOP (39 mg, 7.5×10^{-5} mol, 1 equiv) and NMM (25 μL, 0.91 mmol, 3 equiv) in dry DMF (3 mL) was stirred for 20 min at RT. Afterwards, the free amine from **23a** (30 mg, 7.5×10^{-5} mol, 1 equiv) was added and the solution was stirred at RT overnight. It was then poured into water and the suspension was stirred at 0°C for 2 h. The precipitate was filtered off, washed several times with water and lyophilised. 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; m.p. 250°C (decomposition); ^{1}H NMR ([D$_6$]DMSO, 400 MHz): $\delta=11.54$–11.11 (brs, 1H; pyrrole NH), 11.11–10.65 (brs, 1H NH), 9.29 (brs, 1H; NH), 8.95 (t, $J=5.7$ Hz, 1H; pyreneCON*H*), 8.68 (t, $J=5.6$ Hz, 1H; NH), 8.60 (d, $J=9.2$ Hz, 1H;

pyrene H), 8.54 (brs, 1H; NH), 8.48 (d, $J=9.3$ Hz, 1H; pyrene H), 8.39 (d, $J=7.5$ Hz, 1H; Gly NH), 8.34–8.06 (m, 18H; pyrene H and 2NH), 6.77 (brs, 2H; pyrrole H), 4.55 (q, $J=6.6$ Hz, 1H; CH), 4.58–4.00 (m, 2H; Gly CH_2), 3.73–3.39 (m, 6H; $3\times CH_2$), 1.44 ppm (s, 9H; $C(CH_3)$); ^{13}C NMR ([D_6]DMSO, 100 MHz): $\delta=169.4$ (CONH), 169.1 (CONH), 168.7 (CONH), 168.6 (CONH), 157.9 (CONH), 131.2 (2×pyrene Cq), 131.0 (pyrene Cq), 130.7 (pyrene Cq), 130.1 (2×pyrene Cq), 129.6 (2×pyrene Cq), 127.8 (pyrene CH), 127.7 (pyrene CH), 127.5 (2×pyrene CH), 127.4 (pyrene Cq), 127.2 (pyrene Cq), 126.6 (2×pyrene CH), 126.0 (2×pyrene CH), 125.2 (2×pyrene CH), 125.0 (2×pyrene CH), 124.8 (2×pyrene CH), 124.3 (pyrene CH), 124.2 (pyrene CH), 123.8 (2×pyrene CH), 123.2 (2×pyrene 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 ppm ($C(CH_3)_3$); HRMS (ESI^+): m/z: calcd for $C_{53}H_{47}N_9O_8+Na^+$: 960.344; found: 960.344±0.005 [$M+Na^+$].

Spectroscopic studies: Polynucleotides were purchased as noted: poly dA–poly dT, poly dAdT–poly dAdT, poly dGdC–poly dGdC, poly A–poly U, poly A, poly G, poly C, poly U (Sigma), calf thymus (ct)-DNA (Aldrich). Polynucleotides were dissolved in 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 spectroscopically as concentration of phosphate.

The electronic absorption spectra were obtained with a Varian Cary 100 Bio spectrometer, CD spectra were collected with a Jasco J-810 spectrometer and fluorescence spectra were recorded with 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, $I=0.03$ mol dm^{-3}). Under the experimental conditions used the absorbance and fluorescence intensities of studied compounds were proportional to their concentrations. Relative fluorescence quantum yields (Q) were determined by the standard procedure.[32] All samples were purged with argon to displace oxygen, and emission spectra were recorded from 350–600 nm and corrected for the effects of time- and wavelength-dependent light-source fluctuations by use of a rhodamine 101 standard, 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 L-*N*-acetyltryptophanamide (NATA, Fluka, Buchs, Switzerland) with published fluorescence quantum yield $Q=0.14$.[33] In fluorimetric titrations an excitation wavelength of $\lambda_{exc}>320$ nm was used to avoid inner filter effects caused by absorption of excitation light by added polynucleotide. The binding constant (K_s) and [bound compound]/[polynucleotide phosphate] ratios (n) were calculated by use of the Scatchard equation by nonlinear least-squares fitting, giving excellent correlation coefficients (>0.999) for obtained values for K_s and n. Thermal melting curves for ds-polynucleotides and their complexes with studied compounds were determined as described previously, 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 normalised. Obtained T_m values are the midpoints of the transition curves, determined from the maximum of the first derivative or graphically by a tangent method. Given ΔT_m values were calculated by subtraction of the T_m of the free nucleic acid from the T_m of the complex. Every ΔT_m value here reported was the average of at least two measurements; the error in ΔT_m is ±0.5 °C.

Ethidium bromide (EB) displacement assay: Ethidium bromide ($c=5\times10^{-6}$ mol dm^{-3}) was added ($r_{([EB]/[polynucleotide]}=0.25$) to polynucleotide solution ($c=2\times10^{-5}$ mol dm^{-3}), and quenching of the EB/polynucleotide complex fluorescence emission ($\lambda_{ex}=520$ nm, $\lambda_{em}=601$ nm) was monitored as function of c(EB)/c(compound). The given IC_{50} values represent 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 addition of polynucleotide.

Proliferation assays: The growth inhibition activities were assessed as described previously,[34] by a 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^{-1}; 10^{-5} for **13**) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent (DMSO) was also tested for possible inhibitory activity by adjustment of its concentration to be the same as under the working concentrations (maximum concentration of DMSO was 0.25%). After 72 h 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} values: the concentrations necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose-response curves by means of linear regression analysis through fitting of 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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