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Asymmetrical Diaromatic Guanidinium/2-Aminoimidazolinium Derivatives: Synthesis and DNA Affinity

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In this paper we report the synthesis of three families of new amidine-based aromatic derivatives as potential DNA minor groove binding agents for the treatment of cancer. The preparation of monoguanidine, mono-2-aminoimidazoline, and asymmetric diphenylguanidine/2-aminoimidazoline derivatives (compounds **1a**−**c** to **8a**−**c**) is presented. The affinity of these substrates and of a family of mono- and bis-isoureas (previously prepared in Rozas' laboratory) for DNA was evaluated by means of DNA thermal denaturation measurements. In particular, compounds **2c**, **5c**, **6c**, **7c**, and **8c** were found to bind strongly both to natural DNA and to adenine−thymine oligonucleotides, showing a preference for the adenine−thymine base pair sequences.

Introduction

The DNA minor-groove is the interaction site for many replicative and repair enzymes and transcription control proteins. It also provides an opportunity to design drugs that are capable of selective binding and hence the potential to interfere with these processes. Minor-groove binding agents typically have long and planar structures that allow them to adopt a crescent shape that fits into the minor-groove, forming close contacts in the deep, narrow space formed between the two DNA strands. These DNA/minor-groove binder complexes are typically stabilized by hydrogen bonds (HBs[a]), van der Waals contacts, and/or ionic interactions.[1] Binding is typically driven by hydrophobic interactions, and if the molecule is positively charged, this is usually accompanied by the liberation of a large number of water molecules. The positively charged natural products distamycin and netropsin were among the first examples of molecules that specifically target the minor-groove. Since their discovery, many drugs that target the minor-groove have been developed. They are highly selective, and they compromise the fundamental biochemistry of a cell through inhibition of the actions of DNA-dependent enzymes, for example, by direct inhibition of transcription.[2−4]

Sophisticated synthesis has yielded oligoamides capable of highly specific binding;[5] further development has also witnessed the emergence of hairpin polyamides to latch around the groove.[6] While many different minor-groove binders have been synthesized and tested as anticancer agents, their severe toxicity has prevented any therapeutic use. However, some dicationic aromatic diamidines such as furamidine (Figure 1)[7] have shown promising antiproliferative activity against different tumor cell lines. The furamidine derivative furimidazoline is one particularly good example.[8] It is worth noting that fluorescence microscopy indicates the rapid nuclear accumulation of some furamidines in tumor cells.[9] These findings together open new possibilities for developing novel aromatic dicationic derivatives with enhanced interaction and selectivity within the DNA minor-groove and possibly with more selective toxicity.

We have previously prepared a large number of symmetric diaromatic bis-guanidinium and bis-2-aminoimidazolinium derivatives, which are structurally related to furamidine, with potential to treat sleeping sickness (Figure 1).[10] These compounds showed good affinity for DNA as indicated by the increment of the melting temperature (ΔT_m) values obtained in thermal denaturation measurements. The results encouraged us to develop new ligands that might more efficiently bind to DNA.

Hence, we have further developed this set by preparing three new additional families (Figure 2), comprising a series of diaromatic monoguanidinium and mono-2-aminoimidazolinium derivatives (family I, compounds **1g**,**h** to **8g**,**h**), a series of diaromatic mono- and bis-isouroniums (family II, compounds **9i**,**j** to **11i**,**j**), and a series of asymmetric diaromatic guanidinium/2-aminoimidazolinium dications (family III:, compounds **1c** to **8c**). Consideration of the properties of each of these families has allowed us to explore the requirements for improved binding to the DNA minor-groove. All of the compounds described here contain the basic furamidine scaffold which has been modified by systematically changing both the terminal cationic arms and the bridging furan moiety.

The cationic groups may bind to DNA in two ways, first through direct H-bonds through the NH groups and second through water-mediated interactions with the bases. These

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[a] Abbreviations: HB, hydrogen bond; ΔT_m, increment in DNA denaturation temperature; AT, adenine−thymine pairs; MES, 2-(*N*-morpholino)ethanesulfonic acid; *P*/*D*, ratio between base pairs and ligand (drug).

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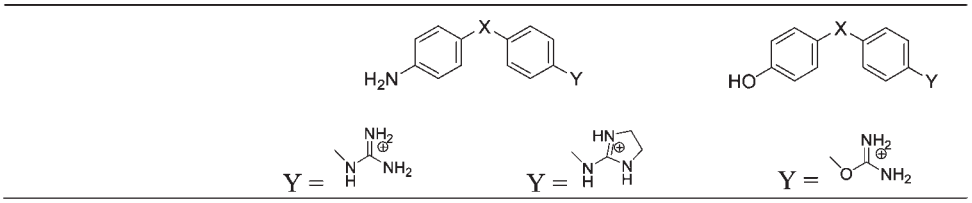
Table 1. Results Obtained from the Thermal Melting Experiments Showing the Change in Melting Temperature in the Presence of the Monoamidine-like Cations (**1g**,**h**−**6g**,**h**, **7g**, **8g**,**h**, **9i**−**11i**) to Salmon Sperm DNA ($\Delta T_{m(SS)}$, °C)[a]



X		$\Delta T_{m(SS)}$, °C		$\Delta T_{m(SS)}$, °C		$\Delta T_{m(SS)}$, °C
CH_2	**1g**	3	**1h**	3	**9i**	2
CH_2CH_2	**2g**	0	**2h**	0		
O	**3g**	2	**3h**	0	**10i**	4
S	**4g**	2	**4h**	0	**11i**	2
NH	**5g**	2	**5h**	2		
Piperazine	**6g**	2	**6h**	1		
CO	**7g**	0				
NHCONH	**8g**	2	**8h**	4		

[a] Melting temperature in phosphate buffer (10 mM) is 69 °C.

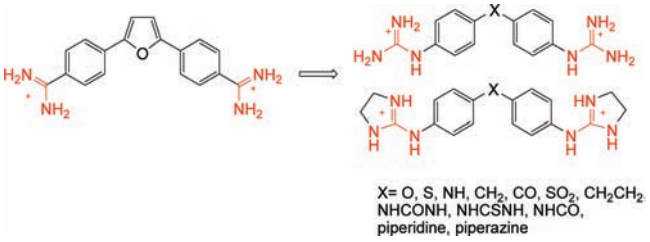


Figure 1. Structure of furamidine (left) and general structure of the related compounds previously reported by Rozas' group[10] that have shown good affinity toward DNA.

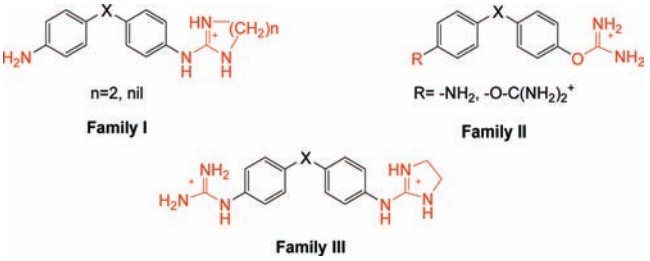


Figure 2. Structures of the three families studied.

two mechanisms of binding mean that different interactions, depending on the nature of the cation, can exist. In this work the amidinium cations have been exchanged with guanidinium, 2-aminoimidazolinium, and isouronium cations. In these cases, we sought to enhance HB contacts in the minor groove by increasing the number of HB donors (NH groups in the guanidinium and 2-aminoimidazolinium cations) or introducing an additional HB acceptor (an O atom in the isouronium cation). Furthermore, these are more articulated cations than amidinium. The structure of the supporting molecular scaffold or linker is very important if we wish to capitalize on these multiple binding interactions. The optimum linker will allow for a better orientation of the positive charges within the groove and introduce the curvature or geometry best suited to fitting. However, it is now known that curvature is not essential to fit into the minor groove, since Boykin et al. have shown that linear molecules are also capable of strongly binding to the minor-groove.[11] Work by Nguyen et al. showed that in the linear molecule CPG-40215A, the *N*,*N*′-diaminoguanidine bridging group served as a "seesaw" hinge facilitating a variety of interactions with the bases through motions that position the terminal amidine cations in a range of binding modes.[12]

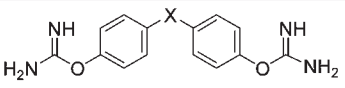
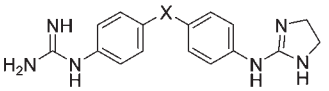
Moreover, to investigate the optimal linking scaffold, we have exchanged the furan moiety of furamidine for a number of smaller functional groups (O, S, NH, CO, CH_2, CH_2CH_2, or NHCONH). We hypothesized that such a substitution should afford a 2-fold advantage by facilitating the interaction with DNA through HBs (as donors or acceptors), and as our cations are larger than in the furamidine series, making this central group smaller should ensure optimization of the distance between the cationic moieties (guanidinium and 2-aminoimidazolinium) for DNA binding.

To assess the binding to DNA, we have performed thermal denaturation experiments in natural DNA (salmon sperm, 68% adenine−thymine base pair (AT) content). Furthermore, since binding to the minor groove requires the presence of a run of five AT base pairs, binding experiments were also conducted in the presence of double stranded AT homopolymers [poly(dA·dT)$_2$ and poly(dA)·poly(dT)] to assess their potential selectivity for the minor groove.

Results and Discussion

Chemistry. On the basis of our previous experience and taking into account the good results obtained by our group,[10,13,14] the guanidinylation and 2-aminoimidazolinylation of the present target molecules were performed following our established methodology. This consists of the reaction of a deactivated aromatic amine with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (Boc protected guanidine precursor) or *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione[13] (Boc protected 2-aminoimidazoline precursor) assisted by mercury(II) chloride in the presence of an excess of triethylamine. Deprotection of the Boc-intermediates of the first stage and further treatment with Amberlyte resin in water lead to the hydrochloride salts of the target molecules.[10,13−15] Preparation of the corresponding monoisouronium (**9i**, **10i**, **11i**, Table 1) and bis-isouronium derivatives (**9j**, **10j**, **11j**, Table 2) has been reported by our group in a

Table 2. Results Obtained in the Thermal Melting Experiments Showing the Change in Melting Temperature of DNA in the Presence of the Bis-isouronium Derivatives (**9j**−**11j**) and Asymmetric Diphenyl Dicationic Compounds (**1c**−**8c**)

X		$\Delta T_{m(SS)}$ (°C) Salmon sperm DNA[a]	$\Delta T_{m(AT)}$ (°C) Poly(dA•dT)$_2$ DNA[b]	Discretion $\Delta T_{m(AT)} - \Delta T_{m(SS)}$ (°C)	$\Delta T_{m(A)(T)}$ (°C) Poly(dA)• poly(dT) DNA[c]	Sequence Specificity
	Cmp		Family II			
CH_2	**9j**	4	–	–	–	–
O	**10j**	6	–	–	–	–
S	**11j**	5	–	–	–	–
	Cmp		Family III			
CH_2	**1c**	6	6	0	17.2	None
CH_2CH_2	**2c**	8	16	8	29	AT
O	**3c**	7	10	3	25.3	AT
S	**4c**	7	9	2	23	AT
NH	**5c**	12	17.5	5.5	35.1	AT
Piperazine	**6c**	9	15	6	31.1	AT
CO	**7c**	8	15	7	32	AT
NHCONH	**8c**	11.5	14	2.5	30	AT
Netropsin		–	39	–	–	–

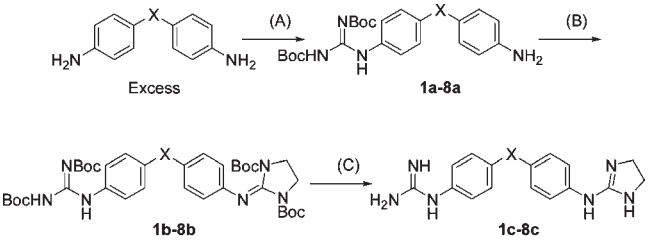
[a] DNA melting temperature in phosphate buffer (10 mM) is 69 °C. [b] $P/D = 10$. Poly(dA·dT)$_2$ melting temperature in phosphate buffer (10 mM) is 47 °C. Experiments were performed in TCD. [c] $P/D = 3$. Poly(dA)·poly(dT) melting temperature in MES buffer (10 mM) is 43 °C. Experiments were performed by W. D. Wilson et al. at Georgia State University, GA.

previous article[16] using a similar synthetic approach to the one just described starting from the corresponding aromatic alcohols.

Synthesis of Asymmetric Guanidine/2-Aminoimidazoline Derivatives (Family III). All our starting materials are symmetric, and hence, the two amino moieties have the same reactivity. As we intend to introduce different groups at both ends of the molecule, an excess of the diamines was required to avoid difunctionalization. Thus, a total of 3 equiv of each of the corresponding diamines was treated with just 1 equiv of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea, 1 equiv of mercury(II) chloride, and an excess of triethylamine to afford the desired monofunctionalized Boc-protected guanidines (**1a**−**8a**) as main products with yields ranging from 29% to 82% (see Scheme 1). Under these conditions, less than 5% of the difunctionalized products was observed.

In the second stage of the synthesis, 1 equiv of each of the previous intermediates was reacted with 1 equiv of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione, a slight excess of mercury(II) chloride, and an excess of triethylamine to afford the asymmetric Boc-protected derivatives (**1b**−**8b**) in moderate to good yields. Standard deprotection of the Boc groups with an excess of trifluoroacetic acid in dichloromethane followed by treatment with Amberlyte resin in water led to the hydrochloride salts of the asymmetric guanidine- and 2-aminoimidazoline containing products (**1c**−**8c**).

Scheme 1[a]

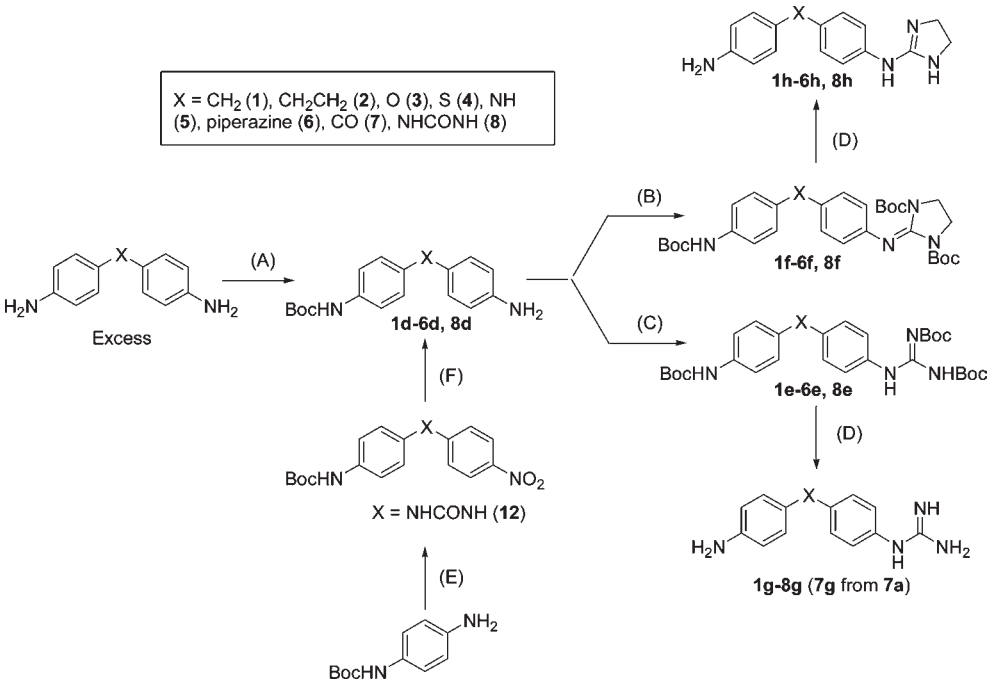


[a] Reagents and conditions: (A) *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea, $HgCl_2$, TEA, DCM or DMF, room temp; (B) *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione, $HgCl_2$, TEA, DCM or DMF, room temp; (C) (i) TFA, DCM, room temp; (ii) Amberlyte resin, H_2O, room temp.

Synthesis of Aminoguanidinium and Amino-2-aminoimidazolinium Derivatives (Family I). Regarding the synthesis of the monoguanidine and mono-2-aminoimidazoline containing derivatives, in the first step we decided to protect one of the amino groups of the starting amines with Boc (see Scheme 2) to increase the solubility of the intermediates and allow a faster development of the column chromatography.

Following the same strategy as mentioned above, a total of 3 equiv of each of the starting diamines was treated with

Scheme 2[a]



[a] Reagents and conditions: (A) Boc_2O, TEA, DCM or DMF, room temp; (B) *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione, $HgCl_2$, TEA, DCM or DMF, room temp; (C) *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea, $HgCl_2$, TEA, DCM or DMF, room temp; (D) (i) TFA, DCM, room temp; (ii) Amberlyte resin, H_2O, room temp; (E) 4-nitrobenzoisocyanate, DCM, 0 °C; (F) H_2 (3 bar), Pd−C, MeOH.

1 equiv of Boc anhydride. The mono-Boc-protected amines (**1d**−**6d**) were obtained as the main products in good yields.

The urea derivative **8d** was prepared following a different route (see Scheme 2). Thus, reaction of 4-nitrobenzoisocyanate with mono-Boc-protected 4-aminoaniline yielded the corresponding Boc protected 4-[3-(4-nitrophenyl)ureido]-aniline (**12**), which upon hydrogenation produced derivative **8d**. However, the carbonyl group proved to be too deactivating and compound **7d** (and subsequently **7e**, **7f** and **7h**) could not be prepared. All these compounds are new except for **1d**[17] and **3d**,[18] which have been already described.

In the second stage, the mono-Boc-protected amines were treated with 1 equiv of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea or *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione, a slight excess of mercury(II) chloride, and triethylamine. The guanidine precursors (**1e**−**6e** and **8e**) were purified by column chromatography in silica gel, whereas for the 2-aminoimidazoline ones (**1f**−**6f** and **8f**), neutral alumina was used instead. Finally, deprotection of the Boc groups afforded the final monoamidinium-like products after three stages in moderate to good overall yields. It should be mentioned that the aminoguanidine derivatives **1g**−**8g** could also be obtained after Boc deprotection of **1a**−**8a**. In fact, this was the only possible route to prepare compound **7g** and proved to be a shorter and more efficient methodology, producing higher overall yields.

All the diamines used as starting materials are commercially available from Aldrich or Fluka except for the 1,4-(bis-4-aminophenyl)piperazine, whose synthesis has been described previously.[19] It is also worth mentioning that the unreacted starting diamines could be recovered from the column eluting with a more polar solvent system.

Biophysical Results: Thermal Denaturation Assays. Thermal denaturation experiments are easy to perform and provide quick and reliable information on the binding of small molecules to DNA. These assays, for example, in unspecific salmon sperm DNA, can be used as a general screening for the suitability of the compounds studied as DNA binders. Afterward, the preference for specific base sequences can be explored.

Denaturation Studies of Families I−III in the Presence of Natural DNA. The interaction of all the molecules with DNA was first examined by performing thermal denaturation experiments using a mixed sequence salmon sperm DNA with a 32% GC content. The melting temperature of salmon sperm DNA ($T_{m(SS)}$) was measured in the presence of the different families of molecules.

The results found for some of the monoamidinium-like derivatives (guanidinium, 2-aminoimidazolinium, and isouronium derivatives from families I and II) are presented in Table 1. The monoamidinium-like compounds tested were found to have a slight stabilizing interaction with natural DNA, as indicated by the moderate increase in melting temperature, with $\Delta T_{m(SS)}$ values of around 3 °C being obtained.

Progressing from the monocationic species to the dicationic symmetric bis-isouronium derivatives (**9j**−**11j**) resulted in moderately greater changes (Table 2). For these compounds, the O linker (**10j**) was found to have the greatest effect on the melting temperature, resulting in an increase of 6 °C in the T_m value.

More pronounced changes were observed when the measurements were repeated for the asymmetric dicationic compounds of family III (**1c**−**8c**). These compounds were found to significantly increase the T_m by a range of 6 and 12 °C, indicating an enhanced stabilization of the duplex structure that is attributed to improved binding.

Comparison of the $\Delta T_{m(SS)}$ values obtained for the symmetric bis-isouronium (**9j**−**11j**) and the asymmetric dicationic (**1c**, **3c**, **4c**) possessing the CH_2, O, or S linker revealed

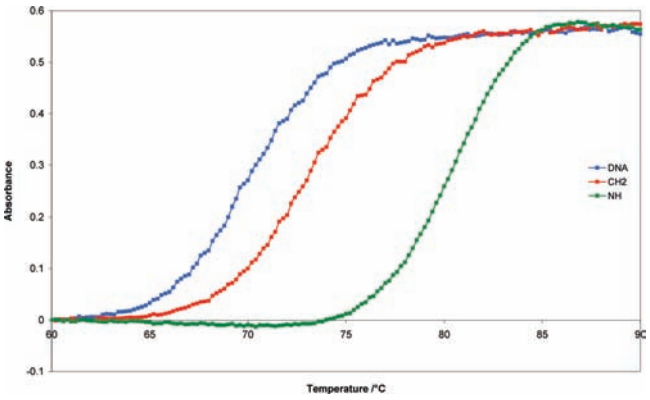


Figure 3. Graph showing the ΔT_m for **1c** (ΔT_m = 6 °C, red) and **5c** (ΔT_m = 12 °C, green) in salmon sperm DNA (blue).

very little difference in the melting temperature between the compounds with different cations.

When we compare the melting results of the molecules with the methylene (**1c**; see thermal melting experiment results in Figure 3) and ethylene (**2c**) bridges in the presence of natural DNA, we observe that the ethylene linker gave a slightly better melting result, which is indicative of improved binding. A possible reason for this is that the extra CH_2 increases hydrophobicity, thereby improving the hydrophobic interactions. Another possible conclusion for this is that the more flexible ethylene linker provides another point of contact with the DNA, allowing for a better binding.

A comparable change was also found in the case of the bulky piperazine linker (**6c**), which yielded intermediate stabilization of DNA. This is interesting given the linear and bulky nature of the piperazine linker, and thus, the combination of both features could play a role in its entry and fitting into the minor groove. The greatest change was observed for the NH (**5c**; see thermal melting experiment results in Figure 3) and NHCONH (**8c**) species (~12 °C). Interestingly, both of these compounds are capable of strong hydrogen bonding interactions. The strong binding of compound **8c** to salmon sperm DNA (Table 2) could possibly be explained because it has a urea linker that could provide enough freedom of rotation, allowing a good induced fit inside the DNA minor groove. However, it is noted that the changes do not seem to reflect the difference in the length of the linker in the cases of **5c** and **8c**.

Denaturation Studies of Family III in the Presence of Poly(dA·dT)$_2$ and Poly(dA)·Poly(dT). The experiments carried out on the asymmetric series in the presence of salmon sperm DNA revealed that, considering the linkers, the order of affinity is −NH− ~ −NHCONH− > −piperazine−, −CO−, $-CH_2CH_2-$, −O−, −S−, $-CH_2-$. In light of the promising results found for family III, the experiments were repeated in the presence of poly(dA·dT)$_2$ using similar experimental conditions. Here, the absence of the G·C base pair reduces the width of the groove, allowing for a tighter fit, with minor groove complexes typically forming with a run of four A·T base pairs. In almost all experiments, a greater change in melting temperature was found in the presence of the asymmetric dications (**2c**−**8c**) in the AT oligonucleotides. This result was taken as a strong indication of a preference for the minor groove binding site afforded in homopolymer DNA and indicates sequence selectivity for the AT domains of DNA (Table 2). The exception was found for the CH_2 species (**1c**) which was found to stabilize the homopolymer to the same extent as natural DNA.

However, in the case of the ethylene linker (**2c**) the melting temperature showed a 2-fold increment compared to salmon sperm DNA. This would imply that while **2c** binds to AT rich domains, which are characterized by a narrow minor groove, **1c** is unlikely to bind in the same fashion, as the increased availability of binding sites in the AT homopolymer is not accompanied by increased binding affinity. Thus, it may be concluded that the restricted degree of rotation conferred by the $-CH_2-$ bridge does not favor insertion into the narrower minor groove present in the AT DNA and the molecule most probably binds more effectively through an alternative mode. However, the higher degree of rotation due to the extra CH_2 in **2c** allows the molecule to wrap around the minor groove better than **1c**.

Next, we considered the influence of the other bridging groups in the selective binding between the different DNA systems. By comparison of molecules **1c**, **3c**, **4c**, and **5c** (linkers $-CH_2-$, −O−, −S−, and −NH−, respectively), it was noted that compound **5c** had the highest melting temperature in both salmon sperm DNA and poly(dA·dT)$_2$. This was followed by molecules **3c** and **4c** that have the same melting temperature in salmon sperm DNA but slightly different in poly(dA·dT)$_2$ DNA (by 1 °C). As noted previously, the smallest change was observed for compound **1c**. These results seem to indicate the importance of hydrogen bonding with a possible HB donation to the O2 of thymine being a potential dominant factor. The fact that **3c** and **4c** gave better melting results in comparison to **1c** illustrates further the order of binding, since the oxygen and sulfur linkers could be HB acceptors, but the methylene bridge is a known weak HB donor.

However, if we consider the relative sensitivity of the melting temperature (ΔT^{rel}) defined to the DNA type ($\Delta T_{m(AT)} - \Delta T_{m(SS)}$; see Table 2), two distinct groupings emerge. The first group contains the compounds **2c** ($-CH_2CH_2-$), **5c** (−NH−), **6c** (−piperazine−), and **7c** (−CO−) and shows a ΔT^{rel} change of about 6 °C. The second grouping shows significantly less sensitivity to the two forms of DNA, **1c** ($-CH_2-$), **8c** (−NHCONH−), **4c** (−S−), and **3c** (−O−), with the ether linker showing the greatest difference of 3 °C.

When the results for molecules **1c**, **5c**, and **7c** (linkers $-CH_2-$, −NH−, and −CO−, respectively) are compared, the primary difference is the HB characteristics of the corresponding linkers, with the methylene bridge being a weak HB donor, the −NH− a stronger HB donor, and the carbonyl group a HB acceptor. The order of binding to DNA and AT oligonucleotides, from best to worst, was found to be as follows: strong HB donor (NH) > HB acceptor (CO) > weak HB donor (CH_2). This indicates that, in the synthesis of future molecules, incorporating more HB donors would be advantageous. The geometry around the bridging linker must also be considered, as it is likely to play an important role. Here, there are two important factors. First, the steric nature of the geometry (tetrahedral $-CH_2-$ vs trigonal planar −CO− and trigonal pyramidal −NH−) will influence the approach to and binding in the pocket. Second, the rotational freedom conferred by the bridging moiety would be expected to influence the binding. There is no freedom of rotation in **7c** (trigonal planar), whereas there is freedom of rotation around the linker for molecules **1c** (tetrahedral linker) and **5c** (trigonal pyramidal linker). It is interesting

to note that compound **6c** shows a medium affinity not only toward natural DNA but also to poly(dA·dT)$_2$. This linker is longer than the rest of the groups considered, and the relatively good results obtained could be justified by the large size and linear characteristics of the piperazine ring. The ΔT_m of netropsin (a well-known minor groove binder) was also measured in this homopolymer for the sake of comparison. Even though this compound shows a 2-fold binding strength compared to the asymmetric dications, this can be explained by the large number of HB donor and acceptor groups present in this molecule.

AT sequence selectivity between different five-base-pair binding sites has previously been reported for the different binding modes (1:1 versus 2:1) of the classic minor groove binder distamycin.[20] In the study by Chen et al., a preference for the ATATA over AAAAA was observed. In addition, small molecules can bind in a 2:1 and a 1:1 fashion in the minor-groove, and this seems to strongly depend on the sequence. It has been found by Vesnaver et al.[21] that the narrowest groove is that of five base pairs of AT and netropsin prefers 1:1 binding in such a system. For this reason, we also considered the interesting results already published by us in collaboration with W. D. Wilson (Georgia State University, GA) on the thermal denaturation of the symmetric bis-guanidines and bis-2-aminoimidazolines[8] and pursued further collaboration with this group using those reported conditions with the asymmetric dications and the poly(dA)·poly(dT) oligonucleotide (Table 2). It was felt that these experiments would help to confirm the selectivity of the asymmetric dications toward the AT sequences.

The most significant differences in conditions were, on the one hand, the higher loading of the drug to DNA, with a ratio between base pairs and ligand (P/D) of 3 rather than that of 10 used above and, on the other hand, the buffer used, 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), as opposed to 10 mM phosphate buffer. Because of these differences, the results obtained cannot be quantitatively compared to the poly(dA·dT)$_2$ results. However, the trend observed is similar and a good correlation was found between both sets of results, as shown in Figure 4. By comparison of the results previously published with Wilson's group on the thermal denaturation of the symmetric bis-guanidines and bis-2-aminoimidazolines[10] and those found now using the same Wilson's conditions (Table 2), it is interesting to observe how the ΔT_m values obtained for the asymmetric compounds are almost an average of those measured for the bis-guanidines (smaller) and those obtained for the bis-2-aminoimidazolines (larger). This would imply a similar binding mode in both AT DNAs and most probably linked to a 1:1 mode, as would be expected given the bis-cationic nature of the compounds under study.

From these studies, it can be concluded that hydrophobic interactions, hydrogen bonding, electrostatic interactions, and molecular conformation are all important factors in DNA minor groove binding for the asymmetric dications. More importantly, these show strong binding to DNA showing AT-sequence specificity.

Conclusions

We have prepared three different families of mono- and bis-amidinium-based derivatives in order to explore their potential as DNA minor-groove binders. First, with respect to family I, the monoguanidinium and mono-2-aminoimidazolinium derivatives have been prepared following the synthetic pathways previously described by us.[10,13,14] Second, regarding family II, the preparation of the asymmetric guanidinium/2-aminoimidazolinium dications has been performed from the corresponding monoguanidinium derivatives and subsequent introduction of the 2-aminoimidazoline group. Third, regarding family III, the preparation of the mono- and bis-isouronium derivatives has been reported in a previous paper from our group.[16]

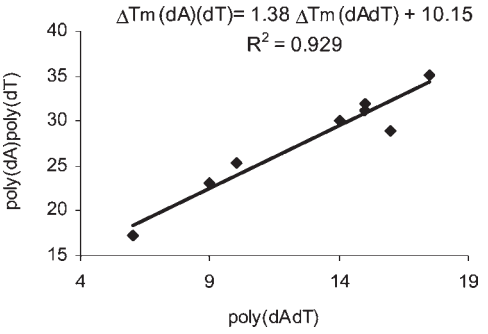


Figure 4. Correlation found between the ΔT_m poly(dA·dT)$_2$ vs ΔT_m poly(dA)·poly(dT).

The ability of these compounds to bind to DNA has been evaluated by thermal denaturation experiments not only with mixed sequence DNA (salmon sperm), which comprises all four bases, but also with poly(dA·dT)$_2$ and poly(dA)·poly(dT) oligonucleotides. Despite showing cationic groups at both ends of the diaromatic moieties, monoamidinium-like derivatives (ammonium and guanidinium or 2-aminoimidazolinium) and bis-isouroniums (two isouronium cations) have been shown to poorly/moderately bind to DNA. Nevertheless, in general, asymmetric bis-amidinium-based derivatives displayed a good affinity toward DNA and a preference for the AT sequences. In particular, compounds **2c**, **5c**, **6c**, **7c**, and **8c** strongly bind to both unspecific and AT oligonucleotides.

Taking into consideration these promising results, we believe that these asymmetric compounds deserve more investigation as DNA targeting agents, and thus, more biophysical studies are ongoing and will be reported soon.

Experimental Section

Chemistry. All the commercial chemicals were obtained from Sigma-Aldrich or Fluka and were used without further purification. Deuterated solvents for NMR use were purchased from Apollo. Dry solvents were prepared using standard procedures, according to Vogel, with distillation prior to use. Chromatographic columns were run using silica gel 60 (230−400 mesh ASTM) or aluminum oxide (activated, Neutral Brockman I STD grade 150 mesh). Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F_{254} silica gel plates or Polygram Alox N/UV_{254} aluminum oxide plates. Visualization was by UV light (254 nm). NMR spectra were recorded in a Bruker DPX-400 Avance spectrometer, operating at 400.13 and 600.1 MHz for ^{1}H NMR and 100.6 and 150.9 MHz for ^{13}C NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker Win-NMR 5.0 software. Electrospray mass spectra were recorded on a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with methanol, water, or ethanol as carrier solvents. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrometer equipped with a Gateway 2000 4DX2-66

workstation and on a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with Universal ATR sampling accessory. Elemental analyses (C, H, N) of the target compounds, which were performed at the Microanalysis Laboratory (School of Chemistry and Chemical Biology, University College Dublin, Ireland), are within ±0.4% of the calculated values, confirming ≥95% purity. Fractional moles of water frequently found in amidinium-like salts could not be prevented despite 24−48 h of drying in vacuum.

General Method for the Synthesis of Monofunctionalized Boc-Protected Guanidines: Method 1 [Scheme 1, Conditions A]. Over a solution containing an excess (9.0 mmol) of each of the starting diamines, 3.0 mmol of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C, a total of 3.3 mmol of $HgCl_2$ was added. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then the mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was washed with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with the corresponding hexane/EtOAc mixture to yield the desired compound as a solid. Less than 5% of the disubstituted substrate was observed.

General Procedure for the Synthesis of Boc-Protected Asymmetric Guanidine/2-Aminoimidazoline, Boc-Protected Amino/2-Aminoimidazolidine, and Boc-Protected Amino/Guanidine Derivatives: Method 2 [Scheme 1, Conditions B; Scheme 2, Conditions B and C]. Each of the corresponding amines was treated in DCM at 0 °C with 1.1 equiv of mercury(II) chloride, 1.0 equiv of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione (for the 2-aminoimidazoline precursors) or *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea (for the guanidine precursors) and 3.1 equiv of TEA. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite to get rid of the mercury sulfide formed. The filter cake was rinsed with EtOAc. The organic phase was washed with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by silica gel (guanidine precursors) or neutral alumina column flash chromatography (2-aminoimidazoline precursors), eluting with the appropriate hexane/EtOAc mixture.

General Procedure for the Synthesis of Mono-Boc-Protected 4,4′-Diphenyldiamines: Method 3. Compounds 1d−6d [Scheme 2, Conditions A]. Over a solution containing an excess (30.0 mmol) of each of the corresponding diamines in DCM (200 mL), 10.0 mmol of TEA and 10.0 mmol of di-*tert*-butyl dicarbonate (Boc_2O) were added at 0 °C. The resulting mixture was stirred at 0 °C for 1 and 16 h more at room temperature. Then the mixture was concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane/EtOAc mixture. The mono-Boc-protected compound was obtained as a solid. The excess of unreacted starting material diamine was recovered from the column. Less than 10% formation of the di-Boc-protected compound was observed.

General Procedure for the Synthesis of the Hydrochloride Salts: Method 4. Each of the corresponding Boc-protected precursors (0.5 mmol) was treated with 15 mL of a 50% solution of trifluoroacetic acid in DCM for 3 h. After that time, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was dissolved in 20 mL of water and treated for 24 h with IRA400 Amberlyte resin in its Cl^- form. Then the resin was removed by filtration and the aqueous solution washed with DCM (2 × 10 mL). Evaporation of the water afforded the pure hydrochloride salt. Absence of the trifluoroacetate salt was checked by ^{19}F NMR.

Dihydrochloride Salt of *N*-{4-[4-(imidazolidin-2-ylideneamino)benzyl]phenyl}guanidine (1c): Method 4. White solid (96%); mp 78−80 °C; ^1H NMR (D_2O) δ 3.58 (s, 4H, CH_2), 3.76 (s, 2H, $PhCH_2Ph$), 6.98 (d, 2H, *J* = 8.0 Hz, Ar.), 7.01 (d, 2H, *J* = 8.0 Hz, Ar.), 7.15 (d, 2H, *J* = 8.0 Hz, Ar.), 7.16 (d, 2H, *J* = 8.0 Hz, Ar.); ^{13}C NMR (D_2O) δ 39.6 ($PhCH_2Ph$), 42.2 (CH_2 Im), 123.3, 125.1, 129.6, 129.7, 131.5, 132.6, 139.7, 140.4 (Ar.), 155.5, 157.6 (CN); HRMS (ESI^+) *m*/*z* 309.1728 calcd $[M + H]^+$; found 309.1732. Anal. ($C_{17}H_{22}Cl_2N_6 \cdot 1.5H_2O$) C, H, N.

Dihydrochloride Salt of *N*-(4-{2-[4-(Imidazolidin-2-ylideneamino)phenyl]ethyl}phenyl)guanidine (2c): Method 4. White solid (94%); mp, decomposes over 215 °C; ^1H NMR (D_2O) δ 2.84 (s, 4H, CH_2), 3.67 (s, 4H, CH_2 Im), 7.03−7.13 (m, 4H, Ar.), 7.15−7.25 (m, 4H, Ar.); ^{13}C NMR (D_2O) δ 35.4, 35.5 ($2CH_2$), 42.2 (CH_2 Im), 123.3, 125.1, 129.4, 129.5, 131.3, 132.3, 140.3, 140.9 (Ar.), 155.6, 157.8 (CN); HRMS (ESI^+) *m*/*z* 323.1984 calcd $[M + H]^+$; found 323.1988. Anal. ($C_{18}H_{24}Cl_2N_6 \cdot 0.8H_2O$) C, H, N.

Dihydrochloride Salt of *N*-{4-[4-(Imidazolidin-2-ylideneamino)phenoxy]phenyl}guanidine (3c): Method 4. Yellowish solid (94%); mp 48−50 °C; ^1H NMR (D_2O) δ 3.70 (s, 4H, CH_2 Im), 6.97−7.06 (m, 4H, Ar.), 7.17−7.26 (m, 4H, Ar.); ^{13}C NMR (D_2O) δ 42.3 (CH_2), 119.5, 119.6, 125.8, 127.4, 129.0, 130.1, 154.8, 155.4 (Ar.), 155.8, 158.1 (CN); HRMS (ESI^+) *m*/*z* 311.1594 calcd $[M + H]^+$; found 311.1595. Anal. ($C_{16}H_{20}Cl_2N_6O \cdot 2.8H_2O$) C, H, N.

Dihydrochloride Salt of *N*-{4-[4-(Imidazolidin-2-ylideneamino)phenylsulfanyl]phenyl}guanidine (4c): Method 4. Yellowish solid (95%); mp 94−96 °C; ^1H NMR (D_2O) δ 3.67 (s, 4H, CH_2), 7.05−7.12 (m, 4H, Ar.), 7.19−7.27 (m, 4H, Ar.); ^{13}C NMR (D_2O) δ 42.2 (CH_2), 123.7, 125.5, 131.5, 132.0, 132.4, 132.9, 133.6, 134.1 (Ar.), 155.3, 157.4 (CN); HRMS (ESI^+) *m*/*z* 327.1293 calcd $[M + H]^+$; found 327.1295. Anal. ($C_{16}H_{20}Cl_2N_6S \cdot 1.8H_2O$) C, H, N.

Dihydrochloride Salt of *N*-{4-[4-(Imidazolidin-2-ylideneamino)phenylamino]phenyl}guanidine (5c): Method 4. Green solid (94%); mp 66−68 °C; ^1H NMR (D_2O) δ 3.60 (s, 4H, CH_2), 6.95−7.09 (m, 8H, Ar.); ^{13}C NMR (D_2O) δ 42.2 (CH_2), 117.8, 118.0, 125.2, 126.0, 126.9, 127.3, 141.3, 142.0 (Ar.), 155.9, 158.1 (CN); HRMS (ESI^+) *m*/*z* 310.1722 calcd $[M + H]^+$; found 310.1724. Anal. ($C_{16}H_{21}Cl_2N_7 \cdot 1.7H_2O$) C, H, N.

Tetrahydrochloride Salt of *N*-(4-{4-[4-(Imidazolidin-2-ylideneamino)phenyl]piperazin-1-yl}phenyl)guanidine (6c): Method 4. Brownish solid (93%); mp 168−170 °C; ^1H NMR (D_2O) δ 3.62 (s, 4H, CH_2 Im), 3.71 (s, 8H, CH_2 Pip), 7.23−7.31 (m, 4H, Ar.), 7.37 (d, 2H, *J* = 9.0 Hz, Ar.), 7.41 (d, 2H, *J* = 8.5 Hz, Ar.); ^{13}C NMR (D_2O) δ 42.3 (CH_2 Im), 50.2, 50.9 (CH_2 Pip), 120.0, 120.3, 125.0, 126.6, 131.4, 133.0, 141.9, 143.1 (Ar.), 155.6, 157.8 (CN); HRMS (ESI^+) *m*/*z* 379.2359 calcd $[M + H]^+$; found 379.2362. Anal. ($C_{20}H_{30}Cl_4N_8 \cdot 1.0H_2O$) C, H, N.

Dihydrochloride Salt of *N*-{4-[4-(Imidazolidin-2-ylideneamino)benzoyl]phenyl}guanidine (7c): Method 4. White solid (94%); mp 99−101 °C; ^1H NMR (D_2O) δ 3.80 (s, 4H, CH_2), 7.38 (d, 2H, *J* = 8.5 Hz, Ar.), 7.42 (d, 2H, *J* = 8.5 Hz, Ar.), 7.78−7.84 (m, 4H, Ar.); ^{13}C NMR (D_2O) δ 42.3 (CH_2), 121.8, 123.5, 131.5, 131.6, 133.7, 134.2, 138.9, 139.5 (Ar.), 155.3, 157.5 (CN), 197.5 (CO); HRMS (ESI^+) *m*/*z* 323.1620 calcd $[M + H]^+$; found 323.1619. Anal. ($C_{17}H_{20}Cl_2N_6O \cdot 2.0H_2O$) C, H, N.

Dihydrochloride Salt of 1-(4-Guanidinophenyl)-3-[4-(imidazolidin-2-ylideneamino)phenyl]urea (8c): Method 4. White solid (94%); mp, decomposes over 200 °C; ^1H NMR (D_2O) δ 3.57 (s, 4H, CH_2), 6.98 (d, 2H, *J* = 8.5 Hz, Ar.), 7.03 (d, 2H, *J* = 8.5 Hz, Ar.), 7.16 (d, 2H, *J* = 8.5 Hz, Ar.), 7.20 (d, 2H, *J* = 8.5 Hz, Ar.); ^{13}C NMR (D_2O) δ 42.1 (CH_2), 120.0, 120.1, 123.4, 125.4, 128.3, 129.3, 136.1, 136.8 (Ar.), 153.4, 155.4, 157.3 (CO, CN); HRMS (ESI^+) *m*/*z* 353.1838 calcd $[M + H]^+$; found 353.1832. Anal. ($C_{17}H_{22}Cl_2N_8O \cdot 2.0H_2O$) C, H, N.

DNA Binding Assays. DNA Salmon Sperm and Poly(dA·dT)$_2$ Experiments. Thermal melting experiments were conducted with a Varian Cary 300 Bio spectrophotometer equipped with a 6 × 6

multicell temperature-controlled block. Temperature was monitored with a thermistor inserted into a 1 mL quartz cuvette containing the same volume of water as in the sample cells. Absorbance changes at 260 nm were monitored from a range of 20 to 90 °C with a heating rate of 1 °C/min and a data collection rate of five points per °C. The salmon sperm DNA was purchased from Sigma Aldrich (extinction coefficient ε_{260} = 6600 cm^{-1} M^{-1} base). A quartz cell with a 1 cm path length was filled with a 1 mL solution of DNA polymer or DNA−compound complex. The DNA polymer (150 μM base) and the compound solution (15 μM) were prepared in a phosphate buffer [0.01 M Na_2HPO_4/NaH_2PO_4], adjusted to pH 7) so that a compound to DNA base ratio of 0.1 was obtained. The thermal melting temperatures of the duplex or duplex−compound complex obtained from the first derivative of the melting curves are reported.

Poly(dA)·Poly(dT) Experiments. Thermal melting experiments were conducted with a Cary 300 Bio UV−visible spectrophotometer (Varian, Inc.) in 1 cm quartz cells. The solutions were prepared in MES buffer containing 10 mM 2-(*N*-morpholino)ethanesulfonic acid, 0.1 M NaCl, 1 mM EDTA, pH 6.25. The DNA polymer poly(dA)·poly(dT) was purchased from Amersham Biosciences (NJ) and used with an extinction coefficient ε_{260} = 6000 cm^{-1} M^{-1} base, room temperature, MES buffer. The samples were mixed with ratio of one compound per two base pairs and scanned up and down a temperature range of 25−95 °C with a heating/cooling rate of 0.5 °C/min. Temperature was recorded with a temperature probe inserted into a cuvette filled with water. The procedure was repeated twice to generate four thermal melting curves for each sample. One strong transition was observed for every curve. The data were processed with a software package included with the instrument.

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Supporting Information Available: Preparation details and ^1H NMR, ^{13}C NMR, and MS data of all new Boc-protected derivatives (**1a**−**8a**, **1b**−**8b**, **1d**−**6d**, **8d**, **1e**−**6e**, **8e**, **1f**−**6f**, **8f**, **12**) and all monoguanidines and mono-2-aminoimidazolines (**1g**−**8g**, **1h**−**6h**, **8h**); a table containing the combustion analysis data of the new target compounds; ^1H and ^{13}C NMR spectra for the final compounds (**1c**−**8c**). This material is available free of charge via the Internet at http://pubs.acs.org.

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