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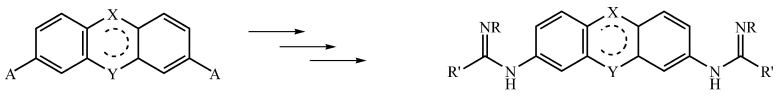

A = H, NO_2, NH_2
X = nil, CH, C=O
Y = CH_2, C=O, N
R = H, CO_2Et
R' = NH_2, NHMe, NHEt, NH^iPr, NHOMe, NHO^tBu, Ph, 2-Py

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Synthesis, DNA Affinity, and Antiprotozoal Activity of Fused Ring Dicationic Compounds and Their Prodrugs

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Dicationic guanidine, *N*-alkylguanidine, and reversed amidine derivatives of fused ring systems (**9a**−**d**, **12a**−**c**, **13a**, and **13b**) have been synthesized from their corresponding bis-amines. DNA binding studies suggest that the diguanidines and the *N*-alkyl diguanidines fluorenes bind in the minor groove in a manner similar to that of the previously reported dicationic carbazole derivatives. The diguanidines and the *N*-alkyl diguanidines showed promising in vitro activity against both *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum.* Promising in vivo biological results were obtained for the dicationic *N*-isopropylguanidino-9*H*-fluorene (**12c**), giving 4/4 cures of the treated animals in the STIB900 animal model for African trypanosomiasis. The *N*-methyl analogue (**12a**) showed high activity as well. In addition, with the goal of enhancing the oral bioavailability, two novel classes of potential guanidine prodrugs were prepared. The *N*-alkoxyguanidine derivatives (**12d**) and (**12e**) were not effective as prodrugs. In contrast, a number of the carbamates (**11a,c**−**e**) showed promising activity. The value of the carbamate prodrugs was clearly demonstrated by the results for (**11c**), which gave 4/4 cures on oral administration in the STIB900 mouse model.

Introduction

Aromatic diamidines and related dicationic molecules have been extensively studied for over 50 years as a consequence of the broad spectrum antimicrobial activity reported for these molecules.[1] Despite these efforts, pentamidine (**1**), first reported in 1942,[2] is the only compound belonging to this class that has found significant human use. Pentamidine is currently used against first-stage human African trypanosomiasis (HAT) and antimony-resistant leishmaniasis and also as a secondary drug for AIDS-related *Pneumocystis jirovecii* pneumonia (PCP).[1] An orally effective prodrug of furamidine (**2**) is currently in phase II clinical trials against malaria, HAT, and PCP and represents a different approach for administration of dicationic molecules.[1,3−6] A key step in the mode of action of these types of dicationic molecules is thought to be binding in the minor groove of DNA at AT-rich sites.[1] It has been postulated that minor groove binding leads to inhibition of DNA-dependent enzymes or possibly direct inhibition of transcription.[1,7−10]

A fundamental element in the design of new potential therapeutics of this type has been that the molecular unit that bears the amidine groups should have a complementary shape to that of the curve of the minor groove of DNA.[11,12] Most of these crescent-shaped molecules consist of unfused aryl and heteroaryl rings linked by various connectors that may result in conjugated or nonconjugated systems (cf. furamidine and pentamidine in Figure 1). Nevertheless, not all minor groove binders have the more commonly encountered crescent shape. Recently, a more linear molecule, **3** (CGP 40215A), has been found to bind strongly to the minor groove of DNA and to exhibit excellent antitrypanosomal activity.[13−15] Thorough investigations of fused ring dicationic carbazoles, benzofurans, and benzothiophenes (**4**), some being more linear molecules, revealed several highly active antimicrobial agents.[16−18] Detailed biophysical studies demonstrated that these types of molecules bind in the minor groove at AT sites by a new type of minor groove binding complex.[19,20] As part of a program focused on the development of novel dicationic antiprotozoan agents, we have now investigated introduction of guanidine and "reversed amidine" cationic centers on some other fused ring frameworks. Earlier studies from these laboratories have demonstrated that the attachment of these cationic centers to classical groove binding frameworks, furamidine-like, can lead to effective antimicrobial agents.[21,22] Others have shown that benzguanidines connected by alkyl and related linkers also show promising antiprotozoal activity.[23]

The p*K* of aromatic amidines and guanidines is greater than 10, and as a result, these type molecules are charged at physiological pH.[24,25] The consequent lack of oral bioavailability of dicationic analogues is well documented.[1] Despite the complexity of metabolism sometimes encountered with prodrugs, they offer an approach to circumvent physicochemical problems inherent with certain classes of molecules.[26−28] Since, in general, an oral dosing regime is preferred over other routes of administration, we also report in this publication the exploration of *N*-alkoxy and carbamate prodrug approaches for the guanidine analogues. We have previously demonstrated that amidoximes and *O*-alkylami-

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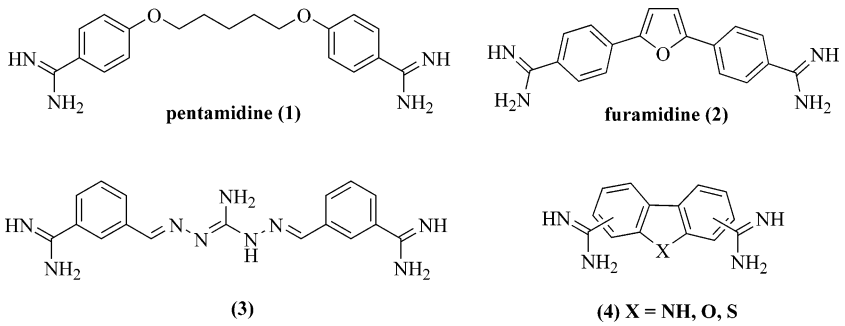
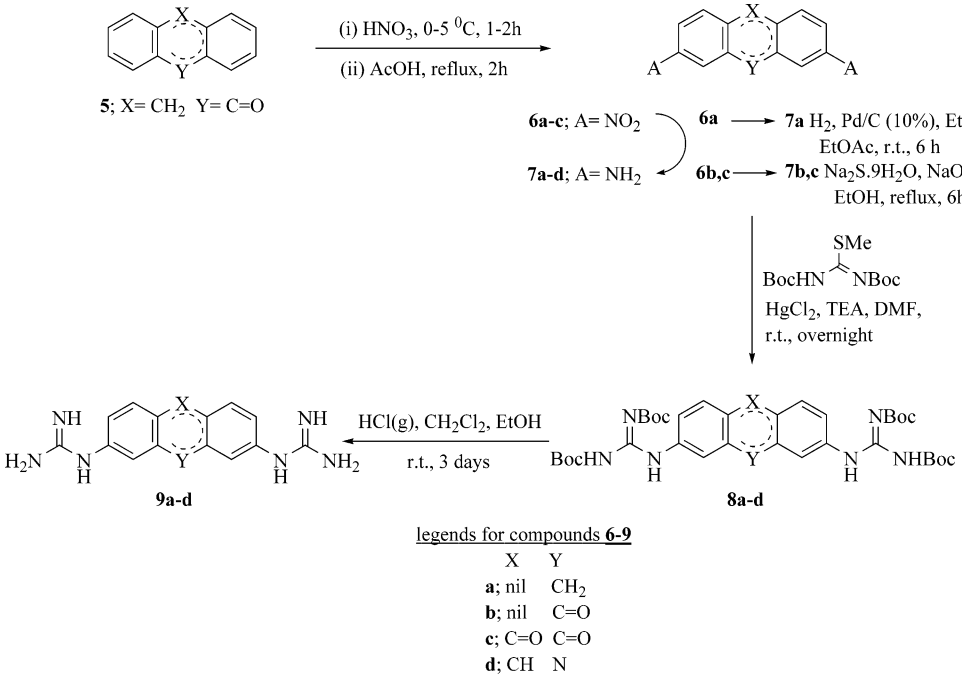


Figure 1. Structures of key dicationic antimicrobial agents.

Scheme 1. Guanidino Derivatives of Fused Ring Systems

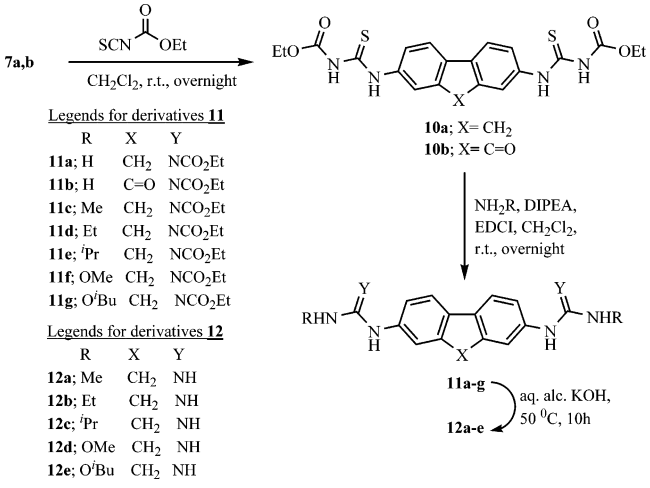


doximes can function as effective prodrugs for aryl diamidines.[28−31] Similarly, carbamates were also shown to be effective prodrugs for diamidines.[32] The study of potential prodrugs of guanidines is limited.[33−36] They are thought to be bioconverted by general esterases,[32−36] and as far as we have been able to determine, this is the first report of prodrugs for diguanidine analogues.

Chemistry

The synthesis of the diguanidino (**9a**−**d** and **12a**−**c**) and reversed diamidino (**13a**,**b**) derivatives of a variety of fused ring systems, viz. fluorene, fluorenone, anthraquinone, and acridine, was achieved starting with the diamino derivatives of tricyclic fused ring pharmacophores (Schemes 1−3). These key precursors were either used directly as purchased or synthesized according to the literature methods by either catalytic or Zinin reduction of the nitro counterparts (Scheme 1).[37] The not previously described reduction (H_2/Pd/C) of 2,7-dinitro-9*H*-fluorene to the diamino derivative (**7a**) was achieved in high yield. As depicted in Scheme 1, synthesis of the guanidines (**9a**−**d**) was accomplished in two successive steps. First, the diamino derivatives (**7a**−**d**) were converted to the Boc-protected bisguanidines (**8a**−**d**) by the $HgCl_2$-promoted reaction with bis-Boc-protected *S*-methylpseudothiourea. The subsequent step was the HCl-assisted deprotection of the Boc-protected guanidines, providing the guanidine hydrochloride salts **9a**−**d**.[38−40]

Scheme 2. Synthesis of Fused Ring System N-Substituted Dicationic Guanidines and Their Potential Prodrugs



Scheme 2 depicts the synthesis of N-substituted guanidines (**12a**−**e**), as well as the corresponding carbamate prodrugs (**11a**−**g**) of several of the synthesized

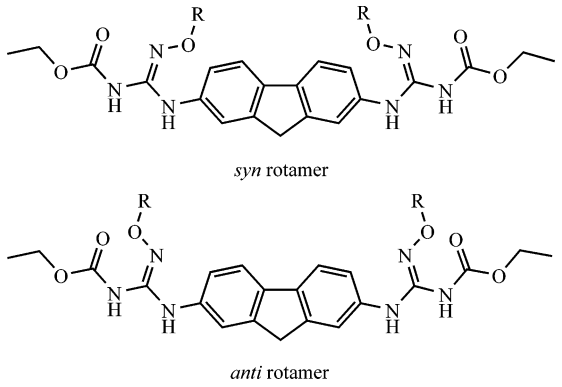
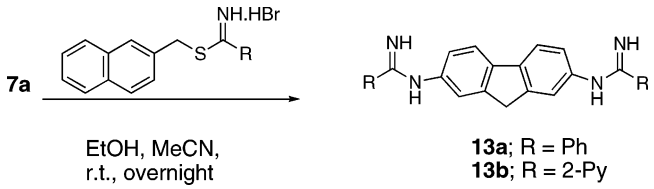


Figure 2. Proposed structures for two possible carbamoyl *N*-alkoxyguanidine isomers.

Scheme 3. 9*H*-Fluorene Reversed Diamidines



DNA-binding dications. Starting with the appropriate diamine, reaction with ethyl isothiocyanatoformate gave the carbamoyl thioureas (**10a**,**b**), which were further reacted with various amines to furnish the carbamoyl guanidines (**11a**−**g**).[41−43] These synthetic intermediates (**11a**−**g**) may function as prodrugs for the final guanidines. The last step of decarbamoylation was performed under basic conditions to obtain the *N*-alkylguanidines (**12a**−**c**) as well as the *N*-alkoxyguanidines (**12d)** and (**12e**).

The ^{1}H NMR and the ^{13}C NMR data for the carbamoyl guanidine precursors of the *N*-alkoxyguanidines (**11f**,**g**) showed two sets of peaks for all the aliphatic and the aromatic functionalities present in the molecule, indicating the presence of a mixture of apparently two inseparable compounds that were otherwise pure. Mixtures were not detected for the other carbamates (**11a**−**e**). The apparent mixtures for **11f** and **11g** were directly subjected to alkaline hydrolysis yielding the corresponding *N*-alkoxyguanidines (**12d**,**e**) as single isomers, as demonstrated by their ^{1}H NMR and ^{13}C NMR and other data. Thus, it can be concluded that the carbamoyl *N*-alkoxyguanidines (**11f**,**g**) likely exist in isomeric forms, possibly syn and anti rotamers arising from restricted rotation around the N−C bond of the carbamate (Figure 2).[44−46] Another plausible explanation is the presence of other tautomeric forms with the carbamoyl group and the alkoxy group on one or the other of the sp^3 and sp^2 terminal nitrogens of the guanidino moiety. Similar isomeric mixtures for carbamoyl *N*-alkoxyguanidines have been observed in related examples.[45]

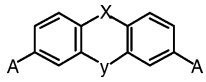
Finally, reversed diamidino derivatives of 2,7-diaminofluorene were prepared according to the reported method[22,23,47] (Scheme 3) applying the reaction with the nonodoriferous reagent phenyl *S*-(2-naphthylmethyl)-thioimidate or 2-pyridyl *S*-(2-naphthylmethyl)thioimidate, thus providing **13a** or **13b**, respectively. The corresponding reaction with the less basic diamines **7b**−**d** failed. It is worth noting that the free bases of these compounds, being sparingly water-soluble, were converted to their hydrochloride salts to improve their aqueous solubility.

Biological Results

Relative Binding Affinity. ΔT_m Measurements. T_m increases for compound complexes relative to uncomplexed DNA (ΔT_m) at specific DNA sequences provide an excellent method for ranking compound binding affinities.[1] The T_m increases caused by the compounds in Table 1 have been determined for their complexes with an AT polymer. The charged compounds have ΔT_m values in the range 6.1−22 °C (Table 1). The uncharged prodrugs, as expected, do not increase the DNA T_m within experimental error. The ΔT_m values for the fused ring dications are modest compared to that of furamidine but are generally in the range of that of pentamidine. Introduction of *N*-alkyl groups on the guanidine moiety (**12a**−**c**) resulted in a slight decrease in the ΔT_m values, whereas introduction of aryl groups increased the values, as in the case of the reversed diamidines (**13a**,**b**). A similar effect was previously noted for N-substitution in unfused triaryl systems.[21,22] While the ΔT_m value for the fluorene parent diguanidine (**9a**) is significantly lower than that of furamidine, it is similar to that of dicationic carbazoles, benzofurans, and benzothiophenes.[16−20] Comparison of the ΔT_m values of **9a** to those of the corresponding fluorenone **9b**, anthraquinone **9c**, and acridine **9d** diguanidines shows a reduction in affinities as a result of the presence of a carbonyl group and six-membered rings in the central core of the fused ring system. The decline in ΔT_m values may reflect steric clash of the central core of these other systems with the floor of the groove.

Binding Mode: Circular Dichroism (CD) Spectroscopy. Binding of **9a**, **13a**, **9b**, and **12a** to poly(d(A-T)$_2$) was characterized by CD spectroscopy in the wavelength range between 220 and 420 nm (Figure 3). The CD spectra monitor the asymmetric environment of the compounds when bound to DNA and therefore can be used to obtain information on the binding mode. The free compounds do not have CD spectra, but they have induced CD when bound to DNA. Minor groove binding is typically characterized by a strong positive CD signal at the maximum absorbance wavelength of the bound compound. Intercalation binding is generally characterized by weak CD signals that are usually negative but can be positive. Addition of **9a** to DNA results in substantial positive CD signals between 270 and 335 nm, where the compound absorbs. Isoelliptic points are observed in the titration of **9a** with DNA at 271 and 253 nm with large positive induced CD signals at 293 and 318 nm. A strong positive CD signal, centered at 330 nm, is also observed for the **13a**−DNA complex with isoelliptic points at 288, 253, and 233 nm. A positive CD signal for the **9a** complex is found at 263 nm where the compound absorbance overlaps with the DNA spectrum. An isoelliptic point is observed in the titration of **12a** with DNA at 278 nm. Although the positive induced CD is characteristic of minor groove binding, the overlap of compound and DNA spectra prevents definitive conclusions for this compound. Strong positive CD signals arise at 280, 293, and 316 nm for **12a** with isoelliptic points at 266, 253, and 224 nm. The large induced CD signals for the **9a**−DNA, **13a**−DNA,

Table 1. DNA Affinities and in Vitro Anti-Protozoan Data for Fused Ring Dicationic Compounds



compd	A	X	Y	ΔT_m [a] (°C) poly(d(A-T)$_2$)	IC$_{50}$ (nM) *Tbr.*[b]	IC$_{50}$ (nM) *P.f.*[b]	TD$_{50}$ [c] (μM) L6 cells
pentamidine				12.6	2.3	34.6	1.5
furamidine				25	4.3	15.5	6.4
9a	NH(C=NH)NH$_2$	nil	CH_2	13.6	24.0	2.3	4.7
11a	NH(C=NCO$_2$Et)NH$_2$	nil	CH_2		814	3700	>201
12d	NH(C=NOMe)NH$_2$	nil	CH_2		47500	3000	136.7
12e	NH(C=NO*i*Bu)NH$_2$	nil	CH_2		21200	351	68.4
12a	NH(C=NH)NHMe	nil	CH_2	10.1	12.6	10	65.2
11c	NH(C=NCO$_2$Et)NHMe	nil	CH_2		2900	10300	95.6
12b	NH(C=NH)NHEt	nil	CH_2	10.0	16.9	13.3	39.2
11d	NH(C=NCO$_2$Et)NHEt	nil	CH_2		8000	4100	173.6
12c	NH(C=NH)NH*i*Pr	nil	CH_2	9.3	40.4	35.7	85.2
11e	NH(C=NCO$_2$Et)NH*i*Pr	nil	CH_2		2500	6300	94.2
13a	NH(C=NH)Ph	nil	CH_2	22	292	481	1.9
13b	NH(C=NH)Py	nil	CH_2	15.2	894	1200	98.7
9b	NH(C=NH)NH$_2$	nil	CO	6.1	7.3	268	41.5
11b	NH(C=NCO$_2$Et)NH$_2$	nil	CO		12900	3200	>199.5
9c	NH(C=NH)NH$_2$	CO	CO	7.2	128	30200	35.3
9d	NH(C=NH)NH$_2$	CH	N	7.7	66.9	227	16.9

[a] Buffer: MES10. Ratio compound/DNA is 0.3; see ref 22. [b] *T.b.r.* (*Trypanosoma brucei rhodesiense*) strain used was STIB900, and the *P.f.* (*Plasmodium falciparum*) strain was K1. Values are of duplicate determinations. See ref 31. [c] Cytotoxicity was evaluated using cultured L6 rat myoblast cells using the same assay procedure as for *Trypanosoma brucei rhodesiense*.

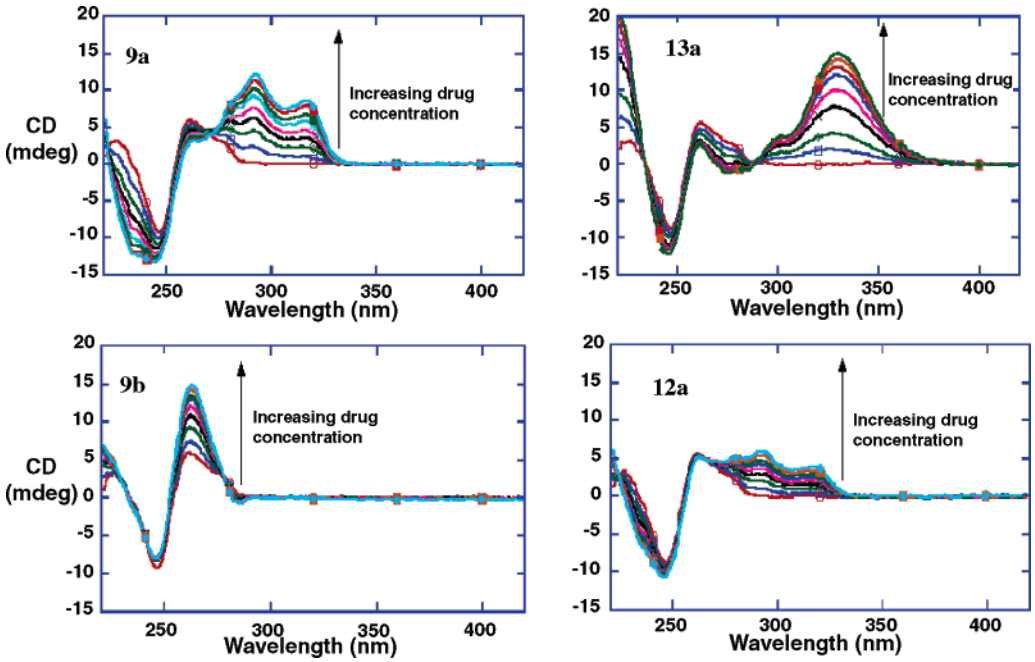


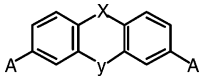
Figure 3. CD spectral titrations of **9a**, **13a**, **9b**, and **12a** with poly(d(A-T)$_2$). Positive induced CD signals are seen for the compound−DNA complexes, above 300 nm for **9a**, **13a**, and **12a**. The **9b** spectrum overlaps with that of DNA near 260 nm. The small molecules exhibit no CD spectra but on binding to DNA show induced CD. A spectrum for DNA without compound is shown for reference and has no CD signal above 300 nm (red). The ratios of compounds to DNA base pairs are 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, and 0.50 as indicated by the arrow direction in the figures. The experiments were conducted in MES10, and the DNA concentration was 2×10^{-5} M in base pairs.

and **12a**−DNA complexes suggest that they bind in the minor groove at AT DNA sequences. The results for **9b** are consistent with minor groove binding, but spectral overlap complicates the interpretation from CD.

The preliminary biophysical studies with these dicationic molecules suggest that they bind in the DNA minor groove, similar to the carbazoles.[19,20] As has been previously noted for the prodrugs, these molecules are essentially uncharged (because of the lower p*K*) and do not bind significantly to DNA.[28,31]

In Vitro Activities. Many of the diguanidines and the *N*-alkyl diguanidines show promising in vitro activity against both *Trypanosoma brucei rhodesiense* (**9a**,**b** and **12a**−**c**) and *Plasmodium falciparum* (**9a**,**c** and **12a**−**12c**) (Table 1) with IC$_{50}$ values ranging from 2 to 40 nM. The number of dicationic compounds[1,21,31] in the STI screen that have shown IC$_{50}$ values versus *Plasmodium falciparum* of less than 5 nM is quite small (approximately 30), which places **9a** in the category of very active compounds. In the fluorene series, the

Table 2. In Vivo Anti-Trypanosomal Activity for Fused Ring Dicationic Compounds in the STIB900 Mouse Model[a]



compd	A	X	Y	dosage for 4 days[b] (mg/kg)	cures[c]	survival[d] (days)
pentamidine				20 ip	0/4	40.8
furamidine				20 ip	0/4	52.5
9a	NH(C=NH)NH_2	nil	CH_2	20 ip	0/4	6
11a	NH(C=NCO_2Et)NH_2	nil	CH_2	100 po	0/4	18.75
12d	NH(C=NOMe)NH_2	nil	CH_2	100 po	0/4	8
12e	NH(C=NO*i*Bu)NH_2	nil	CH_2	100 po	0/4	6.75
12a	NH(C=NH)NHMe	nil	CH_2	20 ip	3/4	>52
				5 ip	2/4	>45.5
11c	NH(C=NCO_2Et)NHMe	nil	CH_2	100 po	4/4	>60
				25 po	2/4	>41.25
12b	NH(C=NH)NHEt	nil	CH_2	20 ip	5/7[e]	>52.7
11d	NH(C=NCO_2Et)NHEt	nil	CH_2	100 po	0/4	>27.75
12c	NH(C=NH)NH*i*Pr	nil	CH_2	20 ip	4/4	>60
11e	NH(C=NCO_2Et)NH*i*Pr	nil	CH_2	100 po	0/4	17.75
9b	NH(C=NH)NH_2	nil	CO	10 ip	2/4	>50.75
				5 ip	0/4	45
11b	NH(C=NCO_2Et)NH_2	nil	CO	100 po	0/4	6
9d	NH(C=NH)NH_2	CH	N	20 ip	2/4	>54.5
				5 ip	0/4	16.25

[a] See ref 31 for details of the STIB900 model. IC_{50} values for **13a**, **13b**, and **9c** did not meet criteria for entry into animal studies. [b] ip = intraperitioneal and po = oral in 10% DMSO water. [c] Number of mice that survive and are parasite-free on day 60 postinfection. [d] Average days of survival; untreated control animals expire between days 7 and 8 postinfection. [e] In two different experiments, on the second day of dosing, one animal died.

addition of an *N*-alkyl group (**12a**−**c**) reduces the antiprotozoan activity and the activity declines with increasing alkyl size. Generally, the reversed diamidines in the fluorene series (**13a**,**b**) and the guanidines with a six-membered central ring (**9c**,**d**) show significantly diminished activity. The prodrugs, as expected, show essentially no in vitro activity. Selectivity for the parasites is generally quite good as judged from their low toxicity for L-6 cells (Table 1).

A general characteristic that has been noted for numerous dicationic minor groove binding systems is the lack of a direct correlation between DNA affinity and in vitro activities.[1,21,31] Some minimum threshold DNA affinity, which varies with class, seems to be required to observe activity because compounds that do not bind show negligible antimicrobial activity. High DNA affinity does not always lead to a correspondingly high antimicrobial activity; however, very low DNA affinity results in loss of biological activity.[1,21,31] A similar result is apparent for the fused ring dications.

In Vivo Activities. Given their high antiparasitic activity and low cell toxicity, several of the diguanidines were advanced to the rigorous STIB900 animal model for African trypanosomiasises (Table 2). Despite the quite good in vitro activity (IC_{50} = 24 nM), the diguanidinofluorene **9a** was inactive on ip dosage. In contrast, the diguanidinofluorenone **9b** and the acridine **9d** analogues showed promising activity, providing 2/4 cures in this model. The *N*-alkylfluorene compounds (**12a**−**12c**) exhibited excellent in vivo activity, providing 3/4, 5/7, and 4/4 cures, respectively. Interestingly **12b**, while showing good activity, apparently was also toxic because one animal death was noted on the second dosing in two different experiments. The toxicity is acute, but the origin is unknown.

The utility of *N*-alkoxy analogues of diamidines as prodrugs has been quite clearly demonstrated,[28−31] and we prepared **12d** and **12e** to test this approach with diguanidines. When these two molecules were evaluated on oral dosing in the STIB900 mouse model, both were found to be ineffective. Further work is required to determine if this result is due to lack of uptake or lack of bioconversion to the parent drug.

The potential prodrug **11a** was also evaluated in the STIB900 model to determine if carbamates are useful as a means to improve oral bioavailability of the diguanidino compounds. While the prodrugs **11a**, **11d**, and **11e** failed to provide cures, they did exhibit a 3-fold or greater increase in survival time of the test animals compared to ip dosing of the parent molecules and thus showed that carbamates of diguanidino analogues can potentially serve as useful prodrugs. The value of carbamate prodrugs is more clearly demonstrated by the results for the *N*-methyl carbamate (**11c**), which gave 4/4 cures on oral administration in this model. Unfortunately, the compound did not yield cures in the chronic mouse model (central nervous system involvement) for African trypanosomiasis (data not presented).

In conclusion, this work demonstrates that diguanidino fused ring systems are potential candidates as antiprotozoan agents. We have shown, for the first time, that carbamate derivatives of diguanidines, which are inactive in vitro and effective in vivo, can function as prodrugs. Other carbamates are being explored with the goal of optimizing oral efficacy of these diguanidines.

Experimental Section

Absorbance Spectroscopy and Thermal Melting (T_m) Experiments. Experiments were done in MES10 buffer (0.01 M MES (2-(*N*-morpholino)ethanesulfonic acid), 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.1 M NaCl with the pH was adjusted to 6.25). DNA polymers were purchased from Pharmacia and characterized by their melting curves. Absorbance and thermal melting experiments were done using a Cary 300 Bio spectrophotometer with the software supplied with the instrument. For absorbance measurements, the buffer was scanned from 400 to 250 nm in 1 cm quartz cuvettes, aliquots of concentrated stock solutions of the compounds were titrated into the buffer, and the solutions were rescanned. The

concentrations of the compounds were 1×10^{-5}, 2×10^{-5}, and 3×10^{-5} M. For thermal melting the concentration of the DNA was about 1×10^{-4} in bases and the ratio of the compound to DNA bases was 0.3.

Circular Dichroism. CD spectra were obtained on Jasco J-810 spectrometer in MES buffer. The software supplied by Jasco provided instrument control, data acquisition, and manipulation. DNA solutions in MES10 buffer were scanned in 1 cm quartz cuvettes. Aliquots of concentrated stock solutions of the compounds were titrated into DNA to give the desired ratio, and the complexes were rescanned.

Synthetic Protocols. Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^{1}H and ^{13}C NMR spectra were recorded employing a Varian GX400 or Varian Unity Plus 300 spectrometer, and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (EI) or a ThermoFinningan LCQ MSD (ESI). Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA). Some compounds were analyzed correctly for fractional moles of dichloromethane, water, and/or ethanol of solvation. In each case ^{1}H NMR showed the presence of the indicated solvent(s). All chemicals and solvents (including anhydrous solvents) were purchased from Aldrich Chemical Co. or Lancaster Synthesis and used as purchased. Acetonitrile and triethylamine were distilled from CaH_2. Synthesis of the bis-aminofluorenone (**7b**) and bis-aminoanthraquinone (**7c**) was achieved as described in Scheme 1 according to the literature.[37] *S*-(2-Naphthylmethyl)thioacetimidate was prepared by adapting the reported procedure.[46]

2,7-Diamino-9*H*-fluorene (7a) (Scheme 1). To a suspension of 2,7-dinitro-9*H*-fluorene (5 g, 19.5 mmol) in EtOAc (50 mL) and EtOH (50 mL) was added Pd/C (1.25 g). The reaction mixture was shaken under hydrogen (55 psi) for 6 h, after which the reaction mixture was filtered through a pad of Celite. The filtrate was eveporated to dryness to give off-white shiny crystals that needed no further purification (3.82 g, quantitative): mp 159−60 °C, ^{1}H NMR (DMSO-d_6) δ 3.56 (s, 2H), 4.89 (br s, 4H), 6.47 (d, J = 8.1 Hz, 2H), 6.67 (s, 2H), 7.24 (d, J = 8.1 Hz, 2H).

Preparation of Bis(*N*′,*N*″-di-Boc-guanidino) Derivatives (General Procedure) (Scheme 1). 2,7-Bis(*N*′,*N*″-di-Boc-guanidino)-9*H*-fluorene (8a). To a solution of 2,7-diaminofluorene (**7a**) (0.49 g, 2.5 mmol) in anhydrous DMF (15 mL) was added 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea (1.52 g, 5.25 mmol), triethylamine (1.52 g, 15 mmol), and mercury(II) chloride (1.56 g, 5.75 mmol). The suspension was kept stirring at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2, washed with Na_2CO_3 solution, and filtered through a pad of Celite. The organic layer was washed with water (3×) followed by brine and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the obtained residue was crystallized from CH_2Cl_2/MeOH, giving a light-yellow solid (1.15 g, 68%): mp >340 °C; ^{1}H NMR ($CDCl_3$) δ 1.52, 1.54 (2s, 36H), 3.91 (s, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.90 (s, 2H), 10.43 (br s, 2H), 11.68 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 163.6, 153.5, 153.4, 144.2, 138.1, 135.3, 120.9, 119.7, 118.9, 83.7, 79.6, 37.2, 28.2, 28.1. Anal. Calcd for $C_{35}H_{48}N_6O_8$ (680.79): C, 61.75; H, 7.11; N, 12.34. Found: C, 61.50; H, 7.11; N, 12.36.

2,7-Bis(*N*′,*N*″-di-Boc-guanidino)fluoren-9-one (8b). Orange solid (1.16 g, 65%), mp >340 °C; ^{1}H NMR ($CDCl_3$) δ 1.51, 1.54 (2s, 36H), 7.42 (d, J = 8.4 Hz, 2H), 7.77 (s, 2H), 7.82 (d, J = 8.4 Hz, 2H), 10.46 (br s, 2H), 11.63 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 192.7, 163.3, 153.5, 153.3, 140.4, 137.4, 135.2, 128.0, 120.6, 118.6, 84.0, 79.9, 28.1, 28.0. Anal. Calcd for $C_{35}H_{46}N_6O_9 \cdot 0.1CH_2Cl_2$ (703.26): C, 59.94; H, 6.62; N, 11.95. Found: C, 59.69; H, 6.67; N, 12.13.

2,7-Bis(*N*′,*N*″-di-Boc-guanidino)anthraquinone (8c). Yellow solid (1.24 g, 82%), mp >340 °C; ^{1}H NMR ($CDCl_3$) δ 1.54, 1.56 (2 s, 36H), 8.24 (s, 2H), 8.30 (d, J = 8.7 Hz, 2H), 8.43 (d, J = 8.7 Hz, 2H), 10.82 (br s, 2H), 11.62 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 182.5, 181.2, 163.1, 153.2, 142.4, 134.5, 129.5, 128.9, 126.7, 119.0, 84.4, 80.3, 28.1, 28.0. Anal. Calcd for $C_{36}H_{46}N_6O_{10} \cdot 0.5CH_2Cl_2$ (765.25): C, 57.28; H, 6.19. Found: C, 57.30; H, 6.09.

3,6-Bis(*N*′,*N*″-di-Boc-guanidino)acridine (8d). Canary-yellow fluffy solid (0.88 g, 73%), mp >340 °C; ^{1}H NMR ($CDCl_3$) δ 1.53, 1.55 (2s, 36H), 7.78 (dd, J = 9.0, 2.1 Hz, 2H), 7.9 (d, J = 9.0 Hz, 2H), 8.45 (d, J = 2.1 Hz, 2H), 8.6 (s, 1H), 10.69 (s, 2H), 11.68 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 163.5, 153.4, 153.3, 149.9, 138.5, 135.1, 128.8, 123.8, 122.3, 118.9, 83.9, 79.7, 28.2, 28.1. Anal. Calcd for $C_{35}H_{47}N_7O_8 \cdot 0.1CH_2Cl_2$ (702.28): C, 60.02; H, 6.77; N, 13.96. Found: C, 59.97; H, 6.88; N, 13.90.

Deprotection of *N*′,*N*″-Di-Boc-guanidines (General Procedure) (Scheme 1). 2,7-Bis-guanidino-9*H*-fluorene Dihydrochloride (9a). The *N*′,*N*″-di-Boc-guanidine (**8a**) (0.25 g, 0.4 mmol) was dissolved in CH_2Cl_2 (10 mL) and diluted with dry EtOH (15 mL), and the chilled solution was saturated with dry HCl. The reaction mixture was then kept stirring at room temperature for 3 days (drying tube), where a precipitate of the product started forming over time. After evaporation of the solvent to dryness, the residue was washed with ether multiple times and was dried under reduced pressure at 50−60 °C overnight to give a whitish-yellow solid of the bis-guanidine dihydrochloride (0.13 g): mp >340 °C; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.45 (s, 2H), 7.58 (br s, 8H), 7.95 (d, J = 8.4 Hz, 2H), 10.23 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 156.1, 144.6, 138.6, 133.9, 123.4, 121.4, 120.9, 36.9; MS (EI) *m/z* (rel intens) 281 ($M^+ + 1$, 5), 252 (100). Anal. Calcd for $C_{15}H_{16}N_6 \cdot 2HCl \cdot 0.25C_2H_5OH$ (364.76): C, 51.04; H, 5.39; N, 23.04, Cl, 19.44. Found: C, 50.74; H, 5.26; N, 22.99, Cl, 19.87.

2,7-Bis-guanidinofluoren-9-one Dihydrochloride (9b). Green solid (0.26 g), mp >340 °C; ^{1}H NMR (DMSO-d_6) δ 7.43−7.46 (m, 4H), 7.68 (br s, 8H), 7.86 (d, J = 8.4 Hz, 2H), 10.25 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 191.58, 156.0, 140.9, 136.5, 134.6, 130.8, 122.4, 119.9; MS (EI) *m/z* (rel intens) 295 ($M^+ + 1$, 23), 278 (100). Anal. Calcd for $C_{15}H_{14}N_6O \cdot 2HCl \cdot 0.35H_2O$ (373.53): C, 48.23; H, 4.51; N, 22.49, Cl, 18.95. Found: C, 48.51; H, 4.55; N, 22.13, Cl, 18.93.

2,7-Bis-guanidinoanthraquinone Dihydrochloride (9c). Orange-red solid (0.22 g), mp >340 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.75 (d, J = 8.4 Hz, 2H), 7.97 (s, 2H), 8.06 (br s, 8H), 8.24 (d, J = 8.4 Hz, 2H), 10.87 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 181.6, 180.4, 155.6, 142.1, 134.2, 129.3, 128.9, 127.6, 119.4; MS (EI) *m/z* (rel intens) 323 ($M^+ + 1$, 100), 162 (59). Anal. Calcd for $C_{16}H_{14}N_6O_2 \cdot 2HCl \cdot 1.66H_2O$ (425.15): C, 45.20; H, 4.58; N, 19.77. Found: C, 45.24; H, 4.58; N, 19.47.

3,6-Bis-guanidinoacridine Trihydrochloride (9d). Orange solid (0.33 g), mp >340 °C; ^{1}H NMR (DMSO-d_6) δ 7.75 (dd, J = 8.4, 2.1 Hz, 2H), 7.96 (d, J = 2.1 Hz, 2H), 8.04 (br s, 8H), 8.23 (d, J = 8.4 Hz, 2H), 8.41 (s, 1H), 10.83 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 181.6, 180.5, 155.6, 142.1, 134.2, 129.3, 128.9, 127.7, 119.4. Anal. Calcd for $C_{15}H_{15}N_7 \cdot 3HCl \cdot C_2H_5OH \cdot 0.33H_2O$ (454.72): C, 44.90; H, 5.46; N, 21.56, Cl, 23.39. Found: C, 45.08; H, 5.10; N, 21.48, Cl, 23.49.

Preparation of Carbamoyl Thiourea Derivatives (Scheme 2). 2,7-Bis(*N*′-ethoxycarbonylthiourea)-9*H*-fluorene (10a). A solution of 2,7-diamino-9*H*-flourene (**7a**) (1 g, 5.1 mmol) in CH_2Cl_2 (10 mL), to which was added ethyl isothiocyanatoformate (1.47 g, 11.2 mmol), was stirred at room temperature overnight. After flash chromatography, the reaction mixture was diluted with hexane and the precipitate formed was collected and dried to yield the bis-carbamoylthiourea as a light-brown solid (2.32 g, quantitative): mp >340 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 6.9 Hz, 6H), 3.95 (s, 2H), 4.23 (q, J = 6.9 Hz, 4H), 7.75 (d, J = 8.4 Hz, 2H), 7.86−7.89 (m, 4H), 11.28 (s, 2H), 11.64 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 178.1, 153.4, 143.7, 138.2, 136.5, 123.4, 121.8, 119.7, 61.9, 36.8, 14.1; MS (EI) *m/z* (rel intens) 459 ($M^+ + 1$, 18), 374.1 (8), 328 (100), 319 (8), 151 (12). Anal. Calcd for $C_{21}H_{22}N_4O_4S_2$ (458.56): C, 55.00; H, 4.83. Found: C, 55.21; H, 4.83.

2,7-Bis(*N*′-ethoxycarbonylthiourea)fluoren-9-one (10b). To a suspension of 2,7-diaminofluorenone (**7b**) (0.3 g, 1.4 mmol)

in toluene (10 mL) was added ethyl isothocyanatoformate (0.41 g, 3.1 mmol). The reaction mixture was heated at reflux for 10 h. After cooling to room temperature, the reaction mixture was diluted with hexanes. The orange precipitate obtained was filtered off and crystallized from aqueous EtOH (0.66 g, 98%): mp >340 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.26 (t, J = 7.2 Hz, 6H), 4.22 (q, J = 7.2 Hz, 4H), 7.69 (dd, J = 8.1, 1.8 Hz, 2H), 7.80 (d, J = 8.1 Hz, 2H), 7.97 (d, J = 1.8 Hz, 2H), 11.39 (s, 2H), 11.60 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 191.6, 178.7, 153.4, 140.8, 139.0, 133.8, 130.9, 121.2, 120.2, 62.0, 14.0. Anal. Calcd for $C_{21}H_{20}N_4O_5S_2\cdot H_2O$ (490.55): C, 51.41; H, 4.52. Found: C, 51.27; H, 4.54.

Preparation of N-Substituted Carbamoyl Guanidines (General Procedure) (Scheme 2). 2,7-Bis(*N*′-ethoxycarbonyl)guanidino-9*H*-fluorene (11a). A stirred solution of carbamoyl thiourea (**10a**) (0.58 g, 1.26 mmol), ammonia (0.5 M solution in dioxane) (10 mL, 5.05 mmol), and diisopropylethylamine (0.98 g, 7.56 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to 0 °C. EDCI (*N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride) (0.96 g, 5.05 mmol) was added, and the solution was stirred at room temperature overnight. The reaction mixture was washed with water (3×) followed by brine and dried over anhydrous Na_2SO_4. The residue remaining after removal of the solvent was crystallized from EtOH/water (0.45 g, 84%): mp >340 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7.2 Hz, 6H), 3.87 (s, 2H), 3.98 (q, J = 7.2 Hz, 4H), 7.33 (d, J = 8.4 Hz, 2H), 7.54 (br s, 4H), 7.65 (s, 2H), 7.72 (d, J = 8.4 Hz, 2H), 9.13 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 163.1, 159.0, 143.4, 136.9, 136.3, 120.4, 119.4, 118.4, 59.4, 36.4, 14.4. Anal. Calcd for $C_{21}H_{24}N_6O_4\cdot 0.5C_2H_5OH$ (447.48): C, 59.04; H, 6.08; N, 18.78. Found: C, 58.96; H, 5.74; N, 18.88.

2,7-Bis(*N*′-ethoxycarbonyl)guanidinofluoren-9-one (11b). Brick-red solid (0.35 g, 75%), mp >340 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, J = 6.9 Hz, 6H), 4.00 (q, J = 6.9 Hz, 4H), 7.47 (dd, J = 8.1, 1.8 Hz, 2H), 7.59 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 1.8 Hz, 2H), 9.31 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 192.9, 162.6, 158.6, 139.6, 138.4, 134.1, 126.5, 120.8, 116.7, 59.9, 14.6. Anal. Calcd for $C_{21}H_{22}N_6O_5\cdot 0.15C_2H_5OH\cdot 0.5H_2O$ (454.35): C, 56.30; H, 5.30; N, 18.49. Found: C, 56.54; H, 5.10; N, 18.47.

2,7-Bis(*N*′-ethoxycarbonyl-*N*″-methyl)guanidino-9*H*-fluorene (11c). With the same procedure for preparation of **11a**, methylamine (2 M solution in THF) was used for the transformation of the thiourea compound (**10a**) into the *N*-substituted guanidine **11c**. The reaction yielded an off-white solid (0.53 g, 93%): mp 157−8 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.2 Hz, 6H), 2.83 (s, 6H), 3.90 (s, 2H), 3.94 (q, J = 7.2 Hz, 4H), 7.32 (d, J = 8.1 Hz, 2H), 7.54 (s, 2H), 7.83 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 161.9, 157.9, 143.7, 137.7, 135.9, 123.1, 121.1, 119.9, 59.9, 36.5, 28.3, 14.5; MS (ESI) *m/z* (rel intens) 453 (M^+ + 1, 100), 407 (39), 323 (15). Anal. Calcd for $C_{23}H_{28}N_6O_4\cdot H_2O$ (470.52): C, 58.71; H, 6.42; N, 17.86. Found: C, 58.81; H, 6.39; N, 17.71.

2,7-Bis(*N*′-ethoxycarbonyl-*N*″-ethyl)guanidino-9*H*-fluorene (11d). Following the general procedure, starting with **10a** and utilizing ethylamine hydrochloride, the target compound was obtained as a beige solid (0.79 g, 96%): mp 220−2 °C; ^{1}H NMR (DMSO-d_6) δ 1.12−1.19 (m, 12H), 3.32 (t, J = 6.9 Hz, 4H), 3.89 (s, 2H), 3.95 (q, J = 6.9 Hz, 4H), 7.31 (d, J = 8.1 Hz, 2H), 7.51 (s, 2H), 7.81 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 163.3, 158.1, 143.7, 137.5, 136.0, 123.1, 121.1, 120.0, 59.7, 36.5, 35.7, 14.8, 14.6; MS (ESI) *m/z* (rel intens) 481 (M^+, 80), 435 (20), 364 (10), 339 (12), 241 (100). Anal. Calcd for $C_{25}H_{32}N_6O_4\cdot 0.25H_2O$ (485.06): C, 61.90; H, 6.75; N, 17.32. Found: C, 61.66; H, 6.79; N, 17.37.

2,7-Bis(*N*′-ethoxycarbonyl-*N*″-isopropyl)guanidino-9*H*-fluorene (11e). With isopropylamine and the same synthetic steps used for preparing **11a**, a beige solid was obtained (0.39 g, 88%): mp 142−4 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, J = 7.2 Hz, 6H), 1.10−1.23 (m, 12H), 3.88 (s, 2H), 3.93 (q, J = 7.2 Hz, 4H), 4.10−4.21 (m, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.52 (s, 2H), 7.80 (d, J = 8.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 163.42, 157.38, 143.54, 137.36, 136.25, 122.93, 120.93, 119.72, 59.60, 42.35, 36.47, 22.53, 14.57; MS (ESI) *m/z* (rel intens) 509 (M^+ + 1, 19), 255 (100). Anal. Calcd for $C_{27}H_{36}N_6O_4\cdot 0.25C_2H_5OH$ (520.13): C, 63.50; H, 7.26; N, 16.15. Found: C, 63.20; H, 7.06; N, 16.35.

2,7-Bis(*N*′-ethoxycarbonyl-*N*″-methoxy)guanidino-9*H*-fluorene (11f). With the general procedure and *O*-methylhydroxylamine hydrochloride, a tan white solid was obtained (0.55 g, 87%): mp 180−2 °C; ^{1}H NMR (DMSO-d_6) δ 0.92−0.95 (m, 6H), 1.20 (t, J = 6.9 Hz, 6H), 3.67 (s, 6H), 3.69 (s, 6H), 3.73−3.86 (m, 8H), 4.10 (q, J = 6.9 Hz, 4H), 6.99 (d, J = 8.1 Hz, 2H), 7.16 (s, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.55−7.62 (m, 6H), 8.34 (br s, 1H), 8.36 (br s, 1H), 8.70 (br s, 2H), 9.13 (br s, 2H), 9.15 (br s, 2H); MS (ESI) *m/z* (rel intens) 485 (M^+ + 1, 100).

2,7-Bis(*N*′-ethoxycarbonyl-*N*″-isobutoxy)guanidino-9*H*-fluorene (11g). Following the general procedure, *O*-isobutylhydroxylamine hydrochloride was used to prepare the target compound, which was obtained as creamy white crystals (0.8 g, 93%): mp 122−5 °C; ^{1}H NMR (DMSO-d_6) δ 0.88−0.99 (m, 30H), 1.21 (t, J = 7.2 Hz, 6H), 1.92−2.02 (m, 4H), 3.65 (d, J = 6.6 Hz, 8H), 3.74−3.78 (m, 4H), 3.85 (q, J = 7.2 Hz, 4H), 4.11 (q, J = 7.2 Hz, 4H), 7.00 (d, J = 8.1 Hz, 2H), 7.17 (s, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.55−7.62 (m, 6H), 8.19 (br s, 1H), 8.21 (br s, 1H), 8.67 (br s, 1H), 8.68 (br s, 1H), 9.01 (br s, 1H), 9.02 (br s, 1H), 9.10 (br s, 1H), 9.11 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 154.0, 153.9, 153.3, 143.4, 143.3, 143.1, 142.9, 142.9, 142.3, 142.2, 138.8, 138.6, 137.9, 137.7, 135.8, 135.6, 134.4, 134.2, 119.7, 119.4, 199.1, 118.8, 117.7, 117.6, 116.6, 114.5, 79.5, 79.3, 61.1, 60.4, 36.6, 36.5, 36.3, 27.3, 27.3, 19.3, 14.4; MS (ESI) *m/z* (rel intens) 569 (M^+ + 1, 100).

Preparation of N-Substituted Guanidines (General Procedure) (Scheme 2). 2,7-Bis(*N*′-methyl)guanidino-9*H*-fluorene (12a). The substituted guanidine **11c** (0.5 g, 1.1 mmol) was suspended in EtOH (5 mL), and 1 N KOH (11 mL, 11 mmol) was then added. The reaction mixture was kept stirring for 10 h, maintaining the temperature at 50 °C. The solvent was evaporated, and the residue was washed multiple times with water and crystallized from aqueous EtOH to give a light-orange solid (0.24 g, 70%): mp 240−2 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 6H), 3.69 (s, 2H), 4.96 (br s, 4H), 5.34 (br s, 2H), 6.70 (d, J = 8.1 Hz, 2H), 6.90 (s, 2H), 7.50 (d, J = 8.1 Hz, 2H).

For preparation of the HCl salt, a solution of the free base in dry EtOH (20 mL) chilled in an ice bath was treated with dry HCl gas for 10 min. The reaction mixture was concentrated under reduced pressure and then diluted with ether. The precipitate formed was collected by filtration to give an orange solid (0.16 g): mp 276−8 °C; ^{1}H NMR (DMSO-d_6) δ 2.84 (s, 6H), 3.96 (s, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.46 (s, 2H), 7.77 (br s, 2H), 7.89 (br s, 2H), 7.95 (d, J = 8.4 Hz, 2H), 10.01 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 155.7, 144.5, 138.6, 134.2, 123.5, 121.5, 120.9, 36.6, 28.3; MS (ESI) *m/z* (rel intens) 309 (M^+ + 1, 100), 155 (9). Anal. Calcd for $C_{17}H_{20}N_6\cdot 2HCl\cdot 0.25C_2H_5OH\cdot 0.75H_2O$ (406.33): C, 51.73; H, 6.20; N, 20.68. Found: C, 51.77; H, 6.24; N, 20.48.

2,7-Bis(*N*′-ethyl)guanidino-9*H*-fluorene (12b). Free Base. Starting with **11d** and following the general procedure, a beige solid was obtained (0.24 g, 85%): mp 155−7 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.07 (t, J = 7.2 Hz, 6H), 3.14 (q, J = 7.2 Hz, 4H), 3.68 (s, 2H), 5.00 (br s, 6H), 6.68 (d, J = 8.1 Hz, 2H), 6.88 (s, 2H), 7.49 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 151.2, 148.9, 143.3, 134.3, 121.4, 119.6, 118.9, 36.2, 35.1, 15.0; MS (ESI) *m/z* (rel intens) 337 (M^+ + 1, 90), 292 (60), 205 (50), 169 (100).

Dihydrochloride Salt. Bright-yellow solid, mp 193−5 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7.2 Hz, 6H), 3.28 (q, J = 7.2 Hz, 4H), 3.95 (s, 2H), 7.24 (d, J = 8.1 Hz, 2H), 7.44 (s, 2H), 7.74 (br s, 4H), 7.95 (d, J = 8.1 Hz, 2H), 8.03 (br s, 2H), 9.96 (br s, 2H). Anal. Calcd for $C_{19}H_{24}N_6\cdot 2HCl\cdot 1.75H_2O\cdot 0.2C_2H_5OH$ (450.09): C, 51.76; H, 6.87; N, 18.67. Found: C, 51.86; H, 6.64; N, 18.68.

2,7-Bis(*N*′-isopropyl)guanidino-9*H*-fluorene (12c). Free Base. Starting with (**11e**) and following the general procedure, a salmon-orange solid was obtained (0.17 g, 79%): mp 247−9 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.11 (d, J = 5.4 Hz, 12H), 3.68

(s, 2H), 3.83−3.87 (m, 2H), 4.93 (br s, 4H), 5.38 (br s, 2H), 6.69 (d, J = 8.1 Hz, 2H), 6.89 (s, 2H), 7.49 (d, J = 8.1 Hz, 2H).

Dihydrochloride Salt. Shiny yellow crystals, mp 308−9 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.18 (d, J = 6.3 Hz, 12H), 3.85−3.94 (m, 4H), 7.23 (d, J = 8.4 Hz, 2H), 7.43 (s, 2H), 7.71 (br s, 4H), 7.93 (d, J = 8.4 Hz, 2H), 8.05 (br s, 1H), 8.07 (br s, 1H), 9.88 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 153.9, 144.8, 138.9, 134.5, 123.7, 121.6, 121.1, 43.8, 22.3; HRMS calcd for MH + $C_{21}H_{28}N_6$: 365.2454. Observed 365.2467. Anal. Calcd for $C_{21}H_{28}N_6 \cdot 2HCl \cdot 1.4H_2O$ (462.63): C, 54.52; H, 7.14; N, 18.17. Found: C, 54.51; H, 6.74; N, 17.79.

2,7-Bis(*N*′-methoxy)guanidino-9*H*-fluorene (12d). Free Base. With **11f** as a starting material, a pink solid was obtained (0.17 g, 79%): mp 200−2 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.60 (s, 6H), 3.76 (s, 2H), 5.46 (br s, 4H), 7.20 (dd, J = 8.4 Hz, 2H), 7.51−7.54 (m, 4H), 7.90 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 151.6, 143.1, 139.5, 133.7, 118.8, 116.1, 114.1, 60.5, 36.5; MS (ESI) m/z (rel intens) 341 (M^+ + 1, 48), 168 (100).

Dihydrochloride Salt. Shiny tan-white solid, mp 248−9 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 6H), 3.96 (s, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.49 (s, 2H), 7.96 (d, J = 8.1 Hz, 2H), 8.24 (br s, 4H), 10.43 (br s, 2H), 11.64 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 156.1, 144.5, 138.8, 133.4, 123.5, 121.5, 120.9, 64.5, 36.6. Anal. Calcd for $C_{17}H_{20}N_6O_2 \cdot 2HCl \cdot 0.5C_2H_5OH$ (436.33): C, 49.64; H, 5.77; N, 19.26. Found: C, 49.95; H, 5.65; N, 19.40.

2,7-Bis(*N*′-isobutoxy)guanidino-9*H*-fluorene (12e). Free Base. Use of **11g** and the general procedure provided a brick-red solid (0.16 g, 62%): mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (d, J = 6.9 Hz, 12H), 1.93−2.02 (m, 2H), 3.55 (d, J = 6.9 Hz, 4H), 3.74 (s, 2H), 5.32 (br s, 4H), 7.21 (dd, J = 8.4, 1.8 Hz, 2H), 7.49−7.52 (m, 4H), 7.82 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 151.5, 143.1, 139.4, 133.8, 118.8, 116.3, 114.3, 79.1, 36.5, 27.3, 19.3; MS (ESI) m/z (rel intens) 425 (M^+ + 1, 100), 245 (10), 156 (56).

Hydrochloride Salt. Pink solid, mp 251−2 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.91 (d, J = 6.9 Hz, 12H), 1.97−2.06 (m, 2H), 3.65 (d, J = 6.9 Hz, 4H), 3.95 (s, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.48 (s, 2H), 7.96 (d, J = 8.4 Hz, 2H), 8.16 (br s, 4H), 10.36 (br s, 2H), 11.65 (br s, 2H). Anal. Calcd for $C_{23}H_{32}N_6O_2 \cdot 2HCl$ (496.21): C, 55.53; H, 6.88; N, 16.89. Found: C, 55.45; H, 6.87; N, 16.70.

Preparation of Reversed Amidines (General Procedure) (Scheme 3). 2,7-Bis[4-(iminobenzylamino)]-9*H*-fluorene (13a). Free Base. A solution of 2,7-diamino-9*H*-fluorene (0.3 g, 1.5 mmol) in dry MeCN (10 mL) was diluted with dry EtOH (15 mL) and chilled in an ice−water bath. The solution was then treated with *S*-(2-naphthylmethyl)thiobenzimidate hydrobromide (1.13 g, 3.15 mmol). The reaction mixture was kept stirring at room temperature overnight, after which the solvent was evaporated to dryness leaving behind an oily residue that was triturated with ether to give a solid of the hydrobromide salt. The solid was then dissolved in EtOH and basified with 1 N NaOH, and the free base was extracted with EtOAc. After the sample was dried over Na_2SO_4, the solvent was evaporated to dryness, giving an off-white solid (0.45 g, 63%): mp 240−2 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 2H), 6.31 (br s, 4H), 6.83−6.85 (m, 2H), 7.05 (s, 2H), 7.39−7.46 (m, 6H), 7.71 (d, J = 8.4 Hz, 2H), 7.96−7.98 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 153.9, 148.5, 143.8, 135.9, 135.7, 129.9, 127.9, 126.9, 120.3, 119.7, 118.3, 36.5.

Hydrochloride Salt. An ice bath cold solution of the free base in dry EtOH was treated with HCl gas for 5−10 min, after which the mixture was concentrated to near dryness and the suspension was diluted with ether to furnish a yellow solid (0.28 g): mp 286−8 °C; ^{1}H NMR (DMSO-d_6) δ 4.09 (s, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.65−7.70 (m, 4H), 7.75−7.79 (m, 4H), 7.95−7.97 (m, 2H), 8.16 (d, J = 8.4 Hz, 2H), 9.16 (br s, 2H), 9.86 (br s, 2H), 11.67 (br s, 2H); ^{13}C NMR δ 163.1, 144.9, 140.1, 133.7, 133.6, 128.9, 128.8, 128.6, 124.5, 122.5, 121.6, 36.9; MS (EI) m/z (rel intens) 402 (M^+, 100), 299 (38), 197 (9), 196 (60), 151 (7), 103 (32), 77 (10). Anal. Calcd for $C_{27}H_{22}N_4 \cdot 2HCl \cdot 0.25C_2H_5OH \cdot H_2O$ (504.94): C, 65.41; H, 5.49; N, 11.09; Cl, 14.04. Found: C, 65.76; H, 5.40; N, 10.87; Cl, 14.09.

2,7-Bis[4-[imino-(2-pyridylmethyl)]amino]-9*H*-fluorene (13b). Free Base. The general procedure was used employing 2,7-diamino-9*H*-fluorene (0.5 g, 2.95 mmol) and *S*-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide (2.22 g, 6.2 mmol) to give the reversed amidine as shiny yellow crystals (0.80 g, 67%): mp 230−2 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 2H), 6.56 (br s, 4H), 6.91 (d, J = 8.4 Hz, 2H), 7.11 (s, 2H), 7.53−7.57 (m, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.93−7.98 (m, 2H), 8.33 (d, J = 7.8 Hz, 2H), 8.63 (d, J = 7.8 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 159.3, 149.7, 144.8, 144.5, 140.2, 138.2, 133.3, 128.5, 124.7, 124.5, 122.7, 121.5, 36.8.

Hydrochloride Salt. Yellow solid (0.33 g), mp 302−4 °C; ^{1}H NMR (DMSO-d_6) δ 4.08 (s, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.74 (s, 2H), 7.83−7.87 (m, 2H), 8.14−8.24 (m, 4H), 8.58 (d, J = 8.1 Hz, 2H), 8.89 (d, J = 7.2 Hz, 2H), 9.39 (br s, 2H), 10.18 (br s, 2H); ^{13}C NMR (DMSO-d_6) δ 159.6, 149.7, 144.0, 143.9, 140.4, 138.5, 133.5, 128.6, 124.9, 124.2, 122.9, 121.8, 36.8; MS (EI) m/z (rel intens) 404 (M^+, 100), 300 (28), 283 (6), 196 (33), 152 (9), 105 (28), 78 (21). Anal. Calcd for $C_{25}H_{20}N_6 \cdot 3.5HCl \cdot 0.33C_2H_5OH \cdot H_2O$ (565.29): C, 54.52; H, 4.89; N, 14.87; Cl, 21.95. Found: C, 54.57; H, 4.74; N, 14.97; Cl, 21.87.

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