Minor Groove DNA Binders as Antimicrobial Agents. 1. Pyrrole Tetraamides Are Potent Antibacterials against Vancomycin Resistant *Enteroccoci* and Methicillin Resistant *Staphylococcus aureus*

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A new series of short pyrrole tetraamides are described whose submicromolar DNA binding affinity is an essential component for their strong antibacterial activity. This class of compounds is related to the linked bis-netropsins and bis-distamycins, but here, only one amino-pyrrole-carboxamide unit and an amidine tail is connected to either side of a central dicarboxylic acid linker. The highest degree of DNA binding, measured by compound-induced changes in UV melting temperatures of an AT-rich DNA oligomer, was observed for flat, aromatic linkers with no inherent bent, i.e., terephthalic acid or 1,4-pyridine-dicarboxylic acid. However, the antibacterial activity is critically linked to the size of the *N*-alkyl substiutent of the pyrrole unit. None of the tetraamides with the commonly used methyl-pyrrole showed antibacterial activity. Isoamyl- or cyclopropylmethylene-substituted dipyrrole derivatives have the minimum inhibitory concentrations in the submicromolar range. In vitro toxicity against human T-cells was studied for all compounds. The degree to which compounds inhibited cell growth was neither directly correlated to DNA binding affinity nor directly correlated to antibacterial activity but seemed to depend strongly on the nature of the *N*-alkyl pyrrole substituents.

Introduction

The rapid emergence of bacterial infections resistant to treatment with standard antibiotics constitutes a serious public health threat[1,2] and a new, yet all too familiar, challenge to medicinal chemistry. Novel antibacterials are needed that are either less prone to give rise to bacterial resistance or are directed toward novel biological targets such that combination therapy becomes more efficient. While many approved antibacterial drugs inhibit parts of the replication machinery, i.e., bacterial polymerases by rifampicin or ribosomes by aminoglycosides,[3] no examples exist that target DNA directly. Targeting bacterial DNA is promising as it would involve early steps in cell proliferation and be less likely to lead to bacterial resistance than targeting enzymes. This approach, however, would also require that bacterial DNA could be targeted preferentially over human DNA to minimize toxicity. The naturally occurring polyamide antibiotics netropsin and distamycin (Figure 1) are well-characterized DNA binding agents.[4−7] These antibiotics bind within the minor groove of DNA at regions with four or five AT base pairs, respectively. Not surprisingly, the cytotoxicity of these compounds precluded clinical use.[8,9] Nevertheless, the pyrrole polyamides netropsin and distamycin were the inspiration for a large body of work aimed at the exploration and improvement of the pharmaceutical potential of DNA minor groove binders (reviewed in refs 6 and 10−12). Besides the many efforts to extend the DNA sequence specificity of peptidic groove binders,[7] considerable work also went into increasing the potency of these compounds by linking together netropsin and distamycin type molecules. Over 20 years of work has been undertaken on the improvement of the synthesis of linked pyrrole polyamides, their DNA binding properties,[13,14] and antitumor[15] or antiviral activity.[16] Lown and coworkers synthesized netropsin and distamycin dimers by joining the N terminal ends with a wide range of dicarboxylic acid linkers (head-to-head linkage). Among them, distamycin dimers with flexible, aliphatic linkers showed increased inhibition of proliferation of several cancer cell lines[15] as compared to distamycin and netropsin alone. These same compounds along with distamycin dimers containing a terephthalic linkage (Figure 1) also showed promising anti-HIV activity, which is related to the inhibition of HIV-1 integrase catalytic activity.[16] This anti-HIV activity has also been connected with the inhibition of viral reverse transcriptase activity. For the latter, dimers with short rigid linkers were clearly more potent. Although most of the linked oligopyrroles exhibit good affinity for AT-rich DNA sequences, a clear correlation between DNA binding and biological activity was not observed.[15] Beyond differential cellular uptake and metabolism obscuring the relationship between DNA binding and antiviral or anticancer activity, one must also consider that these rigidly or flexibly linked dimers encounter different binding modes with a DNA target alone or the ternary complex (binding to DNA−protein complex) in which inhibition is accomplished.

From the lack of biological activity of a short ethylene-linked compound with only one pyrrole unit on each side

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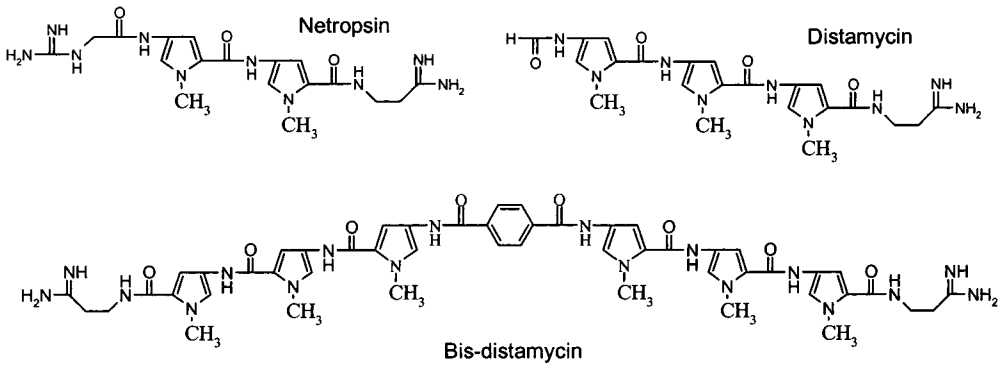


Figure 1. Chemical structures of natural antibiotics netropsin and distamycin as compared to a rigid bis-distamycin (compound **8** from ref *15*).

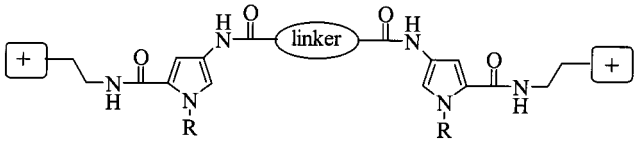


Figure 2. General structure of short pyrrole tetraamides.

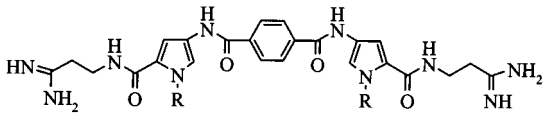
of the linker, it was concluded that activity is tied to at least two pyrrole units.[15] However, considering that the molecular geometry of such a short oligopyrrole with only one pyrrole unit and a short rigid linker should be very similar to that of distamycin itself, one would expect strong DNA binding affinity and possibly biological activity. Recent reports on the antimicrobial activity of short, rigid minor groove binders carrying amidine groups create additional support to those expectations.[17,18] Assuming that smaller compounds are more likely to have suitable cellular uptake and pharmacokinetic properties than bis-netropsins and bis-distamycins, the present study explores the biological activity of a library of short dipyrrole derivatives with the general structure as shown in Figure 2. It is well-known that changes in the overall lipophilicity of a compound can have dramatic effects on its biological activity.[19] This study was driven by the idea that modifications of the pyrrole units could produce significant changes in cellular uptake without detrimental effects on DNA binding and thereby provide antibacterial activity.

In this paper, we report the synthesis of a select group of novel, pyrrole tetraamides, their DNA binding properties, and antibacterial activities. Substantial potency against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) has been observed. We found that strong DNA binding properties are necessary for antibacterial acitivity and that lipophilicity is a strong modulator of activity. We also optimized our library to reduce cytotoxicity toward human T-cells.

Chemistry

Four groups of linked tetraamides shown in Tables 1−4 have been synthesized. Compounds **1**−**3** (Table 1), which have linked pyrroles alkylated with methyl, propyl, and isoamyl groups, respectively, represent the first group. The second group (Table 2), compounds **4**−**14**, presents *N*-isoamylpyrrole units linked with different dicarboxylic acids. Compounds in Table 3, **15**−**21**, are *N*-methyl-cyclopropyl pyrroles linked with aromatic dicarboxylic acids. Table 4 presents compounds

Table 1. In Vitro Antimicrobial Activity, Cell Toxicity, and DNA Binding of Terephthalamides



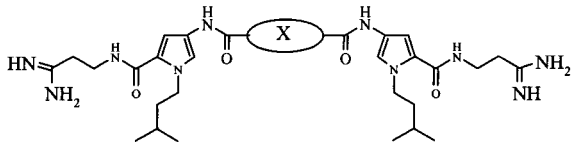
			MIC (μM)			
compd	R	ΔT_m (°C)[a]	MRSA	BM 4147[b]	UCD-3[b]	toxicity (% NCC)[c]
distamycin	methyl	26	>45.5	>45.5	>45.5	79
1	propyl	30	22.7	22.2	>45.5	56
2	3-methyl-butyl	29	11.1	5.6	5.6	61
3		28	1.4	0.7	0.7	4

[a] ΔT_m, changes in the DNA melting temperature (ΔT_m) of an AT-rich dodecamer, (CG**ATTATTAA**GC)·(GC**TTAATAAT**CG), upon binding of an equimolar amount of compound. [b] MIC, minimal inhibitory concentration. [c] MRSA, methicillin resistant *Staphylococcus aureus*. [d] BM4147 and UCD3, two strains of VRE. [e] Toxicity is measured as a fraction of growth as compared to no compound control (percent).

22−**28** with variations in the positively charged terminal groups.

Synthesis of *N*-alkylated pyrrole units, the crucial intermediates for assembly of target tetraamides, was accomplished starting with 4-nitro-pyrrole 2-carboxylate **29**[20] (Scheme 1). After the sodium derivative of **29** was alkylated with alkyl bromides, the corresponding *N*-alkyl derivatives **30**−**33** were hydrolyzed and the liberated acids **34**−**37** were converted into the chlorides by refluxing in thionyl chloride, as described earlier for the *N*-methyl derivative.[21] The reaction with cyclopropylmethyl bromide required a longer reaction time and a large excess of the alkylating agent, as compared with nonbranched alkyl halides. The condensation of acid chlorides **34**−**37** with aminopropionitrile afforded derivatives **38**−**41** as white crystalline products. Cyanoethylamides **38**−**41** were treated under Pinner reaction conditions to generate amidine hydrochloride terminal groups by reaction with hydrochloric acid in ethanol, followed by treatment with ammonia in ethanol. Amidines **42**−**45** were obtained with excellent yield and were used without further purification. When the ethanolic solution of cyanoethylamide **41** was saturated with HCl, followed by alkylamine treatment at 0−5 °C, monoalkyl amidine derivatives **46**−**48** were formed. The same reaction at an elevated temperature led to the formation of dialkylated amidine terminal groups. Thus, the reaction of imino ester derivative of **40** with methyl-

Table 2. In Vitro Antimicrobial Activity, Cell Toxicity, and DNA Binding for *N*-(3-Methyl-butyl)-1H-pyrrole Dimers with Different Linkers



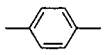
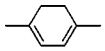
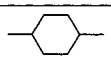
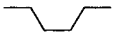
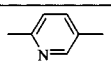
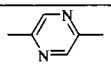
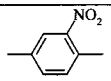
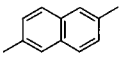
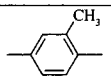
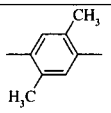
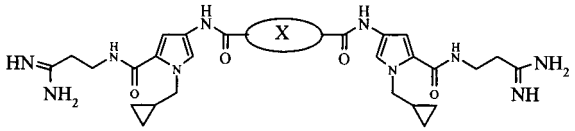
No	X	ΔTm (^{0}C)	MIC (μM)			Toxicity % NCC
			MRSA	BM 4147	UCD-3	
3		28	1.4	0.7	0.7	6
4		26	0.7	1.4	1.4	23
5		11	11.4	>45.5	>45.5	88
6		10	>45.5	>45.5	>45.5	87
7		25	1.4	0.7	1.4	3
8		11	2.8	22.7	22.7	6
9		20	2.8	2.8	2.8	32
10		25	1.4	1.4	1.4	42
11		20	2.8	2.8	5.7	15
12		24	>45.5	>45.5	>45.5	0
13		24	0.7	1.4	1.4	38
14		12	11.4	>45.5	>45.5	75

Table 3. In Vitro Antibacterial Activity, Cell Toxicity, and DNA Binding of Linked *N*-(Cyclopropylmethyl)-1H-pyrrole Derivatives

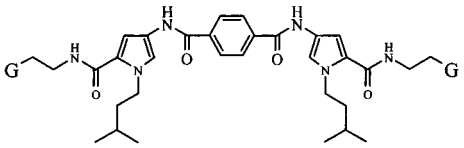


No	X	ΔTm (^{0}C)	MIC (μM) MRSA	MIC (μM) BM 4147	MIC (μM) UCD-3	Toxicity % NCC [1)]
15		28	1.4	2.8	0.7	72
16		28	1.4	2.8	2.8	87
17		27	1.4	1.4	2.8	82
18		23	2.8	2.8	5.7	90
19		26	2.8	2.8	2.8	87
20		27	1.4	1.4	2.8	65
21		26	2.8	5.7	5.7	88

amine in ethanol at 60 °C yielded dimethyl amidine **49**, while the reaction with 1,3-diaminopropane under the same conditions afforded cyclic amidine **50.**[22] Most of the amidine analogues were isolated by precipitation from methanol with ether and were used without additional purification; only cyclic product **50** had to be isolated by preparative high-performance liquid chromatography (HPLC). Aminolysis of the *N*-isoamyl pyrrole derivative **32** with ethylenediamine at 60 °C for 50 h provided (2-amino-ethyl)amide **51** with 62% yield after chromatographic purification. To make this unit compatible with the coupling reaction, we had to protect the amino moieties with Boc-protecting groups.

The novel linked pyrroles designed and evaluated in this paper required the coupling of pyrrole units with dicarboxylic acids as outlined in Scheme 2. We have used commercially available dicarboxylic acids as central linkers; only 2-methylterephthalic and 2,5-dimethylterephalic acids were synthesized from corresponding bromobenzenes as described in the literature.[23] Treatment of 1,4-dibromo-2-methylbenzene or 1,4-dibromo-2, 5-dimethylbenzene with *tert*-butyllithium, followed by saturation with CO_2, yielded mono- and dimethylterephthalic acids, respectively, in moderate yields. Dicarboxylic acids were activated via formation of an

Table 4. In Vitro Antimicrobial Activity, Cell Toxicity, and DNA Binding for *N,N′*-Bis-[1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide with Different Positively Charged Tails



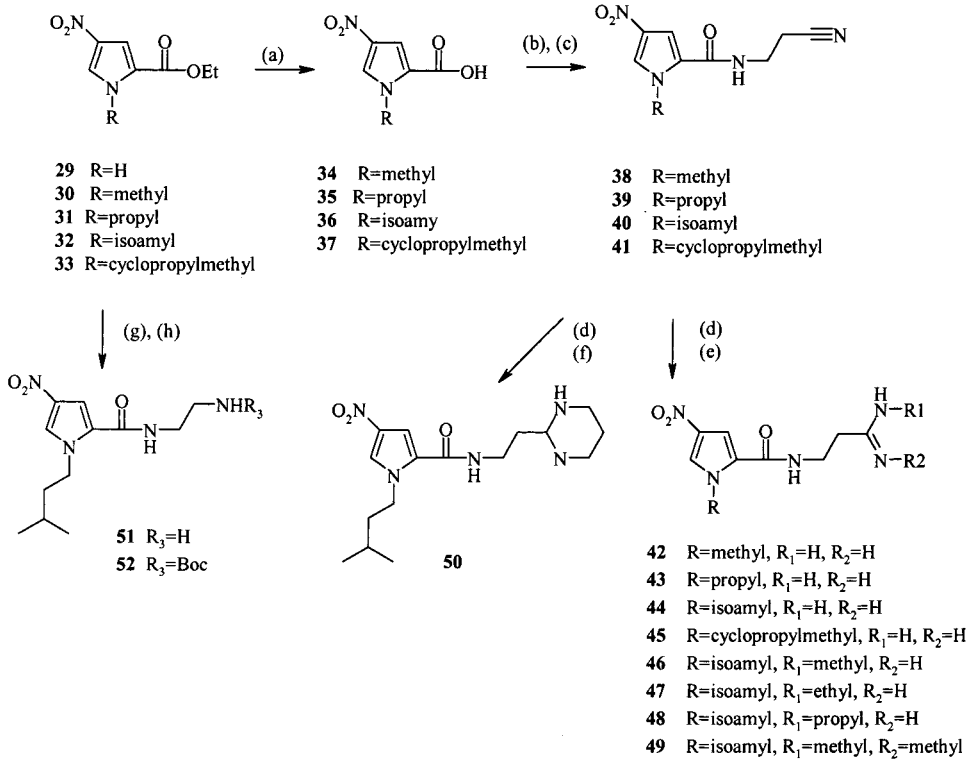
Compound No	G	ΔTm (^{0}C)	MIC (μM) MRSA	MIC (μM) BM 4147	MIC (μM) UCD-3	Toxicity % NCC
3		28	1.4	0.7	0.7	8
22		28	0.7	1.4	1.4	78
23		27	1.4	1.4	1.4	74
24		26	1.4	1.4	1.4	15
25		26	1.4	1.4	1.4	50
26		28	0.7	0.7	0.7	51
27	NH_2	20	2.8	11.4	11.4	24
28		25	0.7	1.4	1.4	5

activated ester with pentafluorophenol.[24] The product of the reaction of the corresponding dicarboxylic acid and pentafluorophenyl trifluoroacetate in dimethylformamide (DMF) was purified by flash chromatography on silica gel in toluene and stored at room temperature. Amino components **42a**−**50a** and **52a** were obtained by the catalytic reduction (Pd/C) of the corresponding nitro compounds **42**−**50** and **52**.[16] Coupling was carried out at 60 °C for 10 hours and resulted in the desired tetraamides **1**−**26**. Reaction between activated terephthalic acid and Boc-protected **51a** yielded in Boc-protected derivative, which was isolated by chromatography on silica gel and then deprotected with HCl in ethanol to get the target compound **27**. The amino functions of **27** were converted to guanidines with pyrazole-1-carboxamidine, as described earlier,[25] to get pyrrole dimer **28** with guanidinium tails. The final products were purified by preparative reverse phase HPLC and transformed into hydrochlorides by precipitation with ether from HCl/ethanol. All final products were homogeneous by HPLC; structures were confirmed with ^{1}H nuclear magnetic resonance (NMR) and high-resolution mass spectroscopy (HRMS) spectra.

Results and Discussions

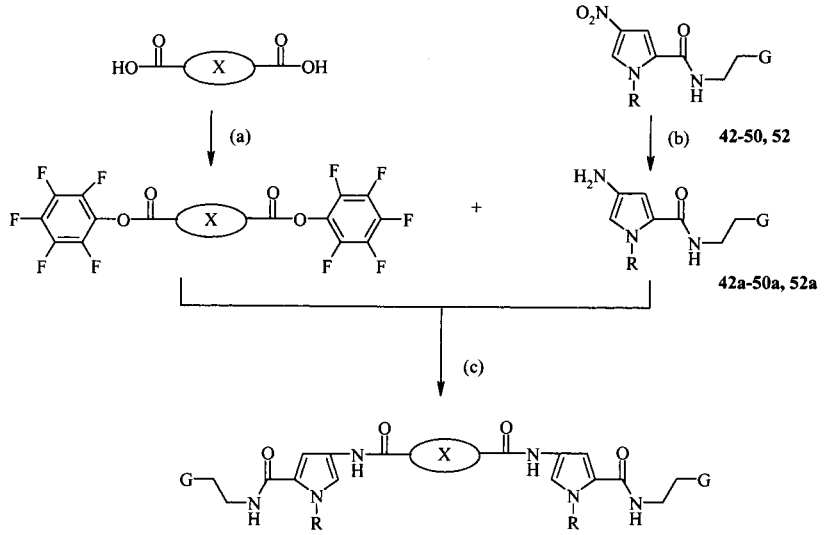
DNA Binding. DNA binding affinities were evaluated using changes in the DNA melting temperature (ΔT_m) of an AT-rich dodecamer, (CG**ATTATTAA**GC)·

Scheme 1. Synthesis of Pyrrole Flanking Units[a]



[a] Reaction conditions: (a) 2 M NaOH, EtOH, 55 °C. (b) $SOCl_2$, 80 °C. (c) Aminopropionitrile (2.1 equiv), toluene. (d) HCl (gas) at 5 °C in ethanol. (e) NH_3 (gas) at 5 °C in ethanol for **42**−**45**, monoalkylamine at 5 °C in ethanol for **46**−**48**, methylamine at 60 °C in ethanol for **49**. (f) 1,3-Propanediamine at 60 °C in ethanol. (g) Ethylenediamine, 50 h, 60 °C. (h) DiBoc-carbonate, DMF.

Scheme 2. Coupling Scheme for Synthesis of Linked Pyrroles[a]



[a] Reaction conditions: (a) Pentafluorophenyltrifluoroacetate (2.2 equiv), diisopropylethylamine (2.2 equiv), DMF. (b) H_2, 5% Pd/C (Degussa type), MeOH, 25 psi, 30 min. (c) DMF, 20 h, 55 °C.

(GC**TTAATAAT**CG), upon binding of an equimolar amount of compound. The values of ΔT_m are listed in Tables 1−4. The T_m for free DNA at 5 μM was 35 °C. The range of ΔT_m for these compounds is from 10 to 30 °C with compound **1** having the highest ΔT_m and compound **6** having the lowest value. The ΔT_m value is related to binding constants via base pair dissociation enthalpy and the temperature dependence of the binding enthalpy.[26,27] For several compounds, we also determined the apparent binding constant (K_D) by a fluorescent ethidium displacement assay (data not shown)[28,29] such that values of ΔT_m can also be interpreted in a coarse but absolute manner. For example, compounds **1**−**3** in Table 1 showed apparent dissociation constants (K_D) of 30−200 nM (data not shown). Compounds with some of the lower ΔT_m values, e.g., **5** and **8** (11 °C), gave values of 1 and 3 μM, respectively.

Biological Assays. Antibacterial activity was tested against a wide panel of species, and values for minimal inhibitory concentrations (MIC) for two strains of VRE, BM4147 and UCD3, and a strain of MRSA are reported in Tables 1−4. The compound concentrations tested for the pyrrole dimers ranged from 0.7 to 45.5 μM (see Experimental Section).

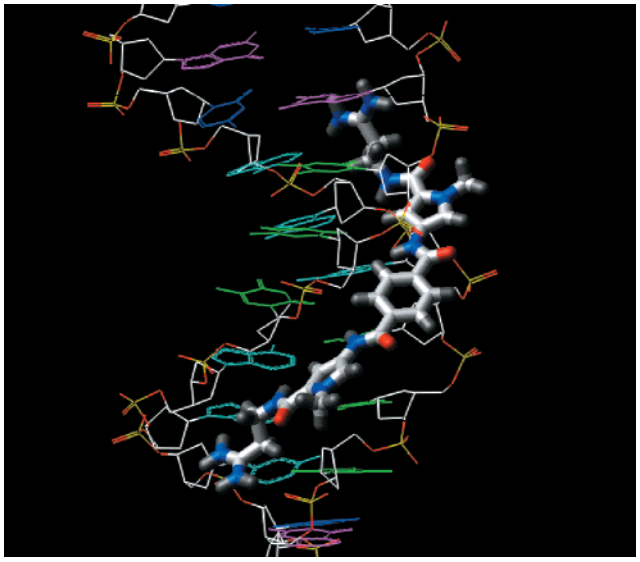


Figure 3. Molecular model of compound **1** docked into the distamycin binding site in DNA dodecamer with sequence GCGAAATTTCGC. Compound **1** is shown in stick format with ICM atom type colors. Color coding: A:T pairs are blue:green, G:C pairs are magenta:purple.

Prompted by the known cytotoxicity of similar compounds,[15] we assessed compound toxicity in human T-cells using a colorimetric cell proliferation assay.[30] These data are reported in Tables 1−4 as the fraction of cellular activity to cleave a tetrazolium salt (WST-1) as compared to untreated cell cultures. Although the T-cell assay is only a simple, cursory predictor of the potential toxicity in humans, the results revealed interesting chemical trends and enabled further compound optimization.

Structure−Activity Relationship (SAR) of Pyrrole Tertraamides. Prompted by the antiviral activity and DNA binding properties of netropsin and distamycin dimers with a terephthalic linkage,[15] we synthesized compound **1** (Table 1), which, not surprisingly, turned out to exhibit excellent DNA binding properties. Molecular modeling suggests that this particular scaffold fits well into the narrow minor groove of a DNA AT tract. Figure 3 shows a model of compound **1** docked into the distamycin receptor site (AAATTT) generated with the flexible docking program.[31] Our approach of docking into the minor groove of the distamycin DNA cocrystal structure allowed a semiquantitative comparison with distamycin itself. Compound **1** not only has improved van der Waals interactions with the groove but head-to-head geometry of this compound is more suitable for hydrogen bonding to minor groove acceptor atoms. Consistent with molecular modeling, ΔT_m for compound **1** was 30 °C, which is 4 °C higher than the value for distamycin. From the model in Figure 3, it was also clear that the pyrrole nitrogens, projecting away from the groove, lend themselves for modification toward increasing compound lipophilicity. Pyrrole dimers **2** and **3**, *N*-alkylated with propyl and isoamyl groups, respectively, (Table 1) indeed retain excellent DNA binding properties, but most importantly, they become potent antibacterials. Whereas compound **1** is only mildly active against MRSA and one of the VRE strains, compound **3** exhibits MIC of 1.4 μM against MRSA and is active at submicromolar concentrations against both VRE strains. This trend parallels the lipophilicity of the pyrrole side chains with the isoamyl substituent being the most active. Compound **3** represents the first linked monopyrrole with good activity against Gram-positive microorganisms.

Lipophilicity of potential drugs is strongly related to their capacity to cross cellular membranes, and it is conveniently expressed as the partioning coefficient for a water−octanol system, log $P_{o/w}$.[19] We determined log $P_{o/w}$ for compounds **1**−**3** experimentally because the prediction of this parameter for highly charged compounds is notoriously difficult. Whereas compounds **1** and **2** are not lipophilic, with log $P_{o/w}$ values of −2 and −1.1, respectively, compound **3** is clearly lipophilic, with a log $P_{o/w}$ value of +1.4, and is in a range where the majority of bioavailable drugs are found.[32] It is likely that the stronger antibacterial activity of compound **3** is related to improved cellular uptake, given that the DNA binding capacity is essentially unchanged. However, the possibility that the pyrrole side chains play a direct role in the inhibition of a ternary complex cannot be ruled out but such discussion is beyond the scope of this paper.

Next, we focused on optimization of the motif presented by compound **3** beginning with the central linker. Results for compounds **4**−**14** are presented in Table 2. It is evident that good DNA binding properties (ΔT_m > 20 °C) are a prerequisite for antibacterial activity. Data for compounds **3**−**6** show that abandoning the flat geometry for bulkier, aliphatic linkers destroys DNA binding and activity. While the cyclohexa-1,3-diene-1,4-linked compound **4** appears very similar to parent compound **3**, cyclohexane-1,4-linked compound **5** demonstrated only moderate activity against MRSA and was not active against VRE. Compound **6** with the flexible adipic acid linker has lost significant antibacterial activity. Our modeling studies suggest that the more bulky aliphatic linkers would produce van der Waals clashes with the narrow minor groove of AT-rich regions. The observed SAR for compounds **3**−**6** demonstrates that DNA binding is necessary for antibacterial activity.

The result for compound **6** is surprising in light of earlier results for similarly linked distamycin dimers for which good binding to calf thymus DNA and antileukemic activity has been reported.[15] We must consider that binding mode and the preferred sequence of bis-distamycins are unclear and elusive. These dimers might bind with only one-half of the molecule, or they could be binding to partially mixed sequences, which better accommodate the aliphatic linker.

Table 2 shows other effects resulting from structural variation of the linker. Substitution in the aromatic ring of the linker affects DNA binding and antibacterial activity to a much smaller degree than the geometry of the linker. Compound **7**, linked with 2,5-pyridine dicarboxylic acid, showed mildly reduced DNA binding and MIC values similar to parent compound **3.** Using pyrazine dicarboxylic acid as a linker, DNA binding again slightly drops, but antibacterial activity is retained. The change of linker geometry, as in compound **8**, caused a sharp drop in DNA binding and anti-VRE

activity. Similar effects of this meta-linker have been reported for the bis-netropsin and bis-dystamicin dimers.[16]

On the other hand, the potency of dimers **10** and **13** indicates that one exocyclic substituent on the aromatic unit of the linker, i.e., nitro or methyl groups, respectively, is well-tolerated. Modeling studies suggest that the small reduction in DNA binding activity is related to a minor van der Waals clash between the exocylic substiutent and the adjacent carbonyl oxygen, since the preferred, docked conformation requires near coplanarity. This steric effect would be even more aggravated for the linker of compound **11**. The two ortho-substituents of 1,4-naphthalene dicarboxylic acid indeed cause slightly reduced activity and DNA binding. The hypothesis that only linker geometries compatible with good minor groove binding confers activity is again confirmed by compound **14**, which exhibits two exocyclic methyl groups at positions 2 and 5. Because they appear on either side of the ring, one would always prohibit the molecule from penetrating the groove deeply enough to engage in sufficient hydrophobic interactions. The activity and DNA binding pattern are essentially that of compound **5**, which has the bulky cyclohexane linker. The most surprising results came from compound **12**, which has a 2,6-naphthalene dicarboxylic acid linker that should be compatible with good groove binding, as seen by modeling and verified by its high ΔT_m value. The fact that compound **12** does not exhibit any activity against VRE and MRSA suggests that the length of the linking unit, which shapes the overall geometry, is also related to antibacterial activity.

The results presented in Table 2 show that the general framework of two *N*-isoamylpyrroles linked with terephthalic or substituted terephthalic acids provides antibacterial activity in the 0.7−2.8 μM ranges. However, the toxicity against human T-cells remained significantly high. For the most active compounds, e.g., **3**, **4**, and **7**, cells grew less than 25% in the presence of compound as compared to the no compound control. Adding a nitro or methyl group to the linker, compounds **10** and **13**, somewhat improves toxicity. It is not surprising that most of the poor minor groove binders barely inhibit T-cell growth. However, toxicity of compound **8** (6% NCC) is an exception and suggests that the meta-geometry of the linker leads to a different, yet unknown, mode of action in human T-cells. Unexpectedly, the highest toxicity is seen for inactive compound **12**, which also suggests that multiple targets might be involved.

To reduce cell toxicity but retain antibacterial activity along two different paths, we further optimized the structure of the most active compounds. The first utilized additional modifications of the *N*-alkyl moiety of pyrrole units, which yielded the interesting group of compounds **15**−**21** (Table 3) that all carry a cyclopropylmethyl group. This group was chosen as a more restrained, smaller mimic of the isoamyl moiety. Compounds **15**−**19** all exhibit the successful linking units introduced in Table 2. In addition to the 1,4-linked aromatic geometries, we also introduced two five-membered ring aromatic dicarboxylic acid linker, thiophene and 3,5-pyrazole dicarboxylic acid. This new linker geometry was very compatible with good minor groove binding, as evidenced by the high ΔT_m values of compounds **20** and **21**. Among the compounds in Table 3, thiophene-linked compound **20** is among those with the best activity profile. A comparison of the group of cyclopropylmethyl compounds **15**−**19** (Table 3) with the isoamyl compounds in Table 2 shows that ΔT_m values are slightly but systematically increased, whereas antibacterial activity is only mildly reduced. Most noticeable, however, is the dramatically reduced toxicity against T-cells with most compounds in Table 3 exhibiting percent NCC values above 80%.

The second approach to optimization of the parent compound **3** was modification of the tail groups bearing positive charges (Table 4). In addition to the alkylated amidine derivatives, compounds **22**−**25**, tails with a primary amine and a guanidinium moiety were evaluated as well (compounds **27** and **28**, respectively). Alkylation of the amidine moiety had very little effect on DNA binding and antibacterial activity. Interestingly, compounds **22** and **23** with small alkyl groups, methyl and ethyl, respectively, show a large reduction in toxicity. In contrast, analogue **24**, containing the longer propyl groups, showed a high toxicity more similar to that of the parent compound **3**. Cyclic, bis-alkylated compound **26** is the most active in the entire pyrrole tetraamide series and showed reasonable toxicity. The other charged tails tested here did not lead to improved compound properties. The primary amine tail for compound **27** caused a significant loss in DNA binding and antibacterial activity. Transformation of the amino terminus to guanidine restored activity, but toxicity against T-cells became very high indicated by a percent NCC value of 5%. For compounds **26** and **27**, the correlation between activity and DNA binding is obvious.

Conclusions

The present study demonstrates that short pyrrole tetraamides with head-to-head geometry strongly bind to the minor groove of AT tracts of DNA. In comparison with distamycin, molecular models of our head-to-head scaffold showed improved hydrophobic interactions as well as improved hydrogen bonding to the floor of the groove. Good DNA binding properties were found to be a requirement for good antibacterial activity. The most revealing conclusion to be drawn from the present data is that lipophilic, alkyl substituents on the nitrogen atom of the pyrroles produce strong antibacterial activity indicated by submicromolar MIC values against both MRSA and VRE. Among the various *N*-substituents evaluated, isoamyl and cyclopropylmethyl groups led to the most active compounds. From data reported in this study, it appears that it is the lipophilicity of the compounds, probably related to improved cellular uptake, that modulates antibacterial activity for those compounds that exhibit sufficiently strong DNA binding. As uptake would also increase for other cells than the bacterial targets, significant toxicity would be expected. Strong toxicity against human T-cells was indeed observed for some compounds, but this toxicity did not correlate with antibacterial activity or DNA binding. However, the degree of toxicity was found to be very sensitive to the alkyl substituents on the pyrrole units. The cyclopropylmethyl group dramatically reduced tox-

icity against T-cells without a concurrent drop in antibacterial activity. This observation leaves room for the speculation that activity is not a simple matter of lipophilicity and DNA binding but that a more complex mechanism is at work, which might involve a specific interaction of the pyrrole substituents in a ternary DNA−protein complex.

This study has produced a series of compounds with promising activity against antibiotic resistant strains of *Staphylococcus aureus* and *Enterococci*. It is noteworthy that the putative mode of action of these compounds involves DNA binding and has not been previously exploited for antibacterial pharmaceutical design. The responsiveness of compound design to alter toxicity against human T-cells is particularly promising.

Experimental Section

Chemistry. All chemicals used were of reagent grade. Melting points were determined on a Mel-temp apparatus (Laboratory Devices, Inc.) and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 MHz. Chemical shifts are reported in parts per million relative to the solvent residual signal. The samples were prepared in methylsulfoxide-d_6 unless otherwise specified. The peaks were assigned based on gCOSY experiments. Electrospray mass spectra were recorded on a spectrometer Mariner-EMS (PE-Biosystems). HRMS (fast atom bombardment (FAB)) were recorded on a VG-ZAB by the UCR Mass Spectrometry department. HPLC purification was carried out on a Vydac 12 μm C_{18} (2.2 cm × 25 cm) column using a solvent gradient with two solvents: 0.1% trifluoroacetic acid (TFA) in water (A) and 0.1% TFA in acetonitrile (B). Unless otherwise stated, the applied conditions for purification were 10−70% eluent B gradient over 40 min with a flow rate of 10 mL/min. The monitoring was at 254 nm.

1-Cyclopropylmethyl-4-nitro-1H-pyrrole-2-carboxylic Acid Ethyl Ester, 33. 4-Nitro-1H-pyrrole-2-carboxylic acid ethyl ester **29**, prepared as in ref 20 (5 g), was dissolved in 50 mL of anhydrous EtOH, and 50 mL of 1 M sodium ethylate was added and then followed with the addition of 10 mL of cyclopropylmethyl bromide. The reaction mixture was heated at 80 °C. Sodium methylate (25 mL) and cyclopropylmethyl bromide (5 mL) were added twice, at 5 h and at 10 h. The reaction mixture was heated for 5 more hours, cooled to room temperature, and partitioned between water and chloroform. The organic phase was washed with water, dried with sodium sulfate, and evaporated. The residue was purified on a silica gel column using toluene/ethyl acetate (9:1 v/v) as the eluant to afford 4.8 g (74%) as white crystals; mp 55−56 °C. H^1 NMR: δ 0.37−0.42 and 0.65−0.72 (m, 2H, CH_2), 1.22−1.28 (m, 1H, CH), 1.37 (t, 3H, CH_3), 4.23 (d, 2H, CH_2−N), 4.32 (q, 2H, CH_2−ethyl), 7.44 and 7.81 (d, 1H, pyrrole).

1-Cyclopropylmethyl-4-nitro-1H-pyrrole-2-carboxylic Acid, 37. Ethyl ester **33** (4.5 g, 19 mmol) was suspended in 20 mL of methanol, 2 M NaOH (10 mL) was added, and the mixture was stirred at 50 °C for 2 h. The clear solution was diluted with water (50 mL), and 1 N HCl was added dropwise to get pH 2.5. The white residue was filtered, washed with water, and dried to get 3.8 g (96%) of **37** as white crystals; mp 216 °C. H^1 NMR: δ 0.37−0.41 and 0.45−0.52 (m, 2H, CH_2), 1.26−1.32 (m, 1H, CH), 4.18 (d, 2H, CH_2−N), 7.27 and 8.28 (d, 1H, pyrrole).

1-(Cyclopropylmethyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Cyano-ethyl)amide, 41. The acid **37** was suspended in $SOCl_2$ (20 mL), and the mixture was refluxed for 4 h until a clear solution was obtained, cooled, evaporated, and dried by coevaporation with toluene (10 mL × 3). The obtained chloroanhydride, without purification, was dissolved in toluene (10 mL), and 3-aminopropionitrile (2.7 mL, 38 mmol) was added. The mixture was kept for 1 h at ambient temperature and evaporated. The white precipitate was suspended in 0.1 N HCl, filtered, washed with water, and dried to yield 3.7 g (78%); mp 124−125 °C. ES MS: 263.97 (M + H$^+$). H^1 NMR: δ 0.02−0.04 and 0.09−0.12 (m, 2H, CH_2), 0.89−1.00 (m, 1H, CH), 2.37−2.41 (t, 2H, CH_2−CN), 3.06−3.11 (dd, 2H, CH_2−NH), 3.86−3.88 (d, 2H, CH_2−N), 7.09 and 7.87 (d, 1H, pyrrole), 8.45 (t, 1H, NH).

1-(Methyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Cyano-ethyl)amide, 38. A literature procedure[20] gave **38** in 74% yield based on pyrrole **29**; mp 134−136 °C (literature[20] mp 135−136 °C).

1-(Propyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Cyano-ethyl)amide, 39. A literature procedure[20] gave **39** in 71% yield based on pyrrole **29**; mp 120−122 °C (literature[20] mp 120.5−121 °C).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Cyano-ethyl)amide, 40. The title compound was synthesized from 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid **36**[21] (4.5 g, 20 mmol) as described above for compound **41**. The yield of **40** was 3.7 g (68%); mp 112 °C. ES MS: 279.32 (M + H$^+$). H^1 NMR: δ 0.83−0.86 (m, 6H, CH_3), 1.40−1.51 (m, 1H, CH), 1.51−1.61 (m, 2H, CH_2−CH), 2.68−2.72 (m, 2H, CH_2−CN), 3.35−3.42 (m, 2H, CH_2−NH), 4.33−4.37 (m, 2H, CH_2−N), 7.38 and 8.15 (d, 1H, pyrrole), 8.56 (t, 1H, NH).

General Procedure for the Synthesis of Amidine Derivatives. A solution of (2-cyano-ethyl)amidine **38**−**41** (5 mmol) in 50 mL of dry ethanol was cooled to 0−5 °C and saturated with HCl gas. The mixture was sealed and refrigerated for 20 h. The mixture was allowed to warm to room temperature, and ethanol was evaporated. The resulting imino ester was dissolved in 50 mL of anhydrous ethanol and saturated with ammonia gas to get amidine **42**−**45**. The treatment of the imino ester with 30 mmol of alkylamine or alkyldiamine resulted in the formation of mono- (**46**−**48**) or bis-substituted (**49**, **50**) amidines. The sealed mixture was kept overnight at 0−5 °C to get amidines or monoalkylated amidines **42**−**48**. Bis-substituted derivatives **49** and **50** were formed under heat overnight at 60 °C. The solvent was evaporated. The solid was dissolved in 5 mL of methanol, and ether (35 mL) was added to precipitate the target product. This procedure was used for the preparation of the following compounds.

1-Methyl-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Carbamimidoyl-ethyl)amidine, 42. This compound was prepared from *N*-methylpyrrole derivative **38**. The corresponding imino ester was treated with ammonia gas to afford 1.1 g (92%) of amidine **42**; mp 269−274 °C (literature[20] mp 268−272 °C).

1-Propyl-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Carbamimidoyl-ethyl)amidine, 43. This compound was prepared from *N*-propylpyrrole derivative **39**. The corresponding imino ester was treated with ammonia gas in ethanol to afford 1.25 g (95%) of amidine **43**; mp 215 °C (literature[20] mp 215−216 °C).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Carbamimidoyl-ethyl)amidine, 44. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding iminoester was treated with ammonia gas in ethanol to afford 1.38 g (96%) of amidine **44**; mp 182−184 °C. ES MS: 296.38 (M + H$^+$). H^1 NMR: δ 0.88−0.86 (d, 6H, CH_3), 1.43−1.61 (m, 3H, CH and CH_2−CH), 2.61−2.65 (m, 2H, CH_2−CN), 3.49−3.55 (m, 2H, CH_2−NH), 4.33−4.38 (m, 2H, CH_2−N), 7.51 and 8.18 (d, 1H, pyrrole), 8.73 (t, 1H, NH).

1-(Cyclopropylmethyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Carbamimidoyl-ethyl)amidine, 45. This compound was prepared from *N*-(cyclopropylmethyl)pyrrole derivative **41**. The corresponding imino ester was treated with ammonia gas in ethanol to afford 1.25 g (90%) of amidine **45**; mp 242 °C. ES MS: 280.31 (M + H$^+$). H^1 NMR: δ 0.36−0.47 (m, 4H, CH_2), 1.21−1.38 (m, 1H, CH), 2.51−2.60 (t, 2H, CH_2−CN), 3.48−3.52 (m, 2H, CH_2−NH), 4.19 (d, 2H, CH_2−N), 7.46 and 8.20 (d, 1H, pyrrole), 8.56, 8.67 and 8.98 (bs, 1H, NH).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid [2-(*N*-Methylcarbamimidoyl)ethyl]amidene, 46. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding imino ester was treated with 1 M solution of aminomethane in methanol at 5 °C to afford 1.25 g (82%) of amidine **46** as colorless foam. ES MS: 310.94 (M +

H^+). H^1 NMR: δ 0.86−0.88 (d, 6H, CH_3−isopentyl), 1.43−1.61 (m, 3H, CH and CH_2−CH), 2.60−2.65 (t, 2H, CH_2−amidine), 3,37 (s, 3H, CH_3−NH), 3.49−3.55 (m, 2H, CH_2−NHCO), 4.33−4.38 (t, 2H, CH_2−N), 7.51 and 8.18 (d, 1H, pyrrole), 8.73 (t, 1H, NHCO).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid [2-(*N*-Ethylcarbamimidoyl)ethyl]amidene, 47. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding imino ester was treated with a 1 M solution of aminoethane in methanol at 5 °C to afford 1.3 g (80%) of amidine **47** as a colorless foam. ES MS: 324.79 (M + H^+). H^1 NMR: δ 0.88−0.86 (d, 6H, CH_3−isopentyl), 1.07−1.12 (t, 3H, CH_3−ethyl), 1.43−1.61 (m, 3H, CH and CH_2−CH), 2.52−2.56 (t, 2H, CH_2 CH_2−amidine), 3.12−3.21 (m, 2H, CH_2−ethyl), 3.49−3.55 (m, 2H, CH_2−NHCO), 4.33−4.38 (t, 2H, CH_2−N), 7.39 and 8.20 (d, 1H, pyrrole), 8.54−8.60 (NH−amidine), 9.38 (t, 1H, NHCO).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid [2-(*N*-Propylcarbamimidoyl)ethyl]amidene, 48. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding imino ester was treated with 1-aminopropane at 5 °C to afford 1.25 g (77%) of amidine **48** as a colorless foam. ES MS: 338.44 (M + H^+). H^1 NMR: δ 0.88−0.86 (d, 9H, CH_3−isopentyl, CH_3−propyl), 1.07−1.12 (t, 3H, CH_3−ethyl), 1.43−1.61 (m, 3H, CH_2−CH), 2.50−2.53 (m, 2H, CH_2−amidine), 3.08−3.14 (m, 2H, CH_2−propyl), 3.29−3.36 (m, 2H, CH_2−propyl), 3.43−3.50 (m, 2H, CH_2−NHCO), 4.33−4.38 (t, 2H, CH_2−N), 7.43 and 8.20 (d, 1H, pyrrole), 8.62−8.90 (m, 2H, NH−amidine), 9.39 (t, 1H, NHCO).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid [2-(*N*,*N*′-Dimethylcarbamimidoyl)ethyl]amide, 49. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding imino ester was treated with 1 M solution of methylamine in methanol at 50 °C to afford 1.25 g (77%) of amidine **49** as a colorless foam. ES MS: 324.74 (M + H^+). H^1 NMR: δ 0.85−0.87 (d, 6H, CH_3−isopentyl), 1.43−1.61 (m, 3H, CH and CH_2−CH), 2.76−2.79 (m, 5H, CH_2−amidine and CH_3−NH), 2.96−2.98 (d, 3H, CH_3−NH), 3.46−3.55 (m, 2H, CH_2−NHCO), 4.34−4.39 (t, 2H, CH_2−N), 7.51 and 8.18 (d, 1H, pyrrole), 9.76 (t, 1H, NHCO).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid [2-(1,4,5,6-Tetrahydropyrimidin-2-yl)ethyl]amide, 50. This compound was prepared from *N*-(3-methyl-butyl)pyrrole derivative **40**. The corresponding imino ester was treated with 1,3-diaminopropane at 50 °C. The crude reaction mixture was evaporated and purified by preparative HPLC to get 1.36 g (61%) of amidine **50** as TFA salt. ES MS: 364. 37 (M + H^+). H^1 NMR: δ 0.85−0.87 (d, 6H, CH_3), 1.42−1.61 (m, 3H, CH_2−CH of pyrrole), 1.79−1.84 (m, 2H, $CH_2CH_2CH_2$), 2.55−2.60 (t, 2H, CH_2−amidine), 3.20−3.30 (m, 4H, $CH_2CH_2CH_2$), 3.40−3.52 (m, 2H, CH_2−NHCO), 4.33−4.38 (t, 2H, CH_2−N), 7.48 and 8.18 (d, 1H, pyrrole), 8.15 and 9.78 (bs, 1H, NH−amidine), 8.89 (t, 1H, NHCO).

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic Acid (2-Amino-ethyl)amide, 51. Compound **32** [21] (1.3 g, 5 mmol) was dissolved in diethylamine (20 mL). This solution was kept for 50 h at 60 °C and evaporated. The chromatography on silica gel ($CHCl_3$/CH_3OH/NH_4OH 9:1:0.05 v/v/v) provided the title product **51** (830 mg, 62%) as a clear oil. ES MS: 269.33 (M + H^+).

2-({1-[1-(3-Methyl-butyl)-4-nitro-1H-pyrrol-2-yl]methanoyl}amino)ethyl]carbamic Acid *tert*-Butyl Ester, 52. Amine **51** was dissolved in DMF (20 mL), and diBoc-carbonate (2.18 g, 10 mmol) was added. The reaction was kept for 1 h at ambient temperature and evaporated. The residue was dissolved in chloroform (30 mL), washed with 0.1 M HCl (10 × 2 mL), 5% $NaHCO_3$ (10 × 2 mL), and water, dried over sodium sulfate, and evaporated. The crude compound **52** was crystallized from toluene/hexane (4:1 v/v) to afford 1.27 g (69%) of white crystals; mp 170−172 °C. H^1 NMR: δ 0.85−0.87 (d, 6H, CH_3), 1.34 (s, 9H, Boc), 1.42−1.61 (m, 3H, CH_2−CH of pyrrole), 3.03−3.08 and 3.18−3.22 (each m, 2H, $NHCH_2CH_2NH$), 4.33−4.38 (t, 2H, CH_2−N), 6.85 (t, 1H, NHBoc), 7.48 and 8.18 (d, 1H, pyrrole), 8.36 (t, 1H, NHCO).

2-Methyl-terephthalic Acid. A stirred solution of 1,4-dibromo-2-methyl-benzene (1.0 g, 4.0 mmol) in anhydrous tetrahydrofuran (40.0 mL) and tetramethylethylenediamine (4.8 mL, 31.8 mmol) was cooled to −70 °C (CO_2/acetone bath) under an argon atmosphere. *tert*-Butyllithium (18.8 mL, 32.0 mmol) as 1.7 M pentane solution was added, and the reaction mixture was stirred for 2.5 h. The yellow reaction solution was transferred to a 1000 mL round-bottom flask containing excess solid CO_2. After the CO_2 sublimated, the reaction was quenched with 5% NH_4Cl (50 mL) and acidified with 1 M HCl to reach pH 3. The resulting mixture was extracted with ethyl acetate, dried with Na_2SO_4, and evaporated. The mixture of mono- and dicarboxylic acids was crystallized from MeOH to give the 2-methyl-terephthalic acid (240 mg, 33% yield). MS: 179.01 (M − H). ^{1}H NMR: δ 13.2 (bs, 2H, COOH), 7.85 (m, 3H, C_6H_4), 2.58 (s, 3H, CH_3).

2,5-Dimethyl-terephthalic Acid. The synthesis was performed using 1,4-dibromo-2,5-dimethyl-benzene as described above for 2-methyl-terephthalic acid. The reaction mixture was extracted with ethyl acetate (20 mL × 4). The ethyl acetate solution was washed with 1 M NaOH (3 × 20 mL). The combined aqueous fractions were acidified to pH 3 with 1 M HCl. The white precipitate was filtered and crystallized from aqueous methanol to afford 444 mg (40%) of 2,5-dimethyl-terephthalic acid. MS: 193.03 (M − H). ^{1}H NMR: δ 13.09 (bs, 2H, COOH), 7.69 (s, 2H, C_6H_4), 2.48 (m, 6H, C_6H_4).

General Method for the Preparation of Activated Dicarboxylic Acids. Dicarboxylic acid (2 mmol) was suspended in 4 mL of dry DMF, and diisopropylethylamine (4.8 equiv) was added followed with pantafluorophenyltrifluoracetate (4.8 mmol). The reaction was kept at room temperature for 1 h and evaporated. The flash chromatography on silica gel (toluene/ethyl acetate 9:1 v/v) provided the corresponding dipentafluorophenyl ester as white crystals or a clear oil. All of the compounds were homogeneous on thin-layer chromatography (toluene/ethyl acetate 7:3 v/v) and had similar ^{19}F NMR spectra. For example, the dipentafluorophenul ester of 1,4-naphthaline dicarboxylic acid ^{19}F NMR: −45 692.62 (t), −44 406.44 (t), −42 983.05 (d).

General Method for the Synthesis of Linked Pyrrole. To a stirred solution of nitropyrrole derivatives **42**−**50** (0.15 mmol) in methanol (20 mL) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated, flushed three times with hydrogen, and finally filled with hydrogen at 25−30 psi. The resultant suspension was stirred vigorously at ambient temperature for 30 min. The suspended material was filtered; the filtrate was evaporated to dryness. The resulting aminopyrrole derivatives **42a**−**50a** were used for the next step without purification. The solution of freshly prepared aminopyrrole derivatives **42a**−**50a** in 2 mL of dry DMF was added to the dipentafluorophenyl ester of dicarboxylic acid (0.07 mmol). The reaction mixture was stirred for 15 h at 55 °C, cooled, and purified by HPLC (Vydac 12 μm C_{18} 2.2 cm × 25 cm column, 10−70% acetonitrile gradient over 40 min, flow rate 10 mL/min) to give corresponding linked dipyrrole as a bis-trifluoroacetate salt. This salt was dissolved in 2 mL of methanol saturated with HCl, 35 mL of diethyl ether was added, and the precipitate of linked dipyrrole as bis-HCl salt was separated and dried. The average yield was 45−55%.

***N*,*N*′-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]terephthalamide, 1.** The reaction of reduced nitropyrrole **42** with the dipentafluorophenyl ester of terephthalic acid gave **1**. H^1 NMR: δ 2.63 (t, 4H, CH_2−amidine), 3.43−3.54 (m, 4H, CH_2NH), 3.83 (s, 6H, CH_3), 6.99 and 7.31 (d, 2H, pyrrole), 8.05 (s, 4H, C_6H_4), 8.03 (t, 1.5H, CONH), 8.69 and 9.02 (s, 3H, amidine), 10.52 (s, 1.3 H, CONH). HRMS (FAB): *m*/*z* 549.2709 [(M + H)$^+$ calcd for $C_{26}H_{33}N_{10}O_4$, 549.2686].

***N*,*N*′-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]terephthalamide, 2.** The reaction of reduced nitropyrrole **43** with the dipentafluorophenyl ester of terephthalic acid gave **2**. H^1 NMR: δ 0.79 (t, 6H, CH_3), 1.62−1.69 (m, 4H, CH_2CH_3), 2.61−2.65 (t, 4H, CH_2−amidine), 3.47−3.51 (m, 4H, CH_2−NH), 4.20−4.25 (t, 4H, CH_2N), 6.99 and 7.35

(s, 2H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.28 (t, 2H, NHCO), 8.69 and 9.01 (s, 6H, amidine). HRMS (FAB): *m/z* 605.3298 [(M + H)$^+$ calcd for $C_{30}H_{41}N_{10}O_4$, 605.3312].

***N,N′*-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]terephthalamide, 3.** The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of terephthalic acid gave **3**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.42−1.55 (m, 6H, CH_2CH of pyrrole), 2.61−2.65 (t, 4H, CH_2−amidine), 3.48−3.51 (m, 4H, CH_2NH), 4.30−4.32 (t, 4H, CH_2N), 6.97 and 7.36 (d, 2H, pyrrole), 8.05 (c, 4H, C_6H_4), 8.29 (t, 1.5H, CONH), 8.70 and 9.02 (s, 3H, amidine), 10.52 (s, 1.3H, CONH−pyrrole). HRMS (FAB): *m/z* 661.3920 [(M + H)$^+$ calcd for $C_{34}H_{49}N_{10}O_4$, 661.3938].

Cyclohexa-1,3-diene-1,4-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]amide}, 4. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of cyclohexa-1,3-diene dicarboxylic acid gave **4**. H^1 NMR: δ 0.86 (d, 12H, CH_3), 1.40−1.53 (m, 6H, CH_2CH of pyrrole), 2.50 (s, 4H, CH_2 of cyclohexadiene), 2.61−2.65 (t, 4H, CH_2−amidine), 3.48−3.51 (m, 4H, CH_2NH), 4.23−4.28 (t, 4H, CH_2N), 6.87 and 7.26 (d, 2H, pyrrole), 6.90 (s, 2H, cyclohexadiene), 8.20−8.22 (m, 1.5H, CONH), 8.64 and 9.02 (s, 3H, amidine), 9.94 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 663.4105 [(M + H)$^+$ calcd for $C_{34}H_{51}N_{10}O_4$, 663.4105].

Cyclohexane-1,4-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]amide}, 5. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of cyclohexane-1,4-dicarboxylic acid gave **5**. H^1 NMR: δ 0.86 (d, 12H, CH_3), 1.40−1.60 (m, 10H, CH_2−CH of pyrrole and CH_2 of cyclohexane), 1.80−2.00 (s, 4H, CH_2 of cyclohexane), 2.52−2.67 (t, 4H, CH_2−amidine), 3.48−3.51 (m, 4H, CH_2NH), 4.23−4.28 (t, 4H, CH_2N), 6.69−79 and 7.12−7.20 (m, 2H, pyrrole), 8.16−8.22 (m, 1.5H, CONH), 8.62 and 8.98 (s, 3H, amidine), 9.66 and 9.79 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 667.4429 [(M + H)$^+$ calcd for $C_{34}H_{55}N_{10}O_4$, 667.4408].

Hexadioic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]amide}, 6. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of adipic acid gave **6.** H^1 NMR: δ 0.86 (d, 12H, CH_3), 1.40−1.54 (m, 10H, CH_2CH of pyrrole and $2CH_2$ of adipic acid), 2.19−2.23 (m, 4H, CH_2 of adipic acid), 2.61−2.65 (t, 4H, CH_2−amidine), 3.48−3.51 (m, 4H, *CH*$_2$NH), 4.19−4.24 (t, 4H, CH_2N), 6.70 and 7.14 (d, 2H, pyrrole), 8.18−8.21 (m, 1.5H, CONH), 8.62 and 8.98 (s, 3H, amidine), 9.82 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 605.3298 [(M + H)$^+$ calcd for $C_{30}H_{41}N_{10}O_4$, 605.3312].

Pyridine-2,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}, 7. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of pyridine-2.5-dicarboxylic acid gave **7**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.40−1.51 (m, 6H, CH_2-CH of pyrrole), 2.61−2.65 (t, 4H, CH_2−amidine), 3.48−3.51 (m, 4H, CH_2NH), 4.28−4.35 (t, 4H, CH_2N), 6.98, 7.12, 7.39, and 7.41 (d, 1H, pyrrole), 8.19 (d, 1H, pyridyl−H4), 8.20−8.45 (m, 1.5H, CONH), 8.51 (dd, 1H, pyridyl−H3), 8.64 and 9.02 (d, 3H, amidine), 9.16 (d, 1H, pyridyl−H6), 10.29 and 10.31 (s, 0.5 H, CONH−pyrrole). HRMS (FAB): *m/z* 662.3901 [(M + H)$^+$ calcd for $C_{33}H_{48}N_{10}O_4$, 662.3891].

Pyridine-2,4-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}, 8. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of pyridine-2,5-dicarboxylic acid gave **8**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.48−1.55 (m, 6H, CH_2-CH of pyrrole), 2.61−2.66 (t, 4H, CH_2−amidine), 3.48−3.52 (m, 4H, CH_2NH), 4.28−4.35 (t, 4H, CH_2N), 7.00, 7.11, 7.39, and 7.42 (d, 1H, pyrrole), 8.10−8.12 (d, 1H, pyridyl), 8.25−8.40 (m, 1.5H, CONH), 8.55 (s, 1H, pyridyl), 8.84−6.86 (d, 1H, pyridyl), 8.71 and 8.04 (s, 3H, amidine), 10.75 and 10.93 (s, 0.5 H, CONH−pyrrole). HRMS (FAB): *m/z* 662.3870 [(M + H)$^+$ calcd for $C_{33}H_{48}N_{11}O_4$, 662.3891].

Pyrazine-2,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}, 9. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of pyrazine-2,5-dicarboxylic acid gave **9**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.42−1.55 (m, 6H, CH_2-CH of pyrrole), 2.37−2.43 (t, 4H, CH_2−amidine), 3.17−3.25 (m, 4H, CH_2NH), 4.27−4.32 (t, 4H, CH_2N), 7.05 and 7.42 (d, 2H, pyrrole), 8.21−8.25 (t, 1.5H, CONH), 8.63 and 9.04 (s, 3H, amidine), 9.26 (s, 2H, pyrazine), 11.00 (s, 1.3H, CONH−pyrrole). HRMS (FAB): *m/z* 663.3821 [(M + H)$^+$ calcd for $C_{33}H_{48}N_{11}O_4$, 663.3843].

***N*^{1},*N*^{4}-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-2-nitro-terephthalamide, 10.** The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of nitroterephthalic acid gave **10**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.48−1.59 (m, 6H, CH_2CH of pyrrole), 2.61−2.66 (m, 4H, CH_2−amidine), 3.48−3.52 (m, 4H, CH_2NH), 4.28−4.35 (t, 4H, CH_2−N), 6.88, 6.99, 7.24, and 7.37 (d, 1H, pyrrole), 7.83−7.86 (d, 1H, C_6H_4), 8.25−8.32 (m, 1.5H, CONH), 8.32−8.40 (d, 1H, C_6H_4), 8.60−8.71 (m, 5H, amidine and C_6H_4), 9.04 (s, 3H, amidine), 10.77 and 10.81 (s, 0.5H, CONH−pyrrole). HRMS (FAB): *m/z* 706.3777 [(M + H)$^+$ calcd for $C_{34}H_{48}N_{11}O_4$, 706.3789].

Naphthalene-1,4-dicarbocylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]amide}, 11. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of naphthalene-1,4-dicarboxylic acid gave **11**. H^1 NMR: δ 0.90 (d, 12H, CH_3), 1.50−1.58 (m, 6H, CH_2CH of pyrrole), 2.59−2.64 (t, 4H, CH_2−amidine), 3.43−3.56 (m, 4H, CH_2NH), 4.29−4.33 (t, 4H, CH_2N), 6.92 and 7.38 (d, 2H, pyrrole), 7.60−7.63 (q, 2H, naphthalene), 7.69 (s, 2H, naphthalene), 8.18−8.21 (q, 2H, naphthalene), 8.23−8.26 (m, 1.5H, CONH), 8.60 and 8.97 (s, 3H, amidine), 10.60 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 711.4067 [(M + H)$^+$ calcd for $C_{38}H_{51}N_{10}O_4$, 711.4095].

Naphthalene-2,6-dicarbocylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]amide}, 12. The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of naphthalene-2,6-dicarboxylic acid gave **12**. H^1 NMR: δ 0.90 (d, 12H, CH_3), 1.50−1.58 (m, 6H, CH_2CH of pyrrole), 2.59−2.64 (t, 4H, CH_2−amidine), 3.43−3.56 (m, 4H, CH_2NH), 4.29−4.33 (t, 4H, CH_2N), 7.00 and 7.38 (d, 2H, pyrrole), 8.06−8.15 (m, 4H, naphthalene), 8.26−8.32 (m, 1.5H, CONH), 8.59 (s, 2H, naphthalene), 8.66 and 9.00 (s, 3H, amidine), 10.61 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 711.4084 [(M + H)$^+$ calcd for $C_{38}H_{51}N_{10}O_4$, 711.4095].

***N*^{1},*N*^{4}-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-2-methyl-terephthalamide, 13.** The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of 2-methylterephthalic acid gave **13**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.48−1.55 (m, 6H, CH_2CH of pyrrole), 2.40 (s, 3H, methyl), 2.60−2.66 (t, 4H, CH_2 amidine), 3.48−3.52 (m, 4H, CH_2NH), 4.24−4.32 (t, 4H, CH_2N), 6.86, 6.95, 7.27, and 7.33 (d, 1H, pyrrole), 7.48 (d, 1H, terephthalyl), 7.80−7.84 (m, 2H, terephthalyl), 8.20−8.38 (m, 1.5H, CONH), 8.64 and 8.99 (s, 3H, amidine), 10.34 and 10.40 (s, 0.5 H, CONH−pyrrole). HRMS (FAB): *m/z* 675.4113 [(M + H)$^+$ calcd for $C_{35}H_{51}N_{10}O_4$, 675.4095].

***N*^{1},*N*^{4}-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-2,5-dimethyl-terephthalamide, 14.** The reaction of reduced nitropyrrole **44** with the dipentafluorophenyl ester of 2,5-dimethylterephthalic acid gave **14**. H^1 NMR: δ 0.87 (d, 12H, CH_3), 1.41−160 (m, 6H, CH_2CH of pyrrole), 2.40 (s, 3H, CH_3−C_6H_4), 2.53−2.60 (m, 4H, CH_2−amidine), 3.43−3.56 (m, 4H, CH_2NH), 4.22−4.32 (t, 4H, CH_2N), 6.86, 6.95, 7.27 and 7.33 (d, 1H, pyrrole), 7.47−7.49 (d, 1H, C_6H_3), 7.80−7.84 (m, 2H, C_6H_3), 8.20−8.30 (m, 1.5H, CONH), 8.64 and 8.99 (s, 3H, amidine), 10.34 and 10.40 (s, 0.5H, CONH−pyrrole). HRMS (FAB): *m/z* 689.4241 [(M + H)$^+$ calcd for $C_{36}H_{53}N_{10}O_4$, 689.4251].

***N,N′*-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]terephthalamide, 15.** The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of terephthalic acid gave **15**. H^1 NMR: δ 0.28−0.47 (m, 8H, CH_2−cyclopropyl), 1.13−1.28 (m, 2H, CH−cyclopropyl), 2.61−2.65 (t, 4H, CH_2−amidine), 3.49−3.53 (m,

4H, CH_2NH), 4.13−4.16 (d, 4H, CH_2−N), 6.99 and 7.40 (d, 1H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.28−8.32 (t, 2H, CONH), 8.67 and 9.00 (s, 3H, amidine), 10.50 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 629.3296 [(M + H)$^+$ calcd for $C_{32}H_{41}N_{10}O_4$, 629.3312].

***N,N′*-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]-2-nitroterephthalamide, 16.** The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of 2-nitroterephthalic acid gave **16**. H^1 NMR: δ 0.19−0.22 and 0.31−0.39 (d, 4H, cyclopropyl), 1.00−1.20 (m, 2H, CH), 2.45−2.60 (m, 4H, CH_2−amidine), 3.43−3.56 (m, 4H, CH_2NH), 4.18 (d, 2H, CH_2N), 6.58, 6.69, 6.97 and 7.10 (d, 1H, pyrrole), 7.55 (d, 1H, C_6H_3), 8.11−8.28 (m, 2.2H, CONH and C_6H_3), 8.30−8.35 (m, 4H, amidine and C_6H_3), 8.69 (s, 3H, amidine), 10.45 and 10.47 (s, 1H, CONH−pyrrole). HRMS (FAB): *m/z* 674.3183 [(M + H)$^+$ calcd for $C_{32}H_{40}N_{11}O_4$, 674.3163].

Pyridine-2,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]amide}, 17. The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of pyridine-2,5-dicarboxylic acid gave **17**. H^1 NMR: δ 0.20−0.35 (m, 8H, CH_2−cyclopropyl), 1.13−1.28 (m, 2H, CH−cyclopropyl), 2.58−2.65 (m, 4H, CH_2−amidine), 3.49−3.53 (m, 4H, CH_2NH), 4.13−4.16 (d, 4H, CH_2N), 6.90, 7.02, 7.33 and 7.37 (d, 1H, pyrrole), 8.05 (d, 1H, C_6H_3), 8.18−8.32 (m, 2H, CONH), 8.41 (d, 1H, C_6H_3), 8.59 and 8.92 (s, 3H, amidine), 9.07 (s, 1H, C_6H_3), 10.50 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 630.3261 [(M + H)$^+$ calcd for $C_{31}H_{40}N_{11}O_4$, 630.3265].

Pyrazine-2,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]amide}, 18. The reaction of reduced nitropyrrole **46** with the dipentafluorophenyl ester of pyrazine-2,5-dicarboxylic acid gave **18**. H^1 NMR: δ 0.29−0.46 (m, 8H, CH_2−cyclopropyl), 1.16−1.28 (m, 2H, CH−cyclopropyl), 2.61−2.65 (t, 4H, CH_2−amidine), 3.50−3.54 (m, 4H, CH_2NH), 4.13−4.16 (d, 4H, CH_2N), 7.09 and 7.50 (d, 1H, pyrrole), 8.28−8.32 (t, 2H, CONH), 8.64 and 8.99 (s, 3H, amidine), 9.27 (s, 2H, pyrazine), 11.02 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 631.3211 [(M + H)$^+$ calcd for $C_{30}H_{39}N_{12}O_4$, 631.3217].

***N,N′*-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropyl-methyl-1H-pyrrol-3-yl]-2-methylterephthalamide, 19.** The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of 2-nitroterephthalic acid gave **19**. H^1 NMR: δ 0.29−0.32 and 0.41−0.49 (m, 4H, cyclopropyl), 1.10−1.30 (m, 2H, CH), 2.42 (s, 3H, CH_3), 2.52−2.68 (m, 4H, CH_2−amidine), 3.45−3.56 (m, 4H, CH_2NH), 4.13−4.15 (d, 2H, CH_2N), 6.89, 6.99, 7.32 and 7.40 (d, 1H, pyrrole), 7.48−7.50 (d, 1H, C_6H_3), 7.81−7.85 (m, 2H, C_6H_3), 8.27−8.28 (t, 1.5H, CONH), 8.63 and 8.97 (d, 3H, amidine), 10.34 and 10.40 (s, 1H, CONH−pyrrole). HRMS (FAB): *m/z* 643.3499 [(M + H)$^+$ calcd for $C_{33}H_{43}N_{10}O_4$, 643.3469].

Thiophene-2,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]amide}, 20. The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of thiophene-2,5-dicarboxylic acid gave **20**. H^1 NMR: δ 0.28−0.47 (m, 8H, CH_2−cyclopropyl), 1.13−1.28 (m, 2H, CH−cyclopropyl), 2.61−2.65 (t, 4H, CH_2−amidine), 3.49−3.53 (m, 4H, CH_2NH), 4.13−4.16 (d, 4H, CH_2−N), 6.96 and 7.33 (d, 1H, pyrrole), 7.96 (s, 2H, thiophene), 8.28−8.32 (t, 2H, CONH), 8.64 and 8.99 (s, 3H, amidine), 10.50 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 635.2869 [(M + H)$^+$ calcd for $C_{30}H_{39}N_{10}O_4$, 635.2876].

1H-Pyrazole-3,5-dicarboxylic Acid Bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(cyclopropyl-methyl)-1H-pyrrol-3-yl]amide}, 21. The reaction of reduced nitropyrrole **45** with the dipentafluorophenyl ester of pyrazole-3,5-dicarboxylic acid gave **21**. H^1 NMR: δ 0.19−0.22 and 0.31−0.39 (m, 4H, cyclopropyl), 1.00−1.20 (m, 2H, CH), 2.45−2.60 (m, 4H, CH_2−amidine), 3.43−3.56 (m, 4H, CH_2NH), 4.18 (d, 2H, CH_2N), 6.62, 6.69, 7.05 and 7.15 (d, 1H, pyrrole), 7.06 (s, 1H, pyrazole), 0.8.21−8.38 (t, 1.2H, CONH), 8.54 and 8.89 (s, 3H, amidine), 10.13 and 10.45 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 619.3200 [(M + H)$^+$ calcd for $C_{29}H_{39}N_{12}O_4$, 619.3217].

***N,N′*-Bis-{1-(3-methyl-butyl)-5-[2-(*N*-methylcarbamimidoyl)ethylcarbamoyl]- 1H-pyrrol-3-yl}terephthalamide, 22.** The reaction of reduced nitropyrrole **46** with the dipentafluorophenyl ester of terephthalic acid gave **22**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.44−1.58 (m, 6H, CH_2CH of pyrrole), 2.59−2.63 (t, 4H, CH_2−amidine), 2.78 (d, 6H, methyl), 3.48−3.51 (m, 4H, CH_2NH), 4.26−4.30 (t, 4H, CH_2−N), 6.96 and 7.35 (d, 2H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.27 (t, 1.5H, CONH), 8.58 and 9.15 (s, 2H, amidine), 9.57 (d, 2H, amidine), 10.51 (s, 1.3H, CONH−pyrrole). HRMS (FAB): *m/z* 689.4253 [(M + H)$^+$ calcd for $C_{36}H_{53}N_{10}O_4$, 689.4251].

***N,N′*-Bis-{1-(3-methyl-butyl)-5-[2-(*N*-ethylcarbamimidoyl)ethylcarbamoyl]- 1H-pyrrol-3-yl}terephthalamide, 23.** The reaction of reduced nitropyrrole **47** with the dipentafluorophenyl ester of terephthalic acid gave **23**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.05−1.11 (m, 6H, CH_2CH_3), 1.44−1.58 (m, 6H, CH_2CH of pyrrole), 2.59−2.63 (t, 4H, CH_2−amidine), 2.78 (d, 6H, methyl), 3.13−3.20 (m, 4H, CH_2CH_3), 3.48−3.51 (m, 4H, CH_2NH), 4.26−4.30 (t, 4H, CH_2N), 7.55 and 8.13 (d, 2H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.70−8.80 (t, 1.5H, CONH), 8.67, 9.23 and 9.64 (bs, 1H, amidine), 10.51 (s, 1.3H, CONH−pyrrole). HRMS (FAB): *m/z* 717.4548 [(M + H)$^+$ calcd for $C_{38}H_{57}N_{10}O_4$, 717.4564].

***N,N′*-Bis-{1-(3-methyl-butyl)-5-[2-(*N*-propylcarbamimidoyl)ethylcarbamoyl]- 1H-pyrrol-3-yl}terephthalamide, 24.** The reaction of reduced nitropyrrole **48** with the dipentafluorophenyl ester of terephthalic acid gave **24**. H^1 NMR: δ 0.84−0.95 (m, 18H, CH_3), 1.42−1.60 (m, 10H, CH_2CH of pyrrole and CH_2 of propyl), 2.61−2.65 (t, 4H, CH_2−amidine), 3.09−3.19 (m, 4H, CH_2 of propyl), 3.48−3.51 (m, 4H, CH_2NH), 4.30−4.32 (t, 4H, CH_2N), 6.97 and 7.35 (d, 2H, pyrrole), 8.05 (c, 4H, C_6H_4), 8.24 (t, 1.5H, CONH), 8.62 and 9.11 (s, 3H, amidine), 9.50 (m, 2H, amidine), 10.51 (s, 1.3H, CONH−pyrrole). HRMS (FAB): *m/z* 745.4884 [(M + H)$^+$ calcd for $C_{40}H_{61}N_{10}O_4$, 745.4877].

***N,N′*-Bis-{5-[2-(*N,N′*-dimethylcarbamimidoyl)ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl}terephthalamide, 25.** The reaction of reduced nitropyrrole **49** with the dipentafluorophenyl ester of terephthalic acid gave **25**. H^1 NMR: δ 0.87 (d, 12H, CH_3), 1.41−1.52 (m, 6H, CH_2CH of pyrrole), 2.61−2.65 (t, 4H, CH_2 CH_2−amidine), 2.77 and 2.99 (d, 3H, CH_3−amidine), 3.48−3.51 (m, 4H, CH_2NH), 4.27−4.31 (t, 4H, CH_2N), 6.96 and 7.35 (d, 2H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.33−8.38 (t, 1.2H, CONH), 8.74 and 9.52 (d, 1.5H, amidine), 10.52 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 717.4587 [(M + H)$^+$ calcd for $C_{38}H_{57}N_{10}O_4$, 717.4564].

***N,N′*-Bis-{1-(3-methyl-butyl)-5-[2-(1,4,5,6-tetrahydropyrimidin-2-yl)ethylcarbamoyl]-1H-pyrrol-3-yl}-terephthalamide, 26.** The reaction of reduced nitropyrrole **50** with the dipentafluorophenyl ester of terephthalic acid gave **26**. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.45−1.55 (m, 6H, CH_2CH of pyrrole), 1.78−1.89 (m, 4H, tetrahydopyrimidinyl), 2.61−2.65 (t, 4H, CH_2 -amidine), 3.20−3.40 (m, tetrahydopyrimidinyl and water), 3.48−3.51 (m, 4H, CH_2NH), 4.27−4.31 (t, 4H, CH_2N), 6.99 and 7.36 (d, 2H, pyrrole), 8.04 (s, 4H, C_6H_4), 8.33−8.38 (t, 1.3H, CONH), 9.67 (s, 3H, amidine), 10.52 (s, 1H, CONH−pyrrole). HRMS (FAB): *m/z* 741.4569 [(M + H)$^+$ calcd for $C_{40}H_{57}N_{10}O_4$, 741.4564].

***N,N′*-Bis-[5-(2-aminoethylcarbamoyl)-1-(3-methylbutyl)-1H-pyrrol-3-yl]terephthalamide, 27.** To a stirred solution of nitropyrrole derivative **52** (0.15 mmol) in methanol (20 mL) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated, flushed three times with hydrogen, and finally filled with hydrogen at 25−30 psi. The resultant suspension was stirred vigorously at ambient temperature for 30 min. The suspended material was filtered; the filtrate was evaporated to dryness. The resultant aminopyrrole derivative **52a** was used for the next step without purification. The solution of freshly prepared aminopyrrole derivative **52a** in 2 mL of dry DMF was added to the dipentafluorophenyl ester of dicarboxylic acid (35 mg, 0.07 mmol). The reaction mixture was stirred for 15 h at 55 °C, cooled, and evaporated. The dark solid was dissolved in 5 mL of methanol, 2 mL of 4 N HCl in dioxane was added, and the reaction was kept for 40 min at

ambient temperature. The reaction mixture was evaporated and purified by HPLC (Vydac 12 μm C_{18} 2.2 cm × 25 cm column, 10−70% acetonitrile gradient over 40 min, flow rate 10 mL/min) to give the corresponding linked dipyrrole **27**. This salt was dissolved in 2 mL of methanol saturated with HCl, 35 mL of diethyl ether was added, and the precipitate of linked dipyrrole **27** as bis-HCl salt was separated and dried to yield 25 mg (52%) based on the dipentafluorophenyl ester of terephthalic acid. H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.50−1.61 (m, 6H, CH_2CH of pyrrole), 2.84−2.95 (m, 4H, CH_2-NH_2), 3.39−3.43 (m, 4H, CH_2NH), 4.27−4.32 (t, 4H, CH_2-N), 6.99 and 7.36 (d, 2H, pyrrole), 7.90−8.10 (bs, 4H, NH_2), 8.04 (s, 4H, C_6H_4), 8.21−8.25 (t, 1.2H, CONH), 10.51 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 607.3719 [$(M + H)^+$ calcd for $C_{32}H_{47}N_8O_4$, 607.3720].

***N,N′*-Bis-[5-(2-guanidino-ethylcarbamoyl)- 1-(3-methyl-butyl)-1H-pyrrol-3-yl]terephthalamide, 28.** The TFA salt of amine **27** (42 mg, 0.05 mmol) was dissolved in DMF (3 mL), pyrazole-1-carboxamidine (55 mg, 0.5 mmol) was added, and the reaction was stirred at ambient temperature overnight. The title product **28** was isolated by HPLC and transformed to hydrochloride as described above to afford 25 mg (65%). H^1 NMR: δ 0.88 (d, 12H, CH_3), 1.48−1.58 (m, 6H, CH_2CH of pyrrole), 3.20−3.40 (m, 8H, $2CH_2$), 4.27−4.32 (t, 4H, CH_2N), 6.96 and 7.35 (d, 2H, pyrrole), 7.58−7.65 and 8.15−8.23 (t, 2H, CONH and guanidine), 8.04 (s, 4H, C_6H_4), 10.51 (s, 2H, CONH−pyrrole). HRMS (FAB): *m/z* 691.4136 [$(M + H)^+$ calcd for $C_{34}H_{51}N_{12}O_4$, 691.4156].

Docking and Model Building. Molecular models of candidate molecules were generated using the ab initio, flexible docking procedure of the ICM 2.8 molecular modeling program by Molsoft.[31,33] Structural coordinates for the "receptor" were derived from the cocrystal structure of distamycin[34] bound to the minor groove of the DNA duplex $(GCCAAATTTCGC)_2$ (PDB entry 2dnd) from which the ligand was removed. A grid potential map for the receptor was created for the inner eight base pairs involving electrostatic, van der Waals, and hydrogen-bonding energy terms at a grid size of 0.5 Å. For the ligands, 2D to 3D conversion is followed by energy minimization using the MMFF option as a force field. Starting from random ligand positions, the first step of docking involves a restrained energy minimization, which brings the ligand into the center of the receptor, which, here, was set at the center of the minor groove in the middle of the AT tract. The second step is comprised of 100 independent Monte Carlo searches of 10 000 steps each in torsion angle internal coordinate space to find the best ligand conformation to interact with the receptor. The top 20 conformations are saved and rescored with hard van der Waals potentials. When this procedure is used to dock distamycin with full torsional flexibility into its native site, deviation from the crystal structure coordinates is only 0.7 Å. Most of that is related to a 0.5 Å 5′ to 3′ shift of the ligand in the groove and a flipped formyl group. The van der Waals component of the intermolecular energy was used as an indicator for the quality of the shape fit of a new ligand. Typically, good DNA binders (indicated by $\Delta T_m > 20$ °C) showed van der Waals contributions to interaction energy better than that of distamycin.

Experimental Log $P_{o/w}$. The apparent octanol/water partition coefficients at pH 7.5 (log $P_{o/w}$) were determined experimentally for selected compounds using the shake-flask method and subsequent quantitation of UV-detected reverse phase HPLC traces.[35] Typically, 50−80 μg were subjected to equilibration between 0.3 mL of potassium phosphate buffer (1 mM, pH 7.5) and 0.3 mL of buffer-saturated octanol at 30 °C. To avoid saturation artifacts, compounds were assayed over a 50−100-fold concentration range through dilution of the initial samples. The precision of our experimental log $P_{o/w}$ values is about 0.5 units.

DNA Binding Methods. Ligand−DNA interactions were monitored in a buffer containing 10 mM HEPES, pH 7.2, 0.1 mM EDTA, and 50 mM NaCl. DNA thermal melting was monitored by UV absorbance at 260 nm on a Cary 100 Bio UV/vis spectrophotometer. A 12 base pair AT-rich DNA oligonucleotide (Oligo1: CGATTATTAAGC) was used at 5 μM and mixed with compounds at various ratios. The temperature was typically varied from 15 to 95 °C with a ramp rate of 0.2 °C/min. To determine the melting temperature (T_m) where half of the double-stranded DNA molecules dissociate into two separated strands,[26,27] the first-order derivatives of the absorption−temperature curve were calculated using the Varian software, and the maximum of derivatives corresponds to the melting temperature. The melting temperatures determined by the derivative method were verified using a standard hyperchromicity method provided by the Varian software. The ΔT_m value was reported as the difference between melting temperatures in the presence and in the absence of compounds. All experiments were at least in duplicate.

Estimation of Drug−DNA Binding Constants. An ethidium bromide (EtBr) displacement assay was used to determine the dissociation constant for binding of compounds to oligo1.[28,29] Briefly, increasing amounts of compounds were titrated to preformed fluorescent EtBr−DNA complexes. The dissociation of EtBr−DNA due to compound competition gives rise to decreases in the fluorescent signal. The competition was allowed to equilibrate for at least 2 h. The fluorescence was monitored at an excitation wavelength of 530 nm and emission wavelength of 620 nm using a 96 well plate fluorimeter (CytoFluor Series 4000, PerSeptives Biosystems). Values of K_D were determined using a standard competition analysis.

Antibacterial MIC Assays. The MIC assays were performed using the microtiter method according to NCCLS protocol M7-A4.[36] Briefly, test compounds were freshly dissolved in 100% dimethyl sulfoxide to a stock concentration of 10 mM and diluted in the BHI for VRE or Mueller-Hinton media for MRSA. Seven 1:2 serial dilutions of compound in appropriate media were prepared such that the compound test concentrations ranged from 45.5 to 0.7 μM. MIC values were called after visual inspection of the wells after 2 days.

T-Cell Based WST Toxicity Tests. Compound toxicity was tested using the CCRF-CEM Human Leukemia cell line (ATCC Manassas, Virginia) and cell proliferation reagent WST-1 (Roche Molecular Biochemicals, Mannheim, Germany).[30] Growth medium was RPMI-1640 (ATCC Manassas, Virginia) supplemented with 10% of fetal bovine sera (Gibco BRL, Grand Island, NY) and Gentamicin 0.1 mg/mL (Gibco BRL, Grand Island, NY). A total of 4×10^4 cells/well was applied to 96 well plates and grown over several days. Concentrations of compound added to cells at the beginning of the experiment were 50, 25, 12.5, and 6.3 μM. After the cells were incubated with WST-1 reagent, absorbance at 440 nm was measured with a Spectra Max 340 PC microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) 24, 48, and 72 h after adding the compound. The toxicity value reported in the present study is the lowest fraction (%) of WST-1 cleavage activity left in treated cells as compared to the culture with no compound (% NCC; % of no compound control), which typically reflects the value taken 72 h after treatment with 50 μM compound. Experiments were done at least in triplicate.

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