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Design, Synthesis, and Structure−Activity Relationships of Benzophenone-Based Tetraamides as Novel Antibacterial Agents

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The increase in the incidence of both hospital- and community-acquired antibiotic-resistant infections is a major concern to the healthcare community. There have been only two new classes of antibiotics approved by the FDA over the past 40 years, and clearly there is a growing need for additional antimicrobial agents. In this paper, we present our work on the discovery of a class of benzophenone containing compounds that possess good activity against MRSA, VISA, VRSA, and VRE and moderate activity against *E. coli*. These compounds display MIC values in the 0.5−2.0 mg/L range and are not cytotoxic against mammalian cells. Extensive structure−activity relationship studies revealed that the benzophenone was absolutely essential for antibacterial activity as was the presence of a cationic group. Although these agents display DNA binding activity, we observed that these compounds do not inhibit any macromolecular synthesis reliant upon DNA nor do they inhibit lipid or cell wall biosynthesis. Instead, we found that these agents cause membrane depolarization, indicating that the bacterial membrane was the primary site of action for these agents. Our studies suggest that caution should be taken in assigning the mechanism of action for DNA binding antibiotics.

Introduction

The rapid emergence of bacteria resistant to treatment with standard antibiotics constitutes a serious public threat.[1−6] Recent studies have shown an increase in antibiotic resistance of Gram-positive bacterial strains, and resistance has been detected to antibiotics ranging from the β-lactams, macrolides, and quinolones to the glycopeptides and oxazolidinones.[7] While the incidence of antibiotic-resistant infections continues to increase, the pharmaceutical industry has decreased its research on new antibacterials.[5,8] Indeed, in past 40 years, only two antibiotics (linezolid and daptomycin) with novel mechanisms of action have made it to the market.[9] Clearly, there is an increasing need for novel antibacterial agents that are less prone to bacterial resistance and directed toward novel biological targets.

Beginning in 2002, a series of reports were published on a new set of molecules that bound to DNA and display potent antimicrobial activity.[10−19] These DNA binding antibiotics (DBA[a]) were based upon the natural products distamycin A and netropsin and consisted of a series of *N*-methylpyrrole amino acids connected via an amide bond. All of the active agents contained at least one cationic group usually at the C-terminus, which interacted with the negatively charged phosphate groups in DNA. In some cases, additional groups at the N-terminus of the molecule were frequently observed to enhance binding affinity.[11−13,19]

Given the fact that these agents possessed DNA binding, most researchers postulated that the antimicrobial activity of these agents was due to disruption of critical biochemical processes involving DNA (i.e., replication, transcription, gene regulation). Surprisingly, however, few studies have been directed at validating this mechanism of action.[20,21] In this paper, we describe the results of our studies on a unique set of benzophenone containing tetraamides with DNA binding affinity and potent antibacterial activity. These compounds are active against antibiotic-resistant Gram-positive microbes with an MIC value of ~0.5 mg/L, have moderate activity against Gram-negative microbes, and are not cytotoxic to mammalian cells. Mechanistic studies on these compounds reveal that they do not inhibit bacterial growth by binding to DNA. Instead, these agents result in depolarization of the bacteria leading to cell death. Our results indicate that care should be taken in assigning the mechanism of action for DBA.

Results

Discovery of a New Antibacterial Agent. Previous work in our laboratory focused on the role that the three-dimensional structure of DNA played on gene expression.[22] One portion of these studies focused on the development of minor groove DNA binding agents with the potential to bend DNA. The design hypothesis of these agents consisted of

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[a] Abbreviations: DBA, DNA binding antibiotic; MIC, minimal inhibitory concentration; VRSA, vancomycin sensitive *Staphylococcus aureus*; VISA, vancomycin intermediate *Staphylococcus aureus*; GISA, glycopeptide intermediate *Staphylococcus aureus*; VRE, vancomycin resistant *Enterococci*; MRSA, vancomycin resistant *Staphylococcus aureus*; MOA, mechanism of action; MHB, Mueller−Hinton broth; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; diSC3(5), 3,3′-dipropylthiadicarbocyanine iodide.

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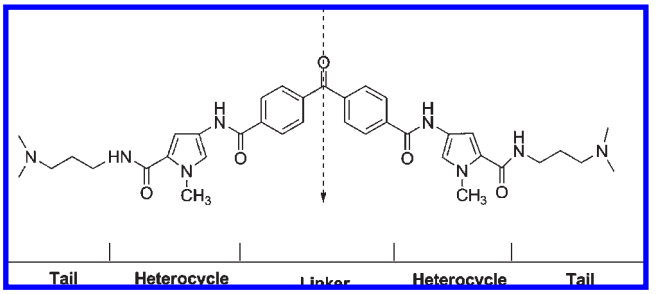


Figure 1. Conceptual breakdown of potent compound **1**. The arrow indicates the axis of symmetry for the molecule.

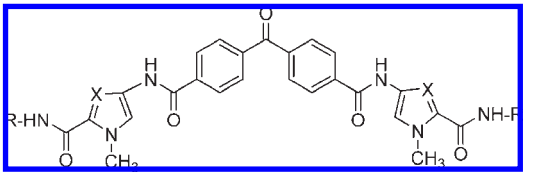
connecting the sequence recognition properties of *N*-methylpyrroles to sterically bulky, hydrophobic groups that could force open the minor groove of DNA, resulting in a bend. As part of this project, approximately a dozen compounds were prepared, and on the basis of previous reports of antimicrobial activity of DNA binding agents, we examined these compounds for antibacterial activity against both Gram-positive and Gram-negative bacteria. Most of the agents displayed no antibacterial activity up to a concentration of 32 mg/L. However, one compound, **1**, gave MIC values in the 8−16 mg/L range against VRSA, MRSA, and GISA. No activity was observed against Gram-negative bacteria. This result is similar to published examples of DBA.[10−19] Although **1** did display antibacterial activity, the MIC values were too high to be useful. Thus, we decided to prepare a series of analogues of the lead agent in an effort to identify more potent molecules and to elucidate some of the key structural features of this molecule that were necessary for activity.

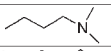
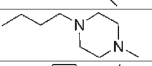
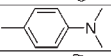
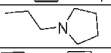
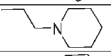
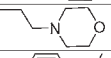
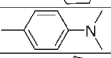
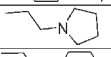
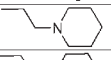
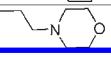
Design of Compounds. The lead agent, **1**, can conceptually be divided into distinct regions (Figure 1). The first, called the tail region, is the portion of the molecule that contain cationic groups. At the time, we reasoned that the positive charge of the tail interacted with the negative charge on the phosphate backbone of DNA. We also were interested in the hydrophobic character of this region, since the tail region also interacts with the hydrophobic portion of the sugar residue located in the minor groove of DNA. Previous studies have shown that enhancing the hydrophobicity of the cationic tail could increase the binding affinity of the molecule to DNA and aid in drug transport.[14,16,18] In addition, the work of others has shown tremendous differences in antibiotic activity depending upon the chemical nature of the tail substituents.[10−14,19] On the basis of previous investigations, we chose to change the tail region to a methylpiperazine, dimethylaniline, morpholine, piperidine, or pyrrolidine (Table 1).

The second region is the heterocycle region. Previous researchers have shown that the nature of the heterocycle can change the antibacterial activity of the molecule.[11,13,16,23] We decided to investigate inclusion of *N*-methylimidazole, since previous researchers have shown that the inclusion of imidazole into DNA binding agents would result in improved antibacterial activity (Table 1). We also examined the role that the heterocycle played in activity by removing this portion of the molecule.

The final region is the linker region, which is unique to our molecule. To investigate this region, we reduce the ketone and replaced the linker with an aliphatic linker of approximately the same length as the benzophenone linker (Table 2).

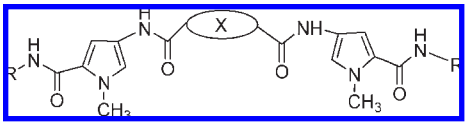
Table 1. DNA Binding Affinity and in Vitro Antimicrobial Activity of **1**−**10**

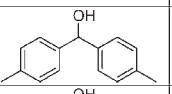
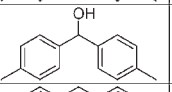
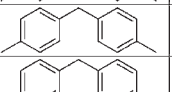
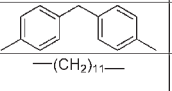
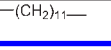


Sl. No	R	X	Ka AT[a] 10^6 M^{-1}	VRSA (mg/L)	MRSA (mg/L)
1		CH	1.0	16	8
2		CH	.108	>32	>32
3		CH	.391	>32	>32
4		CH	.679	2	1
5		CH	.931	1	.5
6		CH	ND	8	4
7		N	ND	>32	>32
8		N	ND	32	32
9		N	ND	8	4
10		N	ND	>32	>32

[a] Association constant for binding to AT hairpin (5′-CGAAAAA-CAAAAAGTTTTTCG-3′) as determined by the ethidium bromide displacement assay.

Table 2. DNA Binding Affinity and in Vitro Antimicrobial Activity for **11**−**16**



Sl. No	X	R	Ka AT[a] 10^6 M^{-1}	VRSA (mg/L)	MRSA (mg/L)
11			.175	>32	>32
12			.0098	>32	>32
13			.154	>32	>32
14			.0238	>32	>32
15	—$(CH_2)_{11}$—		.021	>32	>32
16	—$(CH_2)_{11}$—		.0125	>32	>32

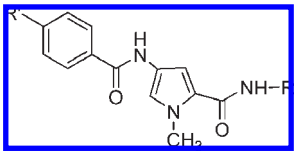
[a] Association constant for binding to AT hairpin (5′-CGAAAAA-CAAAAAGTTTTTCG-3′) as determined by the ethidium bromide displacement assay.

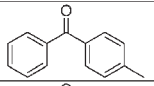
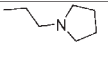
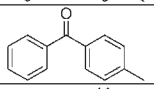
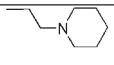
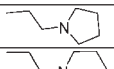
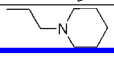
Finally, **1** is symmetrical about the ketone (Figure 1). We wondered whether this symmetry was necessary for activity, and therefore, we prepared a series of molecules that essentially mimicked one-half of the molecule. These compounds should allow us to determine whether both parts of the molecule are needed for antibacterial activity (Table 3).

Chemistry. We prepared 21 new compounds clustered into three categories based upon the regions discussed above.

Synthesis of these molecules was done essentially according to literature protocols and as outlined in Schemes 1−5. The key heterocycle building blocks, *N*-methylpyrrole (**22a**) and *N*-methylimidazole (**22b**), were synthesized according to literature methods.[24,25] The synthesis of compounds **1**−**10** were done according to Scheme 1. Reaction of **22a**,**b** with commercially available amines **23a**−**f** resulted in the desired amides (**24a**−**j**) via the haloform reaction.[26] The nitro group was reduced by hydrogenation, and the resulting amine was used without further purification.[26] Coupling was accomplished by activating the appropriate diacid followed by coupling with amine to generate the desired final products. *N*-Methylaminopyrroles were coupled to the diacids using predominantly two methods. The first was the pentafluoroacetate methodology as described by Dyatkina et al.[18] The second method was the use of a carbodiimide coupling method (EDC, DMAP).[27] Coupling of *N*-methylaminoimidazoles was achieved using HBTU coupling conditions as described in Scheme 1.[16] Finally, the ketone group of benzophenone was reduced to hydroxyl group by using sodium borohydride and Pd/C chemistry (Scheme 2).[28]

Table 3. DNA Binding Affinity and in Vitro Antimicrobial Activity of Compounds **17**−**20**



Sl. No	R'	R	Ka AT[a] 10^6 M^{-1}	VRSA (mg/L)	MRSA (mg/L)
17			.106	>32	>32
18			.227	>32	>32
19	H		.110	>32	>32
20	H		.173	>32	>32

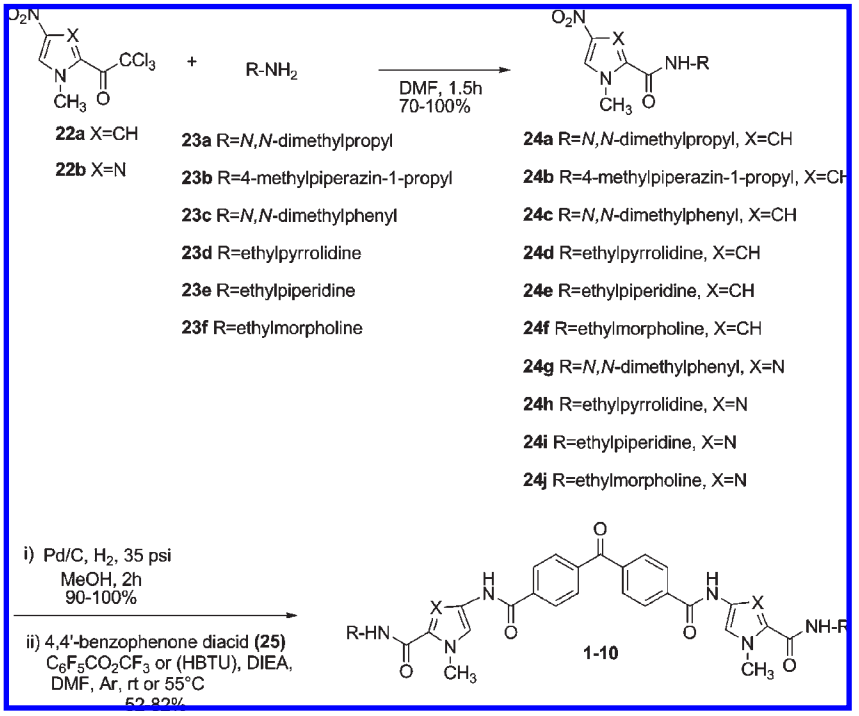
[a] Association constant for binding to AT hairpin (5′-CGAAAAA-CAAAAAGTTTTTCG-3′) as determined by the ethidium bromide displacement assay.

Structure−Activity Relationships. We first prepared a series of analogues in which the cationic "tail" region of the molecule was altered. The substitutions that we chose were based upon work conducted by previous researchers which showed that the nature of the cationic tail had a profound influence on antibacterial activity.[10−13] Two compounds lost all antibacterial activity, while two showed dramatic improvements in activity. Compounds **4** and **5** possessed MIC values in the 0.5−2 mg/L range against VRSA, MRSA, and GISA and gained activity against VRE (4 mg/L) and *E. coli* (16 mg/L). This activity is comparable to vancomycin and linezolid against MRSA but better than these antibiotics against VRSA and GISA. Daptomycin is roughly 2−4 times as potent as our agents.

Replacement of *N*-methylpyrrole with *N*-methylimidazole did result in active compound, but the antibacterial activity was roughly 8-fold lower than for the pyrrole containing agents (**7**−**10**, Table 1). This suggested that the heterocycle portion of the molecule could tolerate modification. Next, we removed the heterocycle portion and found that activity is completely lost (**21**), suggesting that heterocycle portion is required for the activity.

We also examined the role that the linker played in antibacterial activity. Reduction of the ketone to an alcohol resulted in complete loss of activity (**11** and **12**, Table 2). Examination of one of the alcohols revealed a substantial loss in DNA binding activity (compare **12** to **5**). Complete reduction to the methylene (**13** and **14**) also resulted in loss of activity (Table 2). Replacement of the benzophenone with a long, flexible linker of approximately the same length as the

Scheme 1. Synthesis of Benzophenone-Based Compounds Containing *N*-Methylpyrrole and *N*-Methylimidazole Units



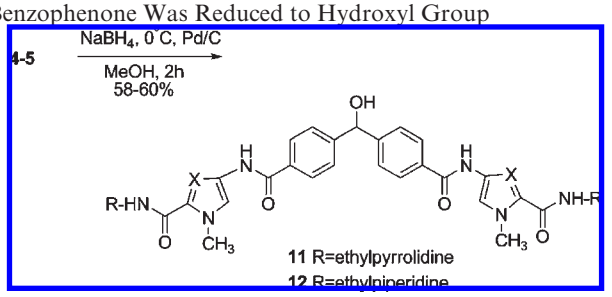
benzophenone (**15** and **16**) resulted in loss of antibacterial and DNA binding activity (Scheme 3, Table 2). Thus, we concluded that the benzophenone linker is important for both the DNA binding and the antibacterial activity of the compounds.

Compound **1** can be thought of as symmetrical molecules with an axis of symmetry bisecting the ketone group of the benzophenone linker. We wondered if both halves of the molecule were necessary for activity. To address this issue, we prepared several compounds (**17**−**20**, Scheme 4) that consisted of only one-half of the lead agent **1**. These agents bound to DNA with affinities comparable to that of the lead agent; however, none of the compounds displayed activity against MRSA and VRSA (Table 3). Thus, we concluded that both halves of the molecule were necessary for antibacterial activity but not required for DNA binding affinity.

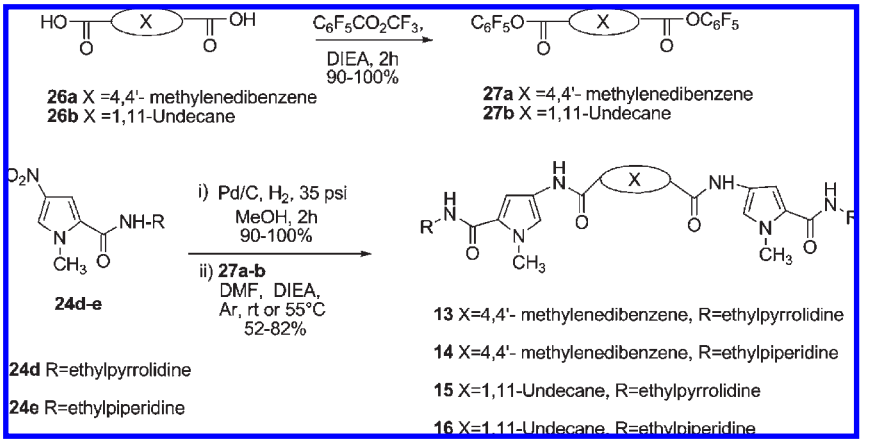
Cytotoxicity Studies. Compounds **4** and **5** display good activity against a variety of Gram-positive, antibiotic-resistant bacteria. These agents also displayed moderate activity against some Gram-negative microbes including *E. coli*. We were interested in determining whether these agents were cytotoxic against mammalian cells. To do this, we incubated **4** and **5** against CHO cells at concentrations ranging from 25 to 100 × MIC levels (using MRSA as the standard MIC value). After incubation for 24 h, we measured cell viability using the MTS assay (Figure 2). Incubation of compounds **4** and **5** with mammalian cells results in no change in cell viability for either compound. These studies indicate that the antibacterial activity of the compounds is not due to general cytotoxic but rather due to selective killing of bacterial cells.

Studies of DNA Displacement in Bacteria. An examination of the data presented in Tables 1−3 reveals that there is no correlation between DNA binding affinity and antibacterial activity. Previous researchers studying DBA have also observed this lack of correlation and have posited that this was due to differences in transport of these molecules into the bacterium.[12,16,27] To examine whether this was the case with our agents, we examined the ability of our compounds to bind to DNA in vivo using a Hoescht dye competition assay. Hoescht dye was incubated with bacteria, resulting in fluorescent bacteria. Individual agents were titrated into a solution containing the fluorescent bacteria, and the change in fluorescence due to Hoechst displacement was measured as a decrease in total fluorescence. As shown in Figure 3, all agents resulted in approximately the same degree of competition, with the Hoechst dye indicating that these agents are transported into the bacteria. Importantly, all compounds, including those lacking antibacterial activity, resulted in a fluorescence decrease. Furthermore, approximately the same degree of competition was observed for both Gram-positive and Gram-negative bacteria even though our agents are approximately 16 times as potent against Gram-positive

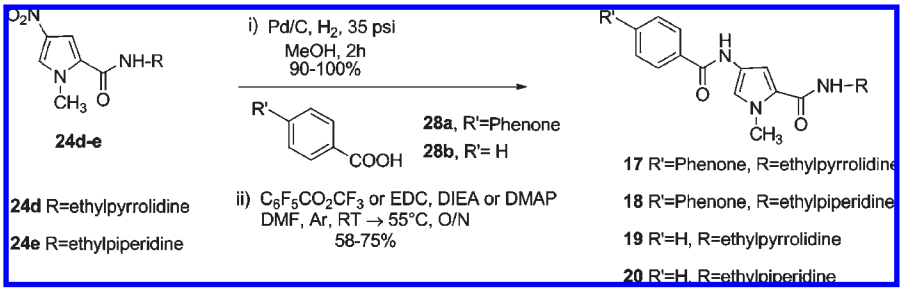
Scheme 2. Synthesis of Compounds in Which Ketone Group of Benzophenone Was Reduced to Hydroxyl Group



Scheme 3. Synthesis of Compounds with Different Linkers



Scheme 4. Synthesis of Compounds Mimicking Only One-Half of Compound **5**



microbes. Taken together, these results gave us misgivings that DNA was the target responsible for antibacterial activity.

Compound 5 Does Not Inhibit DNA, RNA, Protein, Lipid, or Cell Wall Biosynthesis. Of the compounds reported here, **5** displayed the most potent antibacterial activity, and thus, we chose this as our lead agent. To investigate the mechanism of action (MOA) of **5**, we examined the effect of **5** on macromolecule synthesis using radiolabel incorporation assays.[29] *Staphylococcus aureus* (ATCC 29213) was grown to early log phase and treated, in triplicate, with various concentrations of **5** ((0.5−8) × MIC) or a DMSO (10%) control. The cells were incubated for 5 min before radiolabeled precursors were added ([^{3}H]thymidine (DNA synthesis), [^{3}H]uridine (RNA synthesis), [^{3}H]leucine (protein synthesis), [^{3}H]*N*-acetylglucosamine (cell wall), [^{3}H]glycerol (lipid synthesis). After 30 or 60 min (for lipid synthesis), cells were treated with TCA and the amount of radiolabel in the acid precipitate was determined by a scintillation counter.

Treatment of *Staphylococcus* with **5** up to a concentration of 8 × MIC resulted in no dose-dependent inhibition of any of the macromolecular syntheses examined (Figure 4). In contrast, all positive controls gave the appropriate results. This indicates that **5** exerts its antibacterial effects on some pathway other than those commonly employed by antibiotics. This result is important because it establishes that our compounds have a MOA distinct from that of commonly used antibiotics.

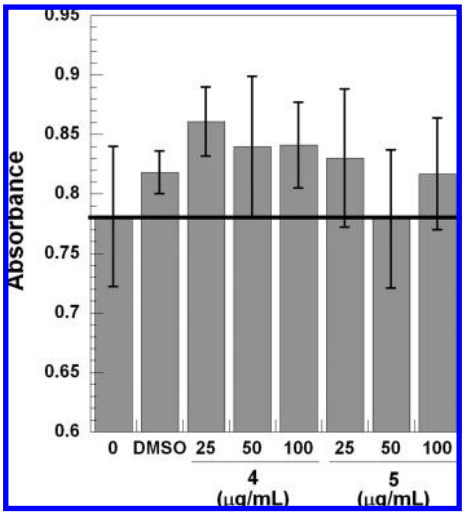
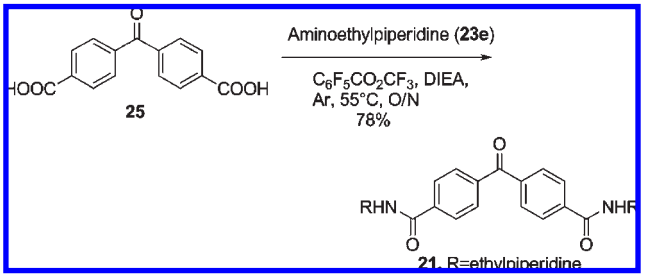


Figure 2. MTS assay of compounds **4** and **5** at three different concentrations (25, 50, and 100 mg/L) in CHO cell line. Untreated cells (with 5% DMSO) were used as control. Error bars are the standard deviation of each triplicate experiment.

Compounds 4 and 5 Depolarize Staphylococcal Cells. Since our studies indicated that **5** does not affect metabolic pathways commonly targeted by antibiotics, we began to examine other potential targets. One potential target is the bacterial membrane itself.[30] The bacterial membrane is the target for a number of cationic peptides, and previous researchers have produced antibacterial peptide mimetics.[31,32] Frequently, these compounds are composed of amphipathic molecules containing a cationic residue that interacts with negatively charged membrane components.[31,32] Although our compounds have no structural relationship to existing antibacterial peptide mimetics, we hypothesized that the cationic nature of **5** might enable it to bind to the membrane. This hypothesis was supported by the fact that removal of the cationic tail from **5** destroyed antibacterial activity.

Many compounds that target bacterial membranes induce pore formation resulting in the depolarization of the cell.[31,33,34] Thus, we examined whether **4** and **5** depolarized bacterial membranes.[35] To conduct these studies, *Staphylococcus aureus* was grown in MHB supplemented with $MgSO_4$ to early log phase, the cells collected by centrifugation and resuspended in 5 mM HEPES buffer (pH 7.2) containing 5 mM glucose. The cells were diluted to an OD_{600} of 0.05, and

Scheme 5. Synthesis of Null Molecule with Respect to the Heterocycle Unit



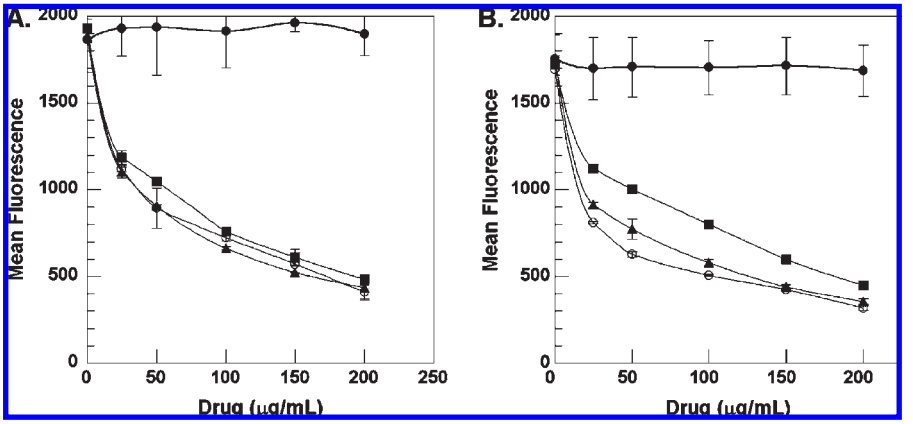


Figure 3. In vivo competition of Hoescht dye with active and inactive antibacterial compounds. Compounds **4** (○) and **9** (■) are active, while **2** (▲) is inactive. All compounds were tested at five different concentrations (25, 50, 100, 150, and 200 mg/L). The background fluorescence of cells not treated with the agents is also shown (●). Error bars are the standard deviation of two independent experiments: (A) competition of compounds with Hoescht dye in Gram-positive bacteria (*S. aureus*); (B) competition of compounds with Hoescht dye in Gram-negative bacteria (*E. coli*).

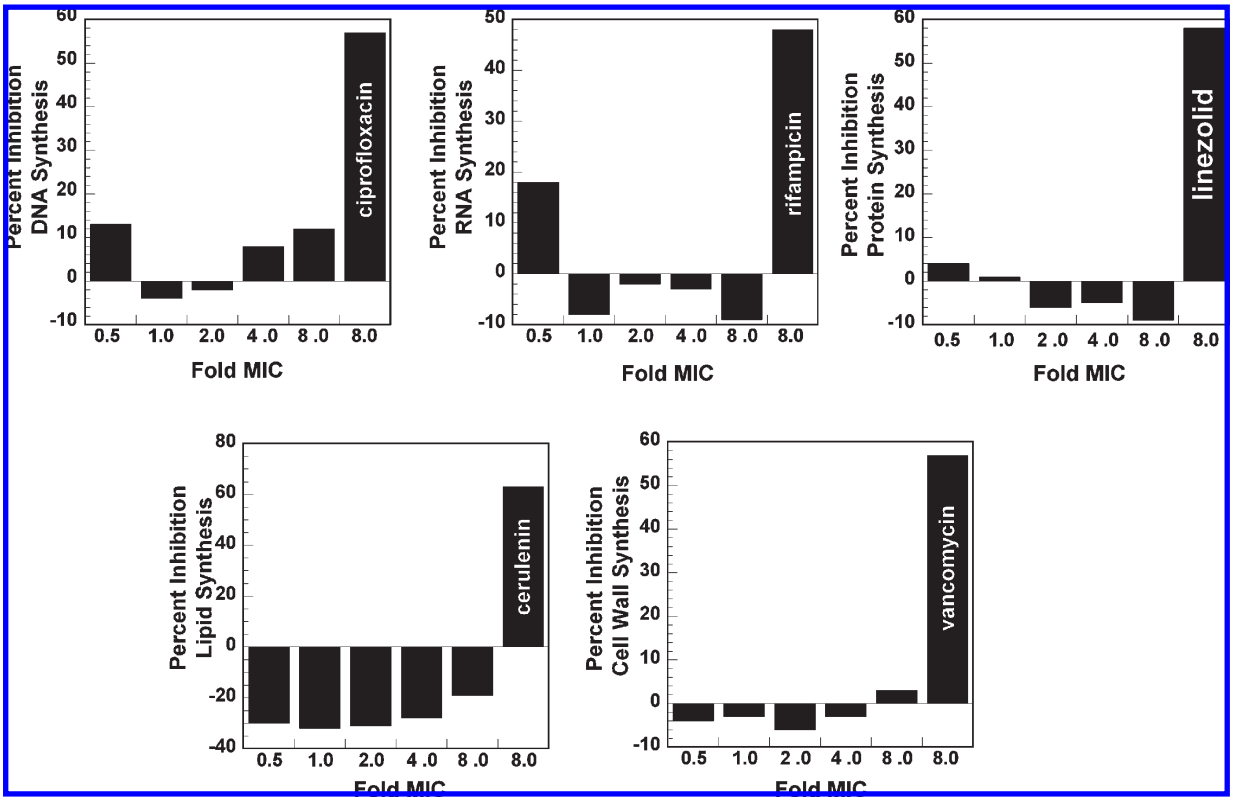


Figure 4. Effect of compound **5** on inhibition of macromolecular synthesis in *S. aureus* cells. Compound **5** was tested at five different concentrations ((0.5−8.0) × MIC), while the respective control inhibitor was used at 8.0 × MIC.

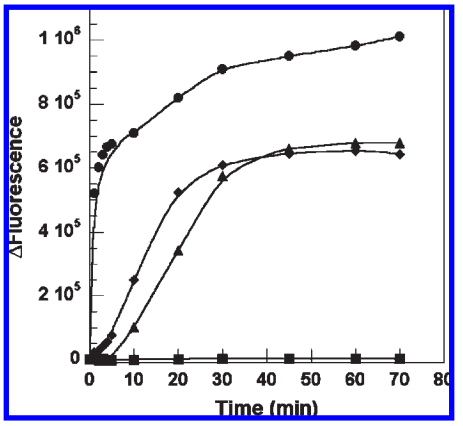


Figure 5. Depolarization studies in *S. aureus,* as measured by the membrane potential-sensitive dye diSC3(5). Compounds **4** (▲, 8.0 mg/mL), **5** (◆, 4.0 mg/L), **15** (■, 32 mg/L), and nisin (●, 25 mg/L) were used. Cells without any drug were used as a negative control. Changes in membrane potential were calculated as a percentage of the nisin control which was set to 100%.

400 nM membrane potential-sensitive dye diSC3(5) was added. The cells were placed into a 37 °C fluorescence cuvette, stirred, and equilibrated for 5 min before addition of 100 μM KCl to equilibrate the intra- and extracellular potassium levels. The compounds to be tested were added (8 × MIC), and the change in fluorescence was monitored over time. As seen in Figure 5, **4** and **5** resulted in depolarization whereas a structurally related compound (**15**) did not. These compounds induced about 50% of the depolarization observed for nisin, which was used as a positive control (Figure 5). In addition, the time to reach a fixed level of depolarization was slower for our agents and there appeared to be a correlation between antibacterial activity and the kinetics of depolarization. We are conducting further studies to examine the depolarization caused by our agents.

Discussion

In this paper, we have presented our work on the discovery of a class of benzophenone-containing DNA binding agents that display potent antibacterial activity against both Gram-positive and Gram-negative bacteria. We have not been the first group to identify potent antibacterial DNA binding antibiotics. Suckling and colleagues as well as several companies have published extensively on the identification of DNA binding agents that display potent antimicrobial activity.[10−19] These groups have elucidated a number of the key structural features necessary for activity, and in a few cases, detailed pharmacokinetic studies of the compounds have also been reported. One area that has not been adequately addressed is the mechanism of action of the compounds. Most researchers have postulated that these compounds bind to DNA and prevent DNA replication, transcription, or gene expression.[20,21] However, a number of groups, including ours, have noted that there is no correlation between DNA binding affinity and antibacterial activity.[12,16,27] While some researchers have proposed that transport differences account for this lack of correlation, we have shown in this report (via the in vivo DNA displacement assay) that there are no qualitative differences in transport between antibacterial versus inactive agents. This suggests that the interaction with DNA is not responsible for the antibacterial activity of these agents, a result validated by studies showing that our agents do not disrupt critical biochemical pathways dependent upon DNA. Instead, we found that our agents result in membrane depolarization, suggesting that the membrane was the primary target for these

compounds. This indicates that simply being able to bind DNA does not clearly provide the mechanism for antibacterial activity. Our results suggest caution is warranted regarding the mechanism of action of antimicrobial agents that bind DNA.

Why are our agents selective for bacterial versus mammalian membranes? Bacterial membranes contain a number of unique characteristics that make bacteria more disposed to the action of membrane targeting drugs.[32] First, and most importantly, the lipid composition of prokaryotes is different from that of eukaryotes.[32,36,37] Prokaryotic membranes contain a large amount of negatively charged phospholipids, whereas eukaryotic membranes contain zwitterionic phospholipids.[34] Furthermore, prokaryotic membranes are frequently decorated with polyanionic groups such as lipoteichoic acids and lipopolysaccharides, further increasing the negative charge of the membrane.[38] Thus, cationic compounds are selectively targeted to bacterial membranes because they form favorable electrostatic interactions with the negatively charged membrane (see ref 32 for a discussion). In addition, bacteria possess a large transmembrane potential and this has been shown to help insertion and pore formation of membrane binding compounds.[39] Indeed, it has been suggested that the electronegativity of the membrane could actually attract cationic molecules to the membrane.[40] This literature evidence indicates that a positive charge is a requirement for selective association to bacterial versus mammalian membranes.

The evidence presented within this paper indicates that a cationic charge is required for activity by our benzophenone-containing antibiotics but is not the sole determinant of antibacterial efficacy. For example, compound **3**, which is inactive, is not charged at physiological pH and yet **2**, which is charged, is also inactive. Other factors must be involved. One of these is likely hydrophobicity, since our data indicate that these agents interact with the membrane. Like charge, the activity of our agents is not simply correlated to the hydrophobicity of the compound (as measured by cLogP). For example, compounds **4** and **9** have similar cLogP values (2.69 vs 2.58) and yet display a 4-fold difference in MIC values. We consider it probable that the relationship between the antibacterial activity of these agents and their physicochemical properties will likely be due to a combination of charge, hydrophobicity, and as yet unknown factors.

The mechanism of action of the compounds outlined in this report, as well as the difficulty in understanding the structure−function-activity relationships of these agents, is similar to that proposed for cationic antimicrobial peptides.[32] These peptides, which are commonly found in nature, have been shown to depolarize bacterial membranes by pore formation or by destabilizing or disintegrating the membrane.[33,34,41] In the case of our agents, we currently do not have direct evidence indicating whether our agents result in pore formation. However, we have observed that treatment of bacteria with our agents does not lead to the release of intracellular ATP, indicating that the compounds do not dissolve the bacterial membrane (S. Firestine, unpublished data). Thus, at the present time, we propose that the benzophenone-containing antibiotics described in this report are coordinated into a pore complex within the membrane and the resulting pore leads to membrane depolarization.

Finally, we note that it was an unexpected finding to observe that DNA binding agents could have direct effects on the bacterial membrane. However, an examination of pharmacophoric features for both DNA binding agents and membrane active antibiotics indicates that they share similar features. Both classes of compounds require a cationic region for interacting with negatively charged phosphate groups present in either the backbone of DNA or the lipid bilayer. Hydrophobic groups are frequently seen in DNA binding agents to maximize contacts with deoxyribose, and these groups are obviously necessary for interaction with the lipid tail. Finally, both classes utilize hydrogen bonding. In the case of DNA binding agents, hydrogen bonding is used to recognize specific base pairs within DNA, whereas in membrane targeted antibiotics, hydrogen bonds are frequently used to form specific secondary structures or to aid in supramolecular assembly of pore complexes. Other researchers have also noticed the similarity between agents that bind DNA and agents that interact with negatively charged membrane components.[42]

Table 4. HPLC Conditions Used To Verify Purity of Compounds **1**−**21**[a]

compd	isocratic method		purity, %
	solvent A, %	solvent B, %	
7, **16**−**18**	70	30	>95
1, **4**, **5**, **6**, **9**, **12**− **15**, **19**, **20**	75	25	>95
8, **10**	78	22	>95
2, **11**, **21**	80	20	>95

[a] Solvent A: 0.1% TFA in water. Solvent B: 0.1% TFA in acetonitrile.

Conclusion

We have described the synthesis, structure−activity relationships, and microbiological and mechanistic studies of a novel class of antibacterial compounds that have potent activity against both Gram-positive and Gram-negative bacteria. These compounds display MIC values in the range of 0.5−1.0 mg/L and are active against a wide range of antibiotic-resistant bacterial including MRSA, VRSA and VRE. The compounds do not display toxicity against mammalian cells. Mechanistic studies revealed that although these agents have modest DNA binding affinity, their antibacterial activity is not related to any biochemical process that involves this macromolecule. Instead, these agents appear to selectively interact with the bacterial membrane, resulting in membrane depolarization. Our findings suggest that others should be cautious in assigning a mechanism of action for agents that interact with DNA. We are actively working on understanding the nature of the membrane interaction and hope to report on these findings in due course.

Experimental Section

Chemistry. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Varian DRX400 at 400 and 100 MHz, respectively. Mass measurements were carried out at the central instrumentation facility at Wayne State University, Detroit, MI, using a electrospray mass spectrometry (Waters-Micromass ZQ qudrupole). The radiolabeled incorporation assays were conducted by Micromyx (Kalamazoo, MI). Purity of all final compounds was determined by HPLC using a PRP-1, 100 Å column from Hamilton (4.1 mm × 150 mm, 10 μm) with an isocratic gradient of 0.1% TFA in water and 0.1% TFA in acetonitrile (see Table 4 for details and percent purities). All compounds were more than 95% pure.

General Method for Coupling Amines with 22a,b[26]. To a solution of **22a** (1.0 mmol) in DMF under argon, 1.2 mmol of

the amine (**23a**−**f**) was added dropwise, and the reaction mixture was stirred at room temperature for 2 h to obtain the desired products (**24a**−**f**). Compound **22b** was also reacted in similar way with amines (**23c**−**f**) to obtain products **24g**−**j**.

***N*-(3-(Dimethylamino)propyl)-1-methyl-4-nitro-1*H*-pyrrole-2-carboxamide (24a).** This compound was prepared from **22a** and **23a** in 95% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1H, NH), δ 7.51 (d, J = 2.0 Hz, 1H, Ar−H), 6.96 (d, J = 1.5 Hz, 1H, Ar−H), 3.99 (s, 3H, CH_3), 3.48 (q, J = 5.2 Hz, 2H, CH_2), 2.55 (t, J = 6.4 Hz, 2H, CH_2), 2.35 (s, 6H, 2 × CH_3), 1.74−1.68 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.7, 135.1, 131.1, 128.1, 117.9, 58.7, 47.0, 36.3, 26.4.

3-(4-Methylpiperazin-1-yl)propan-1-amine)-4-nitro-1*H*-pyrrole-2-carboxamide (24b). This compound was prepared from **22a** and **23b** in quantitative yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.4). ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, J = 2.0 Hz, 1H, Ar−H), 7.26 (d, J = 2.0 Hz, 1H, Ar−H), 3.93 (s, 3H, CH_3), 3.39−3.25 (m, 4H, 2 × CH_2), 2.8−2.41 (m, 6H, 3 × CH_2), 1.80−1.69 (m, 2H, CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 160.7, 135.1, 131.1, 128.1, 117.9, 57.6, 53.7, 46.6, 36.3.

***N*-(4-(Dimethylamino)phenyl)-1-methyl-4-nitro-1*H*-pyrrole-2-carboxamide (24c).** This compound was prepared from **22a** and **23c** in 70% yield. TLC (1:1 ethyl acetate/hexane, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, J = 1.6 Hz, 1H, Ar−H), 7.50 (s, 1H, NH), 7.38 (d, J = 9.2 Hz, 2H, Ar−H), 7.17 (s, 1H, NH), 6.72 (d, J = 8.8 Hz, 2H, Ar−H), 4.01 (s, 3H, CH_3), 2.93 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.64, 148.61, 127.07, 126.93, 126.62, 122.47, 113.07, 107.11, 40.96, 38.125.

1-Methyl-4-nitro-*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (24d). This compound was prepared from **22a** and **23d** in 95% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, J = 1.6 Hz, 1H, Ar−H), 7.15 (d, J = 2.0 Hz, 1H, Ar−H), 3.80 (s, 3H, CH_3), 3.30 (t, J = 6.4 Hz, 2H, CH_2), 2.52 (t, J = 6.0 Hz, 2H, CH_2), 2.52−2.41 (m, 4H, 2 × CH_2), 1.71−1.65 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.58, 126.81, 126.7, 107.14, 54.51, 54.06, 38.20, 38.05, 23.71.

1-Methyl-4-nitro-*N*-(2-(piperidin-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (24e). This compound was prepared from **22a** and **23e** in 90% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.6). ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, J = 2.0 Hz, 1H, Ar−H), 7.29 (d, J = 2.0 Hz, 1H, Ar−H), 3.94 (s, 3H, CH_3), 3.46 (t, J = 7.2 Hz, 2H, CH_2), 2.59−2.45 (m, 6H, 3 × CH_2), 1.68−1.56 (m, 4H, 2 × CH_2), 1.58−1.46 (m, 2H, CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 160.78, 127.20, 107.44, 57.56, 54.34, 36.73, 36.01, 25.34, 23.92.

1-Methyl-4-nitro-*N*-(2-(morpholino-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (24f). This compound was prepared from **22a** and **23f** in 90% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (d, J = 2.0 Hz, 1H, Ar−H), 7.04 (d, J = 2.0 Hz, 1H, Ar−H), 6.54 (s, NH, 2H), 3.98 (s, 3H, CH_3), 3.73 (t, J = 4.4 Hz, 4H, 2 × CH_2), 3.47 (q, J = 5.6 Hz, 2H, CH_2), 2.57 (t, J = 6.4 Hz, 2H, CH_2), 2.56−2.43 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.52, 126.81, 126.71, 106.98, 67.16, 56.97, 53.56, 38.07, 35.76.

***N*-(4-(Dimethylamino)phenyl)-1-methyl-4-nitro-1*H*-imidazole-2-carboxamide (24g).** This compound was prepared from **22b** and **23c** in 78% yield. TLC (1:1 ethyl acetate/methylene chloride, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (s, 1H, Ar−H), 7.50 (d, J = 2.0 Hz, 2H, CH_2), 6.74 (d, J = 2.0 Hz, 2H, Ar−H), 4.14 (s, 3H, CH_3), 2.9 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.50, 148.66, 126.99, 125.14, 121.86, 113.31, 40.86, 37.14.

1-Methyl-4-nitro-*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-imidazole-2-carboxamide (24h). This compound was prepared from **22b** and **23d** in 91% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H, Ar−H), 7.61 (s, 1H, NH), 4.16 (s, 3H, CH_3), 3.51 (q, J = 6.0 Hz, 2H, CH_2), 2.68 (t, J = 6.4 Hz, 2H, CH_2), 2.59−2.46 (m, 4H, 2 × CH_2), 1.84−1.70 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.99, 137.69, 129.24, 128.43, 54.94, 54.27, 38.54, 37.27, 23.71.

1-Methyl-4-nitro-*N*-(2-(piperidin-1-yl)ethyl)-1*H*-imidazole-2-carboxamide (24i). This compound was prepared from **22b** and **23e** in 82% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H, Ar−H), 7.65 (s, 1H, NH), 4.10 (s, 3H, CH_3), 3.45 (q, J = 6.0 Hz, 2H, CH_2), 2.48 (t, J = 6.4 Hz, 2H, CH_2), 2.42−2.32 (m, 4H, 2 × CH_2), 1.59−1.48 (m, 4H, 2 × CH_2), 1.46−1.33 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.93, 137.71, 124.43, 57.52, 54.67, 37.20, 36.59, 26.05, 24.51.

1-Methyl-4-nitro-*N*-(2-(morpholin-1-yl)ethyl)-1*H*-imidazole-2-carboxamide (24j). This compound was prepared from **22b** and **23f** in 83% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.45). ^{1}H NMR (400 MHz, CD_3OD): δ 7.78 (s, 1H, Ar−H), 7.60 (s, 1H, NH), 4.14 (s, 3H, CH_3), 3.71 (t, J = 4.4 Hz, 4H, 2 × CH_2), 3.50 (q, J = 6.0 Hz, 2H, CH_2), 2.55 (t, J = 6.0 Hz, 2H, CH_2), 2.52−2.43 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.99, 137.57, 124.37, 67.08, 57.33, 53.70, 37.24, 36.14.

General Method for the Activation of Dicarboxylic Acids (25/27a,b)[18]. The dicarboxylic acid (1.0 mmol) was suspended in 4.0 mL of dry DMF followed by the addition of diisoproylethyleneamine (DIEA, 2.2 mmol). The solution was stirred for 5 min before the addition of pentafluorotrifluoroacetate (2.2 mmol). The mixture was stirred at room temperature for 1 h, and solvent was evaporated. Flash silica gel chromatography (toluene/ethyl acetate, 9:1) provided the corresponding pentafluorodiester.

Bis(perfluorophenyl) 4,4′-Methylenedibenzoate (27a). This compound was prepared from **26a** in quantitative yield. TLC (95:5 hexane/ethyl acetate, R_f = 0.75). ^{1}H NMR (400 MHz, DMSO): δ 8.16 (d, J = 6.8 Hz, 4H, Ar−H), 7.38 (d, J = 7.2 Hz, 4H, Ar−H), 4.19 (s, 2H, CH_2). ^{19}F NMR (400 MHz, $CDCl_3$): −152.78 (m), −157.50 (t), −162.23 (m). ^{13} C NMR (100 MHz, $CDCl_3$): δ 165.2, 146.7, 142.4, 142.0, 140.1, 139.3, 130.8, 129.2, 127.7, 41.3.

Bis(perfluorophenyl) Undecanedioate (27b). 27b was prepared from **26b** with quantitative yield. TLC (95:5 hexane/ethyl acetate, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.71−2.58 (m, 4H, 2 × CH_2), 1.85−1.72 (m, 4H, 2 × CH_2), 1.40−1.20 (m, 12H, 6 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.81, 33.55, 29.61, 29.52, 29.31, 29.04, 24.97.

General Method for the Coupling of the *N*-Methylpyrrole Amines with Dicarboxylic Acids[18,26]. To a stirred solution of nitropyrrole derivatives (**24a**−**f**, 0.5 mmol) in methanol (30 mL), 10% Pd/C (15% weight of the nitro derivative) was added. The flask was evacuated, flushed three times with hydrogen, and filled with hydrogen to 35−40 psi. The resultant suspension was shaken vigorously for 1.5 h. The suspended material was filtered through Celite, and the filtrate was evaporated to dryness. The resulting aminopyrrole derivatives were used immediately in the next reaction. Freshly prepared aminopyrrole derivatives were dissolved in 3 mL of dry DMF, and a dipentafluorester (0.2 mmol) generated from **25/26a/26b** was added. The mixture was stirred for 15 h at 55 °C and cooled, and the DMF was evaporated. The final product was purified by silica gel using column chromatography.

4,4′-Carbonylbis(*N*-(*N*^{1},*N*^{1}-dimethylpropane-1,3-diamino)-1*H*-pyrrole)benzamide (1). 1 was prepared from **25** and **24a** with 78% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, J = 8.4 Hz, 4H, Ar−H), 7.90 (d, J = 8.4 Hz, 4H, Ar−H), 7.32 (d, J = 2.0 Hz, 1H, Ar−H), 6.99 (d, J = 1.6 Hz, 1H, Ar−H), 3.99 (s, 6H, 2 × CH_3), 3.41 (t, J = 6.4 Hz, 4H, 2 × CH_2), 3.13 (t, J = 7.2 Hz, 4H, 2 × CH_2), 2.87 (s, 12H, 4 × CH_3), 2.03−1.93 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, CD_3OD and $CDCl_3$): δ 164.04, 162.76, 139.41, 138.35, 130.25, 127.66, 122.83, 122.18, 120.02, 104.82, 62.93, 56.32, 52.90, 43.70, 36.75, 36.39, 25.32. ES-MS [M + 1] found 683.3, calculated for $C_{37}H_{46}N_8O_5$ 682.4.

4,4′-Carbonylbis(*N*-(2-(4-methylpiperazin-1-yl)propyl)-1*H*-pyrrole)benzamide (2). 2 was prepared from **25** and **24b** in 70% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.45). ^{1}H NMR

(400 MHz, $CDCl_3$): δ 9.13 (s, 2H, NH), 7.87 (d, J = 8.4 Hz, 4H, Ar−H), 7.64 (d, J = 8.0 Hz, 4H, Ar−H), 7.34 (t, J = 8.4 Hz, 2H, NH), 7.18 (d, J = 1.6 Hz, 2H, Ar−H), 6.81 (d, J = 1.6 Hz, 2H, Ar−H), 3.83 (s, 6H, 2 × CH_3), 3.45−3.37 (m, 4H, 2 × CH_2), 2.50−2.39 (m, 16H, 8 × CH_2), 2.22 (s, 6H, 2 × CH_3), 1.72−1.66 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.34, 164.03, 162.05, 139.20, 138.58, 130.22, 127.45, 124.25, 121.78, 119.07, 104.20, 57.59, 55.10, 53.35, 46.05, 39.46, 36.76, 25.54. ES-MS [M+1] found 793.3, calculated for $C_{43}H_{56}N_{10}O_5$ 792.4.

4,4′-Carbonylbis(*N*-(*N*^{1},*N*^{1}-dimethylbenzene-1,4-diamine)-1*H*-pyrrole)benzamide (3). 3 was prepared from **25** and **24c** in 60% yield. TLC (94:5:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.35). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.04 (s, 1H, NH), 8.05 (d, J = 7.6 Hz, 4H, Ar−H), 7.88 (d, J = 8.0 Hz, 4H, Ar−H), 7.48−7.39 (m, 4H, Ar−H), 7.32 (s, 2H, Ar−H), 6.98 (s, 2H, Ar−H), 6.78 (d, J = 8.8 Hz, 4H, Ar−H), 3.91 (s, 6H, 2 × CH_3), 2.9 (s, 12H, 4 × CH_3). ES-MS [M+1] found 751.1, calculated for $C_{43}H_{42}N_8O_5$ 750.3.

4,4′-Carbonylbis(*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrole)benzamide (4). 4 was prepared from **25** and **24d** in 80% yield. TLC (92:7:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.45). ^{1}H NMR (400 MHz, CD_3OD): δ 8.03 (d, J = 7.6 Hz, 4H, Ar−H), 7.90 (d, J = 8.0 Hz, 4H, Ar−H), 7.31 (d, J = 1.6 Hz, 2H, Ar−H), 6.91 (d, J = 1.6 Hz, 2H, Ar−H), 3.88 (s, 6H, 2 × CH_3), 3.49 (t, J = 6.8 Hz, 4H, 2 × CH_2), 2.88−2.70 (m, 12H, 6 × CH_2), 1.93−1.82 (m, 8H, 4 × CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 195.63, 13.33, 161.73, 139.40, 138.86, 130.59, 128.31, 123.90, 122.47, 119.02, 104.87, 55.69, 54.31, 38.48, 36.74, 23.80. ES-MS [M + 1] found 707.3, calculated for $C_{39}H_{46}N_8O_5$ 706.4.

4,4′-Carbonylbis(*N*-(2-(piperdin-1-yl)ethyl)-1*H*-pyrrole)benzamide (5). 5 was prepared from **25** and **24e** in 82% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, CD_3OD): δ 8.04 (d, J = 8.4 Hz, 4H, Ar−H), 7.88 (d, J = 8.4 Hz, 4H, Ar−H), 7.30 (d, J = 2.0 Hz, 2H, Ar−H), 6.85 (d, J = 2.0 Hz, 2H, Ar−H), 3.89 (s, 6H, 2 × CH_3), 3.46 (t, J = 6.4 Hz, 4H, 2 × CH_2), 2.64−2.50 (m, 12H, 6 × CH_2), 1.70−1.59 (m, 8H, 4 × CH_2), 1.52−1.42 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.25, 161.63, 139.31, 138.77, 130.27, 128.23, 123.86, 122.39, 118.91, 104.68, 58.41, 54.66, 46.26, 36.76, 36.44, 26.19, 24.63. ES-MS [M+1] found 735.3, calculated for $C_{41}H_{50}N_8O_5$ 734.4.

4,4′-Carbonylbis(*N*-(2-(morpholino-1-yl)ethyl)-1*H*-pyrrole)-benzamide (6). 6 was prepared from **25** and **24f** in 80% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (s, 2H, NH), 7.91 (d, J = 8.4 Hz, 4H, Ar−H), 7.74 (d, J = 8.4 Hz, 4H, Ar−H), 7.97 (d, J = 1.6 Hz, 2H, Ar−H), 6.70 (d, J = 2.0 Hz, 2H, Ar−H), 6.48 (s, J = 8.4 Hz, 2H, NH), 3.90 (s, 6H, 2 × CH_3), 3.70 (t, J = 4.4 Hz, 8H, 4 × CH_2), 3.50 (q, J = 5.6 Hz, 4H, 2 × CH_2), 2.54 (t, J = 6.0 Hz, 4H, 2 × CH_2), 2.55−2.39 (m, 8H, 4 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.44, 164.05, 161.88, 139.43, 138.51, 130.39, 127.38, 124.09, 121.56, 119.42, 103.69, 103.62, 67.19, 57.19, 53.54, 36.92, 36.85, 35.72. ES-MS [M+1] found 739.2, calculated for $C_{39}H_{46}N_8O_7$ 738.3.

4,4′-Methylenebis(*N*-(2-(pyrrolidin-1-yl)ethyl)benzamide (13). 13 was prepared from **27a** and **24d** in 70% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.40). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H, NH), 7.71 (d, J = 8.0 Hz, 4H, Ar−H), 7.23 (s, 1H, Ar−H), 7.11 (d, J = 7.6 Hz, 4H, Ar−H), 6.73 (s, 2H, NH), 6.66 (s, 2H, Ar−H), 3.95 (s, 2H, CH_2), 3.82 (s, 6H, 2 × CH_3), 3.43 (q, J = 5.2 Hz, 4H, 2 × CH_2), 2.64 (t, J = 5.6 Hz, 4H, 2 × CH_2), 2.62−2.49 (m, 8H, 4 × CH_2), 1.79−1.66 (m, 8H, 4 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.98, 162.09, 144.26, 129.26, 127.62, 123.68, 121.86, 119.32, 103.90, 55.12, 54.15, 37.94, 36.78, 23.64. ES-MS [M+1] found 693.3, calculated for $C_{39}H_{48}N_8O_4$ 692.4.

4,4′-Methylenebis(*N*-(2-(piperdin-1-yl)ethyl)benzamide (14). 14 was prepared from **27a** and **24e** in 65% yield. TLC (92:7:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.45). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (s, 2H, NH), 7.70 (d, J = 8.0 Hz, 4H, Ar−H), 7.22 (s, 2H, Ar−H), 7.15 (d, J = 8.0 Hz, 4H, Ar−H), 6.59−6.51 (m, 2H, NH), 6.51 (d, J = 1.2 Hz, 2H, Ar−H), 3.98 (s, 2H, CH_2), 3.82 (s, 6H, 2 × CH_3), 3.68 (q, J = 5.6 Hz, 4H, 2 × CH_2), 2.43 (t, J = 6.0 Hz, 4H, 2 × CH_2), 2.40−2.28 (m, 8H, 4 × CH_2), 1.58−1.46 (m, 8H, 4 × CH_2), 1.43−1.36 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.81, 161.89, 144.31, 132.89, 129.40, 127.98, 127.54, 123.92, 121.60, 119.27, 103.39, 57.35, 54.47, 41.76, 36.84, 35.98, 26.14, 24.50. ES-MS [M+1] found 721.4, calculated for $C_{41}H_{52}N_8O_4$ 720.4.

***N*^{1},*N*^{1}-Bis(1-methyl-5-(2-(pyrrolidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-yl)undecanediamide (15). 15** was prepared from **27b** and **24d** in 69% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.35). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (s, 1H, CONH), 7.09 (d, J = 2.0 Hz, 2H, Ar−H), 6.49 (d, J = 1.6 Hz, 2H, Ar−H), 3.87 (s, 6H, 2 × CH_3), 3.45 (q, J = 5.6 Hz, 4H, 2 × CH_2), 2.64 (t, J = 6.4 Hz, 4H, 2 × CH_2), 2.61−2.49 (m, 8H, 4 × CH_2), 2.27 (t, J = 7.2 Hz, 4H, 2 × CH_2), 1.71−1.64 (m, 4H, 2 × CH_2), 1.41 −1.20 (m, 12H, 6 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.86, 162.01, 123.76, 121.48, 118.89, 103.38, 54.95, 54.13, 38.10, 37.14, 36.76, 29.38, 29.35, 29.30, 25.89, 23.73. ES-MS [M + 1] found 681.4, calculated for $C_{37}H_{60}N_8O_4$ 680.4.

***N*^{1},*N*^{11}-Bis(1-methyl-5-(2-(piperidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-yl)undecanediamide (16). 16** was prepared from **27b** and **24e** in 64% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.50). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (s, 2H, CONH), 7.14 (d, J = 6.0 Hz, 2H, Ar−H), 6.62−2.54 (m, 2H, CONH), 6.43 (d, J = 1.6 Hz, 2H, Ar−H), 3.88 (s, 6H, 2 × CH_3), 3.43 (q, J = 5.2 Hz, 4H, 2 × CH_2), 2.49 (t, J = 6.4 Hz, 4H, 2 × CH_2), 2.49−2.34 (m, 8H, 4 × CH_2), 2.27 (t, J = 7.6 Hz, 4H, 2 × CH_2), 1.74−1.64 (m, 4H, 2 × CH_2), 1.62−1.50 (m, 8H, 4 × CH_2), 1.49−1.39 (m, 4H, 2 × CH_2), 1.35 − 1.21 (m. 12H, 6 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.76, 161.90, 123.75, 121.45, 119.00, 103.07, 57.4, 54.48, 37.17, 36.78, 35.96, 29.36, 29.31, 26.18, 25.90, 24.54. ES-MS [M+1] found 709.4, calculated for $C_{39}H_{64}N_8O_4$ 708.5.

General Method for Coupling Amino-*N*-methylimidazole with Dicarboxylic Acid[16]. To a stirred solution of nitroimidazole derivatives **24g−j** (3.0 mmol) in methanol (30 mL), 10% Pd/C (15% weight of the nitro derivative) was added. The flask was evacuated, flushed three times with hydrogen, and filled with hydrogen to 35−40 psi. The resultant suspension was shaken vigorously for 1.5 h. The suspended material was filtered through Celite, and the filtrate was evaporated to dryness. The resulting aminoimidazole derivative was used immediately without purification. To a flame-dried flask, **25** (1.0 mmol), HBTU (3.0 mmol), and DIEA (5.0 mmol) were added followed by addition of the aminoimidazole. The mixture was stirred under argon overnight at room temperature before the solvent was removed by evaporation. The final product was obtained by silica gel column chromatography.

4,4′-Carbonylbis(*N*-(*N*^{1},*N*^{1}-dimethylbenzene-1,4-diamine)-1*H*-imidazole)benzamide (7). 7 was prepared from **25** and **24g** in 60% yield. TLC (1:1 ethyl acetate/$CHCl_3$, R_f = 0.6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 2H, NH), 8.63 (s, 2H, NH), 8.01 (d, J = 8.0 Hz, 4H, Ar−H), 7.88 (d, J = 8.0 Hz, 4H, Ar−H), 7.60 (s, 2H, Ar−H), 7.47 (d, J = 8.0 Hz, 4H, Ar−H), 6.72 (d, J = 8.0 Hz, 4H, Ar−H), 4.09 (s, 6H, 2 × CH_3), 2.92 (s, 12H, 4 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.07, 163.37, 156.49, 139.96, 137.33, 135.83, 134.80, 130.56, 127.50, 121.61, 114.93, 113.19, 36.14. ES-MS [M+1] found 753.1, calculated for $C_{41}H_{40}N_{10}O_5$ 752.3.

4,4′-Carbonylbis(*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-imidazole)-benzamide (8). 8 was prepared from **25** and **24h** in 52% yield TLC (94:5:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.45). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 2H, NH), 8.04 (d, J = 8.8 Hz, 4H, Ar−H), 7.91 (d, J = 8.8 Hz, 4H, Ar−H), 7.59 (s, 2H, NH), 7.56 (s, 2H, Ar−H), 4.06 (s, 6H, 2 × CH_3), 3.53 (q, J = 6.0 Hz, 4H, 2 × CH_2), 2.73 (t, J = 6.4 Hz, 4H, 2 × CH_2), 2.66−2.55 (m, 8H, 4 × CH_2), 1.85−1.76 (m, 8H, 4 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.14, 163.44, 159.16, 139.95, 137.48, 135.93, 134.68, 130.55, 127.52, 114.44, 55.13, 54.25, 38.00, 35.94, 23.73. ES-MS [M+1] found 709.2, calculated for $C_{37}H_{44}N_{10}O_5$ 708.3.

4,4′-Carbonylbis(*N*-(2-(piperdin-1-yl)ethyl-1*H*-imidazole)-benzamide (9). 9 was prepared from **25** and **24i** in 57% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.5 ^{1}H NMR (400 MHz,

CD_3OD): δ 8.08 (d, J = 8.0 Hz, 4H, Ar−H), 7.91 (d, J = 8.4 Hz, 4H, Ar−H), 7.55 (s, 2H, Ar−H), 4.02 (s, 6H, 2 × CH_3), 3.69 (t, J = 6.0 Hz, 4H, 2 × CH_2), 3.26−3.14 (m, 12H, 6 × CH_2), 1.90−1.81 (m, 8H, 4 × CH_2), 1.69−1.63 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 164.75, 160.77, 139.79, 137.71, 136.71, 136.73, 133.95, 130.23, 127.74, 115.38, 57.29, 53.94, 35.32, 34.36, 23.57, 21.87. ES-MS [M + 1] found 737.3, calculated for $C_{39}H_{48}N_{10}O_5$ 736.4.

4,4′-Carbonylbis(*N*-(2-(morpholin-1-yl)ethyl-1*H*-imidazole)benzamide (10). 10 was prepared from **25** and **24j** in 65% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 2H, NH), 8.04 (d, J = 8.0 Hz, 4H, Ar−H), 7.92 (d, J = 8.8 Hz, 4H, Ar−H), 7.58 (s, 2H, NH), 7.57 (s, 2H, Ar−H), 4.07 (s, 6H, 2 × CH_3), 3.78 (m, 8H, 4 × CH_2), 3.55 (q, J = 5.6 Hz, 4H, 2 × CH_2), 2.71−2.58 (m, 12H, 6 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.30, 159.15, 140.02, 137.40, 130.59, 127.47, 114.53, 66.82, 57.52, 53.64, 35.98, 35.50. ES-MS [M + 1] found 741.2, calculated for $C_{37}H_{44}N_{10}O_7$ 740.3.

4-Benzoyl-*N*-(2-(pyrrolidin-1-yl)ethyl)benzamide (17). To a dry flask, **28a** (113 mg, 0.5 mmol) was activated using pentafluorotrifluoroacetate according to methods described above. The resulting activated ester was coupled with the amine derived from **24d** (141.6 mg, 0.6 mmol) in DMF for 15 h. The solvent was evaporated and the residue was purified by column chromatography to generate the final product in 75% yield. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H, CONH), 7.97 (d, J = 8.0 Hz. 2H, Ar−H), 7.87 (d, J = 8.0 Hz. 2H, Ar−H), 7.85−7.78 (m, 2H, Ar−H), 7.63 (s, 1H, Ar−H), 7.54−7.49 (m, 2H, Ar−H), 7.35 (d, J = 1.6 Hz, 1H, Ar−H), 6.82−6.76 (m, 1H, CONH), 6.71 (d, J = 1.6 Hz, 1H, Ar−H), 3.94 (s, 3H, CH_3), 3.52 (q, J = 5.6 Hz, 2H, CH_2), 2.77 (t, J = 5.6 Hz, 2H, CH_2), 2.71−2.60 (m, 4H, 2 × CH_2), 2.59−2.52 (m, 2H, CH_2), 1.85 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.47, 164.26, 162.24, 140.60, 138.17, 137.49, 133.43, 130.74, 130.595, 128.97, 127.45, 124.12, 121.64, 119.81, 104.12, 55.48, 54.55 38.01, 37.21, 23.98, 23.94. ES-MS [M + 1] found 445.1, calculated for $C_{26}H_{28}N_4O_3$ 444.2.

4-Benzoyl-*N*-(2-(piperidin-1-yl)ethyl)benzamide (18). 18 was prepared using **28a** and **24e** in a manner similar to that of **17**. The final product was obtained in 62% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H, CONH), 7.95 (d, J = 7.6 Hz, 2H, Ar−H), 7.85 (d, J = 8.0 Hz, 2H, Ar−H), 7.80 (d, J = 8.8 Hz, 2H, Ar−H), 7.64−7.58 (m, 1H, Ar−H) 7.52−7.47 (m, 2H, Ar−H), 7.33 (d, J = 1.2 Hz, 1H, Ar−H), 6.67−6.61 (m, 1H, CONH), 6.60 (d, J = 1.6 Hz, 1H, Ar−H), 3.93 (s, 3H, CH_3), 3.44 (q, J = 6.0 Hz, 2H, CH_2), 2.5 (t, J = 6.0 Hz, 2H, CH_2), 2.45−2.39 (m, 4H, 2 × CH_2), 1.16−1.11 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.21, 164.02, 161.83, 140.33, 133.97, 137.21, 133.18, 130.46, 130.22, 128.71, 127.17, 124.17, 121.30, 119.40, 109.99, 103.41, 57.38, 54.5, 46.44, 36.92, 36.02, 26.24, 24.56. ES-MS [M + 1] found 459.2, calculated for $C_{27}H_{30}N_4O_3$ 458.2.

4-Benzamido-1-methyl-*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (19). To a dry flask under argon, **28b** (128 mg, 0.5 mmol), EDC (143 mg, 0.75 mmol), and DMAP (91.5 mg, 0.75 mmol) were added followed by 4 mL of dry DMF. The mixture was stirred for 30 min, and then the freshly prepared **24d**, dissolved in 2 mL of dry DMF, was added dropwise to the activated acid. The mixture was allowed to stir at room temperature for 15 h. The DMF was evaporated, and the residue was dissolved in methylene chloride (10 mL) and washed with brine and saturated sodium bicarbonate solution. The organic layer was concentrated in vacuo. The product was purified using column chromatography to yield the final product in 60% yield. TLC (94:5:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H, CONH), 7.82−7.77 (m, 2H, Ar−H), 7.42−7.37 (m, 3H, Ar−H), 7.19 (d, J = 0.8 Hz, 1H, Ar−H), 6.91 (s, 1H, CONH), 6.61(s, 1H, Ar−H), 3.84 (s, 3H, CH_3), 3.45 (q, J = 5.6 Hz, 2H, CH_2), 2.71 (t, J = 5.6 Hz, 2H, CH_2), 2.65−2.59 (m, 4H, 2 × CH_2), 1.82−1.77 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.95, 162.06, 134.65, 131.76, 128.88, 127.18, 121.64, 119.46, 103.87, 55.22, 54.22, 37.66, 36.89, 23.66. ES-MS [M + 1] found 341.2, calculated for $C_{19}H_{24}N_4O_2$ 340.2.

4-Benzamido-1-methyl-*N*-(2-(piperidin-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (20). 20 was prepared using **28b** and **24e** in a manner similar to that of **19**. The final product was obtained in 58% yield. TLC (94:5:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.50). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88−7.81 (m, 2H, Ar−H), 7.76 (s, 1H, CONH), 7.52−7.44 (m, 3H, Ar−H), 7.29 (d, J = 1.6 Hz, 1H, Ar−H), 6.53 (s, 1H, CONH), 6.52 (d, J = 2.0 Hz, 1H, Ar−H), 3.92 (s, 3H, CH_3), 3.43 (q, J = 6.0 Hz, 2H, CH_2), 2.50 (t, J = 6.0 Hz, 2H, CH_2), 2.45−2.37 (m, 4H, 2 × CH_2), 1.62−1.55 (m, 4H, 2 × CH_2), 1.47−1.43 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.94, 161.81, 134.71, 131.83, 128.95, 127.09, 121.44, 119.25, 103.22, 57.58, 54.47, 36.86, 36.03, 26.32, 24.62. ES-MS [M+1] found 355.2, calculated for $C_{20}H_{26}N_4O_2$ 354.2.

4,4′-(Hydroxymethylene)bis(*N*-(2-(pyrrolidin-1-yl)ethyl)benzamide (11). Compound **4** (0.065 mmol, 30 mg) was dissolved in 3 mL of MeOH. Pd/C (3.0 mg) was added. The solution was cooled to 0 °C, and sodium borohydride (0.162 mmol, 6 mg) was added. The mixture was stirred for 1 h under argon. The excess hydride was neutralized with glacial acetic acid and then filtered through Celite. The filtrate was concentrated in vacuo, to yield the product (58%). TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f=0.3). ^{1}H NMR (400 MHz, CD_3OD & $CDCl_3$): δ 7.83 (d, J = 7.6 Hz, 4H, Ar−H), 7.47 (d, J = 7.6 Hz, 4H, Ar−H), 7.24 (d, J = 1.6 Hz, 2H, Ar−H), 6.82 (d, J = 1.6 Hz, 2H, Ar−H), 5.86 (s, 1H, CH), 3.85 (s, 6H, 2 × CH_3), 3.46 (t, J = 6.8 Hz, 4H, 2 × CH_2), 2.73 (t, J = 6.8 Hz, 4H, 2 × CH_2), 2.72−2.69 (m, 6H, 3 × CH_2), 1.87−1.79 (m, 6H, 3 × CH_2). ^{13}C NMR (100 MHz, CD_3OD and $CDCl_3$): δ 165.92, 162.96, 147.94, 133.65, 127.47, 126.69, 123.31, 122.18, 119.71, 104.98, 74.93, 55.23, 54.06, 37.69, 36.06, 23.13. ES-MS [M + 1] found 709.3, calculated for $C_{39}H_{48}N_8O_5$ 708.4.

4,4′-(Hydroxymethylene)bis(*N*-(2-(piperdin-1-yl)ethyl)benzamide (12). Compound **12** was prepared in a manner similar to the preparation of **11** but using **5** as the starting material. TLC (89:10:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.25), 60% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.85 (d, J = 8.4 Hz, 4H, Ar−H), 7.49 (d, J = 8.4 Hz, 4H, Ar−H), 7.27 (d, J = 1.6 Hz, 2H, Ar−H), 6.85 (d, J = 1.6 Hz, 2H, Ar−H), 5.88 (s, 1H, CH), 3.84 (s, 6H, 2 × CH_3), 3.43 (t, J = 6.8 Hz, 4H, 2 × CH_2), 2.56−2.41 (m, 12H, 6 × CH_2), 1.66−1.52 (m, 8H, 4 × CH_2), 1.49−1.41 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 179.26, 165.87, 162.89, 148.18, 133.61, 127.38, 126.59, 123.46, 122.24, 119.54, 104.90, 74.81, 57.94, 54.35, 55.93, 35.65, 25.38, 23.95, 23.07. ES-MS [M + 1] found 737.3, calculated for $C_{41}H_{52}N_8O_5$ 736.4.

4,4′-Carbonylbis(*N*-(2-(piperidin-1-yl)ethyl)benzamide) (21). In an argon flushed flask, **25** (81 mg, 0.3 mmol) was dissolved in 4.0 mL of dry DMF. To this solution, **24e** (177 mg, 0.75 mmol) was added under argon, and the mixture was heated at 55 °C overnight. The solvent was evaporated and the residue purified by column chromatography to give the final product in 78% yield. TLC (97:2:1 CH_2Cl_2/MeOH/Et_3N, R_f = 0.55). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 7.2 Hz, 4H, ArH), 7.81 (d, J = 8.0 Hz, 4H, ArH), 3.52 (q, J = 5.6 Hz, 4H, 2 × CH_2), 2.56 (t, J = 4.8 Hz, 4H, 2 × CH_2), 2.48−2.36 (m, 8H, 4 × CH_2), 1.61−1.55 (m, 8H, 4 × CH_2), 1.46−1.41 (m, 4H, 2 × CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.51, 166.50, 161.40, 139.52, 138.57, 130.73, 127.30, 57.12, 57.01, 54.45, 36.73, 34.81, 26.22, 26.13, 24.47. ES-MS [M+1] found 491.2, calculated for $C_{29}H_{38}N_4O_3$ 490.3.

Ethidium Bromide Displacement Assay. DNA hairpin oligonucleotide 5′-CGAAAAACAAAAAGTTTTTCG-3′ was purchased from Trilink Biotechnologies, San Diego, CA, and prepared for use according to the literature procedures.[43] To a 1.0 mL quartz cell containing Tris buffer (0.1 M Tris HCl, 25 mM, NaCl, pH 7.0), ethidium bromide (4.0 μM) and the AT hairpin oligonucleotides (8.0 μM) were added. The fluorescence was determined using an excitation wavelength of 545 nm and emission wavelength of 595 nm. Various concentrations of the agent were added, the solution was mixed, and the change in the

fluorescence at 595 nm was recorded after a 5 min incubation period. The data were corrected for differences in volume due to addition of the drug, and binding constants were determined using a competitive binding model: $K = K_{EB}[E]/[agent]$, where K is the binding constant, K_{EB} is the binding constant for ethidium bromide, [E] is the total concentration of ethidium bromide, and [agent] is the concentration of the drug that reduces fluorescence by 50%. K_{EB} for the AT hairpin is 0.27×10^{-6} M^{-1}.

MTS Assay. Chinese hamster ovary cell line (ATCC, Manassas, VA) and growth medium RPMI-1640 (ATCC, Manassas, VA) were used. Cells were seeded in 96-well plates at a seeding density of 5000 cell/well per 0.1 mL of medium and allowed to attach overnight. Cells were then treated with medium containing the agent in different concentrations (25, 50, and 100 mg/L) and incubated for 24 h. MTS assay reagent (20 μL) was added to each well and incubated for 120 min, and the absorbance was measured at 505 nm using a microplate reader (Molecular Devices, kinetic microplate reader, Sunnyvale, CA). In this assay, the absorbance is proportional to the number of viable cells. Untreated cell (with or without 5% DMSO in medium) were used as controls. Results were presented as percentage cytotoxicity compared to controls. Experiments were repeated at least three times.

Depolarization Studies[35]**.** *S. aureus* bacteria were grown at 37 °C with shaking to mid-logarithmic phase ($OD_{600} = 0.4-0.5$). Bacteria were collected by centrifugation, washed with buffer (5 mM HEPES, 5 mM glucose, pH 7.2), and suspended in the same buffer to an OD_{600} of 0.05. The cell suspension was incubated with 0.4 μM diSC3(5) for an hour at 37 °C followed by the addition of 100 mM KCl to equilibrate the cytoplasmic and external K^+ concentrations. Cell suspension (1 mL) was placed in a plastic cuvette with a stir bar and placed into a thermostated cuvette holder (heated to 37 °C). The desired concentration of the test agent was added, and the fluorescence readings were monitored for 60 min using a FluoroMax 3 fluorescence spectrophotometer (Horiba Jobin Yvon, Edison, NJ) set to an excitation wavelength of 622 nm and emission wavelength of 670 nm. Nisin was used as a positive control and a blank with only cells, and dye was used to subtract the background.

In Vivo Competition Studies with Hoescht Dye. *S. aureus* bacteria were grown at 37 °C with shaking to mid-logarithmic phase ($OD_{600} = 0.4-0.5$). Cells were collected by centrifugation and suspended in PBS pH 7.2 buffer. Hoescht dye was added to a final concentration of 1.0 μg/mL, and the cells were incubated for 30 min at 37 °C. The bacteria were then placed into a microtiter plate such that each drug concentration was done in triplicate. Test compounds (25, 50, 100, 150, 200 mg/L) were added, and the fluorescence was measured using microplate reader set to an emission wavelength of 461 nm (Molecular Devices, kinetic microplate reader, Sunnyvale, CA). Cells incubated with Hoescht but not treated with the test compounds were used as a control.

Incorporation Studies for the Inhibition of Macromolecular Synthesis[29]**.** *S. aureus* cells were grown at 35 °C overnight on TS agar. An small amount of bacteria was taken from the plate and used to inoculate 10 mL of Mueller−Hinton broth II (MHB). The culture was grown to early exponential growth phase ($OD_{600} = 0.2-0.3$) in a shaker incubator set to 35 °C.

For DNA, RNA, cell wall, and protein synthesis the following protocol was used. When cells reached early exponential phase, 100 μL of culture was added to triplicate wells containing various concentrations of (0.25−4.0 μg/mL) of compound **5**, and control antibiotics (5 μL) at 20× final concentration in 100% DMSO. A 5% DMSO treated culture served as the "no drug" control for all experiments. Cells were added in MHB at 105% to account for the volume of drug added to each mixture or in M9 minimal medium for protein synthesis reactions. Following a 5 min incubation at room temperature, [^{3}H]thymidine (DNA synthesis), [^{3}H]uridine (RNA synthesis), [^{3}H]*N*-acetylglucosamine (cell wall synthesis), or [^{3}H]leucine (protein synthesis) was added at 0.5−1.0 μCi per mixture, depending on the experiment. Reactions were allowed to proceed at room temperature for 15−30 min (60 min for cell wall synthesis) and then stopped by adding 12 μL of cold 5% trichloroacetic acid (TCA) or 5% TCA/2% caesamino acids (protein synthesis only). Mixtures were incubated on ice for 30 min, and the TCA precipitated material was collected on a 25 mm GF/A filter. After being washed three times with 5 mL of cold 5% TCA, the filters were rinsed two times with 5 mL of 100% ethanol, allowed to dry, and then counted using a Beckman LS 3801 liquid scintillation counter. The following compounds served as positive control agents for the macromolecular synthesis assays: DNA (ciprofloxacin), RNA (rifampicin), cell wall (vancomycin), protein (linezolid).

For lipid synthesis, the following method was employed. Cells (0.1 mL) in early exponential growth phase were added to a 1.5 mL Eppendorf tube (in triplicate) containing various concentrations (0.25−4.0 μg/mL) of compound **5**, or control antibiotic (cerulenin). Following a 5 min incubation at room temperature, [^{3}H]glycerol was added at 0.5 μCi per mixture. Reactions were allowed to proceed at room temperature for 15 min and then stopped through the addition of 375 μL of chloroform/methanol (1:2) followed by vortexing for 20 s after each addition. Chloroform (125 μL) was then added to each mixture and vortexed followed by the addition of 125 μL of distilled H_2O and vortexing. Mixtures were centrifuged at 13 000 rpm for 10 min, and 150 μL of the organic phase was transferred to a scintillation vial and allowed to dry in a fume hood for at least 1 h. Samples were then counted via liquid scintillation counting.

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