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Synthetic Mimics of Antimicrobial Peptides from Triaryl Scaffolds

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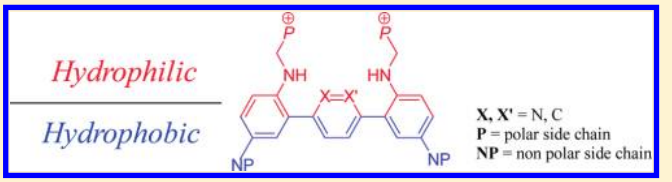
Supporting Information

ABSTRACT: In this report, we describe the synthesis of a new series of small amphiphilic aromatic compounds that mimic the essential properties of cationic antimicrobial peptides using Suzuki−Miyaura coupling. The new design allowed the easy tuning of the conformational restriction, controlled by introduction of *intra*molecular hydrogen bonds, and the overall hydrophobicity by modifications to the central ring and the side chains. This approach allowed us to better understand the influence of these features on the antimicrobial activity and selectivity. We found that the overall hydrophobicity had a more significant impact on antimicrobial and hemolytic activity than the conformational stiffness. A novel compound was discovered which has MICs of 0.78 μg/mL against *S. Aureus* and 6.25 μg/mL against *E. Coli*, similar to the well-known antimicrobial peptide, MSI-78.



INTRODUCTION

The discovery and development of antibiotics and antibacterial agents for treatment of bacterial infections were some of the most profound medical advances of the 20th century. The use of antibiotics has significantly reduced illness and death caused by bacterial infection. However, over the past few decades, there has been an alarming increase in bacterial resistance to even our best antibiotics. The evolution and spread of these multidrug resistant bacteria have become a major threat to global health care.[1] Consequently, there has been increased interest in identifying and developing novel compounds that can act as suitable antibiotics.

Antimicrobial peptides (AMPs) have been investigated as potential antibiotics because of their broad spectrum activity, immunomodulatory response, and unique mode of action.[2−5] AMPs are found in almost all multicellular organisms and form the core of the innate immune system. Most AMPs show direct antimicrobial activity against a variety of bacteria, fungi, protozoa, and viruses.[6] Hundreds of AMPs that exhibit a large variety of sequences and structures have been isolated and identified. AMPs can be broadly classified into α-helical and β-sheet peptides, although other secondary structures like extended coils or loops are also present.[7] Despite their large sequence diversity, AMPs do share some common structural characteristics. They are generally short, composed of 12−50 amino acids, with a net positive charge ranging from +2 to +9, mainly because of the presence of lysine and/or arginine, and have hydrophobic residues. They generally adopt an amphiphilic structure where hydrophilic and hydrophobic residues segregate onto opposite regions, either in the presence of a solvent or upon interaction with the cell membrane.[2,3,8] For many of these cationic AMPs, the mechanism of action has been suggested to primarily involve interaction with the negatively charged components of the bacterial cell membrane, leading to increased permeability and eventually cell death. Because of the difference in membrane phospholipid composition, bacterial membranes have been proposed to be more negatively charged than mammalian ones, and this enables AMPs to be selective toward bacteria. Several models have been proposed to describe the mechanism of interaction between AMPs and bacterial membranes, although the exact mechanism is still not clear.[4,8,9] In addition, some AMPs are also known to kill bacteria by interacting with intracellular macromolecules.[3] Since AMPs target the fundamental feature of bacteria, unlike conventional antibiotics which have very specific binding sites, resistance development has proven to be more difficult.[10,11]

AMPs, with all their unique features, appear to be quite promising as antibacterial drug candidates, but they do have some disadvantages when considered for clinical use. AMPs usually have high cytotoxicity, poor tissue distribution and are susceptible to proteolysis and hydrolysis. The high cost involved in the synthesis of AMPs is another factor hampering their use as drug candidates.[12−14] This has led several research groups to focus on the design and synthesis of unnatural backbones that mimic the structure and activity of AMPs.[15,16] A number of studies, based on this peptidomimetic approach, have reported synthetic mimics of antimicrobial peptides (SMAMPs) including peptoids,[17] β-peptides,[18−20] cyclic peptides,[21] synthetic polymers,[22−26] oligo-acyl lysines,[27,28] and aromatic oligomers.[29−32] The ability to recapitulate AMP activity in SMAMPs has allowed many of the problems plaguing peptide based drug development to be overcome such that a SMAMP is in phase I clinical trials for pan-staph infections.[33]

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Previously, our research group designed a series of aromatic oligomers based on arylamide,[22,30] urea,[31] and phenylene−ethynylene[24,34] backbones with broad spectrum antimicrobial activity and selectivity. The class of arylamide oligomers was designed de novo using molecular dynamics and utilized hydrogen bonding to add conformational rigidity to the backbones. Detailed analysis of these oligomers revealed that replacing the central benzene ring with pyrimidine further rigidified the conformation due to *intra*molecular hydrogen bonding and led to a more potent structure.[30] These oligomers with hydrogen bonding also displayed enhanced antibacterial activities toward *S. aureus* and *E. coli*.[30] The class of phenylene−ethynylene (PE) oligomers, with strictly hydrocarbon backbone, demonstrated excellent antibacterial activity and some selectivity. The PE oligomers had no hydrogen bonds but still could adopt facially amphiphilic conformations via rotation around single bonds in the backbone.[35] The study of all these oligomers summarized above has shown that a formal secondary structure, such as an α-helix, is not critical as long as there is a correct balance and segregation of hydrophobic and hydrophilic groups. It also demonstrated that oligomers with and without restricted conformations could be potent SMAMPs. When a rigid scaffold is used, the design must lead to the correct conformer for maximum potency; if a flexible conformation is employed, many conformers are available but an entropic penalty is incurred when the SMAMP binds to the membrane.[36]

This report describes a novel series of aryl oligomers synthesized by Suzuki−Miyaura coupling. The general design principle of the molecule is shown in Figure 1. This new design is advantageous because of its synthetic versatility that allows the facile construction of a library of compounds with different backbones and side chains. We have altered the central ring providing a systematic study of *intra*molecular hydrogen bonding and thus conformational restriction. We have also explored the effect of hydrophobicity via modifications of both polar and nonpolar side chains. The results demonstrated that antimicrobial and hemolytic activities of this particular class of SMAMPs are more responsive to changes in hydrophobicity than conformational stiffness.

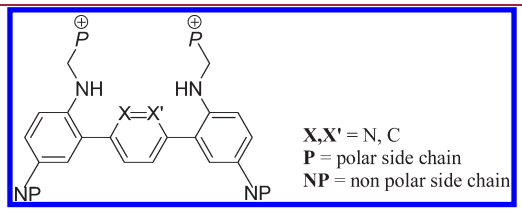

Figure 1. Representative scaffold showing design principles.

RESULTS

Design. Several amphiphilic aryl oligomers were synthesized using Suzuki−Miyaura coupling and evaluated to develop a structure−activity relationship (SAR) of antimicrobial potency. Initially, the central ring of the backbone was varied to observe the effect of different degrees of rotational restriction on the antimicrobial efficiency of those compounds. With this aim, three series of compounds carrying pyridazine, pyridine, or benzene as the central ring and β-alanine as the polar side group were built (Figure 2). We expected the molecule with pyridazine ring (**1a**−**d**) to have the most rigid conformation because of its ability to lock the conformation via two hydrogen bonds (H-bonds), compared to the presence of only one H-bond in the pyridine ring (**2a**−**c**) or none in the case of the benzene ring (**3a**−**e**). To evaluate the effect of different nonpolar and polar groups on both structural and biological properties, the side chains were varied as well. For the nonpolar groups, two different substituents were used, i.e., *tert*-butyl (*t*-butyl), which is hydrophobic and electron-donating, and CF_3, which is a smaller hydrophobic group and electron-withdrawing. The molecule without a nonpolar side group **1c** was also synthesized and compared to **1a** and **1b** to explore the effect of having nonpolar side groups in the molecule.

To test the effect of the spacer length between the aromatic backbone and the cationic amine, β-alanine and aminovaleric acid polar side groups containing three and five carbons in the side chain, respectively, were employed (Figure 3). Compound **3e** was synthesized to test the effect of guanidine versus primary amine, since the guanidinium group is present in many natural AMPs and has been shown to improve antimicrobial activity of SMAMPs.[29]

Synthesis. Scheme 1 shows a general example of the synthesis of oligomers **1a**−**c**. The biaryl carbon−carbon bond of the backbone was constructed using modified Suzuki−Miyaura coupling conditions[37] between 3,6-dibromopyridazine and a 4-substituted aniline boronic ester (**4a**−**c**), which was prepared via the borylation of the corresponding commercially available bromoaniline.[38,39] Polar side chains were added by EDC/HOBT coupling to the oligomers where R is the electron-donating *tert*-butyl or H, but this synthetic strategy was not effective for oligomers with the electron-withdrawing CF_3 group because of its deactivating effect on the amine. For those compounds, the amide coupling was carried out in moderate yield using $POCl_3$/pyridine conditions (see Experimental Section). The final product of all oligomers was obtained as a salt by deprotection of the terminal amine Boc functionality using DCM/trifluoroacetic acid. Oligomers **2a**−**c** and **3a**−**c** were obtained in comparable yields from 2,5-dibromopyridine and 1,6-dibromobenzene,

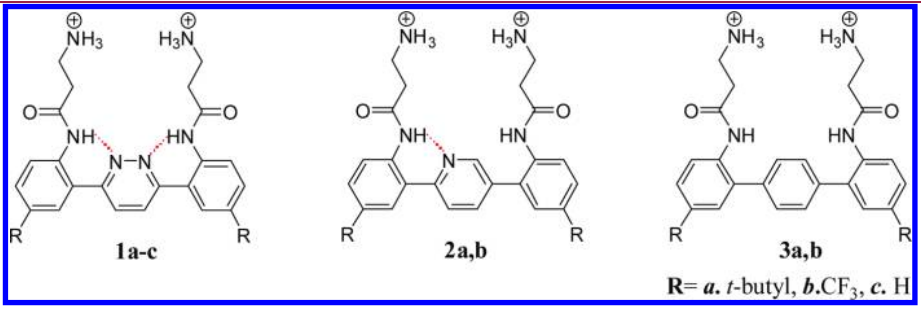

Figure 2. Aryl oligomers with β-alanine polar side chain and different central rings.

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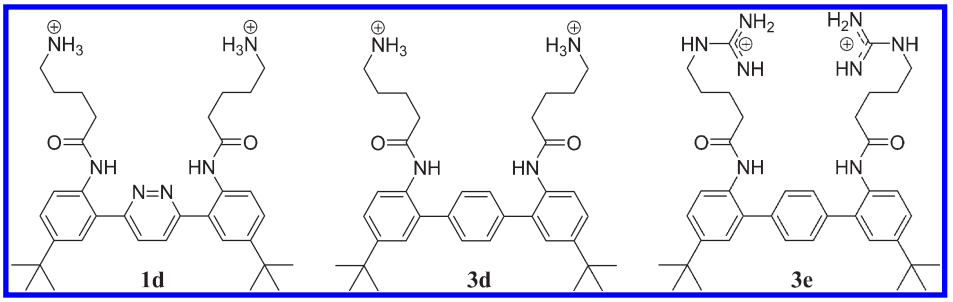
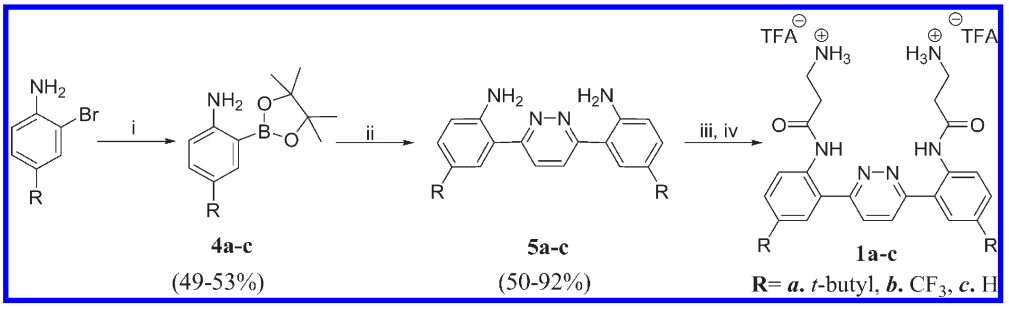


Figure 3. Aryl oligomers prepared for investigating the effect of different polar side groups.

Scheme 1. Synthetic Pathway for Pyridazine Oligomers[a]



[a] (i) Pinacolborane, $PdCl_2(dppf)\cdot CH_2Cl_2$, Et_3N, dioxane, 100°C, 3 h; (ii) 3,6-dibromopyridazine, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3 (aq), DMF, 90°C, 18 h; (iii) (a, c) Boc-β-Ala-OH, EDC/HOBT, CH_2Cl_2, room temp, overnight; (b) Boc-β-Ala-OH, $POCl_3$, pyridine, 0°C, 1 h; (iv) TFA/CH_2Cl_2 (1:3), room temp, 1 h.

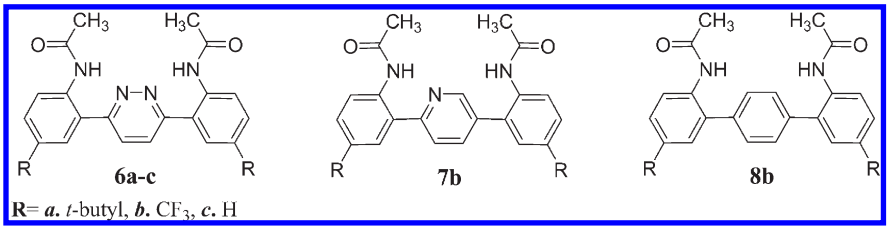


Figure 4. Acetyl derivatives of oligomers used for H-bond investigation.

respectively, using the same synthetic pathway. The synthesis and characterization of all compounds is reported in detail in the Experimental Section.

H-Bond Investigation. Conformational analysis of the compounds was performed using acetyl derivatives as models (Figure 4), assuming that variable polar side chains do not affect the establishment or strength of the intramolecular H-bonding and thus the related backbone rigidity. Acetylation was performed via iodine catalysis according to the literature[40] (see Experimental Section for detailed synthesis.)

We evaluated the presence and strength of the intramolecular H-bond between the nitrogen of the central ring and amide group involved as the H-donor. It is well-known that H-bonding is typically associated with a downfield shift of the ^{1}H NMR signals corresponding to the involved proton and with a shifting of the IR stretching band of the donor group toward lower frequencies.[41,42] Linear correlations between the NMR and IR data have been reported in the literature.[43,44] Here we compared the solvent effect on the ^{1}H NMR amide signal in different backbones while gradually changing the solvent composition of diluted samples (~2.5 mM) from $CDCl_3$ to DMSO-d_6 using tetramethylsilane (TMS) as standard. This analysis provides information about the presence and strength of H-bonding in solution. Table 1 summarizes the final shifts observed for the amide protons in pure solvents. In neat $CDCl_3$, the pyridine nitrogen acts as a better amide proton acceptor than pyridazine, since the H-bonded amide proton is shifted further

Table 1. Spectroscopic Data of Model Compounds

	^{1}H NMR[c]			IR[d]
compd	δ_{N-H} in $CDCl_3$ (ppm)	δ_{N-H} in DMSO-d_6 (ppm)	$\Delta\delta_{(\delta DMSO)-(\delta CDCl3)}$ (ppm)	ν_{N-H} (cm^{-1})
6a	11.33	10.40	−0.93	2924
6b	11.88	10.92	−0.96	2924
6c	11.68	10.75	−0.93	2922
7b	12.24,[a] 8.78[b]	12.11,[a] 9.72[b]	−0.13,[a] 0.94[b]	2917,[a] 3302[b]
8b	8.52	9.39	0.87	3259

[a] N−H involved in H-bond with pyridine ring. [b] N−H not involved in H-bond with pyridine ring. [c] Tetramethylsilane (TMS) was used as the internal standard for H-bond studies. [d] In solid state.

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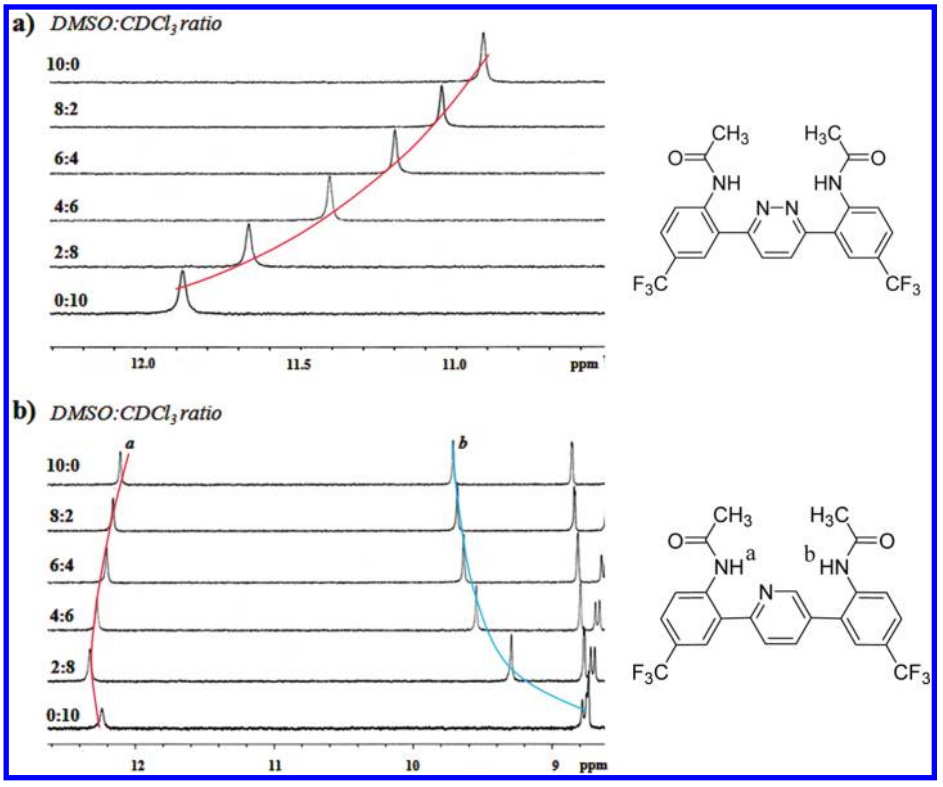

Figure 5. Influence of the solvent composition on the amide proton chemical shift: (a) **6b** vs (b) **7b**.

downfield (δ = 12.24 ppm in **7b**[a] instead 11.88 ppm in **6b**). Both compounds, however, show the presence of *intra*molecular H-bonding when compared to the negative control **8b** with benzene as the central ring (δ = 8.52 ppm). Similar chemical shifts observed in the cases of compounds **6a**−**c** indicate that the ring substituent, either electron-withdrawing or electron-donating, has no significant impact on the H-bond.

The difference in H-bonding strength is more evident when the ratio DMSO-d_6/$CDCl_3$ is increased (Figure 5). Because DMSO is a H-bonding solvent with its oxygen atom as the acceptor, its presence leads to competition between *intra*molecular and *inter*molecular H-bond formations, resulting in a upfield displacement of the proton chemical shift. However, compounds with stronger *intra*molecular H-bonding are known to be less affected by these changes in solvent composition.[45] Upon an increase of the DMSO-d_6/$CDCl_3$ ratio, a substantial upfield shift of the amide proton's resonance for compounds **6a**−**c** ($\Delta\delta_{(\delta_{DMSO})-(\delta_{CDCl3})}$ = −0.96 ppm) was observed, consistent with a reduction in *intra*molecular H-bonding (Figure 5a), whereas the amide proton in **7b**[a] was only slightly sensitive to the solvent composition ($\Delta\delta_{(\delta_{DMSO})-(\delta_{CDCl3})}$ = −0.13 ppm). In contrast, our negative controls (compound **8b** and amide proton in **7b**[b] without H-bonds) showed downfield shifts of the proton amide resonance (Figure 5b) due to the change in solvent dielectric constant. The ^{1}H NMR data are supported by the differences in the IR values of the N−H stretching frequencies for the various amide protons (see Supporting Information for IR data). All these data showed that pyridine can establish a stronger *intra*molecular H-bond than the pyridazine ring, in agreement with studies reported in the literature.[46]

Two-dimensional NOESY (nuclear Overhauser effect spectroscopy) experiments were also performed on the same model compounds to further investigate the effect of *intra*molecular H-bonding on the main conformation adopted in solution. No NOE signal was observed between the amide proton of the side chain and the aromatic protons of the pyrizadine central ring, indicating that those *intra*molecular H-bonds are strong enough to prevent rotation around the aryl carbon−carbon bonds of the backbone and therefore lock the structure into the predicted facially amphiphilic conformation (see Supporting Information Figure S1). Figure 6 shows the NOESY spectra of compound **7b** in DMSO-d_6 solvent in which the asymmetry of the molecule allows the differentiation between the two different amide protons (*a* and *b*). There is a NOE signal between *b* and *d* protons, indicating that the ring is involved in free rotation. However, the absence of signal between *a* and *c* protons indicates that this side of the molecule is conformationally locked because of the presence of H-bond.

Antimicrobial Activity. All the compounds were tested against three different pathogenic bacteria: two Gram-negative (*E. coli* and *K. pneumoniae*) and one Gram-positive (*S. aureus*). Their antimicrobial activity was quantified in terms of minimum inhibitory concentration (MIC), i.e., the lowest concentration of compound that inhibits bacterial growth by more than 90%. These values were determined according to the Hancock method for cationic antimicrobial peptides, which is a modification of the classical microbroth dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI).[47,48] The results are shown in Tables 2 and 3.

In general, all compounds shown in Table 2 have activity comparable to that of magainin analogue compound **17** (MSI-78),[49] an antimicrobial α-helical peptide with peptide sequence G-I-G-K-F-L-K-K-A-K-K-F-G-K-A-F-V-K-I-L-K-K-NH_2, and show better activity against Gram-positive (*S. aureus*) than Gram-negative bacteria (*E. coli* and *K. pneumoniae*). Among the *tert*-butyl containing SMAMPs, **3a**, with the benzene central ring, shows the best activity with MIC values of 3.13 μg/mL for *S. aureus* and

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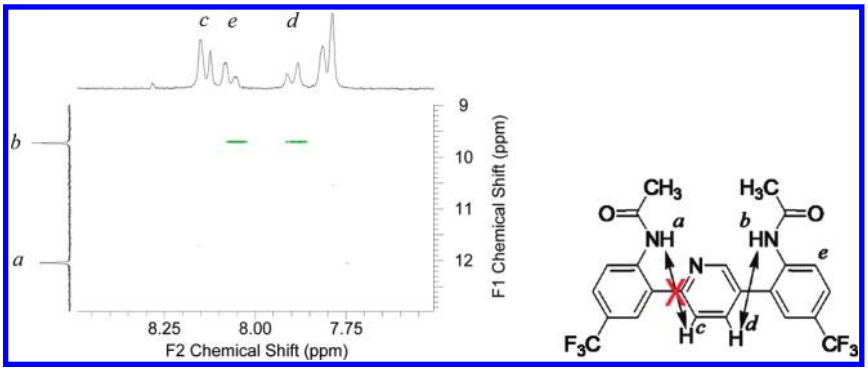


Figure 6. Partial NOESY spectra of compound **7b** in DMSO-d_6 solvent. Only NH[b] shows a NOE effect with ArH[d]. Meanwhile there is no signal between NH[a] and ArH[c], which proves the ability of the intramolecular H-bonding to restrict the rotation around the biaryl bond of the backbone.

Table 2. Biological Activity and Hydrophobicity

	MIC (μg/mL)					
compd	*S. aureus*	*E. coli*	*K. pneumoniae*	HC_{50} (μg/mL)	RT[a] (min)	log K_{ow}[b]
1a	12.5	50	100	82.53	39.3	1.89
2a	12.5	12.5	25	39.02	38.1	2.76
3a	3.13	6.25	12.5	6.46	43.3	3.95
1b	25	50	50	190.7	35.9	0.00
2b	25	50	>100	356.3	37.9	0.86
3b	3.13	12.5	25	36.46	38.9	2.05
1c	50	50	>50	>1000	20.5	−1.93
17	8−16[c]	16−32[c]	8−16[c]	120[d]		

[a] Measured by HPLC using C8 column with a gradient of 1% acetonitrile/min starting with 100% water. [b] According to KOWWIN's estimation method. [c] See ref 49. [d] See ref 32.

Table 3. Biological Activity and Hydrophobicity

	MIC (μg/mL)					
compd	*S.aureus*	*E.coli*	*K.pneumoniae*	HC_{50} (μg/mL)	RT (min)	log K_{ow}
1a	12.5	50	100	82.53	39.3	1.89
1d	12.5	25	25	nd[a]	39.9	3.86
3a	3.13	6.25	12.5	6.46	43.3	3.95
3d	3.13	6.25	12.5	34.46	44.2	5.91
3e	0.78	6.25	12.5	12.78	46.3	4.86

[a] nd = not determined.

6.25 μg/mL for *E. coli*. Changing the central ring to pyridine and pyridazine leads to a decrease in activity. In comparison to compound **3a**, compound **1a** shows 8-fold and 4-fold decreases in activity against *E. coli* and *S. aureus*, respectively. A similar trend is observed with compounds having CF_3 as the side group.

The nature of the nonpolar side group seems to impact the antimicrobial activity of the compounds as well. Within the pyridazine series, changing the side group from *tert*-butyl to CF_3 leads to a 2-fold decrease in activity against *S. aureus* but no change against *E. coli*. Compared to compound **2a**, compound **2b** with CF_3 shows a 2-fold decrease in activity against *S. aureus* and 4-fold decrease against *E. coli*. Compound **1c**, which does not have any nonpolar side group, shows poor activity for both *S. aureus* and *E. coli*. The better activity showed by the *tert*-butyl series can be attributed to the higher hydrophobicity and bulkiness of the *tert*-butyl group.

Since the compounds containing *tert*-butyl, in general, showed better activity than their CF_3 counterparts, we further expanded this series by changing the polar side group of SMAMPs **1a** and **3a** from β-alanine to aminovaleric acid. This led to the two corresponding new compounds, **1d** and **3d**, containing two intramolecular hydrogen bonds and no intramolecular hydrogen bonds, respectively. This allowed us to evaluate the effect of the polar side chain's flexibility alone or in combination with backbone rigidity on antimicrobial activity (Table 3). The incorporation of aminovaleric acid slightly improves the activity in the case of the pyridazine oligomer **1d** compared to **1a**, but this was not true in the case of benzene oligomer **3d** where no change was observed in activity. However, **1d** is still not as potent as its benzene counterpart **3d**. Compound **3e**, which replaced the primary amine with guanidines, shows no increase in activity against *E. coli* but has maximum potency against *S. aureus* with an MIC of 0.78 μg/mL.

Hydrophobicity. In order to further examine the impact of hydrophobicity, we measured the retention time (RT) of the compounds by HPLC using a C_8 column and determined log K_{ow} by software calculations. ECOSAR software (by the USA E.P.A.) was used to calculate the log K_{ow} value according the KOWWIN library. The results are listed in Tables 2 and 3. The data shows that the central ring influences the overall hydrophobicity of the molecule, with oligomers containing the central benzene ring being the most hydrophobic and those with the pyridazine ring being the least hydrophobic. For instance, compound **3b** (RT = 38.9 min and log K_{ow} = 2.05) is more hydrophobic than **1b** (RT = 35.9 min and log K_{ow} = 0.00). The major effect on hydrophobicity, however, is due to the presence of nonpolar side groups. In all the cases, compounds with *tert*-butyl side groups are more hydrophobic than their CF_3 counterparts. However, the type of polar side group (β-alanine vs aminovaleric acid) does not have a significant impact on the overall hydrophobicity of the molecule. For example, compounds **1a** and **1d** have very close retention times, 39.3 and 39.9 min, respectively.

Hemolytic Activity. In order to evaluate the cytotoxicity of these compounds toward mammalian cells, the ability to induce lysis in human erythrocytes was measured as an HC_{50} value, i.e. the lowest concentration that causes the hemolysis of 50% of red blood cells. The SMAMPs showed hemolytic activity consistent with the RTs obtained from HPLC, thereby indicating a correlation between HC_{50} and hydrophobicity. Compound **3a**, with the benzene central ring and *tert*-butyl side groups (RT = 43.3 min), is the most hemolytic in the series with HC_{50} of 6.46 μg/mL,

whereas compound **1a** (RT = 20.5 min) showed no measurable hemolysis within the given concentration range.

DISCUSSION

The design and synthesis of new peptidomimetics with potential therapeutic applications have attracted attention in recent years.[15,36] Although the exact conformational aspects responsible for the activity of SMAMPs are not known, all these compounds resemble the AMPs in terms of their charge and amphiphilicity. In our current study, we designed a novel scaffold to investigate a structure−activity relationship between the various structural and physicochemical parameters (hydrophobicity, conformational restriction) and antimicrobial activity. The difference between our scaffold and the previously studied aryl oligomers is the use of Suzuki−Miyaura coupling for the formation of a direct carbon−carbon bond between the aryl groups, instead of amides (in the case of arylamide oligomers),[22,29] ureas (in the case of urea oligomers),[31] or triple bonds (in the case of phenylene−ethynylene oligomers)[24,34] between the aryls. In this series of molecules, three aryl groups were used and the charge was kept constant at +2, which has been suggested to be the minimum requirement for antimicrobial activity.[50,51] The effect of conformational restriction and hydrophobicity was studied by varying the central ring and side chains, respectively.

Previous studies on the arylamide and arylurea oligomers showed that conformationally restricted molecules, as a result of *intra*molecular H-bonding, improved activity and selectivity.[30] Additionally, increased conformational stiffness of the compound led to better activity in vivo.[29] With this background, we decided to evaluate the effect of changing the number of H-bonds by varying the central ring from pyridazine to pyridine and then to benzene. ^{1}H NMR and NOESY studies confirmed that the pyridazine central ring locks the conformation of the molecule by two *intra*molecular H-bonds with the amide hydrogens, whereas the benzene central ring allows free rotation around the C−C aryl bonds. However, in contrast to our expectations and previous studies, pyridazine-based oligomers were less active than the benzene oligomers, while compounds with pyridine had an intermediate activity. This anomaly can be attributed to the increase in hydrophobicity of benzene-based compounds compared to pyridazine ones. Also it can be assumed that the benzene compounds, being more flexible, orient themselves better at the bacterial membrane leading to increased antibacterial activity. The comparison between compounds **1a** and **3b** in Table 2 seems to support the latter hypothesis. These two compounds in fact have similar hydrophobicity (expressed as both RT and log K_{ow}), but **1a**, with two *intra*molecular H-bonds, is less active than **3b** (MIC of 12.5 μg/mL vs 3.13 μg/mL against *S. aureus*). On the other hand, the increased potency displayed by compounds with *tert*-butyl and the fact that the benzene-based oligomers are the most hydrophobic indicate that in this series of SMAMPs, hydrophobicity is more important than conformational stiffness.

The series of compounds was further extended by modifying the polar side chains. However, the modification of the polar side chain from β-alanine to aminovaleric acid did not have a significant impact on the hydrophobicity of the compound. Compounds **1a** and **1d**, as well as **3a** and **3d**, with similar RT values, showed the same antimicrobial activity, confirming that overall hydrophobicity plays a fundamental role in controlling the activity for this class of compounds. Compound **3e** showed the best antimicrobial activity against *S. aureus* (MIC of 0.78 μg/mL), as expected because of the presence of a guanidinium group.

The hydrophobicity of all the compounds was calculated as the theoretical log K_{ow} value and compared to RT values measured by HPLC. K_{ow} is the *n*-octanol/water partition coefficient which is a common measure of compound hydrophobicity, and it has been used in various structure−activity studies for correlating many solute properties.[52] The theoretical log K_{ow} value was calculated to determine if a robust correlation between the software calculations and experimental HPLC values existed. This would enable the design of new molecules with optimum hydrophobicity using only calculations. In general, a linear correlation between the log K_{ow} and the RT values for compounds was observed in Table 2. However, some deviation was observed when the polar side chains of the molecules became more flexible (see Supporting Information Figure S2). For example, **1a** and **1d** have similar RT values (39.3 and 39.9 min) but have a considerable difference in their log K_{ow} values (1.89 and 3.86). At this point, further studies are necessary to establish the value of correlations between RT and log K_{ow} for this class of compounds. Therefore, for the present study, we chose to follow the experimental RT values to associate hydrophobicity with the activity of these SMAMPs. Plots were made with MIC vs RT for both *S. aureus* and *E. coli* (see Supporting Information Figure S3). A relatively linear trend between the activity and hydrophobicity was noticed only in the case of *S. aureus*.

Previous studies on aryl oligomers have shown that the antimicrobial and hemolytic activity is a result of a proper balance of several parameters including charge, amphiphilicity, hydrophobicity, etc. The molecules discussed in this paper have antimicrobial activities comparable to the magainin analogue compound **17**. Improving the selectivity of these compounds would require fine-tuning one or more of the parameters described above. Additional investigations are ongoing to evaluate the influence of increasing the size and number of charges on the trends observed for the present compounds. This class of molecules provides an easy synthetic tool to make new antimicrobial agents with all the advantages of abiotic structures over peptides in terms of stability and scale-up production cost for drug development.

CONCLUSION

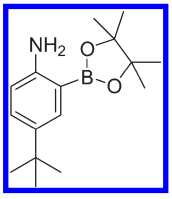
A new series of SMAMPs have been synthesized using Suzuki−Miyaura coupling in which the backbone and the side chains were systematically varied to evaluate the impact of conformational stiffness and overall hydrophobicity on the antimicrobial and hemolytic activity. The presence of *intra*molecular H-bonding and its stabilization of the oligomer conformation were demonstrated by NMR and IR spectroscopy, while hydrophobicity was evaluated by HPLC and software calculations. The data set obtained for the complete series was compared with the corresponding antimicrobial and hemolytic activity trend, expressed as MIC and HC_{50}, respectively, in order to establish a correlation between all these parameters. Analysis of the data leads to the conclusion that, for this class of molecules, the overall hydrophobicity has a more significant impact on the antimicrobial and hemolytic activity than the conformational stiffness. This is observed in particular with Gram-positive bacteria, which are more sensitive to these molecular alterations than Gram-negative bacteria. However, further investigations are ongoing to evaluate the importance of molecular size and number of positive charges, considering that a proper balance of all these features is essential for the biological activity of these SMAMPs.

EXPERIMENTAL SECTION

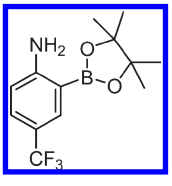
Materials. All the chemicals (reagent grade) were purchased from Aldrich, VWR, Acros, or Fisher and used as received unless otherwise indicated. Dichloromethane (DCM), pyridine, and triethylamine (TEA) were distilled over CaH_2 under nitrogen prior to use. 1,4-Dioxane was distilled from sodium/benzophenone. Column chromatography was carried out using a Combiflash-ISCO column machine.

Measurements. 2D-NOESY and ^1H and ^{13}C NMR spectra were obtained at 300 MHz or 75 MHz, using a Bruker DPX-300 NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The abbreviations for splitting patterns are the following: s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. Mass spectral data including results from high resolution mass spectrometry (HRMS) were obtained at the University of Massachusetts, Mass Spectrometry Facility. IR values were measured using a Perkin-Elmer Spectrum 100. Analytical HPLC was carried out on a Waters system using an Agilent Zorbax SB-C_8, 80 Å, 4.6 mm × 150 mm i.d. (5 μm) column, eluted by water and acetonitrile, both containing 0.1% of TFA. Detection was by UV detector at 254 nm wavelength. The elution was performed by gradually increasing the ratio of acetonitrile in water by 1%/min, starting with 100% water, with a flow rate of 1 mL/min. The purity of the final compounds as determined by analytical HPLC was, in general, greater than 95%.

Synthesis and Compound Data. *A. General Procedure for 4-Substituted 2-(Pinacolboronic ester)aniline (Borylation).* In a flame-dried Schlenk tube, to a mixture of 4-substituted 2-bromoaniline (5.81 mmol, 1 equiv) and 1,1′-bis(diphenylphosphino)ferrocenepalladium(II) dichloride dichloromethane complex (0.3 mmol, 0.05 equiv) in dry 1,4-dioxane (10 mL), TEA (23.28 mmol, 4 equiv) and pinacolborane (17.4 mmol, 3 equiv) were added dropwise under nitrogen atmosphere. The mixture was heated to 100 °C and stirred at that temperature for 3 h. The mixture, cooled to room temperature, was then quenched with saturated NH_4Cl solution (10 mL) and extracted with ethyl acetate (30 mL × 3). The combined organic layers were washed with brine (30 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography (hexanes/ethyl acetate, 90:10) to give a white powder. According to this procedure, the following compounds were synthesized.

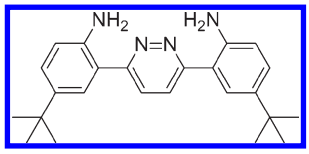


Synthesis of 4-tert-Butyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (4a). Pure compound was obtained as a white solid with 53% yield. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.33 (d, J = 2.4 Hz, 1H, ArH), 7.19 (dd, J = 2.4, 8.6 Hz, 1H, ArH), 6.53 (d, J = 8.6 Hz, 1H, ArH), 5.34 (br s, 2H, NH), 1.28 (s, 12H, Me), 1.19 (s, 9H, t-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 152.43, 136.78, 131.59, 129.97, 114.34, 83.06, 33.33, 31.40, 24.65. m/z = 275.2 (calcd), 275.3 (obtained).

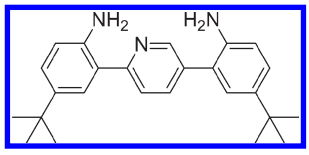


Synthesis of 2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)aniline (4b). Pure compound was obtained as a white solid with 49% yield. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.58 (d, J = 2.1 Hz, 1H, ArH), 7.42 (dd, J = 2.1, 8.7 Hz, 1H, ArH), 6.72 (d, J = 8.7 Hz, 1H, ArH), 6.14 (br s, 2H, NH), 1.30 (s, 12H, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.25, 134.34 (d, J_{CF} = 3.5 Hz), 129.64 (d, J_{CF} = 3.5 Hz), 125.12 (q, J_{CF} = 268.5 Hz), 118.52 (q, J_{CF} = 32.3 Hz), 114.26, 84.12, 24.99. m/z = 287.1 (calcd), 287.3 (obtained).

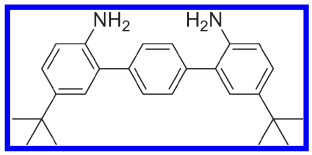
B. General Procedure for Oligomerization (Suzuki Coupling). In a Schlenk tube, Na_2CO_3 (8.4 mmol, 10 equiv) dissolved in water (~8 mL) was added to a solution of aniline boronic ester (2.1 mmol, 2.5 equiv), dibromoaryl (0.84 mmol, 1 equiv), and $PdCl_2$-(dppf)·CH_2Cl_2 catalyst (0.04 mmol, 0.05 equiv) in DMF (HPLC grade, 10 mL) at room temperature. The Schlenk tube was degassed by three freeze−pump−thaw cycles, then purged with nitrogen. The mixture was stirred at 90 °C for 18 h. The reaction mixture, cooled to room temperature, was then quenched with water (50 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexanes/ethyl acetate (80:20) eluent to give pure compound as a solid. According to this procedure, the following compounds were synthesized.



Synthesis of 2-[6-(2-Amino-5-tert-butylphenyl)pyridazin-3-yl]-4-tert-butylaniline (5a). Starting from compound **4a** and 3,6-dibromopyridazine, compound **5a** was obtained with a yield of 86%. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.15 (s, 2H, ArH), 7.52 (d, J = 2.1 Hz, 2H, ArH), 7.24 (dd, J = 2.1, 8.5 Hz, 2H, ArH), 6.81 (d, J = 8.5 Hz, 2H, ArH), 6.47 (br s, 4H, NH), 1.29 (s, 18H, t-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 158.65, 145.20, 138.12, 127.61, 126.04, 125.16, 116.65, 116.46, 33.53, 31.22. m/z = 374.3 (calcd), 375.2 (obtained).

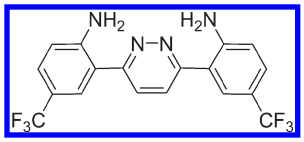


Synthesis of 2-[6-(2-Amino-5-tert-butylphenyl)pyridin-3-yl]-4-tert-butylaniline (9a). Starting from compound **4a** and 2,5-dibromopyridine, compound **9a** was obtained with a yield of 78%. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.64 (d, J = 3.0 Hz, 1H, ArH), 7.92 (dd, J = 3.0, 9.0 Hz, 1H, ArH), 7.81 (d, J = 9.0 Hz, 1H, ArH), 7.48 (d, J = 3.0 Hz, 1H, ArH), 7.14 (m, 2H, ArH), 7.05 (d, J = 3.0 Hz, 1H, ArH), 6.73 (dd, J = 3.0, 6.0 Hz, 2H, ArH), 6.29 (br s, 2H, NH), 4.80 (br s, 2H, NH), 1.28 (s, 9H, t-Bu), 1.25 (s, 9H, t-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 157.13, 147.34, 144.93, 143.01, 139.00, 137.86, 137.01, 132.69, 126.54, 126.47, 125.53, 125.20, 121.47, 119.91, 116.18, 115.30, 33.43, 33.38, 31.27. m/z = 373.3 (calcd), 374.3 (obtained).

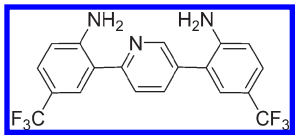


Synthesis of 2-[4-(2-Amino-5-tert-butylphenyl)phenyl]-4-tert-butylaniline (10a). Starting from compound **4a** and 1,6-dibromobenzene, compound **10a** was obtained with a yield of 88%. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.47 (s, 4H, ArH), 7.09 (dd, J = 2.1, 8.4 Hz, 2H, ArH), 7.02 (d, J = 2.1 Hz, 2H, ArH), 6.70 (d, J = 8.4 Hz, 2H, ArH), 4.68 (br s,

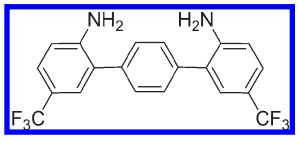
4H, NH), 1.24 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 142.48, 138.77, 138.30, 128.86, 126.36, 124.89, 124.86, 114.99, 33.39, 31.36. m/z = 372.3 (calcd), 372.3 (obtained).



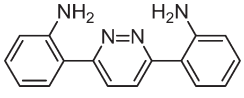
Synthesis of 2-{6-[2-Amino-5-(trifluoromethyl)phenyl]pyridazin-3-yl}-4-(trifluoromethyl)aniline (5b). Starting from compound **4b** and 3,6-dibromopyridazine, compound **5b** was obtained with a yield of 50%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.35 (s, 2H, ArH), 7.97 (br s, 2H, ArH), 7.47 (br s, 6H, ArH + NH), 7.00 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 157.92, 151.01, 127.22 (d, J_{CF} = 3.5 Hz), 126.61 (d, J_{CF} = 3.5 Hz), 126.38, 125.14 (q, J_{CF} = 268.6 Hz), 116.74, 115.78 (q, J_{CF} = 32.1 Hz), 115.79. m/z = 398.1 (calcd), 399.1 (obtained).



Synthesis of 2-{6-[2-Amino-5-(trifluoromethyl)phenyl]pyridin-3-yl}-4-(trifluoromethyl)aniline (9b). Starting from compound **4b** and 2,5-dibromopyridine, compound **9b** was obtained with a yield of 76%.^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.68 (br s, 1H, ArH), 7.97 (br s, 2H, NH), 7.86 (br s, 1H, ArH), 7.42 (d, J = 8.2 Hz, 2H, ArH), 7.31 (m, 3H, ArH), 6.90 (t, J = 8.2 Hz, 2H, ArH), 5.77 (br s, 2H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.62, 149.76, 147.94, 147.00, 137.72, 131.70, 127.80 (m), 127.05 (m), 126.66 (m), 122.81, 122.08, 120.38 (q, J_{CF} = 32.7 Hz), 120.10, 119.21 (q, J_{CF} = 32.6 Hz), 117.04, 115.41. m/z = 397.1 (calcd), 398.1 (obtained).

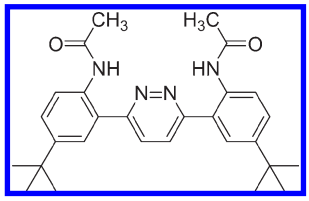


Synthesis of 2-{4-[2-Amino-5-(trifluoromethyl)phenyl]phenyl}-4-(trifluoromethyl)aniline (10b). Starting from compound **4b** and 1,6-dibromobenzene, compound **10b** was obtained as a white solid with a yield of 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.50 (s, 4H, ArH), 7.38 (dd, J = 1.8, 8.5 Hz, 2H, ArH), 7.27 (d, J = 1.8 Hz, 2H, ArH), 6.88 (d, J = 8.5 Hz, 2H, ArH), 5.61 (br s, 4H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 149.07, 137.21, 129.36, 127.10, 126.75 (d, J_{CF} = 3.8 Hz), 125.39, 124.81, 123.52, 116.32 (q, J_{CF} = 31.7 Hz), 114.64. m/z = 396.1 (calcd), 396.1 (obtained).

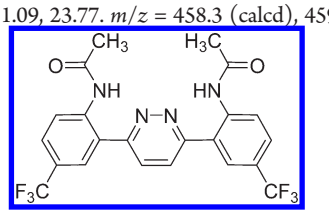


Synthesis of 2-[6-(2-Aminophenyl)pyridazin-3-yl]aniline (5c). Starting from compound **4c** (commercially available) and 3,6-dibromopyridazine, compound **5c** was obtained with a yield of 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.17 (s, 2H, ArH), 7.66 (d, J = 7.9 Hz, 2H, ArH), 7.16 (t, J = 7.1 Hz, 2H, ArH), 6.85 (m, 6H, ArH + NH), 6.67 (t, J = 7.1 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.11, 147.23, 131.03, 129.12, 126.00, 118.37, 117.72, 117.56. m/z = 262.1 (calcd), 263.1 (obtained).

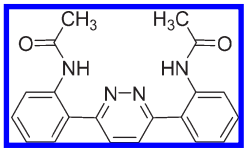
C. General Procedure for N-Acetylation. A large excess of acetyl chloride was added to a mixture of oligomer and 20% iodine. The mixture was stirred at room temperature, and the reaction time was monitored by thin-layer chromatography (TLC). After the completion of the reaction, iodine was quenched by a saturated aqueous solution of $Na_2S_2O_3$ and the product extracted using ethyl acetate (30 mL × 3). The combined organic layer was washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography and eluted with hexanes/ethyl acetate (60:40) to give pure compound as a solid. According to this procedure, the following compounds were synthesized.



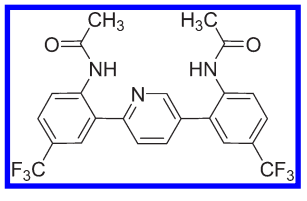
Synthesis of N-{4-tert-Butyl-2-[6-(5-tert-butyl-2-acetamidophenyl)pyridazin-3-yl]phenyl}acetamide (6a). Starting from compound **5a**, compound **6a** was obtained with 55% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.40 (br s, 2H, NH), 8.08 (s, 2H, ArH), 7.82 (d, J = 8.5 Hz, 2H, ArH), 7.69 (d, J = 2.2 Hz, 2H, ArH), 7.54 (dd, J = 2.2, 8.5 Hz, 2H, ArH), 1.97 (s, 6H, Me), 1.33 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.39, 158.45, 147.29, 133.85, 127.77, 127.13, 126.58, 124.41, 34.32, 31.09, 23.77. m/z = 458.3 (calcd), 459.3 (obtained).



Synthesis of N-(2-{6-[2-Acetamido-5-(trifluoromethyl)phenyl]pyridazin-3-yl}-4-(trifluoromethyl)phenyl)acetamide (6b). Starting from compound **5b**, compound **6b** was obtained with 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.86 (br s, 2H, NH), 8.8 (d, J = 8.7 Hz, 2H, ArH), 8.15 (s, 2H, ArH), 7.90 (br s, 2H, ArH), 7.76 (d, J = 8.7 Hz, 2H, ArH), 2.25 (s, 6H, Me). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.96, 157.74, 140.18, 128.49, 127.19 (m), 126.96, 125.91, 124.70 (q, J_{CF} = 32.4 Hz), 123.86, 122.31, 24.19. m/z = 482.1 (calcd), 482.2 (obtained).

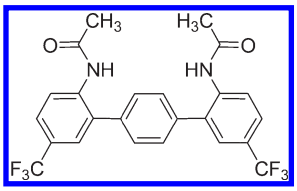


Synthesis of N-{2-[6-(2-Acetamidophenyl)pyridazin-3-yl]phenyl}acetamide (6c). Starting from compound **5c**, compound **6c** was obtained with 65% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.75 (br s, 2H, NH), 8.15 (s, 2H, ArH), 8.03 (d, J = 7.7 Hz, 2H, ArH), 7.81 (dd, J = 1.3, 7.7 Hz, 2H, ArH), 7.52 (t, J = 7.1 Hz, 2H, ArH), 7.35 (t, J = 7.1 Hz, 2H, ArH), 2.02 (s, 6H, Me). ^{13}C NMR (75 MHz. DMSO-d_6) δ: 168.41, 158.29, 136.56, 130.28, 130.03, 127.84, 127.20, 124.72, 123.94, 23.99. m/z = 364.1 (calcd), 346.2 (obtained).



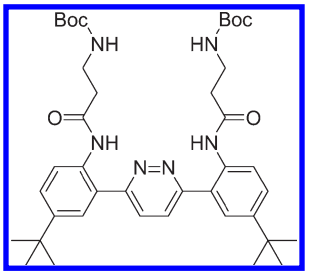
Synthesis of N-(2-{6-[2-Acetamido-5-(trifluoromethyl)phenyl]-pyridin-3-yl}-4-(trifluoromethyl)phenyl)acetamide (7b). Starting from compound **9b**, compound **7b** was obtained with 51% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.11 (br s, 1H, NH), 9.72 (br s, 1H, NH), 8.86 (d, J = 2.1 Hz, 1H, ArH), 8.57 (d, J = 8.7 Hz, 1H, ArH), 8.18

(m, 2H, ArH), 8.09 (dd, J = 2.1, 8.4 Hz, 1H, ArH), 7.93 (d, J = 8.7 Hz, 1H, ArH), 7.81 (d + s, J = 8.4 Hz, 3H, ArH), 2.16 (s, 3H, Me), 1.98 (s, 3H, Me). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.43, 169.22, 154.99, 148.33, 141.02, 139.74, 138.82, 132.98, 132.16, 127.73, 126.45(m), 123.76, 122.46, 25.22, 23.73. m/z = 481.1 (calcd), 481.2 (obtained).

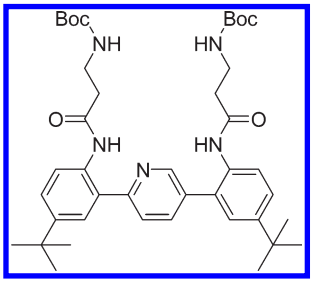


Synthesis of N-{2-{4-[2-Acetamido-5-(trifluoromethyl)phenyl]phenyl-4-(trifluoromethyl)phenyl}acetamide (8b). Starting from compound **10b**, compound **8b** was obtained with 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.39 (br s, 2H, NH), 7.93 (d, J = 8.8 Hz, 2H, ArH), 7.74 (d, J = 8.8 Hz, 2H, ArH), 7.64 (br s, 2H, ArH), 7.57 (s, 4H, ArH), 1.98 (s, 6H, Me). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.03, 138.95, 136.83, 135.04, 129.25, 126.75, 126.30, 125.95, 125.36, 124.86, 122.35, 23.32. m/z = 480,1 (calcd), 480.2 (obtained).

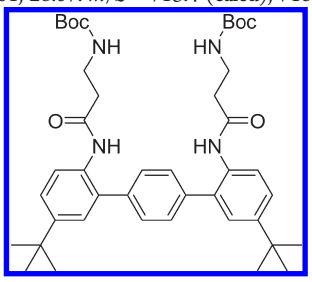
D. General Procedure for EDC/HOBT Coupling. In a round-bottom flask under nitrogen atmosphere, the proper diaminearyl oligomer (0.5 mmol, 1 equiv), Boc-β-alanine (1.5 mmol, 3 equiv), and HOBT (1.5 mmol, 3 equiv) were dissolved in dry DCM (10 mL). The mixture was cooled to 0 °C, and EDC (1.5 mmol, 3 equiv) was added. The reaction mixture was stirred at room temperature overnight, then quenched with water (10 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography and eluted with hexanes/ethyl acetate (60:40). According to this procedure, the following compounds were synthesized as solids.



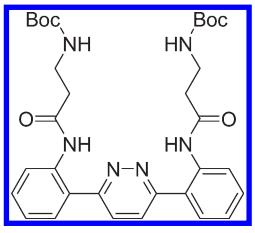
Synthesis of tert-Butyl N-{2-[(2-{6-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-tert-butylphenyl]pyridazin-3-yl}-4-tert-butylphenyl)carbamoyl]ethyl}carbamate (11a). According to the procedure described above, using compound **5a** as starting oligomer, compound **11a** was obtained with 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.46 (br s, 2H, NH), 8.06 (s, 2H, ArH), 7.84 (d, J = 8.4 Hz, 2H, ArH), 7.70 (br s, 2H, ArH), 7.55 (d, J = 8.4 Hz, 2H, ArH), 6.81 (m, 2H, NH), 3.16 (m, 4H, CH_2), 2.40 (t, J = 6.9 Hz, 4H, CH_2), 1.34 (s, 18H, t-Boc), 1.31 (s, 18 h, t-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.31, 159.34, 156.03, 147.22, 134.69, 128.57, 127.87, 125.78, 122.87, 79.18, 37.75, 36.63, 34.63, 31.38, 28.42. m/z = 716.4 (calcd), 717.9 (obtained).



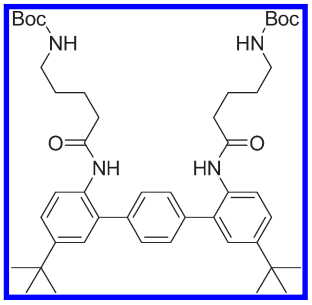
Synthesis of tert-Butyl N-{2-[(2-{6-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-tert-butylphenyl]pyridin-3-yl}-4-tert-butylphenyl)carbamoyl]ethyl}carbamate (12a). According to the procedure described above, using compound **9a** as starting oligomer, compound **12a** was obtained with 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.58 (br s, 1H, NH), 9.46 (br s, 1H, NH), 8.71 (s, 1H, ArH), 8.16 (d, J = 8.7 Hz, 1H, ArH), 7.95 (s, 2H, ArH), 7.74 (s, 1H, ArH), 7.46 (m, 3H, ArH), 7.40 (s, 1H, ArH), 6.85 (m, 1H, NH), 6.79 (m, 1H, NH), 3.23 (m, 2H, CH_2), 3.12 (m, 2H, CH_2), 2.47 (t, J = 7.2 Hz, 2H, CH_2), 2.33 (t, J = 7.2 Hz, 2H, CH_2), 1.34 (s, 18H, t-Boc), 1.32 (s, 18H, t-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.76, 168.95, 155.63, 155.36, 148.53, 147.45, 146.03, 137.66, 134.28, 133.41, 132.52, 132.19, 126.89, 126.64, 126.29, 125.71, 125.44, 122.49, 122.08, 77.47, 37.35, 36.51, 36.06, 34.19, 34.10, 31.01, 28.07. m/z = 715.4 (calcd), 716.9 (obtained).



Synthesis of tert-Butyl N-{2-[(2-{4-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-tert-butylphenyl]phenyl}-4-tert-butylphenyl)carbamoyl]ethyl}carbamate (13a). According to the procedure described above, using compound **10a** as starting oligomer, compound **13a** was obtained with 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.25 (br s, 2H, NH), 7.44 (s, 4H, ArH), 7.39 (s, 4H, ArH), 7.30 (s, 2H, ArH), 6.77 (m, 2H, NH), 3.15 (m, 4H, CH_2), 2.33 (t, J = 7.5 Hz, 4H, CH_2), 1.34 (s, 18H, t-Boc), 1.32 (s, 18H, t-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.01, 155.53, 148.40, 138.19, 135.78, 132.28, 128.81, 127.22, 126.75, 124.70, 77.63, 36.71, 36.27, 34.29, 31.22, 28.26. m/z = 714.4 (calcd), 715.9 (obtained).



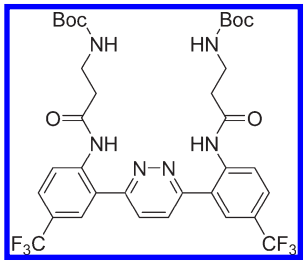
Synthesis of tert-Butyl N-{2-[(2-{6-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)phenyl]pyridazin-3-yl}phenyl)carbamoyl]ethyl}carbamate (11c). According to the procedure described above, using compound **5c** as starting oligomer, compound **11c** was obtained with 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.82 (br s, 2H, NH), 8.14 (s, 2H, ArH), 8.04 (d, J = 7.2 Hz, 2H, ArH), 7.82 (d, J = 6.8 Hz, 2H, ArH), 7.52 (m, 2H, ArH), 7.35 (m, 2H, ArH), 6.83 (br s, 2H, NH), 3.19 (m, 4H, CH_2), 2.45 (m, 4H, CH_2), 1.32 (s, 18H, t-Boc). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.59, 158.29, 155.52, 136.49, 130.29, 130.04, 127.82, 127.19, 124.79, 124.03, 77.64, 36.99, 36.51, 28.19. m/z = 604.3 (calcd), 605.2 (obtained).



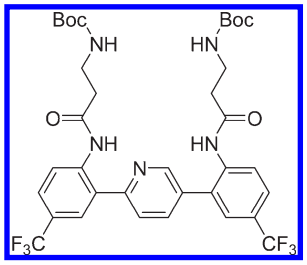
dx.doi.org/10.1021/jm101410t |J. Med. Chem. 2011, 54, 2241–2254

Synthesis of tert-Butyl N-{4-[(2-{4-[2-(5-{[(tert-Butoxy)carbonyl]amino}pentanamido)-5-tert-butylphenyl]phenyl}-4-tert-butylphenyl)carbamoyl]butyl}carbamate (14). By use of the same procedure, but using *N*-Boc-5-aminovaleric acid instead of alanine, compound **14** was synthesized with 60% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.15 (s, 2H, NH), 7.44 (s, 4H, ArH), 7.39 (s, 4H, ArH), 7.30 (s, 2H, ArH), 6.80 (br s, 2H, NH), 2.90 (m, 4H, CH_2), 2.18 (m, 4H, CH_2), 1.50 (m, 4H, CH_2), 1.35 (s, 22H, CH_2 + *t*-Boc), 1.32 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 171.43, 155.31, 148.00, 137.90, 135.49, 132.14, 128.52, 126.92, 126.43, 124.44, 77.05, 54.67, 34.99, 33.96, 30.90, 28.86, 27.99, 22.19. m/z = 770.5 (calcd), 771.8 (obtained).

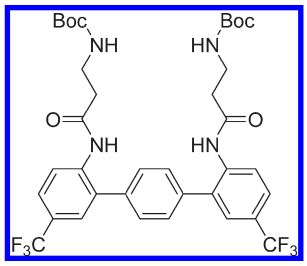
E. General Procedure for $POCl_3$ Coupling. In a round-bottom flask under nitrogen atmosphere, a specific diamine aryl oligomer (0.5 mmol, 1 equiv) and Boc-β-alanine (1.25 mmol, 2.5 equiv) were dissolved in dry pyridine (5 mL). Once the temperature was cooled to 0 °C, $POCl_3$ (1.25 mmol, 2.5 equiv) was added dropwise. The reaction mixture was stirred at that temperature for 1 h. Ethyl acetate was added and the organic layer washed with brine. Pyridine was removed by washing quickly with 1 M HCl. The organic phase was then washed with a saturated solution of $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash column chromatography with hexanes/ethyl acetate (60:40) eluent. According to this procedure, the following compounds were obtained as solids.



Synthesis of tert-Butyl N-{2-[(2-{6-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-(trifluoromethyl)phenyl]pyridazin-3-yl}-4-(trifluoromethyl)phenyl)carbamoyl]ethyl}carbamate (11b). According to the procedure described above, using compound **5b** as starting oligomer, compound **11b** was obtained with a purity of about 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.99 (br s, 2H, NH), 8.81 (d, J = 8.7 Hz, 2H, ArH), 8.16 (s, 2H, ArH), 7.92 (s, 2H, ArH), 7.78 (d, J = 8.7 Hz, 2H, ArH), 5.30 (br s, 2H, NH), 3.52 (m, 4H, CH_2), 2.74 (m, 4H, CH_2), 1.35 (s, 18H, *t*-Boc).

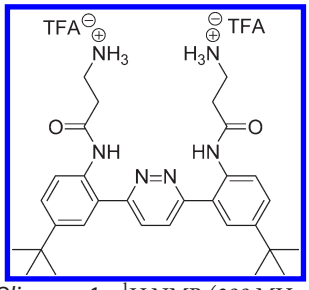


Synthesis of tert-Butyl N-{2-[(2-{6-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-(trifluoromethyl)phenyl]pyridin-3-yl}-4-(trifluoromethyl)phenyl)carbamoyl]ethyl}carbamate (12b). According to the procedure described above, using compound **9b** as starting oligomer, compound **12b** was obtained with 50% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.30 (br s, 1H, NH), 9.74 (br s, 1H, NH), 8.85 (br s, 1H, ArH), 8.59 (d, J = 8.7 Hz, 1H, ArH), 8.17 (d, J = 8.3 Hz, 2H, ArH), 8.09 (dd, J = 2.0, 8.3 Hz, 1H, ArH), 7.95 (d, J = 8.3 Hz, 1H, ArH), 7.80 (m, 3H, ArH), 6.91 (m, 1H, NH), 6.84 (m, 1H, NH), 3.27 (m, 2H, CH2), 3.14 (m, 2H, CH2), 2.55 (t, J = 6.8 Hz, 2H, CH2), 2.39 (t, J = 6.8 Hz, 2H, CH2), 1.33 (s, 9H, *t*-Boc), 1.29 (s, 9H, *t*-Boc). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.11, 169.91, 155.54, 154.58, 147.81, 140.66, 139.21, 138.43, 132.52, 131.73, 127.13, 126.59, 126.47, 126.01, 125.90, 125.77, 125.68, 124.04, 123.61, 123.10, 122.41, 122.29, 122.07, 77.65, 37.93, 36.57, 36.47, 28.18, 28.13. m/z = 739.3 (calcd), 739.3 (obtained).

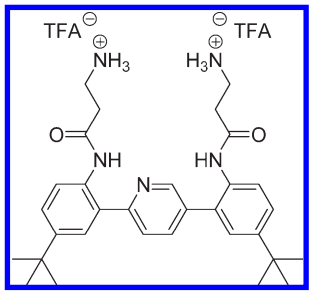


Synthesis of tert-Butyl N-{2-[(2-{4-[2-(3-{[(tert-Butoxy)carbonyl]amino}propanamido)-5-(trifluoromethyl)phenyl]phenyl}-4-(trifluoromethyl)phenyl)carbamoyl]ethyl}carbamate (13b). According to the procedure described above, using compound **10b** as starting oligomer, compound **13b** was obtained with 50% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.45 (br s, 2H, NH), 7.93 (d, J = 8.6 Hz, 2H, ArH), 7.74 (d, J = 8.6 Hz, 2H, ArH), 7.63 (br s, 2H, ArH), 7.57 (s, 4H, ArH), 6.83 (m, 2H, NH), 3.17 (m, 4H, CH_2), 2.40 (t, J = 6.9 Hz, 4H, CH_2), 1.35 (s, 18H, *t*-Boc). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.19, 155.57, 138.79, 136.89, 135.19, 129.29, 126.82, 126.57, 125.99, 124.84, 122.34, 77.65, 36.50, 28.21. m/z = 738.3 (calcd), 739.3 (obtained).

F. General Procedure for Boc Deprotection. *t*-Boc protected oligomer (0.16 mmol) was dissolved in dry DCM (1.5 mL), and trifluoroacetic acid (TFA) was added (0.5 mL). After 1 h the product was precipitated by a mixture of cold hexane and ethyl ether and filtrated. The pure product, achieved as a salt with TFA, was dried under vacuum overnight. The following compounds were obtained in a quantitative yield.

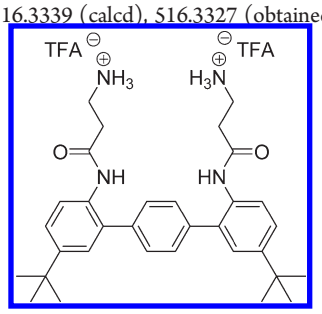


Synthesis of Oligomer 1a. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.58 (br s, 2H, NH), 8.09 (s, 2H, ArH), 7.78 (m, 10H, ArH + NH), 7.59 (d, J = 8.4 Hz, 2H, ArH), 3.02 (m, 4H, CH_2), 2.63 (t, J = 6.5 Hz, 4H, CH_2), 1.35 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.50, 158.45, 147.83, 133.29, 128.41, 127.77, 127.21, 126.72, 124.72, 35.02, 34.42, 33.22, 31.14. HRMS m/z = 517.3291 (calcd), 517.3293 (obtained).

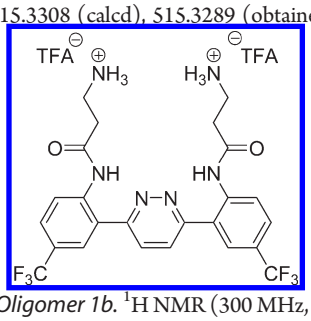


Synthesis of Oligomer 2a. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.44 (br s, 1H, NH), 9.71 (br s, 1H, NH), 8.72 (s, 1H, ArH), 7.99 (m, 3H, ArH), 7.74 (br s, 7H, ArH + NH), 7.49 (m, 3H, ArH), 7.41 (s, 1H, ArH), 3.06 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 2.69 (t, J = 6.6 Hz, 2H, CH_2), 2.55 (t, J = 6.9 Hz, 2H, CH_2), 1.33 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.93, 168.2, 155.72, 148.99, 147.78, 146.76, 137.79, 133.95, 133.57, 132.32, 127.49, 127.07, 126.92, 126.51, 126.06,

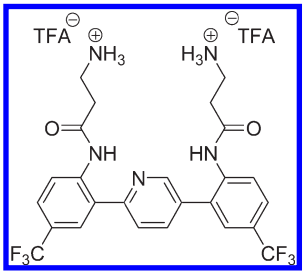
125.70, 122.86, 119.22, 115.25, 35.15, 34.42, 34.34, 33.87, 32.63, 31.18. HRMS m/z = 516.3339 (calcd), 516.3327 (obtained).



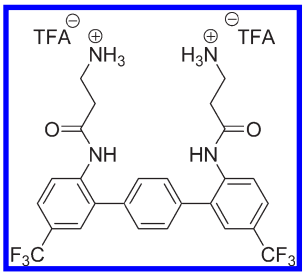
Synthesis of Oligomer 3a. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.53 (br s, 2H, NH), 7.76 (br s, 6H, NH), 7.43 (m, 8H, ArH), 7.32 (s, 2H, ArH), 3.01 (m, 4H, CH_2), 2.56 (t, J = 6.6 Hz, 4H, CH_2), 1.33 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.81, 148.59, 137.96, 135.49, 131.66, 128.64, 127.15, 126.58, 124.67, 34.99, 34.16, 32.37, 31.03. HRMS m/z = 515.3308 (calcd), 515.3289 (obtained).



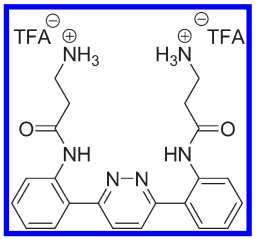
Synthesis of Oligomer 1b. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.02 (br s, 2H, NH), 8.32 (s, 2H, ArH), 8.29 (d, J = 8.6 Hz, 2H, ArH), 8.11 (br s, 2H, ArH), 7.96 (d, J = 8.6 Hz, 2H, ArH), 7.77 (br s, 6H, NH), 3.07 (m, 4H, CH_2), 2.71 (t, J = 6.6 Hz, 4H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.98, 157.63, 139.67, 128.33, 127.82, 127.23 (m), 125.87, 125.16 (q, J_{CF} = 32.3 Hz), 124.44, 122.27, 34.79, 33.58. HRMS m/z = 541.1787 (calcd), 541.1768 (obtained).



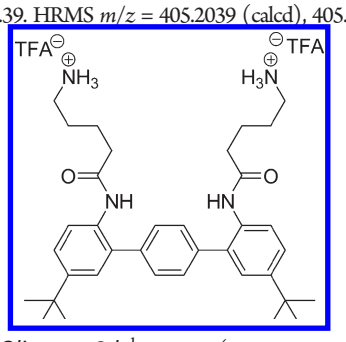
Synthesis of Oligomer 2b. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.11 (s, 1H, NH), 10.02 (s, 1H, NH), 8.83 (s, 1H, ArH), 8.52 (d, J = 8.6 Hz, 1H, ArH), 8.15 (m, 3H, ArH), 7.96 (d, J = 8.6 Hz, 1H, ArH), 7.83 (m, 9H, ArH + NH), 3.10 (m, 2H, CH_2), 3.03 (m, 2H, CH_2), 2.80 (t, J = 6.6 Hz, 2H, CH_2), 2.62 (t, J = 6.5 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.02, 168.84, 154.48, 147.88, 140,08, 138.80, 138.35, 132.49, 131.91, 127.23 (br s), 126.86, 126.61 (br s), 126.25 (br s), 125.74 (br s), 125.48, 125.38, 124.51, 124.19, 123.24, 122.74, 34.89, 34.84, 32.81. HRMS m/z = 540.1834 (calcd), 540.1843 (obtained).



Synthesis of Oligomer 3b. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.83 (br s, 2H, NH), 7.85 (m, 10H, ArH + NH), 7.61 (m, 6H, ArH), 3.05 (m, 4H, CH_2), 2.65 (m, 4H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.10, 138.36, 136.83, 135.28, 129.21, 126.87, 126.22, 125.91, 124.89, 122.3, 34.87, 32.78. HRMS m/z = 539.1882 (calcd), 539.1900 (obtained).

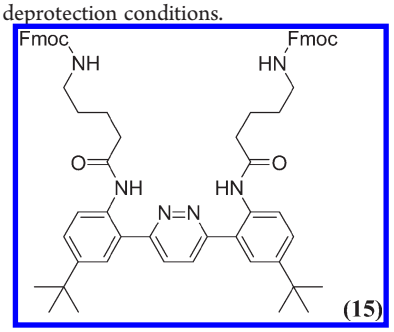


Synthesis of Oligomer 1c. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.84 (br s, 2H, NH), 8.13 (s, 2H, ArH), 7.97 (d, J = 7.9 Hz, 2H, ArH), 7.81 (d, J = 6.6 Hz, 8H, ArH + NH), 7.55 (dd, J = 6.6, 7.5 Hz, 2H, ArH), 7.38 (t, J = 7.5 Hz, 2H, ArH), 3.04 (br s, 4H, CH_2), 2.66 (m, 4H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.49, 158.25, 135.94, 130.3, 130.15, 128.1, 127.76, 125.28, 124.47, 34.97, 33.39. HRMS m/z = 405.2039 (calcd), 405.2012 (obtained).



Synthesis of Oligomer 3d. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.26 (s, 2H, NH), 7.75 (br s, 6H, NH), 7.46 (s, 4H, ArH), 7.39 (br s, 4H, ArH), 7.31 (s, 2H, ArH), 2.76 (br s, 4H, CH_2), 2.22 (br s, 4H, CH_2), 1.56 (br s, 8H, CH_2), 1.33 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 171.24, 148.23, 137.92, 135.61, 132.06, 128.51, 127.07, 126.49, 124.51, 34.59, 34.02, 30.93, 26.40, 21.72. HRMS m/z = 571.4012 (calcd), 571.3987 (obtained).

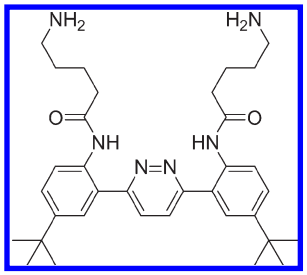
G. Synthesis of Compound ***1d***. Compound **1d** was obtained using Fmoc chemistry because of an unexpected instability toward the common *t*-Boc deprotection conditions.



Synthesis of 9H-Fluoren-9-ylmethyl N-{4-[(4-tert-Butyl-2-{6-[5-tert-butyl-2-(5-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}pentanamido)phenyl]pyridazin-3-yl}phenyl)carbamoyl]butyl}carbamate (15). In a round-bottom flask under nitrogen atmosphere, compound **5a** (0.5 g, 1.34 mmol), Fmoc-5-aminopentanoic acid (1.36 g, 4 mmol), and HOBT (0.54 g, 4 mmol) were dissolved in dry THF (20 mL). The mixture was cooled to 0 °C, and EDC (0.77 g, 4 mmol) was added. The reaction mixture was stirred at room temperature overnight, then quenched with water (10 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product

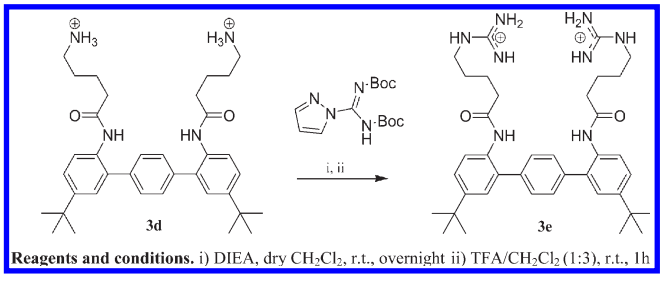
was filtered over a pad of basic alumina to remove the amino acid in excess and then purified by flash column chromatography using hexanes/ethyl acetate (60:40) eluent to obtain compound **15** in 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.39 (br s, 2H, NH), 8.45 (d, J = 8.4 Hz, 2H, ArH), 7.99 (s, 2H, ArH), 7.71 (d, J = 7.5 Hz, 4H, ArH), 7.54 (m, 8H, FmocH), 7.34 (t, J = 7.5 Hz, 4H, FmocH), 7.25 (m, 4H, FmocH), 5.15 (br s, 2H, NH), 4.31 (d, J = 6.9 Hz, 4H, FmocH), 4.12 (t, J = 6.9 Hz, 2H, FmocH), 3.19 (m, 4H, CH_2), 2.44 (m, 4H, CH_2), 1.77 (m, 4H, CH_2), 1.57 (m, 4H, CH_2), 1.37 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.43, 159.51, 156.62, 147.12, 144.08, 141.37, 134.99, 128.69, 127.95, 127.73, 127.12, 125.79, 125.18, 122.93, 122.83, 120.03, 66.63, 47.32, 40.74, 37.68, 34.66, 31.32, 29.56, 22.56. m/z = 1016.5 (calcd), 1018.4 (obtained).

The Fmoc group was then removed using literature procedure[53] to obtain **1d**.

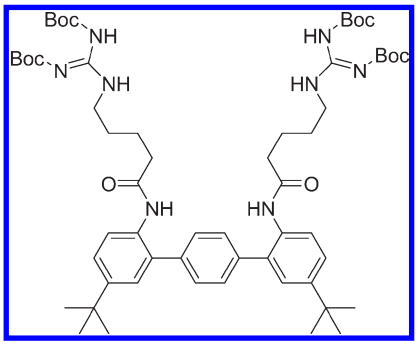


Synthesis of 5-Amino-N-(2-{6-[2-(5-aminopentanamido)-5-tert-butylphenyl]pyridazin-3-yl}-4-tert-butylphenyl)pentanamide (1d). Compound **15** (0.6 g, 0.6 mmol) was dissolved in THF (10 mL) with 1-hexanethiol (0.8 mL, 6 mmol). Then DBU (4.5 μL, 0.03 mmol) was added dropwise and the mixture allowed to stir for 24 h. After removal of solvents under reduced pressure, compound **1d** was precipitated from ethyl ether as a white solid (60% yield). ^{1}H NMR (300 MHz, CD_3OD) δ: 8.11 (s, 2H, ArH), 7.87 (d, J = 8.5 Hz, 2H, ArH), 7.78 (d, J = 2.1 Hz, 2H, ArH), 7.62 (dd, J = 2.1, 8.5 Hz, 2H, ArH), 2.65 (t, J = 7.1 Hz, 4H, CH_2), 2.38 (t, J = 7.2 Hz, 4H, CH_2), 1.66 (m, 4H, CH_2), 1.51 (m, 4H, CH_2), 1.42 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, MeOD) δ: 174.27, 160.73, 150.09, 134.68, 129.57, 129.48, 128.83, 127.91, 125.80, 41.71, 37.55, 35.55, 32.10, 31.68, 23.82. HRMS m/z = 573.3917 (calcd), 573.3892 (obtained).

H. Synthesis of Compound ***3e***. Compound **3e** was directly derived from **3d** by addition of guanidinium group to the terminal amines, followed by *t*-Boc deprotection.

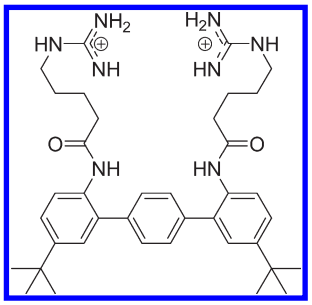


Reagents and conditions. i) DIEA, dry CH_2Cl_2, r.t., overnight ii) TFA/CH_2Cl_2 (1:3), r.t., 1h



Synthesis of tert-Butyl N-[(1E)-({4-[(2-{4-[2-(5-{[(1E)-{[(tert-Butoxy)carbonyl]amino}({[(tert-butoxy)carbonyl]imino})methyl]-amino}pentanamido)-5-tert-butylphenyl]phenyl}-4-tert-butylphenyl)carbamoyl]butyl}amino)({[(tert-butoxy)carbonyl]imino})-methyl]carbamate (16). Compound **3d** (1 g, 1.25 mmol) was dissolved in dry CH_2Cl_2 under nitrogen atmosphere and *N*,*N*-diisopropylethylamine (i.e., DIEA) (1 mL, 5.6 mmol) was added. After few minutes, *N*,*N*′-bis-Boc-1-guanylpyrazole (0.85 g, 2.75 mmol) was added and the mixture stirred overnight at room temperature. After addition of ethyl acetate, the solution was washed with an aqueous solution of 10% $KHSO_4$ and extracted three more times with ethyl acetate. The organic layers were washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. Compound **16** was purified by flash column chromatography (hexanes/ethyl acetate 60:40) and obtained as a solid with 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.47 (s, 2H, NH), 8.35 (br s, 2H, NH), 8.03 (d, J = 8.5 Hz, 2H, ArH), 7.49 (s, 4H, ArH), 7.41 (dd, J = 2.1, 8.5 Hz, 2H, ArH), 7.33 (m, 4H, ArH+NH), 3.39 (d, J = 6.9 Hz, 4H, CH_2), 2.29 (t, J = 6.9 Hz, 4H, CH_2), 1.71 (d, J = 7.0 Hz, 4H, CH_2), 1.63 (d, J = 7.0 Hz, 4H, CH_2), 1.45 (s, 18H, *t*-Boc), 1.44 (s, 18H, *t*-Boc), 1.35 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 171.58, 171.55, 170.36, 163.15, 155.25, 152.13, 138.17, 132.42, 128.84, 126.68, 124.71, 82.88, 78.07, 66.95, 35.22, 34.19, 31.15, 28.28, 27.97, 27.59. m/z = 1054.7 (calcd), 1055.6 (obtained).

Then the *t*-Boc group was removed according to procedure F to give compound **3e** as a salt in a quantitative yield.



Synthesis of Oligomer 3e. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.23 (s, 2H, NH), 7.54 (br s, 2H, NH), 7.42 (s, 4H, ArH), 7.37 (d, J = 6.5 Hz, 4H, ArH), 7.28 (s, 2H, ArH), 6.5−7.5 (br s, 6H, NH), 3.06 (d, J = 5.9 Hz, 4H, CH_2), 2.19 (m, 4H, CH_2), 1.51 (m, 4H, CH_2), 1.43 (m, 4H, CH_2), 1.30 (s, 18H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 156.91, 148.69, 138.30, 136.16, 132.40, 128.82, 127.51, 126.84, 124.90, 40.57, 35.06, 34.35, 31.26, 28.13. HRMS m/z = 655.4448 (calcd), 655.4460 (obtained).

Antimicrobial Activity. All biological testing was conducted by Polymedix, Inc. (Philadelphia, PA) using a modified microbroth dilution assay recommended by the Clinical and Laboratory Standards Institute (CLSI) which has been developed for determining in vitro antimicrobial activities of cationic agents. Modifications were made to minimize loss of the antimicrobial agent due both to adsorption onto glass or plastic surfaces and to the precipitation at high concentrations. Bacteria were grown in Mueller−Hinton broth (MH broth) at 37 °C overnight, and the bacterial growth was measured by turbidity as optical density at λ = 600 (OD_{600}) using an Eppendorf BioPhotometer. Compounds were first dissolved in DMSO and Hancock solution (0.01% acetic acid, 0.2% bovine serum albumin) to make 2-fold dilution stock series and then diluted 10-fold to cell culture in 96-well plates to be tested in duplicate at 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.2, 0.1, and 0.05 μg/mL. Minimal inhibitory concentrations (MICs) were obtained by measuring cell growth at OD_{600} after incubation with compounds for 18 h at 37 °C. Each compound was tested as a di-TFA salt, except for compound **1d** which was tested as diamine, against ATCC bacterial strains (*E. coli* 25922, *S. aureus* 27660, and *K. pneumonia* 13883).

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Hemolytic Activity. HC_{50} was determined by measuring the quantity of hemoglobin released from red blood cells (RBCs) after their lysis. RBCs collected by centrifugation from human whole blood were diluted in a TBS solution to obtain a 0.22% RBC stock suspension. In a 96-well plate, serial 1:2 dilutions of each compound in water were added to the RBC solution (final concentrations tested: from 1000 μg/mL to lower) and the plate was incubated in a shaker at 37 °C for 1 h. After centrifugation at 3000 rpm for 5 min, 30 μL of supernatant was removed and added to 100 μL of H_2O in a sterile polystyrene 96-well flat bottom plate. Hemoglobin concentration in the supernatant was read at OD_{405}. Melittin was used as a positive control, and the most concentrated sample (200 μg/mL) was used as a reference for 100% hemolysis. A control solution without compound was used as a reference for 0% hemolysis.

■ ASSOCIATED CONTENT

Supporting Information. Correlation between log K_{ow} and retention time (RT), activity relation between antimicrobial activity and hydrophobicity, and partial NOESY data of compound **6b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AMPs, antimicrobial peptides; SMAMPS, synthetic mimics of antimicrobial peptides; PE, phenylene ethynylene; SAR, structure−activity relationship; TMS, tetramethylsilane; NOESY, nuclear Overhauser effect spectroscopy; MIC, minimum inhibitory concentration; RT, retention time; TEA, triethylamine; DCM, dichloromethane; HRMS, high resolution mass spectrometry

■ REFERENCES

(1) Neu, H. C. The crisis in antibiotic resistance. *Science* **1992**, *257*, 1064–1073.

(2) Brown, K.; Hancock, R. Cationic host defense (antimicrobial) peptides. *Curr. Opin. Immunol.* **2006**, *18*, 24–30.

(3) McPhee, J. B.; Hancock, R. E. W. Function and therapeutic potential of host defence peptides. *J. Pept. Sci.* **2005**, *11*, 677–687.

(4) Giuliani, A.; Pirri, G.; Bozzi, A.; Giulio, A.; Aschi, M.; Rinaldi, A. C. Antimicrobial peptides: natural templates for synthetic membrane-active compounds. *Cell. Mol. Life Sci.* **2008**, *65*, 2450–2460.

(5) Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **2002**, *415*, 389–395.

(6) Mookherjee, N.; Hancock, R. E. W. Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cell. Mol. Life Sci.* **2007**, *64*, 922–933.

(7) Hancock, R. E.; Lehrer, R. Cationic peptides: a new source of antibiotics. *Trends Biotechnol.* **1998**, *16*, 82–8.

(8) Hancock, R. E. W.; Sahl, H.-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* **2006**, *24*, 1551–1557.

(9) Brogden, K. A. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. *Nat. Rev. Microbiol.* **2005**, *3*, 238–250.

(10) Perron, G. G.; Zasloff, M.; Bell, G. Experimental evolution of resistance to an antimicrobial peptide. *Proc. R. Soc., Ser. B* **2006**, *273*, 251–256.

(11) Mor, A. Peptide-based antibiotics: a potential answer to raging antimicrobial resistance. *Drug Dev. Res.* **2000**, *50*, 440–447.

(12) Koczulla, A. R.; Bals, R. Antimicrobial peptides: current status and therapeutic potential. *Drugs* **2003**, *63*, 389–406.

(13) Marr, A.; Gooderham, W.; Hancock, R. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Curr. Opin. Pharmacol.* **2006**, *6*, 468–472.

(14) Bradshaw, J. Cationic antimicrobial peptides: issues for potential clinical use. *BioDrugs* **2003**, *17*, 233–240.

(15) Rotem, S.; Mor, A. Antimicrobial peptide mimics for improved therapeutic properties. *Biochim. Biophys. Acta, Biomembr.* **2009**, *1788*, 1582–1592.

(16) Som, A.; Vemparala, S.; Ivanov, I.; Tew, G. N. Synthetic mimics of antimicrobial peptides. *Biopolymers* **2008**, *90*, 83–93.

(17) Chongsiriwatana, N. P.; Patch, J. A.; Czyzewski, A. M.; Dohm, M. T.; Ivankin, A.; Gidalevitz, D.; Zuckermann, R. N.; Barron, A. E. Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2794–2799.

(18) Liu, D.; DeGrado, W. F. De novo design, synthesis, and characterization of antimicrobial β-peptides. *J. Am. Chem. Soc.* **2001**, *123*, 7553–7559.

(19) Porter, E. A.; Weisblum, B.; Gellman, S. H. Mimicry of host-defense peptides by unnatural oligomers: antimicrobial beta-peptides. *J. Am. Chem. Soc.* **2002**, *124*, 7324–7330.

(20) Porter, E. A.; Wang, X.; Lee, H. S.; Weisblum, B.; Gellman, S. H. Non-haemolytic beta-amino-acid oligomers. *Nature* **2000**, *404*, 565.

(21) Dartois, V.; Sanchez-Quesada, J.; Cabezas, E.; Chi, E.; Dubbelde, C.; Dunn, C.; Granja, J.; Gritzen, C.; Weinberger, D.; Ghadiri, M. R.; Parr, T. R., Jr. Systemic antibacterial activity of novel synthetic cyclic peptides. *Antimicrob. Agents Chemother.* **2005**, *49*, 3302–3310.

(22) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. De novo design of biomimetic antimicrobial polymers. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110 5114.

(23) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. Mimicry of antimicrobial host-defense peptides by random copolymers. *J. Am. Chem. Soc.* **2007**, *129*, 15474–15476.

(24) Arnt, L.; Nusslein, K.; Tew, G. N. Nonhemolytic abiogenic polymers as antimicrobial peptide mimics. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3860–3864.

(25) Lienkamp, K.; Madkour, A. E.; Musante, A.; Nelson, C. F.; Nusslein, K.; Tew, G. N. Antimicrobial polymers prepared by ROMP with unprecedented selectivity: a molecular construction kit approach. *J. Am. Chem. Soc.* **2008**, *130*, 9836–9843.

(26) Kuroda, K.; DeGrado, W. F. Antimicrobial synthetic polymers: amphiphilic polymethacrylate derivatives. *Abstr. Pap.—Am. Chem. Soc.* **2004**, *228*, U356–U356.

(27) Radzishevsky, I. S.; Kovachi, T.; Porat, Y.; Ziserman, L.; Zaknoon, F.; Danino, D.; Mor, A. Structure−activity relationships of antibacterial acyl-lysine oligomers. *Chem. Biol.* **2008**, *15*, 354–362.

(28) Zaknoon, F.; Sarig, H.; Rotem, S.; Livne, L.; Ivankin, A.; Gidalevitz, D.; Mor, A. Antibacterial properties and mode of action of a short acyl-lysyl oligomer. *Antimicrob. Agents Chemother.* **2009**, *53*, 3422–3429.

(29) Choi, S.; Isaacs, A.; Clements, D.; Liu, D.; Kim, H.; Scott, R. W.; Winkler, J. D.; DeGrado, W. F. De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6968–6973.

(30) Tang, H.; Doerksen, R.; Jones, T.; Klein, M.; Tew, G. Biomimetic facially amphiphilic antibacterial oligomers with conformationally stiff backbones. *Chem. Biol.* **2006**, *13*, 427–435.

(31) Tang, H.; Doerksen, R. J.; Tew, G. N. Synthesis of urea oligomers and their antibacterial activity. *Chem. Commun.* **2005**, 1537.

(32) Liu, D.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; WinklerJ. D.; Klein, M. L.; DeGrado, W. F. Nontoxic membrane-active antimicrobial arylamide oligomers. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158–1162.

(33) Scott, R. W.; DeGrado, W. F.; Tew, G. N. De novo designed synthetic mimics of antimicrobial peptides. *Curr. Opin. Biotechnol.* **2008**, *19*, 620–627.

(34) Arnt, L.; Rennie, J. R.; Linser, S.; Willumeit, R.; Tew, G. N. Membrane activity of biomimetic facially amphiphilic antibiotics. *J. Phys. Chem. B* **2006**, *110*, 3527–3532.

(35) Tew, G. N.; Clements, D.; Tang, H.; Arnt, L.; Scott, R. W. Antimicrobial activity of an abiotic host defense peptide mimic. *Biochim. Biophys. Acta* **2006**, *1758*, 1387–1392.

(36) Tew, G. N.; Scott, R. W.; Klein, M. L.; Degrado, W. F. De novo design of antimicrobial polymers, foldamers, and small molecules: from discovery to practical applications. *Acc. Chem. Res.* **2010**, *43*, 30–39.

(37) Poriel, C.; Liang, J. J.; Rault-Berthelot, J.; Barriere, F.; Cocherel, N.; Slawin, A. M.; Horhant, D.; Virboul, M.; Alcaraz, G.; Audebrand, N.; Vignau, L.; Huby, N.; Wantz, G.; Hirsch, L. Dispirofluorene-indenofluorene derivatives as new building blocks for blue organic electroluminescent devices and electroactive polymers. *Chemistry* **2007**, *13*, 10055–10069.

(38) Baudoin, O.; Guenard, D.; Gueritte, F. Palladium-catalyzed borylation of ortho-substituted phenyl halides and application to the one-pot synthesis of 2,2′-disubstituted biphenyls. *J. Org. Chem.* **2000**, *65*, 9268–9271.

(39) Horn, J.; Marsden, S. P.; Nelson, A.; House, D.; Weingarten, G. G. Convergent, regiospecific synthesis of quinolines from *o*-aminophenylboronates. *Org. Lett.* **2008**, *10*, 4117–4120.

(40) Phukan, K.; Ganguly, M.; Devi, N. Mild and useful method for N-acylation of amines. *Synth. Commun.* **2009**, *39*, 2694–2701.

(41) Symons, M. C. R. Structure in solvents and solutions. NMR and vibrational spectroscopic studies. *Chem. Soc. Rev.* **1983**, *12*, 1–34.

(42) Del Bene, J. E.; Perera, S. A.; Bartlett, R. J. Hydrogen bond types, binding energies, and ^{1}H NMR chemical shifts. *J. Phys. Chem. A* **1999**, *103*, 8121–8124.

(43) Reeves, L. W.; Allan, E. A.; Stromme, K. O. Nuclear shielding parameters for protons in hydrogen bonds. II. Correlation of chemical shifts in intramolecular hydrogen bonds with infrared stretching frequencies. *Can. J. Chem.* **1960**, *38*, 1249–1254.

(44) Dudek, G. O. Spectroscopic studies of keto−enol equilibria. VIII. Schiff base spectroscopic correlations. *J. Org. Chem.* **1965**, *30*, 548–552.

(45) Lomas, J. S.; Adenier, A.; Cordier, C.; Lacroix, J.-C. Hydrogen bonding and solvent effects in heteroaryldi(1-adamantyl)methanols: an NMR and IR spectroscopic study. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2647–2652.

(46) Rusu, V. H.; Ramos, M. N.; Da Silva, J. B. P. Hydrogen bonds between hydrogen fluoride and aromatic azines: an ab initio study. *Int. J. Quantum Chem.* **2006**, *106*, 2811–2817.

(47) Steinberg, D. A.; Hurst, M. A.; Fujii, C. A.; Kung, A. H.; Ho, J. F.; Cheng, F. C.; Loury, D. J.; Fiddes, J. C. Protegrin-1: a broad-spectrum, rapidly microbicidal peptide with in vivo activity. *Antimicrob. Agents Chemother.* **1997**, *41*, 1738–1742.

(48) Yan, H.; Hancock, R. E. Synergistic interactions between mammalian antimicrobial defense peptides. *Antimicrob. Agents Chemother.* **2001**, *45*, 1558–1560.

(49) Ge, Y.; MacDonald, D. L.; Holroyd, K. J.; Thornsberry, C.; Wexler, H.; Zasloff, M. In vitro antibacterial properties of pexiganan, an analog of magainin. *Antimicrob. Agents Chemother.* **1999**, *43*, 782–788.

(50) Hansen, T.; Alst, T.; Havelkova, M.; Strom, M. B. Antimicrobial activity of small beta-peptidomimetics based on the pharmacophore model of short cationic antimicrobial peptides. *J. Med. Chem.* **2010**, *53*, 595–606.

(51) Gabriel, G. J.; Tew, G. N. Conformationally rigid proteomimetics: a case study in designing antimicrobial aryl oligomers. *Org. Biomol. Chem.* **2008**, *6*, 417–423.

(52) Sangster, J. *Octanol−Water Partitioning Coefficients: Fundamentals and Physical Chemistry*; Wiley: Chichester, U.K., 1997.

(53) Sheppeck, J. E.; Kar, H.; Hong, H. A convenient and scaleable procedure for removing the Fmoc group in solution. *Tetrahedron Lett.* **2000**, *41*, 5329–5333.