Solution-Phase Parallel Synthesis of Novel Membrane-Targeted Antibiotics

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The increase in the incidence of antibiotic-resistant infections is a major concern to healthcare workers and requires the development of novel antibacterial agents. Recently, we described a series of benzophenone-containing antibiotics which displayed activity against antibiotic-resistant bacteria. We have shown that these agents function by disrupting the bacterial membrane. To further explore these compounds, a practical and efficient solution-phase parallel synthesis method was developed which allowed us to prepare combinatorial libraries of these agents. Using this method, we prepared 218 compounds in 58 reactions. All of the compounds were characterized by HPLC and MALDI-TOF mass spectrometry. Analysis of this library for antibacterial activity identified six compounds which displayed MIC values of 2.0 mg/L against *Staphylococcus aureus*. Examination of the structure−function relationships of these agents revealed that cationic groups were required and that cyclic, aliphatic amines were crucial for activity. Using the information generated here, we speculate on how the various structural features of the molecule are necessary for the interaction with the bacterial membrane.

Introduction

The incidence of antibiotic-resistant bacterial infections has increased considerably over the last 20 years and now poses a major challenge for physicians. Of the Gram-positive pathogens, the most common antibiotic-resistant infection is MRSA.[1] MRSA infections result in prolonged hospitalization and increases mortality by at least 2.5-fold.[2] The rise of community-acquired MRSA (CA-MRSA) also poses significant, new challenges for clinicians.[3] Currently, vancomycin is the drug of choice for the treatment of antibiotic-resistant infections; however, the rise of vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA), and vancomycin-resistant *enterococci* (VRE) foreshadow a time in which this drug will no longer be useful.[2,4,5] Currently, there are only two new classes of antibiotics approved for the treatment of antibiotic-resistant infections; however, both have significant clinical problems and resistance to these agents has already been detected.[2,4,6] This, coupled with the reduction of antimicrobial research within the pharmaceutical industry, highlights the critical need for academic development of new antimicrobial agents.[7,8] One validated target in antibacterial drug discovery is the bacterial membrane.[9] Previous research has shown that agents targeting bacterial membranes possess activity against a wide range of microorganisms including MRSA, VRSA, VRE, and *E. coli*.[9] Unfortunately, while there has been significant work directed toward antimicrobial peptides (AMP) and natural product lipopeptides, work on small, nonpeptide membrane-targeted antibiotics (MTAs) has been rather modest.[10–13]

Recently, our lab discovered a novel MTA with excellent potency against resistant strains such as MRSA and VRSA (Figure 1).[14] The antibacterial activity of these benzophenone-containing agents was discovered serendipitously, and to date, a rather limited set of derivatives of this molecule have been prepared and examined for antimicrobial activity. Our previous studies, however, have indicated that the amine-containing region of the molecule highlighted in Figure 1 is a critical modulator of activity. To date, we have examined only six different amine substitutions in this region of the molecule and all of the molecules investigated have contained the same amine in both tail regions. Given the importance of discovering new antibiotics and the limited library of derivatives previously explored, we sought to examine the antibacterial activity of a larger library of compounds related to our lead agent. In this paper, we describe the library design, solution-phase parallel synthesis, characterization, screening, and deconvolution of a combinatorial library of benzophenone-containing membrane-targeted antibiotics. Using this approach, we found six new compounds with minimum inhibitory concentrations (MIC) similar to those

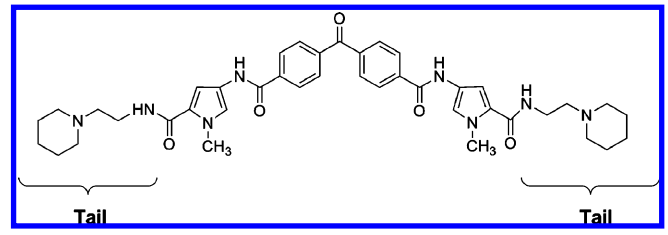

Figure 1. Potent antibacterial compound **SV-7** with amine portions highlighted.

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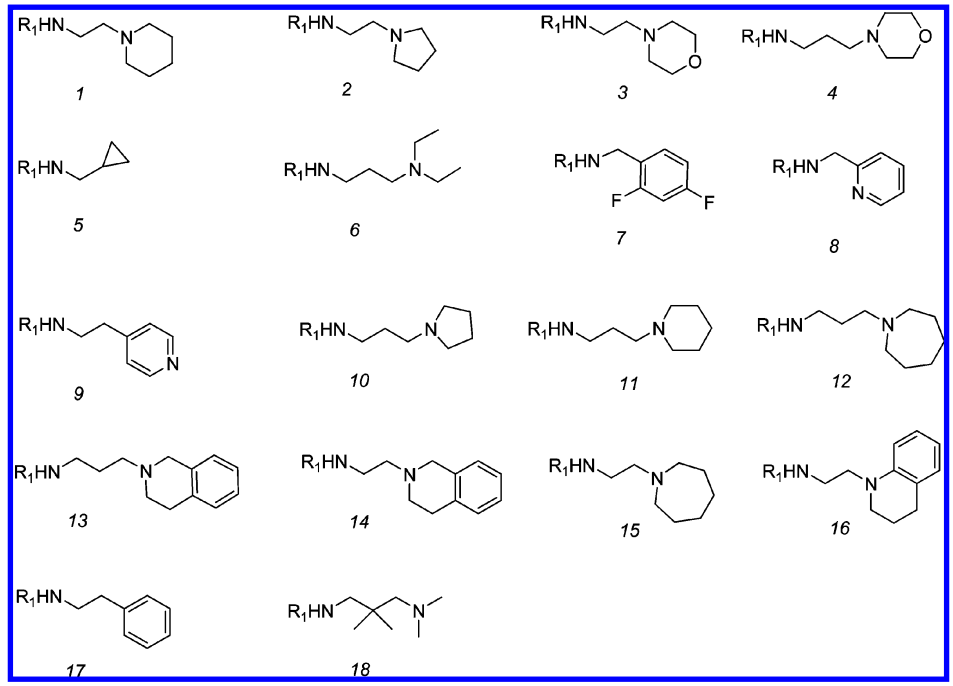


Figure 2. Diversity structures **13**{*1*−*18*}.

determined for the lead agent. These agents are mostly unsymmetrical with respect to the tail region and an examination of the activity of the library members has provided us with additional information regarding the structure−activity relationships of this class of antibiotics.

Results

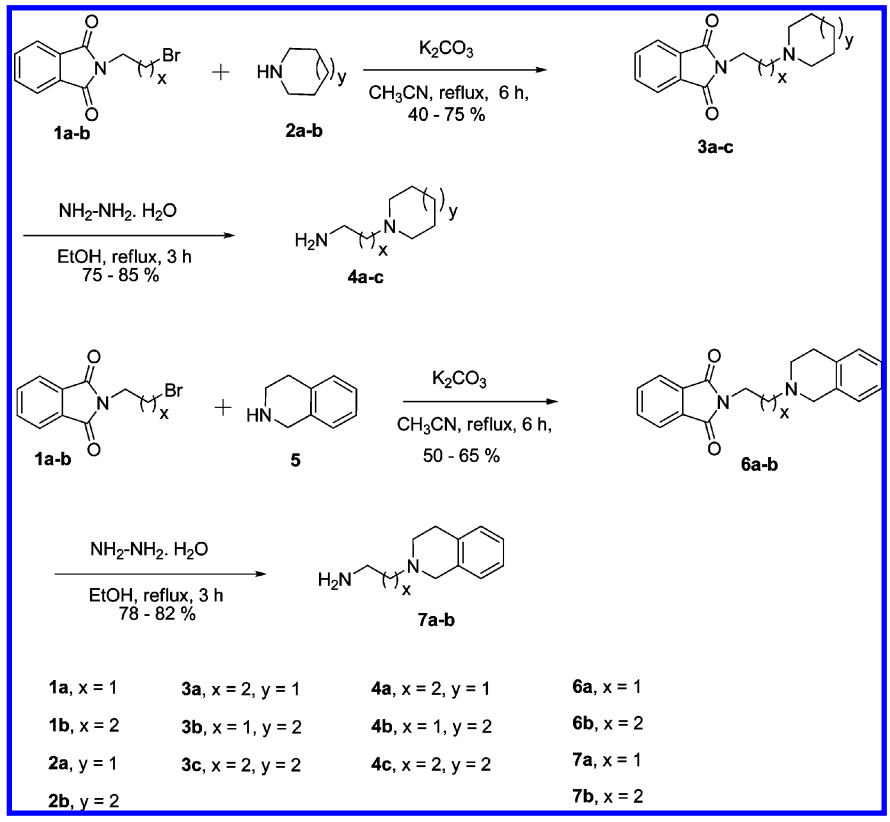
Library Design. Previous studies conducted in our group revealed that the tail region of **SV-7** was a critical determinant for antibacterial activity. To examine the structural requirements of the region more thoroughly, we chose to generate a library of compounds related to **SV-7** in which various amines were located in the tail region of the molecule. We selected 18 different amines to examine (Figure 2). These amines were chosen based upon their basicity and hydrophobicity since our previous investigations showed that these properties were critical for activity. The specific amines were chosen for a number of reasons. Amines *1*, *2*, and *3* were included because they were found in biologically active molecules as determined in our previous investigation[14] whereas compounds *4*, *10*, and *11* were selected to explore the linker length between the core of the molecule and the amine. Cyclic amines with a seven-membered ring (*15* and *12*) were selected to examine the effect of ring size on activity, while acyclic amines (*6* and *18*) were included because of their enhanced hydrophobicity and they allowed us to examine whether acyclic amines were also tolerated in active molecules. We also chose to include aromatic amines (*8*, *9*, *13*, *14*, and *16*) because of their different pK_a values and their ability to form stacking interactions. Stacking or self-association of the molecule may be critical for activity of these classes of molecules. Finally, we also chose to include three nonbasic amines (*5*, *7*, and *17*) to serve as negative controls and also to address questions regarding whether two cationic groups were required for activity.

We were also interested in investigating whether symmetry was required for activity. To date, all of the active compounds are symmetrical about the axis running through the carbonyl group of the benzophenone. However, we were interested to determine if asymmetric molecules (i.e., different tails on each end of the molecule) were also active. To accomplish this, the synthesis of the combinatorial library must be able to generate both symmetric and asymmetric molecules. One potential method for the synthesis of both classes of molecules would be to simply incubate mixtures of amines with an activated acid. However, we felt that such an approach would generate complex mixtures which would be difficult to evaluate. Thus, we chose to generate the combinatorial library by explicitly synthesizing a set of monosubstituted molecules and then reacting these compounds with a mixture of amines to generate the library.

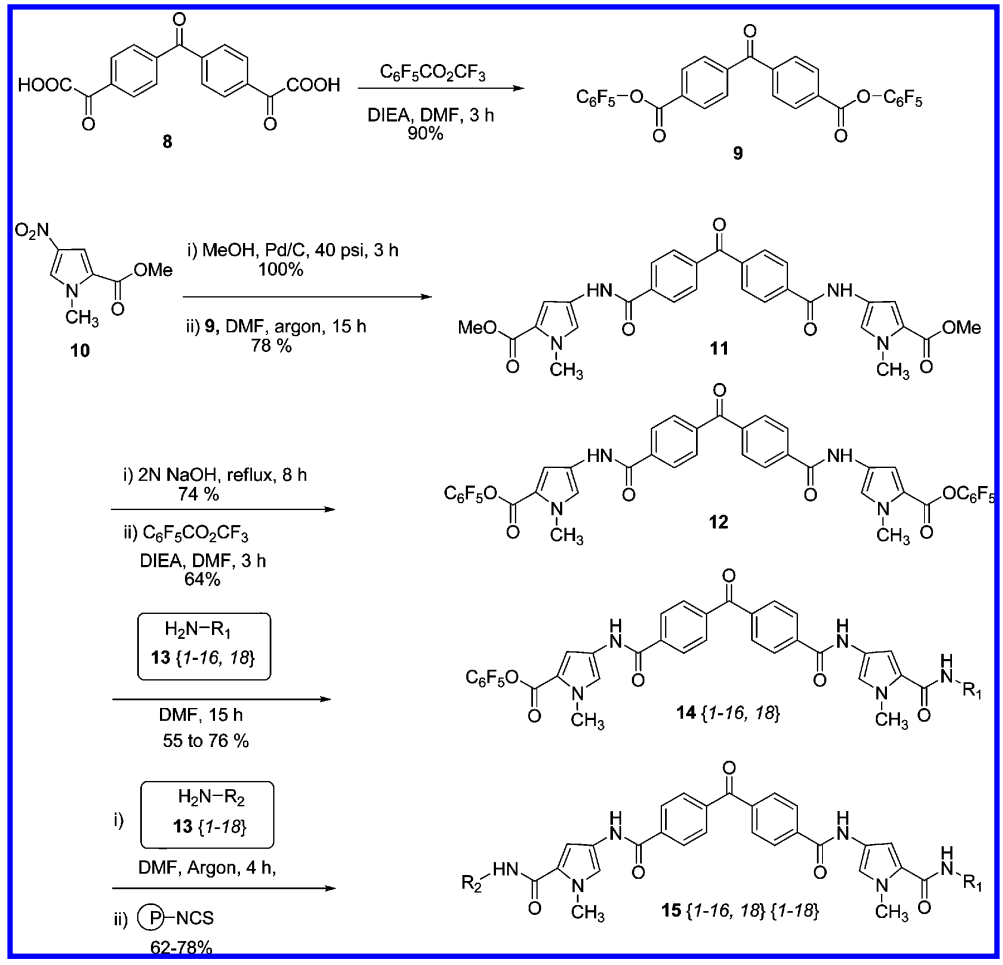
Synthesis of Amines Used in Library Synthesis. Most of the 18 amines shown in Figure 2 are commercially available; however, 6 of 18 amines were not available for purchase. The synthesis of these amines is given in Scheme 1. The synthesis begins by reacting the appropriate pthalimide protected amino alkyl bromides (**1a**−**b**) with the desired secondary amine (**2a-b/5**) to yield the protected intermediate (**3a-c/6a-b**). Deprotection of the intermediate using hydrazine reveals the desired amine (**4a-c/7a-b**) in good yield.[15]

Library Synthesis and Characterization. The solution-phase mixture libraries were synthesized according to Scheme 2. Synthesis began with the conversion of 4,4′-benzophenone diacid (**8**) to the activated pentafluorophenol diester **9**.[16] Addition of the heterocyclic portion of the molecule was done by reduction of the known pyrrole **10** into the corresponding aminopyrrole followed by reaction with **9** to give the intermediate **11**.[17] The diester was converted into the diacid by refluxing in the presence of base and the resulting diacid was activated with pentafluorophenoltrifluoroacetate to yield the activated diester **12**.

Scheme 1. Synthesis of Amines Used in Library Construction



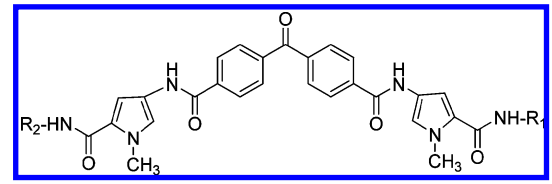
Scheme 2. Solution-Phase Synthesis of the Library



Compound **12** was partitioned into 17 different reaction vessels where each reaction contained only one amine from the panel (**13**{*1*−*16, 18*}). Coupling of **12** with **13**{*1*−*16, 18*} was done under conditions where the stoichiometry of the amine was limiting (0.27 equiv) in order to maximize the synthesis of the mono-coupled product (**14**{*1*−*16, 18*}). Each mono product was purified by column chromatography to remove any diaddition product or unreacted **12**. Once the 17 mono-coupled derivatives (**14**{*1*−*16, 18*}) were synthesized, a parallel solution-phase synthesis strategy was used to make the combinatorial mixtures of the final compounds. Each of the 17 mono-coupled derivatives was partitioned into reaction vessels where they were then coupled with a mixture composed of 3−4 amines. In these reactions, excess of the amine mixture (2.5 equiv) was used to drive the reaction to completion. Once the reaction was completed, unreacted amine and the product fluorophenol were scavenged by addition of 3.0 equiv of methylisothiocyanate polystyrene HL resin.[18] The resulting products (**15**{*1*−*16, 18*}{*1*−*18*}) were prepared in good yield and excellent purity. Overall 218 (54 repeated and 164 nonrepeated) compounds were synthesized in 58 reactions. All reaction mixtures were characterized by HPLC and MALDI-TOF to determine the purity of the reaction mixture and the composition of the products produced in the reaction (Supporting Information). MALDI-TOF measurements of samples were prepared by solvent free technique enabling us to analyze the samples qualitatively and quantitatively.[19] This analysis confirms the distribution determined by HPLC and also validates that the desired compounds were prepared in the combinatorial library. In general, the mixtures were produced in 62 to 78% yield and the distribution of products within the mixtures was equivalent (±10%) except for the following cases: (i) In the case of mixtures **15**{*17, 5, 6, 10*}, yields of the products containing *5* and *17* amines were less compared to the products containing *6* and *10*. (ii) In the case of mixtures containing {*17, 7, 8, 9*}, yields of products containing *7* were low due to the instability of amine *7*. (iii) In the case of the mixtures synthesized using **14**{*16*}, the corresponding products were not observed due to the instability of the starting material (**14**{*16*}).

Antibacterial Screening and Deconvolution. Each mixture was screened at two different concentrations (2.0 mg/L and 8.0 mg/L) for antibacterial activity against *Staphylococcus aureus* (MSSA 1199). This organism was chosen based upon previous studies which indicated that **SV-7** was more selective for Gram-positive over Gram-negative microbes. Four sets of mixtures (**15**{*1*}{*1, 2, 3, 4*}, **15**{*3*}{*1, 2, 3, 4*}, **15** {*15*}{*1, 2, 3, 4*}, **15**{*15*}{*10, 11, 12, 15*}) completely inhibit bacterial growth at 2.0 mg/L. To determine which compound(s) within the mixture were responsible for antibacterial activity, each mixture was subjected to the following deconvolution procedure. Each monosubstituted derivative (**14**) used in the synthesis of the active mixture was reacted individually with each of the amines used to synthesize the mixture. The prepared, individual compounds were then characterized and the minimum inhibitory concentration (MIC) of each compound was determined using a serial dilution method.[20] Of the 16 compounds examined, six (**16**−**21**) were found to have an MIC of 2.0 mg/L (Table 1).

Table 1. Active Compounds Identified from the Combinatorial Library



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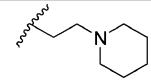
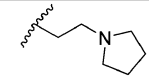
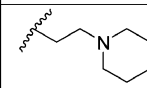
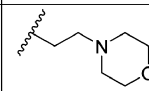
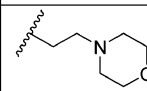
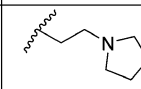
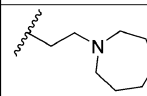
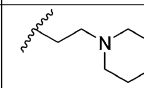
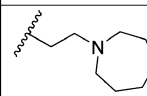
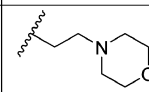
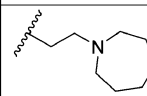
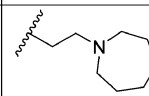
Compound[a]	R_1	R_2	MIC (mg/L)
16 (**15** {*1*} {*2*})			2.0
17 (**15** {*1*} {*3*})			2.0
18 (**15** {*3*} {*2*})			2.0
19 (**15** {*15*} {*1*})			2.0
20 (**15** {*15*} {*3*})			2.0
21 (**15** {*15*} {*15*})			2.0

[a] Column 1: Final compound numbers are in the bold and below are the corresponding numbers from combinatorial library (Scheme 2).

Discussion

Previous studies on benzophenone-containing antibiotics developed in our laboratory have shown that these agents induce membrane depolarization, thus pointing to the membrane as the site of action for these agents.[14] There is currently only one clinically used antibiotic that is thought to target the bacterial membrane (daptomycin), but there are a large number of naturally occurring antimicrobial peptides (AMP) that also target membranes.[10,11] Although AMPs have been studied extensively, a detailed understanding of their mechanism of antibacterial activity still has not been resolved. In addition, one area that has been especially challenging in the study of membrane-targeted antibiotics has been the lack of clear structure−function correlations.[21] This has lead researchers to postulate that these agents do not interact with the membrane in specific binding orientations (like those proposed for ligand−protein interactions) but rather exert

their effects by displaying "interfacial activity" which disrupt the lipid packing of the membrane.[21,22] However, it is unclear what properties and/or structural components of the molecule lead to interfacial activity and to date no definitive formula has been created that would allow for the prediction of interfacial activity within a molecule.

In this article, we have examined over 200 different analogs of a potent membrane-targeted antibiotic. Our results conclusively reveal that at least two cationic groups are required for activity and cyclic amines are preferred in the tail region of the molecule. Beyond these simple observations, structure−function relationships are difficult to determine because antibacterial activity is very sensitive to structural changes in the molecule. For example, addition of one methylene group (i.e., **13**{*15*} vs **13**{*12*}) renders the compound inactive in our assay. Explorations of calculated properties (see the Supporting Information) of the molecules prepared here reveal no correlation between hydrophobicity (as measured by logP), aqueous solubility (as measured by logS), conformation or polar surface area (as measured by TPSA), and antibacterial activity. Thus, it seems unlikely that the agents reported here display a correct balance of the properties defined as being part of interfacial activity.

Despite the lack of clear structure−function data, we can speculate on how the structure of the benzophenone antibiotics prepared here may affect the mechanism of membrane depolarization. Conceptually, membrane-targeted antibiotics would likely require at least three steps for activity. The first would be an association of the antibiotic with the extracellular surface of the membrane. The second step would be an insertion of the antibiotic into the membrane and the third would be either a self-association or an association of the antibiotic with lipids to generate a pore or defect leading to depolarization.

In the case of the benzophenone antibiotics described here, the association with the membrane is likely dominated by electrostatic interactions between the positive charge present in the antibiotic and the negatively charged bacterial membrane.[11] Support for this comes from the studies presented here which indicate that compounds containing nonbasic groups (**13**{*5*}, **13**{*17*}, **13**{*7*}) are inactive. Similar conclusions have been observed for AMPs, although the presence of a cationic group is not always required for some classes of membrane targeted antibiotics.

Insertion into the membrane is the second step. The benzophenone agents are approximately the same length (∼36 Å) as the width of the bacterial membrane (∼29 Å), and thus, these agents are capable of spanning the membrane.[23–25] This ability allows for the two cationic tail groups of the benzophenone agents to interact with the phosphate headgroup located on the inner and outer faces of the membrane. However, before the agent can span the membrane, it must be able to disengage from the negatively charged components located on the outside of the membrane and then insert between the lipid tails of the bilayer. This would require that the compound to be hydrophobic but also possess some degree of hydrophilicity in order to associate with the polar headgroups. An examination of our library reveals that there is no correlation between hydrophobicity (as measured by logP) and activity. For example, the active agents identified in this study display logP values ranging from 5.15 to 2.42. Yet, inactive agents also span the same range. Thus, we conclude that hydrophobicity is required for activity, but it is not the sole determinant for activity.

Finally, many membrane targeted antibiotics are thought to self-associate.[26,27] We have shown by NMR experiments that benzophenone compounds are capable of self-assembly under hydrophobic conditions.[28] Molecular modeling suggests that this self-assembly occurs via stacking of the benzophenone core with a close packing of the tail groups. In general, cyclic, aliphatic tails appear to pack better than acyclic aliphatic tails. Furthermore, the close packing of the cyclic tails facilitates hydrophobic interactions with other molecules in the stack. Thus, if the tail group does pack well, this could lead to an energy penalty which would disrupt the formation of an assembled, active pore. We are currently investigating whether acyclic aliphatic tails prevent self-assembly in these agents.

Conclusion

In this work, we have successfully prepared a solution-phase parallel combinatorial library of benzophenone antibiotics. In total, we synthesized 218 different compounds and found six compounds that display good MIC values against *Staphylococcus aureus*. We observed that at least two cationic groups are required for activity and cyclic amines are preferred in the tail region of the molecule. Both unsymmetrical and symmetrical compounds display good activity and the tail region is very sensitive to modifications. We did not observe any correlation between properties such as logP, logS, or TPSA and antibacterial activity. Finally, we propose that these agents act by forming pores in which self-association of the agents is a prerequisite.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian DRX400 at 400 and 100 MHz, respectively. Mass measurements were carried out at the Central Instrumentation Facility in the Chemistry Department at Wayne State University. Mass measurements were conducted using a Bruker MALDI-TOF.

General Method for Preparation of Amines (13{*11*}−13{*15*}).[15] A 5.0 mmol solution of secondary amine (**2a**−**b** or **5**) in 20 mL of acetonitrile was treated with 6.25 mmol of *N*-bromopthalimide (**1a**−**b**) and K_2CO_3 (15.0 mmol). The resulting mixture was refluxed for 6 h. After the reaction was completed, 30 mL of sat $NaHCO_3$ was added followed by extraction with ethyl acetate. The combined organic layers were acidified with 2 N HCl and washed with water. The pH of the aqueous layer was adjusted to pH 12 using 4 N NaOH and then extracted with methylene chloride. The organic solution was dried over Na_2SO_4 and evaporated to yield the product, which is used in the next reaction without further purification.

The *N*-alkylated pthalimide (**3a**−**c** or **6a**−**b**, 2 mmol) obtained above was dissolved in 20 mL of ethanol and hydrazine hydrate (6.0 mmol, 0.3 mL) was added. The reaction was refluxed for 3 h and cooled to room temperature,

and the resulting precipitate was removed by filtration. The filtrate was concentrated and the residue was diluted with 20 mL of EtOAc. The resulting precipitate was removed by filtration and the filtrate was concentrated to dryness to give the desired product.

3-(Piperidin-1-yl)propan-1-amine (4a). ^{1}H NMR (400 MHz, CD_3OD): δ 2.59 (t, $J = 7.6$ Hz, 2H, CH_2), 2.48−2.40 (m, 4H, 2 × CH_2), 2.36 (t, $J = 7.6$ Hz, 2H, CH_2), 1.71 (t, $J = 7.2$ Hz, 2H, CH_2), 1.64−1.57 (m, 4H, 2 × CH_2), 1.52−1.44 (m, 2H, CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 57.3, 54.4, 48.0, 25.8, 25.4, 24.0.

2-(Azepan-1-yl)ethanamine (4b). ^{1}H NMR (400 MHz, CD_3OD): δ 2.35−2.30 (m, 2H, CH_2), 2.30−2.24 (m, 4H, 2 × CH_2), 2.17 (t, $J = 6.4$ Hz, 2H, CH_2), 1.32−1.18 (m, 8H, 4 × CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 59.0, 55.2, 38.2, 27.2, 26.6.

2-(Azepan-1-yl)propan-1-amine (4c). ^{1}H NMR (400 MHz, CD_3OD): δ 2.68−2.62 (m, 6H, 3 × CH_2), 2.54−2.48 (m, 2H, CH_2), 1.72−1.60 (m, 10H, 5 × CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 56.0, 55.5, 40.0, 30.0, 27.1, 26.9.

2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)ethanamine (7a). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14−7.04 (m, 3H, Ar-H), 7.04−6.98 (m, 1H, Ar-H), 3.62 (s, 2H, CH_2), 2.91−2.83 (m, 4H, 2 × CH_2), 2.80−2.68 (m, 2H, CH_2), 2.63−2.53 (m, 2H, CH_2), 1.49 (s, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.1, 134.6, 128.9, 126.8, 126.3, 125.8, 61.2, 56.4, 51.3, 39.4, 29.4.

3-(3,4-Dihydroisoquinolin-2(1*H*)-yl)propan-1-amine (7b). ^{1}H NMR (400 MHz, CD_3OD): δ 3.61 (s, 2H, CH_2), 2.92−2.86 (m, 2H, CH_2), 2.76−2.68 (m, 4H, 2 × CH_2), 2.55 (t, $J = 7.2$ Hz, 2H, CH_2), 1.80−1.72 (m, 2H, CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 128.5, 126.5, 126.3, 125.7, 56.0, 50.9, 39.9, 29.3, 28.5.

Bis(perfluorophenyl) 4,4′-Carbonyldibenzoate (9).[16] 4,4′-Carbonyldibenzoic acid (**8**, 25.0 mmol, 8.15 g) was suspended in 25.0 mL of dry DMF followed by the addition of diisoproylethyleneamine (DIEA, 55.0 mmol, 9.6 mL). The solution was stirred for 15 min before the addition of pentafluorotrifluoroacetate (55.0 mmol, 9.4 mL). The reaction was stirred at room temperature for 3 h followed by evaporation of the solvent. The residue was purified by flash silica gel chromatography (toluene/ethyl acetate 9:1) to give the desired pentafluorodiester in 90% yield.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, $J = 8.0$ Hz, 4H, Ar-H), 7.96 (d, $J = 8.0$ Hz, 4H, Ar-H).

4-(4-(4-(5-(Methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-5-methoxycarbonyl (11). To a 250 mL pressure flask, methyl *N*-methyl-4-nitropyrrole-2-carboxylate (**10**)[17] (34.2 mmol, 6.29 g) was dissolved in 50 mL of MeOH and 650 mg of Pd/C was added. The reaction was subjected to hydrogenation at 40 psi of hydrogen for 3 h. The reaction mixture was filtered through Celite to remove the catalyst and the filtrated was concentrated in vacuo. The resulting amine was used immediately in the next reaction without purification. To a flask containing the activated benzophenone acid (**9,** 13.7 mmol, 8.25 g) dissolved in dry DMF, the amine was added and the reaction was placed under argon. The reaction vessel was wrapped with aluminum foil to exclude light and reaction was stirred at room temperature for 15 h. At the completion of the reaction, solvent was removed in vacuo and the resulting product was purified by flash column chromatography using 3:1 EtOAC/hexanes to generate the desired material in 78% yield. TLC (3:1 EtOAC/hexane, $R_f = 0.6$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 2H, NH), 8.08 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.87 (d, $J = 8.4$ Hz, 4H, Ar-H), 7.55 (d, $J = 2.0$ Hz, 2H, Ar-H), 6.96 (d, $J = 2.0$ Hz, 2H, Ar-H), 3.85 (s, 6H, 2 × N−CH_3), 3.73 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.6, 163.5, 161.4, 139.5, 138.6, 130.4, 128.3, 123.3, 121.9, 119.6, 109.3, 51.7, 37.0. MS calculated for $C_{29}H_{26}N_4O_7$ (M + H) 543.19, found 543.14.

4-(4-(4-(2-(Carboxypentafluorophenyl)-1-methyl-1*H*-pyrrol-4- ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxy pentafluoro phenyl (12). To a 250 mL flask, **11** (9.78 mmol, 5.30 g) was dissolved in 70 mL of MeOH followed by addition of 27.0 mL of 2N NaOH. The reaction was refluxed for 8. The excess base was neutralized with amberlyst H^+ resin, and the yellow turbid solution was decanted and evaporated. The residue was dried in vacuo to generate the product in 74% yield. TLC (20:80 MeOH/CH_2Cl_2, $R_f = 0.4$). The diacid was used without further purification. The diacid (6.84 mmol, 3.51 g) was dissolved in 35 mL of dry DMF and DIEA (13.26 mmol, 2.31 mL) was added to the solution. The solution was placed under argon and allowed to stir for 30 min. To the solution, pentafluorotrifluoroacetate (13.0 mmol, 2.23 mL) was added dropwise and the reaction was stirred at room temperature for 3 h. The DMF was evaporated and the product purified by flash column to give the desired product in 64.5% yield. TLC (1:1 EtOAc/hexane, $R_f = 0.5$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 2H, NH), 8.11 (d, $J = 8.0$ Hz, 4H, Ar-H), 7.90 (d, $J = 8.8$ Hz, 4H, Ar-H), 7.82 (d, $J = 1.6$ Hz, 2H, Ar-H), 7.36 (d, $J = 1.6$ Hz, 2H, Ar-H), 3.91 (s, 6H, 2 × N−CH_3). MS calculated for $C_{39}H_{20}F_{10}N_4O_7$ (M + H) 847.13, found 847.33.

General Procedure of Coupling Amines with Activated Diacid.[16] Activated diacid (**12**, 0.592 mmol, 0.5 g) was dissolved in 15 mL of dry DMF and the desired amine (**13**{*1*−*16, 18*}, 0.15 mmol) was added to the above solution under argon. The reaction was placed under argon and stirred at 55 °C overnight. At the completion of the reaction, the solvent was removed and the product was purified with flash silica column chromatography using the TLC solvent conditions listed below for each compound.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(2-(piperidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{1}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.13 (s, 1H, NH), 8.62 (s, 1H, NH), 7.90 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.80 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.65 (d, $J = 8.0$ Hz, 4H, Ar-H), 7.24 (s, 2H, Ar-H), 7.21 (s, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.94 (s, 3H, N−CH_3), 3.85 (s, 3H, N−CH_3), 3.42 (q, $J = 5.6$ Hz, 2H, CH_2), 2.44−2.33 (m, 6H, 3 × CH_2), 1.73 (t, $J = 5.6$ Hz, 2H, CH_2), 1.64−1.57 (m, 4H, 2 × CH_2), 1.45−1.36 (m, 2H, CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, $R_f = 0.50$) with 62% yield. MS calculated for $C_{40}H_{35}F_5N_6O_6$ (M + H) 791.26, found 791.33.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(2-(pyrrolidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{2}). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD): δ 7.99−7.93 (m, 4H, Ar-H), 7.78 (d, J = 8.0 Hz, 4H, Ar-H), 7.67 (s, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 6.69 (s, 1H, Ar-H), 3.88 (s, 3H, N−CH_3), 3.46 (s, 3H, N−CH_3), 3.52−3.84 (m, 6H, 3 × CH_2), 2.76 (t, J = 5.6 Hz, 2H, CH_2), 2.72−2.68 (m, 4H, 2 × CH_2), 1.86−1.80 (m, 4H, 2 × CH_2). TLC (89:10:1, CH_2Cl_2: MeOH: Et_3N, R_f = 0.50) with 68% yield. MS calculated for $C_{39}H_{33}F_5N_6O_6$ (M + H) 777.25, found 777.32.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(2-morpholinoethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{*3*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H, NH), 8.16 (s, 1H, NH), 7.99 (d, J = 8.0 Hz, 2H, Ar-H), 7.95 (d, J = 8.0 Hz, 2H, Ar-H), 7.84 (d, J = 4.0 Hz, 2H, Ar-H), 7.83 (d, J = 4.0 Hz, 2H, Ar-H), 7.77 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.21 (d, J = 1.6 Hz, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 3.97 (s, 3H, N−CH_3), 3.92 (s, 3H, N−CH_3), 3.72 (t, J = 4.4 Hz, 4H, 2 × CH_2), 3.47 (q, J = 5.6 Hz, 2H, CH_2), 2.57 (t, J = 5.6 Hz, 2H, CH_2), 2.54−2.47 (m, 4H, 2 × CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.60) with 75% yield. MS calculated for $C_{39}H_{33}F_5N_6O_7$ (M + H) 793.24, found 793.31.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(3-morpholinopropylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{*4*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.36 (s, 1H, NH), 9.06 (s, 1H, NH), 7.89 (d, J = 8.0 Hz, 2H, Ar-H), 7.81 (d, J = 8.0 Hz, 2H, Ar-H), 7.76 (d, J = 1.6 Hz, 1H, Ar-H), 7.61 (t, J = 8.4 Hz, 2H, Ar-H), 7.59 (d, J = 8.0 Hz, 2H, Ar-H), 7.43 (t, J = 4.8 Hz, 1H, NH), 7.29 (d, J = 1.6 Hz, 1H, Ar-H), 7.22 (d, J = 1.6 Hz, 1H, Ar-H), 6.83 (d, J = 1.6 Hz, 1H, Ar-H), 3.91 (s, 3H, N−CH_3), 3.81 (s, 3H, N−CH_3), 3.71 (t, J = 4.0 Hz, 4H, 2 × CH_2), 3.41 (q, J = 5.6 Hz, 2H, CH_2), 2.51−2.48 (m, 6H, 3 × CH_2), 1.75−1.70 (m, 2H, CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.50) with 60% yield. MS calculated for $C_{40}H_{35}F_5N_6O_7$ (M + H) 807.26, found 807.32.

Perfluorophenyl 4-(4-(4-(5-(Cyclopropylmethylcarbamoyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1H-pyrrole-2-carboxylate (14{*5*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H, NH), 8.25 (s, 1H, NH), 7.94 (d, J = 8.8 Hz, 2H, Ar-H), 7.89 (d, J = 8.8 Hz, 2H, Ar-H), 7.77 (d, J = 8.4 Hz, 4H, Ar-H), 7.22 (d, J = 1.6 Hz, 1H, Ar-H), 7.19 (d, J = 1.6 Hz, 2H, Ar-H), 6.73 (d, J = 1.6 Hz, 1H, Ar-H), 6.05 (t, J = 4.8 Hz, 1H, Ar-H), 3.95 (s, 3H, N−CH_3), 3.87 (s, 3H, N−CH_3), 3.21 (t, J = 6.4 Hz. 2H, CH_2), 1.06−0.96 (m, 1H, CH), 0.52 (q, J = 6.4 Hz, 2H, CH_2), 0.22 (q, J = 5.2 Hz, 2H, CH_2). TLC (95:5, CH_2Cl_2: MeOH, R_f = 0.40) with 68% yield. MS calculated for $C_{37}H_{28}F_5N_5O_6$ (M + H) 734.21, found 734.14.

Perfluorophenyl 4-(4-(4-(5-(3-(Diethylamino)Propylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*6*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.55 (s, 1H, NH), 9.15 (s, 1H, NH), 8.11 (t, J = 4.8 Hz, 1H, NH), 7.90 (d, J = 8.0 Hz, 2H, Ar-H), 7.81 (d, J = 8.8 Hz, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 7.61−7.57 (m, 5H, Ar-H), 7.32 (d, J = 1.6 Hz, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.91 (s, 3H, N−CH_3), 3.80 (s, 3H, N−CH_3), 3.40 (q, J = 4.8 Hz, 2H, CH_2), 2.57−2.52 (m, 6H, 3 × CH_2), 1.67 (t, J = 6.0 Hz, 2H, CH_2), 0.99 (t, J = 7.6 Hz, 6H, 2 × CH_3). TLC (89:10:1, CH_2Cl_2: MeOH: Et_3N, R_f = 0.45) with 59% yield. MS calculated for $C_{40}H_{37}F_5N_6O_6$ (M + H) 793.28, found 793.32.

Perfluorophenyl 4-(4-(4-(5-(2,5-Difluorobenzylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*7*}). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 1H, NH), 10.53 (s, 1H, NH), 8.61 (t, J = 5.6 Hz, 1H, NH), 8.11 (d, J = 7.2 Hz, 4H, Ar-H), 8.09 (d, J = 7.2 Hz, 2H, Ar-H), 7.89 (d, J = 8.0 Hz, 4H, Ar-H), 7.87 (d, J = 7.2 Hz, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 7.40−7.32 (m, 3H, Ar-H), 7.23−7.17 (m, 1H, Ar-H), 7.10−7.01 (m, 2H, Ar-H), 4.38 (d, J = 6.0 Hz, 2H, CH_2), 3.91 (s, 3H, N−CH_3), 3.81 (s, 3H, N−CH_3). TLC (3:1, ethyl acetate:hexane, R_f = 0.60) with 71% yield. MS calculated for $C_{40}H_{26}F_7N_5O_6$ (M + H) 806.19, found 806.30.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(pyridin-2-ylmethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)-benzamido)-1*H*-pyrrole-2-carboxylate (14{*8*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.43 (s, 1H, NH), 9.22 (s, 1H, NH), 8.39 (d, J = 5.2 Hz, 1H, Ar-H), 7.86 (d, J = 8.4 Hz, 2H, Ar-H), 7.81 (d, J = 8.0 Hz, 2H, Ar-H), 7.73 (d, J = 2.4 Hz, 1H, Ar-H), 7.6−7.55 (m, 4H, Ar-H), 7.31 (t, J = 4.8 Hz, 1H, NH), 7.28−7.24 (m, 2H, Ar-H), 7.22 (s, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.12−7.09 (m, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 4.56 (d, J = 4.8 Hz, 2H, CH_2), 3.88 (s, 3H, N−CH_3), 3.76 (s, 3H, N−CH_3). TLC (90:10, CH_2Cl_2:MeOH, R_f = 0.50) with 65% yield. MS calculated for $C_{39}H_{27}F_5N_6O_6$ (M + H) 771.20, found 771.30.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(2-(pyridin-4-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{*9*}). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD): δ 9.71 (s, 1H, NH), 9.41 (s, 1H, NH), 8.31 (s, 2H, NH), 7.84 (d, J = 8.8 Hz, 2H, Ar-H), 7.72 (d, J = 8.0 Hz, 2H, Ar-H), 7.54 (d, J = 8.4 Hz, 2H, Ar-H), 7.49 (d, J = 8.0 Hz, 2H, Ar-H), 7.25 (d, J = 1.6 Hz, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.04 (s, 2H, Ar-H), 6.63 (s, 1H, Ar-H), 6.58 (t, J = 4.8 Hz, 1H, NH), 3.88 (s, 3H, N−CH_3), 3.77 (s, 3H, N−CH_3), 3.55 (q, J = 5.6 Hz, 2H, CH_2), 2.81 (t, J = 6.4 Hz, 2H, CH_2). TLC (90:10, CH_2Cl_2:MeOH, R_f = 0.50) with 55% yield. MS calculated for $C_{40}H_{29}F_5N_6O_6$ (M + H) 785.22, found 785.35.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(3-(pyrrolidin-1-yl)propylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{*10*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.40 (s, 1H, NH), 8.92 (s, 1H, NH), 7.92 (s, 1H, Ar-H), 7.90 (d, J = 7.2 Hz, 2H, Ar-H), 7.81 (d, J = 7.2 Hz, 2H, Ar-H), 7.62 (d, J = 5.2 Hz, 4H, Ar-H), 7.61 (d, J = 4.8 Hz, 2H, Ar-H), 7.29 (s, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 6.65 (s, 1H, Ar-H), 3.91 (s, 3H, N−CH_3), 3.80 (s, 3H, N−CH_3), 3.44−3.38 (m, 2H, CH_2), 2.60 (t, J = 5.6 Hz, 2H, CH_2), 2.53−2.46 (m, 4H, 2 × CH_2), 1.81−1.74 (m, 4H, 2 × CH_2), 1.70 (t, J = 5.6 Hz, 2H, CH_2). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.40) with 76% yield. MS calculated for $C_{40}H_{35}F_5N_6O_6$ (M + H) 791.26, found 791.35.

Perfluorophenyl 1-Methyl-4-(4-(4-(1-methyl-5-(3-(piperidin-1-yl)propylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxylate (14{*11*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (s, 1H, NH), 8.68 (s, 1H, NH), 7.92 (d, J = 8.4 Hz, 2H, Ar-H), 7.83 (d, J = 8.0 Hz, 2H, Ar-H), 7.77 (s, 1H, Ar-H), 7.68 (d, J = 8.0 Hz, 4H, Ar-H), 7.28 (s, 2H, Ar-H), 7.21 (s, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.93 (s, 3H, N−CH_3), 3.83 (s, 3H, N−CH_3), 3.42 (q, J = 5.6 Hz, 2H, CH_2), 2.48−2.31 (m, 8H, 4 × CH_2), 1.70 (t, J = 5.6 Hz, 2H, CH_2), 1.61−1.53 (m, 4H, 2 × CH_2), 1.47−1.35 (m, 2H, CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.50) with 68% yield. MS calculated for $C_{41}H_{37}F_5N_6O_6$ (M + H) 805.28, found 805.39.

Perfluorophenyl 4-(4-(4-(5-(3-(Azepan-1-yl)propylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*12*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.92 (s, 1H, NH), 9.46 (s, 1H, NH), 8.01−7.94 (m, 4H, Ar-H), 7.90 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.70−7.64 (m, 3H, Ar-H), 7.29−7.22 (m, 2H, Ar-H), 6.72 (s, 1H, NH), 3.88 (s, 3H, N−CH_3), 3.80 (s, 3H, N−CH_3), 3.39−3.31 (m, 2H, CH_2), 2.65−2.60 (m, 4H, 2 × CH_2), 2.57−2.52 (m, 4H, 2 × CH_2), 1.67 (t, J = 5.6 Hz, 2H, CH_2), 1.61−1.53 (m, 6H, 3 × CH_2), 1.52−1.47 (m, 4H, 2 × CH_2). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.55) with 62% yield. MS calculated for $C_{42}H_{39}F_5N_6O_6$ (M + H) 819.30, found 819.43.

Perfluorophenyl 4-(4-(4-(5-(3-(3,4-Dihydroisoquinolin-2(1*H*)-yl)propylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*13*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H, NH), 8.64 (s, 1H, NH), 8.00 (d, J = 8.0 Hz, 2H, Ar-H), 7.84 (d, J = 8.0 Hz, 2H, Ar-H), 7.76 (m, 4H, Ar-H), 7.32 (s, 1H, Ar-H), 7.24 (s, 1H, NH), 7.07 (m, 3H, Ar-H), 6.87 (m, 1H, Ar-H), 5.59 (s, 1H, NH), 3.93 (s, 3H, N−CH_3), 3.18 (s, 3H, N−CH_3), 3.72 (s, 2H, CH_2), 3.50 (m, 2H, CH_2), 2.92 (t, J = 5.6 Hz, 2H, CH_2), 2.80 (t, J = 5.6 Hz, 2H, CH_2), 2.75 (t, J = 6.0 Hz, 2H, CH_2), 1.82 (m, 2H, CH_2). TLC (97:2:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.50) with 70% yield. MS calculated for $C_{45}H_{37}F_5N_6O_6$ (M + H) 853.28, found 853.45.

Perfluorophenyl (4-(4-(4-(5-(2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)ethylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*14*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (s, 1H, NH), 9.35 (s, 1H, NH), 7.97−7.92 (m, 2H, Ar-H), 7.84 (d, J = 6.4 Hz, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.64−7.54 (m, 4H, Ar-H), 7.51 (d, J = 7.2 Hz, 1H, Ar-H), 7.34−7.31 (m, 2H, Ar-H), 7.15−7.10 (m, 3H, Ar-H), 6.99−6.93 (m, 1H, Ar-H), 6.83 (s, 1H, NH), 4.01 (s, 2H, CH_2), 3.86 (s, 3H, N−CH_3), 3.73 (s, 3H, N−CH_3), 3.64−3.58 (m, 2H, CH_2), 3.20−3.11 (m, 2H, CH_2), 2.99−2.92 (m, 2H, CH_2), 1.22−1.18 (m, 2H, CH_2). TLC (97:2:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.60) with 63% yield. MS calculated for $C_{44}H_{35}F_5N_6O_6$ (M + H) 839.26, found 839.62.

Perfluorophenyl 4-(4-(4-(5-(2-(Azepan-1-yl)ethylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*15*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.11 (s, 1H, NH), 8.67 (s, 1H, NH), 7.93 (d, J = 6.8 Hz, 2H, Ar-H), 7.86 (d, J = 7.6 Hz, 2H, Ar-H), 7.76 (s, 1H, NH), 7.72−7.66 (m, 4H, Ar-H), 7.29 (s, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 3.93 (s, 3H, N−CH_3), 3.85 (s, 3H, N−CH_3), 3.38−3.34 (m, 2H, CH_2), 2.65−2.57 (m, 6H, 3 × CH_2), 1.63−1.52 (m, 8H, 4 × CH_2). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.60) with 72% yield. MS calculated for $C_{41}H_{37}F_5N_6O_6$ (M + H) 805.28, found 805.24.

Perfluorophenyl (4-(4-(4-(5-(2-(3,4-Dihydroquinolin-1(2*H*)-yl)ethylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*16*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.21 (s, 1H, NH), 8.73 (s, 1H, NH), 7.87 (d, J = 8.0 Hz, 2H, Ar-H), 7.78 (d, J = 8.0 Hz, 2H, Ar-H), 7.74 (s, 1H, NH), 7.62 (d, J = 8.0 Hz, 4H, Ar-H), 7.27 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.21 (s, 1H, NH), 7.16 (s, 1H, Ar-H), 6.96 (t, J = 7.2 Hz, 1H, NH), 6.89 (d, J = 7.2 Hz, 1H, Ar-H), 6.57−6.48 (m, 3H, Ar-H), 6.37−6.31 (m, 1H, Ar-H), 3.90 (s, 3H, N−CH_3), 3.79 (s, 3H, N−CH_3), 3.40 (q, J = 6.4 Hz, 2H, CH_2), 3.27 (t, J = 7.2 Hz, 2H, CH_2), 3.18 (t, J = 6.4 Hz, 2H, CH_2), 2.67 (t, J = 6.8 Hz, 2H, CH_2), 1.90−1.78 (m, 4H, 2 × CH_2). TLC (3:1, ethyl acetate:hexane, R_f = 0.35) with 66% yield. MS calculated for $C_{44}H_{35}F_5N_6O_6$ (M + H) 839.26, found 839.31.

Perfluorophenyl (4-(4-(4-(5-(3-(Dimethylamino)-2,2-dimethylpropylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1-methyl-1*H*-pyrrole-2-carboxylate (14{*18*}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (s, 1H, NH), 9.37 (s, 1H, NH), 8.99 (s, 1H, NH), 7.89 (d, J = 8.0 Hz, 2H, Ar-H), 7.78 (d, J = 8.0 Hz, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.58−7.50 (m, 4H, Ar-H), 7.31 (d, J = 1.6 Hz, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 3.88 (s, 3H, N−CH_3), 3.78 (s, 3H, N−CH_3), 3.24−3.16 (m, 2H, CH_2), 2.28 (s, 6H, 2 × CH_3), 2.28−2.24 (m, 2H, CH_2), 0.90 (s, 6H, 2 × CH_3). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.45) with 70% yield. MS calculated for $C_{40}H_{37}F_5N_6O_6$ (M + H) 793.28, found 793.46.

General Method for Preparing Combinatorial Mixtures 15({*1*−*16*, *18*}{*1*−*18*}). In a test tube, several of mono derivatives (**14**{*1*−*16*, *18*}, 0.0128 mmol) were dissolved in 1.0 mL of dry DMF. A stir bar was added to each tube followed by 0.032 mmol of an amine mixture (three to four amines). The tube was capped with a septum, the reaction vessel placed under argon, and the reaction was stirred overnight. Once the reaction was complete, 3.0 equiv of methylisothiocyanate resin were added to scavenge the excess amines and fluorophenol. The reaction was gently stirred for 24 h, the resin was filtered, and washed with THF, and the resulting filtrate was dried under vacuo to yield the desired combinatorial mixture.

Synthesis of Active Agents (16−21) Identified from the Combinatorial Library. All active antibacterial agents were identified by deconvolution of the combinatorial library mixture. To accomplish this, the corresponding mono derivative (**14**, 0.0128 mmol) was dissolved in DMF and the respective amine (**13**, 0.019 mmol) was added. The reaction was stirred at room temperature for 6−8 h and then worked-up according to the procedures listed above.

1-Methyl-4-(4-(4-(1-methyl-5-(2-(piperidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-*N*-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrole-2-carboxamide (16). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 2H, NH), 7.89 (d, J = 8.0 Hz, 4H, Ar-H), 7.71 (d, J = 8.0 Hz, 4H, Ar-H),

7.31 (d, J = 1.6 Hz, 1H, Ar-H), 7.28 (d, J = 1.6 Hz, 1H, Ar-H), 6.73 (d, J = 1.6 Hz, 1H, Ar-H), 6.69 (d, J = 1.6 Hz, 1H, Ar-H), 6.67−6.62 (m, 2H, Ar-H), 3.88 (s, 3H, N−CH_3), 3.87 (s, 3H, N−CH_3), 3.48−3.40 (m, 4H, 2 × CH_2), 2.65 (t, J = 4.8 Hz, 2H, CH_2), 2.56−2.51 (m, 4H, 2 × CH_2), 2.48 (t, J = 4.8 Hz, 2H, CH_2), 2.43−2.34 (m, 4H, 2 × CH_2), 1.78−1.72 (m, 4H, 2 × CH_2), 1.58−1.52 (m, 4H, 2 × CH_2), 1.45−1.38 (m, 2H, CH_2). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.50) with 65% yield. MS calculated for $C_{40}H_{48}N_8O_5$ (M + H) 721.38, found 721.59.

1-Methyl-4-(4-(4-(1-methyl-5-(2-(piperidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-*N*-(3-morpholinopropyl)-1*H*-pyrrole-2-carboxamide (17). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (s, 1H, NH), 8.23 (s, 1H, NH), 7.94 (d, J = 7.6 Hz, 4H, Ar-H), 7.80 (d, J = 7.2 Hz, 4H, Ar-H), 7.34 (s, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 6.65 (s, 2H, Ar-H), 6.61 (s, 1H, Ar-H), 6.44 (t, J = 4.4 Hz, 1H, NH), 3.88 (s, 3H, N−CH_3), 3.81 (s, 3H, N−CH_3), 3.73−3.68 (m, 4H, 2 × CH_2), 3.50−3.40 (m, 4H, 2 × CH_2), 2.54 (t, J = 4.8 Hz, 2H, CH_2), 2.52−2.51 (m, 2H, CH_2), 2.50−2.45 (m, 4H, 2 × CH_2), 2.44−2.39 (m, 4H, 2 × CH_2), 2.38−2.34 (m, 2H, CH_2), 1.62−1.53 (m, 4H, 2 × CH_2), 1.50−1.40 (m, 2H, CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.45) with 72% yield. MS calculated for $C_{40}H_{48}N_8O_6$ (M + H) 737.38, found 737.52.

1-Methyl-4-(4-(4-(1-methyl-5-(2-(pyrrolidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-*N*-(2-morpholinoethyl)-1*H*-pyrrole-2-carboxamide (18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H, NH), 8.62 (s, 1H, NH), 7.96−7.90 (m, 4H, Ar-H), 7.79 (s, 2H, Ar-H), 7.77 (s, 2H, Ar-H), 7.34 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 6.73 (s, 1H, Ar-H), 6.70 (s, 1H, Ar-H), 3.90 (s, 3H, N−CH_3), 3.89 (s, 3H, N−CH_3), 3.73−3.68 (m, 4H, 2 × CH_2), 3.51−3.43 (m, 4H, 2 × CH_2), 2.70 (t, J = 4.8 Hz, 2H, CH_2), 2.64−2.57 (m, 4H, 2 × CH_2), 2.54 (t, J = 4.8 Hz, 2H, CH_2), 2.50−2.45 (m, 4H, 2 × CH_2), 1.84−1.78 (m, 4H, 2 × CH_2). TLC (89:10:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.65) with 70% yield. MS calculated for $C_{39}H_{46}N_8O_6$ (M+H) 723.36, found 723.49.

***N*-(2-(Azepan-1-yl)ethyl)-1-methyl-4-(4-(4-(1-methyl-5-(2-(piperidin-1-yl)ethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxamide (19).** ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H, NH), 8.00 (s, 1H, NH), 7.94 (d, J = 8.0 Hz, 4H, Ar-H), 7.76 (d, J = 8.0 Hz, 4H, Ar-H), 7.50 (s, 1H, NH), 7.39 (s, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 6.76 (s, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.89 (s, 6H, 2 × N−CH_3), 3.57−3.51 (m, 4H, 2 × CH_2), 2.94−2.86 (m, 4H, 2 × CH_2), 2.71 (t, J = 5.6 Hz, 2H, CH_2), 2.66−2.58 (m, 4H, 2 × CH_2), 1.77−1.70 (m, 4H, 2 × CH_2), 1.70−1.60 (m, 10H, 5 × CH_2), 1.52−1.47 (m, 2H, CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.5) with 62% yield. MS calculated for $C_{42}H_{52}N_8O_5$ (M + H) 749.42, found 749.58.

***N*-(2-(Azepan-1-yl)ethyl)-1-methyl-4-(4-(4-(1-methyl-5-(2-morpholinoethylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)benzoyl)benzamido)-1*H*-pyrrole-2-carboxamide (20).** ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H, NH), 9.00 (s, 1H, NH), 7.92 (d, J = 8.4 Hz, 4H, Ar-H), 7.67 (d, J = 8.0 Hz, 4H, Ar-H), 7.36 (s, 1H, Ar-H), 7.30 (s, 1H, NH), 6.87 (s, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.61 (s, 1H, NH), 3.88 (s, 3H, N−CH_3), 3.82 (s, 3H, N−CH_3), 3.68−3.64 (m, 4H, 2 × CH_2), 3.64−3.58 (m, 2H, CH_2), 3.45 (q, J = 5.6 Hz, 2H, CH_2), 3.10−3.02 (m, 6H, 3 × CH_2), 2.53 (t, J = 5.6 Hz, 2H, CH_2), 2.48−2.42 (m, 4H, 2 × CH_2), 1.87−1.74 (m, 4H, 2 × CH_2), 1.66−1.60 (m, 4H, 2 × CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.50) with 75% yield. MS calculated for $C_{41}H_{50}N_8O_6$ (M + H) 751.40, found 751.65.

4, 4′-Carbonylbis(*N*-(2-(azepan-1-yl)ethyl)1*H*-pyrrole) Benzamide (21). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (s, 2H, NH), 7.96 (d, J = 8.0 Hz, 4H, Ar-H), 7.71 (d, J = 8.0 Hz, 4H, Ar-H), 7.40 (s, 2H, Ar-H), 6.87 (s, 2H, Ar-H), 3.87 (s, 6H, 2 × N−CH_3), 3.61−3.54 (m, 4H, 2 × CH_2), 3.00−2.92 (m, 12H, 6 × CH_2), 1.78−1.71 (m, 8H, 4 × CH_2), 1.66−1.57 (m, 8H, 4 × CH_2). TLC (94:5:1, CH_2Cl_2:MeOH:Et_3N, R_f = 0.55) with 68% yield. MS (M + H) calculated for $C_{43}H_{54}N_8O_5$ (M+H) 763.43, found 763.63.

HPLC Validation of Combinatorial Mixtures. All the combinatorial mixtures **15**({*1*−*16, 18*}{*1*−*18*}) and final compounds (**16**−**21**) were characterized by HPLC using Water's column (XBridge, C-18, 4.6 × 150 mm, 3.5 μM). Separation required a gradient elution using 0.1% TFA in water and 0.1% TFA in acetonitrile. Gradients were varied for each mixture to ensure the best separation.

Antibacterial Assay. Each compound or mixture was dissolved in DMSO before the assay. Antibacterial assays were conducted. To a well in a sterile, clear 96-well plate, 10 μL of an overnight culture of MSSA 1199 was added to 190 μL of Mueller-Hinton broth containing 0, 2, or 8 mg/L of the agent/mixture to be tested. Each agent/mixture was tested in triplicate along with a DMSO negative control. The resulting plate was covered and placed into a 37 °C incubator. Bacterial growth in each well was examined using reflected light under the plate and the concentration of drug which gave no visible growth was taken as the MIC value.[20] For the serial determination of MIC values for each antibiotic, the above procedure was repeated except that five concentrations of each agent were tested instead of two.

Supporting Information Available. Biological and analytical characterization data of all the combinatorial mixtures; calculated properties of the amines; and calculated properties of all the individual library members. This material is available free of charge via the Internet at http://pubs.acs.org/.

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