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Amphipathic β-Strand Mimics as Potential Membrane Disruptive Antibiotics

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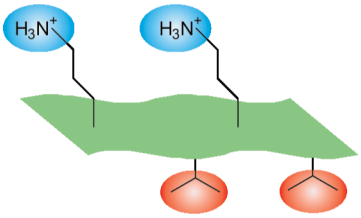

In recent years, there have been increasing numbers of bacterial strains emerging that are resistant to the currently available antibiotics. In the search for new antibiotics, attention has been focused on natural antimicrobial peptides that act by selectively disrupting the membranes of bacterial cells, a mechanism that is thought to be nonconducive to the development of resistance. It is desirable to mimic the structures and activities of these peptides while introducing properties such as resistance to proteolytic degradation, which make molecules more ideal for development as drugs. Described here is the design and synthesis of β-strand mimetic oligomers based on alternating α-amino acids and azacyclohexenone units that segregate cationic lysine and hydrophobic valine side chains on opposite faces of the β-strand. ^{1}H NMR dilution studies demonstrated that despite the incorporation of alternating D- and L-amino acids in order to obtain facial amphiphilicity, these oligomers are capable of dimerizing to β-sheet mimics in a manner similar to the oligomers containing all L-amino acids. The ability of the molecules to disrupt phospholipid vesicles mimicking the membranes of both bacterial and mammalian cells was investigated using a fluorescent dye leakage assay. Several of the oligomers were found to exhibit activity and selectivity for the bacterial over mammalian membranes. Overall, these studies demonstrate the promise of this class of molecules for the development of new potential antibiotics and provide information on the structural features that are important for activity.

Introduction

The increasing emergence of bacterial strains that are resistant to available antibiotics is currently motivating significant interest in new approaches to the treatment of bacterial infections. In natural systems, antimicrobial peptides are often the first line of defense against bacteria.[1,2] These molecules, including families such as the cecropins,[3] magainins,[4] protegrins,[5] and defensins,[6] exhibit a diverse range of structures and activities, but a common feature underlying these classes is the ability of the molecules to adopt conformations in which hydrophobic and cationic amino acids are spatially clustered in discrete regions or faces of the molecules.[1] This structural feature is referred to as amphipathicity. It is proposed that the cationic groups interact selectively with anionic phospholipids,[7] lipopolysaccharides,[8] or teichoic acids[9] on the surfaces of bacterial cells while the hydrophobic groups

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facilitate membrane penetration and disruption via toroidal pore,[10] carpet,[11] or barrel stave mechanisms,[12] leading to the leakage of the cell contents and ultimately resulting in bacterial cell death. This mode of action is of particular interest for the development of new therapeutics as it is thought to be relatively nonspecific and thus not very conducive to the development of resistance.[13]

Inspired by their structures and activities, there has been significant interest in the development of new synthetic mimics of the naturally occurring antimicrobial peptides. These synthetic molecules are providing important insights into the mechanism of action of membrane-disruptive peptides[14−16] while at the same time introducing simplified sequences and resistance to proteolytic degradation,[17] a problem that plagues natural α-peptides as potential drug candidates. Thus far, synthetic oligomers based on α- and β-amino acids[18−23] as well as peptoids[24] that adopt helical amphipathic structures in the presence of membranes have been developed, providing good antibiotic activity and selectivity. Aromatic oligomers based on amides[25] and ureas,[26] as well as oligo(phenylene ethynylene)s[27] that exhibit extended conformations, have also been investigated, providing promising results. Recent work has also shown that through careful balancing of their cationic charge and hydrophobicity, amphiphilic polymers can provide desirable activities.[28−32]

Despite the significant progress in the development of synthetic membrane-disruptive oligomers, the mechanism of action and structure−property relationships are still not fully understood, and new antibiotics are still badly needed. In addition, there has been very little work thus far on peptide-based oligomers that assume amphipathic linear or β-strand-like conformations. Prior successes using the linear aromatic oligomers[25−27] as well as an example of a highly active α-peptide capable of adopting an amphipathic β-sheet structure[33] suggest that such a design would be successful. Although synthetic analogues of protegrins have been developed to mimic their rigid antiparallel two-stranded β-sheet structure which is stabilized by disulfide bonds, these structures have focused on the incorporation of the new turn inducing elements, and like the protegrins, they contain segregated domains of cationic groups at each end of the β-sheet.[34,35] Reported here is the first example of an amino acid based β-strand peptidomimetic that is designed to have an amphipathic structure in which cationic amino acid side chains are directed to one face of the strand and hydrophobic groups are directed toward the opposite face, with the aim of developing a new scaffold for membrane-disruptive antibiotics.

Several synthetic oligomers designed to mimic an elongated β-strand conformation have been developed, although a limited number of these allow for the incorporation of the functional side chains required to access the target amphipathic structures.[36−39] For the current work, oligomers based on alternating α-amino acids and azacyclohexenone (Ach) units (Figure 1a), developed and termed @-tides by Bartlett and co-workers, were selected as the backbone.[39] The replacement of alternating amino acids with the Ach units provides conformational restriction which favors elongated conformations and the tertiary amide limits hydrogen bonding to one edge of the strand, preventing uncontrolled aggregation. They also possess attractive features such as resistance to proteolytic degradation[40] and the easy incorporation of the Ach unit by a flexible, modified peptide synthesis.[39,41] Similar analogues have been demonstrated to bind to the PDZ domains of proteins more strongly than their natural β-sheet ligands.[40] In the work described here, new amphipathic @-tides were designed and synthesized, and their abilities to potentially serve as membrane active antibiotics were investigated by studying the release of encapsulated fluorescent dye molecules from phospholipid vesicle models of both bacterial and mammalian cell

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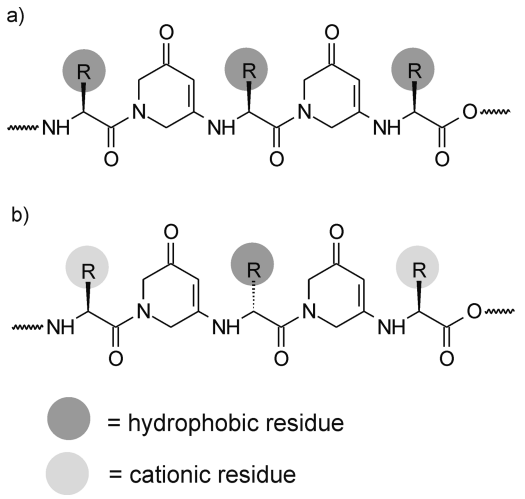

FIGURE 1. Structure of an @-tide comprising (a) all L-amino acids alternating with azacyclohexenone units and (b) hydrophobic D-amino acids and hydrophilic L-amino acids alternating with azacyclohexenone units.

membranes. In addition, the propensity of these modified oligomers to assemble into β-sheet structures in solution was investigated and compared to the results obtained for the original @-tides described by Bartlett and co-workers.[39] Several of the oligomers were found to exhibit membrane disruptive activity as well as selectivity for the bacterial over mammalian cell membrane models. These studies therefore introduce a promising new template for the development of potential antibiotics and provide important insights into the structural features that are critical for activity in this class of molecules.

Results and Discussion

Design. The common structural feature of most membrane disruptive antimicrobial peptides is the segregation of hydrophobic and cationic groups into different regions of the molecule, such as on opposite faces of a helix.[1] In a β-strand based entirely on L-α-amino acids, the side chain groups will naturally diverge to opposite faces of the strand. However, in the @-tides, as every second amino acid is replaced by an azacyclohexenone unit, all of the side chains are expected to be directed to the same face of the strand in its linear conformation as shown in Figure 1a.[39] Therefore, the main design modification made to the previously reported @-tide template was to alternate L- and D-amino acids. By subsequently alternating hydrophobic and cationic amino acids as shown in Figure 1b, it was anticipated that an amphipathic structure should be presented in the @-tide's linear conformation. As a cationic residue, L-lysine was selected because aliphatic amines have been one of the most commonly and successfully used cations in the development of antimicrobial peptidomimetics.[19,25,29] D-Valine was selected as the hydrophobic residue because of its propensity to form β-strands and β-sheets.[42,43] The previously reported antimicrobial peptidomimetics have generally consisted of larger fractions of hydrophobic than hydrophilic residues.[20,21,23] However, it was anticipated that the azacyclohexenone units in the @-tides would introduce significant hydrophobicity to the structures, so in order to obtain at least the minimal water solubility required for the subsequent evaluation of the molecules, structures with equal or greater numbers of lysine relative to valine groups were targeted.

Synthesis. The target molecules were synthesized by a convergent solution-phase approach based on modifications to the method reported by Phillips et al.[41] First, as shown in Scheme 1, the previously reported benzyl carbamate (Cbz) protected @-tide unit **1**[41] was reacted with either D-valine or ε-Boc-L-lysine to provide the corresponding condensation products **2** and **3**, respectively, which will be referred to as dimers throughout this discussion. Without further purification, the L-lysine derivative **3** was converted to the methyl ester **4**. After removal of the Cbz group by catalytic hydrogenolysis in methanol, the resulting dimer amine **5** was coupled with dimer acid **2** using *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) as the coupling agent in *N,N*-diisopropylethylamine (DIPEA) and DMF to provide the tetramer **6**.

The Cbz protecting group on the tetramer **6** was then removed by hydrogenolysis, providing **7**, which was then coupled to α-acetyl-ε-Boc-L-lysine[44] to provide the pentamer **8** as shown in Scheme 2. Alternatively, the tetramer was coupled with the dimer **3** to provide the hexamer **9**. Deprotected versions of the pentamer and hexamer were prepared for evaluation of the membrane disruptive capabilities. The Boc protecting group on the pentamer **8** was removed by treatment with a 1/1 TFA/CH_2Cl_2 solution to provide the dicationic pentamer **10**. The Boc groups on the hexamer **9** were removed under the same conditions to provide the dicationic oligomer **11**.

In addition, it was of interest to investigate the effect of incorporating the alternating D- and L-amino acids to potentially provide facially amphiphilic structures in the linear β-strand conformations, in comparison with oligomers containing all L-amino acids. Thus, molecules corresponding to **2**, **6**, **8**, **9**, **10**, and **11**, but containing only L-amino acids were prepared by the same methods described above. These molecules will be referred to as **2′**, **6′**, **8′**, **9′**, **10′**, and **11′** respectively. It is also noteworthy that while none of the oligomers described above are very long and do not possess highly charged states, high antibiotic activity and selectivity have been previously observed for aromatic amide- and urea-based oligomers of similar lengths.[15,25,26] Therefore, this initial series of molecules was expected to give valuable insights into the activity of this class of molecules.

Oligomers up to the tetramer length (**6**, **6′**) were characterized by the standard methods used for small molecules, using techniques including ^{1}H NMR, ^{13}C NMR, and IR spectroscopy as well as HRMS. All characterization data were consistent with the proposed structures. Due to the increasing broadness and complexity of the NMR spectra with increasing length and solubility constraints, the pentamers and hexamers were characterized mainly by HPLC and HRMS, techniques that are standard for the characterization of oligopeptides, along with ^{1}H NMR spectroscopy.

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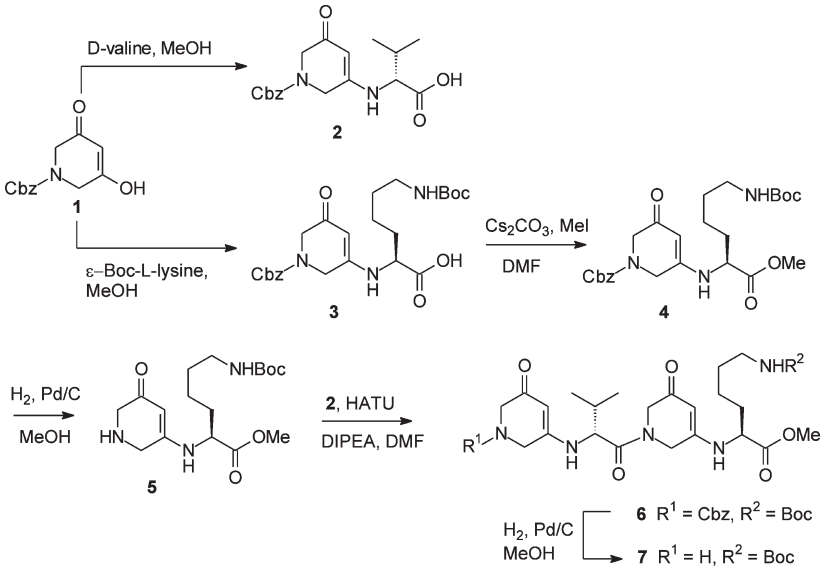
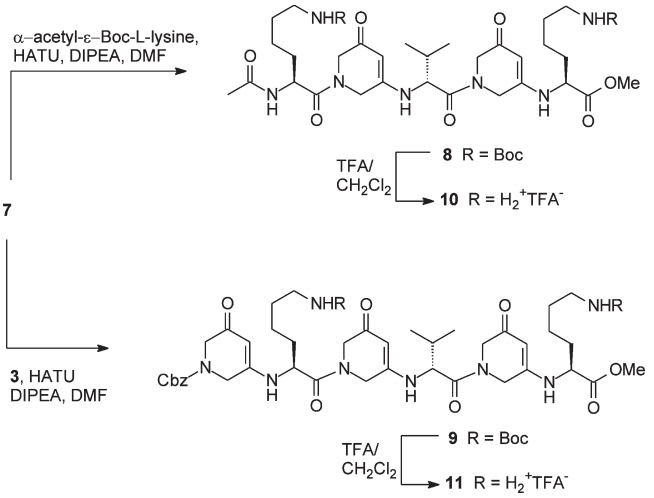
SCHEME 1



SCHEME 2



Self Association of @-tides in $CDCl_3/CD_3OH$ Mixtures. Phillips et al. have reported extensive studies to demonstrate that @-tides of various lengths and compositions assemble in organic and aqueous solutions to form β-sheets.[39,45] While the formation of such assemblies is probably not critical to achieving membrane disruptive activity, it is nevertheless of interest to investigate the effect of alternating the amino acid stereochemistry on this potential dimerization, as it can provide some insight into the conformational preferences of the molecules and their potential to assume amphipathic conformations in the presence of membranes. Therefore, the approximate self-association constants for several oligomers in $CDCl_3$ and $CDCl_3/CD_3OH$ mixtures were determined.

These studies were carried out using the NMR dilution method.[46] First, a solution of **8** was prepared and was gradually diluted. The chemical shifts of the most downfield N-H proton were measured in pure $CDCl_3$, 99/1 $CDCl_3/CD_3OH$, and 97.5/2.5 $CDCl_3/CD_3OH$ as a function of concentration and are shown in Figure 2. Unfortunately, despite the use of 2D NMR experiments including NOESY, COSY, and HMBC it was not possible to unambiguously assign this peak to a specific N-H of **8** due to the high symmetry of the molecule relative to those previously reported,[39] as well as the broadness and overlap of many of the peaks in the spectra. However, to determine the dissociation constant K_d for the dimerization, the NMR chemical shift data was fit to eq 1 using a nonlinear curve fitting procedure,[46] where δ_s is the chemical shift of the nondimerized oligomer, $\Delta\delta$ is the difference in chemical shifts between the dimerized and nondimerized oligomer, and c_o is the concentration of the oligomer. This provided K_d's of 0.2 ± 0.4, 0.7 ± 0.4, and 1.6 ± 0.6 mM in pure $CDCl_3$, 99/1 $CDCl_3/CD_3OH$, and 97.5/2.5 $CDCl_3/CD_3OH$, respectively. The increasing K_d's with increasing CD_3OH content are consistent with the disruptive effect of CD_3OH on the expected hydrogen-bonded dimers. To investigate the effect of oligomer length, the K_d's for both the tetramer **6** and hexamer **9** were also measured using the same method. In 97.5/2.5 $CDCl_3/CD_3OH$ their respective K_d's were 34 ± 3 and 5.7 ± 1.3 mM. The higher K_d for the tetramer **6** was expected because its shorter length allows for the formation of a maximum of four hydrogen bonds in the dimerized state. In contrast, both the pentamer **8** and the hexamer **9** are capable of forming a maximum of six hydrogen bonds in the dimerized state and were therefore expected to exhibit similar K_d's, which was indeed observed. These values are of approximately the same order of magnitude as those obtained by Phillips et al. for the dimerization of @-tides of similar lengths in these solvents.[39]

Overall, the magnitudes of the K_d's, their dependence on the CD_3OH concentration, and their dependence on the oligomer length suggest that the oligomers containing alternating D- and L-amino acids are capable of dimerizing to form β-sheet mimics in the same manner as the previously well-characterized @-tides containing all L-amino acids. This indicates that under some conditions these oligomers should be capable of forming elongated amphipathic

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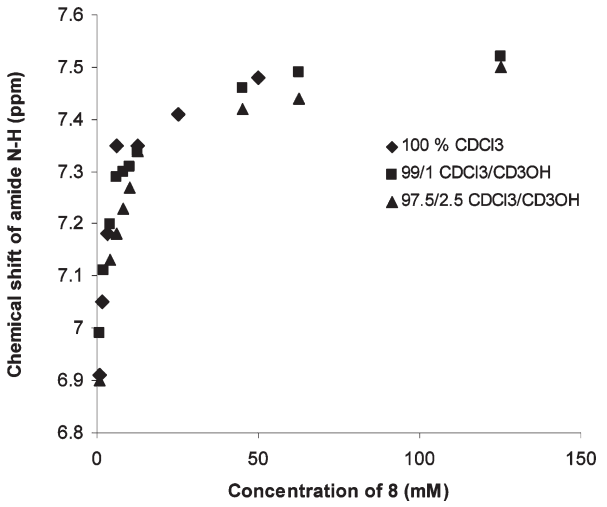

FIGURE 2. Chemical shifts of the farthest downfield N−H proton of pentamer **8** in ^{1}H NMR spectroscopy as a function of solvent and concentration. The concentration dependence is indicative of dimerization of **8** to β-sheet mimics.

conformations. However, the relatively high association constants and the strong effect of hydrogen-bonding solvents observed in our studies and previously reported by Phillips et al.[39,45] for oligomers of these relatively short lengths suggests that they would not spontaneously assemble into dimers in aqueous solution at the concentrations relevant for antimicrobial activity, prior to their interactions with membranes. In addition, it should be noted that while circular dichroism (CD) spectroscopy has been previously used to elucidate the extent of @-tide dimerization in a variety of solvents, this technique was not suitable for the analysis of oligomers containing D- and L-amino acids due to the requirement of having two L-amino acids surrounding the @-unit in order to observe the characteristic signal near 280 nm.[39,45]

$$\delta_{obs} = \delta_s + \Delta\delta\left[1 + \frac{K_d}{2c_0} - \sqrt{\left(\frac{K_d}{2c_0}\right)^2 + \left(\frac{K_d}{c_0}\right)}\right] \quad (1)$$

Assessment of Membrane Disruptive Potential Using a Vesicle Leakage Assay. Numerous studies have demonstrated that the ability of membrane-disruptive antimicrobial peptides to kill bacterial cells via membrane lysis generally coincides with their ability to disrupt and lyse the phospholipid membranes of small unilamellar vesicles (SUVs).[15,16,22,25] Such studies have provided insight into the mechanism of action of these molecules. In addition, in order to predict the selectivity of the molecules for bacterial over mammalian cell membranes, it is possible to choose SUVs that mimic either bacterial or mammalian cell membranes.[15,16] In this study, as previously reported by Yang et al.,[15] an 80/20 ratio of the lipids 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)/1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]-(sodium salt) (DOPG) were selected to mimic bacterial cell membranes. Most Gram-negative bacterial membranes are rich in PE lipids, which have a relatively small headgroup and therefore a tendency to promote negative curvature.[16] DOPG is anionic, which is characteristic of bacterial membranes that contain negatively charged phospholipids, lipopolysaccharides, and teichoic acids on their surfaces.[47] The phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was chosen for the preparation of SUVs mimicking mammalian cell membranes as eukaryotic cell membranes are rich in PC lipids.[15]

Thus far, several techniques have been used to investigate the disruption of phospholipid membranes by antimicrobial peptides and peptidomimetics,[14,15] but by far the most widely used methods involve fluorescence.[15,16,22,25] Generally, a water-soluble dye is entrapped in the vesicle core during vesicle formation at a concentration that is sufficiently high to provide fluorescence quenching. Upon the addition of the membrane-active molecules, and rapid disruption of the membranes, the dye molecules are released from the vesicles, resulting in a significant dilution and the recovery of their fluorescence. In the current work, the 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) and *p*-xylene-bis(*N*-pyridinium bromide) (DPX) fluorescent probe system was selected.[48] HPTS undergoes efficient self-quenching at moderately high concentrations in the presence of DPX, a collisional quencher.[49] Thus, the new oligomers were added to the HPTS-DPX-loaded vesicles at varying concentrations from DMSO solutions. Small volumes of DMSO were used to dissolve the oligomers due to difficulties in dissolving some of the more hydrophobic molecules directly in the aqueous buffer. However, none of the oligomers were found to precipitate upon addition of the DMSO solutions to the aqueous buffer at the concentrations evaluated. The initial fluorescence intensity was taken as 0% HPTS-DPX release, and it was verified that the small quantities of DMSO that were used to dissolve the @-tides for their addition to the vesicles did not lead to any changes in fluorescence. At the end of each experiment, Triton X-100, a well-known membrane disruptive surfactant, was added to completely lyse the vesicles and the observed fluorescence intensity was used to indicate 100% HPTS-DPX release.

In preliminary work, it was found that @-tide oligomers with a carboxylic acid terminus did not lyse either the DOPE/DOPG or the DOPC vesicles to any measurable degree. This may be due to an insufficient cationic charge as the terminal carboxylic acid would cancel the charge of one of the two lysines, leaving an overall positive charge of only +1 on the molecule. Alternatively, the presence of the charged carboxylate ion may make the molecule insufficiently hydrophobic for membrane disruptive activity. Therefore, the current efforts focused on only the evaluation of the methyl ester derivatives. As shown in Figure 3a, the pentamer **10**, with an overall positive charge of +2, exhibited a significant degree of DOPE/DOPG membrane lysis at 100 μg/mL, with greater than 50% of the dye molecules released in 3 min. Hexamer **11**, also having an overall charge of +2, exhibited a similar degree of membrane lysis after 3 min, but the kinetics of dye release were somewhat slower than for the pentamer **10**. To investigate the role of the cationic ε-amines of lysine in the membrane disruptive activity, the protected tetramer **6**,

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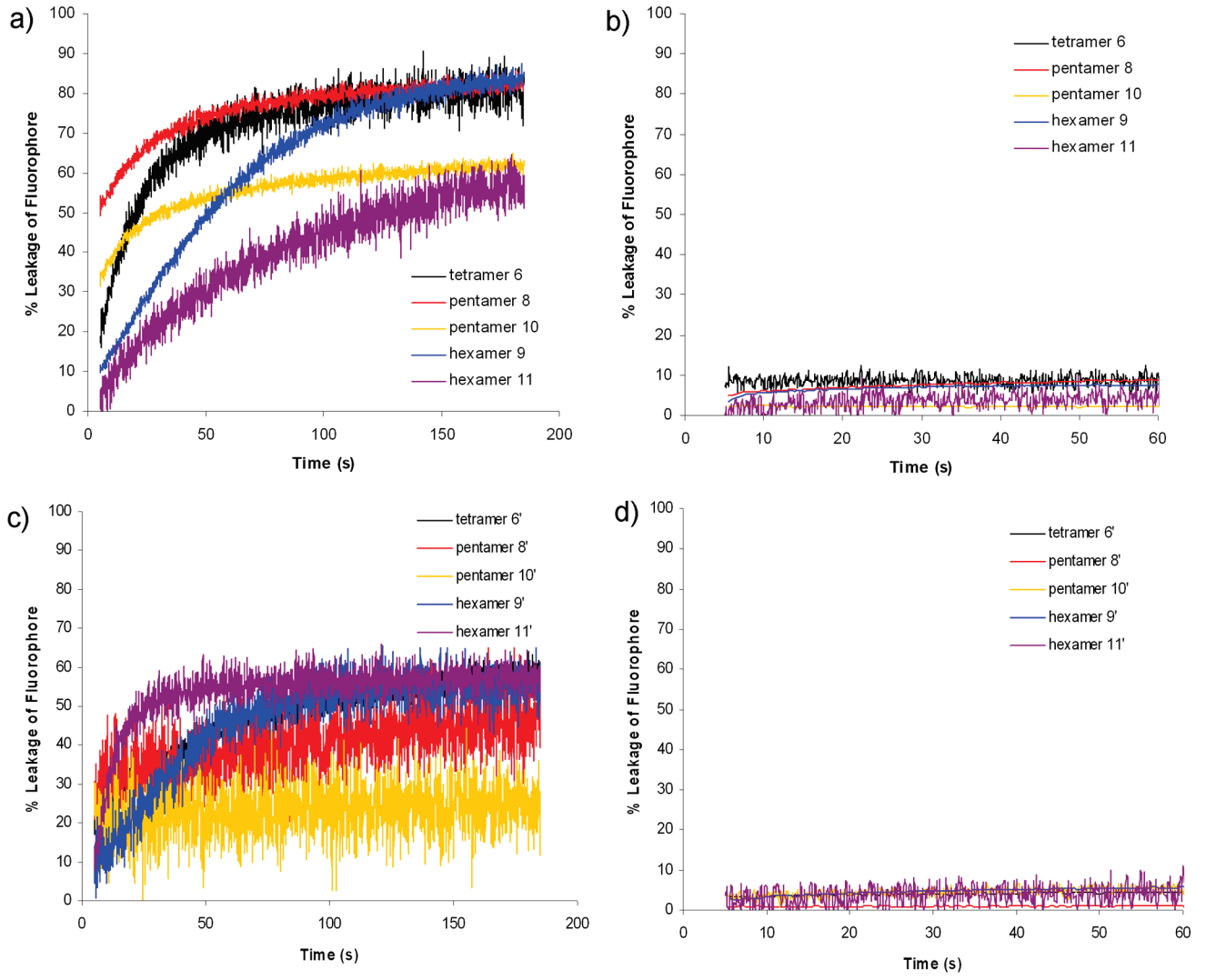

FIGURE 3. Detection of membrane disruption based on the leakage of encapsulated HPTS and its quencher DPX from vesicles: (a) oligomers based on alternating D- and L-amino acids assessed in vesicles of 80/20 DOPE/DOPG (bacterial mimic); (b) oligomers based on alternating D- and L-amino acids assessed in vesicles of DOPC (mammalian mimic); (c) oligomers based on all L-amino acids assessed in vesicles of 80/20 DOPE/DOPG; (d) oligomers based on all L-amino acids assessed in vesicles of DOPC. The oligomer concentration was 100 μg/mL in each case.

pentamer **8**, and hexamer **9** were also investigated. Quite unexpectedly, all of these molecules were found to be active in DOPE/DOPG vesicles. All three molecules provided approximately 80% release of HPTS-DPX after 3 min, with the hexamer again exhibiting slower release kinetics than the tetramer or pentamer. Overall, these results indicate that a cationic charge is not essential for activity in this class of molecules and that perhaps their activity may be enhanced by increasing their hydrophobicity. While **6**, **8**, and **9** are not cationic, they may still be amphipathic with the hydrogen-bonding edge of the molecule being relatively hydrophilic and the opposite edge with the valine side chain and the Boc- protected amine groups being hydrophobic in the β-strand conformation. The results thus far also suggest that there would not be significant benefits to the preparation of longer @-tide oligomers in terms of the degree of membrane disruption or the rate.

While the abilities of the molecules to lyse the DOPE/DOPG-based mimics of bacterial membranes is critical to their potential applications as antibiotics, it is also important to evaluate their abilities to lyse the DOPC-based mimics of mammalian membranes, as this may provide early indications of their potential toxicity to mammalian cells. Therefore, all of the molecules described above, which exhibited activity in the DOPE/DOPG vesicles, were evaluated in DOPC vesicles with encapsulated HPTS-DPX. As shown in Figure 3b, it was found that at a concentration of 100 μg/mL, all of the molecules exhibited greatly reduced membrane disruptive activity in the DOPC vesicles, with less than 10% of the HPTS-DPX released during the course of the experiment. The reduced activities of the molecules in the DOPC vesicles may be due partly to the lack of anionic charge on these vesicle membranes. However, for the non-cationic @-tides **6**, **8**, and **9**, the reduced activity cannot be attributed only to charge effects but may be due at least partly to the intrinsic negative curvature of the DOPE lipids relative to the DOPC lipids and thus their increased susceptibility to lysis.

In order to evaluate the role of the three-dimensional structure on the membrane disruptive activity, the analogues **6′**, **8′**, **9′**, **10′**, and **11′**, containing only L-amino acids, were also tested in the vesicle lysis assay. As illustrated in Figure 1 and described above, it was not expected that these molecules would display facially amphiphilic conformations, as all of the amino acid side chains would be directed to the same side of the β-strand in the linear conformation. As shown in Figure 3c, all of these all L-amino acid analogues exhibited greatly reduced lysis activity in DOPE/DOPG vesicles relative to their alternating D,L counterparts, with the exception of the hexamer **11′** which had similar activity to **11**. This suggests that the designed amphipathicity of the molecules does have an important role in their activity and that while the molecules would not be expected to preorganize into β-sheet mimics in aqueous solution, they may be able to assume conformations resembling β-strands in the presence of membranes. In the case of natural antimicrobial peptides such as magainin, it has been found that while the molecules are capable of displaying amphipathic conformations, they are often unstructured in aqueous solution in the absence of membranes or membrane mimics.[1,50] Nevertheless, this result is surprising in light of the high activities of the protected oligomers **6**, **8**, and **9**, which suggested that the presence of hydrophobic and cationic residues on opposite faces of the molecule was not essential for activity and that instead an amphipathicity based on the hydrogen bonding edge and the edge presenting the amino acid side chains might be sufficient. Compounds **6′**, **8′**, **9′**, **10′**, and **11′** were all also evaluated in the DOPC vesicles to probe their selectivity for bacterial over mammalian cell membranes, and as observed for the alternating D,L analogues, less than 10% of the HTPS-DPX was released.

Overall, these vesicle lysis assays have shown that many of the @-tides described here have moderate membrane disruptive activity and also promising selectivity for bacterial over mammalian membranes. It is also apparent that for membrane disruptive activity there is an advantage to the preparation of @-tides from alternating D- and L-amino acids such that alternating side chains diverge to opposite sides of the molecule in the linear conformation. The surprising activities of the protected oligomers suggest that the azacyclohexenone units of the @-tides may not contribute as much hydrophobicity as expected and that higher levels of activity might be achieved by the use of higher ratios of hydrophobic/hydrophilic amino acid side chains as well as the incorporation of amino acids or terminal groups with higher hydrophobicity.

Conclusions

β-Strand mimetic oligomers based on alternating α-amino acids and azacyclohexenone units were designed as potential membrane disruptive antibiotics. D-Valine and L-lysine were incorporated in an alternating manner with the aim of obtaining amphipathic structures having a cationic and a hydrophobic face in the linear β-strand conformation. The molecules were successfully synthesized by a solution phase convergent approach. Using NMR dilution studies, it was demonstrated that these new oligomers containing both D- and L-amino acids could dimerize to β-sheet mimics in $CDCl_3/CD_3OH$ solutions with similar affinities to the oligomers containing all L-amino acids, which were previously reported. This indicates that the incorporation of D-amino acids likely does not dramatically alter the conformational preferences of the molecules and that they are capable of exhibiting amphipathic conformations. In vesicle leakage assays using membranes designed to mimic those of bacteria, it was found that several oligomers exhibited moderate membrane disruptive activity. No significant effects based on oligomer length were observed in this series of molecules ranging from tetramers to hexamers, although the hexamer exhibited slower leakage kinetics. Surprisingly, the oligomers with the ε-Boc protecting groups on the lysine units were as active or more active than the corresponding deprotected oligomers with pendant cationic amines, demonstrating that a cationic charge was not essential for activity in this class of molecules and that there may be some advantage to the increased hydrophobicity of the protected oligomers. In addition, it was found that oligomers based on alternating D- and L-amino acids generally exhibited significantly higher activity than the corresponding oligomers containing all L-amino acids. This indicates that there is some advantage to having the amino acid side chains diverging to opposite sides of the strand. Furthermore, much lower activity was observed for all of the oligomers with membranes mimicking those of eukaryotic cells in comparison with those of bacterial cells, suggesting that this class of molecules may be capable of selectively killing bacteria in the presence of mammalian cells. Overall, this work represents the first example of membrane disruptive oligomers developed from a β-strand mimic based on α-amino acids. Although the membrane-disruptive activities obtained thus far are relatively modest, several important insights were gained into the structural features that are important for activity, providing the groundwork for further exploration of @-tides as potential antimicrobials. These structures are highly tunable as a diverse range of α-amino acids and oligomer terminal functionalities can be readily incorporated using the same synthetic routes described here. Thus, the careful design, syntheses, and evaluation of additional series of @-tides based on the discoveries described here can likely lead to new molecules with high antimicrobial activity and selectivity.

Experimental Section

Synthesis of Dimer 3 and Representative Dimer Synthesis. Compound **1**[41] (4.5 g, 18 mmol, 1.0 equiv) and ε-Boc-L-lysine[51,52] (4.8 g, 20 mmol, 1.1 equiv) were dissolved in dry MeOH (270 mL) under a N_2 atmosphere. The reaction was heated to 60 °C and maintained at this temperature overnight. The solution was then cooled to room temperature and concentrated. The crude product was redissolved in EtOAc/MeOH (220 mL/9 mL) and extracted with 1 M $KHSO_4$ followed by brine. The organic layer was isolated, dried with $MgSO_4$, filtered, and concentrated. The product was purified by silica gel chromatography using a gradient of EtOAc/hexane (95/5) to remove impurities followed by EtOAc/MeOH (90/10) to elute the product (5.97 g, 68%) as a viscous oil.

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The product was taken to the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2/1)): δ 1.31−1.57 (m, 13H), 1.68−1.95 (m, 2H), 3.02 (t, 2H, J = 6.3 Hz), 3.83−3.94 (m, 1H), 3.96−4.12 (m, 2H), 4.25−4.37 (m, 2H), 5.04−5.11 (m, 1H), 5.12−5.18 (m, 2H), 7.29−7.39 (m, 5H).

Synthesis of Dimer 4. The acid **3** (6.0 g, 13 mmol, 1.0 equiv) was dissolved in THF/H_2O (386 mL/45 mL) with stirring. A 20% solution of Cs_2CO_3 was added to the reaction mixture slowly until a pH of 7 was obtained. The mixture was then concentrated, redissolved in THF, and concentrated again. Methyl iodide (0.94 mL, 15 mmol, 1.2 equiv) in DMF (162 mL) was added to the resulting oil, and the reaction was stirred at room temperature for 30 min. The solvent was evaporated, and the viscous oil was triterated with distilled water to remove salts. The product was filtered and taken back up in MeOH and then concentrated. The product was further purified by silica gel chromatography using a gradient of CH_2Cl_2 to CH_2Cl_2/MeOH (98/2) to remove the impurities, followed by CH_2Cl_2/MeOH (90/10) to elute the product (2.9 g, 48%) as a glassy solid. ^{1}H NMR (600 MHz, CD_3OD): δ 1.26−1.51 (m, 13H), 1.69−1.90 (m, 2H), 2.97−3.07 (m, 2H), 3.71 (s, 3H), 3.96 (t, 1H, J=6.9), 4.00−4.07 (m, 2H), 4.24−4.33 (m, 2H), 5.04 (s, 1H), 5.12 (s, 2H), 7.26−7.37 (m, 5H). ^{13}C NMR (150 MHz, $CDCl_3/CD_3OD$ (2/1)): δ 23.9, 29.0, 30.5, 32.2, 40.9, 48.9, 51.4, 53.1, 56.7, 68.7, 79.7, 95.2, 128.9, 129.2, 129.6, 137.5, 156.1, 158.2, 173.1, 193.3. IR (cm^{-1}, film from CH_2Cl_2): 3269, 3056, 2925, 2859, 1739, 1658. HRMS: calcd for $[M + H]^+$ ($C_{25}H_{35}N_3O_7$) 489.2475, found (ES+) 489.2480.

Synthesis of Tetramer 6 and General Coupling Procedure. The dimer **2** (1.0 g, 2.9 mmol, 1.1 equiv), dimer **5** (0.93 g, 2.6 mmol, 1.0 equiv), HATU (3.6 g, 9.5 mmol, 3.3 equiv), and DIPEA (1.1 mL, 6.4 mmol, 2.2 equiv) were dissolved in dry DMF (84 mL) under N_2, and the reaction mixture was stirred for 24 h. The reaction progress was monitored by thin-layer chromatography (100% EtOAc), and upon completion the solution was concentrated, redissolved in EtOAc (124 mL), and washed with 1 M $KHSO_4$ followed by satd $NaHCO_3$. The organic layer was isolated, dried over $MgSO_4$, filtered, and evaporated to yield a glassy solid. The product was purified by silica gel chromatography using EtOAc to elute the impurities, followed by EtOAc/MeOH (95/5) to elute the product (1.1 g, 63%) as a glassy solid. ^{1}H NMR (600 MHz, CD_3OD): δ 0.81−1.09 (m, 6H), 1.25−1.55 (m, 15H), 1.71−1.95 (m, 2H), 2.02−2.16 (m, 1H), 2.96−3.09, (m, 2H), 3.66−3.78 (m, 3H), 3.94−4.14 (m, 3H), 4.14−4.25 (m, 1H), 4.28−4.62 (m, 5H), 4.73 (t, 1H, J=17.0), 5.06−5.28 (m, 4H), 6.52 (br s, 1H), 7.24−7.38 (m, 5H). ^{13}C NMR (150 MHz, CD_3OD): δ 18.7, 19.6, 24.0, 28.9, 30.5, 32.5, 41.0, 43.7, 45.1, 51.5, 53.1, 56.9, 59.0, 68.8, 79.8, 95.0, 95.5, 129.0, 129.3, 129.7, 137.6, 156.4, 158.4, 163.4, 164.9, 170.8, 171.2, 173.0, 173.2, 192.4, 193.7. IR (cm^{-1}, film from CH_2Cl_2): 3283, 3057, 2960, 2927, 2862, 1736, 1689. HRMS: calcd for $[M + Na]^+$ $(C_{35}H_{49}N_5O_9Na)^+$ 706.3422, found (ES+) 706.3428. HPLC: t_R 9.7 min (MeCN/H_2O (20/80)).

^{1}H NMR Dilution Experiment. The pentamer **8** was dissolved in $CDCl_3$ at a concentration of 50 mM, and serial 2-fold dilutions were carried out to a concentration of 0.78 mM. ^{1}H NMR spectra were obtained at 600 MHz for each concentration. The upper concentration was limited by broadness of peaks at higher concentrations in $CDCl_3$. The same procedure was carried out for pentamer **8** in 99/1 $CDCl_3/CD_3OH$ and 97.5/2.5 $CDCl_3/CD_3OH$ but at concentrations ranging from 125 to 1 mM. The tetramer **6** and hexamer **9** were evaluated in 97.5/2.5 $CDCl_3/CD_3OH$ at concentrations ranging from 125 to 1 mM.

Vesicle Leakage Assay. Vesicles formed from DOPC were used as models for mammalian cell membranes, and vesicles formed from an 80/20 mixture of DOPE/DOPG were used as models for bacterial membranes. HPTS and its quencher DPX were encapsulated in these vesicles. The following solutions were used in the vesicle experiments: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer (10 mM HEPES, 145 mM NaCl, 0.1 mM EDTA, $NaHCO_3$ pH 7.25), HPTS (10 mM) in HEPES buffer, DPX (100 mM) in HEPES buffer, and HPTS/DPX/HEPES buffer (3.0, 1.8, and 5.2 mL, respectively, of the HPTS, DPX, and HEPES buffers). A 1.1 mL portion of a DOPC stock solution (25 mg/mL) was dried under N_2 and then dried under high vacuum for 2 h. A 0.88 mL portion of a DOPE stock solution (25 mg/mL) in chloroform was mixed with 0.22 mL of a DOPG stock solution (25 mg/mL) in chloroform to a total volume of 1.1 mL, and then the resulting solution was dried under N_2 followed by under high vacuum for 2 h. The resulting films were hydrated with 0.6 mL of HPTS/DPX/HEPES buffer for 2 h. The suspensions were then subjected to five freeze−thaw/sonication cycles and were subsequently extruded through a 1 μm Whatman polycarbonate membrane >10 times yielding about 0.5 mL, which was then diluted to 2.5 mL with HEPES buffer. The excess dye was removed by gel filtration chromatography (Nap-10 columns, GE Healthcare) using HEPES buffer, resulting in 4.5 mL of vesicle solution. Twenty microliters of this solution was added to 1.98 mL of HEPES buffer in a quartz fluorescence cuvette. Solutions of the oligomers in DMSO (5−40 μL of a 5 mg/mL solution) were added to provide final oligomer concentrations ranging from 12.5 to 100 μg/mL in the 2 mL of vesicle solution. The solutions were stirred, and the fluorescence emission intensities I_t (λ_{em} = 520 nm, λ_{ex} = 460 nm) were monitored as a function of time (t). Twenty microliters of 20% Triton X-100 in DMSO was then added to provide complete vesicle lysis. The curves were normalized to percent leakage $[(I_t - I_0)/(I_\infty - I_0)] \times 100$. I_0 is the emission intensity before the addition of any of the polymers, and I_∞ is the emission intensity after the addition of Triton X-100.

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Supporting Information Available: General procedures and materials section, syntheses of oligomers **2**, **5**, **7**, **8**, **9**, **10**, **11**, **2′**, **6′**, **8′**, **9′**, **10′**, and **11′**, NMR spectra of compounds **2**/**2′**, **4**, **6**, **6′**, **8**, 8′, **9**, **9′**, **10**, **10′**, **11**, and **11′**, and HPLC chromatograms of compounds **6**, **6′**, **8**, **8′**, **9**, **9′**, **10**, **10′**, **11**, and **11′**. This material is available free of charge via the Internet at http://pubs.acs.org.