Letter

# Peptoid Library Agar Diffusion (PLAD) Assay for the High-Throughput Identification of Antimicrobial Peptoids

Kevin J. Fisher, Jeremy A. Turkett, Ashley E. Corson, and Kevin L. Bicker

*ACS Comb. Sci.*, **Just Accepted Manuscript** • DOI: 10.1021/acscombsci.6b00039 • Publication Date (Web): 17 May 2016

# Peptoid Library Agar Diffusion (PLAD) Assay for the High-Throughput Identification of Antimicrobial Peptoids

Kevin J. Fisher, Jeremy A. Turkett, Ashley E. Corson, Kevin L. Bicker[†]

Middle Tennessee State University, Department of Chemistry, 1301 E. Main St., Murfreesboro, TN 37132.

**Abstract**

Rapid emergence of antimicrobial resistant organisms necessitates equally rapid methods for the development of new antimicrobial compounds. Of recent interest have been mimics of antimicrobial peptides known as antimicrobial peptoids, which exhibit similar potency to the former but with improved proteolytic stability. Presented herein is a high-throughput method to screen libraries of antimicrobial peptoids immobilized on beads embedded into solid media. Termed the Peptoid Library Agar Diffusion (PLAD) Assay, this assay allows for individual chemical manipulation of two identical peptoid strands. One strand can be released to diffuse out from a solid support bead and interact with the microorganism during screening. The other strand can be cleaved after screening from beads showing strong antimicrobial activity and analyzed by mass spectrometry to deconvolute the structure of the peptoid. This method was applied to a small library of peptoids to identify an antimicrobial peptoid with modest efficacy against the ESKAPE pathogens.

**Keywords**

peptoids, high-throughput, antimicrobial, combinatorial library

The increasing prevalence of multi-drug-resistant (MDR) bacterial infections in the clinic necessitates methods to rapidly identify potent new antimicrobial agents that are effective against MDR bacteria. Antimicrobial resistance (AMR) is considered by the World Health Organization to be a major threat to global public health, resulting in a significant rise in global mortality rates and a significant decline in economic growth due to the growing cost of bacterial infection treatment.[1] A recent study predicted that by the year 2050, AMR will result in 10 million premature deaths per year worldwide and roughly $100 trillion USD in lost economic output.[2] Bacterial resistance is a growing problem due to increasing and improper use of antibiotics combined with the ability of bacteria to readily transmit information from one microbe to another.[3, 4] Common mechanisms of bacterial resistance include drug efflux pumps and enzymes that break down common antibiotics, such as β-lactamases and aminoglycosides.[3, 4] There is now a need for antimicrobial compounds that are not susceptible to these drug resistance mechanisms.

One such class that has drawn particular interest lately is antimicrobial peptides (AMPs). AMPs serve as a natural part of the host-defense innate immune system of several organisms.[5, 6] There is little known antibiotic resistance to AMPs, likely due to their non-specific mode of killing.[7] It is believed that most AMPs cause membrane permeabilization, resulting in leakage of cytoplasmic components and cell death.[5, 7] Other evidence indicates that some AMPs may bind to and disrupt DNA or RNA, which is not surprising given the amphipathic structure of these compounds.[5, 7] Although promising, AMPs have not been developed into legitimate therapeutics due to the poor proteolytic stability and low bioavailability of peptides.[8, 9] Several mimics of AMPs have been developed with the goal of preserving their advantages while circumventing their shortcomings.[10] One promising class of these have been based on N-substituted glycines

(termed peptoids) which are similar to peptides, but with the side chain shifted from the *alpha*-carbon to the amide-nitrogen.[9, 11] Peptoids are similar to peptides in function, yet they are not recognized by proteases and hence have a prolonged lifetime *in vivo* as well as improved bioavailability, making them excellent candidates as therapeutics.[9]

The development of antimicrobial peptoids has relied on the mimicry of known AMPs and the generation of small (<20 compound) subsets of peptoids.[12-17] This work has generated antimicrobial peptoids that are effective against *M. tuberculosis* and *P. aeruginosa* biofilms.[13, 14] The rapid development of MDR bacterial strains demands novel antibiotics, and the above mentioned efficacy of peptoids demonstrates their potential as therapeutics. The need now is to develop methods to screen very large libraries of peptoid compounds against any bacteria of interest in a rapid fashion, thereby identifying antimicrobial peptoids that can treat new strains of MDR bacteria. Combinatorial libraries, generated by split-and-pool synthesis, are a way to generate large cohorts of potential therapeutic compounds in a relatively short period of time.[18, 19] Combinatorial libraries of peptoids, first synthesized by Zuckermann *et al.*,[20] have been used to identify inhibitors of VEGFR[21] and antibody ligands,[22] among other applications. These libraries are typically synthesized on the solid-phase to provide easy manipulation during synthesis and subsequent screening.[18] Combined with high-throughput screening methods, combinatorial libraries represent a powerful tool for drug discovery. The work detailed here introduces a Peptoid Library Agar Diffusion (PLAD) assay, which takes advantage of a solid-phase combinatorially produced library of peptoids on a chemically cleavable linker that can be screened within solid agar plates to readily identify potent antimicrobial peptoids against microbes of interest. This PLAD assay relies on a unique branched system with a disulfide linker that can be chemically cleaved after embedding the library into the agar. In contrast to

previous bead diffusion assays, which have used photolabile linkers,[23-25] the disulfide linker allows for cleavage after the beads are embedded in the agar, negating the need for irradiation optimization and reducing cross contamination that would arise from irradiating the beads in one large batch and then spreading them across the agar. Also, since the beads are surrounded by agar instead of spread on top, the compounds have better contact with bacteria, creating zones of inhibition that are easier to read.

The key to the PLAD Assay is a C-terminal linker system that results in two identical peptoid strands, termed the *alpha* and *beta* strands, that can be individually chemically manipulated. During the assay, soft agar is inoculated with the microorganism of interest before addition of compound beads and a small amount of reducing reagent. The soft agar mixture is then poured onto a hard agar Petri dish and allowed to solidify, resulting in an even distribution of compound beads embedded in inoculated soft agar (**Figure 1; Stage 1**). The plate is then incubated overnight, which allows for the bacteria to grow into a lawn and also results in cleavage of the disulfide bond with reducing reagent, releasing the *beta*-strand peptoid from the bead (**Figure 1; Stage 2**). A peptoid compound that is an effective antimicrobial agent kills the microorganism surrounding the bead it was released from, generating an easily read zone of inhibition. This bead can then be removed from the plate manually, and the *alpha*-strand peptoid cleaved to analyze by mass spectrometry (MS) and MS/MS (**Figure 1; Stage 3**).

Synthesis of the C-terminal linker in the PLAD Assay was done on TentaGel resin, a solid support which consists of polyethylene glycol grafted onto a polystyrene matrix. This feature allows swelling in both organic solvents, for synthesis, and aqueous solutions, for screening. The amino acid methionine is added first to the resin (**Figure 2**), which provides a way to orthogonally cleave the peptoid from the resin post-screening using cyanogen bromide.

Resulting in a homoserine lactone, use of a methionine for orthogonal release of the compound from the resin is now common in combinatorial library synthesis and screening.[26, 27] After methionine, β-alanine is added as a spacer to help move the rest of the linker system and peptoid away from the resin. The disulfide linker is then introduced via peptoid submonomer methods[11] using bromoacetic acid followed by mono-Boc protected cystamine. Lastly, Fmoc-aminohexanoic acid was added to the N-terminus of the peptomer followed by removal of the Boc and Fmoc protecting groups. This produces a linker system with two free amino groups ready for peptoid synthesis to generate identical sequences with orthogonal chemical manipulation. Aminohexanoic acid was chosen to use at the branch point in order to space out the two amino groups. Without this spacer, cyclization of the two branches was observed during subsequent peptoid synthesis (data not shown).

Once the initial linker design was completed, a test peptoid was synthesized onto it to confirm the chemical manipulability of the PLAD linker. The submonomer sequence of the test peptoid was NVal-NMeo-NPhe. Nomenclature for peptoid submonomers uses standard three letter codes, as for amino acids, but prefixes the code with "N" to denote the placement of the side chain on the amide nitrogen. Synthesis was accomplished by peptoid submonomer methods[11] using bromoacetic acid, diisopropylcarbodiimide, and the amines isopropylamine (NVal), 2-methoxyethylamine (NMeo), and benzyl amine (NPhe). The test peptoid was analyzed by mass spectrometry (MS) to show the complete mass (**Figure S1**) as well as tested to ensure that treatment with tris(2-carboxyethyl)phosphine (TCEP), a reducing reagent, effectively cleaved the disulfide bond, yielding the β-strand peptoid (**Figure S2**). Lastly, the remaining α-strand peptoid after TCEP treatment was cleaved from the resin with cyanogen bromide then analyzed by MS (**Figure S3**) and MS/MS (**Figure S4**). The resultant spectra confirmed the

peptoid sequence and demonstrated the ability to deconvolute the sequence of a library peptoid after screening. Although all these analyses were successful, the conditions of cyanogen bromide cleavage had to be optimized. After multiple cyanogen bromide cleavages were unsuccessful for hydrophobic compounds in 0.1 M HCl in water, the hydrophobicity of the cleavage solution was altered with addition of acetonitrile. After trying several different ratios (data not shown), it was determined that an optimal ratio of 80:20 acetonitrile:water containing 0.1 M HCl resulted in the highest quality compound analysis by mass spectrometry. This could be in part due to the swelling properties of TentaGel resin as well as a more non-polar solution aiding diffusion of the hydrophobic peptoid out of the bead and into solution.

With conditions optimized for synthesis, chemical manipulation, and mass spectroscopic analysis of the PLAD Assay linker and peptoid strands, we next set out to optimize PLAD Assay screening conditions. A known antimicrobial peptoid was synthesized on the PLAD Assay linker as a proof-of-concept compound for bacterial screening (**Figure 3A**). The peptoid of choice, termed $C13_{4mer}$, was designed by Barron *et al.*[13, 14] and involves the use of hydrophobic alkyl tails to mimic the antibacterial properties of lipopeptides.[28] The addition of an alkyl tail allows the peptoid of interest to be shortened, while still retaining useful antimicrobial behavior. As shown by Barron *et al.*,[28] incorporation of 10 or 13 carbon alky tails onto pentameric antimicrobial peptoids yields similar potency to peptoids that are 12 to 16 submonomers in length but without long alkyl tails. One benefit of the shortening involves limiting the number of reactions occurring in the peptoid process, allowing for a higher yield. Another benefit is the reduction in molecular weight of the compound when changing from a 10-15 submonomer length peptoid (~1250 Da on average) to a 3-5 submonomer length peptoid (~400 Da on average). The sequence of the $C13_{4mer}$ peptoid synthesized on the PLAD Assay linker was NTri-

NLys-NPea-NPea-NLys. Synthesis was again accomplished by peptoid submonomer methods[11] using bromoacetic acid, diisopropylcarbodiimide, and mono-Boc-1,4-diaminobutane (NLys), (±)-phenylethylamine (NPea), and 1-tridecylamine (NTri). The acid sensitive Boc protecting group was used on 1,4-diaminobutane, as any unprotected NLys sub-monomers would act as branching points during bromoacylation. The Boc group was shown to still be attached after subsequent treatments with bromoacetic acid (data not shown) demonstrating its stability to weak acids and usefulness as a protecting group for this synthetic method. After the final coupling of the tridecyl alkyl tail, the Boc groups were removed with triflouroacetic acid (TFA) and the resin washed thoroughly to ensure residual acid was removed. The synthesized $C13_{4mer}$ compound was analyzed by mass spectrometry (MS) to show the complete mass (**Figure S5**) as well as tested to ensure that treatment with TCEP effectively cleaved the disulfide bond, yielding the β-strand of this compound (**Figure S6**).

PLAD linked $C13_{4mer}$ was subsequently used to evaluate the most effective reducing reagent for the PLAD Assay (**Figure 1**) against relatively non-pathogenic *E. coli* (ATCC 25922). Several common reducing reagents were examined at varying concentrations (0, 2, 6, 10, and 14 mM) to identify the most suitable reagent to effectively cleave the disulfide linker without significantly affecting microorganismal growth. The reagents tested were dithiothreitol (DTT), β-mercaptoethanol (BME), and tris-(2-carboxyethyl)phosphine (TCEP). Effectiveness at cleaving the disulfide linker and releasing the *beta*-strand peptoid was evaluated by measuring the zone of inhibition, defined as the area around the bead with no bacterial growth and measured from the edge of the bead to the start of bacterial lawn growth. Effect of reducing reagent on bacterial lawn growth was evaluated by measuring the luminosity of the light reflected by the bacterial lawn when illuminated from an angle, with denser bacterial lawns

resulting in greater luminosity. All three reducing reagents resulted in concentration dependent zones of inhibition (**Figure 3B, Supporting Figure S7**). Comparatively, TCEP provided the clearest zones of inhibition, with the largest zones not surprisingly observed at 14 mM. Evaluation of the bacterial lawn density indicated that no significant effect on bacterial growth was observed for any of the reducing reagents at the concentrations tested (**Figure S8**). Given this data, TCEP at a concentration of 14 mM was used for any subsequent PLAD Assays.

To evaluate the usefulness of the PLAD Assay in identifying antimicrobial peptoids, a very small proof-of-concept library was synthesized on the PLAD linker using semi-combinatorial chemistry (**Figure 4A**). Three aromatic submonomers (furfurylamine, benzylamine, and 1-phenylethylamine) were randomly incorporated into the first C-terminal position of this library, two cationic submonomers (mono-Boc-diaminoethane and mono-Boc-diaminobutane) were randomly incorporated into the second position, and three hydrophobic submonomers (isopropylamine, 1-aminodecane, and 1-aminotridecane) were randomly incorporated into the third position. These submonomers were chosen for this proof-of-concept library because previous studies have shown that peptoids comprised of cationic and hydrophobic submonomers exhibit strong antimicrobial activity.[15, 28, 29] This produced a library with 18 unique peptoid sequences (**Figure S9**) that could be screened to demonstrate the utility of the PLAD Assay and identify a novel antimicrobial agent.

This library was screened against non-pathogenic *E. coli.* (ATCC 25922) as described previously with the known $C13_{4mer}$ antimicrobial peptoid and zones of inhibition were measured using a Leica M165FC microscope. In total, roughly 800 beads were screened, representing 44 replicates of the theoretical diversity. Multiple replicates were evaluated in one screening to gain a better understanding of antimicrobial peptoid sequence homology and to give statistical

credence to the relationship between peptoid sequence and zone of inhibition. Representative images from this screening can be seen in **Figure S10**. Hits, defined as beads with a measurable zone of inhibition, were isolated manually with surgical tweezers and placed into individual tubes. These beads were boiled in 1% sodium dodecylsulfate (SDS) to remove bacterial and media debris from the beads. The *alpha*-strand of the peptoid was cleaved from the bead using cyanogen bromide then analyzed by MS and MS/MS to identify the structure of the unknown peptoid. Representative spectra are given in **Figures S11** and **S12**. In total 34 hits were identified (24% hit rate) and 31 sequences were successfully obtained by MS and MS/MS (**Figure S13**). A homology chart was generated to determine which residues were most prevalent at particular positions in the identified hits (**Figure S14**). In the first position, most antimicrobial peptoids contained benzylamine or 1-phenylethylamine in equal prevalence, while very few contained furfurylamine. There was also little difference in the abundance of diaminoethane and diaminobutane in the second position of identified hits. Interestingly, in the third position, all antimicrobial peptoids identified contained a 1-aminotridecane submonomer. Improved antimicrobial activity with a long hydrophobic residue in this position is not surprising given previously published results. The size of the zone of inhibition, presumably a measure of the peptoid's antimicrobial potency, was also correlated with peptoid sequence (**Figure 4B**). The peptoid with the largest average zone of inhibition was sequence K15 (NTri-Nae-NPea). Note that all but one of the hits identified from this screening had a larger zone of inhibition than $C13_{4mer}$, demonstrating the ability of even a small peptoid library in identifying potent antimicrobial agents. Interestingly, the hits with the smallest zones of inhibition correlated to those containing furfurylamine in position 1, confirming the homology data which showed very little prevalence of this submonomer in identified hits.

To evaluate the efficacy of an antimicrobial peptoid identified from the PLAD Assay, the tripeptoid K15 (**Figure S15**) was synthesized and its MIC against the ESKAPE pathogens determined (**Figure 4C**). The ESKAPE pathogens (*Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) represent a cohort of bacteria that are resistant to most clinically used antibiotics.[30] We note that the *E. coli* tested here was the same strain of *E. coli* used during library screening (ATCC 25922). K15 displayed modest efficacy against six of the seven pathogens tested with the strongest antimicrobial efficacy against *A. baumannii* and *E. faecium* (25 μg/mL). This modest efficacy is undoubtedly due to the limited diversity of the proof-of-concept library. However, these results demonstrate the capability of the PLAD Assay to identify compounds with antimicrobial activity, even against pathogens with modes of antimicrobial resistance. Subsequent studies will focus on screening more diverse libraries via the PLAD Assay against each of the ESKAPE pathogens.

In summary, we have demonstrated a high-throughput screening system to identify antimicrobial peptoids, which we believe to be modular enough to screen any cohort of combinatorially synthesized molecules. By designing a branched linker with orthogonal chemical manipulability, we have shown that we can release the *beta*-strand of a peptoid using TCEP to cleave a disulfide bond during screening, producing an easily read zone of inhibition in response to effective antimicrobial peptoids, while leaving the *alpha*-strand still attached to the bead. MS analysis of test peptoids and a small proof-of-concept library demonstrate the feasibility of deconvoluting the *alpha*-strand peptoid sequence of strong antimicrobial peptoids after screening, allowing researchers to rapidly screen large cohorts of potential compounds without knowing their structure. The optimal reducing reagent conditions were determined for

this assay and a proof-of-concept library was synthesized and screened, subsequently identifying K15, a peptoid with modest efficacy against the drug resistant ESKAPE pathogens. Current efforts are focused on screening more diverse peptoid libraries against both antimicrobial resistant bacterial and fungal pathogens.

## Supporting Information

Materials, submonomer synthesis, peptoid synthesis, and screening procedures as well as supplemental figures can be found in the supporting information.

## Author Information

†*Corresponding Author* – kevin.bicker@mtsu.edu

*Author Contributions* – KJF, JAT, AEC, and KLB designed experiments. KJF and AEC performed method development and validation. JAT performed K15 synthesis and ESKAPE testing. KJF, JAT, and KLB co-wrote the manuscript.

*Notes* – The authors declare no competing financial interests.

## Acknowledgments

This work was supported by start-up and Faculty Research and Creative Activity Committee (FRCAC) funds provided by MTSU and by the National Institutes of Health R03 AI112861. The authors thank Dr. Mary Farone for aiding and advising with ESKAPE pathogen MIC assays.

## Abbreviations

PLAD, Peptoid Library Agar Diffusion; MDR, multidrug resistant; AMR, antimicrobial resistance; AMP, antimicrobial peptide; BME, β-mercaptoethanol; DTT, dithiothrietol; TCEP, tris(2-carboxyethyl)phosphine.

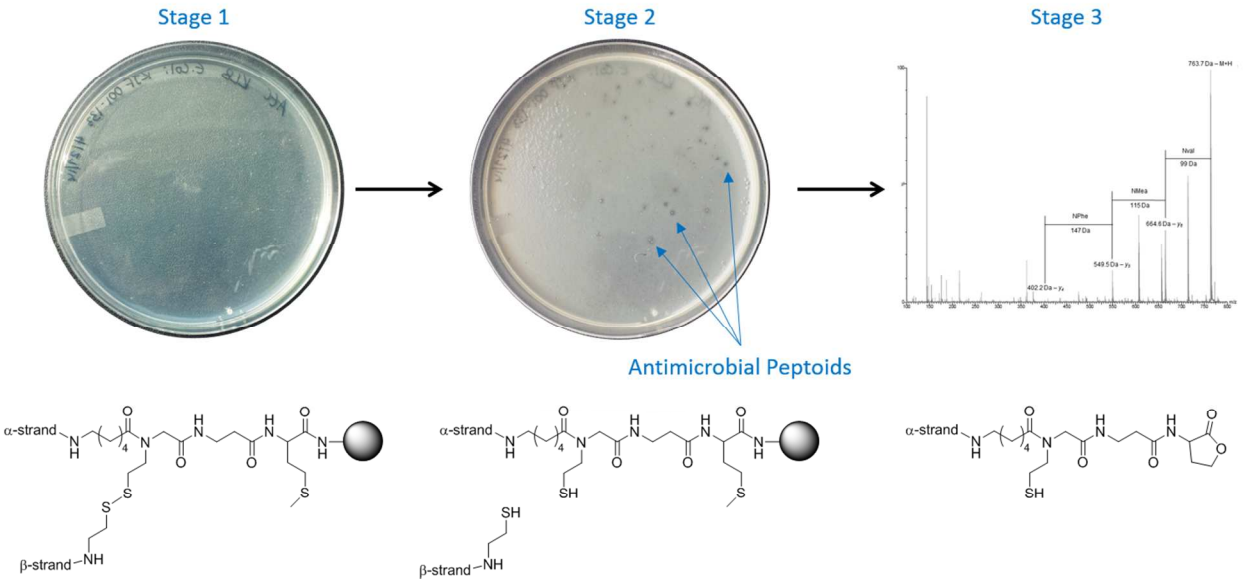



**Figure 1.** The general PLAD Assay screening schematic. (*Stage 1*) PLAD linked peptoids immobilized on beads are imbedded into soft agar containing a small amount of reducing reagent and inoculated with the microorganism of interest. (*Stage 2*) Overnight incubation results in growth of a microorganismal lawn, reduction of the disulfide, and release of the *beta*-strand peptoid from the bead. Peptoids with antimicrobial properties generate a zone of inhibition around the bead. (*Stage 3*) Beads showing zones of inhibition are removed from the plate, *alpha*-strand peptoids are cleaved from the bead, and the peptoid structure is determined by MS/MS.

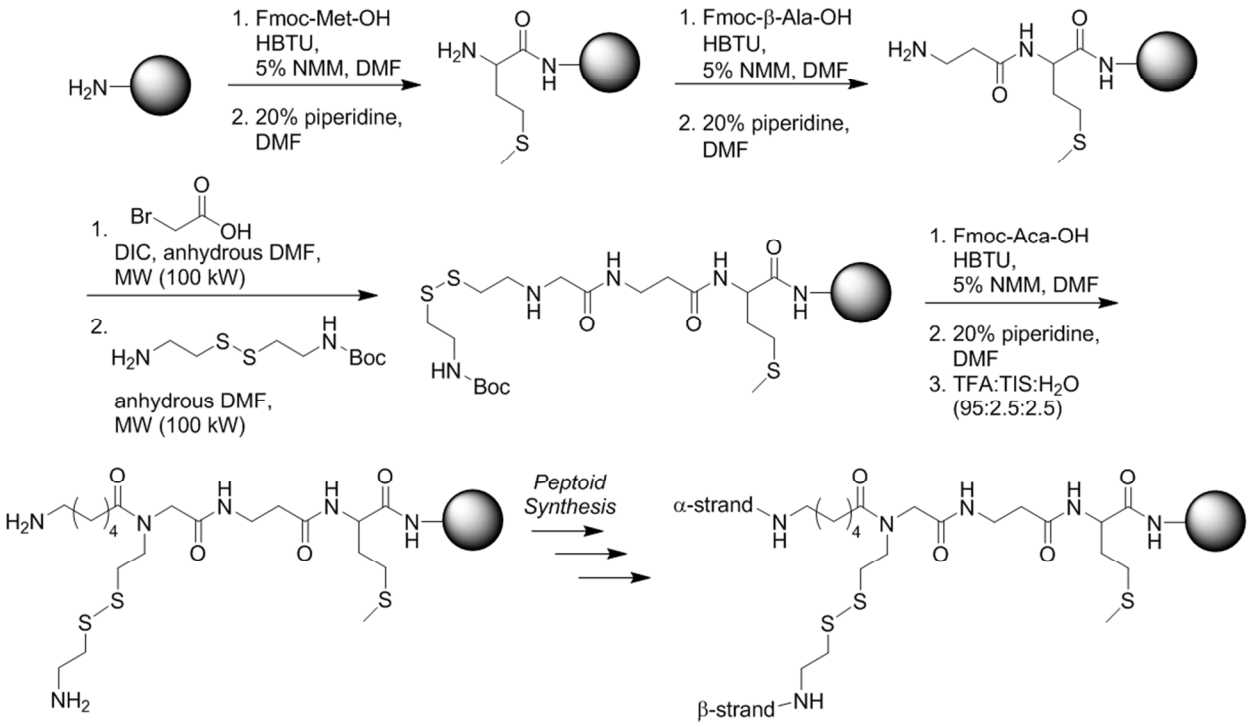



**Figure 2.** Synthesis of the PLAD linker, resulting in identical peptoid strands that can be chemically manipulated orthogonally. The *beta*-strand peptoid can be released using a reducing reagent to cleave the disulfide, followed by release of the *alpha*-strand using cyanogen bromide to cleave at the C-terminal methionine.

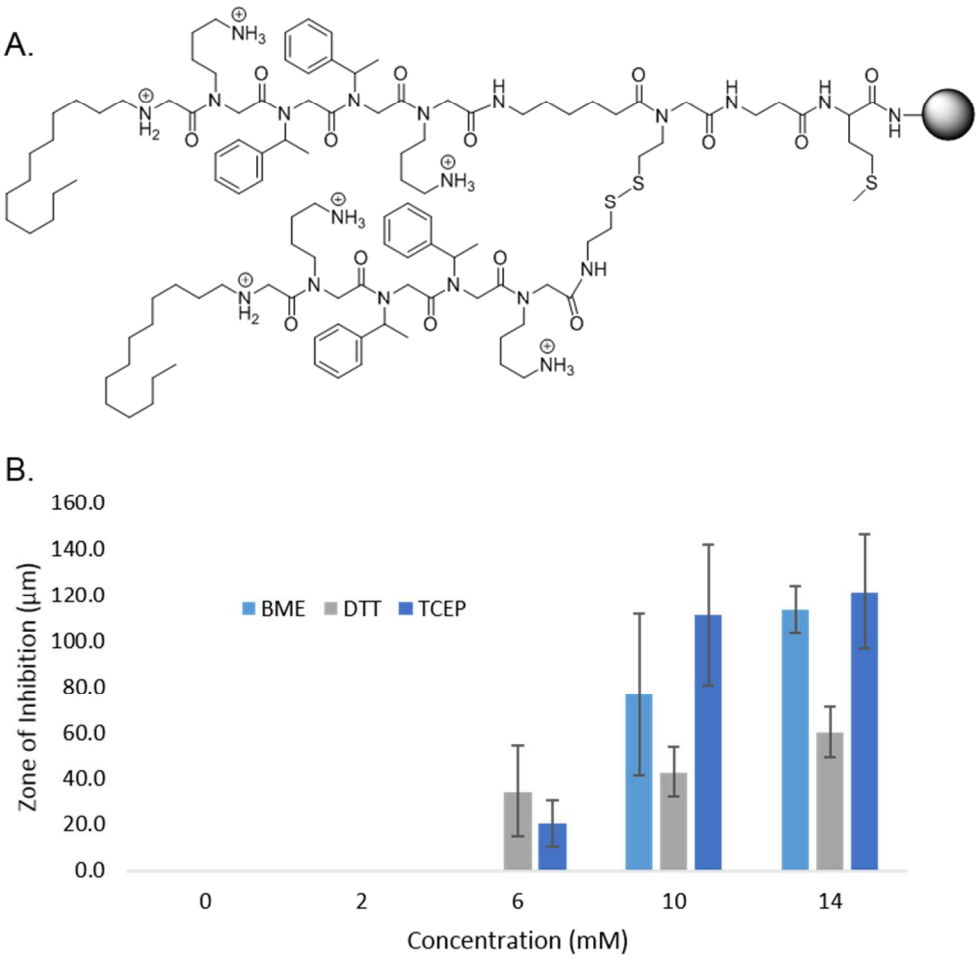
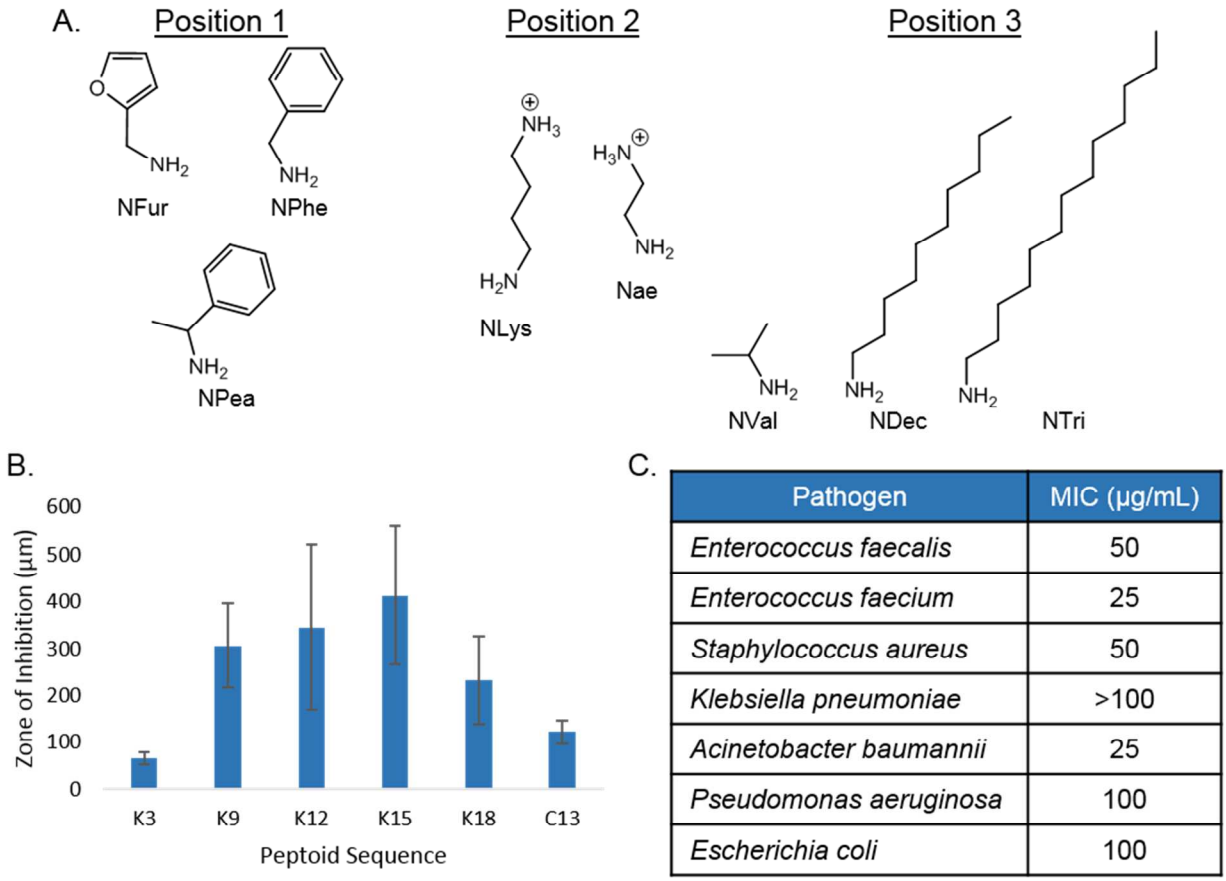



**Figure 3.** (**A**) A proof-of-concept known antimicrobial peptoid, $C13_{4mer}$ immobilized on the PLAD linker. (**B**) Evaluation of the zone of inhibition around a bead in response to varying concentrations of three reducing reagents; β-mercaptoethanol (BME), dithiothreitol (DTT), and tris(2-carboxyethyl)phosphine (TCEP).




C.

| Pathogen | MIC (µg/mL) |
|---|---|
| *Enterococcus faecalis* | 50 |
| *Enterococcus faecium* | 25 |
| *Staphylococcus aureus* | 50 |
| *Klebsiella pneumoniae* | >100 |
| *Acinetobacter baumannii* | 25 |
| *Pseudomonas aeruginosa* | 100 |
| *Escherichia coli* | 100 |

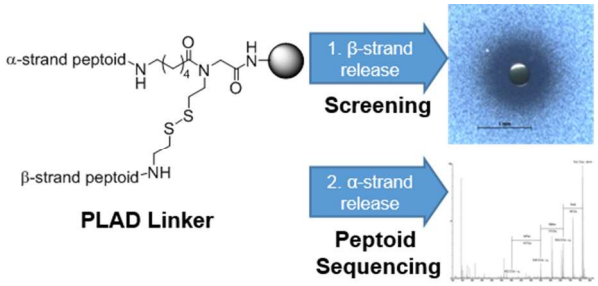
**Figure 4.** (**A**) Amines incorporated into a low diversity, proof-of-concept library on the PLAD linker. (**B**) Average zones of inhibition for the antimicrobial peptoids identified from screening the proof-of-concept library. Minimum inhibitory concentration (MIC) values for peptoid K15 tested against the ESKAPE pathogens.

## References


1. *Antimicrobial resistance: report on global surveillance.*; World Health Organization: France, 2014.
2. *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations.*; The Review on Antimicrobial Resistance: London, 2014.
3. Tenover, F. C., Mechanisms of Antimicrobial Resistance in Bacteria. *Am. J. Med.* **2006,** *119*, (6, Supplement 1), S3-S10.
4. Blair, J. M. A.; Webber, M. A.; Alison J. Baylay; Ogbolu, D. O.; Piddock, L. J. V., Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **2015,** *13*, 42-51.
5. Brogden, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* **2005,** *3*, 238-250.
6. Zasloff, M., Antimicrobial peptides of multicellular organisms. *Nature* **2002,** *415*, (6870), 389-95.
7. Jenssen, H. v.; Hamill, P.; Hancock, R. E. W., Peptide Antimicrobial Agents. *Clin. Microbiol. Rev.* **2006,** *19*, (3), 491-511.
8. Hancock, R. E.; Sahl, H. G., Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* **2006,** *24*, (12), 1551-7.
9. Culf, A. S.; Ouellette, R. J., Solid-phase synthesis of N-substituted glycine oligomers (alpha-peptoids) and derivatives. *Molecules* **2010,** *15*, (8), 5282-335.
10. Giuliani, A.; Rinaldi, A. C., Beyond natural antimicrobial peptides: multimeric peptides and other peptidomimetic approaches. *Cell Mol. Life Sci.* **2011,** *68*, (13), 2255-66.
11. Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H., Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **1992,** *114*, (26), 10646-10647.
12. Chongsiriwatana, N. P.; Wetzler, M.; Barron, A. E., Functional synergy between antimicrobial peptoids and peptides against Gram-negative bacteria. *Antimicrob. Agents Chemother.* **2011,** *55*, (11), 5399-402.
13. Kapoor, R.; Eimerman, P. R.; Hardy, J. W.; Cirillo, J. D.; Contag, C. H.; Barron, A. E., Efficacy of antimicrobial peptoids against Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **2011,** *55*, (6), 3058-62.
14. Kapoor, R.; Wadman, M. W.; Dohm, M. T.; Czyzewski, A. M.; Spormann, A. M.; Barron, A. E., Antimicrobial peptoids are effective against Pseudomonas aeruginosa biofilms. *Antimicrob Agents Chemother.* **2011,** *55*, (6), 3054-7.
15. 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*, (8), 2794-9.
16. Patch, J. A.; Barron, A. E., Helical peptoid mimics of magainin-2 amide. *J. Am. Chem. Soc.* **2003,** *125*, (40), 12092-3.
17. Hein-Kristensen, L.; Knapp, K. M.; Franzyk, H.; Gram, L., Bacterial membrane activity of alpha-peptide/beta-peptoid chimeras: influence of amino acid composition and chain length on the activity against different bacterial strains. *BMC Microbiol.* **2011,** *11*, 144.
18. Kennedy, J. P.; Williams, L.; Bridges, T. M.; Daniels, R. N.; Weaver, D.; Lindsley, C. W., Application of Combinatorial Chemistry Science on Modern Drug Discovery. *J. Comb. Chem.* **2008,** *10*, (3), 345-354.

19. Lam, K. S.; Lebl, M.; Krchnak, V., The "One-Bead-One-Compound" Combinatorial Library Method. *Chem. Rev.* **1997,** *97*, (2), 411-448.
20. Figliozzi, G. M.; Goldsmith, R.; Ng, S. C.; Banville, S. C.; Zuckermann, R. N., Synthesis of N-substituted glycine peptoid libraries. In *Method. Enzymol.*, Academic Press: **1996,** Vol. *267*, 437-447.
21. Udugamasooriya, D. G.; Dineen, S. P.; Brekken, R. A.; Kodadek, T., A Peptoid "Antibody Surrogate" That Antagonizes VEGF Receptor 2 Activity. *J. Am. Chem. Soc.* **2008,** *130*, (17), 5744-5752.
22. Gao, Y.; Kodadek, T., Synthesis, Screening and Hit Optimization of Stereochemically Diverse Combinatorial Libraries of Peptide Tertiary Amides. *Chemistry & Biology* **2013,** *20*, (3), 10.1016/j.chembiol.2013.01.013.
23. Fluxa, V. S.; Maillard, N.; Page, M. G. P.; Reymond, J.-L., Bead diffusion assay for discovering antimicrobial cyclic peptides. *Chem. Commun. 47*, (5), 1434-1436.
24. Oldenburg, K. R.; Vo, K. T.; Ruhland, B.; Schatz, P. J.; Yuan, Z., A Dual Culture Assay for Detection of Antimicrobial Activity. *J. Biomol. Screen.* **1996,** *1*, (3), 123-130.
25. Silen, J. L.; Lu, A. T.; Solas, D. W.; Gore, M. A.; Maclean, D.; Shah, N. H.; Coffin, J. M.; Bhinderwala, N. S.; Wang, Y.; Tsutsui, K. T.; Look, G. C.; Campbell, D. A.; Hale, R. L.; Navre, M.; DeLuca-Flaherty, C. R., Screening for Novel Antimicrobials from Encoded Combinatorial Libraries by Using a Two-Dimensional Agar Format. *Antimicrob. Agents Chemother.* **1998,** *42*, (6), 1447-1453.
26. Chen, X.; Tan, P. H.; Zhang, Y.; Pei, D., On-Bead Screening of Combinatorial Libraries: Reduction of Nonspecific Binding by Decreasing Surface Ligand Density. *J. Comb. Chem.* **2009,** *11*, (4), 604-611.
27. Kappel, J.; Barany, G., Methionine anchoring applied to the solid-phase synthesis of lysine-containing "head-to-tail" cyclic peptides. *Lett. Pep. Sci.* **2003,** *10*, (2), 119-125.
28. Chongsiriwatana, N. P.; Miller, T. M.; Wetzler, M.; Vakulenko, S.; Karlsson, A. J.; Palecek, S. P.; Mobashery, S.; Barron, A. E., Short Alkylated Peptoid Mimics of Antimicrobial Lipopeptides. *Antimicrob. Agents Chemother.* **2011,** *55*, (1), 417-420.
29. Mojsoska, B.; Zuckermann, R. N.; Jenssen, H. v., Structure-Activity Relationship Study of Novel Peptoids That Mimic the Structure of Antimicrobial Peptides. *Antimicrob. Agents and Chemother.* **2015,** *59*, (7), 4112-4120.
30. Pendleton, J. N.; Gorman, S. P.; Gilmore, B. F., Clinical relevance of the ESKAPE pathogens. *Expert Rev. Anti-Infe.* **2013,** *11*, (3), 297-308.

## TOC Graphic


α-strand peptoid
β-strand peptoid
PLAD Linker
1. β-strand release
Screening
2. α-strand release
Peptoid Sequencing