SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL ASSESSMENT OF A MACROCYCLE CONTAINING LYSINE

by

Trevon Jelinek

Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Chemistry and Biochemistry

Texas Christian University

Fort Worth, Texas

May 2, 2021

SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL ASSESSMENT OF A MACROCYCLE CONTAINING LYSINE

Project Approved:

Supervising Professor: Shauna McGillivray, Ph.D.

Department of Biology

Supervising Professor: Eric Simanek, Ph.D.

Department of Chemistry and Biochemistry

David Minter, Ph.D.

Department of Chemistry and Biochemistry

Giridhar Akkaraju, Ph.D.

Department of Biology

ABSTRACT

The synthesis of the lysine macrocycle begins with the step-wise substitution of cyanuric chloride with nitrogenous nucleophiles. The three chlorine atoms are replaced with BOC-hydrazine followed by protected D-lysine and finally an auxiliary amine such as morpholine or dimethylamine. The protecting groups explored were a butyl (BOC) or benzyl (Z) carbamate. To arrive at this first intermediate, a carboxylic acid, the temperatures were varied to control reaction kinetics in order to deliberately mono substitute and obtain desired compound (6.8% yield). After purification and characterization, this acid undergoes a coupling reaction with an amino acetal to provide the monomer for macrocyclization in 10% yield. Treating the monomer with acid leads to dimerization. The desired macrocycle is obtained in quantitative yield and used without purification. Throughout the synthesis, one- and two-dimensional ^{1}H spectroscopy is used to establish structure.

The antibacterial activity of this macrocycle (and others) relied on evaluating the minimum inhibitory concentration (MIC) against both Gram-positive (*Escherichia coli*) and Gram-negative (*Staphylococcus aureus*) bacteria. The macrocycle did not affect bacterial growth: no MIC could be measured at the maximum concentration of 500 μg/mL.

ACKNOWLEDGEMENTS

I would like to show appreciation for the following for their contributions to the completion of my project:

- Dr. Shauna McGillivray

Your patience and encouragement throughout this process was much appreciated, and I enjoyed the time I spent as a member of the lab.

- Dr. Eric Simanek

I am grateful for all the support you showed me throughout the tedious synthesis process. The humorous approach you took to mishaps or undesirable results always lightened the mood. Thank you for making me feel welcome on the chemistry side of things.

- Review Committee

Dr. Akkaraju and Dr Minter, I appreciate you taking the time to serve on my review committee. I have thoroughly enjoyed my time with both of you inside and outside of the classroom.

- John V. Roach Honors College

- Science and Engineering Research Center

Thank you for the funding a received from my SERC grant to make my research possible.

- Graduate and Undergraduate Students in both labs

The support, wisdom, and friendship of all of my fellow lab mates undoubtably carried my through this project.

- All other supporters including my friends and family

I could not have made it this far in life and academic accomplishment if it were not for the support and motivation shown by the people closest to me.

TABLE OF CONTENTS

Page

ABSTRACT……...iii

ACKNOWLEDGEMENTS……iv

TABLE OF CONTENTS……v

FIGURES, CHARTS, AND SCHEMES……...vi

INTRODUCTION……...1

EXPERIMENTAL……...4

Synthesis……..4

MIC Assay……...6

RESULTS AND DISCUSSION……..8

Synthesis and Characterization……...8

Biological Analysis……...12

CONCLUSION……..16

REFERENCES……..17

FIGURES, CHARTS, AND SCHEMES

Page

Chart 1. Chemical structure of the lysine macrocycles…… 2

Figure 1. Free Energy Surface and Probability Map……. 3

Figure 2. Depiction of MIC assay methods……7

Scheme 1. Reaction scheme for macrocycle…….. 9

Figure 3. Proton NMR for acid intermediate……10

Figure 4. Proton NMR for acetal intermediate…….11

Figure 5. Proton NMR for Macrocycle…… 12

Figure 6. Growth of *S. aureus and E. coli* in (D)Lys-Lys – morpholine……. 13

Figure 7. Growth of *S. aureus* and *E. coli* in (L)Lys-Lys – DMA……13

Figure 8. Growth of *S. aureus* and *E. coli* in (D)Lys-Lys – DMA ……. 14

Figure 9. Chemical structure differences in macrocyclic compounds tested……14

Figure 10. Growth of *S. aureus* and *E. coli* in additional compounds……. 15

INTRODUCTION

Antibiotics are heavily relied upon by modern medicine to treat many infectious diseases, which is concerning when considering their rapid loss of effectiveness against evolving microorganisms [1]. Antibiotics are usually naturally occurring compounds or derivatives of compounds found in nature. These drugs are most commonly obtained from actinomycetes, bacteria, and fungi [2]. Here, the molecules described were assessed against Gram-negative and Gram-positive bacteria. *Escherichia coli* and *Staphylococcus aureus* were used because of their abundance and prevalence as pathogenic causes of disease. Moreover, the structural differences between Gram-positive and Gram-negative bacteria, namely the outer membrane in Gram-negative bacteria can lead to differing or specialized efficacy in antibiotics.

The rate of discovery for antibiotic compounds, especially naturally occurring ones, has decreased since the 1950s although antibiotic resistance has increased over this period of time. Therein lies the antibiotic dilemma that the scientific and medical community faces, one that grows more urgent every year [1]. Therefore, this ever-apparent threat of antibiotic resistance has hastened the need for synthetic compounds capable of antimicrobial activity so antibiotic discovery and design can outpace resistance or find a way to deter resistance [3]. As always, the issue calls for innovative solutions.

Unnatural macrocycles represent a potential source for antibiotics. Macrocycles are organic compounds containing a large ring of at least 12 linked atoms [4]. Interestingly, there are already antibiotics in use that have a macrocyclic structure including erythromycin, rapamycin, and cyclosporin. Macrocycles, and specifically macrocyclic peptides, have been a growing area of research, especially in relation to potential pharmaceuticals, because of interest in the binding capabilities of these molecules to large protein surfaces. The evidence for this growth is the increase in publications and citations mentioning this type of molecule [5].

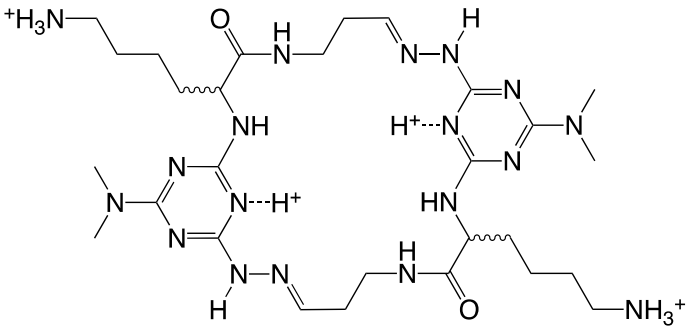
The synthetic approach utilized to build the target macrocycle in this paper relied on a dimerization wherein two monomers react to create a symmetrical molecular structure. One challenge was to find the most efficient method of synthesis for these monomers.

The macrocycles explored in the Simanek group comprise a triazine ring that presents a hydrazine group and amino acid-linked acetal. Lysine was chosen as the amino acid of choice for this project due to its cationic nature. Cationic peptides are commonly used to kill microbes.

Many are naturally occurring [6]. Therefore, integrating a cationic amino acid into the macrocycle could allow an increase in antimicrobial activity. This work also explores the impact of chirality on biological activity. There are many molecules that have drastically different functions in biological systems which vary only at one chiral center. Here, macrocycles containing both R- and S-lysine are explored.

Chart 1 shows the desired target. The acetal comprises three carbons to provide flexibility to the structure. The auxiliary amine is dimethylamine which aids in structure determination studies using $_1$H NMR.

Chart 1. Chemical structure of the lysine macrocycles explored in this study. Both D- and L-lysine were incorporated.



The therapeutic strategy of large macrocyclic compounds, especially macrocyclic peptides, is different than that for small molecule drugs. Small molecules typically bind to structurally well-defined sites on the target protein such as the catalytic center of an enzyme. In contrast, macrocycles can bind to the less-defined, fluid protein surface and potentially disrupt protein-protein interactions associated with important biological processes [7,8]. This behavior requires that macrocycles change conformation and thus overall shape. That is, macrocycles must adopt a certain conformation in the hydrophilic extracellular environment, change to become hydrophobic when passing into the cell through the cell membrane, and readopt an extended hydrophilic structure to allow it to bind to a target.

Computational models suggested that the proposed lysine macrocycle would behave in this manner. Figure 1 shows the free energy surface (and probability surface) of the macrocycle. The surface identifies four different conformations (A-D) that are folded (A and B), partly extended (C) or fully extended (D). This surface is generated using two distance constraints, D1 and D2, that define the x- and y-axes.

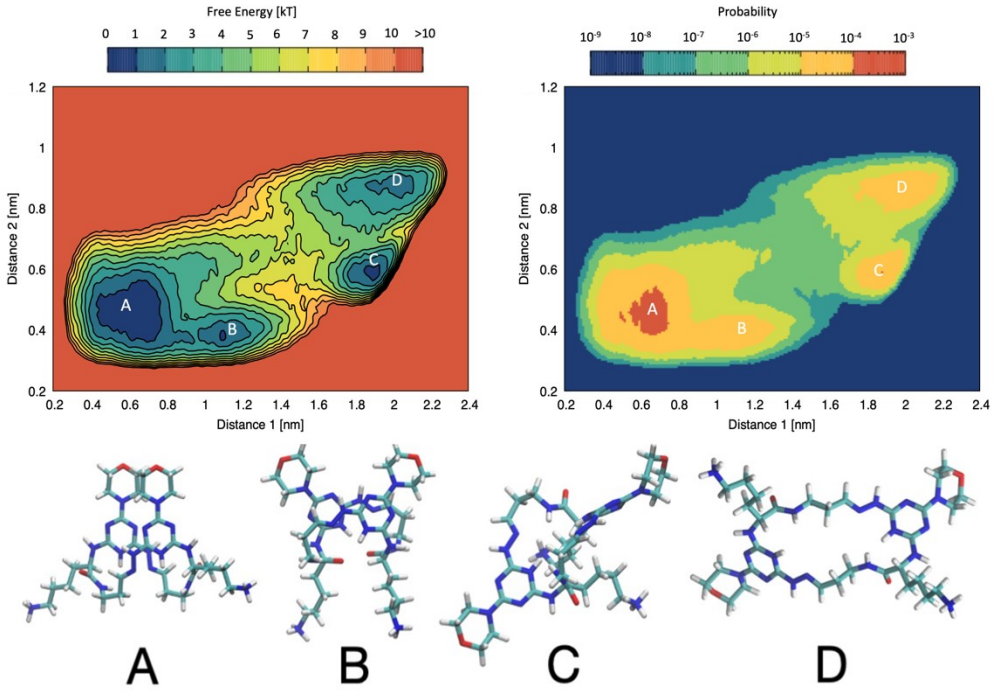


Figure 1. Free Energy Surface (left) and Probability Map (right) created by coworkers (G. Pavan, SUPSI) that allows us to calculate conformations of a lysine macrocycle. A,B,C, and D represent the three dimensional shapes of the four least energetic and most common confirmations.

The axes of the plots reflect the two distances to estimate folding behavior. The first distance is the space between the two aromatic rings measured in nanometers. The second is the distance between the auxiliary groups. When both distances are small (the bottom left corner), we find a molecule (A) that is completely folded. The aromatic rings engage in π–stacking and the auxiliary groups are right next to each other. In contrast, when both distances are large (the top right-hand corner of the graph), we observe a completely extended molecule (D). The free energy diagram on the left illustrates the most energetically favorable conformations and importantly, the relatively low barriers that separate them. The probability map on the right allows us to calculate the relative populations of these species. Folded conformation (A) is shown to be the most abundant.

EXPERIMENTAL

SYNTHESIS

Intermediate 1 (Lysine acid): To the clear solution of cyanuric chloride (1g, 100 mmols, 1 eq) dissolved in 54 mL of THF and chilled with an acetone and ice bath to -10 °C, BOC-hydrazine (0.713 g, 100 mmols, 1 eq) was added dropwise using a pressure equilibrating addition funnel in a solution of 54 mL THF. The solution turned bright yellow upon addition. Subsequently, 1 mL of 5M NaOH (100 mmols, 1 eq) was added, and the pH was measured at 3. Thus, 1.5 mL more 5M NaOH was added to reach the desired 5-6 pH range. A single product was confirmed by thin layer chromatography (10% methanol in dichloromethane) under short wave UV irradiation or by ninhydrin stain ($R_f = 0.7$) 1.5 hours after BOC addition started.

A solution of benzyl chloroformate protected D-lysine amino acid (2.27 g, 150 mmols, 1.5 eq) and 81 mL of DI water was prepared, and 2.4 mL of 5M NaOH (3eq) was added. The solution was heated slightly to promote dissolution of the amino acid. The amino acid solution was then added dropwise using a pressure equilibrating addition funnel (2 drops/sec). Two hours after addition begun another more amino acid was added (0.75 g, 50 mmols, 0.5 eq) in the same manner. After the reaction stirred overnight (16 hours) the yellow color persisted. Thin layer chromatography (10% methanol in dichloromethane) under short wave UV irradiation or by ninhydrin stain showed reactants with little products. The solution was acidified using 1M HCl from a pH of 11 to a pH of 9 and left to continue stirring. After 3 days of stirring and reacting, a white cloudy solution was observed with a pH of 9. Thin layer chromatography showed disappearance of the starting material ($R_f = 0.7$) and appearance of the product ($R_f = 0.3$).

A solution of 40% dimethylamine (1.222 g, 200 mmols, 2 eq) was added dropwise by pipet. Immediately following addition, the solution changed to a clear colorless solution with pH of 10. The solution was stirred at room temperature for 5 h. The reaction was deemed complete from thin layer chromatography (10% methanol in dichloromethane) under short wave UV irradiation and ninhydrin stain.

The solution was then acidified to a pH of 6 and a brine solution was added (~300 mL) to the ~200 mL reaction solution. The solution became cloudy after acidification and addition of brine solution did not result in separation as expected. Ethyl acetate was added (100 mL) and solution separated into three layers: bottom aqueous layer, middle white precipitate/suspension layer, and top organic layer. The precipitate was partially in both the aqueous and organic layers. The aqueous layer was washed 3 times with ethyl acetate which resulted in a solution of the organic layers with some traces of white precipitate. This solution was decanted and then dried with Mg_2SO_4. The solvent was removed via rotary evaporation. Column chromatography was performed with a 10% methanol in dichloromethane solvent. 20 fractions were collected with 6 having a one-spot product purity by TLC. The pure fractions were combined and evaporated multiple times. This yielded 197 mg of product (6.8% yield). NMR was then taken on this product.

^{1}H (DMSO-D_6, 400 MHz): d8.62-8.42 (m, 1H), 8.31-8.17 (m, 1H), 7.38-7.33 (m, 5H), 7.30-7.26 (m, 1H), 6.68-6.49 (m, 1H), 5.00 (s, 2H), 4.35-4.15 (m, 1H), 3.36 (m, 2H), 2.97 (s, 6H), 1.71-1.64 (m, 2H), 1.40 (s, 9H), 1.37-1.25 (m, 2H), 1.19-1.08 (m, 2H).

Intermediate 2 (Lysine acetal): A 0.4 M solution of the acid intermediate (1 eq) in dichloromethane was made and cooled to 0 °C in an ice bath. A 0.4 M solution of EDC.HCl (1.144 mL, 110 mmols, 1.1eq) in dichloromethane was prepared and added rapidly over 1 minute via a pipet. Diisopropylethylamine (0.108 g, 200 mmols, 2 eq) was added dropwise over 1 minute via a pipet. After 1 h, thin layer chromatography (10% methanol in dichloromethane) confirmed disappearance of the starting material (R_f = 0.3) and appearance of a new intermediate at R_f = 0.4 using short wave UV irradiation and ninhydrin stain.

A 0.2 M solution of dichloromethane containing 1-amino-3,3-diethoxypropane (0.1225 g, 200 mmols, 2 eq) was added dropwise over a minute to the 0 °C iced reaction mixture. Then, the reaction was allowed to warm to room temperature and stirred overnight. Thin layer chromatography (5% methanol in dichloromethane) confirmed that all starting material had been consumed. Multiple UV spots were present before extraction. The reaction mixture was transferred to a separatory funnel and washed three times with equal volumes of water. Then, the

organic phase was dried with Mg_2SO_4. Solvent was removed using rotary evaporation and the yield was 0.220 g before further purification. The crude product was then purified by silica gel chromatography (5% methanol in dichloromethane). The final lysine acetal product had a yield of 20 mg. Product was then characterized using NMR.
^{1}H (DMSO-D_6, 400 MHz): d8.56-8.51 (m, 1H), 8.38-8.18 (m, 1H), 7.75-7.68(m, 1H) 7.36-7.32 (m, 5H), 7.30-7.26 (m, 1H), 6.70-6.57 (m, 1H), 5.00 (s, 2H), 4.50-4.44 (m, 1H) 4.28-4.15 (m, 1H), 3.54-3.50 (m, 4H), 3.08-3.06 (m, 2H), 3.03 (s, 6H), 3.00-2.93 (m, 2H), 1.63-1.61 (m, 2H) 1.61-1.60 (m, 2H), 1.40 (s, 9H), 1.35 (m, 2H), 1.29-1.23 (m, 2H), 1.12-1.06 (m, 6H).

Macrocycle: The monomer (20 mg) was dissolved in 0.5 mL of dichloromethane in a small screw cap vial. Trifluoroacetic acid (0.5 mL) was added to the vial. The reaction mixture was stirred with a magnetic flea stir bar until the solvent was completely evaporated. After approximately 2 weeks, an NMR of the sample in MeOD revealed an incomplete reaction. The NMR tube was poured back into the vial and about 3 mL of MeOH was added and allowed to evaporate overnight. The sample was then dissolved in 0.5 mL of dichloromethane and 0.5 mL of trifluoroacetic acid again. It was allowed to completely evaporate again resulting in the desired product in quantitative yield. NMR analysis confirmed the desired product.
^{1}H (DMSO-D_6, 400 MHz): d12.42-12.37 (m, 2H), 12.19 (s, 2H), 9.01 (s, 2H), 8.81 (s, 2H), 7.76 (s, 2H), 7.50 (s, 2H), 4.12-4.10 (m, 2H), 3.60-3.23 (m, 4H), 3.12-3.05 (m, 6H), 2.80-2.78 (m, 4H), 2.51 (m, 4H) 1.76-1.73 (m, 4H) 1.58-1.52 (m, 4H), 1.44-1.40 (m, 4H).

MIC ASSAY

Bacteria cultures of both *Staphylococcus aureus* and *Escherichia coli* were grown overnight (37 °C) in 1 mL MHB media. The next day, 35 µl of SA overnight culture and 70 µl of EC overnight was put into 3 mL of MHB and grown to early log phase (37 °C) with an optical density (OD) around 0.4 at wavelength of 600 nm. These cultures were diluted 1:100 in MHB. 100 µl of both bacteria dilutions were transferred to 6 wells in a 96 well plate with both bacteria species having its own row. Concentrations of the macrocyclic compound being tested were made using serial dilutions in 1.7 ml microcentrifuge tubes. The five concentrations used were: 500, 250, 125,

62.5, 31.25 µg/ml with a positive control (0 µg/ml of compound) and a negative control (no bacteria). The first concentration (500 µg/ml) was achieved by putting 24 µl of a 25 mg/ml stock solution of the compound in water into 576 µl of MHB making 600 µl of 500 µg/ml solution. Then, 4 successive half volume dilutions were carried out to make solutions of the 5 different concentrations. Next, 100 µl of each of the solutions was placed into both rows in its corresponding well. The 96 well plate was placed in the incubator (37 °C) overnight (about 14 h) along with a reference dilution plate and the next day the optical density (OD) was read at a wavelength of 600 nm. The reference dilution plate was used to estimate the relative growth of the bacteria overnight in colony forming units per milliliter (cfu/ml). This value was obtained to ensure consistent amount of growth among multiple assays. The value had to be at least $5x10^5$ cfu/ml.

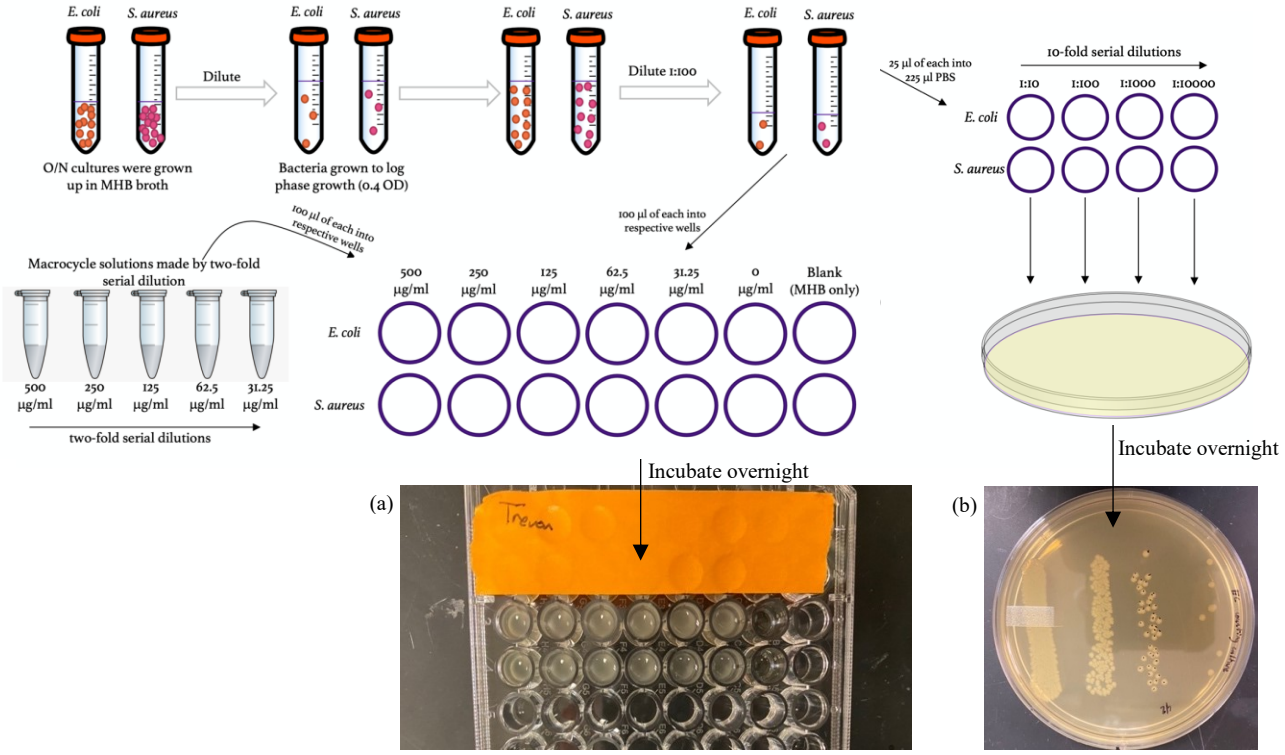


Figure 2. Depiction of MIC assay methods with (a) 96 well plate for separating different experiment conditions and (b) counting plate for calculating relative growth.

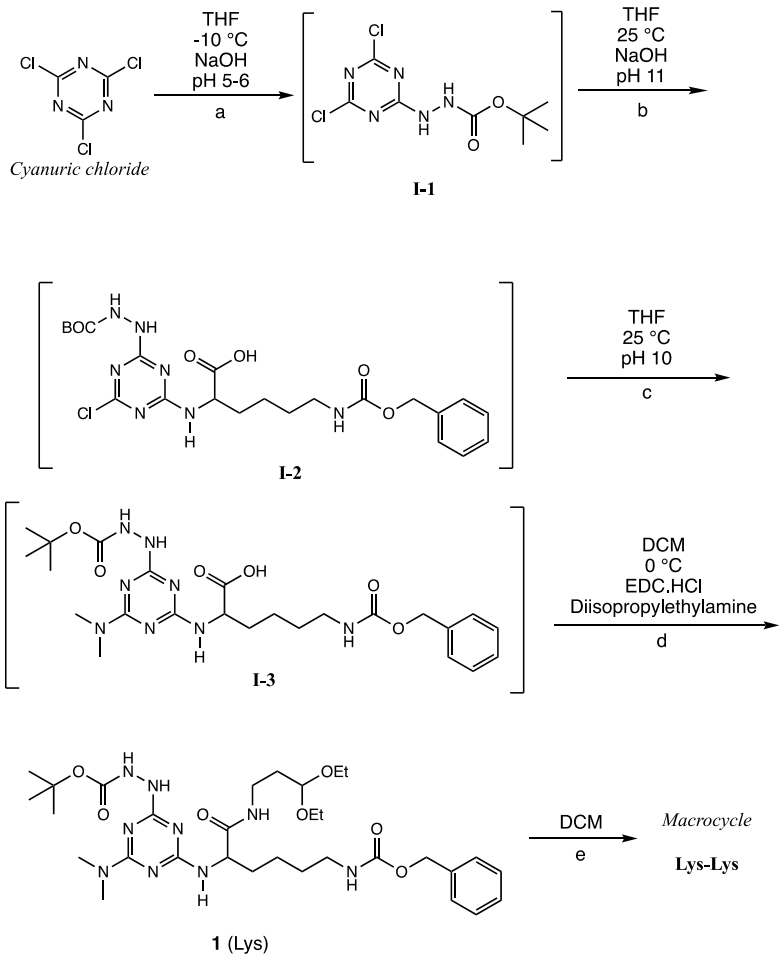
The dilution plate was made by filling every other well on 96 well plate with 225 ul of PBS and then putting 25 ul of 1:100 diluted bacteria solution into the first well. Then, 3 serial dilutions are performed down the line with 25 ul of previous well solution to achieve a 1:10, 1:100, 1:1000,

and 1:10000 dilutions of the original 1:100 diluted solution. Finally, 25 ul of each well was smeared onto a standard agar plate and the number of colonies that formed the next day were counted to determine the starting cfu/ml.

RESULTS AND DISCUSSION

SYNTHESIS AND CHARACTERIZATION

Scheme 1 shows the synthetic approach taken to create the lysine macrocycle. Here Z-protected lysine is shown. BOC-protected lysine was also incorporated.



Scheme 1. The reaction scheme for macrocycle (Lys-Lys). (a) BOC-hydrazine (b) benzyl chloroformate protected D-lysine amino acid (c) dimethylamine (d) 1-amino-3,3-diethoxypropane (e) trifluoroacetic acid

Cyanuric chloride was the starting material for the synthesis of the acid intermediate. It was the electrophilic foundation to which three nucleophilic substituents were substituted subsequentially in one pot under conditions that would allow for controlled, stepwise substitution. BOC-hydrazine was reacted first resulting in the first intermediate (**I-1**). Then, the Z group protected (D) lysine

was reacted resulting in the disubstituted monochlorotriazine (**I-2**) followed finally by the dimethylamine substitution resulting in the acid molecule (**I-3**).

The ^{1}H NMR spectrum shown in Figure 3 confirms the presence of the desired acid molecule, **I-3**. The dimethylamine peak at 2.9 (DMA) was used as a reference peak that was set to 6 protons. The alpha proton cluster of peaks at 4.2 (α) was also used to confirm the presence of the lysine amino acid. There are three peaks of decreasing intensity which indicates varying conformations of the same molecule resulting in different environments for the α proton. The phenyl peaks at 7.3 (phenyl) showed preservation of the Z protecting group. Finally, the BOC-hydrazine substituent was confirmed by the peak at 1.4 (BOC).

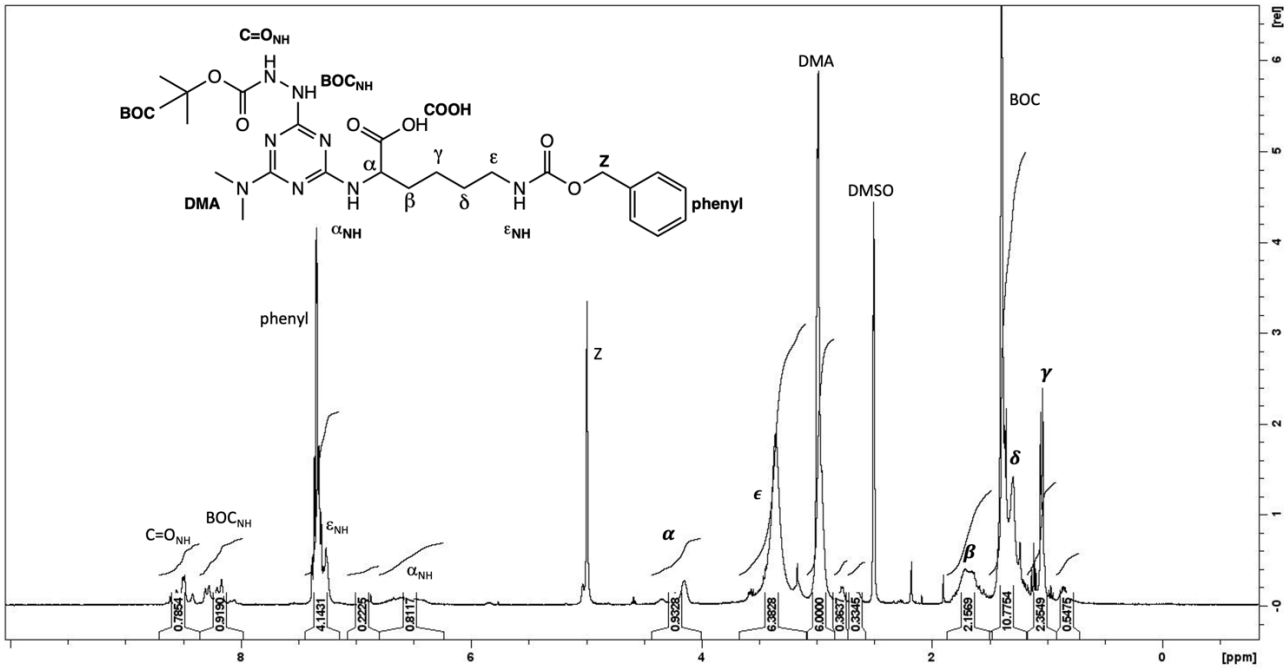


Figure 3. Proton NMR for acid intermediate (**I-3**) in DMSO-d_6

Molecule **I-3** then underwent an EDC-mediated coupling reaction with the acetal (1-amino-3,3-diethoxypropane). After workup, the acetal monomer molecule **1** was obtained. The ^{1}H NMR shown in Figure 4 confirms the presence of the desired acetal monomer molecule **1**. Again, the dimethylamine peak at 2.9 (DMA) was used as a reference peak at an integration of 6. Persistence of the resonances for the α proton, phenyl group, and BOC group is observed. Appearance of the resonances at 3.4 ppm and 7.7 ppm confirm the successful acetal formation. The resonance at 7.7 ppm indicates amide formation by signaling the presence of the nitrogen proton of the amide.

Furthermore, the rest of the acetal group presence is indicated by the alkene resonance at 3.4 ppm. The alkyl protons A (4.4 ppm), B (1.6 ppm), C (2.6 ppm), and CH_2CH_3 (1.1 ppm) also provide evidence for an intact acetal.

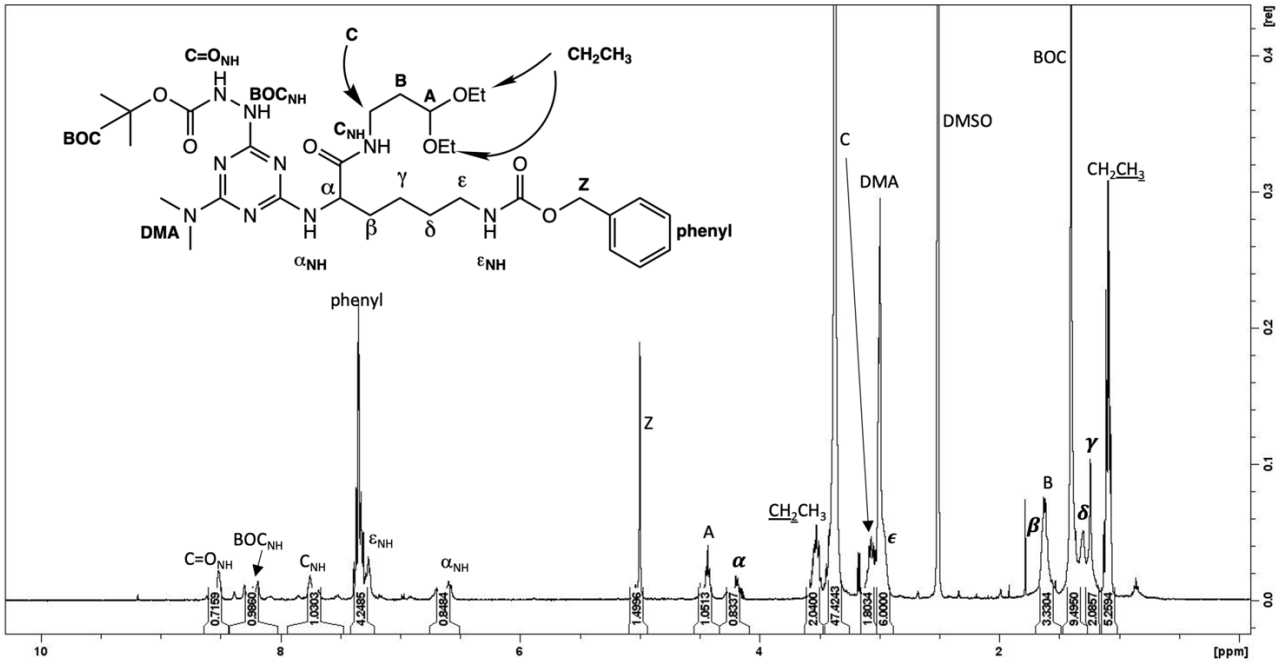


Figure 4. Proton NMR for acetal intermediate (**1**) in DMSO-d_6

*Synthesis and Characterization of Macrocycle, (**Lys-Lys**).*

To obtain the macrocycle, monomer **1** is dissolved in dichloromethane with an equal amount of trifluoroacetic acid and allowed to stir in an open vial until evaporation is complete. This process was repeated until the NMR showed completion of the reaction and the successful synthesis of the desired macrocycle product (**Lys-Lys**).

The ^{1}H NMR spectrum is shown in **Figure 5**. It was obtained following the third treatment of trifluoroacetic acid to confirm the presence of the desired macrocycle, **Lys-Lys**. The α proton resonances at 4.1 are used as a reference and assigned an integration of 1. Key resonances to confirm the formation of the dimerized macrocycle appear in the downfield region between 13 ppm and 7 ppm. They include A, the extremely down field H+ and the N_{NH} resonances. The (H+) peak at 12.4 indicates the strong electron withdrawing forces deshielding this proton. The C_{NH} resonance shifts from 7.8 ppm to 9.1 ppm . The A resonances at 7.4 ppm and N_{NH} at 12.2 ppm

give good indication of the formation of the imine bond. The number of resonances indicate symmetry within the molecule. Further, the disappearance of the ethyl peaks from the acetal shows the formation of the macrocycle.

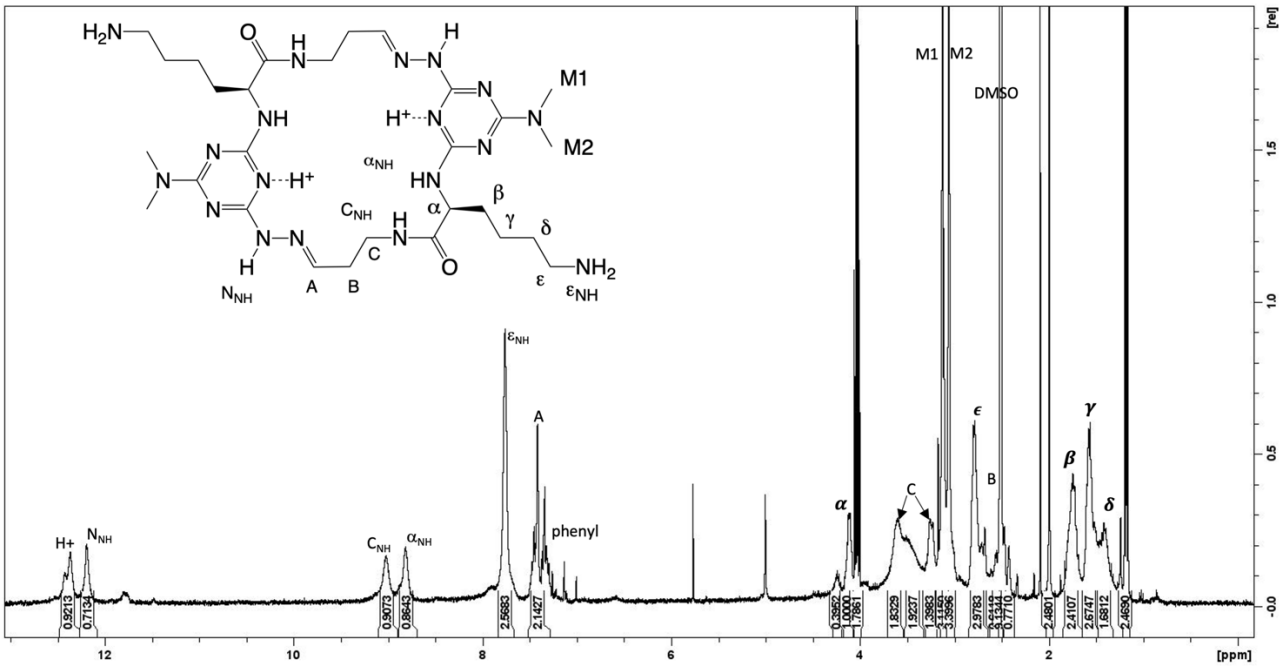


Figure 5. Proton NMR for Macrocycle (**Lys-Lys**) in DMSO-d_6

BIOLOGICAL ANALYSIS

Gram-negative bacteria (*E. coli*) and gram-positive bacteria (*S. aureus*) were subjected to the various synthesized macrocyclic compounds at concentrations ranging from 31.25-500 μg/ml using an MIC Assay. Three trials were completed for each compound tested, and the optical densities were averaged. The growth of the bacteria treated with macrocycle (D)Lys-Lys - morpholine is shown below in Figure 6. There was no statistically significant inhibition in growth for any of the concentrations of (D)Lys-Lys - morpholine in either *S. aureus* or *E.coli*.

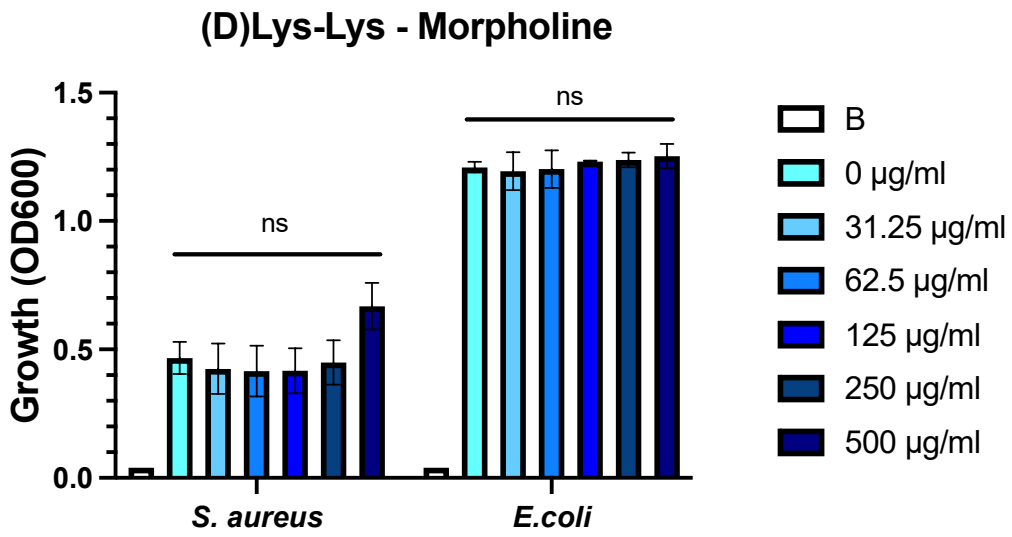


Figure 6. Growth of *S. aureus and E. coli* in increasing concentrations of compound, (D)Lys-Lys - morpholine. The assay was repeated 3-independent times and presented as the mean +/- standard deviation.

A similar result was obtained when (L)Lys-Lys - DMA was assayed (Figure 7).

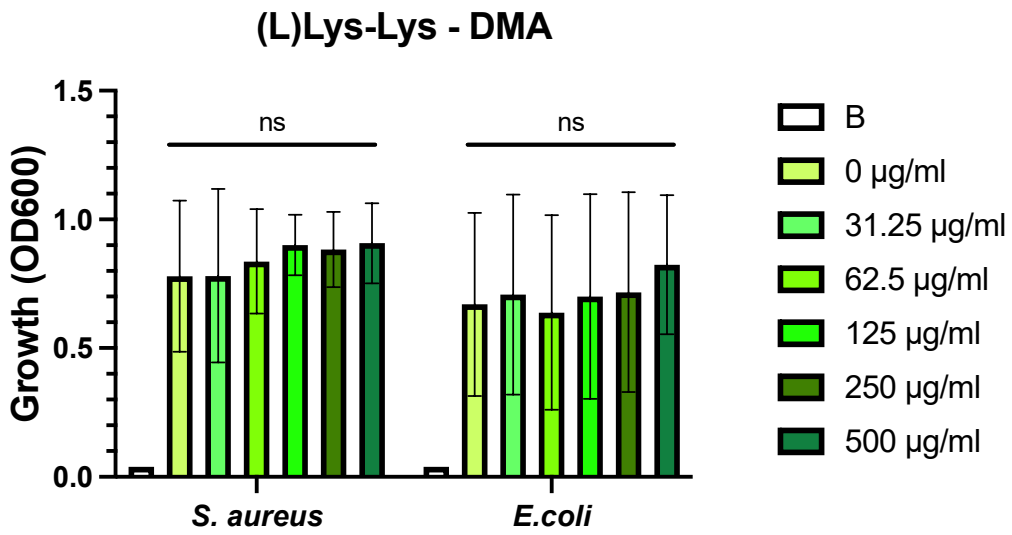


Figure 7. Growth of *S. aureus* and *E. coli* in increasing concentrations of compound, (L)Lys-Lys - DMA. The assay was repeated 3-independent times and presented as the mean +/- standard deviation.

A similar result was obtained when (D)Lys-Lys - DMA was assayed (Figure 8). (D)Lys-Lys – DMA was the product of the specific synthesis mechanism delineated in this paper (labeled above as (Lys-Lys)).

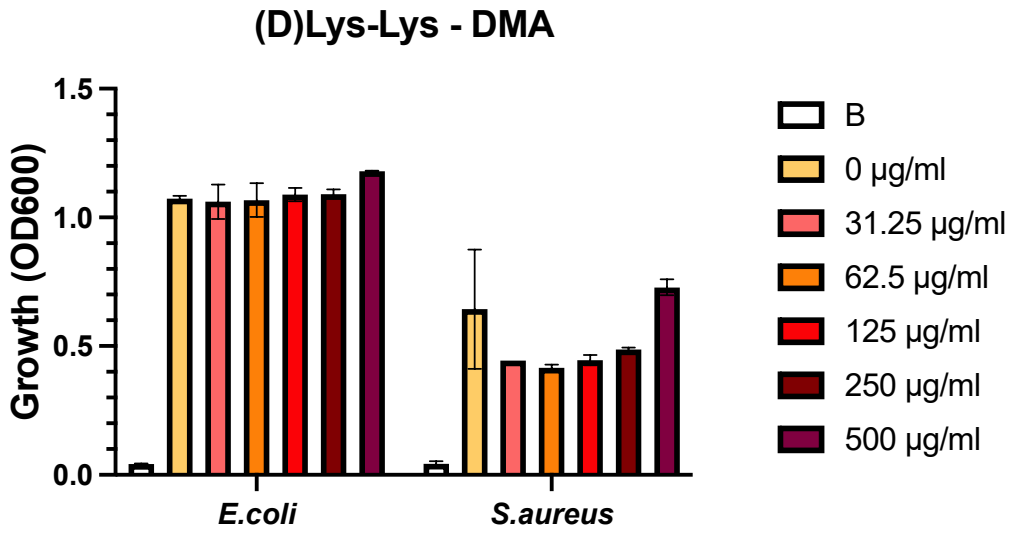


Figure 8. Growth of *S. aureus* and *E. coli* in increasing concentrations of compound, (D)Lys-Lys - DMA. The assay was repeated 2-independent times and presented as the mean +/- standard deviation. No statistical analysis performed.

After the lysine macrocycles were tested and showed no significant inhibition of growth, the scope of testing macrocycles was broadened to include more macrocycles which differ in N-alkylation and amino acid integration. A pictural representation of the varying pieces on each macrocycle was made to illustrate the chemical structure differences (Figure 9).

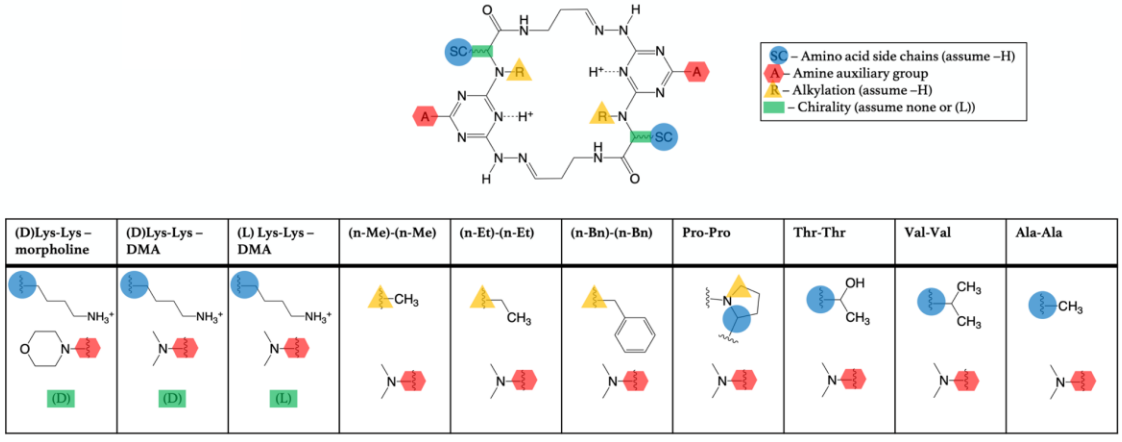


Figure 9. Constant macrocycle structure used with color coded variable groups in the table below.

A single replicate MIC assay was performed with the additional macrocycles in the same manner as with the Lysine macrocycles (Figure 10). Similar results were observed.

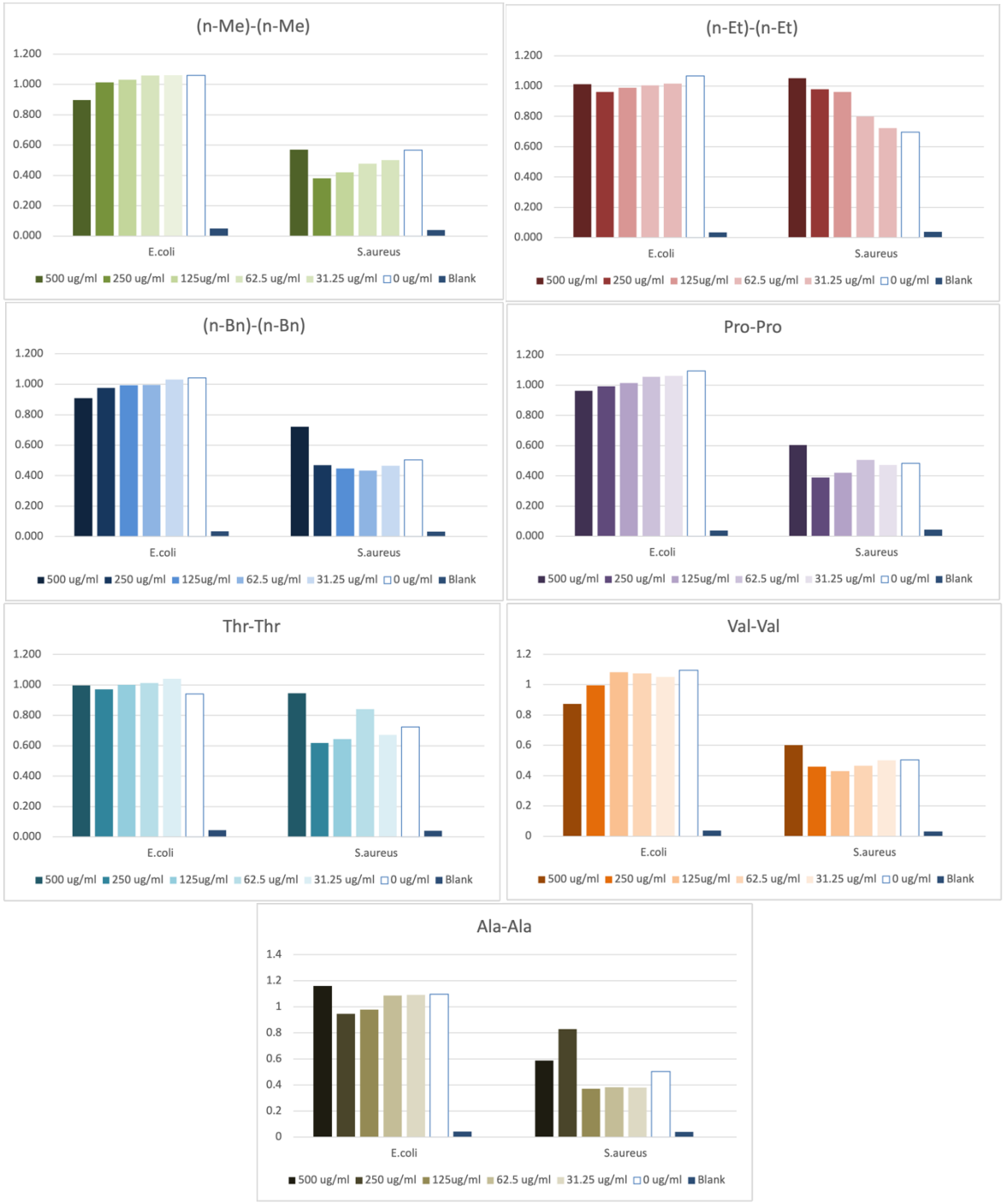


Figure 10. Growth of *S. aureus* and *E. coli* in increasing concentrations of additional compounds. No statistical analysis performed.

CONCLUSION

Successful synthesis of lysine macrocycles was achieved through successive substitution of three nitrogen nucleophiles, a coupling reaction, and finally acidification. Two lysine containing macrocycles were synthesized to completion, a BOC protected lysine macrocycle with morpholine side group, and a benzyl protected lysine macrocycle with dimethylamine side group. Reaction conditions and some work-up steps differed in the methods of the two compounds. However, synthesis of the latter was focused on in this paper. Yield of this macrocycle using the procedure disclosed was low likely due to solubility difficulty and side reactions throughout the synthesis steps. The synthesis should be optimized if further research is to be done on this compound.

No evidence was found to support the hypothesis that the synthesized macrocycles containing lysine amino acids would display toxicity and inhibit the growth of bacteria. Moreover, it was found that none of the tested macrocycles, which varied in respect to amino acid type, amino acid chirality, and auxiliary amine group displayed inhibition of growth at any concentration compared to a control.

This finding does not seem to be surprising since designing fully synthetic antibiotics has proven to be difficult for the pharmaceutical research industry. Resultantly, most antibiotics used in pharmaceutics are natural or semi-synthetic which means they are found in nature or derived from a naturally occurring compound [2]. However, with a constant need for new antibiotics and a diminishing discovery of natural and semi-synthetic antibiotics, synthetic antibiotics should receive more focus from research efforts. It is important to continue to screen synthetic molecules even if they were developed for other targets in the hopes of discovering a new antibiotic molecule or a “scaffold” to develop off further. Moreover, using genomic data to find drug targets is important to synthetic antibiotic development [9].

Further research is required to find if any of the macrocycles that have been produced which were not tested would be successful in inhibiting growth of bacteria. Other research should be done test the binding affinity of the compounds to any array of proteins. This further research can be used to find if the compounds have binding affinity to any proteins of interest. If any of them do have binding affinity to a certain protein of interest, that would open many more opportunities for research and development of the compound as a pharmaceutical therapeutic.

REFERENCES

1. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance, 11, 1645–1658. https://doi.org/10.2147/IDR.S173867
2. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. Current Opinion in Microbiology, 51, 72-80. https://doi.org/10.1016/j.mib.2019.10.008
3. Mitcheltree, M.J., Pisipati, A., Syroegin, E.A. et al. A synthetic antibiotic class overcoming bacterial multidrug resistance. Nature 599, 507–512 (2021). https://doi.org/10.1038/s41586-021-04045-6
4. Yudin, Andrei K. (2015) Macrocycles: lessons from the distant past, recent developments, and future directions. https://doi.org/10.1039/c4sc03089c
5. Alexander A. Vinogradov, Yizhen Yin, and Hiroaki Suga *Journal of the American Chemical Society* **2019** *141* (10), 4167-4181 https://doi.org/10.1021/jacs.8b13178
6. Daniela Ciumac, Haoning Gong, Xuzhi Hu, Jian Ren Lu, Membrane targeting cationic antimicrobial peptides, Journal of Colloid and Interface Science, Volume 537, 2019, Pages 163-185, ISSN 0021-9797, https://doi.org/10.1016/j.jcis.2018.10.103.
7. Tyagi, Mohit. Drug Syntheses Beyond the Rule of 5. https://doi.org/10.1002/chem.201902716
8. Dougherty, Patrick G. Macrocycles as protein–protein interaction inhibitors. Biochemical Journal (2017) 474 1109–1125 https://doi.org/10.1042/BCJ20160619
9. Fischbach, Michael A., and Christopher T. Walsh. “Antibiotics for Emerging Pathogens.” *Science*, vol. 325, no. 5944, 2009, pp. 1089–1093., https://doi.org/10.1126/science.1176667.