Note: Guidelines were changed from 5000 characters to 2-pages

STAR 2024 Project Statement

Synthesis of Lysine-Derived Monomers as Building Blocks for Antimicrobial Polymers

1) Introduction

Antimicrobial resistance (or AMR) occurs when human bodily systems reject antibiotics used to treat infections. AMR is largely due to the overprescription and therefore overuse and misuse of antibiotic medications. AMR is a rapidly increasing global issue; in fact, the National Institutes of Health has deemed it one of the principal public health problems of this century (Pantosti et al.).

Scientists are in the process of researching viable and effective solutions to the resistance of antibacterial drugs. Host defense peptides (HDPs) show promising antibacterial activity and biocompatibility, according to Palermo et al. HDPs are naturally occurring and synthetic peptides used to combat infections by disrupting pathogenic membranes. However, HDPs are expensive to synthesize, and the process of synthesizing HDPs is also quite tedious, as they're difficult to purify.

Rather, antimicrobial polymers (AMPs) could serve as an alternative to HDPs. Inspired by the structural design of HDPs, AMPs possess structural features, such as cationic charge and hydrophobicity (Palermo et al.). These structural features are important in the mode of action of HDP and AMPs. In turn, these features allow for antimicrobial activity and membrane selectivity. The optimized cationic charge of these molecules interacts with the anionic cell membrane. The hydrophobic group allows for the insertion and disruption of the bacterial cell membrane. AMPs offer advantages over HDPs, such as ease of synthesis, more chemically versatile, and cheaper. These synthetic polymers will serve as an efficient alternative to antibiotics.

2) Aims, Goals, and Research Question

The research objective of this work is to develop amino acid-based HDP-mimicking polymers as antimicrobial agents. To accomplish this, three phases are involved: 1) monomer synthesis; 2) polymerization of the monomers; and 3) evaluation of the antimicrobial activity of the polymers. My research task falls within phase one, which is the synthesis of six lysine-derived monomers (Figure 1). Positively charged amino acids, such as lysine, have special cationic groups, which have been investigated to optimize AMP bioactivity (Phuong et al.). Several structural features of lysine-derived monomers (e.g. cationic spacer arms, length of the cationic side chain, and cationic charge density) have been manipulated (Phuong et al.). These changes show a lead lysine monomer that displayed high antimicrobial activity against Gram-negative bacteria. My work will focus on optimizing the working conditions and synthesizing the six monomers shown in Figure 1 to expand the library of lysine-derived polymers for structure-activity relationship evaluation. The panel of monomers will allow us to evaluate the polymers' structural differences, such as the size of the hydrophobic groups (Cbz(Z) versus Fmoc) and cationic position (α versus ε) concerning their antimicrobial activity.

3) Approach

The synthesis of the six monomers will be accomplished following a reaction known as nucleophilic acyl substitution using different coupling agents (Scheme 1). To accomplish this, we follow the general procedure outlined in Figure 2. First, we set up the reaction (steps 1-5). Second, we isolate the product via extraction (step 6). Third, we analyze the product, which verifies the structure of the product and the efficiency of the reaction (steps 7 and 8). This process will be repeated for each of our six monomers.

Last summer, I synthesized Cbz(Z)2 (Figure 1). For the reaction setup, I used dichloromethane (DCM) as the solvent. I added the Boc and Cbz-protected lysine starting reagent, triethanolamine (TEA), and diisopropyl carbodiimide (DIC)/ethylene dichloride (EDC) as the coupling agents to the solution. The mixture was stirred in an ice bath followed by adding TEA and AEMA to the solution. The reaction mixture was kept on a stir plate at room temperature for 24 hours. An acid-base extraction was performed using sodium bicarbonate and hydrochloric acid. Thin-layer chromatography (TLC), using 100% ethyl acetate as the mobile phase, confirmed product formation and was further confirmed by ^{1}H NMR. A cleaner NMR spectrum was obtained with the EDC solution, as shown by the disappearance of the urea impurity at an Rf value of 0.77 on the TLC plate. There was an 88% yield for the EDC and a 67% yield for the DIC. Through this process, I've identified a more effective coupling procedure using EDC. The next step is to perform large-scale reactions and utilize these optimized conditions for the synthesis of the other target monomers. If time allows, given this goal is accomplished, I will proceed to learn the chemistry and techniques involved in phase 2, the polymerization of the monomers.

Character Count: 4761

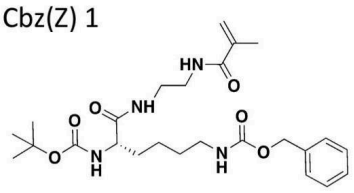
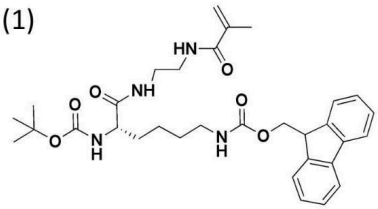
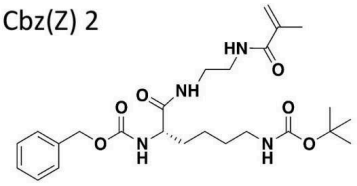
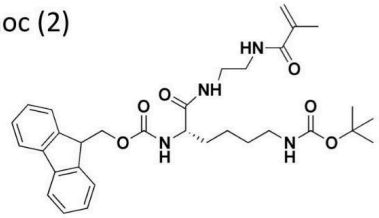
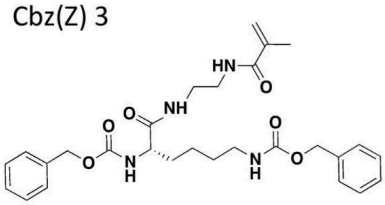
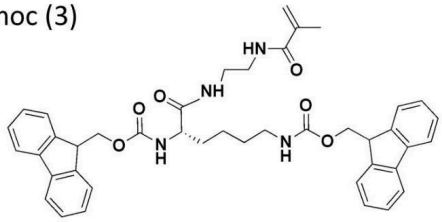
	Lysine(K)-based monomers	
	Cbz(Z)	Fmoc
α-Boc	Cbz(Z) 1 	Fmoc (1) 
ε-Boc	Cbz(Z) 2 	Fmoc (2) 
No-Boc	Cbz(Z) 3 	Fmoc (3) 

Figure 1. List of each lysine(K)-derived monomer to be synthesized in this research study.

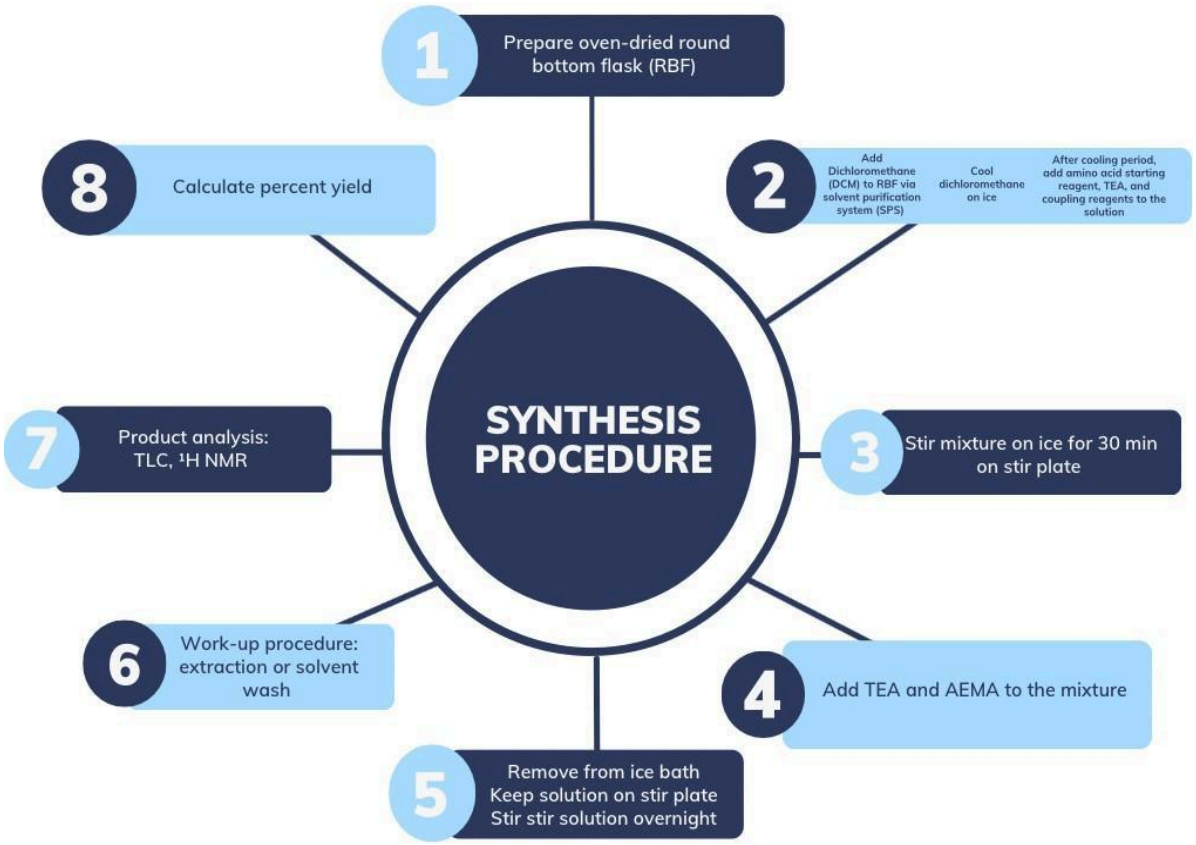
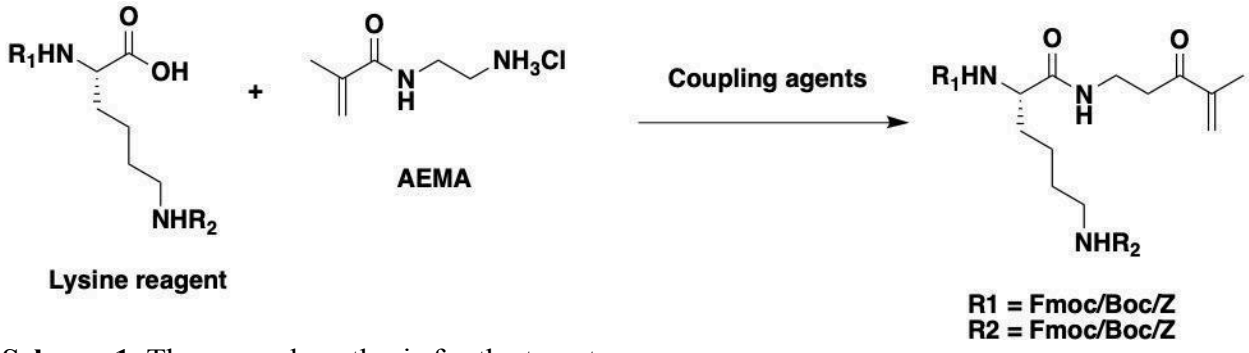


Figure 2. The general workflow for the synthesis and characterization of the lysine-derived monomers.



Scheme 1. The general synthesis for the target monomers.

References

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Pham, Phuong, et al. "Effect of cationic groups on the selectivity of ternary antimicrobial polymers." *Macromolecular Rapid Communications*, vol. 43, no. 21, 2 Aug. 2022, https://doi.org/10.1002/marc.202200377.