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Synthesis and analysis of the antimicrobial peptoid N-LfB6

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Synthesis and Analysis of the Antimicrobial Peptoid N-LfB6

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Abstract

The increasing presence of drug resistant bacteria has led to research of new methods to combat bacteria. A part of the natural peptide Lactoferricin, LfB6 (RRWQWR-NH_2), has been shown to be effective against a variety of gram positive and gram negative bacteria. However, there are several problems associated with peptides. Peptides can quickly degrade in the body which causes larger doses to be effective. Large doses of some peptides are cytotoxic to the host. Peptoids are a class of compounds that can potentially mimic the function of peptides and circumvent the problems associated with them. The backbone of peptoids is similar to that of peptides but functional groups are bonded to nitrogen instead of the alpha-carbon. Peptoids are protease resistant, which allows for lower dosages while retaining equal effectiveness. This research strives to make the peptoid compliment of LfB6, N-LfB6, and determine the effectiveness of N-LfB6 as an antimicrobial agent. The peptoid N-LfB6 has been synthesized and purified. Future research will determine the effectiveness of the peptoid against bacteria. Research into N-LfB6 could prove to be valuable in terms of antimicrobial effectiveness, stability, and low cytotoxicity to the body.

Introduction

Bacterial infections are a commonplace in today's world. Many bacterial strains are becoming drug resistant, making it more difficult to treat infections. The increase of these drug resistant bacteria has led to the research into new methods to defend against bacteria. This has opened up the study for naturally occurring antimicrobial peptides (AMPs) (1,2). However, the use of peptoids, poly-N-substituted glycines, could prove to be more effective at killing bacteria.

The goal of this research is to synthesize the peptoid compliment of the AMP LfB6 and to determine its effectiveness as an antimicrobial agent.

Background

AMPs are a naturally occurring defense mechanism in multicellular organisms. They are effective against a broad spectrum of both gram-positive and gram-negative bacteria. For this reason, they can provide an alternative to traditional antibiotics. AMPs kill bacteria by attacking the bacterial cell wall. The bacterial cell wall is composed of many negatively charged phospholipids heads with a hydrophobic core. AMPs typically contain a combination of positively charged and hydrophobic side chains. The interaction of the AMP with the cell wall causes degradation and perforation to the cell wall which ultimately causes death to the bacterial cell (7).

Though effective antimicrobial agents, AMPs have problems associated with their use. AMPs are very susceptible to proteolytic degradation, reducing the availability of AMPs to combat bacteria. Because of this, it is often necessary to use high doses of AMPs to counteract this problem. However, high concentrations of AMPs can be cytotoxic to the host's body (1). Peptides also have problems with absorption into the body, preventing them from being readily available to the body (4). The use of peptoids could help circumvent these problems.

Peptoids differ from peptides in that the functional group of the peptide is attached to the amide nitrogen instead of the α-carbon (see Figure 1). Peptoids retain similar function to peptides. This movement of the side chain to the nitrogen allows the peptoid to be protease resistant, increasing the bioavailability. Research has shown that peptoids are effective against a wide range of gram-positive and gram-negative bacteria (1, 3). It has also been shown that

peptoids exhibit less cytotoxicity than peptides. In some cases, peptoids show cytotoxicity up to 20 times less that currently used AMPs (1). Peptoids are easily synthesized, often using the same equipment used to make peptides (1). This allows for the relative ease of the adjustment of peptoid side chains. Peptoids could be synthesized to have the most desired properties (3). This includes adjustment of the number and type of functional groups composing the peptoid.

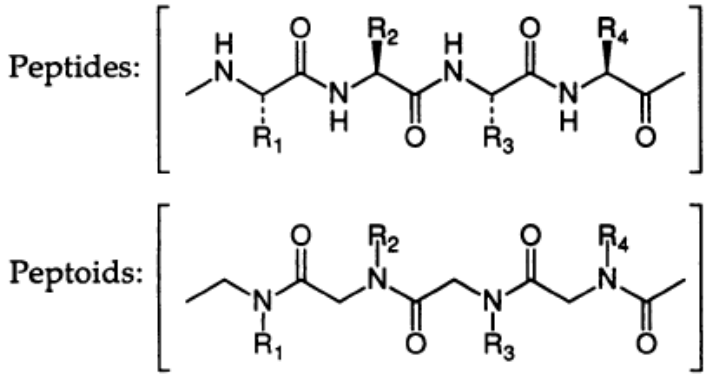

Figure 1: Comparison between peptides and peptoids (4).

The mechanisms for the bactericidal effects are similar to AMP however the exact mechanism is unknown. It has been shown that peptoids cause a rupture within the cell membrane, which causes slow leakage from the cell (1, 3). The presence of cationic functional groups increases this method of antimicrobial activity. This is likely due to the interaction with anionic proteins found in the bacterial membrane. Replacing a functional group on a peptoid with one that was cationic showed increased effectiveness in antimicrobial activity. Antimicrobial activity was also increased when side chains were replaced with bulky, hydrophobic side chains (1).

Lactoferricin (LfB) is a naturally occurring immunity peptide found in bovine protein lactoferrin. The peptide LfB6 is considered to form the antimicrobial active site of lactofericin.

LfB6, RRWQWR-NH_2, has been shown to be an effective AMP against a variety of bacteria (2). Through methylation of the second tryptophan, RRWQ[1-Me-W]R-NH_2, the effectiveness of LfB6 is further increased three times. LfB6 is a good candidate to make into an antimicrobial peptoid (N-LfB6) for many reasons. The presence of the catatonic arginine groups will help to allow for antimicrobial activity. The presence of the two tryptophan side chains was found to be necessary for antimicrobial activity of the peptide (2). The bulky, hydrophobic tryptophan groups could help to increase the antimicrobial activity (1). The presence of tryptophan can also allow the peptoid to retain secondary helical structures similar to the peptide. Hydrogen bonding in peptoids is lost due to the presence of the side chain on the nitrogen, however, the presence of bulky, aromatic side chains could allow the peptoid to form stable helices due to steric interactions (4, 5). The structure of N-LfB6 is shown in Figure 2.

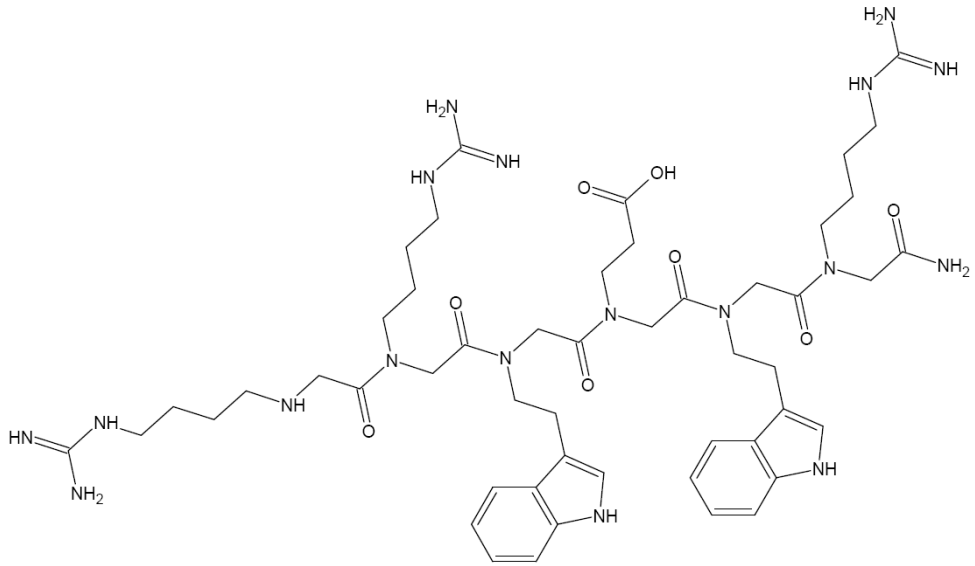

Figure 2: Structure of the peptoid N-LfB6.

Materials and Methods

N-LfB6 requires the side chains N_{Trp}, N_{Gln}, and N_{Arg} in order to be synthesized. The side chains N_{Trp} and N_{Gln} are reliably available and can be purchased. N_{Arg}, however, must be synthesized. N_{Arg} can be synthesized using a published protocol (9) with protecting groups on the terminal nitrogens as shown in Figure 3. This prevents side reactions with the functional group when acylating with a haloacetic acid to create the backbone of the peptoid. The side chain can then be purified using column chromatography. Synthesis conformation can be determined using ESI-MS and purity is determined using H-NMR. The protecting groups are removed after the peptoid is synthesized.

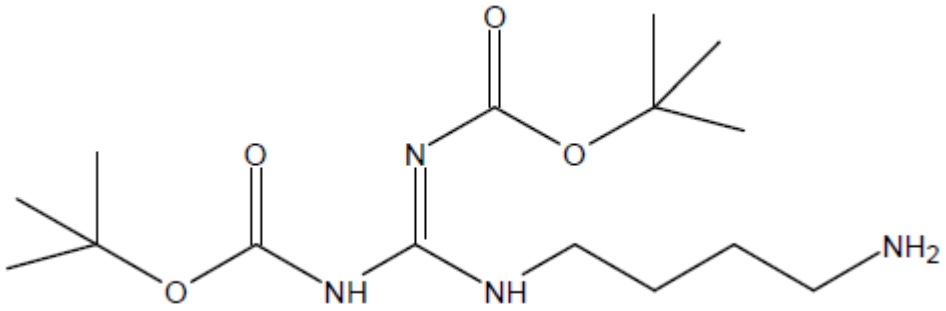

Figure 3: N_{Arg} structure.

The peptoid is synthesized using an ABI 433A automated peptide synthesizer. In this process, an amine on a monomer is attached to a resin. The amine then bonded to a haloacetic acid (bromoacetic acid). The halogen is then replaced to bind to the amine group of a second monomer (6). This process is repeated to create the peptoid as shown in Figure 4 (8).

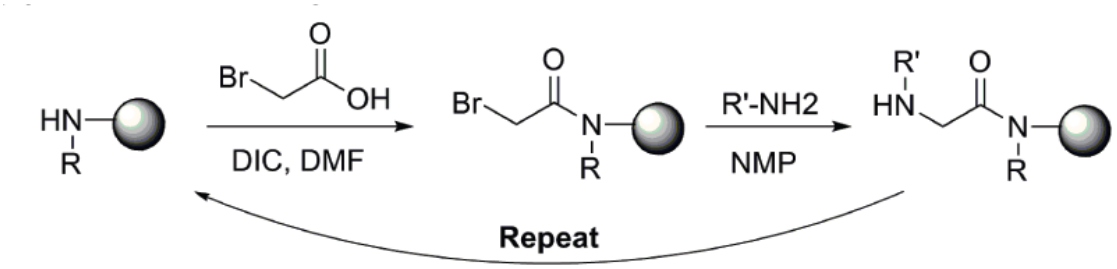

Figure 4: Synthesis of a peptoid. (8)

Once the peptoid is synthesized, it can be separated from the resin using acid. The excess solvents can then be removed using a lyophilizer. The desired peptoid purified using preparative HPLC using a gradient of acetonitrile and water (5-95%) in conjunction with a C18 column. The mass and purity is determined using MALDI.

Antimicrobial investigation will be conducted using a microtitre broth dilution method modified by Denise Greathouse. Effectiveness against both gram-positive bacteria (*Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*) will be tested.

Results

The side chain N_{Arg} was successfully synthesized. The estimated molecular weight of 330.4 was found using ESI-MS as shown in Figure 5. The side chain was then purified using column chromatography. The H-NMR shown in Figure 6 matches the estimation for the side chain structure.

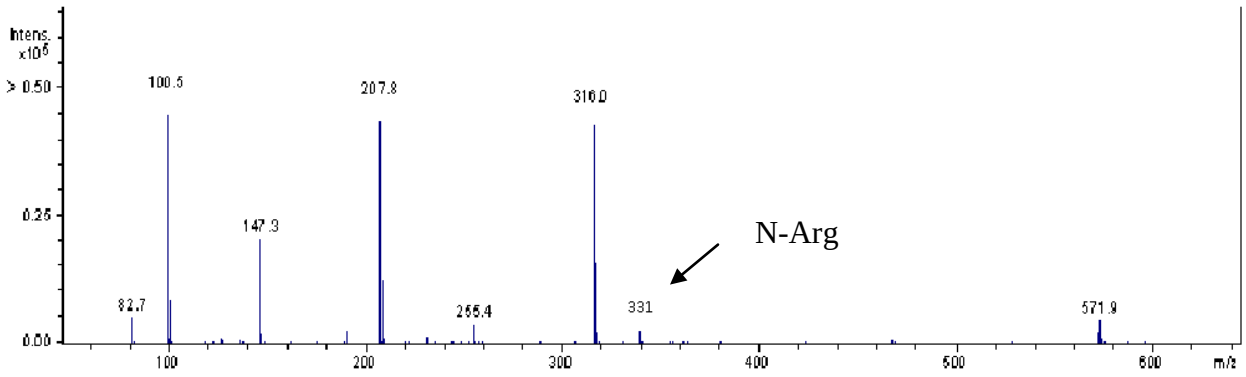

Figure 5: ESI-MS of N_{Arg} synthesis.

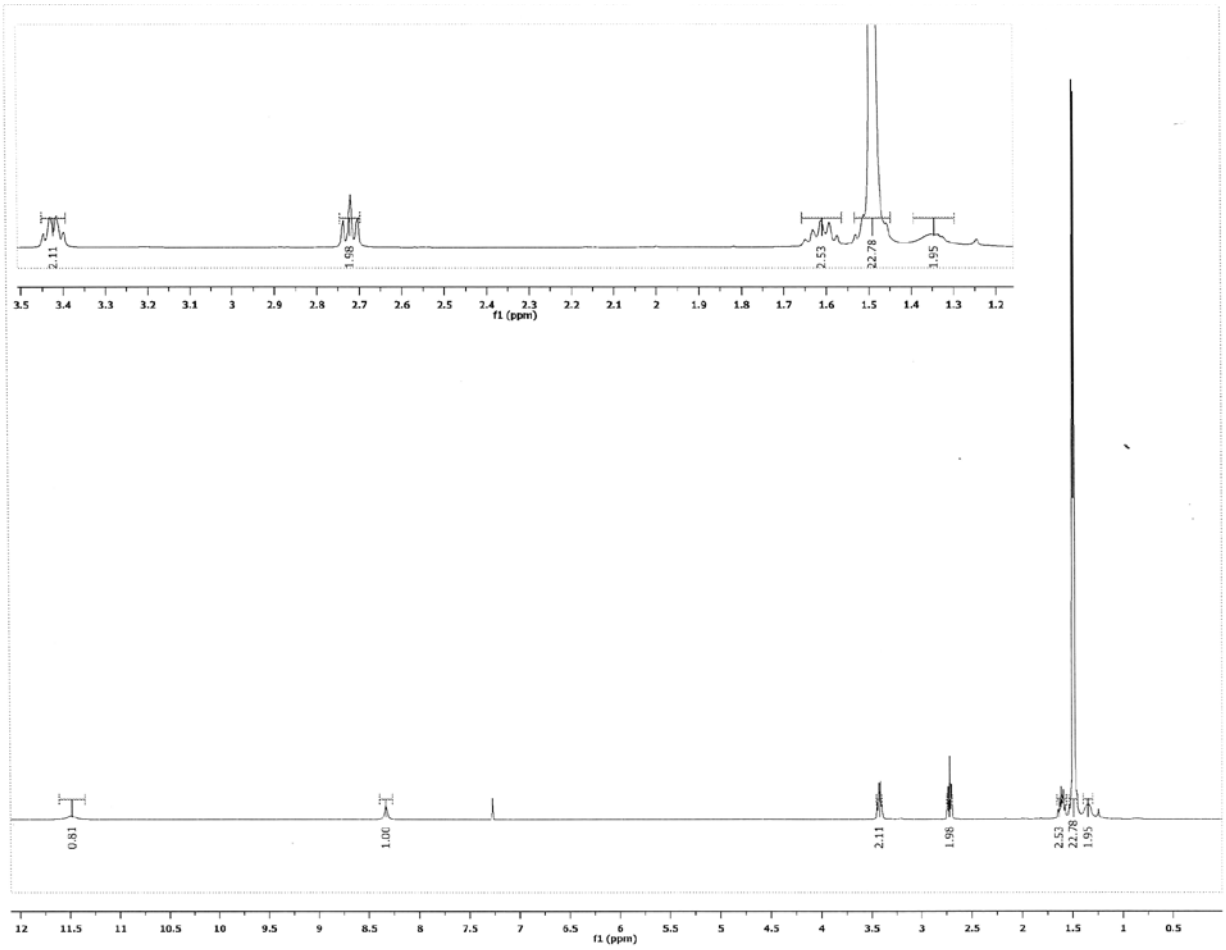

Figure 6: NMR of N_{Arg}.

The peptoid N-LfB6 (Figure 2) was successfully synthesized. The desired molecular weight of 1057.7 was confirmed using MALDI (Figure 7). The peptoid was purified using

preparative HPLC. Analytical HPLC (Figure 8) along with mass spectroscopy shows the peptoid to be 97% pure.

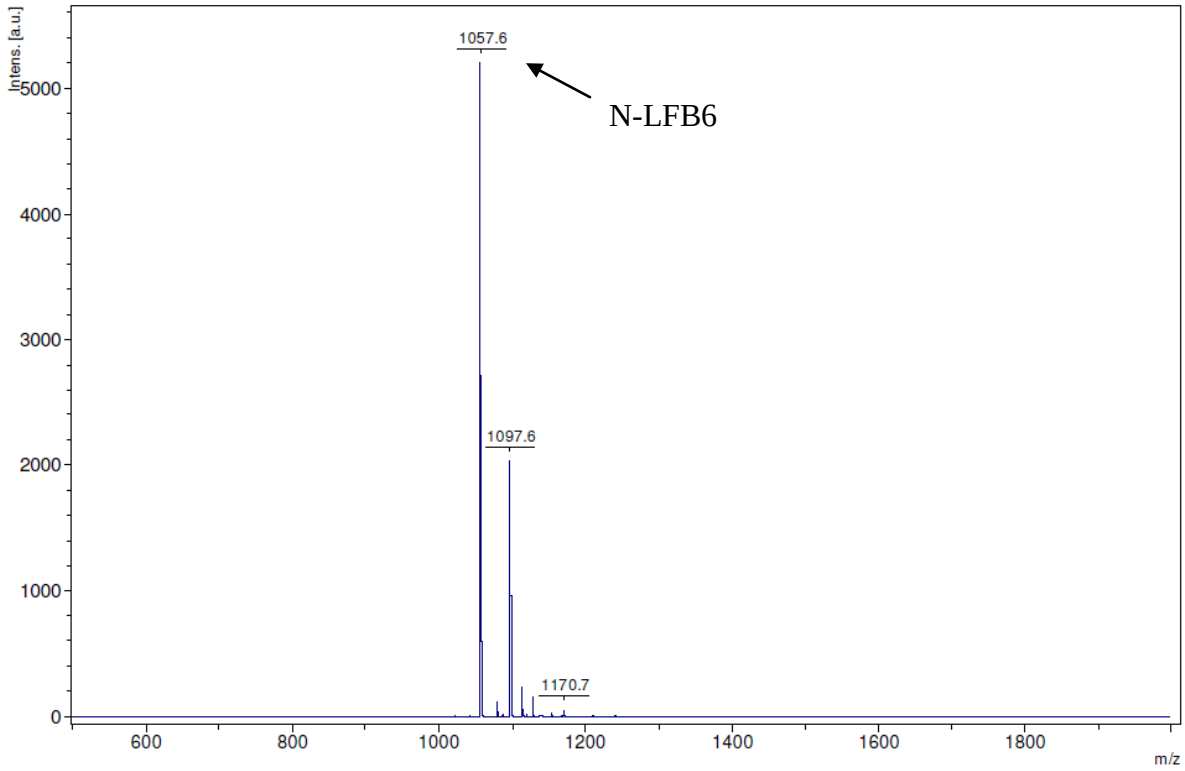

Figure 7: MALDI-MS of synthesized N-LfB6.

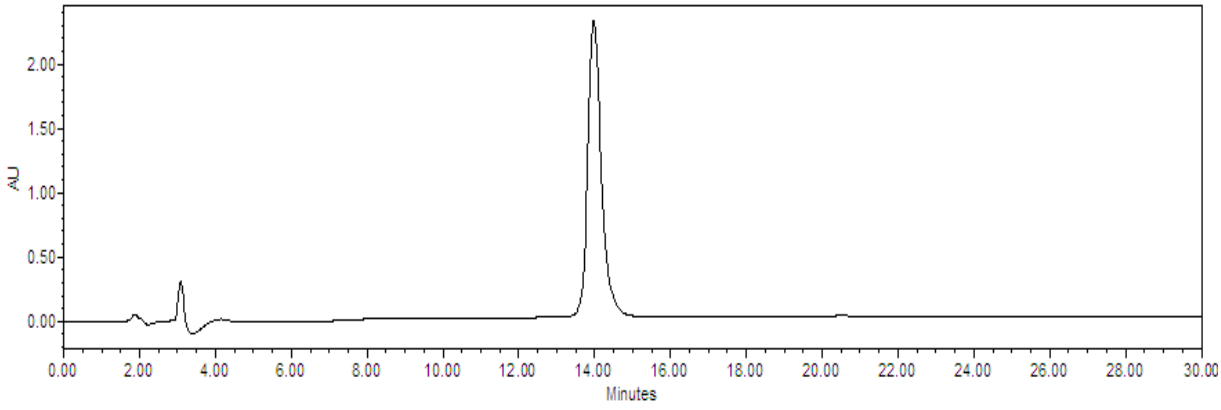

Figure 8: Analytical HPLC results of purified peptoid.

Conclusion

The peptoid N-LfB6 was successfully synthesized and purified. Further investigation into the effectiveness of N-LfB6 is needed. Specifically, the effectiveness of N-LfB6 as an antimicrobial peptoid will be determined via microtitre bacterial assays. LfB6 has been shown to be an effective AMP both with and without the methylated tryptophan. The effective functional group sequence coupled with the benefits of a peptoid could allow N-LfB6 to be a new, effective antibacterial agent.

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