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Synthesis of T4 lysozyme substrate

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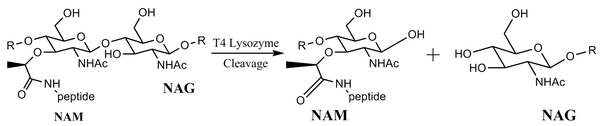
Introduction

One of the most studied enzymes, T4 lysozyme, is produced by T4 bacteriophage and destroys cell walls of bacteria[1]. It works by cleaving bacterial cell walls to release virions (individual viral particles) which leads to infection of other cells[5]. The bacterial cell wall consists of long chains of alternating N-Acetylglucosamine (NAG) and N-Acetylmuramic acid (NAM) units (Scheme 1).

Scheme 1. Hydrolysis of NAM-NAG linkages by T4 lysozyme



Along with being attached to NAG units, the NAM units are also attached to peptides that are crosslinked to other NAG-NAM chains, forming a covalent net around the cell.

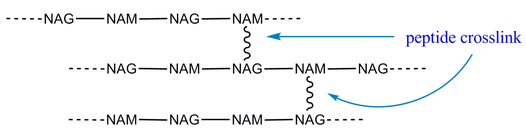


Figure 1. Schematic of bacterial cell wall--the natural substrate for T4 lysozyme

T4 lysozyme disrupts the bacterial cell wall by cleaving the NAG-NAM bonds. Although T4 lysozyme is one of the most thoroughly studied enzymes structurally, has hundreds of mutants made and crystal structures determined, there is little kinetic data due to the lack of a simple substrate. Our goal is to synthesize a simple, convenient substrate.

Due to the heterogeneous nature of the cell wall, it is difficult to clearly define the kinetics (enzyme reaction rate) and substrate specificity of T4 lysozyme. Instead, synthesis of a smaller, well-defined substrate will allow us to study the kinetics and mechanisms of this enzyme in greater detail[4]. This also allows the ability to modify the substrate synthetically to explore the role of various parts of the substrate in binding and cleaving chains to help us study and understand the kinetics and mechanism of T4 lysozyme[6].

The substrate we propose is the muramic acid derivative **1**. T4 lysozyme requires the N-acetylmuramic acid (NAM) to be substituted with a peptide side chain in order for cleavage to occur[2]. Specifically, binding studies on cell wall fragments indicate that muramic acids substituted with Ala-D-Glu(Lys) tripeptide bind to the enzyme[2,4]. The nitrophenol-substituted sugars are commonly used as substrates for glycosidases (enzymes that catalyze hydrolysis of sugars). When hydrolyzed, the released nitrophenol produces an absorption in the visible spectrum.

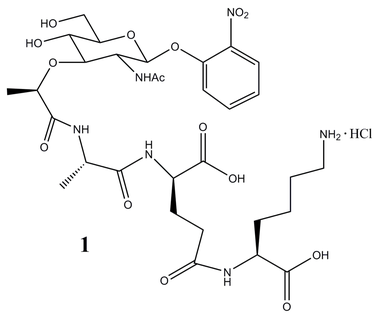
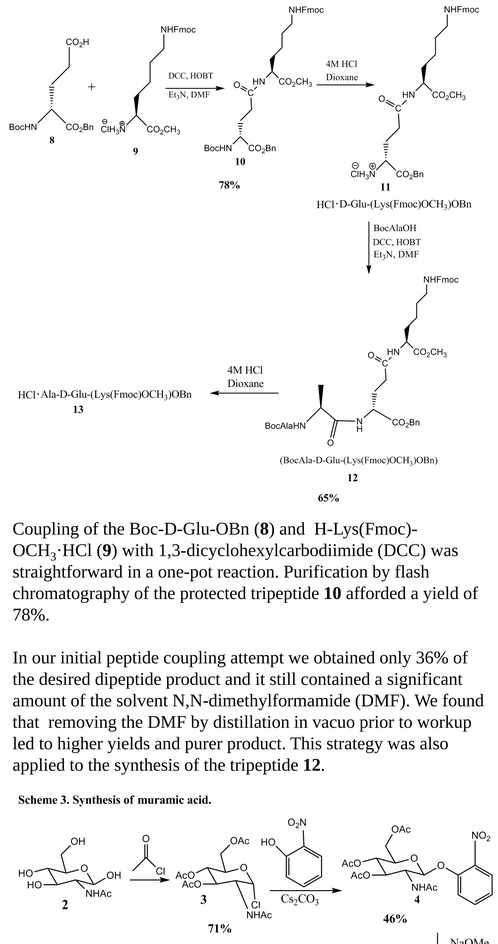


Figure 2. Proposed T4 lysozyme substrate

Synthesis

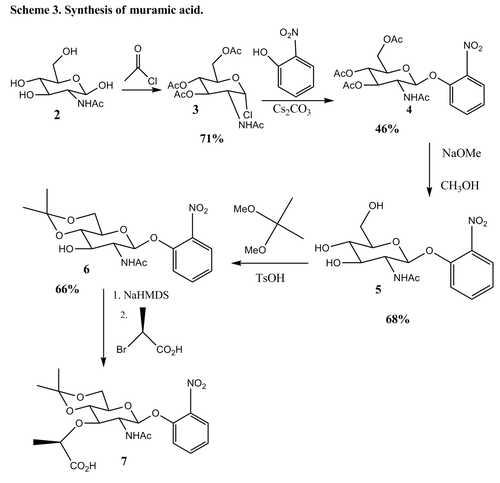
Scheme 2. Synthesis of protected peptide



Coupling of the Boc-D-Glu-OBn (**8**) and H-Lys(Fmoc)-OCH_3·HCl (**9**) with 1,3-dicyclohexylcarbodiimide (DCC) was straightforward in a one-pot reaction. Purification by flash chromatography of the protected tripeptide **10** afforded a yield of 78%.

In our initial peptide coupling attempt we obtained only 36% of the desired dipeptide product and it still contained a significant amount of the solvent N,N-dimethylformamide (DMF). We found that removing the DMF by distillation in vacuo prior to workup led to higher yields and purer product. This strategy was also applied to the synthesis of the tripeptide **12**.

Scheme 3. Synthesis of muramic acid.



The synthesis of the muramic acid began on a large scale of 30 g with the commercially available N-acetylglucosamine. Acetyl chloride was used to acetylate the oxygens and to convert the alcohol at the anomeric position to a chloride leaving group. The result was 71% of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucosyl chloride (**3**).

Coupling of 2-nitrophenol to the chloride sugar **3** with cesium carbonate ($CsCO_3$) proved to be trickier. Product could have been lost during purification by recrystallizing with warm methanol. It was difficult to dissolve the crude product, so a large quantity of methanol was required , but not all of the crude dissolved. Although the large amount of methanol used didn't completely dissolve the crude product, it might have been enough to hinder the product from precipitating out of the methanol during recrystallization.

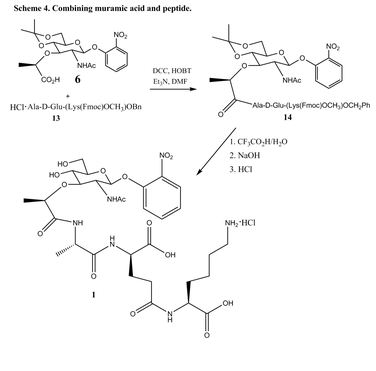
Current Work

Addition of (S)-(-)-2-bromopropionic acid to (2-nitrophenyl) 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**6**) is currently being worked out. When this reaction was previously carried out, cleavage of the nitrophenyl group was occurring and the bromopropionic acid was not being added. To work out these problems, we have been using similar, simpler sugar, Diacetone-D-glucose.

We've also been working on the synthesis of the muramic acid with the 4-nitrophenyl group.

Future Work

Scheme 4. Combining muramic acid and peptide.



Scheme 4 shows the target molecule, T4 lysozyme substrate, of the project. Once the muramic acid derivative and the peptide side chain are synthesized, DCC will be used to couple them together to produce the molecule of interest, 2-nitrophenyl muramic acid with Ala-D-Glu-Lysine **1.**

Bibliography

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Acknowledgements

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