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Characterization of the MalA Enzyme from Bdellovibrio bacteriovorus through the Synthesis and Analysis of Maltose Derivatives

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Joey Jepson and John Hanson

Background

- MalA is an α-glucosidase found in *Bdellovibrio bacteriovorus*, a predatory bacteria that preys on gram-negative bacteria.[1]
- The MalA enzyme binds to and cleaves a maltose sugar into two glucose sugars.

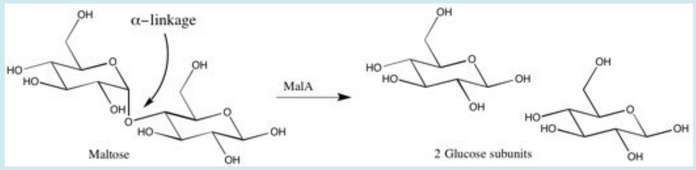


Figure 1. Cleavage of maltose by MalA

- MalA will also hydrolyze longer maltose analogs such as maltotriose and maltotetrose, along with the chromaphoric substrate pNPG.
- MalA will bind to several different disaccharides composed of a glucose α-linked to a variety of sugars (isomaltose, trehalose, turanose, sucrose, etc.), but it does not cleave them.

Objective

- The objective of our research is to investigate how the various parts of maltose contribute to binding and cleavage.
- In order to do this, we will develop a route to synthesize maltose analogs with specific changes to the primary alcohol at the 6th position on the +1 site.
- Methyl α-D-glucopyranoside, a more simple sugar, will be used as a model system in the development of our synthesis. It will also be tested with the MalA enzyme, as it contains a glucose and an α-linkage.
- A positive result of using this particular model system is that we will gain insight on how the other primary alcohol (that which is located on the -1 site) contributes to binding and cleavage by MalA.

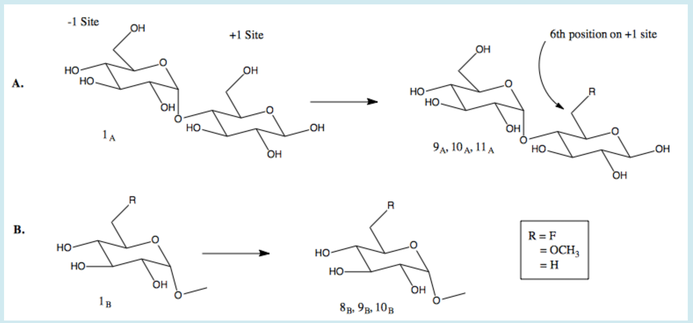
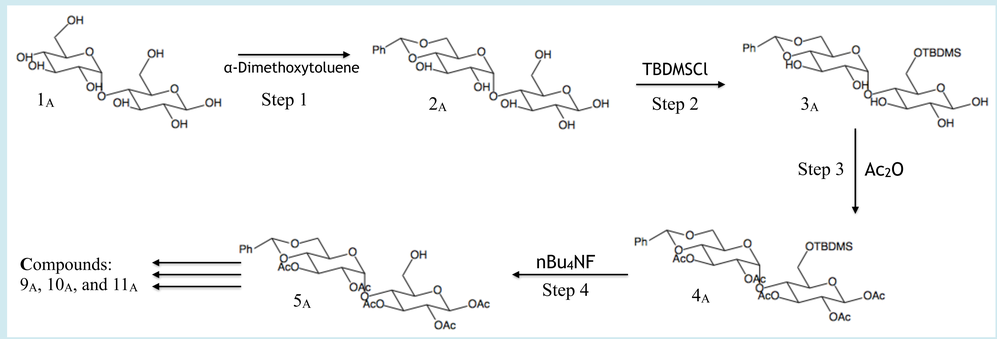


Figure 2. (A) Maltose sugar with changes being made to the 6th position on the +1 site (compounds 9_A, 10_A, and 11_A).
(B) Methyl α-D-glucopyranoside with changes being made to the 6th position (compounds 8_B, 9_B, and 10_B).

- In place of the targeted alcohol, the desired analogs will contain either a fluorine atom, a methoxy group, or a hydrogen.
  - The fluorine atom will act as an H-bond acceptor, but not an H-bond donor.
  - The methoxy group will be a bulky replacement for the alcohol that may impact cleavage due to its size.
  - The deoxygenation will remove the presence of the alcohol that acts as an H-bond acceptor and H-bond donor.
- Once the sugar analogs are produced, they will be tested with MalA to determine how each targeted alcohol contributes to binding and cleavage.

Synthesis of Maltose Derivatives

- In order to selectively act on the alcohol of interest, all alcohols except the primary 6-OH of the +1 site must be protected.
- The first four steps of the synthesis were successfully completed:



Step 1. Addition of benzylidene protecting group:

- To protect the primary alcohol on the -1 site of the maltose compound, a benzylidene acetal functional group was added.
- The reaction was followed by thin-layer chromatography (TLC), which indicated the presence of the aromatic ring under ultra-violet light.

Step 2. Addition of TBDMS protecting group:

- The alcohol on the +1 site was then protected with a TBDMS ether so that this alcohol would not be acetylated in the following step.
- While we were performing this reaction for the first time, it was discovered that the TBDMS was not being added properly, which prompted the use of a simpler compound (methyl α-D-glucopyranoside) to probe out what was wrong.
- We found that the solution to this obstacle was to perform the reaction in an inert atmosphere using a newer TBDMS bottle, and drying the solvent with 3Å molecular sieves.
- By analyzing through H-NMR, it was clear that the TBDMS had been added to our compound.
- The use of this simpler compound led to the commencement of a side project that is to perform homologous changes to this new system (methyl α-D-glucopyranoside and to also test its derivatives with malA, as they contain a glucose sugar with an α-linkage too.

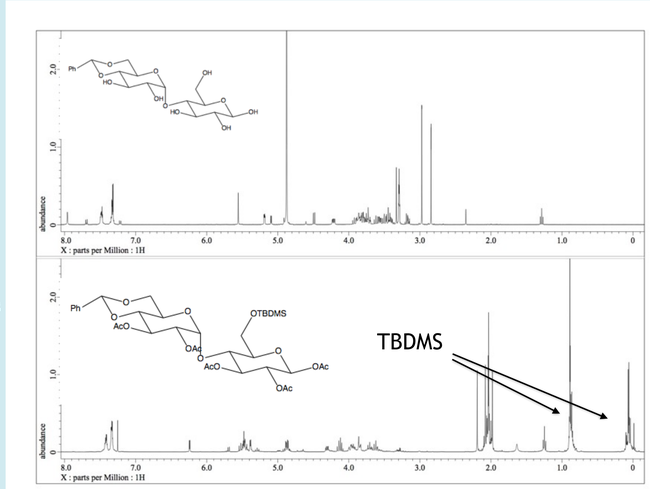


Figure 3. H-NMR of products 2_A and 4_A (before and after the addition of TBDMS).

Step 3. Acetylation of the remaining unprotected alcohols:

- The remaining alcohols were protected, allowing us to specifically react the target alcohol.
- Several methods were attempted, however the best involved adding acetic acid under reflux.
- By analyzing through C-NMR, it was determined that the acetyl groups were successfully attached

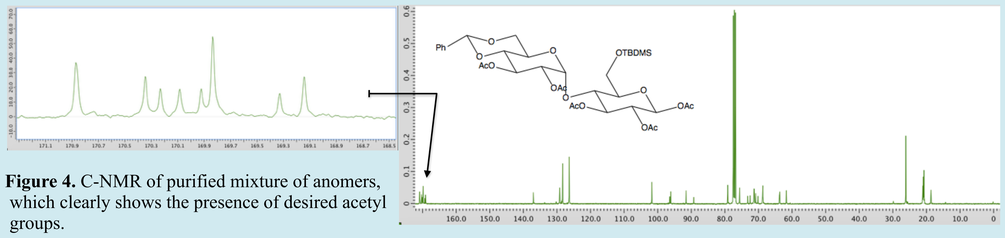


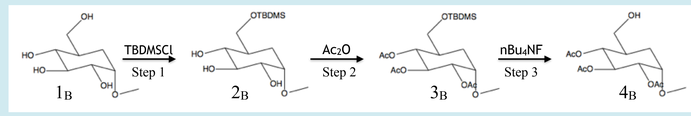
Figure 4. C-NMR of purified mixture of anomers, which clearly shows the presence of desired acetyl groups.

Step 4. Removal of TBDMS protecting group:

- The TBDMS was removed from the +1 primary alcohol so the desired changes can be made to this alcohol only.
- As before, by using H-NMR analysis, it was determined that the TBDMS was removed from our compound.

Synthesis of Methyl α-D-glucopyranoside Derivatives

- Due to the complexity of the maltose compound, along with the presence of a mixture of anomers, NMR analysis was quite challenging. By using methyl α-D-glucopyranoside, a simpler compound that is not anomeric, we are more readily able to conclude whether or not a reaction has gone to completion. In addition to this, because of the similarity of these two compounds, we can use the spectra obtained for one as a reference for the other.
- A benzylidene acetal does not need to be added to this compound in the first step, as there is only one primary alcohol.
- The first three steps of the synthesis of methyl α-D-glucopyranoside analogs were successfully completed:



Step 1. Addition of TBDMS protecting group:

- Just like with maltose, the TBDMS ether was added to the primary alcohol of the compound.
- The completion of this reaction was confirmed by H-NMR analysis.

Step 2. Acetylation of remaining unprotected alcohols:

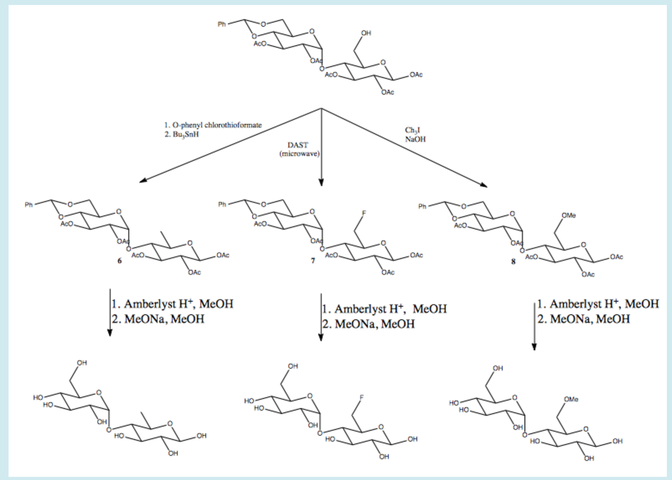
- The remaining alcohols were acetylated, allowing us to perform specific changes to the targeted primary alcohol without reacting with undesired alcohols.
- The completion of this reaction was confirmed by C-NMR analysis.

Step 3. Removal of TBDMS protecting group:

- The TBDMS ether was removed leaving a single alcohol to be reacted with and changed into our desired products.
- The completion of this reaction was confirmed by H-NMR analysis.

Future Plans

- Now that we have produced enough of the protected and acetylated sugars, we can complete the final steps of the syntheses.
  - This involves selectively acting on the remaining unprotected alcohol and deprotecting the rest of each compound:



- Spectral analysis:
  - MalA will be tested with each maltose and methyl α-D-glucopyranoside derivative for its ability to bind and cleave them.
  - This process will be analyzed using NMR analysis and mass spectroscopy.

References

1. Cheng, H. Bdellovibrio bacteriovorous. https://microbewiki.kenyon.edu/index.php/Bdellovibrio_bacteriovorus (accessed February 8, 2016).