Synthetic Approaches Towards a Marine Natural Product, Anomoian B

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Abstract

Since the 1980s, no new classes of antibiotics have been discovered[1]. Anomoian B is compound isolated from Verongida sea sponges that has shown some antibacterial properties[2]. The goal of this project is to synthesize anomoian B in the lab and analyze its bactericidal effects. Researchers have determined the chemical structure of the compound with mass spectrometry and ^{1}H NMR spectroscopy. Because this is a natural product, synthesizing anomoian B under laboratory conditions will ensure no further harm to Verongida sponges as this compound is further studied. Using the data provided by previous researchers, a retrosynthesis procedure was created that separates anomoian B into three fragments. The first fragment has already been synthesized by prior researchers. This project successfully synthesized the second fragment, and nearly finished the third fragment.

Introduction

Microbial resistance, partly caused by the inappropriate use of antibiotics, is one of the most concerning problems faced by the healthcare industry. Currently, most naturally derived drugs are from terrestrial animals. However, marine animals have been found to produce more biologically active substances than any terrestrial group[3]. While much research has been dedicated to finding cancer fighting properties in marine animals, research regarding the antibacterial properties of these organisms is relatively recent. In 2010, a sea sponge from the order Verongida (Hexadella genus), was collected in Indonesia[2]. Several dibromotyramine compounds, anomoian B, aplyzanzine B, and aplysamine 4, were isolated from this sample and their chemical structures were determined. All three compounds showed cytotoxic activity against human cancer cell lines and possibly some antibacterial properties. Anomoian B is the subject of this research project because it exhibits the most cytotoxic effects of the isolated compounds. Before further research can be performed on anomoian B, an effective synthesis procedure must be created to limit further endangerment of Verongida sponges. The proposed synthesis will be accomplished using retrosynthesis which divides the compound into three fragments (Figure 1.). ^{1}H NMR spectroscopy and mass spectrum data will be used for the analysis of each reaction as the compound is synthesized. Two of the fragments are dibrominated tyramine and tyrosine derivatives, and the third fragment is (3-amino)propyl-methansulfonate. Once each fragment has been synthesized, they will be connected using SN2 reactions between highly reactive chemical groups and peptide coupling. Fragment 1 has already been synthesized by previous researchers. The goal of this project is to synthesize the remaining fragments and couple all three pieces together to make the final product. After the compound has been successfully created, we will perform biological testing to evaluate its bactericidal effects against different bacterial species.

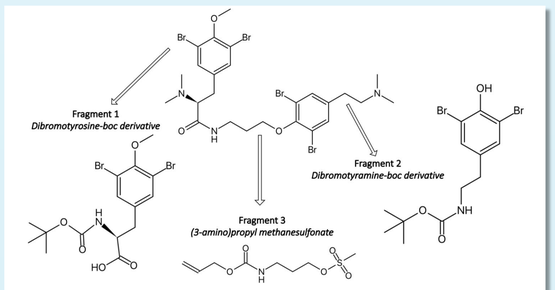

Figure 1. *Anomoian B retrosynthetic strategy dividing the compound into three fragments.*

Fragment 2 Synthesis

The strategy created to synthesize fragment 2 used readily available tyramine as a starting material and bromine and Boc_2O as reactants (Figure 2). The boc protecting group on the amine will force the alcohol to react with the methanesulfonate group on fragment three when the coupling reaction occurs. The bromination procedure utilized a mixture of bromine and tyramine dissolved in acetic acid. ^{1}H NMR spectroscopy (Figure 3) and mass spectrometry (Figure 4) confirmed the solid obtained from this reaction was dibromotyramine. As seen in the ^{1}H NMR data (Figure 3), some monobrominated tyramine is present composing approximately 9% of the product. The percent yield for this reaction was 94%. Our purification attempts using various extraction techniques were not successful. Pushing forward with the synthesis procedure, the boc protecting group was added to the amine which had an 89% yield. ^{1}H NMR spectroscopy (Figure 5) and mass spectrometry (Figure 6.) confirmed the reaction was successful, however, an unexpected splitting pattern was observed for the two hydrogens on the aromatic ring (Figure 5). It appears the addition of the boc protecting group caused these two hydrogens to lose equivalence to each other. We are still investigating this observation and hypothesize it could be due to a folding of the molecule and increased intramolecular attractions. Because the ^{1}H NMR and mass spectrometry data confirmed that the synthesis of fragment 2 was successful, the project continued with the synthesis of fragment 3.

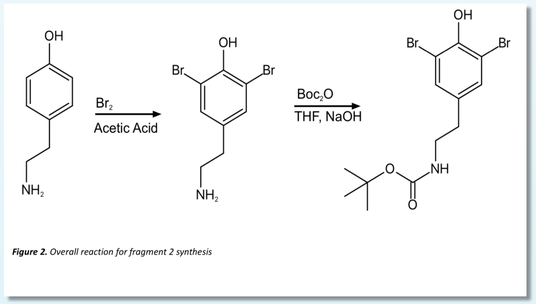

Figure 2. *Overall reaction for fragment 2 synthesis*

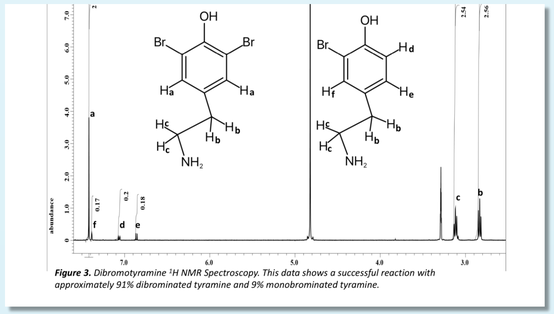

Figure 3. *Dibromotyramine ^{1}H NMR Spectroscopy. This data shows a successful reaction with approximately 91% dibrominated tyramine and 9% monobrominated tyramine.*

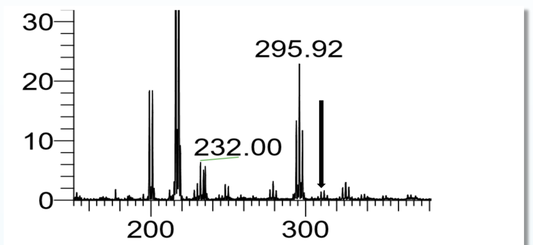

Figure 4. *Dibromotyramine mass spectrometry. The mass of the compound is 307.003 amu. The small signal at this value, indicated by the black arrow, confirms the presence of the desired compound. The pattern of peaks is close to the expected pattern for a dibrominated compound.*

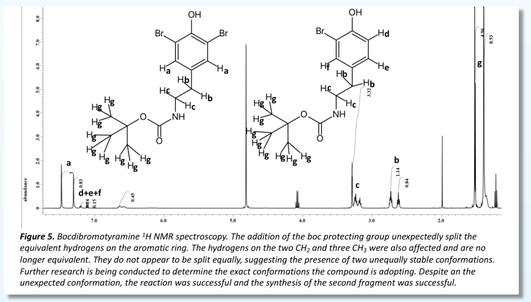

Figure 5. *Bocdibromotyramine ^{1}H NMR spectroscopy. The addition of the boc protecting group unexpectedly split the equivalent hydrogens on the aromatic ring. The hydrogens on the two CH_2 and three CH_3 were also affected and are no longer equivalent. They do not appear to be split equally, suggesting the presence of two unequally stable conformations. Further research is being conducted to determine the exact conformations the compound is adopting. Despite an the unexpected conformation, the reaction was successful and the synthesis of the second fragment was successful.*

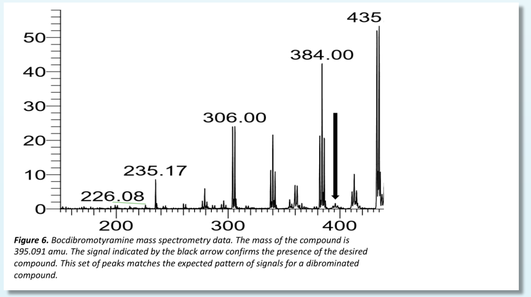

Figure 6. *Bocdibromotyramine mass spectrometry data. The mass of the compound is 395.091 amu. The signal indicated by the black arrow confirms the presence of the desired compound. This set of peaks matches the expected pattern of signals for a dibrominated compound.*

Fragment 3 Synthesis

The strategy used to synthesize fragment 3 started with 3-amino-1-propanol (Figure 7). The amino group was protected with an allyl group so that the methanesulfonyl group could be added to the alcohol in preparation for the coupling reaction. The addition of the allyl group was accomplished using allyl chloroformate and triethylamine in THF. This reaction only had a 53% yield. The ^{1}H NMR data obtained was not very clean, but the reaction was pushed forward to add the methanesulfonyl. After this reaction was performed, the ^{1}H NMR data was analyzed and a mass spectrometry was performed (Figure 8.). The data for this reaction was messy and the integration of the methyl group on the sulfur was not large enough to suggest an efficient reaction. However, the mass spectrometry data did indicate the presence of the desired product. Because the procedure was not very effective, the reaction will be repeated in the future using NaH as a base instead of triethylamine.

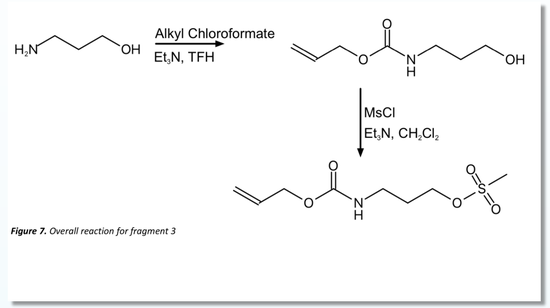

Figure 7. *Overall reaction for fragment 3*

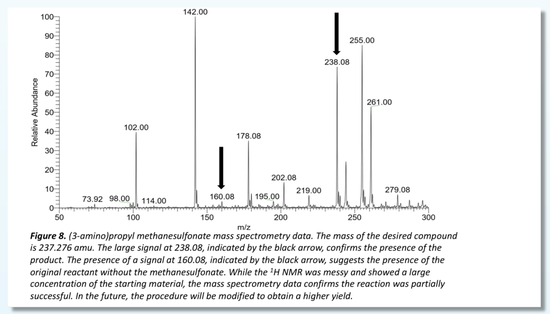

Figure 8. *(3-amino)propyl methanesulfonate mass spectrometry data. The mass of the desired compound is 237.276 amu. The large signal at 238.08, indicated by the black arrow, confirms the presence of the product. The presence of a signal at 160.08, indicated by the black arrow, suggests the presence of the original reactant without the methanesulfonate. While the ^{1}H NMR was messy and showed a large concentration of the starting material, the mass spectrometry data confirms the reaction was partially successful. In the future, the procedure will be modified to obtain a higher yield.*

Conclusion

While a complete synthesis of anomoian B has yet to be accomplished, this project has shown it is possible. The successful synthesis of fragment 2 and nearly successful synthesis of fragment 3 support this conclusion. Further research is needed to investigate the effects that adding the boc protecting group had on the aromatic hydrogens in fragment 2. Additionally, the synthesis strategy of fragment 3 needs to be revised to ensure a successful addition of the methanesulfonyl to the alcohol. Once anomoian B has been synthesized, the antibacterial properties and mode of action will need further analysis. This project is important because the creation of a novel class of antibacterial compounds has the potential to revolutionize the pharmaceutical industry.

References

[1]https://www.reactgroup.org/toolbox/understand/how-did-we-end-up-here/few-antibiotics-under-development/
[2]Tarazona, G., Santamaria, G., Crux, P. G., Fernandez, R., Perez, M., Martinez-Leal, J. F., Rodriguez, J., Jimenez, C., Cuevas, C. ACS Omega 2017, 2, 3494-3501.
[3]https://oceanexplorer.noaa.gov/facts/medicinesfromsea.html

Acknowledgements

Thank you to the following funding sources:
CSU Council on Ocean Affairs, Science & Technology (COAST)
CSU STAN Research, Scholarship and Creative Activity (RSCA)
Thank you Michele Gordon and Chad Stessman for their time spent supporting this project
This research activity is funded all or in part by the Stanislaus State ASPIRE program through a U.S. Department of Education Title III Grant #P031C210159