Sample Scientific Paper

Semi-synthetic modification of TRPM7 modulator, Waixenicin A; & synthesis of complex ring systems using oxime ether tethers in intermolecular diels alder reactions

Celeste Cravalho, Jesus Arriola, Gideon Berger, David Chambers, Mizhael Dones, Dylon Goodson, Leia Hasegawa, Adam Swanson

College of Natural & Computational Sciences, Hawaiʻi Pacific University, 45-045 Kamehameha Hwy, Kāneʻohe, Hawaiʻi, 96744 USA.

Personal Reflection

Spring 2017 was my first semester of being a research assistant and being a part of the INBRE Student Research Experience (SRE) program. Being a part of the INBRE SRE program has allowed me to use the skills I had acquired from the lab classes I have taken previously and it allowed me to gain valuable skills that were not taught in the lab classes. During my semester, I learned numerous techniques organic chemists use and I also learned how to operate various machines critical to our experiments. Not only did I learn techniques and how to operate the machines, but I also developed my communication skills by conversing with lab workers and professors and turning in notebook pages to my supervisor at the end of each lab day. The INBRE SRE program has been a valuable program that has allowed me to blossom into a better scientist.

Introduction

Two projects were being worked on at the same time. The first project involved the semisynthetic modification of Waixenicin A. Waixenicin A is a strong inhibitor of the transient receptor potential melastatin 7 (TRPM7) cation channel [1]. TRPM7 is responsible for intracellular homeostasis of magnesium and cellular proliferation and differentiation. Preliminary studies investigating the characteristics of TRPM7 have demonstrated its implications on pathological conditions such as cancer, organogenesis, cardiovascular disease, and ischemic stroke [2]. The purpose of the semisynthetic modification of Waixenicin A was to create analogues that were more potent and selective towards TRPM7 (Figure 1). The second project involved the click-tethered Diels Alder reaction to create nitrogenous heterocyclic compounds. Nitrogenous heterocyclic compounds are often used in pharmaceuticals. Previous attempts using Diels Alder reactions were limited due to low percent yields, which provided motivation to reevaluate our approach by using oxime ethers derived from cyclohexenecarboxaldehyde (Figure 2).

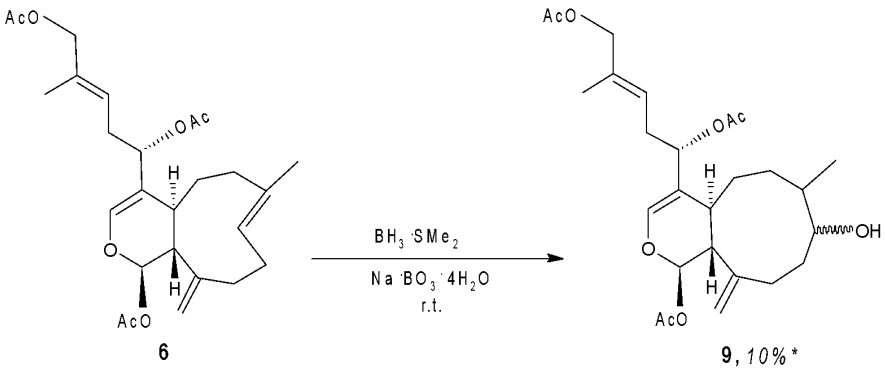

Figure 1. Hydroboration oxidation of Waixenicin A (left) to Waixenicin A analog (right).

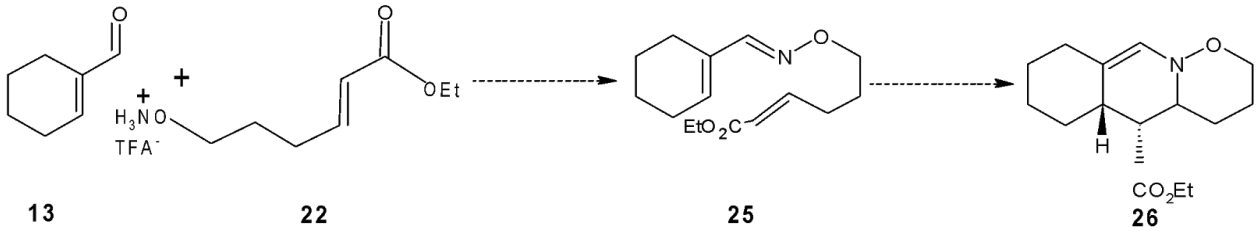

Figure 2. Condensation and Diels Alder reaction with revised aminooxy sidearm.

<u>Methods and Materials</u>

Dr. Horgen provided the Waixenicin A samples. Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) was used to analyze the structure of Waixenicin A analogues. Liquid chromatography mass spectrometry (LCMS) was used to analyze fragments of Waixenicin A analogues, which can lead to the determination of the molecular formula and structure of Waixenicin A analogues. Dr. Andrea Fleig of the Queen's Medical Center will be testing the Waixenicin A analogues modulation using the patch clamp technique. Several hydroboration oxidation reactions were used on Waixenicin A, but one hydroboration reaction had no reaction, while the second provided isomers (Figure 1).

An aminooxy sidearm was prepared by subjecting 5-bromo-1-pentene to displacement conditions using N-BOC hydroxylamine. The BOC protecting group was removed using acidic conditions to provide an aminooxy alkene. The aminooxy alkene was condensed with 1-cyclohexenecarboxaldehyde to provide an oxime ether. An electron withdrawing group was introduced to the aminooxy sidearm. 4-chloro-1-butanol was subjected to a Finklestein Reaction to create a more effective leaving group. 1-iodobutanol resulted from the Finklestein Reaction and it underwent the displacement reaction to introduce the BOC protected aminooxy functionality. The sidearm was reacted with the Dess-Martin oxidizing reagent, which produced an aldehyde. An ylide was used to convert the aldehyde into an

ester alkene using the Wittig reaction. Lastly, the BOC protecting group was removed using trifluoro acetic acid, which provided the target sidearm (Figure 3).

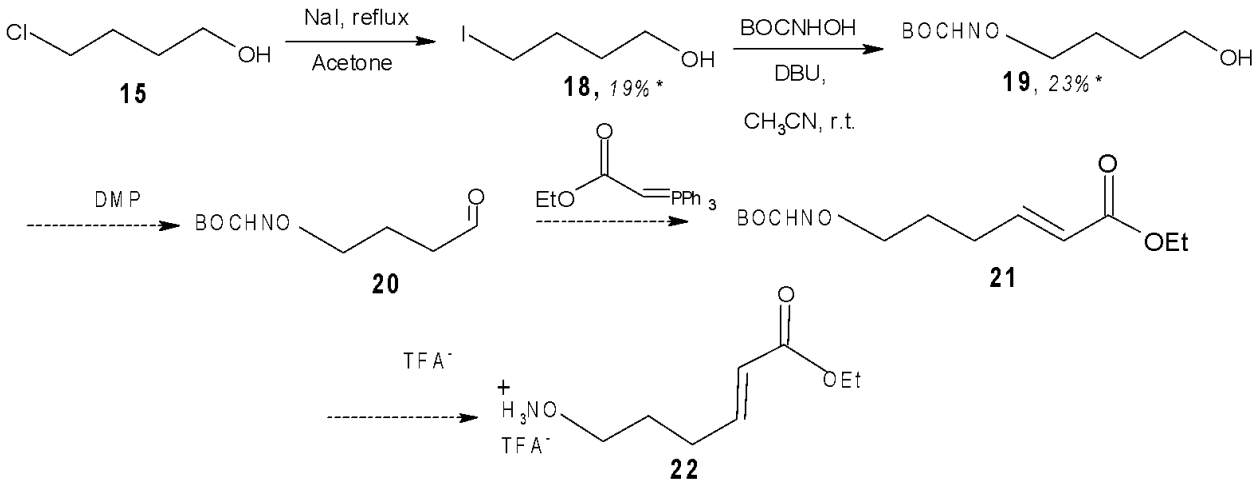

Figure 3. Second attempt of revised side arm for Diels Alder reaction.

Results

^{1}H-NMR and LCMS have provided evidence of a hydroxyl group addition to Waixenicin A. The hydroboration oxidation reaction of Waixenicin A provided a mixture of isomers. The epimers created will be evaluated by Queen's Medical Center for TRPM7 modulation. A sidearm was created using the click-tethered Diels Alder reaction. The creation of sidearms provides pharmaceutical chemists more tools to create nitrogenous heterocyclic compounds.

Discussion

In the future, we will attempt to create more Waixenicin A analogues. The Grignard reagent will be utilized, because it will introduce a bulky nucleophile that could allow us to selectively cleave the primary acetoxy group (Figure 4). Since the sidearm is synthesized, we will attempt to form an oxime ether and then we will strive for our key cycloaddition reaction (Figure 2).

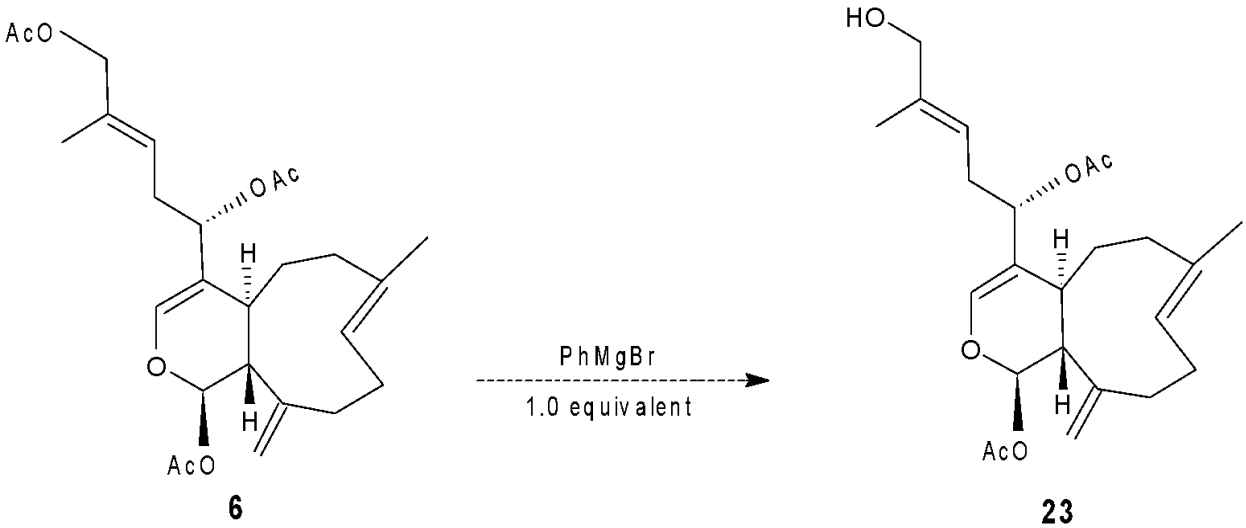

Figure 4. Grignard reaction.

References

1. Zierler, S., Yao, G., Zhang, Z., Kuo, W.C., Porzgen, P., Horgen, F.D., Fleig, A. "Waixenicin A inhibits cell proliferation through magnesium-dependent block of transient receptor potential melatatin 7 (TRPM7) channels." *J. Biol. Chem.* **286**(45), 39328-39335 (2011).
2. Visser, D., Middelbeek, J., van Leeuwen, F. N., & Jalink, K. (2014). Function and regulation of the channel-kinase TRPM7 in health and disease. *European Journal Of Cell Biology*, ***93***(10-12), 455-465. doi:10.1016/j.ejcb.2014.07.001