Study of β-amino Thioamide Sidechains in PPII Mimetic Peptoids
Jack Sharland, 2018

Cell signaling protein-protein interactions govern the way cells behave in living organisms. Specifically the Gorske lab is interested in designing a mimic for the polyproline type 2 (PPII) helix. The lab is interested in studying this motif due its association with a protein binding site, the WW domain. The interaction between these two motifs has been linked with the progression of diseases like cancer and Alzheimer's. If a biological probe could be designed to mimic the PPII helix we could begin to better understand its functions within the cell and how it contributes to unregulated cell growth. In searching for viable biological probe it is important to design a probe that is both biostable and biomimetic. Peptoids are an excellent fit for these criterion. Peptoids are *N*-substituted glycine oligomers and due to their similarity to peptides they have some unique properties. Peptoids are biostable since biological organisms cannot readily digest them with enzymes like the proteases that break down peptides and are highly customizable foldamers, meaning that they can mimic the complex three-dimensional structures of biological compounds like the peptides that make up proteins. Peptoids easy to synthesize. This can be done on solid phase however during the first four weeks of the summer our lab was able to develop a novel synthetic scheme that allowed us to make peptoids on gram scale in over 50% yield for a 12 step synthesis. This makes it simple to generate lots of pure product and thus more easily examine peptoid structure and chemistry. Peptoids are a good choice of biological probe because their secondary structures can be customized depending on the sidechains attached to the backbone. Previous work in the Gorske lab it was demonstrated that thionated sidechains were more effective than carbonyl side chains for imposing the trans-configuration in the peptoid backbone, mimicking a PPII helix, by promoting sidechain-to-backbone n to π* interactions. Upon completion of his honors thesis Tim Boit (class of 2016) was able to synthesize several thiopeptoids trimers but was unable to their study secondary structures due to yield problems.[1] The synthesis was extremely inefficient because sidechains had to be thionated after they were attached to the peptoid backbone due to an intramolecular interaction with sulfur during backbone oligomerization. If the sidechain could be thionated before adherence to the backbone the synthesis would be far more efficient. To solve this problem, I proposed using β-amino thioamides because their chemical structure would provide not only a way around cyclisation but, due to the increased number of stereocenters, rigidity can also be tailored to the task at hand meaning that the strength of the n to π* interaction can be preserved and n to π* interaction pairs can be customized. β-amino thioamides have the potential to interact with backbone carbonyl moieties inaccessible to α-analogues due to their greater length meaning they may adopt previously undescribed peptoid secondary structures. These compounds have never been synthesized before so this research provides opportunities to learn about their unique structure and chemistry. Last summer I researched synthetic pathways that would generate high, enantioselective yields of β-amino amides from β-amino acids. I was able to successfully synthesize a β-amino amide for incorporation into a peptoid trimer in high yield. At the start of this summer I worked with my fellow lab mates to fully develop the solution phase synthetic method for gram scale peptoid synthesis. Using this scheme I was able to make a mixed sidechain peptoid hexamer at an average 72% yield per step. I then applied this synthesis to my research project to build a peptoid incorporating the β-amino amide as the middle sidechain with S-(+)-cyclohexylethylamine as the terminal sidechains. I started from *tert*-butyl-2bromoacetate to

discourage backbone-backbone n to π* interactions at the C-terminus by using an ester cap. After the peptoid was synthesized, I capped the product with a trifluoroacetyl group to make it easier to isolate and discourage backbone-backbone n to π* interactions at the *N*-terminus. I was able to synthesize some product with my first two attempts however the yield was low. This was likely due to difficulties with the addition of the β sidechain to the backbone. I retried the synthesis, this time freebasing the amine and increasing the quantity of base at each amine addition to make the amines more nucleophilic. The modified synthesis gave excellent yield and I ended up with about 0.5g of pure product which was quickly and easily isolated in one step on a Biotage. Product purity was assessed by HPLC and the identity of the product was confirmed by LCMS and NMR. I had enough of this material to run two thionation reactions and a structural study on the unthionated product which is currently in progress. The thionation trial in which the reaction vessel was heated for several days displayed product formation based on analytical HPLC data. In the future I will isolate thionated product by prep-HPLC and finish the structural study and compare the overall secondary structures of both the thionated and un-thionated β-amino amide containing peptoids. Then I will explore the incorporation of different β sidechains and assess the viability of thionation prior to peptoid sidechain addition.

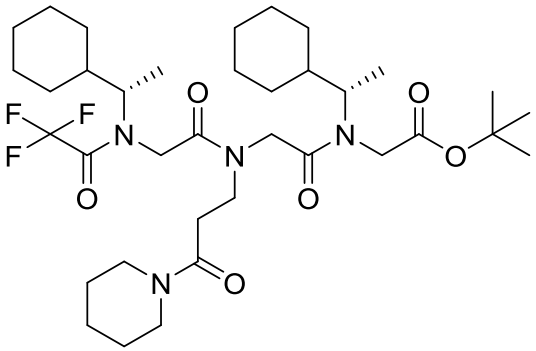

Figure 1: Unthionated version of successfully synthesized β-amino amide containing peptoid.

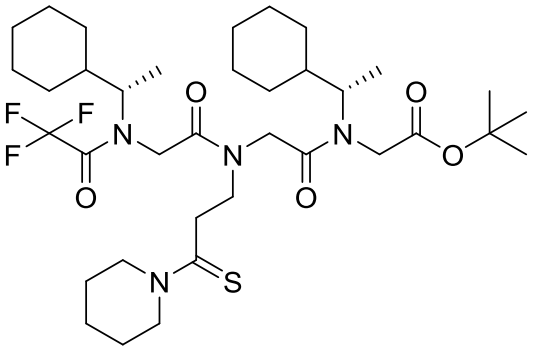

Figure 2: Thionated version of β-amino amide containing peptoid.

Faculty Mentor: Benjamin Gorske

Funded by the Kufe Family Research Fellowship

References: 1. Boit, Tim "Study of Amide and Thioamide Side Chain Influence on cis/trans Isomerism of Peptoid Backbone Amides", Bowdoin College 2016.