Electronic Control of Peptoid Structure for Binding to Proteins

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The rise of complex diseases, antibiotic resistance, and rapidly evolving pathogens furthers the need for new medicines.[1,2] Cancer and Alzheimer's disease are some of the leading causes of death worldwide, and their complexities have greatly slowed the development of drug treatments. These diseases, although different in their symptoms, manifest through dysregulated protein-protein interactions in signaling pathways, such as the Hippo pathway. This specific pathway has not been studied extensively but is suspected to be involved in both diseases. The Hippo pathway involves proteins that contain an unusually high number of specific binding motifs called WW domains, that when bound to, facilitate interactions in the pathway.[3] Proper binding relies in part on the extremely specific shape of the protein, reflected in the binding interaction's "key-and-lock" model. Successful binding can function as an "on/off" switch for a particular protein, allowing a pathway to continue to propagate. This makes the WW domain and the molecules that bind to it subjects of interest to learn more about the Hippo pathway and related diseases.

The WW domain recognizes and binds to a specific shape of molecule, the polyproline type II (PPII) helix.[4] The PPII helix gets its shape by having all *trans*-amide bonds, allowing for shape complementarity with the WW domain and successful binding.[4,5] An exact copy of the PPII helix motif would not be useful to prod signaling pathways because they are typically destroyed by enzymes before they can bind to WW domains. A class of biomolecules called peptoids, which are very structurally and chemically similar to peptides, could be a solution to this problem. Peptoids are more biostable *in vivo* and are easily customizable, both in shape and in chemical properties. This project aimed to synthesize a peptide mimic of the PPII helix to be used to further understand the WW domain and the Hippo pathway.

I aimed to synthesize a four-residue (tetramer) peptoid containing sidechains that can be customized to favor the *trans*-amide bond conformation needed to achieve a PPII helix mimic. Alanine (an amino acid, a peptide building block) derivatives were synthesized as the sidechains/residues and analyzed via liquid chromatography-mass spectroscopy (LC-CMS) and hydrogen nuclear magnetic resonance (^{1}H NMR) to confirm a successful synthesis.

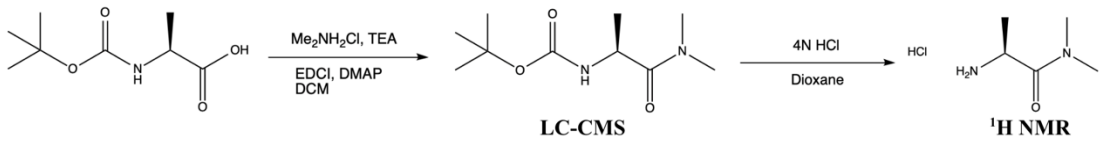

Figure 1. Synthetic and accompanying analytical processes to make the alanine derivative sidechain (rightmost).

The alanine residues were used in peptoid synthesis (per the Gorske Lab) to create a peptoid tetramer. This product is currently undergoing analysis via LC-CMS to confirm its presence. Alanine derivatives were used as sidechains for their relative ease to be selectively modified by a process called thionation, which is believed to increase the likelihood or "preference" for the peptoid to adopt the desired *trans*-amide backbone conformation. In future work, I hope to confirm the synthesis of the alanine tetramer, purify the product, thionate the peptoid, and ultimately analyze the conformations of the tetramers via 2D NMR methods.

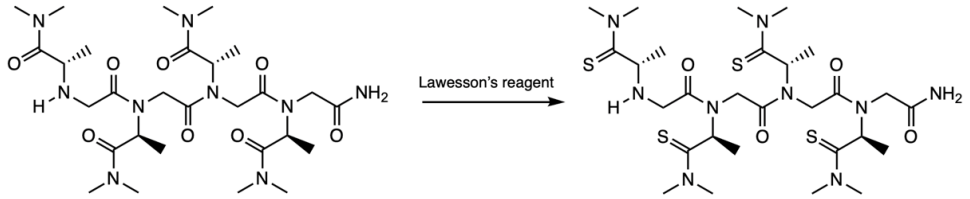

Figure 2. Peptoid tetramer containing alanine sidechains (left) and product after thionation (right).

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References

(1) *Alzheimer's Disease Fact Sheet*. National Institute on Aging. **2023**.

(2) *Cancer Statistics.* National Cancer Institute. **2023**.

(3) Pan, D., The Hippo Signaling Pathway in Development and Cancer. *Developmental Cell* **2010,** *19* (4), 491-505.

(4) Macias, M. J.; Wiesner, S.; Sudol, M., WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. *FEBS Letters* **2002,** *513* (1), 30-37.

(5) Woody, R. W., Circular Dichroism Spectrum of Peptides in the Poly(Pro)II Conformation. *Journal of the American Chemical Society* **2009,** *131* (23), 8234-8245.