The Synthesis of a Thionated Peptoid to Adopt a PPII Helix in Hopes of Binding the WW Domain

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INTRODUCTION:

Any given American citizen is effectively guaranteed to know someone who has been personally affected by either Alzheimer's disease or cancer. That statement makes sense considering that in the United States alone, 1 in 3 seniors will be affected by Alzheimer's or another dementia when they pass, and in 2018, an estimated 1,735,350 newly diagnosed cancer cases will arise, along with 609,640 cancer deaths[1,2]. These brutal diseases are thought to arise from aberrant cell signalling. In Alzheimer's, the primary cause stems from plaque accretion, a process defined by build-up of amyloid β-peptide oligomers[3]. Normally, these peptides occur as a consequence of healthy cell signalling, yet they can prove lethal when the pathway goes awry. Similarly, the hallmark of cancer stands as uncontrolled cell division, another example of a healthy cellular process, moderated by cell signaling, going horribly awry[4].

Considering that these diseases, with the tremendous scientific challenges and emotional tragedy they pose, fundamentally stem from cell signaling, research on how these signal transduction pathways operate in healthy cells—and how, where, and why they go wrong when diseased—remains imperative. One methodology in particular, known as selective interference, intrigues the biomimetic chemist in particular. Biomimetic chemistry seeks to understand the fundamental mechanisms and composition of the chemistry behind Nature's macromolecules (i.e. polypeptides, nucleic acids, lipids, and fatty acids), and to play her at her own game: to mimic biomolecules. To this end, biomimetic chemistry seeks, in part, to mimic protein structure and the consequent function that structure generates, and use these mimics to probe cellular pathways through selective interference. This, of course, stands to be easier said than done.

One class of protein mimic (or "peptidomimetic") in particular has shown significant success in past applications[6], namely the peptoid. Peptoids consist of amino acids wherein the typical side-chain group has been moved from the backbone alpha carbon to the nitrogen (hence poly-N-substituted glycines). Like many peptidomimetics, these subunits are not constrained by the side-chains Nature provided us; indeed, among the quintessential features of peptoids stands their ease of synthesis, low immunogenicity[7], and vast array of combinatorial possibilities, including man-made and natural side-chains alike[8]. Furthermore, the noncovalent interactions that govern the folding mechanics giving rise to secondary structure (and, again, consequent function), have been increasingly well characterized in peptoids. Examples of these noncovalent interactions include the hydrophobic effect, salt bridges, and in peptoids especially, hydrogen bonding, steric considerations, and the $n \rightarrow \pi^*$ interaction.

The $n \rightarrow \pi^*$ interaction, in particular, has proven to be of particular use in the discovery of rationally-designed foldamers. As covalent chemistry fundamentally entails orbital overlap between the highest occupied molecular orbital of one species and the lowest unoccupied molecular orbital of the other (a so-called HOMO-LUMO interaction), the $n \rightarrow \pi^*$ interaction entails a less-severe HOMO-LUMO overlap between a π^* orbital and a lone pair orbital within the same molecule. Indeed, as has been shown in peptides[9] and peptoids[10] alike, the quantum mechanical nature of these surprisingly influential interactions has been studied quite well. To demonstrate the quantum effects (as opposed to electrostatics) yielding this interaction, the carbonyl oxygen (the source of the "n" or lone pair) was replaced by sulfur, a less polarizing but more polarizable species in peptides[9]. The interaction grew stronger under these conditions, proving that the effects were indeed quantum mechanical in nature.

Later, the same technique was employed on peptoids, this time to purposefully increase the $n \rightarrow \pi^*$ interaction for foldameric reasons. Indeed, a major structural design challenge in peptoids has been controlling the cis/trans ratio of the backbone amide bond; the cis conformation yields a polyproline type I helix (PPI), and the trans conformation yields a PPII helix—both very common protein binding motifs. Depending on the target binding substrate, one helix may promote binding, while one may not. Similarly, depending on the actual side-chain structure, sterics, the $n \rightarrow \pi^*$ interaction, and hydrogen bonding may combine to promote one helix over another. By thionating peptoids, or replacing the carbonyl oxygens with sulfurs, the strength of the $n \rightarrow \pi^*$ interaction will increase, promoting one helix over another.

The target of my research this semester has been to develop a peptoid capable of binding to the WW binding domain, a well-researched domain with direct relevance to cancer and Alzheimer's alike[3,11]. Seeing as the WW domain preferentially binds PPII helices, which are three-residue-per-turn and left-handed in nature, the goal of my research centered on synthesizing a peptoid hypothesized to form a PPII helix when thionated[12]. As such, I sought to synthesize a peptoid trimer with a (*S*)-2-amino-*N*,*N*-dimethylpropanamide side group. The first phase of the synthesis consisted of fabricating the actual amine side-chain, or the *α–aminoamide sidechain*. Next, the amine side-chain was added via an S_N2 mechanism to *tert*-Butyl bromoacetate, to cap the oligomer. This product was then bromo acetylated to create an S_N2 electrophilic site for the second amination. The amination and bromo acetylation steps were then repeated sequentially to create a peptoid trimer. The trimer will later be protected with trifluoric acetic anhydride, and thionated with Lawesson's reagent. The product compositions and purities

were inferred by H^1 NMR spectroscopy, and LC-MS in the later stages. The analysis of the synthesized peptoid is still underway, and likely will be completed sometime this winter.

EXPERIMENTAL METHODS:

All reagents were 99% purity, ACS-grade or HPLC-grade, and were obtained from Sigma-Aldrich Chemicals (St. Louis, MO). Solvents were purchased from Fisher Scientific (Fair Lawn, NJ) and Sigma Aldrich and were not subjected to distillation. All products were stored in airtight conditions at room temperature.

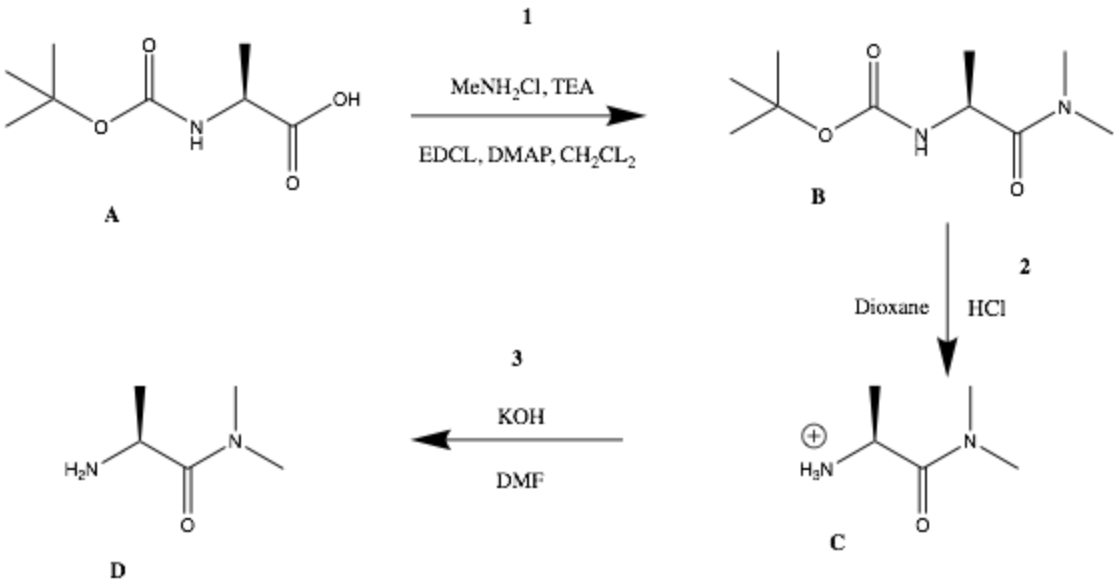
Instrumentation:

Nuclear Magnetic Resonance Spectroscopy (NMR). A Bruker Spectrospin 400 MHz Ultrashield Spectrometer (Bruker, Billerica, MA) was used for all H^1 nuclear magnetic resonance (NMR) spectra. The solvent used was deuterated chloroform ($CDCL_3$). Topsin (Bruker) software was used to analyze the spectra. The chemical shifts for the spectra were recorded in parts per million (ppm) units; tetramethylsilane (TMS) was used as the 0.0 ppm reference, and $CDCL_3$ as the 7.26 ppm reference.

(LCMS/Biotage have yet to be employed at the time of this report, but will be utilized in further analysis of the peptoid)

PROCEDURES:

Part I: ***Synthesis of α–aminoamide sidechain***



Scheme 1. Synthesis of α–aminoamide side chain in three steps: amination, deprotection, and neutralization

Step 1. Amination of A into B

Protocol adapted from T.Boit, Honors Thesis (pg. 19, 2016)

Boc-Ala-OH (1 eq) was dissolved in dichloromethane and stirred at room temperature as EDCl (1.2 eq), DMAP (0.15 eq), and dimethylamine hydrochloride (2.5 eq) were added. After dissolution, a 3M solution of triethylamine (3.7 eq) in dichloromethane was added dropwise to the stirring solution over 30 minutes. During the addition of TEA, the reaction flask was vented with a needle. The reaction was stirred at three days at room temperature. The reaction mixture was washed with 10% citric acid and saturated aqueous sodium bicarbonate. The organic layer was dried over anhydrous magnesium sulfate and then filtered. The solvent was removed in vacuo. H^1 NMR was used to confirm identity.

Step 2. Deprotection of B into C

Protocol adapted from S. Conwell, Notebook (pg 112, Book I)

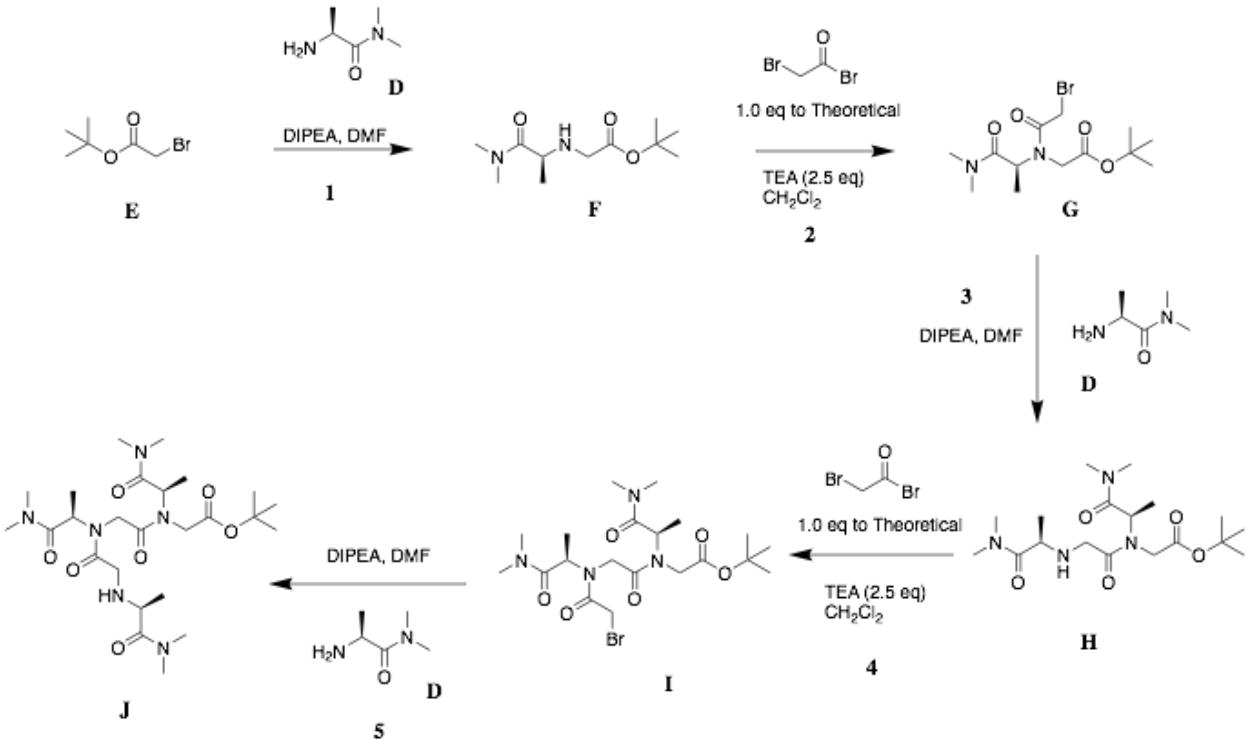
The reaction was conducted in a 50 mL recovery flask with stirrer. The product from the previous step (0.01023 mol **B**) was added to 15 mL of HCl in dry dioxane. The reaction was left to stir for 2 hours, and was quenched by rotary evaporation. H^1 NMR was used to confirm identity.

Step 3. Neutralization of C into D

Protocol adapted from J. Sharland, Honors Thesis (pg 24, 2018).

5 mL of DMF was used to dissolve the deprotected amine (0.01234 mol **C**). This solution was transferred to a 25 mL recovery flask with stirrer. KOH (0.95 eq) was added to the amine. The precipitate was removed with vacuum filtration, and dried with molecular sieves.

Part II: ***Sequential and Repeated Bromo Acetylation and Amination Steps***



Scheme 2. Synthesis of peptoid trimer in five steps: amination 1, bromo acetylation 1, amination 2, bromo acetylation 2, amination 3.

Step 1. Amination Phase 1 of E into F.

In a 20 mL vial with stirrer, the amine (0.2 grams of **D**) was dissolved in dry DMF to give a 1.5 M solution. The vial was capped with an inverted 24.40 septum, and the vial was flushed with nitrogen gas via needles. In a separate vial, the bromide (1 eq) was dissolved in the same amount of dry DMF aforementioned, after which is was added dropwise to the stirring solution of the amine via syringe. A small amount of dry DMF was used to rinse the vial and was subsequently also added. The DIPEA (1 eq) was added dropwise via syringe. After 23 hours, the reaction mixture was transferred to a separatory funnel, and 35 mL of ethyl acetate were added. The reaction vial was rinsed with 5 mL of ethyl acetate that was also transferred. The organic layer was washed with 1M NaOH (3 x 25 mL). The organic layer was dried over sodium sulfate and filtered into a tared 100 mL recovery flask. The sodium sulfate was rinsed with 5 mL of ethyl acetate, also added. The solvent was removed via rotary evaporation. H^1 NMR was used to confirm identity.

Step 2. Bromo Acetylation Phase 1 of F into G.

Protocol taken directly from S. Conwell, Honors Thesis (pg. 17, 2018)

To prepare for the addition of the second monomer, bromoacetyl bromide (1 eq.) was dissolved in 3 mL dichloromethane in a 25 mL recovery flask. While stirring, the flask was capped, cooled in an ice bath, and flushed with nitrogen for 5 minutes. The nitrogen needle remained installed throughout the reaction, but the needle for flushing was removed after 5 minutes. F was dissolved in 3 mL dichloromethane and added dropwise. TEA (2.5 eq) was added dropwise

while stirring. The reaction was run at 0°C for 30 minutes after addition of TEA. The solution was diluted with 10 mL dichloromethane and washed twice 10 mL sodium bicarbonate. The organic and aqueous layers were separated, and the organic layer was dried over anhydrous magnesium sulfate. The solvent was removed in vacuo and the produced assed via H^1 NMR.

Step 3. Amination Phase 2 of G into H.

Same as Scheme 2 Step 1, except 1.5 eq of **D** relative to the theoretical yield of the last step were used; 1 eq of DIPEA, and all of **G**. The reaction ran for 47 hours. Brine was required during aqueous workup.

Step 4. Bromo Acetylation Phase 2 of H into I.

Same as Scheme 2 Step 2, except theoretical yield of previous step served as basis for molar equivalents. Reaction ran for 30 minutes.

Step 5. Amination Phase 3 of I into J.

Same as Scheme 2 Step 1 and 3, except 1.5 eq of **D** relative to the theoretical yield of the last step were used; 1 eq of DIPEA, and all of **G**. The reaction ran for 24 hours. Brine was required during aqueous workup.

DISCUSSION AND FUTURE WORK:

While the thionation steps and secondary structural analysis have yet to be completed at the time of this report, the yield data and H^1 NMR are promising. For the synthesis of the α–aminoamide side chain (Product **D**, Schemes 1 and 2), three separate trials were required (the first had too low a yield, the second was lost in a vacuum filtration accident). The trial that ultimately made its way into a peptoid had a 65% yield with adequate purity determined via H^1 NMR. The yield data is difficult to track for the Scheme 2 reactions, as solvents accumulated through incomplete

rotary evaporation (as was expected), but the data suggest approximate 65% yields for the amination steps, and approximate 40% yields for the bromo acetylation steps. The ultimate mass of peptoid produced (product **J**) is 0.37 grams of 0.24 expected grams, again due to solvents. The immediate future work for this project entails the remaining thionation steps, i.e. trifluoric acetic anhydride protection and thionated via Lawesson's reagent. Upon completion of the thiopeptoid synthesis, the secondary structure shall be analyzed computationally. From there, we hope to be able to ascertain the folding tendencies of this peptoid — especially if it takes the PPII helix form, as that structure binds the WW binding domain, which is the ultimate target of our biomimetic probe.

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