Design and Synthesis of Thiopeptoids as Biological Probes for Protein-Protein Interactions

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A key strategy for studying diseases involving protein-protein interactions, such as Alzheimer’s disease, is through the use of biological probes that mimic natural ligands. While this field of peptidomimetics has traditionally involved synthetic peptides, this strategy has some practical drawbacks when applied in vivo, as human proteolytic enzymes readily degrade peptides.

Peptoids, which are structurally similar to peptides, present a solution to this problem. Nonetheless, their structural differences from peptides allow for greater conformational flexibility, which poses a challenge to the design and use of peptoids, because if they are to serve as effective biological probes or therapeutics, they must fold predictably and adopt a specific structure in order to interact with their target protein.

Thus, research has focused on how to promote greater conformational control, one method being the use of thiopetoids, which are essentially peptoids with the carbonyl oxygen replaced with sulfur. This substitution of oxygen with sulfur stabilizes peptoid conformation by strengthening specific noncovalent interactions. Nevertheless, these interactions can be further strengthened, and thus potentially allow for greater structural stability, by synthesizing mixed peptoid-thiopeptoid oligomers.

The purpose of this study was to investigate methods to selectively thionate peptoids in order to produce these mixed peptoid-thiopeptoid oligomers. As thionation, or the replacement of oxygen by sulfur, generally does not occur in interior residues adjacent to bulky side chains, this study focused on whether steric interactions play a role in regulating this process. This hypothesis is significant because if sterics—the spatial arrangement of atoms in a molecule—do play a role in determining where thionation occurs, then using side chains of varying bulkiness could be a way to achieve selective thionation, thereby facilitating the synthesis of mixed peptoid-thiopeptoid oligomers.

To test this hypothesis, peptoid trimers and tetramers with side chains of varying bulkiness were synthesized using the solid-phase submonomer protocol developed by Zuckerman et. al, 1992. In this procedure, a rink amide resin with a protecting group is first deprotected, followed by a series of alternating acylations and aminations until a peptoid of desired length is attained. As side chains are added to the peptoid during amination, this step allows for the structural diversity of these oligomers. The peptoids were then cleaved from the resin with a strong acid and thionated in solution using Lawesson’s reagent.

Following thionation, the oligomers were analyzed using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). The former identified and purified the samples, while the latter generated the masses of oligomer fragments, from which the location of where thionation occurred could be determined.

While LC-MS results are still pending, this study has optimized analysis techniques and determined thiopetoid-peptoid sensitivity to specific solvents. Future work would involve continuing the structural characterization of the oligomers using LC-MS, as well as other techniques, such as X-ray crystallography. Moreover, a long-term goal for this study would be the assessment of the binding properties of these thiopetoid-peptoid oligomers to native proteins.
Figure 1. Illustration of a ligand binding to the WW domain of Fe65, a protein involved in Alzheimer’s disease. Reproduced from Meiyappan et. al, 2007.

Figure 2. (From left to right) Chemical structures of a peptide, peptoid, and thiopeptoid. The R group represents the variable side chain, which allows for the structural diversity of these molecules.

Figure 3. Solid-phase submonomer protocol (Zuckerman et. al, 1992) for the synthesis of peptoids, followed by solution-phase thionation. The arrow between (1) and (2) represents an acylation, while the arrow between (2) and (3) represents an amination.
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References


