CHEM 551 Literature Summary Guide and Examples

Using the questions below as a guide, write a summary of each assigned paper.

Short Project Description: A 2 – 3 sentence description of your project. An example is shown below:

> "The project is directed toward the development of a novel approach to the synthesis of isoxazolopyridines. This approach is based on the intramolecular cyclization of appropriately activated 3-acylpyridine N-oxide oximes."

Summary: Each reference summary should address the following:

1. Citation/Reference – Include the citation in ACS format (Author, F.; Author, S. Title of Paper. *Journal Abbreviation*, **Year**, *Volume*, Pages)
    - An exception is the journal *Biochemistry*. If that is the major journal in your field, consult with your mentor for the correct citation formatting.
2. *Question* – What specific question(s) do the authors wish to answer?
3. *Significance* – Why are the research questions important to answer?
4. *Approach* – What experiments did the authors carry out to answer their research question? – Did they encounter any problems? How did they solve them?
5. *Results* – What data did the authors generate as a result of their experiments?
6. *Conclusions* – What were the major findings?

Several examples are included on the following pages. The first example came from a student in my (J. Hanna) research group several years ago, the other two examples were adapted from the literature (Schepmann, H. G.; Hughes, L. A. *J. Chem. Educ.* **2006** *83*, 1024)

1. Poręba, K.; Wietrzyk, J. The Synthesis of 3,5,6,7-Tetrasubstituted Isoxazolo[4,5-*b*]Pyiridines and an Evaluation of Their *In Vitro* Antiproliferative Activity. *Adv. Clin. Exp. Med.* **2012,** *21*, 563-571.

Isoxazolopyridines are important because they have been shown to exhibit several interesting biological properties, including anticancer, antibacterial, antiviral, analgesic, and anticonvulsant effects, and only a few syntheses of isoxazolo[4,5-*b*]pyridines have been reported in the literature. In this paper, the authors synthesized a series of novel isoxazolo[4,5-*b*]pyridines via a modified Friedländer condensation of 4-amino-5-benzoylisoxazole-3-carboxamide with several active methylene compounds, using catalytic amounts of either zinc or indium salts.

They investigated several different conditions, but the reaction proceeded smoothly only when $ZnCl_2$ or $In(OTf)_3$ catalysts were employed in the absence of solvent using either conventional or microwave heating. Under this protocol, the authors reported yields from 63 – 86%, with microwave heating giving somewhat better yields than conventional heating. Of the compounds prepared using this method, the ones shown in Figure 1 exhibited the highest level of antiproliferative activity against a range of cancer cell lines.

Figure 1. Antiproliferative isoxazolo[4,5-*b*]pyridines.

2. Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergström, M.; Savitcheva, I.; Huang, G. F.; Estrada, S.; Ausén, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Långström, B. Imaging Brain Amyloid in Alzheimer's Disease with Pittsburgh Compound-B. *Ann. Neurol.* **2004**, *55*, 306-319.

Until recently the detection of amyloid plaques within the brain thought to be linked to Alzheimer's Disease (AD), was not possible in vivo. A new radioactive probe, N-methyl-[11C]2-(4-methylaminohenyl)-6-hydroxybenzothiazole, termed Pittsburgh compound B, (PIB) has been proven effective in detecting amyloid plaques in sixteen AD patients via positron emission tomography (PET) imaging. PIB, a modification of a previously studied radiotracer Thioflavin-T, was chosen because of its ability to cross the blood-brain barrier, due to its small molecular size and lipophilicity. This compound was also proven to have no toxic effects.

In this study sixteen AD patients and nine subjects not diagnosed with AD were administered PIB intravenously. PET scans were performed using a Siemens ECAT HR+ camera. PIB retention, was significant within the AD patients, within the frontal cortex, parietal, temporal, and occipital cortex, and the striatum. The healthy group of subjects showed significantly lower PIB retention in these regions of the brain. However, significant PIB retention, similar to that found in the AD patients, was found in the oldest subject of the healthy group. This result raises the question as to whether amyloid formation is part of normal aging, and is unrelated to whether one will develop AD.

Within the AD subjects the region of highest PIB uptake was the frontal cortex, which also has lower cerebral blood flow in comparison with other the regions examined in this study. This finding was not consistent with in vitro studies, and thus raises the question of whether cerebral blood flow has an effect on PIB uptake or if amyloid plaques are actually found in more abundance in this area. PIB will potentially assist in detecting Alzheimer's before its onset, and will also be able to aid in evaluating antiamyloid treatments.

3. Cuthbertson, A.; Indrevoll, B. Regioselective Formation, Using Orthogonal Cysteine Protection, of an α-Conotoxin Dimer Peptide Containing Four Disulfide Bonds. *Org. Lett*. **2003**, *5*, 2955-2957.

Peptides are amino acid polymers, and proteins are peptides of more than 75 amino acids. Proteins each have a unique shape, and these shapes allow for a high specificity in protein/ substrate interaction. One of the features that proteins use to maintain their shapes is disulfide bond "bridges," which are formed between different cysteine residues on the amino acid polymer. A major challenge that chemists face when synthesizing proteins is the proper pairing of cysteine residues. If the wrong residues form a disulfide bond, the protein folds improperly, and it does not react the same way as would its natural counterpart. One solution is for protecting groups to be added to the thiol side chains on paired cysteines, which can later be removed in order to allow proper disulfide bond formation. As the complexity of the molecule increases, it becomes more difficult to ensure the selective removal of protecting groups and proper pairing of cysteines. To this point, methods for synthesis of peptides with two and three disulfide bonds have been developed. Peptides with four disulfide bonds, however, remain a significant challenge.

This article presented a method for the synthesis of a peptide with four disulfide bonds. Proper folding of the peptide was orchestrated by use of protecting groups on orthogonal, or alternating, cysteine residues. It also introduced a new folding procedure that minimized loss of peptide due to sample transfer, thus saving time, effort, sample, and money.

Using an α-conotoxin (a toxin produced by marine snails of the genus *Conus*) as a blueprint for the monomer, a dimer was assembled. The monomers were separated by a glycine-lysine-glycine linkage. The entire peptide contained eight cysteine amino acid residues.

Residues 1 and 3 were unprotected, residues 2 and 4 were protected with a *t*-butyl (*t*-Bu) protecting group, residues 5 and 7 were protected with acetamidomethyl (Acm) protecting group, and residues 6 and 8 were protected with a methylbenzyl (MeBnz) protecting group.

The novel folding procedure was then introduced. The peptide was dissolved in DMSO (dimethylsulfoxide) and water. This allowed a disulfide bond to form between the unprotected residues. In the same "pot," acetic acid and then iodine were added, which removed the Acm protecting groups, allowing residues 6 and 8 to form a disulfide bond. This was confirmed by LC/MS (Liquid Chromatography/Mass Spectrometry). Purification was performed using HPLC (High Performance Liquid Chromatography). In a second "pot," DMSO/TFA (dimethyl-sulfoxide/trifluoroacetic acid) oxidation was utilized for removal of the remaining protecting groups and subsequent disulfide bond formation. Under this, the *t*-Butyl protecting group cleaved from the cysteine at room temperature, forming a bond between residues 2 and 4, while the MeBzl protecting group cleaved from the cysteine at higher temperatures (45 ^{o}C), forming a bond between residues 6 and 8. LC/MS analysis confirmed the product, which was subsequently purified via HPLC. This two-pot method not only reduced the number of steps required for cleavage and disulfide bond formation, but also reduced the loss of peptide due to transfer of sample from one container to another.

One potential problem involved possible reshuffling of disulfide bonds between residues. In order to confirm whether or not this had happened, the peptide was exposed to trypsin. Trypsin is an enzyme that cleaves peptides between lysine and the next amino acid in a peptide chain. If incorrect disulfide bond formation had occurred across the glycine-lysine-glycine linkage, the peptide could not then be cleaved. Cleavage did occur, which was confirmed via HPLC. An impurity was detected during the HPLC analysis. This was found to be caused by cleavage of a portion of the MeBzl at room temperature, with incorrect disulfide bond formation. Better optimization of reaction conditions will be required to prevent this in the future, although the article did not state exactly what optimizations would be used.

The synthesis procedures proved to be effective, although it was unclear as to their effectiveness when utilized for more complex structures, such as cyclic peptides.