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A Better Understanding of Protein Structure and Function by the Synthesis and Incorporation of Selenium- and Tellurium Containing Tryptophan Analogs

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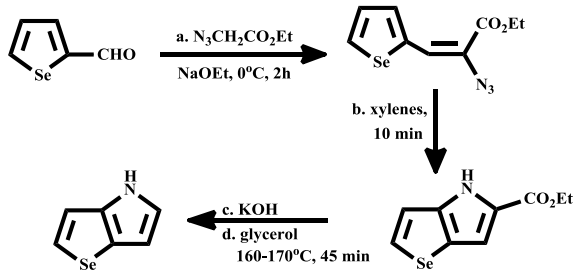
Abstract: Unnatural heavy metal-containing amino acid analogs have shown to be very important in the analysis of protein structure, using methods such as X-ray crystallography, mass spectroscopy, and NMR spectroscopy. Synthesis and incorporation of selenium-containing methionine analogs has already been shown in the literature however with some drawbacks due to toxicity to host organisms. Thus synthesis of heavy metal tryptophan analogs should prove to be more effective since the amino acid tryptophan is naturally less abundant in many proteins. For example, bio-incorporation of β-seleno[3,2-*b*]pyrrolyl-L-alanine ([4,5]SeTrp) and β-selenolo[2,3-*b*]pyrrolyl-L-alanine ([6,7]SeTrp) has been shown in the following proteins without structural or catalytic perturbations: human annexin V, barstar, and dihydrofolate reductase. The reported synthesis of these Se-containing analogs is currently not efficient for commercial purposes. Thus a more efficient, concise, high-yield synthesis of selenotryptophan, as well as the corresponding, tellurotryptophan, will be necessary for wide spread use of these unnatural amino acid analogs. This research will highlight our progress towards a synthetic route of both [6,7]SeTrp and [6,7]TeTrp, which ultimately will be used to study the effect on the catalytic activity of Lignin Peroxidase (LiP).

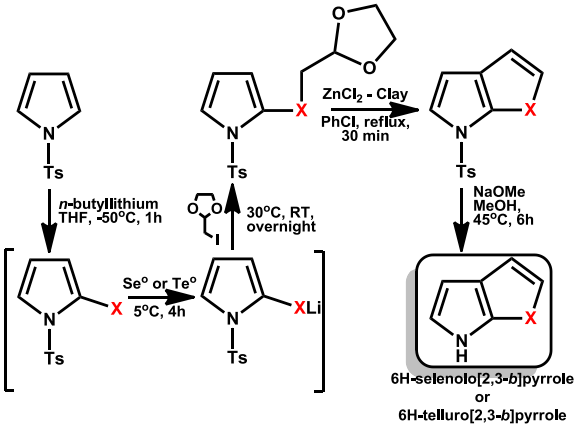
Macromolecular structures such as enzymes and proteins have continued to be uncovered by the techniques of x-ray scattering with anomalous dispersion in the past decade. This has been made possible by the incorporation of heavy atom amino acid analogs into the protein of interest. So far the most predominant heavy atom amino acid analog has been selenomethionine. However, tryptophan analogs have also been created and incorporated into proteins as well. For example [6,7]-selenotryptophan has been incorporated into dihydrofolate reductase.[1] This has revealed that a toolbox of multiple heavy atom amino acid analogs must be discovered. Syntheses of such amino acid analogs must also be progressively more efficient and effective. Thus in the case where one amino acid analog cannot incorporate without disrupting the protein structure another might be able to. We report an improved and more effective synthesis of [6,7]-selenotryptophan. Also we have proposed a synthetic route for tellurotryptophan.

These heavy atom amino acid analogs increase the effectiveness of x-ray crystallography by solving the "phase problem." When an x-ray diffracts through the crystal protein it has an amplitude and a phase. The phase cannot be determined without the position of a heavy atom which can be acquired from anomalous dispersion. Once the amplitude and phase are both determined they can be used to form an electron density map which then can be interpreted to create the protein model. This can only be completed however if a heavy atom amino acid analog can be successfully synthesized and incorporated into the protein of interest. This incorporation requires a bacterium such *Escherichia coli* to utilize the amino acid analog instead of the natural amino acid. Thus the bacterium must be able to grow in the presence of the analog alone. This is not always successful since such amino acid analogs can be toxic or ineffective once incorporated into the protein. Tryptophan has a lower frequency of occurrence in proteins than does methionine thus it makes sense that a tryptophan analog would be more efficient in bio-incorporation and less toxic to the organism[3]. The earliest synthesis of 6-(4H-selenolo[3,2-b]pyrrolyl)-L-Alanine was the Paulmier synthesis which was low yielding and did not allow for ^{77}Se isotope labeling. That synthesis has been improved (Scheme 1) but still does not allow for isotope labeling and is low yielding. The final step of this synthesis includes a decarboxylation step which must be done at high temperatures and thus decomposes the fragile final pyrrole product.



Scheme 1: Phillips Modified Paulmier Synthesis[2]

The syntheses (Scheme 2) proposed in this study for the selenophene and tellurophene pyrroles has less harsh conditions and are higher yielding.



Scheme 2: LANL synthesis of selenophene and tellurophene pyrroles. X= Se or Te

The synthesis is comprised of three steps. The Tosyl-Pyrrole is metallated and alkylated by using n-butyllithium and then the corresponding heavy atom. Then annulation is performed with a Lewis acid. Finally the Tosyl group is cleaved using methoxide in methanol.

Materials and Methods

Metallation/Alkylation: Tosyl pyrrole was added to a round bottom flask with tetrahydrofuran as the solvent. Flask was purged with argon and fitted with argon balloon. Reaction was brought to -78°C in a

dry ice bath with acetone. N-butyllithium was carefully injected slowly via syringe dropwise. Reaction stirred for one hour at -78°C then let warm to -60°C. Selenium or tellurium were quickly added and the reaction was re-purged with argon. The reaction was then left to stir for four hours at -60°C. Then 2-iodomethyl-[1,3]dioxolane was injected via syringe dropwise. Reaction was then left to stir overnight and warm up to room temperature.

Annulation: Ts-Selenide was added to a round bottom flask along with 1,4 dioxane. Reaction was purged with argon and brought to reflux with a condenser fitted with an argon balloon around 110°C. After one hour amberlyst-15 was added to the reaction. Then left to stir overnight at 110°C. Annulation was also tried with $ZnCl_2$ and Chlorobenzene however it did not afford annulated product.

Tosyl-Group Cleavage: Ts-Selenophene was added to a round bottom flask with methoxide in methanol solution. Reaction was then warmed to 45°C and fitted with argon balloon. It was then left to stir for 6 hours.

Results

Thin layer chromatography

Reaction progresses were all monitored by TLC. Selenide Rf = 0.3, Ts-Selenophene Rf = 0.4, Selenophene Rf = 0.34. TLC was run in a 20:80 Ethyl Acetate: Hexanes solution.

Characterization by NMR

Selenide: ^{1}H-NMR (300MHz, $CDCL_3$): 5.1 (t 1H), 3.3 (m 4H), 2.9 (d 2H).

Ts-Selenophene: ^{1}H-NMR (300MHz, $CDCL_3$): 7.9 (d 2H), 7.3 (m 2H), 1.9 (s 3H).

Selenophene: ^{1}H-NMR (300MHz, $CDCL_3$): 7.42 (d 1H), 7.18 (q 1H), 7.04 (t 1H), 5.35 (m 1H)

Telluride: ^{1}H-NMR (300MHz, $CDCL_3$): 5.1 (t 1H), 3.3 (m 4H), 2.9 (d 2H).

Ts-Tellurophene: ^{1}H-NMR (300MHz, $CDCL_3$): 7.8 (d 2H), 7.3 (m 2H), 2.3 (s 3H).

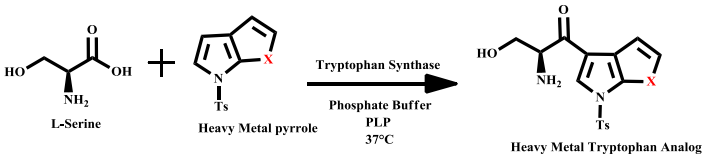
The yields for each step of the synthesis is shown below in Table 1. The tosyl group cleavage was not able to be performed with the tellurium compound.

Heavy Metal	Metallation/Alkylation	Annulation ZnCl2/Clay	Annulation Amberlyst-15	Ts-Cleavage
Selenium	82%	63%	54%	80%
Tellurium	60%	55%	N\A	N\A

Table 1: Yields for synthesis by step and heavy atom

Discussion

The synthetic strategy outlined in Scheme 2 successfully produces the selenophene pyrrole required for serine side chain coupling (Scheme 3) to produce selenotryptophan.



Scheme 3: Serine side chain coupling X = Se or Te

Our synthesis has shown to be higher yielding and less harsh allowing for a more effective production of such heavy atom amino acid analogs. Thus we have added to the toolbox of unnatural amino acid analogs for the determination of protein structure and

function. Also since our synthesis allows for the isotope labeling of the selenium and tellurium atom, NMR spectroscopy can be performed on the respective proteins that incorporate these amino acids. Also since the isotopes of selenium and tellurium are more polarizable they may be able to enhance the catalytic activity of the protein they are incorporated into. We have also come to find that selenotryptophan is more stable than selenomethionine thus increasing shelf life. The study of macromolecular structures such as proteins will continue to evolve and thus by the incorporation of these heavy atom amino acid analogs more and more protein structures will be determined and functions maybe even enhanced.

Acknowledgements

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References

1. Boles, J.O.; Henderson, J.; Hatch, D.; Silks, L.A. *Biochem. Biophys. Res. Comm.* **2002**. 257-261
2. Welch, M.; Philips, R.S., *Heterocyl. Com.*, **1999**, 5, 305-310.
3. Hatch, D. M. et al. *Current Org. Chem*. **2004**. 47-64.
4. Hatch, Duane. Tenn. Tech. Univ. **2003**. pp. 4-8, 19-70.