Improved synthesis of a genetically encoded non-natural amino acid

Ryan Curtis
Indiana University - Purdue University Fort Wayne

Seja Culpepper
Indiana University - Purdue University Fort Wayne

Jackie Kelty
Indiana University - Purdue University Fort Wayne

Follow this and additional works at: http://opus.ipfw.edu/stu_symp2014

Part of the Chemistry Commons

Recommended Citation
http://opus.ipfw.edu/stu_symp2014/63

This Poster is brought to you for free and open access by the IPFW Student Research and Creative Endeavor Symposium at Opus: Research & Creativity at IPFW. It has been accepted for inclusion in 2014 IPFW Student Research and Creative Endeavor Symposium by an authorized administrator of Opus: Research & Creativity at IPFW. For more information, please contact admin@lib.ipfw.edu.
Synthesis of a Nonnatural Amino Acid and Characterizing its Genetic Incorporation
Ryan Curtis, Seja Culpepper, Jackie Kelty, and Eric Tippmann*
Department of Chemistry
Indiana-Purdue University, Fort Wayne

Introduction
All known organisms use a common set of 20 natural amino acids to build all the proteins and enzymes that make life possible. Recently, model organisms, such as E. coli and yeast were engineered to incorporate nonnatural amino acids into specified target proteins. The ability to manufacture proteins and enzymes with additional amino acids has many applications in medicine and biotechnology. However, the previous synthesis of ferrocenyl cysteine involved five synthetic transformations (Scheme 1), making it beyond the means of many of the biologists that would like to use the nonnatural amino acid. The synthesis is also low yielding. Thus, a new, more expedient synthesis was devised (Scheme 2). Preliminary results show spectra consistent with the previous product suggesting a successful synthesis.

Synthetic Organic Chemistry
The previous synthesis of ferrocenyl cysteine involved five synthetic transformations (Scheme 1). This synthesis is beyond the means of many of the biologists that would like to use the nonnatural amino acid. The synthesis is also low yielding. Thus, a new, more expedient synthesis was devised (Scheme 2). Preliminary results show spectra consistent with the previous product suggesting a successful synthesis.

Scheme 1. Synthesis of ferrocenyl cysteine 1. Reagents and conditions: (a.) NaBH₄ 1/3 equivalent, THF/MeOH 50:1, 12 - 24 h RT (98% yield); (b.) oxalyl Chloride 1.5 equiv. CH₂Cl₂, 4 h RT (98%); (c.) Cs₂CO₃/BOC-(L)-cysteine Methyl ester, DMF, 12 h RT (40%); (d.) 4 M HCl in dioxane THF, 40 °C 6-12 h (45%); (e.) LiOH in THF/H₂O 1 h RT (55%).

Scheme 2.

Figure 1. Top Panel shows the 20 common amino acids; Bottom panel shows some Nonnatural amino acids.

Molecular biology and protein expression
The second goal for the project is to understand how nonnatural amino acid 1 reacts with the Leucyl aminoacyl tRNA synthetase (aaRS) enzyme that specifically recognizes ferrocenyl cysteine 1. The gene coding for the aaRS was cloned into pET 28b; the 97 kiloDalton enzyme was expressed in E. coli and purified (Figure 3.).

Figure 3. Lanes 1-2 Molecular weight ladder; Lanes 3-6 fractions following Hexahistidine affinity purification.

Summary
A more efficient synthesis of nonnatural amino acid 1 will increase its availability and future application. Our goal is to now understand how the aaRS previously evolved to recognize 1 does so in the presence of its canonical target leucine. To this end, the enzyme will be studied both biochemically, using classic Michaelis-Menten Kinetics and structurally using protein crystallography.

Figure 2. Top: Liquid chromatography; Bottom: Mass spectrometry showing Parent ion 319.

References