Synthesis of 2,2′-dimethyldodecanoyl ACP to understand substrate specificity in LasI catalyzed Pseudomonas aeruginosa quorum sensing.

Susan Knox1, Aubrey Johnston, Dr. Rajesh Nagarajan

1The College of New Jersey, 2Boise State University

Abstract

Bacteria use signal molecules called autoinducers to estimate local cell population densities. This mechanism, referred to as quorum sensing, aids bacteria to form biofilms. The LasI AHL (acylated homoserine lactone) synthase enzyme in Pseudomonas aeruginosa uses 3-oxododecanoyl-ACP and S-Adenosyl-L-methionine substrates to make 3-oxododecanoyl homoserine lactone autoinducer. Our project is focused on understanding the importance of a carbonyl group in C3 position of acyl-ACP substrate in LasI catalyzed AHL synthesis. Therefore, we decided to compare the catalytic efficiency (kcat/Km) of 2,2′-dimethyldodecanoyl ACP substrate with 2,2′-dimethyl-3-oxododecanoyl ACP to understand substrate specificity at the 3-oxo position. We used a Meldrum’s acid procedure to make the beta-ketoester, dimethylated at C2 position, reduced the carbonyl at C3 via a hydrazine intermediate, and then prepared acyl-CoA through an N-acyl imidazole intermediate. The final acyl-ACP purification will be done using phosphopantetheinyl transfer enzyme. We will use a colorimetric assay to determine the catalytic efficiency of this substrate.

Introduction

In P. aeruginosa, autoinducers are produced through LasI AHL synthase. Once an autoinducer is released by a bacteria, another bacteria will receive the communication signal and also produce an autoinducer. This process continues, and once the local cell population density is high enough, a rise in autoinducers tells the bacteria to form a biofilm. Biofilms are extracellular polysaccharide matrices, difficult to kill with antibiotics.

Materials

Decanoic acid, N,N′-Dicyclohexylcarbodiimide (DCC), 4-Dimethylaminopyridine (DMAP), Meldrum’s Acid, methanol, tetrahydrofuran (THF), sodium hydride, methyl iodide, dichloromethane (DCM), ethanol, p-toluene sulfonyl hydrazine, dimethylformamide (DMF), sulfolane, sodium cyanoborohydride, p-toluene sulfonic acid (pTSA), sodium borohydride, benzene, acetonitrile, and ethylene glycol are all commercially available through SigmaAldrich. NMR spectra were acquired using a Varian 300 MHz NMR spectrometer.

Methods

Synthesis of 2,2′-dimethyldodecanoyl ACP

Figure 4. Synthesis Map of 2,2′-dimethyldodecanoyl ACP. Starting with decanoic acid, 2,2′-dimethyldodecanoyl ACP can be synthesized using the above set of reactions.

Alternative Methods for the Reduction of methyl-2,2′-dimethyl-3-oxo-dodecanoate

Starting with methyl-2,2′-dimethyl-3-oxo-dodecanoate, alternative intermediates can be made in order to synthesize methyl-2,2′-dimethyl-dodecanoate. Figure 6 illustrates two different pathways. In the lab, experiments to create both intermediates have been conducted.

Results and Discussion

Figure 5. 1H1 NMR 300 MHz Images. A. Decanoyl-Meldrum’s Acid. B. Methyl-3-oxo-dodecanoate. C. Methyl-2,2′-dimethyl-3-oxo-dodecanoate. D. Methyl-2,2′-dimethyl-3-hydroxy-dodecanoate.

Conclusions

• We can currently synthesize up through methyl-2,2′-dimethyl-3-oxo-dodecanoate successfully.
• Methyl-2,2′-dimethyl-3-hydroxy-dodecanoate was produced in hopes of further reduction.

Future Works

• Synthesize methyl-2,2′-dimethyl-dodecanoate using alternative methods.
• Synthesize 2,2′-dimethyldodecanoyl ACP.
• Measure kcat/Km of LasI for 2,2′-dimethyldodecanoyl ACP.

Running colorimetric assays on the catalytic efficiency of the substrate would allow us to determine the specificity of the 3-oxo position on the substrate.

Acknowledgements

• Dr. Rajesh Nagarajan, Boise State University
• Boise State University Department of Chemistry and Biochemistry
• National Science Foundation, Grant No. CHE-1005159
• BSU Start Up Fund to Dr. Nagarajan
• Dr. Eric Brown, Boise State University
• Dr. Don Warner, Boise State University
• Aubrey Johnston, Boise State University
• Dr. Dastagir Dudekula, Boise State University
• Marie Claudio, BSU Chemistry REU 2013
• Ryan Brecht, INBRE 2013

References