

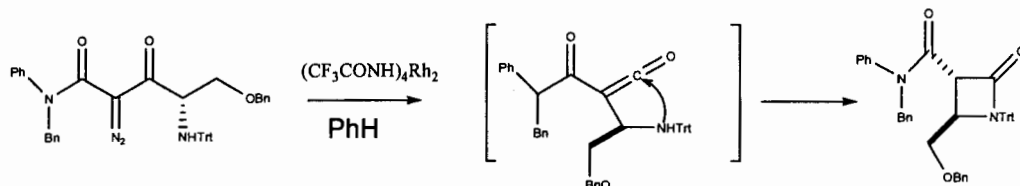
Progress Toward Beta Lactam via Intramolecular Closure

Abstract: β -Lactams are of interest to the pharmaceutical industry as a pre-cursor to the formation of antibiotics. The formation of β -lactams is examined through an intramolecular ring closure through a Wolff ketene like mechanism. The formation of a diketone derivative of serine is examined as a precursor to the β -lactam formation.

Penicillin, first isolated in the 1930's and shortly after used as a clinical antibiotic, has been the cornerstone for a network of future antibiotics¹. Penicillin's relative instability and the human body's efficiency in breaking down the chemical have led to efforts to manipulate the drug to maximize its effectiveness against bacteria². Manipulation of the drug includes the formation of salts with amines to control release of the drug in the body and chemically altering the side chains of the naturally occurring species to maximize the potency of the drug¹. Cephalosporin, another family of antibiotics, differs in that a six member ring is fused to the azetidone ring rather than five member ring that occurs in penicillin¹. The structural entity that the cephalosporin and penicillin have in common is the azetidone ring or β -lactam³.

The β -lactam is effective against bacteria by disrupting the cross linkage of peptides used to form peptidoglycans, which are an integral component to the cell wall of the bacteria⁴. While the β -lactam is effective against bacteria, it is essentially inconsequential to eukaryotic species because the cell's structure consists of a cell membrane, not a cell wall¹. Bacteria are able to fight the mechanism of the β -lactam by cleaving the β -lactam ring with the enzyme β -lactamase³. The action of the enzyme can be fought by attacking the enzyme itself or creating a more robust β -lactam ring by manipulating the side chains¹. The diversity of the β -lactam ring has led to a multifaceted area of chemistry⁴.

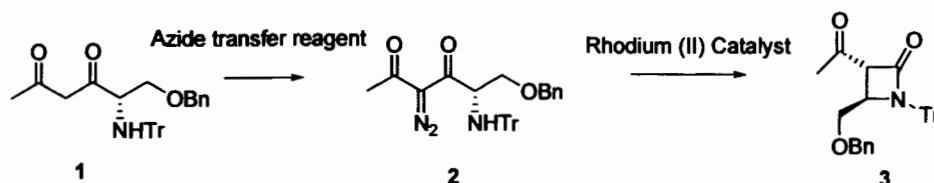
An effective and efficient method for forming the β -lactam backbone, imperative to the antibiotic's mechanism, must be established before creating a diverse range of antibiotics through manipulating various functional groups on the β -lactam ring. Previous work toward the formation of an oxindole led to the serendipitous discovery of a β -lactam⁵. The formation of the β -lactam is thought to proceed through a Wolff-ketene like rearrangement, with retention of stereochemistry, resulting in a trans configuration of the side chains as shown in scheme 1⁵.



Scheme 1

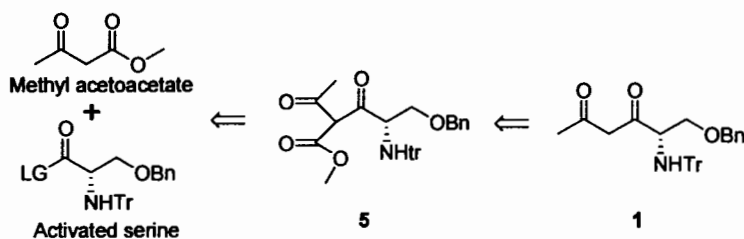
Once the trans β -lactam is created, the formation of naturally occurring antibiotics and a plethora of variations are available after few synthetic steps; furthermore, with the altering of the starting amino acid there are potentially 20 different side chains available to begin the manipulations of the β -lactam. Clearly an intramolecular formation of the β -lactam is a highly valuable synthetic tool.

If it were possible to obtain the diketone, **1**, from N-trityl-O-benzyl-protected serine, the β -lactam would be accessible within two synthetic steps. The reaction scheme **2**, shown below, demonstrates this possibility by incorporating previously known azide transfer chemistry with the Rhodium(II) catalyst ring formation. Once **1** is made an azide transfer reagent such as methyl azide or acetamide benzylsulfonyl azide can be used to place the diazo functionality between the two carbonyl groups shown as **2**. Once compound **2** is formed the Wolff ketene ring closure can be attempted. Unfortunately, the formation of compound **1** proved to be problematic and the remainder of the discussion will be limited to the progress toward the formation of compounds **1** and **2**.



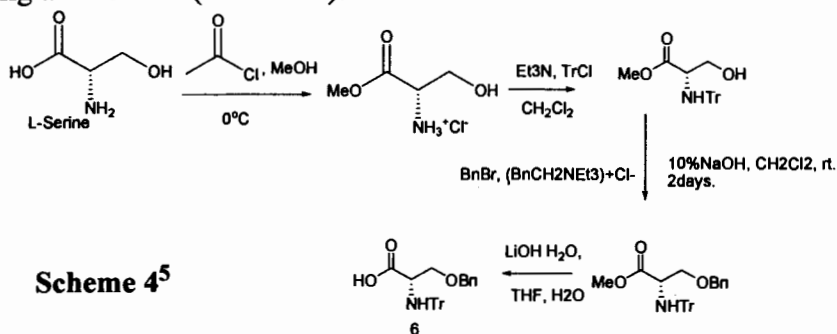
Scheme 2⁵

One retrosynthetic route to obtain **1** is shown below in scheme 3. The activated carboxylic acid of the N-trityl-O-benzyl protected serine is reacted with methyl acetoacetate to yield the tricarbonyl compound **5**. Compound **5** would then be decarboxylated using lithium hydroxide to yield the diketone species **1**.



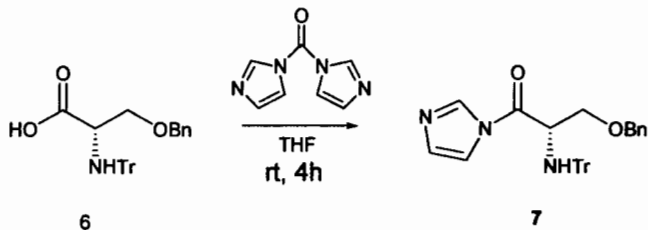
Scheme 3⁵

The N-trityl-O-benzyl protected serine, **6**, was obtained in four steps from the corresponding amino acid (Scheme 4).



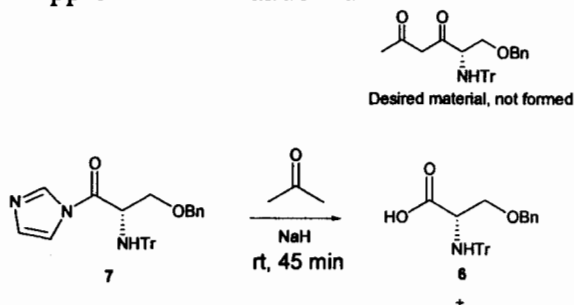
Scheme 4⁵

With compound **6** in hand, the activation of the acid to the imidazole proceeded as shown in Scheme 5. Typically, when imidazole is used as a leaving group it must be used immediately due to its instability. However, it was found that **7** is stable and this is attributed to the large trityl protection of the amine.



Scheme 5⁵

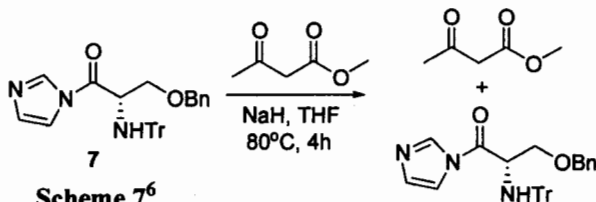
At the suggestion of a colleague, in an attempt to directly access diketone **1**, activated acid **7** was reacted with the sodium anion of acetone. Three spots resulted by TLC and the top spot was found to be mostly hydrocarbon by NMR and was assumed to be the mineral oil from the sodium hydride. The baseline spot was found to be the hydrolyzed product, **6**, as verified by ¹H NMR. The middle spot on the TLC was found to have a mass of 563.7 by mass spec which did not correlate with the desired product and was found in low yields. After a discussion with Professor Konopelski it was suggested that acetone may react with itself forming oligomers and is also very hydroscopic, which is why the free acid may have formed. The acetone reaction is summarized in Scheme 6. Due to complications with the reaction and the absence of desired product, the approach was abandoned.



Scheme 6

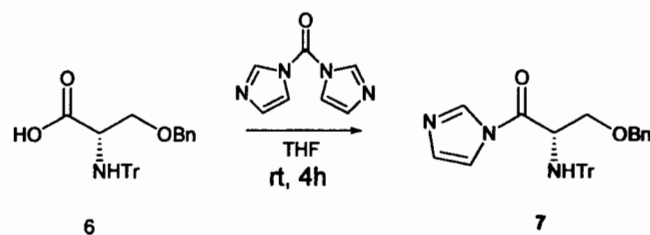
mineral oil + unknown compound of mass 563.7

The next reaction pursued was the attempted formation of the tricarbonyl compound **5**. The activated acid, **7** was reacted with the methyl acetoacetate anion as shown in reaction Scheme 7. Unfortunately, primarily starting material was recovered as depicted in reaction Scheme 7.



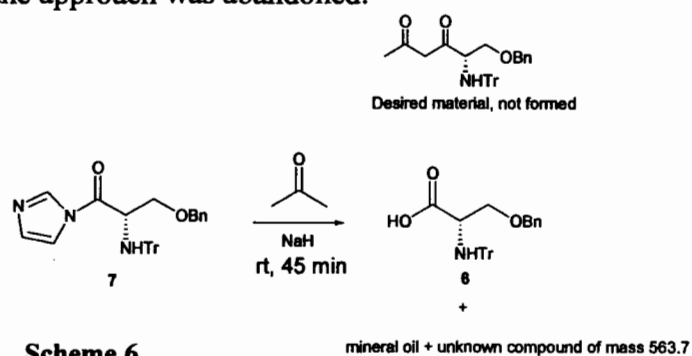
Scheme 7⁶

An alternate base, lithium imidazole, was next used with the same nucleophile, methyl acetoacetate. If the base was equivalent to the leaving group it was thought that the equilibrium might favor the product, resulting in the desired tricarbonyl compound **5**. Regrettably, once again primarily starting material was recovered from the reaction as shown in reaction Scheme 8.



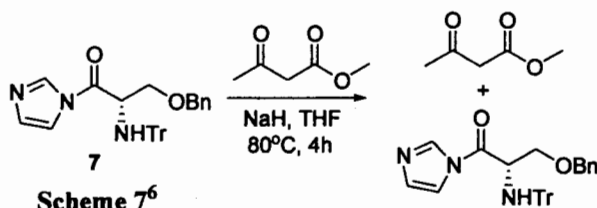
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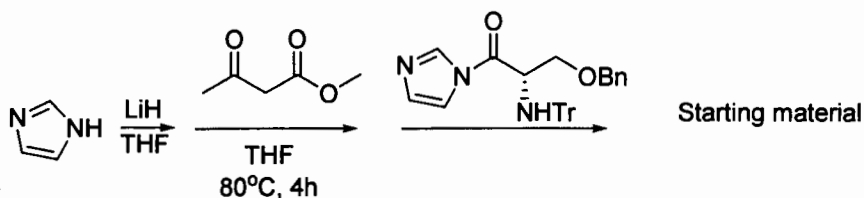
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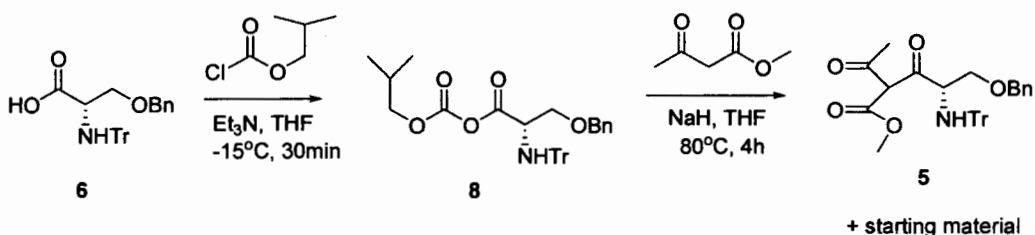
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Scheme 8^{6,7}

Considering that primarily starting material was recovered when the serine imidazolide was used as the reactant, it was thought that the imidazole may not be as good of a leaving group as initially anticipated. As a result, an alternate leaving group was examined. Compound **6** was reacted with isobutyl chloroformate following a deprotonation with triethyl amine in THF to form the mixed anhydride **8**. The formation of the mixed anhydride is shown below in reaction Scheme 9. Following treatment of compound **8** with methyl acetoacetate, several spots were observed by TLC. The third spot from the top was discovered to have the correct molecular weight of the tricarbonyl compound **5** plus the mass of a sodium ion. The resulting yield was quiet low and a carbon NMR was not obtained so the actual structure was not verified. Reaction conditions were examined at 0°C and 80°C. However, insufficient quantity of the desired product and limitations in time dictated that a satisfactory ¹³CNMR has not been obtained. Lacking a ¹³CNMR has inhibited the possible structure elucidation of the unknown compound.



Scheme 9^{7,8}

Experimental: All solids were recrystallized and purity verified by TLC and NMR. Infrared spectra were recorded as thin films on salt plates on a Perkin-Elmer 781 spectrophotometer, with ν_{\max} in inverse centimeters. Proton (¹H-NMR) and carbon (¹³C-NMR) magnetic resonance spectra were obtained in CDCl₃ at 500 MHz. All air and moisture sensitive reactions were carried out under an atmosphere of dry nitrogen using oven-dried or flame-dried glassware and standard syringe techniques. Tetrahydrofuran was distilled from sodium/benzophenone. Flash chromatography was performed on Merck 60, 40-75 mesh silica gel using EtOAc-hexane mixtures as solvent unless otherwise indicated. Thin layer chromatography (TLC) was carried out on Whatman silica gel plates with UV detection and vanillian stain.

Scheme 5: Carbonyl diimidazole was reacted with the free acid **6** at room temperature for 4h. Serine imidazolide or compound **7** was formed in nearly quantitative yield as shown in reaction scheme 5. After passing compound **7** through a silica gel plug and

recrystallizing **7** in hexane, a white yellowish compound remained. The purity of **7** was verified with TLC and NMR before continuing to the next reaction.

Scheme 6: Three equivalents of sodium hydride were dissolved in distilled dry THF. Next, three equivalents of acetone were added to the sodium hydride mixture. After allowing the acetone to react for approximately thirty minutes, compound **7** was added to the reaction vessel. Three prominent spots on TLC were verified after approximately 45 minutes and the reaction was halted.

Scheme 7: Sodium hydride was dissolved in dry distilled THF and heated to 80°C. Next, methylacetoacetate was added to the NaH, THF solution. After the reaction proceeded for approximately 30 minutes compound **7** was added as a solution in THF. The reaction proceeded for approximately 4 hours.

Scheme 8: Lithium hydride was dissolved in THF and imidazole was added as a solid. After about 30 minutes, methyl acetoacetate was added to the imidazolide ion solution. The methylacetoacetate was allowed to react for about 30 minutes when compound **7** was added and then reacted for about 4 hours.

Scheme 9: Compound **6** was dissolved in distilled THF and cooled to -15°C. Triethyl amine was added dropwise to the solution of compound **6**. Isobutyl chloroformate was added dropwise to the mixture of triethyl ammine and compound **6**. The reaction proceeded for approximately 30 minutes at -15°C. The mixed anhydride **8** was assumed unstable and used in-situ. A cannula was used to transfer the reaction mixture to a solution of methyl acetoacetate ion previously prepared. The triethyl ammine chloride salts were transferred over to the reaction flask because literature suggested they would be inert due to limitations of their solubility. Following the addition of compound **8** to the methyl acetoacetate ion the reaction was heated to 80°C and proceeded for approximately 4 hours.

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