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Synthesis of the Azabicyclo[3.1.0]hexane Ring Core of Ficellomycin

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Abstract

Ficellomycin, produced by Streptomyces ficellus, is a naturally occurring secondary metabolite that exhibits antibacterial activity against multidrug resistant Gram-positive bacteria *in vitro*, but shows poor efficacy *in vivo* due to the instability of its azabicyclo[3.1.0]hexane ring core. The effectiveness of ficellomycin against Gram-positive bacteria regardless of resistant strain indicates the possibility of a unique mechanism of action due to the azabicyclo[3.1.0]hexane ring core, compared to currently available antibiotics. To improve ficellomycin's stability and antibacterial activity, a simplified derivative of the azabicylco[3.1.0] ring core was synthesized in a scheme modeled after Shipman et al. by a convergent synthetic pathway characterized by a Lewis Acid promoted double cyclization. Beginning with glyoxylic acid monohydrate undergoing a dehydration and subsequent esterification reaction with benzyl carbamate, methyl 2-(((benzyloxy)carbonyl)amino)-2-methoxyacetate was produced in an 80% overall yield. The methoxyacetate was chlorinated and phosphorylated in a one-pot reaction to produce methyl 2-(((benzyloxy)carbonyl)amino)-2-(dimethoxyphosphoryl)acetate in an average overall yield of 56%. The phosphorylacetate was reacted with pent-4-enal in a basic environment to produce methyl (E)-2-(((benzyloxy)carbonyl)amino)hepta-2,6-dienoate in an average yield of 46%. Epoxidation of the dienoate averaged a 53% yield and subsequent azide addition completed at a 30% yield to afford methyl (E)-7-azido-2-(((benzyloxy)carbonyl)amino)-6-hydroxyhept-2-enoate. The final azide alcohol was reacted with triphenylphosphine in toluene to produce the aziridine and further promote the aziridine to cyclize the alkene chain creating the azabicycle in a single reaction. NMR analysis shows characteristic peaks of the azabicycle product in an impure solution of stereoisomers and reactant contaminants. Purification techniques are being developed. Cyclization trials and synthesis of ficellomycin amino acid derivatives are on going.

Keywords: Ficellomycin, Azabicyclo[3.1.]hexane ring, antibiotic resistant gram-positive bacteria inhibition

1. Introduction

Naturally occurring antibiotics are typically secondary metabolites produced by bacteria or other organisms to inhibit the growth of invading bacterial colonies in order to compete for resources. Bacteria and antibiotics have coexisted in nature since their conception, and ever since they have pushed the evolution of each other continuously. It was not until recent history that scientist have been able to identify these bacteria and utilize these antibiotics to mediate sickness and disease.[1]

In the beginning of the twentieth century, the world crept into a period of biochemical understanding termed the 'golden age of medicine.' From 1880 to 1900 scientists attained an understanding of microbes and went on to identify over 20 different microbes responsible for specific diseases. Counteractive measures to suppress and kill the identified microbes were met with some success. These new developments in medicinal chemistry created a new level of

prestige and respect was given to physicians and medicinal chemists alike and the field of biochemistry was born in the search of therapeutic measures for disease mediation.[2,3]

All bacteria can be split into a dichotomy based on the structural characteristics of their cell wall. Bacteria can either be classified Gram-positive or Gram-negative. Gram-positive bacteria are characterized by a thick peptidoglycan cell wall while Gram-negative bacteria are incased in a lipid membrane. With the different structural constitution of these classifications of bacteria come different metabolic pathways and different methods of inhibition.[4,5] While the peptidoglycan cell wall of Gram-positive bacteria give the microbe protection from many foreign molecules from entering its intracellular environment, the wall becomes a target for antibiotics to inhibit cell growth. Penicillin is a popular antibiotic that is effective against Gram-positive bacteria specifically. Penicillin is able to bind irreversibly to the transpeptidase of bacteria and halt the synthesis of the cell wall killing off the bacteria.[6]

Gram-negative bacteria do not have the exposed peptidoglycan cell wall to target nor protect the bacteria from alien molecules entering the cell. Antibiotics that are able to penetrate the plasma membrane of the bacteria may interact in several antibiotic pathways, from interacting with the DNA and preventing replication and reproduction, to inhibiting the synthesis of vital proteins. Streptomycin is commonly used as an antibiotic against Gram-negative bacteria infections. Streptomycin is able to enter the cell of bacteria and bind to ribosomal subunits responsible for protein synthesis. Streptomycin binds during the beginning stage of protein synthesis and causes the subunit to malfunction and effectively halt protein synthesis causing the bacteria to die off.[7]

As antibiotics have been widely used to better the overall health of societies around the world, antibiotics have unfortunately been overused and misused by many. The abuse and improper handling of antibiotics have given bacteria given a chance to survive and develop resistances to the antibiotics on the market as an evolutionary tool. Today nearly every antibiotic on the market has at least one strain of bacteria resistant to it. Recently there has been a flourishing of multidrug and super resistant strains of bacteria that are nearly impossible to fight with conventional antibiotics on the market. In the USA, one of the leading causes of post-surgical infections is caused by *Staphylococcus* bacteria infections or 'staph' infections for short. MRSA (methicillin resistant *Staphylococcus aureus*) is a particular strain of staph infection that is exceptionally robust bacteria that even the most powerful antibiotics in current circulation have difficulty killing. Such powerful antibiotics are becomingly increasingly toxic and dangerous to the human body as well. In order to halt this spiral of antibiotic resistant strains of bacteria, a new antibiotic mechanism needs to be utilized to combat these bacteria that can be implemented at a concentration and strength that is not toxic to the human body.[1,2,8,9]

Ficellomycin was first isolated from a broth of *Streptomyces ficellus* by Argoudelus *et al.* alongside feldamycin and nojirimycin. Each of the three antibiotics found in this search have widely varying in structural characteristics and antibiotic activity. While each antibiotic shows promise, feldamycin inhibiting Gram-positive bacteria and nojirimycin inhibiting an impressively wide spectrum of bacteria strains even with resistances, ficellomyin shows a particular consistency in effectiveness against Gram-positive bacteria regardless of resistant strains. It can be hypothesized that this effectiveness is due to the fact that ficellomycin has different mechanism of action than drugs currently on market and may prove to be useful as an alternative method to attack multidrug resistant and robust strains of bacteria such as MRSA that plagues developed nations around the world.[10]

Although ficellomycin was isolated and characterized, it wasn't until 1989 that ficellomycin's structure was known, and furthermore revised for more specific stereochemistry in 1993 by Armstrong and Zhao. Ficellomycin was found to have a very rare azabicyclo[3.1.0]hexane ring structure serving as its core. This azabicycle has only been seen in nature once before by a family of anti-tumor agents, the azinomycins. Ficellomycin's structure is shown below as its original and revised structure shows ficellomycin broken into three main components; the azabicyclo[3.1.0]hexane ring, a valine substituent, and a guanidine substituent. The valine substituent is naturally occurring and found in the (S) conformation. The azabicycle system was found in a boat conformation disregarding the higher relative ring strain compared to the more stable chair confirmation. It has been hypothesized that the trisubstituted aziridine is responsible for this stereochemical conformation.[11]

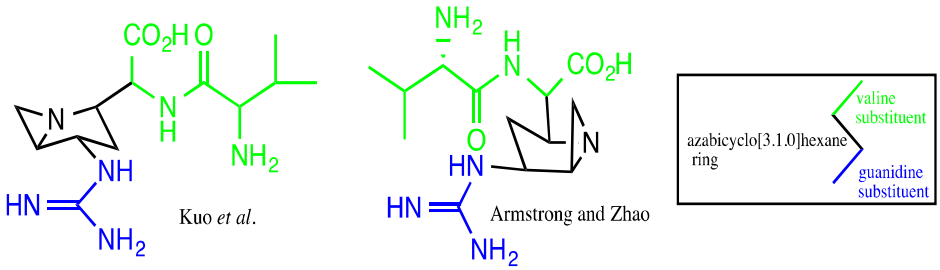


Figure 1. Ficellomycin's originally proposed structure and revised for stereochemistry.[11]

The guanidine plays a particular role in the stability of ficellomycin. While proposed that the guanidine electrochemically stabilizes the azabicyclo[3.1.0]hexane ring, the guanidine also acts as a deactivating agent of ficellomycin. If ficellomycin is subjected to acidic or even neutral environments, the aziridine may become protonated and cause a cascade in which the guanidine performs a nucleophilic ring opening on the azabicycle system, destroying the [3.1.0] azabicycle and forms a nine-member bicycle that deactivates ficellomycin's antibacterial capabilities.[12]

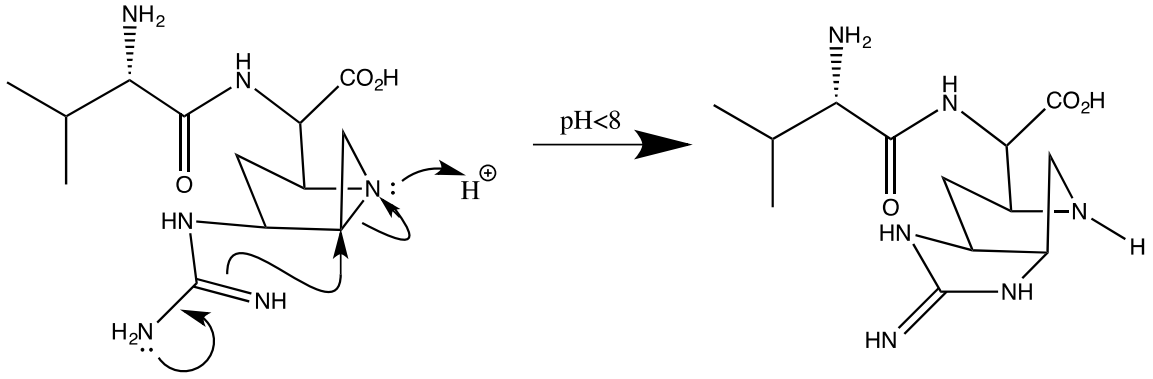


Figure 2. Nucleophilic ring opening of ficellomycin.

While several hypotheses have been evaluated, the exact mechanism of action of ficellomycin is currently unknown. In 1977, Reusser added ficellomycin to toluenized *E. coli* cell culture *in vitro* to analyze interactions with DNA synthesis. The study found that, while ficellomycin does not inhibit the enzymatic polymerization of DNA during semiconservative DNA replication, it does manipulate the polymerization to create defunct strands of DNA averaging in size of 34S that cannot go on to integrate into the DNA synthesis. This buildup of defective DNA segments did lead to cell death. However this mechanism has not been confirmed *in vivo*, but rather remain as a hypothesis and empirically shows that ficellomycin can act as a DNA synthesis inhibitor.[13]

Another hypothesis of an antibacterial mechanism is related to the structurally related azinomycins. Azinomycin B was first discovered in 1954 isolated from *Streptomyces sahachiroi* making it the first azabicyclo[3.1.0]hexane ring found in nature while Azinomycin A as isolated alongside Azinomycin B in 1986 from *Streptomyces griseofuscus.* There are two forms of azinomycins, form A and B, which vary only by the replacement of the methylene in azinomycin A with an enol in azinomycin B (Figure 3).[14]

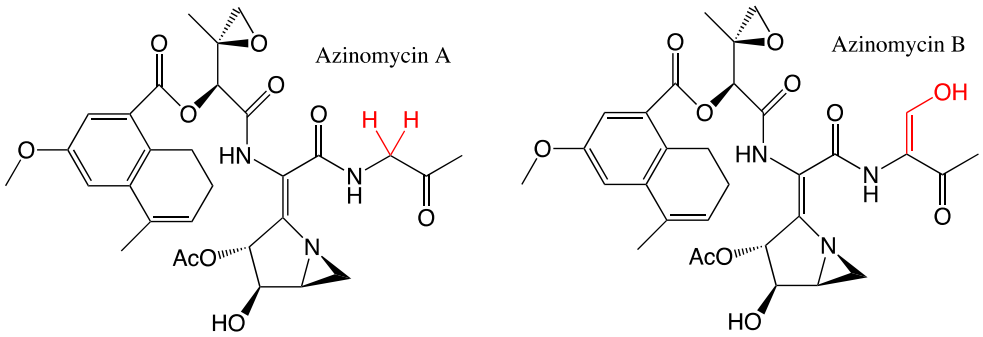


Figure 3. Azinomycin A and B with highlighted structural differences (red).

Azinomycin A and B are known anti-tumor agents that have shown antibiotic activity against both Gram-positive and -negative bacteria. Azinomycin A has an effective MIC of 6.25 μg mL^{-1} and azinomycin B an MIC of 50 μg mL^{-1} against *S. aureus* strains. Through biological testing, azinomycins have been found to bind and crosslink at the epoxide and azabicycle to DNA on major grooves, halting their replicative processes. While ficellomycin is hypothesized to act in a fashion such as this, ficellomycin lacks the intercalating naphthalene that azinomycins utilize to initially anchor into the DNA in order to react. Further research would be required to determine if ficellomycin might overcome its structural shortcoming and maintain the ability to interact with DNA *in vivo*.[15]

While the DNA alkylating and synthesis inhibition characteristics of the azabicyclo[3.1.0]hexane ring has been empirically proven *in vitro*, ficellomycin's particular effectiveness against Gram-positive bacteria and the ability to be attacked by nucleophiles on the aziridine lends viability of the hypothesis that ficellomycin may act in an antibiotic fashion similar to β-lactam antibiotics. Much as penicillin interacts and binds to surface proteins of the cell wall to inhibit cell wall formation, ficellomycin may also utilize a similar mechanistic approach.

Several attempts have been made to synthesize ficellomycin, but no total synthesis has been completed to date. While there are many synthetic options and strategies, this research is based off of the synthetic research of Paumier and coworkers in which a late stage double cyclization cascade of an aziridine was utilized to form the azabicycle in a single cascade of reaction events. Paumier *et al.* were able to synthesize and isolate a simplified analog of ficellomycin containing the azabicyclo[3.1.0]hexane ring core. Synthesis was characterized in its later steps by the epoxidation of a benzyl carbamate-protected alkene, and then use of sodium azide to break the epoxide and afford an azido alcohol. Triphenylphosphine was used to afford the aziridine, and instantaneously the aziridine to perform a nucleophillic attack on the π bond in order to create the cyclized product (Figure 4). While synthesis was completed, yields for the reaction scheme were low with just the latter steps reaching to an overall yield to a mere 20% disregarding preparatory steps to create reagents. Alongside the low yield, purification of ficellomycin from contaminating triphenylphosphine and triphenylphosphine oxide reagents proved to be difficult, so an expensive polymer form, diphenylphosphinoppolystyrene, was utilized in order to circumvent the contamination issues.[12]

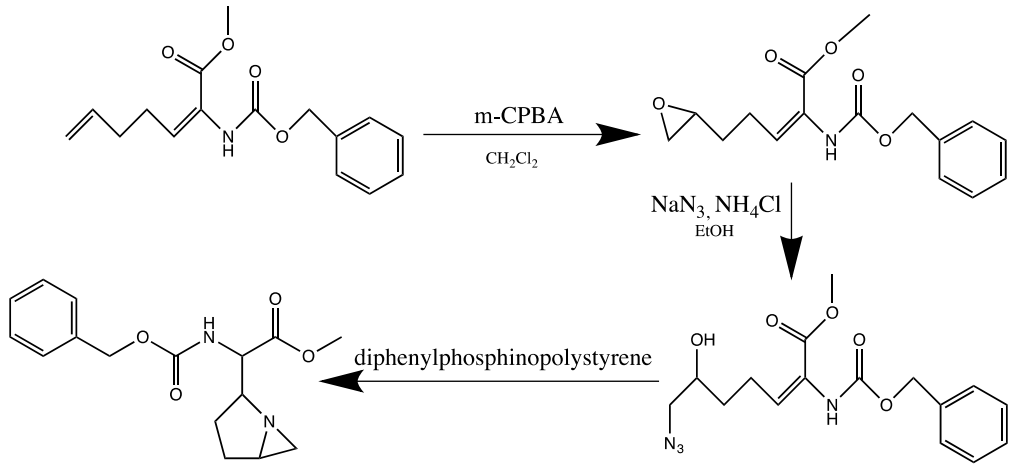


Figure 4. The latter steps in synthesis utilizing azide to promote a double cyclization cascade to form the azabicyclo[3.1.0]hexane ring core of ficellomycin.

While ficellomycin may have low antibiotic efficacy and pose issues with stability in its natural state, the antibiotics ability to inhibit Gram-positive bacteria regardless of resistant strain gives purpose to its development into a better drug candidate. Before becoming a viable drug candidate to be researched on a large scale, there must be a viable synthetic scheme in place in order to complete SAR (structure-activity relationship) analysis and optimize ficellomycin's capabilities.

This research is an attempt to synthetically create the azabicyclo[3.1.0]hexane ring core of ficellomycin and improve the stability and antibacterial activity of ficellomycin's core to promote ficellomycin as a drug candidate and spur further research. The synthesis is based off of the mechanistic pathway of Paumier and coworkers, but emphasizes the efficiency of each step by using cost-effective reagents and reducing waste through the reisolation of unreacted starting materials. During the synthesis, triphenylphosphene will be utilized in an attempt to form the azabicycle as a cheaper, more commercially available reagent rather than the expensive polymer form, diphenylphosphinopolystyrene. By the use of cheaper reagents, more efficient and concise reaction conditions, and reducing waste by reisolating starting materials, the synthesis will be more appealing to industry. Furthermore, this research will attempt to go beyond the scope of Paumier by analyzing a library of analogs formed from the synthesis. First deprotecting the methyl ester and substituting the carbamate with an amino acid, the analogs will be tested against *S. aureus* to determine relative antibiotic efficacies against one another.

This research will also examine the utility of the guanidine substituent and its activity in the antibiotic mechanism. By removing the guanidine, the pH tolerance of ficellomycin's azabicyclo[3.1.0]hexane ring core will become much more versatile. Without having the guanidine acting as a nucleophilic source to react in immediate proximity and the

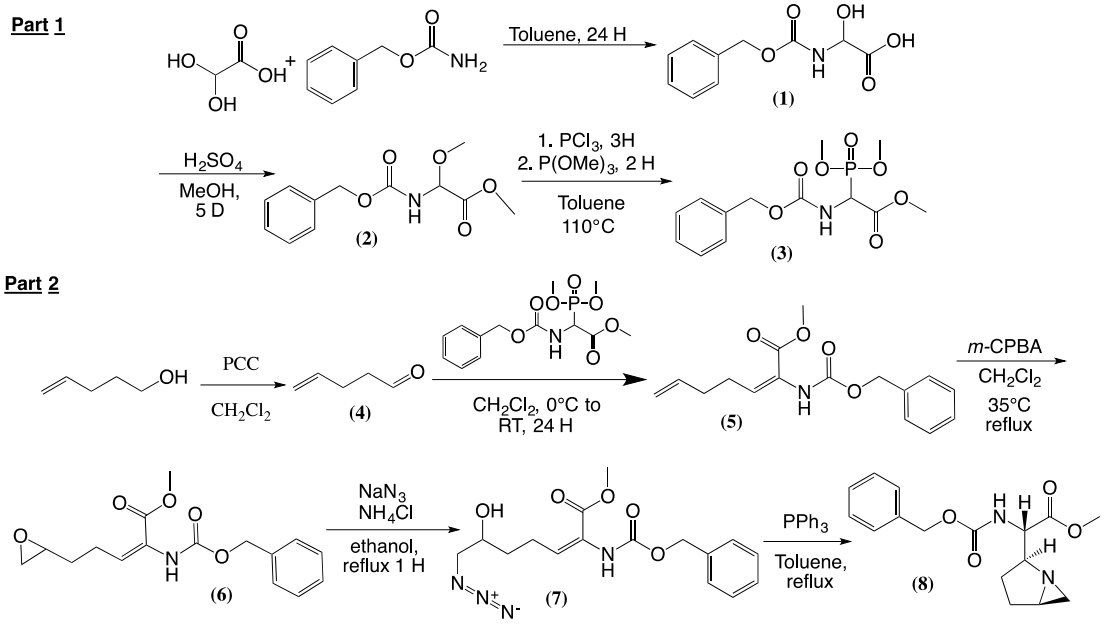
aziridine will be forced to react in an intermolecular fashion much more slowly. The aziridine will persist longer in slightly more acidic environments and may have more time to find the intended biological target to react before deactivating.

2. Methods

Methods have been described in supplemental information.

3. Results and Discussion

The synthetic scheme used in this research was modeled after the synthesis path of Shipman *et al.* with the synthesis of the benzyl carbamate protecting group modeled after Vaswani and Chamberlin. The synthetic pathway used in this research shown in scheme 1 was based off of two converging parts. Part 1 was devoted to the formation of a phosphonate reagent later utilized in part 2 to perform a Horner-Wadsworth-Emmons Wittig reaction, and part 2 was dedicated to form the backbone of the azabicyclo[3.1.0]hexane ring core. A late stage Lewis Acid promoted double cyclization of the azide concluded the synthetic pathway as the azabicycle was formed in one instance. One particular distinction in this synthetic pathway is the Lewis Acid in the final reaction that promotes the double cyclization. Shipman and coworkers utilized the polymer form of triphenylphosphine, diphenylphosphinostyrene. The primary advantage of diphenylphosphinostyrene is that it is easily reacted and removed from solution, but the high price restricts its practical use in industrial chemistry. The synthetic scheme proposed utilizes triphenylphosphine. Triphenylphosphine functions as a competent Lewis Acid, but is difficult to remove from solution without compromising the structure of the cyclized ficellomycin derivative.[11,16]



Scheme 1. Synthesis path, part 1 and 2, towards the synthesis of ficellomycin.

Synthesis began with the creation of a benzyl carbamate to attach to the ficellomycin backbone downstream. Glyoxylic acid monohydrate underwent a condensation reaction with benzyl carbamate in toluene to yield 2-(((benzyloxy)carbonyl)amino)-2-hydroxyacetic acid **(1)**. After filtration, the product was solvated in methanol and reacted with sulfuric acid in an esterification of the primary alcohols to afford methyl 2-

(((benzyloxy)carbonyl)amino)-2-methoxyacetate **(2).** A phosphate was attached through an acid-base reaction to create the final product of part 1, methyl 2-(((benzyloxy)carbonyl)amino)-2-(dimethoxyphosphoryl)acetate **(3)**.

Part 2 of the synthetic pathway began with the oxidation of pent-4-enol by pyridinium chlorochromate (PCC) in dichloromethane to create pent-4-enal **(4)**. The reaction was filtered to remove PCC and immediately react the crude product with methyl 2-(((benzyloxy)carbonyl)amino)-2-(dimethoxyphosphoryl) acetate in a Horner-Wadsworth-Emmons reaction to yield methyl (*E*)-2-(((benzyloxy)carbonyl)amino)hepta-2,6-dienoate **(5)**. Flash chromatography isolated the product from side products and reactants that remain in solution.

Epoxidation of **(5)** was carried out in dichloromethane with *meta*-chloroperbenzoic acid (*m*-CPBA) to yield methyl (*E*)-2-(((benzyloxy)carbonyl)amino) -5- (oxiran-2-yl)pent-2-enoate **(6)**. The product was isolated by flash chromatography. Aziridination of the epoxide **(6)** by sodium azide and ammonium chloride produced methyl (*E*)-7-azido-2-(((benzyloxy)carbonyl)amino)-6-hydroxyhept-2-enoate **(7)** after chromatography purification. The final reaction was the intramolecular double cyclization of the azide promoted by triphenylphosphine as a Lewis acid to produce the cyclized product methyl(*S*)-2-(((benzyloxy)carbonyl)amino)-2-((2*S*,5*R*)-1-azabicyclo[3.1.0]hexan-2-yl)acetate **(8)**.

The overall proposed synthetic scheme was run to completion with spectroscopic data on all purified intermediates to confirm the structures. Purification of the cyclized derivative of ficellomycin has not been completed, but small-scale reactions yield an impure solution of stereoisomers of product as well as side products and reactants. Characteristic signals unique to the cyclized product are present on the ^{13}C NMR with variances showing the presence of stereoisomers. ^{1}H NMR was inconclusive due to excessive noise and contamination that overwhelmed the characteristic signals of the cyclized product. Past literature confirms the utilization of triethylamine pre-treated silica gel to be a viable medium to purify the final azabicycle.[11]

The beginning condensation reaction between glyoxylic acid monohydrate and benzyl carbamate ran consistently in acceptable yields. Solubility of the benzyl carbamate and water contamination of the product were the primary issues with the reaction. The benzyl carbamate solvated much more readily as the scale of the reaction increased, even though the solvent was scaled proportionally with the benzyl carbamate. Water contamination became more of an issue as the reaction scale was increased. The water was removed with additional washing of the organic layer with $NaHCO_3$, H_2O, and NaCl as well as more vigorous evaporation in the rotary evaporator. The esterification of the alcohols also increased in yield as the scale of the reaction increased.

The chlorination and phosphorylation of the carbamate creating the phosphonate (**4**) also increased in yield as scale increased. Completion times of each reaction varied with each trial and were monitored by TLC. Table 1 below depicts the trials and variables concerning each. As trial 8 and 10 depict, sulfuric acid was added in catalytic amount to the reaction. Past literature showed that sulfuric acid assisted in the progression of the reaction and increased percent yields when added into solution at a catalytic amount. Unfortunately, when sulfuric acid was added to solution in this research, the reaction went off course and after purification, no desired product was found.[17]

Table 1. Chlorination and phosphorylation reaction parameters by trial.

Trial	Carbamate (g)	PCl_3 (mL)	$P(OMe)_3$ (mL)	Toluene (mL)	H_2SO_4 (mL)	% Yield
1	0.202	0.08	0.09	2	-	N/A
2	0.205	0.08	0.10	2	-	20
3	0.400	0.19	0.19	5	-	26
4	0.214	0.08	0.11	4	-	43
5	0.403	0.16	0.21	4	-	44
6	0.401	0.16	0.21	5	-	54
7	0.600	0.24	0.32	7	-	47
8	0.599	0.24	0.32	9	.05	-
9	0.803	0.33	0.42	8	-	78
10	0.403	0.17	0.21	4	.05	-
11	0.801	0.33	0.42	8	-	67
12	1.593	0.65	0.84	16	-	61
13	3.000	1.20	1.40	32	-	50
14	2.50	0.95	1.2	27		85

Pent-4-enol was oxidized to 4-penten-1-ol in a PCC oxidation reaction and the crude product went on to react with the phosphonate in a Horner-Wadsworth-Emmons Wittig reaction to yield the dienoate **(6)**. Table 2 below represents the reaction trials and each of the trials conditions along with its percent yield. Each trial only has one yield as the reactions were run in tandem.

Table 2. Tandem PCC oxidation and Wittig reaction trials of 4-penten-1-ol.

Trial	4-penten-1-ol (mg)	PCC* (mg)	DCM (mL)	Phosphonate (mg)	DBN (mL)	DCM (mL)	% Yield
1	100	97	5.8	-	-	-	-
2	50	215	2.9	-	-	-	-
3	50	178	3.5	211	0.08	0.06	13
4	100	238	6.6	428	0.18	0.12	52
5	100	286	5.8	435	0.18	0.2	53
6	200	708	12.1	891	0.35	4.7	32
7	300	1071	17.4	1276	0.52	7.2	14

Trials 1 and 2 of the PCC oxidation afforded no discernable yield of aldehyde product so the subsequent Wittig reaction could not occur. The oxidation of 4-penten-1-ol and handling of PCC have proven tedious and require constant monitoring for reaction progression. The first issue was that, although PCC is an inorganic reactant and will form a heterogeneous solution in organic solvent, the compound would congeal and adhered to the reaction flask in presence of the alcohol. The first solution was to add 4Å molecular sieves to the reaction flask. The PCC still congealed, but rather to the flask, it congealed to the sieves. Even though the PCC was congealed, there was much more surface area in contact with solution than before and the oxidation reaction proceeded further. The next solution was to dilute the reaction to allow more PCC to remain in suspension. This tactic did not have a significant effect on the characteristics of the reaction. Alternative methods to improve the oxidation of the alcohol such as filtering spent PCC to continually replace and push the reaction forward and running parallel reactions at a 100 mg scale were also used with some success.

The following Horner-Wadsworth-Wittig reaction was run with the crude product of the oxidation reaction after the PCC and side products were filtered out. The reaction ran until thin layer chromatography (TLC) tests showed no discernable progress. Purification of this reaction by flash column chromatography proved difficult, as there were many molecules in the crude reaction mixture that needed to be isolated. Reactants from both reactions remained in solution after initial work up and contaminated aliquots of the purified dienoate after column chromatography.

Focus was put on forming an isolation technique to acquire pure samples of the dienoate. Removing the remaining alcohol before the Wittig reaction, either by reacting it with PCC to completion or removing it by column chromatography increased purity. Purification of the product mixture from the Wittig reaction has also been found to be easier by adding a preliminary step of recrystallizing remaining phosphonate ins solution before column chromatography. In order to crystalize the phosphonate, the crude product solution was concentrated by rotary evaporation, then re-solvated in a solution of hexane with small quantities of ethyl acetate. Once the crude product was completely solvated the flask was placed in the freezer and the solution was cooled to -30 °C and left overnight for the phosphonate to crystalize. The product was then removed by syringe and phosphonate stored for future reactions.

Epoxidation of the dienoate was completed with *meta*-chloroperoxobenzoic acid and purified by column chromatography at a consistent yield ranging from 50% to 60%. The epoxide product was purified and brought to the azido alcohol by sodium azide and ammonium chloride. Table 3 below depicts the reaction trials and the parameters of the aziridination reaction.

Table 3. Aziridation of epoxide with sodium azide and ammonium chloride.

Trial	Epoxide (mg)	NaN_3 (mg)	NH_4Cl (mg)	Ethanol (mL)	% Yield
1	50	34	29	0.9	36
2	21	21	15	2	24
3	83	53	44	2	5
4	119.9	104.6	74.4	8	29

Trial 3 from Table 4 was an anomaly as its yield is significantly lower than the other 3 trials. This is believed to be due to the photosensitivity of the azide both in the reactant and the product. During the reaction in trial 3, the product was exposed to light for a portion of the reaction process and degraded over time. The azide degraded in the presence of light to produce nitrogen gas and a nitrene substituent on the parent molecule. In order to prevent this degradation, the reaction was removed from light as much as possible. The reaction flask was covered in aluminum foil for the duration of the reaction and the product was worked up in as dark of an environment as possible. This allowed for the preservation of the azide to continue to the final reaction of the cyclization.

The cyclization of the alkene promoted by triphenylphosphine was the final reaction in the proposed synthesis and forms the simplified protected derivative of ficellomycin. Small-scale synthesis of the reaction has been completed, but due to complications in isolation and purification, there is no definitive yield or spectroscopic confirmation. NMR analysis show characteristic peaks unique to the cyclized product in the form of multiple stereoisomers among contamination of byproducts and remaining reactants. Purification trials are ongoing to obtain a definitive yield and isolated spectroscopic data.

4. Conclusion

Ficellomycin has experimentally proven to be a relatively poor antibiotic with an IC_{50} value of 0.5 mM and stability issues within its molecular structure. Beyond the shortcomings of ficellomycin, its ability to inhibit Gram-positive bacteria regardless of antibiotic resistance gives purpose to researching the antibiotic pathway. As ficellomycin is not readily available on the market nor is it easily synthesized, there is a clear need for a straightforward and concise synthetic pathway to form a stable ficellomycin analog in order to progress research. This research served as a validation of the work of Paumier *et al.* as an effective and reliable synthetic scheme, but more work is needed to make synthesis more cost and time effective in order for further research to be conducted with a consistent stockpile of ficellomycin. [20]

5. Future Work

Synthesis performed in this research further confirms the research of Shipman and coworkers, and provides an acceptable synthetic pathway to create the azabicyclo[3.1.0]hexane ring core of ficellomycin in academic labs. Further synthesis to this azabicycle could be completed deprotecting the ester and performing an amino acid deprotection of the carbamate to yield various amino acid analogs of the azabicycle that may go into *in vitro* analysis by Gram-positive bacterial assays to analyze the antibiotic efficacy of the analogs compared to native ficellomycin.

6. Acknowledgements

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