Proceedings of The National Conference
On Undergraduate Research (NCUR) 2016
University of North Carolina Asheville
Asheville, North Carolina
April 7-9, 2016

Synthetic Approaches towards Azabicyclic Compounds

Emily Lanier
Department of Chemistry
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Amanda L. Wolfe

Abstract

The overuse of antibiotics has contributed to a recent increase in drug-resistant strains of bacteria. Thus, research into new compounds that exhibit novel modes of action will aid in the development of antibiotics that are active against drug-resistant strains. Based on the promising activity of ficellomycin, which contains a unique and highly strained azabicyclo[3.1.0]hexane ring system, this project seeks to improve upon existing methodologies for the synthesis of the azabicycle moiety. This work explores intramolecular reductive amination, via Lewis acid catalysis and subsequent imine hydrogenation, as a viable method of azabicycle synthesis. One possible route utilizes an alkylation reaction of protected pyrrolidine and piperidine methanols followed by the cyclization step. Another route begins with methyl acetoacetate and uses an alkylation followed by epoxidation and aziridination to form the molecule needed for cyclization.

Keywords: Synthesis, Antibiotic, Azabicycle

1. Background

In the field of medicinal chemistry, there is an ever-present need for research into new antibiotics. Bacterial species change and mutate in response to the heavy use of current antibiotics, creating a deficit of available antibiotics that are potent against new resistant strains. Thus, research into new compounds that exhibit novel modes of action will aid in the development of antibiotics that are active against drug-resistant strains. Ficellomycin, shown in Figure 1, is a natural product that shows promise for antibiotic development due to its unique structure and demonstrated activity against drug-resistant *Staphylococcus aureus*. The Upjohn company first isolated ficellomycin in 1976 from a culture of *Streptomyces ficellus*, along with two other antibiotics, feldamycin and nojirimycin.[1] This initial report on ficellomycin gave a partial structure elucidation, as well as demonstrating the compound's antibiotic activity against strains of *S. aureus*, including strains resistant to other antibiotics. Ficellomycin was examined both *in vitro*, in biological assays using *S. aureus*, as well as *in vivo*, by treating mice infected with *S. aureus.* However, the MIC, or minimum inhibitory concentration, of the antibiotic was greater than 1000 μg/mL, meaning that it is not yet as potent of a compound as needed for it to be a viable clinical candidate. Feldamycin and nojirimycin also showed antibiotic properties, but of the three ficellomycin showed the most antibiotic activity and thus is a focus of this work.

In addition to contributing to the medical field, research on ficellomycin is also important to the field of chemistry

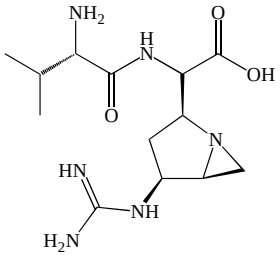


Figure 1. Structure of ficellomycin

for its unique structure (Figure 1). As elucidated by Kuo et al. in 1989,[2] ficellomycin contains a valine group, a guanidine group, and a unique and highly strained azabicyclo[3.1.0]hexane ring system. This specific ring system has only been seen in one other class of natural product compounds, the azinomycins. However, many other natural products exhibit similar azabicyles with differing rings sizes, such as swainsonine[4] and hyacinthacines.[5]

This research explores the synthesis of azabicyclic compounds with varying ring sizes, with the goal of discovering a concise synthesis scheme that will assist in the total synthesis of many biologically active natural compounds. In addition, investigation of the action of ficellomycin's specific azabicycle and its surprising stability in a biological system will add to the current understanding of the use of such structures as possible pharmaceuticals.

1.1 Previous Research

As stated, ficellomycin was first isolated in 1976[1] and the structure fully elucidated in 1989 by Kuo et al.[2] In 1977, Reusser[3] investigated the mode of action of both ficellomycin and its sister compound, feldamycin. The experiments showed that ficellomycin did, indeed, have an effect on semi-conservative DNA replication, the process by which DNA is copied. In this process, there are fragments of DNA formed in the early stages of the replication, called Okazaki fragments, which are later synthesized together to form a complete strand. Reusser showed that ficellomycin does not inhibit the formation of these fragments, but causes them to be formed in such a way that they could not be later synthesized into the full DNA strand.

In a 2011 review, Foulke-Abel et al. summarized research on the mechanism of ficellomycin as well as the structurally related compounds, the azinomycins (Figure 2).[6] No further research has been done into the mode of action of ficellomycin. It has been shown that the azinomycins function by insterting the naphthalene ring system into double-stranded DNA, which positions the molecule to form interstrand crosslinks via attack from the azabicycle and epoxide moieties. This gives evidence for the reactivity of the azabicycle, though ficellomycin's activity may be different than the azinomycin's since it lacks the naphthalene moiety. Azinomycin also differs in that it is mainly investigated for its antitumor effects rather than antibiotic activity. However, it is clear that the unique ring shared by these two compounds merits more research to determine its action in biological systems.

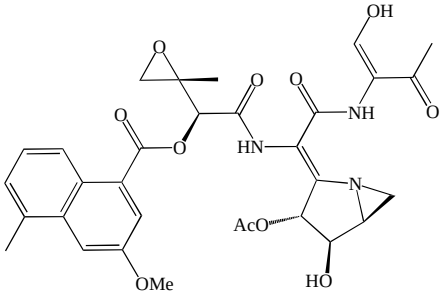


Figure 2. Structure of azinomycin B

In addition to literature on antibiotic modes of action, research pertaining to the synthesis of ficellomycin and other azabicyclic compounds is also of interest to this project. No total synthesis routes have yet been published for ficellomycin; however, routes have been found for the formation of the azabicyclo[3.1.0]hexane core. Paumier first

reported a synthetic pathway for this core in 2004,[7] which Garcia improved upon in the publication of her thesis in 2007.[8] Other research into the formation of azabicycle system include Hartley et al. in 2000,[9] Coleman in 2001,[10] and Ismail et al. in 2009.[11] The most recent attempt by Chen et al. in 2014 utilized bromination of aziridine-containing olefins to promote an S_N2-like cyclization to form the azabicycle (Figure 3). Unlike previous synthetic routes, their approach was able to maintain some stereochemical control by beginning with either *cis*- or *trans*- starting materials.[12]

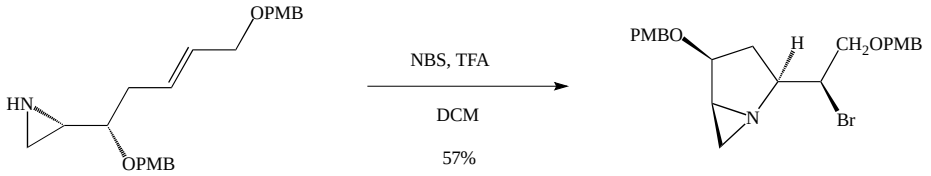


Figure 3. Bromination by NBS followed by nucleophilic attack of aziridine to produce the azabicyclic core of ficellomycin.

This project aims to improve upon the inefficiency and lack of stereochemical control of many of these routes and proposes a new synthetic scheme that uses intramolecular reductive amination to form the azabicyle system. Figure 4 shows the proposed method, which would use nucleophilic attack of the aziridine at a carbonyl group, catalyzed by a chiral Lewis Acid catalyst, to close the azabicycle.

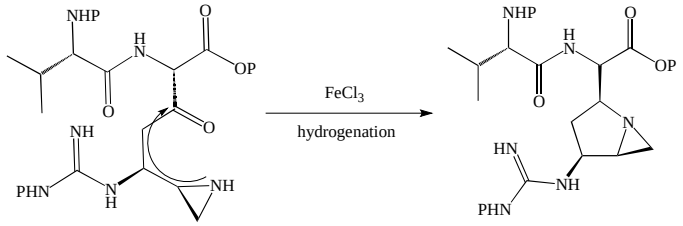


Figure 4. Proposed mechanism for closing the azabicycle of ficellomycin through intramolecular reductive amination.

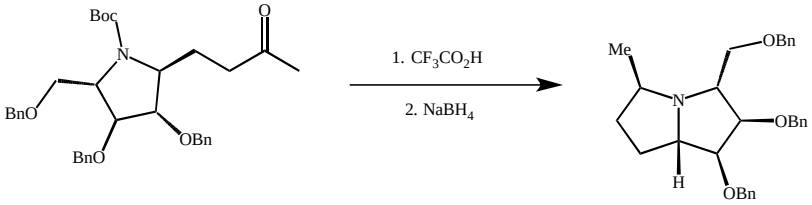


Figure 5. Reductive amination to form hyacinthacine derivatives.

Intramolecular reductive amination has been proven to be effective in the case of hyacinthacines, which contain an azabicyclopentane core. Hu et al. synthesized this core with good yield using CF_3CO_2H as a Brönsted acid and $NaBH_4$ for the hydrogenation step[5] (Figure 5). These results confirm that our approach is viable, at least in the case of an unstrained ring system.

In 2008, Abrams et al. used reductive amination cyclization to form swainsonine, a biologically active compound with a similar core to the hyacinthacines. In their method, the compound was subjected to hydrogenation conditions, with palladium acting as the Lewis acid while hydrogen reduced the resulting imine intermediate.[4]

Other approaches to the synthesis of the 5,5 azabicycle include a method published in 2001 by Lagu et al. who employed an enolate addition to an aldehyde-containing pyrrolidine followed by hydrogenation.[13] The loss of water from the resulting imine causes the aromatization of the system, which yields an unsaturated pyrrole product rather than the desired saturated azabicycle sought in this project.

In summary, the literature thus far demonstrates a lack of efficient and widely applicable synthetic strategies for azabicycle compounds. This project seeks to validate reductive amination as a viable method of azabicycle synthesis, with the goal of adding to the knowledge base for those attempting to synthesize natural products for medicinal use.

2. Methods

All reagents were purchased and used without further purification except for *meta*-Chloroperbenzoic acid, which was washed with pH 7 phosphate buffer and dried under reduced pressure. The instruments used for identification of all compounds were an Oxford Inova 400 MHz NMR and a Thermoscientific Nicolet iS10 FT-IR. Common abbreviations include Tetrahyrofuran (THF), Ethyl acetate (EtOAc), Dichloromethane (DCM), Dimethylformamide (DMF).

2.1 Scheme 1

Methyl 3-oxohept-6-enoate **(2).** Sodium hydride (0.378 g, 9.4 mmol) was dissolved in THF (0.7 M) at -5 °C in an $NaCl/H_2O$ ice bath. Methyl acetoacetate (0.92 mL, 8.61 mmol) was then added dropwise, causing the solution to bubble vigorously. After 20 minutes the solution was clear, and *n*-butyl lithium (3.47 mL, 2.5 M solution in hexanes) was added dropwise. After another 20 minutes of stirring, allyl bromide (0.78 mL, 9.04 mmol) was added. The solution was then allowed to warm naturally to room temperature and react until complete (48 hours). The reaction was quenched with the addition of 1N aqueous HCl. The aqueous solution was then extracted (2×) with diethyl ether. The organic layers were combined, washed with saturated aqueous NaCl, dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified using flash column chromatography (SiO_2, 3 × 14 cm, 10% EtOAc/hexanes). The product was isolated as a yellow oil (839 mg) in 62% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.8 (m,1H), δ 5.05 (m,2H), δ 3.7 (d, 3H), δ 3.46 (s, 2H), δ 2.65 (t, 2H), δ 2.35 (q, 2H).

Benzyl 3-oxohept-6-enoate **(3).** Alkene **2** (1.50 g, 12.92 mmol) was dissolved in toluene (0.15 M). Benzyl alcohol (1.61 mL, 15.50 mmol) was added and the reaction was stirred overnight under nitrogen atmosphere at reflux. The solvent was then removed under reduced pressure and the mixture was purified via flash chromatography (SiO_2, 5 × 30 cm, 5% EtOAc/hexanes). The product was isolated as a clear oil (945 mg) in 63% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (m,5H), δ 5.78 (m,1H), δ 5.2 (s, 2H), δ 5.0 (m, 2H), δ 3.5 (s, 2H), δ 2.61 (t, 2H), δ 2.31 (m, 2H).

Methyl 5-(oxiran-2-yl)-3-oxopentanoate **(4).** Alkene **2** (200 mg, 1.26 mmol) was dissolved in DCM (0.5 M) over 4 *Å* molecular sieves. *Meta*-chloroperoxybenzoic acid (400 mg, 2.32 mmol) was added, and the reaction was allowed to reflux overnight at 35 °C under a nitrogen atmosphere. The reaction mixture was then diluted with ethyl acetate and washed with an saturated aqueous $NaHCO_3$ (2×), H_2O (1×) and an aqueous saturated solution of NaCl (1×). The organic fractions were collected and dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was rapidly purified via flash column chromatography (SiO_2, 40% ethyl acetate/60% hexane, 1.5cm x 10cm column), yielding compound 4 as a white solid (103.1 mg, 47% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.23 (s, 1H), δ 4.53 (m, 1H), δ 3.61 (s, 2H), δ 3.26 (m, 1H), δ 3.0 (m, 2H). Additional reagents are presented in Table 1, following the same procedural outline.

Methyl 7-azido-6-hydroxy-3-oxoheptanoate **(5).** Epoxide **4** (103.1 mg, 0.6 mmol) was stirred with sodium azide (117 mg, 1.8 mmol) and ammonium chloride (96.3 mg, 1.8 mmol) in ethanol (0.12 M) under a nitrogen atmosphere. The reaction was allowed to reflux for 80 minutes at 80 °C. The reaction mixture was then diluted with ethyl acetate and rinsed with H_2O. The organic layer was collected and dried over Na_2SO_4, then concentrated under reduced pressure. The crude reaction mixture (57.7 mg) was carried on to the next reaction.

Methyl 5-(aziridin-2-yl)-3-oxopentanoate **(6).** The crude mixture containing azide **5** was stirred with triphenylphosphine (96.23 mg, 0.37 mmol) in acetonitrile (0.2 M) and allowed to reflux overnight at 80 °C under a nitrogen atmosphere. The reaction mixture was then concentrated under reduced pressure and purified via flash column chromatography (SiO_2, 3 × 14 cm, 30% EtOAc/hexanes elution). ^{1}H NMR did not show the presence of the desired product.

2.2 Scheme 2

tert-butyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate **(9).** Pyrrolidine-2-methanol (1.00 g, 9.88 mmol) was stirred in THF (0.4M) at 0 °C. Triethylamine (2.15 g, 21.26 mmol) was added along with di-*tert*-butyl dicarbonate (2.16 g, 9.88 mmol). The reaction was allowed to stir overnight under a nitrogen atmosphere, warming naturally to room temperature. The reaction was then concentrated under reduced pressure, diluted with saturated aqueous $NaHCO_3$ and extracted with diethyl ether (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The protected pyrrolidine-2-methanol was obtained as a white solid (1.70g, 90% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.95 (m, 1H), δ 3.61 (m, 2H), δ 3.45 (m, 1H), δ 3.3 (m, 1H), δ 3.0 (s, 1H), δ 2.02 (m, 1H), δ 1.8 (m, 2H), δ 1.58 (m, 1H), δ 1.5 (s, 9H).

1-(2-(hydroxymethyl)pyrrolidin-1-yl)ethan-1-one **(10).** Under an inert nitrogen atmostphere pyrrolidine-2-methanol (200 mg, 1.98 mmol) was stirred in acetic anhydride (0.4M) at 0 °C. Triethylamine (0.43 g, 4.25 mmol) was added, followed by 18-crown-6 (0.2 g). The reaction was allowed to stir overnight, warming naturally to room temperature. The reaction mixture was then diluted with diethyl ether, washed with saturated aqueous $NaHCO_3$, washed with saturated aqueous NaCl , and dried over Na_2SO_4. The solvent was then removed under reduced pressure and the desired product was isolated as a brown oil (0.053g, 17% yield). ^{1}H NMR data was unclear.

In the second acetyl protection method attempted, pyrrolidine-2-methanol (200mg, 1.98 mmol) was added to DCM (0.2 M) at 0 °C under an inert nitrogen atmosphere. Acetyl chloride (0.466g, 5.94 mmol) was then added, followed by triethylamine (0.4g, 3.96mmol). The reaction was allowed to stir overnight, warming slowly to 35 °C. After 24 hours, the solvent was removed under reduced pressure and 1N aqueous KOH (10 mL) was added. The reaction continued to stir for 30 minutes. The reaction was then quenched with 1N aqueous HCl , and the aqueous layer was extracted with ethyl acetate (3×). The organic layers were combined, washed with saturated aqueous $NaHCO_3$ (2×) and saturated aqueous NaCl, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was isolated as a brown oil (20 mg, 7%). ^{1}H NMR data was unclear.

2,2,2-trifluoro-1-(2-(hydroxymethyl)pyrrolidin-1-yl)ethan-1-one **(11).** Under an inert nitrogren atmosphere Pyrrolidine-2-methanol (200 mg, 1.98 mmol) was stirred in dry methanol (1.3 M) at 0 °C. Ethyl-triflouroacetate (0.31 g, 1.18 mmol) was added and the reaction was allowed to stir overnight, warming naturally to room temperature. The reaction mixture was concentrated under reduced pressure and then diluted with ethyl ether, washed with ice water, dried over Na_2SO_4, and concentrated under reduced pressure. The product was isolated as a pale yellow oil (0.375g, 96%). While ^{1}H NMR was unclear, thin layer chromatography showed the appearance of a spot less polar than the starting materials and no evidence of starting materials present within the reaction.

2.2.1 procedure for mesylation of protected pyrrolidine:

Under an inert nitrogen atmosphere the protected pyrrolidine (1.7 g, 8.5 mmol) was stirred in DCM (0.5 M) at 0 °C. Dimethylaminopyridine (0.6 molar equivalent) was added portionwise, followed by mesyl chloride (1.2 molar equivalent) added dropwise, and triethylamine (1 molar equivalent). The reaction was allowed to stir overnight, warming naturally to room temperature. The solvent was then removed under reduced pressure, the reaction was diluted with ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate (3×). The organic layers were collected and dried over Na_2SO_4 and then concentrated under reduced pressure.

tert-butyl 2-(((methylsulfonyl)oxy)methyl)pyrrolidine-1-carboxylate **(13).** The product was isolated as a pale orange oil (2.1 g) in 88% yield. ^{1}H NMR data was unclear.

(1-acetylpyrrolidin-2-yl)methyl methanesulfonate **(14).** The product was isolated as a brown oil (48 mg) in 62% yield. ^{1}H NMR data was unclear.

(1-(2,2,2-trifluoroacetyl)pyrrolidin-2-yl)methyl methanesulfonate **(15).** The product was isolated as a pale oil (73 mg) in 26% yield. ^{1}H NMR data was unclear.

(1-phenylpyrrolidin-2-yl)methyl methanesulfonate **(16).** The mixture was purified using flash column chromatography (SiO_2, 3 × 14 cm, 30% EtOAc/hexanes). The product was isolated as a pale oil (226 mg) in 85% yield. ^{1}H NMR data was unclear.

2.2.2 procedure for chlorination of protected pyrrolidine:

The protected pyrrolidine was dissolved in chloroform (0.4 M) with thionyl chloride (2 molar equivalents) The reaction was allowed to reflux for 4 hours. The solvent was then removed under reduced pressure and the reaction residue was diluted with 1N aqueous NaOH and extracted with DCM (2×). The organic layers were combined, washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure.

tert-butyl 2-(chloromethyl)pyrrolidine-1-carboxylate **(17).** No product identified.

1-(2-(chloromethyl)pyrrolidin-1-yl)ethan-1-one **(18).** No product identified.

1-(2-(chloromethyl)pyrrolidin-1-yl)-2,2,2-trifluoroethan-1-one **(19).** The product was isolated as a pale oil (21 mg) in 12% yield. ^{1}H NMR data was unclear.

2-(chloromethyl)-1-phenylpyrrolidine **(20).** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.4 (m, 5H), δ 4.1 (m, 1H), δ 3.58 (s, 2H), δ 3.07 (m, 1H), δ 2.78 (m, 1H), δ 2.08 (m, 3H), δ 1.7 (m, 3H).

2.2.3 procedure for alkylation of pyrrolidine

Protected 1-(3-nitrophenyl)-3-(pyrrolidin-2-yl)propan-1-one **(21).** Sodium hydride (60% in mineral oil, 1.1 molar equivalents) was stirred for 20 minutes in dry DMF (0.5 M) at 0 °C under an inert nitrogen atmosphere. Next, 3-nitroacetophenone (1.1 molar equivalents) was added dropwise to the flask. Upon addition, the reaction bubbled vigorously and turned bright red. After an additional 30 minutes of stirring, the protected pyrrolidine with –Cl or –OMs leaving group was added (200 mg). The reaction was allowed to stir overnight under nitrogen atmosphere, warming naturally to room temperature. The reaction was quenched with 1N aqueous HCl, diluted with saturated aqueous NaCl, and extracted with ethyl acetate (3×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. No desired product was identified.

2.2.4 procedure for alkylation of pyrrolidine with enolate transfer (see Table 2 for variations)

Sodium hydride (60% in mineral oil, 1.1 molar equivalents) was stirred for 20 minutes in dry solvent (0.5 M) at 0 °C under an inert nitrogen atmosphere. Next, acetophenone (3 molar equivalents of pyrrolidine) was added dropwise to the flask. Upon addition, the reaction bubbled vigorously and exhibited a color change to bright red (*m*-nitroacetophenone) or yellow (*o*-methoxyacetophenone). After 30 minutes of stirring, the solution was allowed to settle, and the volume containing 1 equivalent of acetophenone was removed from the top of the solution and added to a separate flask containing the pyrrolidine at the indicated temperature. The reaction was quenched with 1N aqueous HCl, diluted with saturated aqueous NaCl, and extracted with ethyl acetate (3×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. No desired product was identified.

2.2.5 procedure for alkylation of pyrrolidine by Grignard addition

Method (A): The reaction was conducted in flame-dried glass under an inert argon atmosphere. Pyrrolidine **20 (**50 mg, 0.24 mmol) was stirred in THF (0.1M) along with magnesium turnings (0.029 g, 1.19 mmol) and a catalytic amount of I_2 at 60°C for 1 hour. Then, 1-bromo 3',4'5'-trimethoxyacetophenone was added (0.058 g, 0.24 mmol). The reaction was allowed to stir overnight at room temperature, then heated to 50°C for another 24 hours. The reaction mixture was then diluted with ethyl acetate and saturated aqueous NH_4Cl, and the organic layer was extracted and washed with saturated aqueous NaCl and concentrated under reduced pressure. No desired product was identified.

Method (B): The reaction was conducted in flame dried glass under an inert argon atmosphere. 1-bromo 3',4'5'-trimethoxy acetophenone (0.058 g, 0.24 mmol) was stirred in THF (0.1M) along with magnesium turnings (0.029 g, 1.19 mmol) and a catalytic amount of I_2. After stirring overnight, the color changed to a light milky color. Then, Pyrrolidine **20** (50 mg, 0.24 mmol) was added. The reaction was allowed to stir for another 24 hours. The reaction mixture was then diluted with ethyl acetate and saturated aqueous NH_4Cl, and the organic layer was extracted and washed with saturated aqueous NaCl and concentrated under reduced pressure. No desired product was identified.

2.3 Scheme 3

tert-butyl (2-bromoethyl)carbamate **(24).** Under an inert nitrogen atmosphere bromoethylamine (0.45 g, 2.10 mmol) was stirred in THF (0.4 M) at 0 °C. Triethylamine (0.45 g, 4.45 mmol) was added, followed by di-*tert*-butyl dicarbonate (0.45 g, 2.10 mmol). The reaction was allowed to stir overnight, warming naturally to room temperature. The reaction was then diluted with saturated aqueous $NaHCO_3$ and extracted with diethyl ether (2×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The product was isolated as a pink oil (0.44 g, 88%). (^{1}H NMR).

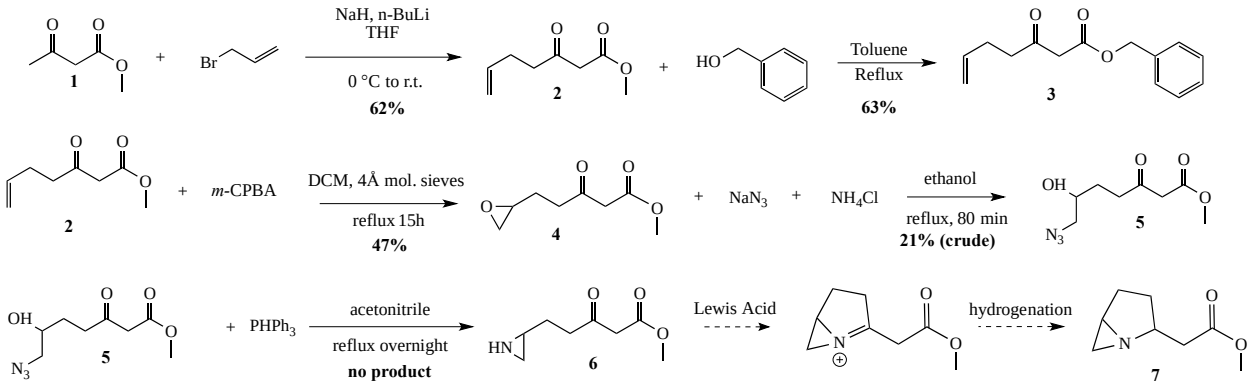
tert-butyl (2-(2-oxocyclopentyl)ethyl)carbamate **(25).** Sodium hydride (60% in mineral oil, 0.116 g, 2.9 mmol) was stirred in THF (0.1 M) at 0 °C under an inert nitrogen atmosphere. After 20 minutes of stirring, cyclopentanone (0.222 g, 2.64 mmol) was added dropwise, causing the reaction to bubble. After an additional 30 minutes of stirring, the protected bromoethylamine **12** (600 mg, 2.64 mmol) was added. The reaction was allowed to stir overnight, warming naturally to room temperature. The reaction was then quenched with 1N aqueous HCl, diluted with saturated aqueous NaCl, and extracted with ethyl acetate (2×). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using flash chromatography (SiO_2, 3 × 14 cm, 10% EtOAc/hexanes elution). The product was isolated as a yellow oil (312 mg, 52%). (NMR)

octahydrocyclopenta[b]pyrrole **(26).** Cyclopentanone **25** was dissolved in DCM (0.18 M) at room temperature. To this, trifluoroacetic acid (0.5 mL, excess) were added. The reaction was allowed to stir for 4 hours. The solvent was then evaporated by reduced pressure and the crude residue was combined with sodium borohydride (27.4 mg, 0.726 mmol) in dry methanol (0.009 M) at 0°C. The reaction was quenched with 0.1 mL acetic acid and allowed to stir for an additional 10 minutes. The solvent was then evaporated under reduced pressure, and the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and diethyl ether .The aqueous layer was confirmed to be neutral and extracted with diethyl ether (3×).The organic layers were combined, washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. No desired product was identified.

3. Results and Discussion

While the goal of this work was to optimize reductive amination conditions for the formation of azabicycles, much of the difficulty was encountered in creating the precursor molecules necessary for testing the cyclization step. A few different approaches were explored in attempting to create azabicyclic cores with various ring sizes.

Scheme 1 shows the steps taken in the first attempt to synthesize the azabicycle core of ficellomycin. In the first reaction, compound 1.1 was synthesized in good yield from commercially available methyl acetoacetate and allyl bromide. However, subsequent reactions proved to be less successful. The first trials of the epoxidation yielded faintly visible spots by TLC, but these could not be reliably visualized during attempted purification via flash column chromatography. From this and further literature research it was concluded that the epoxide was unstable on the SiO_2 column. However, using the epoxidation crude residue in the next reaction without any purification did not yield the desired product. Rapid purification on a smaller column allowed for successful isolation of compound 2. In addition to problems with purification, the highest yield achieved with the epoxidation was only 47%, even with the use of molecular sieves and a nitrogen atmosphere to prevent the *m*-CPBA from reacting with either ambient water vapor or water produced in the reaction. With the small amount of product in hand, the creation of the azide alcohol was attempted. The product of this reaction, compound 3, again proved to be unstable. After trouble with initial visualization of this compound and a seeming lack of product, it was decided that the azide was likely decomposing in the presence of light and the crude product was carried on to the formation of the aziridine, compound 6. Column chromatography of the product mixture yielded 3 spots, but examination using ^1H NMR gave inconclusive elucidation of the compound.



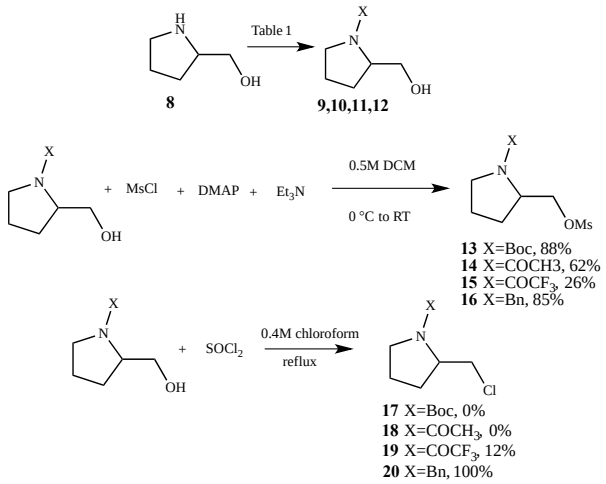
Scheme 1. First attempt at synthesizing ficellomycin azabicycle core, beginning with commercially available methyl acetoacetate.

Continuing work on this scheme has first solved the problem of visibility by replacing the methyl group in the alkene (compound 1.2) with a benzyl group. Optimization of the epoxidation reaction was attempted, with conditions shown in Table 1.

Table 1. Epoxidation conditions

Reaction Conditions	Yield
m-CPBA, DCM (0.2M), reflux	47% (inconsistent)
H_2O_2 (30%), NaOH, methanol (0.05M), 0° C	Resulted in removal of Bz from **3**
H_2O_2 (30%), NaOH, THF (0.05M), 0° C	Not proceeding to completion
H_2O_2 (30%), DBU, Acetonitrile (0.1M), reflux	Not proceeding to completion

Schemes 2 and 3 show the second approach to the general synthesis of a simple, unstrained azabicyclic system (compound 22). In the first reaction, the protection of the amine in pyrrolidine-2-methanol with a di-*tert*-butyl dicarbonate was achieved in good yield. In the next step, both a chlorine and a mesyl substitution at the alcohol were attempted; however, only the mesylate reaction gave good yield for the Boc-protected pyrrolidine. The first attempt at the alkylation of this product with *m*-nitroacetophenone led to a surprising cyclization at the Boc group, forming a carbamate bicycle which has previously been reported[14].



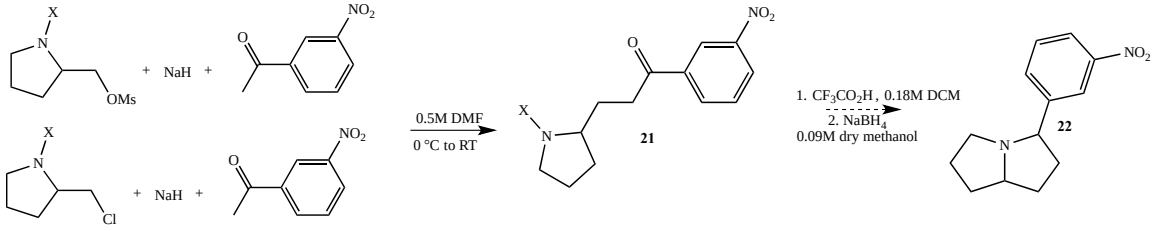
Scheme 2. Synthetic approach to the 5,5 azabicycle beginning with pyrrolidine 2-methanol.

Table 2 shows additional protecting methods attempted after the discovery of the side product formed by the Boc protected pyrrolidine. The triflouroacetyl group gave the highest yield, and the purchase of the commercially available benzyl-protected pyrrolidine eliminated the protection step altogether. The results of the chlorination and mesylation of each protected pyrrolidine are shown in Scheme 2. Mesylation gave the highest yields.

Table 2. Protection conditions

Amine Protecting Group	Reaction Conditions	% Yield
9 Boc	Boc_2O, Et_3N, 0.4M THF	90%
10 Acetyl	Et_3N, 18-crown-6 (cat.), 0.4M acetic anhydride	17-32%
10 Acetyl	Acetyl Chloride, Et_3N, 0.2M CH_2Cl_2, Δ	7%
11 Ethyl Trifluoroacetyl	Ethyl trifluoroacetate, 1.3M anhydrous CH_3OH	96%
12 Benzyl	Commercially Available	n/a

Finally, each of these compounds was subjected to the same alkylation conditions for coupling with *m*-nitroacetophenone (Scheme 3.). However, characterization of the products did not give evidence for the production of compound 10. Side products appeared to be either a hydride substitution at the leaving group site, or a β-elimination at that site.



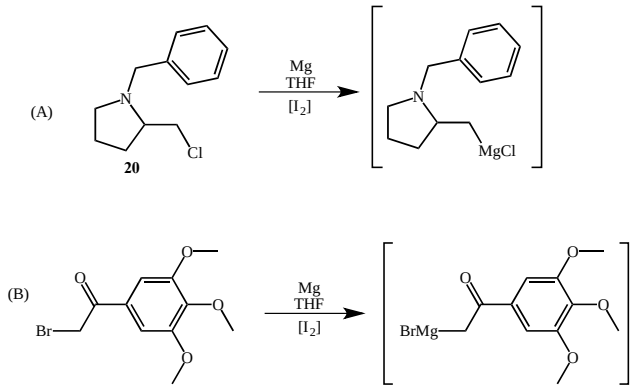
Scheme 3. Alkylation conditions to join the acetophenone to the pyrrolidine starting material.

Due to the lack of success with this method of alkylation, a couple of other approaches were also attempted. First, in order to prevent side reactions such as the hydride substitution, the enolate was formed first, allowing the sodium hydride to settle, and the enolate was then added to a separate flask containing the pyrrolidine starting material. Table 3 shows the different reaction conditions tested with this method. *o*-methoxy acetophenone was also used in this method in hopes of increasing the reactivity of the enolate. However, no desired product (**21**) was observed.

Table 3. Reaction conditions for addition of enolate to pyrrolidine.

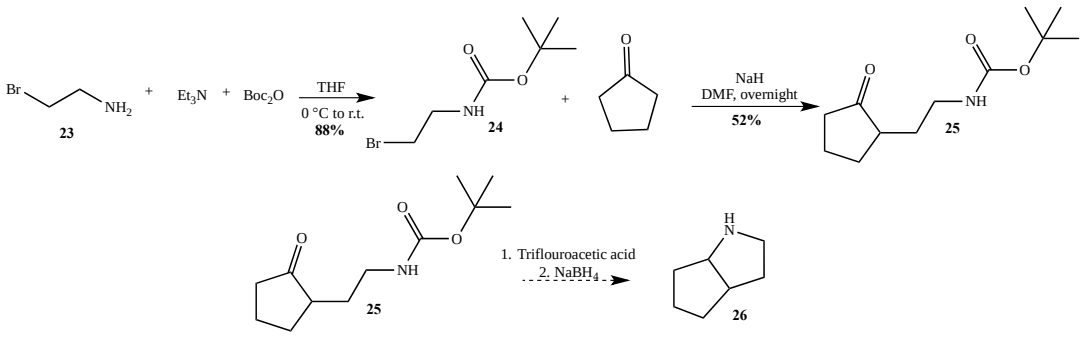
Acetophenone	Leaving Group	Base	Solvent	Temperature
m-nitro	-OMs	NaH	0.1M DMF	0° C to r.t.
m-nitro!	-Cl	NaH	0.1M DMF	0° C to r.t.
m-nitro	-OMs	NaH	0.1M DMF	-78° C to r.t.
m-nitro!	-Cl	NaH	0.1M DMF	-78° C to reflux
o-methoxy	-Cl	NaH	0.1M DMF	-78° C to reflux
o-methoxy	-Cl	NaH	0.1M THF	-78° C to reflux

Additionally, the nucleophile and electrophile reactivities were reversed by attempting the alkylation via formation of a Grignard reagent (Scheme 4). The acetophenone used for this approach was a 1-bromo 2',3'4' trimethoxyacetophenone. Utilizing either this reagent or the chlorinated pyrrolidine as the Grignard reagent did not result in formation of the desired product.



Scheme 4. Formation of Grignard reagents, methoads (A) and (B)

Scheme 5 shows another model system for the study of the intramolecular reductive amination reaction. The commercially available bromoethyl amine was Boc-protected in good yield in the first reaction of this scheme. The second reaction utilized sodium hydride to form the enolate of cyclopentanone, which successfully gave the alkylated compound 13 in moderate yield. The final step was attempted using trifluoroacetic acid and sodium borohydride as the hydrogenating reagent, but the desired compound 14 was not detected within the reaction mixture, possibly due to volatilization of this product.



Scheme 5. Alternate study of intramolecular reductive amination

4. Conclusion

To date the synthesis of the target azabicycles has not been achieved, and the method of intramolecular reductive amination has not been tested for the ring closure step. However, if difficulties in creating the correct precursor molecules can be overcome, this approach may still be applied to construction of these medicinally relevant azabicycles beginning from inexpensive starting materials. Obstacles encountered thus far include the poor yield of the epoxidation reaction and instability of the azide compound in Scheme 1 and the low reactivity of the pyrrolidine compounds used in Scheme 2. Azabicyclic compounds remain an important and elusive synthetic target and further work on these compounds will allow access to many biologically active natural products.

5. Acknowledgements

The author would like to acknowledge funding from the UNC-Asheville Undergraduate Research Program, the UNC Asheville Laurels Fellows Scholarship, and North Carolina Biotechnology Center grant 2015-BRG-1201.

6. References

1. Argoudelix, A.; Reusser, H.; Whaley et al. "Antibiotics produced by Streptomyces ficellus." *J. of Antibiot.* **1976**, *29*, 1001-1019.
2. Kuo, M.; Yurek, D.; Mizsak, S. "Structure elucidation of ficellomycin." *J. of Antibiot.* **1989**, *42*, 357-360.
3. Reusser, F. "Ficellomycin and Feldamycin; Inhibitors of bacterial semiconservative DNA replication." *Biochem.* **1977**, *16*, 3406-3412.
4. Abrams, J.; Babu, R.; Guo, H. et al. "De Novo Asymmetric Synthesis of 8a-epi-Swainsonine." *J. Org. Chem.* **2008**, *73*, 1935-1940.
5. Hu, Xiang-Guo; Jia, Yue-Mei; Xiang, Junfeng; Yu, Chu-Yi. "Exploratory Studies en Route to 5-Alkyl-Hyacinthacines: Synthesis of 5-epi-(–)-Hyacinthacine A3 and (–)-Hyacinthacine A3." *Synlett* **2010**, *6*, 0982-0986.
6. Watanabe Coran M. H., et al. "Mode Of Action And Biosynthesis Of The Azabicycle-Containing Natural Products Azinomycin And Ficellomycin." *Nat. Prod. Rep.* **2011**, *28*, 693-704.
7. Paumier, D. Rapid Assembly of the 1-Azabicyclo[3.1.0]hexane Skeleton of Ficellomycin. *Synlett* **2004**, *12*, 2212-2214.
8. Garcia, Maria. Toward the total synthesis of ficellomycin. Thesis, University of Warwick, 2007.
9. Hartley, J.; Hazrati, L.; Kelland et al. "A synthetic azinomycin analog with demonstrated DNA cross-linking activity: insights into the mechanism of action of this class of antitumor agent." *Angew. Chem. Int. Ed.* **2000**, *39*, 3467-3470.
10. Coleman, Robert S. Total synthesis of natural product DNA cross-linking agents. *Curr. Opin. Drug Disc. Develp.* **2001**, *4*, 435-439.

11. Ismail, F; Levitsky, D.O; Dembitsky, V.M. Aziridine Alkaloids as Potential Therapeutic Agents. *Eur. J. Med. Chem.* **2009**, *44*, 3373-3387

12. Chen, G.; He, Z.; and Yudin, A. K. "Synthesis of functionalized 1-azabicyclo[310]hexanes: Studies towards ficellomycin and its analogs." *Heterocycles* **2014**, *88*, 1299-1310.

13. Lagu, B.; Pan, M.; Wachter, M. "A versatile synthesis of polysubstituted pyrroles." *Tet. Lett.* **2001**, *42*, 6027-6030

14. Curran, Timothy P. et. al. "Loss of the tert-butyloxycarbonyl (Boc) protecting group under basic conditions." *Tetrahedron Lett.* **1994**, *35*, 5409-5412