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Synthetic strategies to combat antibiotic resistance

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Synthetic strategies to combat antibiotic resistance

BY

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Synthetic strategies to combat antibiotic resistance

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on December 12, 2018

Approval signatures are on file with the University of New Hampshire Graduate School.

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-Dedication-

This thesis is dedicated to the instructors who have guided me thought my journey of chemistry. This is a testimony for all the hard work you put into my education and instilling a passion for learning and exploring the wonderful world of chemistry. Each, and everyone, I truly thank you.

-Acknowledgements-

I would like to acknowledge my family for supporting me through this process. No matter what was going on, your support allowed me to push forward. Also, to the friends that I have made at my time at UNH for giving me the support that only a fellow researcher could understand.

I would also like to acknowledge my advisor, Dr. Marc A. Boudreau and fellow Boudreau group members for helping me throughout my time at UNH. You have all moved from being friends to family, and I thank you from the bottom of my heart. Thank you.

-List of Schemes-

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-Abbreviations-

PBP: Penicillin-binding protein

MRSA: Methicillin-resistant *Staphylococcus aureus*

EtOH: Ethanol

HCl: Hydrochloric acid

CH_2Cl_2: Dichloromethane

$(CH_3)_3SI$: Trimethylsulfonium iodide

rt: room temperature

h: hour

NaHMDS: Sodium bis(trimethylsilyl)amide

THF: Tetrahydrofuran

NaH: Sodium hydride

EtOAc: Ethyl acetate

DMSO: Dimethyl sulfoxide

$TiCl_4$: Titanium tetrachloride

Et_3N: Triethyl-amine

CH_3CN: Acetonitrile

PTFE: Polytetrafluoroethylene

NMR: Nuclear magnetic resonance

$LiAlH_4$: Lithium aluminum hydride

$NaBH_4$: Sodium borohydride

Et_2O: Diethyl ether

DMP: Dess–Martin periodinane

PCC: Pyridinium chlorochromate

TBDMSCl: *tert*-Butyldimethylsilyl chloride

Bu_4NF: Tetra-*n*-butylammonium Fluoride

NIS: *N*-iodosuccinimide

MeOH: Methanol

TLC: Thin-layer chromatography

HBr: Hydrobromic acid

LDA: Lithium diisopropylamide

COSY: Homonuclear correlation spectroscopy

HSQC: Heteronuclear single quantum coherence spectroscopy

HMBC: Heteronuclear multiple bond correlation spectroscopy

HRMS: High resolution mass spectrometry

*mec*A: Methicillin resistance gene

p-TSOH: *p*-Toluenesulfonic acid

HOSA: Hydroxylamine-*O*-sulfonic acid

PPh_3: Triphenylphosphine

DIPEA: *N*,*N*-Diisopropylethylamine

-Abstract-

Synthetic strategies to combat antibiotic resistance

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The design and synthesis of β-lactamase inhibitors is a constant area of study to overcome the growing problem of resistance to β-lactam antibiotics. Serine dependent β-lactamase enzymes, through a hydrolysis mechanism, can deactivate many traditional β-lactam antibiotics. Designing drugs to specifically target β-lactamases is of great interest because such drugs could be used in tandem with traditional β-lactam antibiotics. Aza-β-lactam molecules have been theorized to be less vulnerable to hydrolysis than the traditional β-lactams. Nucleophilic attack of the active-site serine of the β-lactamase on the aza-β-lactam ring would lead to a carbamoyl-enzyme intermediate, which would be partially stabilized due to the neighboring nitrogen atom and thus is expected to be more hydrolytically stable than the corresponding acyl-enzyme intermediate of a β-lactam.

The initial attempt to synthesize the aza-carbacepham **1** utilized a rhodium catalyzed N-H insertion as the key step to form the substituted piperidine ring fused to the aza-β-lactam ring. With the use of aldehyde **8,** a Wittig reaction was conducted to extend the carbon chain, but problems arose with the removal of the *N*-acetyl group. Multiple pathways were attempted but all

were unsuccessful in cleaving the *N*-acetyl group, so a different synthetic approach was explored that utilized an aldol reaction to form the respective ring.

The synthesis of aza-carbapenam **33** originally proceeded via enol-ether **34a/b** followed by acid catalyzed conversion to the corresponding alcohol **35**. But due to low yields, a different approach involving hydroboration of alkene **47** was explored. With the use of a model system, the Wittig conditions were optimized and applied to aldehyde **8** to form alkene **47**. Traditional hydroboration conditions were then attempted with the formation of unexpected product **48**.

Rapamycin has been found to potentiate oxacillin activity against several strains of methicillin-resistant *Staphylococcus aureus* (MRSA), but its mechanism of action is unknown. The interaction of rapamycin with oxacillin and MRSA are going to be studied with a photoaffinity labeling process. Diazirine linker **49** was synthesized and initial steps to alkylate rapamycin were attempted. NMR data of rapa-linker **50** is still inconclusive due to low isolated yields.

-Chapter 1-

-Introduction-

The β-lactam antibiotic penicillin was discovered in 1928 by Alexander Fleming.[1] The full potential of penicillin was not understood until the 1940s, when the era of antibiotics truly began as the demand for medication increased drastically. With the demand for large-scale production due to World War II, penicillin became one of the first antibiotics on the market.[2] The effectiveness of penicillin then brought on the discovery of other compounds that had similar bacterial properties. Chemists were eager to apply the antibiotic properties of the β-lactam ring of penicillin and began extensively researching natural product isolation and synthesis. The β-lactam nucleus, 6-aminopenicillanic acid, was discovered to be crucial when synthesizing and modifying penicillin.[3] Cephalosporin C was isolated from a strain of *Cephalosporium acremonium*, and this brought on an entirely new family in the β-lactams.[3] The β-lactam family includes penicillins, cephalosporins, carbapenems, and monobactams represented below in Figure 1.[4]

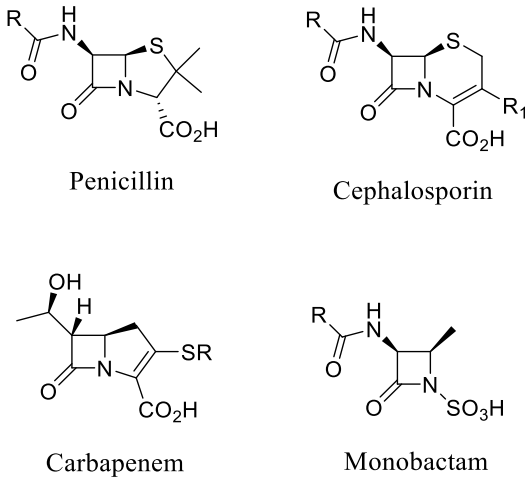
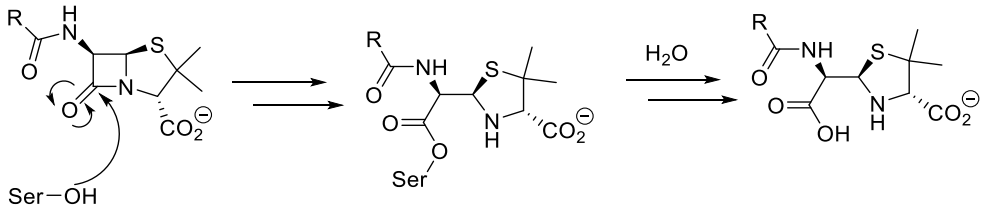


Figure 1. The four common classes of β-lactam antibiotics used in the clinic.

The β-lactams have been a powerful tool as a treatment for bacterial infections leading them to become a commonly prescribed class of antibiotics. The antibacterial property comes from the peptide mimic; penicillin mimics a component of the cell wall which allows it to bind to an enzyme that constructs and strengthens the cell wall.[5,6] A penicillin-derived entity is irreversibly bound by the transpeptidase and carboxypeptidase enzymes essential for bacterial cell wall synthesis. These biosynthetic enzymes are obvious therapeutic targets leading to inhibition of the peptidoglycan cross-linking transpeptidase that cleaves the peptidoglycan D-Ala-D-Ala terminus in the serine acylation half-reaction.[5,6] With loss of the terminal D-Ala, a cross-link is formed in the deacylation half-reaction. Should this enzyme bind a substrate mimic, the initial acylation reaction remains unaffected, but the deacylation mechanism is abolished, the enzyme will fail to complete cell wall synthesis.[5,6]

With the synthesis halted, the cell wall loses its integrity leading to bacterial cell death.[5,6] The crystal structures of these enzymes have been studied to identify the enzyme active sites and determine which interactions are essential to the high binding affinity of β-lactam antibiotics.[7] Due to extensive overuse and misuse, bacteria have developed mechanisms of defense against the β-lactam antibiotics. Only a few years after penicillin was used clinically, reports of penicillin-resistant *Staphylococcus aureus* emerged.[8] This was mainly due to the expression of β-lactamase enzymes. These enzymes hydrolyze the β-lactam ring eliminating the drug's antibacterial activity.

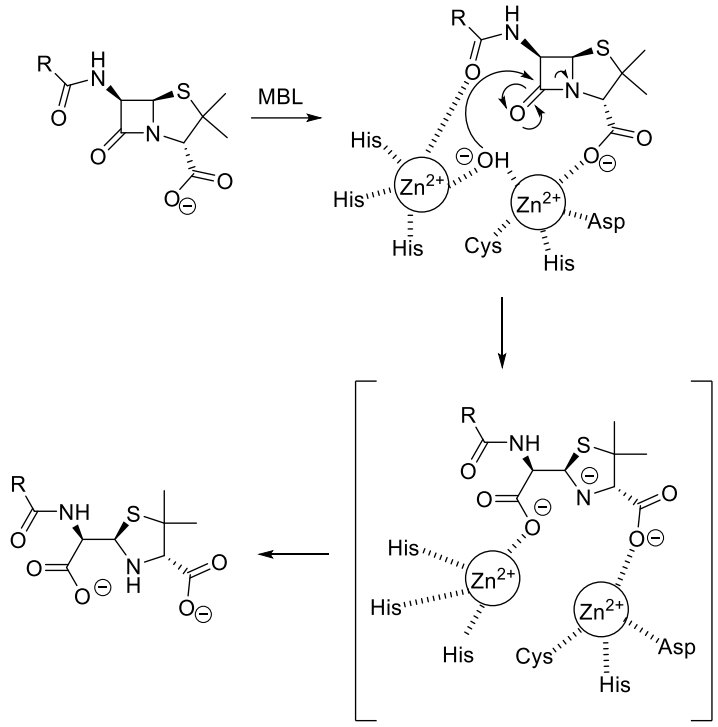
The β-lactamase enzymes are divided into classes A, B, C, and D. Classes A, C, and D utilize an active site serine to initiate β-lactam hydrolysis (Scheme 1).[9] Each follow a similar mechanism of hydrolysis, but differ in the catalytic residue to hydrolyzes the β-lactam ring. Class C utilizes a lysine-tyrosine pair for the catalytic residue, compared with class D, which is activated by a carboxylated-lysine.[10,11]



Scheme 1. The hydrolysis of a β-lactam by a serine-β-lactamase.

With class D, a serine nucleophile attacks the lactam carbonyl yielding a covalent acyl-enzyme intermediate. The tetrahedral oxyanion intermediate for this acylation reaction is stabilized by two main-chain amides and electrostatically by a second active-site lysine. The ester linkage is then hydrolyzed by attack of the α face of the ester by an active-site water affording an inactivated β-lactam.[11] Much progress has been made in attempts to prevent the hydrolysis of the acyl-enzyme intermediate, but more distinct enzyme types continue to be discovered. As of 2017, the Comprehensive Antibiotic Resistance Database (CARD) lists over 1200 different serine-β-lactamase enzymes.[12]

Class B β-lactamases employ one or two Zn^{2+} ions to hydrolyze the β-lactam antibiotic (Scheme 2).[13] The role of the Zn^{2+} ions is to catalytically activate the hydrolytic water to open the β-lactam ring, and simultaneously stabilize the reaction intermediates.[14] Metallo-β-lactamases have been viewed as less of a threat due to their more geographically localized outbreaks of the enzyme, but the enzyme's molecular epidemiology is changing.[13] This change is driven by the emergence of mobile genetic elements containing metallo-β-lactamase genes that are now frequently found in Gram-negative pathogens including *Enterobacteriaceae, Pseudomonas,* and *Acinetobacter* species across the globe.[15,16]



Scheme 2. Mechanism of metallo-β-lactamase catalyzed hydrolysis of β-lactam antibiotic.

In 1959 various penicillin analogues such as methicillin were developed in attempts to sterically protect the β-lactam ring.[17] Shortly after methicillin was brought to the market, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were detected.[18] The increased resistance to methicillin was due to alterations of the β-lactamase enzyme that resulted in a lower affinity for the β-lactam.[19] The restriction to the active site made treatment of the bacteria difficult, and the growing emergence of MRSA identified a need for new treatment options.

Cephalosporins were discovered in the late 1940s, and were found to be less susceptible to deactivation by *staphylococcal* β-lactamase.[8] Early variations of cephalosporins were found to be active against *staphylococci*, but were found to be even more beneficial against gram-negative organisms such as *Pseudomonas aeruginosa*.[17] Carbapenems and monobactams, discovered in 1985, were found to be useful against bacteria typically unaffected by other β-lactam.[20]

Carbapenems have been reserved for treatment of the most difficult bacterial infections, however, resistance to them has become more widespread due to the increased prevalence of carbapenemases.[8] Carbapenemases represent the most versatile family of β-lactamases, with an extensive spectrum of β-lactam-hydrolyzing enzymes. Although known as "carbapenemases," many of these enzymes recognize almost all hydrolyzable β-lactams, and most are resilient against inhibition by many commercially available β-lactamase inhibitors.[21]

The current methods to combat β-lactam resistance include: designing new β-lactam antibiotics that are less susceptible to hydrolysis, or designing new β-lactamase inhibitors to prevent β-lactam hydrolysis.[8] Designing new β-lactam antibiotics is an ongoing process, but the production of β-lactamase inhibitors has been more successful. In 1972 clavulanic acid was found to have limited antibiotic activity despite the β-lactam ring (Figure 2), however it shows a high affinity for β-lactamase enzymes.[22] In 1984, Augmentin, which contains clavulanic acid and amoxicillin, became the first β-lactam/β-lactamase inhibitor antibiotic and in 2001 Augmentin became the best-selling antibiotic on the market.[8]

With much work being done to find potential inhibitors of β-lactamases, diazabicyclooctanes have shown promising results.[23] Mimics of β-lactams, such as Avibactam (Figure 2), have been found to have activity against class A, C, and D β-lactamase.[24] This is due to the unique carbamoyl acyl-enzyme intermediate that does not decompose by a hydrolytic mechanism.[25] In its place, the decarbamylation occurs by a recyclization of the diazabicyclooctanes fused ring to reform the inhibitor to then inactivate more serine-β-lactamase.[25]

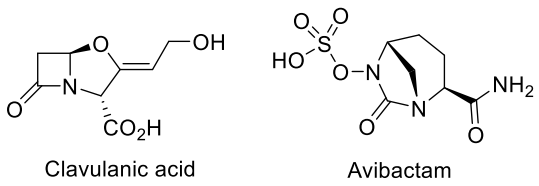


Figure 2. Structure of Avibactam and Clavulanic acid.

Bicyclic imidazolidinones represented in Figure 3, are found to also have interesting inhibition ability.[26] When tested, low antimicrobial activity was observed against bacteria for imidazolidinones **A**, **B**, **C,** and **D** from figure 3.[26,27]

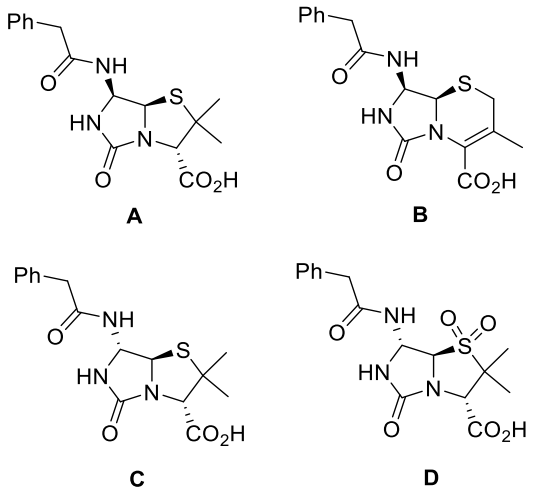
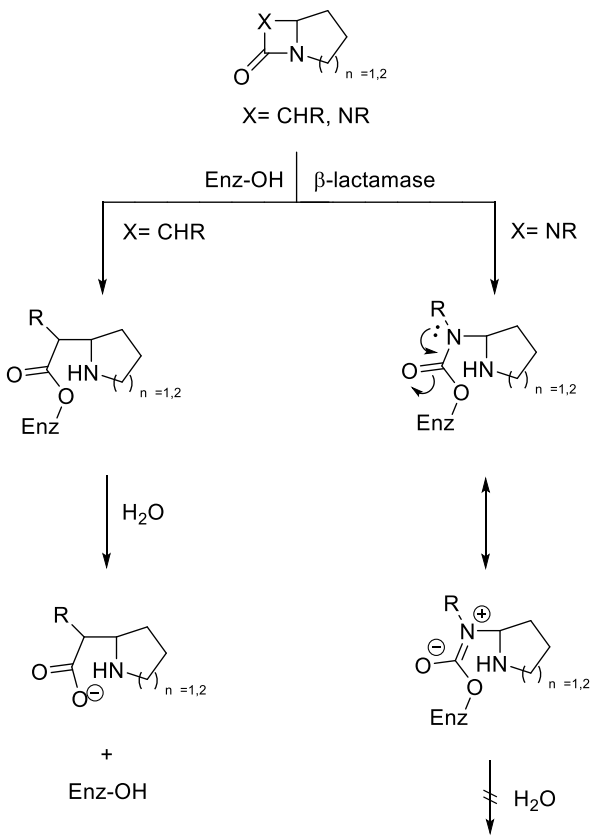


Figure 3. Examples of β-lactam mimics with a bicyclic imidazolidinone framework.

Chandrakala *et al.* explored a synthetic route to make 1,3-diazetidin-2-ones (aza-β-lactams) and proposed that the aza-β-lactam could theoretically have high inhibition ability against β-lactamases.[26] The process begins with the nucleophilic attack of the serine β-lactamase on the non-traditional β-lactam ring, which leads to a carbamoyl-enzyme intermediate that is partially stabilized due to the neighboring nitrogen atom. The carbamate acyl-enzyme intermediate is expected to be more hydrolytically stable than the corresponding ester acyl-enzyme intermediate of a β-lactam (Scheme 3).



Scheme 3. Resonance stabilization of the acyl-enzyme intermediate of an aza-β-lactam with a β-lactamase compared to a traditional β-lactam with a β-lactamase.

In the more traditional β-lactam structure, extra stabilization is not present because the enzyme attacks the β-lactam ring and forms a corresponding ester that can readily be hydrolyzed to leave the β-lactam deactivated. With growing resistance due to serine-β-lactamases and metallo-β-lactamases, new treatment options are of great interest. The aza-β-lactam analogues are close structural mimics of β-lactams and also benefit from resonance stabilization in the acyl-enzyme intermediate. Aza-β-lactam structures have not been examined for activity against β-lactamases, so an efficient synthetic route is required to synthesize a library of derivatives.

-Results and Discussion-

The Johnson research group at University of New Hampshire conducted computational studies to determine whether or not several different acyl-enzyme intermediates were less susceptible to hydrolysis than an ester.[28] The C_6 position of the *β*-lactam was substituted with different heteroatoms, which would form an acyl-enzyme ring linkage.[28] The C_6 position was substituted with nitrogen, oxygen, and a sulfur to study the different strengths of the respective acyl-enzyme intermediate (Figure 4).

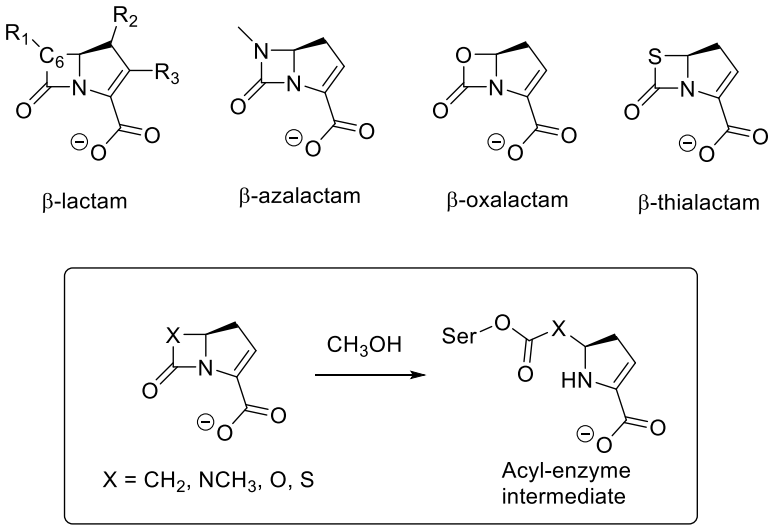


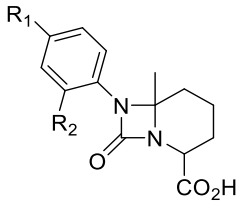
Figure 4. Structures explored computationally for the relative strength of the acyl-enzyme linkage.

The potential energy surface of the methanol-assisted hydrolysis of the acyl-enzyme intermediate was compared for each substituted variation. Based on the computational results, all derivatives have a carbamate acyl-enzyme linkage that is less susceptible to hydrolysis compared to the ester of the typical β-lactam (X= CH_2), except for the oxalactam derivative.[28] The data also suggest that the *N*-acetyl aza-β-lactam would be the most reasonable to form acyl-enzyme intermediate that is less susceptible to the methanol-assisted hydrolysis.[28] With promising

computational data, investigation to efficiently synthesize various aza-β-lactam derivatives was explored.

In order to test the aza-β-lactam compounds for inhibitory activity against β-lactamases, a simple and efficient synthetic pathway is needed. Ideally the pathway would utilize the same chemistry but have minor variations to produce multiple derivatives. The first compound of interest was an aza-carbacepham (Figure 5, **1**). After a general synthetic pathway is determined, the phenyl ring will be functionalized with an electron withdrawing or donating group. Due to the conjugation of the structure, the addition of select groups could change the electronics of the aza-β-lactam ring, which may affect the acylation or deacylation rates during the reaction with β-lactamases.

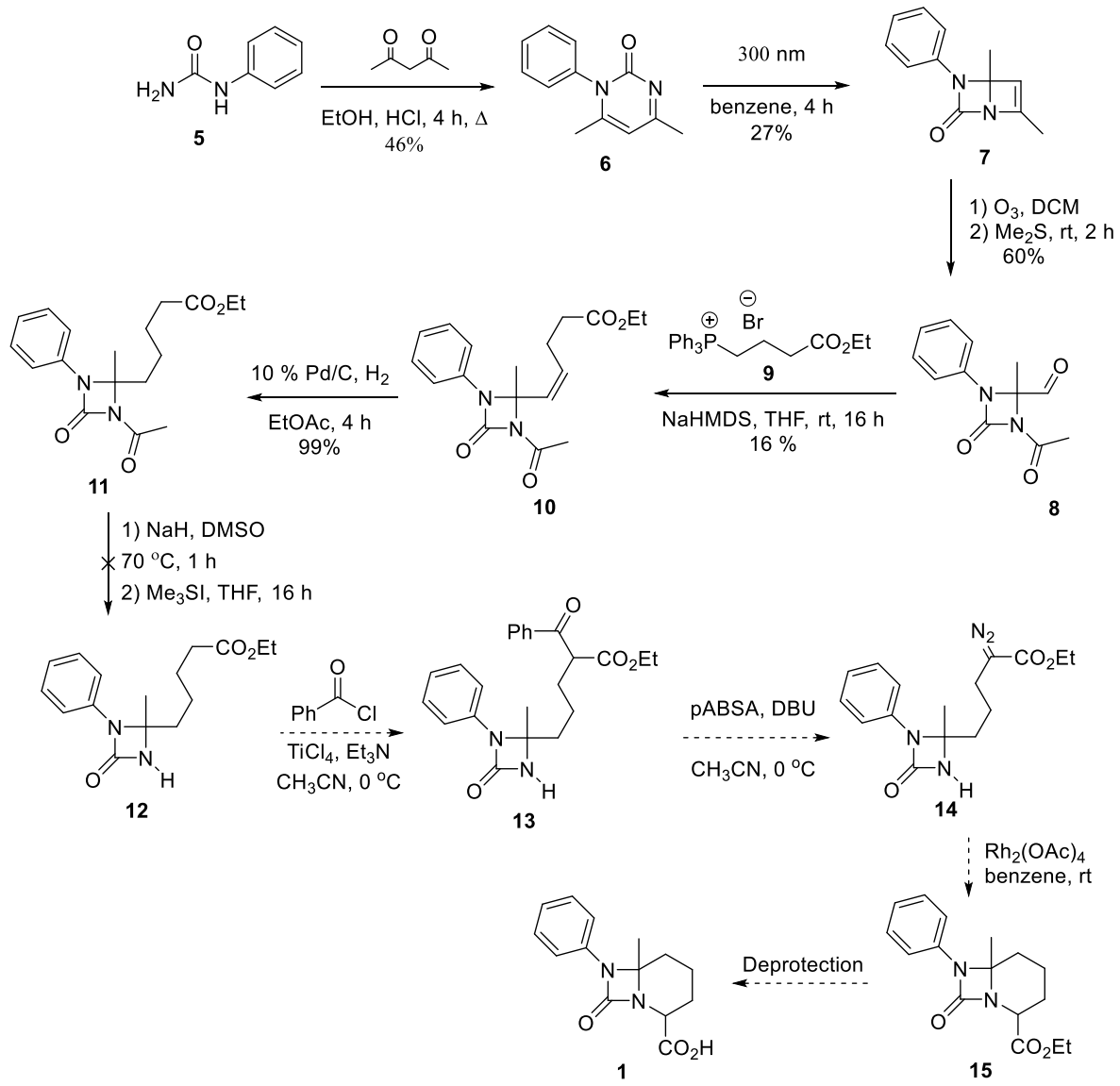
The initial pathway utilized a rhodium-catalyzed N-H insertion for the formation of the respective ring attached to the aza-β-lactam ring (Scheme 4).[29] The proposed method would require minimal steps that utilized mild reaction conditions to efficiently synthesize aza-carbacepham **1** in Figure 5.



1 R_1= H R_2= H
2 R_1= F R_2= H
3 R_1=OCH_3 R_2=H
4 R_1=H R_2=F

Figure 5. The target molecules of the aza-carbacepham **1-4**.

The synthetic pathway began with the formation of pyrimidin-2-one **6** from phenyl urea **5** and acetylacetone.[30] Compound **6** was then exposed to photochemical conditions to form the respective aza-β-lactam ring in **7**, initially with a low yield of 27%.[26]



Scheme 4. Initial synthetic steps to form aza-carbacepham **1** utilizing a Rh catalyzed N-H insertion.

To combat the low yield, flow chemistry was used to optimize the photochemical 4π electrocyclization. Flow chemistry is useful to the synthetic chemist because of such advantages as reduced reaction times, increased heat management, increased product purity and atom efficiency.[31] The high surface-to-volume ratio allows for more efficient mixing and photoreactive conversion, and the use of automated systems allow continuous production of compounds with high efficiency. For the photochemical 4π electrocyclization of **6** to **7**, the initial concern with the

photochemical reaction was the heat generated by the photoreactor, which from long reaction times might promote the reverse reaction. A simple photo-flow-reactor was constructed in attempts to minimize this issue. Polytetrafluoroethylene (PTFE) tubing was coiled around a large glass tube that had the ability to be cooled. The reaction mixture could then flow through the coil with the use of a syringe pump. With this design, various flow rates could be used to allow for longer reaction times and still minimize the heat generated in the solution. The reaction was monitored by ^{1}H NMR, and the conversion ratio of starting material **6** to product **7** was observed (Table 1).

The flow rate was varied to determine how the reaction times affect the percent conversion (Table 1). By decreasing the flow rate and thus increasing the reaction time, the starting material is given more exposure to the radiated light, which increases the efficiency of the reaction. Decreasing the flow rate from 0.322 mL/min (30 minutes) to 0.161 mL/min (60 minutes) results in a large increase in product formation. Varying the concentration was next investigated. The original concentration of 0.016 M was increased to 0.032 M and 0.048 M; with the increase in concentration, a decrease in product formation was observed (Table 1). This is likely due to the low solubility of **6** in benzene. The syringe pump had a difficult time pushing the solution through the reactor. As the solution was pushed through the photo-reactor, solid would form on the syringe preventing the consistent flow of the syringe pump.

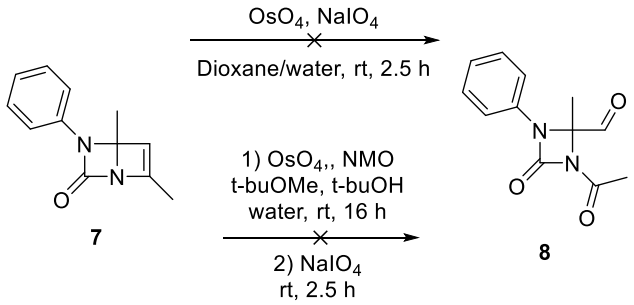
Table 1. Optimization of 4π electrocyclization using flow chemistry.

Concentration (M)	Time (min)	Flow rate (mL/min)	Temperature (°C)	% Conversion of 6 to 7
0.016	30	0.322	0	21
0.016	60	0.161	0	44
0.032	30	0.322	0	23
0.032	60	0.161	0	36
0.048	30	0.322	0	15
0048	60	0.161	0	32
0.016	30	0.322	rt	33
0.016	120	0.080	rt	92
0.016	150	0.060	rt	95

Cooling the reaction mixture was examined to determine if it was necessary. When the reaction was conducted at room temperature, an increase in the conversion of **6** to **7** from 21% to 33% was observed. Nishio *et al.*, also reported that longer reaction times dramatically increase the overall conversion of pyrimidin-2-one to the corresponding diazabicyclo-ene.[32] Much slower flow rates were used, 0.080 mL/min (120 min) and 0.060 mL/min (150 min), to yield 92% and 95% conversion, respectively. Unfortunately, with large scale reaction mixtures and long reaction times, a side product was observed by thin-layer chromatography (TLC). Due to the alkene on the newly formed ring, an additional cyclization occurred causing an oligomer to form when examined my NMR. The formation of the oligomer decreased the isolated yield of **7**, but the increase from 20% to 68% is a respectable gain in yield.

The oxidative cleavage of alkene **7** to form the corresponding aldehyde **8** was first explored using a modified procedure from Inoue *et al*.[33,34] Osmium tetroxide (OsO_4) was used in a 1:1 stoichiometric ratio to convert the alkene to a diol, which could be converted to the respective

aldehyde with the use of sodium periodate ($NaIO_4$), (Scheme 5). When this was attempted with **7,** no product formation was observed.[33] In a different attempt, OsO_4 was used catalytically with *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant (Scheme 5).[34] Unfortunately, no product formation was observed again. It was hypothesized that the second nitrogen atom adjacent to the alkene may interfere with the reaction outcome, so a more direct approach was examined. Compound **7** was subjected to ozonolysis and a reductive workup with dimethyl sulfide to form aldehyde **8** in 60% yield.[26]



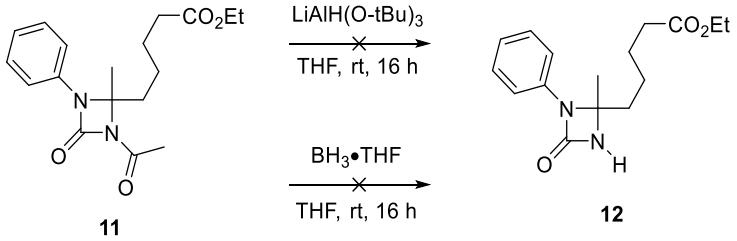
Scheme 5. Oxidative cleavage of **7** with OsO_4 and $NaIO_4$ to form aldehyde **8**.

With the formation of aldehyde **8**, a Wittig reaction was used to extend the carbon chain to the required length for later cyclization. Phosphonium salt **9** was easily synthesized in one step by reacting triphenylphosphine with ethyl-4-bromobutyrate.[35] Phosphonium salt **9** was deprotonated *in situ* with NaHMDS, and the resulting phosphorane was allowed to react with **8** to form **10**.[26,35] The low yield was due to a difficult isolation; multiple attempts afforded only a small amount of pure product **10**. The Wittig product **10** was easily hydrogenated with Pd/C to form **11**.[26] Regrettably, this brought up an issue with the current synthetic pathway; the *N*-acetyl group needed to be removed without reducing the ester on the end of the carbon chain.

Various reducing techniques were explored in an attempt to selectively remove the *N*-acetyl group. The first approach utilized the Corey-Chaykovsky reaction conditions (Scheme 4).[36]

Under these conditions, a sulfur ylide is typically formed that can convert carbonyl compounds such as ketones to their corresponding epoxides. Chandrakala *et al.* observed that when Me_3SI is used to convert aldehyde **8** to an epoxide, simultaneous cleavage of the *N*-acetyl group occurs.[26] When **11** was subjected to these conditions, removal of the *N*-acetyl group was not observed. A modified procedure from Alessandro *et al.* was attempted, which utilized *n*-BuLi in THF for more efficient generation of the sulfur ylide.[37] Even under these conditions, removal of the *N*-acetyl was not observed. Aldehyde **8** was also subjected to the Corey-Chaykovsky conditions in attempts to reproduce the work of Chandrakala *et al.*; interestly no epoxide formation was observed.

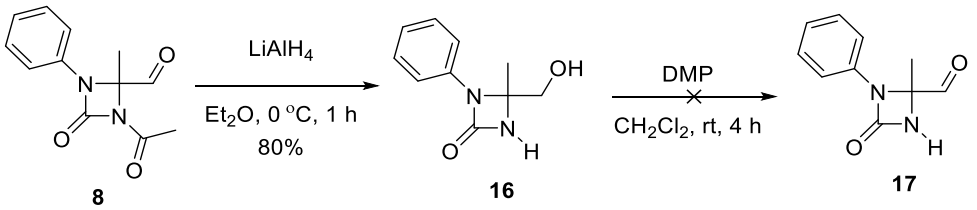
Other reducing agents were also explored to remove the *N*-acetyl group from compound **11** (Scheme 6), lithium tri-*tert*-butoxyaluminum hydride and borane tetrahydrofuran complex were found to be unsuccessful.[38] A new strategy was examined: removal of the acetyl group before the carbon chain is extended.



Scheme 6. Different reductive methods explored to remove *N*-acetyl group.

Chandrakala *et al*. observed that a strong reducing agent such as $LiAlH_4$ is able to reduce the aldehyde to the alcohol and simultaneously cleave the *N*-acetyl group.[26] More mild reducing agents, such as $NaBH_4$, reduced the corresponding aldehyde but do not remove the *N*-acetyl.[26] With a modified procedure from Dess *et al.*, a DMP oxidation was attempted to oxidize alcohol **16** to aldehyde **17** (Scheme 7).[39] Aldehyde **8** was reduced with $LiAlH_4$ to form alcohol **16**, this was then subjected a DMP oxidation in attempt to form **17**. NMR analysis of the crude sample

indicated a small amount of aldehyde formation, but it was unable to be isolated. Another common oxidizing agent , PCC, was used but unfortunately no product formation was observed by TLC, the hindered environment of the alcohol could have prevented the reaction from occurring.[26] With removal of the *N*-acetyl group being problematic and the Wittig reaction of **10** being very low yielding, a different synthetic strategy was explored. The new route used an *N*-alkylated aldol ring closure to form aza-carbacepham **1** (Scheme 8).[26]

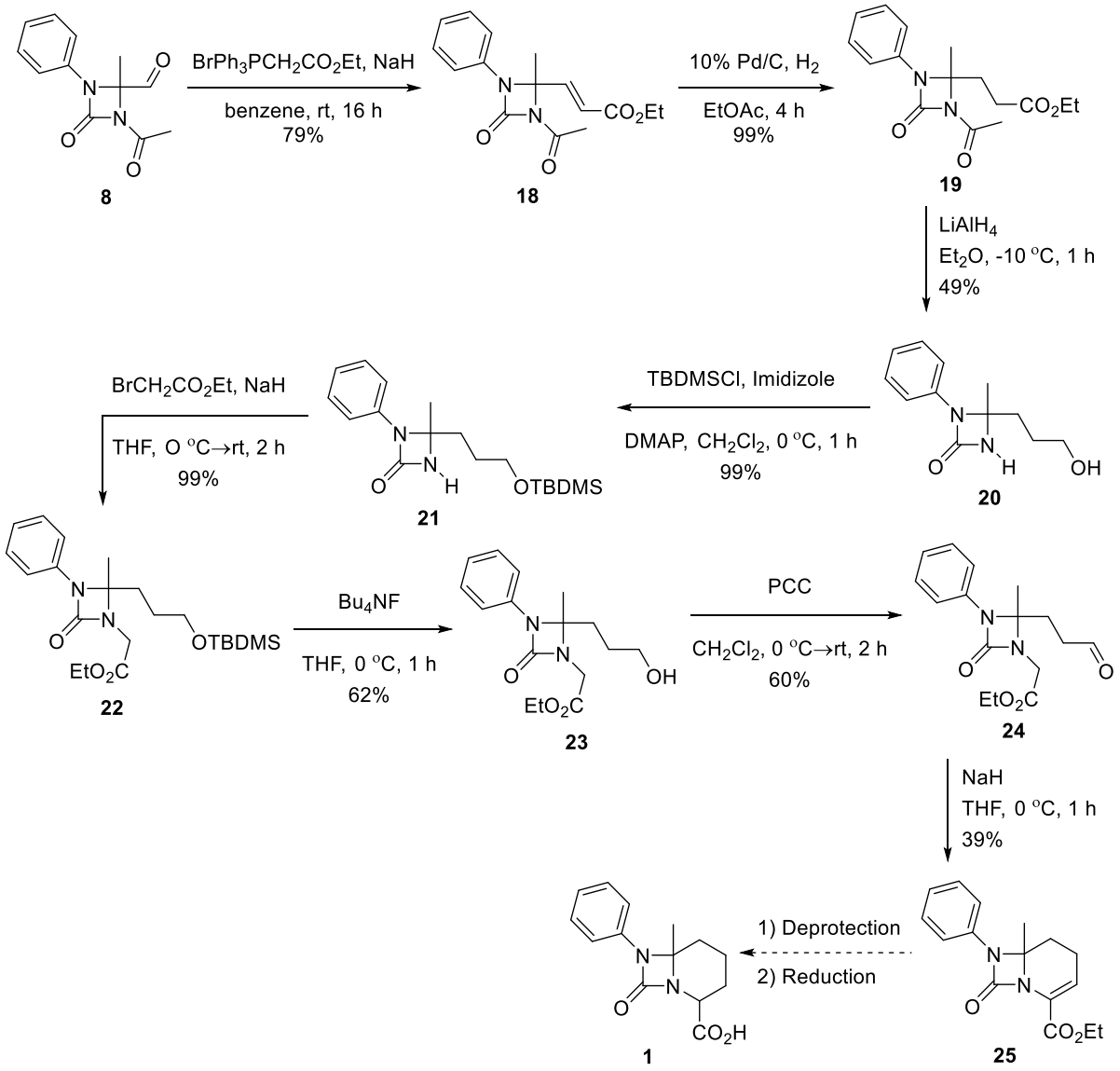


Scheme 7. Early removal of *N*-acetyl with $LiAlH_4$ prior to Wittig reaction.

Treatment of aldehyde **8** with the phosphorane derived from (carbethoxymethyl)triphenylphosphonium bromide formed the Wittig product **18**, which was followed by catalytic hydrogenation of the alkene to produce **19**.[26,40] $LiAlH_4$ was used to reduce the ester to the corresponding alcohol **20** and concurrently cleave the *N*-acetyl group.[26,40] The initial isolated yield was low, therefore the reaction was then cooled to -10 °C in attempt to slow down the rate and diminish the harshness of the reaction conditions. Cooling the reaction more showed a slight increase in yield. The molar equivalents were also reduced from a 3:1 to 1:1 ratio of $LiAlH_4$ to **19**, the corresponding yield was increased to 49%.

A *tert*-butyldimethylsilyl (TBDMS) protecting group was selected for its ability to withstand basic conditions. *tert*-Butyldimethylsilyl chloride (TBDMSCl) was used to protect the primary alcohol of **20** to yield TBDMS ether **21**.[26,40] The primary alcohol was protected to allow

for the nitrogen atom to be alkylated. NaH was used to deprotonate the N-H, which was followed by the addition of ethyl bromoacetate to yield *N*-alkylated TBDMS ether **22.**[26,40]

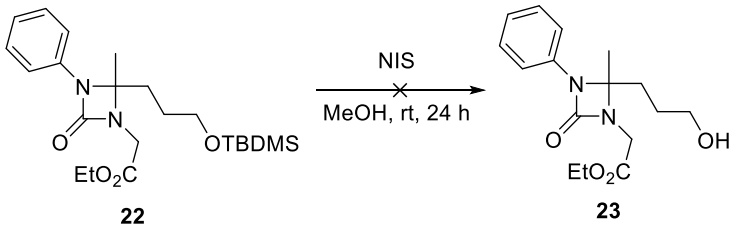


Scheme 8. New synthetic pathway to reach aza-carbacepham **1** through a *N*-alkylated Aldol ring closure.

The deprotection of **22** was attempted with tetra-*n*-butylammonium fluoride (Bu_4NF) and was found to be low yielding.[26,40] The initial attempt at the deprotection gave alcohol **23** in a 62% yield.[26,40] Unfortunately, scaling up the reaction diminished the yield to <10%. It was observed

that the compounds in the reaction undergo degradation when the stoichiometric ratio of Bu_4NF to **22** is high. The initial attempt used a 2:1 ratio of Bu_4NF to **22**, and after 30 min one additional equivalent of Bu_4NF was added until all of **22** was consumed. In attempts to diminish degradation, the molar equivalents were dropped to a 1:1 ratio. Unfortunately, lowering the molar equivalents lengthened the reaction time, 1 h to 3 h, before the disappearance of starting material was observed. After purification, very little alcohol **23** was isolated. In attempts to slow down degradation, the solution was cooled to -10 ^oC. The reduced temperatures resulted in decreased degradation, but the reaction yield was still very low.

Karimi *et al.* reported that *N*-iodosuccinimide (NIS) in methanol is a gentle and efficient means to remove TBDMS protecting groups with subsequent easy purification.[41] When this was attempted with TBDMS ether **22,** the reaction was monitored by TLC and no change was observed (Scheme 9). Karimi *et al*. used simple alkyl chains with minimal functional groups for the study. The various functional groups of **22** could have prevented any reaction from occurring.



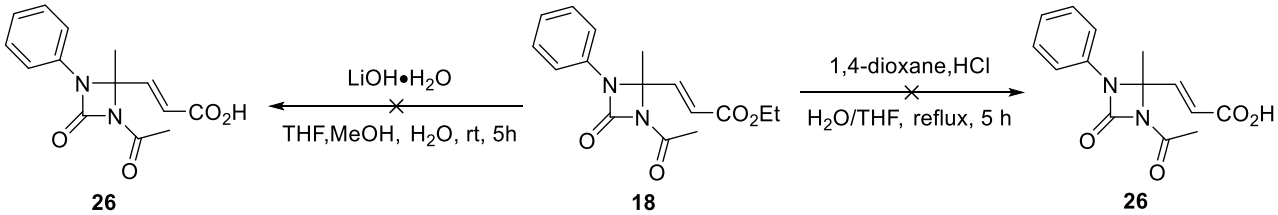
Scheme 9. Attempted TBDMS removal with NIS.

The small quantity of alcohol **23** obtained was nonetheless carried through the pathway to synthesize the final compound. The primary alcohol was oxidized to the corresponding aldehyde with the use of PCC to form aldehyde **24**.[26] With the use of NaH, an aldol cyclization was used to form **25**.[26] With the formation of **25**, the final deprotection to convert the ethyl ester to the corresponding acid was next explored. **25** was not used due to the small amount recovered from

purification, ~2 mg. Instead **18** was used due to the convenience of synthesizing the compound in minimal steps.

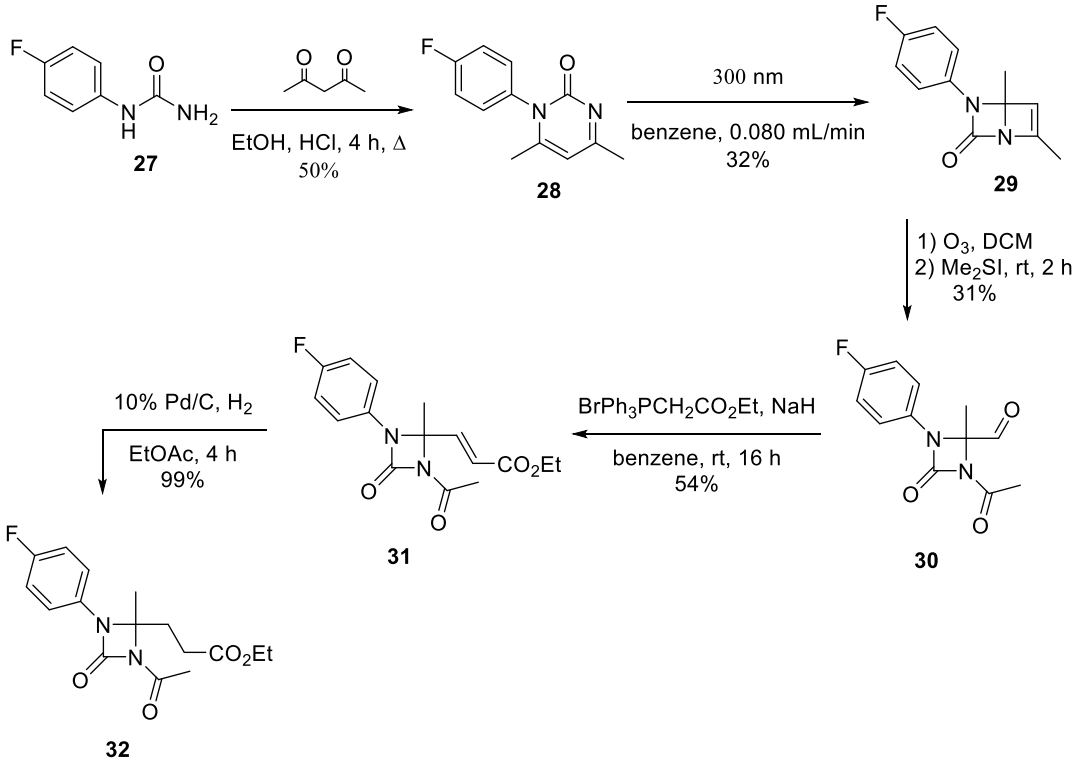
Two methods were explored, a basic and an acidic approach (Scheme 10). Koshikari *et al*. explored hydrolysis of esters under organic solvent-free conditions.[42] From a modified procedure, **18** was subjected to deprotection with LiOH in a methanol/water solution. No change was observed when monitored by TLC, and starting material was recovered. The reaction was attempted again with a 16 h reaction time, and once again no change was observed by TLC.

It has been reported that concentrated HCl in a dioxane/water/THF solution can convert ethyl esters to the respective acids in moderate yields.[43] When these conditions were applied to **18**, some promising results were detected by NMR (Scheme 10). The NMR spectrum of the crude sample indicated the disappearance of the ethyl group of the ester and the presence of the acid proton. But after purification, no isolated compound was identified as **26.** Furthermore, without isolating the product, it was difficult to determine if acid **26** had formed. The same reaction conditions were applied again with a longer reaction time of 16 h, but degradation was observed. The same conditions were applied in a microwave reactor. The reaction was run for 30 min at the temperature of 100 ^{o}C, but unfortunately the solution turned black and degradation was observed.



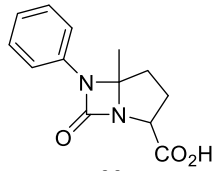
Scheme 10. Attempted ethyl ester deprotection to form corresponding acid under basic or acidic conditions.

Utilizing the optimized steps from Scheme 6, initial steps to form the *p*-fluorophenyl aza-carbacepham **2** were completed (Scheme 11). With the use of 4-fluorophenylurea **27**, the formation of the pyrimidin-2-one **28** was achieved with the same conditions as previously stated. A flow rate of 0.080 mL/min (120 min) was applied to the photo-flow-reactor to generate **29**, which was subjected to ozonolysis and reductive workup to form aldehyde **30**. With the use of (carbethoxymethyl)triphenylphosphonium bromide under strongly basic conditions, the Wittig product **31** was isolated and then hydrogenated in the presence of catalytic Pd/C to make ester **32**. Due to time restraints, the synthesis was stopped at this point. It was observed that the fluoro substituent on the phenyl ring had little effect on the chemistry. Future steps of this study will continue the synthesis forward with *p*-fluorophenyl aza-carbacepham **2** and apply the same conditions to synthesize *p*-methoxyphenyl aza-carbacepham **3** and *m*-fluorophenyl aza-carbacepham **4**.



Scheme 11. Synthetic steps performed to synthesize the *p*-fluorophenyl aza-carbacepham **2**.

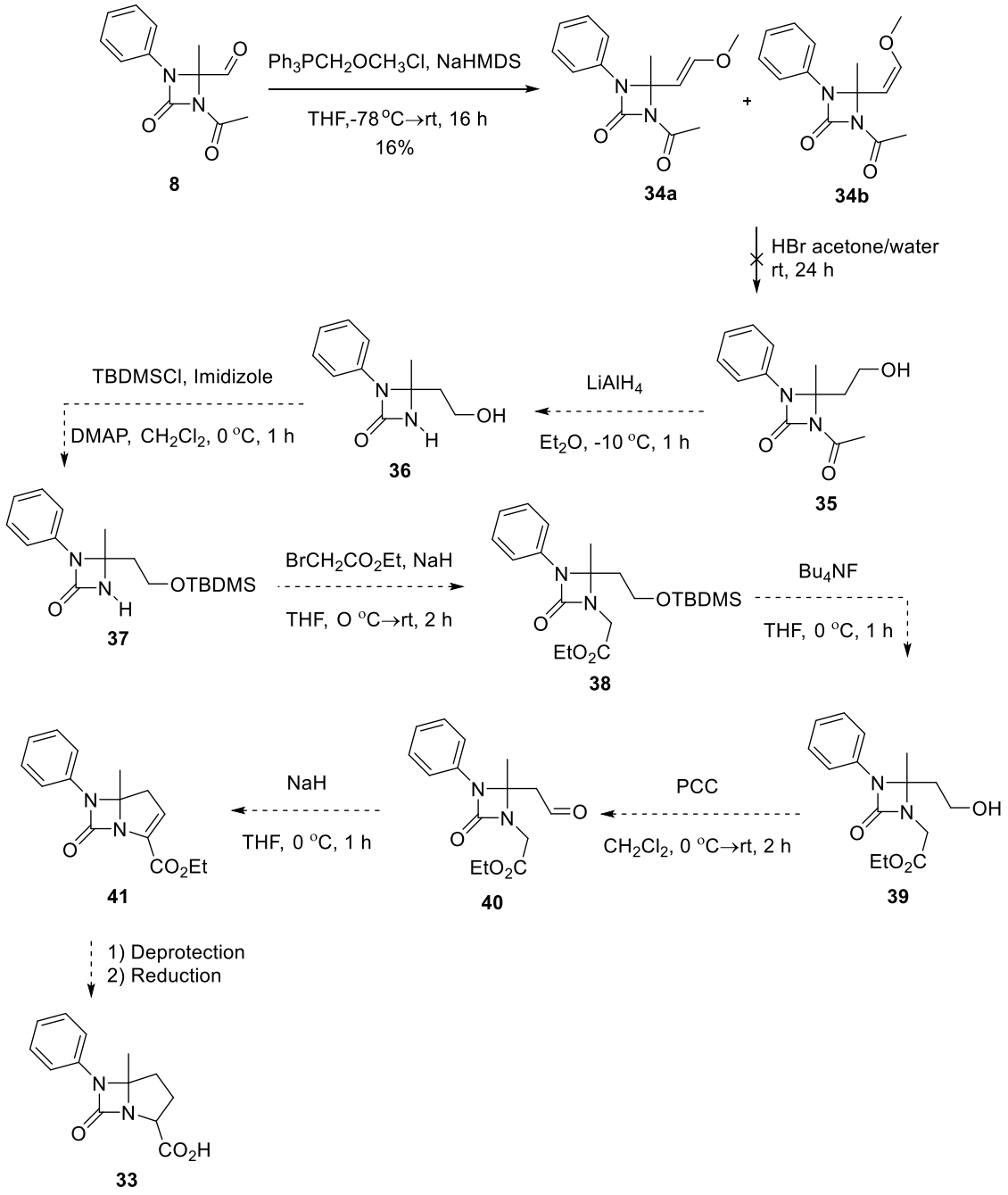
Other derivatives with the aza-β-lactam ring were also attempted, such as aza-carbapenam **33,** the structure of which bears resemblance to the carbapenem class of β-lactam antibiotics (Figure 6). It was hypothesized that much of the same chemistry described in Scheme 8 could be utilized to synthesize aza-carbapenam **33** (Scheme 12).



33

Figure 6. Proposed target molecule aza-carbapenam **33.**

Starting from aldehyde **8**, multiple pathways were explored to homologate the alkyl chain. The formation of alcohol **35** was found to be very difficult. In attempt to synthesize aza-carbapenam **33**, formation of an enol-ether followed by acidic conversion to an alcohol was explored using a modified procedure from Zaytsev *et al*.[44] The reaction was performed using (methoxymethyl)triphenylphosphonium chloride under strongly basic conditions in a Wittig reaction to form 4.3:1 mixture of *E*- and *Z*-enol ethers **34a** and **34b**, respectively (Scheme 12). Unfortunately, isolated yields were low so future exploration of conditions was disregarded. The acidic hydrolysis to form **35** was still attempted but was found to be inconclusive due to the small scale of the reaction.

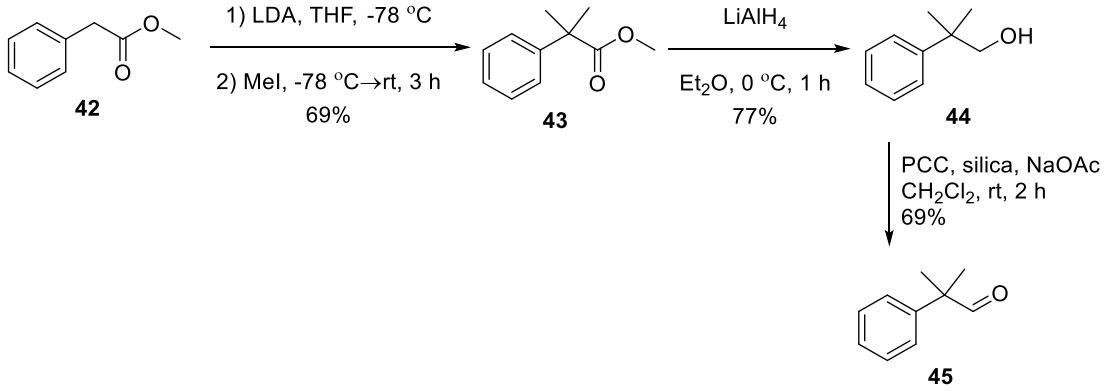


Scheme 12. Theorized synthetic pathway to form aza-carbapenam **33**.

Hydroboration was explored as another potential method to make alcohol **35** (Scheme 15). Utilizing a modified procedure from Della Sala *et al.*, methyltriphenylphosphonium bromide under strongly basic conditions was used in a Wittig reaction.[45] The resulting alkene was then subjected to standard hydroboration conditions to generate the respective alcohol.[45,46] The conditions were

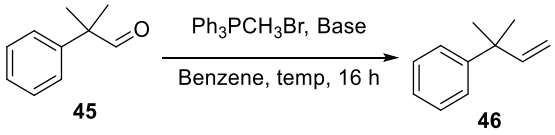
applied to aza-aldehyde **8** and the isolated yield was 9%. Because of the low yield, a sterically hindered aldehyde was used in a model system to optimize the conditions.

For the model system (Scheme 13), a modified procedure was used from Bonneau *et al*.[47] With the use of methyl 2-phenylacetate, two consecutive methylations were completed to yield **43**.[47] $LiAlH_4$ reduction was used to produce the alcohol **44** followed by a PCC oxidation to make the corresponding aldehyde **45**.[44] With the use of **45**, various bases and temperatures were examined to optimize the Wittig reaction for the formation of the hydroboration precursor.



Scheme 13. Synthetic pathway to make hindered aldehyde **45** for hydroboration model system.

Model system conditions were explored to optimize the formation of **46** (Scheme 14). The manner at which the ylide was generated was the first area explored. NaH and NaHMDS were used as methods to generate the ylide, and it was observed that NaHMDS was more efficient resulting in a higher reaction yield (Table 2, entry 3).



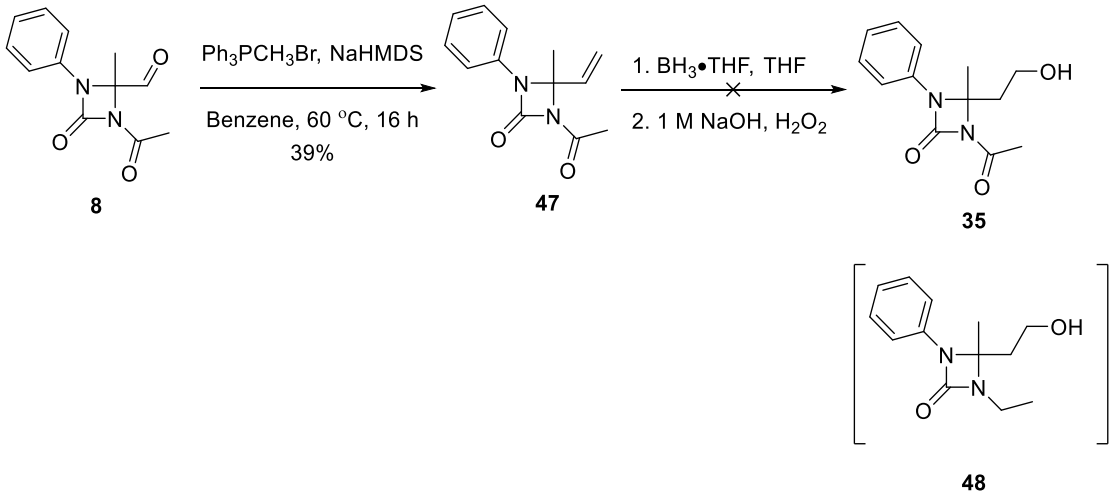
Scheme 14. Hydroboration model system conditions optimization.

In attempt to further increase the reaction yield, gentle heating of the flask was explored. Initially the reaction was performed at 0 ^{o}C, but this was problematic due to the freezing point of benzene (~5.5 ^{o}C). The reaction temperature was warmed to rt, and a small increase of product formation was observed. Finally, the reaction temperature was increased slowly to 60 ^{o}C which resulted in a 42% yield of product **46** (Table 2, entry 5).

Table 2. Model system conditions to form **46** for hydroboration reaction.

Trial	**Base**	**Temperature (^{o}C)**	**Yield (%)**
1	NaH	rt	21
2	NaH	50	4
3	NaHMDS	0 → rt	35
4	NaHMDS	rt	37
5	NaHMDS	60	42

The optimized conditions from the model system were applied to aldehyde **8**. A large increase in isolated yield was observed of 9% to 39%. With the formation of **47**, initial attempts to form **35** were explored. With a modified procedure from Morrill *et al.,* the hydroboration was attempted (Scheme 15).[46] BH_3·THF solution was added to **47** followed by $NaOH/H_2O_2$ and the isolated spot was examined by NMR analysis. It was observed that the alkene was converted to the corresponding alcohol, but the carbonyl of the *N*-acetyl was reduced to yield alcohol **48**.



Scheme 15. Attempted hydroboration to form aza-alcohol **35**.

In conclusion, a synthetic pathway utilizing an *N*-alkylated aldol ring closure was explored in pursuit to produce aza-carbacepham **1**. The 4π electrocyclization was optimized with the use of flow chemistry to form **7**. Deprotection of TBDMS ether **22** with Bu_4NF to form alcohol **23** was found to be low yielding. An additional method was explored with the use of NIS, but no reaction was observed. The small amount of **23** was continued on to make aza-carbacephen **25.** With the general pathway determined, initial steps to form *p*-fluorophenyl aza-carbacepham **2** were completed. The synthetic pathway can then be applied to make the respective *p*-methoxyphenyl aza-carbapenam **3** and *m*-fluorophenyl aza-carbapenam **4**. Synthesis of aza-carbapenem **33** was explored by enol-ether followed by acidic conversion to alcohol **35**, which was found to be unsuccessful. A different pathway utilized hydroboration of **47** to form the unexpected product **48**. Once a large library of aza-β-lactam derivatives have been synthesized, the inhibition ability against β-lactamase will be tested. Positive results could influence the redesign of second generation aza-β-lactam structures.

-Chapter 2-

-Introduction-

Methicillin was introduced to the market in 1959 as a new method for treatment of bacterial infections caused by penicillin-resistant *Staphylococcus aureus*.[18] Unfortunately, isolates were identified in the United Kingdom that had acquired resistance to methicillin in 1961.[18] MRSA isolates were later observed in European countries, Japan, Australia, and the United States.[18] MRSA has become a growing problem in hospitals due to the pathogen's ability to protect itself against many members of the β-lactam antibiotic family.[48] The resistance was brought on by the development of the methicillin resistance gene (*mecA*), which encodes the methicillin-resistant PBP2a to have a low binding affinity for the antibiotic.[18] Glycopeptide antibiotics, such as vancomycin, have been successfully used to treat various MRSA strains. Unfortunately, *Staphylococcus aureus* strains have begun to build resistance to vancomycin. Isolation of vancomycin-intermediate, and most recently vancomycin-resistant *Staphylococcus aureus* (VRSA) carrying the *van*A gene have brought on the need for new treatment options. A new class of antibiotics, oxazolidinones, are synthetic drugs that are active against a large spectrum of Gram-positive bacteria, including methicillin and vancomycin-resistant *staphylococci*, vancomycin-resistant *enterococci*, penicillin-resistant *pneumococci* and *anaerobes*.[49] In 2000 the FDA approved an oxazolidinone, linezolid (figure 7), for treatment of complicated skin infections caused by MRSA.[49] During the subsequent years of clinical use, emergence of resistance to linezolid has been small, but new treatment options need to be explored.

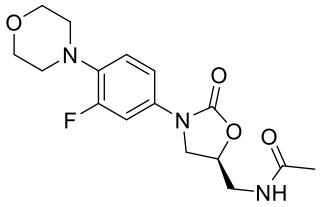


Figure 7. Linezolid, a clinically used oxazolidinone.

The Boudreau research group at University of New Hampshire discovered that rapamycin potentiates the activity of oxacillin against various strains of MRSA (Table 3). In the presence of rapamycin, the minimum inhibitory concentration (MIC) of oxacillin was substantially decreased, but how rapamycin potentiates the activity of oxacillin is unknown.

Table 3. MIC against MRSA strains with and without the presence of rapamycin.

MRSA strain	**MIC (μg/mL)**	
	- Rapamycin	+ Rapamycin
MRSA252	265	256
NRS123	16	1
NRS70	32	8

Rapamycin was discovered in soil samples on Easter island in 1969 and it was originally studied for its antifungal ability, until it was also found to prevent cell multiplication.[50] In 1987, rapamycin was found to also have immunosuppressant abilities, and in 1999, the FDA approved rapamycin as an antirejection drug prescribed to kidney transplant patients.[50] Further studies of rapamycin has discovered the molecule's good permeability across the blood-brain barrier.[51] Rapamycin has been found to have a wide variety of research applications, where it has been engineered to control gene function through rapamycin-induced transcription as well as protein localization, protein degradation, and DNA recombination.[52,53]

From extensive analysis, four chemically accessible locations have been identified for modification of rapamycin (Figure 8). The methoxy group, denoted at position **A**, was found to undergo nucleophilic substitution and β-elimination under acidic conditions.[54] The hydroxyl group at position **B** has been utilized due to the easy protection with silyl groups.[55,56] The lactone at position **C**, can be hydrolyzed and eliminated.[57] The hydroxyl group at position **D** has been found to be easily converted to a carbonate group and esterified.[57] With the various areas for chemical accessibility, methods such as photoaffinity labeling can be utilized to study the interaction of rapamycin with large biomolecules.

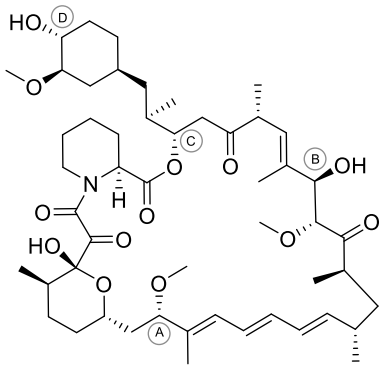
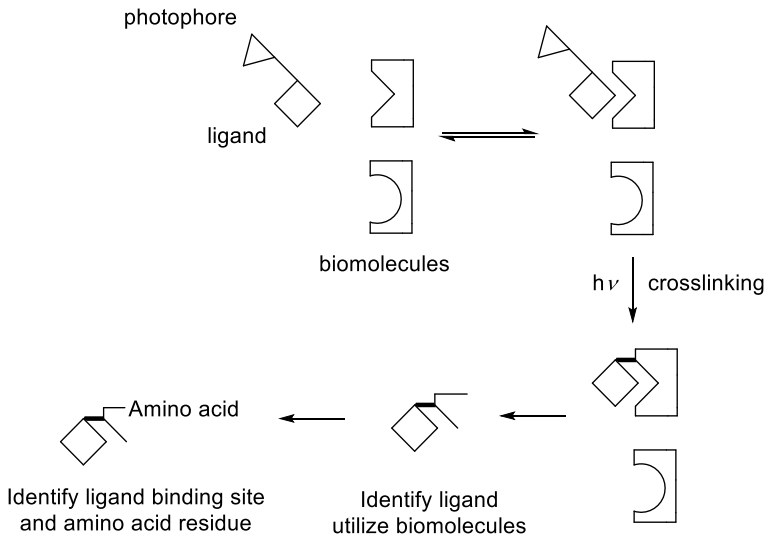


Figure 8. The structure of rapamycin with four chemically accessible locations, denoted A-D.

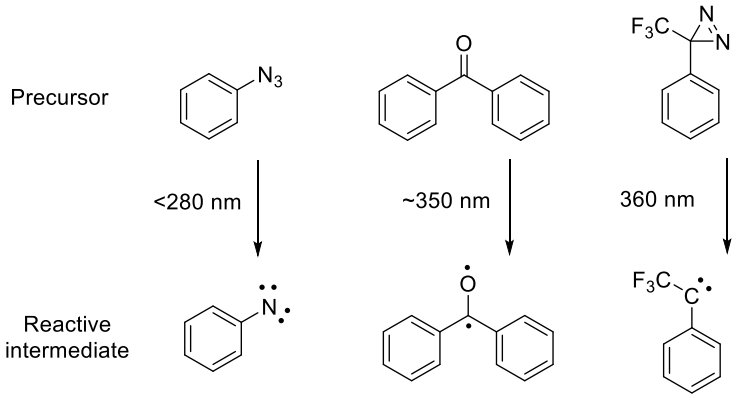
Analyzing the molecular interaction between bioactive ligands and their protein targets is an important step in drug design. Understanding how these interactions take place allows for the discovery of new and potentially more efficient drugs.[58] With the use of a photoaffinity group, a highly reactive species is generated to induce a covalent bond between the ligand and the protein in a nonselective manner.[58] The photophore is affixed to the ligand of interest, and with the use of select wavelengths of light, a reactive species will form a nonselective bond to the nearby biomolecule (Scheme 16). Successful applications of photoaffinity labeling can result in the identification of target biomolecules in crude extracts with the aid of a radioisotope-labeled probe,

which are highly sensitive detection tags.[58] An additional advantage of photoaffinity labeling is the ability to identify the ligand binding site and labeled amino acid. Unfortunately, identification of photolabeled compounds can be difficult due to low photoincorporation yields.[59] Multiple options are available to combat the low photoincorporation yields such as: choice and synthesis of the photophore, irradiation conditions, and choice of tag for the identification of the biomolecule.[58]



Scheme 16. Schematic representation of the process of photoaffinity labeling.

Specific groups such as aryl azides, benzophenones, or aryldiazirines (Scheme 17) have been found to be very useful due to the highly reactive species they generate.[58] The aryl azides are photoactivated to generate a nitrene at wavelengths below 300 nm, but low wavelengths of light have been observed to cause damage to biomolecules, while benzophenones are radiated at 350 nm to generate a reactive triplet carbonyl state.[60] The ground state carbonyl compound can be regenerated so the photoreactive group is reusable, but long photoirradiation times are required for this labeling process.[58] Aryldiazirines generate a carbene when radiated with light at 360 nm, which rapidly forms a covalent bond to biomolecules and does not require long photoirradiation times.[58,61]



Scheme 17. Photochemical groups with generated reactive species that are commonly used in photoaffinity labeling.

It is crucial to limit interference when the photoaffinity probe is attached to the target molecule. The target molecule must act as normal in order to properly study the molecular interactions. Size is a large factor in this consideration, and the aliphatic diazirine linkers have been found to minimize the issue of interference. The benefit of the aliphatic diazirine linker is the overall small size, which will minimize interference with protein binding, and still maintain the short irradiation time.[62] Coupling the small size of the probe with a terminal alkyne as the reporter, the cross-linked protein probe complex can be used for various biochemical analysis, such as pull-down assays and LC-MS/MS, for the identification of the protein target.[62] Since the manner that rapamycin potentiates the activity of oxacillin to inhibit various strains of MRSA is not understood, the molecular interactions can be studied by attaching an aliphatic diazirine linker to rapamycin then examining the interaction.

-Results and discussion-

The mechanism of interaction will be studied by attaching an aliphatic diazirine linker to rapamycin. Li *et al.* explored multiple small aliphatic diazirine linkers that could be used as photoaffinity linkers.[62] With the use of the diazirine linker **49,** the mechanism of interaction with oxacillin and MRSA can be studied. The hydroxyl group on the cyclohexane ring, Figure 8 position **D,** is the location the linker is predicted to be installed to form rapa-linker **50.**[63]

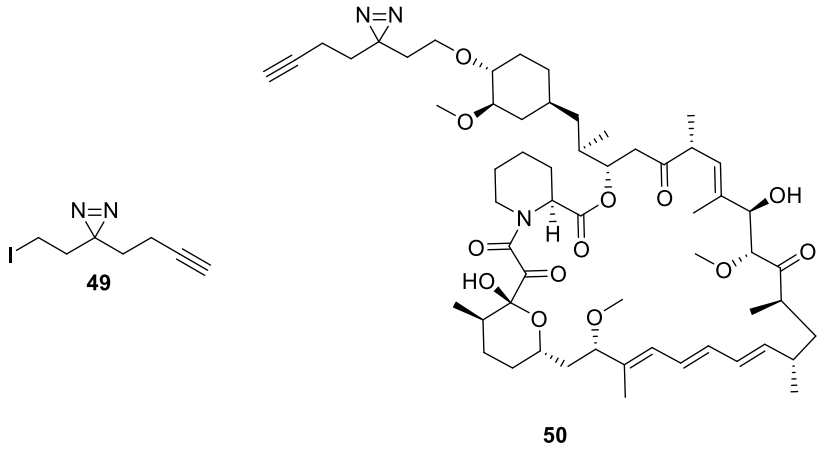
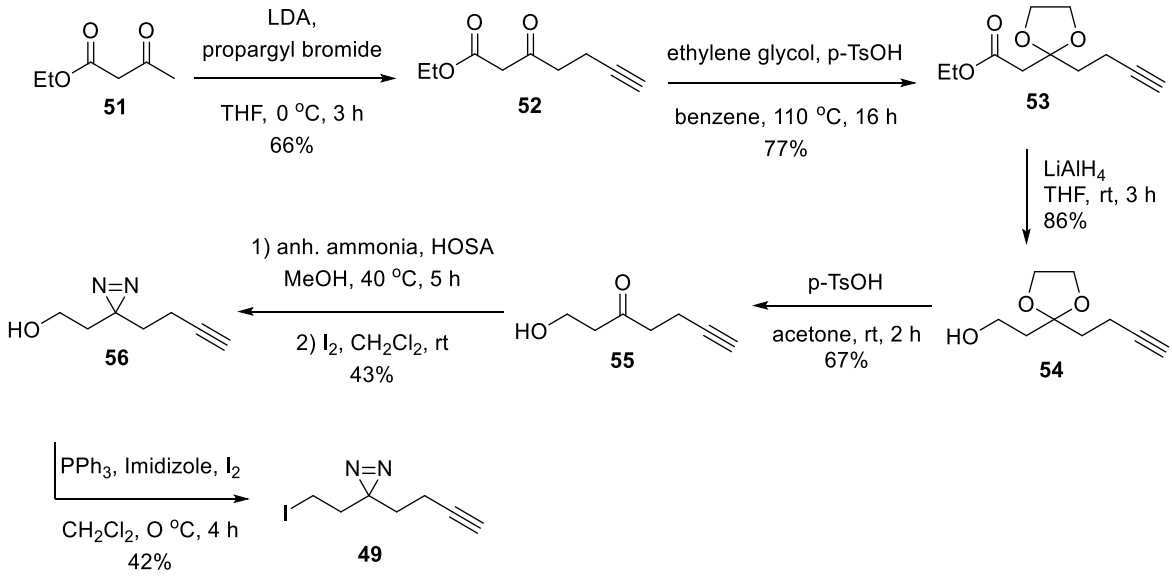


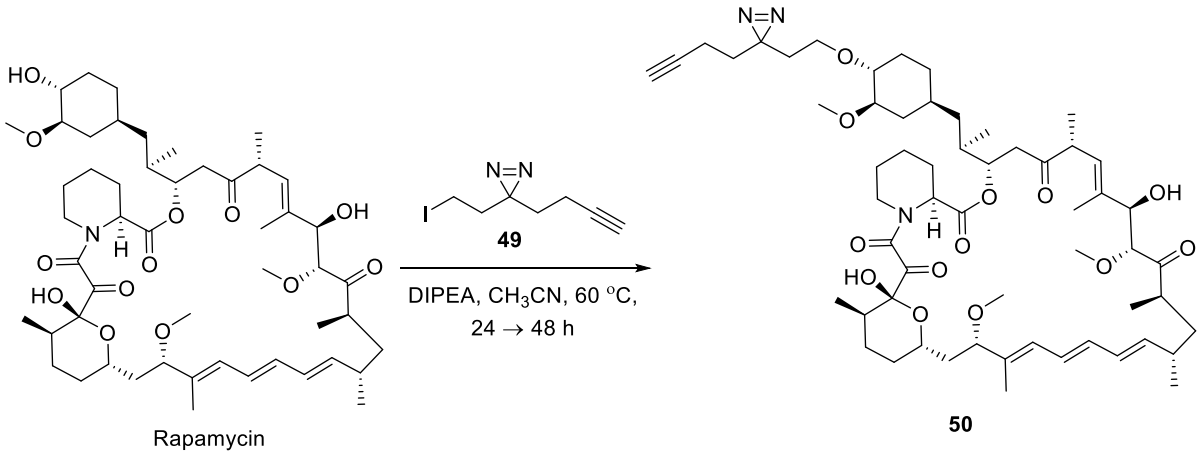
Figure 9. Aliphatic diazirine linker **49** and respective rapa-linker **50.**

Diazirine linker **49** was prepared following the synthetic pathway from Li *et al.* (Scheme 18).[62] The synthesis started with the alkylation of ethyl acetoacetate to form **52**, which was followed by the protection of the ketone with ethylene glycol to form **53**.[62,64] The ester was reduced with $LiAlH_4$ to give the respective alcohol **54**, which was deprotected with *p*-toluenesulfonic acid to give ketone **55**.[62] The formation of the diazirine ring was a multiple step process that began with heating to reflux the ketone in anhydrous ammonia followed by the addition of hydroxylamine-*O*-sulfonic acid to form the intermediate diaziridine. With the use of an iodine solution, the diaziridine ring was oxidized to form corresponding diazirine **56**, which was followed by a halogenation to form iodo-diazirine **49**.[62]



Scheme 18. Synthesis of linker **49** that will be used to alkylate rapamycin.

With the synthesis of linker **49** complete, alkylation attempts of rapamycin were examined. Rodrik-Outmezguine *et al*. studied the functionalization of rapamycin, and modifying the research with linker **49**, initial attempts to alkylate rapamycin were performed (Scheme 19).[63]



Scheme 19. Alkylation of rapamycin to form respective rapa-linker **50**.

The alkylation reaction of rapamycin was monitored by TLC over time, and a new spot formation was observed. The new spot was isolated, then examined by NMR. Unfortunately, the data from the resulting spectra was unclear and difficult to determine if the rapa-linker **50** was

truly formed. Calculations performed with NMR prediction software showed similarities when compared to the ^{13}C NMR of the isolated product, but the data was inconclusive due to the small amount of the sample isolated. In order to fully characterize the product, more would need to be synthesized.

The synthesis of diazirine linker **49** was successfully completed. Initial alkylation attempts of rapamycin have yielded inconclusive results. Future direction of the project would explore other alkylation techniques to attach the linker to rapamycin. With the formation of rapa-linker **50**, the interaction with oxacillin and MRSA can be examined. The rapamycin-protein complex can be isolated from the solution using a pull-down assay, followed by MS/MS analysis to determine the structure of the respective protein.

-Chapter 3-

I. Reagents and Solutions

All reagents and solvents employed were purchased from Acros Organics Ltd. (Hampton, NH), Alfa-Aesar Chemicals. (Haverhill, MA), MilliporeSigma Chemical Company (St. Louis, MO), Oakwood Products, Inc. (Estill, SC), or TCI American Chemicals. (Portland, OR) unless specifically stated.

All reagents and solvents were of American Chemical Society (ACS) grade and were used without further purification unless otherwise stated. All reactions were performed under a positive pressure of nitrogen using oven or flame-dried glassware. All organic solvents when stated dry were prepared by addition of dried over molecular sieves prior to use. For the removal of solvent, *in vacuo* refers to evaporation under reduced pressure below 40 ^{o}C using a rotary evaporator followed by evacuation (< 0.1 mm Hg) to a constant sample mass. Saturated solutions of NH_4Cl, $NaHCO_3$, HCl, and NaOH refer to aqueous solutions. Brine refers to a saturated solution of NaCl.

II. Purification Techniques

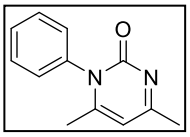
All reactions and fractions obtained from column chromatography were monitored by thin-layer chromatography (TLC) using glass-backed plates (1.5 x 5 cm) pre-coated with silica gel containing a UV fluorescent indicator. Compounds were visualized by exposing the plates to UV light.

III. Instrumentation of Compound Characterization

Nuclear magnetic resonance (NMR, 500 MHz) spectra were obtained on a Varian Mercury 400, 500 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (PPM) relative to tetramethylsilane as the internal standard relative to the solvent of $CDCl_3$ (δ 7.26) and coupling constant values are given in [Hz]. The following abbreviations are given for spin multiplicity: *s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet, *dt* = doublet of triplets, *ddd* = doublets of doublets of doublets, *td* = triplet of doublets, *qd* = quartet of doublets. Additional assignments were made using homonuclear correlation spectroscopy (g-COSY), heteronuclear single quantum coherence spectroscopy (gHSQC) and heteronuclear multiple bond correlation spectroscopy (gHMBC). All literature compounds had ^{1}H NMR spectrum consistent with the assigned structures.

IV. Experimental procedures and Data

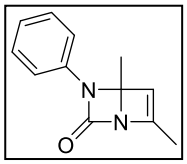
1,2-Dihydro-N-phenyl-4,6-dimethylpyrimidin-2-one (6)



This known compound was prepared according to the procedure from Muthuraman *et al*.[30] Phenyl urea (5.45 g, 40.00 mmol) was dissolved in 95% EtOH (50 mL) with conc. hydrochloric acid (10 mL) and stirred at room temperature for 5 min. Acetyl acetone (4.81 g, 48.00 mmol) was added to the solution and stirred at reflux for 3 h. The reaction mixture was cooled in an ice/water bath and the resulting solid was isolated by vacuum filtration and washed with cold EtOH (3 x 30 mL). The white solid was dissolved in water (40 mL) and then brought to a pH of 8-10 with a sodium hydroxide solution (3.5 M, 5 mL). The

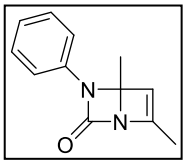
solution was extracted with $CHCl_3$ (3 x 50 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo* to yield a white solid (3.6423 g, 46%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.55 – 7.21 (m, 5H, ArH), 6.20 (d, *J* = 0.8 Hz, 1H, C=CH), 2.42 (s, 3H, CH_3), 1.99 (d, *J* = 0.8 Hz, 3H, CH_3).

3-Phenyl-4,6-dimethyl-2-oxo-1,3-diazabicyclo(2.2.0)hex-5-ene (7)



This known compound was prepared according to the procedure of Chandrakala *et al*.[26] Pyrimidinone **6** (1.20 g, 6.00 mmol) was suspended in benzene (350 mL) and stirred at room temperature for 16 h. The solution was partitioned into 5 equal portions and placed into quartz test tubes then irradiated with light (300 nm) for 3 h. The test tubes were combined, and the solution concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield a yellow solid (0.3227 g, 27%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 – 7.09 (m, 5H, ArH), 6.15 (q, *J* = 1.7 Hz, 1H, vinyl CH), 2.13 (d, *J* = 1.7 Hz, 3H, CH_3), 1.88 (s, 3H, CH_3).

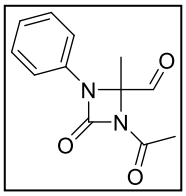
3-Phenyl-4,6-dimethyl-2-oxo-1,3-diazabicyclo(2.2.0)hex-5-ene (7)



This known compound was prepared according to a modified procedure from Chandrakala *et al.*[26] Pyrimidinone **6** (1.00 g, 4.90 mmol) was suspended in benzene (300 mL) and stirred for 16 h. The solution was passed through photo-flow-reactor (50 mL BD glass syringe, 0.080 mL/min, 300 nm) and the solution exited into a collection flask with benzene (~50 mL). The combined solution was concentrated *in vacuo*, and purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield a yellow solid (0.6224

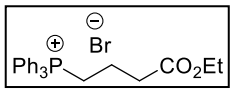
g, 62%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 – 7.09 (m, 5H, ArH), 6.15 (q, *J* = 1.7 Hz, 1H, vinyl CH), 2.13 (d, *J* = 1.7 Hz, 3H, CH_3), 1.88 (s, 3H, CH_3).

1-Phenyl-3-acetyl-4-formyl-4-methyl-1,3-diazetidin-2-one (8)



This known compound was prepared according to the procedure from Chandrakala *et al*.[26] Alkene **7** (0.55 g, 2.70 mmol) was dissolved in CH_2Cl_2 (120 mL) and cooled to -78 °C. A steady stream of ozone was bubbled through the solution at -78 °C until blue color persisted. O_2 was bubbled through the solution until the solution became colorless then was slowly warmed to rt. The mixture was cooled to 0 °C, then dimethyl sulfide (1.00 mL, 0.85 g, 13.60 mmol) was added dropwise and the reaction mixture was warmed to rt then stirred for 2 h. The reaction mixture was concentrated *in vacuo*, then purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield faint yellow solid (0.3785 g, 60%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.45 (d, *J* = 0.5 Hz, 1H, CHO), 7.38– 7.12 (m, 5H, ArH), 2.48 (s, 3H, $COCH_3$), 1.95 (d, *J* = 0.5 Hz, 3H, CH_3).

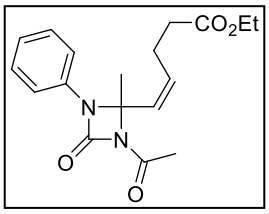
(4-Ethoxy-4-oxobutyl)triphenylphosphonium bromide (9)



This known compound was prepared according to the procedure from Luo *et al*.[35] Triphenylphosphine (3.76 g, 14.34 mmol) was dissolved in dry toluene (20 mL) and stirred at rt for 5 min. Ethyl-4-bromobutyrate (1.47 mL, 2.00 g, 10.25 mmol) was added dropwise to reaction flask and heated to 125 °C for 16 h. The reaction flask was cooled to rt then Ch_2Cl_2 (10 mL) was added, followed by Et_2O (~80 mL) until white precipitate formation stopped. The solid was isolated by vacuum filtration and washed with Et_2O (~100 mL) and dried

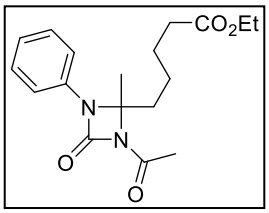
to yield pure white solid (3.1498 g, 81%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94 – 7.68 (m, 15H, ArH), 4.15 – 4.08 (m, 4H, OCH_2 and PCH_2), 2.92 (td, *J* = 6.5, 1.4 Hz, 2H), 1.98 – 1.89 (m, 2H, CH_2), 1.25 (t, *J* = 7.1 Hz, 3H, CH_2CH_3).

(Z)-Ethyl 5-(1-acetyl-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-2-yl)pent-4-enoate (10)



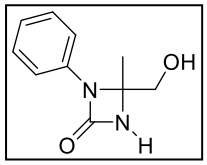
This compound was prepared according to a modified procedure from Luo *et al.*[35] Phosphonium Bromide salt **9** (0.82 g, 1.80 mmol) was suspended in dry THF (10 mL) and cooled to -78 ºC. NaHMDS solution (1 M in THF, 2.25 mL, 2.25 mmol) was added to reaction flask and stirred for 5 min at -78 ºC. Aldehyde **8** (0.35 g, 1.50 mmol) in THF (5 mL) was added dropwise to the reaction flask, then allowed to warm up to rt and stir for 16 h. The reaction mixture was diluted with EtOAc (10 mL) followed by water (15 mL). The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried with $MgSO_4$ and then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 10 % EtOAc/hexanes) to yield yellow oil (0.0770 g, 16%): ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 7.39 – 7.07 (m, 5H, ArH), 5.78 (dt, *J* = 11.9, 7.2 Hz, 1H, vinyl CH), 5.67 (dt, *J* = 11.9, 1.9 Hz, 1H, vinyl CH), 4.09 (qd, *J* = 7.1, 2.1 Hz, 2H, OCH_2), 2.58 – 2.50 (m, 1H), 2.44 – 2.40 (m, 4H, 2 x CH_2), 2.32 – 2.25 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 1.23 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 172.31, 165.31, 154.12, 146.20, 137.82, 129.42, 124.96, 123.71, 115.93, 80.86, 60.51, 33.47, 24.16, 23.06, 22.89, 14.17; HRMS (ES+) calcd for $C_{18}H_{24}N_2O_4Na$ 355.1628 found 355.1634 $[MNa]^+$.

Ethyl 5-(1-acetyl-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-2-yl)pentanone (11)



This compound was prepared according to a modified procedure from Chandrakala *et al.*[26] Ester **10** (0.08 g, 0.23 mmol) was dissolved in EtOAc (10 mL) and flushed with N_2 for 5 min. 10 % Pd/C (0.03 g) was added to reaction flask and flushed with N_2 for additional 5 min. H_2 was bubbled through the solution for 10 min, then the mixture was stirred under H_2 environment for 4 h. The reaction mixture was filtered through a celite pad, then washed with EtOAc (25 mL). The filtrate was concentrated *in vacuo* which afforded a colorless oil (0.0809 g, 99%) with no further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 – 7.09 (m, 5H, ArH), 4.08 (q, *J* = 7.1 Hz, 2H, OCH_2), 2.51 – 2.43 (m, 1H), 2.41 (s, 3H, $COCH_3$), 2.27 – 2.20 (m, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.66 – 1.58 (m, 2H, CH_2), 1.21 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.17, 166.23, 153.95, 146.10, 129.59, 123.75, 116.04, 82.57, 60.29, 34.23, 33.97, 24.30, 22.95, 22.17, 18.62, 14.17; HRMS (ES+) calcd for $C_{18}H_{22}N_2O_4Na$ 353.1474 found 353.1477 $[MNa]^+$.

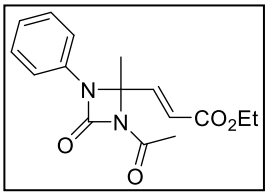
4-(Hydroxymethyl)-4-methyl-1-phenyl-1,3-diazetidin-2-one (16)



This known compound was prepared according to a procedure from Chandrakala *et al.*[26] $LiAlH_4$ (0.02 g, 0.50 mmol) was suspended in Et_2O (8 mL) and cooled to 0 °C. Aldehyde **8** (0.03 g, 0.12 mmol) in Et_2O (3 mL) was added dropwise to reaction flask and stirred for 1 h at 0 °C. The reaction mixture was quenched with NaOH (1 M, 1 mL) followed by water (2 mL) and the resulting solution was filtered through a celite pad. The filtrate was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried with

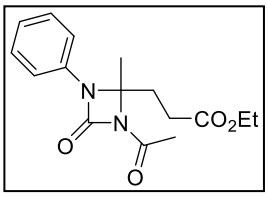
$MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 50 % EtOAc/hexanes) to yield faint yellow oil (0.0184 g, 80%): ^1H NMR ($CDCl_3$, 500 MHz,) δ 7.35 – 7.07 (m, 5H, ArH), 5.02 (s, 1H, NH), 4.03 – 3.87 (m, 2H, OCH_2), 1.75 (s, 3H, CH_3).

(E)-Ethyl 3-(1-acetyl-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-2-yl)acrylate (18)



This known compound was prepared according to the procedure from Chandrakala *et al.*[26] (Carbethoxymethyl)triphenylphosphonium bromide salt (0.85 g, 1.98 mmol) was suspended in dry benzene (9 mL) and stirred at rt for 5 min. NaH (60% in mineral oil, 0.8 g, 2.1 mmol) was added to the suspension and stirred for 5 min. Aldehyde **8** (0.23 g, 0.99 mmol) in dry benzene (6 mL) was added dropwise to reaction flask and stirred for 16 h at rt. The reaction mixture was diluted with water (12 mL) and the resulting solution was separated, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield faint yellow oil (0.2375 g, 79%): ^1H NMR ($CDCl_3$, 500 MHz,) δ 7.35-7.12 (m, 5H, ArH), 7.07 (d, *J* = 15.9 Hz, 1H, vinyl CH), 6.33 (d, *J* = 15.9 Hz, 1H, vinyl CH), 5.31 (s, 1H, NH), 4.23 (qd, *J* = 7.2, 0.8 Hz, 2H, OCH_2), 2.41 (s, 2H, $COCH_3$), 2.07 (s, 3H, CH_3), 1.29 (dt, *J* = 20.6, 7.2 Hz, 3H, CH_2CH_3).

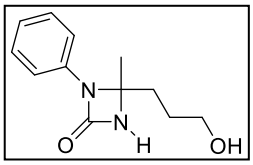
Ethyl 3-(1-acetyl-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-2-yl)propanate (19)



This known compound was prepared according to the procedure from Chandrakala *et al.*[26] Trans ester **18** (0.24 g, 0.79 mmol) was dissolved in EtOAc (30 mL) and flushed with N_2 for 5 min. 10 % Pd/C (0.06 g) was

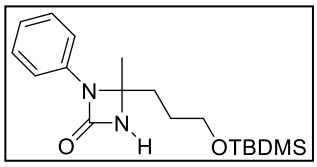
added to the reaction flask and then flushed with N_2 for additional 5 min. H_2 was bubbled through solution for 10 min, then the mixture was stirred under H_2 environment for 4 h. The reaction mixture was filtered through a celite pad and washed with EtOAc (25 mL). The solution was concentrated *in vacuo* to yield colorless oil (0.2745 g, 99%) with no further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 –7.08 (m, 5H, ArH), 4.01 (q, *J* = 7.2 Hz, 2H, OCH_2), 2.77 (ddd, *J* = 15.4, 9.0, 6.5 Hz, 1H, CH_2), 2.48 (ddd, *J* = 15.4, 8.4, 7.0 Hz, 1H, CH_2), 2.40 (s, 3H, $COCH_3$), 2.34 (ddd, *J* = 8.8, 6.6, 3.0 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.18 (t, *J* = 7.2 Hz, 3H, CH_2CH_3).

4-(3-Hydroxypropyl)-4-methyl-1-phenyl-1,3-diazetidin-2-one (20)



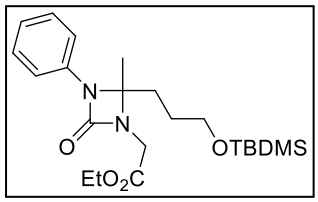
This known compound was prepared according to the procedure from Chandrakala *et al.*[26] $LiAlH_4$ (0.02 g, 0.45 mmol) was suspended in Et_2O (20 mL) and cooled to 0 °C. Ester **19** (0.14 g, 0.45 mmol) in Et_2O (2 mL) was added dropwise to the reaction flask and stirred for 1 h at -10 °C. The reaction mixture was quenched with NaOH solution (1 M, 1 mL) followed by water (5 mL), and the resulting mixture was filtered through a celite pad. The aqueous layer was extracted with Et_2O (3 x 10 mL) and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 80 % EtOAc/hexanes) to yield colorless oil (0.0458 g, 49%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35 –7.04 (m, 5H, ArH), 5.13 (s, 1H, NH), 3.69 (dt, *J* = 10.7, 6.5 Hz, 2H, OCH_2), 2.14 (ddd, *J* = 8.3, 6.5, 1.3 Hz, 2H, CH_2), 1.80 (dt, *J* = 7.2, 1.6 Hz, 1H, CH_2), 1.78 (s, 3H, CH_3), 1.60 – 1.59 (m, 1H, CH_2).

4-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-methyl-1-phenyl-1,3-diazetidin-2-one (21)



This known compound was prepared from a modified procedure from Chandrakala *et al.*[26] Alcohol **20** (0.06 g, 0.26 mmol) was dissolved in CH_2Cl_2 (3 mL) and cooled to 0 °C. Imidazole (0.18 g, 2.60 mmol) was added to the reaction flask, followed by DMAP (0.002 g, 0.02 mmol) and the resulting solution was stirred at 0 °C for 5 min. To the reaction flask, *tert*-butyldimethylsilyl chloride (0.20 g, 1.30 mmol) was added and the mixture was stirred for 1 h at 0 °C. The reaction was diluted with water (3 mL), and the resulting layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 40 % EtOAc/hexanes) to yield colorless oil (0.0985 g, 99%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 – 7.03 (m, 5H, ArH), 5.14 – 5.13 (s, 1H, NH), 3.66 – 3.61 (m, 2H, $SiOCH_2$), 2.10 (dd, J = 8.2, 7.2 Hz, 2H, CH_2), 1.86 – 1.77 (m, 1H, CH_2), 1.75 (s, 3H, CH_3), 1.59 – 1.55 (m, 1H, CH_2), 0.91 (d, J = 17.3 Hz, 9H, $SiC(CH_3)$), 0.12 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$).

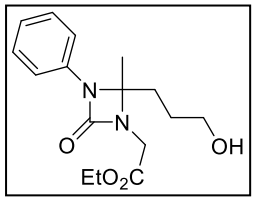
Ethyl 2-(2-(3-((tert-butyldimethylsilyl)oxy)propyl)-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-1-yl) acetate (22)



This known compound was prepared according to the procedure from Chandrakala *et al.*[26] NaH (60% in mineral oil, 0.06 g, 1.56 mmol) was suspended in dry THF (5 mL) and cooled to 0 °C. TBDMS ether **21** (0.13 g, 0.39 mmol) in THF (6 mL) was added dropwise to reaction flask and stirred at 0 °C for 10 min. Ethyl bromoacetate (0.22 mL, 0.33 g, 1.95 mmol) added dropwise, then the reaction flask

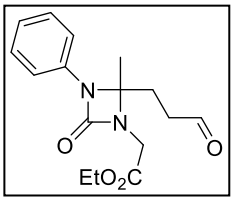
was warm to rt and stirred for 2 h. The reaction mixture was diluted with water (6 mL) and the resulting layers separated. The aqueous layer was extracted with EtOAc (3 x 5 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield colorless oil (0.2077 g, 99%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32 –7.00 (m, 5H, ArH), 4.22 (q, *J* = 7.2 Hz, 2H, OCH_2), 4.01 (d, *J* = 5.8 Hz, 2H, NCH_2), 3.58 (t, *J* = 6.0 Hz, 2H, $SiOCH_2$), 2.15 – 2.02 (m, 2H, CH_2), 1.72 (s, 3H, CH_3), 1.70 – 1.60 (m, 2H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 0.87 (s, 9H, $SiC(CH_3)$), 0.01 (s, 3H, $SiCH_3$), -0.01 (s, 3H, $SiCH_3$).

Ethyl 2-(2-(3-hydroxypropyl)-2-methyl-4-oxo-3-phenyl-1,3-diazetidin-1-yl) acetate (23)



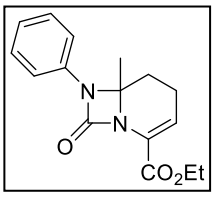
This known compound was prepared according to the procedure from Chandrakala *et al.*[26] *N*-alkylated TBDMS ether **22** (0.02 g, 0.04 mmol) was dissolved in dry THF (1 mL) and cooled to 0 °C. Tetrabutylammonium fluoride (1 M in THF, 0.09 mL, 0.09 mmol) was added dropwise to reaction flask and stirred for 1 h at 0 °C. The reaction mixture was diluted with brine (3 mL) and the resulting layers separated. The aqueous layer was extracted with EtOAc (3 x 5 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 80 % EtOAc/hexanes) to yield colorless oil (0.0082 g, 62%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33 – 7.01 (m, 5H, ArH), 4.23 (qd, *J* = 7.1, 2.3 Hz, 2H, OCH_2), 4.14 (d, *J* = 18.3 Hz, 1H, NCH_2), 3.90 (d, *J* = 18.3 Hz, 1H, NCH_2), 3.71 – 3.55 (m, 2H, OCH_2), 2.16 (ddd, *J* = 9.8, 7.3, 5.3 Hz, 2H, CH_2), 1.87 – 1.77 (m, 1H, CH_2), 1.72 (s, 3H, CH_3), 1.52 – 1.42 (m, 1H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

Ethyl 2-(2-methyl-4-oxo-2-(3-oxopropyl)-3-phenyl-1,3-diazetidin-1-yl) acetate (24)



This known compound was prepared according to the procedure from Chandrakala *et al.*[26] Alcohol **23** (0.008 g, 0.03 mmol) was dissolved in CH_2Cl_2 (1 ml) and then the mixture was cooled to 0 ºC. Pyridinium chlorochromate (0.01 g, 0.06 mmol) was added, then the reaction flask was warmed to rt and stirred for 2 h. The reaction mixture was diluted with Et_2O (2 mL) then filtered through a celite pad and washed with Et_2O (3 x 2 mL). The filtrate was concentrated *in vacuo* to yield a colorless oil (0.0055 g, 60%) with no further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.75 (s, 1H, CHO), 7.31 – 6.99 (m, 5H, ArH), 4.22 (qd, *J* = 7.1, 3.1 Hz, 2H, OCH_2), 4.13 (d, *J* = 18.4 Hz, 1H, NCH_2), 3.82 (d, *J* = 18.3 Hz, 1H, NCH_2), 2.92 – 2.82 (m, 1H, CH_2), 2.45 – 2.32 (m, 3H, 2 x CH_2), 1.74 (s, 3H, CH_3), 1.30 (t, *J* = 7.1 Hz, 3, OCH_2CH_3).

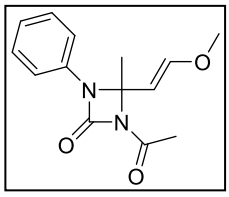
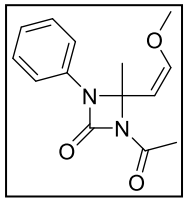
Ethyl 6-methyl-8-oxo-7-phenyl-1,7-diazabicyclo[4.2.0]oct-2-ene-2-carboxylate (25)



This known compound was prepared according to the procedure from Chandrakala *et al.*[26] NaH (60% in mineral oil, 0.005 g, 0.12 mmol) was suspended in dry THF (0.5 mL) and the reaction flask was cooled to 0 ºC. Aldehyde **24** (0.006 g, 0.02 mmol) in THF (1 mL) was added dropwise to the reaction flask and stirred for 1 h at 0 ºC. The reaction mixture was diluted with Et_2O (3 mL), then filtered through a celite pad and washed with Et_2O (3 x 2 mL). The collected filtrate was concentrated *in vacuo*, and purified by preparative thin-layer chromatography (40 % EtOAc/hexanes) to yield aza-carbacephem (0.0019 g, 39%): ^{1}H NMR ($CDCl_3$, 500 mHz) δ 7.34 – 7.04 (m, 5H, ArH), 6.37 –

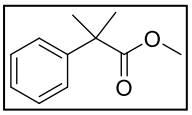
6.33 (m, 1H, vinyl CH), 4.45 – 4.30 (m, *J* = 7.1 Hz, 2H, OCH_2), 2.56 – 2.49 (m, 2H, CH_2), 1.79 (td, *J* = 12.6, 5.1 Hz, 2H, CH_2), 1.67 (d, *J* = 0.8 Hz, 3H, CH_3), 1.39 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

(E/Z)-1-Acetyl-4-(2-methoxyvinyl)-4-methyl-3-phenyl-1-3-diazetidin-2-one (34a, 34b)

These compounds were prepared according to a modified procedure from Della *et al.*[45] (Methoxymethyl)triphenylphosphonium chloride (0.86 g, 2.51 mmol) was suspended in dry THF (8 mL) and the reaction flask was cooled to -78 ºC. NaHMDS (1 M in THF, 2.40 mL, 2.40 mmol) was added dropwise to the reaction flask and stirred for 5 min at -78 ºC. Aldehyde **8** (0.29 g, 1.25 mmol) in THF (4 mL) was added dropwise, then the reaction flask allowed to warm to rt and stir for 16 h. The mixture was diluted with water (8 mL) followed by Et_2O (8 mL). The resulting layers were separated, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield solid in a 4.3:1 mixture of *E/Z* isomers (0.0303 g, 9%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 – 7.09 (m, 5H, ArH), 6.90 (d, *J* = 12.9 Hz, 1H, vinyl CH), 5.03 (d, *J* = 12.9 Hz, 1H, vinyl CH), 3.61 (s, 3H, OCH_3), 2.39 (s, 3H, $COCH_3$), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.55, 153.53, 146.35, 135.82, 129.35, 123.49, 116.06, 110.00, 100.33, 56.52, 23.20, 21.28; HRMS (ES+) calcd for $C_{14}H_{16}N_2O_3Na$ 283.1053 found 283.1059 $[MNa]^+$.

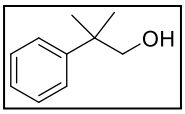
Methyl-2-methyl-2-phenylpropanate (43)



This known compound was prepared according to the procedure from Bonneau *et al.*[47] *N*-Diisopropylamine (16.91 mL, 12.14 g, 120.00 mmol) and bipyridyl (0.003 g, 0.02 mmol) were dissolved in dry THF (120 mL) and the reaction flask was cooled to -78 ºC. *n*-BuLi (1.6 M in THF, 75 mL, 120.00 mmol) was added dropwise to reaction flask and stirred at -78 ºC for 5 min. Methyl phenylacetate (4.22 mL, 4.50 g, 30 mmol) in THF (50 mL) was added to the reaction flask and stirred for 15 min at -78 ºC. Iodomethane (9.34 mL, 21.29 g, 150.00 mmol) was added dropwise and the reaction mixture was allowed to slowly warm to rt and stir for 3 h. The mixture was diluted with water (100 mL) followed by CH_2Cl_2 (100 mL) and the resulting layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 80 mL), and the combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*.

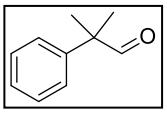
In a new dry flask, additional portion of *N*-diisopropylamine (16.91 mL, 12.14 g, 120.00 mmol) and bipyridyl (0.003 g, 0.02 mmol) were dissolved in dry THF (120 mL) and cooled to -78 ºC. *n*-BuLi (1.6 M in THF, 75 mL, 120.00 mmol) was added dropwise to reaction flask and stirred at -78 ºC for 5 min. The crude sample from before in THF (50 mL) was added dropwise to reaction flask and stirred for 15 min at -78 ºC. Iodomethane (9.34 mL, 21.29 g, 150.00 mmol) was added dropwise and then the reaction flask was warmed to rt and stirred for 3 h. The mixture was diluted with water (100 mL) followed by CH_2Cl_2 (100 mL) and resulting layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 80 mL) and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 5 % EtOAc/hexanes) to yield colorless oil (3.6706 g, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 – 7.24 (m, 5H, ArH), 3.67 (s, 3H, OCH_3), 1.61 (s, 6H, 2 x CH_3).

2-Methyl-2-phenylpropan-1-ol (44)



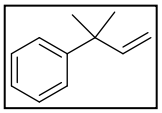
This known compound was prepared according to a modified procedure from Zaysev *et al.*[44] $LiAlH_4$ (0.85 g, 22.40 mmol) was suspended in dry Et_2O (100 mL) and the reaction flask was cooled to 0 ^oC. **43** (1.00 g, 5.60 mmol) in Et_2O (20 mL) was added dropwise and the reaction flask and was stirred for 1 h at 0 ^oC. The reaction mixture was diluted with water (40 mL) and filtered through a celite pad. The filtrate was extracted with Et_2O (3 x 30 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield colorless oil (0.7225 g, 86%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 – 7.20 (m, 5H, ArH), 3.64 (d, J = 6.5 Hz, 2H, OCH_2), 1.36 (s, 6H, 2 x CH_3).

2-Methyl-2-phenylpropanol (45)



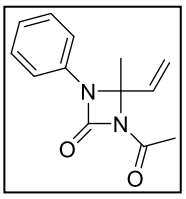
This known compound was prepared according to a modified procedure from Zaysev *et al.*[44] Pyridinium chlorochromate (1.44 g, 6.66 mmol), sodium acetate (0.08 g, 1.00 mmol), and silica (1.03 g, 16.65 mmol) were suspended in CH_2Cl_2 (60 mL) and stirred at rt for 5 min. Alcohol **44** (0.50 g, 3.33 mmol) in CH_2Cl_2 (6 mL) was added dropwise to reaction flask and stirred for 2 h at rt. The reaction mixture was filtered through a silica pad and washed with CH_2Cl_2 (3 x 20 mL). The filtrate was concentrated *in vacuo* and purified by column chromatography (SiO_2, 5 % EtOAc/hexanes) to yield colorless oil (0.3251 g, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.53 (s, 1H, CHO), 7.43 – 7.26 (m, 5H, ArH), 1.49 (s, 6H, 2 x CH_3).

(2-Methylbut-3-en-2-yl)benzene (46)



This known compound was prepared according to a modified procedure from Morrill *et al*.[46] In the reaction flask, methyltriphenylphosphonium bromide (0.97 g, 2.70 mmol) was suspended in dry benzene (8 mL) and stirred at rt for 5 min. NaHMDS (1 M in hexanes, 2.60 mL, 2.60 mmol) was added dropwise and the mixture stirred for 5 min at rt. Aldehyde **45** (0.18 g, 1.23 mmol) in benzene (2 mL) was added dropwise and the reaction flask was heated to 60 ^{o}C for 16 h. The reaction mixture was cooled to rt, then diluted with water (8 mL) and the resulting layers separated. The aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 10 % EtOAc/hexanes) to yield colorless oil (0.0737 g, 41%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40 –7.19 (m, 5H, ArH), 6.06 (ddd, *J* = 17.3, 10.6, 0.5 Hz, 1H, vinyl CH), 5.11 – 5.00 (m, 2H, =CH_2), 1.43 (d, *J* = 0.5 Hz, 6H, 2 x CH_3).

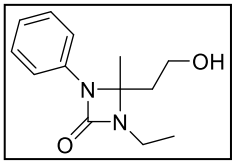
1-Acetyl-4-methyl-3-phenyl-4-vinyl-1,3-diazetidin-2-one (47)



This compound was prepared according to a modified procedure from Morrill *et al.*[46] Methyltriphenylphosphonium bromide (1.67 g, 3.29 mmol) was suspended in dry benzene (15 mL) and stirred at rt for 5 min. NaHMDS (1 M in THF, 3.10 mL, 3.10 mmol) was added dropwise and the reaction flask was stirred for 5 min at rt. Aldehyde **8** (0.38 g, 1.63 mmol) in benzene (5 mL) was added dropwise to the reaction flask and then heated to 60 ^{o}C for 16 h. The reaction mixture was cooled to rt, then diluted with water (15 mL) and the resulting layers separated. The aqueous layer was extracted with EtOAc (3 x 15 mL), and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was

purified by column chromatography (SiO_2, 10 % EtOAc/hexanes) to yield yellow solid (0.1448 g, 39%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 – 7.10 (m, 5H, ArH), 6.11 (dd, *J* = 17.4, 10.7 Hz, 1H, $=CH_2$), 5.72 (d, *J* = 17.3 Hz, 1H, vinyl CH), 5.60 (d, *J* = 10.7 Hz, 1H, $=CH_2$), 2.40 (s, 3H, $COCH_3$), 2.03 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.61, 155.04, 146.24, 135.78, 134.69, 129.36, 123.68, 116.11, 79.73, 23.14, 19.56; HRMS (ES+) calcd for $C_{13}H_{14}N_2O_2Na$ 253.1134 found 253.1129 $[MNa]^+$.

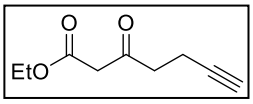
1-Ethyl-4-(2-hydroxyethyl)-4-methyl-3-phenyl-1,3-diazetidin-2-one (48)



This compound was prepared according to a modified procedure from Morrill *et al.*[46] Alkene **47** (0.10 g, 0.46 mmol) in dry THF (4 mL) was cooled to 0 °C, then borane tetrahydrofuran complex (1 M in THF, 0.92 mL, 0.92 mmol) was added dropwise. The reaction flask was warm to rt and stirred for 1 h, then a second portion of BH_3·THF (1 M in THF, 0.50 mL, 0.50 mmol) was added and stirred for additional 30 min at rt. The reaction mixture was cooled to 0 °C, then in a dropwise manner a NaOH solution (1 M, 4 mL) was added which was immediately followed by the addition of H_2O_2 solution (30%, 4 mL) and the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was diluted with brine (10 mL) and the resulting layers were separated. The aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield yellow oil (0.0317 g, 29%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31 –7.15 (m, 5H, ArH), 6.98 (tt, *J* = 7.1, 1.2 Hz, 1H, NCH_2), 3.78 – 3.62 (m, 2H, OCH_2), 3.36 – 3.13 (m, 2H, CH_2), 2.32 – 2.13 (m, 3H), 1.66 (s, 3H, CH_3), 1.25 (t, *J* = 7.3 Hz, 4H, NCH_2CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 153.93, 137.71,

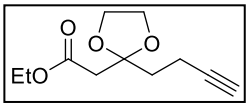
129.34, 122.34, 115.77, 79.68, 57.90, 38.29, 35.19, 22.92, 14.91; HRMS (ES+) calcd for $C_{13}H_{18}N_2O_2Na$ 257.1266 found 257.1266 $[MNa]^+$.

7-Octyne-2,4-dione (52)



This known compound was prepared according to the procedure from Hayakawa *et al.*[64] To the reaction flask, N-diisopropylamine (7.09 mL, 5.12 g, 50.60 mmol) was dissolved in dry THF (50 mL) and then cooled to -78 ºC. *n*-BuLi (1.6 M in THF, 48.7 mL, 63.25 mmol) was added dropwise and stirred at -78 ºC for 5 min. Ethyl acetoacetate (3.22 mL, 3.29 g, 25.30 mmol) in THF (20 mL) was added dropwise to the reaction flask, then stirred at 0 ºC for 30 min. Propargyl bromide (80 % in toluene, 3.10 g, 25.30 mmol) was added in one portion to the reaction flask and stirred at 0 ºC for 1 h. The reaction was quenched with a sat. NH_4Cl solution (4 mL), then diluted with water (50 mL) and Et_2O (50 mL). The resulting layers were separated, and the aqueous layer extracted with Et_2O (2 x 25 mL). The combined organic layers were washed with brine (50 mL) and dried with $MgSO_4$, then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 10 % EtOAc/hexanes) to yield colorless oil (2.8212 g, 66%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.21 (q, 2H, OCH_2), 3.46 (s, 2H, CH_2), 2.82 (t, 2H, CH_2), 2.47 (m, 2H, CH_2), 1.96 (s, 1H, CH), 1.28 (t, 3H, OCH_2CH_3).

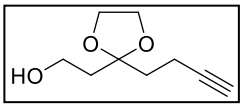
Ethyl-2-(2-(3-butynyl)-1,3-dioxolan-2-yl)acetate (53)



This known compound was prepared according to the procedure from Hayakawa *et al.*[64] Alkyne **52** (2.17 g, 12.90 mmol) was dissolved in benzene

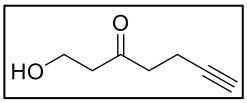
(40 mL) and stirred at rt for 5 min. Ethylene glycol (2.17 mL, 2.41 g, 38.70 mmol) was added to the reaction flask followed by p-toluenesulfonic acid (0.25 g, 1.30 mmol) and the mixture was heated to reflux with a dean-stark trap for 16 h. The reaction was cooled to rt, then poured over a sat. $NaHCO_3$ solution (40 mL) and ice. The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 20 % EtOAc/hexanes) to yield faint yellow oil (2.1101 g, 77%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 4.21 (q, 2H, OCH_2), 4.01 (s, 4H, OCH_2CH_2O), 3.46 (s,2H, CH_2), 2.82 (t, 2H, CH_2), 2.47 (m, 2H, CH_2), 1.96 (s, 1H, CH), 1.28 (t, 3H, $OCH_2\,CH_3$).

2-(3-butynyl)-2-(2-hydroxyethyl)-1,3-dioxolane (54)



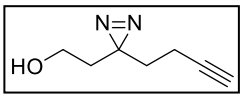
This known compound was prepared according to the procedure from Hayakawa *et al.*[64] $LiAlH_4$ (0.38 g, 10.10 mmol) was suspended in dry THF (35 mL) and cooled to -78 ^{o}C. Protected ester **53** (2.13 g 10.10 mmol) in THF (5 mL) was added dropwise to the reaction flask. The reaction mixture was warm to rt and stirred for 3 h. The reaction mixture was quenched with a 15% NaOH solution (10 mL), then filtered through a celite plug. The filtrate was extracted with EtOAc (3 x 20 mL) and the combined organic layers were dried with $MgSO_4$ then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 50 % EtOAc/hexanes) to yield a colorless oil (1.4512 g, 86%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 4.01 (m, 4H, OCH_2CH_2O), 3.76 (q, 2H, CH_2), 2.66 (t, 1H, CH_2), 2.28 (m, 2H, CH_2), 1.96 (m, 5H, 2 x CH_2 and CH).

1-Hydroxyhept-6-yn-3-one (55)



This known compound was prepared according to the procedure from Hayakawa *et al.*[64] Alcohol **54** (1.36g, 8.00 mmol) was dissolved in acetone (25 mL) and stirred at rt for 5 min. *p*-Toluenesulfonic acid (0.38 g, 2.00 mmol) was added and the reaction flask was stirred at rt for 2 h. The reaction mixture was poured over a sat. $NaHCO_3$ solution (20 mL) and ice, and the resulting layers were separated. The aqueous layer was extracted with Et_2O (3 x 20 mL), and the combined organic layers were washed with brine (20 mL), dried with $MgSO_4$, then concentrated *in vacuo*. The sample was purified by column chromatography (SiO_2, 80 % EtOAc/hexanes) to yield a colorless oil (0.6834 g, 67%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 3.87 (t, 2H, OCH_2), 2.71 (t, 4H, 2 x CH_2), 2.48 (m, 2H, CH_2), 2.38 (br, 1H, OH), 1.97 (t, 1H, CH).

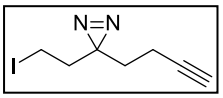
2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethanol (56)



This known compound was prepared according to the procedure form Hayakawa *et al.*[64] With the use of a cold finger trap, anhydrous ammonia (35 mL) was condensed into reaction flask containing ketone **55** (4.43 g, 35.00 mmol). The reaction flask was then heated to 40 ^{o}C and stirred for 5 h maintaining cold finger trap at -78 ^{o}C. Hydroxylamine-*O*-sulfonic acid (4.55 g, 40.25 mmol) in methanol (35 mL) was added dropwise to the reaction flask, then continued to be heated at 40 ^{o}C for 1 h. The cold finger trap was slowly warm to rt and the ammonia was allowed to evaporate overnight. The resulting white solid was filtered through a silica plug and washed with methanol (3 x 25 mL), then condensed *in vacuo*.

The mixture was dissolved in CH_2Cl_2 (17 mL) and triethylamine (6.13 mL, 4.45 g, 43.98 mmol) was added to the reaction flask, and stirred for 30 min at rt. An iodine solution (8 g, 50.4 mmol in 18 mL CH_2Cl_2) was added dropwise to the reaction flask until an orange/brown color persisted, and the resulting solution was concentrated *in vacuo*. The sample was purified excluding light by column chromatography (SiO_2, 80 % CH_2Cl_2/hexanes) to yield a faint yellow oil (2.02 g, 43%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 3.51 (t, 2H, ICH_2), 2.07 (m, 3H, CH_2 and CH), 1.70 (m, 5H, 2 x CH_2 and OH).

3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3H-diazirine (49)



This known compound was prepared according to the procedure from Hayakawa *et al.*[64] Iodine (1.11 g, 4.40 mmol), triphenylphosphine (1.05 g, 4.00 mmol), and imidazole (0.74 g, 10.90 mmol) were dissolved in CH_2Cl_2 (20 mL) and the reaction flask was cooled to 0 ^{o}C. Excluding light, diazirine **56** (0.50 g, 3.60 mmol) in CH_2Cl_2 (4 mL) was slowly added to reaction flask and stirred for 4 h at 0 ^{o}C. The reaction mixture was quenched with a sat. $Na_2S_2O_3$ solution (20 mL) and the resulting layers were separated. The aqueous layer was extracted with EtOAc (3 x 25 mL), and the combined organic layers were washed with brine (30 mL), dried with $MgSO_4$, then concentrated *in vacuo*. The sample was purified excluding light by column chromatography (SiO_2, 8 % EtOAc/hexanes) to yield a yellow oil (0.3822 g, 42%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 2.89 (t, 2H, CH_2), 2.12 (t, 2H, CH_2), 2.01 (m, 3H, CH_2 and CH), 1.69 (t, 2H, CH_2).

-Chapter 4-

-References-

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