The Design, Synthesis, and Testing for Inhibition of Polymyxins Resistance Protein, ArnA, and the Conversion of Esters to Acid Chlorides Using Thionyl Chloride

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Has been approved for the Department of Chemistry and Biochemistry

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Tarek Sammakia

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Date _____

The final copy of this thesis has been examined by the signatories, and we find that Both the content and the form meet acceptable presentation standards of scholarly Work in the above mentioned discipline.

Jacob Arthur Greenberg (Ph.D., Chemistry)

The Design, Synthesis, and Testing for Inhibition of Polymyxins Resistance Protein, ArnA, and the Conversion of Esters to Acid Chlorides Using Thionyl Chloride

Thesis directed by: Professor Tarek Sammakia

Abstract:

One of the most commonly found drug resistant Gram-negative bacteria is *Pseudomonas aeruginosa*, and more than 80% of CF patients die of progressive lung disease caused by *P. aeruginosa*. It has been found that 44% of isolates express a resistance mechanism for the innate immune system's Cationic Antimicrobial Peptides (CAMPs) from as early as 3 months old. This resistance is accomplished through the addition of positively charged moiety 4-aminoarabinose to the lipid-A portion of lipopolysaccharide, which is ordinarily negatively charged. The addition of this moiety decreases the overall negative charge associated with the cell, therefore the effectiveness of CAMPs and antibiotics that act in this fashion. The goal of this project was to design and synthesize selective inhibitors of ArnA a protein in this modification pathway. A synthetic scheme with modular approach in our design was employed and several compounds using this strategy were synthesized. At concentrations of 1 mmol none of the synthesized compounds displayed inhibitory activity. This led to the redesign of our modular strategy to incorporate new, robust functionality in the designed inhibitors.

The reaction of *tert*-butyl esters with $SOCl_2$ at room temperature provides acid chlorides in unpurified yields of 89% or greater. Benzyl, methyl, ethyl, and isopropyl esters are essentially unreactive under these conditions, allowing for the selective conversion of *tert*-butyl esters to acid chlorides in the presence of other esters.

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Contents

List of Tables

List of Figures

List of Schemes

Chapter 1

The Design, Synthesis, and Testing for Inhibition of Polymyxins Resistance Protein, ArnA

1.1 Introduction to Antibiotic Resistance

Bacteria are complex, single-celled organisms that live almost everywhere on Earth, including within other organisms. Most of these bacteria go unnoticed by their hosts and are necessary for survival.[1] However, some strains of bacteria are pathogenic, causing infection and disease. Most of these infections can be treated using traditional antibiotics that target any of several essential bacterial pathways that are absent in human cells.[2] The pathways that have been successfully targeted include: protein synthesis, nucleic acid synthesis, cell wall synthesis, and several others (Figure 1.1).[3] Although antibiotics have saved many lives throughout history, an inherent problem emerges soon after the introduction of a new class of antibiotics, the well-publicized problem of antibiotic-resistant bacterial infections (Figure 1.2).[4] In the United States, the economic burden of antibiotic-resistant infections has been estimated to be as high as $20 billion in health care costs and $35 billion in lost productivity.[5]

In order to combat this public health risk, a comprehensive understanding of these drug resistance mechanisms is essential for the development of new antimicrobials or other alternative treatment strategies.[5] This understanding allows us to predict underlying mechanisms, as well as predict unknown mechanisms of resistance in other emerging pathogens.[4] There are four main mechanisms for drug resistance (Figure 1.1).[3] The first is a decrease in cell permeability, such that the concentration of the antibiotic able to penetrate into the cell is too low to cause cell death. Second is a removal of the antibiotic from the cytosol via efflux pumps. Third is enzymatic inactivation of the antibiotic through chemical modification, and the fourth is the chemical alteration of the antibiotic target site (Figure 1.1).[3]

Figure 1.1 Left, Antibiotic Targets of Bacteria, Right, Mechanisms of Antibiotic Resistance, Image Adapted from Mulvey and Simor 2009[3]

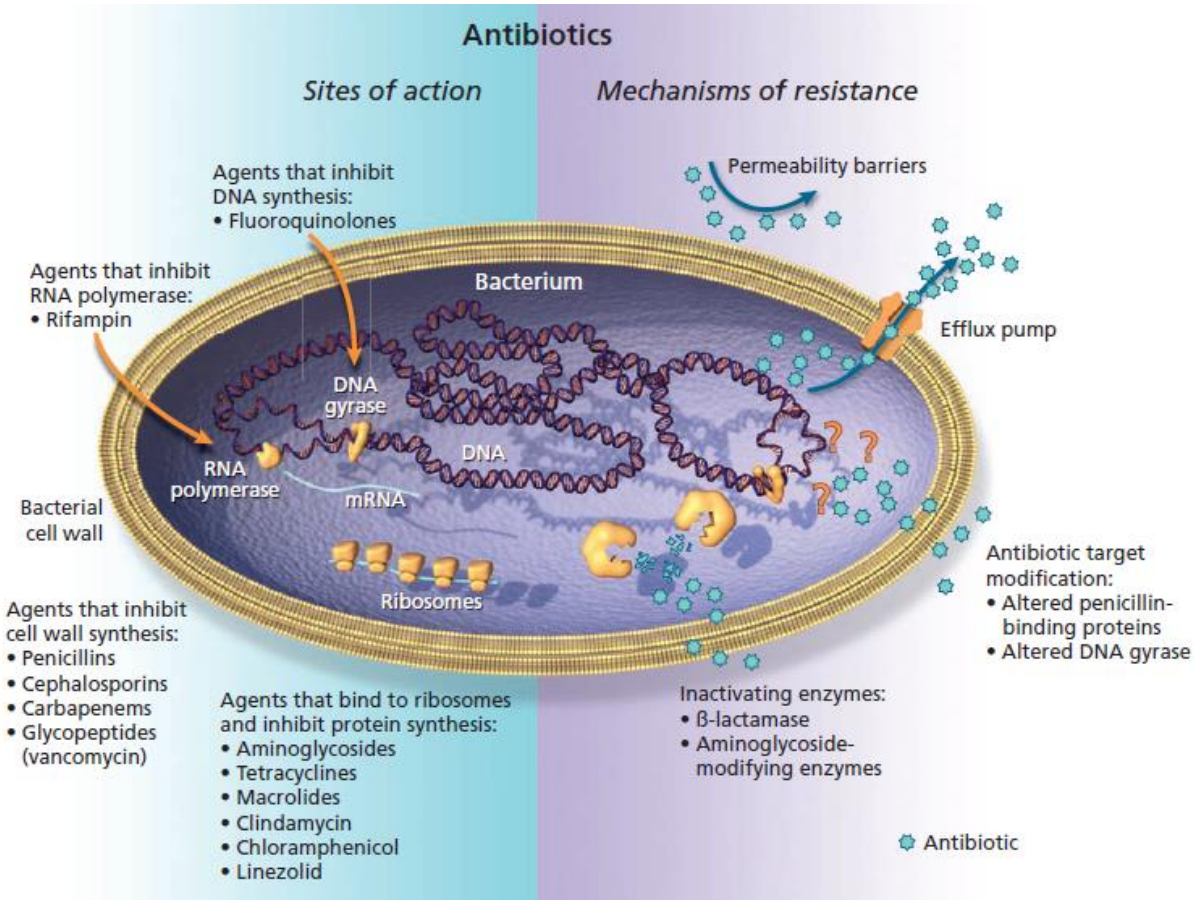
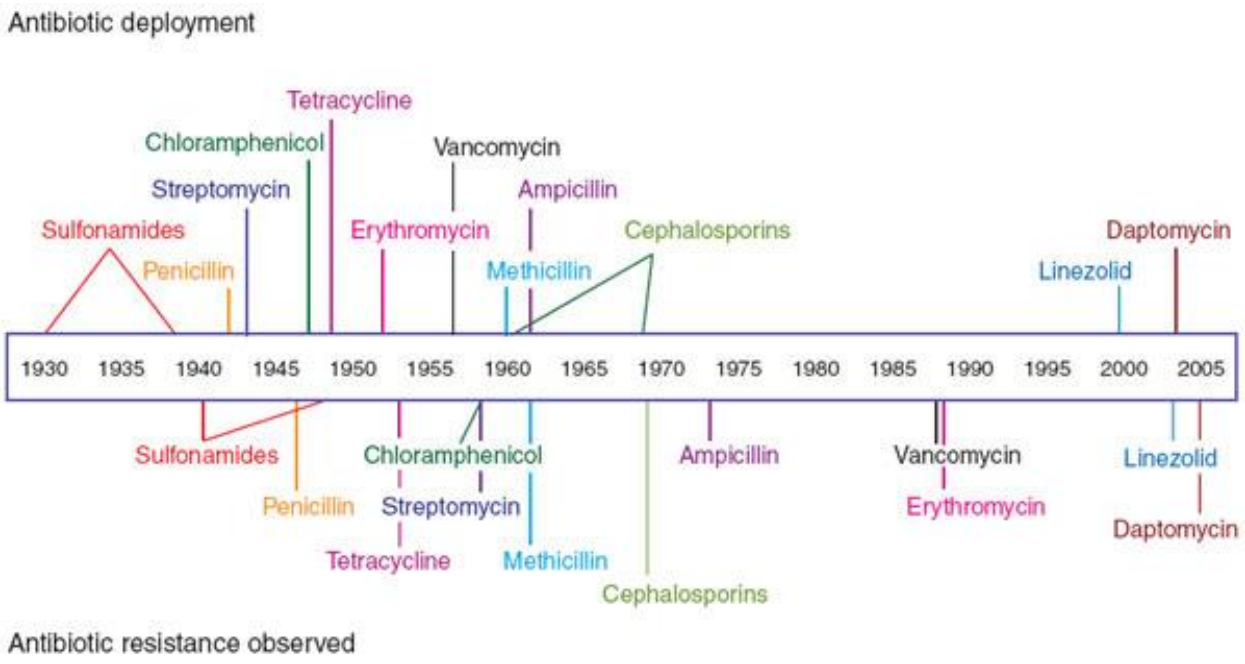


Figure 1.2 Above, A Timeline of the Deployment of Antibiotics, Below, When Antibiotic Resistance was Detected. Image adapted from Clatworthy *et. al.* 2007[3]



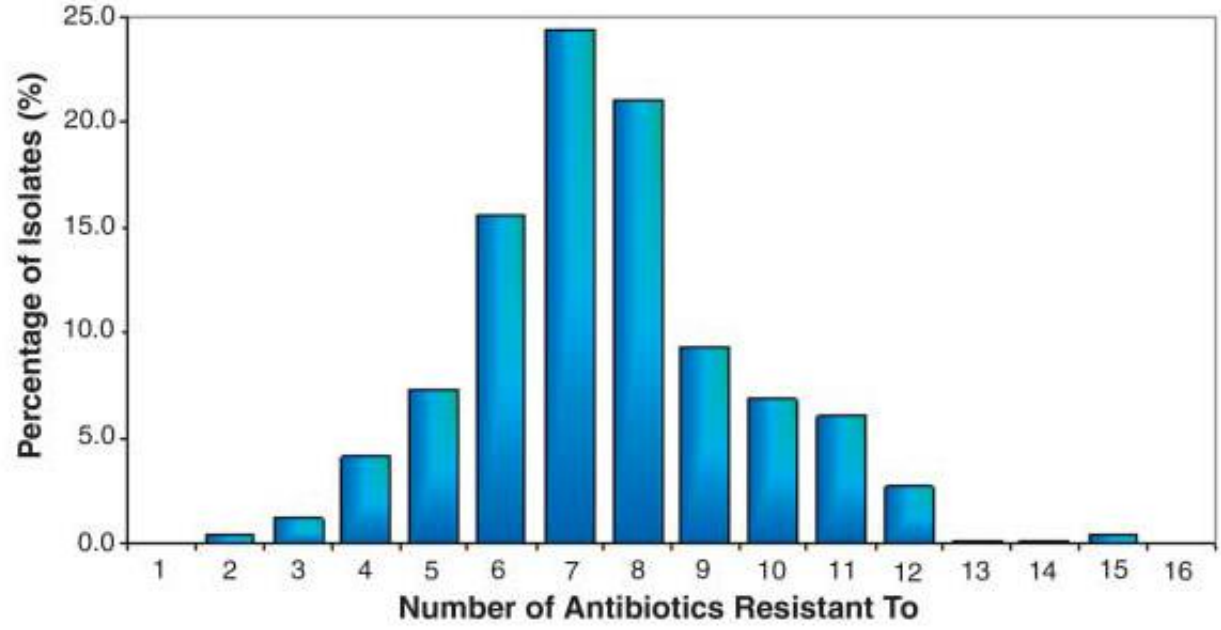
Currently, multidrug resistant infections are among the top threats to global public health. This is primarily due to excessive and inappropriate use of antibiotics.[6] ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) is an acronym that has been used to describe the 6 most well recognized organisms that are known to have multidrug resistant strains.[6] These multidrug resistant organisms are medically important because the number of drugs that can be used to treat these infections is becoming limited, and in some cases all known antibiotic treatments are ineffective.[5,6]

It was once believed that antibiotic resistance emerged because of widespread human use. There is now much evidence suggesting that antibiotic resistance genes co-evolved with the genes responsible for natural products antimicrobial biosynthesis.[7] In 2006, Gerard Wright coined the term "resistome,"[8] which is the gene reservoir responsible for resistance to antimicrobials.

Targeted investigation of the resistome has revealed that at least some drug resistance has been around for thousands of years and arose independently of wide spread human use of antibiotics.

Actinomycetes are a genus of bacteria that are prolific natural product producers,[9] and among these natural products is the source of the most medically relevant antibiotics.[10] It has been suggested that these organisms may provide a wealth of new antimicrobial structures. Because these organisms produce many antibiotics, there must have been evolutionary pressure to retain these genes, and therefore there must also be defense mechanisms to protect against other antimicrobials. To test this hypothesis, the resistome of these organisms was probed. Soil actinomycetes were cultured and the isolates were tested for drug resistance against 21 different antibiotics.[8] It was found that most isolates were inherently resistant to 7-8 different antibiotics (Figure 1.3). Many isolates were resistant to many different types of antibiotics, notably all of the strains were resistant to natural product antibiotics as well as semi-synthetic natural products. Similar studies have been conducted on isolates from a 30,000 year old frozen layer of the arctic tephra,[11] an isolated cave system,[12] the human gut,[13] and monitored throughout the process of cattle production.[14] All of these studies draw similar conclusions, antibiotic-resistance is ancient and naturally occurring in the environment.[7]

Figure 1.3 Histogram of Soil Actimomycetes Isolates and the Number of Antibiotics Each Was Resistant To, Image Adapted from D'Costa *et al*. 2006[8]

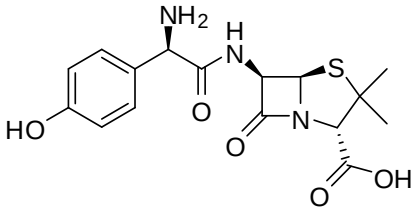


1.2 Treatments for Multi Drug Resistance Infections, Drug Resistance Modifying Agents

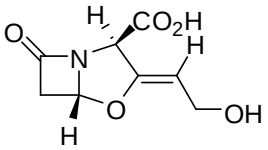
One strategy to combat antibiotic resistance is to employ compounds that resensitize drug-resistant cells to traditional antibiotics. These compounds which are known as resistance modifying agents (RMAs), do not show antibiotic activity of their own. However, when used in conjunction with traditional antibiotics, render the antibiotic effective at treating disease.[15] Often times when treating a bacterial infection, physicians will prescribe a cocktail consisting of 2 compounds. For example, Augmentin is a cocktail consisting of amoxicillin (**1.01**) and clavulanic acid (**1.02**).[16] Amoxicillin is a β-lactam antibiotic whose mechanism of action involves inhibition of the final cross-linking of the peptidoglycan cell wall, resulting in cell death. However, bacterial cells have evolved a protein known as β-lactamase which will inactivate β-lactam antibiotics via one of several different known mechanisms.[17] Clavulanic acid (**1.02**), the other component of Augmentin, shows no antimicrobial activity by itself, but is a potent β-lactamase inhibitor.[17]

Clavulanic acid, when taken in conjunction with amoxicillin, allows the antibiotic to retain its bactericidal activity even in organisms that express β-lactamase. One successful strategy to find RMAs is through the screening of large chemical libraries that show structural diversity.[18] The other type is the targeted design and synthesis of inhibitors of drug resistant proteins, which is the strategy employed in this chapter.

Figure 1.4 Components of Augmentin



Amoxicillin **1.01**



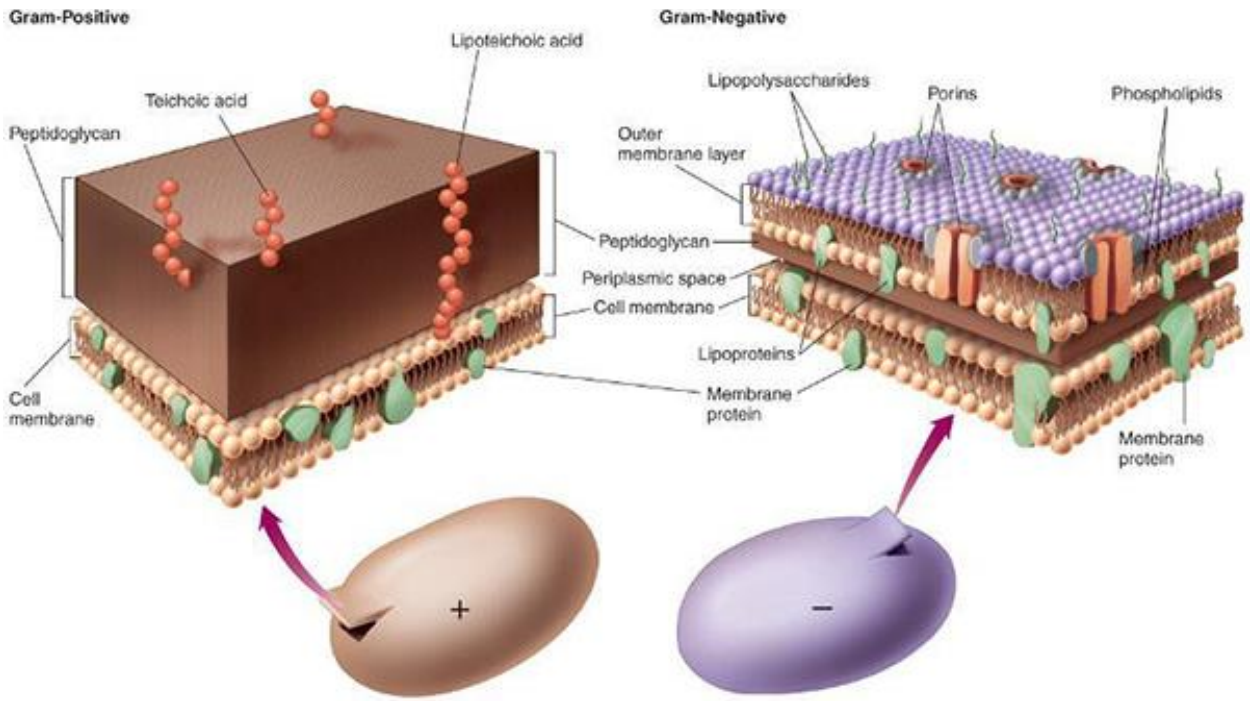
Clavulanic acid **1.02**

1.3 Bacterial Outer Membrane Structure

Although many species of bacteria have been described, they are categorized into 2 major classes according to their outer membrane structure. Hans Christian Gram was the first microbiologist to recognize this striking difference and developed a robust staining assay for differentiating between the two categories.[19] The bacterial outer membrane structural elements were found to be responsible for this difference in staining (Figure 1.5).[20] The Gram positive bacterial outer-membrane contains one phospholipid bilayer surrounded by a thick layer of peptidoglycan, while Gram negative bacterial outer-membranes contain 2 lipid bilayers with a periplasmic region between the two layers containing a thin peptidoglycan cell wall. The inner membrane of Gram negative organisms is symmetric with both layers being comprised mainly of phospholipids. The outer membrane of Gram negative bacteria is asymmetric with the inner leaflet of the outer membrane being composed primarily of phospholipids, and the outer leaflet of the

outer membrane being comprised of lipopolysaccharide (LPS). Gram negative bacteria are known to be more difficult to treat because of enhanced stability of the LPS in the outer membrane. As such, the development of new treatments for these infections is essential.[21]

Figure 1.5 Bacterial Outer Membrane: Left, Gram positive, Right, Gram negative. Image Adapted from McGraw-Hill Microbiology[20]

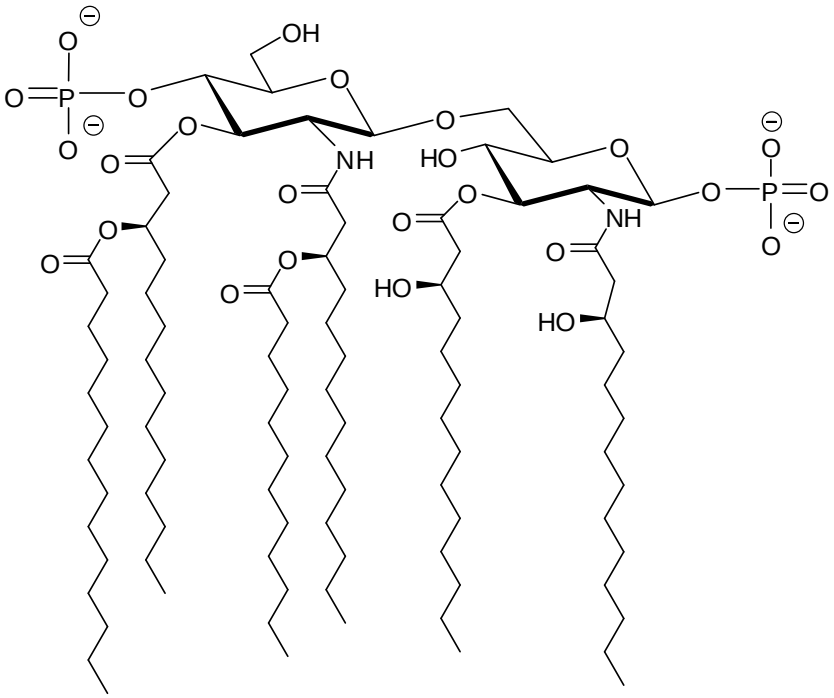


1.4 LPS Structure, Function, and Biosynthesis

LPS is a much more rigid structure that is not as permeable to small molecules, and it is this structural feature that explains why Gram negative infections are more difficult to treat.[21] LPS is an amphipathic molecule that contains 3 major structural elements; the lipid A, the core oligosaccharide, and the O-antigen. Lipid A, also known as endotoxin, is the molecule responsible for the human innate immune system response to Gram-negative bacteria.[22] Lipid A is comprised of a central disaccharide, two glucosamine units in a β(1→6) linkage, decorated with acyl chains and one negatively charged phosphate group on each sugar (Figure 1.6).[23] The core oligosaccharides can further be categorized into an inner and outer core. It has been found that the

number of acyl chains on lipid A, the identity of the core sugars, and in particular the O-antigen can vary between organisms.[22-24] The inner core usually consists of 8-12 sugar units that are often branched, and the outer core consists usually of 6 sugar units that are also often branched. The O-antigen chains attached to a polysaccharide containing up to 200 sugars.[24]

Figure 1.6 Structure of *E.coli* Lipid A

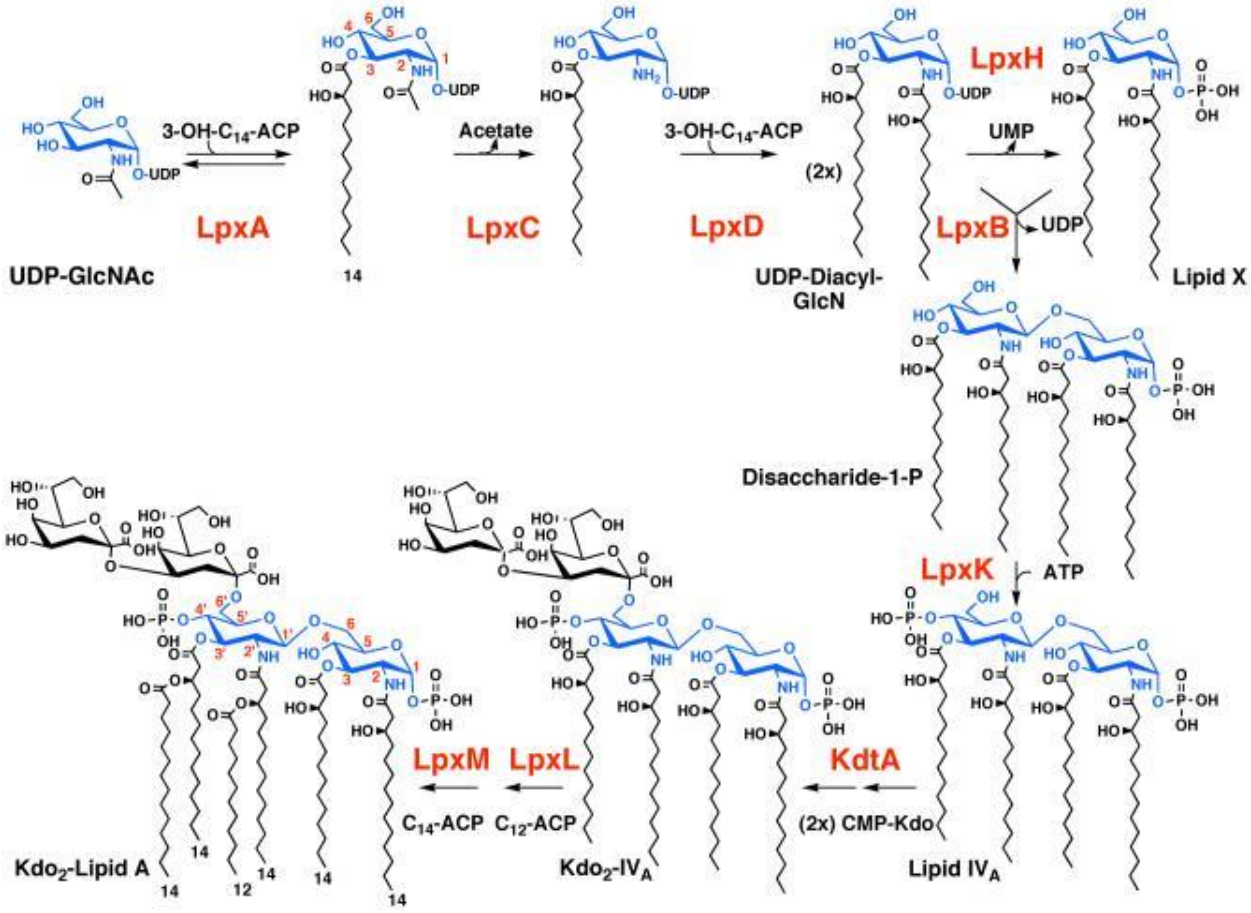


The biosynthesis of LPS has been extensively studied. It begins with nine enzymes that comprise the constitutive Kdo_2-lipid A pathway. Kdo_2 is a second disaccharide unit, composed of two 3-deoxy-D-manno-octulosonic acid units that are biosynthesized in conjunction with lipid A (Figure 1.7). Kdo_2-lipid A is essential for almost all Gram-negative bacteria. Consequently, the enzymes for its biosynthesis have been found to be conserved in almost all Gram-negative bacteria as well.

LPS biosynthesis begins in the cytosol where UDP-glucosamine gets extensively functionalized on its way to the inner leaflet of the inner membrane (Figure 1.7).[25] LpxA, LpxC and LpxD are all cytosolic,[26] while LpxB and LpxH are peripheral lipoproteins located on the inner

leaflet of the inner membrane.[27] LpxK, KdtA, LpxL, and LpxM are integral inner membrane proteins, with cytosolic facing active sites.[28] Kdo_2-lipid A is normally further functionalized with the core oligosaccharide and the O-antigen before it is eventually shuttled to the outer leaflet of the outer membrane.

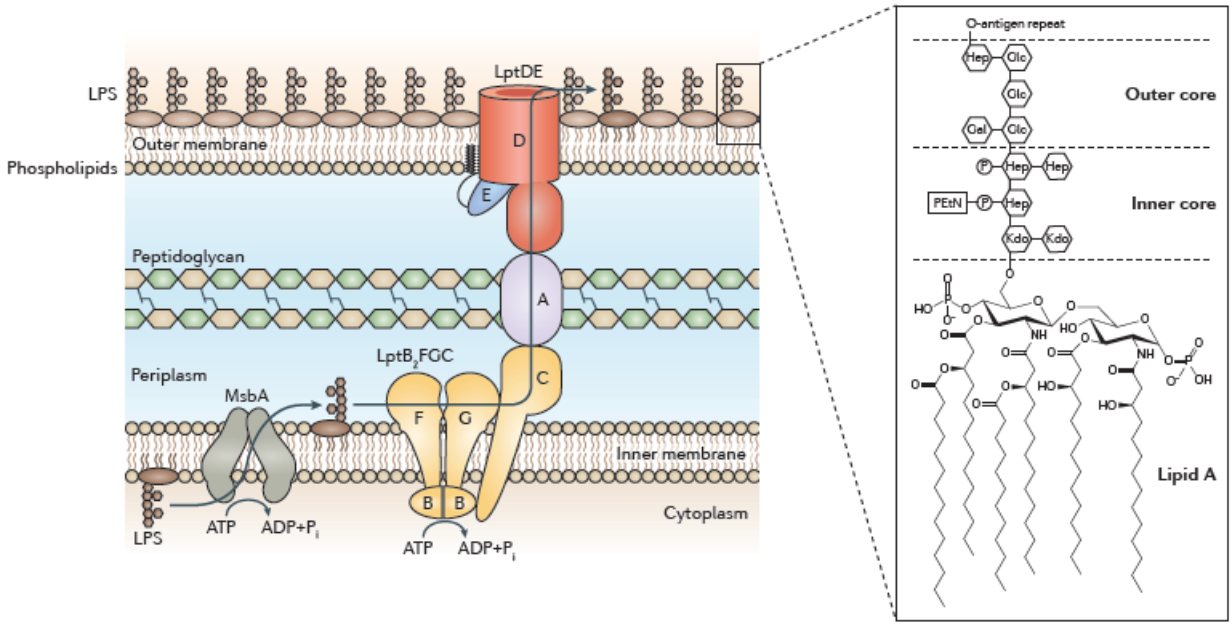
Figure 1.7 The Biosynthesis of Kdo_2-lipid A, Figure Adapted from Raetz *et al.* 2007[25]



The core oligosaccharide is attached to the Kdo_2-lipid A while it is on the inner leaflet of the inner membrane.[29] Newly synthesized core-lipid A is transferred from the inner leaflet of the inner membrane to the outer leaflet of the inner membrane via flippase MsbA. (Figure 1.7).[30] Once facing the periplasmic region, the core-lipid A can be further functionalized with the O-antigen via

ligase, WaaL.[31] The LPS is then shuttled to the outer leaflet of the outer membrane using the lipopolysaccharide transport (lpt) machinery (Figure 1.8).[30]

Figure 1.8 Left, Outer Membrane Machinery, Right, Structure of LPS. Image Adapted from Okuda *et al.* 2016[30]



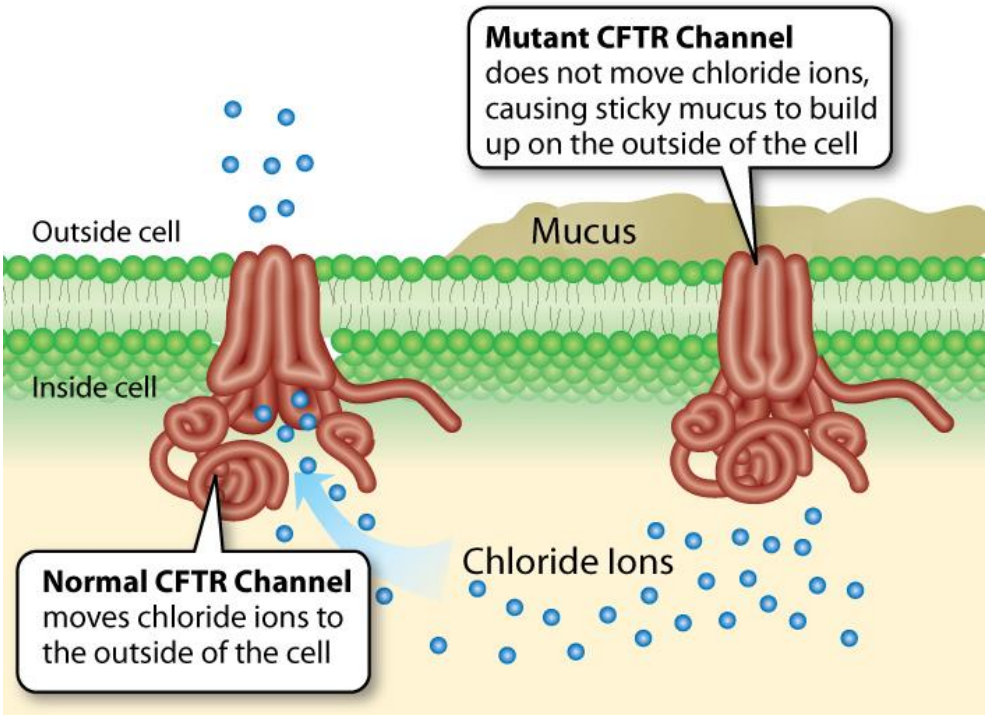
1.5 Multi-drug Resistant *Pseudomonas aeruginosa* Infections in Cystic Fibrosis Patients

The outer membrane structure of Gram negative bacteria is responsible for the paucity of known antibiotics that can treat these infections. [21] Out of the 6 ESKAPE pathogens 4, are Gram-negative. One of these organisms, *Pseudomonas aeruginosa*, has been found to be especially difficult to treat in cystic fibrosis (CF) patients.[32] CF is one of the most common, lethal hereditary disorders affecting about 1 in 3,900 newborns in the United States.[33] More than 30,000 people have CF in the US, with about 1,000 new cases diagnosed each year.[33] The median survival age for individuals with CF is just over 33 years.[33] To improve both the quality of life and the survival

of CF patients it is crucial that new strategies are developed to manage their drug resistant infections.

CF is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene which encodes a chloride ion channel. About 90% of CF patients that have the ΔF508 mutation wherein 3 nucleotides are deleted resulting in the loss of phenylalanine to position 508 of the CFTR protein. This mutation causes serious defects in electrolyte transport which results in the symptoms CF patients experience (Figure 1.9).[34] The most well-known and recognizable symptom is the accumulation of thick, dehydrated mucus in the airways of the lungs which become colonized by opportunistic pathogens, especially *P. aeruginosa*.[35]

Figure 1.9 CFTR Protein with and without the Mutation[36]

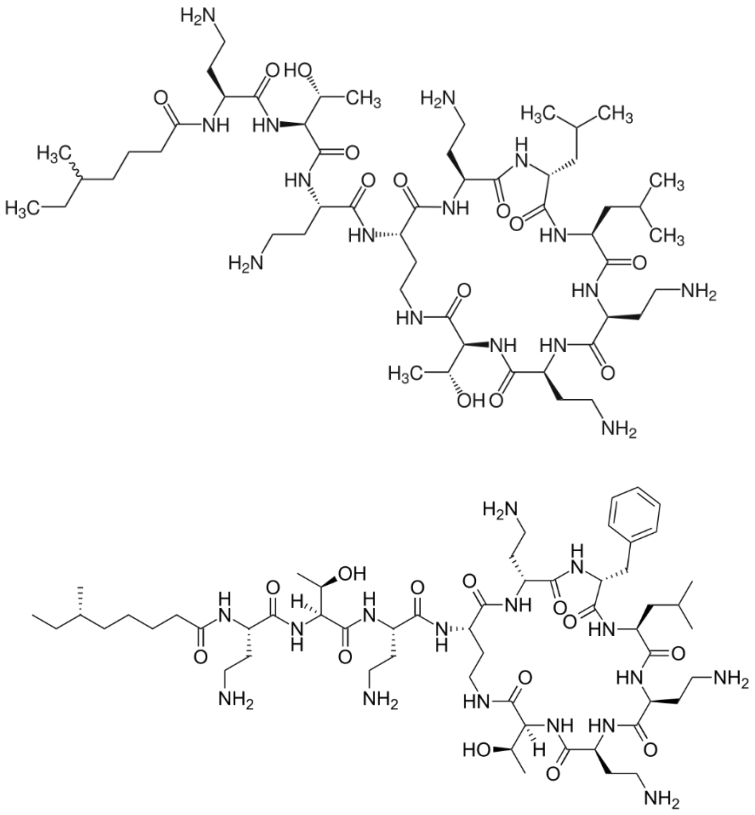


P. aeruginosa adapts to avoid immune clearance and resist antibiotics via efflux pumps, β-lactamase expression, reduced porins, and switching to a biofilm phenotype.[37] Many different treatment methods must be administered over the course of these infections, and even with drug

therapy, these infections often persist in CF patients.[37] Physicians then resort to the use antibiotics of "last resort" to treat these infections.[38] These "last resort" medications are polymyxins, which are in the class of compounds known as the *C*ationic *A*nti*M*icrobial *P*eptides (CAMPs). Polymyxins were first discovered in 1947, and polymyxin b was first isolated in 1954 through the fermentation of different strains of *Paenibacillus polymyxa* (previously *Bacillus polymyxa).*[39] One common structural element in the CAMPs that have been approved for use in humans, colisten (Figure 1.10 top) and polymyxin b (Figure 1.10 bottom), is a cyclic peptide with a longer hydrophobic tail that is hypothesized to interact with the acyl chains of LPS, thus disrupting the outer membrane.

Polymyxins are biosynthesized via nonribosomal peptide synthetase enzyme complexes,[40] and they were widely prescribed to treat Gram-negative infections until the 1970's when their use became unpopular because of the neurotoxicity and nephrotoxicity associated with higher doses of the drug.[41] Polymyxins have now come back into use as a "last resort" drug treatment for multidrug resistant organisms.[42] But even as a "last resort" antimicrobial, it can be ineffective in the treatment of some multidrug resistant organisms, including *P. aeruginosa*, as they have evolved resistance mechanisms to combat the action of CAMPs.

Figure 1.10 Polymyxins Approved as Antimicrobials: Top, Colistin, Bottom, Polymyxin B



P. aeruginosa is the most frequently cultured bacteria from CF patient lung samples, being found in 61% of patients.[43] These infections have been found to become multidrug resistant in patients as young as 6 months.[44] More than 44% of CF patients die of progressive lung disease caused by multidrug resistant *P. aeruginosa* that express a resistance mechanism for the innate immune CAMPs.[45] This project focuses on an approach to new drug therapies of bacterial targets that mediate resistance to polymyxins and endogenous CAMPs.

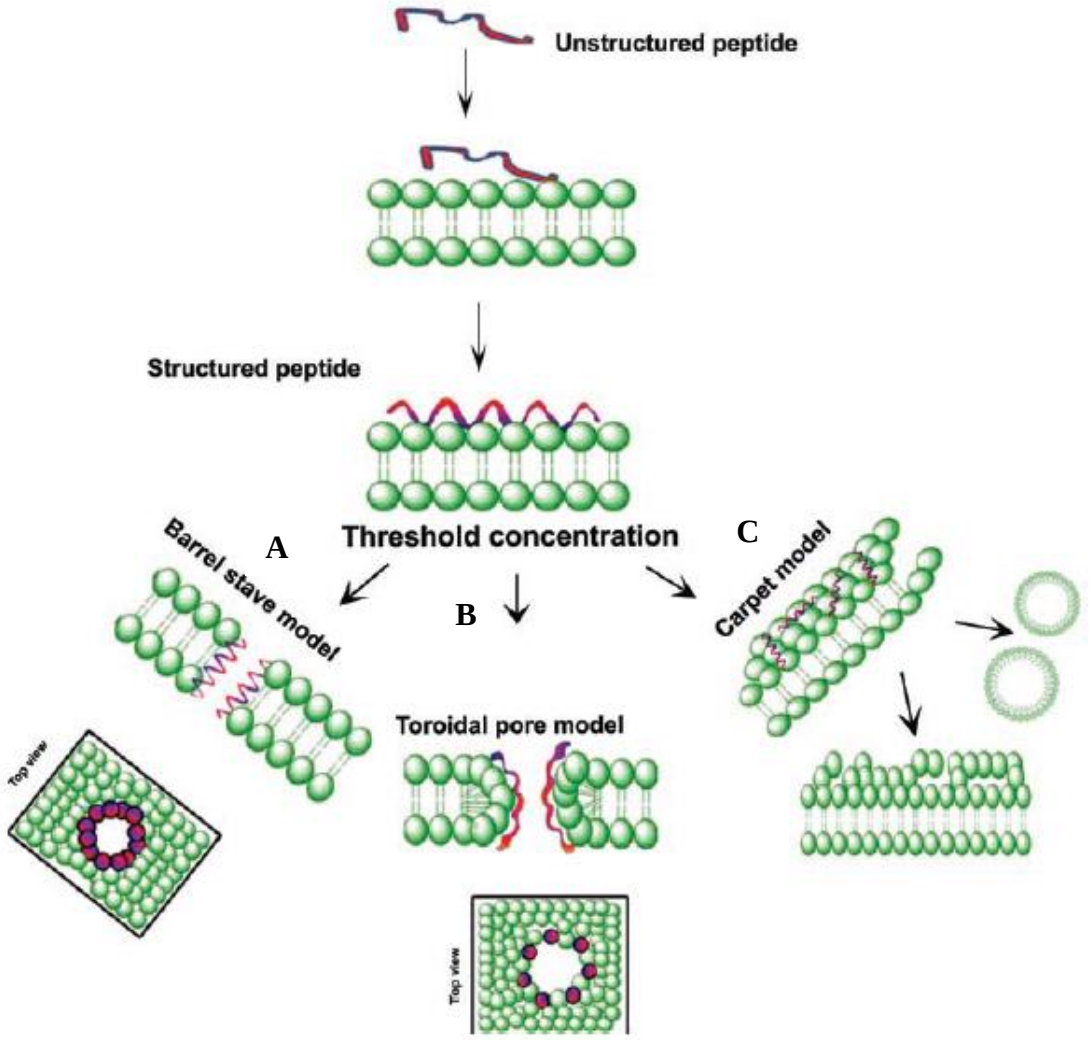
1.6 Cationic Antimicrobial Peptides and Polymyxins

The LPS of Gram negative bacteria is rigid and difficult to penetrate.[21] The human innate immune system has evolved a protection strategy. Host defense against Gram negative bacterial membranes is accomplished through CAMPs. It is hypothesized that these CAMPs have co-evolved with the evolution of the Gram negative outer membrane.[46] CAMPs are amphipathic

and cationic in nature at physiological pH and therefore exhibit an electrostatic attraction to the negative charge associated with the phosphate groups of the lipid A portion of LPS.[47] Once CAMPs are electrostatically bound, they are then able to go on to disrupt the outer membrane.

Several mechanisms of how membrane disruption by CAMPs is accomplished have been proposed, including, the barrel and stave model (Figure 1.11A), the toroidal pore model (Figure 1.11B), and the carpet model (Figure 1.11C). In the barrel-stave model, the CAMPs aggregate and insert themselves into the membrane bilayer so that the hydrophobic peptide regions align with the lipid core region and the hydrophilic peptide regions form the interior region of the pore (Figure 1.11A).[48] The toroidal-pore method hypothesizes that antimicrobial peptide helices insert into the membrane which induces the lipid monolayers to bend continuously through the pore so that the hydrophilic core is lined by both the inserted peptides and the lipid head groups (Figure 1.11B).[49] In the carpet model, CAMPs are electrostatically attracted to the LPS at numerous sites covering the surface of the membrane in a carpet-like manner (Figure 1.11C).[50] At sufficiently high peptide concentrations, surface-oriented peptides are thought to disrupt the bilayer in a detergent-like manner, eventually leading to the disintegration of the outer membrane through the formation of micelles after disruption of the bilayer curvature.[50]

Figure 1.11 The Proposed Mechanisms of CAMPS. Image Adapted from Pasupuleti *et al.* 2012[47]



The number of human innate immune system CAMPs is large with over 100 different peptides known to exhibit broad-spectrum antibacterial activity identified.[51] With this variety in structures it is not surprising that evidence of several different mechanisms of action have been observed. Currently, two CAMPs from bacterial sources are approved for use in the treatment of bacterial infections (Figure 1.9), and there are several that are in clinical trials for use to treat drug-resistant Gram negative bacterial infections.[40,41]

1.7 LPS Modifications for Resistance to CAMPs

Several mechanisms of resistance have been identified and each individually has been found to increase resistance to CAMPs.[25] It has been found that the upregulation of polymyxins resistance genes occurs in low Mg^{2+} concentrations, and can be activated through two separate two component systems, the PhoP/PhoQ and the PmrA/PmrB systems.[52] One mechanism of resistance is the addition of a palmitate moiety to one of the glucosamine units of lipid A; increasing overall hydrophobicity of the outer membrane and altering the CAMPs penetration into the hydrophobic layer.[53] In addition to increasing the hydrophobicity, the negative charge associated with the LPS can be neutralized through the addition of positively charged moieties whose genes are also upregulated via the PhoP/PhoQ and the PmrA/PmrB systems (Figure 1.11).

Two positively charged moieties have been identified, phosphoethanolamine and 4-amino arabinose ($Ara4N^{+}$).[25] Both add a positively charged moiety to LPS and are responsible for altering the overall net negative charge associated with the outer membrane.[54] The overall neutralization of the net negative charge decreases the affinity of CAMPs to the outer membrane, thus evading the innate immune system's defense mechanisms (Figure 1.12). The pathways associated with these additions have been elucidated.[27] Phosphoethanolamine is added to the LPS on the outer leaflet of the inner membrane by EptA.[55] The addition of $Ara4N^{+}$ was found to be much more complex involving an 8 enzyme pathway.[25]

Figure 1.12 Modifications to Lipid A, Image Adapted from Raetz *et al.* 2007[25]

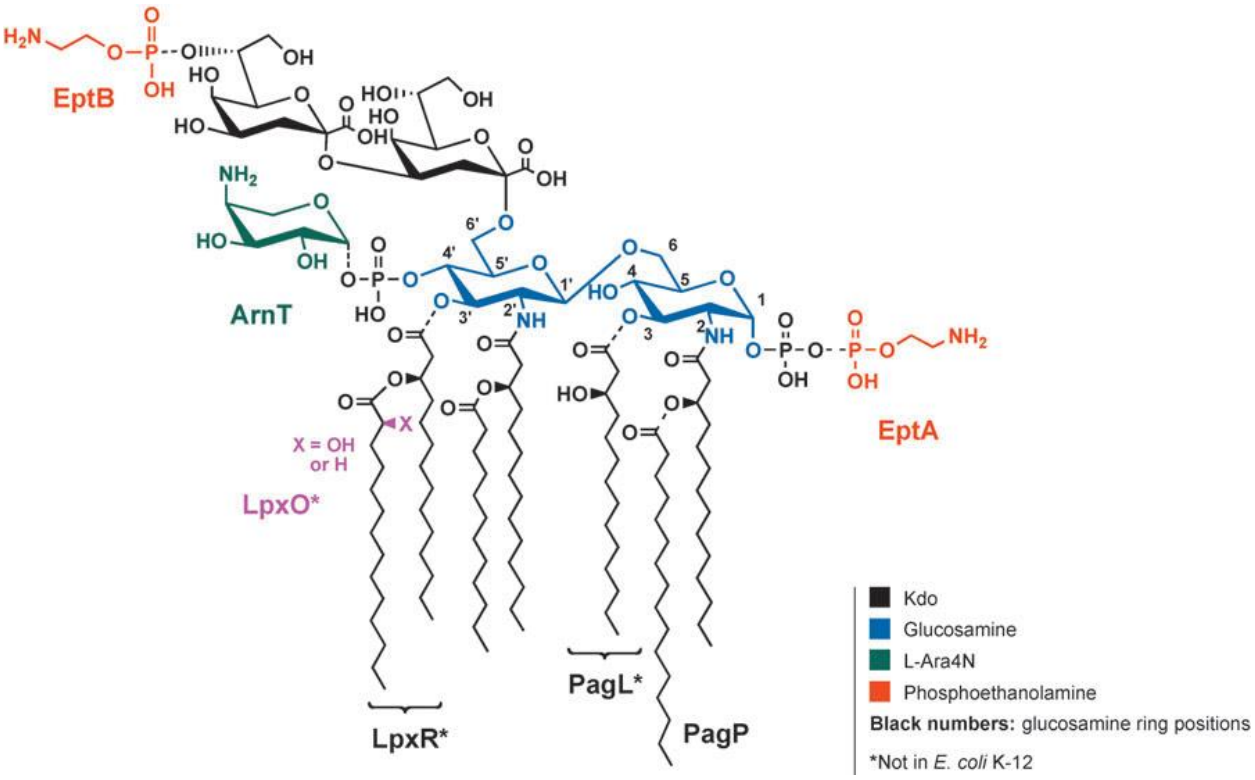
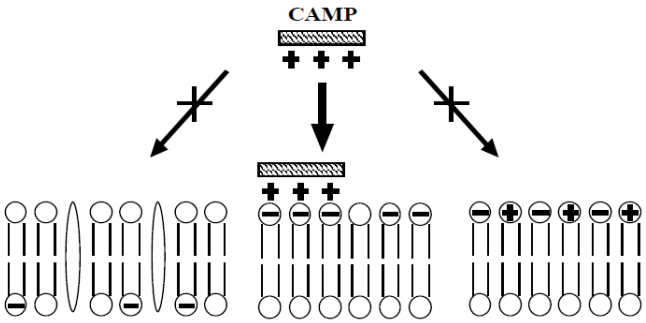


Figure 1.13 Left, Diagram of Outer Membrane Charges Associated with Human, Center, Gram Negative Bacteria, and Right, Gram Negative Bacteria with a Modified Outer Membrane

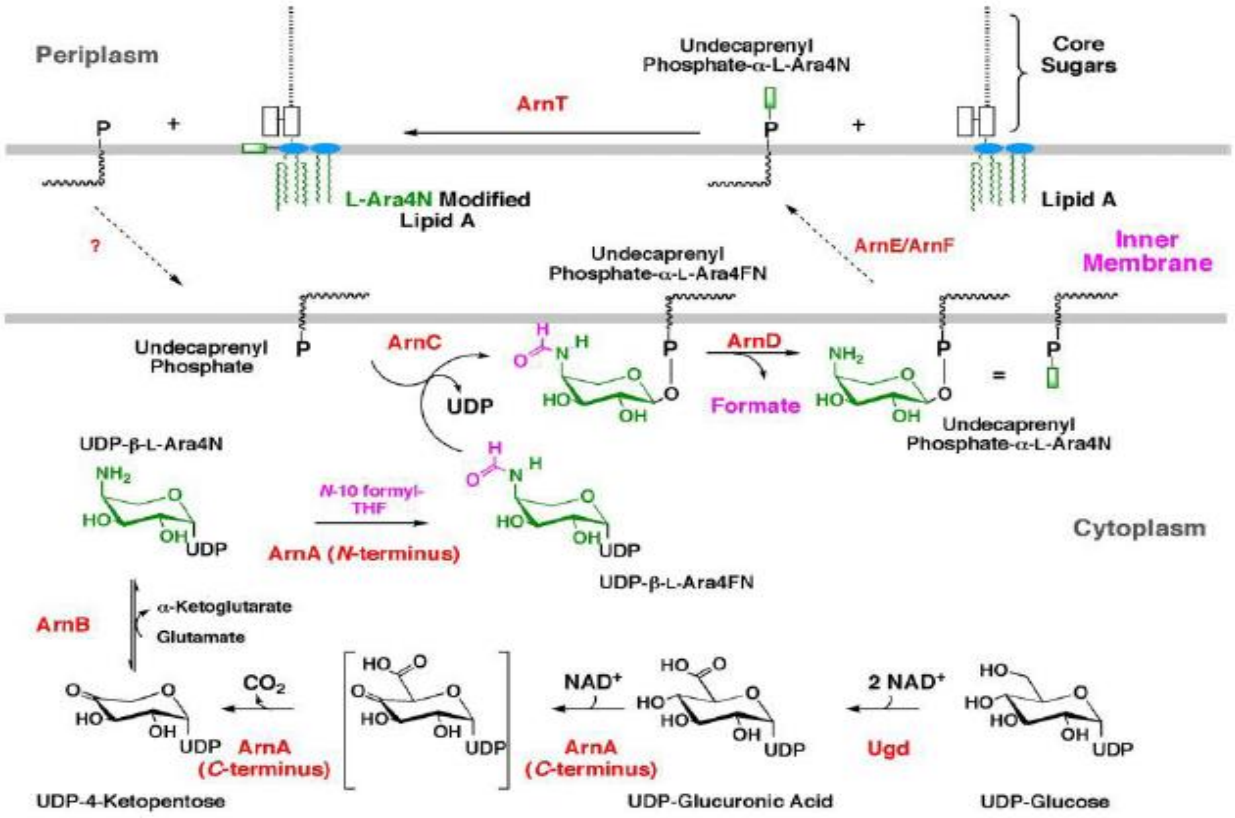


The genes responsible for the biosynthesis of LPS-Ara4N^{+} are encoded on two loci in the Gram negative bacterial genome.[56] One loci encodes for a single protein, PmrE (for *P*oly*M*yxin *R*esistance), a cytosolic UDP-glucose dehydrogenase (Ugd). Ugd is the first enzyme in the

biosynthetic pathway and is responsible for the oxidation of UDP-glucose to UDP-glucuronic acid (Figure 1.13). The remaining 7 enzymes are located on the second loci, previously named PmrHFIJKLM, since renamed ArnBCADTEF (4-amino*AR*abi*N*ose modification) for the function of these genes. In the *Salmonella* ArnBCADTEF operon, all of these genes, with the exception of ArnF, are essential for the [Ara4N$^+$]-Lipid-A pathway,[57] however there is evidence that ArnF is also essential in *E. coli*.[58]

The second step in the pathway is the NAD$^+$ assisted oxidative decarboxylation of UDP-glucuronic acid to UDP-4-ketopentose catalyzed by the C-terminal domain of the bifunctional ArnA.[59] UDP-4-ketopentose is then subjected to reductive amination by the transaminase ArnB, yielding the novel sugar-nucleotide UDP-4-aminoarabinose.[60] However, the equilibrium constant of the reaction catalyzed by ArnB has been found to be unfavorable at about 0.1.[60] To drive the reaction to product, the N-terminal domain of ArnA formylates UDP-4-aminoarabinose, using N_{10}-formyl-tetrahydrofolate as the formyl group donor.[61] No evidence of formyl-4-aminoarabinose-lipid A products have been detected in the outer membrane, suggesting that this formylation compensates for the unfavorable equilibrium constant of the reaction catalyzed by ArnB.[60] This transiently formylated product is then transferred to undecaprenyl-phosphate with release of UDP by the transferase, ArnC.[61] ArnD then proceeds to deformylate the undecaprenyl-phosphate-L-4-formylaminoarabinose, which is then flipped to the periplasmic side of the inner membrane by the heterodimeric ArnE/ArnF.[58,61] Finally, ArnT transfers Ara4N$^+$ from the undecaprenyl intermediate to lipid A to yield the final product [Ara4N$^+$]-lipid A. [62] This functionalized Lipid A is then shuttled to the outer membrane through the lpt machinery.[30]

Figure 1.14 Ara4N^{+}-lipid A Biosynthesis

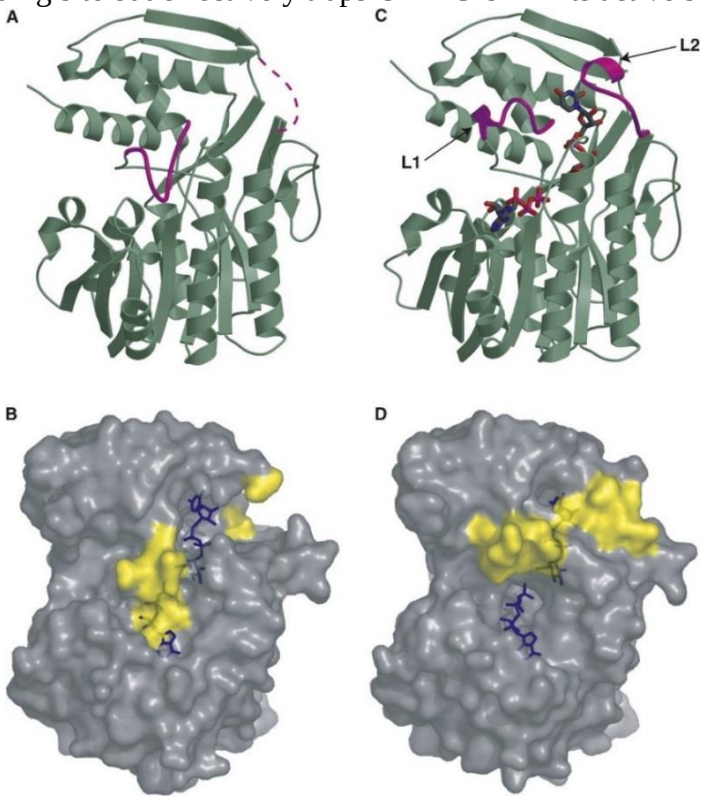


1.8 ArnA C-terminal Domain, Structure, and Mechanism

The second step in the pathway, catalyzed by the C-terminal domain of ArnA, is the first pathway specific reaction in the biosynthesis of [Ara4NH$_4^+$]-Lipid A and is essential for polymyxins resistance. As previously discussed, The C-terminal domain of ArnA catalyzes the NAD$^+$ assisted oxidative decarboxylation of UDP-glucuronic acid (UDP-GlcA) to UDP-keto-pentose.[63] Crystal structures of ArnA were obtained with and without ligands bound and were found to have striking differences.[63] In the ligand-free structure, the UDP-GlcA site is open whereas the NAD$^+$ binding site is partially closed by inhibitory residues.[63] The residues responsible for this allosteric shift are 500-509 and 605-616. The Cα atom of residue I_{506} which resides in the center of the 500-509 loop (L1, Figure 1.14) shows a 17.5 Å shift between the two structures. Residues 605-616 (L2, Figure 1.14) are disordered in the ligand-free structure and were

found to adopt ordered helix loop conformation in the bound structure that now makes several contacts with the uracil ring of UDP-GlcA. The shift of these 2 regions on the protein both reveals the NAD^+ binding site, as well as effectively "locking" the UDP-GlcA in place for the reaction to take place. These differences in the enzyme structure allow us to draw conclusions about the enzymes mechanism, supported by kinetic data.[61, 63]

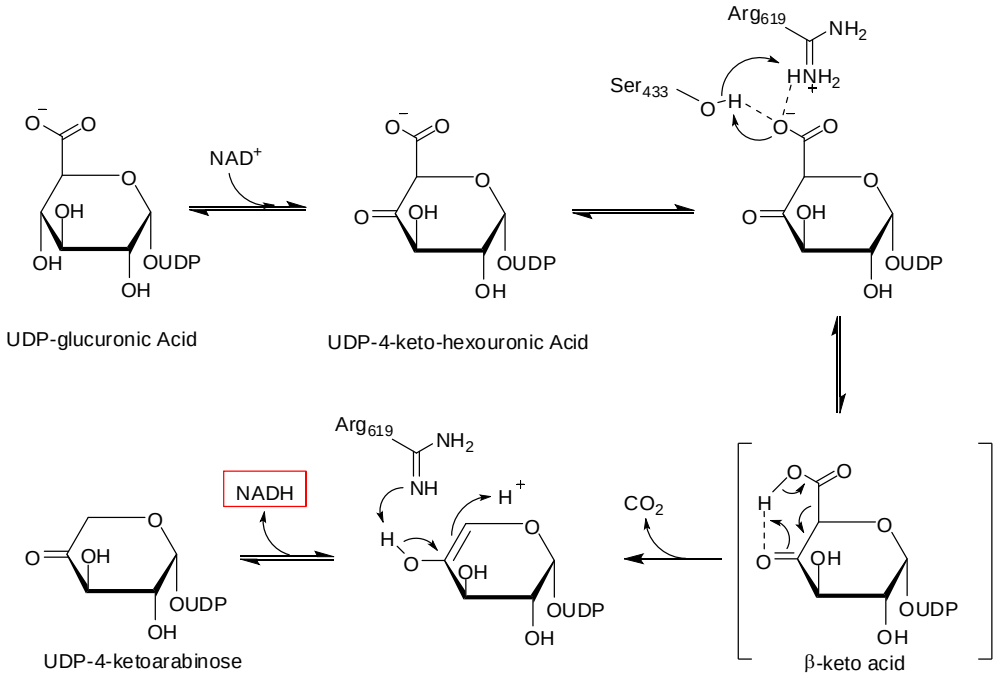
Figure 1.15 Crystal Structures of ArnA with and without Ligands. Image from Gatzeva-Tolalova *et al.* 2005.[61] (**A**) Structure of the ligand-free ArnA C-terminal domain (PDB_ID: 1U9J). The 500–509 loop that blocks the NAD+ binding site is colored in magenta. The disordered loop, loop 605–616, is shown as a dotted line colored in magenta. (**B**) Structure of the ATP/UDP-GlcA ligand bound ArnA domain. The 500–509 loop (L1) is displaced 17 Å (measured at the Cα of the central residue I506) compared to the ligand-free structure, opening the NAD+ binding site. The 605–616 loop becomes ordered into a one-turn helix, followed by a loop that lines the UDP-GlcA binding site (L2). (**C**) Transparent surface model of the ligand-free ArnA C-terminal domain. For reference, the positions of the two ligands from the ligand bound structure are shown as blue sticks. The 500–509 loop is colored in yellow, blocking the NAD+ binding site, while the UDP-GlcA binding site appears open. Residues 604 and 617, which represent the ends of the disordered 605–616 loop, are also colored yellow. (**D**) Transparent surface model of the ATP/UDP-GlcA bound structure. Both ligands are presented as blue sticks. A large conformational change in the 500–509 region, as well as stabilization of the 605–616 region, opens the NAD+ binding site but effectively traps UDP-GlcA in its active site.



The oxidation of UDP-glucuronic acid occurs via an ordered mechanism with a two substrate binding active site. Once UDP-GlcA is bound to the active site, the conformational change reveals the site that binds NAD^+. This allows for the oxidation of the 4' position of the sugar ring to form UDP-4-ketohexo-uronic acid which will then lose byproducts, NADH and CO_2. The evolution of NADH is of note because it can be monitored spectrophotometrically at 254 nm, and it is this piece of data that can be used to determine reaction kinetics.

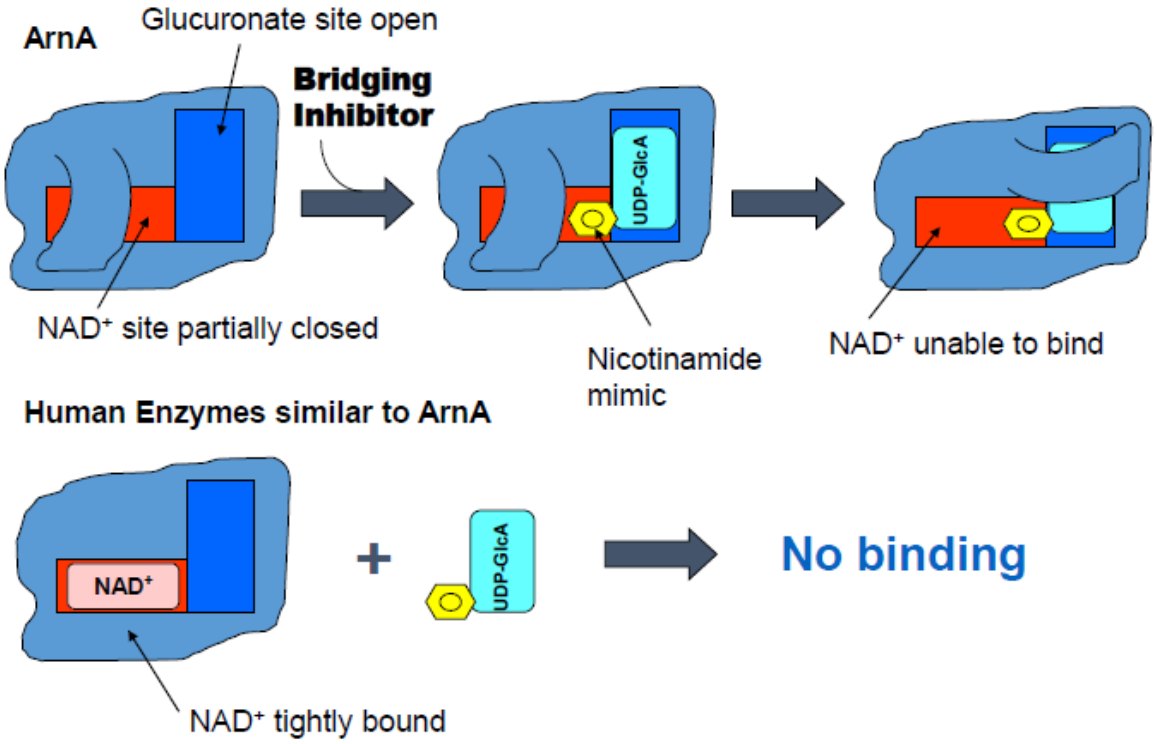
Once UDP-4-ketohexouronic acid is formed, it is capable of being protonated which results in loss of CO_2 via a proton shuttling mechanism involving residues R_{619} and S_{433}.[63] These 2 residues were mutated and 3 point mutations (R619Y, S433T, and R619E) were found to result in a loss of enzymatic activity.[61] In order to rule out misfolding as an explanation for the loss of activity, these mutants were also crystallized and all were found to fold properly.[61] After the irreversible loss of CO_2, the resultant products, NADH and UDP-4-ketoarabinose are released from the enzyme.

Figure 1.16 ArnA Mechanism



This reaction has been found to be unique to bacteria; no described eukaryotic enzymes catalyze identical reactions to an appreciable extent.[64] The human enzyme that is the most similar in structure and function to ArnA is Human UDP-xylose Synthase. In this enzyme NAD^+ is buried deeply in the protein structure with extensive electrostatic contacts; indicating NAD^+ is tightly bound to the enzyme.[65] Thus, ArnA is an excellent target for the development of selective inhibitors. We will take advantage of the enzyme's unique ordered mechanism and allosteric shift to develop selective inhibitors that have potential to behave as RMAs mediating the effectiveness of polymyxins in multidrug resistant Gram negative infections.

Figure 1.17 Selective Inhibition of ArnA and the Incompatibility of the Designed Inhibitors with Human *UDP-xylose Synthase*



1.9 Modular Design of Inhibitors for the Arn Pathway

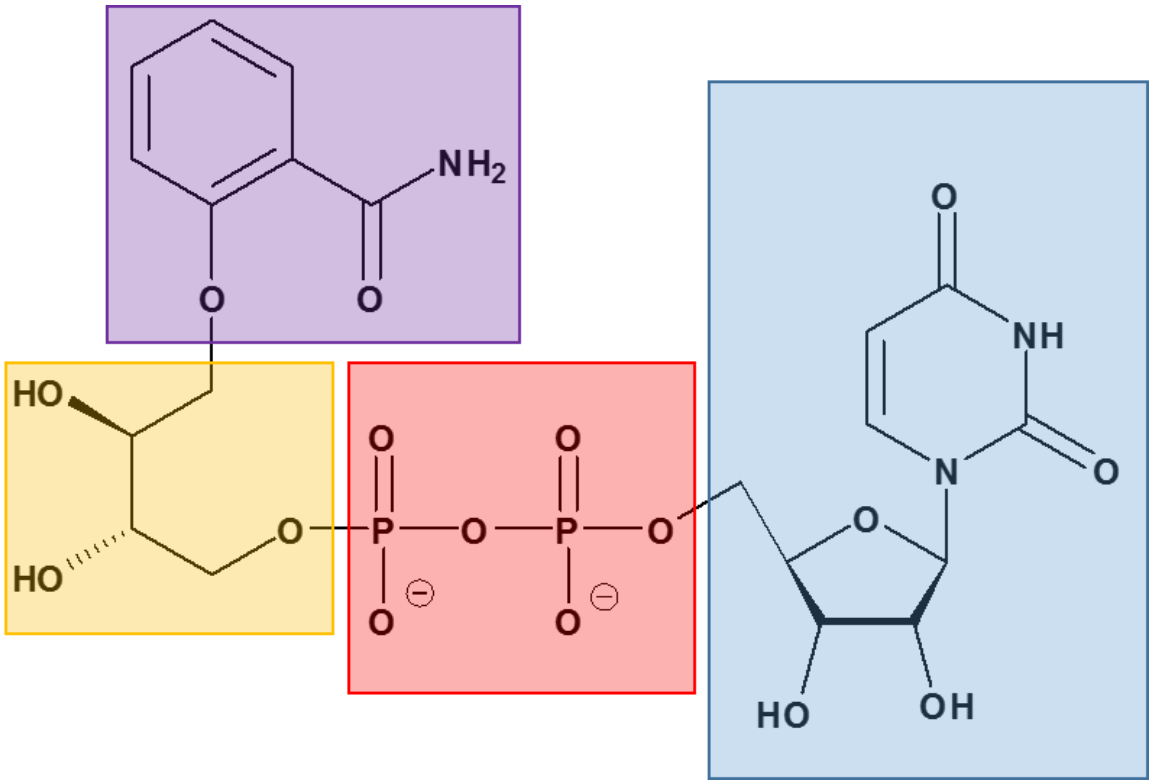
We have adopted a modular approach in our inhibitor design consisting of three modules: a uridine module, a linker module, and a substrate-specific module. The first two modules are common to both endogenous substrates of ArnA and ArnB and are therefore common to inhibitors of both enzymes. The role of these modules is to provide binding energy and affinity to both enzymes. The role of the substrate-specific module is to differentiate between ArnA, ArnB, and human enzymes by taking advantage of specific interactions and catalytic residues found in the two active sites. Details of all 3 modules are provided below (Figure 1.18).

In our first-generation design, the uridine module will simply be uridine without modification. The linker module, however, will consist of either the parent pyrophosphate, a triazole, or a diphosphonate (i.e., compounds containing P-C-P linkages) analog. These selections are based on the structures of the enzyme-substrate complexes. In general, the UDP moiety is held in place by side chain interactions and a hydrogen bonding network in both enzymes.[63,66] For example, the crystal structure of ArnA bound to UDP-glucuronic acid (with ATP in place of NAD^+) shows the uracil moiety making hydrogen bond contacts to the main chain atoms of K256 and I528 while the ribose is held in place by hydrogen bonding to the side chains of Q533 and Y613.[63] In addition, the β-phosphate is held in place by hydrogen bonds to R460, R535, and to the catalytic R619. As such, it would be advantageous to design an inhibitor that would also maintain these electrostatic contacts.

In the case of ArnB, which is a dimer, the uracil ring is sandwiched between the side chains of P16, A186, and I187 from one subunit, and the indole ring of W34 from the opposite subunit.[66] There is also a hydrogen bond between the 4-oxygen of uracil and the main chain amide of I35 from the second subunit. In addition, the side chain of H185 contacts the β-phosphate, whereas the

α-phosphate forms a hydrogen bond with the side chain of H329. In both ArnA and ArnB, the phosphate contacts are all with the non-ethereal oxygen of the diphosphate, and as such we propose the preparation phosphonate linkers wherein a methylene group replaces the ethereal oxygen. These analogs should lack the hydrolytic lability of diphosphates but should retain the binding interactions and affinity of the endogenous substrates.

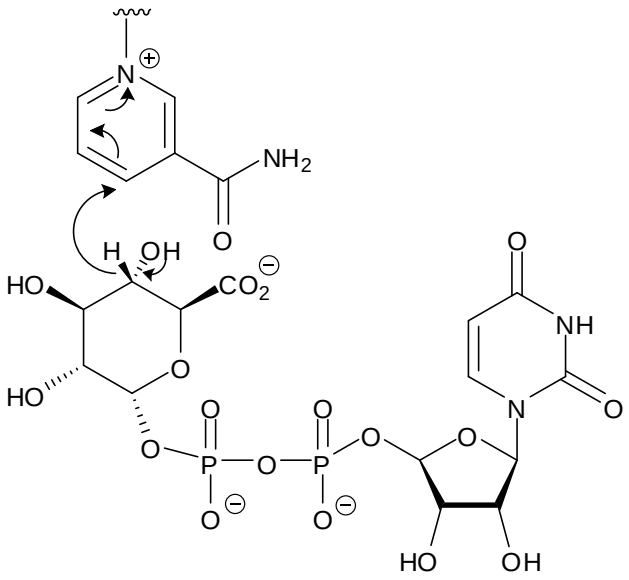
Figure 1.18 Arn Pathway Modular Inhibition Design Strategy: purple and yellow boxes on left represent the substrate specific NAD^+ and glucuronic acid mimic respectively for ArnA inhibition, or the substrate specific module of the ArnB inihibitor, the red box in center indicates the pyrophophate or mimic thereof, common to both inhibition strategies, and the blue box on right represents the uridine moiety which is also common to both inhibition strategies.



The oxidative decarboxylation catalyzed by ArnA C-terminal domain is unique to Gram-negative bacteria with no described eukaryotic enzyme catalyzing identical reactions. Further, structure-function studies have identified specific features in the structure and mechanism of ArnA, [61,63] that we will exploit to develop selective inhibitors. As stated previously, the crystal

structures of ArnA C-terminal domain in the presence and absence of ligands reveal a striking conformational change induced by substrate binding. This mechanism and associated conformational change is fundamentally different from eukaryotic dehydrogenases that catalyze a similar, but non-identical, reaction. Eukaryotic UDP-GlcA decarboxylases use NAD^+ to oxidize the 4" hydroxyl of UDP-GlcA followed by decarboxylation, just as in ArnA; however, these enzymes re-reduce the decarboxylated 4-keto sugar with NADH to generate UDP-Xylose without net consumption of NAD^+. Therefore, NAD^+ works as a coenzyme that remains bound to the enzyme at all times (Figure 1.17, bottom).[64] In the case of ArnA, while the inhibitory loop blocks much of the NAD^+ binding site, the pocket corresponding to the nicotinamide portion of NAD^+ is accessible in ligand-free ArnA. Our inhibitor design, therefore, incorporates truncated nicotinamide mimics, bridging a UDP-glucuronic acid mimic such that the binding sites of both the UDP-glucuronic acid and nicotinamide are occupied, or partly occupied, respectively. In our design, the NAD^+ and GlcA are linked by one atom (C, O, or S) allowing the molecule to adopt a geometry mimicking that of the hydride transfer in the first step of the enzymatic mechanism (Figure 1.18). We anticipate that our compounds will bind tightly to ArnA; functioning as competitive inhibitors. However, they should not bind tightly to the eukaryotic enzymes that have NAD^+ bound at all times as the truncated nicotinamide will not displace the tightly bound NAD^+, thereby providing a mechanism for selectivity.

Figure 1.19 The First Step in the ArnA Mechanism: NAD^+ Assisted Oxidation of UDP-GlcA to UDP-4-Ketohexouronic Acid

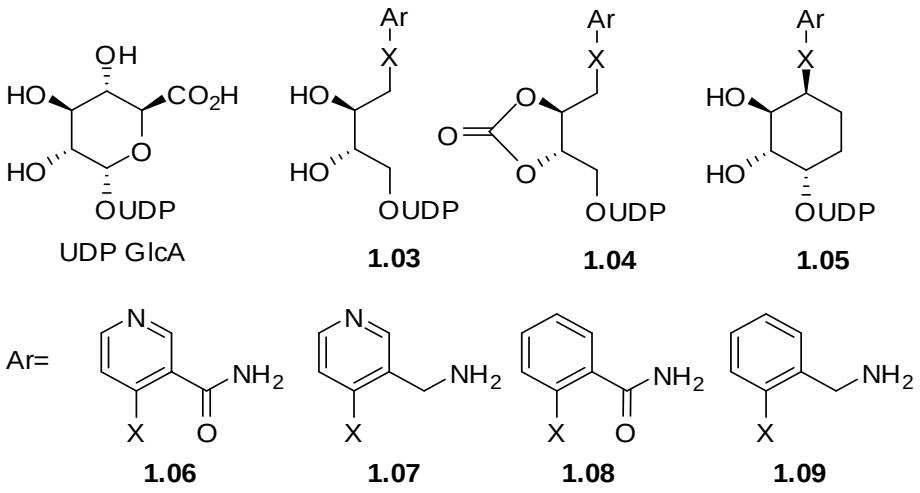


In our initial inhibitor design, we have carved out two of the carbon atoms and an oxygen of the glucuronic acid to provide an acyclic diol, which we will synthesize bound to UDP (**1.03**). This simplifies the synthesis, especially with respect to controlling the stereochemistry at the anomeric carbon of UDP-glucuronic acid, which can be difficult, but maintains the functional groups that we believe are important for binding to the two sites in the enzyme. The structure and stereochemistry of the diol was chosen to mimic the *trans*-geometry of the hydroxyl groups of the glucuronic acid, with conformational control in the ArnA active site of the acyclic structure being derived from hydrogen bonding of the two hydroxyl groups. This design is similar to the anti-viral acyclovir, which has a linear structure mimicking a deoxyguanosine lacking the 2'and 3' carbons in the ribose ring.[67]

If this inhibitor displays excessive flexibility, the corresponding carbonate, which restricts rotation about the diol, will be prepared. This gain in stabilization, however, is at the cost of diminishing the H-bonding on the *trans* diol (**1.04**). In addition to this acyclic molecule, a

carbocyclic compound bearing a *trans* diol that more closely mimics the glucuronic acid but lacks the difficulties of controlling the stereochemistry at the anomeric center can also be envisioned (**1.05**). These compounds will also bear the nicotinamide portion of NAD^+ in order to occupy nicotinamide pocket of the NAD^+ binding site of the enzyme. The nicotinamide mimics include nicotinamide derivative **1.06** as well as the derivative wherein the amide is reduced to the amine (**1.07**). Simpler derivatives wherein the pyridine is replaced with a benzene are also feasible (**1.08** and **1.09**). We chose to first synthesize **1.10** for its synthetic simplicity via an easily modifiable route for increasing complexity (Scheme 1.1).

Figure 1.20 Initial ArnA Inhibition Library Design X= CH_2, O, or S

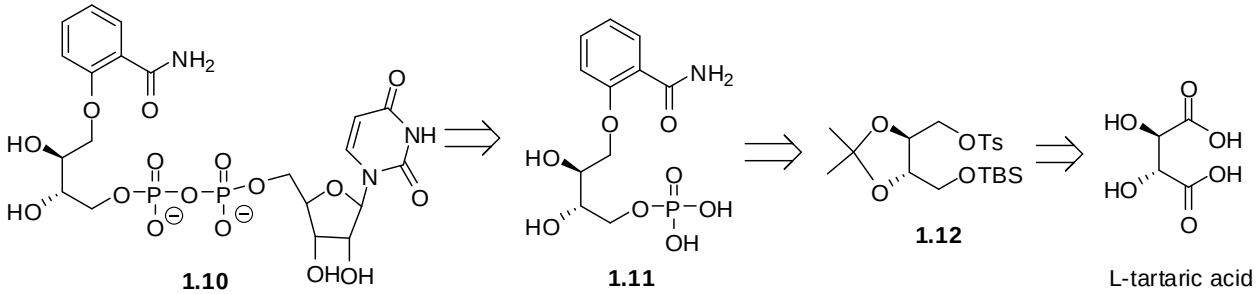


1.10 Synthesis of Diphosphate Inhibitors

While developing the retrosynthesis we were mindful to take advantage of a convergent synthetic strategy to generate intermediates that could be employed in other Arn pathway enzymes, consistent with our modular design. Glycosyltransferase inhibitors have been synthesized via an approach involving diphosphate couplings from 2 monophosphate precursors.[68] We employed this same strategy when designing our initial retrosynthesis for **1.10** (Scheme 1.1). The first major

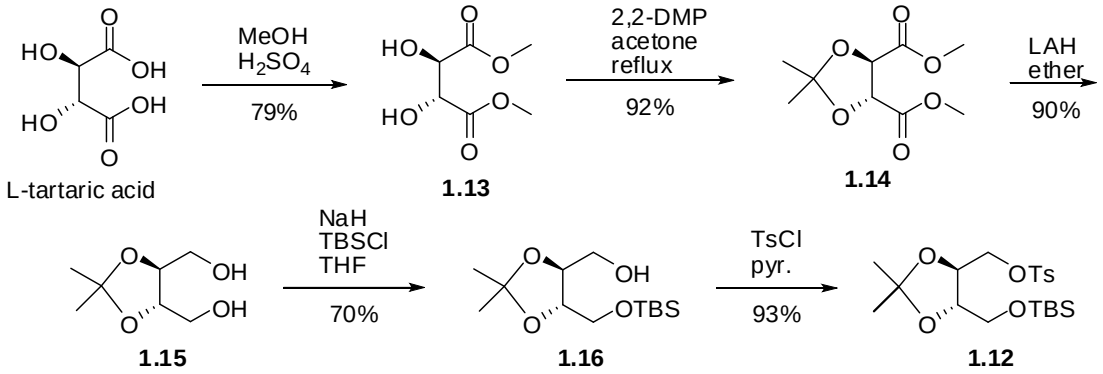
disconnect for compound **1.10** is between the phosphate groups; allowing for the most convergent synthesis. Wong and co-workers were successfully able to perform diphosphate couplings on a variety of monophosphates with the use of uridine 5′-monophosphomorpholidate 4-morpholine-*N,N′*-dicyclohexylcarboxamidine salt and 1-*H*-tetrazole.[69] This same strategy can be applied to the synthesis of the monophosphate precursor **1.11**. The sugar-NAD^+ mimic portion of the molecule can be synthesized from a known, versatile protected *trans*-diol **1.12**. This intermediate can be synthesized in 5 steps via a known synthetic pathway from L-tartaric acid.[70]

Scheme 1.1 Retrosynthesis of Compound 1.10



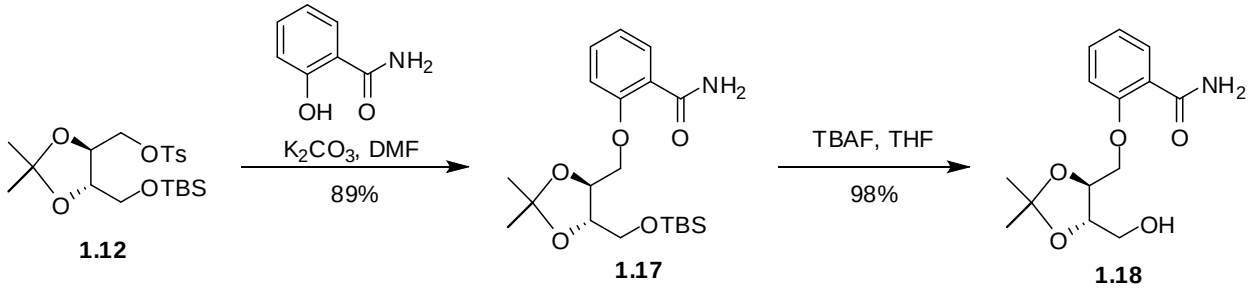
The synthesis began with commercially available L-tartaric acid (Scheme 1.2). The dimethyl ester **1.13** was formed with a Fischer esterification using methanol in 79% yield.[71] The diol of the dimethylester was then protected using 2,2-dimethoxypropane to form acetonide **1.14** in 92% yield. Compound **1.14** was then reduced to a diol **1.15** using LAH in 90% yield. Diol **1.15** was mono-protected through the use of 1 equivalent of NaH followed by 1 equivalent of TBSCl to form compound **1.16** in 70% yield.[72] Compound **1.16** was then tosylated with 4-tolunenesulfonyl in pyridine to provide intermediate **1.12** in a 93% yield.

Scheme 1.2 Synthesis of Versatile Intermediate 1.12



Compound **1.12** is a versatile intermediate that can be used to prepare a variety of potential inhibitors, and with this compound in hand, we then added the NAD^+ mimic portion to the molecule. As such, **1.12** was treated with salicylamide in the presence of potassium carbonate while heating in DMF to provide **1.17** in 89% yield (Scheme 1.3). A TBAF deprotection was employed to remove the TBS group, allowing for further functionalization of alcohol **1.18** in 98% yield.

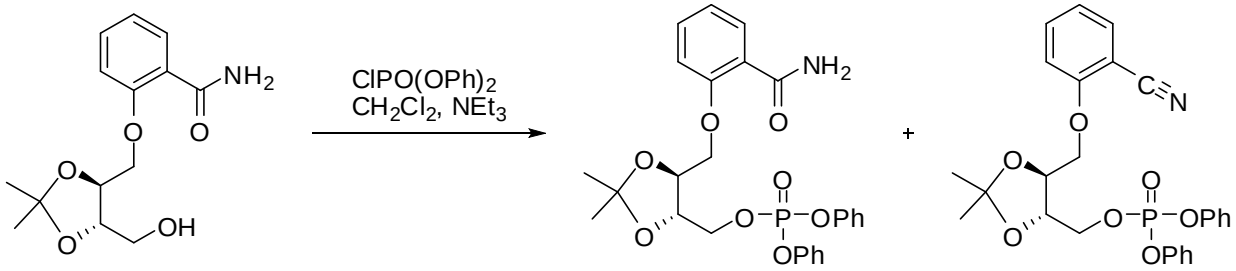
Scheme 1.3 Installation of the NAD^+ Mimic



With a reliable method to install the NAD^+ mimic, we then studied the installation of the phosphate group. When alcohol **1.18** was phosphorylated using 2 equivalents diphenylchlorophosphate in the presence of excess NEt_3, a 7:10 ratio of desired product (**1.19**) to dehydrated benzonitrile side product was observed (**1.20,** Table 1.1, entry 1). This reaction was unexpected and reaction conditions were explored to minimize this side reaction (Table 1.1). We were able to push the reaction to completely form benzonitrile compound **1.20** in 91% yield after

purification when 3 equivalents of diphenylchlorophosphate weres used in the reaction. We hypothesized that the reaction of the primary alcohol is faster than amide dehydration, as no nitrile bearing the primary hydroxyl group without phosphorylation was detected. We therefore studied the use of 1.1 equivalents of diphenylchlorophosphate and obtained a 25:3 ratio of desired product (**1.19**) to the benzonitrile side product (**1.20**), and after purification obtained compound **1.20** in 78% yield.

Table 1.1 Dehydration of Primary Amide to Nitrile with Diphenyl Chlorophosphate[a]



1.18 **1.19** **1.20**

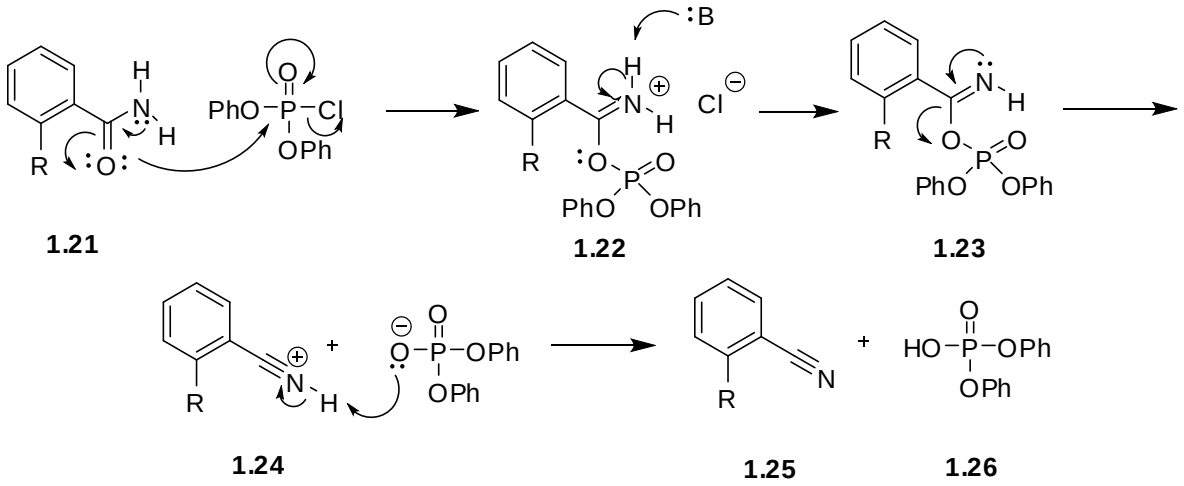
Entry	Equiv of $ClPO(OPh)_2$	Ratio[b] 1.19:1.20
1	2	7:10
2	1.1	25:3
3	3	0:1

Table 1.1 [a]The reaction conditions were 1 equiv **1.18** (2.0 mmol) was dissolved in DCM (20 mL) and 5 equiv of NEt_3 followed by addition of specified amount of diphenylchlorohosphate. The reaction was stirred at room temperature for 2 h before filtration of the solids and concentrated down. [b]The ratio was determined via NMR analysis.

It is known that $POCl_3$, along with other strongly dehydrating compounds such as $SOCl_2$, P_2O_5, and $TiCl_4$, are capable of dehydration of primary amides to nitriles.[73] It is, therefore, reasonable that in our reaction conditions diphenylchlorophosphate is also capable of dehydrating primary amides to nitriles. We suspect the mechanism is similar to that of $POCl_3$ with the difference being that the final phosphorous by-product in our case is diphenylphosphate **1.26**. Mechanistically, the reaction proceeds by the attack of the amide oxygen onto the phosphorous of

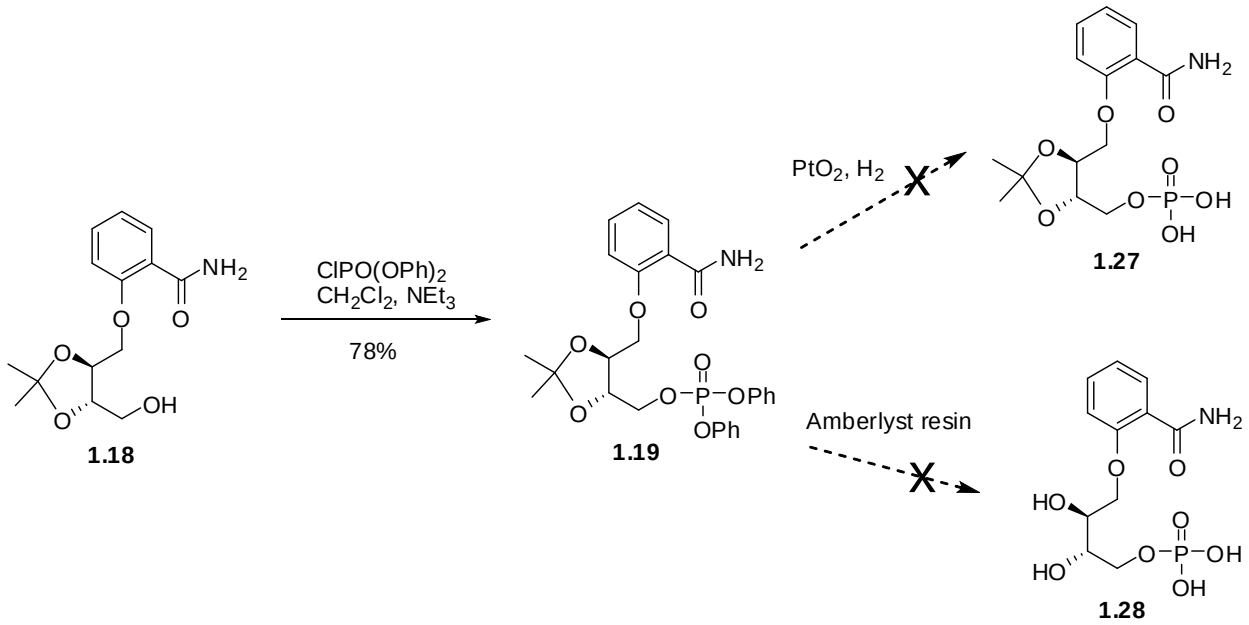
diphenylchlorophosphate, displacing chloride and forming intermediate **1.22** (Scheme 1.4). Loss of a proton on nitrogen and generates compound **1.23**, which is followed by the loss of diphenylphosphate to reveal the newly formed protonated nitrile **1.24**. Loss of a proton from **1.24** generates benzonitrile product **1.25**.

Scheme 1.4 Benzonitrile Formation via Diphenylchlorophosphate



With conditions for successful phosphorylation discovered, deprotection to prepare **1.27** or **1.28** was studied (Scheme 1.5). To deprotect the phenyl phosphate esters, PtO_2 (Adams' catalyst) in the presence of hydrogen gas is commonly employed.[74] When the method of H_2 in the presence of PtO_2 was employed we did not obtain any of the desired product **1.27**, rather we found the NAD^+ mimic had been reduced off of our starting material. With the failure of the PtO_2 method we sought other deprotection methods that would not interact with the aromatic rings. We attempted to use acidic conditions to employ a global deprotection strategy via acidic amberlyst resin to remove the acetonide as well as the phenyl groups on the phosphorous. While monitoring this reaction by TLC, a disappearance of starting material is observed. We were however, unable to purify product **1.28**. When silica gel chromatography was attempted none of the traditional solvents attempted eluted the product from the column. When a cellulose column was employed we were unable to obtain any separation of the desired product from the by-products.

Scheme 1.5 Attempted Deprotection of Phenyl Phosphate



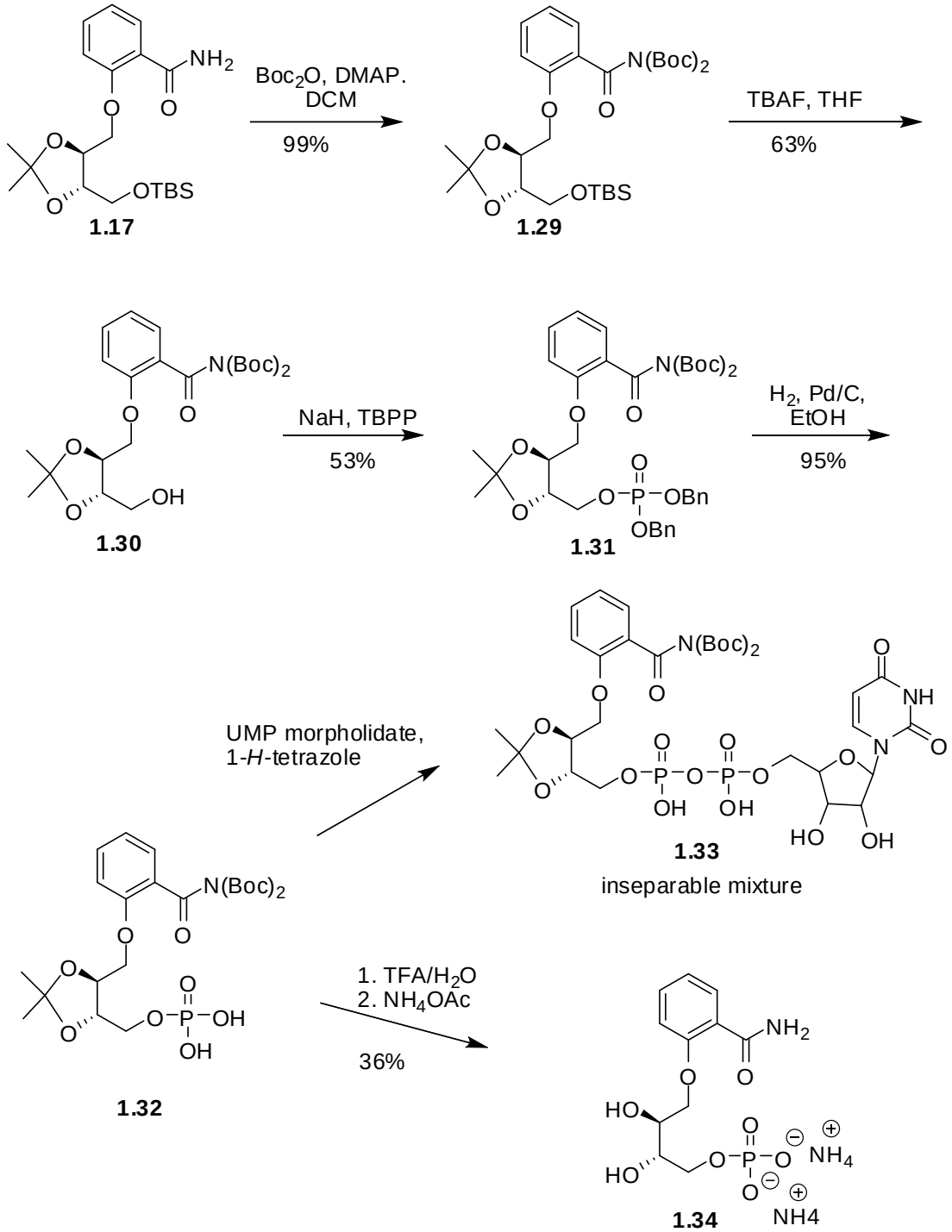
Due to the problems encountered in the deprotection of **1.19** we then studied a different protecting group strategy. In order to avoid the dehydration of the primary amide upon phosphorylation experienced in the previous synthesis (Scheme 1.4), **1.17** was bis-boc protected to provide **1.29** in 99% yield (Scheme 1.6). The TBS group of **1.29** was then removed using TBAF to provide **1.30** in 63% yield. The primary alcohol **1.30** was then deprotonated with NaH and treated with tetrabenzylpyrophosphate (TBPP) to provide **1.31** in 53% yield. TBPP was used over other phosphorylating reagents because it is readily synthesized via a DCC coupling with dibenzylphosphate and is stable at -20 °C.[75] Although this route proceeded in moderate yield, deprotection of **1.31** with H_2, Pd/C was facile and afforded compound **1.32** in 95% yield.

A diphosphate coupling was then attempted on **1.32** with uridine 5′-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt, but we observed a complex mixture. An HRMS sample of the crude reaction product revealed that compound **1.33** was present; however, all efforts to isolate **1.33** were unsuccessful. Employment of a variety of conditions on silica gel chromatography, reverse phase silica gel chromatography, and size

exclusion chromatography were unsuccessful, as the product was co-eluted with impurities in all cases. The monophosphate **1.32** was instead subjected to acidic deprotection conditions followed by purification via silica gel chromatography (*i*PrOH:1M ammonium acetate aqueous, 2:1), and upon lyophilization compound **1.34** was isolated in 36% yield. Although **1.34** does not contain the uridine moiety hypothesized to be necessary for binding specificity, it awaits biological testing as a control.

We have developed an easily modifiable synthetic strategy en route to synthesize compound **1.33**. However, due to the co-elution of **1.33** with impurities during the purification, alternative linker strategies were explored. A triazole linker in place of the pyrophosphate has been studied for use in glycosyltransferase inhibition,[76] and intermediate **1.18** easily allows for the installation of the propargyl group necessary for the triazole formation. Therefore, we proceeded to synthesize an inhibitor with a triazole linker to uridine.

Scheme 1.6 Benzyl Phosphate Protection Strategy

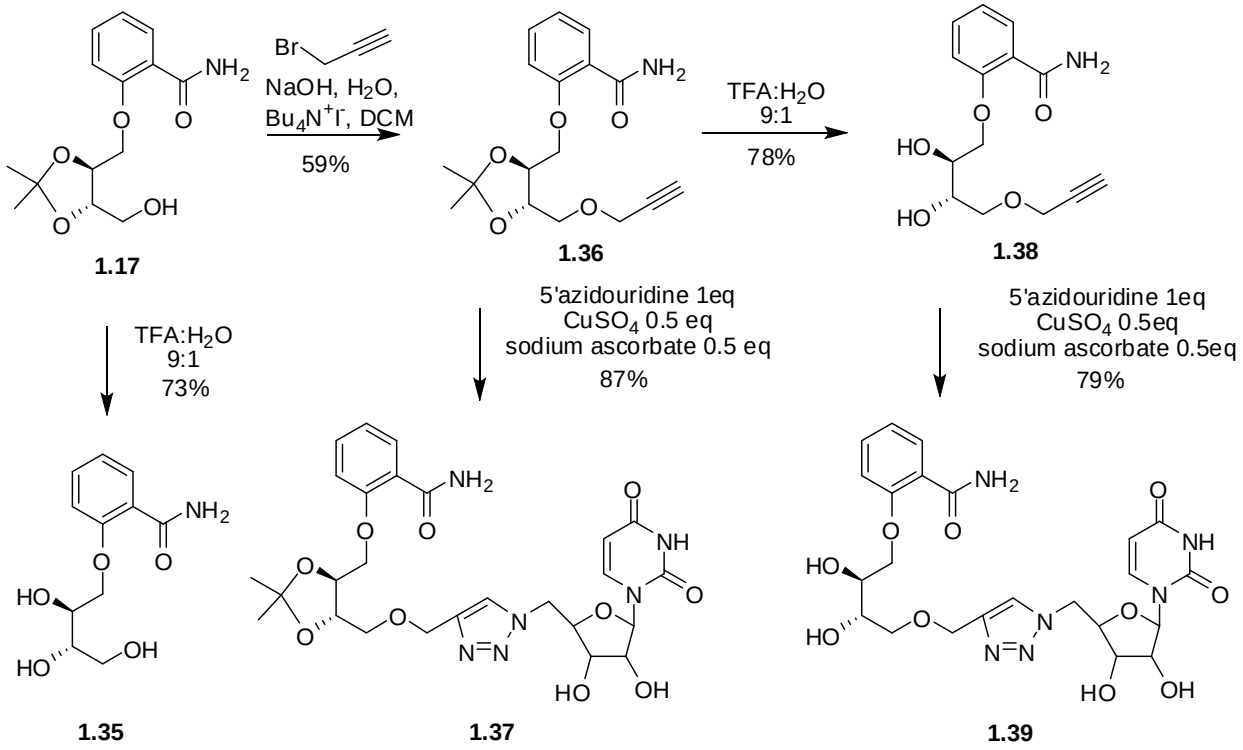


1.11 Synthesis of Triazole Inhibitors

Glycosyltransferase inhibition has been explored utilizing a triazole linker in place of the pyrophosphate.[76] Thus, we devised a strategy in hopes that this linker could successfully bridge the uridine and substrate specific modules in an ArnA inhibitor. In the process of designing this substrate, we also wished to confirm the importance of the uridine diphosphate moiety for inhibition. Therefore, a compound composed of just the ArnA binding module (NAD^+-GlcA mimic, **1.35**) was designed to determine if it would inhibit ArnA (Scheme 1.7).

Compound **1.35** was prepared by removing the protecting group on **1.18** with TFA and water in 73% yield. To generate the triazole linker an alkyne must be installed on **1.18**. Towards this end, **1.18** was propargylated (propargyl bromide, 4M NaOH, dichloromethane, with tetrabutyl ammonium bromide) in a phase transfer catalyzed process to generate compound **1.36** in 59% yield. Compound **1.36** was treated with 5'azidouridine and copper sulfate (0.5 equiv) and sodium ascorbate (0.5 equiv) in a Huisgen/click reaction to produce **1.37** in 87% yield.[77] Although the structure will lack the binding of the hydroxyl groups of the glucuronic acid module the conformational stability of the acetonide ring will provide rigidity and could promote binding to the active site. Compound **1.36** was deprotected with TFA in water to provide compound **1.38** in 78% yield. Compound **1.38** was then used in a click reaction (0.5 equiv $CuSO_4$, 0.5 equiv sodium ascorbate in *t*BuOH:H_2O 1:1) with 5'azidouridine to provide compound **1.39** in 79% yield. The successfully synthesized NAD^+-GlcA mimic (**1.35**) and the triazole linker compounds (**1.37**, and **1.39**) were then screened for inhibitory activity against the C-terminal domain of ArnA.

Scheme 1.7 Click Chemistry Inhibitors



1.12 ArnA Inhibition Assay

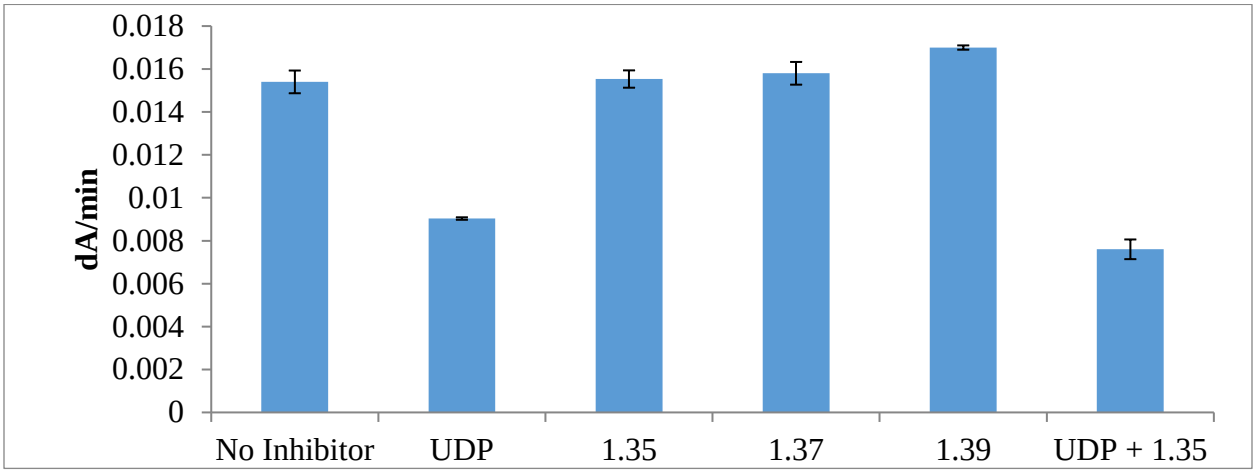
The activity of ArnA has been evaluated *in vitro* by an UV assay that detects the formation of NADH.[63] It has been shown that the two domains of ArnA can function independently *in vitro.*[61,63] To avoid the potential competing interactions of the N-terminal domain in an inhibition assay, the C-terminal domain of ArnA (residues 306-660) was purified to homogeneity as a His-tag fusion protein according to Gatzeva-Topalova *et al.* 2004.[61] Upon protein purification, a control reaction was performed to ensure that active enzyme was isolated, and when it was found to have comparable activities to the literature our inhibition assay was developed.

The conditions chosen for the assay were: 200 nM C-term ArnA, 100 mM KCl, 10% glycerol, 0.2 mg/ml BSA, 25 mM Tris pH = 8.0, 5 mM beta-mercaptoethanol, 4 mM NAD^+, 0.25 mM UDP-GlcA and 1 mM inhibitor. The buffer was selected as it was identical to the literature,

and was also found to behave reliably under these conditions throughout the purification procedure.[63] The relatively high concentration of 4 mM NAD^+ was chosen to ensure saturating conditions, such that the reaction would not be impeded by the absence of NAD^+. The lower concentration of 0.25 mM UDP-GlcA was selected because it is near the K_M and will provide reaction rates that have been found to be reproducible, providing larger rate variations if inhibited, as compared to higher concentrations.[61,63] The concentration of inhibitor at 1 mM, 4 times that of the UDP-GlcA, was chosen to purposefully outcompete the substrate in the active site and determine if further study is needed on that compound.

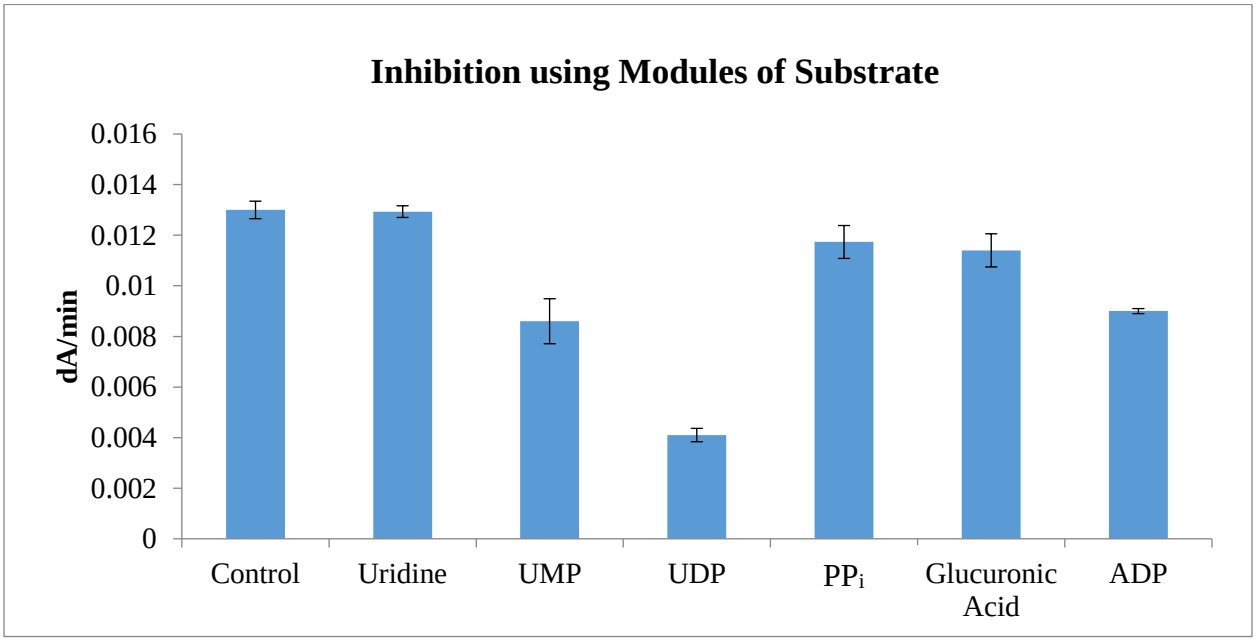
The reaction was initiated through the addition of UDP-GlcA to the reaction, to allow for the inhibitor to come to an equilibrium with the protein before the reaction began. The assay was conducted at 37 $^{\circ}$C in a spectrophotometer, and the generation of NADH at 340 nm was monitored for 5 minutes. Initial rates of NADH formation (change in absorbance/min) were obtained and compared in quadruplicate for each sample. As a negative control no inhibitor was added, and UDP was used as a positive control. All compounds were tested under these conditions, and it was found that none of the synthesized compounds showed inhibitory activity (Figure 1.20). Further, we tested the combination of **1.35** (1 mM) with UDP (1 mM). We hypothesized if we observed inhibition greater than that of the control UDP in our initial control experiment, compound **1.35** is likely binding to the active site in tandem with UDP. However, the inhibition observed was not significantly lower than the control experiment.

Figure 1.21 Results of ArnA Inhibition with Triazole Linkers: y-axis, the initial rate of NADH production, x-axis, compounds. Error bars represent 1 standard deviation from mean.



With purified protein and a reliable inhibition assay we sought out to test fragments of the native substrate to determine what portions of the molecule are important for binding. We tested uridine, UMP, UDP, pyrophosphate, glucuronic acid, and ADP. Upon testing we found the initial rate of the reaction was slower when UDP, UMP, and ADP were added to the reaction. All of these compounds contain a negatively charged phosphate group which suggests that these electrostatic interactions are important for binding and should be included in our inhibitor design. Importantly, it was found that pyrophosphate (PP_i) by itself does not cause inhibition. This suggests that the negative charge alone is not sufficient enough to cause inhibition, and that the nucleotide moiety is required for binding affinity. Notably, uridine is not a competent inhibitor, providing further evidence that the negatively charged phosphate groups are necessary for binding to the active site. With this information, we sought to design other linkers that would maintain the phosphate group's electrostatic interactions.

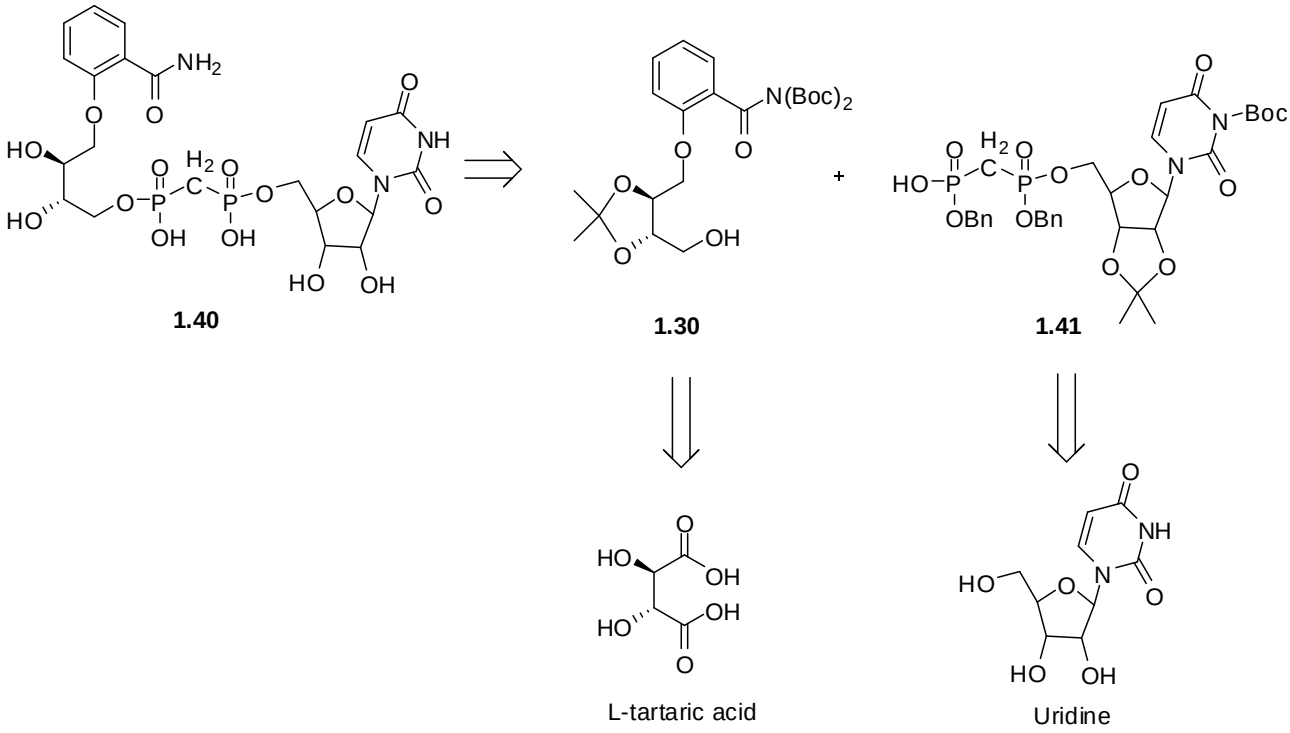
Figure 1.22 Results of ArnA inhibition with Control Compounds: y-axis, the initial rate of NADH production, x-axis, compounds. Error bars represent 1 standard deviation from mean.



1.13 Synthesis of Bisphosphonate Analogue

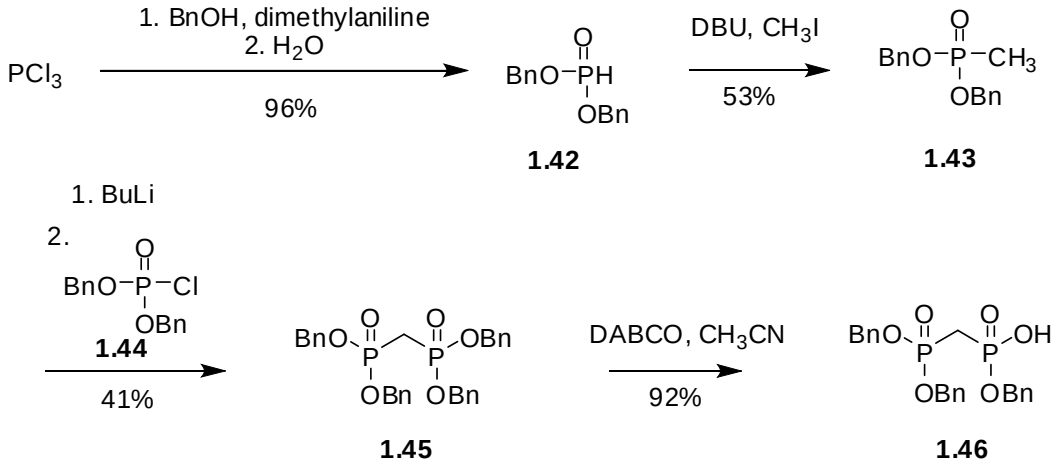
In our new linker design, we sought a molecule that, in addition to bearing the required charges, would be less susceptible to hydrolysis. A bisphosphonate linker can accomplish both these objectives, and can be synthesized separately as its own modular unit in our synthetic plan. Therefore, a retrosynthesis for the preparation of **1.40** from 2 separate components, **1.30** and **1.41**, via a modified Mitsunobu reaction (Scheme 1.8) was designed. With the synthesis of **1.30** in hand, we now required the synthesis of **1.41**.

Scheme 1.8. Retrosynthesis of Bisphosphonate Linker



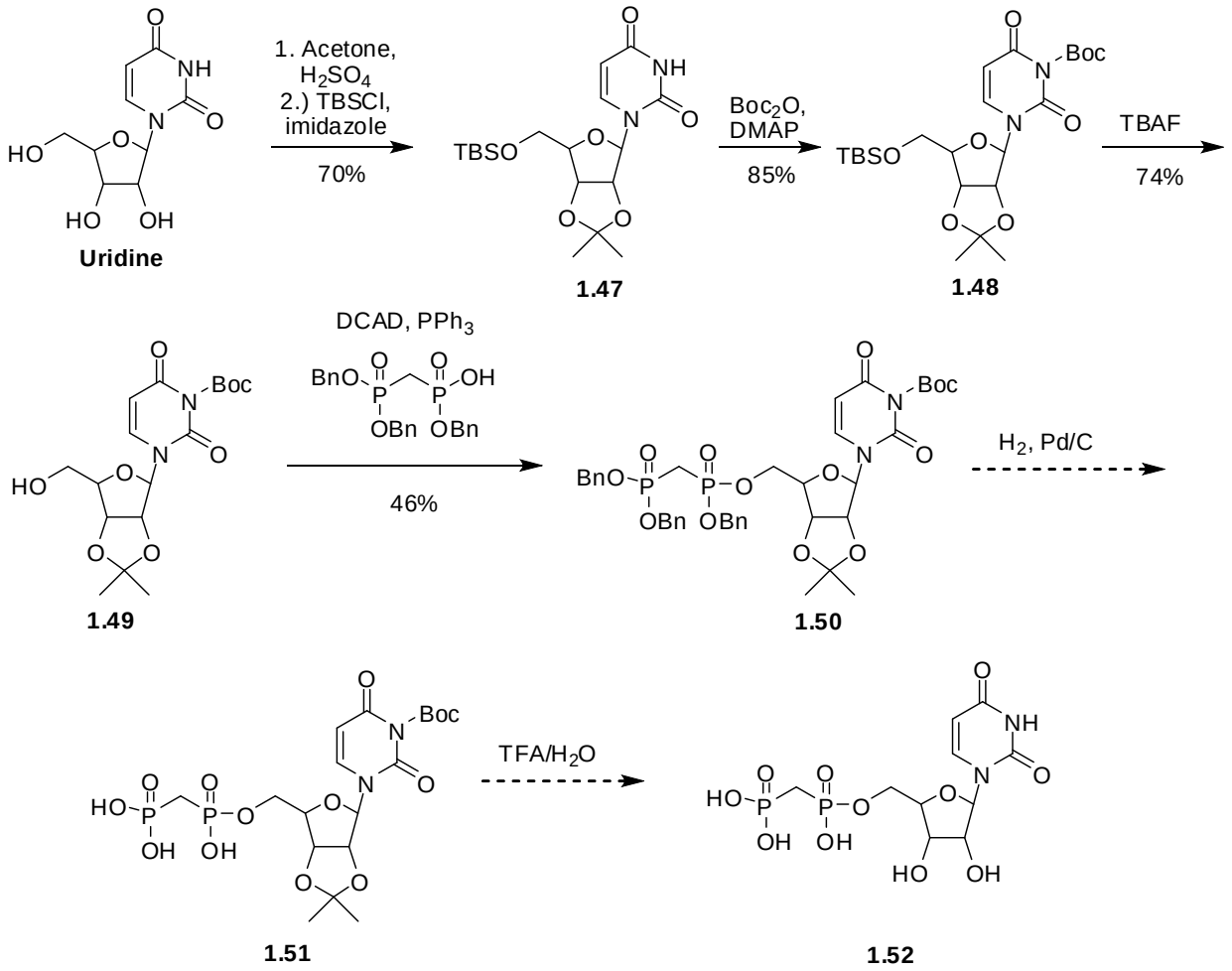
Starting from PCl_3, dibenzyl H-phosphonate (**1.42)** was synthesized in 90% yield via the addition of 2 equivalents of benzyl alcohol in base, followed by water (Scheme 1.9). This H-phosphonate is a versatile intermediate that can be chlorinated to provide the phosphoryl chloride **1.44** with N-chlorosuccimide in 96% yield,[78] or can be deprotonated with BuLi and subjected to methylation with methyl iodide to afford dibenzyl methylphosphonate (**1.43**) in 53% yield. Compound **1.43** was deprotonated and further reacted with **1.44** to generate tetrabenzyl bisphosphonate **1.45** in 41% yield. Compound **1.45** was subjected to monodeprotection with DABCO in refluxing acetonitrile to afford **1.46** in 92% yield. Compound **1.46** is a versatile intermediate that can be used as a linker between the different modules of our inhibitors, including protected uridine **1.49** (Scheme 1.10).

Scheme 1.9 Synthesis of the Bisphosphonate Linker



With compound **1.46** in hand, we first targeted the synthesis of compound **1.50** wherein uridine is bound to our bisphosphonate linker to establish the efficacy of this linker as an inhibitor compared to the native phosphate. Towards this end, uridine was protected as the acetonide with acetone, and the 5' alcohol was then protected as the TBS ether to afford **1.47** in an overall 70% yield over 2 steps. The imide on the uracil moiety of **1.47** was then protected with a boc group using boc anhydride and DMAP to generate **1.48** in 85% yield. The primary alcohol was then revealed via a TBAF deprotection to afford **1.49** in 74% yield. Compound **1.44** was then coupled to **1.46** via a modified Mitsunobu reaction to provide **1.50** in 46% yield. Debenzylation of **1.50** can be accomplished through the employment of palladium on carbon to afford **1.51**. The final deprotection of the acetonide and the boc can then be accomplished using TFA in water or through the use acidic amberlyst resin. Once the efficacy of the bisphosphonate linker is established, the synthesis of the complete inhibitor bearing all of the modules can be pursued.

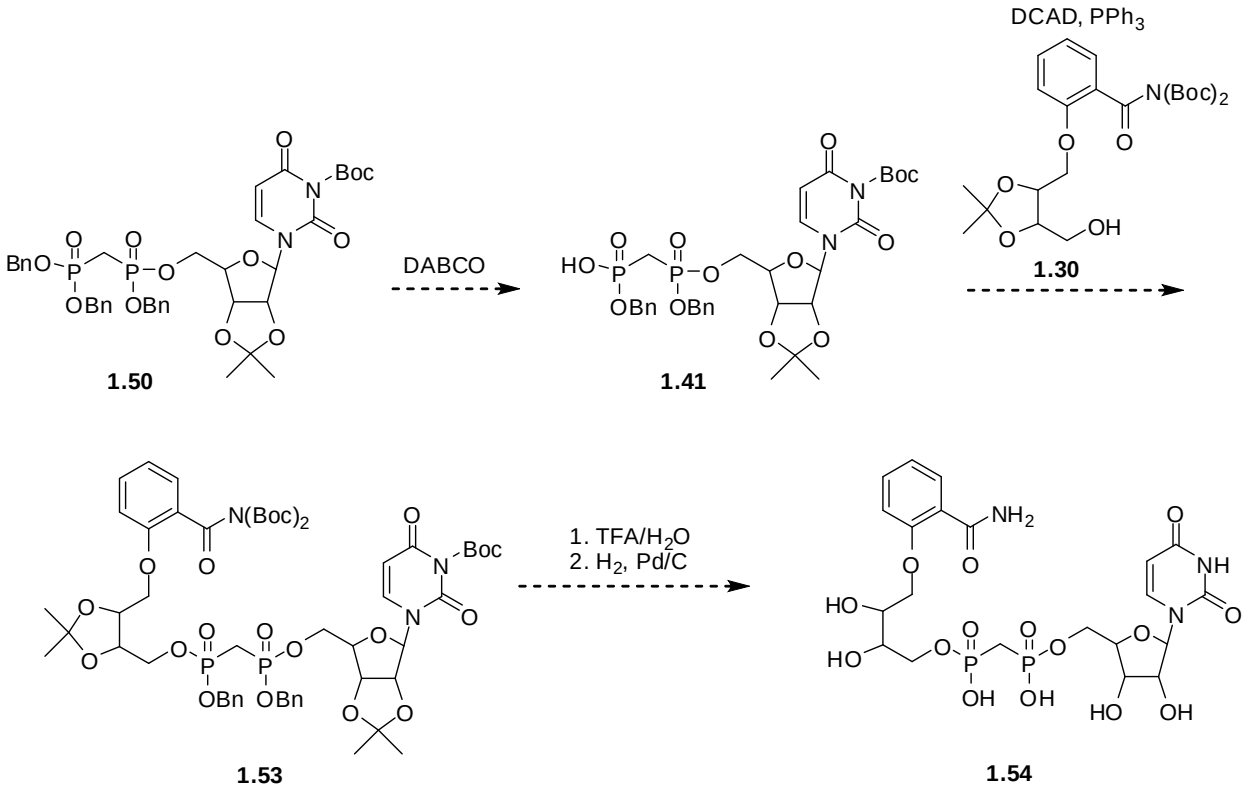
Scheme 1.10 Synthesis of the Designed Bisphosphonate Compound



If we find that the inhibition of the C-terminal of ArnA with the bisphosphonate in place of the pyrophosphate is comparable, it would then be reasonable to explore the synthesis of the complete inhibitor with all modules in place. This can be accomplished by the use of compound **1.50**, which can be monodeprotected utilizing DABCO to generate **1.41** (Scheme 1.11). This product can now undergo functionalization with a variety of adducts to provide UDP-sugar mimic products for inihibition of the Arn pathway enzymes. For ArnA inhibition specifically, **1.41** can be functionalized with the sugar/NAD^+ mimic **1.30** via a modified Mitsunobu reaction to generate compound **1.53**. This compound can then be deprotected with TFA/water, followed by H_2 in

presence Pd/C, to generate the designed bisphosphonate inhibitor **1.54**. Once fully synthesized this molecule will be tested for ArnA C-terminal inhibition. With a reliable synthetic strategy to for the monodeprotected uridine bisphosphonate **1.41** the other conceived sugar/NAD^+ mimics from our initial library can then be installed to generate a library of inhibitors to test for ArnA inhibition.

Scheme 1.11 Future Synthesis of the Complete ArnA Inhibitor



1.14 Conclusions and Future Directions

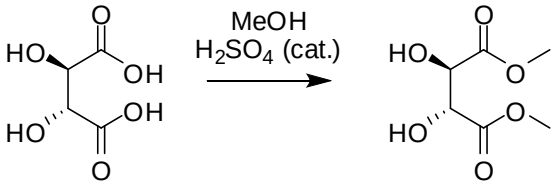
P. aeruginosa is a significant health threat to cystic fibrosis patients, as it is well known to become multidrug resistant. With so few options for drug therapy for these infections, it is imperative that new treatment strategies be developed. One drug resistance modification that confers resistance to polymyxins is the addition of $Ara4N^+$ to the lipid A portion of LPS. ArnA, the first pathway specific enzyme in this modification, is a good target for resistance modifying agent (RMA) development because it is unique to Gram negative bacteria and no human enzymes

catalyzed the reaction to any appreciable extent.[64] The strategy adopted in our inhibitor design is modular in nature. Upon encountering problems with the pyrophosphate linker synthesis, a triazole linker to UDP was proposed. Through this research it has been found that when a triazole linking module is employed, as in compound **1.39**, no inhibition of the C-terminal domain of ArnA was observed. This data suggests that **1.39** may adopt a geometry that is incompatible with the ArnA active site, this may have to do with the rigid 5-membered ring in the triazole moiety, or perhaps more to do with the loss of the electrostatic interactions experienced on the native substrates. We hypothesize successful design for inhibition of ArnA must include a negatively charged species to generate the electrostatic forces needed to bind where the pyrophosphate binds to the active site. Therefore compound **1.52** was designed with a bisphosphonate linker in place of the pyrophosphate which would allow for the negative charge at physiological pH. This compound awaits completed synthesis and biological testing. Once a successful inhibitor is found it will be soaked into the crystal structure of ArnA to determine its binding geometry and interaction in order to guide further drug design.

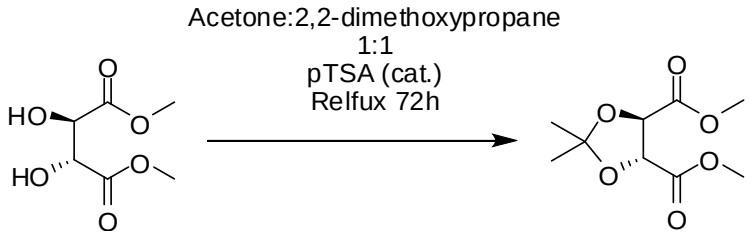
Mindful to adopt a modular strategy, several key intermediates have been successfully synthesized that will allow for inhibitor synthesis for several of the other Arn pathway enzymes. Intermediate **1.12** can be used to synthesize an acyclic *trans* diol moiety that is present in all of the native substrates for Ara4N^{+}-lipid A biosynthesis. The monodeprotected bisphosphonate **1.46** allows for the addition of a bisphosphate moiety to an alcohol via the modified Mitsunobu. Structural and enzymatic mechanism studies of ArnC and ArnD are currently being studied in the Sousa lab and will provide a wealth of material for future inhibitor design of the Arn pathway.

1.15 Organic chemistry experimental

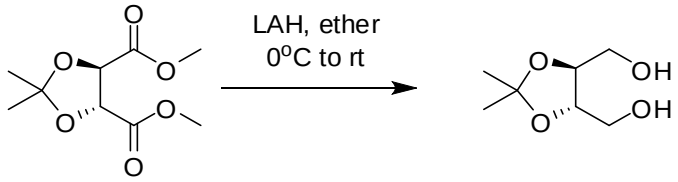
General Information. All reactions were carried out in flame dried glassware under a dry nitrogen atmosphere or sealed in 2 dram (7.4 mL) vials with teflon lined caps as indicated. DCM, diisopropylamine, methanol, ethanol, and isopropanol were distilled from CaH_2 under nitrogen and stored over 3Å molecular sieves prior to use. THF was distilled from Na benzophenone ketyl under nitrogen prior to use. All other reagents were used as received from the supplier. Flash chromatography was performed using 60Å silica gel (40-63 μm). ^{1}H NMR spectra were recorded at 300, 400, or 500 MHz in $CDCl_3$ using residual $CHCl_3$ (7.26 ppm) as an internal reference. ^{13}C NMR spectra were recorded at 75 or 100 MHz in $CDCl_3$ using the center line of the $CDCl_3$ triplet (77.16 ppm) as an internal reference. Infrared (IR) spectra were obtained as thin films on NaCl plates. Exact mass was determined using electrospray ionization (ESI-TOF).



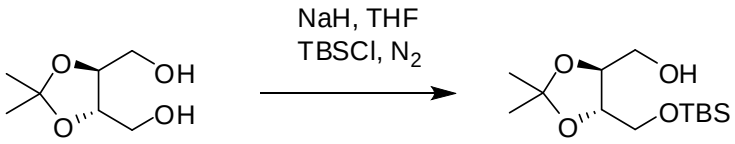
(2R,3R)-dimethyl 2,3-dihydroxysuccinate (**1.13**) Commercially available L-tartaric acid (10.98 g, 80.0 mmol, 1 equiv) was dissolved in anhydrous methanol (250 mL) under nitrogen atmosphere, to which 0.4 mL of Acetyl chloride was added to reaction. The reaction was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and a thick colorless oil was obtained. The crude oil was chromatographed on silica gel with 1:1 hexanes:ethyl acetate to give 13.95 g of product **1.13** (13.95 g, 98% yield) as a clear oil. NMR is in accord with published spectra.[79]



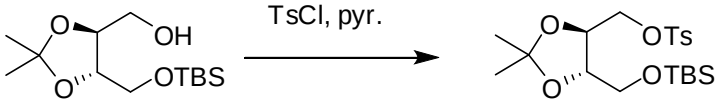
(4R,5R)-dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (**1.14**) Starting material **1.13** (13.95 g, 78.3 mmol) was dissolved in 1:1 acteone:2,2-dimethoxypropane (250 mL), to which 4-toluenesulfonic acid (0.010 g) was added. The reaction was heated to reflux under nitrogen atmosphere for 72 h. During the reflux the reaction turns dark purple in color. The solvent was removed under reduced pressure and a crude purple oil was obtained. The crude material was purified via silica gel chromatography (10:1 hexanes:EA) to obtain **1.14** (15.72 g, 92% yield) as a viscous yellow oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[79]



((4S,5S)-2,2-dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (**1.15**) Compound **1.14** (9.95 g, 45.6 mmol, 1 equiv) was dissolved in dry ether (350 mL), and cooled to 0° C. Lithium aluminum hydride (6.92 g, 182 mmol, 4 equiv) was added in portions over 1 hour, upon addition the evolution of H_2 gas was observed. The reaction was allowed to warm to room temperature slowly and stirred an additional 10 h. The reaction was cooled to 0 °C and quenched through the slow, dropwise addition of H_2O (7 mL), followed by 15% NaOH (7 mL), then H_2O (21 mL). The reaction was allowed to warm to room temperature and stirred for an additional 1 h. The reaction was filtered, solid was washed with ethyl ether (2x), and concentrated to obtain product **1.15** as a clear oil (5.25 g, 71% yield). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[80]

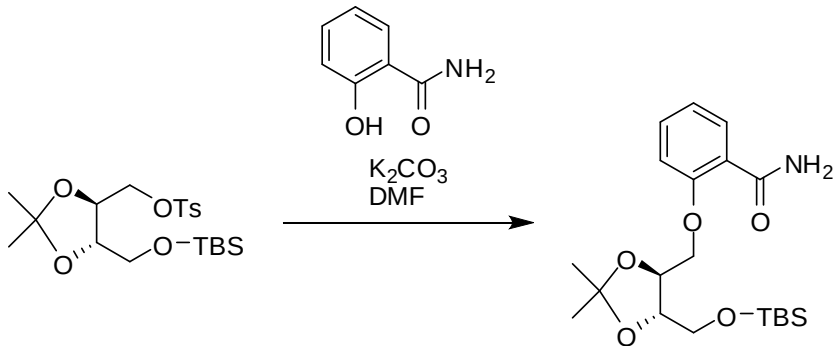


((4S,5S)-5-((tert-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**1.16**) Starting material **1.15** (5.23 g, 32.2 mmol, 1 equiv) dissolved in THF (100 mL) was added to a suspension of sodium hydride at 0 °C (1.55 g, 60% dispersion in oil, 38.7 mmol, 1.2 equiv, washed with anhydrous hexanes, 2x, in 100 mL of THF). The reaction was stirred for 1 h at 0 °C to allow anion to form. TBSCl (4.86 g, 32.2 mmol, 1.0 equiv) dissolved in THF (50 mL) was added to the reaction via cannulation over the course of 1 h. The reaction was slowly warmed to room temperature and allowed to stir for 48 h, until the reaction was found to be void of starting materials via TLC. The reaction was quenched with hexanes followed by brine. The aqueous layer was extracted with 1:1 hexanes:ethyl acetate followed by 100% ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to obtain crude product as a clear oil. The product was purified via silica gel chromatography (1:1 hexanes:EA to 100% EA) to provide product **1.16** as a clear oil (8.40 g, 94% yield) ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[80]



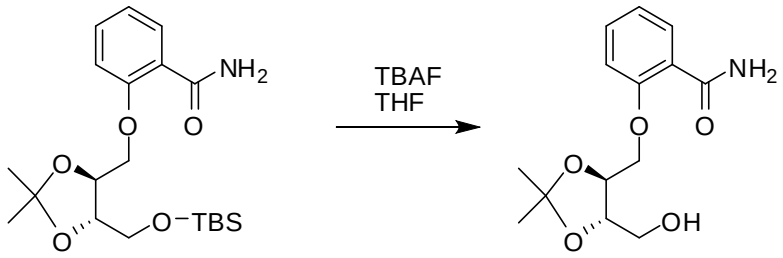
((4S,5S)-5-((tert-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl4-methylbenzenesulfonate (**1.12**) Starting material **1.15** (8.40 g, 30.4 mmol, 1 equiv) was dissolved in dry pyridine (200 mL) and cooled to 0 °C. Tosyl chloride (11.58 g, 60.8 mmol, 2 equiv), dissolved in 100 mL, was added to the reaction via cannula. The reaction was allowed to warm to room temperature overnight until the reaction was found to be complete by TLC. Reaction was concentrated to 50 mL and poured into a seperatory funnel containing 150 mL of H_2O. The

aqueous layer was extracted with ethyl acetate (150 mL, 2x). The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated to obtain crude product as a clear oil which was purified via flash chromatography (5:1 hexanes:ethyl acetate) to obtain product as a clear oil (10.81 g, 83% yield). ^1H and ^{13}C NMR are in accord with those reported in the literature.[81]



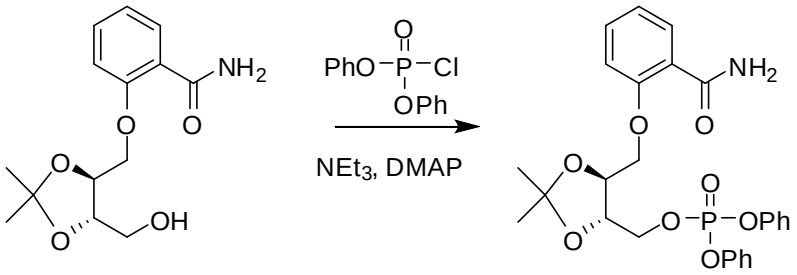
2-(((4S,5S)-5-((tert-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)benzamide (**1.17**) Starting material **1.12** (9.759 g, 22.7 mmol, 1 equiv), salicylamide (3.923 g, 22.7 mmol, 1 equiv) and potassium carbonate (5.90 g, 45.3 mmol, 2 equiv) was dissolved in dry DMF (220 mL) and heated to 80°C for 24 h until the starting material was no longer visible by TLC. Reaction was diluted with water and was extracted with ethyl acetate (3x). The organic layers were combined, washed with brine (3x), dried over $MgSO_4$, filtered and concentrated to obtain crude product as a dark brown oil. The product was purified via silica gel chromatography (4:1 hexanes:EA) to obtain product **1.17** as a crystalline white solid (8.65 g, 96% yield).

^1H NMR (500 MHz, Chloroform-*d*) δ 8.23 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.97 (s, 1H), 7.47 (ddd, *J* = 8.0, 7.3, 1.9 Hz, 1H), 7.16 – 7.09 (m, 1H), 6.99 (dd, *J* = 8.3, 1.0 Hz, 1H), 5.71 (s, 1H), 4.45 – 4.34 (m, 2H), 4.20 (dd, *J* = 9.8, 6.0 Hz, 1H), 4.03 – 3.89 (m, 2H), 3.74 (dd, *J* = 10.3, 7.0 Hz, 1H),), 0.91 (s, 9H), 0.09 (s, 3H), 0.09 (s, 3H).

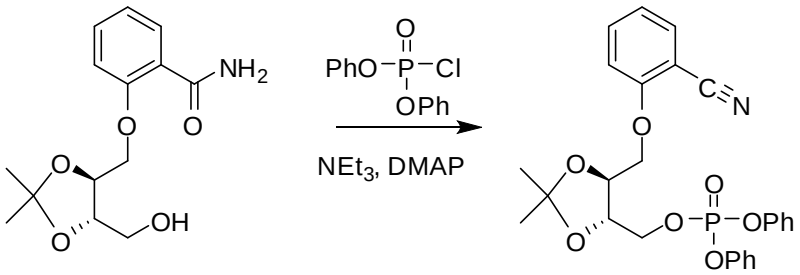


2-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)benzamide (**1.18**)

Starting material **1.17** (3.38 g, 8.54 mmol, 1 equiv) was dissolved in THF (85 mL) and tetrabutylammonium fluoride (5.50 g, 21.36 mmol, 2.5 equiv) was added to the reaction. The reaction was left to stir for 16 h until the reaction was complete by TLC. The reaction was concentrated to 10 mL before being diluted in hexanes:EA (1:1, 100 mL). The organic layer was washed with water (2x, 50 mL), washed with brine (1x, 50 mL), dried over $MgSO_4$, filtered and concentrated to obtain crude product as a clear oil. The crude product was purified via flash chromatography (1:1 hexanes:EA to 100% EA) to obtain product (2.15 g, 90% yield) as a white foam. R_f = 0.40 (100% ethyl acetate); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.20 (dd, *J* = 7.8, 1.9 Hz, 1H), 7.87 (s, 1H), 7.48 (ddd, *J* = 8.2, 7.3, 1.9 Hz, 1H), 7.16 – 7.08 (m, 1H), 6.99 (dd, *J* = 8.4, 1.0 Hz, 1H), 5.91 (s, 1H), 4.42 (ddd, *J* = 8.0, 5.6, 3.7 Hz, 1H), 4.33 (dd, *J* = 9.9, 3.7 Hz, 1H), 4.23 (dd, *J* = 9.9, 5.6 Hz, 1H), 4.09 (dt, *J* = 8.2, 4.1 Hz, 1H), 3.90 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.80 (dd, *J* = 11.9, 4.0 Hz, 1H), 1.50 – 1.45 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.52, 156.77, 133.35, 132.24, 121.61, 121.05, 112.66, 109.81, 78.23, 77.41, 77.09, 76.77, 76.18, 69.19, 62.13, 27.17, 27.10, 27.03, 25.65; IR (thin film): NH_2 and OH, 3451, 3340, C=O, 1656 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{14}H_{19}NO_5$ $[M+Li]^+$, 288.1424; found, 288.1430.

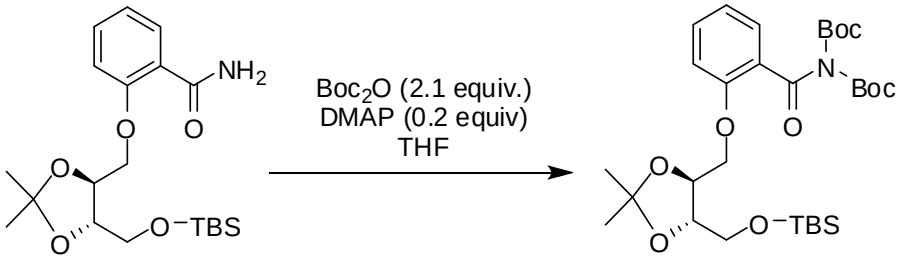


((4S,5S)-5-((2-carbamoylphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl-diphenyl phosphate (**1.19**) Starting material (**1.18**) (0.602 g, 2.1 mmol, 1 equiv) and DMAP (0.005 g) were dissolved in DCM (20 mL) under nitrogen. NEt_3 (1.46 mL, 10.5 mmol, 5 equiv) followed by diphenyl chlorophosphate (0.49 mL, 2.35 mmol, 1.1 equiv) was added to the reaction via syringe. The reaction was stirred for 2 h until found to be complete by TLC, during which forms a white precipitate. The reaction mixture was filtered and the white precipitate was washed with EA. The flintrate was concentrated to afford a clear oil which was subjected to silica gel chromatography (2:1 EA:hexanes). TLC - R_f = 0.58 (2:1 EA:hexanes) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (dd, *J* = 7.8, 1.9 Hz, 1H), 7.72 (s, 1H), 7.47 (ddd, *J* = 8.2, 7.3, 1.9 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 4H), 7.28 – 7.09 (m, 7H), 6.91 (dd, *J* = 8.5, 1.0 Hz, 1H), 5.73 (s, 1H), 4.53 – 4.31 (m, 3H), 4.28 – 4.09 (m, 4H), 2.07 (s, 1H), 1.44 (d, *J* = 9.3 Hz, 6H), 1.28 (t, *J* = 7.1 Hz, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ -11.88.



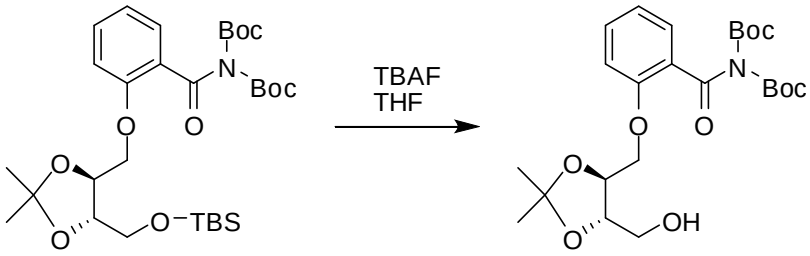
((4S,5S)-5-((2-cyanophenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl diphenyl phosphate (**1.20**) Starting material **1.18** (0.602 g, 2.1 mmol, 1 equiv) and DMAP (0.005 g) were dissolved in

DCM (20 mL) under nitrogen. NEt_3 (1.46 mL, 10.5 mmol, 5 equiv) followed by diphenyl chlorophosphate (0.49 mL, 2.35 mmol, 1.1 equiv) was added to the reaction via syringe. The reaction was stirred for 2 h until found to be complete by TLC, during which forms a white precipitate. The reaction mixture was filtered and the white precipitate was washed with EA. The filtrate was concentrated to afford a clear oil which was subjected to silica gel chromatography (2:1 EA:hexanes). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.61 – 7.47 (m, 2H), 7.33 (t, *J* = 7.8 Hz, 4H), 7.28 – 7.15 (m, 6H), 7.05 (td, *J* = 7.6, 0.9 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 4.57 – 4.47 (m, 2H), 4.45 – 4.37 (m, 1H), 4.29 (dt, *J* = 8.2, 4.2 Hz, 1H), 4.19 (dd, *J* = 4.2, 1.1 Hz, 2H), 1.48 (s, 3H), 1.43 (s, 3H).

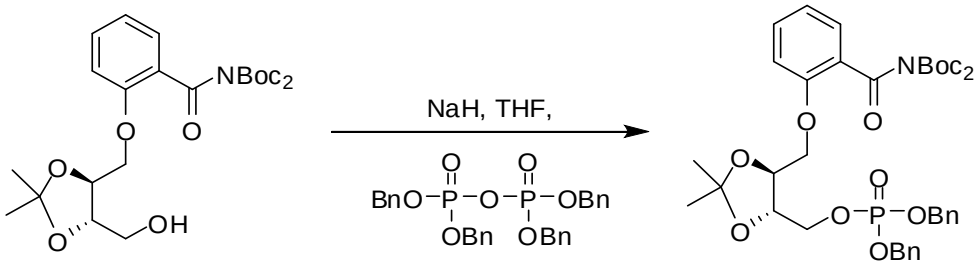


Compound (**1.29)** Starting Material **1.17** (0.103 g, 0.26 mmol), DMAP (0.006 g, 0.052 mmol), and Boc Anhydride (0.119 g, 0.546 mmol) was dissolved in THF under nitrogen atmosphere. The reaction was heated to reflux for 4 hours. Reaction was found to be completed by TLC after 4 h. Reaction was diluted with Ethyl Acetate:Hexanes 1:1, then subsequently washed with water two times followed by washing with brine. The organic layer was dried with MgSO4 filtered and dried under reduced pressure to afford a crude clear oil. Crude Material was purified through silica gel chromatography (5:1 hexanes:EA) to yield product (0.141 g, 92%) as a clear oil. TLC - R_f = 0.66 (5:1 Hexanes: EA); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.54 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.45 (ddd, *J* = 8.2, 7.4, 1.8 Hz, 1H), 7.06 – 6.94 (m, 2H), 4.31 (ddd, *J* = 8.0, 5.2, 4.0 Hz, 1H), 4.26 – 4.12 (m, 2H), 4.07 (ddd, *J* = 7.9, 5.0, 4.2 Hz, 1H), 3.84 (qd, *J* = 11.0, 4.6 Hz, 2H), 1.58 (t, *J* = 0.8 Hz, 4H),

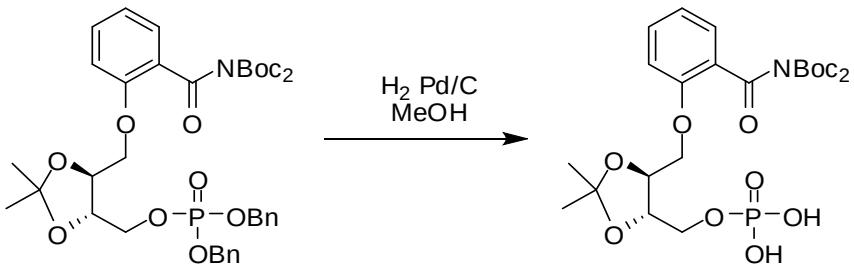
1.49 (s, 2H), 1.43 (s, 6H), 1.33 – 1.24 (m, 2H), 0.89 (s, 9H), 0.07 (d, *J* = 7.1 Hz, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.62, 149.90, 133.11, 130.18, 125.34, 121.09, 112.60, 109.64, 84.17, 78.35, 76.59, 69.44, 63.49, 28.01, 27.62, 27.28, 27.15, 26.03, 18.46, -5.23, -5.31; IR (thin film): 3340, C=O, 1782, 1687, 1601 cm^{-1}



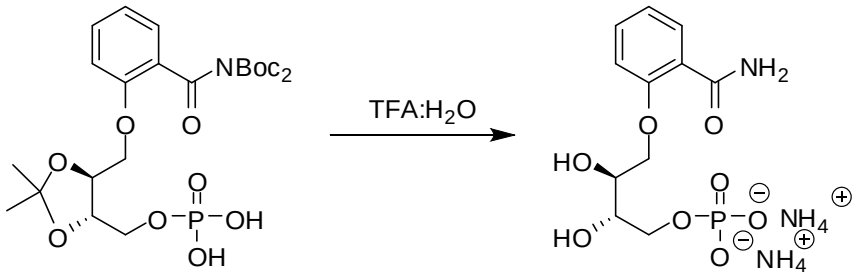
Compound (**1.30**) Starting material **1.29** (3.91 g, 6.56 mmol, 1 equiv) was dissolved in THF (65 mL), TBAF (3.43 g, 13.13 mmol, 2 equiv) was added to the reaction, upon which the reaction turned a pale yellow color. The reaction was let stir for 1 hour until no starting material was visible by TLC. The reaction was diluted with hexanes:EA (1:1 100 mL). The organic layer was washed with water (2x), washed with brine, dried over $MgSO_4$, filtered and concentrated to afford crude material as a pale yellow oil. The crude material was purified via silica gel chromatography (2:1 EA:hexanes) to obtain **1.30** (1.98 g, 63% yield) as a white foam. TLC – R_f = 0.23 (2:1 hexanes:EA) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.56 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.52 – 7.45 (m, 1H), 7.07 (td, *J* = 7.5, 0.9 Hz, 1H), 6.99 – 6.93 (m, 1H), 4.32 – 4.23 (m, 2H), 4.20 – 4.07 (m, 3H), 3.97 – 3.89 (m, 1H), 3.86 – 3.78 (m, 1H), 2.44 (d, *J* = 6.8 Hz, 1H), 2.06 (s, 1H), 1.52 – 1.41 (m, 25H), 1.28 (t, *J* = 7.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.03, 150.16, 133.31, 130.52, 121.56, 112.44, 109.65, 84.94, 80.60, 74.99, 69.40, 62.35, 27.62, 27.26, 27.19.



Compound (**1.31**) Sodium hydride (0.038 g, 1.619 mmol, 3 equiv, 60% dispersion in mineral oil) was washed with hexanes (2x) before being suspended in THF(5 mL). Starting material (0.260 g, 0.540 mmol, 1 equiv) and tetrabenzylpyrophosphate (0.872 g, 1.619 mmol, 3 equiv) were dissolved in THF (5 mL) and added via cannula to the sodium hydride suspension. The reaction was stirred for 24 h at room temperature followed by a quench with water (1 mL) the reaction was then diluted with ethyl acetate (30 mL)and washed with water (30 mL - 2x) followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated to provide crude product as a clear oil. The crude material was purified via flash chromatography (3:1 hexanes:ethyl acetate) to provide **1.31** (0.212 g, 53% yield) as a clear oil. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.54 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.45 (ddd, *J* = 8.3, 7.4, 1.8 Hz, 1H), 7.38 – 7.29 (m, 10H), 7.05 (td, *J* = 7.5, 0.9 Hz, 1H), 6.94 – 6.88 (m, 1H), 5.11 – 5.01 (m, 4H), 4.32 – 4.17 (m, 4H), 4.18 – 4.04 (m, 3H), 2.06 (s, 1H), 1.38 (s, 24H), 1.28 (t, *J* = 7.1 Hz, 1H), 1.17 (s, 1H).

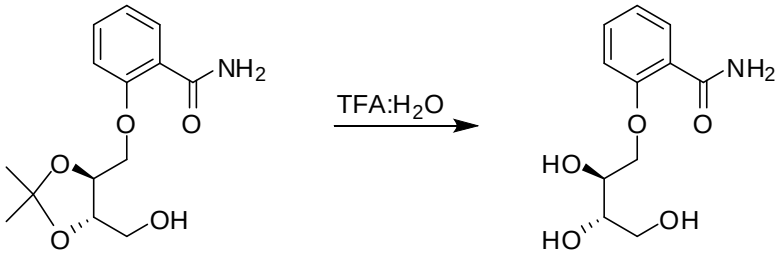


Compound (**1.32)** Starting material **1.31** (0.028 g, 0.038 mmol, 1 equiv) and palladium on carbon (0.040 g, .377 mmol, 10 equiv) were dissolved in MeOH (1 mL). The reactions was purged with H_2 (2x) and was stirred for 15 minutes at room temperature. Reaction was filtered over a pad of silica with MeOH to remove Pd/C. The reaction was concentrated to provide product **1.32** (0.020 g, 95% yield) as a white foam. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.58 – 7.49 (m, 2H), 7.16 (d, J = 8.3 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 4.37 – 4.25 (m, 3H), 4.22 – 4.08 (m, 3H), 3.62 (q, J = 7.1 Hz, 1H), 1.41 (d, J = 13.2 Hz, 23H), 1.19 (t, J = 7.0 Hz, 1H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 156.6, 150.0, 133.7, 130.4, 124.7, 121.3, 113.0, 110.4, 84.9, 68.8, 31.1, 27.8, 27.6, 27.2, 27.0, 24.8. ^{31}P NMR (122 MHz, Methanol-d_4) δ 1.12.

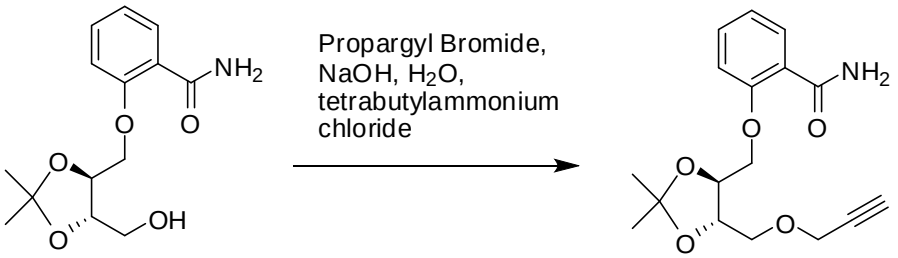


Ammonium (2S,3S)-4-(2-carbamoylphenoxy)-2,3-dihydroxybutyl phosphate **1.34** Starting material (**1.32)** (0.129 g, .230 mmol, 1 equiv) was dissolved in trifluoroacetic acid:water (9:1), Let stir at room temperature for 20 minutes until complete by TLC. The reaction was concentrated and dissolved in *i*PrOH:1M ammonium acetate (2:1, 0.020 mL) and subsequently purified via silica gel chromatography (*i*PrOH:1M ammonium acetate aqueous, 2:1). The isolated product was then lyophilized (to remove residual ammonium acetate and water, 0.14 mBar, -87 °C for 24 h) to obtain product (0.029 g, 36% yield) as a white amorphous solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (d, J = 2.9 Hz, 2H), 7.87 (dd, J = 7.7, 1.8 Hz, 2H), 7.55 (d, J = 3.0 Hz, 2H), 7.46 (ddd, J = 8.7, 7.3, 1.9 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H), 7.02 (t, J = 7.6 Hz, 2H), 4.26 (dd, J = 9.8, 3.8 Hz, 2H), 4.04 (dd, J = 9.7, 7.8 Hz, 2H), 3.95 (dt, J = 7.5, 3.4 Hz, 2H),

3.84 – 3.59 (m, 6H), 3.16 (s, 1H), 1.84 (s, 1H); ^{13}C NMR (75 MHz, DMSO) δ 166.49, 157.51, 133.13, 131.53, 122.85, 121.00, 113.91, 71.74, 71.12, 69.27, 65.07; ^{31}P NMR (122 MHz, DMSO) δ 1.43. HRMS (TOF-ESI) *m/z* calc'd for $C_{11}H_{14}NO_8P$ $[M+Na]^+$, 344.0511; found, 344.0503.

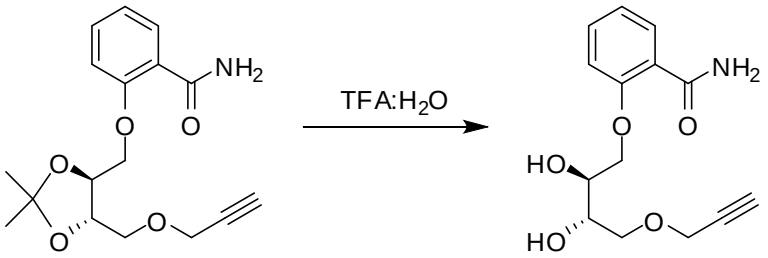


2-((2S,3S)-2,3,4-trihydroxybutoxy)benzamide (**1.35**) Starting material **1.18** (0.349 g, 0.349 mmol, 1 equiv) was dissolved in TFA (10 mL). The reaction was stirred for 15 minutes until found to be complete by NMR. The reaction was concentrated and diluted with EA upon which a white precipitate crashes out of solution. The solid was filtered and subsequently washed with EA (2x) to afford product **1.35** (0.299 g, 73% yield) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.92 (s, 1H), 8.71 (s, 1H), 8.14 – 8.08 (m, 1H), 7.63 – 7.55 (m, 1H), 7.22 – 7.12 (m, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 6.15 (s, 2H), 4.57 – 4.52 (m, 1H), 4.38 (td, *J* = 10.1, 3.6 Hz, 1H), 4.33 – 4.13 (m, 2H), 3.91 (m, 2H).



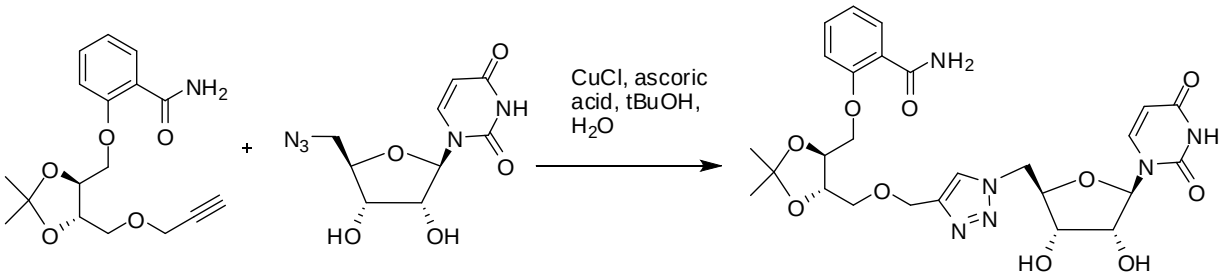
2-(((4S,5S)-2,2-dimethyl-5-((prop-2-ynyloxy)methyl)-1,3-dioxolan-4-yl)methoxy)benzamide (**1.36**) Starting material **1.18** (0.600 g, 2.13 mmol) was dissolved in CH_2Cl_2 (10 mL), tetrabutyl ammonium iodide (0.1 mmol 0.05 equiv, 0.040 g) was added to the reaction followed by aqueous

NaOH (10 mL, 50% wt/v). The reaction was stirred for 30 minutes before propargyl bromide was added dropwise over the course of 1 h. Reaction was vigorously stirred for 2 days at room temperature until no more starting material was observed by TLC. Water was added to the reaction and was extracted with DCM (2x) the organic layers were dried over $MgSO_4$, filtered and concentrated to obtain crude material as a white amorphous solid. Crude material was purified via column chromatography (5% MeOH in $CHCl_3$) to obtain product **1.36** (0.533 g, 78% yield) as a white amorphous solid. TLC – R_f = 0.45 (1:1 Hexanes:EA). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.23 (dd, *J* = 7.8, 1.9 Hz, 1H), 7.89 (s, 1H), 7.52 – 7.45 (m, 1H), 7.13 (td, *J* = 7.6, 1.0 Hz, 1H), 7.01 (dd, *J* = 8.4, 1.0 Hz, 1H), 5.74 (s, 1H), 4.40 – 4.32 (m, 2H), 4.27 – 4.13 (m, 4H), 3.81 (dd, *J* = 9.7, 4.9 Hz, 1H), 3.71 (dd, *J* = 9.7, 6.1 Hz, 1H), 2.49 (t, *J* = 2.4 Hz, 1H), 1.47 (d, *J* = 4.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.91, 156.73, 133.19, 132.57, 121.73, 121.39, 112.72, 110.21, 79.02, 77.36, 77.23, 77.04, 76.73, 76.12, 75.23, 70.06, 69.24, 58.81, 27.17, 27.01; IR (thin film): NH_2, 3458, 3262, CH alkyne, 2114, C=O, 1661 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{17}H_{21}NO_5$ $[M+Li]^+$, 326.1580; found, 326.1528.



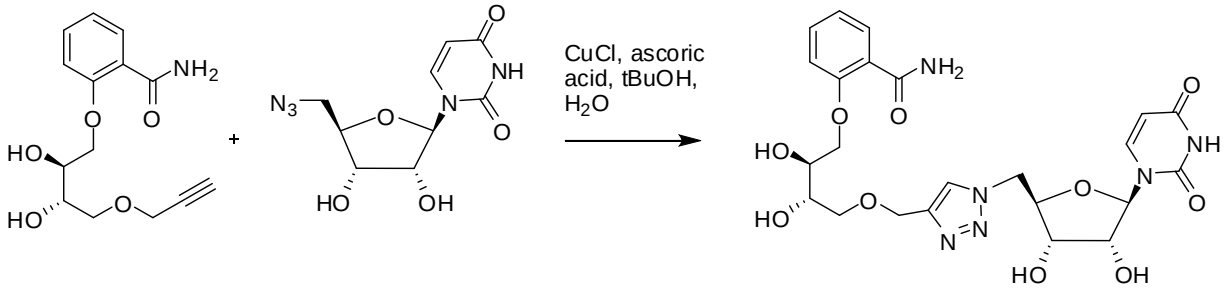
2-((2S,3S)-2,3-dihydroxy-4-(prop-2-ynyloxy)butoxy)benzamide (**1.38**) Starting material **1.36** (0.130 g, 0.407 mmol, 1 equiv) was dissolved in trifluoroacetic acid:water (9:1, 4 mL total). The reaction was stirred for 5 minutes until the reaction is complete by TLC. The reaction was concentrated to provide a pale pink oil. The crude product was purified via silica gel

chromatography (5% MeOH in $CHCl_3$ to 10% MeOH in $CHCl_3$) to provide product **1.38** (0.103 g, 90% yield) as a white amorphous solid. R_f = 0.20 (5% MeOH in $CHCl_3$). ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.97 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.51 (ddd, *J* = 8.1, 7.3, 1.8 Hz, 1H), 7.16 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.07 (td, *J* = 7.5, 1.0 Hz, 1H), 4.33 (dd, *J* = 9.9, 4.0 Hz, 1H), 4.28 – 4.13 (m, 3H), 4.08 (dt, *J* = 7.3, 3.8 Hz, 1H), 3.90 (ddd, *J* = 6.1, 5.1, 3.6 Hz, 1H), 3.72 (dd, *J* = 9.7, 5.2 Hz, 8H), 3.66 (dd, *J* = 9.7, 6.1 Hz, 1H), 3.36 (s, 0H), 2.88 (t, *J* = 2.4 Hz, 1H), 1.34 – 1.24 (m, 1H). ^{13}C NMR (101 MHz, cd$_3$od) δ 168.67, 157.35, 133.12, 131.06, 121.31, 120.71, 112.83, 79.15, 74.69, 70.53, 70.34, 70.18, 69.67, 57.84; IR (thin film): OH and NH_2, 3302, CH alkyne, 2075, C=O, 1610 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{14}H_{17}NO_5$ $[M+H]^+$, 280.1185; found, 280.1162.



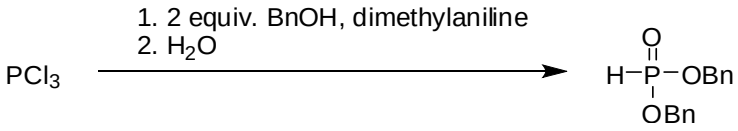
2-(((4S,5S)-5-(((1-(((2R,3S,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)benzamide (**1.37**) Compound **1.36** (0.066 g, 0.208 mmol, 1 equiv), 5'-azidouridine (0.056 g, 0.208 mmol, 1 equiv), sodium ascorbate (0.021 g, 0.104 mmol, 0.5 equiv), and copper (II) sulfate (0.026 g, 0.104 mmol, 0.5 equiv) were all dissolved in *t*-BuOH: water: acetone (1:1:1, 2 mL total). The reaction mixture slowly changed from pale yellow to green over the course of 2 h upon which the reaction found to be complete by TLC. The reaction was concentrated and purified via silica gel chromatography (6:3:1, *i*PrO:ethyl acetate:water) to provide product **1.37** as a pale yellow solid (0.060 g, 49% yield).

R_f = 0.55 (6:3:1, *i*PrOH:ethyl acetate:water); ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.00 (s, 1H), 7.96 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.50 (ddd, *J* = 8.7, 7.4, 1.9 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.14 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.09 (td, *J* = 7.5, 1.0 Hz, 1H), 5.73 (d, *J* = 3.8 Hz, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 4.82 (dd, *J* = 14.6, 3.6 Hz, 1H), 4.73 (dd, *J* = 14.6, 6.6 Hz, 1H), 4.69 (s, 2H), 4.37 (dd, *J* = 10.1, 3.3 Hz, 1H), 4.31 (ddd, *J* = 7.9, 5.6, 3.3 Hz, 1H), 4.24 (ddd, *J* = 11.4, 9.9, 5.9 Hz, 2H), 4.19 – 4.10 (m, 3H), 3.76 (qd, *J* = 10.3, 5.1 Hz, 2H), 3.32 (p, *J* = 1.7 Hz, 2H), 1.42 (s, 3H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, cd$_3$od) δ 169.89, 165.88, 158.26, 151.97, 145.79, 143.08, 134.52, 132.45, 126.55, 122.87, 122.43, 114.37, 111.39, 111.14, 103.10, 92.99, 82.87, 78.11, 77.75, 74.16, 71.87, 71.75, 70.25, 65.29, 52.38, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 27.43, 27.29, 25.25. IR (thin film): OH and NH_2, 3448 and 3341, C=O, 1691 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{26}H_{32}N_6O_{10}$ $[M+H]^+$, 589.2258; found, 589.2255.



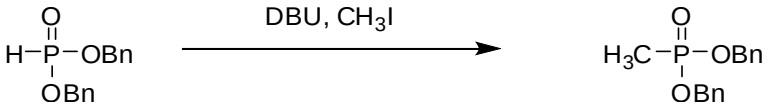
2-((2S,3S)-4-((1-(((2R,3S,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-2,3-dihydroxybutoxy)benzamide (**1.39**) Compound **1.38** (0.055 g, 0.200 mmol, 1 equiv), 5'-azidouridine (0.054 g, 0.200 mmol, 1 equiv), sodium ascorbate (0.019 g, 0.100 mmol, 0.5 equiv), and copper (II) sulfate (0.024 g, 0.100 mmol, 0.5 equiv) were all dissolved in *t*-BuOH: water: acetone (1:1:1, 2 mL total). The reaction was stirred at room temperature for 4 h after found to be complete by TLC. A visible change in color from pale yellow to green was observed during the

reaction period. The reaction was concentrated and purified via silica gel chromatography (6:3:1, *i*PrO:ethyl acetate:water) to provide product as a pale yellow solid (0.087 g, 79% yield). R_f = 0.47 (6:3:1 *i*PrOH:ethyl acetate:water); ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.98 (s, 1H), 7.96 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.50 (ddd, *J* = 8.8, 7.4, 1.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.14 (d, 1H), 7.07 (t, 1H), 5.72 (d, *J* = 3.8 Hz, 1H), 5.69 (d, *J* = 8.1 Hz, 1H), 4.82 (dd, *J* = 14.6, 3.6 Hz, 1H), 4.73 (dd, *J* = 14.6, 6.4 Hz, 1H), 4.66 (d, *J* = 1.5 Hz, 2H), 4.30 (dd, *J* = 9.8, 4.0 Hz, 1H), 4.28 – 4.23 (m, 1H), 4.21 – 4.15 (m, 2H), 4.12 (t, *J* = 5.9 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.90 (ddd, *J* = 6.2, 5.2, 3.5 Hz, 1H), 3.71 (dd, *J* = 9.8, 5.2 Hz, 1H), 3.64 (dd, *J* = 9.8, 6.1 Hz, 1H). ^{13}C NMR (101 MHz, cd$_3$od) δ 168.67, 164.58, 157.32, 150.54, 144.52, 141.84, 133.10, 131.01, 125.00, 121.32, 120.67, 112.83, 101.59, 91.85, 81.41, 72.71, 71.19, 70.40, 70.28, 70.21, 69.64, 63.60, 50.89. IR (thin film): OH and NH_2, 3337, C=O, 1686 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{23}H_{28}N_6O_{10}$ $[M+Na]^+$, 571.1765; found, 571.1764.

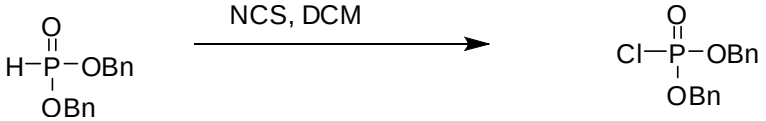


Dibenzyl H-phosphonate **(1.42)** PCl_3 (6.35 mL, 72.8 mmol, 1 equiv) was added to toluene (80 mL) and cooled to 0°C under nitrogen atmosphere. Slowly over 1 h benzyl alcohol (15.1 mL, 145.63 mmol, 2.16 equiv) and N,N-dimethylaniline (19.9 mL, 157.2 mmol, 2.16 equiv) dissolved in toluene (20 mL) and cooled to 0°C was added to the PCl_3 via cannula. As the solutions were combined a white precipitate formed in the reaction. After all of the benzyl alcohol, N,N-dimethylaniline solution was added to the PCl_3 the reaction was stirred for an additional 1 h and was warmed to room temperature. Water (20 mL) was added dropwise over 15 minutes with a

vent to a sat. $NaHCO_3$ solution. The reaction was then diluted with diethylether (100 mL) followed by water (100 mL). The aqueous layer was extracted with ether (2x), the organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to provide crude product as a clear oil. The crude product was purified via flash chromatography (3:1 hexanes:ethyl acetate) to provide product (17.19 g, 90% yield) as a clear oil.

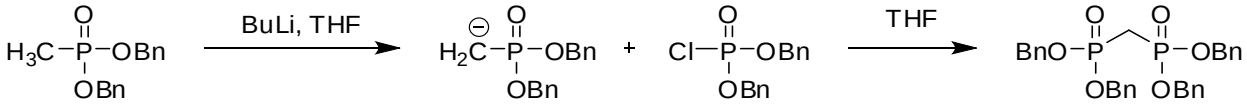


Methyl dibenzyl phosphonate **(1.43)** Starting material (5.425, .0207 mmol, 1 equiv) was dissolved in acetonitrile (41 mL) and cooled to 0°C under nitrogen atmosphere. Methyl iodide (1.42 mL, 22.8 mmol, 1.1 equiv) followed by diazobicycloundecane (3.41 mL, 22.8 mmol, 1.1 equiv) were added to the reaction dropwise via syringe. The reaction was slowly warmed to room temperature and let stir for 16 h. The reaction was diluted with hexanes (30 mL) and was subsequently washed with hexanes (3x). The acetonitrile layer was dried over $MgSO_4$, filtered and concentrated to provide crude product as a thick deep yellow oil. The crude product was purified via flash chromatography (100% hexanes to 1:1 hexanes:EA) to obtain product as a clear oil (4.719 g, 83% yield). ^1H and ^{13}C NMR are in accord with those reported in the literature.

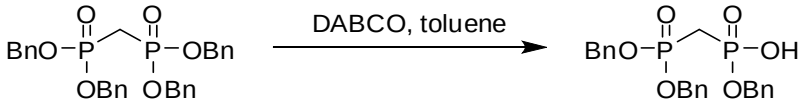


Dibenzyl chlorophosphate. **(1.44)** *N*-chlorosuccinimide (0.332 g, 2.50 mmol, 1.1 equiv) was dissolved in toluene (10 mL). Starting material (0.50 mL, 2.30 mmol, 1 equiv) was added to the reaction via syringe. The reaction was stirred for 2 h at room temperature. The reaction was filtered

over oven dried celite and concentrated to obtain product (0.655 g, 96% yield). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[78]

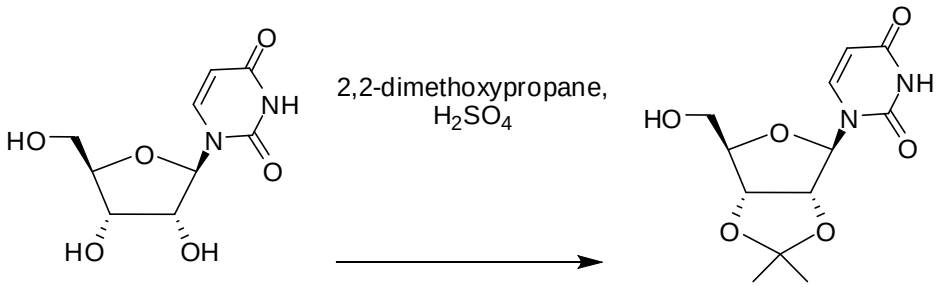


Dibenzyl Methylenediphosphonate (**1.45**) Methyl dibenzyl phosphonate (0.831 g, 3.00 mmol, 2 equiv) was dissolved in THF (10 mL) and cooled to -78°C. BuLi (1.94 mL, 1.54 M, 3.00 mmol, 2 equiv) was added to the reaction dropwise. The reaction was stirred at -78°C for 30 minutes. Dibenzyl chlorophosphate (0.446 g, 1.50 mmol, 1 equiv) dissolved in THF (5 mL) was added to the reaction via cannula. The reaction was stirred at -78°C for 1 h. The reaction was quenched through the addition of sat. NH_4Cl (20 mL) and the reaction was warmed to room temperature. The reaction was extracted with ethyl acetate (2x), the combined organic layers were washed with brine (1x), dried over $MgSO_4$, filtered, and concentrated to obtain crude product as a clear oil. The crude product was purified via flash chromatography (1:1 hexanes: to 1:2 hexanes:ethyl acetate) to obtain product (0.329 g, 41% yield) as a clear oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[82]

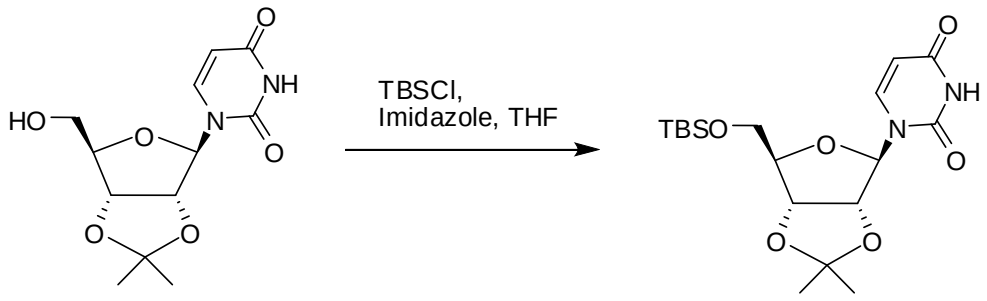


Benzyl Hydrogen (bis(benzyloxy)phosphoryl)methylphosphonate (**1.46**) Starting material **1.45** (0.046 g, 0.86 mmol, 1 equiv) and DABCO (0.010 g, 0.86 mmol, 1 equiv) were dissolved in toluene (8 mL). The reaction was refluxed for 5 h until found to be complete by TLC. The reaction was concentrated, then diluted with 5% HCl (5 mL) upon which a white precipitate crashes out of solution. The acidic layer was extracted with EA (2x), the combined organic layers were washed

with brine, dried over $MgSO_4$, filtered and concentrated to obtain product **1.46** (.035 g, 92% yield) as a colorless oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[83]

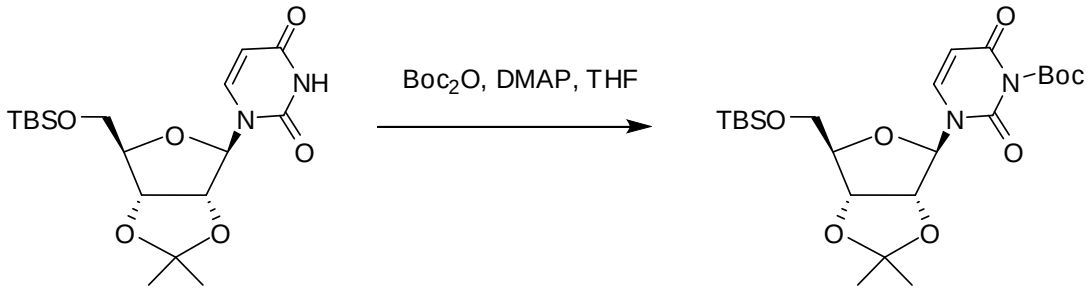


1-((3aR,4R,6R,6aR)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)pyrimidine-2,4(1H,3H)-dione. Uridine (5.00 g, 20.5 mmol, 1 equiv) was dissolved in acetonitrile:2,2-dimethoxypropane (10:1, 200 mL) to which catalytic 4-toluenesulfonic acid (cat. 0.15 g) was added. The reaction was left to stir for 30 minutes. After which the reaction was concentrated to provide crude product as a clear oil. The crude product was purified via flash chromatography (100% ethyl acetate) to provide product (5.35 g, 92% yield) as a white amorphous powder. The NMR spectra are in accord with the literature values.[84]

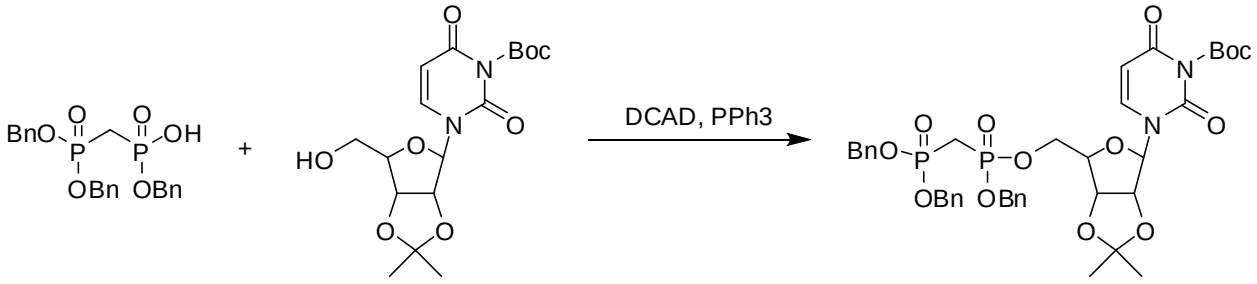


Compound (**1.47**) (4.73 g, 16.64 mmol, 1 equiv) and imidazole (2.26 g, 33.28 mmol, 2 equiv) was dissolved in DCM (120 mL) and cooled to 0°C. TBSCl (2.51 g, 16.64 mmol, 1 equiv) dissolved in DCM (40 mL) was added to the reaction via cannula. It was let warm to room temperature over

the course of 1 h. The reaction was allowed to stir at room temperature until complete by TLC. The reaction was concentrated to 15 mL and diluted with ethyl acetate (100 mL) and washed with water (2x). The reaction was washed with brine, dried over $MgSO_4$, filtered, and concentrated to obtain product (6.50 g, 98% yield) as a clear oil. [85]



Compound (**1.48)** Starting material (4.719 g, 11.84 mmol, 1 equiv) and DMAP (.072 g, 0.592 mmol, 0.05 equiv) were dissolved in THF (120 mL). Boc Anhydride (2.36 mL, 12.43 mmol, 1.05 equiv) was added via syringe under nitrogen atmosphere and was stirred at room temperature for 16 h until found to be complete by TLC. The reaction was concentrated to 20 mL and diluted with hexanes:ethyl acetate (1:1, 200 mL). The organic layer was washed with water (2x), washed with brine (1x), dried over $MgSO_4$, filtered, and concentrated to provide crude product as a clear oil. The crude product was purified via flash chromatography to provide product (5.01 g, 85% yield) as a white foam.



Compound **1.50.** Starting material **1.46** (0.086 g, 0.193 mmol, 1 equiv), compound **1.49** (0.081 g, 0.212 mmol, 1.1 equiv), and triphenylphosphine (0.126 g, 0.483 mmol, 2.5 equiv) were dissolved

in DCM (3 mL) under nitrogen. To which DCAD (0.141 g, 0.386 mmol, 2 equiv), dissolved in DCM (1 mL) was added to the reaction via cannula. The reaction was stirred at room temperature overnight, where a white precipitate crashes out of solution. The reaction is filtered over celite and diluted with EA and water. The aqueous layer was basified to pH 10 and extracted with EA (2x), the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated to obtain crude product as a colorless oil. The crude product is purified via silica gel chromatography (2:1 EA:hexanes) to obtain product **1.50** as a mixture of 2 diastereomers (0.071 g, 46% yield). ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.70 – 7.59 (m, 3H), 7.57 – 7.23 (m, 24H), 5.80 – 5.70 (m, 1H), 5.64 (t, *J* = 8.6 Hz, 1H), 5.15 – 4.88 (m, 7H), 4.83 – 4.76 (m, 1H), 4.69 – 4.62 (m, 1H), 4.29 – 4.08 (m, 4H), 2.45 (td, *J* = 21.1, 4.3 Hz, 2H), 1.54 (d, *J* = 23.2 Hz, 15H), 1.34 – 1.21 (m, 5H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 160.4, 160.3, 148.4, 147.6, 140.6, 135.8, 135.7, 133.0, 132.2, 132.1, 132.1, 132.0, 132.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.5, 128.3, 128.3, 128.2, 128.2, 114.8, 114.8, 102.3, 102.2, 93.0, 92.8, 87.1, 87.1, 84.7, 84.6, 84.4, 84.2, 80.1, 80.0, 68.8, 68.7, 68.7, 68.6, 68.5, 68.5, 68.4, 68.4, 68.3, 68.3, 68.2, 65.7, 65.3, 65.2, 27.5, 27.2, 25.4.

1.16 Biochemistry Experimental

Protein Purification

Purification of ArnA decarboxylase domain. The plasmid pMS159 was transformed into *E. coli* Rosetta (DE3) cells (Novagen) and plated on LB media with 50 μg/mL kanamycin. A 100 mL overnight culture from a single colony containing 50 μg/mL kanamycin was used to inoculate 6x 1L LB medium supplemented with 50 μg/ mL kanamycin. Cultures were grown at 37°C to an

OD_{600} to 0.6 and cooled to 4°C before induction with 0.4 mM isopropyl-*β*-D-thiogalactopyranoside. Cultures were allowed to grow overnight at room temperature.
and the cell pellet was resuspended in lysis buffer containing 25 mM Tris-HCl at pH 8.0, 5 mM 2-mercaptoethanol, and complete EDTA-free protease inhibitor cocktail used at 1 tablet per 100 mL of buffer (Roche). Cells were lysed on ice by sonication. After lysis, KCl was added to a final concentration of 300 mM and cell debris was removed by centrifugation at 16,000 rpm for 30 min at 4°C. The supernatant was applied to a 10 mL Ni-NTA column (Qiagen) pre-equilibrated with the lysis buffer containing 300 mM KCl. The column was washed with 5 column volumes of the above buffer, followed by 5 column volumes of wash buffer (25 mM HEPES-KOH at pH 7.5, 300 mM KCl, 10% glycerol, 5 mM 2-mercaptoethanol, and 25 mM imidazole at pH 8.0). The protein was eluted using a 25-300 mM imidazole gradient at pH 8.0 (70 mL final volume of 5 mL fractions). Fractions containing the protein were loaded on a size-exclusion (HiLoad 26/60 Superdex 200, Amersham Pharmacia Biotech) column pre-equilibrated with 25 mM Tris-HCl at pH 8.0, 150 mM KCl, 10% glycerol, 1 mM EDTA at pH 8.0, and 5 mM 2-mercaptoethanol and eluted in the same buffer. Elution was monitored by measuring the absorption at 280 nm. Fractions containing the protein were dialyzed against 25 mM Tris-HCl at pH 8.0 and 5 mM 2-mercaptoethanol and loaded on a MonoQ HR5 column (Pharmacia Biotech equilibrated in the same buffer. The protein was eluted in buffer containing 25 mM Tris-HCl at pH 8.0, 5 mM 2-mercaptoethanol, and 0-1 M NaCl gradient. The fractions containing protein were combined, and the 6-His tag was removed by overnight incubation at 4 °C with TEV protease (1:50 TEV protease/ArnA) and 10 mM dithiothreitol. The protein was separated from the protease and the cleaved tag by size-exclusion chromatography with the column and buffers specified above. The ArnA C terminus was eluted as a monomer from the column. The fractions

containing protein were combined, and the protein was concentrated to approximately 10 mg/mL (Bio-Rad Protein Assay, Bio-Rad Laboratories). The protein was stored at -80 °C until needed.

Inhibition assay

The conditions used for this assay were: 400 nM enzyme, 100 mM KCl, 10% glycerol, 0.2 mg/ml BSA, 25 mM Tris pH = 8.0, 5 mM beta-mercaptoethanol, 4 mM NAD^+, 0.25 mM UDP-GlcA, and 1 mM inhibitor, with a final volume of 800 µl. The reaction was conducted at 37ºC in a spectrophotometer and the generation of NADH at 340 nm was monitored for 5 minutes. The reaction was begun with the addition of UDP-GlcA. Initial rates of NADH formation were collected (change in absorbance/min) and compared in quadruplicate for each sample. As a negative control no inhibitor was added, and UDP was used as a positive control inhibitor.

1.17 References

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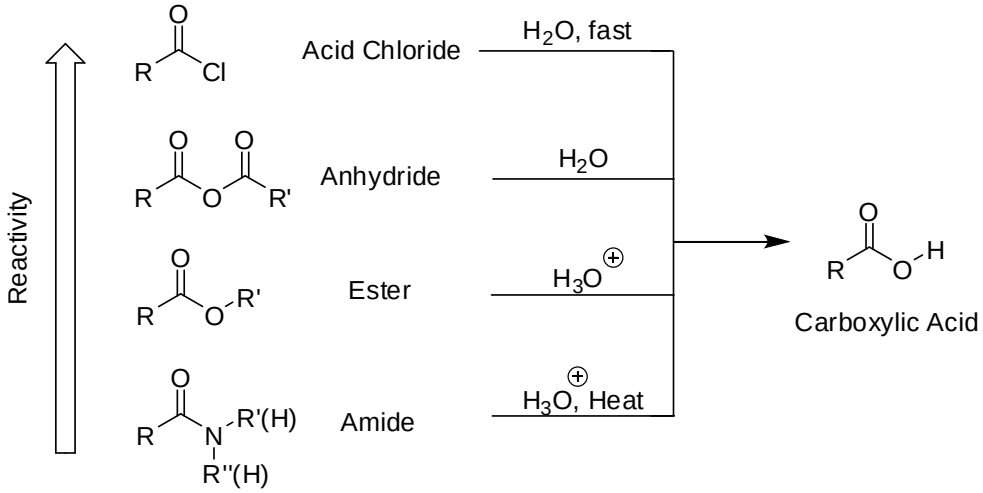
Chapter 2

The Conversion of Esters to Acid Chlorides Using Thionyl Chloride

2.1 Introduction to Acid Chlorides

Acid chlorides are useful intermediates with applications as active esters for the synthesis of carboxylic acid derivatives. This is due to their enhanced reactivity compared to other common carboxylic acid derivatives. Typically, the rate limiting step in the hydrolysis of carboxylic acid derivatives is the attack of the carbonyl.[1] Acid chlorides are more reactive due to the enhanced electrophilicity of the carbonyl group due to the poor π-donation when compared to other carboxylic acid derivative heteroatoms (Figure 2.1). Anhydrides are less reactive than acid chlorides, but they are more reactive than esters and amides. This is because the oxygen lone pair is donating into both carbonyl groups in the anhydride causing less overall π-donation into the carbonyl of interest compared to the ester. The amide nitrogen is a better π-donor to the carbonyl relative to the ethereal oxygen of an ester, reducing the rate of hydrolysis.

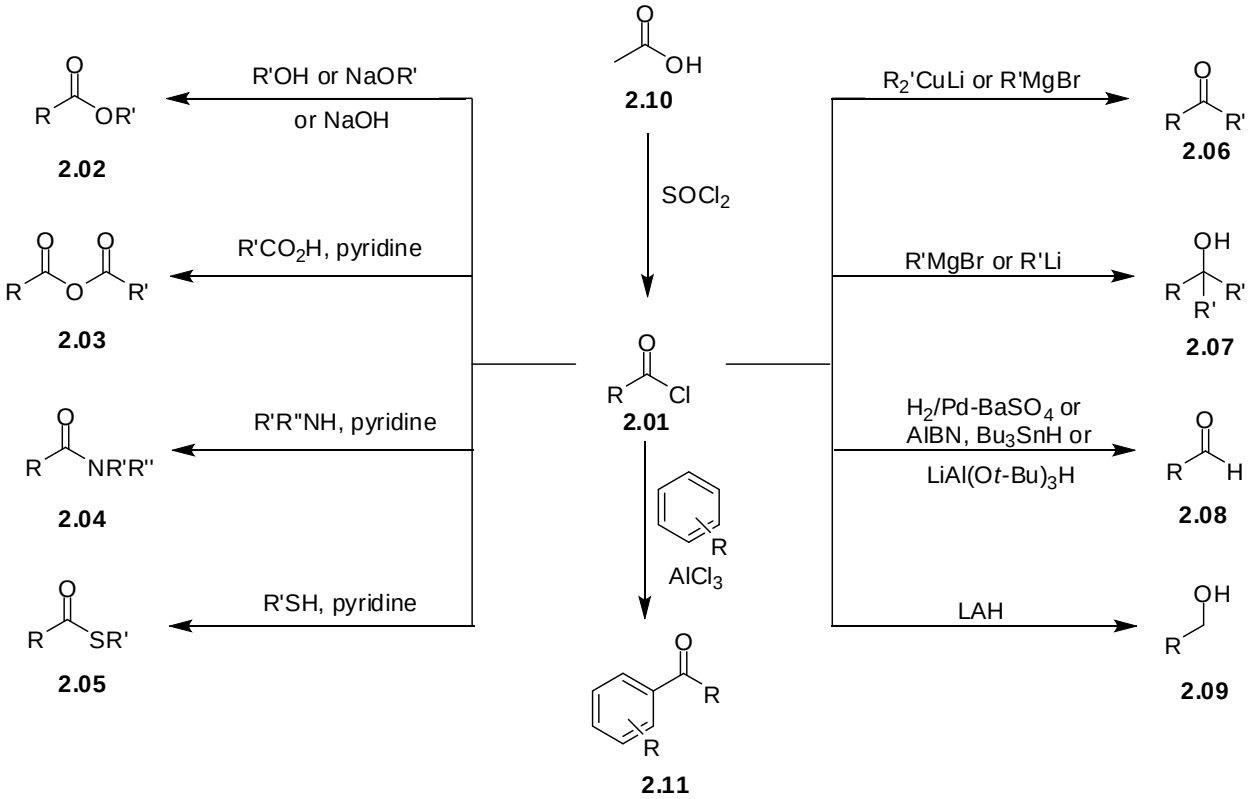
Figure 2.1 General Reactivity of Carboxylic Acid Derivatives



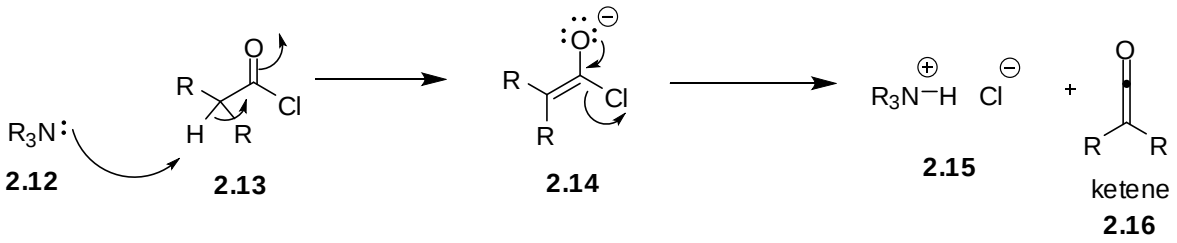
Acid chlorides have been employed in many synthetic transformations that take advantage of their electrophilicity to provide an array of products. Carboxylic acid derivatives can be formed

when reacting an acid chloride with alcohols to form esters (**2.02**), carboxylic acids to form anhydrides (**2.03**), amines to form amides (**2.04**), or thiols to form thioesters (**2.05**) (Figure 2.2).[2] Other reactions acid chlorides can undergo include the addition of 1 equiv of a carbon nucleophile including cuprates and Grignards to provide ketones (**2.06**);[3] treatment with 2 or more equivs of Grignards or organolithium reagents to provide tertiary alcohols (**2.07**);[4] reduction with lithium aluminum hydride to primary alcohols (**2.09**);[5] reduction to aldehydes (**2.08**) via the Rosenmund reaction (H_2/Pd-$BaSO_4$),[6] a radical process using tributyl tin hydride,[7] or through the use of $LiAlH(Ot\text{-}Bu)_3$.[5] Acid chlorides are also used in the Friedel-Crafts acylation to generate aryl ketones (**2.11**), and can react with tertiary amines (**2.12**) or other bases to generate ketenes (**2.16**) taking advantage of the acidity of the α-proton (Scheme 2.1).[8] It is the latter transformation that motivated the research in this chapter as described below.

Figure 2.2 Several Common Acyl Chloride Transformations



Scheme 2.1 The Formation of Ketenes from Acid Chlorides



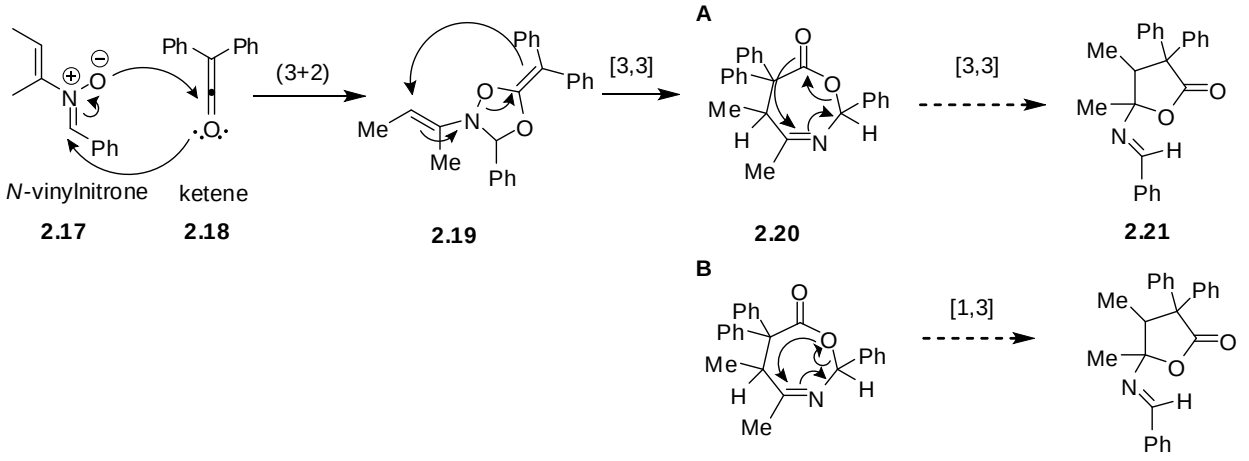
2.2 The Use of Acid Chlorides in the Use of Synthesis Towards the Skeleton of Morphinan

The Sammakia lab is studying the use of ketenes in a novel cascade reaction with *N*-vinyl nitrones.[9] Former group members, Dr. Katelyn Chando and Dr. Ryan Michael, found that ketenes will undergo a (3+2) cycloaddition[10] with *N*-vinyl nitrones to form 5-membered heterocycle **2.19**. This compound then undergoes a [3,3]-sigmatropic rearrangement[11] to form 7 membered ring heterocycle (**2.20**),[9] which then undergoes either a [1,3]- or [3,3]- rearrangement to provide lactone aminal derivative **2.21** with high levels of diastereoselectivity (Scheme 2.2).[12] The two possible mechanistic paths of the last step of this process differ in that the [3,3]-sigmatropic rearrangement involves the carbonyl oxygen of the lactone, an allowed process involving a 6-electron suprafacial transition state (Scheme 2.2 A), while the [1,3]-rearrangement involves the ethereal oxygen of the lactone in a process that can either be allowed, or not, depending on the orbital interactions described below.

The Woodward–Hoffmann rules dictate that a [1,3] sigmatropic rearrangement would proceed either in an antarafacial fashion, or via inversion of the migrating atom, both of which would be predicted to have a high energy barrier.[13] The cascade reaction being explored proceeds at low temperatures and thus, we have hypothesized this reaction may proceed through a [1,3] pseudopericyclic rearrangement rather than the typical pericyclic rearrangement.[14] A pseudopericyclic reaction is "a concerted transformation whose primary changes in bonding

compass a cyclic array of atoms, at one (or more) of which nonbonding and bonding atomic orbitals interchange roles". [15] In this scenario the pair of bonding electrons on the ethereal oxygen will change from a non-bonding pair of electrons into a bonding pair of electrons (Scheme 2.2 B) while the electrons in the C-O σ-bond will become a non-bonding lone pair.

Scheme 2.2 The Ketene, *N*-vinylnitrone Cascade Proposed Mechanism

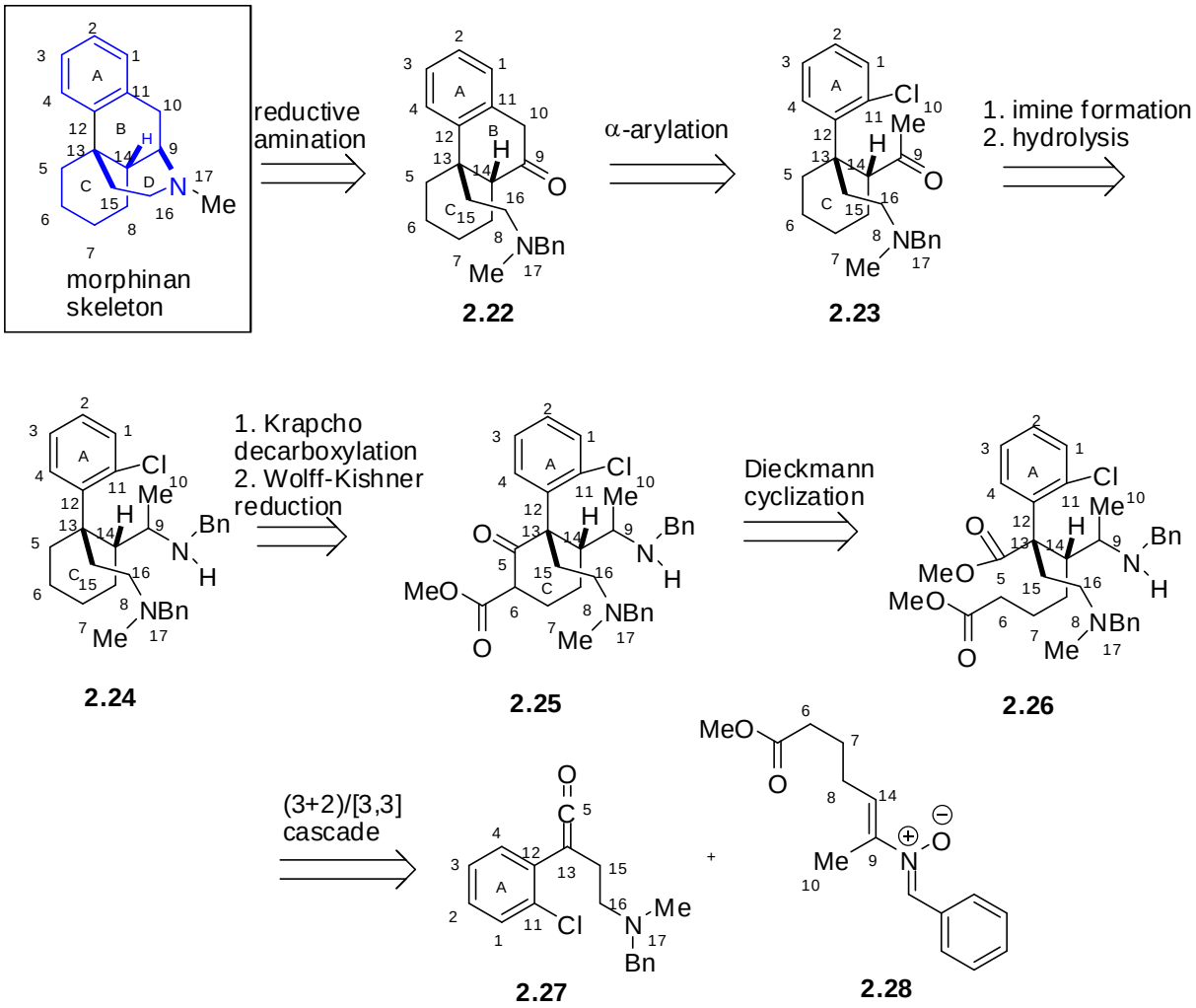


This reaction can be applied to the synthesis of natural products that contain hindered quaternary-tertiary carbon centers, such as the morphinan skeleton (**2.22**). The morphinan skeleton is composed of a tetracyclic structure with a benzylisoquinoline core, and 3 contiguous stereocenters at C-9, C-13, and C-14 (Scheme 2.3). The synthesis of this structure is challenging due to the steric hindrance at C-13, especially with the proximal all-carbon tertiary stereocenter at C-14. Our approach is capable of the synthesis of vicinal stereocenters, including the quaternary stereocenter, C-13, and would allow for the installation of C-13 and C-14 in a single transformation. It also sets up the installation of the C-9, stereocenter as described below.

In our retrosynthesis (Scheme 2.3), we envisioned forming ring D via an intramolecular reductive amination from **2.22**. This compound can be prepared via the α-arylation of compound **2.23**. The methyl ketone **2.23** can be prepared from the benzyl amine **2.24.** Compound **2.25** could

be prepared via a Dieckmann cyclization,[16] which then in the forward direction, would allow for a Krapcho decarboxylation[17] followed by Wolff-Kishner reduction[18] to provide **2.24**. Intermediate **2.26** can then be prepared via the novel ketene/*N*-vinyl nitrone cascade reaction on compounds **2.27** and **2.28**.

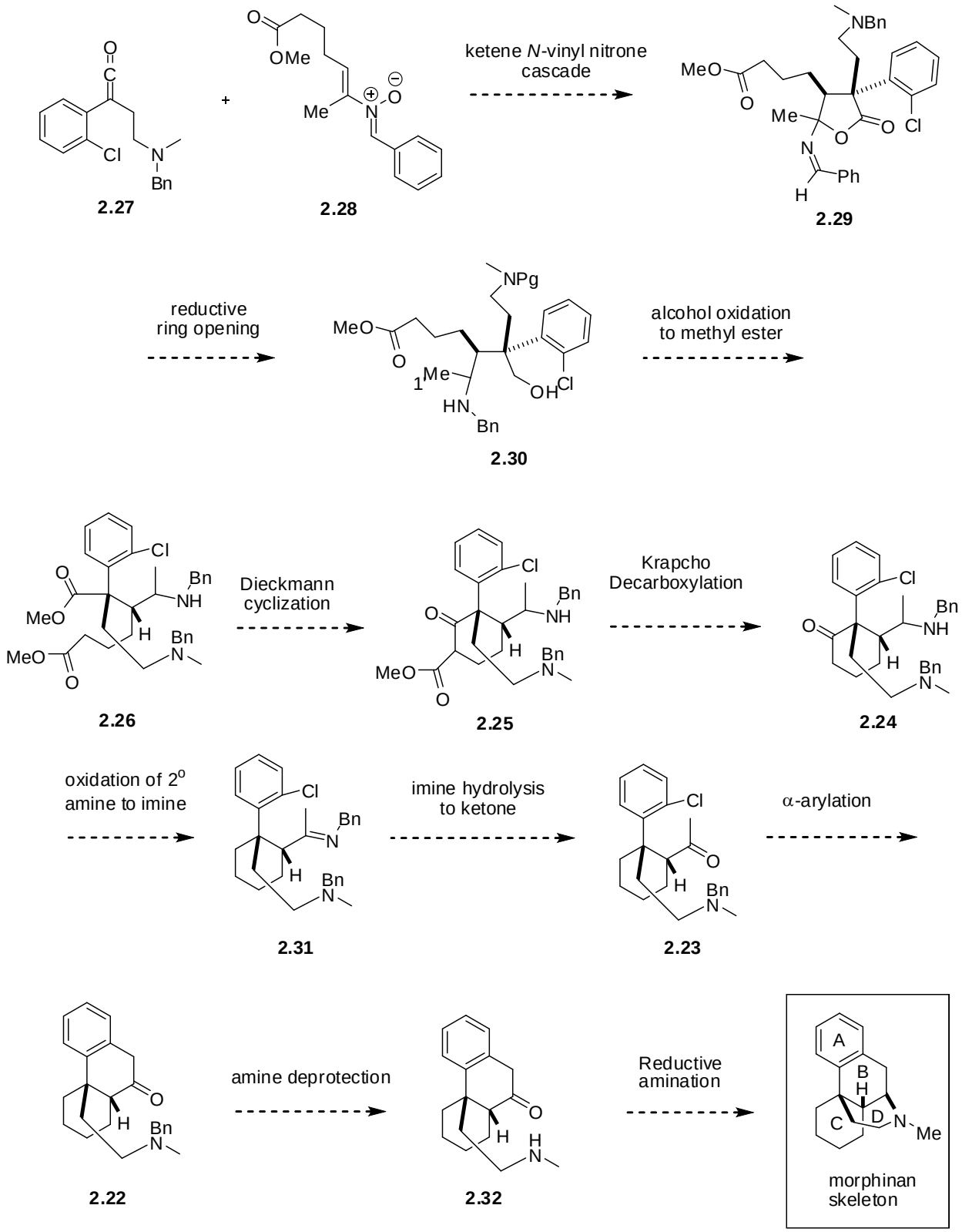
Scheme 2.3 Retrosynthesis of Morphinan Skeleton via Ketene/N-vinylnitrone Cascade, benzylisoquinoline core of the morphinan skeleton is highlighted in blue, and includes all bonds with the exception of C-11 to C-12



In the forward direction, our proposed synthesis is as follows: reaction of ketene **2.27** and *N*-vinyl nitrone **2.28** would provide the cyclic hemiaminal lactone **2.29** (Scheme 2.4). This intermediate can be reduced with LAH to form structure **2.30**. The primary alcohol on **2.30** could

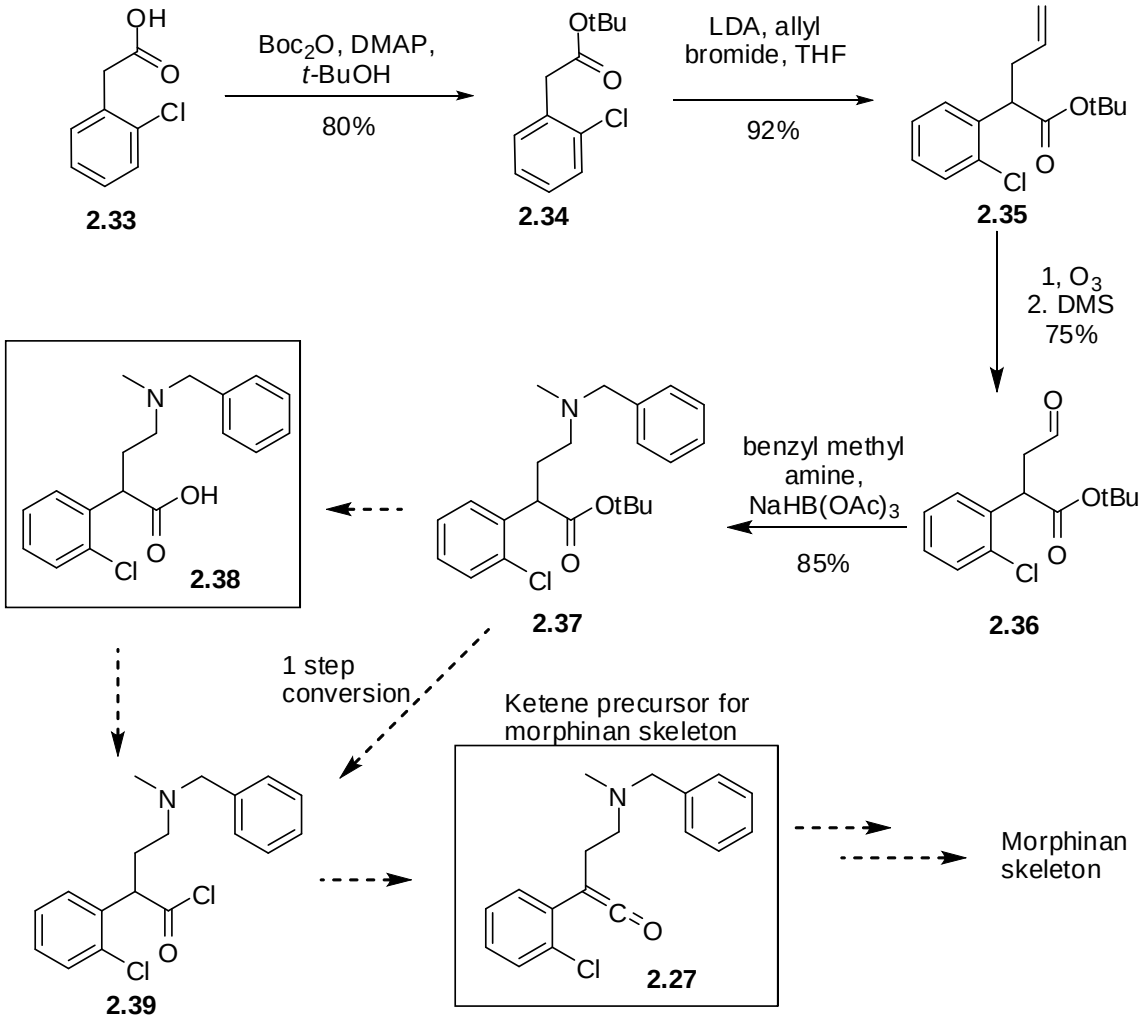
then be oxidized to the methyl ester directly via a heterogeneous Pd/Bi/Te catalyst in presence of O_2 in methanol to generate compound **2.26**.[19] This sets up the cyclization of ring C through a Dieckmann cyclization to form product **2.25**. This compound can then undergo a Krapcho decarboxylation followed by a Wolff-Kishner reduction to generate **2.24**. The secondary amine **2.24** could then be oxidized to the imine with IBX in DMSO to form structure **2.31**.[20] This would allow for an acidic hydrolysis to generate ketone **2.23**. The formation of ring B could then be accomplished via an α-arylation to form **2.22**.[21] After the formation of ring B, compound **2.22** could be deprotected and subjected to intramolecular reductive amination. This reaction can only form the desired diastereomer due to the geometric constraints of the ring system to provide the complete morphinan skeleton.

Scheme 2.4 Proposed Synthesis of the Morphinan Skeleton



This scheme required the ketene **2.27**, and **a**ttempts towards the synthesis of this compound from 2-chlorophenyl acetic acid (**2.33**) proceeded as follows (Scheme 2.5). The carboxylic acid was esterified to obtain *tert*-butyl ester **2.34** in 80% yield. Compound **2.34** was treated with LDA followed by allyl bromide to obtain terminal olefin **2.35** in 92% yield. Compound **2.35** was then subjected to ozonolysis conditions to provide aldehyde **2.36** in 75% yield. Reductive amination of aldehyde **2.36** (benzyl methyl amine/triacetoxyborohydride) provided amino ester **2.37** in 85% yield.

Scheme 2.5 Synthesis of Ketene Intermediate via a *tert*-Butyl Ester

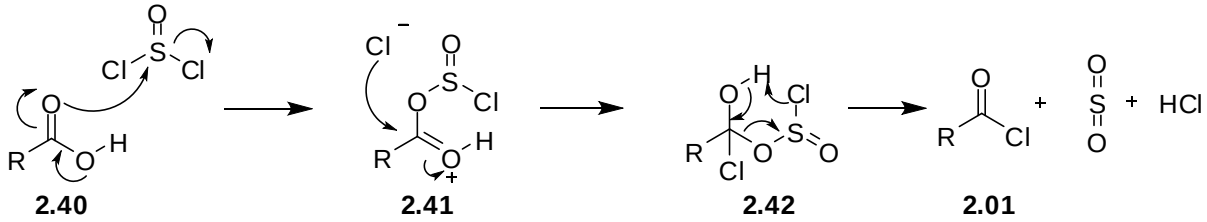


The next step involved the deprotection of the *tert*-butyl ester of **2.37** to provide the amino acid intermediate **2.38**. On initial attempts to deprotect compound **2.37** (TFA/DCM) were unsuccessful; upon deprotection, a complex mixture was observed. Later in the course of this project we discovered conditions that provide the products cleanly (TFA/H_2O); however, the difficulties we were having at the time led us to consider the direct conversion of *tert*-butyl ester **2.37** into acid chloride **2.39**. It is this *tert*-butyl ester to acid chloride conversion that is explored throughout the rest of this chapter.

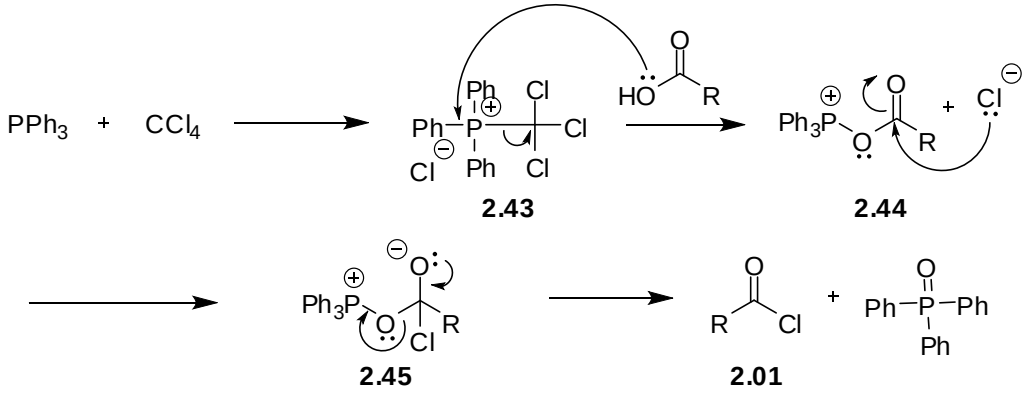
2.3 Preparation of Acid Chlorides from Carboxylic Acids

One of the most common chlorinating agents used to prepare acid chlorides from the corresponding carboxylic acid is thionyl chloride.[22] The mechanism begins with the attack of the carbonyl oxygen of the carboxylic acid (**2.40**) onto the sulfur of thionyl chloride, and displacement of a chloride ion (Scheme 2.6). The product (**2.41**) is subsequently attacked by the chloride at the carbonyl carbon to form tetrahedral intermediate **2.42**. Compound **2.42** then undergoes a pericyclic decomposition to generate SO_2, HCl gas, and the product acid chloride (**2.01**). Many chlorinating agents have been identified for this reaction; PCl_5, $POCl_3$, oxalyl chloride, phosgene, cyanuric chloride, activated triphenylphosphine reagents **2.43** (Scheme 2.7), or α,α-dichloro ethers **2.46** (Scheme 2.8), among others.[23] These reagents all react by a related mechanism which proceeds by, activation of the carboxylic acid (**2.44, 2.47**) followed by attack of the chloride to form a tetrahedral intermediate (**2.45**, **2.48**) which decomposes to form the product acid chloride **2.01**.

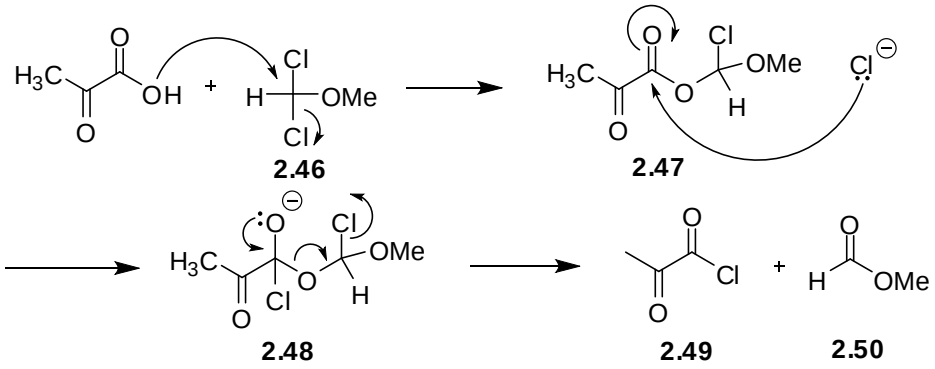
Scheme 2.6 Mechanisms of Acid Chloride Formation with Thionyl Chloride



Scheme 2.7 Triphenylphosphine and Carbon Tetrachloride Formation of Acyl Chlorides



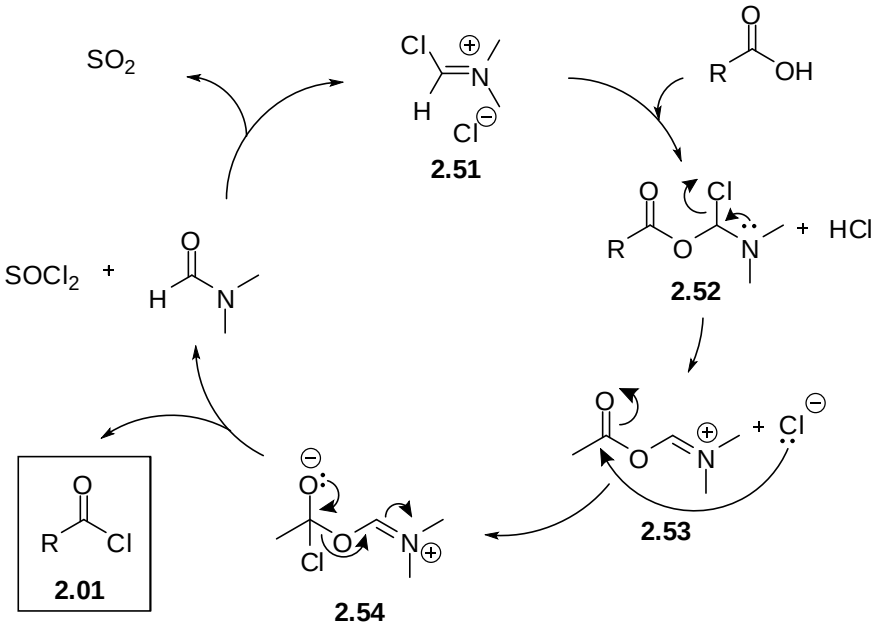
Scheme 2.8 α,α-Dichloromethyl Methyl Ether to Transform Pyruvic Acid to the Acid Chloride



Thionyl chloride reacts with a wide variety of carboxylic acid substrates; however, its reactivity is dependent on the nature of the acid. It was found that when the carboxylic acid is more acidic the reaction proceeds at a slower pace or, in some cases, does not proceed.[23] To

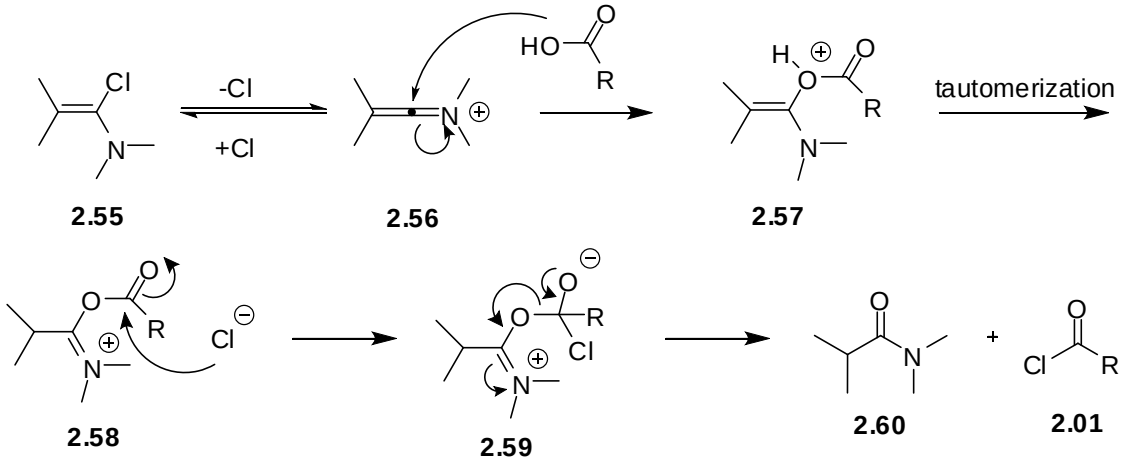
drive the reaction to completion, acid chloride formation can be catalyzed by the addition of DMF to generate the Vilsmeier-Haack reagent **2.51**,[24] which serves as the active chlorinating agent (Scheme 2.9). The Vilsmeier-Haack reagent creates a more electrophilic carbonyl compared to thionyl chloride, thereby allowing this transformation to proceed with less reactive substrates, including the more acidic carboxylic acids mentioned above.[24]

Scheme 2.9 Activation and Catalytic Cycle of Carboxylic Acids with Vilsmeier-Haack Reagent



This procedure works well for the conversion of less reactive carboxylic acids to acyl chlorides, while it is not ideal due to the generation of HCl as a by-product which can cause problems on substrates with acid-sensitive functional groups. To avoid this, the Ghosez reagent, tetramethyl-α-chloroenamine (**2.55**), was developed. This reagent allows the reaction to proceed without the generation of HCl and can be employed in place of DMF (Scheme 2.10).[25]

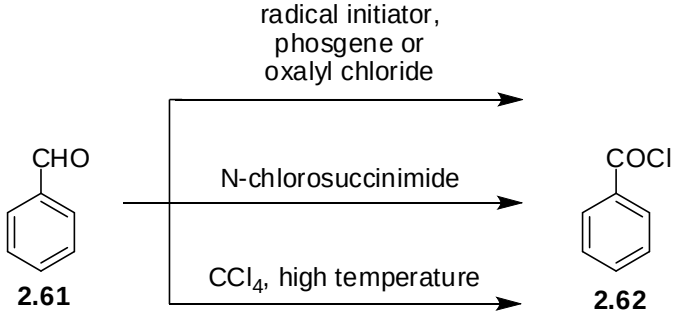
Scheme 2.10 Acid Chloride Formation in Neutral Conditions Using the Ghosez Reagent



2.4 Acyl Chloride Formation Using Aldehydes and Alkenes

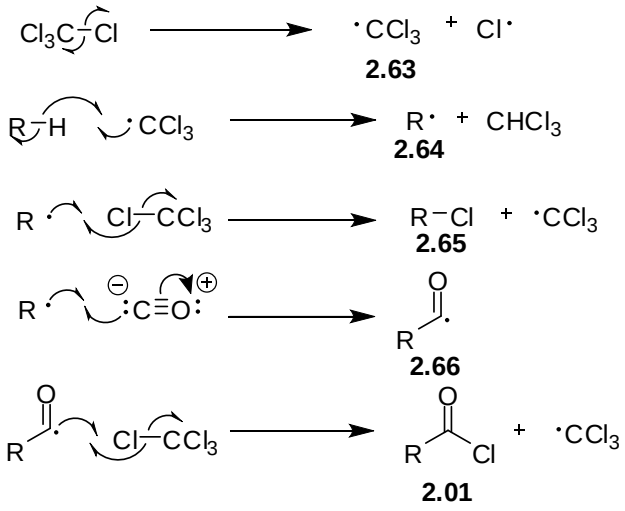
While carboxylic acids are perhaps the best-known functional group that can be transformed into acid chlorides, the transformation of several other functional groups to acid chlorides have also been studied and used by several research groups for decades. In 1832, Liebig and Wohler were successful in the transformation of benzaldehyde **2.61** to benzoyl chloride **2.62**.[23,26] Although not much about the reaction was known then, we now understand the reaction proceeds via a free-radical mechanism and can be employed with most radical halogen sources, such as ethyl hypochlorite,[27] or *tert*-butylhypochlorite.[28]

Scheme 2.11 Benzaldehyde Directly to Benzoyl Chloride, using radical initiators, NCS, or CCl_4 at high temperatures



Although attempts to form benzoyl chloride from benzaldehyde were successful, few examples of the conversion of aldehydes directly to acid chlorides have been reported. The scope of this reaction is relatively small and if the aryl ring is too electron deficient or too electron rich, the reaction fails.[23] This may, in part, be due to the carbon monoxide extrusion known to occur with carbonyl radical species **2.66** (Scheme 2.12).[29] Benzoyl chloride can also be prepared from benzaldehyde in carbon tetrachloride, however this requires very high temperatures, 160-205°C, and the reaction is incompatible with alkyl side chains around the aromatic ring, as they will be halogenated in this process.[30] It has also been reported that this transformation can proceed through the use of *N*-chlorosuccinimide, although similarly this reagent can cause a variety of side reactions on substrates with reactive functional groups.[31] Acyl chlorides may be obtained from alkanes and cycloalkanes through a radical chain reaction with CCl_4 and carbon monoxide.[32] The scope of this reaction is limited as this reaction generates a variety of side products, however the reaction can still be employed successfully on relatively simple or symmetric starting materials.[23,32]

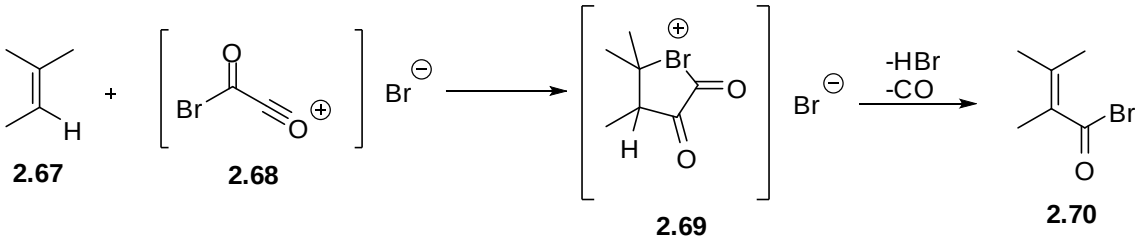
Scheme 2.12 Radical Chain Reaction to Form Acid Chlorides, note that step 4 is a carbonyl radical which can also decompose into an alkyl radical and carbon monoxide



2.5 Acid Halide Formation through Halocarbonyl Addition

In addition to functional group transformations to form acid chlorides the entire carbonyl halide moiety can be inserted into a molecule. For example, cyclohexane can be converted into the cyclohexanecarbonyl chloride in 30% yield, using oxalyl chloride, in the presence of a radical initiator. This reaction is not employed regularly as the regiospecificity and yields are both poor (6-50% yields).[33] The scope of the reaction is also limited as simple straight chain alkanes do not react. Other than simple cycloalkanes the only other functional group capable of the addition of a halo carbonyl group is an alkene. However, the yields of the reaction with alkenes is also poor and are not regioselective. The similar reaction of oxalyl bromide has been reported and the mechanism proposed proceeds through a 5-membered ring intermediate **2.69** (Scheme 2.13).[34]

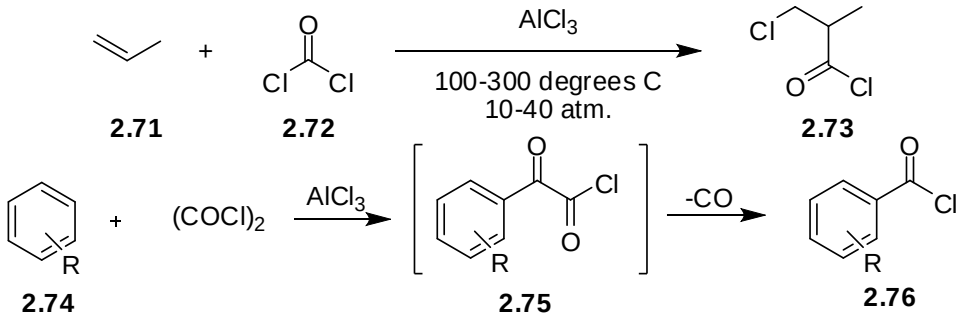
Scheme 2.13. Halocarbonyl Addition to a Double Bond



2.67 **2.68** **2.69** **2.70**

At high temperatures and pressures alkenes and the use of aluminum trichloride can also react with phosgene to form chloro-acid chlorides.[35] Along with insertion to alkenes it is also possible to add a chlorocarbonyl to substituted benzene, naphthalene, or anthracene rings without the need for high temperatures and pressures, and without the need for the toxic reagent, phosgene.[2] This reaction has its drawbacks as the product can further react to form benzophenone derivatives.

Scheme 2.14. Alkene and Aromatic Addition of Chlorocarbonyl Moiety

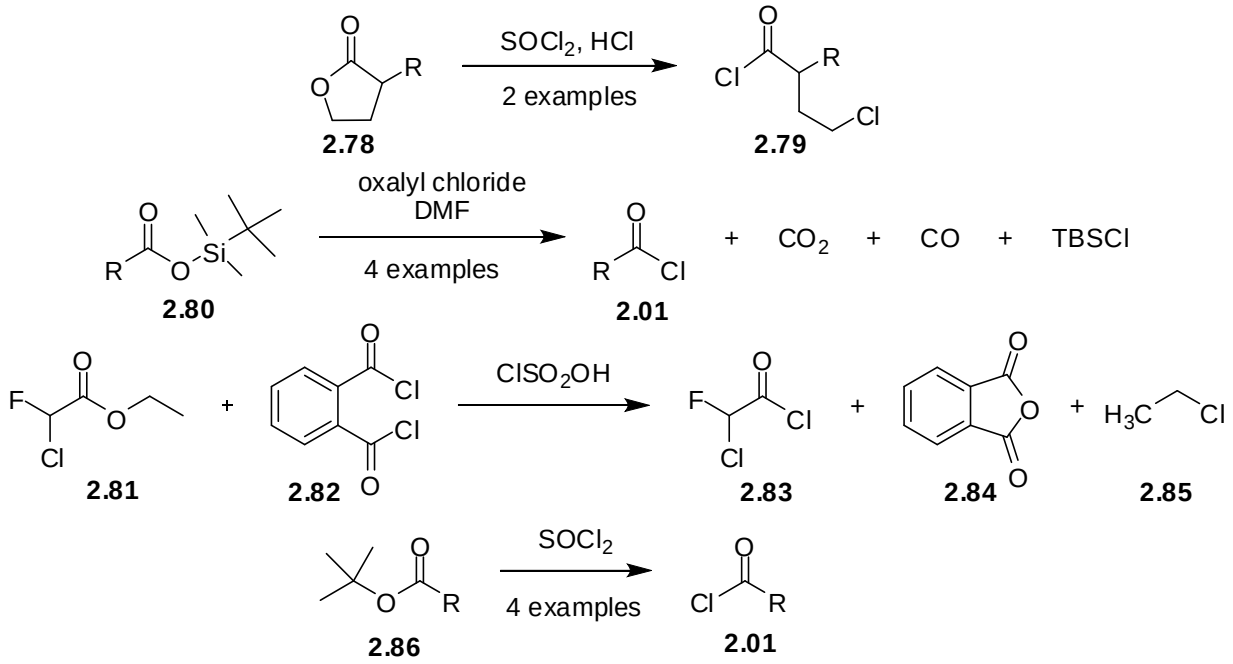


2.6 Making Acid Chlorides from Esters, Silyl Esters, and Lactones

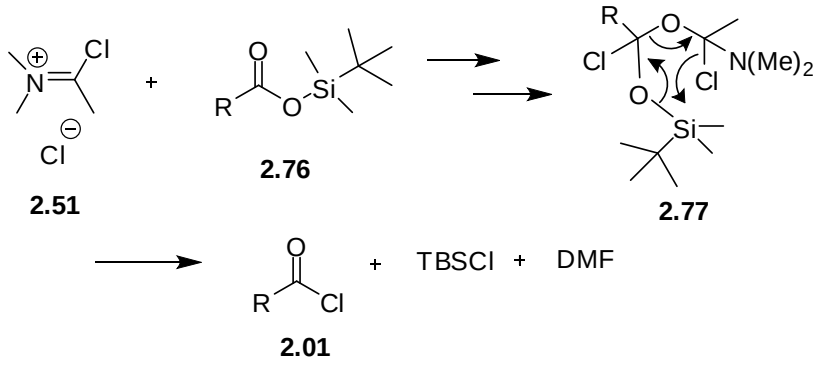
Other than the widely known conversion of carboxylic acids to acid chlorides there are relatively few instances in the literature of other functional group transformations that can yield acid chlorides directly. It has been challenging for chemists to transform other heteroatom carbonyl species, in particular esters, into acid chlorides.[23] Most of these methods employ harsh conditions and only work on a limited set of substrates. Examples include the conversion of two different substituted lactones to form the *in situ* chloro-acid chlorides **2.79** (Scheme 2.16).[36]

Ethyl chlorofluoroacetate **2.81** was converted in a 50% yield to the corresponding acid chloride **2.83** through the use of chlorosulfonic acid and phthaloyl chloride **2.82** (Scheme 2.16).[37] *Tert*-butyldimethylsilyl esters (**2.80**) are transformed to acid chlorides using oxalyl chloride in DMF, taking advantage of the Vilsmeier-Haack reagent (**2.51**) as the reaction rate is significantly slower without the use of DMF (Scheme 2.15).[38] Examples of the conversion of *tert*-butyl esters (**2.86**) to acid chlorides are limited in the literature.[39] In all these cases, $SOCl_2$ was used without any investigation of the mechanism or scope of the reaction (Scheme 2.16). This was the basis for our investigation into the transformation of esters into acid chlorides.

Scheme 2.15 Carbonyl Heteroatom Transformation into Acid Chlorides



Scheme 2.16 Proposed Mechanism of *tert*-Butyldimethyl Silyl Ester to Acid Chloride



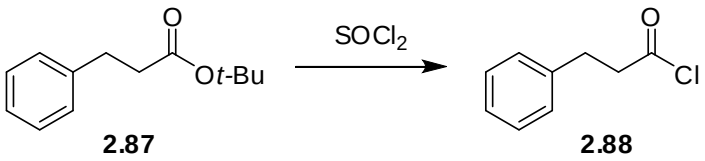
2.7 Initial Studies of the Conversion of *tert*-Butyl Esters to Acid Chlorides

To study the transformation of *tert*-butyl esters to acid chlorides a model substrate, *tert*-butyl dihydrocinnamate (**2.87**) was synthesized. This substrate was subjected to a variety of reaction conditions for the conversion to dihydrocinnamoyl chloride (**2.88**) as described in Table 2.1. The reaction was run at room temperature in a sealed vial, and it was found that after 30 minutes there was a 9% conversion to the acid chloride (Table 2.1, entry 1). After 16 h in a sealed

vial at room temperature it was found that the reaction had an 86% conversion (Table 2.1, entry 2). It is known that thionyl chloride contains trace amounts of hydrogen chloride among other impurities,[40] and we sought to determine if this contaminant was useful in the reaction.

In the presence of 1 equiv of water, which reacts with $SOCl_2$ to provide HCl and SO_2, the reaction was found to proceed to completion in 30 min at room temperature in a sealed vial (Table 2.1, entry 3). On larger scale sealed vessels are less convenient, in particular with reactions that evolve gas, and as such we studied this reaction open to the atmosphere, with water as an additive (Table 1, entries 4-6). The reactions performed open to the atmosphere were qualitatively slower, requiring 16 h to proceed to completion (Table 2.1, entries 5 and 6). We attribute the decreased rate of reactions in open vessels to the loss of HCl from the mixture, leading to lower concentrations of acid than in sealed vessels from which the HCl cannot escape (*We note that the buildup of gasses in the sealed vessels requires caution when opening the vessels upon completion of the reaction!*). This reaction is amenable to scale up and provides similar results on 20 mmol scale when conducted open to the atmosphere in the presence of 1 equiv HCl (a concentrated aqueous solution was used; 91% yield after purification by distillation; Table 2.1, entry 6).

Table 2.1 Optimization of *tert*-Butyl Ester to Acid Chloride Conditions



2.87 **2.88**

Entry	Conditions[a]	Time	Yield[b]
1	Sealed, no additives	0.5 h	9%[c]
2	Sealed, no additives	16 h	83%
3	Sealed, H_2O	0.5 h	91%
4	Open, H_2O	1 h	60%[c]
5	Open, H_2O	16 h	90%
6	Open, HCl, 20 mmol scale	16 h	91%[d]
7	Sealed, 2,6-di-*tert*-butyl-4-methylpyridine	16 h	0%[c]

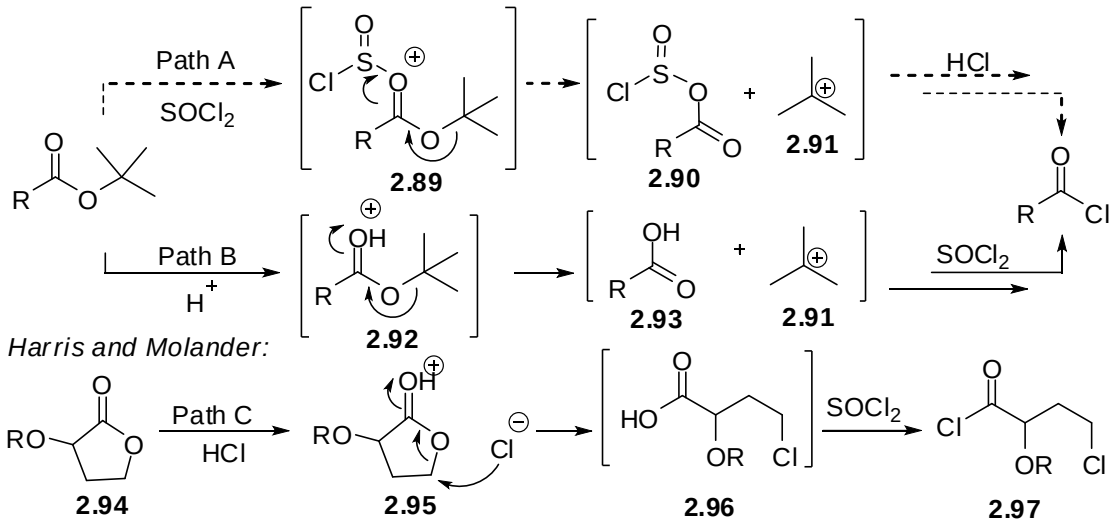
[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The ester (0.5 mmol, 1 equiv) was dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with additive (1 equiv), then sealed (or left open as indicated) and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. [b]Yield refers to isolated crude product unless otherwise indicated. [c]Percent conversion was calculated from the ratio of Product:SM by ^{1}H NMR. [d]Yield refers to product isolated after vacuum distillation; 1 equiv of HCl and 20 equiv $SOCl_2$ were used in this experiment.

2.8 Mechanism studies

Two mechanistic options for this reaction were considered, one in which the ester carbonyl is activated by $SOCl_2$ via complexation (**2.89**) followed by loss of *tert*-butyl cation (**2.91**) (Scheme 1, path A), the other in which the ester carbonyl is protonated (**2.92**), also resulting in loss of *tert*-butyl cation (Scheme 1, path B).[41] The resulting intermediates, **2.90** and **2.93**, would then undergo chlorination with either HCl (path A) or $SOCl_2$ (path B). The addition of a non-nucleophilic base, 2,6-di-*tert*-butyl-4-methylpyridine, can provide evidence in determining which of these mechanisms is most likely. In the presence of this reagent, no reaction is observed after 16 h (Table 2.1, entry 7), suggesting that HCl is playing a crucial role in the reaction, consistent with path B.

Note that this is mechanistically distinct from the $SOCl_2$-mediated chlorination of lactones (**2.94**), first described by Harris and Molander, which likely proceeds via an S_N2 pathway to provide a chloro-acid, such as **2.96**, that subsequently undergoes chlorination (Scheme 1, path C).[36]

Scheme 2.17 Path A describes the activation via thionyl chloride, Path B describes the mechanism via activation with acid, and Path C describes the proposed mechanism of the lactone opening

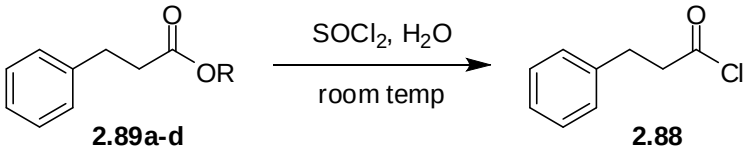


2.9 Selectivity of the Reaction for *tert*-Butyl Esters

To explore the selectivity of the reaction, the methyl, ethyl, isopropyl, and benzyl dihydrocinnamate esters were synthesized. Each was exposed to the optimized reaction conditions for *tert*-butyl esters (H_2O, sealed vials, 0.5 h), as well as with extended reaction times to 16 h. After 30 minutes, no conversion to the acid chloride with any substrates tested was observed (Table 2.2, entries 1-4). With an extended reaction time to 16 h no conversion to product was observed, with the exception of the benzyl ester, which provided 1% product after 16 h (Table 2.2, entry 4). These results show that the reaction is highly selective for *tert*-butyl esters under the optimized conditions. However, the 1% conversion of the benzyl ester is of note, because this shows that the

reaction does proceed to some extent albeit at a much slower rate, and this must be taken into consideration when employing this reaction in the presence of benzyl esters.

Table 2.2 Ester Selectivity with Optimized *tert*-Butyl Ester Conditions[a]



2.89a-d **2.88**

entry	R	Conversion at 30 min	Conversion[b] at 16 h
1	Me (**2.89a**)	NR	NR
2	Et (**2.89b**)	NR	NR
3	*i*-Pr (**2.89c**)	NR	NR
4	Bn (**2.89d**)	NR	1%

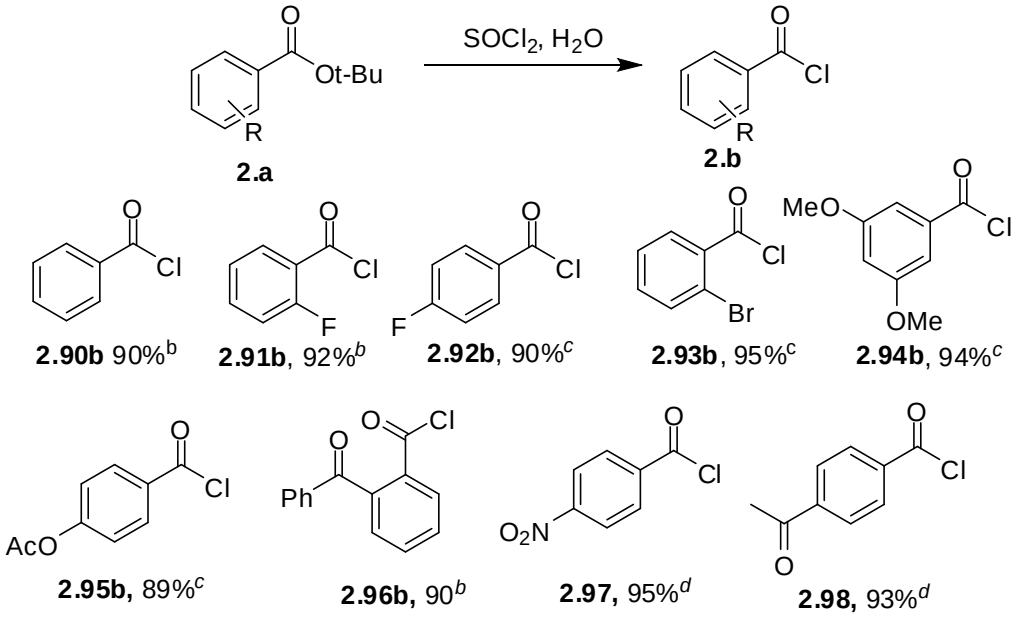
[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The ester (0.5 mmol, 1 equiv) was dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with water (1 equiv), then sealed and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. [b]Percent conversion was calculated from the ratio of Product:SM by ^{1}H NMR.

2.10 Scope of the Reaction

Once it was determined that the reaction conditions are selective for *tert*-butyl esters, the scope of the reaction was studied, and a variety of *tert*-butyl esters were synthesized (Table 2.3 and 2.4). In addition to the aliphatic dihydrocinnamoyl chloride, it was found that aromatic acid chlorides can be prepared from electron poor and electron rich aryl *tert*-butyl esters (**2.90a**-**2.98a**). Aromatic acid chlorides were found to require longer reaction times, and it was found if the reaction was subjected to work-up early, the intermediate carboxylic acid was isolated along with the acid chloride. This indicates that when the carboxylic acid is electron poor, the formation of the acid chloride from the carboxylic acid is the rate-limiting step in the mechanism. We found that strongly electron deficient substrates (*p*-acetyl benzoate and *p*-nitro benzoate esters **2.95a** and **2.97a**), provided complete conversion to the corresponding carboxylic acid at room temperature,

but required heating to 100°C in toluene to provide the corresponding acid chlorides, **2.95b** and **2.97b**.

Table 2.3 Scope of Chlorination of Aromatic *tert*-Butyl Esters[a,b]



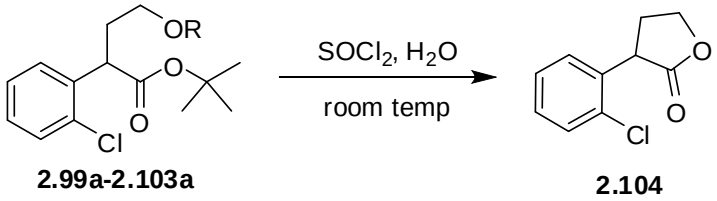
[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The ester (0.5 mmol, 1 equiv) was dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with water (1 equiv), then sealed and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. Yields are of isolated crude products without further purification. [b]Reaction was stirred at 23 °C for 5 h. [c]Reaction was stirred at 23 °C for 16 h. [d]Reaction was performed in toluene (0.1 M) in a sealed 2 dram (7.4 mL) vial at 100 °C for 16 h

Aliphatic *tert*-butyl esters were synthesized with a variety of functional groups (Table 2.5). Most of the aliphatic substrates tested were found to completely react in under 30 minutes (**2.107a-2.112a**). However, it was found that several substrates required extended reaction times for complete conversion at room temperature (**2.113a-116a**). This extended reaction time is likely due to steric, as well as, electronic effects associated with the functional groups, in particular those that can become coordinated with the carbonyl either an intra- or intermolecular fashion. The compounds requiring additional time each contain an additional carbonyl or sulfonyl group that

may play a role in the electronic effects, and they all contain a relatively bulky functional group, tosylate, Cbz, Fmoc, and pivolate, which may be responsible for steric constraints.

The reaction conditions were found to be compatible with terminal olefins (**2.110a**), phthalate protected amino acids (**2.108a**), and acetyl functional groups (**2.109a**). This acetyl functional group is particularly useful as it shows that the reaction is selective for *tert*-butyl ester in the presence of a secondary ester. The subjection of γ-hydroxy ester **2.99a** to $SOCl_2$ provides the corresponding lactone **2.104** (Table 2.4, entry 1), suggesting that the rate of acid chloride formation and lactonization is faster than chlorination of the starting alcohol or of the product lactone. Protection of the alcohol **2.99a** as ethers, either benzyl or silyl, also provided the lactone **2.104** (Table 2.4, entry 2-5). Acid catalyzed deprotection of silyl ether is well known[42] and we speculate that either the silyl ethers underwent deprotection to reveal the alcohol which then went on to provide the lactone faster than the reaction with $SOCl_2$, or that the silyl ether attacks the acid chloride to provide a siloxonium species that undergoes desilylation (Scheme 2.18). It was surprising that the benzyl ether was not retained under these conditions as it is not expected that a benzyl ether would be deprotected to an alcohol under our conditions. The proposed mechanism for this transformation is via attack of the benzyl ether onto the acid chloride to provide activated oxonium intermediate (**2.107**). Attack of the Cl^- onto **2.107** would then provide the lactone and the benzyl chloride (Scheme 2.18).

Table 2.4 Sequential Chlorination, Oxonium Formation, and Lactonization[a]

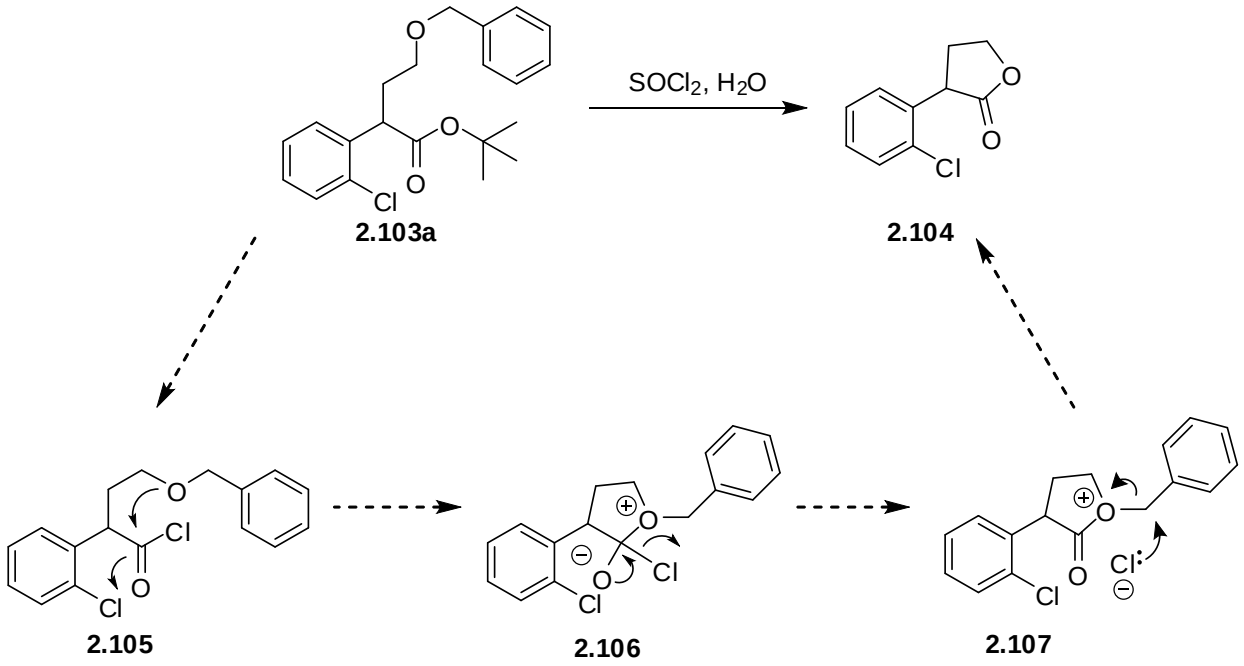


2.99a-2.103a **2.104**

Entry	Compound	R=	Yield[b]
1	**2.99a**	H	98%
2	**2.100a**	TES	84%
3	**2.101a**	TBS	97%
4	**2.102a**	TIPS	97%
5	**2.103a**	Bn	94%

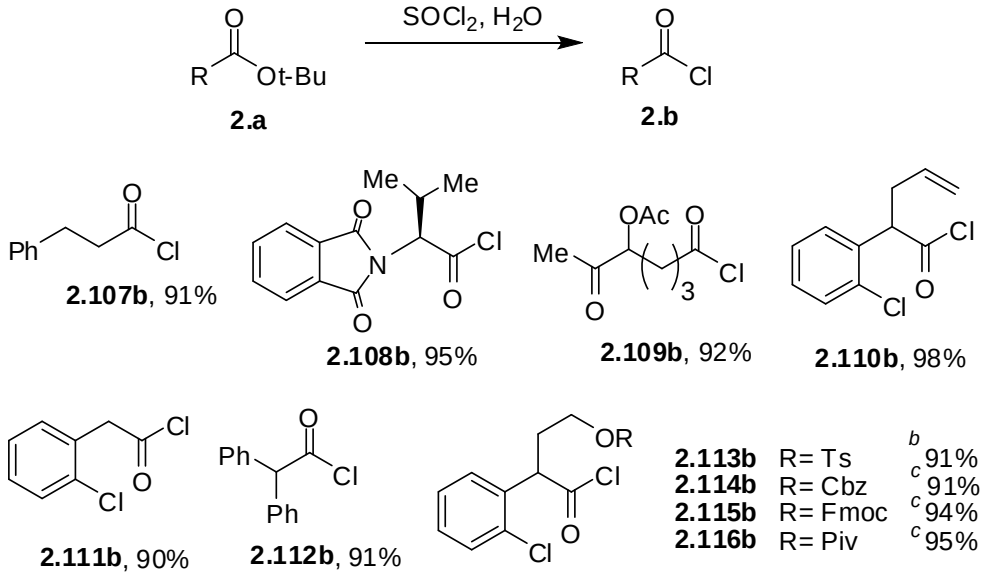
[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The ester (0.5 mmol, 1 equiv) was dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with water (1 equiv), then sealed and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. [b]Yields are of isolated crude products without further purification.

Scheme 2.18 Proposed Mechanism for the Transformation of the Benzyl Ether into the Lactone



To circumvent this, we prepared derivatives that are less prone forming oxonium structure **1.07** and less prone to attack by halide if they do form such oxonium intermediates. We found that protection of the γ alcohol as the tosylate, pivalate, CBZ, or Fmoc carbonate provides the desired acid chlorides after azeotropic distillation of thionyl chloride with toluene (2x) (Table 3, **2.113b-116b**). It is of note all of the examples of the protection of the alcohol group the lone pair on oxygen is conjugated to the sulfone or carbonyl.

Table 2.5 Scope of Aliphatic *tert*-Butyl Esters to Acid Chlorides[a]



[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The ester (0.5 mmol, 1 equiv) was dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with additive (1 equiv), then sealed and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. Yields are of isolated crude products without further purification. [b]Reaction was stirred at 23 °C for 5 h. [c]Reaction was stirred at 23 °C for 16 h.

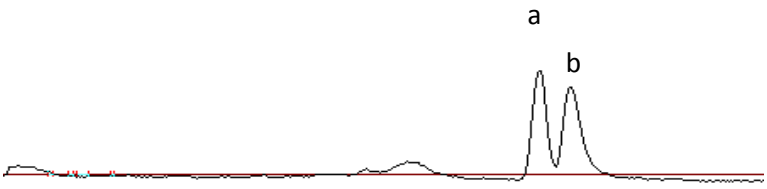
2.11 Stereochemical Integrity under Our Reaction Conditions

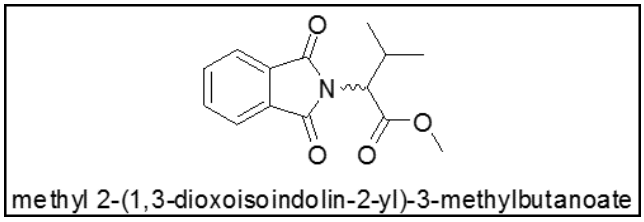
With the scope of compatible functional groups established, the stereochemical integrity of stereocenters α-to the carbonyl was of interest and was studied under our standard reaction conditions. Compound **2.108a**, derived from D-valine, was prepared and subjected to the reaction conditions to provide acid chloride **2.108b**, which was then immediately and carefully converted to the corresponding methyl ester mild under basic conditions (**2.108c**; methanol, 10 equiv; Hünig's base, 1.5 equiv; DCM) to ensure no racemization of the stereocenter in the subsequent step. We observed the product in a 98.4 to 1.6 ratio of enantiomers, indicating that there is minimal erosion of stereochemistry at the α-center in this substrate.

Figure 2.3 HPLC Data for Stereochemical Integrity (a) Racemic valine methyl ester HPLC trace (b) Enantiopure valine methyl ester HPLC trace

(a) Racemic
1.108c

Concentration	1 mg/mL	
Signal	254 nm	
Elution	1 mL/min	
Solvent	1:10	iPrOH:hexanes

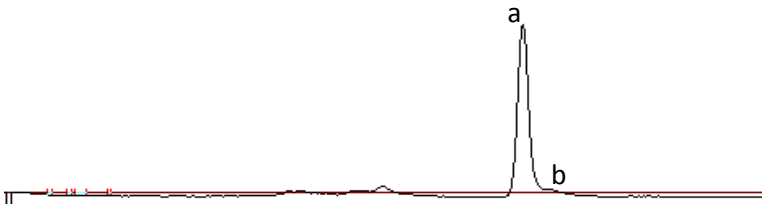


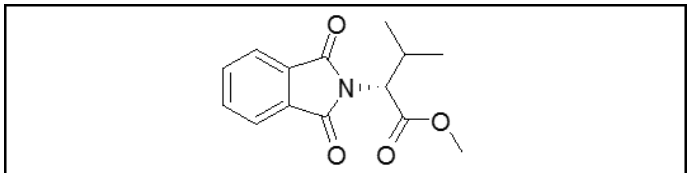


Component	Retention	Area	Height	Area %
a	29.986	1442.0986	30.143	51.53702
b	31.71	1356.0816	23.524	48.46298

(b) Enantiopure-
2.108c

Concentration	1 mg/mL	
Signal	254 nm	
Elution	1 mL/min	
Solvent	1:10	iPrOH:hexanes



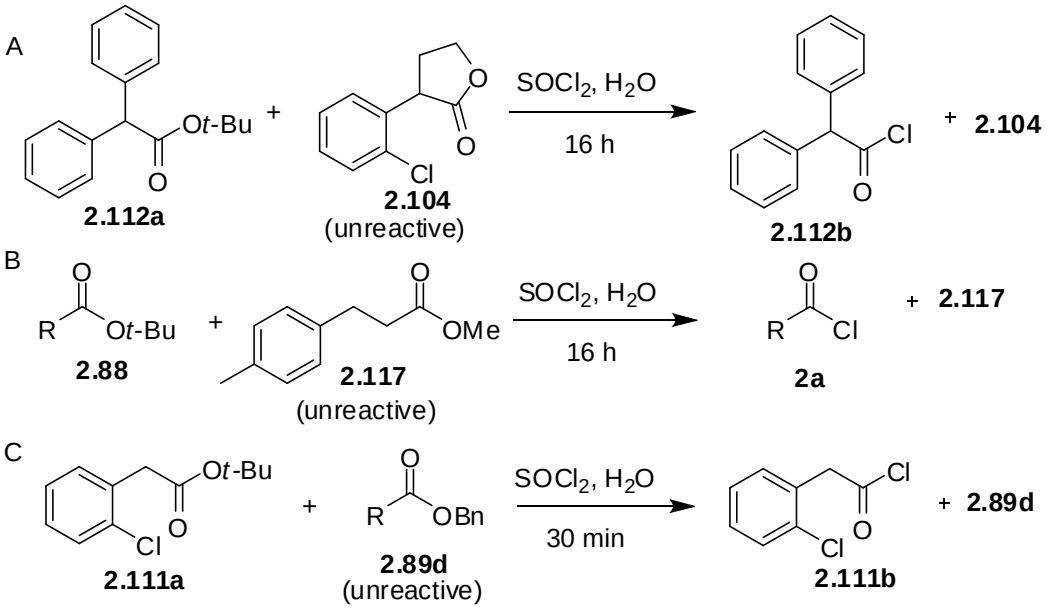


Component	Retention	Area	Height	Area %
a	26.09	2718.716	59.476	98.37514
b	27.216	44.905	2.502	1.624861

2.12 Competition Experiments

The selective conversion of *tert*-butyl esters in the presence of other esters was of interest and competition experiments were performed between substrates bearing *tert*-butyl esters, and substrates with either a lactone, a methyl ester, or a benzyl ester (Scheme 2.19). In all cases, we obtained complete conversion of the *tert*-butyl ester substrates with no observed acid chloride from the lactone, methyl, or benzyl ester substrates. The benzyl ester competition experiment (Scheme 2.19C) was only run for 30 minutes to avoid the slow conversion of benzyl esters into acid chlorides with $SOCl_2$.

Scheme 2.19 Competition Experiments[a,b]



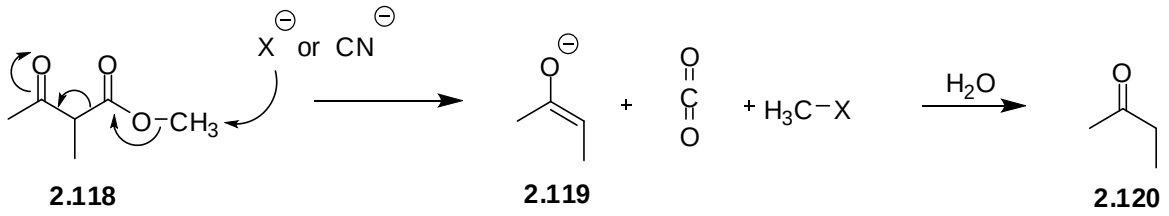
[a]Sealed reactions were performed in 2 dram (7.4 mL) vials containing a stir bar and capped with a teflon-lined cap. The *tert*-butyl ester (0.5 mmol, 1 equiv) and other ester or lactone (0.5 mmol, 1 equiv) were dissolved in $SOCl_2$ (5.0 mmol, 10 equiv), treated with water (1 equiv), then sealed and stirred for the time specified. The vials were then carefully opened, and $SOCl_2$ was removed via azeotropic distillation (2x) with toluene. [b]Conversion was determined by ^1H NMR.

2.13 Conversion of Methyl Esters into Acid Chlorides

Having successfully determined conditions for the selective transformation of *tert*-butyl esters into their subsequent acid chlorides, our efforts were then directed towards the transformation of methyl esters into acid chlorides. When thinking about different types of demethylation mechanisms, one in particular came to mind, the Krapcho decarboxylation that we plan employ in the total synthesis of the morphinan skeleton. The Krapcho decarboxylation proceeds through the use of inorganic salts, such as lithium chloride or sodium cyanide, to promote an S_N2-type reaction involving nucleophilic attack at the methyl carbon of the ester as the initial step (Scheme 2.20). This initial attack causes the decarboxylation process to occur, which may

proceed in a concerted fashion with nucleophilic substitution, which produces an anionic intermediate that is subsequently protonated and tautomerized to afford the final product.[43]

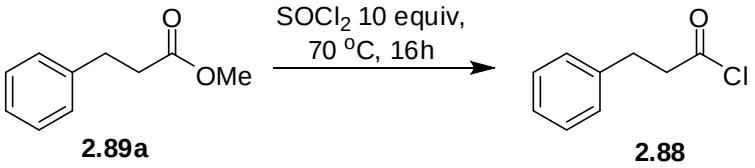
Scheme 2.20 The Krapcho Decarboxylation Mechanism



2.118 **2.119** **2.120**

The initial attack of the methyl group could be employed on methyl esters, and this hypothesis was pursued. Already aware that thionyl chloride with HCl has been employed for a lactone openings and sequential chlorination, [36] we employed these procedures on our methyl ester and were encouraged to find that with a drop of HCl in thionyl chloride we obtained 8% conversion to the corresponding acid chloride overnight (Table 2.6, entry 3). It was reasoned that if the mechanism involves S_N2 displacement similar to the Krapcho mechanism, the addition of the chloride could facilitate the reaction and a variety of chloride sources were screened.[44] The group I salts were not completely soluble in thionyl chloride and did not improve our conversion (Table 2.6, entries 4-7). *Tert*-butyl ammonium chloride (TBAC) and TBAC hydrate were both found to be soluble in thionyl chloride. Surprisingly, only the TBAC hydrate salt was effective and provided the acid chloride in 62% conversion overnight at 70°C. (Table 2.6, entries 8). We were also able to promote the reaction via the use of anhydrous TBAC with added water, and under these conditions we were able to obtain complete conversion to the acid chloride after 1 week (Table 2.6, entry 11).

Table 2.6 Methyl Ester Optimization Conditions[a]



2.89a **2.88**

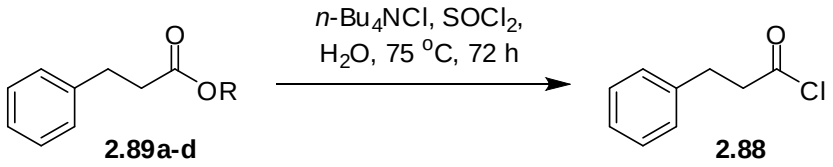
Entry	Conditions	Conversion[b]
1	no additives	>1%
2	H_2O	4%
3	HCl	8%
4	HCl, LiCl	8%
5	HCl, NaCl	8%
6	HCl, KCl	5%
7	HCl, CsCl	7%
8	HCl, *n*-BuN_4Cl[c]	62%
9	*n*-BuN_4Cl[d]	>1%
10	H_2O, *n*-BuN_4Cl[d]	43%[c]
11	H_2O, *n*-BuN_4Cl[d,e]	100%

[a]Reactions were performed in sealed 2 dram vials using 1 equiv of ester and 1 equiv of water or concd HCl, and 2 equiv mmol salt at 70°C for 16 h. Reactions were then diluted with toluene. $SOCl_2$ was then removed via azeotropic two times with toluene prior to NMR analysis. [b]Conversion refers to the ratio of SM:Prod as determined by ^1H NMR. [c]The hydrate of *n*-Bu_4NCl was used. [d]Anhydrous *n*-Bu_4NCl was used. [e]Reaction was run for 1 week in sealed tube at 70°C.

2.14 Conversion of Other Esters to Acid Chlorides with TBAC

Methyl, ethyl, isopropyl and benzyl esters were subjected to the reaction conditions and we were not surprised to see that all of the esters showed some conversion to the acid chloride. It was found that the ethyl ester was not as reactive as the methyl ester (Table 2.7, entry 1-2). This slower rate is expected if the reaction is proceeding via an S_N2 mechanism. However, the isopropyl group reacts faster than the ethyl group, which leads us to believe this is due to competing elimination and S_N2 mechanisms. The benzyl ester reacts at a faster rate in comparison to the other esters, and in this reaction benzyl chloride is also isolated which is consistent with the substitution mechanism for demethylation or debenzylation.

Table 2.7 Percent Conversion of Esters to Acid Chlorides Using Tetrabutyl Ammonium Chloride and Water to Chlorinate[a]



2.89a-d **2.88**

Entry	R	Conversion
1 (**2.89a**)	Me	77%[b]
2 (**2.89b**)	Et	7%
3 (**2.89c**)	i-Pr	30%
4 (**2.89d**)	Bn	97%

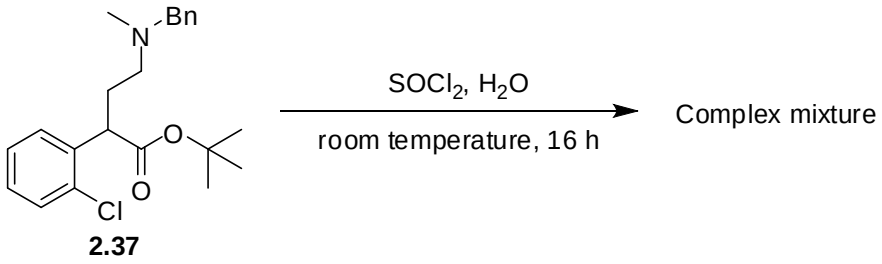
[a]Reactions were performed in sealed 2 dram vials using 1 equiv of ester (0.5 mmol), 1 equiv of water (0.5 mmol), and 2 equiv TBAC (1.0 mmol) at 75°C for 72 h. Reactions were then diluted with toluene. $SOCl_2$ was then removed via azeotropic two times with toluene prior to NMR analysis 0.5 mmol ester, 1.0 mmol of *n*-$Bu_{4N}Cl$, 0.5 mmol H_2O [b]Reaction goes to completion after 1 week.

Although, we are able to form acid chlorides from these esters, the reaction rates are much slower in comparison to the *tert*-butyl ester. Further, the isolation of the product is difficult due to the presence of TBAC, and the long reaction times and sealed reaction vessels make monitoring the reaction challenging. This can be particularly problematic if working with a substrate that has the potential for degredation. The *tert*-butyl ester to acid chloride transformation, on the other hand, proceeds quickly and cleanly at room temperature, open to the atmosphere, and the product can easily be isolated after excess thionyl chloride removal at reduced pressures.

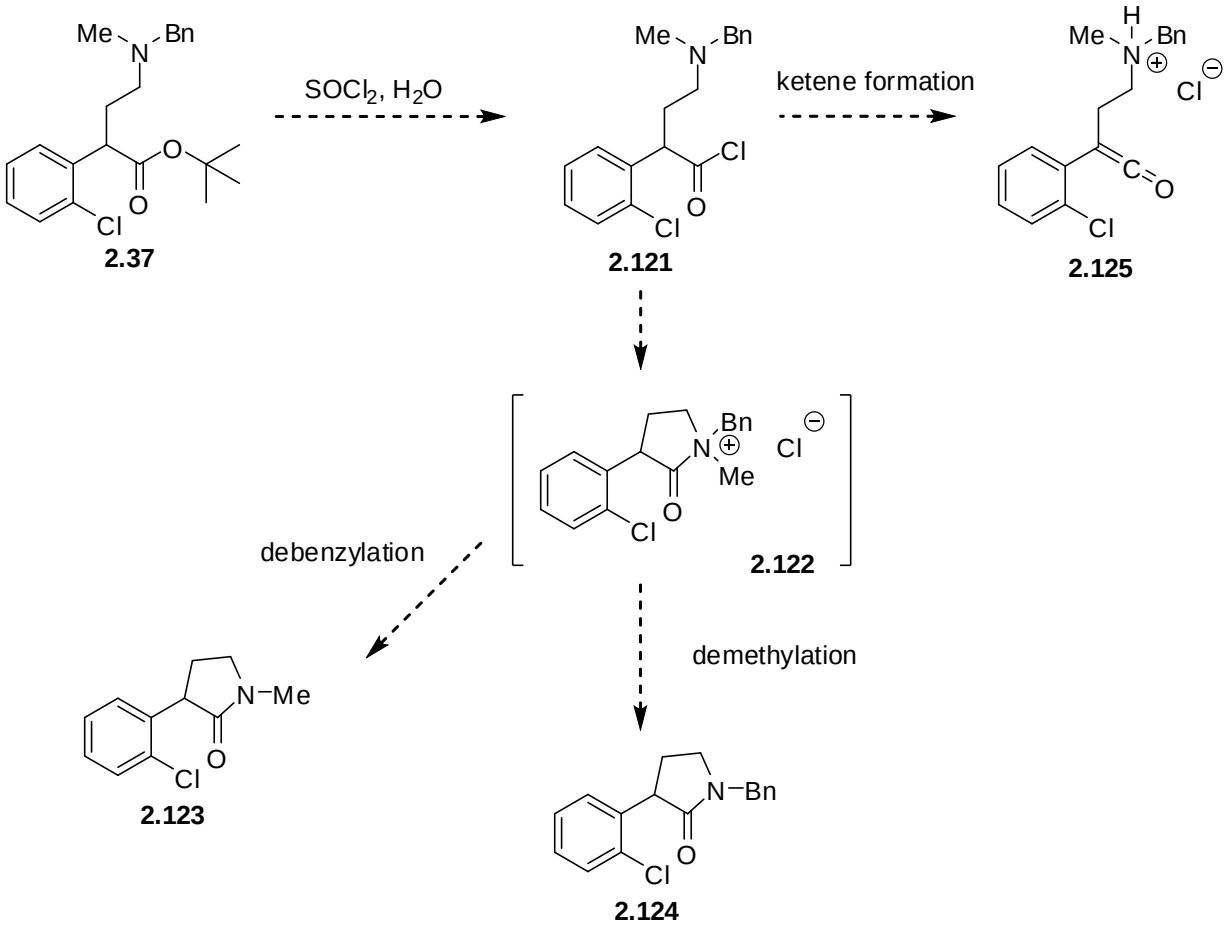
2.15 Future Directions

The synthesis towards the morphinan skeleton via the ketene/*N*-vinyl nitrone cascade via ketene shown in Scheme 2.4 was left off at the formation of *tert*-butyl ester **2.37**. The ketene requires the precursor acid chloride **2.39**, which can be formed via ester **2.37**. However, this reaction resulted in a complex mixture (Scheme 2.21). With a mechanistic understanding of the reaction of the benzyl ether deprotection to form lactones, we speculate that a similar mechanism may be occurring with the amine analog (Scheme 2.22). The formation of the acid chloride would provide compound **2.121** which would be susceptible to attack by the amine (when present as the free base) to provide acyl ammonium species **2.122**. Attack of the halogen can then occur either at the benzylic carbon or the methyl carbon to provide lactam **2.123** or **2.124** respectively. The reaction is further complicated with a tertiary amine moiety capable of behaving as an intramolecular base to provide ketene **2.125**. In short, this particular ketene (**2.30)** is problematic for the total synthesis of the morphinan skeleton. Given that the conversion of *tert*-butyl esters to acid chlorides is compatible with terminal olefins, a new synthesis utilizing this substrate **2.110b** is proposed (Scheme 2.23).

Scheme 2.21 Proposed Acid Chloride Formation for Morphinan Skeleton Synthesis

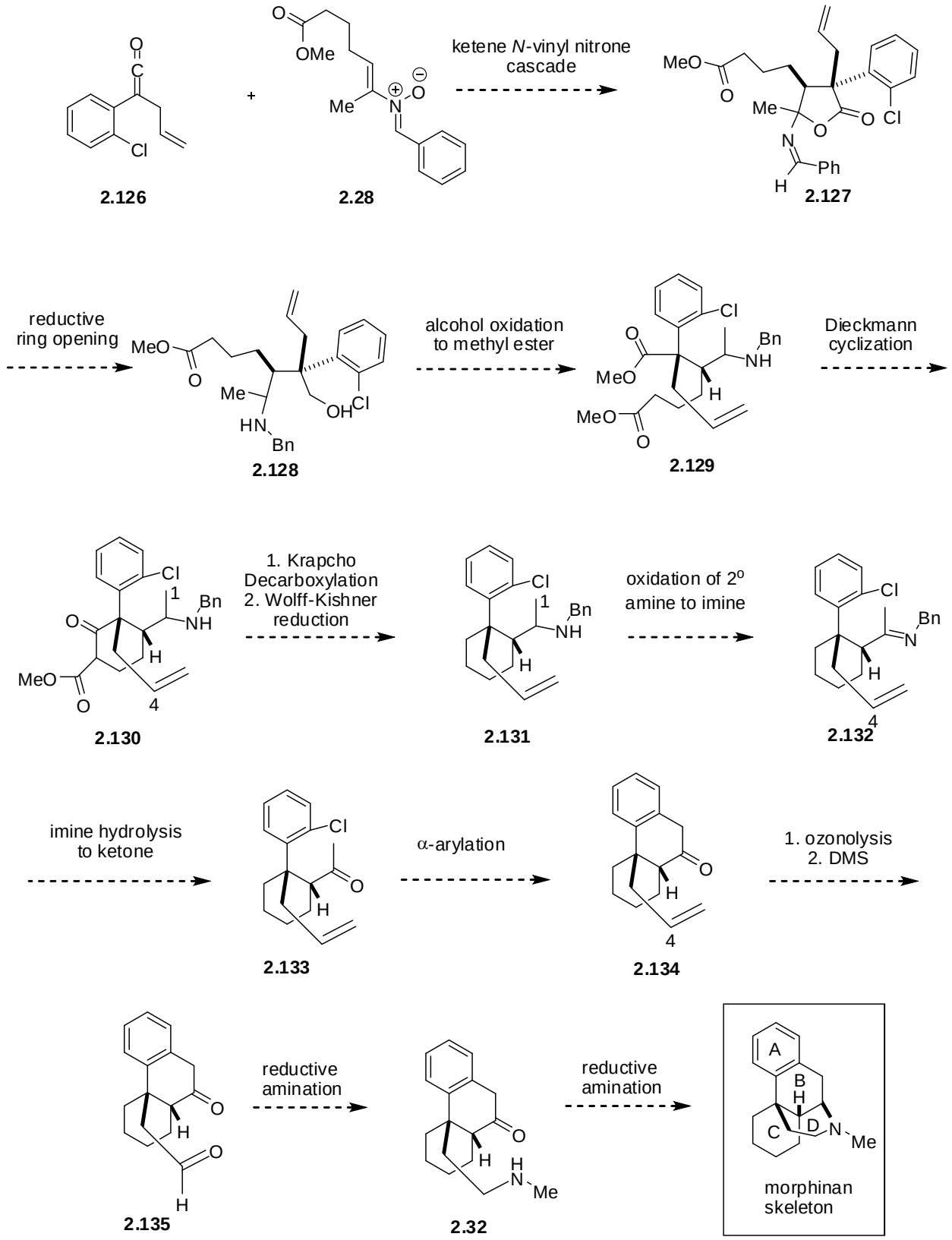


Scheme 2.22 Possible Side Reactions that Occur in the Amine Acyl Chloride



The *tert*-butyl ester to acid chloride reaction can be used in the synthesis of the ketene **2.126**. The overall synthetic strategy is similar to the initial proposal, the difference being that the ketene contains a less reactive olefin compared to the amine. The revised synthesis is as follows: reaction of ketene **2.126** and *N*-vinyl nitrone **2.28** would provide the cyclic hemiaminal lactone **2.127**. A reductive ring opening using LAH to afford **2.128**. The primary alcohol on **2.128** could then be oxidized to the methyl ester **2.129**, setting up for the cyclization of ring C through a Dieckmann cyclization to form product **2.130**. Compound **2.130** can then undergo a Krapcho decarboxylation followed by a Wolff-Kishner reduction to generate **2.131**. The secondary amine **2.131** can then be oxidized to the imine with IBX in DMSO[45] to form imine **2.132**, followed by an acidic hydrolysis to generate ketone **2.133**. The formation of ring B to form compound **2.134** could then be accomplished via an α-arylation.[21] After the formation of ring B, compound **2.134** can be subjected to ozonolysis to afford aldehyde **2.135**. Selective reductive amination (methyl amine/triacetoxyborohydride) of the aldehyde of compound **2.135** would provide amine **2.32** which could undergo a subsequent reductive amination to form the morphinan skeleton.

Scheme 2.23 Synthesis of Morphinan Skeleton via Ketene/*N*-vinylnitrone Cascade



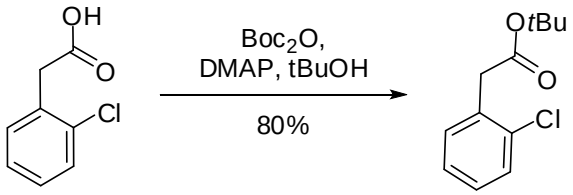
2.16 Conclusion

In conclusion, we describe a simple and efficient method for the conversion of *tert*-butyl esters to acid chlorides on a variety of substrates. Our mechanistic studies suggest that the reaction is promoted by acid, and competition experiments show that the reaction is selective for *tert*-butyl esters in the presence of methyl, 1°, 2°, or benzyl esters, and lactones. We were able to convert methyl, ethyl, isopropyl, and benzyl esters into acid chlorides using tetrabutyl ammonium chloride as a phase transfer catalyst; however, this reaction is slow, requiring a week for methyl esters to convert fully into the corresponding acid chloride. Further the presence of the tetra alkyl ammonium salts presents complications in the work up. The method for acid chloride formation from *tert*-butyl esters described in this chapter is mild and compatible with other functional groups including alkenes, methyl ethers, some protected amines, sulfonates, carbonates, and acetate groups, and provides an alternative to other commonly used methods for acid chloride synthesis.

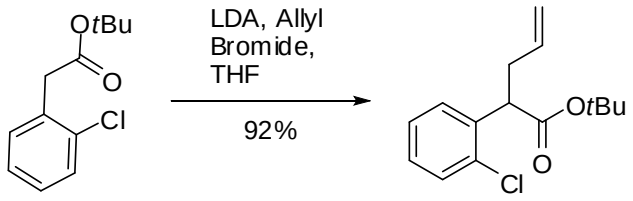
2.17 Organic experimentals

General Information. All reactions were carried out in flame dried glassware under a dry nitrogen atmosphere or sealed in 2 dram (7.4 mL) vials with teflon lined caps as indicated. DCM, diisopropylamine, methanol, ethanol, and isopropanol were distilled from CaH_2 under nitrogen and stored over 3Å molecular sieves prior to use. THF was distilled from Na benzophenone ketyl under nitrogen prior to use. All other reagents were used as received from the supplier. Flash chromatography was performed using 60Å silica gel (40-63 μm). ^{1}H NMR spectra were recorded at 300, 400, or 500 MHz in $CDCl_3$ using residual $CHCl_3$ (7.26 ppm) as an internal reference. ^{13}C NMR spectra were recorded at 75 or 100 MHz in $CDCl_3$ using the center line of the $CDCl_3$ triplet (77.16 ppm) as an internal reference. Infrared (IR) spectra were obtained as thin films on NaCl plates. Exact mass was determined using electrospray ionization (ESI-TOF). We note that in the

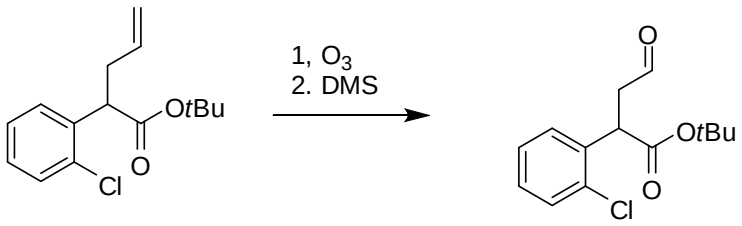
case of several of the acid chlorides, the corresponding lithiated anhydride is present in the HRMS spectrum and data is provided for the $[M\text{-}Cl]^+$ ion. This ion could be derived from the acid chloride or from the anhydride. In the cases where we report $[M\text{-}Cl]^+$, we were unable to observe the metalated (either lithiated or sodiated) acid chloride, or in some cases to find conditions wherein $[M\text{-}Cl]^+$ is the major ion in the spectrum. We attribute this to the reactive nature of the acid chloride functional group, and the fact that the injection solvent is acetonitrile. This solvent was rigorously dried prior to use, but as it is hygroscopic, it can absorb water as it is being used in the MS experiment. Compounds were synthesized according to published procedures.



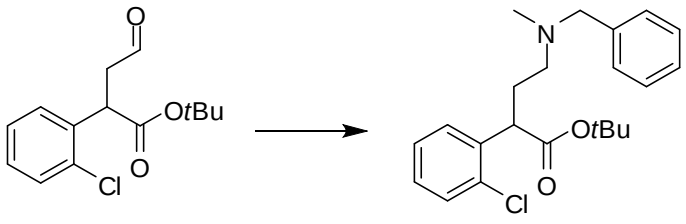
tert-butyl 2-(2-chlorophenyl)acetate (**2.34**) Starting material (5.043 g, 29.5 mmol), Boc anhydride (1.5 equiv 9.677 g, 4.3 mmol), and DMAP (0.1 equiv, 0.36 g, 2.95 mmol) were dissolved in tBuOH, purged with nitrogen and heated to 50 ^{o}C and let stir overnight. The reaction changed in color from yellow to orange. After seen to be completed by TLC the reaction was diluted with Hexanes:EtOAc (5:1) and most of the solvent was removed under reduced pressure. Reaction was then diluted in Hexanes:EtOAc (5:1) and washed with 1M HCl, followed by DI water, then brine. The reaction was dried over $MgSO_4$ filtered and solvent was removed under reduced pressure to obtain crude product as a yellow oil. The crude material was subjected to flash chromatography (gradient 100% hexanes to 10:1 Hexanes:EtOAc). Isolated product as a clear oil (4.82 g, 72% yield). R_f = 0.53 (10:1 Hex:EtOAc). Spectra are in accord with literature**.** [46]



tert-Butyl 2-(2-Chlorophenyl)pent-4-enoate **(2.35)**. Under a nitrogen atmosphere, diisopropylamine (2.06 mL, 14.68 mmol, 1.05 equiv) was dissolved in THF (140 mL) and cooled to -78 °C. *n*-BuLi (10.23 mL of 1.44 M, 1.1 equiv) was added and the reaction was warmed to 0 °C and stirred for 30 min. The reaction was cooled to -78 °C and compound **1k** (3.17 g, 13.98 mmol in 5 mL) was added via cannula and reaction was stirred at -78 °C for 1 h. The reaction turned bright yellow in color. Allyl bromide (1.27 mL, 14.68 mmol, 1.05 equiv) was added to the reaction dropwise over 10 minutes. The reaction was warmed to room temperature and stirred overnight. The reaction was concentrated under reduced pressure then dissolved in hexanes/EA (5:1), washed with sat. NH_4Cl (2x), brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a deep red oil. The crude material was purified via flash chromatography (50:1 hexanes/EA) to provide **2.35** (3.412 g, 92%) as a colorless oil. R_f = 0.60 (10:1 hexanes/EA) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.358 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.353 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.22 (td, *J* = 7.5, 1.6 Hz, 1H), 7.16 (td, *J* = 7.6, 1.8 Hz, 1H), 5.80-5.69 (m, 1H), 5.11 – 4.93 (m, 2H), 4.11 (dd, *J* = 8.3, 6.8 Hz, 1H), 2.78 – 2.69 (m, 1H), 2.53-2.43 (m, 1H), 1.39 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 137.0, 135.2, 134.0, 129.6, 128.5, 128.1, 126.9, 116.8, 81.0, 48.1, 36.7, 27.9; IR (thin film): C=O, 1729 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{15}ClH_{19}O_2$ $[M+ Li]^+$, 272.1225; found, 272.1217.



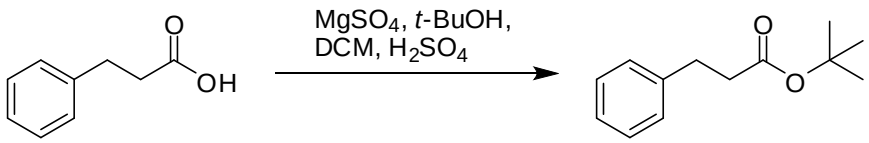
tert-Butyl 2-(2-chlorophenyl)-4-oxobutanoate (**2.36**). Compound **2.35** (4.81 g, 18.0 mmol) was dissolved in MeOH (180 mL) and cooled to -78 °C. Ozone was bubbled through for 30 minutes until the appearance of a light blue color. Oxygen was bubbled through until the blue color dissipated, then dimethyl sulfide (1.73 mL, 23.4 mmol, 1.3 equiv) was added to the reaction. The reaction was warmed to room temperature and stirred overnight under a nitrogen atmosphere. The reaction was concentrated under reduced pressure to provide a pale yellow residue which was subsequently purified via flash chromatography (10:1 hexanes/EA) to provide the corresponding aldehyde **2.36** (3.62 g, 75%) as a clear oil. R_f = 0.36 (10:1 hexanes/EA); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (X of ABMX; broad s, 1H), 7.40 – 7.36 (m, 1H), 7.26 – 7.17 (m, 3H), 4.55 (M of ABMX, *J* = 9.6, 4.5 Hz, 1H), 3.25 (B of ABMX, *J* = 18.3, 9.6, 1.0 Hz, 1H), 2.74 (A of ABMX, *J* = 18.3, 4.6, 0.7 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.4, 171.2, 136.4, 133.6, 129.9, 128.7, 128.5, 127.2, 81.7, 45.9, 43.1, 27.8; IR thin film: C=O, 1726 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{14}H_{17}ClO_3$ [M+ Li]$^+$, 275.1027; found, 275.1016.



tert-butyl 4-(benzyl(methyl)amino)-2-(2-chlorophenyl)butanoate (**2.37**) Aldehyde **2.36** (0.503 g, 1.87 mmol, 1 equiv) and sodium triacetoyborohydride (.594 g, 2.80 mmol, 1.5 equiv) and benzyl

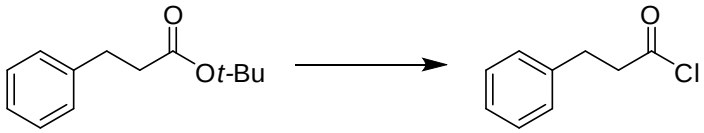
methyl amine (0.253 mL, 1.96 mmol, 1.05 equiv) were dissolved in dichloroethane (20 mL) under nitrogen atmosphere. The reaction was stirred for 3h at room temperature until reaction was found to be complete by TLC. The reaction was diluted with sat. sodium bicarbonate and extracted with EA (2x). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to provide crude product. The crude product was purified via flash chromatography (20:1 hexanes:EA) to provide product **2.37** (0.543 g, 78% yield). R_f = 0.28 (10:1 hexanes:EA); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40 – 7.16 (m, 10H), 4.18 (dd, *J* = 8.0, 6.6 Hz, 1H), 3.50 (d, *J* = 3.0 Hz, 2H), 2.43 (dt, *J* = 7.7, 5.9 Hz, 2H), 2.34 – 2.24 (m, 1H), 2.20 (s, 3H), 2.01 – 1.86 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 137.8, 134.1, 129.8, 129.1, 128.8, 128.3, 128., 127.1, 127.0, 110.2, 81.0, 62.4, 55.2, 46.6, 42.1, 30.4, 28.1; HRMS (ESI-TOF) *m/z* calc'd for $C_{22}H_{28}ClNO_2$ $[M+ Na]^+$, 396.1706; found, 396.1696.

General procedure for the synthesis of *tert*-butyl esters:[47] The starting carboxylic acid (5 mmol; 1 equiv) was dissolved in DCM (12.5 ml; 0.25 M) and $MgSO_4$ (20 mmol; 4 equiv) was added. The flask was then purged with N_2 and concd sulfuric acid (4.75 mmol; 0.95 equiv) was added to the suspension followed by *tert*-butanol (25 mmol; 5 equiv). The suspension was allowed to stir overnight at room temperature. The reaction was quenched by the addition of hexanes/EA (1:1; ~50 mL) followed by saturated $NaHCO_3$ (~50 mL; *gas evolution!*). The layers were separated and the organic layer was washed with water (~50 mL; 2x), brine, then dried over $MgSO_4$. The reaction was filtered and concentrated under reduced pressure to provide the crude product as a colorless oil. The crude product was purified by flash chromatography (10:1 hexanes/EA).



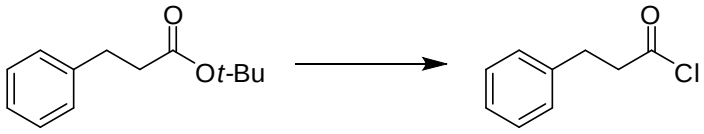
tert-Butyl 3-Phenylpropanoate **(2.87, 2.107a)** This compound was prepared according to the general procedure to provide **2.87** (0.835 g, 59%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [47]

General Procedure for the synthesis of acid chlorides: The *tert*-butyl ester (1 mmol; 1 equiv) was dissolved in $SOCl_2$ (0.70 mL; 10 mmol; 10 equiv) in a 2 dram (7.4 mL) vial equipped with a magnetic stir bar at room temperature. Water (18 μl, 1 mmol, 1 equiv) was then added and the vial was capped with a teflon-lined cap. *Note that the vial was never filled more than 40% full to avoid the possibility of rupture!* The reaction was stirred, at which point, gas evolution was observed. Stirring was continued for the length of time indicated in Table 5. The cap was then carefully removed (*Caution: contents under pressure!*), and toluene (1 mL) was added to the vial and removed at reduced pressure to effect azeotropic removal of $SOCl_2$ and provide the product.

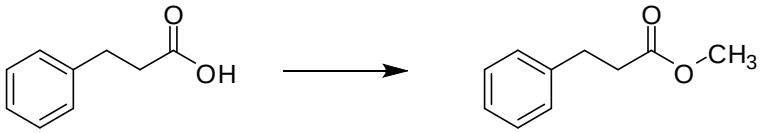


Large scale acid chloride synthesis open to atmosphere: *3-Phenylpropanoyl Chloride* **(2.107b)** The *tert*-butyl dihydrocinnamate (4.174 g, 20.2 mmol, 1 equiv) was dissolved in $SOCl_2$ (20 equiv, 28.5 mL) equipped with a magnetic stir bar at room temperature. Concentrated HCl (1 equiv, 1.66 mL) was then added and the flask was subsequently capped with a polyethylene cap that was punctured with a 21 gauge needle for a vent to the atmosphere. The reaction was stirred, at which point, gas evolution was observed, and stirring continued for 16 h at room temperature. Toluene (5 ml x2) was added to the flask and removed at reduced pressure to effect azeotropic removal of $SOCl_2$. The crude material was isolated in a 98% yield. The crude material was then subjected to

vacuum distillation to isolate product as a clear oil (3.102 g, 91% yield). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[48]

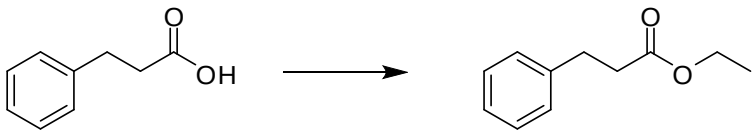


3-Phenylpropanoyl Chloride (**2.88, 2.107b**). This compound was prepared according to the general procedure to provide **2.88** (0.101 g, 91%) as a colorless oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [48]



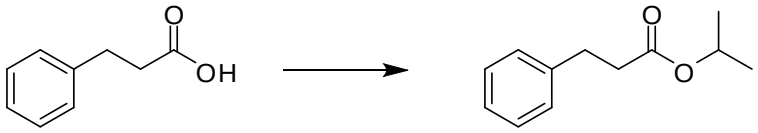
methyl 3-phenylpropanoate

Methyl 3-Phenylpropanoate (**2.89a**) Dihydrocinnamic acid (5.100 g, 34.0 mmol, 1 equiv) was dissolved in MeOH (300 mL) and H_2SO_4 (200 μL) was added to the reaction. The reaction was and stirred for 16 h under a nitrogen atmosphere at room temperature until found to be complete by TLC. The reaction was neutralized by the addition of solid NaOH. The reaction was concentrated to obtain crude product which was purified via flash chromatography (10:1 hexanes:EA) to obtain product **2.89a** (5.190 g, 93% yield) as a clear oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [49]

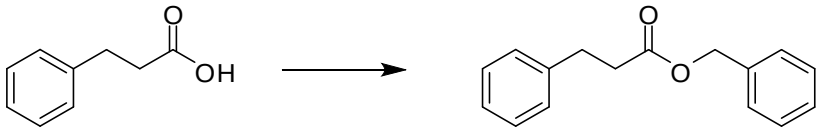


Ethyl 3-Phenylpropanoate (**2.89b**) Dihydrocinnamic acid (0.956 g, 6.40 mmol, 1 equiv) was dissolved in EtOH (65 mL) and H_2SO_4 (100 μL) was added to the reaction. The reaction was

stirred for 16 h under a nitrogen atmosphere at room temperature. The reaction was neutralized by the addition of solid NaOH. Upon neutralization a white precipitate crashed out of solution. The reaction was filtered over a Buchner funnel and with hexanes:EA (10:1) to remove solid. The reaction was concentrated to obtain product **2.89b** (1.768 g, 78% yield) as a white solid. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [50]

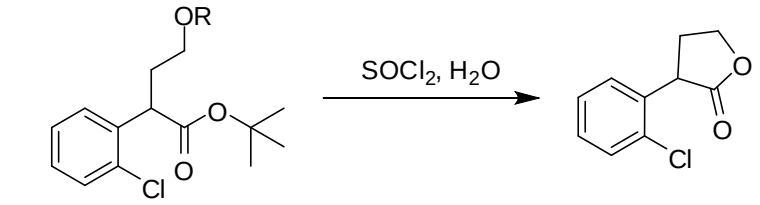


Isopropyl 3-Phenylpropanoate (**2.89c**) Dihydrocinnamic acid (2.060 g, 13.7 mmol, 1 equiv) was dissolved in *i*PrOH (100 mL) and H_2SO_4 (100 μL) was added to the reaction. The reaction was heated to reflux and stirred for 16 h under a nitrogen atmosphere. The reaction was neutralized by the addition of solid NaOH. Upon neutralization a white precipitate crashed out of solution. The reaction was filtered over a Buchner funnel and with hexanes:EA (10:1) to remove solid. The reaction was concentrated to obtain crude product. The crude product was purified via flash chromatography (10:1 hexanes:EA) to obtain product **2.89c** (1.920 g, 73% yield) as a clear oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [51]



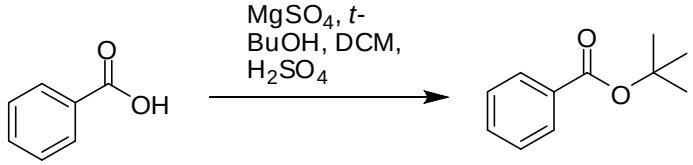
Benzyl 3-Phenylpropanoate (**2.89d**) Dihydrocinnamic acid (2.076 g, 13.8 mmol, 1 equiv) and potassium carbonate (1.911 g, 13.8 mmol, 1 equiv) was suspended in acetone. Benzyl bromide (1.80 mL, 15.18 mmol, 1.1 equiv) was added to the reaction via syringe. The reaction was heated to reflux for 16 h until found to be complete by TLC. The reaction was filtered over celite to

remove potassium carbonate with diethyl ether. The organic layer was washed with water (1x), washed with brine (1x), dried over $MgSO_4$, filtered and concentrated to obtain crude product as a clear oil. The crude product was purified via flash chromatography to obtain **2.89d** (1.877 g, 57% yield. ^1H and ^{13}C NMR are in accord with those reported in the literature. [52]

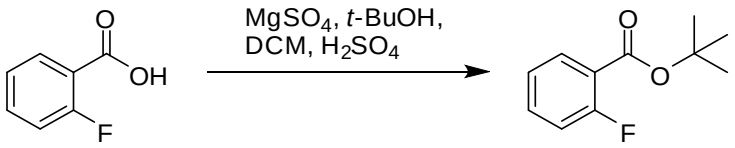


R = H, TES, TBS, TIPS, Bn

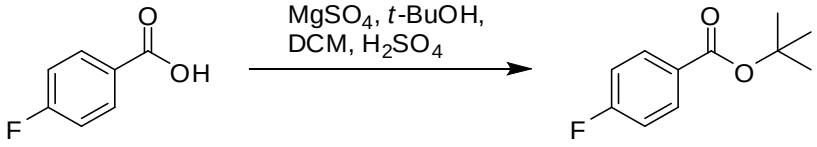
3-(2-Chlorophenyl)dihydrofuran-2(3H)-one (**2.104**). This compound was prepared according to the general procedure with extended reaction time to 16 h to provide **2.104** as a colorless oil, (84-98%), ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (dt, *J* = 7.3, 1.2 Hz, 1H), 7.35 – 7.22 (m, 3H), 4.52 (N of ABMNX, *J* = 8.8, 2.9 Hz, 1H), 4.40 (M of ABMNX, *J* = 9.4, 6.7 Hz, 1H), 4.25 (X of ABMNX, *J* = 10.7, 9.2 Hz, 1H), 2.87 – 2.73 (B of ABMNX, m, 1H), 2.38 (A of ABMNX, *J* = 12.8, 10.7, 9.7, 8.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 135.0, 134.2, 130.1, 129.6, 129.2, 127.6, 66.7, 44.0, 31.0; IR (thin film): C=O, 1771 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{10}H_9ClO_2$ $[M+Li]^+$, 203.0451; found, 203.0453



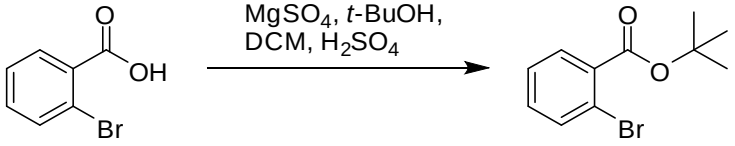
tert-Butyl Benzoate **(2.90a)** This compound was prepared according to the general procedure to provide **2.90a** (2.967 g, 68%). ^1H and ^{13}C NMR are in accord with those reported in the literature.[47]



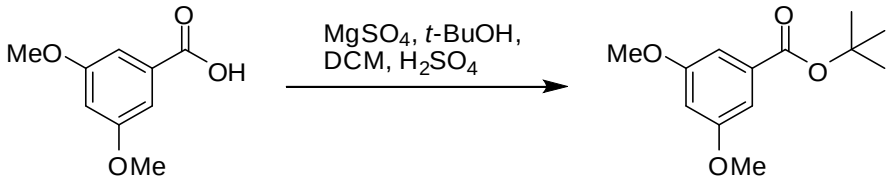
tert-Butyl 2-Fluorobenzoate **(2.91a)** This compound was prepared according to the general procedure to provide **2.91a** (0.835 g, 59%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[53]



tert-Butyl 4-Fluorobenzoate **(2.92a)** This compound was prepared according to the general procedure to provide **2.92a** (1.033 g, 75%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[53]

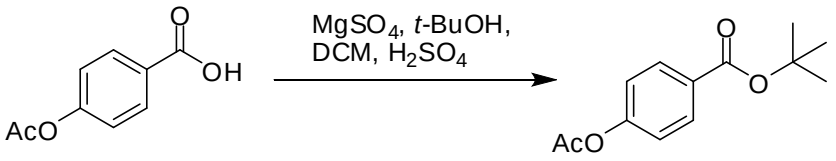


tert-Butyl 2-Bromobenzoate (**2.93a**). This compound was prepared according to the general procedure to provide **2.93a** (1.452 g, 85%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[54]

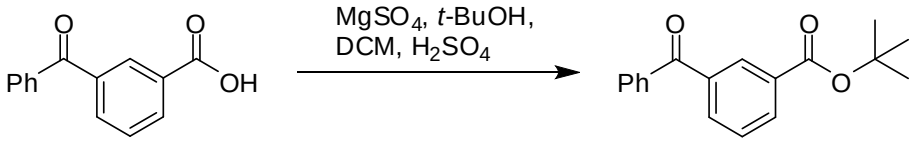


tert-Butyl 3,5-Dimethoxybenzoate (**2.94a**). This compound was prepared according to the general procedure, to provide **2.94a** (0.579 g, 43%) as a white amorphous powder. R_f = 0.40 (10:1

hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (d, *J* = 2.4 Hz, 2H), 6.63 (t, *J* = 2.4 Hz, 1H), 3.84 (s, 6H), 1.60 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.5, 160.5, 133.9, 107.0, 105.0, 81.2, 55.5, 28.1. IR (thin film): C=O, 1713; HRMS (TOF-ESI) m/z calc'd for $C_{13}H_{18}O_4$ $[M+H]^+$, 239.1283; found, 239.1283.

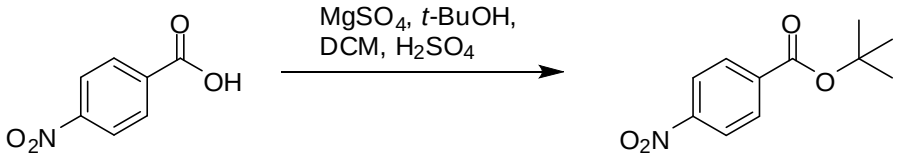


tert-Butyl 4-Acetoxybenzoate (**2.95a**). Under a nitrogen atmosphere, *tert-butyl 4-hydroxybenzoate* (0.642 g, 3.30 mmol, 1 equiv prepared in 1 step following a known procedure)[55] was dissolved in THF-pyridine (1:1, 33 mL) and cooled to 0 °C. Acetyl chloride (0.258 mL, 3.63 mmol, 1.1 equiv) was added dropwise over 15 minutes. A white precipitate forms upon addition. The reaction was allowed to warm to room temperature for 16 h until found to be complete by TLC. Reaction was diluted with hexanes/EA (1:1) and subsequently washed with water (2x), followed by brine, then dried over $MgSO_4$. The reaction was filtered and concentrated to provide **2.95a** (0.775 g, 99% yield) as a yellow oil. R_f = 0.34 (10:1 hexanes/EA); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.7 Hz, 2H), 7.15 (d, *J* = 8.7 Hz, 2H), 2.32 (s, 3H), 1.60 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 165.0, 154.0, 131.0, 129.7, 121.5, 81.2, 28.2, 21.2; IR (thin film): C=O, 1763, 1714 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{13}H_{16}O_4$ $[M+Li]^+$, 243.1209; found, 243.1212.

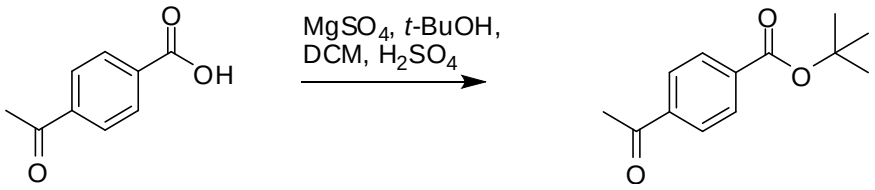


tert-Butyl 2-Benzoylbenzoate (**2.96a**). This compound was prepared according to the general procedure to provide **2.96a** (1.078 g, 86%) as a pale yellow amorphous solid. R_f = 0.34 (10:1

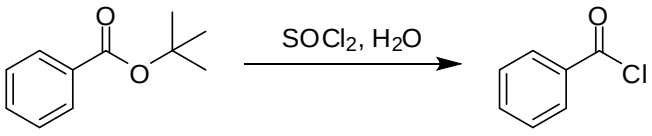
hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 8.00 (m, 1H), 7.82 – 7.77 (m, 2H), 7.60-7.55 (m, 1H), 7.60 – 7.55 (m, 2H), 7.48 – 7.43 (m, 2H), 7.42 – 7.37 (m, 1H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.7, 165.2, 140.9, 137.2, 133.1, 132.0, 131.1, 129.9, 129.6, 129.6, 128.5, 127.6, 82.6, 27.4. IR (thin film): C=O, 1714, 1674 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{18}H_{18}O_3$ $[M+Li]^+$, 289.1416; found, 289.1404.



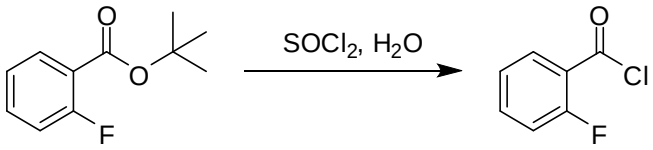
tert-Butyl 4-Nitrobenzoate (**2.97a**) This compound was prepared according to the general procedure to provide **2.97a** (1.315 g, 95%) as a pale yellow amorphous solid. ^{1}H and ^{13}C NMR are in accord with those reported in the literature**.** [47]



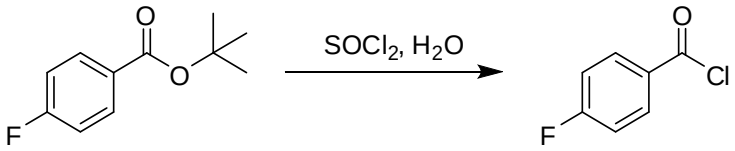
tert-Butyl 4-Acetylbenzoate (**2.98a**). This compound was prepared according to the general procedure to provide **2.98a** (0.645 g, 48%) as a white amorphous powder. ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[56]



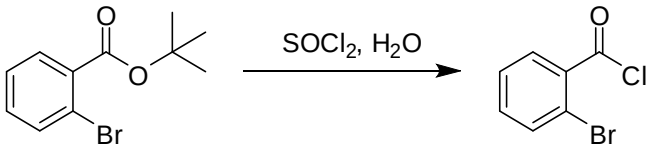
Benzoyl Chloride (**2.90b**). This compound was prepared according to the general procedure with an extended reaction time of 5 h to provide **2.90b** as a colorless oil (0.110 g, 90%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[57]



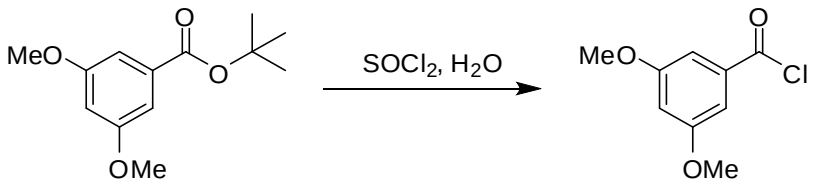
2-Fluorobenzoyl Chloride (**2.91b**) This compound was prepared according to the general procedure with an extended reaction time of 5 h to provide **2.91b** as a pale yellow oil (0.046 g, 92%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[58]



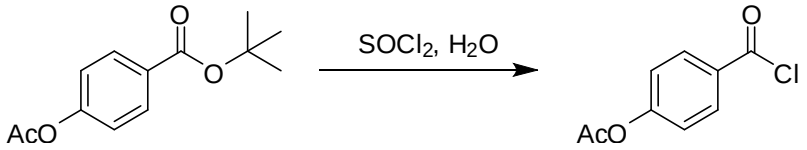
4-Fluorobenzoyl Chloride (**2.92b**) This compound was prepared according to general procedure with an extended reaction time of 16 h to provide **2.92b** as a pale yellow oil (0.062 g, 90%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[59]



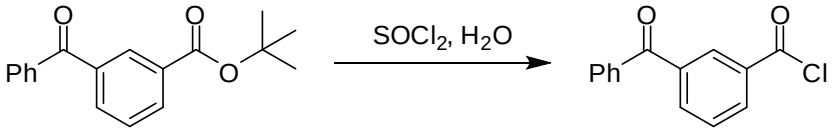
2-Bromobenzoyl Chloride (**2.93b**). This compound was prepared according to the general procedure to provide **2.93b** (0.044 g, 95%) as a colorless oil. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [60]



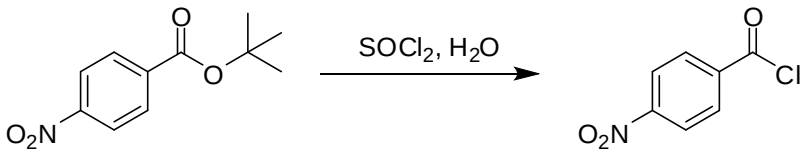
3,5-Dimethoxybenzoyl Chloride (**2.94b**). This compound was prepared according to the general procedure with extended reaction time to 16 h to provide **2.94b** (0.080 g, 94%). ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[61]



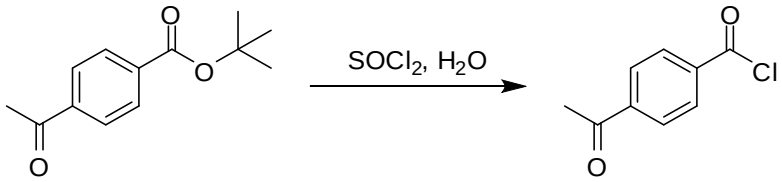
4-(Chlorocarbonyl)phenyl Acetate (**2.95b**) This compound was prepared according to the general procedure with extended reaction time to 16 h to provide **2.95b** (0.054, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.8 Hz, 2H), 7.28 (d, *J* = 8.8 Hz, 2H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 167.5, 156.2, 133.2, 130.7, 122.4, 21.3. IR (thin film): C=O, 1774 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_9H_7O_3$ $[M-Cl]^+$, 163.0395; found, 163.0413.



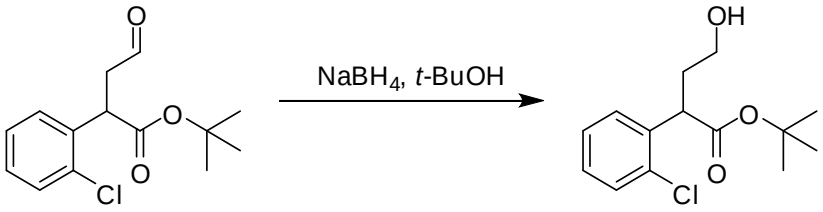
2-Benzoylbenzoyl Chloride (**2.96b**) This compound was prepared according to the general procedure with extended reaction time to 16 h to provide **2.96b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.6 Hz, 1H), 7.79 (apparent t, *J* = 7.5 Hz, 1H), 7.74 – 7.68 (m, 3H), 7.64 (apparent t, *J* = 7.4 Hz, 1H), 7.46 – 7.41 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 151.3, 138.2, 135.5, 130.9, 130.0, 128.8, 126.0, 125.9, 123.6, 123.5, 110.1, 99.9. IR (thin film): C=O, 1791 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{14}H_9O_2Cl$ $[M+Li]^+$, 251.0451; found, 251.0442.



4-Nitrobenzoyl Chloride (**2.97b**) This compound was prepared according to the general procedure with extended reaction time to 16 h and run in toluene (0.1 M), heated to 100 °C to provide **2.97b** (0.080 g, 94%) ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[62]

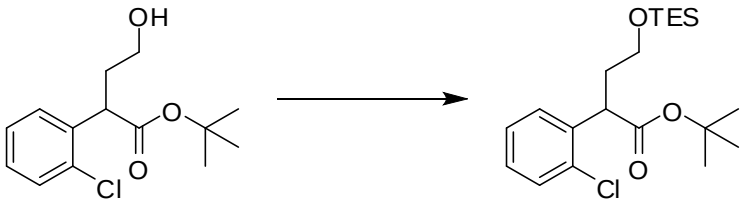


4-Acetylbenzoyl Chloride (**2.98b**) This compound was prepared according to the general procedure with extended reaction time to 16 h and run in toluene (0.1 M) heated to 100 °C to provide **2.98b** (0.054 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 – 8.21 (m, 2H), 8.10 – 8.06 (m, 2H), 2.69 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.1, 168.0, 141.8, 136.6, 131.7, 128.7, 27.2; IR (thin film): C=O, 1773, 1736, 1687 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_9H_7O_2$ [M-Cl]$^{+}$, 147.0446; found, 147.0451.



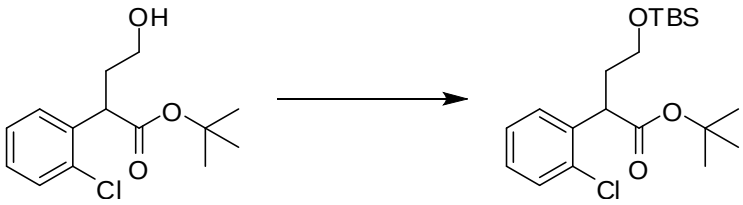
tert-Butyl 2-(2-Chlorophenyl)-4-hydroxybutanoate (**2.99a**) The aldehyde **2.35** (2.288 g, 8.513 mmol) was dissolved in *tert*-butanol (85 mL) and the flask was purged with nitrogen. Sodium borohydride (0.644 g, 17.02 mmol, 2 equiv) was added to the reaction and was stirred for 15 min until starting material was no longer present by TLC. The reaction was quenched by the addition

of water and extracted with EA(2x). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a crude colorless oil. The crude residue was purified via flash chromatography (5:1 hexanes/EA) to provide **1t** (2.02 g, 88%) as a clear oil. R_f = 0.23 (5:1 hexanes/EA) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.34 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.24 (td, *J* = 7.4, 1.3 Hz, 1H), 7.21 – 7.16 (m, 1H), 4.22 (X of ABNMX, apparent t, *J* = 7.3 Hz), 3.76-3.55 (M and N of ABMNX, m, 2H), 2.39 – 2.26 (B of ABMNX, m, 1H), 2.01 – 1.91 (A of ABMNX, m, 1H), 1.76 (s, 1H), 1.40 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 137.2, 133.9, 129.7, 128.7, 128.2, 127.0, 81.2, 60.7, 45.6, 35.2, 27.9; IR (thin film): OH, 3449, C=O, 1727cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{14}H_{19}ClO_3$ [M+ Li]$^+$, 277.1183; found, 277.1182.



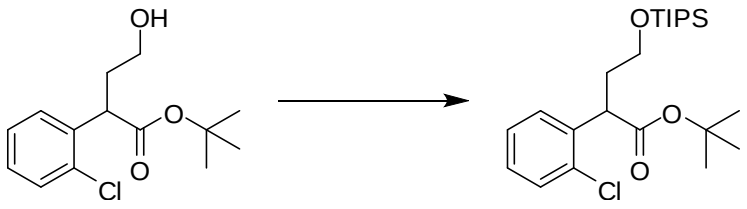
tert-Butyl 2-(2-Chlorophenyl)-4-triethylsilyloxy)butanoate (**2.100a**). Alcohol **2.99a** (0.197 g, 0.727 mmol) was dissolved in DCM (7 mL) and cooled to 0 °C under a nitrogen atmosphere. Triethylamine (0.202 mL, 1.45 mmol, 2 equiv) then triethylsilyl trifluoromethanesulfonate (TESOTf) (0.164 mL, 0.272 mmol, 1 equiv) were added to the reaction dropwise via syringe. The reaction was stirred at 0 °C for 1 h until starting material could no longer be observed by TLC. The reaction was diluted with hexanes/EA (1:1), washed with $NaHCO_3$ (sat.), water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a light pink oil. The crude residue was purified via flash chromatography (10:1 hexanes/EA) to provide **2.100a** (0.197 g, 70%) as a colorless oil. R_f = 0.48 (10:1 hexanes/EA); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.33 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.22 (td, *J* = 7.5, 1.6 Hz, 1H), 7.17 (td, *J* = 7.5,

1.9 Hz, 1H), 4.22 (X of ABMNX, *J* = 8.0, 6.8 Hz, 1H), 3.68 – 3.52 (M and N, of ABMNX, m, 2H), 2.29 (B of ABMNX, *J* = 12.8, 8.0, 6.3 Hz, 1H), 1.94 (A of ABMNX, *J* = 13.4, 6.6 Hz, 1H), 1.39 (s, 9H), 0.94 (t, *J* = 8.0 Hz, 9H), 0.57 (q, *J* = 7.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 137.9, 134.5, 130.1, 129.2, 128.4, 127.3, 81.3, 60.9, 45.4, 35.8, 28.4, 7.2, 4.8; IR (thin film): C=O,1729 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{20}H_{33}ClO_3Si$ $[M+Li]^+$, 391.2048; found, 391.2047.

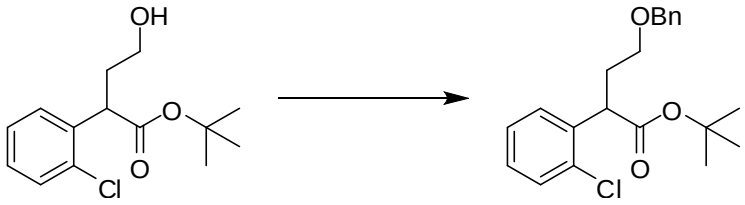


tert-Butyl 4-(tert-Butyldimethylsilyloxy)-2-(2-chlorophenyl)butanoate (**2.101a**). Alcohol **2.99a** (0.478 g, 1.76 mmol) was dissolved in THF (18 mL), imidazole (0.359 g, 5.28 mmol, 3 equiv) was added, and the flask was purged with nitrogen. *tert*-Butyldimethylsilylchloride (TBDMSCl) (0.346 g, 2.3 mmol, 1.3 equiv, in 2 mL of THF) was then added via cannula and the reaction was stirred at room temperature. After a few seconds a white precipitate formed in the reaction. After 30 minutes the reaction was found to be complete by TLC. The reaction was diluted with hexanes/EA (1:1) washed with water (2x), brine, dried over $MgSO_4$, filtered, and concentrated to provide a colorless oil. The crude product was purified via flash chromatography to provide **2.101a** (0.450 g, 66%) as a colorless oil. R_f = 0.31 (10:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.35 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.23 (td, *J* = 7.5, 1.5 Hz, 1H), 7.19 (td, *J* = 7.6, 1.8 Hz, 1H), 4.26 (X of ABMNX, *J* = 8.1, 6.7 Hz, 1H), 3.66 – 3.55 (M and N of ABMNX, m, 2H), 2.27 (B of ABMNX, *J* = 14.0, 8.0, 6.0 Hz, 1H), 1.94 (A of ABMNX, *J* = 13.4, 6.6 Hz, 1H), 1.41

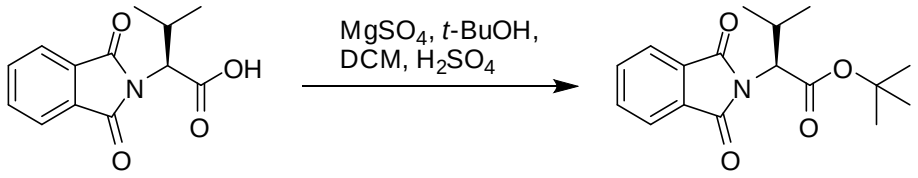
(s, 9H), 0.90 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 137.5, 134.0, 129.6, 128.8, 127.9, 126.8, 80.8, 60.6, 44.8, 35.3, 27.9, 25.9, 18.2, -5.4, -5.5; IR (thin film): C=O, 1729 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{20}H_{33}ClO_3Si$ [M+ Li]$^+$, 391.2048; found 391.2043.



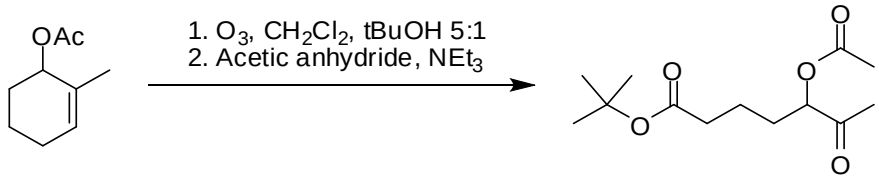
tert-Butyl 2-(2-Chlorophenyl)-4-(triisopropylsilyloxy)butanoate (**2.102a**). Alcohol **2.99** (0.336 g, 1.24 mmol) was dissolved in THF (12 mL) at room temperature, imidazole (0.253 g, 3.72 mmol, 3 equiv) was added, and the flask was purged with nitrogen. Triisopropylsilyl chloride (TIPSCl) (0.345 mL, 1.61 mmol, 1.3 equiv) was added to the solution dropwise via syringe. A white precipitate was observed and the reaction was stirred overnight at room temperature until starting material was no longer present by TLC. The reaction was diluted with hexanes/EA (1:1) and subsequently washed with water (2x), brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a colorless oil. The crude product was purified via flash chromatography (20:1 hexanes/EA) to provide **1w** (0.280 g, 53%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 6.2, 1.3 Hz, 1H), 7.34 (dd, *J* = 6.4, 1.2 Hz, 1H), 7.22 (td, *J* = 7.5, 1.5 Hz, 1H), 7.16 (td, *J* = 7.6, 1.8 Hz, 1H), 4.31 (X of ABMNX, *J* = 8.3, 6.4 Hz, 1H), 3.69 (M and N of ABMNX, *m*, Hz, 2H), 2.27 (B of ABMNX, *J* = 14.0, 8.3, 5.8 Hz, 1H), 1.94 (A of ABMNX, *J* = 13.4, 6.6 Hz, 1H), 1.39 (s, 9H), 1.12 – 0.97 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 137.6, 134.0, 129.6, 128.8, 127.9, 126.8, 80.7, 60.9, 44.8, 35.5, 27.9, 18.0, 11.9; IR (thin film): C=O, 1729 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{39}ClO_3Si$ [M+ Li]$^+$, 433.2517; found 433.2517.



tert-Butyl 4-(Benzyloxy)-2-(2-chlorophenyl)butanoate (**2.103a**). Under a nitrogen atmosphere, alcohol **2.99a** (0.286 g, 1.06 mmol, 1 equiv) was dissolved in 1,4-dioxane (10 mL). Benzyl 2,2,2-trichloroacetimidate (0.392 mL, 0.212 mmol, 2 equiv) and triflic acid (0.019 mL, 0.212 mmol, 0.2 equiv) were added to the reaction via syringe and stirred at room temperature for 16 h. Reaction was diluted with hexanes/EA (1:1), and the organic layer was washed with $NaHCO_3$ sat. (2x), brine, dried over $MgSO_4$, filtered, and concentrated to provide a tan solid. The crude product was purified via flash chromatography (50:1 hexanes/EA) to provide **2.103a** (0.118 g, 31%) as a colorless oil. R_f = 0.21 (50:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.35 – 7.31 (m, 5H), 7.29-7.27 (m, 1H), 7.22 (td, *J* = 7.5, 1.6 Hz, 1H), 7.18 (td, *J* = 7.6, 1.9 Hz, 1H), 4.48 (B of AB, *J* = 11.9 Hz, 1H), 4.45 (A of AB, *J* = 11.9 Hz, 1H), 4.26 (X of ABMNX, apparent t, *J* = 7.4 Hz, 1H), 3.50 (N of ABMNX, *J* = 9.5, 6.0 Hz, 1H), 3.39 (M of ABMNX, *J* = 9.5, 7.3, 5.8 Hz, 1H), 2.40 (B of ABMNX, *J* = 13.5, 7.4, 6.0 Hz, 1H), 2.04 (A of ABMNX, *J* = 13.6, 7.5, 5.9 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 138.5, 137.4, 134.2, 129.8, 129.0, 128.5, 128.2, 127.8, 127.7, 127.1, 81.0, 73.1, 67.9, 45.6, 32.5, 28.0; IR (thin film): C=O, 1726 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{21}H_{25}ClO_3$ $[M+Li]^+$, 367.1653; found, 367.1654.

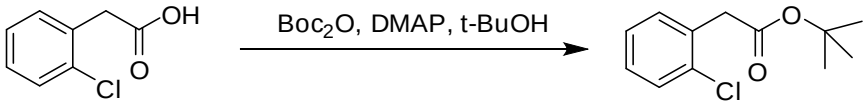


(R)-tert-Butyl 2-(1,2-Dioxoisoindolin-2-yl)-3-methylbutanoate (**2.108a**). This compound was prepared according to the general procedure to provide **2.108a** (0.823 g, 64%) as a white amorphous solid. [α] = 28.2° R_f = 0.32 (10:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.75 (dd, *J* = 5.5, 3.0 Hz, 2H), 4.51 (d, *J* = 8.2 Hz, 1H), 2.75 (dhept, *J* = 8.2, 6.8 Hz, 1H), 1.43 (s, 9H), 1.15 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 167.8, 134.0, 131.7, 123.4, 82.2, 58.6, 28.5, 27.9, 21.0, 19.6; IR (thin film): C=O, 1778, 1720 cm^{-1}; [α] = 28.2°; HRMS (TOF-ESI) *m/z* calc'd for $C_{17}H_{21}NO_4$ $[M+Na]^+$, 326.1368; found 326.1370.

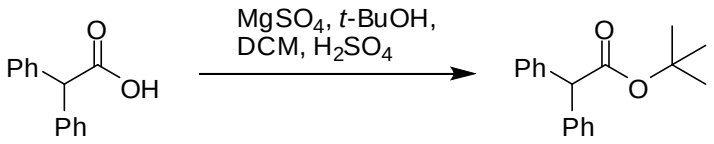


tert-Butyl 5-Acetoxy-6-oxoheptanoate (**2.109a**). 2-Methylcyclohex-2-enyl acetate (0.517 g, 3.35 mmol, 1 equiv, prepared in 4 steps following a known procedure)[63] was dissolved in DCM/*t*BuOH (5:1, 33 mL) and sodium bicarbonate (2.17 g, 25.92 mmol, 4 equiv) was added. The suspension was cooled to -78 °C forming a slurry through which ozone was bubbled for 2 h until starting material was no longer present by TLC. The cold bath was dropped and the reaction was purged with nitrogen. Acetic anhydride (1.84 mL, 19.4 mmol, 3 equiv) and triethylamine (1.36 mL, 9.72 mmol, 1.5 equiv) were added to the reaction via syringe and and the reaction was stirred overnight at room temperature. The reaction was filtered over celite then washed with 0.1 M HCl, saturated sodium bicarbonate, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure

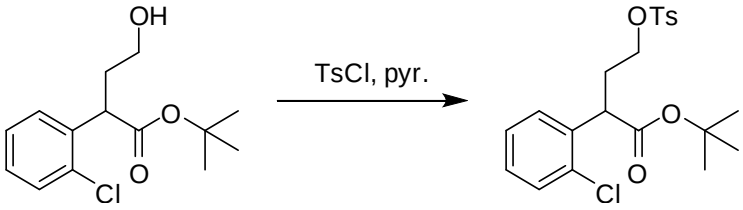
to provide a dark red oil. The oil was purified via flash chromatography (4:1 hexanes/EA) to provide **1n** (0.634 g, 38%) as a light yellow oil. R_f = 0.18 (4:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.01 (dd, *J* = 8.2, 4.2 Hz, 1H), 2.27 (td, *J* = 7.2, 1.7 Hz, 2H), 2.18 (s, 3H), 2.17 (s, 3H), 1.89 – 1.63 (m, 4H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.0, 172.3, 170.5, 80.5, 78.3, 34.8, 29.5, 28.1, 26.1, 20.7, 20.7; IR (thin film): C=O, 1729 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_{13}H_{22}LiO_5$ $[M+ H]^+$, 265.1628; found, 265.1625.



tert-butyl 2-(2-chlorophenyl)acetate (**2.111b**) Starting material (5.043 g, 29.5 mmol), Boc anhydride (1.5 equiv 9.677 g, 4.3 mmol), and DMAP (0.1 equiv, 0.36 g, 2.95 mmol) were dissolved in tBuOH, purged with nitrogen and heated to 50 ^{o}C and let stir overnight. The reaction changed in color from yellow to orange. After seen to be completed by TLC the reaction was diluted with Hexanes:EtOAc (5:1) and most of the solvent was removed under reduced pressure. Reaction was then diluted in Hexanes:EtOAc (5:1) and washed with 1M HCl, followed by DI water, then brine. The reaction was dried over $MgSO_4$ filtered and solvent was removed under reduced pressure to obtain crude product as a yellow oil. The crude material was subjected to flash chromatography (gradient 100% hexanes to 10:1 Hexanes:EtOAc). Isolated product as a clear oil (4.82 g, 72% yield). R_f = 0.53 (10:1 Hex:EtOAc). Spectra is in accord with literature.[46]; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.33 (m, 1H), 7.27 – 7.24 (m, 1H), 7.23 – 7.17 (m, 2H), 3.66 (s, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 169.9, 134.5, 133.1, 131.4, 129.4, 128.4, 126.8, 81.1, 40.4, 28.0; IR (thin film): 2979, 2932, 1735, 1476, 1446, 1393, 1368, 1338, 1280, 1257, 1227, 1149, 1054, 1040, 749 cm^{-1}.

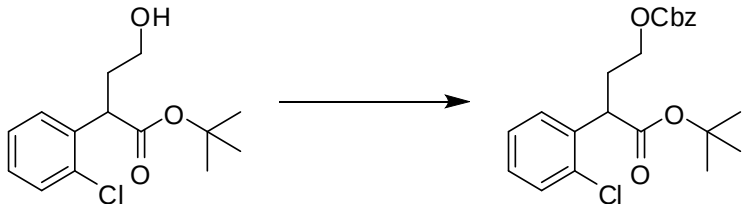


tert-Butyl 2,2-Diphenylacetate (**2.112a**). This compound was prepared according to the general procedure to provide **2.112a** (0.588 g, 46%) as a white amorphous solid. ^{1}H and ^{13}C NMR are in accord with those reported in the literature.[64]

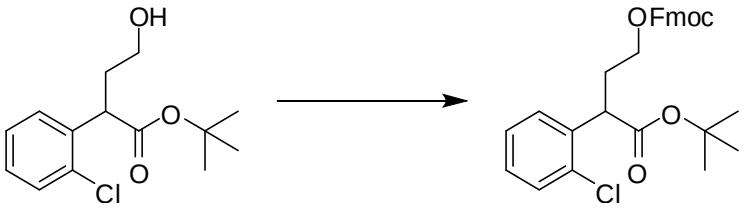


tert-Butyl 2-(2-Chlorophenyl)-4-(tosyloxy)butanoate (**2.113**). Alcohol **2.99a** (0.315 g, 1.16 mmol) was dissolved in anhydrous pyridine (12 mL) and *p*-toluenesulfonyl chloride (0.443 g, 2.32 mmol, 2 equiv) was added. The flask was purged with nitrogen and stirred at room temperature overnight. The reaction was quenched with the addition of water (100 mL) and subsequently extracted with hexanes/EA (5:1, 2x). The combined organic layers were washed with water (2x), brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a crude colorless oil. The crude material was purified via flash chromatography (10:1 hexanes/EA) to provide **2.113** (0.305 g, 62%) as a colorless oil. R_f = 0.20 (10:1 hexanes/EA); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.2 Hz, 2H), 7.35 – 7.28 (m, 3H), 7.20 – 7.13 (m, 3H), 4.13 – 4.02 (X and N of ABMNX, m, 2H), 3.92 (M of ABMNX, *J* = 9.9, 7.1, 5.6 Hz, 1H), 2.45 – 2.32 (B of ABMNX, m, 4H), 2.10 – 1.96 (A of ABMNX, m, 1H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 144.9, 136.3, 134.1, 133.0, 130.0, 130.0, 128.9, 128.6, 128.0, 127.2, 81.6, 68.2, 45.0, 31.3, 28.0, 21.8; IR (thin

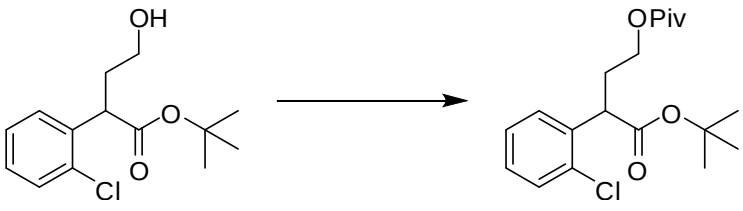
film): C=O, 1726 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{21}H_{25}ClO_5S$ $[M+Li]^+$, 431.1272; found, 431.1268.



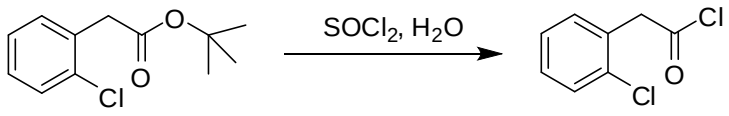
tert-Butyl 4-(Benzyloxycarbonyloxy)-2-(2-chlorophenyl)butanoate (**2.114**). Under a nitrogen atmosphere, alcohol **2.99a** (0.224 g, 0.83 mmol, 1 equiv) and DMAP (0.009 g, 0.083 mmol, 0.1 equiv), were dissolved in DCM (8 mL). Triethylamine (0.115 mL, 0.83 mmol, 1 equiv) was then added via syringe, and cooled to 0 °C. Benzylchloroformate (0.236 mL, 1.06 mmol, 2 equiv) was then added to the reaction dropwise via syringe over 15 minutes. The reaction was warmed to room temperature and was stirred for 16 h. The reaction was diluted with hexanes/EA (1:1, 15 mL), washed with water (2x 10 mL), brine, dried over $MgSO_4$, filtered and concentrated to provide a crude colorless oil. The crude residue was purified via flash chromatography (20:1 hexanes/EA) to provide **2.114** (0.080 g, 24%) as a colorless oil. R_f = 0.20 (20:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.32 (m, 6H), 7.30 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.22 (td, *J* = 7.5, 1.7 Hz, 1H), 7.19 (td, *J* = 7.5, 1.9 Hz, 1H), 5.15 (s, 2H), 4.22 – 4.09 (M, N, and X of ABMNX, m, 3H), 2.44 (B of ABMNX, *J* = 13.6, 7.3, 6.1 Hz, 1H), 2.09 (A of ABMNX, *J* = 13.7, 7.4, 6.2 Hz, 1H), 1.39 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 155.1, 136.7, 135.3, 134.1, 129.9, 128.7, 128.7, 128.6, 128.5, 128.4, 127.2, 81.4, 77.2, 69.7, 65.9, 45.2, 31.4, 28.0; IR (thin film): C=O, 1747, 1728 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{22}H_{25}ClO_5$ $[M+Li]^+$, 410.1542; found, 410.1535.



tert-Butyl 4 (((9H-fluoren-9-yl) methoxy)carbonyloxy)-2-(2-chlorophenyl)butanoate (**2.115**). Under a nitrogen atmosphere, alcohol **2.99a** (0.272 g, 1.00 mmol) dissolved in pyridine (5 mL) was added dropwise via cannula to 9-fluorenylmethoxycarbonyl (Fmoc) chloride (0.312 g, 1.20 mmol, 1.2 equiv) dissolved in pyridine (5 mL). The reaction was stirred at room temperature overnight. Water (15 mL) was added to quench the reaction and was subsequently extracted with EA (2x). The combined organic layers were washed with brine (2x), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a brown oil. The crude residue was purified via flash chromatography (20:1 hexanes/EA) to provide **2.115** (0.180 g, 37%) as a colorless oil. R_f = 0.20 (10:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.36 (m, 3H), 7.37 – 7.30 (m, 3H), 7.26 – 7.14 (m, 2H), 4.39 (d, *J* = 7.8 Hz, 2H), 4.30 – 4.17 (X and N of ABMNX, m, 2H), 4.13 (M of ABMNX, *J* = 10.8, 7.2, 6.0 Hz, 1H), 2.48 (B of ABMNX, *J* = 13.6, 7.3, 6.1 Hz, 1H), 2.13 (A of ABMNX, *J* = 13.8, 7.5, 6.2 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 155.2, 143.5, 143.5, 141.4, 136.8, 134.2, 130.0, 128.7, 128.6, 128.0, 127.3, 127.3, 125.3, 125.3, 120.2, 81.5, 69.9, 66.0, 46.9, 45.2, 31.5, 28.0; IR (thin film): C=O, 1747, 1728 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{29}H_{29}ClO_5$ $[M+Li]^+$, 499.1864; found, 499.1862.



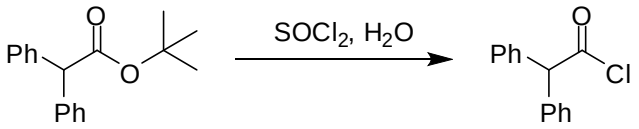
tert-Butyl 2-(2-Chlorophenyl)-4-(pivaloyloxy)butanoate (**2.116**). Under a nitrogen atmosphere, alcohol **2.99a** (0.265 g, 0.979 mmol, 1 equiv) and DMAP (0.002 g, 0.019 mmol, 0.2equiv) were dissolved in DCM (10 mL). Triethylamine (0.177 mL, 1.27 mmol, 1.3 equiv) was added via syringe, and the reaction was cooled to 0 °C. Trimethylacetyl chloride (0.145 mL, 0.117 mmol, 1.2 equiv) was added dropwise via syringe to the reaction over 10 minutes. The reaction was warmed to room temperature and stirred for 16 h. The reaction was diluted with hexanes/EA (1:1, 15 mL), washed with water (2x), brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a yellow oil. The crude material was purified via flash chromatography (20:1 hexanes/EA) to provide **2.116** (0.262 g, 76%) as a colorless oil. R_f = 0.52 (10:1 hexanes/EA); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.33 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.24 (td, *J* = 7.5, 1.5 Hz, 1H), 7.19 (td, *J* = 7.6, 1.8 Hz, 1H), 4.20 (X of ABMNX apparent t, *J* = 7.5 Hz, 1H), 4.08 (N of ABMNX, *J* = 11.1, 7.3, 5.7 Hz, 1H), 3.99 (M of ABMNX, *J* = 11.1, 6.2 Hz, 1H), 2.40 – 2.30 (B of ABMNX, m, 1H), 2.07 (A of ABMNX, *J* = 14.3, 7.3, 5.9 Hz, 1H), 1.40 (s, 9H), 1.20 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.6, 172.1, 136.9, 134.2, 129.9, 128.7, 128.5, 127.3, 81.4, 62.2, 45.2, 38.9, 31.6, 28.0, 27.3; IR (thin film): C=O, 1729 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{19}H_{27}ClO_4$ $[M+Li]^+$, 360.1749; found, 360.1747.



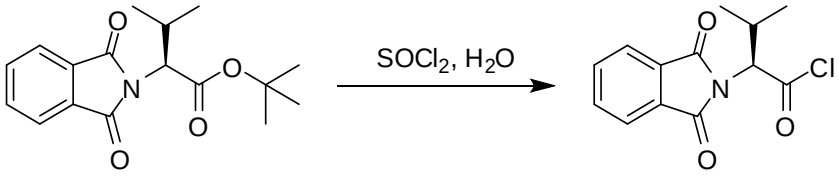
2-(2-chlorophenyl)acetyl chloride

2-(2-Chlorophenyl)acetyl Chloride. (**2.111b**). This compound was prepared according to the general procedure to provide **2k** (0.072 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.38 (m, 1H), 7.32 – 7.23 (m, 3H), 4.28 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 134.7, 131.6, 130.2,

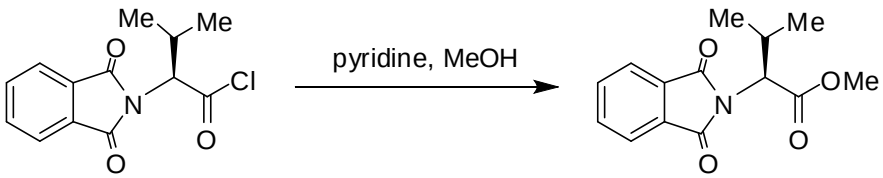
129.9, 129.8, 127.2, 50.8; IR (thin film): C=O, 1798 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for C_8H_6OCl $[M-Cl]^+$, 153.0107; found 153.0104 (minor component).



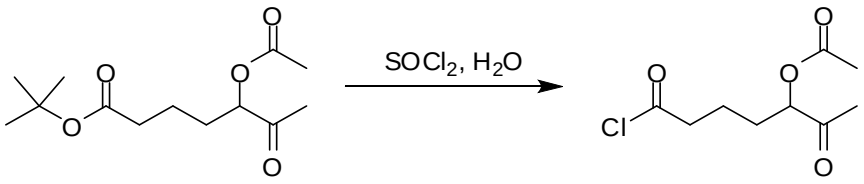
2,2-Diphenylacetyl Chloride (**2.112b**). This compound was prepared according to the general procedure to provide **2.112b** as an off-white amorphous solid (0.064 g, 91%). ^1H and ^{13}C NMR are in accord with those reported in the literature.[65]



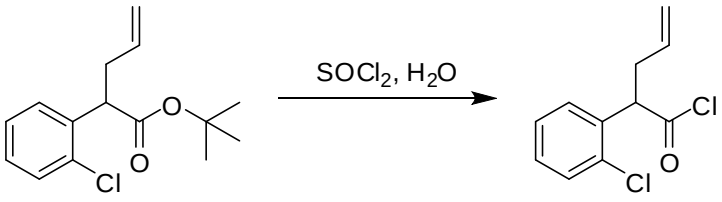
(R)-2-(1,3-Dioxoisoindolin-2-yl)-3-methylbutanoyl Chloride (**2.108b**). This compound was prepared according to the general procedure to provide **2.108b** as a white solid (0.070 g, 95%). m. pt. = 117-119 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.82 (dd, *J* = 5.5, 3.0 Hz, 2H), 4.76 (d, *J* = 8.4 Hz, 1H), 2.77 (dhept, *J* = 8.5, 6.8 Hz, 1H), 1.18 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 167.1, 134.8, 131.5, 124.1, 77.2, 66.5, 29.3, 20.5, 19.2. IR (thin film): C=O, 1805, 1784, 1723 cm^{-1}; [α] = 101.6°; HRMS (TOF-ESI) *m/z* calc'd for $C_9ClH_{13}NO_4$ $[M-Cl]^+$, 230.0817; found, 230.0811.



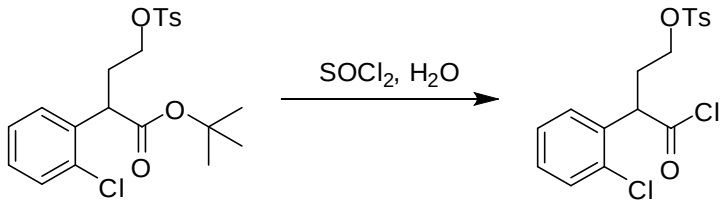
(R)-methyl 2-(1,3-dioxoisoindolin-2-yl)-3-methylbutanoate (**2.108c**). Compound **2.108b** (0.026 g, 0.098 mmol,1 equiv) dissolved in DCM (0.5 mL), was added via cannula to a solution of MeOH (.040 mL, 0.98 mmol, 10 equiv), Hünig's base (0.025 mL, 1.5 equiv) in DCM (0.1 M). The reaction was left to stir for 1h until there was no change by TLC. Reaction was diluted with 1:1 hexanes:EtOAc then subsequently washed with sat. sodium bicarbonate (2x), brine, dried of over $MgSO_4$, filtered, and concentrated to obtain crude material as a colorless oil. The crude product was purified via flash chromatography (10:1 hexanes/EA) to provide **2.108c** as a colorless oil (0.022 g, 85% yield). ^1H and ^{13}C NMR are in accord with those reported in the literature.[66]



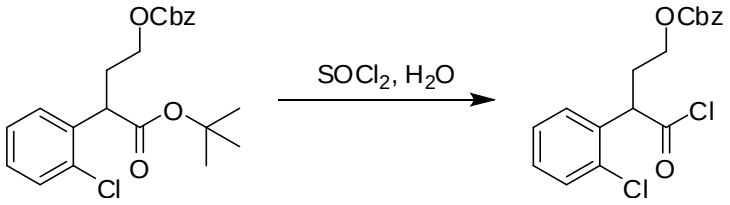
7-Chloro-2,7-dioxoheptan-3-yl Acetate (**2.109**). This compound was prepared according to the general procedure to provide **2.109** (0.093 g, 95%) as a brown oil. ^1H NMR (500 MHz, $CDCl_3$) δ 5.02 (m, *J* = 7.5, 4.0 Hz, 1H), 2.97 (t, *J* = 6.8 Hz, 2H), 2.19 (s, 3H), 2.18 (s, 3H), 1.93 – 1.76 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.6, 173.3, 170.4, 77.7, 46.3, 28.6, 26.1, 20.7, 20.6; IR (thin film): C=O, 1799, 1759, 1729 cm^{-1}; HRMS (TOF-ESI) *m/z* calc'd for $C_9ClH_{13}O_4$ $[M+ Li]^+$, 227.0663; found, 227.0663.



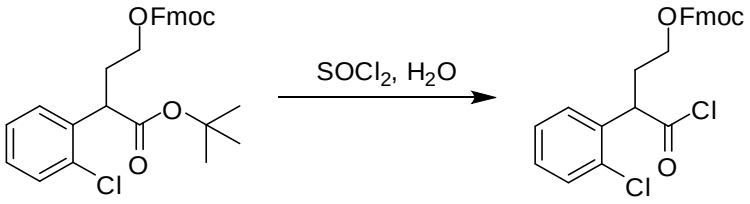
2-(2-Chlorophenyl)pent-4-enoyl Chloride (**2.110b**). This compound was prepared according to the general procedure to provide **2.110b** (0.082 g, 98%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.40 (m, 1H), 7.32 – 7.22 (m, 3H), 5.70 (ddt, *J* = 17.0, 10.2, 6.9 Hz, 1H), 5.12 – 5.03 (m, 2H), 4.66 (apparent t, *J* = 7.4 Hz, 1H), 2.92 – 2.84 (m, 1H), 2.62 – 2.53 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 134.4, 133.7, 133.1, 130.1, 129.5, 129.0, 127.4, 118.5, 59.0, 36.6; IR (thin film): C=O 1793 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{11}Cl_2H_{10}O$ [M+ Li]$^+$, 234.0260; found, 234.0265.



4-Chloro-3-(2-chlorophenyl)-4-oxobutyl 4-Methylbenzenesulfonate (**2.113b**). This compound was prepared according to the general procedure with extended reaction time to 5 h to provide **2.113b** (0.042 g, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.0 Hz, 2H), 7.42 – 7.37 (m, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.30-7.22 (m, 2H), 7.16 – 7.11 (m, 1H), 4.60 (X of ABMNX, *J* = 7.8, 6.7 Hz, 1H), 4.06 (N of ABMNX, *J* = 10.3, 6.4, 5.0 Hz, 1H), 3.88 (M of ABMNX, *J* = 10.3, 7.7, 4.7 Hz, 1H), 2.51 (B of ABMNX, *J* = 14.7, 7.8, 6.8, 5.0 Hz, 1H), 2.43 (s, 3H), 2.15 (A of ABMNX, *J* = 14.5, 7.9, 6.4, 4.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 145.2, 134.4, 133.1, 132.6, 130.6, 130.1, 130.0, 129.7, 128.0, 127.8, 66.8, 55.8, 31.4, 21.8; IR (thin film): C=O, 1796 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{17}H_{16}Cl_2O_4S$ [M+ Li]$^+$, 393.0307; found, 393.0323.

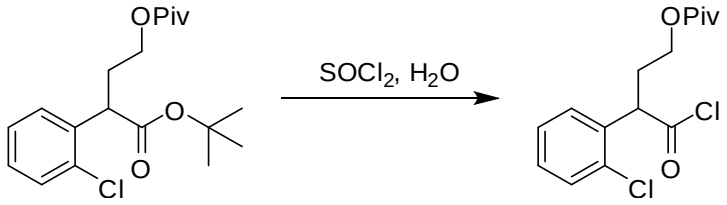


Benzyl 4-Chloro-3-(2-chlorophenyl)-4-oxobutylcarbonate (**2.114b**). This compound was prepared according to the general procedure with extended reaction time to 16 h to provide **2.114b** (0.009 g 91%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (dt, *J* = 6.0, 3.4 Hz, 1H), 7.40 – 7.33 (m, 5H), 7.32 – 7.27 (m, 2H), 7.25 – 7.21 (m, 1H), 5.16 (s, 2H), 4.72 (X of ABMNX, *J* = 8.2, 6.4 Hz, 1H), 4.17 (N of ABMNX, *J* = 11.4, 5.7 Hz, 1H), 4.02 (M of ABMNX, *J* = 11.1, 8.0, 5.1 Hz, 1H), 2.57 (B of ABMNX, *J* = 14.5, 7.9, 6.5, 5.5 Hz, 1H), 2.19 (A of ABMNX, *J* = 14.4, 8.2, 6.0, 5.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 154.9, 135.2, 134.6, 133.5, 130.6, 130.0, 129.4, 128.8, 128.5, 127.9, 77.2, 69.9, 64.7, 56.1, 31.5; IR (thin film): C=O, 1794, 1747 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{16}Cl_2O_4$ $[M+Li]^+$, 372.0577; found, 372.0576.

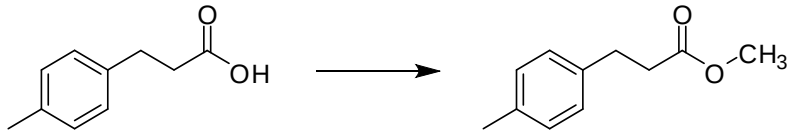


(9H-fluoren-9-yl)methyl 4-Chloro-3-(2-chlorophenyl)-4-oxobutanylcarbonate (**2.115b**). This compound was prepared according to the general procedure to provide **2.115b** (0.016 g, 94%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (dt, *J* = 7.5, 0.9 Hz, 2H), 7.62 (ddd, *J* = 7.5, 2.2, 1.0 Hz, 2H), 7.49 – 7.45 (m, 1H), 7.43 – 7.40 (m, 2H), 7.37 – 7.29 (m, 4H), 7.27 – 7.23 (m, 1H), 4.77 (X of ABMNX, *J* = 8.2, 6.5 Hz, 1H), 4.42 (B of AB, *J* = 1.6 Hz, 1H), 4.40 (A of AB, *J* = 1.0 Hz, 1H), 4.20 (N of ABMNX, *J* = 11.3, 5.7 Hz, 1H), 4.05 (M of ABMNX, *J* = 11.1, 8.0, 5.1 Hz, 1H), 2.61 (B of ABMNX, *J* = 14.6, 8.1, 6.5, 5.4 Hz, 1H), 2.22 (A of ABMNX, *J* = 14.4, 8.2,

6.0, 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 155.0, 143.4, 143.4, 141.4, 134.7, 133.5, 130.6, 130.0, 129.3, 128.1, 127.9, 127.3, 127.3, 125.3, 125.3, 120.2, 70.1, 64.8, 56.1, 46.9, 31.5; IR (thin film): C=O, 1791 cm^{-1}; HRMS (ESI-TOF) *m/z* calc'd for $C_{25}H_{20}Cl_2O_4$ $[M+Li]^+$, 461.0899; found, 461.0902.



4-Chloro-3-(2-chlorophenyl)-4-oxobutyl pivalate (**2.116b**). This compound was prepared according to the general procedure to provide **2.116b** (0.036 g, 95%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 – 7.44 (m, 1H), 7.35 – 7.27 (m, 3H), 4.76 (X of ABMNX, *J* = 7.1 Hz, 1H), 4.14 (bs, 1H), 4.00 (bs, 1H), 2.54 (bs, 1H), 2.17 (bs, 1H), 1.23 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.5, 174.1, 134.5, 133.6, 130.5, 129.9, 129.1, 127.8, 61.4, 56.3, 38.9, 31.8, 27.3; IR (thin film): C=O 1793, 1729 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{15}H_{18}ClO_3$ $[M-Cl]^+$, 281.0945; found, 281.0934.



Methyl 3-p-Tolylpropanoate (**2.117**) Starting material (2.010 g, 12.8 mmol, 1 equiv) was dissolved in MeOH (130 mL) and H_2SO_4 (100 μL) was added to the reaction. The reaction was stirred for 16 h at room temperature under a nitrogen atmosphere. Reaction was neutralized with NaOH (1 pellet), as the reaction neutralized a white precipitate crashes out of solution. The reaction was filtered over a Buchner funnel with hexanes:EA (10:1) to remove solid. The reaction was

concentrated to obtain product **2.117** (1.768 g, 78% yield) as a white solid. ^{1}H and ^{13}C NMR are in accord with those reported in the literature. [67]

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