Aziridinations in aqueous solutions using DNA templating; Towards sustainable asymmetric catalysis

Sydnee Elmore

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Aziridinations in aqueous solutions using DNA templating;
Towards sustainable asymmetric catalysis

By
Sydnee D’Laine Elmore

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in the Department of Chemistry

Mississippi State, Mississippi
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Aziridinations in aqueous solutions using DNA templating;

Towards sustainable asymmetric catalysis

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Modern organic synthesis typically centers around the use of expensive, complex, homogeneous catalyst systems in organic solvents which often generate copious amounts of hazardous waste. Therefore, the development of water-tolerant catalysts capable of performing reactions in aqueous solutions has become a growing area of scientific inquiry. To this end, we have designed and optimized a water-stable catalyst (Mn[TMPyP₄]I₅) capable of generating aziridines from olefins in aqueous solutions. Aziridines are valuable synthetic building blocks that have been used to generate various biologically active compounds, though synthetic techniques for aziridine synthesis are not well-established. Our ultimate goal was developing a catalytic system, which could be paired with DNA in order to perform asymmetric transformation in aqueous solutions. Herein we report the optimization of reaction conditions using Mn[TMPyP₃]I₅ paired with various DNA types, in the hopes of generating chiral aziridines from several olefinic substrates.
DEDICATION

If I could, I’d write a book solely devoted to the people who made this accomplishment possible, but unfortunately, I’m not eloquent enough to do them justice. So, this will have to suffice…

TLDR: Carl, Mom, Mammaw, Grandma

After finishing my Associates degree in English in 2012, I decided to take some time off from school, and worked full time as a waitress. If you know anything about the restaurant business, you know it’s a literal cesspool for bad habits, binge drinking, and petty drama. I was pretty much estranged from most of my family during this period, which only exacerbated my bad habits and terrible coping mechanisms. My entire family was convinced I’d never go back and finish my Bachelors if I continued down the path I was on—can’t say I blame them, but I knew I’d prove them wrong, I just needed to figure some things out the hard way first. I was in a hole, a hole I’d dug for myself and I’d cut all the lines with the only people who could help pull me back out. Not to say I regret any of these choices, but surely, if I hadn’t been so utterly pigheaded, I could have had an easier time getting back on my feet. I knew I’d go back to school, I really did, but I also knew I’d do it on my own terms.

Fast forward to late 2013, I was trying to make amends with my family, so I went home for Thanksgiving. I couldn’t take my dog, Maggie, with me, so I had to drop her off with a friend for the day. It did NOT go well. Immediately when Maggie met my friend’s two dogs, full-blown chaos erupted. Knowing I had to break them up, I heedlessly shove my arm in to grab my dog, and thus
became collateral damage. Showing up to Thanksgiving lunch with a dog bite wound on my arm was not exactly the “Hey family, nice to see you again” I had planned on.

Soon after, I went to the health department to get a tetanus shot, and decided I might as well get my medical records while I was there. At this point, I didn't tell anyone what my plan was. Once I got my records, I applied to start at MSU in Spring 2014, and going out on a limb, declared my major as chemistry. The day of orientation I asked my mom to come over to Starkville, without giving her any real reason, and if I could have captured the look on her face when she realized I’d finally decided to get my life back on track, I would have. I was terrified to start school again, especially as a chemistry major, and for the next 5 years, I dealt with my fair share of imposter syndrome. But in the end, I realized that if you love something enough, even if you aren’t the best or smartest, even if you struggle in every single class you take and wonder constantly “what am I doing here”, if you love it….you’ll do whatever it takes to make it a reality.

Now I say all that to preface to what comes next—I promise I’ll get to the thanks you’s soon!

When I started at MSU, I answered a Craigslist ad for a random roommate since I wouldn’t be able to afford living alone while dividing my time between school and work. I had my apprehensions about it, but I knew the roommate through mutual friends and just hoped it would work out, atleast temporarily, until I could figure out another option. Now as if everything that happened up to this point wasn’t absolutely insane enough, the roommate became my best friend and confidant. We bonded over our love for Miyazaki films and Skyrim among other things. We spent most nights cooking together, playing video games, talking about anything and everything we could come up with. I’d be up doing calculus homework til 5am, and he’d stay up with me until he finally couldn’t hold his eyes open any longer. He pushed me to do better, to be better, and to do it for myself and
to realize that even though I was struggling, the struggle itself was progress. We’ve been dating for almost 6 years now, and to say that answering that Craigslist ad was one of the best decisions of my life would be an understatement.

I would be an idiot to dedicate this work to anyone but you, Carl. You have loved me and supported me when I couldn’t love or support myself. You have sacrificed without question or reward on so many occasions it’s hard to keep track. When I wanted to leave for a month for Study Abroad, but had my own apprehensions about leaving for so long, you told me “Don’t think about any of that, we’ll be ok here, you have to do this; if you don’t go, you’ll never forgive yourself”. When I didn’t know if I’d cut it as a chemistry major (much less a chemistry grad student), you held me up and made me realize I was worthy of the challenge. Looking back, I don’t know if I was worthy because I was so stubbornly persistent, or because you made me realize that if I loved something, it was possible to make it real. You are the kindest, most selfless, tenacious, hardworking, hilarious person I’ve ever had the privilege of knowing and none of this, absolutely NONE of this, would have been possible without you by my side every step of the way. I can’t thank you enough for all you do and all you’ve done. Shekh Ma Shieraki Anni, I love you 13 million.

Mom—When I think about how to define compassion, empathy, and perseverance, I think of you. I can’t begin to explain how grateful I am to be your daughter, and how honored I am to make you proud. Without your love, I could never have done any of this. Without your love, I wouldn’t be the person I am today. Thank you for always nurturing my curiosity, for giving me my weird humor, for all the good music and many many concerts, and mostly, for always being my biggest fan, even if I didn’t deserve it. We may not have always seen eye to eye, but in spite of that, I have never once doubted your endless, unfathomable love for me. Things weren’t always perfect between us, but as you know… “It went the way it had to, the way it was always going to.” I will never be
able to thank you enough for putting up with me, and pushing me to be who I was always capable of being.

Mammaw and Grandma—I feel like anything I say to you two wouldn’t do it justice. To the toughest, kindest, funniest women I know, I can’t thank you enough. Anyone would be lucky to have one amazing grandmother, but I hit the lottery when I was graced with two. I’m so grateful that you were both with me through it all, to Mammaw for being here in person, and to Grandma for being my angel. Without you both, I’d have never picked up all those broken pieces and tried to start putting them back together again. Thank you for being mine.
ACKNOWLEDGEMENTS

I was very lucky to have a terrific support system during my years at MSU, both from my advisor and my professors, as well as the friends I’ve made along the way; this work wouldn’t have been possible without their encouragement and input. I can say with some degree of certainty that I would have left grad school after the first year if Dr Joseph P. Emerson hadn’t taken a chance on me. The first time I sat down to talk with him, he listened to me air my grievances and concerns about my future for 2 hours, and on many occasions since then, he’s done the same. I was worried I wasn’t cut out for graduate school because I lacked the research experience of my peers, but Dr Emerson took the time to foster my interests and help me develop the synthetic skills necessary for me to be a competent chemist. I’d also like to thank my other committee members, Dr Xin Cui, Dr Todd Mlsna, and Dr Sean Stokes, for their support and interest in my research throughout the course of my graduate career. They were always helpful and attentive to my questions and concerns, and their input was invaluable in shaping the direction of this work. In addition, I’d like to acknowledge Dr Whitnee Nettles, for all her expertise, encouragement and kindness; her drive to make us the best educators possible was infectious and I’m lucky to have had the opportunity to work closely with her during my TA duties. I’d like to also thank Dr David Wipf for giving me kind advice when I was struggling with the pressures of graduate school, especially during my first year.

Grad school has been one of the most stressful and mentally taxing periods of my life, and the people you surround yourself with can have a major impact on your grad school experience. Without the incredible friends I’ve made along the way, I’d have lost my mind a long time ago.

To Danny—Little did we know when we walked into into Analytical lab as undergrads that being lab
partners would become our permanent dynamic. I can honestly say I wouldn’t have made it here without you. Thanks for the endless nights of studying for Zhang’s and Scott’s exams, for always taking the time to explain things to me (even if I’m slow on the uptake), and for giving me a place to belong in Emerson’s lab. Looking back, I never could have predicted you’d become one of my best friends, both within school and without, and I can’t thank you enough for always being there, even through the worst of the worst. PS... “APE HANDS!” I’d also like to thank my other labmates, Kayla D. McConnell, Henry U. Valle, Prakash Khanal, ASM Saem, Zhenyu (Tony) Zhu, Ranganath Wahalathantrige Don, and James Cope, for making grad school a little more enjoyable.

Finally, I would be remiss if I didn’t acknowledge my cat, Noodle, for all the company and cuddles during all those late night study sessions. Additionally, Thom Yorke and Ramin Djawadi for basically giving me an endless playlist of study music, and George R. R. Martin for giving me an entire world to escape into when the pains of grad school became overwhelming. Valar Morghulis.
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CHAPTER I
INTRODUCTION

1.1 Sustainable Organic Synthesis

Modern organic synthesis centers around the use of homogeneous catalysts in organic solvents, which generates copious amounts of hazardous waste. Organic solvents have been a staple of synthesis throughout the last 150 years, though recent global legislation has restricted their heavy use due to concerns over their environmental impact. A major source of organic pollution can be attributed to excessive solvent use, the resulting waste, and its inadequate disposal. One new idea is to develop or adapt water-tolerant catalysts capable of performing reactions in aqueous reaction media, where the process of organic synthesis can become more sustainable and less environmentally hazardous.

1.1.1 Organic Solvents

Organic solvents are often highly flammable and volatile and the improper disposal of these chemicals can lead to increased levels of air and water pollution. In addition, human exposure to such solvents can have various detrimental health effects. In recent decades and with the global shift towards more environmentally conscious processes, numerous examples of organic synthesis performed in aqueous solutions have been described. Due to its natural abundance and environmentally benign properties, water has recently been used recently to perform reactions, which traditionally require organic solvents.
Water is one of the most abundant molecules of the surface of our planet. It is considered to be environmentally compatible, nontoxic, nonflammable solvent that possesses many unique properties. Its low viscosity and high polarity can aid in the extraction and purification of organic products. Though water offers many advantageous properties, its usage as a solvent has been sparse due to the generally low solubility of organic materials in this medium.

1.1.2 Catalysis in Water

Catalysts can be used to lower activation energy thereby accelerating organic reaction rates. Catalytic reactions contribute to sustainable chemistry as they streamline synthetic pathways and reduce waste from stoichiometric methods. Over the last two decades, much research has focused on carrying out catalysis in water instead of typical organic solvents.

Since Breslow’s report illustrating that the Diels-Alder reaction in water showed increased rate and selectivity, catalytic reactions in water have become more common.(1) The use of aqueous solutions as a solvent for catalysis greatly lends itself to sustainable organic synthesis by offering a lower cost solvent which has a reduced environmental impact over traditional organic solvents. Several classes of catalysts have been studied over the past decade using water as a reaction medium, including nonmetal and metal-based catalysts, as well as metal nanoparticles.

Various metal-based catalysts have also shown good catalytic activity in water since multidentate ligands, such as salen-type ligands and porphyrin ligands, provide an effective means of protecting the metal center from hydrolysis.(2) First row transition metals are commonly used as they capable of adopting various oxidation states and are relatively cheap and abundant. Additionally, transition metal catalysts can provide high selectivity by limiting access to the reactive center, which leads to both regio- and enantioselectivity through ligand effects. Together these processes offer a more direct means of controlling product formation.
In 2002, Jacobsen and coworkers were able to form chiral epoxides and the corresponding chiral diol from a racemic mixture of epoxides in water using a hydrodynamic resolution catalyst, in this case a Co(salen) complex. (3) (Scheme 1.1) Adequate yields of isolated epoxidation products with high enantiomeric excess for terminal epoxides were reported. However, conjugated epoxides such as butadiene monoeoxide gave lower selectivity. (3) Later, Jacobsen and coworkers investigated a broader range of substrates including conjugated, aliphatic, and halogenated terminal epoxides in addition to epoxides containing various functional groups such as ethers, esters, and ketones. (4) They report decent yields and very high enantioselectivity for both the chiral epoxide and chiral diol. (4) A major drawback of this synthetic process is that the conversion of substrates is divided between formation of the epoxide and diol products.

Scheme 1.1  Jacobsen’s Co(III) hydrodynamic resolution catalyst
Manganese-dependent catalysts, often bearing porphyrin or salen ligands, can exhibit oxidation states from +2 to +7 giving them a wide range of redox activities. (2) Manganese-substituted sulfonated Halterman’s porphyrin ([Mn(HaltS)]Cl) is an example of a chiral water-soluble porphyrin used to generate asymmetric epoxides from hydrogen peroxide. Using Halterman’s porphyrin in water/methanol and buffer/methanol solvents, Srour and coworkers report high conversions and reasonable enantiomeric excess. Using only water as a solvent, 100% conversion was still obtained, but required slightly longer reaction times and was less enantioselective. (5) (Figure 1.1)

Figure 1.1 Manganese-substituted sulfonated Halterman’s porphyrin ([Mn(HaltS)]Cl)

In 2003, Xia and coworkers found that chiral Mn(salen) derivatives in water were capable of forming ketones and chiral secondary alcohols from racemic secondary alcohols. (6) They reported low to moderate (25-62%) conversions and variable (2-88%) enantioselectivity of the secondary
alcohols due to the insolubility of substrates in water. In order to overcome this, they used tetraethylammonium bromide as a phase transfer catalyst and achieved slightly improved yields and enantioselectivity. (6) (Scheme 1.2)

Along with various other metal-based catalysts, supported metal hybrid catalysts have also attracted attention recently for their potential use as durable heterogeneous catalysts. They offer high reusability, large surface area and durability making them promising catalysts for reactions in water.

Scheme 1.2   Xia et al Mn(III) salen derivative catalysts used for synthesizing chiral secondary alcohols

1.2   Aziridines

1.2.1   Three-Membered Ring Systems

Three-membered rings, such as cyclopropanes, epoxides, and aziridines are highly valued molecules in modern organic chemistry. Due to the highly strained nature of these ring systems,
they offer a balance between reactivity and stability, making them important synthetic precursors in multistep syntheses. The molecular tension caused by their unfavorable bond angles is a major contributing factor to their rich chemical reactivity.

![Common examples of three-membered ring systems](image)

Figure 1.2 Common examples of three-membered ring systems

Three-membered ring systems containing only carbon atoms are referred to as cyclopropanes, while three-membered heterocycles containing an oxygen atom or a nitrogen atom are called epoxides and aziridines, respectively. (Figure 1.2) While cyclopropanation and epoxidation chemistry have been thoroughly studied, the synthesis of aziridines remains a challenging and open area of chemical research. Due to the considerable difficulty of their synthesis, aziridines have generated far less attention than other three-membered ring systems. New methods for generating chiral aziridines from olefins in aqueous solutions will be the focus of this thesis.

Aziridines are considered to be valuable synthetic intermediates, which can be used to generate various biologically active compounds due to their propensity to ring-open in the presence of various nucleophiles. In addition, there are a growing number of biologically active compounds that contain an aziridine functional group. Instances of biologically active aziridine-containing molecules are the Azinomycin family derived from *Streptomyces griseofuscus* S42227 and the Mitosanes family derived from *Streptomyces verticillatus*. The pharmacological activity of both drugs is due
in part to the presence of the aziridine ring, which when in the presence of a nucleophile is capable of modifying biological entities such as DNA via DNA cross linking and alkylation reactions. (11-14) In addition, these drugs have shown strong antitumor activity against solid tumors and other cancers. (10) (Figure 1.3)

Figure 1.3  Examples of biologically-active compounds containing aziridines

1.2.2  Physical Properties

The most fundamental class of saturated nitrogen containing heterocycles, aziridines have been difficult to synthesize in satisfactory yields. (17-20) Many aziridines are typically colorless, distillable, and tend to be water-soluble. (21,22) The earliest instance of aziridine synthesis is thought to be in 1888 when Gabriel demonstrated the formation of aziridines from 2-bromoethylamine under basic conditions. (23) (Scheme 1.3)
The delocalized π-like nature and strong C-H and N-H bonding interactions present in cyclopropanes and aziridines can be attributed to the bending of the inner bonding orbitals which reduces the bond strain and distorts the traditionally sp$^3$ hybridization of these atoms to something between sp$^2$ and sp$^3$. (24-27) The distortion of the bonding hybridization contributes to the unique reactivity of these ring systems and causes the aziridinium ion (pKa=7.98) to exhibit stronger basicity than related arylamines but weaker basicity than alkylamines. (17,22,24,28-31)

Aziridines react with alkylating agents in a similar fashion to secondary amines. Due to the increased strain caused by the trigonal ring system, the energy barrier for inversion of the N-H bond is increased when compared to other acyclic compounds. (17,31,32) For instance, the inversion energy of the N-H in 2-methylaziridine (~70 kJ/mol) is much greater than other secondary amines, but still not high enough to prevent racemization at room temperature. (17,31,33,34) By changing the electronics of the aziridine via substitution on the N atom, the inversion barrier can be raised. For example, substituents on the nitrogen atom can generate aziridines which are stereochemically stable at room temperature. This substitution grants increased stability and allows for the separation of substituted aziridine enantiomers. (17,35)

The unique electronic and structural features of aziridines grant high chemical reactivity in addition to the capacity for C-N bond cleavage under both acidic and basic conditions. (36-38) The substituents on the nitrogen atom can dramatically impact the reactivity of the aziridines. Therefore, aziridines are typically separated into “activated” and “non-activated” species based on whether
nucleophilic ring opening reactions proceed in the absence of a positive charge on the nitrogen, and which is closely tied to the nature of the substituents on the nitrogen atom. Substituents on the nitrogen atom can also stabilize the amide anion formed during nucleophilic ring opening reactions, where electron withdrawing groups stabilize the anionic transition state which makes the aziridine more apt to undergo nucleophilic attack. Protonation of the nitrogen is often the easiest method of activating aziridines, however this technique is not practical for more basic nucleophiles, such as carbon-centered nucleophiles.

![Common functional groups used to activate aziridines](image)

**Figure 1.4** Common functional groups used to activate aziridines

Oxygenated functional groups such as carbonyls, sulfonyls, phosphinyls, and phosphoryls are common activating groups for aziridines. There is very little resonance between the nitrogen’s non-bonded pair and the X=O bond due to the increased angle strain on the aziridine ring in the amidate-like resonance structure of this moiety. Inductive effects are the driving force in ring opening reactions due to this ring strain. The nature of these substituents alters
the polarization of the C-N bonds within the ring structure. Nitrogen is more electronegative than carbon, additional electron withdrawing groups tend to further weaken the C-N bond strength.(17,51-53) During ring cleavage, the amide-like anion is stabilized primarily by the inductive effects of the sulfonamide, phosphinamide and phosphonamide transition states while the carbonyl groups stabilize the anion through resonance.(54-58) (Figure 1.5) However cleavage of aziridine rings bearing more electrophilic acyl groups by carbon-centered nucleophiles is uncommon and instead proceeds through acyl transfer to the nucleophile.(17, 59)

Figure 1.5 Stabilization of amide anion using oxygenated functional group

N-sulfonyl groups are often used to activate the aziridine ring in both ring cleavage and ring forming reactions. The ability for sulfonyl groups to stabilize the negative charge facilitates ring cleavage while the tendency for N–sulfonyl aziridines to be more stable when compared to N-H aziridines allows for facile ring formation. These substituted aziridines are typically highly crystalline compounds making them well suited for large-scale production Jacobsen’s Co(III) hydrodynamic resolution catalyst and isolation, although the N-S bond can be cleaved under mild conditions. Due to this limitation, the use of this group of aziridines as synthetic intermediates must be carefully conducted. Tosylsulfonate (Ts) groups can be removed from the aziridine under strong acidic
conditions or under reductive conditions. (60,61) Other modified sulfonamide groups such as nitrobenzene sulfonamides, 2,2,5,7,8-pentamethylchroman-6-sulfonamide, and β-
(trimethylsilyl)ethylsulfonyl (SES) groups can be used to mitigate this limitation. (17,62-65) A number of alkyl ring cleavage reactions have been reported using N-sulfonyl protected aziridines, but it is also necessary to protect the electrophilic groups on the target molecule during these reactions. (66,67)

Due to these constraints, it is important to develop an appropriate catalyst system for aziridine synthesis. The possibility of unprotected N-H product formation in either aziridine products or the ring-opened byproducts should also be considered. It is crucial to choose a nitrogen substituent that is appropriate for the reaction conditions, in addition to planning for further reactions aimed at ring opening or expanding these products.

1.2.3 Mechanisms to Generate Aziridines

Aziridines can be produced using several synthetic methods including α-elimination of metal halides from metal N-arenesulfonyl-N-haloamines, decomposition of organic azides, oxidation of primary amines, and α-elimination of HXN from an amide or amine. (Scheme 1.4)
1.2.3.1 1,2-azidoalcohols/1,2-aminohalides

1,2-Azidoalcohols can be used to form aziridines. These azidoalcohols can be readily made by reacting epoxides with sodium azide. This method of preparation is of great appeal as enantiomerically pure epoxides are widely available. The Staudinger reaction has garnered increased attention and is one example of this technique, which demonstrates N-heterocyclic ring formation of azidoalcohols, catalyzed by phosphines. This reaction is used to form aziridines from chiral epoxides via ring opening with an assortment of azide sources. This method utilized triaryl- or trialkylphosphines reacted with a hydroxyazide to form an oxazaphospholidine species, which is then heated in acetonitrile to eventually form the unprotected aziridine. The reaction shows promise as a wide range of achiral and chiral epoxides can be used to generate the stereochemically
related aziridine products. Both asymmetric centers are predictably and neatly inverted during this process. (Scheme 1.5)

![Scheme 1.5](image)

**Scheme 1.5** Staudinger reaction for generating chiral aziridines

The oldest reported instance of reactions forming aziridines used 1,2-aminoalcohols and 1,2-aminohalides.(69) Reported by Gabriel in 1888, this two-step method of forming aziridines used thionyl chloride to chlorinate ethanolamines then proceeded through an alkali-mediated cyclization. In 1935, Wenker developed another method for aziridine synthesis, which used six hundred grams of ethanolamine in more than one kilogram of 96% sulfuric acid at high temperature. This process yielded two hundred eighty-two grams of the cyclized intermediate, $\beta$-aminoethyl sulfuric acid. The $\beta$-aminoethyl sulfuric acid was then distilled from aqueous base to yield 23 grams of the aziridine product. The intermediate that leads to product formation is unclear, and any substitution alpha to the hydroxy group leads to a mixture of elimination of cyclized product.(70) Because of this, the range of aminoalcohols that can be used for aziridine formation is limited. From this preliminary reaction, an extensive scope of conditions for activating the hydroxy group have been developed,
including Mitsunobu-like oxyphosphonium, allowing for a wider range of enantiomerically pure and achiral aziridines.\(^{71,72}\)  (Scheme 1.6)

Scheme 1.6  Wenker aziridine synthesis, shown with possible intermediates

### 1.2.3.2  Bromoacrylates

The Gabriel-Cromwell reaction is another method for synthesizing aziridines can be performed by reacting amines with a wide array of chiral α-bromoacrylates and related derivatives. Using this method, even ammonia is capable of acting as a nitrene source to form chiral unprotected aziridines.\(^{71}\)  (Scheme 1.7)

Scheme 1.7  Gabriel-Cromwell aziridination reaction
1.2.3.3  Imines

When using a nitrene source for aziridine synthesis, it is typically necessary to form two C-N bonds concurrently. Alternatively, aziridines can be formed by reacting an imine with either a carbene or an ylide to form a C-N and a C-C bond. This method of synthesis has only recently gained consideration.(17) In 1995, Jacobsen and Finney achieved acceptable stereoselectivity for the addition of metallocarbenes to N-arylaldimines.(73) Ethyl diazoacetate was reacted with copper(I) hexafluorophosphate to form the metallocarbene species. They reported decent diastereoselectivities and a 44% enantiomeric excess.(73) A related copper(I) catalyzed reaction reported increased enantioselectivity (72%). This was achieved by reacting N-tosylimines with trimethylsilyldiazomethane in the presence of (R)-Tol-BINAP.(74) (Scheme 1.8) In 2000, Wulff and coworkers investigated a different approach to this reaction.(75) They report the use of a “vaulted” axilly-chiral, boron-based Lewis acid to catalyze the addition of ethylidiazooacetate to an imine capable of very high enantioselectivity.(75)

![Scheme 1.8](image.png)

Scheme 1.8  Cu(I) catalyzed aziridine synthesis using (R)-Tol-BINAP

Another method for generating aziridines is carbene transfer to sulfur or iodine ylides.(76) Ylides formed from β-sulfonium or β-iodonium amide anions can produce azirdines via ring closure.
Recently, Aggarwal and coworkers have expanded the use of sulfonium amide anions for aziridine synthesis by employing chiral sulfides to catalyze the addition of metal carbenoids to imines. This method eliminates the safety hazards associated with using diazoesters by generating them \textit{in situ} from more stable tosylhydrazones and is capable of using a range of $N$-substituted imines such as tosyl, SES, and Dpp.\textsuperscript{(77)} In addition, this method gives high enantioselectivity for a broad scope of aziridination reactions.

\subsection*{1.2.3.4 Nitrenes}

Nitrene transfer reactions are another method for synthesizing aziridines and amines. Transition metal-dependent catalytic systems have been employed to increase the selectivity and efficiency of these reactions. This type of reaction is generally initiated by the formation of a metal-nitrene intermediate, which in turn attacks olefins to produce the amination and aziridination products. (Scheme 1.9) After the nitrogen is transferred to the substrate, the catalyst can be regenerated allowing for effective turnover. (Scheme 1.10)

\begin{center}
\textbf{Scheme 1.9} \hspace{0.5cm} Metal-catalyzed nitrene transfer to olefins
\end{center}
There are two competing mechanisms for nitrene transfer reactions: a redox and non-redox related pathway. The redox pathway proceeds through the formation of a metal-imido complex by nitrogen transfer to the metal center, thereby changing the metal's oxidation state. The oxidized metal complex then transfers the nitrene group to the substrate. This process reduces the metal complex back to its original nitrene-reactive species or requires an external electron source to allow further reactivity. The stability of the oxidized metal complex is crucial to product formation. For example, if the complex is overly stable, it lowers the efficiency for nitrene transfer resulting in lower yields, while if the complex is too unstable, it can decompose through a non-productive mechanism.

The non-redox mechanism is described as the formation of a metal-nitrene complex without a clear oxidative transfer from nitrogen source to catalyst. The nitrene group is then transferred to the substrate by this metal-nitrene complex. The direct aziridination of alkenes has been carried out by the addition of metal-based nitrenes, M=NR, to unsaturated alkenes, but the harsh reaction conditions and lack of stereoselectivity have been major limitations which have impacted the level of interest in this method.

Typically, nitrene species have been formed by a thermally or photochemically induced decomposition of azides. This method of decomposition can lead to either a singlet or triplet metal-
nitrene species. The singlet species can be thought of as containing two lone pairs of electron in one sp\textsuperscript{2} orbital and one p orbital. The triplet species can be described as containing non-bonded electrons in three orbitals, a lone pair in the p orbital and two electrons, of parallel spins, in each sp\textsuperscript{2} orbital. While the singlet metal-nitrenes tend to be more reactive and react stereospecifically with 1,2-disubstituted alkenes, the triplet metal-nitrenes are more stable. The singlet species forms both C-N bonds concertededly, while the triplet species forms each C-N bond sequentially. (Scheme 1.11)

Scheme 1.11  Reactivity of singlet vs. triplet nitrene transfer

The most common techniques for aziridines synthesis include the intramolecular cyclization of amines, reduction of azirines, carbon transfer to imines, and nitrogen transfer to olefins.(42,75,78-82) Although synthetic methods for epoxidation and cyclopropanation are established, techniques
for preparing aziridines have not been as thoroughly studied. The lack of research into aziridines synthesis has been stymied by the inadequate growth of reliable methods and activated N-transfer reactions.

In 1995, Jacobsen and coworkers reported aziridine synthesis through ring opening of an epoxide.\(^{(83,84)}\) Using Jacobsen’s hydrolytic kinetic resolution catalyst, amines bearing two protecting groups can be reacted with racemic epoxides to form the corresponding amino alcohols. The amino alcohols may then be activated to form the aziridine. They report high yields and enantiomeric excesses, but this reaction only utilizes approximately half of the reactions starting material.\(^{(83,84)}\)

In 2002, Ishihara and coworkers reported formation of aziridines using chiral diaziridines.\(^{(85)}\) They found that aldehyde-based diaziridines could produce the trans-aziridine, while large amide-based diaziridines favored the cis-conformation.\(^{(85)}\) Although this method offers complete diastereoselective control and high yields, the substrate scope is severely limited by the chiral reagent.\(^{(85)}\) (Scheme 1.12)

![Scheme 1.12 Ishihara et al. olefin aziridination using diaziridines](image)

Consequently, transition metal catalyzed nitrogen atom transfer reactions have been demonstrated to be more efficient than the methods established by Ishihara. These transition metal
catalysts provide enantioselectivity by coordination of chiral ligands and offer a more direct means of controlling the aziridines synthesis. While Jacobsen's stepwise ring opening and closing reactions are suitable, nitrogen transfer to olefins does not necessarily require highly functionalized ligands.\(17,83,86\)

1.3 Asymmetric Catalysis

An important aspect of modern organic synthesis is the ability to preferentially form products, whether through enantio-, diastereo- or regioselective transformations. Typically chemical reactions yield a mixture of isomers, and this can be undesirable if a target molecule is a specific isomer. As such, asymmetric catalysis has become a prominent field of scientific inquiry due to its relevance in pharmaceutical development. Selectively forming products, especially enantiopure products, can be a challenging task, however several catalytic systems have demonstrated effective methods for achieving chiral transformations.

1.3.1 Importance of Chirality

Enantiomers are molecules that are non-superimposable mirror images of one another. They rotate plane-polarized light in opposite directions, but by the same degree. Interestingly, enantiomers can often possess very different chemical and biological activities. Living things are exceptionally chiral, and the production of biomolecules by organisms typically yields specific stereoisomers. The response to a particular molecule is often contingent upon both its chemical nature and whether it fits into a given receptor. Differences in chirality can dramatically affect the binding of a substrate to their intended receptor, and can lead to unexpected outcomes when both enantiomers or the wrong enantiomer are present. When designing drugs, it is vital to consider both the active and inactive forms that can arise from these chiral molecules. However, the isolation of enantiomers from racemic mixtures can be difficult and costly. This poses a challenge to organic
chemists when only one form of a compound is desired. As such, methods for synthesizing and isolating enantiopure compounds are of paramount importance in pharmaceuticals and drug development.(87,88)

1.3.2 Traditional Approaches

Traditional approaches to asymmetric chemistry typically involve the use of chiral auxiliaries or chiral catalyst systems. These catalyst systems usually rely on the use of expensive transition metals and complex ligand architectures. Due to this, the use of chiral auxiliaries has gained much attention in recent decades in part due to their ability to mitigate the need for costly catalyst systems. Until recently, satisfactory approaches for asymmetric aziridinations have been less developed than their analogous ring systems like cyclopropanes and epoxides. Significant challenges to develop atom-economical and environmentally friendly catalytic processes with high turnover and selectivity are contributing factors to the lack of progress in aziridination chemistry. However, great advances have been made to prepare these chiral aziridines using various synthetic strategies.
Scheme 1.13  Evans and Jacobsen’s Cu-based nitrene transfer to olefins

In 1993, Evans and Jacobsen reported metal-catalyzed nitrene transfer to olefins. They found that copper, supported by chiral bis(oxazoline) (BOX) or diimine ligands, were capable of performing asymmetric aziridinations in hexane or acetonitrile using [N-(p-toluenesulfonyl)imino]phenyliodonane (PhINTs) as a nitrogen source. These reactions supported yields between 50-70% with very good reported enantiomeric excess (58-97%).(89) Later, they successfully applied this methodology to the total synthesis of various natural and biologically active compounds such as (β)-agelastatin.(90-92) In 2012, Katsuki and coworkers used a chiral Ru(CO)salen catalyst to perform enantioselective aziridinations in CH₂Cl₂. Using sulfonyl azides, they were capable of achieving impressive (up to 99%) yields and excellent enantioselectivity (up to 90%).(93) Although these methods are sufficient for the production of chiral aziridines, the substrate scopes are limited to styrenes and other terminal olefins.
Scheme 1.14  Katsuki *et al.* synthesis of aziridines using chiral Ru(CO)salen catalyst

In 2008, Zhang *et al.* reported the first co-catalyzed asymmetric aziridination of olefins using D$_2$-symmetric chiral cobalt porphyrins. Using diphenylphosphoryl azide (DPPA) as a nitrogen source, this reaction yields the corresponding N-phosphorylated aziridines in good yields and moderate enantioselectivity (up to 53%). This cobalt complex can be applied to a wide range of aromatic olefins and substituted styrene derivatives.(94-96) Following that, in 2017 Schomaker and coworkers developed a method for chemo- and enantioselective intramolecular aziridinations. These reactions were carried out in CH$_2$Cl$_2$ using a silver(I) complex bearing a diverse range of N-chelating ligands. In this case, using homoallylic carbamates, the corresponding di- and trisubstitutes aziridines could be formed in good yields and moderate to excellent enantiomeric excess.(97)
Scheme 1.15  Zhang and coworkers aziridinations using D$_2$-symmetric chiral cobalt porphyrin (Co(TPP))

Despite the efficiency of the above methods, they all possess several disadvantages. Their dependence on costly catalyst systems or chiral auxillaries to perform asymmetric catalysis leaves room for further development. In addition, all reactions necessitate the use of organic solvents, as these catalyst systems are incompatible in other more benign solvents. Due to these issues, some researchers have begun to explore other avenues for achieving asymmetric transformations including studies in aqueous solutions, and the use of biomolecules or hybrid catalyst systems to induce chirality. These new techniques can mitigate some of the expenses associated with traditional approaches and allow for the use of more environmentally benign solvents.

1.3.3  Biomolecule-based Catalysis

Biocatalysis is a rapidly growing field of study, which is driven by the ability for biomolecules, such as enzymes, to operate under mild, aqueous conditions with high turnover frequencies and selectivities. These methods afford sustainable catalytic transformations with a significant reduction in costs and industrial waste when compared to traditional organic methods and stoichiometric syntheses. In 2013, Coelho and Arnold reported that cytochrome P450$_{BM3}$ and
other heme-containing proteins could catalyze the reaction of styrene with ethyldiazoacetate to form cyclopropanes. It is well established that “biomimetic” metalloporphyrin complexes can catalyze various atom transfer reactions.\(^{(98)}\) They furthered their investigations in 2014 by mutating the protein to enhance the selectivity of this system. These variants were capable of performing sulfimidation, intramolecular C-H amination and aziridination reactions. In 2015, Arnold and coworkers also demonstrated an enzyme-catalyzed enantioselective aziridination using active site evolved cytochrome P450. The intermolecular aziridination was carried out using various styrene-based substrates and tosyl azide as a nitrene source. The reaction was capable of rapidly generating chiral aziridines with very high enantioselectivity (up to 99\%).\(^{(98-100)}\)

![Scheme 1.16](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme</th>
<th>% Yield</th>
<th>% ee (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no enzyme</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Pd11BM3-C15-T438S</td>
<td>1.1</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>P-1263F</td>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>P-1263F-A328V</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>P-1263F-L437V</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>6</td>
<td>P-1263F-A328V-L437V</td>
<td>55</td>
<td>43</td>
</tr>
</tbody>
</table>

Scheme 1.16  Arnold et al. active-site evolved cytochrome P450 enzyme catalyzed aziridinations
1.3.4 Hybrid Catalysis

Recently, the development of hybrid catalyst systems has gained much attention. Although similar to biocatalysts, hybrid catalysts combine the catalytic capacity of metal-coordination complexes with the inherent chirality of biomolecules like DNA. These systems offer an alternative route to generate chiral molecules and allow for these transformations to be carried out in water or buffered solutions. The application of DNA-based hybrid catalysts for enantioselective synthesis has only recently emerged, but shows results comparable to their traditional metal-based counterparts. As DNA is one of the most stable and highly organized chiral molecules known in nature, and when paired with metal complexes, allows for a chiral microenvironment to steer molecular interactions in catalysis. Furthermore, these hybrid catalysts systems lend themselves to sustainable transformations by using water and buffered solutions as solvents, which lowers the cost of these processes and reduces excessive solvent waste. The use of DNA for hybrid catalysis also lowers the cost of these synthetic techniques since commercially available DNA are generally inexpensive when compared to other chirality-inducing ligands.

In 2005, Roelfes and Feringa developed a DNA-based hybrid catalyst for asymmetric Diels-Alder reactions. Their copper(II) catalyst was based on the supramolecular assembly of a catalytically active metal complex anchored to a DNA scaffold through a binding, nonchiral ligand. The Cu(dmbpy) and st-DNA based system afforded the highest enantioselectivity for a variety of organic reactions.(101) Furthermore, in 2013, Sugiyama and coworkers showed that cationic silica-immobilized DNA hybrid catalysts provided a recoverable chiral source for highly efficient copper(II)-catalyzed asymmetric Diels-Alder reactions in water. This system shows high reusability (up to 10 cycles) without any substantial decrease in yield or enantiomeric excess.(102) In 2015, Roelfes and coworkers again demonstrated a hybrid catalyst system capable of performing asymmetric catalysis. Using a self-assembled cationic iron(III) porphyrin complex with st-DNA,
they performed asymmetric cyclopropanations with decent yields and moderate ee values. (103) These artificial biocatalysts heavily inspired the work presented in this thesis.

Scheme 1.17  Asymmetric Diels-Alder reactions using Cu(II) DNA-based hybrid catalyst developed by Roelfes and Feringa

1.4  Our Approach

Inspired by the works of Roelfes and others, our goal was to design a catalyst system capable of performing asymmetric catalysis in aqueous media. By utilizing a water-soluble porphyrin-based catalyst, we were able to use DNA as a chiral auxilary to induce enantioselectivity. Porphyrin complexes have already been documented as the active site in many biomolecules in addition to their use as catalysts for the synthesis of various organic compounds. These complexes have known binding affinities for various types of DNA and have previously been used to perform asymmetric catalysis. Our aim is to use Mn[TMPyP₄]I₅ to carry out aziridination reactions in water and buffered
solutions while using DNA to create a chiral microenvironment for inducing chirality. Herein, we report our steps toward optimizing this catalyst system, in addition to various hurdles that should be addressed moving forward.
CHAPTER II
RESULTS AND DISCUSSION

2.1 Mn[TMPyP₄]I₅

[MnTMPyP₄]I₅ was synthesized using a slight modification of the previously reported synthesis by Gros and Kadish (Scheme 2.1).(104) Initial complexation was performed by adding 0.445 mmol of the initial 5,10,15,20-tetra-4-pyridinyl-21H,23H-porphine (1 equivalent) to a 250 mL round bottom flask with 0.51 mmol manganese(II) chloride (1.15 equivalent) and 50 mL of dry DMF. The reaction mixture was covered with aluminum foil to shield the reaction from UV light, then flushed with argon and heated to 90 °C. The reaction was stirred for three hours, then the mixture was cooled to 40 °C and 9 equivalents of methyl iodide were added to the reaction dropwise. The reaction was stirred at 40 °C without exposure to light for twenty-four hours. The reaction mixture was then cooled to room temperature, and the product was precipitated out of the DMF by adding 100 mL of diethyl ether slowly. The resulting solid was collected by filtration, washed with diethyl ether, and recrystallized in hot water/acetone.

The finished product was then characterized by UV-Vis and ESI-TOF-MS. The mass and UV-Vis spectra is shown below (Figures 2.1, 2.2) and match well with those reported by Gros and Kadish. The UV-Vis spectra were taken in both acetonitrile and 18 MΩ water, the UV-Vis spectrum in 18 MΩ water shows absorption transitions consistent with both manganese(III) to manganese(V) species being present in solution; these data are consistent with reported spectral data of [Mn(TMPyP₄)]I₅. A decrease intensity of the 420 nm transition that is typically associated with
manganese(III) species and the increase of the band at 462 nm which is consistent with a manganese(V) species.\textsuperscript{105} It has been shown that the active catalytic species is a manganese(V) species.\textsuperscript{105,106}

Scheme 2.1 Synthesis of Mn[TMPyP\textsubscript{4}]\textsubscript{5} from 5,10,15,20-tetra-4-pyridinyl-21H,23H-porphyrin
Figure 2.1 Experimental and theoretical ESI-TOF-MS of 2.45 μM [MnTMPyP₃]I₅ in 18 MΩ water. [C₄₄H₆₅MnN₈]⁴⁺: Calculated: m/z 182.8105. Found: m/z 182.81107
Figure 2.2  UV-Vis spectra of 2.45 μM [MnTMPyP₄]I₅ in acetonitrile (blue) showing only manganese(III) at 420 nm and 18 MΩ water (red) showing both distinctive manganese(III) species at 420nm and manganese(V) species at 460nm
2.2 Aziridinations in Buffered Solutions

Table 2.1 Preliminary substrate scope in buffered solutions with and without DNA

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Yield (without DNA)\textsuperscript{a,b}</th>
<th>% Yield (with DNA)\textsuperscript{a,c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>styrene</td>
<td>32</td>
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</tr>
<tr>
<td>methylstyrene</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>chlorostyrene</td>
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</tr>
<tr>
<td>methoxystyrene</td>
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<td>&lt; 1</td>
</tr>
<tr>
<td>1-hexene</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>trans-2-hexen-1-ol</td>
<td>59</td>
<td>17</td>
</tr>
<tr>
<td>cyclopentene</td>
<td>71</td>
<td>21</td>
</tr>
<tr>
<td>cyclohexene</td>
<td>93</td>
<td>60</td>
</tr>
<tr>
<td>cyclooctene</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

\textsuperscript{a} based on toluene internal standard (GC-MS analysis)
\textsuperscript{b} in 75 mM KH\textsubscript{2}PO\textsubscript{4} buffer solution (pH 7.0)
\textsuperscript{c} in 75 mM KH\textsubscript{2}PO\textsubscript{4} buffer solution (pH 7.0) with 128µM pUC19

An initial substrate scope including aromatic and aliphatic olefins was performed in buffered solutions at pH 4, 7 and 10. Styrene and \(p\)-substituted styrenes were included to determine if inductive or resonance effects had any bearing on product formation. These reactions contained 0.3 mmol chloramine T trihydrate, 3 mmol substrate, and 0.015 nmol [MnTMPyP\textsubscript{4}]I\(_{5}\) (5 mol\% vs nitrogen transfer agent) in 2 mL of buffered solution within a 4 mL vial. The products were extracting 4 times using 2 mL of diethyl ether, and 10 µL toluene was added as an internal standard. These reactions were analyzed using GC-MS. (Table 2.1) In addition, a substrate scope was
performed in 75 mM KH$_2$PO$_4$ buffer (pH 7.0) using 128 µM pUC19 and products were extracted and analyzed using the same method. For reactions at pH 7 without pUC19, substituents on the styrene derivatives may play a role in the stability of the aziridine product in aqueous solutions, and no real inductive effects were observed. It should be noted that styrene derivatives tended to form a large number of side products, whether through alternative reactions pathways or due to the break down of the aziridine product. The aliphatic substrates tended to have higher yields, and generally formed less side products than the corresponding aromatic substrates, which suggests these products were less prone to ring opening. \textit{trans}-2-Hexen-1-ol was the only olefin that was neither terminal nor in the \textit{cis} confirmation; the yields were comparable to olefins in the \textit{cis} confirmation. This suggests that a wider range of substrates could be used for these reactions. With the addition of pUC19 DNA, similar trends were observed. Again, the aliphatic substrates generally produced higher yields than the aromatic olefins. This suggests again that the aziridines formed from aliphatic substrates are less prone to ring opening. Of the styrene-based olefins, styrene supported the highest yield and was used as a model substrate in further optimization reactions. The aziridination product of styrene was isolated from the reaction solution by column chromatography using 90:10:1 (hexanes:ethyl acetate:triethylamine) and fractions containing only product were combined and solvent removed by rotary evaporation. The products were then recrystallized in diethyl ether/hexanes. The aziridination product of this reaction (N-tosyl-2-phenylaziridine) was characterized by GC-MS and $^1$H-NMR. Some of the crystals were removed from solution and dissolved in deuterated chloroform and analyzed by NMR spectroscopy. (Figures 2.3, 2.4)
Figure 2.3  Mass spectrum of N-tosyl-2-phenylaziridine obtained by GC-MS
Figure 2.4 ¹H-NMR of N-tosyl-2-phenylaziridine in deuterated chloroform. Residual solvent (diethyl ether, in red box) present in the sample.
Traditionally, metal nitrene-based aziridine synthesis has used the highly activated N-tosyliminobenzyliodinane (PhINTs) compound as a nitrogen source. However, there are several drawbacks associated with this compound including high cost, short shelf life, and the tendency to form phenyl iodide as a byproduct. Due to these issues, other nitrogen transfer agents were explored. Organic azides have been used recently as an alternative to PhINTs, but still possess various drawbacks such as their stability and relatively high cost. (99,107,108) Recently, haloamine T’s have been used as a viable low-cost alternative. Typically, nitrene transfer reactions require high excess of olefin compared to nitrogen transfer agent. However, studies using haloamine T’s, such as chloramine T, have demonstrated higher yields with the nitrene transfer agent in excess. (108,109) Both chloramine T and bromamine T are highly water-soluble, inexpensive, environmentally-benign, and are capable of catalyzing nitrene transfer reactions efficiently. For our purposes, we compared a set of nitrogen transfer agents, including PhINTs, tosyl azide (TsN₃), and chloramine T, to determine which works best with our system. These reactions were performed in pH 4 buffer as well as in pH 7 buffer with 128 µM pUC19 DNA, and contained 3 mmol of styrene, 0.3 mmol of

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Nitrogen transfer</th>
<th>% Yield (without DNA)ᵃᵇ</th>
<th>% Yield (with DNA)ᵃᶜ</th>
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<tr>
<td>styrene</td>
<td>chloramine T</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>styrene</td>
<td>PhINTs</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>styrene</td>
<td>TsN₃</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

ᵃ based on toluene internal standard (GC-MS analysis)
ᵇ in 75 mM NaC₂H₃O₂ buffer solution (pH 4.0)
ᶜ in 75 mM KH₂PO₄ buffer solution (pH 7.0) with 128 µM pUC19

Table 2.2 Determination of nitrogen transfer agent in buffered solutions with and without DNA
nitrogen transfer agent (chloramine T, N-tosyliminobenzylidinane, or tosyl azide), and 0.015 nmol Mn[TMPyP₄]I₅ (5 mol % vs N-transfer agent) were added to 2 mL buffer. (Table 2.2) These reactions were extracted and analyzed by the same protocols as previously stated above. Both with and without DNA, chloramine T gave the highest yields and was chosen as the nitrogen transfer agent for this study.

Table 2.3  Catalyst loading study in buffered solutions with and without DNA

<table>
<thead>
<tr>
<th>Catalyst (Mn[TMPyP₄]I₅)</th>
<th>% Yieldᵃᵇ</th>
<th>% Yieldᵃᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>34</td>
<td>29 (&lt; 1)</td>
</tr>
<tr>
<td>1%</td>
<td>55</td>
<td>23 (&lt; 1)</td>
</tr>
<tr>
<td>2.5%</td>
<td>--</td>
<td>13 (&lt; 1)</td>
</tr>
<tr>
<td>5%</td>
<td>56</td>
<td>29 (&lt; 1)</td>
</tr>
</tbody>
</table>

ᵃ based on toluene internal standard (GC-MS analysis)
ᵇ in 75 mM NaC₂H₃O₂ buffer solution (pH 4.0)
ᶜ in 75 mM KH₂PO₄ buffer solution (pH 7.0) with 2 mg st-DNA, % ee shown in parenthesis

Until this point, all reactions had been performed using 5 mol% Mn[TMPyP₄]I₅ catalyst vs the limiting reagent, so to optimize the reaction conditions further, a catalyst loading study was performed using 0.5, 1, 2.5 and 5 mol% catalyst vs limiting reagent. The control reactions (without DNA) were prepared using 0.6 mmol chloramine T, 0.3 mmol styrene in 2 mL pH 4 buffer. For the aziridinations using DNA, the reactions were performed using 0.6 mmol chloramine T, 0.3 mmol styrene, and 2 mg of st-DNA in 75 mM KH₂PO₄ buffer (pH 7.0). (Table 2.3) Both the control and DNA-based reactions were extracted and analyzed as previously stated. The control reactions show no significant difference in yields between 1 and 5 mol %, and very little difference at 0.5 mol %. For the DNA-based reactions, 0.5 and 5 mol % gave the highest yields, which 1 and 2.5 mol % gave slightly lower yields. Despite no discernable difference in the yields, no enantioselectivity was
achieved. It could stand to reason that at equilibrium between the DNA and catalyst, the catalyst bound to DNA is unlikely to contribute to product formation even at higher catalyst loadings, while the unbound form is responsible for the aziridination products generated. The unbound form generates a racemic mixture of enantiomers so no enantioselectivity is observed. This could explain the similarity in yields across the different catalyst loadings.

Table 2.4  Varying ratios of styrene:chloramine T using different DNA

<table>
<thead>
<tr>
<th>DNA</th>
<th>% Yield&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>% ee&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Yield&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% ee&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>st-DNA</td>
<td>21</td>
<td>&lt; 1</td>
<td>8</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>ct-DNA</td>
<td>14</td>
<td>&lt; 1</td>
<td>19</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>pUC19</td>
<td>12</td>
<td>&lt; 1</td>
<td>9</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

<sup>a</sup> based on toluene internal standard (GC-MS analysis)
<sup>b</sup> 1:1 ratio of styrene:chloramine T
<sup>c</sup> 1:2 ratio of styrene:chloramine T

Generally, nitrene transfer reactions require high excess of the olefin, however, studies with chloramine T and bromamine T have demonstrated higher yields with the N-transfer agent in excess. (109) In pH 4 buffer without DNA, reactions using various ratios of chloramine T:styrene were conducted, and it was found that the 2:1 ratio gave the highest yields for our catalytic system. In a similar system, Zdilla et al. reported that a Mn-corrole system catalyzed the aziridination of styrene. The rate with respect to styrene is zero-order, therefore, this suggests that the rate is dependent on the binding between nitrogen transfer agent and catalyst. They propose that two nitrene transfer agents must bind with the metal center to form product, so excess nitrogen transfer agent is needed.(106) For the DNA-based aziridinations, a 1:1 and 1:2 ratio of styrene:chloramine T were explored. These reactions contained .3 mmol of styrene, either .3 mmol or .6 mmol of
chloramine T, and either 128 µM pUC19, 2 mg ct-DNA or st-DNA. (Table 2.4) They were extracted and analyzed following the previously established protocols. While neither the 1:1 nor 1:2 ratio gave any discernable enantioselectivity, the 1:1 ratio gave slightly higher yields. This could suggest that when these reactions are performed in the presence of DNA, that at the 1:1 ratio, although higher yields are observed, the enantioselectivity is hindered as the unbound catalyst is forming the racemic product. While at the 1:2 ratio, the yields decrease slightly, and some observable enantioselectivity is seen. This could suggest that more of the DNA-bound catalyst is forming chiral products.

Table 2.5  Temperature study in buffered solution with and without DNA

<table>
<thead>
<tr>
<th>Temperature</th>
<th>% Yield (without DNA)\textsuperscript{a,b}</th>
<th>% Yield (with DNA)\textsuperscript{a,c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 °C</td>
<td>10</td>
<td>&lt; 1 (&lt; 1)</td>
</tr>
<tr>
<td>22 °C</td>
<td>23</td>
<td>29 (&lt; 1)</td>
</tr>
<tr>
<td>45 °C</td>
<td>24</td>
<td>&lt; 1 (7)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} based on toluene internal standard (GC-MS analysis)  
\textsuperscript{b} in 75 mM NaC2H3O2 buffer solution (pH 4.0)  
\textsuperscript{c} in 75 mM KH2PO4 buffer solution (pH 7.0) with 2 mg st-DNA,  
% ee shown in parenthesis

All reactions thus far have been performed at room temperature, and due to the nature of DNA, higher temperatures had been avoided to lessen the possibility of destroying the DNA. While this may be true, supercoiled DNA is capable of being uncoiled when heat is applied. Therefore, temperature studies were performed to see if the formation of aziridine products was temperature dependent. The control reactions were performed in pH 4 buffer using 5 mol % Mn[TMPyP\textsubscript{4}]\textsubscript{5}, 0.6 mmol of chloramine T, and 0.3 mmol of styrene. In these reactions, lower temperatures supported
lower aziridination yields, while there was little difference between room temperature and 45 °C. The DNA-based aziridinations were performed using 5 mol% catalyst, 0.3 mmol of chloramine T, 0.3 mmol of styrene, and 2 mg st-DNA in 75 mM KH₂PO₄ buffer (pH 7.0). Again, it was noted that the lower temperature gave lower yields, and no enantioselectivity. The room temperature reactions gave the highest yields, but no enantioselectivity. Finally, the reactions performed at 45 °C gave very low yields, but did give an increase in the enantioselectivity of product formation. This suggests that by heating the DNA to uncoil it, more catalyst is able to bind with DNA to generate the chiral environment that yields enantioselective formation.

Table 2.6 DNA/catalyst equilibration trials

<table>
<thead>
<tr>
<th>DNA</th>
<th>Equilibration Temp</th>
<th>Reaction Temp</th>
<th>% Yieldᵃ</th>
<th>% eeᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>st-DNA</td>
<td>45 °C</td>
<td>45 °C</td>
<td>1</td>
<td>&lt; 3</td>
</tr>
<tr>
<td></td>
<td>70 °C</td>
<td>RT</td>
<td>5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>70 °C</td>
<td>70 °C</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>ct-DNA</td>
<td>45 °C</td>
<td>45 °C</td>
<td>&lt; 1</td>
<td>&lt; 3</td>
</tr>
<tr>
<td></td>
<td>70 °C</td>
<td>RT</td>
<td>7</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>70 °C</td>
<td>70 °C</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

ᵃ based on toluene internal standard (GC-MS analysis)
ᵇ obtained on chiral HPLC

To further study the effect of temperature on enantioselectivity, a method for increasing the likelihood of catalyst-DNA binding was developed; this was done by equilibrating the catalyst and DNA prior to performing the reaction. For example, 5 mol% Mn[TMPyP₄]I₅ was mixed with 2 mg of st- or ct-DNA in 75 mM KH₂PO₄ buffer (pH 7.0), and the mixture was heated
to 45 °C or 70 °C while stirring for 24 hours. After equilibration, the homogenous catalyst-DNA solution was transferred to a clean vial leaving the insoluble components behind. The mixture was then cooled to room temperature and 0.3 mmol chloramine T, and 0.3 mmol styrene were added. The reactions were then run at either room temperature (~22 °C), 45 °C, or 70 °C. For all trials, the yields were significantly lower than previous trails, but we did see a subtle increase in the enantioselectivity. This suggests that by pre-equilibrating the catalyst and DNA, we were able to generate more of the bound form, which is responsible for the enantioselectivity of product formation. Heating the catalyst-DNA mixture to 70 °C is enough to uncoil the DNA, allowing more catalyst to bind, without completely destroying the DNA itself.

![Chromatogram of N-tosyl-2-phenylaziridine using ct-DNA obtained on chiral HPLC](image)

**Figure 2.5** Chromatogram of N-tosyl-2-phenylaziridine using ct-DNA obtained on chiral HPLC
Figure 2.6  Chromatogram of N-tosyl-2-phenylaziridine using st-DNA obtained on chiral HPLC

Figure 2.7  Chromatogram of N-tosyl-2-phenylaziridine using pUC19 DNA obtained on chiral HPLC
3.1 Challenges of organic synthesis in aqueous solutions

An emerging area of organic method development is the optimization of organic reactions in aqueous solutions. The use of water as a solvent greatly lends itself to sustainable chemistry, as water is one of the most abundant molecules on our planet, and a nontoxic alternative to traditional organic solvents. Reactions performed in aqueous solutions have therefore gained considerable interest recently. In addition, the use of aqueous solutions allows for the use of inherently chiral biomolecules, such as DNA or enzymes, to be used to induce enantioselectivity without the use of expensive or complex ligand structures. However, major issues, such as the solubility of catalysts, organic reagents, and reaction products, have hindered suitable method development efforts.

Furthermore, reactions performed in aqueous solutions can be made more difficult as water itself can be reactive. During a typical aziridination reaction in water, water itself can be nucleophilic enough to induce ring-opening of the aziridine produced. During substrate studies, it was found that aromatic substrates, such as styrene, generated numerous side products, which could be caused by degradative reaction pathways or through ring-opening of the aziridine. (Figure 3.1) While most side products are in yields much lower than 5%, these alternative products can significantly decrease yields of the desired product and further complicate isolation methods.
Figure 3.1  Side products formed during the aziridination of styrene determined by GC-MS

Our research goal was to design a water-stable catalyst capable of performing aziridinations in aqueous media. We hoped to generate a low-cost, sustainable method for performing asymmetric aziridinations by designing a catalyst that could be paired with DNA. While we were able to optimize the reactions conditions for the use Mn[TMPyP₄]I₅ in aqueous solutions to generate aziridines in good yields (43-93%), there are still many issues that should be addressed when this system is paired with DNA. Although the enantioselectivity we observed for the DNA-based reactions was low, further optimization of these reaction conditions could enhance the selectivity of this system.
3.2 The nature of the nitrene transfer agent

In order for asymmetric catalysis to occur, the metal complex must be bound or intercalated into the DNA, and the reactive metal center must be accessible to the nitrene transfer reagent. The nitrene species must be able to react in the axial positions of the metal complex. If the nitrene transfer event cannot approach the metal center while it is in proximity to the DNA, then the reaction will produce the racemic mixture of products. It is therefore paramount that the metal complex remains bound to the DNA while this nitrogen transfer occurs. In our system, chloramine T was used as a nitrene transfer agent, and produced the best yields for these reactions performed in buffered solutions with and without DNA.

Figure 3.2 Chloramine T binding Mn[TMPyP4]I5 paired with DNA

However, there are a few reasons chloramine T may not be appropriate for this catalytic system when paired with DNA, as the chemical nature of chloramine T may not be conducive to the parameters mentioned above. Since the nitrene transfer species must approach the metal center in
the axial position, the bulky tosyl group on chloramine T may hinder the catalyst’s ability to remain within the DNA. As it approaches the bound catalyst-DNA form, the bulky tosylated amine disrupts the catalyst affinity for stacking within the DNA base pairs. Furthermore, the sulfonyl group present on chloramine T has some inherent partial negative charge, which further repels the negatively-charged backbone of DNA. Therefore, these considerations must be addressed in future research and a more suitable N-transfer agent must be found.

3.3 The complex equilibrium between catalyst and DNA

In addition to the issues the nitrene transfer agent presents, there appears to be a complex equilibrium established between the catalyst and DNA. The unbound form is responsible for generating a racemic mixture of products, while the bound form establishes a chiral microenvironment for asymmetric catalysis to occur. As the N-transfer agent (Chloramine T) is added to the reaction mixture, it prevents the metal complex from sufficiently binding with DNA, and therefore lowers the enatioselectivity of this synthesis. We tried a number of reaction conditions
in order to force this equilibrium forward towards the bound catalyst-DNA form, but ultimately were not successful in fully optimizing the conditions needed to foster an environment mainly dominated by the bound form. Further studies should investigate the kinetics and binding affinity of this system with the hope of developing a system where the nitrene transfer event can occur on the bound catalyst-DNA form.
CHAPTER IV
METHODS AND MATERIALS

4.1  Chemicals

All chemicals were purchased commercially and used as received without additional purification. Calf thymus (ct) and salmon testes (st) DNA were purchased commercially (Sigma Aldrich) and prepared by addition to a 75mM KH₂PO₄ pH 7.0 buffer solution.

4.2  Chromatography

Reactions were monitored by GC-MS using a Shimadzu QP-2010S GC-MS with a Rxi-5 ms 30 m column with an internal diameter of 0.25 mm. The GC was equipped with an electron impact ionization, quadruple MS detector, where reaction yields were calculated in reference to an internal standard (toluene) added to each extraction.

4.2.1  Additional organic spectroscopy

[Mn(TMPyP₄)]⁺ mass spectra were obtained using a Brüker microTOF-Q II High Resolution MS system operating in ESI ionization mode. UV-Vis spectra were obtained using a Shimadzu UV-2550 spectrophotometer. NMR spectra were obtained using a Brüker AVANCE III 500 MHz spectrometer at room temperature (20°C). HPLC spectra were obtained using an Agilent Technologies 1200 series system, Chiralpak IC column (4.6 x 250mm, 5µm), with a flow rate of 1 mL/min using 85:15 hexane:2-propanol.
4.3 Synthesis of [MnTMPyP$_4$]I$_5$

[MnTMPyP$_4$]I$_5$ was prepared by combining 5,10,15,20-tetra-pyridinyl-21H,23H-porphine (0.485 mmol) and MnCl$_2$ (0.51 mmol) in a 250 mL round bottom flask. These solids were dissolved in 50 mL of DMF and then the flask was flushed with argon gas. The round bottom flask was shielded in aluminum foil, stirred, and refluxed under argon for 3 hours. The reaction was allowed to cool to 40 ºC and 3x methyl iodide (4.8 mmol) was added dropwise over 2 minutes. The reaction was stirred for an additional 24 hours at 40 ºC. The reaction was then cooled to room temperature, where the [MnTMPyP$_4$]I$_5$ was precipitated from solution by slowly adding 100 mL of diethyl ether into the round bottom flask. The solid was isolated by vacuum filtration and washed with approximately 20 mL of diethyl ether. [MnTMPyP$_4$]I$_5$ was further purified by dissolving it in a 1:2 ratio of hot water:acetone, where upon cooling a dark brown solid precipitated. The solid was filtered and dried under vacuum for 2 days. The weight of the isolated product was 190 mg (0.149 mmol, 30 %). [MnTMPyP$_4$]I$_5$ was characterized by high resolution mass spectrometry and UV-Vis spectroscopy. Mass spectra were obtained using a Bruker microTOF-Q II High Resolution MS system using ESI ionization. UV-Vis spectra was obtained using a Shimadzu UV-2550 spectrophotometer.

4.4 Preparation and isolation of pUC19 plasmid DNA

XL1 Blue *E. coli* cells containing pUC19 were grown on an LA plate and incubated at 37 ºC overnight. For a 1L culture, 25 g of LB Broth was added to 1 L of 18 MΩ water. This LB media was then sterilized by autoclaving. After cooling to room temperature, 1 mL of 1 mg/mL ampicillin was added before addition of the pUC19-bearing *E. coli* cells. This solution was shaken at 37 ºC overnight. The culture was spun down to collect the pelleted cells. The cells were stored at -80 ºC until further needed. The pUC19 plasmid was isolated from cells by a modification to the “Crude
Lysate by Alkaline Lysis\textsuperscript{\textregistered} method found in the Current Protocols in Molecular Biology.\textsuperscript{(110)} This adapted method was performed as follows: First the \textit{E. coli} cells were resuspended in 8 mL of a glucose/Tris/EDTA solution (50 mM glucose, 25 mM pH 8 Tris, 10 mM EDTA). The resuspended cells were placed in centrifuge tubes, and 2 mL of 25 mg/mL egg white lysozyme in glucose/Tris/EDTA solution was added, where this solution was allowed to stand at room temperature for exactly 10 minutes. Next, 10 mL of 10 M NaOH, 10 % SDS solution was added to the cell mixture and stirred gently until the solution turned homogeneous and clear. The solution was allowed to stand on ice for an additional 10 minutes. Next, 15 mL of a 3 M sodium acetate solution was added and stirred gently before standing on ice for 10 minutes. The mixture was then centrifuged at 13,000 rpm for 10 minutes at 4 \textdegree C. Afterwards, the supernatant was decanted into a clean centrifuge tube and 0.6 volumes of isopropanol was added. The solution was mixed by inversion and allowed to stand for 5-10 minutes at room temperature. The DNA was recovered by centrifuging at 11,500 rpm at room temperature for 10 minutes. After, the collected DNA pellet was washed with 4 mL of 70 % ethanol. The ethanol was evaporated off using rotary evaporation and dried under vacuum. The collected plasmid DNA was stored at 4 \textdegree C until further use. The concentration of pUC19 was determined by UV-Vis spectroscopy. The absorbance at 260 nm was recorded and the concentration is back-calculated using Beer-Lamberts law, given the molar absorptivity of $4.4 \times 10^7$ M$^{-1}$ cm$^{-1}$ at 260 nm.

4.5 \textbf{Catalytic generation of N-tosyl-2-phenylaziridine in buffer}

The synthesis of N-tosyl-2-phenylaziridine was performed in 75 mM KH$_2$PO$_4$ (pH 7.0) buffer. Initial reactions were performed with 0.3 mmol Chloramine T trihydrate, 3 mmol styrene, and 0.015 nmol [MnTMPyP]$_5$ (5 mol\% vs nitrogen transfer agent) in 2 mL of buffered solution within a 4 mL vial. These reactions were run in triplicate. The vials were capped and stirred for 24
hours at room temperature (~22 °C). The reaction mixture was extracted 4 times with 2 mL of diethyl ether. The ether extractions were combined, reduced in volume by rotary evaporation, and stored at -20 °C until further analysis. All future reactions were extracted using the protocol stated above.

4.6 Catalytic generation of \(N\)-tosyl-2-phenylaziridine in buffered solution using DNA

The synthesis of \(N\)-tosyl-2-phenylaziridine was performed in 75 mM KH\(_2\)PO\(_4\) (pH 7.0) buffer solution. Initial reactions were performed using 0.6 mmol Chloramine T trihydrate, 0.3 mmol styrene and 0.015 nmol \([\text{MnTMPyP}_4]I_5\) (5 mol% vs styrene), and 128 µM pUC19 in 2 mL of buffered solution within a 4 mL vial. These reactions were run in triplicate. The vials were capped and stirred for 24 hours at room temperature (~22 °C). The reaction mixture was extracted 4 times with 2 mL of diethyl ether. The ether extractions were combined, reduced in volume by rotary evaporation and stored at -20 °C until further analysis. Further reactions were performed using various DNA types.

4.6.1 Nitrogen transfer scope

Alternative nitrogen transfer agents were also examined to determine the best nitrogen transfer agent for our system. A reaction vial containing 0.015 nmol \([\text{MnTMPyP}_4]I_5\), 0.3 mmol styrene, 0.6 mmol nitrogen transfer agent (Chloramine T trihydrate, \(N\)-tosyliminobenzylidionane, or tosyl azide), 128 µM pUC19, and 2 mL 75 mM KH\(_2\)PO\(_4\) (pH 7.0) was stirred at room temperature (~22 °C) for 24 hours. After extraction, the samples were analyzed by GC-MS.

4.6.2 Catalyst loading study

A catalyst loading study was also performed using 0.5, 1.0, or 2.5 mol% of catalyst. Different amounts \([\text{MnTMPyP}_4]I_5\) were added to 4 mL reaction vials containing 2 mL of 1 mg/mL ct-DNA or
st-DNA. A stirbar was placed in the vial and the mixture was equilibrated at 70 ºC overnight. After equilibration, the soluble catalyst mixture was placed in a clean vial and 0.3mmol styrene and 0.6mmol Chloramine T trihydrate were added. The reaction mixture was then stirred at room temperature (~22 ºC) for 24 hours. After extraction, the samples were analyzed by GC-MS to determine catalyst activity.

4.6.3 Temperature studies

The temperature dependence of this N-transfer process was also optimized. A 4 mL reaction vial was prepared by adding a stirbar, 0.015nmol [MnTMPyP₄]I₅, and 2 mL of 1 mg/mL ct-DNA or st-DNA. The vial was heated to 70 ºC, and stirred overnight to equilibrate. The mixture was then placed into a clean vial leaving behind any insoluble solids. Once at room temperature, 0.6mmol Chloramine T trihydrate and 0.3mmol styrene were added to the vial. The reactions were allowed to react with stirring for 24 hours at 3 ºC, room temperature (~22 ºC), and 70 ºC. After the reaction, the mixtures were allowed to cool to room temperature and each reaction was extracted 4 times with 2mL of diethyl ether. These extractions were reduced in volume by rotary evaporation, and analyzed by GC-MS.

4.6.4 DNA/catalyst equilibration trials

Further optimization of these reactions was performed by pre-equilibrating the DNA and catalyst, in an effort to increase the likelihood of DNA-catalyst binding events. These reactions were performed by placing 18 mg of [MnTMPyP₄]I₅ in a vial with 2 mL of either ct-DNA or st-DNA. The samples were stirred overnight at either 45 ºC or 70 ºC overnight. After the equilibration period, the vials were then cooled to room temperature and the homogeneous DNA-catalyst solution was decanted into a clean vial, leaving behind any precipitated catalyst behind. After the soluble DNA hybrid catalyst was placed in a clean vial, 0.6mmol Chloramine T trihydrate and
0.3 mmol styrene are added to the vial and the reaction was run at either room temp (≈22 °C), 45 °C or 70 °C for 24 hours. After extraction, the samples were analyzed by GC-MS.
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