LEVERAGING AUTOMATION TO ELUCIDATE REACTION MECHANISMS

by

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Abstract

Understanding chemical processes facilitates reaction optimization to make synthetic procedures more efficient while also enabling reaction discovery. Temporal profiling of chemical reactions provides the gold standard for increasing mechanistic understanding. Unfortunately, obtaining time-course information reproducibly, accurately, and also minimizing analyst intervention is a significant challenge. Combining in situ spectroscopic methods with automated sampling techniques provides a robust method to generate kinetic profiles enabling increased mechanistic understanding. This thesis explores the development and application of online HPLC as an analytical technique to obtain concentration data while minimizing workload for the analyst. By utilizing commercially available laboratory equipment and software we have created a sampling device capable of automatically monitoring both homogeneous and heterogeneous reactions, as well as those performed under an inert atmosphere. The ability of the platform to sample, dilute, mix, and analyze reaction aliquots reproducibly has been validated, thereby ensuring accuracy of acquired time-course data. This automated reaction monitoring device has been used to delineate reaction mechanisms for a series of chemically distinct transformations. The Kinugasa reaction for the synthesis of beta-lactams was investigated. A novel retrocycloaddition step accounts mechanistically for byproducts associated with the transformation. A telescoped synthesis yielding cyanoimidazoles via combining an imidazole forming condensation annulation with a functional group conversion was also investigated. A series of Buchwald-Hartwig aminations performed within a glovebox using various aryl halide components were explored. Lastly, the mechanism of a synthetic procedure to synthesize Spiro-OMeTAD, a state-of-the-art organic material used in modern solar cells, was probed. By
leveraging automated reaction monitoring devices, mechanistic understanding for each transformation was increased, ultimately making these transformations more efficient.
Lay Summary

Understanding reaction mechanisms is the key to making chemical transformations more efficient and discovering new methods to synthesize molecules of interest. This statement is broadly applicable to areas of chemical research including pharmaceuticals, organic materials, agrochemicals, and polymers. Increasing mechanistic understanding is best done by monitoring changes in concentration of reaction components as a function of time. Unfortunately, collecting temporal profiles for reactions is often laborious or inaccurate. Therefore, we have developed automated methods to generate reaction profiles that are broadly applicable to many classes of reactions. This methodology has enabled increased understanding for the synthesis of several molecules of interest. By leveraging newly found mechanistic information, yields of these molecules has greatly increased, thereby improving efficiency by saving time and money.
Preface

Chapter 1 serves an introduction to the topics covered through this thesis. I wrote this chapter with feedback from my supervisor, Prof. Jason E. Hein.

Chapter 2 is adapted from a published article titled “Real-time HPLC-MS reaction progress monitoring using an automated analytical platform” (Malig, T. C.; Koenig, J. D. B.; Situ, H.; Chehal, N. K.; Hultin, P. G.; Hein, J. E. React. Chem. Eng., 2017, 2, 309 – 314). Mr. Henry Situ was instrumental in early stage development of the reaction monitoring platform. Prof. Hein and I designed the experiments described therein. The Suzuki cross coupling reaction data as well as characterization data was collected by Ms. Navneet Chehal with help from her supervisor Phil G. Hultin. I performed the remainder of the experiments with help from Mr. Joshua Koenig. I analyzed the data and wrote the manuscript with feedback from Prof. Hein., Prof. Hultin, and Mr. Koenig.

Chapter 3 is adapted from a published article titled “A Revised Mechanism for the Kinugasa Reaction” (Malig, T. C.; Yu, D. N.; Hein, J. E. J. Am. Chem. Soc. 2018, 140, 9167 – 9173). Preliminary mechanistic investigations were completed by Ms. Diana Yu. Prof. Jason. E. Hein and I designed the experiments designed herein. I performed the experiments and interpreted the data. I wrote the manuscript with feedback from Prof. Hein.

Chapter 4 is adapted from an article in press titled “Development of a Telescoped Synthesis of 4-(1H)-Cyanoimidazole Core Accelerated by Orthogonal Reaction Monitoring” (Malig, T. C.; Tan, Y.; Wisniewski, S. R.; Higman, C. S.; Carrasquillo-Flores, R.; Ortiz, A.; Purdum, G. E.; Kolotuchin, S.; Hein. J. E. React. Chem. Eng. 2020, Advance Article.). The research conducted in this chapter was conceived out of a joint effort from collaborators at Bristol Myers Squibb (BMS). Preliminary research not discussed in this thesis were completed by Dr Yichen Tan and Dr Steven
Wisniewski. Design of Experiments (DoE) was collected by collaborators at BMS. Prof. Hein and I designed the experiments herein with feedback from our collaborators. I performed the experiments and interpreted the data. Collaborators at BMS helped write the introduction of the manuscript. I wrote the remainder of the article with feedback from Prof. Hein as well as our collaborators listed on the manuscript.

Chapter 5 is unpublished. Both Dr. Sebastian Steiner and Dr. Lars Yunker played integral roles in the development of the automated reaction monitoring platform, specifically for their help in writing the python script to sequence the sampling events. Prof. Hein and myself designed the experiments described and I performed them. I analysed and interpreted the data and wrote the chapter with feedback from Prof. Hein.

Chapter 6 is unpublished. Prof. Hein, Ms. Kea Legard, and I designed the experiments. I performed the experiments with the assistance of Ms. Legard. Kea played a pivotal role in the early stages of the project by finding high-yielding reaction conditions conducive to automated sampling. Mr Brian Patrick helped with the XRD analysis and interpretation. I analysed and interpreted the data and wrote the chapter with feedback from Prof. Hein.
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</thead>
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<tr>
<td>Å</td>
<td>Ångstrom ($10^{-10}$ m )</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>AE</td>
<td>activation energy</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>AU</td>
<td>absorbance units</td>
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<td>butyl</td>
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<tr>
<td>cat</td>
<td>catalyst</td>
</tr>
<tr>
<td>CuAAC</td>
<td>copper(I)-catalyzed azide-alkyne cycloaddition</td>
</tr>
<tr>
<td>COPASI</td>
<td>COmplex PAthway SImulator</td>
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<td>DoE</td>
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<tr>
<td>EDG</td>
<td>electron donating group</td>
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<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>HTM</td>
<td>hole transport material</td>
</tr>
<tr>
<td>iPr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>iPrOH</td>
<td>isopropyl alcohol</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
</tbody>
</table>

xxxv
$J$  coupling constant (NMR spectroscopy)

KIE  kinetic isotope effect

L  liter

LC  liquid chromatography

LiHMDS  lithium hexamethyldisilazane

LUMO  lower unoccupied molecular orbital

m  multiplet

M  molar

Me  methyl

MeCN  acetonitrile

MeOH  methanol

mg  milligram

MHz  megahertz

min  minute

mL  milliliter

mM  millimolar

mmol  millimole

mol  mole

MS  mass spectrometry

m/z  mass-to-charge ratio

NMR  nuclear magnetic resonance

nm  nanometer

Nu  nucleophile
-o ortho substituted

-p para substituted

PAT process analytical technology

PCE power conversion efficiency

PEEK polyether ether ketone

Ph phenyl

ppm parts per million

R organic substituent

R universal gas constant

r.t. room temperature

RPKA reaction progress kinetic analysis

rxn reaction

s second

Spiro-OMeTAD \( N^2,N^2,N^7,N^7,N^7,N^7 \)octakis (4methoxyphenyl)-9,9'-spirobi[fluorene]-2,2',7,7'-tetraamine

T temperature

tBu tert-butyl

TCPTA \( tris((1\text{-cyclopentyl-1H-1,2,3-triazol-4-yl})\text{methyl})\text{amine} \)

TFA trifluoroacetic acid

THF tetrahydrofuran

TLC thin-layer chromatography

TOF time-of-flight

µL microliter
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHPLC</td>
<td>ultra-high-performance liquid chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>Vis</td>
<td>visible</td>
</tr>
<tr>
<td>VTNA</td>
<td>variable time normalization analysis</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>xs</td>
<td>excess</td>
</tr>
<tr>
<td>ZPE</td>
<td>zero-point energy</td>
</tr>
</tbody>
</table>
Acknowledgements

First and foremost, I would like to thank my supervisor, Professor Jason E. Hein for without your guidance none of this would have been possible. I am truly grateful for your mentorship, friendship, and support. During my graduate studies I learned to see the world differently and realize that no problem is unsolvable. I’ll carry this mentality forward to try and make a positive difference in the world.

Next, I would like to thank all my fellow Hein lab group members both past and present. You all made coming into the lab a truly enjoyable experience where I could learn and discuss ideas all while finding humour and enjoying the little things. The memories I have of us playing board games at Gargoyles and gallery are some I will cherish always. Specifically, I would like to thank Joshua Koenig, Kea Legard, and Jessica Li for their help with projects. Also, I would like to thank Dr Lars Yunker and Dr Sebastian Steiner for their contributions towards writing the python script used in the automated sampling and for being so helpful in answering my questions. Without their help Chapters 5 and 6 would not have been possible.

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Last but certainly not least, I would like to thank all my friends and family. Your ongoing support throughout this journey has been pivotal to my success in graduate school, and for that I am forever grateful.
Dedication

For my parents, your love and guidance has encouraged me to follow my curiosities and pursue what is most meaningful to me.
“Your assumptions are your windows on the world. Scrub them off every once in a while, or the light won't come in.”

— **Isaac Asimov**
Chapter 1: Introduction

1.1 Importance of Chemical Synthesis

Synthetic organic chemistry has provided benefits to humankind since the first half of the 19th century when Friedrich Wöhler synthesized the natural product urea from inorganic materials (Scheme 1.1). This discovery is often reported as the starting point of synthetic organic chemistry, leading to countless innovations and discoveries to make useful molecules that have shaped civilization for the better. Using organic synthesis, high-value substances can be generated from abundant natural resources such as coal and biomass. Some examples in which organic synthesis impacts our daily lives include the development of new pharmaceuticals to treat diseases and finding novel strategies to harness alternative energy enabling the transition away from fossil fuels.

![Scheme 1.1. Wohler synthesis of the natural product urea from inorganic materials.](image)

1.1.1 Impactful Chemical Discoveries

An example of a synthetic discovery that has had resonating impact on civilization is the synthesis of aspirin, a widely used medicine for the reduction of pain, fever, and inflammation. A precursor to aspirin can be found in the leaves of willow trees, which has been used for its health effects for over 2400 years. It was not until 1853 that Charles Frédéric Gerhardt first synthesized aspirin from the natural precursor (Scheme 1.2a), a historic discovery eventually leading to the
widespread usage observed today with global consumption of aspirin exceeding 40 000 tons each year.\(^5\)

A second chemical discovery which has had lasting effects on humanity was the synthesis of the polymer polyethylene (Scheme 1.2b). Over 100 million tons of polyethylene are produced annually, accounting for more than one third of all manufactured plastics.\(^5\) Used in everyday commodities such as grocery bags and plastic bottles it is now difficult to imagine life without it. Unfortunately, the high stability of these polymers results in a low rate of degradation, causing polyethylene plastics to accumulate in landfills and oceans.\(^6\) This undesired property of polyethylene now fuels investigation for efficient methods to achieve controlled polyethylene degradation into reusable materials that can be performed at scale.\(^7\)

Scheme 1.2. Impactful synthetic discoveries. a) Synthesis of aspirin. b) Ethylene polymerization to form polyethylene. c) Haber-Bosch process to synthesize ammonia from elemental hydrogen and nitrogen.
A third chemical discovery that has had a lasting impact on humanity is the synthesis of ammonia from elemental nitrogen and hydrogen via the Haber-Bosch process (Scheme 1.2c). Fritz Haber was awarded the Nobel prize in 1918 for his work allowing for the production of ammonia on industrial scale. Before this discovery the global supply of ammonia was insufficient to provide fertilizer to the world’s growing population. It is estimated that this discovery alone has prevented more than 2 billion people from starving annually. While undoubtedly useful, the Haber process is performed at high pressures and temperatures exceeding 200 atm and 400 °C, respectfully, making this an extremely energy intensive process. Modern alternatives to reduce nitrogen under milder conditions is an active area of chemical research.

The advent of these discoveries has undoubtedly altered the course of humanity by allowing access to chemicals to better improve our quality of life. As technology continues to develop, and our global demand for energy increases, it is imperative that the field of chemical research continues to strive for innovation. Whether these innovations come through the discovery of novel transformations or through the modification of existing chemical processes to rendering them more efficient. As such, discovering novel or improved methods to synthesize molecules of interest while minimizing cost and maximizing yield is a worthwhile goal for any chemical researcher. The optimal approach for rendering any chemical transformation more efficient is by elucidating the underlying reaction mechanism.

1.2 Reaction Mechanisms

Reaction mechanisms describe the sequence of interactions of molecules that occur to create products from starting materials. Reaction mechanisms often consist of many elementary reactions which describe how molecules react with each other in a single step. The rate of any
elementary reaction is always written according to the proposed equation and can be described by the number of molecules reacting in that step. Take for example the reaction to form C and D from A and B (Scheme 1.3). The net reaction provides no information pertinent to the reaction rate, and as such the rate assumes the generic form Rate = \( k[A]^a[B]^b \) where \( k \) is a rate constant and \( a \) and \( b \) are the orders in starting materials A and B, respectively. Only through experimentation can elementary reactions 1 and 2 be proposed. Elementary reactions can be categorized by their molecularity: unimolecular reactions have a single substrate, bimolecular reactions have two substrates, and termolecular reactions have three.

### Scheme 1.3. Depiction of a net reaction and its elementary reactions.

Reactions intermediates are species that are formed in one step and consumed in another of the mechanism (i.e. E in Scheme 1.3). The overall reaction rate is governed by the slowest elementary reaction, commonly referred to as the rate determining step. If the bimolecular reaction of A with itself is rate determining, the observed rate law would take on the form Rate = \( k[A]^2 \). If the combination of intermediate E with substrate B is rate limiting, then the rate would law instead express the form Rate = \( k[E][B] \). Unfortunately, expressing rates in terms of intermediates such as E is problematic as their concentration is seldom known due to the difficulty of obtaining the measurement. To circumvent this complication techniques such as the steady state approximation and the pre-equilibrium approximation are used to express rate laws in more meaningful terms.\textsuperscript{11,12}
The importance of understanding reaction mechanisms to facilitate optimization cannot be overstated and is the primary focus of this Thesis. There are many approaches for interrogating reaction mechanisms, with each experiment either further validating a proposed mechanism or refuting a step. Therefore, elucidating reaction mechanisms is an ongoing process that lies at the interface between synthetic, organometallic, and physical chemistry.

1.2.1 Delineating Reaction Mechanisms

The elucidation of underlying reaction mechanisms is the key to optimizing any transformation as it delineates the role of each reaction component on the overall transformation. This information can enable a rational approach to reaction optimization resulting in milder reaction conditions, decreased reaction times, and increased yield. Take for example the generic example where starting materials A and B combine to form product, P (Scheme 1.4).

\[
\begin{align*}
A + B & \longrightarrow P
\end{align*}
\]

Scheme 1.4. Generic reaction scheme.

If the goal of this transformation was to maximize the rate of the forward reaction, first an understanding of the effects of the concentrations of each reagent must be understood which can be completed by performing a small subset of experiments. Table 1.1 shows the effects of the concentrations of A and B on the initial reaction rate.

| Table 1.1. Effects of concentrations of A and B on the initial reaction rate |
|-----------------------------|-------------|-------------|-----------------------------|
| Experiment | [A]₀ (M) | [B]₀ (M) | Rate Ms⁻¹ |
| 1 | 0.1 | 0.1 | 1 |
| 2 | 0.2 | 0.1 | 2 |
| 3 | 0.1 | 0.2 | 2 |
| 4 | 0.2 | 0.2 | 4 |
The experiments in Table 1.1 show that both \([A]_0\) and \([B]_0\) influence the reaction rate. When the \([A]_0\) is doubled between Experiments 1 and 2, the rate also doubles. Similarly, when the \([B]_0\) is doubled between experiments 1 and 3, the rate doubles. Comparison of Experiments 1 and 4 further validates these observations as doubling both the \([A]_0\) and \([B]_0\) results in a 4-fold rate increase. Therefore, we can conclude that the observable rate law takes on the expression outlined in Equation 1.1

\[
Rate = k[A]^1[B]^1
\]  
(eq. 1.1)

Equation 1.1 states that the rate is a product of the concentration of \(A\), the concentration of \(B\), and a rate constant \(k\). Therefore, this reaction is bimolecular because the rate is a function of the concentrations of \([A]\) and \([B]\). Components \(A\) and \(B\) can each be described as first order as their exponent in the rate equations are both equal to 1. Elucidating the underlying rate law (Equation 1.1) is useful as we now know that the combination of \(A\) and \(B\) constitutes a kinetically relevant step. This example represents well an elementary reaction where each reaction component as drawn is kinetically relevant.

An example of a second order reaction is bimolecular nucleophilic substitution (\(S_n2\)) (Scheme 1.5). Because the concentrations of the nucleophile (\(\text{Nu}^(-)\)) and electrophile (\(\text{CH}_3\text{X}\)) affect the reaction rate, it can be deduced that the combination of both represents the rate determining step of the reaction. Therefore, a transition state (highest energy species on the reaction coordinate diagram) of this transformation can be proposed. Because this process only has one transition state, we can describe it as concerted, therefore no intermediates are involved in the process. This
information is useful as we now know that modifying the concentration of either reagent will affect
the rate.

\[ \text{Nu}^{-} + \text{CH}_3\text{X} \rightarrow \text{CH}_3\text{Nu} + \text{X}^{-} \quad \text{Rate} = k[\text{Nu}^{-}]^{1}[\text{CH}_3\text{X}]^{1} \]

Scheme 1.5. \(S_n2\) reaction with rate law and mechanism

1.3 Catalysis

1.3.1 Background

One approach to creating synthetic methods that use less resources, are cheaper, and overall
more efficient is through catalysis. A substance that increases the rate of a chemical reaction that
is not consumed throughout the process is a catalyst. This decrease in the reaction rate is facilitated
by the catalyst because it provides an alternate reaction pathway with a lower activation energy
than the non-catalyzed pathway (Figure 1.1).
This relationship between the decreased activation energy (Ea) and the rate constant (k) of the catalyzed pathway can be translated back to rate via the Arrhenius equation (Equation 1.2), which also depends on the temperature in Kelvin (T), the universal gas constant (R), and a pre-exponential factor, (A).

\[ k = Ae^{\frac{-Ea}{RT}} \]  
(Eq. 1.2, Arrhenius Equation)

Catalysts may be classified as either homogeneous or heterogeneous depending on whether the catalyst is either in the same phase as the reactants or in a different phase. Heterogeneous catalysts are advantageous because they are more easily recycled than their homogeneous counterparts, although catalyst optimization becomes more difficult as they are often less well defined.\textsuperscript{13,14}
Because a catalyst is not consumed over the course of the reaction’s progress it is possible to use substoichiometric (catalytic) amounts. Understanding catalytic reaction mechanisms differs from stoichiometric processes because the catalyst is involved in the transformation to make products but is used in such low quantities. Therefore, to make quantitative amounts of product, the catalyst must be used multiple times in a process called “catalyst turnover”. An ideal catalyst would have an infinite turnover number as the species is not consumed throughout the reaction. Unfortunately, catalysts turnover number is finite as deactivation is an inevitable occurrence which limits the efficacy of the catalyst.\textsuperscript{15} In recent years high turnover catalysis, defined as using catalysts at 0.1\% loading or lower while achieving quantitative conversion, has emerged as an extremely powerful and environmentally friendly form of catalysis.\textsuperscript{16,17} Some processes are capable of using less than ppm levels of catalyst while still observing exceptional effects on the reaction rates and selectivities.\textsuperscript{18}

\subsection*{1.3.2 Modeling Catalytic Cycles}

To model one individual catalyst turnover the reaction mechanism takes on the form of a catalytic cycle (Scheme 1.6).
In this cycle, the catalyst first combines with A to form an intermediate I. Next, I combines with substrate B to form a second intermediate II. This intermediate then fragments releasing the product P while simultaneously liberating the catalyst where it can again react with A to repeat the cycle. This catalytic cycle is an idealized reaction mechanism and disregards other elementary steps and equilibria that can affect the reaction rate and selectivity. Additionally, attempts to propose a reaction mechanism for a catalytic cycle before collecting experimental data is often a worthless endeavor. For instance, it is possible that the catalyst first interacts with B to form I. It is additionally possible that two molecules of A must interact with the catalyst before B enters the catalytic cycle. Therefore, rigorous experimentation must be employed before a reaction mechanism can be proposed.

Research on mechanistic elucidation of catalytic reactions is an evolving field that facilitates reaction discovery and optimization. Because of the inherent complexity of catalysis reaction mechanisms are seldom understood, and minor alterations such as changing a reagent, ligand, or solvent can have dramatic effects on the entire mechanism by adjusting the turnover limiting step or introducing additional equilibria or off-cycle reactivity. Therefore, each chemical...
transformation must be investigated independently as to not make erroneous assumptions about underlying processes.

1.3.3 Evolution of a Catalyst

The delineation of reaction mechanisms has allowed for generations of catalysts to be synthesized each with additional beneficial properties to allow for transformations to be more robust, occur under milder conditions, tolerate a wider substrate scope, be less toxic, or improve chemoselectivity. An example of an evolution of a catalyst is the ruthenium-based Grubbs catalyst used in olefin metathesis (Scheme 1.8).

Scheme 1.7. Olefin metathesis reaction scheme and development of catalysts generation 1-3 for the olefin metathesis.

The first generation reported in 1995 demonstrated great potential for metathesis of acyclic olefins. Several years later, the second generation of Grubbs catalyst was reported that was both...
air- and water-tolerant while also demonstrating increased ring-closing metathesis activity. Catalyst loadings as low as 0.05 mol % could be used. In 2002 a modified Grubbs catalyst was reported that is capable of performing cross metathesis on demanding substrates with the greatest activity observed yet. These advancements in catalyst design have allowed for a wide range of transformations to become more practical and efficient including ring-closing metathesis, cross metathesis, and metathesis polymerization.

1.4 Reaction Monitoring Techniques

The discovery of revolutionary catalytic methodologies for creating molecules of interest is a combination of both chemical understanding and serendipity. By maximizing understanding of chemical reactivity and the impact each reagent has on the transformation, catalytic methods can be optimized, and novel transformations can be realized.

The optimal way to investigate any chemical transformation is via the generation of high-density temporal reaction progress information, ideally for each individual component that has a fluctuational concentration. Collection of these kinetic profiles is no trivial task, and as such methods to accomplish this will comprise a significant portion of this Thesis. Techniques to collect orthogonal temporal data for different species represents the gold standard for delineating reaction mechanisms.

Many forms of process analytical technologies (PAT) exist for measuring the changes in concentration of reaction species over time. Each form of PAT has its own set of inherent advantages and disadvantages, therefore no truly universal approach for reaction monitoring exists. Selection of the appropriate reaction monitoring technology is therefore at the discretion of the analyst and arriving at a decision is dependent on parameters such as species concentration,
reaction pressure and temperature, homogeneity, and substrate functional groups. Different technologies can effectively monitor different reaction components, therefore by combining technologies a more accurate depiction of the underlying mechanism can be delineated as opposed to relying on a single technique. Reaction monitoring technologies can be divided into two main classes: in situ techniques (often spectroscopic), and sampling-based techniques. The former is non-destructive whereas the latter involves aliquot removal from the reaction where it is often quenched before subsequent analysis.

1.4.1 Spectroscopic Techniques

1.4.1.1 Infrared Spectroscopy

Infrared (IR) spectroscopy is an attractive technique to collect time-course data as it is non-destructive and straightforward to implement. For example, the Mettler Toledo ReactIR (Figure 1.2a) uses a flexible fiber-optic cable which allows infrared light to travel through the probe tip where it is projected into solution where spectra can then be recorded. Simply inserting a ReactIR probe into the reaction of interest is sufficient to begin collecting time-course IR spectra as often as every 15 seconds (Figure 1.2b). Functional group conversion throughout the reaction’s progress is reflected as changes of intensity of bands in the IR region. By plotting these fluctuional signals rates of consumption or formation for starting materials, intermediates, and products can be extracted (Figure 1.2c). The use of either diamond or silicon-based windows on the probe makes ReactIR broadly applicable for a wide range of chemical conditions without compromising the probe or reaction. Additionally, through the use of proper fittings and glassware even air sensitive reactions can be monitored. For these reasons ReactIR and other in situ IR methods have become increasingly common for interrogating chemical systems.23–34
A major limitation to ReactIR is that only solution phase measurements can be taken and heterogeneous components cannot be quantified. Additionally, sufficient resolution must exist when quantifying peaks time to extract accurate rate data. Often peaks of interest for molecules will overlap with others compromising the integrity of the quantification. Fortunately, software-based mathematical manipulations can be applied to increase peak resolution for broad or overlapping bands.

1.4.1.2 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy is an invaluable tool for the analysis of reaction mixtures. NMR provides a method to quantify reaction species and simultaneously provide detailed structural information via characteristics such as multiplicity and chemical shift. The simplest way to leverage NMR as a process analytical technology is via static tube experiments. In these experiments all reaction components are combined in a standard NMR tube that is inserted into the spectrometer where spectra are subsequently recorded as the reaction
progresses. This approach requires no specialized equipment making it a commonly employed technique for reaction monitoring to increase mechanistic understanding.\textsuperscript{35–40}

Alternatively, time course NMR experiments can be performed using a flow type apparatus. While this is more difficult to implement than a static tube experiment, it provides additional benefits such as incorporating mixing, temperature adjustments, ease of introducing additional reagents, as well as combining other in situ or offline techniques. Additionally, the accuracy of temporal data for static tube experiments has been brought into question via a duplicate experiment performed both in flow and in a static tube.\textsuperscript{41} Therefore NMR flow systems have been gaining traction as useful alternatives to utilize NMR to gather temporal information.\textsuperscript{42–44}

Despite these advantages, NMR spectroscopy cannot quantify heterogeneous reaction components. Also, reactions performed at high temperatures or pressures might not be amenable for time-course NMR analysis if the reaction is completed in an NMR tube and not in a specialized reactor. Additionally, signal overlap resulting in low peak resolution is often observed in multicomponent reactions complicating quantification. Lastly, the presence of paramagnetic species can interfere with nuclear resonances compromising quantitative capabilities.

1.4.2 Sampling based Techniques

1.4.2.1 Manual Sampling

Manual sampling represents a simple technique for temporal profiling of chemical reactions. This approach requires minimal equipment and is compatible with a range of analytical techniques such as NMR, high performance liquid chromatography (HPLC), and gas chromatography (GC). Analysis using chromatographic techniques such as HPLC and GC have
the added benefit that modifications to either the mobile phase or stationary phase can result in increased signal resolution which might not be possible with spectroscopic techniques.

While useful, manual sampling is plagued with limitations that can raise concerns regarding the accuracy of the kinetic data. Timing discrepancies between collecting reaction aliquots and quantification represents a significant challenge and often manifests as poor reproducibility. Additionally, to ensure accurate quantification an internal standard is often necessary to account for differences in either aliquot or dilution volumes when collecting a sample. The internal standard must be selected based on a demonstrated ability to be both inert to the reaction conditions and have an unchanging concentration throughout the experiment. Additionally, introduction of a needle or cannula into the reaction to allow for aliquot removal can cause fluctuations in both reaction temperature and pressure, therefore effecting the reactions progress. Finally, manual sampling represents a laborious and tedious process. Lengthy reactions can result in days of invested analyst time to profile the entire reaction. Often manual sampling is combined with initial rate measurements to offset the time and labour intensive nature of this approach. However, initial rate studies are prone to large error, as well as their failure to model complex chemical processes such as activation, deactivation, or autocatalysis.

1.4.2.2  Automated Sampling

Automated sampling-based techniques provide an attractive option for collecting data-dense temporal profiles of chemical reactions with minimal analyst intervention. A common tactic is to employ commercially available liquid handlers which facilitate aliquot collection, dilution, and delivery into a vessel for subsequent offline analysis. One example of such liquid handler is the Gilson GX281 commonly used in the Hein lab (Figure 1.3).
The Hein Lab has expanded upon the utility of Gilson liquid handlers to monitor reactions that are either homogeneous or heterogeneous. Heterogeneous sampling is realized by employing a Mettler Toledo EasySampler slurry probe (Figure 1.4). The Easysampler is compatible with heterogeneous reactions because it uses a fixed pocket volume (~20 µL) to collect a sample rather than withdrawing a sample via capillary and syringe type methods. This sampling technique has allowed for the quantification of both solution phase and solid phase measurements to study the diastereo- and enantioselectivity of catalyzed aldol reaction.

Figure 1.3. Gilson liquid handler used for sample aliquotting, diluting, and delivery to vials for subsequent offline analysis

Figure 1.4. Easysampler slurry probe. a) Internal position of probe pocket used for backfilling sample lines and sample delivery. b) External position of probe pocket used for collecting a sample aliquot.
1.5 Investigating Chemical Mechanisms

Upon selecting a reaction monitoring technique that is amenable to collecting temporal data for the reaction of interest, the next task for investigating chemical mechanisms is to decide which experiments need to be completed. There are numerous routes of experimentation that increase mechanistic understanding, several of which will be discussed here and applied throughout this Thesis.

One approach is measuring kinetic isotope effects (KIE’s) which facilitates proposals to be made regarding the turnover limiting step of the reaction. KIE studies have been utilized to investigate transformations such as C-H activations by multiple metals, hydrogen additions reactions, and transition metal mediated cycloadditions.

A second approach is using graphical analysis techniques such as reaction progress kinetic analysis (RPKA) and variable time normalization analysis (VTNA). These analysis techniques provide a workflow outlining a small set of experiments to probe the robustness of a reaction, and allow for the elucidation of the observed rate law. Once the rate law has been delineated inferences regarding the catalyst resting state and turnover limiting step can be made enabling reaction optimization.

1.5.1 The Kinetic Isotope Effect

The kinetic isotope effect (KIE) is the measurement of the changes in the reaction rate when an atom is replaced with an isotope. Typically, a hydrogen atom (H) is replaced with a deuterium atom (D) and the KIE is calculated by comparing the ratio of the two rate constants for the unlabeled \( k_H \) and labelled \( k_D \) substrates (Equation 1.3).
The magnitude of the KIE provides mechanistic information. If the KIE is equal to 1 (unity), no kinetic isotope effect is observed, and it can be assumed that the labelled bond is not involved in the turnover limiting step of the reaction. A KIE value > 1 is termed a normal KIE, whereas a value < 1 is defined as an inverse kinetic isotope effect. A primary kinetic isotope effect occurs when the isotope labelling occurs at a bond that is broken during the turnover limiting step, and a value for $k_H/k_D > 1$ is expected. Secondary isotope effects occur when the labelled atom is near the bond being broken during the turnover limiting step. $k_H/k_D$ values for secondary KIE’s are typically much lower in magnitude than the primary KIE’s and can be either normal ($k_H/k_D \sim 1.1 - 1.2$) or inverse ($k_H/k_D \sim 0.8 - 0.9$).⁶⁴

### 1.5.1.1 Origin of the Kinetic Isotope Effect

The difference in zero-point energies (ZpE’s) between labelled (X-D) and unlabeled (X-H) bonds is the reason kinetic isotope effects exist. When considering a bond breaking event, the stretching vibrational frequency ($v$) is related to the force constant ($k$) and the reduced mass ($m_r$) (Eq 1.3)

\[
v = \frac{1}{2\pi} \sqrt{\frac{k}{m_r}} \text{ where } m_r = \frac{m_1 m_2}{m_1 + m_2}
\]  

(eq. 1.4)
When hydrogen is replaced with deuterium, the reduced mass is affected considerably which also affects the stretching frequency. The ZpE also relates to the stretching frequency, which manifests as a higher activation energy to break a C-D bond (AE\textsubscript{2}) as opposed to C-H bond (AE\textsubscript{1}) (Figure 1.5).

![Figure 1.5. Effects of isotopic labelling on zero point energies and activation energies](image)

The maximum \(k_H/k_D\) isotope effect would be expected in the scenario where the C-H bond is completely broken at the transition state. If this scenario described is the rate limiting step of the reaction a primary kinetic isotope effect is observed and would have an estimated value \(~7.64\) When isotopic labelling is done on atoms other than hydrogen, lower KIE’s are expected due to the smaller difference in zero point energies via differences in the reduced mass.

### 1.5.1.2 Secondary Kinetic Isotope Effects

A secondary kinetic isotope effect is observed when the labelled bond is not being broken in the rate determining step, but instead experiences a change in hybridization. For example, if a
C-D bond changes from sp³ to sp² through the rate determining step a secondary kinetic isotope effect would be observed. The magnitude of these secondary KIEs are typically smaller than primary KIEs.

When considering the magnitude of a secondary KIE it is no longer sufficient to consider the differences in ZpEs of the starting materials; the differences in ZpEs of the transition states must also be accounted for.

![Potential Energy Diagram](image)

**Figure 1.6. Potential energy diagram for a secondary kinetic isotope effect.** C-H/C-D bonds are not being broken during the process but the carbon atoms experience a hybridation change from sp³ to sp².

The magnitude of the difference of ZpE’s for sp³ hybridized atoms is larger than that of sp² hybridized atoms resulting from the differences in energies of the different bending modes.⁶⁴
Therefore, if a late transition state is observed throughout a sp³ to sp² hybridization change the \( \Delta ZpE^R \) is greater than \( \Delta ZpE^{TS} \) (Figure 1.6).

This difference in the magnitude of zero-point energies manifests as a difference in activation energies as well, where \( AE^D > AE^H \). Therefore, throughout this transformation a normal KIE (>1) would be observed classifying this as a normal secondary kinetic isotope effect. The estimated maximum theoretical value for a normal secondary KIE is 1.4, although values ranging between 1.1 – 1.2 are typically observed.⁵⁶

If instead the labelled atom changed from sp² to sp³ hybridization, \( AE^D < AE^H \) due to a large difference in ZpE’s at the transition state. In this scenario the isotopically labelled substrate would react faster than the unlabeled material resulting in an inverse secondary kinetic isotope effect. Typical values for secondary kinetic isotope effects range between 0.8 – 0.9.⁵⁶

### 1.5.2 Graphical Analysis Methods

The increase in accessibility of techniques to acquire robust temporal reaction data has ushered in a modern era which uses chemical kinetics to provide mechanistic insight for simple and complex reactions. Data-dense concentration vs time plots allows reactions to be categorized based on the shape of the profile (Figure 1.7). Examples of these classifications include classical-first order kinetics, autocatalysis, and catalyst activation.
Figure 1.7. Experimental kinetic profiles comparing conventional first order kinetics (red), to sigmoidal type profiles observed via autocatalysis (blue) or catalyst activation (purple).

First order kinetics is defined by the following arbitrary rate law: \( \text{Rate} = k[A]^1 \) where the rate is only dependent upon one species concentration. As the reaction progresses and the \([A]\) decreases, the rate also decreases. This relationship between concentration and time manifests as a reaction profile where the reaction rate is highest at the reaction’s onset but then decreases exponentially until the reaction reaches completion (Figure 1.7).

Autocatalysis is observed if a product of the reaction is catalytically active. Often a latent period exists at the start of these reactions, but as product (i.e. catalyst) is generated as the reaction turns over, the rate increases dramatically resulting in a sigmoidal reaction profile (Figure 1.7). An example of autocatalysis is observed in the Soai reaction where the product (1.3) from alkylating a pyrimidyl aldehyde (1.1) with diisopropyl zinc (1.2) is a competent catalyst in the transformation (Scheme 1.8).\(^{65-67}\)
Reactions that experience catalyst activation are governed by trends that begin with an induction period before a maximum reaction rate is observed (Figure 1.7). An example of this phenomenon occurs in palladium catalyzed amination reactions of aryl bromides where a significant induction period was observed corresponding to catalyst activation via necessary ligand displacement.\(^6\)

Sigmoidal type profiles as observed with catalyst activation and autocatalytic processes are especially difficult to study using classical kinetics (initial rates measurements). The latent periods at the reaction’s onset are prone to large errors in the initial rate measurements and can be difficult to reproduce. Therefore, inferring relationships between substrate concentrations and initial reaction rates to determine the observed rate law is problematic, necessitating for more sophisticated analysis techniques. Reaction progress kinetic analysis (RPKA) and variable time normalization analysis (VTNA) provide modern solutions to understanding reactions which display complex kinetic profiles.

1.5.2.1 Reaction Progress Kinetic Analysis

The generation of reaction progress curves provides a means of probing the entire story, whereas classical methods (i.e. initial rate) focus merely on a snapshot. Reaction progress kinetic analysis (RPKA) is a methodology to maximize mechanistic understanding from a minimal
number of experiments performed at synthetically relevant conditions via graphical manipulations of time-course data.\textsuperscript{59,60} Using RPKA, the underlying rate law of the reaction can be delineated via different [xs] experiments (Section 1.5.2.1.1). Additionally, RPKA can probe the overall reactions robustness by determining if either catalyst deactivation or product inhibition are occurring via the same [xs] protocol (Section 1.5.2.1.2).

1.5.2.1.1 Different [xs] experiments

For a given catalytic network such as Scheme 1.6, RPKA explores the relationships between substrates A and B, and is dependent on the reaction stoichiometry and “excess”. The term “excess” [xs] is defined as the difference between initial concentrations of both substrates (Equation 1.5). If the stoichiometry of the reaction is 1:1 as depicted in Scheme 1.6, [xs] is a constant with units of concentration that allows [A]$_t$ to be calculated from [B]$_t$ and vice versa.

\[ xs = [B]_0 - [A]_0 = [B]_t - [A]_t \]  \hspace{1cm} (eq. 1.5)

RPKA provides a method of determining the reaction orders of [A] and [B] in just three experiments run at synthetically relevant conditions (Table 1.2). By comparing the magnitude of the changes in rates between Experiments 1-3, the orders of [A] and [B] can be elucidated. For example, if the reaction rate decreases between Experiments 1 and 2, the order of [A] is >0, as reducing the [A]$_0$ reduces the overall rate of the reaction.
Table 1.2 Conditions of reactions to allow for the elucidation the orders of A and B

<table>
<thead>
<tr>
<th>Experiment</th>
<th>[A]₀ (mM)</th>
<th>[B]₀ (mM)</th>
<th>[cat] (mM)</th>
<th>[xs]</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>Standard conditions</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>100</td>
<td>1</td>
<td>40</td>
<td>Solve for order in A</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>60</td>
<td>1</td>
<td>-40</td>
<td>Solve for order in B</td>
</tr>
</tbody>
</table>

1.5.2.1.2 Same [xs] Experiments

RPKA also provides a means of probing the overall robustness of a reaction by assessing for participation of either catalyst deactivation or product inhibition. Most kinetic studies of catalytic reactions assume that the concentration of active catalyst remains constant over the course of the reaction and is equal to the amount of catalyst employed. This assumption can be unsubstantiated by either participation of the catalyst in off cycle equilibrium processes or if catalyst death occurs. These nonidealized processes manifest themselves as reaction progress curves that are non-classical (not zeroth, first, or second order).

The “same excess, [xs]” protocol provides a means for probing the active catalyst concentration over the lifetime of the reaction. Additionally, the same [xs] protocol provides a method to probe the effects that [P] has on the overall reaction rate. By performing three experiments (Table 1.3), one can deduce whether catalyst deactivation or product inhibition is occurring.
Table 1.3. Conditions of “same [xs]” reactions of Figure 1.8

<table>
<thead>
<tr>
<th>Experiment</th>
<th>[A]₀ (mM)</th>
<th>[B]₀ (mM)</th>
<th>[P] (mM)</th>
<th>[xs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>60</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1.8. a) Kinetic profiles of “same [xs]” experiments described in Table 1.3; b) time-adjusted profiles for Experiments 5 and 6.

The three experiments in Table 1.3 are carried out with the same [xs] (0), but each experiment represents the same reaction started from different time points. Experiment 4 represents standard conditions. Experiment 5 represents duplicating Experiment 4 but beginning the reaction after the catalyst has turned over 40% of the starting materials. Experiment 6 represents duplicating Experiment 5, but also including product at the reaction’s onset. Figure 1.8a displays representative data for Experiments 4-6.

To identify if either catalyst deactivation or product inhibition are occurring, a horizontal time shift is applied to Experiments 5 and 6 to the timepoint where they should exhibit identical behavior (Figure 1.8b). If Experiments 4 and 5 do not overlay, it is either because the catalyst has undergone a number of turnovers affecting its activity, or because the reaction in Experiment 4 contains 40 mM of P. Experiment 6 provides a means to distinguish whether catalyst deactivation
or product inhibition is the reason the time shifted Experiment 5 does not overlay with Experiment 4. Applying the same time-shift to Experiment 6 results in exceptional overlay between Experiments 4 and 6 confirming that the origin of the decrease in rate over time is product inhibition.

Therefore, by applying the same [xs] protocol the robustness of any catalytic reaction can be assessed by determining if either catalyst deactivation or product inhibition are occurring. Once this information is known additional inferences regarding the reaction mechanism can be proposed to make the process more efficient, perhaps by finding ways to mitigate catalyst deactivation. This ultimately can improve catalytic activity to make these methods more efficient and practical to employ at scale.

### 1.5.2.2 Variable Time Normalization Analysis

Variable time normalization analysis (VTNA), reported by Burés in 2016, represents another breakthrough in kinetic analyses.\(^{61,62}\) Both graphical analysis methods RPKA and VTNA allow for the order of substrates and catalysts to be elucidated, but while RPKA does this using rate data, VTNA accomplishes this using concentration data. This increases the scope of reaction monitoring techniques which can be used to elucidate the rate law by allowing data from spectroscopic methods to be treated directly with VTNA to solve the underlying rate law without first converting to rate data.

#### 1.5.2.2.1 Solving Catalyst Order

The order of a catalyst can be elucidated by plotting concentration data [A] against a normalized time scale \(t(cat)^n\). By adjusting the time scale experiments with different catalysts
loadings can be compared directly using concentration data. The normalization assumes that the catalyst concentration is constant over the course of the reaction. This method compresses each reaction profile proportionally to the initial catalyst loading without altering their shape to allow for a straight-forward, visual comparison to be made. Each time point is multiplied by the concentration of catalyst used in the experiment raised to an adjustable variable. When the variable exponent matches the order of the catalyst, graphical overlay between experiments is observed (Figure 1.9c).

![Diagram](image)

**Figure 1.9.** Time normalization method to determine that the order in catalyst is 1 via observing curve overlay between experiments performed with difference catalyst loadings.

A key advantage of this methodology is that it compares several points over the reaction profile as opposed to just one as in initial rate measurements. Therefore, fewer experiments with small data sets can be used successfully to elucidate the order in catalyst. This methodology has been successfully applied to elucidate the order of various organometallic and organic catalyzed reactions to aid in mechanistic understanding.$^{69-74}$
1.5.2.2 Solving Substrate Orders

VTNA utilizes graphical interrogation of kinetic data by exploiting human’s visual capacity to accurately identify patterns (i.e. graphical overlay) to solve for the orders of substrates in as few as three experiments. Concentration data from the experiments described in Table 1.3 can be used to solve the substrate orders of both A and B by plotting the [P] against a normalized time axis.

Profiles of experiments that differ in one concentration (A) will only overlay when the time axis is substituted by the time integral of the concentration of A raised to the correct power, α (Equation 1.6). All of the values needed to solve equation 1.6 are known from the concentration vs time profiles.

\[ \int_{t=0}^{t=n} [A]^a \, dt = \sum_{i=1}^{n} \left( \frac{[A]_i+[A]_{i-1}}{2} \right)^\alpha (t_i - t_{i-1}) \]  

(eq. 1.6)

Figure 1.10 shows the application on a generic reaction where A and B form P which is catalyzed by cat. Modifying the α value from 0 to 1 affects the normalized x axis from Σ[A]0Δt to Σ[A]1Δt. An α value of 1 results in exceptional overlay between the two experiments evidencing that the order of [A] is 1. By performing one additional experiment and reapplying VTNA the order of the B component can also be elucidated. This methodology has been successfully employed to solve the underlying rate law for a wide range of catalytic transformations.75–79
Figure 1.10. VTNA profile indicating that order of [A] is 1 based on the graphical overlay when the time axis is normalized

More recently the utility of VTNA has been expanded to allow for the removal of induction periods from reaction profiles to simplify the overall progress curves. Additionally, catalyst activation or deactivation profiles can be estimated when the order of the substrates is known.80

1.6 Overview of Chapters

The combination of reaction monitoring technologies with experimental methods to interrogate chemical mechanisms presented in this chapter will play a central role throughout this thesis. Chapters 2-6 discuss case studies that demonstrate the utility of temporal profiling for increasing mechanistic understanding to ultimately make reactions more efficient.

Chapter 2 presents the development and application of an automated reaction monitoring platform which utilizes online HPLC as an analytical tool. The platforms ability to sample and analyze aliquots reproducibly was demonstrated. Three different catalytic reactions were then profiled to showcase the utility and versatility of the device for increasing mechanistic understanding of chemical reactions while minimizing analyst intervention.
Chapter 3 describes mechanistic investigations completed on the Kinugasa reaction used to synthesize beta-lactams. Automated sampling combined with online analysis played an integral role in these investigations and ultimately allowed for the proposal of a novel reaction mechanism which accounts for the undesired byproducts often associated with the reaction.

Chapter 4 explores the development of a telescoped procedure to synthesize cyanoimidazoles via a condensation annulation proceeded by a functional group conversion. Orthogonal reaction monitoring technologies including in situ IR, offline HPLC, static tube NMR, and temporal pH measurements were integral to increasing mechanistic understanding. We report conditions to afford the desired cyanoimidazole in high yields via a telescoped process.

Chapter 5 presents modifications to the reaction monitoring platform discussed in Chapter 2 which allowed for the system to monitor reactions performed within a glovebox. After the sampling reproducibility of the system was demonstrated, a series of Buchwald-Hartwig aminations were profiled. Each reaction profile observed was unexpected when compared to COPASI models and previous kinetic data, thereby highlighting the importance of profiling entire reactions as opposed to performing initial rate measurements. This chapter emphasized the importance that mechanistic assumptions made for similar catalytic systems can lead to faulty understanding, again signaling the importance of monitoring each reaction individually.

Finally, Chapter 6 discusses mechanistic investigations for an optimized synthesis of the hole-transport material Spiro-OMeTAD. Reactions were performed and monitored from within an inert environment using the automated sampling device described in Chapter 5. Remarkable chemoselectivity was observed throughout the reactions progress as only one intermediate was observed during the coupling cascade. Kinetic data lead to the proposal of a reaction mechanism which accounts for the chemoselectivity and identifies that catalyst resting state. These studies
allowed for the synthesis of Spiro-OMeTAD in >90% yields on gram scale which can be isolated without column chromatography.
Chapter 2: Developing an Automated Reaction Monitoring Platform

2.1 Introduction

The innate complexity of chemical catalysis makes it a difficult area of study due to the ambiguity of species in a reaction. The optimal way to increase mechanistic understanding of catalytic reactions is to monitor changes in starting materials, intermediates, and products as the reaction proceeds. Typically, this involves manual sampling by an analyst, where timed aliquots are withdrawn, quenched, and transferred to a dedicated analytical instrument (NMR, HPLC, GC) for offline analysis. When using this approach, an internal standard is necessary to allow accurate quantification. While a greater deal of information can be gathered using manual sampling than using end point analysis, there are several inherent disadvantages to this approach. First, inconsistencies in sampling (variability between time points, sample volume withdrawn, etc.) is a key disadvantage that can lead to inaccurate concentration vs time data and poor reaction understanding, thereby decreasing reproducibility. Second, manual sampling by introducing a cannula or needle can give fluctuations in reaction conditions, such as temperature and pressure, creating disruptions in the reaction behaviour. Last, and most importantly, manual sampling is an extremely laborious and time-consuming process, especially when high data density is required over multiple reaction conditions.

Strides have been taken towards moving away from manual sampling and incorporating multiple orthogonal in situ monitoring techniques in tandem by leveraging automated sampling technology. By utilizing laboratory robotic platforms, it is possible to achieve consistent and reproducible sampling with minimal need for analyst intervention. The most common workflow for this approach involves employing an automated liquid handling device, which withdraws a predetermined aliquot from the reaction mixture at set intervals and quenches this sample for off-
line analysis once the process has completed. This disconnect between sampling and analysis creates several limitations. Most significantly, an appropriate quenching protocol must be identified and validated. This quench must stop the reaction without affecting sensitive reagents or intermediates, such that each timed aliquot remains a true representative sample of the process under investigation. In addition, the delay between sample acquisition and analysis can result in changes in analyte concentrations due to evaporation, precipitation and sample degradation.

Kinetic analysis on heterogeneous reactions presents an especially difficult challenge. Inhomogeneity often leads to variable amounts of the solid or liquid component being extracted, thus producing an inaccurate representation of the overall reaction. Advances in pump technology have improved this situation, but changes in viscosity and particle size over the course of the reaction lead to major variations between sample compositions. In 2017 the Hein Lab demonstrated a slurry sampling approach capable of quantifying both solution and solid state components of a heterogeneous reaction.\textsuperscript{45}

A system for gathering kinetic data from any chemical process that utilizes both automated sampling and immediate chromatographic analysis would be optimal for increasing mechanistic understanding. This combination of technologies would provide accurate and detailed analysis of a complex reaction mixture in real-time and allows for traditionally offline techniques like HPLC-MS to be used similar to more traditional operando techniques like ReactIR or flow NMR. A major hurdle to realizing this goal is the mismatch between operational reaction concentrations and the dilution required for offline techniques, such as HPLC-MS.

In this chapter, we report a prototype robotic system which couples automated reaction sampling, in-line sample dilution, and direct analysis by HPLC-MS. This new device is capable of rapid, unattended reaction analysis, and can deliver kinetic profiles for complex reaction mixtures.
This system can be easily used to monitor both homogenous and heterogeneous catalytic reactions by interfacing with either a syringe pump or slurry sampling device respectively.

To demonstrate the device’s accuracy and broad applicability, it has been employed in real time reaction monitoring for a series of catalytic processes. Each reaction studied presents a particular challenge to conventional analysis using aliquoting and off-line HPLC such as continued reactivity after dilution, heterogeneity, and presence of multiple structurally similar compounds. These include a Cu(I)-catalysed azide-alkyne cycloaddition, a Suzuki cross coupling reaction utilizing a heterogeneous base, and a Lewis-acid catalyzed substitution reaction between 2-furylcarbinol (2.8) and multiple nucleophiles (Scheme 2.1).

Scheme 2.1. Homogeneous (a and c) and heterogeneous (b) reactions capable of being monitored using our automated reaction monitoring platform.
2.2 System Description

Both homogeneous and heterogeneous sampling methods are based on a Gilson GX-281 liquid handling robot. Gilson’s Trilution software packaged can be used to script all actions and events, resulting in excellent reproducibility and complete control in sample timing.

![Diagram of the automated device](image)

**Figure 2.1. Schematic of the two possible configurations of the automated device. A) Configuration for homogeneous sampling. B) Configuration for heterogeneous sampling**

The sampling process for a homogeneous reaction begins with a syringe pump (New Era 1000) withdrawing a 50 µL aliquot from the reaction vial. The sample is pulled through a polyether ether ketone (PEEK) capillary, using an excess volume to ensure the first sample loop is entirely filled. The first Rheodyne 6-port 2-position valve switches positions, placing the sample loop in line with the diluent pump (Valco M6). A predetermined volume of dilution solvent is then pumped from the solvent reservoir through the sample loop and into the mixing block. High sheer and turbulent flow rapidly mixes the sample with the diluent, resulting in highly reproducible in-line
dilution (Figure 2.2). The now diluted sample is then delivered via a length of polytetrafluoroethylene (PTFE) tubing to an injection loop on a second Rheodyne 6-port 2-position valve. The GX-281 then switches the valve position, putting the final sample in-line with the analytical HPLC column. Simultaneously, a trigger signal is sent to the HPLC pump to initiate a separation and quantification method. The system resets to its initial state and a wash cycle is performed to ensure no residual sample remains in the tubing or sample loops.

![Image](image-url)

**Figure 2.2.** Schematic of in-line mixing to produce variable dilution profiles based on differential volumes of diluent. 

- **a)** Sample (in red) is introduced in the fluid lines as a focused band.
- **b)** Flowing diluent through an in-line turbulent mixer changes the focused sample band into an elongated segment.
- **c)** The leading edge (highest sample concentration) can be loaded into the injection loop when a lower diluent volume was applied.
- **d)** Lower concentrations can be injected by increasing the diluent volume.

Sampling for heterogeneous systems utilizes an EasySampler probe (Mettler-Toledo). The sequence begins by extrusion of the sampling head from the EasySampler probe into the reaction mixture, revealing a nitrogen-filled, 20 μL sampling pocket. The pocket fills with sample and then is withdrawn into the probe head. Dilution solvent capable of solubilizing reaction components is then pumped from the solvent reservoir through the sample pocket, where the species are dissolved and diluted. Turbulent flow in the probe head produces a similar in-line dilution effect as the static
mixing element used in the homogeneous sampling circuit. The diluted sample is delivered to the second injection loop and the HPLC run is triggered as described previously. As to not introduce any unwanted solvent into the reaction mixture, nitrogen gas is passed through the EasySampler probe, thereby flushing the lines before the next sample is taken.

2.3 In-line Dilution and Reproducibility

A major obstacle in incorporating real-time HPLC-MS analysis is addressing the large mismatch between reagent concentrations in the reaction vessel and those compatible with the analytical instrument. Moreover, if the system is to be robust, we require a means of dynamically diluting the sample in order to accommodate a wide range of potential concentrations. This challenge is exacerbated by the fixed sampling elements (sample loop, EasySampler pocket, injection loop) in our design, which afford excellent metering accuracy but lack the ability to change volumes on the fly. Our solution was to utilize the dilution profile from the turbulent mixing element (in-line static mixer or EasySampler pocket). When a concentrated sample is passed through the turbulent mixing region, a diffuse band is produced containing a concentration profile along the axis of the fluid transfer line (Figure 2). By controlling the delivery volume used to pass this segment to the injection valve, variable sample concentrations can be introduced for HPLC analysis.

The reproducibility of the in-line dilution method was tested by using a standard solution containing a mixture of reagents. An aliquot was generated using our solution sampling circuit, where the volume of diluent used to deliver the sample to the injection valve was varied before the contents of the injection loop was analyzed by HPLC. By varying the delivery volume and measuring the contents of the injection loop a diagnostic profile is created. As larger volumes of
diluent were employed, we observed a sharp increase in sample concentration until an apex concentration value near 500 μL of diluent is reached. The sample concentration then tails with a lower slope, returning to baseline beyond ~1 mL. (Figure 2.3A). A similar profile was also observed when using the heterogeneous sampling circuit, however, the apex concentration was observed closer to 850 μL. It should be noted that these particular solvent volumes are fully tuneable by varying the length and internal diameter of the fluid lines.

Data for each chosen delivery volume was acquired in triplicate proving that the mixing effect using turbulent flow is highly reproducible. This allows “on-the-fly” concentration optimizations over a wide dynamic range by simply altering the applied diluent volume.

Figure 2.3. Relationships between diluent volume and concentration for the automated sampling device. a) Relationship when using the homogeneous sampling method. b) Relationship when using the heterogeneous sampling method. Each data point represents the average of a triplicate series. Error bars represent ± one standard deviation of the series.
2.4 Applications to Reaction Monitoring

2.4.1 Profiling a Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) reaction

To demonstrate the ability of our automated sampling device in monitoring catalytic reactions we first turned our sights to the Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) reaction.84,85 This reaction has been heavily investigated because of its virtually quantitative yields and synthetic usefulness with relevance to in vivo tagging,86 biomolecular ligation,87 and as a polymerization reaction for the synthesis of linear polymers.88 However, the reaction’s efficiency creates issues in monitoring the kinetics. Simply diluting a timed aliquot from the reaction does not arrest the product formation. Thus, off-line HPLC analysis will not represent the true conversion vs. time profile. Our system does not suffer from this limitation, as the reaction aliquot is diluted in-line and applied to the HPLC column in less than one minute, minimizing the potential for subsequent reactivity after aliquoting.

The reaction between phenylacetylene (2.1) and benzylazide (2.2) catalyzed by CuI/TCPTA (5 mol%) in acetonitrile at 25 °C was monitored using our system (Figure 2.4). A nearly continuous HPLC trend was generated over the course of the two-hour reaction. Concentration profiles for both starting materials 2.1 and 2.2 are nearly identical as indicated by the almost perfectly mirrored curves. The mass balance of the reaction is also in excellent agreement which is expected due to the high yielding nature of the CuAAC “click” reaction. The reaction appears to exhibit a complex kinetic profile and does not obey simple first-order kinetics under these conditions. Instead the reaction displays a distinct sigmoidal curve indicative of in situ catalyst activation. This kinetic profile is common for systems where the catalyst partitions between a major off-cycle inactive reservoir.89
Figure 2.4. Progress curves for CuAAC reaction monitored using the homogenous sampling system and HPLC-MS, TCPTA = tri((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine.

2.4.2 Monitoring a Heterogeneous Reaction

The Pd(0) catalyzed Suzuki reaction has emerged as one of the most important tools for the construction of diverse chemical libraries. This has been made possible by the reaction’s ability to unite a wide array sp³ and sp²-functionalized boronic acids and (pseudo)halides. While many protocols for this reaction have been developed, the reaction typically utilizes a heterogeneous inorganic base, such as K₂CO₃, K₂PO₄, CsF, or Cs₂CO₃. This heterogeneous component complicates on-line analysis techniques such as NMR and interferes with sampling. Furthermore, the efficiency of this system leads to the same complication as observed with the CuAAC reaction; simply diluting timed aliquots does not effectively arrest the reaction progress.
In our test system, vinyl chloride 2.4 was coupled with \( p \)-methoxyphenyl boronic acid (2.5) using Pd\(_2\)(dba)\(_3\) and Cs\(_2\)CO\(_3\) in dioxane (Figure 2.5). Despite being a heterogeneous slurry, the reaction progress could be readily captured using the EasySampler probe and methanol as diluent to solubilize the solid components. This substrate gives exclusive cross coupling at the terminal chloride, producing two isomers of the arylated product. While the Suzuki reaction on vinyl halides generally proceeds with retention of configuration, reports have indicated observing isomerization.\(^{93,94}\) In this example, our conditions gave nearly exclusive retention of configuration, yielding the more crowded (not thermodynamically stable) tri-substituted alkene product.

\[
\begin{align*}
\text{Ph} & \quad \text{O} \quad \text{Cl} \\
\text{2.4} & \quad + \quad \text{ArB(OH)}_2 \\
\text{Pd}_2\text{(dba)}_3 & \quad \text{2.5} \\
\text{Cs}_2\text{CO}_3 & \quad \text{Dioxane, 40°C} \\
& \quad \rightarrow \\
\text{Ph} & \quad \text{O} \quad \text{Cl} \\
\text{2.6} & \quad + \quad \text{Ph} \quad \text{Cl} \\
\text{2.7} & \quad + \quad \text{Cl} \quad \text{Ar}
\end{align*}
\]

Figure 2.5. Progress curves for Suzuki reaction monitored using the heterogeneous sampling system and HPLC-MS. Ar = \( p \)-methoxyphenyl.

It is noteworthy that the reaction trends do show a higher degree of scatter than the analogous homogenous systems. The high reproducibility observed in the in-line dilution
calibration (Figure 2.3b), which was completed using the EasySampler probe on a homogenous solution, indicates that this point-to-point error is not a failing of the probe design. Instead the error is likely due to slight variations in the slurry composition at each time point, containing greater or less volume of heterogeneous base relative to the solution phase. Thus, while the apparatus does offer new capability, some optimization is still required.

2.4.3 Monitoring a Complex Multicomponent Reaction

On-line HPLC analysis is particularly well suited for deconvoluting complex multicomponent reactions due to the ability to rapidly resolve and quantify many chemically similar components (regio- and stereoisomers). To demonstrate the capability of our system to profile chemically similar components, we examined a Lewis-acid catalyzed substitution and rearrangement of 2-methylfurylcarbinol (2.8) in the presence of multiple aniline nucleophiles (Scheme 2.1).95,96 The reaction proceeds via activation of the alcohol by Dy(OTf)₃, forming key oxocarbenium intermediate 2.12 (Scheme 2.2). Aniline can add reversibly to form amine 2.10a. Alternately, nucleophilic addition to the furan ring produces N,O-acetal 2.13, which then undergoes rearrangement involving ring fragmentation and 4π-electrocyclic ring closure to generate cyclopentenone 2.11a. The highly electrophilic nature of 2.12 also makes it susceptible to Friedel-Crafts arylation of electron-rich aromatic compounds.81,97
Scheme 2.2. Network of reactions possible in the Piancatelli rearrangement

The complexity of this reaction is further magnified when multiple nucleophiles are introduced. To demonstrate our system's ability to profile multicomponent reaction mixtures, a competition experiment was carried out between carbinol 2.8 (2 equiv.) aniline (2.9a, 1 equiv.) and p-nitroaniline (2.9b, 1 equiv.). The HPLC profile (Figure 2.6) reveals that both anilines 2.9a and 2.9b are initially converted to their corresponding exocyclic amines (2.10a and 2.10b respectively). However, their fate after this point is quite different. While 2.10a undergoes rearrangement to give cyclopentenone 2.11a, exocyclic amine 2.10b decomposes to release p-nitroaniline 2.9b. This demonstrates that while 2.9b can participate in the hydroxyl substitution, it is too electron poor to engage in the rearrangement reaction, possibly due to rapid fragmentation of the required N,O-aminal.
Figure 2.6. HPLC progress curves for the nucleophilic substitution competition reaction of furylcarbinol (2.8), aniline (2.9a) and nitroaniline (2.9b) catalysed by Dy(OTf)$_3$. Sampling was performed using the homogenous sampling method.

2.5 Conclusions

We have developed an automated system which is capable of real-time reaction monitoring using HPLC-MS. This sampling system is capable of monitoring both homogeneous and heterogeneous reactions by either utilizing a syringe pump sampling method or using a EasySampler sampling probe. Utilization of an in-line turbulent mixer allows the reaction aliquot to be to dynamically diluted, enabling real-time separation and quantification of reaction components by HPLC-MS. The reproducibility of the in-line mixing technique was also validated and shown to be capable of delivering a wide concentration range to an analytical method of choice. These features allow our design to readily adapt and respond to a diverse array of reaction components or conditions. We demonstrated the ability of our newly developed automatic reaction
monitoring system to measure the progress of three distinct reactions. The three reactions profiled were a CuAAC reaction, a heterogeneous Suzuki cross coupling reaction, and a multicomponent competition reaction of the substitution of \(p\)-nitroaniline and aniline on a furylcarbinol.

2.6 Experimental

2.6.1 General Remarks

2.6.1.1 Reagents

All reagents and solvents were purchased from Fisher Scientific, Alfa Aesar, Sigma-Aldrich, and VWR and were used without further purification. All other reagents and solvents were purchased from conventional suppliers and used as received unless otherwise stated. Silica gel was purchased from Silicycle (60 Å, 230 x 400 mesh).

2.6.1.2 Analytical Methods

NMR spectra were recorded on a Bruker spectrospin 300 Instrument (300MHz and 75MHz for \(^1\)H and \(^{13}\)C, respectively), an Agilent (400MHz and 100 MHz for \(^1\)H and 13C, respectively), a Bruker Avance 300 (300MHz and 75MHz for \(^1\)H and \(^{13}\)C, respectively), or a Bruker AvanceIII 500 (500MHz and 125MHz for \(^1\)H and \(^{13}\)C, respectively) and were calibrated with the solvent (CDCl\(_3\): 7.26ppm for \(^1\)H NMR and 77.16 for \(^{13}\)C NMR). The abbreviations s, t, m signify singlet, triplet, multiplet respectively. NMR spectra were analyzed by using the software MNova.

The LC samples were analyzed by HPLC/MS conducted on an Agilent 1200 HPLC with the following configuration:

Agilent G1379B degasser, G1312A binary pump, G1316A thermal column compartment, diode array detector and 6120 single quad mass spectrometer.
Analytical setting for the detectors are:

DAD – 200 – 400 nm collected at 20 Hz storing all spectra for offline analysis. Peak area for quantification varies depending on the experiment, see calibration curves for details

ESI-MSD – positive mode scan for m/Z 110 – 1500 running at 0.8sec/cycle. drying gas = 7.0 l/min, nebulizer pressure = 20 psi, gas temperature = 300 °C, capillary voltage = 4000 V

HPLC column and mobile phase method include one of the following conditions (see individual experiment for details):

(1) Poroshell 120 Phenylhexyl-C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = water, 0.05 % trifluoroacetic acid; Solvent B = acetonitrile, 0.05 % trifluoroacetic acid; Flow Rate = 0.625 mL/min; Starting Conditions = 90 % A, 10 % B; 0.0-4.0 min linear gradient to 20% A, 80 % B.

(2) Poroshell 120 EC-C18, 2.1 x 30 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = water, 0.05 % trifluoroacetic acid; Solvent B = acetonitrile, 0.05 % trifluoroacetic acid; Flow Rate = 0.75 mL/min; Starting Conditions = 35 % A, 65 % B; 0.0-3.0 min linear gradient to 20% A, 80 % B.

(3) Poroshell 120 EC-C18, 2.1 x 30 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = water, 0.05 % trifluoroacetic acid; Solvent B = acetonitrile, 0.05 % trifluoroacetic acid; Flow Rate = 0.625 mL/min; Starting Conditions = 65 % A, 35 % B; 0.0-3.0 min linear gradient to 30% A, 70 % B.
Figure 2.7. Labelled photo of the automated sampling device. A) Rheodyne 6 port, 2 position valve; B) Easymax reactor for heating / stirring. C) Inline mixer. D) Syringe pump used for homogeneous reaction sampling. E) Gilson liquid handler. F) HPLC for online analysis.

Figure 2.8. Zoomed in, labelled photo of the automated sampling device. A) Rheodyne 6 port, 2 position valve. B) Easymax reactor for heating / stirring. C) Inline mixer. D) Syringe pump used for homogeneous reaction sampling.
2.6.2 Synthetic Procedures

2.6.2.1 (Azidomethyl)benzene (2.2)

\[
\begin{align*}
\text{N}_3 & \quad \text{Ph}
\end{align*}
\]

Compound 2.2 was synthesized using a literature procedure and characterization data was consistent with that of the literature.\(^{98}\)

\(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta 7.50 - 7.37 \text{ (m, 5H)}, 4.38 \text{ (s, 2H)};\)

\(^{13}C\{^1H\} \text{ NMR} (101 MHz, CDCl}_3\) \(\delta 135.4, 128.8, 128.2, 128.2, 54.7.\)

2.6.2.2 1-Benzyl-4-phenyl-1H-1,2,3-triazole (2.3)

\[
\begin{align*}
\text{N} &= \text{N} \\
\text{N} &= \text{N} \\
\text{Ph} &\quad \text{Bn}
\end{align*}
\]

To a 16 mL glass vial equipped with PTFE-silicon septum, open-top screw cap, and magnetic stir bar was added 9.5 mL of acetonitrile, (azidomethyl)benzene (0.067 g, 0.50 mmol), and phenylacetylene (0.051 g, 0.50 mmol). CuI (4.8 mg, 0.025 mmol) and TCPTA (12 mg, 0.025 mmol) dissolved in 0.5 mL of acetonitrile was injected via syringe. The reaction was stirred for 3 hours and then quenched with saturated aqueous NaHCO\(_3\) solution. The mixture was then extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over MgSO\(_4\), filtered, and then concentrated \textit{in vacuo}. The crude material was recrystallized from petroleum ether:ethyl acetate to afford 1-benzyl-4-phenyl-1H-1,2,3-triazole (0.0716 g. 0.305 mmol, 61% yield) as colourless crystals.

\(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta 7.80 \text{ (d, } J = 7.3 \text{ Hz, 2H)}, 7.66 \text{ (s, 1H)}, 7.43 - 7.29 \text{ (m, 10H)}, 5.57 \text{ (s, 2H)}\)

\(^{13}C\{^1H\} \text{ NMR} (101 MHz, CDCl}_3\) \(\delta 148.3, 134.8, 130.7, 129.3, 128.9, 128.89, 128.3, 128.2, 125.8, 119.6, 54.3\)

\textbf{MS ESI}+ (calc. for C\(_{15}\)H\(_{13}\)N\(_3\), 235.11): \textit{m/z} = 236.2 [M+H]\(^{+}\), 258.2 [M+Na]\(^{+}\).
2.6.2.3 **Tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (TCPTA)**

This compound was prepared through adaptation of a previous report.\(^9^9\)

\(\text{\(^1\)H NMR (400 MHz, CDCl}_3\)} \(\delta\) 7.77 (s, 3H), 4.90 (p, \(J = 7.0\) Hz, 3H), 3.74 (s, 6H), 2.29 – 2.16 (m, 6H), 2.08 – 1.97 (m, 6H), 1.94 – 1.82 (m, 6H), 1.78 – 1.65 (m, 6H);

\(\text{\(^{13}\)C\{'\(^1\)H\} NMR (101 MHz, CDCl}_3\)} \(\delta\) 143.5, 122.4, 61.73, 47.1, 33.4, 24.1

**MS ESI+** (calc. for C\(_{24}\)H\(_{36}\)N\(_{10}\), 464.31): \(m/z = 465.2\) [M+H]\(^+\), 487.2 [M+Na]\(^+\).

2.6.2.4 **(E)-1,2-Dichlorovinyl phenyl ketone (2.4)**

The synthetic procedure described by Jonczyk et al.\(^1^0^0\) for 2.4 did not give complete conversion. However, another procedure from the same laboratory for methyl 2-formylphenylacetate gave full conversion and was used for the synthesis of 2.4.\(^1^0^1\) The \(^1\)H chemical shifts we observed for 2.4 were not consistent with those reported by Jonczyk et al. but we confirmed that we had indeed made 2.4 by subsequent chemistry. The \(E\)-configuration of compound 2.4 was validated by synthesizing its \(Z\) isomer and comparing their \(^3J_{C,H}\) values.

Compound 2.4 was isolated as a yellow oil in 85% yield.

\(\text{\(^1\)H NMR (500 MHz, CDCl}_3\)} \(\delta\) 6.69 (s, 1H), 7.51-7.54 (m, 2H), 7.64-7.67 (m, 1H), 7.96-7.97 (m, 2H) ppm;

\(\text{\(^{13}\)C\{'\(^\text{\(\text{\(^1\)H}\)}\} NMR (125 MHz, CDCl}_3\)} \(\delta\) 118.9, 127.7, 129.0, 129.8, 133.5, 134.7, 188.2 ppm;

**HRMS:** Calculated for C\(_9\)H\(_6\)OCl\(_2\): 199.9796, Found: 199.9798
2.6.2.5  (E)-2-chloro-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (2.6)

Compound 2.6 was synthesized using a literature procedure and was isolated as a yellow solid in 78% yield. Characterization data was consistent with that of the literature.\(^\text{102}\)

\(^1\text{H} \text{NMR} \ (500 \text{ MHz, CDCl}_3) \ \delta \ 3.72 \ (s, \ 3 \text{H}), \ 6.69-6.72 \ (m, \ 2 \text{H}), \ 7.09-7.12 \ (m, \ 2 \text{H}), \ 7.14 \ (s, \ 1 \text{H}), \ 7.40-7.44 \ (m, \ 2 \text{H}), \ 7.53-7.57 \ (m, \ 1 \text{H}), \ 7.96-7.98 \ (m, \ 2 \text{H}) \ ppm; \)

\(^{13}\text{C} \{\text{H}\} \text{NMR} \ (125 \text{ MHz, CDCl}_3) \ \delta \ 55.2, \ 114.0, \ 124.8, \ 126.0, \ 128.8, \ 129.9, \ 130.0, \ 132.5, \ 134.1, \ 134.2, \ 159.8, \ 191.8 \ ppm; \)

HRMS: Calculated for C\(_{16}\)H\(_{13}\)O\(_2\)Cl: 272.0604, Found: 272.0605

2.6.2.6  (Z)-2-chloro-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (2.7)

Compound 2.7 was synthesized using a literature procedure and was isolated as a white solid in 78% yield.\(^\text{102}\)

\(^1\text{H} \text{NMR} \ (500 \text{ MHz, CD}_3\text{OD}) \ \delta \ 3.85 \ (s, \ 3 \text{H}), \ 7.00-7.02 \ (m, \ 2 \text{H}), \ 7.50 \ (s, \ 1 \text{H}), \ 7.51-7.55 \ (m, \ 2 \text{H}), \ 7.61-7.64 \ (m, \ 1 \text{H}), \ 7.72-7.74 \ (m, \ 2 \text{H}), \ 7.88-7.90 \ (m, \ 2 \text{H}) \ ppm; \)

\(^{13}\text{C} \{\text{H}\} \text{NMR} \ (125 \text{ MHz, CD}_3\text{OD}) \ \delta \ 54.5, \ 113.8, \ 125.2, \ 127.6, \ 128.2, \ 128.9, \ 132.1, \ 132.7, \ 137.3, \ 140.6, \ 161.8, \ 191.8 \ ppm; \)

HRMS: Calculated for C\(_{16}\)H\(_{13}\)O\(_2\)Cl: 272.0604, Found: 272.0604

2.6.2.7  1-(furan-2-yl)ethanol (2.8)

1-(furan-2-yl)ethanone (4.536 g, 41.2 mmol) was added to methanol (100 ml) and cooled to 0 °C. Sodium borohydride (1.870 g, 49.4 mmol) was then added in batches.
After the borohydride addition was complete, the reaction was warmed to room temperature and stirred for one additional hour. The reaction mixture was quenched with water and extracted with dichloromethane. The combined organic layers were dried over MgSO₄, filtered, and then concentrated *in vacuo* to afford 1-(furan-2-yl)ethanol (3.933 g, 35.1 mmol, 85 % yield) as a light yellow oil.

**1H NMR** (400 MHz, CDCl₃) δ 7.38 – 7.36 (m, 1H), 6.33 (dd, *J* = 3.1, 1.7 Hz, 1H), 6.23 (dd, *J* = 3.2, 0.6 Hz, 1H), 4.88 (p, *J* = 6.4 Hz, 1H), 1.97 – 1.94 (m, 1H), 1.54 (d, *J* = 6.6 Hz, 3H);

**13C{H} NMR** (75 MHz, CDCl₃) δ 135.4, 128.9, 128.3, 128.2, 54.8, 30.9;

**MS ESI+** (calc. for C₆H₈O₂, 112.05): m/z = 95.2 [M-H₂O+H]⁺

### 2.6.2.8 N-(1-(furan-2-yl)ethyl)aniline (2.10a)

1-(furan-2-yl)ethanol (187 mg, 1.67 mmol) and aniline (157 mg, 1.67 mmol) were treated with Dy(OTf)₃ (51 mg, 0.083 mmol) in acetonitrile (10 mL). The resulting reaction mixture was heated to 65 °C for 2 hours. The reaction mixture was then quenched with saturated aqueous NaHCO₃ solution and extracted with dichloromethane. The combined organic layers were dried over MgSO₄, filtered, and then concentrated *in vacuo*. The residue was purified on silica gel (petroleum ether/ethyl acetate 19:1) to afford N-(1-(furan-2-yl)ethyl)aniline (74 mg, 0.395 mmol, 23.7 % yield) as an oil.

**1H NMR** (400 MHz, CDCl₃) δ 7.34 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.19 – 7.13 (m, 2H), 6.74 – 6.68 (m, 1H), 6.63 (dd, *J* = 8.5, 0.9 Hz, 2H), 6.29 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.16 (d, *J* = 3.2 Hz, 1H), 4.64 (q, *J* = 6.3 Hz, 1H), 3.87 (s, 1H), 1.56 (d, *J* = 6.8 Hz, 3H);

**13C{H} NMR** (101 MHz, CDCl₃) δ 157.4, 147.1, 141.6, 129.3, 117.9, 113.6, 110.2, 105.2, 47.4, 21.0.
MS ESI+ (calc. for C_{12}H_{13}NO, 187.10): m/z = 95.2 [M-C_6H_7N+H]^+

2.6.2.9 N-(1-(furan-2-yl)ethyl)-4-nitroaniline (2.10b)

1-(furan-2-yl)ethanol (193 mg, 1.721 mmol) and 4-nitroaniline (238 mg, 1.721 mmol) were treated with Dy(OTf)$_3$ (52.5 mg, 0.086 mmol in acetonitrile (10 mL). The resulting reaction mixture was heated to 65 °C for 20 minutes. The reaction mixture was quenched with saturated aqueous NaHCO$_3$ solution and extracted with dichloromethane. The combined organic layers were dried over MgSO$_4$, filtered, and then concentrated in vacuo. The residue was purified on silica gel (petroleum ether/ethyl acetate, 4:1) to afford N-(1-(furan-2-yl)ethyl)-4-nitroaniline (364 mg, 1.567 mmol, 91 % yield) as a yellow solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.09 – 8.04 (m, 2H), 7.35 (dd, $J = 1.7$, 0.7 Hz, 1H), 6.60 – 6.54 (m, 2H), 6.31 (dd, $J = 3.2$, 1.9 Hz, 1H), 6.18 (d, $J = 3.2$ Hz, 1H), 4.79 – 4.68 (m, 2H), 1.61 (d, $J = 6.5$ Hz, 3H);

$^{13}$C{H} NMR (101 MHz, CDCl$_3$) δ 155.3, 152.2, 142.2, 138.4, 126.4, 111.8, 110.4, 105.9, 47.0, 20.5;

MS ESI+ (calc. for C$_{12}$H$_{12}$N$_2$O$_3$, 232.08): m/z = 233.2 [M+H]$^+$, 255.2 [M+Na]$^+$. 
2.6.3 Reaction Monitoring Experiments

2.6.3.1 Copper Catalyzed Azide-Alkyne Cycloaddition Reaction

\[ \text{H} \quad \text{Ph} \quad 2.1 \quad + \quad \text{BnN}_3 \quad \xrightarrow{\text{Cul (5 mol\%)} \quad \text{TCPTA (5 mol\%)} \quad \text{MeCN, 25 °C}} \quad \text{Ph} \quad \text{N} = \text{N} \quad \text{Bn} \quad 2.3 \]

To a 16 mL glass vial equipped with PTFE-silicon septum, open-top screw cap, and magnetic stir bar was added 9.5 mL of acetonitrile, (azidomethyl)benzene (0.067 g, 0.50 mmol), and phenylacetylene (0.051 g, 0.50 mmol). CuI (4.8 mg, 0.025 mmol) and TCPTA (12 mg, 0.025 mmol) dissolved in 0.5 mL of acetonitrile was injected via syringe to initiate the reaction. For HPLC/MS analysis, sampling of the reaction began immediately after injection of the catalyst. A total of 40 samples were collected at a rate of one sample every five minutes. An aliquot of 50 µL was taken during each sampling event at a rate of 2.0 mL/min. A dilution volume of 500 µL of methanol was used to deliver the aliquot to the second sample loop. HPLC analysis was performed using HPLC method (1).

2.6.3.2 Suzuki Cross-Coupling Reaction

\[ \text{Ph} \quad \text{Cl} \quad 2.4 \quad + \quad \text{Ar(B(OH))}_2 \quad \xrightarrow{\text{Pd}_2(\text{dba})_3 \quad (0.25 \text{ mol}\%)} \quad \text{Dioxane, 40 °C} \quad \text{Ph} \quad \text{Cl} \quad 2.6 \quad + \quad \text{Ph} \quad \text{Cl} \quad 2.7 \]

The compound 2.4 (100.525 mg, 0.5mmol, 1 eq), p-MeOPhB(OH)_2 (75.98 mg, 0.5 mmol, 1 eq), Pd_2(dba)_3 (1.145 mg, 0.00125 mmol, .0025 eq), and Cs_2CO_3 (488.73 mg, 1.5 mmol, 3 eq) were placed in a thick glass tube with a screw cap. Anhydrous dioxane (5 ml, 0.1M with respect to 2.4) was added to the glass tube. The stirred solution was heated at 40 °C. For HPLC/MS
analysis, sampling of the reaction began immediately after addition of the dioxane. An aliquot of 50 µL was taken during each sampling event (1 per five minutes) at a rate of 2.0 mL/min. A dilution volume of 500 µL of methanol was used to deliver the aliquot to the second sample loop. HPLC analysis was performed using HPLC method (2).

### 2.6.3.3 Carbinol Substitution Competition Reaction

[![Chemical structure](image)](image)

To a 16 mL glass vial equipped with PTFE-silicon septum, open-top screw cap, and magnetic stir bar was added 4.75 mL of acetonitrile, 1-(furan-2-yl)ethanol (0.056 g, 0.5 mmol), 4-nitroaniline (0.035 g, 0.250 mmol), and aniline (0.023 g, 0.250 mmol) and heated to 65 °C. Dy(OTf)$_3$ (15 mg, 0.025 mmol) dissolved in 0.5 mL of acetonitrile at 65 °C was injected via syringe to initiate the reaction. For HPLC/MS analysis, sampling of the reaction began immediately after injection of the catalyst. A total of 40 samples were collected at a rate of one sample every seven minutes. An aliquot of 50 µL was taken during each sampling event at a rate of 2.0 mL/min. A dilution volume of 500 µL of methanol was used to deliver the aliquot to the second sample loop. HPLC analysis was performed using HPLC method (3).
2.6.4 Calibration Curves

Calibration curves correlating LC chromatogram peak areas and concentration were constructed. For each calibration curve a stock solution (2.0 mL in acetonitrile) of known concentration containing the starting materials and products was prepared. The stock solution was sampled in triplicate using the general reaction monitoring procedure. The stock solution was then diluted (2:1 stock to diluent) and the diluted stock solution was then sampled in triplicate. This sampling and diluting protocol was repeated until 6 triplicate series had been completed. The average peak area of each triplicate series was plotted against their calculated concentration. Fitting by linear regression was applied to determine activity coefficient to allow for quantitation of each analyte at any time point.

![ Calibration curves for compounds quantified in the CuAAC reaction](image)

Figure 2.9. Calibration curves for compounds quantified in the CuAAC reaction
Figure 2.10. Calibration curves for compounds quantified in the Dy(OTf)$_3$ catalyzed competition reaction between furylcarbinols and competing nucleophiles.
Chapter 3: A Novel Reaction Mechanism for the Kinugasa Reaction

3.1 Introduction to the Kinugasa Reaction

β-lactams represent a valuable scaffold in organic synthesis in addition to their important role as the core motif in β-lactam antibiotics such as penicillins, cephalosporins, monobactams, and carbapenems. More recently, molecules containing a β-lactam subunit have demonstrated therapeutic effects by functioning as antifungal, anticancer, and antiviral agents. Furthermore, β-lactams make valuable synthons as they are readily amenable to subsequent functionalization to yield other chemical building blocks such as β-amino acids and amino alcohols. High-yielding, convergent methods for the enantio- and diastereoselective syntheses of β-lactams are therefore of great value.

One method for the direct syntheses of β-lactams was reported in 1972 by Kinugasa where it was observed that stoichiometric treatment of copper acetylides with nitrones formed cis β-lactams. The first catalytic modification of the Kinugasa reaction using a terminal alkyne was reported in 1993 by Miura et al (Scheme 3.1). The utility of the reaction was further increased in 2002 when Fu et al reported a diastereo- and enantioselective variant by utilizing chiral bis(azaferrocene) ligands with reported enantiomeric excesses (ee’s) ranging from 72-91%. The high atom economy and readily-accessible starting materials make the Kinugasa reaction an attractive option for the synthesis of β-lactams.
Scheme 3.1. The Kinugasa reaction and associated byproducts.

The often-reported modest yields of the desired azetidinones due to uncontrolled formation of byproducts remains a significant obstacle (Scheme 3.1).\textsuperscript{114} Despite the apparent usefulness of the Kinugasa reaction, it still remains widely unadopted as a synthetic tool for the synthesis of $\beta$-lactams.

3.1.1 Mechanism of the Kinugasa Reaction

The mechanism of the Kinugasa reaction has been a topic of study for decades and still remains an active area of research. The first reaction mechanism was proposed by Ding and Irwin in 1976.\textsuperscript{115} They suggested the reaction proceeded via a two-step cascade involving a 1,3 dipolar cycloaddition between copper acetylide 3.7 and nitrone 3.2, followed by a rearrangement (Scheme 3.2). In the rearrangement step it was suggested that protonation of the isoxazoline intermediate 3.8 facilitates intramolecular cyclization by the nitrogen to generate a strained bicyclic oxaziridinium intermediate 3.9. Nucleophilic attack by a hydroxide anion onto copper forms the amide functional group of 3.3, while also releasing copper to complete the cycle.
Nearly 30 years later, an alternative mechanism for the Kinugasa reaction was proposed by Tang et al (Scheme 3.3). Their proposed mechanism begins in a similar fashion leading up to the formation of the isoxazoline intermediate 3.8, although the mechanism differs with respect to the fate of 3.8. Instead of proceeding through the strained bicyclic intermediate 3.9, they propose ring opening of 3.8 via N-O bond cleavage generates ketene 3.10. Intramolecular acylation of 3.10 affords copper coordinated enolate 3.11. Proton transfer affords the $\beta$-lactam and regenerates the copper catalyst.

The mechanism for the Kinugasa reaction was further expanded upon in 2015 when a computational study using density functional theory was reported by Himo (Scheme 3.4). Himo’s mechanism resembles more closely that proposed by Tang. Coordination of copper to 3.1 facilitates deprotonation of the alkyne, resulting in formation of $\sigma$-copper acetylide 3.7. It is then proposed that a second copper coordinates to 3.7 forming bis-copper acetylide 3.12. The formal (3 + 2) dipolar cycloaddition between 3.2 and 3.12 proceeds via a stepwise fashion, with the first step...
being C-C bond formation. Cycloaddition results in the formation of the intermediate 3.13, which contains two copper centers. Reductive elimination from the copper (III) center results in ring contraction to form the cuprated heterocycle 3.8. Protonation of 3.8 at the nitrogen center facilitates N-O bond cleavage, forming ketene containing intermediate 3.14. Intramolecular cyclization of 3.14 yields the copper-coordinated enolate 3.15. Ligand exchange at copper, coupled with a proton transfer, affords a mixture of the desired β-lactam diastereomers 3.3. Himo noted that the reaction pathway involving two copper ions proceeded with better overall energetics than if one copper was involved.

Scheme 3.4. Kinugasa reaction mechanism proposed by Himo117

An important distinction was made to compare the feasibility of the two previously proposed mechanisms (Schemes 3.2 and 3.3). It was computed by Himo that the bicyclic
intermediate 3.9 is 31 kcal/mol higher in energy than metallated isoxazaline 3.8, and as much as 80 kcal/mol higher in energy than ketene 3.14, arguably due to the exceptional degree of ring strain. Himo therefore concluded that 3.9 is unlikely to be an intermediate in the reaction.117

While the contributions made by Himo were valuable for differentiating between the two previously proposed mechanisms, there was no mechanistic proposal for the formation of the byproducts associated with the Kinugasa reaction (Scheme 3.1). Also, in their computed efforts towards the enantioselective variant of the Kinugasa reaction, they calculated that the absolute barrier for that of a two-copper mechanism was 27.8 kcal/mol, which is comparable to the uncatalyzed barrier. This exceptionally high barrier associated with enantioselectivity coupled with a lack of rationale for the formation of byproducts left many unanswered questions regarding the mechanism of the Kinugasa reaction.

To the best of our knowledge there existed no detailed kinetic analysis for the Kinugasa reaction despite the current understanding and synthetic usefulness of the transformation. In this chapter we report the use of reaction progress kinetic analysis (RPKA)59,60 in conjunction with VTNA61,62 to help delineate the underlying mechanism with the goal to increase the yield of lactams and provide insight into the pathways that form the undesired byproducts often associated with the reaction. Reactions were monitored using a prototype robotic sampling apparatus coupled to HPLC-MS as described in Chapter 2 of this thesis.118 This device provides real-time quantitative reaction progress data, without the need for collection and quenching of aliquots, that would then be analyzed off-line following reaction completion.
3.2 Critical Importance of the Base Additive

Our investigation began by selecting a model reaction, utilizing phenylacetylene (3.1) and nitrone 3.2 as substrates (Figure 3.1). We selected tetrakis(acetonitrile)copper(I) tetrafluoroborate as the Cu(I) source, tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (TCPTA) as the ligand, and diisopropylamine (DIPA) as the base. This choice of base was based on previous reports, which found that sterically encumbered secondary amines gave superior yields of the desired β-lactam.116,119

![Figure 3.1. Reaction profile when DIPA was used as the basic additive. Reaction conditions: [3.1]₀ = [3.2]₀ = [DIPA]₀ = 0.1 M; [Cu(MeCN)₄BF₄] = [TCPTA] = 0.01 M in acetonitrile at room temperature.](image)

The reaction profile shows the formation of both cis and trans β-lactams (3.3a and 3.3b, respectively), with the cis diastereomer being favoured (Figure 3.1). Unfortunately, the desired β-
lactams were not the major product under these conditions, with ca. 80% of the initial starting materials being converted into the corresponding imine 3.4 and amide 3.16.

Imines are often reported as a major byproduct of the Kinugasa reaction; their formation has been attributed to Cu-catalyzed deoxygenation of the nitrone reagent.\textsuperscript{112,120} Interestingly, 3.4 and 3.16 were consistently generated in a 1:1 ratio throughout the reaction. The observation of the uniform byproduct ratio is not consistent with the off-cycle deoxygenation hypothesis, and instead points to a common intermediate for generation of both lactam and imine/amide products.

The conserved 1:1 ratio between byproducts 3.4 and 3.16 likely stems from decomposition of the Cu-dihydroisoxazolide 3.8 intermediate (Scheme 3.5). This decomposition pathway would involve cleavage of 3.8 via a formal (3+2) retrocycloaddition, giving imine 3.4 and ketene 3.17, the latter of which could be rapidly captured by a nucleophile (such as DIPA) to generate generic product 3.18. Such a pathway is similar to the proposed rearrangement of N-sulfonyl Cu-triazolides 3.19, which decompose to give the corresponding ketenimines (3.20) and nitrogen gas.\textsuperscript{121}

\begin{center}
\textbf{Scheme 3.5. Proposed retrocycloaddition of 3.8 and analogous reaction observed with N-Sulfonyl-1,2,3-triazoles (3.19)}
\end{center}
It should be noted that the formation of imine 3.4 and ketene 3.17 (by way of a Cu-ynoate intermediate) was originally proposed by Miura, who invoked a direct $O$-insertion between nitrone and Cu-acetylide.\textsuperscript{112} However, our reaction progress and KIE measurements (vide infra) do not support this proposal.

Given this new mechanistic insight, unproductive formation of imine 3.4 and amide 3.16 may be arrested by using a stronger, non-nucleophilic amine base, such as DBU (1,8-diazabicyclo-(5.4.0)undec-7-ene). This switch immediately led to an increased yield of $\beta$-lactam (from 17\% to 78\%), with a concomitant decrease in imine 3.4 formation (Figure 3.2).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{reaction_profile}
\caption{Reaction profile when DIPA was used as the basic additive. Reaction conditions: $[3.1]_0 = [3.2]_0 = [DBU]_0 = 0.1 \text{ M}; [Cu(MeCN)_4BF_4] = [TCPTA] = 0.01 \text{ M} \text{ in acetonitrile at room temperature.}}
\end{figure}
It was also noted that DIPA and DBU conferred complementary diastereoselectivities of the β-lactam products. Using DBU the trans β-lactam (3.3b) was favored in a >4:1 ratio, while DIPA gives the cis β-lactam (3.3a) preferentially. This change in diastereoselectivity has been reported by other researchers,\textsuperscript{122} and has been attributed to epimerization from the cis lactam to the thermodynamically favored trans geometry, made possible by the increased basicity of DBU.

Using DBU eliminates the formation of the undesired amide 3.16; however, the generation of imine 3.4 was not fully suppressed (Figure 3.2), and alkynyl imine 3.6 appeared as a new byproduct (Scheme 3.6). While 3.6 is structurally unique from amide 3.16, we believe the mechanism for their formations are closely related. We propose that in the absence of stronger nucleophiles, the highly electrophilic ketene 3.17 is captured via the oxygen center of nitrone 3.2 (Scheme 3.6). This acyl-nitron (3.2) then fragments to give nitrilium 3.23 and 2-phenyl acetate (3.5a), consistent with a proposal by Heine.\textsuperscript{123} Finally, the highly electrophilic nitrilium 3.23 is captured by copper acetylide 3.7 to afford alkynylimine 3.6.

Scheme 3.6. Proposed Reaction Pathway for the Formation of Alkynyl Imine 3.6
3.3 Kinetic Analysis to Identify the Catalyst Resting State

To identify the catalyst resting state and test for any potential catalyst deactivation or product inhibition, a series of same and different excess experiments were carried out.\textsuperscript{60,59} Using the same excess protocol (Section 1.5.2.1.2), there is a clear overlap in the kinetic profiles for the decay of [3.2] over time by applying a positive time shift to the experiment with lower substrate loading (Figure 3.3a). This correlation confirms that neither product inhibition or catalyst deactivation is occurring under these conditions and indicates the high degree of robustness of the Kinugasa reaction.

Next, a series of different excess experiments were conducted to probe the driving force in each reagent. While the overall reaction profile indicates the rate is nearly constant over time, varying the initial concentration of either alkyne 3.1 or nitrone 3.2 has a substantial impact on the observed rate (Figure 3.3b). Specifically, increasing the initial concentration of 3.2 accelerates the reaction, indicating a positive order in [nitrone], while an increase in 3.1 leads to a decrease in rate consistent with a negative order in [alkyne]. These observed kinetic parameters suggest that interaction with 3.2 is involved in the turnover limiting step, while 3.1 participates in an unproductive equilibrium producing an off-cycle reservoir which modulates the concentration of available catalyst. Finally, a series of experiments were carried out where the concentration of catalyst was varied from 5 – 20 mM (Figure 3.3c). This series confirms the system displays a positive order in catalyst. However, initial inspection of the progress curves indicates that the response is greater than first order.
Figure 3.3. RPKA/VTNA Experiment Data. a) Same excess data for the reaction described in Figure 3.2 used to probe for catalyst deactivation and/or product inhibition. b) Data from different excess experiments used to solve for order of 1 and 2. c) Kinetic data used of reactions outlined in Figure 3.2 but with various catalyst loadings, used to solve for order of catalyst. d) VTNA profile of data from c) indicating that order of catalyst is 2.

Analyzing the reaction progress data for both varied initial substrate and catalysts (Figures 3.3b and c) using the variable time normalization analysis (VTNA) method allows the order for catalyst (Figure 3.3d) and both substrates to be elucidated (Figure 3.7). Under our reaction conditions the system obeys the rate law given in Equation 3.1.

\[
Rate = k_{obs}[3.1]^{-1}[3.2]^1[cat]^2
\]  

(eq. 3.1)
These kinetic parameters allow key features of the mechanism to be elucidated. In particular, the second order dependence on the catalyst implies that two molecules of copper are required in a kinetically important step of the catalytic cycle. This is consistent with the proposal by Himo, where cycloaddition between the nitrone and σ-Cu-acetylide was found to preferentially involve the bis-copper intermediate 3.12. Invoking bis-copper complex 3.12 allows the cycloaddition to pass through the 6-membered transition state 3.13, which was predicted to be 4.6 kcal/mol lower in energy than the mono-copper equivalent. Himo’s calculations further predicted that the cycloaddition between bis-Cu complex 3.12 and nitrone 3.2 represented the highest energy barrier on the reaction coordinate, and thus is likely the turnover limiting step.

By adapting Himo’s proposal it is possible to construct a simplified kinetic model to recapitulate the observed kinetic behaviors in the Kinugasa reaction (Scheme 3.7). First, the free Cu(L) complex would bind to alkyne 3.1, whereby deprotonation with DBU forms σ-Cu-acetylide 3.7. Coordination of a second Cu(L) species leads to π-activation of the alkynyl system and gives bis-copper complex 3.12. Alternatively, capture of the σ-Cu-acetylide 3.7 with a second equivalent of alkyne would generate an off-cycle unreactive polyacetylide complex (3.7poly). Productive catalysis would involve coordination of the nitrone to bis-copper complex 3.12. Next, turnover-limiting cycloaddition via Himo et al’s proposed 6-membered transition state produces a transient bis-copper intermediate, containing both a Cu(I) and Cu(III) center (3.13). Rapid reductive elimination would liberate Cu-isoxazolide 3.8 and ultimately yield the β-lactam.
Scheme 3.7. Simplified mechanism representing the kinetically relevant steps in the overall pathway.

### 3.4 Deriving a Steady State Raw Law

Deriving a steady state rate law for the proposed mechanism is possible by applying the quasi-equilibrium approximation for the three major processes involving the catalyst ($K_{eq1}$, $K_{eq2}$ and $K_{eq3}$). Using this approximation, expressions for each intermediate to be obtained as shown in Equations 3.2, 3.3 and 3.4.

\[
K_{eq1} = \frac{[3.7]}{[Cu][3.1]} \quad [3.7] = K_{eq1}[Cu][3.1] \quad \text{(eq. 3.2)}
\]

\[
K_{eq2} = \frac{[3.7_{poly}]}{[3.7][3.1]} \quad [3.7]_{poly} = K_{eq1}K_{eq2}[Cu][3.1]^2 \quad \text{(eq. 3.4)}
\]

\[
K_{eq3} = \frac{[3.12]}{[Cu][3.7]} \quad [3.12] = K_{eq1}K_{eq3}[Cu]^2[3.1] \quad \text{(eq. 3.3)}
\]
Expressing the steady state rate law requires the free catalyst term [Cu] to be related to the total catalyst added via the mass balance equation (Equation 3.5). Evaluating Equation 3.5 is complicated as the complete form takes a quadratic form. However, the equilibrium between alkyne 3.1 and σ-Cu-acetylide 3.7 is anticipated to be strongly product favored due to the inclusion of 1 equivalent DBU, while the coordination of the second Cu will be comparatively much weaker. The catalyst mass balance equation can therefore be simplified to the form shown in Equation 3.6.

\[
[Cu]_{total} = K_{eq1}K_{eq2}[Cu]^2[3.1] + [Cu](1 + K_{eq1}[3.1] + K_{eq1}K_{eq3}[3.1]^2) \quad \text{(eq. 3.5)}
\]

\[
[Cu]_{total} \cong [Cu] + K_{eq1}[Cu][3.1] + K_{eq1}K_{eq3}[Cu][3.1]^2 \quad \text{(eq. 3.6)}
\]

The first order relationship in [nitrone] indicates the cycloaddition between 3.12 and 3.2 represents the first irreversible step, and all subsequent rearrangements are not kinetically meaningful. Thus, the rate of reaction can be written with respect to this turnover limiting event (Equation 3.7) Solving for the unbound catalyst term [Cu] and substituting into the rate law gives the simplified rate law, Equation 3.8.

\[
Rate = k_3[3.2][3.12] \cong k_3K_{eq1}K_{eq2}[3.1][3.2][Cu]^2 \quad \text{(eq. 3.7)}
\]

\[
Rate \cong \frac{k_3K_{eq1}K_{eq2}[3.1][3.2][Cu]^2}{(1 + K_{eq1}[3.1] + K_{eq1}K_{eq3}[3.1]^2)^2} \quad \text{(eq. 3.8)}
\]

The negative order relationship with respect to [alkyne] may reflect that, under strongly basic conditions of this reaction, the equilibrium between 3.1 and 3.7 is strongly product favored, while generation of the off-cycle polyacetylide complex involves a comparatively weaker
interaction ($K_{eq1} >> 1$ while $K_{eq3} << 1$ respectively). By applying these assumptions, the rate equation can be simplified to Equation 3.9, which reconciles the experimentally observed kinetic dependencies. It should be noted that the observation of an overall apparent zero-order reaction profile and second order in [Cu] is specific to the particular reagent concentrations from our study. Significant deviation from our simplified power law is expected when large excesses in either [alkyne] or [nitrone] are employed.

$$Rate \approx \frac{k_3 K_{eq2}[3.2][Cu]^2_{total}}{K_{eq1}[3.1]}$$

(eq. 3.9)

3.5 Kinetic Isotope Experiments

As kinetic studies (vide supra) suggest that numerous equilibrium binding events precede the turnover limiting coupling with nitrone 3.2, a secondary interrogation of the key transition state was sought. By creating both H and D isotopologues of the nitrone, a series of kinetic isotope experiments (KIE) could be performed, monitoring both reaction rate and product selectivity using $^1$H NMR. As the KIE experiments were to be performed utilizing NMR time-course analysis, the reaction behavior must match the HPLC analysis if the results were to be compared.41 Performing the reaction in a static NMR tube produced a profile which is nearly identical to the HPLC trend (Figure 3.4), confirming that data acquisition using the on-line HPLC monitoring prototype accurately captures the reaction profile. Furthermore, the strong correlation between these two analytical methods validates the application of the NMR time course for KIE investigations.
A competition reaction was performed between proteo- and deuterio- nitrones, 3.2 and 3.24 respectively, with alkyne 3.1. The $^1$H signals from the TCPTA ligand were clearly resolved and could be used as an internal standard, allowing the rate of change for each isotopologue of nitrone, $\beta$-lactam (both cis and trans diastereomers) and imine to be delineated. The ratio of nitrone isotopologues was plotted as a function of time, indicating that the rate of consumption of D-nitron 3.24 is greater than the corresponding rate for H-nitron 3.2 (Figure 3.5a). Thus, the catalytic reaction exhibits an inverse secondary KIE under these reaction conditions.
Figure 3.5. Kinetic Isotope experiment data. a) Comparing the ratio of isotopologues 3.2 and 3.24 over the course of the first KIE competition experiment \([3.1]_0 = [3.2]_0 = [3.24]_0 = [DBU]_0 = 0.1\text{M}; \ [Cu(MeCN)_4BF_4] = [TCPTA] = 0.01\text{M}. \) b) Consumption profiles of both isotopologues used to solve for KIE from Equation 3.10. \([3.1]_0 = [3.2]_0 = [DBU]_0 = 0.1\text{M}; \ [3.24]_0 = 0.06\text{M}; \ [Cu(MeCN)_4BF_4] = [TCPTA] = 0.01\text{M}. \)

To quantify the magnitude of the inverse KIE, another competition reaction was carried out, varying only the initial concentrations of H- and D-nitroene isotopologues. Assuming the reaction of both 3.2 and 3.24 obey the simplified rate law given in Equation 3.9, rates of reaction for each isotopologue will be modified both by [nitroene] but also the independent rate constants for the turnover limiting step associated with each isotopologue \((k_i\) in Scheme 3.8). By manipulating Equation 3.9, the KIE for any experiment with varied initial concentrations of 3.2 and 3.24 can be expressed as Equation 3.10.

\[
KIE = \frac{k_H}{k_D} = \frac{\text{Rate}_H[\text{Nit}_H]}{\text{Rate}_D[\text{Nit}_D]} = 0.95 \quad \text{(Eq. 3.10)}
\]
The rate of consumption for each isotopologue follows a zero-order profile, and therefore is a constant given by the slope of reaction profile (See Section 3.8.9 for derivation). The reaction was found to display a secondary inverse KIE of 0.95, which suggests that the turnover limiting step involves a change in hybridization from sp$^2$ to sp$^3$ at the carbon bearing the isotopic label. Applying the same analysis to our preliminary experiment with equimolar concentrations of both isotopologues (from Figure 3.5a) we observe an identical KIE of 0.95. These observations are consistent with the previous RPKA kinetic data, confirming that the initial (3+2) cycloaddition representing the turnover limiting step in the catalytic cycle.

3.6 Expanded Mechanistic Model

By combining our reaction progress and kinetic data, it is now possible to propose a complete mechanistic model that explains both the productive and unproductive reaction pathways (Scheme 3.8). First, the cycloaddition begins with the generation of the key σ-Cu(I) acetylide 3.7 from the terminal alkyne 3.1. At high concentration of alkyne an off-cycle reservoir is created (3.7_poly), restricting the Cu(I) available for productive cycloaddition and manifests as the negative order in [alkyne]. Further activation of the σ-Cu acetylide 3.7 by way of a second Cu(I) is required to effect cycloaddition and gives rise to the second order behavior in [Cu]. Coordination of the nitrone dipole and cycloaddition to produce metallocyclic intermediate 3.13 constitutes the turnover-limiting step, which is consistent with both the positive order in [nitrone] and inverse secondary KIE observed with isotopologue 3.24.
Scheme 3.8. Proposed reaction mechanism based on kinetic data highlighting all key equilibria and binding events.

From intermediate 3.13, rapid reductive elimination followed by protonation and (3+2) cycloreversion gives ketene and imine (3.26 and 3.4, respectively). The fate of these key intermediates governs the observed chemoselectivity of the reaction. If sufficiently nucleophilic reagents are present (such as DIPA or nitrone) capture of the highly electrophilic ketene dominates, diverting the reaction towards byproducts such as amide 3.16, alkynylimine 3.6 and imine 3.4. Alternatively, recombination of 3.4 and 3.26 via a Lewis acid catalyzed (2+2) cycloaddition yields the desired β-lactams. Under this revised mechanism, Cu(I)-catalyzed β-lactam generation
between terminal alkynes and nitrones is better classified as a cascade reaction, terminating with a formal (2+2) cycloaddition, more classically known as the Staudinger synthesis of β-lactams.\textsuperscript{124} This revised pathway is analogous to the formation of N-sulfonylazetidin-2-imines via capture of ketenimine 3.19 with imines.\textsuperscript{121}

Our revised mechanism is not only consistent with the available kinetic data but also resolves two important inconsistencies with previous models. First, the intermediacy of the ketene 3.17 provides the first consistent model rationalizing both the formation of target β-lactams and common byproducts, including imine 3.4, amide 3.16, alkynylimine 3.6, and benzyl carboxylate 3.5a. While similar byproducts have been documented, underlying mechanistic rationale for their appearance has been variable.\textsuperscript{112} For example, Miura found that alkynylimines could be chemoselectively formed by using dppe (1,2-bis(diphenylphosphino)ethane). However, their proposed pathway required direct addition of the σ-Cu(I) acetylide to the nitrene sp\textsuperscript{2} carbon, followed by deoxygenation to give Cu\textsubscript{2}O. Our RPKA data is not consistent with Miura’s proposal, as there is no evidence of catalyst deactivation or change in catalyst activity (Figure 3.3a) which would be apparent if CuBF\textsubscript{4} was being transformed to Cu\textsubscript{2}O during the reaction.

A second important consequence of our modified mechanism relates to the stereocontrolling step. The geometry of the asymmetric center in the β-lactam is set by the (2+2) cycloaddition between ketene 3.17 and imine 3.4, not the initial (3+2) cycloaddition involving the nitrone and σ-Cu(I) acetylide as is conventionally proposed. Thus, ligands, such as PyBOX, generate a Cu(I) complex that acts as a chiral Lewis acid to give stereodiscrimination akin to an asymmetric Staudinger synthesis. This modification resolves an issue noted by Himo, who found that incorporating chiral ligands onto the key bis-Cu-intermediate 3.12 creates numerous steric
penalties, raising the energy of the chiral transition state above that of the uncatalyzed thermal (3+2) Huisgen dipolar cycloaddition.

One final consideration relates to the work by Shintani and Fu, who designed conditions to trap the proposed Cu-enolate, which would be formed after intramolecular cyclization of intermediate 3.14.125 This result is still consistent in the context of our modified mechanism, as both Fu’s conditions and ours operate under stoichiometric strong base additives. Thus, it is possible that the allylation occurs after the formation of the β-lactam via base promoted enolate formation.

3.7 Conclusions

To summarize, we have reported the use of RPKA to delineate the underlying mechanism of the Kinugasa reaction. These experiments reveal that the system displays an overall zero-order profile, brought about by a near integer order of +1 and -1 for [nitrone] and [alkyne] respectively. Furthermore, the kinetic data reveal that an initial (3+2) cycloaddition between doubly activated bis-Cu(I) acetylide and nitrone constitutes the turnover limiting step. This conclusion is supported by the second order behavior with respect to [Cu] as well as a secondary inverse kinetic isotope effect of 0.95 for deuterated nitrone isotopologues. The observed kinetic and chemoselectivity data allow us to propose a modified mechanism, involving a novel (3+2) cycloreversion followed by Lewis acid catalyzed (2+2) cycloaddition. Most importantly, our new catalytic pathway, which proceeds via a common ketene intermediate, provides the first consistent mechanistic model for the generation of all commonly observed byproducts of the Kinugasa reaction. By understanding the pivotal role of the cycloaddition reaction cascade and the intermediacy of the reactive ketene
we have been able to derive reaction conditions using non-nucleophilic bases (such as DBU), which improves the yield of the desired β-lactam products to ~80%.

3.8 Experimental

3.8.1 General Remarks

All reagents and solvents were purchased from Fisher Scientific, Alfa Aesar, Sigma-Aldrich, and VWR and were used without further purification. NMR spectra were recorded on a Bruker spectrospin 300 Instrument (300 MHz and 75 MHz for $^1$H and $^{13}$C, respectively), an Agilent (400 MHz and 100 MHz for $^1$H and $^{13}$C, respectively), or a Bruker Avance 300 (300MHz and 75MHz for $^1$H and $^{13}$C, respectively), and were calibrated with the solvent (CDCl$_3$: 7.26ppm for $^1$H NMR and 77.16 for $^{13}$C NMR). The abbreviations s, d, t, m signify singlet, doublet, triplet, and multiplet, respectively. NMR spectra were analyzed by using the software MNova.

The Liquid Chromatography (LC) samples were analyzed by HPLC/MS conducted on an Agilent 1200 HPLC with the following configuration:

Agilent G1379B degasser, G1312A binary pump, G1316A thermal column compartment, diode array detector and a 6120 single quad mass spectrometer.

Analytical setting for the detectors are:

DAD – 200 – 400 nm collected at 20 Hz storing all spectra for offline analysis. Peak area for quantification varies depending on the experiment, see calibration curves for details

ESI-MSD – positive mode scan for m/Z 110 – 1500 running at 0.8sec/cycle. drying gas = 7.0 l/min, nebulizer pressure = 20 psi, gas temperature = 300 °C, capillary voltage = 4000 V

HPLC column and mobile phase method used the follow conditions:

(1) Poroshell 120 Phenylhexyl-C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = water, 0.05 % trifluoroacetic acid; Solvent B = acetonitrile, 0.05 % trifluoroacetic acid; Flow Rate = 0.625 mL/min; Starting Conditions = 78 % A, 22 % B; 0.0–1.7 min linear gradient to 30% A, 70 % B.

3.8.2 Synthetic Procedures

N-benzylaniline (3.27):

Compound 3.27 was synthesized using a literature procedure and characterization data was consistent with that of the literature.126

(Z)-N,1-diphenylmethanimine oxide (3.2):

To a cooled mixture (0 °C) of N-benzylaniline (3.27) (3.76 g, 20.5 mmol) in dichloromethane (100 ml) was added mCPBA (10.12 g, 41.0 mmol) in portions. After the addition, the mixture was quenched with aqueous sodium bicarbonate and extracted with dichloromethane. The organic extracts were washed with aqueous sodium bicarbonate solution three times, dried over magnesium sulfate, and the solvent was removed under reduced pressure. The crude material was recrystallized from a mixture of petroleum ether:ethyl acetate to afford 3.2 (1.78 g, 9.04 mmol, 44% yield) as light yellow crystals. Spectral data was consistent with that of the literature.127

(3S,4S)-1,3,4-triphenylazetidin-2-one (3.3a):

Compound 3.3a was prepared using the general Kinugasa reaction monitoring procedure. The crude material was purified using flash chromatography (0 – 10% ethyl acetate in petroleum ether) to give 3.3a and 3.3b as a mixture of diastereomers (2.3:1). Characterization data was consistent with that of the literature.116
(3R,4S)-1,3,4-triphenylazetidin-2-one (3.3b):

Compound 3.3b was prepared using the general Kinugasa reaction monitoring procedure. The crude material was purified using flash chromatography (0 – 10% ethyl acetate in petroleum ether) to give 3.3b as a white solid. Characterization data was consistent with that of the literature.\(^\text{128}\)

(E)-N,1-diphenylmethanimine (3.4):

A solution of aniline (0.901 ml, 9.9 mmol) and benzaldehyde (1 ml, 9.9 mmol) in methanol (20 mL) was heated at 65 °C for 3 hours and then the solvent was removed under reduced pressure. The crude material was recrystallized from a mixture of petroleum ether:ethyl acetate to afford 3.4 (1.54 g, 8.50 mmol, 86% yield) as white crystals. Spectral data was consistent with that of the literature.\(^\text{129}\)

N,N-diisopropyl-2-phenylacetamide (3.16):

To a biphasic solution of diisopropylamine (875 μL, 6.14 mmol) in dichloromethane (50 mL) and sodium carbonate (1.952 g, 18.42 mmol) in water (50 mL) was added phenylacetyl chloride (900 μL, 6.75 mmol) slowly. The mixture was stirred for three hours at room temperature. The aqueous layer was discarded, and the organic layer was washed with aqueous 1N HCl and with aqueous sodium bicarbonate solution. The organic extracts were dried over magnesium sulfate and the solvent was removed under reduced pressure to afford 3.16 (1.30 g, 5.93 mmol, 97% yield) as white crystals. Spectral data were in accordance with literature reported data.\(^\text{130}\)
(E)-N,1,3-triphenylprop-2-yn-1-imine (3.6):

Compound 3.6 was synthesized using a literature procedure and characterization data was consistent with that of the literature.\textsuperscript{112,131}

Tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (TCPTA):

This compound was prepared through an adaptation of a previous report.\textsuperscript{99}

1,2-Diphenylethane-1,2-d\textsubscript{2}-1,2-diol (3.28)

Benzil (420 mg, 2 mmol) was added to a mixture of tetrahydrofuran (4 ml) and D\textsubscript{2}O (200 µL) and cooled to (0 °C). Sodium borodeuteride (100 mg, 2.389 mmol) was added in small batches while stirring. The mixture was warmed to room temperature and stirred overnight where it was then quenched with water. Saturated aqueous ammonium chloride solution was added to the mixture to bring the pH to neutral. The aqueous components were then extracted three times with ethyl acetate. The organic extracts were dried over MgSO\textsubscript{4}, filtered, and the solvent was removed under reduced pressure to afford product 3.28 as a white solid which was used in the next step without further purification.

Deuteriobenzaldehyde (3.29)

The diol (3.28) was oxidized overnight at room temperature in a mixture of DCM (2 mL) and water (2 mL) by adding sodium periodate (427 mg, 2 mmol). The aqueous layer was extracted three times with DCM. The organic extracts were washed with brine, dried over MgSO\textsubscript{4}, and the solvent removed under reduced pressure to afford deuteriobenzaldehyde (3.29) (329 mg,
3.07 mmol, 77% over two steps) as a colourless oil. Characterization data was consistent of that the literature.\textsuperscript{132}

\begin{center}
\textbf{N-phenylhydroxylamine (3.30)}
\end{center}

N-phenylhydroxylamine (3.30) was prepared from a modified literature procedure and characterization data was consistent with that of the literature.\textsuperscript{133}

\begin{center}
\textbf{(Z)-N,1-diphenylmethanimine oxide-d (3.24)}
\end{center}

N-phenylhydroxylamine (3.24) (120 mg, 1.1 mmol), magnesium sulfate (240 mg, 1.994 mmol), and deuteriobenzaldehyde (118 mg, 1.1 mmol) were added to DCM (5 ml) and stirred at room temperature overnight. The mixture was filtered to remove the magnesium sulfate, and the solvent was then removed under reduced pressure. The crude material was recrystallized from a mixture of petroleum ether: ethyl acetate to afford deuterionitrone (3.24) (160 mg, 0.807 mmol, 73% yield) as off-white crystals. Characterization data was consistent of that the literature.\textsuperscript{134}
3.8.3 Reaction Progress Data

3.8.3.1 General Procedure for HPLC Kinugasa Reaction Monitoring with DIPA

To a 2 dram glass vial equipped with PTFE-silicon septum, open-top screw cap, and magnetic stir bar was added acetonitrile (1.8 mL), phenylacetylene (3.1) (0.022 mL, 0.2 mmol), (Z)-N-benzylideneaniline oxide (3.2) (0.039 g, 0.2 mmol), tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (9.29 mg, 0.02 mmol) and diisopropylamine (DIPA) (0.029 mL, 0.200 mmol) under an atmosphere of nitrogen. Tetrakis(acetonitrile)copper(I) tetrafluoroborate (6.29 mg, 0.02 mmol) in acetonitrile (200 µL) was injected via syringe to initiate the reaction. For HPLC/MS analysis, sampling of the reaction began immediately after injection of the catalyst. A total of 25 samples were collected at a rate of one sample every 10 minutes. An aliquot of 70 µL was taken during each sampling event at a rate of 2.0 mL/min. A dilution volume of 600 µL of methanol was used to deliver the aliquot to the second sample loop. HPLC analysis was performed using HPLC method (1).
3.8.3.2 General Procedure for HPLC Kinugasa Reaction Monitoring with DBU

To a 2 dram glass vial equipped with PTFE-silicon septum, open-top screw cap, and magnetic stir bar was added acetonitrile (1.8 mL), phenylacetylene (3.1) (0.022 mL, 0.2 mmol), (Z)-N-benzylideneaniline oxide (3.2) (0.039 g, 0.2 mmol), tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (9.29 mg, 0.02 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (0.030 mL, 0.200 mmol) under an atmosphere of nitrogen. Tetrakis(acetonitrile)copper(I) tetrafluoroborate (6.29 mg, 0.02 mmol) in acetonitrile (200 µL) was injected via syringe to initiate the reaction. For HPLC/MS analysis, sampling of the reaction began immediately after injection of the catalyst. A total of 25 samples were collected at a rate of one sample every 15 minutes. An aliquot of 70 µL was taken during each sampling event at a rate of 2.0 mL/min. A dilution volume of 600 µL of methanol was used to deliver the aliquot to the second sample loop. HPLC analysis was performed using HPLC method (1).
Figure 3.6. Sample HPLC chromatograms taken at varying time points from a monitored Kinugasa reaction. A) t = 0 minutes, before addition of catalyst. B) t= 90 minutes after addition of catalyst. C) t = 240 minutes after addition of catalyst. Unlabelled peak at 2.2 minutes is from ligand (TCPTA).

3.8.4 Different Excess Experiments

Following the different excess experiment protocol from RPKA\textsuperscript{60} the following three experiments were performed following the general Kinugasa reaction monitoring procedure.

Table 3.1. Initial reagent conditions for determination of reaction rate dependence upon substrate concentration.

<table>
<thead>
<tr>
<th>Trial</th>
<th>[3.1]\textsubscript{0} (M)</th>
<th>[3.2]\textsubscript{0} (M)</th>
<th>[DBU] (M)</th>
<th>[Cu(CH\textsubscript{3}CN)\textsubscript{4}BF\textsubscript{4}] (M)</th>
<th>[TCPTA] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07</td>
<td>0.07</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Figure 3.7. a) Variable time normalization analysis plot to solve for order of alkyne (3.1) using data from Table 3.1. b) Variable time normalization analysis plot to solve for order of nitrone (3.2) using data from Table 3.1.

3.8.5 Same Excess Experiments

Following the same excess experiment protocol from RPKA\textsuperscript{60} the following two experiments were performed following the general Kinugasa reaction monitoring procedure. Results are plotted in Figure 3.3a.

Table 3.2. Initial reagent conditions for determination of either product inhibition or catalyst deactivation using the same excess protocol

<table>
<thead>
<tr>
<th>Trial</th>
<th>[3.1]_0 (M)</th>
<th>[3.2]_0 (M)</th>
<th>[DBU] (M)</th>
<th>[Cu(CH\textsubscript{3}CN)\textsubscript{4}BF\textsubscript{4}] (M)</th>
<th>[TCPTA] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.07</td>
<td>0.07</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>
3.8.6 HPLC Calibration Curves

Calibration curves correlating LC chromatogram peak areas and concentration were constructed. For each calibration curve a stock solution (2.0 mL in acetonitrile) of known concentration containing the starting materials and products was prepared. The stock solution was sampled in triplicate using the general reaction monitoring procedure. The stock solution was then diluted (7:3 stock to diluent) and the diluted stock solution was then sampled in triplicate. This sampling and diluting protocol was repeated until 4, 5, or 6 triplicate series had been completed. The average peak area of each triplicate series was plotted against its calculated concentration. Fitting by linear regression was applied to determine the activity coefficient to allow for quantitation of each analyte at any time point.

Figure 3.8. a) HPLC Calibration curves to allow for quantification of 3.1, 3.2, 3.4, and 3.16 directly from peak area; integration of all UV peaks completed at a wavelength of 270 nm.
b) HPLC calibration curves to allow for quantification of lactams 3.3a and 3.3b directly from peak area; integration of the UV peaks were taken at 270 nm.

3.8.7 Experiments to Solve Catalyst Order

Table 3.3. Experimental conditions for the Kinugasa reaction at different catalyst concentrations to determine order in cat.

<table>
<thead>
<tr>
<th>Trial</th>
<th>[3.1]₀ (M)</th>
<th>[3.2]₀ (M)</th>
<th>[DBU] (M)</th>
<th>[Cu(CH₃CN)₄BF₄] (M)</th>
<th>[TCPTA] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.005</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Figure 3.9. Online HPLC time-course data for the Kinugasa reaction at different catalyst concentrations. a) Formation of the major product vs time. b) consumption of nitrone vs time.
3.8.8 NMR Time course Experiments

Phenylacetylene (3.1) (7.7 µl, 0.07 mmol), (Z)-N-benzyldieneaniline oxide (3.2) (13.8 mg, 0.07 mmol), TCPTA (3.3 mg, 0.007 mmol), and DBU (10.6 µl, 0.07 mmol) in CD$_3$CN (630 µL) were added to an NMR tube sealed with a teflon screw cap under an atmosphere of nitrogen. The sample was added to the NMR spectrometer where a preliminary spectrum was recorded. Cu(CH$_3$CN)$_4$BF$_4$ (2.2 mg, 0.007 mmol) in CD$_3$CN (70 µL) was added to the NMR tube to initiate the reaction. An NMR time-course experiment was performed whereby a proton NMR spectrum was obtained at a rate of one spectrum every 2 minutes for a total of 150 spectra.

Figure 3.10. a) $^1$H NMR time-course spectra overlay of the aromatic region used to quantify 3.2 and 3.4. b) $^1$H NMR time course-spectra overlay highlighting region used to quantify 3.3a and 3.3b.
Figure 3.11. Reaction time-course data for the Kinugasa reaction from NMR time-course experiment.

First KIE Experiment

Phenylacetylene (3.1) (7.7 µl, 0.07 mmol), (Z)-N-benzylideneaniline oxide (3.2) (13.8 mg, 0.07 mmol), (Z)-N,1-diphenylmethanimine oxide-d (3.24) (13.8 mg, 0.07 mmol), TCPTA (3.3 mg, 0.007 mmol), and DBU (10.6 µl, 0.07 mmol) in CD$_3$CN (630 µL) were added to an NMR tube sealed with a teflon screw cap under an atmosphere of nitrogen. The sample was added to the NMR spectrometer where a preliminary spectrum was recorded. Cu(CH$_3$CN)$_4$BF$_4$ (2.2 mg, 0.007 mmol) in CD$_3$CN (70 µL) was added to the NMR tube to initiate the reaction. An NMR time-course experiment was then performed whereby a proton NMR spectrum was obtained at a rate of one spectrum every 2 minutes for a total of 200 spectra.
Second KIE Experiment

Phenylacetylene (3.1) (7.7 µl, 0.07 mmol), (Z)-N-benzylideneaniline oxide (3.2) (13.8 mg, 0.07 mmol), (Z)-N,1-diphenylmethylamine oxide-d (3.24) (8.3 mg, 0.04 mmol), TCPTA (3.3 mg, 0.007 mmol), and DBU (10.6 µl, 0.07 mmol) in CD$_3$CN (630 µL) were added to an NMR tube sealed with a teflon screw cap under an atmosphere of nitrogen. The sample was added to the NMR spectrometer where a preliminary spectrum was recorded. Cu(CH$_3$CN)$_4$BF$_4$ (2.2 mg, 0.007 mmol) in CD$_3$CN (70 µL) was added to the NMR tube to initiate the reaction. An NMR time course experiment was then performed whereby a proton NMR spectrum was obtained at a rate of one spectrum every 90 seconds for a total of 150 spectra.

Table 3.4. Signals used to quantify all isotopologues during the NMR time course experiment

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>ppm</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\cdot$O$^+$.Ph</td>
<td>1H</td>
<td>Singlet</td>
<td>7.92</td>
<td>$\frac{\text{Integration}}{\varepsilon}$</td>
</tr>
<tr>
<td>Ph</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\cdot$O$^+$.Ph</td>
<td>2H</td>
<td>Multiplet</td>
<td>7.81 - 7.74</td>
<td>$\frac{\text{Integration}}{2\varepsilon} - [3.2]$</td>
</tr>
<tr>
<td>Ph</td>
<td>3.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph</td>
<td>3.3b</td>
<td>Doublet</td>
<td>5.16</td>
<td>$\frac{\text{Integration}}{\varepsilon}$</td>
</tr>
<tr>
<td>Ph</td>
<td>3.3b</td>
<td>Doublet</td>
<td>4.35</td>
<td>$\frac{\text{Integration}}{\varepsilon} - [3.3b]$</td>
</tr>
<tr>
<td>Ph</td>
<td>3.25b</td>
<td>Doublet</td>
<td>5.61</td>
<td>$\frac{\text{Integration}}{\varepsilon}$</td>
</tr>
<tr>
<td>Ph</td>
<td>3.3a</td>
<td>Doublet</td>
<td>5.61</td>
<td>$\frac{\text{Integration}}{\varepsilon}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Integration</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-------------</td>
<td>---</td>
</tr>
<tr>
<td><img src="image1" alt="Ph-N=O" /></td>
<td>1H</td>
<td>Doublet</td>
<td>5.09</td>
<td>$\frac{\varepsilon}{\varepsilon} - [3.3a]$</td>
</tr>
<tr>
<td><img src="image2" alt="Ph-N=H" /></td>
<td>1H</td>
<td>Singlet</td>
<td>8.57</td>
<td>$\frac{\varepsilon}{\varepsilon}$</td>
</tr>
<tr>
<td><img src="image3" alt="Ph-N=Ph" /></td>
<td>2H</td>
<td>Multiplet</td>
<td>7.48 – 7.40</td>
<td>$\frac{\varepsilon}{2\varepsilon} - [3.4]$</td>
</tr>
</tbody>
</table>

Figure 3.12. Sample integration region taken from KIE time course experiment. Signals labelled were used to quantify 3.3a, 3.3b, 3.25a', 3.25b'

### 3.8.9 KIE Derivations

To Solve for KIE we will make the following two assumptions:
1) When nitrone is used in excess with respect to alkyne, the reaction follows an overall zero order profile before complete consumption of alkyne, therefore:

\[ Rate = -slope \]  
\[ (Eq. 3.11) \]

2) Because the reaction is a competition reaction, [Alkyne] and [Cat] are the same with respect to [Nitrone_H] and [Nitrone_D]. We can therefore solve for KIE using the following equations (Assuming the preequilibrium constants – \( K_{eq1} \) and \( K_{eq2} \) for the H and D reactions receptively are identical)

\[ Rate_H = K_{eq1}k_H[Nitrone_H][Alkyne]^{-1}[cat]^2 \]  
\[ (Eq. 3.12) \]
\[ k_H = \frac{K_{eq1}Rate_H}{[Nitrone_H][Alkyne]^{-1}[cat]^2} \]  
\[ (Eq. 3.13) \]
\[ Rate_D = K_{eq2}k_D[Nitrone_D][Alkyne]^{-1}[cat]^2 \]  
\[ (Eq. 3.14) \]
\[ k_D = \frac{K_{eq2}Rate_D}{[Nitrone_D][Alkyne]^{-1}[cat]^2} \]  
\[ (Eq. 3.15) \]

Substituting Equations 3.13 and 3.15 into the original KIE formula affords Equation 3.16, which can be simplified to Equation 3.17

\[ KIE = \frac{k_H}{k_D} = \frac{\frac{K_{eq1}Rate_H}{[Nitrone_H][Alkyne]^{-1}[cat]^2}}{\frac{K_{eq2}Rate_D}{[Nitrone_D][Alkyne]^{-1}[cat]^2}} \]  
\[ (Eq. 3.16) \]
\[ KIE = \frac{k_H}{k_D} = \frac{Rate_H[Nitrone_D]}{Rate_D[Nitrone_H]} \]  
\[ (Eq. 3.17) \]

Because the reaction is overall 0 order:

\[ Rate_H = -slope_H \]  
\[ (Eq. 3.18) \]

and

\[ Rate_D = -slope_D \]  
\[ (Eq. 3.19) \]
Therefore, substituting Eq. 3.18 and 3.19 into Eq. 3.17 affords Equation 3.20 which is solvable using the acquired kinetic data.

\[
KIE = \frac{k_H}{k_D} = \frac{-slope_H[NitroN_D]}{-slop_D[NitroN_H]}
\]

(Eq. 3.20)

\[
KIE = \frac{6.53 \times 10^{-4} \text{ M/min} (0.0589 \text{ M})}{3.87 \times 10^{-4} \text{ M/min} (0.105 \text{ M})} = 0.95
\]

3.8.10 Rate Equations Derivations

Scheme 3.9. Simplified mechanism representing the kinetically relevant steps in the overall pathway to allow for derivation of the rate equation.
Quasi Equilibrium:

\[ K_{eq1} = \frac{[3.7]}{[Cu][3.1]} \]  
\[ [3.7] = K_{eq1}[Cu][3.1] \]

\[ K_{eq2} = \frac{[3.12]}{[Cu][3.7]} \]  
\[ [3.12] = K_{eq1}K_{eq2}[Cu]^2[3.1] \]

\[ K_{eq3} = \frac{[3.7_{poly}]}{[3.7][3.1]} \]  
\[ [3.7_{poly}] = K_{eq1}K_{eq3}[Cu][3.1]^2 \]

\[ [Cu]_{total} = [Cu] + [3.7] + [3.7_{poly}] + [3.12] \]

\[ [Cu]_{total} = [Cu] + K_{eq1}[Cu][Alkyne] + K_{eq1}K_{eq2}[Cu]^2[Alkyne] + K_{eq1}K_{eq3}[Cu][Alkyne]^2 \]

To remove the \([Cu]^2\) term we can assume \(K_{eq2} \gg 1\)

\[ [Cu]_{total} \cong [Cu] + K_{eq1}[Cu][Alkyne] + K_{eq1}K_{eq3}[Cu][Alkyne]^2 \]

\[ [Cu]_{total} \cong [Cu](1 + K_{eq1}[Alkyne] + K_{eq1}K_{eq3}[Alkyne]^2) \]

Rate \(= k_3[3.7][Nitronе]\)

Rate \(= k_3K_{eq1}K_{eq2}[Cu]^2[Alkyne][Nitronе]\)

\[
\text{Rate} \cong \frac{k_3K_{eq1}K_{eq2}[Alkyne][Nitronе][Cu]^2}{(1 + K_{eq1}[Alkyne] + K_{eq1}K_{eq3}[Alkyne]^2)^2}
\]

Assuming (under strongly basic conditions (1equivalent of DBU))

\(K_{eq1} \gg 1\)

And off-cycle binding is weak

\(K_{eq3} \ll 1\)
\[
Rate \approx \frac{k_3 K_{eq}^2 [\text{Nitron}e][\text{Cu}]^2_{total}}{K_{eq}^1 [\text{Alkyne}]}
\]

Scheme 3.10. Simplified mechanism to allow for derivation of the rate equation via a quasi-equilibrium process.

Quasi Equilibrium:

\[
K_{eq}^1 = \frac{[3.7]}{[\text{Cu}][\text{Alkyne}]}
\]

\[
[3.7] = K_{eq}^1 [\text{Cu}][\text{Alkyne}]
\]

\[
K_{eq}^2 = \frac{[3.32]}{[\text{Cu}][\text{Nitron}e]}
\]

\[
[3.32] = K_{eq}^2 [\text{Cu}][\text{Nitron}e]
\]
$$K_{eq}^3 = \frac{[3.7_{paly}]}{[3.7][Alkyne]}$$  
$$[3.7] = K_{eq}^1 K_{eq}^3 [Cu][Alkyne]^2$$

$$[Cu]_{total} = [Cu] + [3.7] + [3.32] + [3.7_{paly}]$$

$$[Cu]_{total} = [Cu] + K_{eq}^1 [Cu][Alkyne] + K_{eq}^2 [Cu][Nitrone] + K_{eq}^1 K_{eq}^3 [Cu][Alkyne]^2$$

$$[Cu]_{total} = [Cu](1 + K_{eq}^1 [Alkyne] + K_{eq}^2[Nitrone] + K_{eq}^1 K_{eq}^3 [Alkyne]^2)$$

Rate = $k_3[3.7][3.32]$

Rate = $k_3 K_{eq}^1 [Cu][Alkyne] K_{eq}^2 [Cu][Nitrone]$

Rate = $k_3 K_{eq}^1 K_{eq}^2 [Alkyne][Nitrone][Cu]^2$

$$Rate = \frac{k_3 K_{eq}^1 K_{eq}^2 [Alkyne][Nitrone][Cu]^2_{total}}{(1 + K_{eq}^1 [Alkyne] + K_{eq}^2[Nitrone] + K_{eq}^1 K_{eq}^3 [Alkyne]^2)^2}$$

Assuming (under strongly basic conditions (1 eq of DBU))

$$K_{eq}^1 \gg 1$$

And both off-cycle binding and nitrone association are weak

a) $K_{eq}^2 \ll 1$

b) $K_{eq}^3 \ll 1$

$$Rate \approx \frac{k_3 K_{eq}^2 [Nitrone][Cu]^2_{total}}{K_{eq}^1 [Alkyne]}$$
Chapter 4: A Telescopied Synthesis of 4-Cyanoimidazoles

4.1 Introduction

Multicomponent one-pot reactions are becoming increasingly popular for the synthesis of pharmaceuticals because of the efficiency and sustainability they provide.\textsuperscript{135–137} Practical methods for the synthesis of substituted imidazoles represent a key strategy in the development of pharmaceutical drug candidates.\textsuperscript{138–144} This heterocyclic motif has been investigated for a number of diseases, specifically Alzheimer’s,\textsuperscript{145,146} Parkinson’s,\textsuperscript{147} Chagas,\textsuperscript{148} influenza,\textsuperscript{149} as well as disease areas, including the treatment of cardiovascular, oncology, and antimicrobials. The 4-trifluoromethyl substitution for imidazoles represents an especially useful motif because of the ability of fluorine to enhance biological activity and increase metabolic stability.\textsuperscript{150–152} Therefore, we sought to increase mechanistic understanding of a one-pot process for the preparation the 4-trifluoromethyl substituted imidazoles motif.

Several methods exist for the installation of a trifluoromethyl group on to an imidazole core although they suffer from limitations regarding their use on process scale. Examples include CF$_3$ installation via copper catalysis,\textsuperscript{153} irradiation with UV or visible light,\textsuperscript{154} or electrocatalysis.\textsuperscript{155} We were pleased to find the often-used but less studied condensation reaction between a trifluoromethyl ketones and benzaldehyde which we predict could be conducted at process scale.\textsuperscript{156} Interestingly, the 4-trifluoromethyl imidazole 4.3 could be easily converted to the corresponding nitrile 4.4 (Scheme 4.1) to afford a second family of substituted imidazoles\textsuperscript{157} that could serve as products or substrates for further condensation rings to build molecular complexity. It is worth noting that this methodology allows for the installation of a cyano- group obviating the need for toxic cyanide reagents or the use of metal catalysts. We sought to understand the
fundamental reactivity and develop mechanistic understanding for these transformations towards the goal of creating a telescoped procedure.

![Scheme 4.1. Simplified scheme for the one-pot generation of cyanoimidazole 4.4. Ar = p-tolyl.](image)

To achieve these goals, we relied on the use of several orthogonal reaction monitoring techniques to gain detailed kinetic information. Studying the kinetics of a reaction is fundamental to furthering mechanistic understanding, and reaction optimization. The most thorough method of probing mechanistic features in a chemical process is by interrogating the reaction as it progresses, at several points in time. This type of time-course analysis is enabled by the use of robust in-line and on-line instrumentation to obtain a highly-dense data set.

### 4.2 The Reaction Monitoring Platform

To aid in our mechanistic investigations we incorporated multiple orthogonal reaction monitoring techniques, namely: offline HPLC/MS, ReactIR, and time course pH measurements (Figure 4.1). Offline HPLC analysis was realized by a slurry probe (Easysampler). The probe actuations were controlled via Trilution software to script sampling events as often as every 90 seconds until the desired number of samples had been taken. Reaction aliquots (20 µL) were quenched with methanol (1.00 mL) into an LC vial for subsequent analysis. Reactions were carried out in an Easymax 102 to enable online control of temperature, stir rate, and dosing via iControl software.
Figure 4.1. Cartoon schematic of the reaction monitoring platform used to gather orthogonal time-course reaction data.

A ReactIR probe was utilized to gather IR spectra as often as every 15 seconds. While the ReactIR probe is valuable for generating data dense reaction profiles of peaks of interest, the identity of such peaks remains ambiguous. Therefore, the combination of both offline LCMS and ReactIR maximizes confidence by cross correlation to correctly identify trends of species of interest over the course of the reaction. Lastly, a pH probe was used to generate pH profiles. We hypothesized that time course pH measurements would be a useful technique to approximate reaction rates during the cascade reaction to convert trifluoromethyl imidazole 4.3 to the cyanoimidazole 4.4. The combination of these three reaction monitoring techniques, plus the discreet digital control of reaction parameters such as dosing, temperature, and stir rate, enabled us to maximize our chemical understanding per experiment.

4.3 Probing the Imidazole Condensation Reaction

4.3.1 Understanding the reactivity of hemiaminal intermediate

A model system using p-tolylbenzaldehyde (4.2) was selected to synthesize trifluoromethyl imidazole 4.3 (Scheme 4.2). Both ketone 4.6 and glyoxal 4.1 have been utilized as commercially
available substrates to synthesize trifluoromethyl imidazoles.\textsuperscript{161–163} In general, methods utilizing ketone 4.6 are more common, possibly due to access of large-scale quantities of this reagent at high purity (4.1 is only available as technical grade aqueous solutions). Ketone 4.6 is typically converted to 4.1 immediately prior to use via hydrolysis in aqueous sodium acetate. Duplicating a synthetic procedure disclosed by Pitts, but modified to use \textit{p}-tolylbenzaldehyde (4.2) instead of benzaldehyde, afforded 4.3 with a 63 % yield, comparable to their yield of 59%.\textsuperscript{162}

\begin{center}
\includegraphics[width=\textwidth]{scheme4.2.png}
\end{center}

\textbf{Scheme 4.2. Proposed mechanism for formation of imidazole 4.3 from key hemiaminal intermediate 4.8 and aldehyde 4.2, as well as routes to access intermediate 4.8. Ar = \textit{p}-tolyl}

Treatment of 4.6 with concentrated NH\textsubscript{4}OH initiates imidazole formation and is envisioned to proceed via hemiaminal 4.8 and hydrated imidazole 4.9. In our early examination, reactions initiated with either aqueous solutions of ketone 4.6 or glyoxal 4.1 (both commercially available solutions or formed via hydrolysis of 4.6 with NaOAc and heat) did not display any markedly different behaviour. Regardless of the choice of starting material (4.1 or 4.6) hydration of electron-poor alkyl ketones and aldehydes is strongly favoured, resulting in geminal diol 4.7\textsuperscript{164} or tetrol 4.5 as the preferred equilibrium starting material for the initiation of imidazole formation. This result seems to indicate that while the simplified continuum of compounds (Scheme 4.2) belies the
underlying complexity inherent to this condensation cascade, rapid equilibrium between O and N nucleophiles erases any difference in reactivity between hydrates 4.5 and 4.7. For simplicity, we opted to initiate synthesis directly, using an aqueous solution of ketone 4.6, obviating pre-treatment and hydrolysis. Understanding the formation and reactivity of the hemiaminal intermediate 4.8 became our first priority, and we utilized time course $^{19}$F NMR spectroscopy to aid in our investigations.

Time course $^{19}$F NMR spectroscopy revealed that addition of 4.7 to a solution containing NH$_4$OH at 25 °C generates a complex mixture, as evidenced by the number of $^{19}$F NMR signals (Figure 4.2). The broad distribution of $^{19}$F NMR signals near -81 ppm upon mixing 4.7 and NH$_4$OH are indicative of a number of chemically similar oligomers. We hypothesize 4.7 and NH$_4$OH can react to form diimines, tetrols, hemiaminals, diols, and geminal amines. These hypothesized transient nucleophiles can react at the electrophilic centers of 4.7 to form oligomers. After one hour of reaction time four major $^{19}$F signals emerged, which we attribute to 4.10 (2 signals), 4.11, and 4.12.

The $^{19}$F time course experiment was repeated but in the presence of $p$-tolyl benzaldehyde (4.2) to probe the effects that 4.2 has on the rates of formation of byproducts 4.10, 4.11, and 4.12. The same byproduct-$^{19}$F NMR signals were observed, as well as a new major peak, which we attribute to formation of imidazol 4.3 (Section 4.7.3.2). By summing the integrals of the three major byproducts (4.10, 4.11, and 4.12) formed in the absence and presence of 4.2, we plotted a trend that describes the effects 4.2 has on the rate of byproduct formation (Figure 4.3). Specifically, Figure 4.3 shows that the initial rate of byproduct formation when exogenous aldehyde 4.2 is added was 0.55 times lower than in its absence. It is clear that undesired reactivity of 4.8 occurs during the reaction conditions, but inclusion of 4.2 depresses the rate of byproduct formation by providing
alternative reactivity pathways. Consequently, limiting the undesired reactivity of key hemiaminal intermediate 4.8 will play an important role in reaction understanding and optimization.

Figure 4.2. Time-course $^{19}$F NMR data for the reactivity of 4.7 in a solution of aqueous ammonia in MeOD/D$_2$O (6:1) over the course of 70 minutes. [4.7]$_0$ = 0.40 M, [NH$_4$OH]$_0$ = 1.8 M.
4.3.2 Dosing Reactions

The $^{19}$F NMR time-course experiments demonstrated that dimerization of intermediate 4.8 is a competitive process. To maximize the formation of the desired imidazole 4.3 despite this competitive process, superstoichiometric amounts of substrate 4.7 can be employed, although this will ultimately result in a higher concentration of dimerization byproducts. Therefore, we were hesitant to employ starting material 4.7 in large excess as the concentration increase of byproducts could be detrimental to a telescoped synthesis to form cyanoimidazole 4.4.

To favour the desired capture of intermediate 4.8 with aldehyde 4.2, we aimed to restrict the in-situ concentration of 4.7 via dosing experiments (Figure 4.4a), thus providing some measure of control over the imidazole product distribution.
Figure 4.4. a) HPLC data for dosing reactions. Expt 1: 4.7 (3.7 mmol) was dosed into a flask at 0 °C over 2 h containing 4.2 (3.3 mmol) and NH₄OH (20 mmol) and was heated to 40 °C after 3 h. Expt 2: 4.7 (3.7 mmol) was dosed into a flask at 40 °C over 2 h containing 4.2 (3.3 mmol) and NH₄OH (20 mmol). b) Time course ¹⁹F spectroscopy data comparing the effects of temperature on the ratio of product 4.3 vs byproducts 4.10, 4.11, and 4.12.

Reaction progress data, which was acquired by automated sampling via the EasySampler followed by offline HPLC-MS, corroborated the in-situ IR trends. The high degree of overlay for
the product curves with both IR and sampling techniques validates the integrity of the LC data (see Section 4.7.5.1).

In the first experiment (Figure 4.4a, Expt 1), 4.7 was added to a flask containing 4.2 and aqueous ammonia in isopropanol at 40 °C over two hours. HPLC measurements revealed that consumption of aldehyde 4.2 and formation of product 4.3 is primarily controlled by the rate of addition (and thus instantaneous concentration) of 4.7. This is evident from the dramatic drop in the rate of product formation at ~120 min when dosing ceased. Formation of 4.3 does continue after this point, albeit at a significantly reduced rate. Furthermore, total conversion of aldehyde 4.2 to 4.3 never exceeded 55%.

In a second experiment, the same dosing rate was employed while holding the reaction at a temperature of 0 °C. When dosing of 4.7 had been completed after two hours, the reaction temperature was elevated to 40 °C. This temperature adjustment resulted in a significant acceleration in the reaction progress. However, the reaction profile and degree of conversion ultimately mirrored the previous experiment where dosing occurred at 40 °C (Figure 4.4a, Expt 1 vs. Expt 2).

These data suggest that coupling between 4.7 and aldehyde 4.2, as well as dimerization of 4.7, may proceed via dynamic equilibrium involving a very fluctualional and dynamic population of hemiaminals, where irreversible condensation to the respective imidazole products requires elevated temperature. While the disposition and relative concentration of the various aminal intermediates may be controlled via the rate of dosing and temperature of the reaction, reaction efficiency requires elevated temperature. Therefore, any control afforded by manipulating the concentration of these aminal intermediates is ineffective.
To further investigate the effects of temperature on the divergence of \textbf{4.7} to either form \textbf{4.3} or dimerize, additional time course $^{19}$F NMR experiments were performed at elevated temperatures (40 °C). When comparing the ratio of the sum of byproducts \textbf{4.10, 4.11, and 4.12} vs the desired product \textbf{4.3} at 25 and 40 °C, the profiles appear to plateau ~ 1 (Figure 4.4b). Adjusting the reaction temperature between 25 °C and 40 °C is therefore insufficient to favour productive imidazole formation over the unproductive pathways, and we will instead use superstoichiometric amounts of \textbf{4.7} to maximize the yield of \textbf{4.3}.

\textbf{4.3.3 Design of Experiments (DoE) data}

Collaborators at Bristol-Myers Squibb (BMS) conducted a design of experiments study to investigate the effects of various reaction parameters. The equivalents of \textbf{4.6}, equivalents of NH$_4$OH, and reaction temperature were identified as key factors (Table 4.1). 1.25 equivalents of \textbf{4.6} was selected as the minimum since excess is required to account for the formation of oligomers and dimerization byproducts. 6 to 18 equivalents of NH$_4$OH was used to ensure enough NH$_3$ was present for the formation of desired product and various possible byproducts under any given conditions (especially with high equivalence of \textbf{4.6}). The temperature range was set between 40 and 60 °C, and pre-mixing of all reagents was performed at low temperature (0 °C) to ensure precise temperature control.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Parameter} & \textbf{High} & \textbf{Mid} & \textbf{Low} \\
\hline
Equiv. of 4.6 & 2.0 & 1.5 & 1.25 \\
Equiv. of NH$_4$OH & 18 & 12 & 6 \\
Temperature & 60 & 50 & 40 \\
IPA/water ratio & 3:2 & 1:1 & 1:2 \\
Total Volume & 61 & 44 & 27 \\
\hline
\end{tabular}
\caption{Range of reaction parameters in DoE experiment}
\end{table}
The combination of high equivalents of 4.6 and high equivalents of NH₄OH at 40 °C were the ideal conditions. The reaction temperature was found to be impactful since a higher temperature always led to a lower conversion under otherwise similar conditions. This relationship suggests that the dimerization is much faster than the desired pathway under high temperatures. The requirement of high equivalence of 4.6 further evidences that the dimerization of 4.8 is the key side-reaction responsible for the incomplete conversion of the aldehyde.

Finalized conditions using 2 equivalents of 4.6, 18 equivalents of NH₄OH and a solvent ratio of 1.2/1: IPA/water at 40 °C were determined through further optimization. A 4-gram reaction was conducted to test the conditions. We were pleased to observe 92% conversion of 4.2, a significant improvement compared to the previous 63%.

4.4 Investigating the Elimination Cascade

We began our mechanistic investigations of the conversion of 4.3 to 4.4 through intermediate 4.13 (Scheme 4.3) by exploring the effects of temperature, substrate concentrations, and pH on both rates and selectivity.

Scheme 4.3. Formation of cyanoimidazole 4.4 from trifluoromethyl imidazole 4.3 via intermediate 4.13.

We first probed the effects of temperature on the rate of conversion of 4.3 by monitoring the reaction pH (Scheme 4.4). Both offline HPLC and pH measurements indicate that treatment of
4.3 with excess NaOH at room temperature effectively deprotonates imidazole 4.3, and forces the pre-equilibrium to imidazolate 4.3a, but does not result in formation of fulvene 4.13.

![Scheme 4.4. Temperature promoted E1cB mechanism Ar = p-tolyl](image)

By increasing the temperature of the imidazole / NaOH solution by 10 °C increments, we established that conversion of imidazolate 4.3a to the fulvene 4.13 was negligible until a temperature of 50 °C had been reached. Above 50 °C, the pH of the solution continues to decrease as a function of time, evidencing continuous formation of HF and, by inference, 4.13. By increasing the temperature from 50 to 60 °C we observed a near two-fold increase in the reaction rate (Section 4.7.7.1). This relationship between temperature and reaction rate indicates that heating a strongly alkaline solution containing 4.3 to 60 °C can be an easily implemented technique to initiate and maintain the elimination cascade to generate 4.13.

Previous studies by Cohen indicated that formation of the desired cyanoimidazole 4.4 is complicated by the generation of by-products 4.14, 4.15, 4.16 due to competitive nucleophilic capture of intermediate 4.13 by alkoxide or hydroxide nucleophiles (Figure 4.5). To ascertain the process conditions that favour the desired cyanoimidazole 4.4, HPLC reaction profiles were generated via automated aliquoting for a series of experiments with varying initial concentrations of NH₄OH in alkaline methanolic solutions (Figure 4.5).
Figure 4.5. HPLC data showing the effects of [NH₄OH]₀ on the reaction rates and speciation of the elimination cascade. a) Plot of the consumption of 4.3 over time. b) Plot of the formation of 4.4 over time c) Plot of the formation of byproducts over time. In all 3 Experiments [4.3]₀ = 0.12 M; [NaOH]₀ = 0.72 M. Expt 1: [NH₄OH]₀ = 0 M; Expt 1 w/ NaF: [NaF] = 0.12 M; Expt 2: [NH₄OH]₀ = 0.60 M; Expt 3: [NH₄OH]₀ = 1.2 M. Ar = p-tolyl.

These data provided several key insights into the behaviour of this cascade. First, stoichiometric addition of fluoride has a negligible effect on the reaction rate (Figure 4.5a, Expt 1,
indicating that the elimination reaction converting imidazolate \(4.3a\) to fulvene \(4.13\) is irreversible under these conditions. This supports the hypothesis that the rate determining step is the formation of the diazafulvene \(4.13\) via a thermally-promoted E1cB mechanism, consistent with the proposal from Cohen,\(^{165}\) and evidencing the highly electrophilic nature of \(4.13\). Second, increasing \([\text{NH}_4\text{OH}]_0\) from 0.60 M to 1.2 M (Figure 4.5, Expt 2 vs Expt 3) decreased the chemoselectivity of by-products: \(4.4\) of the cascade from 6:1 to 4:1, respectively. Curiously this result is not due to an increase in the rate of formation of cyanoimidazole \(4.4\) (Figure 4.5b) but rather a decrease in the rate of by-product production (Figure 4.5c), accompanied by a concomitant reduction in the rate of consumption of starting material \(4.3\). This result reinforces our conclusion that elimination from \(4.3a\), and not the nucleophilic capture of \(4.13\), is the rate limiting step in the overall cascade, as increasing the concentration of the \(N\)-nucleophile (ammonia) does not increase the rate of cyanoimidazole formation.

Interpretation of the divergence in rates of product formation observed when manipulating the concentration of ammonium hydroxide requires a more nuanced examination of the relevant equilibria at play in the reactive network. Assuming a sequential mechanism for the cascade (Scheme 4.5), the reaction rate will be governed by elimination, which can be expressed assuming fast pre-equilibrium control as in Equation 4.1. Product selectivity, by contrast, is the result of irreversible capture of the key fulvene intermediate, \(4.13\), and can be expressed approximately as Equation 4.2. Thus, increasing the concentration of \([\text{NH}_4\text{OH}]\) will have a complex impact due to the interplay between acid/base processes involving imidazole, ammonia, hydroxide, and MeOH/water.
\[
Rate = K_{eq}k_{E1cB}[4.3][OH^-] \\
\frac{[4.4]}{[Byproducts]} \approx \frac{k_1[NH_3]}{k_2[RO^-]}
\]  
(eq. 4.1)  
(eq. 4.2)

Scheme 4.5. Minimal kinetic model capturing rate and selectivity-determining steps in the elimination cascade. \( \text{Ar} = p\text{-tolyl}. \)

To assist our analysis, reaction progress data was modeled in COPASI.\textsuperscript{166} Using the parameter estimate function to fit our experimental data produced excellent agreement with the proposed model from Scheme 4.5 (Section 4.7.7.3) In particular, the model correctly replicated the key kinetic features from our reaction progress analysis, including the reduction in rate of consumption of 4.3, with increased chemoselectivity for cyanoimidazole 4.4 upon increased concentration of ammonium hydroxide.

Despite our increased understanding of the elimination cascade from kinetic experiments, low yields of 4.4 (< 40%), due to the significant amount of undesired diazafulvene capture by competing O-nucleophiles, prompted us to explore different reaction conditions. To favour the formation of the cyanoimidazole 4.4 from 4.3 over the by-products we turned our attention to utilizing isopropanol as a solvent in place of methanol.

We predicted that this change would reduce the propensity for formation of by-products because of a change in nucleophilicity (reduce \( k_2 \), Scheme 4.5) and result in a demonstrable change in selectivity (Figure 4.6). Indeed, simply changing the reaction solvent from MeOH to iPrOH inverts the observed chemoselectivity from 0.2:1 to 5:1 (4.4:byproducts), respectively.
Figure 4.6. Time-course HPLC data showing the effects of changing the solvent from methanol to isopropanol on product distribution of the elimination reaction. In both reactions, $[4.3]_0 = 0.12$ M; $[\text{NaOH}]_0 = 0.72$ M; $[\text{NH}_4\text{OH}]_0 = 1.2$ M.

While employing iPrOH dramatically suppresses the capture of the fulvene intermediate 4.13 by hydroxide or alkoxide nucleophiles, the final purity of the desired cyanoimidazole 4.4 remained unacceptable as in situ yields did not exceed 80%. To fully suppress formation of the by-products, we sought an optimal pH capable of limiting the concentration of competing anionic $O$-nucleophiles, while still maintaining a basic enough pH to form 4.3a from 4.3. We envisioned that dosing aqueous NaOH to a heated reaction solution containing NH$_4$OH and 4.3 would allow for facile deprotonation and elimination forming 4.13, while having the benefit of limiting the concentration of competing $O$-nucleophiles. To test this hypothesis, a solution of 4.3 and NH$_4$OH in iPrOH/water was heated to 60 °C, then treated with progressive doses of a 15 M NaOH solution. The conversion of 4.3 to 4.4 was monitored by aliquoting and HPLC analysis, while the reaction pH was recorded in parallel via an in-situ probe (Figure 4.7).
Figure 4.7. Time-course HPLC and online pH data showing effects of dosing NaOH (15 M, 3.8 mmol each dose) on conversion of 4.3 to 4.4 into a flask containing 4.3 and NH₄OH in iPrOH/H₂O at 60 °C.

The conversion of 4.3 to 4.4 at 60 °C was negligible while the solution pH remains below 9.0. After ca. 50 minutes, dosing aqueous NaOH initiates rapid conversion of 4.3 to 4.4. Each of six progressive charges of NaOH is accompanied by a rapid increase in solution pH, followed by a decline as the elimination cascade generates three stoichiometric equivalents of HF per mol of cyanoimidazole 4.4.

Pausing the addition after the sixth charge allowed the pH to drop below 10 after about 100 min, at which point conversion to 4.4 appears to cease. As only ~75% conversion to 4.4 had been realized, additional NaOH was charged (ca. 140 minutes), raising the pH, and reinitiating the elimination, resulting in complete conversion. Most importantly, undesired by-products were not detectable when employing the dose-wise NaOH addition strategy. Thus, selective formation of the cyanoimidazole can be easily implemented, using pH control via NaOH dosing to initiate the second step of our telescoped synthesis.
4.5 Telescoping the Synthesis

With our increased understanding of key reaction parameters, a new telescoped one-pot synthesis protocol was designed to afford both high conversion of 4.2 and high in-situ yield of 4.4 (Figure 4.8).

This sequence was initiated with two equivalents of dibromide 4.7 with respect to aldehyde 4.2, to maximize the conversion to 4.3. Time-course $^{19}$F NMR spectroscopy experiments indicated that elevated temperatures resulted in the formation of byproducts not observed at 25 °C throughout the imidazole forming reaction. Additionally, pH vs temperature experiments indicate that temperatures above 50 °C are necessary for continuous conversion of 4.2 to 4.3.

![Telescoping the Synthesis](image)

Figure 4.8. LC Data for the one pot conversion of 4.7 and 4.2 to 4.3 and then to 4.4. a) 4.2 (2.8 mmol), 4.7 (5.6 mmol), NH$_4$OH (48 mmol) iPrOH/H$_2$O 6:1, 0 to 60 °C over 3 h. b) NaOH (aq, 15 M, 2.8 mmol), dosed sequentially six times at 288, 370, 390, 413, 430, and 460 minutes. Ar = p-tolyl
We chose to linearly ramp the temperature from 0 °C to 60 °C over the course of three hours to establish a temperature conducive to the elimination cascade, while minimizing by-product formation. The data in Figure 4.7 suggests that by simply dosing concentrated aqueous NaOH into the reaction mixture containing 4.3, the deprotonation, elimination, and nucleophilic capture to convert 4.3 to 4.4 would occur in rapid succession while having the added benefit of limiting the concentration of competing anionic O-nucleophiles. After five hours of reaction time (88% conversion of 4.2) we began dosing NaOH (15 M) sequentially until six equivalents had been added. By leveraging our developed understanding of each transformation >85% of the desired cyanoimidazole was achieved in our telescoped synthesis using these conditions.

4.6 Conclusions

In conclusion, a convenient telescoped synthesis of cyanoimidazoles has been developed. A suite of reaction monitoring techniques was employed to interrogate each transformation independently. Key mechanistic insights regarding the reactivity of 4.7 in the presence of aqueous ammonia to generate key proposed hemiaminal intermediate 4.8 were delineated via time-course $^{19}$F NMR experiments. Dimerization of 4.7 when exposed to ammonium hydroxide was observed, but addition of 4.2 reduces the rate of dimerization by creating alternate reactivity pathways. DoE experiments indicated that high yields of 4.3 can be achieved by using superstoichiometric quantities of 4.7. pH measurements during the elimination cascade of trifluoromethyl imidazole 4.3 evidenced a key temperature dependence on reaction rate and conversion. Undesired capture of the electrophilic diazafulvene intermediate 4.13 was observed in methanol, but utilization of isopropanol resulted in higher yields of the desired cyanoimidazole 4.4. By leveraging mechanistic information of each transformation realized by time course measurements, we report synthetic
conditions yields >85 % of cyanoimidazole 4.4 from carbonyl containing substrates in one pot, a significant improvement from our single-step yield of 63% to synthesize 4.3.

4.7 Experimental

4.7.1 General Remarks

4.7.1.1 Reagents

4-methylbenzaldehyde was purchased from Sigma Aldrich and used as received. 3,3-dibromo-1,1,1-trifluoropropan-2-one was purchased from Oakwood Chemicals and used as received. Ammonium Hydroxide (28 wt %) was purchased form VWR and used as received. NaOH was purchased from Fischer and used as received. All other reagents and solvents were purchased from conventional suppliers and used as received unless otherwise stated. Silica gel was purchased from Silicycle (60 Å, 230 x 400 mesh).

4.7.1.2 Analytical Methods

NMR spectra were recorded on a Bruker AV-400 for $^1$H, $^{13}$C, and $^{19}$F and were referenced to the residual solvent peak. The abbreviations s, d, q, m signify singlet, doublet, quartet, and multiplet, respectively. NMR spectra were analyzed by using the software MNova.

The Liquid Chromatography (LC) samples were analyzed by HPLC/MS conducted on an Agilent 1200 HPLC with the following configuration:

Agilent G1379B degasser, G1312A binary pump, G1316A thermal column compartment, diode array detector and a 6120 single quad mass spectrometer.

Analytical setting for the detectors are:
DAD – 200 – 400 nm collected at 20 Hz storing all spectra for offline analysis. Peak area for quantification varies depending on the experiment, see calibration curves for details.

ESI-MSD – positive mode scan for m/Z 110 – 1500 running at 0.8sec/cycle. drying gas = 7.0 l/min, nebulizer pressure = 20 psi, gas temperature = 300 °C, capillary voltage = 4000 V

HPLC column and mobile phase method used the follow conditions:

(1) Poroshell C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = Water, 0.1 % Formic Acid; Solvent B = acetonitrile; Flow Rate = 0.650 mL/min;
Starting Conditions = 90 % A, 10 % B; 0.0 - 0.8 min isocratic; 0.8 – 3.5 min gradient to 10% A, 90 % B

(2) Poroshell C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = Water, 0.1 % Formic Acid; Solvent B = acetonitrile; Flow Rate = 0.650 mL/min;
Starting Conditions = 99 % A, 1.0 % B; 0.0 - 2.00 min gradient to 80% A, 20 % B; 2.0 – 3.0 min gradient to 0.0% A, 100 % B.

4.7.1.3 Instrumentation

Experiments were performed in a Mettler-Toledo Easymax 102 Advanced Synthesis Workstation. Temporal HPLC data was obtained using an automated reaction sampling platform similar to what was previously reported in our group.45,81,118

All commands were executed by Trilution software working in conjunction with a Gilson Liquid Handler. At fixed time points, ~20 µL samples were automatically taken via the Easysampler probe. Methanol (1.00 mL) was used to deliver through the reaction aliquot into a vial for subsequent HPLC analysis. The sampling lines were then flushed with nitrogen gas for 2 minutes before reinitiating the sampling sequence.

4.7.2 Synthetic Procedures and Characterization Data

\[
\begin{align*}
\text{F}_3\text{C} & \text{H} \\
\text{N} & \text{N} \\
\text{F}_3\text{C} & \text{H} \\
\text{N} & \text{N} \\
\text{F}_3\text{C} & \text{H} \\
\text{N} & \text{N} \\
\text{F}_3\text{C} & \text{H} \\
\text{N} & \text{N} \\
\end{align*}
\]

2-(\(p\)-tolyl)-4-(trifluoromethyl)-1H-imidazole (4.3):

4-methylbenzaldehyde (300 mg, 2.50 mmol) was added to a flask containing 2-propanol (10 mL) and aqueous (28 wt %) ammonium hydroxide (3.5 mL, 25.0 mmol). 3,3-dibromo-1,1,1-trifluoropropan-2-one (1.35 g, 5.0 mmol) was hydrated by adding to water (1.0 mL) slowly and the resulting aqueous solution was then transferred into the reaction flask where it was then heated at 40 °C for six hours. The solvent was removed under reduced pressure and the resulting crude was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO\(_4\), and concentrated under reduced pressure. After the
Solvent was evaporated, the crude product was purified by column chromatography (petroleum ether/ethyl acetate 4:1) to afford imidazole 4.3 (435 mg, 1.92 mmol, 77% yield) as a white solid.  

$^1$H NMR (400 MHz, MeOD) $\delta$ 7.77 (d, $J = 8.3$ Hz, 2H), 7.59 (q, $J = 1.3$ Hz, 1H), 7.27 (d, $J = 7.8$ Hz, 2H), 2.37 (s, 3H);  

$^{13}$C{$^1$H} NMR (101 MHz, MeOD) $\delta$ 148.8, 139.7, 131.4 (q, $J = 39.3$, 37.4 Hz), 129.2, 126.3, 125.5, 122.0 (q, $J = 265.8$ Hz), 117.5, 19.9;  

$^{19}$F NMR (377 MHz, MeOD) $\delta$ -63.45.  

HRMS (EI-TOF) $m/z$ calculated for C$_{11}$H$_9$F$_3$N$_2$, $[M]^+$ = 226.07178; found 226.07189.  

2-(p-tolyl)-1H-imidazole-4-carbonitrile (4.4):  

2-(p-tolyl)-4-(trifluoromethyl)-1H-imidazole (100 mg, 0.44 mmol) was added to 2-propanol (6.0 mL) and aqueous (28 wt %) ammonium hydroxide (6.1 mL, 4.4 mmol) and heated to 60 °C. Aqueous (10 M) sodium hydroxide (0.27 mL, 2.6 mmol) was added to the reaction in 6 equal portions over the course of 90 minutes. The reaction solution was neutralized with saturated-aqueous ammonium chloride and the isopropanol was removed under reduced pressure. The crude was extracted with ethyl acetate. The combined organic layers were washed with saturated ammonium bicarbonate solution, then brine, dried over MgSO$_4$, and concentrated under reduced pressure to afford 4.4 (71 mg, 0.44 mmol, 88% yield) as a tan solid.  

$^1$H NMR (400 MHz, MeOD) $\delta$ 7.90 (s, 1H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.28 (d, $J = 7.9$ Hz, 2H), 2.37 (s, 3H);  

$^{13}$C{$^1$H} NMR (101 MHz, MeOD) $\delta$ 150.5, 141.5, 130.7, 128.9, 127.12, 126.9, 115.9, 113.8, 21.3;  

HRMS (EI-TOF) $m/z$ calculated for C$_{11}$H$_9$N$_3$, [M]$^+$ = 183.07965; found 183.07944
3,3-dibromo-1,1,1-trifluoropropane-2,2-diol (4.7):

3,3-dibromo-1,1,1-trifluoropropan-2-one was hydrated by adding 3,3-dibromo-1,1,1-trifluoropropan-2-one (989 mg, 442 µL, 3.67 mmol) over the course of 15 minutes to distilled water (1.0 mL) in an ice bath while stirring to afford 4.7 in quantitative yield.

$^1$H NMR (400 MHz, D$_2$O) $\delta$ 5.84;

$^{13}$C$^1$H NMR (101 MHz, D$_2$O) $\delta$ 121.4 (q, $J = 289.9$ Hz), 92.6 (q, $J = 31.0$ Hz), 43.7;

$^{19}$F NMR (377 MHz, D$_2$O) $\delta$ -78.0.

4-(trifluoromethyl)-1H-imidazole (4.11):

Compound 4.11 was prepared as per the following procedure.\textsuperscript{168}

$^1$H NMR (400 MHz, MeOD) $\delta$ 7.82 (s, 1H), 7.59 (p, $J = 1.3$ Hz, 1H);

$^{13}$C$^1$H NMR (101 MHz, MeOD) $\delta$ 136.9, 130.6 (q, $J = 38.4$ Hz), 121.9 (q, $J = 265.5$ Hz), 116.9;

$^{19}$F NMR (377 MHz, MeOD) $\delta$ -63.34.\textsuperscript{169}

HRMS (EI-TOF) $m/z$ calculated for C$_4$H$_3$F$_3$N$_2$, [M]$^+$ = 136.02483; found 136.02473.

4.7.3 Time course $^{19}$F Experiments

4.7.3.1 Dimerization of 4.7 Experiments

Aqueous (28 wt%) ammonium hydroxide (0.17 mL, 1.2 mmol) was added to MeOD (400 µL) in an NMR tube sealed with a Teflon screw cap. The sample was placed in the NMR
spectrometer where a preliminary $^{19}$F NMR spectrum was recorded. 3,3-dibromo-1,1,1-trifluoropropane-2,2-diol (72 mg, 0.27 mmol) in D$_2$O (66 µL) was added to the NMR tube to initiate the reaction. An NMR time-course experiment was initiated by obtaining a $^{19}$F NMR spectrum at a rate of one spectrum every 75 seconds for a total of 56 spectra.

This experiment was then repeated but instead a temperature of 40 °C was maintained in the spectrometer. A $^{19}$F NMR spectrum was obtained at a rate of one spectrum every 75 seconds for a total of 56 spectra.

**Figure 4.10.** Time-course $^{19}$F NMR spectra for the reaction of 4.7 in NH$_4$OH displaying signals used to quantify 4.10, 4.11, and 4.12.
Aqueous (28 wt %) ammonium hydroxide (0.15 g, 0.17 mL, 1.2 mmol) was added to a solution of MeOD (400 µL) and 4-methylbenzaldehyde (16.2 mg, 16 µL, 0.135 mmol) in an NMR tube sealed with a Teflon screw cap. The sample was placed in the NMR spectrometer where a preliminary $^{19}$F spectrum was recorded. 3,3-dibromo-1,1,1-trifluoropropane-2,2-diol (72 mg, 0.27 mmol) in D$_2$O (66 µL) was added to the NMR tube to initiate the reaction. An NMR time-course experiment was performed whereby a $^{19}$F NMR spectrum was obtained at a rate of one spectrum every 240 seconds for a total of 160 spectra.

This experiment was then repeated but instead a temperature of 40 °C was maintained in the spectrometer. A $^{19}$F NMR spectrum was obtained at a rate of one spectrum every 59 seconds for a total of 175 spectra.
Figure 4.11. Time course $^{19}$F spectra for the reaction of 4.2 and 4.7 in aqueous NH$_4$OH and signals used to quantify 4.3, 4.10, and 4.11.

4.7.4 Calibration Curves

A stock solution of each compound being calibrated (4.2, 4.3, and 4.4) was created by adding a known mass of material to 1.00 mL of methanol. This stock solution was then sampled three times using the Easysampler sampling method, and the peak areas were quantified using HPLC-method A. The stock solution (600 µL) was then added into methanol (400 µL) and the diluted stock solution was sampled again three times. This diluting and sampling protocol was repeated an additional two times.
Figure 4.12. HPLC Calibration curves to allow for quantification of 4.2, 4.3, and 4.4 directly from peak area. Integration of all UV peaks completed at a wavelength of 254 nm.

4.7.5 Dosing Experiments

4.7.5.1 Dosing Experiment 1

4-methylbenzaldehyde (400 mg, 3.33 mmol) was added to a solution of isopropanol (10 mL) and aqueous (28 wt%) ammonium hydroxide (2.8 mL, 20 mmol) and the temperature was increased to 40 °C. The ReactIR probe was blanked in air, then NH₄OH/IPrOH (4:1), and was inserted into the reaction flask. Data acquisition using iC IR (React IR software) was initiated recording one spectrum every two minutes. 3,3-dibromo-1,1,1-trifluoropropan-2-one (989 mg, 3.67 mmol) was hydrated by adding 4.6 slowly to distilled water (1 mL) in an ice bath with stirring.
The aqueous solution of 4.7 was then dosed into the reaction flask over the course of 2 hours while maintaining a reaction temperature of 40 °C. Once dosing had begun a sampling sequence was initiated. ReactIR data analysis was performed on the double derivative of the IR spectra to allow for better peak deconvolution. Imidazole 4.3 was trended via the peak at 1357 cm⁻¹. HPLC samples were analyzed using HPLC method 1.

![Graph](image)

**Figure 4.13.** a) HPLC conversion profiles of 4.2 and 4.3 for dosing experiment 1. b) Overlay of normalized conversion of 4.3 via temporal FTIR and HPLC measurements.

### 4.7.5.2 Dosing Experiment 2

![Reaction](image)

4-methylbenzaldehyde (400 mg, 3.33 mmol) was added to a solution of isopropanol (10 mL) and aqueous (28 wt %) ammonium hydroxide (2.8 mL, 20 mmol). The resulting solution was
cooled to 0 °C while stirring. The ReactIR probe was blanked in air, then NH₄OH/IPrOH (4:1), and was inserted into the reaction flask. Data acquisition using iC IR (React IR software) was initiated recording one spectrum every two minutes. 3,3-dibromo-1,1,1-trifluoropropan-2-one (989 mg, 3.67 mmol) was hydrated by adding 4.6 slowly to distilled water (1.0 mL) in an ice bath while stirring. The aqueous solution of 4.7 was then dosed into the reaction flask over the course of 2 hours while maintaining a reaction temperature of 0 °C. A sampling sequence was initiated once dosing had begun. After 150 minutes the reaction temperature was increased to 40 °C. ReactIR data analysis was performed on the double derivative of the IR spectra to allow for better peak deconvolution. Imidazole 4.3 was trended via the peak at 1357 cm⁻¹. HPLC samples were analyzed using HPLC method 1.

Figure 4.14. a) HPLC conversion profiles of 4.2 and 4.3 for dosing experiment 2. b) Overlay of normalized conversion of 4.3 via temporal FTIR and HPLC measurements.
4.7.6 Design of Experiments

Reaction Experimental designs were formulated using the statistical software package JMP (SAS Institute) as custom designs. The DoE was run with 24 experiments including 4 center points and 5 factors using a D-Optimal design criteria as shown in Table 4.2. The experiments were blocked in groups of 3 based on reaction temperature.

Table 4.2. DoE for imidazole Formation Step

<table>
<thead>
<tr>
<th>NH4OHequiv</th>
<th>Ketoneequiv</th>
<th>Volume</th>
<th>Temperature</th>
<th>Amount of Water (IPA/H2O)</th>
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</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>1.25</td>
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<td>40</td>
</tr>
<tr>
<td>A2</td>
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<td>2</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
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<td>6</td>
<td>1.25</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
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</tr>
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<td>40</td>
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<td>2</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
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<td>1.25</td>
<td>27</td>
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<td>2</td>
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<td>40</td>
</tr>
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<td>50</td>
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<td>C3</td>
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<td>C5</td>
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<tr>
<td>D6</td>
<td>6</td>
<td>2</td>
<td>47</td>
<td>60</td>
</tr>
</tbody>
</table>

Procedures: At 0 °C, 4-methylbenzaldehyde was added to 8 mL vials followed by addition of aqueous (28 wt %) NH4OH. Then, 1,1-dibromo-3,3,3-trifluoroacetone solution in isopropanol was added to the reaction mixture at 0 °C, followed by the addition of extra isopropanol or H2O to achieve the desired IPA/water ratio and total volume. The vials were then transferred to a heating block at desired reaction temperature and stirred at 300 rpm.
Figure 4.15. Results from the Design of Experiments probing the effects of stoichiometry, temperature, solvent composition and volume on the imidazole formation.

4.7.7 Investigating the Elimination Cascade

4.7.7.1 Time course pH Experiment

\[
\text{F}_3\text{C} \quad \text{4.3} \quad \text{Ar} \quad \text{NaOH} \quad \text{rt} \quad \text{F}_3\text{C} \quad \text{4.3a} \quad \text{Na} \quad \text{Temp} \quad - \text{F} \quad \text{4.13} \quad \text{Ar}
\]

2-(p-tolyl)-4-(trifluoromethyl)-1H-imidazole 4.3 (100 mg, 442 µmol) was added to a solution of isopropanol/water (7:1, 15 mL) containing NaOH (88 mg, 2.2 mmol) at 23 °C. The pH probe was inserted into the reaction flask and time-course pH measurements were initiated. After 12 minutes, the reaction temperature was increased to 30 °C. The temperature was then increased to 40 °C, 50 °C, and 60 °C after 22, 30, and 38 minutes, respectively. The rate of change of pH was calculated by measuring the initial rate upon temperature adjustment.
Figure 4.16. Time-course pH and temperature measurements of a basic solution of 4.3 demonstrating the effects of increasing the reaction temperature on the rate elimination cascade.

4.7.7.2 Measuring the effects of [NH₄OH] on rate and selectivity

Table 4.3. Experimental values for Experiments 1-3 to measure the effects of concentration of NH₄OH and reaction rate and selectivity.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Volume NH₄OH</th>
<th>Volume H₂O</th>
<th>Volume MeOH</th>
<th>Volume NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 mL</td>
<td>1.60 mL</td>
<td>8.0 mL</td>
<td>0.44 mL</td>
</tr>
<tr>
<td>2</td>
<td>0.80 mL</td>
<td>0.80 mL</td>
<td>8.0 mL</td>
<td>0.44 mL</td>
</tr>
<tr>
<td>3</td>
<td>1.60 mL</td>
<td>0.0 mL</td>
<td>8.0 mL</td>
<td>0.44 mL</td>
</tr>
</tbody>
</table>

General Procedure (Expt 2):

2-(p-tolyl)-4-(trifluoromethyl)-1H-imidazole (271 mg, 1.20 mmol) was added to a solution of methanol (8.00 mL) and deionized water (0.80 mL). Aqueous (28 wt %) ammonium hydroxide
(0.70 g, 0.80 mL, 6.0 mmol) and aqueous (15 M) sodium hydroxide (288 mg, 7.20 mmol) were then added. The reaction temperature was increased to 60 °C and a sampling sequence was initiated. HPLC samples were analyzed using HPLC method 2.

The same general procedure was repeated for both Expts 1 and 3 using the experimental values listed in Table 4.3.

The same general procedure was repeated for Expt 1 but NaF (50 mg, 1.20 mmol) was added to the reaction before heating to investigate the effects of fluoride on the rate of the reaction (Expt 1 w/ NaF).

HPLC calibration curve data was used to calculate the mole fraction of both 4.3 and 4.4 at each time point.

To calculate the concentration and therefore Mole Fraction of the byproducts at each time point the following assumption was made: 

\[ [\text{Byproducts}]_t = [4.3]_0 - [4.3]_t - [4.4]_t \]

Temporal HPLC data for Experiments 1-3 can be found in Figure 4.5.

### 4.7.7.3 COPASI Model

The following model was created in COPASI comprised of the following components

A) Acid – Base reactions: This series of equilibria (Schemes 4.6 - 4.9) recapitulates the rapid proton exchange in the reaction and allows the pH to vary dynamically in response to the changing reaction composition.
Scheme 4.6. Acid dissociation constant of imidazole – unknown value to be estimated from model, \( \text{Ar} = p\text{-tolyl} \)

\[
\mathrm{CF}_3\textbf{N} = \text{Ar} + \mathrm{H}_2\mathrm{O} \rightleftharpoons \mathrm{CF}_3\textbf{N} = \text{Ar} + \mathrm{H}_3\text{O}^+ \quad K_a (4.3) = ??
\]

Scheme 4.7. Base dissociation constant for ammonia in water

\[
\mathrm{NH}_3 + \mathrm{H}_2\mathrm{O} \rightleftharpoons \mathrm{NH}_4^+ + \mathrm{HO}^- \quad K_b (\text{NH}_3) = 1.8 \times 10^{-5}
\]

Scheme 4.8. Autoionization of water

\[
\mathrm{H}_2\mathrm{O} + \mathrm{H}_2\mathrm{O} \rightleftharpoons \mathrm{H}_3\mathrm{O}^+ + \mathrm{HO}^- \quad K_w = 1.0 \times 10^{-14}
\]

Scheme 4.9. Base dissociation constant for fluoride in water

\[
\mathrm{F}^- + \mathrm{H}_2\mathrm{O} \rightleftharpoons \mathrm{HF} + \mathrm{HO}^- \quad K_b (\text{F}^-) = 1.5 \times 10^{-11}
\]

Scheme 4.10. Dissociation of sodium hydroxide – assumed to be very large and irreversible

\[
\text{NaOH} \rightarrow \mathrm{OH}^- + \text{Na}^+ \quad K_{diss} (\text{NaOH}) = 1 \times 10^{10}
\]

B) Reaction pathway

The reaction sequence is assumed to follow the following simplified mechanism (Scheme 4.11).
This sequence assumes that:

a) The initial pre-equilibrium \((K_a)\) to give imidazolate will impact the rate of consumption of imidazole \((d[4.3]/dt)\) and will be impacted by the pH of the solution.

b) Elimination from imidazolate to fulvene represents the first irreversible step in the reaction sequence \((k_1)\). This assumption is supported by the lack of kinetic sensitivity to \([F^-]\).

c) The final product selectivity is determined by competitive capture of the electrophilic fulvene by either oxygen (\('OH) or nitrogen (NH_3) nucleophile. This is approximately given Equation 4.3.

\[
\frac{[O]}{[N]} = \frac{k_2[OH^-]}{k_3[NH_3]} \tag{Eq. 4.3}
\]

d) No cross-over occurs following capture by either oxygen or nitrogen to give intermediates 4.17 or 4.18, respectively. Subsequent replacement of fluoride
likely proceeds via a sequential elimination-nucleophilic attack cascade, however, the lack of other byproducts suggests that these steps are very rapid for our test case.

This model was applied to the parameter estimation workflow in COPASI, where the time-course concentrations of starting imidazole 4.3 and products (from oxygen and nitrogen capture) were fit using an evolutionary programming method with a population size of 400 and 200000 generations. The weighting factor for the experimentally determined concentration of starting material was kept low, reflecting the fact that our HPLC method is unable to measure the instantaneous concentration of both imidazole and imidazolate – rather the composite which reports the sum of these two species upon protonation during aliquoting. Input experimental data was obtained from three independent reactions, holding the concentration of imidazole and NaOH constant, allowing only $[\text{NH}_4\text{OH}]_0$ to be varied. Furthermore, the magnitudes of the literature acid-based equilibria constants (equations 4.7, 4.8, 4.9 and 4.10) were fixed.

Key results: The model is in good agreement with experimental data and recapitulates the changes in rate and selectivity as a function of varied $[\text{NH}_4\text{OH}]_0$.

a) Estimated pKa of imidazole = 9.2, compare to pKa of 2-phenyl-4-(trifluoromethyl)-1H-imidazole = 11.170

b) Relative selectivity factor for O vs N is 21.6:1 for hydroxide in MeOH.

Individual comparison of experimental (marks) and fitted (line) data are displayed in Figures 4.17 – 4.19.
Figure 4.17. Experiment 1: \([4.3] = 0.13\text{M}; [\text{NaOH}] = 0.72\text{M}; [\text{NH}_4\text{OH}] = 0\text{M}\n
\rightarrow = [\text{Imidazole}]; \star = [\text{O-Product}]; \bigstar = [\text{CN}]

Figure 4.18. Experiment 2: \([4.3] = 0.13\text{M}; [\text{NaOH}] = 0.72\text{M}; [\text{NH}_4\text{OH}] = 0.6\text{M}\n
\rightarrow = [\text{Imidazole}]; \star = [\text{O-Product}]; \bigstar = [\text{CN}]

Figure 4.19. Experiment 3: \([4.3] = 0.13\text{M}; [\text{NaOH}] = 0.72\text{M}; [\text{NH}_4\text{OH}] = 1.2\text{M}\n
\rightarrow = [\text{Imidazole}]; \star = [\text{O-Product}]; \bigstar = [\text{CN}]

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4.7.7.4 Effects of solvent on selectivity

General Procedure

\[
\begin{align*}
\text{H} & \quad \text{Ar} \\
\text{CF}_3 & \quad \text{N} \\
4.3 & \quad \text{byproducts} \\
& \quad \text{R = Me or H}
\end{align*}
\]

2-\((p\text{-tolyl})\)-4-\((\text{trifluoromethyl})\)-1H-imidazole (271 mg, 1.20 mmol) was added to solvent (8.00 mL), aqueous (28 wt %) ammonium hydroxide (1.6 mL, 12 mmol), and aqueous (15 M) sodium hydroxide (288 mg, 7.20 mmol). The reaction temperature was increased to 60 °C and a sampling sequence was initiated. HPLC samples were analyzed using HPLC method 2.

The general procedure was repeated using isopropanol and methanol as the selected solvents. HPLC time-course data can be found in Figure 4.6.

4.7.7.5 Effects of Dosing NaOH on the conversion of 4.3 to 4.4

\[
\begin{align*}
\text{Br} & \quad \text{HOH} \\
\text{OH} & \quad \text{CF}_3 \\
4.7 & \quad \text{Ar} \\
& \quad \text{Ar = p-tolyl}
\end{align*}
\]

Isopropanol (10.0 mL), aqueous (28 wt %) ammonium hydroxide (6.67 mL, 48 mmol), and 4-methylbenzaldehyde (333 mg, 327 µL, 2.77 mmol) were added to a three necked flask held in an ethylene glycol bath cooled to 0 °C. 3,3-dibromo-1,1,1-trifluoropropan-2-one (1.87 g, 6.9 mmol) was hydrated by adding to water (2.0 mL) cooled in an ice bath over the course of 20 minutes while stirring vigorously to form an aqueous solution of 3,3-dibromo-1,1,1-
trifluoropropane-2,2-diol. The aqueous solution of 3,3-dibromo-1,1,1-trifluoropropane-2,2-diol was added to the reaction flask over the course of 20 minutes. Once the addition was complete, the reaction temperature was increased 40 °C (Reaction \( t_0 \)). A sampling sequence and pH time course measurements were initiated after 225 and 290 minutes of reaction time, respectively. Aqueous (15 M) sodium hydroxide (0.24 g, 3.75 mmol) was added to the reaction after 292, 298, 307, 314, 321, 386, 393, and 412 minutes of reaction time. HPLC samples were analyzed using HPLC method 1. HPLC time-course data can be found in Figure 4.7.

### 4.7.8 Telescoped Synthesis

Isopropanol (10.0 mL), aqueous (28 wt %) ammonium hydroxide (6.67 mL, 48 mmol), and 4-methylbenzaldehyde (333 mg, 2.77 mmol) were added to a three necked round bottomed flask held in an ethylene glycol bath cooled to 0 °C. 3,3-dibromo-1,1,1-trifluoropropan-2-one (1.50 g, 669 µL, 5.54 mmol) was hydrated by adding to water (1.67 mL) cooled in an ice bath over the course of 20 minutes while stirring vigorously. The aqueous solution of 3,3-dibromo-1,1,1-trifluoropropane-2,2-diol was added to the reaction over the course of 20 minutes. Once the addition was complete, the temperature was linearly ramped from 0 to 60 °C over 180 minutes via iControl and a sampling sequence was initiated. After 300 minutes the pH probe was inserted into the reaction and time course pH measurements were initiated. Aqueous (15 M) sodium hydroxide (110 mg, 2.77 mmol) was added to the reaction via syringe to promote the elimination cascade after 288, 370, 390, 413, 430, and 460 minutes of reaction time. HPLC samples were analyzed using HPLC method 1. HPLC time-course data can be found in Figure 4.8.
Chapter 5: Automated Glovebox Sampling: Applications Towards Monitoring Buchwald-Hartwig Aminations

5.1 Introduction

The combination of automated sampling with online, chromatographic analysis represents an optimal method for gathering temporal reaction data with minimal analyst intervention. In Chapter 2 of this Thesis, a reaction monitoring platform capable of autonomous aliquoting, sample delivery, and online analysis using HPLC was presented.\textsuperscript{118} The resolving ability of HPLC allows for the quantification of starting materials, intermediates, products, and byproducts from complex mixtures which makes it an invaluable tool for reaction profiling. In Chapter 3, this automated reaction monitoring platform played a pivotal role throughout our mechanistic investigations of the Kinugasa reaction.\textsuperscript{171} We envisioned that we could expand the reaction monitoring scope of the system to allow for profiling of air-sensitive reactions performed within a glovebox.

The ubiquity of useful, air sensitive transformations, coupled with the difficulty of gathering temporal data without compromising their inert atmosphere, prompted us to modify our sampling device. While this platform has demonstrated its ability to generate reaction profiles of catalytic systems, application towards monitoring air sensitive reactions remains a nontrivial task. With careful analyst technique it is possible to gather time-course data from air sensitive reactions performed on the benchtop without disrupting the inert atmosphere via inserting a capillary for aliquoting through a pierceable septum, although this process is fallible. In this chapter, we present our modified reaction monitoring platform that is capable of automated sampling and online HPLC analysis from reactions performed under an inert atmosphere (inside a glovebox).
We first probed the system’s ability to sample, dilute, mix, and analyze aliquots reproducibly for aliquots collected within an inert environment. We then employed the platform to monitor a series of Buchwald-Hartwig amination reactions, a catalytic transformation for forming C-N bonds.\textsuperscript{172} This useful reaction has become a fundamental tool in organic synthesis for the formation of C(sp\textsuperscript{2})-N bonds.\textsuperscript{172–176} Since the seminal reports by Buchwald\textsuperscript{177} and Hartwig,\textsuperscript{178} the Buchwald-Hartwig amination has seen widespread use in the synthesis of pharmaceuticals,\textsuperscript{179–183} natural products,\textsuperscript{184,185} organic materials,\textsuperscript{186} and agrochemicals.\textsuperscript{187}

A simplified catalytic cycle for a Buchwald-Hartwig amination using Pd(OAc)\textsubscript{2} as a precatalyst is depicted in Scheme 5.1. First, activation of Pd(OAc)\textsubscript{2} via a reducing agent forms the catalytically active Pd(0) species \textbf{5.1}. The cross-coupling catalytic cycle proceeds through four key intermediates (\textbf{5.1-5.4}). Phosphine ligated palladium \textbf{5.1} undergoes oxidative addition with aryl halide \textbf{5.5} to generate complex \textbf{5.2}. Coordination of the amine forms N-bound palladium complex \textbf{5.3} which reacts with base to form the amido complex \textbf{5.4}. Reductive elimination of \textbf{5.4} forms the key C-N bond simultaneously liberating both the cross-coupled product \textbf{5.6} and the Pd(0) species \textbf{5.1}, where it can reenter the catalytic cycle. This simplified model does not account for effects of off-cycle species\textsuperscript{188,189} or palladium aggregates each of which can impact the overall reaction rate.
Scheme 5.1. Generic catalytic cycle for the palladium-catalyzed coupling of aryl halides with arylamines.

5.2 The Reaction Monitoring Platform

The following is a list of the components and their functions that comprise the automated sampling platform (Figure 5.1).

- Arduino microcontroller (to coordinate and initiate sampling events)
- Computer with Python (to script events and communicate with the microcontroller)
- HPLC-MS (for separation and quantification of reaction species in the collected aliquot)
- Mettler Toledo Easysampler™ Sampling Probe and Actuator (to collect the reaction aliquot)
- Cavro 3 port syringe pump (for sample dilution and delivery)
- Nanovalve (HPLC injection sample loop)
- PTFE tubing (for liquid handling/sample delivery)
The sampling sequence begins by priming all fluidic lines with diluent (often the reaction solvent is selected as the diluent). Next, the Easysampler is actuated to extend the sampling pocket into the reaction vial. After five seconds, the Easysampler pocket containing reaction solution is retracted into the probe.

Figure 5.1. Cartoon schematic of the reaction monitoring platform used to gather time-course data from reactions run within a glovebox.

The sample aliquot is then delivered with an adjustable diluent volume and flow rate outside the glovebox via an air-tight union to an injection loop. An inline mixer was not used for this variant of the reaction monitoring platform. The microcontroller then switches the valve position aligning the sample loop between the HPLC pump and column for immediate analysis. The system resets to its initial state and a wash cycle is performed (2.0 mL of diluent) to flush any residual sample from the fluidic lines and injection loop into the waste while also backfilling the probe pocket with clean solvent before the next sample is taken.

An important consequence of backfilling the lines with diluent before collecting the next reaction aliquot is that the next time the sampling probe extends the pocket into the flask the reaction becomes diluted proportional to the volume of the pocket (Figure 5.2).
Figure 5.2. a) Easysampler in internal position backfilled with diluent. b) Easysampler after actuation; the reaction has become diluted proportional to the pocket volume

5.2.1 Sample Reproducibility

We probed the sampling reproducibility by sampling a vial of toluene within the glovebox 50 times and measuring the HPLC response on the benchtop. The sampling sequence begins with a dilution proportional to the volume of the sample pocket. We would therefore anticipate a negative trend in the diode array detector (DAD) response as a function of increasing injection number because of this dilution. If observed, this trend would validate the ability of the platform to sample, dilute, and mix aliquots reproducibly, and has the additional benefit of allowing us to calculate the Easysampler pocket volume.

Sampling a vial of toluene in the glovebox 50 times resulted in a linear trend ($R^2 = 0.99$) with a negative slope (Figure 5.3).
By using the initial response, slope of the line, and initial volume we calculated that the pocket volume of the Easysampler is 20.2 µL (Section 5.7.3.1), which is in excellent agreement with product specifications. This similarity between expected and actual pocket volume demonstrates the ability of the platform to reproducibly collect, mix, dilute, and deliver an aliquot from within the glovebox to an HPLC on the benchtop for real-time analysis.

Previous prototypes of our automated sampling platform displayed a unique relationship between diluent volume and detector response (Figure 2.3).\textsuperscript{118} Because of the similarity in design regarding sample mixing and delivery, we would anticipate a similar trend with this modified platform assembled in the glovebox. By adjusting the diluent volume between 400 – 1200 µL and measuring the response at the detector, we observed a trend shape as anticipated (Figure 5.4).

Figure 5.3. Online HPLC data displaying the relationship between detector response and sample number when a vial of toluene is sampled 50 times from within a glovebox.
Figure 5.4. Online HPLC data displaying the relationship between diluent volume and detector response when a vial of toluene is sampled from within a glovebox. Each data point represents the average of a triplicate series. Error bars represent ± one standard deviation of the series.

A volume in excess of 400 μL (the approximate dead volume between the sample pocket and injection loop) is required to deliver the sample from the reaction vial to the injection loop. Volumes between 400 – 700 μL correspond to a region of growing signal response until a maximum response was observed with a volume of 700 μL. For diluent volumes >700 μL we observe a downward trending response until no signal is received at the detector (1200 μL), indicating that the entire sample has been flushed into the waste. The DAD response for each diluent volume was collected in triplicate and the standard error was plotted (Y error bars). We were pleased to observe low relative standard deviations (often < 1%) further evidencing the high degree of reproducibility the sampling platform can achieve. Therefore, we have demonstrated that by adjusting the diluent volume we can perform on-the-fly, reproducible dilutions. This dynamic dilution data can be utilized to optimize signal response at the detector depending on solution concentration; concentrated reactions can be diluted more by using volumes either before or after
the apex volume, whereas reactions of low concentration can still receive maximum signal by using the apex delivery volume (700 µL).

5.3 Monitoring Buchwald-Hartwig Aminations

5.3.1 Outline / COPASI modeling

Upon confirming that the reaction monitoring system is capable of reproducibly analyzing samples from within the glovebox, we devised a series of experiments to demonstrate the platforms ability to profile catalytic reactions. The Buchwald-Hartwig aminations we chose to monitor is outlined in Scheme 5.2. We sought to answer the following fundamental questions. First, how does varying the aryl halide between iodobenzene (5.7) and bromobenzene (5.8) effect the reaction profiles of the starting materials and products (Scheme 5.2a)? Next, what would the reaction progress of a competition reaction using both iodobenzene and bromobenzene look like (Scheme 5.2b)? Finally, what temporal profile would we observe for the cascade reaction using the dihalogenated substrate 1-bromo-4-iodobenzene (5.11) (Scheme 5.2c)?

Before any reaction profiles were acquired, we utilized COPASI modeling software to generate progress curves. The COPASI models provide a useful starting point to approximate trends based off assumptions made from literature precedence. Previous studies comparing oxidative additions of iodo- and bromobenzene to Pd(0) complexes occur with different kinetic behaviours (aryl iodides undergo oxidative addition more rapidly than aryl bromides) indicating that the mechanism for oxidative addition differs based on the halide identity. Additionally, Buchwald demonstrated that reductive elimination of electron rich amines, such as 5.9, is not a kinetically difficult step in certain Pd catalyzed C-N aminations, further supporting a paradigm where oxidative addition could be turnover limiting. Other mechanistic studies on aminations
of aryl bromides revealed that the secondary amine component does not exhibit positive order kinetics, while a dependence of the rate on [aryl halide] was observed.\textsuperscript{195} Previous kinetic studies and energy differences between Ph-Br and Ph-I bonds\textsuperscript{196,197} lead us to the assumption that oxidative addition is turnover limiting. Therefore, differences in bond strengths between Ph-I and Ph-Br would manifest in a larger rate of consumption of PhI than PhBr.

Scheme 5.2. Buchwald-Hartwig amination reactions to be investigated utilizing the automated reaction monitoring platform. Temporal profiles generated from reactions performed under an inert atmosphere (inside a glovebox). R = \textit{p}-methoxyphenyl.

For the reactions run in parallel (Scheme 5.2a), we assume that the oxidative addition of the aryl halide represents the turnover limiting step to form complex 5.2 (Scheme 5.1). To exemplify these differences in reactivity of aryl halides, a simplified model in COPASI was
constructed (Figure 5.5a) that assumes that $k_1$ with iodobenzene is threefold larger than $k_1$ with bromobenzene (Scheme 5.1a). Figure 5.5a shows first order decay of iodobenzene (5.7) and bromobenzene (5.8) with the rate of consumption of 5.7 being greater. Therefore, we anticipate that the kinetic profile for the parallel reactions described in Scheme 5.2a would resemble the temporal profile visualized in Figure 5.5a.

![Graph](image_url)

**Figure 5.5.** COPASI modelling for the reactions outlined in Scheme 5.2. a) Predicted reaction progress curves for competition / parallel aminations (Scheme 5.2 a) and b). b) Predicted reaction progress curves for the cascade reaction using 1-bromo-4-iodobenzene (Scheme 5.2c).

For the competition reaction using two aryl halides (Scheme 5.2b), we would anticipate simultaneous consumption of PhI and PhBr. Differences in lability of Ph-I and Ph-Br would result in a larger rate of consumption of iodobenzene vs bromobenzene. Therefore, we expect minimal differences in the kinetic profiles for the parallel and competition reactions described in Scheme 5.2a and 5.2b, and that both should resemble COPASI generated profile displayed in Figure 5.5a.
The temporal profile for the cascade reaction using the dihalogenated substrate 5.11 (Scheme 5.2c) would be an extension of the same hypothesis, where the Ph-I bond engages more readily than Ph-Br, leading to a buildup of intermediate 5.12. A model was made in COPASI (Figure 5.5b) that assumed that coupling would occur exclusively at the C-I position of 5.11 due to differences in bond strengths of the two halogens. We also assumed that the rate of oxidative addition for iodobenzene is threefold larger than for bromobenzene. The COPASI model displayed first order decay of 5.11 as monocoupled intermediate 5.12 is formed. Eventually, 5.12 hits a maximum concentration as 5.11 exceeds 85% conversion. Intermediate 5.12 then enters a new regime where its rate of consumption exceeds its rate of formation, causing an inflection point in the [5.12]. The model predicts a sigmoidal profile for the formation of the dicoupled product 5.14. The rate of formation of 5.14 is low at the reactions onset, which corresponds to a low [5.12]. The rate of formation of 5.14 increases as the [5.12] increases and vice versa. Therefore, we predict the reaction profile for the Buchwald-Hartwig amination described in Scheme 5.2c) would resemble the trends in Figure 5.5b.

5.4 Monitoring Parallel Buchwald Hartwig Reactions

To demonstrate the ability of our automated sampling device to monitor reactions performed from within a glovebox, we acquired progress curves for a series of palladium catalyzed Buchwald-Hartwig aminations with various aryl halides. Many developments have been made in optimizing the Buchwald-Hartwig amination to expand the scope and utility of this transformation, but ultimately the selection of the optimal solvent, base, Pd source, and ligand mostly depend on the nature of the substrates.198
For our studies we selected the diarylamine bis(4-methoxyphenyl)amine (5.9) as our amine coupling partner. We opted to use Pd(OAc)$_2$ as a precatalyst, as after in situ reduction, a highly active Pd catalyst is generated providing superior results to Pd$_2$(dba)$_3$, Pd(OAc)$_2$/PhB(OH)$_2$ or [(allyl)PdCl]$_2$ for the arylation of anilines.$^{199}$ We chose the sterically hindered alkyl phosphine P($t$-Bu)$_3$ for our ligand as it is competent for coupling aryl halides and arylamines.$^{200,201}$ The pyrophoricity and ease of oxidation of P($t$-Bu)$_3$ often necessitate its usage under an inert atmosphere, providing an excellent test case for our glovebox sampling system. Commonly employed bases in the Buchwald-Hartwig amination include NaO$t$Bu,$^{202}$ LiHMDS,$^{203}$ and Cs$_2$CO$_3$. We selected the base/solvent combination of LiHMDS and THF to improve reproducibility, as physical properties such as base particle size can affect the reaction rate in heterogeneous palladium-catalyzed aminations.$^{205}$ The moisture sensitive nature of LiHMDS further incentivized the use of an inert atmosphere when performing these coupling reactions.

We began our reaction monitoring studies using a model system to compare the rates and reaction profiles of C-N coupling with diarylamine bis(4-methoxyphenyl)amine (5.9) and both iodobenzene (5.7) and bromobenzene (5.8) as aryl halide coupling partners. Performing two coupling reactions using identical experimental conditions but with different halide substituents (iodobenzene 5.7 or bromobenzene 5.8) produced two distinct reaction profiles (Figure 5.6). Concentrations for 5.7, 5.8, 5.9, and 5.10 were calculated from external calibration curves created for each component (Section 5.7.5).
Figure 5.6 Online HPLC Data for the parallel coupling reactions using aryl halides 5.7 or 5.8. a) Consumption of 5.9 vs time for each coupling reaction. b) Formation of 5.10 vs time for each coupling reaction. Reaction conditions: \([5.7]_0\) or \([5.8]_0\) = \([5.9]_0\) = 0.11 M; \([\text{LiHMDS}]_0\) = 0.165 M; \([\text{Pd(OAc)}_2]_0\) = 2.8 mM; \([\text{P(t-Bu)}_3]\) = 5.6 mM in THF/toluene (17:1) at 60 °C in a glovebox.

The data in Figure 5.6 shows that neither reaction displays a simple kinetic profile. Interestingly, after the first 30 minutes the rates for both reactions were comparable, which contests our straightforward hypothesis that lability of the C-X bond will dictate the reaction rate. After 3 hours the rate of consumption of bromobenzene decreased until a constant reaction rate was observed corresponding to 33% conversion of 5.8. This unchanging rate despite continual reaction progress could indicate a change in the catalyst resting state as a function of turnover for the bromobenzene reaction. Alternatively, the reaction progress when iodobenzene is employed displays a relatively constant reaction rate indicating that the halide identity effects the reaction.
mechanism. This conclusion is within agreement by rate studies completed by Hartwig, which indicated that the mechanism of oxidative addition differs when iodobenzene and bromobenzene are subjected to identical reaction conditions.\textsuperscript{191}

The non-classical behavior observed in both cases could be a result of catalyst activation/deactivation, formation of aggregates, or a change in the catalyst resting state. Simply performing initial rate measurements would result in the oversimplification of complex kinetic behaviour as observed here. Therefore, the ability of our reaction monitoring platform to observe trends over the entire reaction has unparalleled value for increasing mechanistic understanding especially in systems that display complex kinetics.

5.5 Competition Reaction Data

To further probe the ability of the reaction monitoring platform to profile complex multicomponent reactions, we devised a Buchwald-Hartwig competition reaction using two aryl halides. Competition reactions can be a useful strategy for delineating mechanisms, as they can provide insight to multistep catalytic pathways which cannot be obtained via completing reactions singly.\textsuperscript{206}

Both iodobenzene (5.7, 0.5 equiv.) and bromobenzene (5.8, 0.5 equiv.) were included as coupling partners with arylamine 5.9 (1 equiv.) to form the single triarylamine product 5.10. Finding a means to profile each major species for competition reactions such as this is challenging. More routine reaction monitoring techniques such as IR and NMR would likely be incapable of accurately quantifying the reaction species due to signal overlap of structurally similar species. Our system, however, is ideally suited for monitoring this multicomponent reaction because it is
capable of quantifying 5.7, 5.8, 5.9, and 5.10 throughout the entire reactions progress by leveraging online HPLC as an analytical technique (Section 5.7.4.2).

Utilizing the parallel coupling data and COPASI modelling as templates to predict reactivity for the competition reaction we anticipated simultaneous consumption of both 5.7 and 5.8, with the rate of consumption of 5.7 being greater (Figure 5.5a). Instead, we observed a reaction profile with two distinct reactivity regimes (Figure 5.7). Complete consumption of iodobenzene was observed in under four hours and displayed exponential decay. During this time, the consumption of bromobenzene was minor, indicating high selectivity for coupling 5.7 over 5.8 when both are present in similar concentrations. Upon exhaustion of iodobenzene, the rate of consumption of bromobenzene first increases, then decreases until complete consumption was observed after ~14 hours. The sigmoidal profile of 5.8 in Figure 5.7 was unexpected based off the reactions performed in parallel, and further reinforces the importance of robust time-course data for understanding reaction mechanisms. Often chemical processes are more complex than initially expected and making mechanistic proposals for systems based solely on initial rate or endpoint analysis can lead to faulty understanding.

We are confident in the accuracy of the acquire time-course data as we observed a conservation of mass balance throughout the reactions progress. Additionally, the near overlaid trends of 5.8 and 5.9 at times >4h exemplify the quantitative accuracy of the platform by accounting for the 1:1 stoichiometry of the reaction.
Figure 5.7. Online HPLC data displaying progress curves for the competition reaction using coupling partners 5.7 and 5.8. Reaction conditions: $[5.7]_0 = [5.8]_0 = 0.055 \text{ M}$; $[5.9]_0 = 0.11 \text{ M}$; $[\text{LiHMDS}]_0 = 0.165 \text{ M}$; $[\text{Pd(OAc)}_2]_0 = 2.8 \text{ mM}$; $[\text{P(} t \text{-Bu)}_3]_0 = 5.6 \text{ mM}$ in THF/toluene (17:1) at 60 °C in a glovebox.

The high selectivity for depleting iodobenzene over bromobenzene at the early stages of the reaction is indicative of catalyst monopoly and can be rationalized by either comparing the rates of oxidative addition or the reactivity of downstream intermediates. The competition reaction mechanism can be described as two separate catalytic cycles, one for each aryl halide, and with each beginning at $\text{L}_n\text{Pd}(0)$ (Scheme 5.3). Interestingly, both catalytic cycles form the same amido-complex 5.19, so the rate of reductive elimination to form the final product 5.10 should have negligible effects on the reaction’s progress.
Scheme 5.3. Reaction mechanism for the competition reaction using iodobenzene (5.7) and bromobenzene (5.8).

Differences in the facileness of the oxidative addition of iodobenzene and bromobenzene could account for the observed catalyst monopoly. If the magnitude of $k_5 \gg k_6$, then oxidative addition complex 5.15 would form preferentially over 5.16. However, this discrepancy of rate constants between oxidative addition is not supported by the parallel studies where initial rates for PhBr and PhI were comparable, although the reaction rates did differ significantly at the later stages of the reaction.

Reactivity differences of intermediates after the oxidative addition complexes could also account for the observed monopoly. For example, differences in the rate constants $k_7$ and $k_8$ to form coordination complexes 5.17 and 5.18, respectively, could manifest as a higher rate of consumption of 5.15 over 5.16. Similarly, reactivity differences between coordination complexes
and therefore rate constants \( k_9 \) and \( k_{10} \), could also result in higher rates of consumption of 5.15 over 5.16. The observed monopoly could, therefore, arise either from differences in energies of the transition states of the oxidative addition, or stem from increased reactivity of downstream intermediates selectively depleting iodobenzene oxidative addition complex 5.15 where it is subsequently regenerated.

A final consideration regarding the catalyst monopoly is the possibility that oxidative addition of the C-X bond is reversible. Several examples have been reported indicating that oxidative addition of C-Br is reversible when the bulky ligand P(tBu) is used.²⁰⁷–²⁰⁹ If formation of 5.16 from 5.1 and 5.8 is reversible, and 5.15 is more reactive than 5.16, the Curtin-Hammet principle could explain the observed monopoly (Figure 5.8). Therefore, differences in the stability of oxidative addition complexes 5.15 and 5.16 becomes less relevant, as the reactivity of these intermediates is the main driving force for the observed selectivity. If \( \Delta G^{\ddagger}_{\text{i}} < \Delta G^{\ddagger}_{\text{Br}} \) (i.e. \( \Delta \Delta G^{\ddagger} \neq 0 \)), 5.15 will be preferentially depleted to form 5.10. After consumption of 5.15, it can be regenerated from 5.16 via first reforming 5.1 via reductive elimination of 5.16.
5.6 Difunctionalized Aryl Halide Reaction Data

To further solidify the capability of the automated sampling platform to generate reaction profiles of complex chemical systems, we chose to monitor an additional C-N coupling reaction using the difunctionalized aryl halide coupling partner 1-bromo-4-iodobenzene (5.11). Data from the previous parallel and competition reactions would suggest that C-N coupling at the iodo-position of 5.11 would out-compete coupling at the bromo-position to accumulate a detectable amount of mono-coupled intermediate 5.12 (Scheme 5.2). We would then expect a second regime to predominate involving the coupling of intermediate 5.12 with an additional molecule of 5.9 to afford the dicoupled product 5.14 (Figure 5.5b).

Combining equimolar amounts of 5.9 and 5.11 under coupling conditions instead resulted in another unexpected reaction profile (Figure 5.9). Strikingly, we observed the dicoupled product 5.14 on our first collected data point. This result further exemplifies that proposing reaction
mechanisms using temporal data of similar catalytic systems is insufficient and can lead to simplified or erroneous understanding. Collecting time course data of each modified system is the only way to elucidate mechanistic subtleties. We observed overall zero order decay for the consumption of both starting materials 5.9 and 5.11. The consumption rates of 5.9 and 5.11 were 4.4 mM/h and 2.9 mM/h respectively. This discrepancy between consumption rates can be rationalized mechanistically as 5.11 reacts only with 5.9 to form 5.12, whereas 5.9 also reacts with 5.12 to form 5.14.

![Chemical reaction diagram]

Figure 5.9. Online HPLC data displaying progress curves for when 1-bromo-4-iodobenzene (5.11) is used as the aryl halide coupling partner. Reaction conditions: \([5.9]_0 = [5.11]_0 = 0.11\) M; \([\text{LiHMDS}]_0 = 0.165\) M; \([\text{Pd(OAc)}_2]_0 = 2.8\) mM; \([\text{P}(t\text{-Bu})_3] = 5.6\) mM in THF/toluene (17:1) at 60 °C. \(R = p\text{-methoxyphenyl.}\)

To account for the observed kinetic profile when 1-bromo-4-iodobenzene (5.11) was selected as the halogenated substrate (Figure 5.9), a linear reaction network was assumed
comprising two sequential catalytic cycles. Using COPASI, three models were generated where the magnitudes of $k_{12}$ and $k_{13}$ were adjusted to attempt to match the model with the observed profile (Figure 5.10). First, $k_{12} = k_{13}$ (Figure 5.10a), then $k_{12} = 10k_{13}$ (Figure 5.10b), and finally $10k_{12} = k_{13}$ (Figure 5.10c).

![Diagram of reaction network](image)

**Figure 5.10.** COPASI models for a linear reaction network predicting the reactivity of 5.11. $k_{12}$ and $k_{13}$ values were varied to attempt to match the trends in Figure 5.9. a) $k_{12} = k_{13} = 0.1 \text{ M s}^{-1}$; b) $k_{12} = 10k_{13} = 1 \text{ M s}^{-1}$; c) $10k_{12} = k_{13} = 1 \text{ M s}^{-1}$

The COPASI models which assume a linear reaction network (Figure 5.10) provide a simple but useful approach to delineating the coupling cascade depicted in Figure 5.9. When the magnitude of the rate constants for the first and second catalytic cycle are equal ($k_{12} = k_{13}$, Figure 5.10a) the dicoupled product 5.14 forms only after an appreciable quantity of intermediate 5.12 has been generated. Upon completion of the reaction the concentrations of the mono- and dicoupled products are equal, [5.12] = [5.14]. These discrepancies between reaction kinetics and
product distributions lead us to discount this model as an accurate representation of the observed data in Figure 5.9.

When the magnitude of the rate constant of the first catalytic cycle \((k_{i2})\) is tenfold larger than that of the second cycle \((k_{i3}, \text{Figure 5.10b})\), we would observe \(5.12\) as the major product. Upon completion of catalyst turnover less than 20\% of the dicoupled product \(5.14\) had formed. The large discrepancy of concentrations between \(5.12\) and \(5.14\) at the end of the reactions progress, as well as the kinetic profiles lead us to also rule out this scenario to model observed reactivity.

Finally, When the magnitude of the rate constant of the second catalytic cycle \((k_{i3})\) is tenfold larger than that of the first cycle \((k_{i2}, \text{Figure 5.10c})\), we observe a change in the selectivity of the major product. In this scenario, formation of the dicoupled product \(5.14\) predominates while the concentration of intermediate \(5.12\) remains low. A maximum \([5.12]\) is observed corresponding to \(~10\%\) in situ yield. The nonzero-order profiles predicted in this scenario coupled with the product distribution leads us to believe that this model also does not accurately account for the observed trends.

To more accurately model the temporal profile observed in Figure 5.9, we propose an alternate reaction network invoking \(\pi\)-coordination complex \(5.20\) which is formed after C-N coupling at the iodo- position (Scheme 5.4). We propose intermediate \(5.20\) is in equilibrium with \(5.12\) and Pd(0).
Scheme 5.4. Alternate reaction network invoking ring walking as a means to synthesize 5.14.

Invoking 5.20 creates the possibility that 5.14 can be generated directly via a catalyst transfer type mechanism, therefore directly entering the second catalytic cycle without liberating 5.12. This intramolecular catalyst transfer has been implicated in multiple examples of Pd catalyzed polymerizations of multifunctionalized aryl halides and is commonly referred to as catalyst-transfer polymerization (CTP). This methodology is especially useful for the synthesis of π-conjugated polymers with narrow polydispersity. The key difference between step-growth polymerization and CTP is ring-walking, wherein the catalyst remains bound to the π-system (5.20) before migrating to the next C-X terminus. After migration intramolecular oxidative addition at the C-X bond then repeats the catalytic cycle. This cycle continues until all C-X sites have been coupled. The ring-walking behavior of CTP systems has also been supported by both experimental and computational data. CTP is often used to polymerize thiophene monomers with Ni(dpdp)Cl₂, although recent reports demonstrate that comparatively Pd outperforms Ni at CTP.

Creating a mechanistic model in COPASI using the reaction network in Scheme 5.4 which invokes 5.20 as a key intermediate allows the formation of reaction profiles not possible using the previous linear pathway (Figure 5.11a). Accounting for the equilibrium process between 5.12 and 5.20 resulted in the closest fits yet to match the trends associated with 5.12 and 5.14 (Figure 5.11b).
This updated model better recapitulates the observed profiles but does not accurately match the zero order profiles of each species. To more accurately model the observed reaction profile additional mechanistic inferences must be included in the model such as off-cycle equilibria or product effects.

Therefore, considering whether the linear reaction network in Figure 5.10 or the catalyst transfer process (CTP) in Scheme 5.4 best described the cascade reaction with substrate 5.11, we believe the CTP pathway is likely. The combination of a better COPASI fit and literature precedence lead us to this conclusion. To the best of our knowledge, this catalyst transfer type behavior has not been reported in Buchwald-Hartwig aminations, or for any coupling reaction using a heteroatom.

Figure 5.11. Comparing observed kinetic data with a COPASI model for the coupling cascade reaction using 5.11. a) COPASI model generated using reaction network in Scheme 5.4. b) Reaction profile described in Figure 5.9.
5.7 Conclusions

We have developed a modular reaction monitoring platform capable of automated sampling from reactions performed within a glovebox and coupled it with online HPLC analysis. The ability of the platform to sample, dilute, deliver, and analyze reaction mixtures was demonstrated to be highly robust and reproducible by trending the response when a vial of toluene was sampled from within a glovebox and analyzed 50 times. We also observed a characteristic relationship between diluent volume and detector response which allows for a wide range of reaction concentrations to be monitored.

We then used the platform to generate reaction profiles for a series of Buchwald-Hartwig aminations. Reactions with aryl halides iodobenzene and bromobenzene performed in parallel demonstrate distinct profiles neither of which follows classical kinetics. A competition reaction using both aryl halides displayed unexpected selectivity towards coupling iodobenzene over bromobenzene when both were present in similar concentrations. An additional unexpected reaction profile was observed when the dihalogenated substrate 1-bromo-4-iodobenzene was employed. The temporal profiles of each component displayed 0 order kinetics. Additionally, the product after two sequential aminations was observed at the first data point demonstrating the formation of the dicoupled product is extremely facile. We invoke ring-walking behavior of the Pd catalyst in this system to rationalize the observed trends. To the best of our knowledge this is the first example of ring walking behavior in coupling reactions involving heteroatoms.
5.8 Experimental

5.8.1 General Remarks

5.8.1.1 Reagents

Bis(4-methoxyphenyl)amine was purchased from AKScientific and used as received. Palladium(II) acetate, tri-tert-butylphosphine, iodobenzene, bromobenzene, 1-bromo-4-iodobenzene, and LiHMDS were purchased from Sigma Aldrich and were used as received. All other reagents and solvents were purchased from conventional suppliers and used as received unless otherwise stated. Silica gel was purchased from Silicycle (60 Å, 230 x 400 mesh).

5.8.1.2 Analytical Methods

NMR spectra were recorded on a Bruker AV-400 for $^1$H, and $^{13}$C and were referenced to the residual solvent peak. $^{167}$ The abbreviations s, d, q, m signify singlet, doublet, quartet, and multiplet, respectively. NMR spectra were analyzed by using the software MNova.

The Liquid Chromatography (LC) samples were analyzed by HPLC/MS conducted on an Agilent 1200 HPLC with the following configuration:

Agilent G1379B degasser, G1312A binary pump, G1316A thermal column compartment, diode array detector and a 6120 single quad mass spectrometer.

Analytical setting for the detectors are:

DAD – 200 – 400 nm collected at 20 Hz storing all spectra for offline analysis. Peak area for quantification varies depending on the experiment, see calibration curves for details.

ESI-MSD – positive mode scan for m/Z 110 – 1500 running at 0.8sec/cycle. drying gas = 7.0 l/min, nebulizer pressure = 20 psi, gas temperature = 300 °C, capillary voltage = 4000 V.
HPLC column and mobile phase method used the follow conditions:
Poroshell C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 ºC;
Solvent A = Water, 0.1 % Formic Acid; Solvent B = acetonitrile; Flow Rate = 0.625 mL/min;
Starting Conditions = 70 % A, 30 % B; 0.00 – 5.00 min gradient to 30% A, 70 % B; 5.00 – 5.80 min isocratic 30% A, 70% B; 5.80 – 8.00 min gradient to 0% A, 100% B.

5.8.1.3 Default Sampling Method

Temporal HPLC data was obtained using a modified reaction monitoring platform similar to which has been reported in our group previously. All sampling events were executed by an Arduino microcontroller which was controlled via a Python script. The sampling sequence begins by actuating the Easysampler to extend the sampling pocket into the reaction mixture. After 5 seconds the pocket is retracted and THF (standard volume = 700 µL) is delivered (default flow rate = 1.0 mL/min) through the Easysampler, out of the glovebox, and onto the injection valve for analysis by HPLC. The injection valve is then triggered aligning the reaction aliquot with the HPLC pump and column allowing for online analysis. The sampling lines are then flushed with THF (2.0 mL, 1.0 mL/min) before reinitiating the sampling sequence.
5.8.2 Synthetic Procedures

4-methoxy-N-(4-methoxyphenyl)-N-phenylaniline (5.10)

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 mmol), iodobenzene (204 mg, 1.00 mmol), THF (8.4 mL), and toluene (300 µL) and the resultant solution was heated to 60 °C. Palladium(II) acetate (5.6 mg, 25 µmol) and tri-tert-butylphosphine (10.1 mg, 50.0 µmol) in toluene (200 µL) were added via syringe to initiate the reaction. A sampling sequence was then initiated. After 8 hours, the reaction was cooled to room temperature and quenched with saturated aqueous ammonium chloride. The crude was
extracted with ethyl acetate (10 mL, 3x). The organic extracts were washed with brine, dried 
(Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The product was obtained after flash 
chromatography (95:5, petroleum ether / ethyl acetate) to afford 5.10 as a white solid (245 mg, 
0.802 mmol, 80 % yield). Characterization data is consistent with that in the literature.$^{216}$

$^1$H NMR: (400 MHz, CDCl$_3$) $\delta$ 7.17 (t, $J = 7.8$ Hz, 2H), 7.05 (d, $J = 8.8$ Hz, 4H), 6.94 (d, $J = 8.2$ Hz, 2H), 6.89 – 6.80 (m, 5H), 3.80 (s, 6H).

$^{13}$C($^1$H) NMR: (101 MHz, CDCl$_3$) $\delta$ 155.8, 148.9, 141.3, 129.1, 126.5, 121.1, 120.70, 114.8, 55.6

MS ESI+: ($m/z$ calc. for C$_{20}$H$_{19}$NO$_2$, [M + H] = 306.2); found = 306.2

---

4-bromo-N,N-bis(4-methoxyphenyl)aniline (5.12)

To a 4 dram vial under an inert atmosphere was added bis(4-
methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 
mmol), 1-bromo-4-iodobenzene (283 mg, 1.00 mmol) THF (8.4 mL) 
and toluene (300 µL) and the resulting solution was heated to 60 °C. Palladium(II) acetate (5.6 
mg, 25.0 µmol) and tri-tert-butylphosphine (10.1 mg, 50.0 µmol) in toluene (200 µL) were added 
via syringe to initiate the reaction. After 30 hours the reaction was cooled to room temperature and 
quenched with saturated aqueous ammonium chloride. The crude was extracted with ethyl acetate 
(10 mL, 3x). The organic extracts were washed with brine, dried (Na$_2$SO$_4$), filtered, and 
concentrated under reduced pressure. The crude residue was purified via flash chromatography 
(petroleum ether/ethyl acetate; 9:1) to afford 5.12 (62 mg, 0.16 mmol, 16 % yield) as a white solid. 
Characterization data is consistent with that in the literature.$^{217}$

$^1$H NMR: (400 MHz, C$_6$D$_6$) $\delta$ 7.20 – 7.16 (m, 2H), 6.99 – 6.93 (m, 4H), 6.80 – 6.72 (m, 2H), 6.72 
– 6.65 (m, 4H), 3.29 (s, 6H)
To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (446 mg, 1.94 mmol), LiHMDS (444 mg, 2.65 mmol), 1-bromo-4-iodobenzene (250 mg, 0.88 mmol), THF (8.4 mL) and toluene (300 µL) and the resulting solution was heated to 60 °C. Palladium(II) acetate (5.6 mg, 25.0 µmol) and tri-tert-butylphosphine (10.1 mg, 50.0 µmol) in toluene (200 µL) were added via syringe to initiate the reaction. After 30 hours the reaction was cooled to room temperature and quenched with saturated aqueous ammonium chloride. The crude was extracted with ethyl acetate (10 mL, 3x). The organic extracts were washed with brine, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Methanol (150 mL) was added to the crude residue to precipitate out the product. The resulting heterogeneous solution was triturated via sonicator. The mixture was then vacuum filtered and the cake was washed with methanol followed by petroleum ether, then dried under vacuum to afford the purified product 5.14 (310 mg, 0.58 mmol, 66%) as a white solid. Characterization data is consistent with that in the literature.²¹⁸

**¹H NMR:** (400 MHz, C₆D₆) δ 7.15 – 7.11 (m, obscured by solvent signal), 7.07 (s, 4H), 6.74 – 6.70 (m, 8H), 3.29 (s, 12H).

**¹³C{¹H} NMR:** (101 MHz, C₆D₆) δ 156.0, 143.5, 142.2, 126.0, 123.8, 115.1, 55.0.

**MS ESI+:** m/z calc. for C₃₄H₃₂N₂O₄, [M]⁺ = 532.1, found 532.3.
5.8.3 Reproducibility Tests

5.8.3.1 Calculating the Easysampler Pocket Volume

Toluene (10.0 mL) was added to a sealed vial inside of the glovebox. The Easysampler was then introduced into the vial through a hole in the Teflon screw cap. The default sampling method was used to collect a total of 50 samples. The toluene peak response (254 nm) was plotted as a function of injection number. HPLC data is depicted in Figure 6.2. The Easysampler pocket volume was calculated according to Equation 6.2

\[
\text{Dilution Factor} = \frac{\text{Peak Area}(V_0 + \text{Inj#}(0.02015\text{mL}))}{V_0}
\]

(eq. 6.2)

5.8.3.2 Determining Relationship Between Diluent Volume and Detector Response

Toluene (20.0 mL) was added to a sealed vial inside of the glovebox. The Easysampler was then introduced into the vial through a hole in the Teflon screw cap. The default sampling method was to collect a sample in triplicate with the following diluent volumes: 400, 500, 600, 650, 700, 750, 800, 850, 900, 1000, 1200 µL. The average peak area of each triplicate series was plotted against the diluent volume, error bars are ± one standard deviation of the series. HPLC data is depicted in Figure 6.3

5.8.4 COPASI Modeling

5.8.4.1 Early Models

The following models were created in COPASI to approximate kinetic behaviour before experimentation.
5.8.4.2 Updated Model

The following model was created in COPASI to better account for the observed kinetic behaviour after experimentation.
5.8.5 Reaction Monitoring Data

5.8.5.1 Coupling Iodobenzene

\[
\begin{array}{ccc}
H & R^-N\cdot R & L_iHMDS \\
5.9 & 5.7 & Pd(OAc)_{2}/ P(r-Bu)_3 \\
& & THF / Toluene \\
& & 60 \, ^\circ C \\
& & R = p\text{-}-methoxyphenyl \\
\end{array}
\]

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 mmol), iodobenzene (204 mg, 1.00 mmol) THF (8.4 mL) and toluene (300 µL). The stirred solution was heated at 60 °C. Palladium(II) acetate (5.6 mg, 25 µmol) and tri-|tert|butylphosphine (10.1 mg, 50.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate and a sampling sequence was then begun. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) to the injection valve. Samples were analyzed using the general HPLC method.

![Sample HPLC chromatogram during a monitored Buchwald/Hartwig reaction coupling 5.7 and 5.9 to form 5.10.](image-url)

Figure 5.13. Sample HPLC chromatogram during a monitored Buchwald/Hartwig reaction coupling 5.7 and 5.9 to form 5.10.
Figure 5.14. Reaction time-course data for the Buchwald-Hartwig amination coupling 5.7 and 5.9 to form 5.10. Sample aliquots were collected using the automated sampling platform and analyzed using online HPLC.

5.8.5.2 Coupling Bromobenzene

\[
\begin{align*}
&\text{H}^+ \quad \text{LiHMDS} \\
&\quad \text{Pd(OAc)}_2 / \text{P(t-Bu)}_3 \\
&\quad \text{THF / Toluene} \\
&\quad 60 \ ^\circ \text{C} \\
&\quad \text{R} = p\text{-methoxyphenyl} \\
&\text{5.9} \quad \text{5.8} \quad \text{Br} \quad \text{Ph} \\
&\quad \text{5.10}
\end{align*}
\]

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 mmol), bromobenzene (157 mg, 1.00 mmol) THF (8.4 mL) and toluene (300 µL). The stirred solution was heated at 60 °C. Palladium(II) acetate (5.6 mg, 25 µmol) and tri-tert-butylphosphine (10.1 mg, 50.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate the reaction and a sampling sequence was then begun. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) onto the injection valve. Samples were analyzed using the general HPLC method.
Figure 5.15. Sample HPLC chromatogram during a monitored Buchwald/Hartwig reaction coupling 5.8 and 5.9 to form 5.10.

Figure 5.16. Reaction time-course data for the Buchwald/Hartwig amination coupling 5.8 and 5.9 to form 5.10. Sample aliquots were collected using the automated sampling platform and analyzed using online HPLC.

5.8.5.3 Iodobenzene and Bromobenzene Competition Reaction

\[
\begin{align*}
\text{H} & \quad \text{N} \quad \text{R} \\
\text{R} & \quad \text{N} \quad \text{R} \\
5.9 & \quad 5.7 & \quad 5.8
\end{align*}
\]

\[
\text{LiHMDS} \quad \text{Pd(OAc)}_2 / \text{P(t-Bu)}_3 \quad \text{THF / Toluene} \quad \text{60 °C}
\]

\[
\begin{align*}
\text{R} & = \text{p-methoxyphenyl} \\
\text{Ph} & \quad \text{N} \quad \text{R} \\
5.10
\end{align*}
\]
To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 mmol), bromobenzene (79 mg, 0.50 mmol), iodobenzene (102 mg, 0.50 mmol) THF (8.4 mL) and toluene (300 µL). The stirred solution was heated at 60 °C. Palladium(II) acetate (5.6 mg, 25 µmol) and tri-tert-butylphosphine (10.1 mg, 1.23 molar, 0.05 Eq, 50.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate the reaction and a sampling sequence was then initiated. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) onto the injection valve. Samples were analyzed using the general HPLC method. Reaction progress profile is depicted in Figure 5.7.

![HPLC chromatogram](image)

**Figure 5.17.** Sample HPLC chromatogram during a monitored Buchwald/Hartwig competition reaction coupling 5.7 and 5.8 with 5.9 to form 5.10.

5.8.5.4 Coupling 1-bromo-4-iodobenzene

![Reaction scheme](image)

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (229 mg, 1.00 mmol), LiHMDS (251 mg, 1.50 mmol), 1-bromo-4-iodobenzene (283 mg, 1.00 mmol) THF (8.4 mL) and toluene (300 µL). The stirred solution was heated at 60 °C. Palladium(II)
acetate (5.6 mg, 25 µmol) and tri-tert-butylphosphine (10.1 mg, 1.23 molar, 0.05 Eq, 50.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate the reaction and a sampling sequence was then initiated. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL/min) onto the injection valve. Samples were analyzed using the general HPLC sampling method. Reaction progress profile is depicted in Figure 5.9.

Figure 5.18. Sample HPLC chromatogram during a monitored Buchwald-Hartwig reaction coupling 5.9 and 5.11 to form 5.12 and 5.14.

5.8.6 Calibration Curves

A stock solution (5.00 mL) of each compound being calibrated (5.7 – 5.12 and 5.14) was created by dissolving a known mass of material in THF/toluene (19:1). This stock solution was then sampled three times using the Easysampler sampling method. Peak areas were normalized by toluene (internal standard, unchanging concentration between sampling). The stock solution (2.5 mL) was then diluted into THF/toluene (19:1, 2.5 mL) and the resultant diluted stock solution was sampled and quantified again three times. This diluting and sampling protocol was repeated an additional two times to collect a total of four data points in triplicate. HPLC calibration curve data was used to calculate the [5.12] based using the following assumption:

\[ [5.12]_t = [5.11]_0 - [5.11]_t - [5.14]_t \]
Figure 5.19. HPLC Calibration curves to allow for quantification of 5.7, 5.8, 5.9, 5.10, 5.11, and 5.14 directly from normalized peak area. Integration of all UV peaks completed at a wavelength of 215 nm.
Chapter 6: Delineating the Mechanism of an Optimized Synthesis of Spiro-OMeTAD

6.1 Introduction

As the global demand for energy continues to grow, the need for efficient, affordable, and robust alternatives to fossil fuels becomes increasingly important. Solar photovoltaics provide a practical and abundant source of alternative energy to help meet our increasing energy demands. Silicon based solar cell technologies have been used for decades, but historically have had problems related to high material costs and complicated manufacturing techniques. These challenges have prompted researchers to find alternative materials for solar photovoltaics, such as perovskites.219

A perovskite is class of compounds which has the generic crystal structure “ABX₃”. Some perovskite combinations, such as methylammonium lead halides (CH₃NH₃PbX), possess impressive light harvesting properties. These perovskites have been used as the active layer in solar cells (Figure 6.1). Cells using organic-inorganic hybrid perovskites are capable of converting solar power to usable energy, and have become a prominent area of research.220,221

Perovskite solar cells (PSC’s) have seen remarkable increases in power conversion efficiencies (PCE’s) ranging from 3.8% since their inception in 2009222 to over 22% currently.184 These devices also have the additional benefit of being easy to fabricate via solution based processes. For these reasons PSC’s are regarded as the most promising optoelectronic materials for future applications.225
Figure 6.1. Schematic of an organic/inorganic perovskite solar cell.

PSCs contain five layers (Figure 6.1): a conductive glass substrate; an electron transport material (ETM); a perovskite, light-harvesting layer; a hole transport material; and a metal counter-electrode. The perovskite layer absorbs solar energy to generate electron-hole pairs. The ETM extracts the photo-excited electrons from the perovskite and transfers them to the conductive glass anode. The HTM layer transports holes (positive charges) from the perovskite to the metal counter electrode.

One way to increase the performance of perovskite solar cells is to optimize the hole transport material (HTM) layer. Spiro-O-MeTAD (2,2′,7,7′-tetrakis-(N,N-di-4-methoxyphenylamino)-9,9′-spirobifluorene) is a champion HTM used in PSC’s that has gathered considerable interest since the first time it was used in a perovskite solar cell. The ionization potential of Spiro-O-MeTAD is in good agreement with that of the perovskite light absorbing layer. Additionally, remarkable glass forming properties make Spiro-O-MeTAD an extensively studied organic HTM for hybrid organic-inorganic solar cells. High costs from commercial sources
coupled with moderate yielding synthetic procedures of Spiro-OMeTAD (6.3, Scheme 6.1) mandating chromatographic purification prompted us to explore alternate methods to synthesize 6.3 at scale.\textsuperscript{233,234} One synthetic pathway for the formation of Spiro-OMeTAD involves four sequential Buchwald-Hartwig aminations coupling $2,2',7,7'$-tetrabromo-9,9'$'$-spirobi[fluorene] (6.1) with 4 equivalents of bis(4-methoxyphenyl)amine (6.2, Scheme 6.1). A generic catalytic cycle for the palladium catalyzed Buchwald-Hartwig amination is shown in Scheme 5.1.

\begin{center}
\includegraphics[width=\textwidth]{scheme_6_1.png}
\end{center}

\textbf{Scheme 6.1.} Synthetic conditions by Yeon to form Spiro-OMeTAD (6.3) from starting materials 6.1 and 6.2.\textsuperscript{233}

Considering the reaction mechanism and the temporal profiles for the major species creates interesting questions regarding relative rates and selectivities. Throughout the reactions progress, we anticipated the formation of multiple intermediates: monocoupled intermediate 6.4, dicoupled intermediates 6.5 and 6.6 (regioisomers), and the tricoupled intermediate 6.7 (Scheme 6.2). We sought to investigate how modifying the electronic and steric properties throughout the coupling cascade affect the magnitudes of $k_{1-4}$. To aid us in our synthetic studies, we used our recently reported automated reaction monitoring platform capable of generating temporal profiles of species for reactions performed under an inert atmosphere discussed in Chapter 5. Our platform is
ideally suited for monitoring complex, multistep reactions such as this because it can quantify starting materials, intermediates, and products throughout the reaction’s entirety. This level of resolution would be extremely difficult to achieve using spectroscopic reaction monitoring techniques, as the structural similarities between starting materials 6.1 and 6.2, intermediates 6.4, 6.5, 6.6, 6.7, and Spiro-OMeTAD (6.3) would likely result in unresolved signals.

![Scheme 6.2. Proposed cascade of coupling reactions to synthesize Spiro-OMeTAD from starting materials 6.1 and 6.2. R = p-methoxyphenyl.](image)

Before beginning our mechanistic investigations, a COPASI model was generated that assumes that the synthesis of Spiro-OMeTAD follows the linear reaction network described in Scheme 6.2. We assumed that only one of the regioisomers 6.5 and 6.6 is formed throughout the reactions progress to simplify the model. Additionally, we assumed that electronic and steric modifications that result as the reaction proceeds to higher order intermediates has a negligible effect on the observed rate of each coupling event, i.e. $k_1 = k_2 = k_3 = k_4$ (Scheme 6.2). A detectable
quantity of intermediates 6.4, 6.5, and 6.7 is formed in this scenario, and each intermediate displayed similar reaction profiles (Figure 6.2).

![Figure 6.2](image.png)

**Figure 6.2.** COPASI model generated for the linear reaction network described in Scheme 6.2 to make Spiro-OMeTAD (6.3). The model was build with the following assumptions: [6.1]₀ = 1 M; [6.2]₀ = 4 M; k₁ = k₂ = k₃ = k₄ = 0.1 M/s.

### 6.2 Temporal Profile of Optimized Conditions

Our first priority was to find reaction conditions to afford high yields of Spiro-OMeTAD (6.3) from starting materials 6.1 and 6.2 via four sequential Buchwald-Hartwig aminations. The selection of base, catalyst source, ligand, and solvent for this useful class of catalytic transformations depends mostly on the nature of the substrates. We report that the combination of Pd(OAc)₂, P(tBu)₃, LiHMDS, and THF resulted in near quantitative in situ yields of Spiro-OMeTAD after 24h. A temporal profile using these conditions was generated using our online-HPLC reaction monitoring platform (Figure 6.3). Concentrations of the reaction components were calculated via external calibration curves (Section 6.7.5).
Figure 6.3. HPLC reaction progress curves for the optimized synthesis of Spiro-OMeTAD. a) Entire reaction profile showing consumptions of 6.1 and 6.2, and formations of 6.3 and Int. b) Zoomed in region highlighting the profiles of 6.1, 6.3 and Int. Reaction conditions: [6.1]₀ = 40 mM; [6.2]₀ = 180 mM; [LiHMDS]₀ = 200 mM; [Pd(OAc)₂]₀ = 2 mM, [P(t-Bu)₃]₀ = 4 mM in THF/toluene (9:1) at 60 °C performed in a glovebox.

The reaction progress profile in Figure 6.3 displayed several interesting characteristics. Strikingly, we observed the formation of only one intermediate (Int) over the course of the reaction. Throughout the first 6 hours we observed consumption of 6.1 and 6.2 and concomitant formation of Int and Spiro-OMeTAD. After 6 hours the [Int] declines as its rate of consumption now exceeds its rate of formation. The limiting aryl bromide component becomes completely exhausted after 16h. For reaction times exceeding 16 hours we observed exclusive conversion of 6.2 and Int to form 6.3. After 22 hours, we observed 94% in situ yield of Spiro-OMeTAD. By
scaling up this procedure, Spiro-OMeTAD (1.12 g, 91% yield) was isolated without chromatography, a significant improvement to alternative methods.\textsuperscript{233}

The detection of only a single intermediate is in stark contrast with the expected mechanistic pathway, which we anticipated to proceed through three observable intermediates (Scheme 6.2). Rationalizing the unexpected chemoselectivity became a priority, but first \textbf{Int} needed to be isolated, characterized, and identified.

\section*{6.3 Isolating and Identifying the Intermediate}

A reaction solution containing the highest possible [\textbf{Int}] would increase its ease of isolation via extraction and chromatographic techniques. The data in Figure 6.3 shows that highest in situ yield of \textbf{Int} is \textasciitilde20\%, which corresponds to 6 hours of reaction time. Therefore, repeating the synthesis but stopping the reaction after 6 hours provides a means to maximize the in situ [\textbf{Int}] to increase the ease of its isolation. Alternatively, by starving the reaction of a necessary component, such as the basic additive, the turnover will cease once the limiting reagent has been depleted. This method provides the benefit of not having to stop the reaction at the optimal time, and was therefore chosen as the procedure for intermediate isolation.

A reaction was performed using a limiting amount of the basic additive (LiHMDS), only 3 equivalents were used instead of the standard 5 equivalents, to attempt to isolate \textbf{Int}. After one day of reaction time, the crude was treated using liquid/liquid extraction methods to remove the water-soluble components. It was discovered that the residual arylamine reagent (\textit{6.2}) could be removed by exploiting its solubility in methanol; the other crude components remained insoluble. Careful chromatographic separation allowed for the isolation of \textbf{Int} (120 mg) from the crude mixture which also contained starting material \textit{6.1} and Spiro-OMeTAD (\textit{6.3}).
Characterization of the isolated intermediate was attempted using NMR spectroscopy but it was discovered that Int decomposes in CDCl$_3$ and acetone-d$_6$. Instead, C$_6$D$_6$ was selected as the NMR solvent allowing characterization of Int by $^{13}$C and $^1$H spectroscopy (Figure 6.4). The ratio of integrations of the $^1$H NMR spectrum coupled with the presence of 18 $^{13}$C signals in the $^{13}$C spectrum evidenced that the intermediate was a product of 2 C-N couplings between 6.1 and 6.2.

![Figure 6.4. NMR spectra of isolated Int evidencing it is one of either regioisomer 6.5 or 6.6. a) $^1$H NMR Spectrum of Int. b) $^{13}$C NMR spectrum of Int.](image)

Determining if the intermediate was either regioisomer 6.5 or 6.6 was attempted using NMR techniques but it was difficult to assign absolutely Int to either regioisomer. Therefore, we employed X-ray diffraction to unequivocally identify the structure of the intermediate. We report
that Int is the product of two aminations of 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (6.1) on the same fluorene moiety and is therefore structure 6.5 (Figure 6.5).

6.4 Rationalizing the Intermediate Chemoselectivity

The unexpected chemoselectivity resulting in the formation of a single intermediate, identified as 6.5, was a curious discovery that warrants some additional explanation. A modified reaction network that accounts for this observed selectivity is shown in Scheme 6.3. The actual reaction network takes on a more simplified form than originally anticipated by circumventing the formation of intermediates 6.4, 6.6, and 6.7.

Figure 6.5. Confirmation that Int is structure 6.5 using x-ray diffraction. a) Chemdraw of 6.5. b) Crystal structure of isolated Int (6.5).
Scheme 6.3. Comparing the expected and observed mechanistic pathways for the synthesis of Spiro-OMeTAD. a) Expected pathway with four sequential couplings events. b) Observed, simplified pathway. $R = p$-methoxyphenyl.

We attribute the observed selectivity to an intramolecular catalyst transfer mechanism, similar to what was proposed in Section 5.5 of this Thesis. Using this mechanism, the Pd after the first reductive elimination migrates through the π-system of the fluorene to then oxidatively add at the C-Br position across the ring (Scheme 6.4). This ring-walking type behavior has been invoked in polymerization systems of polyfunctionalized aryl halides where exhaustive substitution was observed rather than a statistical mixture of substituted products.\textsuperscript{235–237} We believe our system is analogous to these because of the polyhalogenated starting material 6.1, although a key difference in our system is the spirocentre of the bisfluorene 6.1.
Scheme 6.4. Catalyst transfer mechanism observed via ring-walking of polyhalogenated aryl halides. \( R = p \)-methoxyphenyl.

An expanded catalytic cycle that accounts for the observed chemoselectivity regarding intermediate formation is presented in Scheme 6.5. Synthesis of 6.5 begins with oxidative addition at one of the C-Br positions of 6.1 to form intermediate 6.8. Coordination of 6.2 followed by deprotonation by LiHMDS, loss of LiBr, and reductive elimination forms \( N \)-coordinated complex 6.9. Coordination of the Pd to the \( \pi \)-system generates complex 6.10. At this point, the mechanism bifurcates to either dissociate palladium releasing 6.4 (Path 1, not observed), or the palladium can migrate across the \( \pi \)-system. After catalyst migration, intramolecular oxidative addition at the C-Br terminus across the same fluorene moiety results in the second oxidative addition intermediate 6.11 (Path 2). Reductive elimination to form 6.12 occurs in a similar fashion as with 6.8. At this point the Pd can no longer participate in catalyst transfer as both C-Br positions on one fluorene have been coupled. The catalyst cannot migrate to the other fluorene moiety because of the orthogonal geometry. Therefore, both 6.5 and Pd are liberated where the catalyst can then undergo
oxidative addition with either 6.1 or 6.5. The observed chemoselectivity to form 6.5 indicates the strength of the Pd π-system interaction because ring walking was observed exclusively.

Scheme 6.5. Simplified scheme rationalizing the observed chemoselectivity for the formation of intermediate 6.5. R = p-methoxyphenyl.
In summary, bisfluorene 6.1 enters the catalytic cycle and two coupling events occur across the same fluorene via an inner sphere type mechanism before the Pd dissociates forming 6.5. Dicoupled intermediate 6.5 can reenter the catalytic cycle where the remaining two C-Br positions are coupled with the bisamine 6.2 via this ring-walking mechanism. The high level of competence of the Pd to undergo catalyst transfer in conjunction with the orthogonal nature of the two fluorene moieties explains why intermediates 6.4, 6.6, and 6.7 were not observed throughout the reactions progress.

This catalyst ring-walking behavior has interesting implications regarding creating modular analogues of Spiro-OMeTAD with differing substitution patterns. If conditions could be discovered that exclusively couple the starting material to make a dicoupled intermediate that does not proceed to product, a second coupling partner could be introduced to break symmetry and create hole transport materials with modified physical or electronic properties.

6.5 **Kinetic Analysis to Identify the Catalyst Resting State**

To probe the effects of catalyst concentration on the overall reaction rate, two experiments were conducted with varying $[\text{cat}]_0$. Reaction profiles of these experiments were generated using our online reaction monitoring platform (Figure 6.6a). These experiments confirmed that the system displays a positive order in catalyst. By applying VTNA, the catalyst order can be approximated to be 1 (Figure 6.6b).\textsuperscript{62} These experiments evidence that the catalyst is involved in the turnover limiting step of the cycle, and that the system is well-behaved by displaying an integer order.
A series of different excess experiments were conducted to probe the driving force of each reagent. We plotted the formation of Spiro-OMeTAD using the optimized reaction conditions (Figure 6.2). Reproducing the experiment but varying the initial concentration of either 6.1 or 6.2 has an observable effect on the reaction rate (Figure 6.7a). Decreasing the initial concentration of 6.1 reduces the rate of the reaction (Figure 6.7a). This relationship between concentration and reaction rate is indicative that interaction with 6.1 is involved in the turnover limiting step which we propose is oxidative addition. Conversely, decreasing the initial concentration of 6.2 results in a significant increase in the reaction rate, indicative of a negative order of 6.2.

The resolving power of HPLC allowed us to also monitor the effects modifying [6.1] or [6.2] has on the reaction profile of the intermediate 6.5 (Figure 6.7b). Halving the [6.1]₀ from 40 mM to 20 mM resulted in a slower rate of formation of 6.5 and a maximum in situ [6.5] of nearly
half. Decreasing the $[6.2]_0$ resulted in a higher rate of intermediate formation but interestingly the maximum in situ concentration of both experiments was comparable.

Figure 6.7. HPLC temporal progress data of different [xs] experiments used to solve for the orders of 6.1 and 6.2. a) Effects of changing the [6.1] and [6.2] on the reaction rate. b) Effects of changing the [6.1] and [6.2] on the reaction profile of the intermediate 6.5. Reaction conditions for each experiment: $[\text{LiHMDS}]_0 = 200$ mM; $[\text{Pd(OAc)}_2] = 2$ mM, $[\text{P(t-Bu)}_3] = 4$ mM in THF/toluene (9:1) at 60 °C.

We attributed the negative order of 6.2 to participation in unproductive equilibrium process forming coordination complex 6.13, which modulates the concentration of the active catalyst via producing an off-cycle reservoir (Scheme 6.6). This observed negative order has been documented in similar systems where nucleophilic bases displayed orders <0 via binding palladium affecting the catalyst resting state.\textsuperscript{238,239} By applying VTNA, the orders for substrates 6.1 and 6.2 are approximated to be -1 and -2, respectively (Section 6.8.4.2), allowing us to propose the following rate law (Equation 6.1).

\[
Rate = k[\text{cat}]^1[6.1]^1[6.2]^{-2}
\] (eq. 6.1)
Scheme 6.6. Simplified mechanistic model including off cycle equilibrium between 6.2 and the catalyst to account for the negative order of 6.2. \( R = p\)-methoxyphenyl.

6.6 Increasing the Reaction Rate by Restricting the Arylamine Concentration

Different excess experiments indicated that the order of arylamine is <0, which we attribute to off-cycle binding of 6.2 with the catalyst minimizing the active \([\text{cat}]\). By minimizing the \([6.2]\) via dosing, we anticipated that we could accelerate the reaction rate by mitigating this off-cycle binding, thereby maximizing catalyst activity. We performed control experiments to probe the reactivity in the absence of arylamine 6.2 before investigating the effects dosing 6.2 has on the reaction rate. Specifically, we wanted to assess whether unproductive coupling between LiHMDS and aryl bromide 6.1 will occur in the absence of amine. This reactivity has been reported in similar systems (Scheme 6.7)\(^{240,241}\).

Scheme 6.7. LiHMDS coupling with aryl bromides.
A solution of arylbromide 6.1 was heated to 60 °C in a glovebox and sampled three times using the automated reaction monitoring platform to ensure sampling reproducibility (Figure 6.8a). Next, a premixed solution of Pd(OAc)$_2$ and P(tBu$_3$) was added to the heated solution containing 6.1. The data in Figure 6.8a shows a clear decrease in the [6.1] corresponding to a dilution when the catalyst solution was added. The resultant mixture was sampled for > 100 minutes, where it was observed that the [6.1] remained constant. This unchanging concentration indicated negligible reactivity between 6.1 and the catalyst under these conditions.

![Chemical diagram of reaction](image)

Figure 6.8. Probing reactivity of aryl bromide 6.1 via sequential additions of reaction components. a) Online HPLC data for a solution of 6.1 in the presence and absence of catalyst solution. b) Effects of adding LiHMDS and 6.2 to a solution of 6.1 and catalyst.

Next, a solution of LiHMDS in THF was added to the reaction solution containing 6.1 and the catalyst. The resultant solution was monitored over the course of 100 minutes using online
HPLC (Figure 6.8b). The constant decrease in [6.1] over time after LiHMDS was introduced, combined with the appearance of new growing signals on the LC trace, evidenced that 6.1 and LiHMDS can react in the presence of the catalyst. We attribute this reactivity to C-N coupling with LiHMDS as previously reported.\textsuperscript{240,241}

After monitoring the heated solution containing LiHMDS, 6.1, and catalyst for 120 minutes, arylamine 6.2 was introduced into the reaction to compare the rates of C-N coupling of LiHMDS and 6.2 with 6.1. Gratifyingly, we observed that adding 6.2 arrests the C-N coupling with LiHMDS. The rate of consumption of 6.1 increased via turning on formation of Spiro-OMeTAD (6.3). After 170 minutes, 6.2 had been exhausted therefore resulting in a plateau in the formation of 6.3. We continued to observe consumption of 6.1 presumably via continued C-N coupling with LiHMDS. Arylamine 6.2 was added to the reaction a second time after 230 minutes, which corresponded to an increase in the rate of consumption of 6.1 and concomitant formation of 6.3. The data in Figure 6.8b clearly demonstrates that the combination of 6.1, LiHMDS, and catalyst is capable of consuming 6.1. Fortunately, inclusion of 6.2 modifies the chemoselectivity by generating 6.3. Understanding this reactivity paradigm is critical for finding the optimal rate of dosing to maximize yield of Spiro-OMeTAD and the reaction rate.

To validate the hypothesis that we can increase the reaction rate by limiting [6.2] via dosing, a stock solution of 6.2 in THF was introduced slowly into a flask containing 6.1, LiHMDS, and catalyst via linear dosing using a syringe pump. By dosing the arylamine into the reaction flask, we minimize the [6.2], wherein the ideal scenario the reaction rate is equal to the rate of dosing of 6.2. This dosing procedure is complicated by the unproductive coupling that occurs between LiHMDS and the aryl bromide in the absence of 6.2 (Figure 6.8b). Fortunately, this unproductive coupling pathway is not observed when arylamine 6.2 is present. If the arylamine is introduced
into the flask too slowly, undesired coupling between 6.1 and LiHMDS will predominate. On the other hand, introducing 6.2 into the flask too quickly will effectively sequester the catalyst decreasing the reaction rate. A delicate balance therefore exists between reaction rate and chemoselectivity that is dependent on the rate of dosing of the arylamine.

Preparing a 1.5 M solution of arylamine 6.2 in THF and dosing 1.0 mL of the solution at a rate of 0.02 mL/min afforded the desired rate acceleration, while also avoiding coupling between 6.1 and LiHMDS (Figure 6.9). Introducing 6.2 via dosing resulted in a >7 fold increase of the initial reaction rate from 4.0 mM/h to 28.5 mM/h for the non-dosing and dosing experiments, respectively.

![Graph](image)

**Figure 6.9.** HPLC reaction progress when 6.2 was introduced stoichiometrically or via linear dosing using a syringe pump. Reaction conditions for both experiments: [6.1]₀ = 40 mM; [LiHMDS]₀ = 200 mM; [Pd(OAc)₂] = 2 mM, [P(t-Bu)₃] = 4 mM in THF/toluene (9:1) at 60 °C; 6.2 not dosed: [6.2]₀ = 180 mM; 6.2 dosed: 6.2 in THF (1.0 mL, 1.5 M) was dosed into the reaction linearly at 0.02 mL / min.

It was observed that the rate enhancement achieved did not equal the theoretical rate maximum (the rate of dosing of 6.2). Introducing the amine at a slower rate could result in an even
higher rate of reaction, although the likelihood of coupling between LiHMDS and 6.1 also increases. Given the choice, we prefer an increased yield of Spiro-OMeTAD over an increased reaction rate. We believe the conditions disclosed provide an optimal balance of both. Therefore, we have demonstrated that introducing 6.2 into the reaction via dosing is an effective method to increase the reaction rate, capable of significantly reducing the necessary reaction times for complete conversion to 6.3. This dosing experiment further validates the proposal that the order of 6.2 is negative, and could be attributed to offcycle binding of 6.2 with the catalyst.

6.7 Proposing a Mechanism

By using the product distributions for the synthesis of Spiro-OMeTAD and intermediate 6.5, as well as the data acquired when probing the catalyst resting state, we propose a reaction mechanism that recapitulates the observed kinetic behavior (Scheme 6.6). First, activation of Pd(OAc)$_2$ by P(tBu)$_3$ generates ligand bound Pd(0), PdL which enters the catalytic cycle. An off-cycle reservoir of catalyst exists via competitive binding of arylamine 6.2 and PdL forming coordination complex 6.13. This off-cycle equilibrium manifests as an observed order of 6.2 to be approximately -2.

The oxidative addition between PdL with aryl bromide 6.1 forms intermediate 6.8 and represents the turnover limiting step of the catalytic cycle, as an order of +1 was observed for both the catalyst and 6.1. Coordination of 6.2 with the oxidative addition complex 6.8 followed be deprotonation, loss of LiBr, and reductive elimination forms the C-N bond of 6.9. We propose that after the reductive elimination the catalyst remains bound to the nitrogen of the triarylated amine forming coordination complex 6.9. The catalyst can then bind to the π-system of the bisfluorene generating intermediate 6.10 via an inner-sphere type process.
Scheme 6.8. Proposed reaction mechanism for the formation of 6.5 / Spiro-OMeTAD accounting for all observed kinetic behaviour. R = p-methoxyphenyl.

Migration of the catalyst across the π-system via a catalyst-transfer mechanism results in intramolecular oxidative addition at the aryl bromide position on the same fluorene molecule. It is important to distinguish that this catalyst transfer is only possible via migration through a planar
\pi\text{-}system, which rationalizes the observed chemoselectivity to form only a single reaction intermediate (6.5). After intramolecular oxidative addition, the subsequent steps of the catalytic cycle proceeds as described previously. Upon formation of the second C-N bond after reductive elimination, the Pd can not oxidatively add intramolecularly as the second bis fluorene has an orthogonal geometry. Therefore, the Pd dissociates completely, ending the catalytic cycle. Oxidative addition at either of the remaining two aryl bromide positions repeats the cycle until Spiro-OMeTAD is formed.

6.8 Conclusions

We report novel reaction conditions capable of synthesizing the champion hole transport material Spiro-OMeTAD in excess of 90% isolated yield. This synthesis is especially useful, as the Spiro-OMeTAD can be isolated on >1g scale without using column chromatography. By leveraging our automated reaction monitoring platform, online-HPLC progress curves of the optimized synthesis were generated from a reaction conducted from within a glovebox. We observed unanticipated chemoselectivity via formation of a single intermediate throughout the reactions progress. We attribute this selectivity to a catalyst transfer type mechanism. In this proposal, the Pd ring-walks across the \pi\text{-}system after the first reductive elimination, before performing an intramolecular oxidative addition. Additional kinetic studies using the automated reaction monitoring platform elucidated the orders of catalyst, aryl bromide, and amine to be approximately 1, 1, and -2, respectively. Therefore, we propose that the oxidative addition represents the turnover limiting step of the catalytic cycle. We attribute the negative order of the aryl amine substrate to off-cycle binding of the catalyst lowering its activity. By introducing the amine into the reaction via dosing, a >7 fold rate increase was observed. We hope these studies
will allow for easier access to larger quantities of Spiro-OMeTAD for materials chemists without having to rely on commercial sources. Studies to exploit the observed chemoselectivity to allow for modular synthetic analogs of Spiro-OMeTAD with different coupling partners are currently being explored in the Hein lab.

6.9 Experimental

6.9.1 General Remarks

6.9.1.1 Reagents

Bis(4-methoxyphenyl)amine was purchased from AKScientific and used as received. Palladium (II) acetate, tri-tert-butylphosphine, 2,2',7,7'-tetrabromo-9,9'-spirobifluorene, and lithium hexamethyldisilazide (LiHMDS) were purchased from Sigma Aldrich and were used as received. Optima-grade UHPLC solvents were purchased from Fisher and used as received. All other reagents and solvents were purchased from conventional suppliers and used as received unless otherwise stated. Silica gel was purchased from Silicycle (60 Å, 230 x 400 mesh).

6.9.1.2 Analytical Methods

NMR spectra were recorded on a Bruker AV-400 for \( ^1\text{H} \), and \( ^{13}\text{C} \) and were referenced to the residual solvent peak.\(^{167}\) The abbreviations s, d, q, m signify singlet, doublet, quartet, and multiplet, respectively. NMR spectra were analyzed by using the software MNova.

The Liquid Chromatography (LC) samples were analyzed by HPLC/MS conducted on an Agilent 1200 HPLC with the following configuration:

Agilent G1379B degasser, G1312A binary pump, G1316A thermal column compartment, diode array detector and a 6120 single quad mass spectrometer.
Analytical setting for the detectors are:

DAD – 200 – 400 nm collected at 20 Hz storing all spectra for offline analysis. Peak area for quantification varies depending on the experiment, see calibration curves for details.

ESI-MSD – positive mode scan for m/Z 110 – 1500 running at 0.8sec/cycle. drying gas = 7.0 l/min, nebulizer pressure = 20 psi, gas temperature = 300 °C, capillary voltage = 4000 V.

HPLC column and mobile phase method used the follow conditions:
Poroshell C18, 2.1 x 50 mm, 2.7-Micron Column; Temperature = 25 °C;
Solvent A = Water, 0.1 % Formic Acid; Solvent B = acetonitrile; Flow Rate = 0.625 mL/min;
Starting Conditions = 60 % A, 40 % B; 0.00 – 2.35 min isocratic; 2.35 – 5.00 min gradient to 0% A, 100% B.

6.9.1.3 Default Sampling Method

Temporal HPLC data was obtained using a modified reaction monitoring platform similar to which has been reported in our group previously. All sampling events were executed by an Arduino microcontroller which was controlled via a Python script. The sampling sequence begins by actuating the Easysampler to extend the sampling pocket into the reaction mixture. After 5 seconds the pocket is retracted and THF (standard volume = 700 µL) is delivered (default flow rate = 1.0 mL/min) through the Easysampler, out of the glovebox, and onto the injection valve for analysis by HPLC. The injection valve is then triggered aligning the reaction aliquot with the HPLC pump and column allowing for online analysis. The sampling lines are then flushed with THF (2.0 mL, 1.0 mL/min) before reinitiating the sampling sequence.
6.9.2 Synthetic Procedures

\[
\text{N}_2\text{N}_2\text{N}_2',\text{N}_2',\text{N}_7\text{N}_7,\text{N}_7',\text{N}_7'\text{octakis} (4\text{methoxyphenyl})-9,9'\text{-spirobi[fluorene]-}2,2',7,7'\text{-tetraamine} \text{ (Spiro-OMeTAD, 6.3)}
\]

To a vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (1.03 g, 4.50 mmol), LiHMDS (837 mg, 5.00 mmol), 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (632 mg, 1.00 mmol), THF (18.0 mL), and toluene (1.6 mL) and the resultant solution was heated to 60 °C. Palladium(II) acetate (11 mg, 50 µmol) and tri-tert-butylphosphine (20 mg, 100 µmol) in toluene (400 µL) were added via syringe to initiate the reaction. After 30 hours, the reaction was cooled to room temperature and quenched with saturated aqueous ammonium chloride. The crude was extracted with ethyl acetate (20 mL, 3x). The organic extracts were washed with brine, dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and concentrated under reduced pressure. Methanol (150 mL) was added to the crude residue to precipitate out the product. The resulting heterogeneous solution was triturated via sonicator. The mixture was then vacuum filtered and the cake was washed with methanol followed by petroleum ether, then dried under vacuum to afford the Spiro-OMeTAD (1.12 g, 0.91 mmol, 91%) as an off-white solid. Characterization data is consistent with that in the literature.\textsuperscript{234}

\textbf{\textsuperscript{1}H NMR}: (400 MHz, DMSO) \(\delta\) 7.47 (d, \(J = 8.3\) Hz, 4H), 6.88 – 6.77 (m, 32H), 6.69 (dd, \(J = 8.3, 1.9\) Hz, 4H), 6.18 (d, \(J = 1.9\) Hz, 4H), 3.71 (s, 24H)

\textbf{\textsuperscript{13}C\textsuperscript{\textsuperscript{1}H} NMR}: (101 MHz, DMSO) \(\delta\) 155.3, 149.4, 147.2, 140.4, 134.0, 125.6, 120.9, 115.7, 114.7, 55.2, 40.2, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9.
**MS ESI+:** (m/z calc. for C_{81}H_{86}N_{4}O_{8} [M + H] = 1225.4); found = 1225.4

\[ \text{2',7'-dibromo-N2,N2,N7,N7-tetrakis(4-methoxyphenyl)-9,9'-spirobi[fluorene]-2,7-diamine (6.5)} \]

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (1.03 g, 4.50 mmol), LiHMDS (502 mg, 3.00 mmol), 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (632 mg, 1.00 mmol), THF (18.0 mL) and toluene (1.6 mL) and the resulting solution was heated to 60 °C. Palladium(II) acetate (17 mg, 75.0 µmol) and tri-tert-butylphosphine (30 mg, 150 µmol) in toluene (400 µL) were added via syringe to initiate the reaction. After 30 hours the reaction was cooled to room temperature and quenched with saturated aqueous ammonium chloride. The crude was extracted with ethyl acetate (20 mL, 3x). The organic extracts were washed with brine, dried (Na_{2}SO_{4}), filtered, and concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether/ethyl acetate; 5.7:1) to afford 6.5 (120 mg, 0.13 mmol, 13 % yield) as an off-white solid. The purified product was then recrystallized from toluene using methanol an antisolvent to afford crystals of suitable quality for X-ray diffraction.

**\(^1\)H NMR:** (400 MHz, C_{6}D_{6}) \( \delta \) 7.50 (d, \( J = 8.4 \) Hz, 2H), 7.28 (d, \( J = 1.6 \) Hz, 2H), 7.14 – 7.08 (m, 4H), 6.92 – 6.88 (m, 8H), 6.75 (d, \( J = 2.0 \) Hz, 2H), 6.72 (d, \( J = 8.1 \) Hz, 2H), 6.60 – 6.56 (m, 8H), 3.20 (s, 12H)

**\(^{13}\)C\(^{1}\)H NMR:** (101 MHz, C_{6}D_{6}) \( \delta \) 156.3, 151.6, 148.9, 148.8, 141.3, 139.7, 135.3, 131.2, 127.3, 126.5, 122.2, 122.1, 122.0, 120.5, 117.3, 115.0, 66.3, 54.9.
6.9.3 COPASI Modeling

The following model was created in COPASI to approximate kinetic behaviour before experimentation.
6.9.4 Reaction Monitoring Data

6.9.4.1 Spiro-OMeTAD Synthesis

![Chemical structures](image)

**General Method**

To a 4 dram vial under an inert atmosphere was added bis(4-methoxyphenyl)amine (413 mg, 1.80 mmol), LiHMDS (335 mg, 2.00 mmol), 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (253 mg, 0.40 mmol) THF (9.0 mL) and toluene (800 µL). The stirred solution was heated at 60 °C. Palladium(II) acetate (4.5 mg, 20 µmol) and tri-tert-butylphosphine (8.1 mg, 40.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate the reaction and a sampling sequence was then begun. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) to the injection valve. Samples were analyzed using the general HPLC method. HPLC progress curves are depicted in Figure 6.1.
Figure 6.10. Sample HPLC chromatogram during a monitored reaction to synthesize Spiro-OMeTAD (6.3) from 6.1 and 6.2

Probing the reactivity of aryl bromide 6.1

\[
\begin{align*}
\text{Br} & \quad \text{Br} & \quad \text{Pd(OAc)}_2 \ / \ P(t-\text{Bu})_3 & \quad \text{THF/Toluene} & \quad 60 \degree \text{C} & \quad \text{LiHMDS} & \quad \text{HMDS} & \quad \text{Coupling} & \quad \text{6.3, Spiro-OMeTAD} \\
\text{Br} & \quad \text{Br} & \quad \text{R} = p-\text{methoxyphenyl} & \quad \text{No Rxn} & \quad \text{R} & \quad \text{NH}_2 & \quad \text{R} & \quad \text{6.2} \\
6.1 & \quad 1 \text{ equiv} & \quad & \quad & \quad & \quad & \quad & \quad & \quad
\end{align*}
\]

To a 4 dram vial under an inert atmosphere was added 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (320 mg, 0.50 mmol) THF (8.0 mL) and toluene (750 µL). The stirred solution was heated at 60 °C. A sampling sequence of three samples was then begun using a diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) to the injection valve. Palladium(II) acetate (4.5 mg, 20 µmol) and tri-tert-butylphosphine (6.5 mg, 32 µmol) dissolved in toluene (250 µL) was injected via syringe and a second sampling sequence consisting of eight samples was initiated. Next LiHMDS (504 mg, 3.00 mmol) dissolved in THF (1.4 mL) was then added to the reaction and another sampling sequence consisting of ten samples was initiated. After 120 minutes after the LiHMDS was added bis(4-methoxyphenyl)amine (116 mg, 0.50 mmol) in THF (300 µL) was added to the reaction and another sampling sequence consisting of 15 samples
was initiated. Bis(4-methoxyphenyl)amine (116 mg, 0.50 mmol) in THF (300 µL) was added to the reaction was added to the reaction a second time 240 minutes after the LiHMDS had been added.

**Dosing Reaction**

To a 4 dram vial under an inert atmosphere was added LiHMDS (335 mg, 2.00 mmol), 2,2',7,7'-tetrabromo-9,9'-spirobi[fluorene] (253 mg, 0.40 mmol) THF (9.0 mL) and toluene (800 µL). The stirred solution was heated at 60 °C. Bis(4-methoxyphenyl)amine (344 mg, 1.50 mmol) in THF (1.0 mL) was linearly dosed into the flask over 50 minutes at a rate of 0.02 mL / min. Once dosing was initiated, palladium(II) acetate (4.5 mg, 20 µmol) and tri-tert-butylphosphine (8.1 mg, 40.0 µmol) dissolved in toluene (200 µL) was injected via syringe to initiate the reaction and a sampling sequence was then begun. A diluent volume of 700 µL was used to deliver the reaction aliquot (1.0 mL / min) to the injection valve. Samples were analyzed using the general HPLC method.
6.9.5 Experiments to Solve the Rate Law

6.9.5.1 Solving Catalyst Order

Two experiments were completed following the general reaction monitoring protocol (Section 6.7.3.1) but modifying the concentration of catalyst (Table 6.1) to allow for elucidation of catalyst order by a graphical analysis method.62

Table 6.1. Initial concentrations of substrates and reagents to allow for identification of the catalyst order

<table>
<thead>
<tr>
<th>Trial</th>
<th>[6.1] (mM)</th>
<th>[6.2] (mM)</th>
<th>[LiHMDS] (mM)</th>
<th>[Pd(OAc)₂] (mM)</th>
<th>[P(t-Bu)₃] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>180</td>
<td>200</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>180</td>
<td>200</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
6.9.5.2 Solving Substrate Orders

Following the different excess experiment protocol from RPKA\textsuperscript{59} the following three experiments were performed following the general reaction monitoring procedure (Section 6.7.3.1.). VTNA was then applied to approximate substrate orders based on graphical overlay.\textsuperscript{61}

Table 6.2. Initial concentrations of substrates and reagents to allow for identification of orders of 6.1 and 6.2

<table>
<thead>
<tr>
<th>Trial</th>
<th>[6.1]\textsubscript{0} (mM)</th>
<th>[6.2]\textsubscript{0} (mM)</th>
<th>[LiHMDS] (mM)</th>
<th>[Pd(OAc)\textsubscript{2}] (mM)</th>
<th>[P(t-Bu)\textsubscript{3}] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>180</td>
<td>200</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>180</td>
<td>200</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>120</td>
<td>200</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 6.12. a) HPLC reaction time-course data for the synthesis of Spiro-OMeTAD with varying initial concentration of catalyst described in Table 6.1. (b) VTNA plot for figure 6.12a indicating that the system is first order in [Pd].
Figure 6.13. HPLC Reaction time-course data for the synthesis of Spiro-OMetAD with varying initial concentrations of substrates as per Table 6.2. a) Variable time normalization analysis plot to solve for order of 6.1 being approximately 1 using data from Table 6.2. b) Variable time normalization analysis plot to solve for order of 6.2 being approximately -2 using data from Table 6.2.

6.9.6 Calibration Curves

A stock solution (5.00 mL) of each compound being calibrated (6.1 – 6.3) was created by dissolving a known mass of material in THF/toluene (9:1). This stock solution was then sampled three times using the general sampling method. Peak areas were normalized by toluene (internal standard) according to Equation 6.2.

\[
\text{Normalized Peak Area for } X = \frac{\text{Peak Area (X)}_t}{\text{Peak Area (Toluene)}_t}
\]  
(eq. 6.2)

The stock solution (2.5 mL) was then diluted into THF/toluene (9:1, 2.5 mL) and the resultant diluted stock solution was sampled and quantified again three times. This diluting and sampling protocol was repeated an additional two times to collect a total of four data points in triplicate.
HPLC calibration curve data was used to calculate the [6.5] based on the following assumption described in equation 6.3:

\[
[6.5]_t = [6.1]_0 - [6.1]_t - [6.3]_t
\]

Equation 6.3

Figure 6.14. HPLC Calibration curves to allow for quantification of 6.1, 6.2, and 6.3 directly from normalized peak area. Integration of all UV peaks completed at a wavelength of 254 nm.
6.9.7 X Ray Data of 6.5

Figure 6.15. Labelled crystal structure of 6.5 obtained by single crystal x-ray analysis.

A colourless blade-shaped crystal with dimensions 0.35 × 0.17 × 0.05 mm\(^3\) was mounted on a mylar loop in oil. Data were collected using a Bruker APEX II area detector diffractometer equipped with a Kryoflex low-temperature device operating at \(T = 100(2)\) K.

Data were measured using \(f\) and \(w\) scans of 0.5° per frame for 10 s using MoK\(_\alpha\) radiation (microfocus sealed X-ray tube, 50 kV, 0.99 mA). The total number of runs and images was based on the strategy calculation from the program APEX3. The maximum resolution that was achieved was \(Q = 30.427°\) (0.70 Å).

The unit cell was refined using SAINT (Bruker, V8.40A, after 2013) on 9731 reflections, 16% of the observed reflections. Data reduction, scaling and absorption corrections were performed using SAINT (Bruker, V8.40A, after 2013). The final completeness is 100.00% out to 30.427° in \(Q\).

A multi-scan absorption correction was performed using SADABS-2016/2 (Bruker, 2016/2) was used for absorption correction. \(wR_2\) (int) was 0.1056 before and 0.0460 after correction. The ratio of minimum to maximum transmission is 0.7767. The \(l/2\) correction factor is
not present. The absorption coefficient $m$ of this material is $1.777 \text{ mm}^{-1}$ at this wavelength ($l = 0.71073\text{Å}$) and the minimum and maximum transmissions are 0.711 and 0.915.

The structure was solved and the space group $P2/c$ (# 13) determined by the XT (Sheldrick, 2015) structure solution program using Intrinsic Phasing methods and refined by full matrix least squares minimisation on $F^2$ using version 2018/3 of XL (Sheldrick, 2015). The material crystallizes with both toluene and MeOH solvent in the lattice. There is one half-molecule of toluene, disordered about a two-fold rotational axis in the unit cell. The MeOH is disorder in two orientations and only partially occupied. Occupancies for each fragment were refined individually. All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model.

### Table 6.3. Crystallographic parameters for 70

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>$C_{56.98}H_{45.93}Br_2N_2O_{4.48}$</td>
</tr>
<tr>
<td>$D_{calc}$ / g cm$^{-3}$</td>
<td>1.401</td>
</tr>
<tr>
<td>$m$/mm$^{-1}$</td>
<td>1.777</td>
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<tr>
<td>Formula Weight</td>
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<tr>
<td>Colour</td>
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</tr>
<tr>
<td>Shape</td>
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</tr>
<tr>
<td>Size/mm$^3$</td>
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</tr>
<tr>
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<td>$c$/Å</td>
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<tr>
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<tr>
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<td>Parameter</td>
<td>Value</td>
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<td>--------------------</td>
<td>--------</td>
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<td>Refl's with I &gt; $</td>
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<td>$wR_2$ (all data)</td>
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<td>$R_1$ (all data)</td>
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<tr>
<td>$R_1$</td>
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</tr>
</tbody>
</table>
Chapter 7: Conclusions and Outlook

This Thesis has provided several examples of reaction case studies that have resulted in increased mechanistic understanding by leveraging automated reaction monitoring technologies. Mechanistic information gained throughout these studies has increased the utility of each reaction by increasing product yield, modifying chemoselectivity, or reducing reaction times.

Central to each mechanistic case study was the collection of data-dense temporal profiles of starting materials, intermediates, byproducts, and products throughout the reaction’s entirety. To collect these reaction profiles, various reaction monitoring techniques were utilized such as in situ spectroscopic methods and novel, automated sampling platforms. These automated devices are especially powerful, as by leveraging HPLC as an analytical tool, increased resolution of species of interest in complex mixtures can be obtained not possible with spectroscopic methods. Additionally, these automated sampling platforms are highly modular and can be tailored to reactions that are both homogeneous and heterogeneous, those performed at elevated temperatures, and even reactions performed under an inert atmosphere.

Chapter 2 explored the development and application of an automated reaction monitoring platform which utilizes online HPLC to generate data dense reaction profiles of reactions with minimal analyst intervention. Characteristic features of the sampling device were presented regarding aliquot mixing, dilution and delivery. High levels of sampling reproducibility were achieved demonstrating that the platform can generate accurate temporal profiles of reactions. The platform was employed to profile a copper catalyzed azide-alkyne click reaction of exceptional reactivity, where traditional quench methods are inefficient to accurately to capture the reactions progress. The platform was also successfully utilized to monitor a heterogeneous Suzuki-Miyaura cross-coupling reaction via interfacing with a slurry sampling probe. Last, a complex,
multicomponent cascade reaction with multiple equilibria was monitored. The resolving power of HPLC was demonstrated in this scenario to quantify each major species at each time point, a difficult task to accomplish using traditional in situ spectroscopic techniques. This technology has played a pivotal role in elucidating reaction mechanisms of complex chemical reactions via the generation of robust, time-course data of chemical species with minimal analyst effort. We hope to see these technologies adopted by other researchers to facilitate mechanistic understanding of catalytic transformation.

Chapter 3 discussed an in-depth mechanistic investigation of the Kinugasa reaction used to synthesize beta-lactams. The reaction monitoring platform presented in Chapter 2 was integral throughout these mechanistic investigations. By creating temporal profiles of byproducts observed in the reaction, we proposed a novel mechanism invoking a retro (3+2) cycloaddition which generates an electrophilic ketene intermediate. This cycloreversion pathway accounts for each of the byproducts previously documented with the Kinugasa reaction. By modifying the basic additive combined yields of the beta-lactams were increased from ~30% to ~80%.

Reaction progress kinetic analysis (RPKA) was used to probe the overall robustness of the Kinugasa reaction. Same excess experiments demonstrated that neither catalyst deactivation nor product inhibition were observed. Variable time normalization analysis (VTNA) allowed the orders of [nitrone] and [catalyst] to be deduced as 1 and -1, respectively. An order of the copper catalyst was determined to be equal to 2. The kinetic data reveal that the (3+2) cycloaddition between the nitrone and doubly activated bis-Cu(I) acetylide represents the turnover limiting step. This conclusion regarding the turnover limiting step was supported additionally via kinetic isotope effects (KIE) studies where a secondary inverse KIE of 0.95 was observed. These mechanistic studies should allow for increased utility of the Kinugasa reaction by allowing researchers to
maximize product yield by understanding the mechanistic pathways accounting for byproduct formation. Additionally, our novel reaction mechanism has interesting applications regarding enantioselective variants of the Kinugasa reaction as the stereocenters are set at a later stage of the catalytic cycle than proposed in previous mechanistic studies.\textsuperscript{117} Current studies regarding impacts of the proposed mechanism on enantioselective variants of the Kinugasa reaction are underway in the Hein lab. We believe that mechanistic information gained on the Kinugasa reaction reported in this chapter will result in more widespread usage to synthesize biologically relevant beta lactams.

Chapter 4 discussed a telescoped procedure for the synthesis of cyanoimidazoles from carbonyl containing substrates via a condensation annulation proceeded by a functional group conversion. The combination of in situ IR, temporal pH measurements, static tube NMR, and offline HPLC analysis were used to create orthogonal reaction profiles to aid in these mechanistic investigations.

Time-course \textsuperscript{19}F NMR spectroscopy experiments demonstrated the complex equilibria that the electrophilic 3,3-dibromo-1,1,1-trifluoropropan-2-one starting material can participate in in the presence of aqueous ammonia ultimately resulting in dimerization. This undesired reactivity pathway competes with productive imidazole formation. Fortunately, inclusion of the aryl aldehyde necessary for productive imidazole formation decreases the rate of dimerization, although it is not entirely suppressed.

Dosing experiments indicated that this dimerization pathway will occur even when the concentration of the electrophilic ketone reagent is limited. Also, manipulating temperature while dosing is also insufficient to mitigate this undesired reactivity pathway. DoE experiments
performed at BMS elucidated that achieving high in situ yields (≥80%) of the desired imidazole is possible when superstoichiometric amounts of the dibromo ketone are included.

Offline HPLC analysis was employed to investigate the chemoselectivity of the elimination cascade to convert the trifluoromethyl functional group to a nitrile. Using temporal pH measurements, a temperature dependence was observed where temperatures exceeding 60 °C were conducive to the elimination under a basic environment. Undesired capture of the electrophilic fulvene intermediate generated after the E1cb step was significant when methanol was selected as the reaction solvent. Switching the solvent to isopropanol greatly improved the chemoselectivity favouring cyanoimidazole formation although byproducts were still observed. By introducing the basic component into the reaction via spiking, the chemoselectivity was further improved.

By leveraging mechanistic information gained from the reaction monitoring experiments, we reported conditions to maximize the yield of the cyanoimidazole via a telescoped process. Yields in excess of 85% of the condensation annulation reaction to form the trifluoromethyl imidazole were achieved. Once conversion had ceased, the reaction temperature was increased to 60 °C to promote elimination of the imidazolate. Hydroxide was introduced into the reaction manually via spiking to enhance chemoselectivity favouring the formation of the cyanoimidazole. This telescoped procedure resulted in an overall yield of cyanoimidazole of 85% and has been reproduced on multigram scale. These mechanistic investigations will allow for a broader range of substituted imidazoles to be prepared via condensation methods. Additionally, cyanoimidazoles can be prepared at scale by understanding the effects of pH and temperature on the functional group conversion. This telescoped procedure should allow for easier access to pharmaceutically relevant imidazole motifs at scale.
Chapter 5 discussed the modifications of the reaction monitoring platform discussed in Chapter 2 to allow for automated sampling and online HPLC analysis for reactions performed from within a glovebox. The system utilizes an Easysampler slurry probe to collect sample aliquots where they are delivered onto a HPLC on the benchtop for online analysis. Aliquoting, sample mixing, delivery, and analysis was demonstrated to be extremely reproducible and has allowed for the slurry probe pocket volume to be calibrated by sampling a vial of toluene 50 times. A similar trend relating diluent volume and detector response was observed as observed in Chapter 2.

The reaction monitoring platform was employed to generate temporal profiles for a series of Buchwald-Hartwig amination reactions performed from within the glovebox. Prior to data collection COPASI models were generated for each reaction. First parallel reactions were completed using iodobenzene and bromobenzene as the aryl halide coupling partners. Both reactions displayed a non-first order profile and had similar initial rates. The reaction profiles differed significantly after ~30% conversion indicating that coupling of each aryl halide proceeds via a unique mechanism. COPASI models generated via assuming oxidative addition is turnover limiting did not accurately match the observed trends.

A competition reaction employing both iodobenzene and bromobenzene displayed unanticipated selectivity and did not match the COPASI models. Catalyst monopoly was observed at the early stages of the reaction when the concentrations of iodobenzene and bromobenzene were comparable. A second regime began upon exhaustion of the iodobenzene resulting in a sigmoidal consumption profile for the bromobenzene substrate. The observed monopoly was rationalized either via differences in energy of the oxidative addition, or via reactivity of downstream intermediates.
Finally, we monitored the cascade coupling reaction using the dihalogenated substrate 1-bromo-4-iodobenzene. Again, we observed unanticipated selectivity from the competition and parallel reaction studies as well as the COPASI modeling. The formation of the mono- and dicoupled products both followed zero order profiles, and the dicoupled product was observed at the first data point. The observed profiles could not be recreated from COPASI models using a linear reaction network of two sequential catalytic cycles. Instead, we rationalize the observed selectivity to a catalyst-transfer type (CTP) mechanism where after the first reductive elimination, the Pd can migrate across the pi system and undergo an intramolecular oxidative addition at the C-Br position. To the best of our knowledge this is the first example of a CTP type mechanism that couples heteroatoms.

The development of this automated reaction monitoring platform represents a significant breakthrough in automated methods to study air sensitive reactions without compromising the inert environment. This device will play a crucial role for delineating mechanisms of reactions that mandate an inert atmosphere. Temporal data of air sensitive reactions can be used to optimize the transformations, or to facilitate reaction discovery.

In Chapter 6, an optimized method for the synthesis of the champion hole transport material Spiro-OMeTAD was disclosed. This method has resulted in Spiro-OMeTAD to be synthesized on gram scale and isolated without column chromatography in >90% yield. Kinetic studies using the automated reaction monitoring platform discussed in Chapter 5 allowed for the proposal of a reaction mechanism accounting for the observed kinetic behavior.

Throughout our mechanistic studies we observed unexpected selectivity regarding intermediate formation. Out of the four potential intermediates through the cascade to form Spiro-OMeTAD only one was observed. By isolating the intermediate and using X-ray diffractometry
we confirmed its identity as the dicoupled regioisomer where both $C-N$ forming events occurred on the same fluorene moiety. We attribute this selectivity to an intramolecular catalyst transfer type mechanism similar to what was presented in Chapter 5. A key difference between the systems is the geometry of the spiro-center of the bisfluorene substrate. This tetrahedral center results in both fluorene moieties being orthogonal to each other, preventing the Pd from migrating between fluorenes and explains the observed intermediate selectivity.

By combining kinetic studies with graphical analysis techniques we elucidated the observed rate law. An order of 1 was observed for both the catalyst and the aryl bromide, indicating that oxidative addition between Pd and the aryl bromide likely represents the turnover limiting step. An unexpected order of -2 for the arylamine was observed. We attribute this less than 0 order to off-cycle binding of the amine with the catalyst, thereby lowering the active catalyst concentration. Leveraging this kinetic information, we completed a dosing reaction where we introduced the arylamine into the reaction linearly over time to limit its concentration. A $>7$ fold increase in the reaction rate was observed, further validating the observed negative order in amine and providing a means to accelerate the reaction rate without employing additional catalyst. We hope that these mechanistic studies will allow for wider access of the hole transport material Spiro-OMeTAD for researchers across the globe. Additionally, analogues of Spiro-OMeTAD using multiple aniline coupling partners with varying electronic properties could be synthesized in a straightforward manner by exploiting the catalyst transfer pathway. We envision this could facilitate the generation of hole transport material libraries via telescoped methods, which could allow for the generation of even more efficient perovskite solar cells.

Future work could involve integrating additional orthogonal reaction monitoring techniques with the automated reaction monitoring platforms presented in this Thesis. For
example, combining in situ IR spectroscopy with the platform presented in Chapter 5 would provide an even more detailed picture of air sensitive transformations to increase mechanistic understanding. The reaction monitoring platform presented in Chapter 4 could incorporate flow NMR as an additional technique to collect temporal data from reactions as they progress.

Another area of future work that could be explored is automating the collection, integration, and presentation of reaction time-course data. The data presented throughout this Thesis was largely analyzed and presented by an analyst. Interpreting temporal data is a time consuming task which could be offset by leveraging web-based storage systems and algorithms. Finally, employing artificial intelligence and machine learning to interpret experimental data could expedite the experimental design process by recognizing important reaction parameters without analyst intervention. This data could then be utilized by automated robotic platforms, where experiments could be performed, interpreted, and designed in a closed loop process. This concept of closed loop reaction automation is currently being explored in the Hein lab.\textsuperscript{160,242,243} Combining the autonomy of self-driving labs with the reaction monitoring platforms described throughout this Thesis would increase mechanistic understanding even more efficiently.
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Appendix A: NMR Spectra for Chapter 2

A.1 Benzyl Azide (2.2)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
A.2 1-Benzyl-4-phenyl-1H-1,2,3-triazole (2.3)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
A.3 TCPTA

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
A.4 (E)-1,2-Dichlorovinyl phenyl ketone (2.4)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C{$^1$H} NMR (126 MHz, CDCl$_3$)
A.5 (E)-2-chloro-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (2.6)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C{$^1$H} NMR (126 MHz, CDCl$_3$)
A.6 (Z)-2-chloro-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (2.7)

$^{1}H$ NMR (500 MHz, DMSO-$d_6$)

$^{13}C\{^1H\}$ NMR (126 MHz, DMSO-$d_6$)
A.7 1-(furan-2-yl)ethanol (2.8)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$)
A.8 N-(1-(furan-2-yl)ethyl)aniline (2.10a)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C\{$^1$H\} NMR (101 MHz, CDCl$_3$)
A.9 N-(1-(furan-2-yl)ethyl)-4-nitroaniline (2.10b)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
Appendix B: NMR Spectra for Chapter 3

B.1 N-benzylaniline (3.27)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.2 (Z)-N,1-diphenylmethanimine oxide (3.2)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.3 (3S,4S)-1,3,4-triphenylazetidin-2-one (3.3a) and (3R,4S)-1,3,4-triphenylazetidin-2-one (3.3b)

$^1$H NMR (400 MHz, CDCl$_3$)

B.4 (3R,4S)-1,3,4-triphenylazetidin-2-one (3.3b)

$^1$H NMR (400 MHz, CDCl$_3$)
B.5 (E)-N,1-diphenylmethanimine (3.4)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.6 N,N-diisopropyl-2-phenylacetamide (3.16)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.7 (Z)-N,1,3-triphenylprop-2-yn-1-imine (3.6)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.8 Deuteriobenzaldehyde (3.29)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
B.9 (Z)-N,1-diphenylmethanimine oxide-d (3.24)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$)
Appendix C: NMR Spectra for Chapter 4

C.1 2-(p-tolyl)-4-(trifluoromethyl)-1H-imidazole (4.3)

$^1$H NMR (400 MHz, MeOD)

$^{13}$C($^1$H) NMR (400 MHz, MeOD)
$^{19}$F NMR (400 MHz, MeOD)
C.2 2-(p-tolyl)-1H-imidazole-4-carbonitrile (4.4)

$^1$H NMR (400 MHz, MeOD)

$^{13}$C($^1$H) NMR (101 MHz, MeOD)
C.3 3,3-dibromo-1,1,1-trifluoropropane-2,2-diol (4.7):

$^1$H NMR (400 MHz, D$_2$O)

$^{13}$C($^1$H) NMR (101 MHz, D$_2$O)
$^{19}$F NMR (400 MHz, D$_2$O)
C.4 4-(trifluoromethyl)-1H-imidazole (4.11)

$^1$H NMR (400 MHz, MeOD)

$^{13}$C($^1$H) NMR (101 MHz, MeOD)
$^{19}$F NMR (377 MHz, MeOD)
Appendix D: NMR Spectra for Chapter 5

D.1 $^1$H-NMR 4-methoxy-$N$-(4-methoxyphenyl)-$N$-phenylaniline (5.10)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C($^1$H) NMR (101MHz, CDCl$_3$)
D.2 4-bromo-N,N-bis(4-methoxyphenyl)aniline (5.12)

$^1$H NMR (400 MHz, C$_6$D$_6$)

$^{13}$C$(^1$H) NMR (101 MHz, C$_6$D$_6$)
D.3 N1,N1,N4,N4-tetrakis(4-methoxyphenyl)benzene-1,4-diamine (5.14)

$^1$H NMR (400 MHz, C$_6$D$_6$)

$^{13}$C$\{^1$H$\}$ NMR (101 MHz, C$_6$D$_6$)
Appendix E: NMR Spectra for Chapter 6

E.1 $N^2,N^2',N^7,N^7',N^7',N^7'$octakis (4methoxyphenyl)- $9,9'$-spirobi[fluorene]-2,2',7,7'-tetraamine) (Spiro-OMeTAD, 6.3)

$^1$H NMR (400 MHz, DMSO-d$_6$)

$^{13}$C{$^1$H} NMR (101 MHz, DMSO-d$_6$)
E.2 2',7'-dibromo-$N_2^2,N_7^2,N_7^7$-tetrakis(4-methoxyphenyl)-9,9'$-$spirobi[fluorene]-2,7'-diamine (6.5)

$^1$H NMR (400 MHz, C$_6$D$_6$)

$^{13}$C($^1$H) NMR (101 MHz, C$_6$D$_6$)