SOLVING DIFFICULT PROBLEMS WITH AUTOMATED TECHNOLOGY:
NEW TOOLS TO UNDERSTAND COMPLEX CHEMICAL
AND PHYSICAL PROCESSES

by

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Abstract

The desire for cheaper, faster, energy-conscious, and more sustainable chemical processes often necessitates the design and development of new catalytic methods. Once a catalytic transformation is conceived, the reaction conditions must be optimized to maximize yield and selectivity. Traditional optimization protocols stipulate the correlation of these end-metrics at a fixed time point to variable reaction parameters such as temperature, time, concentration, stoichiometry, etc. By systematically varying these parameters, researchers hope to develop empirical trends relating properties of the chemical species involved to the observed reactivity. What underpins these efforts is an attempt to account for and control the complex, dynamic, and numerous chemical equilibria within a catalytic environment.

However, while idealized catalytic mechanisms can be easily envisioned, the reality is that these processes are often plagued by off-cycle equilibria and decomposition pathways that lead to reduced yield and efficiency. In order to rapidly assess what inhibits productive chemistry, focus must be redirected towards scrutinizing the mechanisms within a catalytic environment. To facilitate this, the acquisition of high-density, trustworthy, and time-resolved reaction progress information for all observable species present within a chemical transformation (starting reagents, intermediates, by-products, products, etc.) by modern in situ reaction monitoring tools offers unmatched opportunities for mechanistic understanding. Ultimately, these time-course profiles provide temporal signatures of dynamic processes active during the chemical transformation that inform process development. This Thesis reports on case studies in which the construction and application of automated technology enabled the solution to difficult problems in complex chemical and physical processes.
Lay Summary

The desire for cheaper, faster, energy-conscious, and more sustainable chemical processes often necessitates the design and development of new catalytic transformations. Catalysis research represents a large dedication of time and resources for both industry and academia and is motivated by the desire to find chemical reagents (catalysts) that can render a difficult chemical transformation more efficient, cost-effective, and cleaner. However, while idealized catalytic mechanisms can easily be envisioned, the reality is that these processes are often plagued by complications leading to reduced yield and efficiency. Consequently, the design, construction, and deployment of modern technology that allows for the capture of data-rich information and will allow for an in-depth understanding of complex chemical and physical phenomena serves as the motivation for this Thesis.
Preface

Parts of Chapter 1 are adapted from a published review article titled “The More, The Better: Simultaneous In Situ Reaction Monitoring Provides Rapid Mechanistic and Kinetic Insight” (Chung, R.; Hein, J. E. *Top. Catal.* 2017, 60, 594-608). My supervisor, Prof. Jason E. Hein, and I conceived the topic of the article. I wrote the manuscript incorporating feedback and suggestions from Prof. Hein.

Chapter 2 is adapted from a published article titled “Copper-Catalyzed Hydrogen/Iodine Exchange in Terminal and 1-Iodoalkynes” (Chung, R.; Vo, A.; Hein, J. E. *ACS Catal.* 2017, 7, 2505-2510). My supervisor, Prof. Jason E. Hein, and I designed the experiments described therein. I performed the experiments with assistance from Mr. Anh Vo. I analyzed and interpreted the data and wrote the manuscript with feedback from Prof. Hein and Mr. Vo.

Chapter 3 is adapted from a published article titled “Catalyst Activation, Chemoselectivity, and Reaction Rate Controlled by the Counterion in the Cu(I)-Catalyzed Cycloaddition between Azide and Terminal or 1-Iodoalkynes” (Chung, R.; Vo, A.; Fokin, V. V.; Hein, J. E. *ACS Catal.* 2018, 8, 7889-7897). My supervisor, Prof. Jason E. Hein, and I designed the experiments described therein. I performed the experiments with assistance from Mr. Anh Vo. I analyzed and interpreted the data and wrote the manuscript with feedback from Prof. Hein, Mr. Vo, and our collaborator, Prof. Valery V. Fokin.

Chapter 4 is adapted from a published article titled “Automated Solubility and Crystallization Analysis of non-UV-Active Compounds: Integration of Evaporative Light Scattering Detection (ELSD) and Robotic Sampling” (Chung, R.; Hein, J. E. *React. Chem. Eng.* 2019, 4, 1674-1681. I designed and built the automated sampling apparatus described in the manuscript. My supervisor, Prof. Jason E. Hein, and I designed the experiments described in the chapter. I performed the
experiments, analyzed and interpreted the data, and wrote the manuscript incorporating feedback from Prof. Hein.

Chapter 5 is unpublished. My supervisor, Prof. Jason E. Hein, and I designed the experiments. I performed the experiments with the assistance of Ms. Vanessa C. Li, Ms. Jessica Li, and Dr. Joshua S. Derasp. I analyzed and interpreted the data and wrote the chapter with feedback from Prof. Hein.

Chapter 6 is unpublished and serves as a conclusion for the Thesis. I wrote the chapter with feedback from Prof. Hein.
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<td>Å</td>
<td>ångström (10^{-10}) m</td>
</tr>
<tr>
<td>AI</td>
<td>artificial intelligence</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>BCA</td>
<td>bicinechoninic acid</td>
</tr>
<tr>
<td>BINAP</td>
<td>2,2'-bis(diphenylphosphino)-1,1'-binaphthyl</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>C</td>
<td>concentration</td>
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<tr>
<td>cm</td>
<td>centimeter</td>
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<tr>
<td>CPC</td>
<td>continuous preferential crystallization</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper(I)-catalyzed Azide-Alkyne Cycloaddition</td>
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<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DAD</td>
<td>diode array detector</td>
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<tr>
<td>DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<tr>
<td>DIPEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>(N,N)-dimethylformamide</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>$E$</td>
<td>eutectic point (isothermal invariant point)</td>
</tr>
<tr>
<td>e.e.</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>e.r.</td>
<td>enantiomeric ratio</td>
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<tr>
<td>ELSD</td>
<td>evaporative light-scattering detector</td>
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<tr>
<td>equiv.</td>
<td>equivalent(s)</td>
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<tr>
<td>ESI-MS</td>
<td>electrospray ionization-mass spectrometry</td>
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<tr>
<td>Et</td>
<td>ethyl</td>
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<tr>
<td>ETFE</td>
<td>ethylene tetrafluoroethylene</td>
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<td>EtOAc</td>
<td>ethyl acetate</td>
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<td>EtOH</td>
<td>ethanol</td>
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<tr>
<td>FBRM</td>
<td>focused-beam reflectance measurement</td>
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<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
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<td>g</td>
<td>gram</td>
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<td>GC</td>
<td>gas chromatography</td>
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<tr>
<td>h</td>
<td>hour</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>Hz</td>
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<tr>
<td>iPr</td>
<td>isopropyl</td>
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<td>iPrOH</td>
<td>isopropyl alcohol</td>
</tr>
<tr>
<td>$J$</td>
<td>coupling constant (NMR spectroscopy)</td>
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<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LiHDMS</td>
<td>lithium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>xxx</td>
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<td>Definition</td>
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<td>M</td>
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<tr>
<td>Me</td>
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<td>acetonitrile</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTBE</td>
<td>methyl tert-butyl ether</td>
</tr>
<tr>
<td>NAD(P)+</td>
<td>nicotinamide adenine dinucleotide phosphate (oxidized form)</td>
</tr>
<tr>
<td>NAD(P)H</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced form)</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>p</td>
<td>pentet</td>
</tr>
<tr>
<td>PAT</td>
<td>process analytical technology</td>
</tr>
<tr>
<td>PEEK</td>
<td>polyether ether ketone</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PTFE</td>
<td>poly(tetrafluoroethylene)</td>
</tr>
<tr>
<td>PVM</td>
<td>particle view measurement</td>
</tr>
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<td>q</td>
<td>quartet</td>
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</table>
R  organic substituent

rac  racemic

R<sub>f</sub>  retention factor

r.t.  room temperature

RPKA  reaction progress kinetic analysis

t  triplet

T  temperature

tBu  <i>tert</i>-butyl

tBuOH  <i>tert</i>-butyl alcohol

TCPTA  <i>tris</i>((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine

TEA  triethylamine

TFA  trifluoroacetic acid

THF  tetrahydrofuran

TLC  thin-layer chromatography

TOF  time-of-flight

µL  microliter

UCB  Universal Control Box

UHPLC  ultra-high-performance liquid chromatography

UV  ultraviolet

v/v  volume per volume

Vis  visible

XRPD  X-ray powder diffraction

xs  “excess”
Acknowledgements

The work in this Thesis would not have been possible without the support of friends, family, and mentors. Words are insufficient to express the heartfelt gratitude and appreciation that I have for those who have helped me grow not only as a student and scientist, but also as a person. The following words are an attempt to acknowledge those who will have made a lifelong impact. For those inadvertently omitted, know that you are recognized well beyond what is written here.

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A special thanks is deserved to Dr. Diana Yu, formerly of the Hein Lab. Without her recommending the UCM Research Experience for Undergraduates (REU), I would have never joined the Hein Lab and certainly would not have even known about or pursued the research that I have. Her selflessness and care ensured that I was able to participate in the REU and unquestionably changed my personal and academic trajectories for the better.

Many thanks are given towards all members of the Hein Lab, past and present, whose companionship and comradery made the uphill battle of obtaining a Ph.D. that much easier. They were always available to talk about chemistry, technology, or life. Our times spent at Gargoyle’s/Elwood’s will be remembered fondly. Similar thanks are deserved to my fellow graduate
students in Department. There is something to be said about the necessity of having friends who are nominally going through the same experience as you (working towards a Ph.D.), but whose day-to-day lives may differ. From their outside perspective, they allow for those “Aha!” moments which unquestionably drive science further. The individuals with whom I shared five difficult but rewarding years will unquestionably be lifelong friends.

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To my friends, family, and mentors.
Here's to the crazy ones. The misfits. The rebels. The troublemakers. The round pegs in the square holes. The ones who see things differently. They're not fond of rules. And they have no respect for the status quo. You can quote them, disagree with them, glorify or vilify them. About the only thing you can't do is ignore them. Because they change things. They push the human race forward. And while some may see them as the crazy ones, we see genius. Because the people who are crazy enough to think they can change the world, are the ones who do.

— Rob Siltanen
Chapter 1: Introduction

1.1 Motivations for Thesis

The desire for cheaper, faster, energy-conscious, and more sustainable chemical processes often necessitates the design and development of new catalytic transformations. These efforts represent a large dedication of time and resources in the pharmaceutical industry for the synthesis of new drugs,\textsuperscript{1,3} the fine chemicals sector for the manufacture of commodity materials,\textsuperscript{4,6} the fuels industry in the hopes of abating our dependence on limited fossil fuel reserves,\textsuperscript{6-9} and the academic community for the advancement of scientific understanding.

Catalysis research is motivated by the desire to find non-stoichiometric reagents (catalysts) that can render a difficult chemical reaction more efficient, cost-effective, and cleaner. Consider, for example, the desired transformation of a generic reagent $A$ into product $Pdt$. A stoichiometric route (Scheme 1.1a) might enlist the addition of reagent $B$ to generate $Pdt$. Potential characteristics of this reaction might be that the reaction is slow and require the usage of a molar equivalent (or more) of reagent $B$, which would be irreversibly consumed during the reaction. This scheme could also require elevated temperatures or harsh forcing conditions for appreciable reactivity to be observed and/or be unselective, forming an undesired by-product (By-$Pdt$). The formation of extraneous products would reduce the yield and efficiency and may require a separate operation to cleanly isolate $Pdt$. Such deficiencies might also render this method too costly to produce $Pdt$ on the desired scale. As a result, these shortcomings might motivate the development of a catalytic method (Scheme 1.1b) in which some non-stoichiometric reagent can mediate the transformation of reagent $A$ solely into $Pdt$ in a faster and economically-feasible manner. The catalyst might also allow the reaction to run at lower temperatures, milder conditions, and can, in theory, be reused in future runs.
Once a catalytic transformation is designed, the reaction must be optimized to maximize yield and selectivity. Traditional reaction optimization protocols stipulate the correlation of these end-metrics measured at a fixed time point as a function of variable reaction parameters such as temperature, time, concentration, stoichiometry, etc. By systematically changing these parameters, researchers hope to develop empirical trends relating the properties of chemical species (substrates, intermediates, products, solvents, etc.) to the observed final reactivity/selectivity of the process. What underpins these efforts is an attempt to account for and control the numerous, complex, and dynamic, chemical equilibria within a catalytic environment to favor a desired outcome.

If a generic catalytic reaction is considered in which a catalyst (Cat) joins together substrates A and B to generate product Pdt, a possible mechanism (Figure 1.1, inner circle) that could be used to account for the formation of Pdt is one in which substrate A reversibly binds to Cat to form intermediate Int1, which would then be able to reversibly bind substrate B to form Int2. From there, Int2 would irreversibly liberate Pdt and turnover to regenerate Cat, starting the cycle once again. Unfortunately, the reality is that catalytic systems are often plagued by “off-cycle” pathways (Figure 1.1, dashed arrows) which divert chemically-productive species into unproductive states.$^{10,11}$ Such
processes can include, but are not limited to, the siphoning of reagents $\text{A}$ and $\text{B}$ into undesired by-products or the irreparable decomposition of $\text{Cat}$, $\text{Int1}$ and $\text{Int2}$. The need for a pre-catalyst (Pre-Cat) to undergo some type of chemical or physical transformation to generate the productive “on-cycle” $\text{Cat}$ might lead to an induction period (a delay in product formation) when the reaction is first initiated. The product itself could participate in “product effects,” which might be inhibitory (wherein an increasing product concentration might prevent further catalytic turnover) or acceleratory (where the product facilitates faster turnover), or itself could decompose. Moreover, Figure 1.1 does not even consider the effects that chemical additives can have on facilitating the desired reaction by either accelerating productive pathways or mitigating deleterious pathways.\textsuperscript{12}

![Diagram](image)

**Figure 1.1.** Idealized (inner circle) and complex (dashed arrows) catalytic pathways.

In order to rapidly assess what inhibits productive chemistry, focus must be redirected towards scrutinizing the mechanisms within a catalytic environment, instead of focusing on improving end-metrics such as yield and selectivity at an arbitrarily-chosen time point. While “classical” means of
determining reaction mechanisms by way of initial rate kinetics (discussed in further detail in Section 1.4) can provide some information, it is only when a reaction’s progress is monitored over its entire time course in which the temporal concentration profiles of starting materials, products, by-products, catalytic species, etc. are captured can a proposed catalytic mechanism be substantiated. By monitoring the reaction under synthetically-relevant conditions, temporal signatures of all dynamic processes at play can be obtained which consequently allow for informed process development.

Any proposed mechanism that attempts to rationalize the reactivity and selectivity of a catalytic transformation must be corroborated with empirical data. Isolation and characterization of catalytic intermediates is often taken as evidence for a particular catalytic pathway; however, this approach is complicated by the instability and low abundance of many catalytic intermediates. More importantly, the mechanistic relevance of isolated species can be hard to assess as some are not competent precursors when re-subjected to the starting reaction conditions. Consequently, the task of determining the most likely pathways in which chemical species are created can become very difficult if there is no time-resolved data on their formation. The significance of isolated species to productive chemistry, therefore, remains dubious until experiments can inform otherwise. Thus, it becomes necessary to employ modern in situ monitoring tools to elucidate chemical pathways.

The need to capture time-resolved information can further be exemplified by Figure 1.2a-c. Here, we consider the reaction of reagents A and B to form products C and D, respectively. In “competition reactions” such as this, the relative ratio of C and D is compared from the various reaction conditions, allowing for the mechanisms and the structure-activity relationships between the substrates and the catalyst to be potentially inferred. As seen in Figure 1.2a-c, each panel of the figure shows that products C and D form in an approximately 1:1 final ratio. Consequently, if the final product distribution of the three experiments is taken solely, an incorrect conclusion would be that products C and D form via similar pathways in each scenario.
Figure 1.2. (a) C and D form simultaneously under zero-order kinetics. (b) C forms in a zero-order manner prior to D under overall first-order kinetics. (c) C forms under overall first-order kinetics prior to D, which forms following an induction period in a sigmoidal manner. (d) The reaction initially forms C and D simultaneously, but the addition of reagent at time $t_1$ selectively arrests the formation of D but leaves the formation of D unaltered.

Such an error would be less likely if the temporal profiles were inspected. In Figure 1.2a, C and D form simultaneously with linear profiles and C forming faster than D. This may indicate that C and D form via similar pathways, but there are other factors (possibly related to differences in A and B) that cause a discrepancy in their respective reaction rates. In Figure 1.2b, C also forms first in a linear manner; however, only after its formation is near completion around time $t_1$ does D form, in
what appears to be an overall first-order profile. From these observations, there may be a preference for the catalyst to interact with A and produce C, and only once it is consumed can B bind and generate D. Moreover, the difference in the profiles for C and D may indicate that the mechanisms of their formation differ. In Figure 1.1c, C forms first in an overall first-order manner until time $t_1$, followed by a delay before D forms at time $t_2$, now with a sigmoidal profile. The appearance of an induction period (a delay in the appearance of D) and a sigmoidal profile indicate that some other chemical or physical process must occur before D can form. Furthermore, by monitoring the reaction over time, changes in reactivity as a result of dosed reagents can be recognized. Figure 1.1d depicts a reaction in which C and D initially form simultaneously; however, after at a certain time $t_1$, a reagent was added. It is clear that the addition of reagent selectively arrested the formation of D, while C was unaffected.

In a similar vein, Figure 1.3 depicts a scenario in which Pdt forms until time $t_1$, after which the system enters a regime where [Pdt] is decreasing until a plateau at time $t_2$. If a single sample of the reaction is analyzed after $t_1$, (e.g., at $t_2$) a possible conclusion might be that reaction is incomplete and require additional time or reagents. Alternatively, the initial conditions could be redesigned in an attempt to increase [Pdt] at $t_1$. However, these optimization efforts may not mitigate the product decomposition pathways that are detrimental to the reaction and be non-fruitful.

**Figure 1.3.** Temporal concentration profile for a system in which product formation occurs until time $t_1$, followed by a regime where [Pdt] is decreasing until a plateau is reached at time $t_2$. 

---

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This kind of “graphical analysis” of time-course reaction profiles can provide both qualitative and quantitative information on the mechanisms of the chemical transformations under study with an overall reduction in time and resources committed. Consequently, the design, construction, and deployment of modern in situ reaction monitoring technologies that allows for the capture of data-rich information can lead to logical and informed manipulations to favor a desired outcome. The result is an in-depth understanding of complex chemical and physical processes and serves as the motivation for this Thesis. Herein, several case studies will be discussed in which the chemical or physical processes of interest share common themes:

(1) High-density, temporal reaction progress information is fundamental to process understanding.

(2) Using an end-of-reaction metric (e.g., yield or conversion) is misleading and incorrect for process optimization.

(3) The development of new in situ reaction monitoring technology is required for efficient kinetic analysis of the processes of interest.

1.2 In Situ Reaction Monitoring Technologies for Studying Complex Processes

Many process analytical technologies17 (PAT) can potentially be applied to studying concentration changes over time. What is discussed in this section is by no means a comprehensive list but includes some of the most common and familiar methods available to the average chemist. Ultimately, each reaction under study will each present its own unique challenges, with different profiles and signatures. The optimal combination of analytical equipment will vary and must be adapted to the processes of interest.
1.2.1 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is probably the first tool of choice when examining reaction progress. The generally straightforward methods of quantification and data processing make this a useful technique and many groups have utilized NMR spectroscopy for kinetic and mechanistic analysis.\textsuperscript{18-28} In addition to the standard static tube experiment, flow systems promise an alternative mode of presenting the reaction to the magnetic field.\textsuperscript{27-31} This is especially important to consider since a recent report by Foley and co-workers has provided evidence that kinetic information gathered from a static tube environment can differ greatly from a continuous flow system.\textsuperscript{28} Despite its many advantages, NMR spectroscopy is not easily amenable to many systems. Reactions in which high pressure, extreme temperatures, heterogeneous reagents, and/or other complex reaction conditions may prevent analysis. In addition, the absence of NMR-active nuclei or the generation of paramagnetic species can also complicate the application of NMR spectroscopy as a monitoring tool. In these cases, electron paramagnetic resonance (EPR) spectroscopy can be employed, allowing mechanistic and kinetic information, as well as coordination geometry and valence to be potentially inferred.\textsuperscript{32-34}

1.2.2 Infrared (IR) and Raman Spectroscopy

Infrared (IR) and Raman spectroscopy are complementary approaches for reaction monitoring. Recent years have seen a large increase in the use of \textit{in situ} IR to study chemical reactions as the interpretation of data is generally straightforward and familiar to the average researcher.\textsuperscript{27, 33, 35}\textsuperscript{40} This spectroscopy is particularly useful when the chemical species of interest have medium to strong absorption characteristics in the IR frequency range and have a noticeable change in intensity over the course of the reaction. By tracking the signal intensities corresponding to starting material, products, by-products, or intermediates over time, the rate of consumption or appearance can be determined.
For most applications, an attenuated total reflection-Fourier transform infrared (ATR-FTIR) probe connected via an optical conduit or fiber-optic cable is employed. This configuration allows the probe to be directly installed into the reaction environment and requires very little special set-up or modification of conventional reactions. A second benefit to this type of probe is that it is largely insensitive towards solid components in heterogeneous systems, allowing the solution phase chemistry to be directly monitored even in the presence of insoluble species. Likewise, Raman spectroscopy can be similarly employed to track species which are IR inactive but nonetheless contains polarizable bonds that will give a discernable signal. As with the ATR-FTIR setup, a fiber optic interface allows the probe to be installed with minimal perturbation to the reaction setup; however, Raman will capture spectral signatures from dissolved and heterogeneous components.\textsuperscript{33, 41-44}

FlowIR is a related technique that has been applied to monitoring reaction processes.\textsuperscript{45-47} In this approach a reaction mixture is passed through a flow cell, allowing FTIR analysis via a transmission cell in place of the ATR interface. A typical flowIR apparatus can provide superior sensitivity for very dilute analytes and is ideal when reaction volumes are restricted, or the reaction conditions are too demanding to be adequately captured by ATR-IR probes. In addition, these tools can be adapted onto standard benchtop instruments or exist as attachments to dedicated ReactIR instruments. However, while this variant can provide an increased signal-to-noise ratio, engineering must be considered in order to reliably deliver a sample to the instrument. In particular, flowIR instruments utilizing a transmission cell require IR-transparent optical windows, which may be brittle or incompatible with reaction environments. Lastly, in order to obtain a stable spectral signal, the system must maintain a very consistent flow rate to minimize spectral baseline shifts.
1.2.3 Ultraviolet-Visible (UV-Vis) Absorbance Spectroscopy

UV–Vis spectroscopy has been used widely for kinetic studies in contexts where the appearance or disappearance of signal at discrete wavelengths can be definitively assigned and are not masked by other components.\(^{33, 48-50}\) A classic example of this tool used in kinetic analysis involves the monitoring of NAD(P)H oxidation for enzyme-mediated reactions that require this co-factor (Scheme 1.2). Here, the reduced form, NAD(P)H contains two absorbance maxima in its UV-Vis spectrum, at 260 and 340 nm. The oxidized form, NAD(P)+, on the other hand, does not have a response at the longer wavelength. Therefore, the rate of the reaction can be determined by proxy where it is assumed to be equal to the rate of oxidation of the co-factor.

Scheme 1.2. The reversible oxidation of (NAD(P)H) to NAD(P)+, affords a change to the compound’s UV-Vis spectrum, where the absorbance at 340 nm is no longer observed.

The application of UV–vis spectroscopy to monitor reactions is reliant on the UV–vis spectrum being appropriately resolved and the concentrations of the analytes being in the optimal regime for reliable spectral acquisition. Depending the system, absorptions may not be sufficiently
visible or conclusively assigned. Nevertheless, UV–vis can be useful to ascertain a general idea of structure. This is very clear when considering solvent effects (via solvatochromic shifts), interactions between separate chemical species (evidenced, for example, by absorption changes associated to metal-to-ligand charge transfer), and other factors that alter the energies of electronic transitions.

1.2.4 Other Reaction Monitoring Technologies

Although not spectroscopic methods, heat-flow isothermal calorimetry\textsuperscript{20, 51-55} and gas uptake measurements\textsuperscript{56-59} can also provide highly-resolved reaction progress information. Calorimetry is compatible with systems that display large enthalpies that can be correlated to reaction progress. It is unique in its ability to directly record the rate of reaction as a function of time, allowing conversion to be obtained by integrating the total thermal evolution curve. This is in contrast to other spectroscopic methods, where the primary data acquired provides concentration as a function of time, and the derivative of this data provides the rate of change. Unfortunately, reaction calorimetry provides no direct information on chemical speciation and instead only records the global change in heat-flow for a chemical process, encompassing all chemical and physical events. As a result, aliquots of the reaction must be taken and analyzed separately to ensure that the calculated conversion from the heat flow is representative of the reaction. Finally, gas uptake also provides a facile means of online monitoring, however, it has a niche role in tracking reactions that include gaseous reagents or products. By monitoring changes in the gaseous mass transport over time, a measure of stoichiometry is obtained thus providing another measurement to facilitate kinetic analysis.

1.3 Hein Lab Capabilities

\textit{In situ} reaction monitoring technologies can be augmented by the removal and quenching of aliquots at timed intervals for independent analysis by gas chromatography (GC), high-performance
liquid chromatography (HPLC), NMR, or other techniques. For cases in which chromatography is used, there is an added benefit in that co-dissolved species can be separated prior to analysis, potentially allowing for increased data resolution.

This approach provides very detailed reaction progress information but relies on accurate and regular aliquot removal. As a result, a number of factors must be carefully addressed: First, the act of removing material from the reactor must not adversely affect the chemical transformation. (A quick method to verify this involves simply changing the sampling frequency and verifying that this has no noticeable effect on the reaction profiles.) Secondly, an appropriate internal standard must be selected so that its diagnostic analytical signals are well-removed from signals corresponding to the chemical species in the reaction. Accuracy in the standard’s concentration is paramount, as this impacts the quantification of all other components. Further, it is necessary to verify that the presence of the internal standard does not alter the chemical process under study. Finally, the aliquot must be rapidly and thoroughly quenched to represent a true “snapshot.” This requires a fine balance, being effective enough to halt the reaction instantaneously but not overly aggressive such that the concentration and identity of reaction components are perturbed upon quenching.

In response to these caveats, our lab has developed a variety of custom-built, robotic platforms that allow for reaction progress monitoring for either “offline” or “online” analysis through automated sampling of homogeneous or heterogeneous reactions. The operation of these systems relies on orchestration of standard laboratory equipment via custom-built computer software.

1.3.1 Homogeneous Reaction Sampling

Automated monitoring of homogeneous reactions can be achieved by immersing a sampling capillary made from an inert material (e.g., polyether ether ketone, PEEK or ethylene tetrafluoroethylene, ETFE) into the contents of a reactor (Figure 1.4). At fixed intervals, aliquots
of the reaction are withdrawn through the capillary and into a sample loop that is located on the head of a six-port, two-position selection valve. The valve, in turn, is connected to a liquid handing robot and programmable syringe pump. Initially, the sampling capillary is connected opposite the syringe pump through the sample loop (the “Load” position, Figure 1.5a). When the syringe pump is triggered by the software, an aliquot is removed, and the loop is filled. The selection valve is then changed such that the sample loop is now in line with the liquid handling robot (the “Inject” position, Figure 1.5b). A diluent pump is initiated which flushes the sample loop with a quench solvent (usually methanol or another HPLC-compatible solvent) and dilutes and delivers the sample directly into an LC vial located on the bed of a liquid handling robot. (An image of one prototype of the automated system is shown in Figure 1.6.)

Figure 1.4. Representative schematic of the automated homogeneous reaction sampling device used throughout this Thesis. See Figure 1.5 for selection valve diagram.

Individual LC vials that are prepared by the automated technology are transferred manually to the HPLC for “offline” analysis either as they are prepared or at the completion of the sampling period. By ensuring that the pull volume of the syringe pump at each sampling event is larger than the total dead volume of the sampling capillary, sample loop, and valve head, reliable and robust
quantification of reaction components stable in the vial can be made without the use of an internal standard. This technology will be used throughout the Thesis.

Figure 1.5. (a) “Load” and (b) “Inject” positions of the two-position, six-port selection valve used in the automated reaction sampling technologies used in this Thesis. Open circles represent the valve ports and black arcs represent the internal fluidic connections of the valve head.

Figure 1.6. Image of the automated homogeneous reaction sampling system, ReactIR15, and Omnical heat-flow isothermal calorimeter.
Alternatively, if reaction components are too unstable for capture in an offline sample, aliquots of the reaction can be delivered directly onto an LC system for instant analysis, called a “direct inject” system (Figure 1.7). In this configuration, the primary selection valve is the same as the previous vial-based sampler; however, the output of this valve is not the liquid handler and is instead an in-line mixer. The mixer, in turn, is connected prior to a secondary valve that contains an injection loop and is interfaced with the HPLC pump and HPLC column. The use of a mixer and a second valve allows for precise dilution and mixing of the sample prior to its introduction onto the HPLC column to ensure that the sample is homogeneous, and the column is not overloaded. This “online” system allows for reaction analysis in near real-time and was necessary for our study of the copper-catalyzed Kinugasa reaction to form β-lactams from terminal alkynes and nitrones.\(^\text{63}\)

**Figure 1.7.** Schematic of “direct inject” system in which the reaction aliquot is delivered directly onto an HPLC column for separation and analysis.

### 1.3.2 Heterogeneous Reaction Sampling

Monitoring of heterogeneous reactions involves the use of a Mettler-Toledo EasySampler (Figure 1.8, left). The EasySampler is a commercially-available, probe-based device that employs a 20 µL pocket to capture samples from the reactor. For our purposes, the base unit containing the carousel of vials (Figure 1.8, left) offers an insufficient number of samples for data-dense analysis of
processes of interest. Instead, the EasySamplers employed in our lab are interfaced with our custom-built robotic reaction sampling technologies.\textsuperscript{63-65}

Figure 1.8. Metter-Toledo EasySampler (left) interfaced with a Mettler-Toledo EasyMax 402 Advanced Synthesis Workstation (right).\textsuperscript{66}

Figure 1.9a shows a cartoon representation of the EasySampler with the pocket withdrawn in the probe head. At each sampling event, the probe head and pocket are first flushed out with inert gas (usually nitrogen) to prevent cross-contamination of the reactor with remnants of the previous sample. Once this is complete, a motor on the EasySampler body extends the pocket into the reactor and the pocket is filled with a slurry sample (solid + liquid phases). Meanwhile, in this extended position, the internal fluidic pathways of the EasySampler are filled with a quench solvent (Figure 1.9b, yellow lines) such that when the pocket is withdrawn, the sample is placed immediately in contact with the quench. Next, a diluent pump is initiated, and the sample is flushed either to an LC vial (for “offline” analysis) or into an injection loop (for “online” analysis). This cycle can be repeated as many times as necessary so long as the volume of the reactor’s contents is sufficient to allow for the pocket to capture a representative sample.
Figure 1.9. Mettler-Toledo EasySampler pocket (a) withdrawn (b) extended.\textsuperscript{67}

The EasySampler probe is liquid-tight, which allows for the flushing of the probe while the pocket is either in the withdrawn or extended positions without contamination of the reactor. Compared to other technologies, the EasySampler allows for the shortest delay between the sample being removed and quenched since the pocket is in direct contact with the quenching solvent once it is retracted. Moreover, since the probe is submerged in the reaction, this minimal delay also allows for quenching to occur at as close to the reaction conditions as possible. For example, if a sample of a reaction performed at cryogenic temperatures (e.g., -78 °C) is allowed to warm up to room temperature prior to quenching, there is concern that the contents of the sample (speciation, relative concentrations, etc.) might be substantially different than what is present in the reactor, and therefore not represent an accurate “snapshot” of the reaction. This technology will be utilized in Chapter 3.

1.3.3 Parallel Solution and Slurry Sampling

Parallel sampling of both the solid-free solution phase and slurry (solids + liquids) of reactions can be achieved by synchronization of the homogeneous sampling technology and the EasySampler. To obtain an aliquot of the solid-free supernatant, a filter is added to the end of the sampling capillary (Figure 1.4), with all other components remaining the same. For processes in which these parallel data streams are necessary, the simple addition of another valve to the system allows for selection between solid-free solution phase and slurry sampling. This type of system was necessary for our
study of amine-catalyzed aldol reactions. By capturing aliquots of the solid-free supernatant and slurry samples of these reactions in tandem, we were able to determine the cause for the observed diastereoselectivity of these reactions, which was due to precise combination of solution-phase epimerization and crystallization, a feature that would be hard to decipher if only one type of sample (either solid-free solution or slurry sampling) were captured. An adaptation of this technology can be found in Chapter 4.

1.3.4 Other Process Analytical Technologies

Our lab is also equipped with other commercially-available technologies, which are non-destructive in situ monitoring devices. By coupling automated sampling with other in situ methods, the simultaneous analysis by multiple, orthogonal analytical techniques provides cross-validated information, with an overall reduction in time and resources. This includes an Omnical isothermal heat-flow calorimeter (Figure 1.10a) used in Chapter 3, a Mettler-Toledo ReactIR (Figure 1.10b) used in Chapter 4, and an in situ particle sizer from BlazeMetrics (Figure 1.10c) used in Chapter 5.

Figure 1.10. (a) Omnical isothermal heat-flow calorimeter, (b) Mettler-Toledo ReactIR 15, and (c) BlazeMetrics InProcess particle sizer.
1.4 “Classical” Methods for Reaction Kinetic Analysis

If we consider a unimolecular reaction in which substrate $A$ transforms into product $Pdt$ with rate constant $k$ (Scheme 1.3), the “classical” method for determining the order in $A$ and therefore the mechanism by which $A$ converts into $Pdt$ would be to monitor the consumption of $A$ (or the formation of $Pdt$) over time and determine which model bests fits the reaction profile.

Scheme 1.3. Unimolecular reaction of $A$ transforming into product $Pdt$ with rate constant $k$.

$A$ priori, we would expect the rate law that governs this reaction to take the form

$$ \text{rate} = \frac{d[Pdt]}{dt} = \frac{-d[A]}{dt} = k[A]^a $$

(eq. 1.1)

where $k$ is the rate constant, $[A]$ is a concentration, and $a$ is the order in $A$. Table 1.1 gives three values of $a = 0, 1, \text{ or } 2$ and the associated differential and integrated forms of the rate law. While it is possible to take the temporal concentration profiles for Scheme 1.3 and examine the fit of the data to each integrated form in Table 1.1, a simpler method for determining the order in $A$ would be to transform the concentration data until a linear graph is obtained (Figure 1.11a-c).

This operation can be rationalized by examining the integrated forms in Table 1.1. If a plot of $[A]$ vs. time is linear (Figure 1.11a), this suggests that the rate of the reaction does not depend on the value of $[A]$ and the reaction is zero-order in $A$ ($a = 0$). Similarly, if a plot of ln $[A]$ vs. time is linear (Figure 1.11b), this would imply that the reaction is first-order in $A$ and $a = 1$. Lastly, if a plot of $1 / [A]$ vs. time is linear (Figure 1.11c), $a = 2$ and the reaction is second-order in $A$. This analysis involving inspection of the shapes of the graphs is useful as it provides a quick visual cue. Moreover, in the absence of non-linear fitting programs, the slope of the line and the value of the rate constant, $k$, can be obtained in a facile manner.
Table 1.1. Differential rate equations and integrated forms for various kinetic orders of the unimolecular reaction of A transforming into Pdt.

<table>
<thead>
<tr>
<th>Order</th>
<th>Differential Form</th>
<th>Integrated Form</th>
<th>Linear Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$-\frac{d[A]}{dt} = k[A]^0 = k$</td>
<td>$[A]_t = [A]_0 - kt$</td>
<td>$[A]_t$ vs. $t$</td>
</tr>
<tr>
<td>1</td>
<td>$-\frac{d[A]}{dt} = k[A]$</td>
<td>$\ln[A]_t = \ln[A]_0 - kt$</td>
<td>$\ln[A]_t$ vs. $t$</td>
</tr>
<tr>
<td>2</td>
<td>$-\frac{d[A]}{dt} = k[A]^2$</td>
<td>$\frac{1}{[A]_t} = \frac{1}{[A]_0} + kt$</td>
<td>$\frac{1}{[A]_t}$ vs. $t$</td>
</tr>
</tbody>
</table>

Figure 1.11. Plots to determine the order in A. (a) A linear graph of $[A]$ vs. time indicates that the reaction is zero-order in A. (b) A linear graph of $\ln[A]$ vs. time indicates that the reaction is first-order in A. (c) A linear graph of $1/[A]$ vs. time indicates that the reaction is second-order in A.

Scheme 1.4. Generic reaction where reagents A and B combine to form product Pdt.

Unfortunately, this analysis breaks down very quickly when an additional reagent is added. For example, consider Scheme 1.4 where a generic reaction in which two starting materials, A and B react to form product Pdt. For this reaction, the rate equation should take the form

$$\text{rate} = k[A]^a[B]^b \quad (\text{eq. 1.2})$$

where $k$ is the rate constant and $a, b$ are the orders in reagents A and B, respectively. The concentrations of A and B are coupled to each other, making the determination of order less
straightforward. If, however, \([B]\) is in large excess (at least ten-fold) compared with \([A]\), then \([B]\) can be assumed to remain constant during the reaction, rendering the rate equation to

\[
\text{rate} = k_{\text{obs}}[A]^a
\]  

(eq. 1.3)

where the observed rate constant, \(k_{\text{obs}}\), is defined as

\[
k_{\text{obs}} = k[B]^b.
\]  

(eq. 1.4)

The order in \(A\) is first determined like that in the unimolecular case, by determining which plot affords a linear graph (Figure 1.11a-c). Next, to determine the order in \(B\), \([B]\) is varied against a fixed \([A]\) and the initial reaction rate (where less than \(\sim 10\)-15\% of the starting materials have been consumed) is measured for each experiment. From eq. 1.4, we see that if we plot \(k_{\text{obs}}\) (initial rate) vs. \([B]\), the value of \(b\) can be determined by examining the shape of the graph (Figure 1.12a-c). For example, if \(b = 0\), the observed reaction rate does not depend on \([B]\), and therefore the graph will be flat (Figure 1.12a). Likewise, if \(b = 1\), a straight line with slope \(k\) and intersecting the origin would indicate that the reaction is first-order in \(B\). For all other values of \(b\), a plot of \(\ln k_{\text{obs}}\) against \(\ln [B]\) should give a straight line with slope \(b\) and \(y\)-intercept = \(\ln k\).

**Figure 1.12.** Plots to determine the order in \(B\). The reaction is performed under “pseudo-first-order” conditions, where \([B] \geq 10[A]\), and \([B]\) is assumed to be non-changing. (a) A horizontal graph of \(k_{\text{obs}}\) vs. \([B]\) indicates that the reaction is zero-order in \(B\). (b) A linear graph of \(k_{\text{obs}}\) vs. \([B]\) indicates that the reaction is first-order in \(B\). (c) A linear graph of \(\ln k_{\text{obs}}\) vs. \(\ln [B]\) indicates that the reaction is \(b\)-order in \(B\) with the slope of the graph equal to \(b\). (Figure adapted with permission from Meek et al.69)
The situation in which a reagent is added in large excess is referred to as “pseudo-first-order” since the concentration of only one reagent is assumed to be changing. However, this can warrant concern as the reaction environment significantly deviates from the parameters employed when the process is used synthetically. Consequently, the conclusions drawn from kinetic experiments performed with excess reagents may not hold under “real-world” conditions. Fortunately, modern in situ reaction monitoring tools combined with newer “graphical” methods of kinetic analysis have allowed for more precise means of kinetic analysis with fewer experiments.

1.5 “Graphical” Methods for Reaction Kinetic Analysis

In this section, an overview of several “graphical” methods for reaction kinetic analysis are presented, by which inferences on a reaction’s mechanism can be made by analyzing the relative shapes and trends of progress graphs. What is discussed here is not a complete survey; rather, it is to show the relative ease in which mechanistic insights can be made with the aid of simple manipulations to reaction progress data.

1.5.1 Lineweaver-Burk Method

Perhaps one of the oldest and well-known methods in which a “graphical” kinetic analysis yields insight on a reaction mechanism is that of the Lineweaver-Burk method for enzymes. This is applied to systems that follow a Michaelis-Menten-like mechanism\(^7\) (Scheme 1.5), a model that is not restricted to enzyme-mediated reactions, as we shall see in Chapter 3. In this scheme, \(E\) is the enzyme, \(S\) is the substrate, \(E\text{--}S\) is the enzyme-substrate complex, and \(Pdt\) is the product. This mechanism assumes that the binding of \(S\) to \(E\) is subject to rapid equilibrium and the rate of the reaction is predominantly controlled by the turnover of the enzyme-substrate complex, \(E\text{--}S\ (k_2)\).
Scheme 1.5. Michaelis-Menten mechanism for the enzymatic conversion of substrate $S$ to $P_{dt}$.

The rate equation\textsuperscript{71} for this scheme can be easily derived using the steady-state approximation and has the form:

$$v = \frac{k_2 [E]_0 [S]}{[S] + \frac{k_{-1} + k_2}{k_1}} = \frac{V_{\text{max}} [S]}{[S] + K_M}$$  \hspace{1cm} (eq. 1.5)

where $v$ is the velocity (rate) of the reaction, $[E]_0$ is the starting concentration of enzyme, $V_{\text{max}}$ is the maximum velocity for the enzyme, and $K_M$ is the Michaelis-Menten constant, a “pseudo dissociation constant” that represents the affinity of the enzyme for the substrate.\textsuperscript{71} A typical plot of $v$ against $[S]$ for a particular substrate-enzyme combination is shown in Figure 1.13a. We see that the graph is hyperbolic and approaches an asymptote at large values of $[S]$. This means that for a particular enzyme-substrate pair, there is a maximum velocity, $V_{\text{max}}$, for the reaction. (Note that if the variable $[S]$ is replaced with $K_M$ in eq. 1.5, we obtain that $v = V_{\text{max}} / 2$, meaning that the Michaelis-Menten constant can be quantified by determining the substrate concentration at which $v$ is half that of $V_{\text{max}}$.) The values of $V_{\text{max}}$ and $K_M$ are unique for each substrate-enzyme combination. Therefore, a ranking of the efficiency of different enzymes to catalyze the same transformation of $S$ can be obtained by comparing the values of $K_M$, $V_{\text{max}}$, and $(K_M / V_{\text{max}})$.\textsuperscript{71}

In order to determine if a reaction adheres to Scheme 1.5, a series of experiments is performed in which $[S]$ is varied while keeping $[E]$ constant. The initial rate of the reaction at each $[S]$ is then measured and plotted, like that in Figure 1.13b. A non-linear regression can then be applied to the data to obtain values of $K_M$ and $V_{\text{max}}$ and a goodness-of-fit parameter obtained from the regression can be an indicator of how well the enzymatic reaction under study fits the model.
In the absence of non-linear fitting programs, another method can be used to determine $K_M$ and $V_{max}$. If the “double-reciprocal” of eq. 1.5 is taken and terms rearranged, we obtain

$$\frac{1}{v} = \frac{[S] + K_M}{V_{max}[S]} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \left( \frac{1}{[S]} \right)$$

(eq. 1.6)

which is referred to as the Lineweaver-Burk equation. By plotting $(1 / v)$ against $(1 / [S])$, a linear trend (Figure 1.13b) should be obtained if the reaction follows Scheme 1.5. By applying a linear regression, the best-fit line through the data can be obtained with the $y$-intercept equal to $(1 / V_{max})$ and slope $(K_M / V_{max})$. The $x$-intercept of this graph is equal to $-K_M$.

However, it is important to note that the Lineweaver-Burk analysis is not without fault. For example, it is highly reliant on accurate measurement of initial velocity. As a consequence, the values of $K_M$ and $V_{max}$ from this analysis assume that the transformation will proceed via the Michaelis-Menten scheme throughout the entire reaction’s progress. Since the later behavior is ignored, deviations from this model are unobserved. Moreover, due to the reciprocal nature of the technique, errors in $v$ and $[S]$ can be exacerbated. Lastly, at least 4 experiments for each substrate-enzyme combination will have to be performed to obtain enough data for analysis.
In spite of these potential drawbacks, the Lineweaver-Burk analysis is particularly powerful in providing visual representations that indicate the mechanisms by which inhibitors act on an enzyme. Figure 1.14a-c show various Lineweaver-Burk plots for experiments without an inhibitor present (blue squares) and with different inhibitors, I, added to the reaction (green circles). As their name suggests, competitive inhibitors (Ic) compete with the substrate for binding with the enzyme. This will affect the binding equilibrium for the substrate and enzyme (K_M) but given enough substrate, the inhibitor can be outcompeted, and V_max will be unchanged. This is manifest in Figure 1.14a with the y-intercept of the two plots being the same, but the slope and x-intercept are different. An uncompetitive inhibitor, IU, meanwhile, binds to the enzyme-substrate complex, resulting in a decrease in enzyme activity and an apparent increase in the affinity of the substrate reducing the magnitude of both V_max and K_M (Figure 1.14b). Lastly, a non-competitive inhibitor, IN, appears to decrease V_max, but K_M is unchanged (Figure 1.14c). The x-intercepts of the graphs for both sets of experiments are coincident, but not the y-intercepts, suggesting that binding of S is not inhibited and only the reaction efficiency has changed.

![Lineweaver-Burk plots for various enzyme inhibitors.](image)

**Figure 1.14.** Lineweaver-Burk plots for various enzyme inhibitors. (a) Competitive inhibitors, IC, leave V_max unchanged, but increase in K_M. (b) Uncompetitive inhibitors, IU, decrease both V_max and K_M. (c) Non-competitive inhibitors, IN, decrease V_max but K_M is unchanged.

As a result of these insights gleaned from a graphical analysis of the Lineweaver-Burk plots, we can modify the Michaelis-Menten model shown in Scheme 1.5 to account for the role of each type
of inhibitor (Scheme 1.6a-c). For the case of a competitive inhibitor (Scheme 1.6a), an additional equilibrium between $E$ and $I_C$ can be added to illustrate how the inhibitor can interfere with the binding of $S$. For an uncompetitive inhibitor (Scheme 1.6b), the decreases in $K_M$ and $V_{\text{max}}$ suggest that the inhibitor does not bind with the free enzyme $E$ and instead binds with the enzyme-substrate complex $E-S$. Thus, an equilibrium between $E-S$ and $I_{UC}$ can be added to the Michaelis-Menten mechanism. Finally, by a similar logic, the change in $V_{\text{max}}$ but not $K_M$ for a non-competitive inhibitor ($I_{NC}$; Scheme 1.6c) can be rationalized by the addition of three new equilibria involving $E$ and $I_{NC}$, the enzyme-inhibitor complex $E-I_{NC}$ and $S$, and the enzyme-substrate-inhibitor complex $E-S-I_{NC}$ and the enzyme-substrate complex $E-S$.

(a) Competitive Inhibitors

\[
E + S \xrightleftharpoons[k_1, k_{-1}]{k_2} E-S \xrightarrow{Pdt} E
\]

(b) Uncompetitive Inhibitors

\[
E + S \xrightleftharpoons[k_1, k_{-1}]{k_2} E-S \xrightarrow{Pdt} E
\]

(c) Non-Competitive Inhibitors

\[
E + S \xrightleftharpoons[k_1, k_{-1}]{k_2} E-S \xrightarrow{Pdt} E
\]

Scheme 1.6. Modifications on the Michaelis-Menten mechanism by (a) competitive inhibitors, $I_C$, (b) uncompetitive inhibitors, $I_{UC}$, and (c) non-competitive inhibitors, $I_{NC}$. 

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1.5.2 Reaction Progress Kinetic Analysis (RPKA)

The development of reaction progress kinetic analysis (RPKA) by Blackmond\textsuperscript{13-15} represents a more modern method for mechanistic elucidation. At its core are the “graphical rate equations” which are enabled by high-density data collected by modern in situ reaction monitoring tools. By simple arithmetic manipulations to the data, a robust and practical method to understand complex kinetic behavior with a minimal number of experiments, has assisted researchers greatly.

$$\text{rate} = k[A]^a[B]^b[\text{cat}]_0^c$$  \hspace{1cm} (eq. 1.7)

where \( k \) is the rate constant, \([\text{cat}]_0\) is the initial concentration of catalyst, and the values of \( a, b, \) and \( c \) are the orders in \( A, B, \) and \( \text{cat}, \) respectively.

A standard set of RPKA experiments is shown in Table 1.2, where \( [A]_{\text{initial}}, [B]_{\text{initial}}, [\text{cat}]_{\text{initial}} \) represent the starting concentrations of \( A, B, \) and \( \text{cat}, \) respectively. Experiment 1.2.1 represents “standard” conditions, those from which changes will be made to determine the empirical rate law. Experiment 1.2.2, called a “different excess” experiment is designed to determine the order in \( B: \) By increasing \([B]_{\text{initial}}\) while leaving \([A]_{\text{initial}}\) and \([\text{cat}]_{\text{initial}}\) the same, any change to the reaction profile will indicate that there is a kinetic dependence on this reagent. Experiment 1.2.3, meanwhile, is a “same excess” experiment, where the difference in the starting concentrations of the reagents is the same (\([B]_{\text{initial}} - [A]_{\text{initial}} = 0\) for experiment 1.2.1 and 1.2.3); however, the latter experiment is initiated at a different starting point, at twice the concentration (\([A]_{\text{initial}} = 2[A]_0 \) and \([B]_{\text{initial}} = 2[B]_0\)). This means
that at the end of experiment 1.2.3, the catalyst will have had more turnover cycles than experiment 1.2.1. Lastly, in experiment 1.2.4, the order in catalyst is probed by doubling $[\text{cat}]_{\text{initial}}$ to $2[\text{cat}]_0$.

Table 1.2. List of RPKA experiments to determine orders in reagents A, B, and cat.

<table>
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<tr>
<th>experiment</th>
<th>marker</th>
<th>$[A]_{\text{initial}}$</th>
<th>$[B]_{\text{initial}}$</th>
<th>$[\text{cat}]_{\text{initial}}$</th>
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<td>$[B]_0$</td>
<td>$[\text{cat}]_0$</td>
<td>“standard” conditions</td>
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<td>$2[B]_0$</td>
<td>$[\text{cat}]_0$</td>
<td>twice $[B]_0$</td>
</tr>
<tr>
<td>1.2.3</td>
<td>▲</td>
<td>$2[A]_0$</td>
<td>$2[B]_0$</td>
<td>$[\text{cat}]_0$</td>
<td>twice $[A]_0$, twice $[B]_0$</td>
</tr>
<tr>
<td>1.2.4</td>
<td>●</td>
<td>$[A]_0$</td>
<td>$[B]_0$</td>
<td>$2[\text{cat}]_0$</td>
<td>twice $[\text{cat}]_0$</td>
</tr>
</tbody>
</table>

Figure 1.15a gives a representative comparison of the temporal concentration profiles from experiments 1.2.1 (standard conditions; black squares) and 1.2.2 ($[B]_{\text{initial}} = 2[B]_0$; red circles). As we can see from the “simple overlay” of these two profiles, by doubling $[B]_{\text{initial}}$, there is no change in the shape of the product concentration profile and therefore there is no order in $B$ and $b = 0$. Conversely, if the same “simple overlay” were applied a reaction in which there was a positive order in reagent $B$, the graph would look something like that shown in Figure 1.15b, where the reaction in experiment 1.2.2 (red circles) reaches completion in a shorter amount of time. However, in this comparison, the exact order in $B$ cannot be determined by inspection.

To remedy this, RPKA would plot the rate of reaction (obtained from heat-flow calorimetry or by taking the first derivative of the temporal $[\text{Pdt}]$ function) vs. $[A]$, to parse out the orders in $A$ and $B$. Figure 1.15d shows the rate of reaction for experiments 1.2.1 (black squares) and 1.2.2 (red circles) divided by $[B]^b$, where $b$ is the order in $B$. As seen in the figure, the graphical rate equations are straight lines with a non-zero slope, implying that the reaction is first-order in $A$ ($a = 1$) and $b^b$-order in $B$. These conclusions can be rationalized by returning the postulated rate equation (eq. 1.7). If we divide both sides by $[B]^b$, the equation becomes
Therefore, if we consider $[\text{cat}]_0^c$ to be constant for both experiments, then this equation can only represent a straight line if $a = 1$. Consequently, the slope of the graphical rate equation in Figure 1.15d is $k[\text{cat}]_0^c$.

The “same excess” experiment 1.2.3, on the other hand, examines the effect of an increase in the number of turnovers that the catalyst must sustain on the reaction; Figure 1.15c gives the temporal profiles for $[\text{A}]$ for these two experiments. For both experiments, reaction progress data was collected at $t = 0$; however, $[\text{A}]_{\text{initial}}$ is not the same for both cases. To compare the two profiles, a “horizontal time shift” must be applied to the data from experiment 1.2.1 (grey squares, Figure 1.15c) until the first data point is aligned with a corresponding point in experiment 1.2.3 (blue triangles). The adjusted profile (black squares) differs from the original data only by a constant displacement to each time value in the original experiment. If the profiles do not align in the concentration regime in which the two experiments overlap ($[\text{A}]_0 \rightarrow 0$), this indicates that the reaction did not perform the same when it was initiated with a higher starting concentration of $\text{A}$ and $\text{B}$. An observation like this usually points to product inhibition or decomposition of the catalyst. An analogous analysis can be made on the graphical rate equations for the same two experiments (Figure 1.15c). If there is no product inhibition or catalyst decomposition, the graphical rate equations should overlay in the overlapping concentration regimes of $\text{A}$.

Lastly, the the order in catalyst can be determined from experiments 1.2.1 (black squares) and 1.2.4 (green diamonds), where $[\text{cat}]_{\text{initial}} = 2[\text{cat}]_0$. Using a similar algebraic manipulation as the “different excess” experiments, the different amounts of $[\text{cat}]_{\text{initial}}$ between the two experiments can be “normalized” by dividing the rate by $[\text{cat}]_0^c$, where $c$ is the order in catalyst. If graphical overlay occurs, then the reaction is $c$th-order in $\text{cat}$.
Figure 1.15. Various forms of “graphical kinetic analysis” and the conclusions that can be potentially made from the interpretation of the graphs. (See Table 1.2 for legend.)
1.5.3 “Visual” Kinetic Analysis

Recent work from Burés has expanded the toolbox of “visual kinetic analysis,” by the application of simple arithmetic operations applied to the concentration vs. time arrays, rather than rate profiles.\(^{75,78}\) Figure 1.16a,b give separate treatments to selected experiments from Table 1.2 to determine order in catalyst and reagents, respectively.

![Diagram](image)

**Figure 1.16.** “Time normalization analysis” of concentration vs. time profiles developed by Burés\(^{75,77}\) to determine (a) order in catalyst and (b) order in reagent B. (See Table 1.2 for legend.)

Figure 1.16a shows a “time normalization” analysis (TNA) that compares the data from experiments 1.2.1 (standard conditions, black squares) and 1.2.4 ([cat]\(_{initial}\) = 2[cat]\(_0\), green diamonds) to determine the order in catalyst. Here, the concentration of A as a function of time under standard conditions (experiment 1.2.1) is plotted as normal. The same is done for the experiment with twice the original catalyst loading (experiment 1.2.4); however, the time associated with each concentration value is multiplied by the factor [cat]\(_0\). This multiplier represents the starting concentration of the catalyst raised to the power \(c\), the order in catalyst.

Initially, the value of \(c\) is unknown. In order to determine the order in catalyst, its value is varied until graphical overlay of the transformed data (green diamonds) with the untransformed data
from standard conditions (black squares) is obtained and can be rationalized as follows: If the reaction exhibits a positive order in $[\text{cat}]_0$ and the concentration of active catalyst is constant under different conditions, then the reaction with $2[\text{cat}]_0$ (experiment 1.2.4) should reach completion in a shorter time than the standard experiment (experiment 1.2.1). Therefore, if “stretching” the time axis of the faster reaction can create a set of data that overlays the data from standard conditions, then the order in catalyst is the value of the exponent $c$. This process “normalizes” the time axis of the two experiments.\textsuperscript{75-77}

Figure 1.16b, meanwhile, shows a representative “variable time normalization” analysis (VTNA)\textsuperscript{77,78} in order to determine the kinetic dependence of the reaction on $B$. Similar to the previous analysis, this treatment employs temporal concentration data from experiments 1.2.1 (standard conditions; black squares) and 1.2.2 ($[B]_{\text{initial}} = 2[B]_0$; red circles), where $[Pdt]$ at some time $t$ is plotted against the integral of $[B]^b$ from 0 to $t$, where $b$ is the order in $B$. The function that represents $[B]$ over time is unknown \textit{a priori}, but its integral can be approximated by trapezoids (eq. 1.9):

$$\int_0^t [B]^b dt = \sum_{i=1}^n \left( \frac{[B]_i + [B]_{i-1}}{2} \right)^b (t_i - t_{i-1}),$$

(eq. 1.9)

where $[B]_i$ represents the $i^{th}$ concentration value at time $t_i$.

As seen in Figure 1.16b, if overlay of the transformed data from experiments 1.2.1 and 1.2.2 is achieved, it suggests that the order in $B$ is $b$. Such an operation can be rationalized as a “normalization” of the x-axis for $[B]$. That is, if the profiles for $[Pdt]$ between two experiments performed at different $[B]_{\text{initial}}$ and the same $[A]_{\text{initial}}$ can be transformed such that the dependence of $[Pdt]$ on the difference in $[B]_{\text{initial}}$ can be removed, then graphical overlay will occur between the two experiments. If we recall the proposed rate law for the reaction (eq. 1.7) and consider the value of $[\text{cat}]_0$ as constant for the sake of the derivation, then we have
rate = $\frac{d[P_{dt}]}{dt} = k_{obs}[A]^a[B]^b$. \hspace{1cm} (eq. 1.10)

Multiplying both sides of the equation by $dt$, we obtain

$$d[P_{dt}] = k_{obs}[A]^a[B]^b dt$$ \hspace{1cm} (eq. 1.11)

and integrating both sides, we have

$$\int d[P_{dt}] = \int k_{obs}[A]^a[B]^b dt.$$ \hspace{1cm} (eq. 1.12)

In comparing two experiments in which $[A]_{\text{initial}}$ are the same and only the value of $[B]_{\text{initial}}$ has been changed we do not consider $[P_{dt}]$ dependent on $[A]'$ and rewrite the right-hand side of eq. 1.12 as

$$\int d[P_{dt}] = k_{obs}[A]^a \int [B]^b dt.$$ \hspace{1cm} (eq. 1.13)

Therefore,

$$[P_{dt}]_t = k' \int_0^t [B]^b dt,$$ \hspace{1cm} (eq. 1.14)

where $k'$ is some constant. So, $[P_{dt}]$ at some $t$ can be represented as the cumulative integral of $[B]$ from 0 to $t$ and be approximated by the trapezoid rule (eq. 1.9) in the absence of a discrete function for $[B]$. An analogous analysis can be performed to determine the order in reagent $A$.

1.6 Structure of Thesis

The choice of “classical” or “graphical” means for reaction kinetic analysis will ultimately depend on the nature of the system under study, equipment available, and resolution of data. This Thesis will use a combination of both types in order to solve complex chemical or physical problems.

Chapter 2 discusses the development of a new automated reaction sampling and quenching procedure to monitor an unusual copper-catalyzed acetylenic hydrogen-iodide exchange between
terminal alkynes and 1-iodoalkynes. This study was necessary to place this ancillary pathway within the larger context of copper-catalyzed azide-alkyne cycloaddition reactions to form 1,4-diarylsubstituted-1,2,3-triazoles (Chapter 3).

Chapter 3 presents an in-depth kinetic investigation on the Cu(I)-catalyzed cycloaddition of azides and alkynes, identifying previously unrecognized mechanistic pathways, detailing a previously unrecognized counter-ion effect, and answering lingering mechanistic questions on this transformation.

Chapter 4 discusses the development new technology for solubility and dynamic crystallization analysis of non-UV-active species.

Chapter 5 discusses the application of the new technology described in Chapter 4 to study and optimize the resolution of rac-allylalanine monohydrate via continuous preferential crystallization (CPC).

Chapter 6 concludes the Thesis and provides an outlook at future directions of the automated in situ monitoring technology described herein.
Chapter 2: Understanding a Copper-Catalyzed Acetylenic H/I Exchange

2.1 Introduction

1-Haloalkynes (Figure 2.1) are useful synthetic intermediates that offer access to diverse halogenated products primed for further synthetic elaboration. \(^{79,80}\) Recent work has taken advantage of these versatile building blocks through hydration, \(^{81-84}\) nucleophilic addition, \(^{85-87}\) cycloaddition, \(^{88-93}\) and even tandem or multicomponent transformations, \(^{94-97}\) proceeding without scission of the C\(_{sp}^{\prime}\)–halogen bond.

\[ R \quad \text{---} \quad X \]

\( X = \text{Cl, Br, or I} \)

Figure 2.1. Representative structure of 1-haloalkynes.

During our lab’s study of the cycloaddition between 1-idoalkynes and organic azides, \(^{98}\) we observed an exchange of the acetylenic proton/iodide between phenylacetylene 2.1 and 1-idoalkyne 2.2 (Scheme 2.1d) under catalytic conditions. To reveal the mechanistic implications of these observations, we sought to measure the native exchange equilibria in the absence of azide. The results of these studies were necessary to locate this ancillary pathway within the mechanisms of Cu-catalyzed triazole synthesis (Chapter 3).

Previously, Abe and Suzuki observed displacement of bromide at the terminal sp-hybridized carbon atom in 1-bromoalkynes by superstoichiometric CuI (Scheme 2.1a). \(^{99}\) Conversely, the deliberate hydrodehalogenation of 1-haloalkynes to the parent terminal alkyne has been reported (Scheme 2.1b). \(^{100,101}\) In a recent report, Diéz-González and co-workers briefly mentioned an observed exchange between (iodoethynyl)benzene and 1-hexyne (Scheme 2.1c); \(^{102}\) however, no experimental data were provided. To our knowledge, a detailed examination of the thermodynamic and kinetic
features of the dynamic exchange between hydrogen and iodine at acetylenic centers has yet to be provided (Scheme 2.1d). In this chapter, our most recent study profiling this process is described.

Scheme 2.1. Selected examples of halogen substitution in 1-haloalkynes.

2.2 Results and Discussion

The exchange was monitored using HPLC by removing timed aliquots of the reaction mixture, facilitated by an automated sampling apparatus like that we have previously described and discussed in Chapter 1.\(^\text{60}\) (A schematic is shown in Figure 2.2.) However, unlike our previous studies, simple dilution of the aliquots with methanol for HPLC analysis was insufficient to arrest further reaction due to the catalytic efficiency of copper. An insufficient quench was evident when the same sample analyzed multiple times showed variable concentrations. Therefore, a new quench procedure was
developed to ensure that each sample provided representative data of the reaction regardless of the time elapsed before analysis.

The bicinchoninic acid (BCA) protocol developed by Smith and co-workers is a well-established protein concentration assay that relies on the reduction of copper(II) salts by peptidic bonds. The standard procedure involves preparing an aqueous solution of the disodium bicinchoninic acid salt in the presence of CuSO₄ and other additives. Upon exposure to the BCA assay, peptidic bonds will oxidize and reduce copper(II) to copper(I); the concentration of protein then becomes linearly proportional to the absorbance of the purple copper(I)–BCA complex.¹⁰³⁻¹⁰⁵

**Figure 2.2.** Schematic of the automated aliquot removal and quenching used in this study.

The BCA dianion itself is what our quench procedure relies on, since it has been found to be very selective for copper(I).¹⁰³⁻¹⁰⁶ Adapting the biochemical Smith assay, the aliquots were dispensed into a methanol solution of the bis(triethylammonium) bicinchoninic acid salt, which immediately stopped the reaction through tight complexation with CuI (Scheme 2.2). Control experiments ensured that the quench did not perturb the equilibrium quantities and that the chemical speciation and concentrations remained stable over time. BCA was chosen in lieu of an oxidative quench,¹⁰⁷ since oxidizing conditions were found to disrupt the equilibrium concentrations. All attempts to arrest the exchange chemistry by oxidizing the copper or providing a thiolate reagent to induce precipitation
interfered with the alkyne or iodoalkyne concentration, either via promotion of alkyne dimerization or decomposition.

Scheme 2.2. Complexation of Cu\(^{1}\) by 2 equiv. of bichinonic acid (BCA). The BCA–copper complex is characterized by its distinctive purple color. (Counter-ions are not shown for clarity.)

The experiments carried out in this work to interrogate the copper-mediated acetylenic hydrogen-iodide exchange are listed in Table 2.1. The experiments represented by entries 2.1.1, 2.1.2, 2.1.5, and 2.1.6 in Table 2.1 are those in which representative exchange reactions were performed under standard conditions “A” (5 mol % of CuI, 5 mol % of TCPTA ligand, MeCN, 25 °C; TCPTA = \textit{tris}(1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine)). It should be noted that these conditions are nearly identical to those in the original cycloaddition report.\(^{88}\) We have substituted the \textit{tert}-butyl azide-derived tris(triazolylmethyl)amine ligand for the cyclopentyl azide variant used in our original report. Cyclopentyl azide is much more easily synthesized than \textit{tert}-butyl azide, and we notice similar, if not greater, acceleratory behavior from this ligand.

For entries 2.1.3, 2.1.4, 2.1.7 and 2.1.8, exogenous base (2 equiv. of Et\(_3\)N; TEA) was added to the reactions to see if there was any noticeable effect on the exchange from base-assisted deprotonation. However, in these cases, we found the reaction proceeded too quickly for an adequate number of aliquots to be removed for kinetic analysis. Therefore, the catalyst and ligand loading were reduced to 1 mol % (conditions “B”) with all other parameters remaining the same. Under these conditions, we rule out the possibility of complete ligand substitution of TCPTA by TEA due to the
absence of a bright canary yellow and catalytically-inactive copper acetylide precipitate that can form when terminal alkynes are subjected to a copper source and a base. Indeed, attempts to conduct the exchange reactions without TCPTA ligand and only TEA led immediately to an insoluble aggregate.

Table 2.1. Acetylenic hydrogen/iodine exchange experiments.

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</tbody>
</table>

$^a$Conditions A: 2.1 (0.1 M), 2.2 (0.1 M), 5 mol % CuI, 5 mol % TCPTA, MeCN, 25 °C. Conditions B: 2.1 (0.1 M), 2.2 (0.1 M), Et$_3$N (0.2 M), 1 mol % CuI, 1 mol % TCPTA, MeCN, 25 °C. TCPTA = tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methylamine. $^b$.$K_{eq} = ([2.1] [2.2'])/([2.1] [2.2]).$ $^c$In units of 10$^3$ min$^{-1}$; see the Supporting Information for calculations. $^d$Value determined by extrapolation of concentration data. $^e$Exhibits complex kinetics.

To fully capture any electronic factors influencing the exchange, the experiments for each set of conditions were performed in pairs, alternating the substitution of the starting materials. In one trial, the *para* substituent was located on terminal alkyne 2.1 (X = CH$_3$, CF$_3$), leaving iodoalkyne 2.2.
unsubstituted \((Y = H)\). Reciprocally, the reaction was then performed with substituted iodoalkyne 2.2 \((Y = CH_3 \text{ or CF}_3)\) with unsubstituted 2.1 \((X = H)\).

Figure 2.1a (X = CH\(_3\)) or 2.1b (x = H) + 2.2a (Y = H) or 2.2b (Y = CH\(_3\)) \(\rightleftharpoons\) 2.1a or 2.1b’ + 2.2a’ or 2.2b’

Figure 2.3. Exchange equilibrium reaction progress graphs for selected entries of Table 2.1 (a) entry 2.1.1 \((X = CH_3, Y = H)\); (b) entry 2.1.3 \((X = CH_3, Y = H)\); (c) entry 2.1.3, dimeric by-products; (d) entries 2.1.3 and 2.1.4, overlay.

Figure 2.3 represents a selection of data for entries 2.1.1–2.1.4 of Table 2.1. The temporal concentration data of the exchange products, 2.1’ and 2.2’, can be fitted to a first-order exponential
and the observed rate constant, $k_{obs}$, extracted. When Figure 2.3a is compared against Figure 2.3b (entry 2.1.1 cf. 2.1.3), the approach to equilibrium with added base still follows exponential behavior but at a much faster rate ($k_{obs(B)}/k_{obs(A)} \approx 7$) and despite the 5-fold reduction in catalyst. However, under these conditions there was a noticeable amount of by-product formation, contributing to a slight mass imbalance at the end of the reaction (Figure 2.3c). Isolation and independent synthesis revealed these species to be dimeric species 2.5a and 2.6a,b (Figure 2.4); their formation is presumed to occur through a Cadiot–Chodkiewicz-type coupling process.\textsuperscript{115,119} With no base added, these species form only in trace amounts on the exchange time scale.

![Image of dimeric diyne by-products](image)

**Figure 2.4.** Homo- and heterodimeric diyne by-products formed upon addition of base.

Notably, while the terminal concentrations of the by-products represent a statistical mixture of the homo- and heterodimeric species, their formation present an interesting mechanistic aspect. For example, the concentration of heterodimer 2.5a in entry 2.1.3 is consistently the largest but experiences a rapid initial increase (0–20 min) followed by linear growth (Figure 2.3c). For both entries 2.1.3 and 2.1.4 of Table 2.1, the homodimer derived from the starting terminal alkyne 2.1 (i.e., 2.6a for entry 2.1.3) is always secondary in concentration and exhibits a profile similar to that of 2.5a. In contrast, the homodimer of the starting iodoalkyne (2.6b for entry 2.1.3) does not form until after the initial burst and there is no incipient rapid increase in concentration. Alternatively stated, despite
dynamic exchange there is a propensity to form heterodimer 2.5a and homodimer 2.6a over 2.6b, which is unexpected on the basis of predictions from the terminal $K_{eq}$ value. These nuances certainly would have been overlooked had a single end-point analysis been used and reinforce the benefits of highly time-resolved reaction progress analysis.

Although the equilibrium position is identical for all alkyne/iodoalkyne pairs ($K_{eq} = 1$), interesting information can be gathered by comparing $k_{obs}$ values against the electron-donating or withdrawing characteristics of the various para-substituents. When the exchange was carried out under conditions A (no base) and with unsubstituted or electron-rich terminal alkynes (X = H or CH$_3$, respectively), $k_{obs}$ is approximately the same irrespective of the iodoalkyne partner (entry 2.1.1 cf. entries 2.1.2 and 2.1.6). Despite alkyne dimerization in the experiments listed in entries 2.1.3 and 2.1.4, equilibration is still well behaved and displays a much larger $k_{obs}$ in comparison to the same systems without added TEA (entries 2.1.1 and 2.1.2 cf. entries 2.1.3 and 2.1.4). For the alkyne pairs containing electron-donating substituents, the rates of equilibration within each set of conditions were identical, regardless of substitution of the starting materials ($k_{obs}(2.1.1) \approx k_{obs}(2.1.2)$ and $k_{obs}(2.1.3) \approx k_{obs}(2.1.4)$).

As such, it appears that the acidity of the terminal alkyne has the largest effect on the rate of equilibration. On comparison of entries 2.1.1 (X = CH$_3$) and 2.1.5 (X = CF$_3$) (conditions A, no base), it is clear that the rate is larger when an electron-deficient alkyne is employed in comparison to an electron-rich alkyne ($k_{obs}(2.1.5) / k_{obs}(2.1.1) \approx 2$). Intriguingly, exchange of electron-poor alkynes or iodoalkynes (X, Y = CF$_3$; entries 2.1.7 and 2.1.8) in the presence of base exhibited complex profiles, no longer fitting an exponential function (Figure 2.5a). Even so, beginning the reaction with unsubstituted phenylacetylene 2.1b still produces an overall slower exchange process. An overlay of the data depicts stark differences in the concentration profiles between the two entries and, while the positions of the respective equilibria are identical, the profile for entry 2.1.7 exhibits sigmoidal behavior whereas for entry 2.1.8 the exchange is only slightly deviated from exponential.
Figure 2.5. Reaction progress graphs for entries 2.1.7 and 2.1.8 of Table 2.1: (a) overlay of entries 2.1.7 and 2.1.8, (b) entry 2.1.7, dimeric by-products, (c) entry 2.1.8, dimeric by-products.

The rate and chemoselectivity of the dimeric by-products for entries 2.1.7 and 2.1.8 are also very instructive (Figure 2.5b,c). Once again, heterodimer 2.5b is the major by-product and homodimer 2.6c, created from the electron-poor components (X, Y = CF₃), is the next major product. With terminal alkyne 2.1c (X = CF₃) as the starting material, the reaction profiles for both heterodimer...
and homodimer 2.6c mirror each other, displaying an initial faster rate followed by a slower pseudo-zero-order regime. Like the electron-rich alkynes, homodimer 2.6b does not appear until this initial rapid phase is complete (Figure 2.5b). When the reciprocal experiment is conducted, homodimer 2.6b, arising from coupling of the terminal alkyne, is initially the second most abundant species; however, after 10 min the formation of 2.6c overtakes 2.6b (Figure 2.5c).

The reaction progress analysis for the dialkyne byproducts indicates that dimerization likely arises solely from a Cadiot-Chodkiewicz-type coupling process involving a Cu acetylide and an iodoalkyne and not Glaser oxidative dimerization of two terminal alkynes. While we did not rigorously exclude oxygen in these experiments, the observed rate and selectivity of the diyne products support this conclusion. This argument is based on the dominance of heterodimerization, especially early in the catalytic process when the requisite iodoalkyne and terminal alkyne are both present in high concentration. The next most abundant byproduct is the homodimer resulting from the original terminal alkyne component. Again, this reflects the critical role of the σ-acetylide in this cross-coupling pathway. It is also noteworthy that the reaction profile for the homodimer corresponding to the initial terminal alkyne component is very similar to that of the heterodimer, suggesting both are formed via similar chemical pathways. The delay in the formation of the homodimer derived from the starting iodoalkyne further confirms our hypothesis, as H/I exchange must first occur to create sufficient terminal alkyne (2.2’ in Scheme 3) in order to engage in Cadiot–Chodkiewicz coupling.

A proposed catalytic cycle that accounts for all observations is shown in Scheme 2.3. The profiles suggest that the generation of the key σ-acetylide from the terminal alkyne 2.1 (step 1) is rate-limiting and would be aided by the high concentration of terminal alkyne at early time points. The subsequent coordination of the iodoalkyne 2.2 (step 2) now provides two possible pathways: exchange of the alkynyl fragment at copper (step 3) yields the exchange product 2.1’. Next, protonolysis of the newly generated copper acetylide generates the other exchange product 2.2’ (step 4).
Alternatively, coordination of the iodoalkyne to the copper acetylide species can lead to the oxidative addition of iodoalkyne, generating a Cu\textsuperscript{III} diacetylide species (step 3'); irreversible reductive elimination then gives the dimeric byproducts and regenerates CuI (step 4'). While we have ruled out complete ligand substitution of TCPTA by TEA, the stark contrast between the early (< 20 min) and later behaviors suggests some type of change in the catalyst speciation. The initial copper species tends to facilitate rapid alkyne coupling. However, after a threshold amount of exchange has occurred, the active catalyst now affords dimers at a significantly reduced rate. As a final comment, it must be noted that this proposed catalytic cycle does not explicitly depict π-coordination of the terminal alkyne to copper prior to deprotonation; however, we surmise that this also serves as a mode of activation to help facilitate exchange.

2.3 Conclusion

In summary, we have reported the detailed kinetic profiles of the exchange equilibrium between terminal and 1-iodoalkynes. This information yields practical considerations for reaction environments in which terminal alkynes and 1-iodoalkynes are present. A direct connection involves
the family of Cu-catalyzed couplings of alkynes, including Glaser-Hay, Cadiot-Chodkiewicz, and others that have been plagued by undesired by-products.

2.4 Experimental

2.4.1 Chemical Suppliers

Phenylacetylene was purchased from VWR, 1-ethynyl-4-methylbenzene and 1-ethynyl-4-(trifluoromethyl)benzene were purchased from Aldrich. Bicinchoninic acid was purchased from Aldrich. Copper(I) iodide was purchased from Strem. All other reagents were purchased from conventional suppliers. All terminal alkynes were passed through a pipette of dry silica gel prior to use. N-Iodomorpholine hydrogen iodide was prepared as previously described. Silica gel was purchased from Fisher (60 Å, 230 x 400 mesh). Acetonitrile was purified using an Innovative Technologies PS-Micro solvent system. Tetrahydrofuran (THF) and low-boiling petroleum ether were used as received.

2.4.2 Reaction Sampling

All kinetic experiments were conducted with a custom-built apparatus similar to that previously reported by our group. The reactions were maintained at the indicated temperature via an Omnical calorimeter. From the reaction vial, 75 µL samples were automatically taken at a rate of 2 mL/min by a Vici M6 liquid handling pump controlled by custom LabView software (UBC Department of Chemistry, Electrical Engineering Services) at defined time points through a PEEK capillary (1/32” outer diameter, 0.15 mm inner diameter) into a 13 µL sample loop attached on a Gilson 918 Injection Valve (rheodyne). Samples were rerouted to a Gilson 215 automated liquid handler robot, which allowed for the dilution of the samples with 487 µL of methanol directly into LC vials containing 0.5 mL of BCA quench solution (see below). The timing of the sampling
technology was governed by the pump that removed the aliquot, triggered the actuation of the rheodyne, and activated the sample dilution and quenching. These samples were manually transferred to the HPLC-MS for analysis as they were prepared or upon completion of the sampling period.

2.4.3 Analytical Methods

The samples were analyzed by HPLC/MS conducted on an Agilent 1290 Infinity system using the following conditions:

Poroshell 120 SB-C18, 2.1 x 100 mm; 2.7 µm

Solvent A = water, 0.05 % trifluoroacetic acid (TFA); Solvent B = MeCN, 0.05 % TFA

Flow Rate = 0.65 mL/min;

Temperature = 35 ºC

Injection Volume = 2 µL

Pump Program: (0 min, 50 % B), (2.3 min, 100 % B).

Integration of the UV-peaks were taken at 230 nm.

$^1$H-NMR spectra were recorded on Bruker NMR spectrometers (300 or 400 MHz). Data for $^1$H-NMR spectra are listed as follows: chemical shift ($\delta$, ppm), multiplicity, coupling constant (Hz), integration, and are referenced to the residual solvent peak (7.26 ppm for CDCl3). Abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. $^{13}$C{$^1$H}-NMR and $^{19}$F{$^1$H}-NMR spectra were recorded on Bruker spectrometers (101 and 377 MHz, respectively), are listed in terms of chemical shift ($\delta$, ppm).

Fourier transfer infrared total attenuated reflection (FTIR-ATR) spectra were recorded on a Perkin-Elmer Frontier spectrometer in the Department of Chemistry’s Shared Instrument Facility and are listed as wavenumbers ($\nu$, cm$^{-1}$). High-resolution mass spectrometry data were acquired by the UBC Department of Chemistry Mass Spectrometry Center.
The relevant kinetic parameters were determined either (a) by applying an exponential fit of the reaction progress data using OriginPro 2016.

2.4.4 Synthetic Procedures

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

This compound was prepared through adaptation of a previous report.\cite{120} A 100 mL two-neck round bottom flask equipped with a magnetic stir bar, water-jacketed condenser, and argon purge was charged with acetonitrile (45 mL). Cu(OAc)$_2$·H$_2$O (65 mg, 0.3 mmol, 2 mol \%) was added in one portion and the mixture was stirred vigorously until a homogeneous, blue solution was obtained. Tripropargylamine (2.1 mL, 14.8 mmol) and cyclopentyl azide (2.9 g, 26 mmol) were dispensed into separate 4 mL vials. Acetonitrile (5 mL) was added to the vial containing the tripropargylamine followed by the neat azide. The mixture was mixed by pipette aspiration and added to the stirring Cu(OAc)$_2$·H$_2$O. An additional 5 mL of acetonitrile was used to rinse the vial and the wash was added to the round bottom flask. The round bottom flask was then placed into a water bath.

L-Ascorbic acid, sodium salt (88 mg, 0.45 mmol) was dissolved in water (5 mL) and sonicated until a homogeneous solution was obtained. The solution was added in one portion to the reaction mixture, upon which the solution turned bright yellow. After stirring for 30 min at room temperature, the flask was then transferred to an oil bath preheated to 45 °C and allowed to stir for 5 h. After 5 h, an additional portion of azide was added and the reaction was stirred for 21 h. HPLC analysis
indicated incomplete conversion and an additional portion of azide was added (0.5 g, 0.3 equiv.) was added and the reaction was allowed to stir for an additional 20 h.

The volatiles were removed by rotary evaporation to give a crude, yellow/brown solid. Dichloromethane (75 mL) was added and the resulting mixture was treated with concentrated ammonium hydroxide (35 mL) and the mixture was allowed to stir vigorously. After all the solids were dissolved, the organic and aqueous layers were separated, and the aqueous layer was extracted with additional DCM (2 x 35 mL). The combined organic fractions were washed with a solution of concentrated ammonium hydroxide and brine (1:1, 4 x 30 mL). (This wash should continue until the aqueous fraction is colorless.) The organic layer was washed with one more portion of brine (30 mL) and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give a bright, canary-yellow solid. A minimal amount of DCM was added to give a transparent solution and diethyl ether was added to precipitate the solid as a white powder. The solid was collected by filtration and washed with diethyl ether. The solid was dried under high vacuum (5.42 g, 79 % yield).

\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \delta 7.74 (s, 3H), 4.96 - 4.84 (m, 3H), 3.73 (s, 6H), 2.30 - 2.16 (m, 6H), 2.10 - 1.96 (m, 6H), 1.96 - 1.81 (m, 7H), 1.80 - 1.67 (m, 3H) } \]

\[ ^13C\{^1H\} \text{ NMR (101 MHz, CDCl}_3 \delta 143.7, 122.3, 61.8, 47.1, 33.4, 24.1 } \]

\[ \text{FTIR-ATR (neat) 3118, 2967, 2874, 1545, 1443, 1361, 1325, 1217, 1141, 1106, 1085, 1045, 1025, 974, 955, 931, 902, 833, 785, 738, 702, 677, 660 cm}^\text{-1} \]

\[ \text{HRMS (ESI-TOF) m/z calculated for C}_{24}\text{H}_{37}\text{N}_{10}, [M + H]^+: 464.3197, \text{ found } 465.3201. \]

2.4.4.2 (iodoethynyl)benzene

![Structure of (iodoethynyl)benzene]
This compound was prepared as previously reported and exhibited characterization data consistent with the literature.¹

2.4.4.3 1-(iodoethyl)-4-methylbenzene

![Chemical structure of 1-(iodoethyl)-4-methylbenzene]

This compound was prepared as previously reported and exhibited characterization data consistent with the literature.¹

2.4.4.4 1-(iodoethyl)-4-(trifluoromethyl)benzene

![Chemical structure of 1-(iodoethyl)-4-(trifluoromethyl)benzene]

To a 20 mL scintillation vial equipped with a magnetic stir bar and screw-top cap was added THF (8 mL, 0.4 M), 1-ethynyl-4-(trifluoromethyl)benzene (0.5 mL, 3.1 mmol), copper(I) iodide (29.2 mg, 0.153 mmol), and N-iodomorpholine–hydrogen iodide (1.15 g, 3.7 mmol). The heterogeneous mixture was allowed to stir vigorously for 45 min at room temperature, during which a fine white precipitate was produced. The mixture was poured into a fritted funnel containing a plug of silica gel (~100 mL dry silica) wet with low-boiling petroleum ether. The plug was washed with more petroleum ether (2 x 75 mL) yielding a cloudy, slightly yellow filtrate. The solvent was removed via rotary evaporation to give the desired product (0.85 g, 94 % yield) as a bright yellow solid.

¹¹H NMR (400 MHz, CDCl₃) δ 7.59 – 7.49 (m, 4H)
\[ ^{1}{H} \text{NMR} \ (101 \text{ MHz, CDCl}_3) \delta \ 132.6, \ 130.9, \ 130.7, \ 130.3, \ 130.0, \ 127.8, \ 127.0, \ 127.1, \ 127.0, \ 125.2, \ 125.3, \ 125.2, \ 125.2, \ 122.5, \ 119.8, \ 92.9, \ 10.3 \]

\[ ^{19}{F} \text{NMR} \ (377 \text{ MHz, CDCl}_3) \delta \ -62.6 \]

\[ \text{FTIR-ATR} \ (\text{neat}) \ 2168, \ 1921, \ 1614, \ 1572, \ 1403, \ 1318, \ 1232, \ 1188, \ 1163, \ 1124, \ 1105, \ 1066, \ 1016, \ 970, \ 954, \ 840, \ 832, \ 753, \ 735 \text{ cm}^{-1} \]

### 2.4.4.5 1,4-diphenylbuta-1,3-diine

![Chemical structure](image)

To a 20 mL scintillation vial equipped with a magnetic stir bar and screw-top cap was added acetonitrile (8 mL, 0.25 M), phenylacetylene (0.55 mL, 5 mmol), (iodoethyl)benzene (1.14 g, 5 mmol), and triethylamine (700 µL, 5 mmol). CuI (95 mg, 10 mol %) was then added in one portion yielding a bright canary-yellow, heterogeneous mixture. The reaction was allowed to stir vigorously for several hours until a homogeneous, light brown solution was obtained. The mixture was then poured over a pad of silica (~100 mL dry silica, wet with low-boiling petroleum ether). The plug was washed with petroleum ether (2 x 150 mL) yielding a transparent filtrate. The solvent was removed via rotary evaporation to give crude product. Off-white crystals were obtained by recrystallization from boiling ethanol (first crop, 0.25 g, 24% yield; non-optimized). The mother liquor can be concentrated to obtain additional crops of crystals.

\[ ^{1}{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3) \delta \ 7.58 – 7.49 \ (m, 4H), \ 7.43 – 7.30 \ (m, 6H) \]

\[ ^{13}{C}\{^{1}{H}\} \text{NMR} \ (101 \text{ MHz, CDCl}_3) \delta \ 132.6, \ 129.4, \ 128.6, \ 121.9, \ 81.7, \ 74.1 \]
2.4.4.6 1,4-di-p-tolylbuta-1,3-diyne

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} \quad \text{H}_3\text{C} \\
& \quad \quad \quad \quad \quad \quad \quad \text{CuI (10 mol \%)} \\
& \quad \quad \quad \quad \quad \quad \quad \text{Et}_3\text{N (4 equiv.)} \\
& \quad \quad \quad \quad \quad \quad \quad \text{MeCN, r.t.}
\end{align*}
\]

To a 20 mL scintillation vial equipped with a magnetic stir bar and screw-top cap was added acetonitrile (5 mL, 0.2 M), 1-ethynyl-4-methylbenzene (0.125 mL, 1 mmol), 1-(iodoethynyl)-4-methylbenzene (242 mg, 1 mmol), and triethylamine (560 \(\mu\)L, 4 mmol). CuI (19 mg, 10 mol \%) was then added in one portion, yielding a bright canary-yellow, heterogeneous mixture. The reaction was then allowed to stir vigorously overnight. The heterogeneous mixture is then poured over a pad of silica (~100 mL dry silica, wet with low-boiling petroleum ether). The plug was then washed with petroleum ether (2 x 150 mL) to give a transparent filtrate. The solvent was removed via rotary evaporation to give crude product. A high-purity, white solid can be obtained by recrystallization from boiling ethanol (first crop, 61 mg, 26\% yield; non-optimized). The mother liquor can be concentrated to obtain additional crops of solid.

\( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)) \( \delta \) 7.46 – 7.39 (m, 4H), 7.18 – 7.11 (m, 4H), 2.37 (s, 6H)

\( ^{13}\text{C}\{^1\text{H}\} \text{NMR} \) (101 MHz, CDCl\(_3\)) \( \delta \) 139.6, 132.5, 129.3, 118.9, 81.6, 73.6, 21.8

2.4.4.7 1,4-bis(4-(trifluoromethyl)phenyl)buta-1,3-diyne

\[
\begin{align*}
\text{F}_3\text{C} & \quad \text{C} \quad \text{F}_3\text{C} \\
& \quad \quad \quad \quad \quad \quad \quad \text{CuI (10 mol \%)} \\
& \quad \quad \quad \quad \quad \quad \quad \text{Et}_3\text{N (2 equiv.)} \\
& \quad \quad \quad \quad \quad \quad \quad \text{MeCN, r.t.}
\end{align*}
\]

To a 20 mL scintillation vial equipped with magnetic stir bar and screw-top cap was added acetonitrile (13.5 mL, 0.1 M), 1-ethynyl-4-(trifluoromethyl)benzene (230 mg, 1.35 mmol), 1-(iodoethynyl)-4-(trifluoromethyl)benzene (400 mg, 1.35 mmol), and triethylamine (375 \(\mu\)L, 2.70 mmol). CuI (26 mg, 10 mol \%) was added in one portion, yielding a bright canary-yellow
heterogeneous mixture. The reaction was then allowed to stir vigorously for several hours until a homogeneous, light-brown solution was obtained. The mixture was then poured over a pad of silica (~100 mL dry silica, wet with low-boiling petroleum ether). The plug was then washed with petroleum ether (2 x 150 mL) to give a transparent filtrate. The solvent was removed via rotary evaporation to give crude product. Transparent crystals can be obtained by recrystallization from boiling ethanol (first crop, 160 mg, 28% yield; non-optimized). The mother liquor can be concentrated to obtain additional crops of crystals.

\[ ^1H \text{ NMR} \ (400 \text{ MHz, CDCl}_3) \delta \ 7.76 - 7.49 (m, 8H) \]

\[ ^{13}C\{^1H\} \text{ NMR} \ (101 \text{ MHz, CDCl}_3) \delta \ 132.8, 131.6, 131.3, 130.9, 130.6, 127.7, 125.5, 125.4, 125.4, 125.3, 125.3, 125.0, 122.3, 119.6, 81.0, 75.6 \]

\[ ^{19}F \text{ NMR} \ (377 \text{ MHz, CDCl}_3) \delta -62.7 \]

2.4.4.8 1-methyl-4-(phenylbuta-1,3-diyln-1-yl)benzene

![Chemical Structure]

To a 10 mL vial equipped with magnetic stir bar and screw-top cap was added acetonitrile (4.00 mL, 0.25 M), 1-ethynyl-4-methylbenzene (116 mg, 1 mmol), (iodoethynyl)benzene (228 mg, 1 mmol), and triethylamine (400 µL, 4 mmol). CuI (19 mg, 10 mol %) was added in one portion yielding a bright canary-yellow heterogeneous mixture. The reaction was then allowed to stir vigorously for several hours until a homogeneous, light-brown solution was obtained. The mixture was then poured over a pad of silica (~100 mL dry silica, wet with low-boiling petroleum ether). The plug was then
washed with petroleum ether (2 x 150 mL) to give a transparent filtrate. The solvent was removed via rotary evaporation to give a crude mixture of products. The residue was trituted with a minimal amount of methanol to give a white solid containing a pure mixture of dimers. The desired heterodimer was not isolated, but the mixture was used in the calibration curves (see procedure below).

**2.4.4.9  1-(phenylbuta-1,3-diyn-1-yl)-4-(trifluoromethyl)benzene**

![Chemical Structure]

To a 10 mL vial equipped with magnetic stir bar and screw-top cap was added acetonitrile (4.35 mL, 0.25 M), 1-ethynyl-4-(trifluoromethyl)benzene (185 mg, 1.1 mmol), (iodoethynyl)benzene (248 mg, 1 mmol), and triethylamine (300 µL, 2.175 mmol). CuI (21 mg, 10 mol %) was added in one portion yielding a bright canary-yellow heterogeneous mixture. The reaction was then allowed to stir vigorously for several hours until a homogeneous, light-brown solution was obtained. The mixture was then poured over a pad of silica (~100 mL dry silica, wet with low-boiling petroleum ether). The plug was then washed with petroleum ether (2 x 150 mL) to give a transparent filtrate. The solvent was removed via rotary evaporation to give a crude mixture of products. The residue was trituted with a minimal amount of methanol to give a white solid containing a pure mixture of dimers. The desired heterodimer was not isolated, but the mixture was used in the calibration curves (see procedure below).
2.4.5 Equilibrium Measurements

2.4.5.1 Preparation of Bicinchoninic Acid (BCA) Quench Solution

To prepare a 0.025 M solution of BCA quench solution, a 25 mL volumetric flask was charged with [2,2'-bipyridine]-4,4'-dicarboxylic acid (bicinchoninic acid; 215 mg, 0.625 mmol) and approximately 20 mL of MeOH. Triethylamine (175 µL, 1.250 mmol) was added and the mixture was shaken to yield a transparent solution. (If there are remaining solids after the addition, additional triethylamine should be added dropwise until a homogeneous solution is obtained.) The volumetric flask was filled to the calibration line and shaken. The flask was wrapped in Parafilm and kept in the fridge when not required. For reaction quenching, the liquid handler was programmed to deliver 13 µL of quench solution (5 equiv. BCA per equiv. of Cu) followed by 487 µL of methanol into a capped LC vial.

Scheme 2.4. Complexation of Cu¹ by two equivalents of BCA.
**Figure 2.6.** Vial containing 0.5 mL of quench solution (left) and vial containing quench solution + 0.5 mL reaction aliquot (right).

2.4.5.2 Method A: General Procedure for Exchange Equilibrium Measurements with No Added Base

A 16 mL vial equipped with magnetic stir bar, flat PTFE-lined septum, and open-top screw cap was charged CuI (4.76 mg, 0.025 mmol), TCPTA ligand (12.0 mg, 0.025 mmol), and 4.5 mL of acetonitrile. The vial was placed in a well of the calorimeter set to 25 °C for approximately 1 h prior to the reaction. To a 1 mL volumetric flask was added the protioalkyne (1 mmol) and the iodoalkyne (1 mmol). Acetonitrile was added to the calibration line and the contents were mixed well. A 1 mL volumetric syringe was filled with 0.5 mL of the alkyne solution and placed in a separate well of the calorimeter set to 25 °C to allow for thermal equilibrium for approximately 1 h prior to the reaction. To initiate the reaction, the alkyne mixture was injected into the vial containing the catalyst solution through the threaded capillary while simultaneously starting the sampling apparatus.
2.4.5.3 Method B: General Procedure for Exchange Equilibrium Measurements with Added Triethylamine Base

![Chemical reaction diagram]

To ensure accuracy in copper content, a stock solution was prepared. A 5 mL volumetric flask was charged with CuI (9.52 mg, 0.05 mmol) and TCPTA ligand (23.0 mg, 0.05 mmol) and filled to the calibration line. After mixing well, 0.5 mL of the solution (providing 0.005 mmol Cu and TCPTA) and 4.0 mL of acetonitrile was added to a 16 mL vial equipped with magnetic stir bar, flat PTFE-lined septum, and open-top screw cap. The reaction vial was placed in a well of the calorimeter set to 25 ºC for approximately 1 h prior to the reaction. To a 1 mL volumetric flask was added the protioalkyne (1 mmol) and the iodoalkyne (1 mmol). Acetonitrile was added to the calibration line and the contents were mixed well. A 1 mL volumetric syringe was filled with 0.5 mL of the alkyne solution and placed in a separate well of the calorimeter set to 25 ºC to allow for thermal equilibrium for approximately 1 h prior to the reaction. To initiate the reaction, the alkyne mixture was injected into the vial containing the catalyst solution through the threaded capillary while simultaneously starting the sampling apparatus.

2.4.6 Calibration Curves

Calibration curves correlating LC chromatogram peak area and concentration were constructed. For each set of experiments, a 2.0 mL stock solution in CHCl₃ of known concentration containing the starting materials and products (roughly 0.1 M for each species) was prepared. The sampling capillary from the prototype apparatus was inserted into the solution and 400 µL was withdrawn through the
sample loop. The rheodyne and liquid handler were triggered, causing the sample to be delivered to a waiting LC and diluted to a total volume of 1 mL. Next, 400 µL of CHCl₃ was added without removing the sampling capillary and the mixture was vortexed well. The sample preparation was repeated for a total of at least 5 samples. The contents of the resulting vials were analyzed by HPLC using the method previously described with integrations taken at 230 nm. The resulting peak areas for each analyte were plotted against their calculated concentration. The equation of least-squares regression fitting for each of the analytes was used to determine concentration for each of the equilibrium measurements.

Table 2.2. Linear regression equations of best fit for calibration data of alkynes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Equation</th>
<th>R² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhCCH</td>
<td>y = 33561x + 2.1469</td>
<td>0.9992</td>
</tr>
<tr>
<td>p-MePhCCH</td>
<td>y = 29251x + 29.524</td>
<td>0.9995</td>
</tr>
<tr>
<td>PhCCI</td>
<td>y = 25602x + 10.291</td>
<td>0.9995</td>
</tr>
<tr>
<td>p-MePhCCI</td>
<td>y = 22743x + 9.2188</td>
<td>0.9995</td>
</tr>
<tr>
<td>p-CF₃PhCCH</td>
<td>y = 29616x + 9.502</td>
<td>0.9992</td>
</tr>
<tr>
<td>p-CF₃PhCCI</td>
<td>y = 17078x - 7.6186</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

Figure 2.7. Calibration curves for selected alkynes.
Figure 2.8. Calibration curves for selected alkynes.

Figure 2.9. Calibration curves for homodimers.
For the heterodimers, a stock solution of the deliberately synthesized mixtures of homo- and heterodimers (section 2.4.4.8 and 2.4.4.9 above) were prepared by adding a known mass of the mixture to CHCl₃ into a 2 mL volumetric flask. At least 8 samples were prepared in a similar fashion to the previous calibration curves. The concentrations of the homodimeric species present in each LC vial was determined from the previously constructed calibration curves. Through mass balance, the concentration of the heterodimeric species and its relative extinction coefficient were determined by the following formula.

$$\varepsilon_{rel} = \frac{[\text{heterodimer}]}{\text{peak area}}$$

The average of all the determined $\varepsilon_{rel}$ for each heterodimer was calculated and the result was used to determine the concentration of the heterodimer in the equilibrium measurements.

**Table 2.4. $\varepsilon_{rel}$ Values for Heterodimers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\varepsilon_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H, CH₃ Heterodimer</td>
<td>72270</td>
</tr>
<tr>
<td>H, CF₃ Heterodimer</td>
<td>70477</td>
</tr>
</tbody>
</table>

2.4.7 Determination of $k_{obs}$

To determine the value of the observed rate constant, $k_{obs}$, for the exchange reaction, the data was fitted in OriginPro 2016 to the following function:

$$y = y_0 + Ae^{(-k_{obs}t)}$$
### Table 2.5. Calculated kinetic parameters for exchange reaction.

<table>
<thead>
<tr>
<th>entry</th>
<th>species</th>
<th>$k_{\text{obs}}$ (min$^{-1}$)</th>
<th>$k_{\text{obs, avg}}$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.1</td>
<td>2.1a', 2.2a'</td>
<td>0.00906, 0.00927</td>
<td>0.00917</td>
</tr>
<tr>
<td>2.1.2</td>
<td>2.1b', 2.2b'</td>
<td>0.00886, 0.00884</td>
<td>0.00885</td>
</tr>
<tr>
<td>2.1.3</td>
<td>2.1a', 2.2a'</td>
<td>0.06177, 0.06482</td>
<td>0.06330</td>
</tr>
<tr>
<td>2.1.4</td>
<td>2.1b', 2.2b'</td>
<td>0.05902, 0.06199</td>
<td>0.06051</td>
</tr>
<tr>
<td>2.1.5</td>
<td>2.1c', 2.2a'</td>
<td>0.01664, 0.01642</td>
<td>0.01653</td>
</tr>
<tr>
<td>2.1.6</td>
<td>2.1b', 2.2c'</td>
<td>0.00884, 0.00883</td>
<td>0.00884</td>
</tr>
<tr>
<td>2.1.7</td>
<td>2.1c', 2.2a'</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.1.8</td>
<td>2.1b', 2.2c'</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Chapter 3: Interrogating the Role of the Counter-Ion in Copper-Catalyzed Azide-Alkyne Cycloaddition Reactions

3.1 Introduction

In 2009, Hein, Fokin, and co-workers disclosed a general and regiospecific means to obtain 5-iodo-1,4,5-trisubstituted-1,2,3-triazoles from 1-iodoalkynes and organic azides (Scheme 3.1a). This transformation, mediated by CuI and a tris(triazolylmethyl)amine accelerating ligand, was the first efficient method to access these halogenated heterocycles under mild conditions. As an extension of the well-known Copper-catalyzed Azide-(terminal) Alkyne Cycloaddition (“CuAAC”, Scheme 3.1b) that exclusively affords 5-protio-1,2,3-triazoles, the CuI-mediated azide-iodoalkyne cycloaddition provided easy access to a diverse array of triazoles directly amenable to further functionalization via the halogen substituent.

![Scheme 3.1. Copper-mediated azide-alkyne cycloaddition.](image)

Although applications incorporating iodotriazoles are at early stages, they already have prominent roles in multicomponent synthetic methods, halogen-bonding and ion-recognition, materials fabrication, and biomedical research. The limited number of uses of iodotriazoles compared to their 5-protio counterparts might be due to the small number of robust...
methods for iodo-triazole synthesis and can be attributed, in part, to poorly understood mechanistic details and catalyst scope of the reaction.

Despite the time elapsed since our original report, mechanistic studies on copper-mediated iodo-triazole formation remain incomplete. We initially posited two mechanisms for iodo-triazole formation, favoring a pathway in which the iodoalkyne $C_{sp^2}$–I bond was not severed during catalysis. The alternative pathway, where a copper acetylide intermediate resulting from copper insertion into the C–I bond of the iodoalkyne, was disfavored due to the lack of 5-protiotriazole formation from protonolysis of copper-bound intermediates.\textsuperscript{88, 98}

Heaney and co-workers offered an alternative mechanistic interpretation based on their studies of copper acetylide ladderane complexes, though no kinetic data were provided.\textsuperscript{137} Later, Díez-González and co-workers reported the first computational investigation employing copper bearing N-heterocyclic carbene (NHC) ligands as representative catalysts.\textsuperscript{93} The most recent reports by Zhu and co-workers have focused on the formation of iodo-triazoles from terminal alkynes. In their work, an iodoalkyne is generated \textit{in situ} via the stoichiometric reaction of $\text{Cu(ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, KI, and Et$_3$N.\textsuperscript{138, 139} However, a systematic analysis of the factors influencing copper-mediated iodo-triazole formation under catalytic conditions has yet to be reported.

During our mechanistic investigations of the CuI-catalyzed azide-iodoalkyne cycloaddition, we observed confounding phenomena that eluded our understanding. In particular, we were intrigued by the results of competition experiments where both 1-iodoalkyne \textbf{3.2} and terminal alkyne \textbf{3.3} were simultaneously subjected to triazole-forming conditions (Scheme 3.2). Reaction progress monitoring revealed an unusual profile bearing three distinct regimes: (i) rapid cycloaddition between the azide \textbf{3.1} and iodoalkyne \textbf{3.2} affording exclusive and quantitative generation of the corresponding iodo-triazole \textbf{3.4}, (ii) a well-defined period with no product generation and an absence of catalytic activity, and (iii) cycloaddition to generate protiotriazole \textbf{3.5}, with a sigmoidal profile.
Given these observations in the face of the well-known (and well-assumed) reliability of copper-catalyzed “click” reactions, we sought to elucidate the chemical underpinnings of the three regimes. Through delineation of the complex catalytic network that exists within this deceptively simple system, this report provides detailed kinetic data that supports the following conclusions: (i) the copper-catalyzed cycloadditions to form 5-protoptriazoles and 5-iodotriazoles are mechanistically distinct, (ii) the catalyst counter-ion has a linchpin role in facilitating the chemoselective generation of 5-iodotriazoles from 1-iodoalkynes in the presence of terminal alkynes, (iii) “activation” of the requisite catalyst for protiotriazole synthesis is highly influenced by the nature of the catalyst counter-ion, and last (iv) a more nuanced interpretation of the role of copper acetylides in triazole synthesis is required. Ultimately, new knowledge is provided that can be harnessed in the development of new chemoselective methods where 1-iodoalkynes and terminal alkynes are simultaneously present.

![Scheme 3.2](image)

**Scheme 3.2.** Competition reaction of benzyl azide (3.1, 0.1 M), (iodoethynyl)benzene (3.2, 0.05 M), and phenylacetylene (3.3, 0.05 M). (TCPTA = tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine).

### 3.2 Initial Kinetic Analysis

To elucidate the differences between the cycloaddition mechanism of iodoalkynes and that of terminal alkynes, we began with a standard reaction progress kinetic analysis (RPKA). These reactions were monitored by heat-flow calorimetry, offering direct access to the reaction rate. Figure 3.1a gives the rate profile for the individual reaction of benzyl azide (3.1, 0.10 M) and terminal alkyne 3.3 (0.10 M) under standard conditions (Scheme 3.2). Upon initiation, the system is subject to an induction process wherein the rate *increases* until a maximum value at ~40% conversion, which is
followed by a period of overall first-order kinetics. Figure 3.1b (red curve) gives the corresponding heat-flow conversion profile for this reaction. Similar rate behavior had previously been reported by Blackmond and co-workers in a Heck-type reaction.\textsuperscript{140} The data suggest that the active catalyst concentration increases until maximum turnover as a result of an irreversible activation.

![Figure 3.1](image)

**Figure 3.1.** (a) Rate profile for the individual reaction of benzyl azide (3.1, 0.10 M) and phenylacetylene (3.3, 0.10 M) under standard conditions (Scheme 3.2). (b) Conversion profiles for the individual protiotriazole reaction for the first addition of substrate (red curve, right) and the second addition of substrate (blue curve left). (c) Rate profiles for “excess” experiments for the second addition of starting materials as measured by heat-flow calorimetry. (d) Rate profiles for the “different excess” experiments of the iodotriazole cyclization to determine order in azide and iodoalkyne.

To probe this activation process, we then charged the reaction vial with additional starting material (azide 3.1 + terminal alkyne 3.3) and continued to monitor the heat flow. From Figure 3.1b
(blue curve, left), it is clear that the initiation of the second protiotriazole reaction is much more straightforward, exhibiting no induction behavior and obeying overall first-order kinetics. Strikingly, despite the decrease in reaction concentration (nearly half that of the first reaction) following the second addition of substrate, we see that the rate of protiotriazole formation is much larger, with full conversion achieved in almost half of the time of the unactivated system.

“Excess” experiments performed following activation (Figure 3.1c) revealed that the individual protiotriazole reaction has a first-order dependence on terminal alkyne 3.3 and a zero-order dependence on azide 3.1, represented by the rate law in eq. 3.1 below.

\[
\text{rate} = k_H [\text{cat}]_0 [\text{terminal alkyne}] \quad \text{(eq. 3.1)}
\]

In contrast, RPKA of the individual iodotriazole reaction is shown in Figure 3.1d. Different “excess” experiments revealed that this cycloaddition displays saturation kinetics in iodoalkyne 3.2 and first-order kinetics in azide 3.1, affording rate eq. 3.2, where \([xs] = [azide]_0 - [iodoalkyne]_0\).

\[
\text{rate} = k_I [\text{cat}]_0 [\text{azide}] = k_I [\text{cat}]_0 ([xs] + [iodoalkyne]) \quad \text{(eq. 3.2)}
\]

The observation of saturation kinetics in the azide-iodoalkyne cycloaddition can be described by a Michaelis-Menten-like model (Scheme 3.3).

![Scheme 3.3. Michaelis-Menten model of the iodotriazole-forming catalytic cycle.](image-url)
The overall rate of reaction is given by

\[
\text{rate} = k_2[\text{int}][\text{azide}].
\]  \hspace{1cm} (eq. 3.3)

Assuming steady-state conditions, we have

\[
\frac{d[\text{int}]}{dt} \approx 0 = k_1[\text{cat}][\text{iodoalkyne}] - k_{-1}[\text{int}] - k_2[\text{int}][\text{azide}].
\]  \hspace{1cm} (eq. 3.4)

From mass balance,

\[
[\text{cat}]_0 = [\text{cat}] + [\text{int}]
\]  \hspace{1cm} (eq. 3.5)

which means that

\[
[\text{cat}] = [\text{cat}]_0 - [\text{int}].
\]  \hspace{1cm} (eq. 3.6)

Substituting the right-hand side of eq. 3.6 into the steady-state eq. 3.4, we have

\[
0 = k_1 ([\text{cat}]_0 - [\text{int}]) [\text{iodoalkyne}] - k_{-1}[\text{int}] - k_2[\text{int}][\text{azide}]
= k_1[\text{cat}]_0[\text{iodoalkyne}] - k_1[\text{int}][\text{iodoalkyne}] - k_{-1}[\text{int}] - k_2[\text{int}][\text{azide}]
\]  \hspace{1cm} (eq. 3.7)

Rearranging eq. 3.7 gives

\[
k_1[\text{int}][\text{iodoalkyne}] + k_{-1}[\text{int}] + k_2[\text{int}][\text{azide}] = k_1[\text{cat}]_0[\text{iodoalkyne}],
\]  \hspace{1cm} (eq. 3.8)

and factoring gives

\[
[\text{int}] (k_1[\text{iodoalkyne}] + k_{-1} + k_2[\text{azide}]) = k_1[\text{cat}]_0[\text{iodoalkyne}],
\]  \hspace{1cm} (eq. 3.9)

which can be rewritten as

\[
[\text{int}] = \frac{k_1[\text{cat}]_0[\text{iodoalkyne}]}{k_1[\text{iodoalkyne}] + k_{-1} + k_2[\text{azide}]}.
\]  \hspace{1cm} (eq. 3.10)
Substituting the right-hand side of eq. 3.10 into the equation 3.3 gives

$$\text{rate} = \frac{k_2 k_1 [\text{cat}]_0 [\text{iodoalkyne}][\text{azide}]}{k_1 [\text{iodoalkyne}] + k_{-1} + k_2 [\text{azide}]}.$$  \hspace{1cm} (eq. 3.11)

Dividing eq. 3.11 by $k_1$ affords the “one-plus” form, eq. 3.12

$$\text{rate} = \frac{k_2 K_1 [\text{cat}]_0 [\text{iodoalkyne}][\text{azide}]}{K_1 [\text{iodoalkyne}] + 1 + \frac{k_2}{k_{-1}} [\text{azide}]} = \frac{k_2 K_1 [\text{cat}]_0 [\text{iodoalkyne}][\text{azide}]}{1 + K_1 [\text{iodoalkyne}] + \frac{k_2}{k_{-1}} [\text{azide}]}.$$  \hspace{1cm} (eq. 3.12)

If $K_1 \gg k_2 / k_{-1}$, then the last term of the denominator can be neglected, yielding

$$\text{rate} = \frac{k_2 K_1 [\text{cat}]_0 [\text{iodoalkyne}][\text{azide}]}{1 + K_1 [\text{iodoalkyne}]}.$$  \hspace{1cm} (eq. 3.13)

If $K_1[\text{iodoalkyne}] \gg 1$ (saturation kinetics), then

$$\text{rate} = k_2 [\text{cat}]_0 [\text{azide}].$$  \hspace{1cm} (eq. 3.14)

If we define

$$[\text{xs}] = [\text{azide}] - [\text{iodoalkyne}]$$  \hspace{1cm} (eq. 3.15)

then

$$\text{rate} = k_2 [\text{cat}]_0 ([\text{xs}] + [\text{iodoalkyne}]),$$  \hspace{1cm} (eq. 3.16)

which is reflected by the graphical rate equation in Figure 3.1d.
3.3 Unexpected Results from Competition Reactions

The need for further mechanistic study of copper-mediated azide-alkyne cycloaddition was spurred by intriguing kinetic data obtained from competition reactions involving benzyl azide (3.1, 0.10 M), (iodoethynyl)benzene (3.2, 0.05 M), and phenylacetylene (3.3, 0.05 M; Scheme 3.2 and Figure 3.2). The competition was monitored by high-performance liquid chromatography-mass spectrometry (HPLC-MS) by removing aliquots of the reaction at specified intervals via an automated sampling apparatus developed by our group.\textsuperscript{61,64,65} It should be noted that the exceptional fidelity of the copper catalyst makes it impossible to sufficiently arrest the reaction aliquot for off-line analysis by simple dilution. Instead, application of a bicinchoninic acid (BCA) quenching procedure, which we have previously reported\textsuperscript{62} and discussed in Chapter 2, is crucial as it does not destroy or alter any of the reaction components.

![Figure 3.2](image)

**Figure 3.2.** Reaction progress graph of the competition in Scheme 3.2 depicting three distinct regimes. (Reaction conditions: CuI, 5 mol %; TCPTA, 5 mol %, MeCN, 25 °C.)

Upon examination of the reaction progress (Figure 3.2), three distinct regimes were observed: The reaction first proceeds with exclusive and quantitative formation of ioddotriazole (3.4) (0−50 min;
regime I), followed by a period with no apparent change in chemical speciation (50–175 min; regime II). Afterward, the reaction proceeds to completion with the formation of protiotriazole 3.5 (>175 min; regime III). Notably, the sigmoidal profile of 3.5 indicates that some type of in situ activation process is present during the last regime, suggesting that the initial complex formed by simply dissolving the CuI and TCPTA ligand is competent in facilitating cycloaddition with iodoalkyne 3.2 but not terminal alkyne 3.3. With these observations, three questions required answering:

(1) Why does iodotriazole formation precede protiotriazole generation?
(2) What occurs during the “interim” period, regime II?
(3) Why is protiotriazole formation sigmoidal?

3.4 Is the Competition Behavior Due to the Presence of the Iodoalkyne or Iodotriazole?

To rule out the possibility that competition behaviors in Figure 3.2 were due to the presence of iodoalkyne 3.2 or iodotriazole 3.4, we collected reaction progress data on the individual cycloaddition reactions where iodoalkyne 3.2 or terminal alkyne 3.3 was separately subjected to the conditions in Scheme 3.2. As seen in Figure 3.3, graphical overlay of the reaction progress data from individual (open markers) and competition reactions (closed markers) strongly indicates that the respective catalytic reactions to form iodotriazole 3.4 during regime I and protiotriazole 3.5 during regime III (Figure 3.3) are identical to those in the individual reactions where iodotriazole 3.4 or protiotriazole 3.5 are the sole products. Otherwise stated, the mechanisms of iodotriazole formation appear to be invariant regardless of whether or not a terminal alkyne is present and, likewise, the presence of iodoalkyne 3.2 or iodotriazole 3.4 does not change the overall mechanisms of protiotriazole formation.
3.5 Influence of Iodide

We then returned to the competition reaction to determine if the kinetic phenomena of Figure 3.2 were still present following a second charge of starting material. Similar to the heat-flow calorimetry experiments, we allowed a competition reaction identical to that in Scheme 3.2 to go to completion, added a second charge of substrate (azide 3.1 + iodoalkyne 3.2 + terminal alkyne 3.3), and monitored the reaction by HPLC. Strikingly, as shown in Figure 3.4, we now see concomitant consumption of both alkynes and the simultaneous appearance of triazoles 3.4 and 3.5. That is, the observed chemoselectivity in Figure 3.2 is no longer present, implying that the component controlling chemoselectivity during the first competition reaction (after the first charge of substrate) was removed as a direct result of triazole formation.

In order to understand this drastic change in chemoselectivity, we re-examined the concentration data in Figure 3.2. Upon close examination, we see that the concentration of iodo triazole 3.4 shows an increase of exactly 5 mol % by the end of regime III relative to the end of

Figure 3.3. Overlay of reaction progress graphs for individual triazole reactions of azide 3.1 (0.10 M) with iodoalkyne 3.2 (0.05 M) or terminal alkyne 3.3 (0.05 M; open markers) and the competition reaction (Scheme 3.2; closed markers). (The azide concentration is not plotted for clarity.)
regimes I and II. This rise, equal to the amount of iodide from CuI, occurs throughout the protiotriazole generation, indicating that the consumption of the catalyst counter-ion and its corresponding generation of iodotriazole are related to catalyst activation for protiotriazole formation.

Figure 3.4. HPLC conversion profiles for the second addition of starting material (azide 3.1, iodoalkyne 3.2, and terminal alkyne 3.3) following the competition reaction in Scheme 3.2. (The azide conversion is not plotted for clarity.)

Figure 3.5. Reaction progress graph of competition from Scheme 3.2 where AgNO₃ (0.025 mmol, 5 mol %) was added after 90 min, leading to the immediate formation of protiotriazole. (The azide concentration is not plotted for clarity.)
Following this, we then wanted to see if the deliberate removal of the catalyst counter-ion would also change the kinetic behavior of the competition. Figure 3.5 gives the reaction progress of an experiment identical to that in Scheme 3.2; however, after 90 min and during regime II, AgNO₃ was added to deliberately remove iodide by precipitation of AgI. As seen in Figure 3.5, protiotriazole generation begins immediately in the now activated system.

### 3.6 Counter-Ion Effects

The competition was then carried out using a variety of copper salts, revealing that the nature of the counter-ion has a significant impact on both the chemoselectivity and rate of cycloaddition (Figure 3.6a-c). For these experiments, all reaction parameters in Scheme 3.2 were held constant except for the copper source. From Figure 3.6a-c, it seems that Cu(I) salts bearing soft anions (I⁻, PhS⁻) are efficient catalysts of the 1-iodoalkyne cycloaddition but appear to require in situ activation to efficiently catalyze the reaction with terminal alkynes, which is reflected by the sigmoidal curve of the concentration profiles of triazole 3.5 (Figure 3.6a cf. Figure 3.2). This results in the selective generation of iodotriazole 3.4 at the onset of catalysis, followed by formation of protiotriazole 3.5 with induction behavior. In contrast, Cu(I) salts bearing hard anions (OAc⁻, BF₄⁻) allow both triazoles to form simultaneously (Figure 3.6b,c).

As a corollary to our previous experiment with AgNO₃, we then monitored a reaction where iodide was deliberately added to the reaction. Figure 3.6d depicts the reaction progress for the competition reaction catalyzed by (MeCN)₄CuBF₄, which normally affords iodotriazole 3.4 and protiotriazole 3.5 concomitantly. However, here NaI was introduced into the operational reaction, selectively arresting formation of protiotriazole 3.5 but not iodotriazole 3.4. These data indicate that iodide, or similarly soft anions, is uniquely responsible for inhibiting the cycloaddition of the terminal alkyne, while no such inhibitory role is experienced by the iodoalkyne cycloaddition.
3.7 Ruling Out Electrophilic Capture of Iodine Prior to Protiotriazole Generation

To explain the inhibitory nature of iodide, we examined the currently accepted mechanisms for CuAAC. Recent work from Bertrand and co-workers has shown two pathways for the generation of protiotriazoles, with each differing in the nuclearity of copper (Scheme 3.4).\textsuperscript{141} As a result, we envisioned three locations where iodide could interfere with protiotriazole formation: (i) iodide or polyiodides, such as I\textsuperscript{3−}, may intercept copper triazolide 3.8, allowing only for the selective formation of iodotriazole 3.4, (ii) tight binding to copper or insufficient Brønsted basicity of iodide could prevent the formation of acetylide 3.7, or (iii) iodide may inhibit the formation of dicopper complex 3.9, which was identified by Bertrand and co-workers as the key intermediate in the kinetically favored pathway.\textsuperscript{141}
Scheme 3.4. Accepted pathways for protiotriazole formation, adapted from Bertrand et al.\textsuperscript{141}

The presence of copper triazolide 3.8 (Scheme 3.4) in protiotriazole synthesis was originally indirectly validated by Finn, Fokin, and co-workers.\textsuperscript{107} In an analogous treatment, we reacted diazide 3.11 and alkynes 3.2 and 3.3 as a triazolide probe for the CuI system (Scheme 3.5). In Figure 3.7, the first regime (0–70 min) shows exclusive formation of mono(iodotriazole) 3.12 and bis(iodotriazole) 3.13. The system then enters a stasis period of \(~\)125 min, followed by concurrent formation of iodo/protiotriazole 3.14 and bis(protiotriazole) 3.15 during the final regime.

Via the same logic of Finn et al., the sequential appearance of mono(iodotriazole) 3.12 followed by bis(iodotriazole) 3.13 in Figure 3.7 suggests that a copper triazolide is not involved in the CuI-catalyzed cycloaddition of 1-iodoalkynes. However, the direct formation of bis(protiotriazole) 3.15 avoiding mono(protiotriazole) indicates that cycloaddition involving the terminal alkyne does indeed proceed via this intermediate. Thus, the two cycloadditions do not involve common reactive intermediates and are better described as orthogonal pathways. Furthermore, this rules out the possibility that the chemoselectivity during regime I of the competition (Scheme 3.2) is due to capture of the triazolide 3.8. Our conclusions are in agreement with observations by Zhu and co-workers who noted that exogenous electrophiles were not incorporated under their experimental conditions.\textsuperscript{139}
Scheme 3.5. Competition reaction involving 1,3-diazide 3.11 (0.05 M), (iodoethynyl)benzene (3.2, 0.05 M), and phenylacetylene (3.3, 0.05 M).

Figure 3.7. Reaction progress graph for Scheme 3.5.
3.8 Ruling Out Inhibition of Copper Acetylide Formation

Lastly, we studied competition reactions employing differently substituted iodoalkynes and terminal alkynes (3.2 and 3.16a,b, respectively; Scheme 3.6). These reactions catalyzed by CuI still display three discrete reactivity regimes; however, the product distribution is significantly more complicated. Although iodotriazole 3.4 is the major product in the first regime, a separate iodotriazole (3.17a,b) is also observed (Figure 3.8a,b). The generation of the minor iodotriazole occurs with concurrent consumption of corresponding terminal alkyne 3.16a,b and production of phenylacetylene (3.3). After the iodoalkynes are consumed, the reaction pauses before cycloaddition continues with the remaining terminal alkynes, producing 3.18a,b and 3.5 as the major and minor protiotriazoles, respectively. Again, the profiles in this last regime are sigmoidal.

Interestingly, the length of the intermediate period between the first and third regimes seems to track with the acidity of the starting terminal alkyne. Relative to unsubstituted phenylacetylene (3.3), the competition reaction involving electron-deficient p-CF3-substituted terminal alkyne 3.16a experiences a shorter interim period (~75 min; Figure 3.8a cf. Figure 3.2). Conversely, the reaction with electron-rich p-CH3-substituted terminal alkyne 3.16b experiences a longer interim period (~175 min) relative to unsubstituted 3.3 (Figure 3.8b cf. Figure 3.2).

Scheme 3.6. Crossover studies with benzyl azide (3.1, 0.10 M), (iodoethynyl)benzene (3.2, 0.05 M), and para-substituted phenylacetylenes (3.16a,b, 0.05 M).
The formation of 3.17a,b and 3.5 can be explained by exchange between the acetylenic hydrogen/iodine of 3.4 and 3.16a,b to give 3.3 and 3.16a',b' (Scheme 3.7). This process has recently been studied and has been shown to likely proceed via a copper-catalyzed σ-metathesis.\(^\text{62}\) The observation of exchange implies that a copper acetylide is present during the initial regime of the competition and is capable of undergoing exchange but not cycloaddition to give protiotriazole. We conclude that the generation of the copper acetylide is possible during iodonitriazole synthesis; however, it alone is inadequate for CuAAC in the presence of iodide or iodoalkynes.

![Figure 3.8](image)

**Figure 3.8.** Reaction progress graph of the crossover experiments in Scheme 3.6.

3.9 Expanded Catalytic Network

The observations presented thus far combined with the large body of experimental\(^\text{108, 107, 108, 112, 117, 139, 141-147}\) and theoretical\(^\text{111, 148-154}\) investigation on CuAAC can be used to construct a more detailed
reaction manifold (Scheme 3.8). When terminal and iodoalkynes are simultaneously present, coordination of iodoalkyne 3.19 to the copper catalyst to give 3.20 is heavily favored \((K_1 \gg 1)\), and from this intermediate, two pathways are possible: First, coordination of azide 3.21 to complex 3.20 can lead to iodotriazole formation. This equilibrium is thought to also be favored \((K_2 > 1)\) because the iodinated terminal alkyne resulting from exchange \((3.16a,b'\); Scheme 3.7) does not reach measurable concentrations in competition reactions, despite formation of minor triazoles 3.5 and 3.17a,b (Scheme 3.6). This is not the case for Cu(I)-catalyzed terminal alkyne/1-iodoalkyne H/I exchange in the absence of azide.62

Alternatively, if terminal alkyne 3.25 binds to 3.20, dialkyne intermediate 3.26 would form, providing an avenue for H/I exchange. Consistent with our previous study, we expect deprotonation of the \(\pi\)-bound terminal alkyne in 3.26 to afford acetylide 3.27 to be turnover-limiting for exchange \((k_5)\). Subsequently, \(\sigma\)-bond metathesis would afford acetylide 3.28, and protonolysis would give the exchanged terminal alkyne 3.19'. The observed rate of exchange is greater for electron-deficient alkynes than that for electron-rich alkynes, accounting for the larger concentration of minor triazoles 3.17a and 3.18a compared to 3.17b and 3.18b (Figure 3.8a,b).

Once the iodoalkyne is consumed, free catalyst 3.6 becomes the dominant copper species. The preceding discussions have already ruled out triazolide interception and interference with copper acetylide formation as the source for the observed chemoselectivity during the competition. Therefore, it follows that iodide must interfere with the formation of dicopper species 3.30 \((K_9)\), which serves as the linchpin to access the kinetically favored protiotriazole-forming pathway. For the third regime of the competition to begin, we propose oxidation of the anion as a means for catalyst activation. While the exact mechanism remains unclear, the interim can be explained by the need to oxidatively remove the inhibitory counter-ion, possibly from diffusion of oxygen into the system.
Scheme 3.8. Expanded catalytic network detailing competitive cycloaddition and H/I exchange involving 1-iodoalkynes and terminal alkynes.
For CuI, oxidation of the counter-ion leads to the formation of $\text{I}^-_3$ or other electrophilic iodide species, which can be captured by acetylide, generating additional 1-iodoalkyne. In this case, iodide and some of the terminal alkyne will be converted into iodotriazole 3.24, explaining the slight increase in iodotriazole products during the last regime of the competition reaction (Figure 3.3). Analogously, when employing CuSPh as the catalyst, the thiophenolate anion can be sequestered by oxidative dimerization to make diphenyl disulfide. Once these detrimental anions are consumed, dinuclear complex 3.30 can be formed, leading to the protiotriazole-forming cycle.

3.10 Conclusions

This report has provided a detailed kinetic interrogation of the role of the catalyst counter-ion in the copper-catalyzed azide-alkyne cycloaddition of organic azides with either terminal or 1-iodoalkynes. On the basis of a systematic study of competition reactions involving 1-iodoalkynes, terminal alkynes, and organic azides with different copper catalysts, it was discovered that the chemoselective formation of iodotriazoles from 1-iodoalkynes in the presence of terminal alkynes is a function of the disposition and speciation of the copper catalyst within a more complicated reaction network.

Critically, the observed chemoselectivity is not due to a relative rate difference between the two cycloaddition reactions. For the CuI-catalyzed system, the experimental data suggest that iodide inhibits the formation of a key dicopper intermediate in the kinetically favored protiotriazole-forming pathway, explaining the initial exclusive formation of iodotriazole in the first reactivity regime. The consumption of the counter-ion via the formation of extra iodotriazole then allows for protiotriazole formation in the final reactivity regime. Furthermore, a previously unrecognized acetylenic hydrogen/iodine exchange pathway was identified and placed into context within the larger reaction manifold.
Ultimately, this study offers two important advances. First, the added mechanistic and kinetic understanding of this important copper-catalyzed reaction provides new opportunities to develop other chemoselective methods where terminal and 1-idoalkynes are simultaneously present in the reaction. With these two species, judicious choice of the catalyst counter-ion is evidently critical to achieve differential reactivity. Second, this study reinforces the importance of continued developments in modern reaction analytical methodologies; the automated reaction monitoring technology used here has enabled rapid deconvolution of a complex and challenging reaction in reasonable time frames. The rich, impactful, and highly nuanced information revealed by the experiments detailed in this Chapter would have been undoubtedly overlooked had highly resolved, time course measurements not been available.

3.11 Experimental

3.11.1 Chemical Suppliers

Phenylacetylene was purchased from VWR, 1-ethynyl-4-methylbenzene and 1-ethynyl-4-(trifluoromethyl)benzene were purchased from Aldrich. All terminal alkynes were passed through a pipette of dry silica gel prior to use. CuI, CuBr, and CuCl were purchased from Strem Chemicals. CuSPh was purchased from Sigma-Aldrich. (MeCN)$_4$CuBF$_4$ was purchased from TCI Chemicals. Benzyl azide and cyclopentyl azide were prepared as previously described.$^{155}$ N-Iodomorpholine hydrogen iodide was prepared as previously described.$^{88}$ All other reagents were purchased from conventional suppliers and used as received unless otherwise stated. Acetonitrile was purified using an Innovative Technologies PS-Micro solvent system. All other solvents were used as received unless otherwise stated. Silica gel was purchased from Silicycle (60 Å, 230 x 400 mesh). Analytical TLC plates were purchased from Merck KGaA and visualized by UV (254 nm) or potassium permanganate staining.
3.11.2 Reaction Sampling

For homogenous reactions, kinetic experiments were conducted with a custom-built apparatus similar to that previously reported by our group.3 The reactions were maintained at the indicated temperature via an Omnical calorimeter. From the reaction vial, 75 µL samples were automatically taken at 2 mL/min by a Vici M6 liquid handling pump controlled by custom LabView software (UBC Department of Chemistry, Electrical Engineering Services) at defined time points through a PEEK capillary (1/32” outer diameter, 0.15 mm inner diameter) into a 13 µL sample loop attached on a Gilson 918 Injection Valve (rheodyne). Samples were rerouted to a Gilson 215 automated liquid handler robot, which allowed for the dilution of the samples with 487 µL of methanol directly into LC vials containing 0.5 mL of BCA quench solution (see below). The timing of the sampling technology was governed by the pump that removed the aliquot, triggered the actuation of the rheodyne, and activated the sample dilution and quenching. These samples were manually transferred to the HPLC-MS for analysis as they were prepared or upon completion of the sampling period.

For heterogeneous reactions, kinetic experiments were conducted with a custom-built slurry sampling apparatus based off a Mettler-Toledo EasySampler interfaced with a Gilson GX-281 liquid handler.4 The EasySampler contains a motor driven scoop, capturing a nominal 20 µL of the reaction from the reactor. Prior to the reaction, the EasySampler probe was placed in the reaction vial for at least 1 h to ensure thermal equilibration prior to the reaction. The timing and triggering of the probe were conducted by the Gilson GX-281. When sampling, an electrical signal is sent from the Gilson GX-281 to custom-built control circuit (UBC Department of Chemistry, Electrical Engineering Services). The first signal makes the actuator extend down and the second signal makes will retract the sample tip. Upon retraction, scoop was flushed with methanol (750 µL) and dispensed directly into LC vials containing 250 µL of BCA quench (see below).
3.11.3 Reaction Calorimetry

Reaction calorimetry experiments were carried out in an Omnical Insight reaction calorimeter in a 16 mL vial equipped with a magnetic stir bar, open-top screw cap, and PTFE-lined silicone septum. Detailed experimental procedures are listed for each type of experiment below. Generally, the reaction vials were charged with solvent, catalyst, and ligand and allowed to stir at the indicated reaction temperature until thermal equilibrium was reached (as indicated by no change in the reaction’s instantaneous heat flow), upon which the substrate was injected. Reaction conversions determined by the integrated heat flow were verified by offline HPLC-MS analysis of the reaction mixture.

3.11.4 Analytical Methods

NMR spectra were recorded on Bruker NMR spectrometers. Data for $^1$H NMR spectra are listed as follows: chemical shift (δ, ppm), multiplicity, coupling constant (Hz), integration, and are referenced to the residual solvent peak. Abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. $^{13}$C{$^1$H} NMR and $^{19}$F{$^1$H} NMR spectra were recorded on Bruker spectrometers and are listed in terms of chemical shift (δ, ppm),

HPLC/MS analysis was performed on an Agilent 1260 HPLC-MS with the following methods:

Poroshell 120 Phenyl Hexyl Column (2.1 x 50 mm; 2.7-Micron)
Flow Rate = 0.700 mL/min; Temperature = 35 °C; 2 µL injection
Solvent A = water, 0.1% trifluoroacetic acid (TFA); Solvent B = acetonitrile, 0.1% TFA
Method: 0 min, 30% B; 3 min, 55% B

Integration of the UV-peaks were taken at 230 nm for all compounds except for benzyl azide (210 nm). Concentration values were obtained by calibration curves of concentration and peak area.

Fourier transfer infrared total attenuated reflection (FTIR-ATR) spectra were recorded on a Perkin-Elmer Frontier spectrometer in the Department of Chemistry’s Shared Instrument Facility and
are listed as wavenumbers (XX, cm–1). High-resolution mass spectrometry data were acquired by the UBC Department of Chemistry Mass Spectrometry Center.

3.11.5 Synthetic Procedures

3.11.5.1 tris((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (TCPTA)

This compound was prepared as previously reported and exhibited consistent characterization data.62

3.11.5.2 (iodoethynyl)benzene

This compound was prepared as previously reported and exhibited consistent characterization data.2

3.11.5.3 1-(iodoethynyl)-4-(trifluoromethyl)benzene

This compound was prepared as previously reported and exhibited consistent characterization data.62

3.11.5.4 1-(iodoethynyl)-4-methylbenzene
This compound was prepared as previously reported and exhibited consistent characterization data.\(^{62}\)

### 3.11.5.5 General Procedure for the Synthesis of 5-Iodotriazoles

![Chemical Structure]

To a 20 mL scintillation vial equipped with magnetic stir bar and screw cap was added CuI (9.52 mg, 0.05 mmol, 5 mol %) and TCPTA (23 mg, 0.05 mmol, 5 mol %) followed by 9 mL THF. The resulting mixture was allowed to stir vigorously until a homogeneous solution was obtained. Meanwhile, iodoalkyne (1 mmol, 1 equiv.) and benzyl azide (146 mg, 1.1 mmol, 1.1 equiv.) was weighed into an LC vial. (If the reaction is performed at larger scale, the substrates should be weighed into separate vials to reduce the risk of a runaway reaction). The remaining 1 mL of THF was used to rinse the LC vial and added to the reaction mixture (10 mL total reaction volume; 0.1 M). The contents were mixed thoroughly and added directly into the stirring solution of CuI/TCPTA, upon which a brown-orange color developed. The reaction was allowed to stir at room temperature for 3-4 h until complete conversion was indicated by LC. The color of the reaction will gradually turn darker as the reaction nears completion.

The reaction was quenched by the addition of conc. \(\text{NH}_3\text{OH}\) (1 mL) and the volatiles were removed by rotary evaporation to give a crude, yellow/brown solid. To the crude was added \(\text{CH}_2\text{Cl}_2\) (10 mL) and a 1:1 mixture of conc. \(\text{NH}_3\text{OH}\):brine (10 mL). The mixture was allowed to stir vigorously until all solids were dissolved. The layers were separated, and the organic fraction was washed with additional portions of 1:1 conc. \(\text{NH}_3\text{OH}\):brine mixture (2 x 10 mL) until the aqueous layer was clear. The combined aqueous fractions were extracted one final time with \(\text{CH}_2\text{Cl}_2\) (10 mL). The combined
organic fractions were washed with brine (10 mL) and dried over anhydrous Na$_2$SO$_4$. The volatiles were removed \textit{in vacuo} to give a bright yellow solid. The solid was trituated with a minimal amount of cold MeOH and the product was isolated via vacuum filtration as a fine white powder.

\subsection*{3.11.5.6 1-benzyl-5-iodo-4-phenyl-1H-1,2,3-triazole}

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield 228 mg, 68%.

**physical state** white solid

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.98 (d, $J = 7.6$ Hz, 2H), 7.49 (t, $J = 7.4$ Hz, 2H), 7.46 – 7.37 (m, 2H), 7.40 – 7.30 (m, 4H), 5.70 (s, 2H)

$^{13}$C{\textsuperscript{1}H} NMR (101 MHz, CDCl$_3$)

FTIR-ATR (neat) 3089, 3055, 3030, 3006, 2969, 2935, 1497, 1471, 1454, 1446, 1435, 1408, 1332, 1224, 1152, 1075, 1061, 1023, 999, 985, 903, 792, 765, 726, 713, 694, 657

HRMS (ESI-TOF) $m/z$ calculated for C$_{15}$H$_{13}$IN$_3$, [M + H]$^+$ = 362.0149; found 362.0150.

\subsection*{3.11.5.7 1-benzyl-5-iodo-4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole}

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield: 315 mg, 73%.
physical state white solid

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.10 (d, $J = 8.1$ Hz, 2H), 7.72 (d, $J = 8.2$ Hz, 2H), 7.43 – 7.29 (m, 5H), 5.69 (s, 2H)

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$) $\delta$ 148.8, 134.1, 133.7, 130.6, 130.3, 129.0, 128.6, 127.9, 127.5, 125.6, 125.5, 125.5, 125.4, 122.7, 54.5

$^{19}$F{$^1$H} NMR (377 MHz, CDCl$_3$) $\delta$ -62.4

FTIR-ATR (neat) 3108, 2961, 2930, 1619, 1496, 1435, 1419, 1329, 1236, 1190, 1168, 1156, 1122, 1107, 1067, 1014, 988, 844, 791, 724, 713, 698, 691, 666

HRMS (ESI-TOF) $m$/z calculated for C$_{16}$H$_{12}$F$_3$IN$_3$, [M + H]$^+$ = 430.0023; found 430.0031.

3.11.5.8 1-benzyl-5-iodo-4-(p-tolyl)-1H-1,2,3-triazole

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield: 338 mg, 60%.

physical state white solid

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (d, $J = 7.9$ Hz, 2H), 7.41 – 7.25 (m, 6H), 5.67 (s, 2H), 2.40 (s, 3H)

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$) $\delta$ 150.4, 138.6, 134.5, 129.4, 129.0, 128.6, 127.9, 127.5, 76.2, 54.5, 21.5

FTIR-ATR (neat) 3063, 3030, 3007, 2984, 2951, 2922, 1541, 1497, 1475, 1455, 1433, 1400, 1357, 1332, 1306, 1253, 1228, 1184, 1148, 1128, 1078, 1061, 1031, 983, 818, 785, 723, 695, 607
**HRMS (ESI-TOF) m/z** calculated for $\text{C}_{16}\text{H}_{15}\text{IN}_3$, $[\text{M} + \text{H}]^+ = 236.1182$; found 236.1183.

### 3.11.5.9 General Procedure for the Synthesis of Protiotriazoles

The following procedure is a modification of a previously reported method.\textsuperscript{120} To a 20 mL scintillation vial equipped with magnetic stir bar and plastic screw cap was added CuOAc $\cdot$ H$_2$O (13 mg, 0.06 mmol, 2 mol %) and 10 mL MeCN; the mixture was allowed to stir vigorously until a bright blue, homogeneous solution was obtained. Meanwhile, stock solutions of benzyl azide (3.0 M in MeCN) and sodium L-ascorbate (0.09 M in H$_2$O) were prepared. To the stirring solution was added 1.0 mL benzyl azide solution (3 mmol, 1 equiv.) and alkyne (3.0 mmol, 1 equiv.) via syringe to give a slightly green solution. In one quick, smooth motion, 1.0 mL of sodium L-ascorbate solution (0.09 mmol, 3 mol %) was added to the to give a bright, canary yellow solution. The reaction mixture was allowed to stir overnight at room temperature.

For workup, the volatiles were removed by rotary evaporation to give a crude yellow solid. To the crude solid was added DCM (10 mL) and concentrated NH$_4$OH (10 mL). The mixture was allowed to stir until all solids were dissolved and then transferred to a separatory funnel. After separating the layers, the organic fraction was washed with additional concentrated NH$_4$OH (approximately 3 x 10 mL) until the washes were clear. The organic layer was then washed with brine (10 mL) and dried over anhydrous Na$_2$SO$_4$. The solvent was removed via rotary evaporation to give the product as a white solid. If necessary, the solid resulting from evaporation can be triturated with a minimal amount of cold methanol to give analytically pure product after vacuum filtration.
3.11.5.10 1-benzyl-4-phenyl-1H-1,2,3-triazole

\[
\begin{array}{c}
\text{Ph} \\
N^* \quad N \\ \\
\text{Bn} \\
\end{array}
\]

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield: 477 mg, 67%.

**physical state** white solid

\[^1\text{H} \text{NMR}\] (400 MHz, CDCl\(_3\)) \(\delta \) 7.80 (d, \(J = 7.5\) Hz, 2H), 7.67 (s, 1H), 7.44 – 7.35 (m, 5H), 7.35 – 7.27 (m, 3H), 5.56 (s, 2H)

\[^{13}\text{C}[^{1}\text{H}] \text{NMR}\] (101 MHz, CDCl\(_3\)) 148.2, 134.7, 130.6, 129.2, 128.8, 128.8, 128.2, 128.1, 125.7, 119.6, 54.2

**FTIR-ATR** (neat) 3121, 3096, 3065, 1495, 1466, 1454, 1442, 1426, 1357, 1223, 1205, 1074, 1048, 1027, 974, 913, 826, 765, 727, 692

**HRMS** (ESI-TOF) \(m/\chi\) calculated for C\(_{15}\)H\(_{14}\)N\(_3\), [M + H]\(^+\) = 236.1182; found 236.1183.

3.11.5.11 1-benzyl-4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole

\[
\begin{array}{c}
\text{CF}_3 \\
\text{Ph} \\
N^* \quad N \\ \\
\text{Bn} \\
\end{array}
\]

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield: 505 mg, 55%.

**physical state** white solid
\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.91 (d, \(J = 8.1\) Hz, 2H), 7.75 (s, 1H), 7.64 (d, \(J = 8.2\) Hz, 2H), 7.40 (d, \(J = 6.8\) Hz, 3H), 7.37 – 7.29 (m, 2H), 5.59 (s, 2H)

\textsuperscript{13}C\{\textsuperscript{1}H\} NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 146.8, 134.4, 133.9, 129.9 (q, \(J = 32.5\) Hz), 129.2, 128.9, 128.14, 125.9 – 125.6 (m), 125.4, 122.7, 120.2, 54.3

\textsuperscript{19}F\{\textsuperscript{1}H\} NMR (282 MHz, CDCl\textsubscript{3}) \(\delta\) -62.9

FTIR-ATR (neat) 3143, 3018, 2953, 2913, 2855, 1496, 1453, 1431, 1348, 129.9 (q, \(J = 32.5\) Hz), 129.2, 128.9, 128.14, 125.9 – 125.6 (m), 125.4, 122.7, 120.2, 54.3

HRMS (ESI-TOF) \(m/\zeta\) calculated for C\textsubscript{16}H\textsubscript{13}F\textsubscript{3}N\textsubscript{3}, [M + H]\textsuperscript{+} = 304.1056; found 304.1064.

3.11.5.12 1-benzyl-4-(\(p\)-tolyl)-1\(H\)-1,2,3-triazole

\begin{center}
\includegraphics[width=0.3\textwidth]{structure.png}
\end{center}

This compound was prepared using the procedure outlined in section 3.11.5.5 above. Yield: 569 mg, 76%.

**Physical state** white solid

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.69 (d, \(J = 7.8\) Hz, 2H), 7.63 (s, 1H), 7.37 (d, \(J = 6.8\) Hz, 3H), 7.34 – 7.26 (m, 2H), 7.20 (d, \(J = 7.8\) Hz, 2H), 5.55 (s, 2H), 2.36 (s, 3H)

\textsuperscript{13}C\{\textsuperscript{1}H\} NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 148.3, 138.0, 134.8, 129.5, 129.1, 128.7, 128.1, 127.7, 125.6, 119.2, 54.2, 21.3

FTIR-ATR (neat) 3143, 3017, 2987, 2953, 2853, 1495, 1452, 1431, 1348, 1222, 1205, 1181, 1135, 1108, 1066, 1046, 1030, 972, 950, 919, 827, 792, 718, 703, 662

HRMS (ESI-TOF) \(m/\zeta\) calculated for C\textsubscript{16}H\textsubscript{16}N\textsubscript{3}, [M + H]\textsuperscript{+} = 250.1339; found 250.1349.
3.11.5.13 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane

This compound was prepared as previously reported and exhibited consistent characterization data.\textsuperscript{157}

3.11.5.14 5,5-bis(azidomethyl)-2,2-dimethyl-1,3-dioxane

This compound was prepared by modifying a previously reported procedure.\textsuperscript{158} To a 100 mL round bottom flask equipped with magnetic stir bar and water-jacketed reflux condenser was added DMF (45 mL, 0.4 M), 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane (5.35 g, 17.7 mmol), and sodium azide (6.91 g, 106 mmol, 6 equiv.). The flask was placed into a pre-heated aluminum mantle at 130 °C and left to stir vigorously overnight during which the reaction turned yellow. The contents were poured into a fritted funnel while hot. A minimal amount of cold DMF was used to rinse the filter cake.

After cooling to room temperature, the filtrate was transferred to a separatory funnel followed by 150 mL of cold water and the mixture was extracted with diethyl ether (4 x 50 mL). The combined organic fractions were washed with water (50 mL), washed with brine (2 x 50 mL), and dried over anhydrous sodium sulfate. The solvent was removed via rotary evaporation to give the desired product as a pale-yellow oil (4.01 g, quantitative yield).

\textbf{physical state} pale yellow oil
\[^1^H\text{NMR}\ (300 \text{ MHz, CDCl}_3) \ \delta \ 3.64 \ (s, 4H), \ 3.44 \ (s, 4H), \ 1.40 \ (s, 6H)\]

\[^{13}C\{^1^H\} \text{NMR}\ (75 \text{ MHz, CDCl}_3) \ \delta \ 98.6, \ 63.3, \ 52.2, \ 38.2, \ 23.6\]

\textbf{FTIR-ATR} (neat) 2994, 2941, 2870, 2094, 1450, 1373, 1285, 1229, 1195, 1163, 1150, 1083, 1042, 1010, 937, 889, 825, 715.

3.11.5.15 1-((5-(azidomethyl)-2,2-dimethyl-1,3-dioxan-5-yl)methyl)-5-iodo-4-phenyl-1\textit{H}-1,2,3-triazole [mono(iodotriazole)]

![Chemical Structure](3.12)

The mono(iodotriazole) ketal product can be obtained by following the procedure listed in section 3.11.5.16 by not allowing the reaction to proceed to completion and performing the work-up early. The major product is predominantly the bis(iodotriazole) (see below); however, a sufficient amount of the desired mono(iodotriazole) was obtained for characterization.

\textbf{physical state} white solid

\textbf{TLC} \(R_f = 0.33\) (petroleum ether:ethyl acetate = 7:2); UV visualization

\[^1^H\text{NMR}\ (400 \text{ MHz, CDCl}_3) \ \delta \ 7.95 \ (d, J = 7.7 \text{ Hz, 2H}), \ 7.53 \sim 7.37 \ (m, 3H), \ 4.57 \ (s, 2H), \ 3.80 \ (d, J = 12.2 \text{ Hz, 2H}), \ 3.69 \ (d, J = 12.2 \text{ Hz, 2H}), \ 3.65 \ (s, 2H), \ 1.48 \ (s, 3H), \ 1.45 \ (s, 3H)\]

\[^{13}C\{^1^H\} \text{NMR}\ (101 \text{ MHz, CDCl}_3) \ \delta \ 149.7, \ 130.2, \ 128.9, \ 128.7, \ 127.8, \ 99.0, \ 79.3, \ 63.4, \ 52.3, \ 50.3, \ 39.2, \ 24.6, \ 23.0\]

\textbf{FTIR-ATR} (neat) 2988, 2965, 2932, 2868, 2099, 1469, 1446, 1371, 1262, 1234, 1207, 1194, 1116, 1082, 1042, 984, 936, 882, 827, 772, 714, 696

\textbf{HRMS} (ESI-TOF) \(m/\zeta\) calculated for C\(_{16}\)H\(_{20}\)IN\(_5\)O\(_2\), \([M + H]^+ = 455.0687\); found 455.0699.

93
3.11.5.16 1,1'-(2,2-dimethyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(5-iodo-4-phenyl-1H-1,2,3-triazole) [bis(iodotriazole)]

To a 40 mL vial equipped with magnetic stir bar and screw cap was added CuI (38 mg, 0.2 mmol, 10 mol %), TCPTA (93 mg, 0.2 mmol, 10 mol %), and 18 mL of MeCN. The vial was placed in a pre-heated aluminium block set at 40 ºC and the contents were allowed to stir vigorously until a homogeneous solution was obtained. Meanwhile, 5,5-bis(azidomethyl)-2,2-dimethyl-1,3-dioxane (452 mg, 2 mmol, 1 equiv.) and (iodoethynyl)benzene (1.00 g, 4.4 mmol, 2.2 equiv.) were weighed into an LC vial. The contents of the LC vial were mixed via pipette aspiration before being added to the stirring CuI/TCPTA solution, immediately yielding a dark-brown solution. The remaining 2 mL of MeCN (20 mL total, 0.1 M) was used to rinse the LC vial into the reaction. The mixture was allowed to stir for 6 h, after which the volatiles were removed via rotary evaporation. The crude solid was dissolved in a minimal amount of DCM and poured over a small plug of silica (~ 5 mL) wetted with CH₂Cl₂. Copious amounts of DCM and EtOAc were used to flush the plug. The solvent was removed in vacuo and the crude purified via column chromatography (petroleum ether:ethyl acetate = 7:2) to yield the desired product as a white solid (1.122 g, 82% yield).

**physical state** white solid

**TLC** $R_f = 0.15$ (petroleum ether:ethyl acetate = 7:2); UV visualization

**¹H NMR** (400 MHz, CDCl₃) $\delta$ 7.98 – 7.91 (m, 4H), 7.48 (t, $J = 7.4$ Hz, 4H), 7.42 (dd, $J = 8.4$, 6.1 Hz, 2H), 4.84 (s, 4H), 3.91 (s, 4H), 1.44 (s, 6H).
$^1$H NMR (101 MHz, CDCl$_3$) $\delta$ 149.8, 130.1, 128.9, 128.7, 127.8, 99.1, 79.8, 63.3, 51.3, 40.4, 23.8

**FTIR-ATR** (neat) 3068, 3029, 2991, 2931, 2871, 2847, 1472, 1451, 1375, 1252, 1233, 1204, 1147, 1130, 1076, 1040, 1000, 983, 924, 893, 827, 768, 716, 700

**HRMS** (ESI-TOF) $m/z$ calculated for C$_{24}$H$_{25}$I$_2$N$_6$O$_2$, [M + H]$^+$ = 683.0123; found 683.0137.

### 3.11.5.17 1,1'-(2,2-dimethyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(4-phenyl-1H-1,2,3-triazole) [bis(prototriazole)]

![Chemical structure](image)

To a 20 mL vial equipped with magnetic stir bar and screw cap was added Cu(OAc)$_2$·H$_2$O (30 mg, 0.15 mmol, 10 mol %) and 13.5 mL MeCN. The vial was placed in a pre-heated aluminum block set at 35 ºC and the contents were allowed to stir vigorously until a homogeneous solution was obtained. Meanwhile, 5,5-bis(azidomethyl)-2,2-dimethyl-1,3-dioxane (339 mg, 1.5 mmol, 1 equiv.) was weighed into an LC vial and the contents were added to the stirring copper solution. Phenylacetylene (0.362 mL, 3.30 mmol, 2.2 equiv.) was added to the stirring contents, yielding a neon-green mixture. Sodium L-ascorbate (59 mg, 0.30 mmol, 20 mol %) was then weighed into an LC vial and dissolved in 1.5 mL water. The L-ascorbate mixture was added to the reaction, upon which a bright, canary-yellow mixture was obtained. The reaction was allowed to stir for 18 h at 35 ºC.

For work up, concentrated NH$_4$OH (3 mL) was added to the stirring reaction and the volatiles were removed by rotary evaporation affording a crude yellow solid. The crude was redissolved in CH$_2$Cl$_2$ (15 mL) and extracted with a 1:1 mixture of conc. NH$_4$OH:brine (3 x 10 mL). The combined aqueous fractions were washed with an additional portion of CH$_2$Cl$_2$ (10 mL). All organic fractions
were then combined and concentrated by rotary evaporation, affording a crude white solid. The crude was purified via column chromatography (petroleum ether:ethyl acetate = 6:4) to yield the desired product as a white powder (0.506 g, 78% yield).

**Physical state** white solid

**TLC** \( R_f = 0.30 \) (petroleum ether:ethyl acetate = 6:4); UV visualization

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.25 (s, 2H), 7.91 (d, \( J = 7.6 \) Hz, 4H), 7.48 (t, \( J = 7.6 \) Hz, 4H), 7.38 (t, \( J = 7.4 \) Hz, 2H), 4.50 (s, 4H), 3.76 (s, 4H), 1.55 (s, 6H)

\(^{13}\)C\{\(^1\)H\} NMR (101 MHz, CDCl\(_3\)) \( \delta \) 147.7, 130.2, 128.9, 128.4, 125.8, 122.4, 99.2, 63.6, 49.3, 39.4, 23.8

**FTIR-ATR** (neat) 3127, 2096, 2059, 3033, 2992, 2931, 2864, 1612, 1467, 1441, 1380, 1367, 1258, 1228, 1192, 1156, 1122, 1086, 1067, 1042, 996, 976, 933, 891, 839, 819, 761, 692

**HRMS** (ESI-TOF) \( m/\zeta \) calculated for C\(_{24}\)H\(_{27}\)N\(_6\)O\(_2\), [M + H]\(^+\) = 431.2190; found 431.2201.

### 3.11.6 General Procedure for RPKA by Heat-Flow Calorimetry

To a 16 mL vial equipped with magnetic stir bar, open-top screw cap, and PTFE-lined flat silicone septum was added CuI (4.76 mg, 0.05 mmol, 5 mol %) and tris((1-tert-butyl-1H-1,2,3-triazolyl)methyl)amine ligand\(^{88}\) (TTTA; 12 mg, 0.05 mmol, 5 mol %). Solvent (THF or MeCN; 4.5 mL) was added to the reaction vial using a volumetric syringe. The vial was placed in the well of the calorimeter set at 25 °C for approximately 30–45 min prior to the initiation of the reaction. Meanwhile, a stock solution of the desired substrates (alkyne/iodoalkyne and azide) were prepared in THF. A 0.5 mL aliquot of the substrate solution was withdrawn into a 1.0 mL volumetric syringe plugged with a small amount of PTFE. The syringe was then placed in the well of the calorimeter to achieve thermal equilibration for approximately 30–45 min prior to the start of the reaction. The reaction was initiated by injecting the substrate solution.
3.11.7 Reaction Progress Monitoring by HPLC-MS

3.11.7.1 Preparation of Bicinchoninic Acid (BCA) Quench Solution

To prepare a 0.025 M solution of BCA quench solution, a 25 mL volumetric flask was charged with [2,2'-bipyridine]-4,4'-dicarboxylic acid (bicinchoninic acid; 215 mg, 0.625 mmol) and approximately 20 mL of MeOH. Triethylamine (175 µL, 1.250 mmol) was added and the mixture was shaken to yield a transparent solution. (If there are remaining solids after the addition, additional triethylamine should be added dropwise until a homogeneous solution is obtained.) The volumetric flask was filled to the calibration line and shaken. The flask was wrapped in Parafilm and kept in the fridge when not required. For reaction quenching, the liquid handler was programmed to deliver 13 µL of quench solution (5 equiv. BCA per equiv. of Cu) followed by 487 µL of methanol into a capped LC vial.

Scheme 3.9. Complexation of CuI by two equivalents of BCA.
3.11.7.2 General Procedure for Reaction Progress Monitoring of Triazole Reactions by Automated Sampling

To a 16 mL vial equipped with magnetic stir bar, open-top screw cap, and PTFE-lined flat silicone septum was added the desired copper(I) salt (CuI, CuSPh, CuOAc, or (MeCN)$_4$CuBF$_4$; 0.05 mmol, 5 mol %) and TCPTA (12 mg, 0.05 mmol, 5 mol %). MeCN (9.0 mL) was added to the reaction vial using a volumetric syringe. The vial was placed in the well of the calorimeter set at 25 ºC for approximately 30–45 min prior to the start of the reaction. Meanwhile, to ensure accuracy twice the amount of the desired alkynes (iodo, terminal, or both; 1 mmol for each alkyne) were weighed directly into a 1.0 mL volumetric flask. Similarly, twice the amount of benzyl azide required (266 mg, 2 mmol) was weighed directly into a separate 1.0 mL volumetric flask. Each volumetric flask was filled to the calibration line with solvent and mixed well. A 0.5 mL aliquot of the alkyne solution (containing 0.5 mmol of each alkyne; 1.0 equiv. per alkyne) and a 0.5 mL aliquot of the benzyl azide solution (1.0
mmol, 2 equiv.) was withdrawn into separate volumetric syringes. The syringes were plugged with a small amount of PTFE and placed in the well of the calorimeter to obtain thermal equilibration for approximately 30–45 min prior to the start of the reaction. The reaction was initiated by injecting the azide solution first followed by the alkyne solution. The sampling apparatus was initiated immediately after the injection of the alkyne solution.

### 3.11.8 Copper Triazolide Experiments

![Chemical Structure](image)

To a 16 mL vial equipped with magnetic stir bar, open-top screw cap, and PTFE-lined flat silicone septum was added CuI (9.52 mg, 0.05 mmol, 10 mol %) and TCPTA (23 mg, 0.05 mmol, 10 mol%). MeCN (4.0 mL) was added to the reaction vial using a volumetric syringe. The vial was placed in the well of the calorimeter set at 25 ºC for approximately 30–45 min prior to the start of the reaction. To ensure accuracy, twice the amount of phenylacetylene (102 mg, 1.0 mmol) and
(iodoethynyl)benzene (228 mg, 1.0 mmol) were weighed directly into a 1.0 mL volumetric flask. Similarly, twice the amount of 5,5-bis(azidomethyl)-2,2-dimethyl-1,3-dioxane was weighed into a separate 1.0 mL flask. Each volumetric flask was filled to the calibration line with solvent and mixed well. A 0.5 mL aliquot of the alkyne solution (containing 0.5 mmol of each alkyne; 1.0 equiv. per alkyne) and a 0.5 mL aliquot of the azide solution (1.0 mmol, 1 equiv.) was withdrawn into separate volumetric syringes. The syringes were plugged with a small amount of PTFE and placed in the well of the calorimeter to obtain thermal equilibration for approximately 30–45 min prior to the start of the reaction. The reaction was initiated by injecting the azide solution first followed by the alkyne solution. The sampling apparatus was initiated immediately after the injection of the alkyne solution.

3.11.9 Calibration Curves

Calibration curves correlating LC chromatogram peak area and concentration were constructed. For each set of experiments, a 2.0 mL stock solution in CHCl₃ (or 1,2-dichloroethane for ketal species) of known concentration was prepared. The sampling capillary from the prototype apparatus was inserted into the solution and 300 µL was withdrawn through the sample loop. The rheodyne and liquid handler were triggered, causing the sample to be delivered to a waiting LC and diluted to a total volume of 1 mL. Next, 300 µL of solvent was added back to the stock solution without removing the sampling capillary and the mixture was vortexed well. The sample preparation was repeated for a total of at least 5 samples. The contents of the resulting vials were analyzed by HPLC using the method previously described with integrations taken at 230 nm (or 210 nm for BnN₃). The resulting peak areas for each analyte were plotted against their calculated concentration. The equation of least-squares regression fitting for each of the analytes was used to determine concentration of the reaction progress experiments.
Table 3.1. Linear regression equations for calibration curves.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Equation</th>
<th>R² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BnN₃ 3.1</td>
<td>( y = 21598x - 12.335 )</td>
<td>0.99982</td>
</tr>
<tr>
<td>Ph-I 3.2</td>
<td>( y = 25546x - 8.4132 )</td>
<td>0.9987</td>
</tr>
<tr>
<td>Ph-H 3.3</td>
<td>( y = 33339x - 15.991 )</td>
<td>0.9987</td>
</tr>
<tr>
<td>3.4</td>
<td>( y = 28686x - 11.405 )</td>
<td>0.9985</td>
</tr>
<tr>
<td>3.5</td>
<td>( y = 29508x - 9.2887 )</td>
<td>0.99839</td>
</tr>
<tr>
<td>3.12</td>
<td>( y = 28525x - 27.041 )</td>
<td>0.99869</td>
</tr>
<tr>
<td>3.13</td>
<td>( y = 52459x - 16.966 )</td>
<td>0.99951</td>
</tr>
<tr>
<td>3.14</td>
<td>This compound was not isolated. Instead, the following mass balance equation was used to obtain the concentration of compound 3.15. ( [3.14] = [3.2] - [3.12] - 2*[3.13] )</td>
<td></td>
</tr>
<tr>
<td>3.15</td>
<td>( y = 52723x - 17.84 )</td>
<td>0.99964</td>
</tr>
<tr>
<td>CF₃-H 3.16a</td>
<td>( y = 30002x - 7.3853 )</td>
<td>0.99865</td>
</tr>
<tr>
<td>Compound</td>
<td>Equation</td>
<td>R² Value</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>![3.16a']</td>
<td>$y = 16663x - 6.6397$</td>
<td>0.99863</td>
</tr>
<tr>
<td>![3.16b]</td>
<td>$y = 19636x - 5.0933$</td>
<td>0.99835</td>
</tr>
<tr>
<td>![3.16b']</td>
<td>$y = 21770x - 5.7711$</td>
<td>0.99978</td>
</tr>
<tr>
<td>![3.17a]</td>
<td>$y = 19636x - 5.0933$</td>
<td>0.99835</td>
</tr>
<tr>
<td>![3.17b]</td>
<td>$y = 28356x - 7.5642$</td>
<td>0.99961</td>
</tr>
<tr>
<td>![3.18a]</td>
<td>$y = 19636x - 5.0933$</td>
<td>0.99835</td>
</tr>
<tr>
<td>![3.18b]</td>
<td>$y = 25792x - 8.1426$</td>
<td>0.99962</td>
</tr>
</tbody>
</table>
Chapter 4: Development of New Technology for Automated Solubility and Crystallization Analysis

4.1 Introduction

The solubility of a chemical compound in a solvent or solvent mixture is an important physicochemical property that has many ramifications for chemical synthesis, drug discovery and development, as well as agrarian and commercial processes. As such, research has been devoted towards the development of methods to accurately, consistently, rapidly, and easily furnish solubility information. These include gravimetric methods, Fourier-transform infrared (FT-IR) or Raman spectroscopy, calorimetry, high performance liquid chromatography (HPLC) and diode array detection (DAD), potentiometric or titration-based assays, and optical techniques.

Each of these methods is associated with its own unique advantages and disadvantages and is varied in their ability to accommodate analysis of mixtures of chemical compounds. HPLC-DAD for measuring solubilities, in particular, is one of the few techniques by which the solution phase concentration of co-dissolved species can be determined as a result of chromatographic separation. However, this analytical method is only amenable if the analytes contain chromophores that are responsive to ultraviolet or visible light (UV-vis). Consequently, the detection and quantification of minimally- or non-active compounds by UV-vis detection is often difficult or impractical without chemical derivatization. Other so-called “universal detectors” that employ charged aerosol detection (CAD), evaporative light scattering detection (ELSD), chemiluminescent nitrogen detection (CLND), refractive index (RI) measurements, or specialized mass spectrometry instruments have been regarded for their proposed capability in detecting and quantifying all kinds of chemical compounds, regardless of their physical or chemical attributes. The merits and disadvantages of these alternative analytical models have been discussed in previous reviews.
Active research projects in our lab have required the accurate and precise quantification of minimally- or non-UV active compounds obtained from crystallization and we sought to apply an evaporative light scattering detector to this problem. Routinely, ELSD has found application in pharmaceutical settings for the quantification of drug-containing solutions; however, the extension of this detection mode for solubility profiling and kinetic analysis of crystallizations has, to our knowledge, yet to be reported.

While devices exist that allow for solubility information to be gathered in an automated manner, these instruments are rarely applied to dynamic systems where the solubility of a given component is changing over time. In such cases, focused-beam reflectance measurements (FBRM) are often employed in conjunction with light microscopy (commonly referred to as particle view measurement, PVM) which can provide temporal information on the crystallization behavior of solid-liquid slurries. However, the data obtained from these tools do not offer compositional information of systems involving multiple analytes. Thus, these tools are not easily deployed for on-line monitoring and control of selective crystallization processes from mixtures containing co-dissolved species.

In this work, we discuss the development and applications of a new automated system designed to help remedy bottlenecks in solubility and crystallization analysis. By integrating HPLC-ELSD with automated sampling, a robotic platform has been developed that can furnish information-rich and trustworthy temporal solubility information on both systems reflecting thermodynamic solubility equilibrium as well as dynamic systems in which the concentration of dissolved components are changing over time. Validation of this new tool is provided by in situ ReactIR spectroscopy and case studies are presented that demonstrate the utility of the new technology.
4.2 Description of the Automated Sampling Technology

The automated platform is a modification of our previously reported reaction sampling prototypes.\textsuperscript{61,62,64,65} In brief, the concentration of dissolved components in a solid–liquid slurry can be measured by automatic removal of aliquots of solid-free supernatant for offline HPLC-ELSD analysis: at fixed time points, a given volume is withdrawn from the reactor by a syringe pump through a PTFE filter that is affixed to the end of a sampling capillary and submerged into the liquid (see Figure 4.1 for a schematic). The filtered aliquot passes through the capillary and into a 20 μL sample loop located on a 6-port, 2-position injection valve. Once the sample loop is filled, the valve’s position is then switched, placing the loop in line with a diluent pump and allowing for the sample to be diluted directly into an LC vial located on the bed of a Gilson GX-281 liquid handling robot.

\textbf{Figure 4.1.} Schematic of the automated sampling device used for solubility and crystallization analysis.

The timing of sample removal is governed by the Mettler-Toledo iControl software that communicates with the EasyMax 102 Advanced Synthesis Workstation (used for precise temperature and stirring control of the reactor) and triggers the removal of the aliquot by the syringe pump, actuation of the selection valve, and sample dilution. In turn, the EasyMax and Gilson Liquid Handler
are interfaced via a Mettler-Toledo Universal Control Box (UCB), allowing for bi-directional communication between the EasyMax and the robotic platform (see Figure 4.2 for a communication schematic).

**Figure 4.2.** Communication schematic of the automated sampling technology used in this work. Double-headed arrows represent bi-directional communication between modules. (UCB = Universal Control Box.)

The pull rate and volume of the syringe pump were optimized to ensure that the aliquots were withdrawn and diluted consistently and to accommodate mixtures containing different solid–liquid loadings. For solid-liquid slurries at high solids loading, a more viscous mixture is often generated which renders consistent sampling more difficult (e.g., cavitation as a result of filter fouling). For our system, a pull volume of 200 μL at a rate of 2 mL/min displayed the right balance of reproducibility and high sampling frequency. In the present configuration, consistent sampling can be achieved (relative standard deviations less than 1%) at a sampling rate as large as one per min. It should be noted that if material is scarce, the syringe pump parameters can be further optimized to minimize the amount lost from the reactor during each sampling event. The prepared LC vials are manually transferred to the HPLC-ELSD for analysis as they are prepared or upon completion of the sampling period.

The use of HPLC-ELSD is especially advantageous due to its ability to chromatographically separate and quantify multiple minimally- or non-UV-active components that are co-dissolved, including stereoisomers. Evaporative light scattering detectors operate by nebulizing the post-column
HPLC stream by an inert gas, such as nitrogen, and directing the resulting aerosol into a heated drift tube where the solvent is evaporated. Upon crossing the internal light source, the solid particles resulting from solvent removal scatter the incident light onto a photomultiplier, which registers a signal. Critically, for ELSD to be viable the volatility of sample must be less than that of the mobile phase. It is worth mentioning that the ELSD response (chromatogram peak area) to analyte concentration are related via an exponential function, rendering calibration slightly more involved than quantification by UV-vis.\textsuperscript{182, 187-189} A linear calibration can be obtained by analyzing stock solutions of known concentration, plotting \( \log_{10}(\text{peak area}) \) against \( \log_{10}(\text{concentration}) \), and fitting a linear regression to the transformed data.

Lastly, the crystallization reactor is also equipped with an \textit{in situ} Mettler-Toledo ReactIR probe (Figure 4.1). This process analytical tool (PAT) provides an additional means to monitor the concentration of dissolved species in real-time with a high data acquisition rate. Since the infrared spectra are acquired attenuated total reflectance (ATR), only components in solution are measured, and the signal is minimally influenced by suspended solids. The use of this secondary \textit{in situ} monitoring technology allowed us to cross-validate the information gathered from the robotic sampling; the comparison of these data streams revealed important considerations that we will discuss below.

### 4.3 Automated Solubility Measurement

To demonstrate the utility of the newly developed technology, we first began by constructing solubility profiles of compounds dissolved in a solvent or solvent mixture as a function of temperature. Several compounds were selected for investigation due to their roles in active projects in our research labs (Scheme 4.1). These amino acids, \textit{rac}-allylalanine monohydrate (4.1), \textit{rac}-isovaline monohydrate (4.2), DL-alanine (4.3), and DL-proline (4.4) all have minimal UV-vis responses, making them less amenable to rigorous HPLC quantification by diode array detection. While the traditional gravimetric
or spectroscopic means of solubility determination were certainly viable, these assays only allow for the total solubility of both enantiomers to be determined. For our purposes, it was necessary for us to be able to precisely measure the concentration of both enantiomers and the enantiomeric excess in solution.

Scheme 4.1. Compounds selected for analysis in this chapter.

The bi-directional communication between the EasyMax and the robotic technology allows for changes in reactor parameters (temperature, stirring, etc.) to be synchronized with the robotic sampling. For the solubility measurements, a simple graphical program was created in the iControl software that set the internal reactor temperature, an incubation period to be sustained to allow for equilibration, and lastly automatic sampling. As a result, solubility data could be collected over the entire temperature range of interest without intervention by the experimenter. (It should be noted that if unattended solubility measurement is desired, care should be taken to ensure that the initial solids loading of the reactor is sufficient to ensure saturation of the liquid phase at all temperatures.)

As an example, Figure 4.3 shows the solubility profile of both enantiomers of rac-allylalanine monohydrate (4.1) in an ethanol:water mixture (75:25, v/v) collected over 0-40 °C for every 2 °C. Once satisfactory reactor conditions were met for each chosen temperature, at least three samples of the solid-free supernatant were acquired. The solubilities of both the (R)- and (S)-enantiomers of 4.1 in the ethanol:water mixture were then determined by chiral HPLC resolution via an Astec Chirobiotic T column and quantification by ELSD. The solubility data presented in Figure 4.3, therefore,
represent the average value at each temperature. As expected for a racemic compound, the concentration of both the \((R)\)- and \((S)\)-enantiomers dissolved in solution are identical. We note the excellent precision of the solubility profile generated by the automatic sampling technology (the profiles for the other compounds in Scheme 4.1 can be found in the Experimental).

![Figure 4.3. Solubility curve of rac-allylalanine monohydrate (4.1) in ethanol:water = 75:25 (v/v) obtained by automated sampling and HPLC-ELSD quantification.](image)

To corroborate the data acquired by automatic sampling, we also measured the solubility of rac-allylalanine monohydrate (4.1) by gravimetric means; Figure 4.4 gives a comparison of the solubility curves from these two techniques. For the latter, aliquots of the saturated supernatant at each temperature were removed manually by withdrawing the liquid through a PTFE syringe filter and dispensing the sample into LC vials. The mass of the filled vial was measured, and the solvent evaporated by placing the vials on a hot plate. Once the solvent was removed, the vials were reweighed, and the resulting mass difference was taken to be the total solubility of both enantiomers in units of g solute per g solution.
Figure 4.4. Comparison of solubility curves of *rac*-allylalanine monohydrate (4.1) in ethanol:water = 75:25 (v/v) measured by HPLC-ELSD (closed black markers) and the gravimetric technique (open red markers). The total solubility of 4.1 is the sum of the concentrations of the (R)- and (S)-enantiomers in solution.

As seen in Figure 4.4, the good agreement in the shapes of the curves indicates that the solubility measurement by the automated technology is valid. We also note that the point-to-point variation in the ELSD data is much less than that in the gravimetric method. This is most likely due to the fact solubility sampling in the automated platform utilizes filtration of the supernatant *in situ*, thereby eliminating any temperature change that may occur when the aliquot is filtered outside of the reactor. Such a procedure can, and often does, affect the final measured solubility value as a result of the temperature change of the supernatant upon contact with the external filter.

4.4 Automated Crystallization Monitoring

Having established that the automated technology could accurately measure the concentration of dissolved components in systems displaying equilibrium solubility, we then turned our attention towards dynamic systems. The robotic platform is well-suited for capturing temporal information on
systems actively undergoing crystallization since the minimally intrusive method of data collection allows for compositional information of the liquid phase to be acquired without perturbing the reactor.

To verify the trends gathered by automated sampling, we decided to simultaneously monitor the crystallizations by *in situ* ReactIR. Since the EasyMax parameters, ReactIR acquisition, and robotic triggering are all controlled by Mettler-Toledo software, integration of all data (reactor temperature, stir rate, ReactIR trends, and sampling events) into one graph is incredibly facile, allowing for quick assessments to be made. Moreover, the information gathered by the PAT can be cross-referenced to the offline HPLC data.

Figure 4.5 represents the time-course temperature and solubility profiles of a system undergoing crystallization initiated by cooling to induce spontaneous primary nucleation, measured by concomitant automated sampling and *in situ* ReactIR monitoring. Here, a saturated solution of rac-allylalanine monohydrate (4.1) in ethanol:water = 75:25 (v/v) at 45 °C was prepared and filtered into an EasyMax reactor. After an equilibration period was sustained, the internal reactor temperature was lowered in a controlled manner from 45 °C to 0 °C at a rate of 1.5 °C per minute.

Upon examination of the solubility profiles, we see that both analytical methods are able to monitor the entire crystallization process. Crystallization is initiated by spontaneous primary nucleation when the internal reactor temperature reaches ~20 °C. After approximately 60 min, the solution phase concentration has nearly stabilized, but solubility equilibrium has been not been established. Indeed, the measured concentration of 4.1 in solution by both ReactIR and sampling is still decreasing asymptotically.
While both ReactIR and HPLC-ELSD data show good agreement, there are discrepancies between the two profiles even after calibration of the IR signal. In particular, the ReactIR appears to indicate a decrease in the solubility of 4.1 during the first 20 min, prior to primary nucleation while minimal change is observed by HPLC-ELSD. We attribute this observation to temperature effects on the IR signal from the reactor cooling. These effects would also explain the mismatch towards the end of the experiment (after 35 min) where the concentration of 4.1 in solution is measured by ELSD as slightly higher than that by the ReactIR (Figure 4.5). These notions reinforce the importance of having multiple data streams to verify the apparent behaviors of a process under interrogation.\

4.5 Monitoring a Preferential Crystallization by Entrainment

As a case study, we used the automated technology to monitor the progress of a preferential crystallization. Compounds 4.1 and 4.2 in Scheme 4.1 are both conglomerate-forming molecules,
wherein each enantiomer crystallizes into separate, homochiral (enantiopure) solid phases. This property allows for preferential crystallization of one enantiomer upon seeding with enantiopure solids (crystallization by entrainment). Such a method for chiral resolution can be very advantageous, especially on large scale, as it eliminates the need for a chiral auxiliary or exogenous resolving agent and will be discussed in greater detail in Chapter 5.

For the preferential crystallization of allylalanine monohydrate, a saturated solution of 4.1 in ethanol:water = 75:25 (v/v) was prepared at 20 °C and filtered into an EasyMax reactor with a jacket temperature of 35 °C to prevent spontaneous crystallization. The temperature of the filtered liquid was then lowered to 18 °C to yield a supersaturated, racemic solution. Once an equilibration period was sustained, 15 mg of enantiopure (S)-4.1 was added followed by initiation of the automated sampling program. Given time, the preferential crystallization is successful if secondary nucleation of only the (S)-enantiomer occurs with none of the (R)-enantiomer entering the solid phase. This should yield a system with a scalemic solution (e.e. > 0) and enantiopure solids. Figure 4.6 gives a cartoon representation of an ideal preferential crystallization.

![Figure 4.6](image_url)

**Figure 4.6.** Cartoon depiction of an ideal preferential crystallization of (S)-4.1 by seeded growth (entrainment) at constant temperature. Green spheres represent enantiopure, homochiral solids.

The temporal solubility and enantiomeric excess (e.e.) profiles of the preferential crystallization are shown in Figure 4.7. The data indicate that the preferential crystallization of (S)-4.1 from solution
was successful for approx. 225 min, where the solution phase e.e. reaches a maximum and no noticeable decrease in the concentration of the (R)-4.1 was measured. However, after this period, it is clear that the preferential crystallization begins to fail as both the solution phase concentration of (R)-4.1 and solution phase e.e. decrease. Importantly, we observe that the solubility profile of (R)-4.1 during this regime is sigmoidal, indicative of spontaneous primary nucleation of this enantiomer.

Figure 4.7. Temporal solution phase concentration and enantiomeric excess profiles of the preferential crystallization of (S)-4.1 initiated by addition of 15 mg of enantiopure seed to a supersaturated solution of rac-allylalanine monohydrate in ethanol:water = 75:25 (v/v) at 18 ºC. (% e.e. = ([R] - [S])/(R] + [S]) x 100)

Time-course data displaying solution composition are extremely valuable in interrogating and optimizing crystallization processes. The profiles in Figure 4.7 clearly illustrate that there is a kinetic window in which the crystallization of only one enantiomer, (S)-4.1, occurs and indicate a time at which the crystallization must be arrested to ensure isolation of enantiopure solids. These tools can easily be utilized for further interrogation and crystallization process development to generate a dedicated preferential crystallization workflow for optimal resolution of (S)- and (R)-4.1 via batch methods such as "Auto-Seeded Polythermal Programmed Preferential Crystallization" (AS3PC).193,194
4.6 Monitoring a Diastereomeric Resolution

Lastly, we used the automated platform to monitor a resolution of a racemate via the formation of a diastereomeric salt. As a model, we chose the resolution of DL-proline (4.4) using L-tartaric acid (4.5), modified from a previous report by Yamada, Hongo, and Chibata. As shown in Scheme 4.2, DL-proline and L-tartaric acid were dissolved in water and upon addition of ethanol, the L-proline–L-tartaric acid diastereomeric complex (4.6) is preferentially formed (which is less soluble than the analogous D-proline–L-tartaric acid complex) and precipitates from solution.

Scheme 4.2. Resolution of DL-proline with L-tartaric acid yields the less soluble L-proline–L-tartaric acid complex, which precipitates from solution.

Figure 4.8a and Figure 4.8b give the temporal solution phase concentrations of D- and L-proline for two separate trials of the diastereomeric resolution. Figure 4.8a depicts the first trial where 8.0 mL of the proline–tartrate solution was dispensed into an EasyMax reactor with a jacket temperature of 20 °C. Following thermal stabilization of the solution, ethanol (8.0 mL) was added to the reactor under vigorous stirring, followed by initiation of the automated sampling platform. The high initial mass loading (∼130 mg/mL for both D- and L-proline) resulted in rapid precipitation of material from solution, in a largely uncontrolled manner. Prior to addition of the EtOH anti-solvent, the initial concentration of D- and L-proline were identical, however, at the first aliquot (t = 0 in Figure 4.8a) the solution phase displays a discrepancy in the quantity of the two enantiomers, with a deficit of D-proline. The system displays a phase where both enantiomers are crystallizing from solution (Figure 4.8, 0–4 min), after which the solution phase concentration of D-proline stabilizes,
while L-proline continues to crystallize for the remainder of the experiment (Figure 4.8a, 4–32 min). These data indicate that while the L-proline–L-tartaric acid salt (4.6) is less soluble than the D-proline–L-tartaric acid complex, the solid phase is not pure, and will contain a mixture of both diastereomeric salts. Thus, it is immediately evident that this first trial represents a failed classical resolution, giving a mixture of stereoisomers in the solid phase.

Using the information from the first attempt, a second, more dilute crystallization was conducted (Figure 4.8b). This time, 5.5 mL of the proline–tartrate solution was dispensed into an EasyMax reactor, and crystallization was initiated as previously with 8.0 mL of ethanol. Here, the solution phase concentration of the undesired D-proline–L-tartaric acid complex remains constant with almost exclusive formation of the desired 4.6.

From a process development perspective, designing an optimal workflow for a diastereomeric resolution such as that in Scheme 4.1 can represent a significant investment in time and skill. However, the time-course solution phase concentration provided by our automated platform can dramatically expedite this development cycle. With only two experiments, a number of critical process parameters are now evident. First, we can confirm the stereochemistry of the least soluble diastereomeric salt and obtain a quantitative relationship for their equilibrium solubility values. This is a unique advantage of the chiral HPLC-ELSD quantitation that is not possible by achiral analytical techniques such as in situ ATR-FTIR or turbidity. Furthermore, we obtain a parallel measure of the solid phase purity by analyzing the solution phase concentration of each individual enantiomer. Using solution phase optical rotation can miss this feature in cases where the rate of crystallization of both enantiomers is identical, as the initial phase of trial 1. Lastly, we obtain a measurement for both the rate (kinetics of crystallization) and estimated mass recovery (thermodynamic solubility).
Figure 4.8 Diastereomeric resolution of DL-proline with L-tartaric acid, (a) trial 1, a stock solution of 1:1 DL-proline:L-tartaric acid in water (8 mL) was treated with EtOH anti-solvent (8 mL) at 20 °C; (b) trial 2, a stock solution of 1:1 DL-proline:L-tartaric acid in water (5.5 mL) was treated with EtOH anti-solvent (8 mL) at 20 °C.

Access to these process insights with so few experiments allows us to evaluate optimal crystallization processes. For instance, the mass throughput per resolution seems to be larger for trial 1 (Figure 4.8a), where nearly 65 mg/mL of L-proline crystallizes from solution over the course of the...
experiment compared with the approximately 40 mg/mL that crystallizes in trial 2 (Figure 4.8b). However, the isolation of enantiopure L-proline in trial 1 would be complicated by the concomitant crystallization of at least 20 mg/mL of D-proline under the experimental conditions. Clearly, a balance needs to be struck between the throughput of the crystallization and the demands of the workup that are necessary for isolation of enantiopure material.

4.7 Conclusions

This work has described the development and application of new technology coupling robotic sampling and HPLC-ELSD quantification for automated solubility and crystallization analysis. Both static processes (those exhibiting solubility equilibrium) and dynamic processes (undergoing active crystallization) could be readily monitored with a high degree of reproducibility and fidelity. We foresee the new technology having a prominent role in reducing bottlenecks in solubility and crystallization analysis and improve our theoretical understanding of these processes to which we were previously blind. We envision that the system could be augmented for use with “direct-inject” LC systems wherein aliquots of the solid-free supernatant are delivered directly onto an LC column for analysis, eliminating the need for vial preparation and providing a near real-time means of analysis. Lastly, the technology discussed herein is not limited to batch crystallization and could be applied to continuous crystallization processes.

4.8 Experimental

4.8.1 Chemical Suppliers

DL-alanine was purchased from Sigma. rac-Isovaline monohydrate and rac-allylalanine monohydrate were synthesized. Phenyl isocyanate and lithium hexamethyldisilazide (LiHMDS) was purchased from Sigma. Di-tert-butyl dicarbonate (Boc₂O), DMAP, and allyl bromide were purchased
from Alfa Aesar. Optima-grade UHPLC solvents were purchased from Fisher and used as received. L-proline was purchased from Alfa Aesar. D-proline was purchased from AK Scientific. L-tartaric acid was purchased from Sigma. 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). Analytical TLC plates were purchased from Merck KGaA and visualized by UV (254 nm) or potassium permanganate staining.

4.8.2 Equipment

All experiments were performed in Mettler-Toledo EasyMax 102 Advanced Synthesis Workstation glass reactors (50 or 100 mL) equipped with either glass or Teflon reactor covers, submersible thermocouple, and magnetic or overhead stirring and controlled by the Mettler-Toledo software iControl 6.0. The internal reactor temperature ($T_i$) was maintained by the EasyMax and measured by a thermocouple placed directly in contact with the contents of reactor.

Temporal solubility data was obtained using a custom-built automated reaction sampling apparatus similar to that previously reported by our group.\textsuperscript{61, 62, 64, 65} In summary, a PTFE filter was affixed to the end of a ETFE sampling capillary (1/16” outer diameter, 0.020” inner diameter) that is submerged into the liquid of the reactor to allow the solid-free supernatant to be withdrawn. At fixed time points, 200 µL samples were automatically taken at a draw speed of 2 mL/min by a New Era Syringe pump (25 mL SGE syringe) through the filter and sampling capillary into a 20 µL sample loop attached on a 6-port, 2-position Gilson 918 Injection Valve (selection valve). The valve position is then switched, and the sample is delivered directly to a 2 mL LC vial located on the bed of a Gilson 215 automated liquid handler robot, by diluting the captured aliquot with 1.0 mL of water.

The timing of the sampling technology was governed by the Mettler-Toledo iControl software that communicates with the EasyMax and can trigger the removal of the reactor aliquot by the syringe pump, actuation of the selection valve position by the Rheodyne, and sample dilution by a diluent
pump. The EasyMax and Gilson Liquid Handler were interfaced via electrical contacts through a Mettler-Toledo Universal Control Box, “UCB.” The prepared samples were manually transferred to the HPLC-ELSD for analysis as they were prepared or upon completion of the sampling period.

Figure 4.9. Photo of the automated sampling apparatus.

Figure 4.10. Photo of the syringe pump, valve, and EasyMax reactor.
HPLC analysis was performed on a standard Agilent 1290 Infinity HPLC equipped with a 385-ELSD detector. The collected samples were analyzed using one of the following methods:

**Method A:** *rac*-Allylalanine Monohydrate

Astec Chirobiotic T Column, 4.6 x 150 mm; 5 µm

Solvent A = Water; Solvent B = Methanol

Flow Rate = 1.00 mL/min

Column Temperature = 25 ºC

Injection Volume = 8 µL

Pump Program: isocratic, A:B = 10:90

**Method B:** *rac*-Isoleucine Monohydrate

Astec Chirobiotic T Column, 4.6 x 150 mm; 5 µm

Solvent A = Water; Solvent B = Methanol

Flow Rate = 0.6 mL/min

Column Temperature = 25 ºC

Injection Volume = 15 µL

Pump Program: isocratic, A:B = 35:65

**Method C:** DL-Alanine

Astec Chirobiotic T Column, 4.6 x 150 mm; 5 µm

Solvent A = Water; Solvent B = Methanol

Flow Rate = 0.6 mL/min

Column Temperature = 25 ºC

Injection Volume = 5 µL

Pump Program: isocratic, A:B = 85:15
Method D: DL-Proline

Astec Chirobiotic T Column, 4.6 x 150 mm; 5 µm

Solvent A = Water; Solvent B = Acetonitrile

Flow Rate = 1.0 mL/min

Column Temperature = 25 ºC

Injection Volume = 4 µL

Pump Program: isocratic, A:B = 95:5

ELSD parameters were the same for all methods and are as follows: Evaporator = 50 ºC, Nebulizer = 50 ºC, Gas Flow Rate = 1.6 SLM, Data Rate = 80 Hz, LED Intensity = 100%, Smoothing = 50 (5.0 s), PMT Gain = 1.0. Concentration values of the samples removed from the reactors were obtained by constructing calibration curves of concentration against ELSD peak area and a described in further detail below.

NMR spectra were recorded on Bruker NMR spectrometers located within the UBC Department of Chemistry. Data for \(^1\)H NMR spectra are listed as follows: chemical shift (δ, ppm), multiplicity, coupling constant (Hz), integration, and are referenced to the residual solvent peak.\(^{156}\) Abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. \(^{13}\)C{\(^1\)H} NMR, \(^{19}\)F{\(^1\)H}, and \(^{31}\)P{\(^1\)H} NMR spectra are listed in terms of chemical shift (δ, ppm).

High-resolution mass spectrometry data were acquired by the UBC Department of Chemistry Mass Spectrometry Center. In situ FT-IR monitoring was conducted with a Mettler-Toledo ReactIR10 equipped with a SiComp (silicon) ATR probe connected via an AgX (silver halide) 6.3 mm x 1.5 m fiber optic cable. Spectra were recorded over 3000 cm\(^{-1}\) to 650 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution with 1x gain. The solubility of the analytes was monitored in real time by tracking the peak area of a diagnostic signal relative to a two-point baseline.
4.8.4 Synthetic Procedures

4.8.4.1 Overall Synthetic Route to rac-Allylalanine

A three-neck, 1 L round bottom flask equipped with magnetic stir bar, addition funnel, and two glass stoppers was added was charged with KOH (19.6 g, 350 mmol, 1 equiv.) and water (700 mL, 0.5 M) and the resulting solution was cooled to 0 ºC via an ice/water bath. DL-alanine (31.2 g, 350 mmol, 1 equiv.) was added in one portion and stirred until a homogeneous mixture was obtained. Phenylisocyanate (45.6 mL, 420 mmol, 1.2 equiv.) was added dropwise via the addition funnel over a 30 min period. The resulting suspension was removed from the ice/water bath and the addition funnel was replaced with a reflux condenser. The reaction was slowly warmed to 65 ºC using a heating mantle with the temperature monitored by a glass thermometer, after which the reaction was allowed to stir for an additional 30 min. The reaction was cooled until r.t., after which a white precipitate was observed. The reaction was filtered through a fritted funnel and the white solids discarded, yielding a transparent solution. Under vigorous stirring, the solution was acidified with conc. HCl (37 %; ~25-
30 mL, added dropwise) until pH 2, resulting in the formation of a fine white precipitate. The solids were isolated by vacuum filtration through a large fritted funnel and suspended in aqueous HCl (6 M, 530 mL). The suspension was heated to 75 °C and allowed to stir vigorously overnight. The reaction was allowed to cool to r.t., affording a white precipitate. The solid was isolated by vacuum filtration through a large fritted funnel and recrystallized with a minimal amount of boiling ethanol. [If there are insoluble components observed in the flask during the recrystallization, the solution should be filtered while hot to remove these components as they can make clean isolation of the desired allylalanine product difficult if they are carried through subsequent reactions.] After cooling to r.t., the solids were isolated via vacuum filtration and washed with cold ethanol yielding 5-methyl-3-phenylimidazolidine-2,4-dione as a white solid (54.4 g, 82%).

**physical state** white solid

**TLC** $R_f = 0.2$ (petroleum ether:diethyl ether = 2:3); UV visualization

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.59 – 7.44 (m, 2H), 7.44 – 7.26 (m, 3H), 6.82 (s, 1H), 4.21 (q, $J = 6.9$ Hz, 1H), 1.52 (d, $J = 7.0$ Hz, 3H)

$^{13}$C{$_1^1$H} NMR (101 MHz, CDCl$_3$) $\delta$ 173.7, 156.8, 131.6, 129.3, 128.4, 126.3, 53.0, 17.9

**HRMS** (ESI-TOF) $m/z$ calculated for C$_{10}$H$_{11}$N$_2$O$_2$, [M + H]$^+$ = 191.0815; found 191.0815.

4.8.4.3 *tert*-butyl 5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate

To a 1 L round bottom flask equipped with addition funnel and magnetic stir bar was added 5-methyl-3-phenylimidazolidine-2,4-dione (22.82 g, 120 mmol, 1 equiv.), 4-(dimethylamino)pyridine (DMAP; 1.46 g, 120 mmol, 0.1 equiv.), and THF (600 mL, 0.2 M), affording a homogeneous, slightly
yellow solution. Di-tert-butyl dicarbonate (Boc₂O; 31.4 g, 144 mmol, 1.2 equiv.) was added dropwise, during which the release of gaseous CO₂ was observed. The resulting mixture was allowed to stir for 1 h at r.t. until the reaction was deemed to be complete by TLC (petroleum ether:diethyl ether = 2:3). The reaction was quenched by the addition of saturated aq. NH₄Cl (100 mL) and the phases separated. The aqueous phase was extracted with DCM (3 x 50 mL) and the combined organic phases were washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated by rotary evaporation. The resulting pale-yellow oil was redissolved in methanol (30 mL) and placed into a refrigerator overnight, during which a white precipitate formed. The solid was isolated by vacuum filtration, washed with a minimal amount of cold MeOH, and dried under high vacuum, affording tert-butyl 5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate as a white powder (28.6 g, 82%).

physical state white solid

TLC Rₖ = 0.4 (petroleum ether:diethyl ether = 2:3); UV visualization

¹H NMR (400 MHz, CDCl₃) δ 7.56 – 7.43 (m, 2H), 7.43 – 7.27 (m, 3H), 4.56 (q, J = 6.8 Hz, 1H), 1.70 (d, J = 6.8 Hz, 3H), 1.58 (s, 9H)

¹³C{¹H} NMR (101 MHz, CDCl₃) δ 170.8, 151.2, 148.7, 130.9, 129.3, 128.9, 126.5, 84.7, 55.7, 28.2, 17.2

HRMS (ESI-TOF) m/z calculated for C₁₅H₁₈N₂O₄, [M + Na]⁺ = 313.1164; found 313.1173.

4.8.4.4 tert-butyl 5-allyl-5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate
A flame-dried, 250 mL two-neck round bottom flask equipped with gas flow adapter and rubber septum was cooled under vacuum and refilled with argon. The flask was charged with tert-butyl 5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate (43.5 g, 150 mmol, 1 equiv.) and evacuated for approximately 30 min. After refilling with argon, anhydrous THF (550 mL, 0.5 M) was added, followed by allylbromide (12.98 mL, 120 mmol, 1 equiv.), and the flask was cooled to 0 °C using an ice/water bath. Meanwhile, LiHMDS (27.6 g, 165 mmol, 1.1 equiv.) was weighed inside a nitrogen-filled glovebox directly into a 20 mL scintillation vial. The scintillation vial was then capped and sealed with electrical tape before being brought out of the glovebox. A separate two-neck round bottom flask equipped with magnetic stir bar, gas flow adapter, and rubber septum was also cooled under vacuum and refilled with argon. The contents of the scintillation vial were transferred to the empty two-neck round bottom flask and THF (150 mL, approx. 1 M solution) was added, yielding a slightly opaque yellow-brown solution. After the flask containing the starting material was sufficiently cooled, the LiHMDS solution was transferred dropwise via cannula over 30 min. [Note: Care should be taken to prevent rapid addition of the LiHMDS solution as this can generate darkly colored by-products (typically brown) that make isolation of the desired product difficult.] Following complete transfer of the LiHMDS solution, the reaction was allowed to stir overnight in the ice/water bath while warming to r.t. The reaction was quenched with saturated aq. NH₄Cl (100 mL) and the aqueous phase was extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated via rotary evaporation, resulting in a yellow-brown oil. The oil was placed under high vacuum overnight to remove residual solvent, generating a crude, off-white solid. The crude solid was dissolved in a minimal amount of hot iPrOH, cooled to r.t., and the desired product was precipitated with a minimal amount of cold water. The precipitate was collected by vacuum filtration, washed with a cold mixture of iPrOH:H₂O (8:2) and
dried to afford tert-butyl 5-allyl-5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate as a white solid (47.0 g, 95%).

**physical state** white solid

**TLC** \( R_f = 0.6 \) (petroleum ether:ethyl acetate = 85:15); UV visualization

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)) \( \delta \) 7.55 – 7.41 (m, 2H), 7.41 – 7.34 (m, 1H), 7.34 – 7.19 (m, 2H), 5.65 (ddt, \( J = 17.3, 10.3, 7.5 \) Hz, 1H), 5.37 – 5.10 (m, 2H), 3.09 (dd, \( J = 13.8, 7.7 \) Hz, 1H), 2.70 (dd, \( J = 13.8, 7.3 \) Hz, 1H), 1.74 (s, 3H), 1.58 (s, 9H)

**\(^{13}\)C\{\(^1\)H\} NMR** (101 MHz, CDCl\(_3\)) \( \delta \) 173.2, 151.3, 148.7, 130.9, 130.3, 129.2, 128.9, 126.6, 121.4, 84.4, 66.1, 40.0, 28.2, 22.5

**HRMS** (ESI-TOF) \( m/\chi \) calculated for C\(_{18}\)H\(_{22}\)N\(_2\)O\(_4\), [M + Na]\(^+\) = 353.1477; found 353.1471.

### 4.8.4.5 2-amino-2-methylpent-4-enoic acid (allylalanine)

To a high-pressure reaction tube equipped with magnetic stir bar was added tert-butyl 5-allyl-5-methyl-2,4-dioxo-3-phenyl imidazolidine-1-carboxylate (24.8 g, 75 mmol) followed by conc. NH\(_4\)OH (60 mL), affording a heterogeneous suspension. The tube was sealed, shaken to mix the contents, and placed in an oil bath at r.t. The oil bath was slowly heated to 135 °C and the reaction was allowed to stir overnight (16 h). **[CAUTION: EXPLOSION HAZARD! The reaction should be placed behind a blast shield.]** The solids were observed to dissolve over time affording a slightly yellow, homogeneous reaction mixture. The reaction was allowed to cool to r.t. without stirring, during which two distinct phases were observed to develop. The upper, colored phase was separated,
and the remaining mixture was concentrated by rotary evaporation, affording an off-white solid. A large, egg-shaped stir bar was added along with a mixture of methyl tert-butyl ether (MTBE):acetone = 1:1. The contents of the flask were then stirred vigorously until a fine powder was generated. The solid was isolated by vacuum filtration and dried under high vacuum, affording 2-amino-2-methylpent-4-enoic acid (allylalanine; 5.7 g, 58.8%) as a white solid. Analytically pure product can be obtained through vacuum sublimation.

physical state white solid

TLC $R_f = 0.44$ ($n$-BuOH:AcOH:H$_2$O = 3:1:1); KMnO$_4$ visualization

$^1$H NMR (400 MHz, D$_2$O) $\delta$ 5.75 (ddd, $J = 17.6, 15.1, 8.0$ Hz, 1H), 5.44 – 5.09 (m, 2H), 2.66 (dd, $J = 14.5, 6.6$ Hz, 1H), 2.46 (dd, $J = 14.5, 8.3$ Hz, 1H), 1.49 (s, 3H).

$^{13}$C{$^1$H} NMR (101 MHz, D$_2$O) $\delta$ 176.3, 130.6, 121.3, 60.9, 41.3, 21.9.

HRMS (ESI-TOF) $m/z$ calculated for C$_6$H$_{12}$NO$_2$, [M + H]$^+$ = 130.0868; found 130.0873.

4.8.4.6 2-amino-2-methylpent-4-enoic acid hydrate (allylalanine monohydrate)

To an Erlenmeyer flask equipped with magnetic stir bar was added 2-amino-2-methylpent-4-enoic acid (5.2 g, 40 mmol) and H$_2$O (~50 mL) until a homogeneous solution was formed. The resulting solution was passed through filter paper and the water removed by directing a gentle stream of air over the filtrate to afford 2-amino-2-methylpent-4-enoic acid hydrate (rac-allylalanine monohydrate) as a white powder (quantitative yield).
4.8.5 Solubility and Crystallization Measurements

4.8.5.1 General Procedure for Solubility Measurements

To a 50 mL EasyMax reactor equipped with a glass reactor cover, thermocouple, magnetic stir bar, and sampling capillary was charged a given amount of the desired solvent or solvent mixture (approximately 20-30 mL). The reactor was placed into the well of an EasyMax 102 Advanced Synthesis Workstation, the stirring was enabled and set to 500 rpm, and the contents of the reactor were cooled to 0 ºC, as measured by the internal reactor temperature, $T_r$. A portion of the desired analyte (~3-4 g for rac-allylalanine monohydrate, ~1-2 g isovaline monohydrate, ~5-7) was charged directly into the reactor and allowed to stir for at least 1 h. After this equilibration period, at least three samples of solid-free supernatant were taken using the automated sampling apparatus. Following collection of the samples, the internal reactor temperature was then raised by 2 ºC and allowed to equilibrate for at least 1 h before subsequent sampling. If upon the temperature change the contents of the reactor turned homogeneous (i.e., no solids present), then additional portions of solid (0.2-0.5 g) were added until a heterogeneous mixture persisted in the reactor and no more dissolution was observed. The reactor contents were then allowed to reach equilibrium for at least 20 min before sampling. The prepared LC vials were vortexed gently to ensure homogeneity before analysis by HPLC-ELSD. Average peak areas were determined from the samples and converted to solubility (mg/mL) by constructing a calibration curves relating ELSD peak area and solubility.
4.8.5.2 General Procedure for ReactIR Verification of Automated Crystallization Analysis by Sampling

To a 100 mL EasyMax reactor equipped with a Teflon reactor cover, thermocouple, and magnetic stir bar was charged a given amount of the desired solvent or solvent mixture (approximately 50 mL). The reactor was placed into the well of an EasyMax 102 Advanced Synthesis Workstation, the stirring was enabled and set to 400 rpm, and the contents of the reactor were heated to 40 °C, as measured by the internal reactor temperature, $T_i$. A portion of the desired analyte (~2-3 g) was charged directly into the reactor and allowed to stir for at least 1 h. If at any point the solids were seen to completely dissolve, additional portions (0.1-0.5 g) were added and until no more dissolution was observed. After an equilibration period was sustained, the solid-free supernatant of this reactor was transferred into a second EasyMax 100 mL reactor equipped with a Teflon reactor cover,

Figure 4.11. Photos of EasyMax reactor set up for automated solubility measurements.
thermocouple, magnetic stir bar, and ReactIR probe via a peristaltic pump by withdrawing the liquid through an ETFE filter attached to the end of the peristaltic tubing and submerged into the heterogeneous mixture. The jacket temperature of the second reactor, $T_J$, was set to 45 ºC to prevent primary nucleation of the dissolved material as a result of the transfer process. Once a second equilibration period was sustained in the new EasyMax reactor (~20 min), the reactor was cooled to 0 ºC over a period of 30 min while collecting solubility data using the automated sampling apparatus. The solubility of the analytes was monitored in real time by tracking the peak area of a diagnostic signal relative to a two-point baseline. A representative spectrum is given below in Figure 4.13.

![Photo of EasyMax reactor illustrating simultaneous ReactIR and automated sampling for crystallization analysis.](image)

**Figure 4.12.** Photo of EasyMax reactor illustrating simultaneous ReactIR and automated sampling for crystallization analysis.
4.8.5.3 General Procedure for Monitoring a Preferential Crystallization of Allylalanine Monohydrate by Automated Sampling

To a 100 mL EasyMax reactor equipped with a Teflon reactor cover, thermocouple, and magnetic stir bar was charged a given amount of the desired solvent or solvent mixture (approximately 50 mL). The reactor was placed into the well of an EasyMax 102 Advanced Synthesis Workstation, the stirring was enabled and set to 400 rpm, and the contents of the reactor were heated to 20 °C, as measured by the internal reactor temperature, $T_r$. A portion of the desired analyte (~2-3 g) was charged directly into the reactor and allowed to stir for at least 1 h. If at any point the solids were seen to completely dissolve, additional portions (0.1-0.5 g) were added and until no more dissolution was observed. After an equilibration period was sustained, the solid-free supernatant of this reactor was transferred into a second EasyMax 100 mL reactor equipped with a Teflon reactor cover, thermocouple, magnetic stir bar, and ReactIR probe via a peristaltic pump by withdrawing the liquid through an ETFE filter attached to the end of the peristaltic tubing and submerged into the heterogeneous mixture. The jacket temperature of the second reactor, $T_j$, was set to 35 °C to prevent primary nucleation of the dissolved material as a result of the transfer process. Once the transfer was complete, the internal reactor temperature, $T_r$, was lowered to 18 °C over 20 min to yield a supersaturated solution. For the preferential crystallization, 15 mg of enantiopure (S)-allylalanine was added followed by initiation of the sampling program.
Figure 4.13. Representative ReactIR spectrum of rac-allylalanine monohydrate in EtOH:water = 75:25. The highlighted peak (1715-1559 cm$^{-1}$) was used for solubility monitoring, referenced to a two-point baseline.
4.8.5.4 Sample iControl Program for Automated Solubility Sampling

Figure 4.14. Sample iControl program for automated solubility sampling.
### 4.8.6 Diastereomeric Resolution of DL-Proline with L-Tartaric Acid

#### 4.8.6.1 Preparation of Authentic L-Proline—L-Tartaric Acid Diastereomeric Salt

This procedure was performed on 1/10th scale.\(^{195}\) To a 40 mL vial equipped with stir bar was charged L-proline (0.576 g, 5 mmol, 1 equiv.), L-tartaric acid (0.750 g, 5 mmol, 1 equiv.), and water (2 mL). Ethanol (30 mL) was added under vigorous stirring to yield a cloudy solution. The vial was then sonicated briefly, affording a white precipitate.

**Physical state** white solid

\(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 4.69 (s, 2H), 4.20 (dd, \(J = 8.8, 6.5\) Hz, 1H), 3.38 (ddt, \(J = 33.9, 11.6, 7.0\) Hz, 2H), 2.43 – 2.30 (m, 1H), 2.16 – 2.04 (m, 1H), 2.01 (dtd, \(J = 14.5, 7.7, 7.1, 2.4\) Hz, 2H).

\(^1\)C\(\{^1\)H\}\ NMR (101 MHz, D\(_2\)O) \(\delta\) 175.1, 174.2, 72.3, 61.0, 46.3, 29.0, 23.8.

#### 4.8.6.2 Trial 1

This procedure was modified from a previously report.\(^{195}\) To a 150 mL beaker equipped with magnetic stir bar was charged D-proline (5.755 g, 50 mmol, 1 equiv.), L-proline (5.755 g, 50 mmol, 1 equiv.), L-tartaric acid (15.01 g, 100 mmol, 2 equiv.), and 20 mL of water. The beaker was placed on a stir plate and heated gently with vigorous stirring to facilitate complete dissolution of the starting
materials. For the diastereomeric resolution, 8.0 mL of this solution was transferred into a 50 mL EasyMax reactor (jacket temperature, $T_j = 20 ^\circ C$) equipped with magnetic stir bar and sampling capillary. Ethanol (8.0 mL) was added to the reactor under vigorous stirring followed by initiation of the sampling apparatus.

### 4.8.6.3 Trial 2

This trial was performed in a similar fashion to Trial 1, with the exception that 5.5 mL of the proline-tartrate solution and 8.0 mL of ethanol were used.

### 4.8.7 Calibration Curves

#### 4.8.7.1 General Procedure for Calibration of ELSD Response and Solubility

To calibrate the ELSD response for each compound and enantiomer, stock solutions of known concentration (mg solute/mL solution) of each racemic compound were prepared in 10 mL volumetric flasks. A given amount of racemic solute was weighed directly into the volumetric flask and filled to the calibration line with deionized water. After mixing the flask thoroughly, the clean sampling line (without filter attached) was inserted directly into the flask and at least three samples were prepared using the automated apparatus. After analyzing the samples using HPLC-ELSD, the raw peak area was plotted against solute concentration. For compounds that displayed a linear ELSD response in the desired working concentration range, a least-squares regression line was fitted onto the data directly. For compounds in which ELSD response was non-linear in the working concentration range, a plot of $\log_{10}(\text{peak area})$ against $\log_{10}(\text{concentration})$ was generated and a least-squares regression line was fitted to this transformed data.

For the latter case, the resulting equation resulting from linear regression takes the form

$$\log_{10}(\text{peak area}) = a \log_{10}(\text{concentration}) + b$$
where $a$ and $b$ are constants. To obtain concentration from peak area $A$, we rewrite the equation as

$$\log_{10}(A) - b = a \log_{10}(\text{concentration})$$

Dividing both sides by $a$, we obtain

$$\frac{\log_{10}(A) - b}{a} = \log_{10}(\text{concentration})$$

Finally, raising both sides of this equation to the power of 10, we obtain

$$10^{\left(\frac{\log_{10}(A) - b}{a}\right)} = \text{concentration}$$
4.8.7.2 Calibration of ELSD Response for rac-Allylalanine Monohydrate

Figure 4.15. Non-linear ELSD peak area response against concentration for rac-allylalanine monohydrate.

![Graph showing non-linear ELSD peak area response against concentration]

Figure 4.16. Log-log plot of ELSD peak area and concentration for rac-allylalanine monohydrate with least-squares regression line of best fit.

![Graph showing log-log plot with regression lines]
4.8.7.3 General Procedure for Calibration of the ReactIR Signal

To calibrate the ReactIR signal, samples of the solid-free supernatant were taken from the reactor at various temperatures by the automated sampling apparatus once the ReactIR signal was determined to be stable. Upon analysis of these samples, the ReactIR peak was plotted against the measured solubility values to obtain a calibration graph.

![Calibration curve of ReactIR peak area to total solubility of rac-allylalanine monohydrate.](image)

**Figure 4.17.** Calibration curve of ReactIR peak area to total solubility of rac-allylalanine monohydrate.
4.8.7.4 Calibration of ELSD Response for rac-Isovaline Monohydrate

Figure 4.18. Non-linear ELSD peak area response against concentration for rac-isovaline monohydrate.

Figure 4.19. Log-log plot of ELSD peak area and concentration for rac-isovaline monohydrate with least-squares regression line of best fit.
4.8.7.5 Calibration of ELSD Response for DL-Alanine

For alanine, stock solution preparation using only deionized water was limited to the solubility at room temperature. In this case, conc. HCl (37%) was added dropwise to a volumetric flask that was half-filled with water until the solids were dissolved.

**Figure 4.20.** Non-linear ELSD peak area response against concentration for DL-alanine.

**Figure 4.21.** Log-log plot of ELSD peak area and concentration for DL-alanine with least-squares regression line of best fit.
Figure 4.22. Non-linear ELSD peak area response against concentration for DL-proline.

Figure 4.23. Log-log plot of ELSD peak area and concentration for DL-proline with least-squares regression line of best fit.
4.8.8 Solubility Data

4.8.8.1 Solubility Data for rac-Allylalanine Monohydrate

Figure 4.24. Solubility data for rac-allylalanine monohydrate in EtOH:Water = 75:25 (v/v).

Table 4.1. Solubility data for rac-allylalanine monohydrate in EtOH:Water = 75:25 (v/v) by ELSD. Error values listed represent one standard deviation.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>(S)-enantiomer (mg/mL)</th>
<th>(R)-enantiomer (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.82 ± 0.04</td>
<td>24.21 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>25.38 ± 0.17</td>
<td>25.81 ± 0.14</td>
</tr>
<tr>
<td>4</td>
<td>26.90 ± 0.09</td>
<td>27.39 ± 0.17</td>
</tr>
<tr>
<td>6</td>
<td>28.21 ± 0.28</td>
<td>28.76 ± 0.06</td>
</tr>
<tr>
<td>8</td>
<td>30.14 ± 0.06</td>
<td>30.61 ± 0.15</td>
</tr>
<tr>
<td>10</td>
<td>32.08 ± 0.14</td>
<td>32.71 ± 0.20</td>
</tr>
<tr>
<td>12</td>
<td>33.94 ± 0.15</td>
<td>34.49 ± 0.16</td>
</tr>
<tr>
<td>14</td>
<td>35.86 ± 0.25</td>
<td>36.49 ± 0.15</td>
</tr>
<tr>
<td>16</td>
<td>37.71 ± 0.13</td>
<td>38.16 ± 0.14</td>
</tr>
<tr>
<td>18</td>
<td>39.85 ± 0.17</td>
<td>40.45 ± 0.28</td>
</tr>
<tr>
<td>20</td>
<td>42.32 ± 0.32</td>
<td>42.80 ± 0.21</td>
</tr>
<tr>
<td>22</td>
<td>44.34 ± 0.27</td>
<td>45.10 ± 0.13</td>
</tr>
<tr>
<td>24</td>
<td>47.09 ± 0.04</td>
<td>47.60 ± 0.22</td>
</tr>
<tr>
<td>26</td>
<td>50.05 ± 0.14</td>
<td>50.57 ± 0.31</td>
</tr>
<tr>
<td>28</td>
<td>53.23 ± 0.13</td>
<td>53.49 ± 0.19</td>
</tr>
<tr>
<td>30</td>
<td>56.14 ± 0.20</td>
<td>56.51 ± 0.33</td>
</tr>
<tr>
<td>32</td>
<td>59.27 ± 0.22</td>
<td>59.75 ± 0.19</td>
</tr>
<tr>
<td>34</td>
<td>63.01 ± 0.19</td>
<td>63.23 ± 0.34</td>
</tr>
<tr>
<td>36</td>
<td>66.24 ± 0.16</td>
<td>66.98 ± 0.05</td>
</tr>
<tr>
<td>38</td>
<td>70.36 ± 0.32</td>
<td>70.75 ± 0.31</td>
</tr>
<tr>
<td>40</td>
<td>74.62 ± 0.25</td>
<td>74.95 ± 0.33</td>
</tr>
</tbody>
</table>
4.8.8.2 Comparison of Solubility Data rac-Allylalanine Monohydrate in Determined by ELSD and Gravimetric Measurement

Figure 4.25. Comparison of solubility curves of rac-allylalanine monohydrate in ethanol:water = 75:25 (v/v) measured by HPLC-ELSD (closed black markers) and the gravimetric technique (open red markers). The total solubility is the sum of the concentrations of the (R)- and (S)- enantiomers in solution.

Table 4.2. Solubility data for rac-allylalanine monohydrate in EtOH:Water = 75:25 (v/v) by the gravimetric method.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Gravimetric Solubility (mg solute/100 g sol'n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.49</td>
</tr>
<tr>
<td>2</td>
<td>3.72</td>
</tr>
<tr>
<td>4</td>
<td>3.81</td>
</tr>
<tr>
<td>6</td>
<td>3.90</td>
</tr>
<tr>
<td>8</td>
<td>4.04</td>
</tr>
<tr>
<td>14</td>
<td>4.70</td>
</tr>
<tr>
<td>16</td>
<td>4.86</td>
</tr>
<tr>
<td>18</td>
<td>5.13</td>
</tr>
<tr>
<td>20</td>
<td>5.47</td>
</tr>
<tr>
<td>22</td>
<td>5.73</td>
</tr>
<tr>
<td>24</td>
<td>6.08</td>
</tr>
<tr>
<td>30</td>
<td>7.07</td>
</tr>
<tr>
<td>32</td>
<td>7.65</td>
</tr>
<tr>
<td>34</td>
<td>8.07</td>
</tr>
<tr>
<td>36</td>
<td>8.52</td>
</tr>
<tr>
<td>38</td>
<td>9.02</td>
</tr>
<tr>
<td>40</td>
<td>9.68</td>
</tr>
</tbody>
</table>
4.8.8.3 Solubility Data for rac-Isovaline Monohydrate

Figure 4.26. Solubility curve for rac-isovaline monohydrate in EtOH:Water = 95:5 (v/v).

Table 4.3. Solubility data for rac-isovaline monohydrate in EtOH:Water = 95:5 (v/v). Error values listed represent one standard deviation.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>(S)-enantiomer (mg/mL)</th>
<th>(R)-enantiomer (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.17 ± 0.07</td>
<td>12.20 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>12.29 ± 0.05</td>
<td>12.37 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>12.34 ± 0.07</td>
<td>12.42 ± 0.08</td>
</tr>
<tr>
<td>6</td>
<td>12.51 ± 0.07</td>
<td>12.55 ± 0.06</td>
</tr>
<tr>
<td>8</td>
<td>12.64 ± 0.04</td>
<td>12.73 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>12.78 ± 0.04</td>
<td>12.88 ± 0.06</td>
</tr>
<tr>
<td>12</td>
<td>12.88 ± 0.05</td>
<td>12.94 ± 0.05</td>
</tr>
<tr>
<td>14</td>
<td>13.15 ± 0.11</td>
<td>13.19 ± 0.05</td>
</tr>
<tr>
<td>16</td>
<td>13.40 ± 0.06</td>
<td>13.51 ± 0.07</td>
</tr>
<tr>
<td>18</td>
<td>13.73 ± 0.06</td>
<td>13.71 ± 0.08</td>
</tr>
<tr>
<td>20</td>
<td>14.36 ± 0.09</td>
<td>14.46 ± 0.11</td>
</tr>
<tr>
<td>22</td>
<td>15.31 ± 0.15</td>
<td>15.37 ± 0.11</td>
</tr>
<tr>
<td>24</td>
<td>16.23 ± 0.09</td>
<td>16.32 ± 0.07</td>
</tr>
<tr>
<td>26</td>
<td>17.09 ± 0.07</td>
<td>17.14 ± 0.08</td>
</tr>
<tr>
<td>28</td>
<td>17.79 ± 0.09</td>
<td>18.99 ± 0.10</td>
</tr>
<tr>
<td>30</td>
<td>18.59 ± 0.07</td>
<td>18.73 ± 0.10</td>
</tr>
<tr>
<td>32</td>
<td>19.51 ± 0.07</td>
<td>20.30 ± 0.03</td>
</tr>
<tr>
<td>34</td>
<td>20.23 ± 0.10</td>
<td>21.38 ± 0.13</td>
</tr>
<tr>
<td>40</td>
<td>22.54 ± 0.09</td>
<td>22.66 ± 0.09</td>
</tr>
</tbody>
</table>
4.8.8.4 Solubility Data for DL-Alanine

Figure 4.27. Solubility curve for DL-alanine in water.

Table 4.4. Solubility data for DL-alanine in water. Error values listed represent one standard deviation.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>L-alanine (mg/mL)</th>
<th>D-alanine (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>106.04 ± 0.40</td>
<td>104.96 ± 0.49</td>
</tr>
<tr>
<td>4</td>
<td>109.89 ± 0.46</td>
<td>108.63 ± 0.56</td>
</tr>
<tr>
<td>8</td>
<td>116.68 ± 0.59</td>
<td>116.05 ± 0.71</td>
</tr>
<tr>
<td>12</td>
<td>120.95 ± 0.72</td>
<td>120.59 ± 0.21</td>
</tr>
<tr>
<td>16</td>
<td>126.92 ± 0.82</td>
<td>126.94 ± 1.26</td>
</tr>
<tr>
<td>20</td>
<td>135.54 ± 0.73</td>
<td>134.23 ± 0.45</td>
</tr>
<tr>
<td>24</td>
<td>142.71 ± 1.18</td>
<td>143.13 ± 1.22</td>
</tr>
<tr>
<td>28</td>
<td>148.09 ± 1.21</td>
<td>148.10 ± 1.04</td>
</tr>
<tr>
<td>32</td>
<td>155.88 ± 1.18</td>
<td>156.25 ± 0.89</td>
</tr>
<tr>
<td>36</td>
<td>163.29 ± 1.26</td>
<td>164.30 ± 1.58</td>
</tr>
<tr>
<td>40</td>
<td>170.00 ± 0.86</td>
<td>172.16 ± 2.67</td>
</tr>
</tbody>
</table>
4.8.9 X-Ray Data

4.8.9.1 Single-Crystal X-Ray Data for (S)-Allylalanine Monohydrate

![Crystallographic Structure](image)

**Figure 4.28.** Asymmetric unit of the unit cell for (S)-allylalanine monohydrate obtained by single-crystal X-ray analysis.

Crystallographic data for (S)-allylalanine monohydrate were measured using CuKα radiation (microfocus sealed X-ray tube, 45 kV, 0.60 mA) and collected with a Bruker APEX II area detector equipped with an Oxford Cryosystems low-temperature device operating at T = 90(2) K. The total number of runs and images was based on the strategy calculation from the program APEX3. The diffraction pattern was indexed, and the unit cell was refined using SAINT (Bruker, V8.38A, after 2013) on 9736 reflections, 36% of the observed reflections. Data reduction, scaling and absorption corrections were performed using SAINT (Bruker, V8.38A, after 2013). A multi-scan absorption correction was performed using SADABS-2016/2 (Bruker, 2016/2) was used for absorption correction. The structure was solved and the space group P 4₁ (76) determined by the XT¹⁰⁶ structure solution program using Intrinsic Phasing and refined by Least Squares using version 2017/1 of Xl¹⁰⁷. All non-hydrogen atoms were refined anisotropically. Most hydrogen atom positions were calculated geometrically and refined using the riding model, but all N—H and O—H hydrogen atoms were refined freely. The crystal structure was refined as a two-component twin.
Table 4.5. Crystallographic parameters for (S)-allylalanine monohydrate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C₆H₁₃NO₃</td>
</tr>
<tr>
<td>$D_{calc}$ / g cm⁻³</td>
<td>1.212</td>
</tr>
<tr>
<td>$\mu$/mm⁻¹</td>
<td>0.810</td>
</tr>
<tr>
<td>Formula Weight</td>
<td>147.17</td>
</tr>
<tr>
<td>Color</td>
<td>colorless</td>
</tr>
<tr>
<td>Shape</td>
<td>prism</td>
</tr>
<tr>
<td>Size/mm³</td>
<td>0.24×0.12×0.10</td>
</tr>
<tr>
<td>$T$/K</td>
<td>90(2)</td>
</tr>
<tr>
<td>Crystal System</td>
<td>tetragonal</td>
</tr>
<tr>
<td>Flack Parameter</td>
<td>0.01(11)</td>
</tr>
<tr>
<td>Hooft Parameter</td>
<td>0.03(7)</td>
</tr>
<tr>
<td>Space Group</td>
<td>P4₁</td>
</tr>
<tr>
<td>$a$/Å</td>
<td>8.8749(7)</td>
</tr>
<tr>
<td>$b$/Å</td>
<td>8.8749(7)</td>
</tr>
<tr>
<td>$c$/Å</td>
<td>20.4808(17)</td>
</tr>
<tr>
<td>$\alpha$/°</td>
<td>90</td>
</tr>
<tr>
<td>$\beta$/°</td>
<td>90</td>
</tr>
<tr>
<td>$\gamma$/°</td>
<td>90</td>
</tr>
<tr>
<td>$V$/Å³</td>
<td>1613.1(3)</td>
</tr>
<tr>
<td>$Z$</td>
<td>8</td>
</tr>
<tr>
<td>$Z'$</td>
<td>2</td>
</tr>
<tr>
<td>Wavelength/Å</td>
<td>1.54178</td>
</tr>
<tr>
<td>Radiation type</td>
<td>CuKα</td>
</tr>
<tr>
<td>$\Theta_{min}$/°</td>
<td>2.157</td>
</tr>
<tr>
<td>$\Theta_{max}$/°</td>
<td>66.704</td>
</tr>
<tr>
<td>Measured Refl.</td>
<td>26776</td>
</tr>
<tr>
<td>Independent Refl.</td>
<td>2843</td>
</tr>
<tr>
<td>Reflections with I &gt; 2(I)</td>
<td>2830</td>
</tr>
<tr>
<td>$R_{int}$</td>
<td>0.0513</td>
</tr>
<tr>
<td>Parameters</td>
<td>224</td>
</tr>
<tr>
<td>Restraints</td>
<td>1</td>
</tr>
<tr>
<td>Largest Peak</td>
<td>0.266</td>
</tr>
<tr>
<td>Deepest Hole</td>
<td>-0.151</td>
</tr>
<tr>
<td>GooF</td>
<td>1.077</td>
</tr>
<tr>
<td>$wR_2$ (all data)</td>
<td>0.0836</td>
</tr>
<tr>
<td>$wR_2$</td>
<td>0.0834</td>
</tr>
<tr>
<td>$R_f$ (all data)</td>
<td>0.0308</td>
</tr>
<tr>
<td>$R_I$</td>
<td>0.0308</td>
</tr>
</tbody>
</table>
Figure 4.29. Overlay of X-ray powder diffraction patterns for rac-allylalanine monohydrate (red curve, lower) and (S)-allylalanine monohydrate (black curve, upper).
Chapter 5: Understanding and Improving Chiral Resolutions by Continuous Preferential Crystallization (CPC)

5.1 Introduction

Molecules that contain at least one stereogenic center and, as a result, are “chiral” represent valuable building blocks in chemistry. Access to compounds containing stereogenic centers can be achieved through established synthetic methods; however, the efficient isolation of a single enantiomer from a racemic mixture (a “chiral resolution”) is a long-standing challenge for modern chemistry. Consequently, the increasing demand for novel chiral molecules motivates further study of chemical or physical processes that can easily, cheaply, and quickly provide large quantities of enantiopure materials.

![Chemical Structures](image)

*Figure 5.1. Selected examples of stereochemically-pure pharmaceuticals.*

Examples of chiral pharmaceutical drugs that have a significant real-world impact are shown in Figure 5.1. Indeed, it has been estimated that at least 50% of all marketed drugs contain at least one stereogenic center.\(^{198-200}\) Thalidomide has an unfortunate and immutable role in history of demonstrating the dire consequences that a simple change in the spatial arrangement of substituents around a single atom can have. First introduced in 1957, thalidomide was used as a sedative and later administered to pregnant women to help with morning sickness and nausea. However, within five years, it was discovered that this drug was teratogenic and caused birth defects. While later studies
did show that the (S)-enantiomer of thalidomide was the bioactive form and the (R)-enantiomer was responsible for birth defects, thalidomide has the unfortunate property of being able to racemize in the human body, rendering the dosage of a single enantiomer ineffective.\textsuperscript{201-204}

Fortunately, the effects of stereochemistry do not have to be as severe as those observed for thalidomide. Escitalopram (sold under the brand names Cipralex® or Lexapro®) is a selective serotonin reuptake inhibitor (SSRI) antidepressant. The (S)-enantiomer of this active pharmaceutical ingredient (API) has the desired antidepressant effects, while the (R)-enantiomer is benign. As a result, drug formulations containing this API do not need to consist of a single enantiomer, and the racemate can be employed.\textsuperscript{205, 206} Similarly, esomeprazole (Nexium®) is a proton-pump inhibitor (PPI) used to treat gastrointestinal (GI) ailments where, in contrast to the previous two compounds, the stereogenic center is at sulfur, instead of carbon. Although the racemic form of this drug, omeprazole (Prilosec®), is also pharmacologically viable, studies have shown that the administration of only the (S)-enantiomer has advantageous clinical effects.\textsuperscript{207-209}

5.2 Traditional Routes to Access Enantiopure Compounds

5.2.1 Chiral Pool Synthesis

One source of building blocks that chemists can utilize to build stereochemically-pure compounds is the “chiral pool,” a set of molecules that are usually found abundantly in Nature and possess well-characterized stereochemistry (examples are shown in Figure 5.2).\textsuperscript{210-212} However, these compounds are usually subject to “biological homochirality,” meaning that only one stereoisomer can be obtained from natural sources.\textsuperscript{213, 214} If an enantiomer or diastereomer of a compound found within the pool was desired (e.g., a diastereomer of shikimic acid) it is likely that a deliberate stereoselective synthesis would need to be developed.\textsuperscript{215} This would require time and resources and may not be
capable of producing enough material in a cost-effective manner. Therefore, it is sometimes necessary to move “beyond Nature’s chiral pool.”\textsuperscript{216}

![Chemical structures of L-amino acids, D-sugars, (S)-carvone, and shikimic acid](image)

**Figure 5.2.** Selected examples of compounds from the “chiral pool.”

### 5.2.2 Stereoselective Catalytic or Enzymatic Transformations of Prochiral Compounds

As shown in Scheme 5.1a, stereoselective transformations of prochiral compounds can be used to generate chiral products and these reactions can be mediated by transition metal-based catalysts,\textsuperscript{217-225} enzymes,\textsuperscript{226-231} or organocatalysts.\textsuperscript{232-238} The stereoselectivity of the asymmetric transformation is achieved by designing the catalyst or enzyme to allow for the substrate to interact in such a way that its prochiral faces can be distinguished. Preferential reaction of one of these faces then leads to an enantiomeric product. For transition metal-based catalysts, stereoselectivity can be obtained through the deliberate design of the ligands supporting the metal catalyst and the appropriate choice of metal. A similar logic is pursued in asymmetric enzymatic transformations: By altering the primary amino acid sequence that comprises the enzyme, its secondary and tertiary structure can be tailored to allow for the substrate to bind in the enzyme active site in an orientation that favors reaction of one prochiral face. For organocatalysts, prochiral discrimination can result from non-covalent interactions (e.g., from chiral Brønsted acid catalysts on a substrate via hydrogen bonding\textsuperscript{239}) or as a consequence of covalent attachment of organocatalyst to the substrate (e.g., N-heterocyclic carbene-mediated transformations\textsuperscript{28b}).
Scheme 5.1. Non-exhaustive list of chemical and physical methods for the chiral resolution of enantiomers. (The graphic in Scheme 5.1f is reproduced from Welch241 with permission.)
5.2.3  Stereoselective Reactions Involving Stoichiometric Auxiliaries

Enantioenriched compounds can also be accessed through the use of stoichiometric chiral auxiliaries (Scheme 5.1b).242  A classic example of these types of compounds are the 4-substituted oxazolidinones developed by Evans and co-workers, which are derived from easily-procured enantiopure amino alcohols (members of the chiral pool). To generate an enantioenriched product, the oxazolidinone chiral auxiliary is first reacted with an acyl chloride under strong base to afford an \(N\)-acyloxazolidinone intermediate.243, 244  The \(R_1\)-substituent at the 4-position of the oxazolidinone directs the subsequent Lewis-acid-mediated aldol reaction between the \(N\)-acyl substituent and an electrophile (in this case, an aldehyde) to give a \(\text{syn}\) - and diastereoselective \(\beta\)-alkylation. Subsequent cleavage of the auxiliary then liberates the enantioenriched product. The diastereoselectivity of this class of reaction can be predicted by considering the Zimmerman-Traxler-type transition state245-247 through which the reaction is assumed to proceed.

5.2.4  Resolution of Racemates by Diastereomeric Salt Formation

Chiral resolution by diastereomeric salt formation (Scheme 5.1c) involves the selective reaction of one enantiomer with an enantiopure resolving agent, forming an adduct that can be separated from the unreacted enantiomer. (Such processes are usually the result of an acid-base reaction.) Scheme 5.1c shows the resolution of racemic \(\text{trans}\)-1,2-diaminocyclohexane by L-tartaric acid under acidic conditions, as reported by Jacobsen and co-workers.248, 249  Here, the L-tartaric acid selectively forms a salt with the \((R,R)\)-diamine, while the \((S,S)\)-diamine remains in solution. The resulting diastereomeric \((R,R)\)-diamine–L-tartaric acid salt is much less soluble in the reaction solvent than the starting materials and precipitates from solution. A simple filtration and subsequent washing then yield a diastereomERICally-pure solid. If instead the \((S,S)\)-diamine was desired, the resolution could just as easily be performed using D-tartaric acid, which would lead to the selective formation of the \((S,S)\)-
diamine–D-tartaric acid salt, leaving the (R,R)-diamine behind in solution. While Jacobsen and co-workers did not isolate the neutral diamine and instead chose to use the salt directly in the subsequent step, it is possible to obtain the pure enantiomer via a simple salt break.\textsuperscript{248,249}

5.2.5 Kinetic Resolution of Racemates

Kinetic resolution (Scheme 5.1d) refers to a scenario in which a chiral catalyst reacts faster with one enantiomer of a racemate. In general, the stoichiometry of the reaction is adjusted to facilitate the complete conversion of one enantiomer, leaving the other enantiomer unreacted.\textsuperscript{250} Jacobsen and co-workers reported that the reaction of racemic epoxides with cobalt catalysts bearing single enantiomer, tetradentate salen-type ligands under hydrolytic conditions yields a stereoselective epoxide ring-opening reaction to give enantiopure diols (Scheme 5.1d).\textsuperscript{251,252} The Jacobsen catalyst bearing an (R,R)-salen ligand selectively reacts with the (S)-epoxide to give the (S)-diol, leaving the (R)-epoxide unreacted. Alternatively, if the (S,S)-salen cobalt complex was employed, the (R)-epoxide would be selectively hydrolyzed, yielding the (R)-diol.

5.2.6 Dynamic Kinetic Resolution of Racemates

In contrast, a dynamic kinetic resolution (Scheme 5.1e) takes advantage of the substrate’s ability to racemize while simultaneously undergoing a stereoselective transformation. For example, Noyori and co-workers demonstrated that the hydrogenation of \(\alpha\)-ketoesters using ruthenium catalysts bearing a (R)-(+)\textendash{}BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthyl) bidentate ligand, (R)-BINAP-Ru, allowed for the enantioselective synthesis of \(\beta\)-hydroxyketones such as the ones shown in Scheme 5.1e. In this case, the reaction conditions are such that racemization of the substrate is faster than the enantioselective transformation, thus allowing for the conversion of a racemate to a single, stereochemically-pure product.\textsuperscript{253-256} This transformation is notable due to the fact that there are four
possible diastereomers that can form; however, the acidity of the ketone α-proton allows for epimerization of this stereocenter via the enolate form of the ketoester. The inherent chirality of the ruthenium catalyst will ultimately dictate which prochiral face of the starting material will be preferentially reduced and consequently afford one stereoisomeric product.²⁵⁵,²⁵⁶

5.2.7 Chromatographic Separation of Enantiomers

Preparatory chromatographic separation of enantiomers (Scheme 5.1f) is a physical process that employs a chiral stationary phase (CSP) to resolve a racemate. This method relies on the transient adsorption of the chiral molecules onto the CSP, momentarily forming a diastereomeric complex that allows for the separation of the stereoisomers via medium- or high-performance liquid chromatography under reverse phase (RP) or supercritical fluid chromatography (SFC) conditions. To obtain chromatographic resolution of the enantiomers, the difference in the free energy of adsorption of each stereoisomer to the stationary phase (ΔΔG_{ads}) needs to only be on the order of 0.025 kcal/mol.²⁴¹

5.3 Preferential Crystallization

5.3.1 Motivation for Preferential Crystallization as a Means for Chiral Resolution

While the aforementioned methods for accessing chiral molecules have great utility and are still employed in modern laboratories, there are advantages and disadvantages to each that dictate their viability for each stereoisomeric target. By virtue of reaction design to give a stereoselective method, almost all of the methods shown in Scheme 5.1 only offer access to one enantiomer and, as a result, are not readily adaptable to being stereodivergent and offering orthogonal access to all possible stereoisomeric products.²²² If other enantiomers or diastereomers were needed, another catalyst, enzyme, or auxiliary would need to be synthesized and additional reaction optimizations would be
necessary to produce the desired stereoisomer. This can be extremely onerous if large quantities of all stereoisomers of a particular molecule are necessary for an intended application.

Moreover, asymmetric methods (catalytic, enzymatic, or stoichiometric) that are designed to give one stereoisomeric product are frequently limited by narrow substrate scopes and can be highly sensitive to changes in substituents on the starting materials and the reaction conditions. For example, in their dynamic kinetic resolution (Scheme 5.1e), Noyori and co-workers observed significant decreases (and up to complete erosion in some cases) in the enantiomeric excess (e.e.) of the products, depending on the steric nature of the \( R^2 \)-substituent and the polarity of the reaction solvent.\(^ {255} \)

Similarly, diastereoselective salt formations may require highly-involved process optimizations to ensure that only one enantiomer crystallizes from solution (see Chapter 5 for an example with DL-proline). Generally, there is no governing principle that specifies which resolving agent is appropriate based on the chemical properties of the starting racemate, so screens of the resolving agent and reaction conditions are necessary. Moreover, while this type of resolution may be appropriate for obtaining small quantities of enantiopure material, the resolving agent may be too expensive for use on large scale. Exotic resolving reagents may not be available in sufficient quantities or in the correct stereochemistry for the desired resolution target. Lastly, although the resolving agent can theoretically be recycled, an additional step is necessary to break the diastereomeric salt and isolate neutral material, incurring additional time and resources.

Kinetic resolutions such as that in Scheme 5.1d require precise catalyst design in order for enough of a kinetic bias for one stereoisomer of the starting material to react over the other. For these resolutions, the stoichiometry and the reaction time need to be precisely controlled in order to prevent reaction of the undesired enantiomer. If the reaction is left operating for too long and/or under inappropriate conditions, there would be nothing to prevent the catalyst from eventually reacting with the undesired enantiomer. Thus, in these processes, care must be taken to understand
“kinetic window” in which the desired enantiomer is reacted to the greatest extent possible before the reaction is terminated. Significantly, from a cost perspective, the maximum theoretical yield of a kinetic resolution is 50%, meaning that at least half of the starting material is not used. Similarly, for dynamic kinetic resolutions, such as that in Scheme 5.1e the reaction conditions must be designed in manner that allows for rapid racemization of the starting material, and also be compatible with the catalyst for the asymmetric transformation.

Although chromatographic resolutions do allow access to both enantiomers, there is an inherent mass-throughput limitation based on the dimensions and packing of the column and the chromatographic hardware. Chiral columns are expensive, especially on preparatory scales, and the lack of generality of the stationary phase to separate different types of compounds can be restrictive if a large library is not available to screen for the appropriate column and conditions for a given molecule. There is currently no first-principles rationale for why one type of chiral stationary phase allows for the separation of one compound but not another, meaning that screening efforts can be lengthy if a positive hit is not immediately discovered. Columns can be fragile and subject to deterioration from incompatible solvents or reagents, meaning that care must be taken to ensure that the material loaded on to the column is first appropriately purified, which can be limiting for when high-throughput screening is desired. Lastly, with chromatographic methods, the solvents used for separation must be removed from the compound and disposed of.

As a result of these potential drawbacks to the “traditional” methods for accessing enantiopure compounds, preferential crystallization represents an alternative technique for chiral resolution. This method relies exclusively on crystallization (a physical process, rather than a chemical reaction) to separate the stereoisomers of a racemate and does not require laborious syntheses and optimizations of chiral catalysts or auxiliaries or the use of exogeneous resolving agents. Moreover, this is a readily scaleable process capable of providing enough material for industrial applications.\(^{161, 257-259}\)
Scheme 5.2 shows a process in which rac-allylalanine, a very useful amino acid building block, is resolved via preferential crystallization into its constituent enantiomers in a single operation. As shown in the scheme, allylalanine is deliberately synthesized as the racemate from readily available and inexpensive DL-alanine. Following preferential crystallization, the separated enantiomers are then immediately primed for further chemical elaboration into stereochemically-pure commodities. The development of this crystallization process serves as the topic for the remainder of this chapter.

Scheme 5.2. Synthesis of rac-allylalanine and resolution by preferential crystallization.

5.3.2 Solid Forms of Chiral Compounds

The resolution of a racemate via preferential crystallization is only practical for conglomerate-forming compounds. In order to understand the reasons for this, the crystalline (solid state) forms of chiral molecules must first be discussed. This Thesis will follow the nomenclature in the monograph of Jacques, Collet, and Wilen, *Enantiomers, Racemates, and Resolutions* which defines the following:

- “An equimolar mixture of two enantiomers whose physical state is unspecified or unknown is called a racemate.”

- “The most common type of crystalline racemate is that in which the two enantiomers are present in equal quantities in a well-defined arrangement within the crystal lattice. The resultant homogenous solid phase corresponds to a true crystalline addition compound which we call racemic compound.”

- “A conglomerate is an equimolecular mixture of two crystalline enantiomers that are, in principle, mechanically separable.”
• “The third possibility corresponds to the formation of a solid solution between the two enantiomers coexisting in an unordered manner in the crystal. We use the term pseudoracemate to designate this case.”

The relationships between these terms is shown in Figure 5.3.

![Diagram of solid phases](Figure 5.3)

Figure 5.3. The solid phases that can form when a racemic mixture of enantiomers crystallizes.

Historically, the abundance of conglomerates has been frequently quoted as 5-10% of organic compounds, originating from a cursory survey by Jacques, Collet, and Wilen.\textsuperscript{191} Not only has this been a deterrent in the wider utilization of preferential crystallization for chiral resolutions, it has also led to conjectural statements concerning the relative stabilities of racemic compounds and conglomerates:

First, from the higher prevalence of racemic compounds, it has been inferred that these crystals are more “stable” compared to those of a conglomerate. Thermodynamically, this can be represented as a spontaneous process in which two dissolved enantiomers, $R_{(\text{diss})}$ and $S_{(\text{diss})}$, crystallize to form a heterochiral solid, $RS_{(\text{solid})}$, in which the unit cell contains a 1:1 ratio of the two enantiomers (Scheme 5.3a). This “reaction” would be associated with a negative free energy of formation ($\Delta G^0 < 0$).\textsuperscript{191}
(a) Crystallization of a racemic compound.

\[
R_{(\text{diss})} + S_{(\text{diss})} \xrightarrow{\text{crystallization}} RS_{(\text{solid})} \quad \Delta G^\circ < 0
\]

(b) Crystallization of a conglomerate.

\[
R_{(\text{diss})} + S_{(\text{diss})} \xrightarrow{\text{crystallization}} R_{(\text{solid})} + S_{(\text{solid})} \quad \Delta G^\circ = ???
\]

**Scheme 5.3.** Crystallization of a (a) racemic compound and (b) conglomerate from dissolved enantiomers \(R_{(\text{diss})}\) and \(S_{(\text{diss})}\).

The apparent preference for racemic compound formation has been rationalized by considering the interactions between enantiomers within the crystal. Wallach’s empirical rule\textsuperscript{260-262} states that racemic compounds tend to be denser than those of a conglomerate, which is due to an increased number of favorable contacts between molecules of opposite handedness within the crystal (e.g., hydrogen bonding or \(\pi\)-stacking) compared to those in a homochiral crystal. Similar stability arguments have also been based on increase in overall symmetry of the unit cell for heterochiral crystals.\textsuperscript{191} However, these statements are far from rigorous substantiation and have been challenged in the literature.\textsuperscript{192, 263} Work from our lab and others has suggested that the occurrence of conglomerates is potentially much higher than is quoted.

### 5.3.3 Identifying a Conglomerate

The identification of a conglomerate-forming compound represents the first task necessary for developing a preferential crystallization.\textsuperscript{257, 264-266} As the distinguishing properties between conglomerates and racemic compounds are only observable in the solid state, analytical techniques that are sensitive to differences in crystal packing are necessarily employed for this objective.
5.3.3.1 Morphological Inspection

Prior to the advent of spectroscopic or crystallographic methods, the primary method for the identification of conglomerates was the inspection of the three-dimensional morphology of crystals. This relies on the fact that crystals grown from optically pure solutions (composed only of one enantiomer) can have distinct morphologies from those grown from racemic solutions. As a result, the presence of enantiomorphic facets on the homochiral (R)- and (S)-crystals (for an example, see Figure 5.4), can be indicative of a conglomerate when compared against the racemic crystals.

![Representative sketch of the enantiomorphous, hemihedral crystals of sodium ammonium tartrate. Image reproduced with permission from Tobe.](image)

**Figure 5.4.** Representative sketch of the enantiomorphous, hemihedral crystals of sodium ammonium tartrate. (Image reproduced with permission from Tobe.\textsuperscript{267})

Perhaps the most famous example of this type of assessment is that of Louis Pasteur and his recognition of the enantiomorphic, hemihedral forms of the sodium ammonium salts of D- and L-tartaric acid. In working with what was then referred to as “racemic acid” (in French, *l’acide racémique*), Pasteur recognized the mirror-image relationship between separate crystals (Figure 5.4 gives a representative sketch), which were composed exclusively of either D- or L-tartrate.\textsuperscript{267,269} As a result of this “spontaneous resolution,” it is possible to resolve a racemic mixture by manual separation. Indeed, Pasteur has famously been reported to have used tweezers to physically separate the enantiomorphic crystals, a story which has been cemented into chemical history. Although this method can provide a rapid visual indication of a racemate’s solid form, it requires crystals of sufficient
quality and size and appropriate habit in order for an identification to be possible. Nevertheless, this work remains the primary inspiration for the study of molecular chirality.

For allylalanine, enantiomorphic hemihedral crystals like those of Pasteur can be obtained by creating a saturated solution of rac-allylalanine at elevated temperature in a mixture of ethanol:water (75:25). After filtering the solution and allowing slow evaporation at room temperature, crystals with well-defined habit are obtained (Figure 5.5) that are of a conglomerate nature. Thermogravimetric analysis of these solids indicate that these crystals are the monohydrate solvate of allylalanine.

![Enantiomphoric hemihedral crystals of allylalanine monohydrate.](image)

**Figure 5.5.** Enantiomphoric hemihedral crystals of allylalanine monohydrate.

### 5.3.3.2 Melting Point Analysis

The solid form of a racemate can also be identified by constructing melting point binary phase diagrams, as formalized by Roozeboom.\(^{191, 270-272}\) Figure 5.6 shows the different graphs for (a) conglomerates, (b) racemic compounds, and (c) ideal solid solutions, in which the melting point and phase behavior (whether the mixture exists as a solid, liquid, or both) is plotted against the mole fraction (or enantiomeric excess) of the two enantiomers. As can be seen in Figure 5.6, the different
crystal lattices (homochiral or heterochiral) that comprise each of the solid forms will be manifest in the different shapes of the phase boundaries and total number of distinct regions.

![Binary melting point phase diagrams](image)

**Figure 5.6.** Binary melting point phase diagrams for (a) conglomerates, (b) racemic compounds, and (c) ideal solid solutions. (Figure adapted from Jacques et al.191)

For a conglomerate (Figure 5.6a), the melting point of each pure enantiomer (100% R or 100% S) is the same; however, upon addition of its mirror image, the final melting point of the system is lowered, and continues to do so until a minimum is reached at a mole fraction equal to 0.5. This value is referred to as the “eutectic point,” \(E\). In comparing Figure 5.6a with Figure 5.6b,c, it is clear that by examining the phase and melting behavior of various compositions of a compound with unknown crystal form, it is possible to identify the crystal type.

### 5.3.3.3 X-Ray

A more obvious method for identifying a conglomerate involves X-ray analysis. A crystal of sufficient quality of allylalanine monohydrate was selected for single-crystal X-ray analysis to give the structure in Figure 5.7, which was solved in the \(P4_1\) space group. As seen in the figure, two asymmetric units of one enantiomer are present in the unit cell (in this case, \((S)\)-allylalanine monohydrate), thus definitively confirming that allylalanine monohydrate is a conglomerate.
Figure 5.7. Asymmetric units of the unit cell for (S)-allylalanine monohydrate obtained from single-crystal X-ray analysis.

Alternatively, if single crystals cannot be grown, a rapid method that can indicate a conglomerate involves X-ray powder diffraction of an enantiopure solid and a racemate. Conglomerates can only crystallize in 66 out of a possible 230 space groups and what is common to this subset is that no inversion symmetry elements are present within the unit cell, allowing for the formation of homochiral solids. As a result, the crystal lattice of the enantiopure (R)- or (S)-solids should be identical to the crystal lattice for a 1:1 mixture. This is not the case for racemic compounds since the existence of an enantiopure, homochiral solid necessitates that its crystal lattice (and space group) be different than that for a racemic compound, since the opposite enantiomer is not present.

Therefore, if the X-ray powder diffraction patterns of the homochiral solid and the racemate are compared, they should be perfectly superimposable if the solid is a conglomerate. Conversely, the X-ray diffraction pattern for a racemic compound should not overlay with that for enantiopure solid of the same compound. The XRPD patterns for rac-allylalanine monohydrate (red curve, lower) and enantiopure (S)-allylalanine monohydrate (black curve, upper) are shown in Figure 5.8. Overlay of the two patterns suggests that allylalanine monohydrate is a conglomerate.
5.3.3.4 Infrared, Raman, and Solid-State $^{13}$C-NMR Spectroscopy

Along similar lines to X-ray powder diffraction analysis, infrared (IR), Raman, or solid-state $^{13}$C-NMR spectroscopy can be employed in the detection of conglomerates. By comparing the spectrum of the enantiopure solid with that of the racemate, complete overlap of spectral features will suggest the existence of a conglomerate.

5.3.3.5 Optical and Refractory Methods

Due to their inherent chirality, enantiomorphous crystals will respond differently to polarized light, a feature that allowed Pasteur relied upon in his work to physically resolve the enantiomers of tartaric acid. Figure 5.9 shows the same enantiomorphous crystals of allylalanine as in Figure 5.5, but subjected to polarized light. As seen in the figure, the two crystals, each of which are comprised only of a single enantiomer, have differing appearances, with the left crystal exhibiting a green color while that of the right crystal displaying a reddish hue.
Different refractory characteristics are observed for each enantiomorph of allylalanine monohydrate. These images are taken of the same crystals in Figure 5.5, left viewed under polarized light.

A loosely related method involves the use of “second-harmonic generation” (SHG) to identify conglomerates. For those conglomerates that crystallize in non-centrosymmetric space groups (which represents >95% of known conglomerates), a possible non-linear optical effect can occur as a result of light interacting with homochiral solids. If upon irradiation of the crystal with light of wavelength $\lambda$, a diffusion of light is observed at wavelength $\lambda/2$ (resulting from a “second harmonic”), this can be indicative of a conglomerate. Although SHG requires specialized instrumentation, it has the advantage of being rapid, non-destructive, and automated, allowing for high-throughput screening.$^{265, 276-278}$

5.3.3.6 Iterative Dissolution or Recrystallization

Although not a rigorous method for detection, certain observations during the recrystallization or iterative dissolution of an enantioenriched material can be suggestive of a conglomerate.$^{191, 279}$ Specifically, if the enantiomeric excess of an enantioenriched solid increases as a result of recrystallization or washing, it is likely a conglomerate. Figure 5.10 shows schematic of an experiment in which a sample of allylalanine monohydrate enriched in the (S)-enantiomer (e.e. = 30%) was optically purified by repeated solvent addition and filtration, allowing for the recovery of enantiopure material.
Figure 5.10. Isolation of enantiopure (S)-allylalanine monohydrate from an enantioenriched sample via iterative dissolution.

Table 5.1. Purification of an enantiomerically-enriched sample of allylalanine monohydrate.

<table>
<thead>
<tr>
<th>trial</th>
<th>solvent added$^a$</th>
<th>solid phase e.e.$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>start</td>
<td>none</td>
<td>30%</td>
</tr>
<tr>
<td>1</td>
<td>40 mL + 10 mL</td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>10 mL + 5 mL</td>
<td>80%</td>
</tr>
<tr>
<td>3</td>
<td>15 mL + 5 mL</td>
<td>94%</td>
</tr>
<tr>
<td>end</td>
<td>10 mL + 5 mL</td>
<td>100%</td>
</tr>
</tbody>
</table>

$^a$The volume of solvent (ethanol:water = 75:25) added represents the amount used for the dissolution + the volume used for washing the solid during filtration. $^b$% e.e. = ([S] – [R])/([R] + [S]) x 100.

The ability to enantiomerically-purify an enantiomerically enriched solid can only occur for a conglomerate as each enantiomer exists in its own solid phase. Since the solubility of each enantiomer should be identical in an achiral solvent, for a scalemic mixture enriched in the (S)-enantiomer, optically pure material can be isolated by washing away equal portions of both enantiomers until only the solids of the (S)-enantiomer in excess remains. Table 5.1 gives the results from the experiment represented by Figure 5.10 in which a mixture of ethanol:water (75:25) was added in various amounts.
and the associated e.e. of the solid phase following each dissolution. Following the addition of solvent, the mixture was stirred gently to facilitate dissolution, and transferred into a fritted funnel for filtration. A second portion of solvent was used to wash the crystals providing enantiopure solid in a facile manner.

5.3.4 Resolution by Entrainment

5.3.4.1 Basic Concepts

Chiral resolution via preferential crystallization relies on a kinetic phenomenon known as “entrainment,”257-259, 265, 266, 280-282 which was briefly discussed in Chapter 4. One method for the entrainment of a single enantiomer is that of “Seeded Isothermal Preferential Crystallization” (SIPC), represented in Figure 5.11. First, a solid-free saturated solution of the racemate is prepared at a given temperature. The saturated solution is then slowly cooled to avoid spontaneous primary nucleation (crystallization) of the dissolved compound, yielding a supersaturated solution. Enantiopure seed is then added, providing a means for secondary nucleation283, 284 (crystallization induced as a result of added solid) of only the seeded enantiomer. An ideal preferential crystallization is one in which none of the (R)-enantiomer crystallizes, affording a scalemic solution (e.e. > 0) and enantiopure solids.

![Diagram of ideal preferential crystallization](image)

**Figure 5.11.** Cartoon depiction of an ideal preferential crystallization by seeded growth (entrainment) at constant temperature. Green spheres represent enantiopure, homochiral solids.
The entrainment process can be represented quantitatively on graphs that depict solubility as a function of temperature. Figure 5.12a gives a representative solubility diagram where the data points are the thermodynamic solubility values. Above the thermodynamic solubility curve is the “metastability limit” (dashed line), the boundary at which spontaneous primary nucleation occurs. The
area in between these two curves is a kinetic “metastability zone” and it is by working within this zone that preferential crystallization is possible.

A saturated solution at \( T_1 \) is represented by a point on the solubility curve (Figure 5.12b). If a solid-free saturated solution is cooled to \( T_2 \), the amount of dissolved materials will not change, but the system is now in a metastable, \textit{supersaturated} state, where more material is dissolved than what is thermodynamically dictated. The “degree” or “extent” of supersaturation is the difference between the concentration of dissolved material in the metastable state and the solubility of dissolved material at thermodynamic equilibrium, denoted by the value \( \Delta C \) in Figure 5.12b.

If the system is cooled too much to \( T_3 \), the metastability limit will be reached, and primary nucleation of both enantiomers will occur to restore the system to thermodynamic solubility (Figure 5.12c) and the reactor will contain a racemic mixture of solids and liquid. However, if the system is kept in a supersaturated state, the addition of enantiopure (\( S \))-seed allows for secondary nucleation of that enantiomer (Figure 5.12d), while the (\( R \))-enantiomer remains in solution. The system now contains a liquid phase with an excess of the (\( R \))-enantiomer and pure (\( S \))-solid following filtration.

Figure 5.13 gives a representative ternary phase diagrams for (a) conglomerates and (b) racemic compounds that are useful in mapping trajectories of the liquid and solid phases during preferential crystallizations.\textsuperscript{191, 280, 281} Such diagrams depict the relative ratios of the relevant phases within the system: For a conglomerate this includes the solid enantiomers (\( R_{\text{solid}} \) and \( S_{\text{solid}} \)) and the liquid phase (either as pure solvent or a solution containing any ratio of enantiomers). The diagram for a racemic compound will also contain these phases but has an additional heterochiral solid phase, \( RS_{\text{solid}} \). In comparing Figure 5.13a and Figure 5.13b, it is clear that the chirality of the unit cells of the two forms (i.e., homochiral or heterochiral) lead to differing phase behavior. While the interpretation of these diagrams will not be presented in this Thesis, it is important to recognize that for a conglomerate, there are large regions in which the solid phase can be composed of only one enantiomer (liquid +
$R_{\text{solid}}$ or liquid + $S_{\text{solid}}$), compared to those for a racemic compound. It is for this reason that preferential crystallization is generally only practical for conglomerates.

![Schematic ternary phase diagrams](image)

**Figure 5.13.** Schematic ternary phase diagrams of (a) conglomerate and (b) a racemic compound. Figures adapted from Jacques et al.\textsuperscript{191}

### 5.3.4.2 Limitations of “One-Pot” Preferential Crystallization

Preferential crystallization is the result of a kinetic bias. The thermodynamically lowest energy state of a system undergoing entrainment is one consisting of racemic liquid and a slightly enantioenriched solid (the mass of which corresponds to the original extent of supersaturation plus the enantiopure seed). For the unseeded enantiomer, there is a free energy barrier that must be overcome in order for primary nucleation to occur (the creation of its own seed) and crystal growth. This energy is generally larger than that required for crystallization mediated by the addition of seed (secondary nucleation). As a result, there will be a kinetic window where the seeded enantiomer has crystallized before the unseeded enantiomer has nucleated to return to thermodynamic equilibrium.

This window can be elucidated by tracking the temporal change in enantiomeric excess (e.e.) of the solution phase, usually with in-line polarimetry.\textsuperscript{282, 285-287} Figure 5.14a-c give three possible
temporal profiles for the solution phase e.e. during a seeded preferential crystallization. If the solution phase e.e. starts at 0% and increases as a result of the addition of (S)-seed, this suggests that only one enantiomer has begun to crystallize. (The e.e. is defined to be positive if there is an excess of the (R)-enantiomer.) Figure 5.14a represents the best-case scenario since the e.e. increases upon addition of the seed and plateaus at some value. This situation allows the greatest flexibility since there is little risk of contamination of the crystallized solid as a result of primary nucleation of the unseeded enantiomer in the shaded region. Figure 5.14b and Figure 5.14c, on the other hand, represent scenarios in which the kinetic window becomes increasingly shorter. Although these systems may reach the same maximum solution phase e.e. as Figure 5.14a, there is only a finite amount of time in which the solid phase remains enantiopure before the (R)-enantiomer begins to crystallize. Thus, the optical purity of the solid in the latter cases will rely on a precise termination of the crystallization.

![Figure 5.14](image)

**Figure 5.14.** Possible temporal solution phase enantiomeric excess profiles from seeded preferential crystallization. The shaded areas represent a kinetic window for isolation of the crystallized solid. (% e.e. = ([R] - [S])/([R] + [S]) x 100.) Figures adapted from Levilain et al.282

Moreover, batch crystallizations in a single reactor or “pot” will have an inherent mass-throughput limitation, depending on the extent of supersaturation that the system is capable of withstanding. For those systems in which the metastability zone is wide (Figure 5.15a), a greater degree of supersaturation can be achieved without approaching too close to the metastability limit.
compared to systems with narrow metastability zones (Figure 5.15b). Thus, the mass of solid that can theoretically be crystallized will be greater for the first system than for the latter ($\Delta C_1 > \Delta C_2$).

![Diagram showing solubility profiles with (a) wide and (b) narrow metastability zones.](image.png)

**Figure 5.15.** Solubility profiles with (a) wide and (b) narrow metastability zones. The degree of supersaturation possible ($\Delta C$) is larger for systems with larger metastability zones.

From an efficiency standpoint, if the metastability zone is narrow, resolution by entrainment will be an extremely inefficient process. The degree of supersaturation represents a theoretical amount that can be crystallized and, in practice, the yield per crystallization will be a fraction of this due to two main reasons: First, as discussed in the previous paragraph, if the stability of the solution phase e.e. at maximum entrainment is very short, the process should be terminated well before this point to prevent contamination of the solid phase. Second, mechanical losses are almost guaranteed since in order to isolate enantiopure solid, a filtration of the reactor contents must be performed. The solids are usually washed with pure solvent to remove the supernatant, possibly leading to unwanted dissolution.

Lastly, in order to isolate pure solids of both enantiomers, the entrainment must occur in a cyclical manner. That is, if the system was initially seeded with the (S)-enantiomer, an ideal preferential crystallization would have only one enantiomer crystallize; filtration of this mixture would provide the
pure (S)-solid. However, the mother liquor from the filtration still contains (in theory) the supersaturated (R)-enantiomer. Thus, by adding (R)-seed, crystallization of the (R)-enantiomer will then occur. These solids can be filtered off and washed to provide pure (R)-solid. This traditional method requires a significant number of manual operations and attention, which not only is burdensome, but also creates opportunities for failure. Coquerel and co-workers have developed “second-generation” adaptations of the traditional preferential crystallization in response to these drawbacks, such as the “Auto-Seeded Polythermal Programmed Preferential Crystallization” (AS3PC)\textsuperscript{193, 194} or the “Auto-Seeded Preferential Crystallization Induced by Solvent Evaporation” (ASPreCISE);\textsuperscript{288} however, the adaptation of preferential crystallization to a continuous mode affords the most flexibility and efficiency, regardless of the conglomerate’s properties.

### 5.3.5 Continuous Preferential Crystallization (CPC)

To address the limitations for preferential crystallization in batch, our lab has reported the development of a continuous method for the resolution of conglomerates.\textsuperscript{190, 289, 290} Figure 5.16 provides a schematic for the resolution of sodium ammonium tartrate via a “two-pot” Continuous Preferential Crystallization (CPC). As seen in the top part of the figure, the left reactor (the “dissolver”) is initially charged with racemic solid and glass beads (used to facilitate mechanical attrition of the solids) and stirred vigorously. The right reactor (the “crystallizer”), meanwhile, is charged with enantiopure D-tartrate seed crystals under mild stirring. The solids in both reactors are suspended in a saturated solution of the tartrate salts in water. In this scenario, both reactors are held isothermally at 13 °C.

A liquid pump affixed to each reactor circulates the solid-free supernatant to the opposite reactor, allowing for the continuous flow of the liquid between the reactors. For this experiment, the circulation was kept at \(~1 \text{mL/min}\) and after approximately 60 h, the result is a physical separation (resolution) of the enantiomers into the two separate pots, as seen in the bottom part of Figure 5.16.
Figure 5.16. Schematic of the “two pot” resolution of sodium ammonium tartrate via continuous preferential crystallization (CPC). (Figure adapted from Hein et al. \textsuperscript{289} with permission.)

Figure 5.17, meanwhile, shows an analogous “three-pot” system used for the resolution of DL-threonine via CPC. Here, initially the center reactor is charged with racemate and glass beads and subjected to sonication while the left and right reactors are charged with enantiopure seed crystals of L- and D-threonine, respectively and with each kept under mild stirring. The solids in all three flasks are suspended in a saturated solution of DL-threonine in water. In this three-pot resolution, the central dissolver is kept isothermally at 35 °C while the two crystallizers are maintained at 20 °C. Circulation of the solid-free supernatant between all three reactors was maintained in a similar manner to that in the two-pot case, allowing for the resolution of DL-threonine after approximately 56 h.

There are two major advantages of the additional crystallizer. First, isolation of enantiopure material of both enantiomers can occur at any time. In the two-pot scenario, the solid in the dissolver is only enantiopure at the end of the resolution when all of the D-solid has been transferred to the crystallizer. Second, additional charges of racemate can be made to the central dissolver, allowing for a resolution that is (theoretically) limitless and restricted only by reactor size.
Figure 5.17. Schematic of the “three-pot” resolution of DL-threonine via continuous preferential crystallization (CPC). (Figure reprinted from Hein et al.289 with permission.)

The success of CPC can be explained by examining Figure 5.18a-d and considering the basic concepts of entrainment discussed previously. Broadly, there are three main elements in operation:

1. A solubility difference between the dissolver and the crystallizer, providing a driving force for crystallization.

2. Continuous circulation of the solid-free supernatant, allowing for renewal of the liquid in each reactor and the maintenance of a near-equilibrium state.

3. The presence of enantiopure seed crystal in the dissolver allowing for secondary nucleation of only the seeded enantiomer.

Figure 5.18a shows the initial state of the “two-pot” system where the dissolver contains the racemate, the crystallizer contains enantiopure seed, and both reactors contain saturated solution. The dissolver is kept a warmer temperature ($T_{hot}$) than the crystallizer ($T_{cold}$). Since the solubility of both enantiomers are the same, any quantity of solid-free supernatant transferred from the dissolver to the crystallizer will contain an equal amount of both stereoisomers (Figure 5.18b). Upon transfer, the crystallizer will be slightly supersaturated because there is more material in solution than the
equilibrium solubility value of the colder crystallizer. This supersaturation (however small) and presence of seed then provides a driving force for crystallization of the seeded enantiomer (Figure 5.18c). However, unlike the one-pot scenario, primary nucleation is circumvented via circulation of the supernatant back to the dissolver. The system resets close to the initial state, since upon return of the depleted supernatant, the solid racemate can replenish the liquid phase with the missing enantiomer. The result of this iterative process is two reactors containing enantiopure solid (Figure 5.18d). Thus, the role of Pasteur’s tweezers is being served by a concentration difference between reactors. (The same logic used here can be applied for CPC with three reactors).

In Figure 5.17 and Figure 5.18, the concentration difference between the reactors is enabled by a temperature difference. In Figure 5.16, conversely, the concentration difference is caused by attrition (crystal breaking) mediated by glass beads in the dissolver. Through mechanically grinding, smaller crystals are present in the left reactor which are more soluble than the larger crystals in the right reactor. The concentration difference between the two reactors does not need to be large (the temperature difference between the crystallizer and dissolver can be as small as 2 °C); however, its magnitude will influence the rate at which the resolution occurs. Nevertheless, by maintaining a small degree of supersaturation in the crystallizer, preferential crystallization will operate far away from the metastability limit, reducing the chances of failure due to unwanted crystallization of the unseeded enantiomer. It is for this reason that this method is referred to as a “near-equilibrium” process.

This method of continuous preferential crystallization has allowed us to resolve the enantiomers of the relatively sensitive monopotassium salt, diethanol solvate of omeprazole. Other research groups have developed variations on this type of “coupled” continuous preferential crystallization for chiral resolution, which were discussed in a recently published review.
5.4 Preliminary Results on the Resolution of \textit{rac}-Allylalanine Monohydrate via Continuous Preferential Crystallization

5.4.1 \textit{rac}-Allylalanine Monohydrate as a Model Compound for CPC

The non-proteinogenic amino acid \( \alpha \)-allylalanine (Figure 5.19) is a very versatile chemical building block. Due to the alkene moiety, allylalanine is readily derivatized\textsuperscript{295-297} and has found use in biomedical spaces in drugs candidates and therapeutics\textsuperscript{298-300} and radioactive imaging agents\textsuperscript{301}. From
a biochemical perspective, α,α-disubstituted amino acids can confer useful conformational and biochemical properties that afford favorable reactivity. For example, peptides including these amino acids have an increased resistance to enzymatic degradation due, in part, to the fact that the amino acid cannot enolize. Similarly, for peptides that contain at least two amino acids with unsaturated side chains, “peptide stapling” is possible where the alkenes are cross-linked via ring-closing metathesis (RCM). These linkages can favor the formation of α-helices within the peptide, conferring greater overall stability. These motifs are also important in cellular signaling and transport.  

\[ \text{Figure 5.19. Structure of } \textit{rac}-\text{allylalanine monohydrate.} \]

Despite the amino acid’s utility, there are no robust methods for producing large quantities of optically pure material. The racemate can be readily furnished by traditional chemical methods; however, the resolution of this material into is generally performed on smaller scales via selective enzymatic derivatization of one enantiomer and separation from the unreacted stereoisomer. At the time of writing, enantiopure samples of allylalanine sell for $1622/g for the (R)-enantiomer and $1332/g for the (S)-enantiomer. Asymmetric protocols via stoichiometric or catalytic methods have been reported. However, these suffer from low yields, incomplete stereoselectivity, and/or access to only one enantiomer.

A more robust method for accessing enantiopure allylalanine would be via resolution by CPC, as shown in Scheme 5.4. As previously discussed, the monohydrate form of this amino acid is a conglomerate, allowing for the resolution of as large a quantity as necessary via this method. Moreover, the direct result of this process is the resolution of a racemate into its constituent
stereoisomers in one operation, which are each primed for further chemical elaboration in to optically pure, value-added commodities.

Scheme 5.4. Resolution of rac-allylalanine by continuous preferential crystallization (CPC). (The monohydrate solvate is not shown for clarity.)

5.4.2 Synthesis of rac-Allylalanine Monohydrate

Our synthesis begins with inexpensive and readily available DL-alanine (5.1), which is transformed into hydantoin 5.2 via the reaction with phenyl isocyanate under basic conditions, followed by acid-mediated ring closure. The free N–H is protected using standard conditions (Boc anhydride and DMAP) to give N\textsuperscript{1}-Boc,N\textsuperscript{3}-phenyl hydantoin 5.3. This intermediate is then allylated under strong base (lithium hexamethyldisilazide) with allylbromide to give allylhydantoin 5.4. Hydrolytic ring-opening and deprotection under basic conditions followed by trituration gives the crude anhydrous allylalanine, which is then recrystallized from water to give rac-allylalanine monohydrate in decagram-scale as a white powder.

This synthesis is an improvement on other methods since all intermediates are crystalline, improving the purity of the final product. From our experiences, for synthetic routes that involve liquid intermediates\textsuperscript{339} the purity of the intervening compounds can be difficult to control due to the high solubility of contaminants in the liquid phase. In our synthesis, the purity of the intermediates is regularly improved from recrystallization as a part of the work up. Moreover, the use of aq. NH\textsubscript{4}OH for the hydrolysis is critical in order to prevent carry through of water-soluble inorganic salts (such as
those that would form as a result of acid-mediated hydrolysis) to the final rac-allylalanine, as these can prevent the formation of the monohydrate conglomerate.

Scheme 5.5. Synthetic route to rac-allylalanine monohydrate on decagram scale from DL-alanine.

5.4.3 Process Development Goals

As seen in Chapter 4, allylalanine monohydrate does not lend itself well to preferential crystallization in batch because the kinetic window for entrainment is very short. To remedy this, a continuous method for the resolution of rac-allylalanine monohydrate could be used. However, there are many variables that must be considered when developing a continuous preferential crystallization process and most are optimized via trial and error. There have been attempts to predict optimal conditions from first principles;\textsuperscript{191,340-345} however, these models are based on narrow parameter ranges and any deviation can lead to discrepancies between the predicted outcome and reality.

These limitations are due, in part, to the few ways in which preferential crystallizations can be monitored. The most straightforward method involves manually taking samples of the slurry from the crystallizer, filtering off the supernatant, and measuring the e.e. of the solid. For a successful CPC, the crystallizing reactor should always have enantiopure solid, while the dissolver contains enantiopure...
solid at the end of the resolution (for a two-pot CPC). However, by taking slurry samples, material is continuously being removed from the reactor, which can lead to several issues: First, the reactor will be regularly perturbed from sampling, increasing the risk of primary nucleation of the unseeded enantiomer. Second, the kinetics of crystallization can change depending on the amount and frequency of samples. Since entrainment is a result of secondary nucleation, the removal of crystals from the reactor could lead to variation in the crystallization profiles between trials.

In contrast to the one-pot preferential crystallization procedure, the solution phase e.e. is not as informative a metric for tracking the progress of CPC because of the liquid circulation. In the continuous mode, the supernatant is constantly being renewed and at best, in-line solution phase methods such as polarimetry will display a stable, non-zero e.e. of the crystallizer if the rate of entrainment in the crystallizer is constant. The dissolver, meanwhile, should have a racemic solution phase e.e. for the majority of the CPC (provided that restoration of the depleted enantiomer by solid racemate is rapid). More worrisome, however, is that the solution phase e.e. of the crystallizer can also remain constant if the rates of crystallization for both enantiomers are identical. Because polarimetry (usually) does not give concentration information, it would be hard to distinguish between a successful and failed process without offline samples of the solid phase e.e.

With the automated solubility and crystallization monitoring technology that we have developed (and described in Chapter 4) we are poised to look at these systems from a unique angle, with the ultimate goal of a greater understanding of complex physical processes to which we were previously blind. In particular, because our system allows for the quantification of both the enantiomeric excess and the solution phase concentration of the dissolved enantiomers, this allows us to capture temporal profiles of the continuous preferential crystallization process that are otherwise unavailable with presently used technologies. In developing a robust process for the resolution of rac-
allylalanine monohydrate by CPC, we wanted to know if the data obtained with our automated system could provide quantitative information on important process development questions such as:

1. What type of seed (ground, single crystals, or a slurry) and how much should be added to initiate preferential crystallization?
2. What is the ideal pump rate for an efficient continuous preferential crystallization?
3. What is the ideal temperature difference between the crystallizer and the dissolver?
4. If the CPC begins to fail (crystallization of the unseeded enantiomer), are there telltale signs in the temporal solution phase concentration profiles that indicate this?
5. Can we use the solution phase concentration profiles to help correct a failed CPC?

5.4.4 Monitoring CPC using Automated Solution Phase Sampling

Figure 5.20a,b give two possible profiles for the solution phase concentration behavior of the crystallizing reactor during CPC. In this scenario, the crystallizer contains a supersaturated solution of the racemic compound. At time $t_1$, enantiopure ($S$)-seed is added to initiate preferential crystallization, which should lead to a decrease in the solution phase concentration of the ($S$)-enantiomer. At time $t_2$, the pumps are initiated to begin CPC and from here we predicted two extremes of the solution phase behavior. Upon starting the pumps, Figure 5.20a shows a scenario in which crystallization of the seeded enantiomer outcompetes renewal of the depleted enantiomer in the dissolver (CPC is dissolution rate-limited). Alternatively, Figure 5.20b, shows a scenario in which the opposite occurs. Upon starting the pumps, crystallization of the seeded enantiomer is slower than dissolution, since the solution phase concentration of the ($S$)-enantiomer quickly returns to its initial value (CPC is crystallization rate-limited). Moreover, because the solution phase concentration is being monitored, we are able to conclude that in the two scenarios of Figure 5.2, crystallization of the unseeded enantiomer has not occurred since the concentration of the ($R$)-enantiomer the same before
and after the seed was added and during the pumping cycle. The time at which the pumps are started are related to the limit of entrainment for the compound under consideration. This must be performed after a large enough change in the solution concentration of the seeded enantiomer can be reliably detected. Conversely, the pumps should also not be started too close to the limit of entrainment since primary nucleation can then occur, leading to a failed CPC. Throughout the CPC process, it is likely that the observed trends in the solution phase concentration of the enantiomers will change as a result of increasing solids in the crystallizer.

**Figure 5.20.** Possible profiles for the temporal solution phase concentration behavior of the crystallizing reactor during CPC upon starting the pumps: (a) The solution phase concentration of the seeded enantiomer remains approximately the same. (b) The solution phase returns to an approximately 1:1 ratio of both enantiomers.

Figure 5.21 gives preliminary results of the initial phase of a two-pot CPC resolution of rac-allylalanine. A saturated solution of rac-allylalanine was created in the crystallizer at 20 °C, followed by the addition of 50 mg of (S)-allylalanine seed. As we can see from the slight imbalance of the two enantiomers and the negative solution phase e.e. of the system at 0 h, some of the seed has dissolved into the saturated solution. Nevertheless, upon slowly cooling the system to 18 °C, we can see that entrainment has initiated since the solution phase concentration of this stereoisomer has begun to decrease and the solution e.e. has increased. The pumps were started at 380 min, which caused the
solution phase concentration of the $(S)$-enantiomer to return to the same value as the $(R)$-enantiomer by 10 h and the solution e.e. is 0. Over time, the mass of solids in the crystallizer increased and of particular note, is that during the first 16 h of the experiment that is displayed, there seems to be no primary nucleation of the unseeded enantiomer, strongly indicating the success of the CPC resolution.

The following morning, a sample of the slurry of the crystallizer was taken, the supernatant decanted, and the solid was redissolved and analyzed by HPLC-ELSD with chromatographic separation of the enantiomers via an Astec Chirobiotic T column. As shown in Figure 5.22, the ratio of enantiomers is approximately 3:1, an extremely promising value since this indicates that the majority of the sample is $(S)$-allylalanine monohydrate. The contamination of the sample with the $(R)$-enantiomer could be due to two possible reasons: Either primary nucleation of the $(R)$-enantiomer occurred after the time displayed by Figure 5.21 or that the decanting of the supernatant was incomplete, leading to the presence of the $(R)$-enantiomer in the analyzed sample. Nonetheless, these

**Figure 5.21.** Temporal solution phase concentration and percent enantiomeric excess of the $(R)$- and $(S)$-enantiomers of allylalanine monohydrate in the two-pot continuous preferential crystallization. (% e.e. = $(\lbrack R \rbrack - \lbrack S \rbrack)/ (\lbrack R \rbrack + \lbrack S \rbrack) \times 100.$)
results show that the automated sampling technology is capable of monitoring the progress of the CPC and will continue to be used as we come to understand and optimize this process.

Figure 5.22. HPLC-ELSD chromatogram showing the analysis of the solid phase at the end of the CPC experiment depicted in Figure 5.21, with chromatographic separation via an Astec Chirobiotic T column. The enantiomeric ratio of the two isomers is approximately 3:1.

5.4.5 Monitoring CPC Using Particle Size Measurements

Other in situ measurements that are useful for understanding crystallizations are furnished by focused-beam reflectance measurements (FBRM) augmented by in situ light microscopy of the crystals via particle view measurement (PVM). These tools monitoring the crystal counts and distributions of various crystal size ranges, providing temporal trends on the crystallization.

In the trial resolution of rac-allylalanine monohydrate, a BlazeMetrics InProcess particle sizing probe was submerged into the crystallizer. This in situ technology is an adaptation of FBRM that also provides information on the size and number of the crystals in the reactor. Figure 5.23 gives the temporal counts of three different chord length ranges (0-400 μm, 1-20 μm, and 20-50 μm) for a 20 h portion of the CPC resolution. As seen in the figure, there is an upward trend in the number of crystals over time in each of the size ranges. This is indicative of crystallization, meaning that the kinetics of crystal growth can be measured in real-time. Moreover, oscillations are observed in the
figure, which are due to the pump cycles used for this particular CPC experiment (see Experimental). Notably, at approximately 5 h the pump parameters were changed to establish a longer pumping cycle, which was captured by the Blaze. Thus, the in situ Blaze data can also be utilized for the understanding and optimization of the CPC resolution of rac-allylalanine monohydrate.

![Graph showing particle size data over time](image)

**Figure 5.23.** In situ particle size data for the CPC resolution of rac-allylalanine monohydrate for the over a 20 h period. (*Data for the 20-50 μm chord length range were scaled by a factor of 10^4.*)

### 5.5 Conclusions and Future Directions

This chapter has discussed the motivation for and preliminary results from the development of a chiral resolution of rac-allylalanine via continuous preferential crystallization. We have demonstrated that the automated sampling apparatus discussed in Chapter 4 can be used to monitor a resolution by CPC and help answer important questions related to process optimization.

Future work would include additional trials of the CPC resolution as many questions remain to be answered with respect to optimal conditions. Once an efficient process is obtained, it would allow access to large quantities of enantiopure compound, which can be transformed into other
important molecules. Current synthetic targets include the α-methyl variants of the canonical amino acids (Scheme 5.6).

Scheme 5.6. Synthesis of enantiopure α-methyl variants of the canonical amino acids enabled by continuous preferential crystallization (R = amino acid side chain).

The *in situ* monitoring tools are extremely adept at providing temporal trends on the progress of the crystallization and further upgrade would allow for accelerated process optimization. For example, integration of an EasySampler to the system would allow for parallel solution and slurry phase sampling, providing a means for analyzing the solid phase optical purity without manual sampling. The microscopy unit of the Blaze could also be harnessed to report on the purity of the solid phase. Figure 5.24 shows a well-defined enantiomorphic hemihedral crystal of one enantiomer of allylalanine monohydrate that has formed during CPC and captured *in situ* by the BlazeMetrics instrument. With the addition of image recognition protocols, a failure of the CPC can be detected through the observation of the undesired enantiomorph.
Lastly, our lab has begun to incorporate artificial intelligence (AI) to our robotic instruments, allowing for autonomy in addition to automation. Integration of AI with CPC would allow for a self-correcting and/or self-optimizing system. As shown in Figure 5.25, automated samples from the CPC could be passed directly on to an HPLC column (direct inject) and analyzed. An artificial intelligence engine would then interpret the data and determine the progress of the CPC. If a failure was detected, the AI could then change the reactor parameters automatically to correct the system.

![Figure 5.24. Enantiomorphic hemihedral crystal of allylalanine monohydrate captured in situ by the BlazeMetrics instrument.](image)

![Figure 5.25. Artificial intelligence integrated with CPC.](image)
5.6  Experimental

5.6.1  Chemical Suppliers

\textit{rac}-Allylalanine monohydrate was synthesized as previously reported.\textsuperscript{550} All other reagents and solvents were purchased from conventional suppliers and used as received unless otherwise stated.

5.6.2  Equipment

All experiments were performed in Mettler-Toledo EasyMax 102 Advanced Synthesis Workstation glass reactors (50 or 100 mL) equipped with either glass or Teflon reactor covers, submersible thermocouple, and magnetic or overhead stirring and controlled by the Mettler-Toledo software iControl 6.0. The internal reactor temperature ($T_i$) was maintained by the EasyMax and measured by a thermocouple placed directly in contact with the contents of reactor.

Temporal solubility data was obtained using a custom-built automated reaction sampling apparatus similar to that previously reported by our group.\textsuperscript{61, 62, 64, 65} In summary, a PTFE filter was affixed to the end of a ETFE sampling capillary (1/16” outer diameter, 0.020” inner diameter) that is submerged into the liquid of the reactor to allow the solid-free supernatant to be withdrawn. At fixed time points, 200 µL samples were automatically taken at a draw speed of 2 mL/min by a New Era Syringe pump (25 mL SGE syringe) through the filter and sampling capillary into a 20 µL sample loop attached on a 6-port, 2-position Gilson 918 Injection Valve (selection valve). The valve position is then switched, and the sample is delivered directly to a 2 mL LC vial located on the bed of a Gilson 215 automated liquid handler robot, by diluting the captured aliquot with 1.0 mL of water.

The timing of the sampling technology was governed by the Mettler-Toledo iControl software that communicates with the EasyMax and can trigger the removal of the reactor aliquot by the syringe pump, actuation of the selection valve position by the Rheodyne, and sample dilution by a diluent pump. The EasyMax and Gilson Liquid Handler were interfaced via electrical contacts through a
Mettler-Toledo Universal Control Box, “UCB.” The prepared samples were manually transferred to the HPLC-ELSD for analysis as they were prepared or upon completion of the sampling period.

Figure 5.26. Photo of the automated sampling apparatus.

Figure 5.27. Photo of the syringe pump, valve, and EasyMax reactor.
Particle size measurements were performed using a BlazeMetrics InProcess instrument. The image plane was set to 120 µm or 220 µm.

5.6.3 Analytical Methods

HPLC analysis was performed on a standard Agilent 1290 Infinity HPLC equipped with a 385-ELSD detector. The collected samples were analyzed using the following method:

Method A: rac-Allylalanine Monohydrate

Astec Chirobiotic T Column, 4.6 x 150 mm; 5 µm
Solvent A = Water; Solvent B = Methanol
Flow Rate = 1.00 mL/min
Column Temperature = 25 ºC
Injection Volume = 8 µL
Pump Program: isocratic, A:B = 10:90

ELSD parameters were as follows: Evaporator = 50 ºC, Nebulizer = 50 ºC, Gas Flow Rate = 1.6 SLM, Data Rate = 80 Hz, LED Intensity = 100%, Smoothing = 50 (5.0 s), PMT Gain = 1.0. Concentration values of the samples removed from the reactors were obtained by constructing calibration curves of concentration against ELSD peak area and a described in further detail below.

5.6.4 General Procedure for Monitoring a Preferential Crystallization of Allylalanine Monohydrate by Automated Sampling

To a 100 mL EasyMax reactor equipped with a Teflon reactor cover, thermocouple, and magnetic stir bar was charged a given amount of the desired solvent or solvent mixture (approximately 50 mL). The reactor was placed into the well of an EasyMax 102 Advanced Synthesis Workstation, the stirring was enabled and set to 400 rpm, and the contents of the reactor were heated to 20 ºC, as
measured by the internal reactor temperature, $T_r$. A portion of the desired analyte (~2-3 g) was charged directly into the reactor and allowed to stir for at least 1 h. If at any point the solids were seen to completely dissolve, additional portions (0.1-0.5 g) were added and until no more dissolution was observed. After an equilibration period was sustained, the solid-free supernatant of this reactor was transferred into a second EasyMax 100 mL reactor equipped with a Teflon reactor cover, thermocouple, magnetic stir bar, and ReactIR probe via a peristaltic pump by withdrawing the liquid through an ETFE filter attached to the end of the peristaltic tubing and submerged into the heterogeneous mixture. The jacket temperature of the second reactor, $T_j$, was set to 35 °C to prevent primary nucleation of the dissolved material as a result of the transfer process. Once the transfer was complete, the internal reactor temperature, $T_r$, was lowered to 18 °C over 20 min to yield a supersaturated solution. For the preferential crystallization, 15 mg of enantiopure ($\delta$)-allylalanine was added followed by initiation of the sampling program.

Figure 5.28. Photos of EasyMax reactor set up for automated solubility measurements.
5.6.5 Calibration Curves

5.6.5.1 General Procedure for Calibration of ELSD Response and Solubility

To calibrate the ELSD response for each compound and enantiomer, stock solutions of known concentration (mg solute/mL solution) of each racemic compound were prepared in 10 mL volumetric flasks. A given amount of racemic solute was weighed directly into the volumetric flask and filled to the calibration line with deionized water. After mixing the flask thoroughly, the clean sampling line (without filter attached) was inserted directly into the flask and at least three samples were prepared using the automated apparatus. After analyzing the samples using HPLC-ELSD, the raw peak area was plotted against solute concentration. For compounds that displayed a linear ELSD response in the desired working concentration range, a least-squares regression line was fitted onto the data directly. For compounds in which ELSD response was non-linear in the working concentration range, a plot of \( \log_{10}(\text{peak area}) \) against \( \log_{10}(\text{concentration}) \) was generated and a least-squares regression line was fitted to this transformed data.

For the latter case, the resulting equation resulting from linear regression takes the form

\[
\log_{10}(\text{peak area}) = a \log_{10}(\text{concentration}) + b
\]

where \( a \) and \( b \) are constants. To obtain concentration from peak area \( A \), we rewrite the equation as

\[
\log_{10}(A) - b = a \log_{10}(\text{concentration})
\]

Dividing both sides by \( a \), we obtain

\[
\frac{\log_{10}(A) - b}{a} = \log_{10}(\text{concentration})
\]

Finally, raising both sides of this equation to the power of 10, we obtain

\[
10 \left( \frac{\log_{10}(A) - b}{a} \right) = \text{concentration}
\]
5.6.5.2 Calibration of ELSD Response for \textit{rac}-Allylalanine Monohydrate

\textbf{Figure 5.29.} Non-linear ELSD peak area response against concentration for \textit{rac}-allylalanine monohydrate.

\textbf{Figure 5.30.} Log-log plot of ELSD peak area and concentration for \textit{rac}-allylalanine monohydrate with least-squares regression line of best fit.
5.6.6 Solubility Data for rac-Allylalanine Monohydrate

Figure 5.31. Solubility data for rac-allylalanine monohydrate in EtOH:Water = 75:25 (v/v).

Table 5.2. Solubility data for rac-allylalanine monohydrate in EtOH:Water = 75:25 (v/v) by ELSD. Error values listed represent one standard deviation.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>(S)-enantiomer (mg/mL)</th>
<th>(R)-enantiomer (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>23.82 ± 0.04</td>
<td>24.21 ± 0.12</td>
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<tr>
<td>2</td>
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<td>25.81 ± 0.14</td>
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<tr>
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<td>27.39 ± 0.17</td>
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<td>6</td>
<td>28.21 ± 0.28</td>
<td>28.76 ± 0.06</td>
</tr>
<tr>
<td>8</td>
<td>30.14 ± 0.06</td>
<td>30.61 ± 0.15</td>
</tr>
<tr>
<td>10</td>
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<td>32.71 ± 0.20</td>
</tr>
<tr>
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<td>34.49 ± 0.16</td>
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<td>40.45 ± 0.28</td>
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<td>42.80 ± 0.21</td>
</tr>
<tr>
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<td>45.10 ± 0.13</td>
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<td>47.09 ± 0.04</td>
<td>47.60 ± 0.22</td>
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<td>26</td>
<td>50.05 ± 0.14</td>
<td>50.57 ± 0.31</td>
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<td>28</td>
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<td>53.49 ± 0.19</td>
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<td>30</td>
<td>56.14 ± 0.20</td>
<td>56.51 ± 0.33</td>
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<td>59.75 ± 0.19</td>
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<td>36</td>
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<td>66.98 ± 0.05</td>
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<td>38</td>
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<td>70.75 ± 0.31</td>
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<tr>
<td>40</td>
<td>74.62 ± 0.25</td>
<td>74.95 ± 0.33</td>
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5.6.7 Procedure for the Resolution of \textit{rac}-Allylalanine Monohydrate by Continuous Preferential Crystallization in Two Reactors

To a 100 mL Mettler-Toledo EasyMax reactor (the “dissolver”) equipped with a Teflon reactor cover, thermocouple, and magnetic cross-shaped stir bar was charged approximately 8 g \textit{rac}-allylalanine monohydrate and 50 mL of EtOH:water = 75:25. The reactor was placed in the right well of an EasyMax 102 Advanced Synthesis Workstation with a jacket temperature, $T_j = 20 \degree C$. The automated sampling apparatus was attached to this reactor. Next, to a second 100 mL EasyMax reactor (the “crystallizer”) equipped with Teflon reactor cover, thermocouple, and magnetic cross-shaped stir bar was charged approximately 6 g of \textit{rac}-allylalanine monohydrate and 50 mL of EtOH:water = 75:25. This reactor was placed in the left well of the same EasyMax 102 Advanced Synthesis Workstation with a jacket temperature, $T_j = 20 \degree C$. The stir rate of both reactors was set to 500 rpm.

A NewEra NE-9000 Peristaltic Pump equipped with a NE-9000G Green Head was affixed to the system. At the ends of the liquid line connecting both reactors (PTFE tubing, 3 mm OD, 1.5 mm ID) of the peristaltic pump were attached two Quality Lab Accessories Porous Micron (Full Flow) Filters (P/N FIL010-PT-a, 10 µm pores) to allow for transfer of the solid-free supernatant between the reactors. The initial pump parameters were set to pump alternating 25 mL portions of the solid-free supernatant between the two reactors in a semi-continuous manner. I.e., the pump would first transfer 25 mL of solid-free supernatant from the dissolver to the crystallizer. This is followed by a 5 s pause before the pump would return a 25 mL portion of the solid-free supernatant from the crystallizer to the dissolver. This pumping cycle was continued overnight to ensure saturation of the liquid phase with \textit{rac}-allylalanine monohydrate and to verify the continuity and accuracy of the pump.

The next morning, the entire contents of the crystallizer (solids + liquids) were manually transferred to the dissolver, followed by a brief cleaning of the reactor. Following reassembly of the
crystallization reactor, a BlazeMetrics InProcess particle sizing probe was affixed to the reactor to allow for *in situ* particle size monitoring, with the image plane set to 220 µm. Once the reactor was returned to the left EasyMax well \((T_i = 20 \, ^\circ\text{C}, \text{stirring} = 500 \, \text{rpm})\) 25 mL of the solid-free supernatant was transferred from the dissolver to the crystallizer. After allowing for thermal equilibration (approx. 1 h), 15 mg of enantiopure crushed \((S)\)-allylalanine monohydrate was added to crystallizer. After stirring for 1 h, the internal reactor temperature \((T_i)\) of the crystallizer was lowered to 18 °C over 60 min. At the same time the cooling ramp was started, automated sampling of the solid-free supernatant was also initiated to monitor the entrainment at 15 min intervals. After 260 min following the end of the cooling ramp, the pump cycles were started (same parameters as previous) to begin the continuous preferential crystallization. (See Figure 5.21 for annotated graph.) Monitoring of the solution phase of the crystallizer was continued for a total of 16 h. The next morning, a slurry sample (~100 µL) of the crystallizer was removed to check the optical purity of the solid. The slurry sample was dispensed directly into a 2 mL Eppendorf tube and centrifuged briefly. The supernatant was decanted and the solid was redissolved in water and transferred into an LC vial. Following HPLC-ELSD analysis, the enantiomeric ratio of the solid phase was approx. 3:1 \((S:R)\).

The next morning, automated solution phase sampling was initiated once again and at the time indicated on Figure 5.23, the peristaltic pump parameters were changed such that following transfer of the supernatant from one reactor to another, a 30 min pause was sustained prior to the next pump operation.
Chapter 6: Conclusions and Outlook

This Thesis has presented case studies in which a combination of “traditional” and “modern” kinetic analyses of complex chemical and physical processes allowed for an in-depth understanding and solutions to difficult problems. Motivated by the power of modern graphical analyses to shed light on convoluted chemical mechanisms with an overall reduction in time and resources, new automated reaction monitoring tools adapted to each system of interest were constructed and deployed to capture high-density, time-resolved information. The studies discussed in this Thesis contained several reoccurring themes:

1. High-density, temporal reaction progress information is fundamental to process understanding.
2. Using an end-of-reaction metric (e.g., yield or conversion) is misleading and incorrect for process optimization.
3. The development of new in situ reaction monitoring technology is required for efficient kinetic analysis of the processes of interest.

Chapter 2 discussed the development of a new automated reaction sampling and quenching procedure to monitor an unusual copper-catalyzed acetylenic hydrogen-iodide exchange equilibrium between terminal alkynes and 1-iodoalkynes. Although this exchange had been reported in the literature, to our knowledge no in-depth kinetic or mechanistic studies had been performed. By monitoring both the rate ($k_{obs}$) and position ($K_{eq}$) of the equilibrium for exchange reactions involving various combinations of electron-deficient or electron-rich 1-iodoalkynes and terminal alkynes, it was discovered that while the position of the equilibrium is the same for all iodoalkyne/terminal alkyne pairs ($K_{eq} = 1$), the rate of approach towards equilibrium depends mostly on the electronic characteristics of the terminal alkyne.
For exchange reactions in which exogenous base was added, the rate of equilibration increased while maintaining the equilibrium position. Interestingly, with added base the extent of homo- and heterodimeric dialkyne by-product formation was increased, with temporal concentration profiles that depended on the nature of the starting alkynes. A mechanism was proposed that accounted for the main exchange cycle and off-cycle by-product generation. The knowledge gained in our study yielded practical considerations for reactions in which terminal alkynes and 1-iodoalkynes are simultaneously present. A direct connection involves the family of Cu-catalyzed couplings of alkynes, including Glaser–Hay, Cadiot–Chodkiewicz, and others that have been plagued by undesired by-products. Moreover, this study was necessary to place this ancillary pathway within of copper-catalyzed azide-alkyne cycloaddition reactions to form 1,4-dialkylsubstituted-1,2,3-triazoles (Chapter 3).

Chapter 3 presented an in-depth kinetic investigation on the Cu(I)-catalyzed cycloaddition of azides and alkynes to form 1,4-dialkylsubstituted-1,2,3-triazoles in regiospecific manner. Motivated by the observation of unusual kinetic phenomena in competition reactions involving organic azides, terminal alkynes, and 1-iodoalkynes, a systematic mechanistic study was initiated, allowing for the identification of previously unrecognized mechanistic pathways, including a previously unreported counter-ion effect. Moreover, lingering mechanistic questions in the literature on this transformation were settled. It was discovered that the chemoselective formation of iodotriazoles from 1-iodoalkynes in the presence of terminal alkynes is a function of the disposition and speciation of the copper catalyst within a more complicated reaction network. Critically, the observed chemoselectivity is not due to a relative rate difference between the two cycloaddition reactions.

Ultimately, this study offered two important advances. First, the added mechanistic and kinetic understanding of this important copper-catalyzed reaction provides new opportunities to develop other chemoselective methods where terminal and 1-iodoalkynes are simultaneously present. With these two species, judicious choice of the catalyst counter-ion is evidently critical to achieve
differential reactivity. Second, this study reinforced the importance of continued developments in modern reaction analytical methodologies; the automated reaction monitoring technology used in this Chapter enabled rapid deconvolution of a complex and challenging reaction. The rich, impactful, and highly nuanced information revealed by the experiments detailed therein would have been undoubtedly overlooked had highly resolved, time-course measurements not been available.

Chapter 4 discussed the development and validation of new technology for solubility and crystallization analysis of non-UV-active species by coupling robotic sampling and HPLC-ELSD quantification. Both static processes (those exhibiting solubility equilibrium) and dynamic processes (undergoing active crystallization) could be readily monitored with a high degree of reproducibility and fidelity. In response to the limited number of technologies that can furnish accurate and highly-resolved solution phase concentration for systems undergoing crystallization, the construction of the new automated technology allowed for the precise capture of temporal information on these physical processes initiated by spontaneous primary nucleation due to reactor cooling, the addition of seed to enable entrainment via secondary nucleation, or the formation of diastereomeric salts upon the addition of a resolving agent. We foresaw the new technology having a prominent role in reducing bottlenecks in solubility and crystallization analysis and improve our theoretical understanding of these processes to which we were previously blind. Moreover, the system can be augmented for use with “direct-inject” LC systems wherein aliquots of the solid-free supernatant are delivered directly onto an LC column for analysis, eliminating the need for vial preparation and providing a near real-time means of analysis. This technology was necessary for more involved studies on preferential crystallization discussed in Chapter 5.

Chapter 5 discussed the application of the technology described in Chapter 4 to study and optimize the chiral resolution of rac-allylalanine monohydrate, a versatile non-proteinogenic α,α-disubstituted amino acid building block, via continuous preferential crystallization (CPC). A summary
of the advantages and disadvantages of “traditional” methods for the generation of enantioenriched compounds was first provided along with a motivation for the use of preferential crystallization as a means for chiral resolution. A discussion on the various solid forms of chiral compounds (conglomerate, racemic solid, or solid solution) was provided alongside descriptions of the various methods by which conglomerates, the form needed for preferential crystallization, can be detected. A basic description on the principles underlying preferential crystallization by entrainment in batch was then provided and the possible pitfalls of this resolution method were discussed.

Following a brief introduction on a new continuous method for the resolution of conglomerates, this chapter discussed the motivation for and preliminary results from trial developments of a chiral resolution of rac-allylalanine via CPC. We demonstrated that the automated technology discussed in Chapter 4 could be used to monitor a resolution by CPC and help answer important questions related to process optimization. The initial data gathered on the trial resolutions are extremely promising, but further work needs to be accomplished in order to find the optimal conditions on the CPC resolution. Following this, access to large quantities of enantiopure allylalanine would then allow the synthesis of value-added stereochemically pure derivatives. Further integration of the BlazeMetrics InProcess particle sizer would allow for the tracking of the kinetics of crystallization in real time and adaptation of the microscopy unit of the Blaze could also be harnessed to report on the purity of the solid phase via enantiomorphic crystal recognition. Similarly, future integration of the crystallization hardware with artificial intelligence could enable a self-optimizing or self-correcting CPC.

Future work could involve the upgrade of the automated systems described in this Thesis to provide more information (via the addition of additional in situ monitoring devices) or allow an easier or quicker access to reaction progress information. The concept of a “connected lab” in which instruments provide, analyze, and store data in-real time to a central web-based access point has become increasingly attractive within recent years as a means to cope with the large data sets that are
generated with data-rich experiments.\textsuperscript{38} Lastly, as discussed briefly in Chapter 5, our lab has made recent in-roads to incorporate artificial intelligence to our robotic platforms, allowing for both autonomy and automation in problem solving and process development.\textsuperscript{349}
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Appendices

Appendix A  NMR Spectra for Chapter 2

A.1  *tris*(1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amine (TCPTA)

^1^H-NMR (400 MHz, CDCl\textsubscript{3})

^1^C\textsubscript{\{^1^H\}}-NMR (101 MHz, CDCl\textsubscript{3})
A.2  (Iodoethynyl)benzene

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

$^{13}$C$^{(1)}$H$^{}$-NMR (101 MHz, CDCl$_3$)
A.3 1-(iodoethynyl)-4-methylbenzene

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

$^{13}$C-$^1$H-NMR (101 MHz, CDCl$_3$)
A.4 1-(iodoethynyl)-4-(trifluoromethyl)benzene

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

$^{13}$C-$^1$H-NMR (101 MHz, CDCl$_3$)
$^{19}$F{^1}H-NMR (377 MHz, CDCl$_3$)
A.5 1,4-diphenylbuta-1,3-diyne

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

$^{13}$C{$^{1}$H}-NMR (101 MHz, CDCl$_3$)
A.6  1,4-di-\(p\)-tolylbuta-1,3-diyne

\(^1\)H-NMR (400 MHz, CDCl\(_3\))

\[^{13}\)C\((^1\)H\)}-NMR (101 MHz, CDCl\(_3\))
A.7 1,4-bis(4-(trifluoromethyl)phenyl)buta-1,3-diyne

\(^1\)H-NMR (400 MHz, CDCl\(_3\))

\[^{13}\{^1\text{H}\}\}-\text{NMR} (101 \text{ MHz, CDCl}_3)
$^{19}$F-{H}-NMR

$\mu$CF$_3$PhCCH Dimer

2.6c
Appendix B  NMR Spectra for Chapter 3

B.1  1-benzyl-5-iodo-4-phenyl-1H-1,2,3-triazole

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

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

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

![1H NMR spectrum](image)

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

![$^{13}$C{$^1$H} NMR spectrum](image)
B.3 5,5-bis(azidomethyl)-2,2-dimethyl-1,3-dioxane

$^1$H NMR (300 MHz, CDCl$_3$)

$^{13}$C{$^1$H} NMR (75 MHz, CDCl$_3$)
B.4 1-((5-(azidomethyl)-2,2-dimethyl-1,3-dioxan-5-yl)methyl)-5-iodo-4-phenyl-1H-1,2,3-triazole [mono(iodotriazole)]

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

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$)
B.5  1,1'-((2,2-dimethyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(5-iodo-4-phenyl-1H-1,2,3-triazole) [bis(iodotriazole)]

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

$^{13}$C-$^1$H NMR (101 MHz, CDCl$_3$)
B.6  1,1\'-(2,2-dimethyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(4-phenyl-1H-1,2,3-triazole) [bis(protiotriazole)]

\[ \text{bis(protiotriazole)} \]

\[ \text{1H NMR (400 MHz, CDCl}_3\text{)} \]

\[ \text{1C}\{\text{1H}\} \text{ NMR (101 MHz, CDCl}_3\text{)} \]
B.7 1-benzyl-5-iodo-4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole

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

$^{13}$C {$^1$H} NMR (101 MHz, CDCl$_3$)
$^{19}$F\{\textsuperscript{1}H\} NMR (377 MHz, CDCl$_3$)

$\text{pCF}_3\text{PhC}_6\text{H}_4\text{N}}_{3.17a}$
B.8 1-benzyl-5-iodo-4-(p-tolyl)-1H-1,2,3-triazole

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

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$)
B.9  1-benzyl-4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole

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

$^{13}$C$^\{1^\prime$H$\}$ NMR (101 MHz, CDCl$_3$)
$^{19}$F{¹H} NMR (282 MHz, CDCl₃)

N\ce{^\text{\textbullet}}N\text{Bn}

\text{CF}_3

3.18a
B.10  1-benzyl-4-(p-tolyl)-1H-1,2,3-triazole

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

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

C.1  5-methyl-3-phenylimidazolidine-2,4-dione

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

$^1$C{$^1$H} NMR (101 MHz, CDCl$_3$)

RC1-235-Hydantoin
C.2  tert-butyl 5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate

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

$^{13}$C{$^1$H} NMR (101 MHz, CDCl$_3$)
C.3  *tert*-butyl 5-allyl-5-methyl-2,4-dioxo-3-phenylimidazolidine-1-carboxylate

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

$^{13}$C-$^1$H NMR (101 MHz, CDCl$_3$)
C.4 2-amino-2-methylpent-4-enoic acid (allylalanine)

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

$^{13}$C{$^1$H} NMR (101 MHz, D$_2$O)
C.5 L-proline—L-tartaric acid complex

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

$^{13}$C{$^1$H} NMR (101 MHz, D$_2$O)