Enabling the Use of Unstable, Hazardous Reagents with Continuous Flow Synthesis

by

Laurel Millikan Heckman

B.S. Chemistry, summa cum laude
Butler University, 2011

Submitted to the Department of Chemistry in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY IN ORGANIC CHEMISTRY

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2018

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Signature redacted

Signature redacted

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Timothy F. Jamison
Department Head and Robert R. Taylor Professor of Chemistry
Thesis Supervisor

Accepted by: ________________________________

Robert W. Field
Haslam and Dewey Professor of Chemistry
Chairman, Departmental Committee on Graduate Studies
This doctoral thesis has been examined by a committee in the Department of Chemistry as follows:

Professor Stephen L. Buchwald

Professor Timothy F. Jamison

Professor Rick L. Danheiser
For my family, especially my late mother

An unabashed, self-assured, brilliant, successful
Feminist, Democrat, Working Mother

A force of nature
Enabling the Use of Unstable, Hazardous Reagents with Continuous Flow Synthesis

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Doctor of Philosophy in Organic Chemistry

ABSTRACT

Highly functionalized 2-arylindoles were synthesized from 2-alkenylarylisocyanides and arylboronic acids using a simple, inexpensive copper catalyst. The reaction exhibits excellent functional group tolerance for both the arylisocyanide and boronic acid coupling partners. To avoid the direct handling of the pungent arylisocyanide starting materials, continuous flow chemistry is further demonstrated to provide safe and effective access to 2-arylindoles through in situ dehydration and cyclization of easy-to-handle 2-alkenyl-N-formylanilines.

Laurel M. Heckman and Dr. Zhi He contributed equally to initial reaction investigation. Z.H. carried out the arylboronic acid scope. L. M. H. carried out reaction optimization, isocyanide scope and reactions in continuous flow.

ABSTRACT
Despite its utility, monochloramine (NH₂Cl) has not achieved widespread use as a nitrogen transfer reagent due to its unstable and hazardous nature. We developed a continuous flow platform for the safe, reliable, and inexpensive on-demand synthesis of NH₂Cl. Additionally we demonstrate the synthetic utility of NH₂Cl by converting it to valuable NH aziridine and nitrile products in good to excellent yield in exceedingly short reaction times.

Dr. Evan Styduhar developed continuous flow synthesis of NH₂Cl. E.S. also developed the reaction of NH₂Cl to form aziridines and nitriles in batch and continuous flow. Laurel M. Heckman helped optimize the continuous flow setup, performed the reaction scope in continuous flow, and explored additional substrates in batch.

ABSTRACT

A rapid, operationally simple synthesis of 6-TAMRA, an important probe for labeling biomolecules, from 2-carboxycarbonyltetraphthalic acid and 3-dimethylaminophenol is described herein. The intermediate ketoacid was synthesized in a single step from commercially available dimethylacetophenone. Additionally, progress was made towards a facile scalable synthesis in continuous flow.

Dr. Justin A. M. Lummiss carried out the oxidation batch synthesis. Laurel M. Heckman carried out reaction screening and optimization of step 2 of the batch synthesis. L.M.H and J.A.M.L contributed equally to the experiments in continuous flow. Dale Thomas (graduate student, Jensen Research Group, MIT Department of Chemical Engineering) developed the fully automated platform. Bruce Adams (Staff, DCIF of MIT Department of Chemistry) helped with low temperature and 2-D NMR experiments. Peter Müller (Director, Diffraction Facility of MIT Department of Chemistry) carried out the single-crystal X-ray diffraction experiments.

Thesis Supervisor: Timothy F. Jamison

Title: Department Head and Robert R. Taylor Professor of Chemistry
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I am deeply grateful for my time at MIT. It has been both challenging and rewarding, which would not have been possible without the help of many individuals, whom I’d like to thank briefly here.

My initial interest in chemistry was sparked as an undergraduate student at Butler University. I chose a degree in chemistry because the Department of Chemistry and Biochemistry is full of wonderful educators with an infectious passion for science. I would particularly like to thank Dr. Todd Hopkins for his mentorship, allowing me to research all four years of my undergraduate career; Dr. Luanne McNulty and Dr. Stacey O’Reilly for serving as strong and supportive women chemist role models; Dr. John Esteb for making my introduction to organic chemistry challenging and fun; Dr. Jeremy Johnson for his excellent class in biochemistry and support; and, of course, my best friend, freshman roommate, fellow chemist, Mary Andorfer, who continues to inspire and support me.

After graduating college, I was extremely fortunate to work at Howard Hughes Medical Institute Janelia Research Campus for Luke Lavis. I cannot thank him enough for the freedom he gave me to try things on my own, his endless encouragement and kindness, and his irresistible excitement for dye chemistry.

I chose MIT because I was drawn to the amazing science of the Jamison lab. Professor Tim Jamison proved to be a better thesis advisor than I even imagined. Working in the Jamison lab has been a joy. Tim is a gracious listener and a tireless and brilliant problem solver. I deeply appreciate his generosity with his time and am especially grateful for our monthly individual meetings. Some of my favorite moments during my PhD were discussing new scientific proposals with Tim. He supported my chemistry, but also my career and professional activities. Additionally, Tim has been an outspoken advocate for the women in the department and has effected real change for us. I am so proud of his leadership initiating the department wide biyearly sexual harassment prevention training. Thank you Professor Timothy F. Jamison for guiding me as a scientist while making the MIT Department of Chemistry a better place to work and grow.

I would like to thank nearly every Jamison group member that I have had the privilege to work with during my time here. They have been great colleagues and friends. This is a testament to Tim’s dedication to cultivating an inclusive, supportive environment where there are always people invested in your personal growth--eager to answer questions, brainstorm, and provide scientific feedback. For brevity, I will thank only a subset of those individuals here, but know that this list is certainly not comprehensive. Thank you to our lab manager, Rachel, for your helpful edits, kindness, support, and understanding. I deeply appreciate all of your hard work to keep our lab run smoothly. Thank you Ping and Yuan for your essential early mentorship in developing my synthetic skills. Thank you Kurt and Beth for your grounded, sage advice, unwavering kindness, and for motivating me to keep moving forward in my research. Thank you Zhi for working with me on the isocyanide project. You became a role model for me as a chemist, marked by kindness, humor, focus, and grit. You are truly missed. Thank you Justin for your supportive mentorship these past couple years. You helped me become a better writer,
presenter, and scientist. Thank you, too, for your hard work and insightful scientific conversations on rhodamine. I would not be graduating without you. Thank you Evan and Kelley for your work on chloramine. Thank you Allie, Rob, and Anne-Cat for your mentorship and dedication to graduate student education and growth. Thank you to my fellow graduate classmates, Andy and Charles, for your scientific discussions and friendship. Thank you Jessica for your friendship and positivity, and a heartfelt thank you for all of your hard work to make the chemistry department a more inclusive environment. It has definitely paid off. Thank you to Katie, Grace R., and Grace A. for your friendship and for making my last year fun.

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And of course, I’d like to thank my family. Thank you Randy for your positivity, support at home, edits, intellectual contribution, emotional support and unconditional love. I continue to strive to match your hard work and organization. Thank you Dad for being my first scientific role model. I am deeply grateful that I got to see first hand how much fun a career in science can be. You taught me that caring for and about your colleagues is an advantage, not a weakness. And of course thank you for your continued emotional and scientific support. Thank you to my sister Heather, whose efficiency, intellect, and humor I have always admired. Thank you for your amazing editing, career advice, friendship, joy, and for planning the most amazing family vacations. Thank you to my niece Josephine for the regular FaceTime calls and text messages, which always brought a smile to my face. I love you all very much. Finally, thank you to my mother, she would have been incredibly proud of this achievement. She valued higher education and science. She was fierce and I hope I am able to carry on that legacy.
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### Abbreviations

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<td>acetate</td>
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<tr>
<td>Ac₂O</td>
<td>acetic anhydride</td>
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<td>adamantyl</td>
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<td>aryl</td>
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<tr>
<td>BMIDA</td>
<td>N-methyliminodiacetic acid boronic ester</td>
</tr>
<tr>
<td>Bpin</td>
<td>boronic acid pincol ester</td>
</tr>
<tr>
<td>BPR</td>
<td>back pressure regulator</td>
</tr>
<tr>
<td>CSTR</td>
<td>continuous stirred-tank reactor</td>
</tr>
<tr>
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<td>cyclohexane</td>
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<td>DCM</td>
<td>dichloromethane</td>
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<td>dimethylformamide</td>
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<td>HFIP</td>
<td>1,1,1,3,3,3-hexafluoro-2-propanol</td>
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<td>HPFA</td>
<td>high-purity perfluoroalkoxy alkane</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
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<td>KOt-Bu</td>
<td>potassium tert-butoxide</td>
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<td>LiOr-Bu</td>
<td>lithium tert-butoxide</td>
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<td>tributylamine</td>
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<td>N-methylmorpholine</td>
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<td>NMP-</td>
<td>N-methylpyrrolidinone</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance (spectroscopy)</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>PEEK</td>
<td>polyether ether ketone</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch gauge</td>
</tr>
<tr>
<td>R</td>
<td>reactor</td>
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<tr>
<td>t-BuOH</td>
<td>tert-butanol</td>
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<tr>
<td>TAMRA</td>
<td>carboxytetramethylrhodamine</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>tᵣ</td>
<td>residence time</td>
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</table>
Preface

Unstable, Hazardous Reagents in Continuous Flow Synthesis
Continuous flow synthesis technologies have found particular utility in developing new and improved chemistries of unstable, hazardous reagents in both academia and industry. Organometallic reactions, oxidations, and nitrations, in addition to reactions with hazardous phosgene and azides have previously been improved with continuous flow synthesis.\(^1\) Examples of hazardous reagents include unstable intermediates, reagents capable of thermal runaways, and explosive reagents in addition to toxic and injurious reagents. These traits limit the exploration of their chemistry in traditional batch equipment, where increasing scale poses particular difficulty. Despite these drawbacks, in many cases, hazardous reagents are low cost, atom-economical, and possess unique, interesting reactivity.

Continuous flow systems are often greener and safer than their batch counterparts.\(^2\)-\(^3\) Continuous flow systems provide precise control over reaction time,\(^4\) improved scalability, mixing,\(^5\)-\(^6\) and heat transfer, and the ability to telescope multistep reactions.\(^7\) Additionally, full process automation is possible, decreasing the operational complexity of the system, while increasing reaction monitoring and reproducibility.

Continuous flow technologies encompass a range of reactor sizes, however herein, our discussion will be limited to microflow reactors (tubing inner diameter = 0.01–0.063 in.). Furthermore all multistep procedures including purifications described are intended to fit safely inside a standard academic laboratory hood. Examples of continuous flow equipment include syringe pumps to add reagents and PEEK mixers to mix reagents in high-purity perfluoralkane (HPFA) tubing reactors or packed bed reactors. Backpressure regulators allow precise control over the system’s pressure and liquid-liquid separators provide a means for inline purification (Figure 1).
Herein, we describe how continuous flow synthesis technologies enable the chemistry of hazardous reagents in a variety of ways, including safety, scalability, cost, or simplicity. We explore new chemistries of difficult to handle reagents including pungent arylisocyanides and reactive monochloramine, further demonstrating their synthetic utility while minimizing their inherent drawbacks. Additionally, we selectively synthesized 6-carboxytetramethylrhodamine, a popular and exceedingly expensive fluorescent label for biomolecules, in two steps at low cost. We developed its first continuous flow synthesis, providing a safe alternative for scaling reactions with hazardous solvents.

References


Chapter 1.
Synthesis of 2-Arylindoles via Copper-Catalyzed Coupling
of Isocyanides and Arylboronic Acids in Batch and
Continuous Flow

ABSTRACT

Highly functionalized 2-arylindoles were synthesized from 2-alkenylarylisocyanides and
arylboronic acids using a simple, inexpensive copper catalyst. The reaction exhibits excellent
functional group tolerance for both the arylisocyanide and boronic acid coupling partners. To
avoid the direct handling of the pungent arylisocyanide starting materials, continuous flow
chemistry is further demonstrated to provide safe and effective access to 2-arylindoles through in
situ dehydration and cyclization of easy-to-handle 2-alkenyl-N-formylanilines.

Laurel M. Heckman and Dr. Zhi He contributed equally to initial reaction investigation. Z.H.
carried out the arylboronic acid scope. L. M. H. carried out reaction optimization, isocyanide
scope and reactions in continuous flow.
Introduction

Due to their unique reactivity, isocyanides, also known as isonitriles, are versatile functional groups in organic synthesis.\(^1\) Resonance structures of isocyanides have been represented as either carbene or zwitterionic in nature (Figure 1).\(^2\) They generally demonstrate carbonic character, however the CN bond displays linear geometry, similar to a triple bond, due to the resonance stabilization provided from the lone pair of its nitrogen. The ambiphilic character at the terminal carbon of isocyanides allows its use as a connection point for multicomponent reaction sequences. Another synthetic advantage of isocyanides is their unusual ability to react with transition metals,\(^3\) nucleophiles,\(^1\) electrophiles,\(^4\) and radicals.\(^5\) Thus, isocyanides are excellent starting materials for the rapid synthesis of a diverse range of nitrogen-containing molecules.\(^6\)

\[ \text{R-N-C} \quad \rightarrow \quad \text{R-N=C} \]

Figure 1. Zwitterionic and carbonic resonance structures of isocyanides.

Recently, the 2-arylindole motif has been recognized for its ability to bind a wide variety of biological targets with high affinity. It is found in a variety of natural products, therapeutics and drug candidates.\(^7\) Key examples include bazedoxifene,\(^1e\) a treatment for postmenopausal osteoporosis, and the natural product cladoniamide G,\(^8\) which demonstrates anti-breast cancer activity. In addition, 2-arylindoles form the core of several medicines currently in development. Specifically, Kenpaullone has shown anti-cystic fibrosis activity,\(^9\) 2-phenylindole-3-carboxaldehydes have shown high anti-proliferative activity in breast cancer cell lines,\(^10\) and 2-pyridylindole has potential application as an anti-depressant (Figure 2).\(^11\) Despite the vast array of methodologies to synthesize 2-arylindole containing molecules,\(^1c,12\) new methods that
efficiently access highly decorated indoles remain desirable. Due to their unique reactivity, isocyanides in particular have gained in popularity in the preparation of the indole nucleus.

![Diagram of molecules]

**Figure 2.** Representative examples of highly substituted 2-arylindole containing molecules: current therapeutics, natural products, and drugs in development.

Surprisingly, arylisocyanides have not been utilized effectively to access highly substituted indoles. In the case of 2-arylindoles, the two current methods employing arylisocyanides provide modest yields, require stoichiometric amounts of palladium, or utilize difficult to handle 2-alkynylarylisocyanides (Scheme 1a). Alternatively, 2-iodo, 2-stannyl, 2-boryl and 2-silyl substituted indoles have been synthesized from isocyanides (Scheme 1b). Subsequent cross-coupling has provided access to a variety of 2-arylindoles. A general, one-step catalytic approach to highly substituted 2-arylindoles directly from arylisocyanides has yet to be developed.

Despite their unique and promising reactivity, arylisocyanides remain underutilized owing to their offensive odor and the hazardous reagents required for their synthesis. One of the most common and reliable ways to synthesize arylisocyanides is from the corresponding
aniline in two steps (Scheme 2). Aniline can be formylated in a variety of ways; however, the most common is with the mixed anhydride formed from acetic anhydride and formic acid. Then, the N-formylaniline can be dehydrated with phosphorous oxychloride (POCl₃) and an amine base (Scheme 2). Other less common dehydrating reagents include Burgess reagent, triphosgene, and phenylchlorothionoformate. The precursors to isocyanides, namely N-formylaniline and anilines, are bench stable, easy to handle, and frequently commercially available; however, the strong acid and dehydrating reagents also required are often dangerous and can pose difficulty on scale. Thus, developing a telescoped procedure in which the arylisocyanides can be synthesized *in situ* from a benign precursor and immediately reacted, allowing the safe handling of strong acids and dangerous dehydrating reagents, would enable their use in organic synthesis.

**Scheme 1.** (A) Current methods to synthesize 2-arylindoles from arylisocyanides. (B) Two-step synthesis of 2-arylindoles from arylisocyanides.

**(A) Takahashi, 2002**

```
Ar-X
stoich. Pd(OAc)₂
HNEt₂, dppp
Ar
THF, 40 °C
10–42%
```

**(B) Takemoto, 2013**

```
R = H, Cl, OMe
```

**(B) Two Step 2-Arylindole Synthesis**

```
FG
EWG
Ar-X
```

Continuous flow technologies are uniquely suited to enable the safe use of toxic, reactive, and dangerous reagents in organic synthesis. Unlike traditional batch chemistry, continuous
Flow reactors contain narrow diameter tubing that enables improved heat transfer for exothermic reactions, improved mixing, and smaller quantities of dangerous reagents at any given time. Additionally, reactions can be telescoped allowing the safe generation and use of toxic or unstable intermediates.

**Scheme 2.** General, robust synthesis of isocyanides.

![Scheme 2](image)

For many of these reasons, there are a few examples of isocyanide chemistry in continuous flow. The Ley group developed continuous flow synthesis of oxazoles, thiazoles and imidazoles from isocyanides. Recently, the Chen group synthesized 3-aminoimidazo[1,2-a]-pyrimidines in a multicomponent reaction of isocyanides in continuous flow conditions. In these examples, reaction times and substrate scope were improved. However, these continuous flow setups required the synthesis and handling of the isocyanide starting materials.

**Scheme 3.** Synthesis of 2-arylindoles via copper-catalyzed cyclization of 2-alkenylarylisonocyanides.

![Scheme 3](image)

Herein we describe a facile copper-catalyzed cyclization of 2-alkenylarylisonocyanides and readily available boronic acids to access highly substituted 2-arylindoles, and demonstrate the safe use of 2-alkenylarylisonocyanides via a continuous flow setup (Scheme 3).
Reaction Optimization

Inspired by the recent copper-catalyzed borylation of 2-alkenylarylisocyanides, we began to investigate the possibility for a copper-catalyzed reaction between arylisocyanide 1a and a variety of arylboron reagents as a means of directly generating 2-arylindole 2a (Table 1). Gratifyingly, initial reactions using easy to handle BMIDA and Bpin reagents demonstrated the viability of the transformation, albeit with low yields of 2a: 2% and 6% respectively (Table 1, Entry 1 & 2). Moving to a more reactive 4-cyanophenylboronic acid gave indole 2a in good yield: 56% (Table 1, Entry 3). Of note, these reactions were heterogeneous in nature, with the copper catalyst being incompletely solubilized. A more soluble copper (II) triflate was tested and showed no improvement in yield (Table 1, Entry 4). As such, the inexpensive copper (II) chloride (CuCl₂) catalyst was used throughout. In the absence of copper catalyst, product formation was not observed (Table 1, Entry 5). Similarly in the absence of base, product formation was not observed (Table 1, Entry 6). We hypothesize that in solution, the alkoxide base activates the boronic acid, forming a nucleophilic aryl boronate salt, which more readily undergoes transmetallation with the copper catalyst. Consistent with this proposal, use of lithium tert-butoxide (LiOt-Bu), which is known to form a less activated boronate species, resulted in a significantly lower yield of 2a (11%, Table 1, Entry 7). Finally, we were pleased to find that the reaction is only marginally air-sensitive, with only a modest reduction in yield when the reaction is prepared without the use of air-sensitive technique (Table 1, Entry 8).
Table 1. Optimization of a copper-catalyzed cyclization of arylisocyanides with arylboronic acids.\[[a]\]

![Reaction Scheme](image)

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<tr>
<th>Entry</th>
<th>Boron Reagent</th>
<th>Base</th>
<th>Copper Source</th>
<th>Additive</th>
<th>Conversion[[b]]</th>
<th>Yield[[b]]</th>
</tr>
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<tr>
<td>1</td>
<td>Ar–BMIDA</td>
<td>KOt-Bu</td>
<td>CuCl₂</td>
<td>–</td>
<td>87%</td>
<td>&lt;5%[[c]]</td>
</tr>
<tr>
<td>2</td>
<td>Ar–Bpin</td>
<td>KOt-Bu</td>
<td>CuCl₂</td>
<td>–</td>
<td>72%</td>
<td>6%</td>
</tr>
<tr>
<td>3</td>
<td>Ar–B(OH)₂</td>
<td>KOt-Bu</td>
<td>CuCl₂</td>
<td>–</td>
<td>93%</td>
<td>56%</td>
</tr>
<tr>
<td>4</td>
<td>Ar–B(OH)₂</td>
<td>KOt-Bu</td>
<td>CuOTf₂</td>
<td>–</td>
<td>93%</td>
<td>55%</td>
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<td>5</td>
<td>Ar–B(OH)₂</td>
<td>KOt-Bu</td>
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<td>–</td>
<td>42%</td>
<td>&lt;1%[[d]]</td>
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<tr>
<td>7</td>
<td>Ar–B(OH)₂</td>
<td>LiOt-Bu</td>
<td>CuCl₂</td>
<td>–</td>
<td>49%</td>
<td>14%</td>
</tr>
<tr>
<td>8</td>
<td>Ar–B(OH)₂</td>
<td>KOt-Bu</td>
<td>CuCl₂</td>
<td>air</td>
<td>89%</td>
<td>39%</td>
</tr>
</tbody>
</table>

[a] Reaction conditions: arylisocyanide 1a (0.1 mmol), 4-cyanophenylboronic acid (1.5 equiv), potassium tert-butoxide (1.3 equiv), CuCl₂ (0.1 equiv) were stirred in THF (2 mL) for 24 h at 65 °C under an inert atmosphere. [b] Conversion and yield were determined using HPLC with naphthalene as an internal standard. [c] Trace product detected. [d] No product detected.
**Reaction Scope**

Encouraged by these promising results, we explored the scope of this copper-catalyzed cyclization of arylisocyanide 1a with a variety of commercially available arylboronic acids to afford the corresponding 2-arylindole (Table 2). We observed that both electron-donating and electron-withdrawing substituents on the arylboronic acids were tolerated (2a-h). Indoles containing functional handles capable of further elaboration such as alkene, alkyne, bromide, and methyl ketone were synthesized in modest to good yield (2j–m). Notably, our method allowed the synthesis of molecules containing multiple heterocycles in a single step (2n and 2o).

Despite a broad substrate scope for the arylboronic acid coupling partner, some functional group limitations were also observed (Figure 3). Substrates containing protic functional groups such as an amino group (2i) or an alcohol (2p) did not provide indole product. However, nitro substituted boronic acids (an amine surrogate) provided the corresponding indole in good yield (Table 2, 2c). Substrates containing a pyridine (2q) were not competent in the reaction likely due to coordination to the copper catalyst or decomposition of the boronic acid under the reaction conditions. Substrates containing ortho-substituted electrophiles like aldehyde 2r and primary bromide 2s provided no indole product. Surprisingly, silyl-substituted substrates (2t) and its tert-butyldimethylphenylsilyl variant also produced no desired product.
Table 2. Preparation of 2-arylidones from various commercially available arylboronic acids.[a,b]

<table>
<thead>
<tr>
<th>Product</th>
<th>Reaction Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a (55%)</td>
<td>Reaction conditions: arylisocyanide 1a (1 mmol), aryl boronic acid (1.5 equiv), potassium tert-butoxide (1.3 equiv), CuCl$_2$ (0.1 equiv) were stirred in THF (15 mL) for 24 h at 65 °C under an inert atmosphere. [b] All yields in parentheses are isolated product after silica gel chromatography.</td>
</tr>
<tr>
<td>2b (80%)</td>
<td></td>
</tr>
<tr>
<td>2c (64%)</td>
<td></td>
</tr>
<tr>
<td>2d (49%)</td>
<td></td>
</tr>
<tr>
<td>2e (76%)</td>
<td></td>
</tr>
<tr>
<td>2f (64%)</td>
<td></td>
</tr>
<tr>
<td>2g (40%)</td>
<td></td>
</tr>
<tr>
<td>2h (41%)</td>
<td></td>
</tr>
<tr>
<td>2j (43%)</td>
<td></td>
</tr>
<tr>
<td>2k (23%)</td>
<td></td>
</tr>
<tr>
<td>2l (51%)</td>
<td></td>
</tr>
<tr>
<td>2m (47%)</td>
<td></td>
</tr>
<tr>
<td>2n (29%)</td>
<td></td>
</tr>
<tr>
<td>2o (57%)</td>
<td></td>
</tr>
</tbody>
</table>
A variety of 2-alkenylarylisocyanides were synthesized to explore the scope of this methodology (Table 3). Both electron-donating and electron-withdrawing groups on the 5, 6, and 7 position of the aryl ring of isocyanide $1$ were tolerated, affording the corresponding indole in good yield ($3a-g$). To further expand the scope of this methodology, orthogonal electrophilic vinyl components of arylisocyanide $1$ were explored. Both the vinylogous nitrile $1i$ and, more interestingly, a poor Michael acceptor, enamide $1h$, proved competent in the reaction. Unsurprisingly, isocyanide $1j$ containing the non-electrophilic styrenyl functional group provided no yield of indole $3j$, resulting in decomposition. Additionally, our method did not provide azaindoles from the corresponding isocyanide.

Figure 3. Arylboronic acid substrates that did not provide the desired indole products.
Table 3. Preparation of 2-arylindoles from various 2-alkenylarylisocyanides.\textsuperscript{[a,b]}

<table>
<thead>
<tr>
<th>Ar–B(OH)\textsubscript{2} (1.5 equiv)</th>
<th>EWG</th>
<th>THF, 65 °C, 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mol % CuCl\textsubscript{2}</td>
<td>KOt-Bu (1.3 equiv)</td>
<td>Ar = 4-benzonitrile</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ar</th>
<th>FG</th>
<th>EWG</th>
<th>CO\textsubscript{2}Me</th>
<th>CO\textsubscript{2}Me</th>
<th>CO\textsubscript{2}Me</th>
</tr>
</thead>
<tbody>
<tr>
<td>3b (76%)</td>
<td>4-F</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c (68%)</td>
<td>4-F</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3d (29%)</td>
<td>4-F</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3e (66%)</td>
<td>4-Cl</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3f (52%)</td>
<td>4-CO</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3g (63%)</td>
<td>4-Me</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3h (75%)</td>
<td>4-CN</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3i (69%)</td>
<td>4-CN</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3j (0%)</td>
<td>4-CN</td>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} Reaction conditions: arylisocyanide 1 (1 mmol), 4-cyanophenylboronic acid (1.5 equiv), potassium tert-butoxide (1.3 equiv), CuCl\textsubscript{2} (0.1 equiv) were stirred in THF (15 mL) for 24 h at 65 °C under an inert atmosphere. \textsuperscript{[b]} All yields in parentheses are isolated product after silica gel chromatography.
Continuous Flow Synthesis and Cyclization of Isocyanides

Our method featuring a copper-catalyzed cyclization of arylisocyanides with arylboronic acids further demonstrates the potential of arylisocyanides in the synthesis of nitrogen containing heterocycles. However, given their odor and variable toxicity, we developed a telescoped continuous flow setup for their safe handling.

To the best of our knowledge, there are only two examples in which isocyanides are prepared \textit{in situ} and subsequently reacted to form product in continuous flow. An integrated flow system developed by the Kim group in 2013 featured the dehydration of N-formylanilines to form the starting isocyanides in toluene, followed by a simple purification of the organic stream with a water extraction through a membrane separator.\textsuperscript{28} Finally, a multicomponent Ugi reaction and a metal-catalyzed reaction of the organic stream containing the isocyanide were demonstrated (Scheme 4). However, this setup was only demonstrated with very simple N-formylanilines and our attempt to use this setup with our substrates resulted clogging and hydrolysis of the isocyanides in the membrane separator. Recently, the Jamison group developed a three-step continuous flow system where 2-pyrrolyl-arylisocyanides were synthesized in two steps from 2-pyrrolyl-anilines, followed by a photochemical cyclization to provide pyrrolo[1,2-a]quinoxaline (Scheme 5).\textsuperscript{29} This continuous flow setup, though elegant, cannot be expanded to isocyanides with reactive functional groups in the \textit{ortho} position, and furthermore is compatible only with chemistry that tolerates the amine and acid byproducts formed during isocyanide synthesis. Thus we set out to develop a general \textit{in situ} continuous flow synthesis that provides pure isocyanide in an organic stream capable of telescoping with sensitive isocyanide chemistry, like our novel copper-catalyzed cyclization.
Scheme 4. A multistep reaction sequence involving the *in situ* generation and use of isocyanides in continuous flow.

N-formylaniline
DIPEA

POCl₃
Toluene

Sonication

H₂O

Aqueous Waste

Reagent

Final Product

Scheme 5. (A) Telescoped three step synthesis of a pyrrolo[1,2-a]quinoxaline from the corresponding 2-(pyrrol-1yl)aniline. (B) Three-step synthesis in a continuous flow setup.

(A)

HCOOAc
CH₃CN

N

NH₂

N

NH₂

POCl₃
DIPEA

CH₃CN

Phl(OCOCy)₂
photocatalyst
CH₃CN

(B)

HCOOAc/ AcOH
CH₃CN

POCl₃

[Ir(dtbbpy)(ppy)₂]PF₆

CH₃CN

DIPEA

BPR

Water Bath
We explored a two-step and three-step continuous flow setup. The improved two-step setup consists of synthesizing the arylisocyanide 1a via dehydration of arylformamide 4 and then accessing the highly-substituted aryl indole in a single step using the method described herein (Scheme 6a). The three-step setup consists of synthesizing 1a from the corresponding aniline 5, followed by cyclization (Scheme 6b).

Scheme 6. (A) Two-step telescoped synthesis of 2-arylindole 2a from N-formylaniline 4. (B) Three-step telescoped synthesis of 2-arylindole 2a from aniline 5.

In continuous flow, shorter reaction times allow smaller reactors and higher throughput, so we explored conditions to decrease reaction time of the synthesis of 2-arylindoles via coupling of arylisocyanides and arylboronic acids. Initially, we explored a 30 minute reaction time, with our conditions, we observed 8% yield of indole product. Increasing to stoichiometric CuCl₂ provided no product and formed an enormous amount of solid, perhaps copper dimers that crashed out of solution. So, we explored other copper sources. By including a superstoichiometric amount of copper beads as Cu(0) in addition to 10 mol% CuCl₂, we presume we are creating Cu(I) in solution, which we believe to be the active catalyst. For a 10 minute
reaction time, we observed a 55% yield with the addition of copper beads. Copper flow reactors have been utilized to decrease reaction times compared to their batch reaction counterparts.\textsuperscript{30} We initially envisioned using a packed bed of copper beads to enhance the reaction, but the reaction formed small amounts of solids within resulting in clogging and inconsistent flow rates. When we replaced the packed bed with a continuous stirred-tank reactor (CSTR) containing stirred copper beads, the solids were easily handled. Our continuous flow system consists of mixing three streams containing arylisocyanide 1a, arylboronic acid, and potassium tert-butoxide (KOT-Bu) in a continuous stirred tank reactor (CSTR) containing copper beads heated to 65 °C for 30 minutes (Scheme 7). We were pleased to observe indole 2a in 56% yield, providing similar yields to our isolated batch reactions (55% yield). NMP was added to increase solubility and reaction rate.\textsuperscript{31,32}

Scheme 7. Copper-catalyzed cyclization of arylisocyanide 1a to indole 2a in a CSTR.\textsuperscript{[a]}

\[ \text{CO}_2\text{Et} \quad \text{KOt-Bu} \quad (1.3 \text{ equiv})
\text{THF/NMP} \]

\[ 1\text{a} \quad 0.1 \text{ M (1 equiv)}
\text{4-Cyanophenyl boronic acid} \quad (1.5 \text{ equiv})
\text{CuCl}_2 \quad (10 \text{ mol%})
\text{THF/NMP/H}_2\text{O} \]

\[ \xrightarrow{\text{CSTR, Copper Beads}} \quad 65 ^\circ \text{C, 30 min} \]

\[ \text{CO}_2\text{Et} \quad 2\text{a} \quad 56\% \text{ yield}
\text{Throughput: 51 mg/h} \]

\[ \text{[a]} \quad \text{Yield was determined via HPLC relative to naphthalene internal standard.} \]

We then developed the dehydration of N-formyl aniline in continuous flow. Streams of 4, phosphorous oxychloride (POCl\textsubscript{3}), and diisopropylethylamine (DIPEA) were mixed for 2.5 min providing isocyanide 1a in 94% yield (Scheme 8, see Experimental Section for details). Dichloromethane (DCM) was chosen as the ideal solvent for the dehydration of formamide 4 due
to its compatibility with the subsequent copper catalyzed cyclization step, solubility of starting materials, and its good separation from the aqueous streams.

**Scheme 8.** Dehydration of N-formylaniline 4 in continuous flow.[a]

![Scheme 8](https://example.com/scheme_8.png)

[a] Yield was determined via HPLC relative to naphthalene internal standard.

Telescoping the dehydration of N-formylaniline 4 (Module 1, Table 4) directly into the copper-catalyzed cyclization (Module 2, Table 4) in continuous flow yielded no indole product (Table 4, Entry 1, see Experimental Section for details). The acidic and amine byproducts formed in the dehydration step are not compatible with the subsequent cyclization. The addition of liquid-liquid membrane separators helped to remove the byproducts of the dehydration through an aqueous extraction. We first attempted a simple water extraction, however we observed hydrolysis of arylisocyanide 1a back to formamide 4 as the POCl₃ by-products hydrolyze into HCl and H₃PO₄. A solution of ammonium chloride was attempted, but hydrolysis was again observed. The addition of a single in-line basic separation with 0.5 M K₃PO₄ (Module 3, Table 4) provided 1a cleanly. The organic stream of this single separation was then added to Module 2 containing the copper-catalyzed cyclization and resulted in a 9% yield of indole 2a (Table 4, Entry 2). After a single extraction, the stream of 1a retained water, so a drying column consisting of a packed bed of 4Å molecular sieves (Module 5) was added, resulting in an increased, yet modest yield of 19% (Table 4, Entry 3). Finally, a slightly acidic separation with
aqueous ammonium chloride was added after the basic separation to remove any remaining acid or amine byproducts. Thus, after two separations and a drying column, indole 2a was observed in 51% yield over two steps, a comparable yield to both the batch isolated yield of 55% and the single step CSTR yield of 56% (Table 4, Entry 4).

Table 4. Development of a two-step continuous flow synthesis of indole 2a from N-formylaniline 4.[a]

![Diagram of reaction process]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Modular Reactors</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 2</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>1 3 2</td>
<td>9%</td>
</tr>
<tr>
<td>3</td>
<td>1 3 5 2</td>
<td>19%</td>
</tr>
<tr>
<td>4</td>
<td>1 3 4 5 2</td>
<td>51%</td>
</tr>
</tbody>
</table>

[a] Yields were determined using HPLC with naphthalene as an internal standard.

The integrated flow setup with increased throughput (550 mg/h) consists of one PEEK cross-mixer, one high-purity perfluoroalkoxyalkane (HPFA) tubing reactor, two liquid-liquid separators, a packed bed containing 4Å molecular sieves, a surge tank, and a CSTR (35 mL) with
copper beads (Scheme 9). This setup provided 2a in 49% yield over two steps demonstrating the scalability of system.

**Scheme 9.** Two-step continuous flow synthesis of indole 2a from simple N-formylaniline 4.\(^{[a]}\)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Concentration</th>
<th>Time</th>
<th>Isolated Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formylation</td>
<td>0.3 M (0.1 equiv) CH$_2$Cl$_2$</td>
<td>2.5 min</td>
<td>49%</td>
</tr>
<tr>
<td>Aqueous</td>
<td>K$_3$PO$_4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Cyanophenyl boronic acid (1.5 equiv) CuCl$_2$ (10 mol %) THF/NMP/H$_2$O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOt-Bu (1.3 equiv) THF/NMP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R$_2$, CSTR</td>
<td>65 °C, 30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper Beads</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[^{[a]}\] Isolated yield of indole 2a (1.8 mmol).

Additionally we explored adding the formylation of simple ortho-alkenylaniline to our two-step continuous flow setup; unfortunately a low yield of 12% of indole 2a was observed due to the increased complexity of the impurity profile (Scheme 10). Initially, our setup did not feature an aqueous extraction after formylation of aniline 4. Yet, even with a large excess or
amine and/or POCl₃, the maximum yield of isocyanide 1a observed was 33% yield. After addition of a single basic separation, a 91% yield of 1a was observed, but the yield of indole 2a was only 12% (Scheme 10). Again, we explored adding additional POCl₃ and DIPEA, which also did not increase the overall yield. Given that the batch preparation of 4 is straightforward, further inline purification modules were not explored after the formylation step.
Scheme 10. Three–step continuous flow synthesis of indole 2a from simple aniline 5.[a]

[a] Yields were determined using HPLC with naphthalene as an internal standard.
Conclusion

In conclusion, we have developed a catalytic method for the synthesis of highly substituted 2-arylidiones from 2-alkenylisocyanides and aryl boronic acids. Additionally, we have developed a safe and effective approach to the synthesis of 2-arylidiones from readily available 2-alkenyl-N-formylanilines in continuous flow. We have shown that multiple incompatible reaction steps can be telescoped through the use of liquid-liquid separators and a drying column, which has the potential to be adapted to a variety of sensitive reactions with isocyanide conducted in continuous flow.
References


Chapter 1. 
Experimental Section
Materials and Methods

Reactions were performed under an inert argon atmosphere. Commercially available reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) and used without any further purification. Anhydrous tetrahydrofuran (THF), triethylamine, and acetonitrile were purified via an SG Water USA solvent column system (Nashua, NH) before use. Copper beads (30-40 mesh) were activated with 10% HCl, washed with water, acetone, and THF, and finally dried under vacuum over night. All reactions were monitored with thin-layer chromatography (TLC), which was performed using EMD 60-F254 silica glass plates and visualized with a UV lamp (254 nm). Products were purified on SNAP Ultra columns utilizing the Biotage® Isolera™ flash purification system.

For flow experiments, backpressure regulators (BPRs) and liquid-liquid separators were purchased from Zaiput Flow Technologies. PTFE Microfiltration membranes were purchased from Pall Zefluor. The reactors were constructed from high-purity perfluoroalkoxy alkane tubing (HPFA: 1/16” OD and 0.03” ID) and PEEK superflangeless fittings purchased from IDEX Health & Science Technologies. Syrris Asia syringe pumps or Harvard Apparatus PhD Ultra syringe pumps were used to pump reagents. High-pressure stainless steel syringes (8 mL with 1/16” SWAGELOCK®) from Harvard Apparatus or glass gas tight syringes (5, 10 mL) purchased from SGE were used in combination with the Harvard Apparatus PhD Ultra syringe pumps. A drying column was prepared by loading 4 Å molecular sieves into an Omnipit™ Glass column.
\(^1\)H NMR, \(^{13}\)C NMR and \(^{19}\)F NMR spectra were recorded on Bruker Avance-400 spectrometer, Varian XL 300 or Varian Inova 500 NMR. Data are reported as follows: chemical shift (\(^\delta\), parts per million, referenced to the residual solvent peak of the proton and carbon resonances of CHCl\(_3\) in CDCl\(_3\)), multiplicity (\(s =\) singlet, \(d =\) doublet, \(t =\) triplet, \(q =\) quartet, \(dd =\) doublet of doublets, \(dt =\) doublet of triplets, \(ddd =\) doublet of doublet of doublets, \(m =\) multiplet, \(bs =\) broad singlet), coupling constant (\(J\)) in Hertz (Hz), and integration. IR spectra were obtained on an Agilent Cary 630 FT-IR spectrometer equipped with an ATR (attenuated total reflectance) accessory. Spectral features are tabulated as follows: wavenumber (cm\(^{-1}\)); intensity (s-strong, m-medium, w-weak, br-broad). High-resolution mass spectrometry data were acquired on a Bruker Daltonics APEXIV 4.7 Tesla TF-ICR Mass Spectrometer at Massachusetts Institute of Technology Department of Chemistry Instrumentation Facility. Melting points were recorded using a Mel-Temp melting point apparatus and are uncorrected.
Procedure for the Synthesis of Arylisocyanides

Step 1: Heck Reaction of 2-Aniline

The starting aniline (10 mmol, 1 equiv.), palladium acetate (10 mol %), tri-orthotolyl phosphine (20 mol %) were added to a screw-cap pressure vial containing a stir bar. The vial was equipped with a rubber septum and the vessel was evacuated and back-filled with argon three times through a needle. Acetonitrile (degassed, 20 mL), triethylamine (6.0 equiv.) and alkenyl coupling partner (1.2 equiv.) were added. The pressure vial was sealed was a screw-cap (caution: high pressure possible, a safety shield was utilized) and stirred at 90 °C for 16 h. After cooling to room temperature, the crude solution was filtered and washed with ethyl acetate. The filtrate was concentrated in vacuo and the resulting mixture was purified by flash column chromatography on silica gel to afford the corresponding 2-alkenylarylaniline.

Step 2: N-Formylation of Aniline Derivatives

Acetic anhydride (1.5 equiv.) and formic acid (1.6 equiv.) were stirred at 50 °C in a sealed tube for 2 hours. The resulting mixed anhydride was cooled to room temperature and was added dropwise over 10 min to a stirred solution of 2-alkenyl aniline (8 mmol, 1.0 equiv.) in THF (16 mL) cooled to 0 °C. The solution was warmed to room temperature and stirred for 30 min. A saturated aqueous solution of sodium bicarbonate (50 mL) was added slowly to the mixture and then the organic layer was extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried with Na₂SO₄, filtered and concentrated in vacuo. The crude product was utilized in the subsequent step without further purification.
Step 3: Dehydration to Arylisocyanide

A THF solution (16 mL) of the crude formamide (8 mmol, 1.0 equiv) and triethylamine (6.0 equiv.) was cooled to 0 °C. Phosphoryl chloride (3.0 equiv.) was added dropwise while maintaining the reaction temperature at 0 °C. The mixture was stirred for an additional 30 min at 0 °C. The solution was warmed to room temperature and quenched with saturated aqueous ammonium chloride solution (50 mL). The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The crude residue was purified via flash column chromatography on silica gel to afford the corresponding 2-alkenylarylisocyanide.

Scheme 11. Three step synthesis of arylisocyanides (1x) from 2-bromoaniline or 2-iodoaniline.
Characterization of Anilines and Arylisocyanides

3-(2-aminophenyl)ethyl acrylate (1a')

This compound was prepared via a Heck reaction of 2-iodoaniline (40.0 mmol) and ethyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a yellow solid (6.42 g, 83% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 (d, $J = 15.8$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 7.18 - 7.11 (m, 1H), 6.78 - 6.71 (m, 1H), 6.68 (d, $J = 8.0$ Hz, 1H), 6.35 (d, $J = 15.8$ Hz, 1H), 4.25 (q, $J = 6.6$ Hz, 2H), 4.08 (br s, 2H), 1.32 (t, $J = 6.6$ Hz, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 167.3, 145.7, 140.1, 131.2, 128.0, 119.7, 118.7, 117.8, 116.7, 60.4, 14.3 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^1$

3-(2-amino-4-fluorophenyl)methyl acrylate (1b')

This compound was prepared via a Heck reaction of 5-fluoro-2-iodoaniline and methyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a light yellow solid (1.74 g, 89% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.73 (d, $J = 15.7$ Hz, 1H), 7.32 (dd, $J = 8.6$, $^3J_{HF} = 6.4$ Hz, 1H), 6.45 (td, $J = 8.6$, 2.5 Hz, 1H), 6.38 (dd, $^3J_{HF} = 10.5$, $J = 2.5$ Hz, 1H), 6.27 (d, $J = 15.7$ Hz, 1H), 4.17 (br s, 2H), 3.78 (s, 3H) ppm
\(^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}\) \(\delta\) 167.8, 164.9 \((d, J_{CF} = 248.8 \text{ Hz})\), 147.6 \((d, J_{CF} = 11.6 \text{ Hz})\), 139.4, 130.0 \((d, J_{CF} = 10.3 \text{ Hz})\), 117.1, 116.0 \((d, J_{CF} = 2.8 \text{ Hz})\), 106.3 \((d, J_{CF} = 22.6 \text{ Hz})\), 103.0 \((d, J_{CF} = 24.4 \text{ Hz})\), 51.8 ppm

The \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra are in agreement with those reported in the literature.\(^2\)

\[
\begin{align*}
\text{F}_3\text{C} & \quad \text{NH}_2 \\
\text{CO}_2\text{Me} &
\end{align*}
\]

3-(2-amino-4-(trifluoromethyl)phenyl)methyl acrylate (1c’)

This compound was prepared via a Heck reaction of 2-bromo-5-trifluoromethylaniline and methyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a yellow solid (2.26 g, 92% yield).

\(R_F = 0.77, 50\%\) ethyl acetate in hexanes; \(\text{mp} = 129-131 \text{ °C}\)

\(^1\text{H NMR (400 MHz, CDCl}_3\text{)}\) \(\delta\) 7.78 \((d, J = 15.8 \text{ Hz}, 1\text{H})\), 7.43 \((d, J = 8.2 \text{ Hz}, 1\text{H})\), 6.97 \((d, J = 8.2 \text{ Hz}, 1\text{H})\), 6.93 \((s, 1\text{H})\), 6.40 \((d, J = 15.8 \text{ Hz}, 1\text{H})\), 4.18 \((\text{br s}, 2\text{H})\), 3.81 \((s, 3\text{H})\) ppm

\(^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}\) \(\delta\) 167.3, 145.6, 139.0, 132.9 \((q, J_{CF} = 32.2 \text{ Hz})\), 128.7, 123.9 \((q, J_{CF} = 272.3 \text{ Hz})\), 122.8, 120.2, 115.2 \((q, J_{CF} = 3.7 \text{ Hz})\), 113.2 \((q, J_{CF} = 3.8 \text{ Hz})\), 52.0 ppm

\(^{19}\text{F NMR (CDCl}_3\text{, 376 MHz)}\) \(\delta\) = −64.3 \((s, 3\text{F})\) ppm

\(^{\text{IR (neat, cm}^{-1}\text{)}}\) 3476.8 (NH, br), 3365.6 (NH, br), 1704.0 (C=O, s)

\(^{\text{HRMS (DART, m/z)}}\) [M+H]\(^+\) calculated for C\(_{11}\)H\(_{10}\)F\(_3\)NO\(_2\): 246.0736; Found: 246.0736

\[
\begin{align*}
\text{O} & \quad \text{NH}_2 \\
\text{CO}_2\text{Me} &
\end{align*}
\]

3-(4-acetyl-2-aminophenyl)methyl acrylate (1d’)

This compound was prepared via a Heck reaction of 3-amino-4-bromoacetophenone and methyl
acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a neon yellow solid (1.78 g, 81% yield).

\[ R_F = 0.14, \text{25\% ethyl acetate in hexanes}; \text{mp} = 126–128 ^\circ \text{C} \]

\[ ^1H \text{ NMR (400 MHz, CDCl}_3) \delta 7.80 (d, J = 15.8 \text{ Hz, 1H}), 7.45 (d, J = 8.1 \text{ Hz, 1H}), 7.36 - 7.27 (m, 3H), 6.44 (d, J = 15.8 \text{ Hz, 1H}), 4.07 (\text{br s, 2H}), 3.82 (s, 3H), 2.56 (s, 3H) \text{ ppm} \]

\[ ^13C \text{ NMR (100 MHz, CDCl}_3) \delta 197.7, 167.2, 145.5, 139.1, 139.0, 128.3, 124.1, 120.2, 118.8, 116.0, 51.9, 26.7 \text{ ppm} \]

\[ \text{IR (neat, cm}^{-1} \text{)} 3459.5 (\text{NH, br}), 3377.1 (\text{NH, br}), 1699.6 (\text{C=O, s}), 1674.6 (\text{C=O, s}) \]

\[ \text{HRMS (DART, m/z)} [\text{M+H}^+] \text{calculated for C}_{12}\text{H}_{13}\text{NO}_3: 220.0968; \text{Found: 220.0959} \]

\[ \text{3-(2-amino-3-chloro-5-fluorophenyl)ethyl acrylate (1e')} \]

This compound was prepared via a Heck reaction of 2-bromo-6-chloro-4-fluoroaniline and ethyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a dark yellow solid (2.31 g, 95% yield).

\[ R_F = 0.63, \text{25\% ethyl acetate in hexanes}; \text{mp} = 44–46 ^\circ \text{C} \]

\[ ^1H \text{ NMR (400 MHz, CDCl}_3) \delta 7.73 (\text{dd, J = 15.8 Hz, 1H}), 7.09 (\text{dd, }^3J_{HF} = 7.8, J = 2.9 \text{ Hz, 1H}), 7.03 (\text{dd, }^3J_{HF} = 9.0 \text{ Hz, J = 2.9 Hz, 1 H}), 6.35 (\text{d, J = 15.8 Hz, 1H}), 4.28 (\text{q, J = 7.1 Hz, 2H}), 1.55 (\text{br s, 2H}), 1.34 (\text{t, J = 7.1 Hz, 3H}) \text{ ppm} \]

\[ ^13C \text{ NMR (100 MHz, CDCl}_3) \delta 166.6, 154.8 (\text{d, }^1J_{CF} = 239.6 \text{ Hz}), 138.6 (\text{d, }^4J_{CF} = 2.4 \text{ Hz}), 138.5 (\text{d, }^4J_{CF} = 2.6 \text{ Hz}), 121.7 (\text{d, }^3J_{CF} = 7.7 \text{ Hz}), 121.0, 120.8 (\text{d, }^3J_{CF} = 10.6 \text{ Hz}), 118.3 (\text{d, }^2J_{CF} = 26.0 \text{ Hz}), 112.6 (\text{d, }^2J_{CF} = 22.6 \text{ Hz}), 60.9, 14.4 \text{ ppm} \]
\(^{19}\)F NMR (CDCl\(_3\), 376 MHz) \(\delta = -125.9\) (t, \(^3\)J\(_{HF-a}\) = 9.3 Hz, 1F) ppm

IR (neat, cm\(^{-1}\)) 3467.7 (NH, br), 3375.8 (NH, br), 1701.5 (C=O, s)

HRMS (DART, m/z) [M+H]\(^+\) calculated for C\(_{11}\)H\(_{11}\)ClFNO\(_2\): 244.0535; Found: 244.0545

F\(_3\)CO

\(\text{N}H_\text{2}\)

3-(2-amino-5-(trifluoromethoxy)phenyl)methyl acrylate (1f’)

This compound was prepared via a Heck reaction of 2-bromo-4-trifluoromethoxyaniline and methyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a yellow solid (2.54 g, 97% yield).

R\(_F\) = 0.65, 50% ethyl acetate in hexanes; mp = 74–76 °C

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.74 (d, \(J = 15.8\) Hz, 1H), 7.21 (s, 1H), 7.02 (d, \(J = 8.8\) Hz, 1H), 6.67 (d, \(J = 8.8\) Hz, 1H), 6.34 (d, \(J = 15.8\) Hz, 1H), 4.07 (br s, 2H), 3.79 (s, 3H) ppm

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 167.4, 144.4, 141.4 (q, \(^4\)J\(_{CF}\) = 1.8 Hz), 139.0, 124.5, 120.7 (q, \(^1\)J\(_{CF}\) = 255.5 Hz), 120.6, 120.4, 119.3, 117.5, 51.8 ppm

\(^{19}\)F NMR (CDCl\(_3\), 376 MHz) \(\delta = -59.5\) (s, 3F) ppm

IR (neat, cm\(^{-1}\)) 3381.6 (NH, br), 3357.0 (NH, br), 1704.2 (C=O, s)

HRMS (DART, m/z) [M+H]\(^+\) calculated for C\(_{11}\)H\(_{10}\)F\(_3\)NO\(_3\): 262.0686; Found: 262.0687
3-(2-amino-3,5-dimethylphenyl)ethyl acrylate (1g')

This compound was prepared via a Heck reaction of 2-bromo-4,6-dimethylaniline and methyl acrylate described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a yellow solid (1.89 g, 95% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.87 (d, $J = 15.7$ Hz, 1H), 7.08 (s, 1H), 6.92 (s, 1H), 6.34 (d, $J = 15.7$ Hz, 1H), 3.86 (br s, 2H), 3.80 (s, 3H), 2.23 (s, 3H), 2.15 (s, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 167.9, 141.7, 140.8, 133.5, 127.4, 126.0, 123.4, 119.5, 117.5, 51.7, 20.4, 17.7 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.  

3-(2-amino-3,5-dimethylphenyl)ethyl acrylate (1h')

This compound was prepared via a Heck reaction of 2-iodoaniline (5.0 mmol) and acrylonitrile described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a pale brown solid (0.48 g, 66% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.54 (d, $J = 16.4$ Hz, 1H), 7.28 (d, $J = 8.1$ Hz, 1H), 7.21 (t, $J = 7.5$ Hz, 1H), 6.78 (t, $J = 7.5$ Hz, 1H), 6.72 (d, $J = 8.1$ Hz, 1H), 5.79 (d, $J = 16.4$ Hz, 1H), 3.94 (s, 2H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 146.4, 145.3, 132.3, 127.3, 119.5, 119.3, 118.9, 117.3, 95.9 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.
3-(2-aminophenyl)-N,N-dimethylacrylamide (II')

This compound was prepared via a Heck reaction of 2-iodoaniline (5.0 mmol) and N,N-dimethylacrylamide described in the general procedure for the synthesis of arylisocyanides. The aniline was obtained as a yellow oil (0.81 g, 85% yield).

R_F = 0.21, 100% ethyl acetate

^1^H NMR (400 MHz, CDCl_3) δ 7.80 (d, J = 15.2 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.17 – 7.11 (m, 1H), 6.80 (d, J = 15.2 Hz, 1H), 6.78 – 6.72 (m, 1H), 6.69 (d, J = 8.0 Hz, 1H), 3.96 (br s, 2H), 3.16 (s, 3H), 3.06 (s, 3H) ppm

^1^C NMR (100 MHz, CDCl_3) δ 167.0, 145.4, 138.0, 130.7, 127.8, 121.1, 118.8, 118.0, 116.6, 37.5, 36.1 ppm

IR (neat, cm^-1) 3447.0 (NH, br), 3354.0 (NH, br), C=O (1642.7, s)

HRMS (DART, m/z) [M+H]^+ calculated for C_{11}H_{14}N_2O: 191.1179; Found: 191.1175

3-(2-isocyanophenyl)ethyl acrylate (1a)

This compound was prepared from aniline 1a' (33.6 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1a was obtained as a pale brown solid (6.76 g, 88% yield over 2 steps).

^1^H NMR (400 MHz, CDCl_3) δ 7.97 (d, J = 16.0 Hz, 1H), 7.76 – 7.66 (m, 1H), 7.54–7.41 (m, 3H), 6.57 (d, J = 16.0 Hz, 1H), 4.33 (q, J = 7.0 Hz, 2H), 1.40 (t, J = 7.0 Hz, 3H) ppm
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.7, 165.6, 137.2, 130.5, 130.4, 129.5, 127.4, 126.6, 125.6, 122.1, 60.6, 14.0 ppm

The \(^1\)H and \(^{13}\)C NMR spectra are in agreement with those reported in the literature.  

![Image of 3-(4-fluoro-2-isocyanophenyl)methyl acrylate (1b)](image)

3-(4-fluoro-2-isocyanophenyl)methyl acrylate (1b)

This compound was prepared from aniline 1b' (8.9 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1b was obtained as a brown solid (1.32 g, 69% yield over 2 steps).

\(R_F = 0.63, 25\%\) ethyl acetate in hexanes; \(mp = 100-103^\circ\)C

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.92 (d, \(J = 16.0\) Hz, 1H), 7.67 (dd, \(J = 9.7, 4J_{HF} = 5.7\) Hz, 1H), 7.21 - 7.15 (m, 2H), 6.48 (d, \(J = 16.0\) Hz, 1H), 3.84 (s, 3H) ppm

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.5, 166.4, 163.1 (d, \(1J_{CF} = 254.4\) Hz), 136.9, 128.9 (d, \(3J_{CF} = 9.2\) Hz), 127.4 (d, \(4J_{CF} = 4.0\) Hz), 127.0, 121.9, 117.8 (d, \(2J_{CF} = 21.7\) Hz), 115.2 (d, \(2J_{CF} = 25.6\) Hz), 52.2 ppm

\(^{19}\)F NMR (CDCl\(_3\), 376 MHz) \(\delta\) -108.1 (td, \(3J_{HF-o} = 8.0\) Hz, \(4J_{HF-m} = 5.8\) Hz, 1F) ppm

IR (neat, cm\(^{-1}\)) 2122.2 (CN, m), 1707.5 (C=O, s)

HRMS (DART, \(m/z\)) [M+NH\(_4\)]\(^+\) calculated for C\(_{11}\)H\(_8\)FNO\(_2\): 223.0877; Found: 223.0875
3-(4-trifluoromethyl-2-isocyanophenyl)methyl acrylate (1c)

This compound was prepared from aniline 1c’ (9.2 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1c was obtained as a brown solid (1.49 g, 64% yield over 2 steps).

RF = 0.80, 25% ethyl acetate in hexanes; mp = 49–50 °C

1H NMR (400 MHz, CDCl3) δ 7.90 (d, J = 16.1 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.68 – 7.61 (m, 2H), 6.59 (d, J = 16.1 Hz, 1H), 3.80 (s, 3H) ppm

13C NMR (100 MHz, CDCl3) δ 171.3, 166.0, 136.6, 134.3, 132.9 (q, 2JCF = 33.9 Hz), 127.9, 126.5 (q, 3JCF = 3.5 Hz), 125.0 (q, 3JCF = 3.9 Hz), 124.6, 122.8 (q, 1JCF = 272.6 Hz), 52.4 ppm

19F NMR (CDCl3, 376 MHz) δ = –64.3 (s, 3F) ppm; IR (neat, cm⁻¹) 2122.2 (CN, m), 1710.3 (C=O, s)

HRMS (DART, m/z) [M+NH₄]⁺ calculated for C₁₂H₈F₃NO₂: 273.0845; Found: 273.0854

3-(4-methylketone-2-isocyanophenyl)methyl acrylate (1d)

This compound was prepared from aniline 1d’ (8.1 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1d was obtained as a white solid (1.37 g, 74% yield over 2 steps).

RF = 0.28, 25% ethyl acetate in hexanes, mp = 131–132 °C
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.01 – 7.92 (m, 3H), 7.76 (d, $J = 8.8$ Hz, 1H), 6.62 (d, $J = 16.1$ Hz, 1H), 3.84 (s, 3H), 2.62 (s, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 195.4, 170.3, 166.1, 138.6, 136.9, 134.8, 129.1, 127.7, 127.5, 126.4, 124.3, 52.3, 26.8 ppm

IR (neat, cm$^{-1}$) 2126.5 (CN, m), 1726.0 (C=O, s), 1688.9 (C=O, s)

HRMS (DART, m/z) [M+Na]$^+$ calculated for C$_{13}$H$_{11}$N0$_3$: 252.0631; Found: 252.0630

3-(3-chloro-5-fluoro-2-isocyanophenyl)methyl acrylate (1e)

This compound was prepared from aniline 1e' (9.5 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1e was obtained as a yellow-brown solid (0.47 g, 19% yield over two steps).

$R_F = 0.70$, 25% ethyl acetate in hexanes; mp = 87 – 90 $^\circ$C

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.89 (dd, $J = 16.0$, $^5$J$_{HF} = 1.3$ Hz, 1H), 7.40 – 7.16 (m, 2H), 6.52 (d, $J = 16.0$ Hz, 1H), 4.31 (q, $J = 7.1$ Hz, 2H), 1.36 (t, $J = 7.1$ Hz, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 174.2, 165.2, 161.4 (d, $^1$J$_{CF} = 255.1$ Hz), 136.2 (d, $^5$J$_{CF} = 2.5$ Hz), 134.6 (d, $^3$J$_{CF} = 9.2$ Hz), 133.5 (d, $^3$J$_{CF} = 11.3$ Hz), 124.9, 121.5, 118.6 (d, $^2$J$_{CF} = 26.5$ Hz), 112.2 (d, $^2$J$_{CF} = 24.0$ Hz), 61.2, 14.2 ppm

$^{19}$F NMR (CDCl$_3$, 376 MHz) $\delta = -107.2$ (t, $^3$J$_{HF-o} = 9.0$ Hz, 1F) ppm; IR (neat, cm$^{-1}$) 2119.4 (CN, m), 1699.6 (C=O, s)

HRMS (DART, m/z) [M+Na]$^+$ calculated for C$_{12}$H$_9$ClFNO$_2$: 276.0198; Found: 276.0187
3-(3-trifluoromethoxy-2-isocyanophenyl)methyl acrylate (1f)

This compound was prepared from aniline **1f** (9.7 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide **1f** was obtained as a white solid (1.44 g, 55% yield over 2 steps).

RF = 0.31, 25% ethyl acetate in hexanes; mp = 68 °C

^1^H NMR (400 MHz, CDCl_3) δ 7.89 (d, J = 16.1 Hz, 1H), 7.50 - 7.45 (m, 2H), 7.28 - 7.23 (m, 1H), 6.53 (d, J = 16.1 Hz, 1H), 3.82 (s, 3H) ppm

^13^C NMR (100 MHz, CDCl_3) δ 170.5, 165.9, 149.3 (q, J = 3.5 CF = 2.0 Hz), 136.6, 133.0, 129.4, 124.3, 123.7, 122.9, 120.2 (q, J = 3.5 CF = 259.4 Hz), 119.1, 52.2 ppm

^19^F NMR (CDCl_3, 376 MHz) δ = -58.9 (s, 3F) ppm

IR (neat, cm⁻¹) 2125.6 (CN, m), 1713.7 (C=O, s)

HRMS (DART, m/z) [M+NH₄]^+ calculated for C₁₂H₈F₃NO₃: 289.0795; Found: 289.0778

3-(3,5-dimethyl-2-isocyanophenyl)methyl acrylate (1g)

This compound was prepared from aniline **1g** (9.5 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide **1g** was obtained as a cream-white solid (1.53 g, 75% yield over 2 steps).

^1^H NMR (400 MHz, CDCl_3) δ 7.88 (d, J = 16.0 Hz, 1H), 7.22 (s, 1H), 7.07 (s, 1H), 6.43 (d, J = 16.0 Hz, 1H), 3.78 (s, 3H), 2.34 (s, 3H), 2.31 (s, 3H)
\(^{13}\text{C NMR (100 MHz, CDCl} \text{3) } \delta 170.0, 166.5, 139.3, 138.5, 135.6, 132.7, 130.3, 124.7, 123.7, 121.2, 51.9, 21.3, 18.9 \)

The \(^1\text{H and }^{13}\text{C NMR spectra are in agreement with those reported in the literature.}\)

\[
\text{\includegraphics[width=0.2\textwidth]{3-(2-isocyanophenyl)acrylonitrile.png}}
\]

**3-(2-isocyanophenyl)acrylonitrile (1h)**

This compound was prepared from aniline 1h' (13.7 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1h was obtained as a pale brown solid (1.15 g, 54% yield over 2 steps).

\(^1\text{H NMR (400 MHz, CDCl} \text{3) } \delta 7.73 (d, J = 16.7 \text{ Hz, 1H}), 7.64 - 7.58 (m, 1H), 7.53 - 7.43 (m, 3H), 6.04 (d, J = 16.7 \text{ Hz, 1H}) \text{ ppm} \)

\(^{13}\text{C NMR (100 MHz, CDCl} \text{3) } \delta 169.9, 144.1, 131.9, 130.0, 129.8, 128.0, 126.3, 117.3, 100.8 \text{ ppm} \)

The \(^1\text{H and }^{13}\text{C NMR spectra are in agreement with those reported in the literature.}\)

\[
\text{\includegraphics[width=0.2\textwidth]{3-(2-isocyanophenyl)-N,N-dimethylacrylamide.png}}
\]

**3-(2-isocyanophenyl)-N,N-dimethylacrylamide (1i)**

This compound was prepared from aniline 1i' (4.2 mmol) via formylation followed by dehydration described in the general procedure for the synthesis of arylisocyanides. Isocyanide 1i was obtained as a pale yellow solid (0.31 g, 37% yield over 2 steps).

\(^1\text{H NMR (400 MHz, CDCl} \text{3) } \delta 7.80 (d, J = 15.6 \text{ Hz, 1H}), 7.59 (d, J = 7.4 \text{ Hz, 1H}), 7.41 - 7.30 (m, 3H), 7.03 (d, J = 15.6 \text{ Hz, 1H}), 3.16 (s, 3H), 3.05 (s, 3H) \text{ ppm} \)
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 168.7, 166.0, 135.6, 131.8, 129.9, 129.6, 127.9, 127.7, 125.4, 122.5, 37.6, 36.0 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^6$
General Procedure for the Synthesis of 2-Arylindoles

A 20 mL pressure vial containing a stirbar was charged with arylisocyanide 1 (1 mmol), arylboronic acid (1.5 equiv.), and CuCl₂ (0.1 equiv.). The vial was evacuated and back-filled with argon three times. THF (15 mL) and KOtBu (1.0 M in THF, 1.3 equiv.) were added. The vial was sealed and heated to 65 °C for 24 h. Upon completion of the reaction, it was quenched with saturated aqueous ammonium chloride (25 mL). The mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was dried with Na₂SO₄, filtered and solvent was evaporated under reduced pressure. The crude residue was purified via flash column chromatography on silica gel to afford the desired indole.
Characterization of 2-Arylindoles

Ethyl 2-(2-(4-cyanophenyl)-1H-indol-3-yl)acetate (2a)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.167 g, 55% yield).

\[ RF = 0.25, \text{20\% ethyl acetate in hexanes; } mp = 158–159^\circ C \]

\[ ^1H \text{ NMR (500 MHz, CDCl}_3) \delta 8.68 (\text{br s, 1H}), 7.71 – 7.64 (\text{m, 3H}), 7.59 (\text{d, } J = 8.2 \text{ Hz, 2H}), 7.30 – 7.15 (\text{m, 3H}), 4.21 (\text{q, } J = 7.1 \text{ Hz, 2H}), 3.86 (\text{s, 2H}), 1.30 (\text{t, } J = 7.1 \text{ Hz, 3H}) \text{ ppm} \]

\[ ^13C \text{ NMR (126 MHz, CDCl}_3) \delta 172.2, 136.8, 136.3, 133.9, 132.5, 128.8, 128.3, 123.5, 120.4, 119.5, 118.9, 111.3, 110.8, 107.5, 61.3, 31.1, 14.3 \text{ ppm} \]

\[ \text{IR (neat, cm}^{-1}\text{) 3351.2 (NH, br), 2226.8 (CN, m), 1718.5 (C=O, s) } \]

\[ \text{HRMS (DART, m/z) } [\text{M+H}]^+ \text{ calculated for C}_{19}H_{16}N_2O_2: 305.1285; \text{ Found: 305.1293}\]

Methyl 4-(3-(2-ethoxy-2-oxoethyl)-1H-indol-2-yl)benzoate (2b)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-methoxycarbonylphenyl boronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a beige solid (0.273 g, 80% yield).

\[ RF = 0.20, \text{20\% ethyl acetate in hexanes; } mp = 139–140^\circ C \]
Ethyl 2-(2-(3-nitrophenyl)-1H-indol-3-yl)acetate (2c)

The titled compound was prepared via copper-catalyzed coupling of 1a and 3-nitrophenylboronic acid described in the general procedure for the synthesis of 2-arylidinoles and was obtained as a yellow solid (0.206 g, 64% yield).

RF = 0.30, 20% ethyl acetate in hexanes; mp = 88–89 °C

1H NMR (500 MHz, CDCl3) δ 8.68 (br s, 1H), 8.43 (s, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 7.56 – 7.47 (m, 1H), 7.25 – 7.09 (m, 3H), 4.24 (q, J = 7.0 Hz, 2H), 3.85 (s, 2H), 1.32 (t, J = 7.0 Hz, 3H) ppm

13C NMR (126 MHz, CDCl3) δ 172.4, 148.4, 136.2, 134.0, 133.8, 133.4, 129.9, 128.7, 123.4, 122.5, 122.3, 120.4, 119.4, 111.3, 107.1, 61.4, 31.1, 14.3 ppm

IR (neat, cm⁻¹) 3374.8 (NH, br), 1716.5 (C=O, s), 1524.9 (N-O, s), 1345.9 (N-O, s)

HRMS (DART, m/z) [M+H]^+ calculated for C18H16N2O4: 325.1183; Found: 325.1187
Ethyl 2-(2-(4-(methylsulfonyl)phenyl)-1H-indol-3-yl)acetate (2d)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-(methanesulfonyl)phenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a beige solid (0.173 g, 49% yield).

**RF** = 0.05, 20% ethyl acetate in hexanes; **mp** = 136–137 °C

**1H NMR (500 MHz, CDCl$_3$)** $\delta$ 9.06 (br s, 1H), 7.89 – 7.84 (m, 2H), 7.80 – 7.75 (m, 2H), 7.69 (d, $J$ = 8.0 Hz, 1H), 7.36 (d, $J$ = 8.0 Hz, 1H), 7.25 – 7.20 (m, 1H), 7.18 – 7.14 (m, 1H), 4.19 (q, $J$ = 7.1 Hz, 2H), 3.84 (s, 2H), 3.06 (s, 3H), 1.27 (t, $J$ = 7.1 Hz, 3H) ppm

**13C NMR (126 MHz, CDCl$_3$)** $\delta$ 172.2, 138.8, 137.9, 136.4, 133.9, 128.8, 128.7, 127.8, 123.5, 120.3, 119.5, 111.5, 107.5, 61.2, 44.5, 31.2, 14.3 ppm

**IR (neat, cm$^{-1}$)** 3353.8 (NH, br), 1727.9 (C=O, s)

**HRMS (DART, m/z)** [M+H]$^+$ calculated for C$_{19}$H$_{19}$NO$_4$S: 358.1108; Found: 358.1114

Ethyl 2-(2-(4-(trifluoromethyl)phenyl)-1H-indol-3-yl)acetate (2e)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-(trifluoromethyl)phenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.262 g, 76% yield).

**RF** = 0.55, 20% ethyl acetate in hexanes; **mp** = 96–97 °C
Ethyl 2-(2-(3-(methylthio)phenyl)-1H-indol-3-yl)acetate (2f)

The titled compound was prepared via copper-catalyzed coupling of 1a and 3-(methylthio)phenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a yellowish oil (0.208 g, 64% yield).

Rf = 0.53, 20% ethyl acetate in hexanes

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.35 (br s, 1H), 7.74 – 7.71 (m, 1H), 7.59 – 7.57 (m, 1H), 7.41 – 7.34 (m, 2H), 7.32 – 7.26 (m, 2H), 7.25 – 7.17 (m, 2H), 4.21 (q, $J = 7.1$ Hz, 2H), 3.87 (s, 2H), 2.55 (s, 3H), 1.30 (t, $J = 7.1$ Hz, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 172.5, 139.4, 135.8, 135.7, 133.1, 129.2, 128.9, 125.82, 125.78, 124.7, 122.7, 120.1, 119.3, 111.0, 105.8, 61.1, 31.3, 15.6, 14.3; IR (neat, cm$^{-1}$) 3388.9 (NH, br), 1718.4 (C=O, s)
Ethyl 2-(2-(4-methoxyphenyl)-1H-indol-3-yl)acetate (2g)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-methoxyphenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a colorless oil (0.125 g, 40% yield).

RF = 0.40, 20% ethyl acetate in hexanes

$^1$H NMR (500 MHz, CDCl₃) δ 8.27 (br s, 1H), 7.73 – 7.65 (m, 1H), 7.61 – 7.54 (m, 2H), 7.34 – 7.29 (m, 1H), 7.23 – 7.13 (m, 2H), 7.02 – 6.95 (m, 2H), 6.80 – 6.70 (m, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 3.85 (s, 3H), 3.83 (s, 2H), 1.28 (t, $J = 7.1$ Hz, 3H) ppm

$^{13}$C NMR (126 MHz, CDCl₃) δ 172.7, 159.5, 135.7, 129.6, 125.0, 122.2, 119.9, 119.1, 116.1, 114.8, 114.4, 110.9, 104.8, 61.0, 55.4, 31.3, 14.3 ppm

IR (neat, cm⁻¹) 3400.1 (NH, br), 1723.8 (C=O, s)

HRMS (DART, m/z) [M+H]$^+$ calculated for C₁₉H₁₉NO₃: 310.1438; Found: 310.1435
Ethyl 2-(2-(2-(benzyloxy)phenyl)-1H-indol-3-yl)acetate (2h)

The titled compound was prepared via copper-catalyzed coupling of 1a and 2-(benzyloxy)phenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a colorless oil (0.152 g, 41% yield).

$$R_F = 0.53, \text{20\% ethyl acetate in hexanes}$$

$$^{1}H\text{ NMR (500 MHz, CDCl}_3) \delta 8.86 \text{ (br s, 1H)}, 7.84 \text{ (dd, } J = 7.7, 1.7 \text{ Hz, 1H)}, 7.78 \text{ (d, } J = 7.7 \text{ Hz, 1H)}, 7.43 - 7.11 \text{ (m, 11H)}, 5.14 \text{ (s, 2H)}, 4.21 \text{ (q, } J = 7.1 \text{ Hz, 2H)}, 3.93 \text{ (s, 2H)}, 1.30 \text{ (t, } J = 7.1 \text{ Hz, 3H) ppm}$$

$$^{13}C\text{ NMR (126 MHz, CDCl}_3) \delta 172.4, 155.9, 136.6, 135.5, 133.1, 131.6, 129.4, 128.7, 128.4, 128.1, 127.2, 122.3, 121.7, 121.5, 119.5, 119.1, 113.8, 110.7, 106.5, 71.0, 60.8, 31.7, 14.3 \text{ ppm}$$

$$\text{IR (neat, cm}^{-1}) \text{ 3344.8 (NH, br), 1726.1 (C=O, s)}$$

$$\text{HRMS (DART, m/z) [M+H]}^+ \text{ calculated for C}_{25}\text{H}_{23}\text{NO}_3: 386.1751; \text{Found: 386.1761}$$

Ethyl 2-(2-(4-vinylphenyl)-1H-indol-3-yl)acetate (2j)

The titled compound was prepared via a copper-catalyzed coupling of 1a with 4-(vinylphenyl)boronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.131 g, 43% yield).

$$R_F = 0.55, \text{20\% ethyl acetate in hexanes; mp} = 98–99 \text{ °C}$$
Ethyl 2-(2-(4-ethynylphenyl)-1H-indol-3-yl)acetate (2k)

The titled compound was prepared via copper-catalyzed coupling of 1a and 4-(dihydroxyborophenyl)acetylene described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.074 g, 23% yield).

Rf = 0.50, 20% ethyl acetate in hexanes; mp = 74–76 ºC

1H NMR (500 MHz, CDCl3) δ 8.26 (br s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.63 – 7.54 (m, 4H), 7.33 – 7.30 (m, 1H), 7.25 – 7.14 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 3.84 (s, 2H), 1.27 (t, J = 7.1 Hz, 3H) ppm

13C NMR (126 MHz, CDCl3) δ 172.4, 136.0, 132.8, 132.7, 129.1, 128.0, 123.0, 121.7, 120.3, 119.4, 115.5, 111.1, 106.4, 83.4, 78.5, 61.2, 31.3, 14.3 ppm

IR (neat, cm⁻¹) 3388.7 (NH, br), 3291.5 (C=C-H, s), 1726.1 (C=O, s)

HRMS (DART, m/z) [M+H]+ calculated for C20H17NO2: 304.1332; Found: 304.1341
Ethyl 2-(2-(2-bromophenyl)-1H-indol-3-yl)acetate (2l)

The titled compound was prepared via copper-catalyzed coupling of 1a and 2-bromophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a colorless oil (0.184 g, 51% yield).

$R_F = 0.20$, 20% ethyl acetate in hexanes

$^1H$ NMR (500 MHz, CDCl$_3$) $\delta$ 8.27 (br s, 1H), 7.70 - 7.63 (m, 2H), 7.53 (dd, $J = 7.5$, 1.7 Hz, 1H), 7.35 (td, $J = 7.5$, 1.3 Hz, 1H), 7.31 (dt, $J = 8.1$, 0.9 Hz, 1H), 7.24 (ddd, $J = 8.0$, 7.5, 1.7 Hz, 1H), 7.20 (ddd, $J = 8.2$, 7.1, 1.2 Hz, 1H), 7.14 (ddd, $J = 8.1$, 7.1, 1.1 Hz, 1H), 4.13 (q, $J = 7.1$ Hz, 2H), 3.71 (s, 2H), 1.24 (t, $J = 7.1$ Hz, 3H) ppm

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 172.0, 135.6, 134.7, 133.7, 133.1, 130.2, 127.9, 127.4, 124.0, 122.7, 120.0, 119.5, 111.1, 107.4, 60.8, 31.2, 14.3 ppm

IR (neat, cm$^{-1}$) 3389.9 (NH, br), 1723.9 (C=O, s)

HRMS (DART, m/z) [M+H]$^+$ calculated for C$_{18}$H$_{16}$BrNO$_2$: 358.0437; Found: 358.0440

Ethyl 2-(2-(4-acetylphenyl)-1H-indol-3-yl)acetate (2m)

The titled compound was prepared via a copper-catalyzed coupling of 1a and 4-acetylphenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.153 g, 47% yield).

$R_F = 0.20$, 20% ethyl acetate in hexanes; mp = 127–128 °C
Ethyl 2-(2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1H-indol-3-yl)acetate (2n)

The titled compound was prepared via a copper-catalyzed coupling of 1a and 1,4-benzodioxane-6-boronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a colorless oil (0.099 g, 29% yield).

R_f = 0.23, 20% ethyl acetate in hexanes

^1H NMR (500 MHz, CDCl_3) δ 8.18 (br s, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.23 – 7.10 (m, 4H), 6.96 (d, J = 8.3 Hz, 1H), 4.29 (s, 4H), 4.18 (q, J = 7.1 Hz, 2H), 3.83 (s, 2H), 1.27 (t, J = 7.1 Hz, 3H) ppm

^13C NMR (126 MHz, CDCl_3) δ 172.4, 143.9, 143.7, 136.0, 135.6, 129.2, 125.9, 122.4, 121.5, 120.0, 119.2, 117.9, 117.2, 110.9, 105.2, 64.5, 61.0, 31.3, 14.3 ppm

IR (neat, cm⁻¹) 3396.9 (NH, br), 1718.9 (C=O, br)

HRMS (DART, m/z) [M+H]^+ calculated for C₂₀H₁₉NO₄: 338.1387; Found: 338.1388
Ethyl 2-(2-(benzo[b]thiophen-3-yl)-1H-indol-3-yl)acetate (2o)

The titled compound was prepared via a copper-catalyzed coupling of 1a and benzo[b]thien-3-ylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a colorless oil (0.192 g, 57% yield).

\[ R_f = 0.55, \text{20\% ethyl acetate in hexanes} \]

\[ ^1H \text{ NMR (500 MHz, CDCl}_3 \rfloor \delta 8.43 (\text{br s, 1H}), 7.99 - 7.92 (\text{m, 1H}), 7.92 - 7.85 (\text{m, 1H}), 7.82 (\text{s, 1H}), 7.79 - 7.70 (\text{m, 1H}), 7.47 - 7.38 (\text{m, 2H}), 7.37 - 7.33 (\text{m, 1H}), 7.31 - 7.20 (\text{m, 2H}), 4.14 (\text{q, J = 7.1 Hz, 2H}), 3.80 (\text{s, 2H}), 1.25 (\text{t, J = 7.1 Hz, 3H}) \] ppm

\[ ^13C \text{ NMR (126 MHz, CDCl}_3 \rfloor \delta 172.4, 140.3, 138.2, 135.9, 130.8, 128.4, 127.8, 127.1, 124.9, 124.7, 123.0, 123.0, 122.6, 120.1, 119.3, 111.1, 107.5, 61.0, 31.3, 14.2 \] ppm

\[ \text{IR (neat, cm}^{-1}\rfloor \text{3350.3 (NH, br), 1723.9 (C=O, s)} \]

\[ \text{HRMS (DART, m/z) [M+H]}^+ \text{calculated for C}_{20}\text{H}_{17}\text{NO}_2\text{S: 336.1053; Found: 336.1040} \]

2-(2-(4-cyanophenyl)-6-fluoro-3-indolyl)methyl acetate (3b)

The titled compound was prepared via a copper-catalyzed coupling of 1b and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a pale brown solid (0.235 g, 76% yield).

\[ R_f = 0.37, \text{25\% ethyl acetate in hexanes; mp = 171–173 °C} \]
\textbf{\( ^1 \text{H NMR (400 MHz, CDCl}_3 \)} \delta 8.37 (s, 1H), 7.72 (s, 4H), 7.58 (dd, \( J = 8.6, 5.3 \) Hz, 1H), 7.00 (d, \( J = 9.2 \) Hz, 1H), 6.93 (t, \( J = 9.2 \) Hz, 1H), 3.82 (s, 2H), 3.76 (s, 3H) ppm

\textbf{\( ^{13} \text{C NMR (100 MHz, CDCl}_3 \)} \delta 172.4, 160.8 (d, \( J_{\text{CF}} = 240.4 \) Hz), 136.6, 136.4 (d, \( J_{\text{CF}} = 12.5 \) Hz), 134.4, 132.8, 128.3, 125.5, 120.6 (d, \( J_{\text{CF}} = 9.8 \) Hz), 118.8, 111.4, 109.6 (d, \( J_{\text{CF}} = 24.5 \) Hz), 107.7, 97.7 (d, \( J_{\text{CF}} = 26.2 \) Hz), 52.5, 30.9 ppm

\textbf{\( ^{19} \text{F NMR (CDCl}_3, 376 \text{ MHz) \delta} = -119.3 \text{ (td, } J_{\text{HF,o}} = 9.7 \text{ Hz, } J_{\text{HF,m}} = 5.3 \text{ Hz, 1F) ppm} \)

\textbf{IR (neat, cm}^{-1}) 3342.3 (NH, br), 2228.1 (CN, m), 1721.5 (C=O, s)

\textbf{HRMS (DART, m/z)} [M–H]^- calculated for C\textsubscript{18}H\textsubscript{13}FN\textsubscript{2}O\textsubscript{2}: 307.0888; Found: 307.0892

2-(2-(4-cyanophenyl)-6-trifluoromethyl-3-indolyl)methyl acetate (3c)

The titled compound was prepared via the copper-catalyzed coupling of 1c and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as an orange solid (0.243 g, 68% yield).

\( R_F = 0.34, 25\% \text{ ethyl acetate in hexanes; } mp = 166-167 \degree\text{C} \)

\textbf{\( ^1 \text{H NMR (400 MHz, CDCl}_3 \)} \delta 8.74 (br s, 1H), 7.76–7.70 (m, 5H), 7.59 (s, 1H), 7.38 (dd, \( J = 8.4, 1.1 \) Hz, 1H), 3.85 (s, 2H), 3.77 (s, 3H) ppm

\textbf{\( ^{13} \text{C NMR (100 MHz, CDCl}_3 \)} \delta 172.4, 163.6, 136.1, 135.2, 132.9, 131.0, 128.7, 126.4, 125.6 (q, \( J_{\text{CF}} = 32.1 \) Hz), 123.7, 120.0, 118.6, 117.3 (q, \( J_{\text{CF}} = 3.4 \) Hz), 112.0, 108.9 (q, \( J_{\text{CF}} = 4.3 \) Hz), 107.8, 52.6, 30.7 ppm

\textbf{\( ^{19} \text{F NMR (CDCl}_3, 376 \text{ MHz) \delta} = -61.9 \text{ (s, 3F) ppm} \)

\textbf{IR (neat, cm}^{-1}) 3326.9 (NH, br), 2229.9 (CN, m), 1718.4 (C=O, s)
HRMS (DART, \( m/z \)) \([M-H]^–\) calculated for \( C_{19}H_{13}F_3N_2O_2 \): 357.0856; Found: 357.0861

![Chemical Structure](image)

2-(2-(4-cyanophenyl)-6-acetyl-3-indolyl)methyl acetate (3d)
The titled compound was prepared via a copper-catalyzed coupling of 1d and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.098 g, 29% yield).

\( R_F = 0.1, 25\% \text{ ethyl acetate in hexanes; } m_p = 266–268 \, ^\circ \text{C} \)

\(^1H\text{ NMR (400 MHz, CDCl}_3) \delta 8.55 \,(s, 1H), 8.08 \,(s, 1H), 7.83 – 7.78 \,(m, 5H), 7.72 \,(d, J = 8.4 \text{ Hz}, 1H), 3.85 \,(s, 2H), 3.75 \,(s, 3H), 2.68 \,(s, 3H) \text{ ppm} \)

\(^{13}C\text{ NMR (100 MHz, CDCl}_3) \delta ^{13}C\text{ NMR (101 MHz, CDCl}_3) \delta 198.2, 172.0, 137.7, 136.2, 135.8, 133.0, 133.0, 132.6, 128.9, 121.1, 119.5, 118.6, 112.4, 112.1, 108.2, 52.5, 30.9, 27.1 \text{ ppm} \)

IR (neat, cm\(^{-1}\)) 3306.1 (NH, br), 2227.8 (CN, m), 1731.9 (C=O, s), 1685.2 (C=O, s)

HRMS (DART, \( m/z \)) \([M-H]^–\) calculated for \( C_{20}H_{16}N_2O_3 \): 331.1088; Found: 331.1087

![Chemical Structure](image)

2-(7-chloro-2-(4-cyanophenyl)-5-fluoro)-3-indolyl)ethyl acetate (3e)
The titled compound was prepared via a copper-catalyzed coupling of 1e and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a brown solid (0.236 g, 66% yield).
$R_F = 0.63$, 25% ethyl acetate in hexanes; $mp = 125–128 ^\circ C$

$^1H$ NMR (400 MHz, CDCl$_3$) $\delta$ 8.29 (br s, 1H), 7.88 – 7.77 (m, 4H), 7.29 (dd, $J = 8.9, 2.1$ Hz, 1H), 7.09 (dd, $J = 8.9, 2.1$ Hz, 1H), 4.20 (q, $J = 7.1$ Hz, 2H), 3.75 (s, 2H), 1.28 (t, $J = 7.1$ Hz, 3H) ppm

$^{13}C$ NMR (100 MHz, CDCl$_3$) $\delta$ 171.6, 157.6 (d, $^1J_{CF} = 239.5$ Hz), 136.5, 135.9, 132.8, 130.4, 129.8 (d, $^3J_{CF} = 10.4$ Hz), 128.8, 118.6, 116.8 (d, $^3J_{CF} = 12.4$ Hz), 112.03, 111.99 (d, $^2J_{CF} = 29.5$ Hz), 108.9 (d, $^4J_{CF} = 5.2$ Hz), 103.7 (d, $^2J_{CF} = 23.9$ Hz), 61.5, 31.2, 14.3 ppm

$^{19}F$ NMR (CDCl$_3$, 376 MHz) $\delta = -121.8$ (t, $J_{HF-o} = 9.1$ Hz, 1F) ppm

IR (neat, cm$^{-1}$) 3308.7 (NH, br), 2228.8 (CN, m), 1719.1 (C=O, s)

HRMS (DART, m/z) [M–H]$^-$ calculated for C$_{19}$H$_{14}$FClN$_2$O$_2$: 355.0655; Found: 355.0637

2-(2-(4-cyanophenyl)-5-trifluoromethoxy-3-indolyl)methyl acetate (3f)

The titled compound was prepared via a copper-catalyzed coupling of 1f and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a brown solid (0.196 g, 52% yield).

$R_F = 0.31$, 25% ethyl acetate in hexanes; $mp = 152–154 ^\circ C$

$^1H$ NMR (400 MHz, CDCl$_3$) $\delta$ 8.28 (br s, 1H), 7.80 (s, 4H), 7.55 (s, 1H), 7.39 (d, $J = 8.8$ Hz, 1H), 7.21 – 7.04 (m, 1H), 3.81 (s, 2H), 3.75 (s, 3H) ppm

$^{13}C$ NMR (100 MHz, CDCl$_3$) $\delta$ 172.2, 143.6 (q, $^4J_{CF} = 2.0$ Hz), 136.3, 136.0, 134.6, 132.9, 129.2, 128.7, 120.9 (q, $^4J_{CF} = 255.7$ Hz), 118.7, 117.8, 112.3, 112.1, 111.9, 108.1, 52.5, 30.9 ppm
$^{19}$F NMR (CDCl$_3$, 376 MHz) $\delta = -59.1$ (s, 3F ppm)

IR (neat, cm$^{-1}$) 3326.9 (NH, br), 2231.0 (CN, m), 1719.5 (C=O, s)

HRMS (DART, $m/z$) [M–H]$^-$ calculated for C$_{19}$H$_{13}$F$_3$N$_2$O$_3$: 373.0806; Found: 373.0799

2-(2-(4-cyanophenyl)-5,7-dimethyl-3-indolyl)methyl acetate (3g)

The titled compound was prepared via a copper-catalyzed coupling of 1g and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a red oil (0.199 g, 63% yield).

$R_F = 0.73$, 25% ethyl acetate in hexanes

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.16 (br s, 1H), 7.80–7.72 (m, 2H), 7.71 – 7.65 (m, 2H), 7.29 (s, 1H), 6.91 (s, 1H), 3.82 (s, 2H), 3.75 (s, 3H), 2.44–2.48 (m, 6H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.6, 137.2, 134.4, 133.9, 133.0, 132.7, 130.4, 128.9, 128.5, 128.0, 126.2, 120.3, 118.9, 116.7, 111.0, 107.8, 52.4, 31.1, 21.6, 16.7 ppm

IR (neat, cm$^{-1}$) 3351.5 (NH, br), 2228.0 (CN, m), 1730.9 (C=O, s)

HRMS (DART, $m/z$) [M–H]$^-$ calculated for C$_{20}$H$_{18}$N$_2$O$_2$: 317.1296; Found: 317.1296.
4-(3-(cyanomethyl)-2-indolyl)benzonitrile (3h)

The titled compound was prepared via a copper-catalyzed coupling of 1h and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a pale brown solid (0.193 g, 75% yield).

\[ R_f = 0.35, \text{25\% ethyl acetate in hexanes; } \text{mp} = 182–183 \, ^\circ\text{C} \]

\[^1\text{H NMR (400 MHz, CDCl}_3\text{)} \delta\text{ 8.36 (br s, 1H), 7.83 – 7.78 (m, 2H), 7.72 (d, } J = 7.9 \, \text{Hz, 1H), 7.68 – 7.63 (m, 2H), 7.45 (dt, } J = 8.1, 0.9 \, \text{Hz, 1H), 7.34 (ddd, } J = 8.1, 7.1, 1.2 \, \text{Hz, 1H), 7.30 – 7.26 (m, 1H), 3.90 (s, 2H) ppm} \]

\[^{13}\text{C NMR (100 MHz, CDCl}_3\text{)} \delta\text{ 136.2, 136.1, 134.0, 133.2, 128.7, 127.8, 124.4, 121.4, 118.9, 118.4, 117.8, 112.3, 111.6, 103.3, 13.9 ppm} \]

\[ \text{IR (neat, cm}^{-1}\text{) 3351.5 (NH, br), 2228.0 (CN, m), 1730.9 (C=O, s)} \]

\[ \text{HRMS (DART, } m/z\text{) [M-H]}^{-}\text{ calculated for } \text{C}_{17}\text{H}_{11}\text{N}_{3}: 256.0880; \text{ Found: 256.0888} \]

2-(2-(4-cyanophenyl)-6-trifluoromethoxy-3-indolyl)-N,N-dimethylacetamide (3i)

The titled compound was prepared via a copper-catalyzed coupling of 1i and 4-cyanophenylboronic acid described in the general procedure for the synthesis of 2-arylindoles and was obtained as a white solid (0.210 g, 69% yield).

\[ R_f = 0.19, \text{50\% ethyl acetate in hexanes; } \text{mp} = 256–259 \, ^\circ\text{C} \]
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.42 (s, 1H), 7.65 (q, $J = 8.3$ Hz, 4H), 7.56 (d, $J = 7.8$ Hz, 1H), 7.28 (d, $J = 8.1$ Hz, 1H), 7.19 (t, $J = 7.5$ Hz, 1H), 7.12 (t, $J = 7.4$ Hz, 1H), 3.89 (s, 2H), 3.05 (s, 3H), 3.00 (s, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 171.0, 137.3, 132.7, 128.4, 123.6, 120.5, 119.4, 111.4, 111.1, 109.1, 37.8, 36.1, 30.3 ppm

IR (neat, cm$^{-1}$) 3268.9 (NH, br), 1646.0 (C=O, s)

HRMS (DART, m/z) [M+H]$^+$ calculated for C$_{19}$H$_{17}$N$_3$O: 304.1444, Found: 304.1456
Continuous Flow Synthesis of Arylindole 2a

Scheme 7 Experimental Details

Flow Setup: The setup consisted of joining S1 and S2 by a PEEK T-mixer and PFA tubing, and flowing the streams directly into a continuous stirred tank reactor (CSTR). The CSTR was a 2-dram vial with a cap and septum containing copper beads (2 g, 30-40 mesh) heated to 65 ℃ under argon. Stock S1 was prepared by charging isocyanide 1a (1 mmol, 1.0 equiv), 4-cyanophenylboronic acid (1.5 equiv), and CuCl2 (10 mol %) to a 10 mL volumetric flask fitted with a cap containing a septum. The vessel was evacuated and back-filled with argon three times. Solids were dissolved in THF (5 mL) and H2O (0.5 mL). NMP (2.5 mL) was then added, followed by dilution of THF to reach a total volume of 10 mL. Stock S2 was prepared by combining 1 M KOt-Bu in THF (1.3 mL, 1.3 equiv), followed by THF (5 mL), and then NMP (2.5 mL) in a 10 mL volumetric flask under argon. THF was added to reach a total volume of 10 mLs. S1 and S2 was transferred to an 8 mL stainless steel syringe.

Scheme 12. Detailed procedure for the copper catalyzed cyclization of arylisocyanide 1a to indole 2a in a CSTR.
Procedure: Solutions A and B were pumped at a rate of 0.05 mL/min into the CSTR for 30 minutes. After 30 minutes, the CSTR solution was removed by a Syrris pump (S3) at 0.1 mL/min. After an additional 30 minutes, solutions were collected for 10 min and were worked up in the same manner as the batch reactions described in section IV. A 56% yield of 2a was obtained via HPLC with naphthalene as an internal standard (Scheme 12).

Dehydration of N-formylaniline 4 (Module 1) Experimental Details

Reactor 1 Setup: Stock solution S1 was prepared by dissolving formamide 4 (3 mmol, 1.0 equiv) in DCM in a 10 mL volumetric flask. Stock S2 was prepared by combining POCl₃ (2 M, 0.89 mL, 1.25 equiv) and DCM (4.11 mL). Stock solution S3 was neat DIPEA (3.75 equiv). Stock solution S2 was transferred to a 10 mL glass syringe. Solutions S1 and S3 were pumped using a Syrris pump. PFA tubing reactor R1 (38 cm, 0.17 mL) was connected to the three streams with a PEEK Cross mixer.

Scheme 13. Detailed setup for Module 1 containing the dehydration of isocyanide 1a from simple N-formylaniline 4 in continuous flow.
Procedure: S1, S2, and S3 were pumped at a rate of 50 µL/min, 9.9 µL/min, and 9.8 µL/min respectively giving a 2.5 min residence time for R1. The system was equilibrated for 3 residence times prior to collecting for 5 minutes. Collected solutions were worked up in the same manner as the batch reactions. A 94% yield of 1a was obtained via HPLC with naphthalene as an internal standard (Scheme 13).

Table 4 Experimental Details (Scheme 14)

Flow Setup:

Module 1 consists of connecting Solutions S1, S2, and S3 with a PEEK Cross mixer to PFA tubing reactor R1 (38 cm, 0.17 mL). Stock solution S1 was prepared by dissolving formamide 4 (3 mmol, 1.0 equiv) in DCM in a 10 mL volumetric flask. Stock S2 was prepared by combining POCl3 (2 M, 0.89 mL, 1.25 equiv) and DCM (4.11 mL). Stock solution S3 was neat DIPEA (3.75 equiv). Solutions S1 and S3 were pumped using a Syrris pump. S2 was transferred to a 10 mL glass syringe and pumped with a Harvard pump.

Module 2 consists of combining a stream containing 1a with S6 and S7 directly in R2. R2 is a CSTR (V = 9 mL) consisting of a 2-dram vial with a cap and septum containing copper beads (3 g, 30-40 mesh) under argon heated to 65 °C. S6 was prepared by adding 4-cyanophenylboronic acid (0.19 M, 1.5 equiv), and CuCl2 (10 mol %) to a 10 mL volumetric flask fitted with a cap containing a septum. The vessel was evacuated and back-filled with argon three times. Solids were dissolved in THF (5 mL) and H2O (0.5 mL). NMP (2.5 mL) was then added, followed by dilution of THF to reach a total volume of 10 mL. S7 was prepared by combining 1 M KOt-Bu in THF (1.3 mL), followed by THF (5 mL), and then NMP (2.5 mL) in a 10 mL volumetric flask under argon. THF was added to reach a total volume of 10 mL S6 and S7 were
transferred to 8 mL stainless steel syringes and pumped with a Harvard pump. Solution was removed from R2 via a Syrris pump (S8).

Module 3 consists of S4 (aqueous stream of 0.5 M K3PO4), a PEEK T-mixer, and a Zaiput liquid-liquid membrane separator (Membrane = 0.5 μm). S4 was pumped using a Syrris pump.

Module 4 consists of S5 (aqueous stream of NH4Cl), a PEEK T-mixer, and a Zaiput liquid-liquid membrane separator (Membrane = 0.5 μm). S5 was prepared by combining 5 mLs saturated ammonium chloride with 5 mLs water. S5 was pumped using a Syrris pump.

Module 5 (shown in gray) is an Omnifit glass packed bed column of activated 4 Å molecular sieves (2.5 g).

Procedure:

Table 4, Entry 1: Generally, Module 1 is connected to Module 2. S1, S2, and S3 were pumped at a rate of 50 μL/min, 9.9 μL/min, and 9.8 μL/min respectively giving a 2.5 min residence time for R1. Module 1 was equilibrated for 3 residence times. Then, the end of PFA tubing reactor of R1 containing isocyanide 1a of Module 1 is directly added to R2 of Module 2. S6 and S7 were flowed at a rate of 0.12 mL/min. After 30 minutes, the CSTR solution was removed by a Syrris pump at 0.3 mL/min (S8). The system ran for an additional 30 minutes. Solutions were collected for 20 minutes and were worked up in the same manner as previously described in the procedure for the synthesis of 2-arylindoles and a 0% yield was determined via HPLC with naphthalene as an internal standard.

Table 4, Entry 2: Generally, the output stream of Module 1 containing isocyanide 1a in DCM is connected to Module 3 to undergo a single liquid-liquid extraction, before being connected to
Module 2. S1, S2, and S3 were pumped at a rate of 50 μL/min, 9.9 μL/min, and 9.8 μL/min respectively giving a 2.5 min residence time for R1. The end of PFA tubing reactor of R1 containing isocyanide 1a of Module 1 is then mixed with the aqueous stream S4 (0.5 M K₃PO₄, 140 μL/min) of Module 3. The organic and aqueous streams are separated using a liquid-liquid membrane separator. After equilibrating Module 1 connected to Module 3 for three residence times, the organic stream containing 1a is then directly added to R2 of Module 2. S6 and S7 were flowed at a rate of 0.12 mL/min. After 30 minutes, the CSTR solution was removed by a Syrris pump at 0.3 mL/min (S8). The system ran for an additional 30 minutes. Solutions were collected for 20 minutes and were worked up in the same manner as described in section IV and a 9% yield was determined via HPLC with naphthalene as an internal standard.

Table 4, Entry 3: Generally, the output stream of Module 1 containing isocyanide 1a in DCM is connected to Module 3 to undergo a single liquid-liquid extraction. The organic stream of Module 4 is then dried through the drying column of Module 5, before being connected to Module 2. S1, S2, and S3 were pumped at a rate of 50 μL/min, 9.9 μL/min, and 9.8 μL/min respectively giving a 2.5 min residence time for R1. The end of PFA tubing reactor of R1 containing isocyanide 1a of Module 1 is then mixed with the aqueous stream S4 (0.5 M K₃PO₄, 140 μL/min) of Module 3. The organic and aqueous streams are separated using a liquid-liquid membrane separator. After equilibrating the previously described system for three residence times, the organic stream containing 1a is then directly pumped through the drying column of Module 5 before being pumped into R2 of Module 2. S6 and S7 were flowed at a rate of 0.12 mL/min. After 30 minutes, the CSTR solution was removed by a Syrris pump at 0.3 mL/min (S8). The system ran for an additional 30 minutes. Solutions were collected for 20 minutes and
were worked up in the same manner as previously described in the procedure for the synthesis of 2-arylindoles and a 19% yield was determined via HPLC with naphthalene as an internal standard.

**Table 4, Entry 4:** Generally, the output stream of Module 1 containing isocyanide 1a in DCM is connected to Module 3 and Module 4 to undergo two subsequent extractions. The organic stream of Module 4 is dried through the drying column of Module 5, before being connected to Module 2. S1, S2, and S3 were pumped at a rate of 50 μL/min, 9.9 μL/min, and 9.8 μL/min respectively giving a 2.5 min residence time for R1. The end of PFA tubing reactor of R1 containing isocyanide 1a of Module 1 is then mixed with the aqueous stream S4 (0.5 M K3PO4, 140 μL/min) of Module 3. The organic and aqueous streams are separated using a liquid-liquid membrane separator. The organic stream is then connected to Module 4 where it is mixed with a second aqueous stream S5 (aqueous NH4Cl, 70 μL/min) and passed through a second liquid-liquid membrane separator. After equilibrating the previously described system for three residence times, the organic stream containing 1a is then directly pumped through the drying column of Module 5 before being pumped into R2 of Module 2. S6 and S7 were flowed at a rate of 0.12 mL/min. After 30 minutes, the CSTR solution was removed by a Syrris pump at 0.3 mL/min (S8). The system ran for an additional 30 minutes. Solutions were collected for 20 minutes and were worked up in the same manner as previously described in the procedure for the synthesis of 2-arylindoles and a 51% yield was determined via HPLC with naphthalene as an internal standard (Scheme 14).

**S1**

0.3 M in CH₂Cl₂
50 µL/min

**S2**

POCl₃
2 M in CH₂Cl₂
(1.25 equiv)
9.9 µL/min

**S3**

i-Pr₂NEt
(3.75 equiv)
9.8 µL/min

R₁, V = 0.17 mL
2.5 min

**S4**

0.5 M Aq. K₃PO₄
140 µL/min

**S5**

50% Aq. Sat'd NH₄Cl
70 µL/min

**S6**

4-cyanophenyl boronic acid
(1.5 equiv)
CuCl₂
(10 mol %)
2.8:1:0.2 THF/NMP/H₂O
120 µL/min

**S7**

KOT-Bu
(1.3 equiv)
3:1 THF/NMP
120 µL/min

R₂
CSTR, V = 9 mL
65 °C, 30 min
3 g Cu beads

51% yield over two steps
throughput: 0.140 g/h
0.46 mmol/hr

= Liquid Liquid Separator  mol. sieves = Drying Column
Scheme 9 Experimental Details

Flow Setup: Same set-up as Scheme 14 except the following differences described below. Module 1 consisted of PFA tubing reactor R1 (154 cm, 0.73 mL) connecting streams S1, S2, and S3 with a PEEK Cross mixer. Due to the increased flow rates, the Zaiput Liquid-Liquid Separators of Module 3 and Module 4 required a 0.1 μm membrane. A surge tank was added after Module 3 and Module 4 because the increased back pressure from the molecular sieves column made the organic/aqueous separation of Module 4 more challenging. The surge tank consisted of a 2-dram vial under air with 1 mL of 1a in CH₂Cl₂. The stream was removed from the surge tank and pumped through the rest of the setup with a Syrris pump (S9). Module 2 consisted of Reactor 2, a 36 mL CSTR in a 250 mL RBF under argon heated to 65 °C containing copper beads (12 g, 30–40 mesh). S1 and S3 were transferred to 10 mL SGE glass syringes. S2, S4, S5, S6, S7, S8, and S9 were transferred to Syrris syringes.

Procedure: Same procedure as Scheme 14 except the following differences described below. All flow rates were increased by approximately 4X. S1 = 0.2 mL/min, S2 = 47 μL/min, S3 = 45 μL/min, S4 = 0.58 mL/min, S5 = 0.29 mL/min, S6 = 0.48 mL/min, S7 = 0.48 mL/min, S8 = 1.2 mL/min, S9 = 0.25 mL/min. The system was equilibrated before the surge tank for 3 equilibration times. The surge tank was attached and filled to 1 mL. S9 was pulled at 0.25 mL/min using a Syrris pump. The solution is then flowed through the drying column containing molecular sieves. The system was equilibrated for an additional 60 minutes. Collected solutions were worked up in the same manner as the batch reactions. Product was collected for 30 minutes (1.8 mmol total material) providing 49% isolated yield over two steps (Scheme S-5).
Scheme 15. Detailed procedure for the two-step continuous flow synthesis of indole 2a from simple N-formylaniline 4 with increased throughput.

- **S1**: 4-cyanophenyl boronic acid (1.5 equiv) in 3:1 THF/NMP (0.48 mL/min) at 65 °C, 30 min.
- **S2**: NHCHO (0.3 M in CH₂Cl₂, 0.2 mL/min).
- **S3**: i-Pr₂NEt (4.0 equiv) in CH₂Cl₂ (41 µL/min).
- **S4**: POCl₃ (2 M in CH₂Cl₂, 1.3 equiv) in CH₂Cl₂ (40 µL/min).
- **S5**: CuCl₂ (10 mol %) in 3:1 THF/NMP (0.58 mL/min).
- **S6**: KOt-Bu (1.3 equiv) in 3:1 THF/NMP (0.48 mL/min).
- **S7**: CSTR, V = 36 mL, 65 °C, 30 min, 12 g Cu beads.

49% yield over two steps with 65 °C, throughput: 550 mg/h.
Experimental Details for 3-Step Continuous Flow Set-up (Scheme 10)

Flow Setup: Same setup as Scheme 15 except the following differences described below. Stock solution S10 was prepared by dissolving arylaniline (15 mmol, 1.0 equiv) in DCM in a 25 mL volumetric flask. Stock S11 was prepared by adding a solution of acetic anhydride (1.5 equiv.) and formic acid (1.6 equiv.), which had previously been stirred at 50 °C in a sealed tube for 2 hours, to DCM in a 25 mL volumetric flask. R3 consisted of S10, S11, a peek T-mixer, and a PFA tubing reactor (110 cm, 0.5 mL). S12 was prepared in the same manner as S4. R1 consisted of a PFA tubing reactor (160 cm, 0.73 mL) connecting streams organic stream containing N-formylaniline 4, S2, and S3 with a PEEK Cross mixer. S9, S10, and S2 were transferred to 10 mL SGE glass syringes and pumped using a Harvard Pump. All remaining streams were transferred to Syrris syringes.

Procedure: Same procedure as Scheme 15, except the following differences described below. S10 (0.1 mL/min) and S11 (0.1 mL/min) were combined with a PEEK T-mixer and flowed through R3, followed by the addition of S12 (0.2 mL/min) containing an aqueous stream and a Zaiput liquid-liquid separator fitted with a 0.2 μm membrane. Connecting the organic stream from the separator to R1, instead of S1, allowed us to add the additional formylation of simple 2-alkenylaniline. Module 1 mixes the organic stream of R3 with S2 (40 uL/min) and S3 (41 uL/min). The system consisting of S10, S11, R3, S12, and Module 1, 3, and 4 was equilibrated for 3 residence times. After which, a 1 mL surge tank was added, which was flowed (0.24 mL/min) through the drying column followed by flowing directly into Module 2 to undergo the copper catalyzed cyclization. The system was equilibrated for an additional 60 minutes. Collected solutions were worked up in the same manner as the batch reactions. Solutions of product were collected for 30 minutes (1.8 mmol total material) providing 12% yield over three
steps obtained via HPLC with naphthalene as an internal standard and 8% isolated yield over three steps (Scheme 16).

**Scheme 16.** Detailed procedure for the three-step continuous flow synthesis of indole 2a from simple aniline 1a.

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**Diagram Description:**
- **S10:** Reaction 1: 0.6 M in CH₂Cl₂, 0.1 mL/min.
- **S11:** Reaction 2: Ac₂O/HCOOH in CH₂Cl₂, 0.1 mL/min.
- **S12:** Reaction 3: 0.5 M Au₃PO₄ in CH₂Cl₂.
- **S13:** Reaction 4: POCl₃ in CH₂Cl₂.
- **S14:** Reaction 5: 0.5 M Aq. K₂PO₄.
- **S15:** Reaction 6: 4-cyanophenyl boronic acid.
- **S16:** Reaction 7: KOr-Bu.
- **S17:** Reaction 8: CSTR, V = 35 mL.

**Reagents and Conditions:**
- **R₃:** V = 0.5 mL, 2.5 min.
- **R₁:** V = 0.73 mL, 2.5 min.
- **R₂:** CSTR, V = 35 mL, 65 °C, 30 min, 12 g Cu beads.

**Notes:**
- 12% yield over three steps.
- Throughput: 0.13 g/h, 0.43 mmol/hr.

**Diagram Symbols:**
- ○ = Pump
- □ = 1 mL Surge Tank
- mol sieves = Drying Column
- + = Cross Mixer
- T = T-mixer
- □ = Liquid Liquid Separator
Figure 4. Continuous flow setup for the synthesis of indole 2a from simple aniline 1a' before the start of the experiment. Additional information: The labels in the photograph correspond to the labels in Scheme S-6. Before the experiment began, each pump was loaded with reagent, 4 Å molecular sieves were added to the glass column, and 30–40 mesh copper beads were added to the CSTR. Product was collected from S8 into a collection jar off to the right of the picture. Two improvements were made for the final three-step continuous flow setup and are not captured in the above photograph. Since we observed our reaction refluxing into the argon line, we removed the vial and replaced it with a 250 mL RBF heated in an aluminum block under argon. Additionally, we added a surge tank after separator 3 because the backpressure from the drying column disrupted the separation.
Figure 5. Continuous flow setup for the synthesis of indole 2a from simple aniline 1a' before the start of the experiment showing the direction of flow throughout the system.
References


Chapter 1.

$^1$H, $^{13}$C, and $^{19}$F Spectra
The diagram shows a 2D NMR spectrum with the following key points:

- Peaks are labeled with their chemical shifts in parts per million (ppm).
- The spectrum includes resonances at various positions, with some peaks clustered around the 1.00 ppm region.
- The structure labeled as 1a' features a CO₂Et group.
- The solvent used is CDCl₃.

The specific chemical shifts and their corresponding intensities are indicated on the graph, providing a detailed view of the molecular structure's NMR signature.
$\text{NM. 2 } N \text{Me}_2 \text{NH}_2 \text{Ii'}$

$\text{f1 (ppm)}$

$\text{115}$
$\text{F}_3\text{CO} \quad \text{NC}\text{CO}_2\text{Me}$

1f

$\delta$ (ppm)
The diagram shows a 1H NMR spectrum of compound 2c. The spectrum displays peaks at various chemical shifts, indicated by ppm values. Key peaks include:

- Peaks at 8.68, 8.43, 8.12, 7.94, 7.92, 7.97, 7.86, 7.83, 7.75, 7.50, and 7.26 (CDC13) ppm.
- Peaks at 4.26, 4.25, 4.23, and 4.22 ppm.
- Peaks at 1.33, 1.32, and 1.31 ppm.

The spectrum also includes resonances at 6.5, 5.5, 4.5, 3.5, 2.5, 1.5, 0.5, and -0.5 ppm. The compound structure 2c includes a CO2Et group and a NO2 group.
The figure shows a chemical spectrum with peaks at various ppm values. The compound structure is labeled as 2k. The spectrum includes peaks at 8.26, 7.70, 7.66, 7.60, 7.58, 7.56, 7.52, 7.46, 7.42, 7.30, 7.26, 7.24, 7.22, 7.20, 7.19, 7.17, 7.16, 4.20, 4.18, 3.84, -3.19, and others. The labels CO2Et and NH are also present in the structure. The spectrum is labeled as CDCl3.
$\text{CO}_2\text{Et}$

2k

$\text{H}$

$\text{N}$

$\text{N}$

$\text{OH}$
$\text{CN}$

$\text{CO}_2\text{Et}$

$\text{NH}$

$\text{2m}$

$\text{f1 (ppm)}$

$\text{198.04}$ $\text{172.32}$

$\text{137.05}$ $\text{136.25}$ $\text{135.80}$ $\text{134.82}$ $\text{128.93}$ $\text{127.97}$ $\text{123.20}$ $\text{119.44}$ $\text{117.30}$ $\text{107.05}$

$\text{77.41 CDC13}$ $\text{77.16 CDC13}$ $\text{76.91 CDC13}$ $\text{-61.19}$ $\text{-31.27}$ $\text{-26.72}$ $\text{-14.30}$

$\text{163}$
Cf the chemical structure and the corresponding NMR spectrum.
The image contains a chemical structure labeled as 3e, along with a 1H NMR spectrum. The spectrum shows various peaks at different chemical shifts, with annotations for some resonances. The spectrum is labeled with ppm values on the x-axis and intensity values on the y-axis.
Chapter 2.

On-Demand Generation of Monochloramine

ABSTRACT

\[ \text{NaOCl + NH}_4\text{OH} \xrightarrow{\text{Reaction + Organic Extraction}} \text{NH}_2\text{Cl} \]

On-Demand

\[ 0.16 - 0.19 \text{ M} \]

in CH\(_2\text{Cl}_2\)

\[ \text{Simple, safe, fast, low-cost synthesis} \]

Despite its utility, monochloramine (NH\(_2\)Cl) has not achieved widespread use as a nitrogen transfer reagent due to its unstable and hazardous nature. We developed a continuous flow platform for the safe, reliable, and inexpensive on-demand synthesis of NH\(_2\)Cl. Additionally we demonstrate the synthetic utility of NH\(_2\)Cl by converting it to valuable NH aziridine and nitrile products in good to excellent yield in exceedingly short reaction times.

Dr. Evan Styduhar developed continuous flow synthesis of NH\(_2\)Cl. E.S. also developed the reaction of NH\(_2\)Cl to form aziridines and nitriles in batch and continuous flow. Laurel M. Heckman helped optimize the continuous flow setup, performed the reaction scope in continuous flow, and explored additional substrates in batch.
Introduction

The development of mild, low-cost, and effective nitrogen transfer reagents remains an active field of research in organic synthesis. The majority of these reagents are electrophilic nitrogen sources, which are available in a variety of forms (Figure 1). The compounds shown in Figure 1 have found utility in C–N and het–N bond-forming reactions, with many recent applications focusing on alkyl or heterocyclic hydrazine formation. Additionally, their successful application has been widely demonstrated as nitrenoid-transfer type agents in olefin azirdination and C-H insertion. Despite their beneficial applications, they possess a number of undesirable features including poor atom economy, prohibitively high cost, and unstable or explosive nature.

![Figure 1. Electrophilic amination reagents.](image)

Monochloramine (NH$_2$Cl) represents a promising but less well-studied reagent in nitrogen transfer reactions. Chloramine has enjoyed intermittent success in synthesizing alkylamine via reaction with organoborane and Grignard reagents. More recently, NH$_2$Cl has been used as a highly efficient and atom economical electrophilic N-heterocyclic amination reagent, providing the amino-heterocycles in excellent yields.

Chloramine has yet to reach its full potential as a synthetic reagent largely due to its instability. Solutions of chloramine are known to decompose at room temperature in less than
one hour. Additionally, NH<sub>2</sub>Cl is toxic in high concentrations and has been implicated as an intermediate in the synthesis of hydrazine.<sup>3</sup>

**Scheme 1.** Continuous flow synthesis of chloramine.

\[
\text{NaOCl} + \text{NH}_4\text{OH} \xrightarrow{\text{Reaction} + \text{Organic Extraction}} \text{NH}_2\text{Cl} \quad \text{(in CH}_2\text{Cl}_2) \quad \text{On-Demand}
\]

Continuous flow technologies have been shown to enable the safe use of toxic and unstable reagents.<sup>5</sup> Unlike traditional batch chemistry, continuous flow reactors are constructed from narrow diameter tubing resulting in improved heat transfer that allows precise control over reaction temperature and smaller quantities of dangerous reagents at any given time.<sup>6</sup> Additionally, reactions can be telescoped allowing the safe generation and use of toxic or unstable intermediates.<sup>7</sup> Herein we describe the safe, operationally simple on-demand synthesis of NH<sub>2</sub>Cl, enabling its exploration as a valuable reagent for both batch and continuous flow synthesis (Scheme 1).
**Generation of Monochloramine in Continuous Flow**

Monochloramine (NH$_2$Cl) was generated via combination of sodium hypochlorite and ammonium hydroxide with ammonium chloride at 0 °C in continuous flow (Scheme 2). A stream of dichloromethane (DCM) was then mixed with the aqueous stream and the streams were separated using a liquid-liquid separator. The organic stream was collected and titrated iodometrically to afford 0.15–0.18 M NH$_2$Cl in DCM. Maintaining the temperature at 0 °C for the generation and extraction of NH$_2$Cl was essential to maintaining high concentrations of NH$_2$Cl in DCM, in large part due to the increased solubility of gases in water at low temperatures. Generally, traditional batch procedures extract NH$_2$Cl into Et$_2$O, however for our setup, dichloromethane was chosen as the ideal organic solvent due to its better separation from water. This provides a relatively dry organic stream, thereby enabling more subsequent chemistry.

**Scheme 2.** On-demand generation of monochloramine in continuous flow.
Reaction Exploration of Monochloramine

Electrophilic amination reagents have recently been used to make hydrazine derivatives capable of nucleophilic addition to enones to form N-H aziridines. The Armstrong group has an extensive body of work demonstrating the synthesis of a wide variety of aziridines from enones. A representative example of their reaction conditions using O-(diphenylphosphinyloxy)hydroxylamine (DPPHA), N-methylmorpholine (NMM) and NaOH is illustrated in Scheme 3A. They propose that the electrophilic amine reagent DPPHA undergoes nucleophilic attack from NMM to form hydrazinium salt 5, which then undergoes deprotonation in the presence of NaOH to form hydrazinium ylide 6. Then, a Michael addition of ylide 6 to chalcone provides the desired unprotected aziridine. However, this and other previous examples use amination reagents with undesirable features including poor atom economy and high cost.

Scheme 3. (A) Previous aziridination of chalcone. (B) Proposed mechanism for aziridination.

Thus, we investigated whether our simple, low-cost, safe platform for the on-demand generation of NH₂Cl could also provide the desired aziridine in good yield. Using the conditions
from Scheme 3A but substituting NH₂Cl in DCM collected from our continuous flow setup for DPPHA, we were pleased to observe a similar yield of 88% of aziridine 3 in only 1.5 h (Scheme 4A). To further improve scalability and safety of this reaction, we developed conditions that could be utilized in a continuous flow setup. By switching to tributylamine (n-Bu₃N), a more nucleophilic and organic soluble amine, generation of the intermediate hydrazinium salt was accomplished homogenously in 30 minutes at room temperature. The addition of a more organic soluble base, namely tert-butoxide (KO−Bu) in tert-butanol (t-BuOH), provided aziridine 3 in 3 minutes in good yield.

**Scheme 4 (A)** Armstrong aziridination of chalcone with monochloramine. (B) Optimized aziridination of chalcone with monochloramine.

Next, we explored the reaction scope with commercially available enones. Modifying the electrophilic functional groups, we observed no desired aziridine 3 with carboxylic acids, esters, nitriles, and amides (Figure 2, 2a–f). Additionally, enones with enolizable protons were not competent, likely undergoing undesired base-catalyzed dimerization or oligomerization (Figure 2, 2g–i).
Interestingly, aldehyde 7 exclusively provided nitrile 8 in good 71% yield (Scheme 5). Although no aziridine products were detected, we were pleased to identify a different synthetically valuable product. Direct conversion of aldehydes to nitriles with mild, transition-metal free conditions and broad substrate scope remains an active area of research. In these reaction conditions, nitrile 8 likely forms via the competing 1,2-addition of ylide 9 to enal 7 followed by dehydration and deprotonation (Scheme 5).

Scheme 5. Synthesis of cinnamionitrile from cinnamaldehyde.

Finally, other electrophiles were explored. The reaction of ylide 9 with epoxides was attempted. Reactions with trans-stilbene oxide and styrenyl oxide only resulted in recovered starting material.
Continuous Flow Synthesis of Aziridines and Nitriles

The aziridination of chalcone was telescoped with NH₂Cl generation in continuous flow (Scheme 6). In order to decrease the reaction time of hydrazinium salt formation from 1 and tributylamine, reactor R₂ was heated to 80 °C, providing the desired hydrazinium intermediate in a 1-minute residence time. Next, chalcone 2 and t-BuOK were added and mixed for 2.0 minutes in reactor R₃, affording aziridine 3 in 74 % yield. We were pleased to observe good yields of 3 with exceedingly short reaction times, providing a throughput of 3 in 0.75 g/h.

**Scheme 6.** Continuous flow synthesis of aziridine 3.

We were able to rapidly synthesize a variety of nitriles from aldehydes using our multi-step continuous flow platform in excellent yield (Scheme 7, see Experimental Section for more details). We achieved quantitative conversion of 2-napthaldehyde to (Table 1, 8b). We observed good yields of benzaldehydes containing both electron-withdrawing and electron-donating functional groups (8c–e). We were pleased to observe that heterocycles, thiophene 8f and indole 8g, provided nitrile products in 80% and 92% yield respectively. Additionally, nitrile 8h was synthesized in good yield from the terpenoid citral. Aldehyde 7i containing a free NH was not
competent in the reaction (Figure 3). Aldehydes 7j and 7k containing an enolizable proton also resulted in no nitrile product (Figure 3).

Scheme 7. Continuous flow synthesis of various nitriles 8 from aldehydes 7.

![Scheme 7 diagram]

Table 1. Scope of aldehydes.\textsuperscript{[a]}

| Aldehyde | Nitrile | Yield (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>8b</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>8c</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>8d</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>8e</td>
<td>92\textsuperscript{[b]}</td>
<td></td>
</tr>
<tr>
<td>8f</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>8g</td>
<td>91\textsuperscript{[b]}</td>
<td></td>
</tr>
<tr>
<td>8h</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} Yields in parentheses are isolated yields of nitrile product (2.0 mmol) after column chromatography. \textsuperscript{[b]} Required a tube-in-tube mixer to prevent clogging (see Experimental Section for more details, Scheme 11).
Figure 3. Substrates that did not provide nitrile product.
Conclusion

A continuous flow platform for the safe, low-cost on-demand generation of monochloramine was developed. We further demonstrated the synthetic utility of NH₂Cl, via rapid synthesis of valuable aziridine and nitrile products in continuous flow. Continuous flow technologies uniquely enabled the easy use of NH₂Cl, thereby rendering chloramine an accessible, atom-economical nitrogen transfer reagent. We continue to explore new synthetic reactions of NH₂Cl. Additionally, this continuous flow setup could be utilized to provide on-demand generation and synthetic application of other hazardous gases. For example, the combination of inexpensive, bench-stable aqueous solutions of sodium bisulfite (NaHSO₃) and HCl produces SO₂ gas, which can then be extracted into an organic stream.
References


Chapter 2.

Experimental Section
Materials and Methods

Unless stated otherwise, reactions were conducted in flame-dried glassware under an atmosphere of argon using anhydrous solvents (either freshly distilled or passed through activated alumina columns). All commercially available reagents were used as received unless otherwise specified. Potassium tert-butoxide (1.0 M in tert-butanol) was obtained from Acros. Ammonium chloride was obtained from Macron Fine Chemicals. Sodium hypochlorite (obtained as 10–15% in H₂O, exact concentration determined by iodometric titration against a standardized thiosulfate solution), tributylamine, and trans-chalcone were obtained from Sigma Aldrich. Ammonium hydroxide (aqueous, 5.0 N) was obtained from VWR. Unless stated otherwise, reactions were performed at room temperature (RT, approximately 23 °C). Thin-layer chromatography (TLC) was conducted on 0.2 mm coated Science silica gel (EM 60-F254) plates and visualized using a combination of UV, anisaldehyde, iodine, and potassium permanganate staining. Column chromatography was carried out on a Biotage Isolera flash chromatography system using SNAP Ultra-Sil columns (silica gel, average particle size 50 µm, 25 µm, and 25 µm spherical, respectively).

For continuous flow experiments, reactions were conducted using commercially available solvents without any further processing or purification and prepared without exclusion of water or air. backpressure regulators (BPRs) and membrane liquid-liquid separators were purchased from Zaiput Flow Technologies. PTFE Microfiltration membranes were purchased from Pall Zefluor with 0.5 µm pore size. Reactors were constructed from high-purity perfluoroalkoxy (HPFA) tubing (1/16” OD × 0.03” ID or 1/8” OD × 0.063” ID) and PEEK superflangeless fittings purchased from IDEX Health & Science Technologies. A Vapourtec E-Series Integrated Flow Chemistry System pumped reagents and solutions from reservoirs, where Harvard
Apparatus PhD Ultra syringe pumps were used to pump reagents and solutions via glass syringes (5, 10, and 25 mL) purchased from SGE Analytical Science. Cooling and heating was accomplished by submerging tubing in an ice/water or silicone oil bath, respectively.

$^1$H NMR spectra were recorded on Bruker spectrometers (400, 600 MHz). Data for $^1$H spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), integration and are referenced to the residual solvent peak 7.26 ppm for CDCl$_3$. 1,3,5-Trimethoxybenzene was used as an internal standard for quantification. $^{13}$C NMR spectra are reported in terms of chemical shift (at 100 or 150 MHz) and are referenced to the residual solvent peak 77.16 ppm for CDCl$_3$. 
Procedure for NH$_2$Cl Generation in Continuous Flow

Scheme 8. Detailed setup for the on-demand generation of monochloramine in continuous flow.

**Stock Solution Preparation and Reactor Setup:** All solutions were prepared in a chemical fume hood. Stock solution A was obtained directly from a bottle of 10–15% aqueous sodium hypochlorite, by which the exact concentration was determined by iodometric titration against a standardized aqueous sodium thiosulfate solution. Based on the concentration of sodium hypochlorite, Stock solution B was prepared by addition of ammonium hydroxide (1.4 equiv relative to NaOCl) and ammonium chloride (1.2 equiv relative to NaOCl) in a dry 250 mL volumetric flask followed by dilution with DI H$_2$O.

**Chloramine Generation Procedure:** Stock solutions A and B were separately loaded into 100 mL glass jar reservoirs. Dichloromethane C was loaded into a 25 mL SGE glass syringe. Stock solutions A and B were pumped from a Vapourtec E-Series Integrated Flow Chemistry System at a rate of 167 µL/min each and combined for a residence time of 1.66 min in R1 at 0 °C. Dichloromethane (C) was pumped from a Harvard Apparatus PhD Ultra syringe pump at a rate of 500 µL/min to combine with the outlet of R1 at 0 °C before entering a membrane-based liquid-liquid separator. The resulting organic solution of chloramine in CH$_2$Cl$_2$ was collected.
The concentration of chloramine was then obtained by iodometric titration against a standardized 0.2 M aqueous sodium thiosulfate solution, typically giving concentrations of 0.16–0.18 M.
Procedure for Aziridine and Nitrile Synthesis

Aziridine 3. To a solution of chloramine (0.19 M in CH₂Cl₂, 1 mL, 0.19 mmol, 1.1 equiv) was added tri-n-butylamine (42 µL, 0.19 mmol, 1.1 equiv) and allowed to stir at 23 ºC. After 30 min, trans-chalcone (0.032 g, 0.171 mmol, 1.0 equiv) was added, followed by dropwise addition of potassium t-butoxide (1.0 M in t-butanol, 285 µL, 0.285 mmol, 2.0 equiv) and allowed to stir at 23 ºC. After 3 minutes, the reaction was then quenched with a solution of saturated aqueous NH₄Cl (2 mL). The resulting biphasic mixture was then transferred to a separatory funnel with CH₂Cl₂ (1 mL) and extracted with CH₂Cl₂ (1 x 2 mL), followed by extraction with EtOAc (2 x 2 mL). The organic layers were combined, dried over MgSO₄, and evaporated under reduced pressure, and the yield of 89% was determined by ¹H NMR analysis with 1,3,5-trimethoxybenzene as an external standard.

Nitrile 8. To a solution of chloramine (0.18 M in CH₂Cl₂, 2 mL, 0.36 mmol, 1.1 equiv) was added tri-n-butylamine (85 µL, 0.36 mmol, 1.1 equiv) and allowed to stir at 23 ºC. After 30 min, cinmmaldehyde (0.032 g, 0.171 mmol, 1.0 equiv) was added, followed by dropwise addition of
KOt-Bu (1.0 M in t-BuOH, 285 μL, 0.285 mmol, 1.5 equiv) and allowed to stir at 23 °C. After 3 minutes, the reaction was then quenched with a solution of saturated aqueous NH₄Cl (4 mL). The resulting biphasic mixture was then transferred to a separatory funnel with CH₂Cl₂ (2 mL) and extracted with CH₂Cl₂ (1 × 4 mL), followed by extraction with EtOAc (2 × 4 mL). The organic layers were combined, dried over MgSO₄, and evaporated under reduced pressure, and the yield was determined by ¹H NMR analysis with 1,3,5-trimethoxybenzene as an external standard.
Continuous Flow Synthesis of Aziridine and Nitrile Products

Procedure for the Continuous Flow Synthesis of Aziridine 3

Stock Solution Preparation and Reactor Setup: All solutions were prepared in a chemical fume hood. Stock solution A was obtained directly from a bottle of 10–15% aqueous sodium hypochlorite, by which the exact concentration of 1.69 M was determined by iodometric titration against a standardized aqueous sodium thiosulfate solution. Based on the concentration of sodium hypochlorite (1.69 M), stock solution B was prepared by addition of ammonium hydroxide (1.4 equiv wrt NaOCl) and ammonium chloride (1.2 equiv wrt NaOCl) in a dry 250 mL volumetric flask followed by dilution with DI H₂O. Stock solution F was prepared by adding chalcone (6.67 mmol) to a 10- mL volumetric flask followed by dilution with DCM. Stock solutions C, E, and G were obtained directly from the reagent bottles.

Procedure: Stock solutions A and B were separately loaded into 100 mL glass jar reservoirs. Dichloromethane C was loaded into a 25 mL SGE glass syringe. Stock solutions A and B were pumped from a Vapourtec E-Series Integrated Flow Chemistry System at a rate of 167 μL/min each and combined for a residence time of 1.66 min in R1 at 0 ºC. Dichloromethane (C) was pumped from a Harvard Apparatus PhD Ultra syringe pump at a rate of 0.5 mL/min to combine with the outlet of R1 at 0 ºC before entering a membrane-based liquid-liquid separator and the resulting solution of chloramine in CH₂Cl₂ was collected. The system equilibrated for 15 min prior to collecting into a surge a 25 mL flask. After collecting for an additional 5 min, the concentration of chloramine was then obtained by iodometric titration against a standardized 0.2 M aqueous sodium thiosulfate solution, giving a concentration of 0.17 M. The organic solution D containing NH₂Cl (0.5 mL/min, 0.17 M, 1.1 equiv) was then pumped through the system. Flow
rates of solutions E (1.1 equiv, 0.1, 0.02 mL/min), F (0.67 M, 1.0 equiv, 0.11 mL/min), and G (1.0 M, 1.5 equiv, 0.11 mL/min) were calculated by the concentration of chloramine D (1.1 equiv). Using the flow rate and concentration of stream F, a collection time was calculated that would correspond to 2 mmol of starting chalcone pumped through the system (26 min 15s). The product stream flowed into an aqueous saturated ammonium chloride solution (40 mL). When collection was complete, aqueous saturated NaCl (20 mL) and DCM (20 mL) were added. The organic layer was extracted. Next, the aqueous was extracted with EtOAc (2 x 30 mL). The crude residue was purified via flash column chromatography on silica gel (0-15% ethyl acetate in hexanes) to afford the desired azirdine.
Scheme 9. Continuous flow synthesis of aziridine 3 from chalcone and monochloramine.
Procedure for the Continuous Flow Synthesis of Nitriles

Stock Solution Preparation and Reactor Setup: All solutions were prepared in a chemical fume hood. Stock solution A was obtained directly from a bottle of 10–15% aqueous sodium hypochlorite, by which the exact concentration was determined by iodometric titration against a standardized aqueous sodium thiosulfate solution. Based on the concentration of sodium hypochlorite, Stock solution B was prepared by dissolving aldehyde (5 mmol) was added to a 1-mL volumetric flask followed by dilution with DCM. Stock solution D, E, and G were obtained directly from the reagent bottles.

Procedure: Stock solutions A and B were separately loaded into 100 mL glass jar reservoirs. Dichloromethane (C) was loaded into a 25 mL SGE glass syringe. Stock solutions A and B were pumped from a Vapourtec E-Series Integrated Flow Chemistry System at a rate of 167 µL/min each and combined for a residence time of 1.66 min in R1 at 0 °C. Dichloromethane (C) was pumped from a Harvard Apparatus PhD Ultra syringe pump at a rate of 500 µL/min to combine with the outlet of R1 at 0 °C before entering a membrane-based liquid-liquid separator and the resulting solution of chloramine in CH₂Cl₂ was collected. The concentration of chloramine was then obtained by iodometric titration against a standardized 0.2 M aqueous sodium thiosulfate solution, typically giving concentrations of 0.15–0.18 M. Flow rates of solutions E (1.1 equiv), F (0.5 M, 1.0 equiv), and G (1.0 M, 2.0 equiv) were calculated by the concentration of chloramine (1.1 equiv). Using the flow rate and concentration of stream F, a collection time was calculated that would correspond to 2 mmol of starting aldehyde pumped through the system. The product stream flowed into an aqueous saturated ammonium chloride solution (40 mL). When collection was complete, aqueous saturated NaCl (20 mL) and DCM (20 mL) were added. The organic
layer was extracted. Next, the aqueous was extracted with EtOAc (2 x 30 mL). The crude residue was purified via flash column chromatography on silica gel (0-15% ethyl acetate in hexanes) to afford the desired nitrile.

For 4-nitrobenzaldehyde and 1-methylindole-2-carboxyaldehyde, clogging was observed upon addition of KOT-Bu at the Y-mixer of R3. Thus, it was replaced with a tube-in-tube reactor and no further clogging was observed.
Scheme 10. Continuous flow synthesis of nitrile 8 from chalcone and monochloramine.
Scheme 11. Tube-in-tube mixer for aldehydes 7e and 7g prevented clogging. The inner diameter of the outer tube was 0.093" and the inner diameter of the inner tube was 0.03".
Characterization of Aziridine and Nitrile Products

2-Benzoyl-3-Phenylaziridinium (3)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of aziridine and isolated as white solid (332 mg, 74% yield).

\(^1\)H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.03 – 7.97 (m, 2H), 7.64 – 7.58 (m, 1H), 7.52 – 7.45 (m, 2H), 7.42 – 7.29 (m, 5H), 3.52 (dd, \(J = 8.0, 2.1\) Hz, 1H), 3.19 (dd, \(J = 9.3, 2.0\) Hz, 1H), 2.73 – 2.64 (m, 1H) ppm

\(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 195.8, 138.5, 136.0, 133.9, 128.9, 128.6, 128.4, 128.0, 126.3, 44.2, 43.6 ppm

The \(^1\)H and \(^{13}\)C NMR spectra are in agreement with those reported in the literature.\(^1\)

Cinnamonic acid (8a)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles and isolated as a colorless oil (243 mg, 94% yield).

\(^1\)H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.51 – 7.32 (m, 6H), 5.87 (d, \(J = 16.6\) Hz, 1H) ppm

\(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 150.6, 133.5, 131.3, 129.1, 127.4, 118.2, 96.4 ppm

The \(^1\)H and \(^{13}\)C NMR spectra are in agreement with those reported in the literature.\(^2\)
Napthalene-2-carbonitrile (8b)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles and isolated as a white solid (303 mg, 99% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (s, 1H), 7.94 – 7.86 (m, 3H), 7.70 – 7.55 (m, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 134.6, 134.1, 132.2, 129.2, 129.1, 128.4, 128.1, 127.7, 126.3, 119.3, 109.3 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^3$

4-Methoxybenzonitrile (8c)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles and isolated as a white solid (239 mg, 90% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.61 – 7.53 (m, 2H), 6.96 – 6.90 (m, 2H), 3.84 (s, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.9, 134.0, 119.3, 114.8, 104.0, 55.6 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^3$
2,6-Dimethylbenzonitrile (8d)

The titled compound was prepared via the previously described procedure for the continuous
flow synthesis of nitriles and isolated as white solid (185 mg, 71% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34 (t, $J=7.7$ Hz, 1H), 7.11 (d, $J=7.7$ Hz, 2H), 2.52 (s, 6H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 142.2, 132.2, 127.4, 117.4, 113.4, 20.9 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^4$

4-nitrobenzonitrile (8e)

The titled compound was prepared via the previously described procedure for the continuous
flow synthesis of nitriles, required a tube-in-tube mixer, and isolated as white solid (272 mgs, 92%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.35 (d, $J=8.8$ Hz, 1H), 7.89 (d, $J=8.8$ Hz, 1H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 150.1, 133.6, 124.4, 118.4, 116.9 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^2$
Benzo[b]thiophene-2-carbonitrile (8f)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles and isolated as a colorless oil (256 mg, 80% yield).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.96 – 7.82 (m, 3H), 7.57 – 7.45 (m, 2H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 141.1, 137.3, 134.9, 127.8, 125.6, 125.2, 122.2, 114.4, 109.4 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^5$

1-Methylindeole-2-carbonitrile (8g)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles, required a tube-in-tube mixer, and isolated as a white solid (284 mg, 91%)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.67 (d, $J$ = 8.1 Hz, 1H), 7.42 (t, $J$ = 7.7 Hz, 1H), 7.33 (d, $J$ = 8.4 Hz, 1H), 7.23 (t, $J$ = 7.5 Hz, 1H), 7.13 (s, 1H), 3.85 (s, 3H) ppm

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 137.9, 126.1, 125.8, 122.3, 121.4, 113.6, 112.6, 110.2, 110.1, 31.4 ppm

The $^1$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^3$
Geranonitrile (8h)

The titled compound was prepared via the previously described procedure for the continuous flow synthesis of nitriles and isolated as colorless oil (217 mg, 73%, $E/Z = 1.2:1$).

$^{1}$H NMR ($400$ MHz, CDCl$_{3}$) $\delta$ 5.08 (s, 3.2H), 5.00 (t, $J = 5.8$ Hz, 1.2H), 2.40 (t, $J = 7.6$ Hz, 2H), 2.23 – 2.09 (m, 6.8H), 2.02 (s, 3.6H), 1.88 (s, 3H), 1.66 (s, 6.6H), 1.59 (d, $J = 7.5$ Hz, 6.6H) ppm

$^{13}$C NMR ($100$ MHz, CDCl$_{3}$) $\delta$ 165.1, 165.0, 133.3, 133.2, 122.3, 122.2, 117.3, 117.0, 95.9, 95.3, 38.6, 36.3, 26.2, 25.68, 25.67, 25.65, 22.9, 21.0, 17.7, 17.9 ppm

The $^{1}$H and $^{13}$C NMR spectra are in agreement with those reported in the literature.$^{6}$
References


Chapter 2.

$^1$H and $^{13}$C Spectra
\[ \text{\(8a\)} \]
Chapter 3.

Simple Two-Step Synthesis of
6-Carboxytetramethylrhodamine

ABSTRACT

A rapid, operationally simple synthesis of 6-TAMRA, an important probe for labeling biomolecules, from 2-carboxycarbonylterephthalic acid and 3-dimethylaminophenol is described herein. The intermediate ketoacid was synthesized in a single step from commercially available dimethylacetophenone. Additionally, progress was made towards a facile, scalable synthesis in continuous flow.

Dr. Justin A. M. Lummiss carried out the oxidation batch synthesis. Laurel M. Heckman carried out reaction screening and optimization of step 2 of the batch synthesis. L.M.H and J.A.M.L contributed equally to the experiments in continuous flow. Dale Thomas (graduate student, Jensen Research Group, MIT Department of Chemical Engineering) developed the fully automated platform. Bruce Adams (Staff, DCIF of MIT Department of Chemistry) helped with low temperature and 2-D NMR experiments. Peter Müller (Director, Diffraction Facility of MIT Department of Chemistry) carried out the single-crystal X-ray diffraction experiments.
Introduction

Functionalized rhodamine dyes are fluorescent molecules commonly used as tools for measuring biologically relevant small molecules and analytes due to their high sensitivity and ability to provide a rapid, bioorthogonal response. Additionally, these probes are highly tunable to experimental need through variation of alkyl substitution on N of their xanthene core (Scheme 1). This subset of the rhodamine dye family contains a functional group, commonly a carboxylic acid, on the pendant benzene ring to provide a handle for late stage attachment of a biomolecule of choice (Scheme 1a). There are two numbering schemes for substitution on the pendant benzene ring of rhodamine dyes, one for the closed form of rhodamine and one for the open form of rhodamine (Scheme 1a). Most reports and commercial vendors use the closed form numbering system, which for consistency, will be utilized herein. As to not interfere with the open-closed equilibrium, substitution on the pendant benzene ring is generally only available in the 5 or 6 position. This seemingly minor structural addition breaks the symmetry of the molecule, greatly increasing the synthetic challenge to access a single structural isomer, which is required for many biological applications since the two isomers display different biological and photo-physical properties.

The most efficient and cost-effective synthesis of an entire family of N-alkylated rhodamine dyes is a single step Friedel-Crafts type addition of two equiv of N-alkylaminophenol 2 to phthalic anhydride. However, applying this synthesis to access rhodamine dyes capable of bioconjugation provides a mixture of two constitutional isomers, namely the 5- and 6-carboxy-rhodamine (3a and 3b respectively) because addition of phenol 2 can occur at either carbonyl of anhydride 1 (Scheme 2). Furthermore, this reaction uses harsh conditions, often providing the
rhodamine products in low yields and more importantly, separating these two isomers remains challenging and costly.\textsuperscript{4}

**Scheme 1.** (A) General structure of rhodamine dyes containing a carboxylic acid for bioconjugation. (B) Family of commercially available rhodamine dyes.

(A) 
\[
\begin{align*}
\text{Closed Form} & \quad \text{Open Form} \\
\end{align*}
\]

(B) 
Rosamine 5(6)-Carboxytetramethylrhodamine 5(6)-Carboxyrhodamine B
5(6)-Carboxy-X-Rhodamine 5(6)-Carboxyrhodamine 6G 5(6)-Carboxyrhodamine 123

Recently, attention has been focused on developing new synthetic routes to selectively access a single isomer.\textsuperscript{5} The Lavis group demonstrated an elegant synthesis of 5-carboxyrhodamine 3 from the single isomer of fluorescein 4 via C-N cross coupling of a variety of amine partners, including previously difficult to access rhodamines with amide substitution
Although isomerically pure fluorescein is less expensive than their rhodamine counterparts, these are still significantly more expensive than simple starting materials. Furthermore, rhodamines containing cyclic amines like rhodamine B cannot be synthesized through this route.

**Scheme 2.** General synthesis of 5- and 6-carboxyrhodamine mixture from anhydride 1.

Another selective approach involves differentiating one of the carbonyls of anhydride 1 into an aldehyde to provide functionalized rhodamines regioselectively under rosamine synthesis conditions. However, these approaches often provide rhodamine in moderate yields and require lengthy multi-step procedures to access the phthalaldehydic acid. More recently, Levin et al. developed improved conditions that provided 71–87% of a single isomer of rhodamine from phthalaldehydic acid 5a or 5b (Scheme 4a). This reaction features two Friedel Crafts reactions of phenol 2, followed by oxidation with O₂ in a single pot, albeit requiring long reaction times and an O₂-enriched atmosphere (Scheme 4b). Furthermore, phthalaldehydic acid 5a/b were
synthesized in a single step from the corresponding dihalobenzaldehyde via Pd-catalyzed
dydroxycarbonylation. However, when synthesizing 5a/b on large scale, a multistep procedure
was utilized. Additionally, the two-step procedure featured long reaction times.

Reactions developed in continuous flow technologies are more easily scale without
reoptimization compared to traditional batch chemistry. In batch chemistry, increasing the scale
of the reaction often requires increasing the size of the batch reactor. For reactions with
dangerous reagents (corrosive, oxidizing, reactive, etc.), increasing reaction scale is accompanied
with increasing safety concerns due to the large quantities of reagent present. Heating of these
reagents often further increases the danger associated with a given reaction. Continuous flow
technologies have provided an easier, safer means to scale reactions in a standard research
laboratory setting. Other advantages of continuous flow include improved heat and mass
transfer, reproducibility, and sustainability, in addition to the ability to develop full process
automation.

We envisioned using a similar strategy to Levin et al. involving differentiating one of the
carbonyl functional groups of anhydride 1 with a carboxyl functional group instead of a
hydrogen. This would generate rhodamine product rapidly and selectively because it would
avoid the need for the additional sluggish oxidation. The 6-carboxytetramethylrhodamine 13 can
be accessed from tri-ketoacid 11 and 3-dimethylaminophenol 12 via decarboxylation and
Friedel-Crafts acylation (Scheme 5). Ketoacid 11 was an excellent intermediate because it can be
synthesized from commercially available 2,5-dimethylacetophenone via KMnO₄ oxidation.
Scheme 4. (A) Levin synthesis of 5- and 6-X-rhodamine from phthalaldehydic acid 6a/b and aminophenol 2. (B) Levin proposed mechanism of double electrophilic aromatic substitution and O_2 oxidation.

Herein we demonstrate a simple two-step selective synthesis of 6-carboxytetramethylrhodamine (6-TAMRA) via oxidation of 2,5-dimethylacetophenone 10.
followed by condensation with 3-dimethylaminophenol 12, which could easily provide access to an entire family of rhodamine dyes via modulation of the phenol coupling partner (Scheme 5). Also, we describe our progress towards developing the first two-step, selective synthesis of a rhodamine dye in continuous flow for improved safety and scalability.$^{10}$

**Scheme 5.** Two-step selective retrosynthesis of 6-carboxytetramethylrhodamine
Batch Preparation of 6-TAMRA

Ketoacid 11 was synthesized via the only known method, namely a KMnO₄ oxidation from inexpensive 2,5-dimethylacetophenone 10 in good 74% yield (Scheme 6). A facile recrystallization procedure after workup was developed and a crystal structure was obtained (Scheme 6). The desired material was isolated in gram quantities, enabling the exploration of the Friedel Crafts reaction of ketoacid 11 and 3-dimethylaminophenol 12.

**Scheme 6.** Simple, rapid oxidation of dimethylacetophenone 10.

We began exploring Lewis acids and Brønsted Acids commonly utilized in Friedel-Crafts reactions. However heating with AlCl₃, ZnCl₂, or polyphosphoric acid, provided no rhodamine product. We achieved initial success adding sodium bisulfite, a reagent commonly used in combination with strong acid to reduce ketoacid of 11 to an aldehyde, in a mixture of hexafluoroisopropanol (HFIP) and water (H₂O) heated to 175 °C for 30 minutes in the microwave. HFIP is likely an excellent solvent for this transformation due to its inherent properties (e.g. its ability to dissolve very polar compounds, ionizing power, and hydrogen bond donating ability). These conditions provided 13, 6-carboxytetramethylrhodamine (6-TAMRA), selectively in a moderate 41% yield (Table 1, Entry 1).

We next explored the addition of a large excess of phenol 12, and observed that 10 equiv provided an increased yield of 59% of 6-TAMRA (Table 1, Entry 2). Alternatively, increasing the equiv of NaHSO₃ had the opposite effect, providing 13 in a significantly decreased yield of
9% (Table 1, Entry 3). Surprisingly, 6.2 equiv of phenol 12 provided a similar yield of 42% of 6-TAMRA as only 2.5 equiv of phenol 12 (Table 1, Entry 3 vs. Table 1, Entry 1). Due to the high cost of HFIP, we explored conditions that would minimize the volume of HFIP required while still maintaining high yields of 13. Increasing reaction concentration, we observed a significantly decreased yield. When the concentration of ketoacid 11 with 6.2 equiv of phenol 12 was increased from 0.05 M to 0.15 M, a low yield of 23% was observed (Table 1, Entry 5 vs. Table 1, Entry 4). Although increasing concentration caused a decrease in yield, we were pleased to observe that decreasing the ratio of HFIP/H₂O provided a comparable yield of 6-TAMRA, thereby providing desired product at lower cost (Table 1, Entry 6 vs. Table 1, Entry 4).

Table 1. Initial investigation into the synthesis of 6-TAMRA via a Friedel-Crafts reaction of ketoacid 11 and phenol 12.[a]

<table>
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<tr>
<th>Entry</th>
<th>Conc. 11 (M)</th>
<th>HFIP/H₂O</th>
<th>NaHSO₃ (Equiv)</th>
<th>12 (Equiv)</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Yield [%]</th>
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</thead>
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<td>1</td>
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<td>2.3:1</td>
<td>2.5</td>
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<td>30</td>
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<tr>
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<td>2:1</td>
<td>2.3</td>
<td>6.1</td>
<td>170</td>
<td>35</td>
<td>23%</td>
</tr>
<tr>
<td>6</td>
<td>0.15</td>
<td>1:1</td>
<td>2.3</td>
<td>6.1</td>
<td>170</td>
<td>35</td>
<td>19%</td>
</tr>
</tbody>
</table>

[a] Reaction conditions: ketoacid 11 (1.0 equiv), 3-dimethylaminophenol 12, and NaHSO₃ were stirred in HFIP/H₂O (2 mL) under air in the microwave. [b] Yields were determined via HPLC relative to trimethoxybenzene internal standard.
Efficient heating was paramount to achieve good yields of rhodamine 13. The same reaction conditions heated in an oil bath consistently provided lower yields of rhodamine 13 compared to heating in the microwave (Table 2). While many theories exist regarding the differences between reactions run in a microwave vs. an oil bath, the benefits achieved in the microwave are likely due to flash heating, rapid heat exchange, and excellent process controls. All of these parameters can be easier to control in continuous flow than in traditional batch chemistry.

**Table 2.** Effect of heating in a microwave or an oil bath.

<table>
<thead>
<tr>
<th>Entry</th>
<th>MW or Oil Bath</th>
<th>Additive</th>
<th>Yield[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MW</td>
<td>-</td>
<td>30%</td>
</tr>
<tr>
<td>2</td>
<td>MW</td>
<td>HCl</td>
<td>54%</td>
</tr>
<tr>
<td>3</td>
<td>Oil Bath</td>
<td>-</td>
<td>4%</td>
</tr>
<tr>
<td>4</td>
<td>Oil Bath</td>
<td>HCl</td>
<td>32%</td>
</tr>
</tbody>
</table>

[^a]: Reaction conditions: ketoacid 11 (1 mmol, 1.0 equiv), 3-dimethylaminophenol 12 (10 equiv), and NaHSO₃ (2.2 equiv) were heated to 150 °C in HFIP/H₂O (2 mL) under air for 40 min. [^b]: Yields were determined via HPLC relative to trimethoxybenzene internal standard.

While optimization of our reaction conditions were underway, we scaled our initial reaction conditions (Table 1, entry 1) to a 20 mL reaction to obtain 6-TAMRA for characterization. When our initial reaction conditions scaled up to a 20 mL reaction in the microwave, 6-TAMRA was isolated in a low 17% yield. Although increasing the scale of the
reaction saw a significant decrease in yield, we were able to isolate and characterize 6-TAMRA, validating the selectivity of the reaction.

**Table 3. Friedel Crafts reaction investigation.**[^a]

![Chemical structures and reactions](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>NaHSO₃ (Equiv)</th>
<th>Additive</th>
<th>Yield[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2</td>
<td>10</td>
<td>30%</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>10 HCl</td>
<td>54%</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>5 HCl</td>
<td>14%</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>2.5 HCl</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>10 HCl</td>
<td>50%</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>10 HCl</td>
<td>44%[^c]</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>10 HCl</td>
<td>36%[^c]</td>
</tr>
</tbody>
</table>

[^a]: Reaction conditions: ketoacid 11 (1 mmol, 1.0 equiv), 3-dimethylaminophenol 12, and NaHSO₃ were heated to 150 °C in HFIP/H₂O (2 mL) under air for 40 min in the microwave. [b]: Yields were determined via HPLC relative to trimethoxybenzene internal standard. [c]: 5-carboxy-TAMRA detected.

Given the difficulty of scaling this reaction in the microwave and the need for efficient heating, we were interested in developing conditions that would be compatible in both a batch and continuous flow setup. The high pressure and temperatures achieved in the microwave were not compatible with a PFA tubing reactor, which burst. However, decreasing reaction temperature from 170 °C, which provided 59% yield of 13, (Table 1, Entry 2) to 150 °C, provided rhodamine 13 in a low yield of 30% (Table 3, Entry 1). Interestingly, the addition of HCl restored reactivity, providing 13 in a 54% yield (Table 3, Entry 2). This result was
especially serendipitous because ketoacid 11 has limited solubility in a variety of solvents. Ketoacid 11 was uniquely soluble above 0.1 M in 1 M HCl and wet ethyl acetate (EtOAc). These reaction conditions simplify its development in continuous flow since each component (11, 12, and NaHSO₃) can be prepared as separate homogeneous solutions.

Upon identification of reaction conditions compatible with both batch and continuous flow setups, we further investigated the effect of varying the equiv of both NaHSO₃ and phenol 12 on reaction yield. Similar to our previous observations, a large excess of phenol 12 was required to achieve good yield of 13 (Table 3, Entries 2–4). Next, we investigated decreasing equiv of NaHSO₃. Halving the relative amount of NaHSO₃ provided rhodamine 13 in similar yields (Table 3, Entry 2 vs. Entry 5). A decrease in yield is observed for 0.5 equiv and no NaHSO₃ added, 44% and 36% yield of 13 respectively (Table 3, Entries 6 and 7). Although 13 can be synthesized in the absence of NaHSO₃, the use of less NaHSO₃ resulted in a mixture of 5- and 6-TAMRA. Thus, the inclusion of NaHSO₃ is paramount to the desired selectivity of this reaction.

**Scheme 7.** Two-step selective synthesis of 6-TAMRA.

Our final two-step synthesis features the KMnO₄ oxidation of dimethylacetophenone 10. After 90 min, a simple crystallization provides pure ketoacid 11 in good yield. Then, ketoacid 11
is reacted with phenol 12 to provide 6-TAMRA selectively in 54% yield via HPLC and 28% isolated yield (Scheme 7). The lower isolated yield was due to the difficulty in separating an unknown side product, formed from excess aminophenol, and 13 with column chromatography. We are currently developing conditions for the crystallization of 6-TAMRA to improve the isolated yield.
**KMnO₄ Oxidation in Continuous Flow**

Given the improved safety and scalability of continuous flow technologies, we endeavored to develop our two-step synthesis in a continuous flow platform. The first step of our synthesis, an oxidation with KMnO₄, though fairly simple in batch, poses difficulty when developed in a continuous flow setup due to its heterogeneity. In batch, due to the high concentrations of reagents, not only is there solid KMnO₄ at the start of the reaction, as the oxidation proceeds, MnO₂ precipitates out of solution. To simplify the continuous flow setup, a more dilute, homogeneous solution of KMnO₄ (0.5 M) with K₂CO₃ was prepared. This aqueous stream of KMnO₄ was reacted with neat dimethylacetophenone in a PFA tubing reactor (R₁) at 78 °C, providing ketoacid 11 in 24% yield (Scheme 8, see Experimental for details). The reaction was sonicated in large diameter tubing to prevent clogging of the MnO₂ precipitating out of solution. Next, an in-line purification system was developed. A reverse tube-in-tube reactor allowed a mixture of citric acid and EtOAc to be pumped into the output of the R₁ (Scheme 8). Citric acid then rapidly dissolves the MnO₂ solid. The subsequent homogeneous organic/aqueous mixture leaves R₁ and is separated using a liquid-liquid membrane separator. This first separation removes unreacted dimethylacetophenone into the organic layer, while ketoacid 11 remains in the aqueous stream. The aqueous stream is further acidified with 1 M HCl and extracted with EtOAc, providing a 7 mM solution of 11 in EtOAc and 2% overall yield (Scheme 8).

This loss of material during the inline purification was due in part to the significant amount of CO₂ produced when the reaction solution containing potassium carbonate was acidified with citric acid (see Experimental Section for more details, Figure 4). The addition of an open surge tank allowed the CO₂ gas to escape and improved the second separation by
increasing the interaction of the organic/aqueous mixture with the membrane of the liquid/liquid separator (Scheme 9, see Experimental for details). This modest adjustment to the setup provided a 3-fold increase in yield of ketoacid 11 (9%). Additionally, we explored the effect of flow rate of EtOAc of the second separation on yield of 11. Unfortunately, increasing the flow rate of EtOAc, resulted in decreasing concentrations of ketoacid (Table 4).

Table 4. Investigation of the second separation of the synthesis and purification of ketoacid 11 in continuous flow.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Flow Rate (uL/min)</th>
<th>Conc. X (mM)</th>
<th>Yield 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>20</td>
<td>9%</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>11</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>1200</td>
<td>2</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>4</td>
<td>2500</td>
<td>2</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

[a] Yields were determined by NMR relative to trimethoxybenzene internal standard.

We are currently investigating replacing the sonicator with a continuous oscillatory baffled reactor, which is adept at handling solids without clogging. Although pumping dimethylacetophene neat provided a low E-factor, yields of 11 were too low and complicated with inseparable side products of varying oxidation states. One of the major contributing factors to low yield of 11 was poor mixing of dimethylacetophenone and aqueous KMnO₄ due to their
significantly differing flow rates (see Experimental Section for more details, Figure 5). Thus, dilution of dimethylacetophenone with benzene in addition to an increasing flow rate is currently being investigated with the improved setup.
Scheme 8. Continuous flow KMnO₄ oxidation of dimethylacetophenone.

Reverse Tube-In-Tube

Citric Acid Dissolves MnO₂ Solids

Mixing of Homogeneous Solutions

Citric Acid
1 M
500 µL/min

EtOAc
500 µL/min

4 M HCl
EtOAc
300 µL/min

EtOAc
300 µL/min

Organic mixing coil

Sonication, T = 78 °C

H₂O
800 µL/min

KMnO₄ (6 equiv)

K₂CO₃ (0.7 equiv)

Me

Me

10
(1 equiv)
10 µL/min

R₁, V = 64 mL

Waste
V = 1.0 mL

Aqueous waste

7 mM in EtOAc

Yield Before Separations: 24%

Yield After Separations: 3%
Continuous Flow Synthesis of 6-TAMRA

The reaction between ketoacid 11 and phenol 12 requires HFIP (a highly corrosive solvent) at high temperatures and pressures. Excellent heat transfer was important to good yields and poor scalability of 6-TAMRA in the microwave was observed. For these reasons and the benefits of continuous flow over traditional batch chemistry, we developed the Friedel-Crafts reaction of 12 and 11 in a continuous flow setup.

Early on in our investigation, before the discovery of the solubility of ketoacid 11 in 1 M HCl, we tested 11 in wet EtOAc in continuous flow with similar conditions to our initial hit (Table 1, Entry 1). Even at decreased temperature, we were pleased to observe 6-TAMRA 13 selectively, albeit in low 6% yield (Scheme 10). After our optimization in batch, we attempted the synthesis of 13 with ketoacid 11 dissolved in 1 M HCl and with increased equiv of phenol 12 (Table 6, Entry 1). Although an increased yield of 18% was observed, this was significantly lower than the yields we achieved in batch.

Scheme 10. Initial continuous flow synthesis of 6-TAMRA.[a]

[a] Yields were determined via HPLC relative to trimethoxybenzene internal standard.
Mixing in continuous flow can be modulated in a variety of ways, having a large effect on reaction efficiency. One way to improve mixing is decreasing the inner diameter (i.d.) of the PFA tubing reactor. Using a reactor with a smaller i.d. (0.03") resulted in an increased yield of 13 (Table 6, Entry 2 vs. Entry 1). Solutions pumped through coiled reactors with high flow rates and short residence times induce radial directionality due to centrifugal forces. In these cases, Dean vortices contribute significantly to the improved mixing of the system. However, in our continuous flow setup, we are using low flow rates and long reaction times. So, we developed a reactor containing mini-coils with alternating directionality (clockwise and counter-clockwise) to create Dean vortices, increase turbulent flow, and further enhance mixing (Figure 1A). When R1 consisted of a reactor with mini-coils, we were pleased to observe an increased yield of 57%, achieving comparable yields to our previous batch setup.

We also investigated the effect of varying residence time on reaction yield in a fully automated continuous flow platform (see Experimental, Figure 6). Using the same solutions and reaction conditions, flow rate was varied to explore yield for a 5–60 minute residence time (Table 5B). As residence time increases, we observed an increase in yield, and a residence of 60 minutes provided rhodamine 13 in 48% yield (Table 5B). Interestingly, as residence time increases, the increase in yield appears to plateau after 40 minutes (Figure 1B). Increasing residence time beyond 60 minutes will likely result in diminishing yield enhancement.
Table 5. (A) Yield dependence on mixing efficiency.\textsuperscript{[a]} (B) Yield dependence on residence time performed in a fully automated continuous flow setup.\textsuperscript{[a]}

![Chemical structure](image)

**Table A**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Tubing Inner Diameter (in)</th>
<th>Mixing Coils (Y/N)</th>
<th>Yield 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.063&quot;</td>
<td>N</td>
<td>18%</td>
</tr>
<tr>
<td>2</td>
<td>0.03&quot;</td>
<td>N</td>
<td>38%</td>
</tr>
<tr>
<td>3</td>
<td>0.03&quot;</td>
<td>Y</td>
<td>57%</td>
</tr>
</tbody>
</table>

**Table B**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Residence Time (min)</th>
<th>Yield 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>11%</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>43%</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>48%</td>
</tr>
</tbody>
</table>

[a] Yields were determined via HPLC relative to trimethoxybenzene internal standard.

![Diagram](image)

**Figure 1.** (A) PFA tubing reactor containing (i.d. = 0.03") containing mini mixing coils. (B) Chart demonstrating a yield dependence of 6-TAMRA on residence time performed in a fully automated continuous flow setup.


**Conclusion**

Previously, methods to access to a single isomer of 5(6)-carboxyrhodamine dyes were limited by cost and complexity. We have developed a simple, low-cost two-step selective synthesis that is applicable access to a variety of 5(6)-carboxyrhodamine dyes. Other important 6-carboxyrhodamines can by synthesized via reaction of ketoacid 11 with various commercially available \(N\)-alkyl-3-aminophenols. 5-carboxyrhodamines can be accessed via oxidation of 2,4-dimethylacetophenone instead of 2,5-dimethylacetophenone. We also described our progress towards developing the first synthesis of a 6-carboxytetramethylrhodamine in a continuous flow setup for improved safety and scalability.
References


K. F. The role of flow in green chemistry and engineering. *Green Chem.* **2013**, *15*, 1456. (g)


Chapter 3.

Experimental Section
Materials and Methods

Commercially available reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO), Dimethylaminophenol and 6-carboxytetramethylrhodamine from Ark Pharm, Inc. (Arlington Heights, IL), HFIP from Chem-Impex International, Inc. (Wood Dale, IL) and used without any further purification. Reactions were performed in the Biotage Microwave. Analytical thin-layer chromatography (TLC) was performed using EMD 60-F254 silica glass plates and visualized with a UV lamp (254 nm). Products were purified on SNAP UP-SIL columns utilizing the Biotage® Isolera flash purification system.

For flow experiments, backpressure regulators (BPRs) and liquid-liquid separators were purchased from Zaiput Flow Technologies. PTFE Microfiltration membranes were purchased from Pall Zefluor with 0.5 μm pore size. The reactors were constructed from high-purity perfluoroalkoxy (HPFA) tubing with 1/16” OD and 0.03”, 0.04”, 0.063” and 0.093” ID and PEEK superflangeless fittings purchased from IDEX Health & Science Technologies. Syrris pumps and Harvard Apparatus PhD Ultra syringe pumps were used to pump reagents and solutions from 8-mL high-pressure stainless steel syringes with 1/16” SWAGELOCK® from Harvard Apparatus.

\(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on Bruker Avance-400 spectrometer or Varian Inova 500 NMR. Data are reported as follows: chemical shift (δ, parts per million, referenced to the residual solvent peak of the proton and carbon resonances of CHCl₃ in CDCl₃), multiplicity (s = singlet, d = doublet, m = multiplet), coupling constant (J) in Hertz (Hz), and integration. IR spectra were obtained on an Agilent Cary 630 FT-IR spectrometer equipped with an ATR
(attenuated total reflectance) accessory. Spectral features are tabulated as follows: wavenumber (cm$^{-1}$); intensity (s-strong, m-medium, w-weak, br-broad). High-resolution mass spectrometry data were acquired on a Bruker Daltonics APEXIV 4.7 Tesla TF-ICR Mass Spectrometer at Massachusetts Institute of Technology Department of Chemistry Instrumentation Facility.
Procedure for the Synthesis of 6-TAMRA

Scheme 11. Two-step synthesis of 6-carboxytetramethylrhodamine.

Step 1 Procedure: A suspension of 2,5-dimethylacetophenone (1.43 g, 9.66 mmol, 1 equiv) in water (30 mL) was prepared in a 100 mL round bottom flask equipped with a stir bar. Solid KMnO₄ (10.13 g, 64.1 mmol, 6.6 equiv) and K₂CO₃ (1.42 g, 7.24 mmol, 0.75 equiv) were added and the mixture was stirred at 70 °C in a thermocoupled oil bath. The reaction mixture turns from deep purple to deep brown with a significant quantity of dark brown solid (MnO₂) precipitating from solution. Note that attempts to increase the concentration of the reaction or the scale were hindered by issues of mass transfer associated with magnetic stirring of the slurry. After 90 minutes the reaction was removed from oil bath and allowed to cool to room temperature over 5 minutes. Ethanol (4 mL) was added and the reaction was left to stand for an additional 10 minutes to quench remaining KMnO₄. The resulting mixture was filtered over Celite to remove MnO₂ and the filter cake was washed with water (6 x 15 mL). The cloudy filtrate was acidified with concentrated HCl (4 mL) to a pH of less than 0. The solution stood over night at room temperature and the desired ketoacid 11 selectively crystallized.
**Step 2 Procedure:** Three stock solutions were prepared (A containing ketoacid 11, B containing phenol 12, and C containing NaHSO₃). Solution A was prepared by adding ketoacid 11 (0.65 mmol) to a 5 mL volumetric flask and diluting with 1 M HCl. Solution B was prepared by adding phenol 12 (10 mmol) to a 10 mL volumetric flask and diluting with HFIP. Solution C was prepared by adding NaHSO₃ (5 mmol) to a 5 mL volumetric flask and diluting with water. Solutions of ketoacid 11 (0.77 mL of a solution A, 1 mmol, 1.0 equiv), dimethylaminophenol 12 (1.0 mL of solution B, 10 equiv) and NaHSO₃ (0.22 mL of solution C, 2.2 equiv) were mixed in a capped 0.5–2 mL microwave vial. The combined solutions created a biphasic mixture. Four additional 0.5–2 mL microwave vials were prepared in this manner. The 5 vials were queued on the microwave, where each was heated to 150 °C for 40 min. After all vials were heated and cooled, they were combined (0.5 mmol of ketoacid 11). The solvent was removed by rotary evaporation to give purple solid and purple oil. The crude residue was purified via flash column chromatography on silica gel with DCM/MeOH (0-20%). A mixture of product and a side-product was isolated and re-purified in the same manner, providing pure 6-TAMRA in 28% isolated yield.
Characterization of Ketoacid 11 and 6-TAMRA

2-(Carboxycarbonyl)terephthalic acid (11)

The titled compound was prepared from 2,5-dimethylacetophenone via potassium permanganate oxidation described in step 1 of previous section and isolated as a crystalline white solid after crystallization in 74% yield.

Analysis by NMR was complicated by the equilibrium between the open form 11a and hemiacetal 11b. At standard temperature, no carbonyl or acetal peak is visible in the carbon spectra because it is likely equilibrating between open and closed. At low temperature, we observe the hemi-acetal carbon at 104.0 ppm.

Scheme 12. Equilibrium of acetal formation of ketoacid 11.

\[
\begin{align*}
\text{11a} & \quad \text{open form} \\
\text{11b} & \quad \text{hemi-acetal}
\end{align*}
\]

\(^1\text{H NMR (500 MHz, CD}_3\text{OD, 23 °C)} \ & \delta 8.28 (dd, J = 7.9, 1.2 \text{ Hz, 1H}), 8.22 (s, 1H), 7.95 (d, J = 7.9 \text{ Hz, 1H}) \text{ ppm} \\
\text{1H NMR (500 MHz, CD}_3\text{OD, -50 °C)} \ & \delta 8.32 (d, J = 7.9 \text{ Hz, 1H}), 8.24 (s, 1H), 7.99 (d, J = 7.9 \text{ Hz, 1H}) \text{ ppm} \\
\text{13C NMR (125 MHz, CD}_3\text{OD, 23 °C)} \ & \delta 169.9, 169.7, 168.0, 148.5, 137.8, 133.1, 132.3, 126.5, 125.7 \text{ ppm}
\]
C NMR (125 MHz, CD$_3$OD, -50 °C) δ 170.9, 169.5, 167.7, 149.2, 137.8, 133.2, 131.7, 126.3, 124.9, 104.0 ppm

IR (neat, cm$^{-1}$)

HRMS (DART, m/z) [M–H]$^-$ calculated for C$_{10}$H$_6$O$_7$: 237.0041, Found: 237.0045

6-Carboxytetramethylrhodamine (13)

The titled compound was prepared from ketoacid 11 and 3-dimethylaminophenol 12 described in step 2 of the previous section and isolated after column chromatography as a purple solid (28% yield).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.19 (d, $J$ = 8.0 Hz, 1H), 8.06 (d, $J$ = 8.0 Hz, 1H), 7.57 (s, 1H), 6.59 – 6.44 (m, 6H), 2.94 (s, 12H) ppm

$^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 168.2, 166.3, 152.8, 152.2, 152.0, 130.6, 129.3, 128.4, 124.9, 124.3, 109.1, 105.6, 98.0, 39.8 ppm

$^1$H NMR (500 MHz, CD$_3$OD) δ 8.23 (d, $J$ = 8.0 Hz, 1H), 8.12 (d, $J$ = 8.0 Hz, 1H), 7.84 (s, 1H), 7.31 (d, $J$ = 9.5 Hz, 2H), 7.03 (dd, $J$ = 9.5, 2.5 Hz, 2H), 6.92 (d, $J$ = 2.5 Hz, 2H), 3.29 (s, 12H) ppm

HRMS (DART, m/z) [M+H]$^+$ calculated for C$_{25}$H$_{22}$N$_2$O$_5$: 431.1601, Found: 431.1585
The position of the carboxylic acids (*meta or para*) on the pendant phenyl ring of TAMRA were determined using NOE. When resonance $H_a$ was inverted, an enhancement in $H_b$ was observed, confirming the *para* relationship of the two carboxylic acids on the pendant phenyl ring (Scheme 13).

**Scheme 13.** Interaction between $H_a$ and $H_b$ for 5(6)-TAMRA.
Procedure for the Continuous Flow Synthesis of Ketoacid 11

Continuous Flow KMnO₄ Oxidation Experimental Details (Scheme 9 and 14)

**Flow Setup:** Stock solution A (1.0 equiv) was dimethylacetophenone and D and G (EtOAc) were used directly from the reagent bottle. Stock solution B was prepared by dissolving KMnO₄ (6.0 equiv, 200 mmol) and K₂CO₃ (0.7 equiv, 22.4 mmol) in 400 mL H₂O. Stock solution C was prepared by citric acid (300 mmol) in 300 mL water. Stock solution F was a 1 M aqueous solution of HCl. Stock solution A was transferred to an 8 mL Harvard stainless steel syringe. All remaining solutions were pumped with a Syrris pump. Sep 1 and Sep 2 were Zaiput Liquid-Liquid Membrane separators. Streams of A and B were mixed in a PEEK Y-mixer with a 0.063” inner diameter (I.D.) and pumped through R1 (0.093” I.D., 64 mL). The output of R1 was attached to a reverse tube-in-tube to dissolve the MnO₂ solid with 1 M citric acid. The reverse tube-in-tube was constructed of a PEEK T-mixer drilled out to 0.093” and streams C and D were flowed through 0.04” tubing which passed through the drilled out PEEK T-mixer into the back of R1. A substantial amount of CO₂ was formed from acidifying the K₂CO₃ solution (Figure 1). The homogeneous mixture of organic and aqueous solutions then passes through Sep 1 and was collected into a surge tank to degas the aqueous solution. Solution was pumped from the surge tank to mix with solutions F and G with a PEEK Y-mixer (0.01”). The solution mixed in a mixing coil made of PFA tubing (0.063” I.D., 1 mL), followed by separation over Sep 2.
Procedure: Solutions A and B were pumped at a rate of 10 μL/min and 800 μL/min respectively resulting in a residence time of 80 minutes for R1. Solutions C and D were pumped at a rate of 800 μL/min and 400 μL/min respectively. The combined solutions were separated over Sep 1 and collected in a surge tank. The whole system equilibrated for 3 residence times before solution from the surge tank was removed and mixed with solutions F and G (both flowed at a rate of 300 μL/min). After separation over Sep 2 the organic stream was collected containing ketoacid 11 and yield of 9% was determined by NMR relative to anisole internal standard.

A

10 (1 equiv)
10 µL/min

KMnO₄ (6 equiv)
K₂CO₃ (0.7 equiv)
H₂O
800 µL/min

B

1 M Citric Acid
800 µL/min

C

Drilled Out
Reverse
Tube-In-Tube

D

EtOAc
500 µL/min

E

Organic waste
Surge Tank
(CO₂ degas)

F

4 M HCl
300 µL/min

G

EtOAc
300 µL/min

Sep 1

Sep 2

mixing coil
V = 1.0 mL

Aqueous waste

Yield Before Separations: 24%

Yield After Separations: 9%
Figure 2. Continuous flow KMnO$_4$ oxidation of dimethylacetophenone.
Figure 3. Zoom-in of the continuous flow inline purification of ketoacid 11.
Figure 4. Carbon dioxide gas formation after addition of solutions C (citric acid) and D (EtOAc).
Figure 5. Poor mixing and distribution of dimethylacetophenone and aqueous KMnO₄ due to their large difference in relative flow rates.
Procedure for the Continuous Flow Synthesis of 6-TAMRA

Detailed Experimental Procedure for Scheme 10:

**Flow Setup:** Stock solution S1 was prepared by dissolving ketoacid 11 (1 mmol, 1.0 equiv) in wet ethyl acetate (extracted once with water) in a 10 mL volumetric flask. Stock solution S2 was prepared by dissolving dimethylaminophenol 12 (3.1 mmol, 2.5 equiv) in HFIP in a 10 mL volumetric flask. Stock S3 was prepared by dissolving sodium bisulfite (6.3 mmol, 2.5 equiv) in water in a 5 mL volumetric flask. Each stock solution was transferred to an 8 mL stainless steel syringe. PFA tubing reactor R1 (0.063” id, 149 cm, 3 mL) was connected to the three streams with a PEEK Cross mixer.

**Procedure:** S1, S2, and S3 were pumped at a rate of 50 µL/min, 40 µL/min, and 10 µL/min respectively giving a 45 min residence time for R1. The system was equilibrated for 3 residence times prior to collecting for 30 minutes (0.1 mmol of ketoacid 1). Methanol was added until solution was no longer biphasic. Using reactor A, 6-TAMRA was observed in 6% yield via LCMS relative to trimethoxybenzene internal standard.
Detailed Experimental Procedure for Table 5:

Flow Setup: Stock solution S1 was prepared by dissolving ketoacid 1 (1.3 mmol, 1.0 equiv) in 1 M HCl in a 10 mL volumetric flask. Stock S2 was prepared by dissolving sodium bisulfite (9.5 mmol, 2.2 equiv) in water in a 10 mL volumetric flask. Stock solution S3 was prepared by dissolving dimethylaminophenol (10 mmol, 10 equiv) in HFIP in a 10 mL volumetric flask. Stock solutions were transferred to 8 mL stainless steel syringes.

PFA tubing reactor A (0.063" id, 149 cm, 3 mL) was connected to the three streams with a PEEK Cross mixer.

PFA tubing reactor B (0.03" id, 660 cm, 3 mL) was connected to the three streams with a PEEK Cross mixer.

PFA tubing reactor C containing mini-coils for improved mixing (0.03" id, 660 cm, 3 mL) was connected to the three streams with a PEEK Cross mixer. A 1 m coil of mini coils was added to
Reactor C 40 cm and 300 cm away from the beginning of the reactor. The coil of mini coils generally consisting of 5 loops were spaced about 5 cm apart. The first mini coil was built by wrapping the tubing clockwise around a 1 mL plastic syringe to create 5 loops, which are then secured in place with a plastic ziptie. The second mini coil was built exactly the same except counter-clockwise starting 5 cm away from the first minicoil. Alternate clockwise and counterclockwise mini coils until 1 m of tubing has been used.

Figure 5. PFA tubing reactor C containing mini coils for improved mixing.

Procedure: S1, S2, and S3 were pumped at a rate of 25.7 µL/min, 7.7 µL/min, and 33.3 µL/min respectively giving a 45 min residence time for reactors A, B, and C. The system was equilibrated for 3 residence times prior to collecting for 30 minutes (0.1 mmol of ketoacid 1). Methanol was added until solution was no longer biphasic. Using reactor A, 6-TAMRA was observed in 18% yield via LCMS with trimethoxybenzene as an external standard. Using reactor B, 6-TAMRA was observed in 41% yield via LCMS with trimethoxybenzene as an external standard. Using reactor C, 6-TAMRA was observed in 59% yield via LCMS with trimethoxybenzene as an external standard.
Figure 6. Continuous flow synthesis of 6-TAMRA from ketoacid 11 in our automated platform.
Crystallization Data for Ketoacid 11

Figure 7. ORTEP Drawing (50% probability ellipsoid) of 11 showing the extended structure; right (left); and a single ketoacid unit (right). Hydrogen atoms omitted for simplicity.
Table 6. Crystal data and structure refinement for compound 11.

<table>
<thead>
<tr>
<th>Crystal data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>( \text{C}<em>{13.33}\text{H}<em>8\text{K}</em>{1.33}\text{O}</em>{10} )</td>
</tr>
<tr>
<td>Formula weight</td>
<td>380.33</td>
</tr>
<tr>
<td>Crystal system, space group</td>
<td>Orthorhombic, ( \text{Pna2}_1 )</td>
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<tr>
<td>Temperature (K)</td>
<td>100</td>
</tr>
<tr>
<td>( a, b, c ) (Å)</td>
<td>9.6032 (2), 10.6071 (2), 20.3850 (4)</td>
</tr>
<tr>
<td>( V ) (Å(^3))</td>
<td>2076.46 (7)</td>
</tr>
<tr>
<td>( Z )</td>
<td>6</td>
</tr>
<tr>
<td>Radiation type</td>
<td>Mo Ka</td>
</tr>
<tr>
<td>( m ) (mm(^{-1}))</td>
<td>0.54</td>
</tr>
<tr>
<td>Crystal size (mm)</td>
<td>0.24 × 0.14 × 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Data collection</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Diffractometer</td>
<td>Bruker Photon2 CPAD</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Multi-scan ( \text{SADABS} ) (Herbst-Irmer et al., 2015)</td>
</tr>
<tr>
<td>( T_{\text{min}}, T_{\text{max}} )</td>
<td>0.025, 0.061</td>
</tr>
<tr>
<td>No. of measured, independent and observed ( [I &gt; 2s(I)] ) reflections</td>
<td>100044, 10046, 9736</td>
</tr>
<tr>
<td>( R_{\text{int}} )</td>
<td>0.038</td>
</tr>
<tr>
<td>( (\sin q/l)_{\text{max}} ) (Å(^{-1}))</td>
<td>0.833</td>
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</table>

<table>
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<tr>
<th>Refinement</th>
<th></th>
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<tbody>
<tr>
<td>( R(F^2 &gt; 2s(F^2)), wR(F^2), S )</td>
<td>0.032, 0.082, 1.13</td>
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<tr>
<td>No. of reflections</td>
<td>10046</td>
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<tr>
<td>No. of parameters</td>
<td>353</td>
</tr>
<tr>
<td>No. of restraints</td>
<td>5</td>
</tr>
<tr>
<td>H-atom treatment</td>
<td>H atoms treated by a mixture of independent and constrained refinement</td>
</tr>
<tr>
<td>( D_{\text{h, max}}, D_{\text{h, min}} ) (e Å(^{-3}))</td>
<td>0.52, -0.29</td>
</tr>
<tr>
<td>Absolute structure</td>
<td>Refined as an inversion twin.</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>0.40 (2)</td>
</tr>
</tbody>
</table>

Computer programs: \( \text{APEX3 v2017.3.0} \) (Bruker-AXS, 2017), \( \text{SAINT 8.38A} \) (Bruker-AXS, 2017), \( \text{SHELXTL2015} \) (Sheldrick, 2015), \( \text{SHELXL2016/6} \) (Sheldrick, 2016), Bruker \( \text{SHELXL} \).
Chapter 3.

$^1$H and $^{13}$C Spectra
$^1$H NMR at -50 °C
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{./image.png}
\caption{\textsuperscript{13}C NMR at -50 \textdegree C}
\end{figure}