A SMALL MOLECULE SYTHESIZER

BY

STEVEN GRAHAM BALLMER

DISSERTATION

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Doctoral Committee:

Professor Martin D. Burke, Chair
Professor Scott E. Denmark
Professor Jeffrey S. Moore
Professor Paul J. A. Kenis
ABSTRACT

Despite many advances over the last two centuries, small molecule synthesis remains a relatively complex, unsystematized, and time-intensive process practiced almost exclusively by highly trained specialists. Collectively, these limitations represent a major bottleneck in the efforts to study, understand, and harness the vast functional capacity of small molecules. In an effort to shift the rate-limiting step of small molecule science from synthesis to function study, we have designed, constructed, and implemented a fully automated small molecule synthesizer.

The synthesizer uses only one C-C bond forming reaction, the Suzuki-Miyaura cross-coupling (SMC) reaction, to assemble off-the-shelf building blocks into many different types of small molecules including organic materials, pharmaceuticals, natural products, and natural product derivatives. Specifically, this automated synthesis platform utilizes the iterative cross-coupling (ICC) strategy enabled by the capacity of N-methyliminodiacetic acid (MIDA) to reversibly attenuate the reactivity of a boronic acid. MIDA boronates are generally crystalline free-flowing solids and are stable to bench-top storage. Furthermore, it has been discovered that they are universally amenable to catch-and-release column chromatography and are universally susceptible to general mild deprotection conditions. Additionally, MIDA boronates can be prepared by a variety of synthetic methods on multi-gram scale and they demonstrate significantly improved reaction yields with slow-addition cross-coupling (SACC). It was understood that these strikingly general features of MIDA boronates made them highly amenable to an automated ICC platform. Fully automated modules for MIDA boronate purification, deprotection, and cross-coupling were developed and with thousands of applicable building blocks commercially available or easily accessible, this now simple, general, and fully automated synthesis platform stands to afford more efficient access to small molecule targets to specialists and non-specialists alike.
To the two greatest loves of my life,
Meghan Elisabeth Ballmer & Emerson Jeanne Beauregard Ballmer
ACKNOWLEDGEMENTS

I would first like to thank my advisor, Martin D. Burke. Marty’s unwavering commitment to rigorous science has been a constant source of inspiration over the last six years. He approaches big unsolved problems and unanswered questions fearlessly while understanding that all the minor problems and questions, whether predicted or not, that stand in the way will be solved in the process in their own time. This fundamental approach to science is by far the leading quality he, as a mentor, has instilled in me. His ability, too, to view science in the broadest scope by considering the impact of every experiment on the current project and the greater impact of the project on the world has changed the way I approach science. The method of forming global hypotheses and then testing them with the cleanest simplest experiments has become the model upon which I have developed my own scientific process.

I would also like to acknowledge and thank my thesis committee members: Prof. Martin D. Burke, Prof. Scott E. Denmark, Prof. Jeffrey S. Moore, and Prof. Paul J. A. Kenis. Together they have held me to the highest standards of science and academics. The humbling experiences of prelim and ORP alone have had a profound effect on my maturation as a scientist, for which I am extraordinarily grateful. Their insistence that I, at the very least, be held accountable for both the science I do and the science I present has, I hope, helped to mold me into a better chemist and an overall well-rounded scientist.

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or an existing building block, but with an entirely new series of protecting groups or a final
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Greg’s enviable command of the literature and physical organic chemistry shaped not only the
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As a general rule, money is never an easy thing to come by and scientific research and graduate school is no exception to that rule. I gratefully acknowledge the National Science Foundation, National Institutes of Health, Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign Department of Chemistry, and Prof. Martin D. Burke for personal and/or research funding.

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### ABBREVIATIONS

1\textsuperscript{st} gen. SPhos palladacycle  
chloro(2-dicyclohexylphosphino-2’,6’-dimethoxy-1,1’-biphenyl)[2-(2-amo
inoethylphenyl)]palladium(II)

1\textsuperscript{st} gen. XPhos palladacycle  
chloro(2-dicyclohexylphosphino-2’,4’,6’-triisopropyl-1,1’-biphenyl)[2-(2-
 aminoethylphenyl)]palladium(II)

2\textsuperscript{nd} gen. SPhos palladacycle  
chloro(2-dicyclohexylphosphino-2’,6’-dimethoxy-1,1’-biphenyl)[2-(2’-amino-1,1’-
biphenyl)]palladium(II)

2\textsuperscript{nd} gen. XPhos palladacycle  
chloro(2-dicyclohexylphosphino-2’,4’,6’-triisopropyl-1,1’-biphenyl)[2-(2’-amino-1,1’-
biphenyl)]palladium(II)

Ac  
acetate

Boc  
\( t \)-butoxycarbonyl

BrettPhos  
2-(dicyclohexylphosphino)-3,6-dimethoxy-2’,4’,6’-triisopropyl-1,1’-biphenyl
BrettPhos palladacycle\n\[\text{chloro}[2\text{-}(\text{dicyclohexylphosphino})\text{-}3,6\text{-}\text{dimethoxy-}2',4',6'\text{-}\text{triisopropyl-}1,1'\text{-}\text{biphenyl}][2\text{-}(2\text{-}\text{aminoethyl})\text{phenyl}]\text{palladium(II)}\]

Cy\ncyclohexyl

CyJohnPhos\n(2-biphenyl)dicyclohexylphosphine

DavePhos\n2-dicyclohexylphosphino-2’-(N,N-dimethylamino)biphenyl

dba\ndibenzylideneacetone

DCM\ndichloromethane

DMF\nN,N-dimethylformamide

DMAP\n4-(N,N-dimethylamino)-pyridine

DMP\nDess-Martin periodinane

DMSO\ndimethyl sulfoxide

dppf\n1,1’-bis(diphenylphosphino)ferrocene

GC\ngas chromatography

h\nhour(s)
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>ICC</td>
<td>iterative cross-coupling</td>
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<tr>
<td>JohnPhos</td>
<td>(2-biphenyl)di-t-butylphosphine</td>
</tr>
<tr>
<td>Mes</td>
<td>mesityl</td>
</tr>
<tr>
<td>MePhos</td>
<td>2-dicyclohexylphosphino-2'-'methylbiphenyl</td>
</tr>
<tr>
<td>MIDA</td>
<td>N-methyliminodiacetic acid</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MOM</td>
<td>methoxymethyl</td>
</tr>
<tr>
<td>MPLC</td>
<td>medium-pressure liquid chromatography</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>pyr</td>
<td>pyridine</td>
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<tr>
<td>rt</td>
<td>room temperature</td>
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<tr>
<td>RuPhos</td>
<td>2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl</td>
</tr>
<tr>
<td>SACC</td>
<td>slow-addition cross-coupling</td>
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SMC  Suzuki-Miyaura cross-coupling

SPhos  2-dicyclohexylphosphino-2’,6’-dimethoxybiphenyl

SRCC  slow-release cross-coupling

TBAF  tetra-N-butylammonium fluoride

TBDPS  t-butyldiphenylsilyl

TBDPSE  2-(t-butyldiphenylsilyl)ethyl

TBS  t-butyldimethylsilyl

TES  triethyldimethylsilyl

$tBuBrettPhos$  2-(di-$t$-butylphosphino)-2’,4’,6’-triisopropyl-3,6-dimethoxy-1,1’-biphenyl

$tBuDavePhos$  2-di-$t$-butylphosphino-2’-(N,N-dimethylamino)biphenyl

$tBuMePhos$  2-di-$t$-butylphosphino-2’-methylbiphenyl

$tBuXPhos$  2-di-$t$-butylphosphino-2’,4’,6’-triisopropylbiphenyl

TIPS  triisopropylsilyl
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<tr>
<td>TMSE</td>
<td>2-(trimethylsilyl)ethyl</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>XPhos</td>
<td>2-dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl</td>
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Despite its advancements, small molecule synthesis remains a relatively complex, unsystematized, and time-intensive process practiced almost exclusively by highly-trained specialists which ultimately makes it the rate-limiting step in understanding small molecule function. In stark contrast, peptides, oligonucleotides, and oligosaccharides are synthesized, and subsequently studied, with relatively remarkable efficiency. This is largely because their synthesis platforms are now routinely automated, a process which is enabled by a general and modular iterative synthesis strategy for each class of macromolecule. Small molecules, though, are currently synthesized via customized routes and while advances have been made in the design, and even automation in some cases, of these routes, this approach has major limitations which includes the need for many different types of reactions and building blocks and a high degree of specialist expertise. This chapter discusses the pioneering development of automated synthesis platforms for peptides, oligonucleotides, oligosaccharides, and even organic polymers. It also describes the development of an analogous and general small molecule synthesis platform based on the iterative cross-coupling (ICC) strategy employing N-methyliminodiacetic acid (MIDA) boronates and how this general platform is amenable to automation which stands to make small molecule synthesis and study equally as efficient as that of macromolecules.
Many peptides and oligonucleotides can now be readily prepared via general and automated synthesis platforms. In these systems common sets of building blocks, having all of the required functional groups and stereochemical features pre-installed, are assembled using a single reaction in an iterative fashion. Limitations still remain, including requirements for synthesis of some specialty building blocks that are not commercially available and optimization of reaction conditions for more challenging couplings. Nonetheless, these platforms have dramatically increased the efficiency with which these compounds can be prepared, and even extended this access to non-specialists. As a result, the rate-limiting step in these scientific areas has shifted from synthesis to function, which in turn has had a transformational impact on many areas of science, medicine, and technology. Progress in this direction has also been achieved with oligosaccharides and some organic polymers. This section highlights the development of automated iterative synthesis platforms for peptides, oligonucleotides, oligosaccharides, and outlines seminal work in the iterative synthesis of organic polymers.

**Automated peptide synthesis**

The automated synthesis of peptides, oligonucleotides, and increasingly oligosaccharides via an iterative coupling platform is now ubiquitous. This automated iterative platform, however, is arguably most commonly associated with peptide synthesis. In particular, solid-phase peptide synthesis (SPPS), and specifically its automation, represents the classic paradigm for efficient and modular synthetic strategies.

This strategy for modern peptide synthesis is represented schematically in Figure 1.1 below. The paradigm relies wholly on the use of a bifunctional building block, an amino acid in this case, that is orthogonally protected at the nitrogen terminus (the classic fluorenylmethyloxycarbonyl, Fmoc, group is shown in Figure 1.1) to avoid random oligomerization during the coupling event. Even though the first manually synthesized peptide, glycylglycine, was reported in 1901 by Emil Fischer, it was not until more than thirty years later, in 1932, that Max Bergmann reported the carboxybenzyl (Cbz) protecting group that first allowed for the reversible attenuation of reactivity of the nitrogen center by masking the nitrogen as the Cbz carbamate. With a properly protected nitrogen terminus, the peptide bond formation
can proceed with high selectivity. After each bond formation event, the protecting group can be removed, revealing the free reactive nitrogen terminus capable of undergoing another round of coupling. Because any amino acid can be introduced during the coupling reactions, this approach is inherently highly flexible and modular.

Figure 1.1. Schematic representation of the modern paradigm for iterative synthesis of peptides via solid-phase peptide synthesis (SPPS) from amino acid building blocks, the automation of which has been pioneered by Merrifield and coworkers. Fmoc = fluorenylmethyloxycarbonyl.

Enabled by this protecting group strategy, Merrifield first reported the concept of solid-phase synthesis and its implementation in the synthesis of a tetrapeptide in 1963. In SPPS, the carboxy terminus of the peptide is immobilized via linkage to a solid resin. The enabling quality of this strategy is that it provides a highly efficient and, more importantly, general method for purification; all impurities can be removed via simple filtration away from the immobilized growing peptide. Only two years after its initial report, the an instrument for automated SPPS was reported. The simplicity and generality of the SPPS strategy likely contributed heavily to its quick translation to an automated platform. As a striking example at the time, the system was used successfully in the automated synthesis of the nona-peptide, bradykinin, in only 32 hours. By 1967, new designs for less expensive SPPS vessels were making the platform increasingly more efficient and less than 20 years later, fully automated peptide (and oligonucleotide) synthesizers were available as commercial units ultimately bringing the power of peptide synthesis to non-chemists. In a landmark implementation of automated SPPS, interleukin-3 (IL-3), a 140-amino acid protein was synthesized in an automated fashion in 1986. The development of SPPS and the subsequent translation of it to an automated iterative synthesis
platform has had a dramatic effect on the synthesis and thereby the study of peptides and their functions in science.\textsuperscript{12,13}

**Automated oligonucleotide synthesis**

Similar to SPPS, solid-phase methods for the iterative synthesis of oligonucleotides have also been developed.\textsuperscript{14,15} This process is represented schematically in Figure 1.2 below. Like peptide synthesis, this paradigm relies on the use of an appropriately protected bifunctional building block, an $O$-protected nucleotide in this case, to prevent random oligomerization during the coupling event. Figure 1.2 shows a generalized dimethoxytrityl-protected nucleotide where B represents the base, thymine, or appropriately protected bases adenine, cytosine, or guanine. This platform benefits from the same advantages that SPPS does; namely that facile purification can be performed by filtering away impurities.

![Figure 1.2. Schematic representation of the paradigm for the iterative synthesis of oligonucleotides from nucleotide building blocks, the automation of which has been pioneered by Ogilvie and coworkers as well as Caruthers and coworkers. B = thymine or appropriately protected adenine, cytosine, or guanine. DMT = dimethoxytrityl. DCL = dichloroacetic acid.](image)

Analogous to peptide synthesis, this simple and general strategy enabled the rapid development of a fully automated platform for oligonucleotide synthesis nearly concurrently by Ogilvie and coworkers in 1981\textsuperscript{16} and Caruthers and coworkers shortly after.\textsuperscript{17} In fact, this platform was so beneficial to the study of genes that in 1984, an entire facility was developed with a series of automated instruments for the analysis and synthesis of both genes and proteins.\textsuperscript{18} By 1985 an varied series of next-generation automated gene and deoxyribonucleic
acid (DNA) synthesizers were commercially available\textsuperscript{19} which, like automated peptides, ultimately brought the power of synthesis to the hands of non-specialists. Along with the technology capable of performing fully automated syntheses of oligonucleotides came the interest and development of separate automated platforms for DNA sequencing\textsuperscript{20,21} as well as DNA purification and isolation.\textsuperscript{22} Again, similar to automated SPPS, the development of an automated platform for oligonucleotide synthesis has had a dramatic effect on the field of gene and DNA function study. For example, these automated platforms continue to play a critical role in the efficient study of oligonucleotide functions as is evidenced by a recent report of the automated synthesis of oligodeoxynucleotides containing a 3’-S-phosphorothiolate linkages, which are important tools for probing enzyme-catalyzed cleave processes.\textsuperscript{23}

**Automated oligosaccharide synthesis**

Similar to the automated platforms that have been developed for peptide and oligonucleotide synthesis as discussed previously, a fully automated solid-phase synthesis platform for oligosaccharides has more recently been reported by Seeberger and coworkers in 2001.\textsuperscript{24} This platform is represented schematically in Figure 1.3 below and analogous to the peptide and oligonucleotide platforms mentioned previously, this paradigm also relies on the use of an appropriately protected bifunctional building block, an $O$-protected saccharide in this case, to prevent random oligomerization during the coupling event. Figure 1.3 depicts a generalized case wherein the bifunctional saccharide building block is orthogonally protected as the levulinoyl ester in the presence of benzyl ethers and pivaloyl esters. The same facile purification process (simple filtration of the impurities away from the immobilized substrate) as solid-phase peptide and oligonucleotide synthesis is taken advantage of here. The development of an automated synthesis platform for oligosaccharides is relatively new science compared to the analogous platforms for peptides and oligonucleotides, and is actively being optimized. Like the other macromolecular synthesis platforms did for their respective research fields, though, this too will likely create a dramatic impact on the field of carbohydrate research by bringing the power of synthesis to the hands of non-specialists. That is, the study of carbohydrate structure-function relationships is currently of interest in the scientific community\textsuperscript{25} and implementing an
automated synthesis platform as a drug discovery engine can provide facile access, particularly by non-chemists, to varied carbohydrate structures to facilitate these studies.\textsuperscript{26,27}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{schematic_representation}
\caption{Schematic representation of the paradigm for the iterative synthesis of oligosaccharides from saccharide building blocks, the automation of which has been pioneered by Seeberger and coworkers. Piv = pivaloyl. Lev = levulinoyl. Bn = benzyl.}
\end{figure}

**Iterative organic polymer synthesis**

As discussed previously, the synthesis, and in turn the study, of macromolecules like peptides, oligonucleotides, and increasingly oligosaccharides has been dramatically simplified by the automation of a general and modular synthesis platform. Small molecule synthesis, however, is still limited by a lack of an analogous general, modular, and automated synthesis platform. Pioneering work by Moore and coworkers concerning the development of an iterative cross-coupling (ICC) approach to organic polymer synthesis,\textsuperscript{28,29} however, represents a seminal advance, at least conceptually, in the modular synthesis of small molecules. Specifically, Moore and coworkers report the synthesis of phenylacetylene oligomers via iterative Pd-mediated cross-couplings as, initially, a solution-phase process\textsuperscript{28} and two years later as a solid-supported synthesis strategy (Figure 1.4).\textsuperscript{29} Here a strategy of iterative Sonogashira cross-coupling reactions\textsuperscript{30} is employed with the terminal acetylene functionalities protected as the trimethylsilyl (SiMe\textsubscript{3}) acetylens and the terminal aryl halides orthogonally masked as 1-aryl-3,3-dialkyltriazene groups via an immobilized triazene linker. Although this work is not specifically reported as an automated process, it could theoretically be translated, especially as a solid-phase strategy, to known automated systems, thereby streamlining the synthetic process.
Suginome and coworkers have pioneered a similar iterative strategy for the synthesis of oligoarenes in solution phase.\textsuperscript{31} This strategy employs 1,8-diaminonaphthalene (dan) as a protecting ligand that attenuates the reactivity of boron in an iterative Suzuki-Miyaura cross-coupling strategy. This platform represents a new strategy in iterative Suzuki-Miyaura transformations and is effective for the synthesis of a range of oligoarenes. Interestingly, while this particular strategy could also conceivably be employed in the synthesis small molecules, the harsh acidic conditions required to remove the dan ligand are not generally amenable to small molecule synthesis. While Suginome’s approach has not yet been reported as an automated platform, it is conceivable that the chemistry could be translated, either as a solid or solution phase process, to an automated system which would increase the efficiency of the synthesis of the oligoarene targets.

1-2 A GENERAL PLATFORM FOR SMALL MOLECULE SYNTHESIS

Small molecules perform an extraordinary range of important functions in science, medicine, and technology. However, the synthesis of such compounds still represents the rate-limiting step in the effort to study and understand their vast functional capacity. This is largely because most small molecules are currently prepared via customized syntheses. While substantial advances have been made in the design, execution, and even in some cases, tailored automation of such pathways,\textsuperscript{32,33} this specialized approach has major limitations. These include the need for many different types of reactions and building blocks and a high degree of specialist expertise.
In stark contrast, as discussed previously, many peptides, oligonucleotides, oligosaccharides, and to a lesser extent, organic polymers, can now be readily prepared via general and automated synthesis platforms. As described, these systems utilize common sets of building blocks, having all of the required functional groups and stereochemical features pre-installed, and assemble them using a single reaction in an iterative fashion. This strategy of assembling building blocks in a simple iterative fashion is enabled by the inherent modularity of these macromolecules (Figure 1.5, A). That is, even very large and complex peptides such as myoglobin\(^\text{34}\) (Figure 1.5, A, left), oligonucleotides such as a group II intron\(^\text{35}\) (Figure 1.5, A, middle), and oligosaccharides such as β-cyclodextrin\(^\text{36}\) (Figure 1.5, A, right) are composed simply of repeating units of amino acids, nucleotides, and saccharides, respectively. The exact identity of each repeating building block may be different, but each macromolecule is ultimately highly modular in nature.

Unlike the macromolecular structures of peptides, oligonucleotides, and oligosaccharides, small molecules as a general class of molecules are not classically thought to be modular in nature, because their structures vary so dramatically. In fact, however, small molecules, including natural products, pharmaceuticals, and organic materials contain a high level of inherent modularity across the entire class (Figure 1.5, B). Specifically, organic materials like the quaterthiophene and pharmaceuticals like the B-Raf kinase inhibitor and glucagon receptor antagonist shown in Figure 1.5B are inherently modular due to the Csp\(^2\)-Csp2 bond disconnections used in their syntheses. These types of aryl/heteroaryl-aryl/heteraryl connections are ubiquitous among many organic materials and active pharmaceuticals. Furthermore, natural products like amphotericin B, croacin C, peridinin, ratanhine, β-parinaric acid, myxalamide A, and synechoxanthin also demonstrate a high degree of inherent modularity in their construction. Specifically, repeating olefin units forming a polyene are very common across many classes of natural products and similar to the materials and pharmaceuticals mentioned previously, many natural products, like ratanhine, also have repeating Csp\(^2\)-Csp2 bond disconnections adding to their inherent modularity. This is not particularity surprising when one considers the similarities between the biosynthesis of many natural products and the biosynthesis of peptides, oligonucleotides, and oligosaccharides. As mentioned previously, peptides, oligonucleotides, and oligosaccharides are composed of repeating amino acid, nucleotide, and saccharides units,
respectively. Comparatively, the most common small molecule biosynthetic pathways, such as polyterpene, polyketide, and fatty acid synthesis, also consist of iterative modular strategies. For example, polyterpenes are composed of repeating isoprene units while polyketides are synthesized by repeating malonyl-CoA units and fatty acids are made from repeating acetyl-CoA units.

A. Peptide, Oligonucleotide, and Oligosaccharide Modularity

Figure 1.5. Peptides, oligonucleotides, and oligosaccharides are inherently modular, A, which enables their efficient synthesis through automated iterative strategies. Small molecules, though not commonly considered to be modular, also contain inherent modularity based on their modular construction in nature or their modular C-C bond retrosynthesis, B, which can also enable their synthesis via automated iterative strategies.

Collectively, the automated synthesis platforms for peptides, oligonucleotides, and oligosaccharides have dramatically increased the efficiency with which these macromolecules can be prepared, and even extended this access to non-specialists (see previous discussion). As a result, the rate-limiting step in these scientific areas has shifted from synthesis to function study. The same transformation in small molecule science would be enabled by a similarly general and
automated synthesis platform. In fact several fully automated synthesis platforms for small molecule discovery have been already been developed. Specifically, the development of fully-automated synthesis systems for preparing various pharmaceutical compounds was reported in 1994 by Okamoto and coworkers\textsuperscript{37} and commercial units for the automated solid-phase synthesis of small organic molecules were available shortly after.\textsuperscript{38} These automated platforms have been shown to be particularly effective for the synthesis of specific targets like radiolabeled imaging probes\textsuperscript{39} or targeted compound libraries.\textsuperscript{40} Recently, fully integrated and automated platforms for rapid drug discovery have even been reported.\textsuperscript{41} Collectively, these automated small molecule synthesis platforms are designed to automate either 1) very specific chemical transformations, which limits their generality in synthesis scope, or 2) many chemical transformations, which limits the efficiency of the synthesis platform. Ideally, a maximally general and efficient small molecule synthesis platform would be highly modular and iterative.

As a step in this direction, a strategy for making small molecules has recently emerged that is analogous to peptide synthesis (Figure 1.6)\textsuperscript{31,42,43}. As discussed previously, peptides are readily assembled via iterative N–C bond formation between amino acid building blocks (Figure 1.6, A). Small molecules, many of which are alternatively comprised of building blocks connected by C–C bonds as detailed earlier, can now be similarly prepared via iterative cross-coupling of haloboronic acids (Figure 1.6, B).\textsuperscript{31,42,43,44,45,46,47,48,49} The specifically employed Suzuki-Miyaura cross-coupling between a boronic acid and an organohalide\textsuperscript{50} is an increasingly general reaction for making certain types of C–C bonds, especially those that involve sp\textsuperscript{2}-hybridized carbon atoms. Importantly, as shown in Figure 1.5B previously, small molecules that contain such bonds include a wide range of materials, pharmaceuticals, and certain types of natural products.
**A**

**iterative N-C bond formation**

![Diagram of iterative N-C bond formation](image)

This iterative cross-coupling strategy is enabled by the capacity of N-methyliminodiacetic acid (MIDA) to reversibly attenuate the reactivity of a boronic acid (Figure 1.6, B), similar to the way an Fmoc group protects an amine and thereby avoids random oligomerization of amino acids (Figure 1.6, A). This switch in reactivity is fundamentally linked to a MIDA-induced change in the hybridization state of a boron atom from sp² (reactive) to sp³ (unreactive). The resulting MIDA boronates are shelf-stable building blocks that can be readily accessed via many different methods, and more than 140 are now commercially available.

**B**

**iterative C-C bond formation**

![Diagram of iterative C-C bond formation](image)

**Figure 1.6.** Iterative C-C bond formation via iterative cross-coupling (ICC) of MIDA boronates, B, is analogous to iterative N-C bond formation via iterative peptide coupling, A.
These, combined with thousands of commercially available boronic acids and halides, constitute a formidable pool of off-the-shelf building blocks that are readily available for iterative cross-coupling (ICC). Harnessing all of these features, several small molecule natural products, natural product derivatives, and pharmaceuticals have been manually prepared using this strategy.

Transforming this platform into a fully automated process foremost required the development of a general strategy for purifying synthetic intermediates. Peptides, as well as oligonucleotides, and oligosaccharides, all contain common functional group handles for attachment to a solid support (Figure 1.7, A). This enables automated purification by solid-phase synthesis, in which excess reagents and byproducts are removed via simple filtration. An important distinguishing feature of small molecules is that they do not contain such a universal functional group handle (Figure 1.7, B) and thus cannot be generally purified using this approach. Alternatively, we recognized that all of the intermediate coupling products in an ICC-based synthesis contain a MIDA boronate (Figure 1.6, B). We thus sought a general and automatable strategy for purifying compounds that contain this functional group.

Figure 1.7. Iterative synthesis (and its subsequent automation) of peptides, oligonucleotides, and oligosaccharides is enabled by the presence of a common functional group handle in each class of molecules, A. A particular challenge in the iterative synthesis of small molecules including materials, pharmaceuticals, and natural products is the lack of a common functional group handle, B.
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8 Nature 1984, 309, 733-734.
19 van Brunt, J. Biotechnology 1985, 3, 775-782.


CHAPTER 2
DEVELOPMENT OF AN AUTOMATED PURIFICATION PLATFORM

Steven G. Ballmer, Eric P. Gillis, Gregory F. Morehouse, and Martin D. Burke

This chapter describes the development of an automated purification platform for MIDA boronates. Critical to the successful implementation of this automated platform was the discovery that MIDA boronates, while not amenable to solid-phase synthesis, exhibit binary silica gel elution properties that make them generally and universally amenable to catch-and-release chromatographic purification. Furthermore, many MIDA boronates are not soluble in relatively non-polar solvents which makes them amenable to additional precipitation-based purification. For specific details on the hardware and software control of this automated purification platform please see Chapter 5.

Eric P. Gillis contributed heavily to the design, construction, and development of the automated purification platform as well as some of the early solid-phase synthesis experiments. Gregory F. Morehouse performed the catch-and-release TLC experiments in Figure 2.4 and contributed heavily to the mock crude reaction purification experiments in Figure 2.9.
2-1 CATCH-AND-RELEASE PURIFICATION

This section describes the discovery and development of “catch-and-release” purification conditions for MIDA boronates that are amenable to automation and use in iterative cross-coupling (ICC). Specifically, it details initial investigations of solid-phase synthesis with MIDA boronates and then discusses solution-phase chromatographic purification strategies. It is important to note that while the method discussed here represents a general and universal purification method for MIDA boronates, the concept of catch-and-release purification is not novel. In fact, this concept in general has been utilized for nearly four decades in the way of metal chelate affinity chromatography. It is also employed with the use of solid-supported reagents and organic synthesis. Phase-switch synthesis and purification methods, in particular, including fluorous tagging strategies have played a significant role in the continued development of the concept of catch-and-release purification. In general, catch-and-release purification methods are a critical strategy in compound separation during synthesis.

Attempts at solid-phase iterative cross-coupling (ICC) with MIDA boronates

An important early decision concerning the development of the automated iterative cross-coupling (ICC) platform was whether to develop it based on solid-phase or solution-phase methods. As a solution-phase based method, the platform would be able to take advantage of all of the MIDA boronate cross-coupling conditions previously developed in our laboratory. However, it was unclear at the time how the general purification and isolation of MIDA boronate-containing products would be accomplished in a solution-phase based platform. A solid-phase based method, however, where MIDA boronates and their resulting cross-coupling products are immobilized on solid support would benefit from all of the purification and isolation advantages of solid-phase synthesis. That is, the immobilized MIDA boronates and cross-coupling products could be purified and isolated by simple washing and filtration of the solid support. In this vein, then, we initially sought to develop the automated purification platform based on solid-phase chemistry.

Specifically, we targeted the use of a carbamate linker with a traceless silyl chloride functionality to immobilize MIDA boronates (Figure 2.1). Attachment of p-nitrophenyl
carbonate linker 2.2 to aminomethyl resin 2.1 gave immobilized silane 2.3 to which bifunctional MIDA boronate ester 2.4 could be loaded as the silyl ether after formation of the silyl chloride with trichloroisocyanuric acid (TCICA), affording immobilized MIDA boronate 2.5. Despite extensive experimentation, however, evidence of a successfully loaded, resin-bound MIDA boronate ester could not be obtained. Subsequent efforts to directly load the MIDA boronate-containing linker 2.6 were also attempted, but were similarly unproductive. We hypothesized that either the silyl linker motif or the reaction conditions being employed were surreptitiously affecting the successful loading of the MIDA boronate. Thus, we sought to develop a new, simplified, route to an immobilized MIDA boronate.

![Figure 2.1](image_url)

**Figure 2.1.** Initial solid-phase experiments, using a silyl carbamate linker, in attempt at developing solid-supported MIDA boronates for solid-phase iterative cross-coupling (ICC).

In this vein, we targeted a synthetic route to an immobilized MIDA boronate starting with an immobilized pinacol boronate ester (Figure 2.2). We first attempted to immobilize a boronic acid by condensing benzoic acid 2.7 onto 2.1 to afford resin-bound boronic acid 2.8. Subsequent attempts at performing a solid-supported Suzuki-Miyaura cross-coupling with bifunctional MIDA boronate 2.9 to give immobilized MIDA boronate 2.10 were ultimately unsuccessful. It is also known that the condensation of a benzoic acid 2.11 with 2.1 affords, after subsequent borylation, immobilized pinacol boronate ester 2.12. Surprisingly, however, efforts to immobilize 2.9 via a solid-phase Suzuki-Miyaura cross-coupling onto 2.12, again, gave no evidence of successful MIDA boronate loading (2.10).
It was at this point that we questioned whether the possible mismatched polarity of the MIDA boronate building blocks and the solid-phase resins was responsible for the lack of successful loading of MIDA boronates onto solid-support. We knew, for instance, that the MIDA boronate complex was highly polar and hypothesized that the inability to immobilize it onto a solid support may have been due to unfavorable interactions with the highly hydrophobic polystyrene core of the resins we were using. Commercially available solid-phase resins vary in polarity from relatively non-polar polystyrenes to relatively polar polyamides. Applying the conditions of both Figures 2.1 and 2.2 to a series of four resins of increasing polarity (Wang, TentaGel MB, PEGA gel, and polyamide lanterns) again gave no evidence of MIDA boronate immobilization. Having reached the polarity limit of available resins with the polyamide lanterns, we sought to address the possible polarity mismatch by decreasing the polarity of the MIDA boronate. We hypothesized that derivatizing the N-methyl group on the MIDA boronate with increasingly less-polar groups would provide an attenuation of substrate polarity and would furthermore enable a two-dimensional screening process to pinpoint effective combinations of ligands and resins. Inspired by the dual functionality of the fluorenlymethoxyxycarbonyl (Fmoc) protecting group, widely used in solid-phase peptide synthesis, as both a protecting group and as a fluorescent tag we sought to create an analogous iminodiacetic acid derivative that was also less polar. Ideally, this derivative would possess the advantageous protecting properties of MIDA as well as a chromophore, which we realized could also provide the desired attenuation of polarity if designed correctly. Initially, N-(1-pyrenylmethyl)iminodiacetic acid was targeted, but...
its synthetic route was severely limited by the drastically differing solubility of the two starting materials and was unable to be synthesized. We then targeted \(N\)-benzyliminodiacetic acid (BIDA) (Figure 2.3, 2.13). Treatment of benzylamine with aqueous base and chloroacetic acid afforded 2.13 after hydrolysis of its barium chelate.\(^{19}\) BIDA boronates 2.15 and 2.17 were obtained after condensation of 2.13 with boronic acids 2.7 and 2.16, respectively. While the ultraviolet signature of the unfunctionalized benzyl group was unlikely to provide sufficient photometric data for solid-phase methodology, 2.15 and 2.17 represented the first step toward a new hydrophobic chromophoric series of ligands for the attenuation of reactivity of boronic acids in ICC. However, under all of the conditions for solid-phase loading described above, these BIDA analogs did not demonstrate improved loading of the boron center onto solid support.

![Figure 2.3. Synthesis of benzyliminodiacetic acid (BIDA) boronates.](image)

It was clear that developing an automated ICC platform relying on solid-phase synthesis as the method for purification was going to continue to be met with significant limitations and challenges. Furthermore, we anticipated even more difficult challenges arising with the issue of properly mixing the heterogeneous cross-coupling reactions on solid phase. That is, MIDA boronate ICC reactions require anhydrous conditions, which necessitate the use of insoluble inorganic bases (e.g. \(\text{K}_3\text{PO}_4\) and \(\text{K}_2\text{CO}_3\)). It is unlikely that insoluble bases would properly, if at all physically, penetrate the pores of the solid supports to mediate productive coupling. This limitation may have been observed with the results from Figure 2.2. During the course of these investigations, we began to question whether it would be possible to instead adapt our laboratory’s established, expanding, and highly versatile solution-phase methodology for MIDA boronate ICC directly to automation. As mentioned previously, at the time it was unclear how the purification and isolation of MIDA boronates would be accomplished in an automated solution-phase system. We understood that the primary advantage of solid-phase synthesis was the facile purification enabled by immobilizing substrates on resins. For solution-phase chemistry to become a tractable methodology for automation we required an equally facile
process for the purification of MIDA boronates in a solution-phase setting. Quite serendipitously, we realized that a general solution for the automated solution-phase purification of MIDA boronates was available in their unique and universal ability to undergo catch-and-release chromatography.

**Catch-and-release silica gel chromatography of MIDA boronates**

Silica gel chromatography is a standard method for purifying small molecules, but variable affinities of different compounds for this stationary phase often necessitate ad hoc optimization of eluent systems for each purification. In stark contrast, in the course of our laboratory’s investigations and development of the iterative cross-coupling (ICC) platform, we have discovered that MIDA boronates are not only generally and universally stable to silica gel column chromatography, but are also amenable to catch-and-release chromatographic purification.\(^{20,21,22,23,24,25,26,27,28}\) We found that MIDA boronates uniformly possess binary elution properties on silica gel with certain pairs of eluents. Specifically, in the presence of eluents such as diethyl ether or hexanes MIDA boronates display no mobility on silica gel regardless of their formula weight, polarity, or solubility in those eluents. These types of eluents allow MIDA boronates to be “caught” on the silica gel, and allow for indiscriminate elution of non-polar impurities with copious volumes of the eluent. However, the addition of as little as 10% of an eluent such as acetone or acetonitrile, allows for complete and rapid elution, or “release” of the purified MIDA boronate. We recognized that this phenomenon presents a direct parallel to solid-phase synthesis, where substrates are now transiently immobilized on silica gel as opposed to covalently linked to a resin. Collectively, our laboratory has shown that this catch-and-release purification phenomenon is general and applies to every MIDA boronate that the Burke group has synthesized thus far.

Interested in optimizing the catch-and-release eluent system for automation, we understood that it would be ideal to have the “release” solvent also be a solvent directly amenable to cross-coupling reactions. Fortuitously, we found that tetrahydrofuran (THF), a solvent commonly employed in cross-coupling reactions, is a competent “release” solvent. Additionally, we observed that in straight diethyl ether, many boronic acids elute inefficiently, likely due to hydrogen-bonding interactions with the silica gel. Experimentally, we found that a
mixture of methanol in diethyl ether efficiently eluted boronic acids as a single band, but still acted as a “catch” solvent for MIDA boronates. This pairing of methanolic ether and THF thus provided an eluent system amenable to both catch-and-release silica gel purification of MIDA boronates and its automation (Figure 2.4).

Figure 2.4. TLC demonstration of the binary elution properties of the MIDA boronate functional group. This binary elution property enables catch-and-release chromatography purification of MIDA boronates.

Specifically, when performing thin layer chromatography (TLC) with a 1.5% (v/v) solution of methanol (MeOH) and diethyl ether (Et₂O), a polar eluent that causes rapid elution of most compounds and reagents, MIDA boronates 2.18a-t, representing a wide range of different
sizes, polarities, and functional group content, all show zero mobility (Figure 2.4, left). Furthermore, we also found that hexanes:THF (3:1) acts as a “catch” solvent where MIDA boronates shown no silica gel mobility. In contrast, all of the same MIDA boronates are rapidly eluted with THF a “release” solvent (Figure 2.4, right).

Interestingly, while we had initially assumed that the “catch” and “release” requirements for an eluent followed a solvent polarity trend, it actually appears to not necessarily be correlated. For instance, we know that acetonitrile, acetone, ethyl acetate, and THF are all competent “release” solvents and that hexanes and diethyl ether are “catch” solvents. This seems to follow the expected polarity trend; the more polar solvents (acetonitrile, acetone, ethyl acetate, and THF) will elute the MIDA boronate whereas the less polar solvents (hexanes and diethyl ether) will not. However, we have also found that dichloromethane and chloroform are “catch” solvents. When examining these solvents’ polarities (Table 2.1)\textsuperscript{29} it can be seen that dichloromethane (Table 2.1, entry 5) and chloroform (Table 2.1, entry 6), specifically, are more polar than THF (Table 2.1, entry 8) and ethyl acetate (Table 2.1, entry 7), and should therefore be “release” solvents if this phenomenon is correlated to polarity. It is clear that the physical underpinnings of this MIDA boronate catch-and-release phenomenon are currently not well understood, but it has nonetheless shown itself to be general and universal for MIDA boronates and has in turn enabled our development of a general and readily automatable catch-and-release purification strategy.
Table 2.1. List of solvents arranged in order of decreasing $E_N^T$ value, as an empirical parameter of solvent polarity. These are normalized $E_N^T$ values derived from the transition energy at 25 °C of the long-wavelength visible absorption of a standard pyridinium $N$-phenolate betaine dye.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>$E_N^T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>water (H$_2$O)</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>methanol (MeOH)</td>
<td>0.762</td>
</tr>
<tr>
<td>3</td>
<td>acetonitrile (MeCN)</td>
<td>0.460</td>
</tr>
<tr>
<td>4</td>
<td>acetone ((CH$_3$)$_2$CO)</td>
<td>0.355</td>
</tr>
<tr>
<td>5</td>
<td>dichloromethane (CH$_2$Cl$_2$)</td>
<td>0.309</td>
</tr>
<tr>
<td>6</td>
<td>chloroform (CHCl$_3$)</td>
<td>0.259</td>
</tr>
<tr>
<td>7</td>
<td>ethyl acetate (EtOAc)</td>
<td>0.228</td>
</tr>
<tr>
<td>8</td>
<td>tetrahydrofuran (THF)</td>
<td>0.207</td>
</tr>
<tr>
<td>9</td>
<td>diethyl ether (Et$_2$O)</td>
<td>0.117</td>
</tr>
<tr>
<td>10</td>
<td>$n$-hexane</td>
<td>0.009</td>
</tr>
</tbody>
</table>

2-2 DESIGN OF THE AUTOMATED PURIFICATION MODULE$^{30}$

Based on the catch-and-release purification platform for MIDA boronates discussed above, we have developed and constructed a fully automated purification module for MIDA boronates in an iterative cross-coupling (ICC) cycle (Figure 2.5, see Chapter 5 for specific details of the hardware and software control of this module). As can be seen, the platform consists of two major components, a silica gel plug for performing catch-and-release chromatography and a precipitation chamber that provides a secondary precipitation-based purification method.
In practice, we observed that many MIDA boronates are not soluble in the “catch” eluent hexanes:THF (3:1). This property means that these MIDA boronates can be precipitated from THF solution (the solution resulting from a cross-coupling reaction) by adding hexanes in the appropriate ratio. This phenomenon, then, allows for the implementation of a secondary precipitation-based purification strategy\textsuperscript{31,32,33} that is complementary to catch-and-release chromatography. Specifically, TLC experiments indicated that in most cases the impurities of cross-coupling reactions remained in the hexanes:THF (3:1) solution while the MIDA boronate was quantitatively precipitated. This precipitation-based purification strategy might be employed for MIDA boronate purification simply through selective precipitation of the boronate followed by filtration. However, not all MIDA boronates are insoluble in hexanes:THF (3:1), which makes this strategy considerably less general than catch-and-release chromatography. It is still, however, a beneficial secondary purification strategy to be implemented as a compliment to catch-and-release purification.
The automated platform pictured in Figure 2.5 is represented schematically in Figure 2.6. The module is seated on a magnetic stir plate and it is connected to two automated syringe pumps through a series of automated valves and the entire module is controlled remotely by a computer. Collectively, the syringe pumps are connected to: 1) the cross-coupling reaction, which is the source of the crude MIDA boronate in THF, 2) a source of hexanes, which when combined with the reaction THF solution provides the precipitation solvent and a “catch” solvent, 3) a source of 1.5% (v/v) methanol in diethyl ether and straight diethyl ether, which are both “catch” solvents, 4) a source of THF, which is the “release” solvent, and 5) a waste container for disposing of unwanted eluents. The silica gel plug is approximately 1.5 cm X 1.5 cm of standard unfunctionalized silica gel (SiO$_2$) between two fiber frits (20 μm porosity) held in a custom-made Teflon® tube. The precipitation chamber is a 25-mL polypropylene tube with a fiber frit (20 μm porosity), a small magnetic stir bar, Celite® filter aid, and 3-aminopropyl functionalized silica gel (3-AP SiO$_2$). This functionalized silica gel acts as a palladium scavenger for the crude reaction mixtures. The bottom of the precipitation chamber is connected to the top of the silica gel plug through a tee connector. The bottom of the silica gel plug, in turn, is connected to one of the automated syringe pumps, while the other syringe pump is connected to the tee connector between the silica gel plug and precipitation chamber. This same syringe pump is also connected to the top of the precipitation chamber. Please see Chapter 2: Experimental Section for more details on the setup and of both the silica gel plug and the precipitation chamber in this automated purification module. Please see Chapter 5 for more details on the connectivity of the purification module.
The intended process for fully automated MIDA boronate purification based on the platform and strategies described above is shown schematically in Figure 2.7. For a hypothetical cross-coupling of boronic acid 2.19 to bifunctional MIDA boronate 2.20 the resulting cross-coupled product 2.21 is formed as part of a crude reaction mixture containing Pd, ligand, THF, and excess 2.19. Here it is assumed that the inorganic base used in the cross-coupling reaction has been previously removed via filtration (see Chapter 4 for details). This crude sample of 2.21 is then submitted to the automated purification platform described here, affording purified 2.21. Specifically, the precipitation chamber is filled with hexanes. Then the crude reaction mixture, as a solution in THF and containing 2.19 and 2.21, is added to the hexanes. This effects the selective precipitation of MIDA boronate 2.21 (for MIDA boronates that are not soluble in hexanes:THF (3:1)). This “catch” eluent is then removed through the silica gel plug, eluting some of the impurities while leaving any soluble 2.21 caught on the silica gel plug. A subsequent elution with methanolic ether (a “catch” solvent) ensures the complete removal of the remaining impurities, particularly the boronic acid 2.19, leaving behind the now purified 2.21. Although 2.21 is now purified and ready for release, the inclusion of any of the remaining residual methanol in the subsequent cross-coupling reaction would result in detrimental methanolysis of the MIDA boronate. In order to remove the methanol, the purification module is eluted with straight diethyl ether. This affords the purified 2.21, free of methanol, as a precipitated solid in
the precipitation chamber and/or an immobilized silica gel deposition. THF is then used as the “release” solvent to solubilize any 2.21 in the precipitation chamber and elute it from the silica gel plug. This entire sequence occurs in a fully automated fashion and the resulting THF solution of pure MIDA boronate is poised to undergo deprotection (see Chapter 3 for details) or isolation. Please see Chapter 2: Experimental Section and Chapter 5 for more details on the automated purification process specific to the automated operations.

![Diagram of automated MIDA boronate purification process]

Figure 2.7. Schematic representation of the automated MIDA boronate purification process, including the precipitation-based purification and catch-and-release silica gel chromatography.

It should be noted that the actual elution method of the MIDA boronate off of the silica gel plug does not occur exactly as Figure 2.7 schematically indicates. An important criterion for the purification module is that the MIDA boronate be released as a THF solution at the highest possible molarity. In theory, the molarity of the product solution is limited by the solubility of the specific MIDA boronate in THF. In practice, this molarity is not possible to achieve without a step that concentrates the THF solution. Although we have developed an efficient process for
this concentration (see Chapter 3 for details) it is still beneficial to minimize the overall volume of THF used. Therefore, the molarity of the released MIDA boronate solution is directly related to the volume of solvent required to fully elute it from the silica gel plug.

The volume of THF required to elute the product from the silica gel plug is dependent on the R_f of the product multiplied by the volume of the silica gel the product must pass through in order to be fully eluted (the effective column volume). Based on the elution properties observed experimentally with MIDA boronates (and as demonstrated in Figure 2.4), Figure 2.8A schematically represents the most likely situation following the “catch” of a MIDA boronate onto the silica gel plug during automated purification. Because an R_f of zero is predicted for all MIDA boronates eluted with any “catch” solvent, the MIDA boronate will remain immobilized at the top of the silica gel plug. If, after purification, the MIDA boronate were “released” by THF in a normal (top to bottom) direction of flow, then the effective column volume would be at a maximum (Figure 2.8B), resulting in possible streaking of the MIDA boronate through the silica gel plug and necessitating a larger volume of THF to elute it. Further, the total volume of THF required to fully elute a MIDA boronate under this normal flow direction cannot be predicted for every MIDA boronate. Alternatively, if the silica gel plug were eluted in a reversed (bottom to top) direction of flow, then the effective column volume would be as small as theoretically possible (Figure 2.8C), resulting in elution of the MIDA boronate in a minimized solvent volume. Additionally, here the MIDA boronate should always be fully eluted from the silica gel plug as long as the volume of THF used for “release” is equal to or greater than the volume of solvent used in the “catch” step because the effective column volume is smaller in the “release” step than in the “catch” step and the product has a higher R_f value in THF than in any “catch” solvent. In fact, this reversed flow direction is the elution process used in the actual automated purification platform by utilizing a tee connector (shown in Figure 2.6) between the precipitation chamber and the silica gel plug. Impurities are eluted, using a “catch” solvent, through the silica gel plug in the normal flow direction, bypassing the side outlet of the tee connector, and then the purified MIDA boronate is released by reversing the flow direction of the “release” solvent. After elution of the MIDA boronate from the silica gel and dissolution of the MIDA boronate in the precipitation chamber, the THF solution is removed through the side outlet of the tee connector. Furthermore, the purity of the MIDA boronates obtained from this reversed flow
direction approach (Figure 2.8C) is the same as that obtained through the normal approach (Figure 2.8B), but the elution occurs with substantially greater efficiency.

![Figure 2.8](image)

**Figure 2.8.** Schematic representation of the silica gel plug of the automated MIDA boronate purification platform during A. the “catch” of a MIDA boronate during catch-and-release chromatography B. the “release” of a MIDA boronate under normal eluent flow direction during catch-and-release chromatography C. the “release” of a MIDA boronate under reversed eluent flow direction during catch-and-release chromatography.

With the fully automated MIDA boronate platform functioning, we sought to test its purification capability by having it purify a series of mock crude cross-coupling reactions (Figure 2.9, see Chapter 2: Experimental Section for operational details). Specifically, a series THF solutions of aryl (Figure 2.9, entries 2, 4, 9, 10, 12), heteroaryl (Figure 2.9, entries 1, 8, 11, 13), vinyl (Figure 2.9, entries 6, 7, 16), alkyl (Figure 2.9, entries 3, 5, 14), and alkynyl (Figure 2.9, entry 15) MIDA boronates (2.22a–p) was prepared as a mixture simulating a crude, completed cross-coupling reaction. These solutions contained 1 equivalent of p-tolyl boronic acid (2.23) to simulate unreacted excess boronic acid coupling partner and 2.5 mol% palladium as Pd(OAc)₂ and 5 mol% ligand as SPhos to simulate the catalyst system. Each individual mock crude reaction mixture was subjected to the fully automated MIDA boronate purification procedure and the percent recovery of the MIDA boronate and its purity were determined. Encouragingly, in all 16 cases, MIDA boronate 2.22 was recovered in good yield (67-94% recovery) and a duplicate run of these experiments showed good reproducibility across the series. Furthermore, it was determined that the fully automated purification of the mock crude cross-coupling reaction afforded all recovered MIDA boronates 2.22a–p in >90% purity. As expected, the only observable impurities by ¹H NMR were small amounts of p-tolyl boronic acid and SPhos.
Figure 2.9. Mock crude reactions used to test the automated MIDA boronate purification platform. Each experiment was set up manually and then submitted to a fully automated purification cycle. Percent recoveries of the starting MIDA boronates were determined by mass and purities were determined by $^1$H NMR. All entries were recovered in >90 purity. Each experiment was run in duplicate and the percent recoveries are shown.

<table>
<thead>
<tr>
<th>Entry</th>
<th>MIDA Boronate</th>
<th>% Recovery (repeat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.22a</td>
<td>74(81)%</td>
</tr>
<tr>
<td>2</td>
<td>2.22b</td>
<td>87(85)%</td>
</tr>
<tr>
<td>3</td>
<td>2.22c</td>
<td>84(87)%</td>
</tr>
<tr>
<td>4</td>
<td>2.22d</td>
<td>80(85)%</td>
</tr>
<tr>
<td>5</td>
<td>2.22e</td>
<td>84(89)%</td>
</tr>
<tr>
<td>6</td>
<td>2.22f</td>
<td>89(92)%</td>
</tr>
<tr>
<td>7</td>
<td>2.22g</td>
<td>81(92)%</td>
</tr>
<tr>
<td>8</td>
<td>2.22h</td>
<td>72(80)%</td>
</tr>
<tr>
<td>9</td>
<td>2.22i</td>
<td>67(63)%</td>
</tr>
<tr>
<td>10</td>
<td>2.22j</td>
<td>77(69)%</td>
</tr>
<tr>
<td>11</td>
<td>2.22k</td>
<td>94(90)%</td>
</tr>
<tr>
<td>12</td>
<td>2.22l</td>
<td>84(53)%</td>
</tr>
<tr>
<td>13</td>
<td>2.22m</td>
<td>81(80)%</td>
</tr>
<tr>
<td>14</td>
<td>2.22n</td>
<td>91(89)%</td>
</tr>
<tr>
<td>15</td>
<td>2.22o</td>
<td>68(67)%</td>
</tr>
<tr>
<td>16</td>
<td>2.22p</td>
<td>71(94)%</td>
</tr>
</tbody>
</table>

2-3 SUMMARY

In summary, we have developed, constructed, and implemented a fully automated MIDA boronate purification platform. This platform utilizes a series of automated valves and syringe
pumps to purify MIDA boronates, taking advantage of their inherent physical properties. Specifically, the insolubility of most MIDA boronates in hexanes allows for a precipitation-based purification while their general and universal stability to silica gel chromatography and their unique binary elution profile allows them to undergo catch-and-release silica gel chromatographic purification. Together, these properties have enabled a two-part (precipitation/catch-and-release) fully automated purification strategy capable of purifying MIDA boronates from a crude cross-coupling reaction.

2-4 REFERENCES


Gillis, E. P. Iterative Cross-Coupling With MIDA Boronates. Ph.D. Dissertation, University of Illinois at Urbana-Champaign, Urbana, IL.


CHAPTER 2: EXPERIMENTAL SECTION

General materials and methods

Commercial reagents were purchased from Sigma-Aldrich, EMD Millipore, Fisher Scientific, Alfa Aesar, Frontier Scientific, Inc., Oakwood Products, Inc., or Strem and were used without further purification unless otherwise noted. Many of the MIDA boronates used in purification studies are commercially available from Sigma-Aldrich® \[2.18a=2.22n \ (697311), 2.18b=2.22o \ (700231), 2.18c \ (721573), 2.18d \ (710032), 2.18e \ (MIDA071), 2.18f \ (698229), 2.18g \ (697494), 2.18h \ (698164), 2.18i=2.22d \ (698016), 2.18j \ (698148), 2.18k \ (704547), 2.18l \ (MIDA032), 2.18m \ (736600), 2.18n \ (748714), 2.18o \ (723711), 2.18p \ (738514), 2.18q=2.22b \ (699861), 2.18r \ (MIDA020), 2.18s \ (MIDA076), 2.18t \ (704873), 2.22a \ (701106), 2.22c \ (701580), 2.22e \ (699144), 2.22f \ (710024), 2.22g \ (699292), 2.22h \ (708828), 2.22i \ (698164), 2.22j \ (698075), 2.22k \ (701084), 2.22l \ (699853), 2.22m \ (697443), 2.22p \ (707252)\]. Manual syntheses were carried out in oven or flame-dried glassware and performed under a dry inert atmosphere unless otherwise noted. Unless otherwise noted, Celite™ refers to Celite™ 545 filter aid (not acid washed). Solvents were purified via passage through packed columns as described by Pangborn and coworkers¹ (THF, Et₂O, CH₃CN, CH₂Cl₂: dry neutral alumina; hexanes, benzene, toluene: dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves. Water was deionized. Triethylamine, diisopropylamine, diethylamine, pyridine, and 2,6-lutidine were freshly distilled under an atmosphere of dry nitrogen from CaH₂.

Thin layer chromatography (TLC) was performed using the indicated eluent on E. Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by exposure to a UV lamp (λ = 254 and/or 366 nm) and/or a basic solution of KMnO₄ followed by brief heating with a Varitemp® heat gun. Flash chromatography was performed as described by Still and coworkers² using EM Merck silica gel 60 (230-400 mesh). ¹H NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500, Varian VXR 500, or Varian Unity Inova 500NB. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CDCl₃, δ = 7.26; (CD₃)₂CO, δ = 2.05, center line; CD₂Cl₂, δ = 5.32, center line; (CD₃)₂SO, δ = 2.50, center line). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q =
quartet, sept = septet, m = multiplet, br = broad, app = apparent, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets), coupling constant \( (J) \) in Hertz (Hz), and integration. 

\(^{13}\)C NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500 or Varian VXR 500. Chemical shifts (\( \delta \)) are reported in ppm downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent \((\text{CDCl}_3, \delta = 77.16,\) center line; \((\text{CD}_3)_2\text{CO}, \delta = 29.84,\) center line; \(\text{CD}_2\text{Cl}_2, \delta = 53.84;\) \((\text{CD}_3)_2\text{SO}, \delta = 39.52,\) center line). Carbons bearing boron substituents were not observed due to quadrupolar relaxation. Low resolution mass spectra (LRMS) and high resolution mass spectra (HRMS) were performed by Furong Sun, Steve Mullen, and Elizabeth Eves at the University of Illinois School of Chemical Sciences Mass Spectrometry Laboratory.

\[
\begin{align*}
\text{NH}_2 & \quad + \quad \text{HO}_2\text{C} \quad \xrightarrow{\text{DIC, HOBt, DMF, rt, 12 h}} \quad \text{O} \\
2.1 & \quad + \quad 2.11 & \quad \rightarrow & \quad 2.23
\end{align*}
\]

**4-iodobenzamide, resin bound (2.23).** An oven-dried 40-mL glass I-Chem vial is charged with aminomethyl polystyrene resin (1.39 mmol/g, 1.014 g, 1.41 mmol, 1 equiv) followed by dry DMF (15.0 mL) for resin swelling. The vial is sealed with a PTFE-lined screw cap and allowed to stand at room temperature for 30 minutes with periodic manual agitation.

During this time an oven-dried 4-mL glass I-Chem vial equipped with a magnetic stir bar is charged with 4-iodobenzoic acid (1050 mg, 4.23 mmol, 3.00 equiv), HOBt (572 mg, 4.23 mmol, 3.00 equiv), and dry DMF (15.0 mL) giving a clear colorless solution. The vial is sealed with a PTFE-lined septum screw cap and then DIC (655 \( \mu \)L, 4.23 mmol, 3.00 equiv) is added via syringe with stirring. The solution is allowed to stir at room temperature for 10 minutes.

During this time the vial containing the swelled resin is opened and the DMF is removed via syringe through a 25 G needle, being cautious not to extract any resin beads, leaving damp off-white resin. The benzoic acid solution is then transferred to the resin vial via syringe. The vial is sealed with a PTFE-lined screw cap and allowed to stand at room temperature for 12 hours with periodic manual agitation. At the end of 12 hours the reaction is transferred to a fritted polypropylene tube fitted with a stopcock and secured to a parallel vacuum manifold. The
reaction is filtered over vacuum and the resin is washed with dry DMF (5 x 10 mL x 5 min) and dry DCM (5 x 10 mL x 5 min). Residual solvent is then removed at reduced pressure (23 °C, 1 Torr) over 2 hours to give free flowing off white resin. The resin gives a negative result in the ninhydrin (Kaiser) test.

![Chemical Reaction Diagram]

**4-(pinacolboronate)benzamide, resin bound (2.12).** An oven-dried 40-mL I-Chem glass vial in a glovebox is charged with resin resin-bound 4-iodobenzamide (1.05 mmol/g, 200 mg, 0.210 mmol, 1 equiv), bis(pinacolato)diboron (107 mg, 0.421 mmol, 2.00 equiv), potassium acetate (62 mg, 0.632 mmol, 3.01 equiv), PdCl$_2$(dppf)-DCM (17 mg, 0.021 mmol, 10 mol%), and dry DMF (20.0 mL). The vial is sealed with a PTFE-lined screw cap and removed from the glovebox. The reaction is agitated at room temperature manually for 2 minutes. It is then placed in a preheated oil bath at 80 °C and allowed to stand for 15 hours with periodic manual agitation. Approximately 1 minute after exposure to heat the reaction turned black.

At the end of 15 hours the reaction is allowed to cool to room temperature over 20 minutes. It is then transferred to a fritted polypropylene tube fitted with a stopcock and secured to a parallel vacuum manifold. The reaction is filtered over vacuum and the resin is washed with dry DMF (5 x 10 mL x 5 min) and dry DCM (5 x 10 mL x 5 min). Residual solvent is then removed at reduced pressure (23 °C, 1 Torr) over 2 hours to give free flowing black resin.

![Chemical Reaction Diagram]
**N-benzyliminodiacetic acid (BIDA) (2.13).** To a 250-mL single-necked round-bottom flask equipped with a PTFE-coated magnetic stir bar is added chloroacetic acid (9.45 g, 100 mmol, 2 equiv) and water (20.0 mL) giving a clear colorless solution. This is allowed to cool in an ice bath with stirring for 10 minutes. Then an aqueous solution of sodium hydroxide (20 M, 10 mL, 200 mmol, 4 equiv) is added slowly via syringe over 10 minutes with stirring. Upon completion of addition the reaction is allowed to stir at 0 °C for an additional 5 minutes. Then, benzylamine (5.45 mL, 50.0 mmol, 1 equiv) is added via syringe over 5 minutes with stirring. The flask is then removed from the ice bath, capped with a polypropylene cap, and allowed to stir at room temperature for 15 hours.

At the end of 15 hours a hot solution of barium chloride dihydrate (12.849 g, 53 mmol, 1.06 equiv) in water (20.0 mL) is poured slowly into the reaction with stirring, giving a white precipitate immediately. This is set to reflux in a heating mantle with stirring for 30 minutes. At the end of 30 minutes the reaction is allowed to cool to room temperature with stirring and is then filtered through a 120-mL medium porosity glass frit. The barium chelate is hydrolyzed with sulfuric acid to obtain the title compound as a white flaky solid.

$$\text{1H NMR (500 MHz, D}_2\text{O)}$$

$$\delta 7.52 \text{ (s, 5H)}, 4.52 \text{ (s, 2H)}, 3.99 \text{ (s, 4H).}$$

HRMS (ESI+)

Calculated for $\text{C}_{11}\text{H}_{14}\text{NO}_4 [\text{M+H}^+]$: 224.0923

Found: 224.0919
4-carboxyphenylboronic acid BIDA ester (2.15). The glassware for this reaction was not dried and the procedure was performed under ambient atmosphere. A 100-mL, single-necked, round-bottom flask, equipped with a magnetic stir bar is charged with 4-carboxyphenylboronic acid (1.684 g, 10.15 mmol, 1 equiv) and N-benzyliminodiacetic acid (2.13) (2.547 g, 11.41 mmol, 1.12 equiv). To the flask with stirring is then added 50 mL of a freshly prepared mixture of DMSO:benzene 1:1. The flask is then fitted with a benzene-filled Dean-Stark trap topped with a water-cooled Graham condenser. All ground glass joints are sealed with Apiezon H high temperature vacuum grease and secured with Keck clips. The arm of the Dean-Stark trap is wrapped with aluminum foil to facilitate heat transfer.

The stirred reaction mixture is brought to reflux using a heating mantle. The reaction mixture is allowed to reflux for 12 hours. The heating mantle is removed, and the reaction is allowed to cool to 23 °C with stirring for 1 hour. The Dean-Stark trap and magnetic stir bar are removed and the reaction mixture is concentrated in vacuo (40 °C, 15 Torr) to afford the crude product in solution with DMSO. This residue is dissolved in ethyl acetate (250 mL) and transferred to a 500-mL separatory funnel. This organic layer is washed with water (10 x 75 mL) (These successive water washes are crucial for the quantitative removal of DMSO. If not effectively removed, DMSO may result in difficulties with purification), brine (75 mL), dried over anhydrous MgSO₄, filtered through Celite™, and concentrated in vacuo (40 °C, 50 Torr) giving the crude product as a brown solid.

The crude solid is then dissolved in acetone and precipitated as a white solid with diethyl ether which is isolated by vacuum filtration to give 2.15 as a white solid (3.176 g, 89% yield).
\[ ^1 \text{H NMR} (500 \text{ MHz}, \text{CD}_3\text{CN}) \]
\delta 8.04 (d, \text{J} = 8 \text{ Hz}, 2\text{H}), 7.73 (d, \text{J} = 8 \text{ Hz}, 2\text{H}), 7.42 (m, 5\text{H}), 4.20 (2, \text{J} = 17 \text{ Hz}, 2\text{H}),
3.72 (s, 2\text{H}), 3.68 (d, \text{J} = 17 \text{ Hz}, 2\text{H}).

**4-bromophenylboronic acid BIDA ester 2.17.** The glassware for this reaction was not dried and the procedure was performed under ambient atmosphere. A 100-mL, single-necked, round-bottom flask, equipped with a magnetic stir bar is charged with 4-bromophenylboronic acid (3) (2.015 g, 10.03 mmol, 1 equiv) and N-benzyliminodiacetic acid (2.13) (2.465 g, 11.04 mmol, 1.10 equiv). To the flask with stirring is then added 50 mL of a freshly prepared mixture of DMSO:benzene 1:1. The flask is then fitted with a benzene-filled Dean-Stark trap topped with a water-cooled Graham condenser. All ground glass joints are sealed with Apiezon H high temperature vacuum grease and secured with Keck clips. The arm of the Dean-Stark trap is wrapped with aluminum foil to facilitate heat transfer.

The stirred reaction mixture is brought to reflux using a heating mantle. The reaction mixture is allowed to reflux for 12 hours. The heating mantle is removed, and the reaction is allowed to cool to 23 °C with stirring for 1 hour. The Dean-Stark trap and magnetic stir bar are removed and the reaction mixture is concentrated \textit{in vacuo} (40 °C, 15 Torr) to afford the crude product in solution with DMSO. This residue is dissolved in ethyl acetate (250 mL) and transferred to a 500-mL separatory funnel. This organic layer is washed with water (10 x 75 mL) (These successive water washes are crucial for the quantitative removal of DMSO. If not
effectively removed, DMSO may result in difficulties with purification), brine (75 mL), dried over anhydrous MgSO$_4$, filtered through Celite™, and concentrated *in vacuo* (40 °C, 50 Torr) giving the crude product as a brown solid.

The crude solid is then dissolved in acetone and precipitated as a white solid with diethyl ether which is isolated by vacuum filtration to give 2.17 as a white solid (3.275 g, 84% yield).

\[ \text{H NMR (500 MHz, CD}_3\text{CN)} \]

\[ \delta 7.60 (d, J = 8 \text{ Hz}, 2H), 7.54 (d, J = 8 \text{ Hz}, 2H), 7.43 (m, 5H), 4.17 (2, J = 17 \text{ Hz}, 2H), 3.72 (s, 2H), 3.65 (d, J = 17 \text{ Hz}, 2H). \]

**Setup of mock crude reactions.** The following is the procedure for setting up, running, and analyzing the mock crude reaction experiments as shown in Figure 2.9.

*Preparation and installation of the precipitation chamber:* A new, fritted, 12-g Luknova polypropylene cartridge containing a PTFE-coated magnetic stir bar was charged with Celite (ca. 150 mg) and 3-aminopropyl functionalized silica gel (ca. 250 mg) and capped. This cartridge will serve as the precipitation chamber during the purification process. This mixture was suspended in hexanes (ca. 5 mL) and vigorously swirled to mix the solids. The mixed suspension was allowed to settle for ca. 5 seconds, then the liquid was drained by forcing a plug of ambient air through the top of the cartridge by syringe. The stir bar was now firmly embedded in the solids to prevent stirring before the precipitation chamber was filled with the simulated reaction mixture. It has been observed that stirring in the dry chamber causes damage to the frit resulting in increased clogging. The precipitation chamber was now installed into the luer fittings on the purification carousel.

*Preparation and installation of the silica gel plug:* A silica gel plug chromatography cartridge was freshly prepared from custom PTFE fittings using unfunctionalized silica gel. The cartridge was similar to the Luknova cartridges except that the significantly smaller bottom
fitted fitting containing the silica was fashioned of PTFE rather than polypropylene. The cartridge was charged with silica gel by addition of silica gel during vacuum aspiration at the bottom luer fitting. This process ensures that fewer gaps are introduced in the silica gel plug. Excess silica gel was removed by spatula, and a frit was placed on top of the silica plug to ensure retention of all solids in the cartridge during operations where solvent flows from the bottom of the cartridge to the top. This doubly-fritted cartridge was secured with a cap from a 4-g Luknova polypropylene cartridge and placed into the luer fittings on the purification carousel on the automated synthesizer.

Pre-activation of the catalyst solution: Palladium(II) acetate (0.001875 mmol, 2.5 mol%) and SPhos (0.00375 mmol, 5mol%) per purification to be run were combined in an 8-mL scintillation vial equipped with a PTFE-coated magnetic stir bar and placed under an argon atmosphere. THF was added to generate a 0.01 M catalyst stock solution (with respect to palladium(II) acetate), and it was stirred vigorously for 30 min at room temperature to generate an orange, yellow, or clear solution. After this activation process, 1 mL of catalyst stock solution was added to the solution in the polypropylene cartridge containing the mock reaction mixture.

Preparation and installation of the mock reaction chamber: A new fritted 12-g Luknova polypropylene cartridge was charged with MIDA boronate (0.075 mmol, 1equiv), 4-methylbenzene boronic acid (0.075 mmol, 1equiv), and THF (10 mL). After addition of the pre-activated catalyst solution, the cartridge was installed into the luer fittings in the reaction block of the automated synthesizer. Once all cartridges were in place, the automated purification routine was run using the computer interface. The samples were collected as THF solutions into tared 40-mL scintillation vials.

Concentration, azeotropic drying, and analysis of recovered materials from purifications: The THF solutions were concentrated under reduced pressure on a rotary evaporator, then the residue was azeotroped with dichloromethane (3x5 mL) to remove residual solvents. These residues were then placed under vacuum for 12-36 hours, after which the yield and purity were determined by comparison of 1H NMR in acetone-\textit{d6} with a standard sample of the desired MIDA boronate and with a sample taken of a simulated reaction mixture.
Automated purification summary. The following is a detailed list of the operations carried out, in order, by the automated MIDA boronate purification platform for the mock crude reaction experiments shown in Figure 2.9 (see Chapter 5 for more details).

1. In the background, the Auxiliary Pump aspirates 6 mL of hexanes and delivers it to the bottom of the precipitation chamber, through the silica gel column. This is repeated once for a total of 12 mL of hexanes.

2. The Primary Pump aspirates 9 mL of reaction mixture from reaction chamber bottom and returns 6 mL, through the bottom, to ensure no more than 3 mL will be delivered to the precipitation chamber.

3. The Primary Pump delivers 3 mL of reaction mixture to the top of the precipitation chamber containing 12 mL of hexanes. This induces MIDA boronate precipitation from the THF solution. The Primary Pump then delivers two 10-mL plugs of dry nitrogen to bottom of precipitation chamber (bypassing the silica gel column) to dislodge the stir bar.

4. The suspension in the precipitation chamber is aspirated from the bottom and through silica gel column by the Auxiliary Pump. The eluent is sent to waste.

5. Steps 1-4 are repeated three additional times to send all of the reaction mixture to the precipitation chamber.

6. The Primary Pump aspirates 1.5 mL of THF and delivers it to the top of the reaction chamber as a rinse. Steps 1-3 are repeated.

7. The Primary Pump aspirates 1.5 mL of THF and delivers it to the top of the reaction chamber as a rinse. Steps 2-3 are repeated.

8. Step 4 is repeated.

9. Steps 1-4 are repeated.

10. Step 4 is repeated.

11. The Primary Pump aspirates 6.5 mL of 1.5% (v/v) MeOH in Et₂O and delivers it to the top of the precipitation chamber. This is repeated once for a total delivery of 13 mL of solvent.

12. The Primary Pump delivers two 10-mL plugs of dry nitrogen to the bottom of the precipitation chamber (bypassing the silica gel column) to dislodge the stir bar.

13. Step 4 is repeated.
14. Steps 11-13 are repeated. Step 4 is repeated again.
15. Steps 11-13 are repeated twice with Et$_2$O instead of 1.5% (v/v) MeOH in Et$_2$O. Step 4 is repeated twice more to dry out the silica gel column.
16. The Auxiliary Pump is rinsed with 2 x 1 mL of THF to wash away any residual MeOH. The wash THF is sent to waste.
17. The Auxiliary Pump aspirates 6 mL of THF and delivers it slowly to the bottom of the precipitation chamber through the silica gel column. This is repeated once for a total of 12 mL of THF.
18. The Primary Pump aspirates 5 mL of dry nitrogen and delivers it to the bottom of the precipitation chamber (bypassing the silica gel column) to agitate the suspension, thus promoting mixing and MIDA boronate dissolution. This is done 40 times.
19. The THF solution of MIDA boronate is aspirated by the Primary Pump out of the bottom of the precipitation chamber (bypassing the silica gel column). The solution is delivered to the collection tube. This aspiration/delivery is repeated an additional 5 times to ensure full transfer.
20. The Auxiliary Pump pushes residual THF in the silica gel column into the bottom of the precipitation chamber as a rinse.
21. The Primary Pump aspirates 5 mL of dry nitrogen and delivers it to the bottom of the precipitation chamber (bypassing the silica gel column) to agitate the suspension, thus promoting mixing and MIDA boronate dissolution. This is done 5 times.
22. The THF rinse is aspirated by the Primary Pump out of the bottom of the precipitation chamber (bypassing the silica gel column). The solution is delivered to the collection tube.

REFERENCES

CHAPTER 3
DEVELOPMENT OF AN AUTOMATED DEPROTECTION PLATFORM

Steven G. Ballmer, Eric P. Gillis, and Martin D. Burke

This chapter describes the development of an automated deprotection platform for MIDA boronates including the construction of a fully automated deprotection module. Critical to the successful implementation of this automated deprotection platform was the discovery that MIDA boronates universally undergo deprotection under general mild conditions. Specifically, upon treatment with aqueous sodium hydroxide at room temperature for 20 minutes, MIDA boronates are universally and cleanly hydrolyzed to their corresponding boronic acids. Additionally, a simple predictable algorithm for both the aqueous volumes produced in these deprotections and the evaporation rates of the resulting organic solutions have enable the development of a fully automated MIDA boronate deprotection module that operates on a single set of general conditions. For specific details on the hardware and software control of this automated deprotection platform please see Chapter 5.

Eric P. Gillis contributed to the design, construction, development, and implementation of the earlier configurations of the automated deprotection platform as summarized in Figure 3.1.
3-1 HYDROLYSIS OF BORONATES

In the course of our laboratory’s development of iterative cross-coupling (ICC) and MIDA boronates in general it has been found that anhydrous conditions are essential for successful ICC sequences. That is, MIDA boronates are susceptible to hydrolysis in the presence of aqueous base and performing a cross-coupling reaction under any of the commonly used biphasic aqueous conditions provides a source of aqueous base. The resulting system, then, provides conditions for in situ hydrolysis of the bifunctional MIDA boronate coupling partner which leads to competitive random oligomerization over productive coupling. This can even occur as a result of the inclusion of protic solvents like methanol and isopropanol.

Given this necessity of anhydrous conditions, at the conception of the automated MIDA boronate deprotection platform it was understood that there was really only one starting requirement: the boronic acid resulting from the deprotection of the corresponding MIDA boronate had to be in an anhydrous form. This “anhydrous form” did not necessarily mean having the boronic acid as the dehydrated boroxine, but rather simply as the free acid without exogenous water. The most logical and practical source of the anhydrous form of boronic acid would be simply a solution of the boronic acid is dry solvent. This simple goal (a boronic acid in dry solvent), however, presented a significant challenge for an automated platform at the time for. Specifically, the most common method for deprotecting a MIDA boronate is treatment with aqueous sodium hydroxide (NaOH). A dry solution of the resulting boronic acid is easily obtainable by standard manual methods (e.g. phase separation followed by drying over anhydrous drying agents like magnesium or sodium sulfate), but it was unclear at the time how the bulk and resulting residue water could be removed automatically. Furthermore, the partition coefficient of boronic acids between the organic and aqueous layers is not general and it was unclear how the boronic acid could be extracted effectively and generally in an automated fashion. Additionally, at the time there was not a simple and efficient method for the automatic concentration of the boronic acid solution.

With these challenges in mind we targeted new methods for MIDA boronate deprotection that excluded, or at least minimized, the use of bulk water thereby making the method more amenable to automation.¹ The standard aqueous basic deprotection condition is 0.1 M THF and 3 equivalents NaOH as 1 M aqueous NaOH (3.3:1 organic:aqueous). Initial experiments attempted
to minimize the water content by simply using a smaller volume of more concentrated aqueous NaOH. Deprotections run at 5:1, 10:1, and 20:1 organic:aqueous were effective in forming the boronic acid in full conversion, but all remained too wet for further consideration. Specifically, the organic phase was dried over a series of anhydrous drying agents including magnesium sulfate (MgSO$_4$), sodium sulfate (Na$_2$SO$_4$), calcium sulfate (CaSO$_4$), potassium carbonate (K$_2$CO$_3$), and molecular sieves and the efficiency of the drying was determined by Karl-Fischer titration. Molecular sieves were found to be the only effective drying agent, but the large amount of sieves required (in excess of 8-10 grams of sieves to reach < 100 µg/mL water content) caused significant loss of boronic acids via adsorption. It should be noted here that the water content data for the Karl-Fischer titrations were collected on mock deprotections where the MIDA boronate was left out, because Karl-Fischer experiments are not compatible with boronic acids. Furthermore, it should be noted that the activation method for the molecular sieves was found to be crucial. The only acceptable method for activation was heating the sieves to 300 °C under ambient atmosphere and pressure for 24 hours and then cooling to room temperature under dry nitrogen or argon.$^2$ Any other method led to incomplete activation or in some cases (e.g. flame drying under vacuum) destruction of the zeolite matrix.

With the goal of continuing to minimize the bulk water content in the deprotection, three relatively anhydrous methods were investigated (Figure 3.1).$^1$ In a limiting case of using smaller volumes of concentrated aqueous base as described above, 4-tolyl MIDA boronate (3.1) was deprotected in the presence of potassium hydroxide (KOH) and 99:1 THF:water (Figure 3.1, A). These conditions resulted in the formation of a large amount of colorless precipitate. This precipitation is consistent with the formation of the trihydroxy borate salt 3.2.$^3$ While the dipotassium salt of MIDA (3.3) was not isolated or characterized it was the predicted byproduct and was likely a part of the precipitate. Treatment of the crude deprotection reaction with HCl in dioxanes caused the dissolution of some of the precipitate and afforded free 4-tolyloboronic acid (3.4) upon isolation. Although the 3.4 was successfully isolated with a minimum of bulk water, the remaining precipitate was a sticky residue that was not ideal for the intended automated process (any remaining solids, especially sticky residues could cause clogs). Furthermore, when this method was applied to other MIDA boronates, incomplete conversion in the deprotection was observed due to the precipitate preventing efficient phase mixing.
Figure 3.1. Initial conditions explored for automated MIDA boronate deprotection. Minimally wet conditions with KOH afforded an insoluble borate salt 3.2 (A). Use of NaOMe afforded borate 3.6 as part of a thick slurry gel (B). Use of dried basic resin (Amberlyst A26) afforded an immobilized borate 3.2 that required acidification (C).

In an attempt to avoid the organic/aqueous phase separation problem entirely, sodium methoxide (NaOMe) was investigated as a source of base (Figure 3.1, B). Here, 3.1 in THF was treated with NaOMe. This afforded a very thick slurry/gel, assumedly containing trimethyl borate salt 3.6, that actually stopped the stirring process and stalled the conversion of the reaction. It should be noted here that the structure of 3.6 was not confirmed. Although it excludes water, the lack of conversion in this method combined with the formation of potential clog-causing solids made it particularly unattractive for pursuing as an automatable deprotection method.

A final, and ultimately tractable, method for MIDA boronate deprotection under minimized water conditions was the use of hydroxide in the form of an immobilized base source (Figure 3.1, C). Specifically, a THF solution of 3.1 was treated with dried Amberlyst A26 (OH), which is a macroporous resin that is functionalized with ammonium residues with hydroxide counter ions. Although the structure was not confirmed, it is assumed that deprotection proceeded via 3.2 as an immobilized trihydroxy salt. Regardless of the structure, the deprotection proceeded in full conversion of 3.1 and afforded 3.4 after acidification with HCl in dioxanes. Because it utilized only residual water, proceeded in good conversion and yield, and had the
added benefit of being agitated by simple air bubbling of the resin this method stood out as a particularly attractive method to pursue for an automated MIDA boronate deprotection platform. Eric P. Gillis contributed heavily to the development of this method and to further extensive investigations of Amberlyst A26 (OH) in MIDA boronate deprotections.¹

Both HCl in dioxanes and anhydrous acetic acid in THF were examined as acidification reagents and both were found to be effective in the isolation of boronic acids. It should be noted that the developed method for these MIDA boronate deprotections included an acid neutralization step, but residual acid had the potential to be included in downstream manipulations. Quite surprisingly, though, it was observed that neither acid adversely affected downstream chemistry in the presence of acid-sensitive functional groups. Specifically, when using HCl as an acidification reagent in the deprotection of an E,E-dienyl MIDA boronate, no isomerization of the corresponding E,E-dienyl boronic acid was observed. Furthermore, MOM protecting groups were also observed to be stable to treatment with HCl. This method was used effectively on a fully automated system similar to the one described below. However, after prolonged use of HCl in dioxanes, which is an aggressively corrosive reagent, as an acidification reagent considerable damage to the metal and plastic components was observed. All of the metal components that had contact with the HCl began developing corrosion and pitting while the plastic components, including PTFE components, became brittle and developed cracks. Additionally, it was found that even minor changes in the preparation of the Amberlyst resin had significant effects on the yield of the boronic acids.

As an effect of what was likely a combination of the problems discussed above, including unforeseen acid sensitivity with downstream chemistry (although the actual source was never implicitly identified), significant irreproducibility in both the automated formation of boronic acids and their subsequent cross-couplings was observed. It was at this point that we questioned whether the standard basic aqueous conditions for MIDA boronate deprotection (THF and aqueous NaOH) would in fact be a tractable solution even though they were previously deemed to be unattractive for the automated platform.

Our laboratory’s collective data suggested more and more that our standard aqueous conditions for MIDA boronate deprotections were not only mild but also surprisingly general, particularly when compared to other boronic acid derivatives. For instance, Suginome’s 1,8-
diaminonaphthelene (DAN) ligand for boronic acid masking requires treatment with aqueous HCl or H₂SO₄ for cleavage. While these conditions are sufficient for the iterative synthesis of oligoarenes, they are not generally amenable to small molecule synthesis. Furthermore, pinacol boronate esters, which are sufficient coupling partners themselves, often require relatively harsh oxidative conditions for deprotection, conditions that, similar to those for the DAN ligand, are not generally amenable to small molecule synthesis. For pinacol boronate esters in particular, significantly milder cleavage conditions utilizing a transesterification procedure with diethanolamine, followed by mild hydrolysis have been reported. These mild conditions, though, while certainly more amenable to small molecule synthesis in general are less attractive for automation due to the multiple transformations required. A facile process for the conversion of pinacol boronate esters to their corresponding boronic acids via the intermediacy of potassium trifluoroborates is also known. This process, similar to the pinacol boronate ester transesterification process discussed above is less than ideal for automation simply due to its multiple transformations. Furthermore, while good yields are observed with this process for aryl pinacol boronate esters, Lloyd-Jones has shown that the aqueous hydrolysis rate of trifluoroborates is highly dependent on the identity of the carbon unit attached to boron (Figure 3.2). Specifically, treatment of trifluoroborate 3.7 with three equivalents of cesium carbonate (Cs₂CO₃) in THF:water (10:1) at 55 °C affords its corresponding boronic acid 3.8. The hydrolytic half lives, however, vary drastically depending on the identity of 3.7. These half lives are reported to be as short as 1 minute in the case of cyclopropyl trifluoroborate (3.7d) or as long as 77,760 minutes (54 days) in the case of 3,5-di(trifluoromethyl)phenyl trifluoroborate (3.7s). Even relatively minor electronic perturbations effect significant changes as is the case when comparing 4-tolyl trifluoroborate (3.7m) with a hydrolytic half life of 51 minutes and phenyl trifluoroborate (3.7n) with a hydrolytic half life of 100 minutes.
In stark contrast to these varying trifluoroborate hydrolytic half lives, the rates of hydrolysis of MIDA boronates under the standard basic aqueous conditions have been observed to be exceptionally similar, regardless of the identity of the carbon unit attached to boron. Specifically, upon treatment with three equivalents of 1 M aqueous NaOH at room temperature, full deprotection is achieved in less than 20 minutes for all MIDA boronates examined in our laboratory to date. The single exception to this general deprotection condition is aryl MIDA boronate 3.9 (Scheme 3.1), which requires 30 minutes to reach full conversion. We currently hypothesize that this retardation in hydrolysis rate is strictly a result of the sterically encumbered environment around the boron center.
Scheme 3.1. Aryl MIDA boronate 3.9 has been observed to take up to 30 minutes to reach full conversion under the standard aqueous basic deprotection conditions. This is currently thought to be due to the sterically encumbered environment of the boron center.

In addition to demonstrating remarkable generality in hydrolysis rate\textsuperscript{10,11} the standard aqueous basic MIDA boronate deprotection conditions have also proved to be generally free of side reactions; the corresponding boronic acids are easily isolated as pure compounds after the water-soluble MIDA ligand is separated from the organic-soluble boronic acid. With all of the inherent benefits of the aqueous basic conditions we therefore sought to develop the automated MIDA boronate deprotection platform based directly on these general and mild conditions. Confident that the deprotection chemistry would operate generally, our initial concerns (as discussed previously) of 1) how to predict the potentially varying aqueous volumes during the workup for the deprotection reaction, 2) how to deal with unknown and potentially varying partition coefficients during the workup of the deprotection reaction, and 3) how to eliminate bulk and residual water became our primary objectives in the context of automation.

3-2 DESIGN OF THE DEPROTECTION MODULE

Based on the aqueous deprotection platform for MIDA boronates discussed above, we have designed, developed, and constructed a fully automated deprotection module for MIDA boronates in an iterative cross-coupling (ICC) cycle (Figure 3.3, please see Chapter 5 for specific details of the hardware and software control of this module.). As can be seen, the module consists of four major components: the deprotection tube, the pre-drying tube, the drying tube, and the deoxygenation/concentration tube. The module’s base is a custom-made anodized aluminum table that supports a series of luer ports which hold the tubes.
Figure 3.3. Picture of the automated MIDA boronate deprotection module showing the deprotection tube, the pre-drying tube, the drying tube, and the deoxygenation/concentration tube.

The automated platform pictured in Figure 3.3 is represented schematically in Figure 3.4 below. The luer ports mentioned previously are connected to two automated syringe pumps through a series of automated valves and the entire module is controlled remotely by a computer. Collectively, the syringe pumps are connected to: 1) a source of tetrahydrofuran (THF), which is the organic solvent used for the deprotection reaction, 2) a source of diethyl ether (Et₂O), which is the organic solvent used in the separation after the deprotection reaction, 3) a source of 0.5 M aqueous (pH = 6) potassium phosphate buffer, which is the quenching reagent used for the deprotection reaction, 4) a source of water (H₂O), which is the aqueous solvent used for the deprotection reaction, 5) a source of 50% saturated aqueous sodium chloride (NaCl), which is the aqueous solvent used in the separation after the deprotection reaction, and 6) a waste container for disposing of unwanted solutions. Additionally, the bottom of each of the four tubes is connected to one of the automated syringe pumps.
The deprotection tube is a 25-mL polypropylene tube with a fiber frit (20 μm porosity) filled with the starting MIDA boronate and solid sodium hydroxide (NaOH) and topped with a 5-mL polypropylene syringe barrel. This barrel acts as an overflow reservoir in the scenario that the deprotection tube is overfilled or bubbles out of the tube during agitation. With this current system now optimized, we have found that overflowing the deprotection tube is highly unlikely. However, should any malfunction lead to an overflowing situation, the syringe barrel prevents both the loss of potentially value-added building blocks as well as chemical spills and/or contamination of the deprotection module hardware. The bottom of the deprotection tube, as mentioned, is connected to one of the syringe pumps, but is also connected to the other syringe pump via a tee connector between the two pumps. Also as discussed earlier, three equivalents of NaOH (with respect to the MIDA boronate) are always used as a general condition for the deprotection. In the course of our experimentation we have found that this configuration of the module can accommodate deprotections on scales as large as 1.5 mmol of MIDA boronate (4.5 mmol of NaOH) and as small as 0.040 mmol of MIDA boronate (0.121 mmol of NaOH).

The pre-drying tube is a 25-mL polypropylene tube with a fiber frit (20 μm porosity) filled with a mixture of 2.1 g of anhydrous magnesium sulfate (MgSO₄) and 800 mg of Celite® filter aid and topped with a small aluminum foil cap. This foil cap simply minimizes the contact that the anhydrous MgSO₄ has with room atmosphere (and atmospheric moisture), thereby maintaining its anhydrous state as long as possible. Initial designs of the deprotection module actually used only anhydrous MgSO₄ in this tube. We found, however, that the drying agent alone would form hard chunky clathrates upon exposure to water which not only trapped the desired boronic acids but also rendered it a less efficient drying agent. We hypothesized that adding a filter aid like Celite® would prevent the formation of the chunky solids by thinning out the heterogeneous drying agent. Extensive experimental screening of the ratio of anhydrous MgSO₄ to Celite®, as well as the total quantity of drying agent used showed that the 2.1 g of MgSO₄/800 mg of Celite® mixture provided the best balance between maximized yield of the boronic acid (as determined by mass recovery) and minimized water content (as determined by Karl-Fischer titration).

The drying tube is a 25-mL polypropylene tube with a fiber frit (20 μm porosity) filled with 3.6 g of activated 4 Å powdered molecular sieves layered over 300 mg of Celite® filter aid
and topped with a connection to a source of dry nitrogen. This helps to maintain the drying tube under inert atmosphere which provides a starting point for the deoxygenation process. Similar to the pre-drying tube, initial designs of the deprotection module actually used only the molecular sieves in this tube. We found, however, that the drying agent alone formed a thick slurry upon exposure to the wet organic solvent and this caused clogs in the drying tube. We hypothesized that again utilizing Celite® as a filter aid would prevent the thick slurry of molecular sieves from clogging the tube. Like the pre-drying tube, extensive experimental screening of the ratio of molecular sieves to Celite®, as well as the total quantity of drying agent used showed that the 3.6 g of MgSO₄/800 mg of Celite® layering provided the best balance between maximized yield of the boronic acid (as determined by mass recovery) and minimized water content (as determined by Karl-Fischer titration).

The deoxygenation/concentration tube is an empty 25-mL polypropylene tube with a fiber frit (20 μm porosity) topped with a three-way connection to a source of dry nitrogen and room atmosphere. This provides a dual mechanism whereby the deoxygenation process can be maintained under inert atmosphere while the concentration process still has a vent to room atmosphere. The bottom of the deoxygenation/concentration tube is also connected to a source of dry argon via a tee connector between the argon and the syringe pump. The argon, when sparged through the bottom of the tube, provides a method for deoxygenation of the organic solution and, over time, concentrates the solution by evaporating the organic solvent. The delivery of the argon to this tube is controlled by an automated solenoid valve. Please see Chapter 5 for more details of the manual setup and connectivity of the automated MIDA boronate deprotection module.
The intended process for the fully automated MIDA boronate deprotection based on the platform and strategies described above is shown schematically in Figure 3.5. For a hypothetical deprotection of MIDA boronate $3.10$ in the presence of THF and aqueous NaOH at room temperature, boronic acid $3.11$ is afforded. Specifically, to the deprotection tube at room temperature and containing one equivalent of MIDA boronate and three equivalents of NaOH, is added 10 mL of THF and 3 mL of water. Through our experimentation, it was found that 10 mL of THF and 3 mL of water were generally effective for the deprotection transformation independent of the reaction scale. The THF dissolves the MIDA boronate and the water dissolves the NaOH affording a biphasic reaction system. The deprotection reaction is then agitated with argon sparging from the bottom of the deprotection tube. Similar to the
deoxygenation/concentration tube, the delivery of argon here is controlled by an automated solenoid valve. Empirically, it was found that argon at a delivery pressure of 4.5-5.5 psig provided the best results for the deprotection module as a whole. Additionally, in order to avoid overflowing the deprotection tube (into the 5-mL syringe barrel) with overly aggressive argon sparging, delivery of the argon in increasingly long pulses was found to be critical. Specifically, a pattern of 0.5-second pulses (with 1.5-second breaks) for a total of 20 seconds followed by 1-second pulses (with 1-second breaks) for an additional 20 seconds and completed with 2-second pulses (with 2-second breaks) for the final 19 minutes and 20 seconds provides aggressive agitation for 20 minutes without overflowing the tube.

This agitation affords a monophasic crude deprotection reaction containing MIDA ligand (likely as the sodium salt under the basic conditions) and boronic acid 3.11 (likely as the trihydroxy borate under the basic conditions). Then 3 mL of 0.5 M aqueous (pH = 6) potassium phosphate buffer is added to quench the reaction followed by 5 mL of Et₂O. This affords a biphasic mixture with free 3.11 in solution in the ethereal organic layer and the water-soluble free MIDA ligand in solution in the aqueous layer. In practice, TLC’s of both layers indicate insignificant loss of 3.11 to the aqueous layer for all MIDA boronates examined to date. The aqueous layer is disposed of leaving behind the wet ethereal solution of 3.11 in the deprotection tube. This wet solution is then washed with 3 mL of 50% saturated aqueous NaCl which extracts a portion of the bulk water. The aqueous layer is again disposed of leaving behind a drier but still wet solution of 3.11 in the deprotection tube.

This solution is then transferred to the pre-drying tube where the anhydrous MgSO₄ removes the remainder of the bulk water. The resulting drier solution of 3.11 is then transferred to the drying tube where the molecular sieves remove any remaining residual water affording a dry ethereal solution of 3.11. It was found that initially wetting the drying agents with 4 mL of neat THF before adding the boronic acid solution prevented any loss of volume of the organic solvent. The dry solution of 3.11 is then transferred to the deoxygenation/concentration tube where argon sparging removes dissolved oxygen and evaporates the organic solvent. Like the deprotection tube, it was found that delivery of the argon in increasingly long pulses was critical to prevent the overflow of the deoxygenation/concentration tube. Specifically, starting at 0.5-
pulses with 1.5-second breaks and ramping up to continuous flow provided the best protocol for this process.

Initial experiments investigating the deprotection of 3.1, afforded 3.4 in only 64% yield after the automated process just described. It was found, after manual flushing, that an additional 14% of the theoretical mass of 3.4 remained in the pre-drying and drying tubes collectively. Adding two washes of the drying agents with 6 mL of neat THF (12 mL total) afforded 3.4 in a 76% yield total. This washing protocol was incorporated as a general process for quantitative transfer and maximized yield of 3.11. This entire deprotection sequence described occurs in a fully automated fashion and the resulting dry and deoxygenated THF solution of freshly prepared 3.11 is poised to undergo cross-coupling (see Chapter 4 for details) or isolation. Please see Chapter 5 for more details on the automated deprotection process specific to the automated operations.

![Figure 3.5](image)

**Figure 3.5.** Schematic representation of the automated MIDA boronate deprotection process, including the aqueous deprotection, the two-stage drying process, and the deoxygenation/concentration process.

A particularly noteworthy aspect of this platform and module is that the fully automated MIDA boronate deprotection procedure is highly dependent on reproducible, if not predictable volumes. That is, the volume of both aqueous layers during the workup of the deprotection
reaction, as well as the total volume of solution finally transferred to the deoxygenation/concentration tube has to be predictable. The automated module currently has no method with which to measure the volumes in real time and adjust its liquid handling accordingly, so the volume values have to be preset in the software control by the operator. That means that these values have to be at least reproducible and ideally predictable through some sort of functional algorithm.

In the case of the aqueous separations and extractions after the deprotection reaction, if the volume of the aqueous layer intended to be disposed of is underestimated, then not all of the layer is disposed of. The resulting excess aqueous layer is then transferred to the drying process along with the organic layer. If the there is too much excess water, then the drying agents will be ineffective. On the other hand, if the volume of the aqueous layer is overestimated, then a portion of the organic layer will also be disposed of. This will not only result in a loss of the desired boronic acid, but also decrease the volume of the organic layer in downstream manipulations. In order to gain a better understanding of the aqueous volumes produced during the deprotection process a series of MIDA boronates including 3.1, 4-bromophenyl MIDA boronate, and trans-propenyl MIDA boronate were subjected to the fully automated deprotection at varying reaction scales and the aqueous volumes both after the reaction quench and after the brine wash were measured (Table 3.1). For this small collection of MIDA boronates, the aqueous volumes reproduced over multiple runs of each experiment and in all cases the error was never more than 200 μL. This reproducibility of the volumes was encouraging, but what was particularly surprising was that the aqueous volumes seemed to be independent of the MIDA boronate identity and correlated only to reaction scale. Specifically, for instance, when the deprotections were run on 1.0 mmol scale, the aqueous volume after the reaction quench was 4.0 ± 0.2 mL and the aqueous volume after the brine wash was 6.4 ± 0.2 mL (Table 3.1, entry 1), regardless of the MIDA boronate being deprotected. We were initially concerned that this small data set would not be applicable to a wider range of MIDA boronates. Encouragingly, though, we have found in the course of our experimentation that these scale-dependent aqueous volumes are reproducible over every MIDA boronate we have examined. Moreover, because these volumes are now predictable, the volume values can easily be adjusted in the software control based simply on the
scale of the deprotection being performed and the fully automated procedure can be run with confidence that the aqueous layers will be disposed of with maximum efficiency.

Table 3.1. Reproducible aqueous volumes, after reaction quench and brine wash, during the automated MIDA boronate deprotection process. The aqueous volume is correlated to the scale of the deprotection and not the identity of the group attached to boron.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Deprotection Scale (mmol)</th>
<th>Aqueous Volume After Quench (mL)</th>
<th>Aqueous Volume After Brine Wash (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>4.0 ± 0.2</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.333</td>
<td>3.8 ± 0.2</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.111</td>
<td>3.0 ± 0.2</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>0.067</td>
<td>3.0 ± 0.2</td>
<td>6.4 ± 0.2</td>
</tr>
</tbody>
</table>

Similar volume reproducibility and predictability was required for the concentration process in the deoxygenation/concentration tube. If the total volume of boronic acid solution is underestimated, then the concentration time will not be sufficient to reach the desired volume and the subsequent cross-coupling reaction will then be run at a more dilute concentration than intended. If, however, the total volume of the solution is overestimated, then the concentration time will be too long and the boronic acid solution will be over-concentrated. We observed, encouragingly, that the total volume of ethereal solution finally transferred to the deoxygenation/concentration tube was reproducible over multiple experiments with varying MIDA boronates (20 mL total, please see Chapter 5 for experimental details). In most cases the targeted final volume of the boronic acid solution 9 mL. In order to accurately predict the appropriate concentration time to reach this volume we needed to have a better understanding of the concentration rate with our specific argon sparging system. Furthermore, Et₂O is used as an extraction solvent and ends up, with THF, in the deoxygenation/concentration tube, but it is not the desired solvent for subsequent cross-coupling reactions. We understood from the beginning that the concentration process would evaporate the lower boiling Et₂O before the THF, leaving the boronic acid as a solution in just THF eventually. We did not, however, know how long the concentration process would need to be in order to evaporate all of the Et₂O.

A simple experiment wherein we performed the fully automated deprotection process without a MIDA boronate present allowed us to cleanly probe both the overall concentration rate
and the rate of selective evaporation of Et₂O over THF (Table 3.2). Specifically, upon the first full transfer of dried organic solvent to the deoxygenation/concentration tube (before any of the THF washes for quantitative transfer) there are a total of 12.0 mL of solvent (Table 3.2, entry 1). This solvent mixture is theoretically a 2:1 THF:Et₂O mixture, or 33% Et₂O. ¹H NMR analysis of the solution showed it to be 27% Et₂O, well within the expected error of the experiment considering that 8 mL of THF had been used to wet the drying agents and some of the Et₂O was expected to have already evaporated during liquid handling operations. Once the argon sparging concentration process was initiated, the total volume was measure every 10 minutes and the Et₂O content was determined periodically. After a total of 70 minutes of concentration the total volume had been reduced to 5.25 mL with a 8% Et₂O content (Table 3.2, entry 8). An additional 40 minutes of concentration (110 minutes total) reduced the total volume to 3.0 mL with a 2% Et₂O content (Table 3.2, entry 12).

With the addition of the two 6-mL THF washes described previously the Et₂O content was able to be reduced to significantly less than 1% and the total averaged concentration rate was determined to be 0.1 mL/min (1 mL/10 min). This concentration rate has proved to be generally reproducible over all automated MIDA boronate deprotections examined to date and only slight variations, as expected, have been observed with a dependence on the ambient temperature. To temporarily minimize this temperature dependency, the ambient temperature of the laboratory in which the deprotection module is housed is often kept constantly below 23 °C (approximately 18 °C). This provides much more reproducible concentration results and still averages a 0.1 mL/min concentration rate. Like the aqueous volumes discussed earlier, because the concentration rates are now predictable and reproducible, the concentration times can easily be adjusted in the software control based simply on the known starting volume and the desired final volume of the boronic acid solution.
Table 3.2. Reproducible concentration rates during the automated MIDA boronate deprotection process. Concentration is performed at room temperature (23 °C) with dry argon sparging from the bottom of the deoxygenation/concentration tube. The approximate total volume (starting at 12.0 mL) is measured in 10 minute intervals. The percent diethyl ether (Et$_2$O) in tetrahydrofuran (THF), as determined by $^1$H NMR, is measured at various time points. Average concentration rate over entire process, including THF washes is 0.1 mL/min (1 mL/10 min).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration Time (min)</th>
<th>Approximate Total Volume (mL)</th>
<th>Percent Diethyl Ether in THF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>12.0</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10.0</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>9.0</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>8.25</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>7.5</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>6.8</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>6.0</td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>5.25</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>4.0</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>12</td>
<td>110</td>
<td>3.0</td>
<td>2</td>
</tr>
</tbody>
</table>

3-3 SUMMARY

In summary, we have designed, developed, and constructed a fully automated MIDA boronate deprotection module based on a general MIDA boronate deprotection platform. This module utilizes a series of automated valves and syringe pumps to deprotect MIDA boronates, affording their corresponding boronic acids in a fully automated fashion. More importantly, the platform operates on remarkably general aqueous deprotection conditions (THF, aqueous NaOH, room temperature, 20 minutes) to hydrolyze the MIDA boronates. After reaction quenching, aqueous extraction, and drying the boronic acids are isolated as freshly prepared THF solutions. Additionally, the boronic acid solutions are deoxygenated and concentrated to the appropriate volume to prepare them for use in a subsequent cross-coupling reaction (see Chapter 4 for details).
3-4 REFERENCES

1 Gillis, E. P. Iterative Cross-Coupling With MIDA Boronates. Ph.D. Dissertation, University of Illinois at Urbana-Champaign, Urbana, IL


CHAPTER 4
DEVELOPMENT OF AN AUTOMATED CROSS-COUPLING PLATFORM

Steven G. Ballmer, Eric P. Gillis, and Martin D. Burke

This chapter describes and details the development and construction of a fully automated MIDA boronate cross-coupling platform amenable to use in automated iterative cross-coupling (ICC) sequences. Specifically, the platform is designed to fully automate the Suzuki-Miyaura cross-coupling reaction of boronic acids, as prepared from MIDA boronates (see Chapter 3 for details), to bifunctional halo-MIDA boronates or other halides. Critical to the successful implementation of this automated platform is ready access to large quantities and numbers of building blocks suitable for cross-coupling. Access to these building blocks has been aided by the development of methods for the multi-gram synthesis of MIDA boronates. For specific details on the hardware and software control of this automated cross-coupling platform please see Chapter 5.

Eric P. Gillis contributed to the design and construction of early configurations of the automated cross-coupling platform and to the conceptual development of methods for multi-gram MIDA boronate syntheses.

4-1 MULTI-GRAM MIDA BORONATE SYNTHESIS

In order for the fully automated cross-coupling module, or the fully functional small molecule synthesizer, in general, to be maximally efficient, ready access to large quantities of MIDA boronate building blocks is necessary. That is, the increased speed and efficiency of small molecule synthesis offered by the fully automated platform is only obtainable if there is already a stock of a large number and quantity of building blocks available to funnel into the automated synthesis platform. In this vein, we sought to translate our laboratory’s existing methods for MIDA boronate building block synthesis\(^2^3\) to the decagram scale (Figure 4.1).

![Chemical structures and reactions](image)

**Figure 4.1.** Multi-gram synthesis of: A) \(N\)-methyliminodiacetic acid, B) 4-bromophenyl MIDA boronate as a product of complexation of MIDA to a boronic acid, C) 4-(\(p\)-tolyl)phenyl MIDA boronate as a product of cross-coupling a bifunctional MIDA boronate, and D) 4-(\(p\)-tolyl)phenyl boronic acid as a product of MIDA boronate hydrolysis.

A previously reported synthesis of \(N\)-methyliminodiacetic acid (MIDA, 4.2) proceeds in only moderate yield from the reaction of methylvamine and chloroacetic acid.\(^4\) A more effective
preparation, however, utilizes formalin and formic acid to reductively methylate (the Eschweiler-Clarke conditions) iminodiacetic acid (4.1) in good yield.\textsuperscript{5,6} This particular synthetic route represents a significant improvement because not only is the use of toxic chloroacetic acid avoided, but all of the starting materials and reagents are readily available and inexpensive. Specifically, formalin and formic acid are commodity chemicals and tens of thousands of metric tons of 4.1 are produced annually as a starting material for the synthesis of herbicides, surfactants, and chelating agents.\textsuperscript{7} Furthermore, both 4.1 and MIDA (4.2) are environmentally benign and are both biodegradable.\textsuperscript{8}

We found that we were able to further optimize this method to be even more effective. Specifically, on a hundred-gram scale, 4.1 was methylated to afford MIDA (4.2) in 90\% yield (Figure 4.1, A). Even this method has since been improved upon in our laboratory with contributions from Brice Uno and Jenna Klubnick. By switching the order of addition of reagents (now adding formalin dropwise to a pot of 4.1 in formic acid), running the reaction at a higher concentration (3 M), and using acetone rather than ethanol to crash out the product, we are able to synthesize MIDA (4.2) in nearly quantitative yield on a kilogram scale at an estimated cost, including all chemicals and solvents, of only $0.05/g to $0.10/g.

The first synthesis of MIDA boronates was reported by Mancilla and coworkers.\textsuperscript{9,10} In order to obtain larger quantities of the desired MIDA boronate building blocks, though we sought to optimize their synthesis. Specifically, using a standard Dean-Stark apparatus, the complexation between 4-bromophenylboronic acid (4.3) and MIDA (4.2) proceeded in excellent yield (92\%) to afford 4-bromophenyl MIDA boronate (4.4) on 40-gram scale (Figure 4.1, B). While benzene can be used as the azeotroping solvent in these complexations, and has been shown to be equally effective, here toluene was used to mitigate any toxicity concerns. Also, we have since further optimized this method, where the volume ratio of DMSO:toluene used in the complexation is significantly reduced (1:10 from 1:1). This enables more facile isolation of the resulting MIDA boronate. Furthermore, 4.4 was isolated without the need for column chromatography; simple precipitation from acetone and diethyl ether was sufficient for purification. Subsequently, the selective Suzuki-Miyaura cross-coupling between 4-tolylboronic acid and 4.4 proceeded in good yield (85\%), affording 4-(4-tolyl)phenyl MIDA boronate (4.5) on 20-gram scale (Figure 4.1, C). It is worth noting that this cross-coupling was performed without
the use of a glove box. In order to avoid MIDA boronate hydrolysis during the course of the reaction, though, it is critical to utilize rigorous Schlenk techniques and avoid the introduction of water. Freshly grinding anhydrous K\textsubscript{3}PO\textsubscript{4} using a hot mortar and pestle (recently removed from a 60 °C oven) is a convenient way to maintain anhydrous conditions during the reaction setup. In a final step, demonstrating that the hydrolysis of the MIDA ligand can conveniently be performed on scale, 4.5 was treated with aqueous sodium hydroxide in tetrahydrofuran and after a standard separation of the organic and aqueous layers, 4-(4-tolyl)phenyl boronic acid (4.6) was afforded in nearly quantitative yield (98%) on 6-gram scale (Figure 4.1, D). Throughout this sequence, all purifications/isolations were performed using only standard recrystallization or extraction techniques. Combined with the fact, as mentioned, that the cross-coupling may be performed without the use of a glovebox, there is no clear indication that these manipulations could not be performed on even greater scale, to provide larger quantities of MIDA boronate building blocks.

In addition to their capacity for iterative cross-coupling (ICC), there are numerous enabling features that make MIDA boronate building blocks highly attractive intermediates for organic synthesis. In contrast to their boronic acid counterparts\textsuperscript{11}, MIDA boronates are invariably monomeric and highly crystalline free-flowing solids. They have also proven to be extremely stable to benchtop storage and universally compatible with silica gel chromatography. These highly attractive qualities, combined with the methods for multi-gram scale synthesis of MIDA boronate building blocks detailed here and the further developments of MIDA boronate chemistry in our laboratory\textsuperscript{12,13,14,15,16,17,18,19} have collectively led to the commercialization of more than 140 MIDA boronate building blocks\textsuperscript{20}, a number that is actively growing.

4-2 DESIGN OF THE CROSS-COUPLING MODULE

With the methodology in place to readily access sufficient quantities of MIDA boronate building blocks as described above and based on the fully automated MIDA boronate purification and deprotection platforms (see Chapters 2 and 3, respectively) we have developed and constructed a fully automated cross-coupling module for use in iterative cross-coupling (ICC) sequences of MIDA boronates (Figure 4.2, see Chapter 5 for specific details on the hardware and software control of this automated module).
This module consists of, basically, three heating stir plates each with its own aluminum heating block capable of holding a series of reaction vessels or tubes. A single cross-coupling reaction utilizes a reaction tube and a reaction filtration tube connected by a reaction transfer tube. The reaction tube section of the cross-coupling module is shown in greater detail in Figure 4.3. The reaction tube is topped with an air condenser which condenses the reaction solvent in cases where a cross-coupling reaction is run at elevated temperatures. The reaction tube, seated in a heating block is surrounded by stir-bar-containing empty reaction tubes. In practice we found that a single reaction tube, by itself, struggled to maintain constant stirring because the magnetic stir bar often lost magnetic connection with the stir plate. This problem is avoided by using place holding (“dummy”) tubes that contain only a stir bar to fill the rest of the heating block. Here, the matrix of stir bars creates a stabilizing force of its own magnetic field and the individual stir bars collectively maintain contact with the magnetic stir plate.

![Figure 4.2](image_url)

**Figure 4.2.** Picture of the automated MIDA boronate cross-coupling platform showing the cross-coupling reaction vessel and the reaction transfer system which consists of the reaction transfer tube and the reaction filtration tube.
Figure 4.3. Close-up picture of the automated MIDA boronate cross-coupling platform showing the cross-coupling reaction vessel, the placeholder (“dummy”) tubes, and the reaction transfer tube.

The automated cross-coupling module pictured in Figures 4.2 and 4.3 is represented schematically below in Figure 4.4. As discussed, each section of the module is seated on a magnetic stir plate and each magnetic stir plate has its own custom-milled aluminum heating block. The entire module is connected to an automated syringe pump through a series of automated valves and is controlled remotely by a computer. The syringe pump is connected to: 1) the THF solution of boronic acid (see Chapter 3 for details), 2) the top and the bottom of the reaction tube, and 3) the bottom of the reaction filtration tube. The reaction tube is a 25-mL polypropylene tube with a glass frit (20 μm porosity), a magnetic stir bar, a source of palladium, ligand, inorganic base, and the halide cross-coupling partner. This tube, as mentioned, is topped with an air condenser, which is simply a 10-mL polypropylene tube. The top of this condenser tube is connected to an oil bubbler which helps to maintain an oxygen-free atmosphere during cross-coupling reactions. The reaction filtration tube is another 25-mL polypropylene tube, but
with a fiber frit (20 μm porosity), a magnetic stir bar, Celite® filter aid, and Florisil®. The reaction and reaction filtration tubes are connected by a length of tubing, the reaction transfer tube. This tubing specifically connects the bottom interior of the reaction tube to the top of the reaction filtration tube. We knew from the beginning that at the completion of a cross-coupling reaction, the insoluble inorganic base (e.g. K$_3$PO$_4$, K$_2$CO$_3$, Cs$_2$CO$_3$) needed to be removed by some style of automated filtration before submitting in crude reaction solution to automated purification (see Chapter 2 for details). Initially, the automated cross-coupling platform was designed without the reaction transfer or reaction filtration tube and where the reaction tube had only a fiber frit. In theory, at the completion of a cross-coupling reaction, the automated syringe would aspirate the crude reaction solution through the bottom of the reaction tube, thereby filtering out the inorganic base on the fiber frit. In practice, however, we found that the prolonged aggressive stirring of the stir bar on the fiber frit caused significant damage to the frit’s surface, which resulted in severe clogs during filtration. We hypothesized that a more durable glass frit would hold up to the stir bar’s repeated impact, thereby retaining its pore integrity and preventing any clogs. While the implementation of the glass frit did help prevent any surface damage from the stir bar, we subsequently found that many cross-coupling reactions produced a crude mixture so fine and with such a high propensity to clog that the integrity of the frit was irrelevant and severe clogging was observed anyway. At this point we hypothesized that utilizing a filter aid to filter out the insoluble components of the crude cross-coupling reaction was the only general solution to the clogging problem. Specifically, we designed the reaction transfer tube to transfer, under reduced pressure supplied by the automated syringe pump, the heterogeneous crude reaction mixture from the reaction tube to the reaction filtration tube. Empirically, we found that a mixture of Celite® and Florisil® (2.5 g:1.25 g) provided the best filter aid. Once the heterogeneous crude reaction mixture is stirred with the filter aid, the crude reaction solution can be automatically aspirated, without clogging, from the bottom of the reaction filtration tube and can then be submitted to an automated purification. Please see Chapter 5 for more details on the connectivity of the cross-coupling module.
Figure 4.4. Schematic representation of the automated MIDA boronate cross-coupling platform showing its connectivity to the automated syringe pump system.

The intended process for the fully automated Suzuki-Miyaura cross-coupling of a boronic acid with a bifunctional halo-MIDA boronate based on the platform and strategies described above is shown schematically in Figure 4.5. For a hypothetical cross-coupling of boronic acid 4.7 to bifunctional halo-MIDA boronate 4.8 in the presence of a palladium catalyst, base, and solvent, the resulting cross-coupled MIDA boronate product 4.9 is formed (Figure 4.5, top).

Just like in manual synthesis, we understood that ad hoc optimization of the reaction conditions (i.e. palladium source, ligand, base, solvent, temperature, and time) would likely be necessary to obtain optimized yields on automated cross-coupling reactions as well. We also understood, though, that a set of general cross-coupling conditions that would work for all Suzuki-Miyaura cross-couplings would be highly enabling and particularly beneficial to the efficiency of this automated cross-coupling platform. Clearly, an all-encompassing set of general cross-coupling conditions, while theoretically ideal, is an impractically aggressive goal given the total scope of the Suzuki-Miyaura transformation. We questioned, however, whether there might
be a set of generalized conditions that would be effective across at least a small subset of cross-couplings. This would provide, then, at least a small boost in the efficiency of the automated platform.

Tetrahydrofuran (THF) is commonly used as a solvent in the Suzuki-Miyaura cross-coupling reaction. In addition, we had already found it to be effective in the automated MIDA boronate deprotection (see Chapter 3) and purification (see Chapter 2) platforms. Committed to maintaining efficient connectivity of the automated modules, we locked in THF as the primary reaction solvent for automated cross-couplings. Furthermore, in the course of investigating cross-coupling conditions with manual experiments, we found that anhydrous \( \text{K}_3\text{PO}_4 \) demonstrated some generality as an effective base. Additionally, the collective work of our laboratory showed, serendipitously, that Buchwald’s biarylphoshine ligands, particularly XPhos\(^{21} \) and SPhos\(^{22} \) were somewhat generally effective ligands in cross-couplings of halo-MIDA boronates. This was especially encouraging because the well known air-stability of these ligands makes them easy to handle on the bench top without the need for a glovebox or inert-atmosphere equipment.\(^{23} \) This is a highly attractive property for maximizing the efficiency of the automated cross-coupling module. While we had initially considered maximizing the generality of the conditions by mixing XPhos and SPhos in a multiligand-based approach\(^{24} \) we fortuitously found that Buchwald’s second generation XPhos palladacycle\(^{25} \) provided an easily handleable and surprisingly generally effective catalyst system.

These conditions (THF as solvent, anhydrous \( \text{K}_3\text{PO}_4 \) as base, and PdXPhos as catalyst) provided some generality in productive cross-coupling, but they still failed to provide good yields in some cases. We hypothesized that boronic acid decomposition, both before and during the cross-coupling reaction, was a major cause. To address the problem of boronic acid decomposition before the cross-coupling reaction we took advantage of inherent MIDA boronate properties. Specifically, MIDA boronates are very stable, and thus their clean hydrolysis immediately prior to a cross-coupling reaction ensures that every reaction starts with a pure boronic acid (see Chapter 3). To mitigate the problem of in situ boronic acid decomposition, we took advantage of slow addition cross-coupling (SACC). Our laboratory had found that in situ slow release (slow release cross-coupling, SRCC) of boronic acids from their corresponding MIDA boronates at a rate approaching or slower than productive cross-coupling maintains a low
concentration of boronic acid which minimizes competitive boronic acid decomposition pathways. This results in significantly increased yields in some case.\textsuperscript{26} This same study found that simple slow addition of the boronic acid into the cross-coupling (SACC) provided similar boosts in yield. This slow-addition technique was directly amenable to the automated syringe pump system and was used in conjunction with fresh preparation of the boronic acid in the design and execution of the automated cross-coupling platform.

![Figure 4.5](image_url). Schematic representation of the automated MIDA boronate cross-coupling process, including the slow-addition of boronic acid.

As a final step in maximizing the generality of the automated cross-coupling module, the boronic acid (4.7) is always used in excess with respect to the halide coupling partner (4.8). This biases the cross-coupling reaction toward full conversion to 4.9 and also typically results in the presence of excess unreacted 4.7 at the end of the reaction (Figure 4.5, bottom). In the automated event, the reaction tube contains 4.8, second generation XPhos palladacycle, and anhydrous K$_3$PO$_4$. The reaction tube, reaction transfer tube, and reaction filtration tube are purged with argon to remove oxygen. The reaction tube is stirred and heated to the desired temperature in the heating block, and then the syringe pump adds 3 mL of neat THF from the bottom to activate the
catalyst system. Then, the THF solution of 4.7 (9 mL of solution) is added slowly to the reaction tube from the top. Once fully added the cross-coupling reaction is allowed to stir for an additional time period to reach full conversion. The resulting crude reaction mixture containing insoluble K$_3$PO$_4$ and PdXPhos, excess 4.7 (and its associated decomposition products), and 4.9 in THF is transferred to the reaction filtration tube as described above. Here the base is filtered away from the THF solution and the reaction tube, reaction transfer tube, and reaction filtration tube are all subsequently washed with neat THF for quantitative transfer. This entire sequence occurs in a fully automated fashion and the resulting THF solution of the crude MIDA boronate (4.9) is poised to undergo purification in the automated purification module (see Chapter 2). Please see Chapter 5 for more details on the automated cross-coupling process specific to the automated operations.

It is important to note that the stir plate for the reaction filtration tube, like the syringe pump and valves, is also controlled remotely by the same computer and software. As detailed above, the reaction filtration tube contains a magnetic stir bar and filter aid. Specifically, the stir bar is at the bottom of the tube and is covered with the filter aid (Figure 4.6, A). In an early configuration of the automated cross-coupling module, this stir plate was operated only manually. That is, the stir plate was manually turned on at the beginning of the reaction and left on for the duration. This meant that the reaction filtration tube was being agitated for the entire time the cross-coupling reaction was going (up to 24 hours in some cases). By the time the reaction filtration tube was utilized, the stir bar had moved to the top of the filter aid layer and was too far from the stir plate to maintain a magnetic connection (Figure 4.6, B). Because it had lost magnetic connection, the stir bar could not mix the crude reaction mixture with the filter aid, which invariably resulted in a clog at the interface. This movement of the stir bar was a result of the phenomenon of granular convection (also referred to as the “Brazil nut effect”). Specifically, the continuous motion of the stir bar created a vibration in the reaction filtration tube which allowed the relatively smaller, although less dense particles of the filter aid, a small amount at a time, to settle underneath the stir bar. Over a prolonged time, the stir bar literally climbed to the top of the filter aid layer. Conceptually, the granular convection problem would be mitigated by simply stirring the reaction filtration tube only when it was needed. This would maintain magnetic connection between the stir plate and
stir bar thereby enable mixing of the filter aid and crude reaction mixture which prevents clogs (Figure 4.6, C). Not wanting to compromise the fully automated nature of the cross-coupling platform by manually turning on the stir plate during an experiment, we connected the reaction filtration stir plate to the cross-coupling module via a solid-state relay. This enables remote control of the power and therefore stirring of the plate.

![Figure 4.6](image.png)

Figure 4.6. Schematic representation of granular convection (the “Brazil nut effect”) in the reaction filtration tube used in the automated cross-coupling module. A) The unstirred reaction filtration tube before the addition of the crude reaction mixture. The stir bar remains on the bottom of the tube, covered with the Celite/Florisil mixture. B) The continuously-stirred reaction filtration tube after the addition of the crude reaction mixture. The stir bar, under granular convection, has moved to the top of the Celite/Florisil mixture, lost magnetic contact with the stir plate, and will not stir the filter aid which causes clogs. C) The automatically stirred reaction filtration tube after the addition of the crude reaction mixture. The stir bar, not susceptible to granular convection, has stayed at the bottom of the tube and mixes the filter aid with the crude reaction mixture which prevents clogs.

While all liquid handling manipulations and operations in the cross-coupling module are performed in a fully automated fashion, as is the stirring control of the reaction filtration tube as just discussed, the heating stir plate for the reaction tube is still controlled manually. That is, the temperature and stir rate are set and turned on and off manually by the operator. In practice the temperature and stir rate are preset and the plate is turned on at the beginning of an experiment. This way, no manual intervention is required after the initiation of the sequence.

4-3 SUMMARY
In summary, we have designed, developed, and constructed a fully automated cross-coupling platform and module amenable to automated iterative cross-coupling (ICC) sequences of MIDA boronates. Specifically, the module can fully automate the Suzuki-Miyaura cross-coupling reaction of boronic acids to bifunctional halo-MIDA boronates or other halide coupling partners. This platform was made possible by the development of methods for multi-gram synthesis of MIDA boronates as well as the development of somewhat general cross-coupling conditions.

4-4 REFERENCES

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CHAPTER 4: EXPERIMENTAL SECTION

**General methods and materials.** Commercial reagents were purchased from Sigma-Aldrich, EMD Millipore, Fisher Scientific, Alfa Aesar, Frontier Scientific, Inc., Oakwood Products, Inc., or Strem and were used without further purification unless otherwise noted. Manual syntheses were carried out in oven or flame-dried glassware and performed under a dry inert atmosphere unless otherwise noted. Unless otherwise noted, Celite™ refers to Celite™ 545 filter aid (not acid washed). Solvents were purified via passage through packed columns as described by Pangborn and coworkers (THF, Et₂O, CH₃CN, CH₂Cl₂: dry neutral alumina; hexanes, benzene, toluene: dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves. Water was deionized. Triethylamine, diisopropylamine, diethylamine, pyridine, and 2,6-lutidine were freshly distilled under an atmosphere of dry nitrogen from CaH₂.

Thin layer chromatography (TLC) was performed using the indicated eluent on E. Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by exposure to a UV lamp (λ = 254 and/or 366 nm) and/or a basic solution of KMnO₄ followed by brief heating with a Varitemp® heat gun. Flash chromatography was performed as described by Still and coworkers using EM Merck silica gel 60 (230-400 mesh). ¹H NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500, Varian VXR 500, or Varian Unity Inova 500NB. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CDCl₃, δ = 7.26; CD₃CN, δ = 1.94, center line; (CD₃)₂CO, δ = 2.05, center line; CD₂Cl₂, δ = 5.32, center line; (CD₃)₂SO, δ = 2.50, center line) or to add tetramethylsilane (δ = 0.00). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, br = broad, app = apparent, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets), coupling constant (J) in Hertz (Hz), and integration. ¹³C NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500 or Varian VXR 500. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl₃, δ = 77.16, center line; CD₃CN, δ = 1.32, center line; (CD₃)₂CO, δ = 29.84, center line; CD₂Cl₂, δ = 53.84; (CD₃)₂SO, δ = 39.52, center line) or to add tetramethylsilane (δ = 0.00). Carbons bearing boron substituents were not
observed due to quadrupolar relaxation. $^{11}$B NMR were recorded using a General Electric GN300WB instrument and referenced to an external standard of BF$_3$•Et$_2$O. Low resolution mass spectra (LRMS) and high resolution mass spectra (HRMS) were performed by Furong Sun, Steve Mullen, and Elizabeth Eves at the University of Illinois School of Chemical Sciences Mass Spectrometry Laboratory.

![Chemical structure diagram]

$N$-methylinododiacetic acid (4.2). The glassware for this reaction was not dried and the procedure was performed under ambient atmosphere. A 1000-mL, single-necked, round-bottom flask, equipped with a magnetic stir bar is charged with iminodiacetic acid (4.1) (91.74 g, 689.2 mmol, 1 equiv) and formalin (82.0 mL, 89.4 g, 1.04 mol, 1.51 equiv) to give an off-white suspension. The flask is then fitted with a water-cooled Allihn condenser (50 cm) topped with a 125-mL pressure-equalizing addition funnel filled with formic acid (78.0 mL, 95.2 g, 2.07 mol, 3.00 equiv). Use of a shorter Allihn condenser results in loss of the volatile formaldehyde reagent. Failure to condense the formaldehyde also results in significant formation of paraformaldehyde on the condenser and addition funnel. Paraformaldehyde can be easily removed with an alcoholic sodium hydroxide solution. All ground glass joints are sealed with Apiezon H high temperature vacuum grease and secured with Keck clips.

The stirred reaction mixture is brought to reflux using a heating mantle, and then the formic acid is added dropwise over 20 minutes (approx. 4 mL/min). Addition of formic acid results in effervescence of CO$_2$, which can become quite vigorous if the addition is performed too quickly. During this time, the reaction mixture becomes clear and orange. The addition funnel is then removed and the solution is allowed to reflux for 2 hours. At the end of 2 hours the heat source is removed and the solution is allowed to cool to 23 ºC over 20 minutes. The Allihn condenser is then removed and deionized water (50.0 mL) is added. The reaction mixture is then poured into a 2000-mL Erlenmeyer flask equipped with a large magnetic stir bar. Deionized water (2 X 50 mL) is used to quantitatively transfer the contents of the reaction mixture to the
Erlenmeyer flask. To the vigorously stirred reaction mixture is then added 1500-mL of absolute ethanol in six 250-mL portions leading to the precipitation of large white crystals. The precipitate is then collected by vacuum filtration. The 2000-mL Erlenmeyer flask is then rinsed with three 100-mL portions of absolute ethanol, with each washing being poured over the collected precipitate. The precipitate is allowed to air dry on the frit for 5 minutes. The frit is then placed into a large vacuum desiccator and residual solvent is removed under reduced pressure (23 °C, 1 Torr) for 12 hours to give the title compound (4.2) as a free-flowing, air-stable, white powder (91.37 g, 621.0 mmol, 90.1% yield).

![Chemical Structure](image)

**mp (uncorrected)**

216 °C dec.

**H NMR (500 MHz, D₂O)**

δ 3.94 (s, 4H), 2.97 (s, 3H).

**C NMR (125 MHz, 95:5 DMSO-d₆:D₂O w/ TMS)**

δ 168.3, 57.6, 43.0.

**IR (Nujol mull, cm⁻¹)**

679, 691, 721, 886, 905, 959, 980, 1018, 1064, 1129, 1170, 1225, 1277, 1334, 1377, 1417, 1462, 1694, 2853, 2923, 2953 3421.

**LRMS (ESI+) m/z (rel. intensity)**

149 (6), 148 (M⁺, 74), 142 (8), 130 (10), 126 (18), 123 (19), 103 (6), 102 (100), 98 (5).

**HRMS (ESI+)**

Calculated for C₅H₁₀NO₄ [M+H⁺]: 148.0610
**Elemental Analysis**

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<th></th>
<th>Calculated</th>
<th>Found</th>
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<tbody>
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<td>N, 9.52</td>
<td>panel</td>
<td>9.37</td>
</tr>
</tbody>
</table>

**4-bromophenylboronic acid MIDA ester (4.4).** The glassware for this reaction was not dried and the procedure was performed under ambient atmosphere. A 250-mL, single-necked, round-bottom flask, equipped with a magnetic stir bar is charged with 4-bromophenylboronic acid (4.3) (25.049 g, 124.73 mmol, 1 equiv) and N-methyliminodiacetic acid (4.2) (20.176 g, 137.13 mmol, 1.10 equiv). To the flask is then added 50 mL of a mixture of dimethyl sulfoxide:toluene 1:1 to afford a light orange heterogeneous reaction mixture. The flask is then fitted with a toluene-filled Dean-Stark trap topped with a water-cooled Graham condenser. All ground glass joints are sealed with Apiezon H high temperature vacuum grease and secured with Keck clips. The arm of the Dean-Stark trap is wrapped with aluminum foil to facilitate heat transfer.

The stirred reaction mixture is brought to reflux using a heating mantle. The reaction mixture is allowed to reflux for 3 hours, during which time the reaction darkens in color and becomes homogenous, giving a clear, brown solution. The heating mantle is removed, and the reaction is allowed to cool to 23 °C with stirring for 1 hour yielding a chunky, tan precipitate. At this point there is more solid than solvent and as such the reaction appears completely solid. The Dean-Stark trap and magnetic stir bar are removed and the reaction mixture is concentrated on a rotary evaporator (50 °C, 15 Torr and then 100 °C, 1 Torr) to afford the crude product as a dark tan, chunky solid.
The product is quantitatively transferred, using 500 mL of ethyl acetate in several portions, to a 4000-mL separatory funnel containing 600 mL of a saturated aqueous sodium chloride solution. To the separatory funnel is added another 1500 mL of ethyl acetate (2000 mL total) and 50 mL of deionized water to redissolve precipitated salt. The layers are separated, and the organic layer is dried over anhydrous MgSO₄, filtered through Celite™, and concentrated on a rotary evaporator (35 °C, 80 Torr) to afford a yellow-orange solid. To this solid is added 100 mL of hot (55 °C) acetone and the flask is swirled to give a suspension of white precipitate in a clear yellow-orange solution. While the majority of the product dissolves in acetone the suspended white crystals are undissolved product and should not be removed. In one portion, 100 mL of diethyl ether is gently layered over the acetone suspension resulting in the formation of more white precipitate. The flask is sealed with a polyethylene cap and Parafilm® and placed gently into a refrigerator and maintained at 3 °C for 12 hours. The resulting solid product is collected via vacuum filtration and washed with diethyl ether (8 X 25 mL). Residual solvent is removed under reduced pressure (23 °C, 1 Torr) for 6 hours to give the title compound (4.4) as a free-flowing, air stable, white solid (35.802 g, 114.78 mmol, 92.0% yield).

mp (uncorrected)
244-245 °C

$^1$H NMR (500 MHz, CD$_3$CN)
δ 7.55 (dt, $J = 8.5$, 2 Hz, 2H), 7.42 (dt, $J = 8.5$, 2 Hz, 2H), 4.07 (d, $J = 17$ Hz, 2H), 3.89 (d, $J = 17$ Hz, 2H), 2.50 (s, 3H).

$^{13}$C NMR (125 MHz, CD$_3$CN)
δ 169.4, 135.5, 131.9, 124.4, 62.8, 48.5.
$^{11}$B NMR (100 MHz, CD$_3$CN)

$\delta$ 12.0.

IR (thin film, acetone, cm$^{-1}$)

645, 706, 813, 867, 962, 977, 997, 1038, 1060, 1088, 1106, 1155, 1181, 1220, 1238, 1266, 1297, 1339, 1384, 1421, 1445, 1459, 1751, 2951, 2997.

LRMS (EI$^+$) $m/z$ (rel. intensity)

313.9 (9.6), 312.9 (74.4), 311.9 (27.1), 310.9 (M$^+$, 76.7), 309.9 (18.8), 284.9 (32.8), 282.9 (33.6), 256.9 (24.8), 255.9 (99.9), 254.9 (48.7), 253.9 (100.0), 252.9 (24.0), 128.0 (41.5), 100.0 (78.5), 72.0 (21.6), 56.9 (77.6).

HRMS (EI$^+$)

Calculated for C$_{11}$H$_{11}$BBrNO$_4$ [M$^+$]: 310.99645

Found: 310.99634

Elemental Analysis

Calculated:  C, 42.36  H, 3.55  N, 4.49

Found:  C, 42.20  H, 3.48  N, 4.50

4-(p-tolyl)-phenylboronic acid MIDA ester (4.5). An oven-dried 500-mL Schlenk flask equipped with a magnetic stir bar is charged with palladium (II) acetate (361 mg, 1.61 mmol, 0.02005 equiv) and (2-biphenyl)-dicyclohexylphosphine (1.159 g, 3.307 mmol, 0.04124 equiv) and then quickly sealed with a rubber septum and placed under an inert atmosphere through five
cycles of evacuation (1000 mTorr) and purging with dry argon. Although it is possible to perform this type of selective cross-coupling reaction without the use of a glovebox, it is very important that rigorous Schlenk techniques are utilized to exclude water. Failure to exclude water can result in hydrolysis of the MIDA boronate ester.

Tetrahydrofuran (400 mL) is then cannulated into the 500-mL Schlenk flask resulting in a clear, pale yellow-orange solution. Under positive argon pressure the rubber septum is removed and replaced with an oven-dried water-cooled Graham condenser topped with a rubber septum pierced with a 20G (1.5 inch) needle. Argon is allowed to flow through the system for 60 seconds at which point the vent needle is replaced with an argon inlet needle and the Schlenk valve is closed. The reaction vessel is lowered into an oil bath preheated to 65 °C and the solution is allowed to reflux with stirring for 20 minutes, during which time the catalyst solution turns colorless. The heating bath is then removed and the catalyst solution is allowed to cool to 23 °C over 10 minutes. The Schlenk valve is reopened and under a positive argon pressure the Graham condenser is replaced with a rubber septum and the head space is purged for 60 seconds through a 20G (1.5 inch) vent needle.

In parallel with the catalyst preparation, an oven-dried 2000-mL three-neck round bottom flask equipped with a magnetic stir bar and fitted with an oven-dried water-cooled Graham condenser topped with a hose-barb adapter in the center neck and a rubber septa on each side neck is charged with 4,4 (25.000 g, 80.148 mmol, 1 equiv), p-tolylboronic acid (16.391 g, 120.56 mmol, 1.50 equiv), and freshly-ground, anhydrous potassium phosphate (51.043 g, 240.46 mmol, 3.00 equiv). It is very important that the potassium phosphate is finely ground and that it remains anhydrous throughout this process. This is achieved without the use of a glovebox. The reaction flask is then quickly placed under inert atmosphere (through the condenser top) through five cycles of evacuation (1000 mTorr) and purging with dry argon. Tetrahydrofuran (400 mL) is then added into the 2000-mL flask through one of the side necks resulting in a white suspension. Tetrahydrofuran (400 mL) is then added into the 2000-mL flask through one of the side necks resulting in a white suspension. The catalyst solution is then cannulated into the 2000-mL reaction flask with stirring resulting in a yellow suspension. The reaction flask is lowered into an oil bath preheated to 70 °C and allowed to reflux with stirring for 6 hours. The heat source is then removed and the reaction mixture is allowed to cool for 20 minutes. The Graham condenser and septa are removed from the flask necks and the reaction is quenched with 800 mL saturated ammonium chloride giving a
biphasic mixture comprised of a clear colorless bottom layer and a clear yellow-orange top layer. This mixture is poured into a 4000-mL separatory funnel. The reaction mixture is quantitatively transferred to the separatory funnel with 2 X 200-mL of THF:diethyl ether 1:1. The layers are separated, and the aqueous layer is extracted with 400 mL of THF:diethyl ether 1:1. The combined organic layers are washed with 100 mL saturated aqueous sodium chloride, dried over anhydrous MgSO₄, and filtered through Celite™. The solvent is removed via rotary evaporation (35 °C, 20 Torr). Residual solvent is removed at reduced pressure (23 °C, 1 Torr) to afford the crude product as a yellow solid. Ethyl acetate (75 mL) is added to the crude mixture in three equal portions and the resulting mixture is swirled vigorously to give a yellow slurry. Diethyl ether (400 mL) is then added in 4 equal portions with swirling to give a white precipitate in a clear yellow solution. The precipitate is collected via vacuum filtration and allowed to air dry under suction for 5 minutes. The frit is then transferred to a large vacuum desiccator and residual solvent is removed at reduced pressure (23 °C, 1 Torr) to give the title compound as a free-flowing, air stable, off-white powder (22.008 g, 68.104 mmol, 85.0% yield).

\[
\text{mp (uncorrected)}
\]
\[211 ^\circ C \text{ dec.}\]

\(^1\text{H NMR (500 MHz, CD}_3\text{CN)}\]
\[\delta 7.65 (d, J = 8 \text{ Hz, } 2\text{H}), 7.56 \text{ (app d, } J = 7.5 \text{ Hz, } 4\text{H}), 7.28 \text{ (d, } J = 7.5 \text{ Hz, } 2\text{H}), 4.08 \text{ (d, } J = 17 \text{ Hz, } 2\text{H}), 3.92 \text{ (d, } J = 17 \text{ Hz, } 2\text{H}), 2.55 \text{ (s, } 3\text{H}), 2.38 \text{ (s, } 3\text{H}).\]

\(^13\text{C NMR (125 MHz, CD}_3\text{CN)}\]
\[\delta 169.6, 142.5, 138.6, 138.4, 134.0, 130.5, 127.7, 127.1, 62.8, 48.5, 21.1.\]

\(^11\text{B NMR (100 MHz, CD}_3\text{CN)}\]

δ 12.4.

IR (thin film, acetone, cm⁻¹)
708, 745, 804, 835, 866, 887, 993, 1029, 1042, 1197, 1225, 1287, 1334, 1762, 2858, 2951, 3013.

LRMS (EI⁺) m/z (rel. intensity)
324.1 (20.3), 323.1 (M⁺, 100.0), 322.1 (25.0), 313.0 (12.4), 311.0 (12.5), 285.0 (5.4), 283.0 (5.6), 256.0 (16.5), 254.0 (16.7), 223.1 (16.4), 208.1 (25.9), 194.1 (28.6), 181.1 (11.0), 168.1 (16.9), 141.1 (15.9), 128.1 (17.6), 115.1 (13.5), 101.0 (48.0), 100.1 (21.9), 77.1 (25.2), 57.0 (37.1).

HRMS (EI+)
Calculated for C₁₈H₁₈BNO₄ [M⁺]: 323.13289
Found: 323.13287

Elemental Analysis
Calculated: C, 66.90 H, 5.61 N, 4.33
Found: C, 65.35 H, 5.55 N, 4.21

4-(p-tolyl)-phenylboronic acid (4.6). A 1000-mL, single-necked, round-bottom flask equipped with a magnetic stir bar is charged with 4.5 (9.975 g, 30.87 mmol, 1 equiv), tetrahydrofuran (200 mL), and an aqueous solution of 1 M sodium hydroxide (92.6 mL, 92.6 mmol, 3.00 equiv) to give a biphasic system consisting of a clear colorless bottom layer and a cloudy white top layer. The flask is covered with a polypropylene cap and the reaction is stirred
vigorously at 23 °C for 10 min to give a biphasic system consisting of a clear colorless bottom layer and a clear yellow top layer. A saturated aqueous solution of ammonium chloride (250 mL) is then added. The resulting mixture is poured into a 1000-mL separatory funnel and the reaction vessel is rinsed with four 50-mL portions of diethyl ether, each washing being poured into the separatory funnel. The layers are separated and the aqueous layer is extracted with tetrahydrofuran:diethyl ether 1:1 (400-mL). The combined organic layers are dried over anhydrous MgSO₄, filtered through Celite™, and concentrated via rotary evaporation (35 °C, 20 Torr). The residual solvent is removed via three azeotropic cycles with acetonitrile on a rotary evaporator (3 X 50 mL, 35 °C, 20 Torr) and then at reduced pressure (23 °C, 1 Torr) for 6 hours to afford the title compound as a fine, off-white powder (6.43 g, 30.3 mmol, 98.2% yield).

mp (uncorrected)
130 °C dec.

¹H NMR (500 MHz, 95:5 DMSO-d₆:D₂O w/ TMS)
δ 7.87 (d, J = 8 Hz, 2H), 7.62 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 7.28 (2, J = 8 Hz, 2H), 2.35 (s, 3H).

¹³C NMR (125 MHz, 95:5 DMSO-d₆:D₂O w/ TMS)
δ 141.5, 137.1, 136.9, 134.7, 129.5, 126.4, 125.3, 20.6.

¹¹B NMR (95:5 DMSO-d₆:D₂O w/ TMS)
δ 36.3.

IR (thin film, acetone, cm⁻¹)
807, 1003, 1024, 1091, 1158, 1340, 1380, 1653, 3406.
LRMS (EI⁺) m/z (rel. intensity)
213.1 (14.3), 212.1 (M⁺, 100.0), 211.1 (25.5), 193.1 (10.4), 184.1 (16.8), 169.1 (7.6), 168.1 (50.4), 167.1 (54.3), 166.1 (26.2), 165.1 (9.76), 153.1 (14.0), 152.1 (22.4), 115.1 (9.2), 91.1 (10.2), 57.0 (7.3).

HRMS (EI+)
Calculated for C₁₃H₁₃BO₂ [M⁺]: 212.10087
Found: 212.10095

REFERENCES


CHAPTER 5
A SMALL MOLECULE SYNTHESIZER

Steven G. Ballmer, Seiko Fujii, Junqi Li, Eric P. Gillis, Gregory F. Morehouse, Matthew J. Clark, and Martin D. Burke

This chapter describes the construction of a fully automated small molecule synthesizer that utilizes the iterative cross-coupling (ICC) platform with MIDA boronates. The full synthesizer consists of three distinct modules (deprotection, cross-coupling, and purification) and has the capacity to run up eight syntheses in parallel, with each synthesis capable of coupling up to four building blocks together using three sequential cycles of ICC. This chapter also describes the use of the small molecule synthesizer to synthesize, in a fully automated fashion, a series of materials, pharmaceuticals, natural products, and natural product derivatives. Critical to the success of the synthesizer was the discovery of general purification, deprotection, and cross-coupling conditions for MIDA boronates as detailed in Chapters 2-4.

Seiko Fujii, Junqi Li, and Gregory F. Morehouse contributed to the synthesis of many of the non-commercial building blocks and performed many of the manual experiments used to determine and optimize reaction conditions used for automation. Junqi Li performed many of the post-automation manual deprotections. Eric P. Gillis contributed to the development and implementation of the software and to early configurations of the synthesizer. Matthew J. Clark contributed building block 5.37, based on Seiko Fujii’s synthetic route.
5-1 DESIGN OF THE SMALL MOLECULE SYNTHESIZER

Based on the platforms developed for automated MIDA boronate deprotection, cross-coupling, and purification discussed in Chapters 2-4 we constructed a fully automated small molecule synthesizer that operates on the automated iterative cross-coupling (ICC) platform for MIDA boronates (Figure 5.1).\textsuperscript{1,2} This section describes the hardware and software used to construct and control the synthesizer.

\textbf{Figure 5.1.} \textbf{A.} Picture of the full small molecule synthesizer. \textbf{B.} Picture of the small molecule synthesizer, cropping out the deprotection and purification equipment for Pumps 5-8 for clarity.
The full synthesizer is shown schematically in Figure 5.2. It consists of three distinct modules: the Purification Module (Figure 5.2, #1, see Chapter 2 for development details), the Deprotection Module (Figure 5.2, #2, see Chapter 3 for development details), and the Cross-coupling Module (Figure 5.2, #3, see Chapter 4 for development details). This current configuration of the synthesizer is capable of running up to eight syntheses in parallel. Each one of the eight parallel synthesizers has its own dedicated syringe pump for fluid handling (Primary Pump). These eight Primary Pumps are built into a single housing unit (Figure 5.2, #4) that create the physical core of the synthesizer as well as form a support table for the Cross-coupling Module. In addition to the Primary Pumps, two separate syringe pumps are shared by all eight parallel synthesizers. The Auxiliary Pump (Figure 5.2, #5) is responsible for much of the fluid handling for the Purification Module while the Wet Pump (Figure 5.2, #6) handles all aqueous reagents and solutions utilized in the Deprotection Module.

Each syringe pump uses a syringe (Figure 5.2, #11) to transfer fluid (either aqueous and/or organic liquid or gases) through a series of automated valves (Figure 5.2, #10) to its appropriate destination. Critical to the proper flow of all fluids and the maintenance of inert atmosphere throughout the synthesizer are series of automated solenoid valves connected to the inert gas manifold (Figure 5.2, #9) that control the flow of dry argon throughout the synthesizer. There are a total of eight solenoids, one dedicated to each of the Primary Pumps. Both the Purification and Cross-coupling Modules utilize heating magnetic stir plates (Figure 5.2, #7) for a source of agitation. The Cross-coupling Module also uses aluminum heating blocks (Figure 5.2, #8) to maintain elevated reaction temperatures during cross-coupling. All of the necessary liquid reagents are stored in reagent containers connected to the synthesizer and are accessed, automatically, by the synthesizer when required. Any necessary solid reagents are manually added to the synthesizer before its use for a particular experiment.
Figure 5.2. Schematic representation of the full small molecule synthesizer showing the general layout of equipment and its identity.
Hardware control of the small molecule synthesizer

Unless otherwise discussed, all commercial hardware components were purchased from J-KEM® Scientific, Inc. (St. Louis, MO), Norgren® Kloehn USA (Las Vegas, NV), Upchurch Scientific, Inc. (Oak Harbor, WA), or Luknova, Inc. (Mansfield, MA), or IKA® Works, Inc. (Wilmington, NC).

A number of small commercially-available hardware components were also used in the construction of the small molecule synthesizer. These are shown in Figure 5.3.

![Figure 5.3](image)

**Figure 5.3.** Pictures of individual small hardware components used in the construction of the small molecule synthesizer and their respective product numbers from one of the following vendors: J-KEM® Scientific, Inc. (St. Louis, MO), Norgren® Kloehn USA (Las Vegas, NV), Upchurch Scientific, Inc. (Oak Harbor, WA), Luknova, Inc. (Mansfield, MA).

Each of the ten syringe pumps on the small molecule synthesizer is fitted with a 10-mL glass and Teflon® (polytetrafluoroethylene, PTFE) syringe (J-KEM®, SPGS-10000). As part of each syringe pump, each syringe is fitted into an eight-port addressable distribution valve (J-KEM®, SPDV-8). These particular valves have eight addressable ports with an additional port for the syringe. This syringe port is the default inlet port, which then allows the syringe to connect to any one of the eight ports at a given time. This configuration means that each syringe
pump can aspirate/inject from any of eight separate ports or destinations. Critical to the leak-free and reproducible performance of these syringes are the Teflon® sealing washers (Kloehn, NC0024573) used to provide a tight seal between the glass tip of the syringe and the metal housing of the valve. All other valves used in the rest of the fluidic network are another style of eight-port addressable distribution valves (J-KEM®, SPDV-CS8). These valves have eight addressable ports with an additional front default inlet port. Similar to the syringe valves described above, this allows the inlet to connect to any one of the other eight ports at a given time and this configuration means that each inlet stream be distributed to any of eight separate ports or destinations.

The final destination for any fluid on the synthesizer is a container in which either a chemical transformation (in the case of the Deprotection or Cross-Coupling Modules) or a work-up/separation (in the case of the Deprotection or Purification Modules) takes place. In all cases, these containers, referred hereafter as “cartridges” are empty 12-g columns (Luknova, FC003012). These cartridges are fitted with medium-porosity fiber frits and have standard luer tips on the bottom and standard luer ports on the top. In the event of an experiment, each cartridge is pre-filled with its appropriate contents (see Chapter 5: Experiment Section for details). Every cartridge is connected to the synthesizer via a friction-sealed luer port/tip connection. Specifically, the bottom luer tips of all cartridges are plugged into a female luer to ¼-28 female red polyether ether ketone (PEEK) adaptor (Upchurch, P-658). If connected to the synthesizer, the tops of cartridges are connected to a male luer with natural PP locking hub to ¼-28 female red ethylene tetrafluoroethylene (ETFE) adaptor (Upchurch, P-675) or to a male luer to ¼-28 male natural ETFE adaptor (Upchurch, P-625) as part of a natural PEEK “Y”-connector (Upchurch, P-513).

The inert gas manifold, which supplies dry argon and nitrogen to the synthesizer, is constructed from a series of natural PEEK tees (Upchurch, P-714) and 9-port black PEEK manifold (Upchurch, P-191). The manifold also uses flat bottomed perfluoroalkoxy (PFA) polymer resin plugs (Upchurch, P-316) to plug unused ports on each manifold. Each one of the eight Primary Pumps has its own dedicated tee and manifold.

All of the tubing used in the fluidic network is either natural 1/16” O.D., 0.030” I.D. fluorinated ethylene propylene (FEP) tubing (Upchurch, 1520) or green 1/8” O.D., 0.062” I.D. fluorinated ethylene propylene (FEP) tubing (Upchurch, 1520) or green 1/8” O.D., 0.062” I.D.
FEP tubing (Upchurch, 1521GL). All of the connections in the fluidic network are made with the appropriate female adaptor, a blue ETFE flangeless ferrule (Upchurch, P-200), and one of the following male nuts: a red Delrin® (polyoxymethylene, POM) flangeless nut (Upchurch, P-202), a black Delrin® flangeless nut (Upchurch, P-201), a black Delrin® short flangeless nut (Upchurch, P-208), a stainless steel flush nut (Upchurch, XF-358), or a custom-milled red Delrin® flushnut.

The Deprotection Module consists of two V6 programmable syringe pumps (J-KEM®, SYR-1400PC) which are connected to the controlling computer via a RS485 to USB connection. Each syringe pump aspirates and injects from 0.0 mL/min to 70.0 mL/min with a step of 0.0029 mL. One pump (the Primary Pump) is utilized as the organic liquid handling pump and the other (the Wet Pump) is used exclusively as the aqueous liquid handling pump. The module utilizes an additional five eight-port distribution valves (J-KEM®, SPDV-CS8) housed in four separate quad stack KEM select distribution modules (J-KEM®, SYR-CS4) for liquid handling. A source of dry nitrogen and dry argon are used for liquid handling and deoxygenation/concentration processes.

The Cross-coupling Module consists of one V6 programmable syringe pump (J-KEM®, SYR-1400PC), the Primary Pump described above. The module utilizes one additional eight-port distribution valve (J-KEM®, SPDV-CS8) housed in one separate quad stack KEM select distribution module (J-KEM®, SYR-CS4) for liquid handling (shared with the Deprotection Module). A source of dry nitrogen and dry argon are used for liquid handling and deoxygenation processes (shared with the Deprotection Module). Two RET control visc IKAMAG® safety control heating stir plates (IKA®, 3364001) and one RCT basic IKAMAG® safety control heating stir plate (IKA®, 3810001) are used for reaction stirring and temperature control.

The Purification Module consists of two V6 programmable syringe pumps (J-KEM®, SYR-1400PC). One is the Primary Pump described above. The other pump (the Auxiliary Pump) is used exclusively as the column eluent and waste handling pump. The module utilizes an additional six eight-port distribution valves (J-KEM®, SPDV-CS8) housed in three separate quad stack KEM select distribution modules (J-KEM®, SYR-CS4) for liquid handling. Five of these distribution valves are shared with the Deprotection and Cross-coupling Modules. Two RET control visc IKAMAG® safety control heating stir plates (IKA®, 3364001), shared with the
Cross-coupling module, and one RCT basic IKAMAG® safety control heating stir plate (IKA®, 3810001) are used.

The heating blocks and support table used by the Cross-coupling Module and the cartridge support tables used by the Deprotection and Purification Modules are all custom-designed and milled or constructed from aluminum by the University of Illinois at Urbana-Champaign School of Chemical Sciences Machine Shop.

**Hardware connectivity of the small molecule synthesizer**

As a completed unit, this fully automated small molecule synthesizer is comprised of a total of ten syringe pumps, 42 eight-port distribution valves, and over 300 additional outlet ports all connected in a complex fluidic network. As discussed above, some of this equipment, such as the Primary Pumps and their valves, is dedicated to one of the eight individual parallel synthesizers while other equipment, such as the Wet and Auxiliary Pumps and their valves, is shared among all eight of the parallel synthesizer units. In order to maximize the organization of the fluidic network for the operator and to clarify the identity of the equipment for the controlling software (see “Software control of the small molecule synthesizer” below for details) each major piece of equipment (a valve or syringe pump) is assigned a name or number. This identification and assignment system is shown schematically in Figure 5.4.

Each of the eight Primary Pumps in the Primary Pump housing is assigned a number 1-8 from left to right. This number assignment is also used as the identification number for that entire individual synthesizer unit. That is, the leftmost Primary Pump in the Primary Pump housing is assigned the number 1 and is referred to as Pump 1 and any equipment dedicated to that pump is called Pump 1’s equipment. Under this assignment system, each of the eight Primary Pumps (Pumps 1-8) has its own dedicated fluidic system comprised of four valves (Valves 1-4). This dedicated fluidic system is used only by the controlling pump; it cannot physically access (or cross-contaminate) another dedicated fluidic system without using shared equipment.

The shared equipment, as discussed above, is shared among all eight of the parallel synthesizer units. Because this equipment is not dedicated to one of the eight individual parallel synthesizer units, it cannot be assigned a name connected to an individual Primary Pump.
Instead, the shared equipment is simply assigned a name or number of its own. Specifically, the shared syringe pumps are assigned the name Wet and Auxiliary (Aux.) Pumps and their valves are assigned the numbers 11 and 0, respectively. The shared valve equipment is numbered in sequential order starting from the last dedicated valve number. That is, the highest numbered dedicated valve is Pump 1-8’s Valve 4, so the shared valves are numbered starting with 5 (5-10 and 12-13). These name and number assignments are how the operator refers to the equipment and, more importantly, how the software recognizes the equipment.

Figure 5.4. Schematic representation of the full small molecule synthesizer showing the syringe pump and valve equipment assignments.
The fluidic network, built from the hardware and assigned an identity as discussed above is shown schematically in Figure 5.5. Here, for clarity, only the distribution valves are shown; the syringes are omitted. As detailed above, Valves 1, 0, and 11 belong to the Primary Pump, Auxiliary Pump, and Wet Pump, respectively. All dedicated equipment is contained within the dashed box as indicated. That is, every Primary Pump has its own fluidic system as shown inside the dashed box. This figure is a specific example for Pump 1 and its first purification, but can be extrapolated for any of the other seven pumps.

Each valve has eight ports (ports A-H) and each port is connected to a general destination. The general destination can be a resource (e.g. a reagent bottle or gas source), a cartridge location (e.g. deprotection cartridge #1), or another valve. In Figure 5.5, the label next to each port indicates what that specific port is connected to. Destinations not specifically shown in the schematic (e.g. reagent bottles and cartridges) are simply labeled with text boxes. Connections made between valves or another piece of equipment are shown as black lines. In the case where a specific valve port is either not used or left open to be used only to access ambient atmosphere, its label is left blank.

Specifically, for the dedicated equipment fluidic network, Valve 1 is connected to the argon source via an automated solenoid and a tee connector through Port A. Valve 1 is also connected to the nitrogen source, all three reaction cartridge tops, and Valve 4. Valve 4 in turn is connected to all three predrying cartridges as well as the Purification Module top and sides for both purifications. Valve 2 is connected to Valve 1’s Port A through the tee connector and is also connected to all three degassing cartridges, all three reaction cartridge bottoms, and Valve 3. Valve 3 in turn is connected to all three deprotection cartridges and all three drying cartridges. Valve 2’s Port H is connected to shared equipment Valve 12, the valve from which all eight dedicated fluidic networks divert. That is, each of Valve 12’s eight ports is connected to an exact replica of the dedicated equipment fluidic network shown, one for each Primary Pump.

For the shared equipment, Valve 12 is connected to Valve 6. Valve 6 is a reagent resource valve. It is connected to THF, dry nitrogen gas, waste, diethyl ether, and 1.5% methanol in diethyl ether. Separately, Valve 11 (the Wet Pump’s valve) is connected to Valve 13 as well as its reagents (water, THF, 50% saturated aqueous sodium chloride, waste, acetone, and pH = 6 potassium phosphate buffer). Valve 13 in turn is connected to all three deprotection cartridges. It
Figure 5.5. Schematic representation of the valve connectivity on the small molecule synthesizer including shared and dedicated equipment.
should be noted, as discussed above, that Valve 3 is also connected to these cartridges. The two respective matching ports on the two valves are joined with a tee connector to form one line before connecting to the deprotection cartridge. Valve 0 (the Auxiliary Pump’s valve) is connected to its reagents (hexanes, waste, and THF) as well as Valve 5 and 7. These valves are the diverting distribution valves for all of the silica gel plugs in the Purification Module. Specifically, Valve 5 is connected to the silica gel plugs for both purifications for Pumps 1-4 and Valve 7 is used for Pumps 5-8.

The deprotection, predrying, drying, and degassing cartridge connections to Valves 2, 3, 4, and 13 as described above all lead to the Deprotection Module. Specifically, they lead to the Deprotection Module table (see Figure 5.2), a custom-made aluminum table which secures a matrix of luer ports into which the appropriate cartridges are plugged in. There are two of these tables, one on the left hand side of the Primary Pump housing (for Pumps 1-4) and one on the right hand side of the Primary Pump housing (for Pumps 5-8). The top views of these two tables are represented schematically in Figures 5.6 (for Pumps 1-4) and Figures 5.7 (for Pump 5-8). These figures act as maps of the Deprotection Module, where each circle is a luer port into which a luer tip can be plugged in. Using Figure 5.6 as an example, there is a 10 X 8 matrix of luer ports. Those with an “X” through them are unused luer port place holders; these luer ports are not connected to any valve and should not be used for any experiment. Every two rows is a repeating pattern of luer tips for a different Primary Pump. That is, the first two (front/bottom) rows are dedicated to Pump 1, the second two rows are dedicated to Pump 2, and so on. In each pump’s set of luer ports there are three each of deprotection, predrying, drying, and degassing ports, one for each cycle of ICC. These are labeled accordingly and are connected to their respective matching valve ports as shown in Figure 5.5. There are also six additional luer ports labeled as “inert gas”. These ports lead directly to the inert gas manifold and, when connected to their associated drying or degassing ports (as indicated with a wavy line) with a short length of tubing, provide maintenance of inert atmosphere in those cartridges. The Deprotection Module table for Pumps 5-8 (Figure 5.7) follows the same layout as discussed here.
Figure 5.6. Schematic representation of the top view of the Deprotection Module table for Pumps 1-4 (left hand side of synthesizer). Each circle is a luer port, labeled with its usage.
Figure 5.7. Schematic representation of the top view of the Deprotection Module table for Pumps 5-8 (right hand side of synthesizer). Each circle is a luer port, labeled with its usage.
The reaction cartridge top and reaction cartridge bottom connections to Valves 1 and 2 as described above all lead to the Cross-coupling Module. Specifically, they lead to the Cross-coupling Module table (see Figure 5.2), a custom-made aluminum table which secures a matrix of luer ports into which the appropriate cartridges are plugged in. This table sits directly one the Primary Pump housing and acts both as a cover for the inert gas manifold connections and the eight automated solenoids and as a table for the three heating stir plates used by the Cross-coupling Module. The top view of this table is represented schematically in Figure 5.8. Similar to Figures 5.6 and 5.7 for the Deprotection Module, this figure act as a map of the Cross-coupling Module, where each circle is a luer port into which a luer tip can be plugged in. Unlike the Deprotection Module, cartridges are never plugged directly into these luer ports. Rather, each luer port is fitted with a length of tubing, the end of which is fitted with another luer port. It is this, now flexible and movable luer port that is then connected to the appropriate cartridge. As seen in Figure 5.8, the Cross-coupling Module table is a 8 X 9 matrix of luer ports. Each of the eight columns of luer ports is dedicated to a different Primary Pump. Specifically, the leftmost column is for Pump 1, the second leftmost column is for Pump 2, and so on. The nine rows of luer ports are broken down into three sets of three. Each set of three rows is dedicated to a different function and each of the three rows within a set is dedicated to a different cycle of ICC. That is, there are three functions that can be used in a sequence of up to three ICC cycles. Specifically, the front/bottom row is the connection for the first reaction cartridge’s top, the next row is the connection for the second reaction cartridge’s top, and the third row is the connection for the third reaction cartridge’s top. The second set of three rows is the connections for the reaction cartridges’ bottoms. The third (back/top) set of three rows is the connections for the reaction cartridges’ inert gas/vent sources. Similar to the “inert gas” ports of the Deprotection Module, these ports lead directly to the inert gas manifold and, when connected to their associated reaction cartridge, provide maintenance of inert atmosphere for that reaction. These are labeled accordingly and are connected to their respective matching valve ports as shown in Figure 5.5.
**Figure 5.8.** Schematic representation of the top view of the Cross-coupling Module table for Pumps 1-8 (center of synthesizer). Each circle is a luer port, labeled with its usage.
Software control of the small molecule synthesizer

All synthesizer equipment, except for the three IKA® RCT basic heating stir plates and the first IKA® RET control-visc heating stir plate, is controlled by a custom software program which was written in VB.NET and is run on a Window’s based computer. As described above, the synthesizer has the capability of running up to eight syntheses in parallel simultaneously. Each synthesis is executed from the software environment using an independent virtual synthesizer and each virtual synthesizer can be scripted and started/stopped without regard to the state of the other virtual synthesizers. The custom software program ensures that resources are queued appropriately. Additionally, the software program is designed to interpret instructions to the synthesizer written in a simple custom scripting language. This enables the operator to write very general instructions to the synthesizer that are read by the software and then mapped and communicated to the appropriate hardware. The commands used in this scripting language are described and discussed in detail in Appendix A and B. The interface of the software program at rest is shown in Figure 5.9. The buttons across the top of the window labeled “Pump 1”, “Pump 2”, etc. select the virtual synthesizer that is to be modified by the operator. The large text box provides an area in which the scripting commands are typed, copied, and edited. When the “Run” button is pressed the software proof-reads all of the scripting commands entered into the virtual synthesizer. If the syntax of the commands is correct then the virtual synthesizer begins operation of the actual synthesizer equipment. If there is a syntax error, the operator is alerted to the line of code that raised the error and the synthesizer does not begin operation until the error is corrected. Figure 5.10 illustrates the interface of the software program while it is running a script on the synthesizer. The active line of code is highlighted in blue while the lines of code that have been run are indicated in gray and the remaining lines are indicated in black. The “Break” button stops execution of the code and the “Pump Status” label alerts the operator to the current operation of the virtual synthesizer. If the virtual synthesizer is queued for use of shared equipment, then the status is updated in the “Pump Status” label.

An important aspect of the software is that scripts which are written for one virtual synthesizer are applicable to all the virtual synthesizers. That is, the script files are portable even though the actual valves that are operated by the script differ depending on which virtual synthesizer the code is executed from. One mechanism by which the software ensures portability
of the scripts is through the use of a “header” section (Figure 5.11). The header is a set of scripting commands that is specific to each virtual synthesizer and contains instructions on how to operate pieces of equipment that are unique to a particular virtual synthesizer. For example, each virtual synthesizer is mapped to a different port on valve #12 (Figure 5.5). Therefore, scripts that are written to be portable across all virtual synthesizers typically use a placeholder variable to refer to valve #12. The header of each virtual synthesizer includes code which specifies the value that will be used to replace the placeholder variable if the code is executed on that specific virtual synthesizer. The header section is typically hidden from the operator but is run as the first portion of code whenever the virtual synthesizer executes script.

Figure 5.9. The software interface while the virtual synthesizer is not operating. This is the screen from which the virtual synthesizer is scripted.
Figure 5.10. The software interface while the synthesizer is operating. This is the screen where the operator monitors the status of the synthesizer and script.

Figure 5.11. The software interface used to change the “header” section of the script.
From a technical standpoint, the software is built from a collection of programming objects (classes). The most broadly defined object is the virtual synthesizer object, which serves as a container for equipment objects. An equipment object comprises all of the software functions necessary to operate a single valve or syringe pump. Specifically, the equipment object translates relatively simple software instructions into the complex arrangement of ones and zeros that are understood by the hardware. The equipment also stores information on the exact hardware address of the equipment, as well as the route by which commands must be sent to the equipment. The most elementary object is the command object, which interprets a single line of code to determine if the syntax of the code is correct. If the syntax is correct, the command object stores the parameters necessary to perform the requested action. Specifically, the command object stores the name of the virtual synthesizer object and the name of the equipment object that will be required for the requested action, as well as the names of the functions that will be performed by the equipment object. When an operator presses the “Run” button in the software program, each line of the script is sent to its own unique command object which interprets the code. The command objects are then organized in a list and the software program sequentially instructs each command object in the list to execute its action.

Finally, a series of prewritten and confirmed script programs has been prepared. These script programs were written to execute a number of distinct and often-used operations. These include first, second, and third-round deprotections, cross-coupling, and purifications as well as several routine maintenance operations, like washing. Each individual prewritten script program can be loaded using a simple drag and drop method and then the experiment is initiated by pressing “Run”. This enable operators who are not well-versed in the script writing to run experiments by simply loading the appropriate scripts for their intended experiment.

5-2 SINGLE CYCLE OF AUTOMATED DEPROTECTION, CROSS-COUPLING, AND PURIFICATION

Having constructed the fully automated synthesizer as described above based on the results discussed in Chapters 2-4 we sought to test its ability to perform a single cycle of MIDA boronate deprotection ([D]), cross-coupling ([C]), and purification ([P]) in a fully automated
fashion. Encouraged by the generality observed with the conditions discussed for automated purification, deprotection, and cross-coupling in Chapters 2, 3, and 4, respectively, we additionally sought to maximize the generality of the automated process by using these conditions. This process is represented schematically in Figure 5.12. Specifically, the deprotection module adds THF and water to a cartridge containing the starting MIDA boronate and solid NaOH (Figure 5.12, top). This system is agitated using pulses of argon gas at room temperature for 20 minutes. This is followed by a reaction quench with pH = 6 potassium phosphate buffer and diethyl ether and the resulting ethereal solution of the freshly prepared boronic acid is separated from the water-soluble MIDA ligand. The organic layer is dried over a series of drying agents (anhydrous MgSO₄ and molecular sieves) mixed with Celite™ and then concentrated and deoxygenated (via argon sparging) to afford a dry deoxygenated THF solution of freshly prepared boronic acid (the argon sparging/concentration is carried out long enough to completely remove the more volatile diethyl ether, leaving only THF as the solvent). The cross-coupling module then heats and stirs a reaction cartridge containing a coupling partner, PdXPhos, K₃PO₄, THF, and a magnetic stir bar (Figure 5.12, middle). The solution of boronic acid in THF, freshly prepared by the deprotection module, is then slowly added to the cross-coupling reaction. Once fully added, the reaction is allowed to stir. Finally, the purification module executes catch-and-release chromatography on the crude reaction mixture using a series of eluents (Figure 5.12, bottom), starting with a 4:1 mixture of hexanes:THF, then using 1.5% (v/v) methanol in diethyl ether, then straight diethyl ether. This series of eluent removes non-MIDA boronate containing impurities including unreacted boronic acid, decomposition byproducts associated with protodeborylation, and catalyst components. Finally THF is used to release the purified MIDA boronate. The ability to use THF as the release solvent enables transfer of the resulting concentrated solution of purified MIDA boronate product directly into the deprotection module to start the next ICC cycle.
Figure 5.12. Schematic representations of fully automated modules for the deprotection ([D]), cross-coupling ([C]), and purification ([P]) steps of ICC.

We first tested the capacity of this automated platform to execute one full cycle of ICC with a wide range of structurally diverse MIDA boronates, using the same general conditions for each module (Figure 5.13). Specifically, we subjected a series of commercially available aryl (5.1a-5.1c, Figure 5.13, entries 1-3), heteroaryl (5.1d-5.1f, Figure 5.13, entries 4-6), and vinyl MIDA boronates (5.1g-5.1i, Figure 5.13, entries 7-9) to this process and quantified the
conversion at each step as well as yield and purity of the final MIDA boronate products (see Chapter 5: Experiment Section for details). Without any modifications of any of the general conditions (General condition for deprotection: MIDA boronate 5.1 (1 mmol, 3 equiv.), NaOH (9 equiv.), THF (0.1 M), rt, 20 minutes. General condition for cross-coupling: MIDA boronate 5.3 (1 equiv.), PdXPhos (5 mol%), K3PO4 (9 equiv.), THF (0.028 M), 55 °C, 16 hours. General condition for catch-and-release purification: SiO2, MeOH:Et2O (1.5:98.5, 36 mL), Et2O (36 mL), THF (12 mL)) we observed excellent conversions of all MIDA boronates 5.1 to the corresponding boronic acids 5.2 (97-99% conversion), and excellent conversions of MIDA-protected haloboronic acid building block 5.3 (86-99% conversion) to provide good isolated yields of all of the desired cross-coupling products 5.4. Most importantly, all of the final MIDA boronate products 5.4a-i were isolated in good yield and excellent purity (with the exception of 5.4c, all were at least >90% pure).
A range of structurally diverse MIDA boronate building blocks were subjected to one fully automated cycle of deprotection ([D]), cross-coupling ([C]), and purification ([P]) using the same set of general conditions in each module. General condition for deprotection: MIDA boronate 5.1 (1 mmol, 3 equiv.), NaOH (9 equiv.), THF (0.1 M), rt, 20 minutes. General condition for cross-coupling: MIDA boronate 5.3 (1 equiv.), PdXPhos (5 mol%), K$_3$PO$_4$ (9 equiv.), THF (0.028 M), 55 °C, 16 hours. General condition for catch-and-release purification: SiO$_2$, MeOH:Et$_2$O (1.5:98.5, 36 mL), Et$_2$O (36 mL), THF (12 mL). % Conversions and % purity were determined via $^1$H NMR.

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<th>boronic acid 5.2</th>
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5-3 MULTIPLE CYCLES OF AUTOMATED DEPROTECTION, CROSS-COUPING, AND PURIFICATION

We next tested whether the synthesizer could prepare a wide range of structurally and functionally diverse materials, pharmaceuticals, and natural products via multiple ICC cycles (Figures 5.14 and 5.15). This section describes these experiments. Remarkably, a single automated run successfully delivered each of the targeted compounds in milligram quantities, fulfilling the requirements of most functional discovery assays. Moreover, similar to automated peptide, oligonucleotide, and oligosaccharide syntheses, all of the synthesizer-generated products were readily purified using standard chromatographic techniques, and any protecting groups other than MIDA were successfully removed in a separate step. In addition, most of the building blocks required for these syntheses are commercially available. Importantly, while at least 3-4 days of full time commitment by a highly trained specialist would be required to manually synthesize each of these targets from the same building blocks, specialists and non-specialists alike can load the reaction cartridges and start the synthesizer in approximately 1 hour. This difference represents 3-4 days of time that could be committed to many other aspects of the small molecule discovery process.

Materials targets

We first targeted a set of organic materials components that are comprised of repeating heteroaryl or phenylene units (Figure 5.14, entries 1 and 2). Quaterthiophene 5.10 represents an important motif found in organic solar cells. Using the same set of previously described general conditions for the automated deprotection, cross-coupling, and purification modules, building blocks 5.5, 5.6, and 5.9 were readily assembled to generate 7.5 mg of quaterthiophene 5.10 (Figure 5.14, entry 1). As a second example, oligophenylenevinlenes (OPVs) such as 5.16 represent important components of organic light-emitting diodes (OLEDs). These OPV motifs suffer from notoriously limited solubility in many organic solvents and a common strategy used to improve their solubility is alkylating the aryl rings. In that vein, and to avoid potential solubility challenges, we targeted OPV 5.16, a tert-butylated OPV target known to have favorable solubility properties. Encouragingly, and still using the same general conditions, the ethyl ester 5.15 was successfully synthesized in a fully automated fashion from building blocks.
5.11, 5.12, and 5.14 in good yield (84%, Figure 5.14, entry 2). A final manual hydrolysis afforded the targeted free acid OPV 5.16 (see Chapter 5: Experimental Section for details).

Pharmaceutical targets

We next questioned whether the synthesizer could also prepare important pharmaceutical compounds representing increased structural complexity and diversity (Figure 5.14, entries 3-4). The phosphodiesterase inhibitor (PDE4) 5.21, a small molecule currently under investigation for treating asthma, was readily prepared from building blocks 5.17, 5.18, and 5.20 (6.6 mg, Figure 5.14, entry 3.) without modification to the previously described general conditions for automated deprotection, cross-coupling, and purification. Because carbon–heteroatom bonds, which are very common in pharmaceuticals, can also be formed via cross-coupling10, we further targeted the anticancer B-Raf kinase inhibitor 5.26. Remarkably, the same general conditions that were being used for C-C bond formation also enabled efficient C–N bond formation between building blocks 5.22 and 5.23. Another automated iteration of deprotection and cross-coupling with building block 5.25 completed the sequence to afford 12.9 mg of 5.26 (Figure 5.14, entry 4).
Figure 5.14. Fully automated syntheses of a range of structurally and functionally diverse materials (entries 1-3) and pharmaceuticals (entries 4 and 5) were accomplished via multiple cycles of deprotection ([D]), cross-coupling ([C]), and purification ([P]). Yields reflect the quantity of each small molecule target that was generated with a single run on the synthesizer. All % yields are based on one equivalent of the final halide building block. Any protecting groups other than MIDA were removed in a separate step.

Natural product targets

Natural products represent the most structurally complex and diverse class of small molecules and are thus the most challenging targets for a general and automated synthesis platform. Similar to the changes in reaction conditions often employed in the automated synthesis of more complex peptides, minor modifications in some cases to the phosphine ligand, base, and/or reaction temperature and time (see Chapter 5: Experiment Section for details) enabled us to successfully expand the scope of the small molecule synthesizer to include a diverse range of complex natural product targets (Figure 5.15). Importantly, this collection of targets represents all four of the most common small molecule biosynthetic pathways: polyketide, fatty acid, polyterpene, and polyphenylpropanoid.
**Figure 5.15.** Fully automated syntheses of a range of structurally and functionally diverse natural products were accomplished via multiple cycles of deprotection ([D]), cross-coupling ([C]), and purification ([P]). Yields reflect the quantity of each small molecule target that was generated with a single run on the synthesizer. All % yields are based on one equivalent of the final halide building block. Any protecting groups other than MIDA were removed in a separate step.

The crocacins represent a recently discovered series of antifungal natural products derived from both polyketide and non-ribosomal peptide biosynthesis machinery. Preliminary studies have suggested that these natural products inhibit the mitochondrial cytochrome bc1 segment of complex III in the respiratory transport chain, and thus represent excellent targets for further study. One such member of this family of natural products, (+)-crocacin C\textsuperscript{12,13,14,15,16} (5.31), has been previously synthesized in our laboratory via an ICC route.\textsuperscript{17} Due to its interesting structurally complexity we targeted 5.31 as our first natural product for automated synthesis. In a preliminary experiment, examining just the first fully automated cycle of ICC with building blocks 5.27 and 5.28, vinyl MIDA boronate intermediate 5.29 was successfully prepared in good yield (71%) and purity (>90%) (Scheme 5.1).

<table>
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| all-trans-retinal (polymerpene) | light-harvesting cofactor for vision |

| ratanilene (polyphenylpropanoid) | isolate from the medicinal plant Ratanhiae radix |

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In a separate fully automated experiment, a subsequent cycle of deprotection and cross-coupling with building block 5.30 afforded (+)-crocacin C (5.31) in 61% isolated yield after chromatographic purification (Figure 5.15, entry 1). Importantly, all of the functional groups and stereochemistry found in this natural product was pre-installed into the building blocks and more than 14 mg of this natural product was routinely prepared via a single run on the synthesizer, which is more than enough compound to support extensive biological investigations.

We further targeted the synthesis of two natural products containing stereodefined polyene frameworks: the fatty acid β-parinaric acid (5.36)$^{18,19}$ and the polyterpene all-trans-retinal (5.42)$^{20,21,22}$. Both targets have been successfully synthesized via an ICC route previously in our laboratory.$^{23}$ The central challenge in constructing polyene frameworks like those contained in 5.36 and 5.42 involves controlling the stereochemistry of each double bond. A key feature of ICC is that stereochemistry is pre-installed in the building blocks and then faithfully translated into the targeted products during the assembly process. However, it was unclear whether isomerizations would occur during deprotection, cross-coupling and/or purification in this automated format.

β-Parinaric acid, a widely used fluorescent probe of lipid biophysics, has been used for more than three decades in structural and functional studies of lipid bilayer membranes. In a preliminary experiment, examining just the first fully automated cycle of ICC with building blocks 5.32 and 5.33, trienyl MIDA boronate intermediate 5.34 was successfully prepared in good yield (71%) and purity (>90%) (Scheme 5.2), and more importantly, with no observable isomerization of the triene.
In a separate fully automated experiment, a subsequent cycle of deprotection and cross-coupling with building block 5.35 afforded β-parinaric acid (5.36) in 56% isolated yield after chromatographic purification (Figure 5.15, entry 2). As seen in the first cycle of ICC, the pre-installed olefin stereochemistry was faithfully transferred to the target. Additionally, more than 18 mg of the fatty acid was synthesized in one automated experiment; more than enough compound to support extensive biological investigations.

As the chromophore for vision in all vertebrates, the light-harvesting cofactor in the energy transducing bacteriorhodopsin complex, and a key substrate for an oxidation process now linked to age-related macular degeneration in humans, the polyterpene derived natural product all-trans-retinal (5.42) has been and remains one of the most important small molecules in all of chemistry, biology, and medicine. Extensive research has focused on the development of efficient syntheses of this natural product and its structural and/or isotopic derivatives to powerfully enable fundamental studies of its function. Fully-automated synthetic access to such compounds from readily available building blocks stands to have a pervasive enabling impact on life science research. Similar to 5.36, in a preliminary experiment, examining just the first fully automated cycle of ICC with building blocks 5.37 and 5.38, trienyl MIDA boronate intermediate 5.39 was successfully prepared in good yield (79%) and purity (>90%) (Scheme 5.3), and more importantly, with no observable isomerization of the triene.
Scheme 5.3. First automated ICC cycle towards all-trans-retinal ketal (5.41).

In a separate fully automated experiment, a subsequent cycle of deprotection and cross-coupling with building block 5.40 afforded all-trans-retinal ketal (5.41) in 74% isolated yield after chromatographic purification (Figure 5.15, entry 3). Again, as seen in the first cycle of ICC, the pre-installed olefin stereochemistry was faithfully transferred to the target. A separate manual deprotection afforded all-trans-retinal (5.42) as the free aldehyde (see Chapter 5: Experiment Section for details). As with 5.31 and 5.36, quantities commensurate with biological assays were synthesized in a single automated experiment.

Emboldened by these successes, we further targeted the automated synthesis of protected form (5.49) of the polyphenolpropanoid ratanhine (5.50), the largest and most complex natural product isolated from the medicinal plant *Ratanhiae radix.*\(^{24,25}\) What made this target particularly challenging was the presence of several difficult cross-couplings, the intermediacy of some very unstable boronic acids, and more so the additional third cycle of ICC required to form the tetramer. With exception of quaterthiophene target 5.10 discussed above, we had yet to test the capability of the synthesizer to complete complex three-step ICC sequences. A set of preliminary experiments, examining either just the first or second automated cycles of ICC were run. In the first case, one fully automated cycle with building blocks 5.43 and 5.44, afforded aryl MIDA boronate intermediate 5.45 in good yield (78%) and purity (>90%) (Scheme 5.4). Subsequently, one fully automated cycle with building blocks 5.45 and 5.46 afforded aryl MIDA boronate intermediate 5.47 in good yield (80%) and purity (>90%) (Scheme 5.5).
Despite requiring several challenging cross-couplings and the intermediacy of some very unstable boronic acids, building blocks 5.43, 5.44, 5.46, and 5.48 were successfully cross-coupled in a fully automated ICC experiment to afford protected ratanhine (5.49) in 36% yield (Figure 5.15, entry 4). A separate manual deprotection afforded free ratanhine (5.50) (see Chapter 5: Experiment Section for details).

5.4 AUTOMATED SYNTHESIS OF A NATURAL PRODUCT LIBRARY

Finally, the development of new medicines, biological probes, and materials with optimized functional properties often requires access to many structural derivatives of a specific small molecule of interest. Due to its modular nature, the ICC approach is well suited for enabling such access, especially if the required syntheses can be performed automatically. Thus,
as a final test for the small molecule synthesizer, we targeted the automated preparation of many derivatives of our most complex prior target, ratanhine (5.50). Furthermore, in this experiment, we did not permit any optimizations of any of the conditions used to construct protected ratanhine 5.49.

We specifically input four sets of building blocks representing common substructural elements found throughout the neolignan family of medicinal natural products and/or other pharmaceutically relevant motifs (Figure 5.16 A). These building blocks include variations in oxidation states, methylation patterns, fluorine content, aromatic ring identity, and size. They also represent pre-programmed oligomer lengths of 3-4 units based on whether the third building block is a bifunctional halo-MIDA boronate or a capping halide. In the event, the small molecule synthesizer successfully generated 20 out of 20 of the targeted derivatives, collectively representing all possible combinations of this four-component matrix of building blocks (Figure 5.16 B). Additionally, all 20 out of 20 targets were successfully deprotected in a separate manual step (see Chapter 5: Experimental Section for details) to afford the corresponding deprotected, free alcohol targets. Included in the collection of targets are two additional known natural products. Ratanhiaphenol III (5.56), another isolate from Ratanhiae radix, shows promising activity in the inhibition of protein tyrosine phosphate 1B (PTP1B)\(^ {26}\). Target 5.58 (CAS # 143001-80-3) is a known isolate from both Krameria grayi\(^ {27}\) as well as Krameria interior\(^ {28}\), a genus of plants used medicinally in tropical and subtropical America to fight eye infections and body weakness. It is worth noting that if performed independently in a manual fashion, these 20 syntheses would require at least 2-3 months of full time commitment by a specialist. In contrast, executing all 20 of the automated syntheses required a total of only ~20 hours of person time.
Figure 5.16. A. A collection of natural product- and pharmaceutical-inspired building blocks for library synthesis. B. 20/20 targeted library members were successfully synthesized in a fully automated fashion using the same series of deprotection, cross-coupling, and purification conditions. The products include protected versions of the natural products ratanhine (5.49) and ratanhaphenol III (5.56) - isolates from the same medicinal plant Ratanhiae radix – as well as many of their derivatives. Unless otherwise noted, the quantity of material indicated below each product represents the yield from a single run on the synthesizer. All percent yields are based on one equivalent of the final halide building block. All protecting groups other than MIDA (R = TIPS, TBDPSE, TMSE, or Bz) were successfully removed in a separate step.

5-5 SUMMARY

In summary, we have created a fully automated platform for small molecule synthesis. At present, this platform is best able to prepare targets that contain an abundance of sp²-hybridized atoms and can be retrosynthesized into four or fewer building blocks. However, within the bounds of this limitation, this small molecule synthesizer can already prepare compounds of diverse structure and function, including materials, pharmaceuticals, complex natural products, and derivatives. This breakthrough enables specialists to more efficiently make such compounds
and thereby accelerate the small molecule discovery process. It also delivers the power of small molecule synthesis to non-specialists and thereby will stimulate a dramatic expansion in small molecule discovery research. Increases in the scope of this automated platform will continue to be driven by new methods for making and cross-coupling suitable building blocks\textsuperscript{29,30,31,32}, including those containing new types of heterocycles\textsuperscript{29,33,34}, heteroatoms\textsuperscript{10}, and even Csp\textsuperscript{3}-hybridized carbon atoms\textsuperscript{35,36,37,38,39}. Harnessing these collective advances in concert with further improvements in the speed and generality of this small molecule synthesizer will ultimately help shift the rate-limiting step in small molecule science from synthesis to function study.

5-6 REFERENCES


\textsuperscript{3} Gillis, E. P. Iterative Cross-Coupling With MIDA Boronates. Ph.D. Dissertation, University of Illinois at Urbana-Champaign, Urbana, IL


CHAPTER 5: EXPERIMENTAL SECTION

General materials and methods

Commercial reagents were purchased from Sigma-Aldrich, EMD Millipore, Fisher Scientific, Alfa Aesar, Frontier Scientific, Oakwood Products, or Strem and were used without further purification unless otherwise noted. Most of the building blocks used in these studies are available from Sigma-Aldrich® [5.1a (730335), 5.1b (699853), 5.1c (699160), 5.1d (708828), 5.1e (701017), 5.1f (701106), 5.1g (733539), 5.1h (697443), 5.1i (710024), 5.1j (699292), 5.1k (703710), 5.1l (707252), 5.3 (698083), 5.5 (MIDA080), 5.6 (701092), 5.12 (MIDA084), 5.14 (B58407), 5.16 (MIDA083), 5.18 (363774), 5.17 (723711), 5.18 (MIDA081), 5.20 (C70223), 5.23 (MIDA085), 5.27 (698032), 5.32 (MIDA034), 5.38 (MIDA013), 5.43 (701831), 5.44 (MIDA014), 5.46 (MIDA017), 5.51 (MIDA039), 5.52 (MIDA015)], Frontier Scientific, Inc. [5.9 (B1644), 5.25 (B10713), 5.53 (B1851)], or Aurora Fine Chemicals LLC [5.22 (A00.242.706)].

Manual syntheses were carried out in oven or flame-dried glassware and performed under a dry inert atmosphere unless otherwise noted. Unless otherwise noted: Celite™ refers to Celite™ 545 filter aid (not acid washed); Darco® refers to activated carbon, Darco® G-60, -100 mesh, powder; and K$_3$PO$_4$ and K$_2$CO$_3$ were both anhydrous and were freshly and finely ground in a 120 °C mortar and pestle. XPhos 2nd generation palladacycle refers to chloro(2-dicyclohexylphosphino-2’,4’,6’-triisopropyl-1,1’-biphenyl)[2-(2’-amino-1,1’-biphenyl)]palladium(II) (741825, Sigma-Aldrich) (21). Solvents were purified via passage through packed columns as described by Pangborn and coworkers$^1$ (THF, Et$_2$O, CH$_3$CN, CH$_2$Cl$_2$: dry neutral alumina; hexanes, benzene, toluene: dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves. Water was deionized. Triethylamine, diisopropylamine, diethylamine, pyridine, and 2,6-lutidine were freshly distilled under an atmosphere of dry nitrogen from CaH$_2$.

Thin layer chromatography (TLC) was performed using the indicated eluent on E. Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by exposure to a UV lamp ($\lambda$ = 254 and/or 366 nm) and/or a basic solution of KMnO$_4$ followed by brief heating with a Varitemp® heat gun. Flash chromatography was performed as described by Still and coworkers$^2$ using EM Merck silica gel 60 (230-400 mesh). $^1$H NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500, Varian VXR 500, or Varian
Unity Inova 500NB. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CDCl₃, δ = 7.26; (CD₃)₂CO, δ = 2.05, center line; CD₂Cl₂, δ = 5.32, center line; (CD₃)₂SO, δ = 2.50, center line). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, br = broad, app = apparent, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets), coupling constant (J) in Hertz (Hz), and integration.

¹³C NMR spectra were recorded at room temperature on one of the following instruments: Varian Unity 500 or Varian VXR 500. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl₃, δ = 77.16, center line; (CD₃)₂CO, δ = 29.84, center line; CD₂Cl₂, δ = 53.84; (CD₃)₂SO, δ = 39.52, center line). Carbons bearing boron substituents were not observed due to quadrupolar relaxation. High resolution mass spectra (HRMS) were performed by Furong Sun and Elizabeth Eves at the University of Illinois School of Chemical Sciences Mass Spectrometry Laboratory.
Design of the deprotection module

The deprotection module consists of two J-KEM® Scientific, Inc. V6 programmable syringe pumps (part # SYR-1400PC) and are connected to the controlling computer via a RS485 to USB connection. Both are fitted with a 10-mL glass/PTFE syringe (part # SPGS-10000) and an 8-port distribution valve (part # SPDV-8). Each syringe pump aspirates and injects from 0.0 mL/min to 70.0 mL/min with a step of 0.0029 mL. One pump (the Primary Pump) is utilized as the organic liquid handling pump and the other (the Wet Pump) is used exclusively as the aqueous liquid handling pump. The module utilizes an additional five 8-port distribution valves (part # SPDV-CS8) housed in four separate quad stack KEM select distribution modules (part # SYR-CS4) for liquid handling. A source of dry nitrogen and dry argon are used for liquid handling and deoxygenation/concentration processes. Connections between valves are made with FEP tubing (1/16” OD, 0.030” ID).

To the Deprotection Cartridge, the Primary Pump adds THF and the Wet Pump adds water. The reaction is then agitated with pulses of argon gas. The Wet Pump then adds aqueous potassium phosphate buffer (pH = 6) and the Primary Pump adds Et\textsubscript{2}O. The resulting biphasic system is agitated with pulses of nitrogen gas and the aqueous layer is drawn off and disposed of by the Wet Pump. The Wet Pump adds 50% saturated aqueous NaCl. The resulting biphasic
system is agitated with pulses of nitrogen and the aqueous layer is drawn off and disposed of by the Wet Pump. The Primary Pump transfers the wet organic solution to a Predrying, and subsequently Drying Cartridge, containing drying agents and agitates the mixture by repeatedly withdrawing/injecting the solution. The Primary Pump transfers the dried organic solution to a Concentration/Deoxygenation Cartridge and concentrates/deoxygenates the solution with pulses of argon gas.

Design of the cross-coupling module

The cross-coupling module consists of one J-KEM® Scientific, Inc. V6 programmable syringe pump (part # SYR-1400PC), the Primary Pump described above. The module utilizes one additional 8-port distribution valve (part # SPDV-CS8) housed in one separate quad stack KEM select distribution module (part # SYR-CS4) for liquid handling (shared with the deprotection module). A source of dry nitrogen and dry argon are used for liquid handling and deoxygenation processes (shared with the deprotection module). Two IKA® RET control visc IKAMAG® safety control heating stir plates (part # 3364001) and one IKA® RCT basic IKAMAG® safety control heating stir plate (part # 3810001) are used for reaction stirring and
temperature control. Connections between valves are made with FEP tubing (1/16” OD, 0.030” ID).

The Reaction Cartridge, agitated with a magnetic stir bar, is deoxygenated with pulses of argon gas. The Primary Pump adds THF to the reaction cartridge and then slowly adds the dried/deoxygenated THF solution of boronic acid. After the addition, the reaction is allowed to agitate.

**Design of the purification module**

The purification module consists of two J-KEM® Scientific, Inc. V6 programmable syringe pumps (part # SYR-1400PC). One is the Primary Pump described above. The other pump (the Auxiliary Pump) is used exclusively as the column eluent and waste handling pump and is connected to the controlling computer via a RS485 to USB connection. The module utilizes an additional six 8-port distribution valves (part # SPDV-CS8) housed in three separate quad stack KEM select distribution modules (part # SYR-CS4) for liquid handling. Five of these distribution valves are shared with the deprotection and cross-coupling modules. Two IKA® RET control visc IKAMAG® safety control heating stir plates (part # 3364001), shared with the cross-coupling module, and one IKA® RCT basic IKAMAG® safety control heating stir plate (part #
Connections between valves are made with FEP tubing (1/16” OD, 0.030” ID).

The Auxiliary Pump adds hexanes to the Precipitation Cartridge, agitated with a magnetic stir bar. The Primary Pump adds portions of the crude reaction solution to the Precipitation Cartridge. The Auxiliary Pump then withdraws the solvent through the Silica Gel Plug. This process is repeated until the Reaction Cartridge is empty. The Primary Pump then adds 1.5% MeOH in Et₂O to the Precipitation Cartridge and then the Auxiliary Pump withdraws the solvent through the Silica Gel Plug. The Primary Pump then adds Et₂O to the Precipitation Cartridge and then the Auxiliary Pump withdraws the solvent through the Silica Gel Plug. The Auxiliary Pump then adds THF to the Precipitation Cartridge and the Primary Pump removes the resulting solution and transfers it to the next Deprotection Cartridge.

General procedure for setting up an automated synthesis

The following cartridges are prepared (unless otherwise noted, cartridge refers to a 12-g Luknova column capped with a 12-g Luknova column screw cap). Once prepared the cartridges are connected to the synthesizer.

**First Deprotection Cartridges** contain solid NaOH and the starting MIDA boronate.

**Second and Third Deprotection Cartridges** contain solid NaOH.

**Predrying Cartridges** contain Celite™ (800 mg) and anhydrous MgSO₄ (2.1 g). These solids are mixed thoroughly and a plastic 5-mL syringe plunger is placed on top of the mixed solids. This is topped with an aluminum foil cover.

**Drying Cartridges** contain Celite™ (300 mg) with 4 Å molecular sieves (activated, powder, -325 mesh) (3.6 g) layered on top. A plastic 5-mL syringe plunger is placed on top of the layered solids.

**Concentration/Deoxygenation Cartridges** are empty.

**First Reaction Cartridges** contain a PTFE-coated magnetic stir bar, coupling partner, catalyst and ligand, and base. For this cartridge, the factory-supplied fiber frit has been removed and a medium porosity glass frit installed. The cap is pierced with a 1.5-inch 18 G needle and topped with an empty 4-g Luknova column (capped with a 4-g Luknova column screw cap). This cap is
tethered to another cap PTFE tubing (1/16-inch I.D., 1/8-inch O.D.). This additional cap, pierced with a 1.5-inch 18 G needle, is attached to the Reaction Filtration Cartridge. The PTFE tubing is adjusted to place the end of the tubing approximately 5 mm above the frit of the First Reaction Cartridge and approximately 20 mm below the screw cap of the Reaction Filtration Cartridge. The luer ports of both screw caps are packed with a small ball of rolled Kimberly-Clark® Kimwipes™.

**Reaction Filtration Cartridges** contain a PTFE-coated magnetic stir bar and a mixture of Celite™ (2.5 g) and Florisil® (1.25 g). This is tethered to the First Reaction Cartridge as described above.

**Second and Third Reaction Cartridges** contain a PTFE-coated magnetic stir bar, coupling partner, catalyst and ligand, and base.

**Precipitation Cartridges** contain a PTFE-coated magnetic stir bar, Celite™ (150 mg), and 3-aminopropyl functionalized silica gel (250 mg). Hexanes (10 mL) is added and the cartridge is swirled vigorously to suspend and homogenize the mixture of solids. The stir bar and solids are allowed to settle over 30 seconds and the supernatant hexanes is pushed out of the cartridge with an overhead pressure of air. The stir bar is now embedded in the mixture of solids wet with hexanes.

**Silica Gel Plugs** contain silica gel, tightly packed, topped with a 4-g Luknova column frit. This is capped with a 4-g Luknova column screw cap, using four layers of PTFE tape on the sealing insert to ensure a leak-free seal.

![Chemical diagram]

**General automated procedure for one ICC cycle**

In the deprotection module, to a Deprotection Cartridge containing starting MIDA boronates 5.1 (1.0 mmol) and NaOH (3.0 mmol, 120 mg) is added 12 mL THF followed by 3 mL
water. After 20 minutes agitation at 23 °C, 3 mL aqueous potassium phosphate buffer (pH = 6) and 5 mL Et₂O are added and the layers are separated. Then 3 mL 50% saturated aqueous NaCl are added and the layers are separated. The THF/Et₂O solution of boronic acid is dried using a Predrying and Drying Cartridge. The resulting dry THF/Et₂O solution is transferred to a Concentration/Deoxygenation Cartridge. The drying agents are washed with 6 mL THF, which is added to the Concentration/Deoxygenation Cartridge. The organic solution is concentrated to 10 mL (evaporating most of the Et₂O) and the drying agents are washed with an additional 6 mL THF, which is added to the Concentration/Deoxygenation Cartridge. The organic solution (now only THF) is concentrated to 9 mL. An empty Second Deprotection Cartridge is used to receive purified cross-coupling product 5.4.

In the cross-coupling module, to a deoxygenated First Reaction Cartridge agitated and heated at 55 °C and containing bifunctional MIDA boronate 5.3 (0.33 mmol, 104 mg), XPhos 2nd generation palladacycle (0.017 mmol, 13.1 mg, 5 mol%), and K₃PO₄ (3.0 mmol, 637 mg), is added 3 mL THF. The THF solution of boronic acid is added over 4 hours (0.0375 mL/min). At the end of the addition the reaction is stirred for an additional 12 hours.

In the purification module, to a Reaction Filtration Cartridge is added the crude cross-coupling solution and to a Precipitation Cartridge/Silica Gel Plug is added 12 mL hexanes. Then 3 mL of the crude cross-coupling solution (filtered through the Reaction Filtration Cartridge) is added and the solvent is removed. This process is performed ten times, using 3 mL THF to wash the Reaction Cartridge and Reaction Filtration Cartridge for each cycle. Then 12 mL 1.5% MeOH in Et₂O are added and the solvent is removed a total of three times (36 mL total). Then 12 mL Et₂O are added and the solvent is removed a total of three times (36 mL total). Finally, 12 mL THF are added and the resulting purified solution of 5.4 is added to the Second Deprotection Cartridge.

In the event, all cartridges are connected to the synthesizer and the synthesis is initiated. From this point forward, no human intervention is required throughout the completion of the synthesis and purification of targeted product 5.4.

In order to characterize the progress of the automated reaction sequences (as shown in Fig. 2), the following analysis was performed. At the end of the deprotection quench, a 500-µL aliquot of the organic layer is removed manually from the First Deprotection Cartridge. The
sample is concentrated, a $^1$H NMR sample is prepared and a spectrum is acquired immediately. The percent conversion for the deprotection step is determined via integration of the resonances corresponding to the boronic acid product and any remaining MIDA boronate starting material. At the end of the synthesis, the final THF solution of purified 5.4 (in the empty Second Deprotection Cartridge) is concentrated and a $^1$H NMR spectrum acquired. The percent conversion for the cross-coupling is determined via integration of the resonances corresponding to the cross-coupling product 5.4 and any remaining halo MIDA boronate starting material 5.4. The purity of cross-coupling product 5.4 is determined via integration of the resonances corresponding to the cross-coupling product 5.4 and any impurities observed in the spectrum. Because the primary goal of the small molecule synthesizer is to easily prepare and purify the targeted compound, the general conditions were developed to maximize the % conversions for the deprotection and cross-coupling reactions as well as the % purity of the MIDA boronate product of each cycle, rather than the % yield.

Automated synthesis of 5.4a

The general procedure was followed using 251.7 mg (1.02 mmol) aryl MIDA boronate 5.1a and 104.0 mg (0.333 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 99% and the conversion for the cross-coupling step was 98%. The desired aryl MIDA boronate 5.4a was obtained as a colorless solid of >95% purity (65.3 mg, 0.202 mmol, 61% yield).
Figure 5.S1. $^1$H NMRs for automated synthesis of 5.4a.

TLC (20% MeCN in Et$_2$O)

$R_f = 0.37$, visualized by UV and KMnO$_4$ stain.

$^1$H-NMR (500 MHz, DMSO-$d_6$:D$_2$O, 95:5)

$\delta$ 7.64 (d, $J = 8.5$ Hz, 2H), 7.51 (d, $J = 8.5$ Hz, 2H), 7.48 (s, 1H), 7.45 (dd, $J = 7.5$, 0.5 Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.17 (dd, $J = 7.5$, 0.5 Hz, 1H), 4.34 (d, $J = 17.5$ Hz, 2H), 4.13 (d, $J = 17$ Hz, 2H), 2.54 (s, 3H), 2.37 (s, 3H).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$:D$_2$O, 95:5)
δ 169.1, 140.7, 140.1, 138.0, 132.9, 128.7, 128.0, 127.2, 125.9, 123.7, 61.8, 47.6, 21.0.

HRMS (EI+)

Calculated for C_{18}H_{18}BNO_4: 323.13289

Found: 323.13253

Automated synthesis of 5.4b

The general procedure was followed using 284.5 mg (1.00 mmol) aryl MIDA boronate 5.1b and 104.3 mg (0.334 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 99% and the conversion for the cross-coupling step was 99%. The desired aryl MIDA boronate 5.4b was obtained as an off-white solid of >90% purity (77.7 mg, 0.216 mmol, 65% yield).

Figure 5.S2. ¹H NMRs for automated synthesis of 5.4b.
TLC (20% MeCN in Et<sub>2</sub>O)

R<sub>f</sub> = 0.28, visualized by UV and KMnO<sub>4</sub> stain.

<sup>1</sup>H-NMR (500 MHz, DMSO-<em>d</em><sub>6</sub>:D<sub>2</sub>O, 95:5)

δ 8.23 (d, J = 1 Hz, 1H), 7.99 (d, J = 8 Hz, 2H), 7.92 (dd, J = 7.5, 1.5 Hz, 1H), 7.85 (dd, J = 9.0, 2 Hz, 1H), 7.82 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 7.56-7.49 (m, 2H), 4.38 (d, J = 17 Hz, 2H), 4.16 (d, J = 17 Hz, 2H), 2.57 (s, 3H).

<sup>13</sup>C-NMR (125 MHz, DMSO-<em>d</em><sub>6</sub>:D<sub>2</sub>O, 95:5)

δ 169.6, 140.5, 137.6, 133.5, 133.3, 132.4, 128.7, 128.4, 127.6, 126.6, 126.4, 126.3, 125.3, 125.2, 62.0, 47.8.

HRMS (EI+)

Calculated for C<sub>21</sub>H<sub>18</sub>BNO<sub>4</sub>: 359.13289

Found: 359.13333

Automated synthesis of 5.4c

The general procedure was followed using 263.5 mg (1.00 mmol) aryl MIDA boronate 5.1c and 104.4 mg (0.335 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 97% and the conversion for the cross-coupling step was 86%. The desired aryl MIDA boronate 5.4c was obtained as a colorless solid of >80% purity (69.2 mg, 0.204 mmol, 61% yield).
**Figure 5.S3.** $^1$H NMRs for automated synthesis of 5.4c.

TLC (20% MeCN in Et$_2$O)

$R_f = 0.31$, visualized by UV and KMnO$_4$ stain.

$^1$H-NMR (500 MHz, DMSO-$d_6$:D$_2$O, 95:5)

$\delta$ 7.66 (d, $J = 8$ Hz, 2H), 7.52 (d, $J = 7.5$ Hz, 2H), 7.37 (t, $J = 7.5$ Hz, 1H), 7.23 (d, $J = 7.5$ Hz, 1H), 7.18 (s, 1H), 6.93 (dd, $J = 8$, 2.5 Hz, 1H), 4.35 (d, $J = 17.5$ Hz, 2H), 4.13 (d, $J = 17$ Hz, 2H), 3.80 (s, 3H), 2.54 (s, 3H).
\[ ^{13}C\text{-NMR (125 MHz, DMSO-}d\text{\textsubscript{6}:D\textsubscript{2}O, 95:5)} \]
\[ \delta \ 169.7, 160.0, 141.8, 140.7, 133.3, 130.3, 126.3, 119.2, 113.3, 112.3, 62.0, 55.3, 47.8. \]

HRMS (EI+)

Calculated for \( \text{C}_{18}\text{H}_{18}\text{BNO}_5 \): 339.12780

Found: \( \text{339.12732} \)

Automated synthesis of \( 5.4d \)

The general procedure was followed using 242.1 mg (1.01 mmol) aryl MIDA boronate \( 5.1d \) and 104.1 mg (0.3334 mmol) bifunctional MIDA boronate \( 5.3 \). The conversion for the deprotection step was 98% and the conversion for the cross-coupling step was 98%. The desired aryl MIDA boronate \( 5.4d \) was obtained as an off-white solid of >95% purity (78.7 mg, 0.250 mmol, 75% yield).

\[ \text{Figure 5S4. } ^1H \text{ NMRs for automated synthesis of } 5.4d. \]
TLC (20% MeCN in Et₂O)

R_f = 0.38, visualized by UV and KMnO₄ stain.

^1^H-NMR (500 MHz, DMSO-d₆:D₂O, 95:5)

δ 7.64 (d, J = 8.5 Hz, 2H), 7.52 (m, 2H), 7.47 (d, J = 8 Hz, 2H), 7.13 (dd, J = 4.5, 4 Hz, 1H), 4.34 (d, J = 17 Hz, 2H), 4.12 (d, J = 17 Hz, 2H), 2.53 (s, 3H).

^1^3^C-NMR (125 MHz, DMSO-d₆:D₂O, 95:5)

δ 169.7, 143.6, 134.4, 133.4, 128.8, 125.9, 124.9, 124.0, 62.0, 47.8.

HRMS (EI+)

Calculated for C₁₅H₁₄BNO₄S: 315.07366

Found: 315.07412

**Automated synthesis of 5.4e**

The general procedure was followed using 225.5 mg (1.01 mmol) aryl MIDA boronate 5.1e and 104.4 mg (0.335 mmol) bifunctional MIDA boronate 5.3 The conversion for the deprotection step was 97% and the conversion for the cross-coupling step was 93%. The desired aryl MIDA boronate 5.4e was obtained as an off-white solid of >90% purity (72.4 mg, 0.242 mmol, 72% yield).
Figure 5S5. $^1$H NMRs for automated synthesis of 5.4e.

TLC (20% MeCN in Et$_2$O)

$R_f = 0.33$, visualized by UV and KMnO$_4$ stain.

$^1$H-NMR (500 MHz, DMSO-$_d_6$-D$_2$O, 95:5)

$\delta$ 7.73 (d, $J = 2$ Hz, 1H), 7.68 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 8$ Hz, 2H), 6.95 (d, $J = 3$ Hz, 1H), 6.58 (dd, $J = 3.5$, 2 Hz, 1H), 4.34 (d, $J = 17$ Hz, 2H), 4.12 (d, $J = 17.5$ Hz, 2H), 2.51 (s, 3H).
$^{13}$C-NMR (125 MHz, DMSO-$d_6$:D$_2$O, 95:5)

$\delta$ 169.7, 153.3, 143.2, 133.2, 131.0, 123.0, 112.4, 106.3, 62.0, 47.8.

HRMS (EI+)

Calculated for C$_{15}$H$_{14}$BNO$_5$: 299.09650

Found: 299.09715

Automated synthesis of 5.4f

The general procedure was followed using 325.4 mg (1.01 mmol) aryl MIDA boronate 5.1f and 104.4 mg (0.335 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 99% and the conversion for the cross-coupling step was 99%. The desired aryl MIDA boronate 5.4f was obtained as an off-white solid of >95% purity (110.9 mg, 0.278 mmol, 83% yield).
Figure 5.56. $^1$H NMRs for automated synthesis of 5.4f.

![Structural formula of 5.4f](image)

TLC (20% MeCN in Et₂O)

$R_f = 0.46$, visualized by UV and KMnO$_4$ stain.

$^1$H-NMR (500 MHz, DMSO-$d_6$:D$_2$O, 95:5)

δ 7.43 (d, $J = 8$ Hz, 2H), 7.33 (dd, $J = 3$, 1.5 Hz, 1H), 7.30 (d, $J = 8.5$ Hz, 2H), 6.26 (t, $J = 3.5$ Hz, 1H), 6.23 (dd, $J = 3$, 1.5 Hz, 1H), 4.34 (d, $J = 17$ Hz, 2H), 4.13 (d, $J = 17$ Hz, 2H), 2.54 (s, 3H), 1.28 (s, 9H).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$:D$_2$O, 95:5)

δ 169.7, 149.1, 134.7, 134.4, 132.0, 128.2, 123.0, 114.7, 111.2, 83.9, 62.1, 47.9, 27.3.

HRMS (ESI+)

Calculated for C$_{20}$H$_{24}$BN$_2$O$_6$: 399.1727

Found: 399.1723

Automated synthesis of 5.4g

The general procedure was followed using 261.1 mg (1.01 mmol) aryl MIDA boronate 5.4g and 105.1 mg (0.337 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 98% and the conversion for the cross-coupling step was 95%. The desired aryl MIDA boronate 5.4g was obtained as a colorless solid of >90% purity (75.7 mg, 0.226 mmol, 67% yield).
Figure S5. $^1$H NMRs for automated synthesis of 5.4g.

TLC (20% MeCN in Et$_2$O)

$R_f = 0.36$, visualized by UV and KMnO$_4$ stain.

$^1$H-NMR (500 MHz, DMSO-$d_6$:D$_2$O, 95:5)

$\delta$ 7.44 (d, $J = 8.5$ Hz, 2H), 7.38-7.32 (m, 3H), 7.28 (app dd, $J = 8$ Hz, 4H), 5.50 (s, 1H), 5.47 (s, 1H), 4.34 (d, $J = 17$ Hz, 2H), 4.12 (d, $J = 17$ Hz, 2H), 2.54 (s, 3H).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$:D$_2$O, 95:5)

$\delta$ 169.7, 149.4, 141.4, 140.9, 132.7, 128.6, 128.2, 128.1, 127.4, 115.0, 62.0, 47.8.
HRMS (EI+)

Calculated for C₁₉H₁₈BNO₄: 335.13289
Found: 335.13356

Automated synthesis of 5.4h

The general procedure was followed using 265.9 mg (1.00 mmol) aryl MIDA boronate 5.1h and 105.6 mg (0.339 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 98% and the conversion for the cross-coupling step was 99%. The desired aryl MIDA boronate 5.4h was obtained as an off-white solid of >95% purity (77.7 mg, 0.228 mmol, 67% yield).

Figure 5.88 ¹H NMRs for automated synthesis of 5.4h.
TLC (20% MeCN in Et₂O)

Rᵣ = 0.44, visualized by UV and KMnO₄ stain.

¹H-NMR (500 MHz, DMSO-ᵈ₆:D₂O, 95:5)

δ 7.35 (s, 4H), 6.34 (d, J = 16.5 Hz, 1H), 6.25 (dd, J = 16.5, 7 Hz, 1H), 4.31 (d, J = 17 Hz, 2H), 4.08 (d, J = 17 Hz, 2H), 2.47 (s, 3H), 2.13-2.06 (m, 1H), 1.74-1.68 (m, 4H), 1.63-1.60 (m, 1H), 1.31-1.23 (m, 2H), 1.18-1.11 (m, 3H).

¹³C-NMR (125 MHz, DMSO-ᵈ₆:D₂O, 95:5)

δ 169.6, 138.1, 136.8, 132.7, 127.3, 125.4, 61.9, 47.7, 40.6, 32.6, 25.8, 25.6.

HRMS (EI+)

Calculated for C₁₉H₂₄BNO₄: 341.17984
Found: 341.18030

Automated synthesis of 5.4i

The general procedure was followed using 199.4 mg (1.01 mmol) aryl MIDA boronate 5.1i and 104.2 mg (0.334 mmol) bifunctional MIDA boronate 5.3. The conversion for the deprotection step was 99% and the conversion for the cross-coupling step was 98%. The desired aryl MIDA boronate 5.4i was obtained as a colorless solid of >90% purity (64.9 mg, 0.238 mmol, 71% yield).
TLC (20% MeCN in Et₂O)

R_f = 0.36, visualized by UV and KMnO₄ stain.

**¹H-NMR (500 MHz, DMSO-d₆:D₂O, 95:5)**

δ 7.49 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8 Hz, 2H), 5.45 (s, 1H), 5.10 (t, J = 1.5 Hz, 1H), 4.33 (d, J = 17 Hz, 2H), 4.10 (d, J = 17 Hz, 2H), 2.50 (s, 3H), 2.10 (s, 3H).

**¹³C-NMR (125 MHz, DMSO-d₆:D₂O, 95:5)**
$\delta$ 169.7, 142.7, 141.0, 132.7, 124.8, 112.9, 61.9, 47.8, 21.6.

**HRMS (EI+)**

Calculated for $\text{C}_{14}\text{H}_{16}\text{BNO}_4$: 273.11724

Found: 273.11679

**General automated procedure for multiple ICC cycles**

The general procedure for one ICC cycle is followed with these additions:

If three building blocks are being assembled, the Second Deprotection Cartridge contains NaOH (1.0 mmol, 40 mg) and the Second Reaction Cartridge contains the second bifunctional MIDA boronate (0.11 mmol), XPhos 2$^{\text{nd}}$ generation palladacycle (0.0056 mmol, 4.4 mg, 5 mol%), and $\text{K}_3\text{PO}_4$ (1.0 mmol, 212 mg). This cartridge is identical to a First Reaction Cartridge, but is not tethered to a Reaction Filtration Cartridge.

If four building blocks are being assembled, the Third Deprotection Cartridge contains NaOH (0.33 mmol, 13.3 mg) and no Drying Cartridge is used for the third reaction. The Third Reaction Cartridge is a 7-mL glass vial containing a PTFE-coated magnetic stir, the capping building block (0.037 mmol), XPhos 2$^{\text{nd}}$ generation palladacycle (0.00185 mmol, 1.5 mg, 5 mol%), and $\text{K}_3\text{PO}_4$ (0.33 mmol, 71 mg). The vial is sealed under argon with a septum-top screw cap.

At the end of the synthesis, the crude reaction is purified by silica gel chromatography or preparative HPLC.
Automated synthesis of 5.10

The general procedure was followed using 255.7 mg (1.01 mmol) thiophenyl MIDA boronate 5.5, 108.3 mg (0.34 mmol) thiophenyl MIDA boronate 5.6, 36.0 mg (0.11 mmol) thiophenyl MIDA boronate 5.6, and 7.9 mg (0.0385 mmol) bromothiophene 5.9. The crude residue was purified via silica gel chromatography (100% hexanes to 20% EtOAc in hexanes) to
afford quaterthiophene 5.10 as a red/orange solid (5.7 mg, 0.0147 mmol, 38% yield). The mixed fractions were combined and repurified via silica gel chromatography (100% hexanes to 10% EtOAc in hexanes) to afford an additional 1.8 mg of 5.10 as a red/orange solid (combined total of 7.5 mg, 0.0194 mmol, 50% yield).

![5.10](image)

TLC (20% EtOAc in hexanes)

\[ R_f = 0.29, \text{ visualized by longwave UV}. \]

\(^1\)H-NMR (500 MHz, CDCl\(_3\))

\[ \delta 9.81 (s, 1H), 7.54 (s, 1H), 7.24 (d, J = 3.5 Hz, 1H), 7.17 (m, 3H), 7.06 (d, J = 3.5 Hz, 1H), 6.90 (d, J = 5.0 Hz, 1H), 2.48 (s, 3H), 2.43 (s, 3H). \]

\(^13\)C-NMR (125 MHz, CDCl\(_3\))

\[ \delta 182.6, 141.6, 140.6, 139.7, 139.2, 136.7, 135.8, 134.5, 134.2, 131.7, 130.7, 128.2, 126.2, 124.8, 124.2, 123.8, 16.1, 15.7. \]

HRMS (EI+)

Calculated for C\(_{19}\)H\(_{14}\)OS\(_4\): 385.99277

Found: 385.99217
Automated synthesis of 5.19

The general procedure was followed with the following modifications: In the second cross-coupling reaction, the concentration was 0.03 M with respect to 5.18 and addition of the boronic acid was performed over 1 minute.

This procedure was followed using 372.0 mg (1.00 mmol) vinyl MIDA boronate 5.16, 114.1 mg (0.34 mmol) bifunctional vinyl MIDA boronate 5.12, and 25.8 mg (0.113 mmol) ethyl ester 5.18. Oligophenylenevinylene 5.19 was afforded as a yellow solid (44.1 mg, 0.0945 mmol, 84% yield).

TLC (40% DCM in hexanes)

151
R_f = 0.31, visualized by UV.

^1^H-NMR (500 MHz, CDCl3)
\[ \delta 8.06 \text{ (d, J = 8 Hz, 2H)}, 7.59-7.54 \text{ (m, 6H)}, 7.41-7.39 \text{ (m, 3H)}, 7.23 \text{ (d, J = 16.5 Hz, 1H)}, 7.22 \text{ (d, J = 16 Hz, 1H)}, 7.15 \text{ (d, J = 15.5 Hz, 1H)}, 7.12 \text{ (d, J = 16 Hz, 1H)}, 4.40 \text{ (q, J = 7 Hz, 2H)}, 1.42 \text{ (t, J = 7.5 Hz, 3H)}, 1.40 \text{ (s, 18H)}. \]

^13^C-NMR (125 MHz, CDCl3)
\[ \delta 166.5, 151.2, 141.9, 137.8, 136.5, 136.0, 130.9, 130.1, 129.3, 127.5, 127.4, 127.3, 127.0, 126.4, 122.4, 121.0, 61.0, 35.0, 31.6, 14.5. \]

HRMS (ESI+)

Calculated for C_{33}H_{39}O_{2}: 467.2950

Found: 467.2943

**Manual deprotection of 5.19**

To a solution of ethyl ester 5.19 (44.1 mg, 0.0945 mmol) in MeOH/THF 1:1 (2 mL) was added LiOH solution (18 mg, 0.752 mmol in 0.4 mL H_2O) in one portion. The mixture was stirred vigorously at 45 °C for 3.5 hours. The reaction was cooled briefly in an ice-water bath and 0.2 mL of 2 N HCl was added. The mixture was diluted with 5 mL H_2O and extracted with EtOAc (10 mL, then 2 × 5 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by recrystallization from hot toluene. A second crop was obtained by precipitation from toluene/hexanes and combined with the first crop to afford oligophenylenevinylene 5.20 as a bright yellow solid (20.5 mg, 0.047 mmol, 50% yield).
$^1$H-NMR (500 MHz, DMSO-$d_6$)

$\delta$ 12.88 (s, 1H), 7.92 (d, $J = 8$ Hz, 2H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.64 (s, 3H), 7.43 (d, $J = 1.5$ Hz, 2H), 7.40 (d, $J = 16.5$ Hz, 1H), 7.34 (d, $J = 16.5$ Hz, 1H), 7.32 (d, $J = 17$ Hz, 1H), 7.30 (s, 2H), 7.26 (d, $J = 16.5$ Hz, 1H), 1.31 (s, 18 H).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$)

$\delta$ 167.0, 150.5, 141.5, 137.2, 136.2, 135.7, 130.6, 129.7 (2C), 129.4, 127.2, 127.1, 126.8, 126.4, 121.6, 120.8, 34.5, 31.2.

HRMS (EI+)

Calculated for C$_{31}$H$_{34}$O$_2$: 438.25588

Found: 438.25538
Automated synthesis of 5.21

The general procedure was followed with the following modifications: In the second cross-coupling reaction, the concentration was 0.03 M with respect to 5.20, 1.11 mmol of K₃PO₄ were used, and addition of the boronic acid was performed over 1 minute.

This procedure was followed using 277.1 mg (1.01 mmol) benzofurazanyl MIDA boronate 5.17, 114.7 mg (0.34 mmol) bifunctional aryl MIDA boronate 5.18, and 16.7 mg (0.111 mmol) chloropyridine hydrochloride salt 5.20. The resulting crude residue was put into 1.5 mL dimethylformamide and purified via HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = acetonitrile, 0 min: 95% A, 5% B; 20 min: 5% A, 95% B) to afford PDE472 (5.21) as an off-white solid (6.6 mg, 0.0218 mmol, 20% yield).
TLC (EtOAc)

$R_f = 0.30$, visualized by UV.

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 8.63 (br s, 2H), 8.12 (t, $J = 1$ Hz, 1H), 7.97 (dd, $J = 9.5$, 1 Hz, 1H), 7.96 (d, $J = 2.5$ Hz, 1H), 7.93 (dd, $J = 9$, 2.5 Hz, 1H), 7.87 (dd, $J = 9.5$, 1.5 Hz, 1H), 7.74 (app d, $J = 6$ Hz, 2H), 7.35 (d, $J = 9$ Hz, 1H), 3.98 (s, 3H).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$)

$\delta$ 157.3, 150.1, 149.3, 148.1, 146.1, 142.2, 135.8, 129.8, 129.1, 129.0, 128.2, 120.9, 114.9 (2C), 112.7, 56.1.

HRMS (ESI+)

Calculated for $C_{18}H_{14}N_3O_2$: 304.1086
Found: 304.1081
Automated synthesis of 5.26

The general procedure was followed using 149.8 mg (0.99 mmol) pyrimidine 5.22, 106.7 mg (0.33 mmol) chloroisoquinoline 5.23, and 23.7 mg (0.114 mmol) bromoisoquinoline 5.25. The crude residue was purified via silica gel chromatography (50% hexanes in EtOAc to 100% EtOAc) to afford 5.26 as an orange/tan solid (12.9 mg, 0.0318 mmol, 28% yield).
$R_f = 0.25$, visualized by UV.

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

$\delta$ 9.31 (br s, 1H), 9.19 (s, 2H), 8.58 (d, $J = 5$ Hz, 1H), 8.16 (d, $J = 8$ Hz, 1H), 8.10 (s, 1H), 8.05 (s, 1H), 7.95 (d, $J = 8.5$ Hz, 1H), 7.87-7.81 (m, 2H), 7.74 (d, $J = 5.5$ Hz, 1H), 7.68-7.60 (m, 2H), 7.56 (br s, 1H), 1.44 (s, 9H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

$\delta$ 171.2, 152.5, 151.6, 148.5, 143.2, 140.9, 136.9, 135.9, 135.2, 133.2, 132.7, 130.8, 128.9, 128.6, 127.2, 126.8, 126.3, 125.6, 121.9, 120.6, 118.2, 39.0, 29.9.

HRMS (ESI+)

Calculated for $C_{26}H_{24}N_5$:

406.2032

Found:

406.2031
Automated synthesis of 5.31

The general procedure was followed with the following modifications: The First Reaction Cartridge did not have a medium porosity glass frit installed; it had only the factory-supplied fiber frit with a 4-g Luknova column fiber frit secured on top of it. The Reaction Filtration Cartridge and PTFE tether were not used. In the first cross-coupling reaction, 5 mol% (2.2 mg, 0.01 mmol) Pd(OAc)$_2$ and 20 mol% (12.2 mg, 0.04 mmol) P(o-tol)$_3$ were used as palladium source and ligand, respectively, and 166 mg (1.2 mmol) anhydrous K$_2$CO$_3$ and 139 mg (0.6 mmol) Ag$_2$O were used as the base and the reaction was run for 8 hours and in order to fill the space around the 4-g Luknova column fiber frit an additional 800 mg K$_2$CO$_3$ was added. Both deprotection reactions were run for 10 minutes. The second cross-coupling reaction was run for 18 hours in a 7-mL glass vial, 7.5 mol% (3.9 mg, 0.0050 mmol) 2nd generation Buchwald XPhos palladacycle was used as catalyst, and 128 mg (0.6 mmol) K$_3$PO$_4$ was used as base.

This procedure was followed using 140.0 mg (0.60 mmol) phenyl MIDA boronate 5.27, 96.0 mg (0.20 mmol) bifunctional MIDA boronate 5.28, and 14.1 mg (0.0668 mmol) vinyl iodide 5.30.

At the end of the synthesis, the crude reaction was filtered through a pad of Celite™. The filter pad was washed with copious THF (approximately 50 mL). The filtrate was then concentrated via rotary evaporation and the resulting residue was azeotroped with DCM (20 mL). The resulting crude residue was purified via silica gel chromatography (40% EtOAc in hexanes to 50% EtOAc in hexanes) to afford (+)-crocacin C (5.31) as an off-white solid (14.5 mg, 0.0406 mmol, 61% yield).

TLC (20% EtOAc in hexanes)

R$_f$ = 0.08, visualized by UV.

$^1$H-NMR (500 MHz, CDCl$_3$)
\[ \delta 7.39 \ (d, \ J = 7.5 \ Hz, \ 2H), \ 7.32 \ (t, \ J = 7 \ Hz, \ 2H), \ 7.23 \ (t, \ J = 7 \ Hz, \ 1H), \ 6.56 \ (d, \ J = 16.5 \ Hz, \ 1H), \ 6.17-6.01 \ (m, \ 3H), \ 5.63 \ (s, \ 1H), \ 5.38 \ (br \ s, \ 2H), \ 4.08 \ (dd, \ J = 7.5, \ 1 \ Hz, \ 1H), \ 3.54 \ (s, \ 3H), \ 3.32 \ (s, \ 3H), \ 3.19 \ (dd, \ J = 10, \ 2 \ Hz, \ 1H), \ 2.56-2.53 \ (m, \ 1H), \ 2.25 \ (d, \ J = 1 \ Hz, \ 3H), \ 1.56-1.52 \ (m, \ 1H), \ 1.19 \ (d, \ J = 7 \ Hz, \ 3H), \ 0.84 \ (d, \ J = 7 \ Hz, \ 3H). \]

\[ ^{13}C\text{-NMR} \ (125 \ MHz, \ \text{CDCl}_3) \]
\[ \delta 169.4, \ 149.7, \ 137.2, \ 136.8, \ 134.1, \ 132.1, \ 129.3, \ 128.7, \ 127.7, \ 126.5, \ 119.7, \ 86.5, \ 81.1, \ 61.6, \ 56.6, \ 42.7, \ 40.2, \ 18.9, \ 13.9, \ 9.8. \]

HRMS (ESI+)

Calculated for C_{22}H_{32}NO_{3}: \quad 358.2382

Found: \quad 358.2392
Automated synthesis of 5.36

The general procedure was followed with the following modifications: In the first cross-coupling reaction, the reaction was run at room temperature for 24 hours and 5 mol% (3.7 mg, 0.017 mmol) Pd(OAc)$_2$ and 10 mol% (15.9 mg, 0.033 mmol) XPhos were used for palladium source and ligand, respectively, and 637 mg (3 mmol) anhydrous K$_3$PO$_4$ was used for base. The second deprotection reaction was run for 10 minutes, and the second cross-coupling reaction was run in a 20-mL glass vial at room temperature for 40 minutes using 4 mol% (1.1 mg, 0.0049 mmol) Pd(OAc)$_2$ and 8 mol% (4.6 mg, 0.0096 mmol) XPhos as the palladium source and ligand, respectively, and 14.0 mg (0.35 mmol) NaOH with 0.5 mL water was a source of aqueous base. The procedure was also conducted under subdued light conditions to protect against isomerization of the polyene framework.

This procedure was followed using 283.2 (1.34 mmol) butenyl MIDA boronate 5.32, 81.9 mg (0.34 mmol) dienyl MIDA boronate 5.33, and 35.2 mg (0.119 mmol) vinyl iodide 5.35.

At the end of the synthesis, the crude reaction was transferred to a 125-mL separatory funnel and quenched with 50 mL saturated aqueous NH$_4$Cl. It was then diluted with 40 mL Et$_2$O. The layers were separated and the aqueous layer was washed with 50 mL Et$_2$O. The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and filtered through Celite™, washing with copious Et$_2$O (approximately 100 mL). The filtrate was then concentrated via rotary evaporation and the resulting residue was put into 1.5 mL dimethylformamide and purified via HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = acetonitrile, 0 min: 95% A, 5% B; 2 min: 95% A, 5% B; 15 min: 0% A, 100% B; 30 min: 0% A, 100% B) to afford β-parinaric acid (5.36) as a fluorescent solid (18.3 mg, 0.0662 mmol, 56% yield). $^1$H NMR indicated a 10:1 mixture of the desired β-parinaric acid (5.36):9-(Z)-parinaric acid (arising from 10:1 E:Z mixture of starting material vinyl iodide 5.35). $^1$H NMR and $^{13}$C NMR analysis of automatically synthesized 5.36 was fully consistent with the data previously reported for β-parinaric acid$^3$. 

![Diagram of 5.36]
TLC (50% Et₂O in hexanes)
\[ R_f = 0.13, \text{ visualized by UV.} \]

\(^1\)H-NMR (500 MHz, CDCl\(_3\))
\[ \delta 6.21-6.05 \text{ (m, 6H), 5.76-5.63 \text{ (m, 2H), 2.34 (t, } J = 7 \text{ Hz, 2H), 2.15-2.07 \text{ (m, 4H), 1.66-1.60 \text{ (m, 2H), 1.43-1.26 \text{ (m, 8H), 1.01 (t, } J = 7.5 \text{ Hz, 3H).}} \]

\(^{13}\)C-NMR (125 MHz, CDCl\(_3\))
\[ \delta 179.2, 136.7, 135.1, 132.6, 132.6, 131.0, 131.0, 130.8, 129.8, 34.0, 33.0, 29.4, 29.2, 29.1, 29.1, 26.0, 24.8, 13.7. \]

HRMS (ESI+)
Calculated for C\(_{18}\)H\(_{29}\)O\(_2\): 277.2168
Found: 277.2175

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- **NaOH** (1.8 mmol), THF (0.06 M), H$_2$O (0.2 M), 10 min, rt
- **K$_2$PO$_4$** (1.8 mmol), Pd(OAc)$_2$ (2.5 mol%), SPhos (5 mol%), THF (0.017 M), rt, 14 h
- 2nd gen, XPhos palladacycle (5 mol%), C$_8$H$_7$CO$_3$ (0.6 mmol), THF (0.022 M), 40 °C, 6 h
Automated synthesis of 5.41

The general procedure was followed with the following modifications: The First Reaction Cartridge did not have a medium porosity glass frit installed; it had only the factory-supplied fiber frit with a 4-g Luknova column fiber frit secured on top of it. The Reaction Filtration Cartridge and PTFE tether were not used. In the first cross-coupling reaction was run for 14 hours at room temperature, 2.5 mol% (1.1 mg, 0.005 mmol) Pd(OAc)$_2$ and 5 mol% (4.1 mg, 0.01 mmol) SPhos were used a palladium source and ligand, respectively, 382 mg (1.8 mmol) K$_3$PO$_4$ was used as base, and in order to fill the space around the 4-g Luknova column fiber frit an additional 750 mg K$_3$PO$_4$ was added. Both deprotection reactions were run for 10 minutes. The second cross-coupling reaction was run in a 7-mL glass vial at 40 °C for 6 hours, 5 mol% (2.6 mg, 0.0033 mmol) 2$^{nd}$ generation Buchwald XPhos palladacycle was used as catalyst and 195 mg (0.6 mmol) Cs$_2$CO$_3$ was used as base. The procedure was also conducted under subdued light conditions to protect against isomerization of the polyene framework.

This procedure was followed using 207.0 mg (0.60 mmol) vinyl MIDA boronate 5.37, 61.8 mg (0.20 mmol) vinyl MIDA boronate 5.38, 18.8 mg (0.0667 mmol) vinyl iodide ketal 5.40.

At the end of the synthesis, the crude reaction was filtered through a pad of Celite™. The filter pad was washed with copious THF (approximately 50 mL). The filtrate was then concentrated via rotary evaporation and the resulting residue was azeotroped with DCM (20 mL). The resulting crude residue was purified via silica gel chromatography (100% hexanes to 30% EtOAc in hexanes) to afford all-trans-retinal ketal 5.41 as a yellow oil (18.2 mg, 0.0491 mmol, 74% yield).

\[
\text{TLC (petroleum ether:ether 4:1) } \quad R_f = 0.86, \text{ stained by KMnO}_4.
\]

$^1$H-NMR (500 MHz, CDCl$_3$)
δ 6.66 (dd, $J = 14.8$, 11.2 Hz, 1H), 6.27 (d, $J = 15.2$ Hz, 1H), 6.19-6.08 (m, 3H), 5.54 (d, $J = 6$ Hz, 1H), 5.21 (d, $J = 6.4$ Hz, 1H), 3.66 (d, $J = 11.2$ Hz, 2H), 3.53 (d, $J = 10.8$ Hz, 2H), 2.01 (t, $J = 6.4$ Hz, 2H), 1.95 (s, 3H), 1.91 (s, 3H), 1.70 (s, 3H), 1.62-1.59 (m, 2H), 1.47-1.44 (m, 2H), 1.23 (s, 3H), 1.01 (s, 6H), 0.75 (s, 3H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

δ 139.3, 137.8, 137.6, 136.6, 135.8, 130.0, 129.3, 127.2, 127.0, 126.2, 109.8, 98.8, 39.6, 34.2, 33.0, 30.0, 28.9, 23.0, 22.0, 21.7, 19.2, 13.4, 12.7.

HRMS (ESI+)

Calculated for C$_{25}$H$_{39}$O$_2$: 371.2950

Found: 371.2950

Manual deprotection of 5.41

A 7-mL vial charged with 5.41 (18.2 mg, 0.049 mmol, 1.0 equiv.) was sealed with a PTFE-lined cap and purged with N$_2$ and THF (1.0 mL, 0.05M) was added to afford a clear yellow solution. The vial was cooled to 0 °C in an ice bath for 5 minutes. Aqueous HCl (1M, 0.5 mL) was added dropwise to the reaction vial and the reaction mixture was allowed to warm to 23 °C with stirring over 30 minutes. After 30 minutes, the reaction mixture was transferred to a separatory funnel containing aqueous saturated NaHCO$_3$ (5 mL), rinsing with diethyl ether (10 mL) and the phases were separated. The aqueous layer was back-extracted with diethyl ether (5 mL) and the combined organic layers were dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo to afford a yellow-orange oil. The resulting crude material (4:1 ratio of all-trans retinal (5.42):13-cis-retinal) was adsorbed onto Celite™ from an acetone solution and purified by silica gel chromatography (32:1 hexanes:EtOAc) to afford all-trans retinal (5.42) as
an orange solid (8.9 mg, 0.0313 mmol, 64% yield). Characterization matches what is reported in the literature.\(^4\)

\[\text{\begin{align*} &\text{1H-NMR (500 MHz, CDCl}_3\text{)} \\
&\delta 10.11 (d, J = 8 \text{ Hz}, 1\text{H}), 7.14 (dd, J = 15, 11.5 \text{ Hz}, 1\text{H}), 6.37 (d, J = 15 \text{ Hz}, 1\text{H}), 6.34 (d, J = 15 \text{ Hz}, 1\text{H}), 6.19 (d, J = 9.5 \text{ Hz}, 1\text{H}), 6.16 (d, J = 16 \text{ Hz}, 1\text{H}), 5.97 (d, J = 8.5 \text{ Hz}, 1\text{H}), 2.33 (d, J = 1 \text{ Hz}, 3\text{H}), 2.04-2.02 (m, 2\text{H}), 2.03 (s, 3\text{H}), 1.72 (s, 3\text{H}), 1.63-1.60 (m, 2\text{H}), 1.49-1.46 (m, 2\text{H}), 1.03 (s, 6\text{H}).\end{align*}}\]

<table>
<thead>
<tr>
<th>Natural all-trans-retinal(^4)</th>
<th>Synthetic all-trans-retinal</th>
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<tr>
<td>10.12 (1H)</td>
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<tr>
<td>7.15 (1H)</td>
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<tr>
<td>6.37 (1H)</td>
<td>6.37 (1H)</td>
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<td>6.20 (1H)</td>
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<tr>
<td>6.18 (1H)</td>
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<tr>
<td>5.98 (1H)</td>
<td>5.97 (1H)</td>
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<tr>
<td>2.33 (3H)</td>
<td>2.33 (3H)</td>
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<tr>
<td>2.03 (3H)</td>
<td>2.03 (3H)</td>
</tr>
<tr>
<td>1.72 (3H)</td>
<td>1.72 (3H)</td>
</tr>
<tr>
<td>1.04 (6H)</td>
<td>1.03 (6H)</td>
</tr>
</tbody>
</table>
Automated synthesis of 5.49

The general procedure was followed with the following modifications: The first cross-coupling reaction was performed using SPhos as the ligand and K₂CO₃ as the base. The second
and third deprotection reactions were run for 10 and 30 minutes, respectively. The second cross-coupling reaction was run for 14 hours and the third cross-coupling reaction was run for 24 hours using SPhos as the ligand.

This procedure was followed using 297.1 mg (1.51 mmol) trans-1-propenyl MIDA boronate (5.43), 119.8 mg (0.34 mmol) 5-bromo-2-benzofuranyl MIDA boronate (5.44), 42.6 mg (0.068 mmol) bifunctional aryl MIDA boronate 5.46, and 6.4 mg (0.0139 mmol) capping vinyl bromide 5.48.

Protected ratanhine 5.49 was isolated as a colorless oil (4.7 mg, 36% yield), the $^1$H NMR of which contained small amounts of hydrocarbon impurities presumed to represent some leaching from the HPLC column.

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 20% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)

13.4 min

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.67 (s, 1H), 7.64-7.62 (m, 4H), 7.54 (d, $J = 9$ Hz, 2H), 7.50 (s, 1H), 7.45-7.43 (m, 8H), 7.25 (d, $J = 2$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 1H), 6.95 (d, $J = 1$ Hz, 1H), 6.56 (dd, $J = 16$, 2 Hz, 1H), 6.51-6.46 (m, 3H), 6.38 (dq, $J = 15.5$, 6.5 Hz, 1H), 6.35 (s, 1H), 6.26 (dq, $J = 15.5$, 6.5 Hz, 1H), 5.46 (s, 2H), 3.86-3.83 (m, 2H), 3.81 (s, 3H), 3.57-3.54 (m, 2H), 1.91 (dd, $J = 6.5$, 1.5 Hz, 3H), 1.88 (dd, $J = 5$, 1.5 Hz, 3H), 1.20-1.17 (m, 2H), 0.99 (s, 9H), 0.89-0.86 (m, 2H), 0.02 (s, 9H).
Manual deprotection of 5.50

To a 1-mL Reacti-vial™ containing 5.49 and a PTFE-coated magnetic stir bar was added CsF (8.2 mg, 0.054 mmol) and 18-crown-6 (1.1 mg, 0.00416) followed by DMSO (0.15 mL) in a glovebox. The vial was sealed with a cap and stirred at 50 °C for 14 hours. The reaction was then cooled to room temperature, diluted with 8 mL EtOAc and washed with a solution of 1:1 saturated aqueous NH₄Cl/H₂O (8 mL). The aqueous layer was extracted with 4 mL EtOAc. The combined organic layers were washed with H₂O (2 × 4 mL), then with brine (8 mL). The organic phase was dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (30% to 40% EtOAc/pentane) to afford ratanhine (5.50) as an off-white solid (0.2 mg, 0.00036 mmol, 7% yield).

TLC (40% EtOAc/pentane)

R_f = 0.44, visualized by shortwave UV.
$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.83 (dt, $J = 9.0$, 2.0 H, 2H), 7.77 (s, 1H), 7.53 (s, 1H), 7.43 (dd, $J = 8.5$, 2.0 Hz, 1H), 7.37 (d, $J = 8.5$ Hz, 1H), 7.34 (d, $J = 2.5$ Hz, 1H), 7.28 (d, $J = 8.0$, 1.0 Hz, 1H), 7.17 (d, $J = 8.5$ Hz, 1H), 7.14 (s, 1H), 6.83 (dt, $J = 8.5$, 2.0 Hz, 2H), 6.55 (s, 1H), 6.50 (dd, $J = 15.5$, 1.5 Hz, 1H), 6.44 (dd, $J = 16.0$, 1.5 Hz, 1H), 6.31-6.21 (m, 2H), 5.61 (d, $J = 1.5$ Hz, 1H), 5.57 (d, $J = 2.0$ Hz, 1H), 3.96 (s, 3H), 1.87 (dd, $J = 6.5$, 1.5 Hz, 3H), 1.84 (dd, $J = 6.5$, 1.5 Hz, 3H).

HRMS (ESI+)

Calculated for $C_{36}H_{31}O_6$: 559.2121

Found: 559.2128

General Scheme for Automated Synthesis of Library Members 5.56-5.67
protected ratanhiaphenol III (5.56)
(27.8 mg, 75% yield)
TLC (50% DCM in hexanes)

R<sub>f</sub> = 0.51, stained by KMnO<sub>4</sub>.

<sup>1</sup>H-NMR (500 MHz, acetone-<em>d<sub>6</sub></em>)

δ 7.82 (d, J = 8.5 Hz, 1H), 7.74-7.71 (m, 4H), 7.53 (d, J = 1.5 Hz, 1H), 7.47-7.42 (m, 6H), 7.39 (d, J = 8.5 Hz, 1H), 7.29 (dd, J = 8.5, 1.5 Hz, 1H), 7.17 (d, J = 1 Hz, 1H), 6.52-6.46 (m, 3H), 6.24 (dq, J = 15.5, 6.5 Hz, 1H), 4.14-4.11 (m, 2H), 3.93 (s, 3H), 1.88-1.84 (m, 5H), 1.09 (s, 9H).

<sup>13</sup>C-NMR (125 MHz, acetone-<em>d<sub>6</sub></em>)

δ 161.3, 158.8, 153.8 (2C), 136.7, 134.8, 134.0, 132.2, 131.2, 130.3, 128.7, 128.3, 124.5, 122.8, 118.7, 112.8, 111.2, 106.6, 105.0, 100.0, 66.2, 55.9, 28.1, 18.6, 18.5, 12.6.

HRMS (ESI+)

Calculated for C<sub>36</sub>H<sub>39</sub>O<sub>3</sub>Si: 547.2668

Found: 547.2673

TLC (50% DCM in hexanes)

R<sub>f</sub> = 0.23, visualized by UV.

<sup>1</sup>H-NMR (500 MHz, acetone-<em>d<sub>6</sub></em>)

δ 7.84 (dd, J = 8, 0.5 Hz, 1H), 7.74-7.72 (m, 4H), 7.47-7.43 (m, 6H), 7.14 (s, 1H), 7.10 (d, J = 1.5 Hz, 1H), 6.93 (d, J = 1.5 Hz, 1H), 6.50-6.45 (m, 3H), 6.24 (dq, J = 15.5, 6.5 Hz, 1H), 4.14-4.10 (m, 2H), 4.01 (s, 3H), 3.93 (s, 3H), 1.87-1.84 (m, 5H), 1.09 (s, 9H).
$^{13}$C-NMR (125 MHz, acetone-$d_6$)

$\delta$ 161.3, 158.7, 153.7, 145.9, 143.0, 136.7, 135.0, 134.8, 132.6, 132.5, 130.3, 128.7, 128.3, 124.6, 112.8, 111.6, 106.6, 105.2 (2C), 100.0, 66.2, 56.3, 56.0, 28.1, 18.5 (2C) 12.6.

HRMS (ESI+)

Calculated for C$_{37}$H$_{41}$O$_4$Si: 577.2774

Found: 577.2767

TLC (50% DCM in hexanes)

$R_f = 0.49$, stained by KMnO$_4$.

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.83 (d, $J = 8.5$ Hz, 1H), 7.74-7.72 (m, 4H), 7.61 (d, $J = 1.5$ Hz, 1H), 7.47-7.42 (m, 7H), 7.35 (dd, $J = 8.5$, 2 Hz, 1H), 7.19 (d, $J = 0.5$ Hz, 1H), 6.77 (dt, $J = 16$, 1.5 Hz, 1H), 6.51-6.47 (m, 2H), 6.35 (dt, $J = 15.5$, 5 Hz, 1H), 4.47 (dd, $J = 4.5$, 1.5 Hz, 2H), 4.14-4.11 (m, 2H), 3.94 (s, 3H), 1.88-1.84 (m, 2H), 1.22-1.08 (m, 30H).

$^{13}$C-NMR (125 MHz, acetone-$d_6$)

$\delta$ 161.4, 158.8, 154.1, 154.0, 136.7, 134.8, 133.2, 131.3, 130.3, 130.2, 128.8, 128.7, 128.3, 123.3, 119.3, 112.8, 111.3, 106.7, 105.0, 100.0, 66.2, 64.8, 56.0, 28.1, 18.6, 18.4, 12.8, 12.6.

HRMS (ESI+)

Calculated for C$_{45}$H$_{59}$O$_4$Si$_2$: 719.3952
Found: 719.3925

TLC (50% DCM in hexanes)

R\textsubscript{f} = 0.40, visualized by UV.

\textsuperscript{1}H-NMR (500 MHz, acetone-\textit{d}_6)

\(\delta 7.84 \text{ (app d, } J = 9 \text{ Hz, 1H), 7.74-7.71 \text{ (m, 4H), 7.48-7.43 (m, 6H), 7.17 (d, } J = 1 \text{ Hz, 1H), 7.16 (s, 1H), 7.00 (d, } J = 1.5 \text{ Hz, 1H), 6.73 (dt, } J = 15.5, 1.5 \text{ Hz, 1H), 6.50 (s, 1H), 6.50--6.48 \text{ (m, 1H), 6.36 (dt, } J = 16, 4.5 \text{ Hz, 1H), 4.47 (dd, } J = 5, 1.5 \text{ Hz, 2H), 4.14-4.11 (m, 2H), 4.02 (s, 3H), 3.93 (s, 3H), 1.88-1.84 (m, 2H), 1.20-1.09 (m, 30H).}

\textsuperscript{13}C-NMR (125 MHz, acetone-\textit{d}_6)

\(\delta 161.3, 158.7, 153.8, 146.0, 143.2, 136.7, 143.7, 134.2, 132.7, 130.5, 130.3, 128.8, 128.7, 128.3, 112.8, 112.3, 106.6, 105.5, 105.2, 100.0, 66.2, 64.8, 56.3, 55.9, 28.1, 18.6, 18.4, 12.8, 12.6.

HRMS (ESI+)

Calculated for C\textsubscript{46}H\textsubscript{61}O\textsubscript{5}Si\textsubscript{2}: 749.4058

Found: 749.4056

TLC (20% EtOAc in hexanes) 173
R_f = 0.28, stained by KMnO₄.

^1^H-NMR (500 MHz, acetone-\textit{d}_6)
\[
\begin{align*}
\delta & 8.37 \ (dd, \ J = 3, \ 0.5 \ Hz, \ 1H), \ 7.89 \ (dd, \ J = 8.5, \ 0.5 \ Hz, \ 1H), \ 7.63 \ (dd, \ J = 2 \ Hz, \ 1H), \\
& 7.50 - 7.48 \ (m, \ 2H), \ 7.39 \ (dd, \ J = 8.5, \ 2 \ Hz, \ 1H), \ 7.31 \ (d, \ J = 1 \ Hz, \ 1H), \ 6.53 \ (dd, \ J = 16, \\
& 1.5 \ Hz, \ 1H), \ 6.29 \ (dq, \ 15.5, \ 6.5 \ Hz, \ 1H), \ 3.95 \ (s, \ 3H), \ 1.87 \ (dd, \ J = 6.5, \ 1.5 \ Hz, \ 3H).
\end{align*}
\]

\[^{13}^C\text{-NMR} \ (125 \ MHz, \ acetone-\textit{d}_6)\]
\[
\begin{align*}
\delta & 157.0, \ 156.6, \ 155.2, \ 142.5, \ 139.2, \ 134.5, \ 132.0, \ 130.4, \ 125.1, \ 123.7, \ 121.2, \ 120.9, \\
& 119.3, \ 111.9, \ 103.6, \ 56.2, \ 18.6.
\end{align*}
\]

HRMS (ESI+)
Calculated for C_{17}H_{16}NO_{2}: 266.1181
Found: 266.1180

TLC (20% EtOAc in hexanes)
R_f = 0.23, stained by KMnO₄.

^1^H-NMR (500 MHz, acetone-\textit{d}_6)
\[
\begin{align*}
\delta & 8.36 \ (dd, \ J = 3, \ 0.5 \ Hz, \ 1H), \ 7.89 \ (dd, \ J = 8.5, \ 0.5 \ Hz, \ 1H), \ 7.49 \ (dd, \ J = 8.5, \ 3.0 \ Hz, \\
& 1H), \ 7.28 \ (s, \ 1H), \ 7.19 \ (d, \ J = 1.5 \ Hz, \ 1H), \ 7.01 \ (d, \ J = 1.5 \ Hz, \ 1H), \ 6.50 \ (d, \ J = 15.5, \ 1.5 \\
& Hz, \ 1H), \ 6.29 \ (dq, \ J = 16, \ 6.5 \ Hz, \ 1H), \ 4.05 \ (s, \ 3H), \ 3.95 \ (s, \ 3H), \ 1.87 \ (dd, \ J = 6.5, \ 1.5 \\
& Hz, \ 3H).
\end{align*}
\]

\[^{13}^C\text{-NMR} \ (125 \ MHz, \ acetone-\textit{d}_6)\]
δ 156.9, 156.6, 146.3, 144.5, 142.5, 139.2, 135.5, 132.3, 131.8, 125.1, 121.2, 120.8, 112.0, 105.8, 103.9, 56.3, 56.2, 18.5.

HRMS (ESI+)

Calculated for C_{18}H_{18}NO_{3}: 296.1287
Found: 296.1282

Library member 5.62 was isolated as an off-white solid containing 5.62 (5.6 mg, 19% yield) and a small amount of cross-coupling byproduct and was carried on to the manual deprotection without further purification.

TLC (20% EtOAc in hexanes)

R_f = 0.33, visualized by UV.

^1H-NMR (500 MHz, acetone-d_6)

δ 8.37 (dd, J = 3, 1 Hz, 1H), 7.89 (dd, J = 9, 1 Hz, 1H), 7.71 (d, J = 1.5 Hz, 1H), 7.52 (d, J = 8.5, 1H), 7.49 (dd, J = 8.5, 3 Hz, 1H), 7.46 (dd, J = 8.5, 1H), 7.34 (d, J = 1 Hz, 1H), 6.80 (dt, J = 15.5, 2 Hz, 1H), 6.40 (dt, J = 15.5, 5 Hz, 1H), 4.50 (dd, J = 4.5, 2 Hz, 2H), 3.96 (s, 3H), 1.22-1.10 (m, 21H).

HRMS (ESI+)

Calculated for C_{26}H_{36}NO_{3}Si: 438.2464
Found: 438.2468
TLC (20% EtOAc in hexanes)

\[ R_f = 0.26, \text{ visualized by UV.} \]

\(^1\)H-NMR (500 MHz, acetone-\(d_6\))

\[ \delta 8.36 \text{ (dd, } J = 3, 0.5 \text{ Hz, 1H), 7.90 \text{ (dd, } J = 9, 0.5 \text{ Hz, 1H), 7.49 \text{ (dd, } J = 8.5, 3 \text{ Hz, 1H), 7.30 \text{ (s, 1H), 7.26 \text{ (d, } J = 1.5 \text{ Hz, 1H), 7.08 \text{ (d, } J = 1.5 \text{ Hz, 1H), 6.76 \text{ (dt, } J = 15.5, 2 \text{ Hz, 1H), 6.41 \text{ (dt, } J = 16, 4.5 \text{ Hz, 1H), 4.49 \text{ (dd, } J = 5, 2 \text{ Hz, 2H), 4.06 \text{ (s, 3H), 3.95 \text{ (s, 3H), 1.21-1.11 \text{ (m, 21H).}} \]

\(^{13}\)C-NMR (125 MHz, acetone-\(d_6\))

\[ \delta 157.0, 156.6, 146.4, 144.7, 142.5, 139.2, 134.8, 131.9, 130.2, 129.3, 121.2, 120.8, 112.8, 106.0, 103.9, 64.7, 56.4, 56.2, 18.4, 12.8. \]

HRMS (ESI+)

Calculated for \(\text{C}_{27}\text{H}_{38}\text{NO}_4\text{Si} \): 468.2570

Found: 468.2572

TLC (20% DCM in hexanes)

\[ R_f = 0.27, \text{ visualized by UV.} \]
\(^1\)H-NMR (500 MHz, acetone-\(d_6\))
\[ \delta 7.94 (d, J = 9 \text{ Hz}, 1\text{H}), 7.74-7.72 (m, 4\text{H}), 7.62 (d, J = 1.5 \text{ Hz}, 1\text{H}), 7.48-7.42 (m, 7\text{H}), 7.37 (dd, J = 8.5, 1.5 \text{ Hz}, 1\text{H}), 7.10 (d, J = 0.5 \text{ Hz}, 1\text{H}), 6.91 (dd, J = 9, 2.5 \text{ Hz}, 1\text{H}), 6.82 (\text{quint, } J = 1.5 \text{ Hz}, 1\text{H}), 6.51 (dd, J = 16, 2 \text{ Hz}, 1\text{H}), 6.27 (dq, J = 16, 7 \text{ Hz}, 1\text{H}), 4.17-4.14 (m, 2\text{H}), 1.90-1.87 (m, 2\text{H}), 1.86 (dd, J = 6.5, 1.5 \text{ Hz}, 3\text{H}), 1.10 (s, 9\text{H}). \]

\(^{13}\)C-NMR (125 MHz, acetone-\(d_6\))
\[ \delta 160.7, 154.2, 151.7, 147.0, 136.7, 134.6, 134.5, 132.0, 130.4, 130.3, 129.8, 128.7, 125.1, 123.8, 121.5 (J_{C:F} = 257 \text{ Hz}), 119.2, 116.8, 114.2, 111.6, 109.0, 105.7, 67.0, 28.1, 18.6 (2\text{C}), 12.4. \]

HRMS (ESI+)
Calculated for C\(_{36}\)H\(_{36}\)F\(_3\)O\(_3\)Si: 601.2386
Found: 601.2386

TLC (20% DCM in hexanes)
\[ R_f = 0.09, \text{ visualized by UV.} \]

\(^1\)H-NMR (500 MHz, acetone-\(d_6\))
\[ \delta 7.94 (d, J = 9 \text{ Hz}, 1\text{H}), 7.74-7.72 (m, 4\text{H}), 7.48-7.42 (m, 6\text{H}), 7.18 (d, J = 1.5 \text{ Hz}, 1\text{H}), 7.07 (s, 1\text{H}), 7.00 (d, J = 1.5 \text{ Hz}, 1\text{H}), 6.92 (dd, J = 9, 2.5 \text{ Hz}, 1\text{H}), 6.81 (\text{quint, } J = 2 \text{ Hz}, 1\text{H}), 6.48 (dd, J = 16, 2 \text{ Hz}, 1\text{H}), 6.27 (dq, J = 16, 6.5 \text{ Hz}, 1\text{H}), 4.17-4.14 (m, 2\text{H}), 4.03 (s, 3\text{H}), 1.90-1.86 (m, 2\text{H}), 1.86 (dd, J = 6.5, 1.5 \text{ Hz}, 3\text{H}), 1.09 (s, 9\text{H}). \]

\(^{13}\)C-NMR (125 MHz, acetone-\(d_6\))
δ 160.7, 151.5, 146.9, 146.1, 143.6, 136.7, 135.5, 134.6, 132.3, 131.9, 130.3, 129.7, 128.7, 125.1, 121.5 (J_{C-F} = 257 Hz), 116.8, 114.2, 111.8, 109.0, 105.9 (2C), 67.0, 56.4, 28.1, 18.6, 18.5, 12.4.

HRMS (ESI+)

Calculated for C_{37}H_{38}O_{4}F_{3}Si: 631.2491
Found: 631.2488

Library member 5.66 was isolated as a colorless oil containing 5.66 (21.6 mg, 42% yield) and a small amount of a cross-coupling byproduct and was carried on to the manual deprotection without further purification.

TLC (10% DCM in hexanes)

R_{f} = 0.11, visualized by UV.

^1H-NMR (500 MHz, acetone-d_{6})

δ 7.95 (d, J = 9 Hz, 1H), 7.74-7.70 (m, 5H), 7.50-7.41 (m, 8H), 7.13 (d, J = 0.5 Hz, 1H), 6.92 (dd, J = 9, 2.5 Hz, 1H), 6.82 (quint, J = 1.5 Hz, 1H), 6.79 (dt, J = 16, 1.5 Hz, 1H), 6.39 (dt, J = 16, 4.5 Hz, 1H), 4.48 (dd, J = 5, 2 Hz, 2H), 4.18-4.14 (m, 2H), 1.90-1.87 (m, 2H), 1.21-1.08 (m, 30H).

HRMS (ESI+)

Calculated for C_{45}H_{54}F_{3}O_{4}Si_{2}: 771.3513
Found: 771.3550
TLC (20% DCM in hexanes)

R_f = 0.11, visualized by UV.

1H-NMR (500 MHz, acetone-\textit{d}_6)

\[ \delta 7.95 \text{ (d, } J = 8.5 \text{ Hz, 1H)}, 7.74-7.72 \text{ (m, 4H)}, 7.46-7.42 \text{ (m, 6H)}, 7.26 \text{ (d, } J = 1.5 \text{ Hz, 1H)}, 7.09 \text{ (s, 1H)}, 7.06 \text{ (d, } J = 1 \text{ Hz, 1H)}, 6.93 \text{ (dd, } J = 8.5, 2.5 \text{ Hz, 1H)}, 6.81 \text{ (quint, } J = 1.5 \text{ Hz, 1H)}, 6.79 \text{ (dt, } J = 15.5, 2 \text{ Hz, 1H)}, 6.39 \text{ (dq, } J = 16, 4.5 \text{ Hz, 1H)}, 4.47 \text{ (dd, } J = 4.5, 1.5 \text{ Hz, 2H)}, 4.17-4.14 \text{ (m, 2H)}, 4.04 \text{ (s, 3H)}, 1.90-1.86 \text{ (m, 2H)}, 1.20-1.09 \text{ (m, 30H).}

^{13}\text{C-NMR (125 MHz, acetone-\textit{d}_6)}

\[ \delta 160.7, 151.6, 146.9, 146.2, 143.8, 136.7, 134.8, 134.6, 132.0, 130.3, 130.2, 129.8, 129.3, 128.7, 121.5 \text{ (} J_{C:F} = 257 \text{ Hz)}, 116.8, 114.2, 112.6, 109.0, 106.2, 106.0, 67.0, 64.7, 56.4, 28.1, 18.5, 18.4, 12.8, 12.4.\]

HRMS (ESI+)

Calculated for C_{46}H_{57}F_3O_5Si_{2}Na: \hspace{1cm} 825.3594

Found: \hspace{1cm} 825.3600
General Scheme for Automated Synthesis of Library Members 5.69-5.75

1.5 mmol

NaOH (4.5 mmol), THF (0.15 M), H2O (0.5 M), 20 min, rt

Pd(0Ac)2 (5 mol%), SPphos (10 mol%), K2CO3 (4 mmol), THF (0.028 M), 55 °C, 16 h

SiO2, MeOH; Et2O; THF

NaOH (0.8 mmol), THF, H2O, 10 min, rt

Pd(0Ac)2 (5 mol%), XPhos (10 mol%), K3PO4 (0.6 mmol), THF (0.0112 M), 55 °C, 14 h

SiO2, MeOH; Et2O; THF

0.33 mmol

0.0673 mmol

0.0135 mmol

0.24 mmol

NaOH (0.24 mmol), THF, H2O, 30 min, rt

Pd(0Ac)2 (10 mol%), SPphos (20 mol%), K3PO4 (0.081 mmol), THF (0.03 M), 55 °C, 24 h
HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 10% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)
17.1 min

^1^H-NMR (500 MHz, acetone-^d_6^)
δ 7.71 (s, 1H), 7.68-7.61 (m, 6H), 7.60 (d, J = 2.5 Hz, 1H), 7.50 (d, J = 1.5 Hz, 1H), 7.47-7.41 (m, 7H), 7.36 (d, J = 8.5 Hz, 1H), 7.29 (dd, J = 8.5, 1.5 Hz, 1H), 7.17-7.13 (m, 2H), 7.10-7.06 (m, 2H), 6.95 (d, J = 1 Hz, 1H), 6.56 (dd, J = 16, 2 Hz, 1H), 6.51 (dd, J = 16, 1.5 Hz, 1H), 6.38 (dq, J = 16, 6.5 Hz, 1H), 6.34 (s, 1H), 6.25 (dq, J = 15.5, 7 Hz, 1H), 5.50 (d, J = 1.5 Hz, 1H), 5.49 (d, J = 2 Hz, 1H), 3.88-3.86 (m, 2H), 3.79 (s, 3H), 1.90 (dd, J = 6.5, 1.5 Hz, 3H), 1.87 (dd, J = 6.5, 1.5 Hz, 3H), 1.27-1.23 (m, 2H), 0.99 (s, 9H).

HRMS (ESI+)
Calculated for C_{54}H_{53}O_{5}Si: 809.3662
Found: 809.3658

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 10% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)
14.3 min

TLC (70% DCM in hexanes)
R_f = 0.5, visualized by UV.

^1^H-NMR (500 MHz, acetone-\textit{d}_6)
\[ \delta 7.75 \text{ (s, 1H)}, 7.68-7.63 \text{ (m, 6H)}, 7.59 \text{ (d, } J = 2.5 \text{ Hz, 1H)}, 7.47-7.41 \text{ (m, 8H)}, 7.17-7.14 \text{ (m, 2H)}, 7.10-7.07 \text{ (m, 3H)}, 6.93 \text{ (s, 1H)}, 6.55 \text{ (dd, } J = 16, 1.5 \text{ Hz, 1H)}, 6.48 \text{ (dd, } J = 16, 1.5 \text{ Hz, 1H)}, 6.38 \text{ (dq, } J = 16, 6.5 \text{ Hz, 1H)}, 6.34 \text{ (s, 1H)}, 6.26 \text{ (dq, } J = 15.5, 6.5 \text{ Hz, 1H)}, 5.51 \text{ (d, } J = 2 \text{ Hz, 1H)}, 5.50 \text{ (d, } J = 2 \text{ Hz, 1H)}, 4.00 \text{ (s, 3H)}, 3.88-3.85 \text{ (m, 2H)}, 3.78 \text{ (s, 3H)}, 1.90 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 3H)}, 1.87 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 3H)}, 1.27-1.23 \text{ (m, 2H)}, 0.99 \text{ (s, 9H}).

HRMS (ESI+)
Calculated for C_{55}H_{55}O_{6}Si: 839.3768
Found: 839.3772

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 10% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)
48.3 min

^1^H-NMR (500 MHz, acetone-\textit{d}_6)
\[ \delta 7.71 \text{ (s, 1H)}, 7.67-7.58 \text{ (m, 7H)}, 7.47-7.37 \text{ (m, 10H)}, 7.16-7.14 \text{ (m, 2H)}, 7.10-7.07 \text{ (m, 2H)}, 6.97 \text{ (s, 1H)}, 6.78 \text{ (app d, } J = 16 \text{ Hz, 1H)}, 6.56 \text{ (dd, } J = 15.5, 1.5 \text{ Hz, 1H)}, 6.41-6.36
(m, 2H), 6.35 (s, 1H), 5.51 (d, \( J = 1.5 \) Hz, 1H), 5.50 (d, \( J = 1.5 \) Hz, 1H), 4.50 (dd, \( J = 5, 2 \) Hz, 2H), 3.88-3.85 (m, 2H), 3.79 (s, 3H), 1.91 (dd, \( J = 6.5, 1.5 \) Hz, 3H), 1.29-1.07 (m, 23H), 0.94 (s, 9H).

HRMS (ESI+)

Calculated for \( C_{63}H_{73}O_6Si_2 \): 981.4946
Found: 981.4949

Library member 5.72 was isolated as a colorless oil (3.0 mg), the \(^1\)H NMR of which contained small amounts of hydrocarbon impurities presumed to represent some leaching from the HPLC column.

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 10% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)
33.4 min

TLC (50% DCM in hexanes)

\( R_f = 0.18 \), visualized by UV.

\(^1\)H-NMR (500 MHz, acetone-\( d_6 \))

\( \delta \) 7.75 (s, 1H), 7.69-7.62 (m, 6H), 7.60 (d, \( J = 2.5 \) Hz, 1H), 7.47-7.40 (m, 7H), 7.17-7.13 (m, 3H), 7.10-7.06 (m, 2H), 7.00 (d, \( J = 1.5 \) Hz, 1H), 6.95 (s, 1H), 6.76 (dt, \( J = 15.5, 1.5 \) Hz, 1H), 6.56 (dd, \( J = 15.5, 1.5 \) Hz, 1H), 6.40-6.35 (m, 2H), 6.34 (s, 1H), 5.52 (d, \( J = 1.5 \) Hz, 2H), 3.88-3.85 (m, 2H), 3.79 (s, 3H), 1.91 (dd, \( J = 6.5, 1.5 \) Hz, 3H), 1.29-1.07 (m, 23H), 0.94 (s, 9H).
Hz, 1H), 5.50 (d, \( J = 1.5 \) Hz, 1H), 4.49 (dd, \( J = 5, 2 \) Hz, 2H), 4.02 (s, 3H), 3.88-3.85 (m, 2H), 3.78 (s, 3H), 1.90 (dd, \( J = 6.5, 2.5 \) Hz, 3H), 1.29-1.23 (m, 2H), 1.23-1.08 (m, 21H), 0.99 (s, 9H).

HRMS (ESI+)
Calculated for C_{64}H_{74}O_{7}Si_{2}Na: 1033.4871
Found: 1033.4895

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 20% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)
12.2 min

\(^{1}\text{H-NMR (500 MHz, acetone-\textit{d}_6)}\)
\delta 7.71 (s, 1H), 7.64-7.62 (m, 4H), 7.56-7.53 (m, 2H), 7.46-7.41 (m, 8H), 7.10 (d, \( J = 8 \) Hz, 1H), 7.08 (d, \( J = 1 \) Hz, 1H), 6.94 (d, \( J = 1.5 \) Hz, 1H), 6.93 (s, 1H), 6.56 (dd, \( J = 15.5, 1.5 \) Hz, 1H), 6.51-6.47 (m, 3H), 6.38 (dq, \( J = 15.5, 6.5 \) Hz, 1H), 6.34 (s, 1H), 6.27 (dq, \( J = 15.5, 6.5 \) Hz, 1H), 5.46 (s, 2H), 4.02 (s, 3H), 3.87-3.84 (m, 2H), 3.80 (s, 3H), 3.56 (app t, \( J = 8 \) Hz, 2H), 1.90 (dd, \( J = 6.5, 1.5 \) Hz, 3H), 1.88 (dd, \( J = 6.5, 1.5 \) Hz, 3H), 1.23-1.20 (m, 2H), 0.98 (s, 9H), 0.91-0.88 (m, 2H), 0.03 (s, 9H).

HRMS (ESI+)
Calculated for C_{60}H_{67}O_{7}Si_{2}: 955.4425
Found: 955.4437
185
HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 20% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)

26.8 min

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.69 (s, 1H), 7.64-7.61 (m, 5H), 7.57 (s, 1H), 7.55 (d, $J = 9$ Hz, 2H), 7.46-7.34 (m, 9H), 7.10 (d, $J = 8.5$ Hz, 1H), 6.97 (s, 1H), 6.80 (dt, $J = 16$, 1.5 Hz, 1H), 6.56 (dd, $J = 15.5$, 1.5 Hz, 1H), 6.49 (d, $J = 7$ Hz, 2H), 6.41-6.34 (m, 3H), 5.46 (s, 2H), 4.51 (dd, $J = 5$, 2 Hz, 2H), 3.90-3.85 (m, 2H), 3.81 (s, 3H), 3.58 (app t, $J = 8$ Hz, 2H), 1.91 (dd, $J = 6.5$, 1 Hz, 3H), 1.24-1.17 (m, 5H), 1.15-1.12 (m, 18H), 0.99 (s, 9H), 0.88 (m, 2H), 0.03 (s, 9H).

HRMS (ESI+)

Calculated for $C_{68}H_{85}O_7Si_3$: 1097.5603

Found: 1097.5591
Library member 5.75 was isolated as a colorless oil (4.0 mg), the \(^1\)H NMR of which contained small amounts of hydrocarbon impurities presumed to represent some leaching from the HPLC column.

HPLC (Agilent Prep-C18, 10 µm, 30 x 150 mm (product number: 413910-302), 25 mL/min, gradient: A = water, B = 20% THF in MeCN, 0 min: 80% A, 20% B; 5 min: 0% A, 100% B; 50 min: 0% A, 100% B)

22.3 min

\(^1\)H-NMR (500 MHz, acetone-\(d_6\))

\(\delta\) 7.72 (s, 1H), 7.64-7.62 (m, 5H), 7.55 (d, \(J = 8.5\) Hz, 2H), 7.46-7.41 (m, 7H), 7.14 (d, \(J = 1\) Hz, 1H), 7.10 (d, \(J = 8\) Hz, 1H), 7.00 (d, \(J = 1.5\) Hz, 1H), 6.95 (s, 1H), 6.76 (dt, \(J = 15.5, 1.5\) Hz, 1H), 6.56 (dd, \(J = 16, 2\) Hz, 1H), 6.49 (d, \(J = 8.5\) Hz, 2H), 6.42-6.34 (m, 3H), 5.44 (d, \(J = 2\) Hz, 1H), 5.43 (d, \(J = 2\) Hz, 1H), 4.50 (dd, \(J = 5, 2\) Hz, 2H), 4.03 (s, 3H), 3.88-3.82 (m, 2H), 3.80 (s, 3H), 3.59 (app t, \(J = 8\) Hz, 2H), 1.91 (dd, \(J = 6.5, 1.5\) Hz, 3H), 1.24-1.17 (m, 5H), 1.15-1.13 (m, 18H), 0.99 (s, 9H), 0.91-0.88 (m, 2H), 0.03 (s, 9H).

HRMS (ESI+)

Calculated for C\(_{69}\)H\(_{86}\)O\(_8\)Si\(_3\)Na: 1149.5528  
Found: 1149.5552

Deprotection of Library Members

Deprotection condition 1 (for library members 5.56-5.59 and 5.62-5.67)

To a 7-mL vial containing the protected library member and a PTFE-coated magnetic stir bar was added TBAF•3H\(_2\)O (2.2–15 equiv) followed by 1:1 DMSO/DMPU under ambient atmosphere. The vial was sealed with a Teflon-lined cap and stirred at 50 °C for 30 minutes–6 hours. The reaction was then cooled to room temperature and diluted with a solution of 1:1 saturated NH\(_4\)Cl/H\(_2\)O (1.5–2 mL). The layers were mixed and the aqueous layer was removed.
The organic layer was washed with H$_2$O (2 × 1.5 mL). The combined aqueous phase was extracted with EtOAc (3 mL). The organic phase was dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography to give the pure product as a white or off-white solid.

\[
\text{ratanhiaphenol III (deprotected 5.56)}
\]
\[
(7.1 \text{ mg, 50\% yield})
\]

TLC (20\% EtOAc/hexanes)

R$_f$ = 0.14, visualized by shortwave UV.

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 8.77 (br s, 1H), 7.83 (d, $J$ = 8.5 Hz, 1H), 7.54 (d, $J$ = 2.0 Hz, 1H), 7.41 (d, $J$ = 8.5 Hz, 1H), 7.29 (dd, $J$ = 8.5, 2.0 Hz, 1H), 7.16 (d, $J$ = 1.0 Hz, 1H), 6.64 (d, $J$ = 2.0 Hz, 1H), 6.60 (dd, $J$ = 8.0, 2.0 Hz, 1H), 6.51 (d, $J$ = 16, 1.5 Hz, 1H), 6.25 (dq, $J$ = 15.5, 7 Hz, 1H), 3.99 (s, 3H), 1.86 (dd, $J$ = 7.0, 2.0 Hz, 3H).

$^{13}$C-NMR (125 MHz, acetone-$d_6$)

$\delta$ 160.2, 159.2, 154.2, 153.8, 134.0, 134.3, 131.3, 128.5, 124.5, 122.7, 118.7, 111.9, 111.2, 108.5, 104.6, 100.2, 55.9, 18.6.

HRMS (ESI$^+$)

Calculated for C$_{18}$H$_{17}$O$_3$: 281.1178

Found: 281.1181
TLC (30% EtOAc/hexanes)

\[ R_f = 0.3, \text{ visualized by shortwave UV.} \]

\(^1\)H-NMR (500 MHz, acetone-\(d_6\))

\[ \delta 8.73 \text{ (br s, 1H), 7.84 (d, } J = 8.5 \text{ Hz, 1H), 7.13 (s, 1H), 7.11 (d, } J = 1.0 \text{ Hz, 1H), 6.93 (d, } J = 1.0 \text{ Hz, 1H), 6.64 (d, } J = 2.0 \text{ Hz, 1H), 6.60 (dd, } J = 8.5, 2.0 \text{ Hz, 1H), 6.48 (dd, } J = 16, 1.5 \text{ Hz, 1H), 6.25 (dq, } J = 16, 6.5 \text{ Hz, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 1.86 (dd, } J = 6.5, 1.5 \text{ Hz, 3H).} \]

\(^{13}\)C-NMR (125 MHz, acetone-\(d_6\))

\[ \delta 160.2, 159.1, 154.0, 145.9, 142.9, 135.0, 132.7, 132.6, 128.5, 124.6, 111.9, 111.6, 108.5, 105.1, 104.8, 100.2, 56.4, 55.9, 18.6. \]

HRMS (ESI+)

Calculated for C\(_{19}\)H\(_{19}\)O\(_4\): 311.1283

Found: 311.1277

TLC (50% EtOAc/hexanes)

\[ R_f = 0.27, \text{ visualized by shortwave UV.} \]
\textsuperscript{1}H-NMR (500 MHz, acetone-\textit{d}_6)
\[\begin{align*}
\delta & 8.78 \text{ (br s, 1H)}, 7.83 \text{ (d, } J = 8.5 \text{ Hz, } 1\text{H}), 7.61 \text{ (d, } J = 1.5 \text{ Hz, } 1\text{H}), 7.43 \text{ (d, } J = 8.5 \text{ Hz, } 1\text{H}), 7.36 \text{ (dd, } J = 9.0, 2.0 \text{ Hz, } 1\text{H}), 7.18 \text{ (d, } J = 0.5 \text{ Hz, } 1\text{H}), 6.70 \text{ (d, } J = 16.0 \text{ Hz, } 1\text{H}), 6.64 \text{ (d, } J = 2.5 \text{ Hz, } 1\text{H}), 6.60 \text{ (dd, } J = 8.5, 2.5 \text{ Hz, } 1\text{H}), 6.38 \text{ (dt, } J = 16, 5.5 \text{ Hz, } 1\text{H}), 4.25 \text{ (t, } J = 5.5 \text{ Hz, } 2\text{H}), 3.99 \text{ (s, } 3\text{H}), 3.84 \text{ (t, } J = 5.5 \text{ Hz, } 1\text{H}).
\end{align*}\]

\textsuperscript{13}C-NMR (125 MHz, acetone-\textit{d}_6)
\[\begin{align*}
\delta & 160.2, 159.1, 154.2, 153.9, 133.3, 131.3, 130.5, 129.6, 128.5, 123.0, 119.2, 111.8, 111.2, 108.4, 104.5, 100.2, 63.4, 55.8.
\end{align*}\]

HRMS (ESI+)
\[\begin{align*}
\text{Calculated for } & C_{18}H_{17}O_4: \quad 297.1127 \\
\text{Found:} & \quad 297.1128
\end{align*}\]

TLC (60\% EtOAc/hexanes)
\[\text{R}_f = 0.22, \text{ visualized by shortwave UV.}\]

\textsuperscript{1}H-NMR (500 MHz, acetone-\textit{d}_6)
\[\begin{align*}
\delta & 8.75 \text{ (br s, 1H)}, 7.85 \text{ (d, } J = 8.0 \text{ Hz, } 1\text{H}), 7.18 \text{ (d, } J = 1.5 \text{ Hz, } 1\text{H}), 7.15 \text{ (s, } 1\text{H}), 6.99 \text{ (d, } J = 1.0 \text{ Hz, } 1\text{H}), 6.66 \text{ (dt, } J = 17, 1.5 \text{ Hz, } 1\text{H}), 6.64 \text{ (d, } J = 2.0 \text{ Hz, } 1\text{H}), 6.60 \text{ (dd, } J = 8.5, 2.5 \text{ Hz, } 1\text{H}), 6.38 \text{ (dt, } J = 16, 5.5 \text{ Hz, } 1\text{H}), 4.25 - 4.24 \text{ (m, } 2\text{H}), 4.04 \text{ (s, } 3\text{H}), 3.99 \text{ (s, } 3\text{H}), 3.84 \text{ (br s, } 1\text{H}).
\end{align*}\]

\textsuperscript{13}C-NMR (125 MHz, acetone-\textit{d}_6)
δ 160.2, 159.1, 154.2, 146.0, 143.2, 134.4, 132.8, 130.9, 129.7, 128.5, 112.3, 111.9, 108.5, 105.4, 104.8, 100.2, 63.4, 56.4, 55.9.

HRMS (ESI+)
Calculated for C_{19}H_{19}O_{5}: 327.1232  
Found: 327.1223

TLC (60% EtOAc/hexanes)
R_f = 0.36, visualized by shortwave UV.

\[ 1^1 \text{H-NMR (500 MHz, acetone-} d_6) \]
δ 8.37 (dd, J = 2.5, 0.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 1.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.50 (dd, J = 9.0, 3.0 Hz, 1H), 7.45 (dd, J = 8.5, 1.5 Hz, 1H), 7.33 (d, J = 1 Hz, 1H), 6.72 (dt, J = 15.5, 1.5 Hz, 1H), 6.41 (dt, J = 16.0, 5.5 Hz, 1H), 4.26 (t, J = 8.0 Hz, 2H), 3.96 (s, 3H), 3.87 (br t, J = 5.0 Hz, 1H).

\[ 1^3 \text{C-NMR (125 MHz, acetone-} d_6) \]
δ 157.1, 156.6, 155.4, 142.4, 139.2, 133.8, 130.4, 130.2, 130.1, 124.1, 121.2, 120.9, 119.8, 111.9, 103.5, 63.3, 56.2.

HRMS (ESI+)
Calculated for C_{17}H_{16}NO_{3}: 282.1130  
Found: 282.1132

deprotected 5.62  
(3.1 mg, 63% yield)
TLC (60% EtOAc/hexanes)

$R_f = 0.27$, visualized by shortwave UV.

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 8.36 (dd, $J = 2.5, 0.5$ Hz, 1H), 7.90 (d, $J = 8.5$ Hz, 1H), 7.49 (dd, $J = 9.0, 3.0$ Hz, 1H), 7.30 (s, 1H), 7.25 (d, $J = 1.0$ Hz, 1H), 7.08 (d, $J = 1.5$ Hz, 1H), 6.68 (app d, $J = 15.5$ Hz, 1H), 6.41 (dt, $J = 16, 5.5$ Hz, 1H), 4.25 (t, $J = 5.5$ Hz, 2H), 4.06 (s, 3H), 3.95 (s, 3H), 3.86 (br t, $J = 5.5$ Hz, 1H).

$^{13}$C-NMR (125 MHz, acetone-$d_6$)

$\delta$ 159.9, 156.5, 146.3, 144.6, 142.4, 139.2, 134.8, 131.8, 130.4, 130.2, 121.2, 120.8, 112.6, 105.9, 103.8, 63.3, 56.3, 56.2.

HRMS (ESI+)

Calculated for $C_{18}H_{18}NO_4$: 312.1236

Found: 312.1239

TLC (20% EtOAc/hexanes)

$R_f = 0.36$, visualized by shortwave UV.
\(^1\)H-NMR (500 MHz, acetone-\(d_6\))
\[ \delta 9.30 \text{ (br s, 1H), 7.96 (d, } J = 8.5 \text{ Hz, 1H), 7.63 (d, } J = 1.0 \text{ Hz, 1H), 7.47 (d, } J = 8.5 \text{ Hz, 1H), 7.38 (dd, } J = 8.5, 1.5 \text{ Hz, 1H), 7.10 (s, 1H), 7.03 (dd, } J = 8.5, 2.0 \text{ Hz, 1H), 6.98 (m, 1H), 6.52 (dd, } J = 15.5, 1.5 \text{ Hz, 1H), 6.28 (dq, } J = 15.5, 6.5 \text{ Hz, 1H), 1.87 (dd, } J = 6.5, 1.5 \text{ Hz, 3H).} \]

\(^1\)C-NMR (125 MHz, acetone-\(d_6\))
\[ \delta 159.7, 154.2, 152.0, 147.2, 134.4, 131.9, 130.5, 130.0, 125.0, 123.7, 121.5 \text{ (} J_{C,F} = 257 \text{ Hz), 119.1, 115.9, 115.7, 111.5, 109.3, 105.3, 18.5.} \]

HRMS (ESI+)
Calculated for \(C_{18}H_{14}O_3F_3\): 335.0895
Found: 335.0889

TLC (20% EtOAc/hexanes)
\[ R_f = 0.27, \text{ visualized by shortwave UV.} \]

\(^1\)H-NMR (500 MHz, acetone-\(d_6\))
\[ \delta 9.33 \text{ (br s, 1H), 7.96 (d, } J = 9.0 \text{ Hz, 1H), 7.19 (d, } J = 0.5 \text{ Hz, 1H), 7.07 (s, 1H), 7.03 (dd, } J = 8.5, 2.0 \text{ Hz, 1H), 7.00 (d, } J = 1.5 \text{ Hz, 1H), 6.97 (m, 1H), 6.49 (dd, } J = 15.5, 1.5 \text{ Hz, 1H), 6.28 (dq, } J = 16.0, 6.5 \text{ Hz, 1H), 4.04 (s, 3H), 1.86 (dd, } J = 6.5, 3.0 \text{ Hz, 3H).} \]

\(^1\)C-NMR (125 MHz, acetone-\(d_6\))

193
δ 159.7, 151.7, 147.1, 146.0, 135.4, 132.2, 131.9, 129.9, 125.0, 121.5 (J_{C-F} = 256 Hz), 115.9, 115.7, 111.8, 109.2, 105.8, 105.6, 56.3, 18.5.

HRMS (ESI+)
Calculated for C_{19}H_{16}O_{4}F_{3}: 365.1001
Found: 365.0997

TLC (40% EtOAc/hexanes)
R_f = 0.21, visualized by shortwave UV.

^{1}H-NMR (500 MHz, acetone-\textit{d}_{6})
δ 9.30 (br s, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.10 (d, J = 1.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.44 (dd, J = 8.5, 2.0 Hz, 1H), 7.12 (d, J = 0.5 Hz, 1H), 7.04 (dd, J = 8.5, 2.5 Hz, 1H), 6.98 (quint, J = 2 Hz, 1H), 6.72 (d, J = 16 Hz, 1H), 6.41 (dt, J = 16.0, 5.0 Hz, 1H), 4.26 (d, J = 5.0 Hz, 2H), 3.84 (br s, 1H).

^{13}C-NMR (125 MHz, acetone-\textit{d}_{6})
δ 159.8, 154.4, 152.1, 147.2, 133.8, 130.6, 130.1, 130.0 (2C), 124.0, 121.5 (J_{C-F} = 256 Hz), 119.7, 115.9, 115.7, 111.6, 109.3, 105.3, 63.3.

HRMS (ESI+)
Calculated for C_{18}H_{14}O_{4}F_{3}: 351.0844
Found: 351.0849
TLC (40% EtOAc/hexanes)

R<sub>f</sub> = 0.13, visualized by shortwave UV.

<sup>1</sup>H-NMR (500 MHz, acetone-<em>d<sub>6</sub></em>)

δ 9.30 (br s, 1H), 7.97 (d, <em>J</em> = 8.5 Hz, 1H), 7.26 (d, <em>J</em> = 0.5 Hz, 1H), 7.09 (s, 1H), 7.06 (d, <em>J</em> = 1.0 Hz, 1H), 7.04 (dd, <em>J</em> = 8.5, 2.5 Hz, 1H), 6.97 (m, 1H), 6.68 (d, <em>J</em> = 15.5 Hz, 1H), 6.41 (dt, <em>J</em> = 16.0, 5.0 Hz, 1H), 4.26 (d, <em>J</em> = 5.0 Hz, 2H), 4.06 (s, 3H), 3.85 (br s, 1H).

<sup>13</sup>C-NMR (125 MHz, acetone-<em>d<sub>6</sub></em>)

δ 159.7, 151.8, 147.1, 146.1, 143.7, 134.8, 131.9, 130.4, 130.1, 129.9, 121.5 (<em>J</em><sub>C,F</sub> = 256 Hz), 115.9, 115.7, 112.4, 109.2, 106.0, 105.6, 63.3, 56.4.

HRMS (ESI+)

Calculated for C<sub>19</sub>H<sub>16</sub>O<sub>5</sub>F<sub>3</sub>: 381.0950

Found: 381.0946

Deprotection condition 2 (for library members 5.69-5.72)

To a 1-mL Reacti-vial™ containing the protected tetramer library member and a PTFE-coated magnetic stir bar was added TBAF•3H<sub>2</sub>O (15-20 equiv) followed by DMSO under ambient atmosphere. The vial was sealed with a cap and stirred at 50 °C for 5 hours. The reaction was then cooled to room temperature, diluted with 8 mL Et<sub>2</sub>O and washed with a solution of 1:1 saturated NH<sub>4</sub>Cl/H<sub>2</sub>O (4 mL). The aqueous layer was extracted with 4 mL Et<sub>2</sub>O. The combined organic layers were washed with H<sub>2</sub>O (2 × 4 mL), then with brine (4 mL). The organic phase was
dried over anhydrous MgSO$_4$, filtered, and concentrated \textit{in vacuo}. The crude product was purified by silica gel chromatography.

TLC (40\% EtOAc/pentane)

\[ R_f = 0.49, \text{ visualized by shortwave UV.} \]

$^1$H-NMR (500 MHz, acetone-$d_6$)

\[
\begin{align*}
\delta & 7.77 \text{ (s, 1H), } 7.53 \text{ (d, } J = 1.5 \text{ Hz, 1H), } 7.37 \text{ (d, } J = 8.0 \text{ Hz, 1H), } 7.27 \text{ (dd, } J = 8.5, 2.0 \text{ Hz, 1H), } 7.18-7.17 \text{ (m, 3H), } 6.82 \text{ (dd, } J = 6.5, 3.0 \text{ Hz, 1H), } 6.68 \text{ (s, 1H), } 6.50 \text{ (dd, } J = 16.0, 1.5 \text{ Hz, 1H), } 6.31 \text{ (dd, } J = 15.5, 1.5 \text{ Hz, 1H), } 6.24 \text{ (dq, } J = 16.0, 6.5 \text{ Hz, 1H), } 6.05 \text{ (dq, } J = 15.5, 7.0 \text{ Hz, 1H), } 5.69 \text{ (d, } J = 2.5 \text{ Hz, 1H), } 5.66 \text{ (d, } J = 2.5 \text{ Hz, 1H), } 3.99 \text{ (s, 3H), } 1.85 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 1H), } 1.78 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 1H).}
\end{align*}
\]

HRMS (ESI+)

Calculated for C$_{29}$H$_{27}$O$_4$: 439.1909

Found: 439.1904

TLC (40\% EtOAc/pentane)

\[ R_f = 0.46, \text{ visualized by shortwave UV.} \]
\[ ^1\text{H-NMR} \text{ (500 MHz, acetone-\(d_6\))} \]
\[ \delta 7.81 \text{ (s, 1H)}, 7.18-7.14 \text{ (m, 3H)}, 7.10 \text{ (d, } J = 1.0 \text{ Hz, 1H}), 6.90 \text{ (d, } J = 1.0 \text{ Hz, 1H}), 6.82 \text{ (dd, } J = 7.5, 1.5 \text{ Hz, 1H}), 6.67 \text{ (s, 1H)}, 6.46 \text{ (dd, } J = 16, 1.5 \text{ Hz, 1H}), 6.30 \text{ (dd, } J = 15.5, 1.0 \text{ Hz, 1H}), 6.24 \text{ (dq, } J = 15.5, 6.5 \text{ Hz, 1H}), 6.04 \text{ (dq, } J = 15.5, 6.5 \text{ Hz, 1H}), 5.70 \text{ (d, } J = 1.5 \text{ Hz, 1H}), 5.67 \text{ (d, } J = 2.0 \text{ Hz, 1H}), 3.98 \text{ (s, 3H)}, 3.96 \text{ (s, 3H)}, 1.85 \text{ (dd, } J = 7.0, 2.0 \text{ Hz, 3H}), 1.78 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 3H}). \]

\text{HRMS (ESI+)}

\begin{align*}
\text{Calculated for } C_{30}H_{29}O_5: & \quad 469.2015 \\
\text{Found: } & \quad 469.2014 \\
\end{align*}

The yield of the deprotection of \textbf{5.71} was determined by \(^1\text{H-NMR} \text{ using an internal standard.} \]

\[ ^1\text{H-NMR} \text{ (500 MHz, acetone-\(d_6\))} \]
\[ \delta 7.78 \text{ (s, 1H)}, 7.60 \text{ (d, } J = 1 \text{ Hz, 1H}), 7.40 \text{ (d, } J = 8 \text{ Hz, 1H}), 7.33 \text{ (dd, } J = 8.5, 1.5 \text{ Hz, 1H}), 7.19-7.17 \text{ (m, 3H)}, 6.82 \text{ (d, } J = 9 \text{ Hz, 1H}), 6.70-6.68 \text{ (m, 2H)}, 6.37 \text{ (dt, } J = 16, 5.5 \text{ Hz, 1H}), 6.31 \text{ (dd, } J = 15.5, 1 \text{ Hz, 1H}), 6.05 \text{ (dq, } J = 15.5, 6.5 \text{ Hz, 1H}), 5.69 \text{ (d, } J = 2 \text{ Hz, 1H}), 5.66 \text{ (d, } J = 2 \text{ Hz, 1H}), 4.24 \text{ (dd, } J = 5, 2 \text{ Hz, 2H}), 3.99 \text{ (s, 3H)}, 1.78 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, 3H}). \]

\text{HRMS (ESI+)}

\begin{align*}
\text{Calculated for } C_{29}H_{27}O_5: & \quad 455.1858 \\
\text{Found: } & \quad 455.1866 \\
\end{align*}
TLC (40% EtOAc/pentane)

R<sub>f</sub> = 0.48, visualized by shortwave UV.

<sup>1</sup>H-NMR (500 MHz, acetone-<sup>d</sup>6)

δ 7.81 (s, 1H), 7.18 – 7.14 (m, 4H), 6.97 (d, J = 1.0 Hz, 1H), 6.82 (dd, J = 7.0, 1.5 Hz, 1H), 6.67 – 6.63 (m, 2H), 6.37 (dt, J = 16, 5.5 Hz, 1H), 6.30 (dd, J = 15.5, 1.5 Hz, 1H), 6.04 (dq, J = 16.0, 7.0 Hz, 1H), 5.69 (d, J = 2.0 Hz, 1H), 5.66 (d, J = 2.0 Hz, 1H), 4.23 (d, J = 4.5 Hz, 2H), 3.98 (s, 3H), 3.98 (s, 3H), 1.78 (dd, J = 6.5, 2.0 Hz, 3H).

HRMS (ESI+)

Calculated for C<sub>30</sub>H<sub>29</sub>O<sub>6</sub>: 485.1964

Found: 485.1952

Deprotection condition 3 (for library members 5.73-5.75)

To a 1-mL Reacti-vial™ containing the protected library member and a PTFE-coated magnetic stir bar was added CsF (25-30 equiv) and 18-crown-6 (2 equiv) followed by DMSO in a glovebox. The vial was sealed with a cap and stirred at 50 °C for 14 hours. The reaction was then cooled to room temperature, diluted with 8 mL EtOAc and washed with a solution of 1:1 saturated NH<sub>4</sub>Cl/H<sub>2</sub>O (8 mL). The aqueous layer was extracted with 4 mL EtOAc. The combined organic layers were washed with H<sub>2</sub>O (2 × 4 mL), then with brine (8 mL). The organic phase was dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography.
TLC (40% EtOAc/pentane)

$R_f = 0.39$, visualized by shortwave UV.

$^1$H-NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.85 (app d, $J = 9.0$ Hz, 2H), 7.81 (s, 1H), 7.43 (dd, $J = 8.5$, 2.5 Hz, 1H), 7.33 (d, $J = 2.5$ Hz, 1H), 7.18 (d, $J = 8.5$ Hz, 1H), 7.12 – 7.10 (m, 2H), 6.92 (d, $J = 1.5$ Hz, 1H), 6.84 (app d, $J = 9.0$ Hz, 2H), 6.55 (s, 1H), 6.49 – 6.42 (m, 2H), 6.30 – 6.21 (m, 2H), 5.62 (d, $J = 2.0$ Hz, 1H), 5.58 (d, $J = 1.5$ Hz, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 1.86 (dd, $J = 6.5$, 1.5 Hz, 3H), 1.83 (dd, $J = 7.0$, 2.0 Hz, 3H).

HRMS (ESI+)

Calculated for $C_{37}H_{33}O_7$: 589.2226

Found: 589.2224

TLC (60% EtOAc/pentane)

$R_f = 0.24$, visualized by shortwave UV.

199
$^1$H-NMR (500 MHz, acetone-$d_6$)
\[\delta 7.82 \text{ (app d, } J = 8.5 \text{ Hz, } 2H), 7.77 \text{ (s, } 1H), 7.60 \text{ (d, } J = 1.5 \text{ Hz, } 1H), 7.43 \text{ (dd, } J = 8.0, 2.0 \text{ Hz, } 1H), 7.40 \text{ (d, } J = 8.0 \text{ Hz, } 1H), 7.35-7.33 \text{ (m, } 2H), 7.17 \text{ (d, } J = 8.5 \text{ Hz, } 1H), 7.15 \text{ (d, } J = 1.0 \text{ Hz, } 1H), 6.83 \text{ (app d, } J = 9.0 \text{ Hz, } 2H), 6.69 \text{ (app d, } J = 16.0 \text{ Hz, } 1H), 6.56 \text{ (s, } 1H), 6.44 \text{ (d, } J = 16 \text{ Hz, } 1H), 6.38 \text{ (dt, } J = 16.0, 5.5 \text{ Hz, } 1H), 6.27 \text{ (dq, } J = 16, 6.5 \text{ Hz, } 1H), 5.61 \text{ (d, } J = 1.5 \text{ Hz, } 1H), 5.58 \text{ (d, } J = 2.0 \text{ Hz, } 1H), 4.25-4.24 \text{ (m, } 2H), 3.97 \text{ (s, } 3H), 1.84 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, } 3H)\].

HRMS (ESI+)

Calculated for C$_{36}$H$_{31}$O$_7$: 575.2070

Found: 575.2070

TLC (60% EtOAc/pentane)

R$_f$ = 0.23, visualized by shortwave UV.

$^1$H-NMR (500 MHz, acetone-$d_6$)
\[\delta 7.85 \text{ (dt, } J = 8.5, 2.0 \text{ Hz, } 2H), 7.81 \text{ (s, } 1H), 7.43 \text{ (dd, } J = 8.0, 2.0 \text{ Hz, } 1H), 7.33 \text{ (d, } J = 2.0 \text{ Hz, } 1H), 7.18-7.17 \text{ (m, } 2H), 7.13 \text{ (s, } 1H), 6.98 \text{ (s, } 1H), 6.84 \text{ (dt, } J = 9.0, 2.0 \text{ Hz, } 2H), 6.66 \text{ (app d, } J = 16.0 \text{ Hz, } 1H), 6.55 \text{ (s, } 1H), 6.44 \text{ (dd, } J = 16.5, 1.5 \text{ Hz, } 1H), 6.37 \text{ (dt, } J = 16.0, 5.5 \text{ Hz, } 1H), 6.27 \text{ (dq, } J = 16.0, 6.5 \text{ Hz, } 1H), 5.62 \text{ (d, } J = 1.5 \text{ Hz, } 1H), 5.58 \text{ (d, } J = 1.5 \text{ Hz, } 1H), 4.25-4.24 \text{ (m, } 2H), 3.99 \text{ (s, } 3H), 3.96 \text{ (s, } 3H), 1.83 \text{ (dd, } J = 6.5, 1.5 \text{ Hz, } 3H)\].

HRMS (ESI+)
Manual synthesis of building blocks for (+)-crocin C (5.31)

Conditions: (a) 3-(trimethylsilyl)propiolaldehyde, Sn(OTf)$_2$, NEt$_3$, CH$_2$Cl$_2$, -78 °C, 2 h; (b) Me$_2$NBH(OAc)$_3$, MeCN/AcOH 2:1, -25 °C, 16 h; (c) Me$_3$OBF$_4$, proton sponge, CH$_2$Cl$_2$, 23 °C, 2.5 h; (d) (NH$_4$)$_2$Ce(NO$_3$)$_6$, MeCN/H$_2$O, 10:1, 23 °C, 1 h; (e) Dess-Martin periodinane, CH$_2$Cl$_2$, 0 °C to 23 °C, 1.5 h; (f) CH$_3$, CrCl$_3$, THF, 0 °C to 23 °C, 3 h; (g) n-BuLi, THF, B(OH)$_3$, -78 °C; 1N HCl; (h) N-methyliminodiacetic acid, benzene/DMSO 10:1, 80 °C, 2 h; (i) AgNO$_3$, 2,6-lutidine, acetone/H$_2$O 1:1, 0 °C, 2.5 h; (j) H$_2$Bu$_3$, Pd$_2$dba$_3$, RuPhos, CH$_2$Cl$_2$, 23 °C; (k) I$_2$, CH$_2$Cl$_2$, 23 °C.

Manual synthesis of β-hydroxyketone 5.77

To a 500-mL Schlenk flask charged with Sn(OTf)$_2$ (112.5 mmol, 46.9 g) was added CH$_2$Cl$_2$ (500 mL) and triethylamine (120 mmol, 16.7 mL) dropwise. The resulting orange-brown
suspension was cooled to -78 °C. To the stirred mixture was added (S)-1-(p-methoxybenzyl)oxy)-2-methylpentan-3-one (5.76)\(^5\) (75.0 mmol, 17.8 g, 2.00 mL) as a neat liquid over 15 minutes. The mixture was stirred for 2 hours at -78 °C. To the mixture was added 3-(trimethylsilyl)propionaldehyde\(^6\) (112.5 mmol, 14.2 g) as a neat liquid over 20 minutes. The mixture was stirred for an additional 3 hours at -78 °C. The reaction was quenched by pouring the mixture slowly into saturated aqueous NH\(_4\)Cl (300 mL) cooled in an ice bath, rinsing with additional CH\(_2\)Cl\(_2\). The mixture was stirred vigorously for 10 minutes and then filtered through Celite\(^\text{TM}\) to remove the tin(II) salts. The filtrate was transferred to a 2-L separatory funnel and the phases separated. The organic phase was washed with saturated aqueous NH\(_4\)Cl (200 mL). The combined aqueous phases were extracted with CH\(_2\)Cl\(_2\) (200 mL). The combined organic phases were washed with H\(_2\)O (200 mL), dried over anhydrous MgSO\(_4\), filtered and concentrated \textit{in vacuo} to afford a yellow-brown oil. This residue was subjected to flash chromatography on silica gel (EtOAc:hexanes 8:100 → 20:100) to afford 5.77 as a colorless oil (36.71 g, 73%).

\[
\text{TLC (20\% EtOAc/hexanes)}
\]

\[R_f = 0.22, \text{stained by KMnO}_4\]

\(^{1}\text{H-NMR (500 MHz, CDCl}_3\)

\[\delta 8.5 (\text{app d, } J = 8.5 \text{ Hz}, 2\text{H}), 6.87 (\text{app d, } J = 8.5 \text{ Hz}, 2\text{H}), 4.78 (d, J = 3.5 \text{ Hz}, 1\text{H}), 4.43 (d, J = 11.5 \text{ Hz}, 1\text{H}), 4.36 (d, J = 11.5 \text{ Hz}, 1\text{H}), 3.79 (s, 3\text{H}), 3.58 (\text{app t, } J = 9 \text{ Hz}, 1\text{H}), 3.41 (\text{dd, } J = 9, 5 \text{ Hz}, 1\text{H}), 3.14 (m, 1\text{H}), 2.92 (dq, J = 7.5, 3 \text{ Hz}, 1\text{H}), 1.26 (d, J = 7.5 \text{ Hz}, 3\text{H}), 1.01 (d, J = 7 \text{ Hz}, 3\text{H}), 0.17 (s, 9\text{H})\]

\(^{13}\text{C-NMR (125 MHz, CDCl}_3\)

\[\delta 216.1, 159.3, 129.5, 129.3, 113.8, 104.0, 90.2, 73.1, 63.3, 55.2, 51.7, 44.7, 13.5, 10.5, -0.2\]
HRMS (ESI+)

Calculated for C_{20}H_{30}O_{4}NaSi: 385.1811

Found: 385.1808

Manual synthesis of diol 5.78

To a 1-L, 3-neck, round-bottom flask charged with tetramethylammonium triacetoxyborohydride (298 mmol, 78.30 g) was added dry acetic acid (240 mL) and MeCN (400 mL). The resulting homogeneous solution was cooled to -30 °C and stirred for 30 minutes. To the stirred solution was added β-hydroxyketone 5.77 (71.6 mmol, 25.95 g), dropwise over 1 minute, as a solution in MeCN (50 mL + 50 mL rinse). The solution was stirred and allowed to slowly warm to 0 °C over 13 hours. The reaction was then poured into aqueous dibasic sodium tartrate solution (0.5 M, 400 mL) and stirred for 1 hour. The solution was transferred into a separatory funnel, and Et₂O was added. After mixing and phase separation, the aqueous layer was extracted with Et₂O. The combined organic phase was washed with saturated aqueous NaHCO₃ (2 × 200 mL), then brine (20 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Toluene was added to azeotrope most of the remaining acetic acid prior to silica gel purification. The residue obtained after concentration was subjected to flash chromatography on silica gel (EtOAc:hexanes:AcOH 10:90:2 → 20:80:2) to afford 5.78 as a pale yellow oil (18.51 g, 71%). Chromatographic purification was carried out again to obtain an analytically pure sample (colorless oil).
TLC (30% EtOAc/hexanes)

R_f = 0.39, stained by KMnO_4

^1^H-NMR (500 MHz, CDCl_3)

δ 7.22 (app d, J = 8.5 Hz, 2H), 6.87 (app d, J = 8.5 Hz, 2H), 4.65 (d, J = 2.5 Hz, 1H),
4.46 (d, J = 11 Hz, 1H), 4.45 (d, J = 11.5 Hz, 1H), 3.80, (s, 3H), 3.73-3.68 (m, 2H), 3.45
(dd, J = 9.5, 5.5 Hz, 1H), 2.00-1.94, (m, 2H), 1.03 (t, J = 7 Hz, 6H), 0.17 (s, 9H).

^1^3^C-NMR (125 MHz, CDCl_3)

δ 159.4, 129.4, 129.3, 113.9, 105.6, 89.6, 80.1, 73.6, 73.4, 66.0, 55.2, 40.6, 34.9, 14.8,
12.8, -0.1.

HRMS (ESI+)

Calculated for C_{20}H_{33}O_4Si: 365.2148

Found: 365.2152

---

**Manual synthesis of per-methylated diol 5.79**

To a 1-L Schlenk flask charged with diol 5.78 (25.8 mmol, 9.40 g) was added CH_2Cl_2
(450 mL). To the stirred solution was added Proton Sponge (141.9 mmol, 38.70 g) as a solid in
one portion, then trimethyloxonium tetrafluoroborate (180.6 mmol, 38.70 g) as a solid in one
portion. The mixture was stirred at 23 °C for 16 hours. The orange mixture was filtered and the
solid was rinsed with additional CH_2Cl_2 (100 mL). The filtrate was concentrated in vacuo to ~50
mL, during which a solid precipitated. To this suspension was added Et_2O (300 mL), mixed and
filtered, rinsing with 150 mL Et_2O. The filtrate was transferred to a 1-L separatory funnel and
washed with 0.5 M HCl (2 × 250 mL). The aqueous phase was extracted with 100 mL Et_2O. The

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combined organics were neutralized with saturated aqueous NaHCO$_3$ (200 mL) and washed with brine, dried over anhydrous MgSO$_4$, filtered and concentrated to give a yellow oil. The oil was subjected to flash chromatography on silica gel (EtOAc:hexanes 1:20 → 1:10) to afford 5.79 as a colorless oil (5.37 g, 53%).

![Chemical Structure](image)

TLC (10% EtOAc/hexanes)

$R_f = 0.33$, stained by KMnO$_4$

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

$\delta$ 7.25 (app d, $J = 8.5$ Hz, 2H), 6.89 (app d, $J = 8.5$ Hz, 2H), 4.44 (d, $J = 11.5$ Hz, 1H), 4.38 (d, $J = 11.5$ Hz, 1H), 4.21 (d, $J = 3$ Hz, 1H), 3.80, (s, 3H), 3.57 (dd, $J = 9.5$, 5 Hz, 1H), 3.41 (s, 3H), 3.39, (s, 3H), 3.27 (dd, $J = 9.0$, 8.0 Hz, 1H), 3.13 (dd, $J = 10.0$, 2.5 Hz, 1H), 2.10 – 2.04, (m, 1H), 1.94 – 1.88 (m, 1H), 1.09 (d, $J = 7.0$ Hz, 3H), 1.01 (d, $J = 7.5$ Hz, 3H), 0.18 (s, 9H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

$\delta$ 160.0, 130.8, 129.0, 113.7, 104.7, 90.9, 85.1, 72.7, 72.0, 71.3, 61.2, 56.5 (2C), 55.2, 41.7, 35.7, 16.4, 11.3, 0.0.

HRMS (ESI+)

Calculated for C$_{22}$H$_{36}$O$_4$NaSi: 415.2281

Found: 415.2272
Manual synthesis of alcohol 5.80

To a stirred solution of 5.79 in MeCN and H₂O was added ceric ammonium nitrate (1.844 mmol, 1.011 g) portion wise over 15 minutes under ambient atmosphere and temperature. The solution was stirred for 60 minutes at 23 °C, then diluted with Et₂O (100 mL) and washed H₂O (150 mL) and saturated NaHSO₃ (2 × 150 mL). The combined aqueous layer was extracted with Et₂O (100 mL). The organic phase was dried over anhydrous MgSO₄, filtered and concentrated. The resulting oil was subjected to flash chromatography on silica gel (EtOAc:CH₂Cl₂ 0:100 → 20:100) to afford 5.80 as a colorless oil (202 mg, 77%).

TLC (20% EtOAc/hexanes)

Rᵣ = 0.37, stained by KMnO₄

¹H-NMR (500 MHz, CDCl₃)

δ 4.23 (d, J = 3 Hz, 1H), 3.86 (d, J = 11, 3 Hz, 1H), 3.53 (dd, J = 11.5, 4.5 Hz, 1H), 3.46, (s, 3H), 3.40 (s, 3H), 3.24 (dd, J = 10, 2 Hz, 1H), 2.01-1.95 (m, 1H), 1.88-1.82, (m, 1H), 1.18 (d, J = 7.5 Hz, 3H), 0.99 (d, J = 7 Hz, 3H), 0.18 (s, 9H)

¹³C-NMR (125 MHz, CDCl₃)

δ 104.3, 91.4, 87.7, 71.8, 64.4, 61.4, 56.4, 42.1, 35.6, 16.1, 11.2, -0.1

HRMS (ESI+)
Calculated for C\textsubscript{14}H\textsubscript{28}O\textsubscript{3}NaSi: 295.1705
Found: 295.1708

**Manual synthesis of aldehyde 5.81**

To a solution of alcohol 5.80 (15.15 mmol, 4.13 g) in CH\textsubscript{2}Cl\textsubscript{2} (150 mL) in a 300-mL round-bottom flask cooled to 0 °C was added Dess-Martin periodinane (30.3 mmol, 12.86 g) portionwise over 5 minutes, under ambient atmosphere. The reaction was stirred for 10 minutes after the addition was complete, then the ice/water bath was removed. The reaction was stirred for another 75 minutes, and then transferred to a separatory funnel. The mixture was extracted with 1:1 saturated NaHCO\textsubscript{3}/1.5 M Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (6 × 50 mL) until TLC indicated that all byproducts have been removed. The aqueous phase was then extracted with CH\textsubscript{2}Cl\textsubscript{2} (100 mL), and the combined organic phases were washed with brine, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated at 27 °C. The resulting oil (5.81) was dried briefly under high vacuum (~15 minutes), then used immediately for the next reaction.

**Manual synthesis of iodide 5.82**

To a 500-mL Schlenk flask charged with anhydrous CrCl\textsubscript{2} (242.4 mmol, 29.81 g) was added THF (250 mL) and cooled to 10 °C in an ice/water bath. With the exclusion of light, to the stirred suspension was added slowly over 15 minutes via cannula a solution of aldehyde 5.81 (assumed 15.15 mmol) and CHI\textsubscript{3} (75.75 mmol, 29.83 g) in THF (80 mL). The resulting red
mixture was stirred at 23 °C for 2 hours. The mixture was filtered through Celite™ and washed with Et₂O (300 mL). The dark-colored solution was transferred to a separatory funnel and washed with H₂O (2 x 200 mL), brine, then dried over anhydrous MgSO₄, filtered and concentrated to give a black residue. The residue was absorbed onto Celite™ \textit{in vacuo} from an acetone solution. The resulting powder was subjected to flash chromatography on silica gel (hexanes:EtOAc 100:0 → 20:100). The product obtained after chromatography was stirred with saturated Na₂SO₃ and passed through a pad of Darco® to remove residual iodine to afford \textbf{5.82} as a pale yellow oil (4.06 g, 68% over 2 steps).

![5.82](image)

TLC (20% EtOAc/hexanes)

\[ R_f = 0.52, \text{ stained by KMnO}_4 \]

\(^1\)H-NMR (500 MHz, CDCl\textsubscript{3})

\[ \delta 6.55 \text{ (dd, } J = 15, 9.5 \text{ Hz, 1H)}, 6.02 \text{ (dd, } J = 15, 1 \text{ Hz, 1H}), 4.23 \text{ (d, } J = 2 \text{ Hz, 1H}), 3.43, \text{ (s, 3H)}, 3.39 \text{ (s, 3H)}, 3.04 \text{ (dd, } J = 10, 2 \text{ Hz, 1H}), 2.49-2.43 \text{ (m, 1H)}, 1.72-1.65 \text{ (m, 1H)}, 1.13 \text{ (d, } J = 6.5 \text{ Hz, 3H)}, 0.94 \text{ (d, } J = 7 \text{ Hz, 3H)}, 0.19 \text{ (s, 9H)} \]

\(^1^3\)C-NMR (125 MHz, CDCl\textsubscript{3})

\[ \delta 147.3, 104.3, 91.3, 85.1, 75.1, 71.8, 61.3, 56.4, 43.2, 42.1, 18.1, 10.8, -0.0 \]

HRMS (ESI+)

Calculated for C\textsubscript{15}H\textsubscript{27}O\textsubscript{2}NaSi: \hspace{1cm} 417.0723

Found: \hspace{1cm} 417.0741
Manual synthesis of boronic acid 5.83

In an unoptimized procedure, a dry 200-mL Schlenk flask was charged with THF (46 mL) and iodide 5.82 in 10 mL THF. The solution was cooled to -78 °C and n-BuLi (1.6 M in hexanes, 12.3 mmol, 7.7 mL) was added dropwise via cannula over 15 minutes, during which the reaction turned yellow. The reaction was stirred at -78 °C for 25 minutes, then trimethylborate (13.45 mmol, 1.5 mL) was added neat in one portion. Stirring was continued at -78 °C for 20 minutes and then the cooling bath was removed. The reaction was slowly warmed to 23 °C over 2 hours. The reaction was poured into a 1 N HCl solution (200 mL) pre-cooled to 0 °C and stirred for 15 minutes. The mixture was transferred to a separatory funnel, rinsing with 100 mL Et₂O. The mixture was shaken and the phases were separated. The aqueous phase was extracted with Et₂O (2 × 50 mL). The combined organics were washed with H₂O (50 mL), brine, dried over anhydrous MgSO₄, filtered and concentrated to give a yellow oil (5.83), which was diluted with toluene (30 mL) and used directly in the next step.

Manual synthesis of vinyl MIDA boronate 5.84

To a solution of the boronic acid 5.83 in toluene (50 mL) and DMSO (5 mL) was added N-methyliminodiacetic acid (1.45 g, 9.84 mmol). The round-bottom flask was fitted with a Dean-Stark trap and a reflux condenser and refluxed for 4 hours. The reaction was cooled to 23 °C and transferred into 1:1 saturated NaCl/H₂O (50 mL). The organic phase was washed with another 50 mL 1:1 saturated NaCl/H₂O. The combined aqueous phase was washed with Et₂O (2 × 50 mL), and the organic layer was dried over anhydrous MgSO₄, filtered, and concentrated.
The residue was purified by flash column chromatography on silica gel (Et<sub>2</sub>O:acetone 100:0 → 1:2) to give 5.84 as a white solid (1.74 g, 50% over 2 steps).

TLC (acetone/hexane 1:1)

R<sub>f</sub> = 0.47, stained by KMnO<sub>4</sub>

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)

δ 6.24 (dd, <i>J</i> = 18, 8 Hz, 1H), 5.41 (dd, <i>J</i> = 18, 1 Hz, 1H), 4.21 (d, <i>J</i> = 3 Hz, 1H), 3.82 (d, <i>J</i> = 16.5 Hz, 2H), 3.66 (dd, <i>J</i> = 16.5, 3 Hz, 2H), 3.40 (s, 3H), 3.38 (s, 3H), 3.08 (dd, <i>J</i> = 10, 2 Hz, 1H), 2.80, (s, 3H), 2.52-2.49 (m, 1H), 1.68-1.61 (m, 1H), 1.13 (d, <i>J</i> = 7 Hz, 3H), 0.95 (d, <i>J</i> = 7 Hz, 3H), 0.17 (s, 9H)

<sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)

δ 167.6, 167.5, 148.9, 104.8, 91.3, 85.6, 72.1, 61.6, 61.2, 56.8, 46.9, 42.8, 42.1, 18.2, 11.2, 0.2

HRMS (ESI+)

Calculated for C<sub>20</sub>H<sub>35</sub>BNO<sub>6</sub>Si: 424.2327

Found: 424.2329

Manual synthesis of vinyl MIDA boronate 5.85
To a solution of alkyne 5.84 in acetone/H$_2$O/lutidine (10:10:1, 84 mL) at 0 °C was added AgNO$_3$ in one portion. The reaction was gradually allowed to warm to 23 °C over 2.5 hours. The reaction was quenched by transferring the mixture into saturated aqueous NH$_4$Cl (100 mL) and diluted with EtOAc (50 mL). The mixture was filtered to remove the insoluble salts. The filtrate was transferred to a separatory funnel and the phases were separated. The organic phase was washed with 0.5 M HCl (2 × 50 mL) and the aqueous phase extracted with EtOAc (50 mL). The organic layer was washed with brine, dried over anhydrous MgSO$_4$, filtered and concentrated to afford a white foam. The foam was dissolved in Et$_2$O (~10 mL), then 20% Et$_2$O/hexane (30 mL) was added and the suspension was filtered, washing with additional 20% Et$_2$O/hexane (20 mL). The filtrate was concentrated in vacuo to give 5.85 as a white solid (1.22 g, 87%)

TLC (acetone/hexane 1:1)

$R_f =$ 0.34, stained by KMnO$_4$

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

$\delta$ 6.24 (dd, $J = 17.5, 8$ Hz, 1H), 5.41 (dd, $J = 17.5, 1$ Hz, 1H), 4.23 (t, $J = 2$ Hz, 1H), 3.79 (d, $J = 16$ Hz, 2H), 3.66 (dd, $J = 16, 1.5$ Hz, 2H), 3.42 (s, 3H), 3.40 (s, 3H), 3.09 (dd, $J = 10, 2.5$ Hz, 1H), 2.81, (s, 3H), 2.55-2.47 (m, 1H), 2.40 (d, $J = 2$ Hz, 1H), 1.69-1.61 (m, 1H), 1.14 (d, $J = 7$ Hz, 3H), 0.96 (d, $J = 7$ Hz, 3H)

$^{13}$C-NMR (125 MHz, CDCl$_3$)

$\delta$ 168.1, 168.0, 148.1, 85.3, 82.6, 74.3, 71.2, 61.4, 61.0, 56.6, 46.9, 42.5, 41.9, 18.0, 10.7

HRMS (ESI+)

Calculated for C$_{17}$H$_{27}$BNO$_6$: 352.1931
Found: 352.1933
Manual synthesis of vinyl MIDA boronate 5.86

Preparation of catalyst stock solution. A dry 7-mL vial equipped with a PTFE-coated magnetic stir bar was charged with RuPhos (9.3 mg, 0.02 mmol). The vial was brought into the glovebox, and Pd$_2$dba$_3$ (4.6 mg, 0.005 mmol) was added. CH$_2$Cl$_2$ (3 mL) was added, and the mixture was stirred at 23 °C for 25 minutes.

The freshly prepared catalyst stock solution was used immediately for the preparation of 5.86: A dry 20-mL vial equipped with a PTFE-coated magnetic stir bar was charged with alkyne 5.85 (176 mg, 0.5 mmol). The vial was evacuated and filled with argon (x 3), then CH$_2$Cl$_2$ (5 mL) was then added, followed by the catalyst stock solution, rinsing with CH$_2$Cl$_2$ (2 mL). Tributyltin hydride (0.27 mL, 1.00 mmol) was added neat dropwise to the reaction under argon at 23 °C, over 1 hour 25 minutes. The reaction was stirred at 23 °C for 2 hours after the addition was complete. The reaction was concentrated in vacuo and purified by silica gel chromatography (10% to 50% acetone/hexanes). The yellow solid obtained after concentration was dissolved in 10% acetone/hexanes and passed through a pad of Darco® and Celite™ to give a colorless solution. The solution was concentrated in vacuo to give 5.86 as an off-white solid (255 mg, 79%).

TLC (acetone/hexane 1:1)

R$_f$ = 0.47, stained by KMnO$_4$
Manual synthesis of MIDA boronate 5.28

To a solution of stannane 5.86 (1.387 g, 2.16 mmol) in CH₂Cl₂ (20 mL) in a 50-mL round-bottom flask was added dropwise a solution of I₂ (0.576 g, 2.27 mmol) in CH₂Cl₂ (30 mL) via a pressure-equalizing funnel at 0 °C under ambient atmosphere over 1 hour. The funnel was rinsed with CH₂Cl₂ (10 mL) and the solution added portion wise to the reaction. The ice bath was removed and the reaction was allowed to gradually warm to 23 °C over 1 hour. The reaction was transferred into a 250-mL separatory funnel, rinsing with additional CH₂Cl₂. The organic layer was washed with 2 × 30 mL 1 M Na₂S₂O₅ solution, then with 2 × 25 mL 3 M KF solution. The combined aqueous layer was extracted with 25 mL CH₂Cl₂. The organic phase was dried over anhydrous MgSO₄, filtered and concentrated in vacuo. The crude product was purified by silica gel chromatography (1:6 to 2:3 acetone/hexanes) to give 5.28 as a white solid (0.827 g, 80%).
TLC (acetone/hexane 1:1)

\[ R_f = 0.32, \text{stained by KMnO}_4 \]

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

\[ \delta 6.46 \text{ (dd, } J = 14.5, 7 \text{ Hz, } 1\text{H}), 6.26, \text{ (d, } J = 14.5 \text{ Hz, } 1\text{H}), 6.19, \text{ (dd, } J = 17.5, 7.5 \text{ Hz, } 1\text{H}), 5.39 \text{ (d, } J = 17.5 \text{ Hz, } 1\text{H}), 3.94 \text{ (d, } J = 16.5 \text{ Hz, } 2\text{H}), 3.88-3.87 \text{ (m, } 1\text{H}), 3.68 \text{ (dd, } J = 16.5, 4.5 \text{ Hz, } 2\text{H}), 3.42 \text{ (s, } 3\text{H}), 3.26 \text{ (s, } 3\text{H}), 3.07 \text{ (dd, } J = 9.5, 1.5 \text{ Hz, } 1\text{H}), 2.80, \text{ (s, } 3\text{H}), 2.55-2.47 \text{ (m, } 1\text{H}), 1.12 \text{ (d, } J = 7.0 \text{ Hz, } 3\text{H}), 1.50-1.45 \text{ (m, } 1\text{H}), 0.76 \text{ (d, } J = 7.0 \text{ Hz, } 3\text{H}).
\]

$^{13}$C-NMR (125 MHz, CDCl$_3$)

\[ \delta 167.7, 167.6, 148.5, 145.8, 85.8, 82.8, 61.4, 61.0, 56.7, 46.8, 41.8, 41.6, 18.0, 9.8. \]

HRMS (ESI+)

Calculated for $\text{C}_{17}\text{H}_{28}\text{BNO}_6$I: 480.1054

Found: 480.1053

Manual synthesis of building blocks for all-trans-retinal ketal (5.41)

Manual synthesis of MIDA boronate 5.37
The following reaction was run in duplicate. In a glovebox, to a 40-mL vial charged with vinyl iodide 5.87 (914 mg, 3.66 mmol, 1.2 equiv.) and bisborylated diene 5.88 (1064 mg, 3.05 mmol, 1.0 equiv.) was added PdCl$_2$(dpdpf)$\cdot$CH$_2$Cl$_2$ (125 mg, 0.153 mmol, 5 mol%), finely ground anhydrous K$_3$PO$_4$ (3884 mg, 18.3 mmol, 6.0 equiv.), and DMSO (20 mL, 0.15 M). The vial was sealed with a PTFE-lined cap and removed from the glovebox. The vial was placed in a 45 °C aluminum heat block and maintained at that temperature with stirring for 24 hours. The reaction was cooled to 23 °C and transferred to a separatory funnel, diluting with EtOAc (50 mL). The organic layer was washed with brine:H$_2$O (1:1, 2 x 50 mL) to remove DMSO, dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. The crude material was adsorbed onto Celite™ from an acetone solution and purified by silica gel chromatography (hexanes:EtOAc 1:1 → EtOAc) to afford trienyl MIDA boronate 5.37 as a pale yellow solid (1.0 g, 48%).

TLC (EtOAc)
$R_f = 0.45$, stained by KMnO$_4$

$^1$H-NMR (500 MHz, acetone-$d_6$)
$\delta$ 6.17 (d, $J = 16.5$ Hz, 1H), 6.11 (d, $J = 16.5$ Hz, 1H), 5.34 (s, 1H), 4.21 (d, $J = 16.5$ Hz, 2H), 4.04 (d, $J = 17.0$ Hz, 2H), 3.03 (s, 3H), 1.98 (s, 3H), 1.98-2.01 (m, 2H), 1.67 (s, 3H), 1.58-1.62 (m, 2H), 1.45-1.47 (m, 2H), 1.00 (s, 6H).

$^{11}$B-NMR (128 MHz, acetone-$d_6$)
$\delta$ 15.8.

$^{13}$C-NMR (125 MHz, acetone-$d_6$)
Manual synthesis of aldehyde 5.90\textsuperscript{10}

Crotly alcohol 5.89\textsuperscript{11,12} (1.08 g, 5.54 mmol, 1.0 equiv.) and activated MnO\textsubscript{2} (14 g, 30 equiv.) was charged in a 100-mL round-bottom flask equipped with a PTFE-coated magnetic stir bar and topped with a rubber septum and purged with N\textsubscript{2}. Dichloromethane (50 mL, 0.11 M) was added to the round-bottom flask and the resulting reaction mixture was stirred at 23 °C for 45 minutes. After 45 minutes, the reaction mixture was filtered through a plug of silica gel and Celite\textsuperscript{TM} and concentrated \textit{in vacuo} to afford aldehyde 5.90 as a yellow oil (500 mg, 46% yield).

TLC (petroleum ether:diethyl ether 4:1)

R\textsubscript{f} = 0.51, stained by KMnO\textsubscript{4}

\textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3})

δ 9.79 (d, J = 6.5 Hz, 1H), 6.84-6.86 (m, 1H), 3.00 (s, 3H).
\(^{13}\)C-NMR (125 MHz, CDCl\(_3\))
\[ \delta 185.9, 141.8, 126.1, 29.9 \]

HRMS (ESI+)

Calculated for C\(_4\)H\(_6\)OI: 196.9463
Found: 196.9468

Manual synthesis of vinyl iodide 5.40

Aldehyde 5.90 (150 mg, 0.77 mmol, 1.0 equiv.), neopentyl glycol (797 mg, 7.7 mmol, 10.0 equiv.), and \(p\)-toluene sulfonic acid (15 mg, 0.08 mmol, 0.1 equiv.) was charged in a 40-mL vial equipped with a PTFE-coated magnetic stir bar. The vial was sealed with a PTFE-lined cap and purged with N\(_2\). Dichloromethane (15 mL, 0.05 M) was added to the vial and the resulting mixture was stirred for 1 minutes until homogeneous. Activated 4 Å powdered molecular sieves (300 mg, 2:1 weight ratio to aldehyde) were added to the vial to afford a cloudy white solution. The resulting reaction mixture was stirred at 40 °C for 24 hours. After 24 hours, the reaction mixture was filtered through a filter funnel and concentrated \(in\ vacuo\). The resulting white solid was adsorbed onto Celite™ from an acetone solution and purified by silica gel chromatography (4:1 petroleum ether:ether) to afford vinyl iodide 5.40 as a pale yellow oil (150 mg, 69%).

TLC (petroleum ether:diethyl ether 4:1)

\[ R_f = 0.71, \text{stained by KMnO}_4 \]
$^1$H-NMR (500 MHz, CDCl$_3$)
\[ \delta 6.27 (d, J = 6.0 \text{ Hz}, 1\text{H}), 5.02 (d, J = 6.0 \text{ Hz}, 1\text{H}), 3.64 (d, J = 11.5 \text{ Hz}, 2\text{H}), 3.88 (d, J = 11.0 \text{ Hz}, 2\text{H}), 2.51 (s, 3\text{H}), 1.20 (s, 3\text{H}), 0.74 (s, 3\text{H}). \]

$^{13}$C-NMR (125 MHz, CDCl$_3$)
\[ \delta 138.0, 102.0, 97.9, 30.0, 29.1, 22.9, 21.9. \]

HRMS (ESI+)
- Calculated for C$_9$H$_{16}$O$_2$I: 283.0195
- Found: 283.0200

Manual synthesis of building blocks for protected ratanhine (5.49) and derivative library (5.56-5.67, 5.69-5.75)

Manual synthesis of aryl iodide 5.92
A 100-mL round-bottom flask charged with 4-bromo-3-methoxyphenol (5.91) (5000 mg, 24.63 mmol, 1.0 equiv.) was sealed with a septum and purged with N2 and diethyl ether (25 mL, 1.0 M) was added to afford a clear solution. The flask was cooled to 0 °C in an ice bath for 10 minutes. Iodine monochloride (1.3 mL, 1.05 equiv.) was added dropwise to the reaction flask and the reaction mixture was allowed to warm to 23 °C with stirring over 1.5 hours. After 1.5 hours, the reaction mixture was transferred to a separatory funnel containing aqueous saturated Na2S2O3 (50 mL), rinsing with diethyl ether (50 mL) and the phases were separated. The aqueous layer was back-extracted with diethyl ether (50 mL × 3) and the combined organic layers were dried over anhydrous MgSO4, filtered, and concentrated in vacuo to afford crude 5.92 as a yellow-brown oil (7.86 g, 97% crude yield). This oil was used for the next step without further purification.

TLC (hexanes:diethyl ether 2:3)

Rf = 0.32, stained by KMnO4

1H-NMR (500 MHz, CDCl3)

δ 7.73 (s, 1H), 6.62 (s, 1H), 5.23 (s, 1H), 3.86 (s, 3H).

HRMS (EI+)

Calculated for C7H6O2IBr: 327.85961

Found: 327.85939

Manual synthesis of TBDPSE-protected phenol 5.94
Triphenylphosphine (PPh$_3$, 5054 mg, 19.3 mmol, 1.8 equiv.) was charged in a 100-mL Schlenk flask equipped with a PTFE-coated magnetic stir bar and back-filled with N$_2$. THF (57 mL) was added and the reaction mixture was cooled to 0 °C for 10 minutes. Diisopropyl azodicarboxylate (DIAD, 3.8 mL, 1.8 equiv.) was added dropwise to the reaction flask affording a white precipitate. This heterogeneous mixture was stirred at 0 °C for 10 minutes. Alcohol 5.93 (5482 mg, 19.3 mmol, 1.8 equiv.) was added to the reaction flask and THF (4 mL) was added to dissolve all reagents and the resulting mixture was stirred at 0 °C for 10 minutes. Separately, aryl iodide 5.92 (3511 mg, 10.7 mmol, 1.0 equiv.) was charged in a 20-mL vial, back-filled with N$_2$, and THF (6 mL) was added. This solution was added to the reaction flask at 0 °C and stirred at that temperature for 10 minutes. After 10 minutes, the reaction mixture was warmed to 23 °C with stirring for 16 hours. After 16 hours, the reaction mixture was transferred to a 200-mL round-bottom flask, rinsing with diethyl ether (20 mL), and concentrated in vacuo to afford an orange oil. The crude material was adsorbed onto Celite™ from an acetone solution and purified by SiO$_2$ chromatography (20% DCM:hexanes → 30% DCM:hexanes) to afford 5.94 as a white solid (5.23 g, 82%).

![Chemical Structure](image)

TLC (hexanes:diethyl ether 2:3)

R$_f$ = 0.68, stained by KMnO$_4$

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

δ 7.81 (s, 1H), 7.65 (d, $J = 7.0$ Hz, 4H), 7.43 – 1.38 (m, 6H), 6.09 (s, 1H), 4.02 – 3.98 (m, 2H), 3.68 (s, 3H), 1.86 – 1.83 (m, 2H), 1.07 (s, 9H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

δ 157.8, 156.8, 141.2, 135.8, 133.5, 129.5, 127.9, 103.0, 98.0, 75.6, 67.6, 56.2, 27.6, 18.0, 11.7.
**Manual synthesis of aryl MIDA boronate 5.46**

In a glove box, to a 100-mL round-bottom flask equipped with a PTFE-coated magnetic stir bar and containing aryl iodide 5.94 (3572 mg, 6.0 mmol, 1.0 equiv.) was added potassium acetate (1767 mg, 18.0 mmol, 3.0 equiv.), bis(neopentylglycolato)diboron (1355 mg, 6.0 mmol, 1.0 equiv.) and PdCl\(_2\)dppf•CH\(_2\)_Cl\(_2\) (147 mg, 0.18 mmol, 3 mol%). The flask was sealed with a septum cap and removed from the glove box. Outside the glovebox, DMSO (50 mL, 0.12 M) was added to the reaction flask under N\(_2\) atmosphere and the reaction mixture was placed in an 80 °C oil bath and stirred at that temperature for 18 hours. After 18 hours, the reaction mixture was cooled to 23 °C and 1 M aqueous NaOH (18.0 mL, 3.0 equiv) was added dropwise and the resulting heterogeneous mixture was stirred vigorously for 30 minutes at 23 °C. The reaction mixture was transferred to a separatory funnel with EtOAc (100 mL) and water (100 mL). The layers were separated and the aqueous phase was extracted with water (3 x 100 mL). Combined organic layers were dried over anhydrous MgSO\(_4\), and concentrated *in vacuo* to afford a crude sample of boronic acid 5.95 as a brown oil. This crude boronic acid was concentrated in a 40-mL I-Chem vial and charged with *N*-methyliminodiacetic acid (MIDA) anhydride\(^{13}\) (3873 mg, 30 mmol, 5.0 equiv.) and equipped with a PTFE-coated magnetic stir bar. The vial was flushed with N\(_2\) and THF (20 mL, 0.3 M) was added and the reaction mixture was placed in a 70 °C aluminum heat block and stirred at that temperature for 24 hours. After 24 hours, the reaction mixture was cooled to 23 °C and transferred to a separatory funnel with EtOAc (100 mL) followed by deionized water (100 mL). The phases were separated and the aqueous layer was extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (100
mL), dried over anhydrous MgSO₄, and concentrated in vacuo to afford an off-white solid. The crude material was adsorbed onto Celite™ from an acetone solution and purified by SiO₂ chromatography (hexanes:EtOAc 1:1 → EtOAc) and recrystallized from DCM/hexanes to afford **5.46** as a white solid (1.707 g, 46% over three steps).

[Chemical Structure Image]

TLC (EtOAc)

R_f = 0.66, stained by KMnO₄

**¹H-NMR (500 MHz, acetone-d₆)**

δ 7.70 (d, J = 8.0 Hz, 2H), 7.63 (s, 1H), 7.42-7.45 (m, 6H), 6.45 (s, 1H), 4.35 (d, J = 17.0 Hz, 2H), 4.20 (d, J = 17.0 Hz, 2H), 4.11-4.14 (m, 2H), 3.72 (s, 3H), 2.84 (s, 3H), 1.91 – 1.88 (m, 2H), 1.05 (s, 9H).

**¹³C-NMR (125 MHz, acetone-d₆)**

δ 169.3, 163.1, 158.5, 138.9, 136.5, 134.5, 130.3, 128.7, 98.5, 70.1, 67.2, 64.5, 56.3, 48.2, 27.9, 21.7, 18.5, 11.8.

**HRMS (ESI+)**

Calculated for C₃₀H₃₆O₆BNSiBr: 624.1588

Found: 624.1602
Manual synthesis of aryl bromide 5.54

To a stirred solution of triphenylphosphine (1.10 g, 4.2 mmol, 1.2 equiv) in THF (10 mL) at 0 °C was added dropwise diisopropylazodicarboxylate (DIAD; 830 μL, 4.2 mmol, 1.2 equiv) to generate a yellow suspension. In one portion, 4-bromo-3-methoxyphenol (5.91) (711 mg, 3.5 mmol, 1 equiv) and silyl alcohol 5.93 (1.19 g, 4.2 mmol, 1.2 equiv) were added. The mixture was stirred for 15 minutes, after which the ice bath was then removed and the reaction warmed to ambient temperature. After stirring for 1 hour, the reaction was diluted with diethyl ether and adsorbed onto Celite™ in vacuo. The Celite™ pad was loaded onto a silica gel column and eluted with hexanes/DCM gradient (4:1 to 3:7). Fractions containing a minor impurity were re-adsorbed on Celite™ and eluted from a silica gel column using a hexanes/DCM gradient (4:1 to 3:1). Purified fractions were combined to afford 5.54 as a colorless amorphous solid (1.19g, 72%).

\[ \text{H-NMR (500 MHz, acetone-}d_6) \]
\[
\delta 7.74 – 7.72 (m, 4H), 7.50 – 7.44 (m, 6H), 7.34 (d, J = 8.5 Hz, 1H), 6.43 (d, J = 2.5 Hz, 1H), 6.27 (dd, J = 9.0, 3.0 Hz, 1H), 4.08 – 4.05 (m, 2H), 3.81 (s, 3H) 1.86 – 1.83 (m, 2H), 1.11 (s, 9H).
\]

\[ \text{C-NMR (125 MHz, acetone-}d_6) \]
\[
\delta 160.5, 157.5, 136.6, 134.7, 133.8, 130.3, 128.7, 107.7, 102.4, 101.3, 66.3, 56.4, 28.1, 18.5, 12.5.
\]

HRMS (EI+)

Calculated for C_{25}H_{29}O_2SiBr: 468.11202

Found: 468.11118
Manual synthesis of aryl bromide 5.55

To a stirred solution of triphenylphosphate (765 mg, 3.0 mmol, 1.5 equiv) in THF (7.5 mL) at 0 °C was added dropwise diisopropylazodicarboxylate (DIAD; 570 μL, 3.0 mmol, 1.5 equiv) to generate a yellow suspension. In one portion, 4-bromo-3-(trifluoromethoxy)phenol (5.96) (500 mg, 2.0 mmol, 1 equiv) and silyl alcohol 5.93 (830 mg, 3.0 mmol, 1.5 equiv) were added. The mixture was stirred for 15 minutes, after which the ice bath was then removed and the reaction warmed to ambient temperature. After stirring for 16 hours, the reaction was diluted with diethyl ether and adsorbed onto Celite™ in vacuo. The Celite™ pad was loaded onto a silica gel column and eluted with 4:1 hexanes/DCM. The colorless oil from this column was re-adsorbed onto Celite™, loaded onto silica gel, and eluted with a hexanes/DCM gradient (hexanes to 9:1 hexanes/DCM) to afford 5.55 as a colorless oil (785 mg, 77%).

\[ \text{F}_3\text{CO} \begin{array}{c} \text{OH} \\ \text{Br} \end{array} \begin{array}{c} \text{HO} \\ \text{TBDPS} \end{array} \begin{array}{c} \text{5.93} \\ \text{(1.2 equiv)} \end{array} \begin{array}{c} \text{THF, 0 °C, 15 min; 23 °C, 2 h} \end{array} \begin{array}{c} \text{F}_3\text{CO} \begin{array}{c} \text{O} \\ \text{TBDPS} \end{array} \begin{array}{c} \text{5.55} \end{array} \end{array} \]

\[
\begin{align*}
\text{1H-NMR (500 MHz, acetone-d}_6\text{)} \\
\delta 7.71 – 7.69 (m, 4H), 7.55 (d, J = 8.5 Hz, 1H), 7.47 – 7.41 (m, 6H), 6.82 – 6.81 (m, 1H), 6.75 (dd, J = 8.5, 2.5 Hz, 1H), 4.11 – 4.07 (m, 2H), 1.86 – 1.83 (m, 2H), 1.08 (s, 9H).
\end{align*}
\]

\[
\begin{align*}
\text{13C-NMR (125 MHz, acetone-d}_6\text{)} \\
\delta 160.5, 157.5, 136.6, 134.7, 133.8, 130.3, 128.7, 107.7, 102.4, 101.3, 66.3, 56.4, 28.1, 18.5, 12.5.
\end{align*}
\]
Manual synthesis of TMSE-protected phenol 5.99

Triphenylphosphine (PPh₃, 7450 mg, 28.4 mmol, 1.8 equiv.) was charged in a 250-mL round-bottom flask equipped with a PTFE-coated magnetic stir bar and back-filled with N₂. THF (70 mL) was added and the reaction mixture was cooled to 0 °C for 10 minutes. Diisopropyl azodicarboxylate (DIAD, 5.6 mL, 28.4 mmol, 1.8 equiv.) was added dropwise to the reaction flask affording a white precipitate. This heterogeneous mixture was stirred at 0 °C for 10 minutes. TMS ethanol 5.98 (4.1 mL, 28.4 mmol, 1.8 equiv.) was added to the reaction flask and the resulting mixture was stirred at 0 °C for 10 minutes. Phenol 5.97 (2400 mg, 15.8 mmol, 1.0 equiv.) was added to the flask in one portion followed by THF (10 mL) and the resulting reaction mixture was and stirred at 0 °C for 10 minutes. After 10 min, the reaction mixture was warmed to 23 °C with stirring for 16 hours. After 16 hours, the reaction mixture was concentrated in vacuo to afford a clear oil. This crude oil was dissolved in minimum amount of diethyl ether (5
mL). Hexanes (100 mL) were added and the solution was stirred at 23 °C for 5 minutes until white solid precipitated. The solid was filtered through a fritted filter funnel rinsing with hexanes. The filtrate was concentrated in vacuo to afford a clear oil, which was adsorbed onto Celite™ from an acetone solution and purified by SiO₂ chromatography (20% DCM:hexanes → 50% DCM:hexanes) to afford 5.99 as a clear oil (434 mg, 11%).

\[
\text{TLC (dichloromethane:hexanes 1:1)}
\]
\[
R_f = 0.28, \text{ shortwave UV}
\]

\[
^{1}H-\text{NMR (500 MHz, CDCl}_3) \\
\delta 7.97 (d, J = 9.5 \text{ Hz}, 2H), 6.88 (d, J = 9.0 \text{ Hz}, 2H), 4.13 (t, J = 7.5 \text{ Hz}, 2H), 3.88 (s, 3H), 1.15 (t, J = 8.0 \text{ Hz}, 2H), 0.09 (s, 9H).
\]

\[
^{13}C-\text{NMR (125 MHz, CDCl}_3) \\
\delta 216.4, 172.1, 163.5, 132.3, 121.4, 114.2, 65.8, 17.6, -1.35.
\]

HRMS (ESI+)

Calculated for C\textsubscript{13}H\textsubscript{20}O\textsubscript{3}SiNa: 275.1079

Found: 275.1084

Manual synthesis of TMSE-protected phenol 5.100
A 40-mL vial equipped with a PTFE-coated magnetic stir bar was charged with LiOH·H₂O (722 mg, 17.2 mmol, 10 equiv.) and deionized H₂O (4 mL). The vial was placed in a 60 °C aluminum heating block and stirred at that temperature for 5 minutes until a clear solution was afforded. The vial was removed from the heating block and a solution of methyl ester 5.99 (434 mg, 1.72 mmol, 1.0 equiv.) in THF (9 mL, 0.2 M) was added to the reaction vial. The vial was sealed with a PTFE-lined cap and placed in a 60 °C aluminum heating block and stirred at that temperature for 14 hours. The reaction mixture was cooled to 23 °C, then to 0 °C for 10 minutes. 6 N HCl was added dropwise to the crude reaction mixture with stirring until pH ≤ 1. The reaction mixture was transferred to a separatory funnel with H₂O (20 mL) and EtOAc (40 mL). The phases were separated and the aqueous layer was extracted with EtOAc (40 mL x 2). The combined organic layers were washed with brine (50 mL), dried over anhydrous MgSO₄, and concentrated in vacuo to afford a white solid. The crude solid was adsorbed onto Celite™ from an acetone solution and purified by SiO₂ chromatography (1:4 EtOAc:hexanes → 2:1:7 EtOAc:EtOH:hexanes) to afford 5.100 as a white solid (361 mg, 88%).

![Chemical Structure](image)

TLC (EtOAc:hexanes 1:4)

Rᵣ = 0.22, shortwave UV

^1^H-NMR (500 MHz, CDCl₃)

δ 8.03 (d, J = 9.0 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.15 (t, J = 7.5 Hz, 2H), 1.16 (t, J = 8.0 Hz, 2H), 0.09 (s, 9H).

^1^3^C-NMR (125 MHz, CDCl₃)

δ 172.2, 163.5, 132.3, 121.4, 114.2, 65.8, 17.6, -1.35.

HRMS (ESI+)
Calculated for C\textsubscript{12}H\textsubscript{18}O\textsubscript{3}SiNa: 261.0923
Found: 261.0934

**Manual synthesis of ester 5.102**

A 100-mL flask equipped with a PTFE-coated magnetic stir bar was charged with benzoic acid 5.100 (361 mg, 1.51 mmol, 1.0 equiv.) and back-filled with N\textsubscript{2}. Phenol 5.101 (447 mg, 1.82 mmol, 1.2 equiv.), DMAP (222 mg, 1.82 mmol, 1.2 equiv.), and dichloromethane (20 mL, 0.08 M) were added to the reaction flask. The clear, colorless solution was cooled to 0 °C and stirred at that temperature for 10 minutes. DCC (375 mg, 1.82 mmol, 1.2 equiv.) was added in one portion at 0 °C under N\textsubscript{2} and stirred at that temperature for 10 minutes. The ice bath was removed after 10 minutes and the reaction flask was allowed to warm to 23 °C with stirring over 15 hours. After 15 hours, the reaction mixture was concentrated *in vacuo* to afford a yellow sludge. The crude material was adsorbed onto Celite\textsuperscript{TM} from an acetone solution and purified by SiO\textsubscript{2} chromatography (30% DCM:hexanes → 40% DCM:hexanes) to afford 5.102 as a clear oil (575 mg, 81%).

TLC (30% DCM:hexanes)

\[ R_f = 0.21, \text{ shortwave UV} \]

\textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3})
δ 8.14 (d, \( J = 8.5 \) Hz, 2H), 7.92 (s, 1H), 7.61 (d, \( J = 8.5 \) Hz, 1H), 6.96 (d, \( J = 9.0 \) Hz, 2H), 6.92 (d, \( J = 8.5 \) Hz, 1H), 6.70 (dd, \( J = 11.0 \) Hz, \( J = 14.5 \) Hz, 1H), 5.78 (s, 1H), 5.74 (s, 1H), 5.32 (d, \( J = 11.0 \) Hz, 1H), 4.17 (t, \( J = 8.0 \) Hz, 2H), 1.18 (t, \( J = 7.5 \) Hz, 2H), 0.10 (s, 9H).

\(^{13}\)C-NMR (125 MHz, CDCl\(_3\))

δ 164.4, 163.6, 148.2, 137.5, 135.3, 132.8, 132.4, 129.2, 124.9, 120.8, 117.5, 114.4, 90.4, 65.9, 17.5, -1.33.

HRMS (ESI+)

Calculated for C\(_{20}\)H\(_{24}\)O\(_3\)Si: 467.0540

Found: 467.0534

Manual synthesis of vinyl bromide 5.104

A 50-mL flask equipped with a PTFE-coated magnetic stir bar was charged with olefin 5.102 (575 mg, 1.23 mmol, 1.0 equiv.), sealed with a septum, and back-filled with N\(_2\). Dichloromethane (12 mL, 0.1 M) was added to afford a clear, colorless solution. This solution was cooled to 0 °C and stirred at that temperature for 10 minutes. Bromine (0.14 mL, 2.71 mmol, 2.2 equiv.) was added dropwise to the reaction mixture at 0 °C over the course of 20 minutes until a bright red color persisted. The crude reaction mixture was concentrated \textit{in vacuo} and azeotroped with DCM (3 x 15 mL) to afford dibromide 5.103 as a yellow foamy solid (721 mg, 93% crude yield). Dibromide 5.103 (721 mg, 1.15 mmol, 1.0 equiv.) was concentrated in a 50-mL round-bottom flask. The flask was equipped with a PTFE-coated magnetic stir bar, sealed with a septum, and back-filled with N\(_2\). Acetonitrile (11.5 mL, 0.1 M) was added and the reaction mixture was stirred at 23 °C for 5 minutes. DBU (0.2 mL, 1.34 mmol, 1.2 equiv.) was added dropwise and the resulting mixture was stirred for 10 minutes. After 10 minutes,
incomplete conversion was observed by TLC analysis, so another 0.3 mL of DBU was added dropwise. After 20 minutes of stirring at 23 °C, the reaction mixture was cooled to 0 °C and 1 N HCl (10 mL) was added. The reaction mixture was transferred to a separatory funnel with EtOAc (15 mL) and H₂O (15 mL) and the layers were separated. The phases were separated and the aqueous layer was extracted with EtOAc (10 mL x 2). The combined organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo to afford a clear oil. The crude material was adsorbed onto Celite™ from an acetone solution and purified by SiO₂ chromatography (40% DCM:hexanes) to afford 5.104 as a pale yellow oil (503 mg, 75% over two steps).

![Chemical Structure](image)

TLC (1:3 DCM:hexanes)

Rᵢ = 0.25, shortwave UV

**¹H-NMR (500 MHz, CDCl₃)**

δ 8.13 (d, J = 9.0 Hz, 2H), 7.78 (s, 1H), 7.70 (d, J = 8.5 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 5.91 (d, J = 1.5 Hz, 1H), 5.84 (d, J = 2.0 Hz, 1H), 4.17 (t, J = 8.0 Hz, 2H), 1.17 (t, J = 8.0 Hz, 2H), 0.10 (s, 9H).

**¹³C-NMR (125 MHz, CDCl₃)**

δ 164.1, 163.6, 147.8, 138.9, 138.9, 135.4, 132.5, 125.3, 123.0, 122.7, 120.7, 114.4, 89.4, 65.9, 17.5, -1.34.

**HRMS (ESI+)

Calculated for C₂₀H₂₃O₃SiBrI: 544.9645

Found: 544.9646
Manual synthesis of TMSE-protected phenol 5.48

In a glove box, to a 7-mL vial equipped with a PTFE-coated magnetic stir bar and containing aryl iodide 5.104 (20 mg, 0.037 mmol, 1.0 equiv.) and trans-propenyl boronic acid (5.105) (4.73 mg, 0.055 mmol, 1.5 equiv.) was added ground potassium phosphate (23 mg, 0.11 mmol, 3.0 equiv.) and PdCl₂dpff•CH₂Cl₂ (1.5 mg, 0.002 mmol, 5 mol%) followed by THF (0.7 mL, 0.05 M). The vial was sealed with a cap and removed from the glove box. The vial was placed in a 45 °C aluminum heating block and stirred at that temperature for 24 hours. After 24 hours, the reaction mixture was cooled to 23 °C, filtered through a pad of Celite™, and concentrated in vacuo. The crude material was adsorbed onto Celite™ from an acetone solution and purified by SiO₂ chromatography (30% DCM:hexanes) to afford 5.48 as a pale yellow oil (13.5 mg, 80% yield).

TLC (1:1 DCM:hexanes)

Rₜ = 0.31, shortwave UV

¹H-NMR (500 MHz, CDCl₃)

δ 8.15 (d, J = 8.5 Hz, 2H), 7.40 (s, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 6.39 (d, J = 16.0 Hz, 1H), (dq, J = 15.5 Hz, J = 6.5 Hz, 1H), 5.89 (d, J = 1.5 Hz, 1H), 5.82 (d, J = 1.5 Hz, 1H), 4.17 (t, J = 8.0 Hz, 2H), 1.90 (d, J = 7.0 Hz, 3H), 1.17 (t, J = 8.0 Hz, 2H), 0.10 (s, 9H).
\[^{13}\text{C-}\text{NMR}\ (125\ \text{MHz, CDCl}_3)\]
\[
\delta 164.6, 163.4, 146.4, 135.8, 133.2, 132.4, 129.6, 127.6, 127.2, 126.8, 124.6, 123.2, 122.1, 121.2, 114.3, 65.9, 18.5, 17.5, -1.33.
\]

HRMS (ESI+)
Calculated for C\(_{23}\)H\(_{28}\)O\(_3\)SiBr: 459.0991
Found: 459.0987

**Conditions:** (a) Benzoyl chloride, \(\text{iPr}_2\text{NEt, DMAP, CH}_2\text{Cl}_2, \text{rt, 19 h; (b) Br}_2, \text{CH}_2\text{Cl}_2, 0\ ^\circ\text{C, 20 min; (c) 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), MeCN, rt, 30 min; (d), trans-propenylboronic acid, PdCl}_2\text{dppf.CH}_2\text{Cl}_2, K_3\text{PO}_4, \text{THF, 65 }^\circ\text{C}}

**Manual synthesis of benzoate ester 5.106**

A dry 25-mL Schlenk flask equipped with a PTFE-coated magnetic stir bar was charged with the phenol 5.101 (738 mg, 3 mmol) and DMAP (73.3 mg, 0.6 mmol) under N\(_2\). The flask was sealed with a rubber septum and CH\(_2\text{Cl}_2\) (15 mL) was added via syringe. To this solution was added DIPEA (2.1 mL, 12.1 mmol) in one portion. Benzoyl chloride (0.7 mL, 6.03 mmol) was then added neat dropwise over 5 minutes. The reaction was stirred for another 19 hours at room temperature, then transferred into a separatory funnel containing 1 N HCl (10 mL). After mixing and phase separation, the organic layer was washed with another portion of 1 N HCl (10 mL) and then with H\(_2\text{O}\) (20 mL). The combined aqueous layer was extracted with CH\(_2\text{Cl}_2\) (20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous MgSO\(_4\), filtered.
and concentrated. The crude product was purified by silica gel chromatography (30 - 35% CH$_2$Cl$_2$/hexanes) to afford 5.106 as a colorless viscous oil (643 mg, 61%).

![5.106](image)

TLC (50% DCM/hexanes)
$R_f = 0.44$, visualized by shortwave UV.

$^1$H-NMR (500 MHz, CDCl$_3$)
$\delta$ 8.21 (d, $J = 7$ Hz, 2H), 7.94 (d, $J = 2$ Hz, 1H), 7.66 (tt, $J = 7.5$, 1.5 Hz, 1H), 7.63 (dd, $J = 8$, 1.5 Hz, 1H), 7.56 (t, $J = 7.5$ Hz, 2H), 6.94 (d, $J = 8.5$ Hz, 1H), 6.71 (dd, $J = 17.5$, 11 Hz, 1H), 5.77 (dd, $J = 18$, 1 Hz, 1H), 5.34 (d, $J = 11$ Hz, 1H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)
$\delta$ 164.6, 148.0, 137.5, 135.4, 133.9, 132.7, 130.2, 129.0, 128.9, 128.7, 124.7, 117.7, 90.6.

HRMS (ESI+)
Calculated for C$_{15}$H$_{12}$O$_2$I: 350.9882
Found: 350.9890

**Manual synthesis of vinyl bromide 5.108**

A solution of alkene 5.106 (3.37 g, 9.62 mmol) in CH$_2$Cl$_2$ (120 mL) in a 200-mL round-bottom flask was cooled to 0 °C under N$_2$. Bromine (0.49 mL, 9.62 mmol) was added neat
dropwise over 15 minutes. After the addition was complete, the reaction was stirred for a further 5 minutes. The reaction was concentrated in vacuo to give an orange solid (5.107). Residual bromine was removed by azeotroping the residue with CH$_2$Cl$_2$ (15 mL × 2). The round bottom flask containing the crude product and equipped with a PTFE-coated magnetic stir bar was sealed with a rubber septum and back-filled with N$_2$ twice. MeCN (120 mL) was added to dissolve most of the solid. DBU (1.4 mL, 9.5 mmol) was then added neat dropwise via syringe over 15 minutes. The reaction was stirred for 15 minutes before being charged with another portion of DBU (0.2 mL, 1.34 mmol) added neat dropwise into the reaction. After another 15 minutes, another portion of DBU (0.2 mL, 1.34 mmol) was added. The reaction was poured slowly into 2 N HCl solution (100 mL) cooled to 0 °C with vigorous stirring. EtOAc (50 mL) was added and the mixture stirred. After phase separation, the aqueous layer was extracted with EtOAc (50 mL). The combined organic phase was washed with brine (100 mL), then dried over anhydrous MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified by silica gel chromatography (30-35% CH$_2$Cl$_2$/hexanes) to afford 5.108 as an off-white solid (2.67g, 66% over 2 steps).

TLC (40% CH$_2$Cl$_2$/hexanes)

$R_f = 0.47$, visualized by shortwave UV.

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

$\delta$ 8.20 (d, $J = 7.5$ Hz, 2H), 7.79 (d, $J = 2$ Hz, 1H), 7.72 (dd, $J = 8.5$, 2 Hz, 1H), 7.66 (t, $J = 7.5$ Hz, 1H), 7.52 (tt, $J = 8$, 1.5 Hz, 2H), 6.99 (d, $J = 8.5$ Hz, 1H), 5.92 (d, $J = 2$ Hz, 1H), 5.85 (d, $J = 1.5$ Hz, 1H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

$\delta$ 164.4, 147.6, 138.9, 135.4, 133.9, 130.3, 128.8, 128.6, 125.1, 123.1, 122.5, 89.7.
Manual synthesis of 5.68

A dry 40-mL vial equipped with a PTFE-coated magnetic stir bar was charged with the aryl iodide 5.108 (858 mg, 2.0 mmol) and trans-propenyl boronic acid (5.105) (223 mg, 2.6 mmol). The vial was brought into a glovebox and charged with K₃PO₄ (1.27 g, 3 equiv.), PdCl₂dppf.CH₂Cl₂ (849 mg, 4 mmol) and THF (20 mL, 0.1 M). The vial was sealed with a Teflon-lined cap and stirred at 60 °C for 18 hours. The reaction was cooled to 23 °C and filtered through a pad of Celite™ and concentrated in vacuo. The crude product was purified by silica gel chromatography (30-40% CH₂Cl₂/hexanes) to give 5.68 as an off-white solid (268 mg, 39%).

TLC (40% CH₂Cl₂/hexanes)

\[ R_f = 0.43, \text{ visualized by shortwave UV} \]

¹H-NMR (500 MHz, CDCl₃)

\[ \delta 8.22 \text{ (dd, } J = 8.0, 1.0 \text{ Hz, 2H), 7.65} \text{ (t, } J = 7.5 \text{ Hz, 1H), 7.52} \text{ (app t, } J = 8.0 \text{ Hz, 2H), 7.41} \text{ (d, } J = 2.0 \text{ Hz, 1H), 7.36} \text{ (dd, } J = 8.5, 2.0 \text{ Hz, 1H), 7.15} \text{ (d, } J = 8.5 \text{ Hz, 1H), 6.40} \]
(dd, J = 16.0, 1.5 Hz, 1H), 6.26 (dq, J = 15.5, 6.5 Hz, 1H), 5.90 (d, J = 1.5 Hz, 1H), 5.83 (d, J = 2.0 Hz, 1H), 1.90 (dd, J = 7.0, 1.5 Hz, 3H).

$^{13}$C-NMR (125 MHz, CDCl$_3$)

$\delta$ 164.8, 146.2, 136.0, 133.6, 133.3, 130.3, 129.5, 129.3, 128.6, 127.6, 127.2, 127.0, 124.5, 123.1, 122.2, 18.5.

HRMS (ESI+)

Calculated for C$_{18}$H$_{16}$O$_2$Br : 343.0334

Found: 343.0336

Manual synthesis of 5.111

A flame-dried single-necked 200mL round-bottomed flask containing a PTFE-coated stir bar was taken into the glove box, where dicyclohexylborane (464mg, 2.6mmol, 0.1eq) was added. This was capped with a rubber septum and taken out of the glove box, where THF was added (26mL). To this stirring suspension was added portions alkyne 5.109 as a solution in DCM (0.992 M, 17 mL, 26.0 mmol, 1 equiv), which generated a colorless solution and evolved heat. To this solution was added neat catecholborane (5.110) (3.6 mL, 27 mmol, 1.05 equiv) in
portions. After the addition was complete, the reaction was stirred vigorously for 4 hours. The reaction was then opened to ambient atmosphere and 1 M aqueous NaOH (60 mL, 60 mmol, 2.3 equiv) was added and stirred vigorously. The dark brown solution was then poured into 1:1 ethyl acetate:diethyl ether (900 mL), to which was added 1:1 saturated aqueous NH₄Cl:H₂O (150 mL). The mixture was shaken and the layers were separated. The organic layer was rinsed with brine (150 mL), dried over anhydrous MgSO₄, and filtered over Celite™. The solution was concentrated to a blue oil, then dissolved in acetone and adsorbed onto Celite™, then subjected to flash chromatography (SiO₂, 8:2:1 hexanes/diethyl ether/methanol). Fractions containing the product (5.111) were concentrated under reduced pressure, resulting in a yellow oil. This oil was used directly without further purification.

\[ \text{5.111} \]

\[
\begin{align*}
\text{(iPr)₂Si-O-} & \text{B(OH)₂} \\
\end{align*}
\]

\(^1\text{H-NMR (400 MHz, 95:5 DMSO-d6:D}_2\text{O)} \]

\[ \delta 6.47 \text{ (dt, } J = 17.9 \text{ Hz, 3.7 Hz, 1H), 5.59 \text{ (dt, } J = 17.9 \text{ Hz, 1.8 Hz, 1H), 4.23 \text{ (m, 2H), 1.11-0.94 (m, 19H)}} \]

Manual synthesis of 5.51

Boronic acid 5.111 was transferred with benzene (235 mL) to a 500-mL round-bottomed flask equipped with a PTFE-coated magnetic stir bar, and N-methyliminodiacetic acid (5.75 g, 39.1 mmol, 1.5 equiv) and DMSO (26 mL) were added. This flask was fitted with a Dean-Stark trap and a reflux condenser and was heated to reflux under ambient atmosphere in an oil bath. The mixture was stirred for 2 hours, after which it was cooled to ambient temperature. The mixture was concentrated to a residue which was partitioned between ethyl acetate (400 mL) and saturated aqueous NaHCO₃ (300 mL). The layers were separated, and the aqueous layer was
rinsed with ethyl acetate (3 x 100 mL). The combined organic layers were then rinsed with 1:1 brine:H₂O (100 mL), 3:1 brine:H₂O (100 mL), then brine (50 mL). The organic layers were then dried over anhydrous MgSO₄, filtered over Celite™, then concentrated to a yellow oil. This oil was taken up in acetone and adsorbed onto Celite™, then subjected to flash chromatography (SiO₂, diethyl ether → 3:2 diethyl ether/acetonitrile). Fractions containing the product (5.51) were concentrated to a colorless foam (6.10g, 63% over two steps).

![Chemical Structure](image)

**1H-NMR (500 MHz, acetone-\textit{d}6)**

δ 6.19 (dt, \( J = 17.6\)Hz, 3.9Hz, 1H), 5.83 (dt, \( J = 17.6\)Hz, 2.0Hz, 1H), 4.34 (m, 2H), 4.20 (d, \( J = 16.9\)Hz, 2H), 4.00 (d, \( J = 16.9\)Hz, 2H), 3.00 (s, 3H), 1.21-1.03 (m, 19H).

**13C-NMR (125 MHz, acetone-\textit{d}6)**

δ 169.0, 144.7, 65.7, 62.3, 47.3, 18.4, 12.8.

**HRMS (ESI+)**

Calculated for C₁₇H₃₅BNO₅Si: 370.2221

Found: 370.2225
Manual synthesis of **5.113**

To a 1000-mL single-necked round-bottom flask equipped with a PTFE-coated magnetic stir bar is added ethyl benzofuran-2-carboxylate **5.112** (29.916 g, 100.02 mmol, 1.00 equiv) and THF (200 mL). To the resulting stirred clear yellow solution is added 1 M aqueous NaOH (120 mL, 120 mmol, 1.20 equiv). The resulting biphasic mixture was allowed to stir vigorously at room temperature for 2 hours. The reaction is transferred to a 1000-mL separatory funnel and diluted with reagent grade (BHT-stabilized) diethyl ether (250 mL) and water (150 mL). To the funnel is added concentrated HCl until the aqueous phase maintains a pH of approximately 2 by litmus paper. The phases are separated and the organic phase is dried over anhydrous MgSO$_4$, filtered through Celite™, and concentrated **in vacuo** (35 °C, 100 Torr) to give the crude **5.113** as an off white chunky powder.

The crude product was not purified further.

**TLC (hexanes:ethyl acetate 1:1)**

$R_f = 0.13$, stained by KMnO$_4$.

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

δ 7.60 (s, 1H), 7.55 (d, $J = 2$ Hz, 1H), 7.25 (d, $J = 2$ Hz, 1H), 3.98 (s, 3H), 3.33 (bs, 1H).

**$^{13}$C NMR (125 MHz, DMSO-$d_6$)**
\[ \delta 159.6, 147.1, 146.0, 143.5, 129.7, 117.0, 116.2, 113.0, 112.3, 56.4. \]

HRMS (EI+)

Calculated for C_{10}H_{7}BrO_{4}:

Found:

269.95277

269.95341

---

Manual synthesis of 5.114

To a 1000-mL single-necked round-bottom flask equipped with a PTFE-coated magnetic stir bar is added benzofuran-2-carboxylic acid 5.113 (100 mmol, 1 equiv), quinoline (freshly distilled, 300 mL), and copper bronze (20.972 g, 115 mmol, 1.15 equiv). The flask is fitted with a water-cooled Allihn condenser. The reaction system is then vac/filled (1000 mTorr) with dry nitrogen and a nitrogen inlet is placed in the condenser, leaving the top of the condenser open to ambient atmosphere (This set-up allows the reaction to be maintained under oxygen-free conditions while venting lower-boiling residues to an appropriately vented fume hood). The reaction is allowed to reflux with stirring in a heating mantle for 1 hour. The reaction is then allowed to cool to room temperature with stirring over 1 hour and filtered through Celite. The Celite pad is washed with copious amounts of reagent grade (BHT-stabilized) diethyl ether and the combined filtrates are transferred to a 2000-mL separatory funnel. To the funnel is added, diethyl ether (500 mL), hexanes (250 mL), ice (~100 g), and 6 M aqueous HCl (500 mL). The mixture is carefully swirled for several minutes and then shaken. The phases are separated and the aqueous phase is extracted with diethyl ether:hexanes (1:1, 250 mL). The combined organic layers are washed with 6 M aqueous HCl (100 mL), 1 M aqueous NaOH (50 mL), brine (50 mL), dried over anhydrous MgSO₄, filtered through Celite™, and concentrated in vacuo (40 °C, 100 Torr) to give the crude product as a dark red/brown liquid.
The crude product was purified via flash chromatography (SiO$_2$, 8 x 16 cm, hexanes:ethyl acetate 1:0 → 9:1) to give 5.114 as a yellow oil (19.485 g, 85% yield)

![Chemical Structure](image)

TLC (hexanes)

$R_f = 0.09$, stained by KMnO$_4$.

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

$\delta$ 7.61 (d, $J = 2$ Hz, 1H), 7.33 (d, $J = 2$ Hz, 1H), 6.91 (d, $J = 2$ Hz, 1H), 6.70 (d, $J = 2$ Hz, 1H), 3.99 (s, 3H).

$^{13}$C NMR (125 MHz, DMSO-$d_6$)

$\delta$ 147.0, 145.7, 142.6, 130.3, 115.8, 115.3, 109.8, 106.6, 56.2.

HRMS (EI+)

Calculated for C$_9$H$_7$BrO$_2$: 225.96294

Found: 225.96284

Manual synthesis of 5.52

To a 250-mL three-necked round-bottom flask equipped with a PTFE-coated stir bar, an internal thermometer in one side neck, an argon inlet in the center neck, and a rubber septum in
one side neck is added hexanes (40 mL) and \( \text{n-BuLi} \) (1.6 M in hexanes, 17.2 mL, 27.5 mmol, 1.20 equiv). This clear colorless solution is allowed to cool to -10 °C in a dry ice/brine bath with stirring for 10 minutes. To the cooled stirred solution is added diisopropylamine (3.5 mL, 25.0 mmol, 1.09 equiv) and the resulting solution is allowed to stir for 30 minutes, maintaining the dry ice/brine bath. To this cooled stirred solution is added dry diethyl ether (40 mL) to quench excess \( \text{n-BuLi} \). This solution is allowed to stir for 20 minutes, maintaining the dry ice/brine bath. To this cooled stirred solution is added benzofuran 5.114 (5.212 g, 22.96 mmol, 1.00 equiv) dropwise as a solution in dry diethyl ether (40 mL) from a 100-mL single-necked pear-shaped flask via cannula (20G). The cannula transfer is performed at such a rate that the internal reaction temperature does not rise above -10 °C. Additional diethyl ether (16 mL) is used for quantitative transfer, again insuring that the internal reaction temperature does not rise above -10 °C. This reaction solution is stirred for 2 hours, maintaining the dry ice/brine bath. To the cooled stirred solution is then added trimethylborate (5.2 mL, 46.6 mmol, 2.03 equiv) and then reaction is allowed to warm to room temperature with stirring over 30 minutes.

To the stirred reaction is added 5 M aqueous HCl (50 mL) and the reaction is stirred vigorously for 10 minutes during which time a white precipitate forms and then dissolves. This mixture is transferred to a 1000-mL separatory funnel. To the funnel is added water (125 mL) and reagent grade (BHT-stabilized) diethyl ether (62 mL). The phases are separated and the aqueous phase is extracted with diethyl ether (62 mL). The combined organic layers are washed with 5% w/v aqueous NaOH (125 mL). This aqueous layer is then extracted with petroleum ether (82 mL) and is then adjusted to pH ~ 1.5 (by litmus paper) with concentrated HCl. The acidified aqueous layers is extracted with diethyl ether (4 x 62 mL) and these combined organic layers are washed with water (62 mL), brine (62 mL), dried over anhydrous MgSO4, filtered through Celite™, and concentrated in vacuo (40 °C, 100 Torr) to give a sample of crude boronic acid 5.115 as a tan solid: TLC (diethyl ether), \( R_f = 0.46 \), stained by KMnO4; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \( \delta 7.47 \) (d, \( J = 1.5 \text{ Hz}, 1\text{H} \)), 7.40 (s, 1H), 7.08 (d, \( J = 1.5 \text{ Hz}, 1\text{H} \)), 3.95 (s, 3H); 3.003 g, 48% yield.

To a 100-mL single-necked round-bottom flask equipped with a PTFE-coated magnetic stir bar is added the crude boronic acid, toluene (50 mL), DMSO (5 mL), and MIDA (2.435 g, 16.55 mmol, 1.50 equiv based on starting boronic acid). The flask is fitted with a toluene-filled
Dean-Stark trap topped with a water-cooled Dimroth condenser. The reaction is allowed to reflux with stirring in a heating mantle for 12 hours during which time the reaction darkens from tan to brown. The reaction is allowed to cool with stirring to room temperature and is then concentrated in vacuo (40 °C, 50 Torr). The resulting residue is dissolved in ethyl acetate (250 mL) and transferred to a 500-mL separatory funnel. This organic layer is washed with water (6 x 75 mL) (These successive water washes are crucial for the quantitative removal of DMSO. If not effectively removed, DMSO may result in difficulties with purification), brine (75 mL), dried over anhydrous MgSO₄, filtered through Celite™, and concentrated in vacuo (40 °C, 50 Torr) giving the crude product as a dark brown solid.

The crude product is then dissolved in a minimum amount of acetone and precipitated with diethyl ether. The precipitate is isolated by vacuum filtration and residual solvent is removed under vacuum (room temperature, 1000 mTorr) to give 5.52 as an off-white solid (3.873 g, 44% yield over the two steps).

TLC (ethyl acetate)

Rf = 0.51, stained by KMnO₄.

\(^1\)H NMR (500 MHz, CD₂CN)

δ 7.38 (d, J = 1.5 Hz, 1H), 7.05 (s, 1H), 7.02 (d, J = 1.5 Hz, 1H), 4.13 (d, J = 17 Hz, 2H), 3.96 (s, 3H), 3.96 (d, J = 17 Hz, 2H), 2.71 (s, 3H).

\(^13\)C NMR (125 MHz, DMSO-\(d_6\))

δ 169.0, 145.6, 145.1, 130.9, 115.9, 115.0, 114.4, 109.8, 61.7, 56.1, 47.5.

HRMS (EI+)

Calculated for C\(_{14}\)H\(_{13}\)BBrNO\(_6\): 381.00193

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REFERENCES


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APPENDIX A
SCRIPTING COMMANDS USED TO PROGRAM THE SYNTHESIZER

This appendix describes the commands that are used in the custom scripting language that is interpreted by the software that runs the synthesizer. Each command described here is followed by an example of its usage. The scripting language is not case-sensitive.

` (apostrophe)

Description:
Used to designate a comment. The text following an apostrophe is not interpreted by the program as a command.

Usage:
` enter comment text following the apostrophe

Example:
valve 1a 2b ` This is a comment on the same line as a command
` This is a comment on its own line

[ ] (brackets)

Description:
Used to designate a variable. A variable is a place-holder for a value that can be specified with a define command. Using variables allows the code to be more flexible.

Usage:
command [variableName]

Executes a command, represented here generically as command, for which the variable variableName contains the instructions to be interpreted by the command. The value of variableName would be defined earlier in the code using the define command
Example:
valve [Tube]
pump 5 in= [THF] out=a rate=[StdRate]

In the above code the variables are [Tube], [THF], and [StdRate]. Using the
define command the values could be set at the beginning of the code and could be
changed easily before the file is executed. For example:

define Tube=4a THF=b StdRate=45

valve [Tube]
pump 5 in=[THF] out=a rate=[StdRate]
pump 2 in=[THF] out=a
valve 2b [Tube]

In this way the code is more flexible. This same routine could be called for a number of
different tube locations and using just one line of code the entire routine could be
modified for a new tube location. Also, if the location of the THF bottle changed, it
would be easy to specify a new location using just one line of code, even though the
routine accesses the THF bottle on several lines.

DEFINE

Description:
Used to set the value of a variable within a script. NOTE: Due to current code limitations
each variable should only be defined once in a procedure.

Usage:
define variable_name=variable_value

variable_name represents the name of the variable to define
variable_value is the data which will now stand in the place of all instances of the
variable defined by variable_name
Example:
define pumpnum=a
define valve 4[pumpnum]
define valve 5[pumpnum] 8[pumpnum]

All instances of [pumpnum] (the variable) will be replaced with the valve a at runtime. That is, the program will interpret the script as follows:

define pumpnum=a
define valve 4a
define valve 5a 8a

Variables allow flexible code since with a few uses of the define command a general script can be mapped onto any number of equipment combinations.

**PAUSE**

**Description:**
Pauses the script for a specified period of time before executing the next line.

**Usage:**
pause [number of seconds to pause]

**Example:**
pause 5

This pauses the script for five seconds.

**LOG**

**Description:**
Writes a user comment to the file “MachineLog.txt” with a time and date stamp. The comment is added to the end of the file without affecting the previous text in the log file.
The command is useful in logging the time and date when specified portions of code are executed or completed.

**Usage:**

```plaintext
log "text here"
```

Any text can be entered between the two quotation marks.

**Example:**

```plaintext
log "Program Start"
```

Writes “5/28/13 1:00:00 PM: Program Start” to the file “MachineLog.txt” where the system date is 5/28/13 and the system time is 1:00:00 PM.

**SUB**

**END SUB**

**Description:**

The `sub` and `end sub` commands enclose scripting commands that can be executed as a sub routine. That is, a particular sequence of code can be designated as a sub routine and given a unique identifying name. Rather than retyping the code each time the routine needs to be run, the code can be executed using the `run` command. Additionally, using the `background` command sub routines can be executed as background processes (although care needs to be taken in how the code is scripted).

**Usage:**

```plaintext
sub SubName
    enter lines of code here
end sub
```

Define a sub routine with the identifier `SubName`. The code contained within the `sub` and `end sub` lines can be executed by using the `run` command followed by the
identifier. NOTE: Every sub routine within a script must have a unique identifier. If two sub routines with the same identifier are used, then an error with result.

**Example:**

```
sub test
    log “Start time”
    pause 30
    log “End time”
end sub
```

Defines the sub routine `test`. At this point the synthesizer will not actually do anything. Rather, it has saved the instructions defined by the `test` routine for later use. To execute the `test` script the `run` command is used.

```
run test ‘ Run the script once
run test ‘ Run the script again
```

The `test` sub routine is run twice. Each time the sub routine is run it writes the line “Start time” to the log file, waits 30 seconds, and writes the line “End time” to the log file. After the above script has executed, the log file will contain four new lines.

**RUN**

**Description:**

Runs a sub routine that has been specified by the `sub` command structure.

**Usage:**

```
run SubName
```

Executes the code contained within the sub routine represented by the identifier `SubName`. The identifier `SubName` can refer to any sub routine defined earlier in the script using the `sub` and `end sub` commands.
**Example:**
see the above examples for the sub and end sub commands.

**BACKGROUND**

**Description:**
Begins the execution of a sub routine as a background process. After processing this initial event, the script will immediately read the next line of the main code as the background process continues. NOTE: There are important consequences to running a process in the background. It is the script writer’s responsibility to ensure that the background process will not conflict with the foreground process. Specifically, appropriate use of the lock and unlock commands is encouraged to make sure that the same equipment is not being accessed at the same time. Additionally, the program cannot display the progress of the background process, nor can it save the progress of the background process if the code is halted. Thus, the background command should be used sparingly. NOTE: Only one instance of a sub routine per pump is allowed to execute as a background process at any given time.

**Usage:**

```
background SubName
```

Begins background execution of the sub routine with the identifier SubName.

**Example:**

```
sub TestPause
    pause 15
end sub

background TestPause

pause 10
```
The `sub TestPause` portion defines a sub routine that will have the synthesizer pause for 15 seconds. The `background` command then calls the `TestPause` routine to execute in the background while it then immediately processes the next command, `pause 10`. Thus, the total execution time for this script will be 15 seconds, since the synthesizer will simultaneously pause for both 15 and 10 seconds.

**WAIT**

**Description:**
Halts program execution until a condition is met. Specifically, the `wait` command allows other synthesizer processes to catch up to the script before executing the next command line. This is particularly useful in coordinating background-executed sub routines.

**Usage:**
`wait SubRoutineName`

`SubRoutineName` is the identifier of a sub routine that was previously executed using the `background` command. The system will wait until the process for `SubRoutineName` is complete before proceeding to the next line.

**Example:**
```
sub TestPause
    pause 15
end sub

background TestPause

pause 5
wait TestPause

log "Script Complete"
```
Similar to the example of the background command, the sub TestPause portion defines a sub routine that will have the synthesizer pause 15 seconds. The background command then calls the TestPause routine to execute in the background while it then immediately processes the next command line, pause 5. After the pause 5 command completes the synthesizer pauses and waits for the TestPause sub routine that is running in the background to complete. Thus, the line “Script Complete” is not written to the log file until 15 seconds after the background TestPause command is executed. If the wait TestPause command was omitted, the log command would execute 5 seconds after the background TestPause command was executed.

**VALVE**

**Description:**
Used to change the port position of an 8-port valve. The command can also be used to turn a solenoid valve on or off. Multiple valves can be manipulated sequentially in a single valve command.

**Usage:**
valve command1 command2 command3

command1, command2, command3, etc. describes the number of the valve being manipulated and the new state for the valve. Multiple valves can be manipulated using a single valve command. In fact, there is no limit to the number of manipulations that can be performed with a single valve command. Changes to the valves of the system are executed in the order command1, then command2, then command3, etc.

**Example:**
valve 1a

This moves valve 1 to position a
valve 1a 2b

This moves valve 1 to position a then moves valve 2 to position b

cvalve xon

This turns the solenoid on

valve xoff

This turns the solenoid off

**PUMP**

**Description:**
Used to control the syringe pump. This command is used to move specific volumes of fluid from one port to another at a rate specified by the operator.

**Usage:**
pump volume in=PortIn out=PortOut rate=RateOfAspiration/Injection
rateIn=RateOfAspiration rateout=RateOfInjection

volume specifies the amount of fluid that is to be moved by the syringe pump. The volume specified can be greater that the capacity of the syringe only if the syringe is both aspirating and injecting the fluid during the same command. The volume is expressed as a number representing mL. That is, do not include the unit when specifying the volume.

PortIn (optional) specifies the port from which the reagent will be aspirated. If PortIn is not specified the program will use whatever port the syringe pump is already set to.

PortOut (optional) specifies the port to which the fluid will be injected. If PortOut is not specified the program will use whatever port the syringe pump is already set to.
RateOfAspiration/Injection (optional) allows the operator to specify the rate at which the syringe pump will handle the fluid transfer. The rate is specified as a number representing mL/min. That is, do not include the unit when specifying the rate.

RateOfAspiration (optional) (similar to above) allows the rate of aspiration of the syringe to be set independently of the rate of injection.

RateOfInjection (optional) (same as RateOfAspiration) but specifies the rate of injection. NOTE: if the rate of aspiration is specified but the rate of injection is omitted the default rate in injection will be used. This applies vice-versa when only the rate on injection is specified.

**Example:**

pump 5 in=a out=b rate=5

Pumps 5 mL of fluid from port a to port b at a rate of 5 mL/min for both aspiration and injection.

pump 3 in=a out=b ratein=10 rateout=2

Pumps 3 mL of fluid from port a to port b at a rate of 10 mL/min for aspiration and 2 mL/min for injection.

pump 5 in=b out=c

Pumps 5 mL of fluid from port b to port c at the default rate for aspiration and injection.

pump 4 in=a

Aspirates 4 mL of fluid from port a in to the syringe and then waits for the next command. It is the operator’s responsibility to account for the fate of the fluid in the syringe. NOTE: No more than 10 mL of fluid can be aspirated into the syringe with this command (since the syringe has a capacity of 10 mL).
pump 2 out=b

Injects 2 mL of syringe contents to port b. This command assumes that the syringe contains at least 2 mL of fluid that was aspirated with a previous command.

pump out=b

Injects the entire contents of the syringe to port b. This command assumes that the syringe contains fluid that was aspirated with a previous command. NOTE: This command can be used to ensure that the syringe is completely empty before executing additional code.

pump out

Similar to above, however a port is not specified. The syringe injects the entire contents to the currently selected port.

pump 0.5 in out

Aspirates 0.5 mL of fluid from the default port and then injects it back through this port. This command is useful for priming fluid lines or mixing a solution in the syringe.

pump 1 in out=c rate=2

Aspirates 1 mL of fluid from the currently selected port and then injects it through port c at a rate of 2 mL/min.

AUX

Description:
This command follows the same structure as the `pump` command, but sends its instructions to the auxiliary pump.

**Usage:**

Same as the `pump` command

**Example:**

```
aux 3 in=b out=a ratein=10 rateout=2
```

Uses the auxiliary pump to transfer 3 mL of fluid from port a to port b, aspirating at a rate of 10 mL/min and injecting at 2 mL/min.

---

**WETPUMP**

**Description:**

This command follows the same structure as the `pump` command, but sends its instructions to the wet pump.

**Usage:**

Same as the `pump` command.

**Example:**

```
wetpump 3 in=a out=b ratein=10 rateout=2
```

Uses the wet pump to transfer 3 mL of fluid from port a to port b, aspirating at a rate of 10 mL/min and injecting at 2 mL/min.

---

**LOCK**

**Description:**

Used to claim a pump or valve for exclusive access by a particular module. This command is used on synthesizers for which there are several autonomous processes that share a common resource (a common valve or pump). Other processes that try to access
the shared valve or pump while it is locked will get a busy signal and will wait until the resource becomes available. The resource is freed with the `unlock` command.

**Usage:**

```
lock #
lock aux
lock wetpump
```

# indicates the number of the valve that should be locked. To use a single `lock` command to lock many valves, separate each valve name with a space.

aux indicates that the auxiliary pump should be locked

c`wetpump` indicates that the wetpump should be locked

**Example:**

```
lock 3 5 aux
```

Locks valve 3, valve 5, and the auxiliary pump for exclusive access by the calling module.

**UNLOCK**

**Description:**

Used to release a pump or valve from exclusive access by a particular module (see `lock` command).

**Usage:**

```
unlock #
unlock aux
unlock wetpump
unlock all
```

# indicates the number of the valve that should be unlocked. To use a single `unlock` command to unlock many valves, separate each valve number with a space.
aux indicates that the auxiliary pump should be unlocked
wetpump indicates that the wet pump should be unlocked
all indicates that all resources locked by the calling module should be unlocked

Examples:
unlock 3 5 aux

Releases valve 3, valve 5, and the auxiliary pump from exclusive access by the calling module.

unlock all

Releases all resources from exclusive access by the calling module.

STIRON

Description:
Used to control the stirring of the reaction filtration cartridge. The IKA® RET control-visc heating stir plate used to stir the filtration cartridge is powered through a solid state relay. The default position of the relay is off; the stir plate has no power. When it is turned on, the stir plate is receives power and resumes its preset stir rate and temperature (preset by the operator). When combined with the stiroff command the stir plate can be turned on and off during a program.

Usage:
stiron

Example:
stiron

valve 1a
The solid state relay is turned on, providing power to the stir plate, which resumes its preset stir rate and temperature. The script will immediately read the next line of the main code (valve 1a) as the stir plate begins powering up. Once powered up, the stir plate will maintain its preset stir rate and temperature until the stiroff command is called.

**STIROFF**

**Description:**

See description for stiron command above.

**Usage:**

stiroff

**Example:**

valve 1a

stiroff

valve 1b

After executing the valve 1a command the solid state relay is turned off, powering down the stir plate, stopping the stirring of the reaction filtration tube. The script will immediately read the next line of the main code (valve 1b) as the stir plate powers down. This command assumes that the stiron command has been called previously.
APPENDIX B
SAMPLE PROGRAM SCRIPT USED ON THE SYNTHESIZER

This appendix shows an example of a complete program script written with the commands described in Appendix A. Specifically, this program script was written to have the synthesizer execute one deprotection ([D]), cross-coupling ([C]), and purification ([P]) sequence as a single experiment (Scheme B.1). This is the program script exactly as the operator would enter it for the software to interpret it.

Scheme B.1. General scheme for the experiment executed by the program script shown in Appendix B; a sequence of one deprotection ([D]), cross-coupling ([C]), and purification ([P]).

```plaintext
' ===========
' Definitions
' ===========

' Define experiment name
define experiment=experiment_name

' Define addition rates (mL/min)
define additionRate1=0.057
define additionRate2=0.057
define additionRate3=0.057

' Define post-addition stir times(seconds)
' Total reaction time is sum of slow-addition and post-addition stir times
' 1h = 3600  2h = 7200  3h = 10800  4h = 14400
' 5h = 18000  6h = 21600  7h = 25200  8h = 28800
' 9h = 32400  10h = 36000  11h = 39600  12h = 43200
' 13h = 46800  14h = 50400  15h = 54000  16h = 57600
' 17h = 61200  18h = 64800  19h = 68400  20h = 72000
' 21h = 75600  22h = 79200  23h = 82800  24h = 86400
' 25h = 90000  26h = 93600  27h = 97200  28h = 100800
' 29h = 104400  30h = 108000  31h = 111600  32h = 115200
' 33h = 118800  34h = 122400  35h = 126000  36h = 129600
' 37h = 133200  38h = 136800  39h = 140400  40h = 144000
' 45 min = 2700

define stirTime1=44100
define stirTime2=46800
```
define stirTime3=57600

log " start of [experiment] "

valve 13h

log " BEGIN: addition of THF "
' ================================
' Add THF To 1st Deprotection Tube
' ================================
' First portion of THF
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 5 in=a rate=[fastTHF] ' aspirate THF
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastTHF] ' aspirate argon plug to clear lines
pause 10
unlock 12 6 ' unlock common reagents

valve [deprotect]
valve 2g
pump out=a rate=10
pump 2 in=b out=a ratein=60 rateout=20

' Second portion of THF
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 5 in=a rate=[fastTHF] ' aspirate THF
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastTHF] ' aspirate argon plug to clear lines
pause 10
unlock 12 6 ' unlock common reagents

valve [deprotect]
valve 2g
pump out=a rate=10
pump 2 in=b out=a ratein=60 rateout=20

' There should now be 10 mL THF in 1st deprotection tube
log " COMPLETE: addition of THF "

valve 3h ' move valve 3 away from 1st deprotection tube
pause 10 ' give some time for the MIDA boronate to dissolve

' ================================
' Add Water To 1st Deprotection Tube
' ================================
wetpump 3 in=a rate=5
pause 3
valve 13a ' select 1st deprotection tube
wetpump out=d rate=5
wetpump 2 in=e out=d ratein=60 rateout=10
wetpump 2 in=e out=d ratein=60 rateout=10

log " water now added to 1st deprotection tube "

valve 13h ' move valve 13 away from 1st deprotection tube

' ==================================
' Begin Agitation Via Argon Sparging
' ==================================
log " BEGIN: 1st deprotection aggitation "
valve [deprotect]
valve 2g
valve 1h ' move valve 1 away from pressure of argon

lock [gas]
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
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valve xoff
pause 2
valve xon
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valve xoff
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282
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valve xoff
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valve xon
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valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xoff
pause 2
unlock [gas]

log " COMPLETE: 1st deprotection agitation "

' ====================================================
' Quench The Reaction With pH=6 0.5 M Phosphate Buffer
' ====================================================
sub quench
  wetpump 3 in=b rate=5 ' aspirate buffer
  pause 5
  valve 13a
  wetpump out=d rate=5 ' inject buffer
  wetpump 2 in=e out=d ratein=60 rateout=10
  wetpump 2 in=e out=d ratein=60 rateout=10
  valve 13h
end sub

background quench

' =============
' Prime The Ether Line
' =============
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [ether] ' select ether
pump 5 in=a rate=5 ' aspirate ether
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastEther] ' aspirate argon plug to clear lines
pause 3
pump out=d rate=20 ' inject ether to waste flask

valve [me]
valve 2h
valve [ether]
pump 5 in=a rate=5
pause 10
valve [ar]
pump 2 in=a rate=[fastEther]
pause 3
pump out=d rate=20

valve [me]
valve 2h
valve [ether]
pump 5 in=a rate=5
pause 10
valve [ar]
pump 2 in=a rate=[fastEther]
pause 3
pump out=d rate=20

pump 2 in=b out=d ratein=60 rateout=20

' =================
' Add Diethyl Ether
' =================
valve [me]
valve 2h
valve [ether]
pump 5 in=a rate=[fastEther]
pause 10
valve [ar]
pump 2 in=a rate=[fastEther]
pause 3
unlock 12 6 ' unlock common reagents

wait quench

valve [deprotect]
valve 2g
pump out=a rate=10
pump 2 in=b out=a ratein=60 rateout=10
pump 2 in=b out=a ratein=60 rateout=10

valve 3h
valve 13a
wetpump 10 in=e out=d ratein=60 rateout=20
wetpump 10 in=e out=d ratein=60 rateout=20
wetpump 10 in=e out=d ratein=60 rateout=20
log "1st deprotection fully quenched"

' =================
' Aspirate 1st Deprotection Mixture
' =================
wetpump 10 in=d rate=10
pause 30
wetpump 4.0 out=d rate=10
pause 10
wetpump out=f rate=10
wetpump 2 in=e out=f ratein=60 rateout=20
wetpump 2 in=e out=d ratein=60 rateout=20
wetpump 2 in=e out=d ratein=60 rateout=20

' ===========================
' Aspirate 50% Saturated NaCl
' ===========================
wetpump 3 in=g rate=5
pause 5

' ===========================
' Inject 50% Saturated NaCl To 1st Deprotection tube
' ===========================
wetpump out=d rate=5
wetpump 2 in=e out=d ratein=60 rateout=20
wetpump 2 in=e out=d ratein=60 rateout=20

valve 13h
valve [deprotect]
valve 2g
valve 1h
lock [gas]
valve xon
pause 1
valve xoff
pause 5
valve xon
pause 1
valve xoff
unlock [gas]
valve 3h
valve 13a
wetpump 10 in=e out=d ratein=60 rateout=20
wetpump 10 in=e out=d ratein=60 rateout=20
wetpump 10 in=e out=d ratein=60 rateout=20
pause 10

' ============================
' PRE-DRYING OVER MgSO4:Celite
' ============================
sub predryCycleStep1
  pump 10 in=h out=h rate=15
  pump 2 in=b out=h ratein=60 rateout=45
  pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15
pump 2 in=b out=h ratein=60 rateout=45
pump 10 in=h out=h rate=15 ' ten times of agitation
pump 5 in=b out=h ratein=60 rateout=45
end sub

log " BEGIN: predrying "
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 4 in=a rate=10 ' aspirate THF
pause 10
valve [ar] ' select argon
pump 2 in=a rate=10 ' aspirate argon plug
unlock 12 6
valve 4a
pump out=h rate=15
pump 2 in=b out=h ratein=60 rateout=30

' Transfer to predrying tube
valve [deprotect]
valve 2g
valve 4a
pump 9 in=a rate=15
pause 1
pump out=h rate=15
pump 9 in=a rate=15
pause 1
pump out=h rate=15
pump 5 in=b out=h ratein=60 rateout=45

run predryCycleStep1 ' rep1
run predryCycleStep1 ' rep2

lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 4 in=a rate=10 ' aspirate THF
pause 10
valve [ar] ' select argon
pump 2 in=a rate=10 ' aspirate argon plug
unlock 12 6
valve 2g
valve [drying]
pump out=a rate=15
pump 2 in=b out=a ratein=60 rateout=30

log " BEGIN: Transfer from predrying to drying tube "
valve 4a
valve 2g
valve [drying]
pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

' All solution should now be in the drying tube

' ==============
' DRYING/DEGASING MODULE
' ==============
sub dryingCycle1
  valve [drying]
  valve 2g
  pump 8 in=a rate=5
  valve 1b
  pause 1
  pump out=a rate=10
  pump 3 in=b out=a ratein=60 rateout=45
end sub

log " BEGIN: drying "
run dryingCycle1 ' Rep 1
run dryingCycle1 ' Rep 2
run dryingCycle1 ' Rep 3
run dryingCycle1 ' Rep 4
run dryingCycle1 ' Rep 5
run dryingCycle1 ' Rep 6
run dryingCycle1 ' Rep 7
run dryingCycle1 ' Rep 8
run dryingCycle1 ' Rep 9
run dryingCycle1 ' Rep 10
run dryingCycle1 ' Rep 11
run dryingCycle1 ' Rep 12
log " BEGIN: transfer to degassing tube "
' Rep 1
valve [drying]
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Rep 2
valve [drying]
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Rep 3
valve [drying]
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Rep 4
valve [drying]
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Air plug
pump 2 in=b out=a ratein=60 rateout=20

log " BEGIN: first wash of drying agents "
' ============
' First Wash Of Drying Reagents
' ============
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 6 in=a rate=10 ' aspirate THF
pause 10
valve [ar] ' select argon
pump 2 in=a rate=10 ' aspirate argon plug
unlock 12 6
valve 4a
pump out=h rate=15
pump 2 in=b out=h ratein=60 rateout=20

run predryCycleStep1 ' rep1

valve 4a
valve 2g
valve [drying]
pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

run dryingCycle1 ' Rep 1
run dryingCycle1 ' Rep 2
run dryingCycle1 ' Rep 3
run dryingCycle1 ' Rep 4
run dryingCycle1 ' Rep 5

' Rep 1
valve [drying]
valve 2g ' Select drying tube
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas] ' Select degassing tube
pump out=a rate=20

' Rep 2
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Air plug
pump 2 in=b out=a ratein=60 rateout=35

log " all solution (first and wash) should now be in degassing tube "

' there are mL in the tube
log " BEGIN: first concentration/deoxygenation "
valve 2a
pump 6 in=a rate=10
pump 1 in=b out=a ratein=60 rateout=20
valve 1h
' there are mL in the tube

lock [gas]

valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
' 10 sec

valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
delay
valve xon
pause 0.5
delay
valve xoff
pause 1.5
' 20 sec

valve xon
pause 1
delay
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
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valve xon
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valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xon

294
pause 2
valve xoff
pause 2 ' 1 min

valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
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valve xoff
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pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5 ' 2 min

valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min

valve xon
pause 420
valve xoff ' 10 min

unlock [gas]

' there are mL in the tube
pump 2 out=a rate=20
pump 2 in=b rate=60
pause 1
pump 1 out=a rate=20
valve 1h
' there are mL in the tube
lock [gas]

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 10 sec

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec

valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff  
pause 1  
valve xon  
pause 1  
valve xoff  
pause 1  
valve xon  
pause 1  
valve xoff  
pause 1  ' 30 sec  
valve xon  
pause 1  
valve xoff  
pause 1  
valve xon  
pause 1  
valve xoff  
pause 1  
valve xon  
pause 1  
valve xoff  
pause 1  
valve xon  
pause 1  
valve xoff  
pause 1  
valve xon  
pause 1  ' 40 sec  
valve xon  
pause 2  
valve xoff  
pause 2  
valve xon  
pause 2  
valve xoff  
pause 2  
valve xon  
pause 2  
valve xoff  
pause 2  
valve xon  
pause 2  
valve xoff  
pause 2  ' 1 min  
valve xon  
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
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pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5 ' 2 min
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min
valve xon
pause 420
valve xoff ' 10 min
unlock [gas]
' there are mL in the tube
pump 3 out=a rate=20
pump 2 in=b rate=60
pause 1
pump 1 out=a rate=20
valve 1h
' there are mL in the tube
lock [gas]
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff

pause 1.5 ' 10 sec
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 30 sec

valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 40 sec

valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2 ' 1 min

valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
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pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5 ' 2 min
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min
valve xon
pause 420
valve xoff ' 10 min
unlock [gas]

' there are mL in the tube
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=30
valve 1h
' there are mL in the tube

' ==============
' Second Wash Of Drying Reagents
' ==============
log " BEGIN: second wash of drying agents "
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 6 in=a rate=10 ' aspirate THF
pause 10
valve [ar] ' select argon
pump 2 in=a rate=10 ' aspirate argon plug
unlock 12 6
valve 4a
pump out=h rate=15
pump 2 in=b out=h ratein=60 rateout=20

run predryCycleStep1 ' rep1

valve 4a
valve 2g
valve [drying]

pump 10 in=h rate=15
pause 10
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=20

run dryingCycle1 ' Rep 1
run dryingCycle1 ' Rep 2
run dryingCycle1 ' Rep 3
run dryingCycle1 ' Rep 4
run dryingCycle1 ' Rep 5

' Rep 1
valve [drying]
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Rep 2
valve 2g
pump 10 in=a rate=5
pause 10
valve 1b
pause 1
valve [degas]
pump out=a rate=20

' Air plug
pump 2 in=b out=a ratein=60 rateout=35

log " all solution should now be in degassing tube "

' there are mL in the tube
log " BEGIN: second concentration/degassing "
valve 2a
pump 8 in=a rate=10
pump 1 in=b out=a ratein=60 rateout=20
valve lh
' there are mL in the tube

lock [gas]

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 10 sec

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec

valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1

303
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 30 sec
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 40 sec
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2 ' 1 min
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5 ' 2 min

valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min

valve xon
pause 420
valve xoff ' 10 min

valve xon
pause 600
valve xoff ' 20 min

valve xon
pause 600
valve xoff ' 30 min

unlock [gas]

' there are mL in the tube
pump 4.0 out=a rate=20
pump 2 in=b rate=60
pause 1
pump 1 out=a rate=20
valve 1h
' there are mL in the tube

lock [gas]

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 10 sec

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec

valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 30 sec
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1 ' 40 sec
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 2
valve xoff
pause 2 ' 1 min
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5 ' 2 min
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min
valve xon
pause 420
valve xoff ' 10 min
valve xon
pause 600
valve xoff ' 20 min
unlock [gas]
' there are mL in the tube
pump out=a rate=20
pump 2 in=b out=a ratein=60 rateout=30
valve 1h
' there are mL in the tube
lock [gas]

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 10 sec

valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5
valve xon
pause 0.5
valve xoff
pause 1.5 ' 20 sec

valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 1
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
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valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 5
valve xoff
pause 5 ' 2 min

valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 10
valve xoff
pause 10 ' 3 min

valve xon
pause 420
valve xoff ' 10 min

unlock [gas]

' there are mL in the tube

log " COMPLETE: Concentration/degassing "

valve 8a

lock [gas]
pump 5 in=b out=e ratein=60 rateout=20
pump 5 in=b out=e ratein=60 rateout=20
pump 5 in=b out=e ratein=60 rateout=20
pump 5 in=b out=g ratein=60 rateout=20
pump 5 in=b out=g ratein=60 rateout=20
pump 5 in=b out=g ratein=60 rateout=20

valve [rxntube]
pump 8 in=b out=a ratein=60 rateout=20
pump 8 in=b out=a ratein=60 rateout=20
pump 8 in=b out=a ratein=60 rateout=20

log "Purge reaction tube with argon"
valve 1h
valve [rxntube3]
valve xon
pause 0.5
valve xoff
pause 0.5
valve xon
pause 1
valve xoff
pause 1
valve xon
pause 2
valve xoff
pause 2
valve xon
pause 5
valve xoff
pause 5
valve xon
pause 10
valve xoff
pause 10
valve xon
pause 600
valve xoff
unlock [gas]

valve 8b

' ===============
' REACTION MODULE
' ===============
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 3 in=a rate=10 ' aspirate THF
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=10 ' aspirate argon plug to clear the line
pause 3
unlock 12 6
valve [rxntube]
pump out=a rate=10
pump 2 in=b out=a ratein=60 rateout=10
pump 2 in=b out=a ratein=60 rateout=35
valve 2h
pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 4.5 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

log "BEGIN: slow addition to reaction tube"
pump 1.5 out=[rxntop] rate=3
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]
pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]
pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]
pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5
valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

valve [degas]
pump 3 in=a rate=10
pause 15
pump 2 out=a rate=10
pause 3

pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump 0.177 out=[rxntop] rate=[additionRate1]
pump out=[rxntop] rate=[additionRate1]

pump 2.5 in=b out=g ratein=60 rateout=0.5

log " BEGIN: stirring [experiment] for [stirTime1]s "
pause [stirTime1]
log " COMPLETE: stirring [experiment] for [stirTime1]s "

StirOn ' turn on stirring to filter aid tube

sub addRxnSolution
    valve 4e
    pump out=h rate=15 ' Add the rxn solution to the ppt. chamber
    pump 5 in=b rate=45
pump out=h rate=20 ' Air plug to clear the line.
valve 4d
pump 10 in=b out=h ratein=30 rateout=50
pump 10 in=b out=h ratein=30 rateout=50
end sub

sub addHexane
lock aux [Column] ' Lock auxiliary pump and column selector
valve [PptValve1] ' select bottom of the column
' Pump first 6 mL, total = 6 mL
aux 6 in=b rate=35
pause 3
aux out=[ColumnSelect] rate=10
' Pump second 6 mL, total = 12 mL
aux 6 in=b rate=35
pause 3
aux out=[ColumnSelect] rate=10
aux 10 in=h out=[ColumnSelect] ratein=60 rateout=30
aux 10 in=h out=[ColumnSelect] ratein=60 rateout=30
unlock aux [Column] ' Unlock auxiliary pump and column selector
end sub

sub drainColumn
lock aux [Column] ' Lock auxiliary pump and column selector
valve [PptValve1] ' Select bottom of the column
' -- rep1
aux 10 in=[ColumnSelect] rate=70
pause 45
valve 0h
aux out=g rate=70
' -- rep2
aux 10 in=[ColumnSelect] rate=70
pause 45
valve 0h
aux out=g rate=70
' -- rep3
aux 10 in=[ColumnSelect] rate=70
pause 45
valve 0h
aux out=g rate=70
' -- rep4
aux 10 in=[ColumnSelect] rate=70
pause 45
valve 0h
aux out=g rate=70
aux 3 in=h out=[ColumnSelect] rate=45
pause 5
unlock aux [Column] ' Unlock auxiliary pump and column selector
end sub

sub drainColumn_addHexane
This sub merges the two subroutines so that they can be run back-to-back as a background process

run drainColumn
run addHexane
end sub

background addHexane

valve 8b
pump 10 in=[rxntop3] out=d ratein=10 rateout=40
pump 10 in=[rxntop3] out=d ratein=10 rateout=40
pump 10 in=[rxntop3] out=d ratein=10 rateout=40
valve 8a
pause 600 'wait for stirring
StirOff ' TURN OFF STIRRING for filter aid tube
pause 60 ' wait for settling

valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
pump 6 out=a rate=10 ' send back all but 3 mL
' there are mL aspirated into the syringe

wait addHexane

log "Beginning product ppt. procedure"

' Cycle #1
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
pump 6 out=a rate=10 ' send back all but 3 mL, should have all THF removed from filter aid tube now

wait draincolumn_addHexane

' Cycle #2
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve 8b
' -------- Add the THF to the rxn tube, then let stir for 2 minutes.
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 3.0 in=a rate=[fastTHF] ' aspirate THF
pause 3
valve [ar] ' select argon for plug
pump [ar] in=a rate=[fastTHF] ' aspirate argon plug to clear lines
pause 3
unlock 12 6
pump out=[rxntop] rate=10
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pause 120 ' let the mixture stir for 2 minutes
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a
valve [rxntube3]
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
wait draincolumn_addHexane

' Cycle #3
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes
valve 8b
' -------- Add the THF to the rxn tube, then let stir for 2 minutes.
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [thf] ' select THF
pump 3.0 in=a rate=[fastTHF] ' aspirate THF
pause 3
valve [ar] ' select Ar for plug
pump 2 in=a rate=[fastTHF] ' aspirate Ar plug to clear lines
pause 3
unlock 12 6
pump out=[rxntop] rate=10
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pause 120 ' let the mixture stir for 2 minutes
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a ' open the celite tube vent valve
valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #4
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve 8b ' close celite vent valve
' ------------ Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=[fastTHF] ' aspirate THF
  pause 3
  valve [ar] ' select Ar for plug
  pump 2 in=a rate=[fastTHF] ' aspirate Ar plug to clear lines
  pause 3
  unlock 12 6
  pump out=[rxntop] rate=10
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pause 120 ' let the mixture stir for 2 minutes
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pause 120 ' there are mL aspirated into the syringe
  wait draincolumn_addHexane

' Cycle #5
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve 8b ' close celite vent valve
' ------------ Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=[fastTHF] ' aspirate THF
  pause 3
  valve [ar] ' select Ar for plug
  pump 2 in=a rate=[fastTHF] ' aspirate Ar plug to clear lines
  pause 3
  unlock 12 6
  pump out=[rxntop] rate=10
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pause 120 ' let the mixture stir for 2 minutes
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  valve 8a ' open the celite tube vent valve
  valve [rxntube3] ' select rxn tube
  pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
  pause 120
  wait draincolumn_addHexane
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #6
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve 8b ' close celite vent valve
' --------- Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=\texttt{[fastTHF]} ' aspirate THF
  pause 3
  valve [ar] ' select Ar for plug
  pump 2 in=a rate=\texttt{[fastTHF]} ' aspirate Ar plug to clear lines
  pause 3
unlock 12 6
pump out=[rxntop] rate=10
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pause 120 ' let the mixture stir for 2 minutes
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a ' open the celite tube vent valve
  valve [rxntube3] ' select rxn tube
  pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
  pause 120
  ' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #7
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes

valve 8b ' close celite vent valve
' --------- Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=\texttt{[fastTHF]} ' aspirate THF
  pause 3
  valve [ar] ' select Ar for plug
  pump 2 in=a rate=\texttt{[fastTHF]} ' aspirate Ar plug to clear lines
  pause 3
unlock 12 6
pump out=[rxntop] rate=10
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pause 120 ' let the mixture stir for 2 minutes
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a ' open the celite tube vent valve
valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #8
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes
valve 8b ' close celite vent valve
' -------- Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=[fastTHF] ' aspirate THF
  pause 3
  valve [ar] ' select Ar for plug
  pump 2 in=a rate=[fastTHF] ' aspirate Ar plug to clear lines
  pause 3
  unlock 12 6
  pump out=[rxntop] rate=10
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pump 2 in=b out=[rxntop] ratein=45 rateout=30
  pause 120 ' let the mixture stir for 2 minutes
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
  pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a ' open the celite tube vent valve
valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #9
run addRxnSolution ' Now add the rxn solution
background draincolumn_addHexane ' Elute the column and send it to waste, then fill it back up with hexanes
valve 8b ' close celite vent valve
' -------- Add the THF to the rxn tube, then let stir for 2 minutes.
  lock 12 6 ' lock common reagents
  valve [me] ' select syringe pump
  valve 2h ' select common reagents
  valve [thf] ' select THF
  pump 3.0 in=a rate=[fastTHF] ' aspirate THF
pause 3
valve [ar] ' select Ar for plug
pump 2 in=a rate=[fastTHF] ' aspirate Ar plug to clear lines
pause 3
unlock 12 6
pump out=[rxntop] rate=10
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pump 2 in=b out=[rxntop] ratein=45 rateout=30
pause 120 ' let the mixture stir for 2 minutes
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
pump 10 in=[rxntop3] out=d ratein=20 rateout=40
valve 8a ' open the celite tube vent valve
valve [rxntube3] ' select rxn tube
pump 9 in=a rate=1 ' Overfill the syringe w/ rxn solution.
pause 120
' there are mL aspirated into the syringe
wait draincolumn_addHexane

' Cycle #10
run addRxnSolution ' Now add the rxn solution
run draincolumn
run draincolumn

define addnrate=20

sub addEtherMeOH
' Add 12 mL of Ether w/ 1.5% v/v MeOH to the ppt. chamber
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
' ---------- Rep 1
valve [meoh] ' select Et2O w/ MeOH
pump 6.5 in=a rate=[fastEther]
pause 5
valve [ar] ' select Ar for plug
pump 2.5 in=a rate=[fastEther]
pause 3
valve 4e
pump out=h rate=15 ' Add the solution to the ppt. chamber
' ---------- Rep 2
valve [meoh]
pump 6.5 in=a rate=[fastEther]
pause 5
valve [ar] ' select Ar for plug
pump 2.5 in=a rate=[fastEther]
pause 3
pump out=h rate=15 ' Add the solution to the ppt. chamber
pump 5 in=b rate=30
pump out=h rate=20 ' Air plug to clear the line.
unlock 12 6

valve 4d
pump 10 in=b out=h ratein=30 rateout=50
pump 10 in=b out=h ratein=30 rateout=50
end sub

' =============================
' Prime The Ether/Methanol Line
' =============================
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [meth] ' select ether
pump 5 in=a rate=5 ' aspirate ether
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastEther] ' aspirate argon plug to clear lines
pause 3

pump out=d rate=20 ' inject ether to waste flask
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [meth] ' select ether
pump 5 in=a rate=5 ' aspirate ether
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastEther] ' aspirate argon plug to clear lines
pause 3

pump out=d rate=20 ' inject ether to waste flask
valve [me] ' select syringe pump
valve 2h ' select common reagents
valve [meth] ' select ether
pump 5 in=a rate=5 ' aspirate ether
pause 10
valve [ar] ' select argon for plug
pump 2 in=a rate=[fastEther] ' aspirate argon plug to clear lines
pause 3

pump out=d rate=20 ' inject ether to waste flask
pump 2 in=b out=d ratein=60 rateout=20

' Rep #1 (12 mL total washing)
run addEtherMeOH
run drainColumn

' Rep #2 (24 mL total washing)
run addEtherMeOH
run drainColumn

' Rep #3 (36 mL total washing)
run addEtherMeOH
run drainColumn

run drainColumn

sub addEther
lock 12 6 ' lock common reagents
valve [me] ' select syringe pump
valve 2h ' select common reagents
' ------------ Rep 1
valve [ether] ' select Et2O
pump 6.5 in=a rate=[fastEther]
pause 5
valve [ar] ' select Ar for plug
pump 2.5 in=a rate=[fastEther]
pause 3
valve 4e
pump out=h rate=15 ' Add the solution to the ppt. chamber
' ------------ Rep 2
valve [ether] ' select Et2O
pump 6.5 in=a rate=[fastEther]
pause 5
valve [ar] ' select Ar for plug
pump 2.5 in=a rate=[fastEther]
pause 3
pump out=h rate=15 ' Add the solution to the ppt. chamber
pump 5 in=b rate=30
pump out=h rate=20 ' Air plug to clear the line.
unlock 12 6
valve 4d
pump 10 in=b out=h ratein=30 rateout=50
pump 10 in=b out=h ratein=30 rateout=50
end sub

' Rep #1 (12 mL total washing)
run addEther
run drainColumn

' Rep #2 (24 mL total washing)
run addEther
run drainColumn

' Rep #3 (36 mL total washing)
run addEther
run drainColumn
run drainColumn
run drainColumn
run drainColumn

' PRODUCT ELUTION
' Define sub routines:
sub mixPPtx5
  valve 4d
  pause 50
  pump 5 in=b out=h rate=45 ' bubble air through the ppt. chamber to help mix the mixture
' ---
  pause 50
  pump 5 in=b out=h rate=45
' ---
pause 50
pump 5 in=b out=h rate=45
' ---
pause 50
pump 5 in=b out=h rate=45
' ---
pause 50
pump 5 in=b out=h rate=45
end sub

sub transfer
' Send the product solution to 2nd deprotection tube
valve 4d
pump 10 in=h rate=10
pause 45
' Relieve the vacuum via the nitrogen port.

valve 1b
pause 5

' Send the MIDA boronate solution out to 2nd deprotection tube
valve 2g
valve 3b
pump out=a rate=20
pump 3 in=b out=a ratein=30 rateout=20 ' Air plugs to clear the line
pump 3 in=b out=a ratein=30 rateout=20
end sub

lock 12 6 ' Lock common reagents to prevent two processes from using THF at the same time
lock aux [Column] ' Lock auxiliary pump equipment
valve [pptValve1]
'Wash the syringe with THF (2 x 1 mL) to make sure there is no residual MeOH from the ether washes.
' v---- Rep #1
aux 1 in=d rate=10 ' Aspirate 1 mL THF
pause 3
aux 9 in=h rate=20
aux out=g rate=70
' v---- Rep #2
aux 1 in=d rate=10 ' Aspirate 1 mL THF
pause 3
aux 9 in=h rate=20
aux out=g rate=70

'--
aux 6.5 in=d rate=10 ' Aspirate 6 mL THF
pause 10
valve [pptValve1] ' Select bottom of the column
aux out=[ColumnSelect] rate=2 ' Pass the THF slowly through the column to maximize its residence time
'--
aux 6.5 in=d rate=10 ' Aspirate 6 mL THF
pause 10
valve [pptValve1] ' Select bottom of the column
aux out=[ColumnSelect] rate=2 ' Pass the THF slowly through the column to
maximize its residence time

aux 1 in=h out=[ColumnSelect] ratein=45 rateout=2
unlock 12 6 aux [Column]

' Run through 40 reps of air agitation of the ppt. chamber
run mixPtx5 '5
run mixPtx5 '10
run mixPtx5 '15
run mixPtx5 '20
run mixPtx5 '25
run mixPtx5 '30
run mixPtx5 '35
run mixPtx5 '40

' Send the MIDA boronate solution to 2\textsuperscript{nd} deprotection tube
run transfer ' Rep #1
run transfer ' Rep #2
run transfer ' Rep #3
run transfer ' Rep #4
run transfer ' Rep #5
run transfer ' Rep #6

lock aux [Column]
valve [pptValve1]
aux 3 in=h out=[ColumnSelect] ratein=45 rateout=3
aux 5 in=h out=[ColumnSelect] ratein=45 rateout=25
aux 5 in=h out=[ColumnSelect] rate=45
unlock aux [Column]

run mixPtx5

' Transfer the wash solution
run transfer

' Purification procedure complete.
log " COMPLETE: first purification of [experiment] "