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From Synthesis to Function via Iterative Assembly of *N*-Methyliminodiacetic Acid Boronate Building Blocks

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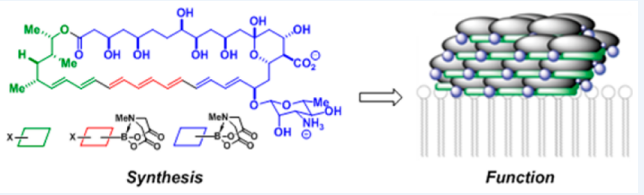
CONSPECTUS: The study and optimization of small molecule function is often impeded by the time-intensive and specialist-dependent process that is typically used to make such compounds. In contrast, general and automated platforms have been developed for making peptides, oligonucleotides, and increasingly oligosaccharides, where synthesis is simplified to iterative applications of the same reactions. Inspired by the way natural products are biosynthesized via the iterative assembly of a defined set of building blocks, we developed a platform for small molecule synthesis involving the iterative coupling of haloboronic acids protected as the corresponding *N*-methyliminodiacetic acid (MIDA) boronates. Here we summarize our efforts thus far to develop this platform into a generalized and automated approach for small molecule synthesis. We and others have employed this approach to access many polyene-based compounds, including the polyene motifs found in >75% of all polyene natural products. This platform further allowed us to derivatize amphotericin B, the powerful and resistance-evasive but also highly toxic last line of defense in treating systemic fungal infections, and thereby understand its mechanism of action. This synthesis-enabled mechanistic understanding has led us to develop less toxic derivatives currently under evaluation as improved antifungal agents.



To access more C_{sp^3}-containing small molecules, we gained a stereocontrolled entry into chiral, non-racemic α-boryl aldehydes through the discovery of a chiral derivative of MIDA. These α-boryl aldehydes are versatile intermediates for the synthesis of many C_{sp^3} boronate building blocks that are otherwise difficult to access. In addition, we demonstrated the utility of these types of building blocks in accessing pharmaceutically relevant targets via an iterative C_{sp^3} cross-coupling cycle. We have further expanded the scope of the platform to include stereochemically complex macrocyclic and polycyclic molecules using a linear-to-cyclized strategy, in which C_{sp^3} boronate building blocks are iteratively assembled into linear precursors that are then cyclized into the cyclic frameworks found in many natural products and natural product-like structures.

Enabled by the serendipitous discovery of a catch-and-release protocol for generally purifying MIDA boronate intermediates, the platform has been automated. The synthesis of 14 distinct classes of small molecules, including pharmaceuticals, materials components, and polycyclic natural products, has been achieved using this new synthesis machine. It is anticipated that the scope of small molecules accessible by this platform will continue to expand via further developments in building block synthesis, C_{sp^3} cross-coupling methodologies, and cyclization strategies. Achieving these goals will enable the more generalized synthesis of small molecules and thereby help shift the rate-limiting step in small molecule science from synthesis to function.

1. INTRODUCTION

Small molecules can perform many important functions, yet the time-intensive and specialist-dependent process typically required for synthesis of such compounds too often limits access to their functional potential. In contrast, automated synthetic platforms now exist for peptides,[1] oligonucleotides,[2] and increasingly oligosaccharides.[3] The resulting expanded access to these molecules has dramatically enabled their discovery and utilization. In each of these cases, automation was achieved by standardizing the synthesis process in the form of a general building block-based strategy. An analogous general and automated platform for making small molecules could similarly shift the bottleneck in small molecule science from synthesis to function and deliver the substantial power of making small molecules to nonspecialists.

Importantly, like peptides, oligonucleotides, and oligosaccharides, most natural products are biosynthesized via the iterative assembly of a small set of building blocks, such as malonyl coenzyme A, isopentenyl pyrophosphate, and pyruvic acid.[4] Additionally, many materials and pharmaceuticals are similarly comprised of collections of aryl and heteroaryl components.[5] This suggests that small molecules also possess an inherent modularity, which would enable their systematic building block-based construction.

With the goal of harnessing this modularity, we developed a small molecule synthesis platform based on the iterative assembly of bifunctional haloboronic acid building blocks.[6]

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Analogous to the synthesis of peptides from amino acids, this approach involves preinstalling all of the required functional groups, oxidation states, and stereochemistry (i.e., olefin geometry and stereogenic centers, where applicable) into the building blocks followed by their assembly via iterative cycles of stereospecific metal-mediated couplings. The precise assembly of bifunctional haloboronic acids through iterative cross-coupling (ICC) is enabled by attenuating the reactivity of boronic acids with the *N*-methyliminodiacetic acid (MIDA) ligand (Figure 1).[7]

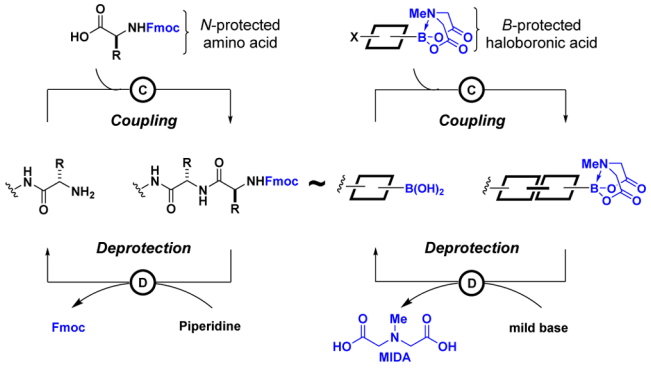


Figure 1. Iterative coupling platform for small molecule synthesis from MIDA boronate building blocks is analogous to peptide synthesis from protected amino acids. D = deprotection, C = coupling.

While this Account focuses on the MIDA boronate platform, other approaches to ICC have also been developed,[8] including a boron-protecting strategy using the 1,8-diaminonaphthalene ligand by Suginome and co-workers.[8c]

Removal of the p-orbital on boron by complexation with MIDA also significantly improves the benchtop stability of boron-containing building blocks. MIDA boronates are generally monomeric, crystalline, air- and temperature-stable, and easily purified through chromatography or recrystallization. The MIDA ligand itself is commercial or can be prepared from commodity chemicals (iminodiacetic acid, formaldehyde, and formic acid).[9] This has enabled the commercialization of many MIDA boronate building blocks. In addition, MIDA boronates are stable toward a variety of reaction conditions, allowing the synthesis of more complex building blocks from simple MIDA boronates.[7] Facile removal of the MIDA ligand under aqueous basic conditions can reveal the free boronic acid during a cross-coupling reaction. When the rate of this release is slower than the cross-coupling reaction, substantial improvements in cross-coupling yield can be achieved. This type of "slow-release cross-coupling" avoids the need for manipulation of sensitive boronic acids.[10,11] These properties collectively make MIDA boronates highly useful building blocks for small molecule synthesis.

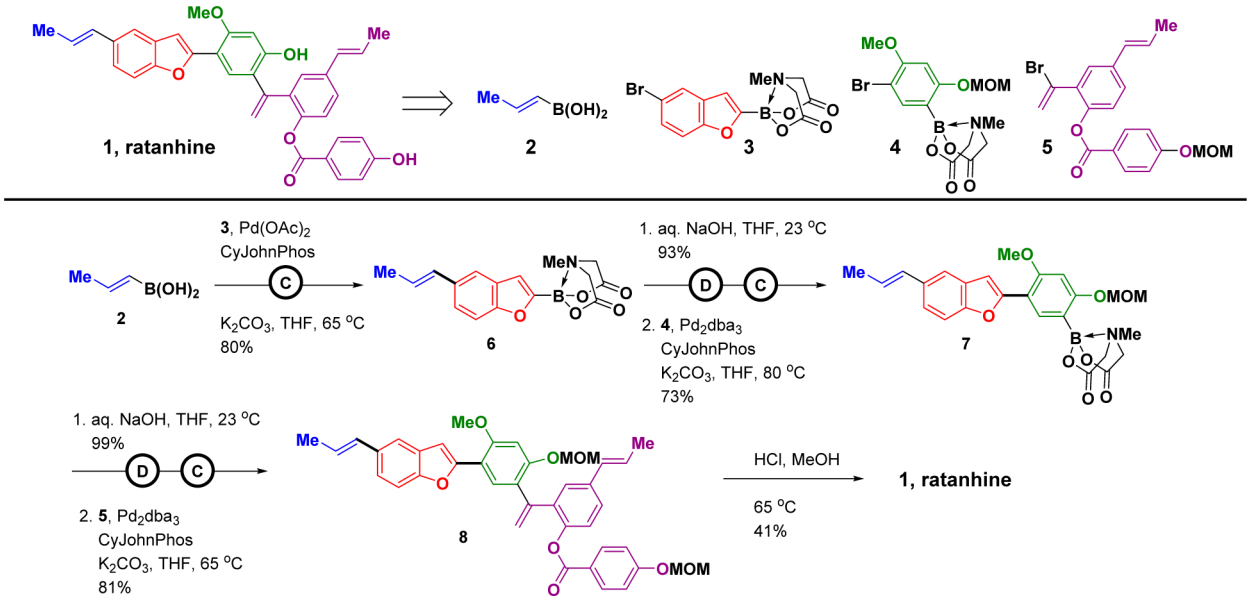
2. ACCESSING LINEAR, C_{sp^2}-RICH SMALL MOLECULES VIA ITERATIVE COUPLING

This platform initially proved capable of accessing a range of linear, C_{sp^2}-rich small molecules, including many polyene natural products. For example, the synthesis of ratanhine (**1**), a polyaryl norneolignan natural product, was achieved via the iterative assembly of building blocks **2**−**5** via recursive cycles of deprotection and coupling (Scheme 1).

The potential to access increased complexity was revealed with a synthesis of (−)-peridinin (Scheme 2).[6c] All of the required functional groups, oxidation states, and stereochemistry present in this complex atypical carotenoid were preinstalled into building blocks **9**−**12**. With use of only stereospecific cross-couplings, these features were faithfully translated into **13** via three iterations of a deprotect-couple sequence. Global deprotection completed the first fully stereocontrolled total synthesis of this complex target.[12]

Encouraged by these results, we questioned whether most polyene motifs found in nature could be synthesized using the same approach. A general retrosynthetic algorithm for systematically deconstructing all known polyene natural product motifs into a minimum number of MIDA boronate building blocks was devised.[13] This analysis predicted that the polyene motifs found in >75% of all polyene natural products can be constructed with

Scheme 1. Total Synthesis of Ratanhine via an Iterative Coupling Approach[a]



[a]Abbreviations: dba = dibenzylideneacetone; CyJohnPhos =2-(dicyclohexylphosphino)biphenyl.

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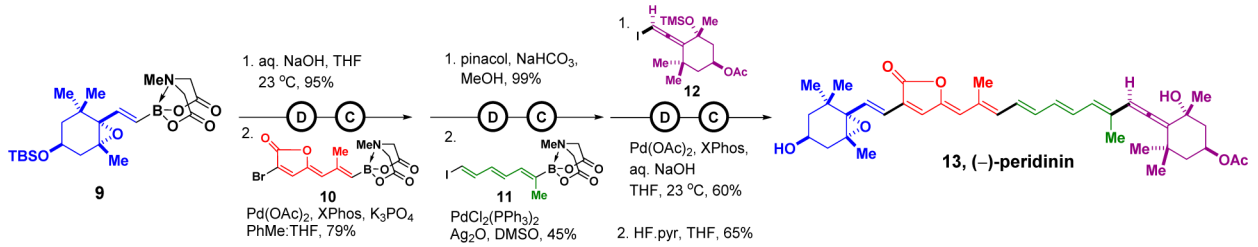
Scheme 2. Completely Stereocontrolled Synthesis of (−)-Peridinin via ICC[a]



[a]XPhos =2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl.

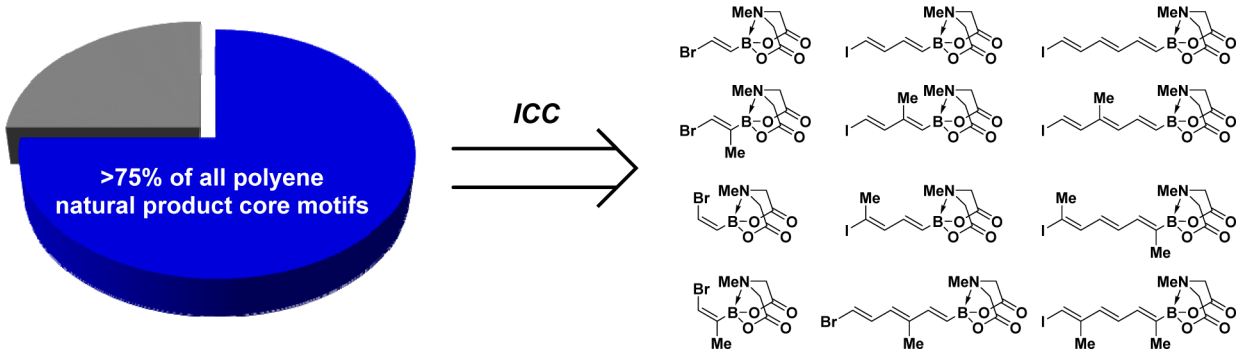


Figure 2. Synthesis of the polyene motifs found in >75% of polyene natural products from 12 MIDA boronate building blocks.

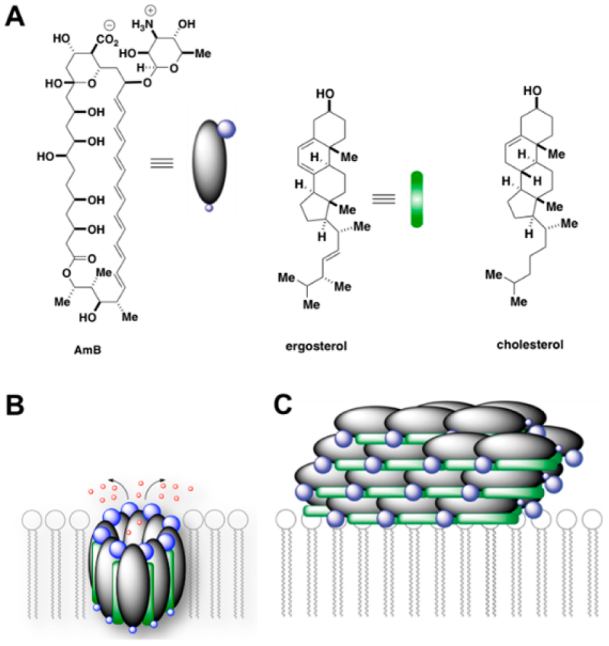


Figure 3. (A) Structures of amphotericin B, ergosterol, and cholesterol. (B) In the presence of ergosterol or cholesterol, AmB spontaneously forms ion channels in lipid membranes. (C) AmB kills yeast by simply sequestering ergosterol.

only 12 MIDA boronate building blocks. This hypothesis was systematically tested and confirmed using standardized Suzuki–Miyaura coupling conditions (Figure 2).

This platform proved capable of enabling a better understanding of small molecule function. Amphotericin B (AmB) is a large and complex polyene macrolide natural product known to form ion channels in yeast and human cells (Figure 3A,B).[14] This feature made it a very attractive starting point for the discovery of small molecules that might replicate the functions of missing protein ion channels, which underlie currently incurable human diseases. AmB has also served as the gold standard in treating life-threatening systemic fungal infections for more than half a century.[15] Remarkably, despite its long-term clinical utilization, AmB has evaded the emergence of pathogen resistance. However, AmB is also highly toxic to human cells, which limits its tolerated dosages. As a result, the mortality rate for invasive fungal infections remains nearly 50%, leading to more than 1.5 million deaths worldwide each year, which is more than malaria or tuberculosis.[16]

It has been widely accepted that the ion channel-forming and cell-killing activities of AmB were inextricably linked. Specifically, it was thought that AmB kills both yeast and human cells via membrane permeabilization. This mechanism discouraged the use of AmB-derived channels to replace missing proteins and encouraged many prior efforts to improve the therapeutic index of AmB to focus on the challenging problem of selectively self-assembling structurally enigmatic ion channels in yeast over human cells.

It had long been suggested but not definitively demonstrated that AmB directly binds both ergosterol and cholesterol (Figure 3A) in the membranes of yeast and human cells, respectively.[17] It was proposed that this small molecule–small molecule interaction was critical for channel self-assembly, which in turn was critical for cell killing. Using AmB derivatives produced through semisynthesis, our early studies suggested that either the putative structural underpinnings of the AmB ion channel were incorrect or ion channel formation was not required for antifungal activity.[18] Interestingly, ergosterol and cholesterol play many critical roles in yeast and human physiology, respectively.[19] We thus proposed that AmB might primarily kill cells not by forming ion channels but by simply binding sterols (Figure 3C). This alternative mechanism would suggest that the channel forming and cell-killing effects of AmB might be separated and that the more rationally approachable selective binding of ergosterol vs

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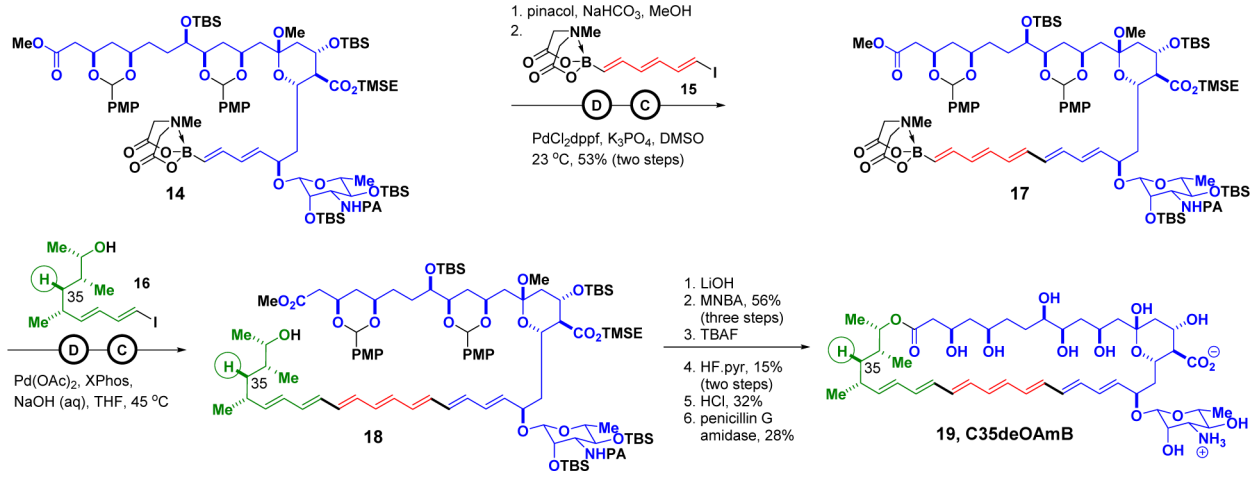
Scheme 3. Synthesis of C35deOAmB through ICC[a]



[a]Abbreviations: MNBA = 2-methyl-6-nitrobenzoic acid; dppf = 1,1′-bis(diphenylphosphino)ferrocene.

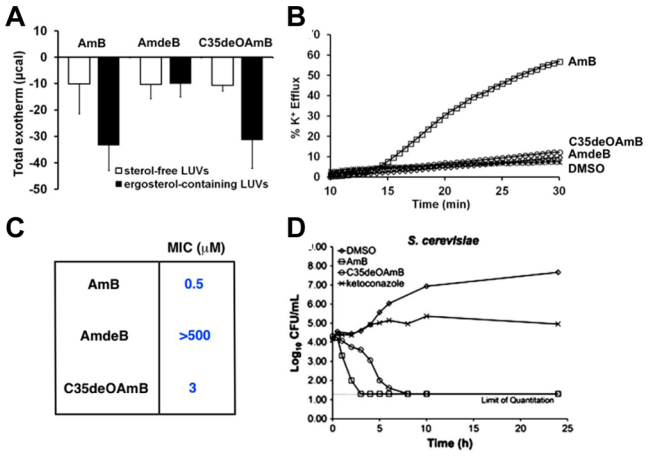


Figure 4. (A) C35deOAmB retains the capacity to bind ergosterol. (B) C35deOAmB is unable to permeabilize yeast while AmB can. (C) C35deOAmB retains potent antifungal activity. (D) AmB and C35deOAmB exhibit potent fungicidal activity. AmdeB = amphoteronolide B.

cholesterol would lead to an improved therapeutic index. Thus, we sought to definitively test this key hypothesis.

A series of modeling studies suggested that the deletion of a single atom from AmB might enable a key experiment. Specifically, it was predicted that the hydroxyl group at C35 was critical for forming ion channels.[20] This suggested that C35-deoxy AmB (C35deOAmB) might bind ergosterol but not permeabilize yeast membranes. The classic membrane permeabilization model would predict the loss of antifungal activity for such a derivative. In contrast, if sterol binding was actually the key cause of cell killing, such a compound would maintain potent antifungal effects.

The synthesis of C35deOAmB required to rigorously test our hypothesis was enabled by the MIDA boronate platform.[21] Specifically, building blocks **14**−**16** were sequentially linked via iterative cycles of MIDA boronate deprotection and coupling to yield linear target **18** (Scheme 3). Subsequent macrocyclization and global deprotection yielded multiple milligrams of C35deOAmB (**19**).

This probe showed retained binding to ergosterol (Figure 4A) but no membrane permeabilizing activity in yeast (Figure 4B). Like AmB, this derivative maintained potent (Figure 4C) and fungicidal (Figure 4D) activity. These data strongly support the conclusion that AmB primarily kills yeast by simply binding ergosterol. Channel formation plays a secondary role that somewhat increases its potency and rate of yeast killing. Extensive SSNMR, transmission electron microscopy, and cell-based experiments further revealed that AmB acts primarily as a large extramembranous sterol sponge that physically extracts ergosterol from the membranes of yeast and thereby exerts its fungicidal effects (Figure 3C).[22] The Carreira group synthesized and studied the methyl ester of C35deOAmB and offered a different interpretation of their results.[23]

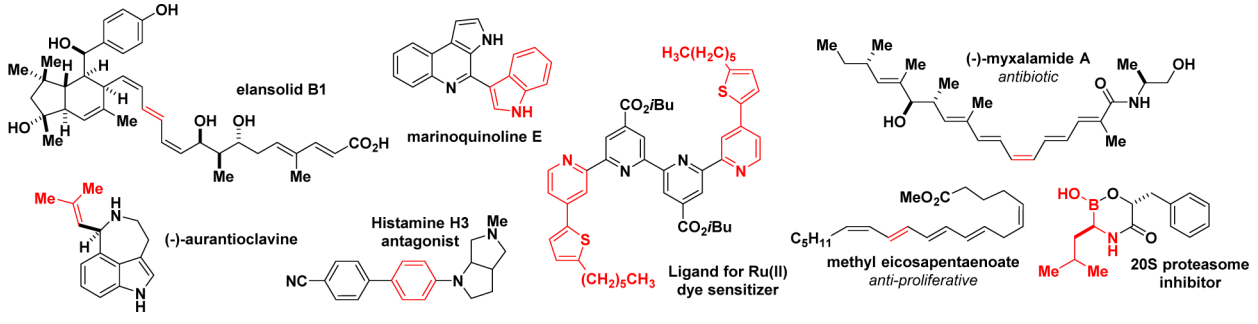


Figure 5. Selected examples of natural products or derivatives synthesized by other groups using MIDA boronates. Portions highlighted in red were derived from MIDA boronate building blocks.

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This synthesis-enabled breakthrough in mechanistic understanding illuminated a simpler and more actionable roadmap for pursuing less toxic amphotericin derivatives focused on the selective binding of ergosterol over cholesterol.[24] Our pursuit of this objective has recently yielded promising new derivatives of AmB that bind ergosterol but, up to the limits of detection, do not bind cholesterol and demonstrate a dramatic improvement in therapeutic index *in vitro* and *in vivo*.[24] Remarkably, we have also found that these new derivatives are no more vulnerable to resistance than AmB, which has managed to evade the emergence of new resistance for over half a century.[24b] These compounds have been licensed to a new biotechnology company committed to developing optimal drug candidates and pursuing clinical studies.

Many MIDA boronates are now commercially available, and this platform has been increasingly employed by other research groups for the syntheses of different types of targets, including natural products, pharmaceuticals, and ligands (Figure 5).[25−29]

3. STEREOSELECTIVE SYNTHESIS AND ITERATIVE COUPLING OF C_{sp^3} BORONATE BUILDING BLOCKS

To more broadly access the functional potential that small molecules possess, we aimed to expand the scope of the iterative coupling platform to include C_{sp^3}-rich and polycyclic small molecules requiring C_{sp^3} couplings for building block assembly.

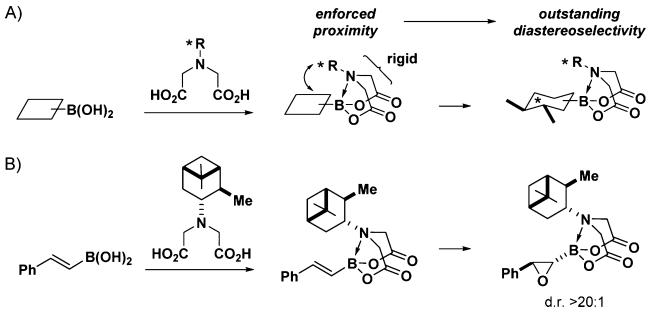

Figure 6. (A) Leveraging the enforced proximity between the chiral R* group and the organic group bound to boron to achieve highly diastereoselective reactions. (B) Diastereoselective epoxidation enabled by a chiral derivative of MIDA.

There have been many recent advances in the stereoselective synthesis of chiral, non-racemic C_{sp^3} pinacol boronic esters.[30] However, the instability of pinacol boronic esters[31] to reactions and/or purifications can make it challenging to convert them into other useful, more complex types of boronic esters. We thus pursued a strategy for the efficient stereoselective synthesis of shelf-stable C_{sp^3} boronates that have the potential to be elaborated into more complex building blocks.

Our previous studies of MIDA boronates have revealed that the *N*-methyl substituent is always closely positioned to the organic group appended to boron and that the iminodiacetic acid framework is conformationally rigid in solution. Collectively, these observations generated the hypothesis that if we changed the *N*-methyl group to a chiral *N*-alkyl group, the enforced proximity between the chiral group and the organic group bound to boron would lead to effective transfer of stereochemical information during functionalizations of the corresponding boronates (Figure 6). In view of the versatility of epoxides in the preparation of other chiral building blocks, we first questioned whether the epoxidation of alkenylboronates can be rendered asymmetric via such modifications of the MIDA ligand.

In this vein, we found that pinene-derived iminodiacetic acid ligand (PIDA) gave >20:1 dr in the epoxidation reaction under standard mCPBA conditions.[32] A variety of alkenyl PIDA boronates with different substitution patterns worked well under the same conditions (Figure 7). Somewhat diminished but still synthetically useful diastereoselectivies were observed with 1,1-disubstituted alkenes (**21i** and **21j**), which are often challenging substrates for asymmetric functionalization.[33,34] These oxiranyl PIDA boronates shared many of the characteristics of MIDA boronates: they are shelf-stable, crystalline solids that can be purified by silica gel chromatography, making them highly desirable chiral building blocks for complex molecule synthesis.

We further found that the epoxide **21a** can undergo a Meinwald rearrangement into an α-boryl aldehyde with complete maintenance of stereochemical purity. The stereochemical out-come of the rearrangement reaction revealed that the rearrangement proceeded with migration of the boronate group during the rearrangement of **21a**. Contemporaneous independent studies by the Yudin group led to codiscovery of the same

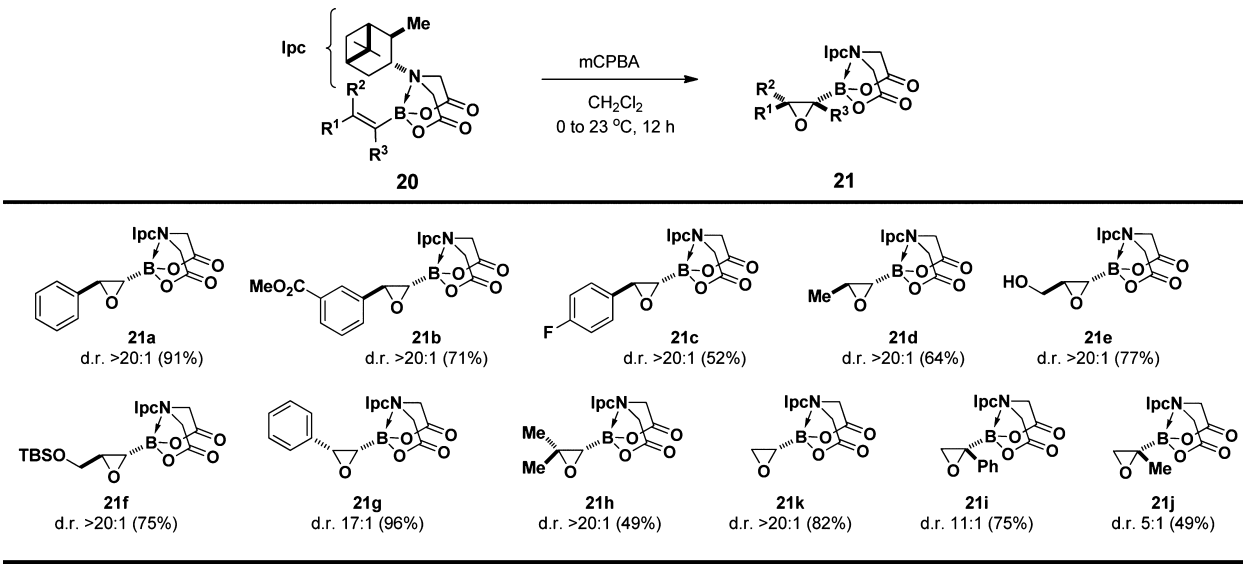

Figure 7. Highly diastereoselective epoxidations of alkenyl PIDA boronates. Numbers in parentheses indicate isolated yields. Ipc = isopinocampheyl.

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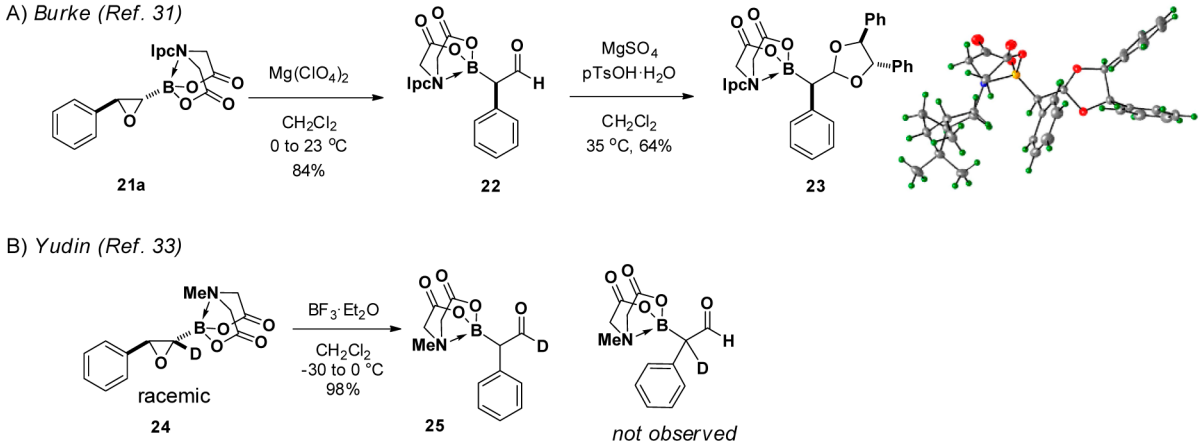
Scheme 4. Determining the Migrating Group by (A) Probing the Stereochemical Outcome of the Rearrangement and (B) Deuterium Labeling



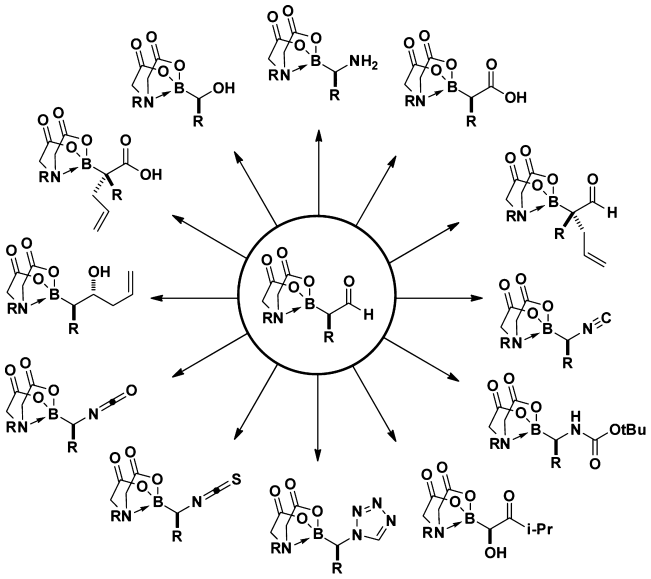
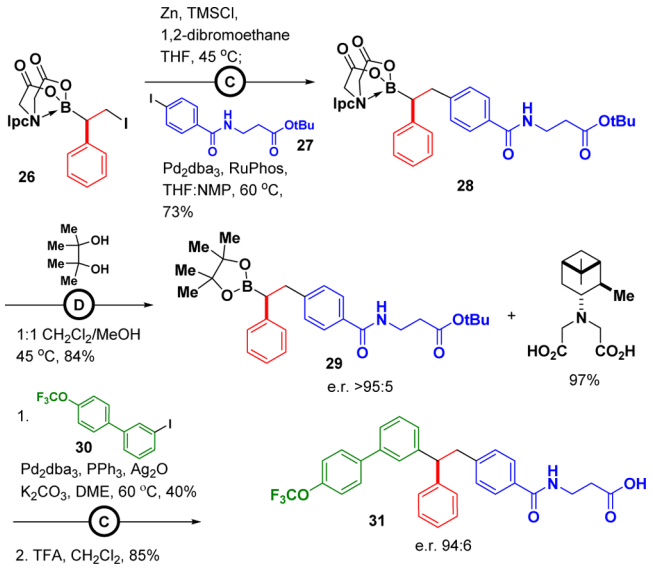


Figure 8. Examples of new types of C_{sp^3} boronates that can be derived from α-boryl aldehydes.

Scheme 5. Iterative C_{sp^3}–C_{sp^2} Cross-Coupling for the Modular Synthesis of 31



rearrangement, and using a deuterium-labeled epoxy MIDA boronate, this group arrived at the same mechanistic conclusion (Scheme 4).[35] Gevorgyan and co-workers recently utilized this 1,2 boryl migration in their synthesis of borylated furans.[36]

The Yudin group also demonstrated that the α-boryl aldehyde can be transformed into a variety of C_{sp^3} boronates, many of which are difficult to access by other methods (Figure 8).[37] The group also demonstrated that these building blocks can be elaborated into boropeptides, which showed nanomolar inhibition of CT-L members of the 20S proteasome.[37b]

The stability of the α-boryl aldehyde **22** allowed us to access a new type of bifunctional building block, **26**, in which both the halide and the boron termini are attached to C_{sp^3} carbons. Preinstalling the required stereochemistry into building block **26** enabled the synthesis of a glucagon receptor inhibitor containing a chiral diarylmethine motif via a concise iterative C_{sp^3}–C_{sp^2} sequence. Negishi coupling with aryl iodide **27** followed by transesterification generates the benzylic pinacol boronic ester, which was coupled with aryl iodide **30** using Crudden's C_{sp^3} coupling method,[38] giving the *t*-butyl ester of **31** with good maintenance of stereochemical purity. Deprotection with TFA then completes the modular and stereocontrolled synthesis of **31** (Scheme 5).

4. LINEAR-TO-CYCLIZED STRATEGY FOR THE SYNTHESIS OF POLYCYCLIC SMALL MOLECULES

Topologically complex polycyclic natural products represent especially challenging targets for the same building block-based approach. However, like linear, C_{sp^2}-rich natural products, many of these natural products are synthesized in Nature via the iterative assembly of a small set of natural building blocks. The resulting linear precursors then undergo different cyclization reactions to yield the (poly)cyclized frameworks. We thus utilized an analogous linear-to-cyclized strategy to access many polycyclic small molecules via iterative MIDA boronate building block assembly. In this approach, building blocks are assembled into linear precursors by iterative cycles of deprotection and coupling, and the linear precursors are then cyclized into the polycyclic framework present in the natural products. The stereochemical information in the building blocks is first translated into linear precursors via stereospecific couplings and then into the targeted products via stereospecific and/or stereoselective

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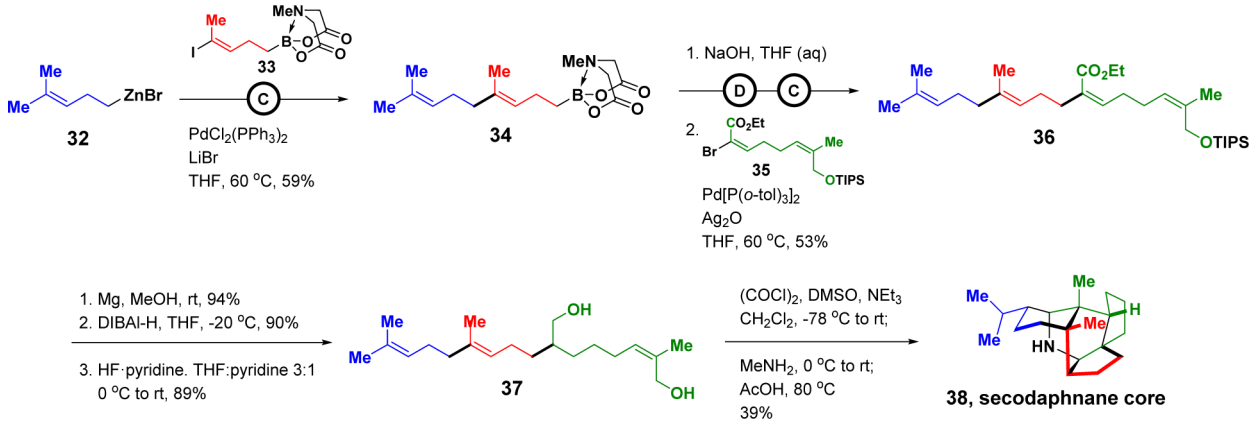
Acc. Chem. Res. 2015, 48, 2297–2307

Scheme 6. Linear-to-Cyclized Approach for the Synthesis of the Secodaphnane Core 38



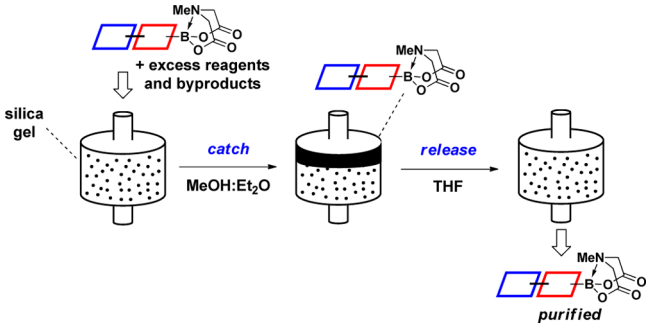


Figure 9. A catch-and-release purification protocol for MIDA boronate intermediates.

(poly)cyclization reactions. To enable such cyclizations, such linear precursors must be suitably flexible and therefore contain multiple C_{sp^3} atoms, which in turn demands challenging C_{sp^3} couplings in their synthesis.[39,40]

We demonstrated the viability of the linear-to-cyclized approach with the synthesis of the pentacyclic secodaphnane core, **38**, of the daphniphyllum alkaloids.[41] A C_{sp^3}–C_{sp^2} couple–deprotect–couple sequence with building blocks **32**, **33**, and **35** generates the linear precursor **36**. Precursor **36** is then elaborated into the diol **37**, which was transformed into the targeted secodaphnane core following the one-pot biomimietic oxidation and iminium ion-triggered cascade cyclization developed by Heathcock and co-workers (Scheme 6).[42]

5. AUTOMATION OF THE ITERATIVE COUPLING PLATFORM

Because small molecule syntheses typically employ strategies and purification methods that are highly customized for each target, efforts in automation have thus far focused on expanding the types of reactions and purification processes that can be implemented on a synthesizer. While the use of advanced engineering and robotics can assist with such an approach,[43] this inherent lack of generality has limited applications and widespread adoption.[44−46]

The key challenge in generally automating a synthesis platform is finding a generalized purification method that can be used for all intermediates. Recognizing that each iteration of building block assembly generates a MIDA boronate as the key intermediate, we sought ways to utilize the MIDA boronate motif as a common handle for purification. We serendipitously discovered that MIDA boronates uniformly possess highly unusual binary affinity for silica gel with certain pairs of eluents. This binary elution profile enabled us to develop a new type of catch-and-release purification protocol for MIDA boronates (Figure 9). A crude reaction mixture is transferred to a short silica gel plug, allowing the MIDA boronate to be caught on silica gel while excess reagents and byproducts are removed from the column by washing with MeOH/Et_2O. The MIDA boronate is then eluted by a solvent switch to THF.

Taking advantage of this generalizable catch-and-release purification protocol, we designed and built a synthesizer,[41] which comprises three modules that sequentially execute the deprotection, coupling, and purification steps required for each synthesis cycle (Figure 10). Each automated synthesis simply requires placing prepacked cartridges onto the synthesizer and pressing "start".

The synthesizer proved to be capable of making a range of natural products, pharmaceuticals, and material precursors via automated carbon–carbon and carbon–heteroatom bond formations, delivering milligram quantities of the desired products, which are sufficient for most functional discovery assays (Figure 11). In addition, the synthesizer was able to

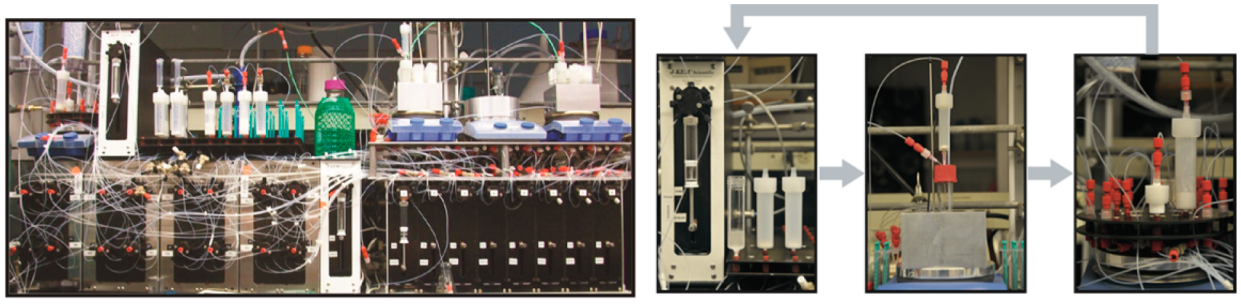


Figure 10. Photograph of the small molecule synthesizer and the three modules for deprotection, coupling, and purification.

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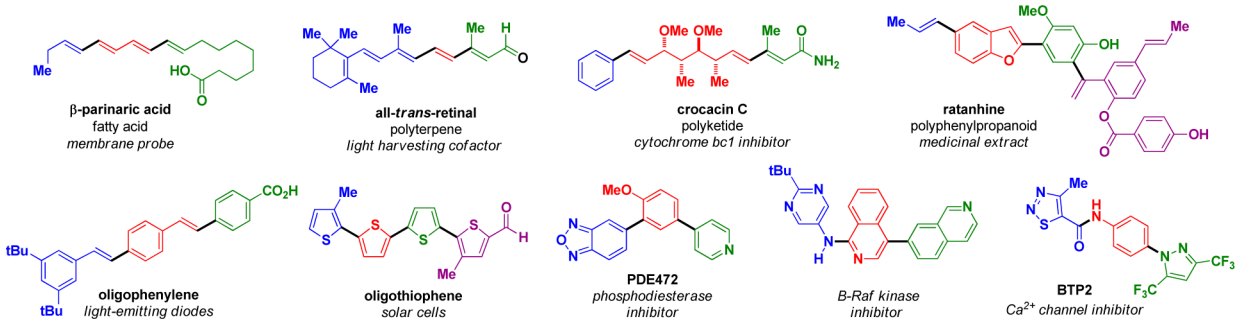

Figure 11. Natural products, pharmaceuticals, and material precursors made on the synthesizer.

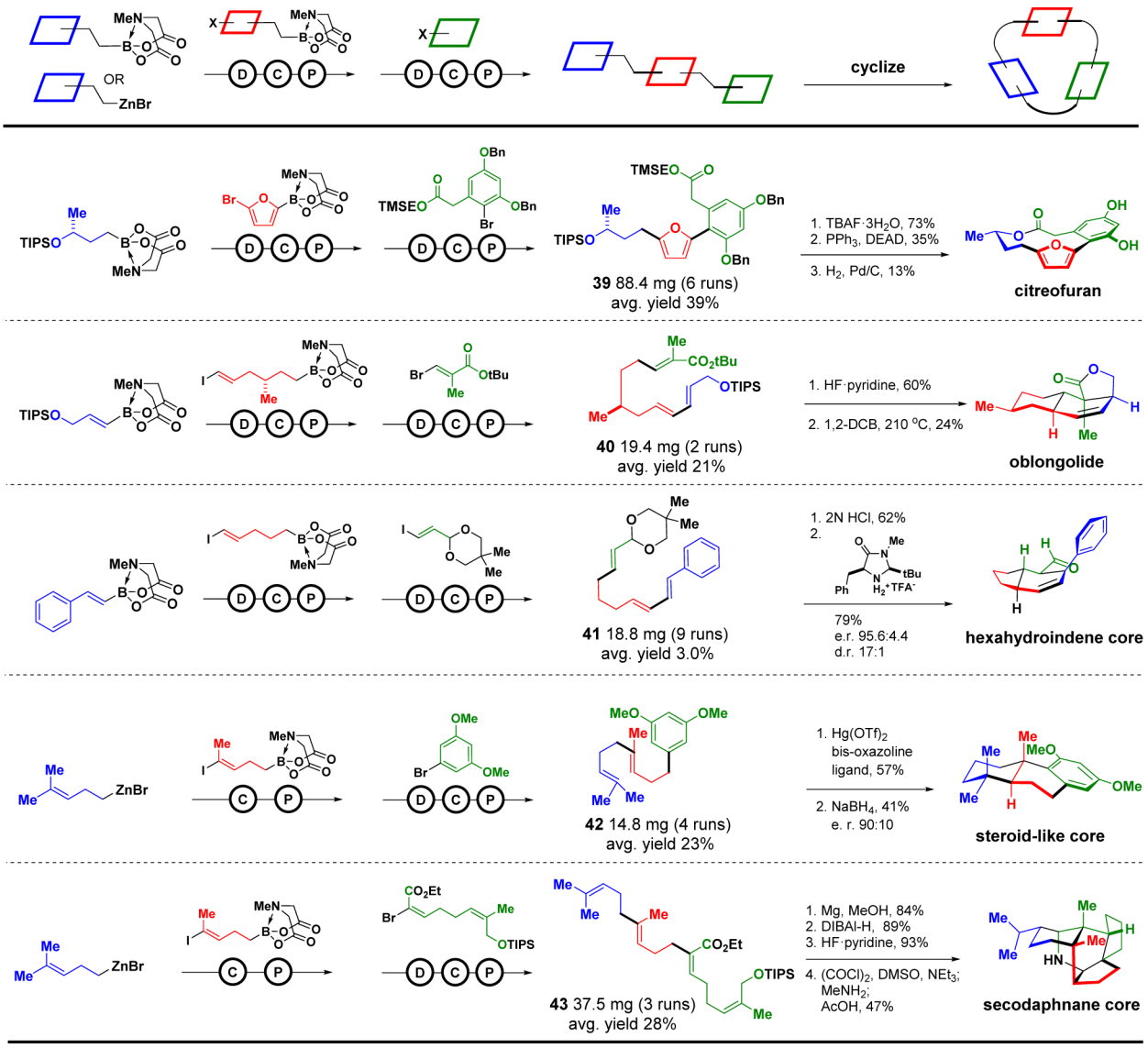

Figure 12. Semiautomated synthesis of macro- and polycyclic natural products and natural product-like cores using the linear-to-cyclized strategy. P = purification; TBAF = *tert*-butylammonium fluoride; DEAD = diethyl azodicarboxylate.

produce a 20-membered library of natural product derivatives based on ratanhine, thus highlighting the use of the building block-based platform in gaining rapid access to structural derivatives of a compound in an automated fashion.

Using the same automated building block assembly process on the synthesizer and interfacing it with the linear-to-cyclized strategy described above, we were able to access topologically complex (poly)cyclized targets. Specifically, the synthesis of linear precursors **39**–**43** via both C_{sp^3}–C_{sp^2} and C_{sp^2}–C_{sp^2} cross-couplings was executed on the synthesizer, generating the corresponding linear precursors in multimilligram quantities (Figure 12). The linear precursors were then successfully cyclized with a macrocyclization (**39**),[47] a Diels–Alder reaction (**40** and **41**),[48,49] a cation–π cyclization (**42**),[50] and a cascade sequence involving

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Acc. Chem. Res. 2015, 48, 2297–2307

hetero-Diels–Alder reactions and aza-Prins-cyclization (**43**).[42] The successful automated synthesis of the linear precursors to complex polycylic targets also has the potential to become a valuable tool for accelerating manual method development for new types of stereoselective cyclizations by providing facile stereocontrolled access to the linear substrates and their analogs.

6. CONCLUSIONS AND OUTLOOK

This building block-based approach has enabled the synthesis of the polyene motifs found in >75% of all polyene natural products from just 12 MIDA boronate building blocks using one coupling reaction. In addition, access to C35deOAmB using the platform allowed us to gain a deeper mechanistic understanding of the fundamental underpinnings of the mechanism of action of AmB. This enabled the rational development of novel, less toxic AmB derivatives currently under preclinical evaluation. This example thus represents an encouraging demonstration of how MIDA boronate-mediated synthesis can impact in the discovery and understanding of small molecule function.

Progress in stereoselective synthesis and iterative coupling of C_{sp^3} boronates has enabled the scope of this platform to begin expanding into the arena of chiral targets. Furthermore, we demonstrated that polycyclic natural products with high C_{sp^3} content could be synthesized using the same platform via a linear-to-cyclized strategy involving the iterative assembly of a linear precursor followed by polycyclization. Finally, the ability of MIDA boronates to undergo catch-and-release purification has allowed the iterative coupling platform to be automated on a synthesizer, which executed the synthesis of 14 classes of small molecules spanning natural products, pharmaceuticals, and materials precursors. Continued expansion in the scope of this platform could help enable a more generalized and automated approach for small molecule synthesis, which in turn could help shift the rate-limiting step in small molecule science from synthesis to function.

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Notes

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Martin D. Burke received his undergraduate degree at Johns Hopkins in 1998, a Ph.D. from Harvard in 2003, and an M.D. from Harvard Medical School in 2005. He is currently a Professor at UIUC and an Early Career Scientist of the Howard Hughes Medical Institute. His lab is focused on the synthesis and study of small molecules with protein-like functions.

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