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From Synthesis to Function via Iterative Assembly of MIDA Boronate Building Blocks

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Abstract

Small molecules can powerfully benefit society, but the study and optimization of their function is too often impeded by the time-intensive and specialist-dependent process that is typically used to make them. In contrast, general and automated platforms have been developed for peptide, oligonucleotide, and increasingly oligosaccharide synthesis, resulting in on-demand access to these molecules, even for non-specialists. A more generalized and automated approach for making small molecules could similarly help shift the rate limiting step in small molecule science from synthesis to function. Targeting this goal, we have developed a fully automated and increasingly general platform for iterative coupling of boronate building blocks. Analogous to peptide synthesis, the process involves iterative coupling of haloboronic acids protected as the corresponding *N*-methyliminodiacetic acid (MIDA) boronates. This platform has enabled us and other groups to access many polyene natural products, including the polyene motifs in >75% of all polyene natural products. It further allowed us to derivatize and thereby understand the powerful but also highly toxic antifungal natural product amphotericin B, which has led to the development of less toxic derivatives currently under evaluation as drug candidates. We also discovered a stereocontrolled entry into chiral, non-racemic α -boryl aldehydes, which are versatile intermediates for the synthesis of many Csp³ boronate building blocks that are otherwise difficult to access. We have also expanded the scope of the platform to include Csp³-rich, polycyclic molecules using a linear-to-cyclized strategy, in which Csp³ 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, 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, Csp³ cross-coupling methodologies, and cyclization strategies.

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The University of Illinois has filed patent applications on various aspects of the work described in this Account, and these have been licensed to REVOLUTION Medicines, a company for which M.D.B. is a founder.

Keywords

MIDA boronates; iterative coupling; automated synthesis; polyenes; Amphotericin B

1. INTRODUCTION

Small molecules perform an array of functions with substantial benefits to society, serving as medicines, biological probes, and as components of frontier technological devices. However, despite nearly 200 years of advances, synthesis remains the rate-limiting step in small molecule science. This impedes efforts to discover and optimize novel small molecule function. 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 accelerated the exploration and utilization of their functional potential. 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 making small molecules to non-specialists.

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/or 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 Analogous to the synthesis of peptides from amino acids, this approach involves pre-installing all of the required functional groups, oxidation states, and stereochemistry into the building blocks followed by their assembly via iterative cycles of stereospecific metal-mediated couplings. Similar to protecting the amine of an amino acid with fluorenylmethoxycarbonyl (Fmoc), 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 While this Account focuses on the MIDA boronate platform, it is noted that other approaches to ICC have also been developed.⁸

Removal of the Lewis acidic 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, are easily purified through chromatography or recrystallization, and can be prepared at a low cost.⁹ This has enabled the commercialization of >200 MIDA boronate building blocks.¹⁰ In addition, MIDA boronates are stable towards 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 reveals the free boronic acid, which can be used directly without necessitating further manipulation of sensitive boronic acids. 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 pre-installed into building blocks 9–12. Using 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.

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.¹¹ This analysis predicted that the polyene motifs found in >75% of all polyene natural products can be constructed with only 12 MIDA boronate building blocks and one coupling reaction. This hypothesis was systematically tested and confirmed (Figure 2).

This platform proved capable of enabling a better understanding of small molecule function. For example, amphotericin B (AmB) is a large and complex polyene macrolide natural product known to form ion channels in yeast and human cells (Figure 3A and 3B).¹² This feature made it a very attractive natural 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.¹³ Remarkably, despite its long-term clinical utilization, AmB has uniquely 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%, resulting in more than 1.5 million deaths worldwide each year.¹⁴

For decades it was 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 model suggested a bleak prospect for transforming a highly toxic channel into a promoter of physiology in the absence of a protein. It also led many efforts to improve the therapeutic index of AmB to focus on the challenging problem of selectively self-assembling structurally enigmatic multimeric ion channels in yeast over human cells.

Intriguing to us, 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.¹⁵ 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 semi-synthesis, our early studies suggested either the putative structural underpinnings of the AmB ion channel were incorrect and/or ion channel formation was not required for antifungal activity.¹⁶ Interestingly, ergosterol and cholesterol play many critical roles in yeast and human physiology, respectively.¹⁷ We thus proposed that AmB might alternatively kill cells via the simple binding of sterols (Figure 3C). This alternative mechanism would suggest that the channel forming and cell-killing effects of AmB might be separated, and illuminate a more actionable roadmap to an improved therapeutic index through selective sterol binding. Thus, we sought to definitively test this key hypothesis.

A series of computational modeling studies suggested that the deletion of a single atom from AmB might enable a key experiment.¹⁸ Specifically, it was predicted that the hydroxyl group at C35 was critical for forming ion channels but not for sterol binding. This suggested C35-deoxy AmB (C35deOAmB) would bind ergosterol but not permeabilize yeast membranes. The classic model would predict no 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 MIDA boronate platform enabled the synthesis of this complex molecular probe.¹⁹ 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), setting the stage for the key experiment. Like AmB, this derivative maintained potent fungicidal activity (Figure 4C), demonstrating that AmB primarily kills yeast by simply binding ergosterol. Further studies revealed that AmB acts primarily as a large extramembranous sterol sponge that physically extracts ergosterol from the membranes of yeast and thereby exerts its cytotoxic effects (Figure 3C).²⁰

This synthesis-enabled breakthrough in mechanistic understanding illuminated a simpler and actionable roadmap to less toxic amphotericins that only required the selective binding of ergosterol over cholesterol.²¹ Pursuit of this objective has 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. These compounds have been licensed to a pharmaceutical company and are currently being evaluated as new drug candidates.

With many MIDA boronates commercially available, this platform has been increasingly employed by other research groups as well (Figure 5). For example, MIDA boronates have enabled the syntheses of (–)-aurantioclavine²² via slow-release cross-coupling,²³ as well as

elansolid B1,²⁴ (–)-myxalamide A,²⁵ eicosapentaenoate methyl ester,²⁶ marinoquinoline C and E,²⁷ and in other applications.²⁸

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

To maximally 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, polycyclic small molecules. There have been many recent advances in the stereoselective synthesis of chiral, non-racemic C_{sp}³ pinacol boronic esters.²⁹ However, the instability of the pinacol boronic esters towards many reaction conditions makes 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 boronate building blocks that have the potential to be elaborated into more complex building blocks.

A chiral diol ligand showed only limited capability of promoting diastereoselective epoxidations of alkenyl boronic esters, presumably because the chiral ligand was distal to the alkene.³⁰ In contrast, the crystal structures of many MIDA boronates have revealed that the *N*-methyl substituent is always closely positioned to the organic group appended to boron. Variable temperature NMR studies have also demonstrated 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 d.r. as determined by ¹H-NMR of the crude reaction mixture in the epoxidation reaction under standard mCPBA conditions.³¹ A variety of alkenyl PIDA boronates with different substitution patterns worked well under the same conditions, giving >20:1 d.r. in most cases (Figure 7). Somewhat diminished but still synthetically useful diastereoselectivities were observed with 1,1-disubstituted alkenes (**21i** and **21j**), which are often challenging substrates for asymmetric functionalization due to the steric similarities between the two diastereotopic faces of the alkene.³² The stereochemical configuration of the epoxides **21a**, **21d** and **21k** were unambiguously confirmed by X-ray crystallography. The remaining product configurations were assigned by analogy. 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 outcome of the rearrangement reaction revealed that the rearrangement proceeded with migration of the boronate group (vs the phenyl group) during the rearrangement of **21a**.

Contemporaneous independent studies by the Yudin group led to co-discovery of the same rearrangement, and using a deuterium-labelled epoxy MIDA boronate this group arrived at the same mechanistic conclusion (Scheme 4).³³ Gevorgyan and co-workers recently utilized this 1,2 boryl migration in their synthesis of borylated furans.³⁴

The Yudin group also demonstrated the synthetic utility of the α -boryl aldehyde in accessing many other types of Csp³ boronates, many of which are difficult to access by other methods.³⁵ The group also demonstrated that these building blocks are useful for the rapid synthesis of stable boropeptides which showed nanomolar inhibition of CT-L members of the 20S proteasome.^{35b}

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 Csp³ carbons. Pre-installing the required stereochemistry into building block **26** enabled the synthesis of a glucagon receptor inhibitor containing a chiral diarylmethine motif via a concise iterative Csp³-Csp² sequence. The PIDA boronate **26** was first transformed into a Negishi reagent which was engaged in a Csp³-Csp² coupling with aryl iodide **27**. Deprotection by pinacol transesterification generates the benzylic pinacol boronic ester which was coupled with aryl iodide **30** using the powerful Csp³ coupling method developed by Crudden and co-workers,³⁶ 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. A LINEAR-TO-CYCLIZED STRATEGY FOR THE SYNTHESIS OF C_{sp}³-RICH POLYCYCLIC SMALL MOLECULES

Topologically complex polycyclic natural products represent especially challenging targets for the same building block-based approach. However, like linear, Csp²-rich natural products, many of these natural product are synthesized in Nature via the iterative assembly of a small set of natural building blocks. The resulting linear precursors that 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 (poly)cyclization reactions. To enable such cyclizations, such linear precursors must be suitably flexible and therefore contain multiple Csp³ atoms, which in turn demands challenging Csp³ couplings in their synthesis. We demonstrated the viability of the linear-to-cyclized approach with the synthesis of the pentacyclic secodaphnane core **38** of the daphniphyllum alkaloids. A Csp³ Negishi coupling between **32** and **33** generates MIDA boronate **34**. Deprotection of **34** yields the corresponding boronic acid which is engaged in another Csp³ coupling to give the linear precursor **36**. **36** is then taken on to the diol **37** by a series of reductions and protecting group removal. Following the one-pot

oxidation and iminium ion-triggered cascade cyclization developed by Heathcock and co-workers, **38** was transformed into the targeted secodaphnane core (Scheme 6).³⁷

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,³⁸ this inherent lack of generality has limited the applications and widespread adoption of this approach.^{39,40,41}

The key challenge in generally automating a synthesis platform is finding a generalized purification method that can be used for all intermediates. Small molecule synthesis typically involves purifications customized for each intermediate, but such customization is incompatible with generalized automated purification. The syntheses of peptides, oligonucleotides, oligosaccharides, and in some cases, organic polymers,⁴² natural products and pharmaceuticals have been aided by solid-phase methods.⁴³ However, small molecules do not possess a common functional group handle for attachment to solid support which precludes generalized application of this approach.

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 surrogate common handle for purification. We serendipitously discovered that MIDA boronates uniformly possess highly unusual binary affinity for silica gel with certain pairs of eluents, and this provided a path forward. Specifically, MIDA boronates show minimal mobility on thin layer silica gel chromatography when eluting with MeOH:Et₂O but are rapidly eluted with THF.

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:Et₂O. 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. The synthesizer comprises three modules that sequentially execute the deprotection, coupling, and purification steps required for each synthesis cycle (Figure 10). All solutions are automatically transferred via computer-controlled syringe pumps running custom designed software. Thus, each automated synthesis simply requires placing pre-packed cartridges onto the synthesizer and pressing “start”.

The synthesizer proved to be capable of making a range of natural products, pharmaceuticals, and materials 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 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 and interfacing it with the linear-to-cyclized strategy described above, the synthesizer further proved capable of enabling access to topologically complex and Csp³-rich (poly)cyclized targets. Specifically, it executed the synthesis of linear precursors **39–43** via both Csp³-Csp² and Csp²-Csp² cross-couplings, generating the corresponding linear precursors in multimilligram quantities (Figure 12). The linear precursors were then successfully cyclized with a macrocyclization (**39**),⁴⁴ Diels-Alder reactions (**40** and **41**),^{45,46} a cation- π cyclization (**42**)⁴⁷ and a cascade sequence involving hetero-Diels-Alder reactions and aza-Prins-cyclization (**43**).³⁷ The successful automated synthesis of the linear precursors to complex polycyclic targets also has the potential to become a valuable tool for accelerating 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 its mechanism of action of AmB. This provided the framework for the development of novel, less toxic AmB derivatives currently under pre-clinical evaluation. This is a powerful demonstration of how MIDA boronate-mediated synthesis can impact in the discovery and understanding of small molecule function.

Progress in the direction of developing the stereoselective synthesis and iterative coupling of Csp³ boronates has enabled us to expand the scope of the platform to include pharmaceutically relevant chiral targets. Furthermore, the inherent modularity in natural products resulting from biosynthesis suggested that polycyclic natural products could be synthesized through the assembly of a linear precursor followed by polycyclization. This linear-to-cyclized strategy was tested and confirmed. 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. 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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Biographies

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Anthony S. Grillo received his B.S. Degrees in Chemistry and Biochemistry from the University of Michigan in 2011 and is currently an NSF Graduate Fellow at the University of Illinois. His research in Martin Burke's laboratory primarily focuses on the prospect of replacing protein deficiencies with small molecules that autonomously perform protein-like functions.

Martin D. Burke received his undergraduate degree at Johns Hopkins in 1998, a PhD from Harvard in 2003, and an MD 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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Figure 1.

The 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

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.

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.

Figure 5. Structures of natural products or derivatives synthesized by other groups using MIDA boronates. Portions highlighted in red come from MIDA boronate building blocks.

Figure 6.

Leveraging the enforced proximity between the chiral R^* group and the organic group bound to boron to achieve highly diastereoselective reactions.

Figure 7.

Highly diastereoselective epoxidations of alkenyl PIDA boronates. Numbers in parentheses indicate isolated yields.

Figure 8.
Examples of new types of Csp^3 boronates that can be derived from α -boryl aldehydes.

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

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

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

Figure 12.

Semi-automated synthesis of Csp³-rich macro- and polycyclic natural products and natural product-like cores using the linear-to-cyclized strategy.

Scheme 1.

Total synthesis of ratanhine via an iterative coupling approach.

Scheme 2.

Completely stereocontrolled synthesis of (-)-peridinin via ICC.

Scheme 3.
Synthesis of C35deOAmB through ICC.

Scheme 4.

Determining the migrating group by A) probing the stereochemical outcome of the rearrangement and B) deuterium labeling.

Scheme 5.
Iterative Csp³-Csp² cross-coupling for the modular synthesis of **31**.

Scheme 6.

The linear-to-cyclized approach for the synthesis of the secodaphnane core **38**.