Automated Stereocontrolled Assembly-Line Synthesis of Organic Molecules

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Abstract

Automation has fuelled dramatic advances in fields such as proteomics and genomics by enabling non-experts to prepare, test and analyse complex biological molecules, including proteins and nucleic acids. However, the field of automated organic synthesis lags far behind, partly because of the complexity and variety of organic molecules. As a result, only a handful of relatively simple organic molecules, requiring a small number of synthetic steps, have been made in an automated fashion. Herein, we report an automated assembly-line synthesis that allows iterative formation of $C(sp^3)$–$C(sp^3)$ bonds with high stereochemical control and reproducibility, enabling access to complex organic molecules. This was achieved on a commercially available robotic platform capable of handling air-sensitive reactants and performing low-temperature reactions, which enabled six sequenced one-carbon homologations of organoboron substrates to be performed iteratively without human intervention. Together with other automated functional group manipulations, this methodology has been exploited to rapidly build the core fragment of the natural product (+)-kalkitoxin, thus expanding the field of automated organic synthesis.

Introduction

Automated synthesis of complex biomolecules has had a major impact in the fields of chemistry, biochemistry, medicine, and healthcare. Indeed, the development of strategies based on iterative coupling of amino acid and nucleotide building blocks heralded the proteomic and genomic revolution.[1,2] Substantial advancement has also been made towards automated polysaccharide synthesis.[3,4] Iteration has been the

cornerstone of all these successful automated synthesis technologies (Fig. 1A), with the building blocks being connected together by a single coupling reaction (e.g., amide, phosphonate, or glycosidic bond formation). In contrast, general, automated methodologies for C–C bond formation have not yet emerged due to the complexity and variety of organic molecules. However, some progress in automated organic synthesis has been achieved but only in the preparation of relatively simple targets;[5-12] the field is still nascent. A notable example from Burke reported the iterative assembly of polyenes, where alkene building blocks were coupled together through Suzuki-Miyaura reactions of MIDA boronates.[13] In this example, $C(sp^2)$–$C(sp^2)$ bonds were formed and a maximum of three iterations were described. Recently, the same group reported $C(sp^3)$–$C(sp^3)$ bond-forming reactions in an automated fashion but, again only a small number (two) of automated iterations were described.[14]

Over the last decade, we have been interested in developing robust, iterative methodologies that have the potential to be automated. In particular, we have developed strategies based on boron homologations with chiral carbenoid building blocks which allow carbon chains to be grown one-atom-at-a-time with complete stereocontrol (Fig. 1B).[15,16] Furthermore, the methodology has been extended to coupling larger building blocks and even applied to late-stage fragment coupling in the pursuit of complex natural products.[17,18] In fact, our methodology was used by Burke, in concert with a new class of hyperstable TIDA boronate-containing building blocks, to achieve automated iterative $C(sp^3)$–$C(sp^3)$ bond formation.[14] We have demonstrated that boron homologations can be performed in an iterative fashion, in a process termed assembly-line synthesis.[19] The advantages of this process include (i) it employs a single set of reaction conditions; (ii) it can be done iteratively without purification of intermediates; (iii) no additional manipulations are required between homologation steps (e.g., protection or redox steps); and (iv) it uses a small set of common repeat building blocks. These features make this chemistry highly suited for automation since all other automated iterative synthesis technologies require additional deprotection steps between each coupling reaction. Whilst these boron homologation reactions are efficient when performed manually in the laboratory, the air-sensitive organometallic reagents and thermally unstable carbenoids

require an inert atmosphere and low temperatures. These conditions are difficult to achieve in automated chemical synthesis since most, but not all,[14] platforms are not designed for use under such extreme environments.

Herein, we describe an automated methodology that allows iterative and stereocontrolled $C(sp^3)$–$C(sp^3)$ bond formation (Fig. C). Successful handling of air-sensitive reactants at low temperatures allowed numerous iterations to be performed in a stereocontrolled fashion. Moreover, functionalization of the elongated carbon chain has also been developed, thus showing how a single robotic platform is capable of performing diverse chemical reactions.

Results

The work presented here has been carried out on an automated workstation manufactured by Chemspeed Technologies AG (See Supplementary Materials 2.1). Our investigation began by exploring the handling of reactive organolithium species on the automated workstation. At the outset, it should be noted that while the Chemspeed software provides a checklist to minimise user errors, some issues related to the chemistry proved challenging and needed to be solved (See Supplementary Material 2.1.4 for a full list of technical issues). In particular, the handling *n*-BuLi sometimes caused syringe blockages (presumably by LiOH), and as such an automated washing of the syringes with dilute HCl, followed by water and then THF was introduced prior to starting the chemistry. We also developed an automated washing and drying of the reaction vessels, followed by purging of the sealed workstation with nitrogen gas for 90 minutes, which enabled the generation of a moisture- and oxygen-free system. Indeed, an automated titration of *n*-BuLi on the platform was conducted by dispensing increasing volumes of the organolithium into *N*-benzylbenzamide solutions at −40 °C and observing the color change at the equivalence point (Fig. S5). Notably, no erosion of *n*-BuLi molarity was observed from the manual laboratory titer.

We then moved our attention to automating the Matteson homologation, a reaction that allows the insertion of a methylene unit into the carbon chain of a boronic ester.[20-23] Using phenethyl boronic acid pinacol ester (**1**) as the model substrate, the Matteson homologation using chloromethyllithium was targeted (Fig. 2A). In the laboratory, this reaction is performed in Et_2O at −78 °C, with the carbenoid generated *in situ* from $BrCH_2Cl$ (3.0 equiv.) by slow addition of *n*-BuLi (2.5 equiv.). Maintaining the low temperature is crucial for minimising decomposition of the thermally unstable carbenoid, whereas the slow addition of *n*-BuLi prevents its competitive reaction with **1** instead of $BrCH_2Cl$. For a successful outcome on the automated platform, we found that a high shaking rate (1000 rpm) during the addition of *n*-BuLi was critical for uniform mixing, with lower rates resulting in poor yields or requiring a further excess of carbenoid (4 equiv.) for full conversion. Other modifications that were introduced to improve efficiency included (i) the reagents were added as stock solutions in anhydrous *t*-butyl methyl ether (TBME) instead of volatile and ignition-hazardous Et_2O; and (ii) at the end of each liquid transfer, an extra 0.2 mL of TBME was added to the reaction vessel to prevent material loss in the lines (see Supplementary Materials 2.1.4). In order for the fully automated synthesis to accommodate all the steps of the synthetic procedure, the work-up of the reaction was also automated. In this case, using the solid-phase extraction module, the reaction mixture was transferred onto silica-loaded capped cartridges, followed by pressurization with inert gas. This allowed for automated silica-plug filtration to remove the LiCl by-product. The filtrates were then transferred back to clean reaction vessels where they were concentrated under reduced pressure. With this setup, the automated Matteson homologation was highly successful, furnishing product **2** in high yield (92% average) and without human intervention. This approaches the highest achievable yield on the platform, as a control experiment in which an inert internal standard was subjected to the same workflow gave back 94% recovery (average). To accomplish an automated assembly-line synthesis, iteration of the homologation steps is required. With product **2** available in the reaction vessel, a second Matteson homologation was performed on this new substrate using an identical workflow. Pleasingly, homologated product **3** could be isolated in an overall 77% average yield (88% yield/step), thus showing high reproducibility over multiple chemical manipulations. Indeed, in contrast to a manual performance in the laboratory, the robot can operate up to

four needles at a time, thus allowing reactions to be run in parallel (ideal to monitor the reproducibility of the methodology).

With iterative Matteson homologations optimised, we then focused on the automated chiral carbenoid homologation with lithiated benzoate ester **4-Li**, using organostannane **4** as the carbenoid precursor (Fig. 2B).[24,25] In the laboratory, **4-Li** is generated by treatment of **4** with equimolar amounts (1.3 equiv.) of *n*-BuLi in Et_2O at −78 °C before the addition of the boronic ester and subsequent warming to promote 1,2-rearrangement. However, as with the Matteson homologation, some adjustments to the laboratory procedure were required for effective translation to the automated platform (see Supplementary Materials 2.3). The major challenge that was encountered involved the formation of a gel-like reaction mixture upon completion of the 1,2-rearrangement. This resulted in significant loss of material during the automated filtration to remove the lithium benzoate (LiOTIB) by-product. Fortunately, the issue could be resolved by concentration of the crude reaction mixture and redissolution in 1,2-dichloroethane (DCE), which provided a homogenous mixture that could be easily handled by the robot. With this modification incorporated into our workflow, the homologation of boronic ester **1** with **4-Li** proceeded with high efficiency on the automated platform, producing product **5** in high yield (92%, average of four parallel reactions) and 99:1 enantiomeric ratio (e.r.). Furthermore, this automated homologation reaction could be performed iteratively, furnishing product **6** in an overall 86% average yield, in 99:1 diastereomeric ratio (d.r.), and with high reproducibility.

With both homologation reactions in hand, the power of the automated assembly-line was demonstrated by applying it to the synthesis of amide **11**, which is a late-stage intermediate previously employed in the synthesis of the neurotoxin (+)-kalkitoxin (Fig. 3A).[26] Remarkably, starting from boronic ester **9**, six consecutive homologations were performed in a continuous sequence that was autonomously completed in 5 days and required only the provision of fresh carbenoid precursors and *n*-BuLi at the start of each homologation (Fig. 3B, Movie S1). Excellent conversions were observed at each step, as evaluated by

offline GC-MS analysis of aliquots collected by the robot, and the final boronic ester **10** was formed in 46% NMR yield (88% yield/step) with complete reproducibility over two parallel runs. Moreover, the whole sequence was carried out with simple automated filtrations of the reaction mixture between the homologation steps, thus, the only chromatographic purification required was that used to purify boronic ester **10**, which was isolated in 41% overall yield. Importantly, no supervision was required during the execution of the program, which enabled the technician responsible for the automated platform (a non-synthesis expert) to run the entire synthesis with minimal intervention.

In order for fully automated syntheses of organic molecules to be achieved, it is important that a single robotic platform has the capability to perform diverse chemical reactions. Therefore, we subsequently investigated the conversion of boronic ester **10** into amide **11**, which requires the transformation of the boronic ester moiety into a primary amine,[27-28] followed by acylation and methylation (Fig. 3C). Upon optimisation (see Supplementary Materials 2.5-2.6), these steps could also be performed on the robotic platform in a fully automated sequence, which provided amide **11** in 48% overall yield after purification by column chromatography. Thus, benzylic boronic ester **9** was successfully transformed into the complex tertiary amide **11** in 20% yield over a sequence of 9 fully automated synthetic steps with only a single intermediate chromatographic purification.

Conclusions

Homologation reactions of organoboron compounds with carbenoid building blocks have been optimized and performed in an automated fashion on a commercially available robotic platform. These reactions, which require low temperature and water- and oxygen-free conditions, enable carbon chains to be grown one-atom-at-a-time with control of chain length and stereochemistry. Six iterative homologations were conducted by , thus establishing the highest number of iterations reported for an automated synthesis of carbon chains. Finally, in addition to iterative homologations, we also demonstrated the automated conversion of boronic esters into amines, subsequent amide formation and alkylation, thereby significantly

broadening the scope of reactions available. Since amines are ubiquitous in natural products and drug discovery, there is considerable potential of our automated synthesis for the preparation of complex molecules, even by non-experts.

Methods: This work has been performed on a Chemspeed Swing Platform developed by Chemspeed Technologies (See section 2 of Supplementary Information). For the Matteson reaction, the robot dispenses boronic ester **1** (1 equiv.) and bromochloromethane (3 equiv.), both as individual stock solutions in TBME, into the reaction vessels which are then cooled down to –70 °C whilst shaking at 800 rpm. After 30 minutes at this temperature, *n*-BuLi (2.5 equiv., 1.6 M in hexane) was dispensed from the top of the reactor vessels at a rate of 0.8 mL/min whilst shaking at 1000 rpm. Upon addition, the system was kept at this temperature for 1 hour, followed by warming of the reactors up to 25 °C. Then, the system was kept at this temperature for another hour before the filtration of the reactions takes place. The full method for the Matteson reaction is reported in section 2.3 of Supplementary Information. For the Lithiation-Borylation reaction, the robot dispensed a TBME solution of organostannane **4** (1.3 equiv.) into the reaction vessels and then cooled the system down to –70 °C whilst shaking at 800 rpm. After 30 minutes at this temperature, *n*-BuLi (1.3 equiv., 1.6 M in hexane) is dispensed from the top of the reactor vessels at a rate of 0.1 mL/min. Upon addition, the system is kept at this temperature for 1 hour to allow complete lithium-tin exchange. A TMBE solution of boronic ester **1** is then added (1 mL/min dispense), keeping the reaction mixture at –70 °C for another hour to allow complete borylation of the carbenoid. Then, the 1,2-migration occurs upon warming the system up to 40 °C for 3 hours, before the filtration of the reactions takes place. The full method for the Lithiation-Borylation reaction is reported in section 2.4 of Supplementary Information.

Data Availability: All experimental procedures and data are available in the Supplementary Information.

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Fig. 1. Automated synthesis development. a, Examples of iterative automated synthesis, including the number of iterations possible, the types of bonds formed, and the years they were developed. In contrast to proteins,[1] nucleic acids,[2] and polysaccharides,[3] the iterative automated synthesis of natural products via C–C bond formation is much less developed.[14] **b**, Assembly-line synthesis by means of homologation of boronic esters, which has been applied to the total synthesis of many natural products, including (+)-hydroxyphthioceranic acid and (+)-faranal.[17,19] The coloured dots show carbon atoms that are introduced by Matteson homologation (green) and homologations with each enantiomer of a chiral carbenoid (blue and red). **c**, Iterative, stereocontrolled automated assembly-line synthesis (this work). The gears denote reactions run on an automated platform. The photograph shows the Chemspeed automated workstation (see section 2.1.1 of the Supplementary Information for a detailed description).

Fig. 2. Automated homologation reactions. A, Automated iterative Matteson homologations. **B**, Automated iterative chiral carbenoid homologations with lithiated benzoate esters. Yields were determined by ^{1}H NMR using an internal standard. Yields in parentheses show the range for 4 parallel runs. Yields in square brackets show average yields for the iterative steps. Bpin = pinacol boronic ester, OTIB = 2,4,6-triisopropylbenzoate, TMBE = *tert*-butyl methyl ether, DCE = 1,2-dichloroethane, rpm = rotations per minute.

Fig. 3. Assembly-line synthesis. a, (+)-Kalkitoxin and its retrosynthetic analysis, leading to **11** as a key target.[24] **b**, Automated assembly-line synthesis of **10** from **9** and stacked GC-MS chromatograms for I_A, I_B, I_C, I_D, I_E and **10** (normalised to the highest peak) after each homologation step, showing the high fidelity of

each reaction. **c**, Automated amination-acylation-methylation sequence to access **11** from **10**. Bpin = pinacol boronic ester, OTIB = 2,4,6-triisopropylbenzoate, THF = tetrahydrofuran.

References

1. Merrifield, R. B. Automated Synthesis of Peptides: Solid-phase peptide synthesis, a simple and rapid synthetic method, has now been automated. *Science* **150**, 178–185 (1965).
2. Caruthers, M. H. Gene synthesis machines: DNA chemistry and its uses. *Science* **230**, 281–285 (1985).
3. Plante, O. J., Palmacci, E. R., Seeberger, P. H. Automated solid-phase synthesis of oligosaccharides. *Science* **291**, 1523–1527 (2001).
4. Joseph, A. A., Pardo-Vargas, A., Seeberger, P. H. Total synthesis of polysaccharides by automated glycan assembly. *J. Am. Chem. Soc*. **142**, 8561–8564 (2020).
5. Burger, B., Maffettone, P. M., Gusev, V. V., Aitchison, C. M., Bai, Y., Wang, X., Li, X., Alston, B. M., Li, B., Clowes, R., Rankin, N., Harris, B., Sprick, R. S., Cooper, A. I. A mobile robotic chemist. *Nature* **583**, 237–241 (2020).
6. Steiner, S., Wolf, J., Glatzel, S., Andreou, A., Granda, J. M., Keenan, G., Hinkley, T., Aragon-Camarasa, G., Kitson, P. J., Angelone, D., Cronin, L. Organic synthesis in a modular robotic system driven by a chemical programming language. *Science* **363**, eaav2211 (2019).
7. Liu, C.; Xie, J.; Wu, W.; Wang, M.; Chen, W.; Idres, S. B.; Rong, J.; Deng, L.-W.; Khan, S. A.; Wu, J. Automated synthesis of prexasertib and derivatives enabled by continuous-flow solid-phase synthesis. *Nature Chemistry* **13**, 451–457 (2021).
8. Mehr, S. H. M., Craven, M., Leonov, A., Keenan, G., Cronin, L. A universal system for digitization and automatic execution of the chemical synthesis literature. *Science* **370**, 101–108 (2020).
9. Coley, C. W., Thomas, D. A., Lummiss, J. A. M., Jaworski, J. N., Breen, C. P., Schultz, V., Hart, T., Fishman, J. S., Rogers, L., Gao, H., Hicklin, R. W., Plehiers, P. P., Byington, J., Piotti, J. S., Green, W. H., Hart, A. J., Jamison, T. F., Jensen, K. F. A robotic platform for flow synthesis of organic compounds informed by AI planning. *Science* **365**, eaax1566 (2019).
10. Tu, N. P., Searle, P. A., Sarris, K. An automated microwave-assisted synthesis purification system for rapid generation of compound libraries. *J. Lab. Autom.* **21**, 459–469 (2016).
11. Li, T., Liu, L., Wei, N., Yang, J.-Y., Chapla, D. G., Moremen, K. W., Boons, G.-J. An automated platform for the enzyme-mediated assembly of complex oligosaccharides. *Nat. Chem.* **11**, 229–236 (2019).

12. Chatterjee, S., Guidi, M., Seeberger, P. H., Gilmore, K. Automated radial synthesis of organic molecules. *Nature* **579**, 379–384 (2020).

13. Li, J., Ballmer, S. G., Gillis, E. P., Fujii, S., Schmidt, M. J., Palazzolo, A. M. E., Lehmann, J. W., Morehouse, G. F., Burke, M. D. Synthesis of many different types of organic small molecules using one automated process. *Science* **347**, 1221–1226 (2015).

14. During the preparation of this manuscript, Burke, Blair, and co-workers reported the automated iterative C(sp^3)-C bond formation. Blair, D. J.; Chitti, S.; Trobe, M.; Kostyra, D. M.; Haley, H. M. S., Hansen, R. L.; Ballmer, S. G.; Woods, T. J.; Wang, W.; Mubayi, V.; Schmidt, M. J.; Pipal, R. W.; Morehouse, G. F.; Palazzolo Ray, A. M. E.; Gray, D. L.; Gill, A. L.; Burke, M. D. Automated iterative Csp^3-C bond formation. *Nature*, DOI: 10.1038/s41586-022-04491-w (2022).

15. Aiken, S. G., Bateman, J. M., Aggarwal, V. K. Boron "ate" complexes for asymmetric synthesis. *Science of Synthesis: Advances in Organoboron Chemistry towards Organic Synthesis* Ch. 13, 393–458 (2019).

16. Casoni, G., Kucukdisli, M., Fordham, J. M., Burns, M., Myers, E. L., Aggarwal, V. K. α-Sulfinyl benzoates as precursors to Li and Mg carbenoids for the stereoselective iterative homologation of boronic esters. *J. Am. Chem. Soc.* **139**, 11877–11886 (2017).

17. Yeung, K., Mykura, R. C., Aggarwal, V. K. Lithiation–borylation methodology in the total synthesis of natural products. *Nature Synthesis* **1**, 117–126 (2022).

18. Fiorito, D., Keskin, S., Bateman, J. M., George, M., Noble, A., Aggarwal, V. K. Stereocontrolled Total Synthesis of Bastimolide B Using Iterative Homologation of Boronic Esters. *J. Am. Chem. Soc.* **144**, 18, 7995–8001 (2022).

19. Leonori, D., Aggarwal, V. K. Lithiation–borylation methodology and its application in synthesis. *Acc. Chem. Res.* **47**, 3174–3183 (2014).

20. Matteson, D. S. α-Halo boronic esters: Intermediates for stereodirected synthesis. *Chem. Rev.* **89**, 1535–1551 (1989).

21. Matteson, D. S., Collins, B. S. L., Aggarwal, V. K., Ciganek, E. The Matteson reaction. *Organic Reactions* (2021).

22. Matteson, D. S., Ray, R. Directed chiral synthesis with pinanediol boronic esters. *J. Am. Chem. Soc.* **102**, 7590–7591 (1980).

23. Matteson, D. S., Ray, R., Rocks, R. R., Tsai, D. J. S. Directed chiral synthesis by way of α-chloro boronic esters. *Organometallics* **2**, 1536–1543 (1983).

24. Burns, M., Essafi, S., Bame, J. R., Bull, S. P., Webster, M. P., Balieu, S., Dale, J. W., Butts, C. P., Harvey, J. N., Aggarwal, V. K. Assembly-line synthesis of organic molecules with tailored shapes. *Nature* **513**, 183–1188 (2014).

25. Leonori, D., Aggarwal, V. K. Reagent-controlled lithiation–borylation. *Synthesis and Application of Organoboron Compounds*, Topics in Organometallic Chemistry, **49**, 271–295 (2015).

26. Balieu, S., Hallett, G. E., Burns, M., Bootwicha, T., Studley, J., Aggarwal, V. K. Toward Ideality: The Synthesis of (+)-kalkitoxin and (+)-hydroxyphthioceranic acid by assembly-line synthesis. *J. Am. Chem. Soc.* **137**, 4398–4403 (2015).

27. Mlynarski, S. N., Karns, A. S., Morken, J. P. Direct stereospecific amination of alkyl and aryl pinacol boronates. *J. Am. Chem. Soc*. **134**, 16449–16451 (2012).

28. Edelstein, E. K., Grote, A. C., Palkowitz, M. D., Morken, J. P. A protocol for direct stereospecific amination of primary, secondary, and tertiary alkylboronic esters. *Synlett* **29**, 1749–1752 (2018).