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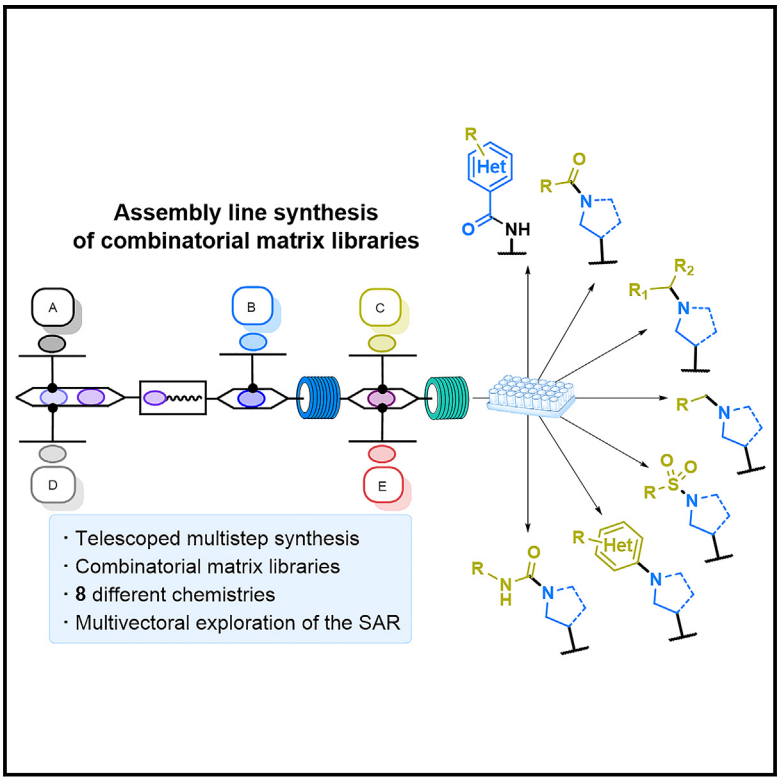
Multistep and multivectorial assembly line library synthesis in flow

Graphical abstract



Highlights

- The system can perform up to eight different chemistries
- Linking three different fragments facilitates multivectorial SAR explorations
- High productivity rate of up to four compounds per hour
- Applications to drug discovery beyond current flow chemistry approaches

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In brief

This study introduces a multistep library synthesis method in continuous flow, enabling greater structural diversity in drug discovery in a single experiment. By incorporating up to eight synthetic methodologies, including modern metallaphotoredox couplings and metal-catalyzed transformations, the approach allows for the rapid exploration of chemical space. This innovation accelerates the development of diverse compounds, enhancing the potential for discovering new drugs with optimized properties.

Pijper et al., 2024, Chem Catalysis *4*, 101118
October 17, 2024
https://doi.org/10.1016/j.checat.2024.101118

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Please cite this article in press as: Pijper et al., Multistep and multivectorial assembly line library synthesis in flow, Chem Catalysis (2024), https://doi.org/10.1016/j.checat.2024.101118

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Multistep and multivectorial assembly line library synthesis in flow

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https://doi.org/10.1016/j.checat.2024.101118

THE BIGGER PICTURE Organic synthesis has traditionally been like an art form, where scientists create complex molecules through a mix of chosen reactions, often relying on trial and error. Our work aims to change this approach by introducing a new method, like how manufacturing transformed production. Just as cars are made on an assembly line, we synthesize compounds in a tube, adding different elements step-by-step to create the final product. In the context of medicinal chemistry, this approach allows for the quick creation of drug analogs, speeding up the discovery of new medicines for diseases that currently have no treatment. For future prospects, we envision combining this methodology with artificial intelligence and machine learning tools to connect the design and synthesis process in a single system, significantly accelerating drug discovery.

SUMMARY

In drug discovery, traditional automated library synthesis has typically involved single-step synthetic procedures targeting a single vector of interest. However, achieving greater structural diversity requires exploring multistep and multivectorial approaches. These methodologies enable the preparation of compounds with varying structures in a single experiment. Here, we present a novel method for multistep library synthesis in continuous flow. This approach offers unique opportunities, such as exploring linkers between two defined vectors or rapidly mapping synergistic structure-activity relationships (SARs) by concurrently exploring multiple vectors. Our method incorporates up to eight different synthetic methodologies, including established chemistries, metal-catalyzed transformations, and modern metallaphotoredox couplings. This broad range of synthetic methodologies ensures a high level of diversity in the compounds generated, providing a powerful tool to accelerate the exploration of the chemical space in drug discovery programs.

INTRODUCTION

Organic synthesis is a crucial part of the multidisciplinary process of drug discovery.[1,2] In recent years, there has been a growing interest in rapidly generating analog libraries in pharmaceutical companies. To accelerate medicinal chemistry programs, automated synthesis protocols have emerged as a promising approach.[3–5] However, most automated library synthesis approaches have been focused on single-step methodologies, exploring one vector at a time.[6–11] There has been limited exploration of automated multistep approaches capable of preparing a diverse range of compounds with variations at several vectors with different transformations.[12,13]

One successful tool for automated multistep synthesis is continuous flow chemistry, which has been used for the preparation of selected active pharmaceutical ingredients (APIs) and sets of close analogs by means of multistep synthesis.[14–21] There are three distinct approaches for multistep synthesis in flow: linear, cyclic, and radial (Figure 1).[22–24] Additionally, continuous flow chemistry offers a unique opportunity to have multiple slugs in line to increase the productivity significantly. Utilizing an automated injection where different components are added, the synthesis of combinatorial libraries in an assembly line fashion becomes possible (Figure 1). This concept was pioneered by Dr. Djuric and colleagues at Abbott Laboratories using Accendo Conjure, demonstrating the potential of plug flow approaches for library synthesis, albeit with some limitations in the diversity of chemistry that could be combined in line.[25–27] Subsequent research by Prof. Jamison showed the feasibility of modular flow for the preparation of pyrazole cores.[28]

Flow chemistry for modular multistep synthesis for library creation offers the potential for the rapid generation of analogs in a

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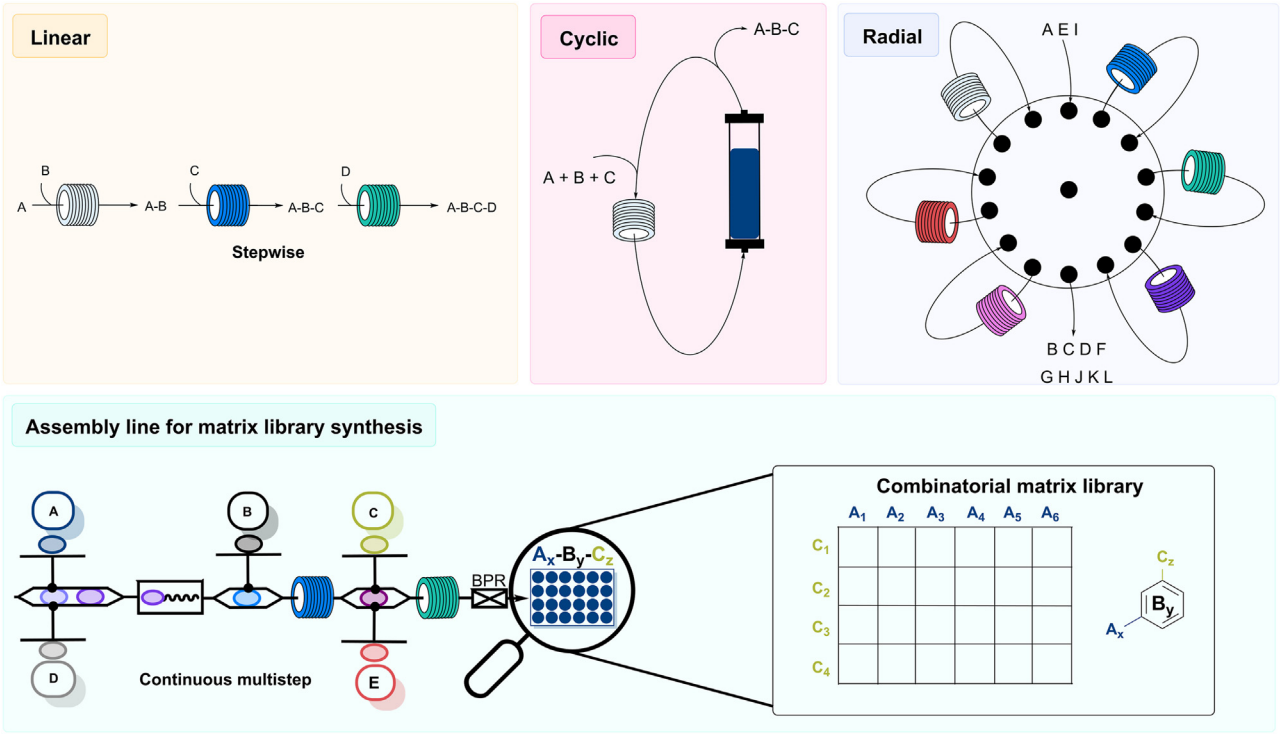

Figure 1. Previous and current work for automated synthesis

Linear: stepwise synthesis with intermediate purification. Cyclic: the synthesis of biopolymers, such as peptide synthesis. Radial: combination of cyclic and linear synthesis approaches where flow modules surrounding a central core require minimal equipment while retaining maximum synthetic versatility. Assembly line: the current approach where a system resembling an assembly line produces molecules and large combinatorial matrix libraries (this work).

combinatorial manner, unlocking new possibilities in drug discovery. Herein, the aim is to develop an iterative modular setup that enables the preparation of combinatorial matrix libraries including diverse chemical transformations, thereby accessing a broader chemical space in a single experiment. This approach allows for the exploration of different substitution patterns and functionalities, assessing both vectors and central core of a hit or lead molecule in an automated manner. The potential applications in medicinal chemistry are significant, as it enables the rapid establishment of structure-activity relationships (SARs) by considering additive effects and the construction of matched molecular pairs of different bioactive compounds.[29] Furthermore, this approach would facilitate linker connectivity exploration for fragment-based drug design[30–32] and proteolysis-targeting chimeras (PROTACs).[33,34]

Figure 2. The ASIA system utilized for assembly line library synthesis

(A) 6 syringe pumps.
(B) Autorim with total of 6 automated injectors.
(C) 2 coil reactors for thermal reactions.
(D) Chip and chip reactor for controlled temperature.
(E) Pressure controller.
(F) Back pressure regulator (BPR).
(G) Automated collector (depicted at the back).[31]

RESULTS AND DISCUSSION

To investigate this approach, we opted for the ASIA flow system from Syrris (Figure 2). This automated flow equipment features 6 automated injection ports that enable the combination of up to six different reagents in line under a nitrogen atmosphere.[35] These ports are paired with chip or tube reactors capable of facilitating thermal reactions. The instrument enables the execution of multiple reactions in line through volumetric calculations, facilitating the addition of reactants or reagents at the appropriate times to enable the modular synthesis, resembling an assembly line.

To validate our synthetic method, we first conducted experiments to determine a suitable carrier solvent for performing sequential reactions. Avoiding cross-contamination is critical for the success of this approach. Inspired by the work of

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Dr. Djuric, we explored various perfluorinated solvents and injected slugs containing a suitable colorant in a mixture of THF and DMF to assess cross-contamination. We observed a wetting effect on the surface of chip and tube reactors, resulting in carryover into the next slug.[36] Similar behavior was observed when higher alkanes were employed as the carrier solvent (see Tables S1 and S2). Pleasantly, when DMF was used as the carrier solvent, no carryover effect was observed, and dispersion could be effectively controlled by the system (see Figure S4).

Building on this discovery, two well-established reactions commonly employed in our group were selected for subsequent combinatorial validation studies: amide formation from esters with lithium hexamethyldisilazane (LiHMDS)[37] and Negishi coupling.[10,38] To assess the cross-contamination of this two-step sequence, we selected 4-amino-2-chloropyridine, while methyl 2-methoxybenzoate and methyl tetrahydro-2*H*-pyran-4-carboxylate were utilized as esters alternatively. The amides formed in line were then coupled with isopropylzinc bromide using $Pd(AcO)_2$ and RuPhos as the catalytic system at intermediate conversion to evaluate the robustness and reproducibility of the system (Figure S3).[39,40] From this experiment, no cross-contamination was observed, and product conversions ranged from 42% to 58%, providing evidence of the reliability of the system (Figure S4).

With the validated system in place, our focus shifted toward the creation of a combinatorial matrix library. Our strategy centered on utilizing regio isomers of methyl bromobenzoate (Figure 3) as the central core, strategically incorporating *ortho*, *meta*, and *para* substitutions to enhance the molecular diversity (cores **1**, **3**, and **4**, Figure 3B). In addition, methyl 6-bromonicotinate (core **2**, Figure 3B) was also included. To modify the ester, we utilized LiHMDS-mediated amide coupling, with four anilines containing electron-withdrawing groups (aniline **1**–**4**, Figure 2B). The (hetero)aryl bromide was modified via Negishi coupling, leveraging three distinct organozinc reagents to modify the (hetero)aryl bromide (**a**–**c**, Figure 3B).

Our efforts resulted in the successful synthesis of an array of 48 compounds, of which 46 were successfully isolated by high-throughput purification (HTP). In HTP, the priority is to obtain sufficient quantities with reverse-phase high-performance liquid chromatography (HPLC) for biological testing with the highest quality and efficiency, often at the expense of recovery and isolation. Therefore, we have included the LC-mass spectrometry (LCMS) conversion data for comparison.[41] This achievement represents a staggering success rate of 96%, translating to a productivity of 4 products per hour with a total run time of 12 h. This combinatorial experiment demonstrated that complex targets can be synthesized by assembling three fragments in a single experiment despite the challenges associated with the use of organometallic reagents in flow.[38,42]

Building upon the success of our initial library, we aimed to incorporate a photochemical transformation into the reaction sequence, recognizing the distinct advantages offered by flow chemistry in such reactions.[43,44] In our recent paper, we addressed reproducibility challenges in high-throughput photochemistry by leveraging amino radical transfer (ART) chemistry.[11,45] Notably, within the scope of our investigation, we discovered the compatibility of this chemistry with free amino alkyl pinacolboranes (amino-Bpins). This discovery offers the prospect of directly functionalizing the amine, thereby unlocking a broad spectrum of possibilities. The envisioned approach involves ART chemistry in the initial step and subsequently harnessing the nucleophilicity of the free amine to be applied in a wide range of transformations, such as amide coupling, reductive amination, alkylation, sulfonamide formation, nucleophilic aromatic substitution (SN_{Ar}), and urea formation with isocyanate, which would allow the exploration of multiple functional groups simultaneously in a single run. If successful, this approach could accelerate the optimization of linkers in PROTACs, because various analogs can be rapidly prepared with varying linker lengths, polarity, and rigidity.[34]

We began assessing the validation and reproducibility of the newly incorporated ASIA photoreactor at intermediate conditions, employing the model ART reaction with two distinct alkyl-Bpins: isopropyl and oxetane. Both reactions exhibited exceptional reproducibility, with standard deviations ranging from 0.35% to 0.46% over 12 and 24 repetitions (Figures S7–S9; Table S3). Additionally, we conducted a comprehensive cross-contamination test, reaffirming our earlier findings, revealing no instances of cross-contamination (Figure S10). To ascertain the system's limits, we examined various injection volumes using two injection ports (Figure S11). Through these experiments, we determined that the minimum injection volume per line is 125 μL, resulting in a total slug of 250 μL of a 0.1 M solution. These findings provided crucial insights into the operational capabilities of the system.

With these parameters in hand, we proceeded to examine the residence time of the ART reaction with the pyrrolidine-Bpin, mindful of the maximum temperature constraint of 25°C due to the cryocontroller integrated into the photoreactor. Our initial investigation revealed that a residence time of 60 min (Table S4) in the first step resulted in the complete consumption of the starting material. Notably, the pyrrolidine-Bpin is supplied as a HCl salt, prompting us to explore whether increasing the morpholine equivalents was required to accommodate the salt and enhance the reaction. However, increasing the morpholine in the mixture showed a slight decrease in conversion (Table S5).

With the optimized conditions for the ART reaction, we proceeded with a direct in-line screening of coupling reagents for the subsequent amide coupling. Our analysis identified both hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) and 1,1′-carbonyldiimidazole (CDI) as superior coupling reagents for this purpose (Figure S12). To ensure complete conversion toward the amide, we employed 3 equiv acid and 6 equiv HATU and diisopropylethylamine (DIPEA), accounting for the excess morpholine (2 equiv) introduced in the previous step.

Having established a fully telescoped in-line sequence, we selected eight different amino-Bpin building blocks (**1**–**8**, Figure 4) to be combined with three acid counterparts to perform a matrix library combining photochemical ART and amide coupling (Figure 4). The library exhibited a success rate of 88%, with 21 out of 24 compounds successfully isolated. **Bpin 4** displayed

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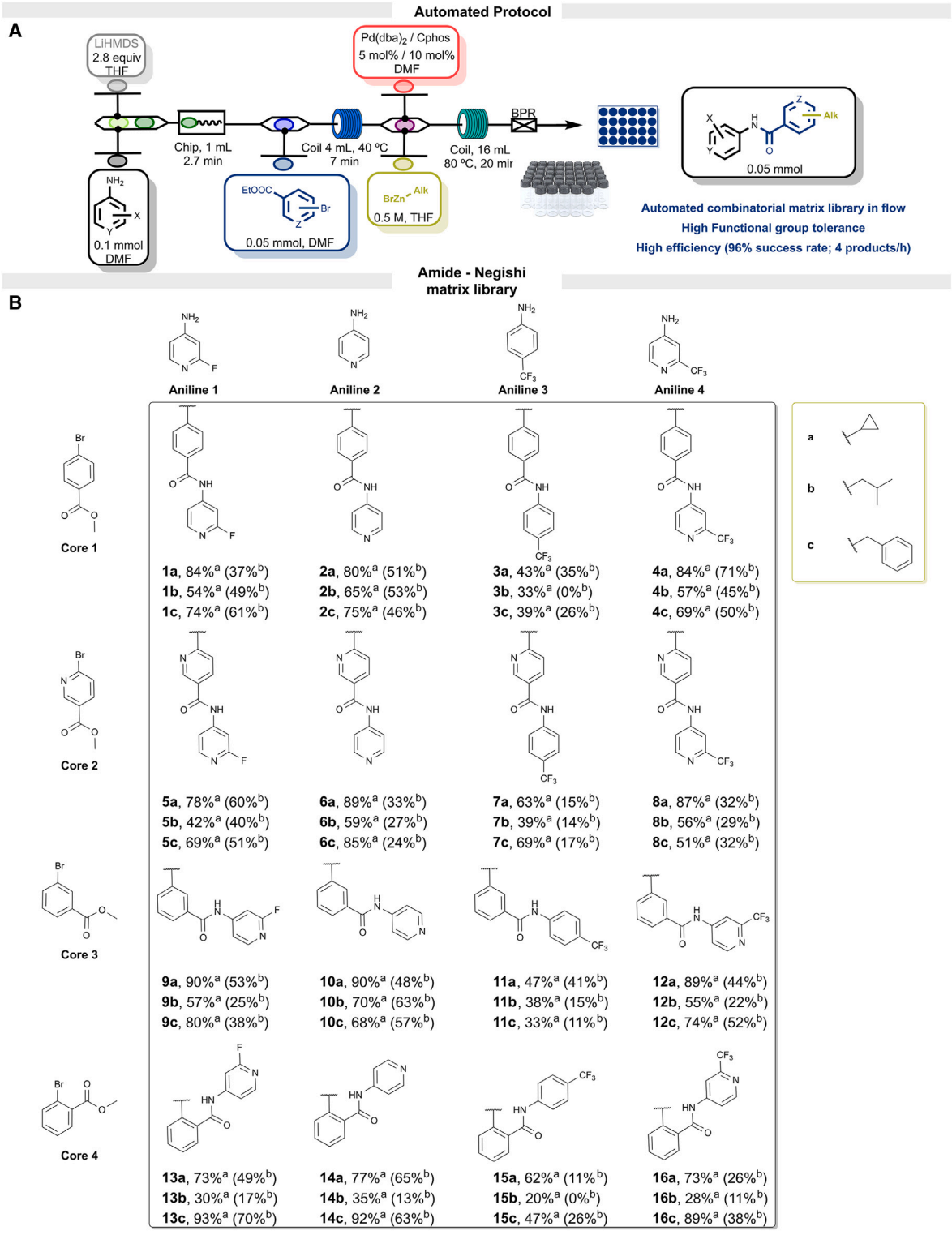

Figure 3. Combinatorial matrix library of 48 compounds with LiHMDS-mediated amide coupling telescoped with Negishi cross-coupling

(A) Schematic overview and picture of the 6-inlet ASIA system with 6 pumps, a chip reactor, 2 heated coil reactors, a BPR, and an automated collector.

(B) The combinatorial matrix library with 4 anilines, 4 bromo (hetero)aryl esters, and 3 organozinc reagents.

[a]LCMS conversion.

[b]Isolated yield by HTP.

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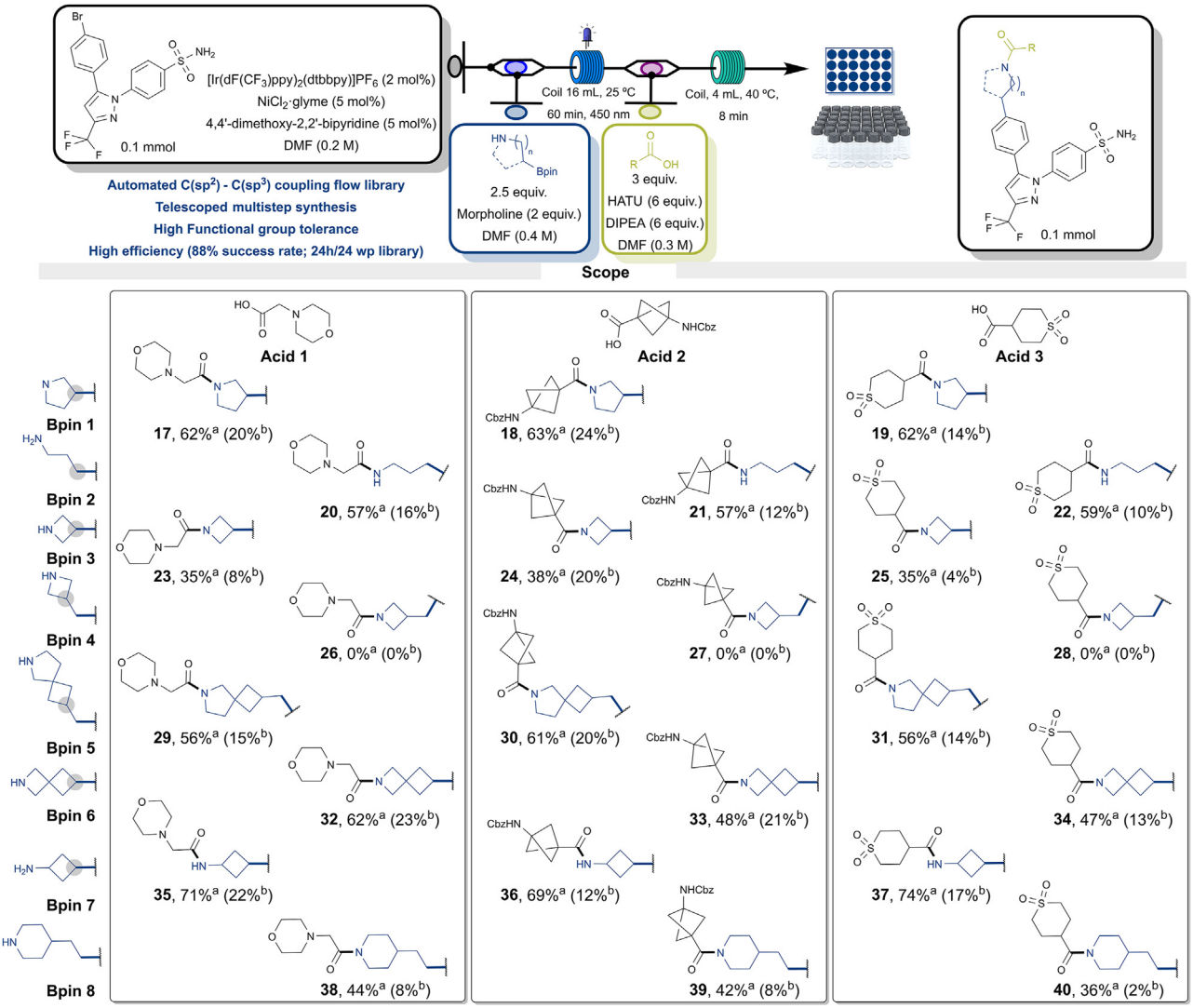

Figure 4. Combinatorial matrix library of ART chemistry telescoped with amide coupling with HATU

8 amine Bpins were combined with 3 acids and resulted in 24 unique products with a success rate of 88%.

[a]LCMS conversion.

[b]Isolated yield by HTP.

no conversion toward the desired product (**27–29**), likely due to the unstable nature of methyl-azetidine radical ring opening via the radical clock reaction.[46] Nonetheless, all the other amino-Bpins demonstrated moderate to good conversions and were isolated successfully.

The total runtime of the library was 24 h, resulting in an average of one product per hour. The primary limitation in productivity was that the second reaction only commenced after the first one entered the second coil reactor. Productivity could be further enhanced upon optimization of the volume of carrier fluid between reaction slugs, allowing the execution of more reactions in a single line.

To further demonstrate the potential of this workflow accessing a broader chemical space, additional chemistry was conducted following the ART step. Utilizing the exact same setup and reaction conditions, with the addition of one more injection line to introduce either the reducing agent or a base, we focused initially on reductive amination. Common reducing agents for both aldehydes and ketones were screened directly in flow (Figure S14). Among these agents, only sodium cyanoborohydride demonstrated full conversion to the desired products, being fully soluble and compatible with DMF. To accommodate the diverse chemistry, the coil reactor was maintained at 60°C.

The reductive amination achieved full conversion for both aldehydes (Figure 5, **41** and **42**) and ketones (**43–48**). Subsequently, utilizing the same setup and reaction conditions, sulfonamide formation (**49–54**), alkylation (**41** and **55–57**), and SN_{Ar} (**59–61**) were tested with DIPEA in the fourth line, demonstrating conversion to the desired product across

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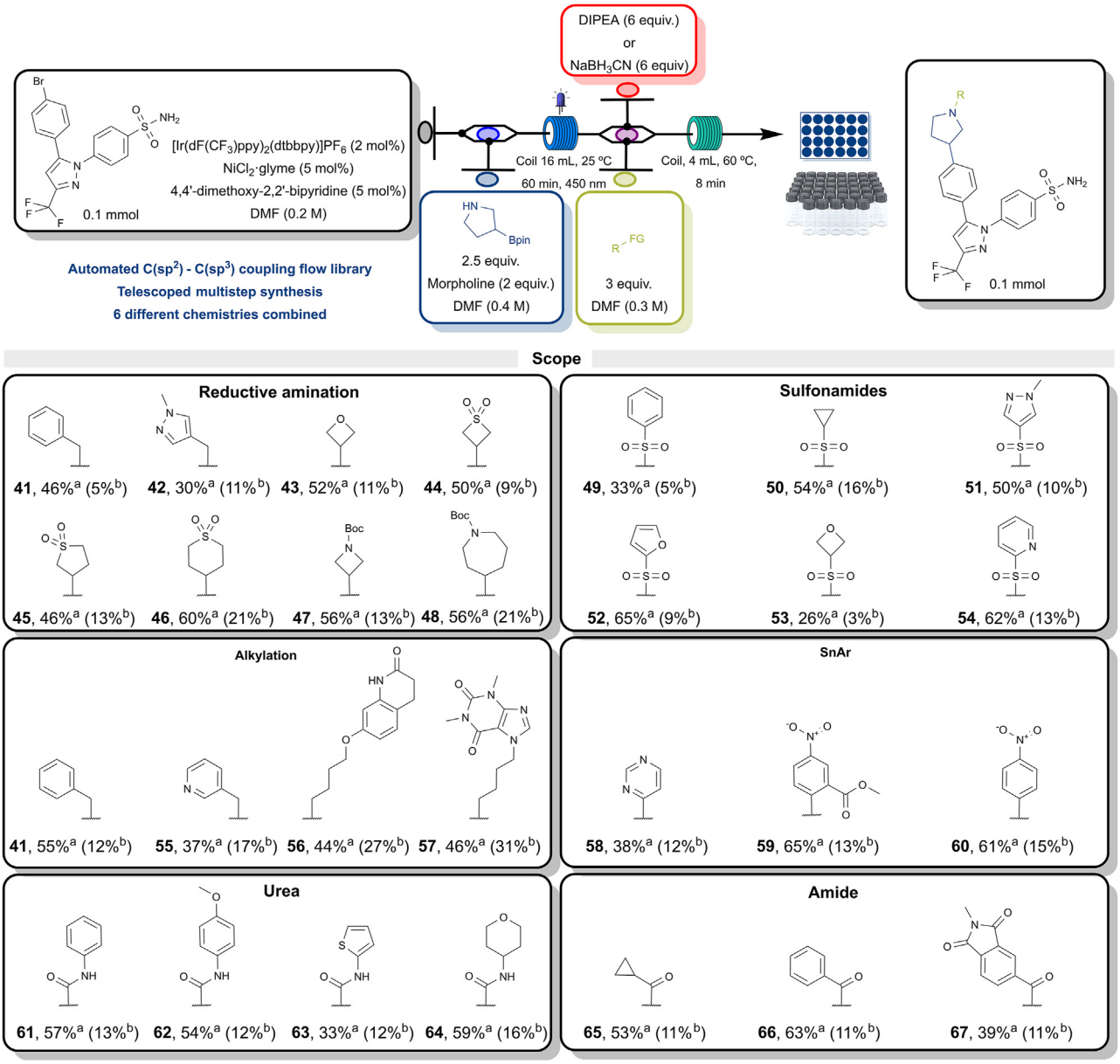

Figure 5. Combinatorial matrix library of ART coupling telescoped with 6 different chemistries

Reductive amination: aldehyde or ketone (3 equiv) with $NaBH_3CN$ (6 equiv). Sulfonamide formation: sulfonyl chloride and sulfonyl fluoride (3 equiv) with DIPEA (6 equiv). Alkylation: alkyl bromide (3 equiv) with DIPEA (6 equiv). Nucleophilic aromatic substitution (SN_{Ar}): chloro- or fluoro-(hetero)aryl (3 equiv) with DIPEA (6 equiv). Urea formation: isocyanate (3 equiv). Amide formation: acid (3 equiv) with HATU and DIPEA (6 equiv).

[a]LCMS conversion.

[b]Isolated yield by HTP.

all the reactions. While urea formation with the isocyanate (**61**–**64**) necessitated shifting one line, the setup remained the same three-line configuration required for amide coupling (**65**–**67**).

This multistep library exemplifies the integration of modern metallaphotoredox catalysis with robust and widely applied transformations in medicinal chemistry, maximizing the potential of the approach and generating structural diversity in a single library (Figure 5).[42] We prepared matched molecular pairs, molecules differing only by a specific structural feature, such as compounds **41**, **49**, **61**, and **66**.[47] In these examples, the aryl group is linked by different functional groups, which could modulate the basicity of the nitrogen and the hydrogen bond donor properties of the molecule. These variations can influence binding properties to the target, providing valuable information for SAR studies.

Additionally, we demonstrated the exploration of different amide bioisosteres, such as sulfonamides, ureas, and

nitrogen-containing heterocyclic systems, prepared via sulfonylation, nucleophilic addition to isocyanate, or SN_{Ar}.[48] This experiment showcases our approach's capability to generate structurally diverse compounds in a single run, enabling the exploration of various properties relevant to building SARs.

Conclusions

In conclusion, we have developed a robust and versatile method for efficiently producing compounds in an assembly line fashion. This approach enables multivectorial exploration and incorporates up to eight different chemistries. By combining different fragments and reactions in a single experiment, we can construct a diverse set of compounds in library format, offering flexibility through the flow setup. This method allows precise modulation of each vector and central scaffold of a hit molecule, making it highly advantageous for exploring a wider chemical space relevant to SAR studies in drug discovery programs. We have demonstrated the broad applicability of this approach through various experiments, including established chemistries, metal-catalyzed transformations, and modern metallaphotoredox couplings. This assembly line synthesis in flow has clear applications in small-molecule drug discovery, PROTAC synthesis, and fragment-based drug discovery, as these areas of research often involve linking fragments. The versatility of our approach makes it particularly valuable in these contexts.

EXPERIMENTAL PROCEDURES

Details regarding the experimental procedures can be found in the supplemental experimental procedures.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jesús Alcázar (jalcazar@its.jnj.com).

Materials availability

Full experimental procedures are provided in the supplemental information.

Data and code availability

All of the data supporting the findings of this study are presented within the article and the supplemental information. Source data are provided with this paper.

ACKNOWLEDGMENTS

We would like to thank Peter Buijnsters, Alexander Jones, Pavel Ryabchuk, and Jennifer Morvan for the meaningful scientific discussion. We would like to express our gratitude to Andrew Mansfield and Leandro Carvalho from Syrris for their support with the system. Additionally, we want to thank the European Union for funding under the PhotoReAct Project, H2020 Marie Sklodowska-Curie grant agreement no. 956324 (MSCA ITN: PhotoReAct).

AUTHOR CONTRIBUTIONS

J.A. managed and supervised the project; B.P. led the project and executed most of the experiments; I.A. performed the initial optimization and cross-contamination studies; S.C., M.L.L., J.M., and J.E.G. contributed to significant scientific discussion and suggestions for the direction of the project; E.P. enabled the first reaction combination of LiHMDS amide coupling with Negishi; R.R. performed the final HTP of compounds; B.C.A. characterized the NMRs of selected compounds; J.A. and B.P. co-wrote the manuscript with input from I.A. All authors discussed the results and reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.checat.2024.101118.

Received: July 31, 2024
Revised: August 6, 2024
Accepted: August 23, 2024
Published: September 23, 2024

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