Assembly line library synthesis in flow: A multistep and multivectorial approach

Brenda Pijper,* Irini Abdiaj, Javier Mazuela, Maria Lourdes Linares, José Enrique Gómez, Raquel Rodriguez, Belén Chaves Arquero, Eduardo Palao, Santiago Cañellas and Jesús Alcázar*

Janssen Research and Development, Janssen-Cilag, S.A., C/ Jarama 75, Toledo, Spain.

Fax: +34 925245771; Tel: +34 925245750; E-mail: jalcazar@its.jnj.com.

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 relationship (SAR) by concurrently exploring multiple vectors. Our method incorporates up to eight different synthetic methodologies, including established chemistries, metal-catalysed 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 exploration of the chemical space in drug discovery programs.

Keywords: Automation, Continuous flow, Library synthesis, Drug discovery, Multistep synthesis, Multivectorial exploration, Photoredox,

Introduction

Organic synthesis is a crucial part of the multidisciplinary process of drug discovery.[1-3] In recent years, there has been a growing interest in rapidly generating analogue libraries in pharmaceutical companies. To accelerate medicinal chemistry programs, automated synthesis protocols have emerged as a promising approach.[4-6] However, most automated library synthesis approaches have been focused on single-step methodologies, exploring one vector at a time.[7-12] 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.[13]

One successful tool for automated multistep synthesis is continuous flow chemistry, which has been used for the preparation of selected active pharmaceutical ingredient (API) and sets of close analogues by means of multistep synthesis.[14-17] There are three distinct approaches for multistep synthesis in flow: linear, cyclic, and radial (Fig. 1).[18-20] 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 (Fig. 1). This concept was pioneered by Dr. Djuric and colleagues at Abbott Laboratories using 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.[21-23] Subsequent research by Prof. Jamison showed the feasibility of modular flow for the preparation of pyrazole cores.[24]

Flow chemistry for modular multistep synthesis for library creation offers the potential for rapid generation of analogues in a 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 (SAR), by considering additive effects and the construction of matched molecular pairs of different bioactive compounds.[25] Furthermore, this approach would facilitate linker connectivity exploration for fragment-based drug design[26-28], and proteolysis targeting chimeras (PROTAC's).[29,30]

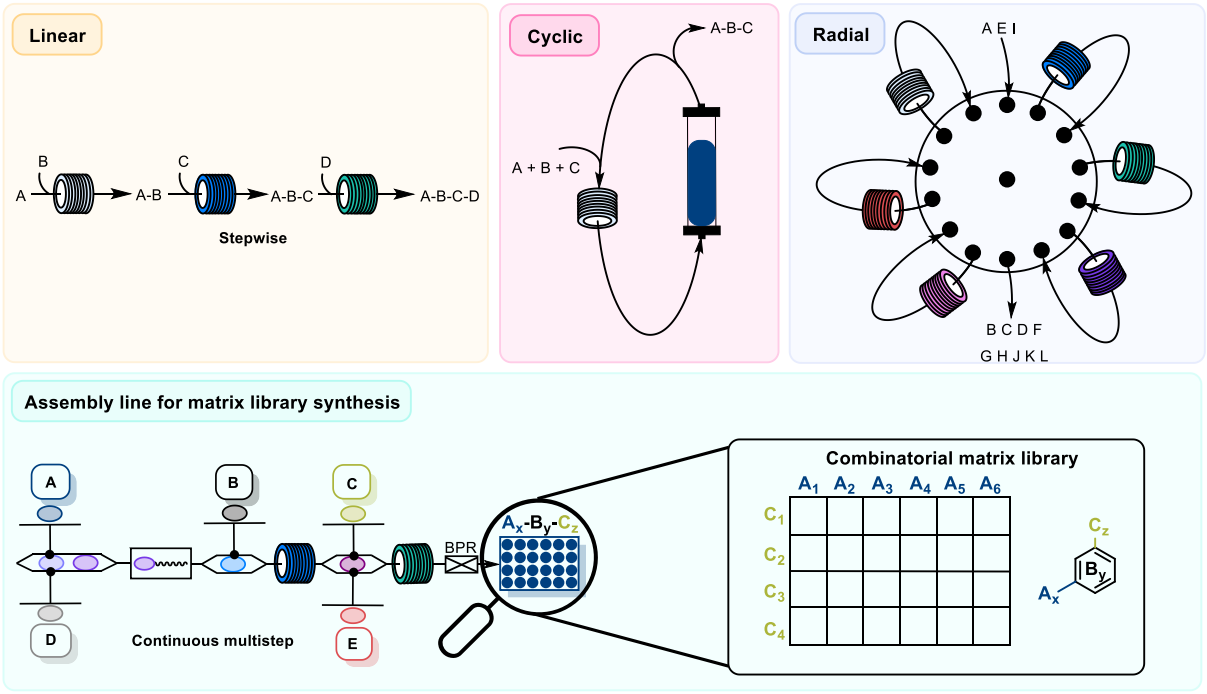

Figure 1, Previous work described for automated synthesis. Linear: Stepwise synthesis with intermediate purification. Cyclic, which is the synthesis of biopolymers such as peptide synthesis. Radial: combination of cyclic and linear synthesis approaches where flow modules surrounding a central core require 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.

Results and discussion

To investigate this approach, we opted for the Asia flow system from Syrris (Fig. 2). This automated flow equipment features 6 injection ports that enable the combination of up to six different reagents in line under nitrogen atmosphere.[31] 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.

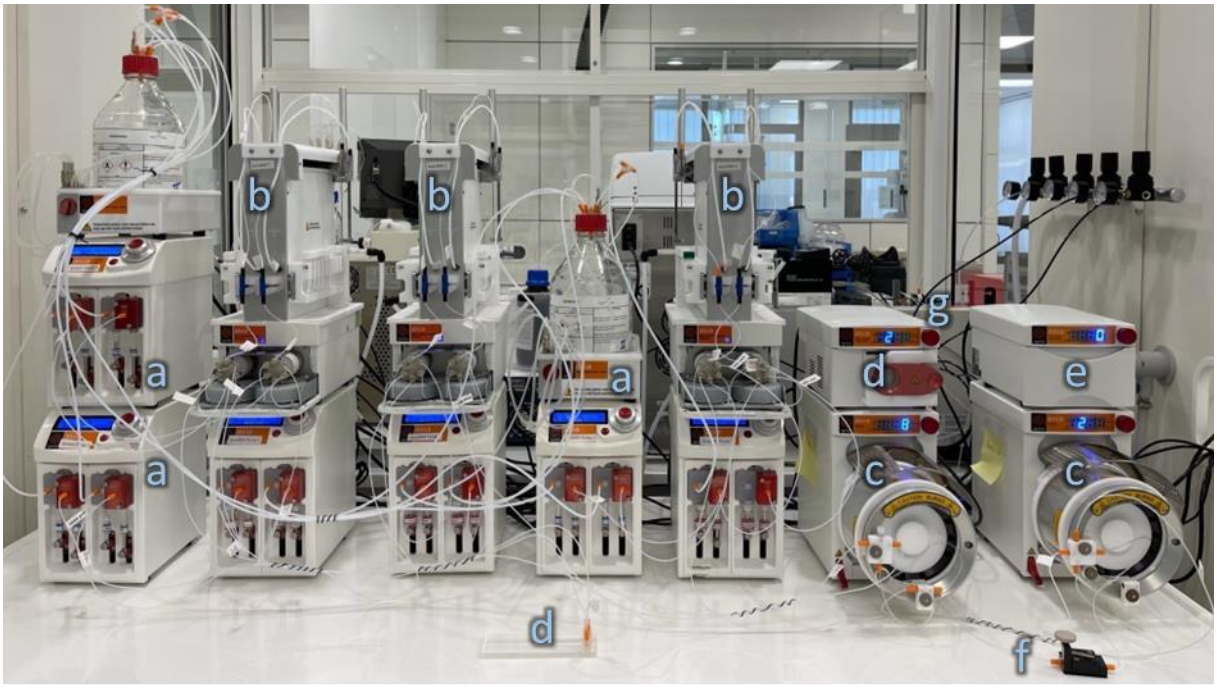

Figure 2, the ASIA system in the setup with (a) 6 syringe pumps, (b) Autorim with total of 6 injectors, (c) 2 coil reactors for thermal reactions, (d) chip and chip reactor for controlled temperature, (e) pressure controller, (f) back pressure regulator (BPR), and (g) automated collector (depicted at the back).[31]

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 Dr. Djuric, we explored various perfluorinated solvents and injected slugs containing 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.[32] Similar behaviour was observed when higher alkanes were employed as carrier solvent (see SI Table S1 and S2). Pleasantly, when DMF was used as carrier solvent, no carryover effect was observed, and dispersion could be effectively controlled by the system (see SI Fig. 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)[33] and Negishi coupling.[11,34] To assess 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 catalytic system at intermediate conversion to evaluate the robustness and reproducibility of the system (SI Fig. S3).[35,36] From this experiment, no cross-contamination was observed, and product conversions ranged from 42-58%, proving evidence of the reliability of the system (SI Fig. S4).

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

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 for biological testing with the highest quality and efficiency, often at the expense of recovery and isolated yield. Therefore, we have included the LCMS conversion data for comparison.[37] 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.[34,38]

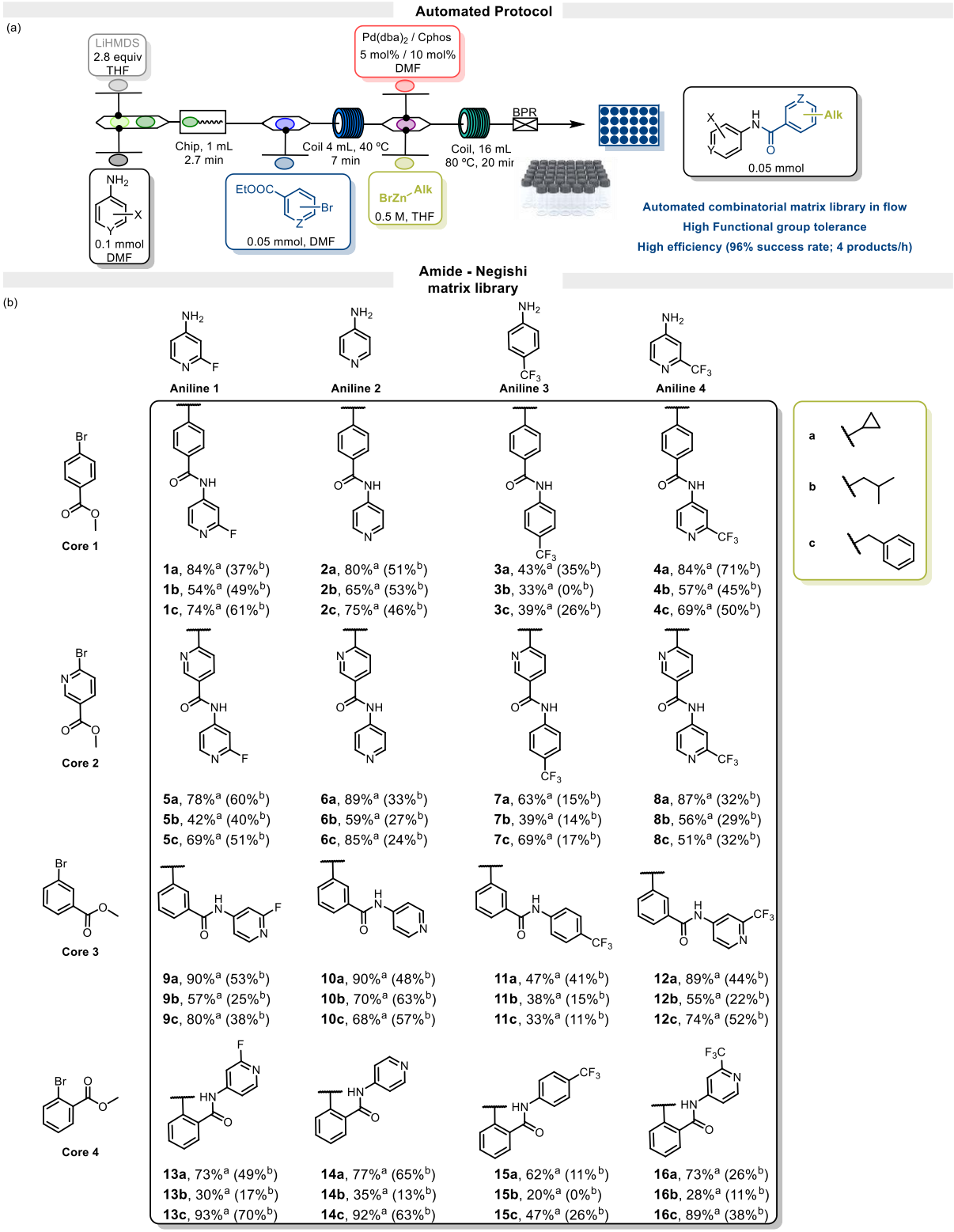

Figure 3, (a) schematic overview and picture of the 6-inlet ASIA system with 6 pumps, a chip reactor, 2 heated coil reactors, back-pressure regulator (BPR) and automated collector. (b) The combinatorial matrix library with 4 anilines, 4 bromo (hetero) aryl esters, and 3 organozinc reagents. [a] LCMS conversion [b] Isolated yield by HTP

Building up on 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.[39,40] In our recent paper, we addressed reproducibility challenges in high-throughput photochemistry by leveraging amino radical transfer (ART) chemistry.[12,41] 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 the 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 (SnAr), and urea formation with isocyanate. Which would allow the exploration of multiple functional groups simultaneous in one single run. If successful, this approach could accelerate optimization of linkers in proteolysis targeting chimeras (PROTACs). Because various analogues can be rapidly prepared with varying linker length, polarity, and rigidity.[30]

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 0.35-0.46% over 12 and 24 repetitions (SI Fig. S7, S8, S9 and table S3). Additionally, we conducted a comprehensive cross-contamination test, reaffirming our earlier findings, revealing no instances of cross-contamination (SI Fig. S10). To ascertain the system's limits, we examined various injection volumes using two injection ports (SI Fig. 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 an 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 ºC due to the cryo controller integrated into the photoreactor. Our initial investigation revealed that a residence time of 60 minutes (SI Table S4) in the first step resulted in 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 would enhance the reaction. However, increasing the morpholine in the mixture showed a slight decrease in conversion (SI 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 (SI Fig. S12). To ensure complete conversion towards the amide, we employed 3 equivalents of acid and 6 equivalents of 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**, Fig. 3) to be combined with three acid counterparts, facilitating the execution of a matrix library utilizing the photochemical ART followed by amide coupling (Fig. 3). The library exhibited a success rate of 88%, with 21 out of 24 compounds successfully isolated. **Bpin 4**, displayed no conversion towards the desired product (**27**-**29**), likely due to the unstable nature of methyl-azetidine radical ring-opening *via* radical clock reaction.[42] Nonetheless, all the other amino-Bpins demonstrated moderate to good conversions and were isolated successfully.

The total run-time of the library was 24 hours, 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.

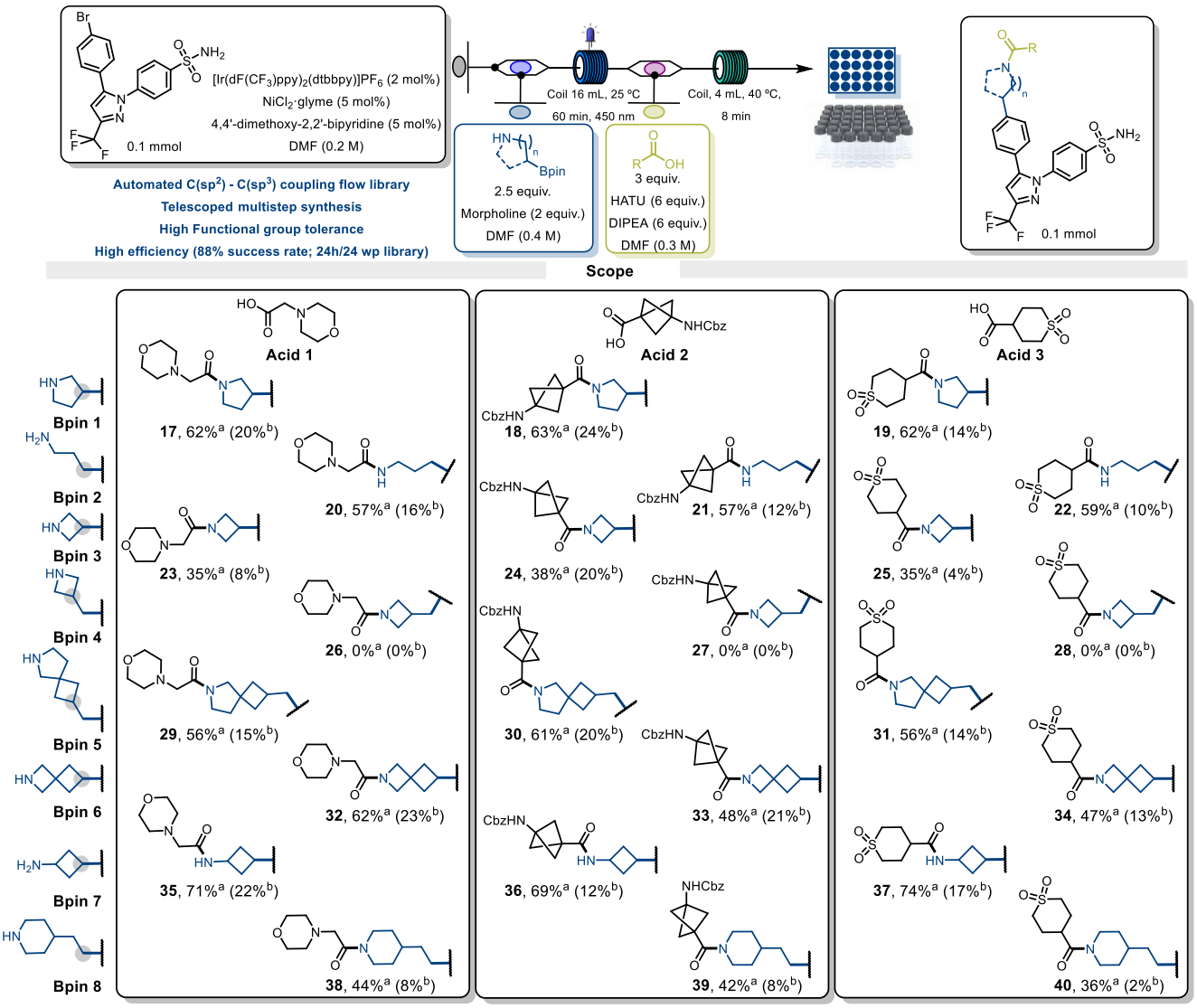

Figure 4, Combinatorial matrix library of ART chemistry telescoped with amide formation with HATU. Where 8 free amine bpins combined with 3 acids resulted in 24 unique products with a success rate of 88%. [a] LCMS conversion [b] Isolated yield by HTP

To further demonstrate the potential of this workflow to access 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 focussed initially on reductive amination. Common reducing agents for both aldehydes and ketones were screened directly in flow (SI Fig. 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 ºC.

The reductive amination achieved full conversion for both aldehydes (Fig. 4, **41, 42**) and ketones (**43**-**48**). Subsequently, utilizing the same setup and reaction conditions, sulfonamide formation (**49**-**54**), alkylation (**41**, **55**-**57**), and nucleophilic aromatic substitution (**59**-**61**) were tested with DIPEA in the fourth line, demonstrating conversion to the desired product across all the reactions. While the urea formation with the isocyanate (**61**-**64**) necessitated shifting one line, the setup remained the same 3-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.[38] We prepared matched molecular pairs, molecules differing only by a specific structural feature, such as compounds **41**, **49**, **61**, and **66**.[43] 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 the 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 nucleophilic aromatic substitution.[44] 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 structure-activity relationships (SAR).

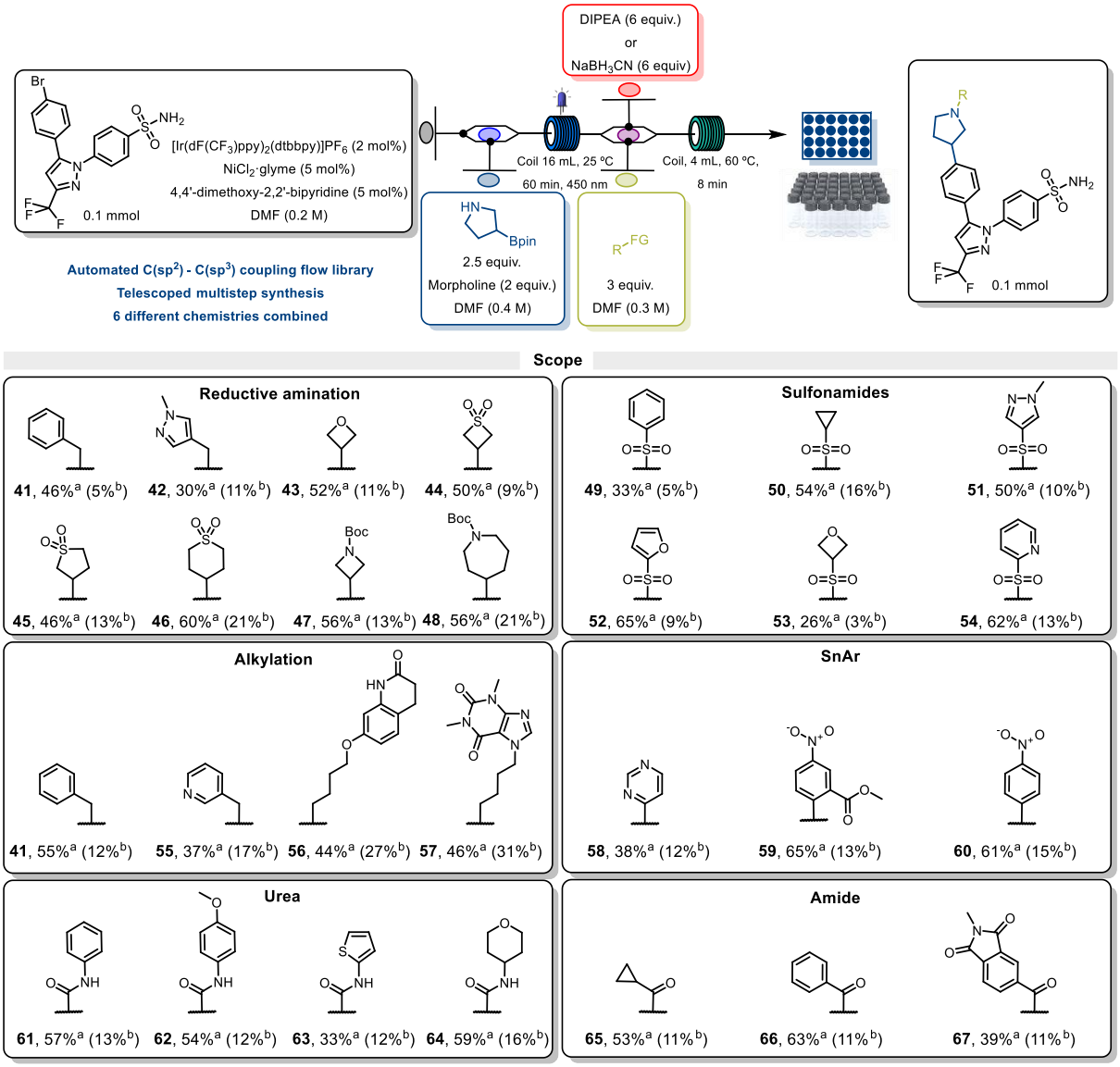

Figure 5, Combinatorial matrix library of ART chemistry telescoped with 6 different chemistries. Reductive amination: aldehyde or ketone (3 equiv.) with $NaBH_3CN$ (6 equiv.). Sulphonamide formation: Sulphonyl chloride and sulphonyl fluoride (3 equiv.) with DIPEA (6 equiv.). Alkylation: alkyl bromide (3 equiv.) with DIPEA (6 equiv.). Nucleophilic aromatic substitution (SnAr): 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

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 structure-activity relationship (SAR) studies in drug discovery programs. We

have demonstrated the broad applicability of this approach through various experiments, including established chemistries, metal-catalysed 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

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, J. 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.

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Data availability

The authors declare that the data to support the findings of this study are available within the paper and the Supporting Information (SI). Source data are provided with this paper.

Author Contributions

J.A. managed and supervised the project; B.P. lead 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., J.E.G., contributed with significant scientific discussion and suggestions for the direction of the project. E.P. enabled first reaction combination of LiHMDS amide coupling with Negishi. R.R. performed final HTP of compounds. B.C.A. characterized the NMRs of selected compounds. J.A. and B.P. co-wrote the manuscript with input of I.A. All authors discussed the results and reviewed the manuscript.

Declaration of interest

The authors declare no competing interests.

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