Symeres

Making Molecules Matter. Together.

DRUG DISCOVERY, SYNTHETIC CHEMISTRY

Accelerating chemical innovation: unveiling Symeres' Parallel Chemistry

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Abstract:
The pursuit of efficiency and speed in drug discovery has fueled a transformation of chemical synthesis methodologies. Parallel chemistry can, when applied with precision, displace traditional linear approaches, which are characterized by sequential experimentation. This shift has enabled researchers to explore vast chemical spaces simultaneously, expediting the discovery of novel compounds for diverse applications. Here, we delve into the philosophy and methodologies of parallel chemistry at Symeres, showcasing its transformative potential in accelerating chemical innovation.

Introduction

In the realm of drug discovery, the quest to accelerate design–make–test cycles continues. Traditional linear approaches to analog synthesis, characterized by sequential experimentation, can require significant time and resource investments to develop robust structure–activity (SARs) and structure–property relationships (SPRs). However, in recent decades, the emergence of parallel chemistry has enabled multiplexing drug-discovery cycles and providing clustered SARs and SPRs with each design–make–test cycle. Thus, parallel chemistry is able to bypass the iterative process of synthesizing individual compounds and screening these in small batches. In contrast, parallel chemistry enables the routine synthesis and screening of tens to hundreds of compounds simultaneously during medicinal chemistry programs.

In this paper, we delve into the philosophy and methodologies of parallel chemistry that we routinely use in Symeres. We describe the typical workflow of a parallel chemistry project, comprising six distinct steps, as depicted in Figure 1. Each of these steps receives detailed elucidation in subsequent sections. Leveraging this parallel chemistry platform, we designed and synthesized an innovative 75,000 compound library for the European Lead Factory (ELF). Today, this extensive collection of compounds, known as SymeGold, is accessible for screening by our strategic collaborator, **Axxam** in Milan. Drawing upon the expertise garnered from the ELF project, as well as numerous standalone initiatives and multiple multidisciplinary medicinal chemistry projects, Symeres has routinely integrated parallel chemistry into the workflow of our projects. Breakthroughs in multiple projects were due to the support of parallel medicinal chemistry. Whether through the serendipitous discovery of exceptionally potent and selective compounds, or the generation of structural-biology-guided arrays in conjunction with computational chemistry, the "more shots on goal" impact of parallel medicinal chemistry can be very powerful.

Figure 1. Workflow of a parallel chemistry project

Design and enumeration

To effectively assemble a compound library, it's crucial to carefully examine the synthetic pathway. Typically, one or more building blocks, or scaffolds, are first synthesized and then modified using parallel chemistry to create a diverse range of compounds (see Figure 2). Ideally, the greatest diversity is achieved in the final stages of synthesis. If this isn't possible with the initial approach, alternative pathways that allow for diversification at a later stage should be considered. In such cases, an expert from the Parallel Chemistry Department can suggest other methods for late-stage modification. These alternative methods can then be tested during a pilot study, known as the validation stage of the project.

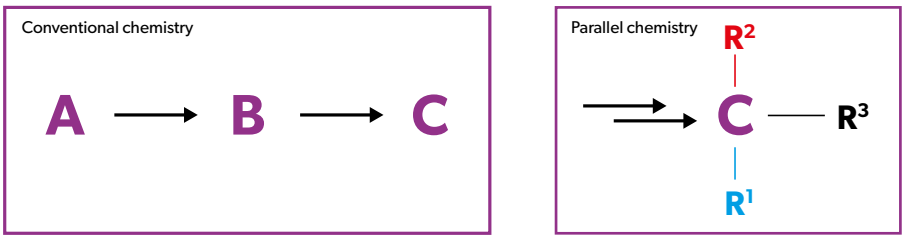


Figure 2. Example of general synthetic route that is highly amenable to parallel chemistry

The second stage in preparing a compound library using parallel chemistry is the establishment of clear objectives and criteria for compound selection. Whether the goal is to identify hit or lead compounds with specific biological activity, explore chemical space to claim intellectual property, or optimize properties such as solubility or stability, defining precise objectives is essential for guiding the design process. Additionally, establishing criteria for compound selection based on structural diversity and synthetic feasibility is crucial for prioritizing compounds within the library. Symeres offers 2,800 diverse building blocks, including nearly 900 amines, 600 carboxylic acids, and 400 aldehydes and ketones, all ready for scaffold decoration. Additionally, more than 20,000 building blocks can be sourced from reputable commercial suppliers to expand this collection. Using an optimized KNIME workflow, we create a virtual library of structurally diverse compounds. Potential target molecules from this virtual library are then evaluated based on their physical and chemical properties or through computer-aided drug design (CADD) with input from our computational scientists. Working with the client, we select target molecules for synthesis, which are then given to the chemist for further development.

Validation stage

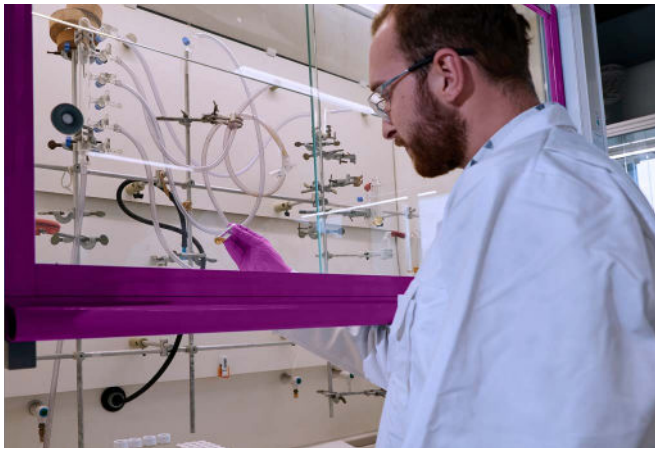
A key requirement is having reliable synthetic methods to efficiently create diverse compound libraries. It's also essential to ensure these synthesis protocols are scalable and reproducible to produce libraries of sufficient size and quality for screening. During the validation stage, the synthetic route for the target compounds is explored and optimized for parallel chemistry. Despite progress in novel parallel synthetic methods and improvements in existing reactions, not all synthetic transformations are easily applicable to robust parallel synthesis. To avoid losing valuable building blocks and to improve success rates in library synthesis, it's crucial to optimize chemistry and thoroughly explore its scope. During validation, various conditions for chemical transformations are tested in parallel, different scaffolds and building blocks are evaluated, and purification methods for final compounds are refined. Where certain building blocks are problematic due to low reactivity or purification issues, they may need to be removed or replaced in the library or treated separately from the main array. The results from the validation stage guide the final design decisions for the library.

Library production stage

While utilizing robotics and automation for compound library preparation is feasible and at times advantageous, there are notable drawbacks associated with such systems. These drawbacks include the considerable time required for setting up, the limited flexibility of these systems, and their susceptibility to equipment failures, especially when encountering poorly soluble building blocks or starting materials. Additionally, while robotics excel in the preparation of very large compound libraries, exceeding say 10,000 compounds, the prevailing trend is toward crafting smaller, focused libraries characterized by improved diversity.

Recognizing these challenges and industry trends, Symeres has adopted a semiautomated approach. In this approach, the setting up of reactions is typically carried out manually, typically in reaction blocks, allowing for greater adaptability and precision. However, the purification process, as detailed in the next section, is executed in a fully automated manner. This hybrid approach strikes a balance between the efficiency of automation and the need for flexibility and adaptability in compound library preparation.

The parallel chemistry infrastructure at Symeres revolves around a modular 24-well plate format, which is tailored to ensure seamless scalability to allow synthesis through hundreds of reactions. Within each well plate position, we use an 8 mL glass vial, optionally sealed with a Teflon-lined cap capable



of withstanding organic solvents and corrosive reagents. The versatility of this setup extends from cryogenic temperatures as low as −78°C to elevated temperatures reaching 150°C, facilitated by directly cooling or heating the plates. Such adaptability renders it an ideal platform for conducting parallel syntheses, yielding final compound quantities ranging from 10 to 100 mg.

In addition to our proprietary 24-well plate format, Symeres is equipped with parallel reaction systems sourced from Radley, which prove invaluable for arrays requiring a slightly larger scale of operation (100 mg or more). Moreover, for air- or moisture-sensitive chemistry, a glovebox is used to ensure the integrity of such sensitive reactions.

In configuring reactions within our 24-well plate format, we have determined that the most convenient approach involves dissolving building blocks and reagents in the solvent of choice, then adding them to the reaction vials by utilizing multichannel pipettes or repeater pipettes. Stirring and heating of the reaction are facilitated by standard laboratory electric heating plates or shakers.

The workup procedure for the reaction is kept to a minimum, typically limited to filtration before proceeding to purification

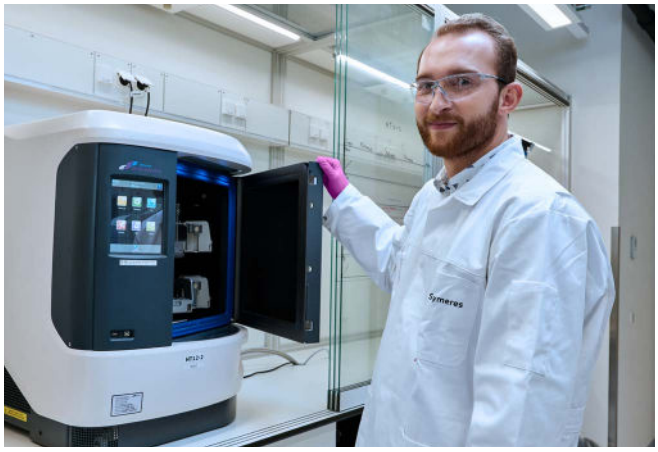
via preparative HPLC. On occasion, an aqueous workup is employed, followed by filtration over a phase separator to eliminate any impurities or residual reagents with high aqueous solubility.

Purification stage

Automation has transformed the purification of molecules, making it more efficient and reliable. These advanced technologies are now essential in modern research labs, enabling scientists to purify molecules quickly, accurately, and consistently.

In earlier combinatorial chemistry, chemists used traditional techniques like precipitation, solid-phase extraction, and solid-phase synthesis. Today, modern methods offer significant advancements in convenience and effectiveness. Automated mass-directed HPLC, for instance, has become a preferred method for purifying compounds, streamlining processes, and improving efficiency.

Symeres has six fully automated preparative HPLC-MS systems alongside two preparative SFC systems, enabling the purification of over 250 molecules per day at maximum capacity. Notably, this setup is also adept at isolating compounds that lack UV activity, further expanding its versatility and applicability.
Following purification via HPLC, product peaks exhibiting the desired m/z values are collected and concentrated under reduced pressure, utilizing centrifugal evaporators that allow for the simultaneous evaporation of hundreds of tubes or vials.



Analytics and QA/QC

Following isolation of the final compounds, aliquots of the purified substances are dissolved and transferred to a 96-well plate and subsequently subjected to liquid chromatography–mass spectrometry (LC-MS) for purity assessment. Compounds lacking sufficient UV absorbance can alternatively undergo analysis using CAD, ELSD, or a combination of methods to ensure accurate purity assessment. This step also involves comparing and confirming the experimental mass-to-charge ratio (m/z) with the calculated value. The structural elucidation of selected molecules, or occasionally all molecules, is then conducted utilizing 1H nuclear magnetic resonance (NMR) spectroscopy or other spectroscopic techniques.
Upon ensuring that the analytical results meet predefined specifications, all final compounds and their corresponding analytical data undergo quality assurance/quality control (QA/QC) procedures before being authorized for shipment. This rigorous QA/QC process involves thorough scrutiny by an independent QA/QC employee, verifying that the compounds adhere to specified criteria and that the scientists have collected and reported data in accordance with our standard operating procedures.

Shipment

The concluding phase of the entire process involves evaluating the potency, metabolic stability, and other properties of the newly synthesized molecules, either at a collaborating facility or within our in-house Admescope ADME-Tox platform. Typically, compounds are lyophilized in barcoded vials and then manually transferred to the designated destination vial, tube, or even a preferred 96-well plate. Any remaining material is carefully stored at −20°C for future use. This procedure ensures the preservation of samples and facilitates their convenient handling and distribution for further analysis.

Cheminformatics

To simplify the management of libraries, Symeres has developed an infrastructure that combines commercial software packages with proprietary tools. This system allows scientists to monitor their chemistry projects effectively. Key to this setup are user-friendly templates that integrate seamlessly with analytical device software and Symeres' Revvity E-notebook system, which is used across all sites.
These templates serve as dynamic tools for monitoring the status of individual reactions and target molecules, including details such as the structure of final compounds, quantities employed in reactions, purity after purification via preparative HPLC, and the chemical yields obtained. Each reaction is labeled with a barcode, facilitating the seamless linkage of template data to the corresponding vials. After purification, the final compounds are once again housed in barcoded vials.
Furthermore, compounds are stored in designated locations, with their barcode information ensuring swift retrieval when needed. This organization not only facilitates efficient internal processes but also enables seamless shipping to external

collaborators and effective compound management for clients during the lifespan of larger projects.

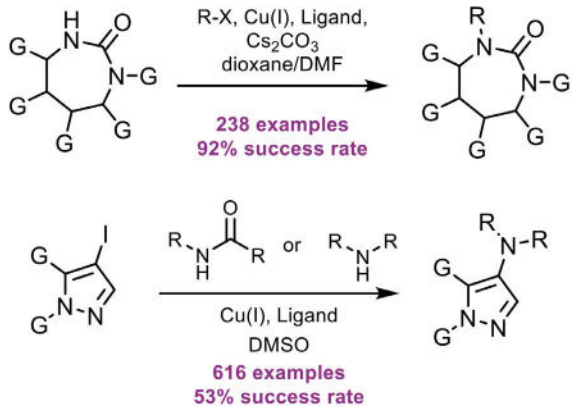
Types of chemistry

Although in theory there are few limitations to the type of chemical transformations that are compatible with parallel chemistry, we usually aim to apply synthetic transformations that are highly robust and known to have a broad scope and afford the desired compounds in high yield. Many reviews have been written on this subject and an extensive summary of this topic is beyond the scope of this paper.[1] However, the most common synthetic transformations can be grouped into one of the categories shown below:

1) **Condensation reactions:** Condensation reactions involve the combination of two or more molecules to form a larger molecule, often accompanied by the elimination of a small molecule such as water or alcohol. Examples of condensation reactions commonly used in parallel chemistry include the formation of amides, esters, and imines.
2) **Alkylation and acylation:** Alkylation and acylation reactions involve the transfer of an alkyl or acyl group, respectively, to a nucleophilic substrate. These reactions are widely used for functionalizing organic molecules and building molecular diversity into parallel chemistry libraries. Examples include Friedel–Crafts alkylation and acylation reactions, but also reductive aminations.
3) **Cross-coupling reactions:** Cross-coupling reactions involve the coupling of two different molecular fragments to form a new carbon–carbon or carbon–heteroatom bond. These reactions are powerful tools for creating molecular complexity and diversity in parallel chemistry libraries. Examples include Suzuki–Miyaura coupling, the Heck reaction, and Sonogashira coupling.
4) **Multicomponent reactions (MCRs):** MCRs involve the simultaneous reaction of three or more starting materials to generate a single product containing multiple functional groups. MCRs are powerful tools for rapidly assembling complex molecular architectures and increasing molecular diversity in parallel chemistry libraries. Examples include the Ugi reaction, the Passerini reaction, and the Biginelli reaction.

Symeres not only possesses expertise across the listed categories, but also has significant experience in executing parallel reactions for chemical transformations that are notorious due to their low yields or scope, such as the Ullmann reaction (Scheme 1). In the first example, substituted seven-membered cyclic ureas were coupled with a series of aryl halides using copper(I) chloride, a ligand, and cesium carbonate to afford the desired arylated final compounds in moderate to good yield and in high purity. Of particular note was the remarkably high success rate, defined as the ratio of shipped compounds to the intended target molecules, achieved within this compound library.

In the second example, a series of (substituted) amines and carboxylic amides were subjected to coupling with numerous 4-iodopyrazoles. Despite the modest success rate, we managed to produce over 600 examples, demonstrating our commitment to overcoming challenges and delivering results.



Scheme 1. Typical examples of Ullmann C–N cross-coupling reactions performed using a parallel approach

Conclusions

Parallel chemistry opens new avenues for speeding up innovations in drug discovery. While Symeres' investment in technology is a key part of our parallel chemistry approach, it's the combination of teamwork and accumulated experience that really determines project success. By nurturing experience, promoting teamwork, and focusing on effective communication, Symeres aims to maximize the potential of its services to achieve project success and deliver impactful outcomes for our clients.

[1]Dombrowski et. al., *J. Org. Chem.* **2022**, *87, 1880-1897*

For further inquiries or collaboration opportunities,
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