Trends in Chemistry

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Review

Multistep automated synthesis of pharmaceuticals

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Multistep automated pharmaceutical syntheses have been a long-term industrial goal. Automation not only enables the rapid and highly efficient preparation of organic compounds by eliminating the physical barriers, but also endow chemists with more time for critical thinking by relieving them from laborious work. With the growing demand for fast and efficient synthesis of active pharmaceutical ingredients (APIs) for drug development, several efforts have been made in various subfields to improve the universality and capability of automation in multistep synthesis. This review highlights the recent development of automated iterative synthesis, automated digitalized batch synthesis, automated solid-phase synthesis (SPS)-flow synthesis, automated radial synthesis, as well as automated multistep continuous-flow synthesis and how they complement each other in the automated synthesis of pharmaceuticals.

Automated synthesis of pharmaceutical: achievements and opportunities

Automation of organic synthesis is not just a rapidly growing research field, but also a long-term goal in chemical and pharmaceutical industries. By liberating chemists from the laborious and repetitive works that machines can handle with better efficiency, the development of lab automation has reformed the way chemists work, granting us more time to explore new ideas. With the removal of potential human errors and physical limitations, not only data with high accuracy and better reproducibility are collected with less time, but also the probability of accidents is reduced significantly, allowing even amateur operators to execute the automated synthesis. More recently, by integrating the thriving artificial intelligence (AI) technologies with automation, even the optimization of reaction conditions [1–3] and discovery of new reactions [4,5] can be automated.

With an automated synthesis platform, the preparation of organic molecules is substantially improved and accelerated, which is critical for drug developments and on-demand synthesis of **pharmaceuticals** (see Glossary), especially in this coronavirus pandemic. Historically, the emergence of automated organic synthesis can be dated back to the last century with the development of automated solid-phase synthesis (SPS) of peptides [6] and oligonucleotides [7]. Since then, prominent advancements were achieved in improving the efficiency of the SPS system and extending it to the synthesis of oligosaccharides [8]. In 2022, the automated synthesis of complex glycans remarkably reached 1080 units via multiplicative synthesis strategy [9]. Contrarily, the establishment of a universal automated synthesis platform for small-molecule pharmaceuticals is much more challenging because, unlike biopolymers with iterative backbones, most of the pharmaceuticals possess complex structures with different bond connections that often require multiple different transformations for their synthesis. Moreover, the underdevelopment of standardized software and versatile hardware hinders the widespread utilization of automated platforms. Despite the challenges, in the past decade, several groundbreaking accomplishments have been made toward the realization of multistep automated pharmaceutical synthesis.

Highlights

The development of iterative synthetic methodologies provides a new angle for retrosynthetic analysis. Remarkably, these methods are powerful for cross-coupling and subtly enable a standard and facile purification protocol to automation.

Universal synthetic software has been developed for automated translation of literature protocols to machine-readable codes. More importantly, it has established a prototype of protocol description and a classified database to record all the reactions, which is a foundation to further combine with AI technologies.

SPS-flow synthesis, on one hand, similar to immobilized reagents and catalyst, is easy to separate and purify using simple filtration, circumventing the incompatibility in continuous-flow synthesis. On the other hand, flow-based synthesis can enhance the efficiency and reproducibility of traditional batch-based SPS and open a facile path toward scale-up and automation.

Continuous-flow synthesis not only allows expansion of the available chemical space and safer handling of hazardous intermediates, but also provides precise control of the reaction, reliable reproducibility, and an end-to-end synthesis fashion, which are especially advantageous to automation.

A radial synthesis approach resolves several challenges in multistep continuous-flow synthesis and also allows both convergent and linear synthesis owing to its non-simultaneous and independent nature in multistep synthesis.

Given the overwhelming growth in this research area over the past decades, it is implausible to cover all examples of automated synthesis in this review. As such, this review aims to provide a big picture on the prospect of automation in multistep pharmaceuticals synthesis for the readers by focusing on the recent progress, with representative works, in different subfields, including iterative synthesis, digitalized batch synthesis, SPS-flow synthesis, radial synthesis, as well as multistep continuous-flow synthesis (MCFS), and how they complement each other towards the apex of automated pharmaceutical synthesis (Figure 1).

Automated iterative synthesis

Synthesizing molecules in an iterative fashion is attractive due to its high adaptability towards automation [10]. In fact, due to the use of similar building blocks, identical linking bonds, and general purification methods, the development of the automated iterative synthesis of peptides and oligonucleotides advanced smoothly and rapidly in the last century [6,11]. To realize the full automated synthesis of small pharmaceutical compounds, which are diverse in structures and bonding type, one promising approach is by developing a universal iterative methodology for C–C bond formation that connects versatile building blocks through an identical transformation under similar reaction conditions and platform setups.

In 2015, an automated iterative synthesis platform for 14 distinct classes of small molecules using the same process was developed by Burke and coworkers [12]. This approach is based on iterative **Suzuki-Miyaura (SM) coupling** between organohalides and boronic acids by using *N*-methyliminodiacetic acid (MIDA) boronates as the masking unit of boronic acids. SM coupling represents an ideal C–C bond formation approach in iterative synthesis due to its good functional group tolerability and universality [13]. To achieve the full automated synthesis, a catch-and-release purification protocol based on the difference of mobility on the silica gel chromatography with different eluent was also developed for the MIDA boronate intermediates. Overall, one automated iterative cycle involves three modules (Figure 2A): (i) a

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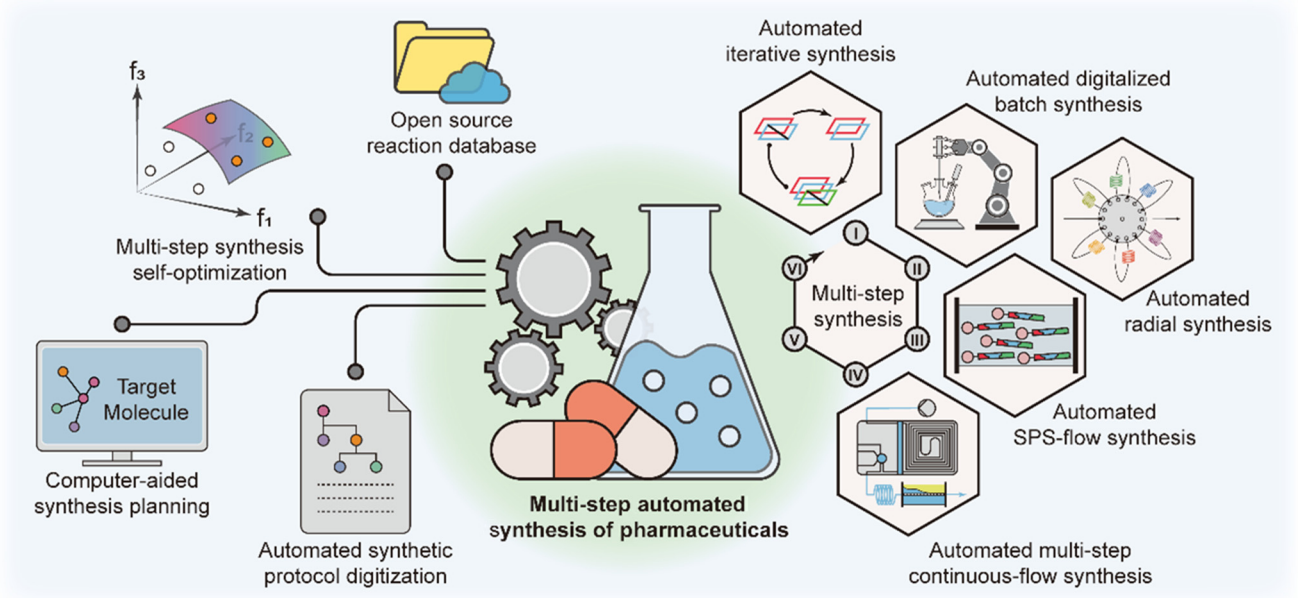

Figure 1. Outline of multistep automated synthesis of pharmaceuticals. Abbreviation: SPS, solid-phase synthesis.

deprotection module that converts MIDA boronate to boronic acid, (ii) a coupling module where boronic acid undergoes iterative SM coupling with organohalide bearing a MIDA boronate motif, and (iii) a purification module that purifies the newly formed MIDA boronate for the next iterative cycle. To demonstrate the practicality of this platform, the author utilized it to synthesize several Csp^2-rich planar pharmaceutical compounds (e.g., **PDE472**) and Csp^2-rich linear precursors that can be converted into Csp^3-rich complex polycyclic structures, such as secodaphnane core (Figure 2A) [12].

Despite the robustness of the SM coupling-based automated platform in iterative synthesis, it is to some extent limited by its C–Csp^2 bond-forming nature. The realization of the Csp^3-rich structure thus far has been achieved solely in a non-automation fashion and usually requires delicate design. In 2022, Aggarwal and coworkers reported an automated stereo-controlled assembly-line synthesis based on iterative boron **homologation reactions** for the construction of organic molecules with a Csp^3-rich backbone [14]. This strategy allows the iterative insertion of a methylene (from $BrCH_2Cl$) or chiral methyne (from organostannane) unit into the carbon chain of a boronic ester for the formation of iterative Csp^3–Csp^3 bond (Figure 2B) [15–17]. As the boronic ester moiety was preserved and transferred to the propagative terminal of the carbon chain to serve as the reactive center for the next iteration, no purification and deprotection of intermediates were required for the intermediates. Only an automated silica-plug filtration was needed before the next iteration to remove the solid by-products (LiBr and LiOTIB). It is worth noting that this automated synthesis was achieved with a commercial Chemspeed Swing Platform that is comparable in size to a glovebox and is connected to inert gas and oil supplies, thus allowing automaton of the air sensitive and low temperature chemistry. Utilizing this protocol, up to six homologations were achieved, with an average yield of 88% per iteration, to assemble the Csp^3–Csp^3 backbone of (±)-**kalkitoxin** (Figure 2B) [14]. Additionally, the subsequent amination, acylation, and methylation to yield the key intermediate of (±)-kalkitoxin were also automated.

Although homologation reaction is powerful for the formation of iterative Csp^3–Csp^3 bond with great stereo-control, the building blocks that were successfully automated are yet limited in types. Moreover, the one-atom-at-a-time addition mode hampers the access to organic molecules that possesses not a single carbon chain but versatile structures. In 2022, Burke and coworkers reported the use of tetramethyl *N*-methyliminodiacetic acid (TIDA) boronates as a hyper stable masking unit for boronic acid, which further expands the scope of automated iterative transformation to C–Csp^3 bond formation [18]. In general, the C–Csp^3 bond-forming reactions with boronates require the use of aqueous bases or highly nucleophilic reagents, which is incompatible with MIDA boronates [19–21]; this would lead to the deprotection of MIDA ligands and, consequently, uncontrollable oligomerization. In this context, the sterically hindered TIDA protecting group is hyper stable under the basic conditions of Csp^2–Csp^3 SM cross-coupling and the strong nucleophilic conditions of 1,2-metallate rearrangements for Csp^3–Csp^3 bond formation and, remarkably, it is even tolerant to *t*-BuLi. In addition, TIDA boronates retain the essences of MIDA boronates in automated iterative synthesis, such as the facile deprotection and the standardized purification [12]. This protocol enables facile automated assembly of increasingly complex small molecules by expanding the scope of accessible building blocks bearing different chemical structures in the iterative C–C bond formation. Using this iterative platform, the core Csp^3–Csp^3 scaffold of macrocyclic antifungal natural product sch725674 was successfully synthesized without human intervention via a sequential stereospecific 1,2-metallate rearrangement with bifunctional sulfoxide-TIDA boronates (Figure 2C). Furthermore, stereospecific Csp^2–Csp^3 cross-coupling reactions were also feasible in this platform and were utilized for the automated synthesis of natural product ieodomycin C.

Glossary

Chemical Assembly (ChASM) code: a digitalized synthetic procedure that is obtained by translating the literature synthetic protocols into an unambiguous and machine-readable scheme using chemical descriptive language (χDL).
ChemPU: Chemical Processing Unit, the latest version of the Chemputer.
GraphML code: the graphical descriptor of the configuration and physical connectivity of the hardware modules.
Grignard reaction: an important organometallic chemical reaction in which alkyl, allyl, vinyl, or aryl-magnesium halides are added to a carbonyl group in an aldehyde or ketone for the formation of carbon–carbon bonds.
Homologation reaction: a reaction that converts the reactant into the next member of the homologous series, for example, a compound with an additional methylene group.
Kalkitoxin: a lipopeptide toxin derived from the cyanobacterium *Lyngbya majuscula*, which can induce neuronal necrosis, block voltage-dependent sodium channels, and induce cellular hypoxia.
Native chemical ligation: covalent condensation of two or more peptide segments to construct a larger polypeptide chain, usually between ionized thiol group of an N-terminal cysteine residue and C-terminal thioester.
Natural language processing: a subfield of artificial intelligence concerning how to program computers to process and analyze large amount of natural language data.
PDE472: a selective inhibitor of the phosphodiesterase PDE4D isoenzyme for the treatment of asthma.
Pharmaceutical: chemical compound used as medicinal drug.
Prexasertib: a small-molecule ATP-competitive selective inhibitor.
(*R*)-tamsulosin: a blockbuster medicine used for dysuria associated with urinary stones and benign prostatic hyperplasia.
Sonidegib: a Hedgehog signaling pathway inhibitor (via smoothened antagonism); a medication to treat cancer.
Suzuki-Miyaura (SM) coupling: a cross-coupling reaction between boronic acid and an organohalide catalyzed by a palladium complex, which is widely used in the chemical and pharmaceutical industry.

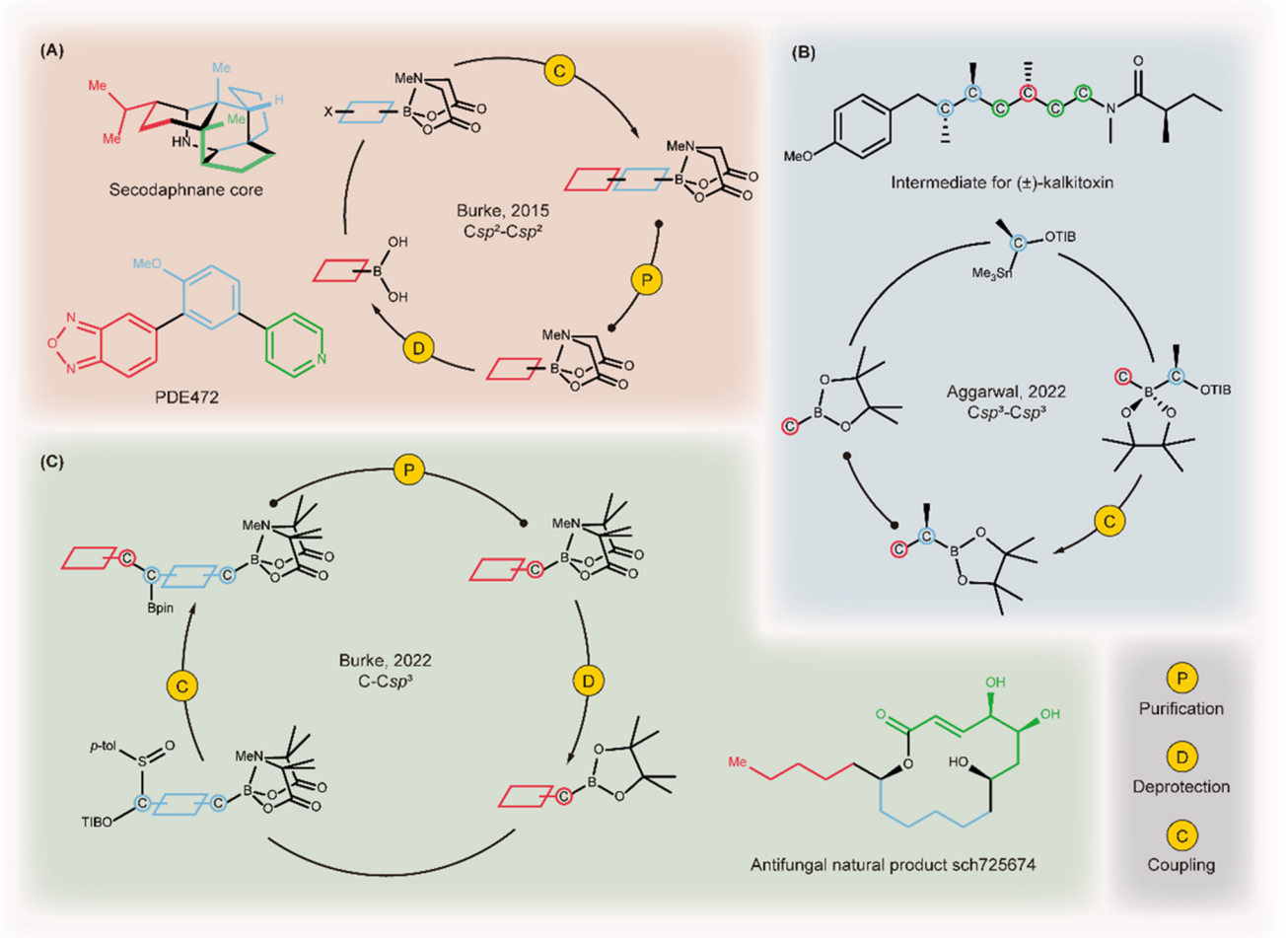

Figure 2. Automated iterative synthesis. (A) Suzuki-Miyaura reaction-based iterative synthesis using MIDA boronates. (B) Boron homologation-based stereocontrolled iterative synthesis in one-atom-at-a-time assembly-line fashion. (C) Hyper stable TIDA boronates enabled iterative synthesis via a universal C–Csp^3 bond formation. Abbreviations: Bpin, pinacol boronic ester; OTIB, 2,4,6-triisopropylbenzoate; *p*-tol, *para*-toluene. See [12,14,18].

Automated digitalized batch synthesis

Great progress was achieved in the automation of chemical synthesis throughout the past decades, including the development of iterative synthesis methodologies [22,23]; however, most of the automated synthesis are still highly task-specific and can only be operated on their corresponding platform. To expand the universality, a versatile robotic hardware platform that can be used for different automated syntheses and a generalized operational software that can conduct standardized chemical reactions are highly sought-after.

In 2019, the Cronin group introduced a modular robotic platform based on a chemical programming language, the Chemputer, and its controlling software, Chempiler [24]. To execute an automated synthesis with the Chemputer, a series of low-level and specific commands for the hardware modules (reactor, filtration, separation, and evaporation) is generated with the Chempiler by combining the **Chemical Assembly (ChASM) code** and **GraphML code**. This binary architecture of the Chemputer enhances the universality of automated organic synthesis because: (i) the workflow written in ChASM code is hardware-independent; therefore, one

elaborate synthetic procedure can be run on various platforms given that the required functions are available; (ii) the Chemputer dissects high-level instructions (e.g., recrystallize) into basic low-level instructions (e.g., heat, stir, cool); hence, it is capable of executing various compositive commands. The robustness of the Chemputer was demonstrated by the multistep automated synthesis of three pharmaceuticals (sildenafil, rufinamide, and nytol) in bench scale with yields comparable with those of manual syntheses. In 2021, the Cronin group increased the hardware modules of Chemputer from four to ten, which includes a deoxygenation module for oxygen-free and moisture-free conditions and a chromatography module for automated purification of the final product [25]. The upgraded Chemputer can execute different reactions, including the existing multistep synthetic paradigms (e.g., SM coupling-based iterative cross-coupling).

Integration of Process Analytical Technology (PAT) in automated synthesis platform has emerged as a powerful strategy for rapid real-time analysis and self-optimization of a reaction [26–28]. In 2021, Cronin, Emmerling, and coworkers integrated a benchtop nuclear magnetic resonance (NMR) spectrometer as an online analytical module into Chemputer, enabling the automated self-optimization of reactions (e.g., **Grignard reaction**) [29]. Correspondingly, in 2022, using online HPLC and Fourier transform infrared spectroscopy (FTIR) as orthogonal PAT tools, the Hein group established a self-adaptive automated synthesizer with custom-built Python script, named AdaptaSyn, which consists of valves, pumps, and probes with liquid/gas samplers all connected by a robotic arm for full-automated operation except for the reagent and solution preparation [30]. Unlike most existing automated synthesis platforms that executed under fixed preset protocols, AdaptaSyn is capable of dynamically adjusting the reaction parameters (e.g., reagent stoichiometries and reaction time) timely based on the data feedback from the online PATs, in the form of concentration versus time trends of each species, throughout the reaction (Figure 3A). The capability of AdaptaSyn in self-optimization of multistep synthesis was demonstrated with one-pot multistep CDI-mediated amidation, an important reaction in pharmaceutical industry, where the reaction parameters of each step can be customized independently for substrates of different reactivities.

The pinnacle of automated synthesis is not just about labor- or time-saving, but the digitization of chemistry [29,31]. In 2020, the Cronin group developed a universal system that automatically converts the imported synthetic protocols from literature into a machine-readable scheme in the form of chemical descriptive language (χDL)-based codes, termed the chemical integrated development environment (ChemIDE) [32]. Using SynthReader, a **natural language processing** algorithm, the χDL codes are generated automatically through tagging, interpreting, and converting the literature procedures into a list of detailed actions for χDL-translation. Furthermore, SynthReader can automatically complement ambiguous process variables (e.g., reflux temperature) based on the physical properties of the reagents. To make ChemIDE a user-friendly system for synthetic chemists with limited programming knowledge, the χDL codes are presented in simple English sentences with highlighted texts that can be easily edited. Of 559 procedures from literature, 523 have been successfully translated with ChemIDE without fatal errors. More recently, in 2022, the Cronin group established an open-source χDL database, which contains 103 prevalent chemical reactions from ten distinct categories (e.g., C–C bond formations, functional group manipulations, and multicomponent reactions) that have been translated from literature [33]. From this database, 53 χDL codes were successfully validated on the **ChemPU** platform with purities and yields comparable with those in the literature. Depending on the results obtained (e.g., yield or/and purity), the χDL file can either be added into the database or pended for further self-optimization aided by machine learning (Figure 3B). As an open-source database, the χDLs are platform-independent and available to anyone for reproduction, validation, version control,

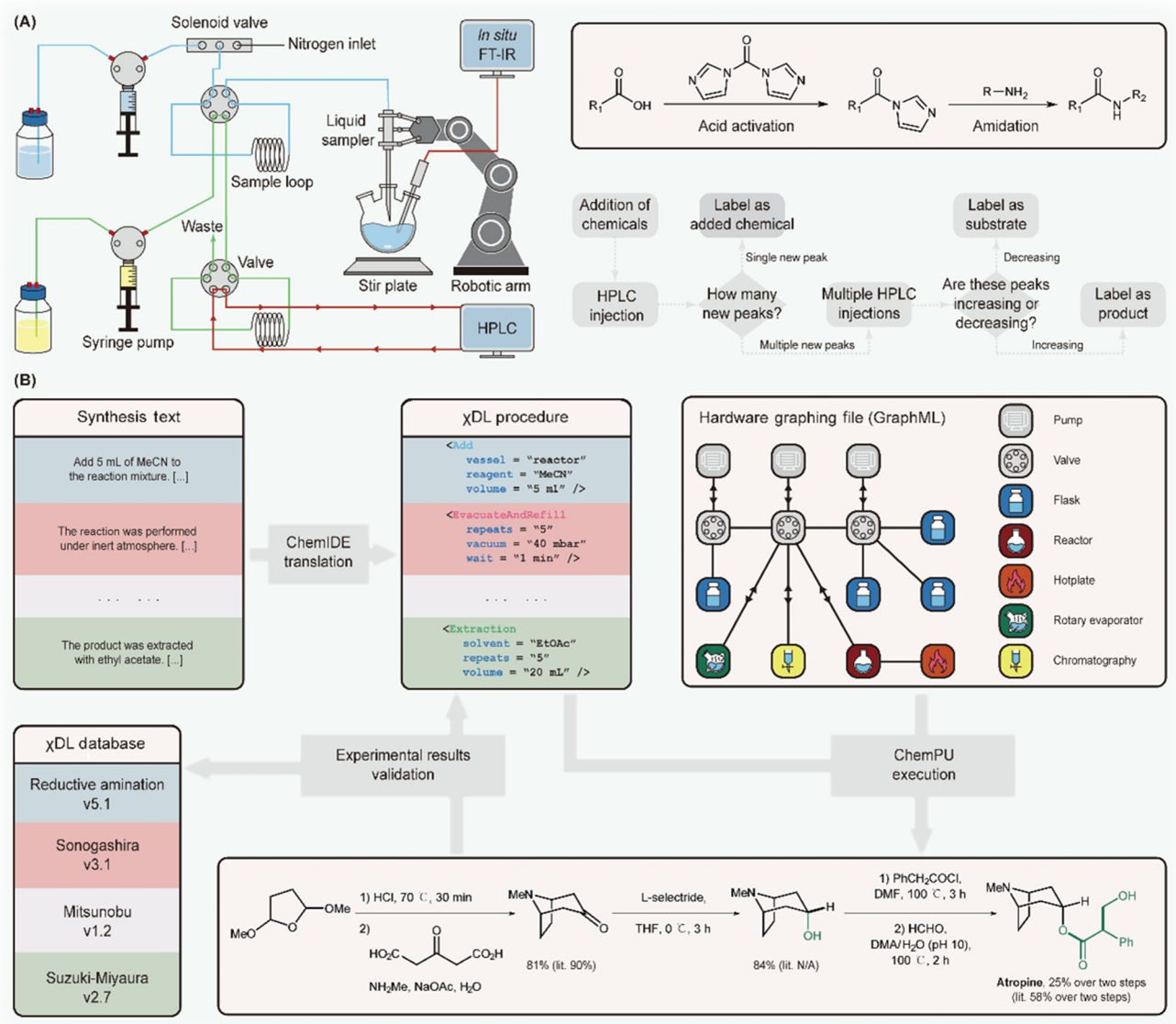

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Figure 3. Automated digitalized batch synthesis. (A) Illustration of AdaptaSyn and peak labeling logic during multistep CDI-mediated amidation. (B) Schematic workflow of ChemPU: from reported protocols in literature to the establishment of χDL database and automated synthesis, which enabled the automated multistep synthesis of atropine.

and update. This feature vastly facilitates the standardization, reproducibility, and automation of organic synthesis [34]. Notably, with the ChemPU platform, the automated multistep synthesis of atropine, an anticholinergic drug, was realized using reaction procedures obtained from different literature for respective steps, showcasing the reliability and universality of this platform in executing well-defined codes translated from literature for multistep synthesis.

Automated SPS-flow synthesis

The well-established automated SPS has revolutionized the synthesis of peptides, oligonucleotides, and oligosaccharides in the past century. In the context of multistep synthesis, SPS is

superior to the solution-phase synthesis in term of efficiency and economy, as no isolation or purification of intermediates is required. In SPS, the substrates are immobilized on a solid supporting material and grow step-by-step by treating with excess reagents in the mobile phase, which is removed by a simple washing protocol, and the final product is then released from the solid-support by facile cleavage. Recently, the merger of continuous-flow synthesis (an innovative and powerful modern technology that possesses great potential in the industrial synthesis of pharmaceuticals with various advantages [35–38]) with SPS technology (termed SPS-flow) has instigated new opportunities in the field of automated multistep synthesis and has led to several notable breakthroughs [39–41].

In 2017, using the SPS-flow based approach, Pentelute and coworkers developed an automated flow peptide synthesis (AFPS) for the expedited synthesis of peptides with high yield and purity, comparable with the standard SPS system, and with minimal epimerization, especially for cysteine and histidine residues [42]. The AFPS system tremendously reduces the reaction time for the coupling to 7 s per amide bond formation and for the entire cycle to 40 s per amino acid (15 to 30 min in previously reported work [43,44]). The platform consists of four modules (storage, mixing, activation, coupling) and an in-line ultraviolet-visible (UV-vis) spectrometer, which was introduced for the in-process assessment of fluorenylmethyloxycarbonyl group (Fmoc) removal for the calculation of deprotection efficiency as well as the coupling yield and to identify any potential undesired on-resin peptide aggregation. The Pentelute group also demonstrated that this AFPS platform serves as a powerful tool in the discovery and development of therapeutic peptides, such as antimicrobial peptides [45] and personalized tumor neoantigen peptides [46].

In 2020, the Pentelute group further extended the AFPS system to the synthesis of proteins that constitute up to 164 amino acids (Figure 4) [47]. Synthesis of peptides longer than 50 amino acids has always been difficult using the traditional SPS method. Even with the development of **native chemical ligation**, the synthesis of protein chains is still non-universal, indirect, and requires elaborated linking strategy from limited peptide segments [48]. Nevertheless, leveraging the AFPS, nine distinct proteins of 86 to 164 amino acids were rapidly synthesized in 3.5 to 6.5 h and the obtained proteins not only possessed identical primary, secondary, and tertiary

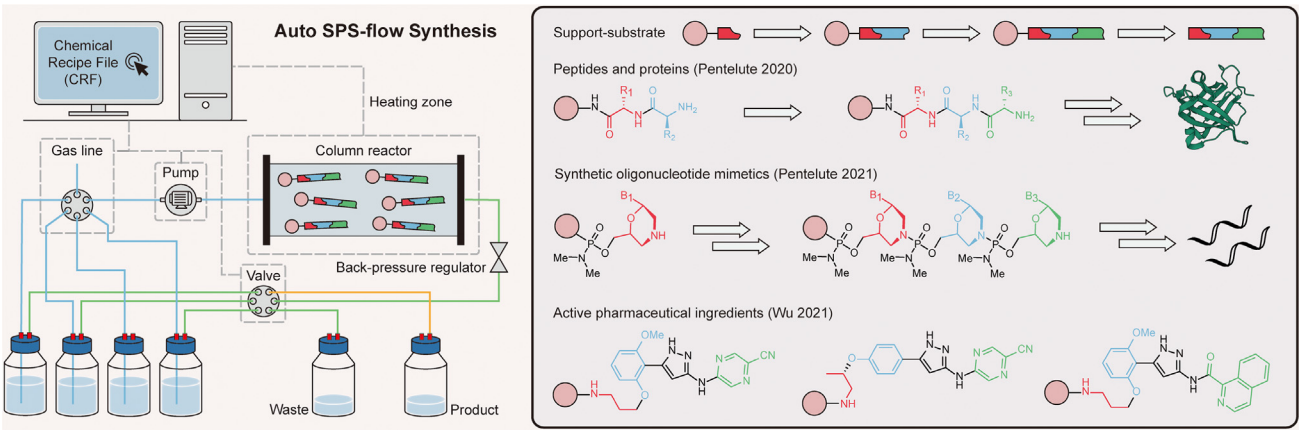

Figure 4. Schematic of automated solid-phase synthesis (SPS)-flow synthesis. Representative SPS-flow synthesis of proteins, oligonucleotide mimetics, and small molecule pharmaceutical ingredients are displayed. See [47,49,51].

structures as the recombinant samples but also showed comparable activity and intact biological functions (e.g., enzymatic catalysis). Furthermore, using automated data collection and analysis with in-line UV-vis detection, the reaction conditions for each distinct amino acid was optimized and a generic amino acid-specific recipe was generated.

Adopting similar construction as the AFPS, in 2021, the Pentelute group designed an automated microscale flow synthesizer for phosphorodiamidate morpholino oligomers (PMOs), an oligonucleotide mimetic used for antisense therapies (Figure 4) [49]. Other than antisense therapeutics, PMOs also stand out as promising antiviral agents against RNA viruses [e.g., Ebola, dengue, influenza, and severe acute respiratory syndrome coronavirus (SARS-CoV) viruses] [50]. However, the development of PMO therapeutics is hindered by long lead optimization times due to the synthetic challenges. Using conventional synthetic method, the coupling time per PMO unit is in the order of 180 min, meaning that a therapeutic PMO sequence of 20 units would take weeks to finish. Nonetheless, by reducing the coupling time per PMO unit to 8 min, the automated SPS-flow synthesizer has substantially expedited the production and development of PMO therapeutics, which is urgently needed under this global coronavirus disease 2019 (COVID-19) pandemic. Notably, using this system, a potential PMO therapeutic for SARS-CoV-2 was synthesized in merely 3.5 h.

Despite the tremendous progress of automated SPS-flow in peptide and PMO synthesis, the implementation of such a system in the synthesis of small-molecule pharmaceuticals was not well-developed, due to their non-iterative structural intricacy. In this regard, in 2021, Wu and coworkers developed a compact SPS-flow platform for the push-button automated six-step synthesis of **prexasertib** and its derivatives (Figure 4) [51]. Using only 1 g of resin, 635 mg prexasertib was synthesized with an isolated yield of 65% after continuous execution of 32 h. To facilitate the facile and highly efficient early- and late-stage diversification, a computer-based chemical recipe file (CRF) for the synthesis of prexasertib was established and utilized to prepare 23 analogs of prexasertib with only minor adjustments. Notably, with a compact and simple hardware framework, this automated SPS-flow platform shows high versatility for other synthetic routes without the need for manual reconfiguration. Moreover, unlike continuous-flow system that often requires meticulous readjustments for the translation, the SPS-flow system offers straightforward translation from solution-batch synthesis. Thus, automated SPS-flow system exhibits great potential in the linear end-to-end synthesis of non-iterative small-molecule active pharmaceutical ingredients (APIs).

Automated MCFS

Compared with conventional batch synthesis, continuous-flow synthesis is generally easier to scale up with high efficiency [52,53]. The ability to perform extremely fast reactions that involve highly reactive and unstable species, which cannot be realized in the batch fashion, with continuous-flow system also opens up unprecedented synthetic routes towards the preparation of complex target products [54]. Furthermore, it is highly desirable to execute multistep synthesis in continuous-flow fashion for advantages that include catalyst compartmentalization and precise control of loading reagents in an uninterrupted reaction network. However, problems arise when combining multiple steps into one single system (e.g., solvent incompatibility, removal of side-products, and clogging due to solid formation). On this basis, the recent development of in-line separation technologies, such as membrane-based liquid–liquid separator [55–58], gravity-based liquid–liquid separator [59–61], immobilized reagents [62,63], and immobilized catalysts [64–66], has partially resolved these issues. These in-line separation technologies have facilitated the MCFS of several APIs with complex chemical structures (selected examples are shown in Figure 5A [57–59,66–72]) in a greener and more straightforward way.

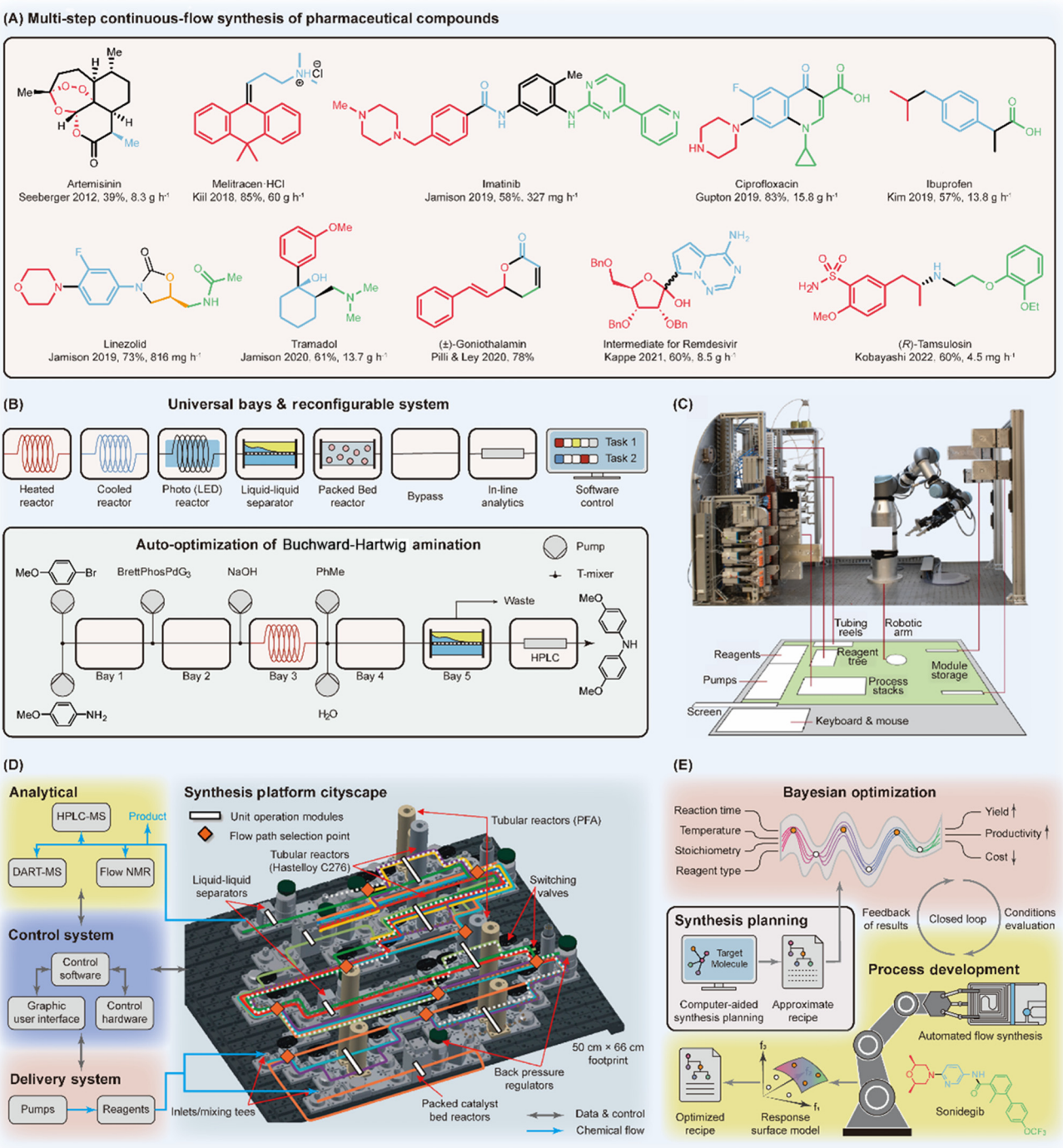

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Figure 5. Automated multistep continuous-flow synthesis (MCFS). (A) Examples of active pharmaceutical ingredients that have been achieved in MCFS. (B) Representative configuration of the components in the reconfigurable continuous-flow synthesis system. Example for auto-optimization of Buchward-Hartwig amination is displayed. (C) Photograph of the robotic flow chemistry platform. (D) Schematic workflow of AutoSyn and construction of the synthesis platform, CityScape. (E) Schematic workflow of a multi-objective Bayesian reaction optimization task, starting from computer-planned recipe to optimized recipe, via a robotic multistep flow synthesis platform. See [57–59,66–72].

Emerging automated flow synthesis platforms

Integrating with PAT and AI technologies, the automated multistep synthesis platforms based on continuous-flow synthesis are booming. In 2018, Jamison, Jensen, and coworkers reported an automated, plug-and-play, continuous-flow chemical synthesis system that consists of five universal bays that can be set up into different modules (heated reactors, cooled reactors, photochemical reactors, packed-bed reactors, membrane-based liquid–liquid separators, and bypasses) using a graphical user interface (Figure 5B) [73]. With a pump equipped in each bay, up to six different reagents or solvents can be introduced in a synthetic task. Through interactions with an algorithm [stable noisy optimization by branch and fit (SNOBFIT)] and feedback from monitoring sensors (pressure sensors, flow meters, etc.), various single- and multistep reactions can be automatically optimized in a matter of hours or days.

In 2019, Jamison, Jensen, and coworkers further developed a robotic platform based on flow synthesis that integrates an AI-aided synthesis planning function to realize the full-automated synthesis of target compound starting from design and retrosynthesis [74]. As the AI-proposed synthesis routes are mostly based on batch synthesis, due to limited literature on flow synthesis, CRFs were generated with additional inputs (e.g., residence time, concentrations, and equivalencies) from chemists to translate the proposed synthesis onto the robotic platform. According to the CRF, a six-axis robotic manipulator will select the required reagents and process modules (laminar flow reactors, packed bed reactors, and membrane separators) and connect them in the designed sequence pneumatically as a sealed linear flow path. The process modules are encapsulated in aluminum shells to enable high temperature and pressure conditions and are integrated with electronics for control. The LEGO-like configuration allows versatile flow synthetic tasks by simply introducing new modules to the platform (Figure 5C). Remarkably, using this platform, the synthesis of 15 APIs or drug-like compounds (e.g., a library of five angiotensin-converting-enzyme inhibitors and a multistep synthesis of a chiral drug substance safinamide) were predicted and achieved automatically.

In 2020, Collins and coworkers reported an automated multistep chemical synthesizer, named AutoSyn, that incorporates four primary components: (i) CityScape, a reconfigurable synthesis platform; (ii) a platform for analytical monitoring and data capture; (iii) a software for automated real-time control; (iv) a mapping tool to generate flow synthesis protocols. [75]. The CityScape is constructed with a base plate and surface-mounted operation modules (reactors, separators, valves, etc.) (Figure 5D). By adjusting the selector valves, up to 3887 unique subway flow paths leading to different target molecules are available. To demonstrate the versatility and multistep capability of AutoSyn, ten small-molecule drugs were successfully synthesized (e.g., imatinib and diphenhydramine).

Automated holistic optimization of MCFS

Self-optimization with PAT and AI technology is a very powerful automated synthesis system [76–78]. The optimization of single variable or single reaction has been widely explored [1,26,28,29,79]; whereas holistic optimization of MCFS, which cannot be realized by simply combining the individually optimized conditions, remains challenging. In 2022, Jensen, Jamison, and coworkers integrated a multi-objective Bayesian optimization algorithm with their robotic platform [74], which enabled the holistic optimization of a three-step computer-aided synthesis of **sonidegib** [80]. The Bayesian optimization algorithm can iteratively propose optimal values of variables, including categorical (reagent type) and continuous (temperature, time, stoichiometry) conditions according to multiple objectives (yield, productivity, cost) (Figure 5E). An inline FTIR was used to monitor the real-time concentration of different species and the yield of each individual step was determined by an LC-MS module. Similarly, in

2022, Bourne, Clayton, and coworkers also reported an automated continuous-flow platform that enables simultaneous optimization of telescoped reactions (e.g., Heck cyclization-deprotection sequence) with a Bayesian optimization algorithm [81]. Notably, using a multipoint sampling strategy, only one on-line HPLC was required to quantify the conversion of each step. The balanced trade-off between exploration of uncertain areas and exploitation of available information offered by the Bayesian method is highly suitable for the automated holistic optimization of MCFS.

Automated radial synthesis

The recent developments in automated flow platforms have addressed some of the challenges and enabled several MCFS of APIs. However, as MCFS mainly rely on linear and sequential process with all steps operating simultaneously, mismatch of time scales between consecutive steps and redundancies of equipment are unavoidable, limiting their versatility.

In this context, in 2020, the Gilmore group developed an automated radial synthesizer for small molecules (Figure 6A), where an array of continuous-flow reactors is arranged around a central switching station (CSS) and operated independently [82]. The radial platform was

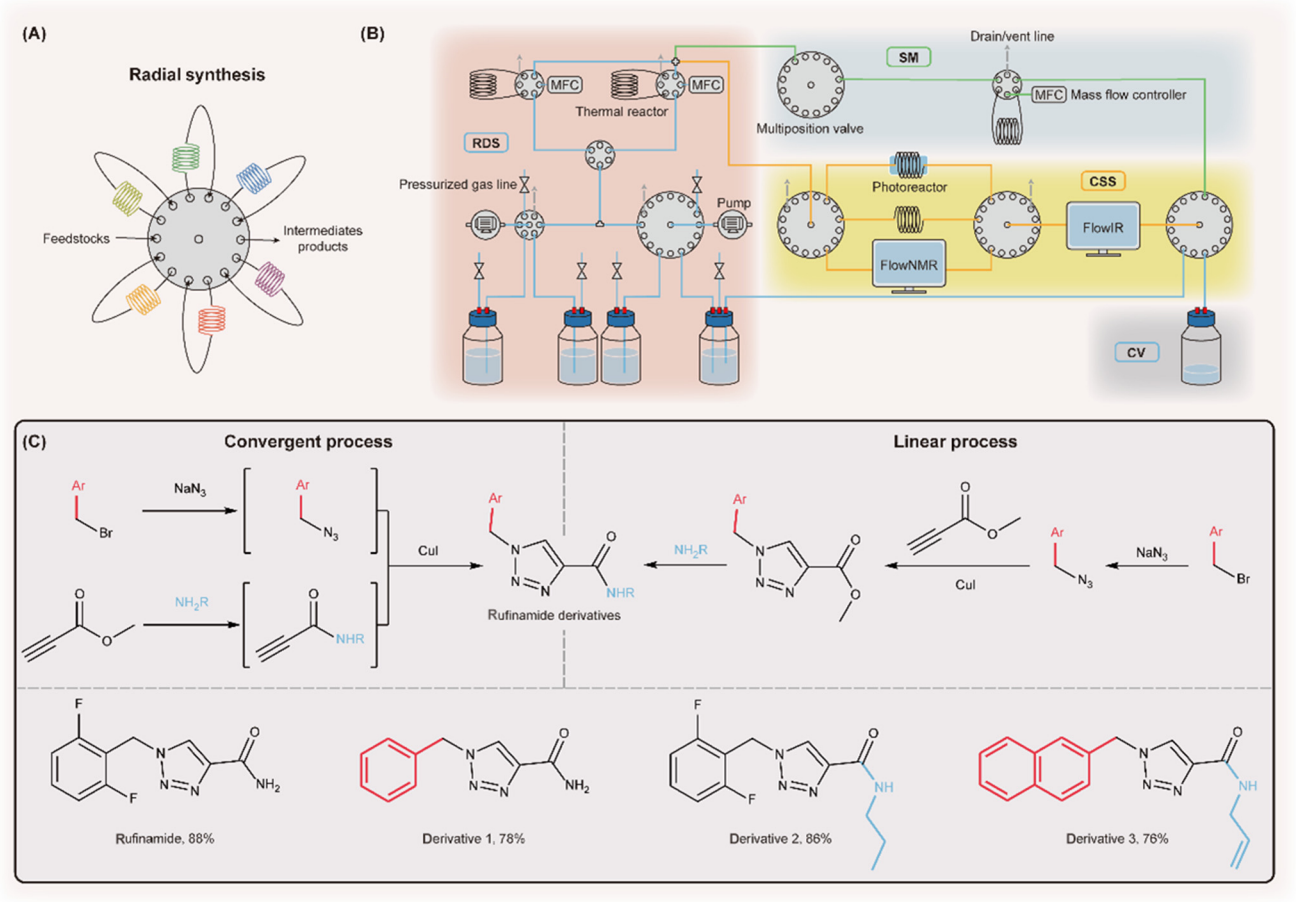

Figure 6. Automated radial synthesis. (A) Schematic of radial synthesis. (B) Schematic of automated radial synthesizer. (C) Generation of rufinamide derivatives. Abbreviations: CV, collection vessel; RDS, reagent delivery system; SM, standby module.

built with commercially available hardware and equipment and consists of four sections (Figure 6B): (i) the solvent and reagent delivery system, which can store up to 12 reagents (neat or solution) and solvents separately and deliver them into the reactor via syringe pump and 16-way valve; (ii) the CSS, which is composed of reactors and in-line analytical instruments (infrared and NMR spectroscopy); (iii) the standby module (SM), where the intermediates are stored for the subsequent transformation; and (iv) the collection vessels (CVs), where the solution of the final product exits the platform and is collected for offline purification and analysis.

With this radial synthesis approach, each step in a multistep synthesis is decoupled and conducted in an asynchronous sequence, thus allowing the reuse of reactors under different flow rate, temperature, and pressure without physical reconfiguration. Notably, aside from linear synthesis, this platform is also effective in convergent synthesis due to its non-simultaneous nature. Furthermore, as the solvents and reagents are stored and delivered separately, the optimization of solvent or concentration via in-line dilution can be done automatically without the need to make new solutions. Using this radial platform, anticonvulsant drug rufinamide and a library of 11 rufinamide derivatives were successfully prepared with good yield via either the linear or convergent approach (Figure 6C).

Concluding remarks

The importance of rapid and efficient synthesis of complex organic molecules for drug development and APIs has been amplified in this pandemic era and automation represents the best option for such feats. Automated synthesis was once thought to be highly hardware- and system-specific, but its capability and universality have improved substantially. The advancement has enabled several automated multistep syntheses of pharmaceuticals and their derivatives, which potentially expedites drug development. It is important to note that such accomplishments were made possible by the development in various subfields (e.g., chemical iterative synthesis, digitalization of chemistry, continuous-flow synthesis, in-line separation technologies, PAT technologies, AI technologies, standardization and commercialization of hardware, etc.).

Although there yet remains some outstanding questions in these subfields, we envision that new advances could be made by integration with other technologies (see Outstanding questions). In the future, the current synthetic platforms could potentially combine with robotics to further decrease manual intervention [74,83]. Furthermore, by utilizing servers in the cloud, the development and renewal of synthetic databases could proceed without spatial and temporal limitations [1]. Besides the development of new synthetic methodologies, engineered optimization of the software and hardware platform is also much desired to target industrial efficiency [23,31,73,82,84–86]. Lastly, to further accelerate the lead optimization process in drug development, automated synthesis could be coupled with computer-guided molecule design and optimization as well as automated biological testing and feedback analysis [87].

Outstanding questions

How can a flow-based platform be made more flexible, such as the independent or parallel operation of several segments?

How can synthetic routes be realized, starting from multiple substrates with final cross-coupling in SPS-flow fashion?

Is it possible to develop an AI-assisted software to guide the condition transformation from reported batch synthesis to undeveloped flow fashion?

How can an efficient and flexible platform be developed for automated SPS-flow synthesis using photochemistry and electrochemistry?

Besides the current PAT technologies, can we make biological testing in the pharmaceutical industry in an automated style?

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Declaration of interests

The authors declare no competing financial interest.

References

1. Fitzpatrick, D.E. *et al.* (2018) Across-the-world automated optimization and continuous-flow synthesis of pharmaceutical agents operating through a cloud-based server. *Angew. Chem. Int. Ed. Engl.* 57, 15128–15132
2. Sagmeister, P. *et al.* (2022) Autonomous multi-step and multi-objective optimization facilitated by real-time process analytics. *Adv. Sci. (Weinh.)* 9, e2105547
3. Perera, D. *et al.* (2018) A platform for automated nanomole-scale reaction screening and micromole-scale synthesis in flow. *Science* 359, 429–434
4. Caramelli, D. *et al.* (2021) Discovering new chemistry with an autonomous robotic platform driven by a reactivity-seeking neural network. *ACS Cent. Sci.* 7, 1821–1830
5. Granda, J.M. *et al.* (2018) Controlling an organic synthesis robot with machine learning to search for new reactivity. *Nature* 559, 377–381
6. Merrifield, R. (1965) Automated synthesis of peptides: solid-phase peptide synthesis, a simple and rapid synthetic method, has now been automated. *Science* 150, 178–185
7. Alvarado-Urbina, G. *et al.* (1981) Automated synthesis of gene fragments. *Science* 214, 270–274
8. Plante, O.J. *et al.* (2001) Automated solid-phase synthesis of oligosaccharides. *Science* 291, 1523–1527
9. Yao, W. *et al.* (2022) Automated solution-phase multiplicative synthesis of complex glycans up to a 1,080-mer. *Nat. Synth.* 1, 854–863
10. Lehmann, J.W. *et al.* (2018) Toward generalization of iterative small molecule synthesis. *Nat. Rev. Chem.* 2, 0115
11. Caruthers, M.H. (1985) Gene synthesis machines: DNA chemistry and its uses. *Science* 230, 281–285
12. Li, J. *et al.* (2015) Synthesis of many different types of organic small molecules using one automated process. *Science* 347, 1221–1226
13. Gillis, E.P. and Burke, M.D. (2007) A simple and modular strategy for small molecule synthesis: iterative Suzuki– Miyaura coupling of B-protected haloboronic acid building blocks. *J. Am. Chem. Soc.* 129, 6716–6717
14. Fasano, V. *et al.* (2022) Automated stereocontrolled assembly-line synthesis of organic molecules. *Nat. Synth.* 1, 902–907
15. Matteson, D.S. (2013) Boronic esters in asymmetric synthesis. *J. Org. Chem.* 78, 10009–10023
16. Matteson, D.S. *et al.* (2004) The Matteson reaction. *Org. React.* Published online March 19, 2021. https://doi.org/10.1002/0471264180.or105.03
17. Burns, M. *et al.* (2014) Assembly-line synthesis of organic molecules with tailored shapes. *Nature* 513, 183–188
18. Blair, D.J. *et al.* (2022) Automated iterative Csp(3)-C bond formation. *Nature* 604, 92–97
19. Ma, X. *et al.* (2020) Stereoselectivity in Pd-catalysed cross-coupling reactions of enantioenriched nucleophiles. *Nat. Rev. Chem.* 4, 584–599
20. Casoni, G. *et al.* (2017) 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
21. Leonori, D. and Aggarwal, V.K. (2014) Lithiation-borylation methodology and its application in synthesis. *Acc. Chem. Res.* 47, 3174–3183
22. Li, Y. *et al.* (2022) Recent advances in autonomous synthesis of materials. *ChemPhysMater* 1, 77–85
23. Gioiello, A. *et al.* (2020) The medicinal chemistry in the era of machines and automation: recent advances in continuous flow technology. *J. Med. Chem.* 63, 6624–6647
24. Steiner, S. *et al.* (2019) Organic synthesis in a modular robotic system driven by a chemical programming language. *Science* 363, eaav2211
25. Angelone, D. *et al.* (2021) Convergence of multiple synthetic paradigms in a universally programmable chemical synthesis machine. *Nat. Chem.* 13, 63–69
26. Sans, V. *et al.* (2015) A self optimizing synthetic organic reactor system using real-time in-line NMR spectroscopy. *Chem. Sci.* 6, 1258–1264
27. Lee, W.G. *et al.* (2020) NMR spectroscopy goes mobile: using NMR as process analytical technology at the fume hood. *Magn. Reson. Chem.* 58, 1193–1202
28. Sagmeister, P. *et al.* (2020) Multivariate analysis of inline benchtop NMR data enables rapid optimization of a complex nitration in flow. *React. Chem. Eng.* 5, 677–684
29. Bornemann-Pfeiffer, M. *et al.* (2021) Standardization and control of Grignard reactions in a universal chemical synthesis machine using online NMR. *Angew. Chem. Int. Ed. Engl.* 60, 23202–23206
30. Liu, J. *et al.* (2022) An adaptive auto-synthesizer using online PAT feedback to flexibly perform a multistep reaction. *Chem. Methods* 2, e202200009
31. Shukla, C.A. and Kulkarni, A.A. (2017) Automating multistep flow synthesis: approach and challenges in integrating chemistry, machines and logic. *Beilstein J. Org. Chem.* 13, 960–987
32. Mehr, S.H.M. *et al.* (2020) A universal system for digitization and automatic execution of the chemical synthesis literature. *Science* 370, 101–108
33. Rohrbach, S. *et al.* (2022) Digitization and validation of a chemical synthesis literature database in the ChemPU. *Science* 377, 172–180
34. Wang, Z. *et al.* (2020) Automated synthesis: current platforms and further needs. *Drug Discov. Today* Published online September 16, 2020. https://doi.org/10.1016/j.drudis.2020.09.009
35. Fülöp, Z. *et al.* (2020) Evolution of flow-oriented design strategies in the continuous preparation of pharmaceuticals. *React. Chem. Eng.* 5, 1527–1555
36. Jiao, J. *et al.* (2021) Multi-step continuous-flow organic synthesis: opportunities and challenges. *Chemistry* 27, 4817–4838
37. Adamo, A. *et al.* (2016) On-demand continuous-flow production of pharmaceuticals in a compact, reconfigurable system. *Science* 352, 61–67
38. Britton, J. and Raston, C.L. (2017) Multi-step continuous-flow synthesis. *Chem. Soc. Rev.* 46, 1250–1271
39. Merrifield, B. (1986) Solid phase synthesis. *Science* 232, 341–347
40. Burgess, K.L. (2000) *Solid-Phase Organic Synthesis*, Wiley Online Library
41. Cankarova, N. *et al.* (2019) Traceless solid-phase organic synthesis. *Chem. Rev.* 119, 12089–12207
42. Mijalis, A.J. *et al.* (2017) A fully automated flow-based approach for accelerated peptide synthesis. *Nat. Chem. Biol.* 13, 464–466
43. Gordon, C.P. (2018) The renascence of continuous-flow peptide synthesis - an abridged account of solid and solution-based approaches. *Org. Biomol. Chem.* 16, 180–196
44. Sletten, E.T. *et al.* (2019) Real-time monitoring of solid-phase peptide synthesis using a variable bed flow reactor. *Chem. Commun. (Camb.)* 55, 14598–14601
45. Albin, J.S. and Pentelute, B.L. (2020) Efficient flow synthesis of human antimicrobial peptides. *Aust. J. Chem.* 73, 380–388
46. Truex, N.L. *et al.* (2020) Automated flow synthesis of tumor neoantigen peptides for personalized immunotherapy. *Sci. Rep.* 10, 723
47. Hartrampf, N. *et al.* (2020) Synthesis of proteins by automated flow chemistry. *Science* 368, 980–987
48. Albericio, F. and El-Faham, A. (2018) Choosing the right coupling reagent for peptides: a twenty-five-year journey. *Org. Process Res. Dev.* 22, 760–772
49. Li, C. *et al.* (2021) Fully automated fast-flow synthesis of antisense phosphorodiamidate morpholino oligomers. *Nat. Commun.* 12, 4396
50. Nan, Y. and Zhang, Y.J. (2018) Antisense phosphorodiamidate morpholino oligomers as novel antiviral compounds. *Front. Microbiol.* 9, 750
51. Liu, C. *et al.* (2021) Automated synthesis of prexasertib and derivatives enabled by continuous-flow solid-phase synthesis. *Nat. Chem.* 13, 451–457
52. Hartman, R.L. *et al.* (2011) Deciding whether to go with the flow: evaluating the merits of flow reactors for synthesis. *Angew. Chem. Int. Ed. Engl.* 50, 7502–7519
53. Breen, C.P. *et al.* (2021) Ready, set, flow! Automated continuous synthesis and optimization. *Trends Chem.* 3, 373–386
54. Yoshida, J. *et al.* (2013) Flash chemistry: flow chemistry that cannot be done in batch. *Chem. Commun. (Camb.)* 49, 9896–9904

55. Kralj, J.G. *et al.* (2007) Integrated continuous microfluidic liquid-liquid extraction. *Lab Chip* 7, 256–263
56. Snead, D.R. and Jamison, T.F. (2015) A three-minute synthesis and purification of ibuprofen: pushing the limits of continuous-flow processing. *Angew. Chem. Int. Ed. Engl.* 54, 983–987
57. Pedersen, M.J. *et al.* (2018) Redesign of a Grignard-based active pharmaceutical ingredient (API) batch synthesis to a flow process for the preparation of melitracen HCl. *Org. Process Res. Dev.* 22, 228–235
58. Russell, M.G. and Jamison, T.F. (2019) Seven-step continuous flow synthesis of linezolid without intermediate purification. *Angew. Chem. Int. Ed. Engl.* 58, 7678–7681
59. Jamison, T.F. *et al.* (2020) Continuous-flow synthesis of tramadol from cyclohexanone. *Synlett* 31, 1888–1893
60. O'Brien, M. *et al.* (2012) A prototype continuous-flow liquid-liquid extraction system using open-source technology. *Org. Biomol. Chem.* 10, 7031–7036
61. Battilocchio, C. *et al.* (2017) Continuous preparation and use of dibromoformaldoxime as a reactive intermediate for the synthesis of 3-bromoisoxazolines. *Org. Process Res. Dev.* 21, 1588–1594
62. Baxendale, I.R. *et al.* (2006) A flow process for the multi-step synthesis of the alkaloid natural product oxomaritidine: a new paradigm for molecular assembly. *Chem. Commun. (Camb.)* 2566–2568
63. Baxendale, I.R. *et al.* (2009) Multistep synthesis using modular flow reactors: Bestmann-Ohira reagent for the formation of alkynes and triazoles. *Angew. Chem.* 121, 4077–4081
64. Tsubogo, T. *et al.* (2015) Multistep continuous-flow synthesis of (R)- and (S)-rolipram using heterogeneous catalysts. *Nature* 520, 329–332
65. Ishitani, H. *et al.* (2020) Enantioselective sequential-flow synthesis of baclofen precursor via asymmetric 1,4-addition and chemoselective hydrogenation on platinum/carbon/calcium phosphate composites. *Chem. Asian J.* 15, 1688–1691
66. Saito, Y. *et al.* (2022) Continuous-flow synthesis of (R)-tamsulosin utilizing sequential heterogeneous catalysis. *Angew. Chem. Int. Ed. Engl.* 61, e202115643
67. von Keutz, T. *et al.* (2021) Flash chemistry approach to organometallic C-glycosylation for the synthesis of remdesivir. *Org. Process Res. Dev.* 25, 1015–1021
68. Tosso, N.P. *et al.* (2019) A consolidated and continuous synthesis of ciprofloxacin from a vinylogous cyclopropyl amide. *J. Org. Chem.* 84, 3370–3376
69. Levesque, F. and Seeberger, P.H. (2012) Continuous-flow synthesis of the anti-malaria drug artemisinin. *Angew. Chem. Int. Ed. Engl.* 51, 1706–1709
70. Lee, H.J. *et al.* (2019) From p-xylene to ibuprofen in flow: three-step synthesis by a unified sequence of chemoselective C-H metalations. *Chemistry* 25, 11641–11645
71. Fu, W.C. and Jamison, T.F. (2019) Modular continuous flow synthesis of imatinib and analogues. *Org. Lett.* 21, 6112–6116
72. Pastre, J.C. *et al.* (2020) Integrated batch and continuous flow process for the synthesis of goniothalamin. *ACS Omega* 5, 18472–18483
73. Bédard, A.-C. *et al.* (2018) Reconfigurable system for automated optimization of diverse chemical reactions. *Science* 361, 1220–1225
74. Coley, C.W. *et al.* (2019) A robotic platform for flow synthesis of organic compounds informed by AI planning. *Science* 365, eaax1566
75. Collins, N. *et al.* (2020) Fully automated chemical synthesis: toward the universal synthesizer. *Org. Process Res. Dev.* 24, 2064–2077
76. Sagmeister, P. *et al.* (2019) Laboratory of the future: a modular flow platform with multiple integrated PAT tools for multistep reactions. *React. Chem. Eng.* 4, 1571–1578
77. Clayton, A.D. *et al.* (2019) Algorithms for the self-optimisation of chemical reactions. *React. Chem. Eng.* 4, 1545–1554
78. Mateos, C. *et al.* (2019) Automated platforms for reaction self-optimization in flow. *React. Chem. Eng.* 4, 1536–1544
79. Clayton, A.D. *et al.* (2020) Automated self-optimisation of multi-step reaction and separation processes using machine learning. *Chem. Eng. J.* 384, 123340
80. Nambiar, A.M.K. *et al.* (2022) Bayesian optimization of computer-proposed multistep synthetic routes on an automated robotic flow platform. *ACS Cent. Sci.* 8, 825–836
81. Clayton, A.D. *et al.* (2023) Bayesian self-optimization for telescoped continuous flow synthesis. *Angew. Chem. Int. Ed. Engl.* 62, e202214511
82. Chatterjee, S. *et al.* (2020) Automated radial synthesis of organic molecules. *Nature* 579, 379–384
83. Burger, B. *et al.* (2020) A mobile robotic chemist. *Nature* 583, 237–241
84. Zhang, P. *et al.* (2018) Advanced continuous flow platform for on-demand pharmaceutical manufacturing. *Chemistry* 24, 2776–2784
85. Zhao, Z. and McBride, C.S. (2020) Evolution of olfactory circuits in insects. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 206, 353–367
86. Jiang, T. *et al.* (2021) An integrated console for capsule-based, automated organic synthesis. *Chem. Sci.* 12, 6977–6982
87. Schneider, G. (2018) Automating drug discovery. *Nat. Rev. Drug Discov.* 17, 97–113