Concepts and Optimization Strategies of Experimental Design in Continuous-Flow Processing

Antimo Gioiello[1]*, Valentina Mancino[1], Paolo Filipponi[1,2], Serena Mostarda[1] and Bruno Cerra[1]

[1]*Laboratory of Medicinal and Advanced Synthetic Chemistry (Lab MASC), Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, I-06122 Perugia, Italy*
[2]*Continuous Manufacturing Unit, Chemical and Analytical Development, Novartis Pharma AG, Novartis Campus St. Johann, CH-4002 Basel, Switzerland*

Received: 26 April 2016; accepted: 01 June 2016

The integration of flow systems with statistical design of experiments is emerging as a valuable strategy to develop new synthetic routes towards relevant building blocks, chemical probes, and drug compounds. Optimization by experimental design incorporates statistical algorithms, mathematical models and equations, predicting tools, feedback control, and validation to generate new optimal conditions. Continuous-flow chemistry is ideally suited for this scope, as the integration of in-line analysis is simple; experimental parameters such as temperature, pressure, and flow rate can be easily controlled and fine-regulated; and automation of reaction screening can be accomplished with software assistance. This review article aims to illustrate how the combination of flow synthesizers and design of experiments can be profitable to speed up the development and optimization of more efficient, safer, and reproducible protocols for modern synthetic methods and manufacturing processes.

1. Introduction

In recent years, medicinal and synthetic chemistry have experienced a significant evolution in the approaches used and general thinking. Several research groups from both academia and within pharmaceutical companies have adopted more technological solutions to help the delivery of compounds from early phases of discovery to (pre)clinical investigations and drug production [1]. Moreover, chemical plants are investing resources to drive a radical innovation in synthetic methodologies, which would enable the conduction of processes that are difficult or impossible to be realized because of safety restriction, timing, and costs.

Within this changing landscape, enabling chemical technologies as flow platforms are significantly supporting these efforts leading, at the same time, to a deep change on how chemists think and perform synthesis [1,2]. In particular, advantages of continuous-flow chemistry have been exploited in the development of safer and greener conditions for existing or novel chemical transformations, in the rapid building of compounds' libraries readily available for biological screenings, as well as in the definition of robust and reliable scale-up processes at lower costs and increased efficiency. The wide applicability of flow synthesis is demonstrated by an increasing number of reports and publications, which qualify flow technology suitable for industrial applications as well as for bench chemists designing new methodologies [2].

Translating a reaction from batch to flow modality can have several potential benefits. During reactions optimization, experimental conditions (temperature, flow rate, pressure, reactant stoichiometry, and concentration) can be varied and carefully controlled in a rapid, automated, and secure way. Moreover, the reaction performance and outcome can be easily evaluated with in-line analytical devices eventually supported by software facilitating the fine-tuning and optimization of experimental parameters and configurations. Labor savings appear determinant, thinking that software-assisted flow devices operate, even along various steps, in automated fashion, thus releasing time to work in other tasks [1]. Along this line, integration of flow synthesizers and chemical computing have been successfully applied to generate diverse chemical libraries with drug-like or lead-like molecular properties that allow the development of fully automated reactions with an increased throughput for screenings [3].

Despite the numerous benefits, the majority of processes in manufacturing industries are still performed in batch. Reasons for these uncertainties are as to whether continuous-flow processing will deliver sufficient benefits to justify the capital investments required to replace existing batch plants. To justify changing a manufacturing process requires a deep understanding and a critical evaluation of the real improvements in terms of quality, efficiency, costs, and ecosustainability of new proposed protocols.

Far from being an extensive review, this article aims to provide the reader with an understanding of concepts, workflow and approaches of statistical design of experiments (DoE), their potential and challenges. In particular, we have selected a number of synthetic processes where the integration of DoE with flow devices has shown its advantages. Self-optimizing continuous-flow reactors, engineering optimization, robotic experiments, and their applications in developing novel functional materials have not been covered. Excellent articles [4] and reviews [5] in these topics have been reported and may be of interest for readers. Herein, studies exploring and demonstrating the potentiality of DoE-assisted flow platform are sampled, with an emphasis placed on the adopted experimental designs and the reasons for their choice discussed along with the main achievements obtained in terms of flow setup and reaction conditions, efficiency, safety, and productivity.

2. Strategies and Potential of Experimental Designs

Optimization of synthetic processes is a major issue for chemists. It is a "long and winding road" that requires high-level scientists, specific chemical knowhow, time, and economical and human resources. To support these efforts, novel strategies and enabling toolboxes as automated equipments and software have shown great potentiality to understand both benefits and risks associated with a chemical process. In this frame,

* Author for correspondence: antimo.gioiello@unipg.it

DOI: 10.1556/1846.2016.00012

First published online: 4 July 2016

DoE provides statistical means and guidelines which can assist researchers on the iterative journey from the working hypothesis, design, and performance of the experiments, to the interpretation and validation of the results obtained (Figure 1) [6].

DoE origins date back to the pioneering work of Ronald A. Fisher [7] and refers to the planning of experiments and analysis of results by statistical methods so that meaningful and objective conclusions can be drawn from the collected data. Specifically, the selection of experiments is based on the following three steps [8]:

(a) Definition of the objectives. Before starting the study, it is essential to define the project goal(s) (e.g., yield, productivity, selectivity, environmental impact, etc.) and select the most appropriate strategy based on the background of the reaction under investigation. For instance, when there are limited literature information and in-house data, it would be appropriate to perform scoping or screening experiments before the optimization study is attempted. On the contrary, if the goal of the project is already well defined, an analysis of robustness of the reaction would be more suitable.

(b) Factor selection. At the early stages, the experimental space should be carefully defined with the identification of those variables important for the response (yield, selectivity, costs, etc.) and the settlement of the relative ranges to be explored. If factor ranges are too small, the optimum conditions can lie outside the area of study. Conversely, large factor ranges could generate a model that is poorly predictive. Since a smaller number of variables implies a reduced number of experiments, it is essential to have an overall view of the process to choose only those variables that have a real impact on final goals, thus to minimize costs and time. Exploratory experiments or screening designs can furnish preliminary hints that can drive the selection.

(c) Choice of the most appropriate design. The design strategy determines the distribution of the experimental points within the experimental space (Tables 1 and 2). Traditionally, the exploration of the reaction space is performed by changing one variable at a time (OVAT). While effective, the OVAT approach is not as efficient as DoE and suffers from a few drawbacks. Indeed, OVAT allows to explore a smaller portion of the reaction space than DoE under the same number of experiments (Figure 2). Moreover, outcomes may be affected by the starting conditions, the interaction among variables is not taken into account, and a high number of experiments is required to acquire sufficient information on the system and optimize the process. On the contrary, DoE study permits to explore combinations of variables simultaneously and to gain a better mapping of the chemical space with a reduced number of experiments [9]. Additionally, DoE algorithms furnish clear interpretation of the data through the building of mathematical models which are able to describe and interpret the functional relation between the response and the experimental variables, to predict the outcome or property not measured, and to depict a response surface.

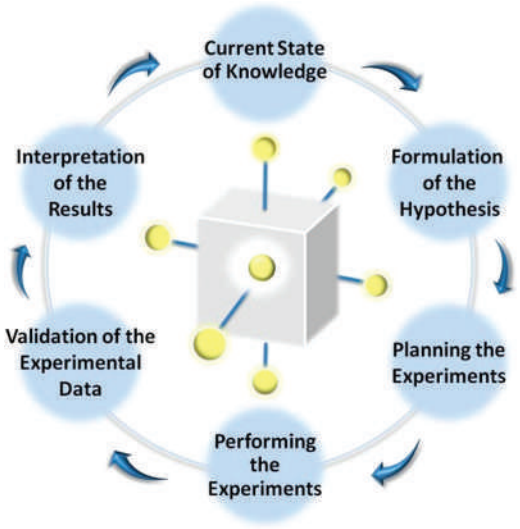


Figure 1. Scientific learning circular flow chart

Response variations can be then expressed as a function of the factors by a polynomial equation (eq. 1) from which it is possible to infer the weight of variables, the direction towards the optimal condition, or the optimal factors settings [10].

$$y = \beta_0 + \sum_i \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j + \sum_i \beta_{ii} x_i^2 + e$$

Equation 1. Mathematical equation based on Taylor model

DoE strategies are generally classified as screening and response surface designs (Table 1) [9]. Screening designs are used to identify those factors that most influence the response and require a low resolution experimentation. This approach is generally applied at the beginning of the study to learn about the process and minimize the efforts required for the optimal solution. The most used are the full factorial (FD), the fractional factorial (FFD), and the Plackett–Burman design (Table 1). Among these, FD is the only screening strategy that enables to calculate interaction mathematical models whose elaboration furnishes important clues on the direction to follow for the optimal reaction conditions. As experiments exponentially increase with the number of variables (k), FD design is wasteful in terms of resources when the number of factors is relatively high. For this purpose, FFD and Plackett–Burman design are more advisable [9a, 10].

Response surface designs are characterized by a higher resolution experimentation furnishing nonlinear mathematical models useful to determine the optimal combination of variables. The building of a response surface involves the exploration of each variable at more than two levels. Central composite design (CCD) and Box–Behnken design are generally used for this scope (Table 1) [9c, 10]. Both methods are suitable for continuous variables leading to symmetric designs in which all the experimental runs are uniformly distributed around the central point [9a, c]. When the reaction space is irregular due to experimental constraints and some regions are impossible to be reached, D-optimal can be used to probe a large number of quantitative and qualitative factors with a "mixed" number of levels [11].

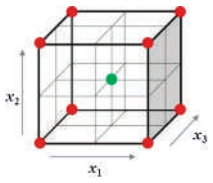
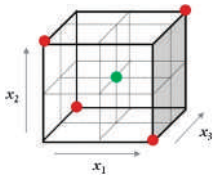
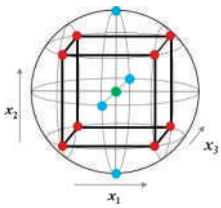
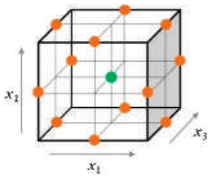
In summary, when little is known about a (synthetic) process and the main effects are not yet fully understood, a screening design is recommended. When the goal is to optimize key experimental parameters analyzing how sensitive are the optimum conditions toward factor variation, a response surface modeling is more appropriate. In general, the choice among the various design strategies depends on the number and the type of the variables under investigation and on the objectives. In Table 2, the guidelines to select the opportune design strategy are illustrated [8].

Nowadays, the available software programs can respond appropriately to different needs that may arise during the reaction optimization process [12]. Depending on the objectives, it is possible to choose a design targeted to (a) feasibility studies, (b) screening of experimental factors, (c) make response surfaces, and (d) more accurate assessment of robustness.

3. DoE-driven Optimization of Continuous-flow Processes

Despite the great potentiality, the integrated use of statistical experimental design with flow systems in the optimization of

Table 1. Main features of DoE strategies

DoE strategy		Independent variable levels	Number of experiments (N)[a]	Experimental space[b]	Mathematical model
Screening design	Two-level full factorial design	Two levels: Low (−1) High (+1)	$N=2^k+n$		Interaction
	Two-level fractional factorial design	Two levels: Low (−1) High (+1)	$N=2^{k-p}+n$ with $(k-p)>k$		Linear
	Plackett–Burman design	Two levels: Low (−1) High (+1)	$N=k+1$[c] with N=multiple of 4	$N\times N$ Plackett–Burman matrix	Linear
Response surface design	Central composite design	Five levels: Axial ($\pm\alpha$) Factorial (±1) Central (0)	$N=2^k+2\,k+n$		Quadratic
	Box–Behnken design	Three levels: Low (−1) Central (0) High (+1)	$N=K^d+n$		Quadratic
	D-optimal design	Mixed number of levels	D-optimal algorithm: selection of the subset of experiments which maximize the determinant of the X′X matrix		Quadratic

[a] k is the number of variables under investigation while n is the number of center points generally included to evaluate the reproducibility.
[b] The three-variable (x_1, x_2, x_3) experimental space can be modeled as a cube whose axes represent the variables while the volume is defined by the union of their possible combinations.
[c] It is strongly recommended that the design has at least six more test runs than the number of variables for a better estimation of the experimental error.
[d] K represents the combination of k variables varied 2 or 3 at a time while the remaining $(k-2)$ or $(k-3)$ is kept at the 0 level.

flow processes is still far from routine though the availability of marketed software accessible also for inexpert users. Yet, the accurate control of reaction parameters and the rapid screening by automation combined with data acquisition and in-line analysis make flow platforms the ideal complement for experimental design. In this section, selected examples about the use of DoE in continuous-flow synthesis found in the literature will be discussed. Major breakthroughs of each example, as well as the optimized synthetic routes to reach the target molecule, will be highlighted.

Early attempts of multivariate screenings using flow microreactors were reported by Yoshida et al. for the bromo–lithium

Table 2. Stepwise approach for design selection

Project goal(s)	Design rationale	Main experimental features	DoE strategy
Scoping, screening or feasibility study	Identification of the main variables	High number of variables	Fractional factorial design
		High number of variables	Plackett–Burman design
	Evaluation of interaction effects between main variables	Low number of variables	Full factorial design
Process optimization or robustness analysis	Identification of the optimal experimental conditions; study of the impact of small experimental changes on the outcome	Preferably quantitative variables; symmetric experimental space	Central composite design
		Preferably quantitative variables; symmetric experimental space with not accessible corners	Box–Behnken design
		Quantitative and/or qualitative variables; irregular experimental space	D-optimal design

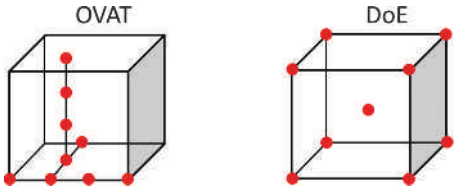
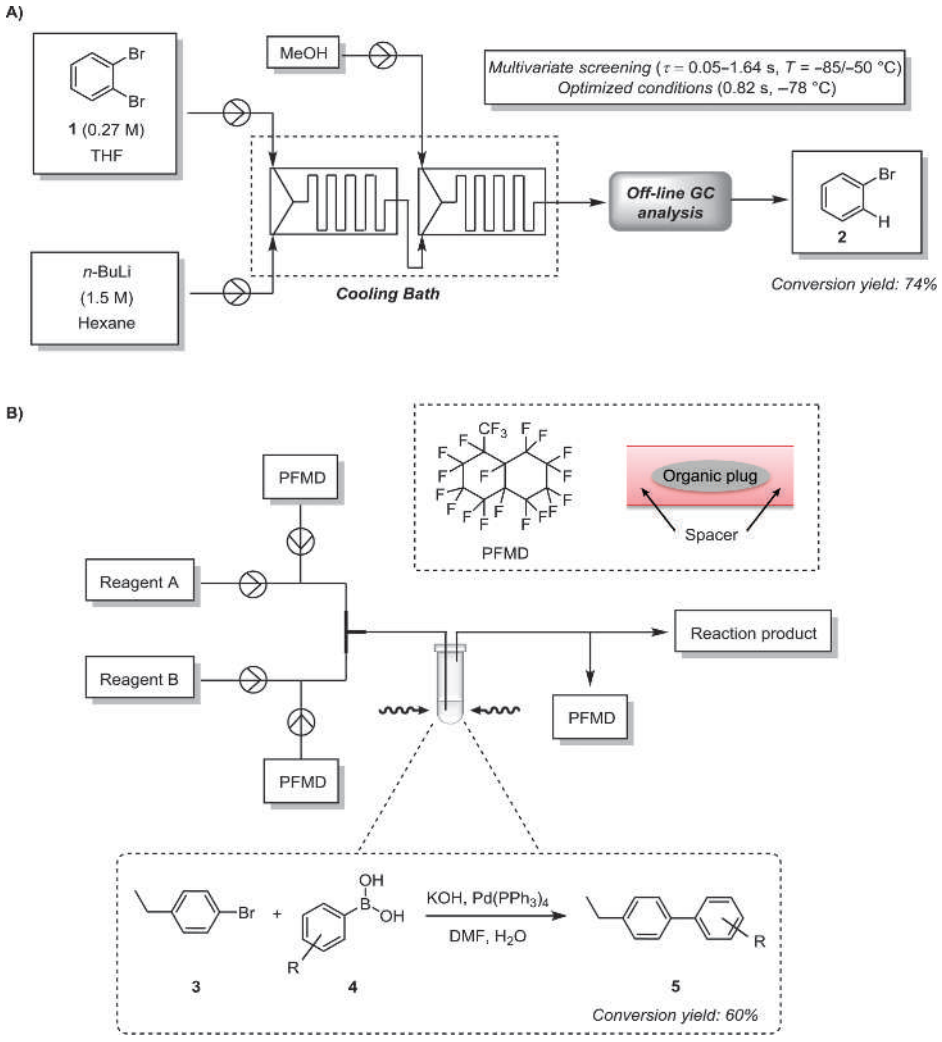


Figure 2. Distribution of the experimental points within the experimental space with OVAT and DoE

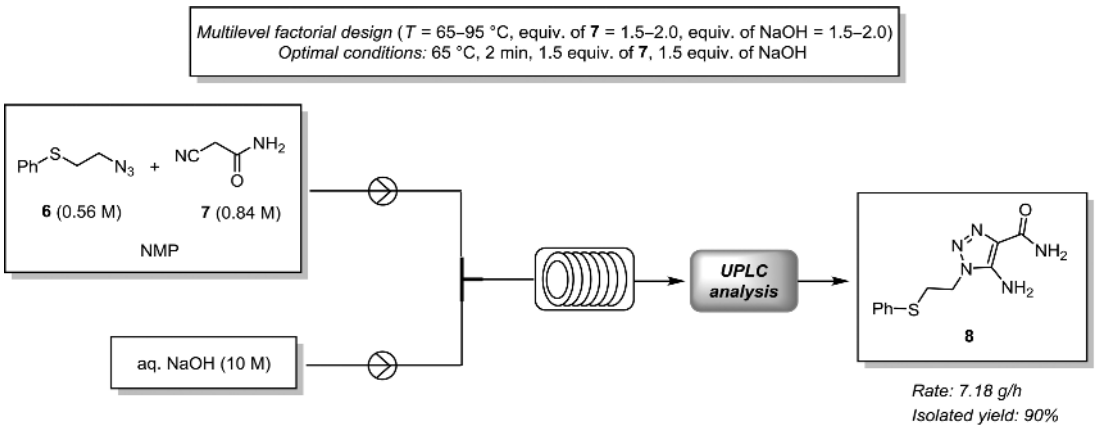
exchange reaction of aryl derivatives (Scheme 1A) [13]. Residence time and temperature were varied and analyzed to unveil the features and mechanistic paths of the reaction. The authors demonstrated that fast heat transfer, which is achievable in microsystems, reduced local deviation of temperature and was extremely powerful for transformations involving unstable organometallic intermediates as aryllithium compounds. The first published example of DoE applied to flow synthesis date back to 2008 when Benali and collaborators at GlaxoSmithKline described a continuous-flow microwave platform for Suzuki–Miyaura cross-coupling reactions (Scheme 1B) [14]. The work showed the advantages of using an immiscible fluorinated spacer (perfluromethyldecalin, PFMD) for the formation of discrete reaction plugs in the system. A DoE methodology was exploited for the translation of the reaction on a series of 500 μL plugs. The optimized conditions were applied to different plug sizes including 200, 300, 500, 1000, and 2000 μL volume yielding comparable conversions (60%) in nearly all experiments.

An early demonstration of the benefits associated with flow chemistry and statistical tools towards the definition of safer synthetic routes is well exemplified by the preparation of 2-azidoethyl phenyl sulfide (**6**), a readily accessible and stable alternative for ethyl azide [15]. The usefulness of this synthon was demonstrated in a [3+2] cycloaddition design space evaluation aimed at developing a straightforward and scalable synthesis of *N*-alkylated 5-amino-1,2,3-triazole carboxamides (Scheme 2). Previously reported synthesis of this scaffold suffered from efficiency and safety hazards due to the use of ethyl azide [16]. The intuition of the authors was that the presence of a large sulfur group at the β position would improve both thermal stability and safety properties of the resulting azide. Readily prepared in situ from β-chloroethyl phenyl sulfide and sodium azide under nonaqueous conditions, **6** was reacted with cyanoacetamide (**7**) in a series of FD experiments examining the interplay of temperature, stoichiometry, and base. Ten molar aqueous NaOH and *N*-methylpyrrolidone (NMP) were used as the base and solvent, respectively. The results showed that the employment of high temperatures and greater amounts of base is detrimental for the yield because of cyanoacetamide (**7**) degradation. The stoichiometry of both **7** and NaOH had a less pronounced effect on the reaction. The best conditions (temperature = 65 °C, residence time = 2 min, 1.5 equiv. of **7**, 1.5 equiv. of NaOH) were applied in a gram-

Scheme 1. Early examples of multivariate screenings. A) Bromo–lithium exchange reaction optimization of aryl derivatives. B) Diagram of the flow-microwave platform for Suzuki–Miyaura cross-coupling reactions



Scheme 2. General flow setup for the optimization of [3+2] cycloaddition between β-azidoethyl phenyl sulfide (**6**) and cyanoacetamide (**7**) under flow conditions

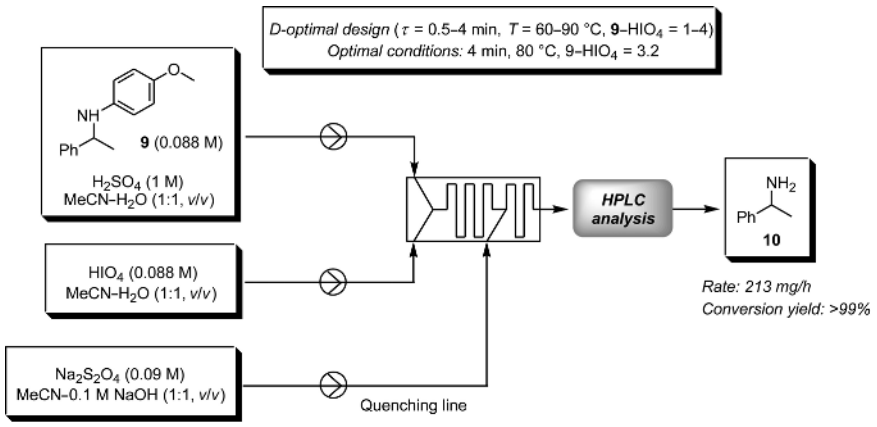


scale experiment, affording **8** in 90% isolated yield with a productivity of 7.18 g/h (Scheme 2).

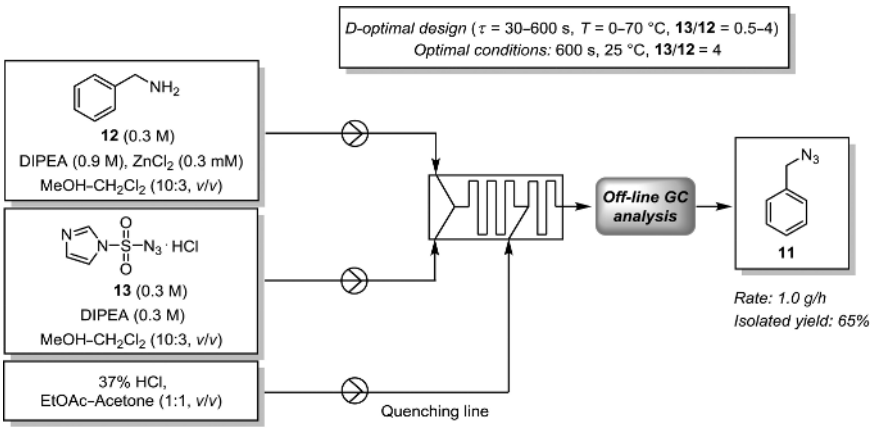
The same year, the DoE optimization of arylamine deprotection was reported by Rutjes group [17]. The objective of the work was to develop a mild protocol for the removal of *p*-methoxyphenyl (PMP) group, which would avoid the use of toxic and expensive reagents particularly needed in scale-up operations. After preliminary batch-screen experiments that allowed to identify the crucial reaction parameters, the model reaction was investigated into a 7-μL flow microreactor using the flow setup depicted in Scheme 3. The effect of the temperature (60–90 °C), residence time (τ) (0.5–4 min), and stoichiometric ratio **9**/HIO_4 (1–4) was assessed by 51 analytical-scale experiments. Contour plots evidenced that reaction time and temperature had a considerable impact on the yield, and the best conditions for a quantitative conversion (>99%) were obtained at 90 °C with a **9**/HIO_4 equivalent ratio of 3.2 and a residence time of 1.3 min. However, the solvents (H_2O and MeCN) boiled at this temperature within the reactor giving rise to an unstable product outflow. To overcome this issue, the scaling up into a 950-μL microreactor was conducted at 80 °C with a reaction time of 4 min using the same reagent stoichiometry. The reaction components were flowed for 4 h, leading to the continuous removal of PMP-protected amine **9** at the rate of 213 mg/h (Scheme 3). Substrate scope and application of the methodology to different scaffolds were not reported.

The efficiency in controlling reaction conditions renders flow chemistry particularly profitable also for the preparation of potentially explosive products as organic azides. Traces of acid or metal salts may indeed catalyze explosive decomposition while exposure to ultraviolet (UV) light or heat is often cause of chemical instability. To circumvent this issue, a flow method for the synthesis of benzyl azide (**11**) from benzylamine (**12**) using imidazole-1-sulfonyl-azide hydrochloride (**13**) as diazotransfer reagent was developed (Scheme 4) [18]. A FutureChemistry FlowScreen system was employed to screen the reaction conditions using two independent pumps for the substrate and reagents whose outflow was quenched by a third line constituted by an organic (EtOAc–acetone) solution of HCl. The product collected in the presence of an internal standard was then analyzed off-line with a gas chromatograph equipped with a flame ionization detector. A solution of MeOH–CH_2Cl_2 was chosen as ideal solvent system being able to avoid the undesired gas evolution responsible for irreproducible gas chromatography (GC) yield determination. Preliminary univariate experiments indicated that resident time, temperature, and reactant stoichiometry were crucial factors for the reaction. A D-optimal design was therefore performed for a multivariate optimization run. The analysis of the 2D-counter plots from a set of 60 data points evidenced the following: (a) high temperatures lowered yields because of imidazole-1-sulfonyl-azide hydrochloride (**13**) decomposition; (b) the optimal temperature range was found between 0 °C and 40 °C; (c) the **13**/**12** stoichiometric ratio was fixed between 3 and 4 to sustain the azide decomposition; and (d) a residence time of about 600 s allowed higher yield, whereas a decrease of the reaction yield was observed for longer residence time. The reaction was then outscaled from a 92-μL chip reactor into a 20-mL steel coil reactor at 25 °C using a stoichiometric ratio of 4 and 10 min of residence

Scheme 3. Flow setup for the reaction optimization of *p*-methoxyphenyl (PMP) removal under flow conditions



Scheme 4. Synthesis of benzyl azide (**11**) by diazotransfer flow reaction



time to give the desired benzyl azide (**11**) in 65% isolated yield with a productivity of 1 g/h (Scheme 4).

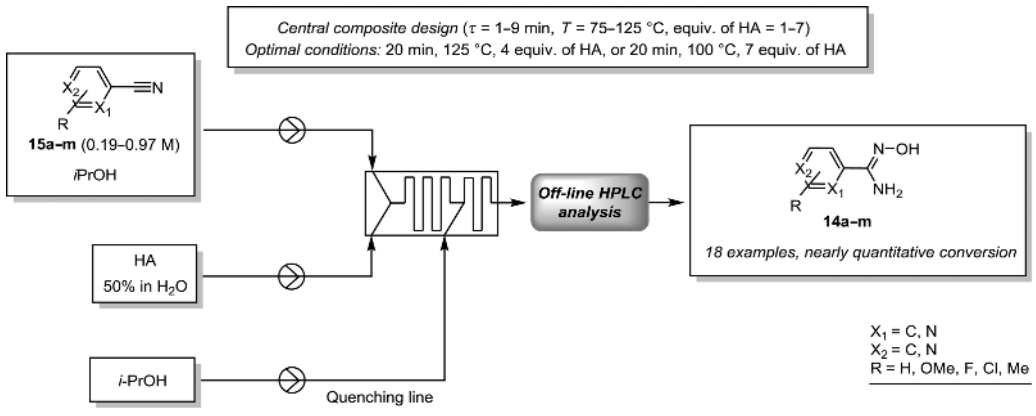
In 2012, the same authors described the multivariate D-optimal optimization of a microreactor-based flow method for the methoxyisopropyl (MIP) protection of cyanohydrin [19] and a new flow protocol for the selective α-monobromination of acetophenone [20].

The safer formation of amidoximes from hydroxylamine (HA) has been described using microflow reactors [21]. In particular, benzamidoxime (**14a**) was obtained from the reaction of a 50% solution of HA in H_2O with benzonitrile (**15a**, X_1 = C, X_2 = C, R = H) (Scheme 5). Having defined the best solvent (*i*PrOH), the crucial experimental parameters were exploited by a CCD (residence time = 1–9 min, temperature = 75–125 °C, HA equiv. = 1–7). Within the established range of residence time, all the parameters had a positive effect and a full conversion of thermostable compounds was accomplished at 125 °C in the presence of 4 equiv. of HA. For temperature sensitive substrates, 100 °C and 7 equiv. of HA were required for a quantitative transformation. These boundary conditions were extended to electron-rich benzonitriles and pyridines **15b**–**m** and for the scale-up of the amidoxime.

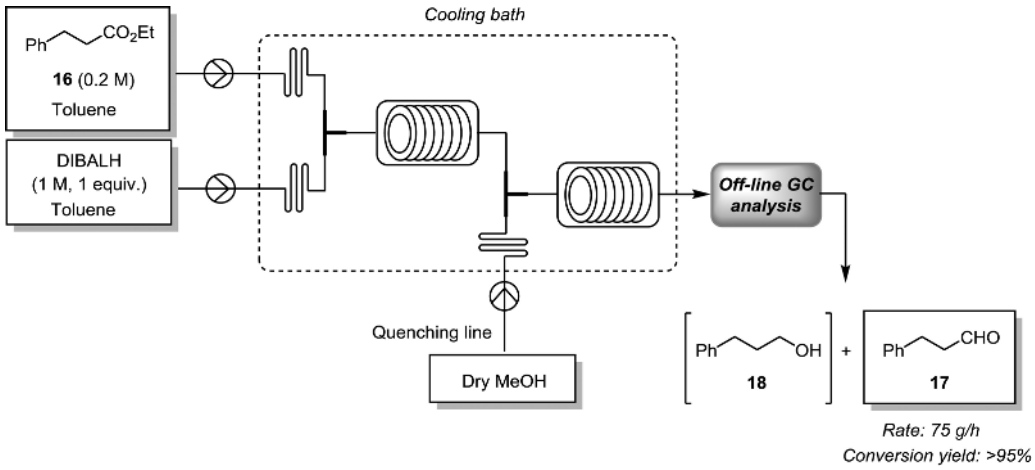
Worthy of note is the DoE-driven optimization for the partial reduction of esters into aldehydes using diisobutylaluminium hydride (DIBALH) as reducing agent [22]. The work of Webb and Jamison was aimed to avoid the over-reduction to alcohols and the use of cryogenic temperatures combined with the slow addition rate of DIBALH as model reaction. These issues that have severely limited the use of this transformation at large scale were overcome using a flow setup composed by three syringe pumps, three precooling loops, two reactors, and two T-shaped mixers submerged into a cooling bath. The authors studied the selective reduction of ethyl hydrocinnamate (**16**) with DIBALH (Scheme 6). The proposed flow setup incorporated a quenching line in which neat dry MeOH was pumped. Preliminary univariate experiments performed at constant temperature (−78 °C) and in presence of 1 equiv. of DIBALH evidenced that high conversions to aldehyde **17** with less than 5% of alcohol **18** were obtained at higher flow rate (36 mL/min). An FD multivariate optimization was then performed to understand the interdependence among variables as the flow rate (from 3.0 to 37.2 mL/min), temperature (from −78 °C to 0 °C), and residence time (from 0.038 to 13.68 s). As expected, over-reduction occurred at higher temperature and lower flow rate, while, at high flow rate, the formation of the aldehyde **17** was still favored even at room temperature, thanks to the more efficient mixing that minimize the accumulation of the reducing agent. Using the optimized conditions (1 equiv. of DIBALH, −78 °C, 36 mL/min of total flow rate), ethyl hydrocinnamate (**16**) was reduced into the corresponding aldehyde **17** with >95% conversion and with a potential productivity of 75 g/h. Unfortunately, the optimized procedure resulted as poorly versatile, requiring a DoE optimization for each substrate.

Nieuwland and collaborators showed how automated microreactor platform can be employed to optimize fast and highly exothermic reactions [23]. In particular, D-optimal designs were exploited to investigate the effect of five parameters including temperature and reaction time for the selective oxidation of benzyl alcohol to benzaldehyde by Swern–Moffatt oxidation. In the conventional batch procedure, the premixing of trifluoracetic anhydride (TFAA, **19**) and dimethylsulfoxide (DMSO) at −78 °C generates trifluoroacetoxy dimethylsulfonium **20** that readily reacts with the primary alcohol **21** to afford the alkoxy dimethylsulfonium salt **22**. Quenching of the crude reaction

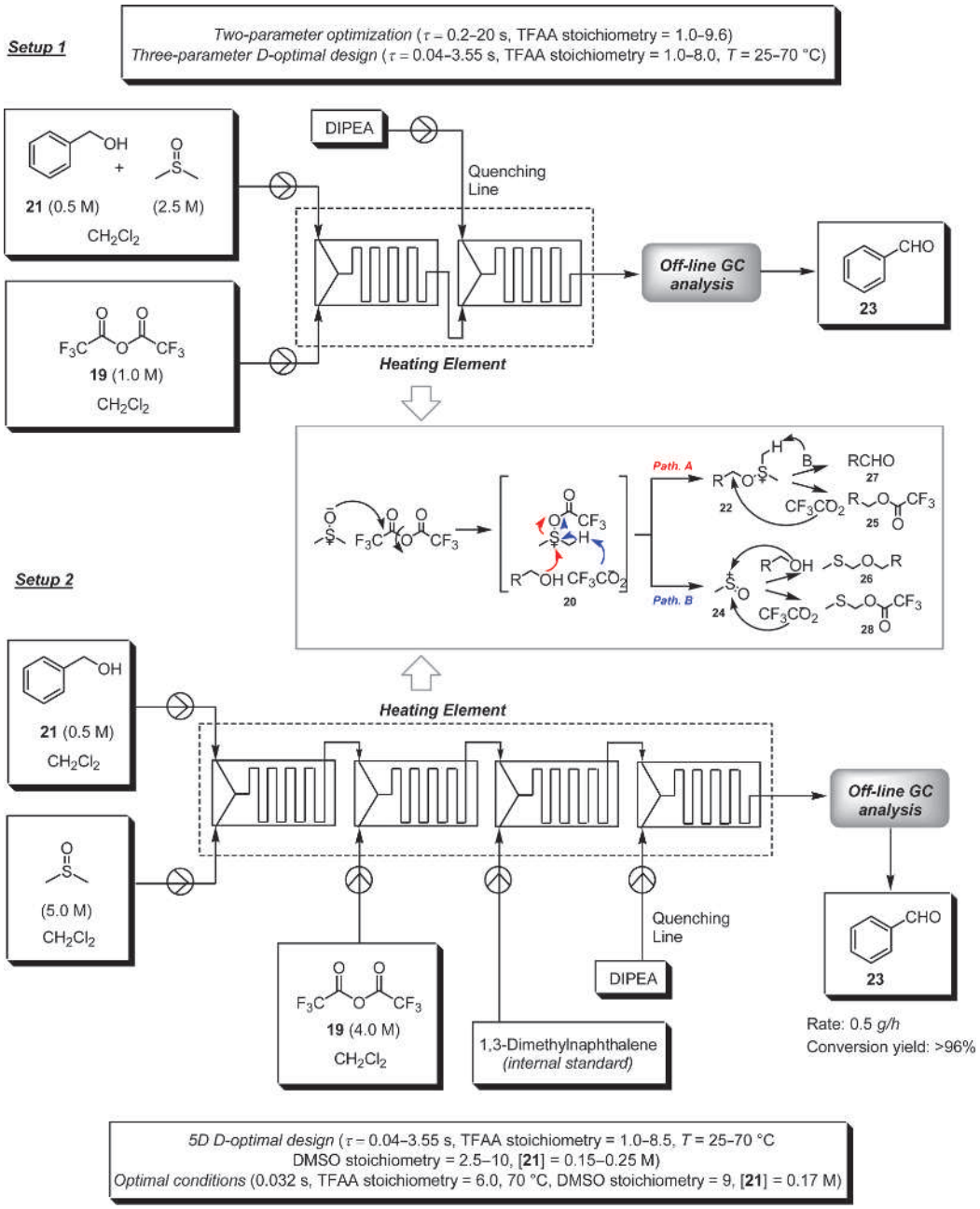
Scheme 5. General flow setup and DoE optimization for the preparation of benzamidoxime derivatives **14a**–**m** in an automated microreactor platform



Scheme 6. General flow setup for the reduction of ethyl hydrocinnamate (**16**) with DIBALH



Scheme 7. Continuous-flow Swern–Moffatt oxidation of alcohols



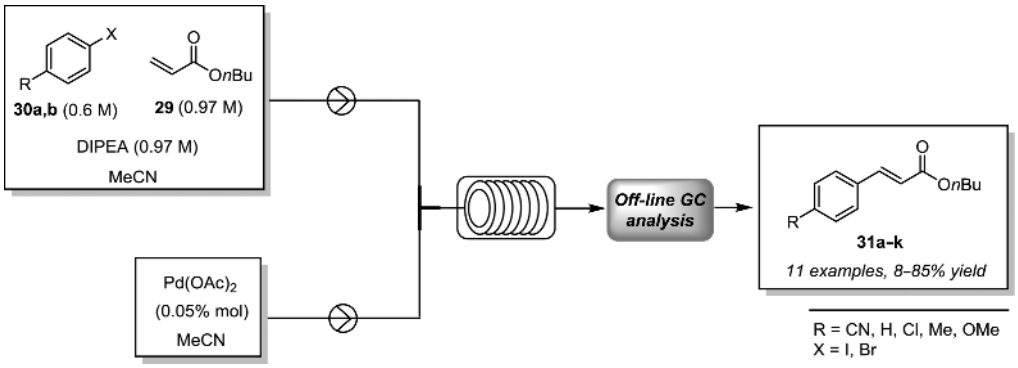
mixture with a tertiary amine gives rise to the corresponding carbonyl derivative **23** (Scheme 7). Despite the high relevance for industry, the necessity to run the reaction at low temperatures severely has limited the application of Swern–Moffatt oxidation in manufacturing processes. Indeed, at higher temperature, intermediates **22** and **24** may undergo the so-called Pummer rearrangement yielding trifluoroacetyl ester **25** and thiomethyl ether **26**, respectively (Scheme 7). Based on previous works [24], initially, the authors studied the reaction in 140-nL flow microtubes by means of a first set of 126 experiments carried out according to Setup 1 (Scheme 7). After interpolation and third-order curve fitting, a polynomial equation was generated from a selection of 30 experiments using a D-optimal model. As a result, high conversion yields to the desired aldehyde were achieved with 1–6 s of residence time using a TFAA–substrate stoichiometric ratio of 6:9. Next, a three-parameter optimization (55 experiments) was performed to unravel the effect of the temperature (25–70 °C) (Scheme 7). Interestingly, the best results were obtained at 45 °C using a TFAA–substrate stoichiometry of 7 and a residence time of 0.5 s. Finally, DMSO stoichiometry relative to alcohol substrate and the relative concentration were also taken into consideration employing a 5-D optimization (180 experiments) (Scheme 7). The three-dimensional slice plot clearly evidenced that residence time, DMSO, and TFAA stoichiometry greatly affected the reaction yield while substrate concentration and temperature were less relevant factors. Remarkably, using a very short mixing and residence time of 32 ms, the optimal temperature was found to be 70 °C, approximately 150 °C higher than under usual conditions. The optimal conditions (residence time = 32 ms, T = 70 °C, TFAA stoichiometry = 6, DMSO stoichiometry = 9, [**21**] = 0.17 M) were then applied to a 500-nL microreactor (yield: ca. 96%), indicating that mixing efficiency was not a limitation up to certain tubing diameter.

In 2012, researchers at Pfizer studied the ligand-free Heck coupling reaction under flow conditions (Scheme 8) [25]. With the aim to minimize the formation of byproducts from reduction (Ar–H) and dimerization (Ar–Ar) reactions, as well as the agglomeration of the catalyst into inactive palladium black, preliminary OVAT experiments were carried out to optimize the loading of $Pd(OAc)_2$. In particular, the efficiency of the reaction as a function of the catalyst loading was investigated, keeping constant the residence time (5 min), the temperature (200 °C), the base (DIPEA, 1.5 equiv.), the equiv. of *n*-butylacrylate (**29**), and the solvent (MeCN, 0.6 M of substrate concentration). *p*-Iodo or *p*-bromobenzonitrile (**30a** or **30b**) were the substrates of choice. As a result, the yield increased using 0.05 mol% of $Pd(OAc)_2$, furnishing **31a** in 85% yield along with a 7% yield of byproducts. A greater amount of byproducts was formed with up to 2 mol% of catalyst. A similar behavior was observed with bromobenzonitrile (**30b**) using a Pd loading between 0.001 and 0.05 mol% and in presence of 0.1 equiv. of tetrabutylammonium bromide (Bu_4NBr) as Pd(0)-stabilizing agent. Interestingly, when the catalyst amount was further increased from 0.05 to 2 mol%, the desired Heck adduct was formed with a slight increase of yield while the formation of side products was negligible [26]. A two-level FD was successfully applied for examining the interplay between temperature and residence time using 0.05 mol% of $Pd(OAc)_2$.

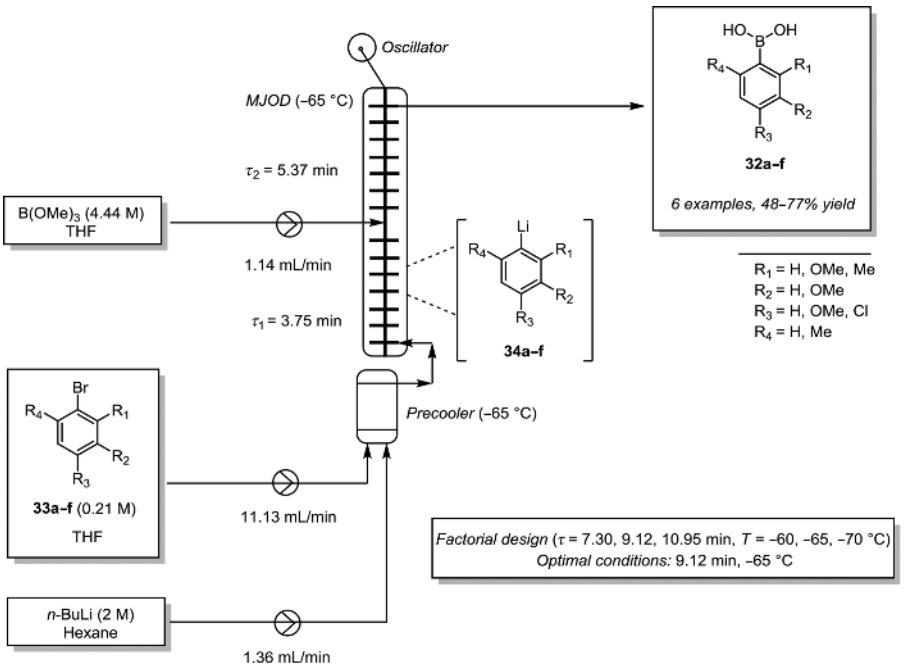
The same year, statistical experimental design was exploited for the synthesis of a small library of phenylboronic acids using a multijet oscillating disc (MJOD) millireactor system [27]. The MJOD reactor is a valuable technology for heterogeneous reactions and flow processes that require cryogenic temperatures. Phenylboronic acids **32a**–**f** were prepared in a continuous two-step telescoped synthesis consisting in the reaction between the corresponding bromobenzene **33a**–**f** and *n*-BuLi to generate phenyllithium **34a**–**f**, which readily reacts with trimethyl borate (Scheme 9). After exploratory experiments aimed at investigating the influence of the temperature for long residence times, an FFD was performed according to the general flow setup depicted in Scheme 8. The matrix was correlated to the response vector using multiple linear regression. The generated contour map predicted a yield of ~100%. However, when the experiment was conducted in the laboratory, the predicted yield was not achieved; instead, the yield was only 69%. This negative outcome was ascribed to the limited screening design that included only two of the experimental parameters leaving out other factors that might be of relevance for the reaction. Further attempt to systematically investigate the process was not performed. Only the effect of **33a** concentration was evaluated during the process intensification (200 mmol scale) using the cryoMJOD flow reactor. The best conditions were then successfully applied to a small selection of bromobenzene derivatives **33b**–**f** (Scheme 9).

The gram-scale flow synthesis of 2,5-dimethyl-pyrroles (**35**) from 2,5-hexanedione (**36**) was achieved by D-optimal algorithms aimed at defining the best set for temperature, stoichiometry of amine–diketone, and reaction time (Scheme 10) [28]. Two solutions of **36** and ethanolamine (**37**)–ethylamine (**38**) in MeOH were used as feedstock, and 2-bromotoluene was added as internal standard to follow the conversion of both the substrate and yield by quantitative GC–flame ionization detection (FID) analysis. A third line of acetone was added as quenching agent to carefully determine the reaction time in combination with off-line analysis. The polynomial equations determined from the raw data and the related contour plots showed that the amine–diketone ratio was determinant for the yield while temperature had a less relevant effect on the reaction. Leave one out cross validation (LOOCV) used to evaluate model predictivity

Scheme 8. Ligand-free Heck coupling reaction under flow conditions



Scheme 9. Telescoped multijet oscillating disk-based flow synthesis of phenylboronic acids

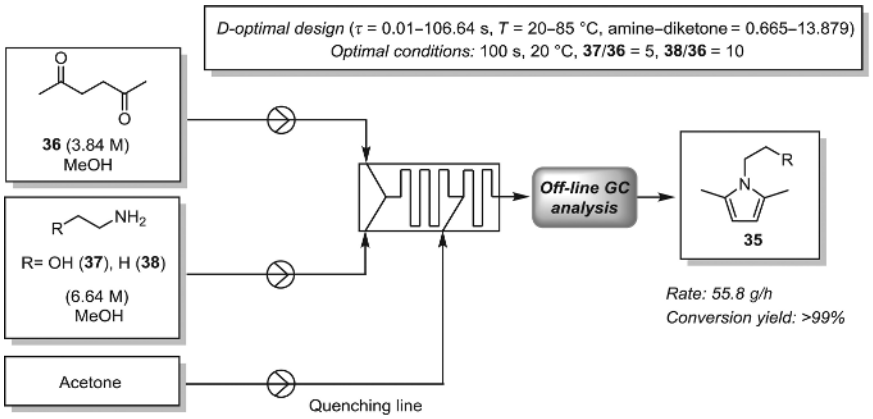


was accomplished at milliliter-scale with a single parallel microstructured flow reactor (internal volume of 9.6 mL). Using the optimal setting (amine–diketone ratio = 5, reaction time = 100 s, T = 20 °C), a quantitative conversion of **37** was observed with an outcome of 55.8 g/h of pyrrole **35** (Scheme 10).

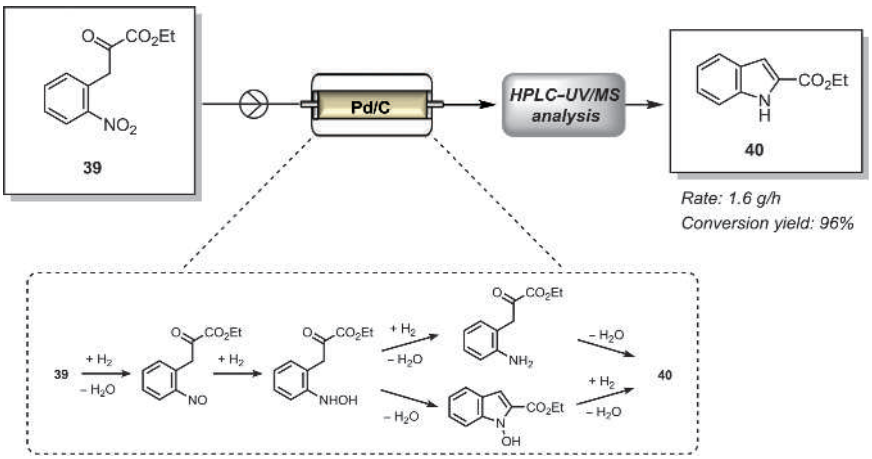
Another application that benefits from DoE concerns the use of hydrogenation flow apparatus. An early study by scientists at Abbott described the synthesis of indole-2-carboxylate and azaindole analogs by Reissert reaction-cascade hydrogenolysis under flow modality [29]. Using *o*-nitrophenylpyruvate (**39**) as the model substrate, 32 experiments (D-optimal design) were performed on a 0.42-mmol scale to screen different parameters (temperature, pressure, catalyst type, solvent, and the presence of AcOH), to identify their main effects and cross-interactions, as well as to verify the robustness of the experiments (Scheme 11). Both the flow rate and substrate concentration were kept constant. The results established Pd/C 10% l-cartridge as the best catalyst, temperature and pressure as significant parameters, and the type of solvent and AcOH not essential to achieve good outcomes. The optimized conditions (catalyst = Pd/C 10% l-cart, solvent = EtOH–EtOAc, flow rate = 3.0 mL/min, substrate concentration = 0.2 M, temperature = 50 °C, pressure = 100 bar) were then applied to long-run experiments that evidenced a decline of reaction efficiency after ~15 min. This lower conversion was attributed to the dissolution of hydrogen on solvent, saturation of hydrogen absorption onto the catalyst, catalyst poisoning, and leaching of palladium. Complementary OVAT experiments were conducted, allowing a stabilized conversion by moving the substrate concentration from 0.2 to 0.05 M. This reduction of **39** concentration was likely to permit catalyst recycle and hydrogen reload. The flow reaction was then performed for 6.75 h using the same catalyst cartridge. Under these conditions, the production output of **40** was 1.6 g/h (96% yield, 88% purity).

More recently, Teva researchers reported the DoE-assisted optimization for the catalytic hydrogenation of methyl 5-[*N*-methyl-*N*-(2,4-dinitrophenyl)amino]-5-oxopentanoate (**41**) to benzimidazole **42** [30], a key intermediate in the synthesis of the antitumor active pharmaceutical ingredient (API) bendamustine hydrochloride (**43**) (Scheme 12). Although the catalytic hydrogenation of the aromatic nitro compound **41** under conventional batch conditions was successful [31], the scale-up presented a number of challenges including byproduct *N*-oxide **44** formation caused by hydrogen starvation, costs, safety concern, and site limitation on hydrogenation at scale. To solve these problems and to increase the overall E-factor of the

Scheme 10. Continuous-flow synthesis of 2,5-dimethyl-pyrroles



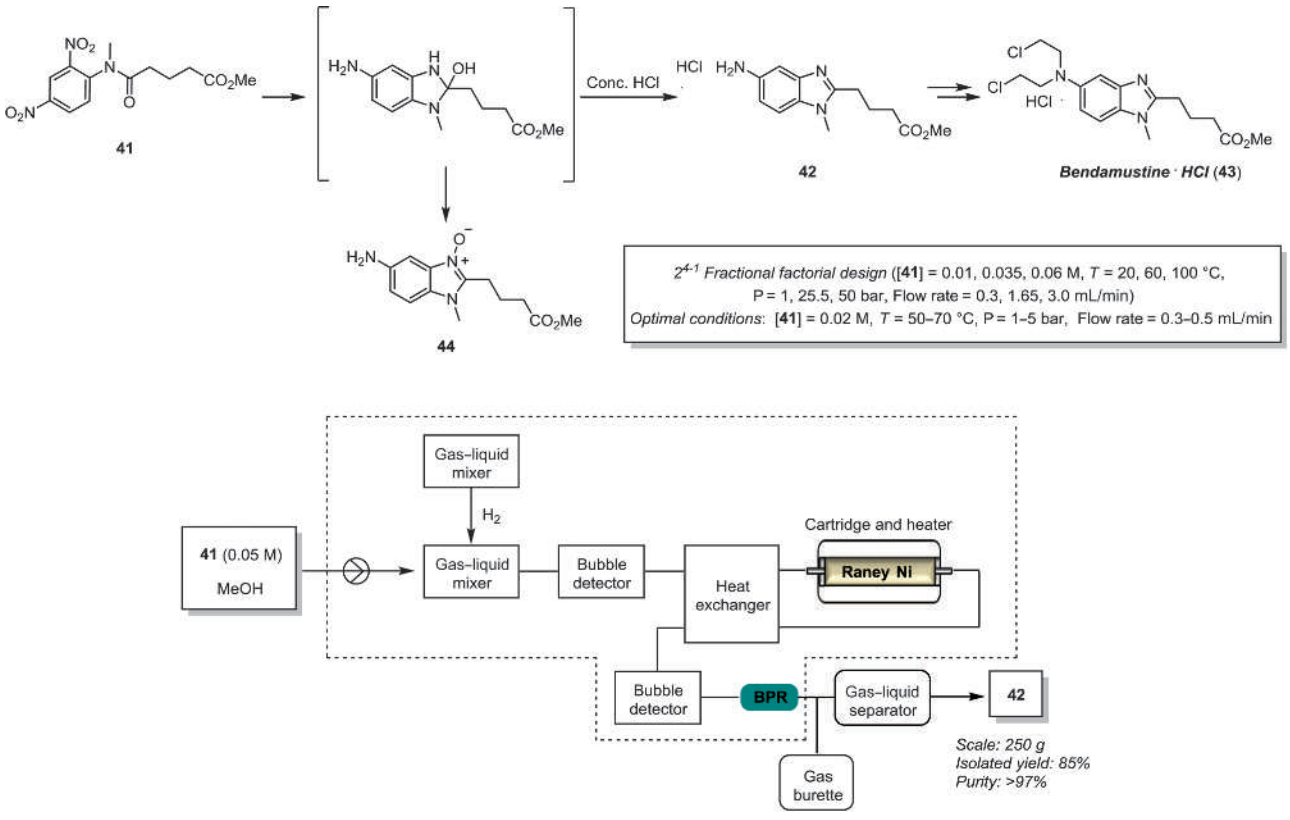
Scheme 11. Mechanism and flow reduction–cyclization of the *o*-nitrophenylpyruvate into ethyl indole-2-carboxylate



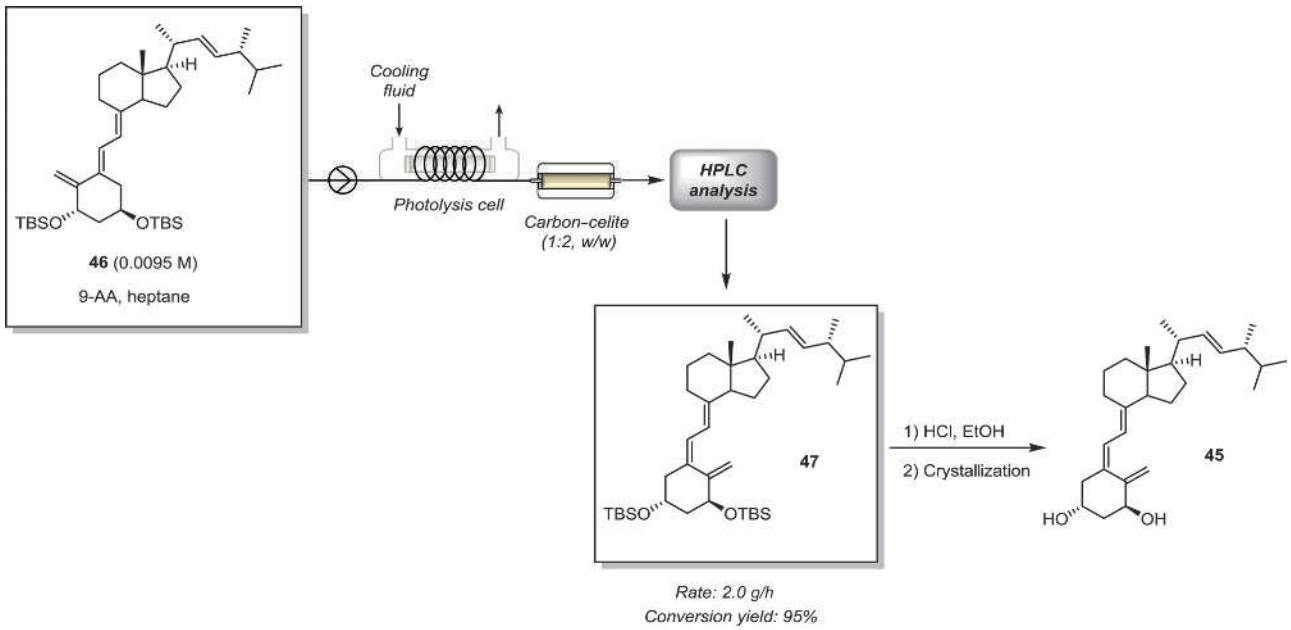
process, a two-level FFD with one center point was applied for the optimization of the reaction in continuous-flow mode. Using Raney nickel as the catalyst and the highest hydrogen rate achievable, four reaction parameters were investigated in the model: temperature (20, 60, and 100 °C), system pressure (1, 25.5, and 50 bar), flow rate (0.3, 1.65, and 3.0 mL/min), and substrate concentration in MeOH (0.01, 0.035, and 0.06 M). Experiments were conducted in a random order, and the yield of each run, determined by HPLC analysis, was plotted versus the flow rate and concentration, as the most significant factors of the reaction. The optimal conditions deriving from the model prediction (Raney nickel, 0.02 M of **41** in MeOH, flow rate 0.3–0.5 mL/min, temperature 50–70 °C, pressure 1–5 bar) were validated, and the reaction was scaled-up on 250 g scale, affording **42** in 85% isolated yield and >97% purity, with 1200 kg/m^3/h^1 space-time yield and 10-fold improvement in catalyst utilization (Scheme 12). Noteworthy, the catalytic performance of Raney nickel was also evaluated by comparing the HPLC analysis at time intervals, showing a constant activity over 48–50 h and demonstrating the sustainability of the process.

A new synthetic approach for the production of doxercalciferol (**45**) from ergocalciferol was developed using the continuous photoisomerization of *trans*-triene vitamin D precursor **46** (Scheme 13) [32]. The scope of this work was to overcome the limitations of batch methods as the nonhomogeneous irradiation of the reaction mixture, which occurs especially with large volumes, and not optimal mixing. Longer reaction times and increased amount of side products may also result in this case. As light exposure can be finely regulated through the flow rate, flow photoreactors are claimed to be endowed with greater efficiency and cleaner reaction. The flow system was constituted by a PTFE tubing coiled around a photolysis cell,

Scheme 12. Synthesis of benzimidazole **42** under continuous-flow conditions and the integrated flow system



Scheme 13. Continuous photochemical production and purification of doxercalciferol (**45**)

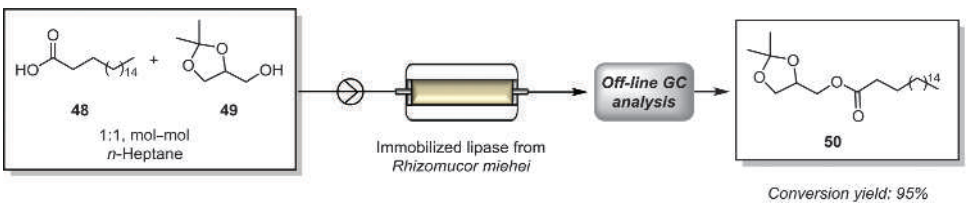


equipped with an immersion well with cooling jacket, a Pyrex sleeve, a 450 W mercury vapor lamp, and a chiller for temperature. The isomerization of **46** in helium-sparged heptane required the presence of 9-acetylanthracene (9-AA) as a photosensitizer agent, which was removed with a column packed with carbon–celite (Scheme 13). The role of the concentration, flow rate, temperature, and 9-AA charge on the reaction outcome was appraised using an FD (19 experiments plus three replicates at the center point). The inspection revealed that higher yields could be achieved by means of higher concentrations of **46** and lower flow rates, or by using more diluted solutions of **46** at higher rates. Both the temperature and the photosensitizer amount were not significant terms. Therefore, supplementary experiments were performed to minimize the amount of photosensitizer and facilitate product purification. Thus, the streamlined photoisomerization coupled with acid deprotection and crystallization gave highly pure doxercalciferol (**45**).

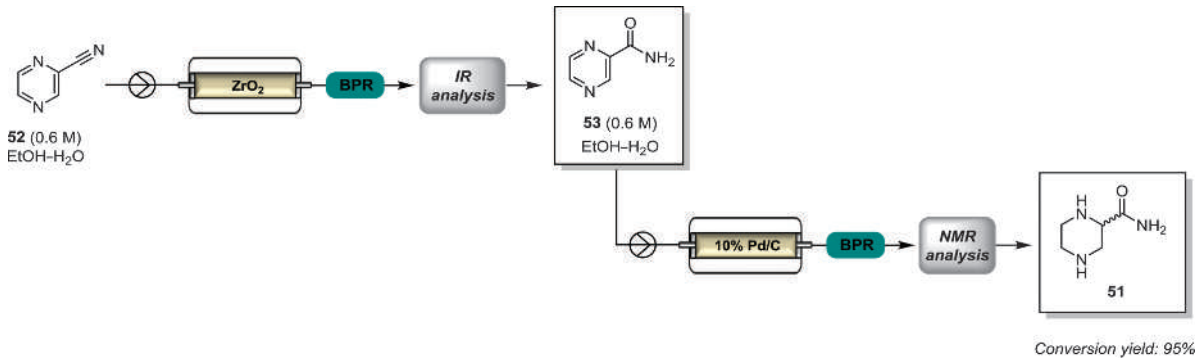
A preliminary FFD and a focused CCD were instrumental to optimize a biocatalytic flow preparation of monoacylglycerols, and eco-friendly and nonionic surfactants [33]. In particular, a packed-bed reactor (0.6 mL volume, 70 mm × 4 mm) containing immobilized lipase from *Rhizomucor miehei* was employed for the esterification of (*R*,*S*)-1,2-isopropylidene glycerol (**48**) with stearic acid (**49**) in *n*-heptane (Scheme 14). FFD 2^{3-1} indicated the flow rate (negative effect) and **49** concentration (positive effect) as the important parameters to be further explored with a CCD (9 experiments plus 3 replicates), while temperature did not show any significance and was kept constant at 60 °C. The reaction data were fitted into a polynomial equation, and the best conditions were successfully applied to the model reaction using two different immobilized lipases (A and C) from *Candida antarctica* (conversion yields = 81% and 91%, respectively).

In 2014, Ley group illustrated the use of an open-source software package integrated with a laboratory hardware to develop and optimize a machine-assisted flow synthesis of pyperazine-2-carboxamide (**51**), a component of the antituberculosis Rifater (Scheme 15) [34]. The reaction consisted in the hydration of nitrile **52** to amide **53** followed by a hydrogenation step. Specifically, a DoE methodology was exploited to improve the synthesis of **51** through the evaluation of the influencing factors and their interplay. Temperature (40 and 100 °C), H_2 pressure (20 bar and full mode), and flow rate (0.1 and 0.2 mL/min) were examined by means of a two-level factorial algorithm (16 experiments, two repeats of eight runs). An automated machine system allowed the fulfilment of up to 9 consecutive reactions. The off-line interpretation of the results disclosed that the effect of H_2 pressure outweighed flow rate and temperature contribution, though a combination of higher temperature and lower flow rate reduced side-products formation. The statistical analysis indicated that 0.1 mL/min of flow rate and 100 °C of temperature under full hydrogen modality could represent the best parameters. Accordingly, experiments repeated in these conditions yielded 95% of the desired product **51** (Scheme 15). The optimized procedure was then combined with the nitrile **52** hydration step using a reservoir flask for the collection of in situ formed **53**. The flask was equipped with a float and a digital webcam for the computerized control of the feedstock so that the reduction stage could start when enough intermediate was collected. Hence, the integrated protocol resulted in a semicontinuous-flow process with a total running time of about 10 h.

Scheme 14. Esterification reaction between of (*R*,*S*)-1,2-isopropylidene glycerol (**48**) and stearic acid (**49**) catalyzed by immobilized lipase from *Rhizomucor miehei* under flow conditions



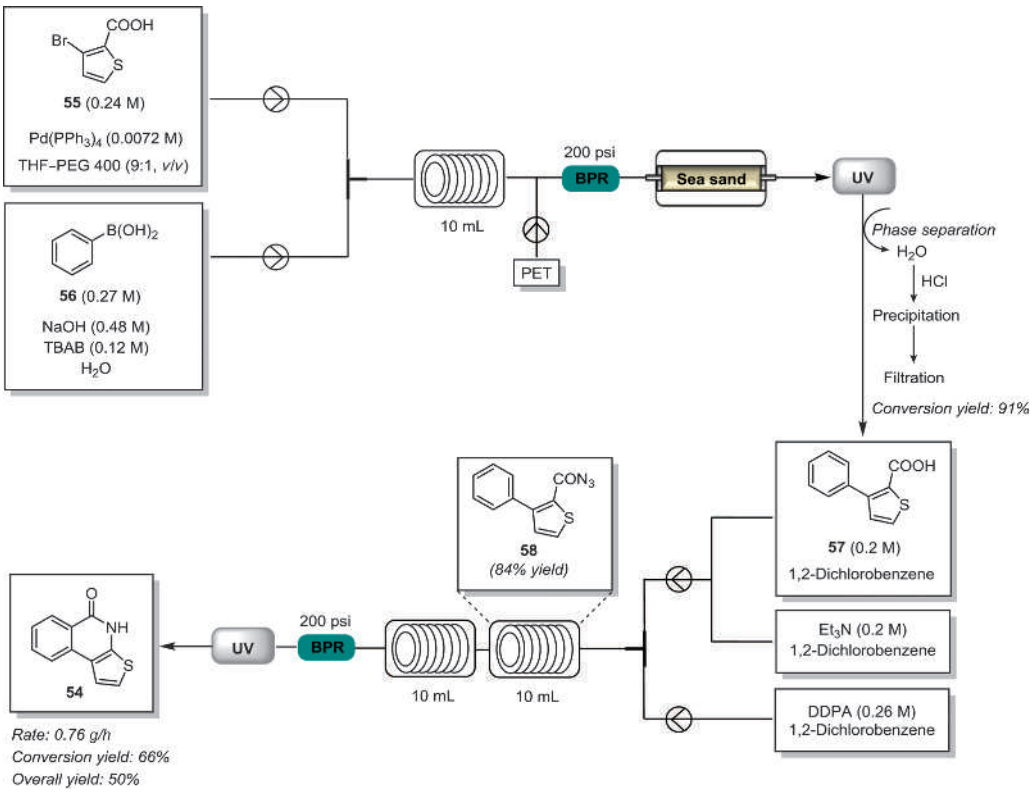
Scheme 15. General setup for the hydrogenation of pyrazine-2-carboxamide (**53**) under flow conditions



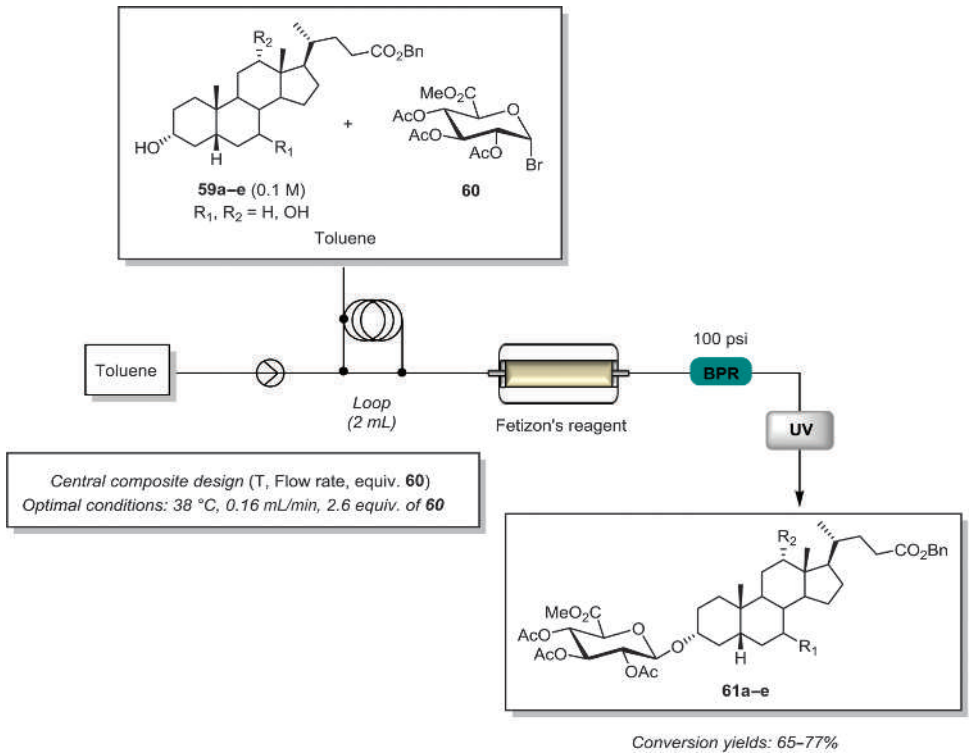
In our group, we demonstrated how DoE principles enabled the telescopic flow synthesis of thieno[2,3-*c*]isoquinolin-5(4*H*)-one-A (TIQ-A, **54**), an important pharmacological tool and building block for the development of PARP-1 inhibitors (Scheme 16) [35]. The batch synthesis for this scaffold suffers from some limitations as the moderate overall yield, the use of hazardous reagents, and a laborious protocol. Each single step was optimized by consequential and integrated phases including the selection of a model reaction and preliminary evaluation of the experimental conditions in batch mode, design of a convenient flow setup, execution and analysis of CCD experiments for the reaction optimization under flow conditions, and scale-up validation. The model reaction for the Suzuki coupling step consisted in the use of 3-bromothiophen-2-carboxylic acid (**55**) and phenylboronic acid (**56**) in the presence of a palladium catalyst ($Pd(PPh_3)_4$), NaOH, and tetrabutylammonium bromide (TBAB) as a phase transfer agent. The data from 12 experiments were fitted into a quadratic equation whose elaboration into a response surface allowed clarifying the dependence of the yield from the temperature and flow rate. The optimal responses (temperature = 173 °C, flow rate = 1.50 mL/min) were validated experimentally with the isolation of intermediate **57** in nearly quantitative yield (Scheme 16). A "one-pot two steps" approach was then accomplished as a means to avoid the isolation of the acyl chloride intermediate and the use of hazardous benzene and $SOCl_2$. Thus, a solution of 3-bromothiophene-2-carboxylic acid (**57**) and triethylamine in 1,2-dichlorobenzene was pumped and reacted at room temperature with a solution of diphenylphosphorylazide (DPPA) in 1,2-dichlorobenzene with a total flow rate of 0.2 mL/min, to give the desired acyl azide **58** in 84% yield. The poor reproducibility and low yield of the thermal cyclization required a CCD optimization whose results evidenced the crucial impact of the temperature (positive effect) and residence time (negative effect), though a lack of predictivity was observed in proximity of the optimal region of the chemical space. The best conditions for the cyclization step reevaluated by a small set of OVAT experiments were applied at gram scale to produce TIQ-A (**54**) in 50% overall yield with a productivity of 0.76 g/h (Scheme 16). The overall flow synthesis was facile, robust, safe, easy to scale-up, and required only a final chromatographic purification.

Another illustration of the power of this approach concerns the flow preparation of bile acid C3-glucuronates [36]. A CCD

Scheme 16. General flow setup and DoE optimization for multigram-scale synthesis of TIQ-A (**54**)



Scheme 17. Regioselective flow synthesis of C3-glucuronidated bile acids



of 19 experiments was employed to set the best conditions for the regioselective Koenigs–Knorr glucuronidation of naturally occurring bile acids (Scheme 17). The model reaction was based on flowing the UV-detectable benzyl ursodeoxycholate (**59a**) with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**60**) in toluene through a column packed with Fetizon's reagent. Temperature (25–80 °C), flow rate (0.1–0.4 mL/min), and stoichiometry of **60** (1.2–3.0 equiv.) were the parameters considered relevant for the conversion yield and regioselectivity of the reaction. As emerged from the analysis of the quadratic equation, the thermal (in)stability of the glucuronyl donor **60** was the main determinant for the reaction outcome: high temperatures correlated with a decrease of yield especially at lower flow rates. The predicted optimal conditions (38 °C, 0.16 mL/min, 2.6 equiv. of **60**) furnished C3-glucuronidated derivative **61a** in 68% yield, with a high degree of regioselectivity and chemoselectivity. Substrate scope was successfully demonstrated, offering the first regioselective strategy for the chemical glucuronidation of naturally occurring bile acids (**59b**–**e**) useful for biological, diagnostic, and pharmacokinetic or absorption, distribution, metabolism, and excretion (PK/ADMET) appraisals.

4. Conclusions

To achieve process optimization, a suitable fine tuning of the reaction conditions is needed. A careful analysis of the impact of relevant experimental parameters and their synergistic interactions is required to ensure the translation of a process from laboratory benches to a manufacturing facility. To this aim, the integration of continuous-flow technology and statistical designs is proving a winning strategy to improve chemical reactions and scale-up operations. In particular, DoE takes advantages from mathematical algorithms to screen reaction parameters, evaluate their effects, and find optimum conditions by a multivariate and broad exploration of the reaction chemical space. Flow devices are suited for such experimentation for their intrinsic characteristics including the rapid and controlled screening of reaction parameters, the possibility to conduct reactions at supercritical conditions, the assistance of in-line purification and analysis, reproducibility, and automation. Through this review, we have illustrated the diverse approaches and applications of DoE in the optimization of flow transformations, demonstrating the value of this strategy in solving synthetic challenges associated with efficiency, safety, costs, and product quality. This was shown to be particularly effective in the development of modern processes for the preparation of target products.

Evolutionary algorithms as efficient global optimization (EGO) [37], Gaussian process (GP) [38], and multi-objective active learner (MOAL) [39], to name a few, their surrogates, and "self-optimizing" systems represent valuable alternatives to DoEs. Although this is an area at nascent state, self-optimization has made significant progress in the field of synthetic optimization and is rapidly growing as a powerful tool for discovery platform [5]. Facilitated by recent advances in laboratory equipments, analytical methods, and computer algorithms, these experimental devices combine automation of reactions, analytical apparatus, and software to generate a closed-loop control that guarantees iterative optimization protocols. More sophisticated machine learning algorithms are also able to predict the outcomes of the future experiments and update the experimental model in a sequential fashion. However, adoption of these approaches involves sophisticated integrated reactors that are expensive and, therefore, may not be available in all labs. In this framework, cloud computing, where networking of synthetic chemists hosted remotely through Internet services, is a novel creative opportunity for chemists to draw benefits and solve challenges in a community and multidisciplinary environment. Evolving from this idea are concepts as "Cloud Chemistry" [40] and "Internet of Things (IoT)" [4a], which envisage the possibility to access equipment remotely, monitor reactions, and acquire real-time data from any Internet-connected device, anywhere in the world.

In conclusion, it is our belief that experimental design is a valuable decision-making toolbox for synthetic chemists in the

critical stage of reaction optimization. Since most of statistical designs lack a priori knowledge of the chemical space under investigation that would better drive the experimental planning, their correct employment cannot leave the scientists' knowledge, skills, attitude, and personal ability to solve synthetic problems out of consideration. Without these qualities, it is nearly impossible to evaluate properly the chemical system, the list of key process factors, and the relative ranges to be explored. Guided by this consciousness, DoE represents a valid option to "traditional" approaches for mapping the experimental space in a multidimensional and interactive level, and for assisting chemists during the tortuous journey of process optimization in both batch and flow conditions.

References

1. Ley, S. V.; Fitzpatrick, D. E.; Ingham, R. J.; Myerset, R. M. *Angew. Chem., Int. Ed.* **2015**, *54*, 3449–3464.

2. For reviews, see: (a) Elvira, K. S.; Casadevalli Solvas, X.; Wootton, R. C. R.; deMello, A. J. *Nature Chem.* **2013**, *5*, 905–915; (b) *Microreactors in Organic Chemistry and Catalysis*, 2nd edition; Wirth, T., Ed; Wiley-VCH: Weinheim, Germany, 2013; (c) Pastre, J. C.; Browne, D. L.; Ley, S. V. *Chem. Soc. Rev.* **2013**, *42*, 8849–8869; (d) McQuade, D. T.; Seeberger, P. H. *J. Org. Chem.* **2013**, *78*, 6384–6389; (e) Wegner, J.; Ceylan, S.; Kirschning, A. *Adv. Synth. Catal.* **2012**, *354*, 17–57; (f) Glasnov, T. N.; Kappe, C. O. *Chem. Eur. J.* **2011**, *17*, 11956–11968; (g) Webb, D.; Jamison, T. F. *Chem. Sci.* **2010**, *1*, 675–680.

3. (a) Cerra, B.; Mostarda, S.; Custodi, C.; Macchiarulo, A.; Gioiello, A. *Med. Chem. Commun.* **2016**, *7*, 439–446; (b) Bryan, M. C.; Hein, C. D.; Gao, H.; Xia, X.; Eastwood, H.; Bruenner, B. A.; Louie, S. W.; Doherty, E. M. *ACS Comb. Sci.* **2013**, *15*, 503–511; (c) Baumann, M.; Baxendale, I. R.; Kuratli, C.; Ley, S. V.; Martin, R. E.; Schneider, J. *ACS Comb. Sci.* **2011**, *13*, 405–413.

4. (a) Fitzpatrick, D. E.; Battilocchio, C.; Ley, S. V. *Org. Process Res. Dev.* **2016**, *20*, 386–394; (b) Amara, Z.; Streng, E. S.; Skilton, R. A.; Jin, J.; George, M. W.; Poliakoff, M. *Eur. J. Org. Chem.* **2015**, 6141–6145; (c) Sans, V.; Porwol, L.; Dragone, V.; Cronin, L. *Chem. Sci.* **2015**, *6*, 1258–1264; (d) Richmond, C. J.; Miras, H. N.; de la Oliva, A. R.; Zang, H.; Sans, V.; Paramonov, L.; Makatsoris, C.; Inglis, R.; Brechin, E. K.; Long, D.-L; Cronin, L. *Nat. Chem.* **2012**, *4*, 1037–1043; (e) Parrott, A. J.; Bourne, R. A.; Akien, G. R.; Irvine, D. J.; Poliakoff, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 3788–3792; (f) Bourne, R. A.; Skilton, R. A.; Parrott, A. J.; Irvine, D. J.; Poliakoff, M. *Org. Process Res. Dev.* **2011**, *15*, 932–938; (g) McMullen, J. P.; Jensen, K. F. *Org. Process Res. Dev.* **2010**, *14*, 1169–1176; (h) McMullen, J. P.; Stone, M. T.; Buchwald, S. L.; Jensen, K. F. *Angew. Chem., Int. Ed.* **2010**, *49*, 7076–7080.

5. (a) Fabry, D. C.; Sugiono, E.; Rueping, M. *React. Chem. Eng.* **2016**, *1*, 129–133; (b) Houben, C.; Lapkin, A. A. *Curr. Opin. Chem. Eng.* **2015**, *9*, 1–7.

6. Seltman, H. J. *Experimental Design and Analysis*; Carnegie Mellon University 2012: Pittsburgh, PA, 2015.

7. (a) Fisher, R. A. *The Design of Experiments*, 8th edition; Hafner Publishing Company: New York, 1966; (b) Fisher, R. A. *Statistical Methods for Research Workers*, 7th edition; Oliver and Boyd: Edinburgh, London, 1970.

8. Owen, M. R.; Luscombe, C.; Lai, L.; Godbert, S.; Crookers, D. L.; Embiata-Smith, D. *Org. Process Res. Dev.* **2001**, *5*, 308–323

9. (a) Leardi, R. *Anal. Chim. Acta* **2009**, *652*, 161–172; (b) Rubin, A. E.; Tummala, S.; Both, D. A.; Wang, C.; Delaney, E. J. *Chem. Rev.* **2006**, *106*, 2794–2810; (c) Tye, H. *Drug Discov. Today* **2004**, *9*, 485–491.

10. (a) Mason, R. L.; Gunst, R. F.; Hess, J. L. *Statistical Design and Analysis of Experiments*, 2nd edition; John Wiley & Sons: New Jersey, 2003; (b) Carlson, R. Design and Optimization in Organic Synthesis. *Data Handling in Science and Technology*, 1st edition; Elsevier Science B. V.: Amsterdam, 1992; vol. 8.

11. Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Wikström, C.; Wold, S. *Design Of Experiments: Principles and Applications*, 3rd edn. Umetrics AB, Umeå Learnways AB: Umeå, Stockholm, 2008.

12. (a) Design-Expert® Software, Stat-Ease, Inc., Minneapolis, Minnesota; (b) MATLAB®, The MathWorks, Inc., Natick, Massachusetts; (c) MODDE®, Umetrics, Umeå, Sweden; (d) Statistica®, StatSoft, Inc., Dell Statistica, Tulsa, Oklahoma.

13. (a) Nagaki, A.; Kim, H.; Yoshida, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 7833–7836; (b) Usutani, H.; Tomida, Y.; Nagaki, A.; Okamoto, H.; Nokami, T.; Yoshida, J. *J. Am. Chem. Soc.* **2007**, *129*, 3046–3047; (c) Tomida, Y.; Usutani, H.; Kim, H.; Takabayashi, N.; Nokami, T.; Okamoto, H.; Yoshida, J. *Chem. Asian J.* **2007**, *2*, 1513–1523.

14. Benali, O.; Deal, M.; Farrant, E.; Tapolczay, D.; Wheeler, R. *Org. Process Res. Dev.* **2008**, *12,* 1007–1011.

15. Tinder, R.; Farr, R.; Heid, R.; Zhao, R.; Rarig, R. S. Jr.; Storz, T. *Org. Process Res. Dev.* **2009**, *13*, 1401–1406.

16. Fisher, R. W.; Misun, M. *Org. Process Res. Dev.* **2001**, *5*, 581–586.

17. Koch, K.; van Weerdenburg, B. J. A.; Verkade, J. M. M.; Nieuwland, P. J.; Rutjes, F. P. J. T.; van Hest, J. C. M. *Org. Process Res. Dev.* **2009**, *13*, 1003–1006.

18. Delville, M. M. E.; Nieuwland, P. J.; Jansen, P.; Koch, K.; van Hest, J. C. M.; Rutjes, F. P. J. T. *Chem. Eng. J.* **2011**, *167*, 556–559.

19. Delville, M. M. E.; van Gool, J. J. F.; van Wijk, I. M.; van Hest, J. C. M.; Rutjes, F. P. J. T. *J. Flow Chem.* **2012**, *2*, 124–128.

20. Becker, R.; van den Broek, S. A. M. W.; Nieuwland, P. J.; Koch, K.; Rutjes, F. P. J. T. *J. Flow Chem.* **2012**, *2*, 87–91.

21. Vörös, A.; Baán, Z.; Mizsey, P.; Finta, Z. *Org. Process Res. Dev.* **2012**, *16*, 1717–1726.

22. Webb, D.; Jamison, T. F. *Org. Lett.* **2012**, *14*, 568–571.

23. Nieuwland, P. J.; Koch, K.; van Harskamp, N.; Wehrens, R.; van Hest, J. C. M.; Rutjes, F. P. J. T. *Chem. Asian J.* **2010**, *5*, 799–805.

24. (a) van der Linden, J. J. M.; Hilberink, P. W.; Kronenburg, C. M. P.; Kemperman, G. J. *Org. Process Res. Dev.* **2008**, *12*, 911–920; (b) Kawaguchi, T.; Miyata, H.; Ataka, K.; Kazuhiro, M.; Yoshida, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2413–2416.

25. Cyr, P.; Deng, S. T.; Hawkins, J. M.; Price, K. E. *Org. Lett.* **2013**, *15*, 4342–4345.

26. Jeffery, T. *Advances in Metal-Organic Chemistry*; Liebeskind, L. S., Ed.; JAI Press Inc: Greenwich, CT, 1996; vol. 5, pp. 153–260.

27. Sleveland, D.; Bjørsvik, H-R. *Org. Process Res. Dev.* **2012**, *16*, 1121–1130.

28. Nieuwland, P. J.; Segers, R.; Koch, K.; van Hest, J. C. M.; Rutjes, F. P. J. T., *Org. Process Res. Dev.* **2011**, *15*, 783–787.

29. Colombo, E.; Ratel, P.; Mounier, L.; Guillier, F. J. *Flow Chem.* **2011**, *2*, 68–73.

30. Chen, J.; Przyuski, K.; Roemmele, R.; Bakale, R. P. *Org. Process Res. Dev.* **2014**, *18*, 1427–1433.

31. Chen, J.; Przyuski K.; Roemmele, R.; Bakale, R. P. *Org. Process Res. Dev.* **2011**, *15*, 1063–1072.

32. Anderson, B. G.; Bauta, W. E.; Cantrell, W. R. Jr. *Org. Process Res. Dev.* **2012**, *16*, 967–975.

33. Junior, I. I.; Flores, M. C.; Sutili, F. K.; Leite, S. G. F.; de M. Miranda, L. S.; Leal, C. R. I.; de Souza, R. O. M. A. *Org. Process Res. Dev.* **2012**, *16*, 1098–1101.

34. Ingham, R. J.; Battilocchio, C.; Hawkins, J. M.; Ley, S. V. *Beilstein J. Org. Chem.* **2014**, *10*, 641–652.

35. Filipponi, P.; Ostacolo, C.; Novellino, E.; Pellicciari, R.; Gioiello, A. *Org. Process Res. Dev.* **2014**, *18*, 1345–1353.

36. Mostarda, S.; Filipponi, P.; Sardella, R.; Venturoni, F.; Natalini, B.; Pellicciari, R.; Gioiello, A. *Org. Biomol. Chem.* **2014**, *12*, 9592–9600.

37. Jones, D. R.; Schonlau, M.; Welch, W. J. J. *Global Optim.* **1998**, *13*, 455–492.

38. Villemonteix, J.; Vazquez, E.; Walter, E. J. *Global Optim.* **2009**, *44*, 509–534.

39. Peremezhneya, N.; Hinesb, E.; Lapkinc, A.; Connaughton, C. *Eng. Optim.* **2014**, *46*, 1593–1607.

40. Skilton, R. A.; Bourne, R. A.; Amara, Z.; Horvath, R.; Jin, J.; Scully, M. J.; Streng, E.; Tang, S. L. Y.; Summers, P. A.; Wang, J.; Pérez, E.; Asfaw, N.; Aydos, G. L. P.; Dupont, J.; Comak, G.; George, M. W.; Poliakoff, M. *Nat. Chem.* **2015**, *47*, 1–5.