Title	Integrating continuous flow synthesis with in-line analysis and data generation
Authors(s)	Baumann, Marcus
Publication date	2018-07-31
Publication information	Baumann, Marcus. "Integrating Continuous Flow Synthesis with In-Line Analysis and Data Generation." Royal Society of Chemistry, July 31, 2018. https://doi.org/10.1039/c8ob01437j.
Publisher	Royal Society of Chemistry
Item record/more information	http://hdl.handle.net/10197/12601
Publisher's version (DOI)	10.1039/c8ob01437j

Journal Name

ARTICLE

Challenges and Opportunities Arising from Integrating Continuous Flow Synthesis with In-line Analysis and Data Generation

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Marcus Baumann*[a]

Continuous flow chemistry has successfully advanced from an academic niche area to a rapidly growing field of its own that directly impacts developments and applications in industrial settings. Whilst the numerous advantages of flow over batch processing are widely recognised and have led to a wider uptake of continuous flow synthesis within the community, we have reached a point where continuous flow synthesis has to transition from a stand-alone enabling technology to a readily integrated synthesis concept. Thus it is paramount to embrace a multitude of in-line analysis and purification techniques to not only allow for efficiently telescoped multi-step sequences but ultimately generate bioactivity data concomittantly on newly synthesised entities. In this short review we wish to summarise the state of the art in this field and present both challenges and opportunities that arise from this ambitious endeavour.

Introduction

Since its advent in research laboratories about 15 years ago, flow chemistry has undertaken a truly remarkable journey from a curiosity driven concept to a widely adopted synthesis mode suitable for the 21st century [1]. Much of this success is based on numerous demonstrations of flow techniques being applied to realise more sustainable chemical syntheses [2] where miniaturisation via micro-reactors enabled fast and safe processes, including many formerly 'forbidden' chemical reactions based on hazardous reagents [3], or highly unstable intermediates [4] that are safely contained within these devices. The accessibility of wider process parameters such as high pressures and temperatures [5] that can be easily accommodated in small footprint flow reactors expanded traditional conditions for many chemical transformations and at the same time reduced reaction times due to faster kinetics [6]. Conversely, superior residence time control at cryogenic temperatures has allowed for flash chemistry [7] to evolve as a subfield in which highly unstable species can be generated and consumed within seconds [8]. These developments have furthermore resulted in the design and application of new reactor types for efficiently handling gases [9] and solids [10] giving rise to their incorporation in effective multi-step flow sequences. The ability to link individual reaction steps into telescoped flow routes [11] necessitated the effective purification of reaction mixtures in-line to ascertain optimal performance during subsequent reactions. With a growing number of powerful in-line purification strategies at hand, the ability of monitor reaction progress by incorporation of in-line spectroscopic techniques [12] has become viable to analyse product composition and identity in real time. Furthermore, several studies have been published linking this data with machine learning algorithms for self-optimising flow reactions [13,14]. Together with the intrinsic power of automated machine-driven processes we have now reached a key stage where flow synthesis of new chemical entities can be linked with data acquisition in an integrated manner [15]. This short review will highlight the current standing of this field and outline challenges to creating key biological data, such as structure-activity-relationships as well as strategies and solutions for overcoming current bottlenecks.

Mastering Multi-Step Sequences in Flow

One of the main disadvantages of conventional batch synthesis is the stepwise execution of chemical reactions requiring each time the charging of a reaction vessel with substrate, reagents and solvent. Similarly, once a reaction is completed a repetitive procedure of quenching, isolation and purification is necessary prior to advancing the obtained product through an iterative sequence of steps into the final target molecule. This process is therefore very labour- and time-intensive and significant amounts of waste are produced at each stage during this effort. Flow chemistry on the other hand enables the chemist to link individual steps into a telescoped sequence to deliver the desired product after careful execution of several linked operations, such as reagent delivery through mixing elements, heating/cooling of the micro-reactor, reaction quenching and in-line purification [16]. Crucial to the success of joining such flow reactions into a telescoped sequence is the optimisation of each individual transformation. The design and streamlining effort that is necessary to enable reaction telescoping is,

[a] School of Chemistry, University College Dublin, Science Centre South, Belfield, Dublin 4, Ireland

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

therefore, a crucial, albeit often underappreciated component of flow chemistry. As such it is not only important to realise the fast and selective transformation of a substrate to the desired product in flow mode, but equally the choice of solvent(s) and reagent(s) is vital to allow for compatibility of subsequent steps including purification steps in between. In-line purification can for instance be accomplished through aqueous extractions [17], crystallisations, [18] or the use of solid-supported scavengers [19] to selectively sequester spent reagents or by-products. However, each of these options needs to be accompanied by further considerations ranging from drying columns to remove traces of water to appropriate dispersion models, when using heterogeneous species such as resins or solid supported scavengers. The use of in-line analysis techniques, [12] such as UV-vis and IR-spectroscopy, have been invaluable in this context, allowing for accurate dosing of subsequent reagent streams needed to minimise reagent use and waste generation. The complexity arising from devising and successfully executing a series of reactions and purifications in a telescoped manner furthermore exemplifies why the adaptation of traceless reagents, as provided through modern photo- [20] and electrochemical [21] reactors, is a vital and highly desirable development in current flow chemistry research. Therefore, it is apparent that mastering the complexity of multi-step flow synthesis, by incorporating efficient in-line purification and analysis techniques, is no simple undertaking and requires careful adaptation of suitable techniques for reaction optimisation assisted by machine learning [13-15,22]. This will result in truly powerful processes that create new and high quality chemical entities and concurrently establish chemical, physical or biological properties to immediately increase the value of these new molecules (Figure 1).

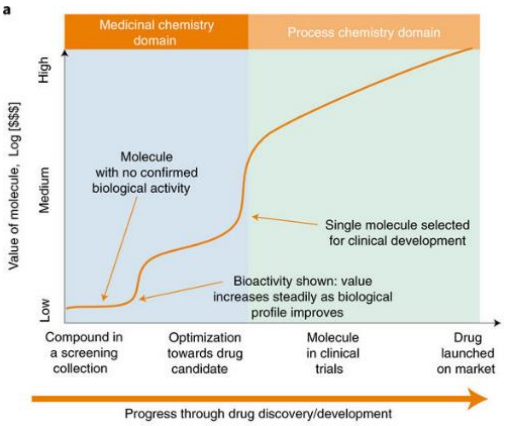
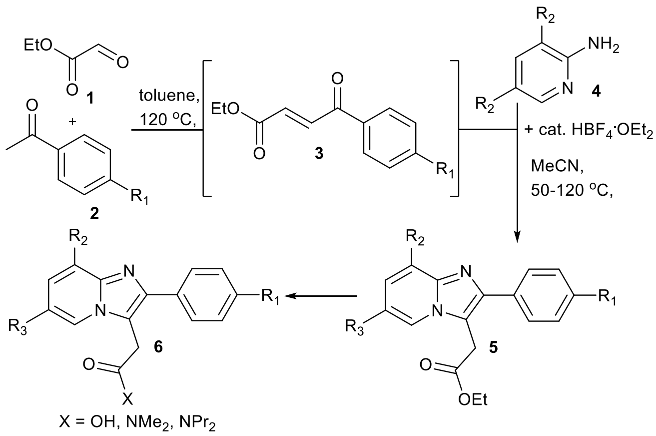


Figure 1: Exemplary value development of a molecule. Figure reprinted with permission from [23].

Key studies on linking chemical synthesis with biological studies

A first study that embraced these challenges was published by the Ley group in early 2013 [24]. In this work a telescoped multi-step construction of several $GABA_A$ agonists is presented which linked flow synthesis with the use of frontal affinity chromatography [25] to evaluate their biological activity. The multi-step synthesis commences with an acid-mediated aldol condensation to give an unsaturated keto ester species (**3**) that is subsequently mixed with different aminopyridine inputs (**4**) using auto-sampling technology (Scheme 1). A variety of different immobilised reagents and scavengers were placed in cartridges to facilitate effective transformations and in-line purification. This enabled the generation of a series of different imidazopyridines (**5**) in pure form within a total of 4 hours synthesis time per compound. To generate the final target structures an autosampler was used to take aliquots of the key intermediate and subject this to either ester hydrolysis, or Me_2AlCl-mediated direct amidation reactions rendering after subsequent purification, a series of acid or amide bearing imidazopyridine structures.



Scheme 1: Simplified synthetic route into target imidazopyridines **5** and **6**.

To evaluate the newly synthesised entities this proof-of-concept study exploited frontal affinity chromatography (FAC) based on immobilised Human Serum Albumin (HSA), the most abundant protein in blood plasma. This biophysical method allows measurement of the binding affinity of small molecules towards a biomolecule upon calibration of the system with a molecule that has no affinity (void marker), as well as strongly binding molecules.

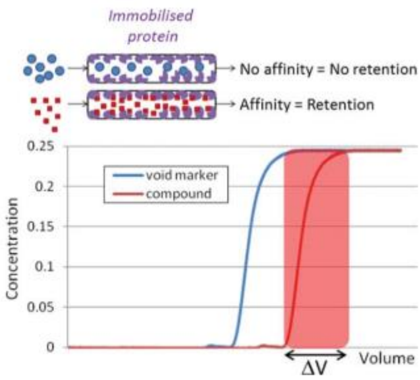


Figure 2: Principle of Frontal Affinity Chromatography (FAC). Reproduced from reference [24] with permission from The Royal Society of Chemistry.

Thus, it was possible to use a flow reactor system to pass aliquots of all imidazopyridine products through a guard column containing immobilised HSA to calculate their binding affinities based on individual retention time. Importantly, this allowed the ranking of these molecules in order of activity as $GABA_A$ agonists. Furthermore, the inclusion of zolpidem and alpidem, two known $GABA_A$ agonists, amongst the synthesised compounds allowed direct comparison and demonstrated good consistency of the data with literature values.
In an analogous fashion the Ley group subsequently demonstrated the use of in-line frontal affinity chromatography to aid in the flow synthesis of several modulators of the histone reader BRD9 [26].

In a subsequent study, a team of researchers from Cyclofluidic recently reported on a technology platform that combines microfluidic synthesis and biological assays with machine learning algorithms to accelerate the hit-to-lead and lead optimisation phases within drug discovery programmes [27]. This approach was demonstrated for the synthesis of novel Abl kinase inhibitors that are important drugs in the treatment of chronic myeloid leukemia. The rapid generation of structure-activity relationships was central to this endeavour and this effort, therefore, highlights a one-step flow synthesis of new lead structures (**7** and **8**, Figure 3) based on a well-studied Sonogashira cross-coupling reaction between alkyne and aryl iodide partners, that were individually synthesised in batch mode.

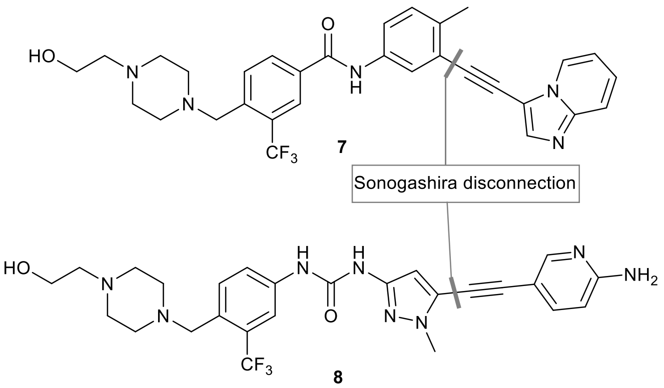


Figure 3: Exemplary structures of benzamide-type (**7**) and pyrazole-urea-type (**8**) Sonogashira products.

The synthesis effort was combined with in-line analysis and purification tools to directly link with an on-line assay, furnishing activity data for each compound (Figure 4). After designing a virtual chemical space for desirable target compounds, it was possible to quickly generate selected structures that in turn would allow for the creation of an SAR heatmap. Using algorithm driven design processes Random Forest activity prediction was employed, via different strategies ('chase potency' or 'most active under sampled'), allowing generation of SAR data based on a subset of compounds synthesised which revealed the most potent structures. The success of this technology platform was demonstrated in the rapid synthesis of only 22 compounds (with a time of ~90 min each) that yielded an SAR heatmap that was remarkably similar to one based on conventionally preparing 270 compounds. Furthermore, this led to the discovery of an unprecedented pyrazole-urea replacement motif (**8**) for the typical benzamide moiety (**7**).

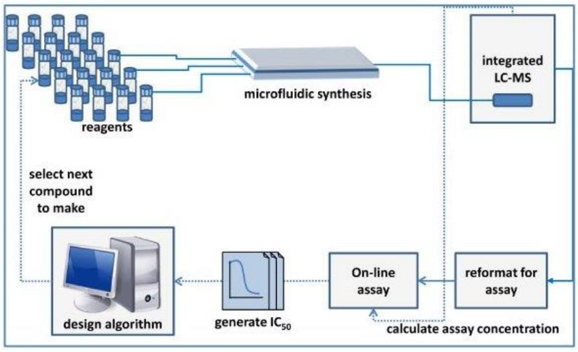
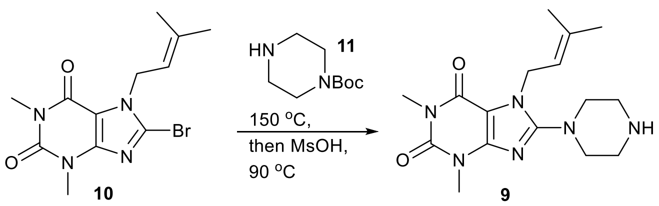


Figure 4: Flow synthesis and on-line assay for Abl kinase inhibitors. Figure reprinted with permission from [27]. Copyright 2013 American Chemical Society.

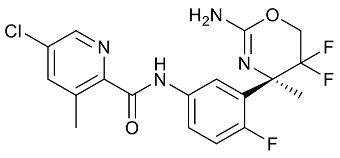
Subsequently, an analogous discovery technology platform was reported under the leadership of Cyclofluidic that integrated the synthesis and testing of amine-substituted xanthine based DPP4 inhibitors **9** [28]. The chemistry involved a high-temperature S_NAr reaction between a bromo-xanthine building block **10** and a mono Boc-protected diamine **11**, followed by acid-mediated Boc-removal (Scheme 2).



Scheme 2: Assembly of exemplary xanthine adduct **9** as potential DPP4 inhibitor.

Within 24 hours 12 compounds were prepared in flow with yields up to 38%. Despite the low yields it was possible to subsequently perform on-line assays furnishing biological data that was found to be in good agreement with data obtained via conventional assays. This effort demonstrated that DPP4 inhibitors can be synthesised, purified and assayed within 2 hours thus presenting options to minimise delays in drug discovery programmes.

In a seminal paper, a team of researchers from Roche reported in early 2014 on an approach for seamlessly integrating micro-fluidic synthesis with microchip-based bioassays [29]. Using commercially available flow reactor technology and liquid handling equipment the efficient one step synthesis of a series of β-secretase (BACE1) inhibitors (**12**), based on an amide forming reaction was realised (Figure 5).



12

Figure 5: Representative structure of a BACE1 inhibitor (**12**).

HPLC and LC-MS systems were used to purify crude reaction mixtures and confirmed the identity of the desired amide products that were subsequently quantified via an ELSD detector. To dilute the desired compound up to six orders of magnitude a neat flow approach was developed, whereby dilution was achieved via Taylor dispersion [30] within a thin capillary (Figure 6, top). Upon addition of the assay reagents (BACE1 enzyme and substrate) to the compound gradient in a distinct ratio, the assay mixture was passed into a micro-chip in which a fluorescence dequenching assay was employed to deliver activity data, which was calibrated against a reference compound (fluorescein; Figure 6, bottom).

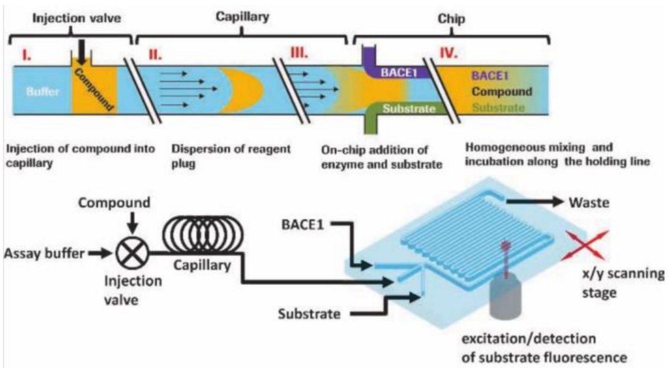


Figure 6: Flow-assay procedure for BACE1 inhibitors. Figure reprinted with permission from [29].

Importantly, this integrated approach demonstrated a remarkable efficiency allowing for a cycle time of 1 h for synthesis, purification and assaying of each compound. This resulted in valuable SAR data that was not only highly consistent with classically established data, but moreover proofed that activities spanning several orders of magnitude could be established with a single technology platform (Figure 7).

Furthermore, owing to the minute quantities of substrates employed and the general versatility of fluorescence-based assays, this approach can easily be applied to many other SAR studies where acceleration of cycle times is crucial.

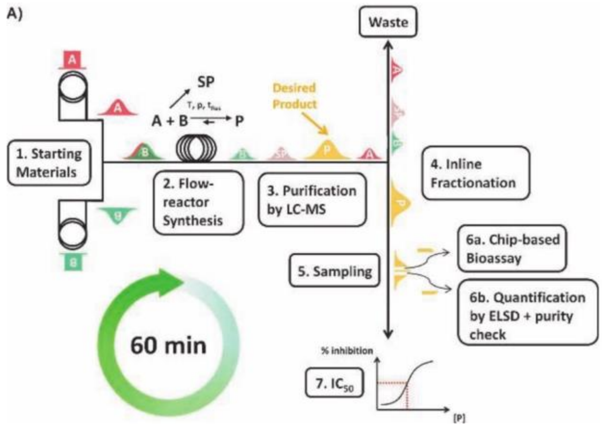


Figure 7: Workflow of synthesis and assay platform. Figure reprinted with permission from [29].

In a final example, a team of researchers at AbbVie have recently reported on their integrated technology platform that combines chemical synthesis with purification and testing modules to expedite generation and evaluation of small molecule libraries [31]. This effort is an important extension of prior work of AbbVie that demonstrated integration of flow synthesis with purification and sample management systems [32]. In this current study two libraries of binders of polycomb protein EED were synthesised either through amidation or Buchwald coupling chemistry (Figure 8).

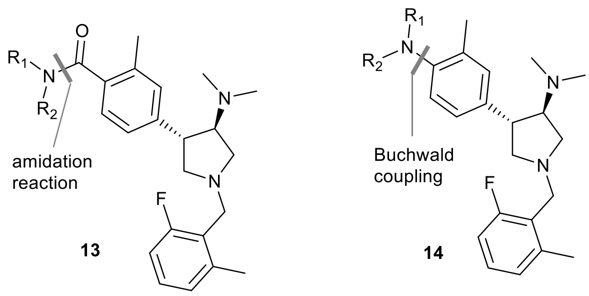


Figure 8: Representative structures prepared by AbbVie through amidation or Buchwald coupling chemistry.

The integrated platform comprises of a combination of commercial equipment and in-house systems to best meet the demands of such campaigns. As such a SWAVE synthesiser was used to prepare the target compounds that are subsequently subjected to MS-triggered preparative HPLC purification and a charged aerosol detector (CAD) for quantification (Figure 9). Following this, two aliquots of each purified compound were transferred to a 96-well plate, one of which was subsequently used for the bioassay. The second was used for confirming purity by NMR spectroscopy. The aliquot destined for the bioassay was evaporated, redissolved in DMSO to a specific concentration and subsequently subjected to serial dilution and a TR-FRET-based binding assay.

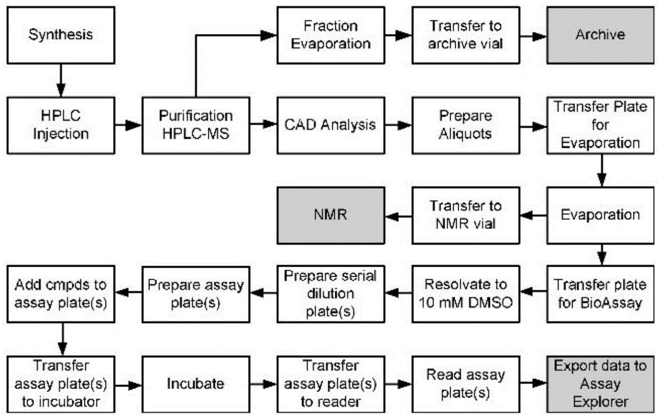


Figure 9: Workflow of AbbVie's integrated platform. Figure reprinted with permission from [31]. Copyright 2017 American Chemical Society.

Based on this integrated approach it was possible to complete the cycle of synthesising, purifying and testing a 22-member amide library in a total time of 15 hours. Similarly, the Buchwald library of 33 amine products was synthesised, purified and tested within 30 h. Importantly, the assay data obtained via the integrated approach showed very good consistency with data obtained through conventional off-line assays, highlighting that no compromise was required when accelerating the traditional turn-around time.

Relevance of Automation Technology

As highlighted in the above case studies and several other recent reports, [33] automation is a central component for linking different enabling technologies resulting in the integration of synthesis, purification and screening modules for bioactive entities [34]. This positively affected the synthesis stage, whereby flow reactors were used to enable the synthesis which was supported by liquid handlers and fraction collectors for sample management and collection. Purification is a key stage for the integration of synthesis and assaying platforms and different approaches have been utilised by different research teams. At this point it appears that HPLC combined with ELSD, or CAD detectors is the preferred option when single step syntheses are envisioned. This can be accounted for by the widespread availability of this technology in industry together with significant expertise in method development and optimisation. On the other hand, more flexibility and expertise are required when performing multi-step synthesis in continuous flow mode, since purification and analysis is crucial at various stages throughout the sequence to ascertain purity of intermediates and removal of contaminants that would otherwise severely impact downstream reactions. Consequently, different techniques such as aqueous washes, crystallisations and immobilised scavenger columns must be evaluated and optimised. Automation of these operations is challenging as it requires careful tuning of various parameters and although this has been largely avoided in industry-led case studies, several academic studies by Ley [35] and others [36] have demonstrated that this can be done through the incorporation of visual aids and suitable software control. Arguably more work is needed to advance these solutions towards hands-on and readily available methods. Additionally, robust and simple solutions are required for automating sample weighing, dissolution and evaporation as this is commonly performed off-line by a chemist and hence takes up considerable amounts of time. Inspiration might be available from high-throughput screening technology that allows rapid processing of samples in a highly automated, albeit cost-intensive process. Crucially, recent studies outlining the successful development of molecule design and SAR predication-validation protocols are indicating effective ways of automating these processes through deep learning algorithms [13] and design of experiment approaches [14].

Future Challenges and Opportunities

Based on the pioneering studies from both academia and industry, key benefits and opportunities can clearly be identified that will pave the way for further developments in this important field. A main driver is the speed at which new molecules can be designed, prepared and tested. In the above studies this ranges from 1-4 hours depending on the complexity of both the synthesis and the biological assay. This compares very favourably with the traditional cycle time which for pharmaceutical applications is reported to be 7-10 days [29]. Furthermore, the ability to considerably accelerate the crucial decision-making process will inevitably improve the overall lead identification and optimisation stages since a wider target area can be considered initially that upon early and rapid feedback can be refined as needed. A further benefit of integrating synthesis and screening technologies is stated in the minute quantities of lead compound needed for performing in-line assays. As this is commonly in the range of only a few milligrams, significant reduction in reagent use and waste generation can be expected, thus dramatically improving on the sustainability of the drug discovery process [29]. Technology-based approaches should furthermore allow for more reliable and reproducible campaign outcomes and the appropriate use of customised instrumentation can be expected to lead to lower maintenance requirements and hence cost savings. Finally, all these considerations taken together will enable the fast and effective exploration of new chemical and patentable space which is expected to enrich drug discovery pipelines in the coming years.

Despite these promising and appealing features, several challenges must be addressed at this stage to realise the desired outcomes from this technology. As the automation applied to the drug discovery process will be welcomed by many, the high degree of control might discourage serendipitous discoveries, unless we allow for a certain degree of freedom. Additionally, the design and synthesis of new molecular structures and their subsequent evaluation via automated SAR-data generation must be able to handle non-linear relationships between chemical structures and data obtained [34]. This will be vital to ascertain a reliable readout of the data generated that must allow for validation by experienced medicinal chemists at key stages of this process. Consequently, the reliability and suitability of biological assays must be regularly evaluated by automated retesting mechanisms to avoid both false-positive and false-negative results to misguide the overall campaign [37]. Despite the current availability of numerous flow synthesis modules that can cater for different needs such as cost, reaction scale, synthesis complexity and homogeneity of inputs, improved and customisable units will continue to be required at low cost to serve the medicinal chemist in such endeavours. This should also include simple chemistry and purification tools that are available 'off-the-shelf' and can be implemented without the need to redesign an initial set-up. Such synthesis tools might include cartridges filled with new catalysts, reagents and valuable enzymes, enabling the performance of more complex and readily telescoped synthesis sequences and thus allowing chemists in industry to extent their synthesis portfolio beyond standard reactions such as amide formations, C-C cross-couplings and common C-N bond formations. Pleasingly, a growing number of applications are already detailing the resulting benefits of flow processing to improve drug development campaigns [38]. Most importantly, it will be crucial to develop and apply such solutions at affordable cost within years rather than decades to enable a growing realisation and uptake of these principles and consequently a rapid transition from today's proof-of-concept studies into routine processes pivotal in drug discovery campaigns.

Conclusions

The availability of numerous modular flow reactor systems that are capable of successfully executing challenging multi-step synthesis covering milligram to kilogram scales, homogeneous and heterogeneous chemistry, as well as in-line analysis and purification operations has expanded the chemist's toolbox in an unprecedented fashion. To control such processes several automation and software aids have been developed and applied, allowing for the integration of synthesis and process tools. As exemplified in this short review, several research groups have taken on the challenge of integrating chemical synthesis with biological evaluation concepts delivering SAR data through automated and accelerated means. Although these proof of concept studies have clearly demonstrated the feasibility of linking chemical synthesis with biological assays, several bottlenecks must be overcome to make this the approach of choice in the future. As such, easily customisable software is required that allows non-experts to set up flow processes that are integrated with in-line purification and analysis tools, prior to a multitude of biological assays. Importantly, the cost of such systems needs to be considered to ensure wider uptake of this advanced technology. Cross-disciplinary training of future scientists will be vital to provide key skills for chemical, engineering and biological challenges. This is believed to be a pivotal element to unite the existing stand-alone techniques into readily integrable technology platforms that will succeed in performing a greater variety of chemical reaction sequences and biological assays than currently demonstrated. If we can advance this field accordingly it will soon be common practice that chemical synthesis will be accompanied by activity evaluation of new entities in an integrated manner. Crucially, this will then empower us to reach the much-anticipated goal of current dial-a-molecule campaigns thus enabling us to deliver tailored molecular structures on demand.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We are grateful for support provided by the School of Chemistry, University College Dublin, Ireland.

Notes and references

1 M. B. Plutschack, B. Pieber, K. Gilmore, P. H. Seeberger, *Chem. Rev.*, 2017, **117**, 11796; D. E. Fitzpatrick, C. Battilocchio, S. V. Ley, *ACS Cent. Sci.*, 2016, **2**, 131; S. V. Ley, D. E. Fitzpatrick, R. M. Myers, C. Battilocchio, R. J. Ingham, *Angew. Chem. Int. Ed.*, 2015, **54**, 10122; K. F. Jensen, *AIChE J.*, 2017, **63**, 858.

2 M. Kakuta, F. G. Bessoth, A. Manz, *Chem. Rev.*, 2001, **101**, 395; V. Hessel, H. Löwe, F. Schönfeld, *Chem. Eng. Sci.*, 2005, **60**, 2479; D. Dallinger, C. O. Kappe, *Curr. Opi. Green Sust. Chem.*, 2017, **7**, 6; J. Yoshida, H. Kim, A. Nagaki, *ChemSusChem*, 2011, **4**, 331; S. V. Ley, *Chem. Record.*, 2012, **12**, 378; S. G. Newman, K. F. Jensen, *Green Chem.*, 2013, **15**, 1456.

3 M. Movsisyan, E. I. P. Delbeke, J. K. E. T. Berton, C. Battilocchio, S. V. Ley, C. V. Stevens, *Chem. Soc. Rev.*, 2016, **45**, 4892; B. Gutmann, C. O. Kappe, *J. Flow Chem.*, 2017, **7**, 65; B. Pieber, C. O. Kappe, *Chim. Oggi/Chem. Today*, 2016, **34**, 38; C. B. McPake, G. Sandford, *Org. Process Res. Dev.*, 2012, **16**, 844.

4 B. J. Deadman, S. G. Collins, A. R. Maguire, *Chem. Eur. J.*, 2015, **21**, 2298; T. Hu, I. R. Baxendale, M. Baumann, *Molecules*, 2016, *21*, 918; G. Glotz, R. Lebl, D. Dallinger, C. O. Kappe, *Angew. Chem. Int. Ed.*, 2017, **56**, 13786; D. Dallinger, C. O. Kappe, *Nature Prot.*, 2017, **12**, 2138; B. Pieber, C. O. Kappe, *Org. Lett.*, 2016, **18**, 1076; F. J. Strauss, D. Cantillo. J. Guerra, C. O. Kappe, *React. Chem. Eng.*, 2016, **1**, 472; A. Harsanyi, A. Conte, L. Pichon, A. Rabion, S. Grenier, G. Sandford, *Org. Process Res. Dev.*, 2017, **21**, 273.

5 J. Hartwig, S. Ceylan, L. Kupracz, L. Coutable, A. Kirschning, *Angew. Chem. Int. Ed.*, 2013, **52**, 9813; A. R. Bogdan, M. Charaschanya, A. W. Dombrowski, Y. Wang, S. W. Djuric, *Org. Lett.*, 2016, **18**, 1732; A. R. Bogdan, N. W. Sach, *Adv. Synth. Catal.*, 2009, **351**, 849; S. Abele, S. Höck, G. Schmidt, J.-A. Funel, R. Marti, *Org. Process Res. Dev.*, 2012, **16**, 1114; M.

Tilley, G. Li, P. Savel, D. Mallik, M. G. Organ, *Org. Process Res. Dev.*, 2016, **20**, 517.

6 V. Hessel, D. Kralisch, N. Kockmann, T. Noël, Q. Wang, *ChemSusChem*, 2013, **6**, 746; B. Gutmann, D. Cantillo, C. O. Kappe, *Angew. Chem. Int. Ed.*, 2015, **54**, 6688; N. Zaborenko, M. W. Bedmore, T. F. Jamison, K. F. Jensen, *Org. Process Res. Dev.*, 2011, **15**, 131.

7 J. Yoshida, Y. Takahashi, A. Nagaki, *Chem. Commun.*, 2013, **49**, 9896; J. Yoshida, *Chem. Rec.*, 2010, **10**, 332; J. Yoshida, *Flash Chemistry. Fast Organic Synthesis in Microsystems*, Wiley-Blackwell, 2008; P. J. Nieuwland, K. Koch, N. van Harskamp, R. Wehrens, J. C. M. van Hest, F. P. J. T. Rutjes, *Chem.-Asian J.*, 2010, **5**, 799.

8 A. Hafner, V. Mancino, M. Meisenbach, B. Schenkel, J. Sedelmeier, *Org. Lett.*, 2017, **19**, 786; M. Ketels, D. B. Konrad, K. Karaghiosoff, D. Trauner, P. Knochel, *Org. Lett.*, 2017, **19**, 1666; L. Kupracz, A. Kirschning, *Adv. Synth. Catal.*, 2013, **355**, 3375; Z. He, T. F. Jamison, *Angew. Chem. Int. Ed.*, 2014, **53**, 3353.

9 M. Brzozowski, M. O'Brien, S. V. Ley, A. Polyzos, *Acc. Chem. Res.*, 2015, **48**, 349; C. J. Mallia, I. R. Baxendale *Org. Proces Res. Dev.*, 2016, **20**, 327.

10 S. L. Poe, M. A. Cummings, M. P. Haaf, D. T. McQuade, *Angew. Chem. Int. Ed.*, 2006, **45**, 1544; J. Sedelmeier, S. V. Ley, I. R. Baxendale, M. Baumann, *Org. Lett.*, 2010, **12**, 3618; T. Horie, M. Sumino, T. Tanaka, Y. Matsushita, Y. Ichimura, J. Yoshida, *Org. Process Res. Dev.*, 2010, **14**, 405; T. Noël, J. R. Naber, R. L. Hartman, J. P. McMullen, K. F. Jensen, S. L. Buchwald, *Chem. Sci.*, 2011, **2**, 287; R. L. Hartman, *Org. Process Res. Dev.*, 2012, **16**, 870; P. Filipponi, A. Gioiello, I. R. Baxendale, *Org. Process Res. Dev.*, 2016, **20**, 371; M. Baumann, I. R. Baxendale, *Eur. J. Org. Chem.*, 2017, 6518; M. Baumann, I. R. Baxendale, P. Filipponi, T. Hu, *Org. Process Res. Dev.*, 2017, **21**, 2052; N. C. Neyt; D. L. Riley, *React. Chem. Eng.*, 2018, **3**, 17.

11 D. Webb, T. F. Jamison, *Org. Lett.*, 2012, **14**, 568; M. Brasholz, J. M. Macdonald, S. Saubern, J. H. Ryan, A. B. Holmes, *Chem. Eur. J.*, 2010, **16**, 11471; I. R. Baxendale, S. V. Ley, A. Mansfield, C. D. Smith, *Angew. Chem. Int. Ed.*, 2009, **48**, 4017; A. A. Desai, E. J. Molitor, J. E. Anderson, *Org. Process Res. Dev.*, 2012, **16**, 160.

12 *For in-line IR, please see:* H. Lange, C. F. Carter, M. D. Hopkin, A. Burke, J. G. Goode, I. R. Baxendale, S. V. Ley, *Chem. Sci.*, 2011, **2**, 765; S. Hübner, U. Bentrup, U. Budde, K. Lovis, T. Dietrich, A. Freitag, L. Küpper, K. Jähnisch, *Org. Process Res. Dev.*, 2009, **13**, 952; T. Brodmann, P. Koos, A. Metzger, P. Knochel, S. V. Ley, *Org. Process Res. Dev.*, 2012, **16**, 1102; J. S. Moore, K. F. Jensen, *Angew. Chem. Int. Ed.*, 2014, **53**, 470. *For in-line MS, please see:* D. L. Browne, S. Wright, B. Deadman, S. Dunnage, I. R. Baxendale, R. Turner, S. V. Ley, *Rapid Commun. Mass Spectrom.*, 2012, **26**, 1999; *For in-line NMR, please see:* B. Ahmed-Omer, E. Sliwinski, J. P. Cerroti, S. V. Ley, *Org. Process Res. Dev.*, 2016, **20**, 1603; M. V. Gomez, A. de la Hoz, *Beilstein J. Org. Chem.*, 2017, **13**, 285; D. A. Foley, E. Bez, A. Codina, K. L. Colson, M. Fey, R. Krull, D. Piroli, M. T. Zell, B. L. Marquez, *Anal. Chem.*, 2014, **86**, 12008; *For in-line UV, please see:* J. Yue, F. H. Falke, J. C. Schouten, T. A. Nijhuis, *Lab Chip*, 2013, **13**, 4855; M. Baumann, I. R. Baxendale, *React. Chem. Eng.*, 2016, **1**, 147.

13 J. P. McMullen, M. T. Stone, S. L. Buchwald, K. F. Jensen, *Angew. Chem. Int. Ed.*, 2010, **49**, 7076; J. E. Hochlowski, P. A. Searle, N. P. Tu, J. Y. Pan, S. G. Spanton, S. W. Djuric, *J. Flow Chem.*, 2011, **2**, 56; D. Reker, G. Schneider, *Drug Discov. Today*, 2015, **20**, 458; L. Zhang, J. Tan, D. Han, H. Zhu, *Drug Discov. Today*, 2017, **22**, 1680.

14 S. A. Weissman, N. G. Anderson, *Org. Process Res. Dev.*, 2015, **19**, 1605; A. Echtermeyer, Y. Amar, J. Zakrzewski, A. Lapkin, *Beilstein J. Org. Chem.*, 2017, **13**, 150.

15 R. J. Ingham, C. Battilocchio, D. E. Fitzpatrick, E. Sliwinski, J. M. Hawkins, S. V. Ley, *Angew. Chem. Int. Ed.*, 2015, **54**, 144; S. V. Ley, D. E. Fitzpatrick, R. J. Ingham, R. M. Myers, *Angew. Chem. Int. Ed.*, 2015, **54**, 3449; V. Sans, L. Cronin, *Chem. Soc. Rev.*, 2016, **45**, 2032; C. A. Shukla, A. A. Kulkarni, *Beilstein J. Org. Chem.*, 2017, **13**, 960.

16 J. Britton, C. L. Raston, *Chem. Soc. Rev.*, 2017, **46**, 1250; P. Bana, R. Örkenyi, K. Lövei, A. Lako, G. I. Turos, J. Eles, F. Faigl, I. Greiner, *Bioorg. Med. Chem.*, 2017, **25**, 6180; P. Pieber, K. Gilmore, P. H. Seeberger, *J. Flow. Chem.*, 2017, **7**, 129; M. D. Johnson, S. A. May, J. R. Calvin, J. Remacle, J. R. Stout, W. D. Diseroad, N. Zaborenko, B. D. Haeberle, W.-M. Sun, M. T. Miller, J. Brennan, *Org. Process Res. Dev.*, 2012, **16**, 1017; S. May, *J. Flow Chem.*, 2017, **7**, 137; H. Zhang, J. Quon, A. J. Alvarez, J. Evans, A. S. Myerson, B. Trout, *Org. Process Res. Dev.*, 2012, **16**, 915; M. Baumann, I. R. Baxendale, *Beilstein J. Org. Chem.*, 2015, **11**, 1194.

17 D. X. Hu, M. O'Brien, S. V. Ley, *Org. Lett.*, 2012, **14**, 4246; A. Adamo, P. L. Heider, N. Weeranoppanant, K. F. Jensen, *Ind. Eng. Chem. Res.*, 2013, **52**, 10802; M. Baumann, I. R. Baxendale, F. Deplante, *Beilstein J. Org. Chem.*, 2017, **13**, 2549.

18 S. Y. Wong, A. P. Tatusko, B. L. Trout, A. S. Myerson, *Cryst. Growth Des.*, 2012, **12**, 5701; A. J. Alvarez, A. S. Myerson, *Cryst. Growth Des.*, 2010, **10**, 2219; J. L. Quon, H. Zhang, A. Alvarez, J. Evans, A. S. Myerson, B. L. Trout, *Cryst. Growth Des.*, 2012, **12**, 3036.

19 R. M. Myers, K. A. Roper, I. R. Baxendale, S. V. Ley, *The Evolution of Immobilized Reagents and their Application in Flow Chemistry for the Synthesis of Natural Products and Pharmaceutical Compounds* in *Modern Tools for the Synthesis of Complex Bioactive Molecules.* J. Cossy and S. Arseniyadis (eds), Wiley-VCH, 2012.

20 D. Cambie, C. Bottecchia, N. J. W. Straathof, V. Hessel, T. Noël, *Chem. Rev.*, 2016, **116**, 10276; K. Loubiere, M. Oelgemöller, T. Aillet, O. Dechy-Cabaret, L. Prat, *Chem. Eng. Processing*, 2016, **104**, 120; J. P. Knowles, L. D. Elliott, K. I. Booker-Milburn, *Beilstein J. Org. Chem.*, 2012, **8**, 2025; M. Nettekoven, B. Püllmann, R. E. Martin, D. Wechsler, *Tetrahedron Lett.*, 2012, **53**, 1363; F. Levesque, P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2012, **51**, 1706; A. Vasudevan, C. Villamil, J. Trumbull, J. Olson, D. Sutherland, *Tetrahedron Lett.*, 2010, **51**, 4007; L. D. Elliott, M. Berry, B. Harji, D. Klauber, J. Leonhard, K. I. Booker-Milburn, *Org. Process Res. Dev.*, 2016, **20**, 1806; L. D. Elliott, J. P. Knowles, P. J. Koovits, K. G. Maskill, M. J. Ralph, G. Lejeune, L. J. Edwards, R. I. Robinson, I. R. Clemens, B. Cox, D. D. Pascoe, G. Koch, M. Eberle, M. B. Berry, K. I. Booker-Milburn, *Chem. Eur. J.*, 2014, **20**, 15226; A. Greb, J.-S. Poh, S. Greed, C. Battilocchio, P. Pasau, D. C. Blakemore, S. V. Ley, *Angew. Chem. Int. Ed.*, 2017, **56**, 16602.

21 M. Yan, Y. Kawamata, P. S. Baran, *Angew. Chem. Int. Ed.*, 2018, **57**, 4149; M. A. Kabeshov, B. Musio, S. V. Ley, *React. Chem. Eng.*, 2017, **2**, 822; A. A. Folgueiras-Amador, K. Philipps, S. Guilbaud, J. Poelakker, T. Wirth, *Angew. Chem. Int. Ed.*, 2017, **56**, 15446; M. A. Kabeshov, B. Musio, P. R. D. Murray, D. L. Browne, S. V. Ley, *Org. Lett.*, 2014, **16**, 4618; K. Watts, W. Gattrell, T. Wirth, *Beilstein J. Org. Chem.*, 2011, **7**, 1108.

22 C. Houben, A. A. Lapkin, *Curr. Opinion Chem. Eng.*, 2015, **9**, 1; H. R. Sahoo, J. G. Kralj, K. F. Jensen, *Angew. Chem. Int. Ed.*, 2007, **46**, 5704; B. J. Reizman, K. F. Jensen, *Acc. Chem. Res.*, 2016, **49**, 1786; C. W. Coley, R. Barzilay, T. S. Jaakkola, W. H. Green, K. F. Jensen, *ACS Cen. Sci.*, 2017, **3**, 434; A. J. Parrott, R. A. Bourne, G. R. Akien, D. J. Irvine, M. Poliakoff, *Angew. Chem. Int. Ed.*, 2011, **50**, 3788; D. C. Fabry, E. Sugiono, M. Rueping, *Isr. J. Chem.*, 2014, **54**, 341; J. P. McMullen, K. F. Jensen, *Annu. Rev. Anal. Chem.*, 2010, **3**, 19; B. J. Reizman, Y.-M. Wang, S. L. Buchwald, K. F. Jensen, *React. Chem. Eng.*, 2016, **1**, 658.

Please do not adjust margins

23 D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson, A. Wood, *Nat. Chem.*, 2018, **10**, 383.
24 L. Guetzoyan, N. Nikbin, I. R. Baxendale, S. V. Ley, *Chem. Sci.*, 2013, **4**, 764.
25 E. Callieri, S. Ceruti, G. Cristalli, C. Martini, C. Temporini, C. Parravicini, R. Volpini, S. Daniele, G. Caccialanza, D. Lecca, C. Lambetucci, M. L. Trincavelli, G. Marucci, I. W. Wainer, G. Ranghino, P. Fantucci, M. P. Abbracchio, G. Massolini, *J. Med. Chem.*, 2010, **53**, 3489.
26 L. Guetzoyan, R. Ingham, N. Nikbin, J. Rossignol, M. Wolling, M. Baumert, N. A. Burgess-Brown, C. M. Strain-Damerell, L. Shrestha, P. Brennan, O. Fedorov, S. Knapp, S. V. Ley, *Med. Chem. Commun.*, 2014, **5**, 540.
27 B. Desai, K. Dixon, E. Farrant, Q. Feng, K. R. Gibson, W. P. van Hoorn, J. Mills, T. Morgan, D. M. Parry, M. K. Ramjee, C. N. Selway, G. J. Tarver, G. Whitlock, A. G. Wright, *J. Med. Chem.*, 2013, **56**, 3033.
28 W. Czechtizky, J. Dedio, B. Desai, K. Dixon, E. Farrant, Q. Feng, T. Morgan, D. M. Parry, M. K. Ramjee, C. N. Selway, T. Schmidt, G. J. Tarver, A. G. Wright, *ACS Med. Chem. Lett.*, 2013, **4**, 768.
29 M. Werner, C. Kuratli, R.E. Martin, R. Hochstrasser, D. Wechsler, T. Enderle, A. I. Alanine, H. Vogel, *Angew. Chem. Int. Ed.*, 2014, **53**, 1704.
30 G. Taylor, *Proc. R. Soc. London Ser. A*, 1953, **219**, 186; J. Ruzicka, *Anal. Chem.*, 1983, **55**, 1040A.
31 A. Baranczak, N. P. Tu, J. Marjanovic, P. A. Searle, A. Vasudevan, S. W. Djuric, *ACS Med. Chem. Lett.*, 2017, **8**, 461.
32 J. E. Hochlowski, P. A. Searle, N. P. Tu, J. Y. Pan, S. G. Spanton, S. W. Djuric, *J. Flow Chem.*, 2011, **2**, 56; J. D. Sutherland, N. P. Tu, T. A. Nemcek, P. A. Searle, J. E. Hochlowski, S. W. Djuric, J. Y. Pan, *J. Lab. Aut.*, 2014, **19**, 176.
33 A. G. Godfrey, T. Masquelin, H. Hemmerle, *Drug Disc. Today*, 2013, **18**, 795; C. A. Nicolaou, I. A. Watson, H. Hu, J. Wang, *J. Chem. Inf. Model.*, 2016, **56**, 1253; J. Li, S.G. Balmer, E. P. Gillis, S. Fujii, M. J. Schmidt, A. M. E. Palazzolo, J. W. Lehmann, G. F. Morehouse, M. D. Burke, *Science*, 2015, **347**, 1221; J. Li, A. S. Grillo, M. D. Burke, *Acc. Chem. Res.*, 2015, **48**, 2297.
34 G. Schneider, *Nature Rev.*,2018, **17**, 97.
35 S. Newton, C. F. Carter, C. M. Pearson, L. de C. Alves, H. Lange, P. Thansandote, S. V. Ley, *Angew. Chem. Int. Ed.*, 2014, **53**, 4915; Z. Qian, I. R. Baxendale, S. V. Ley, *Chem. Eur. J.*, 2010, **16**, 12342.
36 Y.-J. Hwang, C. W. Coley, M. Abolhasani, A. L. Marzinik, G. Koch, C. Spanka, J. Lehmann, K. F. Jensen, *Chem. Commun.*, 2017, **53**, 6649; J. P. McMullen, M. T. Stone, S. L. Buchwald, K. F. Jensen, *Angew. Chem. Int. Ed.*, 2010, **49**, 7076; J. E. Hochlowski, P. A. Searle, N. P. Tu, J. Y. Pan, S. G. Spanton, S. W. Djuric, *J. Flow Chem.*, 2011, **2**, 56; T. Tsubogu, H. Oyamada, S. Kobayashi, *Nature*, 2015, **520**, 329; S. Kobayashi, *Chem. Asian J.*, 2016, **11**, 425.
37 B. A. Posner, H. Xi, J. E. J. Mills, *J. Chem. Inf. Model.*, 2009, **49**, 2202.
38 N. G. Anderson, *Org. Process Res. Dev.*, 2012, **16**, 852; D. L. Hughes, *Org. Process Res. Dev.*, 2018, **22**, 13.

Please do not adjust margins