Drug Discovery

DOI: 10.1002/anie.201400988

Accessing New Chemical Entities through Microfluidic Systems

*Tiago Rodrigues, Petra Schneider, and Gisbert Schneider**

chemical biology · drug discovery · lab-on-a-chip · organic synthesis

Flow systems have been successfully utilized for a wide variety of applications in chemical research and development, including the miniaturization of (bio)analytical methods and synthetic (bio)organic chemistry. Currently, we are witnessing the growing use of microfluidic technologies for the discovery of new chemical entities. As a consequence, chemical biology and molecular medicine research are being reshaped by this technique. In this Minireview we portray the state-of-the-art, including the most recent advances in the application of microchip reactors as well as the micro- and mesoscale coil reactor-assisted synthesis of bioactive small molecules, and forecast the potential future use of this promising technology.

1. Introduction

The advantages provided by continuous flow technology result in the technique having the potential to promote various areas of scientific discovery, specifically synthetic and analytical chemistry, biological research and development.[1] Over the past decade, microfluidic-based applications have soundly matured into relatively low-cost technologies and small-footprint machinery, which enable sensitive analyses with a high resolution and short process times.[1,2] Microfluidic methods operate on small volumes of fluids in geometrically controlled environments that can be subdivided into different functional units, for example, mixers, reactors, and detectors. Typically, converging streams of fluids flow in parallel without turbulence, that is, with a laminar flow, and are characterized by a low Reynolds number (R_e).[3] This unique property of microfluidic systems results in the diffusion-controlled mixing of compounds at the interface of converging fluid streams.[4] The short distances in microfluidic channels enable the rapid and controlled transport of heat and mass. Additionally, mixing in microreactors may be promoted by complex channel geometries, thereby leading to twisting, splitting, and recombining of fluid streams or pulsed flow. The high surface-to-volume ratio of miniaturized channels and reactors can account for the often-observed dramatic increase in throughput and yields in microreactors.[3b,5] Consequently, flow techniques have been widely applied to analytical methods such as mass spectrometry,[6] as well as to protein crystallization,[7] high-throughput screening,[8] single-cell analysis,[9] drug delivery,[10] and several other areas.[11] Depending on the intended application, the envisaged temperature and pressure range, and the corrosivity of fluids, microreactors may be fabricated from a variety of materials including metals, polymers, glass, silicon ceramics, and others. The microfabrication processes of chip reactors are beyond the scope of this Minireview and have been detailed elsewhere.[12]

Despite the evident potential of flow-assisted organic syntheses, only recently have miniaturized reactors been applied extensively to chemistry.[13] For example, de Mello and co-workers used an integrated synthesis/analysis setup on Ugi-type multicomponent reactions,[14] and Kreis et al. used a photochemical microreactor for the synthesis of allylic trifluoromethanes.[15] In another application, aromatic nucleophilic substitution reactions were performed on a chip and coupled to simulated moving bed chromatography for compound purification.[16]

Flow systems are particularly valuable for conducting reactions requiring harsh conditions (e.g. superheating solvents),[17] and for running overlooked chemical reactions, as these reactions can be conducted under flow conditions in a more controlled and safer environment compared to conventional approaches.[18] Hazardous and toxic starting materials or side products are contained and can be trapped

[*] Dr. T. Rodrigues, Dr. P. Schneider, Prof. Dr. G. Schneider
Eidgenössische Technische Hochschule (ETH) Zürich
Departement für Chemie und Angewandte Biowissenschaften
Vladimir-Prelog-Weg 4, 8093 Zürich (Switzerland)
E-mail: gisbert.schneider@pharma.ethz.ch

and destroyed continuously after leaving the flow reactor, thus avoiding any dangerous build-up of large amounts of toxic material. Moreover, microfluidic devices have been applied effectively to radiolabeling small molecules for positron emission tomography.[19] Carbohydrate chemistry,[20] multistep syntheses,[21] and C–C bond-formation methods[22] have benefitted comprehensively from this technique. Buchwald and co-workers expeditiously achieved aromatic trifluoromethylation[23] and the synthesis of chiral β-arylated ketones[24] in a stainless-steel reactor equipped with syringe pumps. Various continuous-flow approaches have been successfully employed in different fields of chemistry for the swift entry into innovative chemotypes with the desired properties.[2] In this Minireview, we focus on recent advances made in microfluidics-assisted organic synthesis as an enabling technology for future drug discovery. We provide selected key examples of on-chip and coil reactor applications to access druglike small molecules with the desired biochemical and biological properties.

Tiago Rodrigues received an MSc in Pharmaceutical Sciences (2006) and a PhD in Medicinal Chemistry (2010) from the University of Lisbon, Portugal, under the supervision of Dr. Francisca Lopes. In 2011 he joined the group of Prof. Gisbert Schneider at ETH Zürich as a postdoctoral researcher. His main research interests are the automated de novo design and microfluidic-assisted synthesis of bioactive compounds.

Petra Schneider received her PhD in Technical Chemistry from the Technical University of Berlin in 1996 with Prof. Dr. Karl-Heinz Reichert. She joined F. Hoffmann–La Roche Diagnostics in Kaiseraugst, Switzerland, and in 1999 transferred to Roche Pharmaceuticals, Basel, Switzerland, as a member of the Global Chemistry Team. She was a consultant in the pharmaceutical industry before she joined ETH Zürich in 2010. She is a cofounder and CEO of inSili.com LLC, Zürich, an ETH spin-off company.

Gisbert Schneider received his PhD in biochemistry from the Free University of Berlin in 1994 in the group of Prof. Dr. Georg Büldt. After several postdoctoral positions, he joined F. Hoffmann–La Roche Pharmaceuticals in Basel, where he headed the cheminformatics group. He completed his Habilitation in Biochemistry and Bioinformatics at the University of Freiburg. 2002–2009 he held the Beilstein Endowed Chair of chem- and bioinformatics at the Goethe-University Frankfurt. In 2010 he joined ETH Zürich as a full professor of computer-assisted drug design.

2. Application to Bioactive Chemical Agents

2.1. Microchip Reactors

The pharmaceutical industry has shown great interest in using microfluidic-assisted syntheses for improving throughput in early lead discovery since the onset of this emerging technology.[25] Short reaction times, the possibility of using only small amounts of starting materials and solvents, the overall reduction of costs, and avoidance of material storage were regarded as crucial advantages in a sector that currently spends billions of dollars on delivering new drugs to patients.[26] It is thus imperative to find economic and sustainable drug-discovery solutions that are focused on rapid synthesis and test cycles, with a molecular design step in the feedback loop.[27]

To this end, in 2003, Warrington and colleagues from GlaxoSmithKline (GSK) reported the use of a microchip reactor to synthesize a small set of pyrazoles from 1,3-dicarbonyl compounds and hydrazines. The semi-automated synthesis of a combinatorial library of pyrazoles using Knorr chemistry was finely tuned to achieve a residence time in the microreactor of 210 s, and ensure near quantitative conversion rates.[28] The integration of synthesis, purification, and biological screening of small molecules was foreseen to dramatically reduce the cycle time per synthesis-assay run, as well as enabling "real-time" design and/or optimization of the next generation of chemical candidates.[29] The group at GSK presented a tightly unified setup comprising the synthesis of both sulfonamides and a methyl ester, which was coupled to their respective assay against T-cell tyrosine phosphatase (**1**, Figure 1A,B). The NS110 Caliper chip containing 12 different channels with widths ranging from 29 to 74 μm and lengths up to 40 mm was used for synthesis. The building-block concentrations, solvent, and flow rates were optimized to afford the target compounds in good yields. The methyl ester derivative **1** was synthesized in a one-pot procedure by sequential S_N2 and transesterification reactions in methanol. Methyl ester **1** and sulfonamides **2**–**4** showed approximately 50% inhibition at a concentration of 60 μM in a T-cell tyrosine phosphatase assay (Figure 1B).[26a] As a consequent further development, and after a series of seminal reports on the use of microfluidic systems for the synthesis and optimization of lead candidates, the Automated Lead Optimization Equipment (ALOE) Platform concept was disclosed. ALOE could significantly reduce time gaps between synthesis-assay-design cycles (Figure 1D). Importantly, its software control contains an algorithm for building predictive bioactivity models and prioritizing the selection of starting materials for subsequent compound generations.[30] Several reactions were screened and optimized with this setup by using T-shaped or Caliper chips.[31] These reactors were suitable for nucleophilic aromatic substitution, nitrostyrene reduction, as well as sulfonamide and amide bond formation. The reactions were unattended and used electro-osmotic flow as the pumping system to deliver building blocks at flow rates of less than 3 $\mu L\,min^{-1}$. This setup resulted in higher yields and considerably lower reaction times (ca. 12 s per reaction) than their batch counterparts. Compounds **5**–**10** were obtained and displayed different target bioactivities, for example, antiapoptotic, matrix metalloproteinase-12 inhibitors.[30]

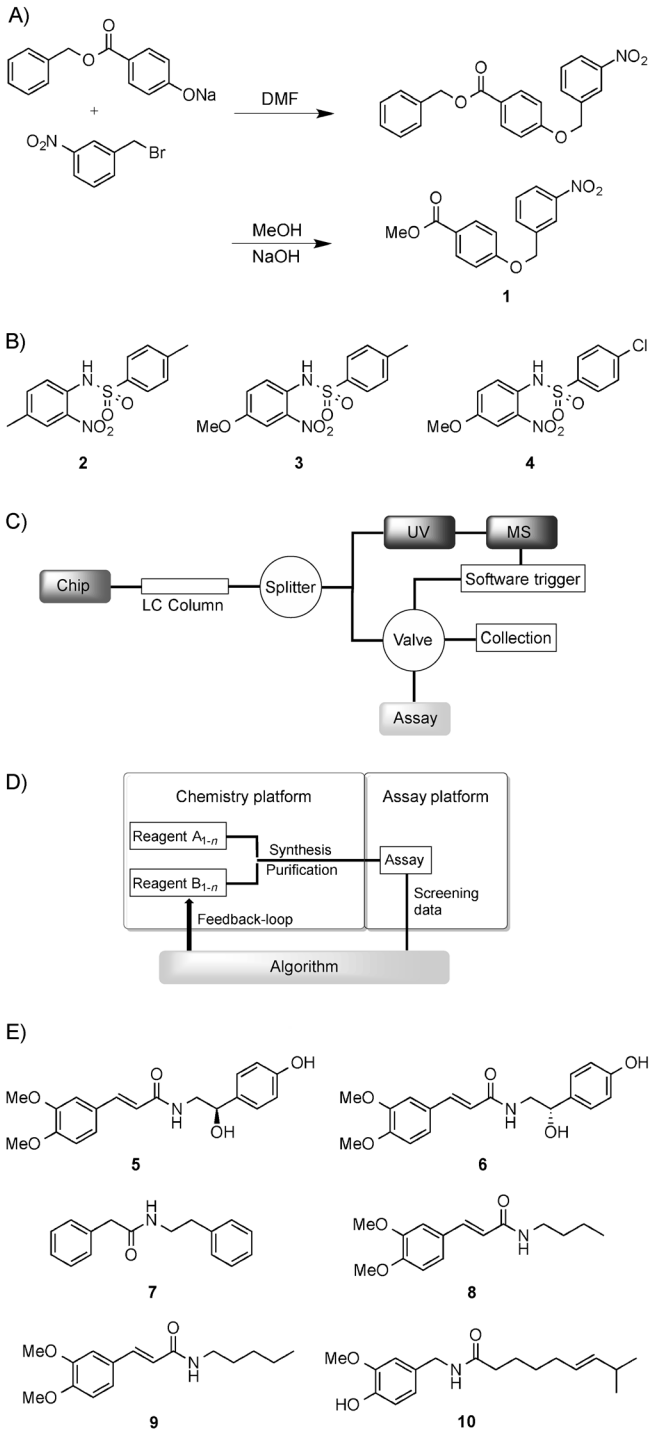

Figure 1. A) Example of a small molecule synthesized using a microchip reactor. B) Sulfonamides with IC_{50} values of about 60 μM against T-cell tyrosine phosphatase. C) Integrated setup based on a microchip-purification-detection assay.[28] D) Schematic representation of ALOE. E) Bioactive amides acquired from microfluidics-assisted synthesis.

Cresset Therapeutics focused on a similar principle and also disclosed an automated platform for the iterative de novo synthesis of new chemical entities.[32]

At the same time, Schwalbe et al. reported a concise and scalable process for the synthesis of ciprofloxacin. This microfluidic system allowed the assembly of a combinatorial fluoroquinolone library with several diversification points. Acylation in flow of β-dimethylamino acrylate with trifluorobenzoic acid chloride afforded the key intermediate for the subsequent diversification through a Michael addition and nucleophilic aromatic substitution.[33]

The on-chip synthesis of bioactive entities has also been increasingly pursued in academia to rapidly and cost-efficiently obtain new chemical entities. Reutlinger et al. reported the swift optimization of the on-chip reaction and assembly of a target-class-focused combinatorial library of imidazopyridines.[34] The small molecule library was acquired through Ugi chemistry, and required only 0.3 s per compound on a microchip with a total reaction volume of 5 μL (Figure 2).

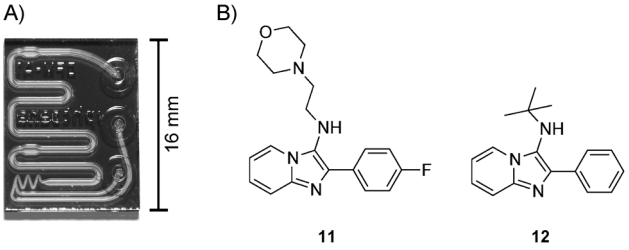

Figure 2. Microreactor chip (A) used for the synthesis of GPCR-modulating compounds **11** and **12** (B).

Off-line coupling to a computational tool for de-orphaning ligand–target associations allowed identification of an innovative GPCR-modulating chemotype, featuring ligand-efficient (LE) adenosine $A_{1/2B}$ and adrenergic $\alpha_{1A/B}$ receptor antagonists. For example, compounds **11** and **12** presented $LE > 0.30$ for adenosine A_{2B} and adrenergic α_{1B} receptors, respectively.[34]

Click reactions continue to be a centerpiece of research in chemical biology.[35] In particular, the Huisgen cycloaddition between an alkyne and an azide has received much attention, and has critically contributed to the development of cutting-edge technology.[36] In 2006, Kolb, Tseng, and co-workers transferred the advantages of in situ click chemistry to microfluidics by integrating it in a parallelized and automated screening platform. Starting from a small diverse set of building blocks, 32 reaction products were screened against bovine carbonic anhydrase II with great success. Importantly, the preparation of the focused library took only 0.5 h with minute amounts of the stock solutions of building blocks.[37] Using a subsequent microchip generation, Wang et al. scaled-up the process to synthesize and screen 1024 reaction products (Figure 3). The integrated microfluidic device included a solid-phase extraction procedure for compound purification and electrospray ionization mass spectrometry, as well as multiple reactions to improve the sensitivity and throughput of the downstream analysis. The method further reduced the reagent consumption, and reduced the process times to 17 s per reaction and 15 s per screening assay in contrast to the 30 min required with the original method.[38]

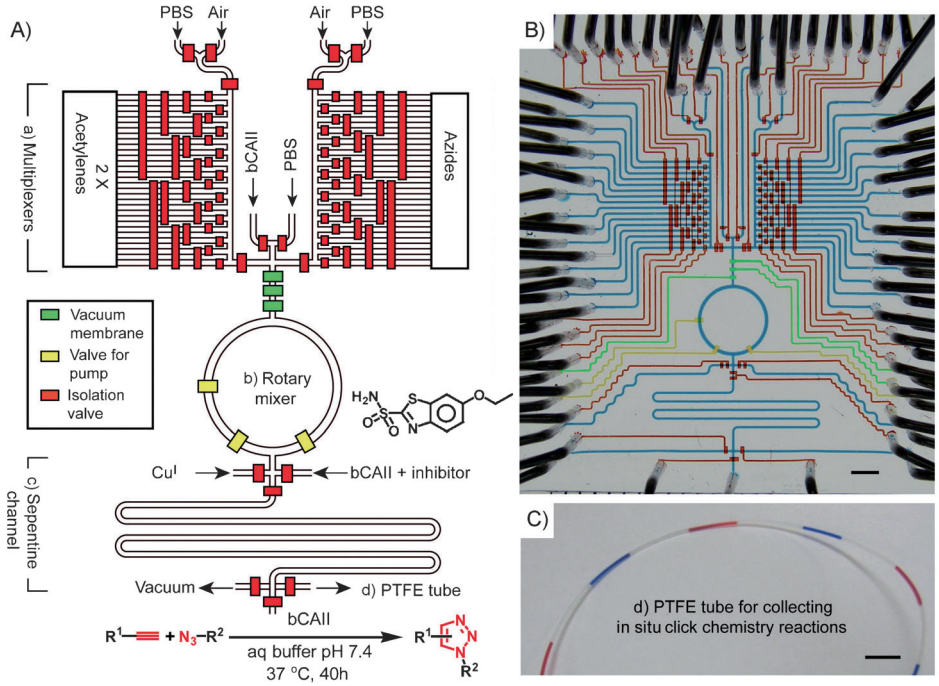

Figure 3. A) An integrated microfluidic platform for in situ click chemistry and screening of bovine carbonic anhydrase II (bCAII) inhibitors. The operation of the circuit was controlled by computer software. B) Image of the actual device, where different channels were loaded with different dyes (see (A)). Fluidic channels in (A) are depicted in blue. C) Tube for collecting reaction products. Black scale bars are 3 mm. (Image reproduced from Ref. [38] with kind permission, Copyright RSC 2009).

2.2. Micro- and Mesoscale Coil Reactors

Compared to microchip reactors, the use of micro- and mesoscale coil reactors has already found broader applicability in drug discovery and development, with the primary aim to solve productivity issues, especially in the industrial setting. In fact, coil reactors can be employed in all phases of drug discovery up to candidate nomination, as reported by Pfizer.[39] Researchers at Abbott have created a synthesis and purification platform in flow for some of the most commonly used reactions in the pharmaceutical chemistry, for example, formation of amides, triazoles, and ureas as well as reductive amination, nucleophilic substitution, and sulfonylation. Reactions were performed in 10 min with a flow rate of 146 $\mu L\,min^{-1}$, which resulted in a throughput of 48-member libraries in less than five days.[40] Access to druglike thiazoles and pyrazoles with a mesoscale flow reactor has also been reported.[41] Coil reactors may be incorporated as part of an intricate platform for performing key reactions. As such, researchers at Amgen have recently reported flow hydrogenation as an intermediate step to acquire a 120-member library of 1-aryl-4-aminopiperidines,[42] while Ley and coworkers described a focused library of druglike pyrrolidines through an integrated flow and batch method.[43]

Building on accumulated experience from previous ventures, researchers at Cyclofuidic used structure–activity relationship (SAR) modeling for the discovery of novel Abl kinase inhibitors. Again, their platform utilizes flow chemistry for rapid inline synthesis, automated purification, and analysis coupled with a bioassay (Figure 4A). The integration of activity prediction using random-forest regression with chemical space sampling algorithms yields an SAR model that refines itself after every synthetic iteration and biological assay cycle. In a particular case, by varying the right-hand heterocycle introduced by Sonogashira coupling, the automated process identified a novel template and hinge-binding motif with $pIC_{50} > 8$ against both wild-type and clinically relevant mutants of Abl kinase in well-plate-based assay format after 21 iterative screening cycles (ca. 24 h, for example, **13**–**15**, Figure 4B).[44] Xanthine-derived dipeptidyl peptidase 4 (DPP4) antagonists, for example, **16** and **17**, were obtained with this platform. Moreover, the bioactivity data obtained in an automated setup and manually obtained assay data were strongly correlated, which further advocates the suitability of a fully integrated microfluidics platform for early drug discovery programs.[45] Czechtizky et al. reported the first functional, purely flow-based assay procedure for obtaining IC_{50} values.[45]

Recently, researchers at F. Hoffmann–La Roche reported a system (Figure 5) that tackles several bottlenecks during lead generation, such as time-consuming compound management and transfer times between synthesis and biological testing. Amidation reactions conducted on a Vapourtec R4 flow synthesizer, coupled to a Gilson liquid handler, permitted the automated purification through preparative HPLC and seamless integration with a screening assay against β-secretase (BACE1).

The complete synthesis-purification-assay cycle took only 60 min per compound, with the biochemical assay taking over 30 min on a glass chip. A split-recombine mixer led to homogeneous mixing of all the assay components, which were injected at a constant flow of 0.8 $mL\,min^{-1}$ each. Several

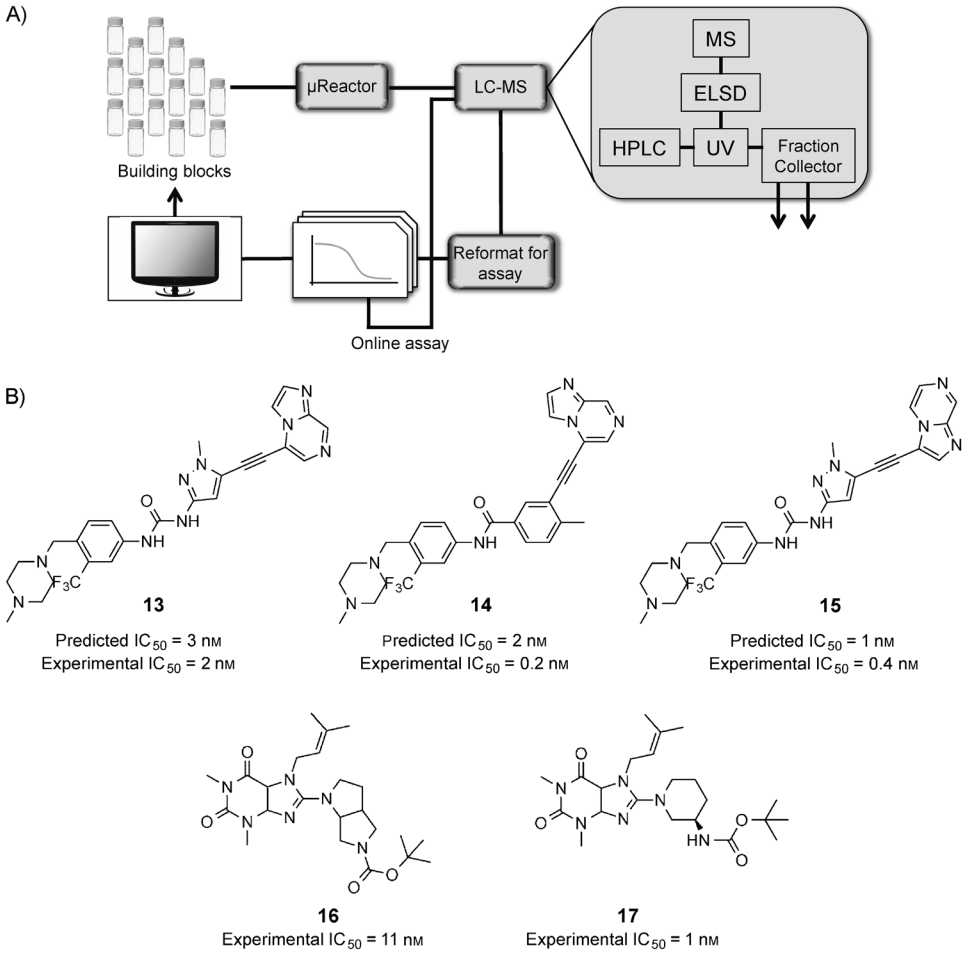

Figure 4. A) Integrated design, synthesis, and screening platform illustrating the fully automated processes implemented for closed-loop drug discovery. After initiation of the process, the system completed multiple iterations of design, synthesis, and screening without manual intervention. ELSD = evaporative light-scattering detector. B) Example of Abl1 kinase inhibitors (**13**–**15**) and DPP4 antagonists (**16**, **17**) obtained through an automated feedback design loop. For further details, see Ref. [44].

compounds were identified as BACE1 inhibitors, among which **18** and **19** showed IC_{50} values of 1327 and 12 nM, respectively. The reported flow-based assay is capable of measuring both very potent and inactive compounds accurately.[46] Finally, multistep continuous flow synthesis (0.15 mL min^{-1}) was applied effectively at Lundbeck to obtain a diverse set of piperazine derivatives as chemokine receptor CCR8-like ligands.[47]

Several research groups have been at the forefront of applying coil reactors to synthesize heterocycles for natural product synthesis and medicinal chemistry applications. A detailed review on the seminal efforts of Ley and colleagues can be found elsewhere.[48] Venturoni et al. exemplified the development of scalable multistep processes for the delivery of pure products by using in-line purification. A series of imidazo[1,2-*b*]pyridazine casein kinase I inhibitors was prepared in four steps. One of the steps in the synthetic route was performed in a combinatorial fashion. Although not fully integrated, the system demonstrated that flow methods, in particular coil reactors, offer a solution for preparing medicinally relevant small molecules through multistep reactions.[49] Further elaboration of this approach led to the development of a fully automated synthetic procedure by which adamantane benzamide derivatives could be obtained (Figure 6 A). Ozonolysis of the starting material, followed by reaction with different nitrogen-containing nucleophiles afforded the target compounds in good yields, without the need for prior isolation of the azalactone intermediate.[50]

In another case study, imidazo[1,2-*a*]pyridines were obtained in a semi-automated fashion. These heterocycles may be regarded as "privileged scaffolds",[51] and flow technologies may assist their access in medicinal chemistry programs.[34] In a first step, the condensation between ethyl glyoxylate and acetophenone building blocks catalyzed by sulfonic acid resin (QP-SA) led to the corresponding unsaturated ketone intermediates after scavenging the excess glyoxylate on a supported benzylamine column (QP-BZA). The unsaturated ketone and an aminopyridine derivative were then reacted to yield the ketimine intermediate, which subsequently underwent cyclization to afford the imidazo[1,2-*a*]pyridines. Finally, the conversion of the ethyl ester into the corresponding amides resulted in the desired target compounds, for example, **20** and **21**, which were screened in-line for binding to human serum albumin, by using a frontal

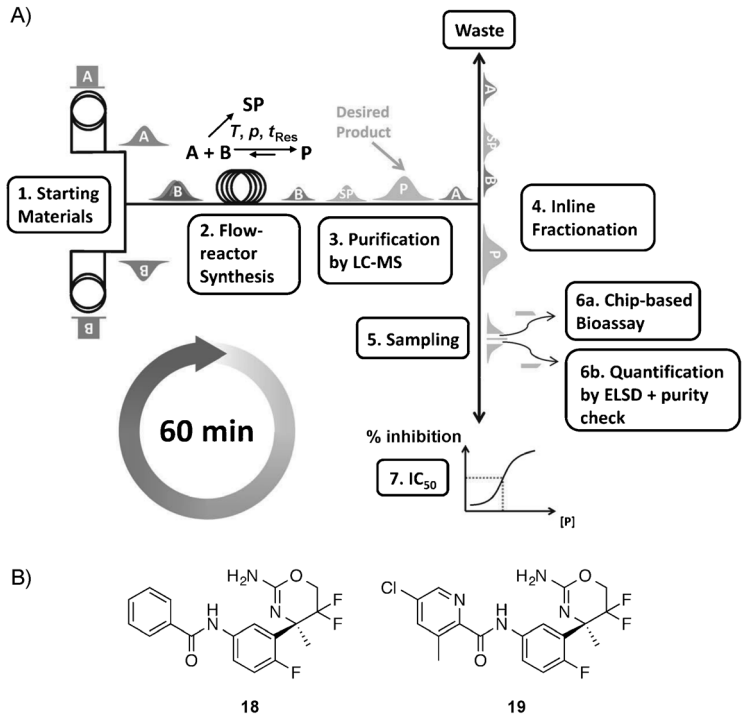

Figure 5. A) Fully automated platform for the generation of SAR data. (Image reproduced from Ref. [46] with kind permission, Copyright Wiley 2014). B) Example of compounds generated with the F. Hoffmann–La Roche platform.

affinity chromatography method (Figure 6B).[52] In a third example, the machine-assisted flow synthesis of a neurotensin receptor-1 probe was accomplished, thereby highlighting how enabling technologies can be used to efficiently solve complex synthetic chemistry problems and drive drug discovery.[53] At the Scripps Institute, a 5×5 library of druglike 3-aminoindolizines was constructed in-flow through tandem Sonogashira coupling and cycloisomerization without handling or isolating any intermediates.[54]

Flow systems have proven their usefulness in the continuous and low-cost production of active pharmaceutical ingredients (APIs) with high market relevance. Artemisinin (Figure 7), a natural product used in antimalarial therapy, has recently been synthesized in-flow,[55] as well as several kinase inhibitors, for example, imatinib and closely related analogues.[56] Quinolone **22** is a potent 5-HT_{1B} antagonist previously developed by AstraZeneca that can be obtained in a six-step flow sequence with an overall yield of 18%.[57] Compound **23** is a selective δ-opioid receptor agonist readily attained under four flow-based steps.[58] Notoriously, olanzapine was also recently synthesized in-flow with a custom-made high-frequency inductor for heating. The Buchwald–Hartwig reaction was performed initially to afford a nitroarene intermediate, which was further processed by reduction of a nitro group followed by acid-catalyzed cyclization to the thieno[1,5]-benzodiazepine intermediate. Olanzapine was isolated in good yield upon reaction of the intermediate with piperazine in the presence of a new silica-supported titanium catalyst.[59]

The first example of an end-to-end integrated continuous manufacturing plant for medicines was reported recently. It represents a technological advance for coping adequately with the high drug demand and shorter production times. In this approach, Trout and co-workers not only obtained a pharmaceutical compound through multistep synthesis, but also formulated it into a tablet that met several quality criteria, for example, uniform visual appearance, comparable size and dosage, and rapid dissolution.[60]

3. Conclusions and Outlook

Flow chemistry technologies have a great influence on how organic synthesis is currently being carried out. Not only have chemical reactions from conventional medicinal chemistry been adapted to continuous flow systems, but new reactions are also being explored. Additionally, the current dearth of new chemical entities for chemical biology and molecular medicine make flow reactors an enabling technology to explore previously inaccessible chemical space. For example, we foresee emerging flow systems playing a key role in diversity-oriented synthesis or simply as a means of performing selected reactions in a complex pathway to obtain novel bioactive molecules. Flow reactors are ideally suited for fully automating several steps of drug discovery. Integrated microfluidics platforms with synthesis-purification-analytics and in-line testing of minute amounts of candidate compounds may alleviate some of the costs of drug discovery and accelerate early stage profiling and hit-to-lead optimization. The automated access to preliminary, yet crucial and accurate, SAR data may guide future drug design. The tight integration of computational methods for designing de novo the next

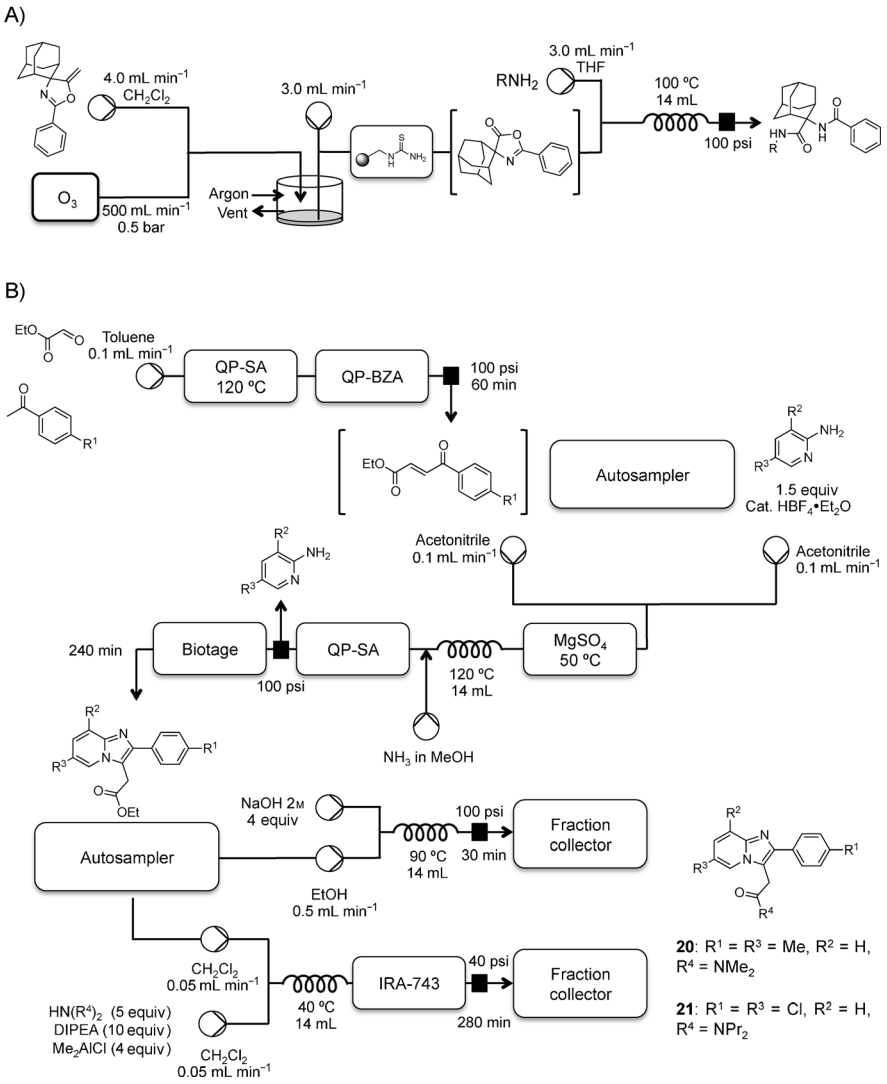

Figure 6. A) Process for the flow synthesis of analgesic adamantane derivatives.[50] B) Process for the flow synthesis of imidazo[1,2-*a*]pyridines.[52]

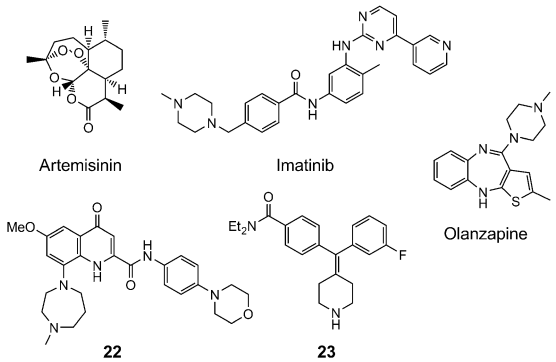

Figure 7. Structures of selected active pharmaceutical ingredients for which a flow synthesis approach has been described.

generation of druglike small molecules in a closed feedback loop platform will undoubtedly assist the discovery of new APIs. In this regard, machine-learning methods applied in adaptive learning schemes are bound to play a pivotal role in the automated design of candidates for synthesis, reaction scheme, and building block selection. Coupled to a microfluidic setup, this concept will enable unsupervised synthesis-assay cycles by active learning.[27,61] We are confident that flow-assisted technologies will be increasingly applied to the discovery of new chemical entities both in academia and industrial settings.

We thank ETH Zürich and the OPO Foundation Zürich for financially supporting our research.

Received: January 29, 2014
Published online: May 6, 2014

[1] G. M. Whitesides, *Nature* **2006**, *442*, 368 – 373.
[2] K. S. Elvira, X. C. Solvas, R. C. R. Wootton, A. J. deMello, *Nat. Chem.* **2013**, *5*, 905 – 915.
[3] a) T. M. Squires, S. R. Quake, *Rev. Mod. Phys.* **2005**, *77*, 977 – 1026; b) R. L. Hartman, J. P. McMullen, K. F. Jensen, *Angew.*

Chem. **2011**, *123*, 7642 – 7661; *Angew. Chem. Int. Ed.* **2011**, *50*, 7502 – 7519; c) C. H. Legge, *J. Chem. Educ.* **2002**, *79*, 173 – 178.
[4] P. S. Dittrich, A. Manz, *Nat. Rev. Drug Discovery* **2006**, *5*, 210 – 218.
[5] a) D. R. Reyes, D. Iossifidis, P. A. Auroux, A. Manz, *Anal. Chem.* **2002**, *74*, 2623 – 2636; b) T. Schwalbe, V. Autze, G. Wille, *Chimia* **2002**, *56*, 636 – 646.
[6] R. S. Ramsey, J. M. Ramsey, *Anal. Chem.* **1997**, *69*, 2617.
[7] C. L. Hansen, E. Skordalakes, J. M. Berger, S. R. Quake, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16531 – 16536.
[8] M. Johnson, C. Li, B. Rasnow, P. Grandsard, H. Xing, A. Fields, *J. Lab. Autom.* **2002**, *7*, 62 – 68.
[9] a) H. Yin, D. Marshall, *Curr. Opin. Biotechnol.* **2012**, *23*, 110 – 119; b) H. N. Joensson, H. A. Svahn, *Angew. Chem.* **2012**, *124*, 12342 – 12359; *Angew. Chem. Int. Ed.* **2012**, *51*, 12176 – 12192.
[10] S. Seiffert, *Angew. Chem.* **2013**, *125*, 11674 – 11680; *Angew. Chem. Int. Ed.* **2013**, *52*, 11462 – 11468.
[11] a) L. Kang, B. G. Chung, R. Langer, A. Khademhosseini, *Drug Discovery Today* **2008**, *13*, 1 – 13; b) P. Neuzil, S. Giselbrecht, K. Lange, T. J. Huang, A. Manz, *Nat. Rev. Drug Discovery* **2012**, *11*, 620 – 632; c) D. Lombardi, P. S. Dittrich, *Expert Opin. Drug Discovery* **2010**, *5*, 1081 – 1094; d) R. Stalder, G. P. Roth, *ACS Med. Chem. Lett.* **2013**, *4*, 1119 – 1123.
[12] *Microreactors in Organic Synthesis and Catalysis* (Ed.: T. Wirth), Wiley-VCH, Weinheim, **2008**, pp. 1 – 41.
[13] a) C. Wiles, P. Watts, *Chem. Commun.* **2011**, *47*, 6512 – 6535; b) P. Watts, C. Wiles, *Chem. Commun.* **2007**, 443 – 467.
[14] M. C. Mitchell, V. Spikmans, A. J. de Mello, *Analyst* **2001**, *126*, 24 – 27.
[15] L. M. Kreis, S. Krautwald, N. Pfeiffer, R. E. Martin, E. M. Carreira, *Org. Lett.* **2013**, *15*, 1634 – 1637.
[16] A. G. O'Brien, Z. Horvath, F. Levesque, J. W. Lee, A. Seidel-Morgenstern, P. H. Seeberger, *Angew. Chem.* **2012**, *124*, 7134 – 7137; *Angew. Chem. Int. Ed.* **2012**, *51*, 7028 – 7030.
[17] R. E. Martin, F. Morawitz, C. Kuratli, A. M. Alker, A. I. Alanine, *Eur. J. Org. Chem.* **2012**, 47 – 52.
[18] S. Sharma, R. A. Maurya, K. I. Min, G. Y. Jeong, D. P. Kim, *Angew. Chem.* **2013**, *125*, 7712 – 7716; *Angew. Chem. Int. Ed.* **2013**, *52*, 7564 – 7568.
[19] a) P. W. Miller, N. J. Long, A. J. de Mello, R. Vilar, H. Audrain, D. Bender, J. Passchier, A. Gee, *Angew. Chem.* **2007**, *119*, 2933 – 2936; *Angew. Chem. Int. Ed.* **2007**, *46*, 2875 – 2878; b) C. C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y. S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. C. Kolb, O. N. Witte, N. Satyamurthy, J. R. Heath, M. E. Phelps, S. R. Quake, H. R. Tseng, *Science* **2005**, *310*, 1793 – 1796.
[20] a) S. Eller, M. Collot, J. Yin, H. S. Hahm, P. H. Seeberger, *Angew. Chem.* **2013**, *125*, 5970 – 5973; *Angew. Chem. Int. Ed.* **2013**, *52*, 5858 – 5861; b) O. Calin, S. Eller, P. H. Seeberger, *Angew. Chem.* **2013**, *125*, 5974 – 5977; *Angew. Chem. Int. Ed.* **2013**, *52*, 5862 – 5865.
[21] a) M. Chen, S. L. Buchwald, *Angew. Chem.* **2013**, *125*, 4341 – 4344; *Angew. Chem. Int. Ed.* **2013**, *52*, 4247 – 4250; b) B. Pieber, S. T. Martinez, D. Cantillo, C. O. Kappe, *Angew. Chem.* **2013**, *125*, 10431 – 10434; *Angew. Chem. Int. Ed.* **2013**, *52*, 10241 – 10244; c) D. Webb, T. F. Jamison, *Chem. Sci.* **2010**, *1*, 675 – 680.
[22] T. Tsubogo, T. Ishiwata, S. Kobayashi, *Angew. Chem.* **2013**, *125*, 6722 – 6737; *Angew. Chem. Int. Ed.* **2013**, *52*, 6590 – 6604.
[23] M. Chen, S. L. Buchwald, *Angew. Chem.* **2013**, *125*, 11842 – 11845; *Angew. Chem. Int. Ed.* **2013**, *52*, 11628 – 11631.
[24] W. Shu, S. L. Buchwald, *Angew. Chem.* **2012**, *124*, 5451 – 5454; *Angew. Chem. Int. Ed.* **2012**, *51*, 5355 – 5358.
[25] E. Garcia-Egido, S. Y. Wong, B. H. Warrington, *Lab Chip* **2002**, *2*, 31 – 33.
[26] a) S. Y. F. W. Hawkes, M. J. V. Chapela, M. Montembault, *QSAR Comb. Sci.* **2005**, *24*, 712 – 721; b) J. W. Scannell, A. Blanckley, H. Boldon, B. Warrington, *Nat. Rev. Drug Discovery* **2012**, *11*, 191 – 200.
[27] *De Novo Molecular Design* (Ed.: G. Schneider), Wiley-VCH, Weinheim, **2013**.
[28] E. Garcia-Egido, V. Spikmans, S. Y. Wong, B. H. Warrington, *Lab Chip* **2003**, *3*, 73 – 76.
[29] V. Hessel, H. Lowe, *Chem. Eng. Technol.* **2005**, *28*, 267 – 284.
[30] a) M. Fernandez Suarez, E. Garcia-Egido, M. Montembault, M. J. Chapela, S. Y. F. Wong-Hawkes, *Proc. ICNMM* **2006**, 997 – 1002; b) S. D. Pickett, D. V. S. Green, D. L. Hunt, D. A. Pardoe, I. Hughes, *ACS Med. Chem. Lett.* **2011**, *2*, 28 – 33.
[31] a) D. M. Hartmann, J. T. Nevill, P.-j. C. Kung, K. I. Peetigrew, B. H. Warrington, H. C. Crenshaw, WO 2007/021815, **2007**; b) I. Hughes, B. H. Warrington, Y. F. Wong, WO 2006/038014, **2006**; c) B. H. Warrington, K. H. Christopher, T. J. Pell, D. A. Pardoe, WO 2007/021813, **2007**; d) I. Hughes, B. H. Warrington, Y. F. Wong, WO 2004/089533, **2004**.
[32] B. Warrington, J. Vinter, M. Mackay, WO 2007/148130, **2007**.
[33] T. Schwalbe, D. Kadzimirsz, G. Jas, *QSAR Comb. Sci.* **2005**, *24*, 758 – 768.
[34] M. Reutlinger, T. Rodrigues, P. Schneider, G. Schneider, *Angew. Chem.* **2014**, *126*, 593 – 596; *Angew. Chem. Int. Ed.* **2014**, *53*, 582 – 585.
[35] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056 – 2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004 – 2021; b) H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128 – 1137.
[36] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708 – 2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596 – 2599.
[37] J. Wang, G. Sui, V. P. Mocharla, R. J. Lin, M. E. Phelps, H. C. Kolb, H. R. Tseng, *Angew. Chem.* **2006**, *118*, 5402 – 5407; *Angew. Chem. Int. Ed.* **2006**, *45*, 5276 – 5281.
[38] Y. Wang, W. Y. Lin, K. Liu, R. J. Lin, M. Selke, H. C. Kolb, N. Zhang, X. Z. Zhao, M. E. Phelps, C. K. Shen, K. F. Faull, H. R. Tseng, *Lab Chip* **2009**, *9*, 2281 – 2285.
[39] L. Malet-Sanz, F. Susanne, *J. Med. Chem.* **2012**, *55*, 4062 – 4098.
[40] J. E. Hochlowski, P. A. Searle, N. P. Tu, J. Y. Pan, S. G. Spanton, S. W. Djuric, *J. Flow Chem.* **2011**, *2*, 56 – 61.
[41] C. M. Thompson, J. L. Poole, J. L. Cross, I. Akritopoulou-Zanze, S. W. Djuric, *Molecules* **2011**, *16*, 9161 – 9177.
[42] M. C. Bryan, C. D. Hein, H. Gao, X. Xia, H. Eastwood, B. A. Bruenner, S. W. Louie, E. M. Doherty, *ACS Comb. Sci.* **2013**, *15*, 503 – 511.
[43] M. Baumann, I. R. Baxendale, C. Kuratli, S. V. Ley, R. E. Martin, J. Schneider, *ACS Comb. Sci.* **2011**, *13*, 405 – 413.
[44] 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 – 3047.
[45] W. Czechtizky, J. e. 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 – 772.
[46] M. Werner, C. Kuratli, R. E. Martin, R. Hochstrasser, D. Wechsler, T. Enderle, A. I. Alanine, H. Vogel, *Angew. Chem.* **2014**, *126*, 1730 – 1735; *Angew. Chem. Int. Ed.* **2014**, *53*, 1704 – 1708.
[47] T. P. Petersen, A. Ritzen, T. Ulven, *Org. Lett.* **2009**, *11*, 5134 – 5137.
[48] M. Baumann, I. R. Baxendale, S. V. Ley, *Mol. Diversity* **2011**, *15*, 613 – 630.
[49] F. Venturoni, N. Nikbin, S. V. Ley, I. R. Baxendale, *Org. Biomol. Chem.* **2010**, *8*, 1798 – 1806.
[50] C. Battilocchio, I. R. Baxendale, M. Biava, M. O. Kitching, S. V. Ley, *Org. Process Res. Dev.* **2012**, *16*, 798 – 810.

[51] M. Reutlinger, C. P. Koch, D. Reker, N. Todoroff, P. Schneider, T. Rodrigues, G. Schneider, *Mol. Inf.* **2013**, *32*, 133 – 138.
[52] L. Guetzoyan, N. Nikbin, I. R. Baxendale, S. V. Ley, *Chem. Sci.* **2013**, *4*, 764 – 769.
[53] C. Battilocchio, B. J. Deadman, N. Nikbin, M. O. Kitching, I. R. Baxendale, S. V. Ley, *Chem. Eur. J.* **2013**, *19*, 7917 – 7930.
[54] P. P. Lange, K. James, *ACS Comb. Sci.* **2012**, *14*, 570 – 578.
[55] a) D. Kopetzki, F. Lévesque, P. H. Seeberger, *Chem. Eur. J.* **2013**, *19*, 5450 – 5456; b) F. Lévesque, P. H. Seeberger, *Angew. Chem.* **2012**, *124*, 1738 – 1741; *Angew. Chem. Int. Ed.* **2012**, *51*, 1706 – 1709.
[56] a) M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Chem. Commun.* **2010**, *46*, 2450 – 2452; b) M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Org. Biomol. Chem.* **2013**, *11*, 1822 – 1839; c) B. J. Deadman, M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Org. Biomol. Chem.* **2013**, *11*, 1766 – 1800.
[57] Z. Qian, I. R. Baxendale, S. V. Ley, *Synlett* **2010**, *4*, 505 – 508.
[58] Z. Qian, I. R. Baxendale, S. V. Ley, *Chem. Eur. J.* **2010**, *16*, 12342 – 12348.
[59] J. Hartwig, S. Ceylan, L. Kupracz, L. Coutable, A. Kirschning, *Angew. Chem.* **2013**, *125*, 9995 – 9999; *Angew. Chem. Int. Ed.* **2013**, *52*, 9813 – 9817.
[60] S. Mascia, P. L. Heider, H. Zhang, R. Lakerveld, B. Benyahia, P. I. Barton, R. D. Braatz, C. L. Cooney, J. M. Evans, T. F. Jamison, K. F. Jensen, A. S. Myerson, B. L. Trout, *Angew. Chem.* **2013**, *125*, 12585 – 12589; *Angew. Chem. Int. Ed.* **2013**, *52*, 12359 – 12363.
[61] G. Schneider, S.-S. So, *Adaptive Systems in Drug Design*, Landes Bioscience, Georgetown, **2001**.