Reaction Chemistry & Engineering

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *React. Chem. Eng.*, 2023, **8**, 1334

Development of a two-phase flow reaction system for DNA-encoded amide coupling†

Robin Dinter, ‡*[a] Suzanne Willems,‡*[b] Mahdi Hachem,[a] Yana Streltsova,[b] Andreas Brunschweiger [b] and Norbert Kockmann[a]

Received 9th January 2023,
Accepted 7th March 2023

DOI: 10.1039/d3re00020f

rsc.li/reaction-engineering

Synthesis platforms are of particular interest to DNA-encoded library (DEL) technologies to facilitate chemistry development, building block validation, and high-throughput library synthesis. A liquid–liquid two-phase flow reactor was designed that enables parallel conduction of reactions on DNA-coupled substrates. The dispersed phase in capillary slug flow contained the DNA reaction mixture and allowed for spatially separated batch experiments in a microchannel. A coiled flow inverter (CFI) tubular reactor with a 3D-printed internal structure on which a capillary is coiled was used for improved mixing and compact setup. An inert continuous phase was introduced, which generated slug flow and prevented backmixing of the individual reactants. In order to enable parallelized reactions, slugs containing a variety of different carboxylic acids were successfully generated to act as individual reaction compartments representing single batch experiments. As a widely used exemplary DEL reaction, the amide coupling reaction was successfully transferred to the tailored flow reaction system and DNA was recovered.

1. Introduction

Drug discovery is an interdisciplinary endeavor that benefits from the application of advanced technologies.[1] DNA-encoded libraries (DELs) are today one of the main technologies for screening of drug-like small molecules on biological targets.[2–4] DEL molecules are chimeric structures consisting of a chemically synthesized compound (small molecule) and its genetic barcode (represented by a DNA strand). They are usually synthesized by iterative cycles of combinatorial chemical reactions and ligation of DNA fragments that encode the chemical building blocks. This process enables the synthesis of large compound collections. Today, there are intense efforts to diversify the chemical space of encoded libraries through novel synthesis methods and technical advances making library synthesis more efficient.[5–8] Although a plethora of reaction conditions can be used to synthesize small molecules on a DNA-sequence, the formation of unwanted side products, incomplete product conversions, and DNA-damage reactions pose common, technology-induced challenges.

Microfluidic reactor technology and flow chemistry are subject to considerable research efforts because reactions in flow often perform much more efficiently due to enhanced mixing and better scalability than batch reactions.[9–11] The fundamental difference between batch and flow chemistry is related to the change of concentration in time and space (reactor length), which is restricted in time along the reactor length related to the volumetric flow rate. Assuming an ideal plug flow reactor (PFR), the length dependence leads to a constant local concentration in the reactor and is reflected as residence time. In practice, a laminar flow profile is present in the microreactor and leads to axial convention and radial diffusion in the flow reactor, and thus causes a residence time distribution (RTD). For increasing the mixing by a secondary flow, a specially designed microfluidic reaction system using bends and curvatures was introduced in the classical tubular reactor design.[10,12,13] A special tubular reactor that offers these characteristics is the coiled flow inverter (CFI). The CFI tubular reactor consists of helically coiled tubes (HCT) with 90° bends between their modules to induce alternating Dean Vortices (secondary flow) in the flowing fluid and a narrow RTD.[10,13] Further advantages of the CFI tubular reactor are rapid fabrication using additive manufacturing for constructing an inner structure, on which a fluorinated ethylene propylene (FEP) tubing is coiled as a capillary microreactor.[9]

A few publications describe the application of microfluidics in DNA-encoded library technology (DELT), for example the work of MacConnell *et al.*[17] and Margulies *et al.*[18] In these

[a] *Department of Chemical and Biochemical Engineering, Laboratory of Equipment Design, TU Dortmund University, Emil-Figge-Str. 68, 44227 Dortmund, Germany*
[b] *Department of Chemistry and Chemical Biology, Medicinal Chemistry, TU Dortmund University, Otto-Hahn-Str. 6, 44227 Dortmund, Germany*
† Electronic supplementary information (ESI) available: Materials, instruments, experimental setup and RP-HPLC chromatograms and MALDI-MS spectra. See DOI: https://doi.org/10.1039/d3re00020f
‡ Robin Dinter and Suzanne Willems contributed equally.

contributions, microfluidic devices were used for the selection screening of DNA-encoded libraries, and DNA sequencing.[15,17,18] In previous work in our groups, Bobers *et al.*[9] validated the iterative process development of a microfluidic CFI liquid–liquid two-phase screening system for organic chemistry. The reaction conditions of this model reaction suggested the application of the microfluidic two-phase system for DNA-encoded chemistry,[9] yet several parameters had to be evaluated and optimized on DNA-tagged model substrates. In addition to the iterative process design, chemical optimization of the reaction with DNA-labeled substrates to the new reaction system was required, too.

Furthermore, flow chemistry shows potential for automation, precise process control, and controlled conduct of reaction.[14] The transfer of traditional laboratory batch DNA-encoded chemistry to continuous flow chemistry systems provides enormous potential for increasing the level of automation of common DEL development processes.[15]

We envisioned, that flow chemistry could offer an intriguing opportunity to facilitate reactions on DNA-tagged substrates. The following key challenges for DNA-encoded chemistry were considered for transfer to flow chemistry: The reactions had to be performed at the μL-scale, whereas previous flow reactors were designed for mL-scale volumes in our group.[9,12,16] Throughout the process, DNA integrity had to be ensured. The recovery of the DNA material from the reactor had to be guaranteed and the product fraction had to be separated from unreacted excess starting materials. Regarding the microfluidic reaction system, the two-phase flow and alternating slugs had to be generated with high reproducibility in a stable two-phase flow system.

Here, we use amide bond formation on a DNA-tagged amine to develop DEL chemistry in flow and compare the performance of the flow reaction system with the batch process, because it is the most common reaction in DEL chemistry.[19–21] As a simple and robust reaction, the amide bond formation allowed us to explore the first steps toward flow chemistry application. We focused in this work on comparing the product formation rate of the reaction mixture in batch and flow. The reactions were carried out in parallel to investigate optimal reaction conditions in flow, establish the robustness of the reactor design, and evaluate the reactivity of a small scope of diverse substituted carboxylic acid (CA) substrates with the reaction system. For the first time, the amide coupling reaction on a DNA-tagged amine was efficaciously transferred from batch to flow chemistry using the tailored CFI tubular reactor.

2. Design of a liquid–liquid two-phase flow reaction system for DNA-tagged substrates

For process intensification, the CFI concept offers flexibility in its design, since many parameters can be adjusted, *e.g.* to achieve enhanced mixing.[12,13,22] The CFI design is detailed in the ESI† section 2 and consists of an FEP tube capillary microreactor with an inner diameter d_i of 1 mm. In order to adapt the CFI to the above-mentioned requirements for DNA-encoded chemistry and DNA-tagged substrates, a number of modifications were made to the previously developed CFI tubular reactor with three bends by Bobers *et al.*,[9] as shown in Fig. 1A. The inner coil diameter d_{ct} was reduced from 20 mm to 16 mm (20% smaller) to achieve a narrower RTD (Fig. 1B). The length of a single coil L_c was reduced from 24.75 mm to 16.50 mm (33% smaller) to increase the number of 90° bends n_b from three to nine. Thus, ten CFI modules instead of four could be used by the same total reactor volume of 4.8 mL to achieve higher Dean numbers (Dn). The Dn number describes flow characteristics in curved tubes and is defined in the ESI† Eqn S3.†[23] This design was selected because the base area of the flow reaction system corresponded to the outer dimensions of a 96-well microtiter plate, that could allow later automation using the automated dosage system (ADoS) developed by Bobers *et al.*[24] The RTD was measured to investigate the single-phase flow behavior of the tailored reaction system for DNA-encoded chemistry, as shown in Fig. 1B. Consequently, the redesigned parameters of the tailored CFI tubular reactor with nine bends ensured enhanced mixing in single-phase flow and a narrower RTD compared to the previously developed reaction system with three bends.[9]

The performance of the liquid single-phase flow was limited due to the total reactor volume and geometry that in turn influences the residence time. As a consequence, the mL-scale CFI tubular reactor applied in single-phase flow does not fulfill the requirements of microscale DNA-encoded reactions. To overcome this limitation, the tailored CFI for DNA-tagged substrates was used with a liquid–liquid two-phase setup, as shown in Fig. 1C. Here, a dispersed phase (DP) was brought into contact with a continuous phase (CP), and both phases are immiscible. Different flow regimes are formed depending on the fluid velocity of the two-phases, their physical properties, and the geometric parameters of the tube.[10] In order to obtain a μL-scale reaction volume according to DNA-encoded chemistry requirements, a slug flow regime was generated *via* a T-junction. In this way, a stable two-phase flow with alternating slugs was produced in which the CP spatially separated the DP.[9,10] Thus, the DNA-reaction mixture was divided into individual reaction compartments called slugs representing spatially separated batch experiments in a microliter FEP-tube. The size of the slugs can be adjusted within a specific range by varying the syringe pump speeds, as shown in ESI† Table S2. Since a CP spatially separates the slugs, there was no RTD in liquid–liquid flow regimes in the CFI but inside every single slug.[25] For the generation of reproducible slugs in this work, both phases need to be pumped at very low volumetric flow rates of 28 μL min^{-1} to produce slugs of approximately 7 μL each. The slugs were fractionated at the end of the CFI in microliter tubes for reproducible reaction volumes that can be compared to the batch experiments, as shown in Fig. 1C. To achieve similar reaction times in the slugs as in the batch

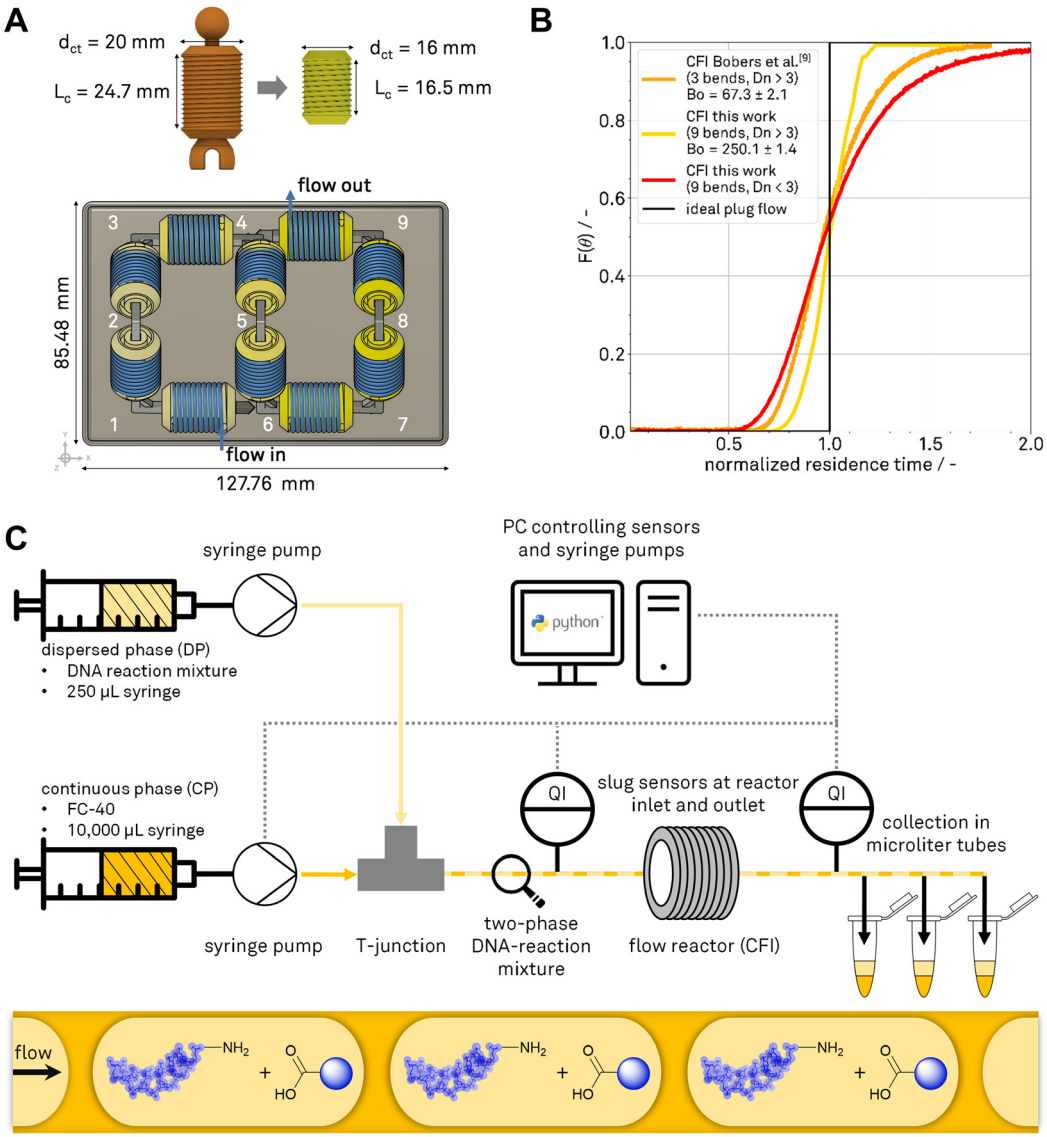


Fig. 1 CFI design and schematic overview of the liquid–liquid two-phase reaction setup. A) Modifying the CFI module with the most important parameters and dimensions. B) Comparison of the RTD between the CFI of Bobers *et al.*[9] with three bends and the tailored CFI for DNA-tagged substrates with nine bends. C) Reaction setup to conduct the DNA-encoded amide coupling reaction. The DP (bright orange) was injected using a 250 µL syringe, and the CP (dark orange) using a 10 mL syringe. Slugs are generated with a T junction. Photosensors at the inlet and outlet of the flow reactor detect the slugs and transmit the signal to a computer controlling the syringe pump of the CP. The slugs are collected in microliter tubes. Based on Dinter *et al.*[33]

experiments, the slugs were pumped back and forth using photosensors developed by Höving *et al.*[12] and placed at the inlet and outlet of the CFI. The detailed experimental setup, the material used, and the operation method can be found in the ESI† section 2.

3. Transfer of a DNA-encoded amide coupling to the flow reactor

With a CFI reactor suitable for µL-scale reaction volume in hand, we tested its applicability for amide coupling reactions on DNA-tagged substrates in a liquid–liquid two-phase flow reaction system.[21] Several reaction conditions are known to enable the amide bond formation between a DNA-tagged amine and a carboxylic acid by coupling agents such as a tetramethyluronium-hexafluorophosphate (HATU), a carbodiimide (EDC), or a morpholinium chloride (DMT-MM), all inducing the formation of an activated ester.[20]

Before transferring the DNA-encoded batch reaction to the newly developed liquid–liquid two-phase flow reactor, we tested the effect of the inert CP on the amide coupling and the recovery rate of a DNA-tagged amine **1** from the flow reactor. The inert fluorocarbon oil FC-40 was chosen as CP, since we considered it immiscible with an aqueous DMSO solvent system. Indeed, we could not observe diffusion of a fluorescence-tagged DNA oligonucleotide from an aqueous

DMSO solution into the inert FC-40 phase after mixing and measuring a UV-vis spectrum of both phases (Fig. 2A). Due to its immiscibility and high density, a complete separation was optically noticeable when FC-40 was brought into contact with an aqueous phase. Since FC-40 has a high global warming potential (GWP) of over 6000, recycling of CP was validated, as shown in ESI† Fig. S1.[26,27]

As a next step, we tested the recovery of a DNA-tagged amine **1** after submission to the developed flow reaction system (Fig. 2B). A fluorescent ssDNA **2** containing aqueous solution was provided as DP and injected as slugs into the CP, as explained in section 2. 10 slugs with an average volume of 7.2 μL ± 0.3 μL each were transported through the CFI reactor and collected as individual slugs. The DNA **2** was precipitated from these slugs, dissolved again and detected using UV-vis absorbance at 260 nm. The fluorescently-labeled DNA **2** was fully recovered (>95%) from the flow reactor with an average concentration of 8.4 μmol L^{-1} ± 0.1 μmol L^{-1}. This allowed us to scale down to perform microscale DNA tagged reactions. For the following experiments, we were able to generate several small DNA containing compartments (slugs) using the previously developed reactor.

For optimizing the amide coupling in a flow reaction system, we started with a reaction protocol utilizing EDC, 1-hydroxy-7-azabenzotriazole (HOAT), and diisopropylethylamine (DIPEA), which is frequently used in our lab for amide synthesis (Table 1).[20] The protocol is generally divided into an initial active ester and a subsequent amide bond formation, with a total reaction time of 16 hours (ESI†). For simplicity, we focused on the amide bond formation and tested whether the reaction time can be reduced due to the high excess of coupling agents and carboxylic acid (Table 1).[28,29] Three chemically diverse carboxylic acids **3a–c**, aromatic, heteroaromatic and aliphatic, were chosen and the reaction time was tested. The reaction was stopped after 5 and 45 min, when the yield of the amide coupling was determined. Whereas the aromatic carboxylic acid **3b** almost fully reacted within 45 min, the heteroaromatic carboxylic acid **3c** did not react at all (Table 1, entry 4 and 6). The aliphatic carboxylic acid **3a** showed a 46% product formation after 45 min and was chosen to perform further flow-optimization studies (Table 1, entry 2).

For the transfer of the batch reaction into a flow reaction system, the pre-activation of the carboxylic acid posed a technical challenge, since it would require the addressing of preformed slugs (7.2 μL) with an additional cannula containing the dissolved DNA. Hence, for a first proof-of-concept of the batch-to-flow transfer, the activation effect was tested in a batch reaction, showing little to no effect on the success of the reaction (ESI† Tables S1 and 1). During our study, we observed variable product yields, which were attributed to the coupling agent EDC (Table 1, entry 7 and 8).

The ability to control the mixing by adapting the flow rate of the individual slugs within the flow reactor is an advantage of using a flow reactor compared to a batch reaction. To achieve similar residence times, the slugs were pumped back and forth and the three different flow rates of 1.6 mL min^{-1} (Dn = 9), 2.4 mL min^{-1} (Dn = 13.5), and 3.2 mL min^{-1} (Dn = 18) were set. In addition, the volumetric flow rates were observed at five reaction times. Surprisingly, little to no effect on product conversions could be observed (Fig. 3, ESI† Table S3). For the batch experiments, increasing conversions of 30% to 50% for longer reaction times were determined. In comparison to this, the flow experiments gave conversions between 20% to 30%. In these initial experiments, the reaction was shown to be inhibited. Apparently, the mixing behavior had an influence on the reaction process, which could not have been investigated in the batch experiments so far and could be the subject to further investigations.

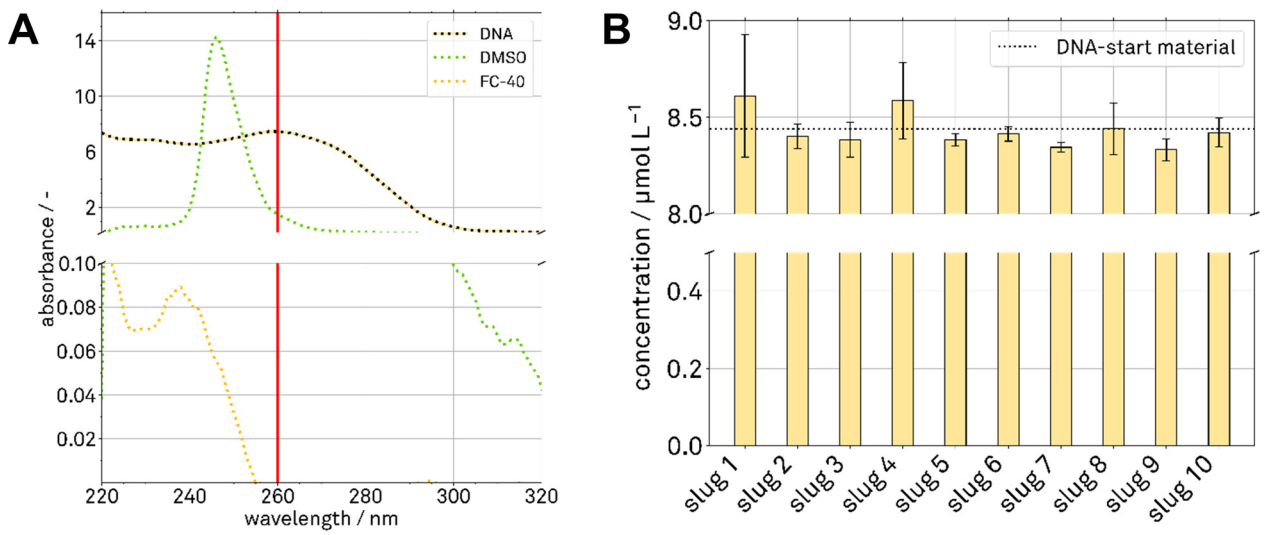
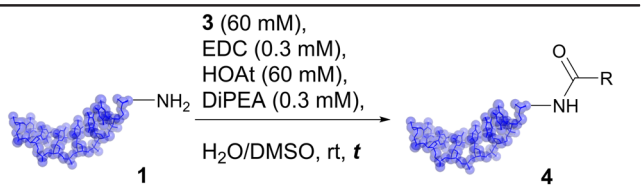


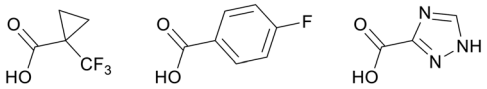
Fig. 2 Experiments that were conducted to transfer a DNA-encoded batch reaction to a liquid–liquid two-phase flow reaction system: A) observed diffusion of fluorescence-tagged DNA oligonucleotide from an aqueous DMSO solution into the inert FC-40 phase after mixing by UV-vis spectroscopy. B) DNA-recovery after submission to the developed flow reaction system. Determined concentrations are shown in yellow columns.

Table 1 Optimization of the batch reaction system for a transfer to a flow reaction system. The conversions of the batch experiments were determined by HPLC. The concentration of the DNA-tagged amine **1** was 3.6 μmol L^{-1}



Entry	3	Conversion [%]	Time [min]
1	**3a**	6	5
2	**3a**	46	45
3	**3b**	70	5
4	**3b**	93	45
5	**3c**	0	5
6	**3c**	0	45
7	**3a**	65	960
8[a]	**3a**	33	960

[a] Coupling agents were stored for one week on bench.



3a **3b** **3c**

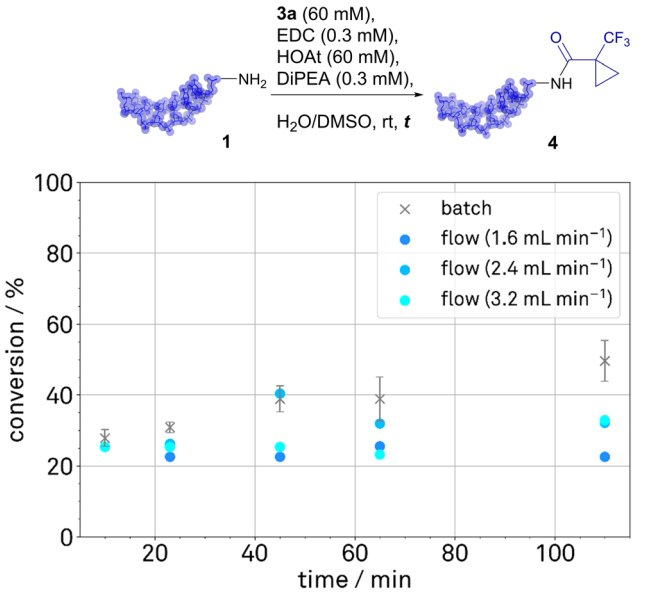


Fig. 3 Plotted results of the flow rate optimization comparing the batch and flow reaction system. The concentration of the DNA-tagged amine **1** was 3.6 μmol L^{-1}.

4. Profiling of carboxylic acids

The profiling of carboxylic acids offers great potential to implement structural diversity to DEL, since more than 20 000 carboxylic acids are commercially available at moderate costs, and helps to identify chemical structures that are suitable for this reaction type.[21] In order to thoroughly examine the amide bond formation in DELs, structurally different carboxylic acids are focused on in this work. This includes the three main categories aromatic, aliphatic, and heteroaromatic carboxylic acids, as shown in Fig. 4. One important objective in the production of a DEL with different building blocks is the avoidance of cross-contamination.

The profiling of nine carboxylic acids was conducted in flow using slightly adapted reaction conditions with a reaction time of 8 hours. All amide coupling reactions were run in one flow setup, dividing each individual reaction into 7 μL slugs. As previously described all slugs were separated using FC-40 as a continuous phase. Only the expected product peaks were observed in the MALDI-MS spectra (ESI† Table S7). No products from other carboxylic acids were detected, meaning that there was no contact of different reaction slugs during their collection at the end, so that we could exclude cross-contamination during compound synthesis and product isolation. To directly compare the performance of the developed flow reactor, a batch reaction was performed in parallel. Both reaction setups, flow and batch gave comparable conversions. Among the chosen carboxylic acids, a primary **3g**, **3k**, secondary **3d**, **3f** and tertiary **3a**, **3e** aliphatic compound were well tolerated in the amide coupling reaction. Nearly full conversion was observed for the difluorinated cyclopropane carboxylic acid **3d**. The hydrochloride amine **3k** was coupled with 33% in the flow reaction and respectively 28% in the batch reaction to the **1**. Heteroaromatic carboxylic acids, such as a thiophene **3i** and indazole **3h** were efficiently coupled to the DNA-tagged amine **1**. However, an *ortho*-positioned hydroxyl-functionality as in **3j** fully inhibited product formation. These results are a successful step towards automatable DEL reactions for high-throughput reaction screening.

5. Conclusions

The motivation for this work was to explore the requirements for the design of a flow synthesis platform for DNA-encoded chemistry. Here, we developed a two-phase flow DEL reaction system and validated this with amide coupling reactions on a DNA-conjugated amine. First, a tailored coiled flow inverter (CFI) reactor was designed for reproducible liquid–liquid two-phase slug flow. FC-40 as an inert continuous phase (CP) was introduced, which generated slug flow of individual slugs with an approximate volume of 7 μL. The slugs were pumped back and forth to achieve similar reaction times as in the batch experiments. Next, the amide coupling reaction with DNA-tagged substrates was efficaciously optimized and conducted in a flow reaction system for the first time. The reactor setup, using varying reaction times without pre-activation of the carboxylic acid at a defined flow rate of 1.6 mL min^{-1}, ensured DNA integrity and comparable batch and flow yields. The robustness of the flow reactor design was demonstrated by evaluating the reactivity of a small scope of diverse substituted carboxylic acid substrates. This study

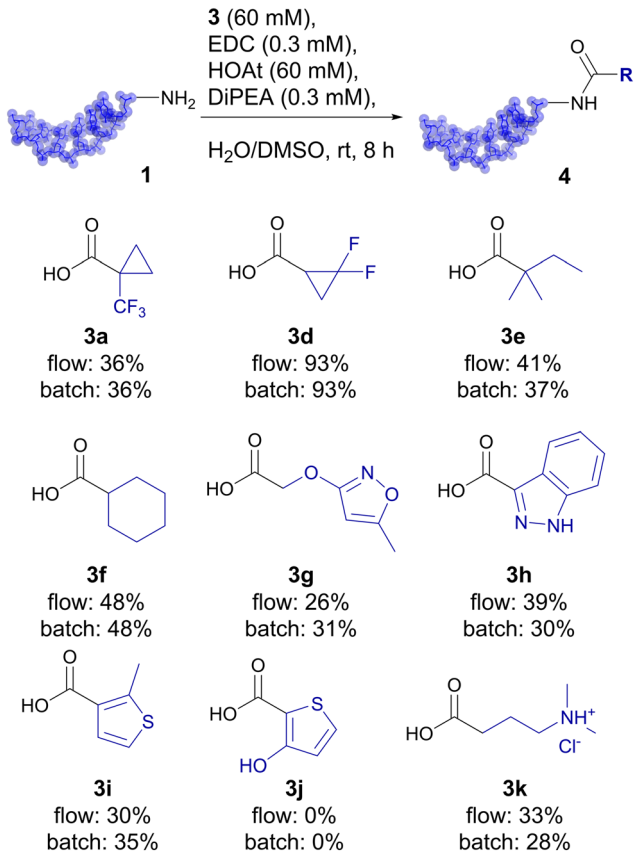


Fig. 4 Results of the profiling of different carboxylic acids in a batch reaction and liquid–liquid two-phase flow reaction system after 8 hours. A volumetric flow rate of 1.6 mL min^{-1} was set for the flow experiments. The concentration of the DNA-tagged amine **1** was 3.6 μmol L^{-1}.

showed that the flow and batch setup deliver comparable results of moderate to full conversions with a diverse set of carboxylic acids. More importantly, FC-40 prevented backmixing of the individual slugs without influencing the amide coupling reaction, and the system allowed for recovery of the DNA-conjugates.

We see further potential for an efficient and automated DEL synthesis platform for the DEL synthesis in replacing the CP by an inert liquid with a lower GWP, but comparable physical properties, such as polydimethylsiloxane (PDMS) or Novec 7500. Further steps in the reaction approach are necessary for a complete flow synthesis platform. Therefore, mixing the reactants in the flow and activating the carboxylic acid will be planned by addressing the slugs. The manual fractioning of the slugs and product separation is a hurdle toward automated DNA-encoded library synthesis. To avoid manual separation, the automatable affinity purification process will be tested for DNA-encoded chemistry developed by Götte *et al.*[30] The CFI flow reaction system shows high potential as a new instrument to conduct further reactions for DEL-synthesis. Reactions of interest have been published by our group, previously, and include protective group chemistry of chemically stabilized DNA[31] or enzymatic ligation of DNA barcodes for encoding a DNA-encoded library.[32] Currently, we are testing the tailored liquid–liquid two-phase reaction system for automated profiling of encoded chemical reactions using the automated reagent-dispensing system.[24]

Author contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank the German Research Foundation (DFG) for financial support under Grants KO2349/18 and BR5049/7-1, and the technician Carsten Schrömges for his support.

Notes and references

1 M. Smietana, J.-J. Vasseur, J. Cossy and S. Arseniyadis, in *Modern Tools for the Synthesis of Complex Bioactive Molecules*, 2012, pp. 539–555.
2 D. Neri and R. A. Lerner, *Annu. Rev. Biochem.*, 2018, **87**, 479–502.
3 A. L. Satz, A. Brunschweiger, M. E. Flanagan, A. Gloger, N. J. V. Hansen, L. Kuai, V. B. K. Kunig, X. Lu, D. Madsen, L. A. Marcaurelle, C. Mulrooney, G. O'Donovan, S. Sakata and J. Scheuermann, *Nat. Rev. Methods Primers*, 2022, **2**, 3.
4 A. Gironda-Martínez, E. J. Donckele, F. Samain and D. Neri, *ACS Pharmacol. Transl. Sci.*, 2021, **4**, 1265–1279.
5 R. M. Franzini and C. Randolph, *J. Med. Chem.*, 2016, **59**, 6629–6644.
6 P. R. Fitzgerald and B. M. Paegel, *Chem. Rev.*, 2021, **121**, 7155–7177.
7 H. Zhu, M. E. Flanagan and R. V. Stanton, *J. Chem. Inf. Model.*, 2019, **59**, 4645–4653.
8 P. Cai, L. A. Schneider, C. Stress and D. Gillingham, *Org. Lett.*, 2021, **23**, 8772–8776.
9 J. Bobers, J. Grühn, S. Höving, T. Pyka and N. Kockmann, *Org. Process Res. Dev.*, 2020, **24**, 2094–2104.
10 M. B. Plutschack, B. Pieber, K. Gilmore and P. H. Seeberger, *Chem. Rev.*, 2017, **117**, 11796–11893.
11 V. Hessel, D. Kralisch, N. Kockmann, T. Noël and Q. Wang, *ChemSusChem*, 2013, **6**, 746–789.
12 S. Höving, J. Bobers and N. Kockmann, *J. Flow Chem.*, 2022, **12**, 185–196.
13 S. K. Kurt, M. Akhtar, K. D. P. Nigam and N. Kockmann, *Ind. Eng. Chem. Res.*, 2017, **56**, 11320–11335.
14 M. P. Marques and N. Szita, *Curr. Opin. Chem. Eng.*, 2017, **18**, 61–68.
15 A. Gioiello, A. Piccinno, A. M. Lozza and B. Cerra, *J. Med. Chem.*, 2020, **63**, 6624–6647.

16 M. Schmalenberg, W. Krieger and N. Kockmann, *Chem. Ing. Tech.*, 2019, **91**, 567–575.
17 A. B. MacConnell, A. K. Price and B. M. Paegel, *ACS Comb. Sci.*, 2017, **19**, 181–192.
18 M. Margulies, M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. I. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley and J. M. Rothberg, *Nature*, 2005, **437**, 376–380.
19 A. Tomberg and J. Boström, *Drug Discovery Today*, 2020, **25**, 2174–2181.
20 Y. Li, E. Gabriele, F. Samain, N. Favalli, F. Sladojevich, J. Scheuermann and D. Neri, *ACS Comb. Sci.*, 2016, **18**, 438–443.
21 T. Kalliokoski, *ACS Comb. Sci.*, 2015, **17**, 600–607.
22 S. Klutz, S. K. Kurt, M. Lobedann and N. Kockmann, *Chem. Eng. Res. Des.*, 2015, **95**, 22–33.
23 S. K. Kurt, M. G. Gelhausen and N. Kockmann, *Chem. Eng. Technol.*, 2015, **38**, 1122–1130.
24 J. Bobers, M. Klika Škopić, R. Dinter, P. Sakthithasan, L. Neukirch, C. Gramse, R. Weberskirch, A. Brunschweiger and N. Kockmann, *ACS Comb. Sci.*, 2020, **22**, 101–108.
25 R. Seemann, M. Brinkmann, T. Pfohl and S. Herminghaus, *Rep. Prog. Phys.*, 2012, **75**, 016601.
26 United States Environmental Protection Agency, Uses and emissions of liquid PFC heat transfer fluids from the electronics sector, **https://www.epa.gov/sites/production/files/2016-02/documents/pfc_heat_tranfer_fluid_emission.pdf**, (accessed 16 November 2022).
27 3M™ Company, 3M™ Fluorinert™ FC-40 Elektronik Flüssigkeit | 3M Deutschland, **https://www.3mdeutschland.de/3M/de_DE/p/d/b40045180/**, (accessed 16 November 2022).
28 L. C. Chan and B. G. Cox, *J. Org. Chem.*, 2007, **72**, 8863–8869.
29 E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606–631.
30 K. Götte, R. Dinter, L. Justen, N. Kockmann and A. Brunschweiger, *ACS Omega*, 2022, **7**, 28369–28377.
31 M. Potowski, V. B. K. Kunig, L. Eberlein, A. Vakalopoulos, S. M. Kast and A. Brunschweiger, *Angew. Chem., Int. Ed.*, 2021, **60**, 19744–19749.
32 M. A. Clark, R. A. Acharya, C. C. Arico-Muendel, S. L. Belyanskaya, D. R. Benjamin, N. R. Carlson, P. A. Centrella, C. H. Chiu, S. P. Creaser, J. W. Cuozzo, C. P. Davie, Y. Ding, G. J. Franklin, K. D. Franzen, M. L. Gefter, S. P. Hale, N. J. V. Hansen, D. I. Israel, J. Jiang, M. J. Kavarana, M. S. Kelley, C. S. Kollmann, F. Li, K. Lind, S. Mataruse, P. F. Medeiros, J. A. Messer, P. Myers, H. O'Keefe, M. C. Oliff, C. E. Rise, A. L. Satz, S. R. Skinner, J. L. Svendsen, L. Tang, K. Van Vloten, R. W. Wagner, G. Yao, B. Zhao and B. A. Morgan, *Nat. Chem. Biol.*, 2009, **5**, 647–654.
33 R. Dinter, S. Willems, M. Hachem, M. Mittelstädt, A. Brunschweiger and N. Kockmann, *Chem. Ing. Tech.*, 2022, **94**, 1213–1234.