Tandem Continuous Flow Curtius Rearrangement and Subsequent Enzyme Mediated Impurity Tagging

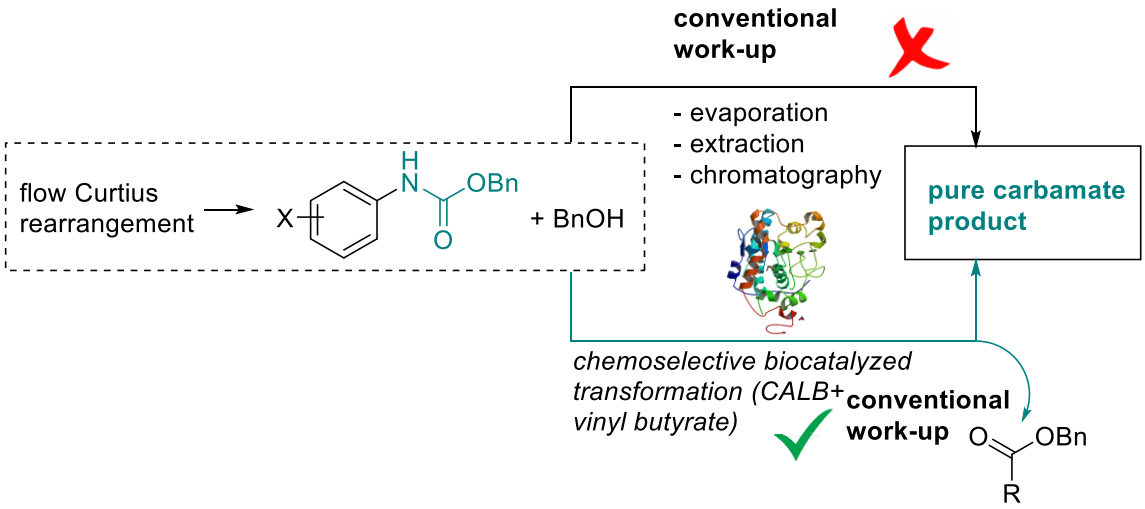
Marcus Baumann[1], Alexander Leslie,[1] Thomas S. Moody[2,3], Megan Smyth[2**] and Scott Wharry[2]*

[1] *University College Dublin, School of Chemistry, Science Centre, South Belfield, D04 N2E2.*

[2] *Almac Group Ltd., 20 Seagoe Industrial Estate, Craigavon, BT63 5QD, United Kingdom.*

[3] *Arran Chemical Company, Unit 1 Monksland Industrial Estate, Athlone, Co. Roscommon, Ireland.*

Table of Contents graphic:



ABSTRACT Continuous flow as an enabling technology within the fine chemical and pharmaceutical industry continues to gain momentum. The associated safety benefits with flow for handling of hazardous or highly reactive intermediates are often exploited to offer industrially relevant and scalable Curtius rearrangements. However, in many cases the Curtius rearrangement requires excess nucleophile for the reaction to proceed to high conversions. This can complicate work procedures to deliver high purity products. However, tandem processing and coupling of the Curtius rearrangement with an immobilized enzyme can elegantly facilitate the chemoselective tagging of the residual reagent resulting in a facile purification process under continuous flow.

KEYWORDS flow synthesis, Curtius rearrangement, biocatalysis, CALB, enzyme impurity tagging.

Introduction: High energy transformations and reactions involving gaseous species are amongst the most frequently exploited reactions in continuous flow mode for industrial applications.[1] This can be rationalized by the multitude of challenges and risks when performing these transformations in batch mode, with such chemistries often referred to as 'forbidden reactions'. The miniaturization of flow reactor components crucially enables effective mass and heat transfer and limits the amount of hazardous species within the reactor at any given time, thus mitigating many of the safety concerns.[2] The Curtius rearrangement is a prime example of a classical 'forbidden reaction' that is invaluable in converting carboxylic acids into amine derivatives with a multitude of applications.[3] As this rearrangement reaction relies on high energy acyl azide intermediates that release nitrogen gas upon heating, the enhanced process control of flow reactors is a vital contribution towards the safe execution of such reactions. Furthermore, continuous processing is

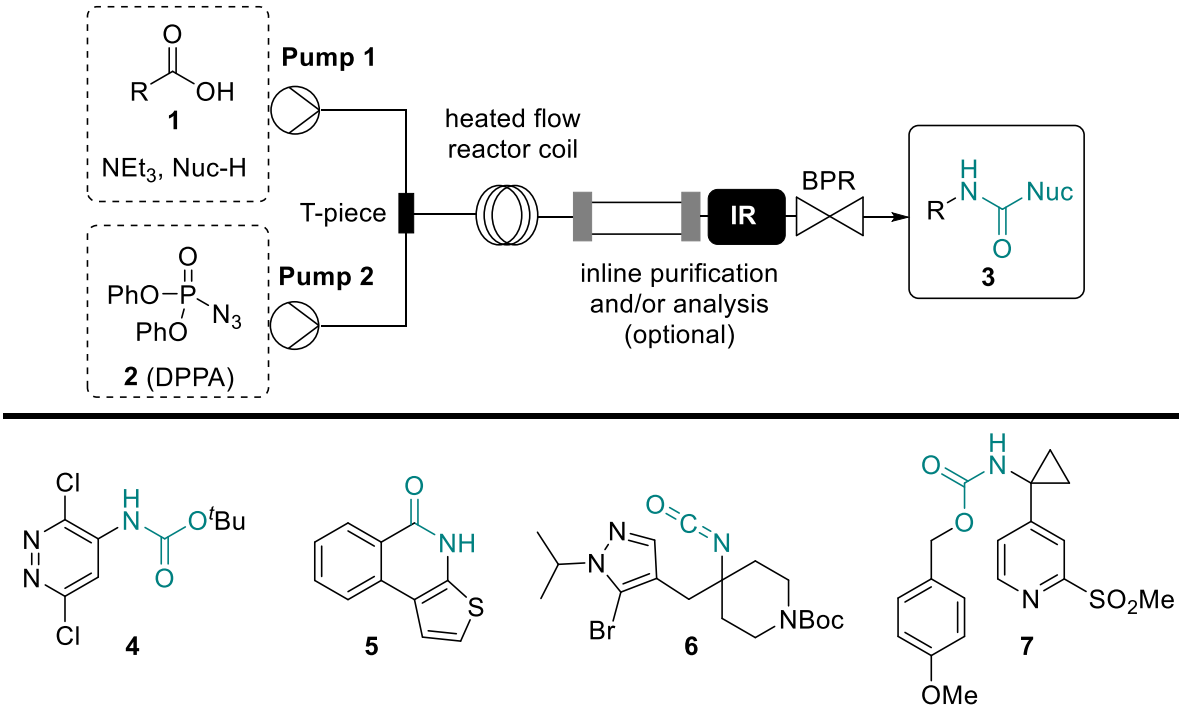
attractive as it offers an effective telescoped means for derivatizing the potentially unstable isocyanate intermediates towards various carbamate products.

Whilst several modes for generating the acyl azide species in flow mode are available, including azidation of acyl chlorides[4] and diazotization of acyl hydrazides,[5] the direct activation of carboxylic acids with diphenylphosphoryl azide (DPPA, **2**)[6] is the most often utilized approach due to the stability and commercial availability of both DPPA and acid substrates, as well as, the feasibility of using a scalable homogeneous liquid phase system.

Early examples of continuous DPPA-mediated Curtius rearrangement reactions exploit both thermal and microwave-assisted heating to generate the intermediate isocyanate species prior to trapping with various nucleophiles. Reaction temperatures of at least 120 °C, in combination with back-pressure regulators (BPR), result in effective transformations in less than 1 hr residence time, which can be complemented by scavenger-based in-line purification (Scheme 1).[7] Related flow procedures were effectively applied towards target structures such as bromosporine analogs (**4**) [8] and PARP inhibitors (**5**) [9] where continuous processing delivered gram quantities of vital building blocks.

Recent work by Pfizer furthermore demonstrates exploitation of a continuous Curtius rearrangement reaction towards the safe and scalable synthesis of the spiropiperidine lactam moiety (**6**) found in a novel ACC inhibitor.[10] Furthermore, researchers from Boehringer Ingelheim have demonstrated a multi-kilogram scale Curtius rearrangement process that alleviated side-product formation observed in batch due to the spatiotemporal processing in flow mode.[11] Coupled with in-line IR monitoring this impressive process achieved a throughput of 0.75 kg/hr (~80%

yield, 48 kg of **7** produced) in a safe and continuous manner that highlights the value of flow processing for this important transformation (Scheme 1).



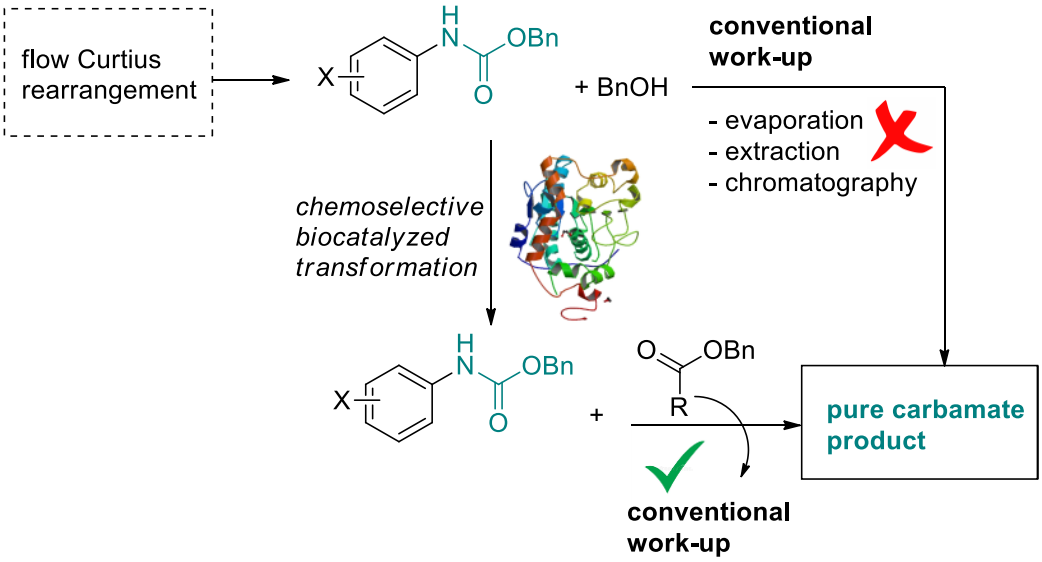
Scheme 1: Common set-up and targets of flow based Curtius rearrangement reactions.

Biocatalysis has widened its scope and applicability for continuous flow through developments in enzyme immobilization.[12] Use of immobilized enzymes have a number of advantages/disadvantages as shown in Table 1 and there are many commercially available immobilized enzymes on the market.[13] Enzyme form is an important consideration to overall process cost contributions on an industrial scale. Flow processing has the potential to increase the rate of biotransformations through enhanced mass transfer facilitating large scale production with significantly smaller architectures allowing tight control of reaction parameters to improve yield and productivies.[14]

Table 1: Enzyme immobilization features.

Advantages	Disadvantages
Amenable to both continuous flow and batch processing	Often immobilization can result in loss of enzyme activity
Economic benefits as a result of reuse over multiple cycles	Can lead to unfavorable alterations in kinetic properties
Improved stability and tolerance to organic solvents compared with soluble enzyme forms	Additional costs/processing time for immobilization process
Easier downstream processing	Mass transfer limitations

Often to push the Curtius rearrangement to reaction completion, an excess of nucleophile is required. This consequently can have an impact on the downstream purification and isolation of the product. The methodology presented herein exploits the chemoselectivity of enzymes to 'tag and modify' impurities or unwanted materials into new products which are easy to purge using conventional purification techniques which do not rely on chromatography. The enzyme mediated introduction of a tag is demonstrated in Scheme 2.[15]



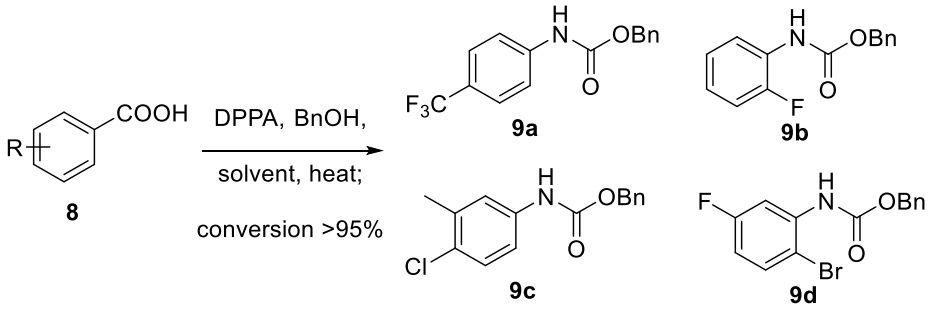
Scheme 2: Enzyme mediated tagging and subsequent purification strategy

While the merits for continuous Curtius rearrangement and biocatalysis reactions are well documented independently, so far, their combination and corresponding downstream processing has not been reported to the best of our knowledge.

The work reported herein demonstrates a continuous flow Curtius rearrangement with a subsequent in-line enzyme-mediated impurity tagging step to facilitate the easy removal of residual BnOH. The methodology was demonstrated and was scaled for the synthesis of Cbz-carbamate **9a**. This work highlights the synergy attainable for a telescoped continuous flow approach coupling a high energy transformation with an enzyme-mediated derivatization protocol as a means of a purification aid.

Results and Discussion: Recent development work of a Curtius rearrangement process using benzyl alcohol (BnOH) as the nucleophile generated a library of products with the Cbz-carbamate moiety (**9a-d**, Scheme 3) from the intermediate isocyanate species. However, removal of residual benzyl alcohol proved challenging on multi-gram scale due to its high boiling point and co-polarity

with the carbamate products **9a-d**. The implementation of a subsequent biocatalytic tagging step, facilitated a simple purification under continuous flow to purge this residual reagent. The development work of the Curtius rearrangement subsequently focused on the conversion of 4-(trifluoromethyl)benzoic acid **8a** into the corresponding Cbz-carbamate **9a** (Scheme 2).

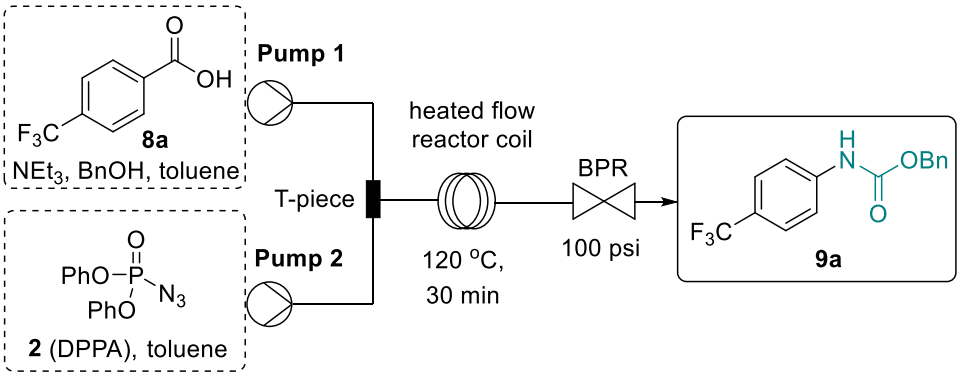


Scheme 3: General Curtius rearrangement process towards carbamates **9a-d**.

Solvent choice was a critical consideration for the development work with initial investigations focusing on acetonitrile and toluene. It was observed that even traces of water in commercial acetonitrile resulted in the formation of insoluble urea side-products. With this observation in mind, toluene was selected with its favorable properties of boiling point (i.e. low vapor pressure at elevated temperature) and low propensity to contain significant amounts of water (ca. 0.033% at 25°C). Reactor fouling due to precipitation of products is one of the fundamental hurdles often experienced by chemists performing reactions under flow and careful consideration about solubility is critical for successful development of a flow process.

Substrate **8a** was only sparingly soluble in toluene, however with addition of triethylamine (1.0 equiv.) and generation of the corresponding salt, **8a** was completely soluble at a concentration of 1 M. Limiting the stoichiometry of DPPA to 0.9 equivalents avoided contamination of the final product with residual azide species. Considering previous reports on flow-based Curtius

rearrangement reactions,[7-11] a flow system was investigated in which a stream containing substrate **8a** (1.0 equiv., 1 M in toluene), NEt_3 (1.0 equiv.) and benzyl alcohol (1.0-2.0 equiv.) was mixed *via* a T-piece (1/8'' PEEK) with a stream of DPPA (0.9 M in toluene). The combined mixture was then reacted in a coiled reactor (PFA, 10 mL) of a Vapourtec E-series flow reactor before passing a BPR (100 psi, Scheme 4). From initial experiments it was determined that a temperature of 120 °C and a residence time of 30 minutes (combined flow rate 0.33 mL/min) yielded full conversion of substrate **8a** (Table 2).



Scheme 4: Flow set-up for continuous Curtius rearrangement towards crude **9a**.

Table 2: Development of Curtius rearrangement on substrate **8a**.

Entry	Residence time	Temperature	Stoichiometry of BnOH	Isolated yield
1	20 min	120 °C	1.0 equiv.	70%
2	30 min	120 °C	1.5 equiv.	79%
3	30 min	120 °C	1.8 equiv.	86%

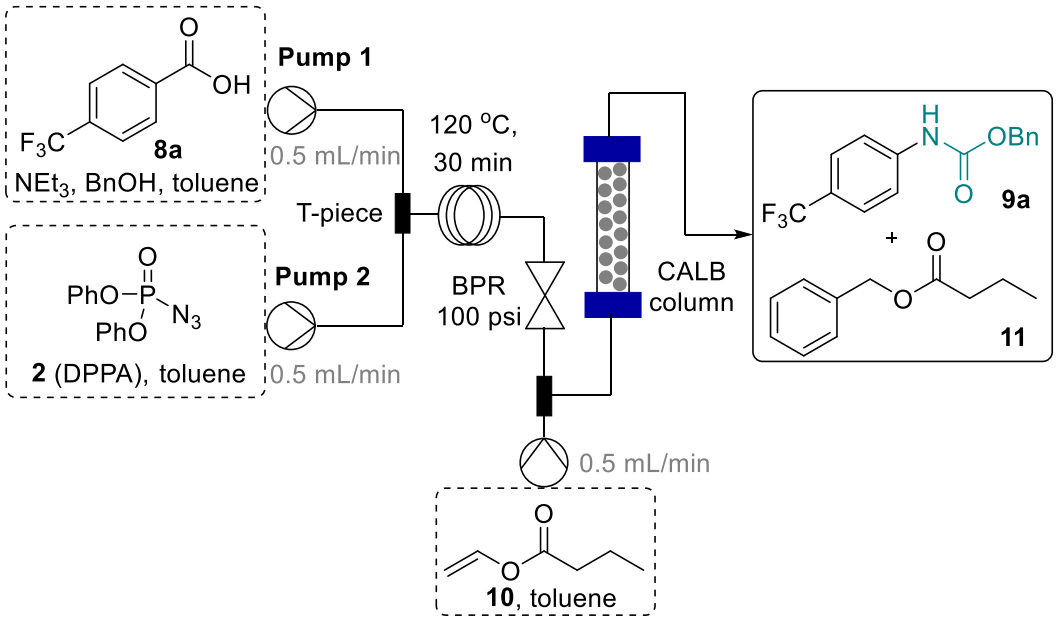
Entry	Residence time	Temperature	Stoichiometry of BnOH	Isolated yield
4	30 min	120 °C	2.0 equiv.	85%

Due to formation of nitrogen gas, a biphasic slug flow regime was observed within the reactor coil (10 mL). It was noted that a slight excess of benzyl alcohol of 1.8 equiv. was necessary for high isolated yields of >80%. The flow process was performed successfully for prolonged periods of time (1-2 hr) to generate gram quantities of the carbamate product **9a** reaching a throughput of ca. 7 mmol/hr (2.1 g/hr). Furthermore, the conditions proved effective in circumventing any observation related to blockages or reactor fouling which could have been associated with precipitation of insoluble materials.

The resultant crude carbamate product **9a** was contaminated with residual benzyl alcohol that proved difficult to purge during work-up due to its high boiling point of ca. 205 °C. To address this, targeted development of a continuous biocatalyzed protocol was carried out utilizing *Candida antarctica* lipase B (CALB) as a robust enzyme to convert benzyl alcohol to benzyl butyrate in the presence of vinyl butyrate. CALB was used in an immobilized form using a hydrophobic carrier (acrylic resin) that provides a loading of ca. 10wt%. CALB has been shown to be a versatile enzyme in chemical processing, amendable to immobilization and protein engineering to tune enzyme properties.[16]

An Omnifit glass column[17] (length: 100 mm, i.d.: 6.6 mm) was packed with CALB and the reaction carried out at ambient temperature. The initial flow set-up was then modified by mixing vinyl butyrate (3 equiv. in toluene) with the crude product stream from the Curtius rearrangement *via* a

T-piece before passing through the CALB column (ca. 2-5 min residence time) and collection of the product solution for analysis by NMR and HPLC. The robustness of CALB,[18] meant it could tolerate the non-purified reaction mixture with no detrimental effect on the performance of the enzyme as a result of spent reagents. Further development experiments demonstrated that 3 equivalents of vinyl butyrate (b.p. 116 °C) was necessary to achieve full conversion of benzyl alcohol to benzyl butyrate, whereas with only 1-2 equivalents typically about 15% of unreacted benzyl alcohol was present (Scheme 5).



Scheme 5: Flow set-up involving high energy Curtius rearrangement and tandem CALB mediated BnOH tagging.

A CALB column with a path length (length of enzyme bed) of about ~8 cm was sufficient to observe full conversion of benzyl alcohol. Furthermore, it was found that the enzyme performance did not deteriorate over several runs (5x1 mmol scale) and a slight discoloration of the enzyme (beige to light brown, Figure 1) was found to be inconsequential. The desired product **9a** was

isolated in pure form after evaporation of all volatiles and extraction (EtOAc/H_2O) to yield a white solid that was crystallized from heptanes. On small scale (1 mmol of **8a**) the isolated yields for the desired carbamate product ranged from 75-82% dependent on isolation procedures.

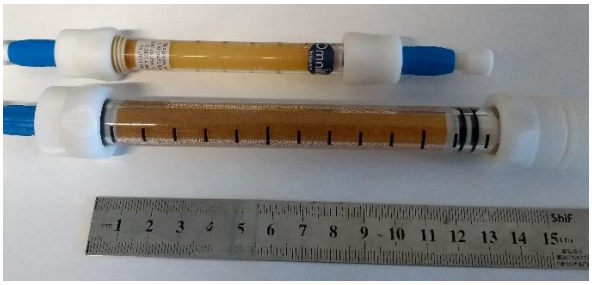


Figure 1: Appearance of used CALB columns.

To demonstrate the scalability and robustness of the flow protocol, the process was carried out to generate the desired product **9a** on 100 mmol scale. The above described flow set-up in combination with a larger Omnifit column (length: 150 mm, i.d.: 10 mm) filled with 3.0 g of immobilized CALB was utilized. Stock solutions of DPPA (0.9 M toluene) and substrate (1 M toluene, 1.0 equiv. NEt_3, 1.8 equiv. BnOH) were pumped with individual flow rates of 0.5 mL/min and directed through three consecutive flow coils (3x10 mL, 120 °C, PFA) after mixing in a T-piece to provide a residence time of 30 minutes. After passing a BPR (100 psi) a stream of vinyl butyrate (3 equiv. in toluene, 0.5 mL/min) was mixed with the crude reaction mixture in a second T-piece prior to passing through the CALB column. The reaction mixture was subsequently collected and evaporated prior to extraction (EtOAc/water) to yield the target product after evaporation and crystallization from heptanes as a white crystalline solid. The isolated yield of 83% parallels previous small-scale reactions and rendered 22 g of pure carbamate **9a**, equivalent to a throughput of 6.6 g/hr. Throughout this scale-up, samples were analyzed by HPLC and ^{1}H-

NMR demonstrating quantitative conversion of residual benzyl alcohol using CALB in all cases throughout the campaign (Figure 2).

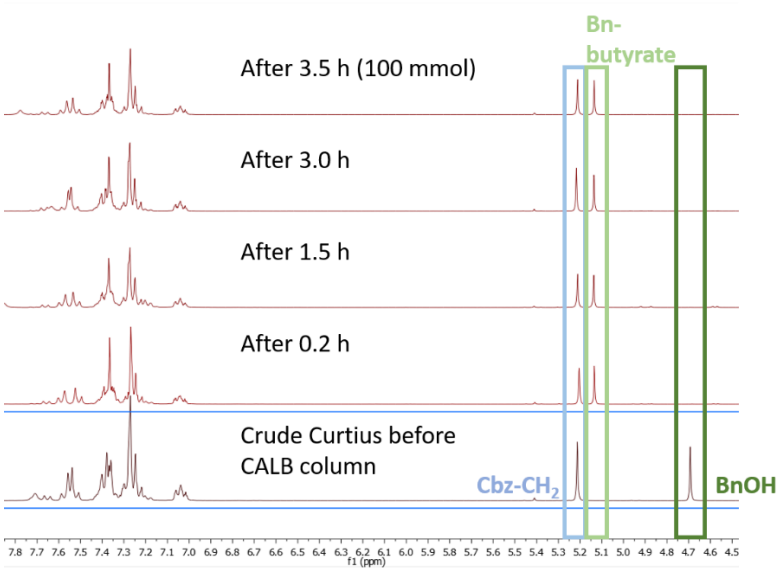


Figure 2: Analysis of reaction performance by ^{1}H-NMR for 100 mmol reaction.

Summary and Conclusions: In conclusion, the integration of a continuous Curtius rearrangement reaction with an efficient biocatalytic transformation in which residual benzyl alcohol is converted to benzyl butyrate has been demonstrated. The tagged impurity is then easily purged during the isolation process. Immobilised CALB packed in a glass column proved very robust during scale-up of the continuous process. The desired carbamate product was isolated in high yield and analytical purity following standard procedures allowing to produce ~22 g of product in less than 4 hours. This approach highlights the value of continuous flow biocatalysis in the effective downstream processing of flow processes and opens the door to a multitude of possible applications to use enzymes as purification tagging tools in the production of chemicals.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge.

Experimental procedures and copies of NMR spectra (PDF)

AUTHOR INFORMATION

Corresponding Author

*Email: marcus.baumann@ucd.ie

**Email: megan.smyth@almacgroup.com

Author Contributions

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

Funding Sources

The authors gratefully acknowledge the financial support from Science Foundation Ireland under the SFI Industry Fellowship program for the project entitled 'Development of Continuous Biocatalysed Processes – Continuous Biocatalysed Chemicals (CATCH)' (19/IFA/7420, to MB).

ACKNOWLEDGMENT

We gratefully acknowledge support from Science Foundation Ireland through a SFI Industry Fellowship (19/IFA/7420, to MB) as well as the Infrastructure Call 2018 (18/RI/5702) and European Regional Development Fund (12/RC2275_P2).

REFERENCES

[1] M. Baumann, T. S. Moody, M. Smyth, S. Wharry, A Perspective on Continuous Flow Chemistry in the Pharmaceutical Industry, *Org. Process Res. Dev.* **2020**, *24*, 1802-1813.

[2] M. Movsisyan, E. I. P. Delbeke, J. K. E. T. Berton, C. Battilocchio, S. V. Ley, C. V. Stevens, Taming hazardous chemistry by continuous flow technology, *Chem. Soc. Rev.* **2016**, *45*, 4892-4928.

[3] A. K. Ghosh, M. Brindisi, A. Sarkar, The Curtius Rearrangement: Applications in Modern Drug Discovery and Medicinal Chemistry, *ChemMedChem* **2018**, *13*, 2351-2373.

[4] a) H. R. Sahoo, J. G. Kralj, K. F. Jensen, Multistep continuous-flow microchemical synthesis involving multiple reactions and separations, *Angew. Chem. Int. Ed.* **2007**, *46*, 5704-5708; b) M. Baumann, I. R. Baxendale, S. V. Ley, N. Nikbin, C. D. Smith, Azide monoliths as convenient flow reactors for efficient Curtius rearrangement reactions, *Org. Biomol. Chem.* **2008**, *6*, 1587-1593.

[5] T. A. Phung Hai, L. J. S. De Backer, N. D. P. Cosford, M. D. Burkart, Preparation of Mono- and Diisocyanates in Flow from Renewable Carboxylic Acids, *Org. Process Res. Dev.* **2020**, 24, 2342-2346.

[6] Preparation of DPPA in flow: S. C. Born, C. E. R. Edwards, B. Martin, K. F. Jensen, Continuous, on-demand generation and separation of diphenylphosphoryl azide, *Tetrahedron* **2018**, *74*, 3137-3142.

[7] M. Baumann, I. R. Baxendale, S. V. Ley, N. Nikbin, C. D. Smith, J. P. Tierney, A modular flow reactor for performing Curtius rearrangements as a continuous flow process, *Org. Biomol. Chem.* **2008**, *6*, 1577-1586.

[8] L. Guetzoyan, R. J. Ingham, N. Nikbin, J. Rossignol, M. Wolling, M. Baumert, N. A. Burgess-Brown, C. M. Strain-Damerell, L. Shrestha, P. E. Brennan, O. Fedorov, S. Knapp, S. V. Ley, Machine-assisted synthesis of modulators of the histone reader BRD9 using flow methods of chemistry and frontal affinity chromatography, *Med. Chem. Commun.* **2014**, *5*, 540-546.

[9] P. Filipponi, C. Ostacolo, E. Novellino, R. Pellicciari, A. Gioiello, Continuous Flow Synthesis of Thieno[2,3-c]isoquinolin-5(4H)-one Scaffold: A Valuable Source of PARP-1 Inhibitors, *Org. Process Res. Dev.* **2014**, *18*, 1345-1353.

[10] K. Huard, S. W. Bagley, E. Menhaji-Klotz, C. Préville, J. A. Southers, Jr., A. C. Smith, D. J. Edmonds, J. C. Lucas, M. F. Dunn, N. M. Allanson, E. L. Blaney, C. N. Garcia-Irizarry, J. T. Kohrt, D. A. Griffith, R. L. Dow, Synthesis of spiropiperidine lactam acetyl-CoA carboxylase inhibitors, *J. Org. Chem.* **2012**, *77*, 10050-10057.

[11] M. A. Marsini, F. G. Buono, J. C. Lorenz, B. - S. Yang, J. T. Reeves, K. Sidhu, M. Sarvestani, Z. Tan, Y. Zhang, N. Li, H. Lee, J. Brazzillo, L. J. Nummy, J. C. Chung, I. K. Luvaga, B. A. Narayanan, X. Wei, J. J. Song, F. Roschangar, N. K. Yee, C. H. Senanayake, Development of a concise, scalable synthesis of a CCR1 antagonist utilizing a continuous flow Curtius rearrangement, *Green Chem.* **2017**, *19*, 1454-1461.

[12] a) M. T. Reetz, Biocatalysis in organic chemistry and biotechnology: past, present, and future. *J. Am. Chem. Soc.* **2013**, *135*, 12480–12496. b) J. Britton, S. Majumdar, G. A. Weiss, Continuous Flow Biocatalysis, *Chem. Soc. Rev.* **2018**, *47*, 5891-5918.

[13] R. DiCosimo, J. McAuliffe, A. J. Poulose, G. Bohlmann, Industrial use of immobilized enzymes, *Chem. Soc. Rev.,* **2013**, *42*, 6437-64674.

[14] L. Tamborini, P. Fernandes, F. Paradisi, F. Molinari, Flow Bioreactors as Complementary Tools for Biocatalytic Process Intensification, *Trends in Biotechnology*, **2018**, *35*, 1, 73-88.

[15] M. Brossat, T. S. Moody, F. de Nanteuil, S. J. C. Taylor, F. Vaughan, Development of an acid washable tag for the separation of enantiomers from bioresolutions, *Org. Process Res. Dev.* **2009**, *13*, 706–709.

[16] CALB can be purchased from Almac.

[17] Omnifit columns were purchased from Kinesis: https://kinesis.co.uk/brands/omnifit-ez-columns-diba

[18] a) J. - W. Shen, J. - M. Qi, X. - J. Zhang, Z. - Q. Liu and Y. - G. Zheng, Efficient Resolution of cis-(±)-Dimethyl 1-Acetylpiperidine-2,3-dicarboxylate by Covalently Immobilized Mutant Candida antarctica Lipase B in Batch and Semicontinuous Modes, *Org. Process Res. Dev.* **2019**, *23*, 1017-1025. b) K. E. Cassimjee, P. Hendil-Forssell, A. Volkov, A. Krog, J. Malmo, T. E. V. Aune, W. Knecht, I. R. Miskelly, T. S. Moody, M. Svedendahl Humble, Streamlined Preparation of Immobilized Candida antarctica Lipase B, *ACS Omega* **2017**, *2*, 8674-8677. c) Y. Cen, W. Singh, M. Arkin, T. S. Moody, M. Huang, J. Zhou, Q. Wu, M. T. Reetz, Artificial cysteine-lipases

with high activity and altered catalytic mechanism created by laboratory evolution, *Nature Commun.* **2019**, *10*, 1-10.