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Article

Tandem Continuous Flow Curtius Rearrangement and Subsequent Enzyme-Mediated Impurity Tagging

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ABSTRACT: The use of continuous flow as an enabling technology within the fine chemical and pharmaceutical industries 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 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 among 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 these transformations are performed in batch mode, with such chemistries often called "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 toward the safe execution of such reactions. Furthermore, continuous processing is attractive because it offers an effective telescoped means for derivatizing the potentially unstable isocyanate intermediates toward various carbamate products.

While several modes for generating 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 because of 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 exploited 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 (BPRs), resulted in effective transformations in residence times of less than 1 h, which can be complemented by scavenger-based in-line purification (Scheme 1).[7] Related flow procedures were effectively applied toward target structures such as bromosporine analogues (**4**)[8] and PARP inhibitors (**5**),[9] where continuous processing delivered gram quantities of vital building blocks.

Recent work by Pfizer furthermore demonstrated exploitation of a continuous Curtius rearrangement reaction toward the safe and scalable synthesis of a spiropiperidine lactam moiety (**6**) found in a novel ACC inhibitor.[10] In addition, researchers from Boehringer Ingelheim demonstrated a multikilogram-scale Curtius rearrangement process that alleviated the side-product formation observed in batch through the spatiotemporal processing in flow mode.[11] Coupled with in-line IR monitoring, this impressive process achieved a throughput of 0.75 kg/h (~80% yield, 48 kg of **7** produced) in a safe and continuous manner, highlighting the value of flow processing for this important transformation (Scheme 1).

Biocatalysis has widened its scope and applicability for continuous flow through developments in enzyme immobilization.[12] The use of immobilized enzymes has a number of advantages and 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

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Scheme 1. Common Setup and Targets of Flow-Based Curtius Rearrangement Reactions

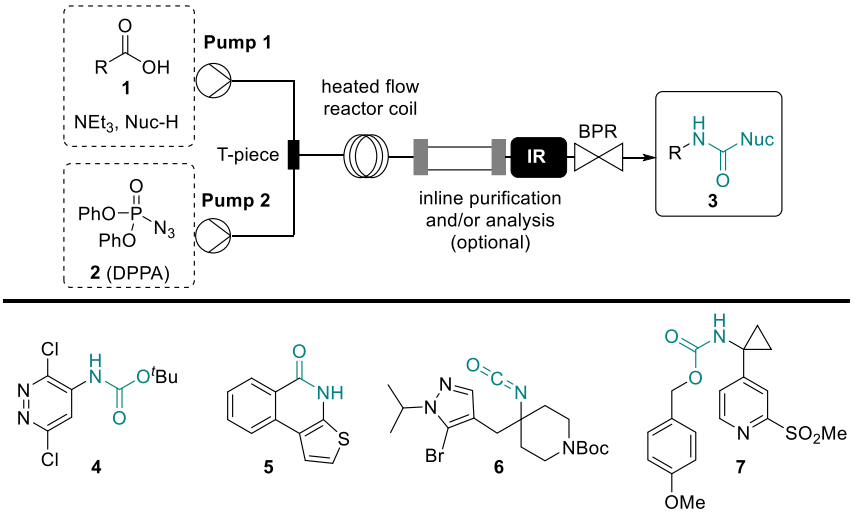
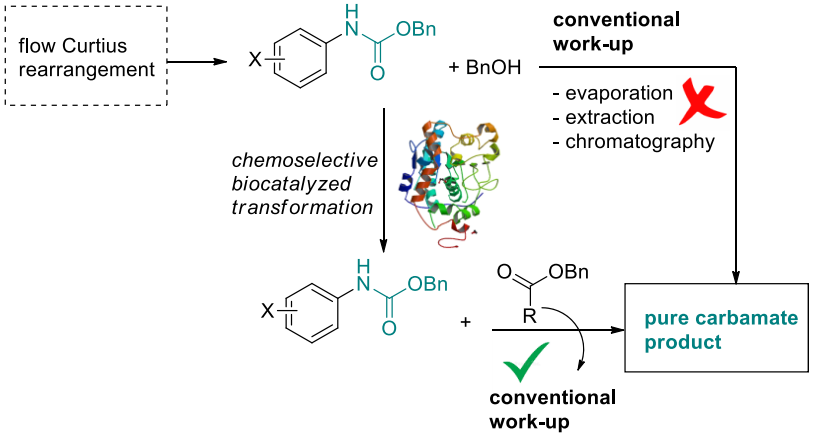


Table 1. Enzyme Immobilization Features

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

Scheme 2. Enzyme-Mediated Tagging and Subsequent Purification Strategy



allowing tight control of reaction parameters to improve the yields and productivities.[14]

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 that are easy to purge using conventional purification techniques that do not rely on chromatography. The enzyme-mediated introduction of a tag is demonstrated in Scheme 2.[15]

While the merits of continuous Curtius rearrangement and biocatalysis reactions are well-documented independently, to date their combination and corresponding downstream processing have 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 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 to aid in purification.

RESULTS AND DISCUSSION

Recent development work on a Curtius rearrangement process using benzyl alcohol (BnOH) as the nucleophile generated a library of products with the Cbz-carbamate moiety (**9a−d**) from the intermediate isocyanate species (Scheme 3). However, removal of residual benzyl alcohol proved to be challenging on the multigram scale because of its high boiling point and copolarity with the carbamate products **9a−d**. The implementation of a subsequent biocatalytic tagging step

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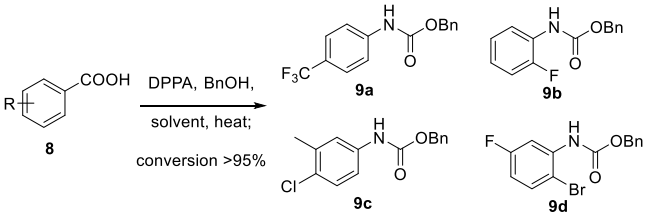
Scheme 3. General Curtius Rearrangement Process toward Carbamates 9a–d



facilitated a simple purification under continuous flow to purge this residual reagent. The development work on the Curtius rearrangement subsequently focused on the conversion of 4-(trifluoromethyl)benzoic acid (**8a**) into the corresponding Cbz-carbamate **9a** (Scheme 4).

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 because of its favorable properties of high 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 of solubility is critical for the successful development of a flow process.

Substrate **8a** was only sparingly soluble in toluene, but with the 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 equiv. 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 min (combined flow rate of 0.33 mL/min) yielded full conversion of substrate **8a** (Table 2).

As a result of the 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 (1.8 equiv) was necessary for high isolated yields of >80%. The flow process was performed successfully for prolonged periods of time (1–2 h) to generate gram quantities of the carbamate product **9a**, reaching a throughput of ca. 7 mmol/h (2.1 g/h). Furthermore, the conditions proved to be effective in circumventing any observation related to blockages or reactor fouling that could have been associated with precipitation of insoluble materials.

Table 2. Development of the Curtius Rearrangement on Substrate 8a

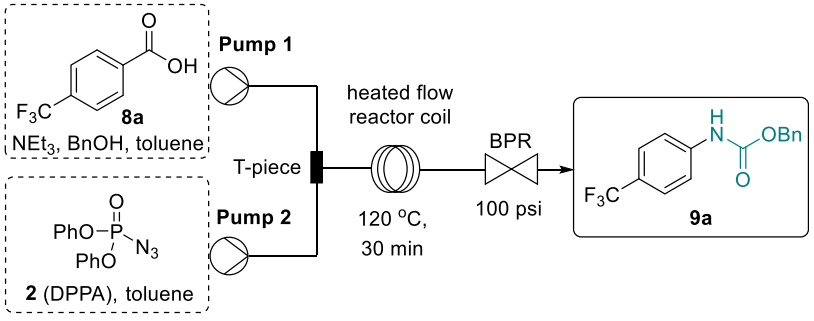
entry	residence time (min)	temperature (°C)	equiv. of BnOH	isolated yield (%)
1	20	120	1.0	70
2	30	120	1.5	79
3	30	120	1.8	86
4	30	120	2.0	85

The resultant crude carbamate product **9a** was contaminated with residual benzyl alcohol, which proved to be difficult to purge during workup because of 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 (**11**) in the presence of vinyl butyrate (**10**). CALB was used in an immobilized form using a hydrophobic carrier (acrylic resin) that provides a loading of ca. 10 wt %. CALB has been shown to be a versatile enzyme in chemical processing that is amenable to immobilization and protein engineering to tune the 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 setup was then modified by mixing **10** (3 equiv. in toluene) with the crude product stream from the Curtius rearrangement via a T-piece before it passed through the CALB column (residence time of ca. 2–5 min) and the product solution was collected for NMR and HPLC analysis. The robustness of CALB[18] meant that it could tolerate the nonpurified reaction mixture with no detrimental effect on the performance of the enzyme as a result of spent reagents. Further development experiments demonstrated that 3 equiv. of **10** (bp 116 °C) was necessary to achieve full conversion of benzyl alcohol to **11**, whereas with only 1–2 equiv. typically about 15% of unreacted benzyl alcohol was present (Scheme 5).

A CALB column with a path length (length of the 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 (5 × 1 mmol

Scheme 4. Flow Setup for Continuous Curtius Rearrangement toward Crude 9a



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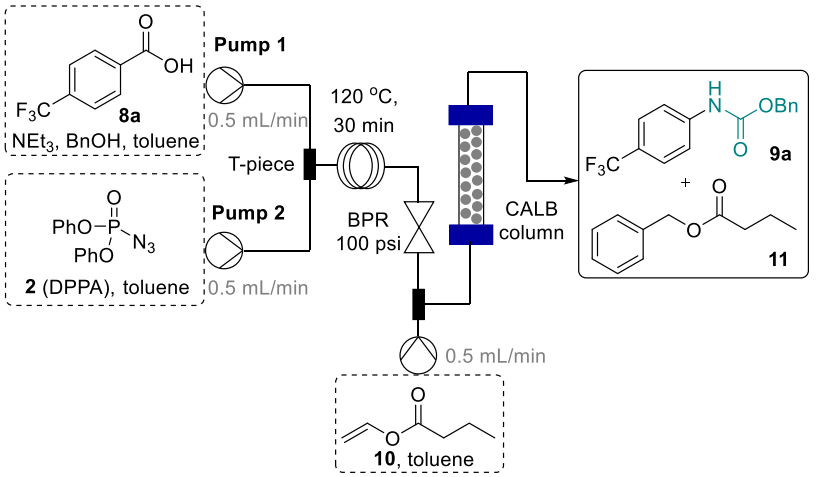
Scheme 5. Flow Setup Involving High-Energy Curtius Rearrangement and Tandem CALB-Mediated BnOH Tagging



scale), and a slight discoloration of the enzyme (from beige to light brown; Figure 1) was found to be inconsequential. The

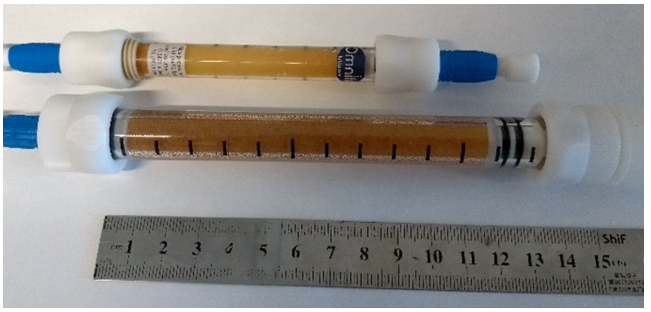


Figure 1. Appearance of used CALB columns.

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 a small scale (1 mmol of **8a**), the isolated yield for the desired carbamate product ranged from 75 to 82% depending on the isolation procedure.

To demonstrate the scalability and robustness of the flow protocol, the process was carried out to generate the desired product **9a** on a 100 mmol scale. The above-described flow setup 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 in toluene) and substrate (1 M in toluene, 1.0 equiv. of NEt_3, 1.8 equiv of BnOH) were pumped at individual flow rates of 0.5 mL/min and directed through three consecutive flow coils (3 × 10 mL, 120 °C, PFA) after being mixed in a T-piece, providing a residence time of 30 min. After the crude reaction mixture passed a BPR (100 psi), it was mixed with a stream of vinyl butyrate (3 equiv. in toluene, 0.5 mL/min) 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 as a white crystalline solid after evaporation and crystallization from heptanes. The isolated yield of 83% parallels those of previous small-scale reactions and rendered 22 g of pure carbamate **9a**, equivalent to a throughput of 6.6 g/h. Throughout this scale-up, samples were analyzed by HPLC and ^1H NMR spectroscopy, which demonstrated quantitative conversion of residual benzyl alcohol using CALB in all cases throughout the campaign (Figure 2).

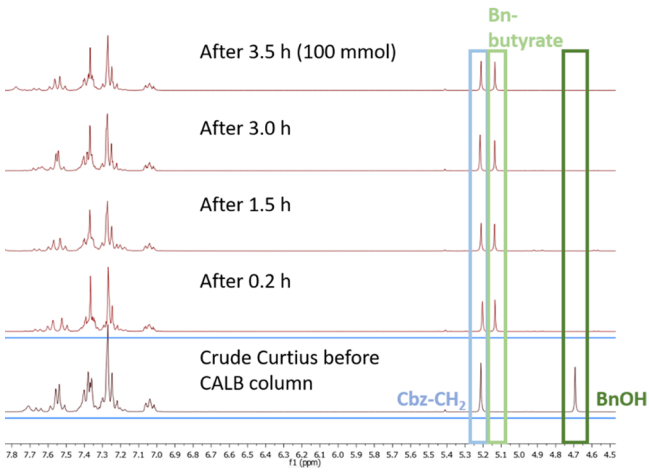


Figure 2. Analysis of reaction performance by ^1H NMR spectroscopy for a 100 mmol reaction.

SUMMARY AND CONCLUSIONS

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. Immobilized CALB packed in a glass column proved to be very robust during scale-up of the continuous process. The desired carbamate product was isolated in high yield and analytical purity following standard procedures, affording ~22 g of product in less than 4 h. 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 Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00420.

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Experimental procedures and copies of NMR spectra (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All of the authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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