



# Development of a multistep reaction cascade for the synthesis of a sacubitril precursor in continuous flow

Katharina Hiebler<sup>1</sup> · Carina Dertnig<sup>1</sup> · Sebastian Soritz<sup>1</sup> · Manuel C. Maier<sup>1</sup> · Theresa R. Hörmann<sup>1</sup> · Bianca Grabner<sup>1</sup> · Heidrun Gruber-Woelfler<sup>1</sup>

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## Abstract

The active pharmaceutical ingredient sacubitril acts as a neprilysin inhibitor in the body and is administered to patients suffering from high blood pressure and chronic heart failure. In this paper, we report the development of a three-step setup for the synthesis of an advanced sacubitril precursor in continuous flow. The key transformation of our cascade is a Suzuki-Miyaura coupling facilitated by a heterogeneous palladium catalyst. Its implementation in a packed-bed reactor and the application of continuous flow methodologies allow intensification of the cross-coupling reaction compared to batch processing. The subsequent steps for the synthesis of the target molecule involve Boc-deprotection as well as *N*-succinylation, which have been optimized using the statistical “Design of Experiments” (DoE) approach. In this way, the individual as well as interactive effects of selected parameters on the output of the reactions could be investigated very efficiently. The consecutive performance of the three reaction steps using an integrated setup enabled the synthesis of a late-stage sacubitril precursor in continuous flow with 81% overall yield.

**Keywords** Continuous flow chemistry · Heterogeneous catalysis · Multistep reaction cascade · Palladium · Sacubitril

## Introduction

Among the different medication options for the treatment of hypertension and related cardiovascular complaints [1], drugs affecting the renin-angiotensin-aldosterone system have proved to be highly effective [1]. One prominent representative of this class of agents is LCZ696 (Novartis International AG, Basel, Switzerland), which was approved by the FDA in 2015 for the treatment of heart failure in patients with reduced

ejection fraction [2]. The fixed-dose combination drug marketed under the brand name Entresto® comprises the active pharmaceutical ingredient (API) valsartan, an angiotensin II-receptor antagonist, and the prodrug sacubitril **1** [3]. The latter compound is metabolized in the body to an active neprilysin inhibitor upon cleavage of its ethyl ester moiety [2]. The synthesis of sacubitril **1** was first reported by Ksander et al. [4] in 1995, starting from an unnatural D-amino acid derivative **3**. Formation of the main chiral intermediate **2** was achieved in 8 linear steps and involved a Wittig reaction as well as stereoselective hydrogenation (Scheme 1, a). An alternative route, also running via formation of intermediate **2**, was developed by Hook et al. [5], who used L-pyroglutamic acid methyl ester **4** as chiral pool for the synthesis of **2** in batch (Scheme 1, b). Recently, Ley et al. [6] published a novel approach applying the concept of continuous flow technology and integrating machine-assisted methods for the synthesis of hydrochloride salt **2\*HCl**. In contrast to the other groups, their strategy employs exclusively achiral starting materials **5–7** and the two chiral centers are installed by both Rh-catalyzed stereoselective hydrogenation and diastereoselective Reformatsky-type carbethoxyallylation. By a combination of two batch steps and five transformations in continuous flow, they obtained the target molecule in 54% overall yield (Scheme 1, c).

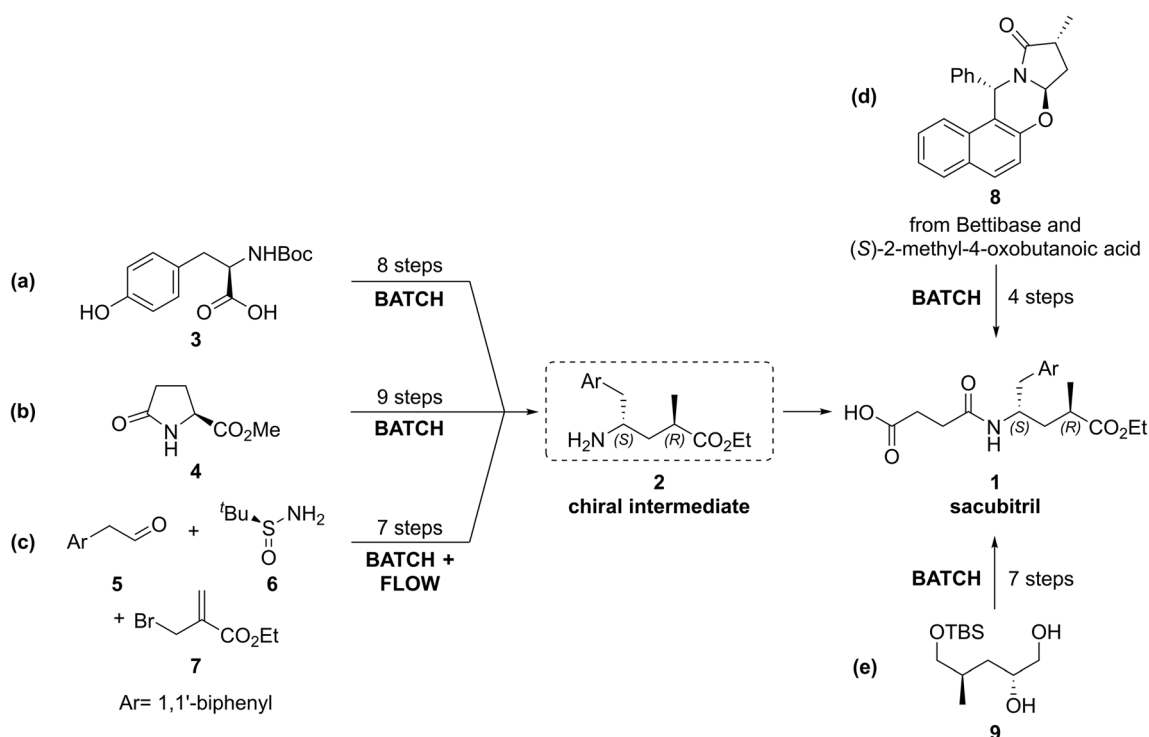
## Highlights

- Development of a multistep setup for the integrated synthesis of a late-stage sacubitril precursor in continuous flow.
- Intensification of Suzuki coupling in continuous flow employing a heterogeneous palladium catalyst implemented in a packed-bed reactor.
- Optimization of Boc-deprotection as well as *N*-amidation using the “Design of Experiments” approach.

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✉ Heidrun Gruber-Woelfler  
woelfler@tugraz.at

<sup>1</sup> Institute of Process and Particle Engineering, Graz University of Technology, Inffeldgasse 13/III, 8010 Graz, Austria



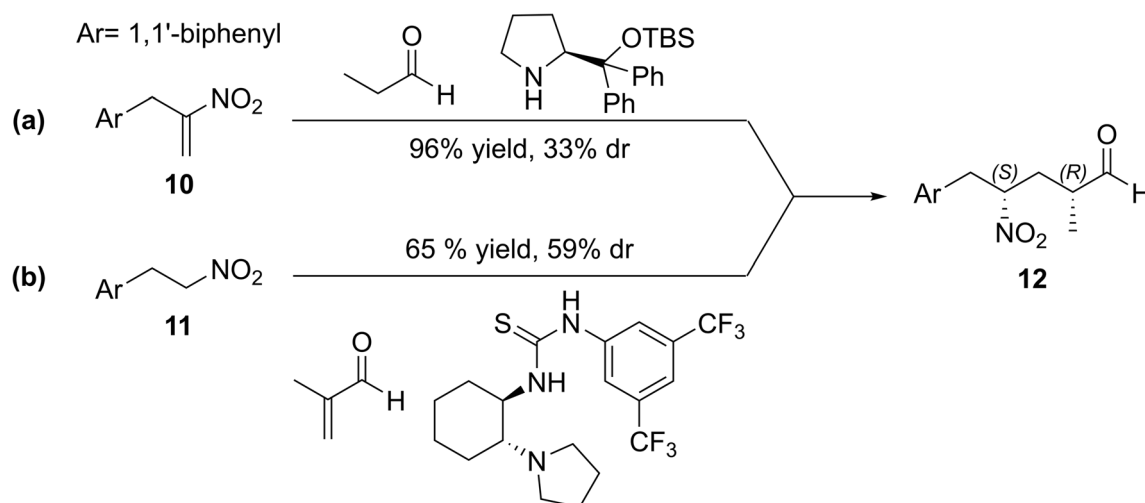
**Scheme 1** Published literature routes for the synthesis of sacubitril **1** [4–8]

Apart from the mentioned approaches involving the formation of chiral intermediate **2**, two alternative strategies for the synthesis of sacubitril **1** have been reported in literature. Firstly, Xu et al. [7] achieved formation of **1** starting from Bettibase and (*S*)-2-methyloxobutanoic acid, which yielded oxazolidine **8**. Upon stereoselective addition of a Grignard reagent to the obtained chiral intermediate **8**, the biphenyl motif is installed and 3 more steps finally give the active pharmaceutical ingredient **1** (Scheme 1, d). Secondly, the group of Wang [8] developed a chiron-based approach relying on the protected triol **9**. The key transformations of their process are epoxide formation as well as an one-flask Staudinger reduction/succinic amide formation, which facilitate formation of sacubitril **1** in a total of 7 steps (Scheme 1, e).

Regarding the synthesis of sacubitril **1** on an industrial scale, Novartis International AG (Basel, Switzerland) holds various patents covering different synthetic routes. Their earlier patents comprise chiral pool approaches, similar to the cascades established by Ksander [9] and Hook [10], for formation of the two chiral centers present in the API **1**. However, researchers at the Swiss pharmaceutical company have recently developed a novel organocatalytic strategy for installation of the stereocenters [11]. The first option described in the patent involves the reaction of Michael acceptor **10** with propionaldehyde employing a proline-derived organocatalyst, which is high-yielding but unsatisfactory in terms of diastereoselectivity (Scheme 2, a). Formation of the desired diastereomer **12** could be improved by the use of nucleophile **11** in combination with methacrolein and a thiourea-derived

catalyst (Scheme 2, b). However, in view of the potential application of organocatalysis for the synthesis of sacubitril **1** on a large scale, diastereoselectivity still needs to be improved to meet the requirements of the pharmaceutical industry.

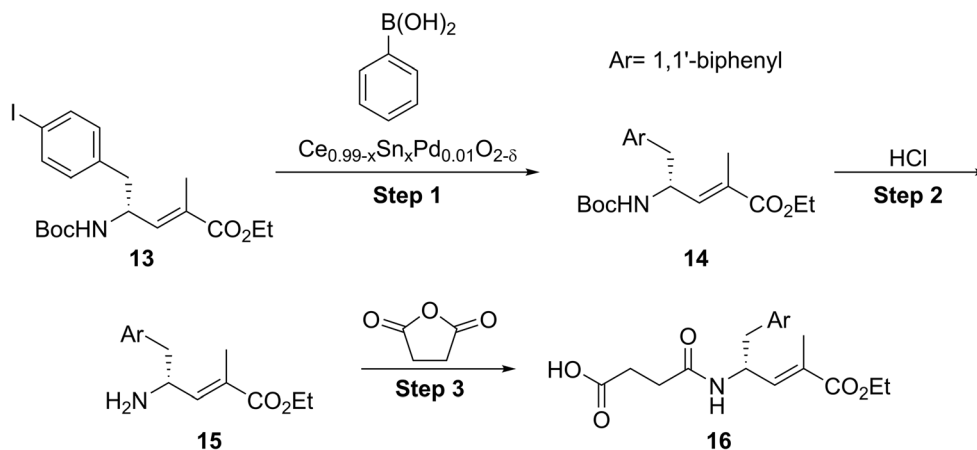
Although apparently a number of different strategies for the synthesis of sacubitril **1** have been developed in the past years, it is conspicuous that almost every approach relies on traditional batch chemistry. As mentioned above, the sole exception is the combined batch and flow process published by Ley and his group [6], involving 5 transformations in continuous flow. Continuous flow methodology offers a plethora of opportunities for the pharmaceutical industry in terms of both process optimization and economics. On the one hand, flow processes benefit from a rapid heat and mass transfer as well as the opportunity for automation and process intensification [exemplarily 12, 13], in this way opening novel process windows [14]. Furthermore, improved energy efficiency, high safety and reduced waste generation associated with continuous processing [13, 15, 16] align with the principles of green and sustainable chemistry [17]. On the other hand, flow chemistry allows to reduce development times and to monitor the product quality in-line, thus saving up to 30% costs in comparison with an equivalent batch process [18–20]. In view of the numerous arguments speaking in favor for the implementation of continuous flow operations, it is not surprising that continuous manufacturing becomes an increasingly important topic for the pharmaceutical industry [21–24] and is also supported by the Federal Drug Administration (FDA) [25, 26].



**Scheme 2** Recent organocatalytic approaches for the synthesis of sacubitril **1** via chiral intermediate **12** patented by Novartis [11]

Accordingly, as part of the ONE-FLOW research project [27], we are aiming for the development of catalyzed cascade reactions for the formation of active pharmaceutical ingredients. Recently, our group has demonstrated the applicability of flow methodology for the multistep synthesis of an advanced valsartan precursor in a continuous fashion [28]. Hence, our next goal was to prove our concept also for the integrated synthesis of a late-stage sacubitril intermediate **16** in continuous flow, which we report in this paper. The key step of our envisaged reaction scheme, inspired by the paper of Ksander et al. [4], is Suzuki-Miyaura cross-coupling of functionalized sacubitril intermediate **13** (obtained via a three-step batch synthesis procedure, see Supporting Information for experimental details) with phenylboronic acid facilitated by the heterogeneous palladium catalyst  $\text{Ce}_{0.99-x}\text{Sn}_x\text{Pd}_{0.01}\text{O}_{2-\delta}$  [28–31]. Subsequently, deprotection of the obtained biphenyl compound **14** and *N*-amidation of the free amine **15** with succinic anhydride yielded the targeted sacubitril precursor **16** (Scheme 3).

**Scheme 3** Targeted three-step approach for the integrated synthesis of sacubitril precursor **16** in continuous flow



## Results and discussion

### Optimization of the individual steps in continuous flow

Palladium-catalyzed C-C cross-coupling reactions have become indispensable in modern organic chemistry [32–34]. Due to its remarkable versatility, functional-group tolerance as well as selectivity, palladium catalysis is long since a key component in the toolbox of the pharmaceutical industry [35–37]. In particular, the Suzuki-Miyaura reaction between aryl halides and boronic acid derivatives plays an important role for the formation of biphenyl motifs [38–40]. In the past couple of years, our group has gained experience with Suzuki-Miyaura reactions in continuous flow facilitated by heterogeneous Pd-Ce-Sn-oxides [28–31]. Reviewing published literature procedures for the synthesis of sacubitril **1**, only the route developed by Ksander et al. [4] comprises a Suzuki coupling step and served as our point of reference. First, we aimed for the identification of a successful cross-coupling reaction facilitated by the class of heterogeneous palladium catalysts with the molecular

formula  $\text{Ce}_{0.99-x}\text{Sn}_x\text{Pd}_{0.01}\text{O}_{2-\delta}$  ( $x = 0, 0.20, 0.495, 0.79, 0.99$ ) [29] developed and available in our lab. Their successful utilization for the Suzuki coupling of aryl halides with boronic acid derivatives in batch as well as in continuous flow has already been reported by our group [28–31]. In previous studies and preliminary experiments, the catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-\delta}$  proved to be the best choice for the Suzuki cross-coupling of advanced chemical intermediates [28]. Therefore, we tested the applicability of the respective palladium catalyst (standard reaction conditions: 1 mol eq. aryl halide, 1.5 mol eq. phenylboronic acid, 1.5 mol eq.  $\text{K}_2\text{CO}_3$ ,  $\text{iPrOH:H}_2\text{O} = 7:3$ ,  $75^\circ\text{C}$ ) for Suzuki couplings employing different cross-coupling partners. In doing so, the C-C bond formation between functionalized aryl iodide **13** and phenylboronic acid (Scheme 3, step 1) emerged to be superior and was chosen to be the key step of our reaction cascade for formation of a sacubitril precursor. Our reaction scheme was completed by Boc-deprotection of the resulting biphenyl derivative **14** as well as reaction of amine **15** with succinic anhydride, giving the sacubitril precursor **16** (Scheme 3).

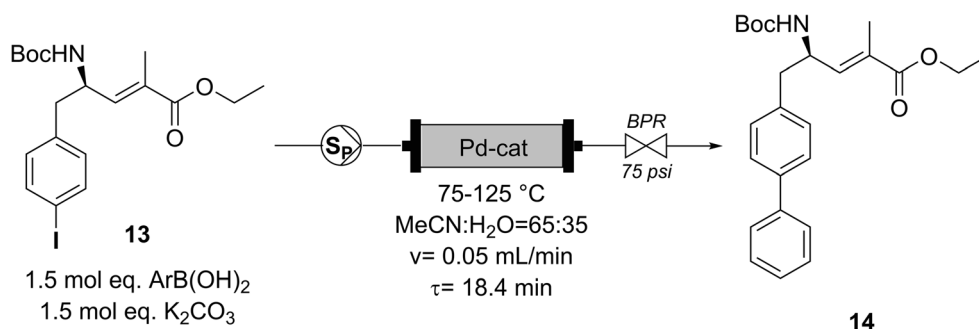
Next, we had to select a suitable solvent for our targeted reaction cascade, meeting different requirements for the individual reaction steps. Concerning the Suzuki coupling step using the chosen type of catalyst, the presence of water is crucial for the reaction to occur [28, 41]. In particular, we experienced that our catalyst favors an aqueous single-phase reaction environment [28]. Regarding the solubility of substrates and reaction intermediates as well as stability of the succinic anhydride reagent in the third step, an aprotic organic cosolvent was needed. For that purpose, acetonitrile (MeCN), tetrahydrofuran as well as dioxane came into question. For toxicity as well as miscibility reasons [42, 43], we decided to use acetonitrile:water as the reaction medium for the synthesis of sacubitril precursor **16**. For the first step of our cascade, Suzuki cross-coupling, a ratio of acetonitrile:water = 65:35 (v/v) turned out to provide sufficient solubility of substrate **13** (25 mM) while keeping the present potassium carbonate dissolved.

After having identified the preferred reaction medium for our targeted synthesis of sacubitril precursor **16**, we aimed to optimize the individual steps of our reaction cascade in continuous flow. Initially, we focused on the first step of the reaction cascade, the Suzuki cross-coupling reaction. Previous studies [28–31] showed that Suzuki couplings employing catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-\delta}$  work best using the respective aryl halide in combination with both 1.5 mol eq. of boronic acid species and potassium carbonate. Hence, we adopted that approach for the targeted reaction of sacubitril intermediate **13** with phenylboronic acid in a continuous fashion. More specifically, we wanted to study the effect of the reaction temperature on measured conversion of substrate **13**. For the experiment, a solution containing the cross-coupling partners and the inorganic base potassium carbonate in a mixture of acetonitrile:water = 65:35 (v/v) was pumped through an HPLC column (L x I.D.  $50 \times 4.6$  mm) filled with particles of palladium catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-\delta}$

(Scheme 4, see Experimental section for details). Using a flow rate of 0.05 mL/min, the mean residence time inside the packed-bed reactor was determined to be 18.4 min regarding the measurement of a tracer-determined residence time distribution curve. According to literature [44], the employed reaction solvent starts boiling at a temperature of approximately  $77^\circ\text{C}$ . However, one of the major benefits of flow chemistry compared to batch processing is the ability to heat the reaction mixture above its boiling point in a pressurized system and thus to accelerate the reaction rate [12, 13]. Hence, we evaluated the performance of our setup at 75, 100 and  $125^\circ\text{C}$ , maintaining a stable pressure of 75 psi with a back pressure regulator. In doing so, a significant increase in measured conversion could be observed with higher temperature. Whereas at a temperature of  $75^\circ\text{C}$  conversion of **13** was determined to be only 59% (Entry 1, Table 1), an increase to  $100^\circ\text{C}$  led to an improved conversion of 84% (Entry 2, Table 1). Best results were obtained at a reaction temperature of  $125^\circ\text{C}$ , giving almost quantitative conversion of **13** using the abovementioned setup (96%, Entry 3, Table 1). Isolation of biphenyl **14** synthesized in continuous flow at a temperature of  $125^\circ\text{C}$  proved that only minor racemization of the chiral center occurs under the employed reaction conditions (95% enantiomeric excess, see Supporting Information for determination of enantiomeric excess by chiral HPLC).

After having demonstrated the applicability of our packed-bed reactor setup for the key Suzuki coupling reaction of our targeted cascade for the synthesis of **16**, we turned our attention to the subsequent steps Boc-deprotection and *N*-amidation. Since we did not have a substantial amount of biphenyl intermediate **14** in hand, we wanted to keep its consumption to a minimum. Therefore, we decided to use readily available surrogate substrates for the optimization of steps 2 and 3 in continuous flow, comprising similar core structures as sacubitril precursor **14**. Thus, we chose to study the Boc-deprotection of Boc-L-phenylalanine methyl ester **17** (Fig. 1) as well as *N*-amidation of L-phenylalanine methyl ester **18** (Fig. 2) using a coil reactor setup. Considering the fact that the outcome (e.g. conversion, yield) of a chemical transformation is the result of a number of interacting reaction parameters, the traditional one-variable-at-a-time optimization is not the most effective method to investigate the experimental space [45, 46]. For this reason, we decided to use an alternative optimization approach employing “Design of Experiments” (DoE), which is a branch of applied statistics. DoE allows the determination of individual as well as combined effects of different influencing factors on the output of an experiment. Furthermore, using DoE the required information for optimization of a given process can be obtained with the minimum amount of experimentation [46]. Being developed already in the 1920s based on the work of R. A. Fisher [47], DoE has been used extensively in various sectors including car manufacturing, biotech and steel industry [46]. In recent years, also the pharmaceutical industry has increasingly taken advantage of DoE for the efficient

**Scheme 4** Suzuki-Miyaura cross-coupling for formation of sacubitril intermediate **14** in continuous flow facilitated by Pd-catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-5}$



identification of optimal process parameters for their manufacturing processes [45, 48–50].

Regarding the Boc-deprotection of compound **17**, we first had to define how we wanted to achieve the removal of the protecting group. In view of the performance of the deprotection step in targeted cascade for the continuous synthesis of sacubitril precursor **16** after the Suzuki coupling step, the transformation had to be performed in an acetonitrile:water solution. Preliminary batch experiments showed that the deprotection of **17** utilizing commonly employed trifluoroacetic acid [51, 52] is relatively slow in aqueous media. Likewise, published deprotection on a strongly acidic ion exchange resin [53, 54] did not prove to be satisfyingly successful in water-containing solvent mixtures. In our case, best results were obtained using aqueous hydrochloric acid for removal of the protecting group. The same strategy has also been reported for achieving Boc-removal on a large scale for the synthesis of APIs in batch [55] as well as in continuous flow [24].

Considering the optimization of our Boc-deprotection step, we decided to examine the influence of the factors *temperature* as well as *mol eq. hydrochloride (HCl)* on the conversion of compound **17**. Applying the Design of Experiments approach, we performed the Boc-deprotection of **17** in continuous flow at three different temperatures (70, 75, 80 °C) and varied the amount of hydrochloric acid (5, 10, 15 mol eq.) in the experiments. As we decided to utilize a full factorial DoE, we determined the conversion of **17** resulting of every possible combination of the two selected reaction parameters. Analysis of the collected experimental data with the software MODDE Pro revealed a statistically significant impact of the two interacting variables on the conversion of **17**, which is visualized in Fig. 1. As expected, the contour plot illustrates an increase in conversion both at higher reaction temperature and using a larger excess of hydrochloric acid.

**Table 1** Optimization of the reaction temperature of step 1 in continuous flow

Entry	T/ °C	Conversion <sup>a</sup> / %
1	75	59
2	100	84
3	125	96

<sup>a</sup> Conversion of **13** after system reached steady-state (determined by HPLC)

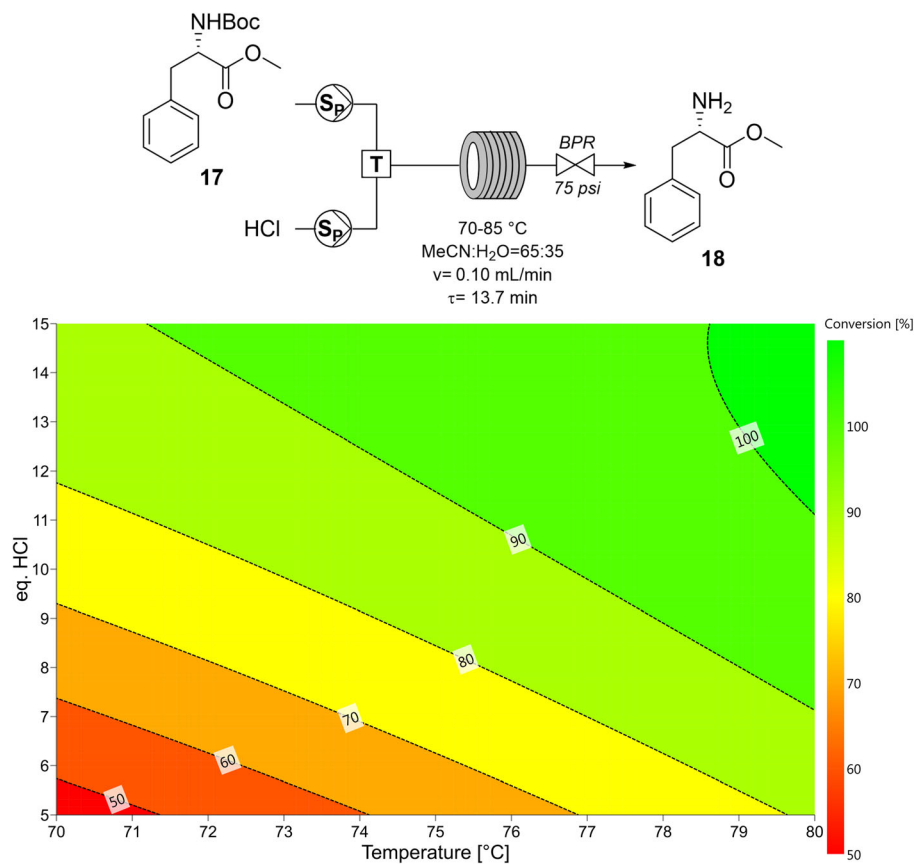
However, the reaction temperature seems to influence the outcome of the reaction more strongly than the excess of hydrochloric acid. Exemplarily, the model suggests that using 5 mol eq. HCl the conversion can be increased from 50% to 80% by raising the temperature only by about 8 °C. To achieve the same boost in conversion at a given temperature of 70 °C, more than double the HCl concentration would be necessary. Regarding our multistep cascade for the synthesis of **16**, we wanted to achieve quantitative conversion utilizing 10 mol eq. of acid. The obtained DoE model led us to expect that quantitative conversion of **17** using 10 mol eq. of HCl could be realized at a temperature of 85 °C. Indeed, the performance of an additional experiment ( $T=85$  °C, 10 mol eq. HCl) confirmed this assumption (see Supporting Information for a detailed description of conducted DoE study, model parameters as well as experimental data).

Concerning the reaction of nucleophiles with succinic anhydride for the synthesis of active pharmaceutical ingredients in continuous flow, *O*-succinylation has already been achieved using the anhydride reagent in combination with a tertiary amine base employing a tube reactor [56]. Apart from that, *N*-succinylation in batch has also been performed in aqueous media at room temperature [57, 58]. However, the presence of water induces the hydrolysis of succinic anhydride, which has a half-life of roughly 5 min (pH 7, RT) [59, 60]. Consequently, the targeted amidation reaction has to be faster than the hydrolysis of the anhydride reagent in order to be successful.

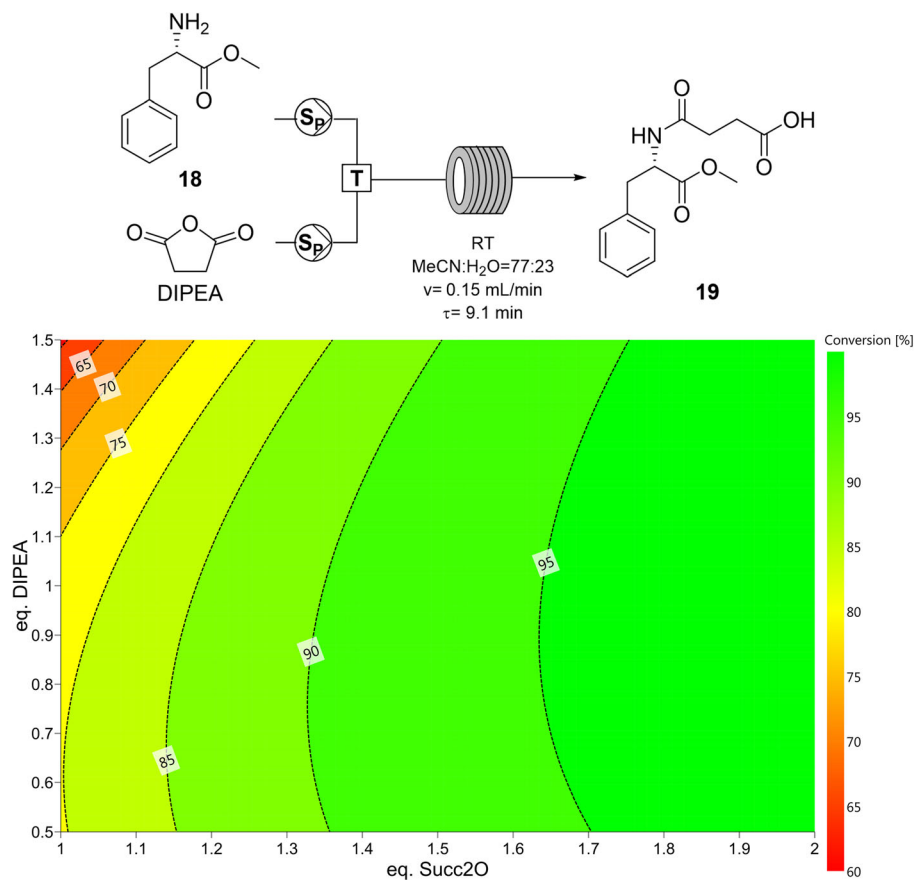
In terms of the continuous *N*-amidation of **18** in an acetonitrile:water medium at room temperature, we chose to determine the impact of the variables *mol eq. succinic anhydride* as well as *mol eq. diisopropylethylamine (DIPEA)* on the reaction outcome. In compliance with full-factorial DoE, we measured the conversion of **18** resulting from every combination of the two influencing factors (1, 1.5, 2 mol eq. succinic anhydride; 0.5, 1, 1.5 mol eq. DIPEA). Evaluation of collected data with MODDE Pro suggested a statistical relevant influence of both parameters on the conversion and revealed also interactive effects. The visual illustration of the calculated mathematical model is depicted in Fig. 2. Within the covered experimental range, maximum conversion is obtained using 2 mol eq. of succinic anhydride along with 1 mol eq. of DIPEA (see Supporting Information for a detailed description of conducted DoE study, model parameters as well as experimental data).



**Fig. 1** Reaction scheme and contour plot obtained by DoE analysis of Boc-deprotection of Boc-L-phenylalanine methyl ester **17** in continuous flow with MODDE Pro



**Fig. 2** Reaction scheme and contour plot obtained by DoE analysis of *N*-amidation of L-phenylalanine methyl ester **18** in continuous flow with MODDE Pro

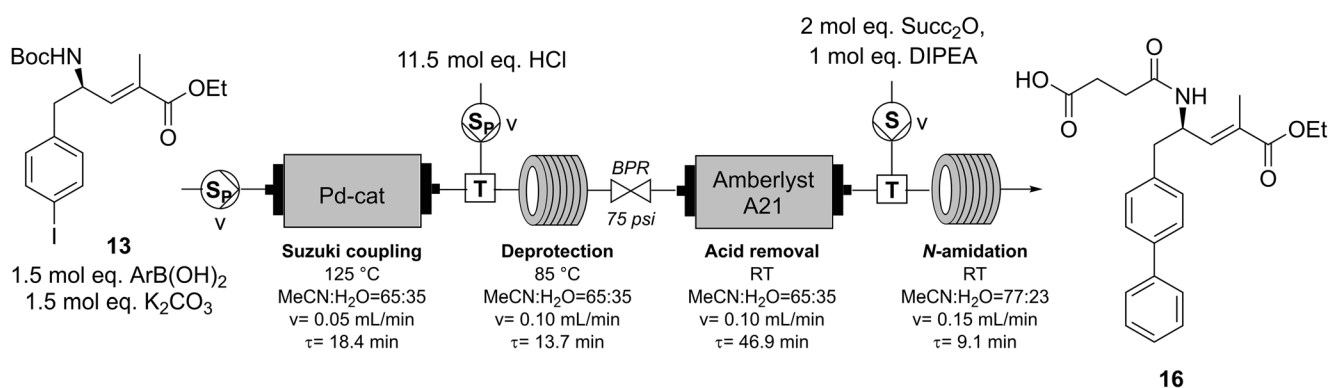


## Multistep continuous setup for the synthesis of 16

Following the intensification of the Suzuki coupling of sacubitril precursor **13** as well as proving the feasibility of Boc-deprotection and *N*-amidation in a continuous fashion, we targeted the integrated synthesis of sacubitril precursor **16** in a fully continuous setup (Scheme 5). As outlined above, Suzuki-Miyaura cross-coupling of **13** was performed at a temperature of 125 °C employing a packed-bed reactor (L x I.D. 50 × 4.6 mm) containing the heterogeneous palladium catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-\delta}$ . Subsequently, Boc-deprotection of obtained biphenyl intermediate **14** was achieved by merging the reaction stream with a solution of hydrochloric acid and introducing it into a coil reactor (L x O.D. x I.D. 3.0 m × 1/16 in. × 0.030 in.). According to the optimization of Boc-removal by DoE, the free amine **15** was generated using 10 mol eq. HCl and a reaction temperature of 85 °C. To take the partial neutralization of hydrochloric acid by present potassium carbonate (1.5 mol eq.) into account, a stock solution containing 11.5 mol eq. HCl was utilized for this purpose. Since the large excess of hydrochloric acid in the reaction solution is detrimental for the following third step of the reaction cascade, the acid was removed deploying anion exchange resin Amberlyst A21 implemented in an HPLC column (L x I.D. 120 × 8 mm). The same approach has already been reported in literature for the removal of trifluoroacetic acid from organic solvents in batch [61]. Finally, as suggested by DoE analysis, *N*-amidation of **15** was accomplished in a coil reactor (L x O.D. x I.D. 3.0 m × 1/16 in. × 0.030 in.) after addition of 2 mol eq. succinic anhydride as well as 1 mol eq. DIPEA. Using this multistep setup (Scheme 5) exhibiting a total residence of about 90 min (including tubing), the synthesis of the late-stage sacubitril precursor **16** was successfully performed for over 5 h yielding the targeted compound with up to 98% yield. After a steady increase in the beginning, the yield of sacubitril precursor **16** was observed to slowly decrease over the course of the experiment and stabilized at approximately 81% (corresponding to a space-time-yield of 4 g/L\*h). after

370 min (see Supporting Information for graphic representation). This can probably be contributed to an initial loss of palladium due to leaching from the employed heterogeneous catalyst, which has already been reported in literature [28, 30]. Determination of the residual levels of palladium, cerium and tin in the reaction solution by ICP-MS strongly support this hypothesis (Table 2). Whereas the reaction outlet collected at  $t = 160\text{--}180$  min exhibits a palladium content of  $563 \pm 13$  µg/kg (Entry 1, Table 2), the concentration of the metal was found to significantly decrease over the course of the continuous experiment. After a run time of 400 min, the amount of palladium was determined to be reduced to  $105 \pm 3$  µg/kg (Entry 4, Table 2), which renders our developed multistep process highly promising to meet the strict regulatory guidelines for the production of pharmaceutical products [62]. Concerning the leaching behavior of tin from the employed heterogeneous catalyst, the residual levels in the outlet flow were measured to be  $\leq 30$  µg/kg (Entries 1–4, Table 2) in all samples. Likewise, the concentration of cerium was very low in analyzed samples ( $\leq 9.7$  µg/kg, Entries 1–4, Table 2).

Apart from that, also the constant reduction of acid scavenging capacity of the deployed ion exchange resin might account for observed course of yield. As far as starting material **13** is concerned, HPLC measurements revealed full conversion throughout the whole experiment. However, it has to be noted that **13** can as well undergo Boc-deprotection and in further consequence *N*-amidation. Regarding the formation of by-products in the three-step continuous cascade, oxidation and homocoupling of phenylboronic acid are reported to occur upon depletion of the aryl halide coupling partner in the Suzuki coupling step [29]. In fact, the HPLC chromatogram of the outlet flow showed a small peak corresponding to biphenyl resulting from the coupling of two molecules of phenylboronic acid. Apart from that, the reaction steps seemed to be rather clean and only small peaks attributable to unidentified side-products could be detected by HPLC ( $\lambda = 237$  nm) at first. However, after an experiment run time of 260 min HPLC analysis showed the appearance of a peak at 0.7 min,



**Scheme 5** Multistep setup for the synthesis of sacubitril precursor **16** in continuous flow ( $\text{S}_\text{p}$  = high-pressure syringe pump,  $\text{S}$  = syringe pump,  $\text{T}$  = T-mixer,  $v = 0.05$  mL/min)

**Table 2** Levels of Pd, Ce and Sn in the outlet flow of the three-step cascade for the synthesis of **16** in continuous flow (determined by ICP-MS)

Entry	Time / min	Pd/ $\mu\text{g kg}^{-1}$	Sn/ $\mu\text{g kg}^{-1}$	Ce / $\mu\text{g kg}^{-1}$
1	160–180	$563 \pm 13$	$24 \pm 5$	$9.7 \pm 0.3$
2	240–260	$312 \pm 2$	$22 \pm 12$	$7.2 \pm 0.5$
3	320–340	$145 \pm 5$	$30 \pm 13$	$9.4 \pm 0.8$
4	400–420	$105 \pm 3$	$29 \pm 8$	$8.9 \pm 0.4$

which seemed to increase with decreasing yield. The molecular structure of the corresponding compound could not have been elucidated so far.

Concerning the absence of other significant by-product peaks in HPLC chromatogram, the use of weakly basic anion exchange resin Amberlyst A21 for the adsorption of excess hydrochloric acid can also be regarded as an in-line purification step. It removes various kinds of acidic materials from the process stream including potentially formed hydroiodic acid [63] resulting from the use of an aryl iodide coupling partner in the Suzuki reaction as well as phenol originating from the oxidation of phenylboronic acid [64]. In addition, we observed that in our multistep continuous cascade Amberlyst A21 acts as a scavenger of unreacted phenylboronic acid too. Moreover, the respective anion exchange resin was reported to adsorb Pd(II) from aqueous solutions [65, 66] and might therefore contribute to the observed low levels of palladium in the outlet flow of the continuous setup.

Besides the reaction yield, another important quality attribute of pharmaceutical processes is the enantiopurity of the obtained target molecule. Enantiomeric excess of compound **16** synthesized in continuous flow utilizing the described integrated setup was determined to be moderate 43% (see Supporting Information for determination of enantiomeric excess by chiral HPLC). As biphenyl intermediate **14** obtained in continuous Suzuki cross-coupling was shown to be almost enantiopure, the partial racemization of the chiral center most likely occurred during the removal of the protecting group. Boc-deprotection was performed in an aqueous and strongly acidic environment at elevated temperature, conditions which presumably caused racemization of the sacubitril precursor to some extent [67, 68]. Therefore, in order to provide a process suitable for pharmaceutical industry, future work will focus on improving the enantiomeric excess of sacubitril precursor **16** obtained in developed continuous cascade by applying milder conditions for the Boc-deprotection step.

## Conclusion

In summary, we have successfully applied continuous flow techniques for the synthesis of a late-stage sacubitril precursor

in three steps. The optimization of interacting reaction parameters was demonstrated to be efficiently performed using statistical “Design of Experiments”. Furthermore, the use of a continuous setup comprising a packed-bed reactor as well as coil reactor modules allowed intensification of the multistep process compared to batch. After an initial equilibration phase, the target molecule was obtained with a steady 81% overall yield and moderate enantiomeric excess using an integrated continuous setup. Moreover, levels of residual palladium in the outlet flow were determined to be in the ppb range, thus proving our developed process to be attractive for pharmaceutical applications. However, in view of a conceivable implementation of developed reaction cascade into an industrial process for API production, its optimization in terms of enantiomeric excess of the final product is indispensable. Nevertheless, we could prove once more that with relatively simple equipment it is possible to synthesize advanced chemical intermediates in continuous flow. With our work, we hope to contribute to the establishment of continuous flow methodologies for process development and operation in pharmaceutical industry.

## Experimental section

### General information

Chemicals and solvents were purchased from commercial suppliers and used as received unless stated otherwise [**Sigma Aldrich**: potassium carbonate (99%), anisole (99%);  $\text{LiAlH}_4$  (1 M in diethyl ether), trifluoroacetic acid (99%), Boc-L-phenylalanine methyl ester (98%), L-phenylalanine methyl ester hydrochloride (98%), Amberlyst A21 (4.6 eq./kg dry weight); **BLDpharm**: Boc-4-iodo-D-phenylalanine (98%); **Fluorochem**: *O,N*-dimethylhydroxylamine hydrochloride (98%), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (99%), ethyl(hydroxyimino)cyanoacetate (98%), (carbethoxyethylidene)triphenylphosphorane (94%), succinic anhydride (99%); **Carl Roth**: triethylamine (99.5%), hydrochloric acid (37%); **Oxchem**: phenylboronic acid (95%); **ACROS organics**: diisopropylethylamine (98%); **ChemLab**: acetonitrile (HPLC grade)]. According to a reported literature procedure [69], L-phenylalanine methyl ester hydrochloride was neutralized by addition of sodium carbonate in water and extraction of the aqueous phase with ethyl acetate yielded free L-phenylalanine methyl ester. Analytical thin layer chromatography was performed on pre-coated aluminium plates (Merck, silica gel 60,  $F_{254}$ ) and spots were visualized with UV light (254 nm) or potassium permanganate stain. Column chromatography purifications were carried out using MN silica gel 60 (70–230 mesh). An Agilent 1100 series



HPLC system was utilized for monitoring of the reaction progress as well as determination of enantiomeric excess (see Supporting Information for HPLC parameters). HPLC-MS measurements were performed on a Waters Acquity H-Class system equipped with a Waters Acquity SQD detector. For determination of Pd, Ce and Sn in the outlet flow of the multistep reaction cascade in continuous flow, an Agilent 7700x ICP-MS system was employed after microwave assisted acidic digestion of the samples. NMR-data were recorded on a Bruker Avance III 300 MHz spectrometer ( $^1\text{H}$ : 300 MHz,  $^{13}\text{C}$ : 75 MHz, see Supporting Information for NMR-data). For the DoE analysis of Boc-deprotection and *N*-amidation in continuous flow, the software MODDE Pro (Version 12.1.0.5491, Copyright © Sartorius Stedim Data Analytics AB) was utilized.

### Optimization of Suzuki cross-coupling in continuous flow

For the Suzuki coupling of intermediate **13** with phenylboronic acid in continuous flow, a packed-bed reactor setup was utilized. Therefore, an HPLC column (L x I.D. 50 × 4.6 mm) was filled with dry Pd-catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-8}$  (602 mg, containing 0.04 mmol Pd) and connected to a back pressure regulator (IDEX BPR Cartridge 75 psi Gold Coat). After equilibration of the reactor setup with the reaction solvent ( $\text{MeCN}:\text{H}_2\text{O} = 65:35$ ), the packed-bed reactor was heated to elevated temperature (75–125 °C) in an oil bath. Then, a solution containing intermediate **13** (25 mM), phenylboronic acid (37.5 mM, 1.5 mol eq.), potassium carbonate (37.5 mM, 1.5 mol eq.) and anisole (62.5 mM) as internal standard was prepared in  $\text{MeCN}:\text{H}_2\text{O} = 65:35$ , degassed in an ultrasonic bath and filtered through a 0.2  $\mu\text{m}$  membrane filter. Obtained stock solution was pumped through the reactor with a flow rate of 0.05 mL/min ( $\tau = 18.4$  min) utilizing a high-pressure syringe pump (VIT-FIT HP, Lambda Instruments). For monitoring of the reaction progress, every 10 min an aliquot (40  $\mu\text{L}$ ) was quenched with  $\text{MeOH}:\text{H}_3\text{PO}_4 = 75:25$  (400  $\mu\text{L}$ ) and analyzed by HPLC (Method B).

### Optimization of Boc-deprotection in continuous flow

The influence of temperature and equivalents of acid on Boc-deprotection was studied using Boc-L-phenylalanine methyl ester **17** as surrogate substrate utilizing a coil reactor setup. Solution A [Boc-L-phenylalanine methyl ester (25 mM), anisole (62.5 mM) as internal standard] and solution B [ $\text{HCl}$  (125–375 mM, 5–15 mol eq.)] were prepared in the reaction solvent  $\text{MeCN}:\text{H}_2\text{O} = 65:35$  and degassed by ultrasonic irradiation. The setup comprising a PEEK coil (L x O.D. x I.D. 3.0 m × 1/

16 in. × 0.030 in.,  $V = 1.37$  mL) connected to a back pressure regulator (IDEX BPR Cartridge 75 psi Gold Coat) was equilibrated with solvent and heated to elevated temperature in a water bath (70–85 °C). The two reagent solutions were merged using a T-mixing element and pumped through the reactor by means of high-pressure syringe pumps (VIT-FIT HP, Lambda Instruments) at a flow rate of 0.05 mL/min each, giving a total flow rate of 0.10 mL/min ( $\tau = 13.7$  min). After certain time points ( $3\tau$  /  $3.5\tau$  /  $4\tau$ ), aliquots (80  $\mu\text{L}$ ) of the outlet flow were quenched with  $\text{MeOH}:\text{H}_3\text{PO}_4 = 55:45$  (400  $\mu\text{L}$ ) and analyzed by HPLC (Method A).

### Optimization of *N*-amidation in continuous flow

The influence of different equivalents of succinic anhydride as well as DIPEA on *N*-amidation of L-phenylalanine methyl ester **18** was examined in a coil reactor setup. Solution A [L-phenylalanine methyl ester (12.5 mM), anisole (31.3 mM) as internal standard] was prepared in  $\text{MeCN}:\text{H}_2\text{O} = 65:35$ , whereas solution B [succinic anhydride (25–50 mM, 1–2 mol eq.), DIPEA (12.5–37.5 mM, 0.5–1.5 mol eq.)] was prepared in anhydrous MeCN to avoid hydrolysis of the anhydride reagent. The stock solutions were degassed in an ultrasonic bath and the reactor setup composed of a PEEK coil (L x O.D. x I.D. 3.0 m × 1/16 in. × 0.030 in.,  $V = 1.37$  mL) was equilibrated with the reaction solvent at ambient pressure and temperature. Stock A (flow rate 0.10 mL/min) and stock B (flow rate of 0.05 mL/min) were merged using a T-mixing element and the reaction solution was pumped through the reactor coil at a total flow rate of 0.15 mL/min ( $\tau = 9.1$  min) using high-pressure syringe pumps (VIT-FIT HP, Lambda Instruments). After certain time points ( $3\tau$  /  $3.5\tau$  /  $4\tau$ ), aliquots (120  $\mu\text{L}$ ) of the outlet flow were quenched with  $\text{MeOH}:\text{H}_3\text{PO}_4 = 55:45$  (400  $\mu\text{L}$ ) and analyzed by HPLC (Method A).

### Integrated synthesis of **16** in continuous flow

For the multistep synthesis of sacubitril precursor **16** in continuous flow, a modular setup comprising a packed-bed reactor and reactor coils was utilized. Stock A [(intermediate **13** (25 mM), phenylboronic acid (37.5 mM, 1.5 mol eq.), potassium carbonate (37.5 mM, 1.5 mol eq.) and anisole (62.5 mM) as internal standard] was prepared in  $\text{MeCN}:\text{H}_2\text{O} = 65:35$ , dissolved by ultrasonic irradiation and filtered through a 0.2  $\mu\text{m}$  membrane filter. Stock B [ $\text{HCl}$  (287.5 mM, 11.5 mol eq.)] and stock C [succinic anhydride (50 mM, 2 mol eq.), DIPEA (25 mM, 1 mol eq.)] were prepared in  $\text{MeCN}:\text{H}_2\text{O} = 65:35$  and MeCN, respectively, and degassed in an ultrasonic bath. After

equilibration of the reactor system, stock A was pumped through the packed-bed reactor (L x I.D. 50 × 4.6 mm) filled with Pd-catalyst  $\text{Ce}_{0.20}\text{Sn}_{0.79}\text{Pd}_{0.01}\text{O}_{2-\delta}$  (594 mg, containing 0.04 mmol Pd) at a flow rate of 0.05 mL/min ( $\tau = 18.4$  min) and a temperature of 125 °C using a high-pressure syringe pump (VIT-FIT HP, Lambda Instruments) for Suzuki-Miyaura cross-coupling. Then, the outlet flow was mixed with stock B, delivered by a second high-pressure syringe pump (VIT-FIT HP, Lambda Instruments), at a flow rate of 0.05 mL/min using a T-mixing element. The resulting reaction solution was introduced into the first coil reactor (PEEK coil, L x O.D. x I.D. 3.0 m × 1/16 in. × 0.030 in., V = 1.37 mL) at a total flow rate of 0.10 mL/min and a temperature of 85 °C ( $\tau = 13.7$  min) for Boc-deprotection. After the first reaction coil, a backpressure regulator (IDEX BPR Cartridge 75 psi Gold Coat) was installed to be able to heat above the boiling point (~77 °C, [44]) of the reaction solution. For removal of excess HCl, the reagent stream was then pumped through a preparative HPLC column (L x I.D. 120 × 8 mm,  $\tau = 46.9$  min) filled with the anion exchange resin Amberlyst A21 (2 g dry weight) at ambient temperature and pressure. Subsequently, the process stream was mixed with stock C, which was pumped into the setup by means of a dual-syringe infusion pump (LA-120, Landgraf) at a flow rate of 0.05 mL/min. *N*-amidation was then performed in a second coil reactor (PEEK coil, L x O.D. x I.D. 3.0 m × 1/16 in. × 0.030 in., V = 1.37 mL) at a total flow rate of 0.15 mL/min ( $\tau = 9.1$  min) to obtain targeted sacubitril precursor **16** (calculated total residence time = 90.2 min). For monitoring of the reaction progress, every 10 min an aliquot of the outlet flow (120 µL) was quenched with  $\text{MeOH}:\text{H}_3\text{PO}_4 = 75:25$  (400 µL) and analyzed by HPLC (Method B). To determine the residual levels of Pd, Ce and Sn in the outlet flow of the continuous setup for synthesis of **16** aliquots of the reaction solution were acidified with equal volumes of 20%  $\text{HNO}_3/\text{HCl}$  (1 + 1, v/v) and analyzed by ICP-MS after microwave assisted acidic digestion.

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