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Development and Optimization of Synthesis Routes for a Sacubitril and a Valsartan Precursor in Continuous Flow

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Abstract

The combination drug Entresto® contains the active pharmaceutical ingredients valsartan (angiotensin II receptor blocker) and sacubitril (neprilysin inhibitor) and is a first-line treatment for heart failure with reduced ejection fraction. With the manufacturing of both active pharmaceutical ingredients still relying on batch manufacturing processes, this thesis focuses on the development and optimization of three-step synthesis routes for advanced precursors of sacubitril and valsartan in continuous flow. Both syntheses feature a Suzuki-Miyaura cross-coupling reaction as a key reaction step, which is catalyzed by a palladium-substituted cerium-tin-oxide with the formula $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$, that is implemented in an HPLC-column as packed-bed reactor. The other two steps of the synthesis routes are *N*-acylation and methyl ester hydrolysis for the valsartan precursor as well as *N*-amidation and Boc-deprotection for the sacubitril precursor. Both three-step setups were successfully utilized to produce the respective advanced precursors, with up to steady-state yields of 88% for the valsartan precursor and 61% for the sacubitril precursor. Parts of the results were published by Hiebler *et al.*[1,2]

Kurzfassung

Das Kombinationsmedikament Entresto®, bestehend aus den beiden Wirkstoffen Valsartan (Angiotensin II Blocker) und Sacubitril (Neprilysin Inhibitor), ist ein Mittel erster Wahl für die Behandlung von Herzinsuffizienz mit reduzierter Ejektionsfraktion. Da die Herstellung beider Wirkstoffe noch auf traditionellen Batch-Produktionsmethoden beruht, liegt der Fokus dieser Arbeit auf der Entwicklung und Optimierung von kontinuierlichen Synthesewegen für die Herstellung von fortgeschrittenen Vorstufen der genannten Wirkstoffe. Beide Syntheserouten beinhalten eine Suzuki-Miyaura Kreuzkupplung, welche mit Hilfe eines Palladium-substituierten Cer-Zinn-Oxid Katalysators der Formel $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ in einer HPLC-Säule als Festbettreaktor durchgeführt wird. Die beiden anderen Syntheseschritte sind *N*-Acylierung und Methylesterhydrolyse für die Valsartanvorstufe sowie *N*-Amidierung und Boc-Entschützung für die Sacubitrilvorstufe. Die dreistufigen kontinuierlichen Herstellungsverfahren erzielten eine „steady-state“ Ausbeute von 88% für die Valsartanvorstufe beziehungsweise 61% für die Sacubitrilvorstufe. Teile der Ergebnisse wurden von Hiebler *et al.* publiziert.[1,2]

Danksagung

Ein besonderer Dank gilt meinen Betreuerinnen Prof. Heidrun Gruber-Wölfer und Katharina Hiebler, für ihre Geduld und ihre Unterstützung. Dank ihnen habe ich Forschung von ihrer besten Seite kennen gelernt, und konnte mich jeden Tag auf die Arbeit freuen.

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Und schließlich danke ich Barbara für all die Unterstützung und Aufmunterung entlang des Weges.

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1. Introduction

The combination drug Entresto® contains the two active pharmaceutical ingredients (APIs) valsartan and sacubitril (as depicted in Figure 1) and is a first-line treatment option for patients suffering from heart failure.[3] Entresto® is classified as an angiotensin receptor-neprilysin inhibitor (ARNi) and has proved to be more effective than angiotensin-converting enzyme (ACE) inhibitors in the treatment of heart failure with reduced ejection fraction (HFrEF).[4]

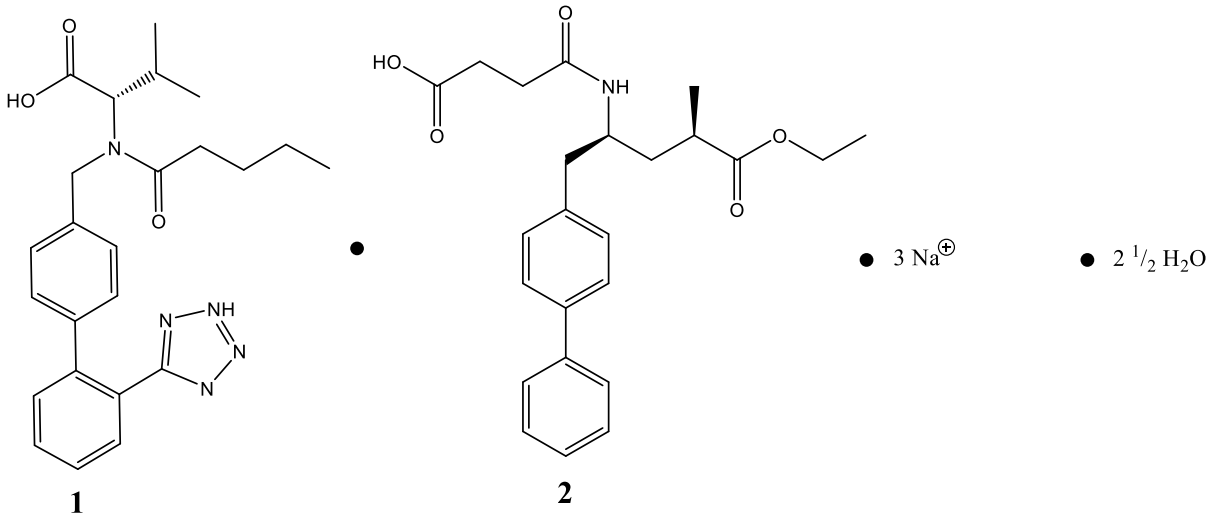


*Figure 1: Molecular structures of the salt forms of the active pharmaceutical ingredients valsartan **1** and sacubitril **2** of the combination drug Entresto®*

Entresto®, which Novartis designated as one of its "key growth drivers", had an 70% increase in sales in the first three quarters of 2019 compared to the first three quarters of 2018, amounting to sales of $1.2 billion.[5] As these numbers are expected to further rise in 2020 and the following years, the manufacturing of Entresto® and potential improvements to the manufacturing process are of high interest.

The current production of the two APIs of Entresto® relies almost exclusively on traditional batch manufacturing processes.[6,7,8] The few papers that deal with continuous flow synthesis of either valsartan or sacubitril are mostly targeting early precursors of these compounds.[9,10,11]

This thesis, as part of the ONE-FLOW project[12], has the goal to develop and optimize syntheses of late-stage precursors of both valsartan and sacubitril, utilizing continuous flow reaction cascades. Both reaction cascades feature a Suzuki-Miyaura cross-coupling step (or "Suzuki-coupling" for short), catalyzed by an in-house developed Pd-Ce-Sn-oxide catalyst with the formula $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ as a key step. The Suzuki coupling, together with the two other coupling reaction types Negishi-coupling and Mizoroki-Heck reaction, was awarded the Nobel prize in chemistry in 2010, showing the importance of "palladium-catalyzed coupling reactions as key methods in organic synthesis".[13]

2. Theoretical Background

2.1. Hypertension and Heart failure

Cardiovascular diseases (CVD) are one of the major causes of mortality worldwide, especially in developed countries. According to the newest statistics, CVD-attributed deaths increased to around 17.6 million (95% confidence interval (CI), 17.3 million to 18.1 million) in 2016, which correlates to an increase of 14.5% (95% CI, 12.1% to 17.1%) compared to 2006.[14] For 2030, a number of 23.3 million CVD-attributed deaths is estimated.[15]

Ischemic heart disease (IHD) and ischemic cerebrovascular disease (stroke) are the leading causes of death, responsible for around 85.1% of CVD-attributed deaths in 2016, with IHD alone accounting for 9.48 million deaths (9.23 million to 9.76 million).[14]

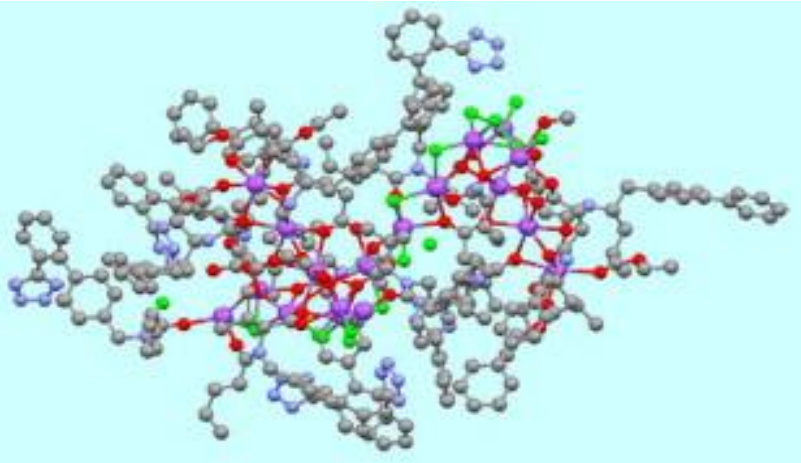
Hypertension (HT) is also a cardiovascular condition that is shown to be the biggest single contributor to both global mortality and global burden of disease. This effect is mostly due to HT leading to the before-mentioned conditions of IHD and stroke.[16] HT and IHD are also risk factors for heart failure (HF).[17]

HF is defined as a condition where the cardiac output of the heart is not sufficient to ensure required organ perfusion. While survival rates for HF have improved in recent years, the chance of dying in the next 5 years after getting diagnosed with HF is still over 50%.[18] Heart failure is becoming more prevalent not only in high-income but also low- and medium-income countries due to the fact that the lifestyle leading to obesity, diabetes and HT is also spreading in those countries.[19] In 2013, the American Heart Association estimated that the prevalence of HF in the United States will rise by 46% and consequently, the costs for medical treatment will increase from $31 to $70 billion.[20]

Treatment options for heart failure are ACE-inhibitors, ARBs (angiotensin II receptor blockers), channel inhibitors, aldosterone antagonists and beta-blockers. A relatively new approach to pharmacological treatment of HF is the combination of the neprilysin inhibitor sacubitril and the ARB valsartan.[3]

2.2. Entresto®

In 2015, Entresto® became the first approved drug containing an angiotensin receptor blocker (valsartan) and a neprilysin inhibitor (sacubitril). Its structure on a molecular level was determined by X-ray crystallographic techniques by Feng *et al.* and was shown to be trisodium-(3-[{1*S*,3*R*}-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl] propionate-[*S*]-3′-methyl-2′-[pentanoyl{2″-(tetrazol-5-ylate)-biphenyl-4′ylmethyl}amino]butyrate) hemipenta-hydrate (Scheme 1).[21]



Scheme 1: Structure of an asymmetric Entresto unit, featuring 6 sacubitril/valsartan pairs, 15 water molecules and 18 sodium ions[21]

Valsartan, an angiotensin receptor blocker with high affinity for AT_1 receptors, induces its effect by competing with angiotensin II to the binding site of the AT_1 receptor and therefore blocking the angiotensin II mediated effects on the renin-angiotensin-aldosterone system (RAAS). The physiological effects of angiotensin II include, among others, vasoconstriction, and sodium and water retention. Therefore, by blocking the effects of angiotensin II through its antagonistic activity at the AT_1 receptors, valsartan achieves a blood-pressure lowering effect.[22,23]

Sacubitril is a prodrug that, after administration, is transformed via de-ethylation into the active metabolite sacubitrilat. The mechanism of action of sacubitril (or, to be more specific, sacubitrilat) is the inhibition of the enzyme neprilysin (NEP), which is the main compound responsible for the degradation of natriuretic peptides. These peptides play an important role in maintaining cardiovascular health by stimulating natriuresis, which is the secretion of sodium through the kidneys. Through their effects they can promote vasodilation and also suppress the RAAS, which, as described above, is also responsible for vasoconstricting effects.[24]

2.2.1. Development of Entresto®

Entresto® was developed by Novartis and was approved for medical use in the US and Europe in 2015. During its development, Entresto was known under the name LCZ696.

A previous attempt to develop a combination drug of a neprilysin inhibitor and an ACE inhibitor was Omapatrilat. Omapatrilat showed to be equally effective when compared to the ACE-inhibitor Enalapril.[25] However, due to an increase in the incidence of angioedema in the Omapatrilat group, the drug was never approved for medical treatment.[26] This was most likely caused by the increase of bradykinin levels induced by the combination of neprilysin- and ACE inhibition.[27] To reduce the risk of angioedema as adverse effect during treatment, a combination drug containing a neprilysin inhibitor and an ARB was developed, as ARBs are generally considered to not increase bradykinin levels.[28,23]

Multiple studies conducted by Gu *et al.*[29] investigated the pharmacokinetics and pharmacodynamics of Entresto® for rats and beagle dogs, respectively, and examined pharmacokinetics, safety and tolerability in human volunteers.

2.2.2. Animal Studies

To evaluate the antihypertensive effect of Entresto®, a study with double transgenic rats in whom the genes for human renin and angiotensinogen were overexpressed, was conducted. The overexpression of those gens induced angiotensin II-dependent hypertension. Oral administration of Entresto in varying doses (2-60 mg/kg) showed a dose-dependent and long-lasting reduction of the mean arterial pressure of the rats.

The NEP inhibition effect of Entresto® in Sprague-Dawley rats was measured by determination of atrial natriuretic peptide immunoreactivity (ANPir) levels, with increasing levels of ANPir indicating NEP inhibition. After oral administration of Entresto (2-60 mg/kg) fast and dose-dependent rising of ANPir levels and therefore NEP inhibition was observed.

The pharmacokinetics study investigated the plasma concentrations of valsartan and sacubitril after administering it either as Entresto® or as a combined administration of valsartan and sacubitril tablets. Resulting t_{max} of valsartan for both groups showed a significantly faster rise in plasma concentration for the Entresto group (t_{max}(Entresto®) = 1.3 h, t_{max}(combined tablets) = 4.0 h). The systemic exposure (Area under curve (AUC) and maximum plasma concentration C_{max}) for valsartan was also higher for the Entresto group by a factor of three.

2.2.3. Clinical trials

2.2.3.1. Dose escalation study

Pharmacokinetic and pharmacodynamic effects of Entresto® were analyzed in the dose escalation study, a randomized, double-blind and placebo-controlled study with healthy individuals. The participants were divided into either a single dose or multiple dose cohort, with each cohort given a different dosage (single-doses ranging from 200 to 1200 mg, multiple doses ranging from 50-900 mg per dose for 14 days). Defined pharmacokinetic primary endpoints were the timespan for peak plasma concentrations (C_{max}) and half-life ($t_{1/2}$) of the prodrug sacubitril, its active form sacubitrilat, and valsartan. Primary endpoints for pharmacodynamic effects for NEP inhibition was cGMP, for angiotensin receptor blockage renin concentration, PRA and angiotensin II.[29]

Table 1: Dose escalation study: peak plasma concentrations and half-life for sacubitril, sacubitrilat and valsartan in healthy individuals

	Time until C_{max} [h] Single-dose	Time until C_{max} [h] Multi-dose	$t_{1/2}$ [h]
Sacubitril	0.5-1.1	0.6-0.9	1.1-3.6
Sacubitrilat	1.9-3.5	1.8-2.7	9.9-11.1
Valsartan	1.7-2.3	1.6-4.9	8.9-16.6

As can be seen in Table 1, both single and multiple dose administration showed same time peak concentrations (1.5-4.5 h), cGMP und RAAS biomarker concentrations also reached maximum concentrations 4 h after administration. Due to a long $t_{1/2}$ for both valsartan and sacubitril, Entresto® was considered to be suitable for a once-daily dosing. Regarding the occurrence of adverse effects, Entresto® was considered safe and well tolerated, even with dosages well above normally prescribed dose ranges (around 25-100 mg per dose).[29]

2.2.3.2. PARADIGM-HF study

In 2014, the Novartis-funded PARADIGM HF study compared Entresto® to the ACE-Inhibitor enalapril (Vasotec) in patients with HF with reduced ejection fraction.[4]

The study showed an overwhelming benefit in favor of Entresto® and was therefore stopped early after 27 months. The primary outcome of the study had been defined as either death from cardiovascular causes or hospitalization for HF. Comparing the two groups of the study showed that 21.8% of the patients that were treated with Entresto® suffered the primary outcome, compared to 26.5% of the patient group that were treated with enalapril.

Taking only death from cardiovascular causes as primary outcome gives a comparison of 13.3% of patients of the Entresto® group versus 16.5% of patients from the enalapril group.

Additionally, comparing the risk of hospitalization due to HF the study showed a reduction of risk by 21% in favor of Entresto® when comparing the two groups (12.8% in the Entresto® group versus 15.6% in the enalapril group).[4]

2.2.3.3. PARAGON-HF study

The PARAGON-HF study[30] is a prospective, multi-centered, randomized, double-blind and active-comparator trial published in September 2019. Solomon *et. al.* compared the effects of Entresto® and valsartan on HF with preserved ejection fraction (HFpEF). The participants were divided into two cohorts and were either treated with Entresto® (97 mg sacubitril and 103 mg valsartan, twice daily) or valsartan (160 mg, twice daily) for a mean period of 36 months. A lower rate of hospitalization and death from cardiovascular causes (CVC) in favor of Entresto® was observed but was not statistically significant.

Table 2: Results of the PARAGON-HF study

	Number of patients	Number of hospitalizations[a]	Incidence of death from CVC [%]
Entresto	526	690	8.5
Valsartan	557	797	8.9

[a]Some participants were hospitalized more than once

Others

Two different studies looked at Entresto® in regard to its ability to lower blood pressure in patients with hypertension. The study by Kario et. al.[31] was conducted as a randomized, double-blind study that compared the effect of Entresto® (in dosages of 100, 200 and 400 mg) and a placebo on blood pressure in Asian patients (n = 457) with HT. During an 8-week period it was demonstrated that Entresto® provided significantly greater reductions in systolic blood pressure (SBP) and diastolic blood pressure (DBP) during the day and overnight, compared to the placebo. Mean differences in SBP and DBP were as follows:

Table 3: Change of BP from the baseline to week 8 in mm Hg

	Placebo	100 mg Entresto®	200 mg Entresto®	400 mg Entresto®
SBP [mm Hg]	-4.97	-16.83	-17.54	-20.35
DBP [mm Hg]	-3.69	-11.53	-10.98	-12.45

The study conducted by Ruilope *et al.*[32] compared the efficacy of the combination drug sacubitril/valsartan (Entresto®) with valsartan on its own in a randomized, double-blind and placebo-controlled study with a duration of 8 weeks (n = 1328). Patients with mild to moderate HT were randomly assigned to an 8-week treatment of either Entresto®, valsartan, sacubitril or placebo. The dosages were varied with either 100, 200 or 400 mg of Entresto, 80, 160 or 320 mg of valsartan, 200 mg of sacubitril or placebo.

Table 4: Comparison of the change of SBP during the 8-week study

SBP [mm Hg]	100 mg Entresto® vs. 80 mg valsartan	200 mg Entresto® vs. 160 mg valsartan	400 mg Entresto® vs. 320 mg valsartan
Entresto®	-6.02	-11.00	-12.50
Valsartan	-4.72	-5.69	-6.44

Table 5: Comparison of the change of DBP during the 8-week study

DBP [mm Hg]	100 mg Entresto® vs. 80 mg valsartan	200 mg Entresto® vs. 160 mg valsartan	400 mg Entresto® vs. 320 mg valsartan
Entresto®	-3.19	-6.14	-6.85
Valsartan	-2.36	-3.17	-4.15

The findings of the study again showed significantly greater reductions in SBP (Table 4) and DBP (Table 5) for Entresto® compared to valsartan.

2.2.4. Synthesis of Entresto

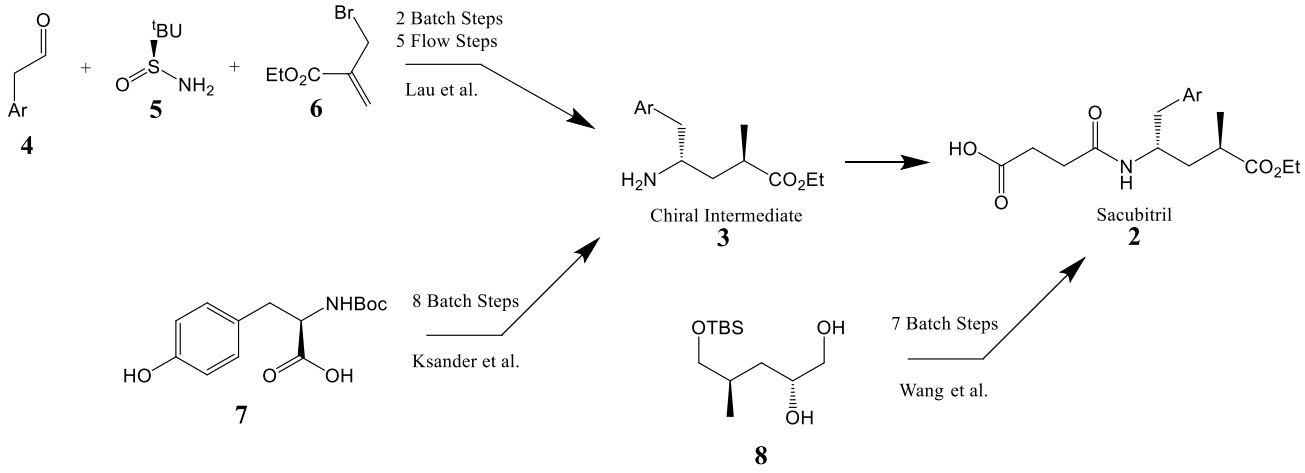
As Entresto® comprises the two APIs sacubitril and valsartan, synthesis routes for both compounds will be discussed below:

Synthesis of sacubitril

First experiments regarding the chemical synthesis of sacubitril **2** were performed in 1995 by Ksander *et al.*[6] To achieve the API synthesis, *N*-Boc-D-tyrosine **7** was used to yield sacubitril **2** after 8 reaction steps including a Wittig condensation, and subsequent Suzuki coupling, Boc-deprotection and *N*-amidation, among others (Scheme 2).

A newer synthesis method for sacubitril **2** was developed by Lau *et al.*[9] in 2015, featuring a zinc-mediated diastereoselective Reformatsky-type carbethoxyallylation and a Rh-catalyzed stereoselective hydrogenation. In their 7-step synthesis they achieved a final yield of 54%, while also using continuous flow chemistry to enhance 5 reaction steps, leaving only 2 batch-relying steps in the reaction cascade (Scheme 2).

Another novel approach for the synthesis of sacubitril **2** was developed by Wang *et al.*[7] in 2016. Their reaction cascade was characterized by a one-step Staudinger reduction/succinic amide formation, starting from a mono-protected chiron **8** and achieving the final compound sacubitril **2** after 4 steps with a yield of 40% (Scheme 2).

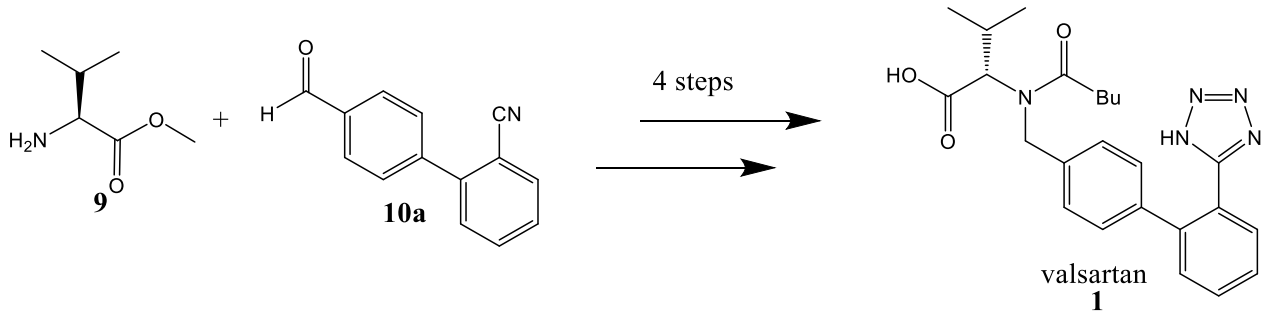


*Scheme 2: Different pathways for the synthesis of sacubitril **2***

Synthesis of valsartan **1**

The first patented synthesis route of valsartan **1** was developed by Bühlmeier *et al.*[33] in 1991, with the patent-holder at the time being Ciba-Geigy, which later merged with Sandoz AG under the name Novartis.

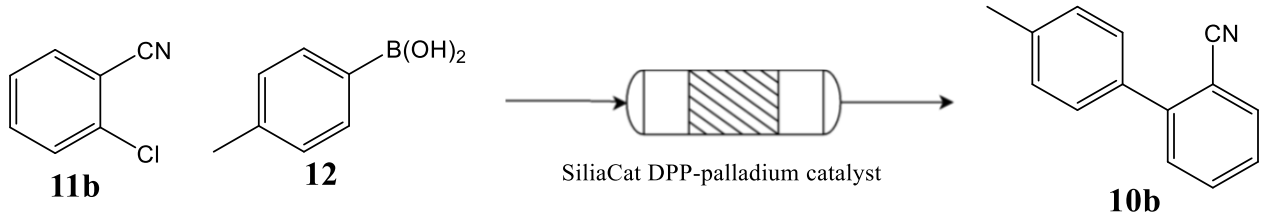
The patented reaction route employs coupling of the substrate L-valine methyl ester **9** with a biphenyl aldehyde **10a** through reductive amination, followed by acylation, triazole formation and saponification of subsequent intermediates. This reaction route still had a lot of room for improvement, considering that the overall yield was less than 10% and it required the use of toxic tin reagents during the triazole formation step (Scheme 3).



*Scheme 3: Synthesis of valsartan **1** as patented by Ciba-Geigy*[33]

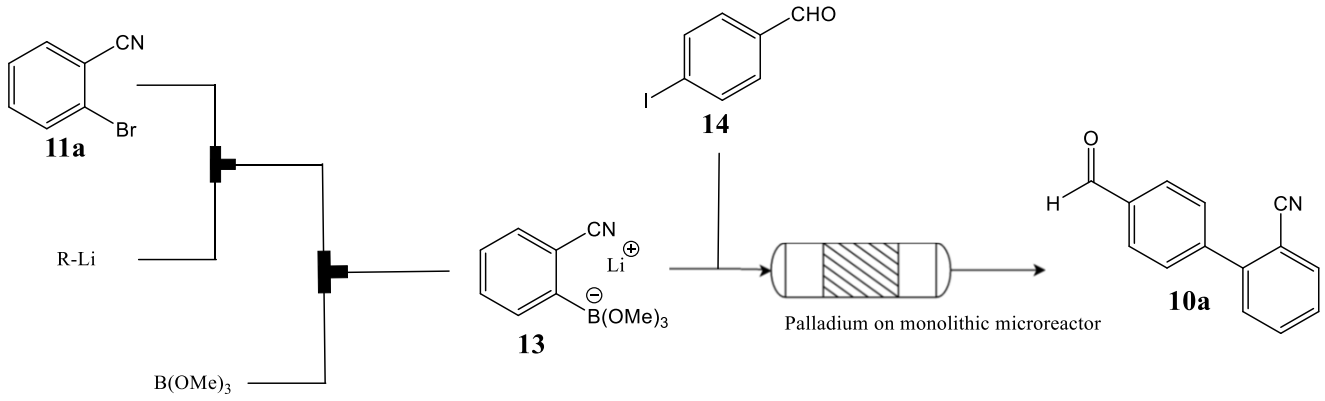
Other synthesis routes were developed to combat those problems. Recent examples for new strategies involved new key reactions, like palladium-catalyzed decarboxylative coupling of biaryles[34], ruthenium-catalyzed C-H activation[35], or palladium-catalyzed Suzuki-Miyaura cross-coupling[8].

Two more potential synthesis route improvements were presented by Pandarus *et al.*[10] in 2015 and Nagaki *et al.*[11] in 2016. Pandarus and coworkers developed an improved synthesis of valsartan precursor **10b** utilizing a Suzuki-Miyaura coupling reaction in continuous flow over sol-gel entrapped SiliaCat DPP-palladium catalyst (Scheme 4). While this gave them the possibility to apply concentrated solutions for their synthesis, the problem of a decrease in conversion over time arose. This was presumably explained by catalyst deactivation due to low stability of palladium(0) in the presence of dissolved oxygen and aryl chloride substrates.



Scheme 4: Synthesis of an early valsartan precursor in continuous flow by Pandarus et al.[10]

The reaction strategy of Nagaki *et al.*[11] utilized split-and recombine flow distributors (SRFD) that were applied in a parallel flow setup of multiple microreactors. For their work they used 5 microreactors and their SRFD to synthesize a valsartan precursor **10a** via Suzuki-Miyaura coupling (Scheme 5).



Scheme 5: Synthesis of an early valsartan precursor in continuous flow by Nagaki et al.[11]

In summary, even though these novel synthesis routes enabled improvement of conditions and overall yield of valsartan and sacubitril for preceding intermediates and the synthesis process, they still rely mostly on "traditional" batch chemistry. The advantages and imperative nature of continuous flow methods, as well as the goal of the ONE-FLOW project will be discussed in detail in the following sections.

2.3. Advantages of continuous manufacturing

At present, the manufacturing methods in the pharmaceutical industry are predominately batch processing based. Due to the fact that pharmaceutical products and intermediates need to satisfy rigorous standards, production costs in this field are generally considered to be very high. To cope with increasing cost-pressures, it is in the best interest of pharmaceutical companies to adopt new manufacturing methods that feature lower production costs with steady product quality while being also environmentally sustainable.[36] In 2005, continuous processing was declared to be one of the most relevant green engineering areas by "the roundtable", a committee founded by the American Chemical Society, the Green Chemistry Institute and multiple global pharmaceutical companies.[36] In 2012, the US Food and Drug Administration (FDA) also voiced their support for the implementation of continuous manufacturing in the pharmaceutical industry.[37]

Continuous processing provides several advantages that will be discussed below:

Economics

Continuous processing has multiple features that can lower production costs, e.g. a higher efficiency in terms of throughput per time and volume unit, which in turn leads to a reduced inventory and less energy consumption. Other factors leading to a cost reduction are the continuous material flow between processing steps by reducing the need of isolated manufacturing modules. Lastly, due to better parameter control in continuous processing, it is possible to carry out selective reaction paths without the necessity of formal protecting groups, which in turn can reduce the number of reaction and work-up steps (such as purification) needed in a reaction pathway. [36,38,39,40]

Schaber *et al.*[41] performed a case-study to compare costs between a batch and continuous process of synthesizing an API and formulating it into a tablet. Taking different scenarios into account, the most favorable continuous process scenario led to a cost reduction between 9 to 40% compared to the batch process, if both scenarios featured equal overall yield.

Quality

Increased quality in continuous manufacturing can be achieved by steady-state working conditions. Steady-state continuous manufacturing compared to dynamically run batch manufacturing features improved heat and mass transfer, allowing for operating processes at e.g. higher temperatures. Due to this feature, a more precise control of process parameters (temperature, pressure, heat and mass transfer) is possible, which also leads to higher yield and, most importantly, higher selectivity and therefore enables reduction of process variance. If

process deviations do occur, a continuous strategy entails less product rework than traditional batch manufacturing due to lower holdup times.

It is also worth mentioning that a reduced holdup time is especially advantageous if APIs or their intermediates are susceptible to degradation over time or due to specific environmental conditions. [36,38]

Safety

As previously mentioned, continuous flow reactors display accurate temperature and mass flow control. Combined with the fact that continuous flow reactors generally need less reactor volume per product output, this leads to higher safety and better risk management compared to traditional batch reactors. Specific safety enhancements are, among others, smaller hold-up volumes of potentially dangerous compounds, smaller containment, safer operation of exothermic reactions, reduced operator exposure and less transport of potentially hazardous compounds. [36,38,40]

Environmental

Waste generation in continuous processing is generally lower than in batch processing, as traditional batch processes frequently employ work-up operations such as isolation and purification. These steps generate further waste and are generally not only time-, but also resource-consuming. Reduction of work-up in continuous technology is achieved by deployment of microreactors. The rapid mixing and heat transfer that is made possible with these reactors enable the use of highly concentrated reagent streams, allowing the performance of reactions with minimal amounts of solvents. Furthermore, process optimization can be sped up and be done with minimal waste due to the short reaction times and small reactor volumes involved.[36,38,42]

2.4. ONE-FLOW Project

The "ONE-FLOW research project Catalyst Cascade Reactions in 'One-Flow' within a Compartmentalized, Green-Solvent 'Digital Synthesis Machinery' – End-to-End Green Process Design for Pharmaceuticals" is a scientific project funded by the Horizon 2020 programme and coordinated by the Eindhoven University of Technology. The vision of this project is to work on translating the vertical hierarchy of chemical multi-step syntheses into horizontal hierarchy *via* integration of compartmentalized flow reactors (Figure 2). ONE-FLOW wants to supersede current state-of-the-art multi-step flow chemistry by developing a flow cascade approach that is able to perform all needed reactions in one step. This is to be enforced by the use of Pickering emulsion/Polymersomes, switchable smart fluids and an overall optimization of reaction conditions, i.e. solvents, reagents, reaction parameters.[12]

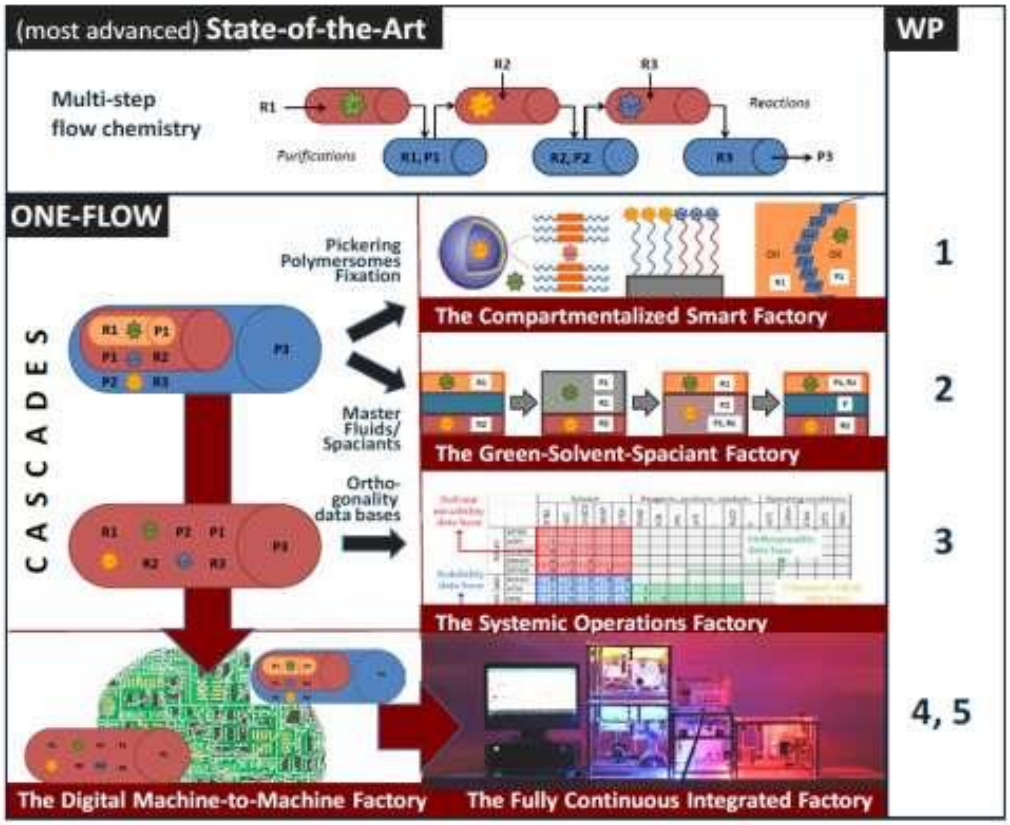


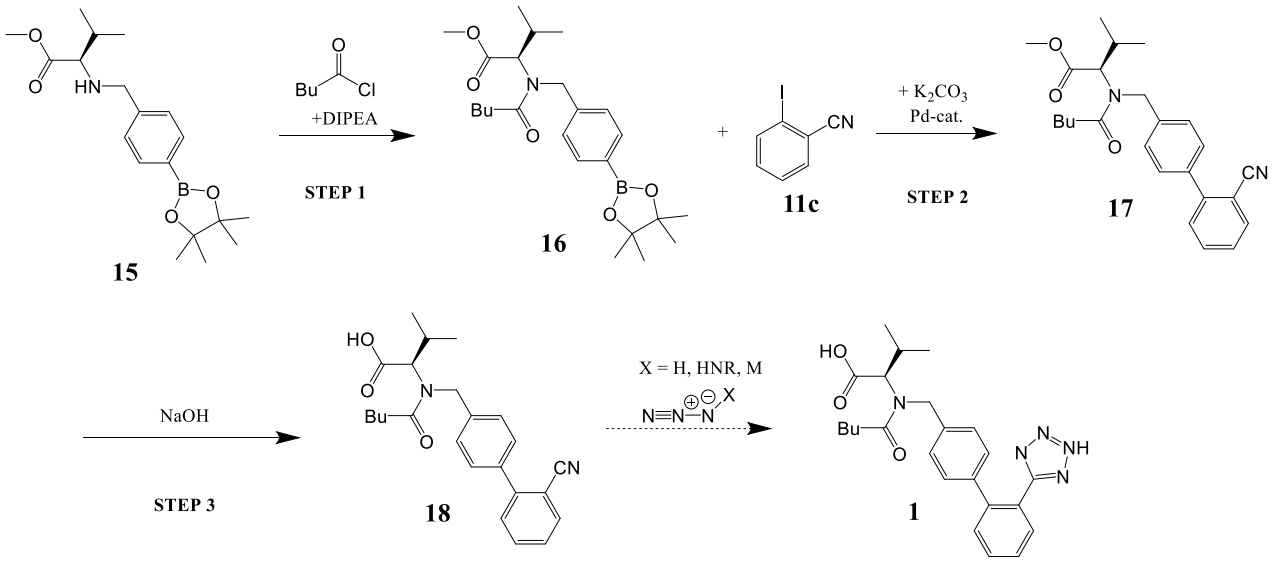
Figure 2: Flowchart of the ONE-FLOW project[12]

2.5. Research Objective

Within the ONE-FLOW project, this thesis focuses on the continuous synthesis of valsartan and sacubitril. According to the vision of this project, the goal is to replace the current batch manufacturing of both APIs with continuous flow cascades. The aim of this thesis is to optimize the batch and continuous flow synthesis of advanced precursors of both APIs using three-step reaction cascades that will be presented below.

2.5.1. Synthesis of a valsartan precursor

The synthesis of valsartan precursor **18** is achieved by a three-step reaction cascade developed by Hiebler *et al.*[1] (see Scheme 6). Step 1 is the *N*-acylation of boronic acid pinacol ester **15** with valeryl chloride to yield intermediate **16**. As second step, a Suzuki-Miyaura cross-coupling reaction with 2-halobenzonitrile **11c** yields intermediate **17**. For this reaction, a heterogenous, in-house prepared palladium catalyst (formula $Ce_{0.99-x}Sn_xPd_{0.01}O_{2-\delta}$) that is implemented in a packed-bed reactor in the so-called "Plug & Play reactor"[43] is used. In the third step, intermediate **17** is converted via methyl ester hydrolysis with NaOH into the advanced valsartan precursor **18**. The final tetrazole formation reaction of the valsartan precursor **18** with organic azides to the API valsartan **1** is not included in this reaction cascade. The goal of this thesis was to develop this three-step reaction cascade in continuous flow.[1]



Scheme 6: Envisioned reaction cascade for the synthesis of a late-stage valsartan precursor and theoretical step for synthesis of valsartan from this precursor

The flowchart below (Figure 3) shows a rough sketch of the research goals needed for the envisioned reaction cascade. The research goal of this thesis is to complete the optimization of the individual steps in continuous flow (see blue square in Figure 3) and start with the development and optimization of a sequential multi-step reaction cascade in continuous flow (see red square in Figure 3). Preliminary experiments including the study of the individual reaction steps in batch as well as sequential batch experiments were conducted by the ONE-FLOW research group at Graz University of Technology in advance.

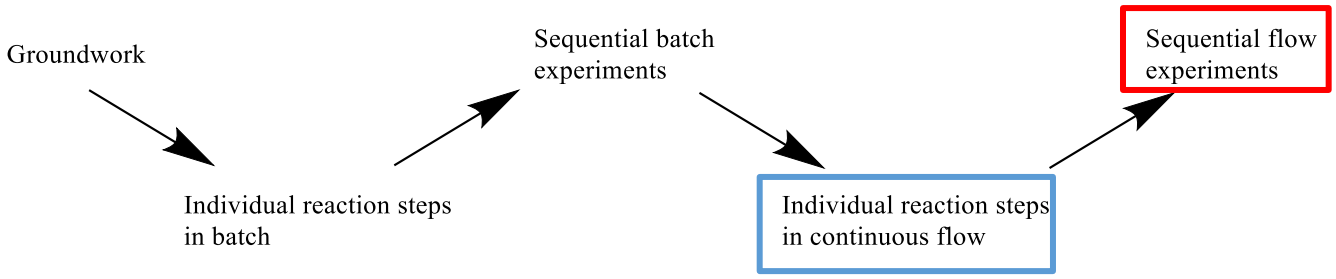
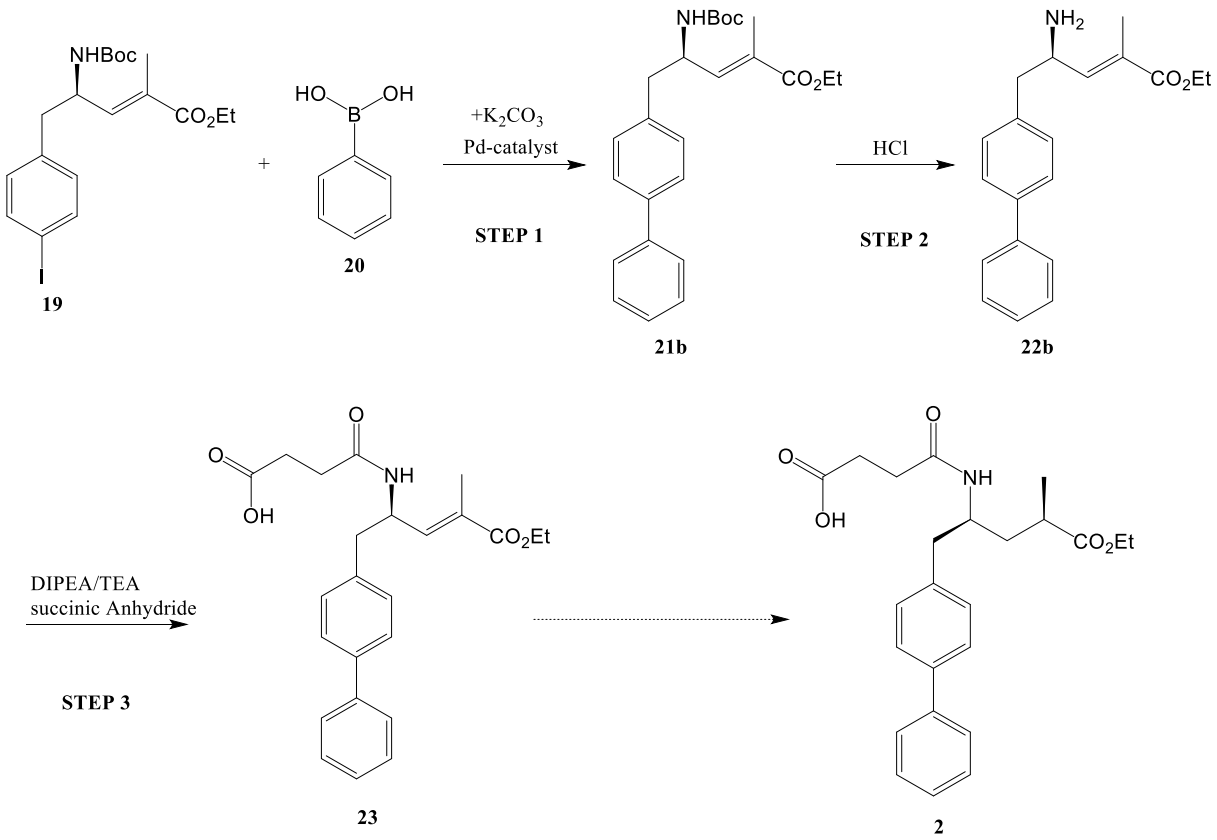


Figure 3: Flowchart for the development of a synthesis route of an advanced valsartan precursor

2.5.2. Synthesis of a sacubitril precursor

The synthesis of an advanced sacubitril precursor was achieved through the development and subsequent optimization of a three-step reaction cascade in continuous flow, which was developed by Hiebler *at al.*[2] and inspired by the method of Ksander *et al.*[6] (see Scheme 7). The first and most important step of the reaction cascade is the Suzuki-Miyaura cross-coupling of an early sacubitril precursor **19** with phenylboronic acid **20**. The Suzuki coupling is catalyzed by an in-house prepared palladium-catalyst with the formula $Ce_{0.99-x}Sn_xPd_{0.01}O_{2-\delta}$ .The second step is the removal of the Boc protection group using HCl and subsequently, *N*-amidation of the generated free amine intermediate with succinic anhydride is performed. All three steps of the reaction cascade are to be carried out in continuous flow. To synthesize the substrate **19** for step 1, a 3-step batch reaction cascade needs to be performed. More details can be found in section 3.2.1.



Scheme 7: Envisioned reaction cascade for the synthesis of an advanced sacubitril precursor and theoretical step for the synthesis of sacubitril from this precursor

The flowchart below (Figure 4) shows the rough development path for the three-step reaction cascade in continuous flow. Within this thesis, two major parts are included. The first part is the completion of the optimization of the individual reaction steps in batch followed by the development of a three-step sequential batch cascade. The second part comprises the optimization of the individual steps in continuous flow and subsequent development of a three-step reaction cascade in continuous flow. The groundwork for the first part in particular was performed by the ONE-FLOW research group of Graz University of Technology.

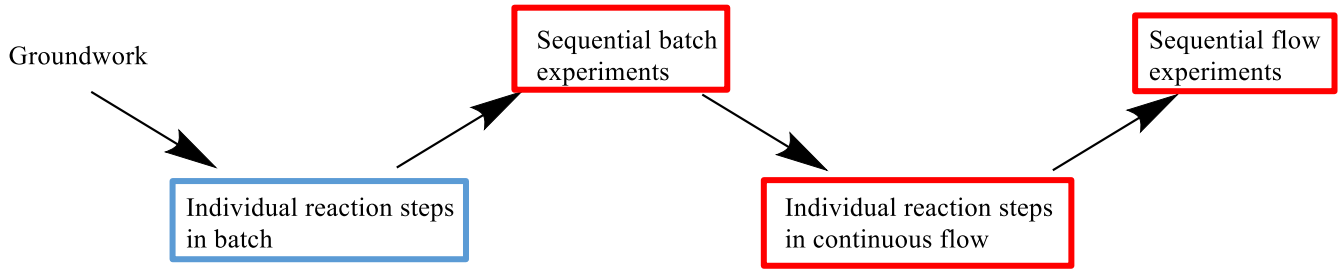


Figure 4: Flowchart for the development of a synthesis route of an advanced sacubitril precursor

3. Results and Discussion

3.1. Valsartan

3.1.1. Previous valsartan experiments

The groundwork for the synthesis of a late-stage valsartan precursor was performed by the ONE-FLOW research group of Graz University of Technology. This included the optimization of the three reaction steps in batch and most of the optimization of the individual reactions in continuous flow. The results will be briefly discussed in the following.

The catalyst for the Suzuki coupling reaction featuring the formula $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ showed the best results.[44] Consequently, the Pd-catalyst used in subsequent experiments refers to the palladium catalyst with this specific formula.

Optimization of individual batch reactions

To reduce the amounts of reagents needed for the optimization in continuous flow, the individual reaction steps were first carried out and optimized in batch. The determined optimal reaction parameters for steps 1-3 as individual reactions can be found in Table 6.[1]

Table 6: Reaction parameters for the individual reaction steps of a late-stage valsartan precursor in batch

Individual Batch reactions	Step 1 *N*-acylation	Step 2 Suzuki coupling	Step 3 Methyl ester hydrolysis
Reagents	2 mol eq. valeryl chloride 2 mol eq. DIPEA 1 mol eq. substrate **15**	0.25 mol% Pd-catalyst 1 mol eq. 2-iodobenzonitrile 1.1 mol eq. substrate **16** 1.5mol eq.K_2CO_3	1 mol eq. substrate **17** 20 mol eq. NaOH
Reaction temperature [°C]	80	80	80
Solvent system	dioxane	dioxane:H_2O = 1:1	dioxane:H_2O = 1:1
Reaction time [min]	5	10	5
Conversion [%]	98.8	88.6	81.3

Optimization of sequential steps 1 and 2 in batch

The determined optimal reaction conditions of the individual batch reactions were used for the sequential performance of steps 1 and 2 in batch. To account for the valeryl chloride added in the *N*-acylation step in sequential batch, the equivalents of K_2CO_3 in the Suzuki coupling step were increased from 1.5 mol eq.to 3.7 mol eq.

Optimization of steps 1 and 3 as individual reactions in continuous flow

Steps 1 and 3 exhibit the same flow setup, which is realized by combining a split-and-recombine (SAR) mixing unit[45] with a subsequent stainless-steel (SS) coil as a reactor. First, the two reagent streams are combined in the SAR unit, which is applicable as mixer for rapid chemical reactions[45]. Second, after the mixing process the reagent stream is pumped through the SS coil reactor, where the reactions take place. The schematics of the flow setup for steps 1 and 3 can be found in section 3.1.5.

The determined optimal reaction parameters for steps 1 and 3 as individual continuous flow reactions are presented in Table 7:[1]

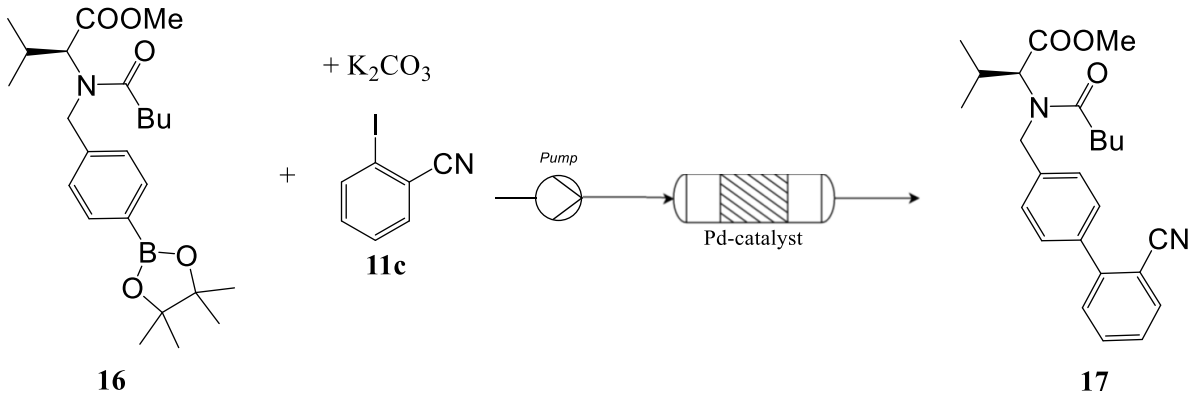
Table 7: Reaction parameters for the individual reaction steps of a late-stage valsartan precursor in continuous flow

Individual Flow reactions	Step 1 *N*-acylation	Step 3 Methyl ester hydrolysis
Reagents	2 mol eq. valeryl chloride 2 mol eq. DIPEA 1 mol eq. substrate **15**	10 mol eq. NaOH
Reaction temperature [°C]	80	80
Solvent system	dioxane	dioxane:H_2O = 1:1
Residence time τ [min]	19	19
Flow rate [mL/min]	0.1	0.1
Flow setup	split-and-recombine unit (SAR) + 3m SS coil	split-and-recombine unit (SAR) + 3m SS coil
Conversion [%]	99.9	99.5

3.1.2. Optimization of the Suzuki coupling reactions as individual steps in continuous flow

Reaction parameters for Suzuki cross-coupling (step 2) in continuous flow were adopted from the results of the individual batch experiments as described in Table 8 (dioxane:H_2O = 1:1 as solvent system, 80°C as reaction temperature and a flow rate of 0.2 mL/min).

As can be seen in Scheme 8 below, the reactor for the Suzuki coupling comprises an HPLC-column filled with the heterogenous Pd-catalyst as a packed-bed reactor, which is implemented in the so-called "Plug & Play reactor" (PPR, Figure 5). First, the reagent solution is heated to the reaction temperature in the PPR. Second, the reagent stream is pumped through the packed-bed reactor, which is installed in the PPR. The PPR is a novel reaction device which features exchangeable modules for mixing, heating/cooling, and carrying out reactions. The use of the PPR has already been successfully demonstrated for Suzuki coupling reactions utilizing Pd-catalysts in continuous flow.[43,46]



Scheme 8: Continuous flow setup for the Suzuki coupling for the synthesis of an advanced valsartan precursor

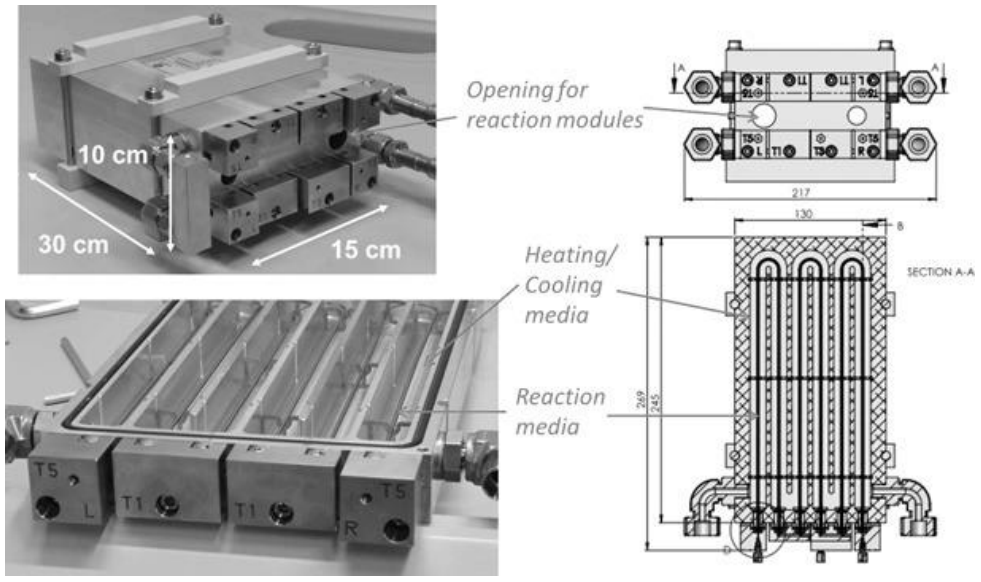


Figure 5: The Plug & Play reactor[43]

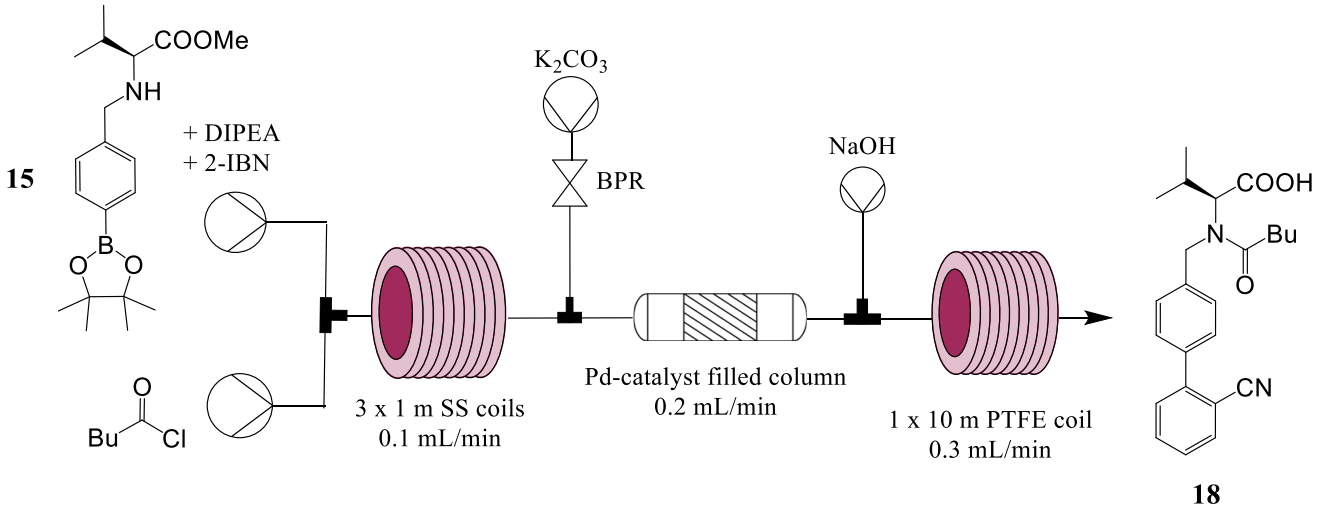
*Table 8: Reaction parameters and conversion of the Suzuki coupling reaction in continuous flow and conversion of **16***

Entry	Solvent	T [°C]	Catalyst [g]	Reagents: mol eq. K_2CO_3 mol eq. substrate **16** mol eq. 2-iodobenzonitrile	Flow rate [mL/min] Reaction time [min]	Column size: L x iD [mm]	Steady-state Conversion [%]
1	dioxane:H_2O = 1:1	80	4.29	1.5 1.1 1	0.2 150	120 x 8	86
2	dioxane:H_2O = 1:1	80	4.9	1.5 0.9 1	0.2 300	120 x 8	74

Both experiments gave good results, with the first experiment featuring a reaction time of 150 min and a steady-state conversion of 86% over a time of 80 minutes. The second experiment with the same reaction conditions but a reaction time of 300 min featured a steady-state conversion of 74% over a time of 230 minutes. The lower conversion obtained in the second case can be explained by a calculation error, due to which the valsartan precursor came to be the limiting reagent in the reaction (0.9 mol eq. instead of 1.1mol eq.), limiting the maximum obtainable conversion. Nevertheless, both experiments showed a steady-state after around 70 minutes of reaction time.

3.1.3. Optimization of the multi-step setup in continuous flow

After optimizing all three steps as individual reactions in continuous flow, a multi-step setup was developed. The three-step reaction cascade in continuous flow was performed twice, with the only change being the concentration of NaOH in Step 3. The flow setup and detailed reaction parameters can be found below (Scheme 9, Table 9).



Scheme 9: Continuous flow setup for the three-step reaction cascade for the synthesis of the late-stage valsartan precursor ***18***

The 3 m SS coil reactor initially used for the individual performance of step 3 was increased to a length of 10 m to account for the higher flowrate of 0.3 mL/min in the combined setup. The other two reaction steps were performed with the same setup as the individual reactions. In step 1, both reagent solutions were pumped at a flow rate of 0.05 mL/min, while the reagent solutions in step 2 and 3 exhibited a flow rate of 0.1 mL/min.

Table 9: Reaction parameters for the three-step continuous flow reaction cascade

	Step 1	Step 2	Step 3
Solvent composition	dioxane	dioxane:H_2O = 1:1	dioxane:H_2O = 14:16
Reactants	1.1 mol eq. substrate **15** 2.2 mol eq. DIPEA 1 mol eq. 2-IBN 2.2 mol eq. valeryl chloride	3.7 mol eq. K_2CO_3 4.3g Pd-catalyst	10 mol eq. NaOH (experiment 1) 15 mol eq. NaOH (experiment 2)
Flow setup	3 x 1 m SS coils	HPLC column 120 x 8 mm in PPR	10 m PTFE coil
Reaction temperature [°C]	80	80	80
Flow rate [mL/min]	0.1	0.2	0.3

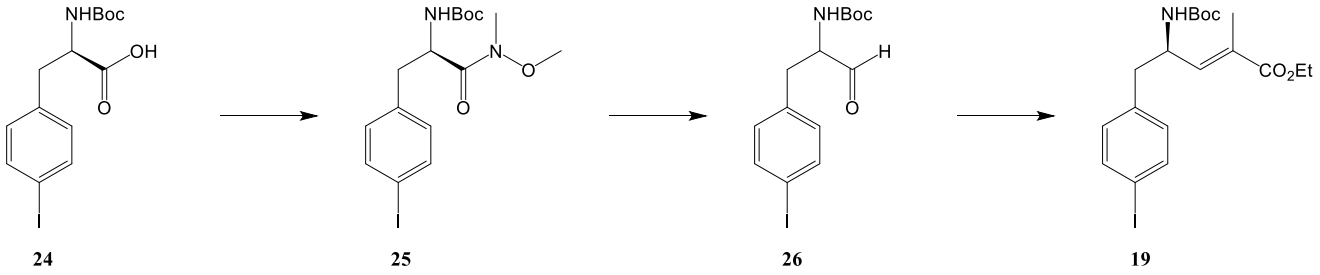
Experiment 1 had a run time of 240 min, whereas experiment 2 was continuously run for 460 min. For the purpose of examining conversion and yield, only experiment 2 will be taken into account. After reaching a steady state, a mean conversion of 99% and a yield of 88% was achieved, showing a significant improvement when compared to the sequential three-step batch experiment, where the yield was only 28%.[1]

3.2. Sacubitril

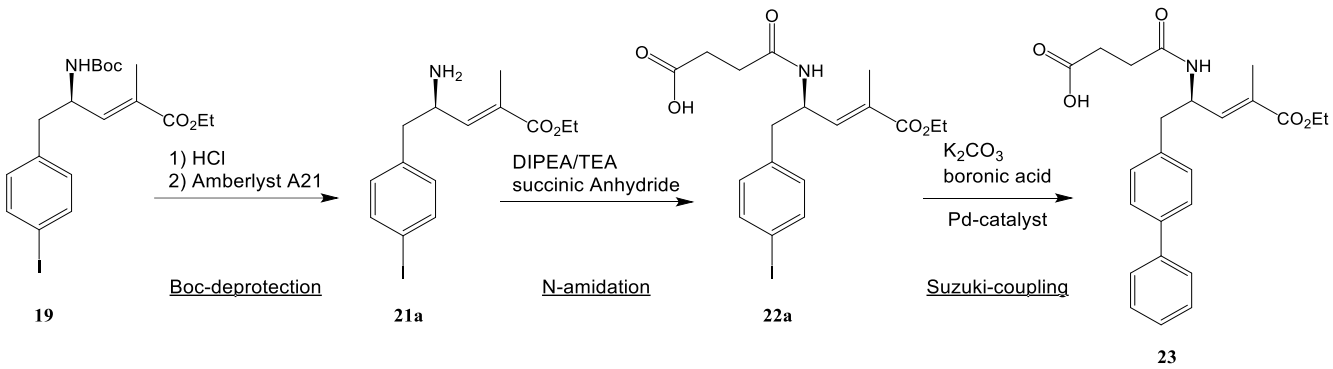
3.2.1. General Information

The overall reaction cascade for the synthesis of an advanced sacubitril precursor comprises two parts. In the first part, compound **19** is synthesized in batch in three individual steps (see Scheme 10). The development and optimization of the reaction cascade yielding compound **19** was performed by the ONE-FLOW research group of Graz University of Technology. It involves the conversion of the substrate **24** into the corresponding Weinreb amide **25**, a subsequent reduction of the amide into the aldehyde **26** and finally a Wittig reaction to give compound **19** with 55% yield. The reagents and reaction parameters of those 3 steps are described in the experimental section (sections 5.2.2.3.1-5.2.2.3.3). The reaction pathway is based on a similar procedure first reported by Ksander *et al.*[6]

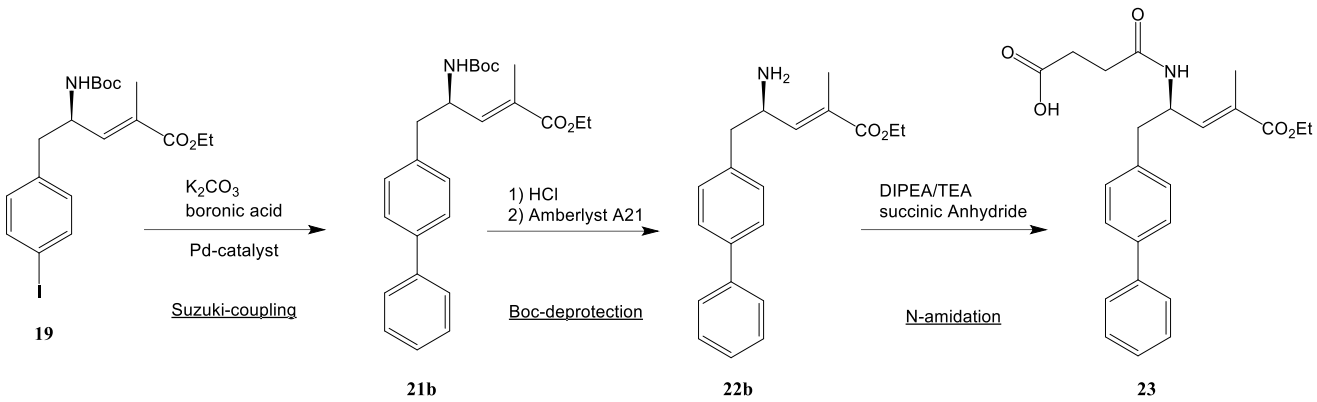
In the second part, compound **19** is then used as starting material for the continuous flow reaction cascade (Scheme 11 and Scheme 12). Regarding the continuous part, two different approaches featuring a different order of the individual reaction steps but yielding the same final product **23** were studied. The reaction sequence in approach 1 was Boc-deprotection, *N*-amidation and Suzuki coupling, for approach 2 the order was Suzuki coupling, Boc-deprotection and *N*-amidation.



*Scheme 10: Reaction scheme for the batch synthesis of compound **19***

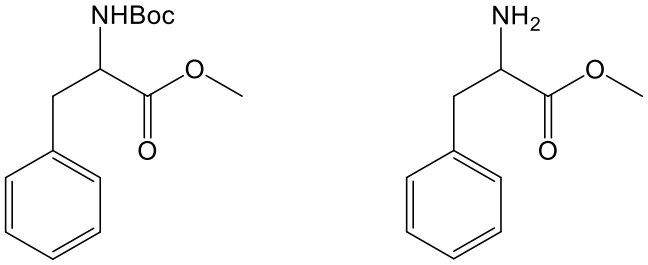


*Scheme 11: Approach 1 for the continuous synthesis of compound **23***



*Scheme 12: Approach 2 for the continuous synthesis of compound **23***

For preliminary tests, easily accessible surrogate substrates featuring similar core structures were used in a multitude of performed batch and flow experiments. To study the Boc-deprotection step Boc-L-phenylalanine methyl ester **27** was used as a surrogate substrate, whereas for examining the *N*-amidation step L-phenylalanine methyl ester **28** was utilized (see Scheme 13). The preparation of the two surrogate substrates is described in section 5.2.2.

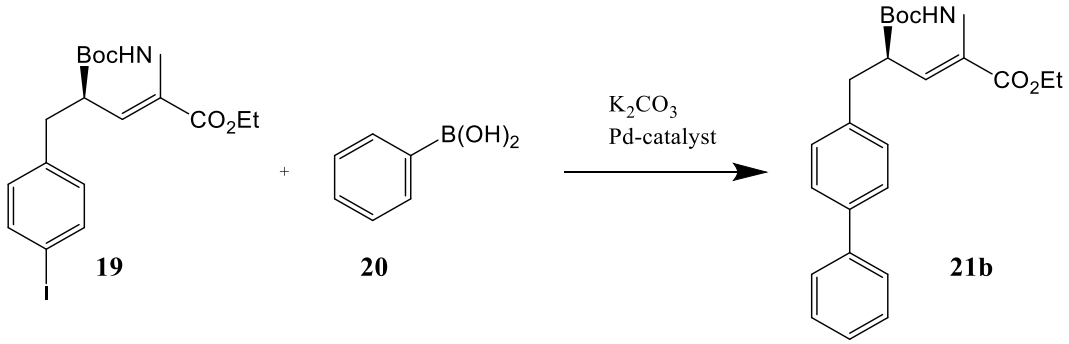


27 28

Scheme 13: Surrogate substrates used to study the Boc-deprotection as well as N-amidation

3.2.2. Optimization of Suzuki cross-coupling as individual step in batch

The Suzuki-Miyaura cross-coupling is the key step of the reaction cascade for the synthesis of sacubitril precursor **23**. In this respect, important to consider were not only the reaction conditions of the Suzuki coupling but also the order of the reaction steps in the cascade. Two different possibilities were investigated in batch. In the first approach, Suzuki coupling was the initial reaction step of the cascade. For optimization of this scenario, the Suzuki coupling was performed in a single-step batch experiment. The results are summarized in Table 10. In the second approach, the Suzuki coupling was the last step of the three-step reaction cascade. The results for optimization of this scenario are discussed in section 3.2.5.



Scheme 14: Studied Suzuki coupling in batch for the synthesis of a sacubitril precursor

Concerning the Pd-catalyst with the formula $Ce_{0.99-x}Sn_xPd_{0.01}O_{2-\delta}$, three different types with x = 0.20, 0.79, 0.99 were tested. Previous studies and preliminary experiments showed that the activity follows the order $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ > $Sn_{0.99}Pd_{0.01}O_{2-\delta}$ >$Ce_{0.79}Sn_{0.20}Pd_{0.01}O_{2-\delta}$ for the Suzuki coupling of advanced chemical intermediates. Consequently, the Pd-catalyst with the formula $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ was used for all further experiments.[1,2]

*Table 10: Tested reaction parameters for the Suzuki coupling step and obtained conversion of **19***

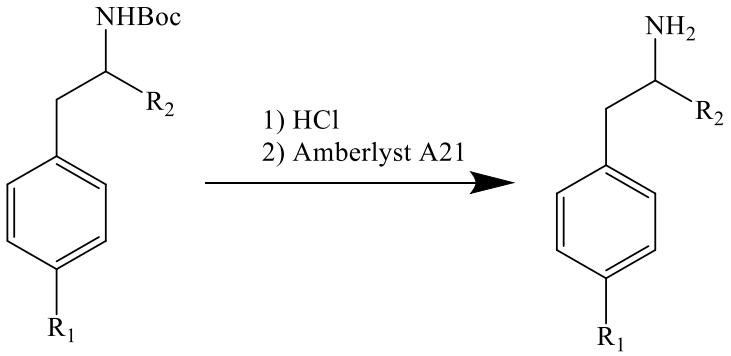
Entry	Educt	solvent	T [°C]	Cat loading [mol%]	Reagents: mol eq. K_2CO_3 mol eq. phenylboronic acid	Reaction time [min]	Conversion [%]
1	**19**	iPrOH:H_2O = 7:3	75	0.25	1.5 3	60	36
2	**19**	Cy:MeOH:H_2O = 1:1:1	60	1	3 3 +2 mol eq. TBAB	120	39
3	**19**	Cy:MeOH:H_2O = 1:1:1	60	1	3 3	120	53
4	**19**	MeCN:H_2O = 1:1	80	0.25	1.5 1.5	60	96
5	**19**	MeCN:H_2O = 7:3	75	0.25	1.5 1.5	60	97
6	**19**	iPrOH:H_2O = 7:3	75	0.25	1.5 1.5	60	99

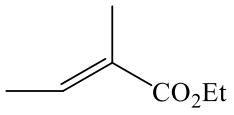
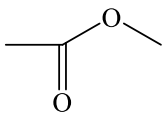
The optimal reaction conditions for the Suzuki couplings with the respective Pd-catalyst have already been investigated, using 1 mol eq. aryl halide, 1.5 mol eq. phenylboronic acid (referring to aryl halide) and 1.5 mol eq. K_2CO_3 with the solvent iPrOH:H_2O = 7:3 and a reaction temperature of 75°C.[2]

Optimizing the Suzuki coupling of **19** with **20** in batch (Scheme 14) showed that employing a reaction temperature of 75°C and using 0.25 mol% of the Pd-catalyst results in >95% conversion after a reaction time of 60 min (entry 5, Table 10). Regarding the choice of the reaction solvent, water is crucial for the reaction to occur[47]. As far as the organic cosolvent is concerned, it was selected considering its compatibility with the other reaction steps. Different solvent mixtures gave equally good results for the Suzuki coupling (entries 4-6, Table 10) and hence the solvent mixture MeCN:H_2O = 75:25 was chosen for further experiments as it showed to be best compatible with *N*-amidation and Boc-deprotection (see sections 3.2.3 and 3.2.4). During one experiment (entry 2, table 10) 2 mol eq. of tetra-*N*-butylammonium bromide (TBAB) were added as a phase transfer catalyst to investigate if this kind of catalyst could potentially speed up the reaction or increase the overall conversion. It has been reported in literature that the addition of TBAB can be potentially beneficial for Suzuki coupling reactions.[47] However, the opposite case was observed (entries 2-3, table 10) for the studied Suzuki coupling reaction.

3.2.3. Optimization of Boc-deprotection as individual step in batch

Optimization of Boc-deprotection included the choice of solvent, choice and equivalents of the used acid, reaction temperature as well as the incorporation of an acid scavenger for better integration into a multi-step setup. To reduce the consumption of compound **19**, several experiments were conducted with the corresponding surrogate substrate **27**.



	—R1	—R2
Original substrate	—I	
Surrogate substrate	—H	

Scheme 15: Studied Boc-deprotection in batch for the synthesis of a sacubitril precursor

The reaction conditions were optimized by performing multiple batch experiments using surrogate substrate **27** with structural similarity to the actual substrate. To achieve optimal reaction conditions that were transferable to continuous flow operations the following experimental parameters were varied:

- Solvent composition
- Reaction temperature (55-75°C)
- Type and equivalents of acid used (HCl in water, MeOH and iPrOH; 5-20 mol eq.)

*Table 11: Tested reaction parameters for Boc-deprotection of surrogate substrate **27** and obtained conversion of **27***

Entry	Educt	Solvent	T [°C]	Equivalents of HCl	Reaction time [min]	Conversion [%]
1	**27**	iPrOH	75	10	60	90
2	**27**	iPrOH:H_2O = 7:3	75	10	60	96
3	**27**	MeOH	55	10	30	99
4	**27**	MeOH	55	16	15	99
5	**27**	iPrOH	75	5	60	99

The findings in Table 11 indicate similar results for variations in temperature, equivalents of acid and solvent systems, with conversions ≥90%. Due to the fact that the Suzuki coupling step needs an aqueous environment, subsequent experiments employing the actual substrate **19** instead of the surrogate substrate **27** were performed using a combined water and organic solvent system.

*Table 12: Tested reaction parameters for Boc-deprotection of sacubitril precursor **19** and obtained conversion of **19***

Entry	Educt	solvent	T [°C]	Equivalents of HCl	Reaction time [min]	Conversion [%]
1	**19**	MeCN	70	5 mol eq. Amberlyst 15	60	100
2	**19**	iPrOH	70	5 mol eq. Amberlite 120	60	<1
3	**19**	iPrOH:H_2O = 7:3	75	10	60	77
4	**19**	MeCN:H_2O = 7:3	75	10	15	97
5	**19**	iPrOH:H_2O = 7:3	75	20	60	98
6	**19**	MeOH:H_2O = 7:3	60	10	15	99
7	**19**	iPrOH	75	10	30	99
8	**19**	MeOH:MeCN = 1:1	65	10	15	100
9	**19**	MeOH:MeCN = 1:1	65	10	15	100

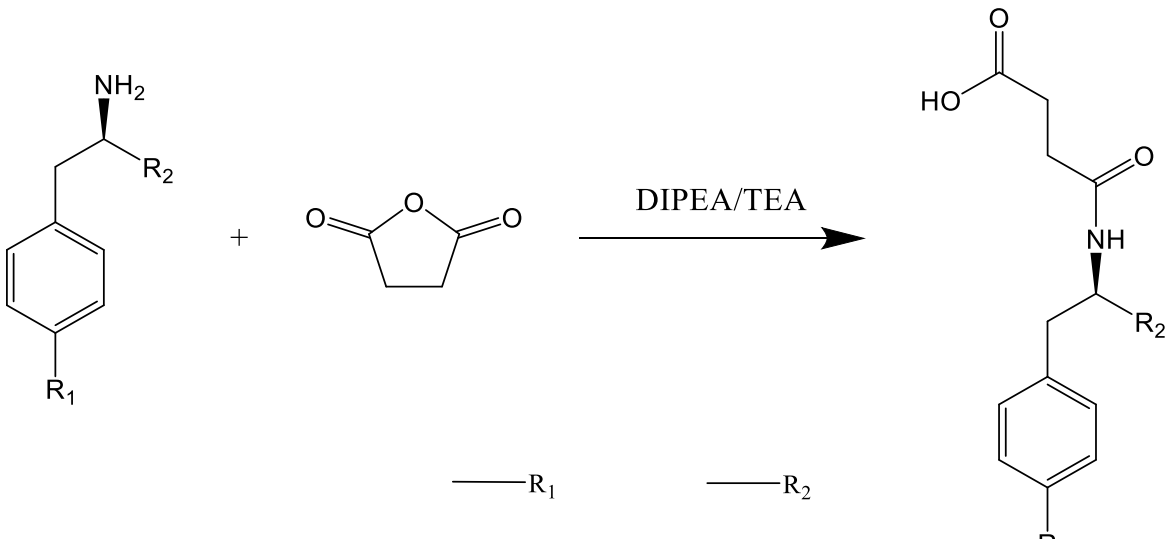
Early experiments with Amberlyst 15 and Amberlite 120 acting as a heterogeneous catalyst for Boc-deprotection, which was already applied for similar experiments[48], showed promising results but were impractical compared to a simple addition of hydrochloric acid. Reaction time needed for a conversion of >95% with Amberlyst 15 as an acid was between 30 and 60 minutes, compared to roughly 15 minutes for 10 mol eq. of HCl, whereas Amberlite 120 showed nearly no conversion after 60 minutes.

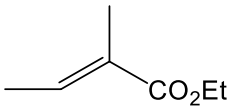
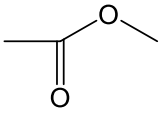
Testing multiple variations of the abovementioned reaction parameters, the best conditions with regard to conversion and applicability in the reaction cascade were MeCN:H_2O = 7:3 as solvent system, a temperature of 75°C and 10 mol eq. of concentrated HCl as acid. These conditions gave a conversion of 97% after a reaction time of 15 minutes.

After the Boc-deprotection step the reaction medium exhibited a low pH, which is not ideal for the subsequent reaction step of *N*-amidation. To remove the excess acid from the reaction solution, the basic ion exchange resin Amberlyst A21 was employed, which was equilibrated in the reaction solvent before use. Multiple experiments showed no negative impact on conversion or interaction between product and Amberlyst A21. Therefore, the use of Amberlyst A21 as an acid scavenger was integrated into the reaction procedure for the deprotection step.

3.2.4. Optimization of *N*-amidation in batch

The reaction step of *N*-amidation proved to be one of the more difficult steps to optimize in batch. One of the problems was the reaction of succinic anhydride with water, leading to a hydrolysis of succinic anhydride with a half-life of about 5 minutes in a pH neutral environment.[49] However, due to the rapid reaction speed of the amidation step[50], this turned out to be less of a problem than initially thought.



	—R1	—R2
Original substrate	—I	
Surrogate substrate	—H	

Scheme 16: Studied N-amidation in batch for the synthesis of a sacubitril precursor

Variations of *N*-amidation in batch that were conducted with surrogate substrate **28** are described in Table 13. All of the experiments noted below were carried out with 1.5 mol eq. of succinic anhydride. Solvent systems used in entries 8-13, Table 13 achieved nearly quantitative conversion. The experiments that were performed at room temperature (RT) did not show significant difference in conversion of 28 than those performed at higher temperatures.

*Table 13: Tested reaction parameters for N-amidation in batch using surrogate substrate **28** and obtained conversion of **28***

Entry	Educt	solvent	T [°C]	mol eq. of Base	Reaction time [min]	Conversion [%]
1	**28**	MeCN:MeOH = 2:1	RT	3 TEA	30	30
2	**28**	MeCN:MeOH = 2:1	RT	3 DIPEA	30	30
3	**28**	MeCN:MeOH = 2:1	RT	1.5 DIPEA	30	40
4	**28**	MeCN:MeOH = 2:1	RT	1.5 TEA	30	42
5	**28**	MeCN:MeOH = 2:1	RT	1.5 TEA	30	60
6	**28**	MeCN:MeOH = 2:1	RT	1.5 DIPEA	30	70
7	**28**	iPrOH:H_2O:MeCN = 7:3:5	RT	1.5 TEA	30	94
8	**28**	iPrOH	RT	3 TEA	30	98
9	**28**	iPrOH:H_2O = 7:3	RT	3 TEA	30	98
10	**28**	MeCN	70	3 TEA	60	98
11	**28**	iPrOH	70	3 TEA	30	99
12	**28**	iPrOH:H_2O = 7:3	70	3 TEA	30	99
13	**28**	iPrOH:H_2O = 7:3	75	1,5 TEA	30	99

After optimizing the reaction step with the surrogate substrate, the step was repeated using two different sacubitril precursors, compound **21a** as well as **22b**.

*Table 14: Tested reaction parameters for N-amidation using two different sacubitril precursors and obtained conversion of either **21a** or **22b***

Entry	Educt	Solvent	T [°C]	mol eq. of Base	Reaction time [min]	Conversion [%]
1	**21a**	MeOH:H_2O = 7:3	60	5 TEA	30	67
2	**21a**	MeOH:MeCN = 25:75	RT	1.5 TEA	30	90
3	**22b**	iPrOH:H_2O = 7:3	75	-	30	35
4	**22b**	MeCN:H_2O = 75:25	RT	1.5 DIPEA	30	96

Regarding the conversion, entries 2 and 4 in Table 14 gave the best results. As water needs to be present in the solvent system due to the performance of the Suzuki coupling step before *N*-amidation, reaction parameters given in entry 4 were determined to be optimal for targeted reaction cascade. It is also noteworthy that the reaction is completed within the first 10 minutes of reaction.

Another optimization attempt was the addition of the nucleophilic catalyst 4-dimethylaminopyridine (DMAP) to the reaction solution before the start of the reaction, as DMAP was already used as a catalyst for similar reactions.[51] However, multiple experiments showed that the addition of DMAP had either no or actually a negative effect of obtained conversion of **28** (Table 15).

*Table 15: Tested reaction parameters for N-amidation in batch using surrogate substrate **28** and DMAP and obtained conversion of **28***

Entry	Educt	Solvent	T [°C]	mol eq. of Base	Addition of DMAP	Reaction time [min]	Conversion [%]
1	**28**	iPrOH:H_2O = 7:3	RT	3 TEA	0.1 mol eq.	30	99
2	**28**	iPrOH:H_2O = 7:3	RT	3 TEA	-	30	98
3	**28**	MeCN	70	3 TEA	0.1 mol eq.	60	60
4	**28**	MeCN	70	3 TEA	-	60	98
5	**28**	THF	60	3 TEA	0.1 mol eq.	60	66
6	**28**	THF	60	3 TEA	-	60	96

3.2.5. Sequential batch experiments

After optimization of the three individual steps, their sequential performance in batch was tested. For the synthesis of target compound **23** starting from compound **19** two different reaction pathways were examined. The first one comprised Boc-deprotection (step 1), *N*-amidation (step 2) and Suzuki coupling (step 3). The second possible path involved Suzuki coupling (step 1), Boc-deprotection (step 2) and *N*-amidation (step 3).

A number of experiments were performed to identify the optimal reaction parameters to achieve maximum conversion over all three steps. Tested ranges of the different reaction parameters are given in Table 16.

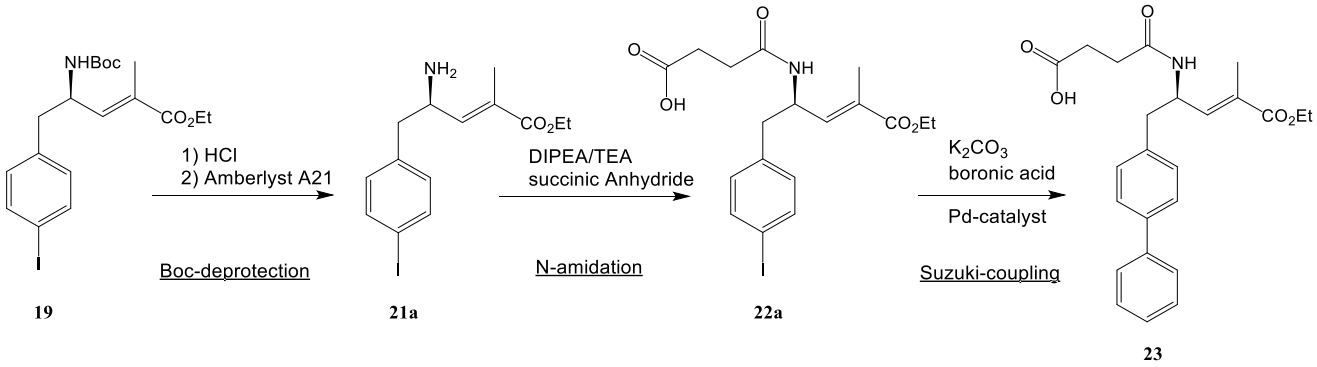
*Table 16: Reaction parameter ranges for sequential batch experiments for the synthesis of precursor **23** (approach 1)*

	Boc-deprotection	*N*-amidation	Suzuki coupling
Solvent compositions	MeCN iPrOH:H_2O = 7:3 MeOH:H_2O= 7:3 MeCN:MeOH = 1:1 MeCN:H_2O = 7:3	MeCN iPrOH:H_2O = 7:3 MeOH:H_2O = 7:3 MeOH:MeCN = 1:2 MeOH:MeCN = 25:75	MeCN:MeOH:H_2O = 50:17:33 iPrOH:H_2O = 7:3 MeOH:H2O = 7:3
Reactants	5-20 mol eq. of HCl	Amberlyst A21 1.5-3 mol eq. DIPEA 1.5-3 mol eq. TEA 1.5-3 mol eq. succinic anhydride	1.5-3 mol eq. K_2CO_3 1.5-3 mol eq. phenylboronic acid 0.25-0.5mol% Pd-catalyst
Reaction temperatures [°C]	55-75	25-70	60-80
Reaction time [min]	15-60	5-60	60-120

Regarding approach 1 for the synthesis of sacubitril precursor **23**, identified optimal reaction parameters are given in Table 17.

*Table 17: Optimal reaction parameters for the sequential batch synthesis of sacubitril precursor **23** (approach 1)*

	Step 1 Boc-deprotection	Step 2 *N*-amidation	Step 3 Suzuki coupling
Solvent composition	MeCN:MeOH = 1:1	MeCN:MeOH = 75:25	MeCN:MeOH:H_2O = 50:17:33
Reactants	5 mol eq. of HCl	Amberlyst A21 3 mol eq. DIPEA 1.5 mol eq. succinic anhydride	1.5 mol eq. K_2CO_3 1.5 mol eq. phenylboronic acid 0.5mol% Pd-catalyst
Reaction temperature	65°C	25°C	75°C
Reaction time	30 min	30 min	60 min



*Scheme 17: Sequential reaction sequence for the synthesis of sacubitril precursor **23** in batch (approach 1)*

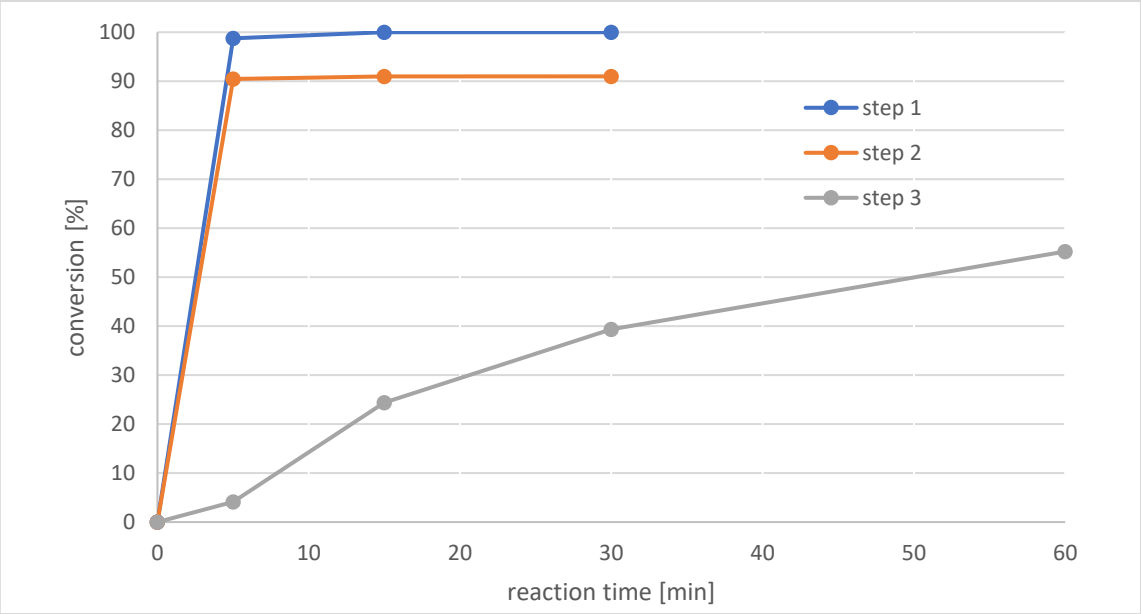


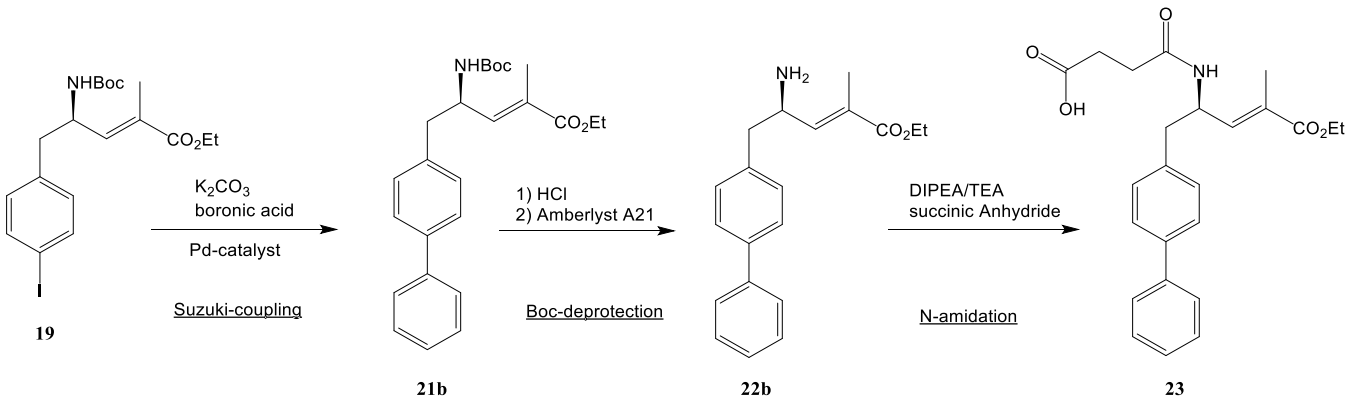
*Figure 6: Conversion of steps 1-3 obtained in the sequential batch synthesis of sacubitril precursor **23** (approach 1)*

Utilizing approach 1, an overall conversion of 48% over three steps was obtained (see Figure 6, step 1: >99% conversion, step 2: 90% conversion, step 3: 54% conversion).

Concerning approach 2 for the sequential performance of the individual steps in batch to synthesize sacubitril precursor **23**, the optimized reaction parameters of the three steps are summarized in Table 18.

*Table 18: Optimal reaction parameters for the sequential batch synthesis of sacubitril precursor **23** (approach 2)*

	Step 1 Suzuki coupling	Step 2 Boc-deprotection	Step 3 *N*-amidation
Solvent composition	MeCN:H_2O = 7:3	MeCN:H_2O = 7:3	MeCN:H_2O = 76:24
Reactants	1.5 mol eq. K_2CO_3 1.5 mol eq. phenylboronic acid 0.25 mol% Pd-catalyst	10 mol eq. HCl	Amberlyst A21 3 mol eq. DIPEA 3 mol eq. succinic anhydride
Reaction temperature	75°C	75°C	25°C
Reaction time	60 min	30 min	30 min



*Scheme 18: Sequential reaction sequence for the synthesis of sacubitril precursor **23** in batch (approach 2)*

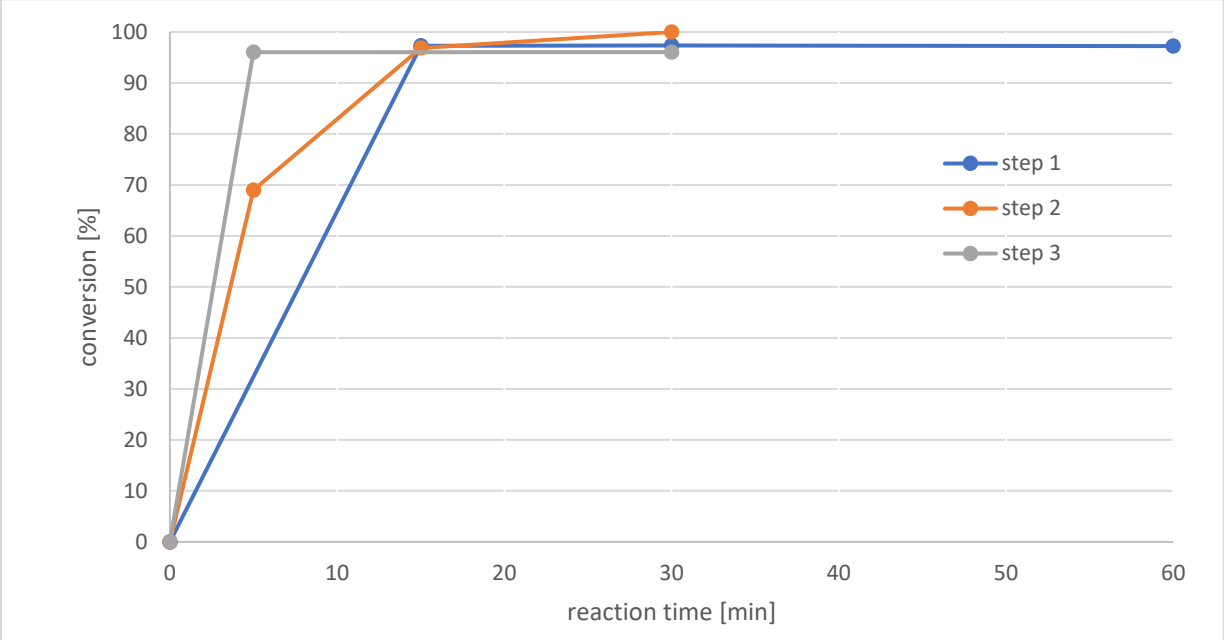


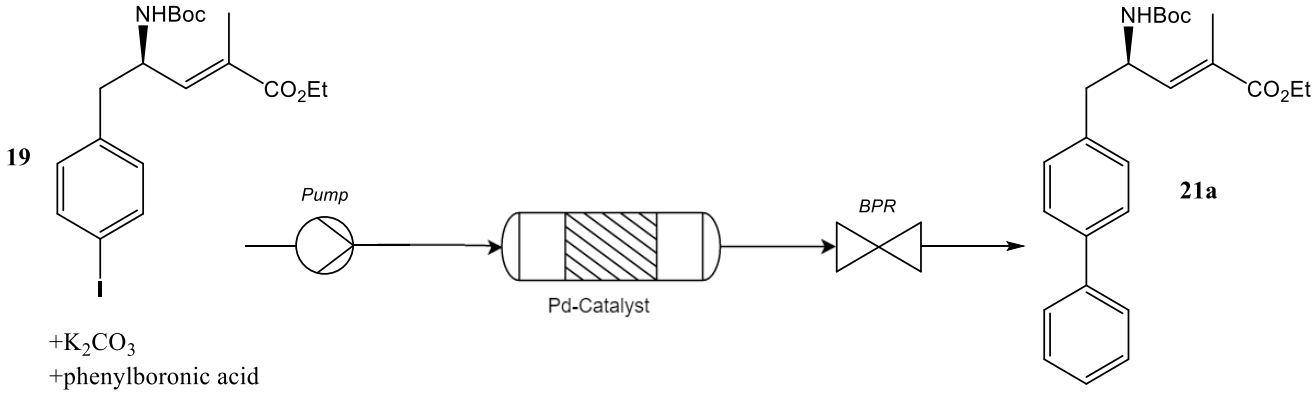
*Figure 7: Conversion of steps 1-3 obtained in the sequential batch synthesis of sacubitril precursor **23** (approach 2)*

Using approach 2, an overall conversion of 97% was achieved (see Figure 7, step 1: 96% conversion, step 2: >99% conversion, step 3: 96% conversion).

Regarding approach 1, the Boc-deprotection as well as the *N*-amidation step reach their maximum conversion after 5 minutes, whereas using approach 2 both steps take 15 minutes. However, approach 2 is superior to approach 1 in terms of the obtained overall conversion and was thus used for the targeted synthesis of sacubitril precursor **23** in continuous flow.

3.2.6. Optimization of Suzuki cross-coupling in continuous flow

The translation of the Suzuki coupling reaction from batch to continuous flow was achieved by using a packed-bed reactor setup. A reaction solution containing substrate **19** as well as the cross-coupling partner phenylboronic acid and the base K_2CO_3 was pumped through a HPLC-column filled with the heterogeneous Pd-catalyst. After the column a back-pressure regulator (75 psi) was installed to maintain a constant pressure over the whole reaction setup. Various solvent systems as well as different column sizes, flow rates and reaction temperatures were tested to optimize the reaction for maximum steady-state conversion over a longer period of time.



Scheme 19: Reaction pathway for Suzuki coupling in continuous flow

First, the use of aqueous mixtures of isopropanol as well as acetonitrile as solvent mixtures was investigated. Both media proved to be applicable for the reaction (78-84% conversion of **19**). However, considering a sequential flow cascade, MeCN:H_2O is favored (Table 19).

Apart from that, the effect of reducing the amount of Pd-catalyst on the conversion of **19** was studied. As expected, reducing it by 90% at a reaction temperature of 75°C led to a decrease of conversion of **19** (59%). However, by increasing the reaction temperature to 125°C almost full conversion of **19** was obtained using a reduced catalyst amount.

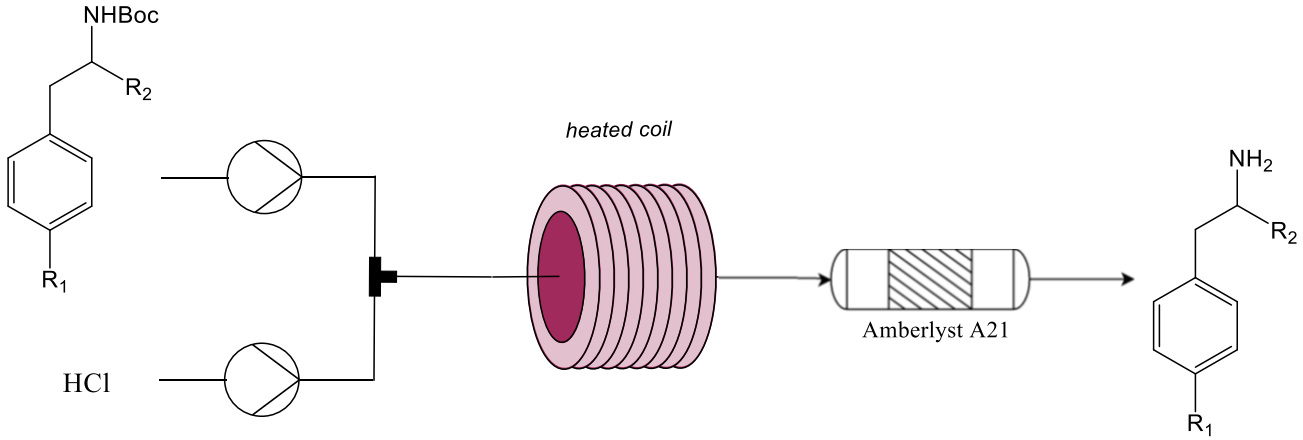
*Table 19: Tested reaction parameters for the Suzuki coupling of **19** with phenylboronic acid in continuous flow and obtained conversion of **19***

Entry	Educt	solvent	T [°C]	Catalyst [g]	Reagents: mol eq. K_2CO_3 mol eq. phenylboronic acid	Flow rate [ml/min] Reaction time [min]	Column size: Length x internal diameter [mm]	Steady-state Conversion [%]
1	**19**	iPrOH:H_2O = 7:3	75	1.26	1.5 1.5	0.1 220	40 x 8	78
2	**19**	MeCN:H_2O = 7:3	75	1.3	1.5 1.5	0.1 240	40 x 8	84
3	**19**	MeCN:H_2O = 65:35	75	0.12	1.5 1.5	0.1 200	50 x 2.1	59
4	**19**	MeCN:H_2O = 65:35	100	0.15	1.5 1.5	0.05 200	50 x 2.1	84
5	**19**	MeCN:H_2O = 65:35	125	0.15	1.5 1.5	0.05 210	50 x 2.1	96

Even though the amount of catalyst needed for a conversion of >95% is higher when using a reaction temperature of only 75°C instead of 125°C, the lower temperature reaction in combination with the bigger HPLC column was still chosen for subsequent experiments for the sequential performance of the three steps in continuous flow. This was done due to the easier assembly of the reaction setup when using temperatures below 100°C.

3.2.7. Optimization of Boc-deprotection in continuous flow

The next step was the optimization of Boc-deprotection in a continuous fashion. The setup (Scheme 20) comprises two syringe pumps, a T-mixer, a heated coil reactor and a subsequent HPLC-column filled with Amberlyst A21 to neutralize the acid in the reaction solution. The coil reactor was placed in a heated water bath.



	—R1	—R2
Original substrate	—I	CO_2Et
Surrogate substrate	—H	O, O

Scheme 20: Setup for the Boc-deprotection in continuous flow

Using the determined optimal solvent system (MeCN:H_2O with a ratio of either 7:3 or 65:35), various equivalents of hydrochloric acid (5,7.5 and 10 mol eq.), reaction temperatures (50, 60, 70°C) and the use of different flow rates (0.1, 0.15 and 0.2 mL/min) were tested to identify optimal reaction parameters. Obtained results are given in Table 20.

*Table 20: Tested reaction parameters for Boc-deprotection of surrogate substrate **27** in continuous flow and obtained conversion of **27***

Entry	Educt	solvent	T [°C]	mol eq. of HCl	Flowrate [ml/min] Reaction time [min]	Conversion [%]
1	**27**	MeCN:H_2O = 7:3	75	10	0.2 20	88[a]
2	**27**	MeCN:H_2O = 7:3	75	10	0.15 30	95[a]
3	**27**	MeCN:H_2O = 7:3	75	10	0.1 40	99[a]
4	**27**	MeCN:H_2O = 65:35	50	5	0.1 55	4[b]
5	**27**	MeCN:H_2O = 65:35	60	5	0.1 55	11[b]
6	**27**	MeCN:H_2O = 65:35	70	5	0.1 55	35[b]
7	**27**	MeCN:H_2O = 65:35	50	7.5	0.1 55	6[b]
8	**27**	MeCN:H_2O = 65:35	60	7.5	0.1 55	18[b]
9	**27**	MeCN:H_2O = 65:35	70	7.5	0.1 55	48[b]
10	**27**	MeCN:H_2O = 65:35	50	10	0.1 55	8[b]
11	**27**	MeCN:H_2O = 65:35	60	10	0.1 55	22[b]
12	**27**	MeCN:H_2O = 65:35	70	10	0.1 55	58[b]

[a]Conversion was measured once after 3τ
[b]Conversion was measured once after 4τ

Finally, two Boc-deprotection experiments using the surrogate substrate 27 were performed and monitored for a longer period of time. In both cases MeCN:H_2O = 7:3 as solvent system, 10 mol eq. of hydrochloric acid, a reaction temperature of 75°C as well as a flow rate of 0.1 mL/min was used. The mean conversions and reaction times can be seen in Table 21.

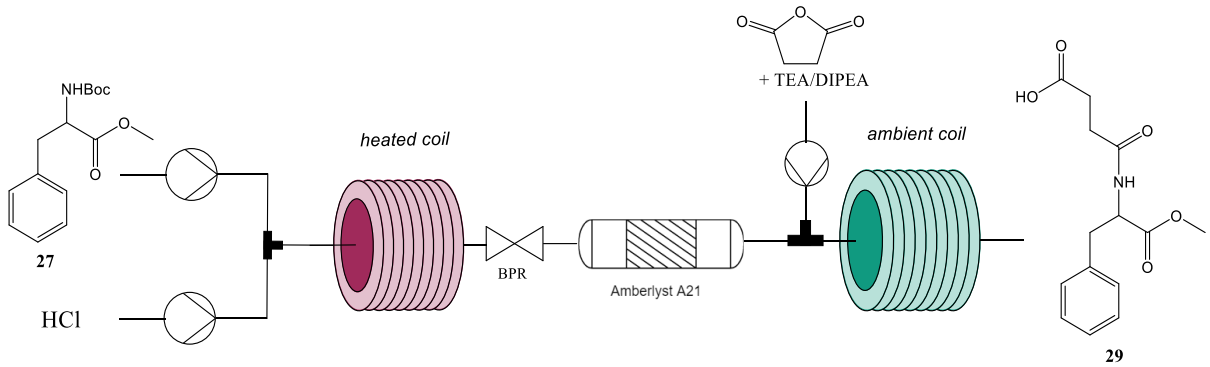
*Table 21: Reaction parameters for different iterations of Boc-deprotection in continuous flow using surrogate substrate **27***

Entry	Educt	solvent	T [°C]	mol eq. of HCl	Flow Setup details	Flowrate [ml/min] Reaction time [min]	Steady-state conversion [%]
1	**27**	MeCN:H_2O = 7:3	75	10	T-mixer + 3 m PTFE coil	0.1 300	98
2	**27**	MeCN:H_2O = 7:3	75	10	T-mixer + 3 m PTFE coil	0.1 350	97

In both experiments, steady state was reached after approximately 100 minutes and almost quantitative conversion was achieved.

3.2.8. Optimization of *N*-amidation in continuous flow

To test the last step of the reaction cascade, *N*-amidation, again a surrogate substrate was used. In order to synthesize the respective compound in situ from surrogate substrate **27**, a Boc-deprotection with a subsequent acid scavenging step was integrated into the flow setup (see Scheme 21).



*Scheme 21: Setup for N-amidation in continuous flow utilizing in situ generation of surrogate substrate **28** via Boc-deprotection of surrogate substrate **27***

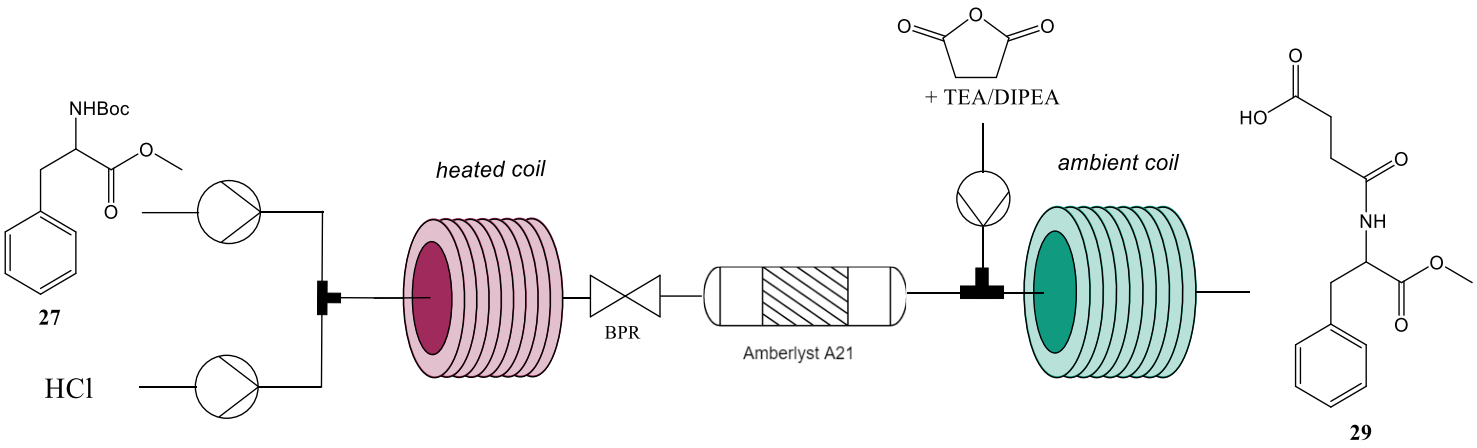
Due to the need of a 2-step sequential flow setup, optimization of *N*-amidation in continuous flow will be discussed further in point 3.2.10.

3.2.9. Optimization of multi-step setups in continuous flow

The final performance of the three reaction steps in a sequential fashion in continuous flow to synthesize sacubitril precursor **23** was done using different attempts. Before doing the full three-step synthesis, several experiments combining only two of the steps were conducted.

3.2.9.1. Combined Boc-deprotection and *N*-amidation

The two steps of Boc-deprotection and *N*-amidation were combined into a single continuous flow setup (reaction parameters can be found in Table 22 below). The setup comprised a 30 psi back-pressure regulator, a column (120 x 8mm) filled with Amberlyst A21 for neutralization and three syringe pumps to pump the respective reaction solutions. The specific *N*-amidation setup comprises a T-mixer and a 2.1 m PTFE coil as reactor.



Scheme 22: Performance of Boc-deprotection and subsequent N-amidation using a two-step continuous flow setup

Using this setup, optimization of *N*-amidation was carried out using different ratios of the MeCN:H_2O solvent system. Using the MeCN:H_2O = 8:2 and 75:25 yielded a mean conversion of 27 of 95% and 94%, respectively, was achieved over a reaction time of 300 and 350 minutes (see Table 22 below). Furthermore, the use of different equivalents of succinic anhydride and DIPEA was examined. Using 3 instead of 2 equivalents of both compounds improved the mean conversion by only 1%, meaning that 2 equivalents are sufficient for this reaction step.

*Table 22: Tested reaction parameters for N-amidation in continuous flow using surrogate substrate **28***

Entry	Educt	solvent	T [°C]	mol eq. of succinic anhydride	mol eq. of base	Flowrate [mL/min] Reaction time [min]	Mean conversion [%]
1	**28**	MeCN:H_2O = 8:2	RT	3	3 DIPEA	0.15 300	95
2	**28**	MeCN:H_2O = 75:25	RT	2	2 DIPEA	0.15 350	94

Next, the effect of varying the amount of base while using 2 mol eq. succinic anhydride was investigated. Obtained results indicate that the amount of DIPEA can be reduced to 1 mol eq. without reducing the conversion.

*Table 23: Tested reaction parameters for N-amidation in continuous flow using surrogate substrate **28** and different equivalents of base*

Entry	Educt	solvent	T [°C]	mol eq. of succinic anhydride	mol eq. of DIPEA	Flow Setup details and Flowrate	Conversion [%]
1	**28**	MeCN:MeOH = 23:7	RT	2	1	T-mixer +3 m PTFE coil 0.15 mL/min	97
					1.5		97
					2		96

The best reaction parameters for this 2-step setup can be found in Table 24.

Table 24: Best reaction parameters for combined Boc-deprotection and N-amidation using a two-step flow setup

	Step 1: Boc deprotection	Step 2: *N*-amidation
Solvent composition	MeCN:H_2O = 7:3	MeCN:H_2O = 75:25
Reactants	10 mol eq. HCl	2 mol eq. succinic anhydride 2 mol eq. DIPEA
setup	T-mixer + 3 m PTFE coil	T-mixer + 3 m PTFE coil
Reaction temperature	75°C	RT
Flow rate	0.1 mL/min	0.15 mL/min
Mean residence time	τ = 13.7 min	τ = 9.1 min
Mean conversion	97%	94%

In both reaction steps mean conversions of >90% were achieved. The mean overall yield of product **22a** was determined to be 84%, with a combined residence time of around 60 minutes.

The figure below shows the substrate conversion and the yield of step 2 during the steady state period of the experiment from minute 110 to minute 300. It can be seen that the yield of the final product steadily increases from around 80% to 98%, while the conversion stays constant at 97% until minute 270, where it drops to around 95% (t= 270 min).

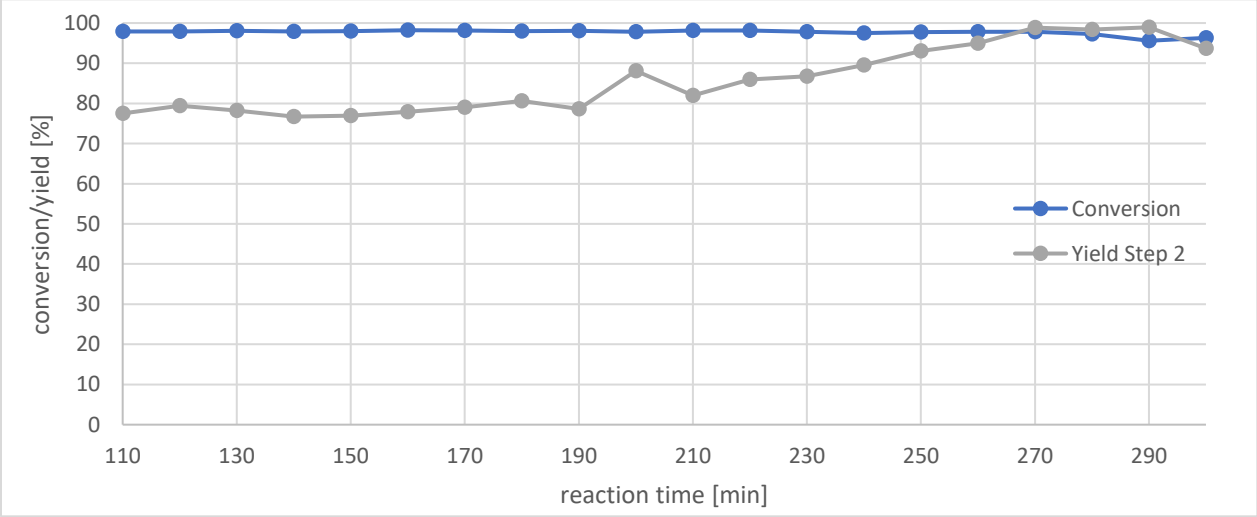
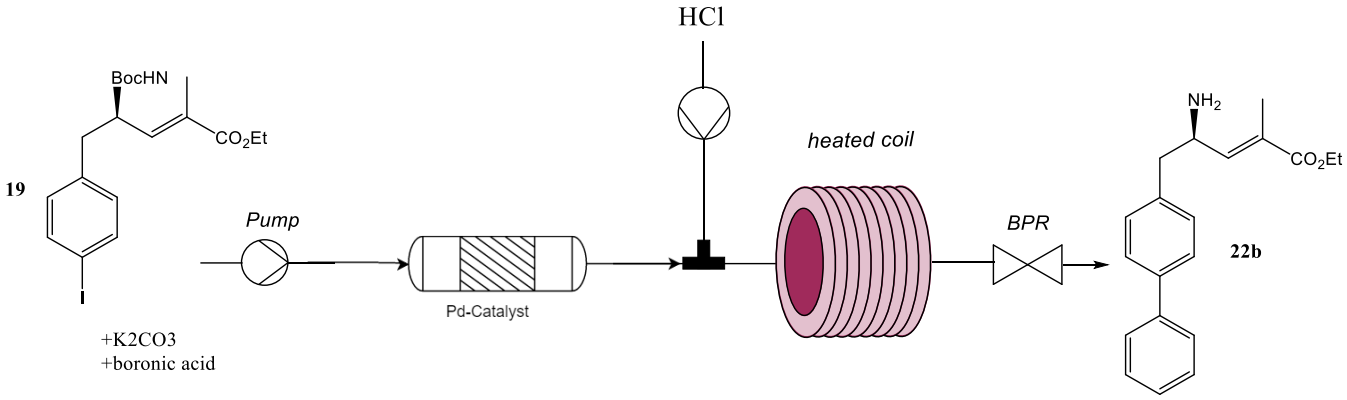


Figure 8: Substrate conversion and yield of step 2 obtained using the optimized two-step flow setup for Boc-deprotection and N-amidation

3.2.9.2. Combined Suzuki coupling and Boc-deprotection

The second combined continuous flow-experiment comprises a Suzuki coupling as first step and a subsequent Boc-deprotection. As the deprotection was the last step in this setup, no subsequent neutralization via Amberlyst A21 was carried out in this setup.



Scheme 23: Performance of Suzuki coupling and Boc-deprotection as two-step continuous flow setup

The setup involves were two high-pressure syringe pumps, a 30 psi back-pressure regulator and the two reactors. One reactor was an HPLC column filled with the heterogeneous palladium catalyst and was heated to reaction temperature using a water bath. The second reactor was a 3 m PTFE coil heated in the same water bath. The overall experiment time was 240 min.

Table 25: Best reaction parameters for combined Suzuki coupling and Boc-deprotection using a two-step flow setup

	Step 1: Suzuki coupling	Step 2: Boc-deprotection
Solvent composition	MeCN:H_2O = 7:3	MeCN:H_2O = 7:3
Reactants	1.5 mol eq. K_2CO_3 1.5 mol eq. phenylboronic acid 1.3 g Pd-cat	10 mol eq. of HCl
setup	HPLC column 40 x 8 mm	T-mixer + 3 m PTFE coil
Reaction temperature	75°C	75°C
Flow rate	0.1 mL/min	0.15 mL/min
Mean reaction time	τ = 9.7 min	τ = 9.1 min
Mean conversion	82%	58%

The mean yield of the final product was around 60%. As can be seen in the figure below, steady-state was reached after a reaction time of around 90 - 110 minutes.

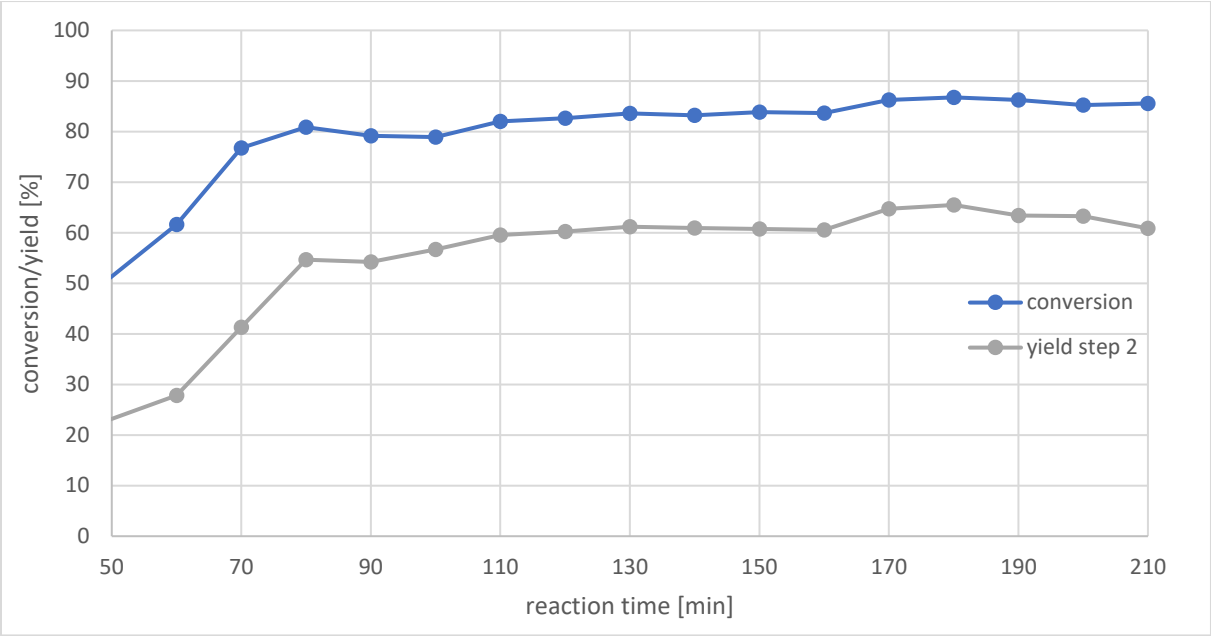
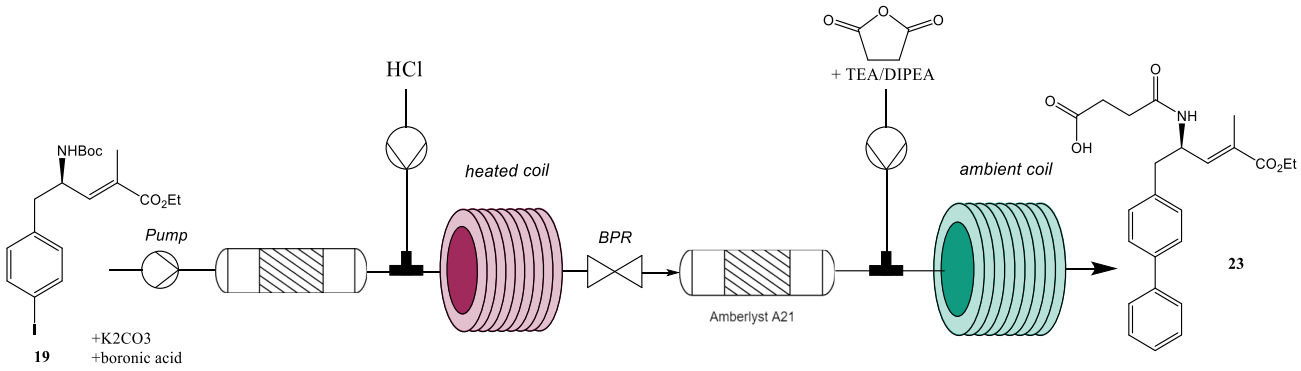


Figure 9: Substrate conversion and yield of step 2 obtained using the optimized two-step flow setup for Suzuki coupling and Boc-deprotection

Due to the fact that the conversion of substrate **19** in the Suzuki coupling step was determined to be 86% in the best case, the setup was slightly changed to ensure a higher conversion in the next multi-step experiment. It was already observed in batch experiments that the reaction speed of the Suzuki coupling was rather slow compared to the two subsequent steps. To reach a higher conversion, the flow rate of the Suzuki coupling step was reduced from 0.1 mL/min to 0.05 mL/min to increase the residence time in the packed-bed reactor. The lower conversion obtained in the Boc-deprotection step compared to the two-step setup described above (combined Boc-deprotection and *N*-amidation) can be explained by the higher flow rate used in this setup (0.15 instead of 0.1 mL/min).

3.2.9.3. Combined three-step reaction setup

Finally, the sequential performance of all three steps of the reaction cascade for the synthesis of sacubitril precursor **23** using an integrated setup in continuous flow was tested. The respective reaction cascade involved Suzuki coupling (step 1) followed by Boc-deprotection (step 2) with subsequent acid scavenging and lastly the *N*-amidation (step 3). The total residence time of the whole setup was determined to be around 100 minutes.



Scheme 24: Final setup for the performance of the three-step cascade for the synthesis of sacubitril precursor 23 in continuous flow

Table 26: Reaction parameters for the optimized three-step flow setup for the synthesis of sacubitril precursor 23

	Step 1: Suzuki coupling	Step 2: Boc-deprotection	Step 3: *N*-amidation
Solvent composition	MeCN:H_2O = 65:35	MeCN:H_2O = 65:35	MeCN:H_2O = 77:23
Reactants	1.5 mol eq. K_2CO_3 1.5 mol eq. phenylboronic acid 1.4g Pd-cat	10 mol eq. of HCl	2 mol eq. succinic anhydride 2 mol eq. DIPEA
setup	HPLC column 40 x 8 mm	T-mixer + 3 m PTFE coil + BPR + Amberlyst A21 column	T-mixer + 3 m PTFE coil
Reaction temperature [°C]	75	75	RT
Flow rate [mL/min]	0.05	0.1	0.15
Mean residence time [min]	τ = 18.4	τ = 13.7	τ = 9.1
Mean conversion	97%	87%	87%

The flow rate of step 1 was lowered to 0.05 mL/min which, as expected, improved the conversion significantly. The mean conversion of the Suzuki coupling over a reaction time of 370 minutes rose from 82% in the 240 min 2-step setup to a nearly quantitative conversion of 97%.

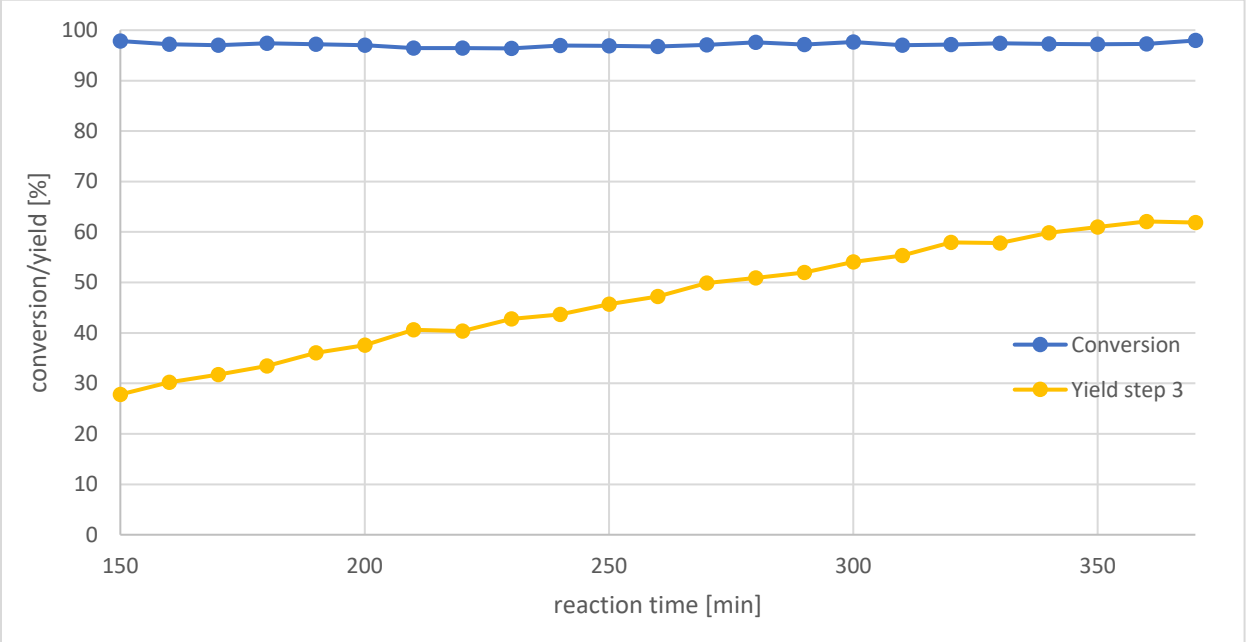


*Figure 10: Substrate conversion and yields of step 3 obtained using the optimized three-step flow setup for the synthesis of sacubitril precursor **23***

As can be seen in the figure above, the yield for the final product **23** was steadily increasing over the course of the reaction, reaching around 61% (t = 350 min), not reaching a steady-state. Conversion of substrate stayed constant at around 97% over the course of the experiment. With the yield of the final product still increasing after 350 minutes, it can be seen that the yield of 61% might still be improved if the reaction were to be run for a longer time.

4. Conclusion and Outlook

The objective of this master thesis was the development and optimization of a reaction cascade for the synthesis of an advanced valsartan as well as a sacubitril precursor in continuous flow, as a part of the ONE-FLOW research project of the Graz University of Technology.

Regarding the synthesis of a valsartan precursor, the three reaction steps *N*-acylation, Suzuki-Miyaura cross-coupling, and methyl ester hydrolysis were first investigated individually in continuous flow. Then, the three steps were sequentially performed using a multi-step flow setup comprising two coil reactors (steps 1 and 3) as well as a packed-bed reactor (step 2). Using this final setup, a steady-state yield of the target compound of 88% over the 3 steps was obtained.

Concerning the synthesis of a sacubitril precursor in continuous flow, the required starting material was successfully synthesized in batch in three steps with an overall yield of 55%. The synthesized intermediate was then employed for the synthesis of an advanced sacubitril precursor in continuous flow via the steps Suzuki Miyaura cross-coupling, Boc-deprotection and *N*-amidation. After proving the applicability of continuous setups for the performance of the individual steps, the development of a combined setup for formation of an advanced sacubitril precursor was targeted. In sequential batch reactions, the reaction order Suzuki coupling/Boc-deprotection/*N*-amidation for was found to be superior to Boc-deprotection/*N*-amidation/Suzuki coupling. Finally, an integrated setup consisting of two coil reactors (steps 2 and 3) and a packed-bed reactor catalyst (step 1) was successfully employed for the three-step synthesis of the sacubitril precursor, giving the target compound with up to 61% yield.

After finishing this master thesis, the synthesis of the advanced sacubitril precursor was further optimized by the utilization of the "Design of Experiments" (DoE) approach. DoE is an applied statistics procedure to determine the influence of different parameters on the reaction output. The full factorial DoE approach was applied for further optimization of the Boc-deprotection step and *N*-amidation step. Moreover, for the Suzuki coupling step a reaction temperature of 125 °C was utilized. Using an improved setup based on these investigations, the targeted sacubitril precursor could be successfully synthesized with a steady 81% yield for over 5 h.[2] Further optimization of the continuous flow synthesis is planned and involves the compartmentalization of the synthesis via implementation of pickering emulsion[52] into the reaction cascades.

5. Experimental Part

5.1. General Information

All chemicals and solvents that were used in the described experiments were obtained from commercial suppliers and were used as received unless stated otherwise.

Ark Pharm: 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2- dioxaborolane [97%]

ACROS organics: diisopropylethylamine [98%]

BLDpharm: Boc-4-iodo-D-phenylalanine [98%]

Carl Roth: dioxane [99.5%], NaOH [99%], triethylamine [99.5%], hydrochloric acid [37%]

ChemLab: acetonitrile [HPLC grade]

Fluorochem: L-valine methyl ester hydrochloride [99%], 2-iodobenzonitrile [98%], *O*,*N*-dimethylhydroxylamine hydrochloride [98%], 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [99%], ethyl(hydroxyimino)cyanoacetate [98%], (carbethoxyethylidene)triphenylphosphorane [94%], succinic anhydride [99%]

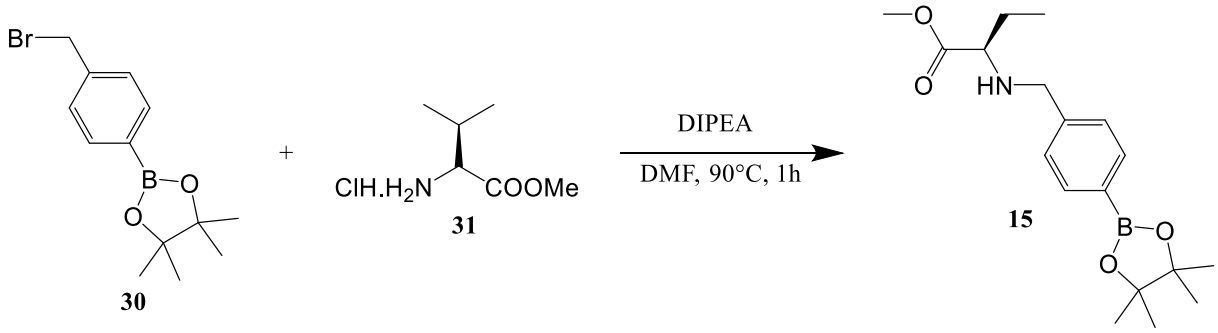
Oxchem: phenylboronic acid [95%]

Sigma Aldrich: potassium carbonate [99%], valeryl chloride [98%], anisole [99%], potassium carbonate [99%], anisole [99%]; $LiAlH_4$ [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]

Thin layer chromatography (TLC) was performed on pre-coated aluminum plates (Merck, silica gel 60, F254), spots were visualized with UV light (254 nm), ninhydrin, or potassium-permanganate stain. Flash column chromatography purifications were carried out using an MN silica gel 60 (70–230 mesh). For monitoring of reaction progress, an Agilent 1100 series HPLC system equipped with a ThermoFischer Scientific Accucore C18 reversed phase column (50 x 4.6mm, iD 2.6µm), a thermostated column compartment, an UV-visible diode array detector, a quaternary pump, an autosampler and an online degasser was utilized. Measurements of the pH were carried out using a Mettler Toledo FiveEasy™ pH bench meter equipped with a Mettler Toledo LE409 probe. If experiments or parts of experiments had to be performed under inert atmosphere, standard Schlenk-technique was employed utilizing Argon as inert gas.

5.2. Chemical synthesis in batch

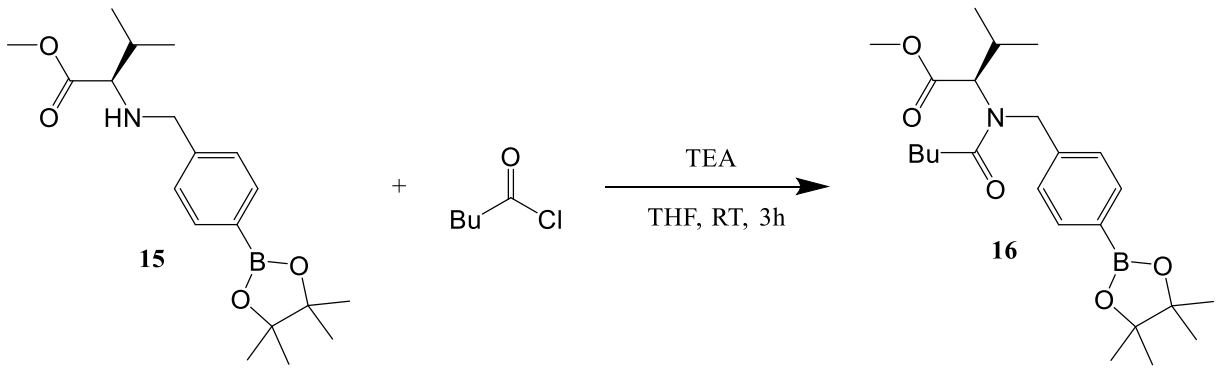
5.2.1.1. Synthesis of compound **15**



*Scheme 25: Reaction scheme for the synthesis of compound **15** in batch*

First, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl bromide **30** (3.00 g, 10.10 mmol, 1 mol eq.), L-valine methyl ester hydrochloride **31** (2.03 g, 12.12 mmol, 1.20 mol eq.) and DIPEA (2.89 g, 22.32 mmol, 2.21 mol eq.) were dissolved in DMF. The reaction mixture was heated to 90°C for 1h and subsequently cooled down to RT. After extraction and purification via liquid-liquid extraction (LLE) with toluene and water, the crude product **15** (2.55 g, 7.34 mmol) was obtained with 73% yield.

5.2.1.2. Synthesis of compound **16**

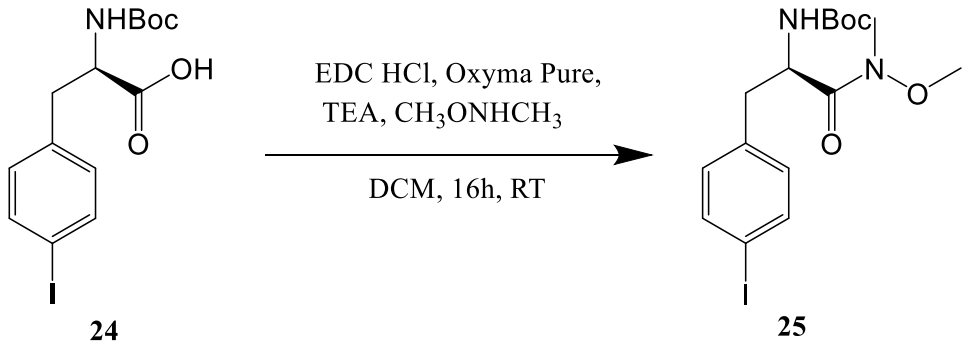


*Scheme 26: Reaction scheme for the synthesis of compound **16** in batch*

For this synthesis step, the crude product **15** (0.93 g, 2.68 mmol, 1 mol eq.) was first dissolved in THF. Then, TEA (0.276 g, 2.73 mmol, 1.02 mol eq.) was added under stirring and the reaction mixture was cooled to 0°C in an ice bath. While keeping the mixture at 0°C, valeryl chloride (0.645 g, 5.36 mmol, 2 mol eq.) was added dropwise. The ice bath was removed and the reaction mixture was stirred for 3h at RT. Finally, the solvent was removed via rotary evaporation and after purification via LLE with EtOAc and water and subsequent flash column

chromatography with n-hexane:EtOAc:toluene = 50:12:50 as an eluent, the crude product **16** (0.68 g, 1.58 mmol) was obtained with 58.6% yield.

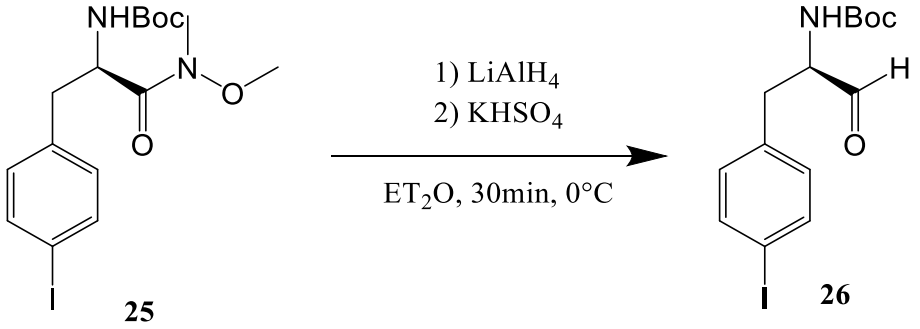
5.2.1.3. Synthesis of compound **19**



*Scheme 27: Reaction scheme for the synthesis of compound **25** in batch*

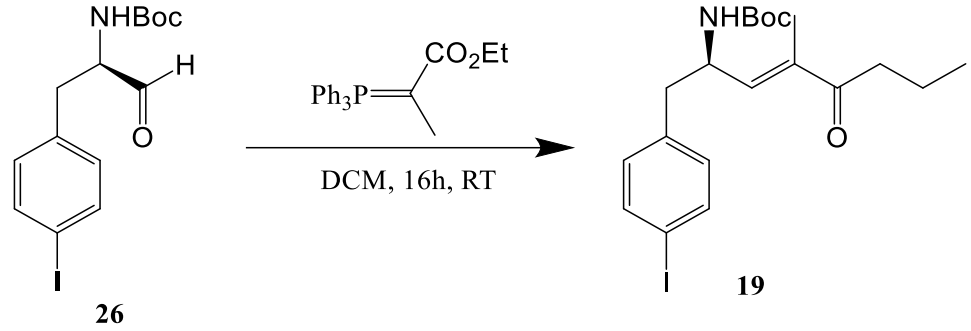
The synthesis of compound **19** was performed based on a procedure reported in literature.[6] It comprises three steps going from compound **24** to **25**, then **26** and lastly compound **19**.

The substrate Boc-4-iodo-D-phenylalanine **24** (3.23 g, 8.25 mmol, 1.15 mol eq.) together with Oxyma pure (1.75 g, 12.34 mmol, 1.72 mol eq.), TEA (0.994 g, 9.83 mmol, 1.37 mol eq.), EDC HCl (3.15 g, 16.43 mmol, 2.29 mol eq.) and *O,N*-dimethyl hydroxylamine HCl (0.7 g, 7.18 mmol, 1 mol eq.) was dissolved in dry dichloromethane (DCM, 25 mL) under Argon gas. The reaction mixture was stirred at room temperature overnight (16 h). The DCM was evaporated, and the crude product **25** (3.32 g) was purified via LLE with EtOAc and aq. $NaHCO_3$, 1M HCl and brine.



*Scheme 28: Reaction scheme for the synthesis of compound **26** in batch*

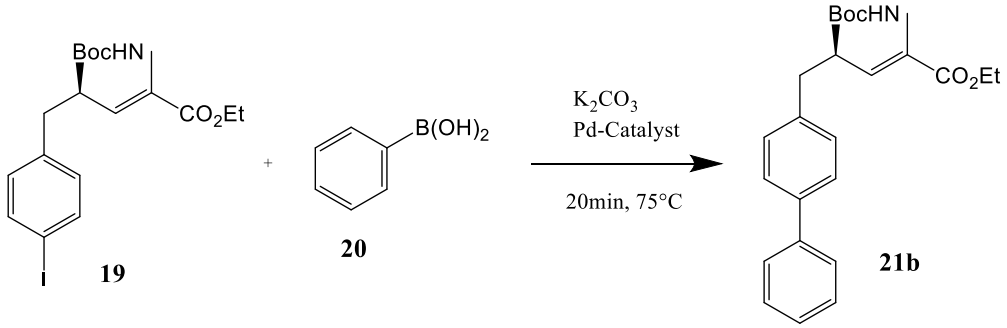
The crude product **25** (3.13 g, 7.18 mmol, 1 mol eq.) was dissolved in diethyl ether (100 mL) and cooled to 0°C. $LiAlH_4$ (0.545 g, 14.37 mmol, 2 mol eq.) was added dropwise to the cooled solution under Argon gas and stirred for 30 min while keeping the reaction solution cooled under 5°C. The reaction was quenched with 2.5 mol eq. $KHSO_4$ as a cooled 5% aq. solution and then warmed up to RT. The crude product **26** (2.96 g) was then extracted via LLE with cold 1M HCl and brine.



*Scheme 29: Reaction scheme for the synthesis of early sacubitril precursor **19** in batch*

The crude product **26** (2.7 g, 10.83 mmol, 1 mol eq.) together with (carbethoxyethylidene)triphenylphosphorane (7.84 g, 21.66 mmol, 2 mol eq.) were dissolved in DCM (110 mL) and the reaction solution was stirred overnight (16 h). The solvent was evaporated, and the crude product **19** was purified via flash column chromatography with toluene:EtOAc = 95:5 to give compound **19** (2.05 g, 5.48 mmol) with an overall yield of 57%.

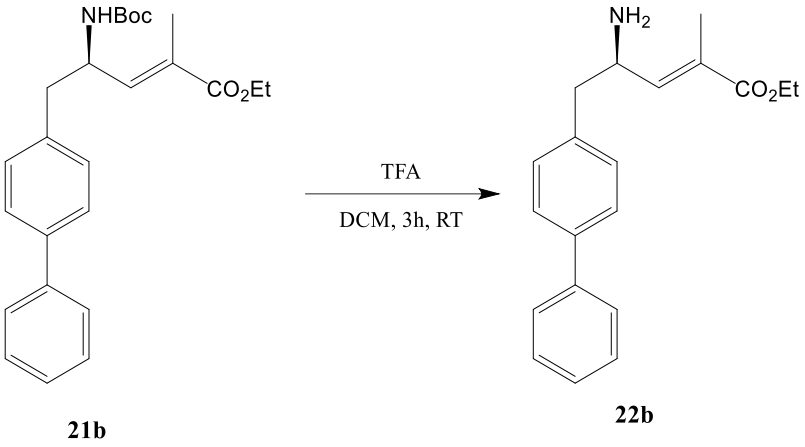
5.2.1.4. Synthesis of compound **21b**



*Scheme 30: Reaction scheme for the synthesis of compound **21b** in batch*

Compound **19** (0.3 g, 0.635 mmol, 1 mol eq.) was added to 26 mL solvent mixture of iPrOH:H_2O = 7:3 and stirred for dissolution at 75°C. Then, K_2CO_3 (0.135 g, 0.98 mmol, 1.5 mol eq.) and phenylboronic acid **20** (0.12 g, 0.984 mmol, 1.51 mol eq.) were added and stirred for dissolution. To start the reaction, Pd-catalyst $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ (0.094 g, 1mol%) was added and the reaction solution was stirred for 20 minutes at 75°C. The solvent was then evaporated at reduced pressure and the crude product **21b** was purified via flash column chromatography, using PE:EtOAc as eluent.

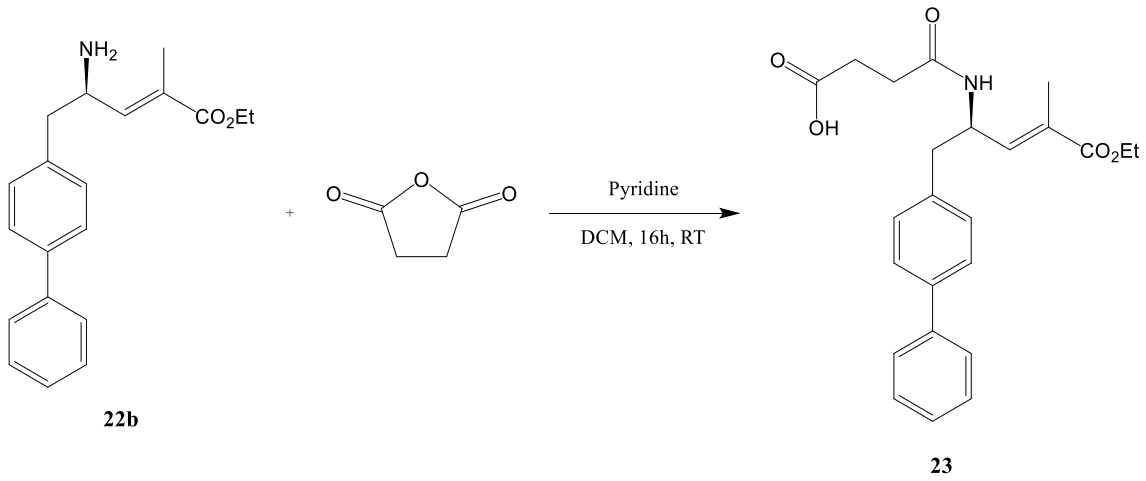
5.2.1.5. Synthesis of compound **22b**



*Scheme 31: Reaction scheme for the synthesis of compound **22b** in batch*

The crude product **21b** from point 5.2.1.4 (0.135 g, 0.331 mmol, 1 mol eq.) was dissolved in DCM (1.5 mL). Next, TFA (2.23 g, 19.6 mmol, 59.2 mol eq.) was added dropwise under stirring. The reaction mixture was then stirred for 3 h at RT. The solvent was evaporated, and the crude product was purified via LLE with EtOAc and Na_2CO_3 and brine to give the purified compound **22b** (0.095 g, 0.307 mmol) with 92% yield.

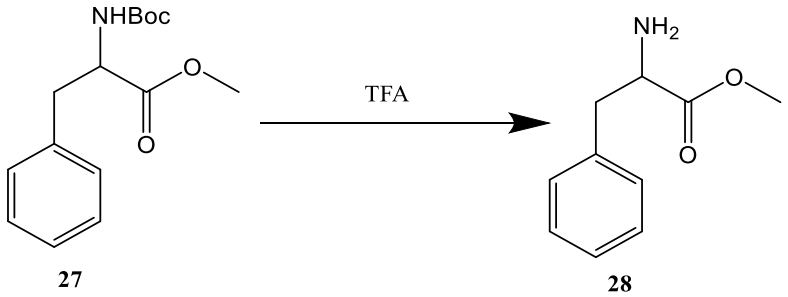
5.2.1.6. Synthesis of compound **23**



*Scheme 32: Reaction scheme for the synthesis of compound **23** in batch*

The crude product **22b** from point 5.2.1.5 (0.095g, 0.307 mmol, 1 mol eq.) was dissolved in 3 mL of a solvent mixture of DCM:pyridine = 1:1. Then, succinic anhydride (0.0461 g, 0.46 mmol, 1.5 mol eq.) were added and the reaction mixture was stirred overnight (16 h) at RT. The crude product was washed via LLE with EtOAc and 1M HCl and sat. aq. NaCl and was subsequently purified via flash column chromatography with first EtOAc and then EtOAc + 0,5% acetic acid as eluents, to give compound **23** (0.085 g, 0.208 mmol) with 68% yield.

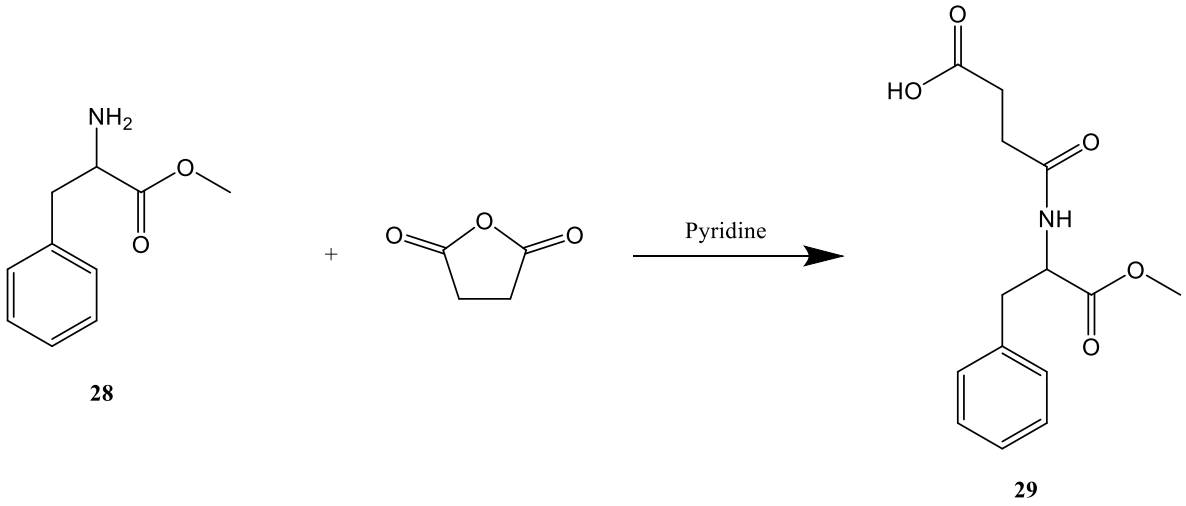
5.2.1.7. Synthesis of compound **28**



*Scheme 33: Reaction scheme for the synthesis of surrogate substrate **28** in batch*

Boc-L-phenylalanine methyl ester **27** (1.05 g, 3.75 mmol, 1 mol eq.) was dissolved in DCM:TFA = 4:1 (25 mL) and the reaction mixture was stirred at RT for 2 h. The solvent was then removed via rotary evaporation and the crude product was purified via LLE using EtOAc, aq. Na_2CO_3 and brine to give compound **28** (0.56 g, 3.11 mmol) with 83% yield.

5.2.1.8. Synthesis of compound **29**



*Scheme 34: Reaction scheme for the synthesis of compound **29** in batch*

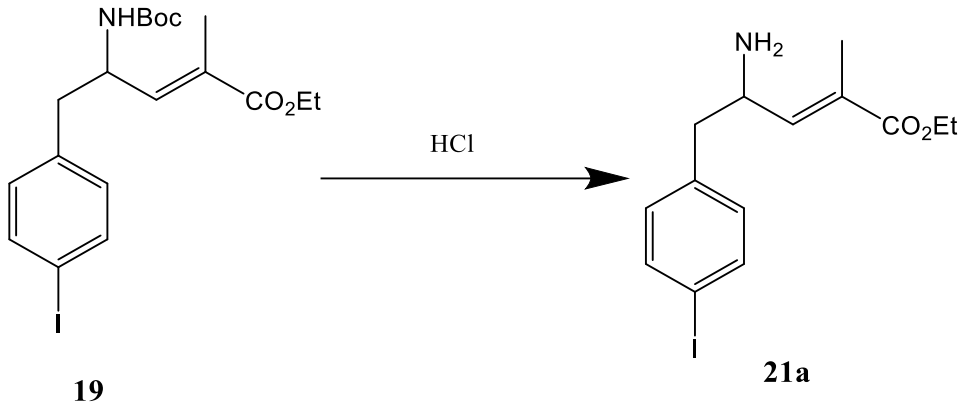
The crude compound **28** (0.1g, 0.557mmol, 1 mol eq.) was dissolved in DCM:pyridine = 1:1 (10 mL). Next, succinic anhydride (0.083 g, 0.837 mmol), 1.5 mol eq.) was added and the mixture was stirred overnight (16 h) at RT. The solvent was then evaporated, and the crude product was washed via LLE with EtOAc, 1M HCl and saturated aq. NaCl. Then, the product was purified via flash column chromatography with first PE:EtOAc = 1:1 and then EtOAc + 0,5% acetic acid as eluents to give compound **29** (0.121 g, 0.433 mmol) with 77% yield.

5.2.1.9. Synthesis of $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$

The palladium catalyst $Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$ was synthesized via a modified[53] solution combustion method from Baidya et al[54]. First, the necessary amounts of $(NH_4)_2Ce(NO_3)_6$ (6.372 g), SnC_2O_4 (9.492 g), $PdCl_2$ (0.102 g) and glycine (10.035 g) were weighed and pestled in a mortar until a finely mixed powder was achieved. In the next step, HPLC-grade water (6 mL) was added to the powder and the mixture was subjected to ultrasonic radiation for 30 min. Then, the mixture was combusted in the oven at 350°C for 1 hour. The particle size of the resulting catalyst was reduced by again using pestle and mortar and the powder was dried in the oven over night at 350°C. The obtained fine powder catalyst was used as is for catalysis.

5.2.2. Batch optimization for the synthesis of a sacubitril precursor

5.2.2.1. Boc-deprotection

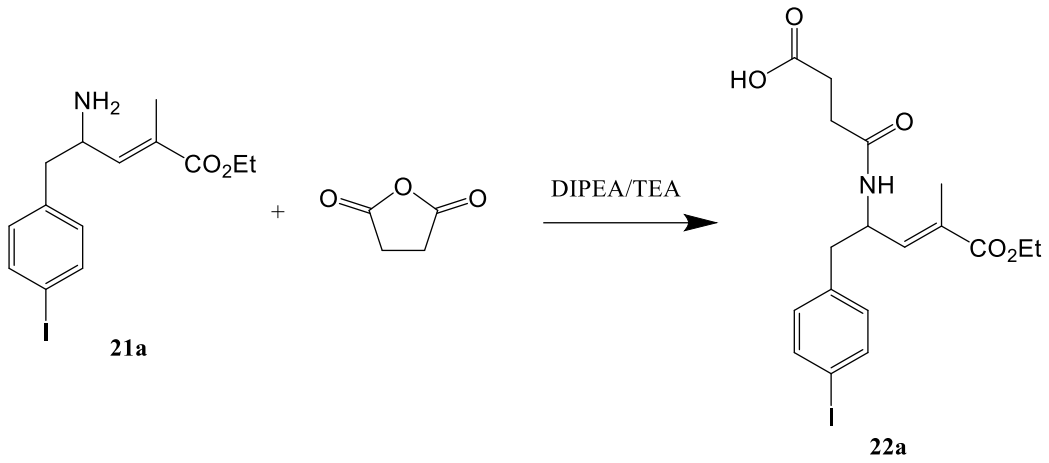


19 **21a**

*Scheme 35: Reaction scheme for Boc-deprotection of compound **19** in batch*

The batch experiments were carried out with 2 mL total reaction solution volume. For the Boc-deprotection, substrate **19** (22.97 mg, 50 µmol, 1 mol eq.) was added to the solvent mixture together with 125 µmol (14 µL) of the internal standard anisole and stirred for dissolution. Then, the reaction solution was heated in a water bath to a specific temperature (55-75°C) and the reaction was started by adding a specific amount of HCl (5-20 mol eq.) to the reaction solution. The reaction was carried out under stirring. To monitor reaction progress, aliquots of the reaction solution (40 µL) were withdrawn at specific times, mixed with 400 µL MeOH:H_3PO_4-buffer = 75:25 for quenching, and analyzed by HPLC (Method C, see section 7.2. for HPLC methods). If the reaction was carried out using the surrogate substrate, the withdrawn aliquot instead gets mixed with 400µL MeOH:H_3PO_4-buffer = 55:45 for quenching, and analyzed by HPLC (Method A-2).

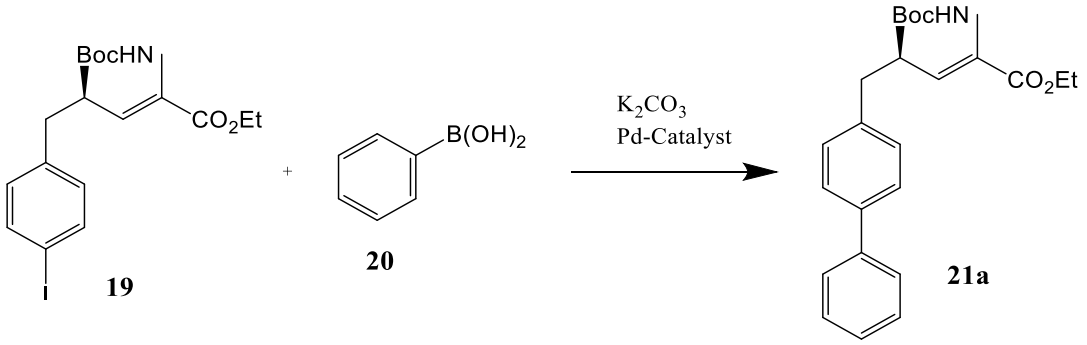
5.2.2.2. *N*-amidation



*Scheme 36: Reaction scheme for N-amidation of compound **21a** in batch*

Substrate **21a** (17.96 mg, 50 µmol, 1 mol eq.) was added to a 2 mL solvent mixture together with 125 µmol (14 µL) of the internal standard anisole and stirred for dissolution. Then, the reaction solution was either heated in a water path to a specific temperature (60-75°C) or was kept at room temperature. The reaction was then started by adding succinic anhydride (1.5-3 mol eq.) and base (TEA or DIPEA, 1.5-3 mol eq.) to the reaction solution. The reaction was carried out under stirring. To monitor reaction progress, aliquots of the reaction solution (40 µL) were withdrawn at specific times, mixed with 400 µL MeOH:H_3PO_4-buffer = 75:25 for quenching, and analyzed by HPLC (Method C). If the reaction was carried out using the surrogate substrate, the withdrawn aliquot instead gets mixed with 400 µL MeOH:H_3PO_4-buffer = 55:45 for quenching, and analyzed by HPLC (Method A-2).

5.2.2.3. Suzuki coupling



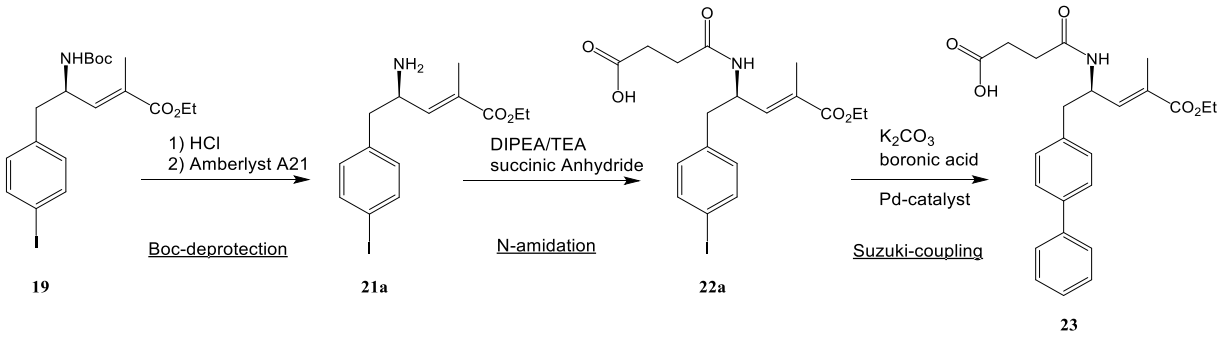
*Scheme 37: Reaction scheme for Suzuki coupling of compound **19** in batch*

For the Suzuki coupling, substrate **19** (22.97 mg, 50 µmol, 1 mol eq.) was added to a 2 mL of solvent mixture together with 125 µmol (14 µL) of the internal standard anisole. Next, a specific amount of K_2CO_3 (1.5-3 mol eq.) and phenylboronic acid **20** (1.5-3 mol eq.) were subsequently

added and the reaction mixture was stirred for dissolution. Then, the reaction solution was heated in a water bath to a specific temperature (60-80°C) and the reaction was started by adding a specific amount of Pd-catalyst ($Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$, 0.25-1mol%) to the reaction solution. To monitor reaction progress, 40 µL aliquots of the reaction solution were withdrawn at specific times, mixed with 400 µL MeOH:H_3PO_4-buffer = 75:25 for quenching, and analyzed by HPLC (Method C).

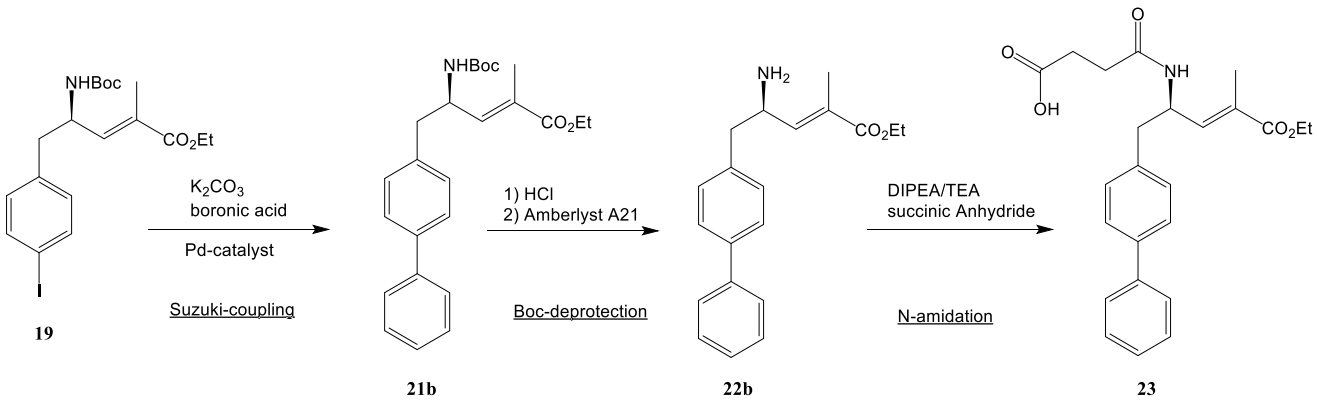
5.2.3. Sequential batch experiments for the synthesis of a sacubitril precursor

For the sequential batch experiments, different reaction steps were subsequently performed with slight modifications to accommodate the different prerequisites for the reaction types. Two different kinds of sequential batch experiments were conducted.



Scheme 38: Reaction scheme of a sequential batch experiment, variation 1

The first type of sequential batch experiments comprised Boc-deprotection with subsequent *N*-amidation, followed lastly by Suzuki coupling. For this, the Boc-deprotection was carried out as described in section 5.2.2.1. After the reaction, 150 mg of the acid scavenger Amberlyst A21 were added to the reaction mixture to achieve a neutral pH. The solution was stirred at room temperature for 10 minutes. The reaction solution (without Amberlyst A21) was then transferred into another vial and the next reaction step was carried out as described in section 5.2.2.2. After the second step, the reaction solution was taken as is and the Suzuki coupling was started as described in section 5.2.2.3.

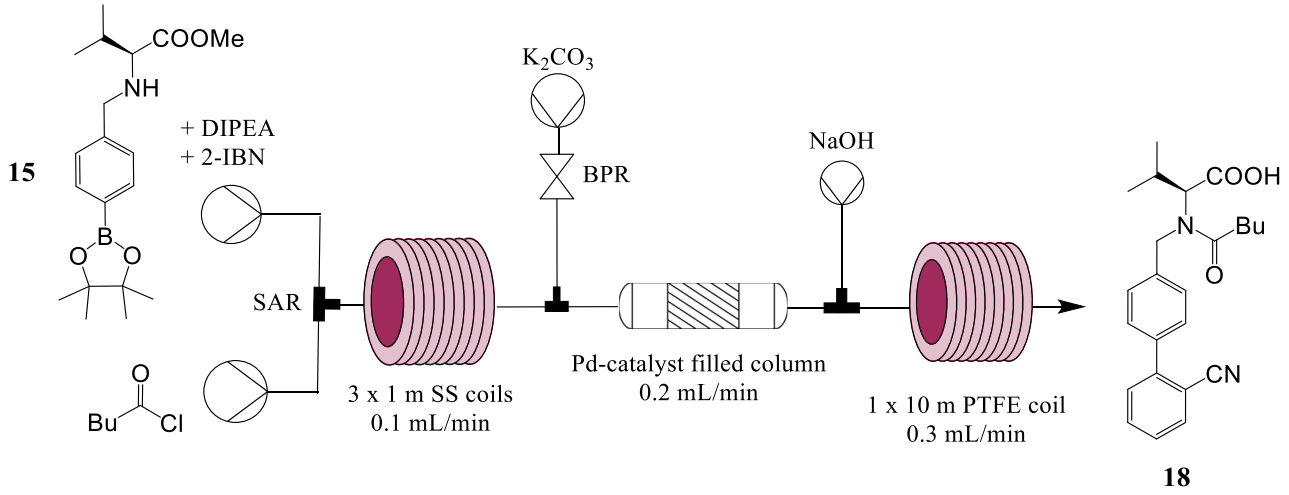


Scheme 39: : Reaction scheme of a sequential batch experiment, variation 2

For the second batch sequence, the Suzuki coupling step was performed as the first step, followed by Boc-deprotection and lastly *N*-amidation. For this, the Suzuki coupling was carried out as described on point 5.2.2.3. After the Suzuki coupling, the two other steps were performed as described in the first set of sequential batch experiments.

5.2.4. Flow experiments

5.2.4.1. Continuous multistep synthesis of valsartan precursor **18**



*Scheme 40: Reaction scheme of the synthesis of valsartan precursor **18** via a three-step reaction cascade in flow*

The three-step synthesis of valsartan precursor **18** in continuous flow was performed using an integrated continuous setup composed of coil reactors as well as a packed-bed reactor unit (Scheme 25).

For the first reaction step, a reaction solution containing the substrate **15** (110 mM, 1.1 mol eq.), DIPEA (55 mM, 2.2 mol eq.), 2-iodobenzonitrile (2-IBN, 25 mM, 1 mol eq.) and the internal standard anisole (240 mM, 2.4 mol eq.) in dioxane was mixed with a solution containing valeryl chloride (220 mM, 2.2 mol eq.) in dioxane using a split-and-recombine

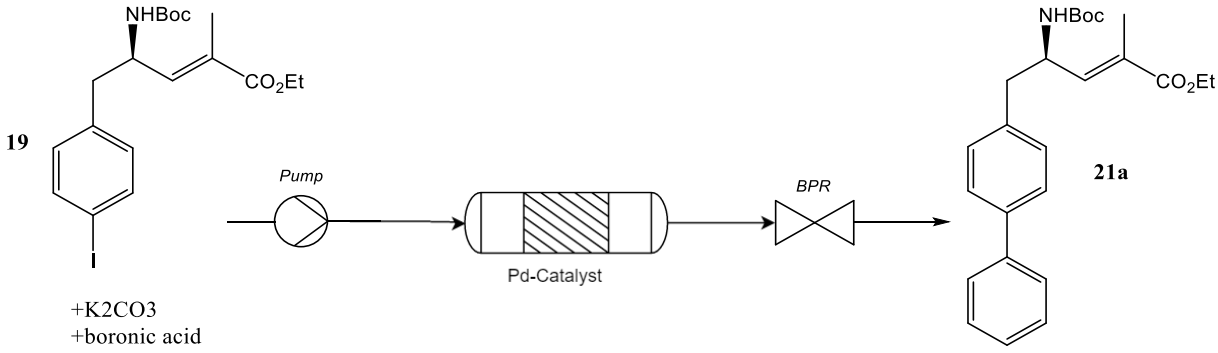
(SAR) mixing element[45]. Both solutions were pumped at a flow rate of 0.05 mL/min using high pressure syringe pumps (VIT-FIT HP, Lambda Instruments). Then, the resulting reagent stream was introduced into 3 SS-coils (length, outer diameter and internal diameter of L x oD x iD 1 m x 1/16in x 0.03 in) , which were heated to 80°C using a water bath, for *N*-acylation.

Next, the reaction solution was merged with a stream of aqueous K_2CO_3 (185 mM, 3.7 mol eq.), which was introduced by an HPLC pump (P4.1S, Knauer) with a flow rate of 0.1 mL/min, using a T-mixer. Additionally, a back-pressure regulator (IDEX BPR cartridge 70 psi) was installed between the HPLC pump and the T-mixer to prevent pressure drop. Then, the resulting reaction mixture with a resulting flow rate of 0.2 mL/min and a solvent composition of dioxane:H_2O = 1:1 was pumped through the PPR featuring an HPLC column (120 x 8 mm) filled with Pd-catalyst at 80 °C.

For the third step, the reaction solution was mixed with a solution of NaOH (500 mM, 10 mol eq.) in dioxane:H_2O = 1:1. The NaOH solution was pumped with a flow rate of 0.1 mL/min using a syringe pump (LA-120, Landgraf) and mixed with the reaction solution using a T-mixer. The resulting solution with a flow rate of 0.3 mL/min was then pumped through a PTFE coil (length, outer diameter and internal diameter of L x oD x iD 10 m x 1/16 in x 0.03 in), which was heated to 80°C using a water bath.

To monitor the reaction progress, aliquots of the resulting reaction solution (40µL) were withdrawn after specific time points, quenched with MeOH:H_3PO_4 = 55:45 (400 µL) and analyzed via HPLC (Method A-1).

5.2.4.2. Continuous Suzuki coupling step for the synthesis of a sacubitril precursor

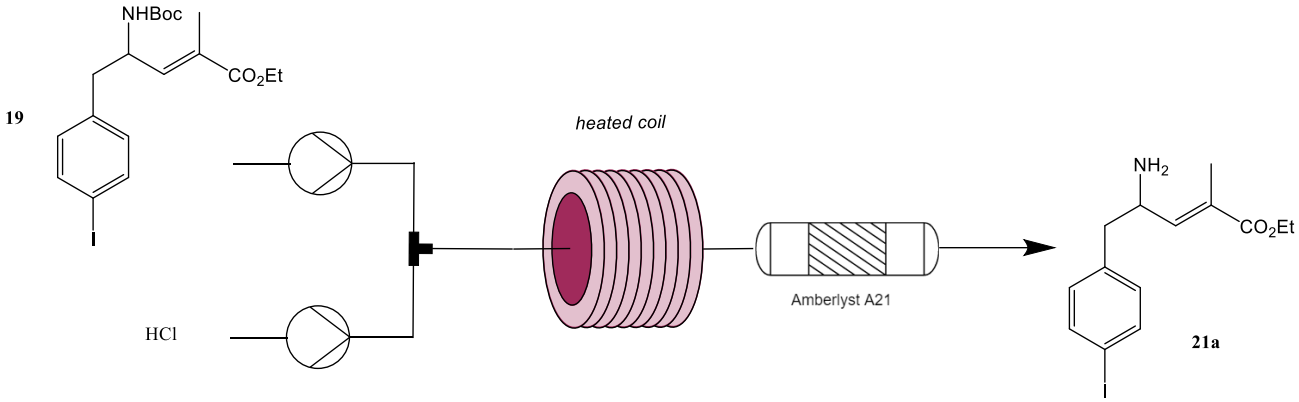


Scheme 41: Reaction scheme of a Suzuki coupling experiment in continuous flow

The Suzuki cross-coupling step in continuous flow was realized by pumping the reaction solution, containing substrate **19** (25mM, 1 mol eq.), phenylboronic acid (37.5 mM, 1.5 mol eq.), K_2CO_3 (37.5 mM, 1.5 mol eq.) and the internal standard anisole (62.5 mM, 2.5 mol eq.), through either an HPLC column (L x iD 40 mm x 8 mm) or a waters HPLC column (L x iD 50

mm x 2.1 mm) filled with around 1.3 g or 0.15 g dry Pd-catalyst ($Ce_{0.20}Sn_{0.79}Pd_{0.01}O_{2-\delta}$), respectively. The filled column was submerged in a heated water bath to reach the required reaction temperature of 75-125°C and had a subsequent back-pressure regulator attached at the outflow. The solution was pumped with a high-pressure syringe pump with a flow rate of 0.05ml/min. The mean reaction time was measured to be τ =18.4 min. A 40 µL aliquot of the outlet flow was collected in a 10-minute interval and was then mixed with 400 µL of MeOH:H_3PO_4-buffer = 75:25 for quenching. The samples were then analyzed via HPLC (Method C).

5.2.4.3. Continuous Boc-deprotection step for the synthesis of a sacubitril precursor

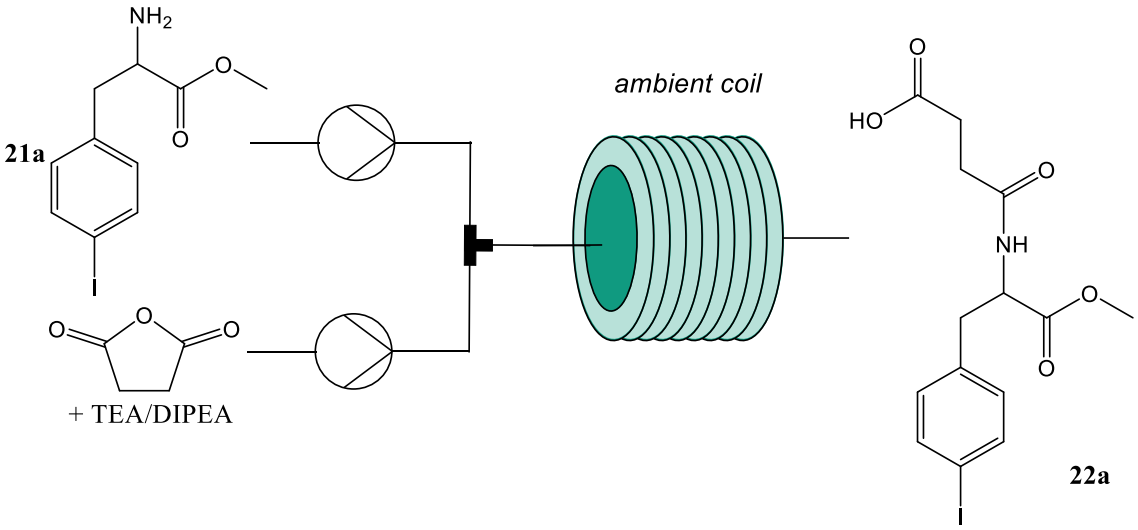


Scheme 42: Reaction scheme of a Boc-deprotection experiment in continuous flow

This step was realized in continuous flow by mixing a solution containing substrate **19** (25mM, 1 mol eq.) and the internal standard anisole (62.5 mM, 2.5 mol eq.) with the acid solution (500 mM, 10 mol eq. acid in solvent) utilizing a T-mixer and a coil reactor setup. The two solutions were pumped with high-pressure syringe pumps at a flow rate of 0.05 mL/min for each pump, giving an overall flow rate of 0.1 mL/min. The utilized coil reactor was made from PTFE with a length, outer diameter and internal diameter of L x oD x iD 3m x 1/16in x 0.03 in.

The reaction temperature was achieved by putting the coil reactor setup in a heated water bath. The coil reactor was further connected to a back-pressure regulator with 75 psi and subsequently an HPLC column filled with Amberlyst A21 as an acid scavenger to provide a neutral pH for the following steps. A 40 µL aliquot of the outlet flow was collected in a 10-minute interval and was then mixed with 400 µL of MeOH:H_3PO_4-buffer = 75:25 for quenching. The taken samples were then analyzed via HPLC (Method C). If the reaction was carried out using the surrogate substrate, the withdrawn aliquot instead gets mixed with 400µL MeOH:H_3PO_4-buffer = 55:45 for quenching, and analyzed by HPLC (Method A-2).

5.2.4.4. Continuous *N*-amidation step for the synthesis of a sacubitril precursor

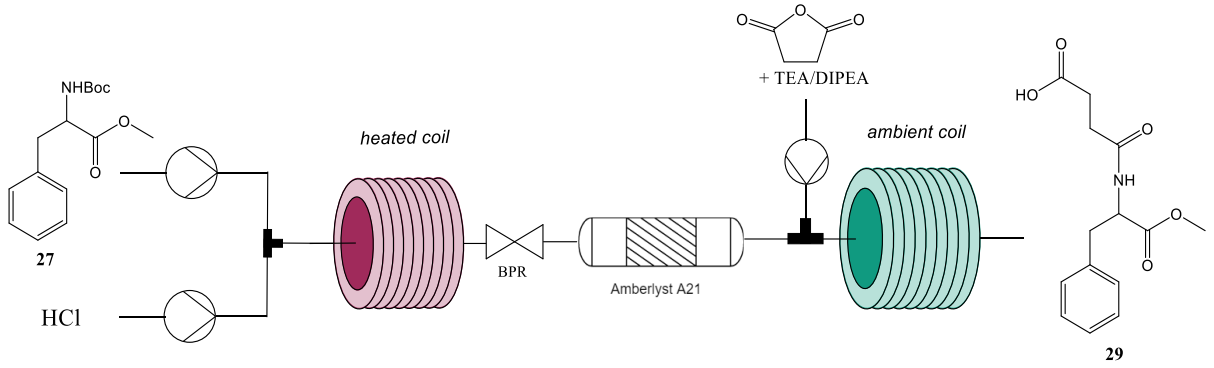


Scheme 43: Reaction scheme of an N-amidation experiment in continuous flow

N-amidation in continuous flow was achieved by mixing the solution containing substrate **21a** (25mM, 1 mol eq.) and the internal standard anisole (62.5 mM, 2.5 mol eq.) with a reaction solution containing succinic anhydride (37.5-75 mM, 1.5-3 mol eq.) and base (37.5-75 mM , 1.5-3 mol eq., TEA or DIPEA). The substrate solution was pumped with a flow rate of 0.1 mL/min while the reaction solution had a flow rate of 0.05 mL/min, giving an overall flow rate of 0.15 mL/min. Both solutions were pumped by high-pressure syringe pumps. After mixing the two solutions in a T-mixer, a PTFE coil reactor with a length of 3 m and an internal diameter of 0.03 in was employed to give a mean reaction time of τ = 9.13min with a reaction volume of V = 1.37mL. A 40 µL aliquot of the outlet flow was collected in 10-minute intervals. The samples were then mixed with 400µL of MeOH:H_3PO_4-buffer = 72:25 for quenching and analyzed via HPLC (Method C). If the reaction was carried out using the surrogate substrate, the withdrawn aliquot instead gets mixed with 400µL MeOH:H_3PO_4-buffer = 55:45 for quenching, and analyzed by HPLC (Method A-2).

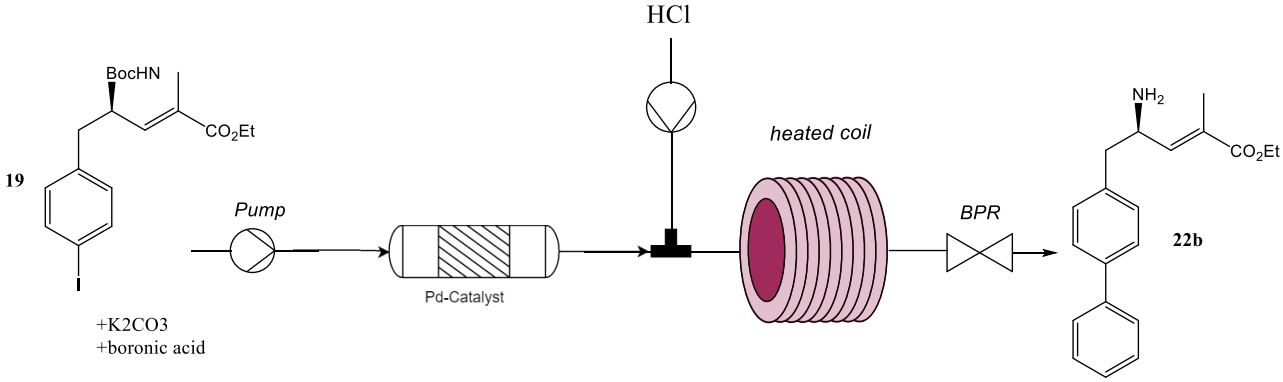
5.2.5. Continuous performance of sequential steps for the synthesis of a sacubitril precursor

Three different multi-step flow setups were carried out:



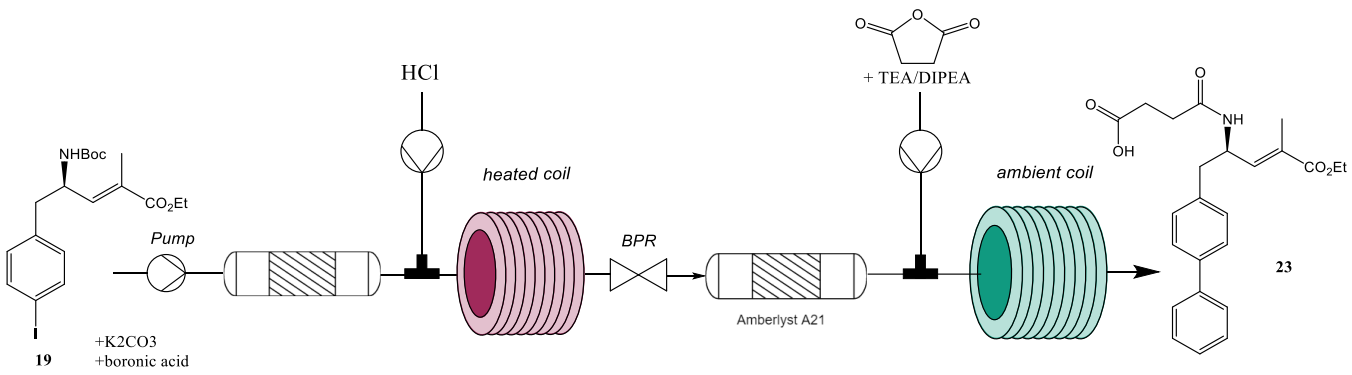
Scheme 44: Reaction scheme of a sequential Boc-deprotection and N-amidation experiment in continuous flow

The first one was Boc-deprotection followed by *N*-amidation. Here, the outlet flow of the Boc-deprotection step was treated as the educt solution for the *N*-amidation. Samples were taken from the outlet flow of the second step and analyzed as described in the points above.



Scheme 45: Reaction scheme of a sequential Suzuki coupling and Boc-deprotection experiment in continuous flow

The next 2-step experiment was composed of Suzuki coupling with subsequent Boc-deprotection. As for the first 2-step experiment, both steps were carried out as described in the points above. The outlet flow of the Suzuki coupling step was treated as the educt solution for the Boc-deprotection step and the samples were again taken from the outlet flow of the second step and analyzed as described in the points above.



Scheme 46: Reaction scheme of a sequential 3-step experiment in continuous flow

The final 3-step setup had the sequence of: first step Suzuki coupling, second step Boc-deprotection, and third step *N*-amidation. As in the 2-step experiments, the outlet flow of the previous step was treated as the educt solution of the next step. Flowrates, reactors and equivalents of reactants were the same as in the single-step flow experiments (see points 5.2.5.1 – 5.2.5.3) and aliquots of the flow were taken from the outlet flow of the last step and analyzed via HPLC similar to the description in the points above.

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7. Appendix

7.1. Residence time distribution

Knowledge of the mean residence time and the residence time distributions of reactors allows reduction of the reaction time while maintaining satisfying conversion. The mean reaction time of coil reactors is mostly preassigned, assessing the residence time distribution of a packed-bed column, however, is more challenging. For the final multistep synthesis of sacubitril precursor **23** two packed-bed columns of different sizes and with fillings were incorporated into the continuous flow setup. A column filled with the Pd-catalyst was used for the Suzuki coupling step, another other column filled with Amberlyst A21 was employed to scavenge excess acid. To determine the residence time distribution of those two columns, a residence time distribution curve was experimentally identified using a tracer-based pulse approach.

The setup for the determination of the residence time distribution curve comprises 2 pumps. One of them pumps a tracerless solvent and the other one pumps a solvent solution containing the tracer anisole. The pumps are connected to a 6-way valve, which in turn is connected to the column. At first the tracerless solvent solution is pumped through the column until the output signal is constant. Then, the 6-way valve is turned to let the tracer solution pass through the column for a specific pulse time. After the determined pulse time the 6-way valve is turned back and the tracerless solvent is pumped through the column until the experiment is finished. The outflow of the column passes through a flow cell, where the tracer concentration is determined via UV-VIS.

Table 27: Determined mean residence times for different columns

Column measurements	Filling	Flow rate [mL/min]	Mean residence time τ [min]
L x iD = 40 x 8 mm	Pd-catalyst	0.05	18.4
L x iD = 120 x 8 mm	Amberlyst A21	0.1	46.9

7.2. HPLC methods

To monitor reaction progress of both batch and continuous flow reactions, aliquots of the reaction were taken at specific time points, quenched with a specific sample diluent, and analyzed via High Performance Liquid Chromatography (HPLC). The samples were measured at 25°C using MeOH and H_3PO_4 buffer (with a buffer composition of H_2O:H_3PO_4 = 300:1, v:v) as mobile phase. The used HPLC methods can be found in Table 28:

Table 28: HPLC methods used for monitoring of the reaction progress

Method	% MeOH	% H_3PO_4 buffer	Run time [min]	Sample diluent	Flow rate [mL/min]
A-1	55	45	15	MeOH:H_3PO_4-buffer = 55:45	1
A-2	55	45	5	MeOH:H_3PO_4-buffer = 55:45	1
B	60	40	15	MeOH:H_3PO_4-buffer = 60:40	1
C	75	25	15	MeOH:H_3PO_4-buffer = 75:25	1

List of Abbreviations

ACE	angiotensin-converting enzyme
ANPir	atrial natriuretic peptide immunoreactivity
API	active pharmaceutical ingredient
ARNi	angiotensin receptor-neprilysin inhibitor
CI	confidence interval
CVC	Cardiovascular causes
CVD	Cardiovascular diseases
DBP	diastolic blood pressure
DCM	dichloromethane
DIPEA	N,*N*-Diisopropylethylamine
DMAP	Dimethylaminopyridine
DMF	dimethylformamide
DoE	Design of experiments
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
HF	heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HPLC	High-performance liquid chromatography
HT	Hypertension
IHD	Ischemic heart disease
iPrOH	Isopropanol
L x iD	Length x internal diameter
LLE	liquid-liquid extraction

MeCN	Acetonitrile
MeOH	Methanol
NEP	neprilysin
PE	Petroleum ether
PPR	Plug & Play reactor
PTFE	Polytetrafluoroethylene
RAAS	renin-angiotensin-aldosterone system
RT	room temperature
SAR	split-and-recombine
SBP	systolic blood pressure
SRFD	split-and-recombine flow distributors
SS	Stainless steel
TBAB	tetra-*N*-butylammonium bromide
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	tetrahydrofuran

7.3. List of Tables

7.4. List of Schemes

7.5. List of Figures