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Organic Synthesis in Continuous Flow
Towards Integrated Multiple Processes

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S. C. J. J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 9 januari 2014
om 10.30 uur precies

door

Mariëlle Minna Elbertine Delville
geboren op 12 augustus 1985
te Hilversum
The work presented in this thesis was conducted in the Synthetic Organic Chemistry and Bio-Organic Chemistry groups, Institute for Molecules and Materials at the Radboud University Nijmegen, Nijmegen, the Netherlands.

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<td>Full Form</td>
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<tr>
<td>[α]</td>
<td>specific rotation</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ACTS</td>
<td>advanced chemical technologies for sustainability</td>
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<tr>
<td>Alloc</td>
<td>alloxycarbonyl</td>
</tr>
<tr>
<td>Ar</td>
<td>Argon</td>
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<td>aryl</td>
</tr>
<tr>
<td>A.U.</td>
<td>atomic unit</td>
</tr>
<tr>
<td>BCN</td>
<td>9-hydroxymethylbicyclo[6.1.0]nonyne</td>
</tr>
<tr>
<td>BPR</td>
<td>back pressure regulator</td>
</tr>
<tr>
<td>br s (NMR)</td>
<td>broad singlet</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celcius (centigrade)</td>
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<td>C</td>
<td>coil</td>
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<td>cumulative energy demand</td>
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<td>cubic centimeter</td>
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<td>CSA</td>
<td>camphorsulfonic acid</td>
</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>d (NMR)</td>
<td>doublet</td>
</tr>
<tr>
<td>dd (NMR)</td>
<td>doublet of doublets</td>
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<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<tr>
<td>DOE</td>
<td>design of experiment</td>
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<td>dt (NMR)</td>
<td>doublet of triplets</td>
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<td>e.g.</td>
<td><em>exempli gratia</em> (for example)</td>
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<tr>
<td>EDA</td>
<td>ethyl diazoacetate</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
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<tr>
<td>equiv</td>
<td>equivalents</td>
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<tr>
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<td>ethyl</td>
</tr>
<tr>
<td><em>et al.</em></td>
<td><em>et alia</em> (and others)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>-------------</td>
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<td>EtOAc</td>
<td>ethylacetate</td>
</tr>
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<td>ethanol</td>
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<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
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<td>Fe(TPP)Cl</td>
<td>meso-tetraphenylphorphyrine iron(III) chloride</td>
</tr>
<tr>
<td>FEP</td>
<td>fluorinated ethylene propylene</td>
</tr>
<tr>
<td>FLLEX</td>
<td>flow liquid-liquid extraction</td>
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<tr>
<td>(g)</td>
<td>gas</td>
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<td>g</td>
<td>gram</td>
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<tr>
<td>G-L</td>
<td>gas-liquid</td>
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<td>hours</td>
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<td>HNL</td>
<td>hydroxynitrile lyase</td>
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<td>high resolution mass spectrometry</td>
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<td>lab</td>
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<td>LCA</td>
<td>life cycle assessment</td>
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<td>L-L</td>
<td>liquid-liquid</td>
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<td>M</td>
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<tr>
<td>(m)-</td>
<td>meta-</td>
</tr>
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<td>M</td>
<td>mixing unit</td>
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<td>multiplet</td>
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<td>m.p.</td>
<td>melting point</td>
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<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
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<tr>
<td>(m^3)</td>
<td>cubic meter</td>
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<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>mbar</td>
<td>millibar</td>
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<td>(m)-CPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
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<td>MeCN</td>
<td>acetonitrile</td>
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<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>MIP</td>
<td>2-methoxyisopropyl</td>
</tr>
<tr>
<td>MJ FU(^{-1})</td>
<td>mega joule per functional unit</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>millimol</td>
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<td>MR</td>
<td>molar ratio</td>
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<td>ms</td>
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<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTBE</td>
<td>methyl tert-butyl ether</td>
</tr>
<tr>
<td>n-</td>
<td>normal-</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>nmol</td>
<td>nanomol</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NWO</td>
<td>Netherlands organization for scientific research</td>
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<tr>
<td>o-</td>
<td>ortho-</td>
</tr>
<tr>
<td>P</td>
<td>pump</td>
</tr>
<tr>
<td>PAA</td>
<td>peroxy acetic acid</td>
</tr>
<tr>
<td>Pd/C</td>
<td>palladium on carbon</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
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<tr>
<td>PG</td>
<td>protection group</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PI</td>
<td>polyimide</td>
</tr>
<tr>
<td>PoaC</td>
<td>process on an chip</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>psi</td>
<td>pound-force per square inch</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>rac</td>
<td>racemic</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
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<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>s (NMR)</td>
<td>singlet</td>
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<tr>
<td>t-</td>
<td>tert-</td>
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<tr>
<td>t</td>
<td>reaction time</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>t (NMR)</td>
<td>triplet</td>
</tr>
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<td>td (NMR)</td>
<td>triplet of doublets</td>
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<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>tetrahydropyranyl</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
</tr>
<tr>
<td>μL</td>
<td>microliter</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
<tr>
<td>μmol</td>
<td>micromol</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume-to-volume ratio</td>
</tr>
<tr>
<td>VH</td>
<td>Vilsmeier-Haack</td>
</tr>
<tr>
<td>Vis</td>
<td>visable</td>
</tr>
<tr>
<td>w/w</td>
<td>weight-to-weight ratio</td>
</tr>
<tr>
<td>wt%</td>
<td>weight percentage</td>
</tr>
<tr>
<td>‰</td>
<td>promile</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>Δ</td>
<td>delta (difference)</td>
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Chapter 1

Introduction

Abstract
Chapter 1 introduces microreactor technology starting with discussing the fundamental differences compared to batch chemistry. Next, the development of single-step flow syntheses into multistep flow processes is described including online and inline analysis methods work-up modules.
1.1. History

Alchemists have been performing chemistry in a traditional batchwise manner for ages (Figure 1.1). Over time, stainless steel and glass reactors have replaced copper ones since they are chemically more robust. Chemical knowledge has increased, sophisticated reactions and reagents have been developed, legislation with respect to conducting chemical reactions, handling, and transport of chemicals in order to create a safe (working) environment was established. These changes led to the development of continuous processes for chemical bulk productions but did not conceptually influenced the way chemists performed their reactions in pharmaceutical and fine chemical productions or in a research setting.

Driven by the societal wish to increase the safety of chemical manufacturing and constant technological developments, approximately one decade ago microreactor technology was introduced to the toolbox of organic chemists. This technology enabled the organic chemist to perform organic reactions in a safe and continuous fashion on a microliter scale, thereby creating opportunities to conduct reactions in a conceptually different manner. Quickly, microreactor technology became more broadly applicable for reactions in the gas, liquid and solid phase. The research described in this thesis will focus on organic flow syntheses performed in the liquid phase.

Figure 1.1. An alchemist’s lab painted by the Flemish painter Jan van der Straet (1523-1605)

The advantages of microreactor technology over batchwise procedures caused a high level of interest among organic chemists. Initially, single-step flow syntheses were performed illustrating the advantages of flow chemistry such as rapid mixing and heat exchange, high control over reaction conditions and the ease of scaling up and numbering up of microreactor modules. Stimulated by the fact that a range of flow synthesis modules
became commercially available, the scope and limitations of flow chemistry have been explored over the past ten years and new developments are still ongoing. Soon it became clear that with continuous product formation, continuous monitoring of the reaction would increase the applications of microreactors either in optimization experiments or in production processes. Analysis using conventional methods such as HPLC, IR, and NMR were explored. After various single-step flow syntheses were developed, microreactor technology was recently taken to a next level; multistep flow processes. In this chapter, all of the aforementioned aspects will be reviewed.

1.2. Advantages and disadvantages of microreactor technology

There are several fundamental differences between continuous flow microreactor synthesis and traditional batch synthesis. In this section, the main advantages and disadvantages of flow chemistry are discussed and illustrated by representative examples.

1.2.1 Heat and mass transport

A microreactor is a small device consisting of one or multiple small channels with a dimension ranging between ten to a few hundred micrometer with a total volume of less than one milliliter. The small reactor volume decreases the fluid layer thickness, causing an increase of surface-to-volume ratio compared to a traditional laboratory flask or a production scale reactor (Table 1.1). Heat is rapidly transferred in or out of the reaction mixture gaining accurate control over the reaction temperature. As a result, the occurrence of hot spots is eliminated in case of highly exothermic reactions. The high area of fluid contact between multiple flows causes mixing of reagents to occur rapidly by diffusion. Furthermore, on the small length scale of a microreactor chip, a laminar flow regime is present which leads to excellent control over reaction times.

Table 1.1. Surface-to-volume ratios of different reactor types

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactor type</th>
<th>Surface-to-volume ratio (cm²/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>microreactor (diameter ~100 μm)</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>flask (100 mL)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>reactor (1 m³)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

An illustrative example is the Swern-Moffatt oxidation of benzyl alcohol (1, Scheme 1.1). In batch, the reaction is typically performed at -50 °C to reduce side product formation to 11%. In flow, it was shown that this reaction could be performed at 20 °C to obtain the product with similar conversion (75%) and side product formation (16%) in only 100 ms. An improvement of the reaction selectivity was achieved at even higher temperatures, 70 °C, where a conversion of 96% was established, yielding only trace amounts of side products in 32 ms. Without high heat and mass transport as reached in the microreactor, this extremely fast reaction with high selectivity would not have been possible at elevated temperatures. Reactions performed in these ultra-short reaction times are called flash
Chapter 1

chemistry reactions.\textsuperscript{15}

\[
\begin{align*}
\text{OH} & \xrightarrow{\text{TFAA, } \text{Et}_3\text{N}} \text{DMSO} \quad \text{O} \\
1 & \quad 2
\end{align*}
\]

In batch: Swern\textsuperscript{12} -50 °C 60 min 84% conversion 11% side products
In flow: Yoshida\textsuperscript{13} 20 °C 100 ms 75% conversion 16% side products
Rutjes\textsuperscript{14} 70 °C 32 ms 96% conversion trace amounts of side products

Scheme 1.1. Swern-Moffat oxidation of benzaldehyde (1)

1.2.2 Selectivity

Having high control over the reaction conditions in the microreactor additionally enables to distinguish between kinetic or thermodynamic products\textsuperscript{16} and mono- or di-additions.\textsuperscript{17} In batch, these reactions need to be slowed down by decreasing temperature, decreasing concentration or by the addition of additives in order to enhance the selectivity. In flow, the reaction can proceed at normal rate or even faster at elevated temperatures while still maintaining high selectivity. The iodination of aromatic compounds illustrates the enhanced selectivity obtained when performing this reaction in flow (Scheme 1.2) due to enhanced mixing.\textsuperscript{18} Identical iodination conditions were applied in both batch and flow set-up namely, 0.625 equivalents of I\textsubscript{2} (precursor of “I”\textsuperscript{+}), and 0 °C. In batch, monoiodo compound 4 was formed in 45% and diiodo compound 5 in 18%. In flow, monoiodo compound 4 was formed in 78% while only 4% of diiodo compound was observed. Additionally, when slower mixing rates were applied in the flow set-up, at some point a steep decrease in selectivity was observed. Both these experiments show that sufficient mixing is necessary in order to obtain higher reaction selectivities.

\[
\begin{align*}
\text{OMe} & \quad \text{OMe} \\
\text{I} & \quad \text{OMe} \\
3 & \quad 4 & \quad 5
\end{align*}
\]

In batch: 45% 18%
In flow: 78% 4%

Scheme 1.2. Iodination in batch and in flow under identical reaction conditions\textsuperscript{18}

1.2.3 Safety

Inherently, microreactor technology increases the safety of chemical processes. The closed system combined with the low reaction volume enables the safe reaction of highly toxic or explosive reagents. The high heat exchange rates eliminate the formation of hot spots and therefore prevent exothermic runaway reactions. One important example is the \textit{in situ} generation of diazomethane (Scheme 1.3). Diazomethane is a highly reactive, toxic,
and, due to the loss of nitrogen gas, highly explosive reagent which cleanly methylates carboxylic acids without byproduct formation. In this set-up, Diazald (6), a commercially available precursor of diazomethane, reacts with potassium hydroxide (KOH) to generate diazomethane in situ. A hydrophobic PDMS membrane causes only the diazomethane to diffuse into the top channel, where it directly reacts with benzoic acid (7) to form methyl benzoate (8) in 99% conversion. Blank experiments revealed that neither benzoic acid nor the product methyl benzoate diffused from the top channel to the aqueous saline bottom channel.

Scheme 1.3. Diazomethane generation, separation and reaction

1.2.4 Environmental benefits and issues

In addition, environmental and economic benefits are created by microreactor technology. An overview of the results obtained by Kreisel et al., who performed a Life Cycle Analysis (LCA) on the highly exothermic m-anisaldehyde two-step synthesis in batch and flow on laboratory and industrial scale, is shown in Figure 1.2. Lab scale was defined as the synthesis of 10 kg of m-anisaldehyde under laboratory conditions. The batch synthesis was conducted at -50 °C to yield m-anisaldehyde in 60%. In continuous mode, the high control over the reaction conditions allowed the reaction to be performed at 20 °C yielding the product in 88%. Transferring this data to the cumulative energy demand (CED) analysis (Figure 1.2a) resulted in a decrease of compounds and solvents usage, electricity consumption, and waste disposal.
For the production of \textit{m}-anisaldehyde at industrial scale, defined as the synthesis of 1 ton of \textit{m}-anisaldehyde, both the batch and flow process yielded the product in 88\%. The main difference between the two processes was the reaction temperature, the batch process operated at -80 °C while the continuous process was run at 20 °C. This directly translates to the CED analysis (Figure 1.2b), which shows a high impact of the liquid nitrogen cooling. Compared to this, the additional energy and material demand during the fabrication step of the continuous flow set-up only plays a minor role.
1.2.5 Scalability

Hazardous and previously unscalable reactions, such as diazomethane generation, are now becoming available. Commercially available microreactors with an internal volume as large as 240 mL are accessible, in which the optimal reaction conditions identified on small scale (10 mL) can directly be implemented. In addition, because of the non-spacious set-ups, reactors are easily parallelized.

A representative example is the Paal-Knorr pyrrole synthesis (Scheme 1.4), in which diketone 9 is condensed with aminoethanol (10) to form the corresponding pyrrole 11. This reaction was optimized in a 7 μL reactor and scaled 1367 times to four parallel mesoscale reactors with a total volume of 9.6 mL to produce pyrrole 11 in 55.8 g/h in 96% yield. In a similar example, the Paal-Knorr synthesis was performed in a 38 mL reactor producing pyrrole 11 in 406 g/h in 99% yield.

![Scheme 1.4. Paal-Knorr synthesis of pyrrole 11](image)

1.2.6 Disadvantages

One of the main disadvantages especially for the smaller channels (< 500 μm) is clogging, generally caused by precipitating solids. Submerging the microreactor in a sonification bath sometimes can prevent this as reported by Yoshida et al. Reactors having larger channels are less prone to fouling, but in these dimensions diffusion is not always rapid enough to obtain fast mixing. Therefore mixing units are incorporated in the chip design regaining fast mixing ability.

Another weakness of flow chemistry is the preparation time needed before starting a synthesis. After preparing the solutions and determining the flows in the system, it has to stabilize for some time to obtain reproducible results. Furthermore, a reliable quenching procedure needs to be developed in order to reliably measure residence and hence reaction times.

During a flow synthesis, it is neither possible to simply prolong the reaction time when no complete conversion is obtained, nor to add additional reagent/catalyst. Conversely, the overall time necessary to perform an optimization is decreased since for analysis only a small amount of reaction mixture is required reducing the production time to a minimum.

Another significant shortcoming of flow chemistry is the decreased versatility of microreactor chips compared to a batch flask or reactor. One chip design is only suitable for a few reactions, limiting its application considerably. Moreover, reactions that take
multiple hours or days to complete cannot be performed in a continuous flow fashion. Either the minimal pump rate is reached when having a fixed channel length or pressure drop problems are encountered while elongating the channel.

Finally, as was mentioned earlier, the costs associated with the production of microreactors are higher compared to batch reactors. The production of microreactors requires high energy and material consuming methods increasing the manufacturing costs and also the CO₂ footprint.

1.3. Online and inline analysis

As in traditional batch synthesis, product analysis is important in flow synthesis. At the outlet of the microreactor, samples can be collected to perform all conventional analysis techniques. However, due to the small scale it might take a considerable amount of time to collect sufficient material depending on the sensitivity of the analysis method. Technological innovations have resulted in highly sensitive devices that are suited for the real time analysis of small aliquots of the reaction mixtures.

There are three types of analysis procedures. The first one is offline analysis: a sample is generated and manually transferred to the analysis apparatus. A second procedure is online analysis: automated systems obtain samples directly out of the reaction line and transfer them to the analysis apparatus. Third is inline analysis: in this procedure, measurements are performed directly into the reaction mixture. In addition, the latter two procedures might comprise systems that directly respond to the outcome of the analysis by adjusting for example pump rates and temperature. In this section, the most commonly used online and inline analysis procedures for microreactor technology are discussed.

1.3.1 GC and HPLC analysis

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are two analysis methods suitable for offline and online analysis. For online analysis, a small aliquot of reaction mixture is funneled into the continuous flow of the chromatograph. GC and HPLC allow the separation of complex reaction mixtures generating relevant information over the selectivity and conversion of a given reaction. Detailed quantitative GC or HPLC analyses are obtained by means of an appropriate internal standard. An internal standard for each flow provides the most accurate information, e.g. by ruling out any deviations resulting from inaccuracy of the pumps.

Jensen et al. reported on rapid reaction kinetics determination by online HPLC analysis (Scheme 1.5). The Diels-Alder reaction of isoprene and maleic anhydride was chosen as a model reaction to test the set-up, which allowed them to successfully model the kinetic data of this reaction based on only 12 experiments.
### 1.3.2 Mass spectrometry

Mass spectrometry is a highly sensitive analytical method used to determine specific mass and (sometimes) chemical structure of a compound. As for GC and HPLC, mass spectrometry is an online analysis method, and sample quantities are low (typically in the order of nmol). In the case of complex reaction mixtures, a mass spectrometer is coupled to an online GC or LC apparatus to separate the different components prior to mass analysis. Online mass spectrometry (MS) has found wide applicability in protein analysis. A fluid feed of protein flows through a microreactor chip containing immobilized trypsin (Figure 1.3). Protein digestion will then result in small peptide fragments, which can directly be sequenced by MS techniques.

![Figure 1.3. A schematic representation of inline mass spectrometry](Image)

### 1.3.3 UV-spectroscopy

UV-spectroscopy can be used as an inline analysis method. This enables the monitoring of compounds and unstable intermediates in their natural environment and therefore the investigation of the reaction mechanism. UV-active molecules can be analyzed based on increasing or decreasing signals in a qualitative manner. Additionally,
since the concentration of a compound is linearly related to the UV-signal, a previously
determined calibration curve enables quantitative analysis as well. A drawback of this
analysis method is the increased overlap between signals because of the small detection
windows. Therefore, this method is not suitable for reaction mixtures containing multiple
UV-active components including solvent.

1.3.4 Raman and IR analysis

Raman and IR analysis methods are widely applied for inline analysis of batch
processes. After some adjustments, several Raman and IR modules for inline flow
analysis became (commercially) available. Raman and IR spectroscopy are used to
monitor product-specific peaks during the conversion. This makes these methods highly
suitable for qualitative analysis.

An example of inline Raman spectroscopy is the monitoring of vinyl chloride
polymerization. Droplets of monomer were analyzed in time providing detailed
information not only on changes in chemical structure (polymer formation), but also on
the concentration within the microdroplet. Polyvinylchloride grains collected at the end
were in good agreement with published data with respect to their dimensions and shapes.

1.3.5 NMR spectroscopy

Nuclear Magnetic Resonance (NMR) is a powerful inline analysis method for
the detection of product and short-lived unstable intermediates enabling structure
determination and elucidation of reaction kinetics. However, the intrinsic insensitivity of
NMR results in high demands on the probe design especially for mass-limited samples
as occur in flow chemistry. By the development of a detection coil in a microfluidic device
with a stripline geometry (Figure 1.4), both resolution and sensitivity were enhanced.

Figure 1.4. (a) The custom-made microfluidic probe. The dashed line indicates the
position of the NMR chip (b) Close-up view of the microreactor holder
mounted on top of the probe (c) Close-up view of the stripline chip
holder (d) Schematic representation of the mechanical arrangement of the
microfluidic chip in the holder
1.4. Multistep synthesis

A decade of research on microreactor technology has provided chemists with extensive knowledge on performing a multitude of single-step reactions in continuous flow.\textsuperscript{5,6,8,13,47,48} However, the synthesis of a target compound usually comprises multiple reactions steps. To take microreactor technology to the next level, these single-step syntheses have to be converted into multistep processes, allowing the preservation of the advantages of flow chemistry such as sustainability, elimination of hazardous compound handling, continuous product formation and the ease of scaling and numbering up. However, direct coupling of single step flow synthesis is limited to only a few examples (Section 1.4.1). In most cases when setting up a successful multistep flow synthesis, solvent incompatibilities and the need for intermediate purification have to be overcome. The latter two are addressed in the overview of available inline work-up modules (Section 1.4.2).

1.4.1 Direct coupling

Multistep flow reactions which comprise of directly coupled single-flow syntheses are rare.\textsuperscript{49,50,51} In the first example, Yoshida et al. reported on the sequential introduction of two substituents in \(o\)-dibromobenzene (21) using a microreactor system consisting of four micromixers and four microtube reactors (Scheme 1.6).\textsuperscript{52} By repeating the same reaction twice, any problems associated with solvent incompatibilities were circumvented. With this set-up, they were able to prepare a series of six different products ranging in yield from 53-74%.

A second approach for direct coupling of two single-step flow reactions makes use of solid-phase reagents. Petersen et al. reported on hydrogenation of a Cbz-protected amine 16 of which the resulting free amine was coupled directly with an isocyanate of interest (Scheme 1.7).\textsuperscript{53} Reported product purities were high (94-99%) and yields were reported as being quantitative. This example allows the use of one solvent, while the relatively flexible reaction time in the hydrogenation module (H-cube) creates the possibility to tune the flow rate as was necessary for the isocyanate addition.
Scheme 1.7. Direct coupling of two single-step flow reactions\textsuperscript{53}

I.4.2 Inline work-up modules

I.4.2.1 Distillation

Solvent incompatibility issues between single-step flow reactions can be overcome by utilization of an inline distillation module. Figure 1.5 shows a microdistillation module as described by Hartman et al.\textsuperscript{54} One year later, in 2010, the same group reported on the first multistep flow synthesis utilizing their distillation set-up to change the solvent from dichloromethane to DMF or toluene.\textsuperscript{55} Other possible applications of microdistillation modules are product concentration and solvent regeneration.

The main obstacles in the development of small-scale distillation modules are the control of heat loss, temperature profile and flow ratio. Therefore, research efforts are ongoing to better understand and optimize the underlying working principles.\textsuperscript{56,57,58}

Figure 1.5. Microdistillation module by Hartman et al.\textsuperscript{54,55}
1.4.2.2 Scavenger agents

Another bottleneck when coupling single-step flow reactions is the need for intermediate purification. The work-up of liquid reaction mixtures can be performed by either solid-liquid or liquid-liquid procedures. Solid-supported scavenger agents are probably the most thoroughly explored method to clean intermediates and products in line with the synthesis.\textsuperscript{5,51,59}

A multistep synthesis utilizing cartridges of immobilized reagent and scavengers is nicely illustrated by the preparation of aniline derivative 20 (Scheme 1.8).\textsuperscript{60} 3-Fluoro-4-nitroanisole (18) and 1-methylhomopiperizine (19) were subjected to nucleophilic aromatic substitution at 135 °C. All HF generated was scavenged by a benzylamine cartridge. The crude intermediate product was immediately reduced by the use of a hydrogenation module (H-cube), followed by scavenging of residual palladium through a thiourea cartridge to provide isolated product 20 in quantitative yield.

Although the use of solid-supported scavenging agents has proven successful for a wide range of flow reactions, they require additional handling.\textsuperscript{53} Since scavenging resins are consumed, they must be either replaced or regenerated after the synthesis of a few compounds. Especially in the case of single-flow multistep synthesis, the dispersion of reagents is problematic.

\begin{center}
\textbf{Scheme 1.8.} Multistep flow synthesis utilizing inline purification by solid-supported scavenging agents\textsuperscript{60}
\end{center}

1.4.2.3 Extraction

A second type of work-up for liquid flows is extraction. In continuous flow, the input of an additional flow containing the washing or extraction fluid is straightforward, but separation of the immiscible liquids requires attention. One of the earliest examples is the microextraction system reported by Kitamori et al. (Figure 1.6a).\textsuperscript{61} A special chip design allows two immiscible liquids to flow in parallel laminar regimes enabling splitting of flows by simple Y-separation. This module is quite limited in terms of flow rate and ratio. However, based on this design, better and more general applicable modules were designed.\textsuperscript{62,63}
A more advanced module is the one reported by Gaakeer et al. (Figure 1.6b).\textsuperscript{64} Instead of using a surface coating, which in time degrades by solvation or chemical irrestance, the module was manufactured from Teflon and glass. These two materials are both highly chemical resistant but since Teflon is hydrophobic and glass hydrophilic, the organic slugs are bend to the left and the aqueous slugs to the right, leading to complete separation of phases. So far, this module is only tested for heptane/water mixtures.

Completely different is the inline separator, which relies on gravitational forces. Two groups simultaneously reported a so-called static collector/phase separator module.\textsuperscript{65,66} Both separation modules were tested by means of a diazotization reaction of which one is shown in Scheme 1.9. A drawback of the static collector/phase separator is that separation can only be achieved when larger amounts of fluids are collected (in the range of mL)

Scheme 1.9. Synthesis of N-arylpyrazole 23 utilizing a static collector/phase separator module\textsuperscript{66}
Aniline 21 was reacted with tert-butyl nitrite (t-BuONO) to form the corresponding water-soluble diazonium salt. After washing with water, the aqueous phase was collected directly in the flask for subsequent reaction with SnCl₂ to form the corresponding hydrazine. When the flow synthesis was completed, keto-enamine 22 was added to the crude reaction mixture to form the desired N-arylpyrazole 23. The drawback of the static collector/phase separator is the hold up of intermediates, which makes this module unsuitable for reactive and instable intermediates.

Jensen et al. were the first to report on the application of membrane technology, in a polycarbonate separation module, utilizing a hydrophobic PTFE-membrane. Scheme 1.10 shows a multistep carbamate synthesis, in which this module is applied. Benzoyl chloride (24) was reacted with sodium azide in a phase transfer reaction to form the corresponding benzoyl azide. Next, the biphasic mixture was separated utilizing their in-house built separation module. Applying a pressure difference across the membrane significantly increased the throughput of the membrane. Upon heating, benzoyl azide was converted to phenyl isocyanate with and without the help of a solid acid catalyst. Subsequently, nitrogen was removed utilizing an altered membrane-based gas-liquid separator. In the final step, carbamates 25 were formed upon reaction of phenyl isocyanate with an alcohol of interest.

Based on this first L-L separation module, similar modules were fabricated from stainless steel and polyimide all utilizing a PTFE-membrane. Additionally, the industrial application of a membrane-based L-L separator was successfully accomplished.
1.5. Research aim and thesis outline

1.5.1 The PoaC project

NWO (Netherlands Organization for Scientific Research) funds and steers the course of Dutch science by means of research programs. One of the subdivisions of NWO is ACTS (Advanced Chemical Technologies for Sustainability). ACTS is a public-private partnership between the Dutch government, universities, research institutes and industry in the field of sustainable chemical technologies. One of the programs under the ACTS umbrella is the Process on a Chip program (PoaC). The ambition of PoaC was to carry out an entire process on a chip, from feeding the raw materials, converting them into the desired chemical entities, carrying out analysis, maintaining control over the process parameters, to purifying the end product using flow chemistry.

The project described in this thesis was part of the NWO-ACTS Process-on-a-Chip (PoaC) program in which microprocessing principles were applied to downstream processing and purification. This particular project, ‘Microreactor chips with integrated work up functionality’, was a collaboration with Prof. Gardeniers and Prof. Dr. Ir. R. G. H. Lammertink (University of Twente) and was supported by the companies DSM, Lionix, Chemtrix, Micronit, FutureChemistry and TNO. The aim of the project was to study the inline integration of work-up steps in continuous flow reactions.

1.5.2 Thesis outline

Chapter 1 provides an introduction on multistep flow synthesis. Chromatographic and spectroscopic analysis procedures are discussed, as well as various separation and purification strategies.

Chapter 2 discusses the single-step flow synthesis of benzyl azide. Due to the explosive nature of azides this reaction represents a safety hazard when performed in large scale batch experiments. The diazotransfer reaction to convert benzylamine in the corresponding azide was translated from a batch to a flow process including a full optimization of the reaction conditions. This more general batch-to-flow translation procedure has been applied in the other chapters as well.

Chapter 3 describes the Prilezhaev dihydroxylation reaction in flow. Organic chemists use this reaction to convert an alkene diastereoselectively via the epoxide into the corresponding trans-diol. After establishing an optimized continuous flow process, the set-up was additionally used to convert five other substrates into the corresponding diols in moderate to excellent yields.

Chapter 4, the first results of application of a liquid-liquid membrane separation module are described. The Vilsmeier-Haack (VH) formylation was chosen as a model reaction. An additional feature of this reaction was that the in situ formation of the highly reactive VH-reagent was monitored real time with inline IR, which was crucial in the optimization process. After optimization of the complete reaction, six other substrates were investigated in the same set-up. Secondly, the formed 2-formylpyrrole was inline extracted utilizing a membrane-based separation module.
Chapter 5 reports on inline phase separation of a biphasic reaction mixture. Ethyl diazoacetate (EDA) formation is a commercially relevant, but dangerous reaction due to the explosive nature of the diazocompound. Since EDA, which is not water soluble, is formed in the aqueous phase, dichloromethane was added for solvation and safety reasons. A membrane-based separation module enabled the collection of the organic EDA layer. Research towards a subsequent cyclopropanation is also described.

In Chapter 6, the separation module was successfully implemented in the first chemoenzymatic cascade flow reaction. An aqueous phase containing an (R)-HNL enzyme lysate was separated from the organic phase containing the intermediate cyanohydrin. In order to prevent fast racemization of these products, a subsequent protection reaction was performed. Thus, the multistep flow synthesis of ten different protected cyanohydrins was investigated.

Chapter 7 provides a general discussion and outlook on the implementation of microreactor technology on labscale and in industrial processes.

I.6. References and notes

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Chapter 2

Continuous flow azide formation
Optimization and scale-up

Abstract
The intrinsically small volumes and highly controlled reaction conditions render continuous flow microreactors ideal systems for the synthesis of potentially explosive compounds such as organic azides. In this chapter, the formation of benzyl azide from benzylamine using imidazole-1-sulfonyl azide hydrochloride as diazotransfer reagent is discussed. In a small scale (semi-automated) continuous flow set-up the diazotransfer reaction was optimized using minimal amounts of reagents; less than 400 mg of benzylamine was required to perform 60 optimization reactions. The optimal reaction conditions were identified to be room temperature, 600 seconds of reaction time and an imidazole-1-sulfonyl hydrochloride to benzylamine molar ratio of 3 to 4. Furthermore, the reaction was successfully scaled with a factor of 200 to gram scale using a single larger continuous flow reactor.

This chapter has been published:
2.1. Introduction

Organic azides possess potentially explosive properties. Trace amounts of acid or specific metal salts may catalyze explosive decomposition due to the formation of molecular nitrogen. In addition, organic azides may also be shock and/or heat sensitive and will generally decompose on exposure to UV light. Nevertheless, organic azides have shown to be valuable and versatile intermediates in organic synthesis. In 2009, Kopach et al. reported the synthesis of 1-(azidomethyl)-3,5-bis-(trifluoromethyl)benzene in batch and in microreactor equipment by substituting a halide with an inorganic azide. This reaction was performed with an aqueous solution of sodium azide at a temperature of 90 °C. Both the high reaction temperature and the requirement of toxic sodium azide significantly decrease the applicability of this method.

An alternative route to organic azides proceeds via diazotransfer onto amine functionalities. The most commonly used diazotransfer reagent is triflyl azide, which in neat form is highly explosive. Due to its reactive nature, it has a relatively short shelf life and needs to be prepared prior to use. In 2007, Goddard-Borger and Stick invented a new diazotransfer reagent, imidazole-1-sulfonyl azide hydrochloride, which is stable and easy to prepare, and eventually cheaper compared to triflyl azide. In addition, reagent can be prepared in large amounts and was reported to be non-explosive. As a result, this diazotransfer reagent can be handled in pure form rather than in solution.

The synthesis of organic azides strongly illustrates the benefits of microreactor technology, in particular the inherently safe way of conducting chemistry due to small hold-up volumes and the closed system. Converting a known batch reaction into a flow synthesis is quite straightforward but does require some additional study. The high surface-to-volume ratio and excellent mass transfer in a microreactor greatly influences the reaction conditions such as temperature and reaction time. In this chapter, a standard protocol is developed to convert a batch process into a continuous flow process including optimization of the reaction conditions.

2.2. Results and discussion

2.2.1 Batch scale

The synthesis of benzyl azide (3) was investigated as a representative procedure for the synthesis of organic azides in microreactors (Scheme 2.1). Nyffeler et al. described the use of two catalysts, zinc chloride and copper sulfate. In some cases, zinc chloride provided better results in yield and reaction time than copper sulfate. Since both catalysts showed no difference in the formation of benzyl azide (3), zinc chloride was arbitrarily chosen as catalyst for the diazotransfer reaction.
In order to ensure well-defined reaction times in the continuous flow system, batch scale experiments were performed to develop a suitable quenching method. Adding a 1M solution of hydrochloric acid in ethyl acetate/acetone (1:1) to the reaction mixture, was identified as an adequate method to quench the reaction. Analysis of the samples was performed using a fast GC method (Section 5.5).

### 2.2.2 Microreactor system

A schematic representation of the microreactor set-up is shown in Scheme 2.2. Initial continuous flow experiments were performed using a water/methanol/dichloromethane (3:10:3) solvent system for both flows. Unfortunately, irreproducible GC yields were obtained probably due to high flow rate deviations. This might be caused by gas formation in the syringe containing solution B (imidazole-1-sulfonyl azide hydrochloride (2) and DIPEA). Changing the solvent system to methanol/dichloromethane (3:10) avoided the undesired evolution of gas.

**Scheme 2.1.** Diazotransfer reaction on benzylamine (1)

**Scheme 2.2.** A schematic view of the microreactor set-up

Preliminary univariate experiments were performed in order to investigate the kinetically critical parameters influencing the GC yield of benzyl azide (3). These experiments indicated the parameters reaction time, reaction temperature and the molar ratio of imidazole-1-sulfonyl azide hydrochloride (2) with respect to benzylamine (1) to be crucial in reaching an optimal yield of benzyl azide (3). In addition, the experiments also provided the range in which the three parameters should be varied in a multivariate optimization run (Table 2.1).
Table 2.1. Overview of kinetically critical reaction parameters and their range screened in a multivariate optimization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time</td>
<td>30 - 600 s</td>
</tr>
<tr>
<td>Reaction temperature</td>
<td>0 - 70 °C</td>
</tr>
<tr>
<td>Molar ratio</td>
<td>0.5 - 4</td>
</tr>
</tbody>
</table>

2.2.3 Reaction optimization

An experimental design was obtained with a D-optimal algorithm. The design was based on the aforementioned three parameters within the respective ranges (Table 2.1) using MATLAB (MathWorks, R2007a). This led to a set of sixty data points of which the corresponding experiments were performed in random order. The resulting GC yields were normalized and fitted to a third order polynomial model. In house-developed FlowFit software\(^1\) was used to calculate the best possible model fit providing a set of optimal values for the reaction parameters varied. The results are visualized in simple 2D-contour plots (Figure 2.1). In the case of benzyl azide (3) synthesis the theoretical set of optimal reaction conditions according to the model fit were 535 seconds of reaction time, a molar ratio of 4.6 while applying a temperature of 1.8 °C.

From the contour plots, three trends can be deduced. The first and most striking observation is that with increasing temperatures, lower yields will be obtained. This is most likely due to decomposition of imidazole-1-sulfonyl azide hydrochloride (2) at higher temperatures which was confirmed with NMR experiments at room temperature to 80 °C. A broad and robust optimal temperature range was found between 0 and 40 °C. This is useful for performing the reaction at production scale since no cooling or heating is required. Decomposition of diazotransfer reagent, which was earlier suggested, implies the need for a large excess of this compound in order to obtain benzyl azide (3) in high yield. This is in compliance with the optimum found for the molar ratio, namely 3 to 4. A third observation was a relatively long optimal reaction time of around 600 seconds required to obtain the product in high yield. In addition, contour plot B showed a decrease in yield at longer reaction times (> 700 s). However, the model fit shows a high uncertainty of the model in this time range. This region should therefore be ignored when making concluding remarks.

For each experiment performed in the optimization screening, an average of 6 mg of benzylamine (1) and 17.5 mg of diazotransfer reagent 2 was used. Consequently, for all sixty experiments less than 400 mg of benzylamine (1) and 1 g of imidazole-1-sulfonyl azide hydrochloride (2) was required to perform a full multivariate reaction optimization.
2.2.4 Continuous flow on gram scale

Based on the results and data interpretation of the small scale multivariate optimization experiments, a gram scale experiment was performed. The reaction was performed at room temperature, requiring no additional cooling or heating. Due to the slightly elevated temperature applied with respect to the optimal set for which 1.8 °C was indicated, a molar ratio of 4 was chosen. Furthermore, this molar ratio is less than in the optimal set to decrease production costs. For the synthesis of benzyl azide (3), a reaction time of 600 seconds was chosen requiring a total flow rate of 2 mL/min in a stainless steel coil reactor of 20 mL. The crude product (3) was collected in 230 mL of quenching solution for 95 minutes. In order to perform the reaction 1.4 g benzylamine (1) and 4.2 g imidazole-1-sulfonyl azide hydrochloride (2) was required, an increase of more than 200 times compared to the optimization experiments. A validation experiment before the actual scale-up, including the use of internal standards, proved to have a GC yield of 97%. After the actual scale-up experiment and a batch work-up procedure, benzyl azide (3) was obtained as a 73% solution in diethyl ether according to ¹H NMR (1.36 g, 65% isolated yield). Most likely, the relatively low yield is due to the low vapor pressure of benzyl azide (3) in combination with the need to evaporate the solvent(s) multiple times.

2.3. Conclusion

In a small scale continuous flow set-up, the reaction conditions for the conversion of benzylamine into benzyl azide were optimized. A semi-automated microreactor system and D-optimal-based design of experiment were used to obtain a multivariate landscape based on the three reaction parameters varied. The optimal reaction conditions were identified to be room temperature, 600 seconds reaction time and an imidazole-1-sulfonyl azide hydrochloride (2)/benzylamine (1) molar ratio of 3 to 4. In addition, we successfully scaled up the reaction by a factor of 200, resulting in the production of approximately 1 gram of benzyl azide (3) per hour using one single flow reactor.
2.4. Acknowledgements

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2.5. Experimental section

2.5.1 Benzyl azide synthesis

Benzyl azide (3) was prepared from benzylamine (1) using imidazole-1-sulfonyl azide hydrochloride (2) as the diazotransfer reagent.10

2.5.2 Microreactor set-up

A schematic representation of the FutureChemistry FlowStart microreactor set-up is shown in Scheme 2.2. All parts within the dotted line consist of a Micronit single glass microreactor with an internal volume of 92 μL, a channel width of 600 μm and a channel depth of 500 μm and an effective channel length of 360 mm. The channel layout contains two mixing units M, being of the folding flow type.12 In case of short reaction times, experiments were performed in a microreactor with an internal volume of 7.0 μL, a channel width of 120 μm, a channel depth of 55 μm and an effective channel length of 1325 mm. This channel layout contained no separate mixing units as the small internal diameter led to sufficient mixing by diffusion. In all cases, the reactor temperature was controlled by Peltier elements and sensed by a Pt1000 temperature sensor.

2.5.3 Reaction optimization

A FutureChemistry FlowScreen was used to perform the screening of reaction conditions. Three glass syringes with an internal volume of 1 mL were used in pumps P1, P2 and P3 as indicated in Figure 2.1. Pump P1 contained solution of benzylamine (1, 328 μL, 3.0 mmol), diisopropylamine (DIPEA, 1.57 mL, 9.0 mmol), ZnCl₂ (183 μL of 2.19 g/L MeOH/CH₂Cl₂ (10:3), 3.0 μmol), and 2-bromotoluene (250 μL, internal standard A) in MeOH/CH₂Cl₂ (10 mL, 10:3 v/v). Pump P2 contained a solution of imidazole-1-sulfonylazide hydrochloride (2, 625 mg, 3.0 mmol), DIPEA (524 μL, 3.0 mmol), and 5-bromo-m-xylene (250 μL, internal standard B) in MeOH/CH₂Cl₂ (10 mL, 10:3 v/v). In order to quench the reaction at the end of the channel, ensuring well-defined reaction times, pump P3 contained a solution of HCl (500 μL, 37% concentrated HCl) in EtOAc/acetone (10 mL, 1:1 v/v), which was added to the reaction after the reaction time channel (shown as meander channels in Scheme 2.2). The product (5 μL) was collected in CH₂Cl₂ (100 μL) containing 2% cyclooctane as an external standard. Table 2.2 shows all 60 experiments obtained from design of experiment (DOE) based on D-optimal algorithm.
<table>
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<th>Temperature (°C)</th>
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<sup>a</sup>The molar ratio of imidazole-1-sulfonyl azide hydrochloride (2) to benzyl amine (1); <sup>b</sup>Exact reaction time and stoichiometry are calculated using flow markers<sup>13</sup>; <sup>c</sup>Molar ratio of imidazole-1-sulfonyl azide hydrochloride (2) to benzyl amine (1); <sup>d</sup>Yields are normalized; <sup>e</sup>Experiments were removed before performing the model fit due to a high flow deviation of >40%.

Model equation:

\[
y = -0.038x_1 + 0.000146x_1^2 - 0.00000014x_1^3 + 0.0000495x_2^2 + 0.466x_3 - 0.00000104x_1x_2^2 + 0.00132x_1x_3
\]

Factors and response are defined as:

- \(y\) = Benzyl azide yield, \(x_1\) = Reaction time
- \(x_2\) = Temperature, \(x_3\) = Molar ratio

The polynomial fit resulting from 58 data points:

BIC-value of the model fit: -28.4

### 2.5.4 Scale-up reaction

A scale-up experiment was performed in a Uniqsis FlowSyn equipped with a 20 mL stainless steel coil reactor. With a flow A of 0.4 mL/min and flow B of 1.6 mL/min, a reaction time of 10 minutes was obtained. The product was collected for 95 minutes after
Continuous flow azide formation

15 minutes of stabilization. Pump P1 continuously pumped a solution of benzylamine (1, 3.28 mL, 30 mmol), DIPEA (15.7 mL, 90 mmol), and ZnCl₂ (1.87 mL of 2.19 g/L MeOH/CH₂Cl₂ (10:3 v/v), 30 µmol) in MeOH/CH₂Cl₂ (100 mL, 10:3 v/v). Pump P2 was used for the solution containing imidazole-1-sulfonyl azide hydrochloride (2, 12.5 g, 60 mmol) and DIPEA (10.5 mL, 60 mmol) in MeOH/CH₂Cl₂ (200 mL, 10:3 v/v). In contrast to the optimization set-up, no quench pump was used because the reaction time in the larger set-up could easily be determined. In order to stop the reaction, the product was simply collected in a quenching solution, 1M HCl in EtOAc/acetone (230 mL, 1:1 v/v). After collecting the product for 95 minutes, the reaction mixture was slowly concentrated under reduced pressure (max 200 mbar in a 40 °C water bath) to 30 mL. Care should be taken during this process due to the low vapor pressure of benzyl azide. The residual yellow oil was filtrated over silica (7 × 10 cm) using Et₂O as the eluent. Bulk solvent was gradually removed under reduced pressure (600 mbar in a 40 °C water bath) up to 50 mL and the residual crude product was washed with 1M HCl (3 × 50 mL) and brine (50 mL), dried over Na₂SO₄, filtrated and concentrated under reduced pressure (600 mbar in a 40 °C water bath). The remaining oil was then diluted with 15 mL Et₂O and again washed with 1M HCl (3 × 10 mL) dried over Na₂SO₄, filtrated and concentrated under reduced pressure (max 180 mbar in a 40 °C water bath) to yield a solution of 993 mg (7.4 mmol) benzyl azide (3) in Et₂O. This is in accordance with a calculated yield of 65% based on ¹H NMR analysis.

2.5.5 Analysis

Off-line GC-analysis was performed with a Shimadzu gas chromatograph (GC-2010) equipped with a Quadrex 007 1701 column (length: 15 m, internal diameter: 0.1 mm, film thickness: 0.1 µm) and a flame ionization detector. An injector temperature of 250 °C and a detector temperature of 325 °C were employed. An initial column temperature of 80 °C for 0.5 minutes was followed by a temperature ramp of 80 °C/min for 2.25 minutes and a final temperature of 260 °C was maintained for 0.25 minutes. The total GC program took 3 minutes. The product sample obtained from the microreactor was collected in dichloromethane containing 2‰ cyclooctane as an external standard. Accurate flow rates were calculated using our recently developed flow marker methodology.¹³

2.6. References and notes

2007, 63, 523-575.
11. www.futurechemistry.com
Chapter 3

Prilezhaev dihydroxylation of olefins in a continuous flow process

Abstract
Epoxidation of both terminal and non-terminal olefins with peroxy acids is a well-established and powerful tool in a wide variety of chemical processes. In an additional step, the epoxide can be readily converted into the corresponding trans-diol. Batchwise scale-up, however, is often troublesome because of the thermal instability and explosive character of the peroxy acids involved. This chapter describes the design and semi-automated optimization of a continuous flow process and subsequent scale-up to preparative production volumes in an intrinsically safe manner.

This chapter has been published:
Chapter 3

3.1. Introduction

The Prilezhaev dihydroxylation is a transformation often used in organic synthesis for the epoxidation of olefins and subsequent hydrolysis into the corresponding trans-diol.\(^1\) In this reaction, a peroxo acid is formed in situ by mixing the carboxylic acid with hydrogen peroxide and sulfuric acid.\(^2\) After addition of the olefin, basic hydrolysis to the diol is performed by addition of sodium hydroxide. This method, however, is laborious, and the thermal instability and explosive character of the peroxides, especially in a basic environment, render the scale-up difficult. Since its first publication in 1909, several attempts have been made to simplify the oxidation, and several alternative oxidizing agents, such as \(\text{m-CPBA}\), have become commercially available.\(^3\)–\(^10\) In addition to oxidation with peroxo acids, selective epoxidation of olefins by alkyl hydroperoxidases catalyzed by \(\text{d}^0\)-metal complexes (Mo\(^{\text{VI}}\), V\(^{\text{V}}\), and Ti\(^{\text{IV}}\)) has been developed for the manufacture of propylene oxide.\(^11\) Both types of oxidizing agents and catalysts are rather expensive, and many of these oxidation processes suffer from the same thermal instability,\(^12\) making them unsuitable for most industrial applications. Continuous-flow microreactors can circumvent the aforementioned problems. In a microreactor, the actual reaction takes place on a microliter scale so that only small amounts of peroxo acid are present during the process. A flow-chemistry approach of the Prilezhaev dihydroxylation would enable an industrial scale application with enhanced safety because the actual active reaction volume remains within several milliliters.

In 2007, Hartung, Keane, and Kraft demonstrated the feasibility of the oxidation of cyclohexene with in situ-prepared peroxy formic acid in a qualitative continuous flow set-up using HPLC-tubing. In an additional step, the epoxide was converted into trans-cyclohexane-1,2-diol.\(^14\) Based on these results, a more widely applicable method was developed for the oxidation of olefins in flow by using the commercially available peroxy acetic acid.

3.2. Results and discussion

3.2.1 Batch scale

The dihydroxylation of cyclohexene (1) (Scheme 3.1) was investigated as a generally applicable procedure for the dihydroxylation of olefins using peroxy acetic acid (2) as the oxidant. In a later stage, the resulting epoxide 3 was then converted into trans-cyclohexane-1,2-diol (4).

\[
\begin{array}{c}
\text{OH} \\
\text{OH}
\end{array} \quad \Rightarrow \quad \begin{array}{c}
\text{O}
\end{array} \quad \Rightarrow \quad \begin{array}{c}
\text{I}
\end{array}
\]

Scheme 3.1. Prilezhaev dihydroxylation of cyclohexene (1)

In the batch procedure, peroxy acetic acid and cyclohexene (molar ratio (MR) = 1) were stirred at 60 °C (Scheme 3.2) and after 4 hours, the reaction was quenched by using
an aqueous solution of sodium sulfite (1M). A quantitative conversion of cyclohexene was observed resulting in epoxide 3 (54%) and trans-diol 4 (36%), as analyzed by GC. In addition, diester 5 and monoester 6 were obtained as minor sideproducts. The large amount of diol observed was probably caused by acidic hydrolysis during the reaction.

**Scheme 3.2.** Epoxidation of cyclohexene

### 3.2.2 Microreactor system

In a continuous flow process (Scheme 3.3), cyclohexene and toluene (internal standard; syringe A) were mixed (M) with a peroxy acetic acid solution (syringe B) for the oxidation reaction to proceed. The reaction was quenched by using an aqueous solution of sodium sulfite (syringe Q), and the reaction mixture was collected in a mixture of acetone/H\textsubscript{2}O (1:1, v/v). In flow, a similar yield of the epoxide (67%) was obtained as described above for the batch procedure, but because of effective heat transfer in the reactor, the reaction time could be significantly decreased to 5 minutes when heated to 60 °C. Moreover, only 10% of diol 4 together with traceable amounts of 5 and 6 was observed under these conditions.

**Scheme 3.3.** Microreactor set-up for epoxide optimization

### 3.2.3 Reaction optimization

A full optimization study was performed based on a reaction time range (t = 60, 108, 180, 300 s), temperature range (T = 25, 36, 48, 60 °C), and molar ratios of peroxy acetic acid to cyclohexene (MR = 0.6, 1.4, 2.2) spread across the optimization region.\textsuperscript{15,16} From these ranges, 50 data points (including duplicates and triplicates) were selected using a D-optimal algorithm. All experiments were performed in random order, and the collected samples were analyzed off line by GC. The GC results were normalized and
fitted to a third-order polynomial model. In house-developed FlowFit software\(^{17}\) was used to calculate the best possible model fit to provide a set of optimal values for the reaction parameters. The results are visualized in 2D-contour plots (Figure 3.1). The optimal reaction conditions according to the model fit led to full conversion of cyclohexene in a reaction time of 300 s, a temperature of 60 °C, and a molar ratio of 1.2. However, from this model, various optimal parameter sets can be selected, depending on the demands that have to be satisfied.

Figure 3.1. Contour plots provided by the model fit of the results obtained in the multivariate optimization

### 3.2.4 Continuous flow on gram scale

Initially, an inline flow approach for epoxide hydrolysis and ester saponification was investigated. Quenching was not required as the reaction was driven to full completion; the quench inlet was used to add an aqueous solution of sodium hydroxide (5 equiv, 3.5M) to the crude reaction mixture to hydrolyze the epoxide along with saponification of the mono- and diesters. Although hydrolysis proceeded quantitatively, the large amount of salts formed in the second reaction led to repetitive clogging of the reactor. Therefore, it was decided to collect the epoxide in a solution of sodium hydroxide (3.5M) (Scheme 3.4). A fast hydrolysis to a nearly quantitative conversion of trans-cyclohexane-1,2-diol (4) was observed when starting from cyclohexene (1). Traceable amounts of mono- and diester were also obtained, but a full conversion to the diol was accomplished after standing for
an additional 2 hours (Scheme 3.5).

Scheme 3.5. Epoxide hydrolysis in flow

By using the optimal conditions obtained from the optimization data, a preparative synthesis of the target compound (trans-cyclohexane-1,2-diol) resulted in continuous production at a 2.46 g/h rate when using a Uniqsis FlowSyn apparatus with a reactor having an internal volume of 650 µL. If required, the production can readily be scaled up to higher rates by using parallel reactors.

3.2.5 Substrate scope

Based on the optimal conditions obtained in the dihydroxylation of cyclohexene (1), a range of different substrates was screened, comprising both terminal and internal olefins (Table 3.1). Initially, substrates 7, 9, and 11 were oxidized by using the previously found optimal conditions. The latter substrates, however, appeared to be less reactive than cyclohexene, which resulted in low to moderate yields ranging from 5–64%. Extended reaction times and slightly higher temperatures were therefore used for the preparative-scale experiments. Dihydroxylation of the terminal olefin 4-phenyl-1-butene (11, entry 3) on a 100 mg scale resulted in a satisfactory isolated yield of 74%.

Table 3.1. Screening of terminal and non-terminal olefins

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<td>9</td>
<td>10</td>
<td>65% t = 10 min, T = 65 °C, MR = 1.2</td>
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<tr>
<td>3</td>
<td>11</td>
<td>12</td>
<td>74% t = 5 min, T = 75 °C, MR = 1.2</td>
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<td>4</td>
<td>13</td>
<td>14</td>
<td>&gt;99% t = 5 min, T = 60 °C, MR = 1.2</td>
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</table>
Conversely, the terminal olefins 1-octene (7, entry 1) and methyl eugenol (17, entry 6) appeared slightly less reactive, resulting in somewhat lower yields of 25 and 31%, respectively, together with a substantial amount of starting material. Oxidation of 1-octadecene (15, entry 5) gave only trace amounts of the product, which can be probably attributed to a low solubility of the lipophilic olefin in the aqueous solvent mixture. The internal olefins trans-4-octene (9, entry 2) and trans-oleic acid (13, entry 4) were readily dihydroxylated in good to high isolated yields of 65 and 99%, respectively.

### 3.3. Conclusion

It has been demonstrated that the Prilezhaev dihydroxylation can be performed on larger scale in a continuous-flow microreactor system for the oxidation of both terminal and internal olefins. A full optimization study for the epoxidation of cyclohexene was performed by using a D-optimal algorithm for the parametric optimization of reaction time (t), temperature (T), and molar ratio (MR). Optimal conditions were reached in a reaction time of 300 seconds at 60 °C with a molar ratio of 1.2. With the optimal conditions found, an off-line procedure was developed to convert the epoxide in the trans-diol. The newly developed method was used to successfully scale-up the dihydroxylation of cyclohexene to continuously produce 2.46 g/h of trans-cyclohexane-1,2-diol (4). The overall yield of 82% showed that the continuous process performs similar to the conventional batch procedure. The major benefits, however, of performing this flow process are better control and therefore less safety risks and a faster overall process, leading to a significantly higher throughput. The process also shows good applicability to some other substituted olefins.

### 3.4. Acknowledgements

Florian Kössl, Bas van den Broek and René Becker (all FutureChemistry, Nijmegen, The Netherlands) are gratefully acknowledged for their work, help, support and fruitful discussion.
3.5. Experimental section

3.5.1 Batch synthesis

A typical procedure for the oxidation in batch: cyclohexene (5.6 mL, 55.0 mmol) was slowly added to peroxy acetic acid (12.8 mL, 61.0 mmol, 32% w/w) at room temperature. The reaction mixture was stirred at 60 °C for 4 hours. For a quantitative analysis, the reaction was quenched with an aqueous sodium sulfite solution (1M). The remaining acid was evaporated, and the residue was treated with an aqueous solution of sodium hydroxide (5M, 20 mL) at 60 °C for 45 min. The solution was neutralized using hydrochloric acid (1M) and concentrated in vacuo. The residue was washed with ethyl acetate (430 mL) to extract the diol and recrystallized from cyclohexene. Yield: 5.5 g, 86%; m.p.: 101 °C; $^1$H NMR (300 MHz, CDCl$_3$, $\delta$): 1.10–1.33 (m, 4H), 1.60–1.75 (m, 2H), 1.90–2.05 (m, 2 H), 2.10–2.60 (br s, 2 H), 2.35–2.40 ppm (m, 2 H). $^1$H NMR data is in agreement with literature.$^{18}$

3.5.2 Microreactor set-up

Schematic representations of the FutureChemistry FlowStart microreactor set-ups are shown in Schemes 3.3 and 3.4. All parts within the dotted lines consist of a Micronit single glass microreactor with an internal volume of 92 μL (Scheme 3.3) or 100 μL (Scheme 3.4), a channel width of 600 μm and a channel depth of 500 μm and an effective channel length of 360 mm. The channel layout contains two mixing units M, of the folding flow type.$^{19}$ The reactor temperature was controlled by Peltier elements and sensed by a Pt1000 temperature sensor.

3.5.3 Reaction optimization

A typical procedure for the oxidation in flow. Peroxy acetic acid (32% w/w; solution B) and an aqueous solution of sodium sulfite (1M, solution Q) were filled into 10 mL syringes. Cyclohexene (3.95 mL, 39.0 mmol) and toluene (803 mL, 7.55 mmol) (internal standard) were mixed together and filled into a 5 mL syringe (solution A). The syringes were connected to the FlowStart system and the flows were actuated. For a quantitative analysis, the reaction mixture (100 mL) was collected in acetone/water (400 mL, 1:1).

The FutureChemistry FlowScreen automated reaction optimization platform was used to perform the screening of reaction parameters. The set-up was identical to the FlowStart B-200, but equipped with a sample collector and software to run automated reaction parameter sequences. A glass syringe with an internal volume of 5 mL and plastic syringes (NORM-JECK) of 10 mL were used, as indicated in Figure 3.1. Pumps P1 and P2 were loaded with cyclohexene (5 mL) and peroxy acetic acid (10 mL), respectively. Pump P3 contained an aqueous solution of sodium sulfite (1M, 10 mL). The quenching and sampling procedure were left unchanged.
Table 3.2. Data set obtained by and results obtained after DOE

<table>
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<td>Experiment #</td>
<td>Molar ratio (PAA/CH)</td>
<td>Reaction time (s)</td>
<td>Temperature (°C)</td>
<td>GC-yield (%)</td>
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<td>46</td>
<td>0.6</td>
<td>300</td>
<td>25</td>
<td>36.63</td>
</tr>
</tbody>
</table>

*Yield determined by GC.

Model equation:

\[ y = \frac{100}{(1 + e^{-y_{\text{logit}}})} \]

\[ y_{\text{logit}} = -1.37 - 0.002094x_1 + 0.0001927x_1x_2 - 0.676x_3 + 0.05182x_3x_2 - 0.007796x_2^2 \]

Factors and response are defined as:

\[ y = \text{cyclohexene oxide 3 yield}, \quad y_{\text{logit}} = \text{logit value of cyclohexene oxide 3 yield} \]

\[ x_1 = \text{Reaction time (s)}, \quad x_2 = \text{Temperature (°C)}, \quad x_3 = \text{Molar ratio peroxy acetic acid to cyclohexene} \]

### 3.5.4 Scale-up reaction

A scale-up experiment was performed by using a Uniqsis FlowSyn equipped with a glass microreactor (0.65 mL) containing folding flow-type mixing units. The general set-up was similar to the one depicted in Scheme 3.3, but the quenching feed was omitted. With a flow rate A (cyclohexene) of 39.1 mL/min and a flow rate B (peroxy acetic acid, 32% w/w in acetic acid/water) of 90.8 mL/min, a reaction time of 5 minutes was set to obtain full cyclohexene conversion. In contrast to the optimization, no inline quenching was used, because the reaction was driven to completion. The reaction mixture was collected for 157 min. Excess peroxy acetic acid from the collected reaction mixture was removed under reduced pressure, and the residue was treated with NaOH (5M, 20 mL) at 60 °C for 45 minutes and neutralized with aqueous HCl. The solvent was removed under reduced pressure, and the product was extracted from the residue by using ethyl acetate (430 mL) and concentrated to yield trans-cyclohexane-1,2-diol (5.81 g, 50.5 mmol) in a 82% isolated yield, which was comparable to the yield obtained in the batch process (86%).

### 3.5.5 Substrate scope

A typical procedure for oxidation of other alkenes in flow: pure alkene (solution A) and peroxy acetic acid (32% w/w, solution B) were filled into a 1 or 5 mL glass syringe depending of the flowrates. The syringes were connected to the FlowStart system and the flows were actuated. Product was collected for 1 hour after which the mixture was concentrated under reduced pressure. To the residue NaOH (5M, 2 mL) was added. The reaction was heated to 60 °C for 45 minutes. The solution was neutralized with aqueous HCl (37% w/w) and concentrated in vacuo until only solid residue was left. The residue was extracted with ethyl acetate (total of ± 25 mL). The corresponding diol was obtained after removing all solvent under reduced pressure and subsequently analyzed by $^1$H NMR.
In case of substrate 14 demineralized water (5 mL) was added to the sodium hydroxide mixture to facilitate stirring. Additionally, substrate 14 and 16 were not concentrated in vacuo but immediately extracted with ethyl acetate (3 x 10 mL) and dichloromethane (3 x 10 mL).

### 3.5.6 Analysis and compound characterization

All product mixtures were analyzed by GC; by a Shimadzu GC2010 apparatus equipped with a Quadrex 007 1701 apolar column (length 15.0 m, inner diameter 0.10 mm) and a flame-ionization detector (T = 325 °C, hydrogen flow rate 60 mL/min, air flow rate 400 mL/min), the following temperature program was used (0-0.8 min: 35 °C; 0.8-4.9 min: 35-200 °C; 4.9-5.4 min: 200 °C), and a 1.0 mL injection with a split ratio of 200 (250 °C injection temperature). GC retention times of the compounds are provided in Table 3.3. NMR spectra were acquired at ambient temperature with a Bruker DMX 300 MHz spectrometer (1H 300 MHz).

**Table 3.3. GC retention times**

<table>
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<tr>
<th>Compound</th>
<th>Function</th>
<th>Retention time (min)</th>
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<tbody>
<tr>
<td>Cyclohexene</td>
<td>Substrate</td>
<td>0.59</td>
</tr>
<tr>
<td>Toluene</td>
<td>Internal standard</td>
<td>1.05</td>
</tr>
<tr>
<td>Cyclohexene oxide</td>
<td>Intermediate</td>
<td>1.63</td>
</tr>
<tr>
<td>Trans-1,2-cyclohexadiol</td>
<td>Product</td>
<td>3.15</td>
</tr>
</tbody>
</table>

**Octane-1,2-diol (8)**

1H NMR (300 MHz, CDCl3) δ = 3.77-3.69 (m, 1H), 3.66 (dd, J = 10.9, 3.0 Hz, 1H), 3.43 (dd, J = 10.9, 7.6 Hz, 1H), 1.56-1.16 (m, 10H), 0.89 (t, J = 6.8 Hz, 3H). 1H NMR was in accordance with literature.

**Anti-octane-4,5-diol (10)**

1H NMR (300 MHz, CDCl3) δ = 3.66-3.57 (m, 2H), 1.82 (br s, 2H), 1.60-1.28 (m, 8H), 0.95 (t, J = 7.1 Hz, 6H). 1H NMR was in accordance with literature.

**4-Phenylbutane-1,2-diol (12)**

1H NMR (300 MHz, CDCl3) δ = 7.38-7.26 (m, 2H), 7.24-7.12 (m, 3H), 3.78-3.69 (m, 1H), 3.66 (dd, J = 18.1, 7.1 Hz, 1H) 3.47 (dd, J = 11.0, 7.5 Hz, 1H), 2.87-2.63 (m, 2H),
1.95-1.63 (m, 2H). $^1$H NMR was in accordance with literature. $^{22}$

\[
\begin{align*}
\text{OH} & \quad \text{O} \quad \text{+} \quad \text{OH} \\
3 & \quad 3 & \quad 3
\end{align*}
\]

**Anti-9,10-dihydroxyoctadecanoic acid (14)**

$^1$H NMR (300 MHz, CD$_3$OD) $\delta = 3.33-3.29$ (dt, $J = 8.3$ Hz, 1H), 2.97-2.87 (m, 1H), 2.27 (td, $J = 7.5, 1.3$ Hz, 2H), 1.67-1.56 (m, 2H), 1.54-1.24 (m, 22H), 0.89 (t, $J = 6.3$ Hz, 3H). $^1$H NMR was in accordance with literature. $^{23}$

### 3.6. References and notes

13. www.futurechemistry.com
17. This software is commercially available www.futurechemistry.com
Chapter 4

Continuous flow production of thermally unstable intermediates

Controlled Vilsmeier-Haack formylation of electron-rich arenes

Abstract
The Vilsmeier-Haack formylation of aromatic compounds is a well-established process in organic synthesis, largely driven by the fact that the resulting aldehydes are generally useful intermediates for the synthesis of fine chemicals and pharmaceutical products. Industrial-scale production, however, is often hampered by laborious procedures requiring the use of hazardous chemicals to produce the highly reactive intermediates. In order to circumvent these issues, a flow chemistry approach was developed including inline analysis and work-up.

Part of this chapter has been published:
4.1. Introduction

The methodologies developed throughout the years for formylation of aromatics are as numerous as they are diverse, indicating not only the intrinsic value of aromatic aldehydes as synthetic intermediates but also the lack of generally applicable routes to synthesize these aldehydes. Hence, a large variety of relatively specific syntheses are well-established, which however appear to be rather laborious.\textsuperscript{1-6} Moreover, hazardous chemicals are often required, rendering scaling up problematic.\textsuperscript{7} Nowadays, the Vilsmeier-Haack (VH) reaction is frequently used for formylation of electron-rich arenes\textsuperscript{8-12} and ketones,\textsuperscript{13} and in addition is used in cyclohaloaddition,\textsuperscript{14-16} cyclization reactions,\textsuperscript{17} and ring annulations.\textsuperscript{18} In the VH reaction a chloroiminium ion is formed as the reactive species by mixing a substituted amide with phosphorous oxychloride.\textsuperscript{19} The highly electrophilic iminium ion then reacts with the arene, after which basic hydrolysis with sodium hydroxide leads to the aldehyde. Albeit that the intermediate chloroiminium ion can be readily prepared, calorimetric studies have demonstrated that its formation poses specific hazards due to thermal instability and generating high and fast temperature rises when heated, possibly resulting in a thermal runaway.\textsuperscript{7} This requires active cooling, and the latter makes batchwise scale-up rather troublesome.\textsuperscript{20} A flow chemistry approach including work-up of the product was envisioned to enable the VH formylation at industrial scale with enhanced safety.

In 2010, Kim \textit{et al.} briefly demonstrated the feasibility of VH formylation of N,N-dimethylaniline as a proof for their newly developed flexible polyimide (PI) film microreactor.\textsuperscript{21} On the basis of these results, in this chapter an extensive investigation is performed towards the opportunities for a more widely applicable flow method for formylation of electron-rich arenes. In addition, the benefits of inline IR analysis is demonstrated by investigating the reactive chloroiminium ion.

4.2. Results and discussion

\begin{equation}
\text{Me}_2\text{NH}_2 \xrightarrow{\text{POCl}_3} \text{Me}_2\text{N}^+\text{HCl}^\circ \xrightarrow{\text{NaOH}} \text{Me}_2\text{N}^+\text{O}^\circ\text{Cl}^\circ
\end{equation}

\begin{equation}
\text{H} \xrightarrow{3} \text{H}^+\text{NMe}_2 \xrightarrow{\text{NaOH}} \text{H}^+\text{O}^\circ\text{Cl}^\circ
\end{equation}

\textbf{Scheme 4.1.} Vilsmeier-Haack formylation of pyrrole

The formylation of pyrrole (5) was chosen as a representative case for VH formylation in a continuous flow process (Scheme 4.1). In batch, 2-formylpyrrole (6) is prepared in a three-step procedure, first allowing the chloroiminium ion 3 to be formed from DMF at low temperature, generally at 0 °C. In a second step, pyrrole is added at
elevated temperatures (typically 60 °C), while in a third step the resulting iminium adduct 5 is hydrolyzed to give product 6 upon heating in a sodium acetate or sodium hydroxide solution.\textsuperscript{22}

4.2.1 Microreactor set-up

Transforming this three-step synthesis into a continuous flow process resulted in a microreactor set-up as is depicted in Scheme 4.2. DMF (1) and phosphorous oxychloride (POCl\textsubscript{3}) are mixed using a T-splitter and transferred into a coil (C1) to allow the chloroiminium ion (3, Vilsmeier-Haack reagent) to be formed. An excess of DMF was used to ensure full conversion of POCl\textsubscript{3} and to retain the intermediate in solution. Reactive intermediate 3 is then delivered into the temperature controlled glass microreactor together with pyrrole (4) and toluene (internal standard) for the formylation reaction to proceed. Finally, the reaction is quenched with a mixture of H\textsubscript{2}O and ethanol. The resulting iminium ion 5 is hydrolyzed offline in a NaOH (2.7M) solution.

\begin{center}
\textbf{Scheme 4.2.} Schematic representation of microreactor set-up
\end{center}

4.2.2 Investigation of the Vilsmeier-Haack reagent by UV and inline IR

Initial experiments showed that it is critical to reach full conversion of POCl\textsubscript{3} in the coil, because it reacts vigorously with pyrrole to form polymers, thereby clogging the reactor. A batchwise spectrophotometric method was developed to determine the reaction time for formation of the chloroiminium ion 3 at room temperature. Measuring the reaction time without additional solvent proved to be impossible. In addition, DMF shows UV absorbance around 300 nm and being used as a solvent, its UV absorbance overlaps with that of the iminium ion (Figure 4.1a). The absorbance was therefore measured at low concentrations of DMF, using acetonitrile as the solvent. Under these conditions, UV absorbance at a wavelength of 304 nm showed that formation of chloroiminium ion 3 was completed within 90 seconds (Figure 4.1b).

The main issue encountered during the UV measurements was the inability to perform real-time analysis of the unstable intermediate without the use of additional solvent (\textit{e.g.} MeCN). This problem was overcome by connecting inline a Mettler Toledo FlowIR infrared flow cell\textsuperscript{23} with the outlet of the microreactor. The data obtained from this inline infrared analysis provided insight not readily available from other sources.
Figure 4.1.  a) UV absorption spectra of DMF, intermediate and POCl₃  b) Formation of VH reagent (3) in MeCN at 304 nm

In order to identify spectral bands unique to the intermediate species, infrared spectra of both substrates DMF and POCl₃ were recorded using the FlowIR system. Next, the formation of the VH reagent was visualized by the relative intensity of a characteristic stretching vibration (C-Cl) at 769 cm⁻¹ (Figure 4.2a), measured at fixed reaction times. The results show an initially fast formation of the phosphonium salt 2 (Figure 4.2), as identified by a characteristic bending frequency (P-O-C, 804 cm⁻¹). The formation of the Vilsmeier-Haack reagent 3 shows a rapid increase between 50 and 100 seconds reaction time, which may be caused by increasing chloride concentration due to decomposition of the dichlorophosphate counterion of 3 (Scheme 4.1). From these results, it was concluded that the formation of the VH reagent was completed within 90 seconds.

Figure 4.2.  a) IR absorption spectra, b) Conversion of the phosphonium salt 2 and the chloroiminium ion 3

4.2.3 Reaction optimization

Next, a full optimization study was designed on the basis of reaction time (t = 10, 30, 60, 120, 180, 300 s), temperature (T = 0, 20, 40, 60 °C), and molar ratio of POCl₃ to the pyrrole substrate (MR = 1, 2, 3, 4). The formation of chloroiminium ion 3 was performed in 90 seconds unless stated otherwise. Iminium ion 5 was quenched with a mixture of water and ethanol, ensuring well defined residence times directly followed by offline hydrolysis by aqueous sodium hydroxide and ethanol in the collection vial. Experiments were performed in random order, and the collected samples were analyzed by HPLC (Figure 4.3). Full conversion was observed in 180 seconds, at 60 °C, using a molar ratio of...
1.5. These conditions were therefore considered as the optimal conditions.

![Figure 4.3](image)

**Figure 4.3.** Reaction optimization. Fixed parameters were 180 s, 20 °C and a molar ratio of 1.5 (chloroiminium ion/pyrrole)

Being able to reach a full conversion, different solvents were evaluated to reduce the use of DMF. The results are presented in Table 4.1, showing that there is an evident difference in the conversion to 2-formylpyrrole (6) in different solvents. The results indicate comparable reaction rates for polar solvents such as DMF (entry 1) and acetonitrile (entry 2) compared to the less polar THF and EtOAc (entries 3-4). Even though the reaction rate is slightly lower in the latter solvents, they clearly show additional value by being less toxic.

**Table 4.1.** Solvent effect

<table>
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<th>Entry</th>
<th>Solvent</th>
<th>Yield (%)a</th>
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<td>DMF</td>
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<tr>
<td>2</td>
<td>Acetonitrile</td>
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<tr>
<td>4</td>
<td>EtOAc</td>
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*aReaction conditions: 180 s, 60 °C and a molar ratio of 1.5

**4.2.4 Continuous flow on gram scale**

Scaling-up of the Vilsmeier-Haack formylation in batch reactors generally poses thermal runaway threats due to the highly exothermic formation of the reactive species. Using the optimal conditions obtained from the optimization data eliminates the need for active cooling completely. A preparative synthesis of 2-formylpyrrole (6) resulted in a continuous production at a 6.0 g/h rate using the Uniqsis FlowSyn equipped with a 0.65 mL glass microreactor and a 2.4 mL stainless steel coil reactor with an internal diameter of 1 mm. The Vilsmeier-Haack reagent was formed in the glass microreactor with a reaction time of 97.5 s, and the outflow was directly pumped into the coil for the formylation of the pyrrole to proceed. Quenching of the reaction was not required since the reaction was driven to full completion. The intermediate iminium ion species was therefore directly hydrolyzed in a solution of NaOH (2.5M) for 60 minutes and subsequently extracted with Et₂O to yield 5.98 g (98%) of 2-formylpyrrole.
4.2.5 Substrate scope

On the basis of the optimal conditions obtained with pyrrole, a range of different substrates were screened (Table 4.2). The conversions were determined by HPLC and confirmed by high resolution mass spectrometry. Isolated yields are depicted in Table 4.2. It was concluded that amine substituted arenes (entries 2-5) are highly reactive towards Vilsmeier-Haack formylation, all showing good conversion within 180 seconds. Di- and triformylation of triphenylamine 13 proved to be less effective, resulting in extended reaction times at elevated temperatures. Anisole (7, entry 1) appeared much less reactive than its amine-substituted derivative 9 since only trace amounts of product were observed.

<table>
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<th>Substrate</th>
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<th>Yield(^a)</th>
</tr>
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<td></td>
<td>MR = 1.5</td>
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<td>MR = 1.5</td>
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</tbody>
</table>

\(^a\)Isolated yield.
4.2.6 Flow Liquid-Liquid Extraction

After collection and offline hydrolysis a batchwise extraction was performed to yield the pure products. In order to increase the industrial application of the Vilsmeier-Haack formylation, an inline hydrolysis and extraction were investigated, (Scheme 4.3). By reducing the concentration of the quenching solution to 0.5M NaOH, inline hydrolysis of imine 5 was established without any clogging. Inline extraction was performed by first addition of the extraction fluid diethyl ether, followed by phase separation. The phase separation was obtained by connecting a Syrris Flow Liquid-Liquid Extraction (FLLEX) module24 to the system.25,26 The module utilizes a hydrophobic Teflon membrane and two backpressure regulators (BPRs) to create a pressure difference across the membrane. After collecting the organic phase, solvent was evaporated to obtain formylpyrrole (2) in 91% yield.

Scheme 4.3. Schematic representation of the Vilsmeier-Haack flow process including the work-up module

4.3. Conclusion

It has been demonstrated that the Vilsmeier-Haack formylation can be readily performed in a continuous flow microreactor system. A full optimization study was carried out involving reaction time (t), temperature (T) and molar ratio (MR) as process parameters. Optimal conditions were obtained at a reaction time of 180 seconds at 60 °C with a molar ration of 1.5 and DMF as a solvent. Other less toxic solvents also showed remarkably high conversions within 180 seconds. Under the optimized conditions, scale-up of the reaction has been successfully realized with a continuous production of 2-formylpyrrole of 5.98 g/h. The newly developed flow synthesis was also shown to be more widely applicable by the successful formylation of several amine-substituted arenes. Introduction of a liquid-liquid separation module yielded via inline extractive work-up the pure product in 91% yield, which is in the same range as for the gram scale flow experiment (98%).

4.4. Acknowledgements

Bas van de Broek (FutureChemistry, Nijmegen, The Netherlands) and Jeroen Leliveld are greatly acknowledged for their help and fruitful discussion during this project.
4.5. Experimental section

4.5.1 Microreactor set-up

DMF (1) and POCl₃ were mixed using a T-splitter and transferred into a coil (C1) to allow the chloroiminium ion (3) to be formed. An excess of DMF was used to ensure full conversion of POCl₃ and to retain the intermediate in solution. The reactive intermediate 3 was then delivered at a constant flow rate into the temperature controlled microreactor (FutureChemistry FlowStart) together with pyrrole and toluene (internal standard) for the formylation reaction to proceed. Finally, the reaction was quenched with a mixture of H₂O and ethanol. The resulting iminium ion 5 was hydrolyzed in a solution of NaOH (2.7 M) and ethanol. Ethanol was added to ensure solubility of the internal standard (toluene). A schematic drawing of the microreactor set-up is shown in Scheme 4.2. All parts within the dotted lines consist of a Micronit single glass microreactor with an internal volume of 92 μL (Scheme 3.3), a channel width of 600 μm and a channel depth of 500 μm and an effective channel length of 360 mm. The channel layout contains two additional mixing units (M) being of the folding flow type. The reactor temperature was controlled by Peltier elements and sensed by a Pt1000 temperature sensor.

4.5.2 Investigation of the Vilsmeier-Haack reagent by UV and inline IR

UV Analysis. To a UV vial containing acetonitrile (1 mL), POCl₃ (5 μL, 55 μmol) and DMF (5 μL, 65 μmol) were added. The vial was stoppered, quickly shaken, and immediately inserted into a UV/Vis spectrometer. The spectrometer measured the UV spectrum with 15 seconds intervals. In between the measurements, the vial was taken out of the spectrometer and shaken to homogenize the sample and promote the reaction. Compound specific vibrations were monitored at 769 cm⁻¹ (C-Cl) and 804 cm⁻¹ (P-O-C).

IR Analysis. A FutureChemistry FlowStart Evo was placed in line with the FlowIR (Mettler Toledo, diamond probe) to analyze the formation of the VH reagent. Two glass syringes with an internal volume of 5 mL were used, and pumps 1 and 2 were loaded with POCl₃ and DMF, respectively. A reactor with an internal volume of 100 μL was connected to the FlowIR to obtain a total system volume of 120 μL, including the internal volume of the flow cell (10 μL). Flows were fixed to corresponding reaction times, and IR spectra were recorded continuously.

4.5.3 Reaction optimization

A FutureChemistry FlowScreen was used to perform the reaction parameter screening. Four glass syringes with an internal volume of either 1 or 5 mL were used, as indicated in Figure 1. Pumps 1 and 2 were loaded with pure POCl₃ and DMF, respectively, for the formation of the Vilsmeier-Haack reagent. Pump 3 contained a solution of pyrrole (4, 1.85 mL, 26.7 mmol) and toluene (1.91 mL, internal standard) in DMF with a total volume of 10 mL. In order to quench the reaction at the end of the microreactor, ensuring well-defined reaction times, pump 4 contained a solution of EtOH/H₂O (5 mL, 1:1, v/v).
The intermediate iminium ion 5 was hydrolyzed in NaOH/EtOH/H₂O (540 μL NaOH (5M) and 460 μL EtOH/H₂O (1:1, v/v) to ensure solubility of the internal standard.

### 4.5.4 Scale-up reaction

A scale-up experiment was performed in a Uniqsis FlowSyn (FCUQ-1020) equipped with a glass microreactor (0.65 mL) containing folding flow type mixing units and a 2.4 mL stainless steel coil reactor with an internal diameter of 1 mm. With DMF flowing at 0.1 mL/min and POCl₃ at 0.3 mL/min, a reaction time for formation of the VH reagent of 97.5 seconds was obtained. It was reacted in the coil with a solution of pyrrole (4, 7.40 mL, 106.8 mmol) in DMF (32.60 mL) at 0.5 mL/min. With a total flow rate of 0.8 mL/min, a reaction time of 180 seconds was obtained. After a stabilization time of 10 minutes, the product was collected for 60 min. In contrast to the optimization, quenching was not necessary since the reaction was driven to full conversion. Instead, the outflow of the reactor was directly collected in a stirred solution of NaOH/H₂O (400 mL, 2.5 M). The product collected was extracted with Et₂O (3 × 200 mL). The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo (700 mbar, 40 °C) to yield 5.98 g of 2-formylpyrrole (6, 98% corrected for some residual DMF) as a viscous oil which solidified upon standing at -18 °C for 16 hours. ¹H NMR (300 MHz, CDCl₃) δ = 10.75 (br s, 1H), 9.48 (s, 1H), 7.18 (td, J = 1.3, 2.5 Hz, 1H), 7.01 (dd, J = 1.4, 3.9 Hz, 1H), 6.35 (dd, J = 2.5, 3.9 Hz, 1H). ¹H NMR data are in agreement with literature.²⁸

### 4.5.5 Substrate scope

A general procedure for the formylation in flow: The pumps containing pure DMF (18.3 µL/min) and POCl₃ (4.6 µL/min) were started 10 minutes prior to the rest of the pumps. Pump 3 contained a glass syringe filled with a 2.7M solution of the substrate in DMF (12.27 µL/min). The reaction was run at 60 °C with a reaction time of 180 seconds unless state otherwise. The reaction was quenched with H₂O/EtOH (1:1, v/v, 18.4 µL/min) and hydrolyzed in the collection fluid (0.4 mg NaOH in 3.6 mL H₂O/EtOH (1:1, v/v)). After stirring for an additional 2 hours at room temperature, the mixture was extracted three times with Et₂O. The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain the desired product. Isolated yields are depicted in Table 4.2. In the case of triphenylamine (13), a solution of 0.27M was prepared due to solubility issues. The corresponding product 14 was after isolation immediately used for a second formylation (entry 5, Table 4.2) of which product 15 was used for the third formylation (entry 6, Table 4.2).

### 4.5.6 Flow Liquid-Liquid Extraction

A schematic representation of this experiment is provided in Scheme 4.3. Pumps 1 and 2 pumping DMF (18.3 µL/min) and POCl₃ (4.6 µL/min) respectively, were started 10 minutes prior to the rest. After starting pumps 3 (pyrrole, 930 µL, 13.4 mmol in 4 mL DMF, 12.27 µL/min), 4 (0.5M NaOH in H₂O, 327.6 µL/min), and 5 (Et₂O, 300 µL/min) the reaction was stabilized for 30 minutes at 60 °C directly followed by collection. The FLLEX module
was set to 1 bar backpressure a pressure difference of 0.18 bar. The organic layer was collected for 40 minutes [crude 1]. The collected water layer [waste 1] was once more extracted with fresh Et₂O (both flows were set to 100 μL/min) to obtain organic layer [crude 2]. Crude 1 and 2 were combined dried over Na₂SO₄ and concentrated under reduced pressure to yield 115 mg of 2-formylpyrrole (6, 91%, corrected for some residual DMF).

### 4.5.7 Analysis and compound characterization

HPLC-analysis was performed using a Shimadzu HPLC system (Adsorbosphere C18 column (length: 100 mm, ID: 4.6 mm)) with a flow rate of 1.0 mL/min; acetonitrile/water: initially (40/60) for 4.0 min; 12.5 min (90/10); 13.0 min (40/60); 17.5 min (40/60); using a UV detector with analysis channels at 210 and 254 nm. Accurate flow rates of the substrate in the optimization experiments were calculated using toluene as an internal standard. NMR spectra were acquired at ambient temperature with a Bruker DMX 300 MHz spectrometer (¹H 300 MHz).

**4-(Dimethylamino)benzaldehyde (10)**

¹H NMR (300 MHz, CDCl₃, δ): 9.75 (s, 1H), 7.74 (d, J = 9.0 Hz, 2H), 6.71 (d, J = 9.0 Hz, 2H), 3.09 (s, 6H). ¹H NMR was in accordance with literature.²⁹

![4-(Dimethylamino)benzaldehyde (10)](image)

**1H-indole-3-carbaldehyde (12)**

¹H NMR (300 MHz, CDCl₃, δ): 10.08 (s, 1H), 8.72 (br s, 1H), 8.36-8.29 (m, 1H), 7.85 (d, J = 3.1 Hz, 1H), 7.48-7.41 (m, 1H), 7.37-7.29 (m, 2H). ¹H NMR was in accordance with literature.³⁰

![1H-indole-3-carbaldehyde (12)](image)

**4-(Diphenylamino)benzaldehyde (14)**

¹H NMR (300 MHz, CDCl₃, δ): 9.80 (s, 1H), 7.67 (d, J = 8.9 Hz, 2H), 7.38-7.30 (m, 4H), 7.19-7.13 (m, 6H), 7.01 (d, J = 8.6 Hz, 2H). ¹H NMR was in accordance with literature.³¹

![4-(Diphenylamino)benzaldehyde (14)](image)

**4,4’-(phenylazanediyl)dibenzaldehyde (15)**

¹H NMR (300 MHz, CDCl₃, δ, mixture with compound 13 (53:47)): 9.90 (s, 2H) 9.81 (s, 1H), 7.78 (d, J = 8.8 Hz, 4H), 7.68 (d, J = 8.9 Hz, 2H), 7.41-7.32 (m, 6H), 7.20-7.14 (m, 12H), 7.01 (d, J = 8.7 Hz, 3H).
4.6. References and notes

23. www.mt.com/FlowIR
24. www.syrris.com
Chapter 5

Ethyl diazoacetate synthesis in flow and integrated cyclopropanation

Abstract

Ethyl diazoacetate is a versatile reagent in organic chemistry and frequently used on lab scale. Its highly explosive nature, however, severely limits its use in industrial processes. The inline coupling of microreactor synthesis and separation technology enables the synthesis of this compound in an inherently safe manner, thereby making it available on demand in sufficient quantities. Ethyl diazoacetate was prepared in a biphasic mixture of an aqueous solution of glycine ethyl ester, sodium nitrite, and dichloromethane. Optimization of the reaction was focused on decreasing the reaction time with the smallest amount of sodium nitrite possible. In addition, a subsequent integrated cyclopropanation reaction was investigated.

Part of this chapter has been published:
5.1. Introduction

Diazo compounds are frequently used versatile building blocks in organic chemistry.1,2 From this class of compounds diazomethane and ethyl diazoacetate (I, EDA) are arguably the synthetically most useful ones. Due to the potentially explosive nature of diazomethane and EDA,3,4,5 however, synthetic routes that involve large scale batchwise handling of such diazo compounds are generally avoided in industrial processes. With the advent of continuous processing over the past decade, new approaches have appeared to conceptually change the way chemical synthesis is performed. While the synthesis of diazomethane has been extensively explored in batch6 and in continuous flow reactors,7,8 EDA is synthesized via different routes in batch,9,10 but relatively little is known about continuous flow approaches.11 Considering the importance of EDA in a wide variety of reactions e.g. cyclopropanation, X-H insertion, cycloaddition, and ylide formation,10,12 and more recently, in the synthesis of valuable compound classes such as β-ketoesters13 and β-hydroxy-α-diazo carbonyl compounds14, the development of an inherently safe continuous flow EDA process is highly desirable. For this process microreactor and separation technology were combined in order to obtain solely EDA.

Ethyl diazoacetate (I) in principle can be synthesized in flow via different pathways. Bartrum et al.15 published a flow synthesis of numerous diazo esters starting from the corresponding arylsulfonylhydrazones, in which the diazo moiety was installed through elimination of the sulfone substituent. An alternative pathway was published by Ley et al.,16 who prepared a range of α-hydroxy acids in flow starting from the corresponding amino acids, involving diazotization of the amine to the diazonium salt in a biphasic system. Inspired by Ley’s approach, which is significantly more atom efficient than the sulfonylhydrazone pathway, we set out to synthesize EDA (1) from glycine ethyl ester (2) using readily available sodium nitrite17 (Scheme 5.1). The first step of Ley’s hydroxy acid synthesis (starting from amino acids) resembles the diazotization of glycine ethyl ester. However, using an amino ester instead of an amino acid allows the diazo product to be isolated, which then can be used for subsequent reactions.

![Scheme 5.1. Synthesis of ethyl diazoacetate (1)](attachment:image)

The optimization process focused on decreasing the reaction time in order to reduce solvent use and gain in throughput. A possibly elevated reaction temperature was considered less of an issue since in an industrial setting energy can generally be efficiently regenerated. Inline phase separation was thought to greatly enhance the usefulness of the EDA flow synthesis. Therefore, the outlet of the microreactor was directly connected to a membrane-based phase separator to obtain EDA in the organic phase, which can then be immediately used for either batch10,12 or continuous flow13,14 follow-up chemistry. In this
case, we investigated cyclopropanation as a follow-up reaction.

5.2. Results and discussion

5.2.1 Microreactor set-up for ethyl diazoacetate synthesis

Ethyl diazoacetate (1) was synthesized from glycine ethyl ester (2) and sodium nitrite in a biphasic system of dichloromethane and an aqueous sodium acetate buffer. Dichloromethane was chosen as the organic phase to dissolve the water insoluble EDA because of its low water uptake, low boiling point, and compatibility with cyclopropanation reactions. In principle, however, any other organic solvent immiscible with water could be used. The pH of the buffer was set to 3.5 which had been identified by Clark et al. as the optimal pH for the reaction.9

A schematic representation of the initial microreactor set-up is shown in Scheme 5.2. At the outlet of the microreactor, a backpressure regulator (BPR, 40 psi) was attached to guarantee a liquid phase. To ensure well-defined reaction times during optimization experiments, neat N,N-diisopropylethylamine (DIPEA) was added by pump 4 to efficiently quench the reaction. The collected product was analyzed by HPLC to establish the conversion of the reaction.

Scheme 5.2. Schematic representation of the microreactor set-up

5.2.2 Reaction optimization of ethyl diazoacetate

Determination of the optimal conditions for the reaction started with investigating the important reaction parameters via a univariate optimization. Based on knowledge obtained from EDA synthesis in batch and flow reactions described in the preceding chapters, reaction time, temperature, and molar ratio of NaNO2 to glycine ethyl ester were chosen as relevant parameters. During the univariate screening one parameter was changed while maintaining the others at a fixed value. Temperature in particular was expected to have a large influence on the rate of the reaction. Shortening the reaction time to a minimum would minimize the risk of side reactions and reduce costs, and the reaction should be performed with the smallest amount of NaNO2 possible. The results of the univariate optimization are shown in Figure 5.1.
Univariate optimization using 30 s, 15 °C and a molar ratio 1.5 as standard

EDA synthesis was shown to be fast, since within 200 seconds complete conversion was obtained at 15 °C. Additionally, raising the temperature from 0-30 °C led to a steep increase in conversion of the reaction, while the molar ratio showed a rather small influence at 30 seconds of reaction time. Based on the univariate optimizations the experimental ranges of the three parameters were defined in order to investigate their interrelationship via a multivariate optimization.

An experimental design based on a D-optimal algorithm was created from the aforementioned three parameters within their respective ranges, namely 5-120 seconds, 0-60 °C, and a molar ratio between 0.7-1.5. Using MATLAB (MathWorks, R2007a), fifty data points were selected of which the corresponding experiments were performed in random order. The resulting HPLC yields were normalized and fitted to a third order polynomial model. In house-developed FlowFit software was used to calculate the best possible model fit. The results are visualized in 2D-contour plots (Figure 5.2).

These plots showed a rather broad optimum for the conversion of glycine ethyl ester (2) into EDA (1). The decrease in the upper left corner of the second contour plot was explained by the high uncertainty of the model at the edge of the plots. As was expected, temperature had a large influence on the reaction rate since the conversion into EDA showed a steep increase with increasing temperature. High temperatures and increasing amounts of NaNO₂ decreased the reaction time to full conversion to a minimum of 20 seconds. Not surprisingly, the minimal molar ratio was one.

The aim was to reach complete conversion into EDA (1) with the smallest amount
of sodium nitrite possible maintaining a short reaction time, possibly using higher temperatures. Based on these boundary conditions, the optimal parameter settings were fixed at 20 seconds reaction time, a temperature of 50 °C and using a molar ratio of 1.5. A triple-experiment was performed to prove that this set of optimal parameters indeed provided complete conversion into EDA. The experiment was performed in alternation with two other sets of parameters to rule out potential memory effects. HPLC yields of 95, 96, and 95% for the triple-experiment demonstrate the high reproducibility of the system.

### 5.2.3 Integrating the Flow Liquid-Liquid Extraction module

Having established a microreactor protocol for the continuous flow synthesis of EDA, the next issue was to separate the product from the biphasic system in which it was collected. In order not to lose the advantages gained by performing the EDA synthesis in flow, the phase separation had to be performed in flow as well. Therefore, a Flow Liquid-Liquid Extraction module (FLLEX) was connected to the system. A schematic representation of the whole set-up is shown in Scheme 5.3.

**Scheme 5.3.** Phase separation using a Flow-Liquid-Liquid-Extraction module (FLLEX) directly coupled to the microreactor

As the conversion into EDA was quantitative, quenching with DIPEA was no longer required. Between the microreactor and the FLLEX module some additional tubing was used to ensure complete partitioning of the compounds over the two phases. The backpressure of the FLLEX was set to 40 psi, similar to the BPR used previously, and the pressure difference was set to 0.14 bar. Direct full separation of phases resulted in a clean organic phase containing 409 mg of EDA (11 wt% solution in CH$_2$Cl$_2$ after 30 minutes of collection) while all salts remained in the water phase. This roughly corresponds to an EDA production of 20 g day$^{-1}$ and a space-time yield of 100 kg day$^{-1}$ dm$^{-3}$ as compared to a reported industrial scale batch process yielding EDA in 48 g day$^{-1}$ dm$^{-3}$.

### 5.2.4 Cyclopropanation of olefins with EDA and Rh$_2$(OAc)$_4$

Direct conversion of EDA into functionalized cyclopropanes in a single flow process (Table 5.1) will further increase safety by decreasing the hold-up of EDA. Three of the most commonly used transition metals in cyclopropanations are rhodium, iron and copper. To test the possibility of conducting both steps in a single process, initial experiments were performed in a batchwise manner using freshly synthesized EDA in combination with dirhodium tetraacetate (Rh$_2$(OAc)$_4$) (Table 5.1).
Table 5.1. \( \text{Rh}_2(\text{OAc})_4 \) catalyzed cyclopropanation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Catalyst loading (mol%)</th>
<th>Temperature (°C)</th>
<th>Reaction time (h)</th>
<th>Yield (%)\text{a,b}</th>
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| 1     | \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{3a.png} 
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0.3    | 21        | 29      | 35                      |
| 2     | \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{3b.png} 
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3b     & \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{4b.png} 
\caption{4b} 
\end{figure} |
0.3    | 21        | 28      | 38                      |
| 3     | \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{3c.png} 
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3c     & \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{4c.png} 
\caption{4c} 
\end{figure} |
0.3    | 21        | 24      | 31                      |
| 4     | \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{3a.png} 
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3a     & \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{4a.png} 
\caption{4a} 
\end{figure} |
0.3    | 45        | 1       | 36                      |
| 5     | \begin{figure}[H] 
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3b     & \begin{figure}[H] 
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\includegraphics[width=0.2\textwidth]{4b.png} 
\caption{4b} 
\end{figure} |
0.3    | 45        | 6       | 60                      |
| 6     | \begin{figure}[H] 
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3c     & \begin{figure}[H] 
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\caption{4c} 
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0.3    | 45        | 2       | 45                      |

\text{aIsolated yield. bProducts were obtained as diastereomers which were not separated.}

For the first set of experiments (entries 1-3), EDA was synthesized in flow, followed by the inline addition of potassium tert-butoxide to neutralize the biphasic reaction mixture, before separating the phases by the FLLEX module. The product was collected in a flask for 30 minutes containing 0.3 mol% \( \text{Rh}_2(\text{OAc})_4 \) and the substrate dissolved in \( \text{CH}_2\text{Cl}_2 \) at room temperature. After additional stirring overnight and purification by column chromatography, the products were obtained in 31 to 38% yield. Increasing the reaction temperature to 45 °C led to products 4a-4c. Since shorter reaction times were expected at elevated temperatures, the reaction time was varied arbitrarily. The corresponding products were obtained in 36 to 60% yield (entries 4-6). In all six experiments, a molar ratio of 3:1 alkene:EDA was used to obtain high conversions of EDA. Crude \( ^1\text{H} \) NMR spectra of entries 1-6 showed presence of EDA and trace amounts of EDA-dimer. 4-Phenyl-1-butene (3a) provided the corresponding cyclopropanation product (4a) under both conditions in similar yield (~35%) despite the shorter reaction time in the second set of experiments. The
two other substrates (3b and 3c) provided the corresponding products in higher yields, showing a clear influence of temperature on the reaction rate.

Next, an inline rhodium-catalyzed cyclopropanation was performed. Van Delft et al. published on the in house-developed synthesis of BCN (9-hydroxymethylbicyclo[6.1.0]nonyne). In order to conduct the first step of BCN synthesis in flow, cyclooctadiene was chosen as substrate. Due to the high flow rates required for the EDA synthesis, a flow of neat cyclooctadiene was necessary in order to keep the molar ratio (alkene:EDA) approximately 3:1. Rh₂(OAc)₄ (0.9 mM, 0.03 mol%) dissolved in cyclooctadiene was directly added to the organic phase after the FLLEX module (40 psi, ΔP = 0.1 bar) to obtain a reaction time of 30 seconds in a 100 μL glass reactor. Performing the cyclopropanation at 60 °C gave, according to 1H NMR, a product ratio of 60 mol% of EDA, 11 mol% of EDA-dimer, and 29 mol% of product. Longer reaction times were envisioned to improve the conversion of EDA. Switching the glass microreactor to larger volume gas permeable tubing (AF 2400) would additionally enable the nitrogen gas to escape from the reaction mixture. Unfortunately, tests with longer reaction times (up to 23 min) and temperatures ranging from 60 to 90 °C did not result in an increased formation of product. Based on these results other transition metals were investigated.

5.2.5 Cyclopropanation of styrene with EDA and an iron-porphyrin complex

Recently, Carreira et al. published on iron-catalyzed cyclopropanation reactions. With meso-tetraphenylporphyrin iron(III) chloride (6, Fe(TPP)Cl) cyclopropanations were performed in an aqueous environment while in situ generating the reagents diazomethane or EDA. The synthesis of Fe(TPP)Cl (6) seemed straightforward starting from meso-tetraphenylporphyrin (5) and FeCl₃. After refluxing the reaction mixture in DMF, it was poured in ice yielding a purple solid product after filtration. Thin layer chromatography and UV-spectroscopy (Figure 5.3a) showed a clean product after flash column chromatography.

![Scheme 5.4. Synthesis of meso-tetraphenylporphyrin iron(III) chloride (Fe(TPP)Cl)](image)

Styrene was chosen as model substrate based on the styrene derivatives investigated by Carreira et al. Since the catalyst is compatible with the reaction conditions applied for EDA synthesis a solution of styrene and Fe(TPP)Cl in CH₂Cl₂ was added to the microreactor set-up via pump 2 (Scheme 5.3). Preliminary experiments showed product
formation at 50 °C but not at room temperature. However, before more thoroughly investigating the reaction conditions, a new batch of catalyst had to synthesized. Applying identical procedures provided a second new batch, which unfortunately never showed any activity in cyclopropanation reactions. Remarkably, a closer look at the UV-spectra of these two catalyst batches showed two different iron-porphyrin complexes (Figure 5.3b). Preparing new batches of catalyst using different bottles of DMF or a different batch of FeCl₃ provided Fe(TPP)Cl complexes with the same UV-spectra as the second batch. Comparing the UV-spectra of all complexes with a commercially available Fe(TPP)Cl complex and literature data, showed a strong resemblance with the second Fe(TPP)Cl batch. Based on this knowledge and the unsuccessful attempts to resynthesize the first phorphyrin complex the investigation towards iron-catalyzed cyclopropanation was stopped.

![Figure 5.3. UV-spectrum of TPP and Fe(TPP)Cl complexes](image)

### 5.2.6 Cyclopropanation of cyclooctene with EDA and copper

A continuous flow copper-catalyzed cyclopropanation reaction was reported by DSM Research, in which thiophene under high temperatures (100-200 °C) and high pressure (up to 100 bar) was converted with EDA in the corresponding product in 10 minutes using the copper piping as catalyst. Based on these conditions, the gas permeable tubing, as used previously, was filled with a string of Cu-wire and fitted behind the FLLEX module. Initial experiments were performed with cyclooctene (3a) at different temperatures for 100 seconds (Table 5.2). After collection, excess cyclooctene was removed under reduced pressure followed by ¹H NMR analysis to determine the product formation, in all cases EDA and EDA-dimer were present. From these experiments, it can be concluded that at by increasing the temperature, the yields increased accordingly (entries 1-3).

Increasing the temperature further (130 and 160 °C, established by submerging the tubing in an oil bath) and additionally elongating reaction times (2 and 5.8 min, by alternating with FEP-tubing) led to product formation in 44 and 48% isolated yields, respectively (entries 4 and 5). However, at temperatures above 100 °C the gas permeable tubing started to deform and became mechanically fragile. Although the gas permeable tubing was not suitable for temperatures of 130 and 160 °C, these experiments do show that cyclopropanation can be performed in flow using copper wire.
Table 5.2. Copper-catalyzed cyclopropanation

<table>
<thead>
<tr>
<th>Entry</th>
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<td>160</td>
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<sup>a</sup>Conversion based on crude NMR data (mol%). <sup>b</sup>Isolated yield.

5.3. Conclusion

EDA can be safely synthesized utilizing microreactor and separation technology starting from cheap and readily available starting materials. Optimization of the reaction was aimed at reaching complete conversion into EDA as fast as possible using a minimal amount of sodium nitrite, possibly applying higher temperatures. The optimal reaction conditions identified based on these criteria were a reaction time of 20 seconds, a temperature of 50 °C and 1.5 equivalents of NaNO<sub>2</sub>. Repeating the EDA synthesis in flow employing the optimal reaction parameters showed complete conversion and high reproducibility of the results. Additionally, a successful combination of a plug-and-play microreactor set-up with a commercially available membrane-based phase separation module was established to perform a direct inline extraction of the product. Even in the small set-up (internal volume 100 μL), EDA was generated in approximately 20 g of pure EDA per day (11 wt% solution in CH<sub>2</sub>Cl<sub>2</sub>). Collecting continuously formed EDA in a round bottom flask for direct cyclopropanation enabled the synthesis of cyclopropanated products of three different substrates up to 60% isolated yield. Integrating a cyclopropanation reaction in the EDA flow process was successful for the copper-catalyzed reactions.

5.4. Acknowledgements

Dr. Pieter Nieuwland (FutureChemistry, Nijmegen, The Netherland), Raf Reintjens and Dr. Ir. Patrick Wenmakers (DSM Research, Geleen, The Netherlands) are kindly acknowledged for fruitful discussions.

5.5. Experimental section

5.5.1 Microreactor set-up for ethyl diazoacetate synthesis
Glycine ethyl ester hydrochloride dissolved in a HCl-acetate buffer of pH 3.5 was premixed with dichloromethane before addition of the sodium nitrate solution in the temperature-controlled microreactor. A schematic representation of the initial FutureChemistry FlowStart microreactor set-up is shown in Scheme 5.2. The box with the dotted line indicates the single glass Micronit microreactor containing two mixing units M of the folding flow type. The reactor temperature was controlled by a Peltier element and sensed by a Pt1000 temperature sensor. At the outlet of the microreactor, a back pressure regulator (BPR, 40 psi) was attached to guarantee a liquid phase. Neat N,N-diisopropylethylamine (DIPEA) was added to ensure well-defined reaction times by efficiently quenching of the reaction. Three different microreactors were used during the experiments:

- Single borosilicate glass quench microreactor with an internal volume of 92 μL, a channel width of 600 μm and a channel depth of 500 μm.
- Single borosilicate glass microreactor with an internal volume of 100 μL, a channel width of 600 μm and a channel depth of 500 μm.
- Single borosilicate glass quench microreactor with an internal volume of 1 μL, a channel width of 120 μm and a channel depth of 50 μm.

5.5.2 Reaction optimization of ethyl diazoacetate

5.5.2.1 Univariate optimization

Solution A: Glycine ethyl ester hydrochloride (40 mmol, 5.6 g) dissolved in 20 mL buffer 1. Solution B: CH₂Cl₂. Solution C: NaNO₂ (60 mmol, 4.1 g) dissolved in 30 mL degassed MilliQ. Solution Q: Neat DIPEA. Buffer 1: NaOAc•3H₂O (132 mmol, 18.0 g) and pyridine (7.5 mL, internal standard) dissolved in 70 mL MilliQ. Concentrated hydrochloric acid (37%, 12M) was added until a pH of 3.5 was reached (17 mL), resulting in a buffer with a total volume of 105 mL.

The flow rates and temperatures were set based on predetermined conditions of reaction times and temperatures (Table 5.3). Experiments were performed in a glass microreactor with an internal volume of 92 μL. Solution Q was set at a flow rate 1/3 of the flow rate of solution A. Each experiment had a collection time equal to 30 μL of solution A. The product was collected in 1 mL of acetonitrile and analyzed by HPLC. Results are visualized in Figure 5.1.

Table 5.3. Conditions of the univariate experiments using 30 s, 15 °C and a MR of 1.5 NaNO₂:glycine as standard

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5.5.2.2 Multivariate optimization

Solution A: Glycine ethyl ester hydrochloride (40 mmol, 5.6 g) dissolved in 20 mL buffer 1. Solution B: CH₂Cl₂. Solution C: NaNO₂ (60 mmol, 4.1 g) dissolved in 30 mL
degassed MilliQ. Solution Q: Neat DIPEA.

The optimization was performed in an automated FlowScreen apparatus. Flow rates and temperatures were set based on predetermined conditions of reaction times and temperatures (Table 5.4). Experiments with a reaction time of 5 seconds were performed in a glass microreactor with an internal volume of 1 μL. For longer reaction times, a microreactor with an internal volume of 92 μL was used. Solution Q was set at a flow rate 1/3 of the flow rate of solution A. Each experiment had a collection time equal to 30 μl of solution A. The product was collected in 1 mL of acetonitrile and analyzed by HPLC. Results are visualized in Figure 5.2 as 2D-contour plots.

Table 5.4. Experiments for the multivariate optimization deduced from a D-optimal experimental design algorithm

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</table>

<sup>a</sup>HPLC yields are normalized; <sup>b</sup>Experiment was removed before performing the model fit due to a high flow deviation.

### 5.5.3 Integrating the Flow Liquid-Liquid Extraction module

Solution A: Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. Solution B: CH₂Cl₂. Solution C: NaNO₂ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. Buffer 2: NaOAc•3H₂O (100 mmol 13.6 g) dissolved in 80 mL MilliQ. Concentrated hydrochloric acid (37%, 12 M) was added until a pH of 3.5 was reached (7 mL). Additional MilliQ was added to obtain a total volume of 100 mL of buffer.

Solution A (86.25 μL/min) was combined in a stainless steel T-splitter with solution B (172.5 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 100 μL) where it was mixed with solution C (86.25 μL/min). The reaction was performed at 50 °C. After the reaction, the mixture was passed through 15 μL of FEP-tubing (ID = 254 μm) before entering the FLLEX module where phases were separated (40 psi, ΔP = 0.14 bar). The set-up was stabilized for 2 minutes before collecting for 30 min. For analysis purposes, the mixture was concentrated under reduced pressure (860 mbar, 40 °C). EDA was obtained as a solution in CH₂Cl₂ (1.52 g). According to 1H NMR analysis,
clean EDA was obtained. Based on the residual solvent peak in the $^1$H NMR spectrum it was calculated to be a 27 wt% solution of EDA in $\text{CH}_2\text{Cl}_2$ meaning 409 mg of pure EDA.

5.5.4 Analysis

HPLC analyses was performed using an Agilent 1120 Compact LC containing a C18 column. Products were analyzed in an eluent of 10% acetonitrile in MilliQ + 0.1% TFA using a UV-detector at 254 nm. Pyridine (internal standard) has a retention time of 1.75 minutes and ethyl diazoacetate of 9.67 minutes.

5.5.5 Cyclopropanation of olefins with EDA and $\text{Rh}_2(\text{OAc})_4$

5.5.5.1 General procedure for the batch synthesis of cyclopropanated products 4a-4c at room temperature

Solution A: Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. Solution B: CH$_2$Cl$_2$. Solution C: NaNO$_2$ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. Solution N: 1M KOt-Bu in MilliQ.

Solution A (21 μL/min) was combined in a stainless steel T-splitter with solution B (43 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 92 μL) where it was mixed with solution C (15 μL/min). The reaction was performed at 50 °C. After the reaction, the mixture was neutralized with solution N (18 μL/min) before entering the FLLEX module where phases were separated (40 psi, $\Delta P = 0.16$ bar). The set-up was stabilized for at least 10 minutes before collecting EDA for 30 minutes in a solution of alkene (3.8 mmol, 3 equivalents) and $\text{Rh}_2(\text{OAc})_4$ (0.0038 mmol, 1.7 mg, 0.003 equivalents) dissolved in 0.5 mL CH$_2$Cl$_2$. After collection was completed, the reaction mixture was stirred overnight.

5.5.5.2 General procedure for the batch synthesis of cyclopropanated products 4a-4c at 45 °C

Solution A: Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. Solution B: CH$_2$Cl$_2$. Solution C: NaNO$_2$ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. Solution N: 1M KOt-Bu in MilliQ.

Solution A (21.51 μL/min) was combined in a stainless steel T-splitter with solution B (43 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 92 μL) where it was mixed with solution C (14.34 μL/min). The reaction was performed at 50 °C. After the reaction, the mixture was neutralized with solution N (18.71 μL/min) before entering the FLLEX module where phases were separated (40 psi, $\Delta P = 0.16$ bar). The set-up was stabilized for at least 10 minutes before collecting EDA for 60 minutes in a solution of alkene (7.7 mmol, 3 equiv) and $\text{Rh}_2(\text{OAc})_4$ (3.4 mg, 0.0077 mmol, 0.003 equiv) dissolved in 5 mL CH$_2$Cl$_2$ at 45 °C. After collection was completed, the reaction mixture was stirred for an additional period (Table 5.1) at 45 °C.

5.5.6 Synthesis of meso-tetraphenylporphyrin iron(III) chloride
Argon gas was bubbled through DMF (15 mL) before dissolving meso-tetraphenylporphyrin (163 mg, 0.27 mmol) and FeCl₃•6H₂O (1.32 mmol, 358 mg, 5 equivalents). The mixture was heated to reflux for 2 hours, after which TLC indicated complete conversion. After cooling the reaction mixture to 30-35 °C, the batch was poured into ice water (400 mL). The precipitate was filtered off and purified by flash column chromatography (silica, MeOH:CH₂Cl₂ gradient), of which the fractions were analyzed by UV-spectrometry.

5.5.7 Cyclopropanation of cyclooctene with EDA and copper

5.5.7.1 General procedure for the flow synthesis of cyclopropanated product 4c in 100 seconds

Solution A: Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. Solution B: CH₂Cl₂. Solution C: NaNO₂ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. Solution D: Neat cyclooctene.

Solution A (75 μL/min) was combined in a stainless steel T-splitter with solution B (150 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 100 μL) where it was mixed with solution C (75 μL/min). The reaction was performed at 50 °C. After passing the FLLEX module where phases were separated (60 psi, ΔP = 0.14 bar), the organic phase was mixed in a stainless steel T-splitter with solution D (59.00 μL/min) in gas permeable tubing (AF 2400, 120 cm, 350 μL) at the desired temperature (Table 5.2). At the end of the gas permeable tubing a 40 psi BPR was connected to guarantee a liquid phase. The set-up was stabilized for 5 minutes before collecting for 1 minute. Volatiles were removed before analyzing the crude mixture by ¹H NMR analysis. Results are depicted in Table 5.2.

5.5.7.2 Flow synthesis of cyclopropanated product 4c in 2 minutes

Solution A: Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. Solution B: CH₂Cl₂. Solution C: NaNO₂ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. Solution D: Neat cyclooctene.

Solution A (75 μL/min) was combined in a stainless steel T-splitter with solution B (150 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 100 μL) where it was mixed with solution C (75 μL/min). The reaction was performed at 50 °C. After passing the FLLEX module where phases were separated (60 psi, ΔP = 0.2 bar) the organic phase was mixed in a stainless steel T-splitter with solution D (59.00 μL/min) in gas permeable tubing (AF 2400, 2 × 30 cm (175 μL) alternating with 1 × 100 cm and 1 × 30 cm FEP tubing (430 μL)), at 130 °C. At the end of the gas permeable tubing a 40 psi BPR was connected to guarantee a liquid phase. The set-up was stabilized for 15 minutes before collecting for 5 minutes. Volatiles were removed before analyzing the crude mixture by ¹H NMR analysis. Results are depicted in Table 5.2.
5.5.7.3 Flow synthesis of cyclopropanated product 4c in 5.8 minutes

**Solution A:** Glycine ethyl ester hydrochloride (10 mmol, 1.4 g) dissolved in 5 mL buffer 2. **Solution B:** Neat CH₂Cl₂. **Solution C:** NaNO₂ (15 mmol, 1.0 g) dissolved in 5 mL degassed MilliQ. **Solution D:** Neat cyclooctene.

Solution A (75 μL/min) was combined in a stainless steel T-splitter with solution B (150 μL/min). The biphasic mixture immediately entered the glass microreactor (internal volume: 100 μL) where it was mixed with solution C (75 μL/min). The reaction was performed at 50 °C. After passing the FLLEX module where phases were separated (60 psi, ΔP = 0.2 bar) the organic phase was mixed in a stainless steel T-splitter with solution D (59.00 μL/min) in gas permeable tubing (AF 2400, 2 × 30 cm (175 μL) alternating with 1 × 190 cm and 1 × 100 cm FEP tubing (1 mL)), at 160 °C. At the end of the gas permeable tubing a 40 psi BPR was connected to guarantee a liquid. The set-up was stabilized for 15 minutes before collecting for 5 minutes. Volatiles were removed before analyzing the crude mixture by ¹H NMR analysis. Results are depicted in Table 5.2.

5.6. References and notes

18. www.futurechemistry.com
19. www.syrris.com


Chapter 6

Chemoenzymatic flow cascade for the synthesis of protected mandelonitrile derivatives

Abstract
A chemoenzymatic two-step cascade process, with both steps having incompatible reaction conditions, was successfully performed in continuous flow. The chemoenzymatic aqueous formation of cyanohydrins was combined with a subsequent organic phase protection step, utilizing a membrane-based phase separation module. The wider applicability of our set-up was demonstrated with the synthesis of nine protected cyanohydrin derivatives, all obtained in good yields and high to excellent enantioselectivity.

This chapter has been published:
Mariëlle M. E. Delville, Kaspar Koch, Jan C. M. van Hest, Floris P. J. T. Rutjes, Chemoenzymatic flow cascade for the synthesis of protected mandelonitrile derivatives, manuscript in preparation.
6.1. Introduction

Cyanohydrins are found in plants, bacteria, fungi, and many insects as part of their defense mechanism, which involves enzymatic release of highly toxic hydrogen cyanide. In addition, cyanohydrins serve as a source of nitrogen for the biosynthesis of amino acids. The natural occurrence of cyanohydrins and their versatile applications render them an interesting compound class for industry. In 1903, Lapworth already reported on the synthetic racemic hydrocyanation of aldehydes. Five years later, Rosenthaler published an enantioselective cyanohydrin synthesis using an enzyme-catalyzed addition reaction. Ever since, the synthesis of (non-)racemic cyanohydrins gained interest resulting in a wide range of synthetic methods. Cyanohydrins contain synthetically strategic functional groups, making them excellent building blocks for more complex structures, and hence, have found widespread application in both academic and more applied research.

Koch et al. reported a chemoenzymatic synthesis of enantiopure cyanohydrins in a continuous flow system. Microreactor technology enables the safe handling of in situ generated and toxic HCN for the enzyme-catalyzed addition to aldehydes. The synthesis was performed using a crude cell lysate containing a hydroxynitrile lyase (HNL). Making use of a biphasic system in a microreactor with suitably designed microchannels, they were able to efficiently form cyanohydrins in high enantiomeric excess (ee) in an uncontrolled slug flow. Since free cyanohydrins tend to racemize, in particular under slightly basic condition, they should be suitably protected directly after formation. For this reason, and also due to the fact that the stability of cyanohydrins may vary with the nature of the substituents, potentially leading to decomposition and release of toxic hydrogen cyanide, the aim was to combine the aqueous cyanohydrin formation with a protection reaction of the hydroxyl function (Scheme 6.1). In such a multistep continuous flow approach, the issues of solvent compatibility and intermediate work-up need to be addressed.

Scheme 6.1. Two-step synthesis of protected cyanohydrins

Formation of protected cyanohydrins generally takes place in the organic phase, which is incompatible with the aqueous conditions of the chemoenzymatic transformation. Introduction of a liquid-liquid phase separation module would enable us to perform this chemoenzymatic cascade in a single continuous flow process. This chapter discusses the first two-step chemoenzymatic flow synthesis of which the incompatible reaction steps are efficiently integrated by utilization of an inline separation module.
6.2. Results and discussion

6.2.1 Optimization of the chemoenzymatic cyanohydrin formation

Initially the separate reactions were optimized, starting from previously identified flow conditions using benzaldehyde 1a (R = Ph). These conditions involved using 10% (v/v) of a crude cell lysate containing an (R)-selective hydroxynitrile lyase (HNL) in a biphasic mixture of methyl tert-butyl ether (MTBE, containing the substrate (0.23M)) and a citrate buffer of pH 5 for 5 minutes at room temperature. Besides the enzyme, the aqueous buffer solution contained KCN (0.69M) to in situ generate HCN. The flow rates of the aqueous and organic solutions were set to 5:1, respectively, and analysis was performed by chiral HPLC.

6.2.1.1 Microreactor set-up

Direct removal of the aqueous phase would eliminate an additional quenching step necessary to accurately determine reaction times. We chose to connect the microreactor to a separation device based on membrane technology, because of its robustness, wide applicability, high chemical resistance and ease of scaling up. More specifically, a FLLEX (Flow Liquid-Liquid Extraction) module was applied to separate the two liquid phases in flow (Scheme 6.2). Dichloromethane (1:4 ratio CH\textsubscript{2}Cl\textsubscript{2} with respect to biphasic buffer) was added to the biphasic reaction mixture to improve phase separation and for solubility reasons in the subsequent protection step. Despite the fact that the aqueous phase contained a crude cell lysate, applying a pressure difference of 0.2 bar over the separation module, efficiently separated both phases without emulsions remaining or clogging of the membrane.

Scheme 6.2. A schematic overview of the microreactor set-up for cyanohydrin formation

6.2.1.2 Reaction optimization

Under the aforementioned reaction conditions 57% conversion into mandelonitrile 2a was observed (Table 6.1, entry 1). Raising the temperature to 40 °C gave an increase in conversion to 74% (entry 2). Additionally, applying a longer reaction time of 12 minutes resulted in a conversion of 83%. Higher conversions are hard to achieve due to the equilibrium of the chemoenzymatic step. In addition, the enantioselectivity of the reactions were determined, which appeared excellent in all cases (product ee >98%).
Table 6.1. Chemoenzymatic formation of mandelonitrile (2a) from benzaldehyde (1a)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Conversion (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ee (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>21</td>
<td>57</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>40</td>
<td>74</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>40</td>
<td>83</td>
<td>98</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined with chiral HPLC (AD-H column).

6.2.2 2-Methoxyisopropyl protection

Having established reaction conditions for the chemoenzymatic reaction, the subsequent protection step was investigated. In literature, a wide range of cyanohydrin protecting groups are known. Not only silyl protecting groups have been applied, but also tetrahydropyranyl (THP), acetyl (Ac), and 2-methoxyisopropyl (MIP)-groups. Based on previous experience in the Rutjes group, MIP-protection was first investigated for optimization in the microreactor.

6.2.2.1 Batch synthesis

Mandelonitrile (rac-2a) was chosen as a model substrate for the MIP-protection of cyanohydrins (Scheme 6.3). 2-Methoxypropene (4) was used as the reagent of choice because it is cheap and has a boiling point of 55 °C, so that excess reagent can be readily removed while evaporating MTBE. The reaction should be carried out using a catalytic amount of acid. Initial experiments were performed by adding phosphoryl chloride (POCl₃), which leads to in situ formation of HCl. Unfortunately, the results were not reproducible, which is probably due to varying amounts of water present in MTBE and hence, different concentrations of acid.

Scheme 6.3. Synthesis of MIP-protected mandelonitrile (rac-3a), starting from mandelonitrile (rac-2a) and 2-methoxypropene (4)

Next, a regular strong acid, camphorsulfonic acid (CSA), was used. Test experiments showed no difference in yield when using a catalytic amount of dry CSA or the corresponding hydrate. When CSA was used to catalyze the batch reactions, thin layer chromatography showed complete consumption of starting material, many spots, and only a small amount of product formation. However, it was envisioned that in the
continuous flow process, side product formation could be avoided by the accurate control over the reaction conditions.\textsuperscript{24}

6.2.2.2 Microreactor set-up

A schematic representation of the set-up used for the flow experiments is depicted in Scheme 6.4. All reagents were put in different syringes preventing undesired (side)-product formation before the start of the reaction. Mandelonitrile (\textit{rac-2a}) and 2-methoxypropene (4) were combined via a T-junction before entering the glass reactor.

![Scheme 6.4](image)

**Scheme 6.4.** A schematic overview of the microreactor set-up for MIP-protection

To ensure well-defined reaction times in the continuous flow system a robust quenching method had to be established. Adding \textit{N,N}-diisopropylethylamine (DIPEA) to the reaction mixture caused the reaction to stop instantaneously. Offline analysis of the reaction mixture was performed using GC-MS or chiral HPLC. For proper analysis, three internal standards (one for each solution) and one external standard were added via the flow marker method previously described by Nieuwland \textit{et al.}\textsuperscript{25} The standards chosen were \textit{ortho}-xylene, nitrobenzene, 4-chloro-3-nitrotoluene, and 2,6-dichlorotoluene.

6.2.2.3 Reaction optimization

The critical process parameters were determined via a univariate optimization. A basic set of reaction parameters was chosen - reaction time, catalyst loading, molar ratio (MR) of 2-methoxypropene (4) to mandelonitrile (\textit{rac-2a}), and temperature - of which one parameter was changed at a time. The results of the univariate screenings are depicted in Figure 6.1. Reaction time has, as expected, a large influence on the formation of MIP-protected mandelonitrile (3a) (Figure 6.1a). At higher acid concentrations a side reaction, possibly 2-methoxypropene polymerization, becomes increasingly important consuming reagent 4 and therefore decreasing the yield of the desired product (Figure 6.1b). As a consequence, the molar ratio should be sufficiently high (>10 equiv) as shown in Figure 6.1c. Raising the reaction temperature led to an increase in conversion until product 3a started to degrade around 60 °C.
Univariate optimization of MIP-protection using 60 s, 3 mol% of CSA, a molar ratio of 7, and 20 °C as standard

Based on the univariate screening, all four process parameters were considered critical for the reaction and used for further optimization in their respective ranges, namely 75-250 s, 1-7 mol% CSA, molar ratio 4-10, and a temperature between 5-60 °C. An experimental design based on a D-optimal algorithm was created using MATLAB (MathWorks, R2007a). This led to a set of seventy two data points, of which the corresponding experiments were performed in random order. The resulting conversions were normalized and fitted to a third order polynomial model. In-house developed FlowFit software was used to calculate the best possible model fit providing a set of optimal values for the reaction parameters.

The results are visualized in the two-dimensional contour plots shown in Figure 6.2. An 80–90% conversion of the reaction is already shown around 40 °C and a molar ratio of 3 (Figure 6.2a). However, decreasing or increasing the temperature has a negative effect on the reaction. In the first case, the reaction is simply not completed. In the second case, at higher temperatures, the rate of polymerization of 2-methoxypropene (4) increased. Raising the molar ratio initially leads to a higher rate of polymerization. At sufficiently high 2-methoxypropene concentration (MR >10), however, product formation is favored over the side reaction. Initially one would have thought that when having a molar ratio above 10, increasing temperatures will not benefit the desired reaction based on the reasons stated above. However, the analysis shows that high temperatures do improve product formation above the critical level of 10. Figures 6.2d and 6.2e underline that a higher molar ratio is required. The amount of CSA, as mentioned earlier for the univariate experiments, has an optimum around 1 mol% (Figures 6.2d and 6.2f). Reaction time seems to have a narrow optimum around 200 seconds, after which product decomposition is observed (Figures 6.2c, 6.2e, and 6.2f). Only minor influences of temperature vs catalyst loading are observed on the reaction in Figure 6.2b although in combination with the parameters reaction time and molar ratio, they are critical for the reaction. Based on these observations, the optimal reaction conditions were determined as follows: reaction time of 200 seconds, temperature at 60 °C using a molar ratio of 11, and 1 mol% of CSA.
6.2.2.4 Continuous flow on gram scale and investigation towards the enantioselectivity

Based on the results and data interpretation of the small scale multivariate optimization experiments, a gram scale experiment was performed using a Uniqsis FlowSyn reactor. For the synthesis of MIP-protected mandelonitrile (rac-3a), the optimal conditions were directly implemented. A 20-mL stainless steel coil required a total flow rate of 6 mL/min at 60 °C. The crude product was collected in 30 mL of quenching solution for 14 minutes. After washing, 1.19 g of MIP-protected mandelonitrile (83% isolated yield) was obtained. Thus, by using this set-up, the reaction was successfully scaled up 200 times.

The stereochemistry was maintained during the reaction when starting from \((R)\)-mandelonitrile (2a). Chiral HPLC analysis of product 3a showed complete retention of configuration on the chiral center.

### 6.2.3 Substrate scope of MIP-protection

To gain further insight in the scope and limitations of this approach, a series of regular alcohols and some additional cyanohydrins were subjected to these optimized flow conditions (Table 6.2). Remarkably, allyl alcohol (5) did not give any product formation according to \(^1\)H NMR analysis. In contrast, menthol (7) did react at 20 °C to produce a mixture of anticipated product 8 and the corresponding elimination product 9 in a 1:1.6 ratio. Raising the temperature to 50 °C resulted in the exclusive formation of elimination product 9. After work-up, enol ether 9 was obtained in 64% isolated yield.
It is hypothesized that the more electron-rich alcohol (compared to the cyanohydrin alcohol) facilitates the elimination process as depicted in Scheme 6.5. In addition, the less electron-rich alcohol function of phenol (10) was under the optimized conditions cleanly converted into the corresponding MIP-product, along with starting material (isolated product yield 23%). The aliphatic substrate acetone cyanohydrin (2b) did not give any product formation according to NMR analysis, probably due to the sterically hindered nature of the alcohol.

To validate the newly established flow conditions for mandelonitrile (rac-2a) on a somewhat larger scale, three mandelonitrile derivatives were tested under the same conditions. MIP-protected (R)-4-chloromandelonitrile (2c), (R)-4-methylmandelonitrile (2d), and (R)-4-methoxymandelonitrile (2e) were all isolated in reasonable yields, albeit that the yields determined by 1H NMR were clearly higher (entries 5-7) meaning that some

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**Table 6.2. Extension of the substrate scope**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\text{--OH}$</td>
<td>$\text{--OMIP}$</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>$\text{HO-CN}$</td>
<td>$\text{MIPO-CN}$</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>57 (82)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>64 (85)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>78 (91)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>$\text{OH}$</td>
<td>$\text{OMIP}$</td>
<td>nd (63)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Isolated yield. <sup>b</sup>Yields determined using 1H NMR.
of the product was lost during work-up. An additional example of protection of a related α-hydroxy ester is shown in entry 8. (R)-Methyl 2-hydroxy-2-phenylacetate (12) showed a fairly reasonable conversion of 63% into the corresponding MIP-protected product 13.

Scheme 6.5. Formation of elimination product 9

6.2.4 Acetyl protection

To enlarge the scope of the cyanohydrin functionalization, acetyl (Ac) and allyloxy carbonyl (Alloc) protecting groups were investigated as well. Unlike MIP-protection, these protecting groups are introduced under basic conditions. Additionally, all three groups have a different mode of cleavage increasing the applicability of the differently protected cyanohydrins in further research.

6.2.4.1 Microreactor set-up

The acetyl protection of cyanohydrins was investigated\textsuperscript{13,14} based on a batchwise literature procedure from Bühler et al. (Ac\textsubscript{2}O, pyridine, 50 °C, 2 h).\textsuperscript{28} A schematic representation of the set-up used for the flow experiments is depicted in Scheme 6.6. In flow, Ac\textsubscript{2}O was added to the mandelonitrile (2a) solution prior to addition of a base preventing instant racemization. Water was used to quench the reaction. All products were isolated and analyzed by \textsuperscript{1}H NMR and chiral HPLC when necessary.

Scheme 6.6. A schematic overview of the microreactor set-up for acetyl protection

6.2.4.2 Reaction optimization

As reported for the batch acetylation reaction, pyridine was used initially as base. Unfortunately, clogging of the microreactor was observed due to the formation of insoluble pyridine salts, so that DIPEA was chosen instead. Initially the acetylation was performed in dry MTBE using Ac\textsubscript{2}O directly from the bottle (Table 6.3, entry 1), providing acetylated mandelonitrile (rac-3f) in a moderate isolated yield of 53%
Table 6.3. Continuous flow acetylation of mandelonitrile (rac-2a)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction time (min)</th>
<th>[H₂O] in MTBE (mM)</th>
<th>Temperature (°C)</th>
<th>Yield (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>&lt; 0.55</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>2b</td>
<td>11</td>
<td>&lt; 0.55</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>3b</td>
<td>11</td>
<td>460</td>
<td>50</td>
<td>79</td>
</tr>
</tbody>
</table>

a Isolated yields. b Ac₂O was purified.

Further purification of Ac₂O before use increased the yield to 90% (Table 6.3, entry 2). Since the protection reaction will be performed subsequently to the chemoenzymatic synthesis, the separated MTBE/CH₂Cl₂ phase will still contain water. Therefore, we also conducted the acetylation in water-saturated MTBE yielding racemic product 3f in 79% yield (entry 3). Karl-Fischer titration experiments made clear that the water concentration in water-saturated MTBE was 460 mM, thereby explaining the relatively low yield for the acetylation. Inline use of a column filled with crushed 4Å molecular sieves, or one with Na₂SO₄ as a drying agent appeared to have insufficient drying capacity to remove substantial amounts of water from MTBE. Another possibility would be to increase the amount of Ac₂O. However, neat acetic anhydride (10.4M) was used and based on the flow rates employed in the chemoenzymatic reaction it was not possible to achieve larger molar ratios.

6.2.5 From aldehyde to protected cyanohydrin in one flow process

6.2.5.1 Flow process set-up

Scheme 6.7. A schematic overview of the flow process for acetyl-protected cyanohydrins

With the different components of the two-step process in place, integration of the two reactions in one single flow process was in order (Scheme 6.7). First the flow process for acetylated cyanohydrins (3) was investigated. In order to keep the solvents in the liquid phase and therefore maintaining control over the flow rates, a 40 psi backpressure regulator (BPR) was used. This additionally led to an increased backpressure necessary for the FLLEX module (80 psi) in order to create a pressure drop over the system to prevent back flushing of the reaction mixture.
6.2.5.2 Process optimization for acetylated cyanohydrins

Using the flow conditions from entries 3 in Tables 6.1 and 6.3, acetylated mandelonitrile (3f) was obtained from this integrated process in an isolated yield of 61% (Table 6.4, entry 1). This is in line with the expected outcome by combining the yields of the individual reaction steps. The ee of the product however, appeared 90% while complete retention of 98% ee was expected. We hypothesized that this partial racemization was caused in the second step under the basic conditions. Upon lowering of the acetylation temperature (entry 2) product 3a was obtained in 60% yield and 95% ee, most likely due to slower racemization. Simultaneous acceleration of the acetylation was achieved by addition of 10% DMAP to the DIPEA flow, which led to a further increase of the enantioselectivity to 98% ee (entry 3).

Table 6.4. Enantioselectivity of the integrated process

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DIPEA</td>
<td>50</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>DIPEA</td>
<td>21</td>
<td>60</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>DMAP/DIPEA</td>
<td>21</td>
<td>64</td>
<td>98</td>
</tr>
</tbody>
</table>

*aIsolated yield over two steps; bDetermined with chiral HPLC (AD-H column).

6.2.5.3 Substrate scope of acetylated cyanohydrins

After successful integration of the two-step chemoenzymatic cascade with benzaldehyde (1a), the set-up was evaluated on a broader range of mandelonitrile derivatives (Table 6.5). The overall yields were in the same range as for acetylated mandelonitrile (3f) except for the more electron-donating substituents shown in entries 2 and 3, which is in line with previously reported results. The same holds for the ee’s reported in Table 6.5, which are all high to excellent except for the aliphatic substrate 1j, which again is in agreement with precedent from literature.15,16

Table 6.5. Two-step flow synthesis of acetylated cyanohydrins (3)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1g</td>
<td>3g</td>
<td>59</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>1h</td>
<td>3h</td>
<td>38</td>
<td>98</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield(^{a}) (%)</th>
<th>ee(^{b}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td><img src="image1.png" alt="Substrate" /></td>
<td><img src="image2.png" alt="Product" /></td>
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<td>86</td>
</tr>
<tr>
<td><img src="image3.png" alt="Substrate" /></td>
<td><img src="image4.png" alt="Product" /></td>
<td>50</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td><img src="image5.png" alt="Substrate" /></td>
<td><img src="image6.png" alt="Product" /></td>
<td>56</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td><img src="image7.png" alt="Substrate" /></td>
<td><img src="image8.png" alt="Product" /></td>
<td>58</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Isolated yield over two steps; \(^{b}\) Determined with chiral HPLC (AD-H or OM column).

6.2.5.4 Introducing the Alloc and MIP-protecting groups in one continuous flow process

The scope of the chemoenzymatic flow cascade was extended to other protecting groups as well (Table 6.6). First, allyloxycarbonyl (Alloc) protection was readily achieved (entry 1) by replacing Ac\(_2\)O with neat AllocCl (9.4M). The chemoenzymatic reaction was performed under the optimal conditions, but upon performing the protection at room temperature in the presence of DMAP the system was clogged. Therefore, the inline protection reaction was performed at 50 °C without addition of DMAP. This gave rise to Alloc-protected cyanohydrin (3m) in 62% yield, but a somewhat lower ee of 87% as probably caused by the elevated temperature (Table 6.4). To minimize waste production and to recover the enzyme solution, the water phase collected from the latter experiment was also directly reused in a second flow cascade. Without the addition of fresh reagents and enzyme, Alloc-protected mandelonitrile (3m) was now obtained in 52% overall yield and 80% ee.

Secondly, the 2-methoxyisopropyl (MIP)-group explored thoroughly in Section 6.2.2 was introduced. The main difference with the acetyl- and Alloc-protection is the acidic conditions used for the introduction of the protecting group. The flow set-ups as depicted in Scheme 6.2 and 6.4, were directly coupled to obtain a new flow process for the integrated synthesis of MIP-protected cyanohydrins. Applying the optimal conditions identified for both set-ups, yielded cyanohydrin 3a in 68% yield and 97% ee.
Table 6.6. Results of Alloc and MIP-protection flow processes.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ee (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OＨ</td>
<td>ＯAlloc</td>
<td>62</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>１a</td>
<td>３m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>OＨ</td>
<td>OMIP</td>
<td>68</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>１a</td>
<td>３a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated yield over two steps. <sup>b</sup> Determined with chiral HPLC (OM column).

6.3. Conclusion

In this chapter the first flow cascade of an aqueous chemoenzymatic reaction with an organic phase protection step was described. The combination of both incompatible reaction steps into a single flow process was enabled by using a membrane-based phase separation module. The flow process has been used for the direct synthesis of MIP- and acetyl-protected cyanohydrins, which are formed in similar yields and ee’s as in the separate reaction steps. Additionally, this approach was extended to carbonate (Alloc)-protected cyanohydrins.

6.4. Acknowledgements

Kaspar Koch (FutureChemistry, Nijmegen, The Netherlands) is kindly acknowledged for his contribution to this chapter. Dr. Pieter Nieuwland (FutureChemistry, Nijmegen, The Netherlands) is kindly acknowledged for support and fruitful discussions. Dr. Martin Schürmann (DSM, Geleen, The Netherlands) is acknowledged for providing the HNL enzyme.

6.5. Experimental section

6.5.1 Chemicals

Benzaldehyde and furfural were freshly distilled before use. Ac<sub>2</sub>O was purified over P<sub>2</sub>O<sub>5</sub> filtered and neutralized with K<sub>2</sub>CO<sub>3</sub>. After a second filtration pure Ac<sub>2</sub>O was obtained after distillation, stored under Ar(g). (R)-HNL was kindly provided to us by DSM Research. The wild type gene encoding for (R)-HNL, originating from bitter almonds (Prunus amygdalus), was cloned and efficiently expressed in the yeast strain Pichia pastoris. The enzyme was secreted from the cells (Pichia pastoris), and was obtained from cell free supernatant by concentration using ultrafiltration/diafiltration. Other chemicals were used as obtained from commercial resources.¹²
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6.5.2 2-Methoxyisopropyl protection

6.5.2.1 Microreactor set-up

All parts within the dotted line of Scheme 6.4, consist of one single glass microreactor with an internal volume of 92 μL, a channel width of 600 μm, a channel depth of 500 μm, and an effective channel length of 360 mm. The channel layout contains two mixing units M, being of the folding flow type. The reactor temperature was controlled by Peltier elements and sensed by a Pt1000 temperature sensor.

6.5.2.2 Reaction optimization of cyanohydrin rac-3a

A FutureChemistry FlowScreen (C-300) was used to perform the screening of reaction conditions. Four glass syringes with an internal volume of 5 mL were used in pumps P1, P2, P3, and P4 as indicated in Scheme 6.4. Pump 1 contained solution of mandelonitrile (rac-2a, 590 μL, 5.0 mmol) and 2,6-dichlorotoluene (130 μL, internal standard A) in 20 mL MTBE. Pump 2 contained a solution of camphorsulfonic acid (46 mg, 0.2 mmol) and o-xylene (300 μL, internal standard B) in 20 mL MTBE. Pump 3 contained a solution of 2-methoxypropene (4, 2.6 mL, 40.3 mmol) and nitrobenzene (600 μL, internal standard C). To quench the reaction at the end of the channel, ensuring well-defined reaction times, pump 4 contained a solution of DIPEA (840 μL, 4.8 mmol) in 19 mL MTBE. The product (50 μL) was collected in CH₂Cl₂ (1 mL) containing 0.15‰ 4-chloro-3-nitrotoluene as an external standard.

6.5.2.3 Continuous flow on gram scale

A scale-up experiment was performed in a Uniqsis FlowSyn (FCUQ-1020) equipped with a stainless steel coil reactor (20 mL). With a flow of 2.0 mL/min for A, B, and C, a reaction time of 200 seconds was obtained. The product was collected for 14 minutes after 6 minutes of stabilization. Pump 1 continuously pumped a solution of mandelonitrile (rac-2a, 1.4 mL, 12 mmol) in 50 mL MTBE. Pump 2 was used for the solution containing camphorsulfonic acid (139 mg, 0.6 mmol) in 200 mL MTBE. Pump 3 continuously pumped a solution of 2-methoxypropene (4, 44 mL, 0.7 mol) in 165 mL MTBE. In contrast to the optimization set-up, no quench pump was used because the reaction time in the larger set-up could easily be determined. In order to neutralize the reaction, the product was collected in a solution of DIPEA (1.5 mL, 8.5 mmol) in 28.5 mL MTBE. After collecting the product for 14 minutes, the reaction mixture was washed with 50 mL demineralized water dried over Na₂SO₄, filtrated, and concentrated under reduced pressure to yield MIP-protected mandelonitrile (rac-3a, 1.2 g, 5.8 mmol, 94 % pure) in 83 %.

6.5.2.4 Analysis

Off-line GC–MS analysis was performed with a Polaris QGC-MS of ThermoFinnigan equipped with a VF1701MS column (length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 μm). An injector temperature of 250 °C was used. The initial column temperature was set to 80 °C increasing to 150 °C using 20 °C/min ramp, directly followed
by a ramp of 40 °C/min to a temperature of 280 °C which was maintained for 2.25 minutes. The total GC program took 10 minutes. Mass spectrometry was performed in electron ionization mode. A two minute delay was set in the detection to cut-off the solvent peak. A spit flow of 50 was used, and the samples were analyzed in a mass range from 20-650. The product sample obtained from the microreactor was collected in dichloromethane containing 0.15‰ 4-chloro-3-nitrotoluene as an external standard. Accurate flow rates were calculated using our recently developed flow marker methodology.  

6.5.3 From aldehyde to protected cyanohydrin in one flow process

6.5.3.1 Flow process set-up

A schematic overview of the flow process set-up is provided in Scheme 6.7. The chemoenzymatic synthesis was performed in FEP-tubing of 576 μL (ID = 0.75 and 0.5 mm). Temperature was maintained at 40 °C by the use of a heated water bath. After 12 minutes of reaction time the biphasic mixture was diluted with CH₂Cl₂ at room temperature before separating the phases using the FLLEX module. The back pressure of the FLLEX was set to 80 psi to prevent back flushing. A pressure difference of 0.2 bar was set over the two channels. The water phase was collected and the organic phase was directly sent to the second reaction.

The protection reaction was carried out in FEP-tubing of 264 μL (ID = 0.75 mm). Temperature of the reaction was set to what was desired by the use of a heated water bath. After the reaction time was finished (controlled by the respective flow rates, the mixture was diluted/quenched before flowing through a back pressure regulator (BPR) of 40 psi.

6.5.3.2 General flow procedure for acetyl-protected cyanohydrins

Solution A: Desired aldehyde (1, 0.69 mmol) in 3 mL MTBE. Solution B: Solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved, 600 μL (R)-HNL enzyme was added. Solution C: Neat Ac₂O (10.6M). Solution D: DMAP (1.1 mmol, 147 mg) dissolved in CH₂Cl₂ (2mL) before adding DIPEA (2mL).

Solution A (8 μL/min) and solution B (40 μL/min) were combined. After 12 minutes of reaction time the reaction mixture was diluted with CH₂Cl₂ (10 μL/min) at room temperature before separating the phases using the FLLEX. The water phase was collected and the organic layer was directly sent to the second reaction. First solution C was added (4 μL/min) directly followed by solution D (8 μL/min). After 9 minutes the reaction mixture was diluted with MilliQ (30 μL/min). The obtained crude product was purified by extraction with CH₂Cl₂ (3 × 3 mL). The combined organic layers were dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane) using an eluent of heptane: ethyl acetate 96:4. The fractions containing product were concentrated under reduced pressure to determine the isolated yield; the products were further characterized by ¹H NMR spectroscopy and chiral HPLC analysis.
6.5.3.3 General batch procedure for the synthesis of racemic acetylated cyanohydrins

KCN (2 mmol, 130 mg, 4 equiv.) was dissolved in 250 μL MilliQ before adding the desired aldehyde (0.5 mmol, 1 equiv.). This was directly followed by the addition of 250 μL AcOH. After stirring for 2 hours (sometimes adding additional portions of KCN to help the reaction reach complete conversion, as determined by TLC) the reaction mixture was diluted with 5 mL H₂O and extracted with Et₂O (3 x 3mL). The combined organic layers were dried over Na₂SO₄, filtrated and concentrated under reduced pressure. To the residue 2 mL toluene was added, followed by evaporation of the solvent. The residue was then dissolved in 1 mL dry CH₂Cl₂ followed by the addition of Ac₂O and DIPEA. The reaction was stirred overnight before diluting with 5 mL H₂O and extraction with 3 mL Et₂O. The organic layer was washed with 5 mL H₂O. The combined organic layers were again extracted with 3 mL Et₂O. The first and second organic layers were combined, dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane) using an eluent of heptane: ethyl acetate 96:4. The fractions containing product were concentrated under reduced pressure to perform analysis by ¹H NMR and chiral HPLC.

6.5.3.4 Flow synthesis of Alloc-protected mandelonitrile (3m)

Solution A: Benzaldehyde (1a, 70 μL, 0.69 mmol) in 3 mL MTBE. Solution B: Solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved, 600 μL (R)-HNL enzyme was added. Solution C: Neat allylchloroformate (9.4 M). Solution D: Neat DIPEA (5.7 M).

Solution A (8 μL/min) and solution B (40 μL/min) were combined. After 12 minutes of reaction time the reaction mixture was diluted with CH₂Cl₂ (4 μL/min) at room temperature before separating the phases using the FLLEX module. The water phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (4 μL/min) directly followed by solution D (8 μL/min). After 9 minutes the reaction mixture was diluted with MilliQ (30 μL/min). The set-up was stabilized for 1 hour and 10 minutes. Product was collected for 1 hour and 20 minutes. The obtained crude product was purified by extraction with Et₂O (3 x 3mL). The combined organic layers were dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane) using an eluent of heptane: ethyl acetate 96:4 followed by a second flash column (silica, CH₂Cl₂) using CH₂Cl₂ as eluent. The fractions containing product were concentrated under reduced pressure to obtain the product (20 mg, 62% yield and 87% ee). Analysis was performed with ¹H NMR, ¹³C NMR, HRMS, IR and chiral HPLC.

6.5.3.5 Recycling of the waterphase of product 3m

From the experiment described above (section 6.5.3.4), 5 mL of water phase was collected: Solution E. Solution A (8 μL/min) and solution E (40 μL/min) were combined. After 12 minutes of reaction time the reaction mixture was diluted with CH₂Cl₂ (4 μL/min) at room temperature before separating the phases using the FLLEX module. The water
phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (4 μL/min) directly followed by solution D (8 μL/min). Temperature was maintained at 50 °C by the use of a heated water bath. After 9 minutes the reaction mixture was diluted with MilliQ (30 μL/min) before flowing through a back pressure regulator (BPR) of 40 psi. The set-up was stabilized for 50 minutes. Product was collected for 45 minutes. The obtained crude product was purified by extraction with Et₂O (3 × 3 mL). The combined organic layers were dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, CH₂Cl₂) using CH₂Cl₂ as eluent followed by a second flash column (silica, heptane) using CH₂Cl₂ as eluent. A third flash column was needed (silica, heptane) using heptane: ethyl acetate 96:4 as eluent, as was a fourth flash column (silica, CH₂Cl₂) using CH₂Cl₂ as eluent. The fractions containing product were concentrated under reduced pressure to obtain the product (9.4 mg, 52% yield, and 80% ee). ¹H NMR and chiral HPLC analysis were performed.

6.5.3.6 Flow synthesis of MIP-protected mandelonitrile (3a)

Solution A: Benzaldehyde (1a, 70 μL, 0.69 mmol) in 3 mL MTBE. Solution B: Solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved, 600 μL (R)-HNL enzyme was added. Solution C: 2-Methoxypropene (523 μL, 8.1 mmol) in 2.5 mL MTBE. Solution D: Camphorsulfonic acid (1.6 mg, 0.0072 mmol) in 3 mL MTBE. Solution Q: DIPEA (146 μL, 0.84) in 3 mL MTBE

Solution A (8 μL/min) and solution B (40 μL/min) were combined. After 12 minutes of reaction time the reaction mixture was diluted with CH₂Cl₂ (4 μL/min) at room temperature before separating the phases using the FLLEX module. The water phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (12 μL/min) directly followed by solution D (11 μL/min). After 200 seconds the reaction mixture was quenched with DIPEA (11 μL/min). The set-up was stabilized for 45 minutes. Product was collected for 1 hour and 50 minutes. The obtained crude product was purified by extraction with Et₂O (1 × 3 mL). The organic layer was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. The product was obtained (28 mg, 68% yield and 97% ee). ¹H NMR and chiral HPLC analysis were performed.

6.5.4 Compound characterization

6.5.4.1 Physical and spectroscopic measurements

NMR spectra were acquired at ambient temperature with a Bruker DMX 300 MHz spectrometer (¹H 300 MHz, ¹³C 75 MHz). Carbon-13 spectra were proton-decoupled. ¹H NMR spectra were referenced to TMS or to the residual solvent peak. ¹³C NMR spectra were referenced to the residual solvent peak. Chiral HPLC was performed using a Shimadzu LC-2010C. Infrared analysis was performed using a Thermo Mattson Nicolet 300 FTIR. Optical rotation was measured using a Perkin Elmer Polarimeter Model 241 MC.
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Electron ionization (EI) mass spectrometry was carried out using a JEOL AccuTOF-GCv. The AccuTOF-GCv contained an Agilent 7890A GC, G4513A, HP-5MS column coupled to a JMS-100GCV system program. Fast Atom Bombardment (FAB) mass spectrometry was carried out using a JEOL JMS SX/SX 102A four-sector mass spectrometer, coupled to a MS-MP9021D/UPD system program. Samples were loaded in a matrix solution (3-nitrobenzyl alcohol) on a stainless steel probe and bombarded with xenon atoms with an energy of 3keV. During the high resolution FAB-MS measurements a resolving power of 10,000 (10% valley definition) was used.

(R)-Mandelonitrile (2a)

Calibration curve for chiral HPLC was prepared with anisole as internal standard. Chiral HPLC: Diace AD-H column, 215 nm, eluent heptane:isopropanol 90:10, elution time 7.0 min (S) and 7.9 min (R). The (R)-enantiomer was bought from Sigma-Aldrich as reference.

MIP-protected (R)-mandelonitrile (3a)

1H NMR (300 MHz, CDCl3, δ): 7.55-7.34 (m, 5H, Ar H), 5.47 (s, 1H, CH), 3.22 (s, 3H, -OCH3), 1.58 (d, J = 0.6 Hz, 3H, CH3), 1.40 (d, J = 0.6 Hz, 3H, CH3). 1H NMR spectrum is in accordance with literature. 31 Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 99.5:0.5, elution time 10.7 min (S) and 11.6 min (R).

MIP-protected (R)-4-chloromandelonitrile (3c)

1H NMR (300 MHz, CDCl3, δ): 7.38 (m, 4H, Ar H), 5.44 (s, 1H, CH), 3.20 (s, 3H, -OCH3), 1.56 (d, J = 0.6 Hz, 3H, CH3), 1.38 (d, J = 0.6 Hz, 3H, CH3). 13C NMR (75 MHz, CDCl3, δ): 135.56 (C), 133.83 (C), 129.40 (2 × CH), 128.52 (2 × CH), 119.03 (C), 103.18 (C), 60.84 (CH), 50.05 (CH3), 25.05 (CH3), 24.51 (CH3). IR(MeOH, cm⁻¹): 2993, 2359, 2339, 1491, 1377, 1215, 1182, 1144, 1093, 1072, 1044, 1014. [α]D20 = + 23 (c = 0.5).

HRMS (FAB⁺) m/z: calcd. (M+H) 240.0791, found 240.0788.

MIP-protected (R)-4-methylmandelonitrile (3d)

1H NMR (300 MHz, CDCl3, δ): 7.37 (s, 2H, Ar H), 7.23 (s, 2H, Ar H), 5.42 (s, 1H, CH), 3.21 (s, 3H, -OCH3), 2.37 (s, 3H, CH3), 1.56 (d, J = 7.6 Hz, 3H, CH3), 1.38 (d, J = 7.6 Hz, 3H, CH3). 13C NMR (75 MHz, CDCl3, δ): 138.35 (C), 130.44 (C), 128.74 (2 CH), 125.85 (2 CH), 118.40 (C), 101.51 (C), 60.24 (CH), 49.18 (CH3), 23.94 (CH3), 23.38 (CH3), 20.20 (CH3). IR(neat, cm⁻¹): 2991, 2304, 2238, 1908, 1513, 1458, 1376, 1257, 1213, 1180, 1143, 1070.
[α]D$^{20}$ = +22 (c = 0.17).
HRMS (FAB$^+$) m/z: calcd. (M$^+$H) 220.1338, found 220.1342.

MIP-protected (R)-4-methoxymandelonitrile (3e)
$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.40 (d, $J = 8.5$ Hz, 2H, Ar H), 6.93 (d, $J = 8.8$ Hz, 2H, Ar H), 5.40 (s, 1H, CH), 3.82 (s, 3H, -OCH$_3$), 3.21 (s, 3H, -OCH$_3$), 1.55 (d, $J = 0.5$ Hz, 3H, CH$_3$), 1.38 (d, $J = 0.5$ Hz, 3H, CH$_3$).
$^1$H NMR spectrum is in accordance with literature.$^{22}$

Ac-protected (R)-mandelonitrile (3f)
$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.52-7.44 (m, 5H, Ar H), 6.41 (s, 1H, CH), 2.17 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{31}$
Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 99:1, elution time 13 min (R) and 16 min (S).

Ac-protected (R)-4-bromomandelonitrile (3g)
$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.65-7.45 (m, 2H, Ar H), 7.44-7.35 (m, 2H, Ar H), 6.37 (s, 1H, CH), 2.17 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{32}$
Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 99:1, elution time 21 min (R) and 26 min (S).

Ac-protected (R)-4-methylmandelonitrile (3h)
$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.40 (d, $J = 8.2$ Hz, 2H, Ar H), 7.25 (d, $J = 8.2$ Hz, 2H, Ar H), 6.37 (s, 1H, CH), 2.39 (s, 3H, CH$_3$), 2.15 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{31,33}$
Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 90:10, elution time 5.9 min (R) and 7.0 min (S).

Ac-protected (R)-4-methoxymandelonitrile (3i)
$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.45 (d, $J = 8.6$ Hz, 2H, Ar H), 6.95 (d, $J = 8.9$ Hz, 2H, Ar H), 6.35 (s, 1H, CH), 3.83 (s, 3H, -OCH$_3$), 2.14 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{31}$
Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 99:1, elution time 20 min (R) and 23.5 min (S).
(R)-1-Cyano-3-phenylpropylacetate (3j)

$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.32-7.16 (m, 5H, Ar H), 5.27 (t, $J = 6.8$ Hz, 1H, CH), 2.87-2.78 (m, 2H, CH$_2$), 2.29-2.91 (m, 2H, CH$_2$), 2.12 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{31}$

Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 95:5, elution time 15 min (R) and 17 min (S).

(S)-Cyano(furan2-yl)methyl acetate (3k)

$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.52 (dd, $J = 1.9$, 0.8 Hz, 1H, Ar H), 6.69 (dd, $J = 2.3$, 1.6 Hz, 1H, Ar H), 6.48 (s, 1H, Ar H), 6.45 (dd, $J = 3.4$, 1.8 Hz, 1H, CH), 2.17 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{33}$

Chiral HPLC: Diacel AD-H column, 215 nm, eluent heptane:isopropanol 95:5, elution time 8 min (R) and 9 min (S).

(R)-Benzo[D][1,3]dioxol-5-yl(cyano)methyl acetate (3l)

$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.03-6.97 (m, 2H, Ar H), 6.84 (d, $J = 8.0$ Hz, 1H, Ar H), 6.31 (s, 1H, CH), 6.03 (s, 2H, CH$_2$), 2.15 (s, 3H, Ac). $^1$H NMR spectrum is in accordance with literature.$^{32}$

Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 99:1, elution time 27 min (R) and 31 min (S).

Alloc-protected (R)-mandelonitrile (3m)

$^1$H NMR (300 MHz, CDCl$_3$, δ): 7.59-7.51 (m, 2H, Ar H), 7.51-7.42 (m, 3H, Ar H), 6.27 (s, 1H, CH), 5.93 (ddt, $J = 17.2$, 10.4, 5.9 Hz, 1H, CH), 5.36 (ddq, $J = 24.9$, 10.4, 1.3 Hz, 2H, CH$_2$), 4.76 - 4.66 (m, 2H, CH$_2$)

$^{13}$C NMR (75 MHz, CDCl$_3$, δ): 153.5 (C=O), 131.3, 130.8, 130.7, 129.5, 128.1, 120.2, 115.8, 70.0, 66.7

IR (neat, cm$^{-1}$): 3069, 3038, 2956, 1753, 1458, 1368, 1234, 943, 914, 784, 764, 695.

$[\alpha]_{D}^{25}$: + 16.4 (c = 0.28)

HRMS (EI$^+$) (m/z): calcd. (M+H) 217.074, found 217.074

Chiral HPLC: Reprosil OM Dr. Maisch column, 215 nm, eluent heptane:isopropanol 90:10, elution time 6.2 min (R) and 7.0 min (S)

6.6. References and notes

17. For a review on HNL-enzymes see e.g.: Hanefeld, U. *Chem. Soc. Rev.* 2013, 12, 6308–6321.
19. www.syrris.com
21. For an extensive research on catalytic THP- and MIP-alcohol protections, see: Kotke, M.; Schreiner, P. R. *Synthesis* 2007, 779-790.
26. www.futurechemistry.com
27. (R)-Mandelonitrile (90 % ee) was purchased from Sigma-Aldrich and used without further purification.
Chapter 7

Where does the flow go...

Abstract
This chapter summarizes my personal view after four years of research on the topic of continuous flow chemistry. My main observation was that continuous flow chemistry is a high-end tool for the organic chemist in addition to the traditional batch chemistry. Flow chemistry has demonstrated to have valuable intrinsic advantages over batch chemistry and with the research performed in the last years by us and others, the feasibility of microreactor technology was extended even further. In addition, I expect some of the limitations of flow chemistry (e.g. clogging and flow rate control) will be tackled in the near future by joint forces of chemists and engineers leading to mainstream applications of continuous flow chemistry in academic research as well as in industrial process development and production.
Challenge and accomplishments

The research described in the preceding chapters has contributed to the rapidly developing field of microreactor technology. A clear indication of the rapid growth is the fact that up to 2002 only 150 papers had been published on microreactors, which increased to almost 1000 papers in the year 2011 as indicated by Hessel et al. Additionally, the increasing number of companies in the field of microreactor technology shows the relevance of continuous flow processes. These developments are largely due to the advantages that are inherent to microreactors such as the intrinsic safety as a result of small reactor volumes, high reproducibility and control over reaction conditions, and the opportunity for low-volume optimization. In this thesis, we focused on one of the applications of microreactor technology, namely performing organic reactions in a continuous flow liquid phase. The objectives that were addressed include (1) the investigation of single-step flow reactions, more particularly design of the flow process, reaction optimization, and scale-up to larger volumes, (2) integration of these reactions into single multistep processes, and (3) exploration of new inline work-up methods.

Since the beginning of microreactor technology, many single-step reactions have been transferred into a continuous flow procedure. We established a standard protocol for this transfer. First, the boundary conditions of the reaction were investigated after which the quenching and analysis methods were determined and the flow process designed. Secondly, the reaction conditions for the flow synthesis were optimized via a univariate optimization study subsequently followed by a multivariate optimization study, which finally led to the production of the desired product utilizing the deduced set of optimal reaction parameters.

At the start of this research project, the majority of microreactor processes that were published in literature, were limited to a single transformation, while less than a handful multistep flow reactions were described. Reasons for the lack of successful multistep flow examples could well be the challenges that are encountered when integrating several reactions in a single process. These comprise solvent incompatibilities, the need for intermediate purification, dilution effects and flow rate control. Owing to the development of liquid-liquid separation modules, microscale distillation devices and immobilized solid-supported scavengers during the past years, a steadily increasing number of multistep flow processes have been reported.

Originally, we wanted to develop our own liquid-liquid separation module. However, with the introduction of the commercially available membrane-based flow liquid-liquid extraction module (FLLEX), we decided to focus our efforts on implementation of this FLLEX module in multistep flow processes. At first, we established the concept of inline extraction by purification of 2-formylpyrrole as discussed in Chapter 4. Subsequently, we addressed solvent mismatches and intermediate work-up with the ethyl diazoacetate (Chapter 5) and cyanohydrin synthesis (Chapter 6). In these two examples, the first reaction step was accomplished in a biphasic reaction mixture, while the second reaction was incompatible with water. The FLLEX module effectively facilitated the separation of the organic and aqueous phase allowing the organic phase
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to be directly transferred into the next (flow) reactor. With the cyanohydrin synthesis, we were the first to report on a two-step flow process in which a biphasic mixture containing an enzyme lysate was successfully separated.

7.2. Considerations and limitations

Part of the challenges encountered in multistep flow synthesis, like solvent incompatibilities and intermediate purification, were successfully addressed in a few illustrative examples. The issues of dilution effects and flow rate control, however, still remain. One possible (partial) solution to excessive dilution might be the distillation device as described by Hartman et al.\textsuperscript{7,8} Although this module was only reported for replacing low boiling for high boiling solvents, excess solvent could potentially also be removed. Flow rate control on the other hand will always be a challenge since for each additional reaction step, a flow of reagents or building blocks has to be pumped into the system. With a fixed channel length, this means that the second reaction has to be completed in shorter residence times compared to the first reaction. Alternatively, when elongating the channel to obtain sufficient residence times, pressure drop problems will be encountered. It goes without saying that every additional reaction step of such a continuous multistep process leads to an even more complex system.

When setting up a flow process the dimensions of all individual modules (e.g. pumps, reactors, work-up and analysis modules, and backpressure regulators) need to operate in the same flow regime. Currently available modules range from μL/h to mL/min and do not always exist in the required operating window.

With the development of multistep flow processes, the question was raised if flow systems could be automated and (time consuming) product handling could be reduced to a minimum by using online and inline analysis procedures. Many different research groups and companies picked up this request and manufactured different online and inline analysis apparatus in different flow regimes. We and others proved the added value of online and inline analysis by optimizing reactions containing highly reactive, \textit{in situ} formed intermediates (e.g. Chapter 4). Additionally, one example is known in which a fully automated optimization set-up was built where the computer directly communicates with the pumps and temperature controller.\textsuperscript{9}

In some instances, a flow process is not possible at all. First, reactions in which solids are present or formed cannot be performed in flow due to fouling and/or clogging of the system.\textsuperscript{10} It was estimated by Roberge et al. that 50% of all reactions performed at Lonza would benefit from flow chemistry, however, 63% of these reactions cannot be carried out in a microreactor due to the presence of solids.\textsuperscript{11} Secondly, reactions that require very long reaction times (order of multiple hours or days) cannot be performed in flow. The enormous length of channels required will take up a large amount of space (larger than a batch reactor of the same production rate) and tremendous pressure drops will be encountered in the system.

Another and economically important point of consideration concerns the costs
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associated with building a continuous flow set-up. As was described in Section 1.2.6, the manufacturing costs of microreactors are higher than for batch reactors. Additionally, a batch reactor can be applied for multiple purposes while a continuous flow set-up is dedicated to one process. This means that the profit margins on a possible flow process need to be sufficient to compensate for the investment.

7.3. Outlook

Overall, I am convinced that continuous flow chemistry has a considerable potential for future applications. It certainly is a valuable tool for the organic chemist in addition to the traditional batch chemistry. The decision as to whether to run a reaction in a flask/reactor or in a microreactor must be made on a case-by-case basis. The advantages and limitations as stated above, in previous chapters, and in literature will help elucidating the pros and cons of continuous flow microreactors for every individual case.

With the research described in this thesis, we contributed to the concept and applicability of flow chemistry in organic synthesis. However, considering the technological aspects of flow chemistry, chemists and engineers will have to bundle their expertise. By joining forces, more complicated flow reactions and processes will be made possible addressing the issues of dilution, incompatible flow modules, and solid handling. Additionally, fully automated optimization in continuous flow has to be explored more extensively. In conclusion, I expect that flow chemistry will progress from academic curiosity and proof of concept to mainstream applications: implementation in industrial research, process development and manufacturing of pharmaceutical and fine chemical companies.

7.4. References and notes

5. Section 1.4.2 of this thesis describes this continuous work-up module.
Summary

Organic chemists have been performing reactions in the traditional batchwise manner for ages. However, the societal wish to increase the safety of chemical manufacturing and constant technological developments led to the introduction of microreactor technology approximately one decade ago. This technology enables the organic chemist to perform reactions in a safe and continuous fashion in micro- to milliliter reactor volumes, thereby creating opportunities to conduct reactions in a conceptually different manner.

In the early days of flow chemistry, the advantages of microreactor technology, such as rapid mixing and heat exchange, were explored and illustrated by single-step flow syntheses. While the research on flow chemistry was progressing, chemists became additionally aware of the fact that with continuous product formation, continuous spectroscopic monitoring would be highly valuable. Analysis by conventional methods such as HPLC, IR and NMR has been explored and successfully implemented, for some techniques even in an inline and real time fashion. A next step in continuous flow developments concerns the investigation of multistep processes including continuous flow synthesis, separation and purification strategies. The aim of the research described in this thesis was to investigate the possibility and applicability of an inline integrated work-up module eventually enabling continuous multistep flow syntheses.

The intrinsically small volumes and highly controlled reaction conditions render continuous flow microreactors ideal systems for the synthesis of potentially explosive compounds such as organic azides. Chapter 2 describes the formation of benzyl azide from benzylamine using imidazole-1-sulfonyl azide hydrochloride as diazotransfer reagent (Scheme 1). In a small scale (semi-automated) continuous flow set-up the diazotransfer reaction was optimized using minimal amounts of reagents; less than 400 mg of benzylamine was required to perform 60 optimization reactions. Furthermore, the reaction was successfully scaled with a factor of 200 to gram scale using a single larger continuous flow reactor.

Scheme 1.1. Benzyl azide synthesis in flow

A second example of a reaction, which is better performed in the closed and highly controlled environment of the microreactor is the Prilezhaev dihydroxylation, a well-established and powerful reaction used in a wide variety of chemical processes. Terminal and non-terminal olefins are epoxidized using peroxyacetic acid, followed by hydrolysis to form the corresponding trans-diols. Batchwise scale-up, however, is often troublesome
because of the thermal instability and explosive character of peroxyacetic acid. In Chapter 3, the design (Scheme 2) and semi-automated optimization of a continuous flow process for the dihydroxylation of cyclohexene is described. Using the optimal conditions identified on small-scale, the corresponding trans-cyclohexane-1,2-diol was successfully synthesized at preparative scale reaching a production rate of 2.46 g/h. Additionally, the wider applicability and limitations of the flow process were probed by investigating six additional olefinic substrates.

Scheme 1.2. Flow design for the synthesis of trans-cyclohexane-1,2-diol

In Chapter 4, the investigation of the Vilsmeier-Haack formylation of pyrrole is described including inline analysis and work-up (Scheme 3). The Vilsmeier-Haack formylation of aromatic compounds is a well-established process in organic synthesis, largely driven by the fact that the resulting aldehydes are generally useful intermediates for the synthesis of fine chemicals and pharmaceutical products. Industrial-scale production, however, is often hampered by the use of hazardous chemicals to produce the highly reactive intermediates. In order to circumvent these issues, a flow chemistry approach was developed. Integrating an inline IR analysis module allowed us to investigate and optimize the formation of the reactive intermediate Vilsmeier-Haack reagent. After optimizing the reaction for pyrrole, a gram scale production process was developed to produce 2-formylpyrrole at a rate of 5.98 g/h. The scope of the flow process was successfully investigated by testing six additional substrates. Unfortunately, all products obtained had to be purified via conventional batchwise extraction procedures, not fully exploiting the advantages of continuous processing. Coupling of a membrane-based extraction module directly after the microreactor chip allowed us to obtain 115 mg 2-formylpyrrole in 91% yield.

Scheme 1.3. Flow synthesis of 2-formylpyrrole including inline work-up
Having established the applicability of the extraction module in a single step flow process, we next investigated the possibility of designing an integrated flow process including inline work-up. Ethyl diazoacetate (EDA) is a versatile intermediate in organic chemistry and frequently used on lab scale. Its highly explosive nature, however, severely limits its use in industrial processes. Chapter 5 describes the inline coupling of microreactor synthesis and separation technology enabling the synthesis of EDA in an inherently safe manner, thereby making it available on demand in sufficient quantities. Ethyl diazoacetate was prepared in a biphasic mixture of an aqueous solution of glycine ethyl ester and sodium nitrite and dichloromethane (Scheme 4). Optimization of the reaction was focused on decreasing the reaction time with minimal amounts of sodium nitrite. In addition, a subsequent integrated cyclopropanation reaction was investigated. Collecting continuously formed EDA in a round bottom flask enabled the synthesis of cyclopropanated products of three different substrates up to 60% isolated yield.

Scheme 1.4. Continuous EDA synthesis, separation, and subsequent batchwise cyclopropanation

The incorporation of an inline extraction module in another multistep flow process is described in Chapter 6. In this case, a chemoenzymatic two-step cascade process was investigated of which both steps have incompatible reaction conditions (Scheme 5). The chemoenzymatic aqueous formation of cyanohydrins was successfully combined with a subsequent organic phase protection step, utilizing the membrane-based extraction module. The wider applicability of our setup was demonstrated with the synthesis of nine protected cyanohydrin derivatives, all obtained in good yields and high to excellent enantioselectivity in a single flow process.
Chapter 7 summarizes my personal view after four years of research on the topic of continuous flow chemistry. My main observation was that continuous flow chemistry is a high-end tool for the organic chemist in addition to the traditional batch chemistry. Flow chemistry has demonstrated to have valuable intrinsic advantages over batch chemistry and with the research performed in the last years by us and others, the feasibility of microreactor technology was extended even further. In addition, I expect some of the limitations of flow chemistry (e.g. clogging and flow rate control) will be tackled in the near future by joint forces of chemists and engineers leading to mainstream applications of continuous flow chemistry in academic research as well as in industrial process development and production.
Samenvatting

Van oudsher voeren organisch chemici reacties op traditionele wijze uit in batchprocessen. Echter de laatste tien jaar heeft de maatschappelijke druk om chemische processen duurzamer en veiliger te maken geleid tot de introductie van microreactortechnologie in de organische synthese. Deze technologie maakt het voor de organisch chemicus mogelijk om reacties op een conceptueel andere wijze selectiever en veiliger uit te voeren onder een continue procesvoering.

In de eerste fase van flowchemie hebben organisch chemici de voordelen van microreactortechnologie zoals snelle menging en warmtewisseling uitvoerig onderzocht en geïllustreerd met enkelvoudige continue flowreacties. Met de vorderingen in het onderzoek naar flowchemie, werden chemici zich ook bewust van het feit dat continue productvorming vraagt om continue analyse. Conventionele analysemethoden zoals HPLC, IR en ook NMR zijn onderzocht en uiteindelijk succesvol geïntegreerd in continue reacties, waarbij het voor sommige technieken zelfs mogelijk was om reacties in-lijn of real-time te volgen. Een volgende stap in de ontwikkeling van continue flowchemie is onderzoek naar multistap processen inclusief meerdere synthesestappen, scheiding- en zuiveringsstrategieën. Het doel van het onderzoek beschreven in dit proefschrift was om continue multistap flowsyntheses te ontwikkelen, gebruikmakend van een in-lijn geïntegreerde opwerkingsmodule.

De van nature kleine volumina en grote controle over de reactiecondities maken dat microreactoren uitermate geschikt zijn voor de synthese van potentiële explosieve moleculen zoals organische aziden. Hoofdstuk 2 beschrijft de vorming van benzylazide uit benzylamine met behulp van imidazool-1-sulfonyl azide hydrochloride, een diazotransfer reagens (Schema 1). Een kleine schaal semi-geautomatiseerde continue flowopstelling is ontworpen, waarin deze diazotransfer reactie geoptimaliseerd is met een minimale hoeveelheid aan reagentia; minder dan 400 mg benzylamine was nodig om 60 optimalisatiereacties uit te voeren. Tevens is de reactie succesvol opgeschaald met een factor 200 tot gramschaal productie gebruikmakend van een enkele grotere flowreactor.

Schema 1. Benzylazide synthese in flow

Een tweede voorbeeld van een reactie die beter in de gesloten en goed gecontroleerde omgeving van de microreactor kan worden uitgevoerd, is de Prilezhaev dihydroxyleringsreactie, een robuuste en krachtige reactie die gebruikt wordt in diverse chemische processen. Zowel eindstandige als interne alkenen worden geëpoxideerd met
samenvatting

Behulp van peroxycarbonzuur, gevolgd door hydrolyse om zo het overeenkomstig trans-diol te vormen. Opschaling van deze reactie via een batchproces geeft vaak problemen vanwege de warmteinstabiliteit en het explosieve karakter van peroxycarbonzuur. In hoofdstuk 3 zijn het ontwerp (Schema 2) en de semi-geautomatiseerde optimalisatie van een continu flowproces beschreven voor de dihydroxylering van cyclohexeen. Gebruikmakend van de optimale condities gevonden op kleine schaal, is het overeenkomstig trans-cyclohexaan-1,2-diol succesvol synthetiseerd op preparatieve schaal met een productiesnelheid van 2.46 g/u. Daarnaast zijn ook de reikwijdte en de beperkingen van het flowproces onderzocht door zes aanvullende olefinische substraten te testen.

Schema 2. Flowopstelling voor de synthese van trans-cyclohexaan-1,2-diol

In hoofdstuk 4 is het onderzoek naar de Vilsmeier-Haack formylering van pyrrool beschreven inclusief het gebruik van in-lijn analyse en opwerking (Schema 3). De Vilsmeier-Haack formylering van aromatische moleculen is een goed beschreven proces in de organische synthese met name doordat de overeenkomstige aldehyden veel worden toegepast in de productie van finechmicaliën en farmaceutische ingrediënten. Industriële schaal productie is echter vaak problematisch door het gebruik van gevaarlijke chemicaliën en de vorming van potentieel explosieve tussenproducten. Om deze problemen te omzeilen is een flowchemische benadering ontwikkeld. Door een in-lijn IR analysemodule te integreren, kon de vorming van het Vilsmeier-Haack reagens goed onderzocht worden. De reactie is vervolgens geoptimaliseerd voor pyrrool en op basis van deze condities is een gramschala productieproces opgezet, waarin het overeenkomstig 2-formylpyrrool is synthetiseerd met een snelheid van 5.98 g/u. Het bereik van dit flowproces is eveneens onderzocht door zes aanvullende substraten succesvol te testen. Desalniettemin zijn alle tot nu toe verkregen producten gezuiverd via conventionele batchextracties, waarmee niet alle voordelen van een continue procesvoering worden benut. Door een membraan-gesteunde extractiemodule direct met de microreactor te koppelen, werd het mogelijk om 115 mg zuivere 2-formylpyrrool in continue flow te synthetiseren in 91% opbrengst.
Samenvatting

Schema 3. Flowsynthese van 2-formylpyrrool inclusief in-lijn opwerking

Met het vaststellen van de toepasbaarheid van de extractiemodule in een éénstaps flowproces, is een volgende stap het onderzoeken van de mogelijkheid om een geïntegreerd flowproces te ontwerpen inclusief een in-lijn scheidingsstap. Ethyldiazoacetaat (EDA) is een veelzijdig tussenproduct in de organische chemie en wordt veelal op labschaal gebruikt. De extreem explosieve aard limiteert echter het gebruik van EDA op industriële schaal. Hoofdstuk 5 beschrijft de in-lijn koppeling van een flowsynthese met een scheidingstechnologie waardoor het veilig synthetiseren van EDA ter plekke mogelijk wordt gemaakt. EDA wordt gesynthetiseerd in een tweefasensysteem van een waterige buffer, waarin glycine ethylester is opgelost, en dichloormethaan (Schema 4). De optimalisatie van de reactie had als doel de reactietijd te minimaliseren gebruikmakend van een minimale hoeveelheid natriumnitriet. Vervolgens is ook een geïntegreerde vervolgreactie getest, namelijk cyclopropanering van een olefine met behulp van een metaalkatalysator en EDA. In flow gesynthetiseerd EDA is opgevangen in een rondbodemkolf, en vervolgens gebruikt om de gecyclopropaneerde producten van drie verschillende substraten te produceren in opbrengsten variërend tussen 31 en 60%.

Schema 4. Continue EDA synthese, fasescheiding en batchgewijze cyclopropanering

Het invoegen van een in-lijn extractiemodule in een ander multistap flowproces is beschreven in hoofdstuk 6. In dit geval is een chemoenzymatische tweeestapscascade onderzocht, waarvan de stappen incompatibele reactiecondities hebben (Schema 5). De chemoenzymatische vorming van cyaanhydrinen in een waterig systeem is succesvol
gecombineerd met een daarop volgende beschermingsreactie in de organische fase, door gebruik te maken van een membraan-gebaseerde extractiemodule. De meer algemene toepasbaarheid van deze opstelling is aangetoond met de synthese van negen beschermde cyaanhydrinderivaten, die allemaal in goede opbrengsten en hoge tot uitstekende enantioselectiviteit zijn verkregen in één geïntegreerd continu flowproces.

**Schema 5.** Multistap flowprocess voor de enantioselectieve synthese van acetyl-beschermd mandelonitril

**Hoofdstuk 7** ten slotte vat mijn persoonlijke mening samen die ik in de afgelopen vier jaar gevormd heb over continue flowchemie. Mijn voornaamste observatie is dat continue flowchemie een belangrijke aanvullende methode is voor de organisch chemicus naast de traditionele batchchemie. Flowchemie heeft bewezen van origine belangrijke voordelen te hebben ten opzichte van batchchemie en met het onderzoek zoals dat de laatste jaren door ons en andere groepen is gepresenteerd, is de toepasbaarheid van microreactortechnologie alleen nog maar verder toegenomen. Daarbij verwacht ik dat sommige van de obstakels die er nu nog zijn (zoals verstopping en controle over de flowsnelheid) de komende jaren succesvol zullen worden opgelost door nauwe samenwerkingen tussen chemici en technologen. Hierdoor zal uiteindelijk de toepassing van continue flowchemie in zowel academisch onderzoek als industriële procesontwikkeling en productie alleen maar verder toenemen.
Dankwoord

Met het afronden van mijn proefschrift wil ik ook graag nog enkele mensen bedanken. Op de eerste plaats wil ik graag mijn promotoren Jan van Hest en Floris Rutjes bedanken. Jan en Floris, jullie hebben mij de mogelijkheid gegeven om een promotie te starten en te voltooien. Dankzij jullie heb ik vier zeer leerzame jaren gehad. Jan, wij zijn de eerste twee maanden van mijn promotie al begonnen met twee zeer geslaagde feesten. Eerst jullie trouwdag in september gevolgd door Jeroens en mijn trouwdag in oktober en ik hoop de afgelopen vier jaar ook al te sluiten met een mooi feest, namelijk mijn promotiefeest! Floris, een van de meest memorabele momenten tijdens mijn promotie die mij is bijgebleven is wel toen jouw auto (VW Polo) was gestolen en wij samen met mijn auto, een Mercedes uit 1980, en jou achter het stuur naar de Universiteit Twente gereden. In de week voorafgaande aan de meeting, heb jij een aantal keer semi-gekscherend gezegd dat je hoopte dat de auto het onderweg niet zou begeven. Maar we hebben het gehaald, zowel de heen- als de terugreis hebben we zonder panne afgelegd, en volgens mij vond je het stiekem toch ook wel leuk om in zo’n “slee” te rijden.

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![Figure 1. ACS spring meeting 2013, met Iria, Mark en Stijn in New Orleans](image-url)
Curriculum Vitae

Mariëlle Delville was born on August 12th 1985 in Hilversum, the Netherlands. She received her VWO-diploma at A. Roland Holst College (Hilversum, NL, 1997-2003) and subsequently studied chemistry at the University of Applied Sciences Utrecht (Utrecht, NL). She performed her first five-month internship at Quest International (Naarden, NL, now Givaudan) under the supervision of Dr. C. Winkel. During her second internship she simultaneously followed courses for her minor both at the University of Amsterdam. This internship was performed in the group of Prof. Dr. H. Hiemstra under the supervision of Dr. R. J. Detz and Dr. J. H. van Maarseveen. After she obtained her Bachelor’s degree in 2007, she continued studying chemistry at Master level at the University of Amsterdam (Mastertrack Molecular Design, Synthesis and Catalysis, 2007-2009). An industrial internship of four months was performed at MercaChem (Nijmegen, NL) under the supervision of H. Veldhuis and Dr. G. Verspui followed by a five-month internship at the University of Wisconsin-Madison (USA) in the group of Prof. R. T. Raines under the supervision of Dr. E. L. Myers. After obtaining her Master’s degree she joined the groups of Prof. Dr. F. P. J. T. Rutjes and Prof. Dr. Ir. J. C. M. van Hest as a PhD student at the Radboud University of Nijmegen in August 2009. The aim of her research was to investigate the possibility and applicability of an inline integrated work-up module eventually enabling continuous multistep flow syntheses. Currently, Mariëlle is working as project officer at the knowledge and technology transfer office at the Radboud Universiteit Nijmegen.
Mariëlle Delville werd geboren op 12 augustus 1985 in Hilversum. Haar VWO-diploma behaalde zij aan het A. Roland Holst College (Hilversum, 1997-2003) en vervolgens studeerde zij scheikunde aan de Hogeschool Utrecht (Utrecht, 2003-2007). Haar eerste stage van vijf maanden voerde zij uit bij Quest International (Naarden, nu Givaudan) onder begeleiding van Dr. C. Winkel. Tijdens haar tweede stage volgde zij ook minor vakken beide voltooid aan de Universiteit van Amsterdam. De stage werd uitgevoerd in de vakgroep van Prof. Dr. H. Hiemstra en was onder begeleiding van Dr. R. J. Detz en Dr. J. H. van Maarseveen. Hierna vervolgde ze haar studie scheikunde aan de Universiteit van Amsterdam (Mastertrack Moleculair Design, Synthesis and Catalysis, 2007-2009). Haar industriële stage van vier maanden voerde zij uit bij MercaChem (Nijmegen) onder begeleiding van H. Veldhuis en Dr. G. Verspui gevolgd door een vijf maanden academische stage aan de Universiteit van Wisconsin-Madison (Verenigde Staten) in de vakgroep van Prof. R. T. Raines onder begeleiding van Dr. E. L. Myers. Na het behalen van haar masterdiploma starte zij medio 2009 in de groepen van Prof. Dr. F. P. J. T. Rutjes en Prof. Dr. Ir. J. C. M. van Hest als promovendus aan de Radboud Universiteit van Nijmegen. Haar onderzoek richtte zich op het ontwikkelen van een continue multistap flowsyntheses, gebruikmakend van een in-lijn geïntegreerde opwerkingsmodule en was voltooid in precies vier jaar. Mariëlle werkt nu als projectmedewerker bij het knowledge and technology transfer office aan de Radboud Universiteit Nijmegen.
List of publications

- **Mariëlle M. E. Delville**, Kaspar Koch, Jan C. M. van Hest, Floris P. J. T. Rutjes, Chemoenzymatic flow cascade for the synthesis of protected mandelonitrile derivatives, manuscript in preparation.