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Combination of Asymmetric Organo- and Biocatalysis in Flow Processes and Comparison with their Analogous Batch Syntheses

Lukas Schober,[a] Fabio Tonin,[b] Ulf Hanefeld,*[b] and Harald Gröger*[a]

In memory of Professor Dr. Herman van Bekkum, pioneer in the field of chemoenzymatic one-pot processes.

A sequential-type as well as a tandem-type chemoenzymatic flow cascade combining an organocatalytic aldol reaction and a biocatalytic reduction to form stereoselectively a 1,3-diol with two stereogenic centers were developed. Initially, a comprehensive screening of 24 alcohol dehydrogenases was carried out and the identified candidates were applied in different multistep flow cascades. All four stereoisomers of the desired 1,3-diol product are accessible *via* a sequential flow approach with product formation-related conversions of up to 76% over two steps, isolated yields of up to 64% and enantiomeric excess of >99% in all cases. In addition, a tandem-type flow process, performing both reaction steps simultaneously, was established leading to 51% conversion with >99% *ee* and 8:1 d.r. and representing a combination of the fields of asymmetric chemocatalysis, biocatalysis and flow chemistry.

Introduction

The development of chemoenzymatic syntheses of in particular chiral building blocks through the combination of suitable stereoselective chemocatalytic and biocatalytic reactions running in a one-pot fashion has received an increasing interest over recent years.[1] Selected advantages of such one-pot cascades without isolation of intermediates are the reduced number of unit operation steps and the reduced amount of solvent consumption. Consequently, formation of less waste can be expected, which makes such one-pot processes attractive in terms of economy as well as sustainability. However, achieving the required compatibility of the involved reactions often represents a challenge as reaction conditions of chemo- and biocatalysis typically differ from each other, thus leading to different "process windows". For example, organic solvents and high temperatures are often used in case of chemocatalysis, whereas biotransformations typically proceed in aqueous reaction media under ambient temperature. Pioneering work in the field of combining chemo- and biocatalysis and demonstrating the opportunities to overcome such hurdles was done in the early 1980s by the group of van Bekkum, who combined an enzymatic isomerization with a heterogenized platinum metal-catalyzed hydrogenation.[2] In the following decades up to now, a broad range of chemoenzymatic one-pot cascades have been realized by numerous groups and several reviews summarize the achievements in this research area.[1] Besides the combination of metal catalysis and biocatalysis (which is the most common combination), more recently we could successfully "merge" organocatalysis and biocatalysis, exemplified for the combination of organocatalytic aldol reactions and a subsequent biocatalytic reductions of the initially formed aldol product to generate chiral 1,3-diols (Scheme 1).[3–6] This 1,3-diol structural motif with two stereogenic centers is of pharmaceutical interest as it represents a substructures in a number of natural products and pharmaceuticals such as in the side chain of the statins Atorvastatin and Rosuvastatin.[7]

However, up to now typically one-pot syntheses using chemo- and biocatalysts are conducted in a "classic" batch mode. In contrast, examples of chemoenzymatic syntheses in flow are still rare although some few examples exist.[8–13] As a selected example, captopril was synthesized in a four-step flow synthesis including one biocatalytic and three chemical steps by Tamborini and co-workers.[11] Most of the given examples make use of immobilization, separation, or packed bed technology to overcome compatibility or stability issues in the reaction cascades, e.g., when utilizing organic solvents.

In spite of the low number of examples of chemoenzymatic synthesis in flow, at the same time flow chemistry has received significant attention over the last years as a promising alternative to the typical batch processes, not only in chemocatalytic[14,15] but also in biocatalytic process development.[16] In addition, flow chemistry addresses the regulatory guidelines of the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) and provides advantages over batch processes in terms of constant product

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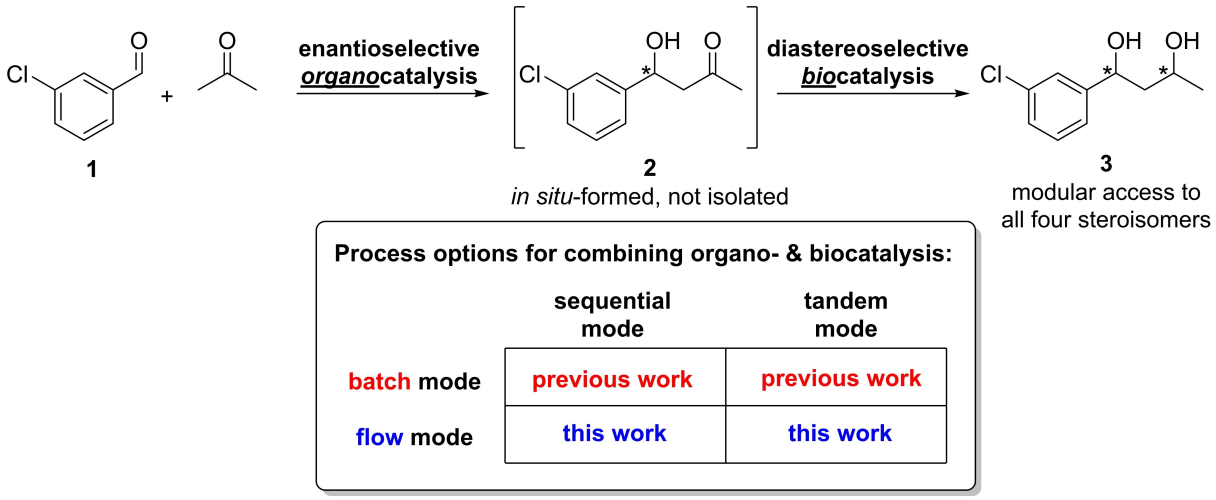


Scheme 1. Concept for combining the organocatalytic aldol reaction and biocatalytic ADH reduction towards all four stereoisomers of the resulting 1,3-diol by means of different process concepts (batch- *versus* flow-mode as well as sequential- *versus* tandem-mode).

quality and access to larger amount of product by numbering up (of the flow reactors) instead of scale-up of the reactor volume as in case of batch reactors.[17] On the other hand, there are different requirements for flow compared to batch processes such as, e.g., homogeneous reaction conditions and avoidance of precipitates in order to avoid "blocking effects".

Addressing this increasing impact of flow chemistry as well as their challenges, we became interested in evaluating the potential of flow processes (and comparing them with analogous batch "counterparts") for chemoenzymatic one-pot syntheses under combination of organo- and biocatalysis. In our study we used homogeneous catalysts within a segmented-flow process, which then could lead to a constant product quality in such a continuous process. Since the above-mentioned first example of a combination of an asymmetric organocatalytic aldol reaction with a biotransformation was studied extensively in the batch mode, thus representing a beneficial benchmark system, we chose this transformation as a "model process" for our flow studies. In detail, this benchmark process consists of an initial enantioselective organocatalytic aldol reaction of acetone with *m*-chlorobenzaldehyde (**1**), followed by a diastereoselective enzymatic reduction of the *in situ*-formed aldol product **2** under formation of the second stereogenic center and the desired 1,3-diol **3** (Scheme 1).

In this contribution, we report the transfer of this chemoenzymatic cascade from a batch into the flow mode as well as the requirements, which have to be fulfilled to run such a cascade in a continuous fashion within a microreactor. It is noteworthy that for both concepts being conceivable for a one-pot process, namely a sequential and tandem process concept, flow-type cascades could be established. These flow processes are based on the use of homogeneously dissolved components (including chemo- and biocatalyst), which theoretically enable a continuous production of products with unchanged quality.

Results and Discussion

In a previous work[18] we showed that the initial step of the cascade, namely the aldol reaction of 3-chlorobenzaldehyde (**1**) and acetone, runs in an aqueous flow setup in the presence of a high amount of isopropanol and excess of acetone. As a homogeneously dissolved organocatalyst, proline-derived peptides have been utilized, which were developed originally for batch applications by the Singh group.[19] Water-miscible, polar co-solvents are indispensable to dissolve the proline based organocatalyst in the reaction mixture, thus enabling to carry out the reaction in a homogenous medium instead of a slurry since a slurry reaction medium would represent a problem for an application in a flow mode.

In order to extend this aldol reaction towards a modular chemoenzymatic flow cascade generating the desired 1-(3-chlorophenyl)-butane-1,3-diol (**3**) over two steps by means of an alcohol dehydrogenase (ADH) as a biocatalyst component, the following features are needed: a) high activity and (dia)stereoselectivity for the enzymatic reduction of the enantiomerically enriched aldol intermediate **2** (meaning no internal asymmetric induction caused by the existing stereogenic center in the aldol adduct); b) no or relatively low activity towards reduction of **1** and acetone, thus avoiding the formation of undesired side-products; c) high solvent tolerance towards isopropanol/acetone; d) ability to perform a substrate-coupled or enzyme-coupled cofactor regeneration. Depending on the enzyme properties the cascade could either be carried out in a sequential mode, which means that initially the aldol reaction is performed and afterwards the aldol product **2** (as an intermediate) is converted to the desired 1,3-diol **3**. Alternatively, the cascade could be conducted in a tandem mode. In such a process, both steps occur at the same time. While both approaches have already been developed as slurry batch processes,[3–6] a modular flow process would increase the value of the reaction system for further applications. With respect to

the compatibility of both reaction steps, the flow tandem option represents the most challenging approach because both catalytic steps have to proceed concurrently under the same reaction conditions. In contrast, in a sequential approach the second biocatalytical step can be adjusted to some extent to the specific needs of the biotransformation as the second step (e.g., the adjustment of pH and solvent after completion of the first step prior to the start of the second step).

In order to extend the narrow range of enzymes, which turned out to be suitable for this purpose (with so far two identified examples in our previous work[3–6]), at first we conducted a biocatalyst screening. Our focus was in particular on commercial alcohol dehydrogenases due to their easy access and readily availability. In total 24 ADHs were screened, and in detail our screening set consisted of 18 commercial ADHs from an enzyme kit from Evoxx and six recombinant, self-expressed ADHs from an in-house collection (ADH-A, Lb-ADH, Sy-ADH, Lk-ADH, Ras-ADH and T-ADH). From the commercial kit, ADH030 and ADH270 showed the highest activities for the reduction of *rac*-**2** among all candidates with 16 $U m \cdot L^{-1}$ (ADH030) and 60 $U m \cdot L^{-1}$ (ADH270, Figure 1). The other commercial enzymes showed no or very low activity in the initial photometric assay (for details see Supporting Information). From the six in-house enzymes, only the T-ADH showed no activity at all. The other five ADHs present activities towards the reduction of *rac*-**2** ranging from 10 to 22 $U m \cdot L^{-1}$ (Figure 1). All activities were determined with a standard photometric assay (see Supporting Information). The two commercial and five in-house enzymes were chosen for further investigations. To evaluate the possibility of any side reactions, the activity of each enzyme towards 3-chlorobenzaldehyde (**1**) and acetone was also determined spectrophotometrically. The calculated activities are almost always higher than for the *rac*-**2** but are still within the same order of magnitude (Figure 1). An exception is the ADH270, which shows the highest volumetric enzyme activity for aldol substrate *rac*-**2**. Even though these results are not completely fulfilling our prerequisite b), it has to be considered that aldehyde **1** is present only in low concentration in case of a sequential "one-pot" flow concept. Acetone is used in higher concentrations due to the required excess amount in the aldol reaction as the first step. However, such a high excess of acetone is a drawback for the second (biotransformation) step since acetone can cause the undesired back reaction of the biotransformation due to the reversibility of the substrate-coupled cofactor regeneration. It should be added that it appeared less likely to find an ADH that exclusively converts the desired aldol product **2** and shows no activity towards the structurally similar, but smaller molecules **1** and acetone. Thus, those ADHs were taken into further consideration, which have a

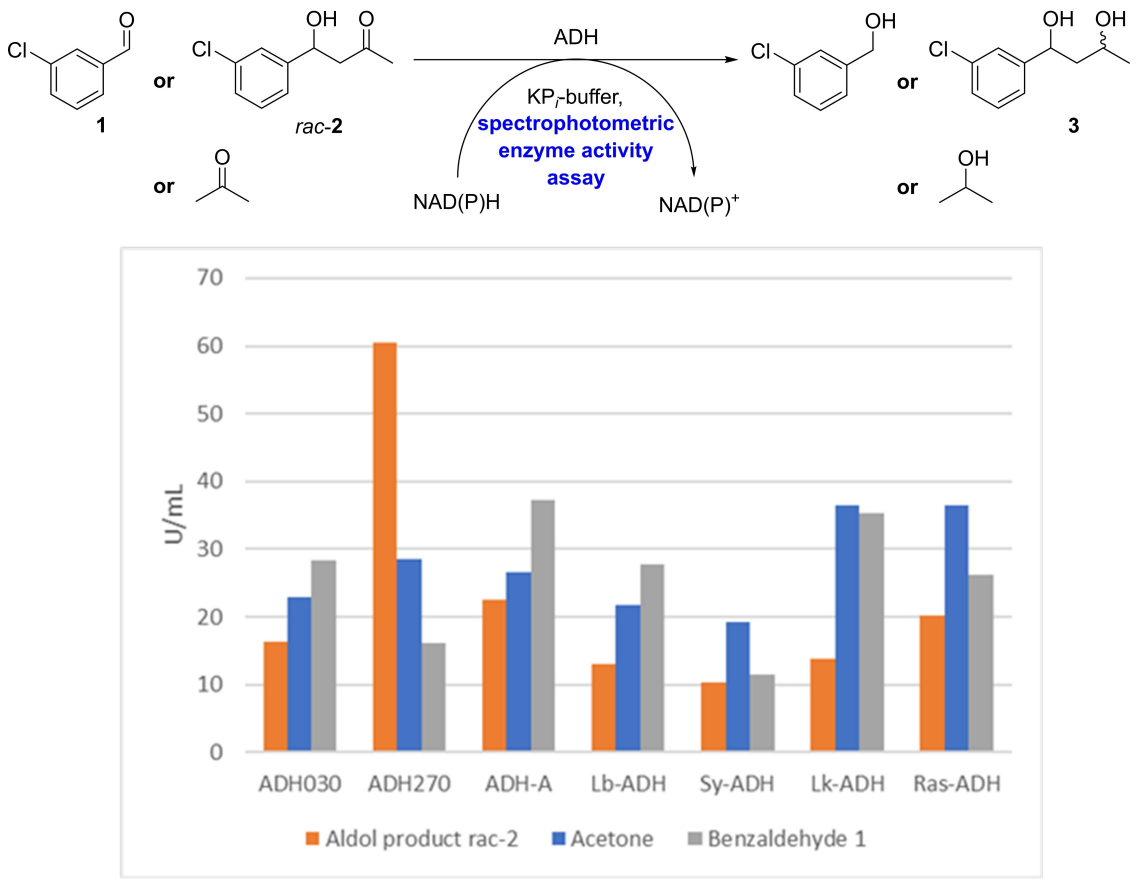


Figure 1. Activity towards aldehyde **1**, aldol product *rac*-**2** and acetone of ADH030, ADH270 (both commercially available), ADH-A, Lb-ADH, Sy-ADH, Lk-ADH and Ras-ADH (all in-house enzymes) determined *via* spectrophotometric assay (for details, see Supporting Information, chapter 2.1.2.).

relatively low tendency for the reduction of acetone as substrate.

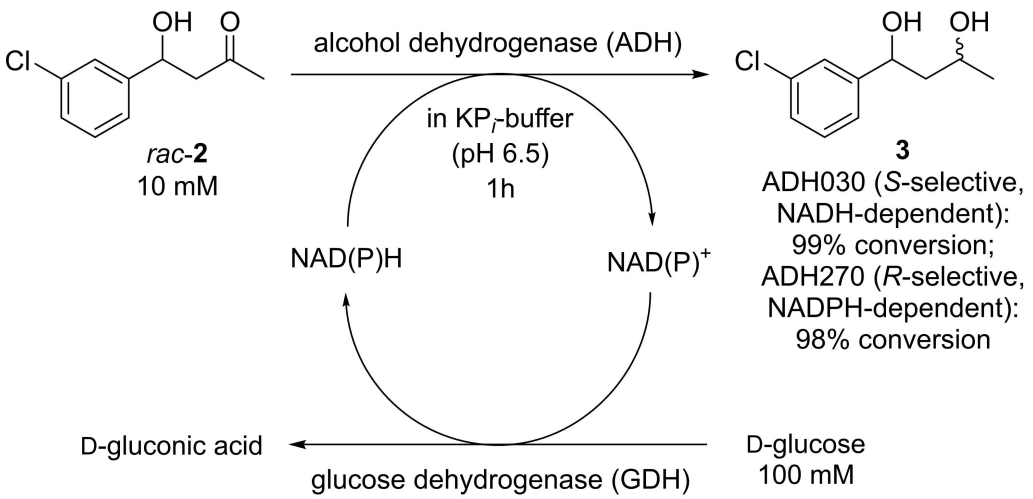
Next, the quantity and overexpression of the enzyme formulations were determined *via* Bradford assay and SDS-PAGE in order to get an insight into the activity per mg of applied protein (which is typically crude extract protein and not purified protein). The results show overexpression of the active enzymes with specific activities ranging between 7.9 and 61.6 $U \cdot mg_{ADH}^{-1}$ (for details see Supporting Information).

After the spectrophotometric characterization of the ADH candidates, synthetic biotransformation experiments with a glucose-based *in situ*-cofactor regeneration were carried out using ADH030 and ADH270 as prioritized enzymes, which are both commercially available and show an opposite enantioselectivity (Scheme 2). The purpose of this study was to demonstrate that both enantiomers of the aldol product **2** can be converted with high conversion to the desired corresponding 1,3-diol of type 3 when using the *R*- as well as *S*-enantioselective commercial ADH. We were pleased to find that almost quantitative conversion of the racemic aldol product **2** towards the desired 1,3-diol **3** at 10 mM substrate concentration was achieved within 1 hour when utilizing these enzymes ADH030 and ADH270. Since these enzymes are "enantio-complementary", in accordance with our previous study[3] all possible four stereoisomers of the 1,3-diol **3** would be available when starting from the corresponding enantiomers of the aldol product **2** (formed in the initial enantioselective organocatalytic step) as a substrate.

Based on these findings and literature data,[20] for the further flow process development of this chemoenzymatic cascade the *S*-enantioselective ADH030 and *R*-enantioselective ADH270 were chosen as enzyme components. It should be added that the commercial enzymes showed no precipitation in presence of higher amounts of water-miscible organic solvents (acetone and isopropanol), which fulfils a further prerequisite to conduct a completely homogenous flow reaction. In the past, we often encountered precipitates when using crude extract of self-expressed enzymes, which is less important for batch but important for flow applications. A further advantage of the choice of these catalysts is that with both types of catalysts (aldol organocatalyst and biocatalyst for enzymatic reduction) being commercially available, now a readily available toolbox exists, which ensures that the process can easily be established also in other laboratories without access to genetically modified organism (GMO) technology and biological preparation of recombinant enzymes.

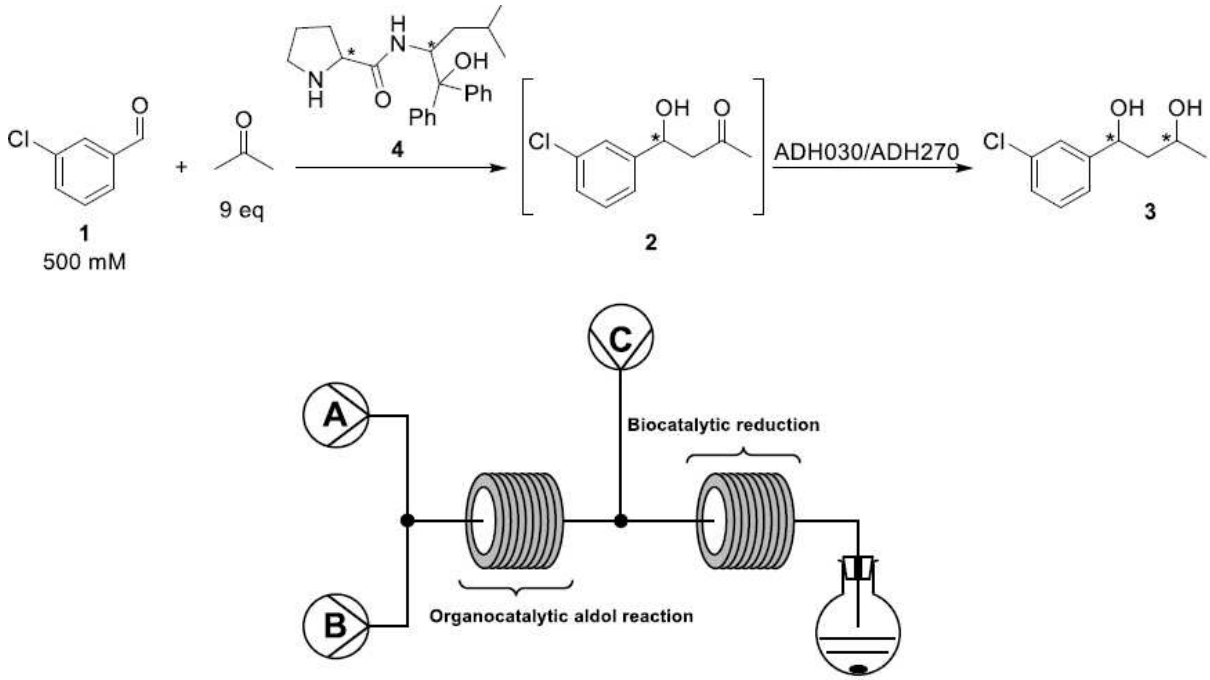
To combine the already existing organocatalytic aldol reaction with the ADHs found in the screening towards a chemoenzymatic cascade in flow, two approaches are conceivable, namely a sequential and tandem mode. We studied both concepts and at first, the sequential approach was investigated according to the set-up shown in Table 1. In such a flow process conducted in a sequential mode, the two reaction steps are carried out one after the other in two series-connected coiled tube-reactors. Two syringes (**A** and **B**) separating the reactive components (aldol catalyst **4** and 3-chlorobenzaldehyde **1**) of the first reaction (aldol reaction) are connected to the first reactor *via* a T-mixer. The outlet of the first reactor and a third syringe (**C**) containing the components for the second biocatalytic step (enzymes, cofactor, co-substrate, buffer) are connected to the second reactor though another T-mixer (Table 1).

The advantage of such a setup is that the initial step is carried out under optimal condition without any interference by the second step. Subsequently, the reaction conditions in the second reactor are changed by addition of a solution through syringe **C**. This setup provides an opportunity to use a second, different solvent system that is more suitable for the biocatalytic step e.g., showing lower co-solvent concentrations. Potentially, both steps could be carried out at different temperatures as well, even though this is not necessary in this case. As there is no purification in between the two reaction steps the process could be seen as a two-step "one-flow" process. A potential disadvantage over the "classic" way to conduct two



Scheme 2. Enzymatic reduction of racemic aldol product *rac*-**2** with *in situ*-cofactor regeneration at a reaction time of 1 hour using the commercially available ADHs ADH030 and ADH270.

Table 1. Flow setup and reaction summary for the sequential aldol-ADH cascade with glucose-based cofactor regeneration for the formation of all four diastereomers of the 1,3-diol **3**. Pump **A** containing 3-chlorobenzaldehyde (**1**), buffer and isopropanol, **B** Aldol catalyst **4**, acetone and isopropanol, **C** ADH, GDH, NAD(P)H, D-glucose and buffer. The initial aldol reaction proceeds at 500 mM substrate concentration and 2 mol% catalyst loading with 1.5 hours residence time in a solvent mixture of isopropanol:acetone:buffer 3:2:1. Prior to the second step the reaction is diluted (1:20) and ADH030 (700 U/mmol of **1**) or ADH270 (2000 U/mmol of **1**), GDH (700 U/mmol of **1**) 0.3 mM NAD(P)H, 100 mM glucose in PPB (50 mM, pH 7) are added. The biocatalytic step proceeds with a residence time of 1 hour, adding up to 2.5 hours total residence time. Both reactions are performed at 25 °C. Product samples are collected at the outlet of the second reactor. For detailed experimental procedures see Table S8.



Entry	Catalyst used	Formed Stereo-isomer[a]	Product-formation related conversion towards **3**[b] [%]	d.r.	*ee* [%]	Remaining aldehyde **1**[b] [%]	Formed 3-chlorobenzyl alcohol[b] [%]	Remaining aldol product **2**[b] [%]
1	(*S*,*S*)-**4** and ADH030	(1*R*,3*S*)	76	7:1	99	0	8	14
2	(*S*,*S*)-**4** and ADH270	(1*R*,3*R*)	51	14:1	99	7	5	36
3	(*R*,*R*)-**4** and ADH030	(1*S*,3*S*)	73	9:1	99	0	14	13
4	(*R*,*R*)-**4** and ADH270	(1*S*,3*R*)	33	4:1	99	5	5	58

[a] Determined via HPLC assigned analogous to [4]. [b] Determined via ^{1}H-NMR.

reactions separately with work-up of the intermediate could be related to the fact that the reagents of the first reaction are still present in the second reactor in such a two-step one-flow process. This could negatively impact the second step or cause side reactions, like further aldol additions/condensations or a racemization of the aldol product. The latter has been reported for long reaction times and high catalyst loadings in a batch mode.[6]

When performing the aldol reaction in such a two-step one-flow process in a solvent mixture of 50 vol% isopropanol, 33 vol% acetone and 17 vol% buffer, almost quantitative conversion of the 500 mM starting material was achieved after 1.5 hours with a product formation-related conversion of up to 90% towards the desired aldol product **2** (Table 1). In this organocatalytic reaction, both enantiomers of the organocatalyst, (*R*,*R*)- and (*S*,*S*)-**4**, can be utilized to generate both aldol enantiomers (*R*)-**2** and (*S*)-**2**. It is noteworthy that the aldol reaction proceeds under kinetic control.[4] Indicated by our aldol reaction optimizations (results not shown) it can be assumed that the aldol reaction does not proceed after diluting the reaction mixture prior to the second reactor. Hence, no racemization of the aldol product is expected during the biocatalytic reaction step. The ADH reduction is carried out in the second reactor after diluting (1:20) the aldol reaction mixture. From the first reaction step, small amounts of acetone (approx. 1.7 vol%) and isopropanol (approx. 2.5 vol%) remain in the reaction. At this point, a certain influence on of the redox system cannot be excluded, however it should favor the desired reductive direction of the ADHs.

Whenever alcohol dehydrogenases are used for preparative syntheses of chiral alcohols the question of efficient co-factor regeneration arises. This key question for sustainable enzymatic synthesis has been reviewed in the past.[21] For this project we considered a glucose-based enzyme coupled or isopropanol-based substrate-coupled cofactor regeneration. Even though the ADH030 can tolerate higher isopropanol concentrations (up to 28 vol%), the ADH270 is not very solvent stable (results not

shown). Therefore, a glucose-based cofactor regeneration was chosen for both enzymes.

This combination of organo- and biocatalysts also enables an access to all four stereoisomers in a modular fashion, and each stereoisomer of 1,3-diol **3** can be formed by means of the right choice of the "enantiocomplementary" organo- and biocatalysts, respectively.[3] However, special attention in the development of a flow process has to be given to the amount of biocatalyst used, we could not increase the biocatalyst concentration significantly above the reported experimental 2.5 g/L (for ADH030) and 3.8 g/L (for ADH270) in stock solution C without observing precipitation in the tubes, which then can cause clogging. We assume the precipitation strongly depends on the formulation of the lyophilizate (which is unknown as it is a commercial sample) and, thus, has to be tested for each enzyme individually. Using either ADH030 or ADH270, which show opposite enantioselectivity, in the enzymatic reduction step then furnished the desired access to all four possible stereoisomers of 1,3-diol **3** in a modular flow reaction setup with good product formation-related conversions ranging between 33 and 76%, good to high d.r. (diastereomeric ratio) and excellent enantiomeric excess of >99% in all cases (Table 1). Unreacted aldehyde **1** from the first reaction step is converted fully to the corresponding benzyl alcohol in case of the ADH030. When using ADH270 however, aldehyde **1** remains in the reaction mixture and is only partially converted to 3-chlorobenzyl alcohol. In all cases, unreacted aldol product **2** remains in the reaction mixture (Table 1), while no aldol condensation product and only small amounts of bis-aldol product (<2%) were observed. The amount of **2** is higher in case of the ADH270 compared to the ADH030, and in both cases this represents the main reason for the incomplete conversion towards the desired 1,3-diol **3**. Therefore, the enzymatic step is rate-limiting under these reaction conditions for both enzymes. Further investigation to enhance the performance of the biocatalysts e.g., by means of enzyme engineering (which, however, is not part of this work), are an interesting option for the future in order to increase the product formation-related conversion to the desired 1,3-diol **3**.

The differences of the enzymes with respect to conversion and d.r. in the biotransformations can further be explained by the properties of the applied biocatalysts. In case of complete conversion towards 1,3-diol **3**, the d.r. is limited to the enantiomeric ratio (e.r.) of **2** after the initial aldol reaction, which is approximately 9:1 (80% ee).[18] Despite the fact that both biocatalysts fully convert a racemic mixture of **2** (meaning there is sufficient activity for both enantiomers) in the chemo-enzymatic flow process only ADH030 showed (nearly) no enantiopreference and converts both enantiomers nearly equally good towards the desired 1,3-diols (1*R*,1*S*)-**3** and (1*R*,3*S*)-**3**.

In contrast, for ADH270 a significant difference in terms of conversion as well as d.r. is observed. This can be explained with a significant enantiopreference of the ADH270 towards the enantiomers of the substrate **2**. In case of the (*R*,*R*)-**4**, the ADH270 preferably converts the aldol *S*-**2** enantiomer, which is also the major enantiomer formed with this organocatalyst. As a result, the d.r. increased to 14:1 with a conversion of 51% over both steps under the given conditions. However, for the opposite organocatalyst (*S*,*S*)-**4** the d.r. as well as the conversion decreases to 4:1 respectively 33%, because *S*-**2**, which is favored by the ADH270, is formed to a minor extent in the organocatalytic step, thus leading to a decrease of both conversion and d.r. ratio. Nevertheless, all four stereoisomers of 1,3-diol **3** could be formed and isolated in good yields (related to the conversion) by collecting the product stream and purifying the crude product *via* automated column chromatography (Table 2).

The second option to combine the organocatalytic aldol reaction and biocatalytic reduction in a flow process is by means of a tandem mode. Unlike the sequential approach here both reactions take place concurrently at the same time in the same reactor. As a consequence, all components of the second step are now present already at the stage of the initial aldol reaction. In theory, this can cause complications for both steps due, e.g., incompatibility of the catalysts with components of the other catalytic transformation. Thus, several additional challenges arise for such a two-step one-flow tandem cascade. First, with respect to the consumption of the initial substrate

Table 2. Comparison of the sequential and tandem aldol-ADH flow cascades towards 1,3-diols **3** with literature-reported batch cascades in terms of reaction/residence time, selectivity, product formation-related conversion (Prc), (space-)time yield and isolated yield. Space-time yield was calculated based on the product formation-related conversion, substrate concentration, batch size and reaction time.

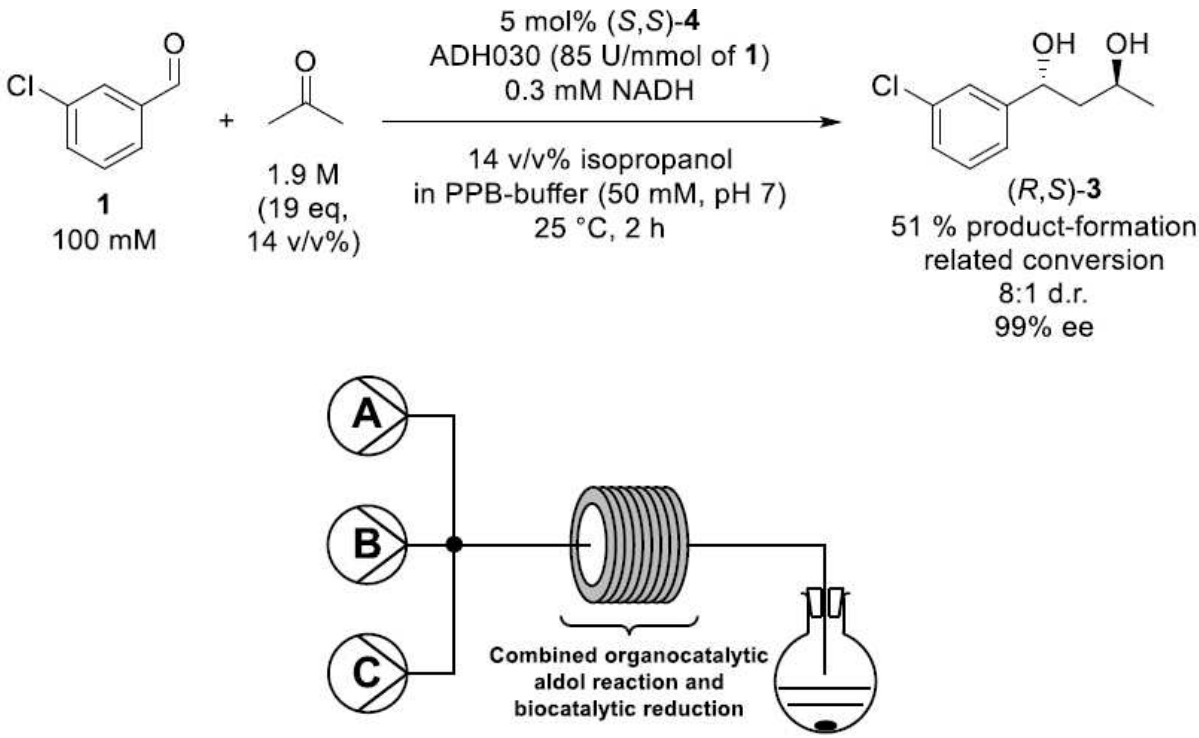
Method	Formed Diastereomer[a]	Prc[b] [%]	Reaction/ residence time [h]	Productivity[c] [μmol·h^{-1}]	Space-time yield (batch size)[c] [μmol·L^{-1}·h^{-1}]	Isolated yield[d] [%] [mg]	d.r.	*ee*[a] [%]
Sequential Flow	(1*R*,3*S*)	76	2.5	182	not applicable	65 (38)	7:1	99
	(1*R*,3*R*)	51	2.5	122		42 (25)	14:1	99
	(1*S*,3*S*)	73	2.5	175		62 (37)	9:1	99
	(1*S*,3*R*)	33	2.5	79		21 (13)	4:1	99
Sequential Batch[4]	(1*R*,3*S*)	89	48	9.3	861 (10.7 mL)	80 (80)	>25:1	99
	(1*R*,3*R*)	72	48	7.5	696 (10.7 mL)	65 (65)	>25:1	99
Tandem flow	(1*R*,3*S*)	51	2	153	not applicable	not deter-mined	8:1	99
Tandem batch[6]	(1*R*,3*S*)	60	24	12.4	11300 (1.1 mL))	not given		
	(1*R*,3*S*)	60	24	138	5500 (25 mL))	33 (660)	not given	

[a] Determined via HPLC assigned analogous to [4]. [b] Determined via ^{1}H-NMR. [c] Calculated from the product-related conversion and flow rates given in Table 1 and Scheme 3. [d] Determined after preparative column chromatography, limited to the corresponding product related conversion.

benzaldehyde **1**, the (desired) aldol reaction now competes against the (undesired) ADH reduction for this substrate. In addition, the biocatalytic step has to proceed at higher solvent concentration compared to the sequential approach, and in particular the high concentration of acetone is disadvantageous for the biotransformation due to the (undesired) re-oxidation of the 1,3-diol product **3** caused by acetone as an oxidation agent.

Regarding the flow setup for such a two-step one-flow process running in a tandem mode, three syringes (**A**–**C**) are used to separate all reagents (Scheme 3). It had to be ensured that no reaction takes place in the syringes (and, thus, reactive components have to be separated) prior to their combination through a 4-way cross piece into the sole, coiled reactor (Scheme 3). Similar to the sequential approach the issue of the most suitable cofactor regeneration for this type of process had to be addressed. First, it can be expected that the ADH-catalyzed reduction of acetone plays a major role, as both ADHs show activity for this compound in addition to the formed aldol product **2** (Figure 1). Because of the resulting undesired ADH-catalyzed reduction of acetone, which is now present at high substrate concentration of 500 mM also at the stage of the biotransformation, a glucose-based cofactor regeneration similar to the sequential process (with only 100 mM of glucose) would result in a main consumption of glucose for the formation of isopropanol. Such an undesired side reaction would consume the redox equivalents provided by the cofactor regeneration for the desired reduction for the formation of the 1,3-diol product **3**. However, this activity of the ADH towards acetone/isopropanol can be taken as an advantage if an isopropanol-based cofactor regeneration is used instead. Alongside the acetone needed for the aldol reaction isopropanol is then added at high concentration to the reaction, which not only provides an elegant cofactor regeneration, but also enhances the solubility of organic compounds in the reaction mixture and has been proven to be compatible with the aldol reaction.

For the tandem-type chemoenzymatic flow process and such a combination of the organo- and biocatalytic reaction steps, several challenges had to be addressed. First, whereas the overall solvent concentration should be lower to ensure a high enzyme activity and stability, for the aldol reaction a high concentration of acetone is needed for a fast reaction. At the same time, for an efficient enzymatic reduction step based on a substrate-coupled cofactor recycling a high excess of isopropanol is typically needed. In addition, the aldol reaction benefits from a high substrate concentration, but increasing the substrate concentration of the aldehyde also requires an increase of the concentrations of all other components (acetone, isopropanol) as well, which can have a negative impact on the biotransformation. Taken these aspects into account, both reaction steps were carefully optimized for the tandem process running in a flow setup, which is shown in Scheme 3. A particular attention was given to the amounts of isopropanol and acetone to ensure also the formation of a completely homogenous solutions before and after the reaction as well as satisfactory reaction rates of each individual reaction



Scheme 3. Tandem aldol-ADH cascade flow setup with isopropanol-based cofactor regeneration for the formation of the (1*R*,3*S*)-**3**. Pump **A** containing 3-chlorobenzaldehyde (**1**) and isopropanol, **B** aldol catalyst (*S*,*S*)-**4** and acetone, **C** ADH030, NADH and buffer (for more information, see Supporting Information, Table S11). The reaction proceeds at 100 mM substrate concentration in a buffer aqueous system containing isopropanol and acetone as cosolvents with a residence time of two hours at room temperature.

step. Similar to the sequential system the amount of enzyme was increased up to a concentration where no precipitate was observed. Unfortunately, during our investigation ADH270 turned out to be not suitable under the found reaction conditions, presumably due to the high amount of acetone that was used. Accordingly, the tandem-type chemoenzymatic two-step one-flow process was then conducted only with the ADH030 as biocatalyst component (Scheme 3).

In the presence of the aldol catalyst (*S*,*S*)-**4** and ADH030 as biocatalyst, the two-step one-flow tandem-type process was then successfully conducted at 100 mM substrate concentration in buffer containing 14 vol% isopropanol and 14 vol% acetone and with 2 hours residence time, leading to a product formation-related conversion of 51% and >99%*ee* for the desired 1,3-diol (1*R*,3*S*)-**3** (Scheme 3). The d.r. of 8:1 is in agreement with the theoretically expected e.r. of the aldol reaction, which could not be measured in this tandem process (since the aldol product as intermediate was not isolated). In contrast to the sequential-type chemoenzymatic one-flow process described above, 3-chlorobenzyl alcohol is now a major side-product (20%) since the ADH reduction of aldehyde **1** competes with the organocatalytic aldol reaction. The other major compound in the reaction mixture of this tandem-type process is unreacted aldol product **2** (24%). Notably, only small amounts of aldol condensation product (<1%) and bis-aldol product (<3%) were observed in all cases. While the biocatalytic reaction seems to be the rate-limiting step in the tandem process, improved results in the future may be obtained by using more (active) enzyme. In addition, within the tandem process an ADH that shows higher activity and affinity towards the aldol intermediate **2** compared to aldehyde **1** is likely to give improved results due to a higher selectivity. To obtain such biocatalysts, enzyme engineering might again be a suitable option. Nevertheless, the results obtained in this work show that a chemoenzymatic cascade combining an organocatalytic aldol reaction and a biocatalytic reduction with an ADH can be realized in a tandem mode running in flow.

It is noteworthy that in these flow processes the reaction medium is completely homogenous, which is needed for a well-defined, stable and reproducible flow reaction. At the same time, ensuring such a homogeneous medium often represents a challenge when working with biocatalyst formulations in aqueous systems in the presence of significant amount of polar organic cosolvent as biomaterials easily precipitates from the dissolved lyophilizate or crude extract under such conditions. Thus, this work also shows that "process windows" can be realized which enables such needed homogeneous reaction conditions even when utilizing precipitation-sensitive biomaterial components.

After having realized both variants (sequential and tandem-mode) of this chemoenzymatic flow synthesis of 1,3-diols under combination of organo- and biocatalysis, we became interested in an evaluation of these flow processes with the previously developed analogous batch processes.[3–6] It should be added that to the best of our knowledge, this combination of an asymmetric organocatalytic aldol reaction with a biocatalytic reduction is up to now the first and only example of a one-pot process, which has been conducted in a sequential and tandem-mode within a batch as well as a flow process. Selected data of this batch *versus* flow evaluation are given in Table 2, and main findings are summarized in the following. When comparing these flow and batch processes, several differences become apparent with respect to various process parameters (see Table 2): Product-formation related conversion for the 1,3-diols (1*R*,3*S*)-**3** and (1*R*,3*R*)-**3** is somewhat higher for the batch process compared to the flow process (in both sequential and tandem mode).

The diasteromeric ratios for the sequential approach are significant lower in the flow process, which might be due to the higher concentration of organocatalyst (2 mol% in flow compared to 0.5 mol% in batch. From our previous investigation[18] of this reaction we know that the enantiomeric excess obtained for the aldol product in such short reaction times at high catalyst loading is between 80 and 90%*ee*, which is in agreement with the observed d.r. of ca. 8:1. However, the 48 h reaction time in batch was reduced to 2.5 h residence time in flow, which resulted in an increase of productivity (product formed per h) for the 1,3-diols (1*R*,1*S*)-**3** and (1*R*,1*R*)-**3**. For the tandem process, the residence time was 2 h (flow) compared to a reaction time of 24 h (batch), which resulted in a significant higher productivity for the flow process also in the tandem-mode.

Summary & outlook

In conclusion, a sequential-type as well as a tandem-type chemoenzymatic flow cascade combining an organocatalytic aldol reaction and a biocatalytic reduction to form stereoselectively a 1,3-diol with two stereogenic centers were developed. Toward this end, a biocatalyst screening was carried out and two commercial and, thus, readily accessible stereocomplementary alcohol dehydrogenases for the diastereoselective reduction of aldol product **2** were found. Both of them were established in a flow setup for the synthesis of 1,3-diols by combining the biotransformations with these enzymes with the needed initial organocatalytic aldol reaction to form the aldol products, which then served as substrates for the enzymatic reduction step. It was shown that the aldol products **2** can be generated in a flow reaction and be converted to the corresponding 1,3-diols **3** without any purification step in between. With a sequential mode flow reaction, all four diastereomers were synthesized and isolated in yields of 33–76% in combination with good to high d.r. and excellent *ee*. Furthermore, a tandem process was realized using the (*S*,*S*)-aldol catalyst and ADH030 as a biocatalyst to form (1*R*,3*S*)-**3** in such a chemoenzymatic flow cascade. To the best of our knowledge, this work together with the previously developed, complementary analogous batch processes represents the first type of combined chemo- and biocatalysis process, for which sequential-type and tandem-type batch as well as flow processes have been developed. With respect to future work, it is envisioned to utilize this platform technology for a broad synthesis of pharmaceutically relevant chiral 1,3-diols and for an

expansion of the scope of this chemoenzymatic toolbox to other types of substrates and reactions running in a flow mode. With respect to further process intensification of both flow cascades and improvement of the process efficiency, optimization of the properties of the biocatalyst, e.g., through enzyme engineering, represents another task for future work.

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Conflict of Interest

The authors declare no conflict of interest.

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