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First Tandem-Type One-Pot Process Combining Asymmetric Organo- and Biocatalytic Reactions in Aqueous Media Exemplified for the Enantioselective and Diastereoselective Synthesis of 1,3-Diols

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Abstract: A suitable "process window" was identified for the combination of an asymmetric organocatalytic aldol reaction and subsequent biocatalytic reduction in aqueous medium, which thus enabled the enantio- and diastereoselective synthesis of 1,3-diols in a tandem-type, one-pot process. A key feature of this one-pot synthesis is the high 500 mM loading of the aldehyde substrate used as a starting material.

Introduction

Although biotechnology has already emerged as a key technology for the industrial production of fine chemicals and pharmaceuticals,[1] it is at the same time often considered a "stand alone" technology, thus being not compatible with "classic" chemical or chemocatalytic reaction steps. As a consequence, biotransformations typically start with purified substrates resulting from chemical processes. Providing such substrates in purified form, however, requires solvent-intensive, time-consuming, and waste-producing downstream-processing steps. Thus, a combination of a "classic" chemical or chemocatalytic reaction step for substrate synthesis with a subsequent biotransformation towards a one-pot process offers unique opportunities with respect to improvement in both process efficiency and sustainability. Running such one-pot processes in water would be of particular interest, as it makes the use of any type of enzyme possible.

Despite the tremendous potential for application, today the number of examples of synthetic one-pot processes under the combination of chemo- and biocatalytic reaction steps is still limited, although in recent years this field has emerged significantly with several contributions from different groups.[2–5] In this context, we recently demonstrated the first example of a combination of an asymmetric organocatalytic reaction with a subsequent biotransformation in aqueous reaction media.[5] This process consisted of an initial organocatalytic aldol reaction and subsequent in situ diastereoselective reduction of the formed β-hydroxy ketone by means of an enzymatic reduction (according to the reaction sequence shown in Scheme 1). The resulting 1,3-diols bearing two stereogenic centers were obtained with excellent diastereoselectivities (*dr* > 25:1) and enantioselectivities (99 % *ee*). In this type of one-pot process, the enzyme (as a catalyst for the second step), cofactor, and 2-propanol as the reducing agent (required in stoichiometric amount) were added after completion of the first reaction step, namely the aldol reaction.

An open question that has remained unanswered over the years is whether this type of one-pot synthesis with two sequential (asymmetric organo- and biocatalytic) steps could be extended towards a tandem-type process in aqueous media. In such a tandem-type synthesis, which has the same reaction sequence as that shown in Scheme 1, both the organocatalyst and enzyme are present at the beginning of the first reaction. Furthermore, suitable reaction conditions (including temperature, pH, substrate, and reagent concentrations) that enable the simultaneous progress of both the organocatalytic and enzymatic reactions have to be found. The unit operations flow of such a concept and its comparison with the previously[5a] developed one-pot process based on two subsequent reaction steps is shown in Scheme 2.

One motivation for carrying out one-pot multistep processes in a tandem mode is that, in principle, thermodynamically unfavorable reaction steps could also be conducted effectively therein, as the resulting product of such a step would be directly converted in an (irreversible) subsequent transformation, which would thus permanently shift the unfavorable thermody-

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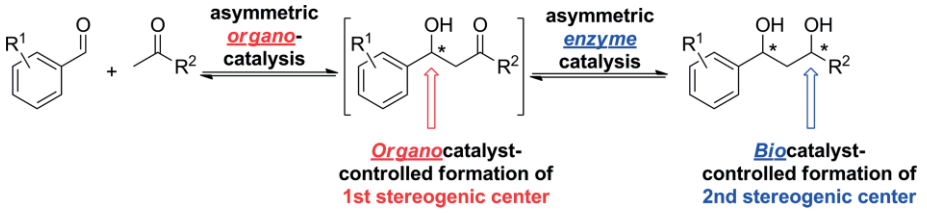
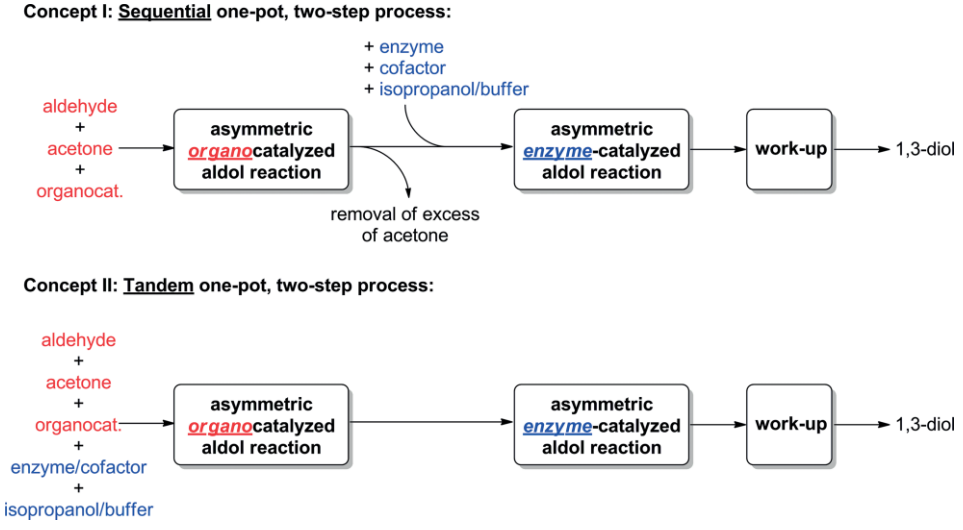


Scheme 1. Combination of an organocatalytic aldol reaction with a diastereoselective enzymatic reduction of the in situ formed β-hydroxy ketone in a one-pot process.



Scheme 2. Comparison of unit operations flow of the previously[5a] developed one-pot process based on two consecutive reaction steps with the tandem-type, one-pot process.

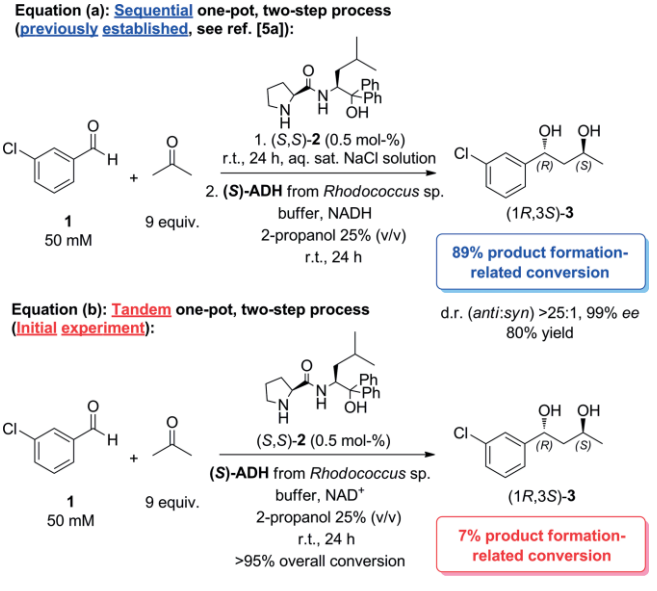
namic equilibrium to the direction of the desired product. Furthermore, tandem processes are an advantageous one-pot option if the concentration of the formed intermediate should be kept low, for example, because of inhibition or stability reasons.

A prerequisite for tandem processes is that both reactions have to proceed under identical reaction conditions such as pH, temperature, substrate concentration (whereas in a sequential one-pot synthesis there is increased "freedom to operate", as reaction conditions for the second step can be adjusted – at least in part and for some reaction parameters such as, e.g., pH, temperature, and substrate concentration – independent of the first reaction step after its completion). Thus, the identification of suitable (and potentially narrow) "process windows"[6] is a key task in the development of tandem-type, one-pot processes.

In the following, we report our results on the development of the first type of a chemoenzymatic tandem-type, one-pot process consisting of asymmetric organocatalytic and biocatalytic transformations in aqueous reaction media, as exemplified for the enantio- and diastereoselective synthesis of 1,3-diols.

Results and Discussion

Taking the previously developed one-pot process based on two consecutive steps without isolation of the intermediate by removal of the excess amount of acetone prior to the addition of the components for the enzymatic reduction step as a starting point [shown in Scheme 3, Equation (a)],[5a] at first we conducted an analogous tandem process by adding both catalysts from the beginning. As components for the initial aldol reaction, 3-chlorobenzaldehyde and acetone were used. For the



Scheme 3. Comparison of the previously developed one-pot process based on two consecutive reaction steps with the initial tandem-type, one-pot process.

subsequent enzymatic reduction, an alcohol dehydrogenase (ADH) from *Rhodococcus* sp.[7] was used as a catalyst, and substrate-coupled cofactor regeneration was done by means of 2-propanol as a reducing agent. However, in contrast to the previous successful one-pot methodology with the two steps done in a consecutive manner, which gave 1,3-diol **3** with a (product-formation-related) conversion of 89 % besides high diastereo- and enantioselectivities [*dr* > 25:1, 99 % *ee*; Scheme 3, Equation (a)], nearly complete loss of product formation of desired 1,3-diol product **3** (with only 7 %) was observed upon changing to a tandem-type, one-pot process in the presence of a buffer/2-propanol reaction mixture as our reaction medium of choice for the biotransformation [Scheme 3, Equation (b)]. This result clearly indicated that besides compatibility of the enzyme and organocatalyst (which was achieved evidenced by the previous successful one-pot, two-step synthesis in aqueous media, see also ref.[5a]), other reaction parameters in this tandem-type, one-pot process were also of high relevance.

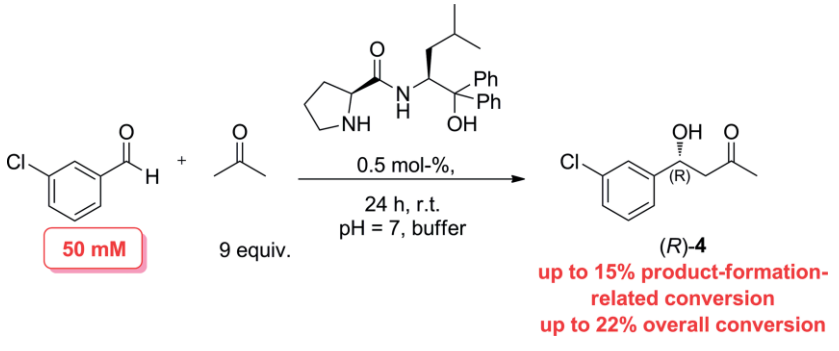
In an attempt to identify the reason for this remarkable and strong difference in product-formation-related conversion upon comparing the sequential one-pot, two-step processes with the tandem-type, one-pot process, we were first interested whether both individual reaction steps (namely, the organocatalytic aldol reaction and enzymatic reduction) proceeded under the reaction conditions chosen for the tandem process. In the previously conducted one-pot process, the aldol reaction (organocatalysis) and ketone reduction (biocatalysis) were done under different reaction conditions adjusted to known "standard reaction conditions" for these two reaction types. For example, the substrate concentration of the organocatalytic aldol reaction (500 mM) was significantly higher than that of the enzymatic transformation (50 mM). In addition, 2-propanol was added after completion of the organocatalytic process [Scheme 3, Equation (a)], and consequently, the organocatalytic reaction in the original one-pot process with two consecutive steps took place in the absence of 2-propanol (which is in contrast to a tandem-type, one-pot process with all components present in the reaction medium from the beginning).

As we changed a variety of reaction parameters for the aldol reaction in a tandem-type, one-pot process relative to the original one-pot process with two consecutive steps (e.g., substrate concentration and reaction medium, as 2-propanol was used as a co-solvent and co-substrate required for the biotransformation), we studied the influence of these reaction parameters on the aldol reaction. The substrate concentration appeared to be of particular interest, as from a kinetic perspective the reaction should proceed in a much more favorable way at elevated substrate concentrations. Given that in the tandem-type, one-pot process the substrate concentration was adjusted to 50 mM (as the biotransformation in the one-pot process with consecutive steps was conducted at 50 mM as a "standard reaction condition"), we first studied if the organocatalytic aldol reaction proceeded at 50 mM of substrate as well. Interestingly, however, we found a strong drop in the conversion upon changing the substrate concentration for the aldol reaction to 50 mM. Thus, instead of a high product-formation-related conversion for the original aldol reaction at a substrate concentration of 500 mM [Scheme 3, Equation (a)], only 15 % conversion related to the formation of product **4** and 22 % overall conversion including side products were found upon operating at a substrate concentration of 50 mM (Scheme 4).

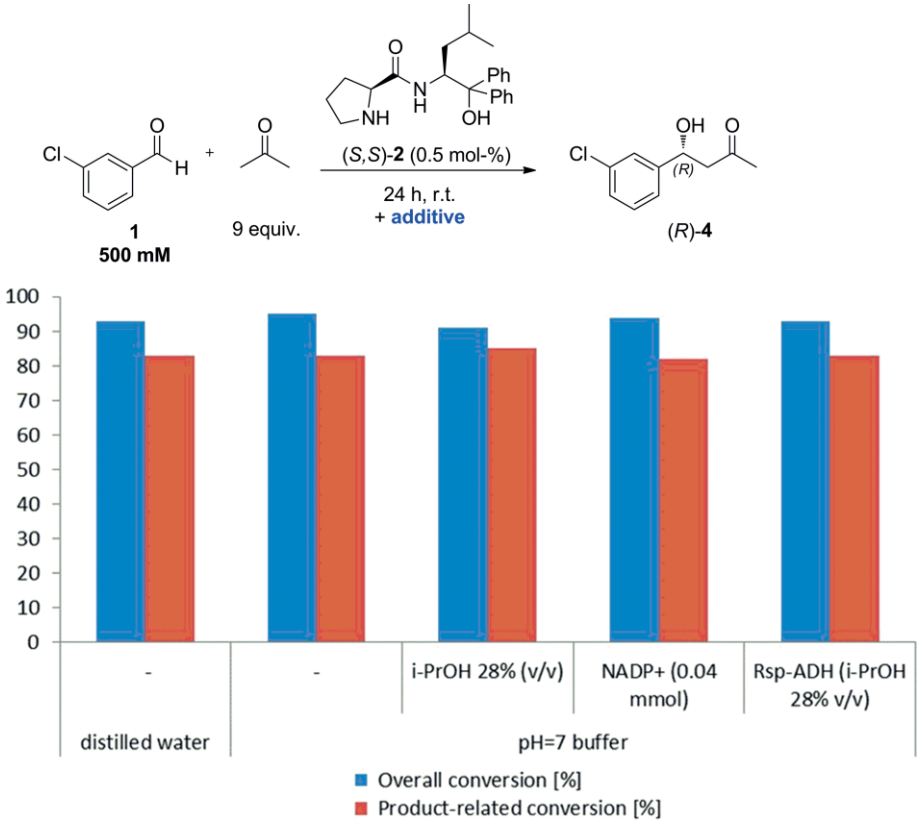
This clearly indicates the need to identify a suitable "process window" and a substrate concentration at which both reactions proceed as a task for the development of an efficient tandem process. The disappointing result of a low conversion upon choosing a 50 mM substrate concentration can be rationalized by the abovementioned kinetic effect. The reaction medium, which is a two-phase system, and an unfavorable distribution coefficient of acetone might also play a role. Accordingly, the organocatalytic aldol reaction should be conducted at an elevated substrate concentration of 500 mM instead of 50 mM in the one-pot process to obtain an excellent conversion related to the formation of aldol product **4** as an intermediate and, thus, subsequently desired 1,3-diol **3**.

As for the influence of other reaction parameters on the tandem process, to our delight we found that neither the use of a buffer instead of distilled water nor the presence of protein (enzyme) or cofactor as an additive had a negative impact on the organocatalytic aldol reaction (Scheme 5). Most notably, there was no negative impact of a large amount of 2-propanol [28 % (*v*/*v*)] in buffer on the aldol reaction, which is of importance owing to the use of 2-propanol as a reducing agent and a co-substrate for in situ cofactor regeneration in the subsequent second step, namely, the enzymatic reduction, in larger amount (to ensure a strong "driving force" for the enzymatic reduction towards formation of the desired 1,3-diol).

Given that this organocatalytic aldol reaction was compatible with all components used in the biotransformation, we next

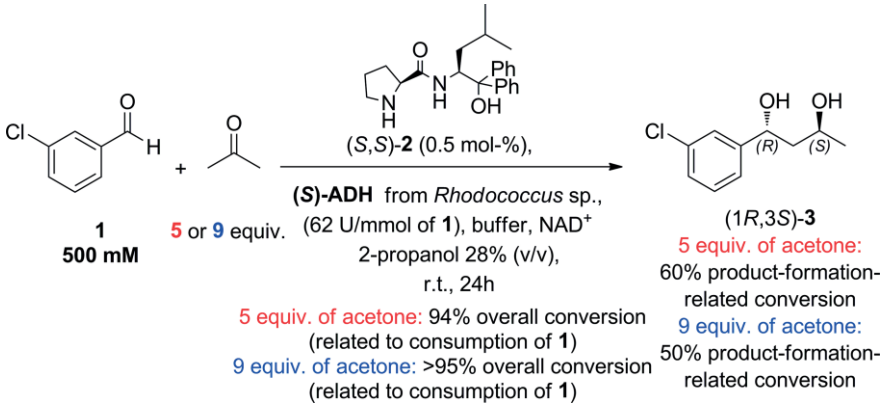


Scheme 4. Impact of different biocompatible reaction media on the organocatalytic aldol reaction.



Scheme 5. Impact of the components of the biotransformation on the organocatalytic aldol reaction.

combined this reaction with enzymatic ketone reduction towards a tandem-type, one-pot process. However, as a high substrate loading was required for the organocatalytic aldol reaction, a prerequisite for a successful tandem reaction was that the biotransformation must also proceed at a high substrate concentration of, for example, 500 mM. We were pleased to find that the enzymatic reduction also proceeded well at a significantly elevated substrate concentration of 500 mM for 3-chlorobenzaldehyde as the substrate for the initial aldol reaction, which led to the formation of desired diol (1*R*,3*S*)-**3** with a conversion of 50 % related to the formation of this product (Scheme 6). The overall conversion was >95 %; consequently, 3-chlorobenzaldehyde was fully consumed. Such a conversion of 50 % for the formation of (1*R*,3*S*)-**3** without process optimization is a remarkable result for this tandem-type, one-pot process, as the initial aldol reaction competes with enzymatic reduction of aldehyde **1** to 3-chlorobenzyl alcohol (which thus leads to decreased formation of the aldol adduct). In addition, owing to the presence of an excess amount of acetone (a required component for the aldol reaction) the "retro-reduction reaction" [= oxidation of (1*R*,3*S*)-**3**] leading to a decomposition product of (1*R*,3*S*)-**3** might also play a role, as well as inhibition or deactivation of the enzyme. However, the desired biotransformation proceeded efficiently in spite of an excess amount of acetone



Scheme 6. Tandem-type, one-pot process combining an organocatalytic aldol reaction with an enzymatic reduction.

in the reaction medium, which thus demonstrates the synthetic utility of the ADH from *Rhodococcus* sp.[7] as a biocatalyst for stereoselective biocatalytic ketone reductions also at elevated substrate loadings and under challenging reaction conditions, such as a high percentage of organic water-miscible components [2-propanol: 28 % (*v*/*v*); acetone: 9 equiv.].

Next, we focused on process optimization to improve the formation of desired diol (1*R*,3*S*)-**3** while minimizing the impact of the undesired side reactions, namely: (1) enzymatic reduction of aldehyde **1**; (2) organocatalytic side reactions such as aldol condensation (see also ref.[5a]); (3) ADH-catalyzed oxidation of (1*R*,3*S*)-**3** with formation of **4** as a result of the presence of an excess amount of acetone from the aldol reaction step, which then serves as a "hydride acceptor". By adjusting the amount of organocatalyst, biocatalyst, stoichiometric amount of acetone and 2-propanol, as well as the reaction time accordingly, the conversion related to the formation of (1*R*,3*S*)-**3** was then further increased to 60 % (Scheme 6; for experimental details, e.g., quantification of byproducts in dependency of selected reaction parameters, see the Supporting Information). To demonstrate the feasibility of this optimized one-pot synthesis on an elevated laboratory scale, a preparative experiment under these conditions was conducted on a 10 mmol scale of 3-chlorobenzaldehyde (**1**), which resulted in a 50 % conversion related to the production of desired (1*R*,3*S*)-diol **3** (see the Supporting Information for experimental details). Subsequent workup and isolation by column chromatography then gave desired purified (1*R*,3*S*)-diol **3** in 33 % yield. A further improvement in the conversion can be expected if acetone as the most volatile component is removed from the reaction mixture continuously, in particular at a later stage of the process after completion of the aldol reaction. Various techniques for the in situ removal of acetone have been developed successfully by Liese et al.[8] and represent a promising tool for future process development and scale up of this tandem-type one-pot process.

Conclusions

In conclusion, we reported the first tandem-type, one-pot process under combination of asymmetric organo- and biocatalytic reaction steps in aqueous medium, as exemplified for the enantio- and diastereoselective synthesis of 1,3-diols. A key challenge was to identify a suitable "process window", namely, reaction conditions under which both reaction steps could proceed efficiently. An additional task was to suppress side reactions caused by reaction components from the other reaction step (e.g., reduction of aldehyde **1** used in the first step by the biocatalyst required for the second step). Thus, besides compatibility of the enzyme and organocatalyst, several reaction parameters in this tandem-type, one-pot process were also deemed to be of high relevance. After process design and optimization, a suitable "process window" was identified that enabled the combination of an asymmetric organocatalytic aldol reaction with enzymatic reduction in a tandem-type, one-pot process in aqueous medium at a high substrate loading of 500 mM of the corresponding aldehyde.

Experimental Section

The experimental protocols and details are given in the Supporting Information.

Acknowledgments

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Keywords: Aldol reactions · Biocatalysis · Diols · Enzyme catalysis · Organocatalysis

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