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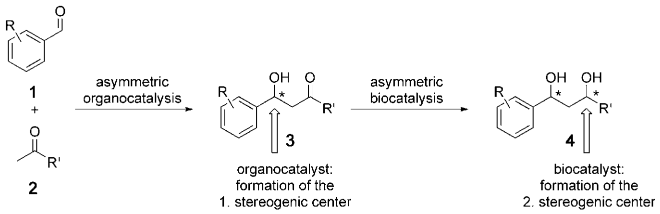
Chemoenzymatic Synthesis

Sequential and Modular Synthesis of Chiral 1,3-Diols with Two Stereogenic Centers: Access to All Four Stereoisomers by Combination of Organo- and Biocatalysis**

*Katrin Baer, Marina Kraußer, Edyta Burda, Werner Hummel, Albrecht Berkessel, and Harald Gröger**

Chiral 1,3-diols with two stereogenic centers are widely used as building blocks in the synthesis of pharmaceutically active compounds.[1] In addition, numerous natural products possess a chiral 1,3-diol subunit.[2] A further interesting application of chiral 1,3-diols is their transformation into chiral 1,3-bisphosphanes, which are often used very successfully as enantiomerically pure ligands in asymmetric catalysis.[3] Accordingly, there is a high demand for stereoselective synthetic processes to generate chiral 1,3-diols with two stereogenic centers (e.g., of the type **4**; Scheme 1), which ideally should allow the synthesis of all stereoisomers in a diastereo- and enantiomerically pure form.[4] To date, efficient synthetic routes[2] are based, in particular, on the enantio- and diastereoselective reduction of 1,3-diketones by hydrogenation with metal catalysts[4a] and enzymes[4b] or chemoenzymatic processes by dynamic kinetic resolution.[4c] A limitation of these methods is often their applicability for only one or two of the four maximum possible stereoisomers. In contrast, methods which offer an approach to all four stereosiomers in an efficient and convenient way are rarely known.[2] In the following we report a modular chemoenzymatic process for the selective synthesis of all four stereoisomers of 1,3-diols **4** through a combination of asymmetric organocatalysis and biocatalysis. A key feature of this synthetic strategy is the sequential construction of the stereogenic centers. Thus, each stereogenic center can be constructed selectively by means of a catalyst which is specifically suitable for this purpose (Scheme 1). This modular synthetic strategy additionally offers the possibility for a two-step one-pot synthesis.



Scheme 1. Synthesis of 1,3-diols by organo- and biocatalysis.

Since organocatalytic syntheses[5,6] are already known for the initial aldol reaction, we first focused on the subsequent diastereoselective enzymatic reduction of β-hydroxy ketones **3** bearing one stereogenic center. Such a study is interesting both from the synthetic perspective of the target molecules **4** and the perspective of the reaction mechanism since chiral ketones are used as substrates. Thus, the effect of the internal stereogenic center of the substrate and the influence of the chiral catalyst (enzyme) on the formation of the second stereogenic center can be compared. Such studies based on the use of redox enzymes are rare,[7] in contrast to the numerous examples of enantioselective reductions of prochiral ketones.[8] A stereoselective reaction course is typically expected when chiral (for example, racemic) substrates are used in an enzymatic reaction, with preferential transformation of only one of the two enantiomers. With respect to a stereoselective construction of all stereoisomers of type **4**, however, we were interested in a diastereoselective reaction course which can be directed exclusively (externally) by the enzyme catalyst. In addition to a highly selective formation of the second stereogenic center—depending only on the biocatalyst—both enantiomeric forms of the aldol product should be tolerated as substrates. We used as enzymes two alcohol dehydrogenases with *S* and *R* enantiospecificity.[9] The suitability of the enzymes as catalysts for the transformation of the ketones **3** (racemic as well as enantiomerically enriched) were first investigated spectrophotometrically. This method is based on the dependence of the decrease in the amount of NAD(P)H as a natural cofactor (reducing agent) on a specific substrate. We found that the enzymes were suitable for the reduction of both enantiomeric forms of the ketones without significant differences.[10]

The enzymatic reduction of ketones **3** was subsequently carried out on a preparative scale according to the concept of

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[*] K. Baer, M. Kraußer, E. Burda, Prof. Dr. H. Gröger
Department of Chemistry and Pharmacy
University of Erlangen-Nuremberg
Henkestr. 42, 91054 Erlangen (Germany)
E-mail: harald.groeger@chemie.uni-erlangen.de
Prof. Dr. W. Hummel
Institute of Molecular Enzyme Technology at the Heinrich-Heine-University of Düsseldorf, Research Centre Jülich
Stetternicher Forst, 52426 Jülich (Germany)
Prof. Dr. A. Berkessel
Department of Chemistry, University of Cologne
Greinstrasse 4, 50939 Cologne (Germany)

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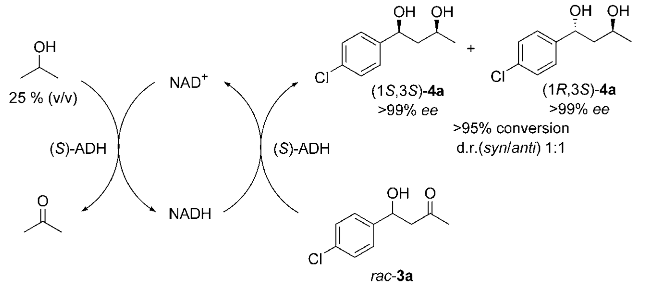
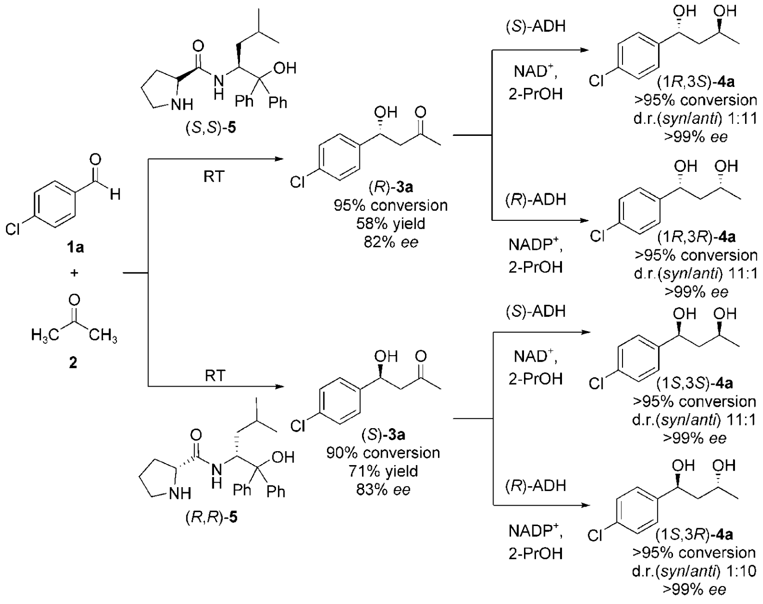
the substrate-coupled regeneration of the cofactor[8] (Scheme 2). Therein, the cofactor NAD(P)H is recycled in situ by means of the oxidation of 2-propanol (2-PrOH) to acetone. Thus, the expensive cofactor is used only in a catalytic amount, and the inexpensive 2-propanol represents the reducing agent required in a stoichiometric amount. The diastereoselective reduction proceeded successfully when the racemic ketone *rac*-**3a** was used. For example, quantitative conversion was achieved in the presence of the (*S*)-alcohol dehydrogenase, and both product diastereomers were obtained in enantiomerically pure form (> 99% *ee*) in a diastereomeric ratio of 1:1. Thus, a diastereoselective reaction has been successfully developed, which—as desired—is controlled exclusively by the "external influence" of the (bio-)catalyst; an undesired internal induction by the stereogenic center in the aldol adduct **3a** was not observed. This result is in contrast to many enzyme-catalyzed reactions, which show a high preference for only the transformation of one of the two enantiomers of a substrate (resulting in an efficient resolution of a racemate through modification of only one enantiomer).[11]



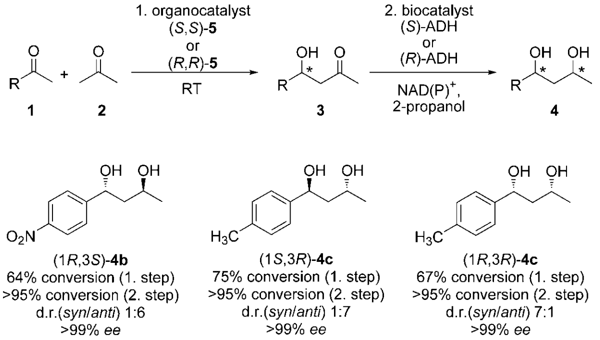
Scheme 2. Diastereoselective enzymatic reduction.

Based on this result, we focused on the envisioned two-step synthesis of all the 1,3-diol stereoisomers by sequential stereoselective formation of both stereogenic centers (Scheme 3). For the initial organocatalytic aldol reaction we oriented ourselves on already known asymmetric organocatalytic processes.[5,6] We were particularly interested in a solvent-free synthesis. On the basis of the work by Singh and co-workers,[5g] aldol products **3a** have been prepared with conversions of up to 95% and enantioselectivities of 82–83% *ee* by using organocatalysts (*S*,*S*)-**5** and (*R*,*R*)-**5** at room temperature. These enantiomerically enriched (*S*)- and (*R*)-aldol adducts **3a** have then been used as substrates in the subsequent enzymatic reduction. When starting from (*S*)-**3a** as the substrate (83% *ee*) in the presence of an *S*-selective alcohol dehydrogenase, the desired 1,3-diol (1*S*,3*S*)-**4a** was formed with a conversion of > 95% and a diastereomeric ratio d.r.(*syn*/*anti*) of 11:1, again in enantiomerically pure form (> 99% *ee*). The enantiomerically pure (1*R*,3*S*) diastereomer (1*R*,3*S*)-**4a** was prepared with > 99% *ee* by using the same enzyme and starting from (*R*)-**3a** (82% *ee*). A high conversion of > 95% and a diastereoselectivity d.r.(*syn*/*anti*) of 1:11 was also obtained in this case. Analogously, starting from enantiomerically enriched substrates (*S*)-**3a** (83% *ee*) and (*R*)-**3a** (82% *ee*) in the presence of an *R*-selective alcohol dehydrogenase afforded the corresponding diastereomers (1*S*,3*R*)-**4a** and (1*R*,3*R*)-**4a**, respectively, with > 99% *ee* and high diastereoselectivities in both cases (conversions of > 95% in both cases). Thus, this chemoenzymatic synthesis is suitable for the synthesis of all four possible stereoisomers of type **4a** in enantiomerically pure form (> 99% *ee*).

In our study of the substrate spectrum, we varied the aldehyde component in particular. We also succeeded in the formation of the corresponding desired 1,3-diol starting from 4-nitrobenzaldehyde (**1b**), as exemplified by the synthesis of (1*R*,3*S*)-**4b** with a (product-related) conversion of 64% (Scheme 4). Besides electron-deficient aromatic aldehydes, electron-rich benzaldehydes can also be used: An organocatalytic aldol reaction starting from 4-methylbenzaldehyde (**1c**; 75% product-related conversion; 76% *ee*), followed by reduction delivered the desired product (1*S*,3*R*)-**4c** with > 95% conversion, a diastereomeric ratio d.r.(*syn*/*anti*) of 1:7, and with > 99% *ee*. The related diastereomer (1*R*,3*R*)-**4c**



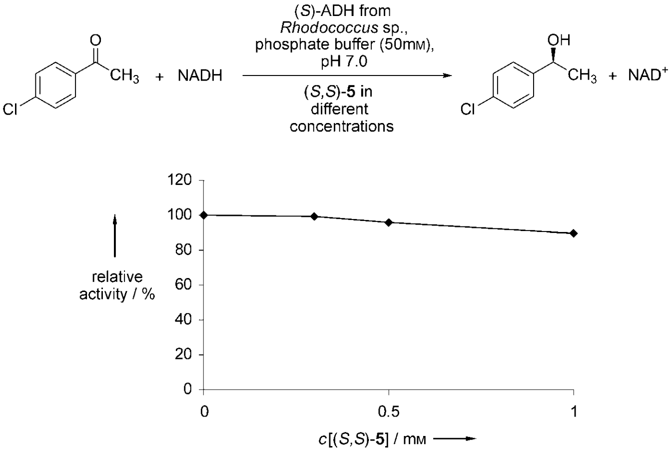
Scheme 3. Chemoenzymatic synthesis of all stereoisomers of **4a**.



Scheme 4. Substrate spectrum of the chemoenzymatic synthesis of **4**.

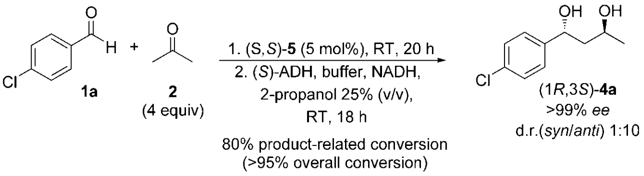
has also been obtained with $>95\%$ conversion and with $>99\%$ *ee*.

Furthermore, we were interested in the combination of both processes to give an economical preparative method in which the reaction product from the first step is used directly without purification for the second reaction. To the best of our knowledge, studies on the combination of organocatalysis and biocatalysis in aqueous reaction media are rare.[12,13] A prerequisite for such a method is a compatibility of the two reaction processes. Accordingly, we studied the impact of the organocatalyst (*S*,*S*)-**5** on the activity of the enzyme by means of spectrophotometrical tests (Scheme 5). Therein, a high enzyme activity of the (*S*)-alcohol dehydrogenase was also found in the presence of organocatalyst (*S*,*S*)-**5**. This result indicates a high compatibility of the organocatalyst with the biocatalyst in aqueous media, thus offering interesting perspectives towards future combinations of organocatalysis and biocatalysis in one-pot multistep syntheses in aqueous media. In addition, the biocompatibility is not limited to peptidic organocatalysts such as (*S*,*S*)-**5**. In further activity tests, the biocompatibility of the L and D enantiomers of proline, which are widely used organocatalysts[5k,6] that also catalyze asymmetric aldol reactions efficiently,[5a,b] has been demonstrated. For example, when using an alcohol dehydrogenase from *Lactobacillus kefir*, irrespective of the proline concentration, the relative enzyme activities are virtually unchanged and high, in comparison with the enzyme activity in the reference reaction without proline as an additive (see also the Experimental Section and schemes in the Supporting Information).



Scheme 5. Enzyme activity as a function of the organocatalyst concentration.

Next, we carried out such a combination of the asymmetric organocatalytic aldol reaction and a subsequent biotransformation with a final work-up step. The aldol reaction was carried out as a solvent-free synthesis and the resulting reaction mixture was passed directly into an aqueous/2-propanol solution of the enzyme. We were pleased to find that such a process proceeds highly efficiently and leads to the desired 1,3-diol (1*R*,3*S*)-**4a** with a product-related conversion of 80% over two steps (at an overall conversion of $>95\%$), a high diastereomeric ratio d.r.(*syn*/*anti*) of 1:10, and an excellent enantiomeric excess of $>99\%$ *ee* (Scheme 6). An advantage of this modified one-pot synthesis over the sequential two-step method with isolation of the intermediate is the avoidance of the critical work-up of product (*R*)-**3a** after the first reaction step (danger of decomposition through dehydration during purification by column chromatography). This offers the possibility for an improved overall economy of the process.



Scheme 6. Combination of organocatalysis and biocatalysis in a modified one-pot synthesis of **4a**.

In summary we have reported the combination of asymmetric organo- and biocatalytic reaction sequences which lead to a sequential construction of both stereogenic centers of 1,3-diols. This modular chemoenzymatic synthetic concept makes possible efficient access to all four stereoisomers in enantiomerically pure form. At the same time, the reaction mixtures resulting from the organocatalytic reaction route are compatible with a direct subsequent enzymatic reduction without the need for a work-up of the aldol reaction. Based on these positive initial results on the compatibility and combination of asymmetric organocatalysis and biocatalysis we are currently working on the development of corresponding one-pot multistep reactions in aqueous reaction media.

Experimental Section

Procedure for the chemoenzymatic two-step synthesis of 1,3-diols (according to Scheme 3):

For the initial step, namely the organocatalytic aldol reaction, 4-chlorobenzaldehyde (**1a**; 1.4 mmol), the corresponding organocatalyst [(*S*,*S*)-**5** or (*R*,*R*)-**5**; 5 mol%], and acetone (5.6 mmol) were sequentially added to a reaction tube. The resulting reaction mixture was then agitated for 18 h at 20–25 °C. After addition of a saturated solution of sodium chloride (5 mL), the resulting mixture was extracted three times with ethyl acetate and the collected organic phases were dried over magnesium sulfate. After removal of the solvent on a rotary evaporator, the resulting crude product **3** was purified by column chromatography [hexane/ethyl acetate, 5:1 (v/v)].

For the second step, namely the enzymatic reduction, the aldol product **3** (0.5 mmol) was first dissolved in 2-propanol (2.5 mL). After addition of phosphate buffer (pH 7; 50 mM; 7.5 mL), magnesium chloride (1 mM, only when using the alcohol dehydrogenase from *Lactobacillus kefir*), and NAD(P)$^+$ (0.02 mmol), the corresponding alcohol dehydrogenase (20–200 U mmol^{-1} of substrate) was added under stirring. After stirring the reaction mixture for 18–67 h at room temperature, it was extracted with ethyl acetate. The collected organic phases were dried over magnesium sulfate, and subsequently the solvent was removed on a rotary evaporator. The crude product was then purified by column chromatography [hexane/ethyl acetate, 5:1 (v/v)], thus affording the product 1,3-diol **4** in a diastereomerically pure and enantiomerically pure form.

Procedure for the combination of the organocatalytic aldol reaction with subsequent enzymatic reduction (according to Scheme 6):

4-Chlorobenzaldehyde (**1a**; 0.5 mmol), acetone (2 mmol), and organocatalyst (*S*,*S*)-**5** (5 mol%) were sequentially added in a reaction tube (2.5 mL) and the resulting mixture was agitated for 20 h at 20–25 °C. Without work-up, the reaction mixture was subsequently decanted into a 25 mL round-bottom flask. Then 2-propanol (2.5 mL), phosphate buffer (pH 7; 50 mM; 7.5 mL), the cofactor NAD$^+$ (0.02 mmol), and the alcohol dehydrogenase from *Rhodococcus* sp. (20 U mmol^{-1}) were added. After stirring the mixture for 18 h at 20–25 °C, subsequent extraction with ethyl acetate (four times), and drying over magnesium sulfate, the solvent was removed on a rotary evaporator. The crude product was then purified by column chromatography [hexane/ethyl acetate, 5:1 (v/v)], to give 1,3-diol (1*R*,3*S*)-**4a** in a diastereomerically pure and enantiomerically pure form.

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