One-Pot Synthesis Hot Paper

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Cooperative Catalysis of Noncompatible Catalysts through Compartmentalization: Wacker Oxidation and Enzymatic Reduction in a One-Pot Process in Aqueous Media**

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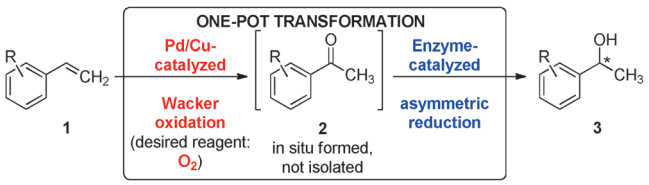
In memory of Erwin Flaschel

Abstract: *A Wacker oxidation using $CuCl/PdCl_2$ as a catalyst system was successfully combined with an enzymatic ketone reduction to convert styrene enantioselectively into 1-phenylethanol in a one-pot process, although the two reactions conducted in aqueous media are not compatible due to enzyme deactivation by Cu ions. The one-pot feasibility was achieved via compartmentalization of the reactions. Conducting the Wacker oxidation in the interior of a polydimethylsiloxane thimble enables diffusion of only the organic substrate and product into the exterior where the biotransformation takes place. Thus, the Cu ions detrimental to the enzyme are withheld from the reaction media of the biotransformation. In this one-pot process, which formally corresponds to an asymmetric hydration of alkenes, a range of 1-arylethanols were formed with high conversions and 98–99 % ee. In addition, the catalyst system of the Wacker oxidation was recycled 15 times without significant decrease in conversion.*

Enantioselective one-pot processes have attracted much attention as sustainable and economically viable methods.[1] One of the particular challenges in this field is the combination of chemical and enzymatic reactions. Often enzymes are deactivated by components of the chemical reaction step, and thus the reactions are not compatible.[2] From a synthetic and industrial point of view, in particular, the combination of chemical catalysts, especially metal catalysts, and biocatalysts in one pot would be valuable for the development of efficient routes towards enantiomerically pure compounds.[2] Only a few examples of combinations of chemocatalytic and enzymatic reactions in aqueous media have been developed by others and our group.[2–4] Furthermore, by means of this chemoenzymatic one-pot approach, access to so-called "dream reactions", for which no efficient catalysts exist, is conceivable when instead of a single catalyst a "catalyst system", consisting of cooperatively acting chemo- and biocatalysts, is used.

One such chemoenzymatic one-pot process of current interest is the enantioselective conversion of styrene or a substituted derivative thereof to give the corresponding 1-phenylethanol. This transformation corresponds to an asymmetric hydration of a styrene, which represents a "dream reaction" in organic chemistry. Up to now, there have been no efficient chemocatalysts for this reaction.[5–8] Recently, however, the Faber group reported the first example of an efficient asymmetric direct hydration based on the use of a decarboxylase as a catalyst and styrenes with a required *p*-hydroxy substituent as suitable substrates.[7] As an alternative, we previously developed a two-step one-pot process for the direct transformation of styrenes into the corresponding chiral secondary alcohols by combination of the Wacker–Tsuji oxidation using benzoquinone as an oxidizing agent and reduction by alcohol dehydrogenase (ADH) from *Lactobacillus kefir*.[4g] Although this method is general with respect to substrate range, one can see a drawback in the requirement of a stoichiometric amount of benzoquinone. The application of the "classic" Wacker oxidation conditions with molecular oxygen as the oxidation agent (according to the process shown in Scheme 1) would represent a more attractive, economically favored, and "greener" alternative. As a catalyst system, palladium chloride and copper chloride are suitable.

Initial studies in our group, however, unfortunately revealed that the presence of Cu salts strongly inhibits the subsequent enzymatic reduction, thus making an efficient combination of the two towards a one-pot tandem process with both reactions in aqueous media impossible.[4g,9] In the following, we report a strategy for how to overcome this limitation, which at the same time represents a general



Scheme 1. Combination of Wacker oxidation and enzymatic reduction without isolation of the intermediate in a one-pot process.

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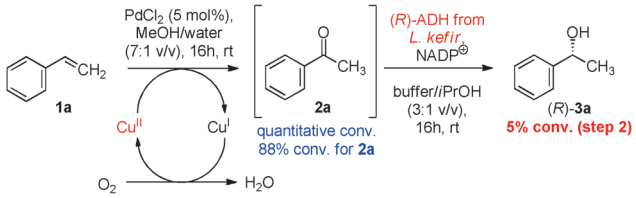
concept for combining incompatible chemo- and biocatalytic reactions conducted in water towards one-pot processes.

To get a more detailed understanding of the impact of metals, in particular Cu ions, on the enzyme first we reinvestigated the one-pot synthesis of **3a** through the combination of Wacker oxidation with $PdCl_2$/CuCl and enzymatic reduction (Scheme 2). Whereas a quantitative

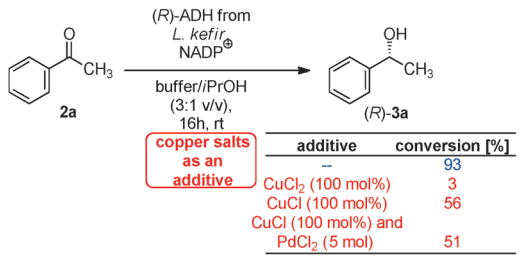


Scheme 2. Combination of Wacker oxidation and enzymatic reduction under "standard" conditions for a one-pot process.

conversion and formation of ketone **2a** with 88% was found for the Wacker oxidation step (when O_2 in a balloon was used, see the Supporting Information), a disappointing conversion of only 5% was observed for the enzymatic reduction of **2a** to **3a**, thus also confirming our initial results.

Next, we studied which metal component is the most critical one for the enzyme in terms of deactivation. For the investigation of the impact of copper ions on ADH, the enzymatic reduction was conducted in the presence of such metal ions. We focused on copper as the assumed critical metal component since in our previous studies[4g,9] we found that $PdCl_2$ has no negative impact on the enzymatic reduction. However, conducting the enzymatic reduction in the presence of a copper component revealed a moderate to strong suppression of the biotransformation with conversions of only 3%, 56%, and 51% in the presence of $CuCl_2$ (100 mol%), CuCl (100 mol%), and a mixture of CuCl (100 mol%) and $PdCl_2$ (5 mol%) as the respective additive (Scheme 3). In contrast, a high conversion of 93% was found for the enzymatic reduction in the absence of such copper additives.

In order to achieve a combination of the Pd- and Cu-catalyzed Wacker oxidation and the enzymatic reduction, we envisioned a compartmentalization strategy as the most promising. Such a compartmentalization should make it possible to conduct both reactions, though not compatible with each other, in a one-pot mode. Attractive options for the compartmentalization of the catalysts include separation by



additive	conversion [%]
--	93
$CuCl_2$ (100 mol%)	3
CuCl (100 mol%)	56
CuCl (100 mol%) and $PdCl_2$ (5 mol)	51

Scheme 3. The impact of copper ions on the enzymatic reduction.

a membrane and encapsulation into a solid support such that they are not in contact with each other. As a particularly promising approach we considered the site-isolation method for the chemocatalysts using polydimethylsiloxane (PDMS) thimbles developed by the Bowden group.[10] Due to the hydrophobic properties of the PDMS membrane, acetophenone formed in situ in the interior could flux through this membrane into the exterior to react in the presence of the enzyme (Figure 1). In contrast, the catalysts in the interior

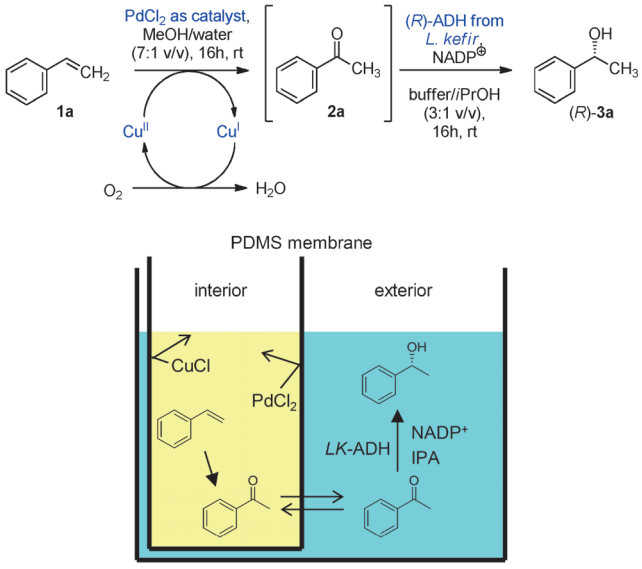


Figure 1. Concept of site-isolation of catalysts using a PDMS thimble for the combination of a Wacker oxidation and an enzymatic reduction (*LK*-ADH: alcohol dehydrogenase from *L. kefir*; IPA: isopropanol).

($PdCl_2$, CuCl) as well as the exterior (enzyme, cofactors) would be kept in their phases, and thus their undesired interaction with each other would be avoided. In their pioneer work the Bowden group[10] used a hydrophobic organic solvent in the exterior for extraction of a range of organic hydrophobic intermediates from the interior into the exterior. We envisioned that this compartmentalization concept might also work with aqueous phases (with significant water content) in both the interior and exterior phases as well as with biocatalysts. The driving force for passing the hydrophobic PDMS membrane would result from the concentration gradient of acetophenone (formed in situ in the interior) between the interior and exterior phases. In addition, recycling of the Wacker oxidation catalyst system in the PDMS thimble should be possible since hydrophilic catalytic species such as metal ions (as the catalyst components for Wacker oxidation) and enzymes are site-isolated and thus retained by the PDMS membrane. In the following, we report a method for the site-isolation of $PdCl_2$/CuCl and ADH from *L. kefir* by a PDMS thimble and recycling of the metal catalysts according to the concept shown in Figure 1 as the first example of a chemoenzymatic one-pot process in water under compartmentalization of water-soluble catalysts.

First, different one-pot concepts for the desired cascade consisting of an initial Wacker oxidation of styrene as a model

substrate in the interior and an enzymatic reduction of in situ formed acetophenone in the exterior (with both reactions conducted at room temperature)[11] were tested (Figure 2 and Figure 3). After finding optimized conditions for the extraction of acetophenone to the exterior as well as the enzymatic reduction there (data not shown), we examined a one-pot process with simultaneous Wacker oxidation and enzymatic reduction (Figure 2). Accordingly, styrene (**1a**) was dissolved in a mixture of methanol and water (7:1) as the optimized solvent[10b] for the Wacker oxidation, and this solution was poured into a PDMS thimble (interior chamber). The PDMS thimble was placed in a flask filled with aqueous buffer, isopropanol, an alcohol dehydrogenase from *Lactobacillus kefir* as a catalyst, and $NADP^+$ as a cofactor (Figure 2). In the resulting process conducted in a "simultaneous mode" the Wacker oxidation in the interior and enzymatic reduction in the exterior proceed in parallel, and the formed acetophenone (**2a**) is extracted into the exterior where it is reduced by the enzyme to alcohol (*R*)-**3a**. However, it turned out that the

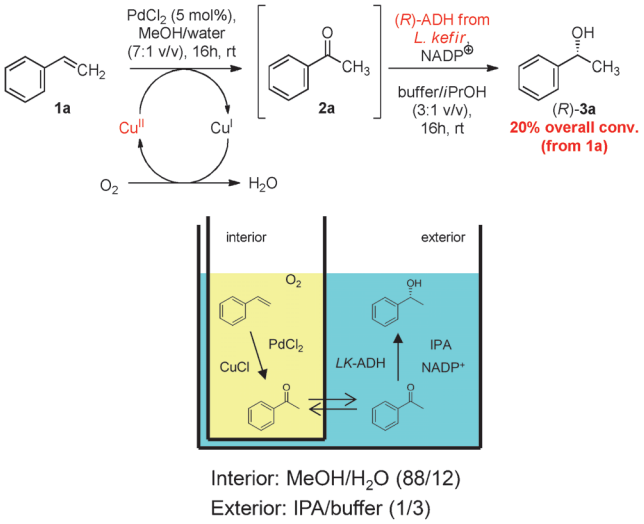


Figure 2. Chemoenzymatic one-pot process in a "simultaneous mode".

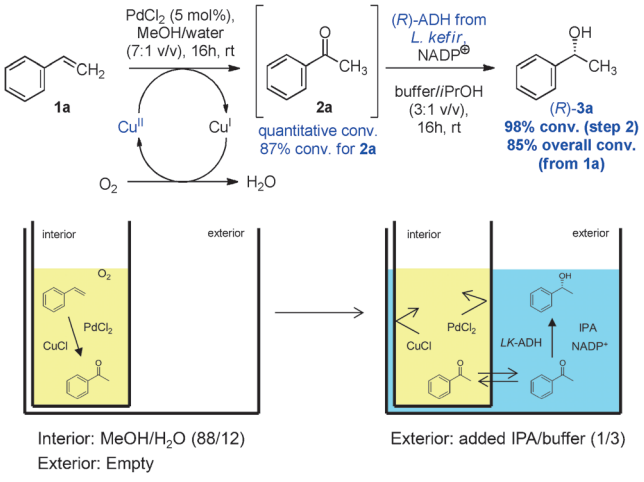


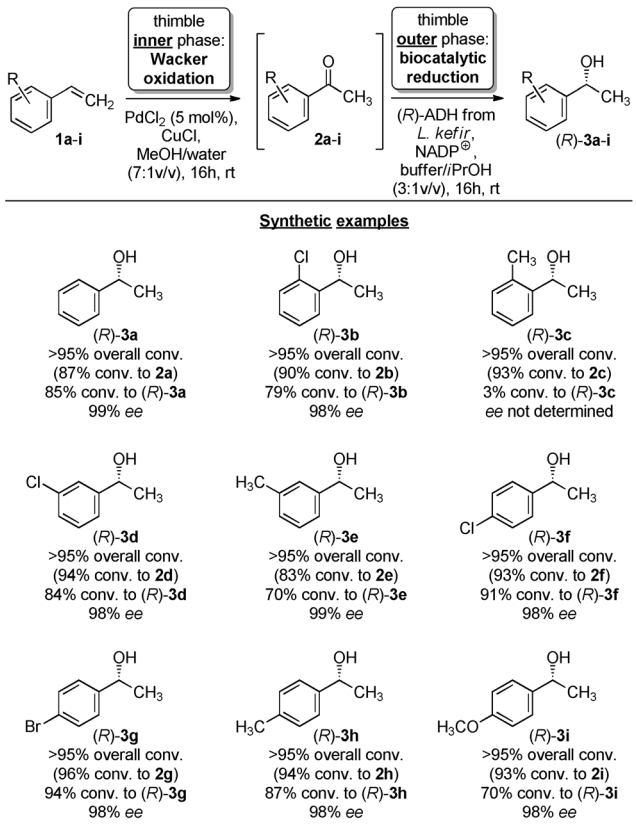
Figure 3. Chemoenzymatic one-pot process in a "sequential mode".

Wacker oxidation in the interior did not proceed efficiently in this case, leading to only 20% conversion (Figure 2). As a reason we found that methanol as the solvent component for the Wacker oxidation is extracted also into the exterior, thus decreasing the methanol content needed for an efficient Wacker oxidation reaction in the interior.

In order to suppress the methanol leaching, an alternative one-pot concept based on conducting the Wacker oxidation and enzymatic reduction in a sequential mode was investigated. In this process the buffer/isopropanol solution with ADH and cofactor was added to the exterior after completion of the initial Wacker oxidation in the interior (Figure 3). We were pleased to find that by means of this one-pot method the accumulated acetophenone was converted successfully into 1-phenylethanol (*R*)-**3a**, leading to an excellent conversion also for the second reaction step with 98% and a high overall conversion of 85% related to the formation of product (*R*)-**3a** (starting from **1a**). This result also emphasizes that diffusion of in situ formed ketone **2a** through the PDMS thimble from the interior to the exterior proceeds highly efficiently. In detail, after the one-pot process was conducted 83% of 1-phenylethanol (*R*)-**3a** and 2% of **2a** was found in the exterior as well as 2% of (*R*)-**3a** in the interior.

Having successfully developed a one-pot procedure for the combination of a Wacker oxidation with molecular oxygen and an enzymatic reduction of the in situ formed Wacker oxidation product **2a** to produce alcohol (*R*)-**3a**, we investigated the substrate scope of this methodology (Scheme 4). Here, almost all investigated styrenes **1** (except 2-methylstyrene (**1c**)) were efficiently converted into the alcohol products **3** with high conversion (over two steps) and excellent enantioselectivities. Thus, this one-pot method is suitable for numerous substrates with different substituents at the aryl moiety in **1**, in particular when these substituents are in the *meta* and *para* position. For example, all of the examined 3- and 4-substituted styrenes with halogen, alkyl, and alkoxy substituents were converted to the corresponding acetophenones **2** and subsequently 1-phenylethanols (*R*)-**3** with high conversion and excellent enantioselectivity. High enantioselectivity was also obtained for the sterically hindered 2-substituted 1-phenyl-ethanol (*R*)-**3b** with 98% *ee* at 79% conversion related to the formation of this product over two steps. An exception within the broad substrate scope, however, is the conversion of 2-methyl-substituted styrene (**1c**) into product **3c**: though Wacker oxidation of **1c** also proceeds successfully in this case, the resulting ketone **2c** is hardly converted by the enzyme (3% conversion).

Next we focused on recycling the catalyst system of the Wacker oxidation (Figure 4). We were pleased to find that by simple reuse of the (aqueous) phase of the interior of the PDMS thimbles for further 15 cycles (which were conducted over a period of 60 days!) no significant drop in conversion and no decrease of enantioselectivity was observed in the one-pot synthesis of alcohol **3a** starting from substrate **1a** (which was added at each new cycle along with with methanol as cosolvent).[12] This indicates that the Pd/Cu catalyst system of the Wacker oxidation in the interior is not deactivated even after multiple recycling and that the ionic components in the interior do not permeate the PDMS membrane.



Scheme 4. Substrate scope of the combined Wacker oxidation and enzymatic reduction in a one-pot process with compartmentalization of the catalysts.

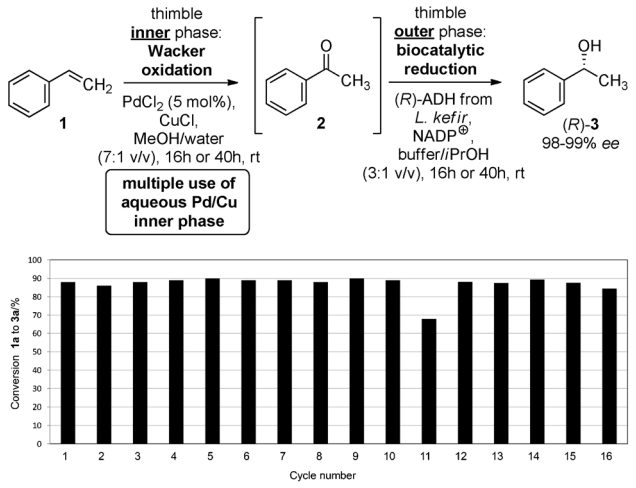


Figure 4. Recycling of the aqueous interior phase (reaction time was 40 h in some reactions for reasons of lab organization, see the Supporting Information).

In summary, the use of a palladium/copper-based catalyst system for a Wacker oxidation in the interior of a PDMS thimble made it possible to combine this reaction with an enzymatic reduction in a one-pot process although copper chloride is highly deactivating to the enzyme. This one-pot method under compartmentalization of the metal and enzyme catalysts enabled the smooth sequential transformation of numerous styrenes **2** into 1-phenylethanols (*R*)-**3** with high overall conversion and 98–99% *ee*. In this one-pot process both reactions are conducted in an aqueous medium, and tedious isolation of the ketone intermediates can be avoided by the transport of the hydrophobic ketones **2** through the PDMS membrane to the exterior solution, while the metal components are retained in the interior. The expensive Pd catalyst can be easily recovered and recycled over at least 15 cycles and remains active for at least 60 days.

Keywords: chiral alcohols · enzyme catalysis · one-pot synthesis · polydimethylsiloxane · Wacker oxidation

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[11] We chose room temperature, a relatively low reaction temperature, to suppress the undesired effects that can be expected at elevated temperatures such as loss of styrene from the reaction mixture due to its evaporation as well as deactivation of the enzyme and the cofactor.
[12] With respect to the recycling of other components, an attempt to recycle methanol was not made due to its low price and readily availability. In contrast, recycling of ADH and the cofactor is in principle an interesting option for future work although modified downstream-processing would be required for this purpose. In the current process based on extractive workup, enzyme and cofactor recycling would be tedious and might also lead to significant enzyme deactivation. In addition, for this process it has to be considered that the ADH can be produced efficiently and inexpensively by means of a recombinant strain, thus making multiple reuse of ADH not a task of highest priority.

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