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Enantioselective One-Pot Two-Step Synthesis of Hydrophobic Allylic Alcohols in Aqueous Medium through the Combination of a Wittig Reaction and an Enzymatic Ketone Reduction

Marina Kraußer,[a] Werner Hummel,[b] and Harald Gröger*[a]

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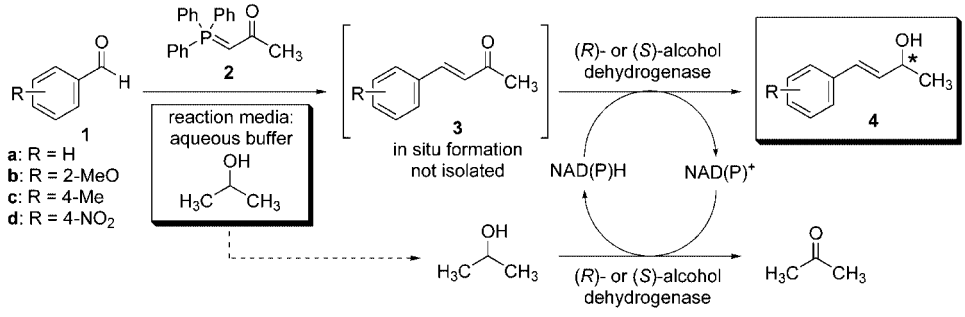
A one-pot two-step process for the enantioselective synthesis of hydrophobic allylic alcohols was developed, which comprises ketone formation by the Wittig reaction and their enzymatic in situ biotransformation into the desired target products. By means of this combined Wittig reaction and bioreduction, the allylic alcohols were prepared with conversions of up to 90 %, and with excellent enantioselectivities of >99 % *ee*.

Introduction

The biocatalytic reduction of ketones represents a highly efficient method for the asymmetric synthesis of chiral alcohols.[1,2] Excellent enantioselectivities in combination with an easily available (bio)catalyst and excellent productivity data make this technology highly attractive for large-scale applications; thus, it is competitive even with the Nobel prize technology of metal-catalyzed asymmetric hydrogenation[3] as an industrially well-established, highly efficient and widely applied reduction route. Surprisingly, however, in spite of its high potential and attractiveness for large-scale processes, the current application range of enzymatic reductive methods (and in general biocatalytic routes) in organic chemistry is still restricted. One reason might be their lack of compatibility with reaction media for "typical" organic synthetic processes. Thus, biocatalytic steps are often treated as "isolated" steps within a multistep organic chemical synthesis, whereas combination of organic chemical reaction steps to multistep one-pot syntheses is widely known.[4,5] Certainly for a broader application range of biocatalysis in organic synthesis the development of compatible chemical and enzymatic reactions is of crucial importance. Whereas successful examples of chemoenzymatic one-pot processes have been already reported for the synthesis of a range of *water-soluble* organic molecules in *aqueous* media[6] and dynamic kinetic resolution of *hydrophobic* alcohols and amines in *organic* media using hydrolases,[7,8] there is still a lack of chemoenzymatic syntheses of *hydrophobic* molecules in *aqueous* media as the most preferred media for biotransformations. In the following, we report this type of reaction. As a representative example for many conceivable reactions, we describe the one-pot synthesis of hydrophobic, water-immiscible alcohols by the oxidoreductase-catalyzed reduction of ketones prepared in situ by the Wittig reaction starting from aldehydes and phosphorus ylides. The concept of such a chemoenzymatic two-step one-pot synthesis is shown in Scheme 1. To the best of our



Scheme 1.

[a] Institute of Organic Chemistry, University of Erlangen-Nuremberg,
Henkestr. 42, 91054 Erlangen, Germany
E-mail: harald.groeger@chemie.uni-erlangen.de
[b] Institute of Molecular Enzyme Technology at the Heinrich-Heine University of Düsseldorf, Research Centre Jülich,
Stetternicher Forst, 52426 Jülich, Germany

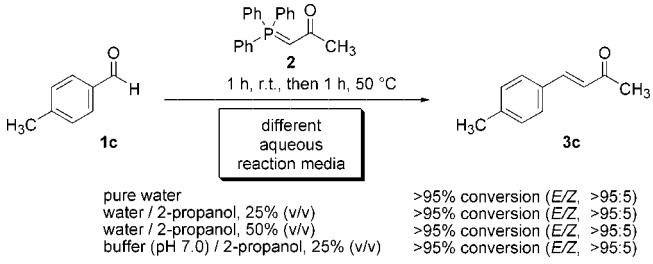
knowledge this chemoenzymatic reaction also represents the first example of a one-pot synthesis of chiral alcohols by the asymmetric alcohol dehydrogenase catalyzed reduction of ketones, which were prepared in situ prior to the enzymatic reduction by means of a non-enzymatic "typical" organic chemical reaction.

Results and Discussion

For the combination of biocatalytic and chemical reactions in one-pot processes, we envisioned the design of chemical and biocatalytic processes, which are *compatible* with each other. Thus, the in situ preparation of ketones **3** was planned to be carried out in reaction media that would be suitable for subsequent enzymatic reduction. Since we chose to recycle the cofactor in the enzymatic reduction process with 2-propanol as a cosubstrate (according to Scheme 1; for this concept of cofactor regeneration, see ref.[1b]), the aqueous buffer/2-propanol reaction media also needed to be suitable for the preparation of ketones **3** by the Wittig reaction. Recently, Bergdahl and coworkers reported a Wittig reaction in pure water as a solvent for the synthesis of α,β-unsaturated esters and ketones of type **3** (e.g., **3a**,**d**) to achieve significantly superior results in water than in organic solvents.[9] In our studies we found that the Bergdahl method was broadly applicable for the synthesis of several other ketones of type **3** (e.g., **3b**,**c**) and led to both high conversion (>95%) and *E*/*Z* ratio (≥95:5). For some ketones **3**, optimization of the reaction time and temperature was carried out (data not shown).

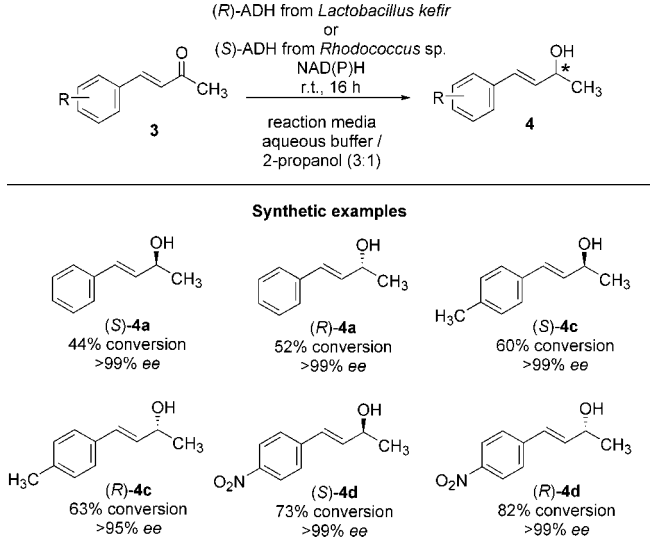
We were in particular interested to learn if the Bergdahl method could also proceed with the same high efficiency with the use of water/2-propanol and aqueous buffer/2-propanol mixtures as reaction media (Scheme 2). When the Wittig synthesis of 4-methylbenzylidene acetone (**3c**) was carried out in mixtures of water/2-propanol with a 2-propanol content of 25% (v/v) and 50% (v/v), respectively, both reactions proceeded with a conversion of >95% and a high *E*/*Z* ratio of >95:5 after a reaction time of 1 h at room temperature, followed by 1 h at 50 °C, thus being in the same range relative to the reaction in pure water (>95% conversion, Scheme 2). A further experiment in a phosphate buffer/2-propanol [25% (v/v)] mixture also gave an excellent result (>95% conversion; *E*/*Z*, >95:5), which shows that a highly efficient Wittig synthesis is also possible in aqueous buffer/2-propanol mixtures. Thus, a negative impact of the organic cosolvent was not observed as one might expect from the literature-known decreased reaction rates when organic solvents are used as reaction media.[9a]

After having prepared ketones **3** in a reaction medium that is also suitable for enzymatic reductions, we focused on the enzymatic reduction of α,β-unsaturated ketones. As enzymes we used an alcohol dehydrogenase (ADH) from *Lactobacillus kefir*[10] as an (*R*)-enantioselective biocatalyst and an ADH from *Rhodococcus* sp.[11] as an (*S*)-enantioselective biocatalyst.[12] In the preliminary photometer tests, activities of both enzymes for the α,β-unsaturated ketone



Scheme 2.

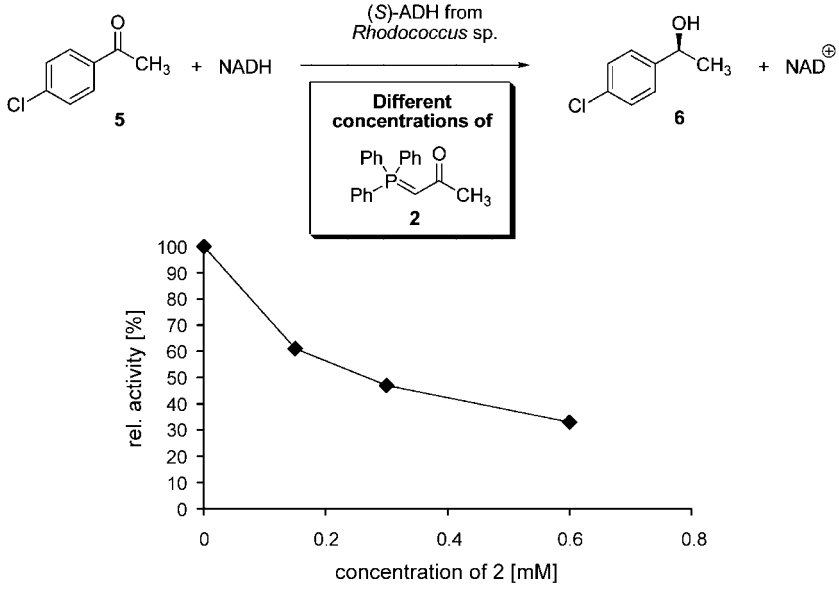
substrates **3**, prepared by the Wittig reaction, were found (data not shown). Subsequently, the preparative biotransformations were carried out on the basis of a substrate-coupled cofactor-regeneration method. Therein, the alcohol dehydrogenases reduce the ketone with formation of the desired alcohol in an enantioselective manner under consumption of the cofactor NAD(P)H, which is used in a low catalytic amount only (according to the concept shown in Scheme 1). The in situ recycling of the cofactor by means of an oxidation of 2-propanol with formation of acetone is catalyzed by the same alcohol dehydrogenase. Thus, 2-propanol acts as a cosubstrate for the alcohol dehydrogenase, and is oxidized to acetone, which makes the reduction of corresponding ketone **3** to desired alcohol **4** possible. When carrying out this biotransformation with the nonsubstituted and *para*-substituted benzylidene acetone substrates **3a**,**c**,**d**, the reactions proceed under formation of allylic alcohols **4a**,**c**,**d**, respectively, with conversions in the range of 44–82% and excellent enantioselectivities of up to >99% *ee* (Scheme 3). Besides its function as a cosubstrate, a further advantage of 2-propanol as a cosolvent might be the improved solubility of the hydrophobic ketone in the reaction mixture.



Scheme 3.

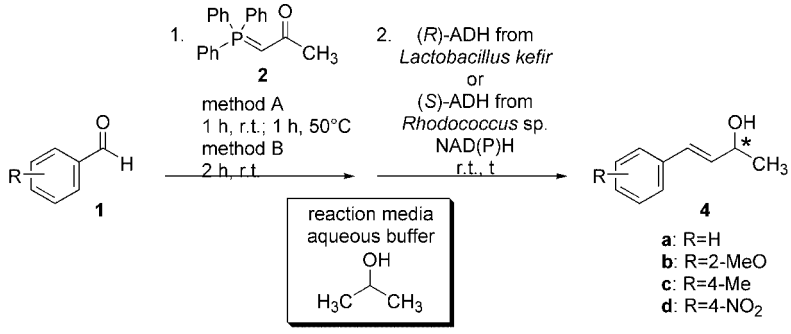
With respect to the desired combination of the Wittig reaction with the biocatalytic reduction, we studied if the presence of ylide **2** (used as the substrate in the Wittig reac-

tion in slight excess) might have a negative impact on the activity of the alcohol dehydrogenase as a result of "cross inhibition". This study was done by means of a photometer assay (according to the assay conditions described in ref.[13]) with the use of (*S*)-ADH from *Rhodococcus* sp. as an enzyme and 4-chloroacetophenone as the "standard substrate" for this enzyme (Scheme 4). Albeit the enzyme activity was influenced and gave a somewhat lower activity in the presence of ylide **2**, we were pleased to find that at an ylide concentration of 0.6 mM, which is close to the solubility limit, 33% of the enzyme activity remained (relative to the enzyme activity in the absence of ylide **2**), and thus it is in a range expected to be sufficient for preparative conversions (Scheme 4).



Scheme 4.

Table 1. One-pot two-step synthesis of allylic alcohols.



Entry[a]	R	Method	ADH	*t* [h]	Conversion [%]	*ee* [%] [c]
1	H	B	(*S*)-ADH	42	58	>99 (*S*)
2	2-MeO	A	(*S*)-ADH	42	48	>99 (*S*)
3	4-Me	A	(*S*)-ADH[b]	16	32	>99 (*S*)
4	4-Me	A	(*S*)-ADH	42	48	>99 (*S*)
5	4-Me	A	(*R*)-ADH	42	31	>99 (*R*)
6	4-NO_2	B	(*S*)-ADH	42	90	>99 (*S*)

[a] The synthetic protocol is given in the experimental section. [b] A reduced catalytic amount of 50% was used in this experiment. [c] The enantiomeric excess was determined by chiral HPLC [Daicel Chiralcel OD column, hexane/2-propanol (95:5) or (99.5:0.5)]. The absolute configuration of (*S*)-**3a** (Entry 1) was determined by comparison of the sign of the optical rotation with the literature data (ref.[14]), and it was in accordance with the expected absolute configuration known from the usual (*S*) selectivity of this (*S*)-ADH. The absolute configurations of alcohols **3b**–**d** (Entries 2–6) were assigned accordingly.

In the subsequent step, the Wittig reaction and the enzymatic reaction, which both were adapted to a compatible reaction media, were combined into a one-pot process. We were pleased to find that starting from 4-methylbenzaldehyde (**1c**) the one-pot two-step synthesis of allylic alcohol **4c** proceeded successfully under the same reaction conditions that were applied before for the separated steps (i) ketone synthesis and (ii) reduction. In this one-pot two-step synthesis, desired allylic alcohol **4c** was also formed with an excellent enantioselectivity of >99% *ee* (Table 1, Entry 3). However, the conversion did not exceed 32% after a reaction time of 16 h; thus, the conversion was somewhat lower relative to the single-step process starting from purified ketone **3c** (60% conversion). This result is in accordance with the above-mentioned observed inhibition of the enzyme in the presence of ylide **2**, albeit other effects might play a role as well. Subsequent process optimization, for example, of the reaction time and adaptation of the biocatalyst amount, led to an improved conversion of 48% after 42 h and an excellent enantioselectivity of >99% *ee* when the (*S*)-ADH from *Rhodococcus* sp. was used as the catalyst with 4-methylbenzaldehyde (**1c**) as the substrate for this chemoenzymatic one-pot two-step synthesis (Table 1, Entry 4).

As a next step, the substrate range of this new one-pot two-step synthesis of hydrophobic allylic alcohols was investigated. A high conversion of 90% was obtained with 4-nitrobenzaldehyde (**1d**) and ylide **2** in the presence of the (*S*)-ADH from *Rhodococcus* sp. as the biocatalyst. Subsequent downstream processing gave resulting allylic alcohol (*S*)-**4d** in 75% yield and with an enantioselectivity of >99% *ee* (Table 1, Entry 6). Notably, good results were obtained with other substrates as well (Table 1, Entries 1,2), which indicates that this chemoenzymatic one-pot two-step synthesis proceeds smoothly with a broad range of substrates. The desired allylic alcohol products (*S*)-**4a**–**d** were formed with conversions of 48–90% and with excellent enantioselectivities of >99% *ee* independent of the substitution pattern. The (*R*)-enantioselective one-pot synthesis of allylic alcohol (*R*)-**4c** was carried out with (*R*)-ADH from *Lactobacillus kefir* as a biocatalyst leading to desired product (*R*)-**4c** in 31% conversion and with a high enantioselectivity of >99% *ee* (Table 1, Entry 5).

Conclusions

A one-pot two-step process for the enantioselective synthesis of hydrophobic allylic alcohols was developed, which comprises ketone formation by the Wittig reaction followed by their enzymatic in situ biotransformation. By means of this combined Wittig reaction and bioreduction, the desired target products were prepared with moderate-to-good conversions of 31–90% and with excellent enantioselectivities of >99% *ee*. To the best of our knowledge this chemoenzymatic reaction also represents the first example of a one-pot synthesis of chiral alcohols by an asymmetric alcohol dehydrogenase catalyzed reduction starting from ketones, which were prepared in situ prior to the enzymatic reduction by means of a "typical" organic chemical reaction. Further process development is planned including the in situ removal of acetone to shift the equilibrium to the direction of the products, which would further improve the conversions. In addition, the development of other chemoenzymatic one-pot multistep reactions is currently in progress.

Experimental Section

Protocol for the Spectrophotometer Assay for the Measurement of the Enzyme Activity in the Presence of Ylide 2: The enzyme activity (see also Scheme 4) was determined spectrophotometrically measuring the consumption of NADH by oxidation to NAD^+ at 340 nm (ε_{340} = 6.3 $mM^{-1} cm^{-1}$) in the presence of 4-chloroacetophenone (**5**) as the substrate and ylide **2** as an additive. The concentration of the ylide was varied between 0 and 0.6 mM. For the spectrophotometric measurement of the enzyme activity, a cuvette (1 mL) was filled with a buffered solution of 4-chloroacetophenone (960 μL, **5**; 10 mM; phosphate buffer: pH 7.0, 100 mM) containing ylide **2** in different concentrations, and a buffered solution of NADH (20 μL, NADH: 12.5 mM; phosphate buffer: pH 7.0, 100 mM). The reaction was started by the addition of a 1:100 diluted enzyme solution (20 μL) of (*S*)-ADH from *Rhodococcus* sp. (partially purified; NADH-dependent; volumetric activity: 488 $U mL^{-1}$ referring to 4-chloroacetophenone as a standard substrate), and subsequently the consumption of NADH was detected. The relative activities were determined by comparison of the detected spectrophotometrical activities in $U mL^{-1}$ with the one in the experiment without the addition of ylide **2** (which was regarded as the reference experiment; relative activity: 100%).

Protocol for the One-Pot Two-Step Synthesis of Allylic Alcohols 4 as Exemplified for the Synthesis of (*S*)-4d: A 20-mL round-bottom flask, fitted with a magnetic stir bar, was charged with ylide **2** (0.6 mmol), 4-nitrobenzaldehyde (**1d**; 0.5 mmol), phosphate buffer (1.875 mL, 100 mM; pH 7.0) and 2-propanol (0.625 mL). The mixture was stirred at room temperature for 2 h. Subsequently, cofactor NADH (0.02 mmol) and an aqueous solution of (*S*)-ADH from *Rhodococcus* sp. (40 μL, partially purified; NADH-dependent; volumetric activity: 488 $U mL^{-1}$ referring to 4-chloroacetophenone as a standard substrate) were added, and the reaction mixture was stirred for a further 42 h at room temperature. The heterogeneous mixture was extracted with ethyl acetate, dried with magnesium sulfate, filtered and concentrated in vacuo. The resulting crude product, which showed a conversion of 90% for desired product (*S*)-**4d**, was purified by flash chromatography (silica gel 60 Å; ø 1.5 cm; length 22 cm; cyclohexane/ethyl acetate, 4:1) to afford isolated allylic alcohol (*S*)-**4d** in 75% yield and with >99% *ee* (see also Table 1, Entry 6).

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