Aqueous Catalysis

DOI: 10.1002/anie.200801341

Modular Chemoenzymatic One-Pot Syntheses in Aqueous Media: Combination of a Palladium-Catalyzed Cross-Coupling with an Asymmetric Biotransformation**

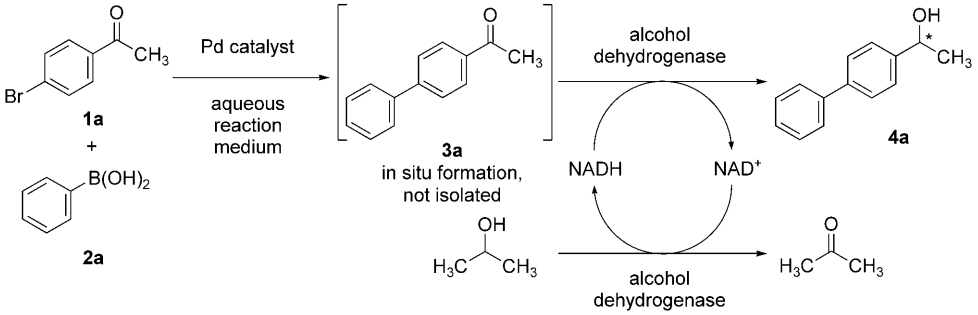
*Edyta Burda, Werner Hummel, and Harald Gröger**

Dedicated to Professor Dr. Jürgen Martens on the occasion of his 60th birthday

Multistep one-pot processes are an attractive synthetic concept for the improvement of overall process efficiency through a decrease in the required number of workup and purification steps. By avoiding such time-, effort-, and solvent-intensive steps, multistep one-pot syntheses contribute to a significantly improved process economy as well as to more sustainable synthetic routes.[1] A key criteria for multistep one-pot processes is the compatibility of the individual reaction steps with one another. Accordingly, most of the multistep one-pot processes known today are based on either chemocatalytic multistep reactions[2] or "pure" biotechnological processes,[3] such as fermentation. In contrast, few successful combinations of chemo- and biocatalytic reactions are known.[4] Remarkable breakthroughs include, in particular, the dynamic kinetic resolutions developed by Williams and co-workers,[5] by Bäckvall and co-workers,[6] and recently by Berkessel et al.[7] These synthetic processes are based on lipase-catalyzed resolution in combination with a simultaneous metal-catalyzed racemization of the substrate in an organic solvent.

However, as most enzymes are incompatible, or at best poorly compatible, with organic solvents, the development of chemoenzymatic multistep one-pot processes in aqueous media is highly desirable. Pioneering studies in this field involved the combination of a glucose isomerase with a heterogeneous platinum catalyst for the conversion of a mixture of D-glucose and D-fructose into D-mannitol.[8] In general, however, chemoenzymatic one-pot processes in aqueous media are still a largely unexplored area of research.[8,9] As palladium-catalyzed cross-coupling reactions[10] are of particular importance in the field of metal catalysis, and enzymatic reduction[11] is very important in biocatalysis, we were interested in the compatibility of these types of reactions in water. As the first example of a one-pot process in which a palladium-catalyzed cross-coupling reaction is combined with a biotransformation in an aqueous reaction medium, we report herein the synthesis of chiral biaryl alcohols **4** through Suzuki cross-coupling and subsequent asymmetric enzymatic reduction (according to the synthetic concept shown in Scheme 1).

Preliminary experiments showed the general difficulty in the development of such a one-pot two-step process, in particular with respect to the compatibility of metal catalysis and biocatalysis. When the Suzuki cross-coupling and enzymatic reduction were carried out separately, both reactions proceeded smoothly (Scheme 2 A). We chose the palladium

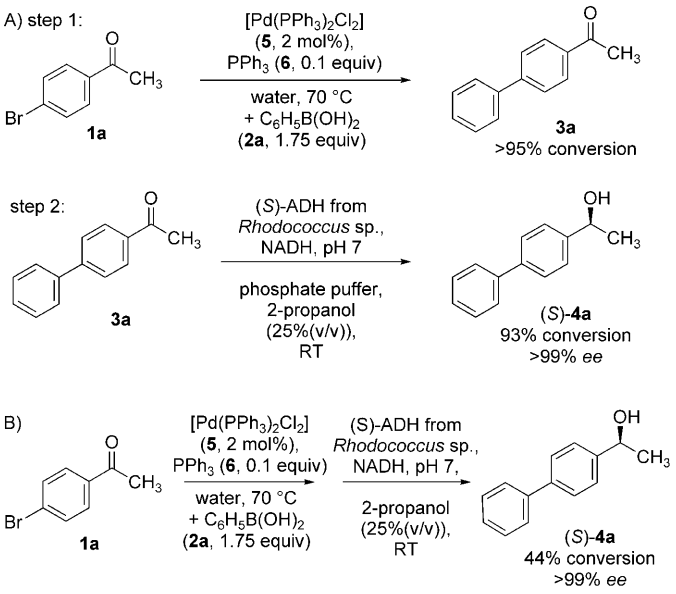


Scheme 1. Concept of the chemoenzymatic one-pot synthesis. NAD^+ = nicotinamide adenine dinucleotide; NADH is the reduced form of NAD^+.

[*] E. Burda, Prof. Dr. H. Gröger
Department of Chemistry and Pharmacy
University of Erlangen–Nuremberg
Henkestrasse 42, 91054 Erlangen (Germany)
E-mail: harald.groeger@chemie.uni-erlangen.de
Prof. Dr. W. Hummel
Institute of Molecular Enzyme Technology
Heinrich Heine University of Düsseldorf
Research Centre Jülich (Germany)

[**] We thank Sonja Borchert for excellent technical support, and Evonik-Degussa, Amano Enzymes, and Oriental Yeast Company, Japan for generous support with chemicals.

complex **5** and phosphane **6** as the catalyst system for the cross-coupling step, as these catalyst components had been applied previously in a Suzuki coupling in an aqueous medium.[12] The Suzuki cross-coupling of the boronic acid **2a** (1.75 equiv) with **1a** gave the biaryl ketone **3a** with a conversion of greater than 95% (Scheme 2 A, step 1). In a second step, enzymatic reduction of the isolated and purified ketone **3a** led to the formation of the desired alcohol (*S*)-**4a** with 93% conversion and >99% *ee* after adjustment of the pH value to pH 7 (Scheme 2 A, step 2). This reaction was catalyzed by an alcohol dehydrogenase (ADH) from *Rhodo-*



Scheme 2. Synthesis in two separate steps (A) versus one-pot two-step synthesis (B).

coccus sp.[13] with in situ substrate-coupled cofactor regeneration with 2-propanol (which is oxidized to acetone). However, when these two processes were combined in a one-pot two-step synthesis with adjustment of the pH value to pH 7 prior to the biotransformation, the desired product (*S*)-**4a** was formed with a significantly decreased conversion of only 44% (Scheme 2B).

To find an explanation for this unsatisfying result, we studied spectrophotometrically the influence of the components of the Suzuki cross-coupling reaction on enzyme activity at concentrations up to the solubility limit, with **3a** as the reference substrate (Figure 1). We first investigated the potential inhibition of the ADH by the palladium complex, as enzyme inhibition by heavy metals is a known phenomenon. To our surprise, however, we found that the palladium complex **5** had only a minor negative impact on the enzyme activity (Figure 1, part A). Even at a metal-complex concentration of 0.63 mM, the enzyme activity remained high at 79%. In contrast, a more significant decrease in the enzyme activity was observed in the presence of the phosphane. For example, the residual activity was only 56% at a 4 mM concentration of triphenylphosphane (**6**; Figure 1, part B). The boronic acid, however, had the strongest negative influence on enzyme activity. In the presence of phenylboronic acid (**2a**) at a concentration of 0.17 M, the residual activity of the enzyme was only 14% (Figure 1, part C). The borate salt formed from the boronic acid in the Suzuki cross-coupling has a much less negative impact on enzyme activity: At a borate-salt concentration of 0.17 M, the residual enzyme activity was 66%.

From these experiments, we deduced the following prerequisites for an enzyme-compatible Suzuki cross-coupling reaction: a) No phosphane additive may be used, b) the boronic acid may not be used in excess, c) conversion must be quantitative with complete consumption of the boronic acid, and d) water must be used as the reaction medium. We

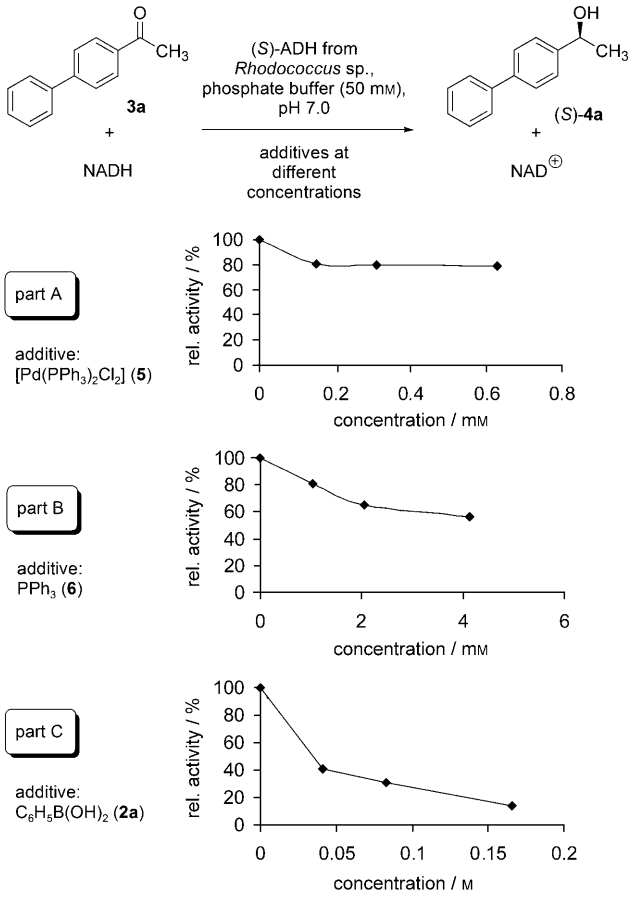
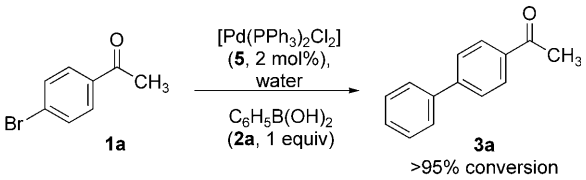


Figure 1. Influence of the components of the Suzuki cross-coupling on enzyme activity.

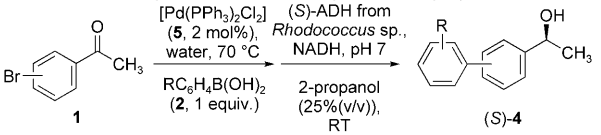
developed such a Suzuki cross-coupling for the synthesis of the biaryl ketone **3a** as a model reaction. In the presence of the catalyst $[Pd(PPh_3)_2Cl_2]$ (**5**) and exactly one equivalent of phenylboronic acid (**2a**), the reaction proceeded successfully in water to give **3a** with a conversion of greater than 95% (Scheme 3).

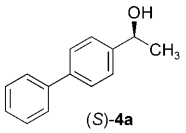
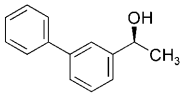
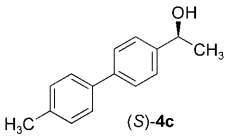


Scheme 3. Optimized Suzuki cross-coupling in water.

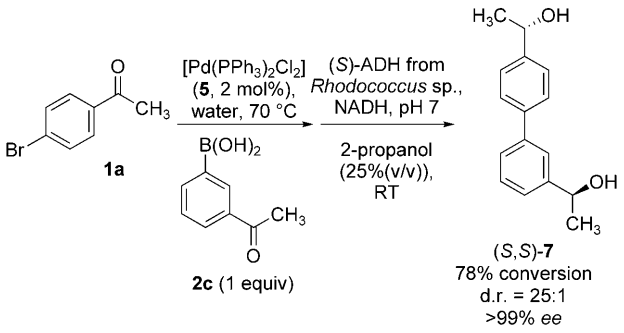
We were pleased to find that the resulting reaction mixture was compatible with a subsequent ADH-catalyzed reaction. When this Suzuki cross-coupling was followed by an enzymatic reduction (after adjustment of the pH value to pH 7) with substrate-coupled cofactor regeneration with 2-propanol, the desired biaryl-substituted alcohol (*S*)-**4a** was formed with 91% conversion and excellent enantioselectivity (>99% *ee*; Table 1, entry 1). This conversion of 91% corresponds almost exactly to the calculated overall con-

Table 1: Substrate spectrum of the one-pot two-step synthesis.[a]



Entry	Product	Conversion [%]	*ee* [%][b]
1	 (*S*)-**4a**	91	>99
2	 (*S*)-**4b**	83	>99
3	 (*S*)-**4c**	67	>99

[a] For the reaction conditions, see the Experimental Section. [b] The *ee* value was determined by HPLC on a chiral phase with a mixture of hexane and 2-propanol (95:5) as the eluent (**4a**: Daicel chiracel OD column; **4b**: Daicel chiracel OJ-H column; **4c**: Daicel chiracel AD-H column).



Scheme 4. Synthesis of the enantiomerically pure diol (*S*,*S*)-**7**.

In conclusion, we have described the one-pot synthesis of chiral biaryl alcohols through Suzuki cross-coupling and subsequent enzymatic reduction. The products were obtained with up to 91% conversion and excellent enantioselectivities (>99% *ee*). To the best of our knowledge, this one-pot two-step synthesis is the first example of the combination of a palladium-catalyzed cross-coupling reaction with an (asymmetric) biotransformation in an aqueous medium. We are currently investigating further one-pot multistep syntheses that combine chemocatalytic and biocatalytic reactions in aqueous media.

version of the two reactions when carried out separately (according to Scheme 2A). Thus, in the one-pot process, the reaction mixture of the Suzuki cross-coupling has minimal negative impact on the subsequent biotransformation, in particular with respect to conversion. Furthermore, this one-pot two-step synthesis is suitable for a broad range of substrates. For example, with the substrate 3-bromoacetophenone, a combination of Suzuki cross-coupling and ADH-catalyzed reduction in an aqueous medium gave the product (*S*)-**4b** with 83% conversion and >99% *ee* (Table 1, entry 2). The boronic acid component can also be varied, as demonstrated by the synthesis of (*S*)-**4c** from 4-methylphenylboronic acid with 67% conversion and >99% *ee* (Table 1, entry 3).

An additional challenge is the synthesis of biaryl diols, such as (*S*,*S*)-**7**. Chiral diols are valuable (monomeric) building blocks for the construction of enantiomerically pure polymers. To date, the only known asymmetric approaches to bis(α-hydroxyethyl)biphenylenes involve a multistep synthesis from (*R*)-3-bromophenylethan-1-ol as a chiral auxiliary,[14] a diastereoselective synthesis,[15] or an enzymatic resolution.[16] In the first asymmetric (bio-)catalytic synthesis of such a diol, we prepared (*S*,*S*)-**7** via a diacetylbiphenyl intermediate (synthesized in situ through Suzuki cross-coupling) with our one-pot two-step synthesis: The Suzuki cross-coupling of the prochiral substrates 4-bromoacetophenone (**1a**) and 3-acetylphenylboronic acid (**2c**) in an aqueous medium, followed by in situ enzymatic reduction of the formed diacetylbiphenyl intermediate, produced the desired diol (*S*,*S*)-**7** with high diastereoselectivity (d.r. = 25:1) and excellent enantioselectivity (>99% *ee*; Scheme 4).

Experimental Section

Spectrophotometric assay for the measurement of enzyme activity (see Figure 1): In analogy with a previous protocol,[17] the consumption of NADH through oxidation to NAD^+ was measured spectrophotometrically at a wavelength of 340 nm in the presence of 4-phenylacetophenone (**3a**) as the substrate and the corresponding additive ($\varepsilon_{340} = 6.3\ mM^{-1}cm^{-1}$). The additives tested and their concentrations are given in Figure 1. A cuvette (1 mL) was filled with 960 μL of a buffered solution of 4-phenylacetophenone (**3a**: 10 mM; phosphate buffer: pH 7.0, 50 mM), which also contained the additive in various concentrations, and 20 μL of a buffered solution of NADH (NADH: 12.5 mM; phosphate buffer: pH 7.0, 50 mM). A solution (20 μL, dilution: 1:100) of (*S*)-ADH from *Rhodococcus* sp. (partially purified; NADH-dependent; volumetric activity: 116 $U\,mL^{-1}$) was then added. The relative activities were determined by comparison of the enzyme activities (in $U\,mL^{-1}$) measured spectrophotometrically with the enzyme activity in the experiment in the absence of an additive (regarded as the reference experiment with a relative activity of 100%). U always refers to **3a** as the standard substrate.

One-pot synthesis of biaryl alcohols (*S*)-**4** (Table 1): The aryl boronic acid **2** (0.25 mmol), the bromoacetophenone component **1** (0.25 mmol), and bis(triphenylphosphane)palladium(II) chloride (**5**, 0.005 mmol, 2 mol%) were added sequentially to a solution of sodium carbonate (10 mmol) in water (7.5 mL) in a 25 mL round-bottomed flask. The reaction mixture was stirred for 17 h at 70 °C and then cooled to room temperature. After adjustment of the pH value to pH 7 by the addition of hydrochloric acid, 2-propanol (2.5 mL), NADH[18] (0.02 mmol), and the ADH from *Rhodococcus* sp. (Table 1, entries 1 and 2: 46 U; Table 1, entry 3: 69 U) were added, and the reaction mixture was stirred for 48 h at room temperature. The aqueous phase was then extracted with dichloromethane (3×20 mL). The combined organic phases were dried over magnesium sulfate, filtered, and concentrated under vacuum. The crude product was purified by flash chromatography (silica gel 60 Å; ∅: 1.5 cm; length:

22 cm; eluent: *n*-hexane/ethyl acetate (5:1)). Alcohols (*S*)-**4a**,**c** were obtained as colorless solids, alcohol (S)-**4b** as a colorless oil.

Received: March 19, 2008
Revised: July 21, 2008
Published online: October 22, 2008

Keywords: chemoenzymatic reactions · chiral diols · cross-coupling · homogeneous catalysis · one-pot synthesis

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[18] The amount of added cofactor has not been optimized. The highest TON value (TON = turnover number) calculated for the experiments carried out to date was 20 (for the synthesis of (*S*,*S*)-**7**, see Scheme 4).

