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Highly Recyclable Chemo-/Biocatalyzed Cascade Reactions with Ionic Liquids: One-Pot Synthesis of Chiral Biaryl Alcohols

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The growing need for more sustainable technologies has placed increased attention on the integration of organic chemistry with biocatalysis to develop effective multistep, one-pot chemical reactions. Cascade reactions, in general, are attractive synthetic strategies because they save time and materials, while producing less waste when compared with the traditional method of carrying out each chemical transformation and its required purification and characterization individually.[1–3] We wished to employ biocatalysis in cascade reactions in combination with metal catalysts because biocatalysis provides the advantages of easily introducing perfect stereo- and regioselectivity. However, integration of biocatalysis in chemical cascade reactions is expected to be problematic because many organic intermediates are poorly soluble in the aqueous reaction medium and/or display toxic effects on the biocatalyst. A solution to these challenges is to exploit a biphasic solvent system with nonmiscible liquid phases in which the biocatalyst is not permanently in contact with the organic molecules. However, using commonly used organic solvents as part of the biphasic system may also damage the biocatalyst itself.[4]

The unique properties of room temperature ionic liquids (ILs), such as nonvolatility, nonflammability, and in many cases, high thermal and chemical stability, have made them an environmentally attractive alternative to organic solvents.[5] The surprising noninvasive effects on cellular membranes of hydrophobic ILs in biphasic IL/water systems make them superior to many organic solvents applied in whole-cell biotransformations.[6,7] In addition, the use of ILs as reaction media for biotransformations has led to higher operational stabilities with enantioselectivities and catalyst activities that are generally equivalent to those observed in organic solvents.[8,9]

We demonstrate herein that performing cascade reactions with biocatalysis in conjunction with transition-metal catalysis, employing ILs in the biphasic system, is possible. Specifically, we report a cascade reaction sequence that is a highly enantio- and diastereoselective synthesis of biaryl alcohols by employing a Suzuki coupling followed by an alcohol dehydrogenase (ADH) catalyzed reduction in a biphasic system containing ILs. Moreover, we demonstrate the recyclability of the biphasic system up to four cycles with only a negligible deactivation of reactivity and selectivity.

The combination of a palladium-catalyzed cross-coupling reaction with an asymmetric enzyme reduction has recently been performed in aqueous medium.[10] Based upon this precedent, we investigated the possibility of performing a one-pot, two-step synthesis of biaryl alcohols in a biphasic system. The first step of the cascade (Suzuki coupling) was performed in the IL phase, since Suzuki couplings can be augmented in terms of catalytic activity and stability when performed in ILs.[7] The second step of the cascade sequence was an ADH-mediated reduction at the aqueous/IL interface. Some precedent exists for the use of whole-cell biocatalysis in ILs. The activity and selectivity of W110A secondary ADH from *Thermoanaerobacter ethanolicus* has been studied in organic solvents and ILs in both mono- and biphasic media.[11] In addition, the asymmetric reduction, in both water and ILs, of hydrophobic phenyl-ring-containing ketones has also been demonstrated by employing the overexpressed, highly organic solvent tolerant ADH-A from *Rhodococcus ruber* DSM 44541.[12] We chose to employ lyophilized cells of *E. coli* containing the overexpressed ADH-A for our purpose. These cells had already demonstrated high specificity and activity in ILs for a broad set of aromatic ketones.

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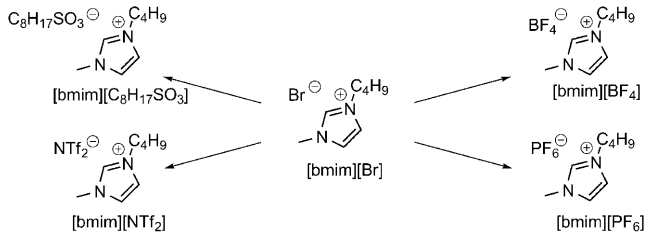
While searching for adequate conditions for a palladium-catalyzed Suzuki reaction that would not interfere with the subsequent biocatalytic reduction, different ILs were investigated (Scheme 1 and Table 1).



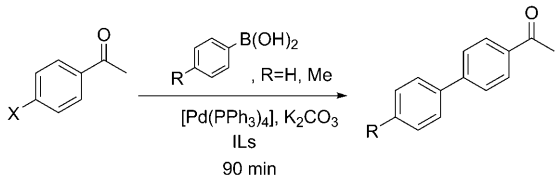
Scheme 1. Ionic liquids employed as solvents for the Suzuki reaction.

Table 1. Suzuki coupling reaction in different ILs.[a]

Boronic Acid	X	Yields [%] [bmim][BF_4]	[bmim][PF_6]	[bmim][$C_8H_{17}SO_3$]	[bmim][Br]	[bmim][NTf_2]
	Br	77	76	75	39	97
	I	73	78	60	40	100
	Br	65	74[b]	60	32	92[b]
	I	77	81[b]	69[b]	45	81[b]

[a] Reaction conditions: Aryl halide (0.48 mmol (or 0.51 mmol in the case of the *p*-tolylboronic acid)), Pd catalyst (1.2 mol%), and boronic acid (0.48 mmol). [b] The homocoupling product was observed at less than 3%.

The use of ILs as solvents for Suzuki couplings of bromo- and iodobenzene with phenyl- and *p*-tolylboronic acids occurred without any apparent catalytic decomposition (Scheme 2). All the Suzuki coupling reactions were performed by stirring $[Pd(PPh_3)_4]$ for 1 h under inert conditions at 110°C in the ILs in the presence of an arylhalide.[13] Subsequently, the base and the boronic acid in aqueous solution were added. Similar results were obtained for the tetrafluoroborate, hexafluorophosphate, and octyl sulfate [bmim] in the presence of K_2CO_3 or NEt_3 as base. However, the anion effect is more pronounced for the bromide anion, with which inferior results were obtained. In contrast, the best coupling yields were obtained when using the bis(trifluoromethanesulfonyl)imide anion. The water-immiscible IL, [bmim][NTf_2], was then chosen as the optimal IL and then investigated in the reductions using ADH in a two-phase system with Tris-buffer.[14]



Scheme 2. The Suzuki coupling reaction.

In a typical experiment, *E. coli* cells containing over-expressed ADH-A (*E. coli*/ADH-A) were employed for the reduction of 4-phenylacetophenone in the presence of 2-propanol for the recycling of the nicotinamide cofactor (NADH) (Table 2). *E. coli*/ADH-A catalyzed the reduction in the biphasic system even at high concentration of IL (70% v/v), but the reaction slowed down with an increasing amount of IL and the enantioselectivity dropped dramatically above 30% of IL.

Because good results were obtained when employing 30% v/v IL, the bioreduction was performed in the presence of each component of the Suzuki coupling that might be susceptible to inhibiting ADH-A, that is, the boronic acid, the palladium catalyst, and the base. Although the palladium catalyst did not impede the activity of the ADH-A, the presence of the boronic acid considerably decreased its activity.[11] When the ionic base K_2CO_3 was used, no bioreduction was observed. However, in the presence of triethylamine, no inhibition of ADH was observed. From these experiments, we deduced the following prerequisites for an ADH-A-compatible Suzuki cross-coupling reaction: 1) the boronic acid may not be used in excess, 2) triethylamine must be used as a base, and 3) Tris-HCl buffer must be used as the IL medium at a final concentration of 30% v/v. To obtain a well-defined profile of the activity of the enzyme at different amounts of *E. coli* cells, the conversion of the reduction was followed. As shown in Figure 1, the reduction at varied concentrations of cells revealed that one milligram of cells for 0.1 mmol of ketone yields to the best reaction time. At all cell concentrations investigated the reduction of the ketone led to the enantiopure alcohol.

Table 2. Reduction of 4-phenylacetophenone using *E. coli*/ADH-A with different amounts of the water-immiscible [bmim][NTf_2].

IL [% v/v]	Yield [%]	*ee*[a] [%]	Reaction time [h]
10	>99	>99	2
20	98	>99	2
30	96	>99	2
50	97	88	2
60	76	87	2.5
70	59	55	2.5

[a] *ee* = enantiomeric excess.

In the presence of the catalyst $[Pd(PPh_3)_4]$ and exactly one equivalent of phenylboronic acid, the cascade sequence (Scheme 3) proceeded successfully in one pot with a global conversion greater than 94% and the final secondary alcohol was obtained in >99% *ee*. The one-pot process has been applied to different substrates (Table 3); thus, a broad range of prochiral substrates obtained by the Suzuki cou-

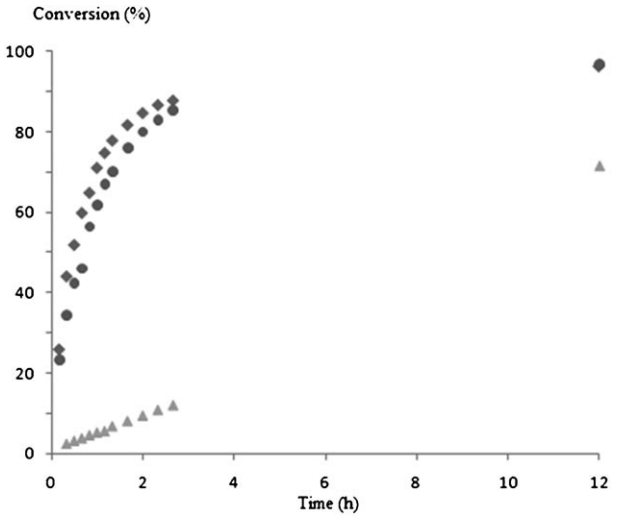
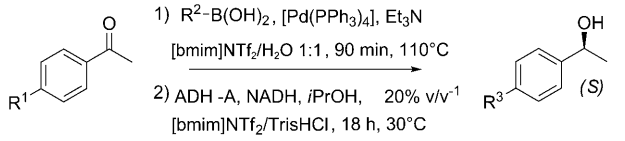


Figure 1. Enzymatic reduction of 4-phenylacetophenone catalyzed by *E. coli*/ADH-A in 30% [bmim][NTf_2]. ◆: 2 mg *E. coli* cells, 0.01 mmol substrate; ●: 1 mg *E. coli* cells, 0.01 mmol substrate; ▲: 1 mg *E. coli* cells, 0.02 mmol substrate.



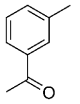
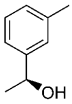
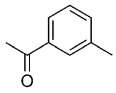
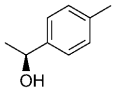
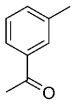
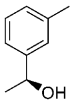
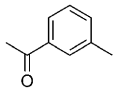
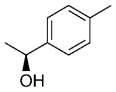
Scheme 3. One-pot chemo-/biocatalyzed cascade.

pling can be reduced by the *E. coli*/ADH-A with excellent enantioselectivities.

The main advantage of these one-pot biphasic reactions is the possibility to reuse both the IL phase,[15] containing the palladium catalyst, in a subsequent catalytic run and also the aqueous phase, containing the rehydrated *E. coli* cells. Since the ILs are insoluble in diethyl ether, the products are easily isolated by simple extraction with diethyl ether. The IL containing the Pd catalyst was recovered and reused in the next cycle without further addition of IL or Pd catalyst. The aqueous supernatant, containing the biocatalyst, was directly removed and reused in a subsequent run. As shown in Table 4, the first four catalytic runs were performed without any loss in conversion or enantioselectivity.

In conclusion, we have performed a cascade sequence combining both an organometallic coupling step and a reusable biotransformation in a biphasic solvent system containing an imidazolium-based IL and an aqueous phase. The procedure leads to an efficient one-pot synthesis of enantiopure biaryl alcohols with high yields and excellent enantioselectivities. Compared with the analogous one-pot reaction in water, the process is faster and both the aqueous phase, containing the cells, as well as the IL phase, containing the Pd catalyst, can be directly reused in a subsequent catalytic run. This study also demonstrated the possibility of integrating biocatalytic whole-cell processes in chemical cascade reactions. As far as we are aware, it is the first cascade reaction ever reported using both organometallic and biotransformation catalysis in a biphasic system with an IL allowing the efficient recycling of both catalysts.

Table 3. One-pot reactions in 30% [bmim][NTf_2] Recycling tests for the one-pot reaction (entry 1 Table 3).

R^1	R^2	R^3	*ee* [%][a]	d.r.	Conversion [%]
I			>99	–	94
I			>99	99:1	56
I			>99	99:1	78
I			>99	–	63
Br			>99	–	83
Br			>99	99:1	81
Br			>99	99:1	55
Br			>99	–	55

[a] All obtained alcohols were of *S* configuration (determined by chiral HPLC).

Table 4. Recycling tests for the one-pot reaction (entry 1 Table 3).

Run	Conversion [%]	*ee* [%][a]
1	94	>99
2	96	>99
3	98	>99
4	94	>99
5	44	>99

[a] The obtained alcohols were of *S* configuration (determined by chiral HPLC).

Experimental Section

General procedure for the Suzuki reaction in IL: Under argon, the aryl halide (0.125 mmol, 1 equiv) and the catalyst (1.5 μmol, 1.2 mol%) were added to IL (0.3 mL) at ambient temperature. The mixture was then slowly heated to 110°C and vigorously stirred for 1 h. The solution was cooled to room temperature and the boronic acid (0.125 mmol, 1 equiv) and triethylamine (0.25 mmol, 2 equiv) diluted in water (0.3 mL; $V_{IL} = V_{H2O}$) were added to the solution and the mixture was heated to 110°C for 30 min. The solution was cooled and extracted with diethyl ether (3× 5 mL). The combined extracts were assembled and washed with brine

(3×5 mL) and water (3×5 mL). The organic extracts were dried over magnesium sulfate, evaporated to give a pale yellow solid, and purified by flash chromatography on a silica gel column (hexane/EtOAc 95:5) to afford the coupling product, which was characterized by ^{1}H NMR spectroscopy and mass spectrometry.

General procedure for the enzymatic reduction of the coupling product: A solution of Tris-HCl buffer (pH 8.3, 50 mM) containing the NADH (10^{-2} M) was prepared. *E. coli*/ADH-A cells (12.5 mg, 1 mg cells/0.01 mmol coupling product) containing the over-expressed ADH-A were rehydrated in NADH-Tris-HCl buffer (0.1 mL) and Tris-HCl buffer (0.4 mL; pH 8.3, 50 mM), for 1 h at 30 °C. The solution was then mixed with the coupling product (0.125 mmol) diluted in [bmim][NTf_2] (0.3 mL) and 2-propanol (0.2 mL, 20 % v/v) was added. The mixture was stirred for 18 h at 30 °C and extracted with diethyl ether (3×5 mL). The combined extracts were dried over magnesium sulfate, evaporated, and purified by flash chromatography on a silica gel column (hexane/EtOAc 95:5) and characterized by ^{1}H NMR spectroscopy and high-resolution mass spectrometry. Conversion rate and enantioselectivity were analyzed by chiral HPLC (Chiralcell OD, 0.46 cm Ø×25 cm, hexane/*i*PrOH 92.5:7.5, 1 mL min^{-1}).

General procedure for the one-pot synthesis of the aryl alcohol: The procedure for the Suzuki coupling reaction was the same as described earlier (V_{IL} = 0.3 mL). Once the coupling reaction was over (intermediate conversion rate based on the halide conversion was followed by HPLC), the reaction medium was cooled to 30 °C, and previously rehydrated *E. coli*/ADH-A cells (0.5 mL) (method described above) were added to the Suzuki reaction media, along with 2-propanol (0.2 mL, 20 % v/v). The solution was then stirred for 18 h at 30 °C, and extracted with diethyl ether (3×5 mL). The combined extracts were dried over magnesium sulfate, evaporated, and purified by flash chromatography on a silica gel column (hexane/EtOAc 95:5) and characterized by ^{1}H NMR spectroscopy and high-resolution mass spectrometry. Conversion rate and enantioselectivity were analyzed by chiral HPLC (Chiralcell OD, 0.46 cm Ø×25 cm, hexane/*i*PrOH 92.5:7.5, 1 mL min^{-1}).

Recycling procedure: After carrying out a one-pot synthesis, supernatant aqueous phase containing the *E. coli* cells were simply removed from the reaction mixture, and were directly re-engaged in another bioreduction step without any further purification. After extraction of the alcohol, the Pd/IL mixture was reused in the one-pot reaction, under the same conditions as described earlier, without addition of IL or catalyst.

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