Novel Insights into the Combination of Metal- and Biocatalysis: Cascade One-pot Synthesis of Enantiomerically Pure Biaryl Alcohols in Deep Eutectic Solvents

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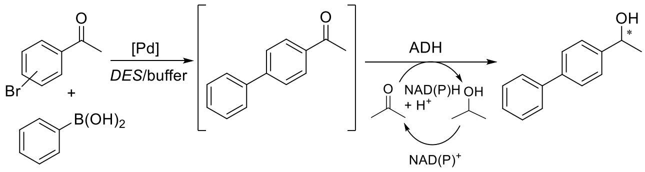
Abstract: One of the pioneering examples of chemoenzymatic cascades in water such as the palladium-catalysed Suzuki-cross coupling followed by an enzymatic reduction has been revisited by the employment of a medium containing *Deep Eutectic Solvents* (*DESs*) for the catalytic performance. Thus, the unique properties of these neoteric solvents enabled to reach high substrate concentration for the overall process. Moreover, both isolated enzymes and whole cells exhibited excellent activities which allowed to obtain a set of chiral biaryl alcohols in good yields and very high enantiomeric excess (>99%).

Introduction

As stated by the burgeoning number of articles, the combination of chemo- and biocatalysts has turned into a pivotal research topic in the catalysis field.[1] Thus, this interest has spurred the development of new methodologies to merge the practical and economic advantages of both catalytic worlds.[2] In this context, one of the pioneering examples combining metal- and enzyme-catalysed transformations in aqueous medium is a sequence consisting of an initial Suzuki cross-coupling of halogenated acetophenones followed by an ADH (alcohol dehydrogenase)-mediated reduction. The original report, dated in 2008, efficiently yielded an enantiopure biaryl alcohol when operating at 33 mM and 70 ºC during the coupling reaction, and 25 mM at room temperature for the biotransformation.[3] Further improvements enabled to conduct the first step at room temperature by the use of water-soluble palladium catalysts and a high percent of propan-2-ol (50% v/v) as co-solvent, although proceeding at a low substrate concentration (up to 40 mM).[4] Alternatively, a biphasic solvent system consisting of water and an ionic liquid (IL) allowed to significantly increase the substrate concentration (210 mM and 125 mM respectively for each step) working at 110 ºC as well as recycling of both catalysts several times.[5]

With these precedents in mind, we turned our attention towards a new class of biorenewable solvents, namely *Deep Eutectic Solvents* (*DESs*), which have been demonstrated as a valuable alternative to volatile organic compounds from the standpoint of sustainability.[6] These solvents are natural mixtures of low-cost biodegradable components such as quaternary ammonium salts (e.g., choline chloride) and uncharged hydrogen-bond donors (HBD) (such as urea, carboxylic acids or polyols), thereby forming an extensive H-bond network throughout the solvent which stabilizes liquid configurations and results in lower melting points than those of their individual components. *DESs* share many unique IL-like solvent properties such as thermal stability, low vapour pressure, non-flammability, easy recycling and high solubility of organic compounds. Furthermore, they are cheaper, readily available, do not require further purification, offer high tunability, and are considered to be less toxic compared to ILs given the nature of its components.[7]

As part of our ongoing interest in the study of chemoenzymatic cascades,[8] we investigated the viability of the abovementioned cascade process, namely the Suzuki cross-coupling followed by bioreduction in a one-pot two-step fashion in mixtures of *DESs* and aqueous buffers (Scheme 1). To the best of our knowledge, there exists only one example of a chemoenzymatic cascade developed in parallel in these neoteric solvents, which is the combination of a ruthenium-catalyzed isomerisation with an enzymatic reduction.[9]



Scheme 1. Devised chemoenzymatic cascade towards chiral biaryl alcohols in *DES*-buffer medium.

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Results and Discussion

Over the past decade *DESs* have found applications in several chemical sciences and technologies such as electrochemistry and metal processing, material chemistry, nanotechnology, photosynthesis and energy technology, separations processes, and stabilisation of DNA. With respect to synthetic purposes, *DESs* have provided examples of improved activity and selectivity in: *i*) organometallic-mediated stoichiometric transformations,[10] and *ii*) metal-,[11] enzyme-,[12] or organo-catalysed reactions.[13] In this sense, processes traditionally restricted to anhydrous solvents such as polar organometallic chemistry could be conducted in *DESs*, establishing a new bridge between main group chemistry and green solvents.[10] Regarding biocatalysis,[14] *DESs* have been successfully implemented as a reaction medium for enzymes such as lipases, epoxide hydrolases, proteases, peroxidases and oxidoreductases so far.[15]

Very recently, several palladium-catalysed cross-coupling reactions (Suzuki-Miyaura, Sonogashira or Heck couplings) were efficiently accomplished in neat *DESs* by using cationic pyridiniophosphine ligands in association with $PdCl_2$.[16] Accordingly, and considering the cationic nature of the ligand to be critical, we set out to investigate the feasibility of the Suzuki coupling in eutectic mixtures by using our previous water-soluble palladium-catalyst system: $PdCl_2$/TPPTS [tris(3-sulfonatophenyl)phosphine hydrate, sodium salt].[3] Thus, the coupling between 4'-bromoacetophenone (**1**) and phenylboronic acid (**2**) to yield 4'-phenylacetophenone (**5a**) was selected as a benchmark reaction, and four choline chloride (*ChCl*)-based eutectic mixtures, namely 1*ChCl*/2*Gly* (*Gly* = glycerol), 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* (*Sorb* = sorbitol) and 1*ChCl*/2Urea, were utilized in this study (Table 1). The reaction medium also contained 20% (v/v) of an buffer solution pH = 8.5 to accomplish the required basic conditions as described in previous reports.[3-5] Preliminary attempts performed according to the reported conditions (40 mM of **1** and **2**, 4 mol% $PdCl_2$, 5 mol% TPPTS, temperature, 24 h) unveiled 1*ChCl*/2*Gly* as the optimal *DES*, leading to a conversion of 92% (entry 4). On the other hand, the reaction did not work in 1*ChCl*/2*Urea* (entry 2) while 1*ChCl*/2H_2O and 1*ChCl*/1*Sorb* displayed conversions higher than 80% (entries 1 and 3).Next, we focused on 1*ChCl*/2*Gly* and explored the effect of parameters such as temperature and catalyst loading. By heating at 70 ºC enabled quantitative conversion towards **5a** (>99%, entry 5). Remarkably, an identical result was obtained at this temperature when using a decreased catalyst loading of only 1 mol% $PdCl_2$ and 3 mol% TPPTS (entry 6). Next, to get more insight about the process, we tested higher substrate concentrations which fit better in an industrial setting (entries 7-11). Thus, it was found that concentrations of 100 mM or greater demanded heating to 100 ºC in the 1*ChCl*/2*Gly*-buffer mixture to reach complete conversion, with the upper limit being 200 mM (entry 8). On the contrary, upon these conditions the analogue mixtures based on 1*ChCl*/1*Sorb* and 1*ChCl*/2H_2O led to poor conversions (<40%, entries 9-10) which made to discard these *DESs* for further optimization. Finally, the parametrization was also extended to other aryl halides. Thus, the aryl chloride turned out to be less reactive (conversion = 65%, entry 12) meanwhile the iodine derivative enabled complete conversion at 200 mM and 100 ºC (entry 13). Based on the results described in the above cited report about cationic phosphine ligands in *DESs*,[16] the reactions with bromine and iodine reagents were essayed with a catalyst load reduced tenfold (entries 14-15). In the case of the aryl iodide the process worked efficiently (entry 14), and despite a slight decreased conversion, the low required catalyst loading could be interesting from a economic point of view for large-scale reactions.

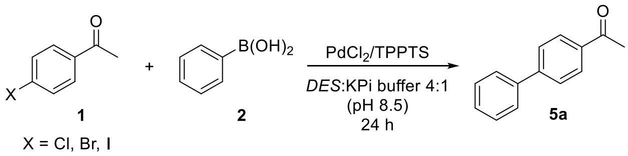
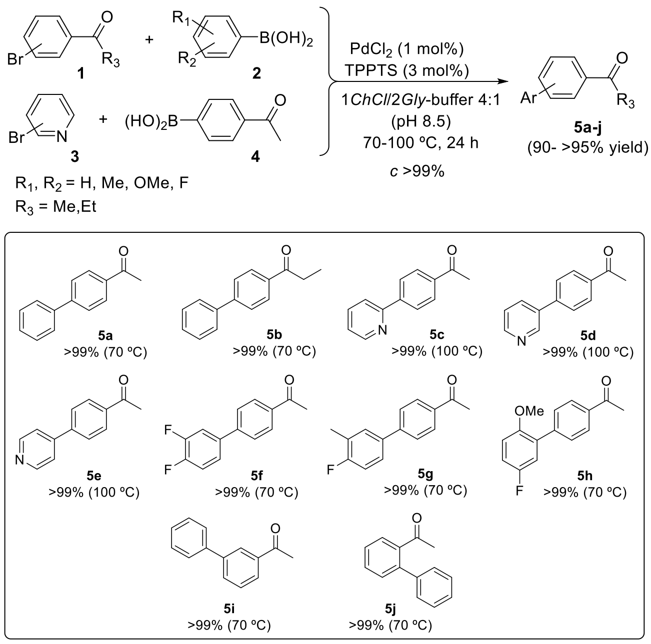


Table 1. Parametrisation of the Suzuki cross-coupling reaction of **1** and **2** in *DES*-buffer (4:1) medium catalysed by $PdCl_2$/TPPTS.[a]

Entry	X	*DES*	[Pd]/ligand (mol%)	T (ºC)	[**1**] (mM)	*C* (%)[b]
1	Br	1*ChCl*/1*Sorb*	4/5	rt	40	82
2	Br	1*ChCl*/2*Urea*	4/5	rt	40	0
3	Br	1*ChCl*/2H_2O	4/5	rt	40	80
4	Br	1*ChCl*/2*Gly*	4/5	rt	40	92
5	Br	1*ChCl*/2*Gly*	4/5	70	40	>99
6	Br	1*ChCl*/2*Gly*	1/3	70	40	>99
7	Br	1*ChCl*/2*Gly*	1/3	70	100	99
8	Br	1*ChCl*/2*Gly*	1/3	100	200	>99
9	Br	1*ChCl*/1*Sorb*	1/3	100	200	40
10	Br	1*ChCl*/2H_2O	1/3	100	200	35
11	Br	1*ChCl*/2*Gly*	1/3	100	300	60
12	Cl	1*ChCl*/2*Gly*	1/3	100	200	65
13	I	1*ChCl*/2*Gly*	1/3	100	200	>99
14	I	1*ChCl*/2*Gly*	0.1/0.3	100	200	92
15	Br	1*ChCl*/2*Gly*	0.1/0.3	100	200	40

[a] Reaction conditions (40 mM): A solution of $PdCl_2$ and TPPTS (previously stirred in 1.0 mL of KPi buffer pH 8.5 during 30 min) was added to a mixture of **1** (0.389 mmol), **2** (0.389 mmol), *DES* (8.0 mL), KPi buffer pH 8.5 (1.0 mL). Then, the pH was adjusted to 8.5 with aq 3 N NaOH and the mixture vigorously stirred at the specified temperature during 24 h. [b] Determined by HPLC.

The substrate scope of the Suzuki coupling under optimised conditions was evaluated for the construction of *ortho*-, *meta*-, *para*-biaryl and arylpyridine ketones, some of them exhibiting different patterns of substitution (Scheme 2). Thus, a set of 10 compounds was prepared by reacting appropriate aryl bromides and arylboronic acids according to a previous report.[17] Thereby, acetyl or propionyl groups were previously present in the aryl bromides (coupling between **1** and **2**; upper synthetic scheme), except for the synthesis of methyl pyridylphenyl ketones, which were obtained from bromopyridines (**3**) and (4-acetylphenyl)boronic acid (**4**, lower synthetic scheme). The products were classified into three groups according to the reactivity exhibited by their precursor reagents: *i*) fluorinated biaryl ketones (**5f-h**), *ii*) unsubstituted biaryl ketones (**5a**,**b**,**i**,**j**), and *iii*) arylpyridine ketones (**5c-e**). First, the fluorinated derivatives reached conversions in the range of 90-95% at room temperature and 40 mM due to the high reactivity of the boronic acids **2** bearing such electron-withdrawing groups. Further heating to 70 ºC enabled quantitative conversion at 200 mM. Similarly, biaryl ketones **5a**,**b**,**i**,**j** underwent quantitative conversion at 200 mM and 70 ºC. Conversely, the pyridine derivatives demanded more drastic conditions since the conversions were lower than 60% at 40 mM and 70 ºC. Thus, a temperature of 100 ºC improved the process dramatically and led to complete conversion even at 200 mM. In all the cases, the resulting biaryl ketones were easily recovered from the reaction medium by adding saturated aqueous NH_4Cl and further extraction with cyclopentyl methyl ether (90- >95%).



Scheme 2. Scope of the Suzuki cross-coupling reaction in *DES*-buffer medium under the optimised reaction conditions [200 mM substrate concentration, 1*ChCl*:2*Gly*-KPi buffer pH 8.5 (4:1), $PdCl_2$ (1 mol%), TPPTS (3 mol%), 70 ºC or 100 ºC, 24 h].

Once assessed the conditions for the Suzuki coupling as the first step of the cascade, next we focused on the reduction of the formed ketones (**5a**-**j**) by using a commercial kit of KREDs and two ADHs overexpressed in *E. coli* with opposite enantioselectivity. In the last years, *DESs* have proved to be an excellent reaction medium for performing bioreductions with whole cells overexpressing oxidoreductases,[13d,e,g,h,j] and very recently applications with purified KREDs were reported as well.[9] Likewise, a fine tuning of the ratio *DES*:water enabled remarkable enhancements of enantioselectivity and even switching the stereochemical outcome.[13d,g] With these premises, the biaryl ketone **5a** was initially tested with a set of engineered KREDs from Codexis (Table 2). In a typical experiment, **5a** (5 mM) was incubated in the presence of a KRED (200% w/w) at 30 ºC and 250 rpm in a mixture of 1*ChCl*/2*Gly*-buffer 1:1.5 (containing 1.25 mM $MgSO_4$ and 1 mM $NADP^+$) supplemented with isopropanol (*i*-PrOH, 10% v/v) for cofactor recycling. The choice of this reaction medium, which contains about 35% (v/v) of *DES*, was based to preserve the stability of the enzymes. Actually, most of purified KREDs considered in this study and the ADH from *L. kefir* were recently reported to display good stability in the bioreduction of propiophenone at 50% *DES* for 1*ChCl*/2*Gly* and 1*ChCl*/1*Sorb* meanwhile only a few ones were active at 80% *DES*.[9] From the series of KREDs of the kit, most of the biocatalysts rendered biphenylethan-1-ol (**6a**) in quantitative conversion and perfect enantioselectivity (>99% *ee*, entries 1-17). Likewise, the two enzymes overexpressed in *E. coli*, namely the (*R*)-selective ADH from *Lactobacillus kefir* DSM 20587 ($NADP^+$ dependent)[18] and the (*S*)-selective ADH from *Rhodococcus ruber* DSM 44541 (NAD^+ dependent),[19] worked efficiently in this reaction medium, forming both enantiomers of **6a** in enantiomerically pure form (entries 18-19). Seeing as the excellent enantioselectivities exhibited by the enzymes at this *DES*-buffer ratio, a further medium engineering optimisation by increasing the percent of *DES* was discarded.

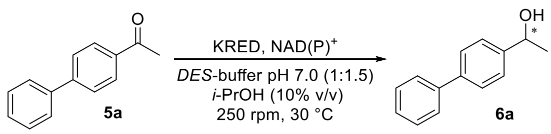


Table 2. ADH-catalysed reduction of 1-(biphenyl-4-yl)ethanone (**5a**) in *DES*-buffer medium.[a]

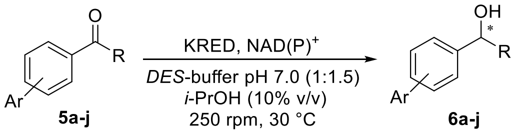
Entry	Enzyme	*Conv.* (%)[b]	*ee* (%)[b]
1	P1-A04	>99	>99 (*R*)
2	P1-B02	>99	99 (*R*)
3	P1-B05	>99	>99 (*R*)
4	P1-B10	>99	>99 (*R*)
5	P1-H08	>99	98 (*R*)
6	P2-G09	>99	>99 (*S*)
7	P2-B02	>99	63 (*S*)
8	P2-C02	>99	90 (*S*)
9	P2-C11	>99	>99 (*R*)
10	P2-D03	>99	93 (*R*)
11	P2-D11	35	99 (*R*)

12	P2-D12	95	95 (*R*)
13	P2-G03	>99	>99 (*R*)
14	P2-H07	>99	>99 (*R*)
15	P3-B03	>99	>99 (*S*)
16	P1-A12	>99	>99 (*R*)
17	P3-H12	95	98 (*S*)
18	*L. kefir* DSM 20587	>99	>99 (*R*)
19	*R. ruber* DSM 44541	>99	>99 (*S*)

[a] Reaction conditions: KRED (1.0 mg) and **5a** (5 mM) were added in a 1*ChCl*:2*Gly* (215 µL)/ KPi buffer 125 mM pH 7.0 (325 µL) mixture (1.25 mM $MgSO_4$, 1 mM $NADP^+$), and *i*-PrOH (60 µL) and shaken at 30 ºC and 250 rpm for 24 h; For *R. ruber* reactions, 5 U were added in a 1*ChCl*:2*Gly* (215 µL)/ KPi buffer 50 mM pH 7.0 (325 µL) mixture (1 mM NAD^+), and *i*-PrOH (60 µL); For *L. kefir* reactions, 15 U were added in a 1*ChCl*:2*Gly* (215 µL)/KPi buffer 50 mM pH 7.0 (325 µL) mixture (1 mM $MgCl_2$ and 1 mM $NADP^+$), and *i*-PrOH (60 µL). [b] Measured by HPLC.

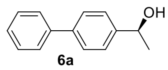
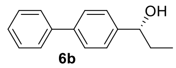
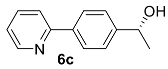
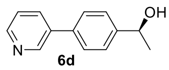
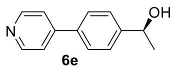
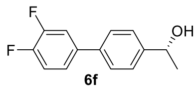
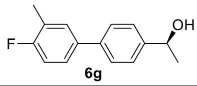
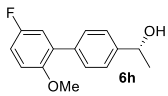
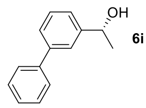
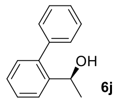
Next, on the basis of the established enzymatic conditions in Table 2, the reduction of ketones **5b-j** was screened with the overexpressed ADHs from *L. kefir* DSM 20587 and *R. ruber* DSM 44541 as well as with four purified enzymes from the Codexis' kit, namely the (*R*)-selective KRED-P1-A04 and KRED-P2-H07 and the (*S*)-selective KRED-P2-G09 and KRED-P3-H12. The choice of these four biocatalysts was based on the results afforded in Table 2 with **5a** and also the good activity recently reported on the bioreduction of propiophenone in *DES*-buffer 1:1 mixtures.[9] As depicted in Table 3, which contains some selected examples of the screening, it was possible to access alternatively both enantiomers of the target alcohols after 24 h with very high conversion (>99%) and enantioselectivity (99->99% *ee*). The only exception was the sterically hindered *ortho*-biarylic ketone **5j**, which led to enantiopure (*R*)-**6j** but with very low conversion (26%, entry 19; see also Table S11 in the SI). Exceptionally, the bioreduction of **5j** was also assayed with a different KRED from the kit, namely P1-B02, which was recently found to be very active towards **5j** in aqueous medium.[20] Pleasantly, this biocatalyst enabled to reach (*S*)-**6j** with complete conversion and >99% *ee* (entry 20).

Interestingly, both ADHs overexpressed in *E. coli* (*L. kefir* and *R. ruber*) were very efficient in terms of reactivity and selectivity towards some of the substrates (*c* >99%, *ee* >99%). This makes these biocatalysts especially attractive for a hypothetical gram-scale process in comparison with the commercial purified ones due to their high cost (for the full panel of enzymatic screenings, see Tables S3-S11 in the SI). A further goal of the project was to enhance the substrate concentration in the biocatalytic step, which could meet the parameters of a manufacture setting. Pleasantly, the good solubilising properties of the *DESs* (being used in an amount of close to 35% v/v) ensured a homogeneous reaction mixture for some selected bioreductions at 75 mM substrate concentration, enabling comparable results to those obtained in the screening at 5 mM.



5a-j → **6a-j**

Table 3. Selection of ADH-catalysed reductions of biaryl ketones **5a-j** in *DES*-buffer medium.[a]

Entry	Ketone	Enzyme	Alcohol	*Conv.* (%)[b]	*ee* (%)[c]
1	**5a**	*L. kefir*		>99	>99 (*R*)
2	**5a**	*R. ruber*	**6a**	>99	>99 (*S*)
3	**5b**	P1-A04		>99	>99 (*R*)
4	**5b**	P3-H12	**6b**	>99	>99 (*S*)
5	**5c**	P1-A04		>99	99 (*R*)
6	**5c**	*R. ruber*	**6c**	>99	>99 (*S*)
7	**5d**	*L. kefir*		>99	>99 (*R*)
8	**5d**	*R. ruber*	**6d**	>99	>99 (*S*)
9	**5e**	*L. kefir*		>99	>99 (*R*)
10	**5e**	*R. ruber*	**6e**	>99	>99 (*S*)
11	**5f**	*L. kefir*		>99	>99 (*R*)
12	**5f**	*R. ruber*	**6f**	>99	>99 (*S*)
13	**5g**	*L. kefir*		>99	>99 (*R*)
14	**5g**	*R. ruber*	**6g**	>99	>99 (*S*)
15	**5h**	P1-A04		>99	>99 (*R*)
16	**5h**	P2-G09	**6h**	>99	>99 (*S*)
17	**5i**	P1-A04	 **6i**	>99	>99 (*R*)
18	**5i**	*R. ruber*		>99	>99 (*S*)
19	**5j**	P2-H07		26	>99 (*R*)
20	**5j**	P1-B02	**6j**	>99	>99 (*S*)

[a] Reaction conditions: **5a-j** (5 mM) and KRED (1 mg) in 1*ChCl*:2*Gly* (215 µL)/ KPi buffer 125 mM pH 7.0 (325 µL) mixture (1.25 mM $MgSO_4$ and 1 mM $NADP^+$), and *i*-PrOH (60 µL). The mixture was stirred for 24 h at 250 rpm and 30 ºC; For *L. kefir* reactions, 15 U and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1mM $MgCl_2$ and 1 mM $NADP^+$; For *R. ruber* reactions, 5 U and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1 mM NAD^+. [b] Determined by HPLC. [c] Determined by chiral HPLC.

With both catalytic steps validated and optimised in terms of substrate concentration and reaction medium composition, the combination in a one-pot fashion with sequential reaction steps was planned as follows: 1) A Suzuki cross-coupling reaction conducted at 200 mM substrate concentration in a *DES*-buffer 4:1 medium; 2) *In situ* enzymatic reduction of the transiently formed ketone previous dilution to 75 mM and *DES*-buffer ~1:1 medium with a solution containing *i*-PrOH, enzyme and cofactor. Accordingly, and based on a recent study about the effect of water in the nanostructure of *DES*,[21] the coupling step can be assumed to be accomplished in a choline chloride/glycerol/water deep eutectic solvent mixture, meanwhile the medium for the bioreduction (containing ~50% H_2O) should be considered an aqueous solution of *DES* components. Thus, a selection of four target alcohols was made in order to show the general applicability of the process, including examples of unsubstituted (**6a**), fluorinated (**6g**) and pyridyl derivatives (**6d**,**e**). The reductions were carried out utilizing the two recombinant ADHs from *L. kefir* DSM 20587 and *R. ruber* DSM 44541, overexpressed in *E. coli*, which turned out as promising biocatalysts in the initial screening (Table 3).

The first synthetic sequence was aimed at obtaining the (*R*)-enantiomer of 1-([1,1'-biphenyl]-4-yl)ethan-1-ol (**6a**, entry 1). Towards this end, the initial Suzuki cross-coupling was accomplished at 200 mM and 100 ºC, according to the optimised reaction conditions described above (Table 1, entry 8). Once the coupling was complete (HPLC analysis), the reaction mixture was diluted to 75 mM with the aqueous buffer for the bioreduction (containing $NADP^+$ and $MgCl_2$) and feeded with the ADH from *L. kefir* DSM 20587 and *i*-PrOH. Thus, the bioreduction of the formed ketone intermediate took place smoothly, (*R*)-**6a** being obtained with good conversion (78%) and >99% *ee* (entry 1). Following an analogous procedure for the coupling step but using the complementary ADH from *R. ruber* DSM 44541 for the bioreduction, (*S*)-**6a** was formed with both high conversion and optical purity (entry 2). Next, similar cascades were established with this couple of enzymes and the required substrates to produce the biarylic alcohols **6d**, **6e** and **6g**. The ADH from *L. kefir* DSM 20587 led to the formation of the corresponding (*R*)-enantiomer of these alcohols with >99% *ee* and conversions of up to 91% (entries 3 and 5). Meanwhile, the ADH from *R. ruber* DSM 44541 gave access to the (*S*)-counterparts with conversions >90% (entries 4, 6 and 7). In all cases, after filtration through silica the target biaryl alcohols were isolated in high yields (>80% with the exception of entry 1). It is worth noting that despite being a stepwise process, the overall methodology is operationally simple and the media coming from the metal-catalyzed reaction was used directly to feed the enzymatic bioreduction, resulting in simplified downstream operations relative to classical multistep reactions with tedious isolation and purification of intermediates.

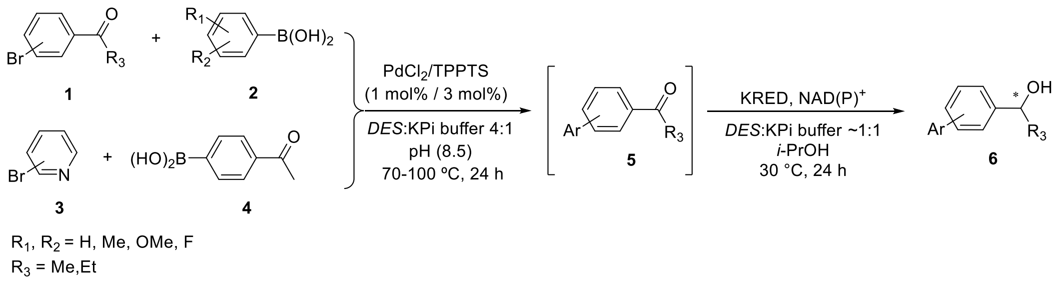


Table 4. One-pot synthesis of biaryl alcohols by palladium-catalysed Suzuki cross-coupling followed by enzymatic reduction.[a]

Entry	Cross-coupling T (ºC)	Enzyme	Product	Overall conv. (%)[b]	Isolated Yield (%)	*ee* (%)[c]	Absolute configuration
1	100	*L. kefir* DSM 20587	**6a**	78	70	>99	(*R*)
2	100	*R. ruber* DSM 44541	**6a**	86	80	>99	(*S*)
3	100	*L. kefir* DSM 20587	**6d**	85	80	>99	(*R*)
4	100	*R. ruber* DSM 44541	**6d**	90	85	>99	(*S*)
5	100	*L. kefir* DSM 20587	**6e**	91	86	>99	(*R*)
6	100	*R. ruber* DSM 44541	**6e**	92	84	>99	(*S*)
7	70	*R. ruber* DSM 44541	**6g**	90	83	>99	(*S*)

[a] Reaction conditions: A solution of $PdCl_2$ (1 mol%) and TPPTS (3 mol%), previously stirred in 1.0 mL of KPi buffer pH 8.5 during 30 min, was added to a mixture of **1** (1.945 mmol, 200 mM), **2** (1.945 mmol, 200 mM), 1*ChCl*:2*Gly* (8.0 mL) and KPi buffer pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 with aq 3 N NaOH and stirred at 70 ºC or 100 ºC during 24 h. For entries 1, 3 and 5, after cooling at rt, KPi buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL), $NADP^+$ (1 mM), $MgCl_2$ (1 mM) and ADH from *L. kefir* DSM 20587 (690 U) were added and the mixture stirred for 24 h at 30 ºC; For entries 2, 4, 6 and 7, after cooling at rt, KPi buffer pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL), NAD^+ (1 mM) and ADH from *R. ruber* DSM 44541 (360 U) were added and the mixture stirred for 24 h at 30 ºC. [b] Determined by HPLC. [c] Determined by chiral HPLC.

Conclusions

A chemoenzymatic cascade consisting on a palladium-catalysed Suzuki cross-coupling followed by an enzymatic reduction mediated by alcohol dehydrogenases has been efficiently implemented in *ad hoc* mixtures of *DESs* and aqueous media. The two catalytic steps took place efficiently and the excellent enantioselectivity displayed by the biocatalysts enabled the preparation of both enantiomers of several chiral biarylic alcohols in enantiomerically pure form. Likewise, the presence of the neoteric solvent in the medium enabled to tackle the solubility hurdles of the substrates, the biotransformation being executed at 75 mM concentration. In summary, this report underlines the advantages of *DESs* for their utilization in the fields of chemocatalysis and biocatalysis and will open up new perspectives for further exploration of chemoenzymatic one-pot processes in these reaction media.

Experimental Section

General procedure for the Suzuki cross-coupling reaction in *DES*-buffer medium

At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol %) and TPPTS (34 mg; 0.06 mmol; 3 mol %) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of arylbromide (1.945 mmol; 1 eq, 200 mM), boronic acid (1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated according to the substrate of choice for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were combined, dried with $NaSO_4$, filtered and concentrated under vacuum providing the crude product.

General procedure for the bioreduction of biarylketones 5a-j in *DES*-buffer medium

In a 2.0 mL Eppendorf tube, ketone (5 mM) and purified KRED (1.0 mg) were added to a *1ChCl*:2*Gly* (215 µL)/125 mM KH_2PO_4 buffer pH 7.0 (325 µL) mixture (containing 1.25 mM $MgSO_4$, 1 mM $NADP^+$), and *i*-PrOH (60 µL, 10% v/v). For *L. kefir* reactions, 15 U of ADH and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1mM $MgCl_2$ and 1 mM $NADP^+$; For *R. ruber* reactions, 5 U of ADH and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1 mM NAD^+. In all the cases, the reaction mixture was shaken at 30 ºC and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase. The mixture was then extracted with AcOEt (2 × 500µL) and aq saturated NH_4Cl (110 µL), the organic layers separated by centrifugation (120 sec, 1300 rpm), combined and dried over Na_2SO_4. The enantiomeric excess of alcohols was measured by chiral HPLC.

Preparative-scale synthesis of (S)-1-(4-(pyridin-3-yl)phenyl)ethanol [(S)-6d] in a one-pot sequential process

A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1mol %) and TPPTS (34 mg; 0.06 mmol; 3mol %) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 3-bromopyridine (1.945 mmol), (4-acetylphenyl)boronic acid (1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 100 ºC for 24 h. After cooling to rt, phosphate buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), NAD^+ (1 mM) and ADH from *Rhodococcus ruber* DSM 44541 [18] (360 U) were added. After stirring for another 24 h at 30 ºC, aq saturated NH_4Cl (25 mL) was added and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1:1) yielded 364 mg of (*S*)-**6d** as a yellowish oil (85%).

Preparative-scale synthesis of (R)-1-(4-(pyridin-4-yl)phenyl)ethanol [(R)-6e] in a one-pot sequential process

A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol %) and TPPTS (34 mg; 0.06 mmol; 3 mol %) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4-bromopyridine (1.945 mmol), (4-acetylphenyl)boronic acid (1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 100 ºC for 24 h. After cooling to rt, 150 mM KH_2PO_4 buffer pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), $NADP^+$ (1 mM), magnesium chloride (1 mM) and ADH from *Lactobacillus kefir* DSM 20587 [17] (690 U) were added. After stirring for another 24 h at 30 ºC, aq saturated NH_4Cl (25 mL) was added and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1:1) yielded 368 mg of (*R*)-**6e** as a white solid (80%).

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Keywords: deep eutectic solvents • alcohol dehydrogenase • Suzuki coupling • cascade reactions • chemoenzymatic synthesis

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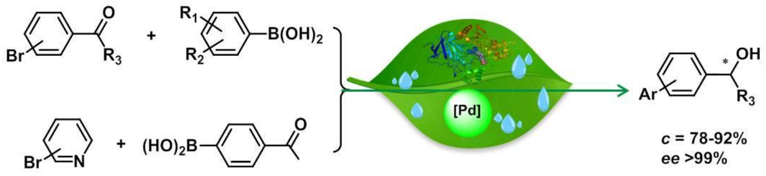

Chemoenzymatic cascade in biorenewable reaction media: A mixture of deep eutectic solvent (*DES*) and aqueous buffer proved to be an optimal medium for performing a chemoenzymatic cascade consisting of a palladium-catalysed Suzuki cross-coupling followed by an enzymatic reduction. Owing to the unique features of *DESs*, the process could be run at high concentration for both steps, enabling a variety of biarylic alcohols with very high conversion and enantiomeric excess.

Juraj Paris, Nicolás Ríos-Lombardía, Francisco Morís, Harald Gröger and Javier González-Sabín**

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Novel Insights into the Combination of Metal- and Biocatalysis: Cascade One-pot Synthesis of Enantiomerically pure Biaryl Alcohols in Deep Eutectic Solvents