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ARTICLE TYPE

Programming Cascade Reactions Interfacing Biocatalysis with Transition-Metal Catalysis in Deep Eutectic Solvents as Biorenewable Reaction Media†

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The first application of *Deep Eutectic Solvents* (*DESs*) in asymmetric bioreduction of ketones has been accomplished for purified ketoreductases (KREDs). The performance of the biocatalysts was enhanced by increasing the percentage of neoteric solvent in *DES*-buffer mixtures. At buffer content of 50% (w/w) and even 20% (w/w), either the combination of choline chloride (*ChCl*)/glycerol (*Gly*) (1:2) or *ChCl*/sorbitol (1:1) proved to be most effective for achieving up to >99% conversion and up to >99% enantiomeric excess of the corresponding secondary alcohols. Moreover, this reaction medium was used to perform the first example of chemoenzymatic cascade process in *DES*-buffer mixtures, namely the ruthenium-catalysed isomerisation of racemic allylic alcohols coupled with a further enantioselective bioreduction, in both sequential and concurrent mode.

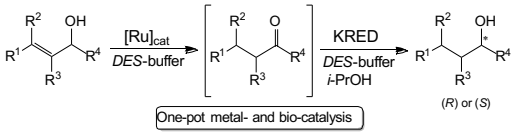
Introduction

As illustrated by the exponential growth of literature over the past decade, considerable attention has been focused on *Deep Eutectic Solvents* (*DESs*) as an increasingly valuable alternative to volatile organic compounds from the standpoint of "greenness" in several fields of sciences such as electrochemistry and metal processing,[1] organic synthesis,[2] material chemistry,[3] nanotechnology,[4] photosynthesis and energy technology,[5] separation processes,[6] and stabilisation of DNA.[7] These eutectic mixtures are usually made from a salt with a cation capable of forming hydrogen bonding and a neutral hydrogen bonding donor molecule, thereby forming an extensive H-bond network throughout the solvent which stabilises liquid configurations and hence results in a large melting point depression with respect to the melting temperature of the individual components. *DESs* share many physicochemical properties with conventional ionic liquids (*ILs*) (e.g., thermal stability, low vapour pressure, non-flammability, easy of recycling),[1] but are cheaper, easier to make (just by heating or gently warming), do not require further purification, offer high tunability, and are believed to be more biodegradable and less toxic compared to traditional *ILs* due to the use of environmentally friendly substances.[8]

Along with these properties, *DESs* have unexpectedly opened the floodgates to new perspectives and broad applications in *i*) polar organometallic chemistry (organolithium and Grignard reagents), thereby contributing to build new bridges between main group chemistry and green chemistry,[9] and *ii*) metal-,[10] bio-,[11] and organocatalysed transformations.[12] Tremendous and revolutionary advances have also been recently made in the field of biocatalysis with several *ad hoc* protocols set up for biotransformations catalysed by both isolated enzymes (lipases, proteases, epoxide hydrolases, peroxidases and lyases) and whole cells in *DESs* and *DES*-buffer mixtures.[11] To the best of our knowledge, however, the use of *DESs* as effective reaction media in *bioreduction processes* has been limited to date to whole cells.[11c,e,h,k-p] A fascinating and intriguing switch in the rate of reaction and enantioselectivity was recently found in baker's yeast-mediated reduction of both β-ketoesters[11c] and aryl-containing ketones [11h] by simply changing the solvent from water to *DES*-water mixtures. Several whole cells overexpressing oxidoreductases were also screened towards aryl-containing ketones in *DES*-water mixtures for the synthesis of chiral key building blocks and Active Pharmaceutical Ingredients (APIs).[11h,k] The design of tandem metal- and bio-catalysed organic processes is also a burgeoning field mainly investigated in water, the natural medium of enzymes. Apart from some exceptions,[13] however, these catalytic networks typically suffer from several drawbacks such as the compatibility of the involved catalysts with reaction conditions or undesired cross-reactivity. In addition, the solubility of most organic substrates is usually low in water, which limits the maximum substrate concentration with the prospect of meeting industrial criteria.[14]

Building on our interest in bioreductions and in *DES* for exploring novel paradigms in biocatalysis,[11h,k,15] herein we present the first successful reaction of *purified ketoreductases* (KREDs) in the asymmetric bioreduction of aryl-containing prochiral ketones directly in *DES*-buffer mixtures as sustainable reaction media.[16] Furthermore, in this work we also describe the first example of one-pot chemoenzymatic cascade by interfacing a metal-catalysed isomerisation reaction of allylic alcohols with a enantioselective KRED-promoted bioreduction in aqueous buffer eutectic mixtures both in sequential and in concurrent fashion. Thus, a practical approach to convert a racemic mixture of allylic alcohols into a

stereodefined, saturated enantiopure secondary alcohol (*R* or *S*) has been set up without isolation/purification steps. The overall transformation involves three consecutive steps: *i*) reduction of the allylic C–C double bond, *ii*) oxidation of the secondary alkyl/aryl carbinol moiety, and *iii*) enantioselective bioreduction of the *in-situ* generated prochiral ketone (Scheme 1).



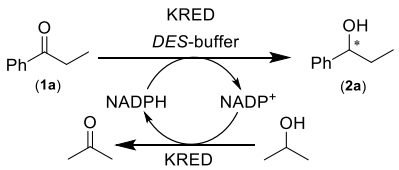
Scheme 1. One-pot Ru-catalysed isomerisation of allylic alcohols combined with an enantioselective bioreduction in *DES*-buffer medium.

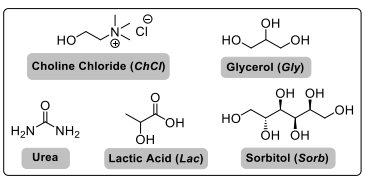
Results and Discussion

KRED-catalysed reduction of ketones in *DES*-buffer mixtures

In order to explore the viability of eutectic mixtures in the bioreduction of prochiral aromatic ketones, we set out to investigate as a bench reaction the bioreduction of propiophenone (**1a**) to 1-phenyl-1-propanol (**2a**) in various *DES* systems catalysed by a set of ten KREDs selected from the Codex® KRED Screening Kit, which are known to exhibit high activity and enantioselectivity towards **1a**.[15b,17] Five choline chloride (*ChCl*)-based eutectic mixtures, namely 1*ChCl*/2*Gly* (*Gly* = glycerol), 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* (*Sorb* = sorbitol), 1*ChCl*/2*Urea* and 1*ChCl*/2*Lac* (*Lac* = lactic acid) were screened at buffer content (phosphate buffer 125 mM pH 7.0, 1.25 mM $MgSO_4$, 1 mM $NADP^+$) from 20 to 50% (w/w) (Table 1). In a typical experiment aimed at evaluating the enzymatic performance, **1a** (20 mM) was incubated in a mixture of *DES* and KPi buffer (15% w/w of *i*-PrOH) at 30 ºC and 250 rpm during 24 h. As reported in Table 1, most of the employed KREDs led to poor conversions in 1*ChCl*/2H_2O at 50% and 80% (w/w) *DES*, and proved to be even inactive in *ChCl*-based *DESs* containing urea or lactic acid as the hydrogen bond donor (HBD) at 50% (w/w) *DES*. Gratefully, *DESs* containing sorbitol or glycerol as HBD and *ChCl* as the hydrogen bond acceptor resulted in high conversions, in particular at 50% (w/w) *DES*. Moving to 80% (w/w) *DES*, more than half of KREDs still remained very active in 1*ChCl*/1*Sorb* (*c* >80%; Table 1, entries 2–7,10), but only KRED-P2-C11 displayed excellent activity both in 1*ChCl*/1*Sorb* and 1*ChCl*/2*Gly*, thereby enabling complete conversion (*c* >99%) at 50% and 80% (w/w) *DES* (Table 1, entry 5). Recently, the commercial supplier of KREDs unveiled the source of some enzymes contained in the kit: 16 out of 24 KREDs variants (six of which are included in Table 1; see details in the ESI)[18] have been derived from the short-chain dehydrogenase of the bacterium *Lactobacillus kefiri* (LKADH).[19] For the sake of comparison, this overexpressed enzyme was similarly submitted to the same panel of experiments. As can be seen in Table 1 (entry 11), *L. kefiri* displayed good activity towards **1a** at 50% and 80% (w/w) *DES* (*c* from 80 to >99%) in the three *DESs* considered, but it was inactive in those containing urea and lactic acid.

Table 1 Effect of different *DESs*-buffer media on the conversion of the KRED-catalysed bioreduction of propiophenone (**1a**).[a,b]





Entry	KRED	1*ChCl*/2*Gly*			1*ChCl*/2H_2O			1*ChCl*/1*Sorb*		
		50%[c] *DES*	80%[c] *DES*	100%[c] *DES*	50%[c] *DES*	80%[c] *DES*	100%[c] *DES*	50%[c] *DES*	80%[c] *DES*	100%[c] *DES*
1	NADH-110	39	-	-	79	50	-	42	25	-
2	P1-A04	>99	33	-	53	50	-	>99	93	-
3	P1-C01	94	7	-	14	25	-	>99	95	-
4	P1-H10	>99	-	-	-	-	-	>99	92	-
5	**P2-C11**	**>99**	**>99**	**50**	**>99**	**>99**	**-**	**>99**	**>99**	**-**
6	P2-D12	90	32	-	19	5	-	>99	88	-
7	P2-H07	>99	-	-	33	12	-	>99	82	-
8	P3-B03	45	-	-	-	-	-	50	33	-
9	P3-G09	42	-	-	-	-	-	45	35	-
10	P3-H12	90	-	-	50	-	-	94	81	-
11[d]	LKADH (*L. kefiri)*	>99	93	-	>99	96	-	87	81	-

[a] Reaction conditions: **1a** (20 mM) in a *DES*-KPi buffer mixture (900 µL, 1.25 mM $MgSO_4$, 1 mM $NADP^+$), KRED (2 mg), *i*-PrOH (190 µL), 24 h at 250 rpm and 30 ºC. Conversion measured by HPLC. [b] No conversion detected both in 1*ChCl*/2urea and 1*ChCl*/2*Lac* at 50% (w/w) *DES*. [c] *DES*-buffer percentages are expressed in w/w. [d] Reaction conditions for *L. kefiri*: **1a** (20 mM) in a *DES*-KPi buffer mixture (900 µL, 1 mM $MgCl_2$, 1 mM $NADP^+$), *L. kefir* (50 U), *i*-PrOH (190 µL), 24 h at 250 rpm and at 30 ºC.

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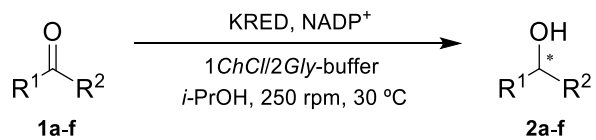
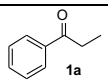
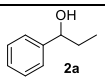
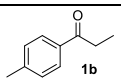
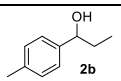
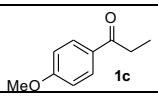
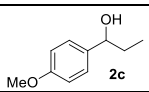
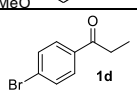
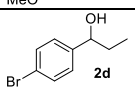
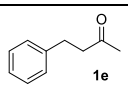
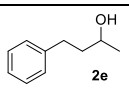
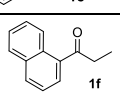
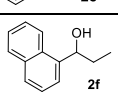
Given that both 1*ChCl*/2*Gly* and 1*ChCl*/1*Sorb* served as excellent eutectic mixtures for the bioreduction of **1a**, we sought to explore the scope of suitable prochiral ketone substrates (**1b**–**f**) employing the glycerol-based *DES* because of the ease of handling. Table 2 shows selected examples of screening with KREDs.[20] In most of cases, KREDs led to very similar conversions at both 50% (w/w) *DES* and buffer. Conversely, KRED-P2-C11 was the only active enzyme at 80% (w/w) *DES* concentration, thereby allowing excellent conversion of all tested ketones with the exception of **1f** (Table 2, entries 2, 3, 5, 7, 9 and 12). As for stereoselectivity, KREDs followed the general trend already observed with whole cells, that is a gradual improvement of enantioselectivity by increasing the percentage of *DES*.[11c,h] KRED-P2-C11, in particular, revealed to be an outstanding catalyst as it enabled an enhancement of the enantiomeric excess (*ee*) from 78% for **2a** or 54% for **2e** in aqueous buffer to >99% by increasing the concentration of *DES* to up to 80% (w/w) (Table 2, entries 2 and 9). In addition, a ketone such as **1e** whose bioreduction had been challenging in aqueous medium (*ee* up to 91%, see Table S5 in the ESI),[20] could now be obtained in enantiopure form (entry 9). Likewise, KRED-P2-H07 (Table 2, entry 10) also exhibited a significant performance in the bioreduction of **1e** by changing the solvent from neat aqueous buffer (**2e**: 32% *ee*) to 50% (w/w) *DES* (**2e**: 98% *ee*). Thus, the increase of *DES* percentage in the buffer medium was generally beneficial on the enantioselectivity for all KREDs. Parallel experiments performed in 1*ChCl*/1*Sorb*-buffer followed the same trend (see Table S7 in the ESI). A *DES* to buffer ratio of 50:50 (w/w) turned out to be the optimal reaction medium for most KREDs as it provided high conversion and high enantioselectivity. Exceptionally, KRED-P2-C11 was able to work even at 80% (w/w) *DES* increasing even more the final *ee*s of the produced alcohols.

Table 2 Selected KRED-catalysed reduction of ketones **1a**–**2a** in 1*ChCl*/2*Gly*-buffer.[a]



Entry	Substrate	Product	KRED	Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
				c (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	 **1a**	 **2a**	P1-A04	>99	>99 (*R*)	>99	>99 (*R*)	33	>99 (*R*)
2			P2-C11	>99	78 (*R*)	>99	96 (*R*)	>99	>99 (*R*)
3	 **1b**	 **2b**	P2-C11	>99	82 (*R*)	98	95 (*R*)	98	97 (*R*)
4			P2-H07	>99	90 (*R*)	96	>99 (*R*)	-	-
5	 **1c**	 **2c**	P2-C11	>99	92 (*R*)	98	96 (*R*)	98	98 (*R*)
6			P2-H07	>99	>99 (*R*)	95	>99 (*R*)	-	-
7	 **1d**	 **2d**	P2-C11	>99	93 (*R*)	>99	95 (*R*)	>99	98 (*R*)
8			P3-H12	>99	99 (*S*)	99	>99 (*S*)	-	-
9	 **1e**	 **2e**	P2-C11	>99	54 (*R*)	>99	93 (*R*)	97	>99 (*R*)
10			P2-H07	>99	32 (*R*)	>99	98 (*R*)	-	-
11	 **1f**	 **2f**	P3-H12	>99	89 (*S*)	>99	90 (*S*)	-	-
12			P2-C11	39	10 (*R*)	6	-	-	-

[a] Reaction conditions: ketone **1** (20 mM) in 1*ChCl*/2*Gly*-KPi buffer mixture (900 µL, 1.25 mM $MgSO_4$, 1 mM $NADP^+$), KRED (2 mg), *i*-PrOH (190 µL), 24 h at 250 rpm and at 30 ºC. [b] Conversion measured by HPLC. [c] Enantiomeric excess (*ee*) measured by chiral-phase HPLC. *R* or *S* refers to the absolute configuration of alcohols **2a**–**2f**.

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Stability of KREDs in *DES*-buffer mixtures

Cell integrity is known to be preserved in *DESs* in the case of whole cells, but there are no data for activity using isolated enzymes.[11c,h] However, as transpires from the data reported in Tables 1 and 2, all the selected, purified KREDs surprisingly remain very active in *ChCl*-based buffer media (e.g., 1*ChCl*/2*Gly*, 1*ChCl*/1*Sorb*). We sought to capitalize on that by getting more insight about the stability of KRED-P2-C11, which is the biocatalyst exhibiting the highest activity in *DES*-buffer media. First, the impact of temperature was evaluated by performing the reduction of **1a** in 1*ChCl*/2*Gly*-buffer 80:20 (w/w) at 30 ºC, 40 ºC, and 50 ºC (Figure 1). Bioreduction took 5 h at 30 ºC to reach complete conversion, while the conversion was no higher than 90% after 1 h. An increase of the temperature to 40 ºC resulted in an acceleration of the process, taking now only 2 h for **1a** consumption (c = 98% after 1 h). On the other hand, a temperature as high as 50 ºC had a detrimental effect in spite of a c = 43% after 1 h; the conversion stayed at 50% after 24 h not evolving further. A similar outcome was observed for the reduction of **1a** catalysed by KRED-P2-C11 in 1*ChCl*/1*Sorb*-buffer 80:20 (w/w) with quantitative biotransformations at 30 ºC or 40 ºC but, again, poor conversion after 24 h at 50 ºC (c = 30%).

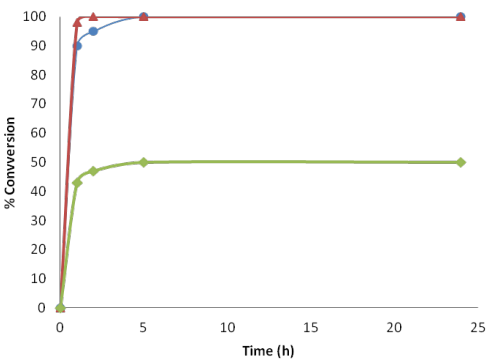


Figure 1. Kinetics of reduction of **1a** in 1*ChCl*/2*Gly*-buffer 80:20 (w/w) catalysed by KRED-P2-C11; Blue: 30 ºC; Red: 40 ºC; Green: 50 ºC

Alternatively, the reduction of **1a** was also carried out in 1*ChCl*/2*Gly*-buffer 80:20 (w/w) at 40 ºC, but incubating for 1 h, 2 h and 3 h the KRED in the reaction medium prior to the addition of the ketone. As depicted in Figure 2, the catalyst was readily deactivated in 2–3 h, which led to poor conversion (up to 25%) after 24 h. However, the residual activity in the KRED (despite concomitant inactivation) after 1 h of incubation was enough to reach a conversion of 93% after only 1 h of reaction, without further evolution. KRED-P2-C11 remained very active in a 1*ChCl*/1*Gly*-buffer 80:20 (w/w) even after incubation at 40 ºC for 24 h after the addition of ketone **1a** leading eventually to a conversion of 93%. For the sake of comparison, the same set of bioreduction of **1a** by KRED-P2-C11 was run in neat aqueous buffer: the biocatalyst turned out to be stable at 50 ºC and led to complete conversion despite a previous incubation of 3 h. In summary, the studied KRED exhibited slightly lower stability in *DESs* than in neat aqueous buffer, although it tolerated temperatures up to 40 ºC.

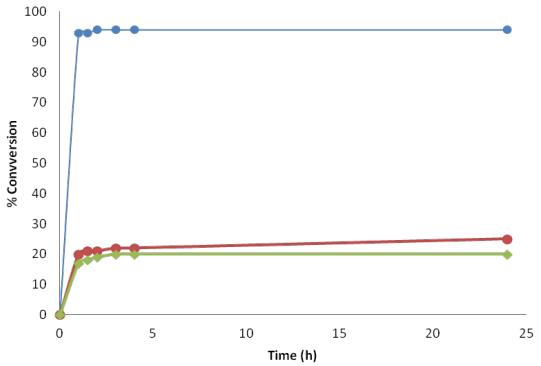
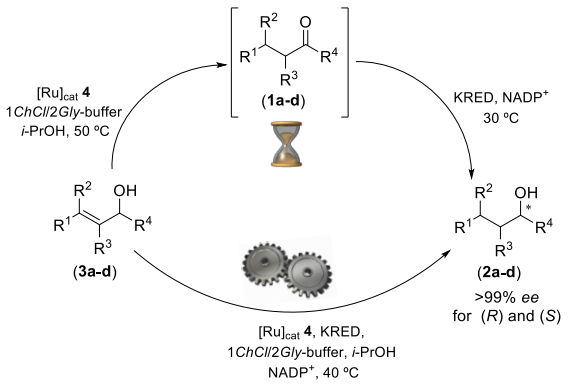


Figure 2. Kinetics of reduction of **1a** catalysed by KRED-P2-C11 at 40 ºC with KRED incubated in the medium [(1*ChCl*/2*Gly*-buffer 80:20 (w/w)] prior to the addition of **1a**; Blue: 1 h; Red: 2 h; Green: 3 h.

One-pot ruthenium-catalysed allylic alcohol isomerisation-asymmetric bioreduction in *DES*-buffer mixtures

In the last years, the ruthenium-catalysed isomerisation of allylic alcohols has been efficiently accomplished not only in water[21] but also in other unconventional solvents such as ionic liquids,[22] glycerol,[23] and *DESs*.[10f] What is more important, this metal-catalysed reaction could be successfully coupled with other biotransformations[13b,15b,24] as well as with organometallic reactions (which are typically restricted to anhydrous solvents) in a one-pot process.[25] We questioned whether the excellent activity displayed by KREDs in the reduction of ketones in neoteric solvents (*vide supra*) could be exploited for setting up a one-pot isomerisation of allylic alcohols coupled with an asymmetric KRED-mediated enzymatic reduction in a *DES*-buffer mixture. We began our studies focusing on a sequential one-pot two-steps methodology as previously reported.[15b,24] The metal-catalysed isomerisation of *α*-vinylbenzyl alcohol **3a** was investigated under the optimised conditions found for the bioreduction of ketones in *DES*-buffer mixtures (see Table 2) working at 200 mM substrate concentration in 1*ChCl*/2*Gly*-buffer 50:50 (w/w), at 50 ºC and using 5 mol% of ruthenium complex **4** as catalyst[26,27] (Scheme 2). Once the isomerisation was complete, KRED-P1-A04 and $NADP^+$ were sequentially added without isolating the intermediate ketone **1a** (Scheme 2), and the resulting mixture stirred for 24 h at 30 ºC (Table 3, entry 1). Saturated alcohol (*R*)-**2a** was obtained with quantitative conversion of substrate, excellent isolated yield (95%), and *ee* >99%. Following an identical protocol, racemic allylic alcohols **3b**–**d** gave rise to the corresponding (*R*) or (*S*)-saturated alcohols **2b**–**d** with very high *ee*s (Table 3, entries 3, 5 and 7) by selecting an adequate KRED. The overall methodology is operationally simple and excellent levels of both conversion

(>90%) and enantioselection (>99%) in the final products have been achieved. On the other hand, the same sequential processes starting from **3a**–**d** and accomplished with *L. kefiri* provided lower conversion values and enantioselectivies than those measured with purified KREDs (Table 3, entries 2, 4, 6 and 8). The only exception was **3a**, which led quantitatively to its saturated analogue (*R*)-**2a** in enantiopure form (Table 3, entry 2). These results are consistent with a compatibility of KREDs with the reaction medium coming from the metal-catalysed step, the impact of the Ru(IV) catalyst **4** on the enzymatic performance being negligible. Although comparable results have been obtained in pure water,[15b] the advantage of using *DES*-buffer mixtures arises from the beneficial effects exerted by such neoteric solvents on the enantioselectivity of KREDs, particularly in the case of challenging substrates unresponsive to be stereoselectively reduced in neat aqueous buffer.



Scheme 2. One-pot cascade synthesis of optically active alcohols by Ru-catalysed isomerisation/ enzymatic reduction in *DES*-buffer medium in both sequential (upper) and concurrent (lower) mode.

Table 3 Chemoenzymatic one-pot process in sequential mode.[a]

Entry	Substrate	Product	KRED	*c* (%)[b]	Yield (%)[c]	*ee* (%)[d]
1	**3a**	**2a**	P1-A04	>99	95	>99 (*R*)
2	**3a**	**2a**	*L. kefiri*	>99	92	>99 (*R*)
3	**3b**	**2b**	P2-H07	95	90	>99 (*R*)
4	**3b**	**2b**	*L. kefiri*	70	64	93 (*R*)
5	**3c**	**2c**	P3-H12	95	90	>99 (*S*)
6	**3c**	**2c**	*L. kefiri*	65	60	93 (*R*)
7	**3d**	**2d**	P2-H07	94	90	>99 (*R*)
8	**3d**	**2d**	*L. kefiri*	80	72	98 (*R*)

[a] Reaction conditions: **3a–d** (200 mM) was dissolved under an Ar atmosphere in a mixture of KPi buffer 125 mM (1.25 mM $MgSO_4$) at pH 7.0 (0.5 mL), *DES* 1*ChCl*/2*Gly* (0.5 mL) and *i*-PrOH (175 µL). Then, complex **4** (5 mol%) was added, and the mixture was stirred at 50 ºC. Once the isomerisation was complete, KRED (100% w/w) and $NADP^+$ (1 mM) were added, and the mixture was shaken for 24 h at 250 rpm and at 30 ºC (entries 1, 3, 5 and 7). For entries 2, 4, 6 and 8, *L. kefiri* (1000 U) and $NADP^+$ (1 mM) were added, and the mixture was shaken for 24 h at 250 rpm and at 30 ºC. [b] Conversion (*c*) measured by HPLC. [c] The yields reported are for products isolated and purified by column chromatography. [d] Enantiomeric excess (*ee*) measured by chiral-phase HPLC.

As for metal-catalysed isomerisation coupled with bioreduction processes run in a concurrent fashion in pure aqueous buffer, that is with the two catalytic systems coexisting from the outset, an open issue was the stability of the KRED in the reaction medium.[15b] These enzymes, indeed, suffered from rapid deactivation, thereby leading to moderate overall conversions in the case of allylic alcohols undergoing slow isomerisation. We selected the 1*ChCl*:2*Gly*-buffer 80:20 (w/w) mixture as the reaction medium for the transformation of the vinylic alcohol **3a** into **2a**, which was catalysed simultaneously and competitively by both KRED-P2-C11 and complex **4**. The load of **4** was optimized to 10 mol%. Indeed, under these conditions, the isomerisation of **3a**–**d** is completed in less of 2 h, and is fast enough to preserve the enzyme's lifetime (see Table S8, ESI). Thus, allylic alcohol **3a** was incubated at 40 ºC and 250 rpm in the above *DES*-buffer medium containing both catalysts. After 24 h, HPLC-analysis revealed that the starting racemic allylic alcohol **3a** was completely consumed and the target saturated alcohol (*R*)-**2a** obtained with an overall conversion of 90% and an *ee* >99%. The extension of the above protocol to substrates **3b**–**d** furnished the saturated chiral alcohols **2b**–**d** with overall conversion ranging from 68 to 96% for the R-configured enantiomer with an *ee* >99% in all cases (Table 4, entries 1-4). Conversely, after running a concurrent process with the overexpressed LKADH of *L. kefiri* and **3a**, we noticed that unsaturated alcohol **3a** remained partially unreacted, as though enzyme exerted some kind of inhibition on the metal complex **4** (Table 4, entry 5). HPLC analysis of the processes carried out with purified KREDs revealed that **4** isomerised quantitatively the starting allylic alcohol in all cases. Thus, the final ketone to saturated alcohol ratio found most probably is related to the significant difference in the catalytic performance exhibited by isolated KREDs according to the nature of the *in situ* formed ketone. Remarkably, the biotransformation of **3d** provided enantiopure (*R*)-**2d** in an overall conversion of 96% (Table 4, entry 4), which improved previous results in aqueous buffer mixtures.[15b]

Table 4 Chemoenzymatic one-pot process in concurrent mode.[a]

Entry	Substr.	Prod.	Product ratio (%)[b]			*ee* (%)[c]
			Allylic alcohol	Ketone	Saturated alcohol	
1	**3a**	**2a**	0	10	90	>99
2	**3b**	**2b**	0	30	70	>99
3	**3c**	**2c**	0	32	68	>99
4	**3d**	**2d**	0	4	96	>99
5[d]	**3a**	**2a**	33	46	21	>99

[a] Reaction conditions (entries 1-4): to a solution under an Ar atmosphere of **3a**–**d** (200 mM) in a mixture of KPi buffer 125 mM (1.25 mM $MgSO_4$) at pH 7.0 (0.5 mL), *DES* 1*ChCl*/2*Gly* (0.5 mL) and *i*-PrOH (175 µL), complex **4** (10 mol%), KRED-P2-C11 (100% w/w) and $NADP^+$ (1 mM) were added, and the resulting mixture was stirred for 24 h at 250 rpm and at 40 ºC; [b] Measured by HPLC. [c] Enantiomeric excess (*ee*) measured by chiral-phase HPLC. The R-enantiomer was obtained in all cases. [d] Reaction conditions (entry 5): to a solution of **3a** (200 mM) in a mixture similar to that reported for entries 1-4, complex **4** (10 mol%), *L. kefiri* (1000 U) and $NADP^+$ (1 mM) were added, and the resulting mixture was stirred for 24 h at 250 rpm and at 40 ºC.

Conclusion

To summarise, purified/overexpressed KREDs showed good chemical stability and exhibited excellent catalytic performance in *ad hoc* mixtures of *Deep Eutectic Solvents* and aqueous buffers. In particular, both the 1*ChCl*/2*Gly*-buffer and the 1*ChCl*/1*Sorb*-buffer mixtures turned out to be the most effective media for carrying out several bioreductions. The higher the percent of *DES*

in the mixture, the greater the enantioselectivity displayed by the selected KRED, which led to a substantial enhancement of the *ee* of the resulting secondary alcohol. Gratifyingly, the above *DES*-buffer mixtures could be successfully used for the first time as suitable reaction media also for setting up a chemoenzymatic cascade process, run both in sequential and in concurrent mode, such as the ruthenium-catalysed isomerisation of racemic allylic alcohols coupled with an asymmetric enzymatic reduction.

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Notes and references

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