Programming cascade reactions interfacing biocatalysis with transition-metal catalysis in *DeepEutectic Solvents* as biorenewable reaction media†

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The first application of *Deep Eutectic Solvents* (DESs) in the 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 a buffer content of 50% (w/w)and even 20% (w/w), the combination of either 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 a 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 modes.

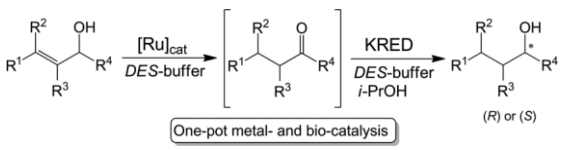
Introduction

As illustrated by the exponential growth of the 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] materials 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 ofthe individual components. DESs share many physicochemical properties (*e.g.*, thermal stability, low vapour pressure, non-flammability, and ease of recycling)[1] with conventional ionic liquids (ILs), 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 biodegradableand 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 the 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 for 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 DESs 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 a one-pot chemoenzymatic cascade by interfacing a metal-catalysed isomerisation reaction of allylic alcohols with an enantioselective KRED-promoted bioreduction in aqueous buffer eutectic mixtures both in a sequential and in a 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).



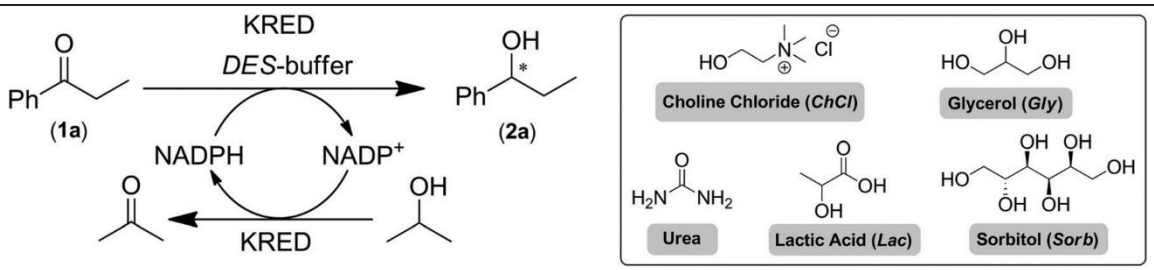
Scheme 1 One-pot Ru-catalysed isomerisation of allylic alcohols combined with an enantioselective bioreduction in DES-buffer medium.

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 cata- lysed 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 1ChCl/2Gly (Gly = gly- cerol), 1ChCl/2H_2O, 1ChCl/1Sorb (Sorb = sorbitol), 1ChCl/ 2Urea and 1ChCl/2Lac (Lac = lactic acid) were screened at buffer contents (phosphate buffer 125 mM, pH 7.0, 1.25 mM $MgSO_4$, and 1 mM $NADP^+$) from 20 to 50% (w/w) (Table 1). In atypical experiment aimed at evaluating enzymatic perform-

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



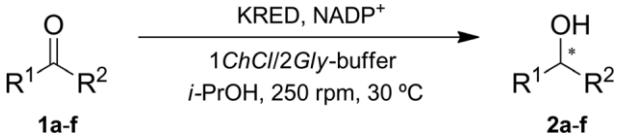
		1ChCl/2Gly			1ChCl/2H_2O			1ChCl/1Sorb		
Entry	KRED	50%[c] DES	80%[c] DES	100%[c] DES	50%[c] DES	80%[c] DES	100%[c] DES	50%[c] DES	80%[c] DES	100%[c] DES
1	NADH-110	39	—	—	79	50	—	42	25	—
2	P1-A04	>99	33	—	53	50	—	>99	93	—
3	P1-C01	94	7	—	14	25	—	>99	95	—
4	P1-H10	>99	—	—	—	—	—	>99	92	—
5	P2-C11	>99	>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 $MgSO_4$, and 1 mM $NADP^+$), KRED (2 mg), i-PrOH (190 µL), 24 h at 250 rpm and 30 °C. Conversion measured by HPLC. [b] No conversion detected both in 1ChCl/2Urea and in 1ChCl/2Lac 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 $MgCl_2$, and 1 mM $NADP^+$), *L. kefiri* (50 U), i-PrOH (190 µL), 24 h at 250 rpm and at 30 °C.

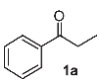
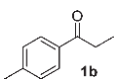
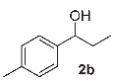
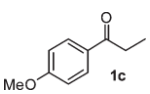
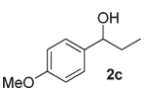
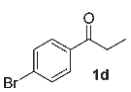
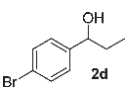
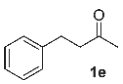
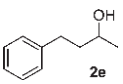
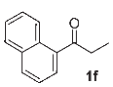
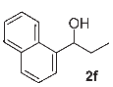
ance, 1a (20 mM) was incubated in a mixture of DES and KPi buffer (15% w/w of i-PrOH) at 30 °C and 250 rpm for 24 h. As reported in Table 1, most of the employed KREDs led to poor conversions in 1ChCl/2H_2O at 50% and 80% (w/w) DES, and proved to be even inactive in ChCl-based DESs containing urea or lactic acid as the hydrogen bond donor (HBD) at 50% (w/w) DES. Gratifyingly, 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 the KREDs still remained very active in 1ChCl/1Sorb (*c* from 81 to >99%; Table 1, entries 2–7 and 10), but only KRED-P2-C11 displayed excellent activity both in 1ChCl/1Sorb and in 1ChCl/2Gly, 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 ofsome enzymes contained in the kit: 16 out of the 24 KRED var-iants (six of which are included in Table 1; see also details in the ESI†)[18] have been derived from the short-chain dehydro- genase 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.

Given that both 1ChCl/2Gly and 1ChCl/1Sorb 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 high viscosity of the sorbitol-based eutectic mixture even at 40 °C (*vide infra*). Table 2 shows selected examples of screening with KREDs.[20] In most of the 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 in whole cells, that is a gradual improvement of enantioselectivity by increasing the percentage of DES.[11c,h] KRED-P2-C11, in particular, was 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

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



1a-f 2a-f

				Neat aqueous buffer		50% w/w DES		80% w/w DES	
Entry	Substrate	Product	KRED	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 1ChCl/2Gly-KPi buffer mixture (900 μL, 1.25 mM $MgSO_4$, and 1 mM $NADP^+$), KRED (2 mg), i-PrOH (190 μL), 24 h at 250 rpm and at 30 °C. [b] Conversion measured by HPLC. [c] Enantiomeric excess (ee) measured by chiral-phase HPLC. *R* or *S* refersto the absolute configuration of alcohols 2a–f.

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 the 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 the DES percen-tage in the buffer medium was generally beneficial for the enantioselectivity for all KREDs. Parallel experiments performed in 1ChCl/1Sorb-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 KREDsas 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 ees of the produced alcohols.

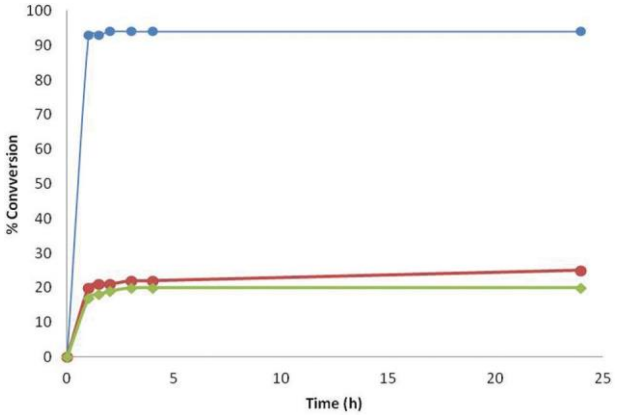


Fig. 2 Kinetics of reduction of 1a catalysed by KRED-P2-C11 at 40 °C with KRED incubated in the medium [(1ChCl/2Gly-buffer 80 : 20 (w/w))] prior to the addition of 1a; blue: 1 h; red: 2 h; green: 3 h.

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.*, 1ChCl/ 2Gly and 1ChCl/1Sorb). 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. Firstly, the impact of temperature was evaluatedby performing the reduction of 1a in 1ChCl/2Gly-buffer 80 : 20 (w/w) at 30 °C, 40 °C, and 50 °C (Fig. 1). Bioreduction took 5 h at 30 °C to reach complete conversion, while the conversionwas no higher than 90% after 1 h. An increase of the tempera- ture to 40 °C resulted in an acceleration of the process, takingnow only 2 h for 1a consumption (c = 98% after 1 h). On the other hand, a temperature as high as 50 °C had a detrimentaleffect 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 1ChCl/1Sorb-buffer 80 : 20 (w/w) with quantitative biotrans-formations at 30 °C or 40 °C but, again, poor conversion after 24 h at 50 °C (c = 30%).

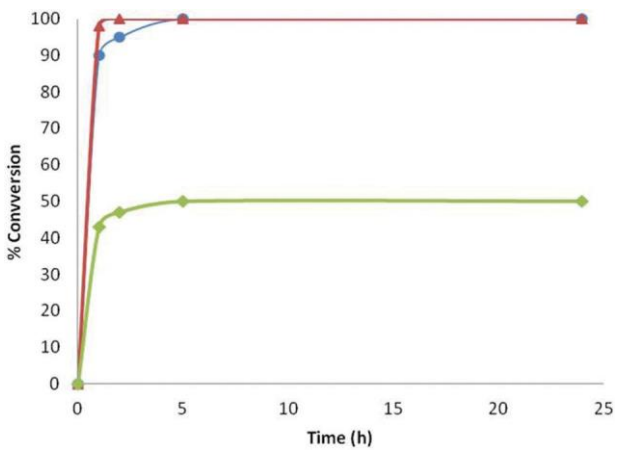


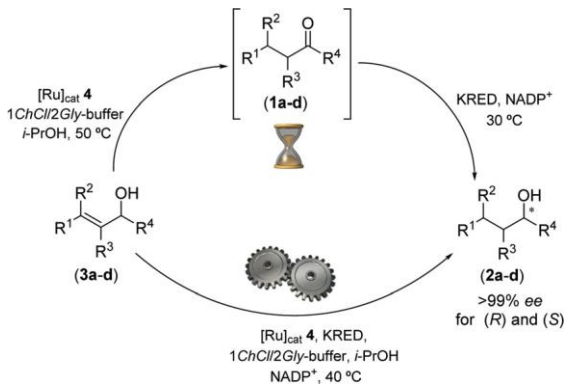
Fig. 1 Kinetics of reduction of 1a in 1ChCl/2Gly-buffer 80 : 20 (w/w) catalysed by KRED-P2-C11; blue: 30 °C; red: 40 °C; green: 50 °C.

Alternatively, the reduction of 1a was also carried out in 1ChCl/2Gly-buffer 80 : 20 (w/w) at 40 °C, but incubating for 1 h, 2 h and 3 h KRED in the reaction medium prior to the addition of the ketone. As depicted in Fig. 2, the catalyst was readily deactivated in 2–3 h, which led to poor conversion (upto 25%) after 24 h. However, the residual activity in 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 1ChCl/1Gly-buffer 80 : 20 (w/w) even after incubation at40 °C for 24 h after the addition of ketone 1a leading even- tually to a conversion of 93%. For the sake of comparison, the same set of bioreduction of 1a by KRED-P2-C11 was run inneat aqueous buffer: the biocatalyst turned out to be stable at 50 °C and led to complete conversion despite a previous incu- bation 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 °C.

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

In the past few 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 organo-metallic 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 the 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-step 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 1ChCl/2Gly-buffer 50 : 50 (w/w) at 50 °C and using 5 mol% of the ruthenium complex 4 as the 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 mixturewas stirred for 24 h at 30 °C (Table 3, entry 1). Saturated alcohol (*R*)-2a was obtained with quantitative conversion of the 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



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

Table 3 Chemoenzymatic one-pot process in the 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) were 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 1ChCl/2Gly (0.5 mL) and i-PrOH (175 μL). Then, complex 4 (5 mol%) was added, and the mixture was stirred at 50 °C. 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 °C (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 °C. [b] Conversion (*c*) measured by HPLC. [c] The yields reported are for the products isolated and purified by column chromatography. [d] Enantiomeric excess (ee) measured by chiral-phase HPLC.

with very high ees (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 enantioselectivities 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 the enantiopure form (Table 3, entry 2). These results are consistent with the 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.

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 KRED in the reac-tion 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 1ChCl : 2Gly-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 optimised to 10 mol%. Indeed, under these conditions, the isomerisation of 3a–d is completed in less than 2 h, and is fast enough to preserve the enzyme's lifetime (see Table S8, ESI†). Thus, the allylic alcohol 3a was incubated at 40 °C and 250 rpm in the above-mentioned 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 was 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 the overall conversion ranging from 68 to 96% for the *R*-configured enantiomer with an ee > 99% in all cases (Table 4, entries 1–4).[28] Conversely, after running a concurrent process with the overexpressed LKADH of *L. kefiri* and 3a, we noticed that the unsaturated alcohol 3a remained partially unreacted, as though the 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 the concurrent mode[a]

			Product ratio[b] (%)			
Entry	Substrate	Product	Allylic alcohol	Ketone	Saturated alcohol	ee[c] (%)
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): Under an Ar atmosphere, to a solution 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 1ChCl/2Gly (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 °C. [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) ina 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 °C.

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 1ChCl/2Gly-buffer and the 1ChCl/1Sorb-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 theenantioselectivity displayed by the selected KRED, which led to a substantial enhancement of the ee of the resulting secondary alcohol. Gratifyingly, the above-mentioned DES-buffer mixturescould be successfully used for the first time as suitable reactionmedia also for setting up a chemoenzymatic cascade process, run both in sequential and in concurrent modes, such as the ruthenium-catalysed isomerisation of racemic allylic alcohols coupled with an asymmetric enzymatic reduction.

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