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

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SUPPORTING INFORMATION

Table of Contents

1. General procedures

Enzymes

-The R-alcohol dehydrogenase from *Lactobacillus kefiri* (LKADH) was provided as a cell-free extract (CFE) by the Group of Prof. H. Gröger (University of Bielefeld). The determination of the protein amount revealed ca. 22 mg/mL. The activity of the enzyme is about 1468 U/mL, with reference to the activity for acetophenone. Codex® KRED Screening Kit was purchased from Codexis. While it is technically possible to ID these enzymes by proteomics and comparison with the databases where Codexis reveals the coded information, such "reverse engineering" would violate the sales agreement. However, in a recent article[1] the commercial supplier revealed the origin of some KREDs involved in this study: KRED-P1-A04, KRED-P1-B05, KRED-P1-C01, KRED-P2-C11, KRED-P2-D12, KRED-P2-H07 have been derived from the short-chain dehydrogenase of the bacterium *Lactobacillus kefiri* (LKADH).[2] On the other hand, KRED-P3-B03, KRED-P3-G09, KRED-P3-H12 have been derived from the *Thermoanaerobacter brockii* alcohol dehydrogenase (TBADH).[3]

Protein and DNA Sequences for LKADH:

MTDRLKGKVAIVTGGTLGIGLAIADKFVEEGAKVVITGRHADVGEKAAKSIGGTDVIRFV
QHDASDEAGWTKLFDTTEEAFGPVTTVVNNAGIAVSKSVEDTTTEEWRKLLSVNLDGV
FFGTRLGIQRMKNKGLGASIINMSSIEGFVGDPTLGAYNASKGAVRIMSKSAALDCALK
DYDVRVNTVHPGYIKTPLVDDLEGAEEMMSQRTKTPMGHIGEPNDIAWICVYLASDES
KFATGAEFVVDGGYTAQHHHHHH

ATGACGGACCGTTTGAAGGGAAAAGTTGCAATCGTCACCGGCGGCACACTTGGTA
TTGGTCTGGCAATCGCAGACAAATTCGTAGAAGAGGGCGCAAAAGTTGTTATCACG
GGTCGCCACGCGGATGTAGGCGAGAAGGCTGCAAAGTCTATTGGCGGAACCGAC
GTTATTCGTTTCGTTCAGCACGATGCCAGTGATGAAGCTGGATGGACTAAGCTGTT
CGATACAACAGAGGAAGCTTTTGGCCCCGTTACGACAGTGGTTAATAACGCCGGT
ATTGCGGTTAGTAAATCAGTCGAAGACACCACAACGGAGGAGTGGCGTAAGCTTTT
AAGTGTAAACCTTGATGGAGTCTTTTTCGGTACACGCTTGGGCATTCAGCGCATGA
AGAATAAAGGTCTTGGCGCGTCGATCATTAATATGTCGTCAATCGAGGGATTTGTG
GGCGATCCCACGTTAGGGGCTTATAATGCCTCGAAGGGAGCGGTTCGCATCATGT
CGAAAAGCGCTGCTCTGGATTGTGCTCTGAAAGATTATGACGTACGCGTGAACACT
GTCCATCCTGGGTATATTAAGACGCCACTGGTAGACGATCTTGAGGGTGCCGAAG
AAATGATGTCCCAACGTACTAAGACTCCTATGGGACATATCGGTGAGCCTAATGAC
ATTGCGTGGATCTGTGTCTACCTGGCCTCAGACGAAAGCAAGTTCGCTACGGGCG
CAGAATTTGTGGTTGACGGAGGATATACTGCTCAG

Reagents

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[1] M. A. Emmanuel, N. R. Greenberg, D. Oblinsky and T. K, Hyster, *Nature*, 2016, **540**, 414–417.

[2] W. Hummel, *Appl. Microbiol. Biotechnol*., 1990, **34**, 15–19.

[3] O. Bogin, M. Peretz and Y. Burstein, *Protein Sci*., 1997, **6**, 450–458.

Ketones **1a**–**f** and allylic alcohols **3a**–**d** were purchased from commercial sources or prepared according to conventional procedures. All the other reagents and solvents were of the highest quality available. Deep eutectic solvents *ChCl-Gly* (1:2 mol/mol), *ChCl*-H_2O (1:2 mol/mol), *ChCl-Sorb* (1:1 mol/mol), *ChCl-Urea* (1:2 mol/mol), *ChCl-Lac* (1:2 mol/mol) were prepared by gently heating under stirring at 60–80 ºC for 1 h the corresponding individual components until a clear solution was obtained.

General methods

^{1}H-NMR spectra ($CDCl_3$) were run on a Bruker DPX-300 (^{1}H, 300.13 MHz) spectrometer using the δ scale (ppm) for chemical shifts. Calibration was made on the signal of the solvent (^{1}H: $CDCl_3$, 7.26).[4]. The degree of conversion was determined by HPLC analyses carried out on an Agilent RR1200 HPLC system using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 18μm, 4.6 × 50 mm, Agilent), whereas those to determine the enantiomeric excess (*ee*) were performed on a Hewlett Packard 1100 LC liquid chromatograph using either Chiracel OJ-H or Chiralpak AD-H columns. For column chromatograpy, Merck silica gel 60 (particle size, 40–63 lm) and solvents of spectrophotometric grade were used. Thin-layer chromatography was performed on precoated TLC plates of Merck silica gel 60F254, using potassium permanganate as a developing reagent.

[4] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.

2. Protocols

2.1. General procedure for the enzymatic reduction of 1a in *DESs*-buffer mixtures.

In a 2.0 mL Eppendorf tube, KRED (2 mg), **1a** (20 mM) and *i*-PrOH (190 μL) were added to 900 μL of a *DES*-KPi buffer (pH 7.0) mixture (1.25 mM $MgSO_4$, 1 mM $NADP^+$). The reaction was shaken at 250 rpm and at 30 ºC for 24 h. Then, NH_4Cl was added (500 μL), the mixture extracted with EtOAc (2×500 μL), the organic layers separated by centrifugation (90 sec, 13000 rpm), combined and finally dried over Na_2SO_4. Conversion was determined directly by HPLC. The *ee* of **2a** was determined by chiral HPLC analysis.

2.2. General procedure for the enzymatic reduction of 1a–f.

In a 2.0 mL Eppendorf tube, KRED (2 mg), ketone (20 mM) and *i*-PrOH (190 μL) were added to 900 μL of a *DES*-KPi buffer (pH 7.0) mixture (1.25 mM $MgSO_4$, 1 mM $NADP^+$). The reaction was shaken at 250 rpm and at 30 ºC for 24 h. Then, NH_4Cl was added (500 μL), the mixture extracted with EtOAc (2×500 μL), the organic layers separated by centrifugation (90 sec, 13000 rpm), combined and finally dried over Na_2SO_4. Conversion was determined by HPLC analysis. The *ees* of the resulting alcohols **2a**–**f** were determined by chiral HPLC analysis. The identity of alcohols **2a**–**f** was confirmed by comparing ^{1}H-NMR spectra with those reported in the literature.[5]

2.3. General procedure for the catalytic isomerisation of allylic alcohols 3a–d into ketones 1a–d.

The corresponding allylic alcohol (**3a–d**, 0.5 mmol) and 0.5 g of the eutectic mixture 1*ChCl*/2*Gly* (previously degassed with Ar) were introduced into a sealed tube under an Ar atmosphere. The acetate ruthenium(IV) complex [Ru(η^3:η^3-$C_{10}H_{16}$)Cl(κ^2-*O*,*O*-CH_3CO_2)] (**4**, 5 mol%) was then added at room temperature under Ar. The reaction mixture was stirred for 2 h at 50 °C giving rise to the corresponding ketones (**1a–d**) in quantitative conversions (99%, GC analysis).

2.4. General procedure for the one-pot sequential ruthenium-catalysed allylic alcohol isomerisation-asymmetric bioreduction using purified KREDs.

Allylic alcohol (**3a**–**d**, 200 mM), 0.5 mL of KPi buffer (previously degassed with Ar) 125 mM at pH 7.0 (1.25 mM $MgSO_4$), 0.5 mL of *DES* 1*ChCl*/2*Gly*, and *i*-PrOH (175 μL) were introduced into a sealed tube under an Ar atmosphere. Complex **4** (5 mol%) was then added at room temperature, and the resulting solution was heated at 50 ºC. Once the isomerisation process was completed (GC analysis), the selected KRED in Table 3 (100% w/w) and $NADP^+$ (1 mM) were consecutively added, and the mixture was stirred at 30 ºC for 24 h. After this time, an aliquot was treated as described in Section 2.2 for

[5] N. Ríos-Lombardía, C. Vidal, E. Liardo, F. Morís, J. García-Álvarez and J. González-Sabín, *Angew. Chem., Int. Ed*., 2016, **55**, 8691–8695.

determining both conversion and enantioselectivity. Then, NH_4Cl (1 mL) was added to the mixture, and the product was extracted with cyclopentyl methyl ether (1 × 1.0 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness in a vacuum concentrator to yield the crude products with overall conversions ranging from 94 to >99%. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate mixtures) yielded the pure alcohols with yields ranging from 85 to 90% and *ee* >99%.

2.5. General procedure for the one-pot sequential ruthenium-catalysed allylic alcohol isomerisation-asymmetric bioreduction using overexpressed LKADH.

An identical procedure to that described in section 2.4 was followed for the initial isomerisation of the allylic alcohols **3a**–**d**. Once the isomerisation was completed (GC analysis), *L. kefiri* (lyophilized; 1000 U) and $NADP^+$ (1 mM) were consecutively added, and the mixture was stirred at 30 ºC for 24 h. After this time, an aliquot was treated as described in Section 2.2 for determining both conversion and enantioselectivity. Then, NH_4Cl (1 mL) was added to the mixture, and the product was extracted with cyclopentyl methyl ether (1 × 1.0 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness in a vacuum concentrator to yield the crude products with overall conversions ranging from 65 to >99%. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate mixtures) yielded the pure alcohols with yields ranging from 60 to 92% and *ee* from 93 to >99%.

2.6. One-pot concurrent ruthenium-catalysed allylic alcohol isomerisation-asymmetric bioreduction of 3a using a purified KRED.

1-Phenylprop-2-en-1-ol (**3a**, 200 mM), 0.2 mL of KPi buffer (previously degassed with Ar) 125 mM at pH 7.0 (1.25 mM $MgSO_4$, 1 mM $NADP^+$), 0.8 mL *DES* 1*ChCl*/2*Gly*, and *i*-PrOH (175 μL) were introduced into a sealed tube under an Ar atmosphere. Complex **4** (10 mol%) and KRED-P2-C11 (27 mg, 100% w/w) were then added at room temperature, and the resulting mixture was heated at 40 ºC for 24 h. After this time, an aliquot was treated as described in Section 2.2 for determining both conversion and enantioselectivity. Then, brine (1.0 mL) was added to the mixture, and the product was extracted with cyclopentyl methyl ether (1 × 1.0 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness in a vacuum concentrator to yield, after filtration on silica gel, pure (*R*)-**2a** in 85% yield and >99% *ee*.

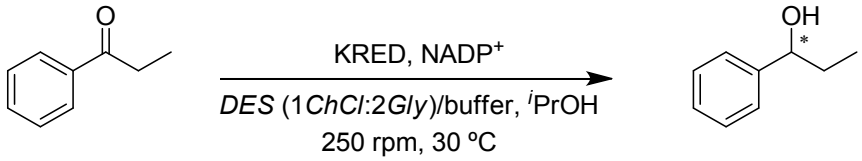
2.7. One-pot concurrent ruthenium-catalysed allylic alcohol isomerisation-asymmetric bioreduction of 3a using overexpressed LKADH.

1-Phenylprop-2-en-1-ol (**3a**, 200 mM), 0.2 mL of KPi buffer (previously degassed with Ar) 50 mM at pH 7.0 (1 mM $MgCl_2$), 0.8 mL *DES* 1*ChCl*/2*Gly*, and *i*-PrOH (175 μL) were introduced into a sealed tube under an Ar atmosphere. Complex **4** (10 mol%), *L. kefiri* (lyophilized; 1000 U) and $NADP^+$ (1

mM) were then added at room temperature, and the resulting mixture was heated at 40 ºC for 24 h. After this time, an aliquot was treated as described in Section 2.2 for determining both conversion and enantioselectivity. The reaction crude was not purified due to the low percentage of the desired **2a**.

3. Enzymatic screening

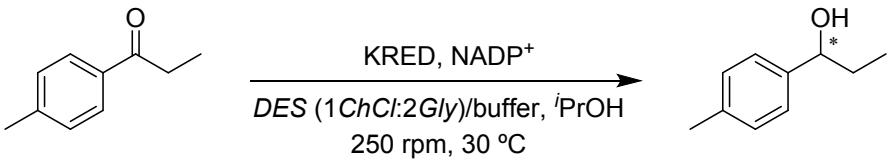
Table S1. KRED-catalysed reduction of **1a** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	>99 (*R*)	>99	>99 (*R*)	-	-
2	P1-C01	>99	25 (*S*)	94	40 (*S*)	-	-
3	P2-C11	>99	78 (*R*)	>99	86 (*R*)	>99	>99 (*R*)
4	P2-D12	>99	16 (*R*)	90	50 (*R*)	-	-
5	P2-H07	>99	>99 (*R*)	>99	>99 (*R*)	-	-
6	P3-H12	>99	92 (*S*)	90	94 (*S*)	-	-

[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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

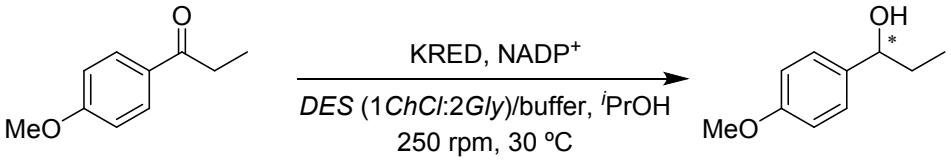
Table S2. KRED-catalysed reduction of **1b** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	98 (*R*)	95	>99 (*R*)	-	-
2	P1-C01	>99	-	82	20 (*S*)	-	-
3	P2-C11	>99	82 (*R*)	98	95 (*R*)	98	97 (*R*)
4	P2-D12	>99	76 (*R*)	80	86 (*R*)	-	-
5	P2-H07	>99	90 (*R*)	96	>99 (*R*)	-	-
6	P3-H12	>99	97 (*S*)	95	>99 (*S*)	-	-

[a] Reaction conditions: **1b** (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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

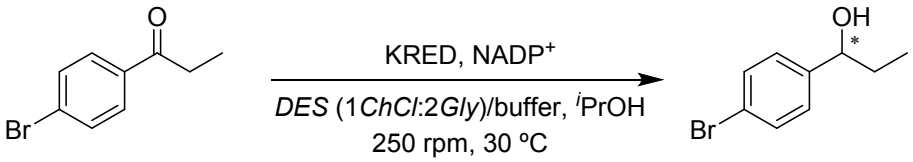
Table S3. KRED-catalysed reduction of **1c** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	>99 (*R*)	93	>99 (*R*)	-	-
2	P1-C01	>99	0	81	28 (*S*)	-	-
3	P2-C11	>99	92 (*R*)	98	96 (*R*)	98	98 (*R*)
4	P2-D12	>99	60 (*R*)	80	68 (*R*)		
5	P2-H07	>99	>99 (*R*)	95	>99 (*R*)	-	-
6	P3-H12	>99	98 (*S*)	87	99 (*S*)	-	-

[a] Reaction conditions: **1c** (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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

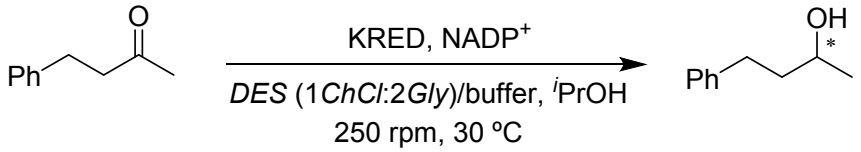
Table S4. KRED-catalysed reduction of **1d** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	99 (*R*)	>99	99 (*R*)	-	-
2	P1-C01	>99	-	>99	10 (*S*)	-	-
3	P2-C11	>99	93 (*R*)	>99	95 (*R*)	>99	98 (*R*)
4	P2-D12	>99	73 (*R*)	97	80 (*R*)	-	-
5	P2-H07	>99	99 (*R*)	>99	99 (*R*)	-	-
6	P3-H12	>99	99 (*S*)	>99	>99 (*S*)	-	-

[a] Reaction conditions: **1d** (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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

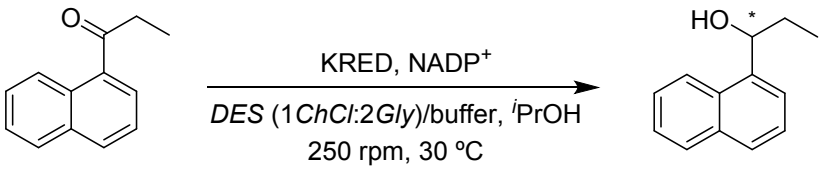
Table S5. KRED-catalysed reduction of **1e** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	84 (*R*)	>99	>99 (*R*)	-	-
2	P1-B10	>99	91 (*R*)	>99	>99 (*R*)	-	-
3	P1-C01	>99	10 (*R*)	>99	3 (*R*)	-	-
4	P2-C11	>99	54 (*R*)	>99	93 (*R*)	93	>99 (*R*)
5	P2-D12	>99	6 (*R*)	>99	3 (*R*)	-	-
7	P2-G03	>99	86 (*R*)	>99	90 (*R*)	-	-
8	P2-H07	>99	32 (*S*)	>99	>98 (*S*)	-	-
9	P3-H12	>99	28 (*R*)	>99	65 (*R*)	-	-

[a] Reaction conditions: **1e** (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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

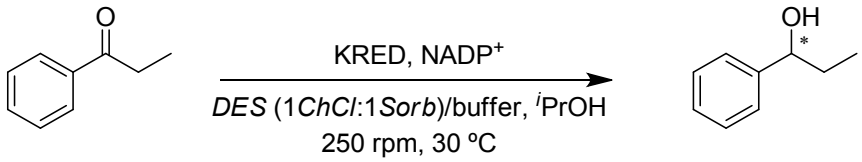
Table S6. KRED-catalysed reduction of **1f** in *DES* 1*ChCl*/2*Gly*-buffer mixtures.[a]



		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	14	20 (*R*)	5	-	-	-
2	P1-C01	>99	90 (*S*)	55	88 (*S*)	-	-
3	P2-C11	39	10 (*R*)	6	-	-	-
4	P2-D12	>99	96 (*S*)	27	97 (*S*)	-	-
5	P2-H07	25	34 (*R*)	6	-	-	-
6	P3-H12	>99	89 (*S*)	>99	90 (*S*)	-	-

[a] Reaction conditions: **1f** (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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

Table S7. KRED-catalysed reduction of **1a** in *DES* 1*ChCl*/1*Sorb*-buffer mixtures.[a]

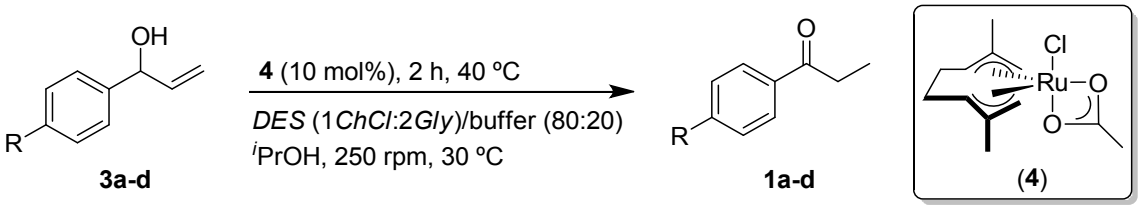


		Neat aqueous buffer		50% w/w *DES*		80% w/w *DES*	
Entry	KRED	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	P1-A04	>99	>99 (*R*)	>99	>99 (*R*)	93	>99 (*R*)
2	P1-C01	>99	25 (*S*)	>99	56 (*S*)	95	66 (*S*)
3	P2-C11	>99	78 (*R*)	>99	96 (*R*)	>99	99 (*R*)
4	P2-D12	>99	16 (*R*)	>99	39 (*R*)	88	40 (*R*)
5	P2-H07	>99	>99 (*R*)	>99	>99 (*R*)	82	>99 (*R*)
6	P3-H12	>99	92 (*S*)	94	86 (*S*)	81	86 (*S*)

[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 at 30 ºC. [b] Conversion measured by HPLC. [c] Measured by chiral phase HPLC.

4. Parametrisation of the allylic alcohol isomerisation

Table S8. Isomerisation of allylic alcohols **3a**–**d** catalysed by complex **4** in a *DES*-buffer medium for concurrent chemoenzymatic processes.[a]



Entry	Alcohol	Mol% [Ru]	t (h)	*c* (%)[b]
1	**3a**	10	2.0	>99
2	**3b**	10	2.0	>99
3	**3c**	10	2.0	>99
4	**3d**	10	2.0	>99

[a] Reaction conditions: **3a**–**d** (0.074 mmol) under an Ar atmosphere at 40 ºC, 10 mol% Ru(IV) catalyst **4** (2.4 mg), in 5 mL of *DES*:KPi buffer 125 mM (pH 7.0) mixture (80:20) which contains 1.25 mM $MgSO_4$ and 1 mM $NADP^+$. Buffer solution was degassed with Ar. [b] Calculated by GC.

5. Analytical data

5.1. Isomerisation of allylic alcohols

5.1.1. GC Analyses for conversion determination

The following column was used: Beta Dex (120 m 0.25 mm 0.25μm).

Table S9. GC analyses data.

Substrate	T programme[a]	Retention time (min)	
		Alcohol	Ketone
1-Phenylprop-2-en-1-ol (**3a**)	170/3/10/220	1.8	1.6
1-(*p*-Tolyl)prop-2-en-1-ol (**3b**)	170/3/10/220	2.2	2.1
1-(*p*-Methoxyphenyl)prop-2-en-1-ol (**3c**)	170/3/10/220	3.9	3.8
1-(*p*-Bromophenyl)prop-2-en-1-ol (**3d**)	170/3/10/220	4.6	3.6

[a] GC programme: initial temp. (ºC) / time (min) / ramp (ºC/min) / final temp. (ºC).

5.2. Enzymatic reduction of ketones

5.2.1. HPLC Analyses for conversion determination

HPLC analyses were carried out on an Agilent chromatographic system, using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 1.8 μm, 4.6 × 50 mm, Agilent) and MeCN and 0.1% trifluoroacetic acid (TFA) in water as the solvents. Samples were eluted with three linear gradients from 10% to 60% MeCN for 5.70 min, followed by another gradient from 60% to 100% MeCN for 0.5 min, and a third one from 100% to 10% MeCN for 1.90 min, at flow rate of 2 ml/min. Detection and spectral characterisation of peaks [UV absorption maxima (from HPLC-diode array) at 210 and 324 nm] were performed with a diode array detector and ChemStation Rev.B.03.01 software (Agilent).

Table S10. HPLC data.

Compound	Retention time (min)	
	ketone	alcohol
Propiophenone (**1a**)	4.0	3.1
1-(*p*-Tolyl)propan-1-one (**1b**)	4.9	4.0
1-(*p*-Methoxyphenyl)propan-1-one (**1c**)	4.2	3.2
1-(*p*-Bromophenyl)propan-1-one (**1d**)	5.5	4.5
4-Phenylbutan-2-one (**1e**)	4.1	3.7
1-(Naphthalen-1-yl)prop-2-en-1-one (**1f**)	5.6	4.7

5.2.2. HPLC Analyses for enantiomeric excess determination

Table S11. HPLC data.[a]

Compound	Column	Eluent (Hex/ *i*-PrOH)	Flow (mL/min)	T (ºC)	Retention time (min)
2a	OJ-H	90:10	0.8	40	7.4 (*S*), 7.8 (*R*)
2b	AD-H	99:1	1.0	40	12.4 (*R*), 13.8 (*S*)
2c	AD-H	97:3	1.0	40	15.4 (*R*), 16.5 (*S*)
2d	OJ-H	97:3	0.8	40	13.4 (*S*), 14.7 (*R*)
2e	AD-H	97:3	0.8	30	11.5 (*S*), 12.1 (*R*)
2f	AD-H	97:3	1.0	40	14.5 (*S*), 16.1 (*R*)

[a] Detection wavelength: 210 nm.