BVMO-catalysed dynamic kinetic resolution of racemic benzyl ketones in the presence of anion exchange resins†‡

Cristina Rodríguez,[a] Gonzalo de Gonzalo,[a] Ana Rioz-Martínez,[a] Daniel E. Torres Pazmiño,[b] Marco W. Fraaije[b] and Vicente Gotor*[a]

Received 30th October 2009, Accepted 30th November 2009
First published as an Advance Article on the web 7th January 2010
DOI: 10.1039/b922693a

4-Hydroxyacetophenone monooxygenase from *Pseudomonas fluorescens* ACB was employed in the presence of a weak anion exchange resin to perform dynamic kinetic resolutions of racemic benzyl ketones with high conversions and good optical purities. Different parameters that affect to the efficiency of the enzymatic Baeyer–Villiger oxidation and racemisation were analyzed in order to optimize the activity and selectivity of the biocatalytic system.

Introduction

Dynamic kinetic resolutions (DKRs) have emerged in the last few years as very useful tools in organic synthesis.[1] By combining a selective kinetic resolution (KR) with an *in situ* efficient racemisation of the starting material, DKRs can theoretically provide single enantiomeric products with 100% yields. Racemisation can be performed by a chemocatalyst or a biocatalyst, or it can occur spontaneously. The use of biocatalysts represents an attractive alternative to conventional chemical methods, due to the mild and environmentally friendly conditions and the high chemo-, regio- and/or enantioselectivities associated with DKR processes catalysed by enzymes.[2] A common approach for enzymatic DKRs is the combination of selective acylation of secondary alcohols or amines catalysed by lipases coupled with transition metal-catalysed racemisation,[3] but lately, several DKRs have been developed employing other biocatalysts.[4]

Baeyer–Villiger monooxygenases (BVMOs) are nicotinamide-dependent flavoproteins able to perform a wide set of oxidative reactions.[5] In the last few years, a great number of BVMOs are becoming available due to progress in genome sequencing and mining.[6] This resulted in a boost in research focusing on their application for asymmetric Baeyer–Villiger oxidations and for the oxygenation of heteroatom-containing compounds (S, Se, P and N). One of the recently cloned and overexpressed BVMOs is 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB.[7] HAPMO represents the first identified BVMO primarily active on aromatic ketones.[8] Studies on substrate specificity have demonstrated that HAPMO accepts preferably acetophenones and benzaldehydes bearing an electron-donating substituent at the *para*-position.[9] The enzyme is also able to catalyze the Baeyer–Villiger oxidation of a wide variety of other ketones and aldehydes, including heteroaromatic and aliphatic compounds. Some years ago, Berezina *et al.* described the first dynamic kinetic resolution applied to a Baeyer–Villiger oxidation using a recombinant *E. coli*/cyclohexanone monooxygenase strain. This was made possible by performing the oxidations at a relatively high pH (at least 8.0), as under these conditions the substrates racemise *via* a keto–enol tautomerisation due to the acidic character of the α-proton.[10] This process was improved by combining the whole cell Baeyer–Villiger oxidation with an ion-exchange resin-catalysed *in situ* racemisation of the substrate.[11]

In the present paper, we describe for the first time the use of an isolated BVMO for catalysing the dynamic kinetic resolution of a range of benzyl ketones yielding the corresponding benzyl esters with high conversions and optical purities.

Results and discussion

All the Baeyer–Villiger reactions were performed employing isolated HAPMO. The oxidations were coupled to a second enzymatic reaction catalysed by glucose-6-phosphate dehydrogenase (G6PDH) in order to regenerate NADPH.[12]

We have previously reported that the HAPMO-catalysed oxidation of (±)-3-phenylbutan-2-one **1a** occurred with high selectivity, yielding (*R*)-**1a** and (*S*)-1-phenylethyl acetate **1b**.[13] This kinetic resolution was found to be pH-dependent, as at relatively high pH, higher enzymatic activity and a loss in the biocatalyst enantioselectivity (*E* value) was observed.[14] Thus, the enzymatic resolution of (±)-**1a** at pH 8.0 (2 h) occurred with 25% conversion and an *E* value of 136, while at pH 10, 48% conversion and a good enantioselectivity ($E = 60$) was obtained.

Due to the acidic character of the α-hydrogen of ketone **1a**, a pH-dependent racemisation can be expected due to the keto–enol tautomerisation. However, when (*R*)-**1a** of 43% ee was dissolved in Tris/HCl pH 8.0 and 25 °C, hardly any racemisation was observed within 120 h ($k_{rac} = 4.9 \times 10^{-4}$ h^{-1}). When the pH was increased to 10.0, only a slight decrease in the ee value of (*R*)-**1a** was observed after 120 h (ee = 34%), with a higher racemisation constant being achieved than at pH 8.0 ($k_{rac} = 0.002$ h^{-1}). Thus, when racemic 3-phenylbutan-2-one was oxidized in the presence of HAPMO at

[a]*Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, C/Julián Clavería 8, 33006, Oviedo, Spain. E-mail: vgs@fq.uniovi.es; Fax: (+34) 985 103448; Tel: (+34) 985 103448*
[b]*Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747, AG, Groningen, The Netherlands*

† This paper is part of an *Organic & Biomolecular Chemistry* web theme issue on biocatalysis

‡ Electronic supplementary information (ESI) available: Experimental data, characterization data of compounds and ^{1}H, ^{13}C and DEPT spectra. See DOI: 10.1039/b922693a

Table 1 Dynamic kinetic resolution of (±)-**1a** in the presence of different anion exchange resins when working at pH 10.0 and 25 °C[a]

Entry	Resin	ee (*R*)-**1a** (%)[b]	ee (*S*)-**1b** (%)[b]	Conv. (%)[b]
1	None	90	21	77
2	Amberlyst A-26	17	75	17
3	Amberlite IRA-440C	≤3	73	40
4	Amberlite IRA-900	≤3	67	22
5	Dowex 1	13	54	33
6	Amberlite IRA-67	31	72	56
7	Lewatit MP62	35	77	50
8	Dowex MWA-1	16	84	86

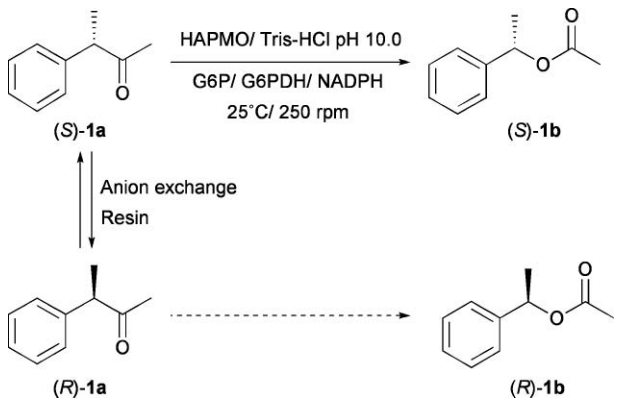
[a] Reaction time: 116 h. For reaction details, see the Experimental part. [b] Measured by GC.

pH 10.0 and 25 °C (Table 1, entry 1), (*R*)-**1a** presented a 90% ee after 120 h in a process with high conversion (c = 77%), indicating that the racemisation of the substrate was inefficient. (*S*)-1-phenylethyl acetate was obtained with a low optical yield (ee = 21%).

For a successful DKR the rate of the racemisation needs to be greater than the rate of the HAPMO-catalysed oxidation, something that does not occur when working under the conditions described above. For this reason, the enzymatic resolution of (±)-**1a** was tested in the presence of different anion exchange resins (10.0 mg resin mL^{-1}), in order to increase the racemisation rate of ketone **1a** (see the ESI‡). First, we analysed the change in the optical purity of (*R*)-**1a** at pH 10.0 in the presence of different anion exchange resins. The use of a strong anionic resin (Amberlite IRA-440C) led to a fast racemisation of the starting ketone (k_{rac} = 0.041 h^{-1}). After 24 h, (*R*)-**1a** was obtained with ee = 5%, while the racemic ketone was recovered after 72 h. When using weak resins, two different behaviours were achieved. While the addition of Lewatit MP62 resulted in a slow drop in the enantiomeric excess of (*R*)-**1a**, with a racemisation constant of 0.016 h^{-1} being measured, this process occurred much faster in the presence of Dowex MWA-1 (ee = 10% after 24 h, k_{rac} = 0.03 h^{-1}). Racemisation of the final product (*S*)-**1b** was also studied, but no change in optical purity was observed when this ester was dissolved in Tris/HCl solutions at pH 10.0 containing different anion exchange resins. Thus, it can be established that the optical purities of (*S*)-**1b** are only due to the HAPMO selectivity in the conditions employed.

After establishing the effect of the exchange resins on the racemisation of ketone **1a**, its HAPMO-catalysed oxidation was performed, as indicated in Table 1. The use of strong anion resins led to a significant deactivation in enzymatic activity (entries 2–5). For this reason, all conversions led to values lower than 40% after 116 h, and (*S*)-**1b** was formed with moderate optical purities.

Subsequently, the reactions were carried out in presence of weak anion resins. Under these conditions, the racemisation process was slower than when employing the strong resins, as is shown by the enantiomeric excesses obtained for the remaining ketone. This effect is clearly seen when using Amberlite IRA-67 or Lewatit MP62 (entries 6 and 7, respectively). (*R*)-**1a** was recovered for both resins with ee values near 30%, while (*S*)-**1b** was obtained with a moderate optical yield and conversions of 50%. The best result was obtained when using Dowex MWA-1, as shown in entry 8. In presence of this weak anion exchange resin, an 86% conversion of (*S*)-**1b** with 84% optical purity can be obtained after 116 h. This demonstrates that under these reaction conditions, a DKR can be performed in order to achieve the chiral ester (Scheme 1). Racemisation in the presence of this resin was better than for other weak resins, as the remaining (*R*)-**1a** displayed an enantiomeric excess of only 16%.



Scheme 1 HAPMO-catalyzed DKR of (±)-**1a** employing anion exchange resins.

Once the best exchange resin had been selected for the preparation of (*S*)-**1b** with the highest yield and optical purity, some of the parameters that can affect dynamic kinetic resolution were analyzed (Table 2). Despite the negative effect of strong anionic resins on the enzymatic activity, we analyzed the DKR of (±)-**1a** using lower amounts of Amberlite IRA-440C and IRA-900A. As shown in entries 1 and 2, complete racemisation of (±)-**1a** was achieved with only a slight increase in (*S*)-**1b** formation. The enzymatic biooxidation of ketone (±)-**1a** was then performed by using different amounts of Dowex MWA-1 (entries 3–5) with the aim of improving the racemisation rate. However, the increase of the anion exchange resin in the medium had a negative effect on the enzymatic activity, and (*S*)-**1b** was obtained with conversions lower than 30% after 120 h (entries 4 and 5). By altering the reaction temperature, differences in the racemisation rate could be observed. As expected, when the HAPMO-catalysed oxidation was performed at 10 °C, a low racemisation was observed (entry 6), yielding 35% of optically active (*S*)-1-phenylethyl acetate. On the other hand, the use of higher temperatures could

Table 2 Enzymatic preparation of (*S*)-**1b** through DKR processes catalyzed by HAPMO and Dowex MWA-1[a]

Entry	[**1a**]/ mM	Resin/ mg	pH	T/°C	t/h	ee ket (%)[b]	ee est (%)[b]	Conv. (%)[b]
1[c]	10	4	10	25	116	≤3	75	47
2[d]	10	4	10	25	116	≤3	69	33
3	10	10	10	25	116	16	84	86
4	10	20	10	25	120	5	72	28
5	10	30	10	25	120	4	73	25
6	10	10	10	10	144	55	80	35
7	10	10	10	40	144	≤3	53	24
8	10	10	8	25	144	12	86	20
9	10	10	9	25	144	10	87	61
10	5	10	10	25	72	46	61	91
11	20	10	10	25	116	5	84	59
12	40	10	10	25	116	≤3	78	44

[a] For reaction details, see the Experimental part. [b] Measured by GC. [c] Reaction performed with Amberlite IRA-440C. [d] Amberlite IRA-900 was employed

improve the racemisation, but the DKR process was negatively affected due to the inactivation of HAPMO (entry 7, 53% ee).[13] Racemic **1a** and (*S*)-**1b** with low optical purity were recovered in a process with 24% conversion after 144 h at 40 °C. In order to enhance both the activity and selectivity of the enzymatic system, lower pHs were used (entries 8 and 9). At pH 8.0 a considerable decrease in enzymatic activity was observed, reaching only 20% conversion after 144 h. When the oxidation was performed at pH 9.0, (*S*)-**1b** can be obtained with 61% conversion (87% ee). Finally, ketone concentration was also optimised. When using 5 mM of **1a**, it was possible to achieve a 91% conversion after 72 h. However, under these conditions, the racemisation was not very efficient, as (*R*)-**1a** was only obtained with 46% enantiomeric excess and (*S*)-**1b** with a moderate optical purity (61%, entry 10). Although conversions were much lower when increasing the substrate concentration, the space time yield (expressed as mmol of (±)-**1a** per L of solution h^{-1}) improved from 0.07 mmol L^{-1} h^{-1} at 5 mM until reaching the highest value (0.15 mmol L^{-1} h^{-1}) when using a ketone concentration of 40 mM. This result indicates a more efficient biooxidation while increasing the substrate amount.

To obtain a better view of the DKR progress of the HAPMO-catalysed oxidation of (±)-**1a** in buffer at pH 10.0 containing Dowex MWA-1 at 25 °C, the reaction was monitored over time. As shown in Fig. 1, the biooxidation initially presented a classical kinetic resolution behaviour, in which the optical purity of ketone (*R*)-**1a** increased while there was only a slight decrease in the enantiomeric excess of (*S*)-**1b**. After 24 h, 64% of (*S*)-**1a** with 90% enantiomeric excess was obtained, while the optical purity of (*R*)-**1a** reached a maximum value (ee = 37%). From this time, racemisation of the starting ketone was predominant, producing an important loss in the optical purity of **1a** while the ee of (*S*)-**1b** decreased to a small extent. Thus, after 72 h, oxidation had reached a 79% conversion, leading to chiral ester with an 86% enantiomeric excess. Prolonged reaction times led to a slight increase in the conversion (86%), while no significant changes in the enantiomeric excesses of substrate and product were measured (t = 116 h).

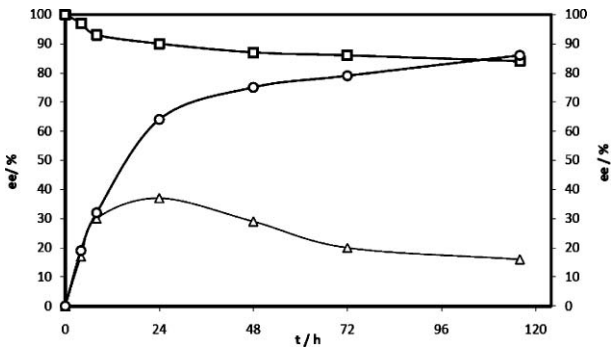


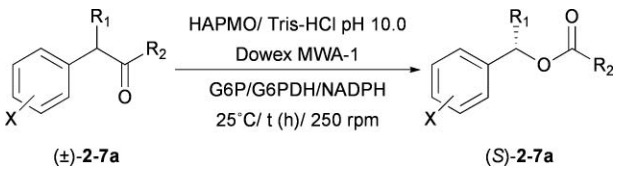
Fig. 1 Time study of the DKR of (±)-**1a** catalyzed by HAPMO in presence of Dowex MWA-1. Conversion (○) and enantiomeric excesses of (*R*)-**1a** (△) and (*S*)-**1b** (□) are represented.

Once the best reaction conditions were established, the applicability of the system using other benzyl ketones was studied (Scheme 2 and Table 3). Racemic 3-(3-methylphenyl)butan-2-one was selectively oxidized to the corresponding ester (*S*)-**2b** with 81% enantiomeric excess and 69% conversion after 120 h. DKRs of benzyl ketones presenting electron-withdrawing groups in the aromatic ring were also tested. Compound (±)-**3a** was previously resolved as a substrate for HAPMO at pH 8.0 with a high E value (E = 112) and 39% conversion after 2 h.[9a] When working at pH 10.0 with Dowex MWA-1, (*S*)-**3b** was obtained with 88% conversion and good optical purity after 96 h. The 4-chloro derivative was oxidized with moderate optical purity (ee = 58%) to (*S*)-1-(4-chlorophenyl)ethyl acetate (*S*)-**4b** in a reaction slower than when using the 3-trifluoromethyl derivative (c = 69% after 120 h). This compound was previously resolved by HAPMO-catalyzed oxidation at pH 8.0 with moderate enantioselectivity (E = 50).

Table 3 HAPMO-catalyzed dynamic kinetic resolution of different benzyl ketones employing Dowex MWA-1[a]

Ketone	R_1	R_2	X	t/h	ee ket (%)[b]	ee est (%)[b]	Conv. (%)[b]
(±)-**2a**	Me	Me	*m*-Me	120	21	81	69
(±)-**3a**	Me	Me	*m*-CF_3	96	17	79	88
(±)-**4a**	Me	Me	*p*-Cl	120	14	58	69
(±)-**5a**	Me	Et	H	96	17	83	66
(±)-**6a**	Et	Me	H	96	13	80	72
(±)-**7a**	Pr	Et	H	144	17	65	46

[a] For reaction details, see Experimental Part. [b] Measured by GC.



Scheme 2 DKR of different benzyl ketones by HAPMO-catalyzed Baeyer–Villiger oxidation to the corresponding optically active benzyl esters.

The same reaction conditions were applied for the DKR of the racemic pentanones (±)-2-phenylpentan-3-one (±)-**5a** and (±)-3-phenylpentan-2-one (±)-**6a**. For both substrates, conversions close to 70% were measured after 96 h, while (*S*)-**5–6b** were obtained with 83 and 80% enantiomeric excess, respectively. Finally, oxidation of a long alkyl chain ketone such as (±)-**7a** led to the formation of (*S*)-**7b** with a conversion lower than 50% after 144 h. This compound was achieved with 65% enantiomeric excess.

Conclusions

In the present paper, the dynamic kinetic resolutions of different benzyl ketones could be performed by combining an isolated BVMO-catalysed Baeyer–Villiger oxidation and a racemisation catalysed by different anion exchange resins. The results obtained revealed that resins with strong character resulted in a relatively fast racemisation but also lead to significant enzyme inactivation. The best results were achieved with Dowex MWA-1, a weak anion resin, for which it was possible to obtain (*S*)-1-phenylethyl acetate with high conversions and optical purities. The optimisation of the DKR was performed by analyzing the effect of the resin concentration, pH and temperature, resulting in the observation that the use of high amounts of resin inactivates the enzyme, the optimal temperature was found to be 25 °C and a pH of 10.0 produces the highest conversion and enantiomeric excess of

(*S*)-**1b**. This resin was finally tested in the effective DKR of a number of benzyl ketones using isolated HAPMO, leading to moderate to good results depending on the substrate structure.

Experimental

Recombinant HAPMO from *Pseudomonas fluorescens* ACB was overexpressed and purified as previously described.[7] Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was obtained from Fluka-Biochemika. Anion exchange resins were purchased from Sigma-Aldrich-Fluka. Before use, they were washed with a NaOH solution (1.0 N), then with water until neutrality and filtrated under reduced pressure. All reagents and solvents were of the highest quality grade available and were obtained from Sigma-Aldrich-Fluka and Acros Organics. The corresponding racemic ketones (±)-**1–7a** were synthesised according to the literature, employing the corresponding alkyl iodide and NaOH in a biphasic system water–CH_2Cl_2.[15] All ketones exhibit physical and spectral properties in accordance with those reported.[9a,13,16] Esters (±)-**1–7b** were prepared by the chemical acylation of the racemic alcohols by using the corresponding anhydride in CH_2Cl_2 and pyridine.

Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F_{254} plates and visualized by UV irradiation. Flash chromatography was carried out with silica gel 60 (230-240 mesh, Merck). ^{1}H-NMR, ^{13}C-NMR and DEPT spectra were recorded with tetramethylsilane (Me_4Si) as the internal standard with a Bruker AC-300 DPX (^{1}H: 300.13 MHz; ^{13}C: 75.5 MHz) spectrometer. The chemical shift values (δ) are given in ppm. APCI$^+$ and ESI$^+$ using a Hewlett Packard 1100 chromatograph mass detector or EI$^+$ with a Hewlett Packard 5973 mass spectrometer were used to record mass spectra (MS). GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1 bar N_2), a Varian CP-Chiralsil-DEX CB (25 m × 0.32 mm × 0.25 μm, 1 bar N_2) or a Mercherey-Nagel Hydrodex-β-TBOAc (30 m × 0.25 mm × 0.25 μm, 1 bar N_2) for chiral determinations or a HP-1 (crosslinked methyl siloxane, 30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) from Hewlett-Packard for measuring the conversions values. For all the analyses, the injector temperature is 225 °C and the FID temperature is 250 °C.

Racemisation studies of 3-phenylbutan-2-one, 1a

Racemisation experiments were performed by dissolving optically active 3-phenylbutan-2-one (*R*)-**1a** (10 mM) isolated from the preparative kinetic resolution of (±)-**1a** performed at pH 8.0 and 20 °C with HAPMO, in Tris/HCl buffer containing the anion exchange resins at the selected conditions (pH and temperature). The solution was shaken for different reaction times and aliquots were taken, extracted with ethyl acetate, dried onto Na_2SO_4 and analyzed by GC in order to determine the enantiomeric excesses. The same experiments were performed for 1-phenylethyl acetate (*S*)-**1b**.

Typical procedure for the BVMO-catalyzed dynamic kinetic resolution of racemic benzyl ketones (±)-1-7a

Unless otherwise stated, the starting racemic ketones (±)-**1–7a** (10 mM) were dissolved in a 50 mM Tris/HCl buffer at pH 10 (1.0 mL) containing glucose-6-phosphate (20 mM), glucose-6-phosphate dehydrogenase (5.0 units), NADPH (0.2 mM), HAPMO (2.0 μM) and the corresponding anion exchange resin (10.0 mg). Reactions were shaken at 250 rpm and 25 °C for the times established. Once finished, the mixtures were extracted with AcOEt (2 × 500 μL). The organic phases were dried onto Na_2SO_4 and analyzed directly by GC in order to determine the conversion and the enantiomeric excesses of the esters (*S*)-**1–7b** and the remaining ketones (*R*)-**1–7a**.

Scale-up of the HAPMO-catalysed Baeyer–Villiger oxidation of ketone (±)-1–7a in presence of Dowex MWA-1

Racemic ketones (±)-**1–7a** (50 mg) were dissolved in a 50 mM Tris/HCl buffer at pH 10 (15 mL) containing glucose-6-phosphate (20 mM), glucose-6-phosphate dehydrogenase (50.0 units), NADPH (0.2 mM) HAPMO (2.0 μM) and Dowex MWA-1 (100 mg). Reactions were shaken at 250 rpm and 25 °C for the times established (116 h for (±)-**1a**; 96 h for compounds (±)-**3a** and (±)-**5–6a**; 120 h for (±)-**2a** and (±)-**4a** and 144 h for (±)-**7a**). Once finished, the crude reactions were extracted with EtOAc (4 × 5 mL). The organic phases were dried onto Na_2SO_4 filtered and evaporated under reduced pressure. The crude residues were purified by flash chromatography on silica gel using hexane–diethyl ether 8 : 2 (compounds **1–2a**, **4a** and **5–6a**), hexane–diethyl ether 7 : 3 (ketone **3a**) or hexane–ethyl acetate 8 : 2 (**7a**) to afford the corresponding (*S*)-esters. For all these reactions, some amount (5–15%) of the alcohols formed by the non enzymatic hydrolysis of esters **1–7a** was observed.

Acknowledgements

C.R. thanks the Principado de Asturias for her predoctoral fellowship. A.R.-M. (FPU program) acknowledges the Spanish Ministerio de Ciencia e Innovación (MICINN) for her predoctoral fellowship, which is financed by the European Social Fund. G.d.G. (Juan de la Cierva Program) thanks MICINN for personal funding. Financial support from MICINN (Project CTQ2007-61126) is gratefully acknowledged. M.W.F. and D.E.T.P. receive support from the EU-FP7 "Oxygreen" project.

Notes and references

1 (*a*) Z. Ding, J. Yang, T. Wang, Z. Shen and Y. Zhang, *Chem. Commun.*, 2009, 571; (*b*) M.-J. Kim, J. Park, Y. K. Choi, in *Multi-Step Enzyme Catalysis*, ed. E. García-Junceda, Wiley-VCH, Weinheim, 2008, pp. 1; (*c*) H. Pellissier, *Tetrahedron*, 2008, **64**, 1563; (*d*) B. Martín-Matute, J.-E. Bäckvall, in *Asymmetric Organic Synthesis with Enzymes*, ed. V. Gotor, I. Alfonso and E. García-Urdiales, Wiley-VCH, Weinheim, 2008, pp. 89; (*e*) Q. Chen and C. Yuan, *Chem. Commun.*, 2008, 5333; (*f*) O. Pàmies and J.-E. Bäckvall, *Chem. Rev.*, 2003, **103**, 3247.

2 (*a*) J. M. Woodley, *Trends Biotechnol.*, 2008, **26**, 321; (*b*) *Asymmetric Organic Synthesis with Enzymes*, ed. V. Gotor, I. Alfonso and E. García-Urdiales, Wiley-VCH, Weinheim, 2008; (*c*) *Biocatalysis in the Pharmaceutical and Biotechnology Industry*, ed. R. N. Patel, CRC Press, Boca Raton, 2007.

3 Some examples of lipase-catalysed DKR: (*a*) A. Traff, K. Bogár, M. Warner and J. E. Bäckvall, *Org. Lett.*, 2008, **10**, 4807; (*b*) S. Wuyts, J. Wahlen, P. A. Jacobs and D. E. De Vos, *Green Chem.*, 2007, **9**, 1104; (*c*) K. Bogár and J.-E. Bäckvall, *Tetrahedron Lett.*, 2007, **48**, 5471; (*d*) A. Berkessel, M. L. Sebastian-Ibarz and T. N. Müller, *Angew. Chem., Int. Ed.*, 2006, **45**, 6567; (*e*) P. Ödman, L. A. Wessjohann and U. T. Bornscheuer, *J. Org. Chem.*, 2005, **70**, 9551; (*f*) M.-J. Kim, H. M. Kim, D. Kim, Y. Ahn and J. Park, *Green Chem.*, 2004, **6**, 471; (*g*) B. Martín-Matute, M. Edin, K. Bogár and J.-E. Bäckvall, *Angew. Chem., Int. Ed.*, 2004, **43**, 6535.

4 For some recent examples: (*a*) S. Lüdeke, M. Richter and M. Müller, *Adv. Synth. Catal.*, 2009, **351**, 253; (*b*) R. M. Haak, F. Berthiol, T. Jerphagnon, A. J. A. Gayet, C. Tarabiono, C. P. Postema, V. Ritleng, M. Pfeffer, D. B. Janssen, A. J. Minnaard, B. L. Feringa and J. G. de Vries, *J. Am. Chem. Soc.*, 2008, **130**, 13508; (*c*) D. Giacomini, P. Galletti, A. Quintavalla, G. Gucciardo and F. Paradisi, *Chem. Commun.*, 2007, 4038; (*d*) D. Kalaitzakis, J. D. Rozzell, S. Kambourakis and I. Smonou, *Org. Lett.*, 2005, **7**, 4799; (*e*) C. Kobler and F. Effenberger, *Tetrahedron: Asymmetry*, 2004, **15**, 3731.

5 (*a*) M. M. Kayser, *Tetrahedron*, 2009, **65**, 947; (*b*) D. E. Torres Pazmiño, M. W. Fraaije, in *Future Directions in Biocatalysis*, ed. T. Matsuda, Elsevier, Dordrecht, 2007, pp. 107; (*c*) M. W. Fraaije, D. B. Janssen, in *Modern Biooxidation*, ed. R. D. Schmid and V. B. Urlacher, Wiley-VCH, Weinheim, 2007, pp. 77; (*d*) M. D. Mihovilovic, *Curr. Org. Chem.*, 2006, **10**, 1265; (*e*) N. M. Kamerbeek, D. B. Janssen, J. H. Van Berkel and M. W. Fraaije, *Adv. Synth. Catal.*, 2003, **345**, 667.

6 (*a*) C. Szolkowy, L. D. Elti, N. C. Bruce and G. Grogan, *ChemBioChem*, 2009, **10**, 1208; (*b*) Heidekamp, R. Fortin and D. B. Janssen, *J. Biol. Chem.*, 2004, **5**, 279; (*c*) M. W. Fraaije, J. Wu, D. P. H. M. Heuts, E. W. van Hellemond, J. H. Lutje Spelberg and D. B. Janssen, *Appl. Microbiol. Biotechnol.*, 2005, **66**, 393.

7 N. M. Kamerbeek, M. J. H. Moonen, J. G. M. van der Ven, W. J. H. van Berkel, M. W. Fraaije and D. B. Janssen, D., *Eur. J. Biochem.*, 2001, **268**, 2547.

8 N. M. Kamerbeek, A. J. J. Olsthoorn, M. W. Fraaije and D. B. Janssen, *Appl. Environ. Microbiol.*, 2003, **69**, 419.

9 (*a*) C. Rodríguez, G. de Gonzalo, D. E. Torres Pazmiño, M. W. Fraaije and V. Gotor, *Tetrahedron: Asymmetry*, 2009, **20**, 1168; (*b*) G. de Gonzalo, D. E. Torres Pazmiño, G. Ottolina, M. W. Fraaije and G. Carrea, *Tetrahedron: Asymmetry*, 2006, **17**, 130; (*c*) M. J. H. Moonen, A. H. Westphal, I. M. C. M. Rietjens and W. J. H. van Berkel, *Adv. Synth. Catal.*, 2005, **347**, 1027; (*d*) G. de Gonzalo, G. Ottolina, F. Zambianchi, M. W. Fraaije and G. Carrea, *J. Mol. Catal. B: Enzym.*, 2006, **39**, 91; (*e*) A. Rioz-Martínez, G. de Gonzalo, D. E. Torres Pazmiño, M. W. Fraaije and V. Gotor, *Eur. J. Org. Chem.*, 2009, 2526.

10 N. Berezina, V. Alphand and R. Furstoss, *Tetrahedron: Asymmetry*, 2002, **13**, 1953.

11 M.-C. Gutiérrez, R. Furstoss and V. Alphand, *Adv. Synth. Catal.*, 2005, **347**, 1051.

12 C.-H. Wong and G. M. Whitesides, *J. Am. Chem. Soc.*, 1981, **103**, 4890.

13 C. Rodríguez, G. de Gonzalo, M. W. Fraaije and V. Gotor, *Tetrahedron: Asymmetry*, 2007, **18**, 1338.

14 *E* (enantiomeric ratio) is an adequate parameter to quantify the selectivity of the biocatalyzed kinetic resolutions. See: C.-S. Chen, Y. Fujimoto, G. Girdauskas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7295.

15 A. J. Fry and J. P. Bujanauskas, *J. Org. Chem.*, 1978, **43**, 3157.

16 (*a*) C. Liu, C. He, W. Shi, M. Chen and A. Lei, *Org. Lett.*, 2007, **9**, 5601; (*b*) N. S. Hatzakis and I. Smonou, *Bioorg. Chem.*, 2005, **33**, 325; (*c*) L. Panella, B. L. Feringa, J. G. de Vries and A. J. Minnaard, *Org. Lett.*, 2005, **7**, 4177; (*d*) C. Rodríguez, G. de Gonzalo, D. E. Torres Pazmiño, M. W. Fraaije and V. Gotor, *Tetrahedron: Asymmetry*, 2008, **19**, 197.