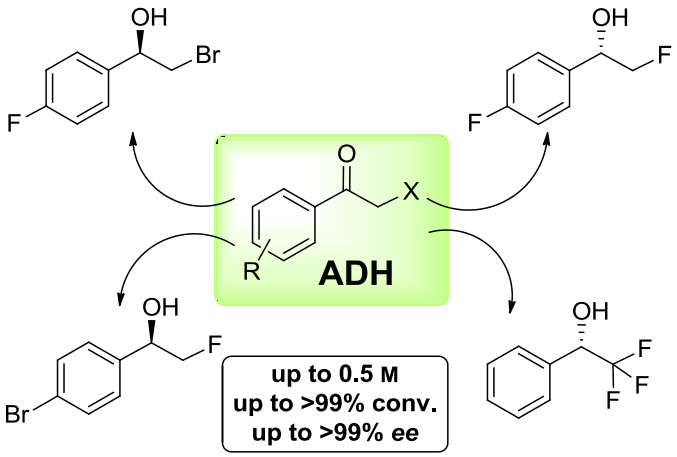
Synthesis of Enantiopure Fluorohydrins Using Alcohol Dehydrogenases at High Substrate Concentrations

Wioleta Borzęcka, Iván Lavandera, and Vicente Gotor**

Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, University of Oviedo, C/Julián Clavería 8, 33006 Oviedo, Spain

Tel.: +34 985 103452 or +34 985 103448; Fax: +34 985 103448; E-mail: lavanderaivan@uniovi.es or vgs@uniovi.es.



Abstract. The use of purified and overexpressed alcohol dehydrogenases to synthesize enantiopure fluorinated alcohols is shown. Performing the bioreductions with ADH-A from *Rhodococcus ruber* overexpressed in *E. coli*, no external cofactor was necessary to obtain the enantiopure (*R*)-derivatives. Employing *Lactobacillus brevis* ADH, it was possible to achieve the synthesis of enantiopure (*S*)-fluorohydrins at 0.5 M substrate concentration. Furthermore, due to the activated character of these substrates, it was not necessary a huge excess of the hydrogen donor.

Fluorinated compounds have a central role nowadays in pharmaceuticals, agrochemicals, radiotracers, and high performance materials.[1] Particularly, vicinal fluoro alcohols (fluorohydrins), also present interesting properties as liquid crystals[2] and as precursors of natural product analogues such as steroids or carbohydrates,[3] since it is well-known that the introduction of C-F bonds can modulate their physicochemical properties, including bioavailability, lipophilicity, and oxidative stability.

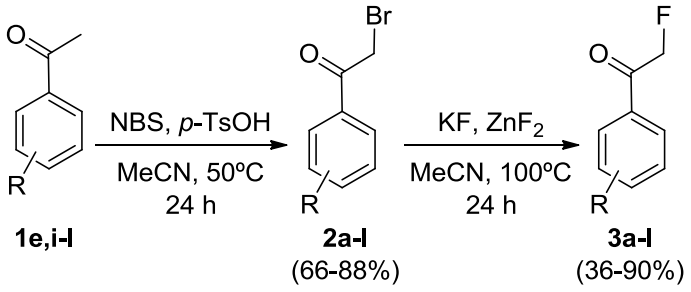
To obtain these derivatives in a selective fashion, recently several strategies have been described[4] and among them, the aperture of racemic or *meso*-epoxides,[5] the C-C formation of (partially) fluorinated building blocks[6] or the reduction of α-fluoro ketones[7] can be highlighted. Although remarkable improvements have been done in these fields in the past years, the use of enzymes[8] to synthesize chiral alcohols is very competitive in terms of selectivity and environmentally friendly conditions. Thus, in the particular case of the bioreduction of fluorinated ketone precursors, historically whole cells were employed to obtain enantioenriched fluorohydrins,[9] but due to the presence of several enzymes with opposite stereopreference, in many examples it was not possible to obtain the enantiopure alcohols. Therefore, in the last few years the use of isolated or overexpressed alcohol dehydrogenases (ADHs)[10] in combination with very efficient nicotinamide recycling systems has been successfully shown to synthesize fluorinated alcohols in excellent yields and *ee*.[11]

Most of the biocatalytic examples achieved until now are related with the reduction of 2-fluoroacetophenone and 2,2,2-trifluoroacetophenone,[9,11] so due to the relevance of chiral aromatic fluorohydrins,[2,12] we became interested in achieving a systematic study about the application of purified and overexpressed ADHs with opposite stereopreference to obtain both enantiopure antipodes of the fluorohydrin derivatives.

A series of α-fluoroacetophenones (**3b-l**) were synthesized through bromination of the corresponding acetophenones **1** with *N*-bromosuccinimide (NBS) and *p*-toluenesulfonic acid (*p*-TsOH) in acetonitrile (MeCN) at 50ºC to obtain the α-bromo ketones **2**,[13] followed by nucleophilic substitution using KF and ZnF_2 in MeCN at 100ºC (Scheme 1).[14] 2-Fluoroacetophenone (**3a**) was prepared as previously

described.[15] The synthesis of the corresponding racemic fluorohydrins was performed by simple treatment of the ketones with $NaBH_4$ in MeOH at 0ºC.

Scheme 1. Synthesis of α-fluoroacetophenone derivatives **3a-l**

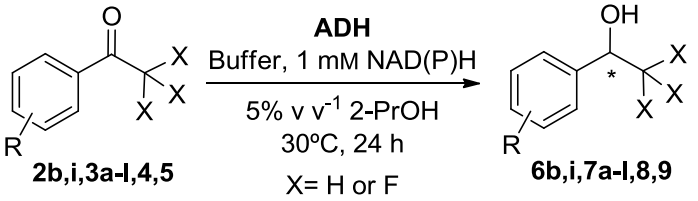


a, R= H; **b**, R= *p*-F; **c**, R= *p*-Cl; **d**, R= *p*-Br
e, R= *p*-I; **f**, R= *p*-Me; **g**, R= *p*-OMe; **h**, R= *p*-NO_2
i, R= *m*-F; **j**, R= *m*-Cl; **k**, R= *m*-OMe; **l**, R= *m*-NO_2

Due to our previous expertise in ADH-catalyzed reductions,[13,16] we selected a series of purified and overexpressed biocatalysts (see the Experimental Section), to achieve the transformations using ketones **3a-l** as substrates, together with fluorinated α-bromoacetophenones **2b** and **2i**, and also 2,2-difluoro- (**4**) and 2,2,2-trifluoroacetophenone (**5**) to compare the activity and selectivity with the monofluorinated compound **3a**. Buffer Tris.HCl 50 mM pH 7.5 was selected as appropriate medium, except for brominated compounds **2b** and **2i** where Tris.H_2SO_4 was employed to avoid undesired S_N2 reactions.[13] Furthermore, 1 mM of the corresponding nicotinamide cofactor was also added and 2-propanol or glucose with glucose dehydrogenase (GDH) to recycle it. After enzymatic screening, overexpressed ADH-A from *Rhodococcus ruber* in *E. coli* (*E. coli*/ADH-A),[17] and commercially available ADH from *Lactobacillus brevis* (LBADH)[18] were chosen as the best biocatalysts in terms of activity and selectivity. The employment of these enzymes is highly desirable since they can work under the 'coupled-substrate' approach using 2-propanol to recycle the cofactor, diminishing processes costs and allowing higher substrate concentrations due to better ketone solubility in the aqueous buffer. Furthermore, due to the opposite stereopreference they show, it was possible to get access to both alcohol enantiomers.

Therefore, substrates **2b**, **2i**, **3a-l**, **4** and **5** (30 mM) were subjected under these biocatalytic conditions obtaining excellent results with both enzymes (Table 1).

Table 1. Bioreductions of fluorinated ketones (30 mM) using *E. coli*/ADH-A and LBADH[a]



entry	ketone	*E. coli*/ADH-A		LBADH	
		c (%)[b]	*ee* (%)[c,d]	*c* (%)[b]	*ee* (%)[c,d]
1	**2b**	>99	>99 (*R*)	>99	>99 (*S*)
2	**2i**	>99	>99 (*R*)	>99	>99 (*S*)
3	**3a**	>99	>99 (*R*)	>99	>99 (*S*)
4	**3b**	>99	>99 (*R*)	>99	>99 (*S*)
5	**3c**	>99	>99 (*R*)	>99	>99 (*S*)
6	**3d**	>99	>99 (*R*)	99	>99 (*S*)
7	**3e**	>99	>99 (*R*)	>99	>99 (*S*)
8	**3f**	>99	>99 (*R*)	>99	>99 (*S*)
9	**3g**	>99	>99 (*R*)	>99	>99 (*S*)
10	**3h**	>99	>99 (*R*)	>99	>99 (*S*)
11	**3i**	>99	>99 (*R*)	>99	>99 (*S*)
12	**3j**	>99	>99 (*R*)	>99	>99 (*S*)
13	**3k**	>99	>99 (*R*)	>99	>99 (*S*)
14	**3l**	>99	>99 (*R*)	>99	>99 (*S*)
15	**4**	>99	>99 (*R*)	>99	>99 (*S*)
16	**5**	>99	>99 (*R*)	>99	>99 (*S*)

[a] For experimental details, see Experimental Section. [b] Measured by GC. [c] Measured by chiral GC. [d] Change in Cahn-Ingold-Prelog (CIP) priority.

Gratefully, in every case the substrate was completely transformed into the enantiopure (*R*)- or (*S*)-alcohol when using ADH-A or LBADH, independently it bore electron withdrawing or electron donating groups at *meta*- or *para*-positions. Also di- or trifluorinated ketones **4** and **5** afforded perfect conversions and *ee* with both biocatalysts.

One additional advantage of working with overexpressed ADH preparations is the possibility to avoid the use of external cofactor when performing the bioreductions.[19] Therefore, some of these ketones were tested at higher substrate concentration with *E. coli*/ADH-A without the addition of external NADH (Table 2). It was interesting to observe that in some cases the enantiopurity of the final alcohols slightly decreased. This could be probably due to the action of other NADP-dependent ADHs from the host organism that in the absence of additional NADH could compete with ADH-A diminishing the overall selectivity. Moreover, it was demonstrated that even at high concentrations such as 200 mM, conversions remained quantitative.

Table 2. Bioreductions of fluorinated ketones using *E. coli*/ADH-A and 2-PrOH (10% v v^{-1}) without the addition of external NADH (*t*= 24 h)[a]

entry	ketone	concentration (mM)	*c* (%)[b]	*ee* (%)[c,d]
1	**3a**	200	>99	>99 (*R*)
2	**3b**	200	>99	97 (*R*)
3	**3c**	200	>99	98 (*R*)
4	**3e**	200	>99	>99 (*R*)
5	**4**	200	>99	94 (*R*)
6	**5**	100	>99	>99 (*R*)

[a] For experimental details, see Experimental Section. [b] Measured by GC. [c] Measured by chiral GC. [d] Change in CIP priority.

In a next step, we decided to increase the substrate concentration keeping constant the quantity of LBADH in the presence of 1 mM of the nicotinamide cofactor and just increasing the amount of 2-

PrOH, although due to the fact that α-halogenated ketones can be quasi-irreversibly reduced,[20] it was not necessary a huge excess of the hydrogen donor. A selection of the results obtained is shown in Figure 1.

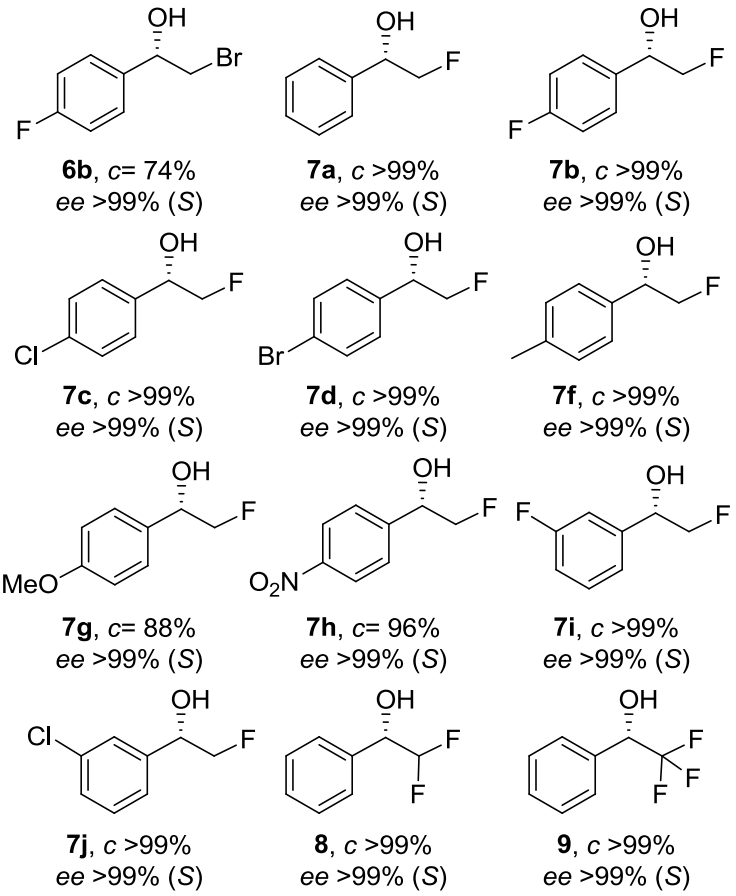


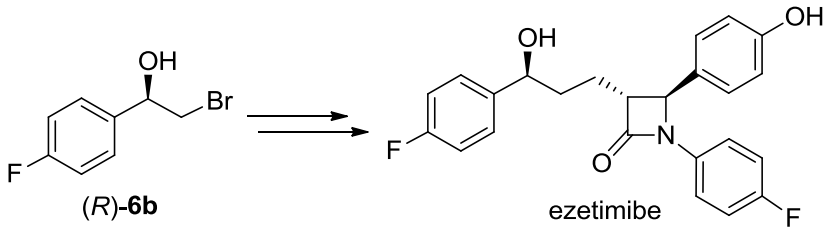
Figure 1. Examples of enantiopure fluorinated alcohols obtained at 0.5 M substrate concentration with LBADH and 2-PrOH (*t*= 24 h). For other results, see Supporting Information.

Several enantiopure fluorinated alcohols were achieved at 0.5 M concentration with excellent conversions in the presence of 3 U of the enzyme and 1 mM $NADP^+$. In these examples, 20% v v^{-1} of the hydrogen donor 2-PrOH was employed, approximately a molar excess of 5:1 with regards to the ketone substrate, confirming that the bioreduction of these derivatives was thermodynamically favored.

(*R*)-**6b** is a precursor of cholesterol absorption inhibitors (CAI) such as AZD4121 or ezetimibe (Scheme 2), a class of compounds that have attracted attention for the treatment of hypercholesterolemia, related to several diseases as atherosclerosis.[21] This alcohol was recently

synthesized *via* asymmetric reduction with (*R*)-2-methyloxazaborolidine in the presence of borane-dimethyl sulfide complex in THF at 0ºC.[22] With regards to biocatalyzed synthesis, Zhu and co-workers obtained the enantiopure (*S*)-alcohol using an isolated ADH from *Candida magnolia* with glucose and GDH to recycle the nicotinamide cofactor.[23]

Scheme 2. (*R*)-**6b**, a precursor of cholesterol absorption inhibitors such as ezetimibe



Due to the relevance of this bromohydrin, the *E. coli*/ADH-A catalyzed bioreduction of **2b** was performed at 260 mg-scale with a 100 mM substrate concentration, obtaining enantiopure (*R*)-**6b** after purification with 70% yield.

Overall, with the systematic study shown here it was demonstrated that ADH-catalyzed hydrogen transfer reduction of α-fluoro ketones is a very convenient method to synthesize both alcohol antipodes in an enantiopure form at high substrate concentrations with excellent conversions. Thus, when performing the bioreduction in the presence of overexpressed enzymes it was also possible to avoid the use of external nicotinamide cofactor. Due to the nature of these activated ketones, it was not necessary the employment of a huge excess of the hydrogen donor.

Experimental Section

General. Acetophenones **1e**, **1i**, **1j**, **1k**, and **1l**, α-bromoacetophenones **2b**, **2c**, **2d**, **2f**, **2g**, and **2h**, difluorinated ketone **4** and trifluorinated derivative **5** were obtained from commercial sources. All other reagents and solvents were of the highest quality available. LBADH from *Lactobacillus brevis* and GDH were obtained from commercial sources. The following overexpressed enzymes in *E. coli* have been

kindly provided by Prof. Wolfgang Kroutil from the University of Graz (Austria): ADH-A from *Rhodococcus ruber* (*E. coli*/ADH-A), ADH-T from *Thermoanaerobium* sp. (*E. coli*/ADH-T), SyADH from *Sphingobium yanoikuyae* (*E. coli*/SyADH), RasADH from *Ralstonia* sp. (*E. coli*/RasADH), and TeSADH from *Thermoanaerobacter ethanolicus* (*E. coli*/TeSADH).

Chemical reactions were monitored by analytical TLC, performed on silica gel 60 F254 plates and visualized by UV irradiation. Flash chromatography was performed using silica gel 60 (230-400 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on an infrared Fourier transform spectrophotometer on NaCl pellets. ^{1}H-, ^{13}C, ^{19}F-NMR, and DEPT were obtained using 300 and 400 MHz spectrometers for routine experiments. The chemical shifts (δ) are given in ppm and the coupling constants (*J*) in Hertz (Hz). ESI^{+} mode was used to record mass spectra (MS) and ESI-TOF for HRMS. Gas chromatography (GC) analyses were performed on a standard GC chromatograph. Calibration curves were performed with α-fluoro ketones and the corresponding fluorohydrins to measure accurately the enzymatic conversions. Optical rotations were measured using a standard polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. For new compounds, the indirect assignation of their configuration was based in two convergent criteria: a) the perfect stereoselectivity shown by ADH-A and LBADH for the other members of this fluorinated family and other similar compounds, and b) the order of the elution peaks in the chiral GC column.

Synthesis of α-bromoacetophenone derivatives 2. To a solution of the acetophenone derivative (15.0 mmol, 1 equiv) in 8 mL of acetonitrile, NBS (2.72 g, 15.3 mmol, 1.02 equiv) and *p*-toluenesulfonic acid (2.85 g, 15.0 mmol, 1 equiv) were added. The reaction mixture was stirred for 24 h at 50˚C. After that time, the solvent was evaporated under reduced pressure. Then, a water solution of saturated $NaHCO_3$ (30 mL) was added and the solution was extracted with dichloromethane (3 x 30 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the residue was subjected to column chromatography (silica gel) using hexanes/CH_2Cl_2 (from 9:1 to 4:1) as eluent. These compounds exhibited physical and spectral data in agreement with those reported.[24] **2e**

(4.30 g, 88% yield), **2i** (2.12 g, 66% yield), **2j** (2.88 g, 82% yield), **2k** (2.45 g, 71% yield), and **2l** (2.60 g, 71% yield).

Synthesis of α-fluoroacetophenone derivatives 3. A mixture of KF (4.8 mmol, 1.5 equiv), ZnF_2 (4.8 mmol, 1.5 equiv) in 7 mL of acetonitrile was stirred and heated at 100˚C in a sealed heavy-walled Pyrex tube for 1 h. Then, a solution of the substrate (3.2 mmol, 1.0 equiv) in acetonitrile (7 mL) was added into the reaction mixture. Then it was heated for 24 h at 100˚C. After that time, the solvent was evaporated under reduced pressure. Then, water (20 mL) was added and the solution was extracted with dichloromethane (3 x 20 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the residue was subjected to column chromatography (silica gel) using hexanes/EtOAc (from 95:5 to 90:10) as eluent. These compounds exhibited physical and spectral data in agreement with those reported.[14,25] **3b** (230 mg, 47% yield), **3c** (255 mg, 47% yield), **3d** (625 mg, 90% yield), **3e** (326 mg, 39% yield), **3f** (274 mg, 56% yield), **3g** (380 mg, 71% yield), **3h** (210 mg, 36% yield), **3i** (265 mg, 53% yield), **3j** (367 mg, 66% yield), **3k** (368 mg, 68% yield), and **3l** (214 mg, 36% yield).

2-Fluoro-4'-iodoacetophenone (3e). White solid; mp: 92.4-95.3˚C; IR (NaCl): 3054, 2987, 1422, 1265, 972, 896, 741, 666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.89 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 5.49 (d, *J* = 46.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.0 (d, *J* = 15.8 Hz), 138.3, 133.0, 129.2 (d, *J* = 2.9 Hz), 102.3, 83.5 (d, *J* = 182.5 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -229.9; MS (ESI^+, *m/z*): 287 [$(M+Na)^+$, 100%], 265 [$(M+H)^+$, 30%]; HRMS (ESI^+, *m/z*) calculated for C_8H_6OFINa $(M+Na)^+$: 286.9365, found: 286.9340.

3'-Chloro-2-fluoroacetophenone (3j). Yellow oil; IR (NaCl): 3057, 2987, 2933, 1711, 1575, 1428, 1266, 1229, 1101, 1087, 998, 739, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.91 (m, 1H), 7.79 (m, 1H), 7.62 (m, 1H), 7.47 (m, 1H), 5.51 (d, *J* = 46.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.4 (d, *J* = 16.0 Hz), 135.2 (d, *J* = 11.6 Hz), 134.1, 130.3, 128.1 (d, *J* = 2.9 Hz), 126.0 (d, *J* = 2.7 Hz), 83.6 (d, *J* = 182.7 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -230.1; MS (ESI^+, *m/z*): 195 [$(M+Na)^+$, 100%], 173 [$(M+H)^+$, 70%]; HRMS (ESI^+, *m/z*) calculated for $C_8H_6OClFNa$ $(M+Na)^+$: 194.9992, found: 194.9983.

2-Fluoro-3'-methoxyacetophenone (3k). White solid; mp: 55.7-58.8˚C; IR (NaCl): 3054, 2987, 1710, 1600, 1584, 1432, 1265, 1092, 738, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (m, 3H), 7.18 (m, 1H), 5.54 (d, *J* = 46.9 Hz, 2H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.2 (d, *J* = 15.2 Hz), 160.0, 134.9, 129.9, 120.7, 120.2 (d, *J* = 2.3 Hz), 112.1 (d, *J* = 2.2 Hz), 83.5 (d, *J* = 181.4 Hz), 55.5; ^{19}F NMR (282 MHz, $CDCl_3$): δ -230.8; MS (ESI^+, *m/z*): 191 [$(M+Na)^+$, 100%], 169 [$(M+H)^+$, 85%]; HRMS (ESI^+, *m/z*) calculated for $C_9H_9O_2FNa$ $(M+Na)^+$: 191.0488, found: 191.0479.

General procedure for the synthesis of the racemic alcohols 6-9. To a solution of the corresponding ketone (4.0 mmol) in methanol (5 mL) at 0˚C, sodium borohydride (1.2 mmol) was added. When the reduction was completed (according to the TLC), a few drops of 1 M HCl were added. The solvent was evaporated under reduced pressure. Then, water (10 mL) was added and the solution was extracted with dichloromethane (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the residue was subjected to column chromatography (silica gel) using mixtures of hexanes/ethyl acetate (from 9:1 to 4:1) as eluents. These compounds exhibited physical and spectral data in agreement with those reported.[23,26] Isolated yields: 71-95%.

2-Bromo-1-(3-fluorophenyl)ethanol (6i). White solid; mp: 66.8-69.0˚C; IR (NaCl): 3054, 2987, 1422, 1265, 1158, 896, 740, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 1H), 7.16 (m, 2H), 7.05 (tdd, *J* = 8.4, 2.5, 0.9 Hz, 1H), 4.95 (dd, *J* = 8.8, 3.3 Hz, 1H), 3.65 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.55 (dd, *J* = 10.5, 8.8 Hz, 1H), 2.69 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.0 (d, *J* = 245.3 Hz), 142.8 (d, *J* = 27.4 Hz), 130.2 (d, *J* = 31.8 Hz), 121.6 (d, *J* = 11.4 Hz), 115.3 (d, *J* = 83.7 Hz), 113.0 (d, *J* = 89.0 Hz), 73.1, 39.9; ^{19}F NMR (282 MHz, $CDCl_3$): δ -112.2; $[\alpha]_D^{20}$ = +41.0 (c 1.13, $CHCl_3$), *ee* >99% (*S*).[27]

1-(4-Chlorophenyl)-2-fluoroethanol (7c).[26d] $[\alpha]_D^{20}$ = +42.9 (c 1.67, $CHCl_3$), *ee* >99% (*S*).

2-Fluoro-1-(4-iodophenyl)ethanol (7e). Light yellow solid; mp: 42.3-45.0˚C; IR (NaCl): 3055, 2987, 1422, 1265, 896, 740, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 5.00 (m, 1H), 4.60-4.28 (m, 2H), 2.52 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8, 137.7, 128.2, 94.0, 86.8 (d, *J* = 173.9 Hz), 72.4 (d, *J* = 20.1 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -229.9;

MS (ESI^+, *m/z*): 289 [$(M+Na)^+$, 100%], 249 [$(M-OH)^+$, 25%]; HRMS (ESI^+, *m/z*) calculated for C_8H_8OFINa $(M+Na)^+$: 288.9484, found: 288.9496; $[\alpha]_D^{20}$ = +30.3 (c 1.04, $CHCl_3$), *ee* >99% (*S*).

2-Fluoro-1-(4-methylphenyl)ethanol (7f). White solid; mp: 33.8-37.5ºC; IR (NaCl): 3054, 2987, 1422, 1265, 1090, 1004, 896, 743, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.02 (m, 1H), 4.61-4.41 (m, 2H), 2.50 (dd, *J* = 2.9, 0.8 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.3, 135.1 (d, *J* = 8.2 Hz), 129.3, 126.3, 87.2 (d, *J* = 173.2 Hz), 72.8 (d, *J* = 19.6 Hz), 21.2; ^{19}F NMR (282 MHz, $CDCl_3$): δ -220.4; MS (ESI^+, *m/z*): 177 [$(M+Na)^+$, 100%], 137 [$(M-OH)^+$, 90%]; HRMS (ESI^+): calculated for $C_9H_{11}OFNa$ $(M+Na)^+$: 177.0675, found: 177.0686; $[\alpha]_D^{20}$ = +51.9 (c 1.34, $CHCl_3$), *ee* >99% (*S*).

2-Fluoro-1-(3-fluorophenyl)ethanol (7i). Light yellow oil; IR (NaCl): 3055, 2986, 1594, 1450, 1266, 1137, 1010, 896, 739, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 1H), 7.16 (m, 2H), 7.05 (tdd, *J* = 8.4, 2.5, 1.0 Hz, 1H), 5.05 (m, 1H), 4.64-4.31 (m, 2H), 2.57 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.0 (d, *J* = 245.1 Hz), 140.7 (d, *J* = 7.5 Hz), 130.2 (d, *J* = 8.1 Hz), 121.9 (d, *J* = 2.7 Hz), 115.3 (d, *J* = 21.0 Hz), 113.4 (d, *J* = 22.2 Hz), 86.9 (d, *J* = 173.6 Hz), 72.3 (d, *J* = 20.1 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -112.4, -221.5; $[\alpha]_D^{20}$ = +42.3 (c 1.21, $CHCl_3$), *ee* >99% (*S*).[27]

1-(3-Chlorophenyl)-2-fluoroethanol (7j). Yellow oil; IR (NaCl): 3060, 2983, 2952, 1600, 1576, 1479, 1433, 1266, 1198, 1079, 1012, 912, 789, 739, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.43 (m, 1H), 7.30 (m, 3H), 5.06 (m, 1H), 4.63-4.30 (m, 2H), 2.64 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.1 (d, *J* = 8.0 Hz), 134.6, 129.9, 128.6, 126.5, 124.5, 86.9 (d, *J* = 173.7 Hz), 72.3 (d, *J* = 20.0 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -221.4; $[\alpha]_D^{20}$ = +42.1 (c 1.65, $CHCl_3$), *ee* >99% (*S*).[27]

2-Fluoro-1-(3-methoxyphenyl)ethanol (7k). Light yellow oil; IR (NaCl): 3056, 2950, 2838, 1603, 1587, 1489, 1467, 1456, 1436, 1320, 1267, 1158, 1043, 1010, 881, 786, 737, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (m, 1H), 6.97 (m, 2H), 6.89 (m, 1H), 5.02 (m, 1H), 4.63-4.32 (m, 2H), 3.84 (s, 3H), 2.39 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.9, 139.7 (d, *J* = 8.2 Hz), 129.7, 118.5, 113.9, 111.8, 87.2 (d, *J* = 173.3 Hz), 72.9 (d, *J* = 19.7 Hz), 55.3; ^{19}F NMR (282 MHz, $CDCl_3$): δ -220.7; MS

(ESI$^+$, *m/z*): 193 [(M+Na)$^+$, 100%], 153 [(M-OH)$^+$, 50%]; HRMS (ESI$^+$, *m/z*) calculated for $C_9H_{11}O_2FNa$ (M+Na)$^+$: 193.0637, found: 193.0635; $[\alpha]_D^{20}$ = +42.3 (c 1.85, $CHCl_3$), *ee* >99% (*S*).

2-Fluoro-1-(3-nitrophenyl)ethanol (7l). Light yellow solid; mp: 49.9-54.8˚C; IR (NaCl): 3054, 2987, 1534, 1422, 1353, 1265, 896, 738, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (ap t, *J* = 2.0 Hz, 1H), 8.22 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.59 (ap t, *J* = 8.0 Hz, 1H), 5.16 (m, 1H), 4.69-4.36 (m, 2H), 2.76 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.4, 140.4 (d, *J* = 7.6 Hz), 132.4, 129.6, 123.3, 121.4, 86.5 (d, *J* = 174.2 Hz), 71.9 (d, *J* = 20.5 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ -222.7; MS (ESI$^+$, *m/z*): 208 [(M+Na)$^+$, 100%], 186 [(M+H)$^+$, 45%]; HRMS (ESI$^+$, *m/z*) calculated for $C_8H_8NO_3FNa$ (M+Na)$^+$: 208.0374, found: 208.0380; $[\alpha]_D^{20}$ = +33.5 (c 1.04, $CHCl_3$), *ee* >99% (*S*).

Enzymatic screening with 3a. In a 1.5 mL Eppendorf vial, LBADH (3 U) or lyophilized cells of the overexpressed ADH (ADH-A, ADH-T, RasADH, SyADH, or TeSADH) in *E. coli* (20 mg), were added in Tris.HCl buffer 50 mM pH 7.5 (570 µL, 1 mM $NADP^+$ for LBADH, ADH-T, RasADH, SyADH, and TeSADH or 1 mM NADH for ADH-A; 1 mM $MgCl_2$ for LBADH), and mixed with **3a** (30 mM) and 2-propanol (30 µL, 5% v v^{-1}, for LBADH, ADH-A, ADH-T, SyADH, and TeSADH) or with glucose (1.5 M) and GDH (5 U) for RasADH. The reactions were shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 0.5 mL). The organic layers were separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions of the corresponding alcohol were determined by GC obtaining the following results: for LBADH (>99%), *E. coli*/ADH-A (>99%), *E. coli*/RasADH (99%), *E. coli*/SyADH (4%), *E. coli*/ADH-T (99%), and *E. coli*/TeSADH (64%).

General procedure for an enzymatic reduction using LBADH from *Lactobacillus brevis.* In a 1.5 mL Eppendorf vial, LBADH (3 U) was added in Tris.HCl buffer 50 mM pH 7.5 (570 µL, 1 mM $NADP^+$, 1 mM $MgCl_2$) and mixed with 2-propanol (30 µL, 5% v v^{-1}) and the corresponding ketone (30 or 50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by GC (see Tables 1

and S1). For α-brominated ketones, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (570 µL, 1 mM $NADP^+$, 1 mM $MgBr_2$) was used.

General procedure for an enzymatic reduction using *E. coli*/ADH-A from *Rhodococcus ruber*. In a 1.5 mL Eppendorf vial, *E. coli*/ADH-A (20 mg) was added in Tris-HCl buffer 50 mM pH 7.5 (570 µL, 1 mM NADH) and mixed with 2-propanol (30 µL, 5% v v^{-1}) and the corresponding ketone (30 or 50 mM). The reactions were shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by GC (see Tables 1 and S1). For α-brominated ketones, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (570 µL, 1 mM NADH) was used.

Enzymatic reduction using *E. coli*/ADH-A in the absence of the nicotinamide cofactor. In a 1.5 mL Eppendorf vial, *E. coli*/ADH-A (20 mg) was added in Tris-HCl buffer 50 mM pH 7.5 (540 µL) and mixed with 2-propanol (60 µL, 10% v v^{-1}) and the corresponding ketone (100 or 200 mM). The reactions were shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by GC.

Enzymatic reduction of ketones at 100 or 200 mM concentration using LBADH. In a 1.5 mL Eppendorf vial, LBADH (3 U) was added in Tris.HCl buffer 50 mM pH 7.5 (540 µL, 1 mM $NADP^+$, 1 mM $MgCl_2$) and mixed with 2-propanol (60 µL, 10% v v^{-1}) and the corresponding ketone (100-200 mM). The reactions were shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 1 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by (chiral) GC (see Table S1). For α-brominated ketones, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (540 µL, 1 mM $NADP^+$, 1 mM $MgBr_2$) was used.

Enzymatic reduction of ketones at 100 or 200 mM concentration using *E. coli*/ADH-A. In a 1.5 mL Eppendorf vial, *E. coli*/ADH-A (20 mg) was added in Tris.HCl buffer 50 mM pH 7.5 (540 µL, 1

mM NADH) and mixed with 2-propanol (60 μL, 10% v v^{-1}) and the corresponding ketone (100-200 mM). The reactions were shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 1 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by (chiral) GC (see Table S1). For α-brominated ketones, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (540 μL, 1 mM NADH) was used.

Enzymatic reduction of ketones at 0.5 M concentration using LBADH. In a 1.5 mL Eppendorf vial, LBADH (3 U) was added in Tris.HCl buffer 50 mM pH 7.5 (480 μL, 1 mM $NADP^+$, 1 mM $MgCl_2$) and mixed with 2-propanol (120 μL, 20% v v^{-1}) and the corresponding ketone (0.5 M). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with ethyl acetate (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and *ee* of the corresponding alcohols were determined by GC (see Figure 1 and Table S1). For α-brominated ketones, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (480 μL, 1 mM $NADP^+$, 1 mM $MgBr_2$) was used.

Scale-up of the bioreductions using LBADH. In a 10 mL glass vial, LBADH (15 U) was added in Tris.HCl buffer 50 mM pH 7.5 (1 mM $NADP^+$, 1 mM $MgCl_2$) and mixed with 2-propanol (10% v v^{-1}) and the corresponding ketone (50 mg, 100 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with diethyl ether (2 x 5 mL). The organic layer was dried over Na_2SO_4 and the solvent was carefully evaporated (CAUTION: fluorohydrins are highly volatile). Conversions and *ee* of the corresponding alcohols were determined by GC and NMR. For **2i**, in order to avoid undesired S_N2 reactions with Tris.HCl buffer,[13] Tris.H_2SO_4 buffer 50 mM pH 7.5 (1 mM $NADP^+$, 1 mM $MgBr_2$) was used. In most cases, the final enantiopure products exhibited excellent purity after solvent evaporation, so no further purification was necessary. To obtain (*S*)-**6i**, (*S*)-**7f** and (*S*)-**7j**, flash chromatography was employed to purify the alcohol derivatives. (*S*)-**6i** (37 mg, 73% yield, >99% *ee*), (*S*)-**7a** (47 mg, 93% yield, >99% *ee*), (*S*)-**7b** (44 mg, 87% yield, >99% *ee*), (*S*)-**7c** (36 mg, 71% yield, >99% *ee*), (*S*)-**7d** (46 mg, 91% yield, >99% *ee*), (*S*)-**7e** (44 mg, 87% yield, >99% *ee*), (*S*)-**7f** (33 mg, 64% yield, >99% *ee*),

(*S*)-**7g** (48 mg, 94% yield, >99% *ee*), (*S*)-**7h** (48 mg, 95% yield, >99% *ee*), (*S*)-**7i** (42 mg, 83% yield, >99% *ee*), (*S*)-**7j** (36 mg, 71% yield, >99% *ee*), (*S*)-**7k** (41 mg, 81% yield, >99% *ee*), (*S*)-**7l** (45 mg, 88% yield, >99% *ee*), (*S*)-**8** (42 mg, 83% yield, >99% *ee*), and (*S*)-**9** (43 mg, 85% yield, >99% *ee*).

Scale-up of the bioreduction of ketone 2b employing *E. coli*/ADH-A. In a 25 mL Erlenmeyer flask, *E. coli*/ADH-A (460 mg) was added in Tris.H_2SO_4 buffer 50 mM pH 7.5 (10.8 mL, 1 mM NADH) and mixed with 2-propanol (1.2 mL, 10% v v^{-1}) and ketone **2b** (260 mg, 100 mM). Reactions were shaken at 30ºC and 250 rpm for 24 h. After that time the pellet was separated by centrifugation (15 min, 6000 rpm) and extracted with ethyl acetate (3 x 20 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the crude was purified by flash chromatography. Isolated yield of (*R*)-**6b**: 184 mg, 70%, >99% *ee*.

2-Bromo-1-(4-fluorophenyl)ethanol (6b).[23] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.08 (ap t, *J* = 8.7 Hz, 2H), 4.93 (m, 1H), 3.65-3.50 (m, 2H), 2.72 (d, *J* = 3.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.6 (d, *J* = 245.3 Hz), 136.0 (d, *J* = 3.0 Hz), 127.7 (d, *J* = 8.3 Hz), 115.6 (d, *J* = 21.4 Hz), 73.2, 40.1; ^{19}F NMR (282 MHz, $CDCl_3$): δ -113.4.

Acknowledgments. W.B. thanks the Principado de Asturias for her predoctoral fellowship (Severo Ochoa Program). I.L. thanks the Spanish Ministerio de Ciencia e Innovación (MICINN) for personal funding (Ramón y Cajal Program). Financial support of this work by the Spanish MICINN (Project MICINN-12-CTQ2011-24237) is gratefully acknowledged.

Supporting Information Available. Enzymatic conversions and *ee* at higher substrate concentrations (Table S1), analytical methods, and copies of ^{1}H-, ^{13}C-, and ^{19}F-NMR for new compounds are described. This material is available free of charge via the Internet at http://pubs.acs.org.

References

1. (a) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320–330. (b) Ametamey, S. M.; Honer, M.; Schubiger, P. A. *Chem. Rev.* **2008**, *108*, 1501–1516.

2. Kusumoto, T.; Hiyama, T. in *Enantiocontrolled Synthesis of Fluoro-Organic Compounds: Stereochemical Challenges and Biomedicinal Targets*, Soloshonok, V. A., Ed.; Wiley: Chichester, 1999, pp. 535–556.

3. For some recent examples, see: (a) Cresswell, A. J.; Davies, S. G.; Lee, J. A.; Morris, M. J.; Roberts, P. M.; Thomson, J. E. *J. Org. Chem.* **2012**, *77*, 7262–7281. (b) Chen, J.-L.; Zheng, F.; Huang, Y.-E.; Qing, F.-L. *J. Org. Chem.* **2011**, *76*, 6525–6533.

4. (a) Hollingworth, C.; Gouverneur, V. *Chem. Commun.* **2012**, *48*, 2929–2942. (b) Furuya, T.; Kamlet, A. S.; Ritter, T. *Nature* **2011**, *473*, 470–477. (c) Haufe, G. *J. Fluor. Chem.* **2004**, *125*, 875–894.

5. Kalow, J. A.; Doyle, A. G. *J. Am. Chem. Soc.* **2011**, *133*, 16001–16012.

6. Arroyo, Y.; Sanz-Tejedor, M. A.; Parra, A.; García Ruano, J. L. *Chem. Eur. J.* **2012**, *18*, 5314–5318.

7. Mohanta, P. K.; Davis, T. A.; Gooch, J. R.; Flowers, R. A., II *J. Am. Chem. Soc.* **2005**, *127*, 11896–11897.

8. (a) *Enzyme Catalysis in Organic Synthesis*, 3rd ed.; Drauz, K., Gröger, H., May, O., Eds.; Wiley-VCH: Weinheim, 2012. (b) Faber, K. *Biotransformations in Organic Chemistry*, 6th ed.; Springer: Berlin, 2011.

9. Some selected examples: (a) Barkakaty, B.; Takaguchi, Y.; Tsuboi, S. *Tetrahedron* **2007**, *63*, 970–976. (b) Nakamura, K.; Yamanaka, R. *Tetrahedron: Asymmetry* **2002**, *13*, 2529–2533. (c) Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65,* 157–163.

10. Recent bibliography: (a) Hollmann, F.; Arends, I. W. C. E.; Holtmann, D. *Green Chem.* **2011**, *13*, 2285−2313. (b) García-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2011**, *111*, PR110−PR180. (c) Musa, M. M.; Phillips, R. S. *Catal. Sci. Technol.* **2011**, *1*, 1311−1323.

11. (a) Xu, G.-C.; Yu, H.-L.; Zhang, X.-Y.; Xu, J.-H. *ACS Catal.* **2012**, *2*, 2566−2571. (b) Itoh, N.; Isotani, K.; Nakamura, M.; Inoue, K.; Isogai, Y.; Makino, Y. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1075–1085. (c) Wang, L.-J.; Li, C.-X.; Ni, Y.; Zhang, J.; Liu, X.; Xu, J.-H. *Bioresour. Technol.* **2011**, *102*, 7023–7028. (d) Grau, B. T.; Devine, P. N.; DiMichele, L. N.; Kosjek, B. *Org. Lett.* **2007**, *9*, 4951–4954.

12. Schirlin, D.; Baltzer, S.; VanDorsselaer, V.; Weber, F.; Weill, C.; Altenburger, J. M.; Neises, B.; Flynn, G.; Rimy, J. M.; Tarnus, C. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 253–258.

13. Bisogno, F. R.; Cuetos, A.; Orden, A. A.; Kurina-Sanz, M.; Lavandera, I.; Gotor, V. *Adv. Synth. Catal.* **2010**, *352*, 1657–1661.

14. Adapted from: Zizhan, C.; Zhu, W.; Zheng, Z.; Zou, X. *J. Fluor. Chem.* **2010**, *131*, 340–344.

15. Mąkosza, M.; Bujok, R. *J. Fluor. Chem.* **2005**, *126*, 209–216.

16. (a) Cuetos, A.; Bisogno, F. R.; Lavandera, I.; Gotor, V. *Chem. Commun.* **2013**, *49*, 2625–2627. (b) Cuetos, A.; Rioz-Martínez, A.; Bisogno, F. R.; Grischek, B.; Lavandera, I.; de Gonzalo, G.; Kroutil, W.; Gotor, V. *Adv. Synth. Catal.* **2012**, *354*, 1743–1749. (c) Kurina-Sanz, M.; Bisogno, F. R.; Lavandera, I.; Orden, A. A.; Gotor, V. *Adv. Synth. Catal.* **2009**, *351*, 1842–1848.

17. This (*S*)-selective enzyme has a strong preference for NADH/NAD^+. See: Edegger, K.; Gruber, C. C.; Poessl, T. M.; Wallner, S. R.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402–2404.

18. This (*R*)-selective enzyme shows a preference for NADPH/$NADP^+$. See, for instance: Wolberg, M.; Hummel, W.; Wandrey, C.; Müller, M. *Angew. Chem., Int. Ed*. **2000**, *39*, 4306–4308.

19. (a) Ma, H.; Yang, L.; Ni, Y.; Zhang, J.; Li, C.-X.; Zheng, G.-W.; Yang, H.; Xu, J.-H. *Adv. Synth. Catal.* **2012**, *354*, 1765–1772. (b) Shen, N.-D.; Ni, Y.; Ma, H.-M.; Wang, L.-J.; Li, C.-X.; Zheng, G.-W.; Zhang, J.; Xu, J.-H. *Org. Lett.* **2012**, *14*, 1982-1985.

20. Bisogno, F. R.; García-Urdiales, E.; Valdés, H.; Lavandera, I.; Kroutil, W.; Suárez, D.; Gotor, V. *Chem. Eur. J.* **2010**, *16*, 11012–11019.

21. Burnett, D. A. *Curr. Med. Chem.* **2004**, *11*, 1873−1887.

22. Karlsson, S.; Sörensen, J. H. *Org. Process Res. Dev.* **2012**, *16*, 586−594.

23. Ren, J.; Dong, W.; Yu, B.; Wu, Q.; Zhu, D. *Tetrahedron: Asymmetry* **2012**, *23*, 497–500.

24. (a) Macharla, A. K.; Nappunni, R. C.; Marri, M. R.; Peraka, S.; Nama, N. *Tetrahedron Lett.* **2012**, *53*, 191–195. (b) Duca, M.; Malnuit, V.; Barbault, F.; Benhida, R. *Chem. Commun.* **2010**, *46*, 6162–6164. (c) Richards, J. J.; Melander, C. *J. Org. Chem.* **2008**, *73*, 5191–5193. (d) Pravst, I.; Zupan, M.; Stavber, S. *Tetrahedron* **2008**, *64*, 5191–5199.

25. Raja, E. K.; Klumpp, D. A. *Tetrahedron Lett.* **2011**, *52*, 5170–5172.

26. (a) Castro, L. C. M.; Bezier, D.; Sortais, J.-B.; Darcel, C. *Adv. Synth. Catal.* **2011**, *353*, 1279–1284. (b) Beier, P.; Alexandrova, A. V.; Zibinsky, M.; Surya Prakash, G. K. *Tetrahedron* **2008**, *64*, 10977–10985. (c) Fuglseth, E.; Sundby, E.; Bruheim, P.; Hoff, B. H. *Tetrahedron: Asymmetry* **2008**, *19*, 1941–1946. (d) Liu, J.; Ni, C.; Li, Y.; Zhang, L.; Wang, G.; Hu, J. *Tetrahedron Lett.* **2006**, *47*, 6753–6756. (e) Stavber, G.; Zupan, M.; Jereb, M.; Stavber, S. *Org. Lett.* **2004**, *6*, 4973–4976. (f) Sibille, S.; Mcharek, S.; Perichon, J. *Tetrahedron* **1989**, *45*, 1423–1428.

27. It was not possible to measure the MS spectra of this compound since it decomposed in all conditions tried.