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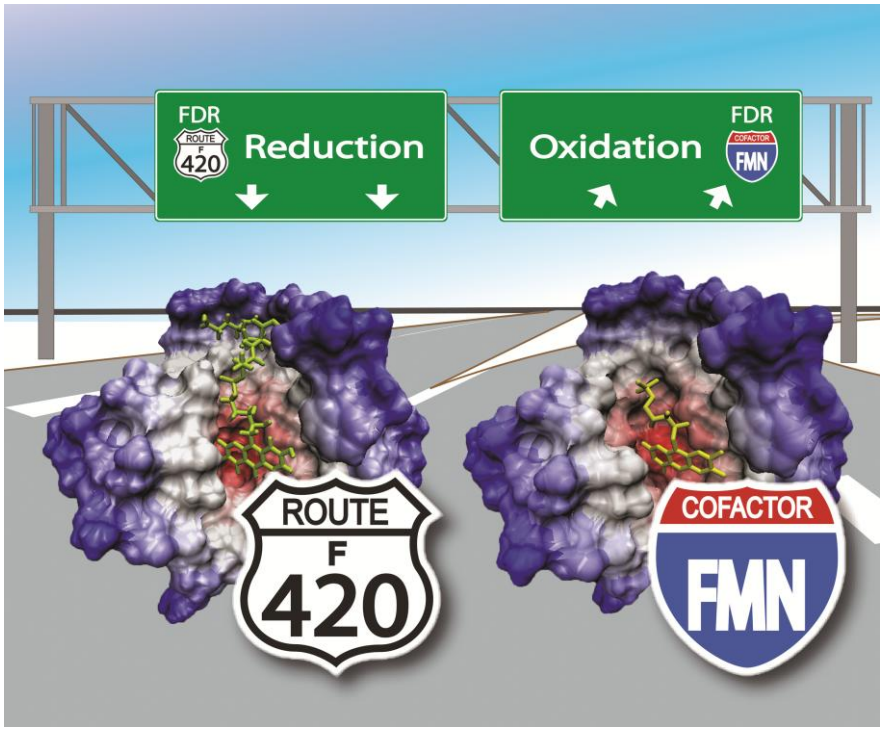

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PAPER

Enantiopure 3-methyl-3,4-dihydroisocoumarins and 3-methyl-1,2,3,4-tetrahydroisoquinolines *via* chemoenzymatic asymmetric transformations†

Juan Mangas-Sánchez, Eduardo Busto, Vicente Gotor-Fernández* and Vicente Gotor*

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A new divergent and asymmetric synthetic route has been developed for the production of enantiomerically pure isocoumarin and isoquinoline derivatives. Stereoselective formation of 2-(2-hydroxypropyl)benzonitriles has been identified as the key step. *Rhizomucor miehei* lipase has displayed from moderate to excellent selectivities in the acetylation of its (*R*)-enantiomers, while ADH-A from *Rhodococcus ruber* catalyzed the bioreduction of the corresponding ketones with excellent activities and stereopreferences. Enantiopure alcohols obtained in this manner have been used for the straightforward synthesis of a series of 3-methyl-3,4-dihydroisocoumarins and 3-methyl-1,2,3,4-tetrahydroisoquinolines with good overall yields.

Introduction

The development of new strategies in heterocyclic chemistry continues to be an attractive and challenging task for synthetic chemists. In this context, the preparation of 3,4-dihydroisocoumarins has been gathering increasing attention in recent years because of their structural implications as basic scaffolds in alkaloids and many natural products[1] that exhibit a broad range of biological activities. Both non-asymmetric and stereoselective methods for the preparation of this family of oxygenated compounds have been extensively reported in recent years.[2] In addition, dihydroisocoumarins are a class of naturally occurring biologically active lactones possessing remarkable antibacterial, anticancer, antifungal or antimalarial properties.[3] Asymmetric synthesis and isolation from plant extracts of 3-substituted-3,4-dihydroisocoumarins have particularly attracted attention.[4] However, only a few chemoenzymatic routes have been reported until now, among which biocatalytic methods for the preparation of 3-methyl-3,4-dihydroisocoumarins only include chemoenzymatic pathways involving ketone transformations such as Baeyer–Villiger oxidations[5] or bioreduction processes.[6]

Here we describe for the first time a divergent synthetic approach for the production of 3-methyl-3,4-dihydroisocoumarins and 3-methyl-1,2,3,4-tetrahydroisoquinolines starting from readily commercially available 2-bromobenzonitriles. The asymmetric actions of several lipases and alcohol dehydrogenases (ADHs) have been considered in the search for the optimal production of enantiopure 2-(2-hydroxypropyl)benzonitriles, which have shown an excellent versatility as key intermediates for the preparation of the target molecules.

Results and discussion

Hydrolase-catalyzed kinetic resolution[7] and dynamic kinetic resolution[8] of racemic 1-phenylpropan-2-ols have been widely reported through acylation processes using a variety of acyl donors and biocatalysts. In spite of the fact that lipases act with a different degree of selectivity in the resolution of 1-phenylpropan-2-ols,[9] their action towards 2-(2-hydroxypropyl)benzonitriles still remains unexplored. The reaction of commercially available 2-bromobenzonitrile (**1a**) with a solution of butyl lithium in THF as solvent, followed by the addition of propylene oxide in the presence of boron trifluoride etherate, afforded racemic 2-(2-hydroxypropyl)benzonitrile (**2a**) in 71% yield (Scheme 1).

The lipase-catalyzed kinetic resolution of (±)-**2a** was then analyzed using 3 equiv. of vinyl acetate and variable biocatalyst loadings and solvents at 30 °C. *Candida cylindracea* lipase (CCL), porcine pancreas lipase (PPL), lipase AK from *Pseudomonas fluorescens*, *Thermomyces lanuginosa* lipase (TLL) and alcalase CLEA all displayed very low conversions and poor enantiodiscrimination values, while *Candida antarctica* lipase type A (CAL-A), *Candida antarctica* lipase type B (CAL-B) and *Burkholderia cepacia* lipase (PSL-IM) led to higher reactivities, with close to 50% conversion, but very poor selectivities ($E < 20$). However, the use of *Rhizomucor miehei* lipase (RML IM) as a biocatalyst led to excellent enantioselectivities ($E > 150$) at different temperatures and variable protein loadings (Table 1, entries 1–3). Optimal results were found when using a substrate : enzyme ratio

Universidad de Oviedo, Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, Avenida Julián de Clavería 8, Oviedo 33006, Spain. E-mail: vicgotfer@uniovi.es, vgs@uniovi.es; Fax: +34 985103451; Tel: +34 985103448

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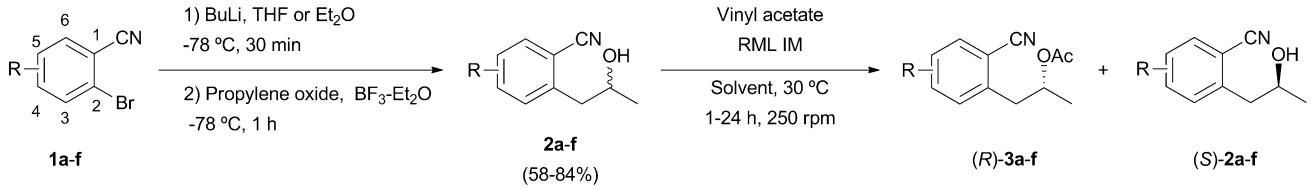


Scheme 1 Chemical synthesis and kinetic resolution of racemic alcohols **2a–f** using *Rhizomucor miehei* lipase.

of 1 : 2 at 30 °C, with both substrate and product being recovered in 96% ee after 8 h (entry 3). Similar results in terms of excellent selectivities were achieved with other solvents, such as 1,4-dioxane, THF, toluene or vinyl acetate as both solvent and acyl donor, although with a lower reactivity (entries 4–7).

To examine the scope of RML IM lipase, the kinetic resolution of the substituted benzene derivatives **2b–f** was studied. Racemic alcohols **2b–e** were prepared in moderate to high yields using an identical synthetic approach. However, for the 4-fluorinated derivative **1f**, 2-bromo-4-fluoro-3-(2-hydroxypropyl) benzonitrile was isolated as the unique reaction product due to the fluorine promoted metallation at the C3-position.[11] The use of Et_2O instead of THF as reaction medium led to the bromine–lithium exchange, the desired alcohol **2f** being obtained in 73% isolated yield. Lipase-catalyzed acetylation led to the production of all the acetates (*R*)-**3b–f** in a range between 92 and 99% ee using TBME as solvent (entries 8–12). However only non-substituted alcohol **2a** (entry 3, $E = 194$) and 4-substituted **2b,f** ($E > 200$, entries 8 and 12) were acetylated with very good to excellent enantioselectivities. On the other hand, the 5-substituted **2c,d** (entries 9 and 10) and the 3-methylated substrate **2e** (entry 11) led to moderate selectivities, the reaction rate being much lower for **2e**, which posses the substitution closer to the reacting centre.

In order to improve the synthetic access to enantiopure precursors of 3-methyl-3,4-dihydroisocoumarins, and motivated by the high potential shown by redox enzymes in the bioreduction of 1-aryl-2-propanones,[12] we decided to explore the potential of alcohol dehydrogenases for the production of enantioenriched **2a–f**. To do this, racemic alcohols **2a–f** were chemically oxidized, the bioreduction of the ketones **4a–f** obtained in this way was then studied. The Dess–Martin reagent was chosen to perform the chemical oxidations due to the mild conditions required, affording **4a–f** in good yields (Scheme 2).

A series of ADHs were tested for the bioreduction of **4a**, including alcohol dehydrogenases from *Rhodococcus ruber* (ADH-A), *Candida parapsilosis* (ADH-CP) or *Lactobacillus kefir* (ADH-LK), and also the very well known Baker's yeast.[13] Initial experiments were carried out in the mg-scale, obtaining alcohol (*S*)-**2a** in high to excellent enantiomeric excess, except for the reaction with ADH-LK which, following the anti-Prelog stereoselectivity rule, led to the opposite enantiomer (Table 2, entries 1–4). On the basis of these results, and bearing in mind the scaling-up of the biocatalytic processes for further synthetic applications, ADH-A overexpressed in *E. coli* was successfully applied in the bioreduction of **4a**, yielding identical results to those of commercially available ADH-A (entry 5).

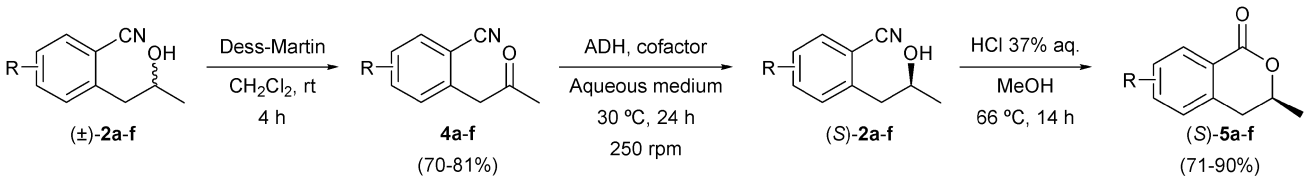
Using our knowledge of the best reaction conditions, an extension of this enzymatic study was done for substituted ketones **4b–f**. The formation of the enantiopure (*S*)-alcohols in complete conversion was detected after 24 h for all the substrates, with the exception of the bioreduction of the 3-methyl derivative (**4e**), which reached 49% conversion (data not shown). This may be explained by the hindering effect of the methyl group which is very close to the carbonyl group. Biocatalytic reactions were subsequently scaled-up to 80 mg of substrate. In all cases complete conversions (entries 6–11) were obtained, longer reaction times (72 h) only being required for ketone **4e** (entry 10). Finally, the intramolecular cyclisation of alcohols (*S*)-**2a–f** in acidic medium led to the enantiopure 3-methyl-3,4-dihydroisocoumarins (*S*)-**5a–f** after purification by extraction with CH_2Cl_2 (65–90%).[14]

In order to expand the applicability of these enantiomerically pure alcohol intermediates, we decided to move towards the asymmetric synthesis of structurally similar compounds. The possibility of using the same alcohol precursors highlights the importance of this divergent synthetic approach, especially

Table 1 Lipase-catalyzed kinetic resolution of 2-(2-hydroxypropyl)benzonitriles **2a–f** using vinyl acetate as an acyl donor

Entry	Substrate	RML IM : **2a–f**[a]	*T*/°C	Solvent	*t*/h	ee_p[b] (%)	ee_s[b] (%)	*c*[c] (%)	*E*[d]
1	**2a**	1 : 1	30	TBME	23	99	63	39	> 200
2	**2a**	1 : 1	45	TBME	8	> 99	35	26	> 200
3	**2a**	2 : 1	30	TBME	8	96	96	50	194
4	**2a**	2 : 1	30	1,4-Dioxane	23	> 99	32	24	> 200
5	**2a**	2 : 1	30	THF	23	> 99	14	12	> 200
6	**2a**	2 : 1	30	Toluene	23	98	85	47	> 200
7	**2a**	2 : 1	30	Vinyl acetate	23	98	44	31	153
8	**2b** (R = 4-Me)	2 : 1	30	TBME	8	96	99	51	> 200
9	**2c** (R = 5-OMe)	2 : 1	30	TBME	24	92	80	47	59
10	**2d** (R = 5-Me)	2 : 1	30	TBME	9	95	61	39	73
11	**2e** (R = 3-Me)	2 : 1	30	TBME	9	94	20	17	39
12	**2f** (R = 4-F)	2 : 1	30	TBME	24	99	57	37	> 200

[a] Ratio enzyme : substrate in weight (w/w). [b] Enantiomeric excesses of product (ee_p) and substrate (ee_s) determined by HPLC. [c] Conversion values calculated as $c = ee_s/(ee_s + ee_p)$. [d] Enantiomeric ratio calculated as $E = \ln[(1 - ee_s)/1 + (ee_s/ee_p)]/\ln[(1 + ee_s)/1 + (ee_s/ee_p)]$.[10]



Scheme 2 Chemoenzymatic synthesis of 3-methyl-3,4-dihydroisocoumarins through bioreduction processes.

Table 2 Bioreduction of ketones **4a–f** in aqueous medium at 30 °C and 250 rpm

Entry	Ketone	ADH[a]	*t*/h	ee_p[b] (%)	*c*[c] (%)
1	**4a**	ADH-A	24	>99	>97
2	**4a**	Baker's yeast	24	96	>97
3	**4a**	ADH-CP	24	>99	>97
4	**4a**	ADH-LK	24	98	38
5	**4a**	*E. coli*/ADH-A	24	>99	>97
6[d]	**4a**	*E. coli*/ADH-A	24	>99	>97 (>97)
7[d]	**4b**	*E. coli*/ADH-A	24	>99	>97 (93)
8[d]	**4c**	*E. coli*/ADH-A	24	>99	>97 (>97)
9[d]	**4d**	*E. coli*/ADH-A	24	>99	>97 (82)
10[d]	**4e**	*E. coli*/ADH-A	72	>99	>97 (62)
11[d]	**4f**	*E. coli*/ADH-A	24	>99	>97 (77)

[a] (*S*)-Alcohols **2a–f** were obtained as final products with the exception of the bioreduction of **4a** with ADH-LK. [b] Determined by HPLC. [c] Determined by GC. [d] Reactions in 80 mg scale of substrate. Isolated yields in brackets.

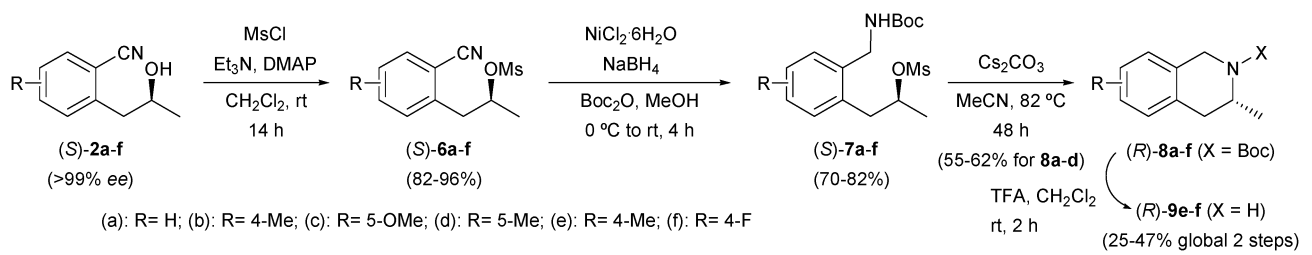
when the desired targets are isoquinoline derivatives, a class of nitrogenated compounds that are widely distributed in Nature and show a potent physiological action.[15] As a result of their importance, a number of research groups have reported the asymmetric synthesis of isoquinoline derivatives.[16] Biocatalytic production of these derivatives is nowadays mainly based upon lipase-catalyzed kinetic resolution,[17] bioreduction of cyclic imines with metalloenzymes[18] or deracemisation experiments using evolved monoamine oxidases combined with non-selective reducing agents.[19] However, little attention has been paid to the asymmetric synthesis of 3-substituted tetrahydroisoquinolines,[20] a class of compounds that not only shows remarkable biological activity[21] but also offers practical applications in asymmetric catalysis.[22]

Taking advantage of the versatility of (*S*)-**2a–f**, our aim was to design a general synthetic pathway for the synthesis of 3-methyl-1,2,3,4-tetrahydroisoquinolines. To do this, alcohols were mesylated in dichloromethane at room temperature, and then different methods to reduce the nitrile group were attempted. The mild reduction procedure with $NiCl_2$–$NaBH_4$,[23] followed by an *in situ* protection of the amine formed, was found to be an ideal strategy for the preparation of the diprotected compounds (*S*)-**7a–f** (Scheme 3). The intramolecular cyclisation of (*S*)-**7a–f** in the presence of cesium carbonate yielded enantiopure *N*-Boc amines (*R*)-**8a–f**, a minimum presence of the corresponding *tert*-butyl 2-((1*E*)-prop-1-en-1-yl)benzyl carbamates (2–10%) being observed in all cases. The chromatographic purification of the reaction crudes afforded the *N*-protected amines **8a–d** in moderate yield.

However, the *N*-Boc tetrahydroisoquinolines **8e,f** decomposed in silica gel, so further transformations were required for the isolation of these amines. Then, (*R*)-**8e,f** were chemically deprotected with trifluoroacetic acid in dichloromethane, yielding (*R*)-**9e** in 47% overall yield after flash chromatography. Purification of (*R*)-**9f** proved unsuccessful, since it shows a high instability in the chromatographic purification step. To avoid this drawback we designed an alternative procedure using *p*-nitrobenzaldehyde as a scavenger for the remaining amounts of 1-(2-((1*E*)-prop-1-en-1-yl)-*p*-fluorophenyl)methanamine, followed by an extraction purification step affording (*R*)-**9f** in high chemical purity and low yield. In order to confirm the absolute configuration of the final products, we have synthesized (*R*)-3-methyl-1,2,3,4-tetrahydroisoquinoline (**9a**) from **8a**. The optical rotation value is in concordance with previously published data for (*R*)-**9a**.[24]

Conclusions

2-(2-Hydroxypropyl)benzonitriles have been found to be adequate building blocks in the divergent asymmetric synthesis of enantiopure substituted 3-methyl-3,4-dihydroisocoumarins and 3-methyl-1,2,3,4-tetrahydroisoquinolines. *Rhizomucor miehei* lipase has displayed the best results in the lipase-catalyzed acetylation of racemic alcohols. However, enantioselectivity was found to be highly dependent on the ring pattern substitution, with moderate stereoselectivities being found for 3- and 5-monosubstituted alcohols, and very high to excellent values being achieved with none or 4-substituted derivatives. The chemical oxidation of racemic alcohols and subsequent bioreduction of the related ketones provided access to a series of novel enantiopure 2-(2-hydroxypropyl)benzonitriles, ADH-A



Scheme 3 Chemoenzymatic synthesis of 3-methyl-1,2,3,4-tetrahydroisoquinolines.

from *Rhodococcus ruber* being found as an excellent asymmetric biocatalyst for this reduction step. The enantiopure alcohols so obtained were treated in acidic medium, leading to a family of substituted (*S*)-3-methyl-3,4-dihydroisocoumarins in moderate to very high yield by intramolecular cyclisation. Taking advantage of their versatility, alcohol precursors were used in the synthesis of enantiopure *N*-Boc-3-methyl-1,2,3,4-tetrahydroisoquinolines through a three-step synthesis in a very straightforward manner and without loss of the optical purity.

Experimental

General procedure for the synthesis of 2-(2-hydroxypropyl)-benzonitriles 2a–f

To a solution of the corresponding commercially available 2-bromobenzonitriles **1a–f** (1 equiv.) in THF (or Et_2O for **1f**, 0.2 M), BuLi (1.1 equiv., 1.6 M in hexane) was added dropwise at −78 °C under an inert atmosphere. The solution was stirred for 30 min at −78 °C and then, propylene oxide (1.5 equiv.) and boron trifluoride etherate (1.5 equiv.) were added dropwise. The mixture was stirred at −78 °C for additional 1 h, until the reaction was quenched with water. The mixture was extracted with Et_2O (3 × 20 mL), organic layers were collected, dried over Na_2SO_4, and the solvent evaporated under reduced pressure. The resulting crude was purified by flash chromatography (50% Et_2O/hexane), obtaining the corresponding alcohols **2a–f** in high yields (69–84%).

General procedure for the synthesis of racemic 1-(2-cyanophenyl)propan-2-yl acetates 3a–f

To a solution of the corresponding racemic alcohols **2a–f** (1 equiv.) in dry CH_2Cl_2 (0.1 M), triethylamine (3 equiv.), 4-(*N*,*N*-dimethylamino)pyridine (0.3 equiv.) and acetic anhydride (2 equiv.) were successively added at rt under an inert atmosphere. The mixture was stirred and the reaction monitored by TLC analysis (50% Et_2O/hexane) until no starting material was detected (30–60 min). Solvent was evaporated afterwards and the crude purified by flash chromatography (50% Et_2O/hexane), obtaining the corresponding 1-(2-cyanophenyl)propan-2-yl acetates **3a–f** in high to excellent yields (82–98%).

General procedure for the synthesis of 2-(2-oxopropyl)-benzonitriles 4a–f

To a solution of the corresponding racemic alcohols **2a–f** (1 equiv.) in CH_2Cl_2 (0.1 M), the Dess–Martin reagent (1.5 equiv.) was added at rt under an inert atmosphere. The mixture was stirred for 4 h. Then, the reaction was quenched with an aqueous saturated 1 : 1 solution of $Na_2S_2O_3$–$NaHCO_3$. The mixture was extracted afterwards with CH_2Cl_2, organic layers were collected, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The resulting crude was purified by flash chromatography (50% Et_2O/hexane), affording the corresponding ketones **4a–f** in high yields (70–81%).

General procedure for the synthesis of 3-methylisochroman-1-ones 5a–f

The corresponding alcohols **2a–f** (1 equiv.) were dissolved in a 1 : 1 solution of MeOH and hydrochloric acid (37%) (0.02 M). The solution was warmed to 66 °C and stirred overnight under reflux. The reaction was quenched with water and extracted with CH_2Cl_2 (3 × 10 mL). Organic layers were collected, dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure, affording the corresponding lactones **5a–f** without further purification in high yields (71–90%).

General procedure for the synthesis of 1-(2-cyanophenyl)propan-2-yl methanesulfonates 6a–f

To a solution of the corresponding alcohols **2a–f** (1 equiv.) in CH_2Cl_2 (0.1 M), triethylamine (2 equiv.), 4-(*N*,*N*-dimethylamino)-pyridine (0.1 equiv.) and methanesulfonyl chloride (2 equiv.) were successively added under an inert atmosphere. The reaction was stirred overnight at rt and then the solvent evaporated under reduced pressure. The resulting crude was purified by flash chromatography (50% Et_2O/hexane), to afford the corresponding 1-(2-cyanophenyl)propan-2-yl methanesulfonates **6a–f** in good yields (82–96%).

General procedure for the synthesis of 1-[2-(((*tert*-butoxycarbonyl)amino)methyl)phenyl]propan-2-yl methanesulfonates 7a–f

To a solution of the corresponding 1-(2-cyanophenyl)propan-2-yl methanesulfonates **6a–f** (1 equiv.) in MeOH (0.13 M), $NiCl_2 \cdot 6H_2O$ (0.1 equiv.), di-*tert*-butyldicarbonate (2 equiv.) were added under an inert atmosphere. Then, the mixture was cooled to 0 °C and $NaBH_4$ (7 equiv.) was added in small portions over 15 min. The reaction was warmed to rt afterwards and stirred for 4 h. Then, the solution was filtered over Celite®, the solvent evaporated under reduced pressure and the resulting crude purified by flash chromatography (50% Et_2O/hexane), affording the corresponding protected amines **7a–f** with good yields (70–82%).

General procedure for the synthesis of *tert*-butyl 3-methyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylates 8a–d

To a solution of the corresponding 1-[2-(((*tert*-butoxycarbonyl)amino)methyl)phenyl]propan-2-yl methanesulfonates **7a–d** (1 equiv.) in dry MeCN (0.1 M), cesium carbonate was added (10 equiv.) under an inert atmosphere. The mixture was stirred at 82 °C for 2 days. Then, water was added and the reaction extracted with CH_2Cl_2 (3 × 10 mL), organic layers were collected, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The resulting crude was purified by flash chromatography (5% EtOAc/hexane), affording the corresponding *tert*-butyl 3-methyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylates **8a–d** in good yields (55–62%).

General procedure for the synthesis of 3-methyltetrahydroisoquinolines 9a,e

To a solution of the corresponding 1-[2-(((*tert*-butoxycarbonyl)-amino)methyl)phenyl]propan-2-yl methanesulfonates **7a,e** (1 equiv.) in dry MeCN (0.1 M), cesium carbonate was added (10 equiv.) under an inert atmosphere. The mixture was stirred at 82 °C for 2 days. Then, water was added and the reaction extracted with CH_2Cl_2 (3 × 10 mL), organic layers were collected, dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The mixture was then dissolved in a 1 : 1 mixture of CH_2Cl_2 and TFA (0.025 M), stirred for 2 h, and then basified with NaOH (3 N) and extracted with CH_2Cl_2.

Organic layers were collected, dried over Na_2SO_4, filtered, the solvent was evaporated under reduced pressure, and the resulting crude purified by flash chromatography (10% MeOH/CH_2Cl_2), affording the corresponding 3-methyltetrahydroisoquinolines **9a,e** in moderate global yields for the two steps (47–54%).

General procedure for the synthesis of (*R*)-6-fluoro-3-methyl-1,2,3,4-tetrahydroisoquinoline 9f

To a solution of (*S*)-1-(2-(((*tert*-butoxycarbonyl)amino)-methyl)-5-fluorophenyl)propan-2-yl methanesulfonate **7f** (53 mg, 0.15 mmol) in dry MeCN (1.5 mL), cesium carbonate was added (478 mg, 1.46 mmol) under an inert atmosphere. The mixture was stirred at 82 °C for 2 days. Then, water was added and the reaction extracted with CH_2Cl_2 (3 × 15 mL), organic layers were collected, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The resulting crude was then dissolved in CH_2Cl_2 (2 mL) and TFA (2 mL) was added. The mixture was stirred for 2 h at rt, and then basified with NaOH (3 N), and extracted with CH_2Cl_2 (3 × 15 mL). Organic layers were collected, dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The mixture was then dissolved in CH_2Cl_2 (10 mL) and 4-nitrobenzaldehyde (15 mg, 0.10 mmol) and $MgSO_4$ (100 mg, 0.83 mmol) were added. The mixture was stirred for 30 min. Then, the mixture was extracted with a pH 4.5 NaOAc/HCl buffer (30 mL). The aqueous phase was afterwards basified with NaOH (3 N) and extracted with CH_2Cl_2 (3 × 10 mL). Organic layers were collected, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure, affording pure **9f** in low yield (6 mg, 25%).

General procedure for the lipase mediated kinetic resolution of alcohols 2a–f

To a suspension of racemic alcohols **2a–f** (0.2 mmol, 1 equiv.) and the adequate lipase in dry solvent (2 mL), vinyl acetate (55 μL, 0.6 mmol) was added under an inert atmosphere. The reaction was shaken at 30 °C and 250 rpm, taking regularly aliquots that were analyzed by HPLC until around 50% conversion was reached. Then the reaction was stopped, and the enzyme filtered with CH_2Cl_2 (3 × 10 mL). The solvent was evaporated and the crude of the reaction purified by flash chromatography on silica gel (50% Et_2O/hexane), obtaining the corresponding enantiomerically enriched acetates (*R*)-**3a–f** and the alcohols (*S*)-**2a–f** (see Table 1 for additional details).

General procedure for the bioreduction of ketones 4a–f with ADHs

Different protocols were used depending on the enzyme and have been listed below. See Table 2 for additional details.

Bioreduction of 4a–f with ADH-A. In an eppendorf tube containing the corresponding ketone (2 mg) in TRIS–HCl 50 mM pH 7.5 buffer (325 μL), isopropyl alcohol (25 μL), 50 μL of a NADH 10 mM solution in TRIS–HCl buffer and 100 μL of an ADH-A solution (3 U) were successively added. Reaction was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4, analyzing the reaction crude by GC (conversion) and HPLC (enantiomeric excess).

Bioreduction of 4a with Baker's yeast. Baker's yeast (2.2 g) was added to a solution of glucose (282 mg) in H_2O (19 mL) stirring the resulting suspension for 15 min at 30 °C and 250 rpm. After this time the corresponding ketone **4a** (0.29 mmol) was added and the suspension was stirred at 30 °C and 250 rpm. Then the reaction was centrifuged and the supernatant extracted with Et_2O (3 × 20 mL). Organic phases were combined, dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure affording a reaction crude containing alcohol (*S*)-**2a**.

Bioreduction of 4a with ADH-CP. In an eppendorf tube containing **4a** (2 mg, 0.012 mmol) in TRIS–HCl 50 mM pH 7.5 buffer (425 μL), isopropyl alcohol (25 μL), 50 μL of a NADH 10 mM solution in TRIS–HCl buffer and 7.5 μL of ADH-CP (3 U) were successively added. Reaction was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4, analyzing the reaction crude by GC (conversion) and HPLC (enantiomeric excess).

Bioreduction of 4a with ADH-LK. In an eppendorf tube containing **4a** (2 mg, 0.012 mmol) in TRIS–HCl 50 mM pH 7.5 buffer (450 μL), 20 μL of glucose-6-phosphate, 10 μL of glucose 6-phosphate dehydrogenase (3 U), 50 μL of a NADPH 10 mM solution in TRIS–HCl buffer and 2 mg of ADH-LK (3 U) were successively added. Reaction was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4, analyzing the reaction crude by GC (conversion) and HPLC (enantiomeric excess).

Bioreduction of 4a–f with ADH-A overexpressed in *E. coli*. 15 mg of *E. coli*/ADH-A cells (15 mg) were rehydrated in TRIS–HCl 50 mM pH 7.5 buffer (500 μL) inside an eppendorf tube. The mixture was shaken at 250 rpm for 30 min. Then, the corresponding ketones **4a–f** (2 mg), isopropyl alcohol (30 μL) and NADH 10 mM solution (60 μL) were added. The reaction was shaken at 250 rpm and 30 °C for 24 h, analyzing the reaction crude by GC (conversion) and HPLC (enantiomeric excess).

Scale up bioreduction of ketones 4a–f with *E. coli*-ADH-A. To a solution containing rehydrated *E. coli*-ADH-A cells (150 mg) in TRIS–HCl 50 mM pH 7.5 buffer (5 mL), isopropyl alcohol (300 μL), NADH 10 mM in TRIS–HCl 50 mM pH 7.5 solution (600 μL) and the corresponding ketones **4a–f** (80 mg) were successively added. The reaction was shaken until no starting material was detected by GC analysis. Then, the mixture was extracted with Et_2O (3 × 10 mL), organic layers were combined, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure, affording the corresponding alcohols (*S*)-**2a–f** in excellent yields without further purification.

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