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Chemoenzymatic synthesis of optically active Mugetanol isomers: use of lipases and oxidoreductases in fragrance chemistry

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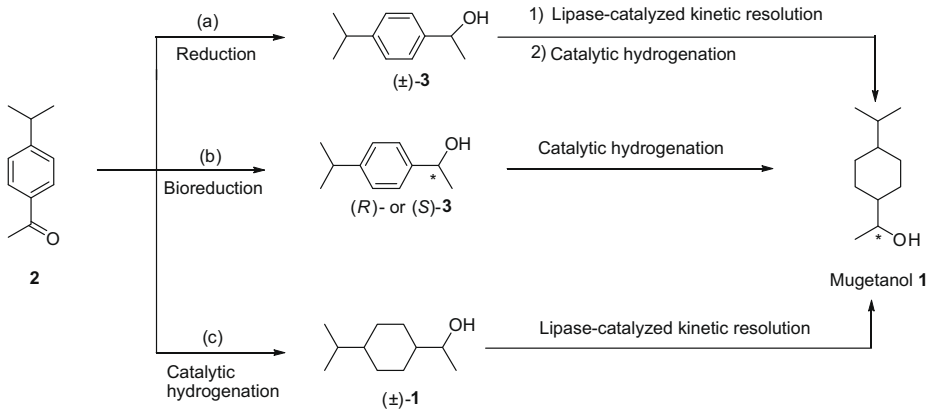
ABSTRACT

Straightforward synthetic strategies for the preparation of optically active Mugetanol isomers have been developed through different independent chemoenzymatic routes implying the use of either alcohol dehydrogenases in aqueous media or lipases in organic solvents coupled with a catalytic hydrogenation process. Among the alcohol dehydrogenases tested, ADH RS1 showed the best activities in the bioreduction of 4-isopropylacetophenone. The lipase from *Pseudomonas cepacia* (PSL-C I) reached high activity values in the lipase-catalyzed transesterification of 4-isopropylphenyl)ethanol, while *Candida antarctica* lipase B showed the best stereopreference in the acetylation of 4-isopropylcyclohexylethanol.

1. Introduction

The synthesis of optically active compounds is a highly challenging task for organic chemists, especially if targeted final products possess remarkable applications for the fine chemical industrial sector.[1] It has been extensively demonstrated along recent years that Biocatalysis offers ecological, economic, scalable, and straightforward methods for the production of fine chemicals with high enantiomeric excess.[2] In fact, the enzymatic preparation of enantiomerically enriched odorants has recently been reviewed,[3] pointing out the importance of using hydrolases for the preparation of different fragrances through chemo- or/and stereoselective acetylation or hydrolytic procedures, but also the effect of the absolute configuration on the odor properties of optically active molecules. For all of that, we have focused our attention in the production of chiral fragrances, looking for the discovery of new odor profiles.



Scheme 1. Chemoenzymatic production of optically active Mugetanol approaches.

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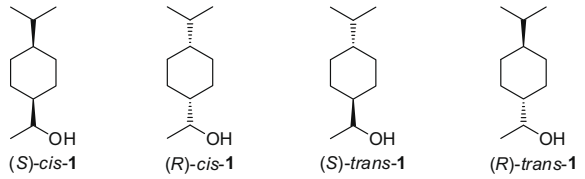


Figure 1. The four stereoisomers of Mugetanol.

Mugetanol [1-(4-isopropylcyclohexyl)ethanol, **1**, Scheme 1] is a fragrance, which possesses four diasteroisomeric forms (Fig. 1), with the (−)-1-(*S*)-*cis* isomer giving the highest odor intensity.[4] In this paper we report the chemoenzymatic stereoselective synthesis of Mugetanol from commercially available 4-isopropylacetophenone using different biocatalysts. Thus, three approaches have been considered depending on the enzyme used in the biocatalytic key step: lipases or alcohol dehydrogenases (ADHs).[5]

2. Results and discussion

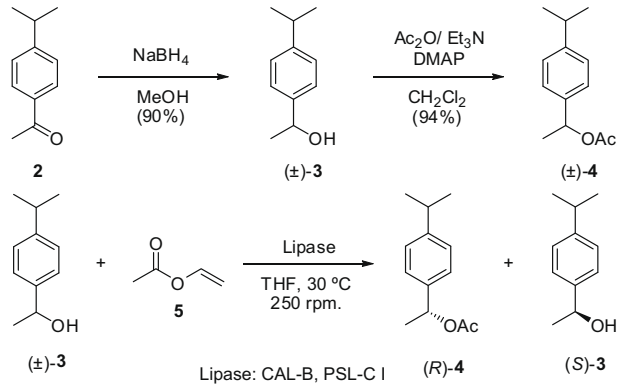
Biocatalytic methods have recently shown a great efficiency in the production of natural flavors and fragrances,[6] highlighting relevant biotransformations using lipases or oxidoreductases. Following this idea, we have considered different stereoselective synthetic routes, for the production of optically active Mugetanol isomers, which are described in Scheme 1:

(a) Chemical reduction of ketone **2**, enzymatic kinetic resolution of alcohol (±)-**3** using lipases and later catalytic hydrogenation to afford optically active **1**.
(b) Bioreduction of **2** using alcohol dehydrogenases and subsequent chemical hydrogenation.
(c) Catalytic hydrogenation of compound **2** producing alcohol (±)-**1** followed by lipase-catalyzed kinetic resolution of this derivative.

Best results have been summarized below, focusing on the use of enzymes for the introduction of chirality in the moiety.

2.1. Method A: Chemical reduction of 4-isopropylacetophenone followed by enzymatic kinetic resolution of (±)-3 and catalytic hydrogenation

Initially, the reduction of ketone **2** was carried out using sodium borohydride in dry MeOH to yield racemic alcohol **3** in 90% yield after flash chromatography (Scheme 2).[7] Next, the chemical acetylation of **3** at room temperature allowed the preparation of racemic acetate **4** with 94% isolated yield using acetic anhydride in the presence of triethylamine, catalytic DMAP, and dry CH_2Cl_2. Adequate chiral HPLC analyses were developed for both alcohol and acetate in order to achieve a reliable method to measure the enantiomeric excesses of both remaining substrate and the final product from the lipase-catalyzed kinetic resolution.



Scheme 2. Synthesis of racemic alcohol **3** and acetate **4**, and lipase-catalyzed kinetic resolution of (±)-**3** using vinyl acetate in THF at 30 °C.

Although over the last few decades many asymmetric transformations have been developed for the preparation of enantiopure compounds, the enzymatic kinetic resolution of racemic mixtures remains the most popular method to prepare enantiomerically pure compounds.[8] For this reason, the lipase-catalyzed transesterification of racemic alcohol **3** was carried out using three equivalents of vinyl acetate **5** (VA) and *Candida antarctica* lipase B (CAL-B) or *Pseudomonas cepacia* lipase (PSL-C I) as biocatalysts.

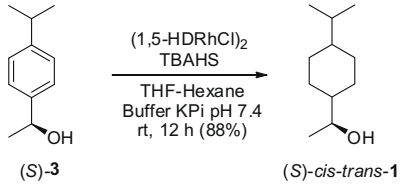
All the experimental data are summarized in Table 1. Reactions were performed at 30 °C observing that both lipases showed a complete stereopreference for the acetylation of the (*R*)-isomer (entries 4 and 7). These results are in accordance with Kazlauskas' rule[9] and previous results obtained by Saville and Kazlauskas.[10] Under the same reaction conditions, a higher reaction rate was observed for PSL-C I (entries 5–7) rather than for CAL-B (entries 1–4), isolating acetate (*R*)-**4** and alcohol (*S*)-**3** with very high yields after flash chromatography.

Table 1
Lipase-catalyzed acetylation of (±)-**3** using vinyl acetate and THF at 30 °C and 250 rpm

Entry	Enzyme	t (h)	ee_S[a] (%)	ee_P[a] (%)	c[b] (%)	E[c]
1	CAL-B	2.5	29	⩾99	22	⩾200
2	CAL-B	4.5	42	⩾99	30	⩾200
3	CAL-B	22	94	⩾99	48	⩾200
4	CAL-B	26	98 (96)	⩾99 (82)	49	⩾200
5	PSL-C I	2.5	75	⩾99	43	⩾200
6	PSL-C I	4.5	92	⩾99	48	⩾200
7	PSL-C I	6	⩾99 (96)	98 (88)	50	⩾200

[a] Enantiomeric excesses were determined by chiral HPLC and isolated yields in brackets.
[b] $c = [ee_S/(ee_S + ee_P)]$.
[c] $E = \ln[(1 - c) \times (1 - ee_S)]/\ln[(1 - c) \times (1 + ee_S)]$.[11]

Next, the catalytic hydrogenation of (*S*)-**3** was carried out in an autoclave at room temperature following a similar procedure to the one described for Januszkiewicz and Alper.[12] Enantiopure (*S*)-**3**, obtained from PSL-C I catalyzed acetylation (Table 1, entry 7), was reacted with hydrogen (80 atm), a catalytic amount of chloro-(1,5-hexadiene)rhodium, and tetrabutylammonium hydrogen sulfate (TBAHS) as a phase transfer catalyst in a mixture of THF/hexane/phosphate buffer, pH 7.4, to give the corresponding alcohol **1** as a mixture (*S*)-*cis*-*trans* in 88% yield after flash chromatography (Scheme 3).



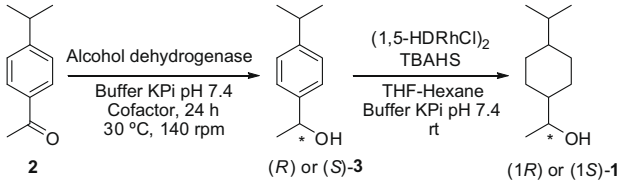
Scheme 3. Catalytic hydrogenation of (*S*)-**3** to afford (*S*)-Mugetanol as a *cis*–*trans* mixture.

Significant diasteroisomeric excess (64%) was observed for optically active Mugetanol (1*S*)-**1**, as a mixture of both *cis*- and *trans*-isomers, with (1*S*)-*cis* obtained as the major isomer. Investigation of the ^{13}C NMR experiment of (1*S*)-**1** allowed the correlation of signals integration with major isomer. The relative configuration of isomers was attributed based on the chemical shift of carbons-1 and 4 located in cyclohexyl moiety. The *cis*-isomer occurs as an equilibrium of two principal conformers. In this case, the substi-

tuted groups were located in axial and equatorial positions to give a γ-gauche effect. Hence, lower chemical shift was observed for carbons-1 and 4 (δ 43.3 and 42.0, respectively) in the *cis*-isomer. No γ-gauche effect occurs for the *trans*-isomer existing as a principal stable conformation with both groups in equatorial position. This feature is responsible for the higher chemical shift values for carbons 1 and 4, at 45.2 and 44.0 ppm, respectively. These data are in accordance with those described in the literature for similar compounds.[13] Likewise, it was possible to attribute *cis* configuration for major isomers for compounds **6** and **7**, see Section 2.3.

2.2. Method B: Bioreduction of 4-isopropylacetophenone and later catalytic hydrogenation

A complementary approach for the synthesis of Mugetanol was studied using different commercial alcohol dehydrogenases for the bioreduction of ketone **2** in an aqueous system and later catalytic hydrogenation (Scheme 4).



Scheme 4. Stereoselective bioreduction of ketone **2** and later catalytic hydrogenation.

All the enzymatic processes were carried under the optimal reaction conditions for each ADH (see Section 4). The results are summarized in Table 2, with opposite stereoselectivies achieved for the different ADHs tested in the bioreduction of **2**. Thus, ADH type T, CP, RS1, and A showed a complete selectivity for the production of (*S*)-**3**, meanwhile ADH type LB and PR2 provided enantiopure (*R*)-**3**. It is noteworthy that the high conversions were achieved for (*S*)-**3** when ADH A and especially ADH RS1 were employed, while (*R*)-**3** was obtained with the highest conversion when using ADH LB.

Table 2
Stereoselective bioreduction of ketone **2** at 30 °C and 140 rpm during 24 h

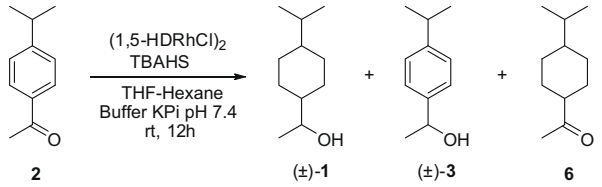
Entry	ADH	ee_p[a] (%)	c[b] (%)	Config.
1	T	⩾99	74	(*S*)
2	LB	⩾99	76	(*R*)
3	CP	⩾99	65	(*S*)
4	PR2	⩾99	17	(*R*)
5	RS1	⩾99	96	(*S*)
6	A	⩾99	76	(*S*)

[a] Enantiomeric excesses of the optically active alcohol were determined by chiral CG.
[b] Conversion values were calculated by ^{1}H NMR of the reaction crude.

As previously described, the catalytic hydrogenation of optically active **3** with H_2 (80 atm), $(1,5\text{-HDRhCl})_2$, and TBAHS at room temperature can lead to optically active Mugetanol isomers.

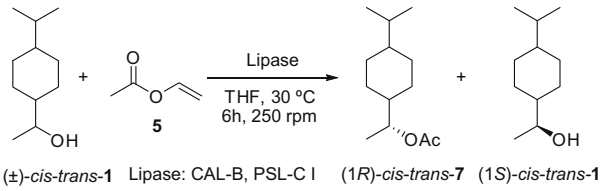
2.3. Method C: Catalytic hydrogenation of 4-isopropylacetophenone and later enzymatic kinetic resolution alcohol (±)-1

Finally, we decided to carry out the catalytic hydrogenation of ketone **2** as the first step in the synthesis of Mugetanol using hydrogen (80 atm), $(1,5\text{-HDRhCl})_2$, and TBAHS. After 12 h, a mixture of four components was obtained: the remaining ketone **2** (20%) and three final products, ketone **6** (16%) and racemic alcohols **1** (32%) and **3** (22%) (Scheme 5). Unfortunately, the separation of the different compounds through flash chromatography of the reaction crude was very difficult due to the similar R_f values of reaction products, which made inadvisable the suggested approach.



Scheme 5. Catalytic hydrogenation of ketone **2**.

However, some alcohol (±)-**1** was recovered and the lipase-catalyzed kinetic resolution was studied in the best experimental conditions previously used for alcohol (±)-**3** (Scheme 6). Data are summarized in Table 3.



Scheme 6. Enzymatic kinetic resolution of (±)-**1** using lipases and vinyl acetate.

Aliquots were regularly analyzed by GC during the time courses of the enzymatic reactions with CAL-B or PS-C I, observing four possible peaks for each compound (acetate and alcohol) because of the appearance of mixture *cis*–*trans*. Mugetanol **1** was obtained with excellent enantiopreference when CAL-B was used in the kinetic resolution system, with 99% ee and 22% de of (*S*)-*cis* (Table 3, entry 1). On the other hand, using PSL-C I Mugetanol **1** was

Table 3
Lipase-catalyzed kinetic resolution of (±)-1 using vinyl acetate at 30 °C and 250 rpm. Reaction time 6 h

Entry	Enzyme	ee **1**[a] (%)		Yield[b] (%)	de[c] (%)	ee **7**[a] (%)		Yield[b] (%)	de[d] (%)	c[e] (%)		E[f]
		(*S*)-*cis*	(*S*)-*trans*			(*R*)-*cis*	(*R*)-*trans*			*cis*	*trans*	
1	CAL-B	⩾99	⩾99	91	22	⩾99	⩾99	87	25	50	50	>200
2	PSL-C 1	60	86	93	16	⩾99	⩾99	74	15	38	46	>200

[a] Enantiomeric excesses were determined by chiral GC.
[b] Isolated yields of a mixture of *cis*- and *trans*-isomers.
[c] Diastereromeric excesses were determined by chiral GC with (*S*)-*cis* being the major isomer.
[d] Diastereomeric excesses were determined by chiral GC with (*R*)-*cis* being the major isomer.
[e] $c = [ee_S/(ee_S + ee_P)]$.
[f] $E = \ln[(1-c)\times(1-ee_S)]/\ln[(1-c)\times(1+ee_S)]$.

obtained with moderate to good enantioselectivity [60% ee to (*S*)-*cis* and 86% to (*S*)-*trans*] and 16% de in favor of (*S*)-*cis*.

Both PSL-C I and CAL-B showed an excellent enantiopreference for the acetylation of the (1*R*)-position, yielding the acetate **7** in 99% ee and with good isolated yields. When CAL-B was used in the kinetic resolution, acetate **7** was obtained with 25% diastereoisomeric excess (de) in favor of *R-cis* isomer (entry 1). In the same conditions, when the reaction was performed using PSL-C I acetate **7** was obtained with 15% de in favor of *R-cis* isomer (entry 2).

3. Conclusions

Three novel chemoenzymatic methodologies have been studied in order to obtain Mugetanol, as well as a great variety of its precursors, starting from commercially available substrates. For this purpose, lipases (CAL-B and PSL-C I) and a set of commercial alcohol dehydrogenases have been employed in the key steps of the different syntheses developed. Excellent enantioselectivities were obtained for the enzymatic kinetic resolution of Mugetanol precursors when using vinyl acetate in biocatalyzed acetylation processes, being acetylated in all cases the *R*-enantiomer. The bioreduction of ketone **2** was also studied to obtain both possible enantiomers of enantiopure alcohol **3** by modifying the biocatalyst employed. The best results were achieved with ADH RS1 for obtaining (*S*)-**3**, while with ADH LB (*R*)-**3** can be obtained with the highest conversion. Thus, by combining some chemical steps with high selective-biocatalyzed processes, the obtention of compounds with high interest in organic chemistry can be achieved.

4. Experimental

4.1. General

C. antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) was a gift from Novo Nordisk Co. *P. cepacia* lipase PSL-C I (1638 U/g) was acquired from Sigma–Aldrich. Alcohol dehydrogenases and glucose dehydrogenases were purchased from Codexis. All other reagents were purchased from Acros, Aldrich or Lancaster and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen. *Flash* chromatographies were performed using Silica Gel 60 (230–240 mesh). High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph UV detector at 210 nm using a Daicel Chiralcel OD or Chirapak IA column (25 cm × 4.6 mm I.D.) varying the conditions depending on the specific substrate. Gas chromatography (GC) analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a CP-Chiralsil DEX CB column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) from Varian for determining the enantiomeric excesses. IR spectra were recorded on using NaCl plates or KBr pellets in a Perkin–Elmer 1720-X FT. ^{1}H, ^{13}C NMR, DEPT, and ^{1}H–^{13}C heteronuclear experiments were obtained using AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) Bruker spectrometers.[14] Inverse gated ^{13}C NMR spectra were recorded on a Bruker Avance DRX-500 (125 MHz) using $CDCl_3$ as solvent. The chemical shifts are given in delta (δ) values. HP1100 chromatograph mass detector was used to record mass spectra experiments (MS) through ESI$^+$ experiments. Measurement of the optical rotation was done in a Perkin–Elmer 241 polarimeter.

4.2. Synthesis of 1-(4-isopropylphenyl)ethanol (±)-3

To a solution under a nitrogen atmosphere of ketone **2** (1.00 g, 12.3 mmol) in dry MeOH (60 mL), sodium borohydride (1.36 g, 36.9 mmol) was slowly added at 0 °C. The reaction mixture was stirred at room temperature for 1 h, [complete consumption of the starting material was observed by TLC analysis (20% EtOAc/hexane)] after which the solvent was evaporated under reduced pressure. The resulting suspension was redissolved in H_2O and extracted with CH_2Cl_2 (3 × 100 mL). Organic phases were combined and dried over Na_2SO_4, the solvent was evaporated under reduced pressure, and the resulting crude purified after flash chromatography (10% EtOAc/hexane) yielding 1.80 g of (±)-**3** as a colorless oil (90%). R_f (20% EtOAc/hexane): 0.25; IR (NaCl): ν 3401, 2963, 2361, 1643, 1460, 1366, 1296, 1209, 1087, 1008, 898, 832 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.31 (d, $^3J_{HH}$ = 6.3 Hz, 2H, H_2, and H_6), 7.22 (d, $^3J_{HH}$ = 6.3 Hz , 2H, H_3, and H_5), 4.85 (q, $^3J_{HH}$ = 6.5 Hz, 1H, $H_{1'}$), 2.92 (m, 1H, $H_{1''}$), 2.20 (s, 1H, OH), 1.49 (d, $^3J_{HH}$ = 6.4 Hz, 3H, $H_{2'}$) 1.27 (d, $^3J_{HH}$ = 6.9 Hz, 6H, $H_{2''}$, and $H_{3''}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 148.0 (C_4), 143.1 (C_1), 126.4 (C_2 and C_6), 125.3 (C_3 and C_5), 70.0 ($C_{1'}$), 33.7 ($C_{1''}$), 24.8 ($C_{2'}$), 23.9 ($C_{2''}$ and $C_{3''}$); MS (ESI$^+$, *m*/*z*): 164 (M$^+$, 34%), 149 [(M−CH_3)$^+$, 100%].

4.3. Preparation of 1-(4-Isopropylphenyl)ethyl acetate (±)-4

Over a solution under a nitrogen atmosphere of alcohol (±)-**3** (52 mg, 0.32 mmol) in dry CH_2Cl_2 (3.0 mL), Et_3N (88.8 μL, 0.64 mmol), DMAP (3.9 mg, 0.032 mmol), and Ac_2O (60 μL, 0.64 mmol) were successively added. The reaction mixture was stirred at room temperature for 2 h until complete consumption of the starting material was observed by TLC analysis (20% EtOAc/hexane), then the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography (5% EtOAc/hexane) yielding 62 mg of (±)-**4** as a colorless oil (94%). R_f (20% EtOAc/hexane): 0.50; IR (NaCl): ν 2962, 1737, 1513, 1459, 1371, 1242, 1065, 1021, 944, 831 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.30 (d, $^3J_{HH}$ = 6.3 Hz, 2H, H_2, and H_6), 7.24 (d, $^3J_{HH}$ = 6.3 Hz, 2H, H_3, and H_5), 5.91 (q, $^3J_{HH}$ = 6.5 Hz, 1H, $H_{1'}$), 2.94 (m, 1H, $H_{1''}$), 2.09 (s, 3H, $H_{4'}$), 1.57 (d, $^3J_{HH}$ = 6.6 Hz, 3H,$H_{2'}$) 1.28 (d, $^3J_{HH}$ = 6.9 Hz, 6H, $H_{2''}$, and $H_{3''}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.3 ($C_{3'}$), 148.4 (C_4), 138.8 (C_1), 126.4 (C_2 and C_6), 126.1 (C_3 and C_5), 72.1 ($C_{1'}$), 33.7 ($C_{1''}$), 23.8 ($C_{2''}$ and $C_{3''}$), 21.9 ($C_{4'}$), 21.3 ($C_{2'}$); MS (ESI$^+$, *m*/*z*): 206 (M$^+$, 17%), 131 [(M−CH_3−AcOH)$^+$, 100%].

4.4. Typical procedure for the enzymatic kinetic resolution of racemic alcohol 3

To a suspension under a nitrogen atmosphere of alcohol (±)-**3** (52 mg, 0.32 mmol) and the corresponding enzyme (ratio 1:1 in weight respect to the alcohol) in dry THF (3.2 mL), vinyl acetate (89.4 μL, 0.96 mmol) was added and the reaction mixture was shaken at 30 °C and 250 rpm. Aliquots were regularly analyzed by HPLC until the conversion value reached around 50% after which the reaction was stopped. The enzyme was then filtered and the solvent evaporated under reduced pressure. The reaction crude was finally purified by *flash* chromatography (eluent gradient 5–10% EtOAc/hexane) affording the corresponding optically active (*R*)-**4** [98% ee, $[\alpha]_D^{20} = +78.5$ (*c* 1.2, CH_2Cl_2)] and (*S*)-**3** [>99% ee, $[\alpha]_D^{20} = +82.2$ (*c* 1.2, $CHCl_3$)].

4.5. Catalytic hydrogenation of alcohol 3: Synthesis of optically active [1-(4-isopropylcyclohexyl)ethanol 1 as a *cis–trans* mixture

A suspension of **3** (507.5 mg, 3.09 mmol) over [1,5-HDRhCl]$_2$ (13.5 mg, 0.031 mmol) and TBAHS (431.2 mg, 1.27 mmol) in a mixture of hexane (10.1 mL), phosphate buffer pH 7.4 (2.5 mL), THF (1.5 mL) was charged with H_2 (80 atm) and the mixture was stirred at room temperature in an autoclave. After 12 h, the reaction mixture was filtered in Celite and the filtrate was then extracted with

EtOAc (3 × 20 mL). The organic phases were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography (5% EtOAc/hexane) to yield 446.1 mg of optically active **1** as a colorless oil (88%). R_f (20% EtOAc/hexane): 0.36; IR (NaCl): ν 3360, 2930, 2865, 1453, 1383, 1070, 932 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ *S-cis* 3.72 (m, 1H, $H_{1'}$), 1.59–1.49 (m, 1H, $H_{1''}$), 1.47–1.41 (m, 8H, $2H_2 + 2H_3 + 2H_5 + 2H_6$), 1.15 (d, $^3J_{HH}$ = 6.3 Hz, 3H, $H_{2'}$), 1.18–1.13 (td, $^3J_{HH}$ = 7.0, 1.1 Hz, 2H, H_1, and H_4), 0.83 (d, $^3J_{HH}$ = 6.6 Hz, 6H, $H_{2''}$, and $H_{3''}$); *S-trans* δ 3.51 (m, 1H, $H_{1'}$), 1.59–1.49 (m, 1H, $H_{1''}$), 1.47–1.41 (m, 8H, $2H_2 + 2H_3 + 2H_5$, and $2H_6$), 1.15 (d, $^3J_{HH}$ = 5.9 Hz, 3H, $H_{2'}$), 1.18–1.13 (td, $^3J_{HH}$ = 7.0, 1.1 Hz, 2H, H_1, and H_4), 0.84 (d, $^3J_{HH}$ = 6.8 Hz, 6H, $H_{2''}$, and $H_{3''}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ *S-cis* 72.2 ($C_{1'}$), 43.3 (C_1), 42.0 (C_4), 32.8 ($C_{1''}$), 26.3 (C_3), 26.1 (C_5), 25.6 (C_2), 24.6 (C_6), 21.1 ($C_{2'}$), 20.4 ($C_{2''}$ and $C_{3''}$); *S-trans* δ 69.4 ($C_{1'}$), 45.2 (C_1), 44.0 (C_4), 29.4 (C_3), 29.3 (C_5), 29.0 ($C_{1''}$), 28.7 (C_2), 28.4 (C_6), 20.4 ($C_{2''}$ and $C_{3''}$), 19.5 ($C_{2'}$); MS (ESI^+, *m*/*z*): 152 [($M-H_2O$), 19%], 45 [($M-{}^i$PrCyhex)$^+$, 100%].

4.6. Procedure for the bioreduction of ketone 2 depending on the ADH used in the enzymatic process

4.6.1. ADH T

In an eppendorf tube were added 132 μL (50 U) of enzyme, 12.0 μL of **2**, and 800 μL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.17 mg of $NADP^+$ was added as cofactor and 200 μL of 2-propanol for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.2. ADH LB

In an eppendorf tube were added 38.5 μL (50 U) of enzyme, 12.0 μL of **2**, and 800 μL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.17 mg of $NADP^+$ was added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.3. ADH CP

In an eppendorf tube were added 3 μL (1 U) of enzyme, 30.0 μL of **2**, and 800 μL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.74 mg of NAD^+ was added as cofactor and 200 μL of 2-propanol for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.4. ADH PR2

In an eppendorf tube were added 7.7 μL (1 U) of enzyme, 30.0 μL of **2**, and 800 μL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.74 mg of $NADP^+$ was added as cofactor and 200 μL of 2-propanol for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.5. ADH RS1

In an eppendorf tube were added 3.6 μL (1 U) of enzyme, 30.0 μL of **2**, 54 mg of glucose, and 1 mL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.74 mg of NAD^+ was added as cofactor and 2 μL (1U) of glucose dehydrogenase for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.6. ADH BS2

In an eppendorf tube were added 1.0 μL (1 U) of enzyme, 30.0 μL of **2**, 54 mg of glucose, 35 mg of $CaCO_3$, and 1 mL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.74 mg of NAD^+ was added as cofactor and 2 μL (1U) of glucose dehydrogenase for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.6.7. ADH A

In an eppendorf tube were added 1.5 mg (9 U) of enzyme, 30.0 μL of **2**, and 800 μL of Tris–HCl 0.1 M buffer of pH 7.0. Then, 0.74 mg of $NADP^+$ was added as cofactor and 200 μL of 2-propanol for cofactor regeneration. The reaction mixture was shaken at 140 rpm and 30 °C, and after 24 h the reaction mixture was extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4.

4.7. Catalytic hydrogenation of ketone 2

A suspension of **2** (1.0 g, 6,1 mmol) over $[1,5\text{-HDRhCl}]_2$ (26,5 mg, 0.061 mmol) and TBAHS (849 mg, 2.5 mmol) in a mixture of hexane (20 mL), buffer KPi pH 7.4 (5 mL), and THF (3 mL) was charged with H_2 (80 atm) and the mixture was stirred at room temperature in an autoclave. After 12 h, the reaction mixture was filtered in Celite and the filtrate was then extracted with EtOAc (3 × 20 mL). Organic phases were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography (5% EtOAc/hexane) yielding a mixture of compounds: 331.8 mg of **1**, 220.1 mg of **3**, and 164.0 mg of **6**. Compound **6** was isolated as a colorless oil. R_f (20% EtOAc/hexane): 0.59; IR (NaCl): ν 2930, 2861, 2361, 1710, 1598, 1451, 1366, 1243, 1177 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ *cis* 2.48 (q, $^3J_{HH}$ = 5.0 Hz, 1H, H_1), 2.13 (s, 3H, $H_{2'}$), 2.04–1.91 (m, 3H, $H_{1'} + H_2$, and H_6), 1.56–1.41 (m, 4H, $H_2 + H_3 + H_5$, and H_6), 1.32–1.21 (m, 3H, $H_3 + H_4$, and H_5), 0.83 (d, $^3J_{HH}$ = 6.8 Hz, 6H, $H_{2''}$, and $H_{3''}$); *trans* δ 2.29–2.20 (m, 1H, H_1), 2.21 (s, 3H, $H_{2'}$), 2.04–1.91 (m, 3H, $H_{1'} + H_2$, and H_6), 1.56–1.41 (m, 4H, $H_2 + H_3 + H_5$, and H_4), 1.32–1.21 (m, 3H, $H_3 + H_4$, and H_5), 0.85 (d, $^3J_{HH}$ = 7.4 Hz, 6H, $H_{2''}$, and $H_{3''}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): *cis* δ 211.2 ($C_{1'}$), 51.7 (C_1), 42.8 (C_4), 30.7 ($C_{1''}$), 27.9 ($C_{2'}$), 26.5 (C_3 and C_5), 25.9 (C_2 and C_6), 20.0 ($C_{2''}$ and $C_{3''}$); *trans* δ 212.4 ($C_{1'}$), 51.7 (C_1), 43.3 (C_4), 32.7 ($C_{1''}$), 28.9 (C_3 and C_5), 28.6 (C_2 and C_6), 27.9 ($C_{2'}$), 19.7 ($C_{2''}$ and $C_{3''}$); MS (ESI^+, *m*/*z*): 168 (M^+, 6%), 43 [($M-{}^i$PrCyhex)$^+$, 100%].

4.8. 1-(4-Isopropylcyclohexyl)ethyl acetate (±)-7

Over a solution under a nitrogen atmosphere of alcohol (±)-**1** (20.5 mg, 0.12 mmol) in dry CH_2Cl_2 (1.2 mL), Et_3N (32.2 μL, 0.24 mmol), DMAP (1.5 mg, 0.012 mmol), and Ac_2O (22.5 μL, 0.24 mmol) were successively added. The reaction mixture was stirred at room temperature for 2 h, until complete consumption of the starting material was observed by TLC analysis (20% EtOAc/hexane), then the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography (5% EtOAc/hexane) yielding 18 mg of (±)-**7** as a colorless oil (71%). R_f (20% EtOAc/hexane): 0.67; IR (NaCl): ν 2933, 2864, 2361, 1736, 1454, 1371, 1246, 1116, 1042, 950 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ *R-cis* 4.93 (m, 1H, $H_{1'}$), 2.03 (s, 3H, $H_{4'}$), 1.81–1.37 (m, 11H, $H_1 + 2H_2 + 2H_3 + H_4 + 2H_5 + 2H_6$, and $H_{1''}$), 1.18 (d, $^3J_{HH}$ = 6.5 Hz, 3H, $H_{2'}$), 0.85 (d, $^3J_{HH}$ = 6.6 Hz, 6H, $H_{2''}$, and $H_{3''}$); *R-trans* δ 4.71 (m, 1H, $H_{1'}$), 2.02 (s, 3H, $H_{4'}$), 1.81–1.37 (m, 11H, $H_1 + 2H_2 + 2H_3 + H_4 + 2H_5 + 2H_6$, and $H_{1''}$), 1.15 (d, $^3J_{HH}$ = 6.3 Hz, 3H, $H_{2'}$), 0.84 (d, $^3J_{HH}$ = 6.8 Hz, 6H, $H_{2''}$, and $H_{3''}$) ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ *R-cis* 170.8 ($C_{3'}$), 72.3 ($C_{1'}$), 42.1 (C_4), 40.4 (C_1), 32.7 ($C_{1''}$), 29.2 (C_2), 28.5 (C_6), 26.1 (C_3), 25.0 (C_5), 20.4 ($C_{2''}$ and $C_{3''}$), 17.9 ($C_{2'}$); *R-trans* δ 170.8 ($C_{3'}$), 74.6 ($C_{1'}$), 43.9 (C_4), 42.6 (C_1), 29.2 ($C_{1''}$), 29.2 (C_2), 28.5 (C_6), 26.1 (C_3), 25.4 (C_5), 19.7 ($C_{2''}$ and $C_{3''}$), 17.1 ($C_{2'}$); MS (ESI^+, *m*/*z*): 152 [($M-AcOH$)$^+$, 19%], 43 [(^{i}Pr)$^+$, 100%].

4.9. Typical procedure for the lipase-catalyzed kinetic resolution of racemic alcohol 1

To a suspension under a nitrogen atmosphere of alcohol (±)-**1** (35.4 mg, 0.21 mmol) and the corresponding enzyme (ratio 1:1 in weight respect to the alcohol) in dry THF (2.1 mL), vinyl acetate (58.7 mL, 0.63 mmol) was added and the reaction mixture was shaken at 30 °C and 250 rpm. Aliquots were regularly analyzed by GC until the conversion value reached around 50%, then the reaction was stopped, the enzyme filtered, and the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography (eluent gradient 5–10% EtOAc/hexane) affording the corresponding optically active (*R*)-**7** and (*S*)-**1** as a mixture of *trans*- and *cis*-isomers, being the *cis* isomers obtained as major components.

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14. Alcohol **1** and acetate **5** are given as examples of the numerical locants used for NMR assignment.