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Lentinus strigellus: a new versatile stereoselective biocatalyst for the bioreduction of prochiral ketones

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ABSTRACT

Growing cells of the basiodiomycete *Lentinus strigellus* in potato-dextrose broth have been used for the first time as a biocatalyst in the stereoselective reduction of aromatic and aliphatic ketones. Most of the aromatic ketones were converted into the corresponding optically active alcohols in up to >99% enantiomeric excess under very mild reaction conditions. Among the aliphatic ketones tested, 2-octanone was enzymatically reduced by this microorganism to enantiopure (*S*)-2-octanol with almost complete conversion.

1. Introduction

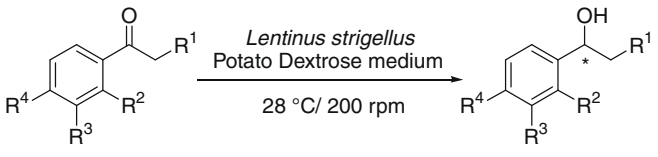
Nowadays, the fine chemical, pharmaceutical, and agrochemical industries are demanding the production of optically active compounds. Chiral alcohols are of great importance to the industrial sector, since these products are normally bioactive compounds and adequate precursors for the synthesis of more complex structures.[1] The asymmetric reduction of prochiral ketones is the most straightforward way to prepare the desired enantiomerically enriched alcohols and traditionally two different approaches have been described: catalytic asymmetric hydrogenation using chiral organometallic complexes[2] or bioreduction reactions catalyzed by oxidoreductases.[3] Both of them have the advantage that optically active alcohols can be theoretically recovered in 100% yield.

Besides well-known chemical methodologies, biocatalytic approaches have gained much attention since they constitute a more environmentally friendly alternative. During the last few years, the development of novel biocatalytic processes has attracted an increasing interest in the field of organic chemistry due to the discovery of new enzymes from Nature or wild-type biocatalysts.[4] This fact has presented biocatalysis as an ideal tool for the production of a wide range of optically active products, which can often be obtained under very mild reaction conditions and in high enantiomeric excesses. In this context, whole-cells from microorganisms and vegetables have been established as efficient biocatalyst in the bioreduction of prochiral ketones.[5]

As part of our ongoing project in the identification, analysis, and application of novel biocatalysts from the Brazilian biodiversity,[6] herein we report an investigation of the basidiomycete *Lentinus strigellus* as reducing agent of aromatic and aliphatic prochiral ketones to the corresponding chiral alcohols. This edible and medicinal mushroom is found in certain parts of South America, and has been reported as a source of bioactive polysaccharides.[7] This is the first report of this fungus as a biocatalyst in organic reactions.

2. Results and discussion

A set of aromatic **1**–**10a** (Scheme 1) and aliphatic **11**–**14a** (Scheme 2) ketones was reduced by growing cells of *L. strigellus* in potato-dextrose broth. Enzymatic reductions of each prochiral



1-10a (*S*)-**1-8b** (*R*)-**9-10b**

1 R^1=H R^2=H R^3=H R^4=H
2 R^1=H R^2=OMe R^3=H R^4=H
3 R^1=H R^2=NO_2 R^3=H R^4=H
4 R^1=H R^2=H R^3=OMe R^4=H
5 R^1=H R^2=H R^3=NO_2 R^4=H
6 R^1=H R^2=H R^3=H R^4=OMe
7 R^1=H R^2=H R^3=H R^4=NO_2
8 R^1=H R^2=H R^3=H R^4=Me
9 R^1=Cl R^2=H R^3=H R^4=H
10 R^1=Cl R^2=H R^3=Cl R^4=Cl

Scheme 1. Bioreduction of prochiral aromatic ketones using *Lentinus strigellus*.

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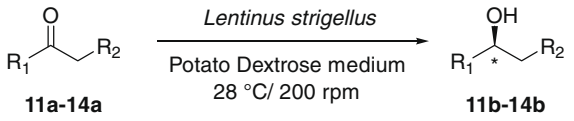

11a R^1=Cy R^2=H
12a R^1=$CH_2(CH_2)_4CH_3$ R^2=H
13a R^1=$CH_2(CH_2)_7CH_3$ R^2=H
14a R^1=CH_2COOCH_3 R^2=Cl

Scheme 2. Bioreduction of prochiral aliphatic ketones using *Lentinus strigellus*.

ketone have been exhaustively investigated by analyzing both substrate and product after 1, 3, and 6 days of reaction, the most relevant data being summarized in Tables 1 and 2. For almost all the ketones studied, *L. strigellus* showed Prelog selectivity, by the insertion of the hydride in the *Re* face of the corresponding ketones. In general, this novel biocatalyst has worked very efficiently in the first 3 days of the enzymatic processes, showing for most of the substrates a decrease in the enantioselectivity at longer reaction times.

Table 1
Lentinus strigellus-biocatalyzed reduction of ketones **1a**–**10a** in potato dextrose medium at 28 °C and 200 rpm[a]

Entry	Ketone	Time (days)	c[b] (%)	ee[b] (%)
1	**1a**	3	83	⩾99 (*S*)
2	**2a**	3	92	⩾99 (*S*)
3	**2a**	6	97	97 (*S*)
4	**3a**	3	48	98 (*S*)
5	**4a**	1	34	97 (*S*)
6	**4a**	3	64	93 (*S*)
7	**5a**	3	⩾99	98 (*S*)
8	**6a**	3	58	⩾99 (*S*)
9	**7a**	1	86	⩾99 (*S*)
10	**7a**	3	98	94 (*S*)
11	**7a**	6	98	62 (*S*)
12	**8a**	3	58	⩾99 (*S*)
13[c]	**9a**	1	85	78 (*R*)
14[c]	**10a**	1	96	91 (*R*)

[a] For more detailed reaction conditions see Section 4.
[b] Conversion (*c*) and enantiomeric excesses (ee) were calculated by HPLC.
[c] Absolute configurations are reversed due to a change in the substituent priority according to the CIP sequence rules.

Table 2
Lentinus strigellus-biocatalyzed reduction of ketones **11a**–**14a** in potato dextrose medium at 28 °C and 200 rpm[a]

Entry	Ketone	Time (days)	c[b] (%)	ee[b] (%)
1	**11a**	3	⩾99	91 (*S*)
2	**12a**	3	97	⩾99 (*S*)
3	**13a**	3	57	63 (*S*)
4	**14a**	1	⩾99	32 (*R*)

[a] For more detailed reaction conditions see Section 4.
[b] Conversion (*c*) and enantiomeric excesses (ee) were determined by GC.

First of all, we selected acetophenone **1a** as a model substrate following the reaction course by HPLC. Excellent enantiomeric excesses (⩾99%) were observed in all reaction times tested. In this case, the optimal conversion (83%) for (*S*)-phenylethan-1-ol, (*S*)-**1b**, which was observed on the third day of reaction (Table 1, entry 1), remained constant during the sixth day.

The influence of an electron-donor (OMe) group and an electron-withdrawing (NO_2) group in the *ortho* position of the aromatic moiety was then investigated, the corresponding alcohols being obtained in almost enantiopure form in both cases (entries 2 and 4) suggesting no influence of these groups on the enzymatic selectivity. Furthermore, the presence of the methoxy group located at the *ortho* position **2a** increased the product formation when compared with acetophenone **1a**. The maximum conversion (97%) was observed in the sixth day of reaction but with a slight reduction of the optical purity of (*S*)-**2b** in comparison with that of the third day (from ⩾99 to 97%, entry 3). The presence of the nitro group at the same position **3a** strongly influenced the product conversion, leading to a 48% of (*S*)-**3b** after three reaction days (entry 4). No increase in the process conversion was observed after 6 days.

Ketones **4a** and **5a** having the methoxy and the nitro groups, respectively, in the *meta* position were also analyzed as substrates of *L. strigellus* (entries 5–7). 3-Methoxyacetophenone **4a** yielded the corresponding alcohol (*S*)-**4b** with the maximum conversion (64%) after 3 days (entry 6). In this case, a slight decrease in (*S*)-**4b** enantiomeric excess (93% ee) was observed when compared with the reaction stopped after one day (97% ee and 34% conversion, entry 5). Reduction of 3-nitroacetophenone **5a** occurred with total conversion after 3 days, indicating that the presence of an electron-withdrawing group in the *meta* position can improve the reaction rate of the *L. strigellus*-biocatalyzed reduction. As shown in Table 1 (entry 7), enantiopure (*S*)-**5b** was achieved.

The influence of two donor substituents (methoxy and methyl) and a withdrawing group (nitro) in the *para* position of the acetophenones was then investigated. The same conversions (58%) and enantiomeric excesses (⩾99%) were observed in the bioreduction of ketones **6a** (entry 8) and **8a** (entry 12) to the corresponding (*S*)-**6b** and (*S*)-**8b**, both presenting electron-donor groups. The enantioselectivity of 4-nitroacetophenone **7a** reduction to (*S*)-**7b** was strongly influenced by the time. After 1 day, (entry 9), enantiopure alcohol can be prepared with 86% conversion. When the reaction was carried out for longer times (3 and 6 days, entries 10 and 11, respectively), a subsequent decrease in the enantiomeric excess of (*S*)-**7b** was observed (62% ee after 6 days). As for the *meta*-derivatives, the presence of an electron-withdrawing group in the *para* position seemed to be better for enzymatic activity when compared with the reduction of ketones with electron-donating substituents.

Hence, acetophenone derivatives series with NO_2 and OMe groups were studied. All nitro derivatives showed maximum conversion at 3 days (entries 4, 7, and 10), the *ortho*-nitro derivative **3a** (48% conversion) being less reactive than the *meta* and *para*-substituted compounds (complete conversions). For these three ketones, the biocatalyst presented a very similar enantioselectivity (98% ee for **3b** and **5b**) while for (*S*)-**7b** a 94% ee was measured. With regard to the methoxy derivatives, all ketones yielded the corresponding alcohols in excellent enantioselectivities, especially for the 2-methoxy and 4-methoxyacetophenones, while concerning the activity, this enzyme presented a clear preference for a methoxy group in the *ortho* position.

After these encouraging results, we decided to extend our studies toward the analysis of 2-substituted ethanones in order to investigate the substrate specificity of *L. strigellus*. Thus, the results obtained from the bioreduction of 2-chloro-1-phenylethanone **9a** and 2-chloro-1-(3,4-dichlorophenyl)ethanone **10a**, both having a chlorine atom in the α-position to the carbonyl group, are presented in entries 13 and 14 of Table 1. It must be highlighted that in comparison with acetophenone **1a**, the presence of the chlorine atom in **9a** produced a slight increase in the product conversion (*R*)-**9b**, with a significant decrease in the enantioselectivity. In the case of compound **10a**, presenting two chlorine atoms in the aromatic ring, (*R*)-**10b** was prepared with higher conversion and enantiomeric excess values when compared to **9a**. No influence of the reaction time was observed in the bioreduction of both chlorinated ketones. The presence of the chlorine atom in the α-position to the carbonyl group in both ketones did not change the direction of the reaction enantioselectivity as the major enantio-

mers were identified as Prelog products (*R* configuration due to CIP priority).

Next, four aliphatic ketones **11a**–**14a** were also subjected to the bioreduction by growing cells of *L. strigellus* (Scheme 2) and the more significant results are summarized in Table 2.

Cyclohexylmethylketone **11a** was completely reduced to the corresponding alcohol **11b** with a very high enantioselectivity (Table 2, entry 1), meanwhile both excellent conversion (97%) and alcohol enantiomeric excess (⩾99%) were measured in the bioreduction of octan-2-one **12a** to the corresponding alcohol (*S*)-octan-2-ol **12b** after 3 days of reaction (entry 2). Significant reduction in conversion and enantiomeric excess values was observed in the bioreduction of the longer chain ketone undecan-2-one **13a** when compared to **12a** (entry 3). The stereochemistry of the major enantiomers obtained from the reduction of ketones **11a**–**13a** was established as (*S*) (Prelog's model) following the order of the elution times of the major enantiomer by GC analysis and comparison with standards previously described.[8] An absolute chemoselectivity was observed in the bioreduction of β-ketoester **14a**. This compound was completely reduced to the corresponding monoalcohol–monoester **14b** after only 1 day, but with low enantioselectivity (32% ee). In this case, the (*R*)-configuration was found for the major enantiomer indicating a Prelog product by comparison with standards previously reported.[9]

3. Conclusions

In summary, a set of prochiral aromatic and aliphatic ketones was reduced to the corresponding enantiomerically enriched alcohols by growing cells of *Letinus strigellus* in moderate to excellent conversions and enantioselectivities, suggesting that this basidiomycete can act as a promising stereoselective biocatalyst. When reducing aromatic ketones, the presence of electron-donor and electron-withdrawing substituents in the aromatic moiety has an important effect in the biocatalytic properties of *L. strigellus*. In case of reducing aliphatic ketones, the size of the alkyl chain has a great effect on both conversions and enantiomeric excess values. The biocatalyst presents a Prelog selectivity, the alcohols of (*S*)-configuration being obtained in all cases, unless for those compounds for which the absolute configuration is reversed due to the CIP rules.

4. Experimental

4.1. General

L. strigellus strain (CC-40) was obtained from Embrapa Recursos Genéticos e Biotecnologia (Brasilia-DF, Brazil). Flash chromatography columns were prepared using Silica Gel 60 (230–240 mesh). High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph, UV detector using a Tracer Spherisorb (25 cm × 4.6 mm), Daicel Chiralcel OB-H (25 cm × 4.6 mm), Chiralpak AS (25 cm × 4.6 mm) or Chirapak IA (25 cm × 4.6 mm) columns, varying the conditions according to the specific substrate. Gas chromatography (GC) analyses were performed on a Hewlett–Packard 6890 Series II chromatograph equipped with a Hewlett–Packard HP-1 (crosslinked methyl siloxane, 30 m × 0.25 mm 0.25 μm, 1.0 bar N_2), a Varian CP-Chiralsil DEX CB (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2), and a Restek RT-βDEXse (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). In all the experiments, the injector temperature was 225 °C and the detector temperature was 250 °C. ^{1}H, ^{13}C NMR and DEPT experiments were obtained using a DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) Bruker spectrometer. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz).[10] Measurement of the optical rotation was done in a Perkin–Elmer 241 polarimeter.

Starting ketones **1**–**2a**, **4a**, **6a**, **8a**, **9a**, **12**–**14a,** and racemic alcohols 1-phenylethan-1-ol (±)-**1b**, octan-2-ol (±)-**12b**, undecan-2-ol (±)-**13b,** and methyl 3-hydroxy-4-chloroacetoacetate (±)-**14b** were purchased from Sigma–Aldrich. Compounds **3a**, **5a**, **7a**, **10a, 11a,** and 1-cyclohexyl ethanol (±)-**11b** were obtained from Alfa Aesar. Racemic acetates from alcohols **11–14b** were synthesized by chemical acetylation of the corresponding alcohols with DMAP and acetic anhydride in ethyl acetate, in order to measure the enantiomeric excesses, with quantitative yields for all the compounds being achieved.[11] All other reagents and solvents were purchased from Aldrich and used without further purification. Absolute configurations of alcohols **1-10b** obtained from the biocatalysis process were determined by comparison of their specific rotation with those previously reported in the literature. For alcohols **11**–**14b**, the absolute configurations were established by comparison of retention times on GC with previously published data.

4.2. Typical procedure for the preparation of racemic alcohols 2–10b[12]

To a solution of the corresponding ketone **2**–**10a** (200 mg) in dry MeOH (4.0 mL) was slowly added sodium borohydride (4 equiv) at 0 °C under nitrogen atmosphere. The reaction was stirred at room temperature during 3 h and followed by TLC analysis (20% EtOAc/hexane) until complete disappearance of the starting ketone. Solvent was evaporated under reduced pressure and the resulting suspension was redissolved in H_2O and extracted with EtOAc (3 × 100 mL). Organic phases were combined and dried over anhydrous Na_2SO_4. After solvent distillation under reduced pressure, the resulting crude product was purified by flash chromatography [10% EtOAc/hexane for **2b** (74% yield), **4b** (74% yield), **6b** (96% yield), **8**–**10b** (94%, 83%, and 90% yield, respectively), and 20% EtOAc/hexane for **3b** (97% yield), **5b** (95% yield), and **7b** (95% yield)].

4.3. General method for the biocatalyzed reduction of ketones 1–14a using *L. strigellus*

Erlenmeyers of 100 mL containing 50 mL of commercial Potato Dextrose (PD) culture medium were previously autoclaved at 121 °C for 15 min and inoculated under aseptic condition with a 7 mm disk of the microorganism (7 days old in PD). After 7 days in a shaker (200 rpm) at 28 °C, 10 mg of the corresponding ketone **1**–**14a** was added to the erlenmeyer. Aliquots of 1 mL were collected after 1, 3, and 6 days reaction from each flask and extracted with ethyl acetate (3 × 2 mL). The organic solvent was evaporated under reduced pressure and the crude products were purified by flash chromatography on silica gel using the adequate eluent mixture. Purified products were analyzed by HPLC or GC in order to determine the enantiomeric purity of the alcohols **1**–**14b**, meanwhile conversion values were calculated from the reaction crudes.

4.3.1. (*S*)-1-Phenylethanol 1b

Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane, 20 °C; t_R **1a** 5.8 min, t_R **1b** 8.3 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 10.2 min, t_R (*R*) 15.4 min. $[\alpha]_D^{25} = -22.9$ (*c* 1.0, CH_2Cl_2) for the (*S*)-enantiomer in >99% ee.

4.3.2. (*S*)-1-(2-Methoxyphenyl)ethanol 2b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.52 (3H, d, J = 6.6 Hz, H_2), 3.88 (3H, s, OCH_3), 5.11 (1H, q, J = 6.6 Hz, H_1), 6.90 (1H, dd, J = 7.5, and 1.5 Hz, $H_{3'}$), 6.98 (1H, dt, J = 8.5, and 1.5 Hz, $H_{5'}$), 7.29 (1H, dt, J = 8.5 and 1.5 Hz, $H_{6'}$), 7.36 (1H, dd, J = 7.5, and 1.5 Hz, $H_{4'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 22.8 (C_2), 55.2 (OCH_3), 66.3 (C_1), 110.3

($C_{3'}$), 120.7 ($C_{5'}$), 126.0 ($C_{6''}$), 128.2 ($C_{4'}$), 133.4 ($C_{1'}$), 156.4 ($C_{2'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R **2a** 6.3 min, t_R **2b** 7.1 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 10.3 min, t_R (*R*) 16.9 min. $[\alpha]_D^{25} = -17.1$ (*c* 0.75, $CHCl_3$) for the (*S*)-enantiomer in >99% ee.[13]

4.3.3. (*S*)-1-(2-Nitrophenyl)ethanol 3b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.55 (3H, d, *J* = 6.3 Hz, H_2), 5.40 (1H, q, *J* = 6.3 Hz, H_1), 7.41 (1H, dt, *J* = 8.1, and 1.2 Hz, $H_{4''}$), 7.63 (1H, dt, *J* = 8.1, and 1.5 Hz, $H_{5'}$), 7.82 (1H, dd, *J* = 8.1, and 1.2 Hz, $H_{6'}$), 7.88 (1H, dd, *J* = 8.1, and 1.5 Hz, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 24.2 (C_2), 65.2 (C_1), 124.2 (C_3'), 127.5 (C'_6), 128.0 (C'_4), 133.5 (C'_5), 140.9 ($C_{1'} + C_{2'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (**3a**) 6.3 min, t_R (**3b**) 8.9 min; Determination of the ee by HPLC: Chiralpak AS, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 24.0 min, t_R (*R*) 22.1 min. $[\alpha]_D^{25} = +18.5$ (*c* 0.23, MeOH) for for the (*S*)-enantiomer in >99% ee.[14]

4.3.4. (*S*)-1-(3-Methoxyphenyl)ethanol 4b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.47 (3H, d, *J* = 6.6 Hz, H_2), 3.81 (3H, s, OCH_3), 4.83 (1H, q, *J* = 6.6 Hz, H_1), 6.81 (1H, dd, *J* = 8.5, and 1.2 Hz, $H_{6'}$), 6.93 (2H, m, $H_{2'}$, and $H_{4'}$), 7.26 (1H, t, *J* = 8.5 Hz, $H_{5'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.0 (C_2), 55.1 (OCH_3), 70.0 (C_1), 110.8 ($C_{2'}$), 112.7 ($C_{4'}$), 117.6 ($C_{6'}$), 129.3 ($C_{5'}$), 147.6 ($C_{1'}$), 159.6 ($C_{3'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R **4a** 5.8 min, t_R **4b** 8.4 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 13.7 min, t_R (*R*) 18.8 min. $[\alpha]_D^{25} = -29.8$ (*c* 0.85, MeOH) for the (*S*)-enantiomer in 97% ee.[15]

4.3.5. (*S*)-1-(3-Nitrophenyl)ethanol 5b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.47 (3H, d, *J* = 6.6 Hz, H_2), 4.96 (1H, q, *J* = 6.6 Hz, H_1), 7.47 (1H, t, *J* = 7.8 Hz, $H_{5'}$), 7.67 (1H, d, *J* = 7.8 Hz, $H_{6''}$), 8.04 (1H, ddd, *J* = 8.3, 2.1, and 0.9 Hz, $H_{4'}$), 8.18 (1H, t, *J* = 1.8 Hz, $H_{2'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.2 (C_2), 69.1 (C_1), 120.2 ($C_{4''}$), 122.1 ($C_{2'}$), 129.3 ($C_{5'}$), 131.6 ($C_{6'}$), 147.8 ($C_{1'}$), 148.1 ($C_{3'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R **5a** 9.2 min, t_R **5b** 12.0 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 13.2 min, t_R (*R*) 14.8 min. $[\alpha]_D^{25} = -20.5$ (*c* 1.0, $CHCl_3$) for the (*S*)-enantiomer in 98% ee.[16]

4.3.6. (*S*)-1-(4-Methoxyphenyl)ethanol 6b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.48 (3H, d, *J* = 6.3 Hz, H_2), 3.82 (3H, s, OCH_3), 4.84 (1H, q, *J* = 6.3 Hz, H_1), 6.88 (2H, d, *J* = 8.7 Hz, $H_{3'}$, and $H_{5'}$), 7.30 (2H, d, *J* = 8.7 Hz, $H_{2'}$, and $H_{6'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 24.3 (C_2), 55.2 (OMe), 69.8 (C_1), 126.6 ($C_{2'}$ and $C_{6'}$), 113.8 ($C_{3'}$ and $C_{5'}$), 137.9 ($C_{1'}$), 158.9 ($C_{4'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (**6a**) 7.5 min, t_R (**6b**) 8.5 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 14.4 min, t_R (*R*) 20.4 min. $[\alpha]_D^{25} = -29.1$ (*c* 1.5, $CHCl_3$) for the (*S*)-enantiomer in 99% ee.[17]

4.3.7. (*S*)-1-(4-Nitrophenyl)ethanol 7b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.50 (3H, d, *J* = 6.6 Hz, H_2), 5.01 (1H, q, *J* = 6.6 Hz, H_1), 7.53 (2H, d, *J* = 8.7 Hz, $H_{2'}$, and $H_{6'}$), 8.17 (2H, d, *J* = 8.7 Hz, $H_{3'}$, and $H_{5'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.3 (C_2), 69.5 (C_1), 123.6 ($C_{2'}$ and $C_{6'}$), 126.0 ($C_{3'}$ and $C_{5'}$), 147.0 ($C_{1'}$), 153.1 ($C_{4'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R **7a** 6.5 min, t_R **7b** 7.7 min; Determination of the ee by HPLC: Chiralpak AS, 0.8 mL/min; 20% 2-propanol/hexane; 20 °C; t_R (*S*) 13.7 min, t_R (*R*) 15.8 min. $[\alpha]_D^{25} = -20.5$ (*c* 1.2, $CHCl_3$) for the (*S*)-enantiomer in >99% ee.[18]

4.3.8. (*S*)-1-(4-Methylyphenyl)ethanol 8b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.47 (3H, d, *J* = 6.6 Hz, H_2), 2.37 (3H, s, CH_3), 4.83 (1H, q, *J* = 6.6 Hz, H_1), 7.17 (2H, d, *J* = 8.1 Hz, $H_{3'}$, and $H_{5'}$), 7.27 (2H, d, *J* = 8.1 Hz, $H_{2'}$, and $H_{6'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 20.9 (Me), 24.9 (C_2), 69.6 (C_1), 125.2 ($C_{2'}$ and $C_{6'}$), 129.0 ($C_{3'}$ and $C_{5'}$), 136.8 ($C_{4'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 3% 2-propanol/hexane; 20 °C; t_R **8a** 5.6 min, t_R **8b** 8.1 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 7.6 min, t_R (*R*) 9.1 min. $[\alpha]_D^{25} = -25.1$ (*c* 0.54, $CHCl_3$) for the (*S*)-enantiomer in >99% ee.[13]

4.3.9. (*R*)-2-Chlorophenylethan-1-ol 9b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 3.56 (1H, dd, *J* = 11.4, and 3.3 Hz, H_2), 3.66 (1H, dd, *J* = 11.4, and 8.7 Hz, H_2), 4.81 (1H, dd, *J* = 8.7, and 3.6 Hz, H_1), 7.30 (5H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, and $H_{6'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 50.8 (C_2), 74.0 (C_1), 126.0 ($C_{2'}$ and $C_{6'}$), 128.4 ($C_{3'}$ and $C_{5'}$), 139.9 ($C_{1'}$). Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R **9a** 4.9 min, t_R **9b** 6.3 min; Determination of the ee by HPLC: Chiralpak IA, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 13.2 min, t_R (*R*) 15.1 min. $[\alpha]_D^{25} = -42.5$ (*c* 0.87, CH_2Cl_2) for the (*R*)-enantiomer in 78% ee.[19]

4.3.10. (*R*)-2-Chloro-1-(3,4-dichlorophenyl)ethan-1-ol 10b

^{1}H NMR ($CDCl_3$, 300.13 MHz): 3.59 (1H, dd, *J* = 11.4, and 8.7 Hz, H_2), 3.72 (1H, dd, *J* = 11.4, and 3.6 Hz, H_2), 4.87 (1H, dd, *J* = 8.7, and 3.6 Hz, H_1), 7.21 (1H, dd, *J* = 8.4, and 1.8 Hz, $H_{6'}$), 7.43 (1H, d, *J* = 8.4 Hz, $H_{5'}$), 7.51 (1H, d, *J* = 1.8 Hz, $H_{2'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 50.4 (C_2), 72.8 (C_1), 125.4 ($C_{6'}$), 128.1 ($C_{2'}$), 130.6 ($C_{5'}$), 132.4 ($C_{4'}$), 132.8 ($C_{3'}$), 140.0 ($C_{1'}$); Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 3% 2-propanol/hexane; 20 °C; 29 bares; t_R **10a** 5.4 min, t_R **10b** 6.4 min; Determination of the ee by HPLC: Chiralcel OB-H, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 13.1 min, t_R (*R*) 17.2 min. $[\alpha]_D^{25} = -32.7$ (*c* 1.0, $CHCl_3$) for the (*R*)-enantiomer in 78% ee.[20]

4.3.11. (*S*)-1-Cyclohexylethanol 11b

Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 150 °C; t_R **11a** 3.7 min, t_R **11b** 4.2 min. Determination of the ee by GC: Chiralsil DEX-CB, 90 °C (5 min), 2.5 °C/min, 105 °C, 5 °C/min, 120 °C, 20 °C/min, 180 °C; t_R (*S*) 13.0 min, t_R (*R*) 13.7 min.[8a]

4.3.12. (*S*)-Octan-2-ol 12b

Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 150 °C; t_R **12a** 3.0 min, t_R **12b** 3.3 min. Determination of the ee by GC: RTβDEXse, 90 °C (5 min), 2.5 °C/min 105 °C, 5 °C/min, 130 °C (2 min) 20 °C/min, 180 °C; t_R (*S*) 14.7 min, t_R (*R*) 16.4 min.[8b]

4.3.13. (*S*)-Undecan-2-ol 13b

Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 100 °C, 5 °C/min, 150 °C; t_R **13a** 8.4 min, t_R **13b** 8.8 min. Determination of the ee by GC: Chiralsil DEX-CB, 90 °C (5 min), 2.5 °C/min, 105 °C, 20 °C/min, 140 °C, 2.5 °C/min, 180 °C; t_R (*S*) 17.0 min, t_R (*R*) 17.7 min.[8c]

4.3.14. (*R*)-Methyl 3-hydroxy-4-chloroacetoacetate 14b

Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 200 °C; t_R (**14a**) 3.6 min, t_R (**14b**) 4.2 min. Determination of the ee by GC: RTβDEXse, 90 °C (5 min), 2.5 °C/min,

105 °C, 5 °C/min, 130 °C (2 min) 20 °C/min, 180 °C; t_R (*S*) 20.3 min, t_R (*R*) 20.6 min.[9]

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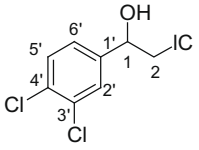
10. Alcohol **10b** is given as example of the numerical locants used for NMR assignment:



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