SAGE-Hindawi Access to Research
Enzyme Research
Volume 2011, Article ID 976368, 8 pages
doi:10.4061/2011/976368

Research Article

Chiral Pharmaceutical Intermediaries Obtained by Reduction of 2-Halo-1-(4-substituted phenyl)-ethanones Mediated by *Geotrichum candidum* CCT 1205 and *Rhodotorula glutinis* CCT 2182

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Received 14 January 2011; Accepted 10 March 2011

Academic Editor: Robert F. H. Dekker

Enantioselective reductions of *p*-R_1-$C_6H_4C(O)CH_2R_2$ (R_1 = Cl, Br, CH_3, OCH_3, NO_2 and R_2 = Br, Cl) mediated by *Geotrichum candidum* CCT 1205 and *Rhodotorula glutinis* CCT 2182 afforded the corresponding halohydrins with complementary *R* and *S* configurations, respectively, in excellent yield and enantiomeric excesses. The obtained (*R*)- or (*S*)-halohydrins are important building blocks in chemical and pharmaceutical industries.

1. Introduction

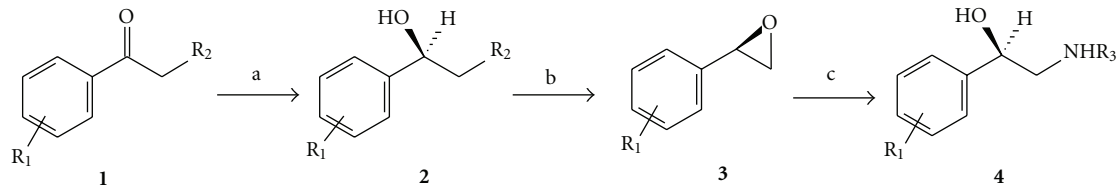
Chiral halohydrins are important and valuable intermediates in the synthesis of fine chemicals and pharmaceuticals as optically active 1,2-aminoalcohols. The halohydrin (*R*)-1-aryl-2-haloethanol may be used for the preparation of (*R*)-1-aryl-2-aminoethanols that are used as α- and β-adrenergic drugs.

An interesting chemoenzymatic synthetic route to obtain optically active 1-aryl-2-ethanolamines is from the enantioselective reduction of the correspondent α-haloacetophenones giving halohydrins that are transformed into an epoxy that reacts with the appropriate amine (Scheme 1) [1, 2].

An enormous potential of the use of microorganisms and enzymes for the transformation of synthetic chemicals with high chemo-, regio-, and enantioselectivity has been increasing in the pharmaceutical industry [3]. The dehydrogenases in the form of whole cells for the production of chiral styrene oxides have been used on a pilot-plant scale [4]. Therefore, a large number of papers have appeared reporting the enantiomeric reduction of α-bromoacetophenone [5–10] and α-chloroacetophenone [4, 6, 7, 11–17] by whole cells of microorganism and also by isolated enzyme [18] giving halohydrins in high enantiomeric excesses (ee).

There are few examples of biocatalytic reduction of α-haloacetophenone having suitable substituted group attached to the aromatic ring for enantioselective preparation of some target 1-aryl-2-ethanolamines [2, 19]. It is known that some examples of biocatalytic reductions of α-haloacetophenone that have substituted groups like 3-chloro [20, 21], 4-nitro [10, 22], and 3,4-methylenedioxy [23–25] were mediated by a number of microorganisms. Also, isolated enzymes have been used to reduce α-haloacetophenone having various kinds of substituted groups [26, 27].

The performances of *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 in bioreduction of α-haloacetophenone have been calling our attention due to the efficiency and complementary enantioselectivity of these microorganisms giving the corresponding (*R*)- and (*S*)-halohydrins in high ee, respectively [8]. Also, those microorganisms show the same efficiency in the reduction of α-azido-*para*-substituted acetophenones [28]. In this work, we use those two microorganisms for reduction of α-bromo- and α-chloroacetophenones having *para*-substituted groups to produce separately both enantiomers of halohydrins that can be used as chiral building blocks for preparations of the corresponding 1,2-aminoalcohols.



R_1 = substituent group; R_2 = Cl or Br; R_3 = H, alkyl or aryl group

Scheme 1: (a) reduction using chiral catalytic reagent or biocatalytic process; (b) base; (c) amine.

2. Materials and Methods

IR spectra were recorded on a Bomem MB Series spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$. Gas chromatographic analyses were performed using a Shimadzu GC/MS Class 5000, with helium as carrier gas. The fused silica capillary columns used were either a Supelco Simplicity ITM (30 m × 0.25 mm × 0.25 μm) and a chiral GC-column CHIRASILDEX (30 m × 0.25 mm × 0.25 μm). Optical rotation was measured with a J-720, VRDM306 JASCO, 589.3 nm (25°C) spectropolarimeter. The melting points were obtained in MQAPF-301-MicroQuímica equipment.

The 2-bromo-1-(4-substituted phenyl)-1-ethanones **1a-e** were *obtained* with brominating 4-substituted acetophenones in CH_2Cl_2 at 0°C, and 2-chloro-1-(4-substituted phenyl)-1-ethanones **1f-j** were prepared applying the Wyman and Kaufman methodology [29] by chlorination of corresponding 4-substituted acetophenones with sulfuryl chloride in CH_2Cl_2 at 0°C. All other reagents and solvents were reagent grade.

The racemic 2-halo-1-(4-substituted phenyl)-ethanols **2a-j**, used as reference for the determination of ee in a GC provided with a chiral column, were obtained by reacting the corresponding **1a-j** with $NaBH_4$ in water/methanol at rt. All other solvents and reagents were reagent grade.

2.1. Growth Conditions for Microorganisms Culture. The microorganisms *Geotrichum candidum* CCT 1205 (isolated from industrial waste water treatment—Preston, United Kington) and *Rhodotorula glutinis* CCT 2182 (isolated from *Psidium guajava*—Atlantic Rainforest, Brazil) were stored at "André Tosello" Research Foundation (Campinas, Brazil) [30]. *G. candidum* was cultivated in 400 mL of nutrient broth **1** (10 g/L malt extract, 5 g/L peptone, 10 g/L glucose, 3.12 g/L K_2HPO_4, and 11.18 g/L KH_2PO_4) at 28°C, and *R. glutinis* was cultivated in 400 mL of nutrient broth **2** (3 g/L Yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L glucose) at 30°C. Both yeasts were incubated for 2 days on an orbital shaker (200 rpm) before use. All materials and medium were sterilized in an autoclave at 121°C before use and the yeasts were manipulated in a laminar flow cabinet.

2.2. General Procedure for Bioreduction of 2-Halo-1-(4-substituted phenyl)-ethanones. The yeasts were incubated for two days (400 mL nutrient broth in Erlenmeyer of 1 L). After that, the ketone **1** (2 mmol) dissolved in 1.5 mL of ethanol was added directly to the suspension where the yeasts grew. The resulting suspension was stirred in an orbital shaker (200 rpm) at 28°C for *G. candidum* and at 30°C for *R. glutinis* until the full conversion of **1** (18 h). The product was extracted with CH_2Cl_2 and purified by flash silica gel column chromatography using hexane/ethyl acetate (7 : 3).

*2.3. (S)-(+)-2-Bromo-1-(4-bromophenyl)ethanol (S)-**2a**.* The bioreduction of ketone **1a** (0.556 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2a** (0.540 g with 96.4%) as colorless solid, m.p. 72°C; $[\alpha]_D^{25}$ +40.0° (*c* 1, $CHCl_3$) [lit. −31.0°, *c* 2.9, $CHCl_3$ for *R* isomer, 94% ee] [31], giving an optical purity of >99% determined by GC using a chiral column; IR (KBr): 3402, 3086, 3064, 3049, 3026, 2958, 2922, 2852, 1593, 1488, 1420, 1402, 1218, 1192, 1071, 1010, 828, 722, 680, 613 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.74 (s, 1H, OH), 3.46 (dd, 1 H, J = 8.4 Hz and 11.3 Hz, CH_2), 3.59 (dd, 1 H, J = 3.6 Hz and 11.3 Hz, CH_2), 4.87 (dd, 1H, J = 3.6 Hz and 8.4 Hz, CH), 7.24–7.31 (m, 2H, Ph), 7.48–7.51 (m, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.72, 72.97, 122.12, 127.43, 131.55, 138.99; MS *m/z* (rel. int. %): 188 (5), 187 (71), 186 (7), 185 (79), 183 (4), 182 (2), 171 (2), 169 (2), 159 (13), 158 (2), 157 (17), 155 (4), 120 (4), 119 (2), 106 (3), 105 (6), 103 (4), 102 (9), 91 (14), 90 (6), 89 (7), 79 (6), 77 (100), 75 (18), 78 (46), 76 (16), 50 (62), 51 (57), 43 (39).

*2.4. (R)-(−)-2-Bromo-1-(4-bromophenyl)ethanol (R)-**2a**.* The bioreduction of ketone **1a** (0.556 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2a** (0.554 g, 99.0% yield) as colorless solid, m.p. 72°C; $[\alpha]_D^{25}$ −40.4° (*c* 1, $CHCl_3$) [lit. −31.0°, *c* 2.9, $CHCl_3$ for *R* isomer, 94% ee] [31], giving an optical purity of >99% determined by GC using a chiral column; ^{1}H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.5. (S)-(+)-2-Bromo-1-(4-chlorophenyl)ethanol (S)-**2b**.* The bioreduction of ketone **1b** (0.467 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2b** (0.448 g, 95.1% yield) as colorless oil; $[\alpha]_D^{25}$ +38.7° (*c* 1, $CHCl_3$) [lit. 38.6°, *c* 1.15, $CHCl_3$ for *S* isomer, 91% ee] [32], giving an optical purity of >99% determined by GC using a chiral column; IR (film): 3392, 3088, 3051, 3030, 3003, 2957, 2896, 1596, 1492, 1428, 1408, 1338, 1310, 1256, 1310, 1256, 1199, 1173, 1089, 1072, 1013, 973, 944, 897, 834, 778, 752, 704, 674 cm^{-1}; ^{1}H NMR

(300 MHz, $CDCl_3$) δ 2.74 (sl, 1H, OH), 3.48 (dd, 1H, J = 7.0 Hz and 11.3 Hz, CH_2), 3.61 (dd, 1H, J = 5.8 Hz and 11.3 Hz, CH_2), 4.87 (dd, 1H, J = 5.8 Hz and 7.0 Hz, CH), 7.24–7.28 (m, 2H, Ph), 7.31–7.51 (m, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.72, 72.97, 122.12, 127.43, 131.56, 139.00; MS *m/z* (rel. int. %): 143 (29), 142 (11), 141 (100), 139 (4), 138 (2), 121 (7), 115 (6), 113 (16), 112 (6), 111 (4), 108 (7), 107 (5), 105 (4), 104 (2), 103 (2), 102 (2), 91 (2), 89 (1), 79 (11), 78 (9), 77 (70), 76 (2), 75 (15), 74 (9), 70 (6), 63 (8), 51 (28), 50 (30), 49 (3), 44 (22), 43 (20), 40 (32).

*2.6. (R)-(−)-2-Bromo-1-(4-chlorophenyl)ethanol (R)-**2b**.* The bioreduction of ketone **1b** (0.467 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2b** (0.457 g, 97.0% yield) as colorless oil; $[\alpha]_D^{25}$ −38.7 (*c* 1, $CHCl_3$) [lit. 38.6, *c* 1.15, $CHCl_3$ for *S* isomer, 91% ee] [32], giving an optical purity of >99% determined by GC using a chiral column; ^{1}H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.7. (S)-(+)-2-Bromo-1-(4-methylphenyl)ethanol (S)-**2c**.* The bioreduction of ketone **1c** (0.426 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2c** (0.413 g, 96.0% yield) as colorless oil; $[\alpha]_D^{25}$ +48.3° (*c* 1, $CHCl_3$) [lit. +41.8°, *c* 1.0, $CHCl_3$ for *S* isomer, 95% ee] [32, 33], giving an optical purity of >99% determined by GC using a chiral column; IR (film): 3378, 3064, 3044, 2971, 2931, 2907, 2836, 1612, 1585, 1511, 1458, 1443, 1368, 1300, 1243, 1205, 1174, 1115, 1087, 1069, 1034, 1004, 898, 830, 807 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.46 (sl, 1H, OH), 3.50 (dd, 1H, J = 8.7 Hz and 10.4 Hz, CH_2), 3.62 (dd, 1H, J = 3.4 Hz and 10.4 Hz, CH_2), 4.87 (dd, 1H, J = 3.4 Hz and 8.7 Hz, CH), 7.18–7.26 (d, 2H, J = 8 Hz, Ph), 7.30 (d, 2H, J = 8.0 Hz, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.16, 40.12, 73.67, 125.91, 129.32, 137.32, 138.16; MS *m/z* (rel. int. %): 217–215 (M^+, 2-2), 202 (2), 200 (2), 138 (7), 137 (93), 136 (16), 135 (13), 134 (45), 123 (1), 122 (2), 121 (2), 120 (3), 119 (5), 118 (5), 117 (8), 115 (4), 110 (4), 109 (49), 108 (4), 107 (4), 105 (4), 104 (2), 103 (4), 102 (2), 95 (3), 94 (34), 93 (43), 92 (9), 91 (38), 90 (2), 89 (5), 81 (2), 79 (9), 78 (8), 77 (30), 76 (4), 75 (3), 74 (4), 68 (5), 67 (3), 66 (13), 65 (20), 64 (11), 63 (21), 62 (8), 61 (3), 55 (4), 54 (1), 53 (10), 52 (8), 51 (30), 50 (19), 49 (1), 45 (8), 44 (6), 43 (100), 41 (12), 40 (10).

*2.8. (R)-(−)-2-Bromo-1-(4-methylphenyl)ethanol (R)-**2c**.* The bioreduction of ketone **1c** (0.426 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2c** (0.410 g, 95.3% yield) as colorless oil; $[\alpha]_D^{25}$ −48.3 (*c* 1, $CHCl_3$) [lit. +41.8°, *c* 1, $CHCl_3$ for *S* isomer] [32], giving an optical purity of >99% determined by GC using a chiral column; ^{1}H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.9. (S)-(+)-2-Bromo-1-(4-methoxyphenyl)ethanol (S)-**2d**.* The bioreduction of ketone **1d** (0.458 g, 2 mmol) by *Geotrichum candidum* CCT 1205 gave (*S*)-**2d** (0.453 g, 98.0% yield) as colorless oil; $[\alpha]_D^{25}$ +19.8° (*c* 1, $CHCl_3$) [lit. −37.7°, *c* 1.0, $CHCl_3$ for *R* isomer, 87% ee] [31, 34], IR (film): 3371, 3062, 3030, 2973, 2928, 2907, 2878, 1616, 1581, 1511, 1458, 1442, 1368, 1300, 1240, 1205, 1174, 1112, 1081, 1069, 1024, 1001, 892, 830, 804 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (sl, 1H, OH), 3.61 (dd, 1H, J = 8.7 Hz and 11.2 Hz, CH_2), 3.70 (dd, J = 3.9 Hz and 11.2 Hz, CH_2), 3.79 (s, 3H, CH_3), 4.86 (dd, 1H, J = 3.9 Hz and 8.7 Hz, CH), 6.89 (d, 2H, J = 8.8 Hz, Ph), 7.31 (d, 2H, J = 8.8 Hz, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.12, 56.28, 78.95, 114.94, 127.71, 133.40, 160.03; MS *m/z* (rel. int. %): 233–231 (M^+, 1-1), 218 (1), 215 (1), 214 (1), 202 (2), 200 (2), 153 (2), 152 (4), 151 (2), 138 (6), 137 (100), 135 (11), 134 (9), 122 (2), 121 (2), 120 (2), 119 (20), 110 (3), 109 (16), 108 (3), 107 (2), 105 (2), 104 (1); 103 (4), 102 (2), 95 (3), 94 (16), 93 (2), 92 (7), 91(18), 90 (2), 89 (4), 81 (2), 79 (6), 78 (5), 77 (21), 76 (2), 75 (3), 68 (2), 67 (2), 66 (5), 65 (12), 64 (9), 63 (6), 55 (1), 54 (1), 53 (8), 52 (4), 51 (12), 50 (14), 45 (3), 44 (2), 43 (79), 41 (10), 40 (8).

*2.10. (R)-(−)-2-Bromo-1-(4-methoxyphenyl)ethanol (R)-**2d**.* The bioreduction of ketone **1d** (0.458 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 gave (*R*)-**2d** (0.452 g, 97.8% yield) as colorless oil; $[\alpha]_D^{25}$ −19.7 (*c* 1, $CHCl_3$) [lit. −37.7°, *c* 1.0, $CHCl_3$ for *R* isomer, 87% ee] [31], ^{1}H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.11. (S)-(+)-2-Bromo-1-(4-nitrophenyl)ethanol (S)-**2e**.* The bioreduction of ketone **1e** (0.488 g, 2 mmol) by *Geotrichum candidum* CCT 1205 gave (*S*)-**2e** (0.480 g, 97.6% yield) a light yellow solid, mp 98°C; $[\alpha]_D^{25}$ +25.0° (*c* 1, $CHCl_3$) [lit. +32.1°, *c* 1, $CHCl_3$ for *S* isomer, 91% ee] [33, 35], giving an optical purity of >99% determined by GC using a chiral column. IR (KBr): 3455, 3109, 3079, 2947, 2924, 2889, 2851, 1601, 1520, 1347, 1291, 1203, 1074, 1012, 855, 760, 730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.83 (sl, 1H, OH), 3.53 (dd, 1H, J = 8.4 Hz and 10.6 Hz, CH_2), 3.68 (dd, 1H, J = 3.5 Hz and 10.6 Hz, CH_2), 5.03–5.08 (m, 1H, CH), 7.45 (d, 2H, J = 8.8 Hz, Ph), 8.22 (d, 2H, J = 8.8 Hz, Ph);^{13}C NMR (75 MHz, $CDCl_3$): δ 39.32, 72.52, 123.60, 126.65, 146.10, 146.90; MS *m/z* (rel. int. %): 153 (8), 152 (100), 149 (2), 141 (1), 139 (1), 136 (2), 127 (1), 125 (2), 122 (5), 106 (10), 105 (9), 102 (4), 95 (5), 94 (11), 91 (8), 78 (13), 77 (17), 66 (6), 51 (17), 50 (13), 43 (20).

*2.12. (R)-(−)-2-Bromo-1-(4-nitrophenyl)ethanol (R)-**2e**.* The bioreduction of ketone **1e** (0.488 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 gave (*R*)-**2e** (0.483 g, 98.0% yield) a light yellow solid, mp 98°C; $[\alpha]_D^{25}$ −25.0° (*c* 1, $CHCl_3$) [lit. +32.1°, *c* 1, $CHCl_3$ for *S* isomer, 91% ee] [33, 36], giving an optical purity of >99% determined by GC using a chiral column; ^{1}H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.13. (S)-(+)-2-Chloro-1-(4-bromophenyl)ethanol (S)-**2f**.* The bioreduction of ketone **1f** (0.467 g, 2 mmol) by *Geotrichum candidum* CCT 1205 gave (*S*)-**2f** (0.468 g, 99.4% yield) as colorless oil; $[\alpha]_D^{25}$ +35.0° (*c* 1, $CHCl_3$) [lit. −35.87°, *c* 1.1072, $CHCl_3$ for *R* isomer, 99% ee] [14], giving an optical purity of >99% determined by GC using a chiral column; IR (film):

3421; 3106; 3087; 3064; 3031; 3006; 2956; 2895; 1593, 1575, 1494; 1453; 1426; 1387; 1336; 1300; 1295; 1248; 1200; 1085; 1064; 1074; 1030; 1012; 972; 944; 917; 869; 824; 768; 724; 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.41 (sl, 1H, OH), 3.61 (dd, 1H, J = 8.7 Hz and 11.3 Hz, CH_2), 3.73 (dd, 1H, J = 3.4 Hz and 11.3 Hz, CH_2), 4.88 (dd, 1H, J = 3.4 Hz and 8.7 Hz, CH), 7.28 (d, 2H, J = 8.4 Hz, Ph), 7.51 (d, 2H, J = 8.4 Hz, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.90, 74.12, 122.04, 127.28, 132.02, 139.46; MS *m/z* (rel. int. %): 238–236 (M^+, 1-1), 202 (1), 200 (1), 158 (1), 156 (3), 108 (7), 107 (100), 105 (5), 104 (2), 103 (4), 102 (1), 91 (6), 89 (1), 79 (60), 78 (8), 77 (42), 76 (2), 75 (2), 51 (31), 50 (13).

*2.14. (R)-(−)-2-Chloro-1-(4-bromophenyl)ethanol (R)-**2f**.* The bioreduction of ketone **1f** (0.467 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 gave (*R*)-**2f** (0.460 g, 97.7% yield) as colorless oil; $[\alpha]_D^{25}$ −34.9 (*c* 1, $CHCl_3$) [lit. −35.87°, *c* 1.1072, $CHCl_3$ for *R* isomer, 99% ee] [14], giving an optical purity of >99% determined by GC using a chiral column; ^1H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.15. (S)-(+)-2-Chloro-1-(4-chlorophenyl)ethanol (S)-**2g**.* The bioreduction of ketone **1g** (0.378 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2g** (0.363 g, 95.0% yield) as colorless oil; $[\alpha]_D^{25}$ +48.3° (*c* 1.25, $CHCl_3$) [lit. $[\alpha]_D^{20}$ 44.2° (*c* 2.1, $CHCl_3$) for *S* isomer, 96,6% ee] [36], giving an optical purity of >99% determined by GC using a chiral column; IR (film): 3387, 3103, 3090, 3067, 3053, 3020, 2956, 2894, 1598, 1492, 1427, 1410, 1338, 1308, 1252, 1198, 1090, 1075, 1013, 970, 947, 895, 871, 833, 776, 751, 704, 673 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.2 (sl, 1H, OH), 3.58 (dd, 1H, J = 8.4 Hz and 11.3 Hz, CH_2), 3.67 (dd, 1H, J = 3.7 Hz and 11.3 Hz, CH_2), 4.84 (dd, 1H, J = 3.7 Hz and 8.4 Hz, CH), 7.27–7.34 (m, 4H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.39, 73.15, 127.16, 128.27, 128.48, 133.82, 138.09; MS *m/z* (rel. int. %): 192-191 (M+, 4), 158 (2), 156 (7), 143 (11), 142 (3), 141 (38), 139 (4), 138 (2), 121 (7), 115 (6), 114 (3), 113 (19), 112 (6), 111 (5), 105 (2), 103 (5), 102 (2), 101 (1), 91 (2), 89 (1), 87 (1), 85 (2), 78 (8), 77 (77), 75 (13), 74 (7), 73 (3), 70 (7), 71 (2), 65 (2), 63 (5), 62 (3), 61 (2), 60 (1), 55 (1), 53 (3), 52 (6), 51 (33), 50 (23), 49 (3), 46 (1), 45 (11), 44 (4), 42 (100), 41 (1), 40 (1).

*2.16. (R)-(−)-2-Cloro-1-(4-chlorophenyl)ethanol (R)-**2g**.* The bioreduction of ketone **1g** (0.378 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2g** (0.36 g, 94.2% yield) as colorless oil; $[\alpha]_D^{25}$ −48.3° (*c* 2.1, $CHCl_3$); [lit. $[\alpha]_D^{20}$ 44.2° (*c* 2.1, $CHCl_3$) for *S* isomer, 96,6% ee] [36], giving an optical purity of >99% determined by GC using a chiral column; ^1H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.17. (S)-(+)-2-Chloro-1-(4-methylphenyl)ethanol (S)-**2h**.* The bioreduction of ketone **1h** (0.337 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2h** (0.329 g, 96.4% yield) as colorless oil; $[\alpha]_D^{25}$ +48.3° (*c* 1.1, $CHCl_3$) [lit. +47.2° (*c* 1.1, $CHCl_3$) for *S* isomer, 92% ee] [32], giving an optical purity of >99% determined by GC using a chiral column; IR (film): 3414, 3094, 3052, 3017, 2970, 2924, 2863, 1611, 1512, 1445, 1411, 1369, 1302, 1280, 1257, 1192, 1181, 1112, 1090, 1071, 1010, 941, 892, 813, 724 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2,34 (s, 3H, CH_3), 2.50 (sl, 1H, OH), 3.63 (dd, 1 H, J = 8.5 Hz and 11.2 Hz, CH_2), 3.74 (dd, 1H, J = 3.9 Hz and 11.2 Hz, CH_2), 4.85 (dd, 1H, J = 3.9 and 8.5 Hz, CH), 7.20 (d, 2H, J = 8 Hz, Ph), 7.29 (d, 2H, J = 8 Hz, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.17, 50.82, 73.98, 126.05, 129.39, 137.13, 138.22; MS *m/z* (rel. int. %): 171-170 (M^+, 4) 158 (1), 156 (3), 137 (2), 136 (16), 135 (1), 122 (4), 121 (50), 119 (5), 118 (5),117 (8), 115 (4), 107 (2), 105 (1), 103 (1), 102 (1), 94 (4), 93 (49), 92 (11), 91 (45), 89 (4), 78 (5), 79 (2), 77 (30), 75 (1), 74 (1), 67 (3), 66 (2), 65 (20), 64 (2), 63 (10), 62 (4), 60 (15), 59 (2), 57 (4), 55 (1), 53 (4), 52 (5), 51 (18), 50 (9), 46 (1), 45 (9), 44 (3), 43 (100), 41 (10), 40 (4).

*2.18. (R)-(−)-2-Chloro-1-(4-methylphenyl)ethanol (R)-**2h**.* The bioreduction of ketone **1h** (0.337 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2h** (0.327 g, 95.7% yield) as colorless oil; $[\alpha]_D^{25}$ −48.3 (*c* 1.1, $CHCl_3$) [lit. +47.2° (*c* 1.1, $CHCl_3$) for *S* isomer, 92% ee] [32], giving an optical purity of >99% determined by GC using a chiral column; ^1H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

*2.19. (S)-(+)-2-Chloro-1-(4-methoxyphenyl)ethanol (S)-**2i**.* The bioreduction of ketone **1i** (0.369 g, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2i** (0.370 g, 99.2% yield) as colorless oil; $[\alpha]_D^{25}$ +41.4° (*c* 1, $CHCl_3$) [lit. +40.2°, for *S* isomer, 90,5% ee] [36], giving an optical purity of >99% determined by GC using a chiral column; IR (film): 3400, 3372, 3062, 3031, 2950, 2931, 2907, 2836, 1610, 1520, 1511, 1458, 1443, 1368, 1300, 1250, 1205, 1174, 1115, 1084, 1069, 1030, 1004, 898; 840, 780 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.70 (sl, 1H, OH), 3.52 (dd, 1H, J = 8.7 Hz and 11.4 Hz, CH_2), 3.61 (dd, 1 H, J = 3.8 Hz and 11.4 Hz, CH_2), 3.80 (s, 3H, CH_3), 4.78 (dd, 1H, J = 3.8 Hz and 8.7 Hz, CH), 6.90 (d, J = 8.7 Hz, 2H, Ph), 7.20 (d, J = 8.7 Hz, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 50.70, 55.34, 73.61, 113.78, 127.10, 132.12, 159.62; MS *m/z* (rel. int. %): 186 (M^+, 6), 152 (21), 153 (2), 151 (2), 138 (7), 137 (100), 135 (13), 134 (45), 122 (2), 121 (2), 120 (3), 119 (27), 110 (4), 109 (23), 108 (4), 107 (4), 105 (4), 104 (2), 103 (4), 102 (2), 95 (3), 94 (26), 93 (3), 92 (9), 90 (2), 91(38), 89 (5), 81 (2), 78 (8), 79 (9), 77 (25), 76 (4), 75 (3), 74 (4), 68 (5), 67 (3), 66 (13), 65 (38), 64 (11), 63 (21), 62 (8), 61 (3), 55 (4), 54 (1), 53 (10), 52 (8), 51 (30), 50 (19), 49 (1), 45 (8), 44 (6), 43 (100), 41 (12), 40 (10).

*2.20. (R)-(−)-2-Chloro-1-(4-methoxyphenyl)ethanol (R)-**2i**.* The bioreduction of ketone **1i** (0.369 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2i** (0.366 g, 98.0% yield) as colorless oil; $[\alpha]_D^{25}$ −41.5 (*c* 1, $CHCl_3$) [lit. +40.2°, for *S* isomer, 90,5% ee] [36], giving an optical purity of >99% determined by GC using a chiral column; ^1H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

Table 1: Asymmetric reduction of 2-halo-1-(4-substituted phenyl)-ethanones **1a-j** mediated by *Geotrichum candidum* CCT 1205 and *Rhodotorula glutinis* CCT 2182[a].

Ketone	Microorganism	T (°C)	Alcohol	Yield (%)	$[\alpha]_D^{25}$[b]
1a	*Geotrichum candidum*	28	(***S***)-**2a**	96.4	+40.0
1b	"	28	(***S***)-**2b**	95.1	+38.7
1c	"	28	(***S***)-**2c**	96.0	+48.3
1d	"	28	(***S***)-**2d**	98.0	+19.8
1e	"	28	(***S***)-**2e**	97.6	+25.0
1f	"	28	(***S***)-**2f**	99.4	+35.0
1g	"	28	(***S***)-**2g**	95.0	+48.3
1h	"	28	(***S***)-**2h**	96.4	+48.3
1i	"	28	(***S***)-**2i**	99.2	+41.4
1j	"	28	(***S***)-**2j**	97.0	+32.6
1a	*Rhodotorula glutinis*	30	(***R***)-**2a**	99.0	−40.4
1b	"	30	(***R***)-**2b**	97.0	−38.7
1c	"	30	(***S***)-**2c**	95.3	−48.3
1d	"	30	(***R***)-**2d**	97.8	−19.7
1e	"	30	(***R***)-**2e**	98.0	−25.0
1f	"	30	(***R***)-**2f**	97.7	−34.9
1g	"	30	(***R***)-**2g**	94.2	−48.3
1h	"	30	(***R***)-**2h**	95.7	−48.3
1i	"	30	(***R***)-**2i**	98.0	−41.5
1j	"	30	(***R***)-**2j**	98.0	−32.6

[a] 18 h, 2 mmol of ketone/1.5 mL of EtOH was added to 15 g of yeast (wet weight)/400 mL of nutrient broth 1 (malt extract, peptone) for *Geotrichum candidum* or nutrient broth 2 (yeast extract, malt extract, peptone) for *Rhodotorula glutinis*. [b] ee>99%. [c] See Materials and Methods for *c* values and solvent.

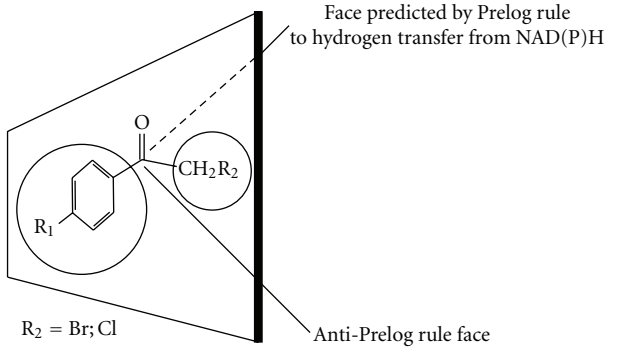


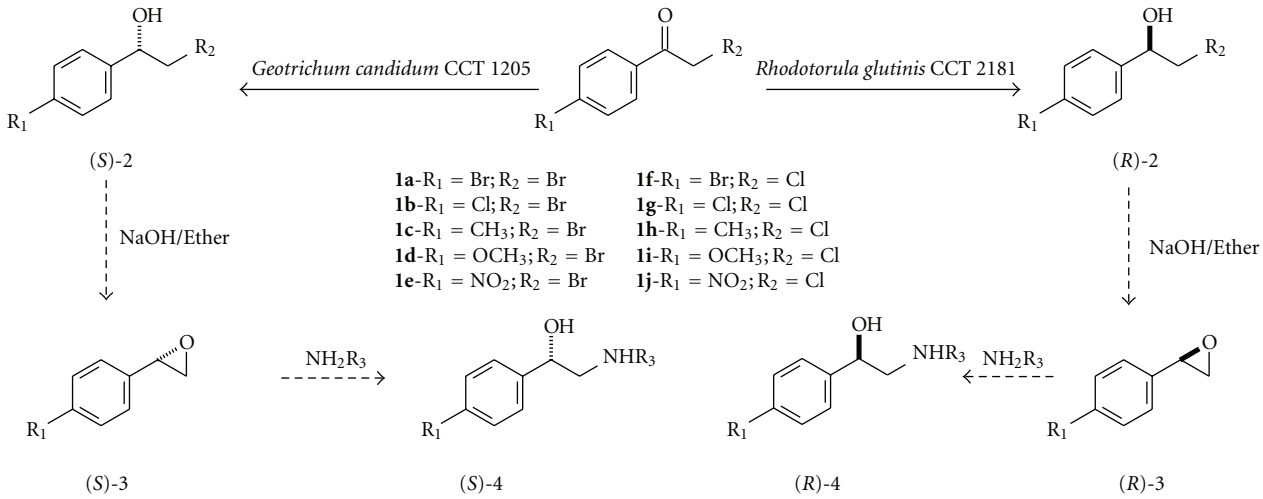
Figure 1: Prelog rule for discrimination of the faces of carbonylic group by the enzymes.

*2.21. (S)-(+)-2-Chloro-1-(4-nitrophenyl)ethanol (S)-**2j**.* The bioreduction of ketone **1j** (0.399, 2 mmol) by *Geotrichum candidum* CCT 1205 furnished (*S*)-**2j** (0.391 g, 97.0% yield) a white solid, mp 87°C (lit. p.f. 87°C) [33]; $[\alpha]_D^{25}$ +32,6° (*c* 1, $CHCl_3$) [lit. +37.2°, *c* 2.0, $CHCl_3$ for *S* isomer, 98,2% ee] [36], giving an optical purity of >99% determined by GC using a chiral column; IR (KBr): 3304, 3051, 3021, 2970, 2923, 2878, 1599, 1506, 1452, 1364, 1323, 1275, 1251, 1204, 1166, 1125, 1075, 1024, 964, 951, 902, 863, 823, 773, 743, 703, 652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.93 (sl, 1H, OH), 3.64 (dd, 1H, J = 8.1 Hz and 11.3 Hz, CH_2), 3.68 (dd, 1H, J = 3.3 Hz and 11.3 Hz, CH_2), 5.03–5.05 (m, 1H, CH), 7.50 (d, 2H, J = 8.7 Hz, Ph), 8.20 (d, 2H, J = 8.7 Hz, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 50.20, 69.38, 123.56, 126.73, 146.59, 146.95; MS *m/z* (rel. int. %): 166 (5), 153 (8), 152 (100), 136 (2), 122 (6), 107 (2), 106 (13), 105 (12), 102 (3), 95 (4), 94 (13), 81 (3), 79 (3), 78 (18), 77 (22), 65 (9), 51 (24), 50 (16), 43 (23), 41 (9).

*2.22. (R)-(−)-2-Chloro-1-(4-nitrophenyl)ethanol (R)-**2j**.* The bioreduction of ketone **1j** (0.399 g, 2 mmol) by *Rhodotorula glutinis* CCT 2182 furnished (*R*)-**2j** (0.395 g, 98.0% yield) a white solid, mp 87°C (lit. p.f. 87°C) [36]; $[\alpha]_D^{25}$ −32,6° (*c* 1, $CHCl_3$) [lit. +37.2°, *c* 2.0, $CHCl_3$ for *S* isomer, 98,2% ee] [33], giving an optical purity of >99% determined by GC using a chiral column; ^1H and ^{13}C NMR and IR spectra and MS analysis were identical to those observed with its (*S*) enantiomer.

3. Results and Discussion

The reduction of ethanones **1a-j** was carried out in 5 mmol/L in a slurry of growing yeast of *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205. These ethanones having substituted groups (electron withdrawing groups—EWG: $-NO_2$, –Br, –Cl; electron donating groups—EDG: $-CH_3$, $-OCH_3$) attached to position 4 of benzene ring were studied in order to investigate the influence of these groups in the bioreduction performed by these two microorganisms.



Scheme 2

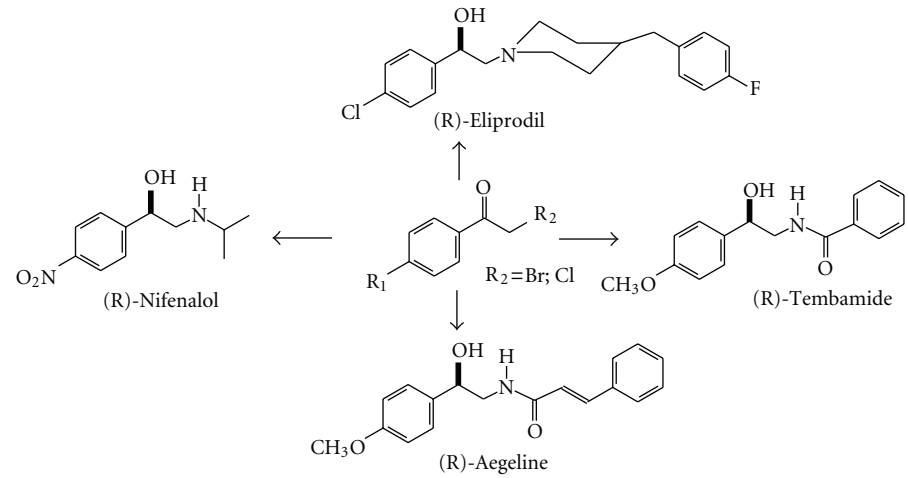


Figure 2: Pharmaceutical useful ethanolamines.

The reaction progress was monitored by GC analysis, and the yields and enatiomeric excesses are shown in Table 1.

The reductions of 2-bromo-1-(4-substituted phenyl)-ethanones **1a-e** and 2-chloro-1-(4-substituted)-ethanones **1f-j** mediated by *Rhodotorula glutinis* CCT 2182 gave the corresponding halohydrins **2a-j** with (*R*) configuration, while the halohydrins **2a-j** with (*S*) configuration were obtained when *Geotrichum candidum* CCT 1205 mediated the reduction of the ethanones **1a-j**.

α-Haloacetophenones have been used as mechanistic probe in the reduction reactions of NADH-dependent horse liver alcohol dehydrogenase [37–40], for identification of reductants in sediments [41] and even in the whole cells [42]. This probe enables the differentiation between reduction processes which proceed through hydride transfer (H^-) or by a multistep electron transfer (e^-, $H^\bullet$ or e^-, H^+, e^- as has been suggested). Acetophenone is the reduction product obtained by electron transfer, while optically active halohydrin is obtained when an enzyme mediates a hydride transfer process. In this work, the reductions of **1a-e** proceed via hydride transfer mediated by an oxireductase, since halohydrins were obtained in high ee and no 4-substituted acetophenone was detected.

Rhodotorula glutinis gives products following the Prelog rule [43], which predicts that, in general, hydrogen transfer from NAD(P)H to the prochiral ethanones **1a-j** occurs to the face of carbonylic group shown in Figure 1, taking into account that the aryl group is larger than the $–CH_2Br$ and $–CH_2Cl$ groups. On the contrary, the *Geotrichum candidum* gives anti-Prelog halohydrins.

The excellent results and complementary enantioselectivities of the produced halohydrins obtained by using *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 in reduction of ethanones **1a-j** are remarkable and highlight the potential of such approach to obtain separately the two isomers of the 1,2-aminoalcohols, by reaction of the easily obtainable epoxy with the appropriated amine (Scheme 2), as an alternative to the approach using the

reduction of α-azido-*para*-substituted acetophenones mediated by those microorganisms [28]. The separate synthesis of two enantiomers is important since the FDA Guidance for Development of New Stereoisomeric Drugs [44] says that "to evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers, manufacturers should develop quantitative assays for individual enantiomers in *in vivo* samples early in drug development." However, the products of biotransformation of **1b-e** and **1g-j** using *Rhodotorula glutinis* CCT 2182 may be used as important starting material for the preparation of the known pharmaceuticals products with (*R*) configuration: Eliprodil from halohydrins **2b** and **2g**; Tembamide from halohydrins **2c** and **2h**; Aegeline from halohydrins **2d** and **2i**; Nifenalol from halohydrins **2e** and **2j** (Figure 2).

4. Conclusions

The use of *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 in bioreduction reaction of 2-halo-1-(4-substituted phenyl)-ethanones results in an important chiral halohydrins in high ee, excellent yield, and complementary enantioselectivity. These halohydrins may be used as intermediates in the synthesis of optically active substituted styrene oxides and aminoalcohols which have numerous industrial applications.

Acknowledgments

The authors thank FAPESP and CNPq for financial support.

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