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Chemoselective screening for the reduction of a chiral functionalised (±)-2-(phenylthio)cyclohexanone by whole cells of Brazilian micro-organisms

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ABSTRACT

The use of whole cells of micro-organisms to bring about the biotransformation of an organic compound offers a number of advantages, but problems caused by enzymatic promiscuity may be encountered upon with substrates bearing more than one functional group. A one-pot screening method, in which whole fungal cells were incubated with a mixture of 4-methylcyclohexanone **1** and phenyl methyl sulfide **2**, has been employed to determine the chemoselectivity of various biocatalysts. The hyphomycetes, *Aspergillus terreus* CCT 3320 and *A. terreus* URM 3571, catalysed the oxidation of **2** accompanied by the reduction of **1** to 4-methylcyclohexanol **1a** and, for strain *A. terreus* CCT 3320, the Baeyer-Villiger oxidation of **1**. The Basidiomycetes, *Trametes versicolor* CCB 202, *Pycnoporus sanguineus* CCB 501 and *Trichaptum byssogenum* CCB 203, catalysed the oxidation of **2** and the reduction **1**, but no Baeyer–Villiger reaction products were detected. In contrast, *Trametes rigida* CCB 285 catalysed the biotransformation of **1** to **1a**, exclusively, in the absence of any detectable sulfide oxidation reactions. The chemoselective reduction of (±)-2-(phenylthio)cyclohexanone **3** by *T. rigida* CCB 285 afforded exclusively the (+)-*cis*-(1*R*,2*S*) and (+)-*trans*-(1*S*,2*S*) diastereoisomers of 2-(phenylthio)cyclohexan-1-ol **3a** in moderate yields (13% and 27%, respectively) and high enantiomeric excesses (>98%). Chemoselective screening for the reduction of a ketone and/or the oxidation of a sulfide group in one pot by whole cells of micro-organisms represents an attractive technique with applications in the development of synthesis of complex molecule bearing different functional groups.

1. Introduction

Enantiomerically pure alcohols, ketones, sulfides or amines are commonly required as starting materials in the synthesis of chiral compounds.[1] Alcohols with high enantiomeric purities can be readily formed in quantitative yields of around 100% through chemical or biocatalytic reduction of prochiral ketones.[2] Alternatively, the kinetic resolution of a racemic alcohol with one stereogenic centre can be achieved, for example, by lipase-catalysed transesterification,[3] although the maximum quantitative yield of the desired enantiomer is only 50%. Biotransformation reactions involving racemic ketones as substrates are not usually employed in biocatalytic methods.[4]

The use of whole cells of micro-organisms to bring about the biotransformation of an organic compound offers a number of advantages over processes involving isolated enzyme systems. In this context, we have recently obtained biotransformation products with high enantiomeric excesses following a biocatalytic protocol involving whole fungal cells in respect of Mannich eliminations of amine salt,[5] the kinetic resolution of seleno-alcohols by biomethylation,[6] the stereo-inversion of racemic alcohols[7] and the stereoselective reduction of prochiral ketones.[8] For substrates bearing several functional groups, however, the use of whole cells may give rise to problems caused by enzymatic promiscuity.[9]

Herein, whole cells of micro-organisms have been selectively screened with regard to their ability to carry out a specific biotransformation of a substrate with more than one functional group. In a preliminary study, cells of six strains of the fungal genera *Aspergillus*, *Trametes*, *Pycnoporus* and *Trichaptum* were subjected to a one-pot screening against a substrate mixture consisting of 4-methylcyclohexanone **1** and methyl phenyl sulfide **2**. Under the reaction conditions employed, only *Trametes rigid*a CCB 285 was able to promote the bioreduction of ketone **1** without the concomitant oxidation of the sulfide group of **2**. The application of whole cells of this micro-organism in the biocatalytic reduction

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of the synthetic racemic substrate (±)-2-(phenylthio)cyclohexanone **3**, which contains a ketone and a sulfide function in the same molecule, was subsequently investigated.

2. Results and discussion

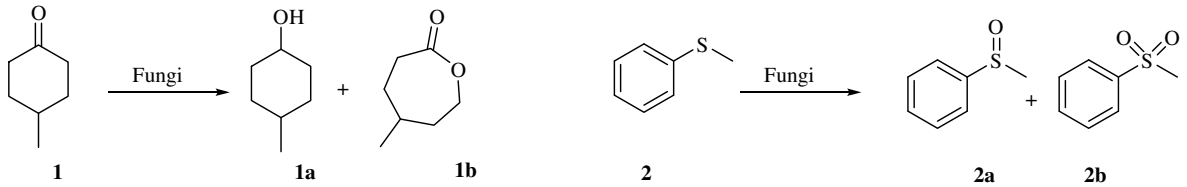
The biotransformation of a mixture of 4-methylcyclohexanone **1** and phenyl methyl sulfide **2** by whole cells of the Basidiomycetes *Trametes versicolor* CCB 202, *T. rigida* CCB 285, *Pycnoporus sanguineus* CCB 501 and *Trichaptum byssogenum* CCB 203), and of the Hyphomycetes *Aspergillus terreus* CCT 3320 and *A. terreus* URM 3571 was studied in a one-pot system. Table 1 shows the progress of the reduction and/or oxidation reactions as a function of incubation time, together with the percentage yields of the products formed. One-pot screening methods using whole cells and several organic substrates have recently been reported for monitoring oxidoreductases.[10]

Under the described conditions, whole cells of *T. versicolor* CCB 202, *P. sanguineus* CCB 501 and *T. byssogenum* CCB 203 promoted the reduction of ketone **1** to 4-methylcyclohexanol **1a** and the oxidation of sulfide **2** to sulfoxide **2a** and sulfone **2b**, all in high conversions (Table 1, entries 1–9). Although these micro-organisms were able to catalyse the oxidation of the sulfide, the Baeyer–Villiger oxidation reaction of **1** to yield 5-methylheptalactone **1b** was not observed. When cells of *T. rigida* CCB 285 were employed as a biocatalyst, ketone **1** was reduced exclusively to the corresponding alcohol **1a** in high conversion (Table 1, entries 10–12), but no oxidation products of the sulfide **2** were detected.

With respect to the strains of *A. terreus* studied, enzymes present in whole cells of *A. terreus* CCT 3320 catalysed the reduction of **1** to exclusively afford alcohol **1a** in very high conversion, while the oxidation of **2** to yield the sulfoxide **2a** and the sulfone **2b** occurred with a high conversion (Table 1, entries 16–18). The microbial sulfoxidation of phenyl methyl sulfide **2** by *A. terreus* CCT 3320 has already been reported.[11] Whole cells of *A. terreus* URM 3571 afforded **2a** and **2b** from **2** with conversion values similar to those obtained with strain *A. terreus* CCT 3320; however, in this case, the Baeyer–Villiger oxidation of **1** to form lactone **1b** was the main biotransformation reaction of the ketone, and only low concentrations of the reduction product **1a** were formed (Table 1, entries 13–15). The biotransformations of cyclic ketones by strains of *A. terreus* have previously been reported.[12] The screening experiments indicated that the enzymes present in whole cells of *T. rigida* CCB 285 were highly chemoselective since the ketone group of **1** was reduced to yield **1a** exclusively, and no reactions occurred at the sulfide group of **2**. This strain of *T. rigida* was therefore considered to be the micro-organism of choice in the study of the biocatalytic transformations of the functionalised (±)-2-(phenylthio)cyclohexanone **3**, a compound containing both ketone and sulfide groups in the same molecule. Racemic **3** was synthesised by the reaction of the nucleophilic thiolate anion (prepared in situ by treating commercial thiophenol with potassium carbonate) with 2-chlorocyclohexanone (Scheme 1).[13]

The product was purified by column chromatography (CC) over silica gel with *n*-hexane–ethyl acetate (8:2) as eluent and was obtained in good yield. Racemic diastereoisomers of the β-hydroxy sulfide **3a** were prepared for use as standards in the subsequent HPLC analysis by the reduction of **3** with $NaBH_4$ in the presence of SiO_2–H_2O (10:3) (Scheme 2).[14] Racemic diastereomers of **3a** were obtained in excellent yields in the form of a mixture with a *cis:trans* ratio of 75:25. The diastereomers were separated by CC over silica gel eluted with *n*-hexane–ethyl acetate (10:1), and were identified by NMR, IR and HRMS analyses. The spectroscopic data of the *cis*- and *trans*-diastereoisomers of **3a** were in agreement

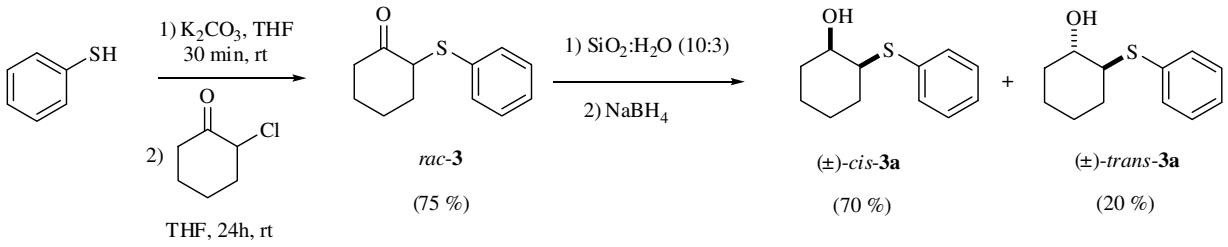
Table 1
Selective one-pot screening for reduction and/or oxidation reactions catalysed by whole cells of micro-organisms[a]



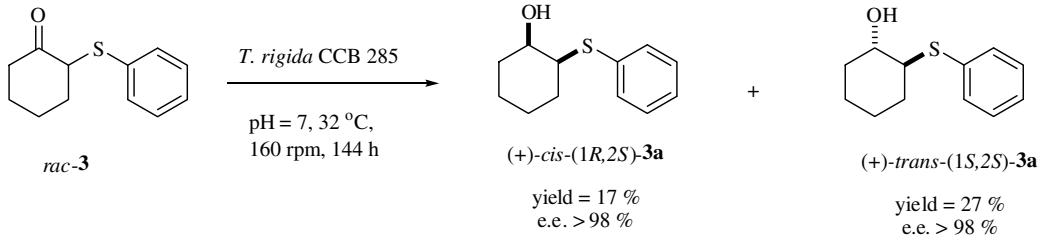
1 **1a** **1b** **2** **2a** **2b**

Entry	Micro-organism	Incubation time (days)	Product concentration[b] (%)			
			Alcohol **1a**	Lactone **1b**	Sulfoxide **2a**	Sulfone **2b**
Basidiomycetes						
1	*Trametes versicolor* CCB 202	1	82	—	74	7
2		4	77	—	90	10
3		6	78	—	81	16
4	*Pycnoporus sanguineus* CCB 501	1	68	—	60	3
5		4	56	—	45	11
6		6	44	—	21	27
7	*Trichaptum byssogenum* CCB 203	1	71	—	32	61
8		4	69	—	42	58
9		6	70	—	38	62
10	*Trametes rigida* CCB 285	1	9	—	—	—
11		4	76	—	—	—
12		6	69	—	—	—
Hyphomycetes						
13	*Aspergillus terreus* URM 3571	1	36	64	16	84
14		4	24	76	9	91
15		6	24	76	10	90
16	*Aspergillus terreus* CCT 3320	1	100	—	14	86
17		4	100	—	12	88
18		6	100	—	8	92

[a] Reaction conditions: 1.0 g (wet weight) of fungal mycelia and 20 μL each of 4-methylcyclohexanone **1** and phenyl methyl sulfide **2** in 50 mL of Na_2HPO_4/KH_2PO_4 buffer (pH 7) incubated at 32 °C on an orbital shaker (160 rpm).
[b] Concentration determined by GC–MS using an achiral column.



Scheme 1. Synthesis of (±)-2-(phenylthio)cyclohexanone **3** and (±)-2-(phenylthio)cyclohexan-1-ol **3a**.



Scheme 2. Chemoselective reduction of (±)-2-(phenylthio)cyclohexanone by whole cells of *Trametes rigida*.

Table 2
Chemoselective biotransformation of (*RS*)-2-(phenylthio)cyclohexanone **3** by whole cells of *Trametes rigida* CCB 285[a]

Entry	Incubation time (h)	Ketone **3**	(+)-*cis*-(1*R*,2*S*)-**3a**		(+)-*trans*-(1*S*,2*S*)-**3a**	
		c (%)[b]	*c* (%)[b]	ee (%)	*c* (%)[b]	ee (%)
1	48	—	39	—	61	—
2	96	—	31	—	69	—
3	144	—	35	>98[c]	68	>98 [c]

[a] Reaction conditions: 1.0 g (wet weight) of fungal mycelia and 20 μL of **3** in 50 mL of Na_2HPO_4/KH_2PO_4 buffer (pH 7) incubated at 32 °C on an orbital shaker (160 rpm).
[b] Concentration of product (%) determined by chiral HPLC analysis.
[c] Enantiomeric excess (%) determined by chiral HPLC analysis of products isolated in the quantitative study.

with those reported in the literature.[15–19] The racemic diastereomers of **3a** could be resolved by HPLC analysis using a Chiralcel OD-H column and *n*-hexane–2-propanol (99:1).

A qualitative study of the biotransformation of (±)-2-(phenylthio)cyclohexanone **3** was performed using cells of *T. rigida* CCB 285 as the source of alcohol dehydrogenase and cofactors required to bring about a chemo- and stereo-selective reduction of the ketone group. The results presented in Table 2 show that the bioreduction of the ketone group of **3** led to the preferential formation of *trans*-β-hydroxysulfide **3a** with 32% diastereoisomeric excess. In this case, the diastereoisomeric ratio obtained by biocatalytic reduction was the opposite of that obtained by chemical reduction. This finding is in agreement with the results recently obtained in our laboratory employing the same fungus in the reduction of seleno-ketones.[20]

A quantitative study of the bioreduction of **3** by whole cells of *T. rigida* CCB 285 was performed in which alcohols **3a** were isolated from the reaction mixture after 144 h of incubation, purified and subsequently characterised by spectroscopic analyses. The yields of *cis*-**3a** and *trans*-**3a** were 13% and 27%, respectively, and both diastereoisomers were obtained in high (>98%) enantiomeric purities (Scheme 2, Fig. 1). The absolute configurations of the products were assigned by comparison with the values in the literature.[16–18]

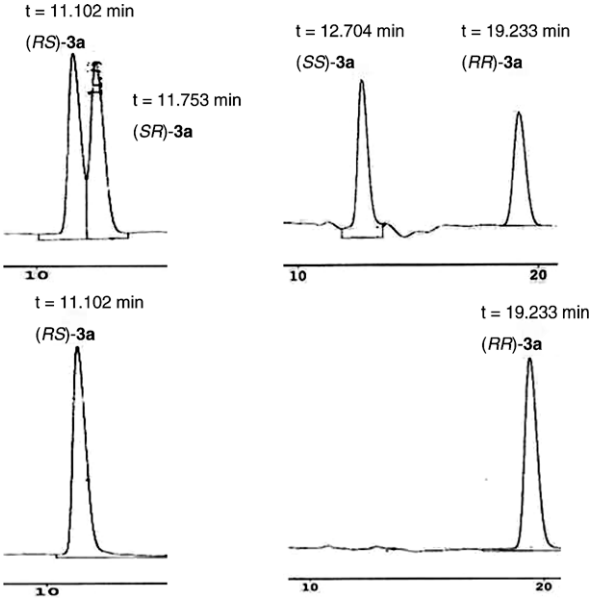


Figure 1. HPLC chromatograms of the chemoselective biotransformations of (±)-2-(phenylthio)cyclohexanone **3** by *T. rigida* CCB 285.

3. Conclusion

The successful chemoenzymatic biotransformation of an organic compound containing several reactive centres by whole fungal cells is not trivial. In this context, the one-pot selective screening technique employing different substrates described herein represents an important biocatalytic methodology with application in the development of syntheses of molecules bearing multiple stereogenic centres in high enantiomeric excesses. Using this screening

method, *T. rigida* CCB 285 was selected to study the chemoselective reduction of the functionalised (*RS*)-2-(phenylthio)cyclohexanone **3**. The biotransformation reaction afforded the chiral sulfide-alcohols (+)-*cis*-(1*R*,2*S*)-**3a** and (+)-*trans*-(1*S*,2*S*)-**3a** in high enantiomeric excesses (ee >98%) and in moderate yields.

4. Experimental

4.1. General methods

4-Methylcyclohexanone, benzethiol and 2-chlorocyclohexanone were purchased from commercial sources and used as supplied, all other reagents and solvents were previously purified and/or dried where necessary using methods described in the literature.[21] Micro-organisms were manipulated in a Veco laminar flow cabinet, and all incubation experiments were carried out using sterile materials. Technal TE-421 or Superohm G-25 orbital shakers were employed in the biocatalysed transformations.

Compound purification was carried out by column chromatography over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and ethyl acetate. Column effluents were monitored by TLC on pre-coated Silica Gel 60 F254 layers (aluminium-backed: Merck) eluted with mixtures of *n*-hexane and ethyl acetate, and visualised by spraying with *p*-anisaldehyde/sulfuric acid reagent followed by heating at ca. 120 °C. Products of the enzyme-catalysed reactions were analysed using a Shimadzu model GC-17A gas chromatograph equipped with a flame ionisation detector (FID) and a J & W Scientific HP5 column (30 m × 0.25 mm i.d.; 0.25 μm). The GC conditions were oven temperature initially at 50 °C and increased at a rate of 10 °C/min; run time 20 min; injector temperature 230 °C; FID temperature 250 °C; injector split ratio 1:20; hydrogen carrier gas pressure 100 kPa. GC–MS analyses were performed on a Shimadzu model QP5050A instrument equipped with a capillary column DB-5 (J & W Scientific DB-5 column, 30 m × 0.25 mm i.d.; 0.25 μm) with helium as the carrier gas. HPLC analyses were carried out using a Shimadzu model SPD-10Av equipped with UV–vis detector and a Chiralcel OD-H column (25 × 0.46 cm i.d.) eluted with *n*-hexane–2-propanol (99:1).

^{1}H and ^{13}C NMR spectra were measured on a Bruker DRX 500 spectrometer (500 MHz, ^{1}H; 125 MHz, ^{13}C) with samples dissolved in $CDCl_3$: chemical shifts are presented in ppm with respect to tetramethylsilane (TMS). IR spectra were recorded in nujol mulls using a Bomem MB 100 spectrometer. HRMS analyses were performed on a Bruker Daltonics Esquire 3000 Plus instrument equipped with an ion trap detector. Optical rotation values were determined with a Jasco DIP-378 polarimeter using a 1 dm cuvette, and reported values refer to the Na-D line.

4.2. Synthesis of standard racemic alcohols[15]

2-(Phenylthio)cyclohexanone **3** (1 mmol, 206 mg) and $NaBH_4$ (1.1 mmol, 42 mg) were added to a stirred mixture of SiO_2 (100 mg) and H_2O (30 mg) contained in a 5 mL two-necked round-bottomed flask equipped with a magnetic stirrer, and stirring was continued for 5 min at room temperature. After work-up with CH_2Cl_2, the solvent was removed under vacuum and the residue purified by CC (silica gel; *n*-hexane–ethyl acetate, 9:2) to afford the racemic alcohols **3a**. The spectroscopic data of these compounds were in agreement with those reported in the literature.[15–19]

4.3. Biotransformation of 1–3

Stock cultures of Basidiomycetes were stored in Petri dishes on a solid medium (agar 20 g/L, peptone 5 g/L, yeast extract 2 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KH_2PO_4 1 g/L, NaCl 0.06 g/L) in a refrigerator at 4 °C. Slices of agar (0.5 × 0.5 cm) containing mycelia were inoculated into 1 L of liquid medium with same composition as the solid medium contained in a 2 L Erlenmeyer flask, and incubated at 32 °C for 8 days on an orbital shaker (160 rpm). Stock cultures of Hyphomycetes were stored on solid medium in a refrigerator at 4 °C. Slants of the cultures were inoculated into 1 L of Oxoid malt extract medium (20 g/L) contained in a 2 L Erlenmeyer flask, and incubated at 32 °C for 6 days on an orbital shaker (160 rpm). Following incubation, fungal cells were harvested by vacuum filtration, and 1.0 g aliquots of the wet cells were suspended in 50 mL portions of Na_2HPO_4/KH_2PO_4 buffer (pH 7) contained in separate Erlenmeyer flasks (250 mL). Biotransformation reactions were initiated by the addition to the cell suspensions of 20 μL each of 4-methylcyclohexanone **1** and phenyl methyl sulfide **2** (all fungal strains), or of 20 μL of (±)-2-(phenylthio)cyclohexanone **3** (*T. rigida* CCB 285), and the reaction mixtures were incubated at 32 °C for 6 days on an orbital shaker (160 rpm). Progress of the biotransformation was monitored every 2 days by GC–MS using an achiral column (for substrates **1** and **2**) or by HPLC using a chiral column (for substrate **3**). In order to determine the yields of (+)-*cis*-(1*R*,2*S*)-**3a** and (+)-*trans*-(1*S*,2*S*)-**3a** formed from **3** by the cells of *T. rigida* CCB 285, appropriate reaction mixtures were set up exactly as described above in 10 separate Erlenmeyer flasks (250 mL) and incubated for 6 days. After this time, the reaction mixtures were filtered, the aqueous phases were bulked and extracted with ethyl acetate, the organic phase was dried over $MgSO_4$ and the solvent removed under vacuum. Alcohols **3a** were isolated by CC from the resulting residue and identified by NMR, IR and HRMS analyses.

4.4. Characterisation of alcohols 3a

4.4.1. (+)-*cis*-(1*R*,2*S*)-2-(Phenylthio)-1-cyclohexanol *cis*-3a

Isolated as a yellow oil; yield: 13%; $[\alpha]_D^{25} = +19.7$ (*c* 0.42, CH_2Cl_2); ee >98%; IR (film) υ/cm^{-1}: 3403, 2942, 2917, 2852, 1579, 1481, 1435, 1066, 733; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.45–7.44 (m, 2H), 7.31–7.23 (m, 3H), 3.78–3.76 (m, 1H), 3.56–3.33 (m, 1H), 1.86–1.65 (m, 6H), 1.53–1.48 (m, 1H), 1.41–1.34 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 134.2, 132.2, 129.1, 127.3, 67.0, 54.4, 31.7, 27.7, 24.9, 20.9; HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{16}NaOS$ 231.0820. Found: 231.0811.

4.4.2. (+)-*trans*-(1*S*,2*S*)-2-(Phenylthio)-1-cyclohexanol *trans*-3a

Isolated as a yellow oil; yield: 27%; $[\alpha]_D^{25} = +61.0$ (*c* 0.44, CH_2Cl_2); ee >98%; IR (film) υ/cm^{-1}: 3392, 2934, 2925, 2851, 1582, 1482, 1354, 1087, 732; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.51–7.46 (m, 2H), 7.32–7.25 (m, 3H), 3.36–3.30 (m, 1H), 2.97 (m, 1H), 2.81–2.75 (m, 1H), 2.17–2.00 (m, 2H), 1.76–1.67 (m, 2H), 1.38–1.21(m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 133.8, 132.5, 128.9, 127.7, 72.0, 56.6, 33.8, 32.7, 26.2, 24.3; HRMS (ESI) $[M+Na]^+$, calcd for $C_{12}H_{16}NaOS$: 231.0820. Found: 231.0815.

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References

1. http://www.sigmaaldrich.com/catalog/search/TablePage/16270415.
2. (a) Gruber, C. C.; Lavandera, I.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* **2006**, *348*, 1789–1805; (b) Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107–117.

3. (a) Ghanem, A. *Tetrahedron* **2007**, *63*, 1721–1754; (b) Ghanem, A.; Aboul-Enein, H. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 3331–3351.
4. (a) Moore, J. C.; Pollard, D. J.; Kosjek, B.; Devine, P. N. *Acc. Chem. Res.* **2007**, *40*, 1412–1419; (b) Goldberg, K.; Schroer, K.; Lutz, S.; Liese, A. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 249–255.
5. Raminelli, C.; Kagohara, E.; Pellizari, V. H.; Comasseto, J. V.; Andrade, L. H.; Porto, A. L. M. *Enzyme Microb. Technol.* **2007**, *40*, 362–369.
6. Da Costa, C. E.; Comasseto, J. V.; Crusius, I. H. S.; Andrade, L. H.; Porto, A. L. M. *J. Mol. Catal. B* **2007**, *45*, 135–139.
7. (a) Comasseto, J. V.; Andrade, L. H.; Omori, A. T.; Assis, L. F.; Porto, A. L. M. *J. Mol. Catal. B* **2004**, *29*, 55–61; (b) Comasseto, J. V.; Assis, L. F.; Andrade, L. H.; Schoenlein-Crusius, I. H. S.; Porto, A. L. M. *J. Mol. Catal. B* **2006**, *29*, 24–30.
8. (a) De Conti, R. M.; Porto, A. L. M.; Rodrigues, J. A. R.; Moran, P. J. S.; Manfio, G. P.; Marsaioli, A. J. *J. Mol. Catal. B* **2001**, *11*, 233–236; (b) Comasseto, J. V.; Omori, A. T.; Andrade, L. H.; Porto, A. L. M. *Tetrahedron: Asymmetry* **2003**, *14*, 711–715.
9. (a) Taglieber, A.; Hobenreich, H.; Carballeira, J. D.; Mondière, R. J. G.; Reetz, M. Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 8597–8600; (b) Hult, K.; Berglund, P. *Trends Biotechnol.* **2007**, *25*, 231–238.
10. (a) Gonçalves, R. A. C.; Porto, A. L. M.; Pinheiro, L.; Cagnon, J. R.; Manfio, G. P.; Marsaioli, A. J. *Food Technol. Biotechnol.* **2004**, *42*, 355–361; (b) Sicard, R.; Chen, L. S.; Marsaioli, A. J.; Reymond, J-L. *Adv. Synth. Catal.* **2005**, *347*, 1041–1050; (c) Pinheiro, L.; Marsaioli, A. J. *J. Mol. Catal. B* **2007**, *44*, 78–86.
11. Porto, A. L. M.; Cassiola, F.; Dias, S. L. P.; Joekes, I.; Gushiken, Y.; Rodrigues, J. A. R.; Moran, P. J. S.; Manfio, G. P.; Marsaioli, A. J. *J. Mol. Catal. B* **2002**, *19*, 327–334.
12. Keppler, A. F.; Porto, A. L. M.; Schoenlein-Crusius, I. H.; Comasseto, J. V.; Andrade, L. H. *Enzyme Microb. Technol.* **2005**, *36*, 967–975.
13. Carreno, M. V. *Chem. Rev.* **1995**, *95*, 1717–1760.
14. Zeynizadeh, B.; Behyar, T. *J. Brazil Chem. Soc.* **2005**, *16*, 1200–1209.
15. Wu, J.; Hou, X. L.; Dai, L. X.; Xia, L. J.; Tang, M. H. *Tetrahedron: Asymmetry* **1998**, *9*, 3431–3436.
16. Crumbie, R. L.; Deol, S.; Nemorin, J. E.; Ridley, D. D. *Aust. J. Chem.* **1978**, *31*, 1965–1980.
17. Fujisawa, T.; Yamanaka, K.; Mobele, B. I.; Shimizu, M. *Tetrahedron Lett.* **1991**, *32*, 399–400.
18. Cohen, T.; Ritter, R. H.; Oullette, D. *J. Am. Chem. Soc.* **1982**, *104*, 7142–7148.
19. White, J. M.; Lambert, J. B.; Spiniello, M.; Jones, S. A.; Gable, R. W. *Chem. Eur. J.* **2002**, *12*, 2799–2811.
20. Piovan, L.; Capelari, M.; Andrade, L. H.; Comasseto, J. V.; Porto, A. L. M. *Tetrahedron: Asymmetry* **2007**, *18*, 1398–1402.
21. Armarego, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*, 4th ed.; Butterworth-Heinemann: Oxford, 1996; pp 1–529.