Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 54 (2008) 50–54
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

1,2-Octanediol deracemization by stereoinversion using whole cells

Lu S. Chen [a,1], Simone M. Mantovani [a], Luciana G. de Oliveira [a], Marta C.T. Duarte [b], Anita J. Marsaioli [a,*]

[a] *Instituto de Química, Universidade Estadual de Campinas – UNICAMP, CP 6154, 13084-971 Campinas, SP, Brazil*
[b] *Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas – CPQBA – UNICAMP, CP 6171, 13083-970 Campinas, SP, Brazil*

Received 26 September 2007; received in revised form 26 November 2007; accepted 29 November 2007
Available online 4 December 2007

Abstract

This paper describes the stereoinversion of (*R*)-1,2-octanediol promoted by *Aspergillus niger* CCT 1435 and *Candida albicans* CCT 0776 from Brazilian collections. Racemic 1,2-octanediol can be converted into (*S*)-1,2-octanediol with 70% isolated yield and 99% ee in 10 h using *C. albicans*. This is one of the best results in the literature and on-going experiments indicate that the reaction rate can be further accelerated.

Keywords: Deracemization; Stereoinversion; Epoxide-hydrolases

1. Introduction

Enantiomerically pure alcohols are important building blocks for the synthesis of bioactive compounds for pharmaceutical and agrochemical applications [1]. The synthesis of optically active alcohols is usually performed by asymmetric reduction of ketones using chiral organometalic reagents or through biocatalytic methods [2]. Highly efficient bioprocess deracemization is a good alternative to the usual enzymatic resolution, allowing the transformation of racemates into chiral building blocks with 100% chemical yield and 100% enantiomeric excess (ee) [3]. Such processes are mainly achieved following three different approaches: dynamic kinetic resolution which combines transition metal-catalyzed racemization with an enantioselective lipase-catalyzed hydrolysis reaction [4], enantioconvergent reactions using two independent pathways [5,6]; or enantioselective stereoinversion of a racemic mixture, yielding a single enantiomerically pure product [7,8].

The latter is the most attractive as theoretically only half of the starting material, for example, the [(*S*)-enantiomer] undergoes a chemical transformation while the other half [(*R*)-enantiomer] remains unaltered, already with the desired configuration. Microbial biotransformation of one enantiomer into its mirror image is not a widespread biosynthetic pathway in Nature. Consequently a limited number of microorganisms capable of performing such reactions have been reported in the literature [9].

While searching for epoxide-hydrolases using whole cells from wild microorganisms [10] we have selected strains that converted 1,2-epoxyoctane (**1**) into (*S*)-1,2-octanediol [(*S*)-**2**] in a cascade process involving ring opening followed by a selective stereoinversion. This paper reports deracemization by stereoinversion using microorganisms from Brazilian culture collections.

2. Experimental

2.1. Analytical and substrates

Merck 60 silica gel (230–400 mesh ASTM) was used for flash chromatography. Dichloromethane was dried over CaH_2 and distilled immediately before use. ^{1}H NMR (300.07 MHz, $CDCl_3$) and ^{13}C NMR (75.50 MHz, $CDCl_3$) spectra were recorded on a Gemini 300P - Varian Instruments using $(CH_3)_4Si$ as internal reference (δ 0.00). Chemical shifts are given in ppm and coupling constants *J* are given in Hz. GC–MS analyses were performed with an Agilent 6890 Series GC System and mass spectra were recorded with a Hewlett-Packard 5973 Mass Selective Detector (70 eV) using a HP-5MS-crosslinked 5% phenyl methyl siloxane fused silica capillary column

* Corresponding author. Tel.: +55 19 35213067; fax: +55 19 35213023.
E-mail address: anita@iqm.unicamp.br (A.J. Marsaioli).
[1] Present address: Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, CP 6111, 13084-971 Campinas, SP, Brazil.

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.11.022

(30 m × 0.25 mm i.d. × 0.25 μm film thickness) and helium as carrier gas (1 mL/min). Chiral GC was performed on an Agilent 6890 Series GC System or on an Agilent 6850 Series GC System with FID detectors, using hydrogen as carrier gas (10 psi) and a Chirasil-Dex CB β-cyclodextrin chiral column (Chrompack, 25 m × 0.25 mm i.d. × 0.25 μm film thickness). Optical rotation was measured in methanol using a J-702 Jeol polarimeter (589.3 nm).

2.2. Microorganisms and culture conditions used in the small-scale biocatalysis

The microorganisms *Aspergillus niger* CCT 1435 and *Candida albicans* CCT 0776 were acquired from the CCT collection (Coleção de Culturas Tropical, Fundação Tropical de Pesquisas André Tosello, Brazil) and *Bacillus pumilus* CBMAI 0008 was purchased from the CBMAI collection (Coleção Brasileira de Microrganismos de Ambiente e da Industria, CPQBA, Unicamp).

The microorganisms were grown in Erlenmeyer flasks (500-mL) containing 100 mL of culture medium A (*A. niger* CCT 1435), B (*B. pumilus* CBMAI 0008) or C (*C. albicans* CCT 0776). Medium A was prepared with malt extract (20 g/L); medium B was prepared with xylan (10 g/L), peptone (10 g/L), Tween 80 (1 g/L), $(NH_4)_2SO_4$ (1.4 g/L), KH_2PO_4 (2.0 g/L), urea (0.3 g/L), $CaCl_2{\cdot}2H_2O$ (0.4 g/L), $MgSO_4{\cdot}7H_2O$ (0.3g/L), 5 mL $FeSO_4{\cdot}7H_2O$ (1 g/L), 1.6 mL $MnSO_4{\cdot}7H_2O$ (1 g/L), 1.4 mL $ZnSO_4{\cdot}7H_2O$ (1 g/L) and 2 mL $CoCl_2$ (1 g/L, pH 10), and medium C contained yeast extract (3.0 g/L), malt extract (3.0 g/L), peptone (5.0 g/L) and D-glucose (1.0 g/L). After appropriate stages of growth, the cells were harvested by centrifugation (5000 rpm, 20 min at 18 °C) or filtration (*A. niger* CCT 1435) and used in the biocatalytic assays.

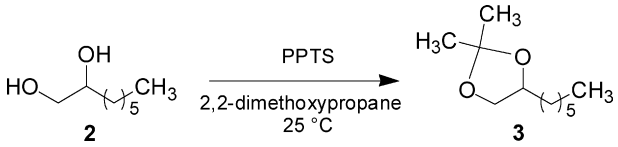
2.3. General procedure for biotransformation reaction with 1,2-epoxyoctane (*1*)

Whole cells (2–4 g) were stirred in a 125-mL Erlenmeyer flask at 150 rpm containing 40 mL of phosphate buffer pH 7.0 and maintained at 28 °C. Then, 40 μL of the racemic epoxide **1** in DMF (20 μL) was added to the medium (final substrate concentration 1 μL/mL). The bioconversion was monitored from time to time extracting aliquots from the bioreaction with organic solvent, after saturation with NaCl. Conversion was based on residual epoxide **1** present in a 0.5 mL sample extracted with 0.5 mL of ethyl acetate (containing pentadecane as internal standard (0.01 mg/mL)) and determined by GC–MS. To determine the ee of the diol **2**, 3 mL aliquots of the reaction medium were saturated with NaCl extracted with diethyl ether and evaporated for direct derivatization to the acetonide (**3**). The ee of the diol **2** was then indirectly determined by chiral GC-FID analysis (Section 2.4).

2.4. General procedure for the diol derivatization

Conversion of the diols **2** into acetonides **3** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate (PPTS) was performed using the diol/epoxide mixtures [11]. The aliquots of the reactions mixtures (3 mL) were extracted with diethyl ether (2× 3 mL), the organic layer was then dried over Na_2SO_4 and evaporated under a nitrogen flow. To the residue, 2,2-dimethoxypropane (1 mL) and catalytic amount of PPTS were added and the reaction was stirred at room temperature for 2 h. After washing with a saturated NaCl solution, the aqueous phase was extracted with hexane, and the organic layer was dried over Na_2SO_4 and evaporated. Hexane (500 μL) was added to the residue and 1 μL of this solution was directly analyzed by chiral GC-FID to determine the diol concentration.

2.5. Synthesis of (±)-4-hexyl-2,2-dimethyl-[1,3]-dioxolane (*3*)



2 **3**

(±)-**2** (25 mg, 0.17 mmol) and 2,2-dimethoxy-propane (3 mL) were stirred in the presence of a catalytic amount of PPTS [12] for 2 h at room temperature. After washing with a saturated NaCl solution, the aqueous phase was extracted with hexane, and the organic layer was dried over Na_2SO_4 and evaporated. Purification of the crude product by flash chromatography (hexane/EtOAc, 9.5:0.5) afforded **3** as a colorless oil, 16.7 mg (0.09 mmol, 53%) ^{1}H NMR (300.07 MHz, $CDCl_3$): δ 4.12–4.00 (m, 2H), 3.50 (t, *J* 7 Hz, 1H), 1.72–1.15 (m, 16H), 0.88 (t, *J* 6.5 Hz, 3H); ^{13}C NMR (75.50 MHz, $CDCl_3$): δ 108.5 (C), 76.2 (CH), 69.6 (CH_2), 33.7 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 27.1 (CH_3), 25.8 (CH_3), 25.8 (CH_2), 22.7 (CH_2), 14.1 (CH_3). EI/MS *m/z*: 172(10), 171(100), 111(10), 101(14), 72(35), 69(96), 59(12), 55(30), 43(67), 42(10), 41(25).

2.6. Standard procedure for small-scale enzymatic deracemization of (±)-1,2-octanediol (*2*)

Racemic **2** (40 μL) in DMF (20 μL) was added to a 125-mL Erlenmeyer flask containing the whole cells (2–4 g) and 40 mL of phosphate buffer (pH 7.0) and maintained at 28 °C with stirring.

Conversion was based on residual diol present in a 0.5 mL sample extracted with ethyl acetate (0.5 mL) containing an internal standard (pentadecane, 0.01 mg/mL) and determined by GC–MS. The ee of **2** was monitored by withdrawing 2 mL of the reaction mixture from time to time, and extracting with diethyl ether (2× 2 mL) after saturation with NaCl, evaporating the organic layer. After direct derivatization to the corresponding **3** the ee of **2** were determined by chiral GC-FID analysis (Section 2.4).

2.7. Standard procedure for preparative-scale enzymatic hydrolysis of (±)-1

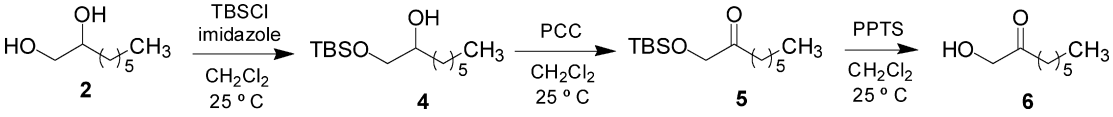
Hydrolysis of 70 μL of 1,2-epoxyoctane by *A. niger* CCT 1435 cells (7.0 g wet weight) in 70 mL phosphate buffer (pH 7.0) as previously described provide 30.7 mg of the crude

1,2-octanediol after 20 h. Purification by silica gel column chromatography (eluted with hexane and increasing amounts of ethyl acetate) furnished pure (*S*)-1,2-octanediol (17.2 mg, 25.6%).

2.8. Absolute configuration of 1,2-octanediol (**2**)

Optical rotations were measured in methanol and the specific optical rotation for 1,2-octanediol was determined and compared with literature data: $[\alpha]_D^{22} = +6.0$ (*ca.* 1.6, methanol; ee = 47.1%), lit.: (*S*)-1,2-octanediol: $[\alpha]_D^{20} = -13.6$ (*ca.* 1.0, methanol; ee > 99%) [12] and (*R*)-1,2-octanediol: $[\alpha]_D^{24} = +12.8$ (*ca.* 0.95, methanol; ee = 80%) [13].

2.9. Synthesis of 1-hydroxy-2-octanone (**6**)



Synthesis of (**4**): To a solution of (±)-1,2-octanediol (**2**) (510 mg, 3.5 mmol) in CH_2Cl_2 (17 mL, previously treated with CaH_2), were added imidazol (262 mg, 3.85 mmol) and TBSCl (527 mg, 3.5 mmol) and the reaction was stirred for 30 min at 25 °C. Brine was added to the reaction mixture and the aqueous phase was extracted with CH_2Cl_2 (three times). The organic phase thus obtained was dried with Na_2SO_4 and evaporated and the crude product was used directly to synthesize **5**.

Synthesis of (**5**): To product **4** (1.02 g) in CH_2Cl_2 (14 mL), were added sodium acetate (60 mg) and pyridinium chlorochromate (2.17 g, 10 mmol). The reaction mixture was stirred for 24 h at 25 °C, after which the reaction mixture was diluted in diethyl ether (50 mL) and washed with diethyl ether (three times). The reaction mixture was filtered through a short Florisil pad, dried over Na_2SO_4 and evaporated. The crude product was further purified by flash chromatography (hexane/ether, 9.8:0.2) affording 533 mg (2.06 mmol, 61%) of 1-(*tert*-butyl-dimethylsilyloxy)-2-octanone (**5**) as a colorless oil.

Synthesis of (**6**): A mixture of the ketone **5** (533 mg, 2.06 mmol) in 10 mL of CH_2Cl_2, water (three drops) and monohydrated *p*-toluenesulfonic acid (157 mg, 0.82 mmol) was stirred at 25 °C for 24 h. After washing the organic layer with a saturated $NaHCO_3$ solution, the aqueous phase was extracted with CH_2Cl; the organic portion was dried over Na_2SO_4 and evaporated. Flash chromatographic purification (hexane/ether, 9:1) afforded 223 mg (1.54 mmol, 75%) of the 1-hydroxy-2-octanone as a colorless oil. ^{1}H NMR (300.07 MHz, $CDCl_3$): δ 4.24 (s, 2H); 2.90 (sl, 1H), 2.41 (t, *J* 7.3 Hz, 2H), 1.63 (q, *J* 7.3 Hz, 2H), 1.2–1.4 (m, 6H), 0.88 (t, *J* 7.2 Hz, 3H); ^{13}C NMR (75.50 MHz, $CDCl_3$): δ 209.9 (C), 68.1 (CH_2), 38.4 (CH_2), 31.4 (CH_2), 28.8 (CH_2), 23.7 (CH_2), 22.4 (CH_2), 15.0 (CH_3). EI/MS *m/z*: 113 (100), 110 (24), 95 (12), 85 (48), 81(11), 57 (40), 55 (64), 53 (17).

2.10. General procedure for biotransformation reaction with 1-hydroxy-2-octanone (**6**)

The prochiral ketone **6** (40 μL) was added to an Erlenmeyer flask (125-mL) containing phosphate buffer (pH 7.0, 70 mmol/L, and 40 mL) and whole cells (2–4 g). This was stirred at 150 rpm at 28 °C. The bioconversion was monitored from time to time by extracting aliquots (2 mL) with organic solvent after addition of NaCl. Conversion was based on GC–MS analyses using the residual ketone **6** present in the 0.5 mL sample extracted with ethyl acetate (0.5 mL), compared to an internal standard (either pentadecane or benzophenone, 0.01 mg/mL). The ee was obtained by chiral GC-FID analysis (Chrompack column) either directly or after derivatization to **3** when the column in use did not discriminate the enantiomers of **2**.

Table 1
Biohydrolysis of (±)-1,2-epoxyoctane by *A. niger* CCT 1435, *C. albicans* CCT 0776 and *B. pumilus* CBMAI 0008 *C. albicans* CCT 0776 resting cells in phosphate buffer (pH 7.0)

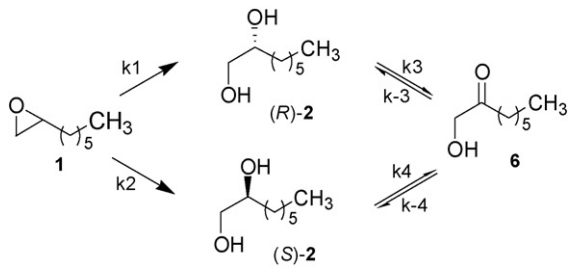
Microorganisms	Time (h)	Conversion (%)	ee (epoxide, %)	Epoxide (absolute configuration)[a]	ee (diol, %)	Diol (absolute configuration)
A. niger	2	77.8	13.1	(*S*)	49.1	(*R*)
	7	92.2	33.7	(*S*)	50.2	(*R*)
	24	>99	25.8	(*S*)	25.3	(*S*)
B. pumilus	2	28.6	5.8	(*S*)	20.5	(*R*)
	7	72.5	9.4	(*S*)	20.3	(*R*)
	24	>99	22.6	(*S*)	76.0	(*S*)
C.albicans	2	95.0	37.0	(*S*)	21.0	(*R*)
	4	>99	–	–	0	–
	9	>99	–	–	>99	(*S*)

[a] Comparison and co-injection with (*R*)-(+)-1,2-epoxyoctane purchased from Aldrich.

3. Results and discussion

Conversion of **1** into **2**, the ee and absolute configuration are summarized in Table 1. The absolute configurations of both enantiomers of **2** were determined by comparison with the specific optical rotation values reported in the literature [13,14]. The bioreactions with each of the three microorganisms showed an interesting time-dependent inversion of compound **2** configuration. The biotransformation using *A. niger* whole cells afforded (*R*)-2 with 51% ee and 92% of conversion after 7 h of reaction. However, this configuration was changed to *S* after 24 h. The same phenomenon occurred with *B. pumilus*, changing racemic **1** into an enriched mixture of **2** (20.3% ee (*R*)-enantiomer) after 7 h that was further converted to the (*S*)-diol **2** in 76.0% ee after 24 h. For *C. albicans*, racemic **1** produced (*R*)-2 in 37% ee and 95% of conversion after 2 h; however, after 9 h only the *S* enantiomer was detected (Table 1).

These results suggest that we are observing a multienzymatic process allowing the hydrolysis and deracemization by stereoinversion of (±)-epoxyoctane (**1**) in cascade biosynthetic pathways. This mechanism was proposed based on the detection of the prochiral intermediate **6** using *C. albicans* and *B. pumilus* (Scheme 1) as determined by the mass fragmentation pattern in the GC–MS spectrum and comparison with a synthetic standard of **6** (co-injection).Therefore, we can propose that (*R*)-**2** could undergo a selective stereoinversion promoted by oxidation to the α-hydroxy-ketone **6**, followed by a selective reduction to (*S*)-**2** (Scheme 1). Meanwhile (*S*)-**2** remains almost unaltered. This rationale fits the biotransformations performed by *A. niger* CCT 1435 and *C. albicans* CCT 0776 which were carried out to yield almost quantitative deracemizations of 1,2-octanediol (**2**). Thus, a preparative reaction of (±)-**2** with *A. niger* CCT 1435 produced chromatographically pure (*S*)-**2** in 48 h, 88.5% yield and 83.5% of ee, and the reaction with *C. albicans* CCT 0776 furnished (*S*)-**2** in 8 h, 65.5% yield and ee over 99% (Table 2).A control reaction with (±)-**2**, in phosphate buffer (pH 7.0) provided the diol in 91.1% yield with no enantiomeric excess. *B. pumilus* also converted racemic **2** to optically pure (*S*)-**2**. However, the optically pure (*S*)-diol was obtained together with an equal amount of 1-hydroxy-2-octanone (**6**), clearly characterizing a kinetic resolution process.



Scheme 1. Sequential action of epoxide-hydrolase and oxidoreductase on (±)-1,2-octanediol resulting in deracemization with *A. niger* CCT 1435 and *C. albicans* CCT 0776 and kinetic resolution in *B. pumilus*. Suggested relative reaction rate constants for *A. Niger* and *C. albicans* are k1 > k2; k3 ≫ k4; k-4 ≫ k-3; and for *B. pumilus* is k1 > k2; k3 > k4 »> k-3 and k-4 (or does not occur at all).

Table 2
Microbial transformation of (±)-1,2-octanediol

Microorganisms	Time (h)	ee (*S*)-1,2-octanediol (%)
A. niger CCT 1435[a]	48	>99.0
A. niger CCT 1435[b]	33	97.6
B. pumilus CBMAI 0008[c]	48	95.6
B. pumilus CBMAI 0008[b]	33	95.5
C. albicans CCT 0776[d]	8	>99.0

[a] The formation of 1-hydroxy-2-octanone was not observed in the reactions with *A. niger* CCT 1435.
[b] Pre-incubation with epoxide for 20 h at 28 °C and shaking at 150 rpm.
[c] The formation of 1-hydroxy-2-octanone was observed with *B. pumilus*. The bioconversion (55.5% in 48 h) of the diol to the ketone was based on residual diol determined by GC–MS.
[d] The formation of 1-hydroxy-2-octanone was detected in small amounts in the reactions with *C. albicans* CCT 0776.

The evaluated microorganisms perform the deracemization of (±)-**2** by two different pathways: either kinetic resolution in which the (*R*)-diol is selectively oxidized to **6** or a stereoinversion in two cascade reactions with a selective oxidation of the (*R*)-diol to 1-hydroxy-2-octanone (**6**) followed by a selective reduction of **6** to the (*S*)-diol. To confirm this, the biotransformation of the prochiral ketone **6** was performed with *A. niger* and *C. albicans* cells, producing (*S*)-**2** with >99% ee after 30 and 18 h, respectively (Table 3). With *B. pumilus* there was no observable reduction of **6** even after 6 days of reaction.

These data can be interpreted as follows: *A. niger*, *B. pumilus* and *C. albicans* express epoxide-hydrolases of low to moderate (*R*)-enantioselectivity. Upon extended incubation, (*R*)-**2** is selectively oxidized to 1-hydroxy-2-octanone (**6**) by all microorganisms, allowing accumulation of (*S*)-**2**. Moreover, **6** is reduced by (*S*)-stereoselective oxidoreductases present in *A. niger* and *C. albicans*, increasing the conversion yield of (*S*)-**2** to 100% and maintaining high ee levels (Scheme 1). In *B. pumilus* the oxidoreductases seems to be absent and 1-hydroxy-2-octanone (**6**) accumulates. The exact mechanism involved in the stereoinversion is a challenging topic [9] to be further investigated.

Table 3
Biotransformation of 1-hydroxy-2-octanone by *A. niger* CCT 1435, *C. albicans* CCT 0776 and *B. pumilus* CBMAI 0008[a] and *C. albicans* CCT 0776 resting cells in phosphate buffer (pH 7.0)

Microorganisms	Time (h)	Conversion (%)	ee (diol, %)	Diol (absolute configuration)
A. niger[b]	2	17.7	79.8	(*S*)
	8	36.0	86.2	(*S*)
	21	87.6	93.1	(*S*)
	30	>99	>99.9	(*S*)
C. albicans	6	83	18	(*S*)
	18	>99	>99	(*S*)

[a] Reactions with *B. pumilus* were monitored for 6 days and conversion to diol was not observed.
[b] Reactions with *A. niger* were monitored for 4 days and after 30 h the conversion and ee values of the diol formed remained unaltered.

4. Conclusion

These experiments confirm that *C. albicans and A. niger* are able to deracemize (±)-**2** by stereoinversion of the (*R*)-**2**, while *B. pumilus* yields the (*S*)-enantiomer by kinetic resolution of (±)-**2**.

Acknowledgements

The authors are indebted to FAPESP (Fundação de Amparo a Pesquisa do Estado de S. Paulo), FAEP (Fundação de Amparo a Extensão e Pesquisa, UNICAMP) and CNPq for grants and scholarships for L.S. Chen and S.M. Mantovani. We thank Prof. Carol Collins for final text revision.

References

[1] K. Faber, Biotransformations in Organic Chemistry, Springer/Verlag, Berlin, 1997.
[2] J.V. Comasseto, L.H. Andrade, A.T. Omori, L.F. Assis, A.L.M. Porto, J. Mol. Catal. B: Enzym. 22 (2004) 55–61.
[3] H. Stecher, K. Faber, Synthesis-stuttgart 1 (1997) 1–16.
[4] O. Pamies, J.-E. Bäckvall, Trends. Biotechnol. 22 (2004) 130–135.
[5] W. Xu, J.-H. Xu, J. Pan, X.-Y. Wu, Org. Lett. 8 (2006) 1737–1740.
[6] A. Steinreiber, S.F. Mayer, R. Saf, K. Faber, Tetrahedron: Asymm. 12 (2001) 1519–1528.
[7] X.Q. Mu, Y. Xu, Y. Nie, J. Ouyang, Z.H. Sun, Process Biochem. 40 (2005) 2345–2350.
[8] S.K. Padhi, D. Titu, N.G. Pandian, A. Cadha, Tetrahedron 62 (2006) 5133–5140.
[9] C.C. Gruber, I. Lavandera, K. Faber, W. Kroutil, Adv. Synth. Catal. 348 (2006) 1789–1805.
[10] Unpublished work: 44 microorganisms were purchased from CCT – Coleção de Culturas Tropicais da Fundação André Tosello and CBMAI - Coleção Brasileira de Microrganismos de Ambiente e Indústrias collections: *Agrobacterium tumefaciens*, *Antrobacter oxydans*, *Antrobacter* sp., Arquea, *Bacillus cereus*, *Bacillus*, *Citrobacter amalonaticus*, *Escherichia coli*, *Micrococus luteus*, *Pseudomonas oleovorans*, *Pseudomonas putida*, *Salmonella thyphimurium*, *Serratia liquefaciens*, *Serratia marcescens*, *Serratia plymuthica*, *Serratia rubidae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus ochracius*, *Aspergillus oryzae*, *Aspergillus terreus*, *Cunninghamella ecchinulata*, *Curvularia eragrostides*, *Curvularia lunata*, *Curvularia pallescens*, *Emericela nidulans*, *Geotrichum candidum*, *Mortirela isabelina*, *Nodulisporum* sp., *Rhyzopus oryzae*, *Trametes versicolor*, *Candida albicans*, *Candida utilis*, *Kluyveromyces marxianus*, *Pachysolen tannophilus*, *Pichia canadensis*, *Pichia kluyveri*, *Pichia stipitis*, *Rhodotorula glutinis*, *Sacharomyces boulardii*, *Sacharomyces cerevisae*, *Sacharomyces* sp., *Trichosporon cutaneum*. Another 37 were isolated from Amazonian soil, but have not been identified: Ama 1 to Ama 37.
[11] T.W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, 2nd ed., Wiley–Interscience, 1991.
[12] M. Kitamura, M. Isobe, Y. Ichikawa, T. Goto, J. Am. Chem. Soc. 106 (1984) 3252–3257.
[13] J. Hasegawa, M. Ogura, S. Tsuda, S. Maemoto, H. Kutsuki, T. Ohashi, Agric. Biol. Chem. 54 (1990) 1819–1827.
[14] C.A.G.M. Weijers, A.L. Botes, M.S. van Dyk, J.A.M. de Bont, Tetrahedron: Asymm. 9 (1998) 467–473.