

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/260114030>

Chemoenzymatic synthesis of fluoxetine precursors. Reduction of β -substituted propiophenones

ARTICLE *in* JOURNAL OF MOLECULAR CATALYSIS B ENZYMATIC · APRIL 2014

Impact Factor: 2.75 · DOI: 10.1016/j.molcatb.2014.01.022

CITATIONS

2

7 AUTHORS, INCLUDING:



Cesar Iglesias

University of the Republic, Uruguay

3 PUBLICATIONS 13 CITATIONS

SEE PROFILE



Paula Rodríguez

University of the Republic, Uruguay

8 PUBLICATIONS 42 CITATIONS

SEE PROFILE



David Gonzalez

University of the Republic, Uruguay

49 PUBLICATIONS 432 CITATIONS

SEE PROFILE

Chemoenzymatic synthesis of fluoxetine precursors. Reduction of β -substituted propiophenones.

Camila Coronel,^a Gabriel Arce,^a Cesar Iglesias,^a Cynthia Magallanes Noguera,^b Paula Rodriguez Bonnacarrère,^a Sonia Rodríguez Giordano^a and David Gonzalez.^{a,*}

^a Laboratorio de Biocatálisis y Biotransformaciones – DQO/DEPBIO – Facultad de Química – Universidad de la República (UdelaR) – CC1157 – Montevideo – Uruguay

^b INTEQUI-CONICET, Área de Química Orgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis (UNSL), San Luis, Argentina.

Abstract

Five endophytic yeast strains isolated from edible plants were tested in the reduction β -chloro- and β -azidopropiophenone for the preparation of optically active fluoxetine precursors. The biotransformation rendered not only the corresponding chiral γ -substituted alcohols, but also unsubstituted alcohols and ketones. The product profile was studied and a plausible mechanism for the reductive elimination of the β -functional group is proposed.

Keywords: Endophytes; Fluoxetine; β -Substituted ketones

Introduction

Medical treatment of depression is based on two types of drugs: classical tricyclic antidepressants and selective serotonin re-uptake inhibitors (SSRIs). Owing to the less significant side effects, SSRIs are used as first line drugs for the treatment of mild to moderate depression. They are also prescribed for the treatment of other disorders such as bulimia, obesity, and anxiety disorders. Fluoxetine (PROZAC®) (**1**) is a representative SSRI and despite the emerging of newer drugs it still ranks among the most prescribed drugs worldwide [1].

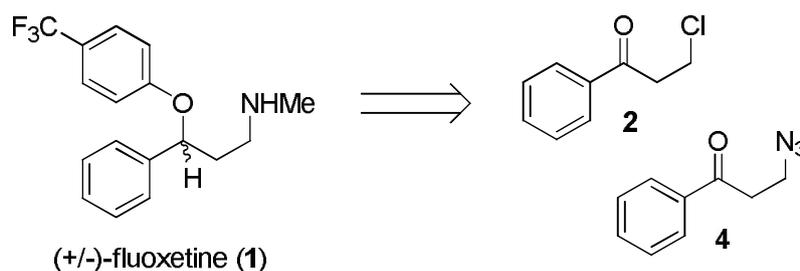
Fluoxetine is a chiral aminoalcohol with a benzylic chiral center. Although the drug is used as a racemate both isomers have been independently tested and patented for the treatment of different disorders. For instance *S*-fluoxetine can be more effective than the *R* isomer for the management of migraine [2] while *R*-fluoxetine has been patented for the treatment of sleep apnea [3].

The synthesis of optically pure benzylic alcohols is usually a good niche for biocatalytic reductions [4] and enzymatic kinetic resolutions [5]. Chiral optically pure secondary alcohols and particularly α - and γ -substituted alcohols are valuable precursors for the synthesis of several biologically active molecules [6-8]. In our laboratory we have researched the biocatalytic diversity of endophytic microorganisms (bacteria, yeast and fungi) in order to

* Prof. Dr. David Gonzalez - Lab. de Biocatálisis y Biotransformaciones - Facultad de Química - CC1157 - Montevideo - Uruguay - Ph: (598) 2924-4543 - email: davidg@fq.edu.uy

identify newer biocatalysts and also to enlighten the interesting phenomena of the stereoselective reduction of ketones by simple plants fragments. These type of enzymatic transformations were originally reported by Mironowicz [9] and Baldassarre [10] at the end of the XX century and several other reports have followed studying the scope and limitations of the reactions [11-28].

As a proof of concept of the biocatalytic capability of endophytic microorganisms we studied the reduction of two related β -substituted ketones by selected strains from our laboratory collection. We have previously isolated a number of endophytic bacteria, yeast and fungi and established their potential as biocatalysts for the reduction of heteroaromatic ketones and α -substituted- β -ketoesters [29-30]. In this opportunity we researched the ability of our collection for the reduction of 3-chloro- (2) and 3-azido-1-phenylpropan-1-one (4) since the corresponding alcohols are viable precursors for the synthesis of fluoxetine [31] (Scheme 1). The *S*-chloro alcohol has been prepared previously by bioreduction [32-34] and is commercially available as a GC standard. The azido alcohol has been prepared by enzymatic resolution[35] but is not commercially available. For the reaction we tested five yeast strains isolated from common plants. The results were also compared to the corresponding reductions utilizing intact *Daucus carota* slices and simple chemical reduction with sodium borohydride.



Scheme 1. Two β -substituted ketones that can be viable precursors for fluoxetine synthesis

Materials and Methods

Biotransformations with Daucus carota roots

Edible carrot (*Daucus carota*) roots were purchased in a local market in Montevideo, Uruguay. The roots were thoroughly washed with running tap water and the external layer was removed with a sterile knife. Then, the plant tissue was washed with sterile distilled water, disinfected with 10% sodium hypochlorite solution for 3 minutes, and rinsed with sterile distilled water. Next, the plant parts were soaked in 70% aq. ethanol for 3 minutes and rinsed three times with sterile distilled water. Disinfected plant tissues were sliced with a sterile knife. Immediately, 90 g of plant fragments were transferred to a 250 mL sterile Erlenmeyer flask and suspended in 100 mL of sterile water containing 90 mg of the appropriate substrate. The culture was incubated at 28 °C and 150 rpm in a ZHICHENG ZHWY-211B orbital shaker for 48 h.

Endophytic yeast

The endophytic yeasts *Pichia* sp. CR and *Rhodotorula* sp. CR were isolated from carrot root. *Aureobasidium pullulans* CQA and *Wickerhamomyces anomalus* Z1 were isolated from kumquat fruit and pumpkin fruit respectively (Table 1) as previously described [29-30].

Rhodotorula glutinis H93 was isolated from fennel root by the following procedure. Roots were thoroughly washed and disinfected as described above for the biotransformations performed with carrot root. In order to confirm that the surface disinfection process was successful, tissue surface impressions and water from the final rinsing steps were used to inoculate Petri dishes of tryptic soy agar (TSA, Sigma) and potato dextrose agar (PDA, Difco) [36]. No contamination was detected after incubation at 28 °C for 72 h. Disinfected plant tissues were sliced with sterile knife and the plant fragments were used to inoculate the appropriate “plant broth” media composed of a sterile suspension of each triturated plant. The culture was incubated at 28 °C and 150 rpm in a ZHICHENG ZHWY-211B orbital shaker for 48 h. Serial dilutions of the culture were performed on physiologic serum, and 100 µL of the 10⁻², 10⁻³ and 10⁻⁴ dilutions were spread onto “agar plant plates” (plant broth supplemented with 2% agar), tryptic soy agar (TSA, Sigma) and potato dextrose agar (PDA, Difco) and incubated at 28 °C for 72 h. The isolated yeast strain was stored as a frozen culture in 15% glycerol at -20 and -70 °C.

The isolated yeast strain was identified by phylogenetic analysis. Extraction of yeast genomic DNA was performed by standard procedures [37]. The ITS1–5.8S–ITS2 region was amplified using primer pair ITS1–ITS4 [38]. Amplification of D1/D2 domain of the LSU rRNA gene was performed with primers ITS1-F (TCCGTAGGTGAACCTGCGG) and NL-4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The resulting PCR products were sequenced with an Applied Biosystems automatic sequencer ABI 3730XL at Macrogen Corp., Seoul, Korea. Sequences were compared with other released sequences in the GenBank database using the BLAST program (National Center for Biotechnology Information) [39]. Based on these analyses the isolated endophytic yeast was identified as *Rhodotorula glutinis* H93.

General procedure for biotransformations with yeast strains

Fresh plates of each yeast strain were streaked from the frozen stock in PDA. A single colony was used to inoculate 100 mL of YM Broth. The culture was incubated at 28 °C and 150 rpm for 48 h and the cells were collected by centrifugation at 4000 rpm and 4 °C for 15 minutes. The pellet was washed three times with 50 mL physiological serum. Afterward, 2 g of yeast cells (wet weight) were suspended in 20 mL of 10% dextrose solution and 30 mg of the appropriate substrate were added. The culture was incubated at 28 °C and 150 rpm in an orbital shaker ZHICHENG ZHWY-211B for 48 h.

Identification of metabolites

After 48 h of incubation, the biomass was separated from the culture media by either filtration (*D. carota* biotransformation) or centrifugation (yeasts biotransformations). The culture media was then extracted with ethyl acetate (3x25 mL), the solvent was dried over anhydrous Na₂SO₄ and removed at reduced pressure. Aliquots of the residues (0.5 µL) were analyzed by chiral GC on a Shimadzu 2010 chromatograph equipped with a Megadex DET-TBS (25 m, 0.25 mm) column (MEGA, Italy) and a FID detector. The operating conditions for the

analysis of 3-chloro-1-phenylpropan-1-one experiments were 60 °C (6 min) / 15 °C/min / 90 °C (10 min) / 45°C/min / 180 °C (15 min) and the conditions for 3-azido-1-phenylpropan-1-one experiments were 70 °C (2 min) / 1 °C/min / 130 °C (5 min) / 10°C/min / 180 °C (10 min).

Metabolites (*S*)-**3**, (*R*)-**3**, and (*S*)-**7** were identified by gas chromatographic coinjection with commercial optically pure samples (Sigma-Aldrich). Since no commercial standard was available for metabolite (*S*)-**5**, the compound was isolated and the structure verified by Nuclear Magnetic Resonance. Absolute configuration was determined by comparison of the measured optical rotation to a literature value. The recorded value: $\alpha_D^{23} = -28$ (c 0.5, MeOH) was lower but in agreement with the literature value: $\alpha_D^{30} = -33.5$ (c 6.0, CHCl₃) reported by Kamal *et al.* for the same compound prepared by a different enzymatic approach [35]. This result confirmed the identity of the metabolite as (*S*)-**5**. Achiral metabolite **6** (propiophenone) was identified by ¹H-NMR and ¹³C-NMR and the spectra were compared to reported data [40].

Chemical synthesis of unsaturated ketone **8**

Chloroketone **2** was stirred in aq. Na₂CO₃ (pH = 9) at 28° C for three days until no more starting material was remaining and a product was formed. The compound was isolated and purified by column chromatography and identified as 1-phenylprop-2-en-1-one (**8**) by comparison of the experimental ¹H-NMR with literature data [41].

NMR data for compounds not identified with commercial standards

Propiophenone (6)[40]

¹H NMR (400 MHz, CDCl₃) δ 8.00 – 7.94 (m, 4H), 7.59 – 7.53 (tt, $J = 7.3, 1.4$ Hz, 1H), 7.49 – 7.43 (tt, $J = 7.6, 1.6$ Hz, 4H), 3.05 – 2.98 (q, $J = 7.2$ Hz, 0H), 1.26 – 1.20 (t, $J = 7.2$ Hz, 1H).
¹³C NMR (101 MHz, CDCl₃) δ 201.01, 137.06, 133.03, 128.70 (overlap), 128.12 (overlap), 31.93, 8.39.

1-phenylprop-2-en-1-one (8)[41]

¹H NMR (400 MHz, CDCl₃) δ 7.99 – 7.89 (m, 2H), 7.61 – 7.55 (m, 1H), 7.54 – 7.43 (m, 2H), 7.16 (dd, $J = 17.1, 10.6$ Hz, 1H), 6.44 (dd, $J = 17.1, 1.7$ Hz, 1H), 5.94 (dd, $J = 10.6, 1.7$ Hz, 1H).

(S)-3-azido-1-phenylpropan-1-ol ((S)-5)[35]

¹H NMR (400 MHz, CDCl₃) δ 7.42 – 7.25 (m, 4H), 4.82 (ddd, $J = 7.9, 4.6, 2.6$ Hz, 1H), 3.49 (ddd, $J = 12.3, 7.6, 6.2$ Hz, 1H), 3.37 (dt, $J = 12.5, 6.3$ Hz, 1H), 2.20 (d, $J = 3.0$ Hz, 1H), 2.10 – 1.87 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 143.91, 128.79, 128.03, 125.82, 77.16, 71.91, 48.49, 37.93.

Results and Discussion

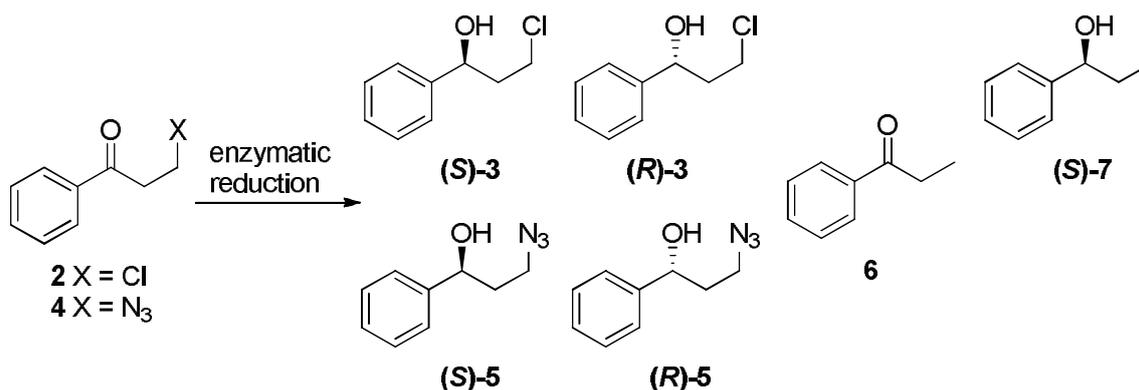
In order to evaluate the feasibility of the chemoenzymatic synthesis we exposed two β -substituted ketones to five endophytic yeast strains from our collection. The strains, detailed in Table 1, were isolated from common edible plants that exhibited reductase activity on β -ketoesters, acetophenone or other ketones. Isolation of the strains was performed during the course of a bioreduction experiment with the corresponding plant under the hypothesis that endophytic microorganisms isolated in the presence of a xenobiotic carbonyl compound are

more likely to exhibit reductase activity. We have previously verified that hypothesis [29-30] and therefore we now use the strategy in a regular basis to search for new biocatalysts, particularly for densely functionalized substrates or those with more than one reducible functional group.

Table 1. Endophytic strains tested for the reduction of β -substituted ketones **2** and **3**.

Plant	isolated strain
Carrot (<i>Daucus carota</i>)	<i>Pichia</i> sp. CR <i>Rhodotorula</i> sp. CR
Pumpkin (<i>Curcubita maxima</i>)	<i>Wickerhamomyces anomalous</i> Z1
Kumquat (<i>Fortunella margarita</i>)	<i>Aureobasidium pullulans</i> CQA
Fennel (<i>Foeniculum vulgare</i>)	<i>Rhodotorula glutinis</i> H93

We studied the reduction of commercial compound **2** and its synthetic derivative **4** which was prepared in our laboratory by nucleophilic displacement of the chlorine atom by azide ion in dimethylformamide [42]. Both ketones were readily reduced by the enzymatic systems but the results were significantly different for each substrate and reducing agent.



Scheme 1. Reduction of β -substituted ketones.

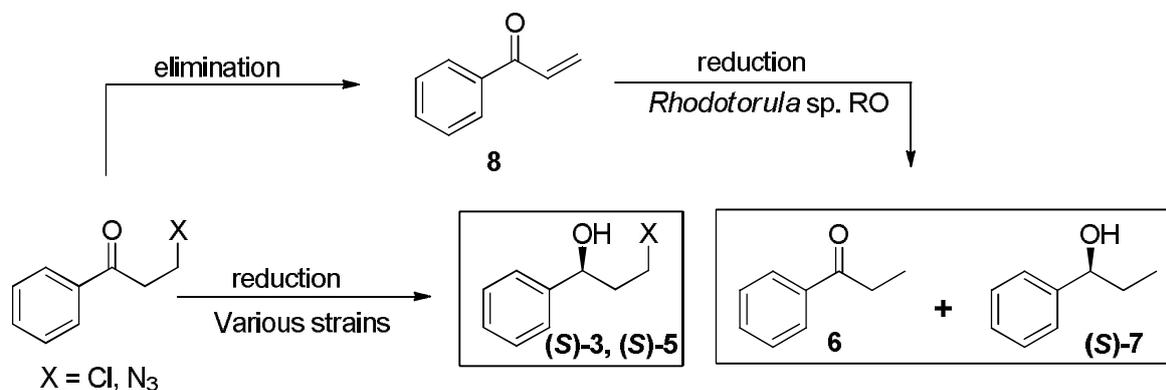
Simple reduction of the carbonyl group was not the main product in some cases. Along with the expected enantiomers of chloro- ((*S*)-**3**, (*R*)-**3**) and azidoalcohols ((*S*)-**5**, (*R*)-**5**), we observed the formation of propiophenone (**6**) as well as the fully reduced (*S*)-1-phenyl-1-propanol ((*S*)-**7**) (Scheme 1). The compound distribution was analyzed by chiral GC and it was demonstrated that the different strains exhibited characteristic metabolite profiles (Table 1). *Aureobasidium pullulans* CQA was a highly selective strain for the production of alcohol (*S*)-**5** by reduction of the corresponding azide **4** (Entry 4). The azidoalcohol accounted for the 93% of the recovered materials and was obtained with high optical purity (98% ee, chiral GC). However, if chloroketone **2** was exposed to the same strain it rendered a significant amount of dehalogenated compounds (alcohol (*S*)-**7**, 17% and ketone **6**, 7%) while the chloroalcohol (*S*)-**3** constituted only a 61% of the mixture. Reduction with the *Pichia* and *Rhodotorula* strains (both isolated from *Daucus carota*) rendered very different results. *Pichia* rendered ketone **6** in about 80% and substituted *S*-alcohols in close to 25% conversion for both substrates (Entry 1). On the other hand *Rhodotorula* provided a high yield of alcohol *S*-

7 from the halogenated ketone but was a very poor reducer for of the azidoketone (Entry 2). We have previously reported the different and complementary behavior of this two strains in the reduction of β -substituted ketoesters [29]. Reduction of **2** with commercial *Daucus carota* root (Entry 6) yielded ketone **6** as the only product in 78% yield; on the other hand, *D. carota* was a very poor reducing agent for azide **4** (90% recovery of the starting material). Interestingly, in both cases the only product was ketone **6** indicating that propiophenone was not a substrate for that enzymatic system.

Table 2. Biotransformation results

Entry	Reducing agent	Compound distribution (%)									
		3-chloro-1-phenylpropanone (2)					3-azido-1-phenylpropanone (4)				
		2	(<i>S</i>)- 3	(<i>R</i>)- 3	6	(<i>S</i>)- 7	4	(<i>S</i>)- 5	(<i>R</i>)- 5	6	(<i>S</i>)- 7
1	<i>Pichia</i> sp. PDA	16	22	2	56	4	25	25	0	47	3
2	<i>Rhodotorula</i> sp. RO	0	1	0	8	91	72	20	0	5	3
3	<i>R. glutinis</i> H93	26	3	0	68	3	72	15	1	12	0
4	<i>A. pullulans</i> CQA	0	61	15	7	17	5	93	1	0	2
5	<i>W. anomalus</i> Z1	23	33	2	39	3	49	30	0	20	1
6	<i>D. carota</i>	22	0	0	78	0	90	0	0	10	0
7	NaBH ₄	2	70 (rac)		0	28 (rac)	3	83 (rac)		2	12 (rac)

The formation of propiophenone (**6**) during the course of a biotransformation has been reported by Hage et al.[43] In their communication, the authors describe the formation of 60% of dehalogenated alcohol (*S*)-**7** and 5% of propiophenone in the reduction of chloroketone **2** by the fungus *Merulius tremellosus* ono991. The authors mentioned the possibility that an enzymatic dehalogenation might be involved. Although this is possible we think that the transformation can also be explained by a two step reaction *via* 1-phenylprop-2-en-1-one (**8**) formed during an elimination process (Scheme 3). As a matter of fact compound **8** was formed in variable amounts during GC analysis of ketones **2** and **3** by thermal elimination inside the gas chromatograph. In order to sustain the participation of α,β -unsaturated ketone **8** as intermediate in the formation of **6** and (*S*)-**7** we exposed a sample of synthetically prepared **8** to a culture of *Rhodotorula* sp. RO and detected the formation of (*S*)-**7** which was also the mayor product of the reduction of chloroketone **2** with this strain (entry 2). Ketone **8** was prepared by stirring chloroketone **2** in a pH=9 buffer at 28 °C for three days. This result supports the hypothesis that formation of **8** (at least from **2**) could take place without enzymatic catalysis.



Scheme 3. Formation of β reduced products *via* elimination and alkene reduction

The elimination of a β -chloroketone is a well described process [44], but the elimination of the azide group deserves further investigation since the latter is a much poorer leaving group. Elimination of related azides has been explained by a photochemical mechanism [45] that is not probably occurring in this case. Alternatively, the formation of ketone **6** from azidoketone **3** may have taken place via a 3,3 sigmatropic rearrangement with assistance of the neighboring enol group as described in Scheme 4. The reaction is formally a retro-Michael process and deserves further experimental study, particularly on secondary azides that can render alkenes with defined stereochemistry.



Scheme 4. Proposed mechanism for the formation of **8** from azide **4**.

Conclusion

This study of the reduction of synthetically relevant β -chloro- and β -azidopropiophenone by *Daucus carota* and five endophytic strains rendered new information on this relatively scantily researched reductase substrates. Simple carbonyl reduction was not the only product and a complex array of metabolites was obtained. The product distribution was highly dependent on the strain and nature of the substituent, suggesting the participation of carbonyl reductases and enoate reductases. Elimination of the substituent giving place to a α,β -unsaturated ketone system could take place solely by a non-catalyzed process but enzyme action cannot be discarded. Further study of these reactions, with a wider array of substrates including isotopically labeled compounds and β -disubstituted ketones will clarify the acting mechanisms.

Acknowledgement

This work was supported by ANII program for undergraduate research scholarships, CSIC-UdelaR and PEDECIBA. The authors thank Horacio Pezaroglo and Verónica Martínez for recording NMR spectra.

Bibliography

- [1] J.M. Grohol, Top 25 Psychiatric Medication Prescriptions for 2011, Psychiatry Central (2012) Retrieved on December 26, 2013, from <http://psychcentral.com/lib/top-25-psychiatric-medication-prescriptions-for-2011/00012586>
- [2] J.W. Young, T.J. Barberich, "Method for treating migraine headaches using optically pure S(+) fluoxetine." US 5,589,511, December 31, 1996
- [3] D.W. Robertson, D.T. Wong, "Selected serotonin subtype receptor agonist to treat sleep apnea." US 5,356,934, October 18, 1994

- [4] W. Kroutil, H. Mang, K. Edegger, K. Faber, *Curr. Opin. Chem. Biol.* 8 (2004) 120-126.
- [5] E. García-Urdiales, I. Alfonso, V. Gotor, *Chem. Rev.* 105 (2005) 313-354.
- [6] D. Zhu, C. Mukherjee, L. Hua, *Tetrahedron: Asymmetry* 16 (2005) 3275-3278.
- [7] R.N. Patel, *Coordin. Chem. Rev.* 252 (2008) 659-701.
- [8] M.C. Núñez, M.E. García-Rubiño, A. Conejo-García, O. Cruz-López, M. Kimatrai, M.A. Gallo, A. Espinosa, J.M. Campos, *Curr. Med. Chem.* 16 (2009) 2064-2074.
- [9] A. Mironowicz, K. Kromer, *Collect. Czech. Chem. Commun.* 63 (1998) 1655-1662.
- [10] F. Baldassarre, G. Bertoni, C. Chiappe, F. Marionni, *J. Mol. Catal. B-Enzym.* 11 (2000) 55-58.
- [11] R. Bruni, G. Fantin, A. Medici, P. Pedrini, G. Sacchetti, *Tetrahedron Lett.* 43 (2002) 3377-3379.
- [12] J.S. Yadav, P.T. Reddy, S. Nanda, A.B. Rao, *Tetrahedron: Asymmetry* 12 (2002) 3381-3385.
- [13] J.V. Comasseto, A.T. Omori, A.L.M. Porto, L.H. Andrade, *Tetrahedron Lett.* 45 (2004) 473-476.
- [14] T. Utsukihara, S. Watanabe, A. Tomiyama, C. Wen, C.A. Horiuchi, *J. Mol. Catal. B-Enzym.* 41 (2006) 103-109.
- [15] L.L. Machado, J.S. Souza, M.C. de Mattos, S.K. Sakata, G.A. Cordell, T.L.G. Lemos, *Phytochemistry* 67 (2006) 1637-1643.
- [16] R. Bruni, G. Fantin, S. Maietti, A. Medici, P. Pedrini, G. Sacchetti, *Tetrahedron: Asymmetry* 17 (2006) 2287-2291.
- [17] L.H. Andrade, R.S. Utsunomiya, A.T. Omori, A.L. M. Porto, J.V. Comasseto, *J. Mol. Catal. B-Enzym.* 38 (2006) 84-90.
- [18] V. Aldabalde, P. Arcia, A. Gonzalez, D. Gonzalez, *Green Chem. Lett. Rev.* 1 (2007) 25-30.
- [19] G.A. Cordell, T.L.G. Lemos, F.J.Q. Monte, M.C. De Mattos, *J. Nat. Prod.* 70 (2007) 478-492.
- [20] A. Pacheco, E. Kagohara, L.H. Andrade, J.V. Comasseto, I.H.S. Crusius, C.R. Paula, A.L.M. Porto, *Enzyme Microb. Tech.* 42 (2007) 65-69.
- [21] T. Matsuda, R. Yamanaka, K. Nakamura, *Tetrahedron: Asymmetry* 20 (2009) 513-557.
- [22] J.S. Yadav, B.V. Subba Reddy, C. Sreelakshmi, A.B. Rao, *Synthesis* (2009) 1881-1885.
- [23] J.S. Yadav, B.V. Subba Reddy, C. Sreelakshmi, G.G. Narayana Kumar, A. Bhaskar Rao, *Tetrahedron Lett.* 49 (2008) 2768-2771.
- [24] H.M.C. Ferraz, G.G. Bianco, F.I. Bombonato, L.H. Andrade, A.L.M. Porto, *Quim. Nova* 31 (2008) 813-817.
- [25] L.L. Machado, G. de Gonzalo, T.L.G. Lemos, M.C. de Mattos, M.d.C. de Oliveira, V. Gotor-Fernández, V. Gotor, *J. Mol. Catal. B-Enzym.* 60 (2009) 157-162.
- [26] A.M. Bizerra, G. de Gonzalo, I. Lavandera, V. Gotor-Fernández, M.C. de Mattos, M. de Oliveira, T.L.G. Lemos, V. Gotor, *Tetrahedron: Asymmetry* 21 (2010) 566-570.
- [27] L.L. Machado, F.J. Monte, M. de Oliveira, M.C. de Mattos, T.L.G. Lemos, V. Gotor-Fernández, G. de Gonzalo, V. Gotor, *J. Mol. Catal. B-Enzym.* 54 (2008) 130-133.
- [28] G.A. Vieira, D.M. de Freitas Araujo, T.L.G. Lemos, M.C. de Mattos, G. de Gonzalo, V. Gotor, *J. Braz. Chem. Soc.* 21 (2010) 1509-1516.
- [29] P. Rodriguez, B. Reyes, M. Barton, C. Coronel, P. Menéndez, D. Gonzalez, S. Rodríguez, *J. Mol. Catal. B-Enzym.* 71 (2011) 90-94.
- [30] P. Rodriguez, M. Barton, V. Aldabalde, S. Onetto, P. Panizza, P. Menéndez, D. Gonzalez, S. Rodríguez, *J. Mol. Catal. B-Enzym.* 49 (2007) 8-11.

- [31] Â. de Fátima, A.A.M. Lapis, R.A. Pilli, *Synthesis* 16 (2005) 495-499.
- [32] Y. Gen-Sheng, X. Jiang-Yan, O. Zhi-Min, Y. Shan-Jing, *Biotechnol. Lett.* 31 (2009) 1879-1883.
- [33] Y. Choi, H. Choi, D. Kim, K.-N. Uhm, H.-K. Kim, *Appl. Microbiol. Biot.* 87 (2010) 185-193.
- [34] H.J. Choi, K.-N. Uhm, H.-K. Kim, *J. Mol. Catal. B-Enzym.* 70 (2011) 114-118.
- [35] A. Kamal, M.S. Malik, A.A. Shaik, S. Azeeza, *Tetrahedron: Asymmetry* 19 (2008) 1078-1083.
- [36] M. Surette, A. Sturz, R. Lada, J. Nowak, *Plant Soil* 253 (2003) 381-390.
- [37] A. Wach, H. Pick, P. Philippsen, in: J.R. Johnston (Ed.), *Molecular Genetics of Yeast: A Practical Approach*, IRL Press at Oxford University Press, New York, 1994, pp. 1-16.
- [38] T.J. White, T. Bruns, S. Lee, J. Taylor, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), *PCR Protocol: A Guide to Methods and Applications.*, Academic Press, San Diego, CA., 1990, pp. 315 - 322.
- [39] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, *J. Mol. Biol.* 215 (1990) 403 - 410.
- [40] B. Zhao, X. Lu, *Tetrahedron Lett.* 47 (2006) 6765-6768.
- [41] M.S. Chen, N. Prabakaran, N.A. Labenz, M.C. White, *J. Am. Chem. Soc.* 127 (2005) 6970-6971.
- [42] P.N. Singh, S. Muthukrishnan, R.S. Murthy, R.F. Klima, S.M. Mandel, M. Hawk, N. Yarbrough, A.D. Gudmundsdóttir, *Tetrahedron Lett.* 44 (2003) 9169-9171.
- [43] A. Hage, D.G.I. Petra, J.A. Field, D. Schipper, J.B.P.A. Wijnberg, P.C.J. Kamer, J.N.H. Reek, P.W.N.M. van Leeuwen, R. Wever, H.E. Schoemaker, *Tetrahedron: Asymmetry* 12 (2001) 1025-1034.
- [44] P.M. O'Neill, E. Verissimo, S.A. Ward, J. Davies, E.E. Korshin, N. Araujo, M.D. Pugh, M.L.S. Cristiano, P.A. Stocks, M.D. Bachi, *Bioorg. Med. Chem. Lett.* 16 (2006) 2991-2995.
- [45] R.F. Klima, A.V. Jadhav, P.N.D. Singh, M. Chang, C. Vanos, J. Sankaranarayanan, M. Vu, N. Ibrahim, E. Ross, S. McCloskey, R.S. Murthy, J.A. Krause, B.S. Ault, A.D. Gudmundsdóttir, *J. Org. Chem.* 72 (2007) 6372-6381.