Highly enantioselective acylation of chlorohydrins using a Amano AK lipase from *P. fluorescens* immobilized in silk fibroin-alginate spheres

Irlon M. Ferreira, Rodolfo H.V. Nishimura, Ana B. dos A. Souza, Giuliano C. Clososki, Sergio A. Yoshioka, André L.M. Porto

PII: S0040-4039(14)01185-X
DOI: http://dx.doi.org/10.1016/j.tetlet.2014.07.032
Reference: TETL 44875

To appear in: *Tetrahedron Letters*

Received Date: 3 June 2014
Revised Date: 7 July 2014
Accepted Date: 8 July 2014

Please cite this article as: Ferreira, I.M., Nishimura, R.H.V., Souza, B.d.A., Clososki, G.C., Yoshioka, S.A., Porto, A.L.M., Highly enantioselective acylation of chlorohydrins using a Amano AK lipase from *P. fluorescens* immobilized in silk fibroin-alginate spheres, *Tetrahedron Letters* (2014), doi: http://dx.doi.org/10.1016/j.tetlet.2014.07.032

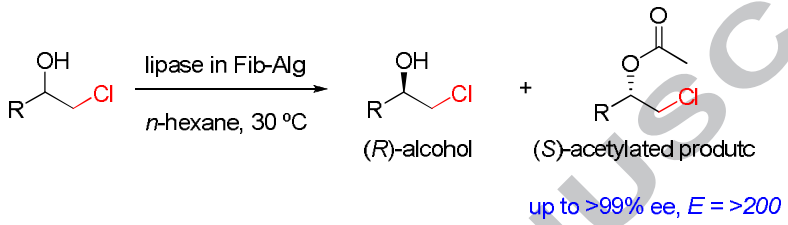
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Highly enantioselective acylation of chlorohydrins using a Amano AK lipase from *P. fluorescens* immobilized in silk fibroin-alginate spheres

Leave this area blank for abstract info.

Irlon M. Ferreira,[a] Rodolfo H. V. Nishimura,[b] Ana B. dos A. Souza,[a] Giuliano C. Clososki,[b] Sergio A. Yoshioka,[a] André L. M. Porto [a,]*



ELSEVIER

Tetrahedron Letters
journal homepage: www.elsevier.com

Highly enantioselective acylation of chlorohydrins using a Amano AK lipase from *P. fluorescens* immobilized in silk fibroin-alginate spheres

Irlon M. Ferreira,[a] Rodolfo H. V. Nishimura,[b] Ana B. dos A. Souza,[a] Giuliano C. Clososki,[b] Sergio A. Yoshioka,[c] André L. M. Porto [a,*]

[a]*Laboratory of Biocatalysis and Organic Chemistry, Institute of Chemistry of São Carlos, University of São Paulo, Av. João Dagnone, 1100, Ed. Environmental Chemistry, Santa Angelina, 13563-120, São Carlos-SP, Brazil.*

[b]*Research Center for Natural and Synthetic Products, University of São Paulo, Av. do Café, S/N, 14.040-903, Ribeirão Preto-SP, Brazil.*

[c]*Laboratory of Biochemistry and Biomaterials, Institute of Chemistry of São Carlos, University of São Paulo, Av. Trabalhador São-carlense, 400, 13560-970, São Carlos, SP, Brazil.*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
biocatalysis
enzymatic kinetic
chiral chlorohydrins
silk fibroin
Pseudomonas fluorescens

ABSTRACT

Aromatic, allylic and aliphatic compounds containing a chlorohydrin group were selected as substrates for the enzymatic kinetic resolution mediated by a Amano AK lipase from *Pseudomonas fluorescens* immobilized in silk friboin-alginate spheres. Thus, the enantioselectivity of the process was sufficient for the production of the desired alcohols and acetates in good yields and high enantiomeric purities. This paper provides a simple, cheap and practical protocol for enantioselective and reinforces the versatility of silk fibroin as supports.

In recent decades the concept of green chemistry has intensified the search for sustainable synthetic methodologies.[1] Among the advantages of the application of enzymes in chemical synthesis, features such as high catalytic activity, high chemo-, regio-, and stereoselectivity.[2] However, low stability and difficulties in recovering and maintaining enzymatic turnover have frequently precluded the application of biocatalysts in chemical synthesis.[3]

As a consequence of the growing importance of the biocatalysis in the industry, the development of techniques for stabilizing biocatalysts has received great attention.[4] Thus, immobilization of enzymes and whole cells is nowadays accepted as a key strategy for developing efficient and economically viable biocatalytical processes. However, in the case of lipases, immobilization frequently promotes the increase of catalytic activity in organic solvents as an extra advantage.[5]

Despite the great importance, some biomaterials used in the immobilization of enzymes and microorganisms are still little studied, such as, the silk fibroin (SF), chitosan nanofibres and chitosan.[6] The silk fibroin is a known class of biopolymers derived from cocoons of the *Bombyx mori* silkworm that presents a good biodegradability and biocompatibility.[7,8]

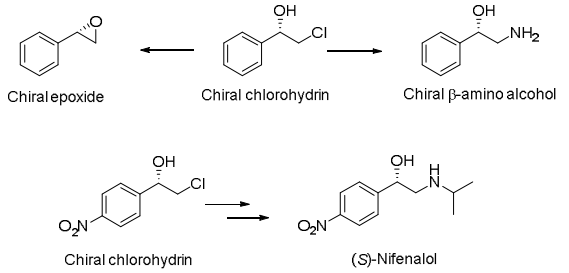
Thus, due to its unique tensile strength and elasticity, good thermal stability, hygroscopicity, and microbial resistance,[9] this biomaterial presents important features for enzymes immobilization.

Studies involving the use of enzymes in the enantioselective preparation of optically active chlorohydrins are relevant since these compounds find a great range of applications in organic synthesis (Figure 1).[10] The kinetic resolution of racemic 2-chloro-1-phenylethan-1-ol has been successfully achieved by Amano lipase from *P. fluorescens* leading to the corresponding (*S*)-ester and (*R*)-alcohol with 92% and 97% *ee* respectively. In addition, a combination of *Pseudomonas cepacia* (PS-C "Amano") and a ruthenium catalyst were used in a dynamic kinetic resolution of aromatic chlorohydrins. Moreover, the resolution of a (*R*)-α-lipoic acid chlorohydrin precursor has been achieved via lipase catalyzed enantioselective transacylation.[11]

Recently, Nishimura and coworkers synthesized several chlorohydrins through the reaction of aldehydes with the mixed lithium-magnesium carbenoid $ClCH_2MgCl.LiCl$.[12] In this work, we have investigated the enzymatic kinetic resolution (EKR) of these compounds using free and silk fibroin-alginate immobilized Amano AK lipase from *Pseudomonas fluorescens*. Some representative aromatic, allylic and aliphatic chlorohydrins were selected as targets for this study.

Figure 1. Some synthetic applications of chiral chlorohydrins.

* Corresponding author. Tel.: +55 16 3373 8103. Fax.: +55 16 3373 9952. E-mail address: almporto@iqsc.usp.br (A.L.M. Porto)



In order to investigate the catalytic behavior of the free and immobilized Amano AK lipase from *P. fluorescens* in the EKR of chlorohydrins, we have chosen the (*R,S*)-2-chloro-1-phenylethan-1-ol ((*R,S*)-**1a**) as a model substrate. In these reactions vinyl acetate was used as an irreversible acyl donor since the leaving group (enol) spontaneously tautomerize to acetaldehyde.

The results are summarized in Table 1. Initial experiments on the resolution of (*R,S*)-**1a** using the free lipase from *P. fluorescens* have provided the desired acetate (*S*)-**2a** in 29% conversion and 97% *ee* (*E* = 98) within 24h (Table 1, entry 1). Interestingly, when the spheres of Fib-Alg-Lip were used under the same reaction time, the reaction conversion dropped to 22% but the enantiomeric excess increased to >99% (*E* = 262, Table 1, entry 2). In contrast, no acetate formation was observed when the reaction was performed with Amano AK lipase from *P. fluorescens* immobilized in calcium alginate (Table 1, entries 3, 7 and 11). These unexpected results could be explained by changes on the active site of the lipase under the immobilization process. It is worth pointing out that the immobilized material was subsequently lyophilized favoring the deactivation of the lipase.

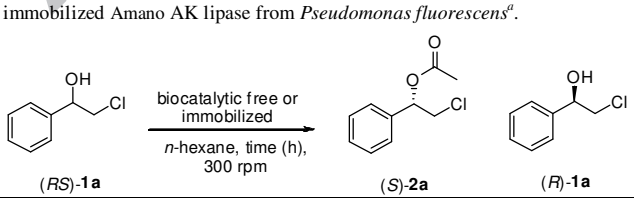
Additionally, no acetylated products were observed in control experiments carried out with spheres of calcium alginate in absence of the lipase (Table 1, entries 4, 8 and 12).

When the EKR of (*R,S*)-**1a** using free and Fib-Alg immobilized Amano AK lipase from *P. fluorescens* were analyzed after 48h, the enantiomeric excesses were very similar to the observed with 24h reaction (95 and 99%) but the conversion increased to 41% and 35%, respectively. Thus, it is evident that the immobilized biocatalyst showed higher enantioselectivity than the free lipase when used *n*-hexane as solvent (*E* = 339 and 80, respectively), (Table 1, entries 5 and 6).

In 96 h of reaction, the immobilized lipase converted the (*R,S*)-alcohol **1a** into the desired (*S*)-**2a** in 48% (*ee* = 98%; *E* = 327) (Table 1, entry 10). A similar conversion (49%) was observed when the lipase was used in its free from, but the selectivity was slightly lower (*ee* = 91, *E* = 124) when compared with 24h and 48h reaction (Table 1, entry 9).

Results of Table 1 suggest that immobilization of Amano AK lipase from *P. fluorescens* onto spheres of silk fibroin-alginate by encapsulation, favored the reaction selectivity in comparison with the free enzyme.

Table 1. Kinetic resolution of the chlorohydrin (*R,S*)-**1a** with free and immobilized Amano AK lipase from *Pseudomonas fluorescens*[a].



Entry	Biocatalyst	Time (24 h)			
		ee (%)			
		(*S*)-**2a**[b]	(*R*)-**1a**[b]	*c* (%)[c]	*E*[d]
1	Free lipase	97	41	29	98
2	Fib-Alg-Lip	>99	28	22	262
3	Alg-Lip	Nr	Nr	Nr	Nr
4	Alg	Nr	Nr	Nr	Nr
		Time (48 h)			
5	Free lipase	95	68	41	80
6	Fib-Alg-Lip	99	53	35	339
7	Alg-Lip	Nr	Nr	Nr	Nr
8	Alg	Nr	Nr	Nr	Nr
		Time (96 h)			
9	Free lipase	91	95	49	124
10	Fib-Alg-Lip	98	94	48	327
11	Alg-Lip	Nr	Nr	Nr	Nr
12	Alg	Nr	Nr	Nr	Nr

[a]General conditions: Immobilized Amano AK lipase from *P. fluorescens* in spheres of silk fibroin-alginate (30 mg), substrate (40 mg), vinyl acetate 75 µL, *n*-hexane (1 mL), 32 °C, 300 rpm.
[b]$c_{CG\text{-}FID} = ee_S/ee_P + ee_S$, as defined in Ref [13].
[c]conversion: $c = ee_S/ee_S + ee_P$.
[d]$E = \ln[ee_P(1 - ee_S)/(ee_P + ee_S)]/\ln[ee_P(1 + ee_S)/(ee_P + ee_S)]$.
Nr = No reaction.

Using Fib-Alg immobilized lipase spheres, the model reaction was optimized by varying solvent and catalyst loadings. In addition, to determine the optimum concentration of biocatalyst, the EKR of (*R,S*)-**1a** were performed by using different immobilized lipase amounts (0.015, 0.02, 0.03, 0.04, and 0.05 g Table 2). After 48 hours reaction, the maximum *ee* (99%) was obtained with 0.05 g of immobilized lipase (Table 2, entry 5). Moreover, after 96 hours reaction, the increase of the catalyst amount from 0.03 g (Table 2, entry 8) to 0.04 g and 0.05 g (Table 2, entries 9-10) had no effect on the yield and enantiomeric excess of the desired product since all were obtained in 49% conversion and 98% *ee*.

Table 2. Effect of the biocatalyst amount on the EKR of (*R,S*)-**1a**with immobilized Amano AK lipase from *P. fluorescens* in spheres of silk fibroin-alginate[a].

Entry	immobilized lipase (mg)	Time (48 h)			
		ee (%)			
		(*S*)-**2a**[b]	(*R*)-**1a**[b]	*c* (%)[c]	*E*[d]
1	15	99	61	38	372
2	20	99	36	27	282
3	30	99	53	35	338
4	40	99	66	40	396
5	50	99	78	44	474
		Time (96 h)			
6	15	99	61	38	371
7	20	97	94	49	234
8	30	98	94	49	354
9	40	98	96	49	392
10	50	98	96	49	392

[a]General conditions: Immobilized Amano AK lipase from *P. fluorescens* on spheres of silk fibroin-alginate (30 mg), substrate (40 mg), vinyl acetate 75 µL, *n*-hexane (1 mL), 32 °C, 300 rpm.
[b]$c_{CG\text{-}FID} = ee_S/ee_P + ee_S$, as defined in Ref [13].
[c]conversion: $c = ee_S/ee_S + ee_P$.
[d]$E = \ln[ee_P(1 - ee_S)/(ee_P + ee_S)]/\ln[ee_P(1 + ee_S)/(ee_P + ee_S)]$.

Biocatalysis in organic solvents offers numerous advantages with a great impact on the enzyme activity which compelled us to study their effect on the resolution of the chlorohydrin (*R,S*)-**1a** by the free and immobilized Amano AK lipase from *P. fluorescens*. The data are summarized in Table 3. A variety of solvents with different polarities was studied. *n*-Hexane was found to be the best solvent providing the desired product in 99% and 98% yields, respectively (Table 3, entries 1 and 7). This result is in agreement with the fact that hydrophobic solvents with higher *log P* values do not have a tendency to strip the tightly bound water molecules from the enzyme surface which are essential for the catalytic activity of lipases. It is well know that in some organic solvents enzymes may suffer from reduced

activity, selectivity or stability. [14] In our study, this effect has been observed when ethyl ether, DMF and DMSO were used (Table 3). Despite that the desired product had been observed with high enantiomeric excesses in DMF and DMSO, the conversions were much lower, even at 96 h of reaction. When ether was used as solvent, not conversion was observed (Table 3, entries 3 and 9). In toluene and chloroform the conversions were moderate when compared with *n*-hexane as reaction solvent (Table 3, entries 10-11). And it can be clearly seen that both the conversion and the enantiomeric excesses are dramatically dependent on the solvent, for lipase immobilized on Fib-Alg. In our study the enzymatic resolution of (*R,S*)-**1a** with ethyl ether and diisopropyl ether were not obtained with free and immobilized lipase from *P. fluorescens* (experiments performed in duplicate). This result was unexpected, since Oda et al.[11]promoted the resolution of (*RS*)-**1a** successfully using the same free enzyme.

After finding the best reaction condition for the EKR of (*R,S*)-**1a**, we have investigated the generality and scope of this protocol through its application on the enzymatic resolution of different chlorohydrins. Thus, compounds (*R,S*)-**1b-h** were reacted with the Amano AK lipase from *Pseudomonas fluorescens* immobilized on beads of silk fibroin-alginate (0,03 g) in hexane at 32 ºC (300 rpm) using vinyl acetate as an acyl donor (Table 4). The consumption of the starting materials was monitored through the CG-FID or HPLC analysis of reaction aliquots, collected every 48 and 96 hours (see Experimental Section).

With the exception of compound (*R,S*)-**1d,** that appeared as a non-reactive substrate after 240 hour reaction (Table 4, entry 3), all other substrates were resolved with $E > 200$ within 120 hours reaction. Thus, the enantioselectivity of the process was sufficient for the production of the most desired alcohols and acetates in good yields and enantiomeric purities. For example, resolution of (*R,S*)-**1b** afforded the corresponding acetate (*S*)-**1b** gave *ee* = 99% and 45% of conversion (Table 4, entry 1 and Figure 2).

Table 3. Effect of solvents in the EKR of (*R,S*)-**1a** with immobilized Amano AK lipase from *P. fluorescens* in spheres of silk fibroin-alginate[a].

Entry	Solvents	48 h *ee* (%)			
		(*S*)-**2a**[b]	(*R*)-**1a**[b]	*c* (%)[c]	*E*[d]
1	Hexane	99	53	35	209
2	DMSO	>99	4	4.3	207
3	Ethyl ether or Disopropyl ether	Nr	Nr	Nr	Nr
4	Toluene	>99	4	4.3	207
5	Chloroform	>99	3	3	205
6	DMF	99	0.5	0.6	200
		96 h			
7	Hexane	98	94	49	354
8	DMSO	96	3	3	50
9	Ethyl ether or Disopropyl ether	Nr	Nr	Nr	Nr
10	Toluene	98	17	15	116
11	Chloroform	98	13	12	112
12	DMF	83	1	1	11

[a]General conditions: Immobilized Amano AK lipase from *P. fluorescens* in spheres of silk fibroin-alginate (30 mg), substrate (40 mg), vinyl acetate 75 µL, *n*-hexane (1 mL), 32 °C, 300 rpm.
[b]$c_{CG\text{-}FID} = ee_S/ee_P + ee_S$, as defined in Ref [13].
[c]conversion: $c = ee_S/ee_S + ee_P$.
[d]$E = \ln[ee_P(1 - ee_S)/(ee_P + ee_S)]/\ln[ee_P(1 + ee_S)/(ee_P + ee_S)]$.

Interestingly, the alcohol (*R,S*)-**1c** was converted into (*S*)-**2c** in 30% with 99% *ee* in 120 h (Table 4, entry 2). Comparison of this result with the one obtained for the alcohol (*R,S*)-**1d** (Table 4, entry 3) suggests that the position of substituent in the aromatic ring has a strong influence on the interaction of the substrate with the active site of the lipase.

Finally, compounds (*S*)-**2e**-**h** were synthesized with 99% *ee* and with enantioselectivity *E* >200 (Table 4, entries 4-7). The absolute configuration of the acetates (**2a-h**) was assigned as *S* configuration by comparison with optical rotation values described in the literature. Consequently, it was possible to observe that the immobilized lipase has stereochemical preference for *S*-acetates esterification. Thus, the observed esterification preference is in agreement with the predictions of the Kazlauskas rule.[15]

In order to evaluate the recycling potential of the lipase immobilized on the Fib-Alg spheres, a series of repetitive EKR experiments was carried out using (*R,S*)-2-chloro-1-phenylethanol **1a** as a substrate. After each process, the catalytic spheres were collected by filtration and used again in a new experiment. As shown in Figure 2, the immobilized enzyme maintained its activity, exhibiting less than 55% decay after six cycles. The conversion value and the enantiomeric excess of product (*S*)-**2a** stabilized after the fourth cycle in 23% and 98% respectively.

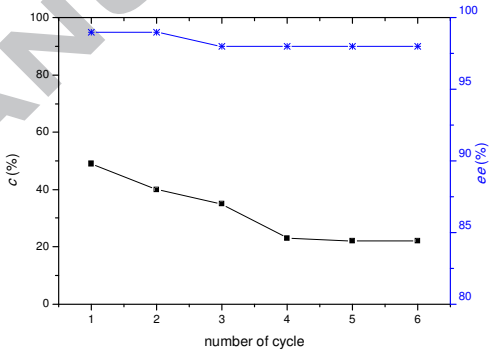


Figure 2. Reuse of Amano AK lipase from *Pseudomonas fluorescens* immobilized in Fib-Alg in the EKR of the (*R,S*)-2-chloro-1-phenylethanol (**1a**). Conversion (▪); Enantiomeric excess (*)

The enzymatic kinetic resolution of chlorohydrins mediated by a Amano AK lipase from *Pseudomonas fluorescens* immobilized in fibroin-alginate spheres has allowed the enantioselective preparation of the desired compounds with high enantioselectivity. In some cases, the enantioselective enzymatic transesterification also provided the expected acetates in high enantiomeric excess (> 99%). In summary, this paper provides a simple, cheap and practical protocol for enantioselective synthesis of chlorohydrins and reinforces the versatility of silk fibroin as supports for heterogeneous catalysts since it is removed easily from the reaction mixture by simple filtration and maintains good yields and excellent enantioselectivity after consecutive reactions.

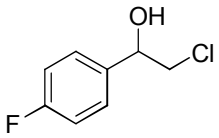
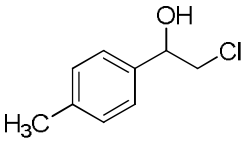
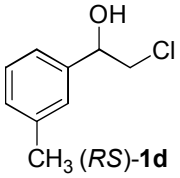
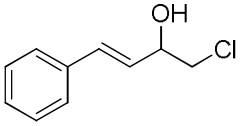
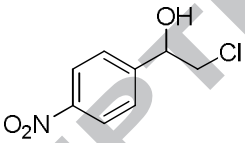
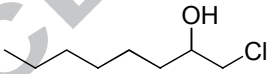
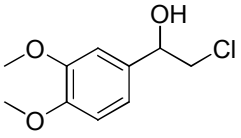
Acknowledgments

I. M. Ferreira thanks to CNPq for scholarship. The authors would like to acknowledge FAPESP, CAPES and CNPq for the financial support provided to this research.

Appendix A. Supplementary data

Supplementary material (^{1}H NMR, ^{13}C NMR spectra, GC and HPLC chiral chromatography) associated with this article can be found, in the online version, at http://dx.doi.org/xxxx

Table 4. Kinetic resolution of chlorohydrins (*R,S*)-**1b-h** mediated by Amano AK lipase from *Pseudomonas fluorescens* immobilized on silk fibroin-alginate spheres[a].

Entry	chlorohydrins	*ee* (%)		E[d]	*c*(%)[e]	Time (h)
		(*S*)-**1**	(*R*)-**2**			
1[b]	 (*RS*)-**1b**	99	82	510	45	120
2[b]	 (*RS*)-**1c**	99	43	304	30	120
3[b]	 (*RS*)-**1d**	Nr	Nr	Nr	Nr	240
4[c]	 (*RS*)-**1e**	99	43	304	30	120
5[c]	 (*RS*)-**1f**	99	47	317	32	120
6[c]	 (*RS*)-**1g**	39	99	292	71	120
7[c]	 (*RS*)-**1h**	99	15	230	13	120

[a]General conditions: immobilized Amano AK lipase from *P. fluorescens* on spheres of silk fibroin-alginate (30 mg), substrate (40 mg), vinyl acetate (75 µL), *n*-hexane (1 mL), 32 °C, 300 rpm.
[b]$C_{CG\text{-}FID} = ee\text{S}/(ee\text{P} + ee\text{S})$, as defined in Ref [12]
[c]$C_{HPLC} = ee\text{S}/(ee\text{P} + ee\text{S})$, as defined in Ref [12]
[d]$E = \ln[ee_P(1 - ee_S)/(ee_P + ee_S)]/\ln[ee_P(1 + ee_S)/(ee_P + ee_S)]$.
[e]Conversion: $c = ee\text{S}/(ee\text{S} + ee\text{P})$.
(Nr) = No reaction.

References and notes

1. (a) Dunn, P. J.; *Chem. Soc. Rev.*, **2012**, *41*, 1452-1461; (b) Cirminna, R.; Pagliaro, M. *Org. Process Res. Dev.,* **2013**, *17*, 1479-1484.

2. (a) Kamal, A.; Chouhan, G. *Tetrahedron Lett*., **2004**, *45*, 8801-8805; (b) Dhake, P. K.; Thakare, D. D.; Bhanage, M. B. *Flavour Gragr. J.,* **2013**, *28*, 71-83; (c) Deasy, R. E.; Mood, T. S.; Maguire, A. R. *Tetrahedron: Asymmetry,* **2013**, *24,*1480-1487; (d) Xie, Z. B.; Wang, N.; Jiang, G. F.; Yu, X. Q. *Tetrahedron Lett*., **2013**, *54,* 945-948; (f) Ramos, J. L.; Neto, W.; Woodley J. M. *Top. Catal.,* **2014**, *57*, 301-320; (e) Fan, Y.; Qian, J. *J. Mol. Catal. B: Enzym.,* **2010**, *66*, 1-7; (f) Raminelli, C.; Santos, A. dos; Porto, A. L. M.; Andrade, L. H.; Comasseto, J. V. *Tetrahedron,* **2005**, *61*, 409-415.

3. (a) Sheldon, R. A.; Pelt, D. V.; *Chem. Soc. Rev.,* **2013**, *42*, 6223-6235; (b) Netto, C. G. C. M.; Andrade, L. H.; Toma, H. E. *Tetrahedron: Asymmetry*, **2009**, *20*, 2299-2304; (c) Rebelo, L. P.; Netto, C. G. C. M.; Toma, H. E.; Andrade, L. H. *J. Braz. Chem. Soc.*, **2010**, *21*, 1537-1542.

4. (a) Bommarius, A. S.; Paye, M, F. *Chem. Soc. Rev.,* **2013**, *42*, 6534-6565; (b) Huisman, W. G.; Collier, J. S. *Curr. Opin. Chem. Biol.* **2013**, *17*, 284-292.

5. (a) Adlercreutz, P. *Chem. Soc. Rev.*, **2013**, *42*, 6406-6436; (b) DiCosimo, R.; M. J.; Poulose, A. J.; Bohlmann, G. *Chem. Soc. Rev.*, **2013**, *42*, 6437-6474; (c) Rodrigues, R. C.; Ortiz, C.; Murcia, A. C.; Torres, R.; Lafuente, R. F. *Chem. Soc. Rev.*, **2013**, *42*, 6290- 6307.

6. (a) Huang, X-J.; Ge, D.; Xu, Z-K. *Eur. Polym. J.,* **2007**, *43*, 3710-3718; (b) Chen, B.; Yin, C.; Cheng, Y.; Li, W.; Cao, Z-a; Tan, T. *Biomass Bioenergy*, **2012**, *39,* 59-66. (c) Rocha, L. C.; Souza, A. L. de; Filho, U. P. R.; Filho, S. P. C.; Setteb, L. D.; Porto, A. L. M. *J. Mol. Catal. B: Enzym.,* **2012**, *84*, 160-165; (d) Zhang, Y. *Biotech. Adv.*, **2002**, *20*, 91.

7. (a) Bini, E.; Knight, D.; Kaplan, D. *J. Mol. Biol.,* **2004**, *335*, 27-40; (b) Moraes, M. A.; Weska, R. F.; Beppu, M. M. *J. Biomed. Mater. Res. B Appl. Biomater.,* **2014**, *102*, 869.

8. Foo, C.; Bini, E.; Huang, J.; Lee, S.; Kaplan, D. *Appl. Phys. A: Mater. Sci. Process.*, 2006, **82**, 193-203.

9. Wang, M.; Jin, H.; Kaplan, D.; Rutledge, G. *Macromolecules*, **2004**, *37,* 6856-6864.

10. (a) Bergeron-Brlek, M.; Teoh, T.; Britton, R. *Org. Lett.* **2013**, *15*, 3554-3557; (b) Zhang, J.; Wang, J.; Qiu, Z.; Wang Y. *Tetrahedron*, **2011**, *67*, 6859-6887; (c) Bentley, A. P.; Mei, Y.; Du, J. *Tetrahedron Lett*., **2008**, *49*, 1425-1427; (d) Usami, Y.; M, K. *J. Nat. Prod*. **2011**, *74*, 877-881; (e) Hodgson, D. M.; Salik, S.; Fox, D. *J. Org. Chem.* **2010**, *75*, 2157-2168.

11. (a) Hiratake, J.; Inagaki, M.; Nishioka, T.; Oda J. *J. Org. Chem.*, **1988**, *53*, 6130-6133. (b) Träff, A.; Bogá K.; Warner, M.; Bäckvall, Jan-E. *Org. Lett.,* **2008**, *10*, 4807-4810. (c) Zhou, W.-J.; Ni, Y.; Zheng, G.-W.; Chen, H-H.; Zhu, Z-R.; Xu, J-H. *J. Mol. Catal. B: Enzym.,* **2014**, *99*, 102-107.

12. Nishimura, R. H. V.; Toledo, F. T.; Lopes, J. L. C.; Clososki, G. C. *Tetrahedron Lett*., **2013**, *54*, 287-290.

13. Reigada, J. B.; Tcacenco, C. M.; Andrade, L. H.; Kato, M. J.; Porto, A. L. M.; Lago, J. H. G. *Tetrahedron: Asymmetry,* **2007**, *18*, 1054-1058.

14. Sakai, S.; Liu, Y.; Watanabe, R.; Kawabe, M.; Taya, M.; Kawakami, K. *J. Mol. Catal. B: Enzym.,* **2012**, *83*, 120-124.

15. Faber, K. *Biotransformation in Organic Chemistry*, 4th ed.; Springer: Berlin, 2000.

*Experimental procedure for kinetic resolution of chlorohydrins (R,S)-**1a-h** by immobilized Amano AK lipase from P. fluorescens:* To a vial flask (3 mL) were added solvent (1 mL), vinyl acetate (0.100 mL), immobilized lipase Fib-Alg (30 mg) and the appropriate chlorohydrins (*R,S*)-**1a-h** (40 mg). The reaction mixture was stirred in a mechanic agitation at 300 rpm, at 32 ºC. The progress of the reactions was followed by removing samples (30 µL of reaction mixture were added to 600 µL of EtOAc) for GC or HPLC analyses, according to the time indicated in Table 4. After the reaction was complete, the immobilized lipase was filtered off. The filtrate was evaporated under reduced pressure and purified by column chromatography on silica gel using hexanes/EtOAc (8:2) as eluent, yielding the enantiomerically enriched (*R*)-alcohols **1a-h** and (*S*)-acetates **2a-h**. *Reusability of the biocatalyst*: At the end of the reaction performed with **1a** compound, following above-mentioned procedure in Supplementary material. In addition, at the end of each recycle the filtered immobilized lipase was recovered, washed with *n*-hexane (3x 1mL) and reused in another reaction by six cycles.