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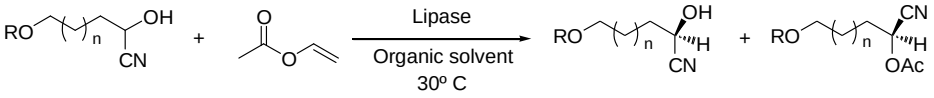
Gonzalo de Gonzalo, Iván Lavandera, Rosario Brieva and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33071, Oviedo, Spain.



R: Ac; Bz; MOM; CPh_3.
n: 1; 2; 3.

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Enzymatic acylation reactions on ω-hydroxycyanohydrins.

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Abstract—The enzymatic acylation of certain ω-hydroxycyanohydrins protected at the primary alcohol has been studied. The best enantioselectivities are obtained with *Pseudomonas cepacia* lipase (PSL-C) and *Candida antarctica* lipase A (CAL-A), for the ω-*O*-tritylated cyanohydrins. The effect of the protecting group in the enzymatic reactions has been studied using molecular modeling.

1. Introduction

Cyanohydrins have a great synthetic potential in organic synthesis. As α-substituted carboxylic acid derivatives, cyanohydrins may serve as stereochemically pure starting materials for the preparation of important classes of compounds.[1] Our interest in the preparation of optically active ω-functionalized cyanohydrins and their application to the synthesis of nitrogen heterocycles,[2] led us to study the (*R*)-oxynitrilase catalyzed addition of acetone cyanohydrin to 4-hydroxybutanal and 5-hydroxypentanal.[3] These aldehydes can be considered difficult substrates for (*R*)-oxynitrilase because of their relatively high water solubility.[4] The main problem of these processes was the competition with the non-enzymatic hydrocyanation. Even though by varying the reaction parameters we could reduce the extent of the undesirable competing reaction and optimize the optical purity of the obtained cyanohydrins, the highest enantiomeric excess obtained was only 62 %. On the other hand, the preparation of the corresponding (*S*)-cyanohydrins using (*S*)-oxynitrilases as catalysts, is more difficult because of the low availability of these enzymes and their more restrictive substrate specificity. In recent years, several reviews on the use of these biocatalysts for the synthesis of chiral cyanohydrins have been reported.[5]

The lipase catalyzed resolution of racemic cyanohydrins is an alternative catalytic method which has been exploited for the preparation of certain optically active cyanohydrins.[6] The main disadvantage of these kinetic resolutions is that the maximum obtainable yield of each enantiomer cannot exceed the 50%. However, several strategies, like a coupled racemization[7] or *in situ* Mitsunobu esterification,[8] have been developed in order to increase the theoretical yield.

Molecular modeling has been used as a potent tool for the explanation of many results in biotransformations.[9] Thus, as a qualitative tool, these methods are greatly useful. They can predict, for example, how to increase the selectivity by site-directed mutagenesis.[10] However, for quantitative predictions, such as the degree of enantioselectivity, they are still not reliable.[11] Modeling is limited by the availability of three-dimensional structures of the enzymes. The force field (FF) methods and molecular mechanics are empirically based. The FF relates the geometry and the potential energy of a molecule using an analytical function. Among them, AMBER is one of the most appropriate for the study of proteins and other natural products.[12]

Here we studied the lipase-catalyzed resolution of ω-hydroxycyanohydrins. The enantioselectivity of the process strongly depends on the protecting group at the primary hydroxyl group, and some of these enzymatic reactions have been examined using molecular modeling, explaining the important effect of the protecting group on the ω-hydroxyl.

2. Results and discussion

In order to prevent the possible competition of the non-enzymatic acylation on the primary alcohol, we used as substrates the *O*-protected derivatives (±)-**3a-f.** Four protecting groups were selected, presenting different sizes and electronic properties, so we could compare the effect of these parameters on the reaction rate and the enantioselectivity of the resolutions. Cyanohydrins (±)-**3a-e** were prepared from the corresponding aldehydes,[3] and subsequent protection of the free hydroxycyanohydrins (±)-**1a-b**. For the synthesis of (±)-**3f**, the less stable 6-hydroxyhexanal was prepared by reduction of ε-

* Corresponding author. Tel.: +34-985-103-448; fax: +34-985-103-448; e-mail: vgs@fq.uniovi.es

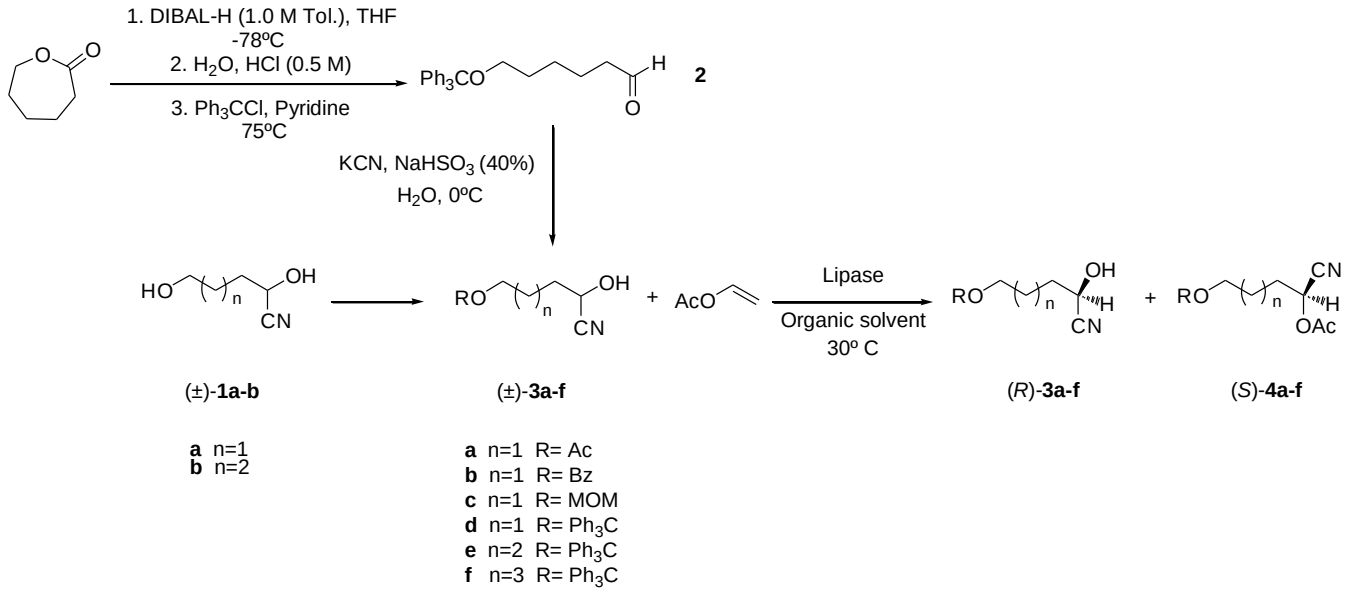
caprolactone under mild conditions. The free hydroxyaldehyde was protected *in situ* by treatment with trityl chloride and pyridine to obtain 7-trityloxyheptanal **2**, and then converted into the racemic *O*-protected hydroxycyanohydrin (Scheme 1).

Our initial experiments were designed to find the most suitable lipase for catalyzing the acylation of 5-acetoxy-2-hydroxypentanenitrile (±)-**3a** with 5 equivalents of vinyl acetate (Table 1). A first set of experiments were carried out at 30ºC in toluene, using vinyl acetate as acylating agent. In all the resolutions, the (*S*)-enantiomer is preferentially acylated by the lipases, while the remaining substrate possesses (*R*)-configuration. Three of the tested enzymes catalyzed the reaction in toluene: Lipases A and B from *Candida antarctica* (CAL-A and CAL-B) and the immobilized form of the *Pseudomonas cepacia* lipase, PSL-C. In all the cases, the enantioselectivities were low. CAL-B showed the highest enantiomeric ratio (***E*** = 9.4, entry 1),[13] but the reaction was very slow. When CAL-A or PSL-C were used (entries 2 and 3), the reaction rates were increased, with similar enantiomeric ratios.

In order to improve the rate and enantioselectivity of these processes, we examined the influence of the organic solvent in the resolution of substrate (±)-**3a** carried with the PSL-C. The reaction in *t*-BuOMe was faster than in toluene but when 1,4-dioxane was used, the reaction rate decreased significantly. In both cases, there was no improvement in the enantioselectivity (entries 4 and 5).

Then, we studied the influence of the protecting group in the processes catalyzed by CAL-A and PSL-C. For 5-benzoyloxy-2-hydroxypentanenitrile, (±)-**3b** reaction rates were faster than for the acetylated cyanohydrin (±)-**3a**, but the enantioselectivities were not significantly modified (entries 6 and 7). Better results were observed when methoxymethyl was used as protecting group. For substrate (±)-**3c**, moderate enantioselectivities can be obtained using CAL-A (***E*** = 16, entry 8) and PSL-C (***E*** = 19, entry 9) with only a small decrease of the reaction rate.

The best results in the lipase catalyzed acetylation of these 2,5-dihydroxypentanenitriles were obtained using trityl as the protecting group (Scheme 1, Table 2). For substrate (±)-**3d**, the resolution catalyzed by PSL-C in toluene showed a very high enantioselectivity (***E*** = 125, entry 2), with a good reaction rate (after only 4 h, 46% conversion was achieved). The use of CAL-A as catalyst in the same solvent (entry 1), resulted in a lower reaction rate and enantioselectivity compared to PSL-C, nevertheless the *E* value obtained was also high (***E*** = 72). In view of these excellent results, we also tested CAL-B as catalyst for this substrate (entry 3), but the reaction was slower and less enantioselective.



Scheme 1. Synthesis and lipase catalyzed resolution of ω-*O*-protected hydroxycyanohydrins using vinyl acetate as acylating agent.

Table 1. Lipase catalyzed acylations of substrates (±)-**3a-c** using vinyl acetate[a] at 30ºC in organic solvents.

Entry	R	Lipase	Solvent	t (h)	*c* (%)[b]	*ee* (%)[c] (*R*)-**3a-c**	*ee* (%)[c] (*S*)-**4a-c**	*E*[d]
1	Ac	CAL-B	Toluene	20	29	30	75	9.4
2	Ac	CAL-A	Toluene	1	26	31	63	5.9
3	Ac	PSL-C	Toluene	2	38	38	62	6.1
4	Ac	PSL-C	^{t}BuOMe	2	52	57	53	3.5
5	Ac	PSL-C	1,4-dioxane	72	12	10	71	6.5
6	Bz	CAL-A	Toluene	1	52	66	59	7.5
7	Bz	PSL-C	Toluene	2	49	43	44	3.8
8	MOM	CAL-A	Toluene	4	45	63	78	16
9	MOM	PSL-C	Toluene	2	42	60	82	19

[a] Reactions were carried using 5 equivalents of vinyl acetate. [b] Conversion, $c = ee_S/(ee_S + ee_P)$. [c] Determined by HPLC or GC.

[d] Enantiomeric ratio, $\boldsymbol{E} = \ln[(1-c)(1+ee_p)]/\ln[(1-c)(1-ee_p)]$.

Table 2. Lipase catalyzed acylations of the *O*-tritylated hydroxycyanohydrins (±)-**3d-f** using vinyl acetate[a] at 30ºC in toluene.

Entry	n	Lipase	t (h)	*c* (%)[b]	*ee* (%)[c] (*R*)-**3d-f**	*ee* (%)[c] (*S*)-**4d-f**	*E*[d]
1	1	CAL-A	6	25	32	96	72
2	1	PSL-C	4	46	82	96	125
3	1	CAL-B	22	22	21	75	8.6
4	2	CAL-A	4	53	91	80	28
5	2	PSL-C	4	27	35	95	51
6	3	CAL-A	4	55	87	71	16
7	3	PSL-C	4	32	44	95	62

[a] Reactions were carried using 5 equivalents of vinyl acetate. [b] Conversion, $c = ee_S/(ee_S + ee_P)$. [c] Determined by HPLC.

[d] Enantiomeric ratio, $\boldsymbol{E} = \ln[(1-c)(1+ee_p)]/\ln[(1-c)(1-ee_p)]$.

Finally, once trityl was found to be the best protecting group for the resolution of these hydroxycyanohydrins, we studied the enzymatic acetylation in toluene of two longer chain ω-hydroxycyanohydrins, (±)-**3e** (entries 4 and 5) and (±)-**3f** (entries 6 and 7). CAL-A and PSL-C showed less enantioselectivity towards the cyanohydrins (±)-**3e** and **3f** than towards the shorter chain cyanohydrin (±)-**3d.** In both cases, the reaction rates were much higher when the processes were catalyzed by CAL-A, although PSL-C showed the higher enantioselectivities, $\boldsymbol{E}$ = 51 for substrate (±)-**3e** and $\boldsymbol{E}$ = 62 for substrate (±)-**3f**.

To rationalize these experimental facts, we used computer modeling. First, we chose a X-ray crystal structure of the *Pseudomonas cepacia* lipase[14] from the Brookhaven Protein Data Bank.[15] Then, we built the phosphonates shown in Figure 1 into the active site of PSL. These intermediates mimic the acetylation of the substrates (±)-**3a-d**.[16] We had to use a chloride instead of cyanide because of the impossibility of the FF for applying a potential to the second group. We thought the first one was the best choice to mimic the real intermediate due to the bond linearity and the similar electronic properties.

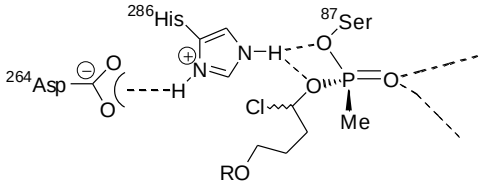


Figure 1. Phosphonate analogue for the PSL-C-catalyzed acetylation of ω-*O*-protected hydroxycyanohydrins.

We studied the extreme cases, with the smallest protecting group (R = Ac), and the biggest one (R = Ph_3C). Thus, for the cyanohydrin **3a**, which had been found to be a bad substrate for lipase-resolution, the structures obtained for both enantiomers are detailed in Figure 2. As we expected, one stable conformation could be found for both cases. The structure of (*S*)-**3a** (Figure 2a), fits well into the medium alcohol-binding site and is pointing to the large acyl pocket. For the enantiomer (*R*)-**3a** (Figure 2b), we observed another disposition of the aliphatic chain. It fits in the alternate hydrophobic pocket perfectly because of the small size of the acetyl protecting group. This site was discovered by Kazlauskas' group and it has been used

for the explanation of the surprising reactivity from this lipase.[17]

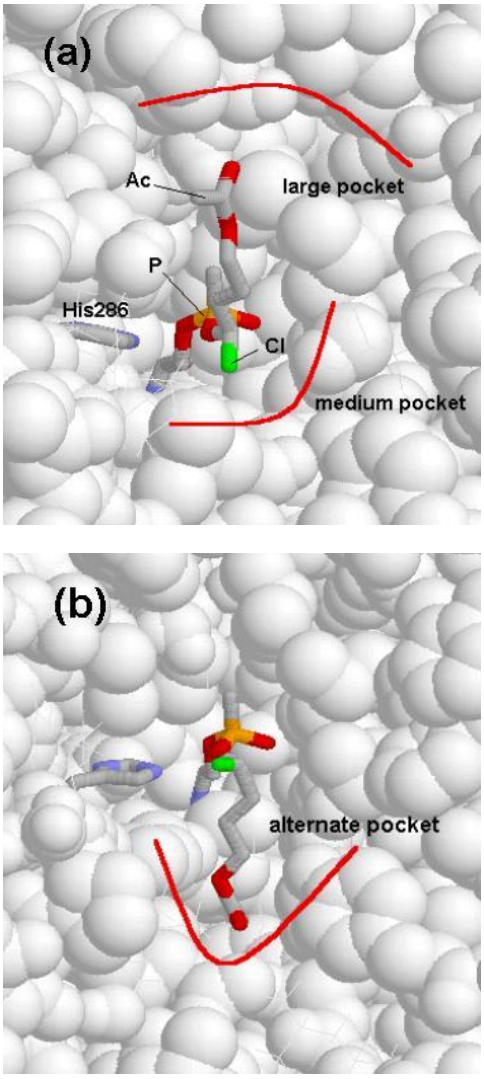


Figure 2. Molecular modeling of the intermediates in the enzymatic acetylation of the cyanohydrins: (a) (*S*)-**3a**. (b) (*R*)-**3a**. In these intermediates, red represents oxygen atoms, blue represents nitrogen atoms, yellow represents the phosphorus atom, and green represents the chlorine atom.

Next, we studied the acylation of substrate **3d**, which had shown a great preference for the reaction with its *S*-enantiomer. Thus, for (*S*)-**3d** (Figure 3a), we obtained a stable-minimized structure, which is relatively rigid, where the chain fits well into the medium pocket, and the trityl protecting group places, one part into the alternate hydrophobic pocket, and the rest is pointing to the organic solvent. In this case, due to the great size of the trityl, we obtained a similar intermediate for (*R*)-**3d** (Figure 3b), but as can be noted, the C-α to the hydroxyl is placed very close to the histidine residue of the catalytic triad, which makes this amino acid moves away from the nucleophile, disrupting the necessary interaction between them.

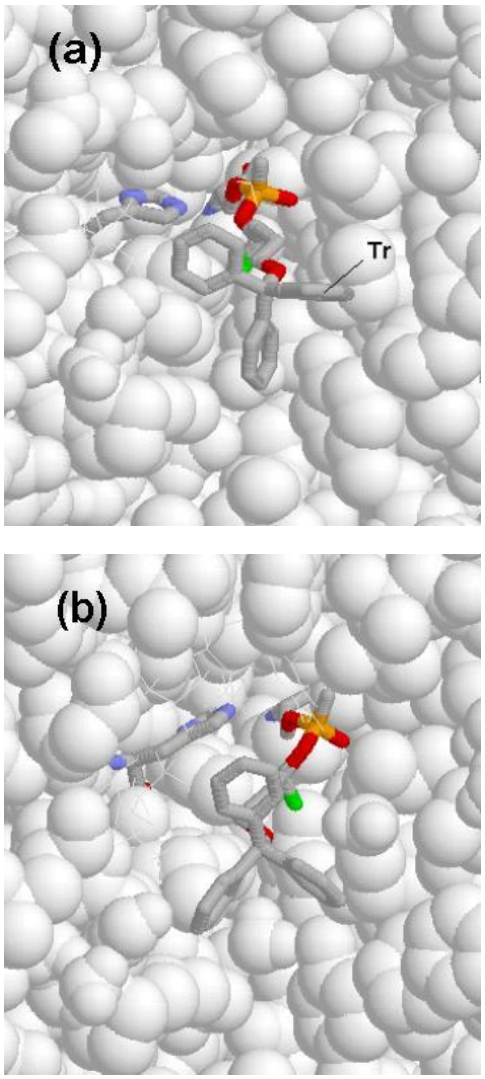


Figure 3. Molecular modeling of the intermediates in the enzymatic acetylation of the cyanohydrins: (a) (*S*)-**3d**. (b) (*R*)-**3d**.

The absolute configuration of the products and the remaining substrates was established as follows. The specific rotation sign of the cyanohydrin ester (*S*)-**3a** was opposite to that reported for the (*R*)-configuration, $[\alpha]_D^{18}$=+2.8 (*c* 1.14, $CHCl_3$).[3] The deprotection of the ω-*O*-protected cyanohydrins (*R*)-**2b-e** afforded the free hydroxycyanohydrins (*R*)-**1a-b**, whose specific rotation signs were in agreement with the values obtain for (*R*)-**1a**, $[\alpha]_D^{18}$=+2.7 (*c* 1.00, acetone) and for (*R*)-**1b**, $[\alpha]_D^{18}$=+3.2 (*c* 1.00, acetone).[3] In the case of substrate **2f**, its specific rotation was compared with the value obtain for (*R*)-**2f** in an enzymatic transcyanation using the (*R*)-oxynitrilase of *Prunus amygdalus*, $[\alpha]_D^{18}$=+4.6 (*c* 1.38, MeOH; e.e. 51%). This *O*-tritylated cyanohydrin possesses (*R*)-configuration.

3. Conclusions

This paper describes the resolution of different size chain ω-hydroxycyanohydrins *via* lipase-catalyzed acylation. High enantioselectivities can be achieved by an appropriate selection of the protecting group at the primary alcohol and the reaction parameters. The best results were obtained using the trityl protecting group in the acylation catalyzed by PSL-C. The process was carried out in toluene using vinyl acetate as acyl donor. The influence of the protecting group has been studied using molecular modeling. When this group is small, as in the case of the acetyl, due to the flexibility of these compounds, they can adopt different conformations in the active site, allowing the reaction for both enantiomers. However, when the protecting group is much bigger, as in the case of trityl, the substrate is more fixed into the lipase, and then the *S*-enantiomer fits better than the *R* one in it.

4. Experimental[18]

General Methods. *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g) was a gift from Novo Nordisk Co. Lipase A from *Candida antarctica* (CAL-A, CHIRAZYME L-5, 1 kU/g) was purchased from Roche Molecular Biochemicals. *Pseudomonas cepacia* lipase immobilized (PSL-C, 1019 units/g) was a product of Amano Co. All these commercial lipases were carrier-fixed products. Other chemicals or solvents were of the highest quality grade available.

Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. Melting points were taken using a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1720-X FT Infrared spectrophotometer. ^{1}H- and ^{13}C-NMR were obtained with TMS (tetramethylsilane) as internal standard, using Bruker AC-200 (^{1}H, 200.13 MHz and ^{13}C, 50.3 MHz), AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers. Mass spectra were recorded on a Hewlett-Packard 1100 Series spectrometer. Microanalyses were performed on a Perkin-Elmer 240B elemental analyzer. Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). The ee's were determined by chiral HPLC on a Shimazdu LC liquid chromatograph or by GC analysis on a Hewlett-Packard 6890 Series II chromatograph. Two well resolved peaks were obtained for all the racemic compounds.

Molecular modeling was performed using Discover, version 2.9.7 (Biosym/MSI, San Diego, CA), with the AMBER force field.[12] We used a distance dependent dielectric constant of 4 and scaled the 1-4 van der Waals interactions by 50%. Results were displayed using Insight II, version 2000.1 (Biosym/MSI). The structure of PSL (3LIP),[19] was obtained from the PDB.[15] Using the Biopolymer module of Insight II, hydrogen atoms were added. The catalytic histidine (His286) was protonated. The corresponding phosphonates were built and covalently linked to Ser87. Energy minimization proceeded in six stages. First, iterations of steepest descent algorithm, with all protein atoms constrained with a force constant of 10 kcal mol^{-1} $Å^{-2}$; second, iterations of conjugate gradients algorithm with the same constraints; third, iterations of steepest descent algorithm with only the backbone of the protein constrained by a 10 kcal mol^{-1} $Å^{-2}$; fourth, iterations of conjugate gradients algorithm with the same constraints; for the fifth stage, minimization was continued using iterations of steepest descent algorithm without any constraints. In all these cases, we used iterations until the rms deviation value reached less than 0.03 Å mol^{-1}. Finally, in the sixth stage, we used iterations of conjugate gradients algorithm without any constraints until the rms deviation reached less than 0.005 Å mol^{-1}. Water molecules and the substrate were not constrained through any of the minimization cycles. Protein structures in Figures 2 and 3 were created using RasMac v2.6.

4.1. Synthesis of (±)-5-acetoxy-2-hydroxypentanenitrile, (±)-3a. Vinyl acetate (1.96 mL, 21.5 mmol) was added to a solution of 2,5-dihydroxypentanenitrile (±)-**1a** (0.5 g, 4.3 mmol) and molecular sieves (4 Å, 800 mg) in ^{t}BuOMe (40 mL). The reaction was stirred at 40ºC and 250 r.p.m. in a rotatory shaker. After 5 days, the mixture was filtered over Celite, washed with ^{t}BuOMe, and the solvent evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel using hexane/EtOAc (1:1) to afford compound (±)-**3a** as a colourless oil (0.49 g, 73%).

4.2. Synthesis of (±)-5-benzoyloxy-2-hydroxypentanenitrile, (±)-3b. Benzoyl chloride (0.60 mL, 5.2 mmol) was added dropwise to a solution of (±)-**1a** (500 mg, 4.3 mmol) in CH_2Cl_2 (20 mL) and pyridine (0.42 mL, 5.2 mmol), and stirred at room temperature for 12 h. The resulting mixture was washed with 2N HCl. The organic fraction was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel, using Et_2O/CH_2Cl_2 (8:2) to afford compound (±)-**3b** as a colourless oil (0.60 g, 64%).

4.3. Preparation of (±)-2-hydroxy-5-(methoxy)methoxypentanenitrile, (±)-3c. Methoxymethyl chloride (0.39 mL, 5.2 mmol) was added dropwise to a 0 ºC solution of (±)-**1a** (0.5 g, 4.3 mmol) and diisopropylethylamine (1.84 mL, 10.7 mmol) in dry CH_2Cl_2 (15 mL). The mixture was refluxed for 8 h. After this time, the reaction was washed with 3N HCl until pH 1-2, and extracted with CH_2Cl_2. The organic fraction was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude residue was purified by flash chromatography using Et_2O/CH_2Cl_2 (5:95) to afford compound (±)-**3c** as a colourless oil (0.40 g, 59%).

4.4. General procedure for the synthesis of the ω-*O*-trityloxyhydroxycyanohydrins (±)-3d-e. To a solution of the hydroxycyanohydrins (±)-**1a-b** (0.5 g, 1 eq) in dry

pyridine (30 mL), trityl chloride (1.2 eq) was added, and the reaction was stirred at 75ºC for 14 h. The mixture was cooled to room temperature and the solvent evaporated under reduced pressure. The crude residue was purified by flash chromatography with hexane/EtOAc (8:2) to give the corresponding products (±)-**3d** (1.00 g, 67%), and (±)-**3e** (0.89 g, 62%) as white solids.

4.5. Synthesis of 7-trityloxyheptanal, 2. A solution of DIBAL-H 1M in toluene (52 mL) was added dropwise to a solution of ε-caprolactone (5.0 g, 43.8 mmol) in THF (40 mL). The mixture was stirred at –70ºC for 8 h and then quenched by slow addition of H_2O. The reaction was warmed up to room temperature and stirred for 15 min. Then, HCl 0.5N was added and the reaction was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. To a solution of the crude residue (2.15 g, 18.5 mmol) in pyridine (40 mL), trityl chloride (6.1 g, 22.2 mmol) was added, and the reaction was stirred at 75ºC for 10 h. The mixture was cooled to room temperature and the solvent evaporated under reduced pressure. The aldehyde was purified by flash chromatography using hexane/EtOAc (8:2) to afford **2** as a white solid (5.92 g, 35%).

4.6. Preparation of 2-hydroxy-7-trityloxyheptanenitrile, (±)-3f. To a solution of **2** (1.0 g, 2.79 mmol) in water (1 mL) containing KCN (0.25 g, 3.89 mmol), an aqueous solution of $NaHSO_3$ 40% (1.5 mL) were added dropwise at 0ºC. The reaction was stirred for 20 h. Then, the mixture was extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated. The crude residue was purified by flash chromatography using hexane/EtOAc (8:2) to give (±)-**3f** (0.61 g, 57%).

4.7. Typical procedure for the enzymatic acetylation of the racemic ω-*O*-protected hydroxycyanohydrins, (±)-3a-f. The lipase (60 mg) and vinyl acetate (1.5 mmol) were added to a solution of the hydroxycyanohydrins (±)-**3a-f** (0.3 mmol) in the corresponding solvent (12 mL). The mixture was stirred at 30 ºC and 250 r.p.m. in a rotatory shaker. Once the reaction was finished, the enzyme was removed by filtration, washed with EtOAc and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel to afford the hydroxycyanohydrin acetates (*S*)-**4a-f** and the corresponding hydroxycyanohydrins (*R*)-**3a-f**.

4.8. Cleavage of the benzoyl group. To a solution of **3b** (0.20 g, 0.91 mmol) in methanol (4 mL), NaOH 1.0% (2 mL) was added. The reaction was stirred at room temperature and monitored by TLC. After this, HCl 2N was added until acidic pH. Then, the mixture was extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude residue was purified by flash chromatography using Et_2O/CH_2Cl_2 (8:2) to obtain **1a** (17 mg, 16%).

4.9. Cleavage of the methoxymethyl group. HCl 6N (0.25 mL) was added to a solution of **3c** (100 mg, 0.62 mmol) in isopropanol (5 mL). The mixture was stirred at 50ºC for 10 h. Then, the reaction was extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude residue was purified by flash chromatography using Et_2O/CH_2Cl_2 (5:95) to obtain **1a** (29 mg, 41%).

4.10. General procedure for the cleavage of the trityl group. The trityloxycyanohydrins **3d-e** (200 mg) were dissolved in CH_2Cl_2 (1.0 mL) and HCl 2N (1.5 mL). The reaction was stirred for 20 h at room temperature. After this, the mixture was extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude residue was purified by flash chromatography using hexane/EtOAc (8:2) to afford **1a** (21 mg, 66%) or **1b** (17 mg, 54%).

6-Trityloxyhexanal, 2. White solid; mp (ºC): 75.5-76.9; IR (KBr): υ 3019, 2936, 1724, 1596, 1490 and 1215 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.42-1.55 (m, 2H), 1.61-1.74 (m, 4H), 2.42 (t, 2H, $^3J_{HH}$ 6.2 Hz), 3.08 (t, 2H, $^3J_{HH}$ 6.3 Hz), 7.24-7.33 (m, 9H), 7.44-7.51 (m, 6H) and 9.74 (s, 1H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 22.1 (CH_2), 26.1 (CH_2), 29.9 (CH_2), 44.0 (CH_2), 63.4 (CH_2), 86.5 (C), 127.1 (CH), 127.9 (CH), 128.9 (CH), 144.1 (C) and 203.0 (C=O); MS (ESI$^+$, *m/z*): 381 [(M+Na)$^+$, 100] and 359 [(M+H)$^+$, 23]; Anal. Calcd. (%) for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31. Found: C, 83.9; H, 7.2.

(*R*)-5-Acetoxy-2-hydroxypentanenitrile, 3a. Colourless oil; $[\alpha]_D^{18}$=+7.5 (*c* 1.36, EtOH; e.e. 57%); IR (KBr): υ 3417, 2963, 2244, 1730, 1442 and 1254 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.99-2.07 (m, 4H), 2.26 (s, 3H), 2.81 (br s, 1H), 4.33 (t, 2H, $^3J_{HH}$ 6.1 Hz) and 5.31 (t, 1H, $^3J_{HH}$ 6.2 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 21.3 (CH_3), 25.7 (CH_2), 33.6 (CH_2), 61.7 (CH), 65.3 (CH_2), 122.1 (C≡N) and 173.3 (C=O); MS (ESI$^+$, *m/z*): 180 [(M+Na)$^+$, 100] and 158 [(M+H)$^+$, 2]; Anal. Calcd. (%) for $C_7H_{11}NO_3$: C, 57.49; H, 7.05; N, 8.91. Found: C, 57.3; H, 7.2; N, 9.1; GC conditions for the analysis after acetylation: column Rt-βDEXse, 1 mL min^{-1} N_2, 100ºC, 5 min; 5ºC min^{-1} until 200ºC, t_R (min): 22.17.

(*R*)-5-Benzoyloxy-2-hydroxypentanenitrile, 3b. Colourless oil; $[\alpha]_D^{18}$=+3.8 (*c* 1.44, EtOH; e.e. 66%); IR (KBr): υ 3421, 3073, 2942, 2246, 1717, 1601, 1434, and 1268 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.03-2.12 (m, 4H), 3.41 (br s, 1H), 4.41 (t, 2H, $^3J_{HH}$ 5.8 Hz), 4.60 (t, 1H, $^3J_{HH}$ 6.1 Hz), 7.46 (t, 2H, $^3J_{HH}$ 7.6 Hz), 7.57 (t, 1H, $^3J_{HH}$ 7.6 Hz) and 8.04 (dd, 2H, $^3J_{HH}$ 7.6, $^4J_{HH}$ 1.4 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 23.9 (CH_2), 31.7 (CH_2), 60.6 (CH), 63.8 (CH_2), 119.5 (C≡N), 128.3 (CH), 129,4 (CH), 129.6 (C), 1331. (CH) and 166.7 (C=O); MS (ESI$^+$, *m/z*): 258 [(M+K)$^+$, 53], 242 [(M+Na)$^+$, 100] and 220 [(M+H)$^+$, 20]; Anal. Calcd. (%) for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.9; H, 5.8; N, 6.6; HPLC conditions after acetylation: column Chiralcel OD, eluent hexane/*i*-propanol (90:10), 0.8 mL min^{-1}, 35ºC; t_R (min): 28.78.

(*R*)-2-Hydroxy-5-(methoxy)methoxypentanenitrile, 3c. Colourless oil; $[\alpha]_D^{18}$=+9.2 (*c* 1.02, EtOH; e.e. 63%); IR (KBr): υ 3390, 2951, 2247, 1445 and 1215 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.86-2.09 (m, 4H), 3.39 (s, 3H), 3.58-3.69 (m, 2H), 4.18 (br s, 1H), 4.54-4.60 (m, 1H) and 4.67 (s, 2H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 24.9 (CH_2), 32.7 (CH_2), 55.3 (CH_3), 60.8 (CH), 66.7 (CH_2), 96.2 (CH_2) and 119.5 (C≡N); MS (ESI^+, *m/z*): 198 [$(M+K)^+$, 12], 182 [$(M+Na)^+$, 100] and 160 [$(M+H)^+$, 2]; Anal. Calcd. (%) for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80. Found: C, 53.0; H, 8.1; N, 8.7; GC conditions after acetylation: column Rt-βDEXse, 1 mL min^{-1} N_2, 100ºC, 5 min; 5ºC min^{-1} until 200ºC, t_R (min): 20.81.

(*R*)-2-Hydroxy-5-trityloxypentanenitrile, 3d. White solid; mp (ºC): 84.2-86.0; $[\alpha]_D^{18}$=+7.0 (*c* 0.98, EtOH; e.e. 91%); IR (KBr): υ 3430, 3019, 2928, 2239, 1596, 1448 and 1217 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.80-2.04 (m, 4H), 3.03-3.14 (m, 1H), 3.28-3.35 (m, 1H), 3.92 (d, 1H, $^3J_{HH}$ 10.2 Hz), 4.53 (t, 1H, $^3J_{HH}$ 6.4 Hz), 7.26-7.38 (m, 9H) and 7.42-7.50 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 25.2 (CH_2), 33.2 (CH_2), 61.1 (CH), 63.2 (CH_2), 87.6 (C), 119.7 (C≡N), 127.1 (CH), 127,9 (CH), 128.4 (CH) and 143.4 (C); MS (ESI^+, *m/z*): 396 [$(M+K)^+$, 19], 380 [$(M+Na)^+$, 100] and 358 [$(M+H)^+$, 2]; Anal. Calcd. (%) for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 80.5; H, 6.6; N, 4.0. HPLC conditions after acetylation: column Chirobiotic T, eluent hexane/*i*-propanol (95:5), 0.3 mL min^{-1}, 20ºC; t_R (min): 26.51.

(*R*)-2-Hydroxy-6-trityloxyhexanenitrile, 3e. White solid; mp (ºC): 87.4-88.8; $[\alpha]_D^{18}$=+5.0 (*c* 1.15, EtOH; e.e. 82%); IR (KBr): υ 3437, 3058, 2979, 2248, 1596, 1490 and 1218 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.60-1.72 (m, 4H), 1.77-1.87 (m, 2H), 2.42 (d, 1H, $^3J_{HH}$ 11.8 Hz), 3.11 (t, 2H, $^3J_{HH}$ 5.9 Hz), 4.44 (dd, 1H, $^3J_{HH}$ 6.6, $^3J_{HH}$ 11.8 Hz), 7.24-7.39 (m, 9H) and 7.43-7.51 (m, 6H); ^{13}C-NMR ($CDCl_3$, 50.4 MHz): δ 21.3 (CH_2), 29.1 (CH_2), 34.8 (CH_2), 61.2 (CH), 62.8 (CH_2), 86.4 (C), 119.7 (C≡N), 126.9 (CH), 127.7 (CH), 128.5 (CH) and 144.1 (C); MS (ESI^+, *m/z*): 394 [$(M+Na)^+$, 100] and 372 [$(M+H)^+$, 5]; Anal. Calcd. (%) for $C_{25}H_{25}NO_2$: C, 80.83; H, 6.78; N, 3.77. Found: C, 81.0; H, 7.0; N, 3.7; HPLC conditions after acetylation: column Chiralcel OD, eluent hexane/*i*-propanol (98:2), 0.3 mL min^{-1}, 20ºC; t_R (min): 56.93.

(*R*)-2-Hydroxy-7-trityloxyheptanenitrile, 3f. White solid; mp (ºC): 90.4-91.7; $[\alpha]_D^{18}$=+7.6 (*c* 0.75, EtOH; e.e. 87%); IR (KBr): υ 3434, 3021, 2936, 2250, 1596, 1448 and 1218 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.45-1.56 (m, 4H), 1.64-1.72 (m, 2H), 1.79-1.90 (m, 2H), 2.70 (d, 1H, $^3J_{HH}$ 9.8 Hz), 3.10 (t, 2H, $^3J_{HH}$ 6.3 Hz), 4.43 (t, 1H, $^3J_{HH}$ 6.6 Hz), 7.22-7.37 (m, 9H) and 7.45-7.53 (m, 6H); ^{13}C-NMR ($CDCl_3$, 50.4 MHz): δ 24.4 (CH_2), 25.7 (CH_2), 29.8 (CH_2), 35.2 (CH_2), 61.3 (CH), 63.2 (CH_2), 86.4 (C), 119.9 (C≡N), 126.9 (CH), 127.8 (CH), 128.7 (CH) and 144.4 (C); MS (ESI^+, *m/z*): 408 [$(M+Na)^+$, 100] and 386 [$(M+H)^+$, 3]; Anal. Calcd. (%) for $C_{26}H_{27}NO_2$: C, 81.01; H, 7.06; N, 3.63. Found: C, 81.2; H, 7.0; N, 3.5; HPLC conditions after acetylation: column Chirobiotic T, eluent hexane/*i*-propanol (97:3), 0.3 mL min^{-1}, 20ºC; t_R (min): 24.68.

(*S*)-2,5-Diacetoxypentanenitrile, **4a**. Colourless oil; $[\alpha]_D^{18}$=-30.9 (*c* 0.90, MeOH; e.e. 75%); GC conditions: column Rt-βDEXse, 1 mL min^{-1} N_2, 100ºC, 5 min; 5ºC min^{-1} until 200ºC, t_R (min): 22.83.

(*S*)-2-Acetoxy-5-benzoyloxypentanenitrile, 4b. Colourless oil; $[\alpha]_D^{18}$=-9.0 (*c* 0.83, MeOH; e.e. 59%); IR (KBr): υ 3020, 2942, 2246, 1753, 1714, 1622, 1454, and 1215 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.93-2.05 (m, 2H), 2.07-2.16 (m, 2H), 2.19 (s, 3H), 4.41 (t, 2H, $^3J_{HH}$ 6.0 Hz), 5.43 (t, 1H, $^3J_{HH}$ 6.2 Hz), 7.48 (t, 2H, $^3J_{HH}$ 7.4 Hz), 7.60 (t, 1H, $^3J_{HH}$ 7.4 Hz) and 8.06 (dd, 2H, $^3J_{HH}$ 7.4, $^4J_{HH}$ 1.4 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 20.2 (CH_3), 24.0 (CH_2), 29.2 (CH_2), 60.6 (CH), 63.4 (CH_2), 116.4 (C≡N), 128.3 (CH), 129.7 (CH), 129.8 (C), 130.1 (CH), 166.3 (C=O) and 169.0 (C=O); MS (ESI^+, *m/z*): 300 [$(M+K)^+$, 21], 284 [$(M+Na)^+$, 100] and 262 [$(M+H)^+$, 5]; Anal. Calcd. (%) for $C_{14}H_{15}NO_3$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.5; H, 5.6; N, 5.4; HPLC conditions: column Chiralcel OD, eluent hexane/*i*-propanol (90:10), 0.8 mL min^{-1}, 35ºC; t_R (min): 16.98.

(*S*)-2-Acetoxy-5-(methoxy)methoxypentanenitrile, 4c. Colourless oil; $[\alpha]_D^{18}$=-29.0 (*c* 0.94, MeOH; e.e. 78%); IR (KBr): υ 2947, 2251, 1752, 1436 and 1215 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.75-1.88 (m, 2H), 2.00-2.11 (m, 2H), 2.16 (s, 3H), 3.37 (s, 3H), 3.59 (t, 2H, $^3J_{HH}$ 5.9 Hz), 4.62 (s, 2H) and 5.41 (t, 1H, $^3J_{HH}$ 6.7 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 19.9 (CH_3), 24.3 (CH_2), 29.1 (CH_2), 54.8 (CH_3), 60.4 (CH), 65.8 (CH_2), 95.9 (CH_2), 116.3 (C≡N) and 168.7 (C=O); MS (ESI^+, *m/z*): 224 [$(M+Na)^+$, 100] and 202 [$(M+H)^+$, 15]; Anal. Calcd. (%) for $C_9H_{15}NO_3$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.7; H, 7.4; N, 7.0; GC conditions: column Rt-βDEXse, 1 mL min^{-1} N_2, 100ºC, 5 min; 5ºC min^{-1} until 200ºC, t_R (min): 21.52.

(*S*)-2-Acetoxy-5-trityloxypentanenitrile, 4d. Hygroscopic solid; $[\alpha]_D^{18}$=-22.4 (*c* 1.28, MeOH; e.e. 96%); IR (KBr): υ 3022, 2934, 2240, 1596, 1448 and 1220 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.78-1.84 (m, 2H), 1.97-2.07 (m, 2H), 2.14 (s, 3H), 3.17 (t, 2H, $^3J_{HH}$ 6.0 Hz), 5.33 (t, 1H, $^3J_{HH}$ 6.4 Hz), 7.26-7.37 (m, 9H) and 7.42-7.47 (m, 6H); ^{13}C-NMR ($CDCl_3$, 50.4 MHz): δ 20.8 (CH_3), 25.1 (CH_2), 29.5 (CH_2), 60.9 (CH), 62.1 (CH_2), 86.6 (C), 116.7 (C≡N), 127.1 (CH), 127,8 (CH), 128.5 (CH), 143.9 (C) and 169.0 (C=O); MS (ESI^+, *m/z*): 438 [$(M+K)^+$, 60], 422 [$(M+Na)^+$, 100] and 400 [$(M+H)^+$, 2]; Anal. Calcd. (%) for $C_{26}H_{25}NO_2$: C, 78.17; H, 6.31; N, 3.51. Found: C, 78.0; H, 6.6; N, 3.4; HPLC conditions: column Chirobiotic T, eluent hexane/*i*-propanol (95:5), 0.3 mL min^{-1}, 20ºC; t_R (min): 29.40.

(*S*)-2-Acetoxy-6-trityloxyhexanenitrile, 4e. Hygroscopic solid; $[\alpha]_D^{18}$=-20.8 (*c* 0.95, MeOH; e.e. 95%); IR (KBr): υ 3058, 2933, 2241, 1753, 1596, 1490 and 1219 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.58-1.72 (m, 4H), 1.83-1.90 (m, 2H), 2.14 (s, 3H), 3.11 (t, 2H, $^3J_{HH}$ 5.9 Hz), 5.31 (t,

1H, $^3J_{HH}$ 6.7 Hz), 7.24-7.37 (m, 9H) and 7.41-7.52 (m, 6H); ^{13}C-NMR ($CDCl_3$, 50.4 MHz): δ 20.5 (CH_3), 21.5 (CH_2), 29.1 (CH_2), 32.1 (CH_2), 61.0 (CH), 62.7 (CH_2), 85.5 (C), 116.9 (C≡N), 127.0 (CH), 127.8 (CH), 128.7 (CH), 144.2 (C) and 169.2 (C=O); MS (ESI$^+$, *m/z*): 452 [(M+K)$^+$, 12] and 436 [(M+Na)$^+$, 100]; Anal. Calcd. (%) for $C_{27}H_{27}NO_2$: C, 78.42; H, 6.58; N, 3.39. Found: C, 78.2; H, 6.7; N, 3.3; HPLC conditions: column Chiralcel OD, eluent hexane/*i*-propanol (98:2), 0.3 mL min^{-1}, 20ºC; t_R (min): 50.31.

(*S*)-2-Acetoxy-7-trityloxyheptanenitrile, 4f. Hygroscopic solid; $[\alpha]_D^{18}$=-21.2 (*c* 1.35, MeOH; e.e. 95%); IR (KBr): υ 3022, 2938, 2240, 1752, 1597, 1448 and 1217 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.46-1.52 (m, 4H), 1.62-1.70 (m, 2H), 1.85-1.92 (m, 2H), 2.11 (s, 3H), 3.09 (t, 2H, $^3J_{HH}$ 6.2 Hz), 5.30 (t, 1H, $^3J_{HH}$ 6.7 Hz), 7.21-7.36 (m, 9H) and 7.43-7.50 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 20.3 (CH_3), 24.3 (CH_2), 25.5 (CH_2), 29.6 (CH_2), 32.1 (CH_2), 60.9 (CH), 63.0 (CH_2), 86.2 (C), 116.8 (C≡N), 126.9 (CH), 127.6 (CH), 128.6 (CH), 144.4 (C) and 169.1 (C=O); MS (ESI$^+$, *m/z*): 450 [(M+Na)$^+$, 100] and 438 [(M+H)$^+$, 2]; Anal. Calcd. (%) for $C_{28}H_{29}NO_2$: C, 81.72; H, 7.10; N, 3.40. Found: C, 81.5; H, 7.2; N, 3.6; HPLC conditions: column Chirobiotic T, eluent hexane/*i*-propanol (97:3), 0.3 mL min^{-1}, 20ºC; t_R (min): 28.40.

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References

1. North, M. *Tetrahedron: Asymmetry* **2003**, *14*, 147-176 and references cited therein.
2. (a) Menéndez, E.; Brieva, R.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Chem. Commun.* **1995**, 989; (b) Monterde, M. I.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 525-528.
3. De Gonzalo, G.; Brieva, R.; Gotor, V. *J. Mol. Cat. B:Enzym.* **2002**, *19-20*, 223-230.
4. Ognyanov, V. I.; Datcheva, K.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6992-6996.
5. (a) Gregory, R. J. H. *Chem. Rev*. **1999**, *99*, 3649-3682; (b) Griengl, H.; Schwab, H.; Fechter, M. *TIBTECH* **2000**, *18*, 425-429; (c) Brussee, J; van der Gen, A. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker, New York, 2000, pp 289-320; (d) Effenberger, F. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker, New York, 2000, pp 321-342.
6. (a) Kanerva, L. T.; Kiljunen, E.; Huuhtanen, T. T. *Tetrahedron: Asymmetry*, **1993**, *4*, 2355-2361; (b) Tellitu, I.; Badía, D.; Domínguez, E.; García, F. J. *Tetrahedron: Asymmetry* **1994**, *5*, 1567-1578.
7. (a) Inakagi, M.; Hiratake, J.; Nishioka, T.; Oda, J. *J. Org. Chem.* **1992**, *57*, 5643-5649; (b) Li, Y.–X.; Straathof, A. J. J.; Hanefeld, U. *Tetrahedron: Asymmetry* **2002**, *13*, 739-743.
8. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779-1786.
9. (a) Kazlauskas, R. J. *Curr. Opin. Chem. Biol.* **2000**, *4*, 81-88; (b) Kazlauskas, R. *Science* **2001**, *293*, 2277-2279.
10. (a) Holzwarth, H. C.; Pleiss, J.; Schmid, R. D. *J. Mol. Cat. B:Enzym.* **1997**, *3*, 73-82; (b) Scheib, H.; Pleiss, J.; Stadler, P.; Kovac, A.; Potthoff, A. P.; Haalck, L.; Spener, F.; Paltauf, F.; Schmid, R. D. *Protein Eng.* **1998**, *11*, 675-682.
11. Hæffner, F.; Norin, T. *Chem. Pharm. Bull.* **1999**, *47*, 591-600.
12. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765-784.
13. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.*, **1982**, *104*, 7294-7299.
14. We assumed that there is no difference between the active sites of the *Pseudomonas cepacia* lipase (PSL) and its immobilized form (PSL-C), because the physical adsorption of the enzyme should not affect the conformation close to the catalytic triad.
15. http://www.rcsb.org/pdb/.
16. Phosphonates have been shown to mimic the tetrahedral intermediates in serine-mechanisms appropriately. To see more: Hanson, J. E.; Kaplan, A. P.; Bartlett, P. A. *Biochemistry* **1989**, *28*, 6294-6305.
17. Tuomi, W. C.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 2638-2647.
18. Compounds **1a**, **1b** and **4a** have been described in ref. 3.
19. Schrag, J. D.; Li, Y.; Cygler, M.; Lang, D.; Burgdorf, T.; Hecht, H. J.; Schmid, R.; Schomburg, D.; Rydel, T. J.; Oliver, J. D.; Strickland, L. C.; Dunaway, C. M.; Larson, S. B.; Day, J.; McPherson, A. *Structure* **1997**, *5*, 187-202.