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# Enzymatic resolution of hindered cyanohydrins, key precursors of muscarinic receptor antagonists

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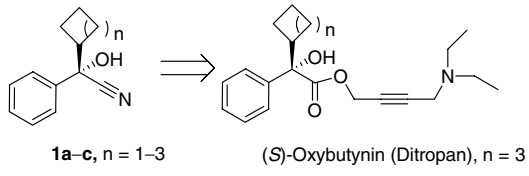
**Abstract**—Enantiomerically pure 1-cycloalkyl-1-hydroxy-1-phenylcyanohydrins, key precursors in the synthesis of (*S*)-oxybutynin and other antimuscarinic agents, have been successfully prepared via lipase-catalyzed kinetic resolutions. *Pseudomonas cepacia* and *Candida antarctica* lipase B have shown excellent enantioselectivities in hydrolysis and acylation processes, depending on the substrate structure and the reaction conditions. Furthermore, molecular modeling of phosphonate transition state analogue for the *C. antarctica* lipase B enzymatic hydrolysis resolution step supports these facts, which were experimentally observed.


## 1. Introduction

Tertiary α-hydroxy acids are constituents of a variety of pharmaceuticals and important natural products.[1] For example, oxybutynin (Fig. 1),[2] such as the majority of muscarinic receptor antagonists, is composed of a tertiary α-hydroxy acid ester.[3] Despite the limited therapeutic applicability of the racemic drug due to side effects, oxybutynin has been widely prescribed for the treatment of urinary tract disorders. However, the (*S*)-enantiomer is proposed to display an improved therapeutical profile compared to its racemic counterpart and a number of derivatives have been synthesized mainly by modification of the ester or the cycloalkyl moieties.[4] Several approaches have been used to construct the quaternary stereocenter of these compounds. Grignard addition using a chiral auxiliary,[5] chiral mandelic acid as a starting material,[6] diastereoselective Michael addition,[3a] Sharpless asymmetric dihydroxylation,[7] or enantioselective cyanosilylation of cycloalkyl phenyl ketones catalyzed by a gadolinium complex,[8] have all been reported to synthesize the key α-hydroxy acid precursor of (*S*)-oxybutynin and analogues.




**1a–c**, n = 1–3 (*S*)-Oxybutynin (Ditropan), n = 3

**Figure 1.**

On the other hand, the resolution of tertiary alcohols by enzymatic methods is an interesting challenge from a theoretical point of view; hydrolytic enzymes are usually unable to accept sterically hindered substrates bearing fully substituted quaternary carbons in the vicinity of the function to be hydrolyzed or acylated.[9]

Taking into account the importance of enantiomerically pure tertiary α-hydroxynitriles as precursors of antimuscarinic agents and the theoretical interest in their resolution, we describe a study of their enzymatic resolution. Some of the results presented here have been rationalized using a computational approach.

## 2. Results and discussion

First, we studied the transesterification of racemic cyanohydrins (±)-**1a–c** catalyzed by several commercially available lipases [*Candida antarctica* lipases A and B (CAL-A and CAL-B), *Pseudomonas cepacia* (PSL-C), *Candida rugosa* (CRL), *Aspergillus Melleus*, porcine pancreatic

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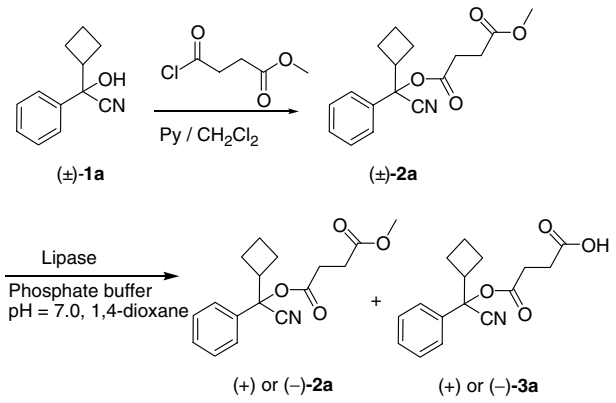
(PPL)], and also pig liver esterase (PLE). As was expected, none of the enzymes tested catalyzed the process. These results are probably due to adverse steric interactions caused by the quaternary stereogenic center in the active site of the enzyme. Consequently, in order to circumvent this problem, we proceeded to modify the substrate by the preparation of several derivatives. First, we prepared the derivative of the cyclobutyl cyanohydrin (±)-**2a** (Scheme 1). Thus, the enzyme catalyzed the hydrolysis of the methyl ester sited five bonds away from the stereogenic center. The strategy to turn tertiary alcohols into suitable substrates for hydrolases was successfully applied by Franssen et al.,[10] nevertheless the processes described by these authors showed low enantioselectivities.




**Scheme 1.** Synthesis and enzymatic hydrolysis of (±)-**2a**.

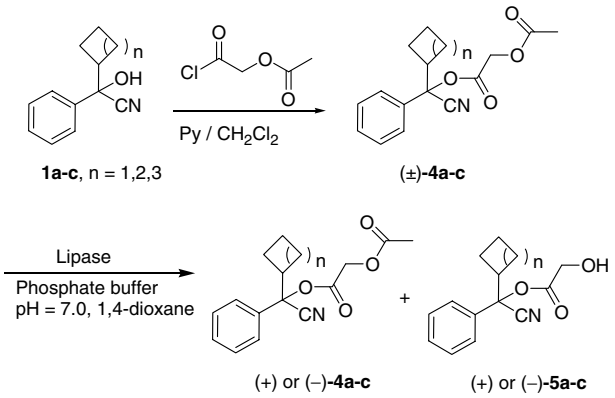
The hydrolysis of (±)-**2a** was carried out at 30 °C in a 1:1 mixture of phosphate buffer 1 M and 1,4-dioxane. Under these conditions, two of the enzymes tested catalyzed the reaction, even though the enantioselectivities were low and only moderate conversions were achieved (Table 1).

**Table 1.** Lipase-catalyzed hydrolysis of (±)-**2a** at 30 °C in phosphate buffer 1 M pH = 7.0/1,4-dioxane 1:1, after 65 h

| Entry | Lipase | $t$ (h) | $c$ (%)[a] | $ee_s$[b] (%) | $ee_p$[b] (%) | $E$[c] |
|---|---|---|---|---|---|---|
| 1 | CAL-B | 65 | 36 | 35 | 63 | 6 |
| 2 | PSL-C | 65 | 37 | 16 | 27 | 2 |

[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by chiral HPLC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.[11]

Next, we examined the behavior of acetoxyacetylated derivatives (±)-**4a**–**c** as possible candidates for the enzymatic hydrolysis resolution (Scheme 2). First, we carried out the hydrolysis of the cyclobutyl derivative (±)-**4a** under the same conditions as for substrate (±)-**2a**, and four of the




**Scheme 2.** Synthesis and enzymatic hydrolysis of (±)-**4a**–**c**.

enzymes tested catalyzed the process. The reactions catalyzed by CAL-B and PSL-C were very fast, reaching in both cases, 100% conversion after 24 h. The reaction rates of the hydrolysis catalyzed by CCL or CAL-A were slower but the obtained products were formed in both cases as racemates. In order to obtain conversions close to 50% in the hydrolysis catalyzed by CAL-B and PSL-C, we carried out these processes using less concentration of the buffer and stopping the reaction after 16 h. The results in both cases were very successful. As shown in Table 2, good rates and very high enantioselectivities were reached, especially for the product that was recovered in enantiopure form.

Depending on the biocatalyst employed, an opposite stereochemical preference in the resolution processes was observed. CAL-B catalyzed the hydrolysis of the (*R*)-(+) enantiomer, while for the PSL-C, product **5a** was obtained with the (*S*)-(−)-configuration, leaving the (*R*)-(+) enantiomer of the unreacted substrate **4a**.

In view of these promising results, we decided to apply the same reaction conditions employed in the resolution of derivative (±)-**4a** with **4b** and **4c**. CAL-B and PSL-C showed high or moderate conversions and the same opposite stereochemical preference as shown in the case of substrate (±)-**4a**; however, low enantioselectivities were found (Table 3, entries 1–4). In order to improve the enantioselectivity of these processes, we analyzed the influence of the organic cosolvent in the hydrolysis of these substrates (entries 5–11), but none of the solvents tested (*t*-BuOMe, toluene, and acetonitrile) improved the results obtained using PSL-C in 1,4-dioxane.

Taking into account that the spacer inserted between the quaternary stereogenic center and the reaction center must be long enough to convert cyanohydrins (±)-**1a**–**c** into suit-

**Table 2.** Lipase-catalyzed hydrolysis of (±)-**4a** at 30 °C in a mixture 1:2 of 1 M phosphate buffer pH = 7.0/1,4-dioxane, after 16 h

| Entry | Lipase | Buffer/solvent | $c$[a] (%) | $ee_s$[b] (%) | Conf. | $ee_p$[b] (%) | Conf. | $E$[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | CAL-B | 1:2 | 42 | 71 | (*S*) | 99 | (*R*) | >200 |
| 2 | PSL-C | 1:2 | 31 | 45 | (*R*) | 99 | (*S*) | >200 |

[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by chiral HPLC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.[11]

**Table 3.** Lipase-catalyzed hydrolysis of (±)-**4b** and (±)-**4c** at 30 °C in a mixture 1:2 of 1 M phosphate buffer pH = 7.0/1,4-dioxane

| Entry | Substrate | Lipase | Cosolvent | $t$ (h) | $c$[a] (%) | $ee_s$[b] (%) | Conf. | $ee_p$[b] (%) | Conf. | $E$[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (±)-**4b** | CAL-B | 1,4-Dioxane | 5 | 89 | 40 | (*S*) | 5 | (*R*) | 1 |
| 2 | (±)-**4b** | PSL-C | 1,4-Dioxane | 24 | 35 | 36 | (*R*) | 66 | (*S*) | 7 |
| 3 | (±)-**4c** | CAL-B | 1,4-Dioxane | 7 | 83 | 40 | (*S*) | 8 | (*R*) | 2 |
| 4 | (±)-**4c** | PSL-C | 1,4-Dioxane | 17 | 56 | 40 | (*R*) | 31 | (*S*) | 3 |
| 5 | (±)-**4b** | CAL-B | *t*-BuOMe | 7 | 56 | 10 | (*S*) | 8 | (*R*) | 1 |
| 6 | (±)-**4b** | PSL-C | *t*-BuOMe | 6 | 79 | 48 | (*R*) | 13 | (*S*) | 2 |
| 7 | (±)-**4b** | CAL-B | Toluene | 9 | 48 | 10 | (*S*) | 11 | (*R*) | 1 |
| 8 | (±)-**4b** | PSL-C | Toluene | 19 | 39 | 13 | (*R*) | 20 | (*S*) | 2 |
| 9 | (±)-**4c** | PSL-C | Toluene | 11 | 11 | 5 | (*R*) | 70 | (*S*) | 2 |
| 10 | (±)-**4b** | CAL-B | Acetonitrile | 5 | 40 | 6 | (*S*) | 9 | (*R*) | 1 |
| 11 | (±)-**4b** | PSL-C | Acetonitrile | 24 | 41 | 43 | (*R*) | 63 | (*S*) | 7 |

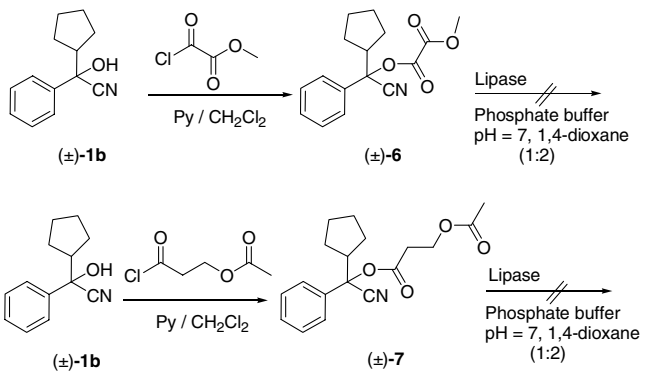
[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by chiral HPLC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.[11]

able substrates to be accepted for the lipases, while on the other hand, that the chain length interferes with the chiral recognition process, we prepared two new derivatives with different chain sizes (Scheme 3).

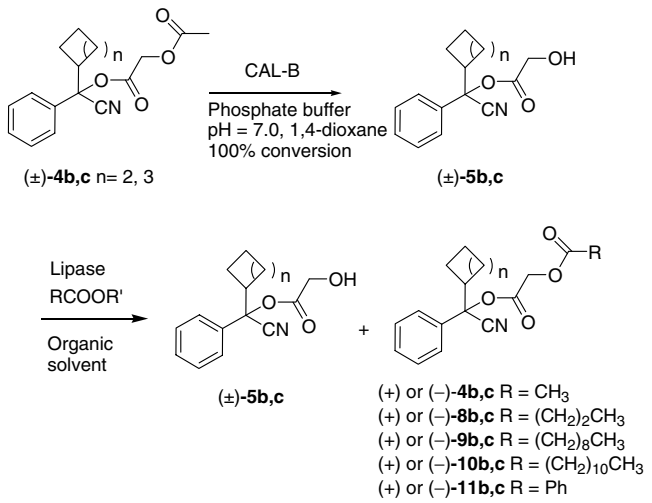
The enzymatic hydrolysis processes catalyzed by CAL-B or PSL-C of substrates (±)-**6** and (±)-**7** were carried out under the same reaction conditions as described above. Unfortunately, none of the examined enzymes catalyzed the hydrolysis of these substrates.

Another common approach for the resolution of racemic alcohols is the enzymatic transesterification reaction. In order to examine these enzymatic processes we used as substrates the racemic derivatives (±)-**5b** and (±)-**5c**, obtained through the enzymatic hydrolysis of (±)-**4b** and (±)-**4c** catalyzed by CAL-B, when the processes were allowed to react until 100% conversion (Scheme 4).

First, we studied the resolution catalyzed by CAL-B or PSL-C of substrate (±)-**5b** using common reaction conditions for an enzymatic transesterification, so vinyl acetate was chosen as acyl donor and the reactions were carried out in toluene at 30 °C (Table 4, entries 1 and 2). The reaction catalyzed by CAL-B was very fast and after 30 min a




**Scheme 3.** Synthesis and enzymatic hydrolysis of derivatives with different chain lengths, (±)-**6** and (±)-**7**.




**Scheme 4.** Synthesis and enzymatic acylation of (±)-**5b**,**c**.

100% of conversion was achieved. Using PSL-C the process was slower but also showed a low enantioselectivity.

Then, we tested non-activated esters, which are less reactive acylating agents, observing that with ethyl acetate, CAL-B showed a moderate rate and low enantioselectivity (entry 3). No reaction was observed with PSL-C (entry 4). When ethyl acetate was used also as the solvent, PSL-C catalyzed the reaction with a moderate rate but a low enantioselectivity (entry 5). Slightly better was the enantioselectivity obtained with ethyl butyrate and PSL-C (entry 7). Using this acylating agent, 23% conversion was obtained after 24 h, with a low but promising enantioselectivity of $E = 10$. The use of longer chain esters seems to lead to better results in the enzymatic process catalyzed by PSL-C. We then tested this reaction using ethyl and vinyl esters of decanoic acid, and also vinyl laurate and vinyl benzoate. The results are summarized in entries 8–11 of Table 4. The best results were obtained using vinyl decanoate since 37% conversion was achieved after only 1.5 h of reaction with good enantioselectivity ($E = 41$, entry 9).

A similar study was carried out for the enzymatic resolution of substrate (±)-**5c** catalyzed by PSL-C. As in the case

**Table 4.** Lipase catalyzed acylation of (±)-**5a**–**c** at 30 °C in toluene, using 5 equiv of the corresponding acylating agent

| Entry | Substrate | Lipase | Acylating agent | $t$ (h) | $c$[a] (%) | ee$_s$[b] (%) | Conf | ee$_p$[b] (%) | Conf | $E$[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (±)-**5b** | CAL-B | Vinyl acetate | 0.5 | 100 | — | — | — | — | — |
| 2 | (±)-**5b** | PSL-C | Vinyl acetate | 0.5 | 14 | 8 | (*S*) | 50 | (*R*) | 3 |
| 3 | (±)-**5b** | CAL-B | Ethyl acetate | 96 | 48 | 51 | (*R*) | 56 | (*S*) | 6 |
| 4 | (±)-**5b** | PSL-C | Ethyl acetate | 120 | — | — | — | — | — | — |
| 5 | (±)-**5b** | PSL-C | Ethyl acetate[d] | 17 | 26 | 20 | (*S*) | 57 | (*R*) | 4 |
| 6 | (±)-**5b** | CAL-B | Ethyl butyrate | 24 | 9 | 6 | (*R*) | 64 | (*S*) | 5 |
| 7 | (±)-**5b** | PSL-C | Ethyl butyrate | 24 | 23 | 23 | (*S*) | 77 | (*R*) | 10 |
| 8 | (±)-**5b** | PSL-C | Ethyl decanoate | 24 | 6 | 5 | (*S*) | 86 | (*R*) | 14 |
| 9 | (±)-**5b** | PSL-C | Vinyl decanoate | 6 | 37 | 54 | (*S*) | 92 | (*R*) | 41 |
| 10 | (±)-**5b** | PSL-C | Vinyl laurate | 6 | 92 | 99 | (*S*) | 9 | (*R*) | 4 |
| 11 | (±)-**5b** | PSL-C | Vinyl benzoate | 48 | 32 | 33 | (*S*) | 70 | (*R*) | 8 |
| 12 | (±)-**5c** | PSL-C | Vinyl acetate | 3 | 48 | 45 | (*S*) | 49 | (*R*) | 4 |
| 13 | (±)-**5c** | PSL-C | Ethyl acetate | 24 | 20 | 17 | (*S*) | 70 | (*R*) | 7 |
| 14 | (±)-**5c** | PSL-C | Ethyl butyrate | 24 | 10 | 10 | (*S*) | 90 | (*R*) | 21 |
| 15 | (±)-**5c** | PSL-C | Ethyl decanoate | 24 | 37 | 42 | (*S*) | 72 | (*R*) | 9 |
| 16 | (±)-**5c** | PSL-C | Vinyl butyrate | 1 | 54 | 51 | (*S*) | 43 | (*R*) | 4 |
| 17 | (±)-**5c** | PSL-C | Vinyl decanoate | 15 | 85 | 81 | (*S*) | 15 | (*R*) | 3 |
| 18 | (±)-**5c** | PSL-C | Vinyl benzoate | 72 | 30 | 40 | (*S*) | 96 | (*R*) | 73 |

[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by chiral HPLC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.[11]
[d] Ethyl acetate was used as solvent and acylating agent.

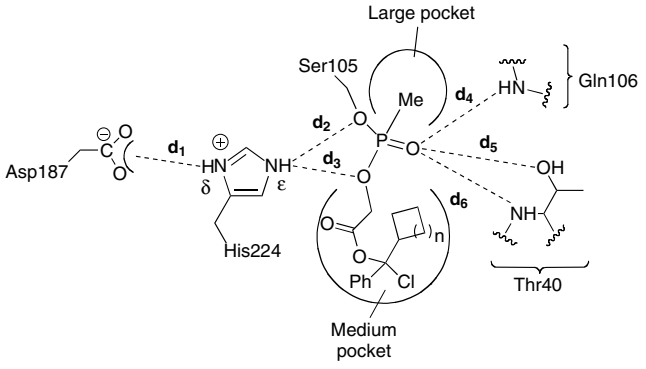
of (±)-**5b** we observed a strong influence of the acylating agent in the rate and enantioselectivity of the reaction. The most significant results are summarized in entries 12–18 of Table 4. The best results were obtained for this substrate using vinyl benzoate as the acylating agent. In this case, a moderate rate and a high enantioselectivity ($E = 73$, entry 18) were achieved.

A further optimization of other reaction parameters such as solvent, acylating agent concentration or temperature could be carried out in order to improve the results obtained for both substrates.

The high stereoselectivity observed with the CAL-B-catalyzed hydrolysis of the cyclobutyl acetoxyacetylated **4a** and not with their homologous cyclopentyl **4b** and cyclohexyl **4c** derivatives motivated us to undertake a modeling study of this reaction to understand better the results. Thus, we obtained the X-ray structure of CAL-B[12] from the Protein Data Bank.[13] This structure presents a phosphonate linked to the active site of the lipase. The catalytic triad is formed by residues Asp187-His224-Ser105 and the oxyanion hole is formed by the main chain NH groups of residues Gln106, Thr40, and the hydroxyl group of the later.

To qualitatively explain the stereoselectivity of CAL-B, we modeled phosphonate analogues of the key intermediates in the hydrolysis of both stereoisomers of each of the three cycloalkyl acetoxyacetylated derivatives (i.e., cyclobutyl **4a**, $n = 1$; cyclopentyl **4b**, $n = 2$; cyclohexyl **4c**, $n = 3$). These phosphonates were able to mimic the key features of the intermediates in hydrolysis reactions and allowed the computationally simpler molecular mechanics approach to be used.[14] This approach focuses on how the substrate fits in the active site of the enzyme, but might omit subtle details about the transition state.

The starting point for modeling was a simple phosphonate, which mimics the transition state for the hydrolysis of methyl acetate. Geometry optimization of this simple phosphonate formed all six essential hydrogen bonds for catalysis ($d_1$–$d_6$ in Scheme 5).[15] To model the cycloalkyl acetoxyacetylated substrates, we added the quaternary carbon and its substituents (cyano, cycloalkyl, and phenyl) sequentially until we obtained the phosphonate shown in Scheme 5 and systematically searched for catalytically productive conformations. We were forced to use the chloride instead of the cyanide because the force field (AMBER[16]) could not apply a potential to the latter.[17] These intermediates mimic the hydrolysis of substrates (±)-**4a**–**c**.[14] We define the catalytically productive conformations as those




**Scheme 5.** Phosphonate analogue intermediates of the transition state for the hydrolysis of cycloalkyl acetoxyacetylated derivatives **4a**–**c** ($n = 1, 2, 3$). Key hydrogen bonds between CAL-B and the tetrahedral intermediate are: one from N$_\delta$ of His224 to the carboxylate of Asp187, two from N$_\varepsilon$ of His224 to the oxygen of Ser105 and the *O*-alkyl group of the tetrahedral intermediate, and three from the oxyanion to Gln106 (one) and to Thr40 (two). For computer modeling the different groups are introduced in a stepwise fashion as described in the text forming both enantiomers.

that (a) contained all six key catalytic hydrogen bonds, (b) avoided steric clashes between the phosphonate and the lipase, and (c) avoided steric clashes within the phosphonate.

When geometry optimization was carried out with the cyclobutyl acetoxyacetylated (*R*)-enantiomer (Fig. 2A), the tetrahedral intermediate for the hydrolysis reaction situates both cyclobutyl and phenyl rings on the right side of the large hydrophobic pocket with the chloride pointing out to the pocket, making it the most productive conformation due to better enzyme substrate interaction, since this structure contains all six catalytically essential hydrogen bonds in the catalytic site. However, when the tetrahedral intermediate for the hydrolysis reaction of cyclobutyl acetoxyacetylated (*S*)-enantiomer was built and minimized, no single productive conformation was obtained. In all structures some of the key H-bond interactions were lost. In the best of them (Fig. 2B), the enzyme situates both cyclobutyl and phenyl rings inside the large hydrophobic pocket but in its edge with the chloride pointing to the right inside the hydrophobic pocket. The binding alkane rings appears to be a key factor in explaining the enzyme enantioselectivity.

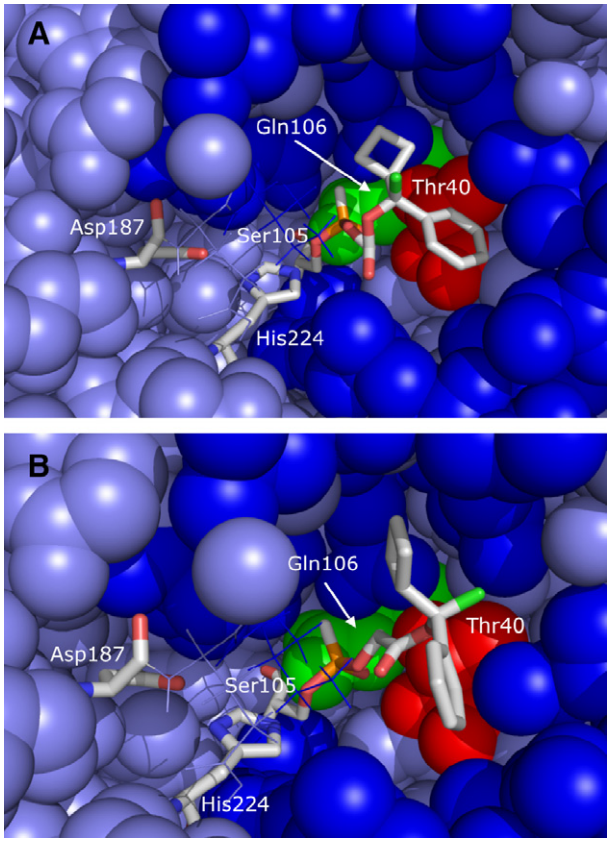



**Figure 2.** The best conformations of cyclobutyl acetoxyacetylated (*R*)-enantiomer (A) and (*S*)-enantiomer (B) intermediates in CAL-B are shown above. The tetrahedral intermediate for the favored (*R*)-enantiomer hydrolysis reaction (A) situates both cyclobutyl and phenyl rings on the right side of the large hydrophobic pocket and the chloride pointing out to the pocket, making it the most productive conformation due to a better enzyme substrate interaction. The tetrahedral intermediate for the (*S*)-enantiomer hydrolysis reaction (B) situates both cyclobutyl and phenyl rings inside the large hydrophobic pocket, but in its edge with the chloride pointing to the right inside the hydrophobic pocket. The binding alkane rings appears to be the key factor in explaining the enzyme enantioselectivity. Darker spheres indicate the amino acids that are the limits of the medium and large hydrophobic size pockets around the CAL-B active site. The above image displays Glu188 and Ile189 in a line representation to allow a better view of the large pocket of the lipase.

Conversely, the geometry optimization of both enantiomers of cyclopentyl or cyclohexyl acetoxyacetylated derivatives yielded structures containing all six catalytically essential hydrogen bonds in the catalytic site, showing no enzyme selectivity for either of the enantiomers in both cases. Thus, modeling explains the experimentally observed facts.

## 3. Conclusions

Herein we have reported an easy methodology for the resolution of three 1-phenyl-1-hydroxy-1-cycloalkylcyanohydrins via a lipase-catalyzed hydrolysis or acylation of a suitable derivative. Thus, among all the lipases tested, CAL-B or PSL-C presented the best results and an opposite stereochemical preference. Enzymatic hydrolysis shows the highest enantioselectivity for the cyclobutyl derivative, whereas the acylation catalyzed by PSL-C is apparently more adequate for the resolution of cyclopentyl and cyclohexyl derivatives. The high enantioselectivity obtained in the acylation process of the cyclohexyl derivative should be noted. Taking into account the collected data, we can conclude that the resolution of tertiary alcohols by enzymatic methods is possible, even though hydrolytic enzymes are usually unable to accept hindered substrates when the quaternary carbons are in the vicinity of the function to be hydrolyzed or acylated. As is usual for enzymatic processes, all reaction parameters must be optimized for each substrate.

The applicability of this method for the preparation of antimuscarinic agents that include a tertiary α-hydroxy acid ester in their structure due to the simplicity and easy scale-up of lipase-catalyzed reactions should be noted.

Results obtained from the molecular modeling study on the CAL-B-catalyzed hydrolysis of cycloalkyl acetoxyacetylated derivatives are in agreement with the observed experimental data. Thus, CAL-B showed high stereoselectivity in the case of the cyclobutyl derivative but failed with cyclopentyl or cyclohexyl acetoxyacetylated derivatives.

This study constitutes another good example of how remote interactions can affect the stereo- and/or regioselectivity of an enzyme.[18] The key difference between the intermediates obtained for both enantiomers is the superior binding of the (*R*)-enantiomer caused at a site remote from the reaction center. Such remote binding can lower the free energy of the corresponding transition state by favoring a catalytically productive orientation, and at the same time disfavoring the intermediate for the observed (*S*)-enantiomer.

## 4. Experimental

### 4.1. General remarks

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *C. antarctica* lipase B, CAL-B Novozym 435 (7300 PLU/g), was a gift from Novo Nordisk co. and immobilized *P. cepacia* lipase (PSL-C, 783 U/g) is commercialized by Amano Pharmaceuticals.

Chemical reagents were commercialized by Aldrich, Fluka, Lancaster or Prolabo. Solvents were distilled over an appropriate desiccant under nitrogen. Flash chromatography was performed using Merck Silica Gel 60 (230–400 mesh). Optical rotations were measured using a Perkin–Elmer 241 polarimeter and are quoted in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. $^1$H NMR, $^{13}$C NMR and DEPT spectra were recorded in a Bruker AC-300, Bruker AC-300 DPX or Bruker NAV-400 spectrometer using $CDCl_3$ as solvent. The chemical shift values ($\delta$) are given in ppm. Positive electrospray ionization ($ESI^+$) was used to record mass spectra on a Hewlett-Packard 110 LC/MSD Series spectrometers. The enantiomeric excesses were determined by chiral HPLC analysis on a Hewlett-Packard 1100, LC liquid chromatograph, using a CHIRALCEL OD for the acylated derivatives **4** and **8**–**11**, and CHIRALPAK AS for hydroxylated derivatives **5a**–**c**. Absolute configurations were determined after refluxing the derivatives **5a**–**c** in HCl 6 M to obtain the corresponding known acid and comparison of their optical rotation with reported values: cyclobutylmandelic acid,[6a] cyclopentylmandelic acid,[19a] and cyclohexylmandelic acid.[19b]

### 4.2. Molecular modeling

The program INSIGHT II, version 2000.1, was used for viewing the structures. The geometric optimizations and molecular dynamics were performed using Discover, version 2.9.7 (*Accelrys*, San Diego, CA, USA), using the AMBER[16] force field. The distance dependent dielectric constant was set to 4.0 to mimic the electrostatic shielding of the solvent and the 1–4 van der Waals interactions were scaled to 50%. The crystal structure for the CAL-B (1LBS[12]) was obtained from the Protein Data Bank,[13] and includes a phosphonate. The methodology followed for the localization of energy minima structures, by means of a systematic search has been described elsewhere.[20] Protein structures in Figure 2 were generated using PyMOL 0.99.[21]

**4.2.1. General procedure for the synthesis of cycloalkyl-1-hydroxy-1-phenylacetonitriles (±)-1a–c.** Tetramethyl-silylcyanide (3.8 mmol) was added dropwise to a stirred solution of the corresponding ketone (3.2 mmol) in dry $CH_2Cl_2$ (12 mL). A catalytic amount of $ZnI_2$ was also added and the reaction mixture was stirred for 24 h at room temperature. The solvent was then evaporated under reduced pressure and the crude residue hydrolyzed with HCl (3 M) and extracted with EtOAc ($3 \times 20$ mL). The organic phases were combined and dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc 7:3) to afford the corresponding product (±)-**1a**–**c**.

**4.2.1.1. (±)-1-Cyclobutyl-1-hydroxy-1-phenylacetonitrile (±)-1a.** White solid. Mp: 73–75 °C. IR (KBr): 2275, 3480 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.71–2.29 (m, 6H), 2.74–2.86 (m, 1H), 2.99 (br s, 1H), 7.31–7.44 (m, 3H), 7.47–7.59 (m, 2H). $^{13}$C NMR ($CDCl_3$, 75.5 MHz): $\delta$ 16.5 ($CH_2$), 23.1 ($CH_2$), 23.4 ($CH_2$), 45.5 (CH), 76.8 (C), 119.9 (CN), 124.8 (CH), 128.7 (CH), 128.9 (CH), 138.5 (C); MS ($ESI^+$, *m*/*z*): 210 [(M+Na)$^+$, 40], 188 [(M+H)$^+$, 10].

**4.2.1.2. (±)-1-Cyclopentyl-1-hydroxy-1-phenylacetonitrile (±)-1b.** Colorless oil. IR (NaCl) 2225, 3654 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.39–1.71 (m, 8H), 2.38–2.43 (m, 1H), 3.87 (br s, 1H), 7.36–7.53 (m, 5H). $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta$ 26.0 ($CH_2$), 26.2 ($CH_2$), 28.6 ($CH_2$), 29.0 ($CH_2$), 52.1 (CH), 78.7 (C), 121.0 (CN), 125.9 (2CH), 129.3 (2CH), 129.5 (CH), 140.6 (C). MS ($ESI^+$*m*/*z*): 224 [(M+Na)$^+$, 100], 202 [(M+H)$^+$, 20].

**4.2.1.3. (±)-1-Cyclohexyl-1-hydroxy-1-phenylacetonitrile (±)-1c.** Colorless oil. IR (NaCl): 2195, 3492 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.11–2.00 (m, 10 H), 2.35 (m, 1H), 3.71 (br s, 1H), 7.35–7.50 (m, 5H). $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta$ 14.3 ($CH_2$), 21.2 ($CH_2$), 26.0 ($CH_2$), 26.1 ($CH_2$), 27.4 ($CH_2$), 27.7 (CH), 49.1 (C), 61.0 (C), 78.6 (CN), 120.6 (CH), 125.9 (CH), 128.7 (CH), 129.1 (CH), 139.5 (C). MS ($ESI^+$ *m*/*z*): 238 [(M+Na)$^+$, 100], 216 [(M+H), 5].

**4.2.2. General procedure for the preparation of derivatives (±)-2a, (±)-4a–c, (±)-6, and (±)-7.** The corresponding acyl chloride (14 mmol) was added dropwise to a stirred solution of the corresponding 1-cycloalkyl-1-hydroxy-1-phenylacetonitrile (±)-**1a**–**c** (7 mmol) in pyridine (14 mmol), and dry $CH_2Cl_2$ (13 mL). The resulting mixture was heated and stirred for 24 h at 60 °C. After this time, the mixture was extracted with $CH_2Cl_2$ ($3 \times 20$ mL). The organic phases were combined and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the crude residue purified by flash chromatography on silica gel (hexane/EtOAc 7:3) to afford the corresponding product.

**4.2.2.1. (±)-(1-Cyano-1-cyclobutyl-1-phenylmethoxy)carbonylmethyl acetate (±)-4a.** White solid. Mp: 89–92 °C. IR (KBr): 3032, 2255, 1739, 1742 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.78–1.85 (m, 3H), 1.92–2.07 (m, 3H), 2.14 (s, 3H), 2.91–3.02 (m, 1H), 4.69 (s, 2H), 7.39–7.42 (m, 5H). $^{13}$C NMR ($CDCl_3$, 100.5 MHz): $\delta$ 16.5 ($CH_3$), 20.2 ($CH_2$), 23.1 ($CH_2$), 23.9 ($CH_2$), 44.6 (CH), 60.2 ($CH_2$), 80.3 (C), 115.9 (CN), 124.4 (CH), 128.8 (CH), 129.1 (CH), 135.0 (C), 165.4 (CO), 170.0 (CO). MS ($ESI^+$, *m*/*z*): 288 [(M+H)$^+$, 100].

(*R*)-(+)-**4a**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL min$^{-1}$, $t_R$ 14.8 min (major); $t_R$ 20.5 min (minor). $[\alpha]_D^{20} = +39$ (*c* 0.10, $CHCl_3$) for 70% ee.

**4.2.2.2. (±)-(1-Cyano-1-cyclopentyl-1-phenylmethoxy)-carbonylmethyl acetate (±)-4b.** White solid. Mp: 78–80 °C. IR (KBr): 2235, 1740, 1745 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.28–1.69 (m, 5H), 1.79–1.93 (m, 3H), 2.12 (s, 3H), 2.58–2.64 (m, 1H), 4.65 (s, 2H), 7.31–7.43 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 21.1 ($CH_3$), 25.9 ($CH_2$), 26.2 ($CH_2$), 28.5 ($CH_2$), 29.4 ($CH_2$), 51.5 (CH), 61.1 ($CH_2$), 82.6 (CN), 116.9 (C), 125.6 (CH), 129.6 (CH), 129.9 (CH), 137.3 (C), 166.1 (CO), 170.8 (CO). MS ($ESI^+$ *m/z*): 324 [$(M+Na)^+$, 100], 302 [$(M+H)^+$, 20], 340 [$(M+K)^+$, 5].

**4.2.2.3. (±)-(1-Cyano-1-cyclohexyl-1-phenylmethoxy)carbonylmethyl acetate (±)-4c.** White solid. Mp: 77–79 °C. IR (KBr): 3140, 2230, 1730, 1690 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.11–1.18 (m, 3H), 121–1.25 (m, 3H), 1.63–1.69 (m, 2H), 1.72–1.84 (m, 1H), 1.85–1.88 (m, 2H), 2.01 (s, 3H), 4.66 (s, 2H), 7.37–7.43 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 25.5 ($CH_3$), 25.6 ($CH_2$), 26.6 ($CH_2$), 27.6 ($CH_2$), 48.3 (CH), 60.2 ($CH_2$), 81.8 (C), 115.8 (CN), 125.2 (CH), 128.6 (CH), 129.1 (CH), 135.4 (C), 165.2 (CO), 169.9 (CO). MS ($ESI^+$, *m/z*): 338 [$(M+Na)^+$, 100], 316 [$(M+H)^+$, 20].

**4.2.3. General procedure for enzymatic hydrolysis.** The reaction mixture containing the corresponding substrate (0.15 mmol), 1 mL of 1 M phosphate buffer (pH 7.0), the lipase (100 mg), and the corresponding organic solvent (2 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 7:3) until the required conversion was obtained. The enzyme was then removed by filtration and washed with $CHCl_3$ (3 × 5 mL). The product was extracted with EtOAc (3 × 5 mL), the organic phases were combined and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the crude residue purified by flash chromatography on silica gel (hexane/EtOAc 7:3).

**4.2.3.1. (±)-1-Cyano-1-cyclobutyl-1-phenylmethyl hydroxyacetate (±)-5a.** White solid. Mp: 74–76 °C. IR (KBr): 3640, 3015, 1735 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.76–1.88 (m, 3H), 2.06 (m, 2H), 2.25–2.32 (m, 1H), 2.54 (br s, 1H), 2.93–3.03 (m, 1H), 4.18–4.26 (m, 2H), 7.61–7.85 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 16.5 ($CH_2$), 23.0 ($CH_2$), 24.02 ($CH_2$), 44.5 (CH), 60.4 ($CH_2$), 80.5 (C), 115.9 (CN), 124.4 (CH), 128.8 (CH), 129.2 (CH), 135.2 (C), 171.1 (CO). MS ($ESI^+$, *m/z*): 256 [$(M+Na)^+$, 100], 234 [$(M+H)^+$, 5].

(*S*)-(−)-**5a**: Determination of the ee: the compound was converted into the acetylated derivative (−)-**4a** and analyzed by HPLC analysis. $[\alpha]_D^{20} = -49$ (*c* 0.12, $CHCl_3$) for 99% ee.

**4.2.3.2. (±)-1-Cyano-1-cyclopentyl-1-phenylmethyl hydroxyacetate (±)-5b.** White solid. Mp: 79–81 °C. IR (KBr) 3652, 1741 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.32–1.62 (m, 7H), 1.93–2.02 (m, 1H), 2.48 (br s, OH), 2.61–2.66 (m, 1H), 4.15 (dd, 1H, $J = 17.46$ Hz), 4.25 (dd, 1H, $J = 17.46$ Hz), 7.37–7.49 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 25.4 ($CH_2$), 25.7 ($CH_2$), 28.1 ($CH_2$), 29.1 ($CH_2$), 50.9 (CH), 60.9 ($CH_2$), 82.4 (C), 116.5 (CN), 125.2 (CH), 129.2 (CH), 129.6 (CH), 137.0 (C), 171.3 (CO). MS ($ESI^+$, *m/z*): 282 [$(M+Na)^+$, 100], 260 [$(M+H)^+$, 20].

(*S*)-(+)-**5b**: Determination of the ee by HPLC analysis: Chiralpak AS, hexane/EtOH 96:4, UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 29.9 min (minor); $t_R$ 34.3 min (major). $[\alpha]_D^{20} = +13$ (*c* 0.10, $CHCl_3$) for 66% ee.

**4.2.3.3. (±)-1-Cyano-1-cyclohexyl-1-phenylmethyl hydroxyacetate (±)-5c.** Pale yellow oil. IR (NaCl): 3647, 1731 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.09–1.25 (m, 4H), 1.65–1.71 (m, 2H), 1.84–1.87 (m, 1H), 1.98–2.14 (m, 4H), 4.07–4.28 (m, 2H), 7.37–7.43 (m, 5H Ar). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 21.3 ($CH_2$), 25.9 ($CH_2$), 25.9 ($CH_2$), 26.9 ($CH_2$), 28.0 ($CH_2$), 48.5 (CH), 60.8 ($CH_2$), 82.3 (C), 116.3 (CN), 125.5 (CH), 129.1 (CH), 129.6 (CH), 136.1 (C), 171.4 (CO). MS ($ESI^+$, *m/z*): 296 [$(M+Na)^+$, 100].

(*S*)-(+)-**5c**: Determination of the ee by HPLC analysis: Chiralpak AS, hexane/EtOH 96:4, UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 31.8 min (minor); $t_R$ 35.1 min (major). $[\alpha]_D^{20} = +7.5$ (*c* 0.10, $CHCl_3$) for 83% ee.

**4.2.4. General procedure for enzymatic acylation.** The reaction mixture containing the corresponding substrate (0.15 mmol), the corresponding acylating agent (0.75 mmol), the lipase (100 mg) and the corresponding organic solvent (4 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 7:3) until the achievement of the required conversion. The enzyme was then removed by filtration and washed with $CHCl_3$ (3 × 5 mL). The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3).

**4.2.4.1. (±)-(1-Cyano-1-cyclopentyl-1-phenylmethoxy)-carbonylmethyl butyrate (±)-8b.** Pale yellow oil. IR (NaCl): 2189, 1732 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 0.96 (t, 3H), 1.35–1.60 (m, 8H), 1.68–1.72 (m, 2H), 2.25 (t, 2H), 2.5 (m, 1H), 5.04 (s, 2H), 7.19–7.25 (m, 5H Ar). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 13.5 ($CH_3$), 18.3 ($CH_2$), 26.6 ($2CH_2$), 27.5 ($2CH_2$), 35.9 ($CH_2$), 40.8 (CH), 61.7 ($CH_2$), 81.6 (C), 118.1 (CN), 126.1 (CH), 128.2 (CH), 128.5 (2CH), 128.9 (CH), 146.3 (C), 172.9 (CO). MS ($ESI^+$, *m/z*): 330 [$(M+H)^+$, 25].

(*R*)-(−)-**8b**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 11.7 min (major); $t_R$ 14.7 min (minor). $[\alpha]_D^{20} = -5.1$ (*c* 0.10, $CHCl_3$) for 64% ee.

**4.2.4.2. (±)-(1-Cyano-1-cyclohexyl-1-phenylmethoxy)carbonylmethyl butyrate (±)-8c.** Pale yellow oil. IR (NaCl): 2193, 1740 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 0.96 (t, 3H), 1.32–1.65 (m, 10H), 1.67–1.71 (m, 2H), 2.24 (t, 2H), 2.51 (m, 1H), 5.05 (s, 2H), 7.19–7.31 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 13.5 ($CH_3$), 18.3 ($CH_2$), 26.6 ($2CH_2$), 27.5 ($2CH_2$), 29.8 ($CH_2$), 35.4 ($CH_2$), 40.7 (CH), 60.7 ($CH_2$), 81.7 (C), 118.0 (CN), 126.1 (CH), 128.2

(CH), 128.5 (CH), 128.9 (CH), 146.1 (C), 173.2 (CO). MS ($ESI^+$, *m/z*): 344 [$(M+H)^+$, 60].

(*R*)-(−)-**8c**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 11.2 min (major); $t_R$ 14.6 min (minor). $[\alpha]_D^{20} = -6.2$ (*c* 0.10, $CHCl_3$) for 90% ee.

**4.2.4.3. (±)-(1-Cyano-1-cyclopentyl-1-phenylmethoxy)-carbonylmethyl decanoate (±)-9b.** Pale yellow oil. IR (NaCl): 2228, 1736 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 0.96 (t, 3H), 1.29–1.68 (m, 22H), 2.25 (m, 2H), 2.49 (m, 1H), 5.04 (s, 2H), 7.18–7.41 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 14.5 ($CH_3$), 22.8 ($CH_2$), 25.1 ($CH_2$), 26.7 ($2CH_2$), 27.7 ($2CH_2$), 29.1 ($CH_2$), 29.4 ($2CH_2$), 29.7 ($CH_2$), 31.9 ($CH_2$), 33.7 ($CH_2$), 40.7 (CH), 61.8 ($CH_2$), 81.9 (C), 118.0 (CN), 126 (CH), 128.2 (2CH), 128.6 (2CH), 146.1 (C), 168.9 (CO), 173.1 (CO). MS ($ESI^+$, *m/z*): 414 [$(M+H)^+$, 100].

(*R*)-(−)-**9b**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 9.1 min (major); $t_R$ 13.0 min (minor). $[\alpha]_D^{20} = -26$ (*c* 0.15, $CHCl_3$) for 92% ee.

**4.2.4.4. (±)-(1-Cyano-1-cyclohexyl-1-phenylmethoxy)carbonylmethyl decanoate (±)-9c.** Pale yellow oil. IR (NaCl): 2192, 1745, 1695 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 0.95 (t, 3H), 12.8–1.65 (m, 24H), 2.21 (m, 2H), 2.47 (m, 1H), 5.10 (s, 2H), 7.17–7.39 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 14.0 ($CH_3$), 22.5 ($CH_2$), 24.6 ($CH_2$), 24.9 ($CH_2$), 25.3 ($CH_2$), 27.7 ($2CH_2$), 28.7 ($CH_2$), 28.9 ($CH_2$), 29.1 ($2CH_2$), 29.3 ($2CH_2$), 31.7 ($CH_2$), 33.8 ($CH_2$), 40.6 (CH), 61.8 ($CH_2$), 82.0 (C), 116.1 (CN), 124.8 (CH), 128.8 (2CH), 129.2 (2CH), 136.6 (C), 170.9 (CO), 173.1 (CO). MS ($ESI^+$, *m/z*): 428 [$(M+H)^+$, 100].

(*R*)-(−)-**9c**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 8.6 min (major); $t_R$ 9.9 min (minor). $[\alpha]_D^{20} = -6.8$ (*c* 0.12, $CHCl_3$) for 72% ee.

**4.2.4.5. (±)-(1-Cyano-1-cyclopentyl-1-phenylmethoxy)-carbonylmethyl benzoate (±)-11b.** Pale yellow solid. Mp: 108–110 °C. IR (KBr): 2232, 1741, 1690 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.37–1.73 (m, 8H), 2.56 (m, 1H), 4.91 (d, 2H), 7.19–7.47 (m, 8H), 7.97 (m, 2H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 25.4 ($CH_2$), 25.7 ($CH_2$), 28.1 ($CH_2$), 28.9 ($CH_2$), 51.1 (CH), 61.2 ($CH_2$), 81.6 (C), 116.6 (CN), 125.7 (CH), 128.8 (CH), 128.9 (CH), 129.2 (CH), 129.5 (CH), 130.2 (CH), 133.9 (CH), 136.9 (C), 141.7 (C), 165.6 (CO), 166.0 (CO). MS ($ESI^+$, *m/z*): 386 [$(M+Na)^+$, 100], 402 [$(M+K)^+$, 20].

(*R*)-(−)-**11b**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 12.7 min (major); $t_R$ 15.8 min (minor). $[\alpha]_D^{20} = -6.1$ (*c* 0.10, $CHCl_3$) for 70% ee.

**4.2.4.6. (±)-(1-Cyano-1-cyclohexyl-1-phenylmethoxy)carbonylmethyl benzoate (±)-11c.** Pale yellow solid. Mp: 115–117 °C. IR (KBr) 3195, 1724 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 1.35–1.74 (m, 10H), 2.56 (m, 1H), 4.91 (d, 2H), 7.19–7.47 (m, 8H), 7.97 (m, 2H). $^{13}C$ NMR ($CDCl_3$, 100.5 MHz): $\delta$ 25.4 ($CH_2$), 25.7 ($CH_2$), 27.6 ($CH_2$), 28.0 ($CH_2$), 28.9 ($CH_2$), 51.1 (CH), 62.1 ($CH_2$), 81.5 (C), 116.6 (CN), 125.3 (CH), 128.9 (CH), 128.8 (CH), 129.2 (CH), 129.7 (CH), 130.3 (CH), 134.0 (CH), 136.8 (C), 141.8 (C), 165.5 (CO), 166.1 (CO). MS ($ESI^+$, *m/z*): 400 [$(M+Na)^+$, 60], 416 [$(M+K)^+$, 5].

(−)-**11c**: Determination of the ee by HPLC analysis: Chiralcel OD, 25 °C, hexane/2-propanol (90:10), UV 210 nm, 0.5 mL $min^{-1}$, $t_R$ 13.014 min (minor); $t_R$ 14.415 min (major). $[\alpha]_D^{20} = -12$ (*c* 0.12, $CHCl_3$) for 96% ee.

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