

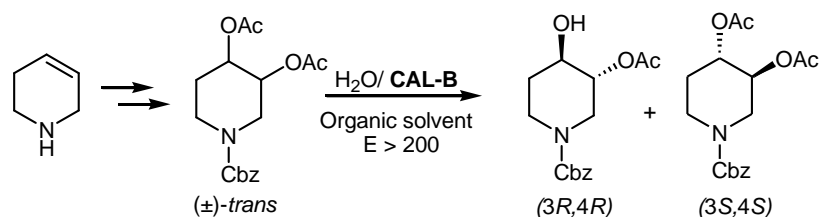
Graphical Abstract

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

Biocatalytic Preparation of Enantioenriched 3,4-Dihydropiperidines and Theoretical Study of *Candida Antarctica* Lipase B Enantioselectivity.

Laura F. Solares, Iván Lavandera, Vicente Gotor-Fernández, Rosario Brieva and Vicente Gotor*

Leave this area blank for abstract info.





Pergamon

TETRAHEDRON

Biocatalytic Preparation of Enantioenriched 3,4-Dihydropiperidines and Theoretical Study of *Candida antarctica* Lipase B Enantioselectivity.

Laura F. Solares, Iván Lavandera, Vicente Gotor-Fernández, Rosario Brieva and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33006-Oviedo, Spain

Abstract— Enzymatic acetylations of *N*-substituted *cis*- and *trans*-3,4-dihydropiperidine and hydrolysis of their diacetylated derivatives have been studied. High enantioselectivities are obtained with *Pseudomonas cepacia* lipase and *Candida antarctica* lipase B for the hydrolysis of the *trans*-derivative, while the *cis*-derivatives are not adequate substrates in the same biocatalytic conditions. The enantiopreference of these processes can be rationalized by means of a molecular modelling study.

1. Introduction

Polyhydroxylated piperidines have attracted great interest due to their biological properties. Some of them are inhibitors of glycosidases and glycoprotein-processing enzymes.¹ These inhibitors have shown a promising therapeutic potential for the treatment of various diseases related to metabolic disorders of carbohydrates such as diabetes,² and viral infections including AIDS³ or cancer.⁴ In this sense, optically pure 3,4-dihydropiperidine is an interesting structure since the (3*R*,4*R*)-*trans* isomer is an inhibitor of β -D-glucuronidase⁵ and has been used as intermediate for the preparation of a xylanase inhibitor (Figure 1).⁶ Also, this structure is a suitable precursor of the gastroprokinetic agent cisapride.⁷ On the other hand, the *cis*-isomer is an intermediate for the preparation of the antidepressant ifoxetine.⁸

The optically pure (3*R*,4*R*)-*trans*-isomer has been synthesized from either D-arabinose⁵ or D-tartaric acid.⁶ Both routes require a large number of steps to afford the desired compound in low overall yields. Recently, Chang et al.⁹ have reported the preparation of the optically pure *trans*-isomers using enantioselective *trans*-dihydroxylations or epoxidations of *N*-substituted 1,2,5,6-tetrahydropyridines, catalyzed by the bacterial strain *Sphingomonas* sp. HXN-200.

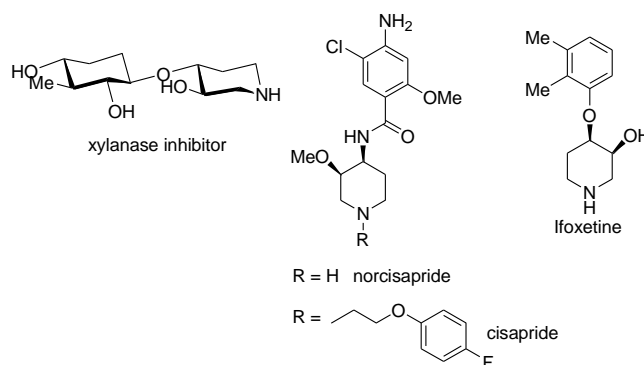


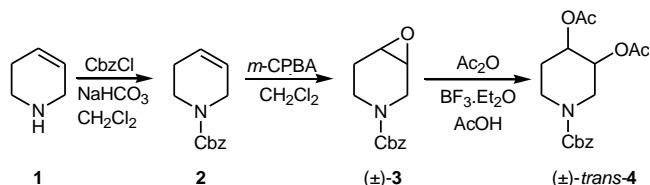
Figure 1. Examples of different chiral 3,4-dihydropiperidine derivatives.

On the other hand, lipase-catalyzed processes in organic solvents are very advantageous alternatives to the conventional chemical reactions, that have been widely used for the preparation of optically pure cycloalkanediols¹⁰ and carbosugars.¹¹ Nevertheless, to the best of our knowledge, the use of this methodology for the preparation of polyhydroxylated piperidines has not been studied until now. This paper describes the acetylation of *N*-protected *cis*- and *trans*-3,4-dihydropiperidine and the hydrolysis of their diacetylated derivatives catalyzed by lipases. Some of the results presented here have been rationalized using a computational approach.

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

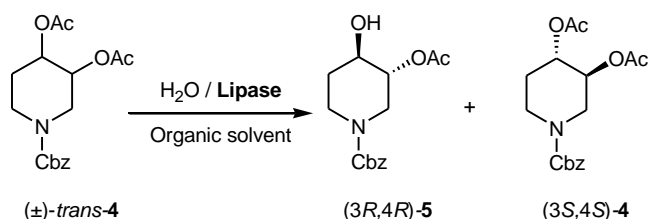
2. Results and discussion

Our initial experiments were designed to find the most suitable lipase for catalyzing the hydrolysis of the diacetylated derivative (\pm)-*trans*-**4**. This substrate has been prepared almost in quantitative yield by treatment of 1-benzyloxycarbonyl-1,2,5,6-tetrahydropyridine (**2**) with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 and subsequent opening of the resulting epoxide (\pm)-**3** with acetic anhydride and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (Scheme 1).



Scheme 1. Synthesis of (\pm)-*trans*-**4**.

First, we studied the hydrolysis of (\pm)-*trans*-**4**, in aqueous media (phosphate buffer, pH 7). In these conditions, the non-enzymatic hydrolysis occurred in competition with the enzymatic reaction. So, we decided to examine the process in organic solvents using a small amount of water as nucleophile (Scheme 2).



Scheme 2. Enzymatic hydrolysis of (\pm)-*trans*-**4** in organic solvents.

Since we had previously obtained good results in these hydrolytic conditions,¹² in a first set of experiments at 30 °C, acetonitrile was chosen as the reaction solvent and

different lipases were used as biocatalysts: *Candida antarctica* A (CAL-A), *Candida antarctica* B in two forms [Novozym 435 (CAL-B) and Chirazyme-L2 (CAL-B-L2)], *Candida rugosa* (CRL) and *Pseudomonas cepacia* (PSL-C). Three of the tested enzymes catalyzed the regioselective hydrolysis of (\pm)-*trans*-**4** affording only the 3-acetyl-4-hydroxy derivative (3*R*,4*R*)-**5** (Table I, entries 1 to 3). In all cases, the enzymatic reactions were regioselective towards the acetyl group at *O*-4 position. This was confirmed by means of a two dimensional ^1H - ^1H -COSY NMR analysis.

The enantioselectivity of the enzymatic hydrolysis was also very high. Both immobilized forms of lipase B from *Candida antarctica*, CAL-B and CAL-B-L-2, showed the highest enantioselectivities ($E > 200$),¹³ with excellent reaction rates, after 24 h a 49 % conversion was achieved. The lipase PSL-C showed also a high enantioselectivity under these conditions, ($E = 98$), but displayed a lower reaction rate. In these conditions, CAL-A and CRL did not show any activity.

The absolute configurations of the product and the remaining substrate were assigned as follows. Hydrolysis of ($-$)-*trans*-**5** using NaOMe in MeOH, afforded the dihydroxy derivative (+)-*trans*-**6**, whose specific rotation sign was in agreement with that reported for (3*R*,4*R*)-(+)-**6**, $[\alpha]_{\text{D}}^{20} = +3.51$ (c 0.76, CHCl_3).⁹

In order to improve the performance of the process, we studied the influence of the organic solvent on the enantioselectivity of the hydrolysis catalyzed by CAL-B. Thus, under the same reaction conditions, similar results were obtained in 1,4-dioxane than in acetonitrile (Table I, entry 4), even though it is apparent that in acetonitrile were slightly higher. Lower reaction rate and enantioselectivity were achieved in toluene (Table I, entry 5). Finally, an increase of the nucleophile concentration up to 10 equivalents furnished lower conversion (Table I, entry 6).

Table 1. Lipase catalyzed hydrolysis of (\pm)-*trans*-**4** in organic solvents at 30°C.

Entry	Lipase	Solvent	H ₂ O (eq)	<i>t</i> [h]	<i>c</i> [%] ^a	ee _s [%] ^b	ee _p [%] ^b	<i>E</i> ^c
1	CAL-B	Acetonitrile	5	24	49	97	>99	>200
2	CAL-B-L2	Acetonitrile	5	24	49	96	>99	>200
3	PSL-C	Acetonitrile	5	24	8	9	98	98
4	CAL-B	1,4-Dioxane	5	48	47	89	>99	>200
5	CAL-B	Toluene	5	24	10	9	83	12
6	CAL-B	Acetonitrile	10	24	36	54	>99	>200

^a Conversion, $c = ee_s / (ee_s + ee_p)$.

^b Determined by chiral HPLC.

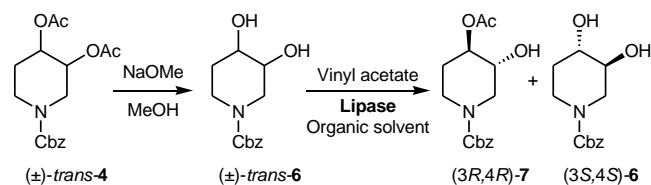
^c Enantiomeric ratio, $E = \ln[(1 - c)/(1 - ee_s)] / \ln[(1 - c)/(1 + ee_s)]$.¹³

Another common approach for the resolution of racemic polyalcohols is the enzymatic transesterification reaction.¹⁴

So, we decided to synthesize the dihydroxypiperidine (\pm)-*trans*-**6**, starting from the diacetate (\pm)-*trans*-**4** and using

NaOMe in MeOH. Taking into account that the best enzymes for the hydrolysis are both CAL-B preparations and PSL-C, we examined the acetylation of the dihydroxy derivative (\pm)-*trans*-**6** catalyzed by these lipases (Scheme 3, Table II).

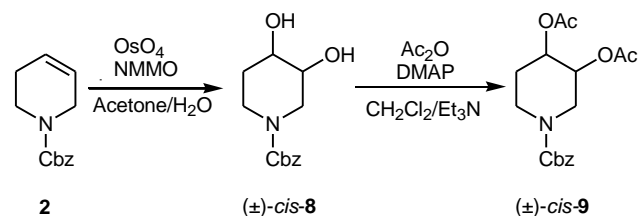
First we carried out the process using 5 equivalents of vinyl acetate as acyl donor and acetonitrile as solvent. In these conditions, CAL-B-L2 and PSL-C catalyzed the processes with good reaction rates (entries 1 and 2), however both showed very low enantioselectivities. Surprisingly, in the process catalyzed by CAL-B, after 72 h of reaction only the unaltered substrate was recovered (entry 3). A fifty-fold excess of the acylating agent was necessary to obtain a moderate reaction rate with this catalyst (entry 4). Also a slightly improvement in the enantioselectivity was observed.



Scheme 3. Enzymatic acetylation of (\pm)-*trans*-**6**.

As expected according to the principle of microscopic reversibility, the regio- and stereochemical preferences of these lipases were the same as for the hydrolytic processes: the hydroxyl group at C-4 position of the piperidine ring was preferentially acetylated to afford the product (3*R*,4*R*)-**7**. In all cases, the formation of only one of the two possible regioisomers was observed. Unfortunately, the enantioselectivities of the enzymatic acylations were very low. Attempts to improve the results obtained in the reaction catalyzed by PSL-C, using different solvents such as toluene (entry 5) or *tert*-butyl methyl ether (entry 6) afforded also poor enantioselectivities, although these processes were faster.

In order to establish the scope of this enantiomer separation methodology, similar biocatalytic conditions were applied for the hydrolysis of (\pm)-*cis*-*N*-benzyloxycarbonyl-3,4-diacetoxypiperidine derivative. The substrate was synthesized according to the Scheme 4. Treatment of 1-benzyloxycarbonyl-1,2,5,6-tetrahydropyridine (**2**) with OsO₄ and *N*-methylmorpholine *N*-oxide (NMMO) yielded the diol (\pm)-*cis*-**8**, that was acetylated to the corresponding diacetate derivative (\pm)-*cis*-**9**.



Scheme 4. Synthesis of (\pm)-*cis*-**8** and (\pm)-*cis*-**9**.

Unfortunately, the enzymatic hydrolysis carried out with this substrate showed, in all cases, very low reaction rates. For instance, the conversion of the reaction catalyzed by CAL-B in acetonitrile at 30 °C, using 5 equivalents of water was less than 5% after 7 days of reaction. An increment of the temperature at 60 °C or an excess of the nucleophile up to fifty-fold did not increase the reaction rate. Other lipases and different organic solvents were tested but no improvement on the reaction rate was obtained. Even more, when we analyzed the products using gas chromatography, we observed a mixture 1:1 of the two possible regioisomers.

Similar results were achieved in the acetylation of the dihydroxyderivative (\pm)-*cis*-**8**. Very low reactivities and regioselectivities were found in all the tested conditions (data not shown).

Entry	Lipase	Solvent	VA (eq)	<i>t</i> [h]	<i>c</i> [%] ^a	ee _s [%] ^b	ee _p [%] ^b	<i>E</i> ^c
1	CAL-B-L2	Acetonitrile	5	15	59	20	14	2
2	PSL-C	Acetonitrile	5	16	30	74	61	2
3	CAL-B	Acetonitrile	5	72	–	–	–	–
4	CAL-B	Acetonitrile	50	22	30	19	13	9
5	PSL-C	Toluene	5	15	62	39	24	2
6	PSL-C	^t BuOMe	5	15	68	25	11	2

Table 2. Lipase catalyzed acetylation of (\pm)-*trans*-**6** with vinyl acetate (VA) in organic solvents at 30 °C.

^a Conversion, $c = ee_s / (ee_s + ee_p)$.

^b Determined by chiral HPLC.

^c Enantiomeric ratio, $E = \ln[(1 - c)/(1 - ee_s)] / \ln[(1 - c)/(1 + ee_s)]$.¹³

Since all these lipase-catalyzed reactions were dramatically influenced by the stereochemistry of the chiral centres, we found of a great deal of interest to rationalize the experimental facts observed in the hydrolysis of the *trans*-derivative (\pm)-**4** using a computational approach. Since the X-ray crystal structure of CAL-B was resolved,¹⁵ several molecular modelling¹⁶ and molecular dynamics studies¹⁷ have been made in order to explain its excellent reactivity and selectivity with chiral alcohols and amines. These reports have been very useful to make rational design of this biocatalyst¹⁸ with improved selectivities,^{17b,19} or catalyzing through novel reaction pathways.²⁰ Thus, engineered CAL-B has been utilized to perform aldol,²¹ Michael-type additions,²² or Baeyer-Villiger reactions.²³

The restricted volume of the active site of this lipase has been used to rationalize its special properties. So, viewed with the catalytic triad Asp187-His224-Ser105 oriented from left to right, CAL-B presents two well-differed subsites: a) the large hydrophobic pocket (or acyl pocket) above the catalytic triad, which is lined by Leu140 and Leu144 at the top, Ile189 and Val190 on the left, as well as Val154 on the far right of the pocket. Deep in this subsite, Asp134 is on the left and Gln157 on the right; and b) the medium-sized pocket (so called nucleophile or stereospecificity pocket) below it, which is crowded by Trp104 at the bottom and the Leu278-Ala287 helix to the right. The acyl chains lie in the large subsite, while nucleophiles bind to the medium-sized pocket. This smaller subsite has been found as the key factor to the enantioselectivity of secondary alcohols.²⁴

Thus, the fast-reacting enantiomer (following Kazlauskas' rule,²⁵ usually the *R*), places the medium-sized substituent into this subsite and the big one into the acyl pocket, while the slow enantiomer has to introduce them in the opposite orientation, existing more unfavourable steric interactions. This general rule for acyclic alcohols, is not so clear for cyclic one, due to although *R*-acylated or hydrolyzed enantiomers are usually obtained, in several cases has been observed that for 1,2-*cis*-cyclohexanols, lipase-catalyzed processes are not achieved or are not selective, in spite of the excellent enantioselectivity for their *trans* counterparts.²⁶ There are no many examples of the use of molecular modelling to study CAL-B-catalyzed acylations or hydrolyses of cyclic alcohols.^{16c,16d,16g} Here we will use this tool to explain qualitatively the experimental results obtained for the hydrolysis at the 4-position in the cyclic diacetate (\pm)-**4**.

We have modelled the first tetrahedral intermediate (TI-1) since in the hydrolysis processes this is the structure where the ester is bound to the lipase, and is the responsible of the enantioselection. Phosphonate analogues were used for mimicking this intermediate using the AMBER force field,²⁷ and several local energy minima were obtained for each enantiomer by means of a systematically search, as described elsewhere.^{16g} The selection of the best structures was based in two main criteria: a) those that presented the most possible of the

six essential hydrogen-bonds [one between Asp187 and N δ -His224; one between N ϵ -His224 and O γ -Ser105; one between N ϵ -His224 and the proton-acceptor oxygen of the acetate; and three between the oxygen of the oxyanion formed with Gln106 (one) and Thr40 (two)]; and b) those that minimized the steric clashes between the phosphonate and the amino acids which surrounded the active site of CAL-B. In general, the nucleophile pocket restricted the binding of the substrate to such an extent that only a few conformations were obtained with the desired properties.

We started with the minimization of the intermediate for the hydrolysis in the 4-position of (3*R*,4*R*)-**4**. This enantiomer was experimentally the fast-reacting one. We used the X-ray structure 1LBS^{16a} obtained from the Protein Data Bank (<http://www.rcsb.org/pdb/>), which presented an inhibitor phosphonate that was manually deleted, and then the substrate was built.^{16g}

When the bulky benzyloxycarbonyl moiety was manually added to the phosphonate and minimized, two families of intermediates were usually got. In both cases this group was accommodated into the narrow tunnel created by Ile189, Leu278, Ala281, Ala282, Ile285, and Val286, but with different orientations. Some of them (usually more stable), presented this moiety on the left, with the phenyl group close to Ile189 and Leu278, and the other one had the phenyl ring on the right side near from Ala282, Ile285, and Val286 pointing to the solvent.

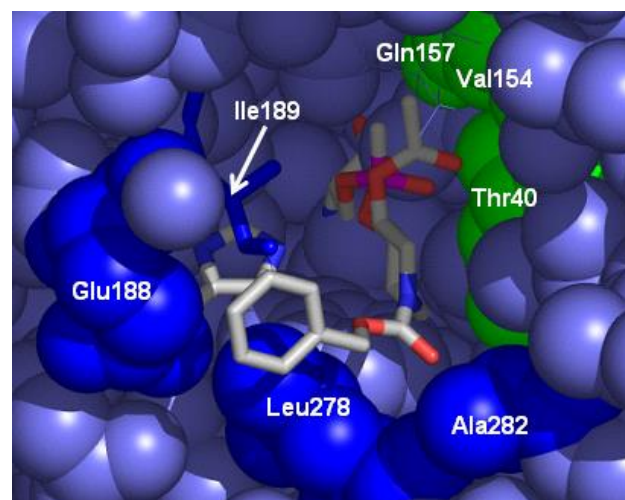


Figure 2. Best intermediate obtained for the CAL-B-catalyzed acylation of (3*R*,4*R*)-**4**. Amino acids which make stabilizing interactions with the 3-*O*-acetyl group of the substrate are depicted in green, and those with the *N*-benzyloxycarbonyl moiety are shown in dark blue. The above image displays Leu144 in a line illustration and Ile189 in a stick representation to allow a better view of the large pocket of the lipase.

The best structure obtained for (3*R*,4*R*)-**4** is shown in Figure 2. As described previously,^{16b,16g} the cycle was placed into the stereospecificity pocket, with the acetyl group in the 3-position fitted into the acyl pocket, generating appropriate interactions between the methyl group with Val154 and the carbonyl moiety with the

hydrophilic side chains of Gln157 and Thr40. The benzyloxycarbonyl group was on the left of the tunnel with the phenyl ring making stabilizing hydrophobic interactions with the side chains of Ile189, Glu188, and Leu278, with the carbonyl group making a H-bond with the NH-backbone of the Ala282 (3.03 Å), and the methylene moiety interacting with the hydrophobic side chain of Ala282. All key-hydrogen bonds were present in this structure.

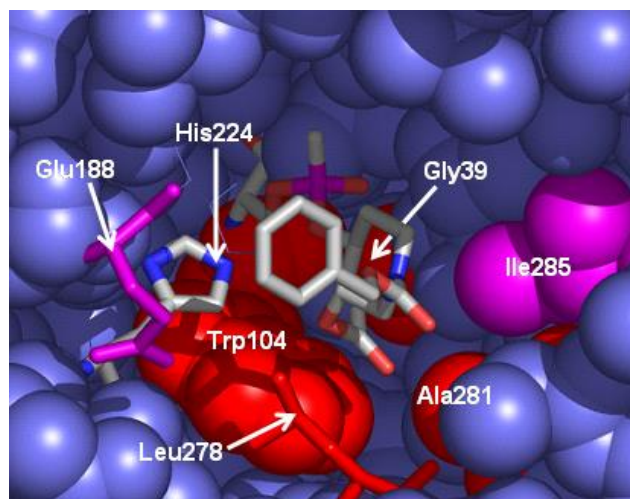


Figure 3. Best intermediate obtained for the CAL-B-catalyzed acylation of (3*S*,4*S*)-**4**. Amino acids which make destabilizing interactions with the 3-*O*-acetyl group of the substrate are depicted in red and with the *N*-benzyloxycarbonyl moiety are shown in violet. The above image displays Ile189 in a line illustration and Glu188 and Leu278 in a stick representation to allow a better view of the phosphonate structure.

When the intermediate for (3*S*,4*S*)-**4** was built and minimized, the best structure obtained presented several structural differences with regards to the (*R,R*)-enantiomer (Figure 3). Although all essential-hydrogen bonds existed too, due to the acetyl moiety in 3-position bound into the medium nucleophile subsite, the carbocycle did not fit in this pocket, and the Cbz was not placed as stable as in the case of (3*R*,4*R*)-**4**. On the one hand, many destabilizing interactions appeared between the acetyl group and Trp104, Ala281 (both with the carbonyl), Gly39 (with the methyl), Leu278 and more importantly, with the catalytic His224 (both with the oxygen of the alcohol); on the other hand, the benzyloxycarbonyl moiety was placed at the top of the tunnel making unfavourable clashes between the carbonyl group and the hydrophobic chain of Ile285, and the phenyl ring with the carbonyl-backbone of Glu188.

Molecular modelling studies agree with the experimental results, explaining the excellent enantioselectivity showed by CAL-B toward *trans*-diacetate **4**. Thus, although all essential hydrogen-bonds were present for both intermediates, better fitting of the (*R,R*)-enantiomer into the active site of the lipase, and more stabilizing interactions with the amino acids which are surrounding it can be the key explanation for this resolution. Furthermore, in the (*S,S*)-structure, the acetyl in 3-

position seemed to be very close from the catalytic His224, what might destabilise its position for the necessary transfer of the proton to the reactive carbonyl.

Looking at these structures, a plausible explanation for the bad enantioselectivity in the acetylation of the diol derivative *trans*-(±)-**6** could be established. Thus, the only difference between the first tetrahedral intermediate in the hydrolysis process, and the second tetrahedral intermediate in the acetylation reaction, is that in the first case at the 3-position there is an acetoxy group, and in the second one there is an hydroxyl moiety at this position. Some of the main structural differences obtained between both enantiomers were based on the altered interactions of the 3-*O*-acetyl group with the amino acids close to the lipase active-site. Thus, while for the (*R,R*) structure they were stabilizing (see green interactions in Figure 2), in the (*S,S*) enantiomer they destabilized the intermediate (see red interactions in Figure 3). Since this moiety is not present in the acetylation intermediates both structures would present less differences.

3. Conclusions

This paper describes an easy methodology for the preparation of both enantiomers of *trans*-1-benzyloxycarbonyl-3,4-dihydropiperidine and the (*R,R*)-3-*O*-monoacetylated derivative *via* a lipase catalyzed hydrolysis. Thus, among all the lipases tested, CAL-B presented the best results, since allows obtaining one of the six possible products of this reaction (four monoalcohols and two diols) with high yield, showing this process total regio- and enantioselectivity at the same time. Attempts to acetylate the *trans*-diol (±)-**6** afforded good regioselectivity but lower reaction rates and enantioselectivities.

Molecular modelling has been used in order to rationalize these experimental facts. Thus, phosphonate analogues of both enantiomers of diacetate **4** were built and minimized, obtaining several structural divergences that can explain the excellent enantioselectivity obtained in the hydrolysis reaction with this substrate.

On the other hand, from the results shown in this paper, it is apparent that the *cis*-isomer is not a suitable substrate for the tested lipases, neither through hydrolysis nor acylation processes.

Taking into account the simplicity and easy scale-up of lipase catalyzed reactions,²⁸ it is noteworthy the applicability of this method for the preparation of the gastroprokinetic agent cisapride and other optically pure structures with highly interesting biological properties.

4. Experimental

General Remarks. Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *Candida antarctica* lipase B, CAL-B Novozym 435 (7300 PLU/g), was a gift from Novo Nordisk co. *Candida antarctica* lipase B (CAL-B-L2, CHIRAZYME L-2, c-f, C3, ≥ 400 U/g), *Candida rugosa* lipase (CRL, CHIRAZYME L-3, >250 U/mg) and *Candida antarctica* lipase A (CAL-A, CHIRAZYME L-5, 1 kU/g) were supplied by Roche Molecular Biochemicals. *Pseudomonas cepacia* lipase lyophilized (PSL, 30000 U/g) and immobilized (PSL-C, 783 U/g) are 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 $\text{cm}^2 \text{g}^{-1}$. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and DEPT spectra were recorded in a Bruker AC-300, Bruker AC-300 DPX and Bruker NAV-400 spectrometer using CDCl_3 as solvent. The chemical shift values (δ) are given in ppm. Positive electrospray ionisation (ESI^+) was used to record mass spectra on an 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 diacetylated derivative **4**. The diol **6** and the monoacetylated products **5** and **7** were first acetylated to convert them into the diacetate derivative **4** and then analyzed.

Molecular Modelling. The program Insight II, version 2000.1, was used for viewing the structures. The geometric optimizations were performed using Discover, version 2.9.7 (Accelrys, San Diego CA, USA), using the AMBER force field.²⁷ 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^{16a}) was obtained from the Protein Data Bank (www.rcsb.org/pdb/). Protein structures in Figures 2 and 3 were generated using PyMOL 0.97.²⁹ The methodology followed for localization of energy minima structures by means of a systematically search has been described elsewhere.^{16g}

4.1. Synthesis of 1-Benzoyloxycarbonyl-1,2,5,6-tetrahydropyridine, (2). Benzyl chloroformate (3.8 mL, 26.3 mmol) was added dropwise to a stirred mixture of 1,2,5,6-tetrahydropyridine **1** (2.0 g, 21.9 mmol) and NaHCO_3 (2.8 g, 26.3 mmol) in H_2O (50 mL) at 0°C , and the mixture was stirred at room temperature for 4 h. After this time CH_2Cl_2 (50 mL) and 5% aqueous Na_2CO_3 (20 mL) were added, the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (50 mL). The combined organic phases were dried over Na_2SO_4 , the solvent was removed under reduced pressure and the

crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 3:1) to afford the Cbz-protected product **2** as a yellow oil (4.4 g, 85%). $^1\text{H-NMR}$ (CDCl_3 , 300.13 MHz): δ 7.36 (m, 5 H), 5.81 (br s, 1 H), 5.64 (br s, 1 H), 5.16 (s, 2 H), 3.96 (m, 2 H), 3.57 (m, 2 H), 2.14 (m, 2 H); $^{13}\text{C-NMR}$ (CDCl_3 , 75.5 MHz): δ 155.2 (CO), 136.6 (C), 128.2 (CH), 127.7 (CH), 127.6 (CH), 125.1 (CH), 124.8 (CH), 66.7 (CH_2), 43.1 (CH_2), 40.1 (CH_2), 24.7 (CH_2); IR (CH_2Cl_2): ν 1713, 1630, 1425 cm^{-1} ; MS (ESI^+ , m/z): 218 [(M + H)⁺, 100%], 240 [(M + Na)⁺, 30].

4.2. Synthesis of (\pm)-1-Benzoyloxycarbonyl-3,4-epoxypiperidine, [(\pm)-3]. A solution of *m*-CPBA (4.3 g, 25.0 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise to a stirred solution of **2** (3.9 g, 18.0 mmol) in dry CH_2Cl_2 (20 mL) at 0°C , and the mixture was stirred at room temperature for 4 h, then washed with 5% aqueous K_2CO_3 solution (20 mL), followed by a saturated aqueous NaCl solution (20 mL). The organic phase was dried over Na_2SO_4 , the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 1:1) to afford the product (\pm)-**3** as a yellow oil (3.8 g, 90%). $^1\text{H-NMR}$ (CDCl_3 , 300.13 MHz): δ 7.36 (m, 5 H), 5.10 (s, 2 H), 3.85-3.72 (m, 2 H), 3.44 (m, 2 H), 3.23 (m, 2 H), 2.01 (m, 2 H); $^{13}\text{C-NMR}$ (CDCl_3 , 75.5 MHz): δ 155.2 (CO), 136.3 (C), 128.4 (CH), 128.0 (CH), 127.8 (2 CH), 67.4 (CH_2), 55.3 (2 CH), 47.5 (CH_2), 41.3 (CH_2), 21.1 (CH_2); IR (CH_2Cl_2): ν 1715, 1428, 1212 cm^{-1} ; MS (ESI^+ , m/z): 234 [(M + H)⁺, 10%], 256 [(M + Na)⁺, 100%].

4.3. Preparation of (\pm)-*trans*-3,4-Diacetoxy-1-benzoyloxycarbonylpiperidine, [(\pm)-*trans*-4]. To a solution of (\pm)-**3** (0.95 g, 4.1 mmol) in acetic acid (35 mL), acetic anhydride was added (1.6 mL, 17 mmol). Boron trifluoride etherate (507 μL , 4.1 mmol) was slowly added, and the mixture was stirred at room temperature for 4 h. An aqueous saturated solution of NaHCO_3 (10 mL) was added, and the mixture extracted with EtOAc (20 mL). The organic phase was washed with saturated aqueous Na_2CO_3 solution (20 mL) followed by saturated aqueous NaCl solution (20 mL), and then was dried over Na_2SO_4 . The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 1:1) to afford the product (\pm)-*trans*-**4** as a yellow oil (1.2 g, 90%). $^1\text{H-NMR}$ (CDCl_3 , 400.13 MHz, 40°C): δ 7.38 (m, 5 H), 5.18 (d, 2 H), 4.98 (s, 1 H), 4.83 (m, 1 H), 3.84 (dd, 1 H, $^3J_{\text{HH}}$ 3.45, $^3J_{\text{HH}}$ 13.80 Hz), 3.71-3.47 (m, 3 H), 2.11-2.01 (m, 1 H), 2.10 (s, 3 H), 1.97 (s, 3 H), 1.70 (m, 1 H); $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz, 40°C): δ 169.7 (CO), 169.5 (CO), 155.1 (CO), 136.2 (C), 128.3 (CH), 127.8 (CH), 127.6 (CH), 68.0 (2 CH), 67.1 (CH_2), 44.1 (CH_2), 40.1 (CH_2), 26.1 (CH_2), 20.8 (CH_3); IR (CH_2Cl_2): ν 1737, 1712, 1425, 1210 cm^{-1} ; MS (ESI^+ , m/z): 358 [(M + Na)⁺, 100%].

4.4. Enzymatic hydrolysis of (\pm)-*trans*-4. The reaction mixture containing (\pm)-*trans*-**4** (50 mg, 0.15 mmol), the appropriate amount of H_2O (see Table I) and the lipase (50 mg) in the corresponding organic solvent (50 mL) was

shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 1:1) until the achievement of the required conversion. Then, the enzyme was removed by filtration and washed with the corresponding organic solvent. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 1:1) to afford the monoacetylated product (3*R*,4*R*)-5 and the remaining substrate (3*S*,4*S*)-4.

(3*S*,4*S*)-3,4-Diacetoxy-1-benzyloxycarbonylpiperidine, [(3*S*,4*S*)-4]. Yellow oil [α]_D²⁵ = + 30.6 (*c* = 1, CHCl₃), ee >99 %.

(3*R*,4*R*)-3-Acetoxy-1-benzyloxycarbonyl-4-hydroxypiperidine, [(3*R*,4*R*)-5]. Yellow oil [α]_D²⁵ = -20.2 (*c* = 1, CHCl₃), ee >99 %. ¹H-NMR (CDCl₃, 400.13 MHz, 40 °C): δ 7.38 (m, 5 H), 5.16 (2s, 2 H), 4.66 (m, 1 H), 4.03 (dd, 1 H, ³*J*_{HH} 3.32, ³*J*_{HH} 13.32 Hz), 3.88-3.80 (m, 3 H), 3.25 (m, 1 H), 2.22 (br s, 1 H, OH), 2.06 (s, 3 H), 2.04-2.00 (m, 1 H), 1.61 (m, 1 H); ¹³C-NMR (CDCl₃, 100.6 MHz, 40 °C): δ 170.6 (CO), 155.3 (CO), 136.6 (C), 128.5 (CH), 128.0 (CH), 127.9 (CH), 72.5 (CH), 69.4 (CH), 67.3 (CH₂), 44.4 (CH₂), 40.8 (CH₂), 30.8 (CH₂), 20.9 (CH₃); IR (CH₂Cl₂): ν 3621, 1728, 1715, 1430, 1213 cm⁻¹; MS (ESI⁺, *m/z*): 316 [(M + Na)⁺, 100%].

4.5. Preparation of (±)-*trans*-1-Benzyloxycarbonyl-3,4-dihydroxypiperidine, [(±)-*trans*-6]. To a solution of (±)-*trans*-4 (1.2 g, 3.7 mmol) in MeOH (35 mL), a solution of a catalytic amount of NaOMe in MeOH (1 mL) was added. The mixture was stirred for 2 h, and then the solvent was evaporated under reduced pressure and the product was purified by flash chromatography on silica gel (hexane/ EtOAc 1:1) to afford the product (±)-*trans*-6 as a yellow oil (0.85 g, 94%); ¹H-NMR (CDCl₃, 400.13 MHz, 40 °C): δ 7.35 (m, 5 H), 5.13 (s, 2 H), 4.25 (dd, 1 H, ³*J*_{HH} 3.23, ³*J*_{HH} 13.10 Hz), 4.11-4.08 (m, 3 H), 3.55-3.47 (m, 2 H), 1.98 (m, 1 H), 1.50 (m, 1 H); ¹³C-NMR (CDCl₃, 100.6 MHz, 40 °C): δ 155.3 (CO), 136.5 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), 71.8 (2 CH), 67.4 (CH₂), 47.8 (CH₂), 42.1 (CH₂), 31.4 (CH₂); IR (CH₂Cl₂): ν 3618, 3447, 1710, 1425, 1204 cm⁻¹; MS (ESI⁺, *m/z*): 252 [(M + H)⁺, 30%], 274 [(M + Na)⁺, 100%].

4.6. Enzymatic acetylation of (±)-*trans*-6. The reaction mixture containing (±)-*trans*-6 (50 mg, 0.15 mmol), vinyl acetate (see table II) and the lipase (50 mg) in the corresponding organic solvent (50 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC using the solvent system hexane/ EtOAc 1:1 until the required conversion was achieved. Then, the enzyme was removed by filtration and washed with the corresponding organic solvent. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 1:1) to afford the monoacetylated product (3*R*,4*R*)-7 and the remaining substrate (3*S*,4*S*)-6.

(3*S*,4*S*)-1-Benzyloxycarbonyl-3,4-dihydroxypiperidine, [(3*S*,4*S*)-6]. Yellow oil [α]_D²⁵ = -3.7 (*c* = 1, CHCl₃), ee >99 %.

(3*R*,4*R*)-4-Acetoxy-1-benzyloxycarbonyl-3-hydroxypiperidine, [(3*R*,4*R*)-7]. Yellow oil [α]_D²⁵ = -12.2 (*c* = 1, CHCl₃), ee >99 %. ¹H-NMR (CDCl₃, 400.13 MHz, 40 °C): δ 7.38 (m, 5 H), 5.15 (2s, 2 H), 4.77 (m, 1 H), 4.13 (dd, 1 H, ³*J*_{HH} 3.16, ³*J*_{HH} 13.52 Hz), 3.97-3.87 (m, 3 H), 3.19-2.92 (m, 1 H), 2.52 (br s, 1H, OH), 2.13 (s, 3 H), 2.12-2.05 (m, 1 H), 1.59 (m, 1 H); ¹³C-NMR (CDCl₃, 100.6 MHz, 40 °C): δ 171.0 (CO), 155.4 (CO), 136.5 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), 74.7 (CH), 68.8 (CH), 67.4 (CH₂), 47.7 (CH₂), 41.4 (CH₂), 28.2 (CH₂), 21.1 (CH₃); IR (CH₂Cl₂): ν 3520, 1717, 1432, 1218 cm⁻¹; MS (ESI⁺, *m/z*): 294.1 [(M + H)⁺, 40%], 316.1 [(M + Na)⁺, 100%].

4.7. Preparation of (±)-*cis*-1-Benzyloxycarbonyl-3,4-dihydroxypiperidine, [(±)-*cis*-8]. To a solution of 2 (1.0 g, 4.6 mmol) in acetone/H₂O (1:1, 8 mL), *N*-methylmorpholine *N*-oxide (0.8 g, 6.8 mmol) was added, followed by a solution of OsO₄ in *tert*-butanol (0.8 mL, 2.5 % w/v), then the mixture was stirred for five days at room temperature. A saturated solution of Na₂S₂O₅ (60 mL) was added and the mixture was extracted with EtOAc (5 × 40 mL); the organic phase was dried over Na₂SO₄, the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 1:2) to afford the product (±)-*cis*-8 as a yellow oil (0.88 g, 76%); ¹H-NMR (CDCl₃, 400.13 MHz, 0 °C): δ 7.35 (m, 5 H), 5.12 (2s, 2 H), 3.84-3.30 (m, 6 H), 1.82 (m, 1 H), 1.65 (m, 1 H); ¹³C-NMR (CDCl₃, 100.6 MHz, 0 °C): δ 155.8 (CO), 136.4 (C), 128.4 (CH), 128.0 (CH), 127.7 (CH), 68.4 (CH), 67.8 (CH), 67.2 (CH₂), 46.1 (CH₂), 40.1 (CH₂), 29.6 (CH₂); IR (CH₂Cl₂): ν 3534, 3427, 1715, 1423, 1210 cm⁻¹; MS (ESI⁺, *m/z*): 252 [(M + H)⁺, 100%].

4.8. Synthesis of (±)-*cis*-3,4-Diacetoxy-1-benzyloxycarbonylpiperidine, [(±)-*cis*-9]. To a solution at 0 °C of (±)-*cis*-8 (0.9 g, 3.6 mmol) in CH₂Cl₂ (8 mL), Et₃N (2.0 mL, 14.3 mmol) and a catalytic amount of DMAP, acetic anhydride (1.4 mL, 14.3 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 8 h. Then the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ EtOAc 2:1) to afford the product (±)-*cis*-9 as a yellow oil (1.1 g, 90%); ¹H-NMR (CDCl₃, 400.13 MHz, 0 °C): δ 7.38 (m, 5 H), 5.22-5.03 (m, 4 H), 4.07-3.95 (m, 2 H), 3.89-3.84 (m, 2 H), 2.08 (s, 3 H), 2.02-1.81 (m, 2 H), 1.95 (s, 3 H); ¹³C-NMR (CDCl₃, 100.6 MHz, 0 °C): δ 170.5 (2 CO), 155.5 (CO), 136.4 (C), 128.7 (CH), 128.3 (CH), 128.0 (CH), 69.5 (2 CH), 67.5 (CH₂), 44.8 (CH₂), 41.2 (CH₂), 26.5 (CH₂), 21.1 (CH₃); IR (CH₂Cl₂): ν 1740, 1716, 1432, 1207 cm⁻¹; MS (ESI⁺, *m/z*): 358 [(M + Na)⁺, 100%].

4.9. Enzymatic hydrolysis and acetylation of (±)-*cis*-8 and (±)-*cis*-9. These reactions were carried out using the same procedures as described as for the *trans* derivatives.

Acknowledgments

This work was supported by the Ministerio de Ciencia y Tecnología (PTR1995-0775-OP). L.F.S. thanks MEC for a predoctoral fellowship. V.G.-F thanks MEC for a personal grant (Juan de la Cierva Program). We express our appreciation to Novo Nordisk Co. for the generous gift of the lipase Novozym 435.

References

- (a) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Chem. Rev.* **2000**, *100*, 4683–4696. (b) Felpin, F.-X.; Bouberkeur, K.; Lebrenton, J. *J. Org. Chem.* **2004**, *69*, 1497–1503 and references cited therein.
- (a) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C.-H. R.; Liu, P. S. *J. Org. Chem.* **1989**, *54*, 2539–2542. (b) Balfour, J. A.; McTavish, D. *Drugs* **1993**, *46*, 1025–1054.
- Ratner, L.; Heyden, N. V.; Dederá, D. *Virology* **1999**, *181*, 180–192 and references cited therein.
- Gross, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W. *Clin. Cancer Res.* **1995**, *1*, 935–944.
- Ichikawa, Y.; Igasashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007–3018.
- Williams, S. J.; Hoos, R.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 2223–2235.
- Cosy, J.; Molina, J. L.; Desmurs, J.-R. *Tetrahedron Lett.* **2001**, *42*, 5713–5715.
- Waldmeier, P. C.; Maitre, L.; Baumann, P. A.; Hauser, K.; Bischoff, S.; Bittiger, H.; Paioni, R. *Eur. J. Pharm.* **1986**, *130*, 1–10.
- Chang, D.; Heringa, M. F.; Witholt, B.; Li, Z. *J. Org. Chem.* **2003**, *68*, 8599–8606.
- (a) Crout, D. H. G.; Gaudet, V. S. B.; Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 808–810. (b) Xie, Z.-F.; Nakamura, I.; Suemune, H.; K. Sakai, *J. Chem. Soc., Chem. Commun.* **1988**, 966–967. (c) Seemayer, R.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1991**, 49–50. (d) Caron, G.; Kazlauskas, J. *J. Org. Chem.* **1991**, *56*, 7251–7256.
- Yu, S.-H.; Chung, S.-K. *Tetrahedron: Asymmetry*, **2004**, 581–584.
- Solares, L. F.; Brieva, R.; Quirós, M.; Llorente, I.; Bayod, M.; Gotor, V. *Tetrahedron: Asymmetry* **2004**, *15*, 341–345.
- Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
- García, J.; Fernández, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Org. Lett.* **2004**, *6*, 3759–3762.
- (a) Uppenberg, J.; Patkar, S. A.; Bergfors, T.; Jones, T. A. *J. Mol. Biol.* **1994**, *235*, 790–792. (b) Uppenberg, J.; Hansen, M. T.; Patkar, S. A.; Jones, T. A. *Structure* **1994**, *2*, 293–298.
- (a) Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838–16851. (b) Hæffner, F.; Norin, T.; Hult, K. *Biophys. J.* **1998**, *74*, 1251–1262. (c) Cuiper, A. D.; Kouwijzer, M. L. C. E.; Grootenhius, P. D. J.; Kellogg, R. M.; Feringa, B. L. *J. Org. Chem.* **1999**, *64*, 9529–9537. (d) Otto, R. T.; Scheib, H.; Bornscheuer, U. T.; Pleiss, J.; Sydlatk, C.; Schmid, R. D. *J. Mol. Cat. B* **2000**, *8*, 201–211. (e) Park, S.; Forró, E.; Grewal, H.; Fülöp, F.; Kazlauskas, R. *Adv. Synth. Catal.* **2003**, *345*, 986–995. (f) Venkatachalam, T. K.; Samuel, P.; Li, G.; Qazi, S.; Mao, C.; Pendergrass, S.; Uckun, F. M. *Bioorg. Med. Chem.* **2004**, *12*, 3371–3381. (g) Levy, L. M.; Lavandera, I.; Gotor, V. *Org. Biomol. Chem.* **2004**, *2*, 2572–2577. (h) Lavandera, I.; Fernández, S.; Magdalena, J.; Ferrero, M.; Kazlauskas, R. J.; Gotor, V. *ChemBioChem* **2005**, *6*, 1381–1390.
- (a) Raza, S.; Fransson, L.; Hult, K. *Prot. Sci.* **2001**, *10*, 329–338. (b) Ottosson, J.; Rotticci-Mulder, J. C.; Rotticci, D.; Hult, K. *Prot. Sci.* **2001**, *10*, 1769–1774. (c) Ottosson, J.; Fransson, L.; Hult, K. *Prot. Sci.* **2002**, *11*, 1462–1471.
- Lutz, S. *Tetrahedron: Asymmetry* **2004**, *15*, 2743–2748.
- Recently, an *S*-selective CAL-B has been designed using a rational redesign approach: Magnusson, A. O.; Takwa, M.; Hamberg, A.; Hult, K. *Angew. Chem. Int. Ed.* **2005**, *44*, 4582–4585.
- Magnusson, A.; Hult, K.; Holmquist, M. *J. Am. Chem. Soc.* **2001**, *123*, 4354–4355.
- Branneby, C.; Carlqvist, P.; Magnusson, A.; Hult, K.; Brinck, T.; Berglund, P. *J. Am. Chem. Soc.* **2003**, *125*, 874–875.
- Carlqvist, P.; Svedendahl, M.; Branneby, C.; Hult, K.; Brinck, T.; Berglund, P. *ChemBioChem* **2005**, *6*, 331–336. Wild-type CAL-B has also achieved this type of transformations: Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 1724–1725; Torre, O.; Gotor-Fernández, V.; Alfonso, I.; García-Alles, L. F.; Gotor, V. *Adv. Synth. Catal.* **2005**, *347*, 1007–1014.
- Carlqvist, P.; Eklund, R.; Hult, K.; Brinck, T. *J. Mol. Mod.* **2003**, *9*, 164–171.
- Magnusson et al. have shown that for wild-type CAL-B there is enough space up to an ethyl moiety into the stereospecificity pocket: Magnusson, A. O.; Rotticci-Mulder, J. C.; Santagostino, A.; Hult, K. *ChemBioChem* **2005**, *6*, 1051–1056.
- Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
- (a) Forró, E.; Kanerva, L. T.; Fülöp, F. *Tetrahedron: Asymmetry* **1998**, *9*, 513–520. (b) Luna, A.; Astorga, C.; Fülöp, F.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 4483–4487. (c) Brunet, C.; Zarevucka, M.; Wimmer, Z.; Legoy, M.-D. *Enzyme Microb. Technol.* **2002**, *31*, 609–614. (d) Levy, L. M.; Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2003**, *14*, 2053–2058. (e) Tanikaga, R.; Matsumoto, Y.; Sakaguchi, M.; Koyama, Y.; Ono, K. *Tetrahedron Lett.* **2003**, *44*, 6781–6783.
- (a) Cornell, W. D.; Cieplak, P.; Bayley, C. I.; Kollmann, P. A. *J. Am. Chem. Soc.* **1993**, *115*, 9620–9631. (b) 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.
- Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Keßeler, M.; Stümer, R.; Zeliński, T. *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824.
- DeLano, W. L. “The PyMOL Molecular Graphics System”; DeLano Scientific LLC, San Carlos, CA, USA; <http://www.pymol.org>.