Kinetic Resolution of (±)-1-Phenylbutan-1-ol by Means of CALB-Catalyzed Aminolyses: A Study on the Role of the Amine in the Alcohol Resolution

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Abstract: The kinetic resolution of (±)-1-phenylbutan-1-ol [(±)-**1**] by means of CALB-catalyzed aminolysis of its acetyl derivative [(±)-**2**] using (±)-1-phenylethanamine [(±)-**3**] as nucleophile is a slower but more enantioselective process (E = 50) than the corresponding CALB-catalyzed transesterification of (±)-**1** with vinyl acetate (E = 19). The use of triethylamine and acetanilide as additives in the transesterification of (±)-**1** enhanced the enantiomeric ratio (E = 43 and 38, respectively), thus showing that both the basic character of the amine as well as its structural nature could be responsible for the enantioselectivity differences observed between the transesterification and aminolysis reactions. We have also carried out the aminolysis of (±)-**2** using different chiral and non-chiral amines. Enantiomeric ratio values varied significantly with the amine employed, but the enzyme always remained more selective towards the R-enantiomer of the substrate. Among all the amines tested, (±)-1-phenylpropan-1-amine [(±)-**5**] was the nucleophile of choice. Analysis of the conversion values for each enantiomer of (±)-**2** showed that the selectivity differences exhibited by the lipase in the aminolysis reactions were due to the different stabilization of the fast-reacting enantiomer of the substrate [(*R*)-**2**] during the catalytic process. The CALB behavior in these reactions could be explained on the basis of substrate imprinting effects, which were corroborated by means of enzyme recycling experiments. Finally, a solvent screening allowed the kinetic resolution of this alcohol for synthetic purposes.

Keywords: alcohols; amines; chiral resolution, enantioselectivity; enzyme catalysis

Introduction

Lipases accept a wide range of racemic substrates which are usually converted with high enantioselectivities. In particular, the lipase-catalyzed kinetic resolution of racemic alcohols has become a powerful technique for the preparation of optically active alcohols and related compounds and it has traditionally been accomplished by means of hydrolysis, esterification, or transesterification reactions.[1] In order to maximize the enantiomeric excesses of both substrate and product, irreversibility of the process has to be achieved.[2] Recently, we took advantage of the lipases' lack of amidase activity[3] to develop a new methodology in which different racemic secondary alcohols were resolved through aminolysis of their corresponding acetates.[4] As racemic amines were used, both the amine and the alcohol were resolved in a single reaction with excellent yields and enantioselectivities. Lipase B from *Candida antarctica* (CALB) was employed as catalyst owing to the excellent catalytic properties that this enzyme has shown in the simple resolution of both types of nucleophiles.[5]

Unfortunately, lipases are not always enantioselective enough for synthetic purposes. To overcome this limitation, modification of reaction conditions (temperature, solvent, acylating agent, etc.),[6] the utilization of additives,[7] and protein engineering techniques[8] have been employed. Surprisingly, when the substrate to be resolved is a nucleophile, the influence that the leaving group of the acyl donor has on the enantioselectivity of the process has almost always been ignored. The same lack of knowledge reigns over the influence of the nucleophile on the enantioselectivity of the resolution of a racemic leaving group. The main reason for this situation could be the fact that, according to the accepted mechanism for lipases,[9] the leaving groups of an acyl donor and the nucleophile are not present at the same time in the transition state responsible for the enantioselection. Nevertheless, some authors have tried to shed

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light on this aspect of lipase catalysis. Thus, Hoff et al.[10] studied the influence of the leaving group on the enantiomeric ratio (E)[11] values obtained in the CALB-catalyzed transesterifications of some secondary alcohols with different butanoates. They concluded that when 2-chloroethyl and 2,2,2-trichloroethyl butanoates were employed, the variation in E values was due to the acylating agent's reactivity but not to the released alcohol. On the other hand, butanoic anhydride and vinyl butanoate made the lipase less specific, probably by acylation of different residues of the enzyme.

CALB-catalyzed kinetic resolutions of secondary alcohols have been extensively studied and rationalized. Thus, the stereochemical outcome of the reaction can be reliably predicted by Kazlauskas' rule.[12] Substrate mapping studies have shown the structural requirements that a secondary alcohol needs to be successfully resolved by CALB: alcohols bearing a medium-sized substituent larger than an ethyl group or a large-sized substituent smaller than a *n*-propyl group would lead to poor enantiomeric ratio values.[13] Moreover, this lipase behaves in essentially the same manner when it catalyzes the transformation of racemic α-substituted primary amines.[14] Therefore, (±)-1-phenylbutan-1-amine [(±)-**6**], an amine bearing an *n*-propyl group as the medium-sized substituent, should be resolved by CALB with a low E value.

We have previously carried out the resolution of (±)-1-phenylbutan-1-amine [(±)-**6**] using ethyl acetate as acyl donor.[15] The E value measured for this reaction (E = 32) turned out to be approximately half the size of the one obtained when (±)-1-phenylethyl acetate was used as acyl donor.[4] Thus, the utilization of different acetyl donors has a clear effect on the resolution of this nucleophile. In order to shed light on the surprising enantioselectivity shown by CALB towards this amine, we have undertaken a study on the enantioselectivity shown by lipase B from *Candida antarctica* in the kinetic resolution of its isosteric secondary alcohol 1-phenylbutan-1-ol [(±)-**1**] by means of aminolysis reactions of its acetyl derivative [(±)-**2**] with different chiral and non-chiral amines. We have focused our attention only on the alcohol resolution process. Thus, when racemic amines were employed, no exhaustive data were obtained concerning their resolution. Our aim was to gain insight into the role played by the amine in the alcohol enantiorecognition process for the purpose of further rationalizing the lipase-catalyzed resolution of racemic nucleophiles. The reason for not using amine (±)-**6** as substrate was the fact that enzymatic reactions of esters and alcohols can be much better monitored than those in which amines and amides are present.

Results and Discussion

Transesterifications of (±)-**1** were carried out using vinyl acetate as acyl donor in the absence or presence of nitrogenated compounds as additives. Aminolyses of (±)-**2** were accomplished with the chiral and non-chiral primary amines **3 – 7**. In all cases, reagents (vinyl acetate, amines, and additives) were employed in a three molar excess relative to the substrate and 1,4-dioxane was used as solvent unless stated otherwise; 4 Å molecular sieves were added to ensure a low water activity in the reaction medium and the temperature was fixed at 30 °C.

Initially, we carried out the CALB-catalyzed transesterification of (±)-**1** (Figure 1). The enzyme reacted faster with the *R*-enantiomer of the substrate but the enantioselectivity measured was low (Table 1, entry 1). Next, we studied the resolution of (±)-1-phenylbutyl acetate [(±)-**2**] through CALB-catalyzed aminolysis using (±)-1-phenylethanamine [(±)-**3**] as nucleophile (Figure 2). Again, (*R*)-**2** was the fast-reacting enantiomer but the degree of conversion (c) achieved (Table 2, entry 1) was lower than that obtained for the acetylation reaction of (±)-**1**. This result was in accordance with the general knowledge that alcohols deacylate the acyl-enzyme intermediate faster than amines.[14] However, it is of note that the aminolysis reaction of (±)-**2** is a much more enantioselec-

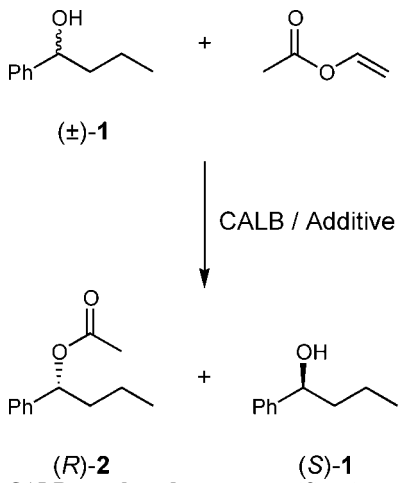

Figure 1. CALB-catalyzed transesterification of (±)-1-phenylbutan-1-ol [(±)-**1**].

Table 1. Effect of different additives on the CALB-catalyzed acetylation of (±)-1.

Entry	Additive	c [%][a]	E
1	None	17.3	19.4 ± 0.4
2	Triethylamine	15.1	42.6 ± 2.0
3	Acetanilide	14.4	38.4 ± 0.7

[a] Reaction time: 163 h.

tive process than the transesterification of (±)-**1** (Figure 1). According to the accepted mechanism by which lipases operate,[9] the enantioselectivity of both reactions should stem from the same transition states, prior or subsequent to the tetrahedral intermediate shown in Figure 3. Moreover, as during this mechanism no ternary complexes are formed, neither amine nor acetaldehyde should be complexed with the active site during the alcohol enantiorecognition steps. Then, how can the aminolysis be a more enantioselective process than the transesterification?

There is no single answer to this question. It has been reported that the liberation of acetaldehyde during the transesterification with vinyl acetate decreases both reactivity and selectivity of lipases. For the case of *Candida rugosa* lipase (CRL), it has been described that this effect is probably due to deactivation of the enzyme because of the formation of Schiff bases with primary amino groups of the enzyme.[16] However, the negative effect of acetaldehyde depends on the lipase used. Thus, the opposite effect has been described[17] for CALB, which has shown to be highly stable when exposed to acetaldehyde formed from vinyl esters.[18] Because the presence of acetaldehyde did not explain the lower enantioselectivity of the transesterification, we decided to focus our attention on the other alternative: the presence of the amine could be responsible for the higher enantioselectivity of the aminolysis. Amines are basic compounds and the beneficial effect of adding non-reactive bases to CALB-catalyzed reactions has already been reported.[19] This effect has been attributed to the removal of traces of carboxylic acids, generated by undesired hydrolysis side reactions of the ester, thereby protecting the enzyme from deactivation. However, the effect of the amine could also be based on other properties apart from its basicity.

With the aim to clarify the role played by the amine in the *E*-enhancement observed herein, we decided to accomplish the transesterification of (±)-**1** (Figure 1) using triethylamine and acetanilide as additives. Triethylamine would represent the basic character of (±)-**3** without participating in the enzymatic reaction and acetanilide, a non-reactive compound with no appreciable basic character, would resemble the (±)-**3** structure. The results obtained (Table 1, entries 2 and 3) showed that both additives had a positive effect over the enantioselectivity of the acetylation reaction. Therefore, the higher enantioselectivity of the aminolysis of (±)-**1** could be a consequence of the two factors: the basic character of the amine and the structure of either the amine or the resulting amide.

In order to study in depth the influence of the structure of the amine, we decided to carry out the aminolysis of (±)-**2** using other chiral and non-chiral amines (Figure 2). Results are summarized in Table 2. Again,

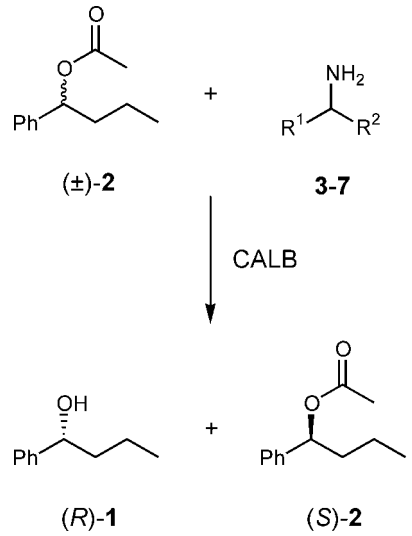

Figure 2. CALB-catalyzed aminolyses of (±)-1-phenylbutyl acetate [(±)-**2**] with amines **3** – **7**.

Table 2. CALB-catalyzed aminolyses of (±)-2.

Entry	Amine	R^1	R^2	*c* [%][a]	*E*
1	(±)-**3**	Ph	Me	6.5	49.7 ± 3.2
2	**4**	Ph	H	5.1	34.7 ± 1.3
3	(±)-**5**	Ph	Et	9.6	68.6 ± 1.3
4	(±)-**6**	Ph	*n*-Pr	5.9	44.8 ± 2.5
5	(±)-**7**	n-C_6H_{13}	Me	4.2	33.7 ± 1.8
6	(±)-**5**[b]	Ph	Et	8.5	53.4 ± 1.1
7	(±)-**5**[c]	Ph	Et	7.6	51.4 ± 1.3

[a] Reaction time: 163 h.
[b] Twofold molar excess with regard to the substrate.
[c] Equimolar amounts of amine and substrate.

the enzyme reacted faster with the *R*-enantiomer of the substrate. In all cases, the reaction rate was lower than for the esterification processes but the *E* values were higher. Both enantiomeric ratio and conversion values varied with the amine used. Even though enantiomeric ratio changes were of greater magnitude than conversion ones, variation of both parameters seemed to be connected. Thereby, the more enantioselective an aminolysis was, the higher degree of con-

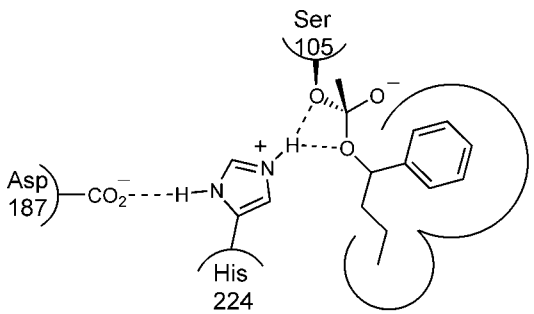

Figure 3. Schematic representation of the tetrahedral intermediate corresponding to the deacylation (transesterification) and acylation (aminolysis) steps of the catalytic sequence.

version it reached. The best result was obtained when (±)-1-phenylpropan-1-amine [(±)-**5**] was used. For the other amines, enantioselectivity and conversions decreased in the order (±)-**3** > (±)-**6** > **4**, (±)-**7**.

The enantiomeric ratio reflects the ratio of reactivity between the fast- and the slow-reacting enantiomers of a racemic mixture. Thus, the differences in the *E* values measured for the aminolyses of (±)-**2** with amines **3** – **7** could be ascribed to different rates of conversion for the slow-reacting enantiomer, the fast-reacting enantiomer, or both of them. As for irreversible processes, the degree of conversion is related to the reaction rate and so, to the activation energy of the reaction, the degree of conversion of each enantiomer has to be related to the energy of the diastereomeric transition states that the enzyme forms with each enantiomer. In Figures 4 and 5 the conversion of each enantiomer of (±)-**2** is plotted against the reaction time. As can be seen, the differences observed for both *E* and *c* values in the aminolyses of (±)-**2** were mainly due to the different stabilization of the fast-reacting enantiomer [(*R*)-**2**].

While this work was in progress, Lee and coworkers reported the resolution of different racemic secondary alcohols by means of CALB-catalyzed transesterifications using enol acetates as acyl donors.[20] They found that better enantioselectivities were achieved when a *substrate matching* approach was utilized (the enol leaving group was isosteric with the secondary alcohol resolved). The authors justified this behavior on the basis that the enzyme would be imprinted by the leaving group during the formation of the acyl-enzyme intermediate. Then, the enzyme would memorize the leaving group structure and this memory would be used for the subsequent recognition of the nucleophile, which would be acetylated with a higher selectivity the more similar the leaving group was to the nucleophile.

When applied to our case, this *substrate matching* approach failed. However, the imprinting principle was still capable of explaining our results. As can be seen from Table 2 (entries 1 – 5), the more the amine resembled the substrate, the higher was the enantioselectivity attained, except when the amine analogous to the substrate [(±)-**6**, entry 4] was employed. In our case, the two enantiomers of the amine could be regarded as two different nucleophiles that compete to deacylate the acyl-enzyme intermediate. Assuming that CALB is more selective towards the *R*-enantiomers of the amines used,[4,14] deacylation of the acyl-enzyme intermediate by the (*R*)-**3** – **7**[21] would modify the CALB active site in such a way that the enzyme would become more selective towards the *R*-enantiomer of **2**. On the contrary, when the *S*-enantiomer of the amines **3** – **7**[21] deacylated the acyl-enzyme intermediate the opposite effect would take place. In addition, both effects would be of greater magnitude the more the amine resembled the alcohol structure. On the other hand, deacylation of the acyl-enzyme intermediate by both enantiomers of the amines would have an almost negligible effect over the deacylation rate when (*S*)-**2** acted as acyl donor as compared to that observed for (*R*)-**2**. Therefore, CALB's selectivity towards (±)-**2** would depend on both the degree of resemblance between the amine and the alcohol and on the amine enantiomeric ratio. Single point estimations of the *E* values for the racemic amines turned out to decrease in the order (±)-**3** and -**5** (*E* > 100) > (±)-**7** (*E* = 70) > (±)-**6** (*E* < 40). Moreover, we can state from a qualitative point of view that the degree of re-

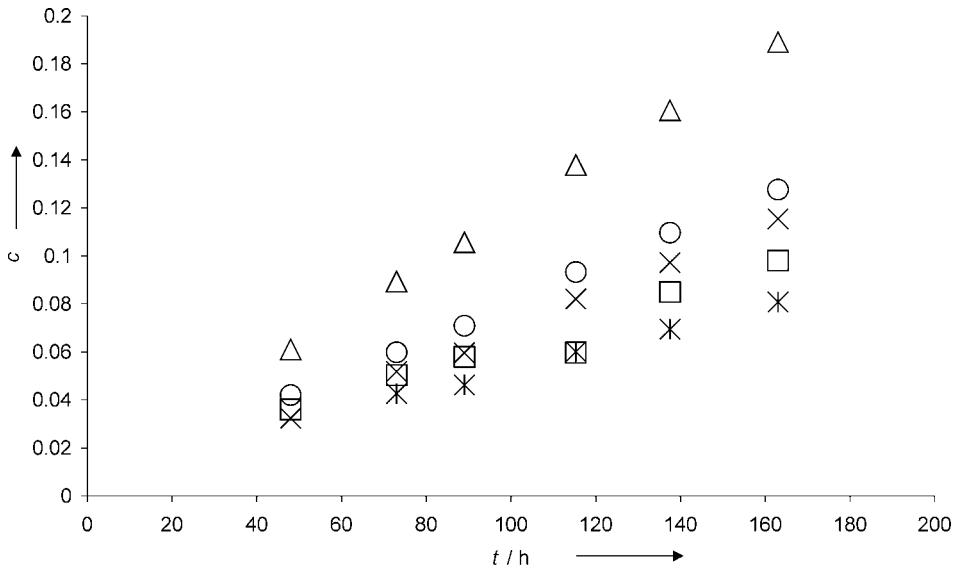

Figure 4. Degree of conversion of (*R*)-**2** versus reaction time in the aminolysis reactions with (±)-1-phenylethan-1-amine (○), benzylamine (□), (±)-1-phenylpropan-1-amine (△), (±)-1-phenylbutan-1-amine (×) and (±)-1-methylheptan-1-amine (✳).

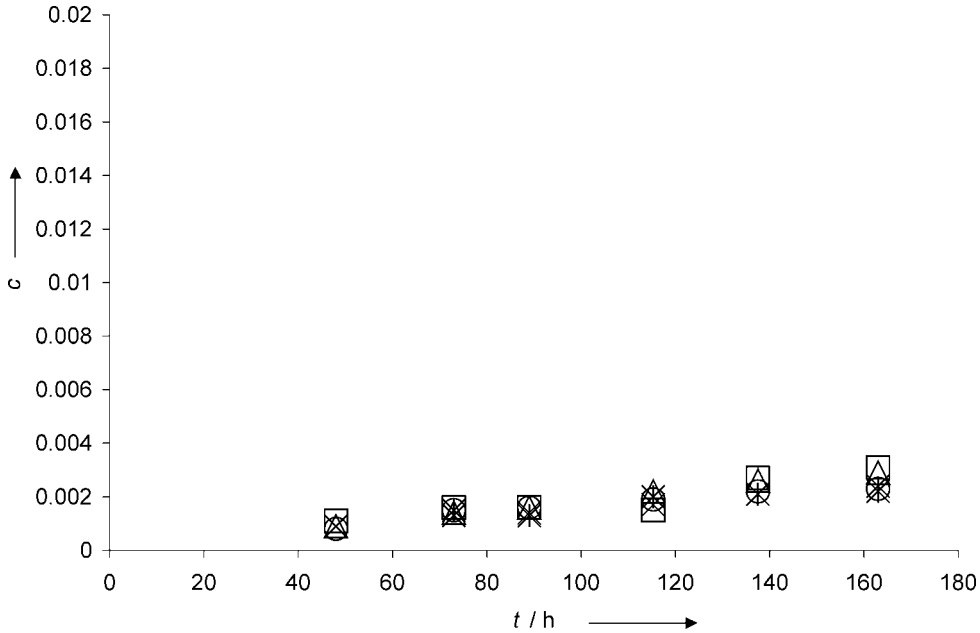

Figure 5. Degree of conversion of (*S*)-**2** versus reaction time in the aminolysis reactions with (±)-1-phenylethan-1-amine (○), benzylamine (□), (±)-1-phenylpropan-1-amine (△), (±)-1-phenylbutan-1-amine (×) and (±)-1-methylheptan-1-amine (✳).

semblance between the amines utilized and substrate (±)-**2** decreases in the order (±)-**6** > (±)-**5** > (±)-**3** > **4**, (±)-**7**. Combination of both parameters explains why the *substrate matching* approach is not the best option for the resolution of (±)-**2**, and, generally speaking, for nucleophiles that are not accepted by the enzyme with a high degree of selectivity.

We have also carried out the aminolysis of (±)-**2** using different concentrations of the amine (±)-**5**. Differences in the nucleophile concentration for a given substrate should not affect the *E* value measured for the alcohol if only the reactive amine were responsible for the enantiomeric ratio changes observed herein. This is due to the fact that, ideally, the enantiomeric ratio for a given substrate does not depend on the concentration of the reagents.[11] Results obtained are summarized in Table 2 (entries 3, 6, and 7). When the molar excess of the amine (±)-**5** with regard to the ester (±)-**2** was decreased from three to two (entries 3 and 6, respectively) the enantiomeric ratio of the reaction decreased as well. Nevertheless, further reduction of the amine concentration did not produce an additional significant worsening of CALB selectivity (entry 7). With regard to the reaction rate, the reduction of the amine concentration also led to a decrease in the degree of conversion. Although the *E* variation was not linearly related to the change in the nucleophile concentration, the results obtained indicated that also non-reactive enzyme-amine complexes could influence the enantiomeric ratio of (±)-**2** aminolysis. Moreover, according to the *c* values measured for each reaction, the produced amide concentration hardly changed with the amine concentration. This is why enzyme-amide complexes were not considered to be responsible for this enantioselectivity fluctuation.

Molecular memory is the phenomenon whereby functional properties of a substance depend on the sample's history.[22] Enzymes in organic solvents are rigid entities due to the absence of the molecular lubricant water. This conformational rigidity is responsible for the molecular memory of enzymes. Thereby, imprinted enzymes should retain their acquired new properties as long as they are not suspended in water or re-imprinted. In order to verify that in our case imprinting phenomena were taking place we decided to accomplish two different experiments. Both of them were acetylation reactions of (±)-**1** using vinyl acetate as acyl donor (Figure 1). The difference between them laid in the different history of the CALB used. Results obtained are summarized in Table 3. For the experiment corresponding to entry 1, the CALB used was the one which had catalyzed the aminolysis of (±)-**2** using amine (±)-**5** as nucleophile. For the other experiment (Table 3, entry 2), the enzyme used was incubated under the same experimental conditions described for the aforementioned aminolysis but without substrate (±)-**2** so that no reaction had taken place. In both cases, the lipase became less active than when the commercial enzyme was directly utilized (Table 1, entry 1) but more enantioselective, thus showing the presence of imprinting effects. Despite the fact that the *E* value measured when recycled CALB was employed was far from that ob-

Table 3. Effect of the history of the CALB on the lipase-catalyzed acetylation of (±)-1.

Entry	c [%][a]	E
1[b]	10.4	38.0 ± 0.2
2[c]	8.2	32.9 ± 0.3

[a] Reaction time: 163 h.
[b] CALB recycled from the aminolysis of (±)-**2** with (±)-**5**.
[c] CALB incubated during the same reaction time and under the same experimental conditions as those described for the aminolysis reactions but without substrate (±)-**2**.

tained for the aminolysis reaction (Table 2, entry 3), it was still approximately twice as much as the one obtained for the acetylation of (±)-**1** (Table 1, entry 1). This partial decrease in CALB enantioselectivity can be ascribed to partial mobility of the enzyme in the organic solvent. However, the thorough solvent washing process that the enzyme underwent when recycled (see experimental section) could make CALB less enantioselective as well. Nevertheless, further experimental work is needed to verify both hypotheses. Comparison of the CALB selectivity for the two preparations used (Table 3) showed that the enzyme recycled from the aminolysis reaction was slightly more enantioselective than the one incubated without substrate. Thereby, both reactive and non-reactive amine-enzyme complexes can imprint the enzyme even though the reactive amine turned out to be a somewhat more effective agent than the non-reactive one.

Once the influence of the amine on the resolution of (±)-1-phenylbutan-1-ol had been established, we focused our attention on the improvement of the reaction rate so that the aminolysis of (±)-**2** with amine (±)-**5** could have a synthetic applicability. So, we decided to carry out a solvent screening. At present, it is widely recognized that the solvent greatly influences both enzymatic activity and selectivity.[6b] In our research group, activity studies over the CALB-catalyzed transesterification of ethyl acetate with *n*-butanol showed that when acyclic ethers were employed as solvents, faster reactions were attained.[23]

Table 4. Solvent effect on CALB-catalyzed aminolysis of (±)-2 with (±)-5 and acetylation of (±)-1.

Entry	Solvent	Reaction	c [%][a]	E
1	1,4-Dioxane	Aminolysis	9.6	68.6 ± 1.3
2	CH_2Cl_2	Aminolysis	<1.0	n.d.
3	^{t}BuOMe	Aminolysis	42.0	130.6 ± 0.9
4	*n*-Hexane	Aminolysis	27.9	61.2 ± 1.1
5	Toluene	Aminolysis	30.2	65.8 ± 0.6
6	1,4-Dioxane	Acetylation	17.3	19.4 ± 0.4
7	^{t}BuOMe	Acetylation	43.5	55.4 ± 0.7
8	*n*-Hexane	Acetylation	41.5	25.4 ± 0.3
9	Toluene	Acetylation	51.4	75.9 ± 3.0

[a] Reaction time: 163 h.

Among all the solvents tested, *tert*-butyl methyl ether (^{t}BuOMe) was the one in which the lipase was more active. Moreover, in a recent report dealing with slow CALB-catalyzed transesterifications of different secondary alcohols, more hydrophobic solvents such as toluene, benzene and *n*-hexane proved to be a good election.[24]

On the basis of these antecedents we decided to use ^{t}BuOMe, *n*-hexane, and toluene as reaction solvents. In addition, we also included dichloromethane in order to check the influence that halogenated solvents could have on the reaction rate. Results obtained are summarized in Table 4. Except for dichloromethane in the other solvents tested, the aminolysis reaction of (±)-**2** (Table 4, entries 3 – 5) proceeded faster than in 1,4-dioxane (entry 1). The enantioselectivity also changed with the solvent used. Thus, in *n*-hexane (entry 4) and toluene (entry 5) the reactions were slightly less enantioselective than in 1,4-dioxane. Nevertheless, the solvent of choice turned out to be ^{t}BuOMe (entry 3). Apart from being the solvent in which the aminolysis reaction was fastest, the E value was almost twice as much as the one obtained in 1,4-dioxane.

For the purpose of checking that CALB remained more enantioselective in the aminolysis of (±)-**2** using amine (±)-**5** as nucleophile than in the acetylation of (±)-**1** regardless of the solvent utilized, we also accomplished the acetylation of (±)-**1** in ^{t}BuOMe, *n*-hexane and toluene (Table 4, entries 7 – 9). As can be seen from Table 4, the reactions proceeded faster than in 1,4-dioxane (entry 6) and than the corresponding aminolysis processes (entries 3 – 5). Only for the case of ^{t}BuOMe (entry 7) this reaction rate improvement was negligible. Moreover, the E values for the acetylation reactions were lower than those measured for the aminolysis ones. The only exception was when toluene was used as solvent. In this case, the E value obtained for the aminolysis (entry 5) was somewhat lower than that measured for the acetylation reaction (entry 9), thus altering the general trend observed in these processes. Again, imprinting effects afforded an explanation for this behavior. It has been reported that the enantioselectivity exhibited by lipase from *Pseudomonas cepacia* (PCL) in the resolution of (±)-sulcatol was greatly influenced by the solvent structure.[25] They attributed this influence to solvent molecules bound to PCL alcohol binding site during the enantiorecognition process of both enantiomers of sulcatol, thus affecting the reaction rate of each enantiomer in a different way. However, CALB has, as compared to other lipases, a very limited amount of available space in the active site pocket.[26] Accordingly, it is not likely that solvent molecules could be present in the alcohol binding site during either the enantiodiscrimination of (±)-**1** or the deacylation of the acyl-enzyme by the amine (±)-**5**.

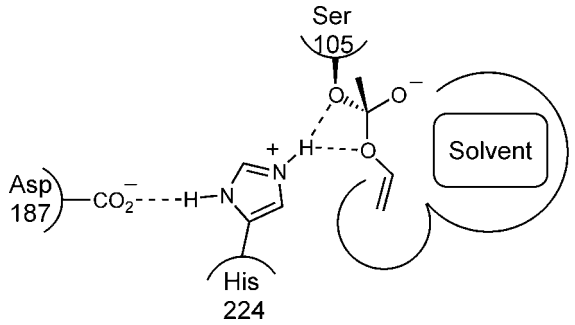

Figure 6. Schematic representation of the tetrahedral intermediate corresponding to the acylation step of the catalytic serine by vinyl acetate in the presence of a solvent molecule.

However, during the acetylation of the catalytic serine by vinyl acetate, solvent molecules could accommodate in the alcohol binding site due to the small size of the leaving group. In Figure 6 a schematic representation of such a binding mode is shown. As can be deduced from this figure, this vinyl acetate-solvent complex could imprint the alcohol binding site the same way amines employed did. And again, the efficacy will depend on the solvent's degree of resemblance with the phenyl ring of the substrate. It is clear that when the solvent is toluene, the structure of the complex vinyl alcohol-toluene greatly resembles the structure of 1-phenylbutan-1-ol. Thus, in this case, the imprinting caused by the solvent in combination with the acylating agent could be more effective than the one exerted by amine (±)-**5**. This situation would result in a higher *E* value for the acetylation than for the aminolysis process. Moreover, this sort of complexes could also explain the result obtained when substrate (±)-**1** was acetylated in the presence of acetanilide (Table 1, entry 3) by simply considering the competitive binding between the solvent and acetanilide to the alcohol binding site in the way shown in Figure 6.

Conclusion

In summary, we have shown that the CALB-catalyzed resolution of (±)-1-phenylbutan-1-ol is much more efficiently achieved by means of the aminolysis of its acetyl derivative than through its acetylation with vinyl acetate. The *E* values measured for the transesterification of (±)-**1** with different additives showed that both the basic character of the amine as well as its structural nature are responsible for this *E*-enhancement. Moreover, this is the first time, to the best of our knowledge, in which an additive such as an amide has been found to increase lipase enantioselectivity. The enantioselectivity of the aminolysis reaction depends on the amine employed and is maximized when the amine utilized greatly resembles the alcohol structure and is accepted by the lipase with a high degree of selectivity. Results obtained were ascribed to imprinting effects and were corroborated by enzyme recycling experiments. The proper selection of the reaction medium has allowed the resolution of this alcohol for synthetic purposes. The methodology described herein widens the range of substrates that can be successfully resolved by CALB as well as further rationalizes the optimization of lipase-catalyzed kinetic resolutions of racemic nucleophiles.

Experimental Section

General

Lipase B from *Candida antarctica*, Novozym 435, was a gift from Novo Nordisk co. and was employed without any previous treatment except when recycled. In this case, it was thoroughly washed with dichloromethane and diethyl ether and remaining organic solvents were removed in vacuum before its use. All reagents were purchased from Aldrich Chemie. (±)-1-Phenylbutan-1-ol [(±)-**1**] and (±)-1-phenylbutan-1-amine [(±)-**6**] were obtained by reduction ($NaBH_4$, MeOH) and reductive amination [$AcONH_4$, $Na(CN)(BH_3)$, MeOH] of the commercially available butyrophenone, respectively. (±)-1-Phenylbutyl acetate [(±)-**2**] was obtained by conventional acetylation (Ac_2O, Py, *N*,*N*-DMAP, CH_2Cl_2) of (±)-**1**. Solvents used in the enzymatic reactions were distilled over an adequate desiccant and stored under nitrogen. Flash chromatography was performed with Merck silica gel 60 (230 – 240). Optical rotations were measured by means of a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 1720-X FT IR spectrometer. Mass spectra were recorded on a VG Autospec. ^1H and ^{13}C NMR spectra were obtained with a Bruker DPX 300 (^1H, 300 MHz and ^{13}C 75.5 MHz). GC analyses were performed in a Hewlett-Packard 5890 Series II chromatograph equipped with an FID detector using the capillary columns RtβDEXse (30 m × 0.25 mm; Restek) and CHIRALDEX-BPH (30 m × 0.25 mm; Astec) as chiral stationary phases and nitrogen as carrier gas (110 kPa).

Typical Procedure for the Enzymatic Reactions

To a solution of the substrate [(±)-**1**, 150 mg, 1.0 mmol or (±)-**2**, 192 mg, 1.0 mmol] in the solvent used (10 mL), vinyl acetate (0.28 mL, 3.0 mmol) or the corresponding amine [(±)-**3** – **7**, 3.0 mmol], 4 Å molecular sieves (250 mg), the additive where appropriate (triethylamine, 0.21 mL, 3.0 mmol; acetanilide, 203 mg, 3.0 mmol) and CALB (200 mg) were added. The resulting mixture was shaken at 30 °C and 250 rpm. during the reaction time (163 h). Six aliquots (10 μL) were withdrawn at regular intervals and analyzed through chiral GC. For reactions carried out in ^{t}BuOMe the enzyme and molecular sieves were filtered and washed with dichloromethane and the organic solvents were evaporated. Flash chromatography of the residue (eluent: hexane/ethyl acetate, 4:1) yielded substrate and product of the reaction. For each enzymatic reaction a control experiment without enzyme was carried and no non-enzymatic reaction was detected through GC analysis.

(*R*)-1-Phenylbutyl Acetate [(*R*)-2]

Isolated from the reaction described in Table 4, entry 7. Colorless liquid, 93% ee; (44% *c*, 80 mg, 95 % yield). R_f = 0.56 (hexane/ethyl acetate, 3:1); GC conditions: RtβDEXse, 90 °C, 45 min hold, 90 – 120 °C, 2 °C/min, 15 min hold, $t_R(S)$ = 55.9 min and $t_R(R)$ = 56.6 min;$[\alpha]_D^{22}$: +78.2 (*c* 0.9 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ = 0.92 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3), 1.32 (m, 2H, CH_2), 1.57 (m, 1H, CH_2), 1.91 (m, 1H, CH_2), 2,08 (s, 3H, CH_3), 5.75 (dd, $^3J_{H,H}$ = 7.7 Hz, $^3J_{H,H}$ = 6.3 Hz, 1H, CH), 7.33 (m, 5H, C_6H_5); ^{13}C NMR (75.5 MHz, $CDCl_3$, rt, TMS): δ = 13.7 (CH_3), 18.7 (CH_2), 21.2 (CH_3), 38.3 (CH_2), 75.8 (CH), 126.4 (CH, C_6H_5), 127.7 (CH, C_6H_5), 128.3 (CH, C_6H_5), 140.7 (C, C_6H_5), 170.3 (C=O); IR (neat): $\tilde{\nu}$ = 1736 cm^{-1} (C=O); MS (70 eV, EI): *m/z* (%) = 192 (29) $[M]^+$, 150 (80), 117 (83), 107 (100).

(*R*)-1-Phenylbutan-1-ol [(*R*)-1]

Isolated from the reaction collected in Table 4, entry 3. Colorless liquid, 97% ee, (42% *c*, 57 mg, 90% yield). R_f = 0.36 (hexane/ethyl acetate, 3:1); GC conditions: RtβDEXse, 90 °C, 45 min hold, 90 – 120 °C, 2 °C/min, 15 min hold, $t_R(S)$ = 63.8 min and $t_R(R)$ = 65.3 min; $[\alpha]_D^{22}$: +49.1 (*c* 0.9 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ = 0.94 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3), 1.37 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 2.14 (brs, 1H, OH), 4.67 (dd, $^3J_{H,H}$ = 7.6 Hz, $^3J_{H,H}$ = 5.9 Hz, 1H, CH),7.34 (m, 5H, C_6H_5); ^{13}C NMR (75.5 MHz, $CDCl_3$, rt, TMS): δ = 13.9 (CH_3), 18.9 (CH_2), 41.1 (CH_2), 74.3 (CH), 125.8 (CH, C_6H_5), 127.3 (CH, C_6H_5), 128.3 (CH, C_6H_5), 144.8 (C, C_6H_5); IR (neat): $\tilde{\nu}$ = 3356 cm^{-1} (O-H); MS (70 eV, EI): *m/z* (%) = 150 (13) $[M]^+$, 117 (8), 107 (100).

Determination of the Enantiomeric Ratio and Conversion Values

The enantiomeric ratio values for the resolutions of either (±)-**1** or (±)-**2** were calculated from the experimental values of ee_s and ee_p measured for each aliquot according to Rakels et al.[27] *E* values for the resolution of amines **3** and **5** – **7** were calculated from single point measurements of *c* and ee_p values according to Chen et al.[11] The degree of conversion was determined through the experimental values of ee_s and ee_p according to Sih et al.[28] Conversion values for each enantiomer were determined by means of the degree of conversion and the ratio of the areas of the peaks corresponding to each enantiomer of the reaction product measured for each aliquot.

Determination of the Absolute Configuration

The absolute configuration of ester **2** was assigned by comparison of the sign of the optical rotation measured for this compound with that published for its *R* enantiomer.[29] Thereby, for each reaction, the absolute configuration of the alcohol **1** was assigned to be opposite to that obtained for ester **2**.

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