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Two Approaches For *CAL-B*-Catalyzed Enantioselective Deacylation of a Set of α - Phenyl Ethyl Esters: Organic Solvent With Sodium Carbonate And Micro-Aqueous Medium.

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Abstract:

Herein, we report an efficient enantioselective cleavage of the acyl- moiety of a set of α- phenyl ethyl esters with different chain-lengths catalyzed by lipase B from *Candida antarctica (CAL-B)* by comparing two reactional approaches: anhydrous media with sodium carbonates and micro-aqueous medium. The deacylation is performed in organic solvent, in the presence of Na_2CO_3 in the first case, and by addition of a drop of phosphate buffer solution pH=7 in the second. The results show the high efficiency of the deacylation in the presence of the sodium carbonate for the enzymatic resolution of all the esters and that in term of reactivity (31% ≤ conv ≤ 50%) and selectivity (E >200). While, during the hydrolysis in micro-aqueous media, the conversion is strongly affected by the length of the acyl-chain side, the conversion decreases from conv =50% with the 1-phenylethyl acetate **1a** to conv =19% with 1-phenyethyl dodecanoate **6a**, and this, even if the selectivity remains high (E>89). In both conditions, the lipase *CAL-B* shows a high enantioselectivities in favor of (*R*)-1-phenyl ethanol enantiomer

(conv >45%, E>200) but the reactivity is modulated by the form and the size of the acyl-chain side.

Introduction:

An impressive range of industrial scale biocatalytic applications keeps developing [1-2]. These tools are highly efficient and commercially available with high selectivities and clean reactions [3]. Enzymatic hydrolysis resolution is one of the most important, fundamental, and practical reactions to have access to optically enriched compounds which can be transformed with various functionalities such as pharmaceuticals, agrochemicals, and natural products [4-9]. Serine hydrolases (EC.3.1.1.3) are considered as promising catalysts due to their advantages, such as mild and environmentally benign reaction conditions not requiring cofactors and especially remarkable *chemo-*, *regio-*, *stereo*selectivities [10-14]. Immobilized lipases are excellent biocatalysts for the enzymatic synthesis of short- and medium-chain fatty esters used as food flavor compounds. The properties of flavor esters make possible a great variety of applications in the food sector in many beverages, candies, jellies, jams, wines and dairy and also in the cosmetic industry, as fragrances in perfumes, deodorants, creams and soaps and flavors in lip cosmetics [15]. Among the employed lipases, *Candida antarctica* fraction B, under an immobilized form, is one of the most popular, it is a very robust biocatalyst in various non-conventional media, with high catalytic efficiency in kinetic resolutions. The exclusive properties of immobilized *CAL-B* such as facile recovery, thermostability, environmental harmlessness, are making it attractive for wide applications in pharmaceuticals, food technology, organic synthesis, paper industry and laundry [4, 7, 9, 16-18].

In our previous work, *Candida antarctica lipase* (*CAL-B*) was successfully used for resolving a set of acetates *via* alkaline enzymatic hydrolysis in the presence of the carbonate salts [19]. The main objective of this process was to circumvent some of disadvantages of the conventional methods for enzymatic hydrolysis, such as the perturbation on the lipase's enantioselectivity due to the *co*-solvent nature as well as the difficulty of controlling the pH value of the aqueous solution during the hydrolysis procedure. The application of these alkaline hydrolysis by means

of *CAL-B* and sodium carbonate in organic media gave an excellent resolutions which have been successfully applied to the deracemization process [20]. Moreover, this procedure has been successfully applied to deacylate numerous substrates with a high stereoselectivity such the *chemo*selective deacylation of *N,O*-protected amino alcohols [21], the enantio- and the ***diastereo***selective deacylation of *cis/trans* -2-aryl-1-cyclohexanol derivatives [22] (Scheme 1). In all these previous works, the leaving group was acyl moiety (*O-Acetyl*).

Scheme 1: *CAL-B* catalyzed deacylation of aryl-ethyl acetates in the presence of Na_2CO_3.

In the continuity of our work, the main objective focused the effect of the carbon chain length of the leaving group, on both reactivity and selectivity, in the *CAL-B*-catalyzed deacylation of a set of α-phenyl ethyl esters in organic medium, without external water, in the presence of the carbonate sodium. The major advantage of this approach is to limit the constraints of the aqueous medium including slow solubility of the substrate and the liquid-liquid extraction step. So, the study has been extended to examine the hydrolysis reaction in the presence of large amount of external water, catalyzed by *CAL-B* and without carbonate salts. The idea was to compare between two environments of hydrolysis with *CAL-B* lipase, one with large amount of external water and the other in micro- aqueous media (Scheme 2, Path A and B).

Scheme 2: Investigated reactions.

2. Experimental section:

2.1. Chemicals and Materials

All reagents and solvents were of analytical grade and were purchased from Sigma-Aldrich. The *Candida antarctica lipase* fraction B immobilized on acrylic resin, recombinant, expressed in *Aspergillus niger* purchased from Sigma-Aldrich with specific activity up to 5000U/g, was used without any pre-treatment. The monitoring of the reactions was conducted using TLC on Silica gel 60F$_{254}$ plates type *MERCK* 5179, 250 *mesh*. The separation of the resulting alcohols and the remaining acetates was performed by column chromatography using Silica gel 60Å, 70-230 mesh 63-200µm.

2.2. Instrumentations:

The spectroscopic characterisation was performed with Brüker spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemical shifts were reported in δ ppm from tetramethylsilane with the solvent resonance as internal standard for ^{1}H NMR and chloroform-d (delta 77.0) for ^{13}C NMR. The enantiomeric excesses of alcohol and esters were measured by a chiral stationary phase HPLC: Chiralcel-ODH column (4.6×250mm) column, by a chiral stationary phase GC: column Astec® CHIRALDEX®B-PM. Retention times are reported in minutes.

2.3. General procedure for the chemical preparation of 1a and 4a:

The racemic esters (1a & 4a) were obtained by standard classical chemical acetylation of 1-phenylethanol, according to the following procedure: to 1 equivalent of racemic 1-phenylethanol, 1.2 equivalent of triethylamine and 0.1 equivalent of 4-dimethylaminopyridine (DMAP) dissolved in 4 mL of ether, 1.5 equivalent of acetic anhydride or *iso*-butyric anhydride were added slowly. The evolution of the reactions was monitored by TLC. The esters are obtained pure after standard work up, in good yields. All spectroscopic analysis were detailed in the supplementary data.

2.4. General procedure for the chemical preparation of [2a-3a-7a]:

To 1mmol of 1-phenyl ethanol dissolved in 10 mL of chloroform, 2eq. of the appropriate carboxylic acid was added followed by the addition of 1.1mmol *N, N'*-dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at room temperature for 5 hours. The solvent was removed in *vacuo*, and the crude reaction mixture was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 8:2) to afford the corresponding esters. All spectroscopic analysis were detailed in the supplementary data.

2.5. General procedure for the chemical preparation of [5a-6a]:

To 1mmol of 1-phenyl ethanol dissolved in 5 mL of diethylether, 1.2 eq. of the appropriate chloride acid was added followed by the addition of 0.1mmol 4-dimethylaminopyridine (DMAP). The suspension was stirred at room temperature. The reaction was monitored by TLC, after total consumption of the alcohol, the solvent was removed in *vacuo*, and the crude reaction mixture was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 8:2) to afford the corresponding esters. All spectroscopic analysis were detailed in the supplementary data.

2.6. General procedure for the deacylation of racemic esters [1a-7a] in the presence of Na_2CO_3:

To 1 mmol of the racemic acetate dissolved in of organic solvent which was used directly from the bottle P.A quality (for analysis), without distillation, 1 mmol of sodium carbonate, previously dried over night, and an appropriate amount of the lipase, are added. The suspension was stirred at 40°C for the indicated time. The reaction mixture is filtered and concentrated *in vacuo*. The remaining acetate and the obtained alcohol were separated by

chromatography on silica gel (petroleum ether / ethyl acetate: 80/20) and analyzed by chiral GC.

2.7. General procedure for the conventional hydrolysis of racemic esters [1a-7a] in biphasic media:

1 mmol of racemic esters **[1a-7a]** were dissolved in 1 mL of organic solvent and added to the appropriate volume of phosphate buffer pH=7. An appropriate amount of *CAL-B* (UA=5000U/g) was then added. The suspension was stirred at room temperature for two days. The reaction mixture was filtered on celite and concentrated in *vacuo*. The furnished ester and the remaining alcohol were separated by flash chromatography on silica gel (petroleum ether/ethyl acetate: 80/20) and analyzed by chiral HPLC or GC.

2.8. Chiral GC analysis and/or Chiral HPLC analysis :

Retention times are reported in minutes. The conditions for the analysis of (*R*) alcohol **1** and acetates (*S*)-**1a-7a** are reported below:

2.8.1. (*R*,*S*)-1-Phenylethanol (1): HPLC (Chiralcel IB , hexane/iPrOH: 95/5, flow 0.5 ml/min) : t_R= 14.22 min, t_S= 15.71 min.

2.8.2. (*R*,*S*)-1-phenylethyl acetate (1a): $C_{10}H_{12}O_2$. Crude oil. Yield = 78%. R_f = 0.65. Eluent (*v*,*v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel OD-H, hexane/iPrOH: 95/5, flow 0.5 ml/min):t_R= 9.25min, t_S= 9.70 min.

2.8.3. (*R,S*)-1-phenylethyl benzoate (2a): $C_{15}H_{14}O_2$. Crude oil. Yield = 75%. R_f = 0.73. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel OD-H, hexane/iPrOH: 95/5, flow 0.5 ml/min) : t_1= 9.37 min, t_2= 9.99 min.

2.8.4. (*R,S*)-1-phenylethyl butyrate (3a): $C_{11}H_{14}O_2$. Crude oil. Yield = 82%. R_f = 0.75. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel ADH, hexane/iPrOH: 95/5, flow 1 ml/min) : t_R= 3.81 min, t_S= 4.23 min.

2.8.5. (*R,S*)-1-phenylethyl isobutyrate (4a) : $C_{12}H_{16}O_2$. Crude oil. Yield = 80%. R_f = 0.80. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel ADH, hexane/iPrOH: 95/5, flow 1 ml/min) : t_R= 3.67 min, t_S= 4.02 min.

2.8.6. (*R,S*)-1-phenylethyl decanoate (5a): $C_{18}H_{28}O_2$. Crude oil. Yield = 74%. R_f = 0.84. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel ADH, hexane/iPrOH: 95/5, flow 1 ml/min) : t_R= 3.49 min, t_S= 3.87 min.

2.8.7. (*R,S*)-1-phenylethyl dodecanoate (6a): $C_{20}H_{32}O_2$. Crude oil. Yield = 82%. R_f = 0.85. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel ADH, hexane/iPrOH: 95/5, flow 1 ml/min): t_R= 3.39 min, t_S= 3.70 min.

2.8.8. (*R,S*)-1-phenylethyl benzoate (7a): $C_{15}H_{14}O_2$. Crude oil. Yield = 75%. R_f = 0.73. Eluent (*v,v*): hexane/ethyl acetate (80/20). HPLC (Chiralcel OD-H, hexane/iPrOH: 95/5, flow 0.5 ml/min) : t_1= 9.37 min, t_2= 9.99 min.

3. Results and discussion:

We have selected the *rac*-α-phenyl ethyl esters [**1a-7a**] with different chain (lengths and congestion) to examine enzymatic hydrolysis resolution of esters (scheme 3).The low molecular weight esters represent an important class of fragrances, consisting of compounds derived from short chain acids, such as acetates, propionates and butyrates, which are often responsible for fruity aroma [23]. Moreover, the odour intensity of esters decreases with the increase of molecular weight; however, the flavour industry needs esters with strong odours and high molecular weight to prolong the duration of the odour of flavourings [24]. Our work could be offer an interesting approach in this area. The enzymatic deacylation of the esters [**1a-7a**] experiments were performed in the presence of *Candida antarctica* lipase fraction B immobilized on acrylic resin (*CAL-B*) at different proportion. For the reaction of *CAL-B*-catalyzed kinetic resolution of α- phenyl alkyl esters in the presence of Na_2CO_3 in non-aqueous media, we have applied the operating protocol previously described [19]. Regarding the *CAL-B*-catalyzed kinetic resolution of *rac*-α-phenyl ethyl esters, the conventional hydrolysis in buffered medium has been studied, from biphasic medium to micro-aqueous medium, under various experimental conditions on 1-phenylethyl acetate (**1a**) as study model.

Scheme 3. *Rac*-α-phenyl ethyl esters [**1a-7a**]

3.1. CAL-B Catalyzed kinetic resolution of α- phenyl ethyl esters in the presence of Na_2CO_3 in non-aqueous media:

We investigated the hydrolysis of phenyl ethyl esters [**1a-7a**] to examine the influence of the acyl part in the hydrolysis in the presence of Na_2CO_3. The enzymatic deacylation reactions were carried out on equimolecular mixture of the racemic esters [**1a-7a**] and sodium carbonate in 2 mL of toluene to which is added 40 mg of *CAL-B*. The mixture is stirred three days at 40°C

without addition of any external amount of water (scheme 2) [19]. Both enantiomers of the remained esters and furnished alcohols were recovered after filtration of the lipase and evaporation in *vaccuo*. Their enantiomeric excesses were evaluated by chiral chromatography (GC or HPLC), and the results are summarized in **table 1.**

Table 1: *CAL-B* Catalyzed kinetic resolution of *rac*-α- phenyl ethyl esters in non-aqueous media.

The result from **table 1** shows that the activity and selectivity of lipase in this basic hydrolysis of racemic esters are related to the effect of the migrating group structure. The nature of acyl moiety is of great importance. We observe that the acyl chain length did not affect the selectivity of the lipase *CAL-B* which remains optimal (E>>200). The *CAL-B* exhibits *R*-enantiopreference [26-28], according to the Kazlauskas rules, where the *R*-enantiomer reacts faster than the *S*-enantiomer during the enzymatic kinetic resolution of secondary alcohols [29].

High enantioselectivities were observed with aliphatic acyl chain side (*ee*>99%), but reactivities varies between good to high depending of their bulkiness (31≤ conv ≤ 50). The deacylation of 1-phenylethyl butyrate **3a** was achieved with conv =31% compared to the 1-phenylethyl propionate **2a** and the 1-phenylethyl acetate **1a** (entry 4 *vs* entries 3-2). Good results were obtained for the deacylation of fatty esters **5a-6a** with conversion varied 44.7< conv <48.2 and excellent selectivity E >200 (entries 5-6). Whilst, using a benzoate as acyl side **7a**, the conversion and the selectivity drops sharply in the three tested solvents, the best, conv =9% and E=12 was noted in TBME (Entry 8 vs Entries 7, 9). This result suggests that the deleterious impact on the conformational changes on lipase due to a aryl group impact more strongly than the effect of chain portion with isopropyl group **4a** [30].

The obtained results are in according of previous results of enzymatic hydrolysis in biphasic medium, which is explained by the shape of the active site of the lipase *CAL-B*. It is more adapted to accept linear substituent, such as decanoate or laurate, which can more easily penetrate into it [31].

In the previous investigation, the basic non-conventional deacylation catalyzed by *CAL-B* on the aryl ethyl acetates, had not shown significant effect of the aryl moiety, on both the reactivity and the selectivity, while here the structure of the acyl side moiety have a significant effect on catalytic performance of the lipase *CAL-B*.

The aryl substituent as a leaving group shows a drastic impact on the reactivity and the selectivity of the *CAL-B* during the deacylation with sodium carbonate. It is not the case with the aliphatic substituent's, which seem the best candidates for this pathway. This easy non-conventional methodology, assisted by sodium carbonates has been applied without any external water addition shows ideal for the cleavage of the aliphatic chain acyl moieties, especially for the deacylation of fatty esters with high enantioselectivities.

3.2. CAL-B Catalyzed kinetic resolution of rac-α- phenyl ethyl esters: from biphasic to micro-aqueous media

The objective of this study was to examine the enzymatic hydrolysis operated in micro-aqueous media. Traditional enzymatic hydrolysis of racemic esters was mostly operated within a large amount of external water (phosphate buffer solution pH 7). To find the optimum conditions, the catalytic amount of the biocatalysts, was first examined. Then, the enzymatic hydrolysis have realized under different conditions of aqueous medium, from large amount of external water until micro-aqueous media. The reactivity and selectivity of the lipase *CAL-B* as catalyst was studied on deacylation of 1-phenylethyl acetate **1a** as study model. A series of experiments of enzymatic deacylation reactions were carried out on 1 mmole of *rac*-phenylethyl acetate (**1a**)

diluted in 2mL of organic *co*-solvent in the presence several proportion of phosphate buffer solution (pH=7) with the appropriate amount of lipase. The reaction mixtures were stirred for 48 hours at room temperature. Both enantiomers of the remained esters and furnished alcohols were recovered after filtration of the lipase and liquid-liquid extraction. The course and selectivity of the enzymatic hydrolysis were quantified by chiral chromatography, and the results are summarized in **table 2**

Table 2: *CAL-B* Catalyzed kinetic resolution of *rac*-**1a** *from biphasic to micro-aqueous media*

The results from **Table 2** indicate the high enantioselectivity of the lipase *CAL-B* under all the experimental conditions applied (E>>200). According to previous experimental conditions for the conventional hydrolysis [31], an important influence of *CAL-B* loading on the lipase reactivity is observed. The first test is carried out in an ethyl ether/water mixture (2 ml / 12 ml) and with 150 mg of *CAL-B* (entry 1). More eco-friendly reaction conditions [32-34] were then introduced, such decreasing to the half, of both phosphate buffer solution also the *CAL-B* amount with the use of *tertio*butylmethyl ether (TBME) as alternative to of the diethylether in term of environmental exigencies [35-36]. A similar result was reached and an ideal EKR is achieved: the conversion value of 50%, the (*R*)-alcohols are obtained with excellent enantiomeric excesses, $ee_s = ee_p = 99\%$ and E > 200 (entries 1 and 2). No disturb on both reactivity and selectivity was recorded by more reducing the amount of *CAL-B* to lower values of 12.5 mg per 1mmole of substrate (entries 6-7).The decreasing of the proportion of the buffer solution/*co*-solvent (*v/v*) from (5/1) to (0.02/1) only a slight drop of the conversion from 50% to 45% (entry 5 *vs* 9). No reactivity without external source of water was observed (entry 10). So, to ensure the hydrolysis reaction, it's sufficient to use micro-aqueous system under the optimum elaborated conditions and a single drop of water was essential to catalytic activity. To

study the scope of the *CAL-B* catalyzed hydrolysis in micro-aqueous media with the lipase *CAL B*, we extended the methodology using our optimized conditions to other substrates. These experimental conditions have been performed on deacylation of various *rac*-α- phenyl ethyl esters [**1a-7a**] in micro-aqueous media with the lipase *CAL B*. The obtained results have been compared to those given by in non-aqueous media in presence of the carbonate salts. The obtained results are detailed in **Table 3**.

Table 3: *CAL-B* catalyzed kinetic resolution of *rac*-α- phenyl alkyl esters in micro-aqueous media

The enzymatic hydrolysis resolution in micro-aqueous media is carried out with an optimal amount of lipase CAL-B, the conditions of 12.5 mg of lipase *CAL-B* in TBME/H_2O (1/0.02) have been selected for the kinetic resolution of *rac*-α- phenyl alkyl esters [**1a** -**7a**] in micro-aqueous media.

The data of **Table 3** show a high selectivity of *CAL-B* lipase (E > 89) under the selected conditions for the reaction in the micro-aqueous system although the reactivity is significantly destabilized. The esters [**2a-6a**] were hydrolyzed with conversions that vary according on the length of the acyl moiety. The best reactivities were achieved with the 1-phenyl ethyl acetate **1a** and the 1-phenyl ethyl propionate **2a** (entries 1 and 2). It has been observed that when a migrating group chain has more than 2 carbon atoms in length, the reactivity decreases. In the hydrolysis of **3a**, the selectivity obtained is E = 89 for a conversion of conv =24.2% (entry 3). A very similar effect was observed in the hydrolysis of **4a** which the butyl group is replaced by the more bulky *iso*-butyl group (entries 3 and 4). The hydrolysis of the fatty esters **5a** and **6a** was done with more moderate conversions (18% ≤ conv ≤ 22%). This reveal that the micro-

aqueous conditions are not really efficient for the long chain esters despite an excellent enantioselectivity factor (E>>200. No hydrolysis of the 1-phenylethyl benzoate **7a**.

The comparison between two deacylations approaches reveals the efficiency of the path A, the protocol involving sodium carbonate in organic solvent (scheme 1), in terms of reactivity and selectivity for the *rac-α-* phenyl ethyl esters, especially for the fatty esters, molecules of high interest in several domains such: pharmaceutics and cosmetics [37]. The path B method, using the traditional hydrolysis in micro-aqueous system (scheme 1 path B) showed once again the limits of enzymatic hydrolysis in biphasic media especially the problems of solubility of the substrates and the difficulty of controlling the pH value of the aqueous solution during the hydrolysis procedure [38].

4. Conclusion:

Deacylation of a set of α- phenyl ethyl esters with different chain-lengths [**1a**-**7a**] *via Candida antarctica* lipase B as catalyst in different conditions was studied: in organic solvent with sodium carbonate and in micro-aqueous medium. The best results recorded shown are significantly related to the nature of the leaving group structure. During the alkaline hydrolysis, high selectivity were observed with aliphatic acyl chain side (E>>200), but, reactivity varied between good to high in function of their bulkiness (31≤ conv≤ 50), the acyl chain length of the esters [**1a**-**6a**] has affects slightly lipase reactivity. The obtained results show the effectiveness of the deacylation in the presence of the sodium carbonate for the enzymatic resolution, especially of the fatty esters. For the conventional hydrolysis in biphasic media, we have optimized the minimum quantity of necessary water, and shown the limits of micro-aqueous conditions for the hydrolysis of esters. Unfortunately, except for **1a** and **2a**, the conversions obtained with other esters [**3a-6a**] are very moderate (18% ≤ conv ≤ 22%). So, the alkaline enzymatic hydrolysis path stills the best alternative for the cleavage of aliphatic long chain acyl

esters, which can be very precious for the enantioselective deacylation of fatty esters. Contrariwise, the micro-aqueous conditions are less adapted and only compatible with short chain esters. A novel hydrolysis process in toluene with sodium carbonate catalyzed by *CAL-B* has been successfully extended to a set of α- phenyl ethyl esters with different chain-lengths which are valuable commodity chemicals widely found in agrochemicals and pharmaceuticals.

Acknowledgements:

References:

1. Breuer M, Ditrich K, Habicher T, Hauer B, Keseler M, Sturmer S, Zelinski T(2004) Angew Chem Int Ed 43: 788-824.

2. Sheldon R A, Brady D (2019) Chem Sus Chem 12: 2859-2881.

3. Shuke W, Radka S, Jeffrey C M, Kai B, Uwe T B (2020) Angew Chem Int Ed 59: 2–34.

4. Pavel RN (2003) Curr Opin Drug Disc Dev 6: 902-920.

5. Faber K, Riva S (1992) J Synth Org Chem 895-910.

6. Zhu B, Panek JS (2000) Org Lett 2(17): 2575-2578.

7. Maugard T, Tudella J, Legoy D (2000) Biotechnol Prog 16:358-362.

8. Maugard T, Legoy M D (2000) J Mol Catal B: Enzym 8: 275-280.

9. Mcconnell O, bach ii A, Balibar C, Byrne N, Cai Y, Carter G, Chlenov M, Di L, Fan K, Goljer I, He Y, Herold D, Kagan M, Kerns E, Koehn F, Kraml C, Marathias V, Marquez B, Mcdonald L, Nogle L, Petucci C, Schlingmann G., Tawa G, Tischler M, Williamson RT, Sutherland A, Watts W, young M D, Zhang M Y, Zhang Y, Zhou D, Ho D (2007) Chirality 19: 658–682.

10. Klibanov AM (1986) Chemtech 16: 354-359.

11. Klibanov AM (1986) J Am Chem Soc 108:2767-2768.

12. Kirchner G, Scollar MP, Klibanov AM (1985) J Am Chem Soc 107:7072-7076

13. Wong CH, Whitesides GM (1994) Baldwin GE, Magnus PD (eds), Enzymes in Synthetic Organic Chemistry." In Tetrahedron Organic Chemistry Series, 1st ed. Vol. 12. London: Pergamon Press

14. Faber, K (2011) Biotransformations in Organic Chemistry, 6th ed.; Springer-Verlag: Berlin Heidelberg.

15. Amanda G A S, Alessandra C de M, Pedro H H de A, Debora de O (2017) Trends in Food Science & Technology 69: 95-105.

16. Idris A, Bukhari A (2012) Biotechnology Advances 30: 550–563.

17. Faber K, Riva R (1992) J Synth Org Chem 895-910.

18. Zhu B, Panek JS (2000) Org Lett 2(17): 2575-2578

19. Merabet-Khelassi M , Houiene Z , Aribi-Zouioueche L, Riant O (2012) Tetrahedron: Asymmetry 23 : 828–833.

20. Houiene Z, Merabet-Khelassi M, Bouzemi N, Aribi-Zouioueche L, Riant O (2013) Tetrahedron: Asymmetry 24 :290-296.

21. Alalla A, Merabet-Khelassi M, Riant O, Aribi-Zouioueche L (2016) Tetrahedron: Asymmetry 27 :1253–1259.

22. Belkacemi FZ, Merabet-Khelassi M, Aribi- Zouioueche L, Riant O (2018), Res Chem Intermed 44 :6847–6860.

23. Arthur L B D, Philipe d S, Julian (2018) Journal of CO_2 Utilization 23: 159–178.

24. Cheng Li, Jingcan S, Caili F, Bin Y, Shao Q L, Tianhu Li, Dejian H (2014) Food Chemistry 145 : 796–801.

 25. Kagan HB, Fiaud JC (1988) , Eliel EL, Wilen SH, Ed.J. Wiley & Sons, Inc. New York Kinetic Resolution Topics in Stereochemistry 18: 249-330.

26. Hayashi T, Matsumoto Y (1991) Tetrahedron: Asymmetry 2: 601-612.

27. Naemura K, Murata M, Tanaka R, Yano M, Hirose K, Tobe Y (1996) Tetrahedron: Asymmetry 7: 1581-1584.

28.   Naemura K, Murata M, Tanaka R, Yano M, Hirose K, Tobe Y (1996) Tetrahedron: Asymmetry 7 : 3285-3294.

29. Kazlauskas RJ, Weissfloch AN, Rappaport AT, Cuccia LA, (1991) J Org Chem 56: 2656-2665.

30. Goto M., Kawasaki M, Kometani, T (2000) J Mol Catal B: Enzym 9 (4-6): 245-250.

31. Melais N, Aribi-Zouioueche L, Riant O (2016) Comptes Rendus Chimie 19 : 971-977.

32. Anastas PT, Warner JC (1998) Green chemistry: theory and practice (Oxford University Press, New York P 3)

33. Anastas PT, Li PT (2010) Water as a Green Solvent (John Wiley & Sons EdsAnastas PT, Eghbali N (2010) Chem Soc Rev 39(1):301-312.

34. Trost BM (1995) Angew Chem Int Ed 107: 285 – 307

35. Byrne FP, Jin S, Paggiola G, Petchey THM, Clark JH, Farmer TJ, Hunt AJ, McElroy CR, Sherwood J (2016) (2016) Sustain Chem Process 4(7):1-24

36. Prat D, Wells A, Hayler J, Sneddon H, McElroy CR, Abou-Shehada S, Dunn PJ (2015) Green Chem 18(1): 288-296.

37. Blasco M A, Gröger H (2014) Bioorg med chem 22(20): 5539-5546

38. Rakels J L L, Straathof A J J, Heijneii JJ (1994) Tetrahedron: Asymmetry 5: 93–100.

Two approaches for *CAL-B*-Catalyzed Enantioselective Deacylation of a Set of α- phenyl ethyl esters: organic solvent with sodium carbonate and micro-aqueous medium.

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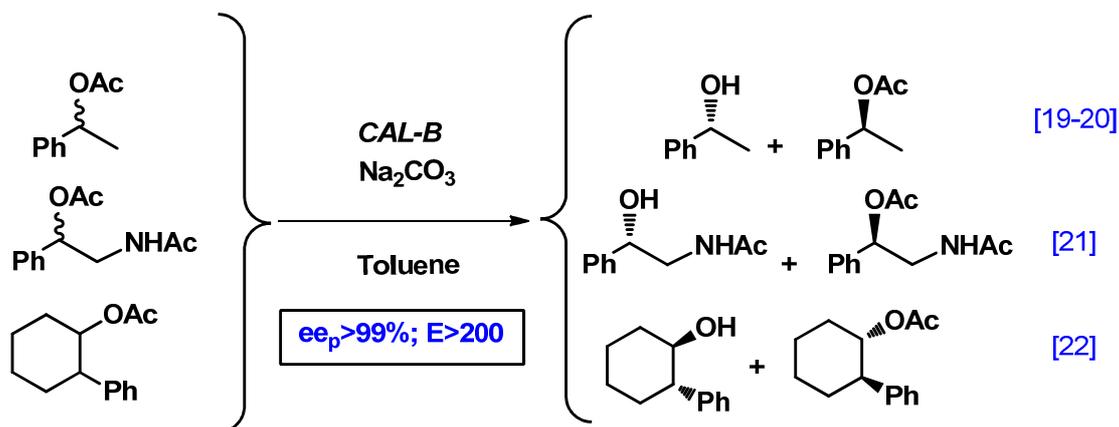
Scheme 1: *CAL-B* catalyzed *O*-deacylation of aryl-ethyl acetates in the presence of Na_2CO_3.
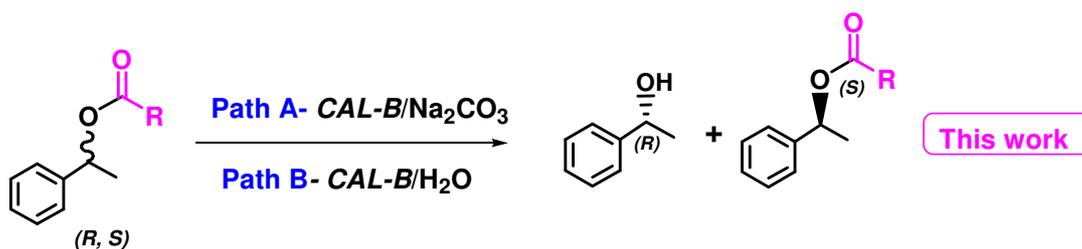
1a: R= -CH₃; 2a: R= -CH₂-CH₃; 3a: R= -(CH₂)₂-CH₃; 4a: R= -*i*Pr;
5a: R= -(CH₂)₈-CH₃; 6a: R= -(CH₂)₁₀-CH₃ ; 7a: R= -Ph

Scheme 2: Reactions investigated.
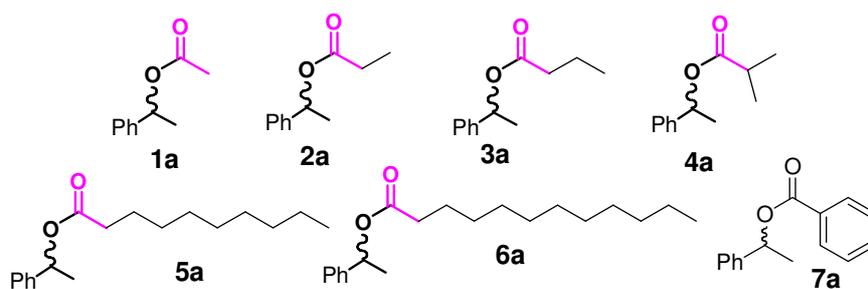
Scheme 3: *Rac-α*-**phenyl ethyl esters [1a-7a]**

Table 1: *CAL-B* Catalyzed kinetic resolution of *rac*-α- phenyl ethyl esters in non-aqueous media.

Entry	Substrate[a]	$ee_s(\%)^b$ (S)	$ee_p(\%)^{b)}$ (R)	C (%)[c]	E[c]
1	1a	> 99	> 99	50	>>200
2	2a	93	> 99	48.4	>>200
3	3a	98	> 99	50	>>200
4	4a	44	> 99	31	>>200
5	5a	92	> 99	48.2	>>200
6	6a	80	> 99	44.7	>>200
7		1	74	1	7
8[(b)]	7a	8	84	9	12
9[(c)]		8	74	10	7

[a] 1mmol of racemic ester, 1 mmol of Na$_2$CO$_3$, 40 mg of *CAL-B* in 2 mL of Toluene at 40°C. [b] 2 mL of hexane. [c] 2 mL of TBME. [d] Enantiomeric excess of recovered alcohols and remainingesters are measured by chiral GC or HPLC. [e] C=ee$_S$/ee$_P$+ee$_s$; Selectivity: E =Ln [(1-C) (1-ee$_{(S)}$)]/ Ln [(1-C) (1+ee$_{(S)}$)] [23]

Table 2 : *CAL-B* Catalyzed kinetic resolution of *rac*-1a: *from biphasic to micro-aqueous media*

Entry[a]	*CAL-B* (mg)	Org. Solv./Buffer phosphate solution (*v/v*) mL	ee$_s$(%)[b] (S)	ee$_p$(%)[b] (R)	C (%)[c]	E[c]
1	150	Et$_2$O / H$_2$O (2/12)	> 99	> 99	50	>>200
2	75		> 99	> 99	50	>>200
3	50		> 99	> 99	50	>>200
4	25	TBME/H$_2$O (1/5)	> 99	> 99	50	>>200
5	12.5	TBME/H$_2$O (1/5)	> 99	> 99	50	>>200
6	12.5	TBME/H$_2$O (1/5)	> 99	> 99	50	>>200
7	12.5	TBME/H$_2$O (1/1)	90.3	> 99	47.7	>>200
8	12.5	TBME/H$_2$O (1/0.5)	88	> 99	47	>>200
9	12.5	TBME/H$_2$O (1/0.02)	80	> 99	45	>>200
10	12.5	TBME/H$_2$O (1/0)	-	-	NR	>>200

[a] 1mmol of rac-**1a**, an appropriate amount of *CAL-B*, in mixture of organic solvent and phosphate buffer, stirred room temperature for 48 hours. [b] Enantiomeric excess of recovered alcohols and remaining acetates are measured by chiral GC. [c] Conversion: C=ee$_S$/ee$_P$+ee$_s$; Selectivity: E =Ln [(1-C) (1-ee$_{(S)}$)]/ Ln [(1-C) (1+ee$_{(S)}$)] [23]. Org. Solv. : Organic Solvent. NR: No Reaction

Table 3: *CAL-B* Catalyzed kinetic resolution of *rac*-α- phenyl alkyl esters in micro-aqueous media
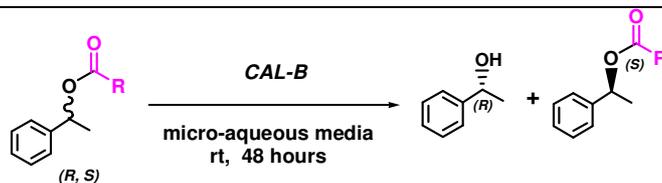
Entry	Substrate[a]	$ee_s(\%)^{[b]}(S)$	$ee_p(\%)^{[b]}(R)$	$C(\%)^{[c]}$	$E^{[c]}$
1	1a	80	> 99	45	>>200
2	2a	87	> 99	46	>>200
3	3a	31	97	24.2	89
4	4a	29	> 99	22	>>200
5	5a	22	98	18	>>200
6	6a	23	98	19	>>200
7	7a	-		[d] NR	-

[a] 1mmol of rac- ester, 12.5 mg of *CAL-B*, in TBME/H_2O (1/0.02) mL, stirred room temperature for 48 hours. [b] Enantiomeric excess of recovered alcohols and remaining acetates are measured by chiral GC or HPLC. [c] Conversion: $C = ee_S/ee_P + ee_s$; Selectivity [23]: $E = Ln[(1-C)(1-ee_{(S)})]/Ln[(1-C)(1+ee_{(S)})]$. [d] NR: No reaction.