Optically active alcohols and esters are important compounds to organic synthesis, since they are highly applicable as chiral building blocks. Among the known methodologies to achieve these optically active compounds, lipase-mediated enzymatic kinetic resolution stands out, via acylation of alcohols or deacylation of corresponding esters. Despite lipases’ ability to hydrolyze long-chain esters, acetyl compounds are usually employed as substrates in synthetic approaches. However, it is known that chain length can influence the activity and enantioselectivity of lipases in enzymatic kinetic resolution reactions. Therefore, the influence of chain length in lipase-mediated reactions has been studied only in batch mode. In this context, we present a study involving the deacylation of carboxylic esters with different acyl groups (2, 3 and 6 carbon atoms) in continuous-flow mode, in order to establish a protocol to achieve optically active esters and alcohols with high enantioselectivity. The influence of chain length was evaluated, showing that no clear tendency was observed in enantioselectivity or conversion rates of studied reactions. However, continuous-flow reactions were more productive presenting values 1.9 up to 10.3-fold higher than batch mode. Moreover, a competitive reaction took place when 2-octyl hexanoate was employed as substrate in batch, which was not favored when the reaction was performed in continuous-flow mode.

Keywords: Chiral resolution; continuous-flow; enantioselectivity; enzymatic catalysis; transesterification

1. Introduction

Optically active alcohols and esters present huge social and economic importance in organic synthesis, since they are widely used as building blocks and chiral intermediates in the synthesis of pesticides, pharmaceuticals, flavorings, and fragrances. Several chemical processes to achieve optically active alcohols and esters have been reported. From the most common processes, the most efficient reactions involve asymmetric transfer hydrogenation of prochiral ketones catalyzed by chiral Ru (II), Rh and Ir complexes, asymmetric aryl transfer reactions to aromatic aldehydes and chiral resolution of racemates.

Among these methodologies, one of the most consolidated approaches are those related to biocatalysis. It is known that the use of enzymes to achieve optically active compounds may present several advantages, such as effectiveness, biodegradability, also enzymes act under mild conditions of temperature and pH and can present excellent stereoselectivity for several substrates. In this regard, the use of lipases (EC 3.1.1.3) stands out in enzymatic kinetic resolution (EKR) reactions via esterification, interesterification, transesterification (acylation and deacylation), hydrolysis, amidation, and synthesis of peracids and peptides.

Lipases naturally catalyze the synthesis of esters of fatty acids (triacylglycerols) in aqueous media and the hydrolysis of these esters in media with high water concentration. Due to this fact, lipases ordinary substrates should be long-chain compounds, however, for practical purposes, acetyl moiety has been the first choice in synthetic approaches of lipase-mediated EKR reactions. In spite of acetyl moiety practicality, the influence of chain length of acyl portion is an important variable to be studied in lipase-mediated deacylation reactions. Reports on this subject are scarce in the literature, but it has been shown that chain length in the acyl portion can affect lipases activity and enantioselectivity in EKR reactions, however, no clear tendency has been observed. Besides that, it is important to highlight that these reactions are still performed in batch mode, despite the known popularity of continuous-flow reactions.
Biocatalyzed reactions in continuous-flow systems can be more efficient, \(^ {27-29}\) since they use less biocatalyst to produce the same amount of product, \(^ {30}\) present shorter reaction times, \(^ {30, 31}\) high reproducibility and productivity, \(^ {32}\) lower costs in optimization of the processes, no enzyme lixiviation from support, reuse of the immobilized enzyme\(^ {33}\) and, mainly, the product is easily and quickly removed from the contact with the biocatalyst. \(^ {30, 33}\)

Concerning the use of continuous-flow systems in biocatalyzed reactions, several examples are already reported in literature, \(^ {34-39}\) including a study from our research group about the EKR of cyanohydrin esters via deacylation reactions. In this study, deacylation was proven to be a better protocol for this EKR than acylation since high conversion rates and enantioselectivity were observed for all substrates. \(^ {40}\) Acylation reaction was not a valuable method to achieve optically active cyanohydrins, presenting low conversion rates in batch and continuous-flow modes.

On the other hand, a study employing benzylic and aliphatic alcohols and their respective acetates in EKR reactions mediated by Novozym 435\(^ {®}\) via acylation and deacylation reactions demonstrated that acylation reactions presented better results than deacylation reactions, although, only acetates were employed as substrates for this reaction. \(^ {41}\)

In order to improve the EKR protocol via deacylation reactions of carboxylic esters, in this work, we discuss our recent results in the evaluation of the influence of chain length in acyl portion of benzylic and aliphatic carboxylic esters in Novozym 435\(^ {®}\)-mediated EKR reactions in both batch and continuous-flow modes.

## 2. Results and Discussion

### 2.1. Selection of substrates

A series of esters (1-6) was planned in order to investigate the influence of chain size in acyl portion of carboxylic esters (Figure 1). For this, aliphatic (2-3) and benzyl (5-6) esters were chosen, with 3 and 6 carbon atoms in the acyl portion. In addition, results were compared to previously reported data from corresponding acetates. \(^ {41}\)

### 2.2. Chemical synthesis

Racemic esters 2, 3, 5, 6 were synthesized from corresponding alcohols using the appropriate anhydrides as acyl donors, 4-(dimethylamino)pyridine (DMAP) as catalyst, and dichloromethane (DCM) as solvent, with yields up to 97\% (see experimental section and Supporting Information for details).

### 2.3. Enzymatic Kinetic Resolution (EKR) Reactions

For EKR reactions in continuous-flow mode, substrates and n-butanol were solubilized in n-hexane (final volume 5 mL) and eluted through a column filled with Novozym 435\(^ {®}\) for two cycles with a flow rate of 0.1 mL min\(^ {¯}\) (see experimental section and Supporting Information for details). EKR reactions in batch mode were also carried out in parallel in order to compare both modes. For this purpose, substrates and n-butanol were solubilized in n-hexane (final volume 2 mL) in a sealed vial and Novozym 435\(^ {®}\) was added to this solution. Reaction medium was maintained under constant magnetic stirring. Periodic aliquots were taken and analyzed via gas chromatography. Results of EKR reactions in continuous-flow and batch mode for esters 1-6 are shown in Table 1.

EKR reaction of 2 presented high enantioselectivity (E > 200) in both systems, but higher conversion in batch (c = 46\%) than in continuous-flow mode (c = 12\%) (Table 1 – Entry 2). These results can be related to higher contact time between substrate and enzyme in batch than in continuous-flow mode, since residence time was only 8.6 min. However, continuous-flow reaction presented productivity almost 2-fold higher than its batch counterpart. Surprising results were observed in EKR of 3 when comparing enantioselectivity in continuous-flow and batch modes (Table 1 – Entry 3). Deacylation of 3 employing n-butanol as nucleophile produces (S)-3, (R)-2-octanol and n-butyl hexanoate (7) (Scheme 1 – A), which is also described as an acylating agent in acylation of secondary alcohols. \(^ {42}\) Due to this fact, a competitive EKR reaction took place, in which 7 is the acyl donor and (R)-2-octanol is the nucleophile of the acylation reaction, giving (R)-3 ( Scheme 1 – B), which results in an apparent decrease in enantioselectivity, since racemate is regenerated in the reaction medium.

The effect of this competitive reaction is minimized in continuous-flow mode (Table 1 – Entry 3), since the solution is almost immediately removed from the contact with biocatalyst. For other substrates, competitive EKR reaction was not observed in any mode. When ester 5
was employed as the substrate, higher enantioselectivity (E = 94) was observed in batch mode than in continuous-flow mode (E = 16; Table 1 – Entry 5). This similar behavior was also observed in our previous work \(^4\) and it was explained by a large amount of enzyme in continuous-flow mode (Flow: 200 mg; Batch: 20 mg), since reactions employing a smaller amount of biocatalyst (100 mg) in continuous-flow mode resulted in a significant increase in enantioselectivity. This phenomenon was not entirely understood, but it results in an apparent decrease of enantiomeric ratio. EKR reaction of ester 6 presented similar results in both modes, regarding enantioselectivity and conversion rates (Table 1 - Entry 6).

Table 1. EKR reactions with esters 1–6 in batch and continuous-flow modes

| Ester / Entry | Continuous-Flow | Batch |
|--------------|-----------------|-------|
|              | Time / h c | ee / % ee / % | r / µmol min⁻¹ g⁻¹ | E f | Time / h c | ee / % ee / % | r / µmol min⁻¹ g⁻¹ | E f |
| 1 a          | 8.6 27 37 >99 j | 6.8 >200 | 24 46 85 >99 j | 1.6 >200 |
| Aliphatic 2  | 8.6 12 14 >99 k | 3 >200 | 24 46 84 >99 l | 1.6 >200 |
| 3            | 8.6 45 82 >99 k | 11.3 >200 | 24 32 32 68 m | 1.1 7 |
| Benzyl 4     | 8.6 46 84 >99 j | 11.5 >200 | 10 49 94 >99 j | 4.1 >200 |
| 5            | 8.6 32 84 n | 7 16 10 48 88 94 o | 1.7 94 |
| 6            | 8.6 25 33 o 63 >99 n | 6.3 >200 | 10 31 44 p | 0.9 >200 |

aReaction conditions: Batch mode: ester (0.1 mmol), n-butanol (0.4 mmol), n-hexane (2 mL), and Novozym 435® (20 mg) at 50 °C. Flow mode: ester (0.1 mol L⁻¹), n-butanol (4 equiv.), n-hexane (5 mL), Novozym 435® (200 mg) at 50 °C, two cycles of elution in 0.1 mL min⁻¹. b Residence time: (reactor volume/flow rate) x number of cycles. c Conversion: ee/(ee + eep); ee = substrate enantiomeric excess, eep = product enantiomeric excess. d Enantiomeric excess: (R - S)/(R + S) × 100 (determined by chiral GC analysis). e Productivity (flow): [P]/m, [P] = product concentration [µmol mL⁻¹]; f = flow rate/number of cycles [mL min⁻¹]; m = amount of enzyme [g]. f Productivity: E = ln{(ee - ee)/(ee + eep)}/ln{(ee + ee)/(ee + eep)}. g Productivity (batch): nP/(t × m), nP = amount of product [µmol]; t = reaction time [min]; m = amount of enzyme [g]. h Determined by derivatization to the corresponding propionate. i Determined by derivation to the corresponding acetate. j Determined by the formula described in footnote c.

A) Interest EKR reaction

![Scheme 1. Competitive EKR reaction of 3 in batch mode](image)

B) Competitive EKR reaction

was employed as the substrate, higher enantioselectivity (E = 94) was observed in batch mode than in continuous-flow mode (E = 16; Table 1 – Entry 5). This similar behavior was also observed in our previous work \(^4\) and it was explained by a large amount of enzyme in continuous-flow mode (Flow: 200 mg; Batch: 20 mg), since reactions employing a smaller amount of biocatalyst (100 mg) in continuous-flow mode resulted in a significant increase in enantioselectivity. This phenomenon was not entirely understood, but it results in an apparent decrease of enantiomeric ratio (Flow: 7; Batch: 94). EKR reaction of ester 6 presented similar results in both modes, regarding enantioselectivity and conversion rates (Table 1 - Entry 6). However, results in continuous-flow mode were obtained...
in 8.6 min while the reaction in batch mode extended up to 10 h.

Concerning the influence of chain length in the acyl portion in continuous-flow EKR, it was not observed any tendency for reactions involving aliphatic compounds (1-3). All EKR reactions presented high enantioselectivity, although, when increasing chain length in one carbon atom, from 2-octyl acetate (1) to 2-octyl propionate (2), a decrease in conversion rate (27% to 12%) was observed. When increasing chain length in three more carbon atoms, from 2-octyl propionate (2) to 2-octyl hexanoate (3), it was noted a significant increase in conversion rates (12% to 45%).

Continuous-flow EKR reactions of benzyl esters (4-6), presented differences in enantioselectivity and conversion rates when increasing the chain length of the acyl portion. A decrease in enantioselectivity and conversion rate was observed when increasing chain length in one carbon atom, from 1-phenyl ethyl acetate (4) to 1-phenyl ethyl propionate (5). When chain length was increased in three more carbon atoms, 1-phenyl ethyl propionate (5) to 1-phenyl ethyl hexanoate (6), it was noted that enantioselectivity increased from 16 to >200, however, both reactions presented low conversion rates (28% and 25%, respectively). Batch mode EKR reactions presented the same behavior, with EKR of 5 presenting the lowest enantioselectivity of benzyl esters (Figure 2).

The efficiency of EKR reactions in each mode (batch and continuous-flow) was measured by productivity parameter ($r$) (Figure 3). From this parameter, continuous-flow was the most productive mode for EKR reactions of all studied compounds, presenting values 1.9 up to 10.3-fold higher than batch mode.

Continuous-flow EKR of ester 4 was the most productive reaction, also presenting the best values for enantioselectivity and conversion rate. Due to this fact, ester 4 was chosen as a substrate for a preparative scale EKR reaction (Scheme 2). The same reaction conditions presented in Table 1 were applied in a preparative scale (2 mmol) and similar results were observed, since ester ($S$)-4 and ($R$)-1-phenylethanol were obtained with high enantiomeric excesses (87% and >99%, respectively) and 47% conversion rate.

In summary, the influence of acyl portion of carboxylic esters in EKR reactions was evaluated, however, no specific pattern was observed. Changes in stereoselectivity were observed on going from short to medium chain length and particularities were observed for reactions in continuous-flow mode, as well as the influence of the amount of enzyme and a competitive reaction for substrate 3. Regarding the productivity parameter, all EKR reactions presented higher values in continuous-flow mode than in their batch counterparts, especially for substrates 3 and 6.
3. Conclusions

The influence of chain length in acyl portion of carboxylic esters in lipase-mediated EKR in batch and continuous-flow mode was evaluated. These results indicated that there is no ideal protocol, batch or continuous-flow mode, to achieve high enantioselectivity for all structures, since no tendency was observed among the series. EKR reaction of substrate 3 was particularly interesting due to a competitive reaction in batch mode that caused a loss in enantioselectivity. This reaction was not favored in continuous-flow mode. Despite the absence of a tendency regarding conversion rates and enantioselectivity, continuous-flow was the most productive mode for all studied substrates.

4. Experimental

4.1. Experimental Procedure for Syntheses of Racemic Esters 2, 3, 5 and 6.

To a solution of corresponding alcohol (2-octanol, 3 mmol, 390 mg; 1-phenylethanol, 3 mmol, 366 mg) in dichloromethane (5 mL), propionic anhydride (4 mmol, 0.51 mL) and DMAP (1 crystal) were added. The reaction mixture was kept under magnetic stirring for 30 min at room temperature, then neutralized with aqueous NaHCO3 (3 x 5 mL). The organic phase was dried over anhydrous MgSO4, filtered through silica and the solvent was evaporated under reduced pressure. Spectroscopic data are in accordance to literature.43-46

2-Octyl propionate (2). Colourless liquid. Yield: 87%, 478 mg.
GC-MS (70 eV), m/z (relative intensity): 129 (8%), 129 (2%), 117 (19%), 112 (16%), 99 (100%), 83 (17%), 71 (37%), 60 (9%), 57 (17%), 43 (27%), 29 (3%). 1H NMR (200 MHz, CDCl3, TMS), δ 0.85-0.93 (9H, 6H); 1.20 (d, J = 6.2 Hz, 3H); 1.28-1.67 (m, 16H); 2.23-2.31 (m, 2H); 4.90 (sext, J = 6.2 Hz, 1H). 13C NMR (50 MHz, CDCl3), δ 13.8; 13.9; 19.9; 22.2; 22.5; 24.7; 25.3; 29.0; 31.2; 31.7; 34.6; 35.9; 70.7; 173.6. IR (KBr) ν/cm-1 2935, 2856, 1731, 1459, 1187.

2-Octyl hexanoate (3). Colourless liquid. Yield: 93%, 637 mg.
GC-MS (70 eV), m/z (relative intensity): 143 (8%), 129 (2%), 117 (19%), 112 (16%), 99 (100%), 83 (17%), 71 (37%), 60 (9%), 57 (17%), 43 (27%), 29 (3%). 1H NMR (200 MHz, CDCl3, TMS), δ 0.85-0.93 (9H, 6H); 1.20 (d, J = 6.2 Hz, 3H); 1.28-1.67 (m, 16H); 2.23-2.31 (m, 2H); 4.90 (sext, J = 6.2 Hz, 1H). 13C NMR (50 MHz, CDCl3), δ 13.8; 13.9; 19.9; 22.2; 22.5; 24.7; 25.3; 29.0; 31.2; 31.7; 34.6; 35.9; 70.7; 173.6. IR (KBr) ν/cm-1 2957, 2930, 2858, 1734, 1464, 1377, 1178.

1-Phenyl ethyl propionate (5). Colourless liquid. Yield: 95%, 508 mg.
GC-MS (70 eV), m/z (relative intensity): 178 (M+, 26%), 122 (100%), 105 (97%), 91 (2%), 77 (30%), 63 (2%), 57 (53%), 51 (10%), 43 (7%). 1H NMR (200 MHz, CDCl3, TMS), δ 1.14 (t, J = 7.6 Hz, 3H); 1.53 (d, J = 6.6 Hz, 3H); 2.30-2.41 (m, 2H); 4.90 (sext, J = 6.2 Hz, 1H); 7.26-7.38 (m, 5H). 13C NMR (50 MHz, CDCl3), δ 9.1; 22.3; 27.9; 72.1; 126.0; 127.8; 128.5; 141.9; 173.7. IR (KBr) ν/cm-1 3064, 3034, 2983, 2935, 1740, 1452, 1369, 1190, 1064, 759, 699.

1-Phenyl ethyl hexanoate (6). Colourless liquid. Yield: 97%, 641 mg.
GC-MS (70 eV), m/z (relative intensity): 220 (M+, 5%), 122 (91%), 115 (7%), 105 (100%), 99 (16%), 91 (2%), 77 (19%), 71 (14%), 55 (4%), 51 (5%), 43 (27%). 1H NMR (200 MHz, CDCl3, TMS), δ 0.84-0.91 (9H, 6H); 1.24-1.32 (m, 4H); 1.53 (d, J = 6.6 Hz, 3H); 1.59-1.70 (m, 2H); 2.28-2.36 (m, 2H); 5.90 (q, J = 6.6 Hz, 1H); 7.26-7.36 (m, 5H). 13C NMR (50 MHz, CDCl3), δ 9.1; 22.3; 27.9; 72.1; 126.0; 127.8; 128.5; 141.9; 173.1. IR (KBr) ν/cm-1 3088, 3065, 3034, 2957, 2932, 2864, 1735, 1453, 1372, 1117, 1057, 760, 698.

4.2. General Procedure for Enzymatic Kinetic Resolution in Batch Mode41

In a sealed vial (4 mL), esters 1–6 (0.1 mmol) were solubilized in n-hexane (2 mL), n-butanol (37 μL, 0.4 mmol) and the supported enzyme CAL-B (Novozym 435®; 20 mg) were then added to this solution. The reaction mixture was stirred at constant temperature (50 °C), and aliquots of 200 μL were periodically taken, diluted in 300 μL of n-hexane, derivatized and analyzed by chiral GC. Details for GC analyses can be found in Supporting Information.
4.3. General Procedure for Enzymatic Kinetic Resolution in Continuous-Flow Mode

Esters 1–6 (0.5 mmol) and n-butanol (0.18 mL, 2 mmol) were solubilized in n-hexane (5 mL) and this solution was eluted through a packed-bed column (74.0 x 4.6 mm) with the biocatalyst (200 mg, internal volume 0.43 mL) with a flow rate of 0.1 mL min⁻¹ for two cycles at 50 °C. Aliquots from each cycle were collected, derivatized and analyzed by chiral GC. Details for GC analyses can be found in Supporting Information.

4.4. Experimental Procedure for Derivatization to Acetate or Propionate

Acetic anhydride or propionic anhydride (5 μL) and DMAP (1 crystal) were added directly to the reaction aliquot and it was maintained under magnetic stirring for 5 min. The aliquot was neutralized with aqueous NaHCO₃ and the organic layer was dried over anhydrous MgSO₄ before analysis. Details for GC analyses can be found in Supporting Information.

4.5. Experimental Procedure for Preparative Scale Enzymatic Kinetic Resolution of 4 in Continuous-Flow Mode

Ester 4 (2 mmol, 328 mg) and n-butanol (8 mmol, 0.75 mL) were solubilized in n-hexane (20 mL) and this solution was eluted through a packed-bed column with the biocatalyst (200 mg) with a flow rate of 0.1 mL min⁻¹ for two cycles at 50 °C. After that, compounds were separated by flash column chromatography (hexanes/ethyl acetate, 8:2) and enantiomeric excesses were determined by chiral GC analysis.

(S)-1-Phenyl ethyl acetate [(S)-4]: [α]₂⁰ 24° - 8.4 (c 1.0, n-hexane, ee 87%). Ref.47: [α]₂⁰ 26° - 59.2 (c 0.5, CHCl₃, ee 76%).

(R)-1-Phenylethanol: [α]₂⁰ 24° 6.7 (c 1.0, n-hexane, ee >99%). Ref.48: [α]₂⁰ 53.1 (c 1.0, CHCl₃, ee >99%).

Supporting Information

Supporting information for this article is available free of charge at https://rvq.sbq.org.br/

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