# Substrate engineering on the enzymatic transesterification of 2-bromobutyric esters: Influence of alcohol moiety.

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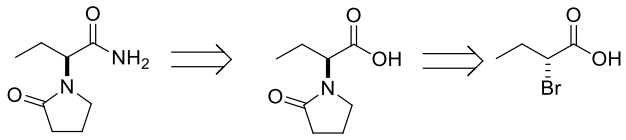
## Abstract

Engineering of ester alcohol moiety was performed as a strategy for improve the enzymatic enantioselectivity.

## Introduction

Lipases-mediated reactions allow the achievement of optically active compounds through enzymatic kinetic resolution (EKR). *Candida antartica* lipase B (CAL-B) is one of the most used lipase in organic synthesis, and it was successfully applied in the resolution of chiral secondary alcohols and amines. However, the range of substrate scope of carboxylic acids/esters that could be resolved using this lipase is limited, which makes substrate engineering a tool for improving the enantioselectivity.[1] Here, we report our efforts towards EKR of 2-bromobutyric acid derivatives, an important chiral building block in the synthesis of of levetiracetam (Keppra®), a blockbuster antiepileptic drug (Scheme 1).




**Levetiracetam**

**Scheme 1.** Retrosynthetic analysis of levetiracetam.

## Results and Discussion

A series of 2-bromobutanoates (**1-9**) was synthesized. Size of alkyl chain, presence of ramifications, unsaturations and heteroatoms in alcohol moiety were evaluated (Figure 1).

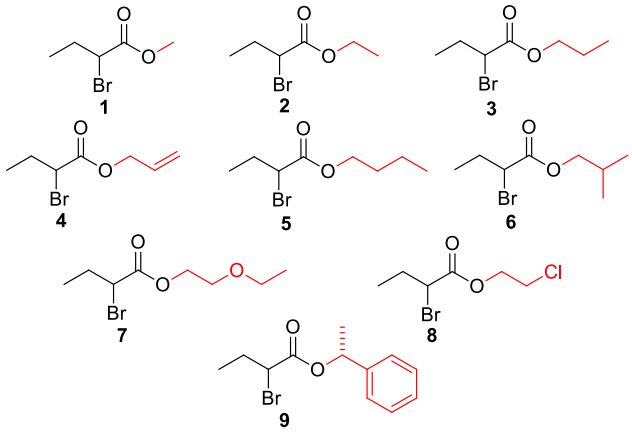
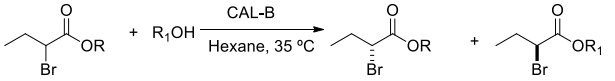



**Figure 1.** Variations on the ester alcohol moiety.

The results of enzymatic transesterification of these substrates are summarized in Table 1:

**Table 1.** Substrate screening for the transesterification reaction.




| Ester | R | $R_1$ | Time/ (h) | c / (%) | e.e.$_s$ / (%) | e.e.$_p$ / (%) | E |
|---|---|---|---|---|---|---|---|
| 1 | Me | *n*-Pr | 12 | 80 | 88 | 22 | 4 |
| 2 | Et | | 12 | 74 | 84 | 30 | 4 |
| 3 | *n*-Pr | Et | 24 | 85 | 80 | 14 | 3 |
| 4 | Alil | | 12 | 85 | 90 | 16 | 3 |
| 5 | *n*-But | | 24 | 75 | 76 | 26 | 3 |
| 6 | *i*-But | | 24 | 48 | 49 | 54 | 5 |
| 7 | 2-EtOEt | | 4 | 82 | 90 | 20 | 4 |
| 8 | 2-ClEt | | 0.5 | 75 | 88 | 29 | 5 |
| 9 | (*R*)-1-phenylethyl | | 36 | n.d | 21 (*S*) | 28 (*R*)[a] | n.d |

Reactions conditions: ester (0.1 mmol), alcohol (4 mmol), CAL-B (20 mg), hexane (2 mL); 35 $^0$C. n.d: not determined. [a]Diasteroisomeric excess.

The alcohol moiety alterations did not change the enantiomeric ratio in a significant way. On the other hand, rate of conversion was very dependent to the alcohol structure. The presence of a branched moiety in **6** decreased the reaction rate in comparison to alkyl esters **1-5**. This suggests a steric hindrance factor. The presence of oxygen on ester **7** appears to imply in a increasing reaction rate. This heteroatom could interact with amino acids through hydrogen bonding, what stabilizes the tetrahedral intermediate. Among all the substrates, 2-chloroethyl ester (**8**) was the one that improved dramatically the reaction rate. The electron withdrawing effect of the chlorine atom on the hydroxyl group makes 2-choloroethanol a better leaving group. An opposite enantiopreference was observed in EKR of ester **9** containing (*R*)-1-phenylethyl substituent, an unusual observation.

## Conclusions

Despite of substrate engineering, none improvement in the enantioselectivity was observed. However, the reaction rate and enaniopreference were dependent of alcohol moiety modifications.

## Acknowledgements

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[1] Tsai, S.-W. *J. Mol. Catal. B: Enzym.* **2014**, available online.