Recombinant pig liver esterase-catalyzed synthesis of (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate with subsequent enantioselective crystallisation

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Introduction

Pig liver esterase (PLE) is an industrially used biocatalyst for the synthesis of a broad spectrum of enantiopure fine chemicals and pharmaceuticals.[1] Unfortunately, enzyme preparations from animal tissue consist of undefined mixtures of isoenzymes with different substrate specificities lowering the synthetic value of PLE. However, commercially available recombinant pig liver esterases from Enzymicals AG (ECS-PLEs) can be used to overcome this limitation.[2, 3]. In this study ECS-PLE06 was investigated for the enantioselective hydrolysis of *cis*-1,4-diacetoxy-2-cyclopentene yielding (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate on preparative scale (Figure 1).

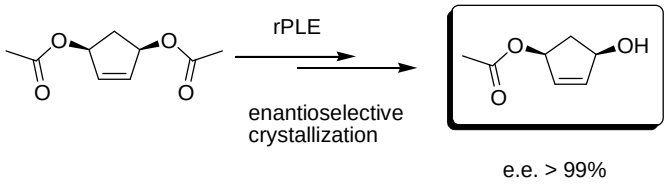

Figure 1. rPLE-catalyzed synthesis of enantiopure (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate.

Results

Significant differences towards the specific activity and enantioselectivity for *cis*-1,4-diacetoxy-2-cyclopentene (Figure 2) were found for the six ECS-PLEs where ECS-PLE03 and ECS-PLE06 show the best results. However, ECS-PLE06 with a half life of 91 h is substantial more stable than ECS-PLE03 (1.7 h).

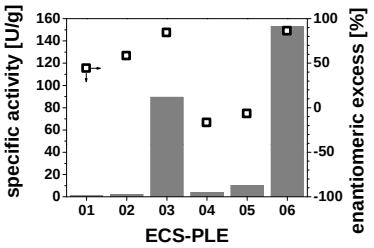

Figure 2. Comparison of the specific activity and enantioselectivity towards *cis*-1,4-diacetoxy-2-cyclopentene (hydrolysis reaction). *Reaction conditions*: 0.25-15.5 mg/mL PLE isoenzyme (lyophilizate), 9 mmol/L substrate, 1.5 mL 100 mM phosphate buffer pH 7.5, 40 °C, 120 min.

The ECS-PLE06-catalyzed hydrolysis of *cis*-1,4-diacetoxy-2-cyclopentene was performed in 100 mM phosphate buffer at pH7.5 without co-solvent at 50 °C facilitating full conversion with an enantiomeric excess of 83 to 89% (Table 1).

Table 1. rPLE-catalyzed hydrolysis of *cis*-1,4-diacetoxy-2-cyclopentene at larger scale with pH-adjustment by an external base solution.

Entry	V [L]	Substrate [mM]	Activity[a] [U/mL]	Reaction time [min]	e.e. [%]	Conversion [%]	STY [g/(L·h)]
1	0.02	100	20.0	11.0	88	98	76.0
2	0.02	100	10.0	36.7	89	>99	23.0
3	0.1	100	10.0	37.5	87	97	22.1
4	0.02	100	5.0	36.7	85	95	22.1
5	0.02	100	2.5	75.0	83	90	10.2
6	0.02	200	5.0	110.0	84	91	14.1
7	0.02	500	10.0	240.0	84	63[b]	11.2

[a] Used volumetric enzyme activity; calculated from specific activity [U/mg] according to standard activity assay and enzyme amount [mg] per volume [mL]. [b] Due to an extremely slowing down of the reaction velocity, the reaction was interrupted after 240 minutes.

For the subsequent enantioselective crystallization further knowledge about binary phase diagram (BPD) including the eutectic composition (x=0.725) was required (Figure 3).

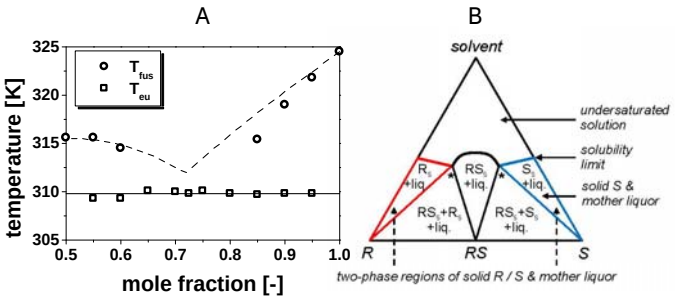

Figure 3. A) Binary melting point diagram of (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate. Theoretical calculation of the liquidus and solidus curve was performed applying the simplified equations of Schröder-van-Laar and Prigogine-Defay. B) Schematic representation of the ternary solution phase diagram of a racemic compound; * - eutectic(s), R_S – solid *R*-enantiomer, S_S – solid *S*-enantiomer, RS_S – solid racemic mixture, liq – liquid

The enantioselective crystallization requires an initial enantiomeric excess between the eutectic and the pure enantiomer in the two-phase-region (Figure 3B). During the enantioselective crystallization the remaining enantiomeric excess of the mother liquor needs to be higher than the eutectic mixture (except for preferential crystallizations).

Table 2. Enantioselective crystallization of (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate.

Entry	Initial e.e. [%]	Final e.e. [%]	Remaining e.e. [%]	Theoretical yield [%]	Practical yield [%]	Melting point [°C]
1	83	≥99.5	71	66	33	51.3
2	86	98.2	54	72	63	51.7
3	91	99	71	84	44	n.d.

The subsequent enantioselective crystallization was performed from *n*-heptane (Table 2). The crystallization process was started at a temperature of 50 °C and afterwards cooled down with 10 K/h to 10 °C.

Summary

The ECS-PLE06-catalyzed enantioselective hydrolysis of *cis*-1,4-diacetoxy-2-cyclopentene yielding (1*S*,4*R*)-4-hydroxy-2-cyclopentenyl acetate was investigated. The enzymatic reaction yielded an enantiomeric excess of only 83-89%, which was improved by enantioselective crystallization to ≥99% (16.7 g after scale-up).[4]

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