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ASYMMETRIC BIOTRANSFORMATIONS IN CONTINUOUS FLOW REACTORS

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ABSTRACT. Asymmetric acetylations of prochiral diols 3a-d with isopropenyl acetate were investigated with different commercial and self prepared lipases. Continuous flow mode reactions were performed in small stainless steel packed-bed reactors which can precisely control temperature (-10-200 °C), pressure (0-150 bar) and flow rate (0.1-3.0 mL/min). The effect of the temperature and flow-rate on the conversion and enantiomer excess of the chiral monoesters was investigated.

Keywords: lipase, continuous flow reactor, asymmetric biotransformation

INTRODUCTION

Biotechnology and biocatalysis are more and more applied to produce optically active intermediates of pharmaceuticals and fine chemicals [1]. Among the available biocatalysts, several characteristics make hydrolases useful for synthetic biotransformations [2, 3, 4, 5]. Besides hydrolysis, hydrolases can also catalyze several related reactions such as condensations (reversal of hydrolysis) and alcoholysis (a cleavage using an alcohol in place of water). Lipases proved to be highly versatile biocatalyst in stereoselective biotransformations such as kinetic resolutions [6], deracemisations and dynamic kinetic resolutions [7]. Examples for using asymmetric acetylations of prochiral diols to produce pharmaceutical intermediates has already been reported [8, 9, 10]. Enzymatic enantioselective processes typically was performed in batch mode [2, 6, 11, 12]. In a few work hydrolase catalyzed enantioselective processes were carried out in continuous flow systems [13, 14].

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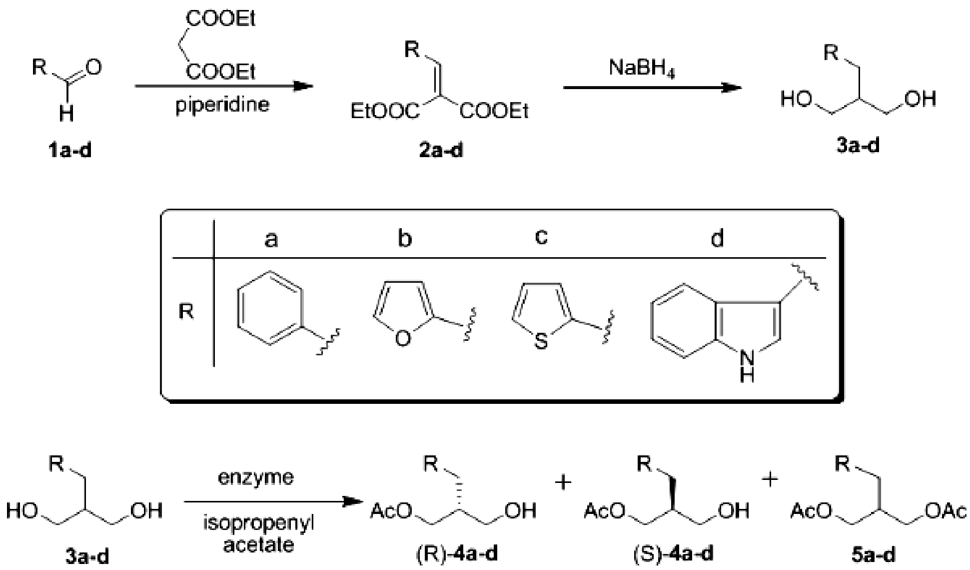
RESULTS AND DISCUSSION

In a previous work, the kinetic resolution of three racemic cyclic secondary alcohols 1-phenylethanol, 1-cyclohexylethanol and 1-phenyl-propane-2-ol were examined [15]. It was found that the productivity (*r*) of the lipase-catalyzed reactions was better in continuous flow reactors than in batch mode systems. On the other hand, the enantiomer selectivity (*E*) of the reactions were similar in the two reaction modes [15]. In the continuous flow reaction the pressure had no effect on the productivity (*r*) and selectivity (*E*). The productivity (*r*) increased monotonously with the temperature. More interestingly, the enantiomer selectivity (*E*) had a local maximum and a local minimum between 0 - 60 °C [15].

In this study, we intended to study the asymmetric acetylation catalyzed by lipases in continuous flow mode. For this purpose, we synthesized an isocyclic **3a** and three heterocyclic **3b-d** prochiral diols (Scheme 1). The asymmetric biotransformations of these prochiral diols **3a-d** were examined by various lipases in batch mode. The target diols **3a-d** were prepared by a Knoevenagel-condensation followed by reduction of the forming unsaturated esters **2a-d** (Scheme 1).



Scheme 1

In the first step, the Knoevenagel-condensation between diethyl malonate and the corresponding aldehydes **1a-d** in toluene at reflux temperature using Dean & Stark trap for 6 h resulted in the corresponding esters **2a-d** in good yields (78-84 %). The next step of the synthesis was more time-consuming. The reductions using sodium tetrahydridoborate needed 3 days to produce the diols **3a-d** in good yields (65-80 %).

For selecting the proper enzymes for the continuous flow mode reaction, a selection of enzymes were screened in batch mode. Among the tested commercially available enzymes, the best enantiotope selectivity (Ee>93%) and conversion (c > 80%) were achieved with Lipase PS (lipase from *Burkholderia cepacia*, formerly *Pseudomonas cepacia)* and isopropenyl acetate as acylating agent in hexane-THF solution for all diols **3a-d** (Scheme 1).

The lipase catalyzed acetylation of 2-benzylpropane-1,3-diol **3a** is a typical asymmetric biotransformation (Figure 1). Because the minor enantiomer (*S*)**-4a** reacts faster in the second acetylation step due to the conserved preference of the enzyme towards its free hydroxyl group, the enantiomeric excess of the monoacetate (*R*)**-4a** can be influenced by the conversion to diacetate **5a**. For synthesis of the enantiomerically pure (*R*)-monoacetate (*R*)-**4a** without formation of diacetate **5a** a highly enantioselective enzyme is required.

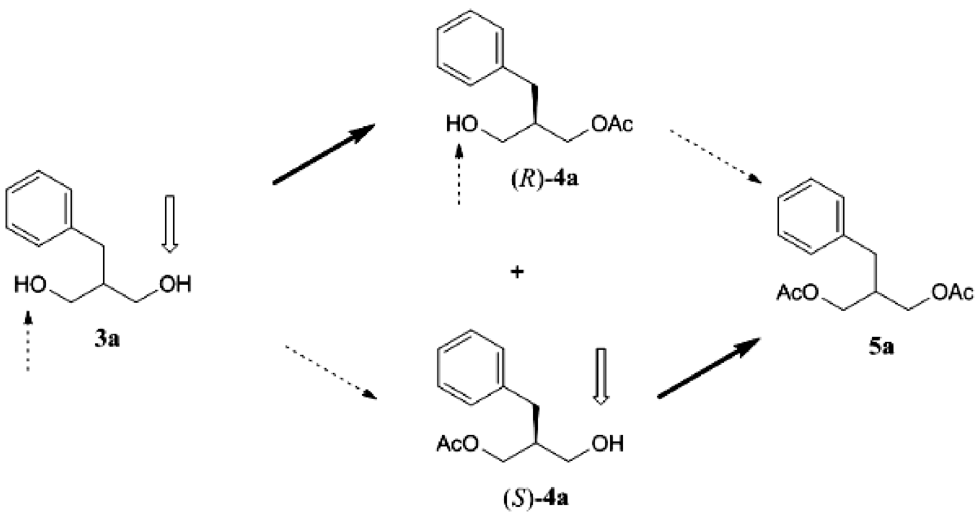


Figure 1. Asymmetric acetylation of the prochiral 2-benzylpropane-1,3-diol **3a**

Next, the effect of temperature on the conversion of the prochiral diol **3a** to monoacetate (*R*)**-4a** in asymmetric acetylation was investigated in a continuous flow reactor filled with Lipase PS (Figure 2).

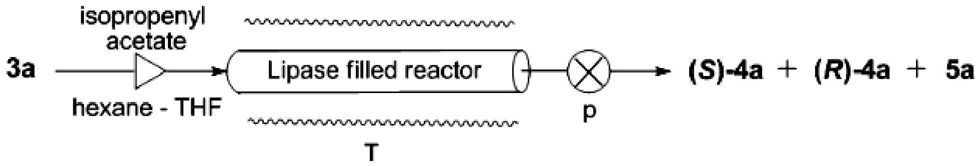


Figure 2. Continuous flow system for the asymmetric acetylation of 2-benzylpropane-1,3-diol **3a**

It was found that the conversion is significantly influenced by the temperature (Figure 3). At lower temperatures (a constant flow rate of 0,1 mL/min) less diacetate **5a** was formed besides the same amount of monoacetate **4a** (~98 %). This imply increased enantiotope selectivity of the biocatalyst at lower temperature.

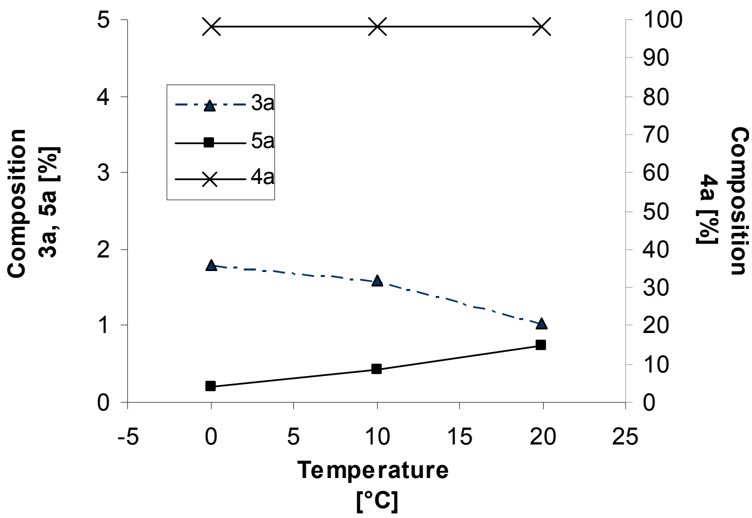


Figure 3. Products of the asymmetric acetylation of 2-benzylpropane-1,3-diol **3a** at different temperatures in Lipase PS-filled continuous flow reactor

Dependence of the effect of temperature on the enantiomeric excess in the asymmetric biotransformation of 2-benzylpropane-1,3-diol **3a** in continuous flow system investigated with a sol-gel immobilized OcTMOS/TMOS/Celite Lipase AK (lipase from *Pseudomonas fluorescens*; prepared by our research group), which has exhibited a good but not extremely high enantioselectivity. Instead of the expected monotonous decrease of the enantioselectivity with increasing temperature, the enantiomeric excess of the monoacetate (*R*)-**4a** had a maximum at approximately 40 °C (*ee* = 90 % at 0,1 mL/min; *ee* = 91 % at 0,2 mL/min) (Figure 4).

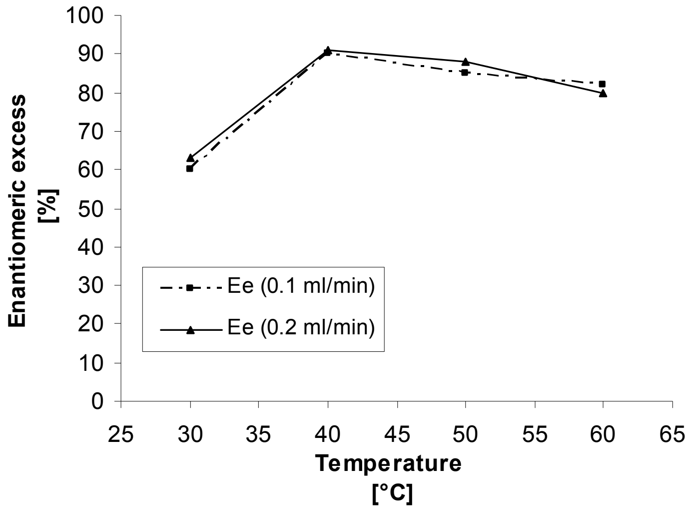


Figure 3. Dependence of the enantiomeric excess of the forming (R)-**4a** in the asymmetric acetylation of 2-benzylproprane-1,3-diol **3a** on the temperature at different flow rates in sol-gel Lipase AK-filled continuous flow reactor

CONCLUSION

Our results indicated that prochiral diols **3a-d** can be effectively transformed to chiral monoacetates **4a-d** by various enzymes. It was also demonstrated that continuous flow mode packed-bed reactors can be effectively used for asymmetric biotransformation of prochiral diol **3a**. Investigating of the effect of the temperature for the conversion and the enantiotope selectivity in the lipase PS-catalyzed process indicated that the amount of diacetate **5a** decreased with the temperature while the amount of the monoacetate **4a** was constant. In the sol-gel Lipase AK-catalyzed process, the enantiomeric excess of (*R*)-**4a** had a maximum at about 40 °C within the investigated temperature range (30-60 °C).

EXPERIMENTAL SECTION

Methods

GC analyses were carried out on ACME 6100 or Agilent 4890D instruments equipped with FID detector and Hydrodex-β-6-TBDAc column (50 m × 0.25 mm × 0.25 μm film with acetylated β-cyclodextrin; Macherey& Nagel) or Hydrodex-β-6-TBDM column (25 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-O-methyl-6-O-t-butyldimethylsilyl)-β-cyclodextrine; Macherey&

Nagel) using H_2 carrier gas (injector: 250 °C, detector: 250 °C, head pressure: 10 psi, 50:1 split ratio). Optical rotations were determined on a Perkin Elmer 241 polarimeter. The continuous flow bioreactions were performed by X-Cube^{TM} laboratory flow reactor (X-Cube^{TM}—trademark of ThalesNano, Inc.; Ser. No.: 002/2006) equipped with enzyme filled CatCart^{TM} columns (CatCart^{TM}—registered trademark of ThalesNano Inc.: stainless steel (INOX 316 L); inner diameter, 4 mm; total length, 70 mm; packed length, 65 mm; inner volume, 0.816 mL).

*Synthesis of the unsaturated diesters **2a-d***

The corresponding aldehyde **1a-d** (50 mmol), diethyl malonate (50 mmol, 8.01 g), piperidine (60 mmol, 5.11 g) and acetic acid (100 mg) were dissolved in toluene (100 mL) in a flask equipped with a Dean & Stark trap. Stirring at reflux for 6 h resulted in water (~1 mL) formation. The reaction mixture was washed with 5% HCl (30 mL), saturated $NaHCO_3$ (30 mL) solution and brine (30 mL) and dried over sodium sulfate. The solvent was distilled off from the resulting solution by rotary evaporation. The residue was separated by chromatography on silica gel to give **2a-d** in good yields (78-84 %).

*Synthesis of the prochiral diols **3a-d***

To a solution of the unsaturated ester **2a-d** (20 mmol) in ethanol (100 mL), $NaBH_4$ (100 mmol, 3.78 g) was portionwise added. The resulting mixture was stirred at room temperature for 3 days. After slow addition of acetic acid (100 mmol, 6.0 g), the ethanol was removed from the reaction mixture under reduced pressure. The residue was dissolved in ethyl acetate, washed with 5% HCl (20 mL), saturated $NaHCO_3$ solution (20 mL) and brine (20 mL) and dried over sodium sulfate. The solvent was distilled off from the resulting solution by rotary evaporation. The residue was separated by chromatography on silica gel to yield the diols **3a-d** in good yields (65-80 %).

*Enantiotope selective acetylation of diols **3a-d** in shake vials*

To a solution of the prochiral diol **3a-d** (20 mg) in hexane-THF-isopropenyl acetate 2:1:1 mixture (2 mL), the enzyme (20 mg) was added in a sealed amber glass vial and the resulting mixture was shaken (1000 rpm) at 25°C for 24h. The reactions were analyzed by GC and optical rotation measurements.

*Enantiotope selective acetylation of diol **3a** in continuous mode*

The solution of prochiral diol **3a** (10 mg mL^{-1}) in hexane-THF-isopropenyl acetate 2:1:1 mixture was pumped through the enzyme-filled (Lipase PS or sol-gel Lipase AK) columns at different temperatures and flow rates without choking. At a run under certain conditions, samples were analyzed by TLC and GC at every 10 min between the start and 60 min.

ACKNOWLEDGMENTS

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