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Lipase-catalyzed Irreversible Transesterification of Secondary Alcohols Using Isopropenyl Acetate

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Summary. Asymmetric acetylation of a set of secondary alcohols with the innocuous acyl donor isopropenyl acetate catalyzed by a lipase from *Pseudomonas cepacia* immobilized on ceramic particles (PSL-C) in toluene as organic medium afforded the chiral alcohols and the corresponding acetates in high enantiomeric excess (up to 99%). An effective baseline separation of the enantiomers of both substrate and product was performed in one analysis without derivatization using gas chromatography on a new chiral stationary phase (CSP) Chirasil-β-Dex containing an undecamethylene spacer (C11-Chirasil-Dex).

Keywords. Enantiomeric resolution; Irreversible transesterification; Lipase; Secondary alcohols; Gas chromatography.

Introduction

Enzymes and especially lipases are biological catalysts of extraordinary selectivity and efficiency. Enantiomerically pure secondary alcohols are useful chiral auxiliaries in organic synthesis and they are preferably synthesized by enzymatic kinetic resolution of the racemates. This biochemical transformation process has become a standard reaction protocol in organic synthesis [1]. In the presence of a suitable acyl donor and enzyme as well as solvent and at the optimum temperature one enantiomer of the racemic mixture is selectively transferred to the corresponding ester leaving the second unreacted enantiomer in optically pure form. The reversible enzymatic process usually requires a long reaction time and a large excess of the ester as acyl donor to achieve a reasonable degree of conversion [2]. In order to render the process irreversible, the use of various activated esters like vinyl acetate

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[3], trifluoroethyl esters, chloroethyl esters [4], cyanomethyl esters [5], and acid anhydrides [6, 7] have been reported. However, these methods have some drawbacks, like the generation of toxic side products, thereby deactivating the enzyme and inhibiting the formation of the required products [8], or the generation of water which causes the undesired hydrolysis of the enantiomerically pure ester, leading to a decrease in the conversion and in the enantiomeric excess. The generation of acetaldehyde resulting from the tautomerization of vinylalcohol when using vinylacetate as acyl donor can enter into reactions with many functional groups, particularly with the lysine side chains in enzymes leading to a deactivation of various lipases, especially *Geotrichum candidum* and *Candida rugosa*. The latter is being one of the most important lipases having a broad applicability to substrates in organic synthesis [9]. In contrast to the transesterification with vinyl acetate, isopropenyl acetate is an innocuous acyl donor releasing acetone as a non-harmful byproduct to enzymes.

The ways in which the efficiency and the practicability of the lipase-catalyzed kinetic resolution of racemates are defined is depending on a large numbers of factors [10, 11]. The development of accurate non-chiroptic methods for the determination of enantiomeric purity has been critical for the development of enantioselective synthesis. The modern and most sensitive methods used in the determination of enantiomeric purity of the outcome of kinetic resolution reactions, allowing a detection as little as 0.1% of one enantiomer in the presence of another, are chiral GC and HPLC methods. For an efficient monitoring of the reaction progress, enantioselective gas chromatography (GC) was the method of choice for the simultaneous determination of the enantiomeric excesses of both substrate and product in enantioselective reactions.

Although a large number of chiral stationary phases (CSPs) have been developed [12, 13], the choice of an appropriate column is still difficult. Modified cyclodextrins (CDs) have been widely used as chiral stationary phases for GC separation of racemic chiral compounds. These CD derivatives are dissolved in polysiloxane phases and are used for preparing efficient capillary columns [14, 15]. Chirasil-β-Dex, a polysiloxane-anchored permethylated β-cyclodextrin with 3, 5, and 8-spacer have been successfully used as CSP in GC [16]. In this contribution, we report on the investigation of Chirasil-β-Dex with a new 11-spacer (C11- Chirasil-Dex) [17] as CSP in GC for the gas chromatographic enantiomers separation of a set of secondary alcohols and their corresponding acetates. The utility of the titled CSP is demonstrated in the analysis of the enantiomeric excesses of substrates and products resulting from the lipase-catalyzed enantioselective transesterification of secondary alcohols using isopropenyl acetate as an innocuous acyl donor in toluene as organic solvent.

Results and Discussion

Chirasil-β-Dex with a new C11-spacer (C11-Chirasil-Dex, Fig. 1) has been synthesized according to Ref. [16]. ^{1}H, ^{13}C NMR, and IR spectroscopy confirmed the chemical link of the cyclodextrin moiety to the polysiloxane backbone, however, the regioselective position of the C11 spacer (O6 *vs.* O2) is still elusive. The new stationary phase was used in GC to separate a set of secondary alcohols and their corresponding acetates. All secondary alcohols (Fig. 2) and their corresponding

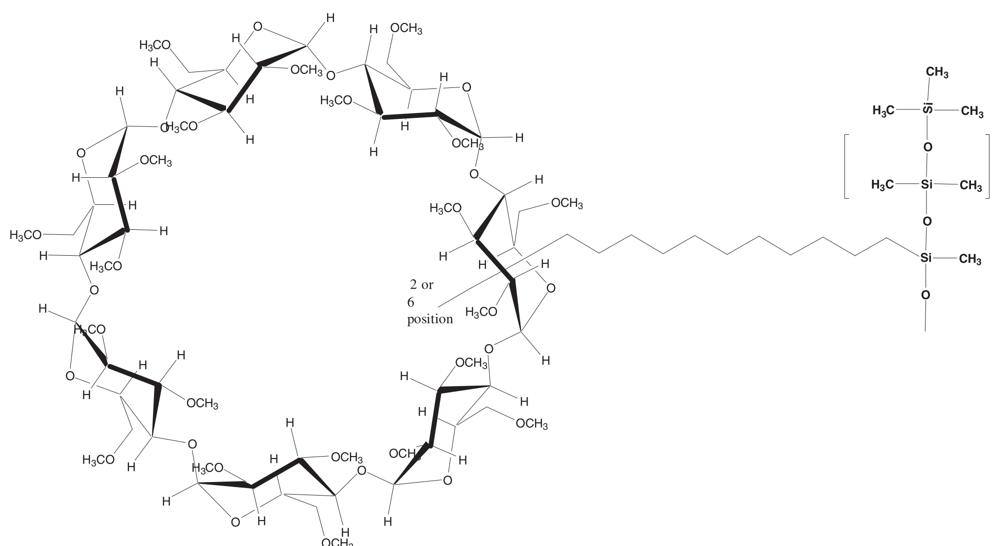


Fig. 1. Structure of the permethylated β-cyclodextrin with a new C11-spacer (C11-Chirasil-Dex)

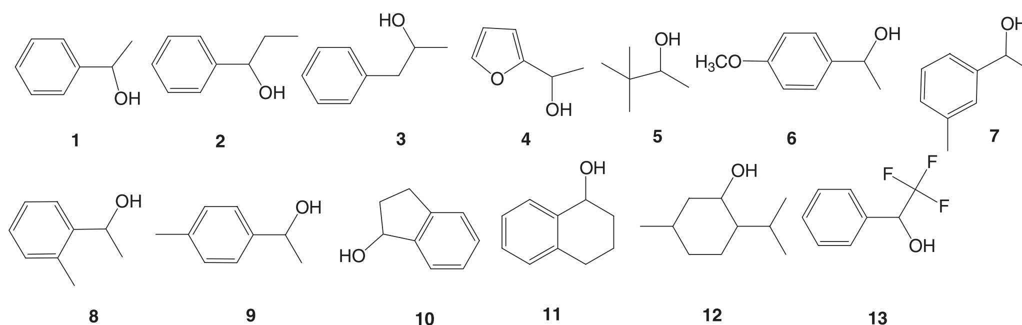


Fig. 2. A set of secondary alcohols tested in lipase-catalyzed resolution using isopropenyl acetate as acyl donor in toluene; all have been base-line separated on Chirasil-β-Dex with a new C11- spacer (C11-Chirasil-Dex)

acetates (racemic mixture or enantiomerically pure resulted from the enzymatic reaction) tested in this investigation were baseline separated (for GC parameters see Table 1).

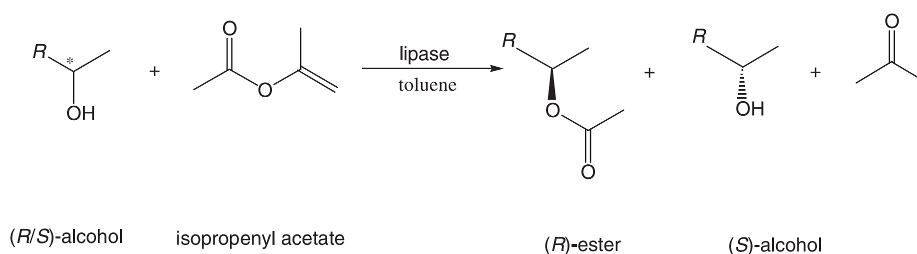
The unusual elution order observed in the resolution of **3** and **5** using the new CSP should be noted. The utility of the new CSP is demonstrated in the analysis of the enantiomeric excesses of substrates and products resulting from the lipase-catalyzed enantioselective transesterification of secondary alcohols using isopropenyl acetate as an innocuous acyl donor in toluene as an organic solvent (Scheme 1). The reaction was carried out at 40°C at a molar ratio of isopropenyl acetate to racemic alcohol of 2:1 to ensure the irreversibility of the reaction.

The results of the lipase-catalyzed transesterification of secondary alcohols are summarized in Table 2. In the transesterification of (*R*,*S*)-secondary alcohols, the (*R*)-alcohol was the faster reacting enantiomer yielding the (*R*)-acetate in

Table 1. Oven temperature (T), retention time (t_R), resolution (R_s) and the separation factor (α) of the simultaneous baseline separation of racemic secondary alcohols and their corresponding acetates

compound[1]	oven temperature/°C[2]	$t_R(R)$/min	$t_R(S)$/min	R_s	α
1	95	10.1	11.2	5.83	1.12
1a		7.3	6.2	9.10	1.19
2	100	14.2	15.2	4.20	1.08
2a		8.0	7.4	4.74	1.09
3	80	27.1	26.1	2.02	1.04
3a		30.2	24.9	11.34	1.22
4	85	4.6	4.8	1.88	1.05
4a		3.6	4.4	9.92	1.26
5	50	5.4	5.8	1.78	1.07
5a		6.1	4.9	7.91	1.26
6	110	20.5	22.1	4.76	1.08
6a		19.0	16.7	8.45	1.14
7	105	10.6	11.5	4.68	1.09
7a		7.2	6.5	5.83	1.12
8	90 for 13 min, then increase	18.2	20.0	10.66	1.10
8a	to 120 at the rate of 10/min	12.0	11.5	2.34	1.04
9	105	9.3	10.5	6.63	1.14
9a		8.4	7.4	7.44	1.14
10	100	22.1	21.4	1.79	1.03
10a		17.9	17.2	2.27	1.04
11	120	16.7	15.9	2.68	1.05
11a		12.8	13.3	2.64	1.04
12	100	10.6	10.3	1.64	1.04
12a		7.9	9.1	7.91	1.16
13	90 for 4 min, then increase	10.9	11.3	3.47	1.04
13a	to 120 at the rate of 10/min	3.4	3.3	2.32	1.06

[1] **a** is assigned to the acetate of the corresponding secondary alcohol; [2] the head pressure is 50 kPa, the injector temperature was 200°C and the FID temperature was 250°C



Scheme 1

high *ee* and leaving (*S*)-alcohol as enantiomerically pure unreacted enantiomer. The absolute configuration of the resulting alcohols and esters were determined by comparison with authentic samples. Molecular sieves (4 Å) were added prior to the enzymatic reaction to scavenge the liberated acetone (as a byproduct from the enzymatic reaction), thereby shifting the equilibrium into the desired direction. In

Table 2. Lipase-catalyzed transesterification of secondary alcohols using isopropenyl acetate as acyl donor in toluene as organic solvent

compound	time/h	ee_s/% (**S**)-alcohol	ee_p/% (*R*)-ester	conversion/%	*E*
1	4	>99	78	56	93
2	4	>99	92	52	287
3	4	>99	93	51	>300
4	4	>99	5	94	4
5	24	94	76	55	26
6	4	>99	87	53	141
7	4	>99	78	56	78
8	15	>99	36	73	16
9	24	>99	49	67	23
10	4	>99	18	84	9
11	4	99	29	77	10
12	15	85	88	49	42
13	15	>99	53	65	29

ee_s: enantiomeric excess of substrate (alcohol); ee_p: enantiomeric excess of product (ester); *E*: enantiomeric ratio

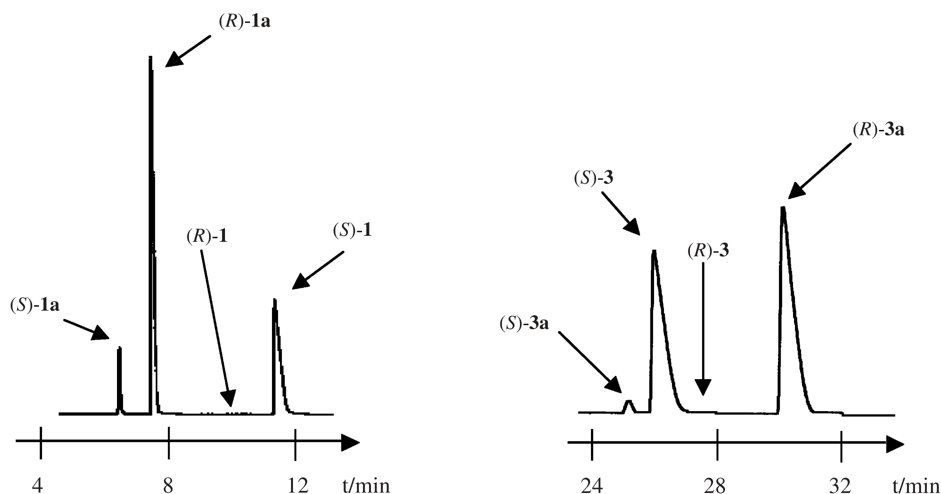


Fig. 3. Lipase-catalyzed transesterification of **1** (4 h) and **3** (15 h) using isopropenyl acetate as acyl donor in toluene

the case of alcohols **4**, **10**, and **11**, the reaction was very fast. This was clear from the fact that the faster reacting enantiomer reached its state of equilibrium more rapidly and prolonged reaction time lead to some further conversion of the slower reacting enantiomer and, thus, giving a less satisfactory enantiomeric excess of the products (Table 2, compounds **4**, **10** and **11**).

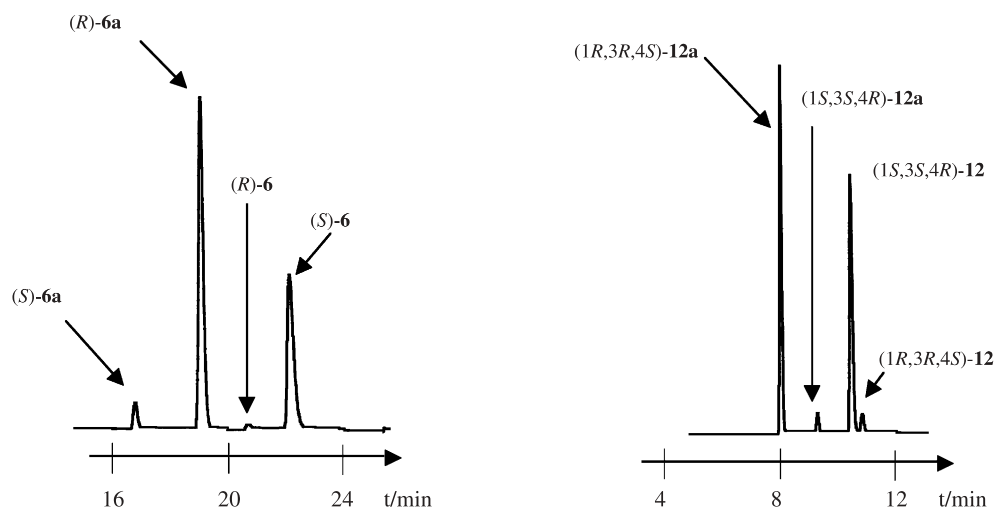


Fig. 4. Lipase-catalyzed transesterification of **6** (4 h) and **12** (15 h) using isopropenyl acetate as acyl donor in toluene

Conclusion

The lipase-catalysed enantioselective transesterification of a set of secondary alcohols was performed using the innocuous acyl donor isopropenyl acetate in toluene. A baseline separation of both substrate and product was achieved using GC equipped with a new CSP consisting of a polysiloxane-anchored permethylated β-cyclodextrin with a new 11-spacer (C11-Chirasil-Dex).

Experimental

Chemicals and Instrumentation

All alcohols were commercially available and dried over molecular sieve prior to use. The racemic esters were synthesized on an analytical scale to optimize a baseline separation of the enantiomers by gas chromatography. Lipase from *Pseudomonas cepacia* immobilized on ceramic particles (PSL-C) was a gift from Amano (Nagoya, Japan).

General Procedure for the Synthesis of Esters (Analytical Scale)

Acetic anhydride (30 mm^3) is added to 20 mm^3 of alcohol in 200 mm^3 of pyridine. The mixture was kept for 1 h at 100°C and injected without further cleanup.

Lipase-catalyzed Irreversible Transesterification of Secondary Alcohols

All reactants (alcohol, ester) were stored over activated molecular sieves (4 Å). Racemic alcohol (0.5 mmol) and 1 mmol of isopropenyl acetate were dissolved in 3 cm^3 of toluene in a 5 cm^3 reaction vial. The reaction mixture was thermostated in an oil bath to 40°C. Then, 100 mm^3 of the reaction mixture were withdrawn for GC analysis ($t = 0$ of sample). Afterwards, 100 mg of lipase (PSL-C) were added followed by the addition of 100 mg of molecular sieves (4 Å). Samples (100 mm^3) were taken after several time intervals and centrifuged to separate the lipase. The organic layer was diluted by

100 mm^3 of solvent and the reaction progress was monitored qualitatively by thin layer chromatography. An aliquot of the supernatant was used for the GC analysis. When maximum conversion was reached, the reaction was terminated by filtration, the enzyme was washed with solvent and then with acetone, and the lipase powder was dried in air for further use.

GC Analysis Using a CSP

Gas-chromatographic analysis was performed on a Hewlett Packard instrument (Waldbronn, Germany) equipped with a flame ionisation detector (FID). The chiral stationary phase C11-Chirasil-Dex was coated on a non-deactivated 19 m × 0.25 mm fused silica capillary column (0.25 μm film thickness) according to Ref. [18]. The analytical conditions were: injector temperature 200°C; FID temperature 200°C; oven temperature varying from 50 to 120°C depending on the analyzed compound for the simultaneous separation of enantiomers of both substrate and product. Hydrogen was used as carrier gas (50 kPa column head pressure). The enantiomeric excess of both substrate and product as well as conversion and enantiomeric ratio E were determined as described previously [10, 11].

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