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Lipase-catalyzed access to enantiomerically pure (*R*)- and (*S*)-*trans*-4-phenyl-3-butene-2-ol

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Abstract—The enzymatic kinetic resolution of (*RS*)-*trans*-4-phenyl-3-butene-2-ol was investigated by screening a range of lipases both for enantioselective transesterification and for enantioselective hydrolysis of its acetate. The lipase from *Pseudomonas cepacia* immobilized on diatomaceous earth (PSL-D)-catalyzed asymmetric transesterification was performed on gram scale using isopropenyl acetate as an innocuous acyl donor in organic media affording the (*S*)-alcohol in high enantiomeric excess (>99% ee) and enantiomeric ratio *E* >150. The lipase (*Candida antarctica B*, CAL-B)-catalyzed asymmetric hydrolysis of the racemic acetate was performed on gram scale in phosphate buffer affording the (*R*)-alcohol in high enantiomeric excess (>99% ee) and enantiomeric ratio *E* >150. The investigation demonstrates that the transesterification of the racemic alcohol in organic solvent was faster than the hydrolysis of the corresponding acetate in phosphate buffer. A GC method was developed to achieve an effective analytical separation of the enantiomers of both substrate and product in one analysis using the chiral stationary phase heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin.

1. Introduction

Enantiomerically pure allylic alcohols are useful chiral auxiliaries in organic synthesis, as many stereoselective transformations can be performed by taking advantage of the 1,3-allylic motif of such systems.[1] They are usually obtained by kinetic resolution of the racemic alcohols using Sharpless' epoxidation[2] or via enzyme-mediated transformations.[3] The latter biochemical process has become a standard reaction protocol in organic synthesis.[4]

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.[5] In order to render the process irreversible, various activated esters such as vinyl acetate,[6] trifluoroethyl esters, chloroethyl esters,[7] cyanomethyl esters,[8] and acid anhydrides[9,10] have been utilized. However, these methods have some drawbacks, including the generation of reactive side products thereby deactivating the enzyme and inhibiting the formation of the required products,[11] and the generation of water which causes the undesired hydrolysis of the enantiomerically enriched ester, leading to a decrease in the conversion and the enantiomeric excess, additionally, the generation of acetaldehyde resulting from the tautomerization of vinyl alcohol when using vinyl acetate as the acyl donor leads to deactivation of various lipases.[12]

The enantiomeric allylic alcohol (*S*)-(−)- and (*R*)-(+)-*trans*-4-phenyl-3-butene-2-ol **1** are frequently used as chiral building blocks for numerous biologically active compounds and they are used for studies of reaction mechanisms.[13] Therefore, it is desirable that both enantiomers of this chiral starting material are available in high enantiomeric purity. Different routes to chiral *trans*-4-phenyl-3-butene-2-ol have been reported using ruthenium-[14] or enzyme-catalyzed asymmetric reduction of the corresponding ketone.[15] The lipase-catalyzed transesterification of (*RS*)-*trans*-4-phenyl-3-butene-2-ol (*RS*)-**1** using vinyl acetate[6] or dimethyl malonate[13] as acyl donor has been reported, however, the use of vinyl acetate as acyl donor in enzymatic reactions is restricted to enzymes which are not sensitive to the acetaldehyde released from the tautomerization of vinyl alcohol. In the case of using dimethyl malonate as the acyl donor, the enantiomeric excess did not exceed 93% ee (*S*)-**1** and in addition, the conditions applied in the kinetic resolution, e.g. reduced pressure (8 Torr) and the use of $KHCO_3$ limit their application at a large scale.

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Herein, we report an efficient method for the gram scale preparation of enantiomerically pure (*R*)- and (*S*)-*trans*-4-phenyl-3-butene-2-ol **1** using two different approaches based on the lipase-catalyzed transesterification of the racemic alcohol (*RS*)-**1** using isopropenyl acetate as acyl donor in toluene as solvent affording the (*S*)-alcohol (*S*)-**1** and (*R*)-ester (*R*)-**2**, and the lipase-catalyzed enantioselective hydrolysis of the corresponding acetate (*RS*)-**2** affording the (*R*)-alcohol (*R*)-**1** and (*S*)-ester (*S*)-**2**, respectively.

The precise determination of the enantiomeric excess of substrates and products is an important prerequisite for the detailed investigation of the enzyme-catalyzed kinetic resolution. For an efficient monitoring of the reaction progress, enantioselective gas chromatography (GC) is the method of choice for the simultaneous determination of the enantiomeric excess of both substrate and product as well as the conversion. Although a large number of chiral stationary phases have been developed,[16] the selection of an appropriate column is still difficult. A baseline separation of both the racemic alcohol (*RS*)-**1** derivatized as the isopropylcarbamate (*RS*)-**3** and the acetate (*RS*)-**2** was achieved using heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as the chiral stationary phase in GC.[17]

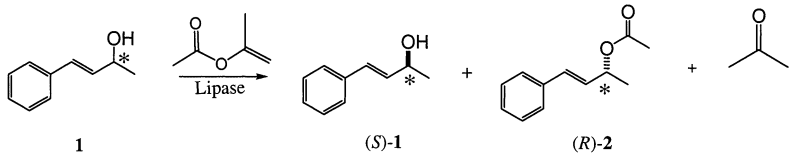
2. Results and discussion

2.1. Lipase-catalyzed kinetic resolution of (*RS*)-*trans*-4-phenyl-3-butene-2-ol (*RS*)-1

Different lipases were screened in resolving racemic *trans*-4-phenyl-3-butene-2-ol (*RS*)-**1** either in the enantioselective transesterification of (*RS*)-**1** using isopropenyl acetate as an innocuous acyl donor in toluene as solvent in non-aqueous medium or the enantioselective hydrolysis of the corresponding acetate (*RS*)-**2** using phosphate buffer (pH 6) in aqueous medium. Among the lipases tested for the enantioselective transesterification of (*RS*)-**1** and hydrolysis of (*RS*)-**2** were PSL, PFL, PSL-C, PSL-D, ANL, CRL, CCL, CAL-B, Novozyme 525 L and Lipozyme RM immobilized (RML). The transesterification of **1** was carried out at 40°C in toluene at a molar ratio of isopropenyl acetate to racemic (*RS*)-**1** of 2:1 to ensure the irreversibility of the reaction (cf. Scheme 1). The results of the lipase-catalyzed transesterification of **1** are summarized in Table 1.

In the transesterification of (*RS*)-**1**, (*R*)-**1** was the faster reacting enantiomer, yielding (*R*)-**3** in high ee and leaving (*S*)-**1** as enantiomerically pure unreacted enantiomer. Both lipases from *Pseudomonas cepacia* immobilized on ceramic particles (PSL-C) or immobilized on diatomaceous earth (PSL-D) displayed high enantioselectivity towards **1** (cf. Table 1). In regard to the ee of the remaining substrate (*S*)-**1** and that of the product (*R*)-**3** as well as the rate of conversion (51% in 4 h) and enantiomeric ratio $E=680$, PSL-D was the best lipase employed in the transesterification of **1**. The immobilized lipase from Novozyme (CAL-B) also showed high enantioselectivity towards **1**, however, the enantiomeric ratio *E* was much lower in comparison with the PSL-D catalyzed transesterification of **1**. Other enzymes showed low to moderate enantioselectivities and reaction rates in the transesterification of **1** (cf. Table 1).

P. cepacia lipase immobilized on diatomaceous earth (PSL-D) was the enzyme of choice used in the transes-



Scheme 1. Lipase-catalyzed asymmetric transesterification of racemic (*RS*)-**1** using isopropenylacetate as acyl donor in toluene as organic solvent.

Table 1. Lipase-catalyzed asymmetric transesterification of (*RS*)-**1** using isopropenyl acetate in toluene

Lipase	Time (h)	[%] ee_s (*S*)-**1**	[%] ee_p (*R*)-**2**	% conversion	*E*
PSL	9	97	86	52	61
PSL-C	4	>99	91	52	227
PSL-D	4	>99	96	51	680
PFL	9	>99	92	52	284
ANL	48	52	41	55	4
CCL	24	50	57	47	6
CRL	48	44	46	49	4
CAL-B	6	99	80	55	93
Novozyme 525 L	9	93	97	49	322
RML	48	46	22	67	2

Ee_s: enantiomeric excess of substrate (alcohol). Ee_p: enantiomeric excess of product (ester).
E: enantiomeric ratio.

terification of **1** using isopropenyl acetate in toluene on a preparative scale. An E value of more than 300 was observed and the reaction was terminated after 4 h yielding (*S*)-**1** with more than 99% ee and the ester (*R*)-**3** was recovered with 86% ee determined by capillary GC after 50% conversion. 4 Å molecular sieves were added in order to scavenge the acetone liberated as a by-product from the lipase-catalyzed reaction using isopropenyl acetate as acyl donor. The beneficial effect of molecular sieves was reported previously in lipase-catalyzed transesterification of 1-(2-furyl)ethanol using isopropenyl acetate in organic solvents.[20,21]

Compared to the transesterification experiments in the non-aqueous medium described above, the enzymatic hydrolysis of the acetate (*RS*)-**2** in aqueous medium proceeded slowly (cf. Scheme 2). Only moderate conversion (45%) and high ee (up to 99% for the alcohol (*R*)-**1** and 80% for the remaining unreacted ester (*S*)-**2**) (cf. Table 2) was achieved after 24 h using Novozyme IM (CAL-B). In all cases, the hydrolysis reaction proceeded quickly until reaching 9 h, afterwards the reaction proceeded very slowly. This is probably due to product inhibition resulting from the accumulation of products in the enzymatic hydrolysis reaction of (*RS*)-**2**, thus competing with the active site of the enzyme. CAL-B (Novozyme) not only showed the best performance in the analytical runs, but also gave an excellent result for the hydrolysis of (*RS*)-**2** on a multigram scale (in 24 h, ee_s 74%, ee_p >99, conv. 43 and E >300).

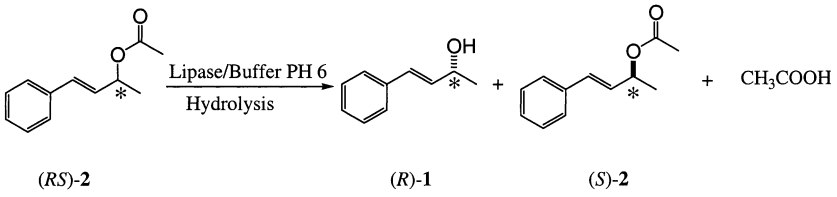
3. Experimental

3.1. Materials and methods

^1H and ^{13}C NMR spectra were recorded on an AC 250 Bruker spectrometer. Specific rotations [α] were measured with a Perkin–Elmer 241 polarimeter operating at the sodium D line at 20°C.

3.2. Chemicals and enzymes

All chemicals were purchased from Fluka (Switzerland). Lipases from *P. cepacia* (PSL), *Pseudomonas fluorescens*, AK (PFL), *P. cepacia* immobilized on ceramic particles (PSL-C), *P. cepacia* immobilized on diatomaceous earth (PSL-D), and *Aspergillus niger* (ANL) were gifts from Amano (Nagoya, Japan). Lipase from *Candida rugosa* type VII (CRL) was from Sigma (Steinheim, Germany). Lipase from *Candida cylindracea* (CCL) was purchased from Fluka (Buchs, Switzerland). Novozyme 435, an immobilized non-specific lipase (*Candida antarctica B*, CAL-B) produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, Lipozyme RM IM, RML, an immobilized 1,3-specific lipase from *Rhizomucor miehei* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and Novozyme 525 L, the non-immobilized liquid version of Novozym 435, were gifts from Novozymes, Bagsvaerd, Denmark.



Scheme 2. Lipase-catalyzed asymmetric hydrolysis of racemic (*RS*)-**2** using phosphate buffer (pH 6) and toluene as organic solvent.

Table 2. Lipase-catalyzed enantioselective hydrolysis of (*RS*)-**2** using phosphate buffer (pH 6)

Lipase	Time (days)	[%] ee_s (*S*)-**2**	[%] ee_p (*R*)-**1**	% conversion	E
PSL	2	57	99	36	>150
PSL-C	4	30	99	23	>150
PSL-D	2	27	99	21	>150
PFL	4	56	>99	36	>150
ANL	2	41	99	29	>150
CCL	4	40	54	43	5
CRL	4	33	99	25	>150
CAL-B	1	80	99	45	>150
Novozyme 525 L	4	59	99	37	>150
RML	4	39	99	28	>150

Ee_s: enantiomeric excess of substrate (ester). Ee_p: enantiomeric excess of product (alcohol).
E: enantiomeric ratio.

3.3. Synthesis and biochemical transformation reactions

3.3.1. Synthesis of (*RS*)-*trans*-4-phenyl-3-butene-2-ol, (*RS*)-1. *trans*-4-Phenyl-3-butene-2-one (benzalacetone, 2.04 g, 0.014 mol) was dissolved in methanol (40 ml) and stirred in a 100 ml round bottom flask placed in an ice-bath. A solution of $NaBH_4$ (0.56 g, 0.015 mol) in water (40 mL) was added dropwise over 1 h, then the reaction was stirred for 3 h in an ice-bath and then for a further 2 h at room temperature. The volume was concentrated to 30 ml. Hot water (20 ml) was added and the reaction mixture was filtered. The residue was washed several times with 20 ml portions of hot water and extracted twice with *n*-hexane/dichloromethane (1:4 v/v). The organic layer was separated and the solvent was removed under reduced pressure yielding a colorless oil (1.9 g, 93%); ^{1}H NMR ($CDCl_3$) δ 7.11–7.27 (m, 5H, aromatic), 6.39 (d, 1H), 6.09 (dd, 1H), 4.31 (m, 1H), 2.39 (s, 1H, OH), 1.23 (d, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 137.15, 134.04, 129.64, 129.0, 128.0, 127.4, 69.20, 23.81.

3.3.2. Synthesis of (*RS*)-2-acetoxy-4-phenyl-but-3-ene, (*RS*)-2. Compound **1** (14.8 g, 0.1 mol) was stirred with acetic anhydride (17 ml, 0.18 mol) in pyridine (65 ml). The mixture was stirred for 24 h at room temperature. Water was added and the extraction was performed using ethyl acetate. The organic layer was washed with 1N HCl followed by water. Drying was performed using anhydrous sodium sulphate. The solvent was removed under reduced pressure. The residue was purified by column chromatography using *n*-hexane/ethyl acetate (9:1 v/v) affording acetate **2** (8.8 g, 60%); ^{1}H NMR ($CDCl_3$) δ 7.18–7.29 (m, 5H, aromatic), 6.48 (d, 1H), 6.06 (dd, 1H), 5.41 (m, 1H), 1.91 (s, 3H, CH_3), 1.32 (d, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 174.75, 131.91, 128.88, 128.80, 128.68, 126.82, 124.52, 71.40, 21.62, 20.4.

3.4. General procedure for the lipase-catalyzed asymmetric transesterification of (*RS*)-1 (analytical scale)

Prior to the transformation, all reactants (alcohol, ester) were stored over activated 4 Å molecular sieves. racemic alcohol (*RS*)-**1** (74 mg, 0.5 mmol) and isopropenyl acetate (108.8 mg, 1 mmol) were dissolved toluene (3 ml) in a 5 ml reaction vial. The reaction mixture was heated at 40°C in a thermostatically controlled oil bath. Then, a 100 μl sample of the reaction mixture was withdrawn and derivatized with 10 μl isopropyl isocyanate at 100°C for 30 min, diluted with toluene and analyzed by GC ($t=0$ of sample). Afterwards, lipase (100 mg) was added followed by the addition of 4 Å molecular sieves (100 mg). Samples of the reaction mixture (100 μl) were taken after several time intervals. The samples were centrifuged to separate lipase. The organic layer was treated with isopropyl isocyanate, heated to 100°C for 30 min then diluted with toluene (100 μl) and analyzed by GC. The reaction progress was monitored qualitatively by thin layer chromatography using *n*-hexane/ethyl acetate (9:1 v/v) as eluent. 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 acetone and then dried in air for further use. Substrate (*S*)-**1** and product (*R*)-**2** were separated by flash chromatography over silica (*n*-hexane/ethyl acetate, 9:1).

3.5. General procedure for the lipase-catalyzed asymmetric hydrolysis of 2-acetoxy-4-phenyl-but-3-ene (*RS*)-2 (analytical scale)

Lipase (100 mg) was dissolved in potassium phosphate buffer (20 mM, pH 6, 2.8 ml) and added to a solution of the pure substrate (*RS*)-**2** (0.5 mmol) in toluene (1 ml) in a 5 ml reaction vial. The reaction mixture was heated at 40°C in a thermostatically controlled oil-bath. Samples (100 μl) of the reaction mixture (organic layer) were withdrawn at several time intervals and derivatized as described before for GC analysis. The reaction progress was monitored qualitatively by thin layer chromatography (*n*-hexane/ethyl acetate, 9:1). When maximum conversion was reached, the reaction was terminated by filtration. The enzyme was washed with acetone and then dried in air for further use. The unreacted acetate (*S*)-**2** and the product (*R*)-**1** were separated by flash chromatography over silica (*n*-hexane/ethyl acetate, 9:1).

3.6. General procedure for the gram-scale lipase-catalyzed production of enantiomerically pure (*S*)-*trans*-4-phenyl-3-butene-2-ol, (*S*)-1

Racemic alcohol (*RS*)-**1** (8.8 g, 0.06 mol) and a solution of isopropenyl acetate (24 g, 0.24 mol) in toluene (500 ml) were placed in a 1 L round-bottomed flask equipped with magnetic stirring bar and heated at 40°C in a thermostatically controlled oil bath. Samples (100 μl) of the reaction mixture were withdrawn and derivatized as described before for GC analysis ($t=0$). Lipase (3.08 g) (PSL-D) (0.35 mass equivalent) was added in one portion followed by the addition of 4 Å molecular sieves (5 g). The progress of the reaction was monitored by thin layer chromatography (*n*-hexane/ethyl acetate, 9:1) and GC. After 4 h the reaction reached 50% conversion, the enzyme was separated by Büchner-filtration and the solution was concentrated under reduced pressure. The residue was chromatographed on silica using *n*-hexane/ethyl acetate (9:1) affording (*S*)-**1** (4.1 g, >99% ee by GC, yield 47%) $[\alpha]_D^{20}$ −19.9 (*c* 1, CH_2Cl_2) [lit. $[\alpha]_D^{20}$ −24.5 (*c* 5.16, $CHCl_3$), 98% ee],[18] and (*R*)-**2** (4.3 g, 87% ee by GC, yield: 49%) $[\alpha]_D^{20}$ +74.2 (*c* 1, CH_2Cl_2).

3.7. General procedure for the gram-scale lipase-catalyzed production of enantiomerically pure (*R*)-*trans*-4-phenyl-3-butene-2-ol, (*R*)-1

Lipase (Novozyme 435, 3.15 g) was dissolved in potassium phosphate buffer (20 mM, pH 6, 250 ml) and added to a solution of (*RS*)-**2** (7.8 g, 40 mmol) in toluene (20 ml) at 40°C. The progress of the reaction was monitored by thin layer chromatography (*n*-hexane/ethyl acetate, 9:1). Samples (100 μl) were taken at several time intervals, the organic layer was derivatized

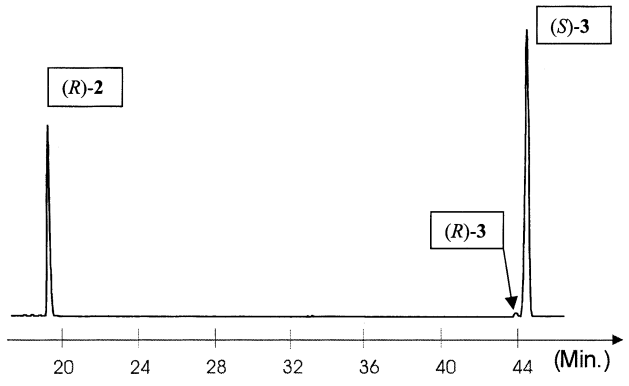


Figure 1. Gas-chromatographic separation of the enantiomer of both substrate **1** as carbamate **3** and product **2** on heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin of the *Pseudomonas fluorescens* lipase (PFL)-catalysed transesterification of **1** in toluene at $t=4$ h. $Ee_s=97\%$, $ee_p=96\%$, conv = 50%, $E=247$. The retention time of (*S*)-**2**, (*R*)-**2**, (*R*)-**3**, (*S*)-**3** were 18.5, 19.4, 43.7, 44.4 min, respectively.

as described before and analyzed by GC. When maximum conversion was reached (44% after 24 h), the enzyme was removed by filtration and the organic layer was separated and concentrated under vacuum. Substrate (*S*)-**2** and product (*R*)-**1** were separated by column chromatography (*n*-hexane/ethyl acetate, 9:1) affording (*R*)-**1** (3.3 g, >99% ee by GC, yield: 43%) $[\alpha]_D^{20}$ +19.9 (*c* 1, CH_2Cl_2), and (*S*)-**2** (3.5 g, 74.5% ee by GC, yield: 46%) $[\alpha]_D^{20}$ −71.2 (*c* 1, CH_2Cl_2).

3.8. Enantioselective gas-chromatographic analysis

The enantiomers of the underivatized allylic alcohol (*RS*)-**1** were not separated on heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin used as chiral stationary phase in GC, however, upon derivatization with acetic anhydride (the acetate (*RS*)-**2**) or isopropyl isocyanate (the carbamate (*RS*)-**3**), a baseline separation has been achieved (cf. Fig. 1) thus, the enantioselective analysis of the racemic alcohol *trans*-4-phenyl-3-butene-2-ol (*RS*)-**1** (substrate) as carbamate (*RS*)-**3** and acetate (*RS*)-**2** (product) were performed simultaneously on a gas chromatograph (Hewlett Packard 580, Waldbronn, Germany) equipped with a flame ionization detector (FID) (Fig. 2).

The chiral stationary phase heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (20% w/w) was dissolved in PS 86 (Gelest, ABCR GmbH & Co., Karlsruhe, Germany) and coated on a 25 m×0.25 mm (i.d.) fused silica capillary column (0.25 μm film thickness) according to the literature.[17] The analytical conditions were: injector temperature, 200°C; FID temperature, 250°C; oven temperature 130°C for 19 min then 30°C/min until 160 for 25 min. Hydrogen was used as the carrier gas (60 KPa column head pressure). The retention time of (*S*)-**2**, (*R*)-**2**, (*R*)-**3**, (*S*)-**3**, were 18.2, 19.1, 42.8, 43.2 min, respectively. Upon derivatization of the racemic alcohol (*RS*)-**1** with acetic anhydride, the elution of the resulting acetate (*RS*)-**2** was in the order (*S*)<(*R*) with 18.2 and 19.1 min, respectively, and a separation factor $\alpha=1.05$ and resolution $R_s=2.83$. Upon derivatization of the racemic alcohol (*RS*)-**1** with isopropyl isocyanate, the elution of the resulting carbamate (*RS*)-**3** was in the reverse order (*R*)<(*S*) with 42.8 and 43.2 min, respectively, and a separation factor $\alpha=1.01$ and resolution $R_s=1.07$ (cf. Fig. 1). Substrate **1** and product **2** were identified by using a GC/MSD-system HP 6890/5973 (Hewlett Packard, Waldbronn, Germany) equipped with an HP 7683 autosampler. The enantiomeric excess ee of both substrate (ee_s) and product (ee_p) as well as conversion (conv.) and enantiomeric ratio (*E*) were determined by the computer program available on the internet http://www.orgc.TUGraz.at/orgc/programs/selectiv/selectiv.htm, developed by Faber et al.[19]

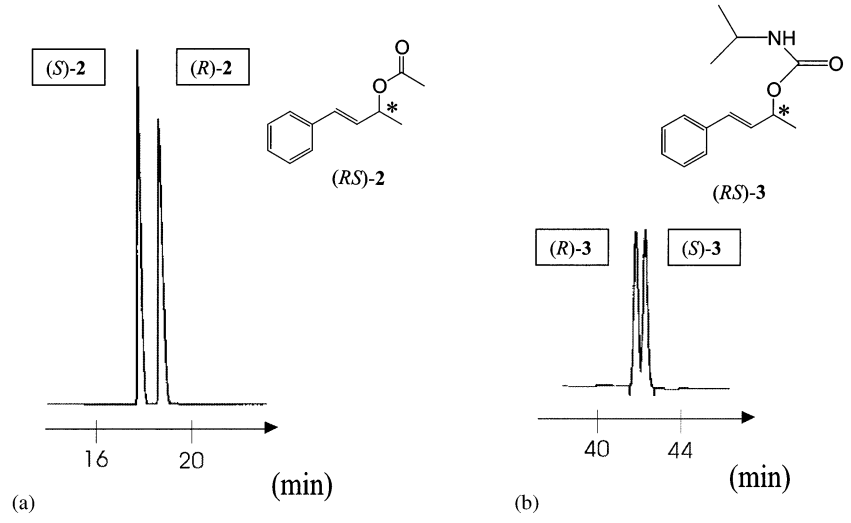


Figure 2. Gas-chromatographic separation of racemic (*RS*)-**1**, the isopropylcarbamate (*RS*)-**3** (Fig. 2b) and the racemic acetate (*RS*)-**2** (Fig. 2a) on heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin with a separation factor $\alpha=1.05$ and resolution $R_s=2.83$ for the ester (*RS*)-**2** and $\alpha=1.01$ and resolution $R_s=1.07$ for the carbamate (*RS*)-**3**. The oven temperature was 130°C isothermal for 20 min, then 30°C/min until 160°C for 25 min.

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