Lipase-Catalyzed Synthesis of Both Enantiomers of 3-Chloro-1-arylpropan-1-ols

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Abstract: The lipase-catalyzed synthesis of both enantiomers of 3-chloro-1-(4-fluorophenyl)propan-1-ol, 3-chloro-1-(4-iodophenyl)propan-1-ol, and 3-chloro-1-phenylpropan-1-ol is described. The procedure is based on the enantiomer-selective acylation of the racemic alcohols in presence of lipase from *Pseudomonas fluorescens* (LAK) followed by the lipase from *Candida rugosa* (CRL) mediated hydrolysis of previously obtained enantiomerically enriched 1-aryl-3-chloropropyl esters. For the production of enantiopure (*S*)-1-aryl-3-chloropropan-1-ols (99% ee, 34–42% yield) the reactions were stopped at higher conversions than the theoretical optimum of 50%, while for enantiopure (*R*)-1-aryl-3-chloropropyl acetates (99% ee) the reactions were stopped at lower conversions. The latter compounds were enzymatically hydrolyzed into the corresponding (*R*)-1-aryl-3-chloropropan-1-ols (97–99% ee, 18–24% yield). The absolute configuration of the resolution products was determined by VCD measurements combined with quantum chemical calculations.

Key words: enzymes, kinetic resolution, enantioselectivity, chiral resolution, alcohols, halide

The need for the production of optically active compounds as single enantiomers is growing rapidly. Classical resolution via diastereomeric salts of chiral compounds is still used widely, but in recent decades many catalytic enantioselective procedures have been developed for the synthesis of enantiopure compounds.

By virtue of their chiral nature, biocatalysts are predominantly preferred for the production of enantiopure stereoisomers. Thus, biocatalysis is widely used for the synthesis of pharmaceuticals and fine chemicals.[1–3] Biotransformations are already applied in industry for the manufacture of various products, such as drugs, agricultural chemicals, fine chemicals, and plastics.[4,5]

Biocatalytic methodologies based on enantiomer-selective kinetic resolutions of racemates and enantiotope-selective transformations of prochiral substrates have been devised for the efficient, economical, and environmentally friendly synthesis of optically active compounds.

Hydrolases and especially lipases, have proved to be versatile biocatalysts for synthetic biotransformations. Lipases can retain their high chemo-, regio-, and stereoselectivity and catalytic activity also in organic media and are versatile biocatalysts for kinetic resolutions,[6,7] deracemizations, and dynamic kinetic resolutions[8] of a broad range of substrates.

The low cost of many lipases makes them very useful catalysts for the kinetic resolution of variously substituted secondary aryl alcohols including 1-aryl-2-chloroethanols[9] and 1-aryl-3-chloropropan-1-ols.[10–15]

1-Aryl-3-chloropropan-1-ols are important racemic building blocks for the synthesis of pharmaceuticals, e.g. antifungal agents that act against different species of *Candida* and exhibit low cytotoxicity.[16] A number of derivatives obtained from the above-mentioned chiral building blocks have turned out to be potent inhibitors of *Candida albicans* strains resistant to Fluconazole, with minimal inhibitory concentrations (MICs) <10 μg mL^{-1}. Against dermatophyte strains (MICs ≤ 5 μg mL^{-1}), they have been found to be equipotent with Ketoconazole, Econazole, and Miconazole.[17] (*R*)- or (*S*)-1-Aryl-3-chloropropan-1-ols have served as starting materials for the synthesis of (*S*)- or (*R*)-oxetanes in enantiopure form.[18] Both enantiomers of 3-chloro-1-phenylpropan-1-ol are commercially available from Pharmacon Technology Co. Ltd., China. Several biocatalytic procedures are known for their preparation. Most of them are based on the enantiomer-selective enzymatic acylation of racemic 3-chloro-1-phenylpropan-1-ol[10] or on the stereoselective hydrolysis of their acylated counterparts.[11] For the Novozyme 435 catalyzed dynamic kinetic resolution, ruthenium complexes were used for the in situ racemization of 3-chloro-1-phenylpropan-1-ol.[12] Microbial biotransformations have also been developed for this purpose. Oxidoreductase from baker's yeast[13] and *Merulius tremellosus*[14] stereoselectively reduce 3-chloro-1-phenylpropan-1-one to give the desired product, but the occurrence of secondary undesired reactions involving the elimination of HCl, followed by the reduction of the in situ formed 1-phenylprop-2-en-1-one, leads to a low overall yield of the target compound. An interesting procedure for deracemization of the racemic substrate through the use of *Sphingomonas paucimobilis*, involving enzymatic redox reactions, fur-

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nished (*R*)-3-chloro-1-phenylpropan-1-ol[15] in 79% yield and with excellent stereoselectivity.

The present paper describes the biocatalytic synthesis of (*R*)- and (*S*)-3-chloro-1-(4-fluoro- and 4-iodophenyl)propan-1-ols and reports a comparison with the results obtained for the unsubstituted phenyl counterpart.

Ketones **1a** and **1c** are commercially available. The reduction of 3-chloro-1-(4-fluorophenyl)propan-1-one (**1a**) was achieved according to a literature procedure.[19] A modified reduction of **1c** was performed in order to obtain *rac*-**2c**. The Friedel–Crafts reaction of 3-chloropropionyl chloride with iodobenzene to obtain ketone **1b** was carried out as described in the literature,[20] while the reduction was performed as in the case of the unsubstituted ketone derivatives (Scheme 1).

For the acylation of *rac*-**2a–c** all the classical methods (acid anhydride or acid chloride in the presence of a base) failed. The yields were poor and several byproducts were formed. Lipase A from *Candida antarctica* (CaLA) is known to be an appropriate catalyst for the nonselective acylation of small racemic secondary alcohols. Thus, with vinyl esters as acyl donors, alcohols *rac*-**2a–c** were quantitatively transformed with CaLA into the corresponding racemic esters *rac*-**3a–c** in *n*-hexane, as shown in Scheme 1 (a).

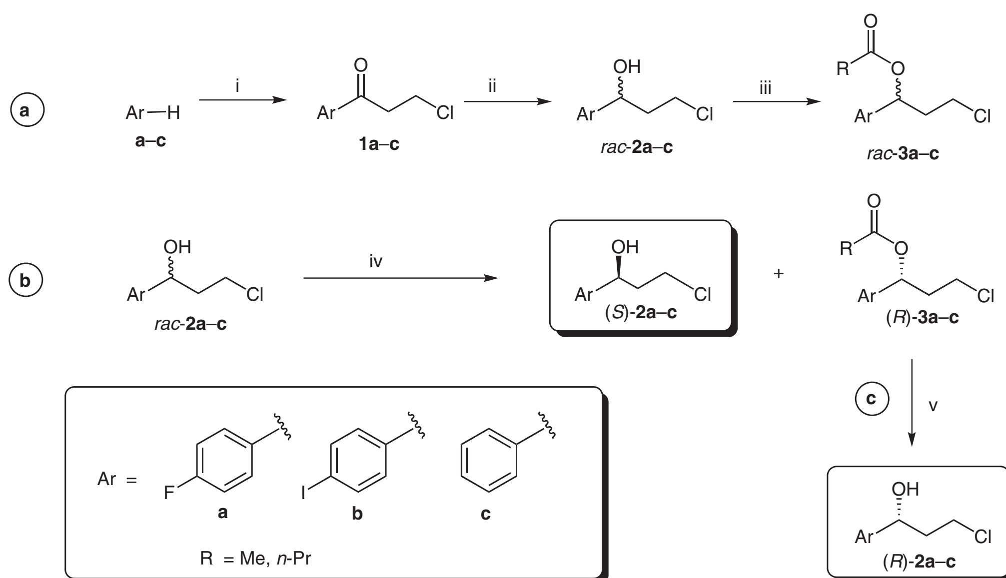
To investigate the stereoselectivity of the enzymatic acylation of racemic 1-aryl-3-chloropropan-1-ols *rac*-**2a–c**, they and their acylated counterparts *rac*-**3a–c** were first chromatographically separated (Table 5).

An approach to the synthesis of the enantiomerically enriched (*R*)-**3a–c** and (*S*)-**2a–c** is based on enantiomeric acylation of *rac*-**2a–c**, as depicted in Scheme 1 (b). For this, the analytical-scale enantiomer selective enzyme-catalyzed acylations in neat vinyl acetate were first tested in the presence of several enzymes. For *rac*-**2a–c** most of the tested enzymes such as lipase A, G, M, N, and P, lipase from *Pseudomonas cepacia* (LPS), lipase B from *Candida antarctica* (CaLB), and Lipozym TLIM were mostly catalytically inactive or displayed low reactivity and moderate selectivity. For the enzymatic kinetic resolution of *rac*-**2a**, only lipase from *Pseudomonas fluorescence* (LAK) and lipase from *Candida rugosa* (CRL) exhibited high selectivity, though their activity in neat vinyl acetate was low ($E = 64–168$, 5–9% conversion). LAK was also the best biocatalyst for the enantioselective transformation of *rac*-**2b**,**c** in neat vinyl acetate (for *rac*-**2b** $E = 45$, 13% conversion, and for *rac*-**2c** $E = 47$, 17% conversion).

It is known that the nature of the solvent and the acylating agent can significantly influence the selectivity and activity of the enantiomer-selective acylation of *rac*-**2a–c**. The acylation of *rac*-**2a** with vinyl acetate was first tested in the presence of LAK and CRL in several solvents, such as aliphatic and aromatic hydrocarbons, cyclic saturated ethers, and acetonitrile. The selectivity and the activity of CRL in all the tested solvents were strongly decreased relative to those in neat vinyl acetate ($E < 25$). *N*-Hexane proved to be the most appropriate solvent for the LAK-mediated acylation ($E = 65$ and 35% conversion after 20 h; Table 1, entry 2), but the enantiomer-selective acylation of *rac*-**2a** in toluene, cyclohexane, or *n*-octane was also satisfactory (entries 1, 3, and 4).

The same solvents were tested for the enzymatic acylation of *rac*-**2b**,**c**. Appropriate catalytic activity and selectivity of the previously selected enzyme were observed in only a few solvents. For both substrates, *n*-hexane proved to be a suitable acylation solvent, with $E > 200$ and 20–28% conversion after 12 hours.

In view of the unsatisfactory result for the LAK-mediated acylation of *rac*-**2a** ($E < 200$), the enzymatic acylation of



Scheme 1 (a) Synthesis of racemic 1-aryl-3-chloropropyl alkanoates; (b) enzymatic enantioselective acylation of *rac*-**2a–c**; (c) enzymatic enantioselective hydrolysis of (*R*)-**3a–c**. *Reagents and conditions:* (i) $Cl(CH_2)_2COCl$, $AlCl_3$, CH_2Cl_2; (ii) $NaBH_4$, EtOH, –15 °C; (iii) CaLA, vinyl acetate or vinyl butanoate, *n*-hexane; (iv) LAK, *n*-hexane, vinyl acetate or vinyl butanoate; (v) CRL in phosphate buffer, pH 8.

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Table 1 Influence of the Solvents upon the Selectivity of the LAK-Mediated Acylation of *rac*-**2a** with Vinyl Acetate[a]

Entry	Solvent	Conv. (%)	ee_s (%)	ee_p (%)	*E*
1	*n*-octane	23	29	96	65
2	*n*-hexane	35	51	95	65
3	cyclohexane	24	29	94	43
4	toluene	26	34	95	54

[a] Substrate (10 mg), enzyme (10 mg), vinyl acetate (10 μL), solvent (1 mL), 20 h.

rac-**2a**–**c** was tested with vinyl butanoate in the previously selected solvents. While the selectivity for the enzymatic transformation of *rac*-**2a** was enhanced (*E* >200, 28% conversion after 19 h) the enantiomeric purity for the resolution products of *rac*-**2b**,**c** was lower as compared with that observed with vinyl acetate as acyl donor (data not given).

As all the enzymatic reactions gave good, but not excellent enantioselectivities, two additional experiments were performed for the synthesis of the highly enantiomerically enriched reaction products (*S*)-**2a**–**c** and (*R*)-**3a**–**c**.

While the reactions were stopped at lower conversions for enantiopure (*R*)-**3a**–**c** (Table 2, entries 1–3), for enantiopure (*S*)-**2a**–**c** the reactions were stopped at higher conversions than the theoretical optimum of 50% (Table 2, entries 4–6).

Table 2 Optimal Conditions for the Resolution of the Racemic Alcohols *rac*-**2a**–**c**[a]

Entry	Substrate	Acyl donor	Time (h)	Conv. (%)	ee_s (%)	ee_p (%)
1	*rac*-**2a**	vinyl butanoate	19	28	39	99
2	*rac*-**2b**	vinyl acetate	22	20	25	99
3	*rac*-**2c**	vinyl acetate	21	28	39	99
4	*rac*-**2a**	vinyl butanoate	70	55	99	80
5	*rac*-**2b**	vinyl acetate	100	59	99	70
6	*rac*-**2c**	vinyl acetate	82	54	99	83

[a] Substrate (10 mg), enzyme (10 mg), vinyl ester (10 μL), *n*-hexane (1 mL).

Lipases usually retain their enantiomer preference in hydrolysis or alcoholysis.[1–3,6] Consequently, such reactions should result in opposite enantiomeric forms of the enantiomerically enriched 1-aryl-3-chloropropan-1-ols **2a**–**c** and 1-aryl-3-chloropropyl acetates or 1-aryl-3-chloropropyl butanoates **3a**–**c** as compared with those found in the kinetic resolution by acylation. The hydrolysis and alcoholysis of the corresponding racemic 1-aryl-3-chloropropyl acetates and 1-aryl-3-chloropropyl butanoates *rac*-**3a**–**c** were also considered.

With regard to their low water solubility, the enzymatic alcoholysis of *rac*-**3a**–**c** was first tested. The same wide selection of commercial hydrolases as used for the enzymatic acylation was screened for the analytical-scale alcoholysis of the racemic esters *rac*-**3a**–**c**. Experiments were first carried out in neat anhydrous methanol, ethanol, propanol, or butanol, followed by experiments using the same enzymes in all of the tested solvents checked for enzymatic esterification; five equivalents of alcohol were added to the reaction mixture. Since the enzymatic alcoholysis proved to be totally inefficient in all cases (yields <5% after 5 d, substrate/enzyme ratio 1:1), the enzymatic hydrolysis of *rac*-**3a**–**c** was studied. When the same enzymes were tested (substrate/enzyme ratio 1:1), it was found that only CRL and pig liver esterase (PLE) exhibited high enzymatic activity toward all *rac*-**3a**–**c**. Experiments were conducted in sonicated double distilled water, in phosphate or Tris buffer (pH 6.5, 7, 7.5, or 8, all 20 mM). Samples were taken every 30 minutes and analyzed via HPLC and GC. In all cases, the complete and nonstereoselective hydrolysis of *rac*-**3a**–**c** occurs in 2–5 hours.

In order to obtain (*R*)-**2a**–**c**, (*R*)-**3a**–**c** obtained from the enantioselective enzymatic acylation of *rac*-**2a**–**c** was subjected to enzymatic hydrolysis [Scheme 1 (c)] under the same conditions as described in the previous paragraph. In the cases of the fluoro and iodo derivatives, partial racemization of the produced alcohols was observed. The level of racemization was strongly dependent on the reaction time, the type of enzyme used, the buffer, and the pH. The highest extent of racemization was observed when water was used as reaction medium. This can be explained by the strong decrease in pH during the reaction, determined by the accumulation of acetic or butanoic acid as a product of the enzymatic hydrolysis. To avoid a long contact of the alcohols formed with the water from the reaction medium, the reaction time was shortened with a higher enzyme/substrate ratio (2:1).

Table 3 The Optimal Conditions for the Enzymatic Hydrolysis of (*R*)-**3a**–**c**[a]

Entry	Substrate	ee_s (%)	Time (h)	Product	ee_p (%)
1	(*R*)-**3a**	99	1.5	(*R*)-**2a**	98
2	(*R*)-**3b**	99	5	(*R*)-**2b**	97
3	(*R*)-**3c**	99	5	(*R*)-**2c**	99

[a] Substrate (10 mg), CRL (20 mg), phosphate buffer (1 mL), pH 8.

The optimal conditions for the enzymatic hydrolysis of (*R*)-**3a**–**c** are presented in Table 3. When CRL was used as biocatalyst and phosphate buffer (pH 8, 20 mM) as reaction medium for the complete hydrolysis of (*R*)-**3a**–**c** (Table 3), the lowest loss of enantiomeric excess was observed for the produced (*R*)-**2c**. Following the sequence depicted in Scheme 1 (b) and (c), the preparative-scale synthesis of both (*S*)- and (*R*)-**2a**–**c** was next performed (Table 4). The dilutions, substrate/biocatalyst ratio and reaction conditions were the same as in the case of the ana-

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Table 4 Yield, Enantiomeric Excess and Optical Rotation

Compound	(*R*)-**3a–c**			(*R*)-**2a–c**			(*S*)-**2a–c**		
	Yield (%)	ee (%)	$[\alpha]_D^{25}$	Yield (%)	ee (%)	$[\alpha]_D^{25}$	Yield (%)	ee (%)	$[\alpha]_D^{25}$
a	26	99	+58.7	24	98	+20	38	99	–22
b	20	99	+52.1	18	97	+ 9	34	99	–10
c	25	99	+59.5	23	99	+21	42	99	–24

lytical-scale reactions. In order to obtain enantiopure (*R*)-**3a–c**, the enzymatic reactions were stopped at low conversions (30–45%). For enantiopure (*S*)-**2a–c**, the reactions were stopped at high conversions (60–75%) by filtering off the enzyme. In both cases, the enantiomeric composition of the reaction counterparts was monitored via GC and HPLC to stop the reaction at conversions and enantiomeric excess levels of (*R*)-**3a–c** and (*S*)-**2a–c** close to those found in the analytical-scale procedures. After the enzyme had been filtered off from the reaction mixture, the solvents were removed in vacuo at room temperature.

It was important to avoid heating of the solution during the distillation because (*S*)- and (*R*)-**2a–b** displayed thermal instability, which leads to racemization. Moreover, the same effect was observed in the presence of polar and protic solvents such as methanol, ethanol, organic acids, or amines in the solution containing the enantiopure products, or a longer contact time with silica gel. For this reason, the preparative-scale separation of the reaction counterparts was performed with vacuum chromatography, using the minimal amount of silica gel. After (*R*)-**3a–c** had been eluted with dichloromethane, the chromatographic column was washed with acetone in order to recover (*S*)-**2a–c** rapidly.

The preparative-scale enzymatic hydrolysis of (*R*)-**3a–c** was also performed under the same conditions as used for the analytical-scale reactions. After the complete hydrolysis of the esters, the reaction mixture was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, followed by evaporation of the solvent at room temperature. Compounds (*R*)-**2a–c** were purified using the procedure described above.

The residual enantiomers formed in the low-conversion and in the high-conversion enzymatic acylations, were used to recover the racemic starting materials *rac*-**2a–c**. First, (*R*)-**3a–c** from the high-conversion reaction was enzymatically hydrolyzed to (*R*)-**2a–c**. These compounds were mixed with the (*S*)-**2a–c** formed in the low-conversion enzymatic acylation. In consequence of their thermal enantiostability, on refluxing mixtures in toluene, the complete racemization occurred in 3–4 hours for **2a**,**b** and 48 hours for **2c**. The recovery rate for all compounds was around 90%.

The absolute configuration of the synthesized enantiomerically enriched 1-aryl-3-chloropropanol derivatives (+)-**2a–c** and (–)-**2a–c** was assigned by vibrational circular dichroism (VCD) measurements combined with quantum chemical calculations at DFT level of theory. VCD, defined as the differential absorption of left- and right circular polarized infrared radiation during vibrational excitation of chiral molecules is today considered a well-established and extremely reliable technique for the determination of the absolute configuration and conformation of small to medium-sized molecules in solution.[21,22]

VCD spectra in $CDCl_3$ of (–)- and (+)-**2a–c**, with unknown absolute configuration, are shown in Figure 1 (top) in comparison with the simulated VCD spectra of their *R*-enantiomers (bottom). The VCD bands of aromatic ring vibrations at ~1600 cm^{-1} were the most intense in the case of the fluorinated derivatives (+)- and (–)-**2a**, while only a very weak band was observed for (+)- and (–)-**2c**, bearing no substituent on the phenyl group. This shows that in this case even substituents situated far from the stereogenic center have influence on the spectrum, and coupling of the aromatic ring vibrations with the vibrations of bonds placed around the stereogenic center cannot be neglected. As the VCD spectra of such chiral molecules may strongly depend on the relative orientation of the aromatic ring, a very careful conformational analysis was needed and no structural simplifications could be done for molecular modeling.

Since the investigated compounds are conformationally very flexible molecules, the calculation of their theoretical VCD spectra was preceded by a systematic conformational analysis, the *R*-enantiomers of **2a–c** were chosen for the quantum chemical calculations. A total of 25–27 conformers were found for each compound, of which ~9 had an estimated population of more than 1%. The simulated VCD spectra of (*R*)-**2a–c** in Figure 1 were obtained as the population-weighted sums of the calculated VCD spectra of their individual conformers with populations of more than 1%, the contributions of other, higher-energy conformers being neglected (for the computational details, see the VCD spectroscopic measurements and quantum chemical calculations).

The VCD spectra of enantiomers are opposite in sign and in general there is very good agreement between the measured spectra of the (+)-enantiomers and the calculated spectra of the *R*-enantiomers of the same compounds, in terms of both the positions, and the signs of the VCD bands, the matching pairs of vibrational bands being labeled with corresponding numbers (Figure 1).

The common spectral feature of (+)-**2a–c** showing up as a +/–/+ band pattern around 1300 cm^{-1} and a positive band at ~1200 cm^{-1}, very well reproduced by the calculations

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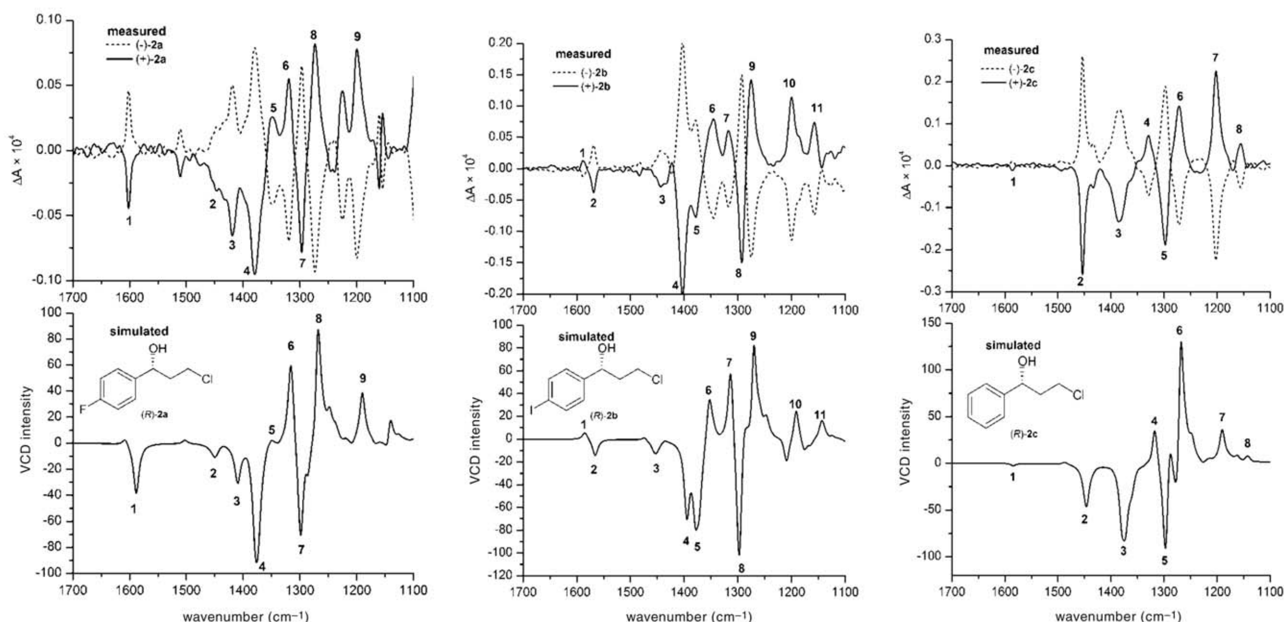

Figure 1 VCD spectra of compounds (–)-**2a** and (+)-**2a**, (–)-**2b** and (+)-**2b**, and (–)-**2c** and (+)-**2c** measured in $CDCl_3$ (top) in comparison with the simulated VCD spectrum of (*R*)-**2a**, (*R*)-**2b**, and (*R*)-**2c** (bottom), obtained as a population-weighted sum of the calculated spectra of individual conformers. Corresponding bands are labeled with identical numbers.

for (*R*)-**2a–c**, suggests that these compounds belong in the same stereochemical series.

The comparison of the measured and calculated VCD spectra and the considerations presented above allow the unequivocal assignment of the absolute configurations of (+)-**2a–c** as *R* and of (–)-**2a–c** as *S*.

All air- or moisture-sensitive reactions were conducted under an atmosphere of dry argon. All starting materials and reagents were purchased from Aldrich, Alfa-Aesar, Fluka, or Merck Company. All solvents were of reagent grade, but anhyd solvents were freshly distilled before use (CH_2Cl_2 was distilled from CaH_2). $CDCl_3$ (99.96% D) used as solvent for VCD spectroscopy was purchased from Aldrich.

Lipases A, G, M, N, P and lipases from *Pseudomonas fluorescens* (LAK) and *Pseudomonas cepacia* (LPS) were acquired from Amano, England. Lipase from *Candida rugosa* (CRL) and pig liver esterase (PLE) were purchased from Fluka. Immobilized lipase B from *Candida antarctica* (CaLB, Novozym 435), lipase A from *Candida antarctica* (CaLA), and lipase from *Thermomyces lanuginosus* (Lipozyme TLIM) were purchased from Novozyms, Denmark.

^{1}H NMR spectra were recorded on a Varian Mercury Plus 400 MHz spectrometer; except *rac*-**3a** and *rac*-**3b** (300 MHz, $CDCl_3$) and ^{13}C NMR spectra were recorded on a Bruker Advance spectrometer operating at 75 MHz; ^{1}H spectra were referenced internally to the solvent signal ($CHCl_3$, δ = 7.26) and ^{13}C spectra to the solvent signal ($CDCl_3$, δ = 77.7).

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Elemental analysis was collected on Carlo Erba Elemental Analyzer, Model 1012. HPLC analyses for the enzymatic reactions and for the racemic and enantiopure forms of **2a–c** and **3a–c** were made with an Agilent 1200 instrument. Enantiomeric separation of *rac*-**2a**,**b** and *rac*-**3a**,**b** was performed with a tandem of ChiralPak-IA and -OJ columns (4.6 × 250 mm) using *n*-hexane–*i*-PrOH (95:5). For the enantiomeric separation of *rac*-**2c** and *rac*-**3c**, a ChiralPak IB column and *n*-hexane–*i*-PrOH (98:2) were used. GC analyses were carried out with an Agilent 7890 A GC system. Enantiomeric separations of *rac*-**2a–c** and *rac*-**3a–c** were run on an Astec-Chiral column (30 m × 0.25 mm, B-DM) at a flow rate of 1 mL min^{-1}, with a split ratio of 80 in isothermic mode at 180 °C (carrier gas N_2; head pressure: 0.276 bar; injector: 250 °C; FID detector: 250 °C; Table 5).

Table 5 Retention Time (t_R) of Compounds *rac*-**2a–c** and *rac*-**3a–c**

Compound	HPLC analysis		GC analysis	
	S	*R*	*S*	*R*
2a	22.8	24.8	11.9	12.6
2b	32.3	36.4	50.9	50.4
2c	14.4	16.5	11.0	11.3
3a	13.1	11.1	12.9	13.1
3b	11.7	10.0	32.6	33.3
3c	6.0	5.7	8.7	8.9

TLC was carried out using Merck Kieselgel $60F_{254}$ sheets. Spots were visualized using a UV lamp and by treatment with 5% ethanolic phosphomolybdic acid soln and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63–200 μm). Yields refer to chromatographically and spectroscopically pure compounds. IR spectra were recorded in KBr pellets on a Perking-Elmer FT-IR 1650 spectrometer. Optical rotations were determined on a Bellingham-Stanley ADP 220 polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Electron impact mass spectra (EI-MS) were taken on a LCQ Advantage spectrometer by Thermo Fisher operating at 70 eV.

The determination of *E* was based on the equations: $E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)]$; $c = ee_s/(ee_S + ee_P)$ using linear regression

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{E as the slope of the line $\ln[(1-c)(1-ee_S)]$ vs $\ln[(1-c)(1+ee_S)]$}}.[23]

The prochiral ketones **1a** and **1c** were commercially available and only the prochiral ketone **1b** was chemically synthesized.

3-Chloro-1-(4-iodophenyl)propan-1-one (1b)

To a suspension of $AlCl_3$ (6.00 g, 45 mmol) and CH_2Cl_2 (10 mL) was added, under argon, 3-chloropropionyl chloride (5 mL, 53.64 mmol) over 15 min and the mixture was stirred at r.t. for a further 15 min. To the cooled soln (0–5 °C), iodobenzene (5 mL, 44.70 mmol) was added dropwise over 30 min and the soln was stirred at r.t. for 3 h. Ice-cold H_2O (30 mL) was then added and the organic layer was washed with H_2O (3 × 30 mL), $Na_2S_2O_3$ soln (15%, 2 × 20 mL), sat. Na_2CO_3 (2 × 30 mL), and finally H_2O (2 × 10 mL). After drying (anhyd $MgSO_4$) and evaporation in vacuo, the crude product was crystallized [CH_2Cl_2 (10 mL) and hexane (40 mL][20] to give **1b** (6.71 g, 22.78 mmol, 51%); mp 83–85 °C (Lit.[20] 83–84 °C); R_f = 0.6 (toluene).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.41 (t, J = 6.7 Hz, 2 H, H2 or H3), 3.91 (t, J = 6.7 Hz, 2 H, H3 or H2), 7.65–7.67 (m, 2 H, H_{Ar}), 7.84–7.86 (m, 2 H, H_{Ar}).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.13, 41.82, 102.44, 130.13, 136.31, 138.82, 196.73.

Anal. Calcd for C_9H_8OClI: C, 36.67; H, 2.72. Found: C, 36.25; H, 2.57.

Racemic Alcohols; General Procedure

A soln of commercially available or chemically synthesized prochiral ketone **1a–c** in 96% EtOH was cooled to –20 °C and $NaBH_4$ (98%) was added in small portions. The suspension was stirred at –15 °C for 3 h. Ice-cold H_2O was then added and the mixture was stirred for 15 min and concentrated under reduced pressure. The inorganic salt was filtered off and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried (anhyd $MgSO_4$), and concentrated under reduced pressure. The residue was purified by vacuum distillation to give the desired product.[21]

3-Chloro-1-(4-fluorophenyl)propan-1-ol (*rac*-2a)

Following the general procedure using a soln of commercially available 3-chloro-1-(4-fluorophenyl)propan-1-one (25.53 g, 0.14 mol) in 96% EtOH (250 mL), 98% $NaBH_4$ (7.80 g, 0.21 mol); workup with ice-cold H_2O (100 mL) and extraction CH_2Cl_2 (3 × 50 mL) and washing H_2O (2 × 30 mL) gave a residue that was purified by vacuum distillation to give *rac*-**2a** (19.80 g, 0.10 mol, 77%) as a colorless oil; bp 116–118 °C/4 mbar (Lit.[20] 101–102 °C/0.13 mbar); R_f = 0.3 (toluene).

IR (KBr): 656, 726, 836, 926, 1013, 1054, 1158, 1225, 1293, 1510, 1603, 2950, 3200 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.99–2.07 (m, 1 H, H2x), 2.14–2.23 (m, 1 H, H2y), 2.35 (s, 1 H, OH), 3.49–3.54 (m, 1 H, H3x), 3.67–3.74 (m, 1 H, H3y), 4.91 (dd, J = 8.4, 4.9 Hz, 1 H, H1), 6.99–7.06 (m, 2 H, H_{Ar}), 7.28–7.33 (m, 2 H, H_{Ar}).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.39, 41.56, 70.61, 115.30 (d, J_{C-F} = 21.4 Hz), 127.35 (d, J_{C-F} = 8.2 Hz), 139.36 (d, J_{C-F} = 3.3 Hz), 160.65 (d, J_{C-F} = 245.9 Hz).

MS (EI, 70 eV): m/z (%) = 190 (^{37}Cl, 4, M$^+$), 188 (^{35}Cl, 27, M$^+$), 125 (100), 97 (61), 77 (31).

Anal. Calcd for $C_9H_{10}ClFO$ (188.63): C, 57.31; H, 5.34; Found: C, 57.48; H, 5.35.

3-Chloro-1-(4-iodophenyl)propan-1-ol (*rac*-2b)

Following the general procedure using 3-chloro-1-(4-iodophenyl)propan-1-one (**1b**, 5.00 g, 17 mmol), 96% EtOH (100 mL), 98% $NaBH_4$ (1.29 g, 34 mmol) with stirring at –15 °C for 2 h. Workup used ice-cold H_2O (50 mL) and the pH was adjusted to 4–5 with 10% HCl; extraction with CH_2Cl_2 (3 × 50 mL) and washing with H_2O (2 × 15 mL) gave an oil residue that was purified by column chromatography (silica gel, $CHCl_3$) to give *rac*-**2b** (3.17 g, 10.69 mmol, 63%) as a colorless oil; (Lit.[24] bp 151–152 °C/0.13 mbar); R_f = 0.4 ($CHCl_3$).

IR (KBr): 661, 715, 818, 927, 1004, 1059, 1195, 1259, 1357, 1399, 1482, 1587, 1718, 1764, 2958, 3410 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.01–2.08 (m, 2 H, H2x, OH), 2.14–2.25 (m, 1 H, H2y), 3.51–3.57 (m, 1 H, H3x), 3.70–3.76 (m, 1 H, H3y), 4.91 (dd, J = 8.6, 4.5 Hz, 1 H, H1), 7.07–7.13 (m, 2 H, H_{Ar}), 7.66–7.70 (m, 2 H, H_{Ar}).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.16, 41.45, 70.56, 93.21, 127.65, 137.59, 143.22.

MS (EI, 70 eV): m/z (%) = 298 (^{37}Cl, 3, M$^+$), 296 (^{35}Cl, 6, M$^+$), 233 (95), 78 (100), 49 (44).

Anal. Calcd for $C_9H_{10}ClIO$ (296.53): C, 36.45; H, 3.40; Found: C, 36.53; H, 3.71.

3-Chloro-1-phenylpropan-1-ol (*rac*-2c)

Following the general procedure using commercially available 3-chloro-1-phenylpropan-1-one (25.28 g, 0.15 mol), 96% EtOH (500 mL), and 98% $NaBH_4$ (19.90 g, 0.52 mol); workup used ice-cold H_2O (250 mL) and the pH was adjusted to 5–6 with concd HCl; extraction with $CHCl_3$ (3 × 150 mL) and washing with H_2O (2 × 50 mL) gave a residue that was purified by vacuum distillation to give *rac*-**2c** (18.30 g, 0.11 mol, 72%) as a colorless oil; bp 115–116 °C/4–5.33 mbar (Lit.[25] 125–130 °C/8–10.6 mbar), R_f = 0.3 (toluene).

IR (KBr): 656, 699, 767, 914, 968, 1019, 1061, 1143, 1201, 1285, 1338, 1452, 1492, 2957, 2985, 3030, 3250 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.03–2.11 (m, 1 H, H2x), 2.17–2.26 (m, 2 H, H2y, OH), 3.51–3.57 (m, 1 H, H3x), 3.69–3.75 (m, 1 H, H3y), 4.91 (dd, J = 8.4, 4.7 Hz, 1 H, H1), 7.25–7.37 (m, 5 H, H_{Ar}).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.31, 41.66, 71.18, 125.70, 127.82, 128.57, 143.59.

MS (EI, 70 eV): m/z (%) = 172 (^{37}Cl, 14, M$^+$), 170 (^{35}Cl, 44, M$^+$), 107 (100), 79 (87), 51 (33).

Anal. Calcd for $C_9H_{11}ClO$ (170.64): C, 63.35; H, 6.50; Found: C, 62.98; H, 6.54.

Acylation Reaction; General Procedure

The chemical acylation of *rac*-**2a–c** was performed, but it was observed that other compounds appeared and we could not observe *rac*-**3a–c** by HPLC analysis. Then enzymatic acylation of *rac*-**2a–c** was performed and the compounds *rac*-**3a–c** were observed by HPLC analysis.

Enzymatic acylation reaction: To a mixture of *rac*-**2a–c** (200 mg) in *n*-hexane (10 mL), CaLA (100 mg) and vinyl acetate (10 mL) or vinyl butanoate (10 mL) were added and the mixture was shaken for 24 h at 250 rpm at r.t. After the reaction was complete (TLC), *rac*-**3a–c** was separated from the enzyme, the solvent was removed in vacuo affording the pure product.

3-Chloro-1-(4-fluorophenyl)propyl Butyrate (*rac*-3a)

Following the general procedure gave, after column chromatography (CH_2Cl_2), *rac*-**3a** a colorless liquid, (273.2 mg, 1.06 mmol, 99.5%); R_f = 0.9 (CH_2Cl_2).

IR (KBr): 739, 836, 939, 966, 1152, 1223, 1267, 1356, 1510, 1605, 1708, 2956, 2988 cm^{-1}.

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^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.93 (t, J = 7.2 Hz, 3 H, H4′$_x$, H4′$_y$, H4′$_z$), 1.61 (m, 2 H, H3′$_x$, H3′$_y$), 2.15 (t, J = 6.8 Hz, 2 H, H2′$_x$, H2′$_y$), 2.29–2.43 (m, 2 H, H2$_x$, H2$_y$), 3.40–3.59 (m, 2 H, H3$_x$, H3$_y$), 5.90 (dd, J = 8.3, 5.3 Hz, 1 H, H1), 7.04–7.00 (m, 2 H, H$_{Ar}$), 7.33–7.29 (m, 2 H, H$_{Ar}$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.54, 18.34, 36.20, 38.94, 40.51, 72.16, 115.64 (d, J_{C-F} = 21.9 Hz), 128.1 (d, J_{C-F} = 8.8 Hz), 135.44 (d, J_{C-F} = 3.3 Hz), 172.55.

MS (EI, 70 eV): m/z (%) = 258.1 (^{35}Cl, 0.04, M$^+$), 188.1 (3.75), 125.1 (100), 97.1 (51.97), 77.1 (24.52), 43 (10.44).

3-Chloro-1-(4-iodophenyl)propyl Acetate (*rac*-3b)

Following the general procedure gave, after column chromatography (CH_2Cl_2), *rac*-**3b** (227.2 mg, 0.67 mmol, 99.5%) as a colorless liquid; R_f = 0.6 (CH_2Cl_2).

IR (KBr): 700, 736, 819, 965, 1005, 1238, 1370, 1484, 1524, 1734, 2957, 2989 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.98 (s, 3 H, H2′$_x$, H2′$_y$, H2′$_z$), 2.04–2.30 (m, 2 H, H2$_x$, H2$_y$), 3.31–3.49 (m, 2 H, H3$_x$, H3$_y$), 5.79 (dd, J = 8.3, 5.3 Hz, 1 H, H1), 6.99 (d, J = 8.3 Hz, 2 H, H$_{Ar}$), 7.58 (d, J = 8.3 Hz, 2 H, H$_{Ar}$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.94, 38.60, 40.34, 72.40, 93.76, 128.18, 128.49, 137.59, 169.74.

MS (EI, 70 eV): m/z (%) = 339.9 (^{37}Cl, 8.1, M$^+$), 337.9 (^{35}Cl, 21.59, M$^+$), 275 (40.98), 232.9 (74.73), 217 (17.90), 203 (13.71), 172.1 (^{37}Cl, 31.05), 170.1 (^{35}Cl, 82.26), 117.1 (100), 107.1 (74.34), 91.1 (41.69), 77.1 (32.51), 43 (43.94).

3-Chloro-1-phenylpropyl Acetate (*rac*-3c)

Following the general procedure gave, after column chromatography (CH_2Cl_2), *rac*-**3c** (248.3 mg, 1.2 mmol, 99.5%) as a colorless liquid; R_f = 0.6 (CH_2Cl_2).

IR (KBr): 660, 699, 760, 971, 1023, 1225, 1284, 1370, 1452, 1495, 1734, 3033 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.07 (s, 3 H, H2′$_x$, H2′$_y$, H2′$_z$), 2.15–2.43 (m, 2 H, H2$_x$, H2$_y$), 3.40–3.60 (m, 2 H, H3$_x$, H3$_y$), 5.90 (dd, J = 8.3, 5.3 Hz, 1 H, H1), 7.30–7.36 (m, 5 H, H$_{Ar}$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.91, 38.84, 40.49, 72.94, 126.19, 128.07, 128.46, 139.34, 169.80.

MS (EI, 70 eV): m/z (%) = 214.1 (^{37}Cl, 0.89, M$^+$), 212.1 (^{35}Cl, 4.35, M$^+$), 172.1 (^{37}Cl, 4.36), 170.1 (^{35}Cl, 14.79), 117.1 (30.68), 105.1 (30.60), 91.1 (37.82), 77.1 (35.62), 43.0 (100).

Analytic-Scale Enzymatic Acylation of *rac*-2a–c; General Procedure

A mixture of enzyme (10 mg), *rac*-**2a**–**c** (10 mg) and vinyl acetate or vinyl butanoate (10 μL) in hexane (1 mL) was shaken at 1000 rpm and r.t. Samples (10 μL) were taken after 24 h and diluted with *n*-hexane (400 μL). Samples (10 μL) were analyzed with HPLC, GC, and TLC.

Analytic-Scale Enzymatic Hydrolysis of (*R*)-3a–c; General Procedure

A mixture of enzyme (10 mg) and *rac*-**3a**–**c** (10 mg) in phosphate buffer (20 mM, pH 8) was shaken at 1000 rpm and r.t. Samples (2 μL) were taken at every 30 min, diluted with *n*-hexane (400 μL), dried (anhydrous Na_2SO_4), filtered, and analyzed with HPLC, GC, and TLC.

Preparative-Scale Enzymatic Synthesis of (*S*)- and (*R*)-2a–c

A mixture of *rac*-**2a**–**c** (400 mg), vinyl butyrate/vinyl acetate (5 equiv), and LAK (300 mg) in *n*-hexane (10 mL) was shaken at r.t. Samples from the mixture (10 μL) were diluted with *n*-hexane (400 μL) and analyzed with HPLC/GC. For enantiomerically pure (*R*)-3-chloro-1-arylpropanoates (*R*)-**3a**–**c**, the reactions were stopped by filtering the enzyme at approx 23–40% conversion, while for enantiopure (*S*)-3-chloro-1-arylpropanols (*S*)-**2a**–**c** at 55–65% conversion. Solvents were removed in vacuo and the crude product was purified by vacuum chromatography (CH_2Cl_2) resulting in both optically active (*R*)-**3a**–**c** and (*S*)-**2a**–**c** as semisolids. The whole amount of the previously isolated (*R*)-**3a**–**c**, CRL, and the phosphate buffer (20 mL) was shaken at 1000 rpm for several hours (2 h for **1a**, 3 h for **1b**,**c**). The dilution and substrate/enzyme ratio was the same as previously described. The mixture was extracted with CH_2Cl_2 (3 × 30 mL), the organic layers were dried (anhyd Na_2SO_4), and the solvent removed in vacuo. The crude product was purified by preparative vacuum-chromatography (CH_2Cl_2). The solvent was removed in vacuo affording the enantiomerically enriched product (*R*)-**2a**–**c**.

VCD Spectroscopic Measurements and Quantum Chemical Calculations

VCD spectra of (+)-**2a**–**c** and (–)-**2a**–**c** at a resolution of 4 cm^{-1} were recorded in $CDCl_3$ soln with a Bruker PMA 37 VCD/PM-IRRAS module connected to an Equinox 55 FTIR spectrometer.

The ZnSe photoelastic modulator of the instrument was set to 1300 cm^{-1} and an optical filter with a transmission range of 1800–800 cm^{-1} was used in order to optimize the sensitivity in the fingerprint region of the spectrum. The instrument was calibrated for VCD intensity with a CdS multiple-wave plate. A BaF_2 cell with a path length of 50 μm and sample concentrations of 100 mg/mL [for (+)- and (–)-**2a**] and 200 mg/mL [for (+)- and (–)-**2b**,**c**] were used. In order to increase the signal/noise ratio of the low-intensity VCD signals, measurement times of 7 h (corresponding to ~24,000 accumulated interferograms) were applied. Since both enantiomers of the investigated compounds were available at comparable enantiomeric purity, the instrumental baseline was calculated as the average of the raw VCD spectra of the opposite enantiomer samples and this average was used for baseline correction (being subtracted from the original VCD spectra). This way all eventual dichroic artifacts caused by the BaF_2 windows of the measurement cell or arising from detector nonlinearity at the absorption band positions could be cancelled.

Geometry optimizations and the computation of vibrational frequencies and VCD rotatory strengths were performed for (*R*)-**2a**–**c** with the Gaussian 03 quantum chemical software package.[26] For (*R*)-**2a** and (*R*)-**2c** at the B3LYP/6-31G(d) DFT level of theory was used, while in the case of (*R*)-**2b** the B3LYP functional was applied in combined with the LANL2DZ basis set for the iodine atom (for which the 6-31G(d) basis set is not defined) and the 6-31G(d) basis set for other atoms (C, H, O, Cl). The vibrational frequencies were scaled by a factor of 0.963. VCD curves were simulated from the calculated wave number and rotatory strength data by using Lorentzian band shape and a half-width at half-height value of 6 cm^{-1}.

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