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Green methodology for enzymatic hydrolysis of acetates in non-aqueous media via carbonate salts

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ABSTRACT

Herein we report a new approach to enantiomerically enriched acetates using a lipase-catalyzed hydrolysis in non-aqueous media by alkaline carbonate salts. The use of sodium carbonate in the enzymatic hydrolysis with *Candida antarctica* lipase B (CAL-B) of racemic acetates shows a large enhancement of the reactivity and selectivity of this lipase. The role of the carbonate salts, the amount and the nature of the alkaline earth metal on the efficiency of this new pathway are investigated. The enzymatic kinetic resolution of acetates **1a**–**9a**, by enzymatic-carbonate hydrolysis under mild conditions is described. In all cases, the resulting alcohols and remaining acetates were obtained in high ee values (up to >99%) while the selectivities reached E >500.

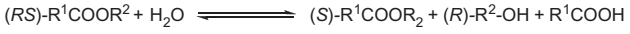
1. Introduction

Hydrolytic enzymes are very attractive and are one of the most useful synthetic tools, which integrate numerous organic transformations in a large variety of catalytic reactions. Their use contributes to the development of the basic principles of green chemistry.[1] Serine hydrolases (EC.3.1.1.3) are the most widely used for their ease of manipulation and for their remarkable chemo-, regio-, and enantioselectivity,[2] especially in kinetic resolutions.[3] They offer access to a wide range of biotransformations, such as the manufacture of pharmaceuticals, cosmetics, agricultural chemicals, and fine chemicals.[4]

Furthermore, their utility has also been reinforced by the possibility of combining enzymatic and homogeneous catalysis. Moreover, efficient methods for deracemizations via biocatalyzed kinetic resolutions have been developed over the last decade, and offer new pathways for the production of enantioenriched molecules with high yields and selectivities.[5]

Lipases are highly active and stable, both in water and organic solvents. They are powerful tools for biotransformations involving either (*trans*)esterification of carboxylic acids and alcohols in organic media, or hydrolysis in aqueous media.[6] Recently, an efficient approach for deacylation of secondary acetates using free ammonia in non-aqueous media has been described.[7]

The most conventional methods for the enzymatic kinetic resolution through hydrolysis, as reported in the literature,[8] were performed in aqueous media,[9] or in a biphasic system: aqueous/organic solvent.[10] The catalytic mechanism of the hydrolysis[11] is held on the interface of the two phases (Scheme 1).



Scheme 1.

However, enzymatic hydrolysis in biphasic media (water, buffer or water saturated) is still limited because of two major disadvantages; the first is the decrease of the lipase's enantioselectivity due to the co-solvent nature; the second is the difficulty of controlling the pH value of the aqueous solution during the hydrolysis procedure.[12] These limitations explain its moderate applications for the resolution of secondary[9b,13] and/or the primary benzylic acetates[14] compared to the enzymatic transesterifications.[15]

Herein we have developed a green and easy pathway for enzymatic hydrolysis in non-aqueous organic media, in the presence of sodium carbonates. We have also applied this procedure to a large range of aromatic acetates.

2. Results and discussion

The enzymatic hydrolysis was carried out on an equimolecular mixture of racemic acetates and sodium carbonate in the presence of catalytic amounts of lipase, in toluene, without the addition of any more water (Scheme 2). We examined various parameters of the reaction and then studied the effect of the sodium carbonate

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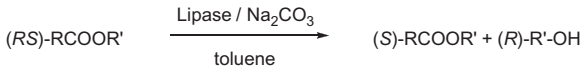
according to these parameters, such as the lipase's nature, its catalytic loading and the temperature.

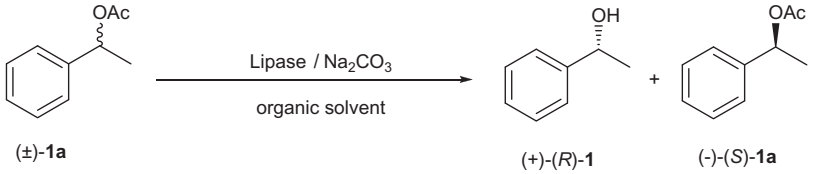


Scheme 2.

2.1. Effect of the amount and the nature of the lipase

In our preliminary kinetic resolution investigation, the racemic phenylethyl acetate **1a** was chosen as a model substrate in order to evaluate the suitable reaction conditions.

Various commercially available lipases were screened: the *Candida cylindracea* lipase (*CCL*; LA = 3.85 U/mg), *Candida rugosa* lipase (*CRL*; LA = 1170 U/mg), *Pseudomonas cepacia* lipase (*PCL*; LA >30,000 U/mg) and an immobilized lipase: the *Candida antarctica* lipase fraction B immobilized on acrylic resin (*CAL-B*; LA >10,000 U/g). The catalytic amount of the biocatalysts, reaction times and temperature were also examined (Scheme 3) in order to determine the appropriate conditions. Hydrophobic solvents, subjected beforehand to molecular sieves, were employed, in order to determine the water source in the hydrolysis reaction media. The course and selectivity of the kinetic resolution were checked by sampling the reaction mixture by chiral GC. The results are summarized in Table 1.



Scheme 3.

Table 1
Effect of the amount and the nature of lipase

Entry[a]	Lipase (mg)	Time (h)	ee_S (%)[i]-(S)[k]	ee_P (%)[i]-(R)[k]	C[i](%)	E[j]
1	—[b]	72	—	—	—	—
2	—[c]	72	—	—	—	—
3	CALB[d,e] (40)	72	9.5	99.5	9	>200
4	CAL-B[e] (40)	72	67	99.5	40	>500
5	CAL-B[e,g] (40)	48	86	99.5	46	>500
6	CAL-B[f] (50)	72	49	99.5	33	>500
7	CAL-B[f] (100)	72	70	99.5	41	>500
8	CAL-B[f,h] (100)	72	99	99.5	50	>500
9	CCL (100)	6 days	0.4	7	5	1
10	CRL (100)	6 days	0.4	12.4	3	1
11	PCL (100)	6 days	6.4	99.5	6	>500

[a] Reaction conditions: 1 mmol of racemic acetate, 1 mmol of Na_2CO_3 in 3 mL of toluene in the presence of lipase at room temperature.
[b] Reaction was performed without lipase or Na_2CO_3.
[c] Reaction was performed without lipase and in the presence of Na_2CO_3.
[d] Reaction was performed in the presence of lipase and without Na_2CO_3.
[e] CALB purchased from Aldrich. Specific activity >10,000 U/g.
[f] CALB purchased from Sigma. Specific activity >10,000 U/g.
[g] Et_2O as the organic solvent.
[h] At 40 °C.
[i] Measured by GC.
[j] Conversion:[16] $C = ee_S/ee_P + ee_s$; selectivity[16] $E = Ln[(1 - C)(1 - ee_{(S)})]/Ln[(1 - C)(1 + ee_{(S)})]$.
[k] The absolute configuration was determined by the sign of the specific rotation of the isolated product with the literature (see Section 4).

The data from Table 1 show that no hydrolysis took place in the absence of both lipase and sodium carbonate (entry 1). Furthermore, none of the corresponding alcohol was obtained when 1 equiv of sodium carbonate but no biocatalyst was used (entry 2).

A slight improvement in the hydrolysis of **1a** occurred, only in the presence of CAL-B, and the conversion reached $C = 9\%$ after 3 days (entry 3). The (*R*)-phenylethanol was obtained with an excellent enantioselectivity (ee >99.5%). With the introduction of both Na_2CO_3 and CAL-B, the conversion achieved $C = 40\%$ while maintaining high enantioselectivity (entry 4). In contrast, the use of free lipases: CCL, CRL, and PCL, gave very low reactivity, with only $C < 10\%$ conversion of starting material being obtained after 6 days (entries 9, 10 and 11), although PCL gave high enantioselectivity (entry 11). With the immobilized lipase CAL-B, our results showed a significant improvement, giving high selectivities $E > 500$ and good conversion $33\% < C < 40\%$ (entries 4 and 6).

When the amount of immobilized lipase was doubled, 40% conversion of the starting acetate was reached after stirring for 72 h (entry 7). While heating at 40 °C, we achieved the optimum conversion, $C = 50\%$, and both the remaining acetate and the resulting alcohol were obtained with ee values of up to 99% (entry 8).

The use of another hydrophobic solvent Et_2O, gave an analogous selectivity $E > 500$ and reactivity (entry 5), than those obtained when toluene was employed as the organic media.

From all of these data, we are able to conclude that since the solvents used were hydrophobic (aromatics and ether), the water implicated in the hydrolysis processes probably originates from the lipase.

Fülöp et al. reported similar phenomena and noted that hydrolysis of an acetate took place without the addition of any H_2O. This fact was attributed to the residual water present in the *Burkholderia cepacia* lipase preparation or in the solvent used.[17]

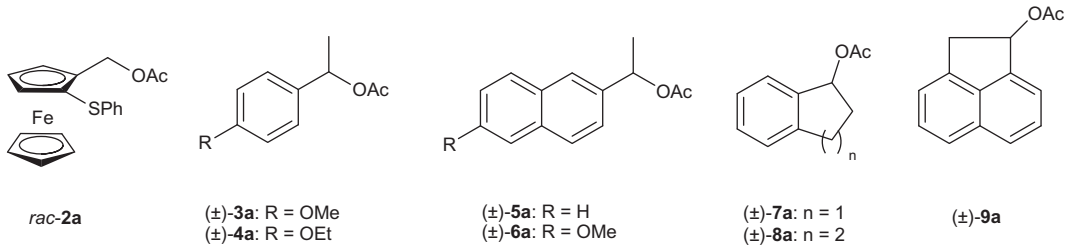
The CAL-B and PCL lipases displayed good enantioselectivities, but only the CAL-B exhibited high reactivity. In contrast, CRL and CCL were inactive for the hydrolysis processes in these cases.

In order to validate this novel approach, we decided to apply the optimized conditions of the enzymatic kinetic resolution via hydrolysis to a series of primary and secondary aromatic acetates **2a**–**9a**.

2.2. Enzymatic-alkaline hydrolysis of racemic acetates 2a–9a

The enzymatic-alkaline hydrolysis reactions of racemic acetates **2a**–**9a** (Scheme 4), under the new reaction conditions, were performed. All experiments were carried out with 1 mmol of substrate, with 1 equiv of sodium carbonate in the presence of a catalytic amount of CAL-B, in 3 mL of toluene for 72 h at 40 °C. The results are summarized in Table 2.

All of the substrates showed good to high selectivities (Table 2), albeit with slight differences in the reactivity of the lipase toward some substrates, in the presence of sodium carbonate in organic media.



Scheme 4.

Table 2
Enzymatic-alkaline hydrolysis of aromatic acetates

Entry[a]	Substrate	CAL-B (mg)	Solvent	ee_S (%)[b]-(*S*)[g]	Yield[c] (%)	ee_P (%)[b]-(*R*) [g]	Yield[c] (%)	*C*[d] (%)	*E*[d] (%)
1	**2a**	40	Hexane	19.5	ND[e]	99.5	ND[e]	16	>500
2	**2a**	40	Et_2O	5	ND[e]	99.5	ND[e]	4	>500
3	**2a**	40	iPr_2O	11	ND[e]	99.5	ND[e]	10	>500
4	**2a**	40	TBME	20.5	ND[e]	99.5	ND[e]	17	>500
5	**2a**	40	CH_2Cl_2	—	—	—	—	NR[f]	—
6	**2a**	40	Toluene	34	ND[e]	99.5	ND[e]	25	>500
7	**2a**	80	Toluene	69	42	99.5	30	41	>500
8	**2a**	100	Toluene	65	40	99.5	35	40	>500
9	**5a**	100	Toluene	95	47	99.5	45	49	>500
10	**5a**	50	Toluene	98	48	98.6	48	50	>500
11	**6a**	100	Toluene	99	32	99.5	34	50	>500
12	**6a**	50	Toluene	93	29	96.3	30	49	>150
13	**8a**	100	Toluene	78	46	99.5	35	44	>500
14	**8a**	50	Toluene	99.5	48	99.5	48	50	>500
15	**3a**	100	Toluene	95	47	97	46	49	>300
16	**3a**	50	Toluene	96	48	97	48	49.6	>200
17	**4a**	100	Toluene	92	45	95.6	40	49	150
18	**4a**	50	Toluene	95	42	97.6	35	49.3	>300
19	**7a**	100	Toluene	95	35	99.5	30	49	>500
20	**7a**	50	Toluene	98.2	36	99	32	50	>500
21	**9a**	50	Toluene	90.3	35	98.2	27	47.9	>300

[a] Reactions were carried out with 1 mmol of racemic acetate, 1 mmol of Na_2CO_3 in 3 mL of solvent at 40 °C, in the presence of a catalytic amount of CAL-B (>10,000 U/g), for 72 h.
[b] Measured by GC or HPLC.
[c] Isolated yields.
[d] Conversion:[16] $C = ee_S/ee_P + ee_s$; selectivity[16] $E = Ln[(1 - C)(1 - ee_{(S)})]/Ln[(1 - C)(1 + ee_{(S)})]$.
[e] Not determined.
[f] No reaction.
[g] Absolute configuration was determined by comparison of the sign of the specific rotation of the isolated product with the literature (see Section 4).

The amount of CAL-B was then reduced to 50 mg (entries 10, 12, 14, 16, 18, 20, and 21); the lipase's reactivity and selectivity remained unaffected, for most of the substrates tested.

In general, lipases showed high enantioselectivity toward secondary alcohols or ester derivatives, but exhibited low enantioselectivity toward primary substrates. The best selectivity E >500 was reached using 80 mg of lipase, at 40 °C, with a high conversion, C = 41% (entry 7).

We were pleased to note that we could obtain the (R_{FC})-2-hydroxymethy-1-phenylthioferrocene **2**, via hydrolysis of the primary acetate **2a**, with excellent enantiomeric excess ee = 99.5%. Furthermore, the other enantiomer, (S_{FC})-2-hydroxymethy-1-phenylthioferrocene was obtained with high enantioselectivity via enzymatic acylation in our previous investigations.[18] Both enantiomers of this primary substrate were thus obtained in high enantioselectivity with the same lipase.

This methodology for enzymatic-alkaline hydrolysis is simple, easy to use, and effective and can be used under environmentally friendly conditions. We have improved upon these novel reaction conditions for a range of various potentially useful substrates with CAL-B. The enantiomerically enriched alcohols and acetates have been obtained in good isolated chemical yields.

2.3. Effect of the sodium carbonate

We next studied the influence of carbonate salts on the outcome of the enzymatic hydrolysis in non-aqueous organic media. The nature of the counter-ion of the carbonate was first investigated. For this, we chose Na^+, K^+ and Ca^{2+}. We also studied the influence of the amount of sodium carbonate on the hydrolysis reaction, which was performed with 1 mmol of phenylethyl acetate **1a** with 50 mg of CAL-B in 3 mL of toluene, for 72 h at 40 °C. The amount of sodium carbonate was varied from 0.1 to 3 mmol. The results are shown in Table 3.

2.4. Attempt to interpret the reaction mechanism

The reaction was performed in toluene, a hydrophobic solvent. Without any added carbonate, it was thought that the hydrolysis was probably triggered by the structural water molecules from the enzyme. Using these conditions, moderate conversion (9%) was obtained (Table 3; entry 1). In the presence of sodium or potassium carbonate, a high increase in the lipase reactivity was noted and a conversion of C = 50% was reached (Table 3; entry 6) without any noticeable change in the selectivity.

Table 3
Effect of the carbonate salts

Entry[a]	Carbonate (mmol)	ee_S[d] (%)	Yield[e] (%)	ee_P[d] (%)	Yield[e] (%)	C[f] (%)	E[f]
1	Without[b]	9.5	59	99.5	7	9	>200
2	Na_2CO_3[c] (1)	—	—	—	—	—	—
3	Na_2CO_3 (3)	99.5	15	98	48	50.5	>500
4	Na_2CO_3 (2)	99.5	18	99.5	24	50	>500
5	Na_2CO_3 (1)	83	43	99.5	30	46	>500
6	Na_2CO_3 (0.5)	97.5	23	98.7	22	49.7	>500
7	Na_2CO_3 (0.4)	99.2	28	>99	15	48	>500
8	Na_2CO_3 (0.3)	75.2	24	98.2	19	43	>300
9	Na_2CO_3 (0.2)	36.2	70	>99	15	27	>250
10	Na_2CO_3 (0.1)	31	26	97	10	24	97
11	K_2CO_3 (1)	95	28	99.5	15	49	>500
12	K_2CO_3 (0.5)	99	24	99	19	50	>500
13	$CaCO_3$ (1)	15	—	>99	—	13	>250
14	$CaCO_3$ (0.5)	15	—	>99	—	13	>250

[a] Reaction conditions: 1 mmol of racemic acetate, appropriate amount of carbonate in 3 mL of solvent at 40 °C, in the presence of 50 mg of CAL-B (>10,000 U/g) for 72 h.
[b] Reaction in the presence of lipase and without a carbonate.
[c] Reaction without lipase and in the presence of a carbonate.
[d] Measured by GC or HPLC.
[e] Isolated yields.
[f] Conversion:[16] $C = ee_S/ee_P+ee_s$; selectivity[16] $E = \mathrm{Ln}[(1 - C)(1 - ee_{(S)})]/\mathrm{Ln}[(1 - C)(1 + ee_{(S)})]$.

Recently, the use of $CaCO_3$ as a stabilizing agent of fungal lipase has been reported. The authors assumed that this stabilization brought about by Ca^{2+} and by safeguarding the medium at pH 6 during the hydrolysis of 2-ethylhexyl acetate in aqueous media.[19]

In our case, maintaining the lipase's excellent enantioselectivity could be attributed to the capture of the leaving group (AcO^-) by the metal ion during the course of the reaction; a minimum of 0.5 equiv was crucial in activating the hydrolysis process. The presence of cations Na^+, K^+, or Ca^{2+}, afforded a salt residue, which was easy to remove by simple filtration with the lipase. The salts formed may shift some equilibrium toward the desired direction. When the reaction is carried out in aqueous media; the by-product formed is an acid. In addition, the literature reports that the addition of metal salts, such as, LiCl or $MgCl_2$ enhanced the enantioselectivity of *Candida rugosa lipase* (CRL) during the course of enzymatic hydrolysis in biphasic media.[20] This enhancement in enantioselectivity was attributed to a change of conformation and the lipase's flexibility. Similar observations have been reported by Salgin et al., who demonstrated that the treatment of CRL with metal ions, or their use as additives, increased the hydrolysis rate of racemic naproxen methyl ester in biphasic media. These additives may ensure electrostatic interactions between metal ions/enzyme, thus inducing conformational changes. The increase in the interaction energy allows better accessibility to the enzyme by the substrate.[21]

In our case, it is noteworthy that in the absence of a carbonate salt, moderate reactivity with an optimum conversion of 9% is reached. The introduction of an increasing amount of Na_2CO_3 has an important effect on the reaction rate and an optimum $C = 50\%$ was finally reached; this was also observed with K_2CO_3. When, a divalent cation Ca^{2+} was introduced, we observed a dramatic decrease of the reaction rate, and the conversion does not exceed 13%, showing almost no improvement compared to the reaction carried out without a carbonate (Table 3, entries 13 and 14); attesting more difficulties that reduced the substrate penetration to the active site. However, the approach of substrates to the active site seemed to be easier in the presence of monovalent cations in the reaction medium.[22]

The resolution of acetates with CAL-B, an immobilized lipase, via hydrolysis in the presence of carbonate salts is applicable on equimolar scale in organic media; optimal conversion was achieved with C >45% with monovalent carbonates. When, in the presence of divalent carbonates, a marked decrease of the reaction rate to $C = 13\%$ was observed. It should be noted that the selectivity was still high with all of the substrates studied E >250.

3. Conclusion

Herein we have reported a novel methodology for kinetic resolution via enzymatic hydrolysis in non-conventional media *via* carbonate salts. The performance of this reaction was improved with various types of primary and secondary aromatic acetates.

Enzymatic hydrolysis was carried out under hydrophobic organic media, in the presence of both sodium carbonate and CAL-B lipase. The effect of the carbonate salts was investigated, and proven to have a significant influence on the enzymatic reactivity, without any perturbation on its selectivity. An equimolar amount of Na_2CO_3 must be present in order to achieve the optimal conversion. Finally, we have developed a novel methodology for the enzymatic hydrolysis, in the presence of sodium carbonate, using simple and green chemistry.

4. Experimental

4.1. General

NMR spectra were recorded on Brucker spectrometers (300 MHz for ^1H, 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm from tetramethylsilane with the solvent resonance as the internal standard for ^1H NMR and chloroform-*d* (δ 77.0 ppm) for ^{13}C NMR. Coupling constants (J) are given in Hertz. The following abbreviations classify the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal. The mass spectra were obtained from the mass-service at the Catholic University of Louvain (FINNIGAN-MAT TSQ 7000 and FINNIGAN-MAT LQC spectrometers). IR spectra were recorded on Shimadzu FTIR-8400S spectrometer. Optical rotations were determined using a Perkin–Elmer 241 Polarimeter at room temperature using a cell of 1 dm length and $\lambda = 589$ nm. The enantiomeric excesses were measured by a chiral stationary phase HPLC on Chiralpak® AD column and Chiralcel®ODH or by gas chromatography (ThermoFinnigan Trace GC) equipped with an automatic autosampler and using a CHIRALSIL-DEX CB column (25 m; 0.25 mm; 0.25 μm). Retention times are reported in minutes.

4.2. Synthesis of 2-hydroxymethyl-1-phenylthioferrocene

The starting racemic 2-hydroxy-methyl-1-phenylthio-ferrocene was prepared as previously described starting from commercially available *N*,*N*-dimethylamino-ferrocene.[18] Ortholithiation by *tert*-butyllithium in diethyl ether followed by electrophilic trapping with diphenyl disulfide afforded the *N*,*N*-dimethylaminomethyl-1-phenylthio-ferrocene. The alcohol was then generated by the reaction of the amine in acetic anhydride followed by methanolysis the resulting the 2-acetoxymethyl-1-phenylthioferrocene.

4.2.1. *N*,*N*-Dimethylaminomethyl-1-phenylthio-ferrocene[18]

Mp: 70 °C, IR (film, cm^{-1}) : ν = 690.4; 736.7; 817.7; 1026; 1080; 1103.2; 1176.5; 1261.3; 1477.3; 1581.5; 2765.7; 2812; 2939.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.02 (s, 6H, N(C***H***$_3$)$_2$); 3.37 & 3.42; 3.43 & 3.47 AB (dd, *J* = 13.3 Hz for each one, 2H , C***H***$_2$N(CH$_3$)$_2$); 4.6 (s, 5H, C_P); 4.32 (s, 1H, C_P), 4.46 & 4.50 (d, 2H, *J* = 13.6 Hz, C_P); 7.01–7.11 (m_a, 5H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 45.1; 57.0; 69.1; 70.3; 71.3; 75.6; 76.0; 88.0; 124.8; 126.2; 128.5; 140.0. MS (D-APCI; *m*/*z*): 242.18 ([FcCH$_2$NMe$_2$]$^+$, 100%); 307 ([FcSPhCH$_2$]$^+$, 49%); 350.08([M−H]$^+$, 35%); 351 ([M]$^+$, 24%); 352.04 ([M+H]$^+$, 6%).

4.2.2. 2-Acetoxymethyl-1-phenylthioferrocene 2a[18]

Mp: 128 °C. IR (film, cm^{-1}): ν = 690.4; 744.4; 817.7; 952.7; 999; 1022.2; 1238.2; 1369.3; 1577.6; 1728.1 (νC=O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.75(s, 3H, O=C–C***H***$_3$); 4.27 (s, 5H, C_P); 4.41(s, 1H, C_P), 4.52 et 4.55 (d, 2H, *J* = 10.17Hz, C_P); 4.90 et 4.94 (d, *J* = 11.38 Hz, 1H) et 5.07–5.11 (d, *J* = 12.39Hz, 1H) C***H***$_2$OH; 7.04–7.2 (m_a, 5H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.61; 61.00; 69.62; 70.21; 71.83; 76.17; 85; 124.95; 126.17; 128.48; 141.5; 172. MS (EI; *m*/*z*): 43.9 ([Ac]$^+$, 86%); 148.8 ([Fc-H]$^+$, 71%); 185.8 ([Fc]$^+$, 53%); 366 ([M]$^+$, 100%); 367.1 ([M+H]$^+$, 24%).

4.2.3. 2-Hydroxymethyl-1-phenylthioferrocene 2[18]

MP: 127 °C. IR (film, cm^{-1}): ν = 690.4; 740.6; 817.7; 987.4; 1006.7; 1076.2; 1107; 1249.7; 1481.2; 1581.5; 3244 (ν OH). ^{1}H NMR (300 MHz, $CDCl_3$): 1.51 (br s, O***H***); 4.27 (s, 5H, C_P); 4.41(s, 1H, C_P); 4.37(m, 2H, C_P), 4.53 (m, 1H, CP + 2H, C***H***$_2$OH); 7.06 (m, 3H, Ph); 7.17 (m, 2H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 59.0; 70.1; 70.0; 75.6; 91.0; 96.2;125.2; 125.8; 128.9; 140.0. MS (EI; *m*/*z*): 185.8 ([Fc]$^+$, 100%); 323.9 ([M]$^+$, 100%).

4.3. General procedure for the synthesis of racemic acetates 1a–9a

The acetates were synthesized by classical chemical acetylations via the corresponding racemic alcohol (1 equiv), using 1.5 equiv of anhydride acetic, 1.2 equiv of Et_3N, and a catalytic amount of 4-dimethylaminopyridine (0.1 equiv) in 4 ml of ether. The acetates were obtained pure after standard work-up. The ^{1}H NMR spectra of these products were in good agreement with the literature.

4.4. General procedure for the hydrolysis of racemic acetates 1a–9a with *Candida Antarctic- B* lipase

A dry Schlenk tube was charged with 1 mmol of the racemic acetate **1a**–**9a** is dissolved in 3 mL of solvent before the addition of 1 mmol of sodium carbonate and 50 mg of CAL-B. The suspension was stirred at 40 °C for the indicated time. The reaction mixture was filtered on Celite and concentrated in vacuo. The acetate formed and the remaining alcohol were separated by flash chromatography on silica gel (petroleum ether/ethyl acetate: 80/20) and analyzed by chiral HPLC or GC.

4.5. Chiral GC analysis and/or chiral HPLC analysis

The chemical analysis was performed by gas chromatography (ThermoFinnigan Trace GC) equipped with an automatic autosampler and using a CHIRALSIL-DEX CB column (25 m; 0.25 mm; 0.25 μm), or by a chiral stationary phase HPLC on Chiralpak-AD column or Chiralcel-ODH column. Retention times are reported in minutes. The absolute configurations of all chiral compounds were determined by polarimetry in comparison with the literature data. The conditions for the analysis of alcohols (*R*)-**1**–**9** and acetates (*S*)-**1a–9a** are reported below.

4.5.1. (*R*)-(+)-1-Phenylethanol 1

GC (Chiralsil-Dex CB,): t_R = 3.9 min, t_S = 4.1 min (T_{column} = 140 °C), $[\alpha]_D^{20}$ = +53 (*c* 0.2, $CHCl_3$) for 99% ee.[15b]

4.5.2. (*R*)-(+)-1-(4-Methoxyphenyl) ethanol 3

GC (Chiralsil-Dex CB): t_R = 12.7 min. t_S = 13.5 min (T_{column} = 135 °C), $[\alpha]_D^{20}$ = +40 (*c* 0.2, $CHCl_3$) for 97% ee.[23]

4.5.3. (*R*)-(+)-1-(4-Ethoxyphenyl) ethanol 4

GC (Chiralsil-Dex CB): t_R = 13.1 min., t_S = 13.9 min (T_{column} = 155 °C). $[\alpha]_D^{20}$ = +41 (*c* 0.5, $CHCl_3$) for 97.6% ee.[24]

4.5.4. (*R*)-(+)-1-(2-Naphthyl) ethanol 5

GC (Chiralsil-Dex CB): t_R = 10.2 min, t_S = 10.5 min. T_{column} = 170 °C, $[\alpha]_D^{20}$ = +38.3 (*c* 0.25, $CHCl_3$) for 99% ee.[15b]

4.5.5. (*R*)-(+)-1-(6-Methoxynaphthalen-2-yl) ethanol 6

GC (Chiralsil-Dex CB): t_R = 17.2 min. t_S = 17.7 min. T_{column} = 180 °C). $[\alpha]_D^{20}$ = +39.8 (*c* 0.15, $CHCl_3$) for 99% ee.[15b]

4.5.6. (*R*)-(−)-1-Indanol 7

GC (Chiralsil-Dex CB), t_R = 51.2 min. t_S = 51.9 min. T_{column} = 80 °C for 7 min, after 135 °C for 5 min. $[\alpha]_D^{20}$ = −16 (*c* 0.1, $CHCl_3$) for 99% ee.[15b]

4.5.7. (*R*)-(−)-1,2,3,4-Tetrahydronaphthalen-1-ol 8

GC (Chiralsil-Dex CB), t_S = 63.7 min. t_R = 65.0 min. T_{column} = 80 °C for 7 min, after 135 °C for 5 min. $[\alpha]_D^{20}$ = −28 (*c* 0.5, $CHCl_3$) for 99% ee).[15b]

4.5.8. (*R*)-(−)-Acenaphthenol 9

Chiral HPLC: *Chiracel* OD-H column, t_R = 38.2 min; t_S = 41.5 min. Eluant (v,v): hexane/*i*-PrOH: 97/3; debit 0.5 mL/min; $[\alpha]_D^{20}$ = −1.4 (*c* 0.5, $CHCl_3$) for 98.2% ee.[13d]

4.5.9. (*RFc*)-(+)-2-Hydroxymethyl-1-phenylthioferrocene 2

Chiral HPLC: Chiralpak AD column, t_R = 30.6 min, t_S = 34.1 min. Eluant (v,v): hexane/EtOH: 97/3; debit: 1 mL/min. $[\alpha]_D^{20}$ = +56 (*c* 1, CH_2Cl_2) for 99% ee).[18a]

4.5.10. (*S*)-(−)-1-(Phenylethyl) acetate 1a

GC (Chiralsil-Dex CB), t_S = 2.9 min. t_R = 3.2 min, T_{column} = 140 °C. $[\alpha]_D^{20}$ = −136 (*c* 0.1, $CHCl_3$) for 99% ee.[15b]

4.5.11. (*S*)-(−)-1-(4-Methoxyphenyl)ethyl acetate 3a

GC (Chiralsil-Dex CB), t_S = 10.9 min. t_R = 12.1 min, T_{column} = 135 °C. $[\alpha]_D^{20}$ = −13 (*c* 0.3, $CHCl_3$) for 96% ee.[23]

4.5.12. (*S*)-(−)-1-(4-Ethoxyphenyl)ethyl acetate 4a

GC (Chiralsil-Dex CB), t_S = 12.2 min. t_R = 13.4 min. T_{column} = 155 °C. $[\alpha]_D^{20}$ = −110 (*c* 0.5, $CHCl_3$) for 95% ee.[24]

4.5.13. (*S*)-(−)-1-(Naphthalen-2-yl) ethyl acetate 5a

GC (Chiralsil-Dex CB), t_S = 8.6 min. t_R = 8.9 min. T_{column} = 170 °C. $[\alpha]_D^{20} = -110.7$ (*c* 0.12, $CHCl_3$) for 98% ee.[15b]

4.5.14. (*S*)-(−)-1-(6-methoxynaphthalen-2-yl) ethyl acetate 6a

GC (Chiralsil-Dex CB), t_S = 16.2 min. t_R = 16.6 min. T_{column} = 180 °C. $[\alpha]_D^{20} = -110$ (*c* 0.1, $CHCl_3$) for 99% ee.[15b]

4.5.15. (*S*)-(−)-2,3-dihydro-*1H*-inden-yl acetate 7a

GC (Chiralsil-Dex CB): t_S = 46.1 min. t_R = 46.9 min. T_{column} = 80 °C for 7 min, after 135 °C for 5 min. $[\alpha]_D^{20} = -101$ (*c* 0.8, $CHCl_3$) for 97% ee.[23]

4.5.16. (*S*)-(−)-1,2,3,4-Tetrahydronaphthalen-1-yl acetate 8a

GC (Chiralsil-Dex CB): t_R = 57.4 min. t_S = 58.6 min. T_{column} = 80 °C for 7 min, after 135 °C for 5 min. $[\alpha]_D^{20} = -107$ (*c* 0.7, $CHCl_3$) for 99% ee.[23]

4.5.17. (*S*)-(−)-1-Acenaphthyl acetate 9a

Chiral HPLC: *Chiracel* OD-H column. t_S = 11.5 min; t_R = 12.8 min. Eluant (v,v): hexane/*i*-PrOH: 99.3/0.7; debit 1 mL/min; $[\alpha]_D^{20} = -78$ (*c* 0.15, $CHCl_3$) for 90% ee.[13d]

4.5.18. (*SFc*)-(−)-2-Acetoxymethyl-1-phenylthioferrocene 2a

Chiral HPLC: *Chiralpak* AD column. t_S = 9.5 min, t_R = 12.8 min. Eluant (v,v): hexane/EtOH: 99/1; debit 1 mL/min.[18a]

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