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Influence of substituents on enantiomeric ratio in transesterification of racemic C-3 synthons using lipase B from *Candida antarctica*

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Abstract

The fluorides, chlorides and bromides of 3-halo-1-phenoxy-2-propanol, 3-halo-1-phenylmethoxy-2-propanol and 3-halo-1-(2-phenylethoxy)-2-propanol have been resolved by transesterification with various butanoates as acyl donors in hexane and lipase B from *Candida antarctica* (Novozyme 435) as catalyst. The enantiomeric ratio *E* depended on the hydroxy protecting groups in 1-position and the halogens in 3-position. For some substrates, the enantiomeric ratio was dependent on the acylating agent.

Keywords: Candida antarctica lipase B; Transesterification; Halohydrins; Enantiomeric ratio; Acyl donor

1. Introduction

Lipase B from *Candida antarctica* (CALB) has been widely used as a catalyst in kinetic resolutions. In particular, high enantiomeric ratios, *E*, have been obtained with secondary alcohols [1–4]. On this basis, it is important to understand what structural features in the substrate controls the enantiomeric discrimination of this enzyme.

The X-ray crystal structure has been refined to 1.55 Å resolution and it reveals that the enzyme has a catalytic triad consisting of Ser–His–Asp [5]. Moreover, CALB does not seem to have a structural element that covers the active site, the so-called lid. This structural feature implies that CALB does not show interfacial activation which has been taken as the most characteristic property of lipases [6,7]. Stereospecificity studies using the monomolecular layer technique has shown that it is 1,3-specific in hydrolysis of glycerides as opposed to another characterized lipase from *C. antarctica*, lipase A, which has preference for the 2-position [8]. Molecular modeling of the butanoate of 1-methoxy-3-[2-phenylethoxy]-2-propanol revealed a stereospecificity pocket in CALB [9]. Fig. 1 shows a model of the fast reacting enantiomer (in this case the *R*-form) bound to the serine of the catalytic triad. The stereospecificity pocket is located at the bottom of the active site cleft close to the serine (Ser 105) of the catalytic triad. The faster reacting enantiomer fits into the active site as a «V», one arm consisting of the largest group ($ROCH_2$–, in Fig. 1, R = $–CH_2CH_2Ph$) the other arm con-

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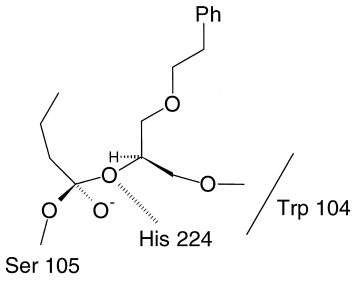


Fig. 1. Model of the fast reacting enantiomer (In this case the *R*-form) of the butanoate of 1-methoxy-3-[2-phenylethoxy]-2-propanol in the active site cleft of CALB. The substrate may be seen as a ''V'' with the acyl group as one wing, the largest group located at the stereocentre being the other wing, and the ester oxygen bound to Ser 105 at the middle. At the bottom of the stereospecificity pocket there is located a tryptophane residue (Trp 104) which limits the size of the small group at the stereocentre. Another important feature is a hydrogen bond between the ester oxygen and His 224.

sisting of the acyl group and the ester oxygen bound to Ser 105 in the middle. At the secondary stereocenter, there is also the medium size substituent ($-CH_2R$, in Fig. 1, $R = -OCH_3$). The latter being positioned in the sterospecificity pocket whose size is limited by a tryptophane residue (Trp 104). Another important feature for catalysis is a hydrogen bond between the ester oxygen and His 224 as indicated in Fig. 1. A slight collision between the OCH_3-group of the substrate and Trp 104 was observed. It has been suggested that also non steric interactions (electronic interactions) in the stereospecificity pocket are important for the preference of the enzyme [10].

Earlier studies with related substrates indicated that when varying the protecting group through the series Ph, CH_2Ph, CH_2CH_2Ph the *E*-value also varied [11]. We wanted to investigate this further using substrates with the same protecting groups (Scheme 1).

Our main objective was to study the preference of the enzyme. However, these compounds are also versatile synthons for β-blockers [12] and anti-viral agents [13–15]. The bromides and chlorides may easily be converted into the corresponding glycidyl ethers which have a broad range of applications.

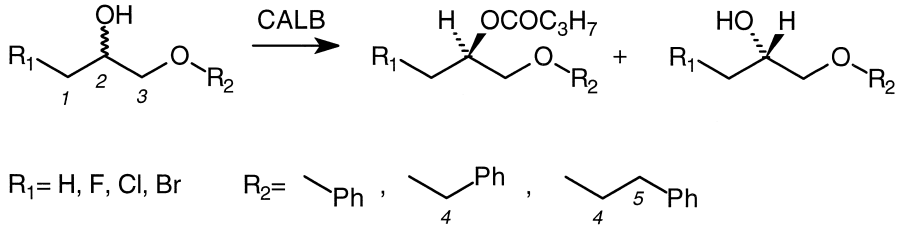
We have previously resolved substrates **5** and **6** by hydrolysis using CALB [16]. In particular, addition of cosolvents led to high *E*-values [2]. Substrates **4** and **7** have been resolved using crude *Pseudomonas* lipase, giving *E*-values of 223 and 56, respectively [17]. Substrate **4** has been resolved with a lipoprotein lipase from *Pseudomonas aeruginosa* with an *E*-value of 58 [18].

2. Results and discussion

The transesterifications were performed using the alcohols **1**–**9** as acyl acceptors (Scheme 1) and vinyl butanoate, 2,2,2-trifluoroethyl butanoate, 2-chloroethyl butanoate and 2,2,2-trichloroethyl butanoate as acyl donors. The acyl donors were used in excess ($5\times$) relative to the racemic alcohols and solvent was hexane. For transesterification of substrates **1**–**9** we have calculated enantiomeric ratios, *E*, and equilibrium constants, K_{eq}, on the basis of ping-pong bi-bi kinetics [19].

2.1. Resolution of fluorohydrins *1*–*3*

Resolution of 3-fluoro-1-phenoxy-2-propanol (**1**) with 2,2,2-trifluoroethyl butanoate gave a



Scheme 1. Resolution of compounds **1**–**9** by transesterifications in hexane catalysed by CALB.

high *E*-value ($E = 490$). When increasing the size of the protecting group (R_2 in Scheme 1) by one CH_2-unit, the enantiomeric ratio decreased to $E = 17$ (substrate **2**). For fluorohydrin, **3**, however, the enantiomeric ratio was high ($E = 375$). Fig. 2 shows a plot of enantiomeric excess vs. conversion for substrate **1**–**3** using this acyl donor. The curves of substrate **1** and **3** almost coincide, indicating that the discrimination of enantiomers by CALB is very similar. With vinyl butanoate as acylating agent the *E*-values were lowered for resolution of **1**, **2** and **3** ($E = 87$, 4 and 37, respectively) and the same trend concerning the effect of the protecting group on the enantiomeric ratio was observed.

There is a large difference in the enantiomeric ratio between substrates **1** and **3** on one hand and **2** on the other (Fig. 2). It is important to realize that the enantiomeric ratio reflects the ratio of reactivity between the fast and slow reacting enantiomer. Therefore, it is not straightforward to explain the reason for a steric effect on *E* on the basis of molecular modeling unless both enantiomers are taken into account. Lower *E*-values for compound **2** may be due to increased rate for the slow reacting enantiomer or a decreased rate for the fast reacting enantiomer. The conversion of each enantiomer can

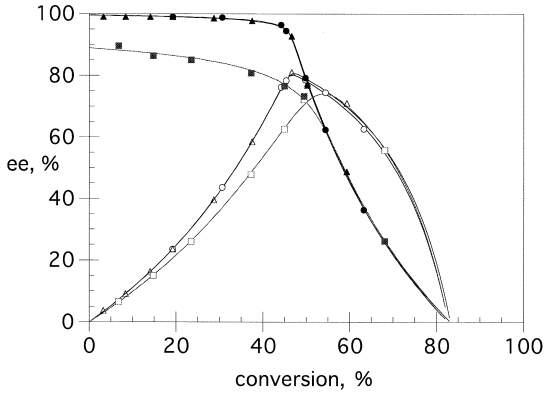


Fig. 2. Resolution of substrate **1**–**3** using 2,2,2-trifluoroethyl butanoate as acyl donor. Enantiomeric excess of product/substrate are plotted against conversion. Circles: **1**, squares: **2**, and triangles: **3**. Filled symbols, product fraction, open symbols, substrate fraction.

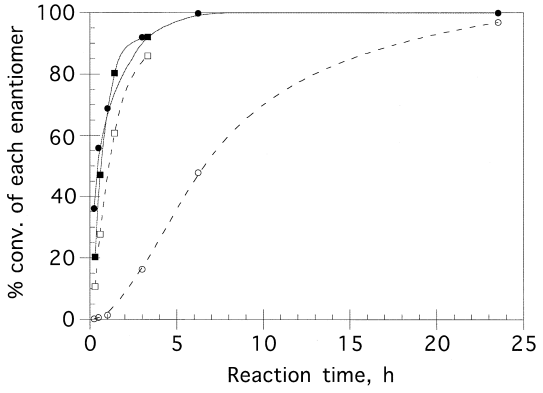


Fig. 3. Conversion of each enantiomer plotted against reaction time for resolution of substrate **1** and **2** with vinyl butanoate as acyl donor, circles, **1**, squares, **2**, filled symbols, fast reacting enantiomer, open symbols, slow reacting enantiomer.

be calculated from ee_p (or ee_s) and the % conversion of the resolution reaction. By plotting the conversion of each enantiomer vs. time for each measurement it can be seen that the lowered *E*-value of substrate **2** is due to a higher reaction rate of the slow reacting enantiomer for this substrate (Fig. 3).

*2.2. Resolution of chlorohydrins **4**–**6***

Resolution of chlorohydrins **4**–**6** gave lower *E*-values than for the corresponding fluorohydrins. Using 2,2,2-trifluoroethyl butanoate as acyl donor *E*-values for **4**, **5** and **6** were 41, 20 and 32, respectively. With vinyl butanoate the *E*-values were low for all of the substrates. Interestingly, the acyl donor 2-chloroethyl butanoate gave higher *E*-values for these substrates ($E = 85$, 23 and 41 for **4**, **5** and **6**, respectively), which correspond with our earlier findings [11]. However, resolutions with this acyl donor have the drawback of very low equilibrium constants, i.e., the equilibrium is shifted towards starting materials. Fig. 4 shows resolutions of 3-chloro-1-phenoxy-2-propanol (**4**) using 2-chloroethyl butanoate and vinyl butanoate as acyl donors. The effect of the acyl donor on *E* and K_{eq} is apparent. Again there is a pronounced effect of the protecting group on the *E*-value, as dis-

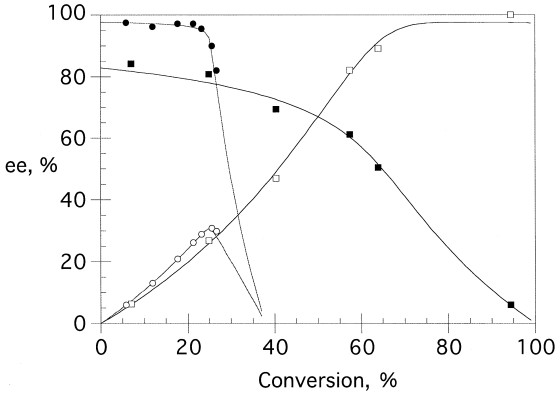


Fig. 4. Effect of acyl donor on E and K_{eq}. Enantiomeric excesses of product and substrate are plotted against conversion. Resolution of 3-chloro-1-phenoxy-2-propanol using vinyl butanoate (squares) and 2-chloroethyl butanoate (circles) as acyl donors, filled symbols, product fraction, open symbols, substrate fraction.

cussed above. This effect is observed in resolution of the chlorohydrins, for all of the acyl donors except for vinyl butanoate.

2.3. Resolution of bromohydrins **7**–**9**

For resolution of the bromohydrins, the situation was reversed concerning the acyl donors. Resolutions using vinyl butanoate gave higher *E*-values than for the same reaction using 2,2,2-trifluoroethyl butanoate. Resolution of 3-bromo-1-phenoxy-2-propanol (**7**) with vinyl butanoate proceeded with a fairly high *E*-value ($E = 58$). Bromohydrin **9** reacted in a similar way to **7**. As can be seen from Fig. 5, where the reaction progress of the resolution of substrate **7**–**9** using vinyl butanoate as acylating agent are plotted, the curves of the two resolutions almost are superimposed. Again for the benzyloxy protected bromohydrin **8** the *E*-value was low ($E = 9$). The reason for this difference in *E*-value between substrates having different protecting groups is that the slow reacting enantiomer of 3-bromo-1-phenylmethoxy-2-propanol (**8**) reacted faster than the slow reacting enantiomer for the other two substrates. The fast reacting enantiomers of substrates **7**–**9** reacted at almost equal rate.

2.4. Effect of the acyl donor

It is well known that the acylating agent has a strong influence of the thermodynamics of the resolution process. Vinyl butanoate is considered irreversible, and the other acyl donors are more or less reversible. Achiral acyl donors which all produce the same acyl enzyme should in theory give the same *E*-value. However, in some cases, the acyl donor have been reported to affect *E* [20,21]. We have previously investigated the effect of different acyl donors and concluded that vinyl butanoate was not well suited as acylating agent for resolution of 1-phenoxy-, 1-phenylmethoxy- and 1-[2-phenylethoxy]-2-propanol using CALB because of low *E*-values [11]. As can be seen from Tables 1 and 2 and Fig. 4 the *E*-value of the resolution process depended on the acylating agent. Moreover, the efficacy of the acyl donor in terms of high *E*, also depend on the substrate that is used. For instance, substrates **1**–**6** give higher *E* with reversible acyl donors while the bromohydrins (**7**–**9**) seemed to give higher *E*-values when vinyl butanoate was used.

When searching for the most suited acyl donor, both the *E*- and K_{eq}-values must be

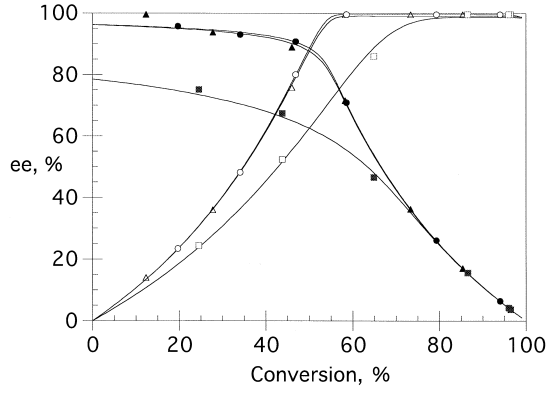


Fig. 5. Resolution of substrate **7**–**9**. Enantiomeric excess of product and substrate plotted against conversion, circles, **7**, squares, **8** and triangles, **9**, filled symbols, product fraction, open symbols, substrate fraction.

Table 1
E- and K_{eq}-values in resolution of substrates **1**–**9** using vinyl butanoate and 2,2,2-trifluroethyl butanoate as acyl donors. K_{eq}-values for reactions with vinyl butanoates were in the range of 10 000

Substrate	R_1	R_2	Vinyl butanoate *E*	2,2,2-Trifluroethyl butanoate	
				E	K_{eq}
1	F	Ph	87	490	0.95
2	F	CH_2Ph	4	17	0.95
3	F	CH_2CH_2Ph	37	375	0.99
4	Cl	Ph	11	41	0.63
5	Cl	CH_2Ph	13	20	0.94
6	Cl	CH_2CH_2Ph	15	32	0.56
7	Br	Ph	58	37	0.48
8	Br	CH_2Ph	9	13	0.59
9	Br	CH_2CH_2Ph	52	26	0.50

taken into account, since for reversible acyl donors the substrate enantiomer can only be obtained in moderate enantiomeric excess. The product enantiomer on the other hand, can be obtained in reasonable good yield with a reversible acyl donor (Fig. 2). A list of acyl donors giving the highest *E*-values for the different substrates is presented in Table 2.

2.5. Effect of the halogens

It has been shown that substrates containing bromine and chlorine have a lower tendency of placing the halogen in the sterospecificity pocket [10]. This leads to lower *E*-value when bromine or chlorine atoms are located in the medium size substituent, and higher *E*-values when bromine and chlorine are a part of the large substituent. Probably, there are unfavorable interactions between the π-electron system of the tryptophane or some other residue inside the pocket and the halogens. In the above-mentioned study, chlorides gave higher *E*-values than bromides. We find that the effect of the different acyl donors are not simple to explain since the effect changes when different substrates are used. However, choosing the highest *E*-value for all of the substrates regardless of acyl donor used, it is shown that highest *E* is obtained when substituents contain F and H and furthermore, that Cl is better than Br (Table 2). It seems that small compact atoms having few available electrons (H and F) give higher *E*-values than the larger more polarizable atoms (Cl and Br). However, there is also an increase in size going through the series H > F > Cl > Br, and the size effect will also contribute. We are currently challenging the size of the stereospecific pocket in order to determine the largest group that it can accommodate.

2.6. Effect of the protecting group

From the above discussion, it may be concluded that the *E*-values are higher with Ph and CH_2CH_2Ph than for CH_2Ph. We have identi-

Table 2
The best acyl donor in terms of *E*-value for resolution of 12 different substrates. Acyl donors tried for substrate **1**–**9** were 2-CEB, 2-chloroethyl butanoate, 2,2,2-TFEB, 2,2,2-trifluoroethyl butanoate, 2,2,2-TCEB, 2,2,2-trichloroethyl butanoate and VB, vinyl butanoate. For substrates **10**–**12** the acyl donors tried were 2-CEB, 2,2,2-TFEB, VB and butanoic anhydride [11]. For the significance of R_1 and R_2, see Scheme 1

Substrate	R_1	R_2	Maximum *E*-value	Acyl donor
10	H	Ph	139	2-CEB
11	H	CH_2Ph	22	2-CEB
12	H	CH_2CH_2Ph	319	2-CEB
1	F	Ph	490	2,2,2-TFEB
2	F	CH_2Ph	30	2-CEB
3	F	CH_2CH_2Ph	375	2,2,2-TFEB
4	Cl	Ph	83	2-CEB
5	Cl	CH_2Ph	23	2-CEB
6	Cl	CH_2CH_2Ph	55	2,2,2-TCEB
7	Br	Ph	58	VB
8	Br	CH_2Ph	16	2,2,2-TCEB
9	Br	CH_2CH_2Ph	52	2,2,2-TCEB/VB

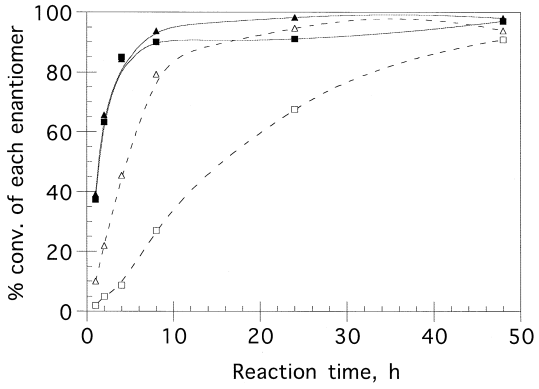


Fig. 6. Conversion of each enantiomer plotted against reaction time, circles, **7**, squares, **8**, filled symbols, fast reacting enantiomer, open symbols, slow reacting enantiomer. Conversion (%) of each enantiomer is plotted vs. time for the reactions using vinyl butanoate as acyl donor. For the sake of simplicity, results for **9** are excluded from the plot. For this substrate, the curve was almost identical with the curve for **7**.

fied the reason for this to be a higher rate for the slow reacting enantiomer in the case of the benzyloxy derivative (Figs. 3 and 6). Earlier studies on related substrates both in hydrolysis [16,22,23] and transesterifications [11] have shown the same effect which seems to be more clear in transesterification than in hydrolysis. What this means on a molecular level is not yet known. Modeling of the slow reacting enantiomer of the butanoate of 1-methoxy-3-[2-phenylethoxy]-2-propanol showed that although it retains the H-bond between the ester oxygen and His 224 (see Fig. 1), the large group is in a very unfavorable strained conformation [9]. Thus, it may seem that the slow reacting enantiomer fits better for the benzyloxy derivative. Interestingly resolution of 1,1,1-trifluoro-5-benzyloxy-2-ol-2-yne gave strong preference for the *S*-enantiomer ($E > 100$) [24]. This substrate has two rather large groups connected to the stereocenter. The preference of the enzyme indicates that the alkyne part probably is not located in the stereospecificity pocket.

3. Conclusion

- Ph and CH_2CH_2Ph as protecting groups give higher *E*-values than CH_2Ph.
- H or F as part of the medium size substituent gives higher *E*-values than Cl or Br.
- When the medium size substituent is H, F or Cl highest *E*-values are obtained with reversible acyl donors.

When the medium sized substituent is Br highest *E*-values are obtained with irreversible acyl donors.

4. Experimental

4.1. General

Immobilized CALB (Novozyme 435, Novo-Nordisk) had an activity of 7000 PLU/g, and a water content of 1–2% w/w. (*S*)-glycidol and (*S*)-epichlorohydrin were purchased from Sigma and Daiso, Osaka, Japan, respectively. HPLC-grade hexane was dried over molecular sieves.

4.2. Analytical methods

Chiral analyses were performed using a Varian 3400 gas chromatograph equipped with a CP-Chirasil-DEX-CB column from Chrompack. All of the substrates **1**–**9** were derivatized and analyzed as the corresponding acetates **1a**–**9a**. The corresponding butanoates **1b**–**9b** were analyzed directly without derivatization. Column pressure: **1**–**3**, 7.5 psi, **4** and **6**, 8 psi, **5**, 7 psi, **7–9**, 9 psi. NMR spectroscopy was performed using Bruker DPX 300, 400 and 600 operating at 300, 400 and 600 MHz for ^1H, respectively. Chemical shifts are in ppm rel. to TMS and coupling constants in Hz. Homo- and heteronuclear COSY experiments were used for assignments.

4.3. Transesterifications

To hexane (3 ml), was added substrate (1.31×10^{-4} mol) and acyl donor (6.55×10^{-4} mol).

The reaction was started by adding CALB (20 mg) to the reaction mixture. The reactions were performed in a shaker incubator at 30°C. Analysis gave ee_s- and ee_p-values from which conversion, c, was determined, $c = ee_s/(ee_s + ee_p)$. The degree of conversion from the measurements of ee_s and ee_p were the same as by using internal standards for one substrate in control experiments. Internal standards were not used since they may influence on the physical and chemical nature of the reaction medium, and in turn the enzyme catalyzed reaction. In control experiments without enzyme, no acylation was observed. Enantiomeric ratios, E and equilibrium constants, K_{eq} were calculated using the computer program *E and K calculator 2.03* [25].

4.4. Preparation of substrates

Racemic substrates and enantiopure reference compounds were prepared from the corresponding glycidyl ethers. (*R*)-phenyl glycidyl ether was prepared from (*S*)-glycidol and phenol under Mitsunobu conditions [23]. (*R*)-phenylmethyl glycidyl ether and (*R*)-2-phenylethyl glycidyl ether were synthesized from epichlorohydrin and benzyl alcohol or 2-phenylethanol with NaOH and tetrabutylammonium hydrogen sulfate [26]. The enantiomeric excess of the fluoro- and bromohydrins were dependent on the enantiomeric excess of the corresponding glycidyl ethers, which was in the range 84–99%. During synthesis of fluorides **1**–**3** using tetrabutylammonium dihydrogen trifluoride, the reported regioselectivity for preparation of **1** was not observed [27]. Products were purified by flash chromatography, eluents: **1** pet. ether/Et_2O: 1.5:1, **2** pet. ether/Et_2O: 2:1, **3** CH_2Cl_2/acetone: 9:1. (*S*)-(+)-3-fluoro-1-phenoxy-2-propanol: ee > 99%, $[\alpha]_D^{18} + 16.7$ (*c* 1.50, EtOH), (*S*)-(+)-3-fluoro-1-phenylmethoxy-2-propanol: ee 91%, $[\alpha]_D^{18} + 10.5$ (*c* 1.52, EtOH), (*S*)-(+)-3-fluoro-1-[2-phenylethoxy]-2-propanol: ee 84%, $[\alpha]_D^{18} + 11.2$ (*c* 1.61, EtOH). The chlorides **4**–**6** were prepared from the corresponding glycidyl ethers using Li_2CuCl_4 [28]. Products were purified by distillation in vacuo. The bromides **7**–**8** were prepared from the corresponding glycidyl ethers using LiBr and AcOH in THF [29]. Products were purified by column chromatography, eluent: pet. ether/acetone 8:2. (*S*)-(+)-3-bromo-1-phenoxy-2-propanol: ee 96%, $[\alpha]_D^{20} + 5.3$ (*c* 1.71, EtOH), (*S*)-(+)-3-bromo-1-phenylmethoxy-2-propanol: ee 89%, $[\alpha]_D^{20} + 2.9$ (*c* 1.70, EtOH), (*S*)-(+)-3-bromo-1-[2-phenylethoxy]-2-propanol: ee 87%, $[\alpha]_D^{20} + 6.5$ (*c* 1.69, EtOH).

4.5. Determination of absolute configurations

The absolute configuration of **1**–**3** and **7**–**9** were verified by synthesis from enantiomerically enriched glycidyl ethers as described above. The absolute configurations of **6** and **7** have been identified previously [16]. The absolute configuration of **4** was not determined directly, but assigned from the known preference of CALB and *Pseudomonas* lipase [17]. The preferred enantiomer had also the same elution order as substrates **1**–**3** and **5**–**9**.

4.6. Chromatographic properties of acetates *1a–9a* and butanoates *1b–9b*

R_{tR} and R_{tS} retention time of (*R*) and (*S*) enantiomer, respectively, R_S resolution.

4.6.1. 3-Fluoro-1-phenoxy-2-propanol (*1*)

Temp. prog. 120–150°C, 1°/min, **1a** R_{tR} 16.64 min, R_{tS} 18.23 min, R_s 4.2, **1b** R_{tR} 27.95 min, R_{tS} 28.68 min, R_s 2.1.

4.6.2. 3-Fluoro-1-phenylmethoxy-2-propanol (*2*)

Temp. prog. 130–142°C, 1°/min, 5 min hold, 142–155°C, 1°/min, **2a** R_{tR}: 15.93 min, R_{tS} 17.12 min, R_s 3.5, **2b** R_{tR} 27.95 min, R_{tS} 28.58 min, R_s 2.6.

4.6.3. 3-Fluoro-1-[2-phenylethoxy]-2-propanol (*3*)

Temp. prog. 120–150°C, 1°/min, 150–157°C, 0.5°/min, **3a** R_{tR} 27.31 min, R_{tS} 28.56

min, R_s 3.5, **3b** R_{tR} 40.93 min R_{tS} 41.63 min R_s 2.3.

4.6.4. 3-Chloro-1-phenoxy-2-propanol (4)

Temp. prog. 110–140°C, 2°C/min, 140–151°C, 0.5°C/min, 5 min hold, **4a** R_{tR} 25.21 min, R_{tS} 26.63 min, R_s 5.9, **4b** R_{tR} 39.46 min, R_{tS} 40.15 min, R_s 2.4.

4.6.5. 3-Chloro-1-phenylmethoxy-2-propanol (5)

Temp. prog. 110–140°C, 2°C/min 140–155°C, 0.5°C/min, 20 min hold. **5a** R_{tR} 32.25 min, R_{tS} 33.50 min, R_s 3.7, **5b** R_{tR} 49.78 min, R_{tS} 50.54 min, R_s 2.0.

4.6.6. 3-Chloro-1-[2-phenylethoxy]-2-propanol (6)

Temp. prog. 105–130°C, 1°C/min, 130–145°C, 0.5°C/min, 20 min hold. **6a** R_{tR} 50.22 min, R_{tS} 52.25 min, R_s 3.7, **6b** R_{tR} 72.36 min, R_{tS} 73.19 min, R_s 1.5.

4.6.7. 3-Bromo-1-phenoxy-2-propanol (7)

Temp. prog. 100–130°C, 2°C/min, 130–150°C, 0.5°C/min, 10 min hold. **7a** R_{tR} 37.94 min, R_{tS} 40.02 min, R_s 5.0, **7b** R_{tR} 57.17 min, R_{tS} 57.94 min, R_s 3.6.

4.6.8. 3-Bromo-1-phenylmethoxy-2-propanol (8)

Temp. prog. 110–140°C, 1°C/min, 140–155°C, 0.5°C/min, 10 min hold. **8a** R_{tR} 44.42 min, R_{tS} 45.97 min, R_s 4.1, **8b** R_{tR} 64.35 min R_{tS} 65.15 min, R_s 2.7.

4.6.9. 3-Bromo-1-[2-phenylethoxy]-2-propanol (9)

Temp. prog. 110–130°C, 1°C/min, 130–158°C, 0.5°C/min, 20 min hold., **9a** R_{tR} 67.26 min, R_{tS} 69.07 min, R_s 3.7, **9b** R_{tR} 96.34 min, R_{tS} 97.32 min, R_s 2.0.

4.7. NMR spectroscopic properties

The ^{1}H and ^{13}C NMR spectra of **1** and **2** were in agreement with their structures and with results reported earlier [27]. Spectra of **5**, **6** and their butanoates have been reported earlier [16]. Spectra of **8** were in agreement with structure and previous results [15]. For carbon numbering, see Scheme 1.

4.7.1. 3-Fluoro-1-[2-phenylethoxy]-2-propanol (3)

^{1}H NMR: 7.35–7.21, (5H, m, aromatic), 2.31 (1H, OH), 3.75 (1H), 3.72 (1H) and 2.91 (2H), ABX_2-system for $-OCH_2CH_2Ph$, $J_{AX} = J_{BX}$ 7.0, J_{AB} 9.4, 4.45 (1H), 4.42 (1H), 3.98 (d of m, 1H), 3.58 (1H) and 3.54 (1H), ^{19}FABMXY system for $FCH_2CH(OH)CH_2O$, $^2J_{HF}$ 47.2, $^3J_{HF}$ 18.3, $^4J_{HF}$ 1.4, J_{AB} 9.6, J_{AM} 4.6, J_{BM} 5.3, J_{XY} 9.4, J_{XM} 4.6, J_{YM} 5.7. ^{13}C NMR: 36.6 (C5), 69.6 (C2, $^2J_{CF}$ 19.8), 71.1 (C1, $^3J_{CF}$ 6.7), 72.9 (C4), 84.3 (C3, $^1J_{CF}$ 169.1), 126.7, 128.8, 129.3, 139.1 (aromatic C).

4.7.2. 3-Chloro-1-phenoxy-2-propanol (4)

^{1}H NMR: 7.33–7.23 and 7.01–6.90 (5H, m, aromatic), 2.13 (1H, OH), 3.79 (1H), 3.74 (1H), 4.23 (1H), 4.11 (1H), 4.08 (1H), ABMXY system, $ClCH_2CH(OH)CH_2O-$, J_{AB} 11.1, J_{AM} 5.2, J_{BM} 5.5, J_{XY} 9.4, J_{XM} 5.2, J_{YM} 5.4. ^{13}C NMR: 46.4 (C3), 68.8 (C1), 70.3 (C2), 114.9, 121.9, 130.0, 158.6 (aromatic C).

4.7.3. 3-Bromo-1-phenoxy-2-propanol (7)

^{1}H NMR: 7.28–7.23 and 6.95–6.84 (5H, m, aromatic), 2.52 (1H, OH), 3.62 (1H), 3.56 (1H), 4.15 (1H), 4.06 (1H), 4.02 (1H), ABMXY system, $BrCH_2CH(OH)CH_2O-$, J_{AB} 10.5, J_{AM} 5.2, J_{BM} 5.6, J_{XY} 9.3, J_{XM} 5.3, J_{YM} 5.1. ^{13}C NMR: 33.1 (C3), 67.0 (C1), 67.8 (C2), 112.2, 119.61, 127.6, 156.0 (aromatic C).

4.7.4. 3-Bromo-1-[2-phenylethoxy]-2-propanol (9)

^{1}H NMR: 7.25–7.14 (5H, m, aromatic), 2.36 (1H, OH), 3.65 (1H), 3.64 (1H), 2.83 (2H), ABX_2 system, OCH_2CH_2Ph, $J_{AX} = J_{BX}$ 6.9, 3.40 (1H), 3.35 (1H), 3.87 (1H), 3.51 (1H), 3.49 (1H), ABMXY system, $BrCH_2CH(OH)CH_2O-$, J_{AB} 10.3, J_{AM} 5.5, J_{BM} 5.7, J_{XY} 9.7, J_{XM} 5.3, J_{YM} 4.8. ^{13}C NMR: 33.2 (C3), 34.5 (C5), 68.3

(C2), 70.4 (C1), 70.8 (C4), 125.0, 127.6, 128.2, 137.3 (aromatic C).

*4.7.5. Butanoate of 3-fluoro-1-phenoxy-2-propanol (**1b**)*

^{1}H NMR: 7.32–6.90 (5H, m, aromatic), acyl part: 0.95 (3H, t), ^3J 7.3, 1.68 (2H, m), 2.36 (2H, t), ^3J 7.7, 4.71 (1H), 4.65 (1H), 4.17 (1H), 4.14 (1H), 5.36 (1H, dm), ^{19}F-ABMXY system, $FCH_2CH(OCOR)CH_2O$–, $^2J_{HF}$ 46.9, $^3J_{HF}$ 20.7, $^4J_{HF}$ 1–1.3, J_{AM} 4.7, J_{BM} 3.5, J_{AB} 10.4, J_{MX} 5.7, J_{MY} 5.5, J_{XY} 10.1. ^{13}C NMR: 13.5 (C7), 18.4 (C6), 36.1 (C5), 65.0 (C1, $^3J_{CF}$ 6.8), 70.2 (C2, $^2J_{CF}$ 20.3), 81.4 (C3, $^1J_{CF}$ 173.0), 172.9 (C4), 114.6, 121.4, 129.6, 158.2 (aromatic C).

*4.7.6. Butanoate of 3-fluoro-1-phenylmethoxy-2-propanol (**2b**)*

^{1}H NMR: 7.37–7.25(5H, m, aromatic), acyl part: 0.95 (3H, t), ^3J 7.4, 1.66 (2H, m), 2.33 (3H, t), ^3J 7.4, 4.56 (1H), 4.54 (1H), AB system, benzylic protons, J_{AB} 12.1, 4.59 (1H), 4.57 (1H), 5.21 (1H, dm), 3.64 (2H, dd), ^{19}F-$ABMX_2$ system, $FCH_2CH(OCOR)CH_2O$– $^2J_{HF}$ 47.1 $^3J_{HF}$ 21.5, $^4J_{HF}$ 1.0, J_{AB} 10.3, J_{AM} 4.7, J_{BM} 3.7, $^3J_{MX}$ 5.5. ^{13}C NMR: 13.6 (C8), 18.4 (C7), 36.1 (C6), 67.4 (C1, $^3J_{CF}$ 7.5), 70.8 (C2, $^2J_{CF}$ 19.4), 73.4 (C4), 81.7 (C3, $^1J_{CF}$ 172.1), 172.9 (C5), 127.6, 127.8, 128.5, 137.7 (aromatic C).

*4.7.7. Butanoate of 3-fluoro-1-[2-phenylethoxy]-2-propanol (**3b**)*

^{1}H NMR: 7.31–7.19 (5H, m, aromatic), acyl part 0.95 (3H, t), ^3J 7.4, 1.67 (2H, m), 2.32 (2H, t) ^3J 7.6, 3.69 (1H), 3.67 (1H) and 2.87 (2H, t), ABX_2 system for $-OCH_2CH_2Ph$, $J_{AX} = J_{BX}$ 7.0, J_{AB} 9.4, 4.51 (2H, dd), 5.15 (1H, dm), 3.60 (2H, d), A_2MX_2 system, ^{19}F splitting for 2 and 3 bond copling, $FCH_2CH(OCOR)CH_2O$–, $^2J_{HF}$ 47.2, $^3J_{HF}$ 21.9, J_{AM} 4.1. J_{XM} 5.5. ^{13}C-NMR: 13.7 (C9), 18.5 (C8), 36.2 (C7), 36.3 (C5), 69.1 (C1, $^3J_{CF}$ 6.8), 70.1 (C2, $^2J_{CF}$ 20.4), 72.6 (C4), 81.8 (C3, $^1J_{CF}$ 172.0), 173 (C6), 126.4, 128.5, 129.0, 138.9 (aromatic C).

*4.7.8. Butanoate of 3-chloro-1-phenoxy-2-propanol (**4b**)*

^{1}H NMR: 7.34–7.28 and 7.03–6.92 (aromatic, m, 5H), acyl part: 2.38 (1H), 2.37 (1H), 1.71 (m, 2H), 0.99 (t, 3H), ABM_2X_3 system, $J_{AM} \approx J_{BM}$ 7.4, J_{XM} 7.4, 3.88 (1H), 3.82 (1H), 5.38 (1H), 4.20 (1H), 4.19 (1H), ABMXY system, $ClCH_2CH(OCOR)CH_2O$–, J_{AB} 11.7, J_{AM} 5.1, J_{BM} 5.3, J_{XY} 10.2, J_{XM} 4.9, J_{YM} 5.2. ^{13}C NMR: 14.0 (C7), 18.8 (C6), 36.5 (C5), 43.0 (C3), 66.4 (C1), 71.2 (C2), 173.3 (C4), 115.0, 121.8, 130.0, 158.6 (aromatic C).

*4.7.9. Butanoate of 3-bromo-1-phenoxy-2-propanol (**7b**)*

^{1}H NMR: 7.31–7.25 and 7.0–6.85 (5H, m, aromatic), acyl part: 2.36 (1H), 2.35 (1H), 168 (2H, m), 0.97 (3H, t), ABM_2X_3 system, $J_{AM} \approx J_{BM}$ 7.5, J_{XM} 7.5, 3.71 (1H), 3.63 (1H), 5.33 (1H), 4.20 (1H), 4.16 (1H), ABMXY system, $BrCH_2CH(OCOR)CH_2O$–, J_{AB} 10.8, J_{AM} 5.2, J_{BM} 5.4, J_{XY} 10.1, J_{XM} 5.0, J_{YM} 5.6. ^{13}C NMR: 14.0 (C7), 18.8 (C6), 31.0 (C3), 36.5 (C5), 67.2 (C1), 70.9 (C2), 173.2 (C4), 121.8, 130.0, 158.6 (aromatic C).

*4.7.10. Butanoate of 3-bromo-1-phenylmethoxy-2-propanol (**8b**)*

^{1}H NMR: 7.35–7.25 (5H, m, aromatic), acyl part: 2.34 (1H), 2.33 (1H), 1.67 (2H, m), 0.96 (t, 3H), ABM_2X_3 system, $J_{AM} \approx J_{BM}$ 7.5, J_{XM} 7.5, 4.57 (1H), 4.55 (1H), J_{AB} 12.1, AB system, benzylic protons, 3.69 (1H), 3.54 (1H), 5.17 (1H), 3.64 (1H), 3.62 (1H), ABMXY system, $BrCH_2CH(OCOR)CH_2O$–, J_{AB} 10.5, J_{AM} 4.9, J_{BM} 5.5, $J_{XM} \approx J_{YM}$ 5.2. ^{13}C NMR: 13.6 (C8), 18.4 (C7), 31.0 (C3), 36.1 (C6), 69.0 (C1), 71.0 (C2), 73.4 (C4), 172.9 (C5), 127.6, 127.9, 128.4, 137.6 (aromatic C).

*4.7.11. Butanoate of 3-bromo-1-(2-phenylethoxy)-2-propanol (**9b**)*

^{1}H NMR: 7.31–7.19 (5H, m, aromatic), acyl part: 2.32 (1H), 2.31 (1H), 1.66 (2H, m), 0.96

(3H), ABM_2X_3 system, J_{AM} 7.4, J_{BM} 7.5, J_{XM} 7.4, 3.70 (1H), 3.68 (1H), 2.88 (t, 2H), ABX_2 system, OCH_2CH_2Ph, $J_{XA} = J_{XB}$ 6.9, 3.54 (1H), 3.46 (1H), 5.11 (1H), 3.65 (1H), 3.60 (1H), ABMXY system, $BrCH_2CH(OCOR)$-CH_2O–, J_{AB} 10.8, J_{AM} 5.0, J_{BM} 5.5, J_{XY} 10.5, J_{XM} 5.0, J_{YM} 5.4. ^{13}C NMR: 13.6 (C9), 18.4 (C8), 31.1 (C3), 36.1 (C7, C5), 69.7 (C1), 70.9 (C2), 72.45 (C4), 172.8 (C6), 126.3, 128.4 128.9, 138.7 (aromatic C).

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