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Lipase-catalyzed solvent-free kinetic resolution of substituted racemic ε-caprolactones

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Abstract—The kinetic resolution of racemic seven-membered lactones is reported, for the first time in bulk (solvent-free) conditions, by lipase-catalysed butanolysis. The lactones studied were substituted at either the 4- or the 6-position. With these lactones, Novozym-435 lipase (from *Candida antarctica*) induced (*S*)-selective butanololysis, and (*R*)-lactones were recovered unreacted with >97% enantiomeric excess (e.e.). The reactions were studied at different reaction temperatures, viz. 0, 23, 40 and 60°C. A comparative study of the effect of temperature on the kinetic resolution is presented.

1. Introduction

Optically active lactones are valuable building blocks in organic synthesis and find wide application as synthons in natural product synthesis[1] and in the preparation of optically active biodegradable polymers, which have several industrial and biomedical applications.[2] Several methods for producing these compounds have been explored, these include multi-step syntheses starting from optically active starting materials,[3] chromatographic separations of diastereomeric precursors,[1b] Baeyer–Villiger oxidation of enantiomerically enriched substituted cyclohexanones,[4] and enantioselective Baeyer–Villiger oxidations of the corresponding cyclohexanones using metal complexes.[5] Unfortunately all of these methods are either experimentally cumbersome or afford the lactones with only modest enantioselectivity.

Enzymes are very useful tools for the synthesis of optically active lactones, and yeast-catalyzed Baeyer–Villiger oxidation reactions have been described in a recent review.[6] Isolated enzymes, such as lipases[7] and esterases,[8] have also been exploited for preparing optically active four-, five- and six-membered lactones by enantioselective ring-opening of racemic lactones and enantioselective lactonisation[9] of racemic hydroxy esters. However, only a few literature reports describe the enzymatic resolution of substituted seven-membered lactones.[8c,10] Fellous et al.[8c] investigated the hydrolysis of seven-membered lactones substituted at the 6-position and reported that the stereoselectivity of the esterase was influenced by the bulk of the substituents. In a recent report, Shioji and co-workers[10] studied the CAL (*C. antarctica* lipase)-catalyzed hydrolysis of 3-, 4-, 5- or 6-methyl substituted seven-membered lactones in water-saturated diisopropyl ether, and they reported that the stereoselectivity of the lipase was influenced by the position of the substituent. Aqueous reaction media offer high enzyme activity but have many drawbacks, including the insolubility of the organic substrates, specific pH requirements, and tedious workup procedures. The use of organic solvents[11,12] for enzymatic reactions addresses some of these concerns, but their use renders an otherwise green technology non-biocompatible. Motivated by the need to limit the use of solvents, especially those that are known to be toxic, the use of enzymes in alternative media, i.e. reverse micelles,[13] ion-pairs,[14] surfactant activated,[15] and in solvent-free[16] environments have been actively pursued. The use of a lipase catalyst in solvent-free (bulk) reactions is the subject of this report.

An important research focus in our laboratories continues to be the synthesis of novel, functional, and biodegradable polymers.[17] Of particular interest has been the synthesis of chiral polyesters because of their potential importance in biomedical applications. Studies in our laboratories and others have exemplified the use of enzymes in bulk for polymerization reactions,[16–18] however, there is no precedent for the kinetic resolu-

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tion of simple lactones under solvent-free conditions. Herein, we report the highly enantioselective, lipase-catalyzed kinetic resolutions of ε-caprolactones substituted at the 4- or the 6-position. To the best of our knowledge, this is the first report on the kinetic resolution of lactones in a solvent-free environment. The reactions were highly enantioselective despite the large distance (three/five bonds) between the stereocenter at the 4/6-position and the lactone carbonyl group. Literature reports describing efficient resolution of lactones containing a stereocenter remote from the carbonyl group, as in these substrates, are rare.

2. Results and discussion

The substrates, (±)-ε-caprolactones **1**, **2** and **3** (Fig. 1) were synthesized by Baeyer–Villiger oxidation of the corresponding commercially available cyclohexanones and characterized from their ^{1}H and ^{13}C NMR spectra.[8b,19] Authentic samples of the racemic hydroxy butyl esters (±)-**4**, (±)-**5** and (±)-**6** were prepared by TFA-catalyzed ring-opening of the lactones **1**, **2** and **3**, respectively with *n*-butanol. Kinetic resolutions were examined using Novozym-435 (immobilized lipase-B from *Candida antarctica*)[20] catalyzed ring-opening of the lactones **1**, **2**, and **3** with 1.5 molar equivalents of *n*-butanol (Scheme 1). All reactions were carried out in bulk and monitored periodically by collecting samples which were then analyzed by gas chromatography (GC) (Fig. 2).

The Novozym-catalyzed ring-openings were investigated at four different reaction temperatures viz. 0, 23, 40 and 60°C. In Fig. 3, the effect of reaction temperature on lactone conversion is shown at 5 h. The results of kinetic resolution of the lactones **1**, **2** and **3** are summarized in Table 1. Generally, the rate of monomer conversion increased with increasing reaction temperatures. For example, in the ring-opening of **1** at 0°C there was no reaction even after 12 h, but at room temperature (23°C) ~45% of the lactone was converted to the (*S*)-enriched hydroxy ester in 8 h. A further increase of 17°C in the reaction temperature to 40°C only affected the rate of the reaction slightly. At 23 and 40°C, there was a noticeable decrease in the rate of lactone conversion beyond ~30%. This could have been due to the high enantioselectivity of the lipase, i.e. as the faster reacting enantiomer is depleted, the rate of the reaction decreases due to the decreasing availability of the substrate to the enzyme active site. In a solvent-free system, the decreased accessibility may also have been a result of the increasing diffusion constraints in the reaction because of the increasing concentration of the ω-hydroxy esters, which are more viscous than the lactones and *n*-butanol. The reactions at 60°C, however, were more efficient. For example, more than 8 h was needed for 50% conversion of **1** at 40°C compared to less than 4 h at 60°C. The higher rate of monomer conversion at a higher temperature, i.e. 60°C, coupled with the observation that the lipase was even more selective at 60°C (Table 1), suggests that the reaction is indeed a diffusion-controlled process. Interestingly, the ring-opening of **1** in solvent-free reactions was still faster than its hydrolysis in water-saturated diisopropyl ether.[10] For instance, in water saturated-diisopropyl ether (at 37°C), 47% of **1** was hydrolyzed in 24 h,[10] while in bulk (at 40°C) 45% of **1** was consumed in 6 h.

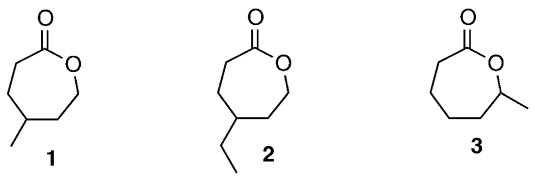
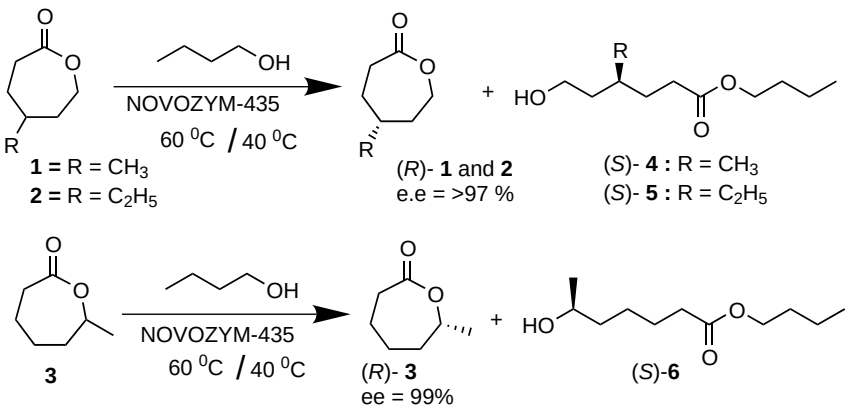


Figure 1. Chemical structures of the lactones **1**, **2** and **3**.

Novozym-435 lipase was highly enantioselective for the resolution of lactones (*RS*)-**1** and (*RS*)-**2**, in which the (*S*)-enantiomers were preferentially esterified and the unreacted (*R*)-lactones were recovered with e.e. of >97%. As expected, to about 50% monomer conversion, the enantiomeric excess of the unreacted lactones **1** and **2** increased with increasing lactone conversion. The corresponding hydroxy esters also had rather high e.e. values (90%) at ~50% lactone conversion. The enantioselectivity in esterification of the lactone (*RS*)-**3**, however, was only moderate. At 60°C, 80% of the lactone had to be converted to the hydroxy ester to recover (*R*)-**3** with 99% e.e.



Scheme 1.

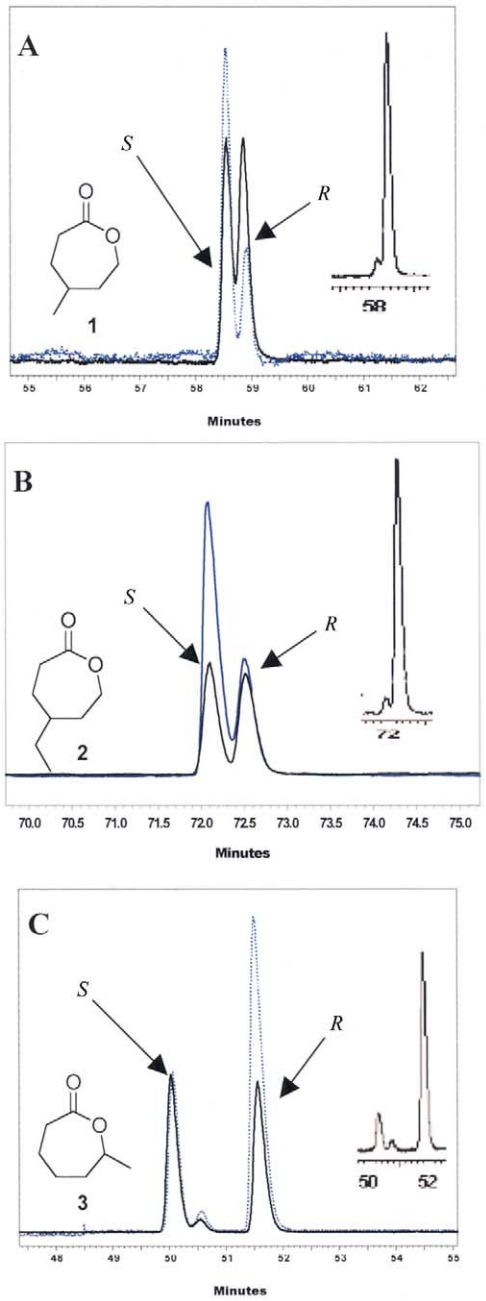


Figure 2. GC Chromatograms of racemic (solid line) lactones and their coinjections with authentic enriched (dashed line) lactones. Inserts: GC chromatograms of the recovered lactones (*R*)-**1** (e.e. >97%), (*R*)-**2** (e.e. >95%), and (*R*)-**3** (e.e. >70%) at ~50% conversion.

The effect of reaction temperature on enantioselectivity, *E* was more intriguing as it varied greatly with temperature (Fig. 4). When the reaction yield was lower than 50%, a temperature drop from 60 to 40°C diminished the *E* and enantiomeric excess, but a similar trend was not observed in the reactions at 23 and 40°C. The enantioselectivity of the lipase in reactions performed at 60°C was higher (for **1**, E >125) than that at 40°C (for **1**, E ~25). Usually an *E* value of 100 is considered a minimum satisfactory value for the industrial application of an enzymatic process. Thus, in the resolution of **1**, a 20°C temperature increment from 40 to 60°C made an otherwise inadequate reaction process a very efficient one. This effect was also observed for **2**, where the *E* value increased from 8 to 60 with an increase in temperature from 40 to 60°C. This finding is in contrast to several papers reporting an inverse correlation between the temperature and the lipase enantioselectivity.[21] Unfortunately, the effect of temperature on lipase selectivity is not very well understood,[22] partly because enzymes are temperature labile and, thus, variation in reaction temperature is a rather less obvious choice to improve lipase enantioselectivity.

For all three lactones, the (*S*)-enantiomer reacted faster than the (*R*)-enantiomer. *C. antarctica* lipase (CALB or Novozym-435), like other lipases, has the same mechanism of action as a serine protease and the Ser_{105}–His_{224}–Asp_{187} triad is responsible for the catalytic action.[23] Interestingly, *C. antarctica* lipase demonstrates *R* stereoselectivity towards a number of secondary alcohols.[24] However, substrates with a stereogenic center on the acyl side have not been studied with equal vigor. Contrary to our observations, it has been suggested[25] that the acyl side of the active site is more spacious and hence possesses much lower stereoselectivity. Based upon the acyl binding models[26] of the lipase and the *S* selectivity of the lipase CALB for lactones, as observed by us and others,[10] a cartoon representation of the active site of the enzyme is shown in Fig. 5. We have concluded that there is a certain steric restriction in the small subsite (stereospecificity pocket) to accept the alkyl group instead of the hydrogen (Fig. 5). We assumed (in Fig. 5) that in the fast reacting (*S*)-lactone, the position of the carbonyl carbon and the oxyanion as well as the oxygen on the lactone must be relatively fixed in order to allow the formation of a hydrogen bond between the catalytic histidine (His_{224}) residue and the oxygen. These constraints and the rigidity of the lactone ring then determine how the remainder of the lactone will fit in the active site. In the (*S*)-enantiomer

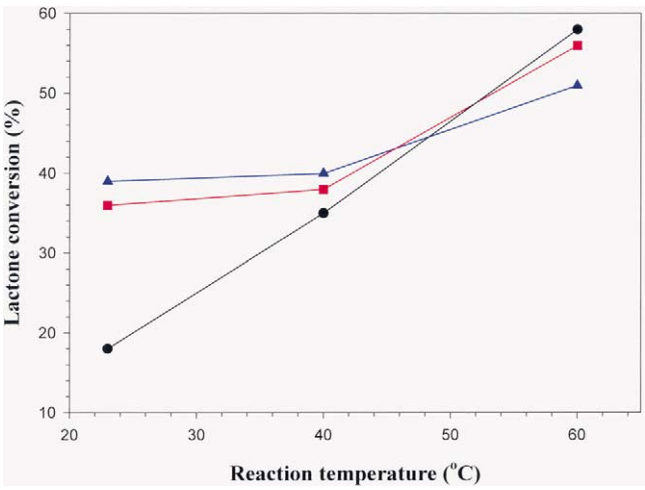


Figure 3. Plots of reaction temperature versus lactone conversion in Novozym-435-catalyzed resolution of lactones **1** (▲), **2** (■) and **3** (●) at 23, 40 and 60°C after 5 h. No reaction was observed at 0°C even after 24 h.

Table 1. Novozym-435 catalyzed solvent-free kinetic resolution of **1**, **2**, and **3** with *n*-butanol[a]

Lactone	Reaction at 60°C				Reaction at 40°C				Reaction at 23°C			
	Conv. % (time/h)	% e.e.[b]		E[c]	Conv. % (time/h)	% e.e.[b]		E[c]	Conv. % (time/h)	% e.e.[b]		E[c]
		Lactone (*R*) ($[\alpha]_D^{23}$ *c* 0.5)	Ester (*S*)			Lactone (*R*)	Ester (*S*)			Lactone (*R*)	Ester (*S*)	
1	25 (1)	70	>99		21 (1)	27	>99		36 (4)	40	71	
	45 (3)	85	>99		35 (3)	41	76		39 (6)	56	88	
	51 (6)	97 (+40)	93	>125	45 (6)	69	84	24	44 (8)	68	86	28
2	12 (1)	17	>99		10 (1)	23	>99		38 (4)	40	65	
	44 (3)	73	93		26 (3)	30	85		45 (6)	56	68	
	54 (4)	99 (+42)	84	60	45 (6)	60	73	12	47 (8)	64	72	12
3	43 (2)	35	46		17 (2)	12	59		12 (4)	8	59	
	59 (5)	80	56		33 (5)	26	53		26 (12)	22	63	
	80 (7)	99 (+18)	25	7	47 (7)	35	40	3	46 (24)	30	35	3

[a] No reaction was observed at 0°C even after 24 h. Enzyme to substrate ratio (w/w) = 0.5 and *n*-butanol to lactone molar ratio = 1.5.
[b] Calculated from chiral gas chromatographic analyses, for details see Section 4.
[c] Calculated from conversion and e.e. values,[29] for details see Section 4.

of lactones **1** and **3**, the methyl group effectively fits into the small 'stereospecificity' pocket. In the (*R*)-enantiomer, the methyl group is pointing away from the 'stereospecificity' pocket and may have many close van der Waals contacts. These steric clashes make this interaction highly unfavorable. The lower lipase enantioselectivity for the 4-ethyl ε-caprolactone compared to the 4-methyl ε-caprolactone suggested that the 'stereosepecificity' pocket is only big enough to accommodate a methyl group effectively. Also, the higher rate of conversion in reactions of **1** over **2** support that a methyl group rather than an ethyl group at the 4-position results in a better fit in the active site of Novozym-435.

The enantioselectivity (E) of the lipase was lowest ($E = 4$ at 60°C) for the 6-methyl-ε-caprolactone **3** compared to that for the 4-ethyl- **2** ($E = 60$) and 4-methyl- **1** ($E > 125$) analogues. This is in contradiction to previous findings in water-saturated ether[10] in which the enantioselectivity of the enzyme increased with decreasing distance between the lactone carbonyl group and the substituent on the lactone ring, i.e. E for substitution at position $6 \sim 2 > 5 \sim 3 > 4$. This may very well be a result of the conformational differences in the enzyme in two very different reaction environments.

3. Summary

Resolutions of substituted ε-caprolactones at the 4- or 6-position catalyzed by lipase Novozym-435 were investigated in the absence of any added solvent. To the best of our knowledge, this is the first report of solvent-free kinetic resolution of substituted lactones catalyzed by a lipase. The resolution reactions were investigated at four different temperatures, i.e. 0, 23, 40 and 60°C. For all compounds studied, Novozym-435 lipase showed (*S*)-selectivity, i.e. the (*S*)-enantiomer reacted more rapidly with *n*-butanol. Considering the fact that the reactions were conducted without added solvents, the enantioselectivity is remarkable. Contrary to a general perception and several literature reports that suggest an inverse relationship between the reaction temperature and enantiomeric excess, in the resolution described here the stereoselectivity increased with increasing reaction temperature. It was demonstrated that 60°C is the desired temperature for these resolutions. The work provides useful examples of the very high enantioselectivities that can be achieved by applying enzyme catalysis in a solvent-free environment. Using this method, enantiomerically enriched (*R*)-lactones having e.e. values of >97% were prepared. This approach is environmentally benign as it eliminates the need for organic solvents in the resolution process.

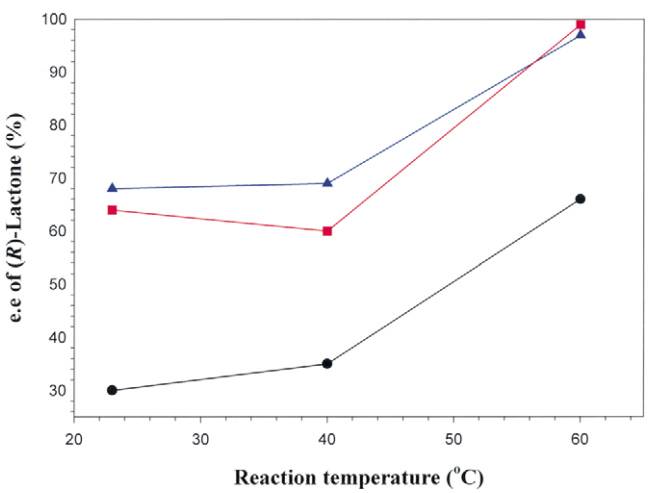


Figure 4. Plots of reaction temperature versus % e.e. of recovered (*R*)-lactone in Novozym-435-catalyzed resolution of lactones **1** (▲), **2** (■) and **3** (●) at 23, 40 and 60°C after 46% (±1%) lactone conversion. No reaction was observed at 0°C even after 24 h.

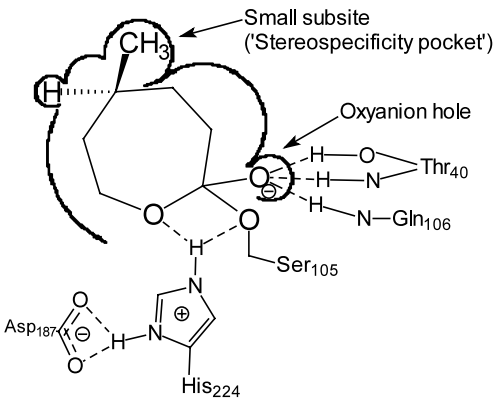


Figure 5. A cartoon representation of the lipase active site and its binding to the lactone.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-360 and Bruker DPX-250 spectrometers. ^{1}H NMR (360 or 250 MHz) chemical shifts (ppm) were reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal standard. The concentrations used were ~4% w/v in chloroform-*d* ($CDCl_3$). ^{13}C NMR (90 or 62.5 MHz) spectra chemical shifts in ppm were referenced relative to the internal standard chloroform-*d* at 77.00 ppm. Gas chromatographic analyses were conducted on a Shimadzu GC-17 system equipped with flame ionization detector (FID) and Cyclodex-B chiral capillary column (permethylated β-cyclodextrin, 30 m×0.25 mm×0.25 μm, J & W scientific). Helium was used as a carrier gas. Mass spectra were recorded on a Shimadzu GCMS-QP 5000 mass spectrometer at 70 eV. Optical rotations were measured on an Autopol IV (Rudolph Instruments) automatic polarimeter at 23°C in $CHCl_3$ at a concentration of 0.5.

Lipase Novozym-435 was kindly provided by Novo Nordisk Bioindustrial Inc. and methyl and ethyl cyclohexanones and *m*-chloroperoxybenzoic acid (*m*-CPBA) were purchased from Acros Chemical Co. and used as received. *n*-Butanol and caprolactones were freshly distilled over anhydrous potassium carbonate and calcium hydride, respectively, and transferred under a nitrogen atmosphere prior to use.

The lactones were prepared by Baeyer–Villiger oxidation of substituted cyclohexanones following the general experimental procedure reported in the literature.[19]

4.2. 4-Methyl-ε-caprolactone[19] 1

76% yield. ^{1}H NMR (360 MHz) δ: 4.25 (m, 2H, -$COOCH_2$), 2.50–2.75 (m, 2H, -CH_2COO), 1.75–2.0 (m, 3H, -CH_2), 1.50 (m, 1H, -CH), 1.30 (q, $J=1.6$ Hz, 1H, -CH_2), and 1.00 (d, $J=7.2$ Hz, 3H, -CH_3). ^{13}C NMR ($CDCl_3$) δ: 175.21 (*C*=O), 67.22 (*C*H_2-O), 36.48, 34.24, 32.35, 30.03, and 21.35.

4.3. Synthesis of 4-ethyl-ε-caprolactone[19] 2

74% yield. ^{1}H NMR (360 MHz) δ: 4.15–4.35 (m, 2H, -$COOCH_2$), 2.6–2.7 (m, 2H, -CH_2COO), 1.90–2.05 (m, 2H, -CH_2), 1.50–1.60 (m, 2H, -CH_2), 1.25–1.40 (m, 3H) and 0.95 (t, $J=7.2$ Hz, 3H, -CH_3). ^{13}C NMR ($CDCl_3$) δ: 175.26 (C=O), 67.13 (*C*H_2-O), 40.64, 34.05, 32.09, 27.62, 27.32, and 10.30.

4.4. 6-Methyl-ε-caprolactone[8b] 3

72% yield. ^{1}H NMR ($CDCl_3$) δ: 4.45–4.50 (m, 1H, -$COOCH_2$), 2.63–2.66 (m, 2H, -CH_2COO), 1.89–1.93 (m, 3H, -CH_2), 1.56–1.67 (m, 3H), and 1.34 (d, $J=5$ Hz, 3H, -CH_3). ^{13}C NMR: δ 175.36 (*C*=O), 76.48 (CH), 35.92 (*C*H_2C=O), 34.67, 27.90, 22.60, and 22.26.

4.5. General procedure for the enzymatic resolution: reaction of 4-ethyl-ε-caprolactone 2 with *n*-butanol

All reactions were carried out in bulk. The lipase was dried in a drying pistol over P_2O_5 at 50°C/0.1 mmHg, 15 h. In a glove bag, maintained under a nitrogen atmosphere, ethyl caprolactone (0.2 g, 1.40 mmol) and *n*-butanol (0.19 mL, 2.1 mmol) were transferred to a 12 mL reaction vial, and then pre-weighed dried enzyme (0.1 g) was added. The reaction vial was sealed with a rubber septum and placed in a thermostatically controlled oil bath maintained at 40 or 60°C. At predetermined times, samples from the reaction vial were collected through the septum and the progress of the reaction was monitored by chiral phase GC analyses. Reactions were terminated by dissolution of the contents of the reaction vial in chloroform and removal of the enzyme (insoluble) by filtration (glass-fritted filter, medium pore porosity). The filtrates were combined and solvent removed in vacuo. The absolute configuration of the recovered lactone was determined by coinjection with the authentic (*S*)-**2** (Fig. 2) in chiral phase GC analysis and also by the specific rotation of the enantioenriched unreacted **2** isolated by column chromatography (e.e. >98%, $[\alpha]_D^{23}=+42$ ($c=0.5$, $CHCl_3$)) with the reported data in the literature.[27]

4.6. General procedure of TFA-catalyzed ring-opening: synthesis of (±)-butyl 4-ethyl-6-hydroxy hexanoate 5

To a mixture of 4-ethyl-ε-caprolactone **2** (0.1 g, 0.7 mmol) and *n*-butanol (0.19 mL, 2.1 mmol) was added a catalytic amount of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12 h and then poured on crushed ice (5 g). The product was extracted with chloroform (2×25 mL), washed with water (2×25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was passed through a silica gel plug (100–200 mesh, eluent = 20% EtOAc in petroleum ether) to afford (±)-**5** as a colorless liquid (0.134 g, 85%). ^{1}H NMR (250 MHz) δ: 4.10 (t, $J=5.2$ Hz, 2H, -$COOCH_2$), 3.70 (t, $J=5.2$ Hz, 2H, -CH_2OH), 2.31 (t, $J=6$ Hz, 2H, -CH_2COO), 1.96 (s, br, 1H, -OH), 1.50–1.66 (m, 7H), 1.30–1.46 (m, 4H), 0.95 (t, $J=6$ Hz, -CH_3, 3H), and 0.88 (t, $J=6$ Hz, -CH_3, 3H). ^{13}C NMR (62.5 MHz) δ:

174.12 (C=O), 64.10 (-$OCOCH_2$), 60.41 (-CH_2OH), 35.73, 34, 31.48, 30.48, 27.90, 25.57, 18.94, 13.50, and 10.39. MS: m/z 216 (M^+, 1%), 186 (3%), 157 (12%), 143 (25%), 125 (40%), 113 (25%), 97 (60%), 83 (75%), 73 (90%) and 57 (100%).

4.7. (±)-Butyl 4-methyl-6-hydroxy hexanoate 4

Prepared by the reaction of lactone **1** with *n*-butanol according to the procedure described for **5** and isolated as a colorless liquid (0.14 g, 90%). ^{1}H NMR (250 MHz) δ: 4.07 (t, $J=5.3$ Hz, 2H, -$COOCH_2$), 3.65 (t, $J=5.2$ Hz, -2H, -CH_2OH), 2.32 (m, 2H, -CH_2COO), 1.64 (m, 1H), 1.53–1.58 (m, 4H), 1.40–1.46 (m, 4H), 0.95 (d, $J=6$ Hz, 3H, -CH_3), and 0.92 (t, $J=5.6$ Hz, 3H, -CH_3). ^{13}C NMR (62.5 MHz) δ: 174.21 (C=O), 64.15 (-$OCOCH_2$), 60.35 (-CH_2OH), 39.26, 31.81, 31.60, 30.50, 28.95, 19.12, 18.97, and 13.51. MS: m/z 202 (M^+, 1%), 129 (30%), 111 (25%), 83 (37%), 69 (45%) and 56 (100%).

4.8. (±)-Butyl 6-hydroxy heptanoate 6

Prepared by the reaction of lactone **3** with *n*-butanol according to the general procedure described and isolated as a colorless liquid (0.13 g, 85%). ^{1}H NMR (250 MHz) δ: 4.07 (t, $J=5.4$ Hz, -$COOCH_2$, 2H), 3.77–3.84 (m, -CHOH, 1H), 2.31 (t, $J=6$ Hz, -CH_2COO, 2H), 1.50–1.60 (m, 4H), 1.40–1.45 (m, 6H), 1.18 (d, $J=4.8$ Hz, -$CHCH_3$, 3H), 0.94 (t, $J=6$ Hz, -CH_3, 3H). ^{13}C NMR (62.5 MHz) δ: 173.80 (C=O), 67.43 (-$OCOCH_2$), 64.03 (-CH_2OH), 38.64, 34.10, 30.50, 25.11, 24.74, 23.22, 18.96, and 13.50. MS: m/z 202 (M^+, 1%), 129 (25%), 111 (40%), 102 (20%), 83 (40%) and 73 (100%).

4.9. Enantiomeric purity

The analytical separation of the two enantiomers of the racemic lactones was achieved by carefully adjusting the analysis parameters of the gas chromatograph equipped with a Cyclodex-B (J&W Scientific) chiral phase capillary column. Chiral separations were performed using the following gradient temperature program: 64°C (2 min) to 130°C (5 min) at 1°C followed by a 10°C/min gradient to 180°C (15 min). Carrier gas flow was 1.8 mL/min for analyses of **1** and **3** and 2.0 mL/min for **2**. The injector and detector temperatures were maintained at 250 and 300°C, respectively.

The absolute configurations of the separated enantiomeric peaks in the racemic lactones were established by their co-injection with an authentic enantiomerically pure (*S*)-**1** or **2** (Fig. 2) and also from comparison of the specific rotation with that reported in literature.[1a,27,28]

The enantiomeric ratios (E), a measure of the enantioselectivity of the lipase (Table 1), can be related to the extent of conversion and the enantiomeric ratio and were calculated using the following equation.

$$E=\frac{\ln[(1-c)(1-\text{e.e.}_s)]}{\ln[(1-c)(1+\text{e.e.}_s)]} \quad (1)$$

Where c is the lactone conversion (determined by GC), e.e.$_s$ is the enantiomeric excess (determined by GC) of the unreacted lactone. The equation is based on the assumptions that resolution proceeds irreversibly, that the two enantiomers compete for the same active site, and that there is no product inhibition.[29]

The enantiomeric excess (e.e.$_p$) of the (*S*)-enriched hydroxy esters **4**, **5**, and **6** was calculated from the lactone conversion, c, and the fractions, viz. $[S/(S+R)]_m$ and $[R/(S+R)]_m$] of the (*S*)- and (*R*)-enantiomers, respectively, in the recovered lactone (Eq. (2)).[18d]

$$\text{e.e.}_p=\frac{(S_p-R_p)}{(S_p+R_p)} \quad (2)$$

$$S_p=0.5-\left[(1-c)\left\{\frac{S}{S+R}\right\}_s\right]$$

$$R_p=0.5-\left[(1-c)\left\{\frac{R}{S+R}\right\}_s\right]$$

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