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ORIGINAL PAPER

Kinetic resolution of α-lipoic acid via enzymatic differentiation of a remote stereocenter

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Abstract Kinetic resolution of α-lipoic acid, a case of remote stereocenter discrimination, was accomplished using lipase from *Aspergillus oryzae* WZ007. Performance of this lipase was investigated for enantioselective esterification of (*S*)-α-lipoic acid, leaving the target product (*R*)-α-lipoic acid in unreacted form. The effects of chain length of alcohol, type of solvent, molar ratio of alcohol:acid, and reaction temperature were studied. The optimum reaction conditions were found to be esterification with *n*-octanol at 50°C in heptane with an alcohol:acid molar ratio of 5:1. The conversion rate of α-lipoic acid was 75.2%, with an enantiomeric excess of 92.5% towards unreacted substrate in a reaction time of 48 h.

Keywords α-Lipoic acid · Remote stereocenter · Lipase · *Aspergillus oryzae* · Kinetic resolution

Introduction

α-Lipoic acid, which has been isolated [1] from natural sources and identified as the *R* configuration [2], is a growth factor for a variety of microorganisms and a cofactor involved in many enzyme-catalyzed reactions, particularly in decarboxylation of α-keto acids [1, 3–5]. Since α-lipoic acid exhibits a high level of biological activity, its use in the treatment of various diseases has been investigated. For example, it shows effects in diabetes mellitus [5] and hepatic diseases [6, 7] as well as anti-oxidative [8, 9], anti-inflammatory [10] and immunological activity [11]. Recently, it has also been reported that α-lipoic acid and its derivatives are highly active as anti-HIV [12, 13] and anti-tumor agents [14].

Generally, the (*R*)-enantiomer is much more active than the (*S*)-enantiomer [15]. Therefore, great attention has been focused on the stereoselective synthesis of pure (*R*)-enantiomer. There are two main specific routes for the synthesis of (*R*)- and (S)-α-lipoic acid by chemical methods, including asymmetric synthesis [16–18] and a strategy starting from 'chiral pool' material [2, 19–23]. Various methods of enzyme catalysis, such as enzyme-catalyzed reactions of key intermediates or precursors [24–28] and kinetic resolution of racemic material [29], have also been developed. Lipase-catalyzed kinetic resolution of α-lipoic acid involves esterifying the carboxylic group located four carbon atoms away from the stereogenic center. Although several examples of enzymatic resolution of key compounds with remote stereocenters have been reported [30–34], in practice, enzymatic or classical resolution involving a remote chiral center remains a difficult task. To date, no enzymes have been reported to enable kinetic resolution of racemic α-lipoic acid except for the commercially available *Candida rugosa* lipase, which shows enantioselectivity towards the (*S*)-enantiomer. However, results obtained with this latter enzyme are unsatisfactory, with a low enantiomeric excess of 23.8% towards (*R*)-α-lipoic acid [29]. In this study, lipase from *Aspergillus oryzae* WZ007 was used to catalyze kinetic resolution of α-lipoic acid. We present evidence to suggest that this enzyme can enantioselectively esterify (*S*)-α-lipoic acid to the corresponding (*S*)-ester (Scheme 1), and that the residual (*R*)-α-lipoic acid can be recovered by extraction with an enantiomeric excess of 92.5%. Various reaction parameters affecting the conversion rate and enantioselectivity were investigated.

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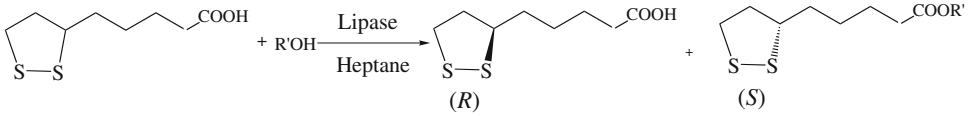
Scheme 1 Kinetic resolution of racemic α-lipoic acid by lipase from *Aspergillus oryzae* WZ007



R' = *n*-Bu, *n*-Pen, *n*-Hex, *n*-Oct, *n*-Dec, *n*-Dodec

Materials and methods

Chemicals and reagents

α-Lipoic acid was purchased from Fluka BioChemika (Buchs, Switzerland). Porcine pancreas lipase (Type II) was purchased from Sigma (St. Louis, MO). Lipases from *Penicillium expansum* and *Aspergillus niger* were purchased from Shenzhen Leveking Bio-engineering (Shenzhen, Guangdong, China). All the other chemicals were obtained from commercial sources and were of analytical reagent grade.

Microorganisms and culture conditions

The strain of *Aspergillus oryzae* WZ007 was newly isolated from lipase-producing strains and deposited with the China Center for Type Culture Collection with the accession number of CCTCC No. M206105. The strain was maintained on slant medium consisting of potato 200 g/l, glucose 20 g/l and agar 17 g/l. The culture was grown aerobically at 30°C and 200 rpm for 48 h in cell growth medium consisting of glucose 10 g/l, peptone 5 g/l, KH_2PO_4 1 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, $FeSO_4 \cdot 7H_2O$ 0.01 g/l, KCl 0.5 g/l and olive oil 10 ml/l. After harvesting by centrifugation, the mycelium was thoroughly washed with distilled water and 100 mM Tris-HCl buffer (pH 7.0) and then dried using a freeze-drying system (Christ, Osterode am Harz, Germany).

Esterification reaction

In a typical experiment, α-lipoic acid (206 mg, 1 mmol), *n*-octanol (0.79 ml, 5 mmol), heptane (20 ml) and lyophilized microbial cells (200 mg) were placed in a conical flask (100 ml). The reaction mixtures were incubated at 200 rpm and 37°C for 48 h on a shaker. Reaction mixtures without microbial cells were also run to exclude any possible spontaneous chemical reaction. The esterifying reaction was quenched by removing mycelium or enzyme powder through centrifugation. Unreacted α-lipoic acid was extracted with 40 ml 0.5% (w/v) sodium bicarbonate and recovered after acidification with 20% (v/v) hydrochloric acid and extraction with dichloromethane. Dichloromethane was removed by vacuum distillation and the recovered α-lipoic acid was dissolved in acetonitrile for subsequent high performance liquid chromatography (HPLC) analysis.

Analytical methods

Conversion rate and enantiomeric excess of α-lipoic acid were assayed by HPLC 1100 (Agilent, Wilmington, DE) with a Chiralpak AS-H column (250 mm × 4.6 mm, 5 μm, Daicel, Hyogo, Japan). The mobile phase was composed of hexane/2-propanol/trifluoroacetic acid at a ratio of 97/3/0.1. The flow rate was 0.8 ml/min. Absorbance of column effluents was monitored at 220 nm. Enantioselectivities (*E* values) were calculated from conversion rate and enantiomeric excess according to the following equation [35].

$$E = \frac{\ln[(1-c)(1-ee_S)]}{\ln[(1-c)(1+ee_S)]}$$

$$c = \frac{c_0 - c_e}{c_0} \times 100\%$$

$$ee_s = \frac{[R]-[S]}{[R]+[S]} \times 100\%$$

where c is the conversion ratio of reaction, c_0 the initial amount of racemic α-lipoic acid, c_e the amount of racemic α-lipoic acid at the end of reaction, and ee_s is the enantiomeric excess of the residual α-lipoic acid; [*R*] and [*S*] are the peak areas corresponding to the (*R*)-α-lipoic acid isomer and (*S*)-α-lipoic acid isomer, respectively.

Results and discussion

Screening of lipase

Lipases from *Penicillium expansum*, *Aspergillus niger*, porcine pancreas and *Aspergillus oryzae* WZ007 were used in this study. Lipase powder from *P. expansum*, *A. niger* and porcine pancreas were commercially available. Lipase from *A. oryzae* WZ007 was produced by fermentation. A strain of *A. oryzae* WZ007 with high activity and enantioselectivity towards biotin intermediate 1,3-dibenzyl-5-(hydroxymethyl)-2-oxo-4-imidazolidinecarboxylic acid was newly isolated from soil samples by our team in a previous study [36]. Esterification reactions of racemic α-lipoic acid using the four lipases demonstrated that only the lipase from *Aspergillus oryzae* WZ007 exhibited high esterification ability and enantioselectivity towards the (*S*)-enantiomer. The other three lipases showed the opposite enantioselectivity. Hydrolysis of the (*R*)-α-lipoic acid esters formed is

thus also a potential alternative route to produce (*R*)-α-lipoic acid. α-Lipoic acid could not be esterified for the reaction system in the absence of enzyme. In this work, lipase from *A. oryzae* WZ007 was chosen for further studies.

Optimization of esterification conditions

Effect of alcohol chain length

To investigate the effect of alcohols with different chain length on the conversion rate and enantioselectivity, esterification reactions of α-lipoic acid in heptane were carried out with *n*-butanol (C4), *n*-pentanol (C5), *n*-hexanol (C6), *n*-octanol (C8), *n*-decanol (C10) and *n*-dodecanol (C12), respectively. The results in Table 1 indicate that there was a marked tendency for the enantioselectivity, together with the conversion rate, to rise as the alcohol chain length increased up to *n*-octanol. Use of alcohols with longer chains, such as *n*-decanol and *n*-dodecanol, did not increase the resolution of α-lipoic acid further. Thus, *n*-octanol was chosen for further study. Similarly, longer alcohols, such as *n*-octanol and *n*-decanol, resulted in a higher initial rate and enantioselectivity than *n*-hexanol when enantioselective esterification of ibuprofen was catalyzed by *Candida rugosa* lipase in isooctane [37]. The results suggested a mode of binding of the acyl donor (acid) in a hairpin conformation that leaves the active site tunnel empty to accommodate the acyl acceptor (alcohol) [38]. The exact location of the alcohol molecule in the tunnel is crucial for the enantioselectivity of the lipase, and the optimal carbon chain length of alcohol would therefore fit the active site tunnel of the lipase to provide both high enzyme activity and enantioselectivity [39].

Effect of solvents

It has been demonstrated that the activity and enantioselectivity of lipases is greatly affected by the nature of the non-aqueous solvent used when catalysis occurs in a nearly anhydrous environment [40, 41]. The effects of various organic solvents, ranging from hydrophobic to hydrophilic, were tested in the typical esterification reaction experiment, replacing heptane with the other organic solvents. The log *P*-value of the solvents was the usual parameter taken to express solvent hydrophobicity and its possible effects on enzyme activity in the non-aqueous phase [41]. The results are shown in Table 2. In hydrophobic solvents, the conversion rate increased with increase in log *P* values, reaching a maximum value of 71.8% in hexane (log $P = 3.5$). Enantioselectivity increased with increasing log *P* values up to 4.3 in heptane. No conversion occurred in hydrophilic solvents such as tetrahydrofuran, acetonitrile and acetone. The highest enantiomeric excess of (*R*)-α-lipoic acid ($ee_s = 74.6\%$), enantioselectivity ($E = 4.3$) was obtained using heptane. The activity and enantioselectivity of an enzyme decreases as the hydrophobicity of the solvent decreases, and hydrophilic solvents may alter or denature enzymes by stripping off essential water from the enzyme [42]. More hydrophilic organic solvents can result in conformational changes in the enzyme that affect the affinity of the substrate-binding site for its ligand and the enantioselectivity of the enzyme [39].

Effect of molar ratio of n-octanol to acid

In enzymatic stereoselective esterification reactions, alcohols act as nucleophiles and their concentration is known to affect reaction rate and enantioselectivity. Enantio-selective esterification reactions were carried out at five different molar ratios of *n*-octanol to acid, i.e., 4:1, 5:1, 6:1, 7:1, and 8:1, respectively. As shown in Fig. 1, upon increasing *n*-octanol concentration in the esterification

Table 1 Effects of alcohol chain length on the conversion rate and enantioselectivity of esterification of α-lipoic acid

Alcohol	*c* (%)	ee_s (%)	*E*
n-Butanol	52.1	14.7	1.5
n-Pentanol	57.3	35.7	2.4
n-Hexanol	65.1	64.5	3.8
n-Octanol	68.0	74.6	4.3
n-Decanol	68.8	73.5	4.1
n-Dodecanol	67.6	72.7	4.2

Reaction conditions: 1 mmol of racemic α-lipoic acid, 5:1 molar ratio of alcohol to racemic α-lipoic acid and 200 mg microbial cells in 20 ml heptane, reaction temperature = 37°C, reaction time = 48 h, shaking at 200 rpm

Table 2 Effects of different organic solvents on the conversion rate and enantioselectivity of esterification of α-lipoic acid

Solvent	Log *P*	*c* (%)	ee_s (%)	*E*
Isooctane	4.5	65.4	68.1	4.1
Heptane	4.0	68.0	74.6	4.3
Hexane	3.5	71.8	72.7	3.6
Cyclohexane	3.2	63.6	51.4	2.9
Toluene	2.5	45.4	27.1	2.5
Tetrahydrofuran	0.49	NA[a]	0	ND[b]
Acetone	−0.25	NA	0	ND
Acetonitrile	−0.34	NA	0	ND

Reaction conditions: 1 mmol of racemic α-lipoic acid, 5:1 molar ratio of *n*-octanol to racemic α-lipoic acid and 200 mg microbial cells in 20 ml organic solvent, reaction temperature = 37°C, reaction time = 48 h, shaking at 200 rpm

[a] No activity

[b] No data

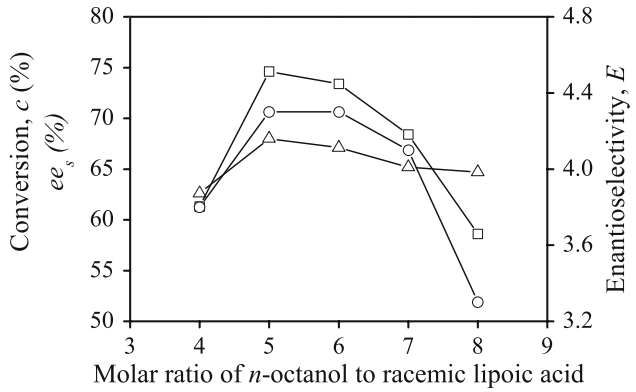


Fig. 1 Effect of the molar ratio of *n*-octanol to racemic α-lipoic acid on the enantioselective esterification of racemic α-lipoic acid. Reaction conditions: 1 mmol racemic α-lipoic acid, different molar ratios of *n*-octanol to racemic α-lipoic acid and 200 mg microbial cells in 20 ml heptane, reaction time = 48 h, reaction temperature = 37°C, shaking at 200 rpm. △ Conversion, □ ee_s, ○ enantioselectivity

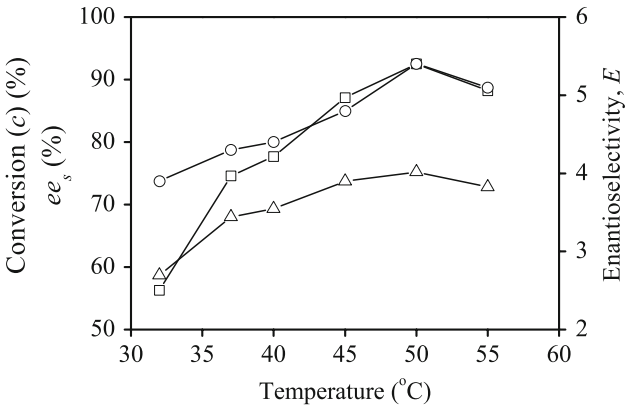


Fig. 2 Effect of reaction temperature on the enantioselective esterification of racemic α-lipoic acid. Reaction conditions: 1 mmol racemic α-lipoic acid, 5:1 molar ratio of *n*-octanol to racemic α-lipoic acid and 200 mg microbial cells in 20 ml heptane, reaction temperature ranging from 32 to 55°C, reaction time = 48 h, shaking at 200 rpm. △ Conversion, □ ee_s, ○ enantioselectivity

reaction, the conversion rate and enantioselectivity increase up to an optimum of 68.0% and $E = 4.3$ (ratios 5:1 or 6:1), respectively. However, when the *n*-octanol concentration increased further (ratios 7:1 and 8:1), enantioselectivity dropped. A good explanation for the observed change in enantioselectivity is that the two diastereomeric acyl-enzymes have different K_m values for the alcohol. A typical case of this would be a situation where both acyl-enzymes are saturated with alcohol at the high concentration, whereas at the low concentration only the *S*-acyl-enzyme is saturated, leading to a higher *E*-value [43]. This is also explained at a molecular level by a molecular modeling study. In contrast to the fast-reacting enantiomer, the slow-reacting *R*-enantiomer leaves the tunnel empty. It is thus suggested that the alcohol coordinates to the tunnel and inhibits the fast-reacting but not the slow-reacting enantiomer. An increased alcohol concentration would therefore decrease the enantioselectivity [38].

Effect of temperature

The esterification reaction of α-lipoic acid and *n*-octanol catalyzed by whole-cell lipase from *A. oryzae* WZ007 was carried out in heptane at temperatures ranging from 32 to 55°C. The results (shown in Fig. 2), demonstrate that both the conversion rate and enantioselectivity increase with increasing reaction temperature up to 50°C. At higher temperatures, the conversion rate and enantioselectivity decreased gradually. A reaction temperature of 50°C was selected as an optimum temperature for the reaction as it exhibited the highest enantioselectivity and appropriate conversion of 75.2% with an ee_s of 92.5% at a reaction time of 48 h.

Conclusions

Lipase from *A. oryzae* WZ007 can enantioselectively esterify (*S*)-α-lipoic acid, leaving the target product (*R*)-α-lipoic acid in the unreacted form, although the reaction center is four carbon atoms away from the stereogenic center. The effects of various reaction parameters on the performance of lipase from *A. oryzae* WZ007 in the preparation of enantiopure (*R*)-α-lipoic acid were studied. When α-lipoic acid was esterified with *n*-octanol at 50°C in heptane for 48 h with a molar ratio of alcohol to acid of 5:1, the enantiomeric excess of (*R*)-α-lipoic acid could reach as high as 92.5%, with a conversion rate of 75.2%.

References

1. Reed LJ, Debusk BG, Gunsalus IC, Hornberger CS Jr (1951) Crystalline α-lipoic acid: a catalytic agent associated with pyruvate dehydrogenase. Science 114:93–94. doi:10.1126/science.114.2952.93
2. Brookes MH, Golding BT, Howes DA, Hudson AT (1983) Proof that the absolute configuration of natural α-lipoic acid is *R* by the synthesis of its enantiomer [(*S*)-(−)-α-lipoic acid] from (*S*)-malic acid. J Chem Soc Chem Commun 19:1051–1053. doi:10.1039/C39830001051
3. Reed LJ (1974) Multienzyme complexes. Acc Chem Res 7:40–46. doi:10.1021/ar50074a002
4. Sigel H (1982) The hydrophobic and metal-ion coordinating properties of α-lipoic acid—an example of intramolecular equilibria in metal-ion complexes. Angew Chem Int Ed Engl 21:389–400. doi:10.1002/anie.198203893
5. Natraj CV, Gandhi VM, Menon KKG (1984) Lipoic acid and diabetes: effect of dihydrolipoic acid administration in diabetic rats and rabbits. J Biosci 6:37–46. doi:10.1007/BF02702855
6. Möller E, Schmitt R (1976) A contribution to the treatment of chronic liver diseases. Med Klin 71:1831–1835

7. Friedrich D, Kirsten E, Achim K, Stefanie W, Arno S, Nina R et al (2007) Protection from hepatic ischemia/reperfusion injury and improvement of liver regeneration by α-lipoic acid. Shock 27:644–651
8. Bast A, Haenen GRMM (1988) Interplay between lipoic acid and glutathione in the protection against microsomal lipid peroxidation. Biochim Biophys Acta 963:558–561. doi:10.1016/0005-2760(88)90326-8
9. Packer L, Witt EH, Tritschler HJ (1995) Alpha-lipoic acid as a biological antioxidant. Free Radic Biol Med 19:227–250. doi:10.1016/0891-5849(95)00017-R
10. Cho YS, Lee J, Lee T-H, Lee EY, Lee K-U, Park JY, Moon H-B (2004) α-Lipoic acid inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. J Allergy Clin Immunol 114:429–435. doi:10.1016/j.jaci.2004.04.004
11. Paetau-Robinson I, Zicker SC (2006) Methods for increasing immune response in an animal. International Patent WO/2006/058248
12. Baur A, Harrer T, Peukert M, Jahn G, Kalden JR, Fleckenstein B (1991) Alpha-lipoic acid is an effective inhibitor of human immuno-deficiency virus (HIV-1) replication. J Mol Med 69:722–724. doi:10.1007/BF01649442
13. Merin JP, Matsuyama M, Kira T, Baba M, Okamoto T (1996) Alpha-lipoic acid blocks HIV-1 LTR-dependent expression of hygromycin resistance in THP-1 stable transformants. FEBS Lett 394:9–13. doi:10.1016/0014-5793(96)00919-2
14. Bingham PM, Zachar Z (2000) Lipoic acid derivatives and their use in treatment of disease. International Patent WO/2000/024734
15. Gunsalus IC, Barton LS, Gruber W (1956) Biosynthesis and structure of lipoic acid derivatives. J Am Chem Soc 78:1763–1766. doi:10.1021/ja01589a079
16. Zimmer R, Hain U, Berndt M, Gewald R, Reissig H-U (2000) Enantioselective synthesis of (*S*)- and (*R*)-6-hydroxy-8-nonenecarboxylates by asymmetric catalysis: a formal synthesis of (*R*)-α-lipoic acid and its (*S*)-antipode. Tetrahedron Asymmetr 11:879–887. doi:10.1016/S0957-4166(00)00018-5
17. Upadhya TT, Nikalje MD, Sudalai A (2001) Asymmetric dihydroxylation and hydrogenation approaches to the enantioselective synthesis of *R*-(+)-α-lipoic acid. Tetrahedron Lett 42:4891–4893. doi:10.1016/S0040-4039(01)00734-1
18. Zhang S, Chen X, Zhang J, Wang W, Duan W (2008) An enantioselective formal synthesis of (+)-(*R*)-α-lipoic acid by an L-proline-catalyzed aldol reaction. Synthesis 3:383–386. doi:10.1055/s-2008-1032022
19. Rao AVR, Gurjar MK, Garyali K, Ravindranathan T (1986) Enantiospecific synthesis of *R*-(+)-α-lipoic acid from D-glucose. Carbohydr Res 148:51–55. doi:10.1016/0008-6215(86)80035-0
20. Rao AVR, Mysorekar SV, Gurjar MK, Yadav JS (1987) Synthesis of (3R, 4R)-1, 5-hexadien- 3,4-diol and its unsymmetricalderivatives: application to (R)-(+)-α-lipoic acid. Tetrahedron Lett 28:2183–2186. doi:10.1016/S0040-4039(00)96076-3
21. Brookes MH, Golding BT, Hudson AT (1988) Syntheses of α-(*R*)- and α-(*S*)-lipoic acid from (*S*)-malic acid. J Chem Soc Perkin Trans 1(1):9–12. doi:10.1039/P19880000009
22. Yadav JS, Mysorekar SV, Pawar SM, Gurjar MK (1990) Synthesis of (3*R*, 4*R*)-1, 2-divinylglycol and its unsymmetrical derivatives: an application to the synthesis of *R*-(+)-α-lipoic acid. J Carbohydr Chem 9:307–316. doi:10.1080/07328309008543834
23. Chavan SP, Praveen C, Ramakrishna G, Kalkote UR (2004) Enantioselective synthesis of R-(+)-α and S-(−)-α-lipoic acid. Tetrahedron Lett 45:6027–6028. doi:10.1016/j.tetlet.2004.06.027
24. Gopalan AS, Jacobs HK (1989) Stereochemical control of yeast reductions: Synthesis of *R*-(+)-α-lipoic acid. Tetrahedron Lett 30:5705–5708. doi:10.1016/S0040-4039(00)76176-4
25. Dasaradhi L, Fadnavis NW, Bhalerao UT (1990) A novel enantiospecific synthesis of (S)-(−)-methyl 6, 8-dihydroxyoctanoate, a precursor of (R)-(+)-α-lipoic acid. J Chem Soc Chem Commun 10:729–730. doi:10.1039/C39900000729
26. Gopalan AS, Jacobs HK (1990) Bakers' yeast reduction of alkyl 6-chloro-3-oxohexanoates: synthesis of (*R*)-(+)-α-lipoic acid. J Chem Soc Perkin Trans 1(7):1897–1900. doi:10.1039/P19900001897
27. Adger B, Bes MT, Grogan G, McCague R, Pedragosa-Moreau S, Roberts SM, Villa R, Wan PWH, Willetts AJ (1995) Application of enzymic Baeyer-Villiger oxidations of 2-substituted cycloalkanones to the total synthesis of (*R*)-(+)-lipoic acid. J Chem Soc Chem Commun 15:1563–1564. doi:10.1039/C39950001563
28. Adger B, Bes MT, Grogan G, McCague R, Pedragosa-Moreau S, Roberts SM, Villa R, Wan PWH, Willetts AJ (1997) The synthesis of (*R*)-(+)-lipoic acid using a monooxygenase-catalyzed biotransformation as the key step. Bioorg Med Chem 5:253–261. doi:10.1016/S0968-0896(96)00234-9
29. Fadnavis NW, Koteshwar K (1997) Remote control of stereoselectivity: lipase catalyzed enantioselective esterification of racemic α-lipoic acid. Tetrahedron Asymmetr 8:337–339. doi:10.1016/S0957-4166(96)00519-8
30. Hughes DL, Bergan JJ, Amato JS, Reider PJ, Grabowski EJJ (1989) Synthesis of chiral dithioacetals: a chemoenzymic synthesis of a novel LTD4 antagonist. J Org Chem 54:1787–1788. doi:10.1021/jo00269a007
31. Hughes DL, Bergan JJ, Amato JS, Bhupathy M, Leazer JL, McNamara JM, Sidler DR, Reider PJ, Grabowski EJJ (1990) Lipase-catalyzed asymmetric hydrolysis of esters having remote chiral/prochiral centers. J Org Chem 55:6252–6259. doi:10.1021/jo00313a010
32. Hedenström E, Nguyen B-V, Silks LA III (2002) Do enzymes recognise remotely located stereocentres? Highly enantioselective *Candida rugosa* lipase-catalysed esterification of the 2- to 8-methyldecanoic acids. Tetrahedron Asymmetr 13:835–844. doi:10.1016/S0957-4166(02)00172-6
33. Angoli M, Barilli A, Lesma G, Passarella D, Riva S, Silvani A, Danieli B (2003) Remote stereocenter discrimination in the enzymatic resolution of piperidine-2-ethanol. Short enantioselective synthesis of sedamine and allosedamine. J Org Chem 68:9525–9527. doi:10.1021/jo035215g
34. Hu S, Kelly S, Lee S, Tao J, Flahive E (2006) Efficient chemoenzymatic synthesis of pelitrexol via enzymic differentiation of a remote stereocenter. Org Lett 8:1653–1655. doi:10.1021/ol0602755
35. Chen CS, Fujimoto Y, Girdaukas G, Sih CJ (1982) Quantitative analyses of biochemical kinetic resolutions of enantiomers. J Am Chem Soc 104:7294–7299. doi:10.1021/ja00389a064
36. Zheng JY, Wang Z, Zhu Q, Zhang YJ, Yan HD (2009) Resolution of biotin intermediate lactone by enzyme-catalyzed stereoselective lactonization in organic solvent. J Mol Catal B Enzym 56:20–23. doi:10.1016/jmolcatb.2008.04.004
37. Won K, Hong J-K, Kim K-J, Moon S-J (2006) Lipase-catalyzed enantioselective esterification of racemic ibuprofen coupled with pervaporation. Process Biochem 41:264–269. doi:10.1016/j.procbio.2005.07.006
38. Holmquist M, Haeffner F, Norin T, Hult K (1996) A structural basis for enantioselective inhibition of *Candida rugosa* lipase by long-chain aliphatic alcohols. Protein Sci 5:83–88
39. Shang C-S, Hsu C-S (2003) Lipase-catalyzed enantioselective esterification of (*S*)-naproxen hydroxyalkyl ester in organic media. Biotechnol Lett 25:413–416. doi:10.1023/A:1022948009889
40. Cambou B, Klibanov AM (1984) Comparison of different strategies for the lipase-catalyzed preparative resolution of racemic acids and alcohols: asymmetric hydrolysis, esterification, and

transesterification. Biotechnol Bioeng 26:1449–1454. doi:10.1002/bit.260261209
41. Laane C, Boeren S, Vos K, Veeger C (1987) Rules for optimization of biocatalysis in organic solvents. Biotechnol Bioeng 30:81–87. doi:10.1002/bit.260300112
42. Zaks A, Klibanov AM (1988) Enzymatic catalysis in nonaqueous solvents. J Biol Chem 263:3194–3201
43. Berglund P, Holmquist M, Hult K, Högberg H-E (1995) Alcohols as enantioselective inhibitors in a lipase catalysed esterification of a chiral acyl donor. Biotechnol Lett 17:55–60. doi:10.1007/BF00134196