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Effects of organic solvents and ionic liquids on the aminolysis of (*RS*)-methyl mandelate catalyzed by lipases

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Abstract—The enzymatic resolution of (*RS*)-methyl mandelate with *n*-butylamine using lipases in organic solvents (*n*-hexane, *tert*-butanol, and chloroform) and ionic liquids [BMIm][BF_4] and [BMIm][PF_6] is reported. The amide configuration is dependent on the organic solvent. When using mixtures of chloroform or *tert*-butanol/ionic liquids (10:1 v/v) with CAL-B as the catalyst, the amides were obtained in high enantiomeric excess (ee_p >99% and E >200).

1. Introduction

Amines and their amide derivatives are important compounds in organic synthesis, because of the presence of these functional groups in many pharmacologically active compounds.[1] Due to an increasing demand for optically active compounds in the pharmaceutical industry, as well as the synthetic applicability of enantiomerically pure amines (such as chiral auxiliaries, bases, and ligands),[2] the design of efficient methods for the preparation of optically active amines is of special interest. Among the resolution-based procedures, enzymatic methods are emerging as a useful alternative to the traditional resolution procedures using an optically active carboxylic acid.[3,4]

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are classified as hydrolases, that catalyze the breakdown of fats and oils with subsequent release of free fatty acids, diacylglycerols, monoacylglycerols, and glycerols, at a water–oil interface. They are the most versatile group of biocatalysts in organic synthesis because of their catalytic ability, commercial availability, low cost, stereoselectivity, and the possibility of using them under a wide range of pH and temperature (20 and 70 °C) conditions.[5] Lipases can catalyze the resolution of esters,[6] amines,[7] acids,[8] and β-hydroxy selenides[9] in organic solvents with a high degree of enantioselectivity.

In recent years, ionic liquids (ILs) have gained a great deal of attention as green solvents in organic synthesis and other chemical processes. Room temperature ionic liquids are non-volatile, thermally stable, and highly polar, and are also moderately hydrophilic solvents. Ionic liquids with melting points at room temperature or below (as low as −96 °C) can now be produced, which is an important reason why ionic liquids are becoming more attractive substitutes for volatile and toxic organic solvents. Furthermore, they are considered environmentally friendly.[10–12] Ionic liquids also have many favorable properties, for example, they are good solvents for a wide range of inorganic, organic, and polymeric materials, showing catalytic effects. Berger et al. reported the determination of the solubilities of molecular hydrogen in imidazolium-ionic liquids and the influence of the hydrogen concentration in asymmetric catalytic hydrogenation.[13] Very recently, the use of neutral organophosphorus reagents as synergists in the extraction of alkali and alkali earth cations by crown ethers into 1-alkyl-3-methylimidazolium was reported.[14]

Ionic liquids have also been used in the study of enzymatic systems, such as lipase-catalyzed kinetic resolution of 1-phenylethanol,[10] esterification of carbohydrates,[10] formation of *Z*-aspartame,[15] and as catalysts in ammoniolysis,[16] and perhydrolysis reactions.[10,17] More specifically, immobilized lipase from *Candida antarctica* B or *Pseudomonas cepacea* (PCL, native) showed a high catalytic efficiency in the resolution of chiral esters, amines, and alcohols both in organic solvents and ionic liquids or in mixtures of the

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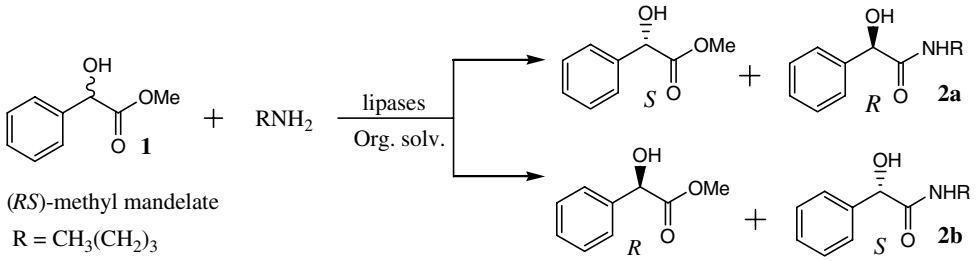

Scheme 1. Aminolysis of (*RS*)-methyl mandelate **1** with *n*-butylamine.

two.[12,18] In general, markedly enhanced regio- and enantioselectivity was achieved in ionic liquids.[19]

Herein we report the enzymatic resolution of (*RS*)-methyl mandelate with *n*-butylamine in organic media and/or organic solvent/ionic liquid mixtures by the use of different lipases free or immobilized in poly(ethylene oxide) (PEO) under different experimental conditions (Scheme 1).

2. Results and discussion

2.1. Screening of lipases

The evaluation of lipases from different sources in the aminolysis of (*RS*)-methyl mandelate **1** with *n*-butylamine in organic media and/or in organic solvent/ionic liquid mixtures was carried out.

As lipases, those from *Pseudomonas cepacia* (PSL), *P. cepacia* (PSL-C and PSL-D), *Rhizomucor miehei* (RML), *Thermomyces lanuginosus* (TLL), and *C. antarctica* (NOVOZYM 435—CAL-B) were selected and used free or immobilized in poly(ethylene oxide) (PEO) films.

When lipases from RML, TLL, PSL, PSL-C, and PSL-D were employed, in their native form or immobilized in PEO, no selectivity, and very low conversion into the corresponding amide was obtained, values being in the range of 0–5% using *n*-hexane, chloroform, or *tert*-butanol as organic solvents at 35 °C until 96 h of reaction.

However, better results were obtained when CAL-B was used as the biocatalyst, regardless of the organic solvent used. The conversion degree was in the range of 17–99%. Based on these results, CAL-B was used in all subsequent experiments.

2.2. Effect of solvent and temperature

The solvent and water content influences the selectivity in a complex way involving several interactions between the reaction medium, both substrates and the enzymes.[20] The strong influence of the nature of the solvent on enantioselectivity has been reported. No clear consensus has yet emerged on the parameters to be used, which quantitatively describe the solvents and their influence on the enzymatic reaction, and the most frequently used is log *P* and the dielectric constant (ε).[21,22]

Using *n*-hexane (log *P* 3.9) in the aminolysis of (*RS*)-**1**, the corresponding racemic amide was formed in a quantitative yield (>99%) after 3.5 h. Figure 1 shows the conversion degree and the enantiomeric excess of the product (ee_p) as a function of time. In the range of 15–180 min, the conversions were 33–96%, with ee_p values between 30% and 70%, and moderate *E* values between 2.8 and 14. These are considered unacceptable for practical purposes.[4] However, these conditions can be used to produce the corresponding (*RS*)-amide in high yield and in a short reaction time.

Using some polar organic solvents such as chloroform and *tert*-butanol, the enantioselectivity increased unless the conversion values decreased. Using these organic solvents, the influence of temperature was evaluated for the resolution of (*RS*)-**1** with *n*-butylamine using CAL-B, in typical experiments at 25, 35, and 45 °C. The results of the conversion into the amide as a function of temperature are given in Figures 2 and 3 for chloroform and *tert*-butanol, respectively. The data showed a considerable effect on the enantioselectivity with different tendencies for the two solvents. The absolute configurations of the products were determined by comparison with the retention times of pure standard compounds.

Using *tert*-butanol (log *P* 1.45), also at 35 °C, a higher conversion degree and lower *E* values were obtained, these being 92% and 10, respectively. In this case, the

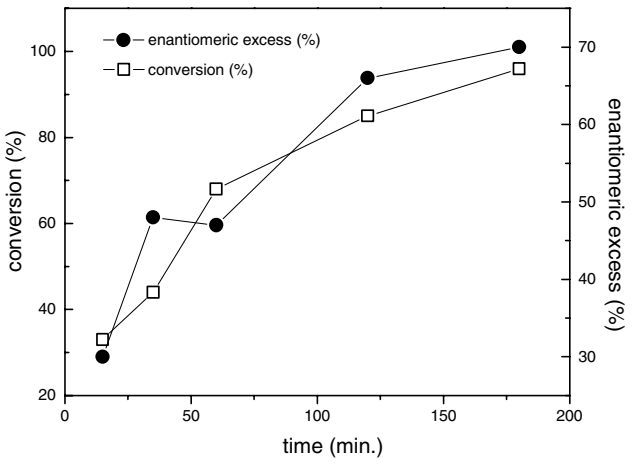

Figure 1. Effect of time on the enzymatic resolution of (*RS*)-methyl mandelate **1** using CAL-B (100 mg) with *n*-hexane (25 mL) at 35 °C.

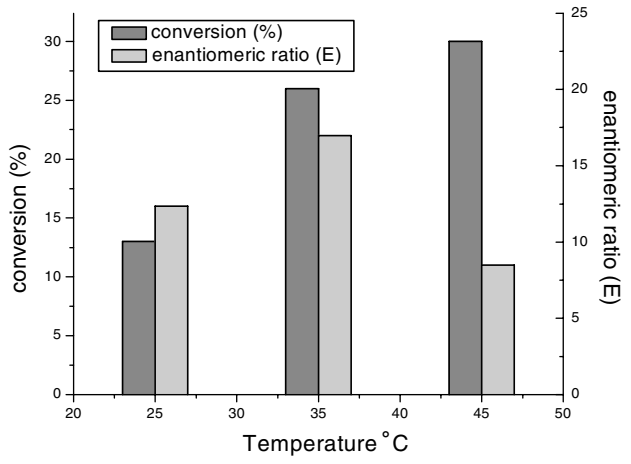

Figure 2. Effect of temperature on conversion (% *c*) and enantiomeric ratio (*E*) of (*RS*)-methyl mandelate **1** with *n*-butylamine catalyzed by CAL-B (100 mg) in chloroform at 24 h.

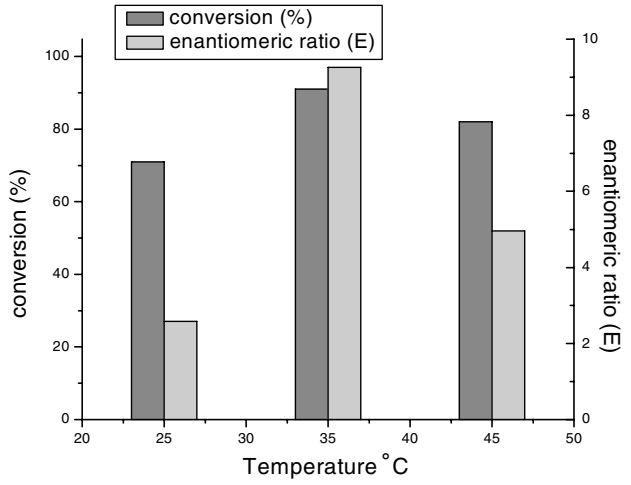

Figure 3. Effect of temperature on conversion (% *c*) and enantiomeric ratio (*E*) of (*RS*)-methyl mandelate **1** with *n*-butylamine catalyzed by CAL-B (100 mg) in *tert*-butanol at 24 h.

product formed was mainly (*R*)-amide **2a**. These data are in agreement with the literature, which shows that this enzyme has a highly pronounced catalytic preference towards *R*-enantiomers.[23–25]

Using chloroform (log *P* 2.00), lower conversions into amide and higher *E* values were obtained when compared with *tert*-butanol. The optimum temperature was 35 °C, forming the (*S*)-amide **2b** in 25% conversion with an *E* value of 22, which is considered moderate to good for synthetic approaches.[4] When the temperature was raised to 45 °C, the conversion increased to 30% but the *E* value decreased to 9. A polar solvent such as chloroform, could strip the essential water layer around the enzyme and thus distort the catalytic conformation inducing an inversion of the product configuration.[26] The solvent-induced inversion of the enantioselectivity of a protease and some lipases was reported by Tawaki and Klibanov,[27] and by Kawasaki et al. for lipase-catalyzed deacetylation in organic solvents.[28]

For both solvents the best temperature was 35 °C. Above this value, both solvent evaporation and enzyme denaturation must be considered. Thus, this temperature was selected for the subsequent experiments.

2.3. Effect of enzyme loading

The influence of the amount of CAL-B was also evaluated in the aminolysis of (*RS*)-**1** in *tert*-butanol at 35 °C for 24 h. The amounts used were 25, 50, 100, and 150 mg. Using 25 and 50 mg, the conversions were 46% and 48%, ee_p 20% and 33% and *E* values 2.4 and 3.1, respectively. Using 100 or 150 mg, better conversions and ee_p values were obtained, these being 91% and 70%, with *E* values of 9.7 and 4.6, indicating the low selectivity of CAL-B under these experimental conditions as discussed above. Considering these results, the amount of 100 mg of CAL-B was selected for the subsequent experiments, which furnished the best results. The data are given in Figure 4.

2.4. Effect of ionic liquid

Ionic liquids are considered to be highly polar solvents, being in the range of lower alcohols and formamide.[10] Generally, enzymes are more stable in solvents with larger log *P* (>3) than lower log *P* (<2) values,[21] but in ionic liquids they have shown enhanced activity, stability, and enantiorecognition. The solvent properties of ionic liquids, their effects on enzyme performance with respect to enzyme activity, stability, and selectivity, and their application in biocatalysis have been well discussed.[10,19]

Herein, both 1-butyl-3-methyl imidazolium tetrafluoroborate [BMIm][BF_4] and 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIm][PF_6] were used in mixtures with *n*-hexane, chloroform, and *tert*-butanol for

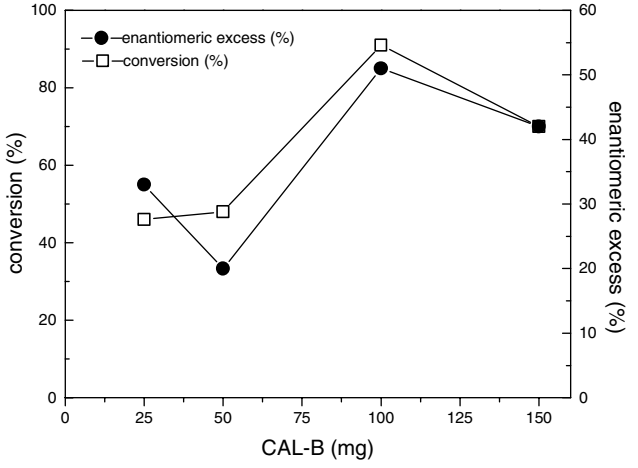

Figure 4. Effect of amount of CAL-B on the enzymatic resolution of (*RS*)-methyl mandelate **1** with *n*-butylamine in *tert*-butanol (25 mL) at 35 °C, 24 h.

the resolution of (*RS*)-methyl mandelate **1** with *n*-butylamine at 35 °C.

When using *n*-hexane/[BMIm][BF_4] (10:1 v/v) or *n*-hexane/[BMIm][PF_6] (10:1 v/v), no improvement in enantioselectivity was observed when compared with the pure solvent, and the conversion into (*RS*)-amide **2a** and **2b** was also quantitative after 3.5 h reaction. Thus, using ionic liquids under these concentrations, there was no apparent influence on the nature of the reaction medium, furnishing results similar to *n*-hexane.

The influence of organic solvents on the enzymatic resolution of (*RS*)-**1** using chloroform or *tert*-butanol catalyzed by 100 mg CAL-B was also studied (Table 1).

As previously observed, the results showed a considerable effect on the enantioselectivity but with different tendencies for the two solvents. Using chloroform, lower conversions into amide and higher *E* values were obtained, these being 26% conversion with an *E* value of 22 (entry 6), mainly forming the (*S*)-enantiomer **2b**. However, when *tert*-butanol was used as the organic solvent, a higher conversion degree, and lower *E* value were obtained, these being 91% and 9.7 (entry 3), respectively, the main product formed now being the (*R*)-enantiomer **2a**.

Much better results were obtained when mixtures of chloroform or *tert*-butanol/[BMIm][BF_4] (10:1 v/v) were used. The conversion degrees were in the range of 14–48, with ee_p >99% and *E* >200 (entries 7–10). The tendency of the two mixtures with respect to enantioselectivity remained the same as that reported above for the pure solvents, with the enantiomers (*S*)- and (*R*)- being formed for the former and latter mixtures, respectively.

When mixtures of chloroform or *tert*-butanol/[BMIm][PF_6] (10:1 v/v) were used, a dependence of the reaction time on the reaction enantioselectivity was also observed. In general, when using this ionic liquid, the reaction formed the enantiopure amide in a shorter time. After using chloroform/[BMIm][PF_6] for 24 h of reaction time the (*S*)-amide was obtained in 21% conversion, with ee_p >99% and *E* >200 (entry 11). After this, a sharp decrease in both the ee_p and the *E* values was observed, these being 17% and 3.3 (entry 12), respectively. Using a *tert*-butanol/[BMIm][PF_6] mixture, just after 7 h of reaction the (*R*)-amide was obtained in 15% conversion with an ee_p >99% and *E* >200 (entry 13). For a longer reaction time, the (*R*)-amide showed a 59% conversion but both the ee_p and the *E* values decreased, these now being 28% and 3.8 (entry 14), respectively.

When using chloroform or *tert*-butanol, pure or in mixtures with the ionic liquid, the configurations of the corresponding amides were the same. However, the use of mixtures with an ionic liquid resulted in the formation of products with a good conversion degree and higher ee_p and *E* values (particularly entry 10).

The difference in the conversion degree and enantioselectivity using these two ionic liquids can be explained in terms of their different properties, such as polarity, hydrophobicity, hydrogen-bond basicity, anion nucleophilicity, and viscosity.[29] These properties may be of primary importance in enzyme-catalyzed reactions, since they are capable of affecting the conformation of the enzymes and consequently their reactivity.[30]

In particular, [BMIm][BF_4] is highly hydrophilic in nature, whereas [BMIm][PF_6] is hydrophobic due to the different anions associated with the common organic cation, similar behavior occurring with the use of pure apolar organic solvents in biocatalytic process.[30]

According to Yang,[19] the enzyme activity is related more to the viscosity and less to the polarity of ionic liquids. Taking these parameters into consideration, it is quite inappropriate to use a single property (such as polarity, hydrophobicity, or viscosity) of ionic liquids to describe their effects on enzyme performance.

Table 1. Effect of solvent on the enzymatic resolution of (*RS*)-methyl mandelate **1** catalyzed by CAL-B

Entry	Solvent[a]	Time (h)	*c* (%)	ee_s[b] (%)	ee_p[c] (%)	*E*[d]	Config.
1	*tert*-Butanol	8	66	66	10	2	*R*
2	*tert*-Butanol	17	68	52	16	2.1	*R*
3	*tert*-Butanol	24	91	93	51	9.7	*R*
4	Chloroform	8	17	77	58	6.5	*S*
5	Chloroform	17	18	86	56	9.3	*S*
6	Chloroform	24	26	92	75	22	*S*
7	Chloroform/[BMIm][BF_4]	24	14	1.5	>99	>200	*S*
8	Chloroform/[BMIm][BF_4]	48	25	22	>99	>200	*S*
9	*tert*-Butanol/[BMIm][BF_4]	72	22	63	>99	>200	*R*
10	*tert*-Butanol/[BMIm][BF_4]	96	48	86	>99	>200	*R*
11	Chloroform/[BMIm][PF_6]	24	21	95	>99	>200	*S*
12	Chloroform/[BMIm][PF_6]	48	45	88	17	3.3	*S*
13	*tert*-Butanol/[BMIm][PF_6]	7	15	74.5	>99	>200	*R*
14	*tert*-Butanol/[BMIm][PF_6]	12	59	78	28	3.8	*R*

Reaction conditions: (*RS*)-methyl mandelate (0.15 mmol); *n*-butylamine (0.3 mmol); CAL-B (100 mg); at 35 °C.

[a] Pure solvent (25 mL), mixtures of organic solvent/ionic liquids (10:1 v/v), at 35 °C, 24 h.

[b] Enantiomeric excess of methyl mandelate **1**.

[c] Enantiomeric excess of amide **2a** or **2b**.

[d] Enantiomeric ratio.

These properties must be considered when explaining the results and as discussed above. Both enantiomers can be obtained through an appropriate choice of solvent and enzyme. Furthermore, the ionic liquids can be readily reused offering another advantage to this methodology.

3. Conclusions

In conclusion, these data show the influence of lipase source and solvent on the resolution of (*RS*)-methyl mandelate **1** via enantioselective aminolysis with *n*-butylamine. The corresponding amides were obtained in high enantiomeric excess (ee_p >99%) using organic solvent/ionic liquid mixtures. The solvent also determined the configuration of the product. Thus, ionic liquids can be used as solvents for lipase-catalyzed aminolysis with the advantage of enhancing the enantioselectivity, suggesting that they have great potential as alternative media for biocatalysis and biotransformations.

4. Experimental

4.1. Materials and methods

Lipases from *P. cepacia* (PSL) (30,000 U g^{-1} solid), *P. cepacia* (PSL-D) (immobilized on diatomaceous earth, 500 U g^{-1}), and *P. cepacia* (PSL-C) (immobilized on ceramic particles chemically modified with a methacryl group, 600 U g^{-1}) were obtained from Amano Pharmaceutical Co. (Nagoya, Japan). Lipases from *R. miehei* (Lipozyme RM IM 5-6 BAUN g^{-1}, RML), *T. lanuginosus* (Lipozyme TL IM 250 IUN g^{-1}, TLL), and *C. antarctica* (NOVOZYM 435—immobilized lipase type B—10.000 PLU g^{-1}, CAL-B) were obtained from Novozymes Latin America Ltda (Brazil). Poly(ethylene oxide) (300.000 g mol^{-1}) and (*RS*)-mandelic acid were purchased from Sigma–Aldrich. *n*-Hexane, *tert*-butanol, chloroform, and *n*-butylamine were obtained from Vetec (Rio de Janeiro, Brazil), and all solvents and other reagents were of analytical grade.

4.2. Immobilization of lipases on PEO

Enzyme immobilization on PEO was performed by dissolving 500 mg of the polymer and 100 mg of the lipases RML and TLL and 50 mg of PSL, PS-D, and PS-C in 25 mL of water. After stirring for 4 h, the solvent was evaporated at room temperature in a Petri dish (Teflon) forming a film, which was then cut into several regular sections of 3 mm^2. Residual water in the film was removed under vacuum conditions.[8]

4.3. General procedure for the synthesis of 1-butyl-3-methyl imidazolium tetrafluoroborate [BMIm][BF_4] and 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIm][PF_6]

These compounds were obtained in 79% and 95% yield, respectively, as described in the literature.[31]

4.4. Preparation of (*RS*)-methyl mandelate

Racemic (*RS*)-(±)-methyl mandelate was prepared by the esterification of (*RS*)-(±)-mandelic acid with methanol and sulfuric acid. In a typical reaction procedure, 0.005 mol of mandelic acid, 2.5 mol of methanol, and drops of concentrated sulfuric acid were kept under reflux for 5 h. The progress of the reaction was monitored by TLC using *n*-hexane/ethyl acetate (8:2) as the mobile phase and the reaction was taken to completion. The racemic mixture of (*RS*)-methyl mandelate was separated from the unreacted mandelic acid by dissolving it in ice-cold ethyl ether and then separating the insoluble mandelic acid out. Ethyl ether was evaporated to yield the colorless solid (*RS*)-methyl mandelate (mp = 53–54 °C). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.75 (s, 1H), 5.17 (s, 1H), 7.25–7.39 (m, 5H).

4.5. General procedure for lipase-catalyzed resolution of (*RS*)-1 with *n*-butylamine

To a solution of (*RS*)-methyl mandelate **1** (0.15 mmol; 25 mg) and *n*-butylamine (0.3 mmol; 0.03 mL), the lipases (25, 50, 100, and 150 mg) free or immobilized in PEO were added to different pure organic solvents (25 mL) or to their mixtures with ionic liquids (10:1 v/v) at temperatures of 25, 35, and 45 °C. The mixture was shaken in a rotary shaker. The reaction progress and enantiomeric excess values were measured with a gas chromatograph (GC) equipped with a chiral column (CP-chirasil-Dex CB, packed β-cyclodextrin, 25 m × 0.25 mm × 0.25 mm, CROMOPACK—Varian). H_2 was used as the carrier gas with a detector, an injector set at 275 °C, and a column set to temperature ramps of 80, 140, and 210 °C (5 °C/min and 3 °C/min). The enantiomeric ratio (*E*) values were calculated from the conversion degree and enantiomeric excess of substrate (ee_s) and products (ee_p), according to the Sih, Sharpless, and Fajans equation.[32] Free shareware programs for the calculation of the enantiomeric ratio can also be obtained via the Internet.[33]

Acknowledgments

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References

1. Henkel, T.; Brunne, R. M.; Reichel, F. *Angew. Chem, Int. Ed.* **1999**, *38*, 643–647.
2. Juaristi, E.; Leon-Romo, J. L.; Reyes, A.; Escalante, J. *Tetrahedron: Asymmetry* **1999**, *10*, 2441–2495.
3. Irimescu, R.; Kato, K. *Tetrahedron Lett.* **2004**, *45*, 523–525.
4. Faber, K. In *Biotransformations in Organic Chemistry*; Springer: Berlin, 1997; Vol. 1, Chapter 1, pp 1–26.

5. Woolley, P.; Petersen, S. B. *Lipases: Their Structure, Biochemistry and Application*; Cambrigde University Press: New York, 1994; Sharma, R.; Chisti, Y.; Banerjee, U. C. *Biotechnol. Adv.* **2001**, *19*, 627–662.
6. Nieto, I.; Rocchietti, S.; Ubiali, D.; Speranza, G.; Morelli, C. F.; Fuentes, I. E.; Alcantara, A. R.; Terreni, M. *Enzyme Microb. Technol.* **2005**, *37*, 514–520.
7. Alcantara, A. R.; de Maria, P. D.; Fernandez, M.; Hernaíz, M. J.; Sánchez-Montero, J. M.; Sinisterra, J. V. *Food Technol. Biotechnol.* **2004**, *42*, 343–354.
8. Nascimento, M. G.; Queiroz, N.; Soldi, V.; Crespo, J. *Process Biochem.* **2005**, *40*, 401–409.
9. Nascimento, M. G.; Comasseto, J. V.; Zanotto, S. P.; Barchesi, H. B.; Clososki, G.; Costa, C. E. *Tetrahedron: Asymmetry* **2004**, *26*, 7–16.
10. van Rantwijk, F.; Lau, R. M.; Sheldon, R. A. *Trends Biotechnol.* **2003**, *21*, 131–138.
11. Kragl, U.; Eckstein, M.; Kaftzik, N. *Curr. Opin. Biotechnol.* **2002**, *13*, 565–571.
12. Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.* **2003**, *14*, 432–437.
13. Berger, A.; Souza, R. F.; Delgado, M. R.; Dupont, J. *Tetrahedron: Asymmetry* **2001**, *12*, 1825–1828.
14. Stepinski, D. C.; Jensen, M. P.; Dzielawa, J. A.; Dietz, M. L. *Green Chem.* **2005**, *7*, 151–158.
15. Erbeldinger, M.; Mesiano, A. J.; Russell, A. J. *Biotechnol. Prog.* **2000**, *16*, 1129–1131.
16. Lau, R. M.; van Rantwijk, F.; Seddon, K. R.; Sheldon, R. A. *Org. Lett.* **2000**, *2*, 4189–4191.
17. Moreira, M. A.; Bitencourt, T. B.; Nascimento, M. G. *Synth. Commun.* **2005**, *35*, 2107–2114.
18. Kim, K. W.; Song, B.; Choi, M. Y.; Kim, M. J. *Org. Lett.* **2001**, *3*, 1507–1509.
19. Yang, Z.; Pan, W. *Enzyme Microb. Technol.* **2005**, *37*, 19–28.
20. Pearsson, M.; Costes, D.; Wehtje, E.; Adlerereutz, P. *Enzyme Microb. Technol.* **2002**, *30*, 916–923.
21. Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30*, 81–87.
22. Salunkhe, M. M.; Nair, R. V. *Enzym. Microb. Technol.* **2001**, *28*, 333–338.
23. Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1998**, *16*, 181–204.
24. Palomo, J. M.; Mateo, C.; Fernández-Lorente, G.; Solares, L. F.; Diaz, M.; Sánchez, V. M.; Bayod, M.; Gotor, V.; Guisan, J. M.; Fernandez-Lafuente, R. *Tetrahedron: Asymmetry* **2003**, *14*, 429–438.
25. Palomo, J. M.; Segura, R. L.; Fuentes, M.; Ortiz, C. C.; Guisan, J. M.; Fernandez-Lafuente, R. *Enzyme Microb. Technol.* **2006**, *38*, 429–435.
26. Klibanov, A. M. *J. Biol. Chem.* **2001**, *409*, 241–246.
27. Tawaki, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1992**, *114*, 1882–1884.
28. Kawasaki, M.; Nakamura, K.; Kawabata, S. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 447–451.
29. Zhao, H. *J. Mol. Catal. B: Enzym.* **2005**, *37*, 16–25.
30. Chiappe, C.; Leandri, E.; Lucchesi, S.; Pieraccini, D.; Hammock, B. D.; Morisseau, C. *J. Mol. Catal. B: Enzym.* **2004**, *27*, 243–248.
31. Cassol, C. C.; Ebeling, G.; Ferreita, B.; Dupont, J. *Adv. Synth. Catal.* **2006**, *348*, 243–248.
32. Sih, C. J.; Wu, S.-H. *Top. Stereochem.* **1989**, *19*, 63.
33. http://www-orgc.tu-graz.ac.at, accessed in January 2006.