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'Gelozymes' in organic synthesis. Part 3: Lipase mediated synthesis of enantiomerically pure (*R*)- and (*S*)-enantiomers of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile ☆

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Abstract—Lipases such as *Candida rugosa* lipase and *Pseudomonas cepacia* (Amano Ps) lipase immobilized in gelatin gels (gelozymes) exhibit very high enantioselectivities ($E > 200$) during alcoholysis of racemic 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile with *n*-butanol in hexane and diisopropyl ether. *C. rugosa* lipase in *n*-hexane shows selectivity towards the (*R*)-ester while *P. cepacia* (Amano Ps) lipase shows selectivity towards the (*S*)-ester producing the corresponding cyanohydrins. After decomposition of the cyanohydrin by treatment with 1 M imidazole solution to cinnamaldehyde and its removal by bisulfite treatment, (*R*)- or (*S*)-enantiomer of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile can be obtained in high yields (90%) and high enantiomeric excess (>99%).

1. Introduction

The enzymatic synthesis of enantiomerically pure cyanohydrins is a reaction of great interest since cyanohydrins are intermediates for several building blocks such as α-hydroxy carboxylic acids, α-amino acids, 1,2-amino alcohols, O-protected α-hydroxyaldehydes, α-mercaptonitriles, 2-amino thiols, etc.[1] Two major enzymatic approaches have been developed for the synthesis of enantiomerically pure cyanohydrins. The first approach is based on the enantioselective addition of HCN to aldehyde or ketone catalyzed by enzyme oxynitrilase.[2–6] The second approach is based on lipase catalyzed enantioselective hydrolysis,[7] or alcoholysis[8–11] of racemic cyanohydrin esters. In continuation of our work on the chemo-enzymatic preparation of enantiomerically pure cyanohydrins[12,13] using lipases immobilized in gelatine matrix by sol–gel technique (gelozymes),[14] herein we report the preparation of both (*R*)- and (*S*)-enantiomers of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile **1**. The enantiomerically pure multifunctional intermediates **2** and **3** can be useful in the synthesis of corresponding (*E*)-2-hydroxy-4-phenyl-3-butenoic acids **7**, which in turn can be used as versatile intermediates for several reactions such as asymmetric epoxidation, dihydroxylation, halogen addition, etc.[15,16]

The (*R*)-enantiomer **2** can also be converted to ethyl (*R*)-2-hydroxy-4-phenylbutyrate (HPB ester) **8**, which has gained significant importance as a raw material for production of a variety of ACE inhibitors.[17] (Scheme 1).

2. Results and discussion

A preliminary screening of some commercially available lipases for the hydrolysis of **1** indicated that lipases from *Pseudomonas cepacia* (Amano Ps), porcine pancreas (PPL) and *Mucor miehei* showed selectivity towards the (*S*)-ester while lipase from *Candida rugosa* (CRL) showed selectivity towards the (*R*)-ester. Among all the lipases, Amano Ps lipase showed highest reactivity towards **1** and was chosen for further studies.

2.1. Enantioselective alcoholysis of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile 1 catalyzed by *P. cepacia* lipase (Amano Ps)

Schneider et al. have reported enantioselective hydrolysis of **1** in phosphate buffer catalyzed by lipase from *Pseudomonas* sp.[18] The enantioselectivity *E* for the reaction was reported to be 17, and the configuration of

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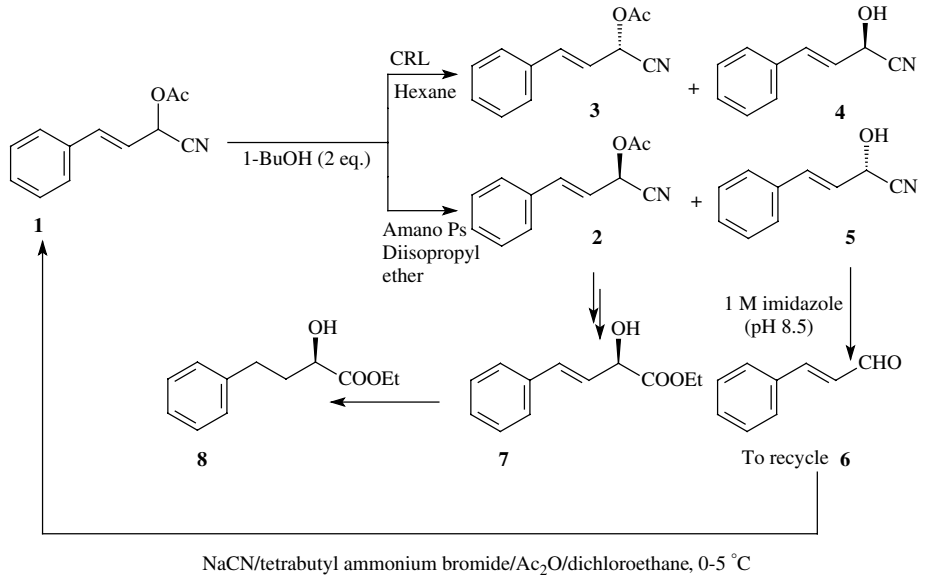

Scheme 1.

the recovered acetate was tentatively assigned as (*R*), which is the desired stereochemistry for the preparation of **5**. The preparation of **8** starting with hydrocinnamaldehyde has been described by Wong et al.[19] *Pseudomonas* lipase catalyzed transesterification of the cyanohydrin derived from hydrocinnamaldehyde with vinyl acetate gave the (*R*)-(−)-cyanohydrin with 98% ee and 39% yield. The enantioselectivity *E* for the reaction was reported to be 24. However, isolation of the cyanohydrin by column chromatography is tedious and the yields are poor due to decomposition to aldehyde.

In our earlier work with cyanohydrins, we observed that the enantioselectivity of the reaction can be greatly improved if alcoholysis of the ester in a water-immiscible organic solvent is carried out instead of hydrolysis in aqueous buffer. Similarly, the preparation of enantiomerically pure cyanohydrin ester can be achieved in excellent yields via destructive resolution where one enantiomer is selectively destroyed.[13] We have tried a similar strategy in the present case.

2.1.1. Effect of alcohol acyl acceptor on enantioselectivity. It has been noted earlier that the carbon chain length of an alcohol affects the enantioselectivity of lipase catalyzed reactions.[13] To optimize the reaction conditions, the alcoholysis reactions were carried out in hexane under identical conditions with alcohols of varying chain length (C_1–C_8) for 10 h. It was observed that the enantiomeric purity of the unreacted ester increased with increasing chain length only up to 1-butanol and then remained constant (Fig. 1). Thus 1-butanol was selected as alcohol of choice for alcoholysis reactions.

2.1.2. Effect of solvent on enantioselectivity and reaction rate. Various solvents such as chloroform, dichloromethane, ethyl acetate, diisopropyl ether, hexane, heptane and isooctane were used for the lipase catalyzed alcoholysis reaction with 1-butanol (2 equiv) as the nucleophile. We observed that the reactions were extremely sluggish in chloroform, dichloromethane and ethyl acetate. Diisopropyl ether and hydrocarbon solvents like hexane, heptane and isooctane were found to be quite suitable. Since the solubility of the substrate was quite high in diisopropyl ether (>10%), this solvent was chosen for further studies.

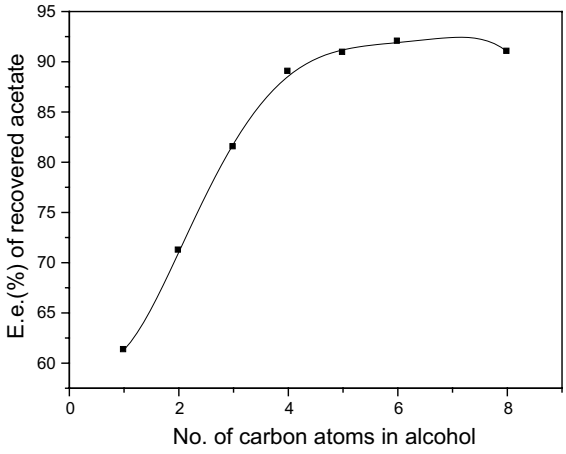

Figure 1. Effect of alcohol chain length on enantiomeric purity of the recovered **2** during alcoholysis of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile **1** catalyzed by *P. cepacia* lipase (Amano Ps) gelozyme (34 mg lipase) at 30 °C in hexane. [**1**] = 0.1 M, [alcohol]/[**1**] = 2, reaction volume 10 mL, reaction period 10 h.

2.1.3. Effect of 1-butanol concentration on enantioselectivity *E*. We have observed only a small effect of 1-butanol concentration on the reaction rate as well as the enantioselectivity of the reaction. We have used a substrate to alcohol molar ratio of 1:2 in all our reactions. A small excess of 1-butanol, however, helps in maintaining the solubility of the product cyanohydrin in the reaction medium and avoiding phase separations

during the reaction. At 50% conversion, the ee of the unreacted ester was 99% while that of product cyanohydrin was 95%. The *E* value was thus calculated to be 210.

2.1.4. Effect of substrate concentration on reaction rate. Increase in substrate concentration is accompanied by a parallel increase in the amount of 1-butanol in a given volume. It was thus important to find out the effect of substrate concentration on the enantioselectivity of the alcoholysis reaction. In these experiments, the molar ratio of alcohol to substrate, and enzyme to substrate ratios (w/w), were kept constant at 1:2 to avoid too many variables. It was observed that the overall rate of the reaction (amount of ester reacted/h/g enzyme) increased with substrate concentration and the enantioselectivity *E* was not adversely affected. Analysis of the data by Michaelis–Menten kinetics gave the value of apparent Michaelis constant $K_{m,app.} = 0.27$ M, and apparent maximum velocity $V_{max,app.} = 80.7$ mM/h/g enzyme.

2.1.5. Effect of particle size of the biocatalyst on reaction rate. Substrate diffusion plays an important role in determining the reaction velocity in case of immobilized enzymes. The scanning electron microscopic studies of the gelozymes prepared in our laboratory showed an ordered porous structure with channels of 30–40 μM diameter.[14] It was expected that substrate pore diffusion would not be a limitation in case of gelozymes. This was borne out by our studies on reaction rates as a function of particle size of the gel. The gel, after enzyme immobilization, was frozen in liquid nitrogen and ground with pestle and mortar. The particles were then sieved with mesh sizes varying from <0.85 to >2.36 mm. Reactions were carried out under identical conditions with the gelozyme of varying particle sizes. With substrate concentration of 0.5 M in the reaction medium, very little variation (<5%) in the typical reaction rate of 53 mM/h/g enzyme was found.

2.1.6. Determination of enantiomeric purity. The course of reaction was followed by chiral HPLC analysis of the reaction mixture on Chiralcel OD (Daicel, Japan). A careful selection of analysis conditions provided a complete resolution of the peaks corresponding to cinnam- aldehyde, the enantiomers of the acetate and of the cyanohydrins in a single run. It was thus easy to follow the progress of the reaction and changes in enantioselectivities quite accurately. The stereochemistry of the products was assigned on the known stereochemical preference of *Pseudomonas* lipase for the (*S*)-enantiomer. The decrease in the peak height of the (*S*)-acetate **3** at 14.56 min was accompanied by a concomitant increase in the peak height of the (*S*)-cyanohydrin **5** at 25.41 min. In case of *C. rugosa* lipase, the peak at 15.63 min corresponding to (*R*-)-acetate **2** decreased while the peak of (*R*)-cyanohydrin **4** at 23.46 min increased as the alcoholysis reaction progressed. For a comparison, the racemic cyanohydrin was prepared by reaction of NaCN with cinnamaldehyde in biphasic system of ethyl acetate–water at pH 4.

After complete disappearance of one enantiomer of the acetate **1** and formation of corresponding cyanohydrin, the cyanohydrin was decomposed to cinnamaldehyde by stirring the reaction mixture with 1 M imidazole (pH 8.5) for 15 min. The cinnamaldehyde was removed as bisulfite adduct by stirring with 30% sodium bisulfite solution. The unreacted enantiomer of the actetate from the organic phase was isolated, purified by column chromatography and its optical rotation was measured on a polarimeter. The unreacted enantiomer recovered after alcoholysis with *P. cepacia* lipase (Amano Ps) had $[\alpha]_D^{25} = -22.0$ (*c* 2.0, chloroform).

2.1.7. Enantioselective alcoholysis of 2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile 1 catalyzed by *C. rugosa* lipase immobilized in gelatin matrix. It has been observed earlier that *C. rugosa* lipase and Amano Ps lipase exhibit opposite enantioselectivities towards cyanohydrin esters.[13] Since the (*S*)-enantiomer **3** is also of considerable significance as an intermediate to (*S*)-**7**, we have studied the enantioselectivity of CRL during the alcoholysis of **1**. In comparison with Amano Ps lipase, the reaction rate for CRL in diisopropyl ether was extremely slow while the reaction rate in hexane was about 10 times slower. The reaction was further inhibited by formation of cinnamaldehyde **6** due to decomposition of the cyanohydrin. By removal of the aldehyde as a bisulfite adduct and subjecting the recovered ester to enzymatic reaction it was possible to obtain (*S*)-**3** with ee >99% in 82% yield. $[\alpha]_D^{25} = +21.8$ (*c* 2.0, chloroform).

2.1.8. Assignment of absolute configuration. From the known stereochemical preference of *P. cepacia* lipase for (*S*)-cyanohydrin esters, the unreacted acetate should have (*R*)-configuration and the cyanohydrin produced should have (*S*)-configuration. The formation of (*S*)-cyanohydrin **5** by stereospecific addition of HCN to cinnamaldehyde catalyzed by oxynitrilase from *Hevea brasiliensis* with 95% ee has been reported by Griengl et al.[21] The reported $[\alpha]_D^{25}$ is −27.9 (*c* 1.95, $CHCl_3$) while the (*R*)-acetate obtained during our experiments with *P. cepacia* lipase has $[\alpha]_D^{25}$ of −22 (*c* 2.0, $CHCl_3$). Since oxynitrilase from *Hevea brasiliensis* was not readily available, we prepared the (*R*)-cyanohydrin using (*R*)-oxynitrilase from almonds (*Prunus amygdalis*) as described by Han et al.[22] in 50% ee. The retention time for the major enantiomer matched with that for the cyanohydrin obtained during CRL catalyzed reaction and the retention time for the minor enantiomer matched with that obtained during *P. cepacia* lipase catalyzed reaction. Thus the recovered acetate after *P. cepacia* lipase catalyzed reaction appears to be (*R*)-**2**. To assign the stereochemistry with more confidence, we sought to convert the enantiomer **2**–**7** of known stereochemistry.

A direct ethanolysis of **2** in 0.3 M ethanolic HCl for 24 h at room temperature gave the required **7**. However, the reaction was accompanied by partial racemization and **7** was obtained with 55% ee as determined by chiral HPLC analysis. The product had a negative rotation

corresponding to (*R*)-configuration with $[\alpha]_D^{25} = -17.1$ (*c* 2.0, $CHCl_3$); lit[16] $[\alpha]_D^{25} = -67.7$ (*c* 1.03, $CHCl_3$ for the corresponding methyl ester). Although the specific rotation of the methyl and ethyl esters cannot be compared directly, the negative sign of rotation for **7** obtained from **2** suggests the (*R*)-configuration of **2**.

3. Conclusion

In conclusion, present work describes the preparation of enatiomerically pure cyanohydrin esters **2** and **3** using lipases of opposing stereoselectivities immobilized in gelatin matrix. Conditions for analysis by chiral HPLC have been established to determine the enantiomeric purity of the products without derivatization. Although preparation of hydroxyester **7** and its antipode via enantiospecific reduction of the corresponding α-keto acid has been well demonstrated,[15,20] the necessity of growing the appropriate cultures in an organic chemist's laboratory is a handicap. Use of commercially available lipases solves these problems. The recycling of the lipases immobilized in gelatine matrix for several recycles has already been demonstrated[12] and thus our procedure can be used for large scale preparations. Although our first experiments with conversion of enantiomerically pure **2**–**7** without racemization have not been completely successful, efforts are being made to develop an appropriate methodology.

4. Experimental

Lipase from *C. rugosa*, porcine pancreas and *M. miehei* were obtained from Sigma, USA. Lipase Ps was obtained from Amano Pharmaceutical Corporation Ltd. Japan. All other reagents were A.R. grade obtained from SD Fine Chem, India. HPLC analyses were carried out on Hewlett Packard HP1090 unit with diode array detector and HP Chem station. The enzymes were immobilized as described earlier.[12,14] A loading of 18% (w/w) was obtained in the gelatin matrix for both the enzymes. Almond meal was prepared from freshly ground almonds, defatted with cold hexane and used as described in literature.[22]

4.1. Preparation of (*RS*)-2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile 1

Sodium cyanide (49 g, 1 mol) and tetrabutyl ammonium chloride (5.55 g, 20 mmol) were dissolved in water (600 mL) and the solution was cooled in ice with stirring. A solution of cinnamaldehyde (92.5 g 0.7 mol) in dichloroethane (500 mL) was added and the reaction mixture was stirred for 4 h at 4–5 °C. Acetic anhydride (71 g, 0.7 mol) in dichloroethane (200 mL) was added dropwise with stirring over 2 h. The reaction mixture was stirred further for 4 h in cold and then allowed to reach room temperature. The layers were then separated and the organic layer was washed with sodium carbonate, water and brine; dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The residual dark yellow oil was extracted with diisopropyl ether at room temperature. The combined extracts on removal of solvent gave a pale yellow viscous oil (129 g, 92%). CAUTION: It was important to maintain the temperatures below 40 °C to avoid formation of polymeric materials during solvent evaporation. The crude material so obtained was sufficiently pure for use in the enzymatic resolution. However, a small portion was purified by column chromatography using hexane–ethyl acetate (98:2) as eluent (r.f. 0.64) for analytical purpose. IR (neat): 3484, 3061, 3029, 2933, 2361, 1753, 1654, 1579, 1495, 1451, 1372, 1293, 1216, 1122, 1071, 1021, 968, 922, 837, 750, 694, 601, 559, 505 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 2.2 (s, 3H), 5.99 (d, 1H $J = 6.8$ Hz), 6.16 (dd, 1H, $J_1 = 6.4$ Hz, $J_2 = 15.9$ Hz), 6.95 (d, 1H, $J = 14.7$ Hz), 7.25–7.45 (m, 5H). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 41.2, 76.12, 94.88, 98.17, 107.12 (2C), 108.55, 109.15 (2C), 114.22, 117.52, 148.25. Anal. calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.96; found C, 71.21; H, 5.48; N, 6.52.

4.2. Enzymatic preparation of (*R*)-2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile 2

(*RS*)-**1** (20.1 g, 0.1 mol) in diisopropyl ether (200 mL) and 1-butanol (14.8 g, 0.2 mol) were stirred magnetically with *Amano Ps* gelozyme (20 g, 3.6 g lipase) at room temperature in a round-bottom flask. The progress of the reaction was monitored by chiral HPLC. The reaction was continued till all the (*R*)-ester had reacted (6 h, 50% conversion). The reaction mixture was then decanted, the filtrate was stirred with 1 M imidazole solution (50 mL, pH 8.5) for 20 min to decompose the cyanohydrin to cinnamaldehyde. The reaction mixture was then stirred with 30% sodium bisulfite solution for 30 min. After separating the bisulfite adduct, the organic layer was washed with water, phosphate buffer (0.1 M, pH 8.2) and brine. After evaporation of the solvent under high vacuum at room temperature, the residue obtained (9.2 g, 92% of theoretical) consisted of >95% (*R*)-**2** (HPLC analysis). A small portion was purified by column chromatography using hexane–ethyl acetate (98:2) as eluent (r.f. 0.64). $[\alpha]_D^{25} = -22.0$ (*c* 2.0, chloroform), ee >99%.

4.3. Enzymatic preparation of (*S*)-2-acetoxy-4-phenyl-(*E*)-but-3-enenitrile 3

(*RS*)-**1** (2.01 g, 0.01 mol) in hexane (100 mL) and 1-butanol (1.48 g, 0.02 mol) were stirred magnetically with *C. rugosa* gelozyme (10 g, 1.8 g lipase) at room temperature in a round-bottom flask. The progress of the reaction was monitored by chiral HPLC. After every 8 h, the reaction mixture was decanted from the immobilized enzyme and vigorously stirred with sodium bisulfite solution (30%, 50 mL) for 10 min. The bisulfite adduct was filtered off, the organic layer was washed with sodium phosphate buffer (3×20 mL, 0.1 M, pH 8.2), dried over anhydrous magnesium sulphate and again subjected to enzymatic reaction after addition of 1 mL 1-butanol to compensate for its loss during aque-

ous washings. The reaction was continued till all the (*S*)-ester had reacted (3 days). The reaction mixture was then decanted, the filtrate was treated as in Section 4.3 to obtain the (*S*)-acetate **3** (0.82 g, 82% of theoretical) after purification by column chromatography using hexane–ethyl acetate (93:7) as eluent. $[\alpha]_D^{25} = +21.8$ (*c* 2.0, chloroform), ee >99%.

4.4. Preparation of ethyl 2-hydroxy-4-phenyl-(2*R*,3*E*)-3-butenoate 7

(*R*)-**2** (2.01 g, 0.01 mol) was dissolved in 0.3 M ethanolic HCl (100 mL) and stirred at room temperature for 24 h. The product **7** was recovered from the reaction mixture after removal of ethanol and column chromatography over silica gel using hexane–ethyl acetate as eluent (1.2 g, 60%). $[\alpha]_D^{25} = -17$ (*c* 2.0, chloroform), ee 55%. IR (neat): 675, 750, 975, 1000, 1105, 1125, 1200, 1350, 1410, 1725, 2975, 3495. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.34 (t, 3H, $J = 6.80$ Hz), 4.22–4.35 (m, 3H), 4.73 (m, 1H), 6.17 (dd, 1H $J_1 = 4.9$ Hz, $J_2 = 15.86$ Hz); 6.77 (d 1H $J = 15.86$ Hz), 7.17–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 14.07, 61.97, 70.93, 125.43, 126.36 (2C), 127.52, 128.41 (2C), 132.0, 136.16, 172.97.

4.5. Chiral HPLC analysis

Enantiomeric purity of **2**, **3** and **7** was determined by chiral HPLC analysis on Chiralcel OD column (5 × 250 mm), Daicel Chemical Industries, Japan. Mobile phase 10% isopropanol in hexane; flow rate 0.7 mL/min; detection wavelength 255 nm; retention times: cinnamaldehyde **6** 10.99 min; (*S*)-acetate **3** 14.56 min; (*R*)-acetate **2** 15.63 min; (*R*)-cyanohydrin **4** 23.46 min; (*S*)-cyanohydrin **5** 25.41 min. The hydroxy ester **7** was also analyzed under similar conditions: (*S*)-ester 12.0 min, (*R*)-ester **7** 13.9 min.

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References and notes

1. Effenberger, F.; Forster, S.; Wajant, H. *Curr. Opin. Biotechnol.* **2000**, *11*, 532–539.
2. Gotor, V. *J. Biotechnol.* **2002**, *96*, 35–42.
3. Li, N.; Zong, M.-H.; Peng, H.-S.; Wu, H.-C.; Liu, C. *J. Mol. Catal. B: Enzym.* **2003**, *22*, 7–12.
4. Gonzalo de Gonzalo, G.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* **2002**, *19–20*, 223–230.
5. Griengl, H.; Hickel, A.; Johnson, D. V.; Kratkey, C.; Schmidt, M.; Schwab, H. *J. Chem. Soc., Chem. Commun.* **1997**, 1939–1993.
6. Schmidt, M.; Griengel, H. *Top. Curr. Chem.* **1999**, *200*, 193–226.
7. Mitsuda, S.; Yamamoto, H.; Uemura, T.; Hiirohara, H.; Nabeshima, S. *Agric. Biol. Chem.* **1990**, 2907–2912.
8. Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1555–1564.
9. Hanefeld, U.; Straathof, A. J. J.; Heijen, J. J. *J. Mol. Cat. B. Enzym.* **2001**, *11*, 213–218.
10. Zhang, T.; Yang, L.; Zhu, Z.; Wu, J. *J. Mol. Catal. B: Enzym.* **2002**, *18*, 315–323.
11. Sakai, T.; Miki, Y.; Tsuboi, M.; Takeuchi, H.; Ema, T.; Uneyama, K.; Utaka, M. *J. Org. Chem.* **2000**, *65*, 2740–2747.
12. Fadnavis, N. W.; Babu, R. L.; Sheelu, G.; Deshpande, A. *Tetrahedron: Asymmetry* **2000**, *11*, 3303–3309.
13. Fadnavis, N. W.; Babu, R. L.; Sheelu, G.; Deshpande, A. *Tetrahedron: Asymmetry* **2001**, *12*, 1695–1699.
14. Fadnavis, N. W.; Koteshwar, K. *Biotechnol. Prog.* **1999**, *15*, 98–104.
15. Schummer, A.; Yu, H.; Simon, H. *Tetrahedron* **1991**, *47*, 9019–9034.
16. Yu, H.; Simon, H. *Tetrahedron* **1991**, *47*, 9035–9052.
17. Herold, P.; Indolese, A. F.; Studer, M.; Jalett, H. P.; Siegrist, U.; Blaser, H. U. *Tetrahedron* **2000**, *56*, 6497–6499.
18. Almick, A.; Buddrus, J.; Honicke-Schmidt, P.; Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1989**, 1391–1393.
19. Wang, Y. F.; Chen, S. T.; Kevin, K.; Liu, C.; Wong, C. H. *Tetrahedron Lett.* **1989**, 1917–1920.
20. Casy, G.; Lee, T. V.; Lovell, H. In *Preparative Biotransformations: Whole Cell & Isolated Enzymes in Organic Synthesis*; Roberts, S. M., Ed.; John Wiley & Sons Ltd: UK, 1993; pp 2:6.1–2:6.17.
21. Schmidt, M.; Herve, S.; Klempier, N.; Griengl, H. *Tetrahedron* **1996**, *52*, 7833–7840.
22. Han, S.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **1998**, *9*, 1838–1853.