Journal of Molecular Catalysis B: Enzymatic 63 (2010) 128–134

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Bacillus alcalophilus MTCC10234 catalyzed enantioselective kinetic resolution of aryl glycidyl ethers

Neeraj Bala [a], Swapandeep Singh Chimni [a,*], Harvinder Singh Saini [b,*], Bhupinder Singh Chadha [b]

[a] *Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[b] *Department of Microbiology, Guru Nanak Dev University, Amritsar 143005, Punjab, India*

ARTICLE INFO

Article history:
Received 16 October 2009
Received in revised form
31 December 2009
Accepted 31 December 2009
Available online 11 January 2010

Keywords:
Epoxide hydrolase
Enantioselective
Kinetic resolution
Aryl glycidyl ethers
Biohydrolysis

ABSTRACT

The phenyl glycidyl ether derivatives have been kinetically resolved with the growing cells of *Bacillus alcalophilus* MTCC10234 yielding (*S*)-epoxides with up to >99% ee and (*R*)-diols with up to 89% ee. The enantiomeric ratio (*E*) of up to 67 has been obtained for biohydrolysis process. The effect of different substituents of phenyl glycidyl ether on the biocatalytic efficiency of *B. alcalophilus* MTCC10234 showed preference for methyl- and chloro-substituted aryl glycidyl ether derivatives whereas nitro-derivatives were transformed at a slower rate. 2,6-Dimethylphenyl glycidyl ether which contains a bulky aryl group having methyl group on both the *ortho* positions was resolved with an $E = 39$.

1. Introduction

The synthesis of enantiopure epoxides, as well as their corresponding vicinal diols is an actively pursued area of research. This is due to the fact that these compounds are essential chiral intermediates for the synthesis of bioactive products in the pharmaceuticals and agrochemical industries. Therefore, the development of efficient and cost effective processes for the preparation of these chiral synthons in enantiopure form are of utmost importance. The biocatalytic hydrolytic kinetic resolution (HKR) of racemic epoxides is one of the most promising methods. The methodology is based on the use of epoxide hydrolases (EH) (EC 3.3.2.3)—a natural, biodegradable and environmentally benign catalyst that provides an important "sustainable chemistry" alternative to the traditional potentially toxic transition metal catalysts. Therefore, this methodology allows minimizing the cost of resources and the production of toxic waste in industrial applications.

In addition to these advantages, epoxide hydrolases from fungal and bacterial sources have been shown to be highly selective, cofactor independent, produced in sufficient quantity by fermentation without the requirement of sophisticated enzyme induction. In the last two decades a few fungal [1–5], bacterial [6–12] and yeast [13–16] strains have been shown to produce epoxide hydrolase enzymes that have found applications in the synthesis of enantiopure epoxides and diols. However, the use of bacterial epoxide hydrolase for kinetic resolution of aryl glycidyl ether is limited. The discovery of new bacterial strains with enantioselective epoxide hydrolase having broad substrate selectivity and high activity is an actively pursued area of research and development. With the objective of finding a new bacterial strain having enantioselective epoxide hydrolase activity, we initiated the screening of bacterial strains available with the working group. This resulted in the discovery of *Bacillus alcalophilus* MTCC10234 (HA-13) having epoxide hydrolase activity for the enantioselective kinetic resolution of aryl glycidyl ether derivatives. Aryl glycidyl ethers and their related compounds are potentially important intermediate for the synthesis of chiral amino alcohols [17] and bioactive compounds such as β-blockers [18]. Bacterial epoxide hydrolase from *Bacillus megaterium* [8], has been shown to resolve phenyl glycidyl ether with high enantioselectivity ($E = 47.8$) but its general applicability for substituted derivative of phenyl glycidyl ethers has not been explored. In this report we present the study of the scope of bacterial epoxide hydrolase from *B. alcalophilus* MTCC10234 for kinetic resolution of phenyl glycidyl ether derivatives, obtaining (*S*)-epoxides with up to >99% ee and (*R*)-diols with up to 89% ee.

* Corresponding authors. Tel.: +91 183 2258809x3204; fax: +91 183 2258820.
E-mail addresses: sschimni@yahoo.com (S.S. Chimni), sainihs@gmail.com (H.S. Saini).

1381-1177/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.12.019

2. Experimental

2.1. General methods

NMR spectra were obtained at 300 MHz (JEOL AL-300) using either $CDCl_3$ or DMSO as solvents with Me_4Si in $CDCl_3$ as the internal standard. The chemical shifts are reported in δ values relative to TMS and coupling constants (J) are expressed in Hertz. Spectral patterns are designated as s = singlet; d = doublet; dd = doublet of doublets; q = quartet; t = triplet; br = broad; m = multiplet. Analytical thin-layer chromatography (TLC) was performed on either (i) aluminum sheets pre-coated with silica gel GF254 (Merck, India) or (ii) glass plates (7.5 cm × 2.5 cm) coated with silica gel GF-254 (Spectrochem, India) containing 13% calcium sulphate as binder and various combinations of ethyl acetate and hexane were used as eluents. Visualization of the spots was accomplished by exposing to UV light or iodine vapors. Column chromatography was performed on 60–120 mesh silica (Spectrochem, India) using mixture of hexane and ethyl acetate as eluents. Organic compounds benzyl alcohols, phenols and epichlorohydrin used for the synthesis of epoxide were obtained from Aldrich, Spectrochem or S. d. fine-chem. Ltd. India and used as received without further purification. The substrate and product concentrations as well as the enantiomeric excess of epoxide and diol were determined by HPLC (Dionex, USA) using chiral columns (Daicel Chiralpak OD-H and AD-H). The racemic epoxides were prepared by the procedure reported in literature [20–21].

2.2. Growth medium

Minimal salt medium (MSM) of following composition was used for biotransformation reaction (in 1 L): Na_2HPO_4 3.6 g, $(NH_4)_2SO_4$ 1.0 g, KH_2PO_4 1.0 g, $MgSO_4$ 1.0 g, ammonium ferric citrate (1%, w/v) 1.0 mL, $CaCl_2{\cdot}2H_2O$ 0.10 g, TES[1] 1.0 mL, tryptone and sucrose were added as required from their respective sterilized stock solution, pH 7.

The stock solution of the tryptone (25%, w/v) and sucrose (50%, w/v) were sterilized separately and added to MSM as per desired supplement.

Nutrient agar medium (NAM) of following composition (in 1 L) was used for preservation of culture on Petri plate: Beef extract 3.0 g, peptones 5.0 g, NaCl 5.0 g and agar 20 g.

2.3. General method for B. alcalophilus MTCC10234 mediated biohydrolysis of epoxides (1–15)

The protocol for conducting the biotransformation involves the activation of the culture in 50 mL sterilized minimal synthetic media (MSM) in Erlenmeyer flasks (250 mL) containing 1.2% (w/v) tryptone and 1% (w/v) sucrose, added from their respective stock solutions. The flask was incubated for 24 h at 30 °C in an orbital shaker at 110 rpm. Then 5% (v/v) of inoculum was used to inoculate 100 mL of sterilized MSM in 500 mL Erlenmeyer flask supplemented with 1.2% (w/v) tryptone and 1% (w/v) sucrose. The flask was incubated for 24 h on an orbital shaker at 110 rpm. Epoxide (100 mg) dissolved in ethanol (300 μl) was added to each flask near a spirit in laminar flow and further incubated in an orbital shaker for time period specified in Table 1. The biotransformation was ceased by the addition of acetone and after saturation with NaCl, the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate and concentrated to obtain the crude product. The product was chromatographed for analysis.

(*S*)-1,2-Epoxy-3-phenoxypropane (1a): Yield 27%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.72 (dd, J = 2.7 and 4.95 Hz, 1H, CH_2), 2.88 (t, J = 4.5 Hz, 1H, CH_2), 3.29–3.34 (m, 1H, CH), 3.95 (dd, J = 5.4 and 10.9 Hz, 1H, CH_2), 4.16 (dd, J = 3.3 and 10.9 Hz, 1H, CH_2), 6.87–6.96 (m, 3H, ArH), 7.22–7.29 (m, 2H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 43.9, 49.6, 68.1, 113.9, 120.5, 128.8, 157.8; enantiomeric excess >99%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 90:10); flow rate 1 mL/min; λ = 218 nm, t_R (*R*) 8.27 min, t_R (*S*) 12.89 min.

(*R*)-3-(Phenoxy)propane-1,2-diol (2a): Yield 73%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.52 (bs, 1H, OH), 3.12 (bs, 1H, OH), 3.60–3.80 (m, 2H, CH_2), 3.95 (d, J = 5.4 Hz, 2H, CH_2), 4.06 (m, 1H, CH), 6.84–6.94 (m, 3H, ArH), 7.12–7.25 (m, 2H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 62.9, 68.4, 69.7, 113.8, 120.6, 128.8, 157.7; enantiomeric excess 68%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 90:10); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 16.25 min, t_R (*S*) 34.52 min.

(*S*)-1,2-Epoxy-3-(4-methylphenoxy)propane (1b): Yield 38%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 2.70 (dd, J = 2.7 and 4.9 Hz, 1H, CH_2), 2.84 (dd, J = 4.8 and 9.6 Hz, 1H, CH_2), 3.26–3.31 (m, 1H, CH), 3.92 (dd, J = 5.4 and 10.9 Hz, 1H, CH_2), 4.10 (dd, J = 3.3 and 10.8 Hz, 1H, CH_2), 6.72–6.82 (m, 2H, ArH), 6.98–7.08 (m, 2H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 20.4, 44.7, 50.2, 68.9, 114.5, 129.9, 130.0, 130.3, 130.4, 156.4; enantiomeric excess >99%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 7.58 min, t_R (*S*) 8.85.min.

(*R*)-3-(4′-Methylphenoxy)propane-1,2-diol (2b): Yield 62%; white solid; m.p. 69–70 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.38 (bs, 1H, OH), 2.89 (bs, 1H, OH), 3.70 (dd, J = 5.1 and 11.4 Hz, 1H, CH_2), 3.79 (d, J = 8.7 Hz, 1H, CH_2), 3.97 (d, J = 5.7 Hz, 2H, CH_2), 4.01–4.05 (m, 1H, CH), 6.73–6.78 (m, 2H, ArH), 7.03 (d, J = 8.7 Hz, 2H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 19.7, 63.0, 68.6, 69.8, 113.7, 129.3, 129.8, 156.6; enantiomeric excess 73%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 30.66 min, t_R (*S*) 45.08 min.

(*S*)-1,2-Epoxy-3-(3′-methylphenoxy)propane (1c): Yield 37%; colourless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.78 (dd, J = 3.0 and 4.96 Hz, 1H, CH_2), 2.89 (dd, J = 4.0 and 4.89 Hz, 1H, CH_2), 3.36–3.38 (m, 1H, CH), 3.96 (dd, J = 5.4 and 12.0, 1H, CH_2), 4.22 (dd, J = 3.0 and 11.1, 1H, CH_2), 6.80 (d, J = 8.28, 1H, ArH), 6.87–6.88 (m, 2H, ArH), 7.13–7.15 (m, 2H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 16.2, 44.7, 50.3, 68.7, 111.3, 120.9, 126.8, 127.1, 130.8, 156.7; enantiomeric excess 98%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 90:10); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 6.78 min, t_R (*S*), 8.91 min.

(*R*)-3-(3′-Methylphenoxy)propane-1,2-diol (2c): Yield 63%; white solid; m.p. 63–65 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.47 (bs, 1H, OH), 2.92 (bs, 1H, OH), 3.70–3.84 (m, 2H, CH_2), 3.96–4.01 (m, 2H, CH_2), 4.05–4.11 (m, 1H, CH), 6.76–6.86 (m, 2H, ArH), 7.08–7.12 (m, 2H, ArH); ^{13}C NMR (100.61 MHz, CD_3OD): 16.0, 64.4, 70.5, 71.9, 112.6, 121.6, 127.8, 131.4, 158.3; enantiomeric excess 86%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 90:10); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 15.75 min, t_R (*S*) 17.54 min.

(*S*)-1,2-Epoxy-3-(2′-methylphenoxy)propane (1d): Yield 38%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 2.74 (dd, J = 2.7 and 4.9 Hz, 1H, CH_2), 2.87 (dd, J = 4.2 and 4.9 Hz, 1H, CH_2), 3.29–3.34 (m, 1H, CH), 3.96 (dd, J = 5.4 and 10.95 Hz, 1H, CH_2), 4.16 (dd, J = 3.3 and 11.1 Hz, 1H, CH_2), 6.73–6.85 (m, 2H, ArH), 7.06–7.11 (m, 2H, ArH). ^{13}C NMR (125.75 MHz, $CDCl_3$): 14.9, 43.4, 49.0, 67.4, 110.0, 119.7, 125.5, 129.5, 153.0; enantiomeric excess >99%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min, λ = 218 nm; t_R (*R*) 7.63 min t_R (*S*) 10.60 min.

[1] Composition of trace element solution (in 1 L): $ZnSO_4{\cdot}7H_2O$ 10 mg, $MnCl_2{\cdot}4H_2O$ 3.0 mg, $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$ 2.0 mg, $Na_2MoO_4{\cdot}2H_2O$ 3.0 mg, H_3BO_3 30 mg and $CuCl_2{\cdot}2H_2O$ 1.0 mg.

Table 1
Bacillus alcalophilus catalyzed enantioselective hydrolysis of different epoxides.[a]

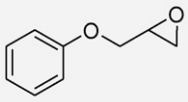
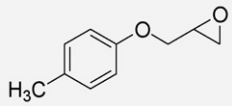
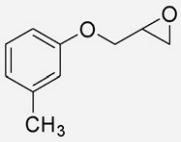
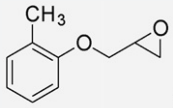
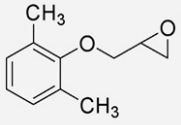
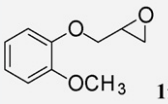
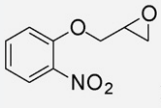
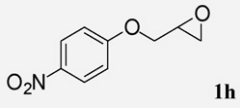
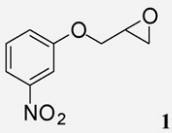
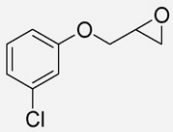
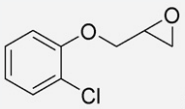
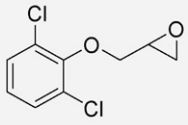
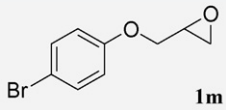
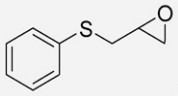
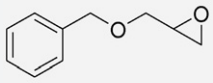
S. No.	Substrate	Yield of epoxide[b] (%)	ee epoxide[b] (%) (*S*)	Yield of diol[b] (%)	ee diol[b] (%) (*R*)	*E*[c]
1	 **1a**	27	>99	73	68	27
2	 **1b**	38	>99	62 (16h)	73	33
3	 **1c**	37	98	63 (16h)	86	67
4	 **1d**	38 44	>99 94	62 (24h) 56 (16h)	84 89	64 61
5	 **1e**	24	93	76	84	39
6	 **1f**	70	28	30	64	6
7	 **1g**	62	5	38	28	2
8	 **1h**	94	2	6	20	–
9[d]	 **1i**	n.t.	–	–	–	–
10	 **1j**	33	>99	67	68	27
11	 **1k**	40	93	60	78	27

Table 1 (*Continued*)

S. No.	Substrate	Yield of epoxide[b] (%)	ee epoxide[b] (%) (*S*)	Yield of diol[b] (%)	ee diol[b] (%) (*R*)	*E*[c]
12[d]	 **1l**	n.t	–	–	–	–
13	 **1m**	70 33[e]	10 >99[e]	30 67[e]	68 72[e]	6 31
14	 **1n**	30	23	70 (10 h)	27	2
15	 **1o**	50	30	50	40	3

[a] Unless otherwise specified the biotransformation reaction time is 24 h in all cases.
[b] Calculated on the basis of HPLC analysis.
[c] *E* values are calculated as given in [19].
[d] No transformation (n.t.) was observed in these cases.
[e] 6% DMSO was used as additive.

(*R*)-3-(2′-Methylphenoxy)propane-1,2-diol (2d): Yield 62%; white solid; m.p. 65–66 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.23 (bs, 1H, OH), 2.74 (bs, 1H, OH), 2.92 (bs, 1H, OH), 3.63–3.85 (m, 2H, CH_2), 4.02 (d, J = 5.1 Hz, 2H, CH_2), 4.09 (m, 1H, CH), 6.77–6.86 (m, 2H, ArH), 7.08–7.13 (m, 2H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 15.5, 63.1, 68.5, 69.8, 110.4, 120.3, 125.9, 126.2, 130.1, 155.7; enantiomeric excess 84%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min, λ = 218 nm; t_R (*R*) 36.68 min t_R (*S*) 42.75 min.

(*S*)-1,2-Epoxy-3-(2′,6′-dimethylphenoxy)propane (1e): Yield 24%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.72 (dd, J = 2.7 and 4.9 Hz, 1H, CH_2), 2.85 (dd, J = 4.2 and 4.9 Hz, 1H, CH_2), 3.28–3.33 (m, 1H, CH), 3.95 (dd, J = 5.4 and 10.9 Hz, 1H, CH_2), 4.13 (dd, J = 3.6 and 11.1 Hz, 1H, CH_2), 6.63 (d, J = 8.4 Hz, 1H, ArH), 6.73 (d, J = 7.5 Hz, 1H, ArH), 6.97 (t, J = 7.8, 1H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 11.6, 20.2, 44.6, 50.4, 69.1, 109.4, 122.8, 125.5, 125.8, 138.1, 156.4; enantiomeric excess 93%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 8.22 min, t_R (*S*) 12.65 min.

(*R*)-3-(2′,6′-Dimethylphenoxy)propane-1,2-diol (2e): Yield 76%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54 (bs, 1H, OH), 2.13 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.64 (bs, 1H, OH), 3.68–3.86 (m, 2H, CH_2), 3.95–4.00 (m, 2H, CH_2), 4.03–4.12 (m, 1H, CH), 6.65 (d, J = 8.1 Hz, 1H, ArH), 6.74 (d, J = 7.5 Hz, 1H, ArH), 6.97 (t, J = 7.8 Hz, 1H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 11.6, 20.0, 63.9, 69.5, 70.6, 109.2, 122.9, 125.1, 125.9, 138.1, 156.2; enantiomeric excess 84%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 98:2); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 36.79 min, t_R (*S*) 49.61 min.

(*S*)-1,2-Epoxy-3-(2-metohxyphenoxy)propane (1f): Yield 28%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.73 (dd, J = 2.7 and 4.9, 1H, CH_2), 2.87 (m, 1H, CH_2), 3.34–3.40 (m, 1H, CH), 3.85 (s, 3H, OCH_3), 4.02 (dd, J = 5.4 and 11.4 Hz, 1H, CH_2), 4.22 (dd, J = 3.6 and 11.4 Hz 1H, CH_2), 6.85–6.97 (m, 5H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 44.4, 49.8, 55.5, 70.1, 112.0, 114.5, 120.7, 121.8, 148.1, 149.7; enantiomeric excess 28%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 8.67 min, t_R (*S*) 9.05 min.

(*R*)-3-(2′-Methoxyphenoxy)propane-1,2-diol (2f): Yield 30%; viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.49–3.59 (bs, 2H, OH), 3.68–3.74 (m, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.97–4.15 (m, 3H, CH_2, CH), 6.80–6.89 (m, 4H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 55.7, 63.8, 70.3, 71.7, 111.9, 114.5, 121.2, 121.9, 148.2, 149.6; enantiomeric excess 64%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 27.5 min, t_R (*S*) 31.2 min.

(*S*)-1,2-Epoxy-3-(2-nitrophenoxy)propane (1g): Yield 62%; yellow solid; m.p. 42–43 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.82 (dd, J = 2.7 and 4.95, 1H, CH_2), 2.87–2.91 (m, 1H, CH_2), 3.32–3.37 (m, 1H, CH), 4.12–4.17 (m, 1H, CH_2), 4.35 (dd, J = 3.0 and 11.2, 1H, CH_2), 7.02–7.13 (m, 2H, ArH), 7.47–7.53 (m, 1H, ArH), 7.79–7.83 (m, 1H, ArH). ^{13}C NMR (75.45 MHz, $CDCl_3$): 44.4, 49.7, 71.0, 115.5, 121.1, 125.7, 133.8, 151.9; enantiomeric excess 5%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 16.9 min, t_R (*S*) 15.88 min.

(*R*)-3-(2′-Nitrophenoxy)propane-1,2-diol (2g): Yield 38%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (bs, 1H, OH), 3.80–3.88 (m, 2H, CH_2), 4.09–4.26 (m, 3H, CH_2, CH), 7.02–7.12 (m, 2H, ArH), 7.52–7.57 (m, 1H, ArH), 7.84–7.88 (m, 1H, ArH). ^{13}C NMR (75.45 MHz, $CDCl_3$ + d_6-DMSO): 63.3, 69.8, 71.0, 114.8, 120.8, 125.8, 134.6, 139.4, 152.1; enantiomeric excess 28%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 95: 5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 30.79 min, t_R (*S*) 41.55 min.

(*S*)-1,2-Epoxy-3-(4′-nitrophenoxy)propane (1h): Yield 94%; yellow solid; m.p. 60–62 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.73 (dd, J = 2.7 and 4.8 Hz, 1H, CH_2), 2.90 (dd, J = 4.2 and 4.8 Hz, 1H, CH_2), 3.30–3.70 (m, 1H, CH), 4.00 (dd, J = 5.7 and 11.2 Hz, 1H, CH_2), 4.33 (dd, J = 3.0 and 11.1 Hz, 1H, CH_2), 6.95–7.00 (m, 2H, ArH), 8.15–8.21 (m, 2H, ArH); ^{13}C NMR (100.61 MHz, $CDCl_3$): 44.2, 49.6, 69.6, 114.8, 125.8, 142.2, 163.5; enantiomeric excess 2%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 90:10); flow rate 1.0 mL/min; λ = 218 nm; t_R (*R*) 20.26 min, t_R (*S*) 21.82 min.

(*R*)-3-(4′-Nitrophenoxy)propane-1,2-diol (2h): Yield 6%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO): δ 3.64–3.76 (m, 1H, CH_2), 4.03–4.19 (m, 3H, CH and CH_2), 4.52–4.62 (bs, 2H, OH), 7.00–7.09 (m, 2H, ArH), 8.14–8.20 (m, 2H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$ + d_6-DMSO): 62.8, 69.5, 69.6, 114.2, 125.3, 140.9, 163.6; enantiomeric excess 20%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 90:10); flow rate 1.0 mL/min; λ = 218 nm; t_R (*R*) 31.32 min, t_R (*S*) 35.78 min.

(*S*)-1,2-Epoxy-3-(3′-chlorophenoxy)propane (1j): Yield 33%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (dd, *J* = 2.7 and 5.0 Hz, 1H, CH_2), 2.86 (t, *J* = 4.5 Hz, 1H, CH_2), 3.26–3.31 (m, 1H, CH), 3.92 (dd, *J* = 5.4 and 11.0 Hz, 1H, CH_2), 4.16 (dd, *J* = 3.3 and 11.1 Hz, 1H, CH_2), 6.75–6.79 (m, 1H, ArH), 6.87–6.94 (m, 2H, ArH), 7.14–7.25 (m, 2H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 44.4, 49.7, 69.0, 113.1, 115.1, 121.4, 130.2, 135.0, 159.2; enantiomeric excess >99%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 8.71 min, t_R (*S*) 9.14 min.

(*R*)-3-(3′-Chlorolphenoxy)propane-1,2-diol (2j): Yield 67%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.03 (bs, 2H, OH), 2.92 (bs, 1H, OH), 3.72 (dd, *J* = 6.0 and 11.5, 1H, CH_2), 3.82 (dd, *J* = 3.0 and 11.4 Hz, 1H, CH_2), 3.98–4.03 (m, 2H, CH_2), 4.06–4.13 (m, 1H, CH) 6.76–6.80 (m, 1H, ArH), 6.89–6.96 (m, 2H, ArH), 7.15–7.20 (m, 1H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 63.5, 69.2, 70.3, 112.9, 115.0, 121.5, 130.3, 134.9, 159.1; enantiomeric excess 68%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 29.84 min, t_R (*S*) 38.88 min.

(*S*)-1,2-Epoxy-3-(2-chlorophenoxy)propane (1k): Yield 40%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (dd, *J* = 2.7 and 5.25 Hz, 1H, CH_2), 2.88 (dd, *J* = 4.2 and 4.95 Hz, 1H, CH_2), 3.32–3.37 (m, 1H, CH), 4.06 (dd, *J* = 5.1 and 11.1 Hz, 1H, CH_2), 4.22 (dd, *J* = 3.6 and 11.1 Hz, 1H, CH_2), 6.86–6.94 (m, 2H, ArH), 7.13–7.19 (m, 1H, ArH), 7.31–7.35 (m, 1H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 44.5, 50.0, 69.7, 114.1, 122.2, 123.1, 127.7, 130.3, 154.0; enantiomeric excess 93%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 10.93 min, t_R (*S*) 11.99 min.

(*R*)-3-(2′-Chlorophenoxy)propane-1,2-diol (2k): Yield 60%; white solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.18–3.19 (bs, 1H, OH), 3.63 (bs, 1H, OH), 3.77–3.86 (m, 2H, CH_2), 4.05–4.13 (m, 3H, CH and CH_2), 3.97 (m, 2H, CH_2), 4.03–4.10 (m, 1H, CH), 6.88–6.91 (m, 2H, ArH), 7.17–7.20 (m, 1H, ArH), 7.32–7.34 (m, 1H, ArH). ^{13}C NMR (125.75 MHz, $CDCl_3$): 63.9, 67.9, 70.5, 113.6, 121.7, 122.6, 127.6, 129.8, 153.5; enantiomeric excess 78%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 98:2); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 35.51 min t_R (*S*) 38.49 min.

(*S*)-1,2-Epoxy-3-(4′-bromophenoxy)propane (1m): Yield 33%; white solid; m.p. 55–56 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.64–2.68 (m, 1H, CH_2), 2.80–2.84 (m, 1H, CH_2), 3.23–3.27 (m, 1H, CH), 4.09–4.15 (m, 1H, CH_2), 4.09–4.15 (m, 1H, CH_2), 6.72–6.76 (m, 2H, ArH), 7.28–7.33 (m, 1H, ArH), 7.31–7.35 (m, 1H, ArH). ^{13}C NMR (100.61 MHz, $CDCl_3$): 44.5, 49.9, 69.3, 113.6, 116.7, 132.3, 157.8; enantiomeric excess >99%; determined by HPLC (Daicel Chiralpak AD-H, hexane/EtOH 99.5:0.5); flow rate 1 mL/min; λ = 228 nm; t_R (*R*) 21.9 min, t_R (*S*) 20.3 min.

(*R*)-3-(4′-Bromophenoxy)propane-1,2-diol (2m): Yield 67%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (bs, 1H, OH), 2.89 (bs, 1H, OH), 3.69 (dd, *J* = 5.1 and 11.3 Hz, 1H, CH_2), 3.80 (dd, *J* = 4.8 and 11.4 Hz, 1H, CH_2), 3.97 (m, 2H, CH_2), 4.03–4.10 (m, 1H, CH), 6.74–6.83 (m, 2H, ArH), 7.17–7.38 (m, 2H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 63.5, 69.4, 70.3, 113.5, 116.3, 130.4, 132.4, 157.5; enantiomeric excess 72%; determined by HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH 90:10); flow rate 1 mL/min, λ = 228 nm; t_R (*R*) 13.4 min, t_R (*S*) 14.8 min.

(*S*)-1,2-Epoxy-3-(phenylthio)propane (1n): Yield 30%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (dd, *J* = 2.4 and 4.9 Hz, 1H, CH_2), 2.72 (dd, *J* = 3.9 and 4.8 Hz, 1H, CH_2), 2.87–2.95 (m, 1H, CH), 3.09–3.16 (m, 2H, CH_2), 7.16–7.23 (m, 3H, ArH), 7.38–7.40 (m, 2H, ArH). ^{13}C NMR (100.61 MHz, $CDCl_3$): 31.6, 41.9, 45.7, 121.5, 123.7, 125.3, 130.4; enantiomeric excess 23%; determined by HPLC (Daicel Chiralpak OD-H, hexane/i-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 7.54 min, t_R (*S*) 7.24 min.

(*R*)-3-(Phenylthio)propane-1,2-diol (2n): Yield 70%; white solid; ^{1}H NMR (500 MHz, CD_3OD): δ 3.00 (dd, *J* = 6.86 and 13.5 Hz, 1H, CH_2), 3.10 (dd, *J* = 5.98 and 13.5 Hz, 1H, CH_2), 3.59 (dd, *J* = 5.89 and 11.3 Hz, 1H, CH_2), 3.66 (dd, *J* = 4.14 and 11.3 Hz, 1H, CH_2), 3.76–3.78 (m, 1H, CH_2), 7.15–7.18 (m, 1H, ArH), 7.25–7.28 (m, 2H, ArH), 7.36–7.38 (m, 2H, ArH); ^{13}C NMR (125.75 MHz, CD_3OD): 37.3, 65.4, 71.2, 126.6, 129.4, 129.7, 136.8; enantiomeric excess 27%; determined by HPLC (Daicel Chiralpak OD-H, hexane/i-PrOH 95:5); flow rate 1 mL/min; λ = 218 nm; t_R (*R*) 34.1 min, t_R (*S*) 31.3 min.

(*S*)-1,2-Epoxy-3-(benzyloxy)propane (1o): Yield 50%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.57 (dd, *J* = 3.0 and 5.0 Hz, 1H, CH_2), 2.75 (dd, 1H, *J* = 4.2 and 5.0 Hz, CH_2), 3.10–3.15 (m, 1H, CH), 3.41 (dd, *J* = 5.7 and 11.4 Hz, 1H, CH_2), 3.71 (dd, *J* = 3.0 and 11.4 Hz, 1H, CH_2), 4.53 (d, *J* = 12.0 Hz, 1H, CH_2), 4.59 (d, *J* = 12.0 Hz, 1H, CH_2), 7.23–7.32 (m, 5H, ArH); ^{13}C NMR (75.45 MHz, $CDCl_3$): 44.0, 50.7, 70.7, 73.2, 127.6, 128.3, 137.9; enantiomeric excess 30%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 93:7); flow rate 0.5 mL/min; λ = 218 nm; t_R (*S*), 17.93 min t_R (*R*) 19.64.

(*R*)-3-(Benzyloxy)propane-1,2-diol (2o): (*RS*)-3-(Benzyloxy) propane-1,2-diol [11] **(2o):** Yield 50%; colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.02–2.56 (bs, 2H, OH), 3.46–3.70 (m, 4H, CH_2), 3.82–3.88 (m, 1H, CH_2), 4.53 (s, 2H, CH), 7.25–7.35 (m, 5H, ArH); ^{13}C NMR (125.75 MHz, $CDCl_3$): 64.0, 70.7, 71.7, 73.5, 127.8, 127.9, 128.4, 128.5, 137.6; enantiomeric excess 40%; determined by HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH 93:7); flow rate 0.5 mL/min; λ = 218 nm; t_R (*R*) 43.29 min, t_R (*S*) 55.0 min.

3. Results and discussions

The different isolates (80) were screened for epoxide hydrolase activity using phenyl glycidyl ether (**1a**) as substrate following the protocol described in Section 2.3. Five isolates designated as MO-10, AO5, AO6, WH-15 and HA-13 were capable of carrying out the hydrolysis of **1a**. The isolates AO6 and AO5 afforded the conversion of 30% and 25%, respectively to the corresponding racemic diol. The isolates MO-10, WH-15 and HA-13 resulted in enantioselective kinetic resolution of phenyl glycidyl ether yielding (*S*)-phenyl glycidyl ether (**1a**) with an ee of 70%, 20% and >99%, respectively, and (*R*)-(phenoxy)propan-1,2-diol (**2a**) with an ee of 45%, 8% and 68%, respectively. The isolate HA-13 was chosen for further studies as it supported the highest enantioselectivity of (*S*)-phenyl glycidyl ether and (*R*)-(phenoxy)propan-1,2-diol out of these five isolates. This isolate was deposited in MTCC, IMTECH, Chandigarh (India) under the accession number 10234 and was identified as *B. alcalophilus*.

The comparison of these results with the literature reports the untransformed phenyl glycidyl ether was assigned (*S*)-absolute configuration and biohydrolysis product 3-(phenoxy)propan-1,2-diol was assigned (*R*)-absolute configuration. We were delighted to obtain these results since the (*S*)-enantiomer of phenyl glycidyl ether (**1a**) is useful for synthesis of bioactive β-blocker [18] and (*R*)-(phenoxy)propan-1,2-diol (**2a**) is an important intermediate in synthesis of various bioactive molecules [22].

In order to study the scope and limitation of this biotransformation process different substituted aryl glycidyl ether derivatives were subjected to hydrolytic kinetic resolution by the growing cells of *B. alcalophilus* MTCC10234 (Fig. 1). In all the cases the enzyme preferentially hydrolyzes (*R*)-enantiomer of racemic phenyl glycidyl ether, resulting in the hydrolytic kinetic resolution of the substrates. The untransformed aryl glycidyl ether derivatives were obtained in high enantioselectivity (up to >99%) and the diol deriva-

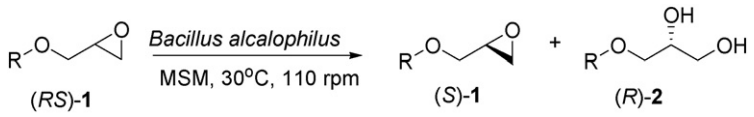


Fig. 1. *B. alcalophilus* catalyzed biohydrolysis of glycidyl ethers.

Table 2
Effect of time on biotransformation of **1c** by *B. alcalophilus* MTCC10234.

Time (h)	Yield of epoxide (%)	(*S*)-Epoxide ee (%)	Yield of diol (%)	(*R*)-Diol ee (%)
4	65	30	35	93
8	54	38	46	93
12	48	66	52	90
16	41	98	59	86
24	37	100	63	77

tives with moderate to high enantioselectivity (up to 89%) (Table 1). Further the same absolute configuration as in phenyl glycidyl ether could be assigned to other derivatives of both epoxide and 1,2-diol since the homologous series of molecules is similarly recognized by the enzyme [23].

The substituents on the aryl group affect the course of hydrolytic kinetic resolution by *B. alcalophilus* MTCC10234. Although, the methyl- and chloro-substituted aryl glycidyl ether derivatives were well recognized by the epoxide hydrolase of *B. alcalophilus* MTCC10234 but the nitro-derivatives were transformed at a slower rate. The biohydrolysis of methyl derivatives **1b**–**1d** (Table 1, Entries 2–4) occurred faster than that of chloro derivatives **1j**–**1k** (Table 1, Entries 10 and 11). A conversion of 63% was obtained in 16 h in case of **1d** where as in case of **1j** a conversion of 67% was obtained in 24 h. An enantiomeric excess of 98%, >99% and 93% for kinetically resolved epoxides **1c**–**1e** (Table 1, Entries 3–5) were obtained, respectively. In case of 3-chloro and 2-chloro-substituted derivatives **1j** and **1k** an enantiomeric excess of >99% and 93%, respectively were obtained for resolved epoxides.

2,6-Dimethylphenyl glycidyl ether (**1e**) which contains a bulky aryl group having methyl group on both the *ortho* positions was well recognized by the enzyme to provide the corresponding diol with 76% yield in 24 h (Table 1, Entry 5). The residual epoxide was observed to have an ee of 93% and the diol ee of 84%.

2-Methoxyphenyl glycidyl ether (**1f**) (Table 1, Entry 6) containing bulky OMe group at *ortho* position was biotransformed slowly. A conversion of 30% was obtained in 24 h with a low ee 28% for the residual epoxide (**1f**) and a moderate ee 64% for the diol (**2f**). 4-Bromophenyl glycidyl ether (**1m**) (Table 1, Entry 13) also reacts slowly to provide lower conversion and lower ee of epoxide. It was observed that this probably originates from the lower solubility of **1m** in the biotransformation medium which decreases its availability. A repeat biotransformation reaction of **1m** using DMSO (6% v/v) as an additive, resulted in enhancing the conversion to 67%. The enantiomeric excess of epoxide and corresponding diol was increased to >99% and 72%, respectively. The nitro-substituted aryl glycidyl ether derivatives **1g**–**1i** were found to be poor substrates for epoxide hydrolase of *B. alcalophilus* MTCC10234. A conversion of 38% and 6% was obtained in case 2-nitro- and 4-nitro-derivatives, respectively. No transformation was observed in the case of 3-nitrophenyl glycidyl ether. We thought that this may be due to low bioavailability of the substrates. The bioavailability of the substrate was increased by using DMSO (6% v/v) as additive, but no improvement in conversion and ee of epoxide and ee of diol was observed.

Further, in order to observe the effect of the ether oxygen on the biotransformation we performed the biohydrolysis of phenyl glycidyl thioether (**1n**) (Table 1, Entry 14) under similar condition, the introduction of sulphur atom resulted in faster transformation however the ee of the epoxide (ee 23%) and diol (ee 27%) were found to be low.

The selectivity of enzyme decreases significantly on introduction of the CH_2 spacer between the phenyl ring and oxirane ring. In benzyl glycidyl ether (**1o**), after 24 h the yield of remaining epoxide was 50% with an enantiomeric excess of only 30% and the yield of 3-(benzyloxy)propan-1,2-diol (**2o**) was 50% with an enantiomeric excess 40% (Table 1, Entry 15).

As the efficiency of an enzymatic kinetic resolution process can be expressed in terms of enantiomeric ratio (*E*). So *E* values have been calculated for all substrates. As shown in Table 1 substrates **1b**–**1e** and **1m** are the best substrates for the biotransformation having *E* value 31–67 while substrates **1a**, **1j** and **1k** having *E* value of 27 are moderate substrates for this biotransformation. *E* values for the **1f**–**1h**, **1l**, **1n** and **1o** are below 15, showing that these are not good substrates for biotransformation.

As 3-methylphenyl glycidyl ether (**1c**) was giving the highest value of *E*, it was chosen for another study, i.e. the variation in time of biotransformation reaction. It is clear from Table 2 that after 4 h the conversion to diol is only 35% and ee of epoxides and diols were 30% and 93%, respectively. As we increase the time of biotransformation reaction from 4 h to 24 h the conversion to corresponding diol as well as the ee of epoxide increases up to 63% and 100%, respectively and the ee of diol decreases to 77%. These results are expected in the case of enantioselective kinetic resolution. Thus by allowing the biotransformation reaction to run for different period both (*S*)-epoxide and (*R*)-diol can be obtained in high ee.

4. Conclusion

In conclusion we have developed a biocatalytic methodology using growing cells of *B. alcalophilus* MTCC10234 for procuring enantiomerically enriched glycidyl ether derivatives and their corresponding diols. The scope and limitation of this methodology has also been explored. The epoxides have been kinetically resolved with up to >99% enantiomeric excess and 1,2-diol with an ee of up to 89%. The enantiomeric ratio (*E*) of up to 67 has been obtained for biohydrolysis process which is considered to be excellent for any biocatalytic resolution process.

Acknowledgments

The authors are grateful to UGC, India for financial assistance [Grant No. F.31-121/2005(SR)] and award of senior research fellowship to NB. The authors thank IIIM-Jammu for providing NMR facility.

References

[1] S. Pedragosa-Moreau, C. Morisseau, J. Zylber, A. Archelas, J. Baratti, R. Furstoss, J. Org. Chem. 61 (1996) 7402–7407.
[2] J. Deregnaucourt, A. Archelas, F. Barbirato, J.M. Paris, R. Furstoss, Adv. Synth. Catal. 349 (2007) 1405–1417.
[3] X.J. Chen, A. Archelas, R. Furstoss, J. Org. Chem. 58 (1993) 5528–5532.
[4] S. Pedragosa-Moreau, A. Archelas, R. Furstoss, Tetrahedron 52 (1996) 4593–4606.
[5] M. Cleij, A. Archelas, R. Furstoss, J. Org. Chem. 64 (1999) 5029–5035;
(f) W.J. Choi, E.C. Huh, H.J. Park, E.Y. Lee, C.Y. Choi, Biotechnol. Tech. 12 (1998) 225–228.
[6] Z. Liu, J. Michel, Z. Wang, B. Witholt, Z. Li, Tetrahedron: Asymmetry 17 (2006) 47–52.
[7] S. Hwang, C.Y. Choi, E.Y. Lee, Biotechnol. Lett. 30 (2008) 1219–1225.
[8] Y.F. Tang, J.H. Xu, Q. Ye, B. Schulze, J. Mol. Catal. B: Enz. 13 (2001) 61–68.
[9] M. Mischitz, W. Kroutil, U. Wandel, K. Faber, Tetrahedron: Asymmetry 6 (1995) 1261–1271.
[10] R.V.A. Orru, S.F. Mayer, W. Kroutii, K. Faber, Tetrahedron 54 (1998) 859–874.
[11] R.V.A. Orru, S.F. Mayer, W. Kroutii, K. Faber, Tetrahedron Lett. 38 (1997) 1753–1754.
[12] J.H.L. Spelberg, R. Rink, R.M. Kellogg, D.B. Janssen, Tetrahedron: Asymmetry 9 (1998) 459–466.
[13] C.A.G.M. Weijers, Tetrahedron: Asymmetry 8 (1997) 639–647.
[14] C.A.G.M. Weijers, A.L. Botes, M.S.V. Dyk, J.A.M. De. Bont, Tetrahedron: Asymmetry 9 (1998) 467–473.
[15] Y. Xu, J.H. Xu, J. Pan, Y.F. Tang, Biotechnol. Lett. 26 (2004) 1217–1221.
[16] Y. Xu, J.H. Xu, J. Pan, L. Zhao, S.L. Zhang, J. Mol. Catal. B: Enz. 27 (2004) 155–159.
[17] A. Kamal, Y. Damayanthi, M.V. Rao, Tetrahedron: Asymmetry 3 (1992) 1361–1364.
[18] M. Kloosterman, V.H.M. Elferink, J.V. lersel, J.H. Roskam, E.M. Meijer, L.A. Hulshof, R.A. Sheldon, Tibtech 6 (1988) 251–255.
[19] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294–7299.
[20] B.K. Pchelka, A. Loupy, A. Petit, Tetrahedron 62 (2006) 10968–10979.
[21] S. Takano, Y. Sekiguchi, M. Setoh, T. Yoshimitsu, K. Inomata, M. Takahashi, K. Ogasawara, Hetrocycle 31 (1990) 1715–1719.
[22] S. Wu, J. Shen, X. Zhou, J. Chen, Appl. Microbiol. Biotechnol. 76 (2007) 1281–1287.
[23] S.S. Chimni, S. Singh, S. Kumar, S. Mahajan, Tetrahedron: Asymmetry 13 (2002) 511–517.