Tetrahedron Letters 52 (2011) 5355–5358

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Lipase-mediated kinetic resolution of (*RS*)-1-bromo-3-[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol to (*R*)-1-bromo-3-(4-(2-methoxyethyl) phenoxy) propan-2-yl acetate

Abhishek Kaler [a], Vachan Singh Meena [a], Manpreet Singh [a], Brahmam Pujala [b], Asit K. Chakraborti [b], Uttam Chand Banerjee [a,*]

[a] Biocatalysis Laboratory, Department of Pharmaceutical Technology, National Institute of Pharmaceutical Education and Research, Sector-67, S. A. S. Nagar, Punjab 160062, India
[b] Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Sector-67, S. A. S. Nagar, Punjab 160062, India

ARTICLE INFO

Article history:
Received 6 July 2011
Revised 2 August 2011
Accepted 4 August 2011
Available online 11 August 2011

Keywords:
Candida rugosa lipase
Metoprolol
Transesterification
Intermediates

ABSTRACT

A novel biocatalytic method for the enantioselective synthesis of (*R*)-bromo-3-[4-(2-methoxy-ethyl) phenoxy]-2-propanol [(*R*)-BMEPP], a precursor for the synthesis of (*S*)-metoprolol, an anti hypertensive drug is described. We have developed kinetic resolution of *rac*-BMEPP by transesterification using *Candida rugosa* lipase and vinyl acetate as the acyl donor affording the product with excellent conversion (49%) and ee (>99%). Various reaction parameters (source of enzyme, reaction media, and concentration of substrate and acylating agent) for the enzymatic kinetic resolution have been reported.

Metoprolol (1-isopropylamino-3-[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol), an antihypertensive drug belongs to the class of β-blockers having a preferential effect on cardiac β_1-adrenoreceptors.[1–3] Studies indicated on metoprolol have revealed the eudismic ratio (ER) of the order of 270 in favor of the (*S*)-enantiomer.[4] Therefore, it is highly imperative to administer this drug in optically pure form.

A retro-synthetic approach reveals that optically pure (*S*)-metoprolol can be synthesized by (*R*)-1-bromo-3-[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol (BMEPP) as the chiral drug intermediate. The existing methods for the chemical synthesis of metoprolol have been reported in literature.[5–9] However, these chemical methods suffer from disadvantages, such as higher cost, hazardous reaction conditions, formation of side products, and poor enantioselectivity. Biocatalysts, on the other hand, exhibit better regio- and stereoselectivity, due to which they are increasingly exploited to produce complex molecules of industrial interest.[10–13] Lipases are known to bring about a range of biocatalytic conversions by different reaction mechanisms.[14–17] Role of lipase as mediator of kinetic resolution of number of racemates to enantiopure products is well established.[18–20] Lipase mediated resolution of various drugs and drug intermediates has been successfully carried out in our laboratory.[21–25] These findings encourage us to use lipase as a tool for the enantioselective synthesis of metoprolol intermediate.

The present study demonstrates a novel approach for the synthesis of enantiopure (*R*)-BMEPP from 2-[4-(2-methoxy-ethyl)-phenoxymethyl]-oxirane. The process involves the chemical synthesis of *rac*-BMEPP from oxirane and the subsequent kinetic resolution using lipase-mediated transesterification. The various parameters for the transesterification reaction have been further optimized to achieve better enantio-selectivity with higher conversion.

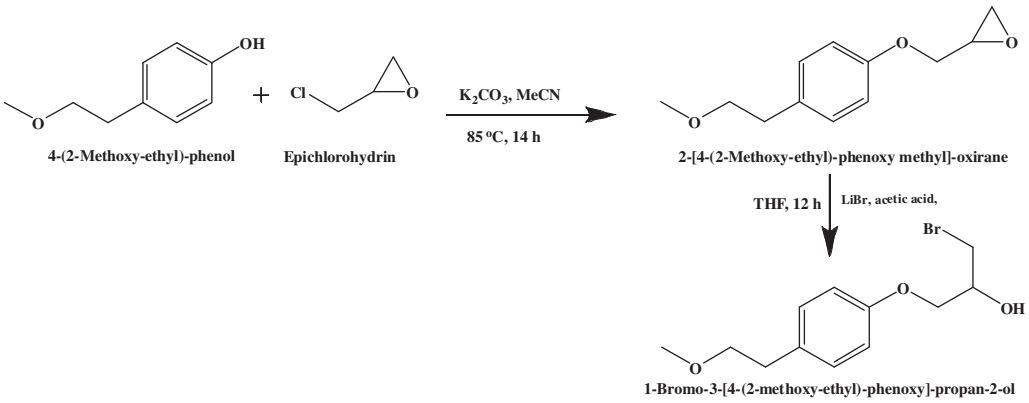
The synthesis of *rac*-BMEPP was achieved in a two-step reaction (Scheme 1). The reaction sequence includes the treatment of 4-(2-methoxyethyl)-phenol with epichlorhydrine under basic conditions to afford the product 2-[4-(2-methoxy-ethyl)-phenoxy-methyl]-oxirane.[26,27] Subsequently epoxide ring was opened by lithium bromide to obtain *rac*-BMEPP.[28,29] Our next objective was to achieve the kinetic resolution of *rac*-BMEPP using different lipases and acetylating agent in various organic solvents.

As a model reaction transesterification of *rac*-BMEPP was tried with lipase from *Pseudomonas aeruginosa* in organic solvent (Scheme 2). Good conversion up to 27% with 97% ee prompted us to screen lipases from commercial sources such as *Candida rugosa* and porcine pancreatic lipase to catalyze the reaction.[30]

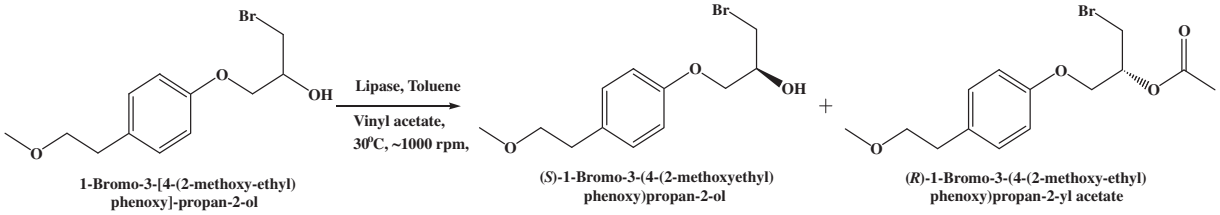
Modified form of the Winkler and Stuckmann method was applied for the quantification of lipase activity.[31] The enzyme activity of the crude preparation of *P. aeruginosa* lipase was 854 U mg^{-1}

* Corresponding author. Tel.: +91 172 2214682 85; fax: +91 172 2214692
E-mail address: ucbanerjee@niper.ac.in (U.C. Banerjee).

0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.08.031



Scheme 1. Synthesis of 1-bromo-3-[4-(2-methoxy-ethyl)- phenoxy]-propan-2-ol via epoxide ring opening.



Scheme 2. Lipase catalyzed enantioselective transesterification of (*RS*)-1-bromo-3-[4- (2-methoxy-ethyl)-phenoxy]-propan-2-ol.

much higher than the enzyme activities of *C. rugosa* (520 U mg^{-1}) and porcine pancreas (325 U mg^{-1}). The reactions were carried out at identical values of the enzyme activity in the reactor. At the end of the reaction, *C. rugosa* lipase-catalyzed reaction had attained a *rac*-BMEPP conversion of nearly 34% (ee ~98%) compared to the conversion of 27% (ee ~97%) obtained with *P. aeruginosa* lipase (Table 1). The porcine pancreatic lipase did not show any conversion. During transesterification reaction, vinyl acetate is known to release acetaldehyde, which in turn deactivates the enzyme.[32,33]

Lipases are highly stable in organic solvents like *n*-dodecane, 1-pentanol, and toluene[34] and retention of more than 80% lipase activity in hexane, toluene, and petroleum ether has already been reported.[35] Hence we tried to study the effect of different organic solvents such as toluene, hexane, cyclohexane etc. on the reaction outcome. It is evident from Table 2 that the best results were obtained using toluene as the solvent affording better conversion and enantiomeric excess as compared to other solvents.

Further to achieve maximum conversion and enantiomeric excess of (*R*)-1-bromo-3-[4-(2-methoxyethyl) phenoxy] propan-2-yl acetate, the amount of the enzyme required to catalyze the reaction was optimized. The concentration of *C. rugosa* lipase was varied (5200–26000 U) while keeping the concentrations of the substrate and vinyl acetate constant (Fig. 1). It was observed that 30 mg *C. rugosa* lipases (15600 U) in the reaction mixture showed maximum conversion (40%). The conversion did not increase with further increase in the enzyme concentration. This may be due to the increased viscosity of the reaction mixture at higher enzyme concentration, which reduced the collision between the enzyme and the substrate.

Table 1
Effect of different sources of enzymes on the transesterification reaction

Source	Conversion (%)	ee (%)
C. rugosa	34	98
P. aeruginosa	27	97
Porcine pancreas	Nil	Nil

Table 2
Effect of solvent on the lipase-catalyzed transesterification of *rac*-BMEPP

Solvents	Conversion (%)	ee (%)
Toluene	34	99
Hexane	5	53
Cyclohexane	10	48
Octane	ND	ND
Tetrahydrofuran	ND	ND
Dioxane	ND	ND
Ethyl acetate	7.5	42

ND: Not detectable.

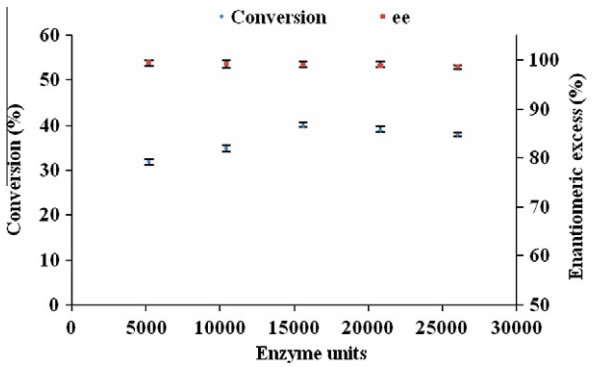


Figure 1. Effect of enzyme concentration on the transesterification of *rac*-BMEPP using *C. rugosa* lipase.

To study the time course, the reaction was allowed to proceed for 36 h and the samples were withdrawn every 6 h and subjected to HPLC analysis.[36] It is evident from the Figure 2 that the conversion of alcohol to ester increased as the reaction proceeded, on the other hand enantiomeric excess decreased with time as commonly observed in such biotransformation. The best conversion (42%) and enantiomeric excess (>99%) were achieved after 24 h.

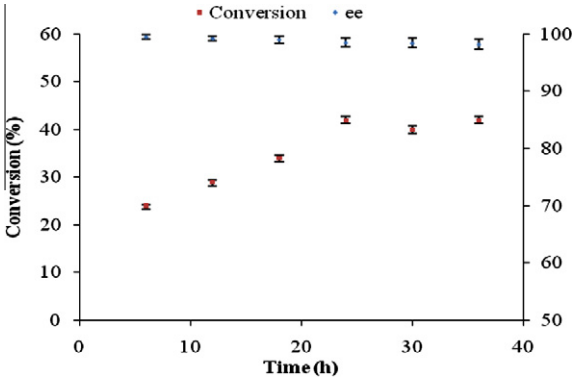


Figure 2. Effect of time-course on the transesterification of *rac*-BMEPP by *C. rugosa* lipase.

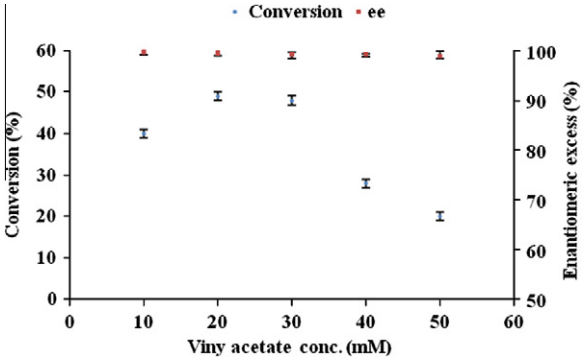


Figure 4. Effect of vinyl acetate concentration on transesterification of *rac*-BMEPP by *C. rugosa* lipase.

The effect of substrate concentration was monitored by varying the amount of *rac*-BMEPP (5–25 mM) in the transesterification reaction catalyzed by *C. rugosa* lipase, keeping the other components of the reaction mixture constant. It was observed that highest conversion (46%) was obtained when the initial concentration of *rac*-BMEPP was 15 mM. Increasing substrate concentration beyond 15 mM decreased the conversion (Fig. 3). The enzyme might have experienced substrate inhibition or this could be due to the formation of the dead-end inhibition complex between lipase and *rac*-BMEPP.

Two acyl donating vinyl esters (vinyl acetate and butyrate) were compared for the transesterification of *rac*-BMEPP. The results showed that vinyl acetate gave excellent conversion (48%) and enantiomeric excess (>99%) compared to vinyl butyrate where both the conversion (30%) and enantiomeric excess (96%) were less. In order to optimize the concentration of acyl donor, reactions were carried out at various concentrations of vinyl acetate (10–50 mM) while keeping the other parameters constant. There was an increase in the conversion (49%) with an increase in vinyl acetate upto 20 mM and then decreased with further increase in its concentration (Fig. 4).

An efficient and practical synthesis method of 1-bromo-3-[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol and their successful enzymatic kinetic resolution using lipase has been demonstrated. The enantiomerically pure isomer may be used for the synthesis of biologically important compounds like metoprolol, a selective β_1-blocker. The reaction conditions have been optimized to provide an economical and greener method for obtaining the enantiopure (*R*)-BMEPP with excellent conversion and enantioselectivity. Stereoselective transesterification of *rac*-BMEPP has been successfully carried out using lipase from *C. rugosa* in this study.

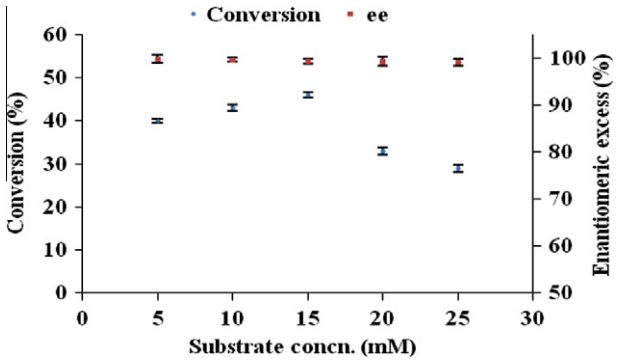


Figure 3. Effect of substrate concentration on the transesterification of *rac*-BMEPP by *C. rugosa* lipase.

Acknowledgments

A.K. and V.S.M. thank the Department of Biotechnology and B.P. thanks the Council of Scientific and Industrial Research, Govt. of India for senior research fellowships.

References and notes

1. Bodor, N.; El-Koussi, A. A.; Kano, M.; Khalifa, M. M. *J. Med. Chem.* **1988**, *31*, 1651–1656.
2. Teerlink, J. R.; Massie, B. M. *Am. J. Cardiol.* **1999**, *84*, 94–102.
3. Liese, A.; Filho, M. V. *Curr. Opin. Biotech.* **1999**, *10*, 595–603.
4. Mostafavi, S. A.; Foster, R. T. *Int. J. Pharm.* **2000**, *202*, 97–102.
5. Di Giuseppe, B.; Antonio, S. *Synthesis.* **1995**, *1995*, 699–702.
6. Manoury, P. M.; Binet, J. L.; Rousseau, J.; Lefevre-Borg, F. M.; Cavero, I. G. *J. Med. Chem.* **1987**, *30*, 1003–1011.
7. Rao, A. V. R.; Gurjar, M. K.; Joshi, S. V. *Tetrahedron: Asymmetry* **1990**, *1*, 697–698.
8. Shetty, H. U.; Nelson, W. L. *J. Med. Chem.* **1988**, *31*, 55–59.
9. Takahashi, H.; Sakuraba, S.; Takeda, H.; Achiwa, K. *J. Am. Chem. Soc.* **1990**, *112*, 5876–5878.
10. Rozzell, J. D. *Bioorg. Med. Chem.* **1999**, *7*, 2253–2261.
11. D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. *Tetrahedron: Asymmetry* **2010**, *21*, 914–918.
12. Pollard, D. J.; Woodley, J. M. *Trends Biotechnol.* **2006**, *25*, 66–73.
13. Patel, R. N. *Curr. Opin. Drug Discov. Devel.* **2003**, *6*, 902–920.
14. Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature* **2001**, *409*, 258–268.
15. Haki, G. D.; Rakshit, S. K. *Bioresour. Technol.* **2003**, *89*, 17–34.
16. Jaeger, K. E.; Ransac, S.; Dijkstra, B. W.; Colson, C.; van Heuvel, M.; Misset, O. *FEMS Microbiol. Rev.* **1994**, *15*, 29–63.
17. Pandey, A.; Benjamin, S.; Soccol, C. R.; Nigam, P.; Krieger, N.; Soccol, V. T. *Biotechnol. Appl. Biochem.* **1999**, *29*, 119–131.
18. Di Nunno, L.; Franchini, C.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Tetrahedron: Asymmetry* **2000**, *11*, 1571–1583.
19. Wünsche, K.; Schwaneberg, U.; Bornscheuer, U. T.; Meyer, H. H. *Tetrahedron: Asymmetry* **1996**, *7*, 2017–2022.
20. Pamies, O.; Backvall, J.-E. *J. Org. Chem.* **2001**, *66*, 4022–4025.
21. Singh, M.; Banerjee, U. C. *Tetrahedron: Asymmetry* **2007**, *18*, 2079–2085.
22. Singh, M.; Singh, R. S.; Banerjee, U. C. *J. Mol. Cat. B: Enzymat.* **2009**, *56*, 294–299.
23. Singh, M.; Singh, S.; Singh, R. S.; Chisti, Y.; Banerjee, U. C. *Bioresour. Technol.* **2008**, *99*, 2116–2120.
24. Singh, M.; Singh, R. S.; Banerjee, U. C. *Process Biochem.* **2010**, *45*(1), 25–29.
25. Banoth, L.; Singh, M.; Tekewe, A.; Banerjee, U. C. *Biocatal. Biotransfor.* **2009**, *27*(4), 263–270.
26. Shivani; Pujala, B.; Chakraborti, A. K. *J. Org. Chem.* **2007**, *72*, 3713–3722.
27. Synthesis of 2-[4-(2-methoxy-ethyl)-phenoxymethyl]-oxirane was carried out using 4-(2-methoxy-ethyl)-phenol, (1.0 g, 3.8 mM), epichlorohydrin (0.52 g, 5.7 mM) and potassium carbonate (1.05 g, 7.6 mM) in acetonitrile (10 ml). The reaction was allowed to proceed for 14 h, under reflux. Acetonitrile was used for work-up and the reaction mixture was purified by silica-gel (60-120 mesh) column chromatography with ethyl acetate/hexane (1:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.72–2.90 (m, 4H), 3.31 (m, 1H), 3.34 (s, 3H), 3.56 (t, 2H, J = 7.2 Hz), 3.94 (dd, 1H, J = 5.6 Hz), 4.18 (dd, 1H, J = 3.2 Hz), 6.85 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.4 Hz).
28. Bajwa, J. S.; Anderson, R. C. *Tetrahedron Lett.* **1991**, *32*, 3021–3024.
29. (*RS*) 1-Bromo-3-[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol (*rac*-BMEPP) was synthesized by treating 2-[4-(2- methoxy-ethyl)-phenoxymethyl]-oxirane (1.56 g, 7.5 mM) with lithium bromide (7.5 mM) in acetic acid (1.35 g, 22.5 mM) and THF (10 ml) at room temperature for12 h. The reaction mixture was diluted with ethyl acetate (15 ml), washed with water, dried over Na_2SO_4, and was concentrated under vacuum to afford (*RS*)-1-bromo-3-

[4-(2-methoxy-ethyl)-phenoxy]-propan-2-ol.^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04 (s, 1H), 2.82 (t, 2H; J = 6.98), 3.35 (s, 3H), 3.54–3.68 (m, 4H), 4.02–4.11 (m, 2H), 4.15–4.20 (m, 1H), 6.85 (d, 2H; J = 8.4) 7.15 (d, 2H; J = 8.4) , APCI MS:209.2 $(M-79)^+$

30. Enzymatic transesterification of *rac*-BMEPP. Crude preparation of *P. aeruginosa* lipase, commercial lipase from *C. rugosa* and porcine pancreas were used for the transesterification of *rac*-BMEPP. The reaction was carried out in triplicate using appropriate quantity of lipase in 10 ml stoppard flask containing substrate *rac*-BMEPP (15 mM) and vinyl acetate (20 mM) dissolved in toluene (5 ml). Reaction mixture was magnetically stirred (~1000 rpm) at 30 °C for 36 h with periodic sampling (6 h). Conversion and enantiomeric excess of the substrate and product were analyzed by Chiral HPLC. Product (*R*-BMEPP-ester) was separated from the reaction mixture and authenticated by NMR. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.12 (s, 3H), 2.82 (t, 2H, J = 7 Hz), 3.34 (s, 3H), 3.56 (t, 2H, J = 7 Hz), 3.62–3.70 (m, 2H), 4.10–4.19 (m, 2H), 5.29 (m, 1H), 6.85 (d, 2H, J = 8.6 Hz), 7.14 (d, 2 H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.9, 30.4, 35.2, 58.6, 66.8, 70.7, 73.7, 114.5, 129.8, 131.9, 156.6, 170.1.
31. Winkler, U. K.; Stuckmann, M. *J. Bacteriol.* **1979**, *138*, 663–670.
32. Castaing, M. D.; Jeso, B. D.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953–954.
33. Wang, Y. F.; Lalonade, J. J.; Momongan, M.; Bergbretter, D. E.; Wong, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.
34. Rahman, R.; Baharum, S. N.; Basri, M.; Salleh, A. B. *Anal. Biochem.* **2005**, *341*, 267–274.
35. Singh, S.; Banerjee, U. C. *J. Mol. Cat. B: Enzymat.* **2005**, *36*, 30–35.
36. High-performance liquid chromatography (HPLC) was performed using Shimadzu 10AVP instrument equipped with an UV detector on a Chiralcel ODH column (0.46 mm × 250 mm, 5 μm, Chiralcel). Elution was done with hexane-isopropyl alcohol at 90:10 (v/v) and at a flow rate of 0.5 ml/min. The conversion and enantiomeric excess (ee%) were quantified at 220 nm. The retention time of (*S*)-ester and (*R*)-ester were 12.5 and 15.32 min, respectively and (*S*)- and (*R*)- alcohol enantiomers were eluted at 25.7 and 29.0 min, respectively. The remaining substrate and the product formed were authenticated by ^{1}H and ^{13}C NMR spectra recorded on a Bruker Advance DPX 300 MHz NMR spectrometer.