

Chiral discrimination by hydrolytic enzymes in the synthesis of optically pure materials

VIRINDER S PARMAR*, KIRPAL S BISHT, AMARJIT SINGH and AMITABH JHA

Department of Chemistry, University of Delhi, Delhi 110 007, India

Abstract. Enantioselection effected by commercially available hydrolytic enzymes reported in recent years from our own laboratory and those published by other groups is described. The discussion is confined to enantioselection in symmetric diols, amino acids and benzopyran derivatives only. The paper describes a variety of substrates accepted by these hydrolytic enzymes to produce compounds in high enantiomeric excess which can be used as chiral building blocks for the synthesis of compounds of pharmaceutical and synthetic interest.

Keywords. Biocatalysis; biotransformation; enzymes; lipases; diols; amino acids; benzopyran derivatives.

1. Introduction

The application of enzymes in organic synthesis is one of the most useful and practical methods for the preparation of optically active compounds in high optical purities (Boland *et al* 1991; Drucekhammer *et al* 1991). Various hydrolytic enzymes, such as pig liver esterase (PLE) (Ohno and Otsuka 1989), porcine pancreatic lipase (PPL) (Hultin *et al* 1991). *Pseudomonas fluorescens* lipase (PFL) (Xie 1991) etc. have been reported by several groups and the active site models of these enzymes were tentatively proposed in an attempt to explain their role in the discrimination of the enantiotopic ester groups of symmetric compounds and the resolution of racemic compounds. A chemical differentiation between two enantiotopic groups in prochiral compounds by enzymes has always been regarded as a major achievement due to obvious reasons, i.e.

- (a) theoretically 100% of the substrate can produce one enantiomer of the product,
- (b) the reaction process in the biotransformation of a *meso* compound involves enantioselective conversion and an inherent kinetic resolution, which enhances the enantioselectivity of the process,
- (c) the resulting product is bifunctional and can be elaborated synthetically at both the ends.

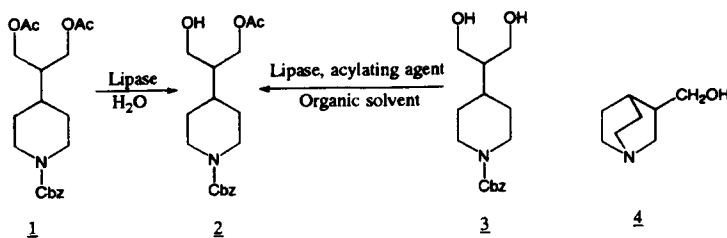
Optically active polyols, and natural and unnatural α -amino acids are important precursors for the synthesis of pharmaceuticals, agro-chemicals and food ingredients (Kleeman *et al* 1985). They have also been amply utilized in synthetic studies as chiral auxiliaries, reagents and catalysts for asymmetric synthesis. Because of their widespread use, intensive research efforts have been directed towards the development of new and

*For correspondence

improved stereoselective synthesis of α -amino acids (Williams 1989). Of particular interest are catalytic methods that offer a high degree of enantioselective control. Here, we intend to give some representative examples of lipase-catalysed reactions, including some of our own findings where enzymes have been used for kinetic resolution of the *racemic* and prochiral substrates to yield enantiomerically pure amino acids and other compounds of synthetic interest.

2. Construction of bifunctional chiral building blocks from symmetric diols

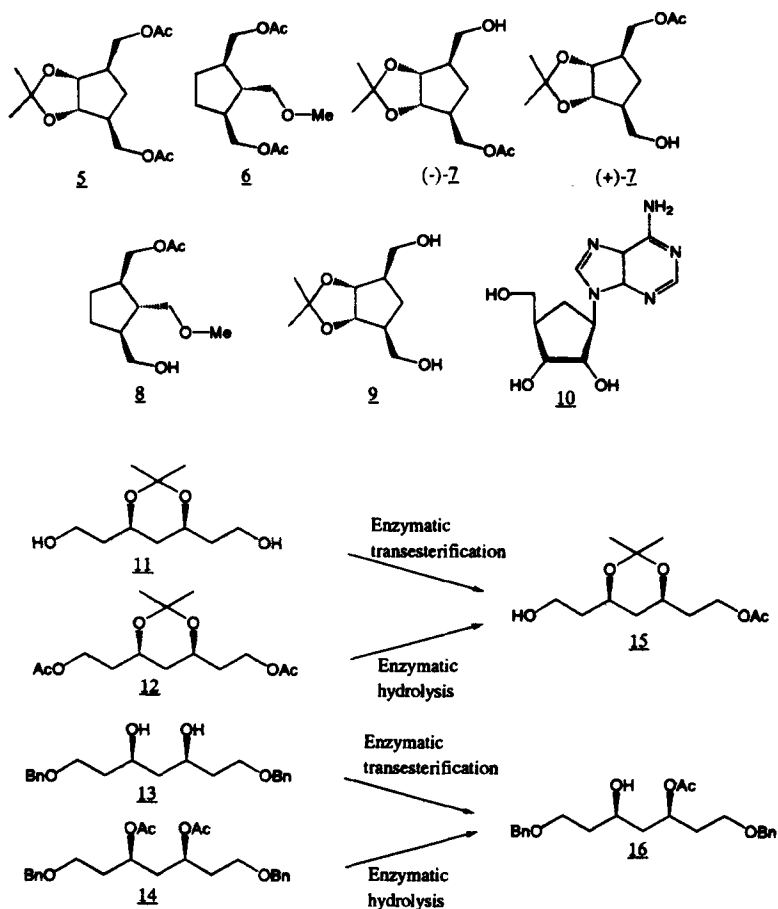
Enantiomerically pure molecules containing a nitrogen-heterocycle are useful units that can be used as starting materials in the synthesis of alkaloids and many other important biologically active targets (Guerrier *et al* 1983; Kawanami *et al* 1984; Fuji *et al* 1990; Guanti *et al* 1990; Nieduzak and Carr 1990; Chenevert and Dickman 1992; Wirz and Walther 1992; Jefford and Wang 1993). Unfortunately their number is rather limited and continuous efforts have been currently made to improve the so-called "heterocycle chiral pool" (Hanessian 1983). Both enantiomers of the monoacetate **2** have been prepared in high *ee* by lipase-catalysed monohydrolysis of the prochiral *meso* diacetate **1** and by monoacetylation of the corresponding *meso* diol **3**. The 3-hydroxymethyl-quinuclidine (**4**) has been synthesized in both the enantiomeric forms with an *ee* up to 98% starting from (+) and (-) **2** (Guanti *et al* 1994), the compound **2** is of great interest as it is an important intermediate in the synthesis of mequitazine and other 3-substituted quinuclidine derived drugs (MacLeod *et al* 1990; Wadsworth *et al* 1992; Clark *et al* 1993).



Rhizopus delemar lipase (RDL)-catalysed hydrolyses of *meso*-1, 3-bis(acetoxymethyl) cyclopentane derivatives **5** and **6** have afforded the chiral monoacetates (-) **7** and **8** respectively in > 99% *ee* (Tanaka *et al* 1993). On the other hand, PFL-catalysed transesterification of *meso*-bis(hydroxymethyl) cyclopentane (**9**) has been shown to give (+) **7** in > 99% *ee*. The (-) **7** and (+) **7** thus obtained have been converted into the natural carbocyclic nucleoside (-) aristeromycin (**10**).

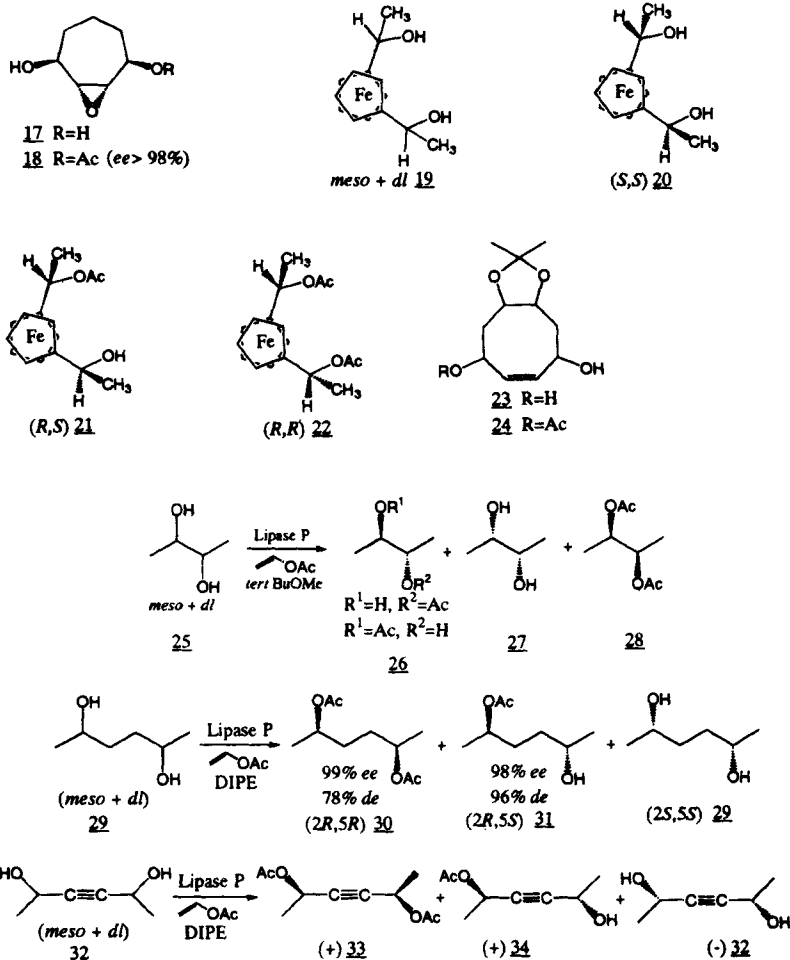
Enzymatic desymmetrization of *meso* compounds **11**, **12**, **13** and **14** have been carried out to form corresponding monoacetyl compounds **15** and **16** (Bomini *et al* 1993). Out of the five enzymes screened [*Candida cylinderacea* lipase (CCL); *Mucor javanica* lipase; PLE; PPL and PFL], PFL has been found to be the most selective enzyme with excellent results with the tested substrates. The monoacetylated compounds **15** and **16**, thus obtained have been used as chiral building blocks in the syntheses of several natural products.

The chemoenzymatic synthesis of enantiomerically pure epoxy-alcohol **18** has been carried out in high chemical yield and excellent enantioselectivity from the corresponding epoxy diol **17** utilizing *Pseudomonas cepacia* lipase (PCL) in organic and aqueous



media (Bis *et al* 1993). Reduction of 1,1'-diacetyl ferrocene with lithium aluminium hydride affords 1,1'-bis(α -hydroxyethyl) ferrocene (19) as an isomeric mixture in which *meso* and *racemic* forms are present in about equal amounts and these can be separated by a combination of repeated recrystallizations from hexane and column chromatography (Yamakawa and Hisatome 1973); PCL has been successfully utilized to convert the whole of the *meso*-form of the compound 19 to the optically active (*R, S*)-form 21; the remaining *dl* mixture then gets converted into the (*S, S*) 20 and (*R, R*) 22 (Lambusta *et al* 1993). Together with the resolution of the enantiomers, desymmetrization of the *meso* form affords an additional optically active compound which may be suitable for further manipulations to give potentially useful chiral compounds. *Meso*-diol 23, derived from 1, 5-cyclooctadiene in the presence of PCL in isoprenyl acetate has been converted to 24, an attractive intermediate useful in the synthesis of sugars and related compounds (Johnson *et al* 1994).

We have carried out reactions by using lipase from *Pseudomonas fluorescens* (Lipase P) which exhibits a high degree of diastereo- and enantioselectivity in the transesterification of *meso* and *dl* mixtures of butane-2,3-diol (25). The reaction yields monoacetates of *meso*-diol 26, and the diacetate of (*2R, 3R*) diol 28. The (*R, R*) diacetate 28 thus obtained has the *ee* > 98% (Bisht *et al* 1993). In the acetylation of *meso*- and *dl*-2,



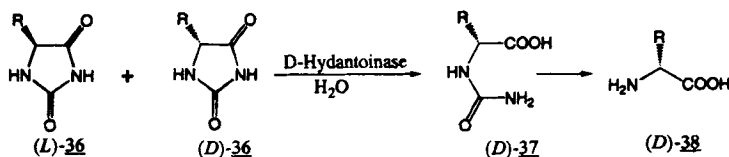
5-hexanediol (29) with Lipase P and vinyl acetate in diisopropyl ether also, the enzyme exhibited high degree of enantio- and diastereoselectivity, 3-hexyne-2,5-diol (32) under similar conditions afforded (+)-diacetate 33 , (+)-monoacetate 34 and (-)-diol 32 (unpublished results from our laboratory).

3. Synthesis of optically pure amino acids

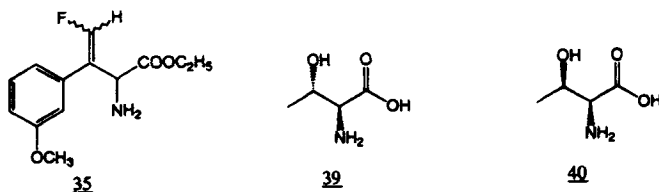
α,α -Disubstituted α -amino acids have attracted increasing attention in recent years. This group of non-proteinogenic amino acids induces dramatic conformational change when incorporated into peptides (Barone *et al* 1985; Paul *et al* 1986). α -Methyl α -amino acids have been studied the most for biological activity; α,α -dialkyl amino acids with both side-chains larger than methyl are also of significant interest because of their steric properties, some of them have been found to induce dramatic structural changes in peptides (Benedetti *et al* 1984; Crisma *et al* 1991). Hence their synthesis in optically pure form using an efficient and convenient method has become mandatory for chemists.

Humicola amino esterase (minor protein component isolated from crude *Humicola langinosa* lipase) has been used for the resolution of substrates like aliphatic, aromatic and cyclic amino acid esters into optically active amino esters and acids with high enantioselectivity (Liu *et al* 1995). The (*Z*) and (*E*) isomers of ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenolate (**35**) have been prepared and the *R* and *S* enantiomers of the biologically active (*E*)-isomer have been separated with an enantiomeric excess > 95% by the kinetic resolution of the *racemic* mixture using the enzyme α -chymotrypsin (Lacan *et al* 1995). The facile isomerisation of the double bond has permitted the isolation of the enantiomers of (*Z*)-ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenolate from the corresponding (*E*)-isomers. The *R* and *S* enantiomers of (*E*)-**35** have been analysed by single crystal X-ray crystallography.

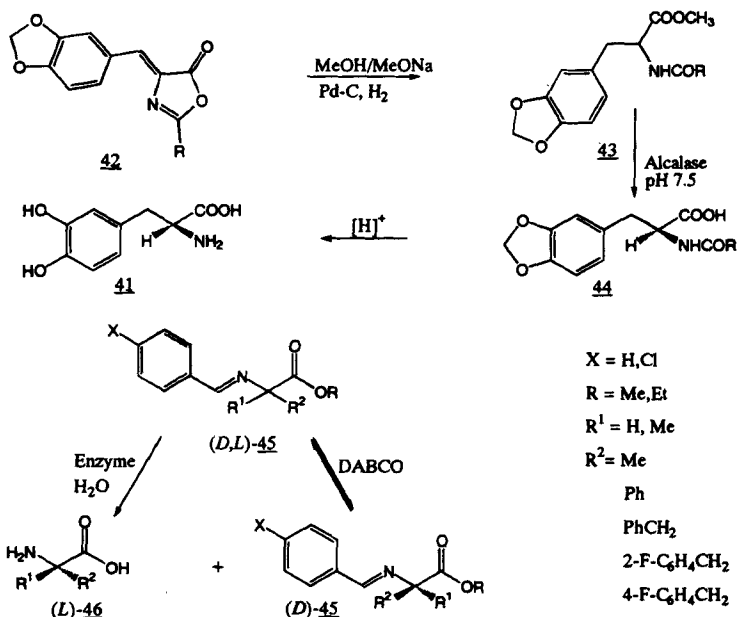
Enantiomerically pure D- α -amino acids are important building blocks for a variety of biologically active pharmaceuticals like peptides (Kamphuis *et al* 1992), semisynthetic β -lactam antibiotics (Durckheimer *et al* 1985) and ACE inhibitors (Patchett *et al* 1980). Enantioselective hydrolytic ring opening of 5-monosubstituted imidazolidin-2,4-diones (**36**) has been carried out to form D-amino acids by using D-specific hydantoinases from thermophilic microorganisms, the N-carbomoyl-D-amino acids (**37**) thus produced have been transformed chemically or enzymatically into the corresponding D-amino acids (**38**) (Keil *et al* 1995).



L-Threonine aldolase from *Candida humicola* has been used for the synthesis of multifunctional β -hydroxy- α -amino acids. L-Threonine aldolase catalyses the condensation of glycine and acetaldehyde to form a mixture of *L-allo*-threonine (**39**) and L-threonine (**40**) in 93:7 ratio (Vassilev *et al* 1995).



We have carried out the synthesis of several optically active α -amino acids, including that of L-DOPA (**41**) in high optical purity (98%) via Erlenmeyer's azalactone method using alcalase in the hydrolytic step (Tyagi *et al* 1992). The azalactone **42** is converted to (*R, S*)-N-acetyl-3,4-methylenedioxyphenylalanine methyl ester (**43**), which on enzymatic resolution by alcalase (subtilisin Carlsberg) yielded (*S*)-N-acetyl-3,4-methylenedioxyphenylalanine (**44**), and this on subsequent acid treatment afforded L-DOPA (**41**). In another novel approach, we have done the enzymatic hydrolysis of *racemic* Schiff's bases (**45**) in aqueous organic solvents using α -chymotrypsin, PPL, CCL and amano 'P' lipase to form α -amino acids (**46**) corresponding to L-ester in good yield (42-87%) and high optical purity (Parmar *et al* 1996). The addition of an organic

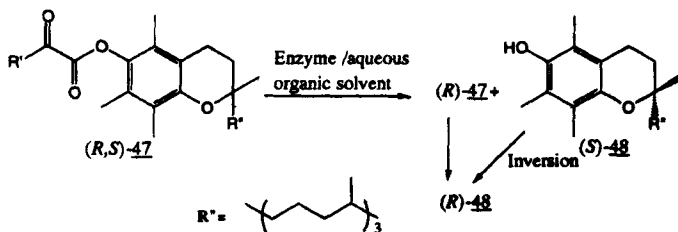


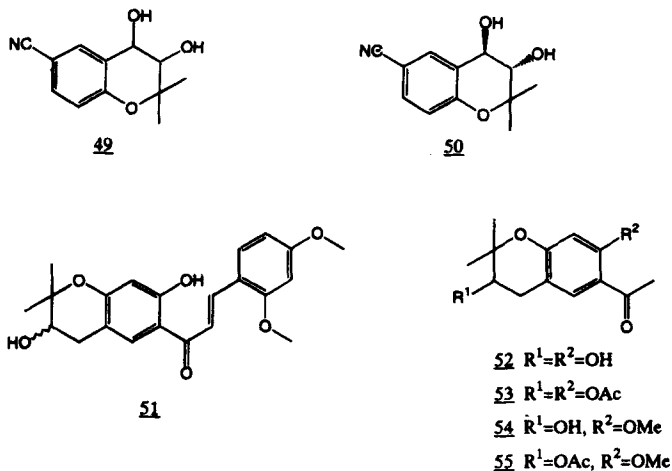
base (DABCO) into the solution resulted in the racemization of the remaining D-ester and the additional hydrolysis of the substrate, thus leading to the effective asymmetric transformation of the initial ester; upto 87.5% of the initial racemate has been converted into the L-enantiomer.

4. Preparation of biologically important optically active benzopyran derivatives

α -Tocopherol (48) has been known as a potent, safe and lipid-soluble antioxidant. Novel resolution of (*RS*)- α -tocopherol oxalate (47) has been carried out using *Candida rugosa* lipase and CCL in aqueous organic solvents to form (*R*)-47 and (*S*)-48, which have been successfully converted to *d*- α -tocopherol (*R*-48) (Mizuguchi and Achiwa 1993).

Stereoselective acetylation of *racemic* 49 has been carried out with both CCL and PCL to yield monoacetylated product and unreacted (3*S*, 4*R*)-*trans*-3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (50) in > 40% yield and > 90% optical purity (Patel *et al* 1995). CCL, *Pseudomonas* species lipase, lipozyme *Humicola lanuginosa*, *Penicillium camemberti* and *Geotrichum candidum* have been screened for the acetylation of *racemic* 4-hydroxychromans. Out of them, CCL has been found to be active and highly enantioselective (Majeric *et al* 1995).





The *racemic* dimethoxycinnamoylbenzopyran **51** has shown good anti-invasive activity against human breast carcinoma cells at test concentrations of 100 and 10 μ M but shows cytotoxic effect as well (Parmar *et al* 1994). It is reasonable to believe that one enantiomer is responsible for the biological activity in question while the other causes cytotoxicity. We then undertook to resolve the starting acetophenone **52** using lipases. The kinetic acetylation of **52** and deacetylation of **53** using PPL and CCL failed to yield the required products. We then methylated **52** to yield **54**, which on acetylation catalysed by CCL in toluene yielded **55** in 40% yield having $[\alpha]_D^{24} = -43.6^\circ$ (unpublished results from our laboratory), the determination of its precise *ee* by chromatographic analysis is being carried out. Further studies to get other optically pure analogues of **55** and **51** are in progress.

5. Conclusions

From examples of the endeavours cited in the text for enantioselection in organic synthesis using enzymes, it is obvious that enzymes are highly efficient and versatile reagents. To exploit their use further, we need to explore many more potential enzymes and microorganisms. Looking at the quantum of work being put in by fellow colleagues around the world in this field, we are sure that this will be realised soon and that enzymes will become a major component in organic synthesis in the preparation of useful optically pure materials, both in the laboratory and in the industry.

Acknowledgement

KSB and AS thank the Council of Scientific and Industrial Research, New Delhi and AJ, the University Grants Commission, New Delhi for fellowships.

References

- Barone V, Lelj F, Bavoso A, Di Blasio B, Grimaldi P, Pavone V and Pedone C 1985 *Biopolymers* **24** 1759
- Benedetti E, Toniolo C, Hardy P, Barone V, Bavoso A, Di Blasio B, Grimaldi P, Lelj F, Pavone V, Pedone C, Bonora G M and Lingham I 1984 *J. Am. Chem. Soc.* **106** 8146

- Bis S J, Whitaker D T and Johnson C R 1993 *Tetrahedron: Asymmetry* **4** 875
- Bisht K S, Parmar V S and Crout D H G 1993 *Tetrahedron: Asymmetry* **4** 957
- Boland W, Froßl C and Lorenz M 1991 *Synthesis* 1049
- Bonini C, Racioppi R, Viggiani L, Righi G and Rossi L L 1993 *Tetrahedron: Asymmetry* **4** 793
- Chenevert R and Dickman M 1992 *Tetrahedron: Asymmetry* **3** 1021
- Clark R D, Miller A B, Berger J, Repke D B, Weinhardt K K, Kowalczyk B A, Eglén R M, Bonhaus D W, Lee C H, Michel A D, Smith W L and Wong E H F 1993 *J. Med. Chem.* **36** 2645
- Crisma M, Valle G, Bonora G M, Toniolo C, Lelj F, Barone V, Fraternali F, Hardy P M and Maia H L S 1991 *Biopolymers* **31** 637
- Drueckhammer D G, Hennen W J, Pederson R L, Barbas III C F, Gautheron C M, Krach T and Wong C H 1991 *Synthesis* 499
- Durckheimer W, Blumbach J, Lattrell R and Scheunemann K H 1985 *Angew. Chem., Int. Ed. Engl.* **24** 180
- Fuji K, Kawabata T, Kiryu Y, Sugiura Y, Taga T and Miwa Y 1990 *Tetrahedron Lett.* **31** 6663
- Guanti G, Narisano E, Podgorski T, Thea S and Williams A 1990 *Tetrahedron* **46** 7081
- Guanti G, Banfi L, Brusco S and Narisano E 1994 *Tetrahedron: Asymmetry* **5** 537
- Guerrier L, Royer J, Grierson D S and Husson H P 1983 *J. Am. Chem. Soc.* **105** 7754
- Hanessian S 1983 In *Total synthesis of natural products: The 'Chiron' approach* (Oxford: Pergamon)
- Hultin P G, Mueseler F J and Jones J B 1991 *J. Org. Chem.* **56** 5375
- Jefford C W and Wang J B 1993 *Tetrahedron Lett.* **34** 2911
- Johnson C R, Harikrishnan L S and Golebiowski A 1994 *Tetrahedron Lett.* **35** 7735
- Kamphuis J, Boesten W H J, Kaptein B, Hermes H F M, Sonke T, Broxtermann Q B, van den Tweel W J J and Schoemaker H E 1992 In *Chirality in industry* (eds) A N Collins, G N Sheldrake and J Crosby (New York: Wiley and Sons) p. 187
- Kawanami Y, Ito Y, Kitagawa T, Taniguchi Y, Katsuki T and Yamaguchi M 1984 *Tetrahedron Lett.* **25** 857
- Keil O, Schneider M P and Rasor J P 1995 *Tetrahedron: Asymmetry* **6** 1257
- Kleeman A, Leuchtenberger W, Hoppe B, Tanner H 1985 In *Ullmann's encyclopedia of industrial chemistry* (Weinheim: VCH) **A2** 57
- Lacan G, Satyamurthy N and Barrio J R 1995 *Tetrahedron: Asymmetry* **6** 525
- Lambusta D, Nicolosi G, Patti A and Piattelli M 1993 *Tetrahedron: Asymmetry* **4** 919
- Liu W, Ray P and Benezra S A 1995 *J. Chem. Soc., Perkin Trans. I* 553
- MacLeod A M, Baker R, Freedman S B, Patel S, Merchant K J, Roe M and Saunders J 1990 *J. Med. Chem.* **33** 2052
- Majeric M, Gelo-Pujic M, Sunjic V, Levai A, Sebok P and Timar T 1995 *Tetrahedron: Asymmetry* **6** 937
- Mizuguchi E and Achiwa K 1993 *Tetrahedron: Asymmetry* **4** 2303
- Nieduzak T R and Carr A A 1990 *Tetrahedron: Asymmetry* **1** 535
- Ohno M and Otsuka M 1989 *Org. React.* **37** 1
- Parmar V S, Jain R, Sharma S K, Vardhan A, Jha A, Taneja P, Singh S, Vyncke B M, Bracke M E and Mareel M M 1994 *J. Pharm. Sci.* **83** 1217
- Parmar V S, Singh A, Bisht K S, Kumar N, Belokon Y N, Kochetkov K A, Ikonnikov N S, Orlova S A, Tararov V I and Saveleva T F 1996 *J. Org. Chem.* **61** 1223
- Patchett A A, Harris E, Tristram E W, Wyvrath M J, Wu M T, Taub D, Peterson E R, Ikeler T J, ten Broeke J, Payne L G, Ondeyka D L, Thorsett E D, Greenlee W J, Lahr N S, Hoffsommer R D, Joshua H, Ruyle W V, Rothrock J W, Aster S D, Maycock A L, Robinson F M, Hirschmann R, Sweet C S, Ulm E H, Gross D M, Vassil T C and Stone C A 1980 *Nature (London)* **288** 280
- Patel R N, Banerjee A, McNamee C G and Szarka L J 1995 *Tetrahedron: Asymmetry* **6** 123
- Paul P K C, Sukumar M, Bardi R, Piazzesi A M, Valle G, Toniolo C and Balaram P 1986 *J. Am. Chem. Soc.* **108** 6363
- Tanaka M, Yoshioka M and Sakai K 1993 *Tetrahedron: Asymmetry* **4** 981
- Tyagi O D, Boll P M, Parmar V S, Taneja P and Singh S K 1992 *Indian J. Chem.* **B31** 851
- Vassilev V P, Uchiyama T, Kajimoto T and Wong C H 1995 *Tetrahedron Lett.* **36** 4081
- Wadsworth H J, Jenkins S M, Orlek B S, Cassidy F, Clark M S G, Brown F, Riley G J, Graves D, Hawkins J and Naylor C B 1992 *J. Med. Chem.* **35** 1280

- Williams R M 1989 In *Synthesis of optically-active α -amino acids* (Oxford: Pergamon) p. 257
Wirz B and Walther W 1992 *Tetrahedron: Asymmetry* **3** 1049
Xie Z F 1991 *Tetrahedron: Asymmetry* **2** 733
Yamakawa K and Hisatome M 1973 *J. Organomet. Chem.* **52** 407