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review

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## Biocatalytic Synthesis of Chiral Pharmaceutical Intermediates

*Ramesh N. Patel*

Process R &amp; D, Bristol-Myers Squibb, New Brunswick, NJ 08903, USA

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### Summary

The production of single enantiomers of drug intermediates has become increasingly important in the pharmaceutical industry. Chiral intermediates and fine chemicals are in high demand from both the pharmaceutical and agrochemical industries for the preparation of bulk drug substances and agricultural products. The enormous potential of microorganisms and enzymes for the transformation of synthetic chemicals with high chemo-, regio- and enantioselectivities has been demonstrated. In this article, biocatalytic processes are described for the synthesis of chiral pharmaceutical intermediates.

*Key words:* biocatalysis, chiral intermediates, antiviral, anticancer, antihypertensive, anti-infective, receptor agonists/antagonists, enantioselective enzymatic de-protection

### Introduction

The production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceutical industry (1). Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. The advantage of biocatalysis over chemical synthesis is that enzyme-catalyzed reactions are often highly enantioselective and regioselective. They can be carried out at ambient temperature and atmospheric pressure, thus avoiding the use of more extreme conditions that can cause problems with isomerization, racemization, epimerization and rearrangement. Microbial cells and enzymes derived from them can be immobilized and reused for many cycles, and enzymes can be over-expressed to make biocatalytic processes economically efficient. A number of review articles (2–8) on the use of enzymes in organic synthesis have been published. The current review provides examples of the use of enzymes for the synthesis of single enantiomers of key intermediates used in the preparation of pharmaceuticals.

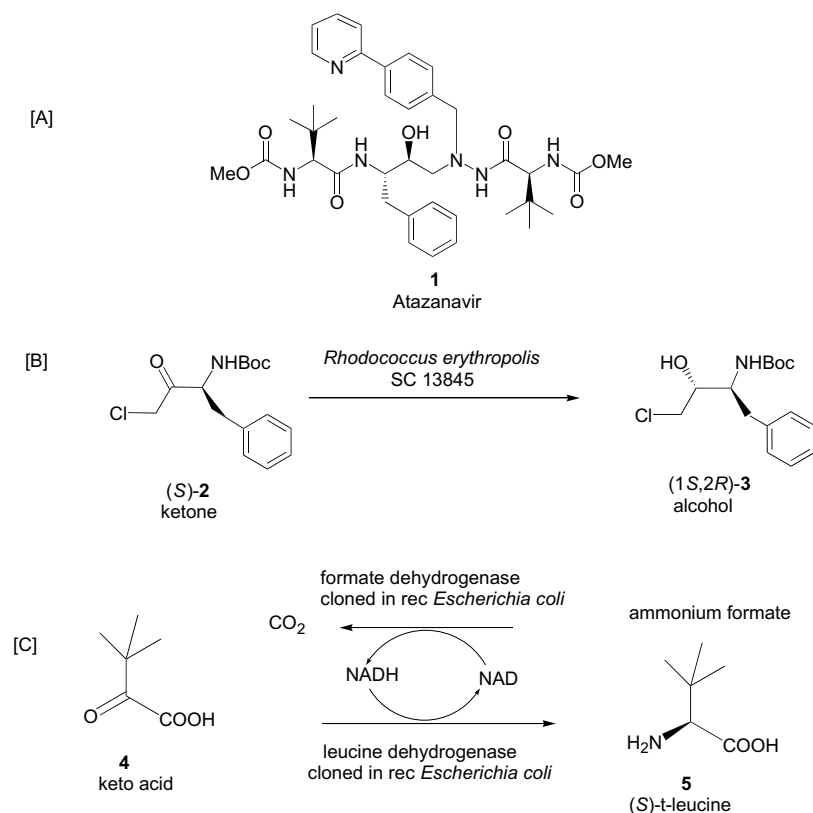
### Antiviral Agents

#### *Atazanavir*

An essential step in the life cycle of human immunodeficiency virus (HIV-1) is the proteolytic processing of its precursor proteins. This processing is accomplished by HIV-1 protease, a virally encoded enzyme. Inhibition of HIV-1 protease arrests the replication of HIV *in vitro*. Thus, HIV-1 protease is an attractive target for chemotherapeutic intervention (9,10). Atazanavir **1** [Scheme 1A] is an acyclic aza-peptidomimetic that is a potent HIV protease inhibitor (11,12). The original process was utilized for small-scale preparation of Atazanavir to support initial development (13) and subsequently an improved process for the large-scale synthesis of Atazanavir was developed (14).

An alternative biocatalytic process was developed for the preparation of a key chiral intermediate required for the synthesis of Atazanavir (15). The diastereoselective microbial reduction of (1S)-[3-chloro-2-oxo-1-(phenylme-

\* Corresponding author; E-mail: ramesh.patel@bms.com



**Scheme 1.** Synthesis of chiral intermediates for antiviral agent **1**: (A) Enantioselective enzymatic reduction of (1S)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester **2** to the corresponding (1S,2R)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester **3**, has been demonstrated [Scheme 1B]. (B) Synthesis of chiral intermediates for antiviral agent **1**: enzymatic reductive amination of keto acid **4** to (S)-t-leucine **5** by leucine dehydrogenase  
Boc – t-butylcarbonyl group

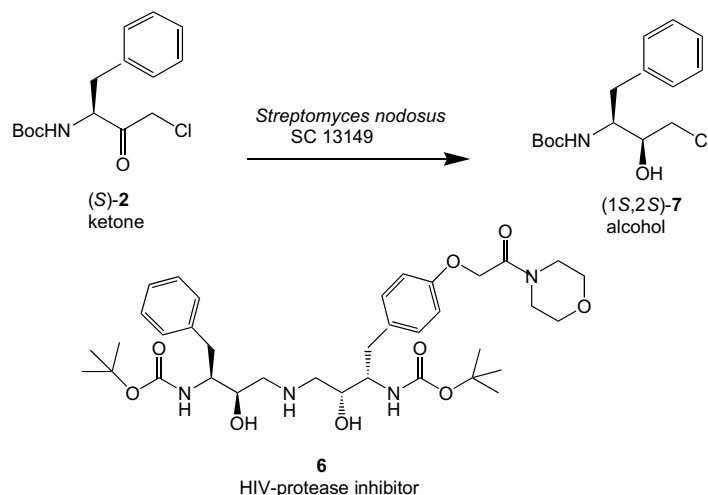
thyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester (S)-2 to homochiral chlorohydrin (1S,2R)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester (1S,2R)-3, has been demonstrated [Scheme 1B]. Among microbial cultures evaluated, *Rhodococcus*, *Brevibacterium* and *Hansenula* strains reduced (S)-2 to (1S,2R)-3. Three strains of *Rhodococcus* gave >90 % yield with a diastereomeric purity of >98 % and enantiomeric excess (e.e.) of 99.4 %. *Rhodococcus erythropolis* SC 13845 and *Rhodococcus* sp. 16002 were grown in a 25-L fermentor for 48 h. The cells were collected and suspended in 70 mM potassium phosphate buffer (pH=7.0), and the resulting cell-suspensions were used to carry out the two-stage process for biotransformation of (S)-2. A reaction yield of >92 %, diastereomeric purities of >98 % and enantiomeric excesses of 99.3–99.4 % for alcohol (1S,2R)-3 were obtained. An efficient single-stage fermentation-biotransformation process was developed for the reduction of ketone (S)-2 with cells of *Rhodococcus erythropolis* SC 13845. A reaction yield of 95 %, diastereomeric purity of 98.2 % and enantiomeric excess of 99.4 % for (1S,2R)-3 were obtained.

Synthesis of Atazanavir also required the (S)-tertiary leucine **5** [Scheme 1C]. An enzymatic process was developed for the synthesis **5**. Enzymatic reductive amination of keto acid **4** to amino acid **5** by leucine dehydrogenase from *Thermoactinomyces intermedius* has been demonstrated. Leucine dehydrogenase from *T. interme-*

*dus* was cloned and over-expressed in *Escherichia coli*. The reaction required ammonia and NADH as a cofactor. NAD produced during the reaction was regenerated back to NADH using formate dehydrogenase from *Pichia pastoris*. Formate dehydrogenase from *P. pastoris* was also cloned and over-expressed in *Escherichia coli*. A reaction yield of >95 % and enantiomeric excess of >99.5 % were obtained for **5** at 100 g/L of substrate input (R. Hanson, S. Goldberg, R. Patel, unpublished results).

#### BMS-186318

Barrish *et al.* (16) reported the discovery of a new class of selective HIV protease inhibitors that incorporate a C-2 symmetric aminodiol core as a key structural feature. Members of this class, particularly compound **6** [Scheme 2], display potent anti-HIV activity in cell culture. The diastereoselective microbial reduction of (1S)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester (S)-2 to (1S,2S)-[3-chloro-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester (1S,2S)-7, a key intermediate in the total chemical synthesis of BMS-186318, has been demonstrated (17). Among 100 microorganisms screened for the reduction, the two best cultures, *Streptomyces nodosus* SC 13149 and *Mortierella ramanniana* SC 13850, were used to convert (S)-2 to (1S,2S)-7 in 60–65 % reaction yields, 99.9 % e.e.'s and >99 % diastereomeric purities. An efficient single-stage fermentation-biotransformation



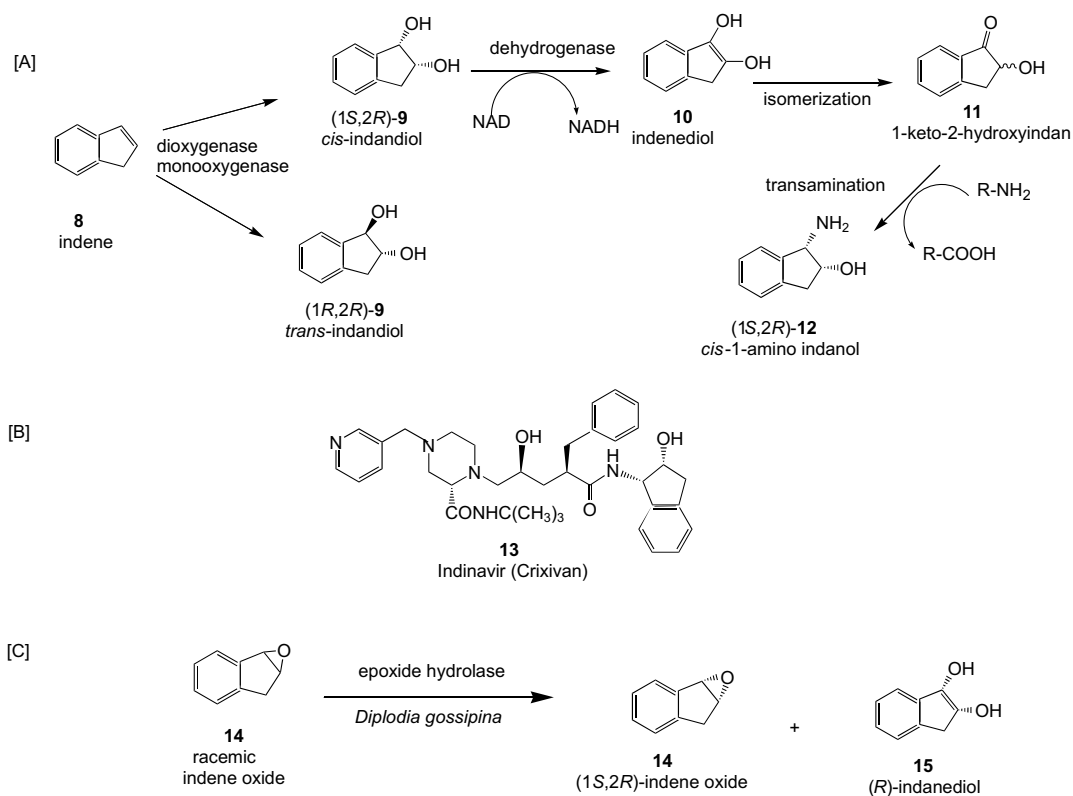
**Scheme 2.** Synthesis of chiral intermediates for HIV-protease inhibitor **6**: enantioselective enzymatic reduction of (1S)-[3-chloro-2-oxo-1(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester **2** to the corresponding (1S,2S) **7** by *Streptomyces nodosus* SC 13149  
Boc – t-butylcarbonyl group

process was developed using cells of *S. nodosus* SC 13149. A reaction yield of 80 %, a diastereomeric purity of >99 % and an e.e. of 99.8 % were obtained for (1S,2S)-**7** at 10 g/L of substrate input.

#### Indinavir

*Cis*-(1S,2R)-indandiol **9** or *trans*-(1R,2R)-indandiol **9** [Scheme 3A] are both potential precursors to *cis*-(1S,2R)-1-aminoindan-2-ol **12**, a key chiral synthon for Cri-

xivan (Indinavir) **13** [Scheme 3B], an HIV protease inhibitor. Enrichment and isolation of microbial cultures yielded two strains, *Rhodococcus* sp. B 264-1 (MB 5655) and I-24 (MA 7205), capable of biotransforming indene **8** to *cis*-(1S,2R)-indandiol and *trans*-(1R,2R)-indandiol respectively (**18**). Isolate MB 5655 was found to have a toluene dioxygenase, while isolate MA 7205 was found to harbor both toluene and naphthalene dioxygenases as well as a naphthalene monooxygenase that catalyzes the



**Scheme 3.** Synthesis of chiral intermediates for Crixivan **13**: (A) Microbial oxygenation of indene **8** to *cis*-indandiol **9** and *trans*-indandiol **9**. (C) Resolution of racemic indene oxide **14** to (1S,2R)-indene oxide **14** by epoxide hydrolase from *Diplodia gossipina*

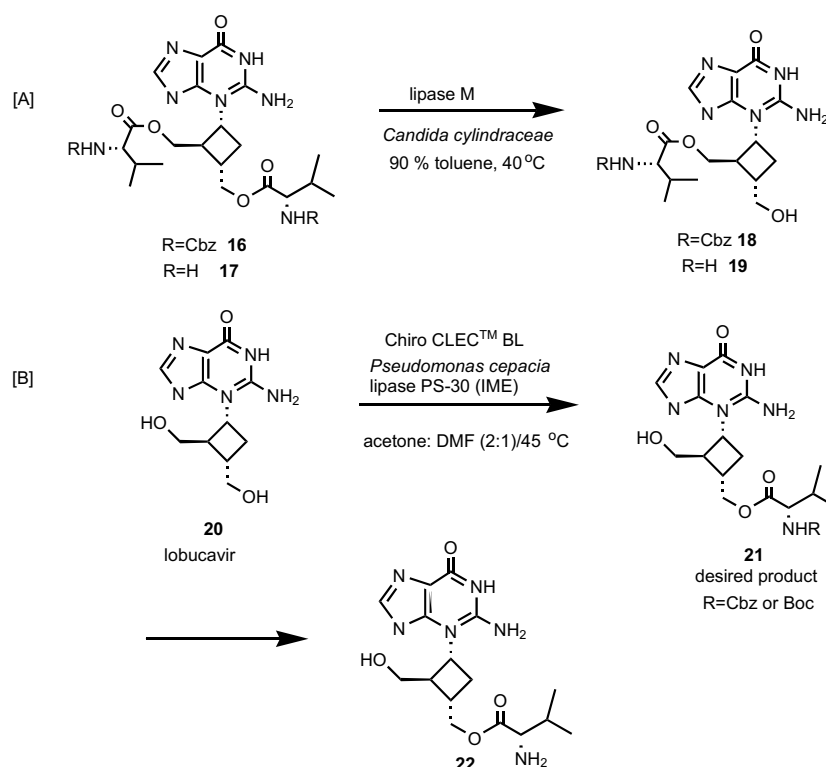
above biotransformation. When scaled up in a 14-L fermentor, MB5655 produced up to 2.0 g/L of *cis*-(1*S*,2*R*)-indandiol **9** with e.e. >99%. *Rhodococcus* sp. MA 7205 cultivated under similar conditions produced up to 1.4 g/L of *trans*-(1*R*,2*R*)-indandiol **9** with e.e. >98%. Process development studies yielded titers of greater than 4.0 g/L of *trans*-(1*R*,2*R*)-indandiol **9** (19). A metabolic engineering approach (20) and a directed evolution technique (21) were evaluated to avoid side reactions, block degradative pathways and enhance the key reaction to convert indene to *cis*-amino indanol or *cis*-indandiol.

Multi-parameter flow cytometry was employed for the measurement of indene toxicity to the strain. Concentrations of indene up to 0.25 g/L (0.037 g (indene)/g (dry cell)) in batch bioconversions did not influence cell physiology. Using this information, a single-phase indene fed-batch bioconversion was carried out. Cytoplasmic membrane integrity and membrane polarization of a large number of cells were measured during bioconversions and compared to a control in order to assess any toxic effects of indene feeding. Indene supply at a rate of 0.1 g L<sup>-1</sup>h<sup>-1</sup> was feasible without any deleterious effects. *Cis*-(1*S*,2*R*)-indandiol **9** production rates were enhanced from 20 up to 200 mg L<sup>-1</sup>h<sup>-1</sup>, in a silicone oil two-liquid-phase system, by a combination of suitable indene feeding rates in the stationary phase and operating with a high biomass concentration (22).

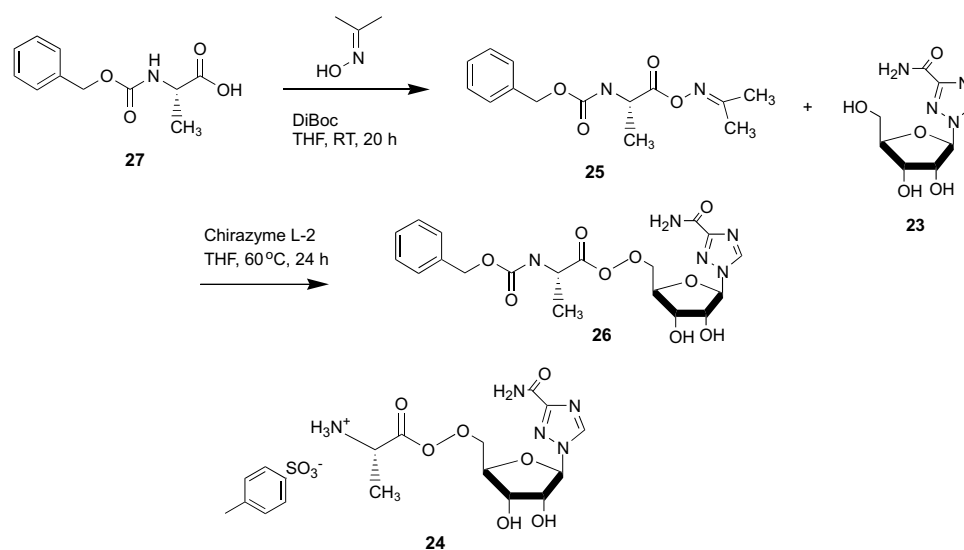
In an alternate process, an epoxide hydrolase from *Diplodia gossipina* was used for the resolution of racemic indene oxide **14** [Scheme 3C]. The desired enantiomer (1*S*,2*R*)-indene oxide **14**, a chiral intermediate for Crixivan, was obtained in 14% yield and >99.9% e.e.

### Lobucavir

Lobucavir **20** [Scheme 4] is a cyclobutyl guanine nucleoside analog that is potentially useful as an antiviral agent for the treatment of herpes virus and hepatitis B (23). The lobucavir pro-drug L-valine [1*S*,2*R*,3*R*]-3-(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)-2-(hydroxymethyl)cyclobutylmethyl ester monohydrochloride **22**, in which one of the two hydroxyls is coupled to valine, has also been considered for development. Regioselective aminoacylation was difficult to achieve by chemical procedures but appeared to be suitable for an enzymatic approach (24). Synthesis of the lobucavir pro-drug **22** required regioselective coupling of one of the two hydroxyl groups of lobucavir with valine. Enzymatic processes were developed for the aminoacylation of either hydroxyl group of lobucavir (24). The selective hydrolysis of *N,N'*-bis[(phenylmethoxy)carbonyl]bis[L-valine], *O,O'*-[(1*S*,2*R*,3*R*)-3-(2-amino-6-oxo-1H-purin-9-yl)cyclobutan-1,2-diyl]methyl ester **16** [Scheme 4] was accomplished with lipase M (Amano Enzyme Company) to yield **18** in 83% yield. When bis[*valine*],*O,O'*-[(1*S*,2*R*,3*R*)-3-(2-amino-6-oxo-1H-purin-9-yl)cyclobutan-1,2-diyl]methyl ester **17** dihydrochloride was hydrolysed with lipase from *Candida cylindraceae*, **19** was obtained in 87% yield. Compound **21**, the final intermediate for the lobucavir pro-drug **22**, could be obtained by transesterification of lobucavir using ChiroCLEC™ BL from Altus Company (61% yield) or more selectively by using the lipase from *Pseudomonas cepacia* immobilized on Accurell polypropylene (84% yield).



**Scheme 4.** Synthesis of chiral intermediates for the lobucavir pro-drug **22**: (A) Regioselective enzymatic hydrolysis of **16** and **17**. (B) Regioselective enzymatic aminoacylation of lobucavir **20**  
Cbz – carbobenzyloxy group



**Scheme 5.** Synthesis of chiral intermediates ribavirin pro-drug **24**: regioselective enzymatic acylation of ribavirin **23** with oxime ester of L-carbobenzyloxy(cbz)-alanine **25** to give desired product **26** using Novozym 435

### Ribavirin pro-drug

Ribavirin **23** [Scheme 5] is an antiviral agent used in combination with  $\alpha$ -2 $\beta$  interferon to treat hepatitis C (25–27). Although this therapy is effective against hepatitis C virus, it has several side effects (28,29). To improve the pharmacokinetics profile and reduce side effects, ribavirin pro-drug was considered for development. In a series of preclinical evaluations, the alanine ester of ribavirin **24** [Scheme 5] showed improved bioavailability and reduced side effects. The synthesis of **24** required the acylation of unprotected ribavirin. Chemical acylation gave a mixture of mono-, di- and triacylated products. An enzymatic process was developed for the regioselective acylation of ribavirin **23** with oxime ester of L-carbobenzyloxy(cbz)-alanine **25** to give the desired **26** using Novozym 435 (*Candida antarctica* lipase B). The chemical deprotection of **26** gave **24**. In a preparative-scale process, the coupling of **27** with acetone oxime in the presence of di-*t*-butyl dicarbonate in THF was carried out giving **25** in greater than 96 % yield. At the end of the reaction, the reaction mixture was diluted three-fold with THF and ribavirin was added and the acylation was initiated by the addition of lipase. Following 24-h reaction at 60 °C, the product **26** was isolated in 85 % yield (30).

### Anti-Infective Drugs

#### Microbial hydroxylation of pleuromutilin or mutilin

Pleuromutilin **28** [Scheme 6] is an antibiotic from *Pleurotus* or *Clitopilus* basidiomycete strains that kills mainly gram-positive bacteria and mycoplasmas. A more active semi-synthetic analogue, tiamulin, has been developed for the treatment of infections of animals and poultry and has been shown to bind to prokaryotic ribosomes and inhibit protein synthesis (31). Metabolism of pleuromutilin derivatives results in hydroxylation by microsomal cytochrome P-450 at the 2- or 8-position and inactivates the antibiotics (32). Modification of the 8-po-

sition of pleuromutilin and analogues is of interest as a mean of preventing the metabolic hydroxylation. Microbial hydroxylation of pleuromutilin **28** or mutilin **30** would provide a functional group at this position to allow further modification at this site to avoid metabolic hydroxylation. The target analogues would maintain the biological activity of the parent compounds but would not be susceptible to metabolic inactivation.

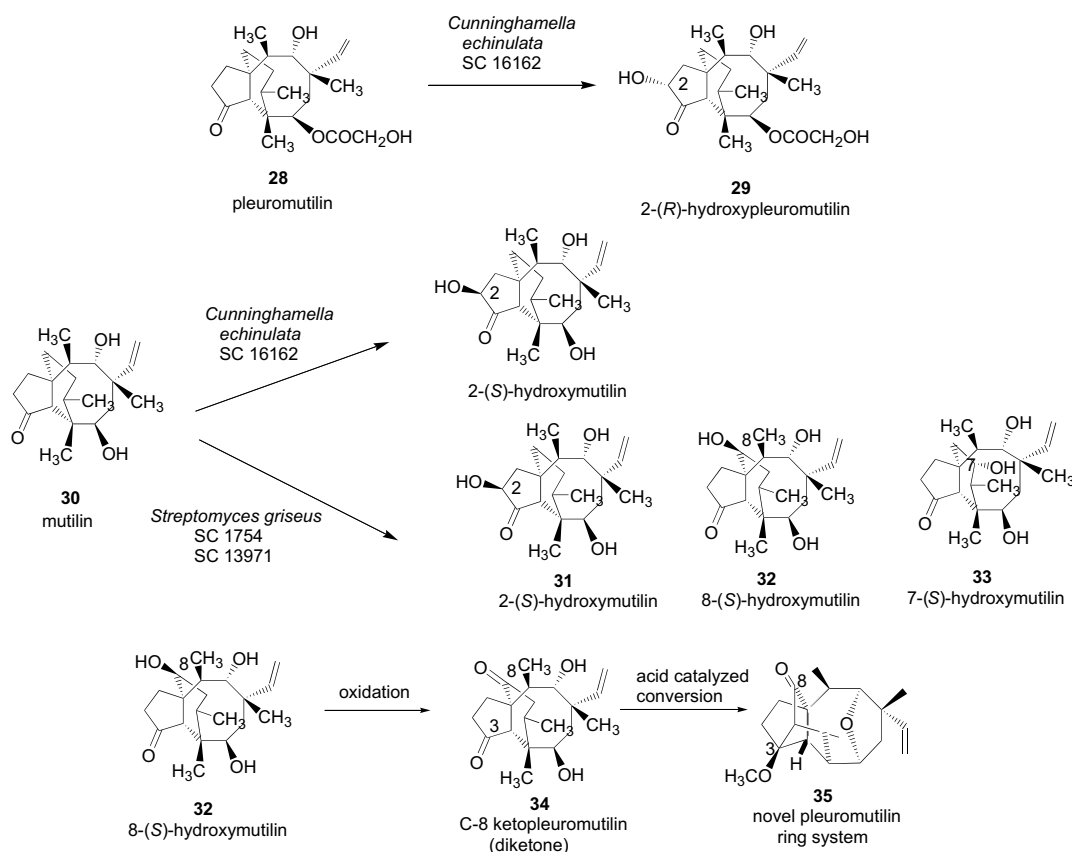
Biotransformation of mutilin and pleuromutilin by microbial cultures has been investigated to provide a source of 8-hydroxymutilin or 8-hydroxypleuromutilin (33). *Streptomyces griseus* strains SC 1754 and SC 13971 (ATCC 13273) hydroxylated mutilin to (2*S*)-hydroxymutilin **31**, (8*S*)-hydroxymutilin **32** and (7*S*)-hydroxymutilin **33** [Scheme 6]. *Cunninghamella echinulata* SC 16162 (NRRL 3655) gave (2*S*)-hydroxymutilin **31** or (2*R*)-hydroxypleuromutilin **29** from biotransformation of mutilin or pleuromutilin, respectively. The biotransformation of mutilin by the *S. griseus* strain SC 1754 was scaled up in 15-, 60-, and 100-L fermentations to produce a total of 49 g of (8*S*)-hydroxymutilin (BMS-303786), 17 g of (7*S*)-hydroxymutilin (BMS-303789) and 13 g of (2*S*)-hydroxymutilin (BMS-303782) from 162 g of mutilin (33).

A C-8 ketopleuromutilin **34** derivative has been synthesized from the biotransformation product 8-(*S*)-hydroxymutilin (34). A key step in the process was the selective oxidation at C-8 of 8-hydroxymutilin using tetrapropylammonium perruthenate. The presence of the C-8 keto group gave an interesting intramolecular chemistry to afford a compound with a novel pleuromutilin-derived **35** ring system, which was achieved by acid-catalyzed conversion of C-8 ketopleuromutilin.

### Anticancer Agents

#### Paclitaxel

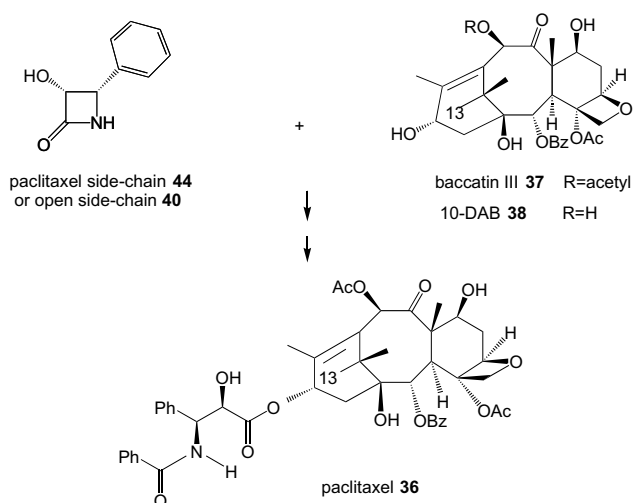
Among the antimetabolic agents, paclitaxel **36** [Scheme 7], a complex, polycyclic diterpene, exhibits a unique mode of action on microtubule proteins responsible for



**Scheme 6.** Microbial hydroxylation of pleuromutilin **28** and mutilin **30**

the formation of the spindle during cell division (35–39). Paclitaxel inhibits the depolymerization process of microtubulin (39,40). Various types of cancers have been treated with paclitaxel and the results in treatment of ovarian cancer and metastatic breast cancer are very promising. Paclitaxel was originally isolated from the bark of the yew, *Taxus brevifolia*, and has also been found in other *Taxus* species in relatively low yield. Paclitaxel was obtained from *T. brevifolia* bark in 0.07 % yield and it required cumbersome purification from the

other related taxanes. It is estimated that about 9000 kg of yew bark (the equivalent of about 3000 trees) are needed to produce 1 kg of purified paclitaxel (41,42). The development of a semi-synthetic process for the production of paclitaxel from baccatin III **37** (paclitaxel without the C-13 side-chain) or 10-deacetyl-baccatin III **38** (10-DAB, paclitaxel without the C-13 side-chain and the C-10 acetate) and C-13 paclitaxel side-chain **40** or **44** [Scheme 7] is a very promising approach. Baccatin III and 10-DAB can be derived from renewable resources



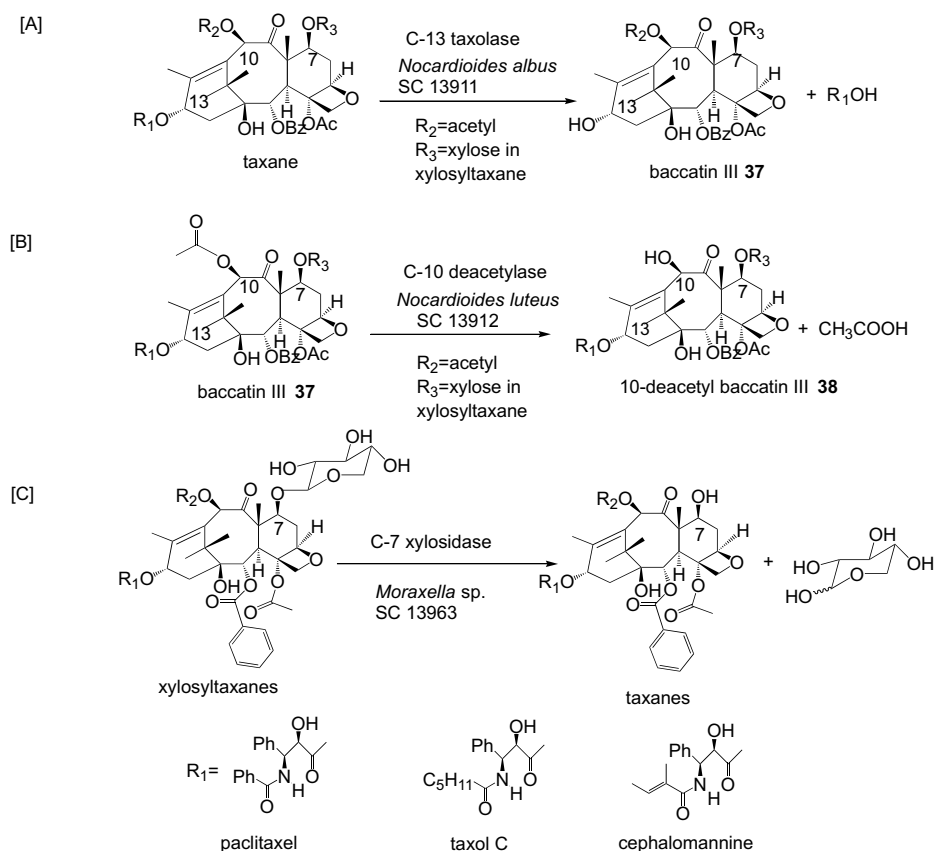
**Scheme 7.** Semisynthesis of paclitaxel **36**, an anticancer agent. Coupling of baccatin III **37** and C-13 paclitaxel side-chain synthons **40** or **44**

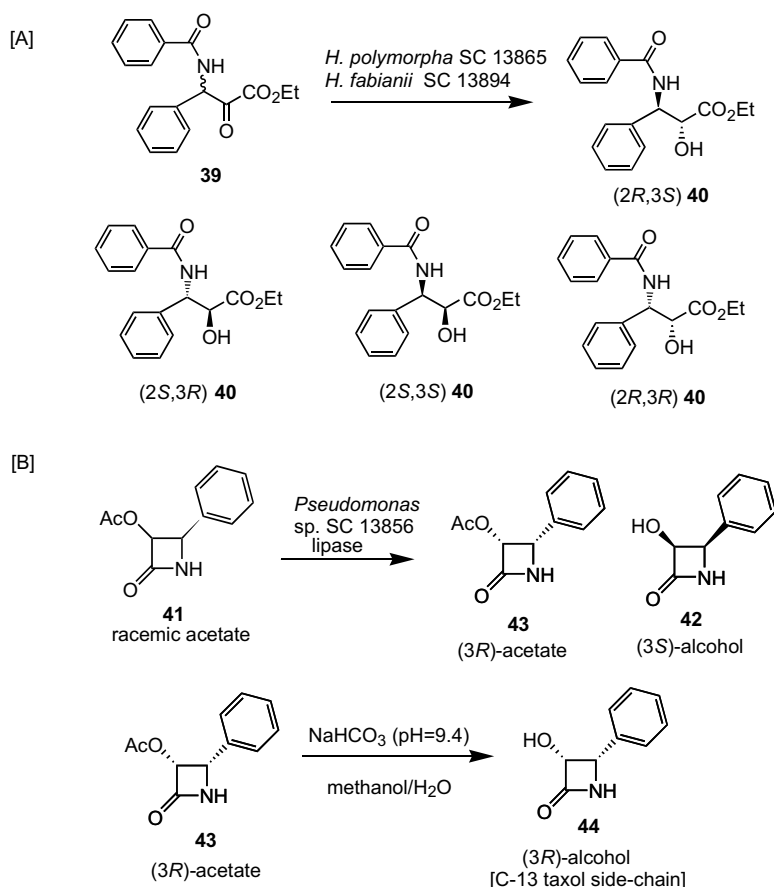
such as extracts of needles, shoots and young *Taxus* cultivars (43). Thus the preparation of paclitaxel by semi-synthetic process eliminates cutting down the large yew trees.

By using selective enrichment techniques, two strains of *Nocardioide*s were isolated from soil samples that contained novel enzymes C-13 taxolase, C-10 deacetylase and C-7 xylosidase (44–46). The extracellular C-13 taxolase derived from the filtrate of the fermentation broth of *Nocardioide*s *albus* SC 13911 [Scheme 8A] catalyzed the cleavage of C-13 side-chain from paclitaxel and related taxanes such as taxol C, cephalomannine, 7- $\beta$ -xylosyltaxol, 7- $\beta$ -xylosyl-10-deacetyltaxol, and 10-deacetyltaxol [Scheme 8A]. The intracellular C-10 deacetylase derived from the fermentation of *Nocardioide*s *luteus* SC 13912 [Scheme 8B] catalyzed the cleavage of C-10 acetate from paclitaxel, related taxanes and baccatin III to yield 10-DAB. The C-7 xylosidase derived from the fermentation of *Moraxella* sp. [Scheme 8C] catalyzed the cleavage of C-7 xylosyl group from various taxanes. Fermentation processes were developed for growth of *N. albus* SC 13911, *N. luteus* SC 13912 and *Moraxella* sp. to produce C-13 taxolase, C-10 deacetylase and C-7 xylosidase, respectively, in 5000-L batches, and a bioconversion process was demonstrated for the conversion of paclitaxel and related taxanes in extracts of *Taxus* cultivars to a single compound 10-DAB using both enzymes. In the bioconversion process, ethanolic extracts of the whole young plant of five different cultivars of *Taxus* were first

treated with a crude preparation of the C-13 taxolase to give complete conversion of measured taxanes to baccatin III and 10-DAB in 6 hours. Whole cells of *Nocardioide*s *luteus* SC 13912 were then added to the reaction mixture to give complete conversion of baccatin III to 10-DAB. The concentration of 10-DAB was increased by 6- to 24-fold in the extracts treated with enzymes. The bioconversion process was also applied to extracts of the bark of *T. braviifolia* to give a 12-fold increase in 10-DAB concentration. The enhancement of 10-DAB concentration in yew extracts was potentially useful in increasing the amount of this key precursor for the paclitaxel semi-synthetic process using renewable resources.

Another key step in the paclitaxel semi-synthetic process is the preparation of chiral C-13 paclitaxel side-chain. Two different enantioselective enzymatic processes were developed for the preparation of chiral C-13 paclitaxel side-chain synthon (47,48). In one process, the enantioselective microbial reduction of 2-keto-3-(N-benzoylamino)-3-phenyl propionic acid ethyl ester **39** to yield (2*R*,3*S*)-(-)-N-benzoyl-3-phenyl isoserine ethyl ester **40** [Scheme 9A] was demonstrated (47). *Hansenula polymorpha* SC 13865 and *Hansenula fabianii* SC 13894 catalyzed the enantioselective reduction of ketone **39** to the (2*R*,3*S*)-ethyl ester **40** in greater than 80 % reaction yield and greater than 94 % e.e. in a preparative-scale bioreduction using cell suspensions in independent experiments. A 20 % yield of undesired anti-diastereomers content was obtained with *H. polymorpha* SC 13865 com-





**Scheme 9.** Enzymatic synthesis of C-13 side-chain of paclitaxel **36**: (A) Enantioselective microbial reduction of 2-keto-3-(N-benzoylamino)-3-phenyl propionic acid, ethyl ester **39**. (B) Enantioselective enzymatic hydrolysis of *cis*-3-(acetyloxy)-4-phenyl-2-azetidinone **41**

pared with a 10 % yield with *H. fabianii* SC 13894. A 99 % e.e. was obtained with *H. polymorpha* SC 13865 compared with a 94 % e.e. with *H. fabianii* SC 13894. In a single-stage fermentation/bioreduction process (15-L scale), cells of *H. fabianii* were grown in a 15-L fermentor for 48 h; the bioreduction process was then initiated by addition of 30 g of substrate and 250 g of glucose and continued for 72 h. A reaction yield of 88 % and an e.e. of 95 % were obtained for (2*R*,3*S*)-ethyl ester **40**.

In an alternate enzymatic resolution process for the preparation of C-13 paclitaxel side-chain, the enantioselective enzymatic hydrolysis of racemic acetate *cis*-3-(acetyloxy)-4-phenyl-2-azetidinone **41** [Scheme 9B] to the corresponding (*S*)-alcohol **42** and the unreacted desired (*R*)-acetate **43** was demonstrated (**43**) by lipase PS-30 from *Pseudomonas cepacia* (Amano International Enzyme Company) and BMS lipase (extracellular lipase derived from the fermentation of *Pseudomonas* sp. SC 13856). Reaction yields of greater than 48 % (theoretical maximum yield 50 %) and e.e. of greater than 99.5 % were obtained for *R*-acetate. BMS lipase and lipase PS-30 were immobilized on Accurel polypropylene (PP) and immobilized lipases were reused (10 cycles) without the loss of enzyme activity, productivity or the e.e. of the product **43** in the resolution process. The enzymatic process was scaled up to 250-L (2.5 kg of substrate input) using immobilized BMS lipase and lipase PS-30, re-

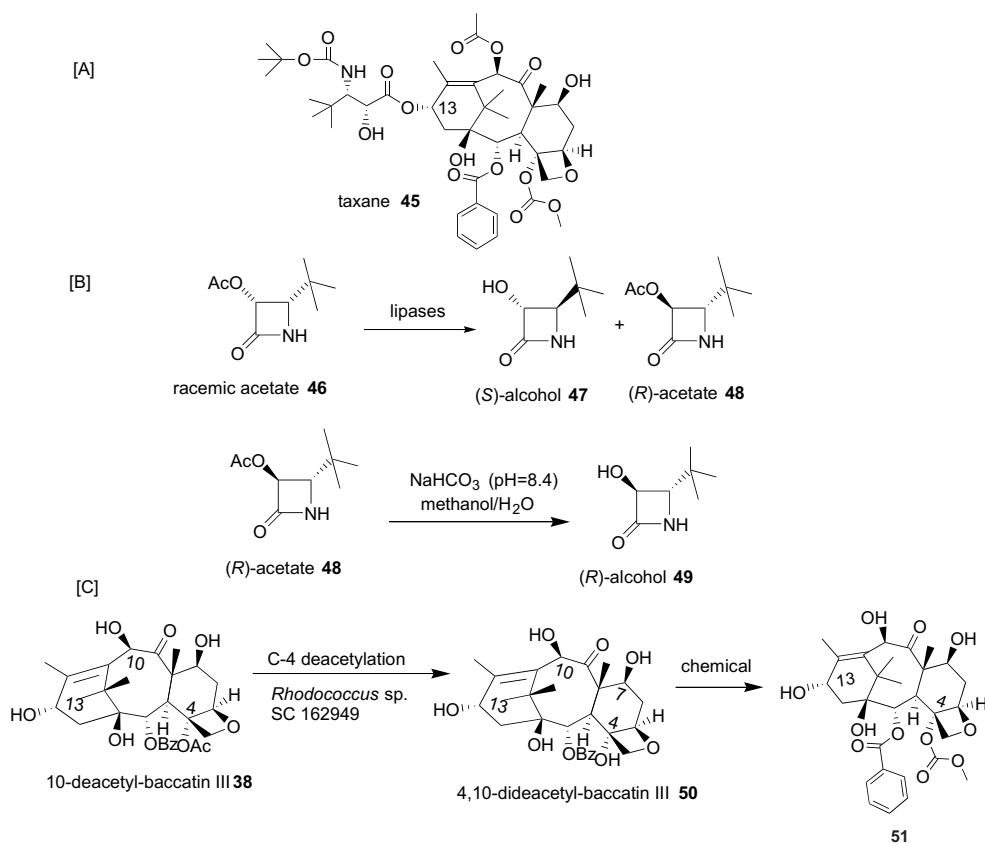
spectively. From each reaction batch, (*R*)-acetate **43** was isolated in mol fraction,  $x=45$  % yield (theoretical maximum yield 50 %) and 99.5 % e.e. (*R*)-acetate was chemically converted to (*R*)-alcohol **44**. The C-13 paclitaxel side-chain synthon (**40** or **44**), produced either by the reductive or resolution process, could be coupled to baccatin III **37** after protection and de-protection to prepare paclitaxel by semi-synthetic process (**49**).

#### Oral taxane

Due to poor solubility of paclitaxel, various groups are developing water-soluble taxane analogs (**50**). Taxane **45** [Scheme 10A] is an anticancer water-soluble taxane derivative given orally, which was as effective as intravenous paclitaxel in five tumor models [murine M109 lung and C3H mammary 16/C cancer, human A2780 ovarian cancer cells (grown in mice and rats) and HCT/pk colon cancer]. Compound **45** was also active in a human, hormone-dependent, prostate tumor model, CWR-22, and just as effective as antiandrogen chemotherapy (**50**).

The enantioselective enzymatic hydrolysis of *cis*-3-(acetyloxy)-4-(1,1-dimethylethyl)-2-azetidinone **46** [Scheme 10B] to the corresponding undesired (*S*)-alcohol **47** and unreacted desired (*R*)-acetate **48** was carried out using immobilized lipase PS-30 or BMS lipase. Reaction yields of greater than 48 % and e.e. of greater than 99 %





**Scheme 10.** (A) Enzymatic synthesis of C-13 side-chain 49 of orally active taxane 45. (B) Enantioselective enzymatic hydrolysis of *cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone 46. (C) Enzymatic C-4 deacetylation of 10-deacetyl-baccatin III 38

were obtained for desired 48. Acetoxy  $\beta$ -lactam 48 was converted to hydroxy  $\beta$ -lactam 49 for semisynthesis of oral taxane 45. Immobilized lipase was reused over many cycles in this process (51).

The synthesis of oral taxane 45 also required 4,10-dideacetyl baccatin 50 [Scheme 10C] as starting material for the synthesis of C-4 methylcarbonate derivative of 10-deacetyl-baccatin III 51. A microbial process was developed for deacetylation of 10-deacetyl-baccatin III 38 to 4,10-dideacetyl-baccatin III 50 using a *Rhodococcus* sp. SC 162949 isolated from soil using an enrichment culture technique (52).

### Retinoid Receptor Gamma Specific Agonists

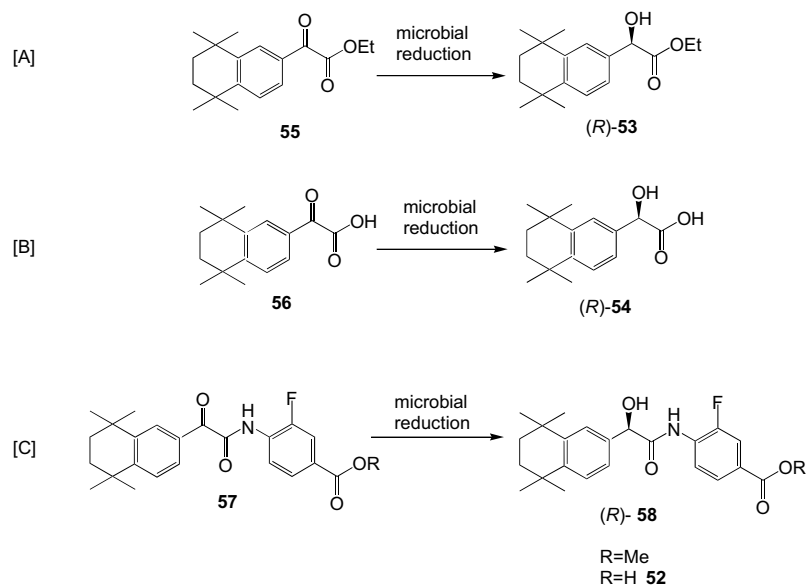
A number of studies have demonstrated that retinoids (vitamin A derivatives) are essential for normal growth, vision, tissue homeostasis, and reproduction (53,54). Retinoic acid and its natural and synthetic analogs (retinoids) exert a wide variety of biological effects by binding to or activating a specific receptor or sets of receptors (55). They affect cellular growth and differentiation and are promising drugs for the treatment of cancers (56). A few retinoids are already in clinical use for the treatment of dermatological diseases such as acne and psoriasis (57). (*R*)-3-fluoro-4-[hydroxy-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-acetyl]amino-benzoic acid 52 [Scheme 11] is a retinoic acid receptor gamma-specific agonist potentially useful as a dermatological and anticancer drug (58).

The chiral intermediate ethyl-2-(*R*)-hydroxy-2-(1',2',3',4'-tetrahydro-1',1',4',4'-tetramethyl-6'-naphthalenyl)acetate 53 and the corresponding acid 54 were prepared as intermediates in the synthesis of the retinoic acid receptor gamma-specific agonist 52 (59). Enantioselective microbial reduction of ethyl-2-oxo-2-(1',2',3',4'-tetrahydro-1',1',4',4'-tetramethyl-6'-naphthalenyl) acetate 55 to alcohol 53 was carried out using *Aureobasidium pullulans* SC 13849 in 98 % yield and with an e.e. of 96 % (59). Among microorganisms screened for the reduction of 2-oxo-2-(1',2',3',4'-tetrahydro-1',1',4',4'-tetramethyl-6'-naphthalenyl)acetic acid 56 to hydroxy acid 54, *Candida maltosa* SC 16112 and two strains of *Candida utilis* (SC 13983, SC 13984) gave greater than 53 % reaction yields with greater than 96 % e.e. At the end of the reaction, hydroxyester 53 was adsorbed onto XAD-16 resin and, after filtration, recovered in 94 % yield from the resin with acetonitrile extraction. The recovered (*R*)-hydroxyester 53 was treated with Chirazyme L-2 or pig liver esterase to convert it to the corresponding (*R*)-hydroxyacid 54 in quantitative yield. The enantioselective microbial reduction of keto amide 57 to the corresponding (*R*)-hydroxyamide 58 by *A. pullulans* SC 13849 was also demonstrated (59).

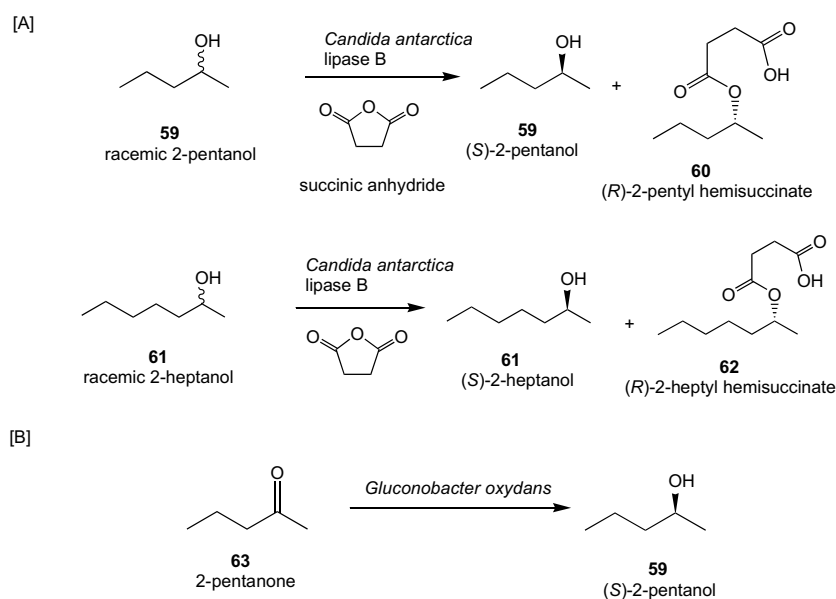
### Anti-Alzheimer's Drugs

#### Resolution of racemic secondary alcohols

(*S*)-2-Pentanol 59 and (*S*)-heptanol 61 [Scheme 12A] are intermediates in the synthesis of several potential anti-Alzheimer's drugs that inhibit synthesis and release



**Scheme 11.** Enzymatic synthesis of chiral synthons for the retinoid receptor gamma specific Agonist 52: (A) Enantioselective microbial reduction of ethyl 2-oxo-2-(1',2',3',4'-tetrahydro-1',1',4',4'-tetramethyl-6'-naphthalenyl) acetate 55 to (R)-hydroxy ester 53. (B) Enantioselective microbial reduction of 2-oxo-2-(1',2',3',4'-tetrahydro-1',1',4',4'-tetramethyl-6'-naphthalenyl) acetate 56 to (R)-hydroxy acid 54. (C) Enantioselective microbial reduction of keto amide 57 to (R) hydroxy amide 58



**Scheme 12.** (A) Synthesis of chiral intermediates for anti-Alzheimer's drugs: enzymatic resolution of racemic 2-pentanol 59 and 2-heptanol 61 by *Candida antarctica* lipase. (B) Enantioselective microbial reduction of 2-pentanone 63 to (S)-2-pentanol

of  $\beta$ -amyloid peptide (60,61). The enzymatic resolution of racemic 2-pentanol and 2-heptanol by lipase B from *Candida antarctica* has been demonstrated (62). Commercially available lipases were screened for the enantioselective acetylation of racemic 2-pentanol in hexane in the presence of vinyl acetate as an acyl donor.

*C. antarctica* lipase B efficiently catalyzed this reaction, giving yields of 49 % (theoretical maximum yield 50 %) and 99 % e.e. for (S)-2-pentanol. In a preparative-scale acetylation (100 g of input), 44.5 g of (S)-2-pentanol was isolated with an e.e. of 98 %. Among the acylating agents tested, succinic anhydride was best due to easy recovery of the (S)-2-pentanol at the end of the reaction.

Using 0.68 mol-equivalent of succinic anhydride [Scheme 12A] and 13 g of lipase B per kg of racemic 2-pentanol, which was present as both substrate and solvent, a reaction yield of mol fraction  $x=43$  % and an e.e. of greater than 98 % were obtained for (S)-2-pentanol. The resolution of 2-heptanol was also carried out using lipase B under similar conditions to give a reaction yield of mol fraction  $x=44$  % and an e.e. of greater than 99 % of (S)-2-heptanol, isolated in 40 % overall yield.

In an alternative approach, the enantioselective reduction of 2-pentanone 63 to the corresponding (S)-2-pentanol [Scheme 12B] was demonstrated for *Gluconobacter oxydans*. Using Triton X-100 treated cells of *G.*

*oxydans*, preparative scale reduction of 2-pentanone was carried out and 1.06 kg of (*S*)-2-pentanol was prepared (63).

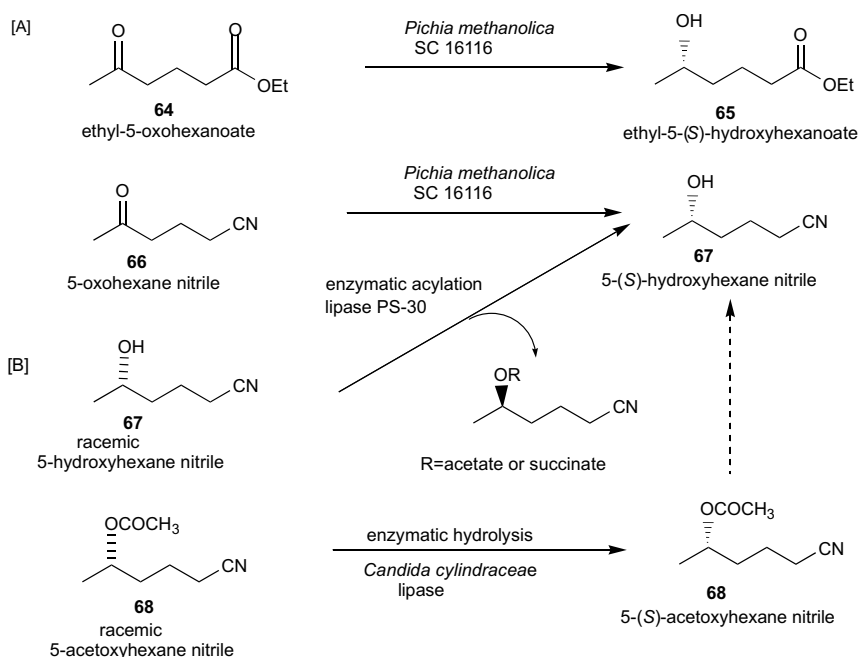
### Enzymatic Reduction of 5-Oxohexanoate and 5-Oxohexane nitrile

Ethyl-(*S*)-5-hydroxyhexanoate **65** and (*S*)-5-hydroxyhexane nitrile **67** [Scheme 13A] are key chiral intermediates in the synthesis of several pharmaceuticals. Both chiral compounds have been prepared by enantioselective reduction of ethyl-5-oxohexanoate **64** and 5-oxohexane nitrile **66** by *Pichia methanolica* SC 16116. Reaction yields of 80–90 % and e.e.'s greater than 95 % were obtained for each chiral compound. In an alternate approach, the enzymatic resolution of racemic 5-hydroxyhexane

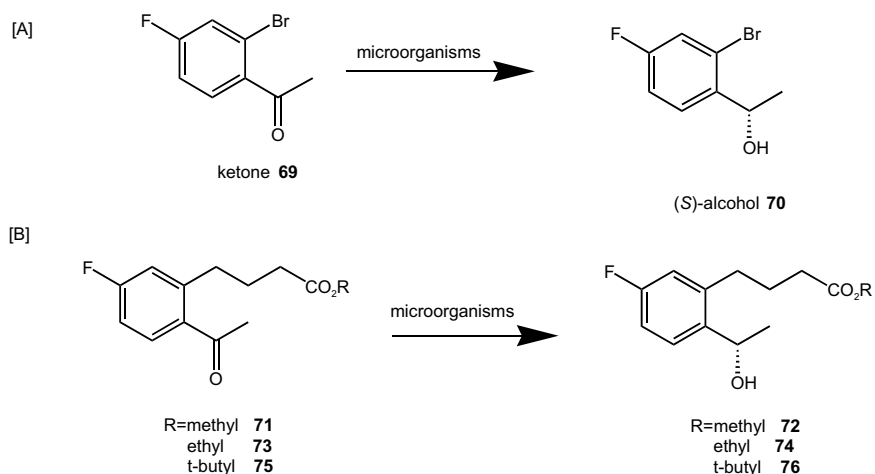
nitrile **67** [Scheme 13B] by enzymatic succinylation was demonstrated using immobilized lipase PS-30 to obtain (*S*)-5-hydroxyhexane nitrile **67** in 35 % yield (maximum yield is 50 %). (*S*)-5-Acetoxyhexane nitrile **68** was prepared by enantioselective enzymatic hydrolysis of racemic 5-acetoxyhexane nitrile by *Candida cylindracea* lipase. A reaction yield of 42 % and an e.e. of greater than 99 % were obtained (64).

### Enantioselective Microbial Reduction of Substituted Acetophenone

The chiral intermediate ethyl-(*S*)-1-(2'-bromo-4'-fluorophenyl)ethanol **70** [Scheme 14A] was prepared by the enantioselective microbial reduction of 2-bromo-4-fluoro acetophenone **69** (65). Organisms from the gen-



**Scheme 13.** (A) Synthesis of chiral intermediates for anti-Alzheimer's drugs: enantioselective microbial reduction of ethyl-5-oxohexanoate **64** and 5-oxohexane nitrile **66**. (B) Enzymatic resolution of 5-hydroxyhexane nitrile **67** and 5-acetoxyhexane nitrile **68**



**Scheme 14.** Synthesis of chiral intermediates for anti-Alzheimer's drugs: (A) Enantioselective microbial reduction of 2-bromo-4-fluoro acetophenone **69**. (B) Enantioselective microbial reduction of methyl-4-(2'-acetyl-5'-fluorophenyl) butanoate **71**

era *Candida*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces* and *Sphingomonas* and baker's yeast reduced **69** to **70** in greater than 90 % yield and 99 % e.e. In an alternative approach, the enantioselective microbial reduction of methyl-, ethyl- and t-butyl-4-(2'-acetyl-5'-fluorophenyl) butanoates **71**, **73**, and **75** [Scheme 13B], respectively, was demonstrated using strains of *Candida* and *Pichia*. Reaction yields of 40–53 % and e.e.'s of 90–99 % were obtained for the corresponding (S)-hydroxy esters **72**, **74**, and **76**. The reductase that catalyzed the enantioselective reduction of keto esters was purified to homogeneity from cell extracts of *Pichia methanolica* SC 13825. It was cloned and expressed in *Escherichia coli* and recombinant cultures were used for the enantioselective reduction of the keto-methyl ester **71** to the corresponding (S)-hydroxy methyl ester **72**. On preparative scale, a reaction yield of 98 % and an e.e. of 99 % were obtained (65).

### Melatonin Receptor Agonist

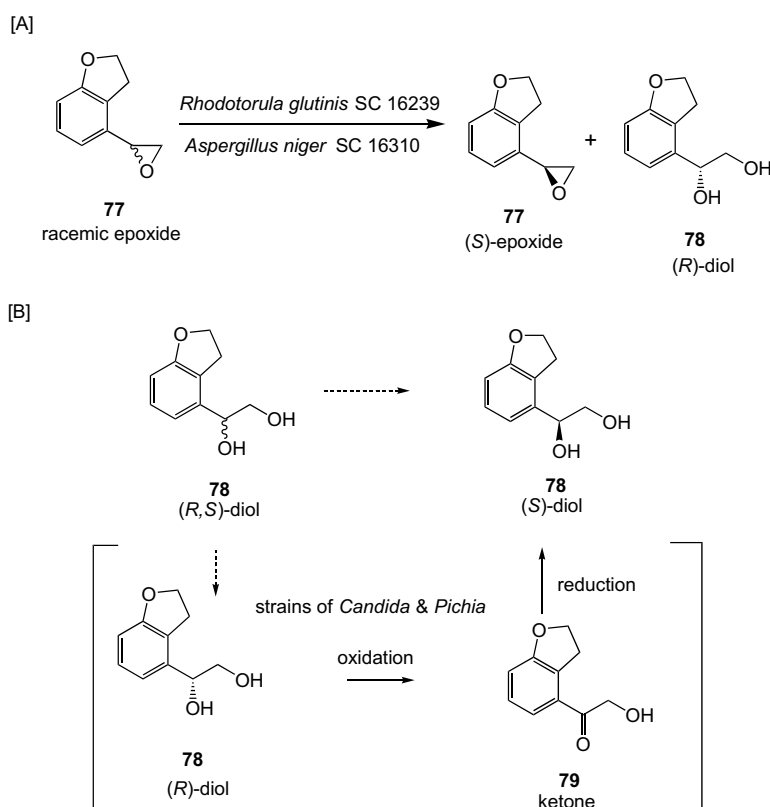
#### Enantioselective enzymatic hydrolysis of 1-[2',3'-dihydro benzo(b)furan-4'-yl]-1,2-oxirane

Epoxide hydrolase catalyzes the enantioselective hydrolysis of an epoxide to the corresponding enantiomerically enriched diol and unreacted epoxide (66,67). The (S)-epoxide **77** [Scheme 15A] is a key intermediate in the synthesis of a number of prospective drug candidates (68). The enantiospecific hydrolysis of the racemic 1-[2',3'-dihydro benzo(b)furan-4'-yl]-1,2-oxirane **77** to the

corresponding (R)-diol **78** and unreacted S-epoxide **77** was demonstrated by Goswami *et al.* (69). Among cultures evaluated, two *Aspergillus niger* strains (SC 16310, SC 16311) and *Rhodotorula glutinis* SC 16239 selectively hydrolyzed the (R)-epoxide, leaving behind the (S)-epoxide **77** in greater than 95 % e.e. and 45 % yield (maximum yield is 50 %). Solvents had significant effects on both the extent of hydrolysis and the e.e. of unreacted (S)-epoxide **77**. Most solvents gave a lower e.e. product and slower reaction rate than those of reactions without any solvent supplement. MTBE (15 % volume fraction) gave a reaction yield of 45 % (theoretical maximum yield 50 %) and an e.e. of 99.9 % for unreacted (S)-epoxide **77**.

#### Biocatalytic Dynamic Kinetic Resolution of (R,S)-1-[2',3'-dihydroenzo(b)furan-4'-yl]-ethane-1,2-diol

Most commonly used biocatalytic kinetic resolutions of racemates often provide compounds with high e.e., however, the maximum theoretical yield of product is only 50 %. In many cases, the reaction mixture contains an approximately 50:50 mixture of reactant and product. The reactant and product often possess only slight differences in physical properties (*e.g.* a hydrophobic alcohol and its acetate), and thus separation may be very difficult. These issues with kinetic resolutions can be addressed by employing a »dynamic kinetic resolution«



**Scheme 15.** (A) Synthesis of chiral intermediates for a melatonin receptor agonist: enantioselective microbial hydrolysis of racemic epoxide **77** to the corresponding (R)-diol **78** and unreacted (S)-epoxide **77**. (B) Stereoconversion of racemic diol **78** to (S)-diol **78** by *Candida boidinii* and *Pichia methanolica*

process involving a biocatalyst or biocatalyst with metal-catalyzed *in situ* racemization (70,71).

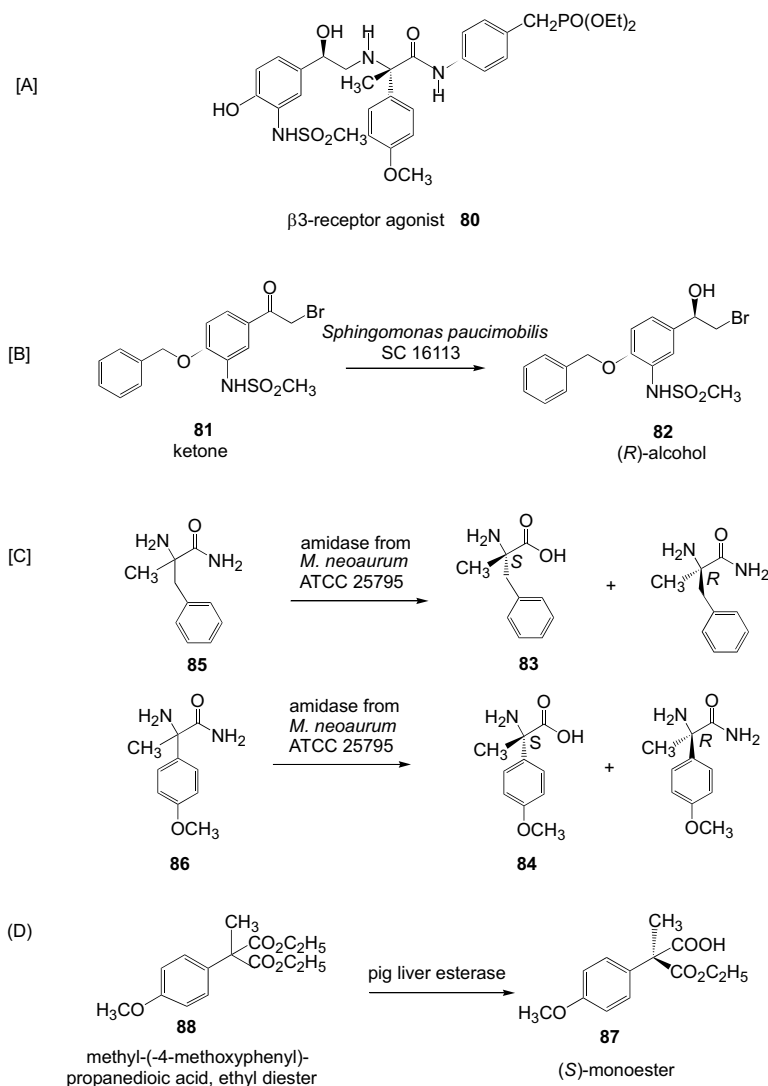
(*S*)-1-[2',3'-dihydrobenzo(b)furan-4'-yl]-ethane-1,2-diol **78** [Scheme 15B] is a potential precursor of (*S*)-epoxide **77** (68). The dynamic kinetic resolution of the racemic diol **78** to the (*S*)-enantiomer **78** has been demonstrated (72) by strains of *Candida boidinii*, *Pichia methanolica*, and *Hansenula polymorpha*. During biotransformation, the relative proportion of (*S*)-diol **78** increased with time and, at the end of one week, the e.e. obtained with these microorganisms was in the range of 87–100 % (yield 60–75 %). A new compound was formed during these biotransformations and was identified as the hydroxy ketone **79**. The area of the HPLC peak for hydroxy ketone first increased with time, reached a maximum, and then decreased, as expected for the proposed dynamic kinetic resolution pathway. *C. boidinii* SC 13822, *C. boidinii* SC 16115, and *P. methanolica* SC 13860 transformed the racemic diol **78** in 3–4 days to (*S*)-diol **78** in yields of 62–75 % and e.e.'s of 90–100 %.

### β3-Receptor Agonists

β3-Adrenergic receptors are found on the cell surface of both white and brown adipocytes and are responsible for lipolysis, thermogenesis, and relaxation of intestinal smooth muscle (73). Consequently, several research groups are engaged in developing selective β3 agonists for the treatment of gastrointestinal disorders, type II diabetes, and obesity (74,75). Three different biocatalytic approaches for synthesis of chiral intermediates required for the total synthesis of β3 receptor agonists **80** [Scheme 16A] have been investigated (76).

#### Microbial reduction of 4-benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone

The microbial reduction of 4-benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone **81** [Scheme 16B] to the corresponding (*R*)-alcohol **82** has been demonstrated (76) using *Sphingomonas paucimobilis* SC 16113. The growth of *S. paucimobilis* SC 16113 was carried out



**Scheme 16.** (A) Enzymatic synthesis of chiral synthon for β3-receptor agonist **80**. (B) Enantioselective reduction of 4-benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone **81** to (*R*)-alcohol **82**. (C) Enantioselective hydrolysis of α-methyl phenylalanine amide **85** and α-methyl-4-methoxyphenylalanine amide **86** by amidase. (D) Enantioselective enzymatic hydrolysis of methyl-(4-methoxyphenyl)-propanedioic acid ethyl diester **88** to (*S*)-monoester **87**

in a 750-L fermentor and cells (60 kg) harvested from the fermentor were used to conduct the biotransformation in 10-L and 200-L preparative batches. The cells were suspended in 80 mM potassium phosphate buffer (pH=6.0) at  $m/V=20$  % (wet cells) concentration, supplemented with compound **81** (2 g/L) and glucose (25 g/L), and the reduction was carried out at 37 °C. In some batches, the fermentation broth was concentrated 3-fold by microfiltration and subsequently washed with buffer by diafiltration and used directly in the bioreduction process. In all the batches, reaction yields of greater than 85 % and e.e.'s of greater than 98 % were obtained. The isolation of alcohol **82** from the 200-L batch gave 320 g (80 % yield) of product with an e.e. of 99.5 %.

In an alternate process, 200 g of frozen cells of *S. paucimobilis* SC 16113 were suspended in 1 L of 80 mM potassium phosphate buffer (pH=6.0) and supplied with 10 g of substrate adsorbed on 50 g of hydrophobic resin XAD-16. In this process, an average reaction yield of 85 % and an e.e. of greater than 99 % were obtained for alcohol **82**. The product formed was adsorbed on to XAD-16 resin. At the end of the biotransformation, the reaction mixture was filtered on a 100-mesh (150  $\mu$ m) stainless steel screen, and the resin retained by the screen was washed with water. The product was then desorbed from the resin with acetonitrile and crystallized, giving an overall  $x=75$  % yield and 99.8 % e.e.

#### Enzymatic resolution of racemic $\alpha$ -methyl phenylalanine amides

The chiral amino acids **83** and **84** [Scheme 16C] are intermediates for the synthesis of  $\beta$ 3-receptor agonists (74,75). These are available via the enzymatic resolution of racemic  $\alpha$ -methyl phenylalanine amide **85** and  $\alpha$ -methyl-4-methoxyphenylalanine amide **86**, respectively, by an amidase from *Mycobacterium neoaurum* ATCC 25795 (76). Wet cells ( $m/V=10$  %) completed reaction of amide **85** in 75 min with a yield of  $x=48$  % (theoretical max. 50 %) and an e.e. of 95 % for the desired (*S*)-amino acid **83**. Alternatively, freeze-dried cells were suspended in 100 mM potassium phosphate buffer (pH=7.0) at 1 % concentration to give complete reaction in 60 min with a yield of  $x=49.5$  % (theoretical max. 50 %) and an e.e. of 99 % for the (*S*)-amino acid **83**.

Freeze-dried cells of *M. neoaurum* ATCC 25795 and partially purified amidase (amidase activity in cell ex-

tracts purified 5-fold by diethyl aminoethyl cellulose column chromatography) were used for the biotransformation of compound **86**. A reaction yield of  $x=49$  % and an e.e. of 78 % were obtained for the desired product **84** using freeze-dried cells. The reaction was completed in 50 h. Using partially purified amidase, a reaction yield of  $x=49$  % and an e.e. of 94 % were obtained after 70 h.

#### Enantioselective hydrolysis of diethyl methyl-(4-methoxyphenyl)-propanedioate

The (*S*)-monoester **87** [Scheme 16D] is a key intermediate for the synthesis of  $\beta$ 3-receptor agonists. The enantioselective enzymatic hydrolysis of diester **88** to the desired acid ester **87** by pig liver esterase (76) has been demonstrated. In various organic solvents the reaction yields and e.e. of monoester **87** depended upon the solvent used. High e.e.'s (>91 %) were obtained with methanol, ethanol, and toluene as a co-solvent. Ethanol gave the highest reaction yield (96.7 %) and e.e. (96 %) for the desired acid ester **87**.

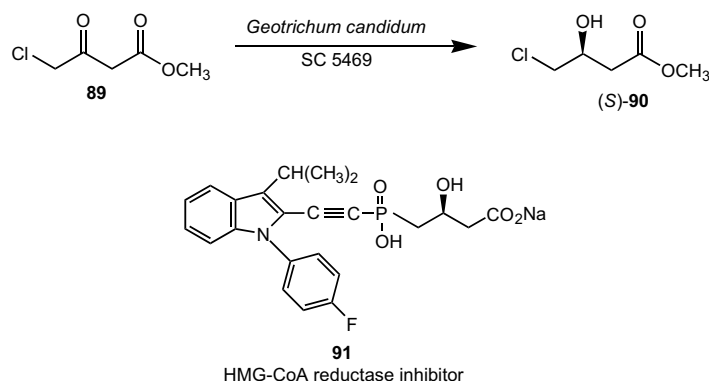
The e.e. of the (*S*)-monoester **87** was increased by decreasing the temperature from 25 °C to 10 °C, when biotransformation was conducted in a biphasic system using ethanol as a co-solvent. A semi-preparative hydrolysis (30 g scale) was carried out using 10 % ethanol as a co-solvent in a 3-L reaction mixture (pH=7.2) at 10 °C for 11 h. A reaction yield of  $x=96$  % and an e.e. of 96.9 % were obtained. From the reaction mixture, 26 g ( $x=86$  % overall yield) of (*S*)-monoester **87** of 96.9 % e.e. was isolated.

### Cholesterol Lowering Agents

#### Hydroxy methyl glutaryl CoA reductase inhibitors

##### Reduction of 4-chloro-3-oxobutanoic acid methyl ester

The reduction of 4-chloro-3-oxobutanoic acid methyl ester **89** to *S*-(-)-4-chloro-3-hydroxybutanoic acid methyl ester **90** [Scheme 17] has been carried out with cell suspensions of *Geotrichum candidum* SC 5469. (*S*)-**90** is a key chiral intermediate in the total chemical synthesis of **91**, a cholesterol antagonist that acts by inhibiting HMG CoA reductase (77). In the biotransformation process, a reaction yield of 95 % and e.e. of 96 % were obtained for (*S*)-**90** by glucose-, acetate- or glycerol-grown cells ( $m/V=10$  %) of *G. candidum* SC 5469 at 10 g/L of sub-



**Scheme 17.** Synthesis of a chiral synthon for the cholesterol-lowering drug **91**: enantioselective microbial reduction of 4-chloro-3-oxobutanoic acid methyl ester **89** to (*S*)-4-chloro-3-hydroxybutanoic acid methyl ester **90**

strate input. The e.e. of (*S*)-**90** was increased to 98 % by heat-treatment of cell-suspensions (55 °C for 30 min) prior to conducting the bioreduction of **89**.

#### Reduction of ethyl 4-chloroacetoacetate

In an alternate approach, the asymmetric reduction of ethyl 4-chloroacetoacetate to (*S*)-4-chloro-3-hydroxybutanoate was demonstrated by a secondary alcohol dehydrogenase (PfODH) from *Pichia finlandica*. The gene encoding PfODH was cloned from *P. finlandica* and over-expressed in *Escherichia coli*. Formate dehydrogenase was used to regenerate cofactor NADH required for this reaction. The recombinant *E. coli*, co-expressing both PfODH and formate dehydrogenase from *Mycobacterium* sp., produced (*S*)-4-chloro-3-hydroxybutanoate in 98.5 % yield and 99 % e.e. at 32 g/L of substrate input (78).

#### Preparation of (*R*)-4-cyano-3-hydroxybutyrate

An enzymatic process was developed for the preparation of 4-halo-3-hydroxybutyric acid derivatives by ketoreductase-catalyzed conversion of 4-halo-3-ketobutyric acid derivatives. Thus the genes encoding halohydrin dehydrogenase from *Agrobacterium tumefaciens*, ketoreductase from *Candida magnoliae*, glucose dehydrogenase from *Bacillus subtilis* and formate dehydrogenase from *Candida boidinii* were separately cloned into *Escherichia coli* BL21. Each enzyme was produced by fermentation, isolated and characterized. Then ethyl (*R*)-4-cyano-3-hydroxybutyrate was prepared from ethyl 4-chloroacetoacetate by the following procedure. Ethyl 4-chloroacetoacetate was incubated at pH=7.0 with ketoreductase and glucose dehydrogenase and NADP for 40 h to produce ethyl (*S*)-chloro-3-hydroxybutyrate. The ethyl (*S*)-chloro-3-hydroxybutyrate was extracted with ethyl acetate, dried, filtered and concentrated to yield a  $\approx$ 97 % pure ester. The dried ethyl (*S*)-chloro-3-hydroxybutyrate was dissolved in phosphate buffer and mixed with halohydrin dehalogenase and sodium cyanide at pH=8.0. After

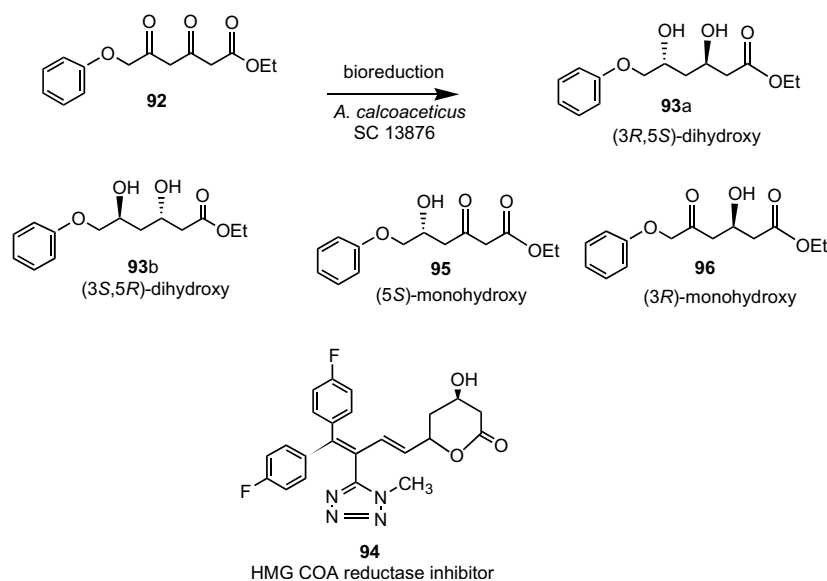
57 h reaction, essentially pure (*R*)-4-cyano-3-hydroxybutyrate was recovered, which is an intermediate used in the synthesis of many HMG-CoA reductase inhibitors (79).

#### Preparation of (*R*)- and (*S*)-ethyl-3-hydroxybutyrate

An efficient two-step enzymatic process for production of (*R*)- and (*S*)-ethyl-3-hydroxybutyrate (HEB) was developed and scaled-up to a multikilogram scale. Both enantiomers were obtained at 99 % chemical purity and over 96 % e.e., with an overall process yield of 73 %. The first reaction involved an acetylation of racemic HEB with vinyl acetate for the production of (*S*)-HEB. In the second reaction, (*R*)-enriched ethyl-3-acetoxybutyrate (AEB) was subjected to alcoholysis with ethanol to derive optically pure (*R*)-HEB. Immobilized *Candida antarctica* lipase B (CALB) was employed in both stages, with high productivity and selectivity. The type of butyric acid ester influenced the enantioselectivity of the enzyme. Thus, extending the ester alkyl chain from ethyl to octyl resulted in a decrease in e.e., whereas using bulky groups, such as benzyl or *t*-butyl, improved the enantioselectivity of the enzyme. The immobilized enzyme was packed in a column and the reactants were circulated through the enzyme bed until the targeted conversion was reached. The desired products were separated from the reaction mixture in each of the two stages by fractional distillation. The main features of the process are the exclusion of solvent (thus ensuring high process throughput) and the use of the same enzyme for both the acetylation and the alcoholysis steps to prepare kilogram quantities of (*S*)-HEB and (*R*)-HEB (80).

#### Enzymatic reduction of 3,5-dioxo-6-(benzyloxy) hexanoic acid, ethyl ester

The enantioselective reduction of a diketone, 3,5-dioxo-6-(benzyloxy) hexanoic acid ethyl ester **92**, to (3*R*,5*S*)-dihydroxy-6-(benzyloxy) hexanoic acid ethyl ester **93a** [Scheme 18] has been demonstrated by cell suspensions of *Acinetobacter calcoaceticus* SC 13876 (81). Compound **93a** is a key chiral intermediate required for the chemi-



**Scheme 18.** Synthesis of a chiral synthon for the cholesterol lowering drug **94**: enantioselective microbial reduction of 3,5-dioxo-6-(benzyloxy) hexanoic acid, ethyl ester **92** to (3*R*,5*S*)-dihydroxy-6-(benzyloxy) hexanoic acid, ethyl ester **93a**

cal synthesis of {4-[4 $\alpha$ ,6 $\beta$ (E)]}-6-[4,4-bis-(4-fluorophenyl)-3-(1-methyl-1H-tetrazol-5-yl)-1,3-butadienyl]-tetrahydro-4-hydroxy-2H-pyren-2-one **94**, an anticholesterol drug that acts by inhibition of HMG CoA reductase (82). A reaction yield of 85 % and an e.e. of 97 % were obtained using glycerol-grown cells at 2 g/L. Cell extracts of *A. calcoaceticus* SC 13876 in the presence of NAD<sup>+</sup>, glucose and glucose dehydrogenase reduced **92** to the corresponding monohydroxy compounds [3-hydroxy-5-oxo-6-(benzyloxy) hexanoic acid ethyl ester **96** and 5-hydroxy-3-oxo-6-(benzyloxy) hexanoic acid ethyl ester **95**]. Both **95** and **96** were further reduced to the (3R,5S)-dihydroxy compound **93a** by cell extracts. A reaction yield of 92 % and an e.e. of 98 % were obtained at 10 g/L of substrate input.

#### Enzymatic preparation of a 2,4-dideoxyhexose derivative

The chiral 2,4-dideoxyhexose derivative required for the HMG CoA reductase inhibitors has also been prepared using 2-deoxyribose-5-phosphate aldolase (DERA). As shown in Scheme 19, the reactions start with a stereospecific addition of acetaldehyde **97** to a substituted acetaldehyde to form a 3-hydroxy-4-substituted butyraldehyde **98**, which reacts subsequently with another acetaldehyde to form a 2,4-dideoxyhexose derivative **99**. DERA has been expressed in *Escherichia coli* (83).

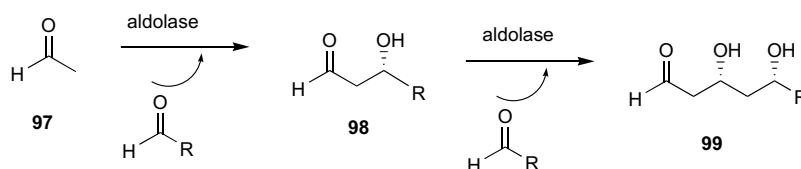
The above process has been improved and optimized for production of key intermediates for the common chiral side chain of cholesterol-lowering drugs such as Lipitor (atorvastatin) and Crestor (rosuvastatin). An improvement of almost 400-fold in volumetric produc-

tivity relative to the published enzymatic reaction conditions has been achieved, resulting in an attractive process that has been run on up to a 100-g scale in a single batch at a rate of 30.6 g L<sup>-1</sup>h<sup>-1</sup>. Catalyst load has been improved by 10-fold as well, from *w*=20 to 2.0 % DERA. These improvements were achieved by a combination of discovery from environmental DNA of DERA with improved activity and reaction optimization to overcome substrate inhibition. The two stereogenic centers are set by DERA with enantiomeric excess at greater than 99.9 % and diastereomeric excess at 96.6 %. In addition, downstream chemical processes have been developed to convert the enzyme product efficiently to versatile intermediates applicable to preparation of atorvastatin and rosuvastatin (84).

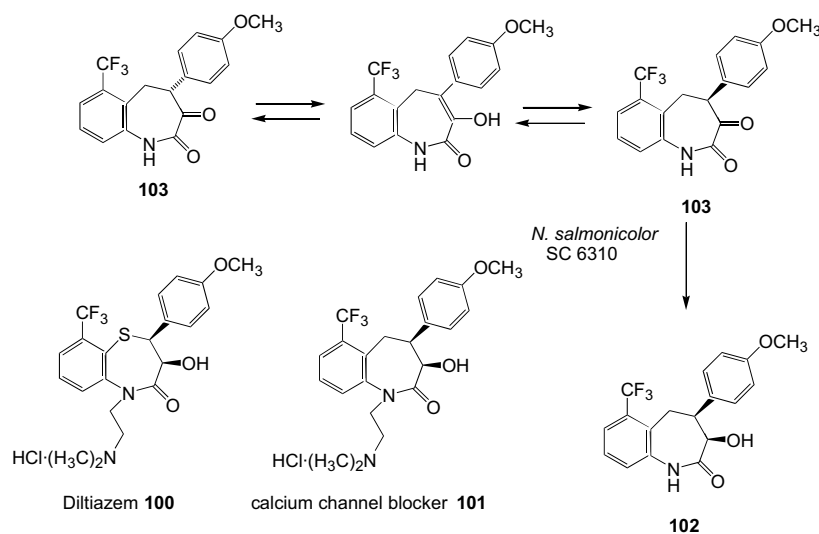
#### Calcium Channel Antagonist

##### *Enzymatic synthesis of [(3R-cis)-1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-6-(trifluoromethyl)-2H-1-benzazepin-2-one]*

Diltiazem **100** [Scheme 20], a benzothiazepinone calcium channel-blocking agent that inhibits influx of extracellular calcium through L-type voltage-operated calcium channels, has been widely used clinically in the treatment of hypertension and angina (85). Since diltiazem has a relatively short duration of action (86), an 8-chloroderivative has recently been introduced into the clinic as a more potent analogue (87). Lack of extended duration of action and little information on structure-activity relationships in this class of compounds led Floyd *et al.*



**Scheme 19.** Enzymatic synthesis of 2,4-dideoxyhexose derivative **99**, a chiral synthon for cholesterol lowering drugs



**Scheme 20.** Synthesis of chiral synthon for calcium channel blocker **101**: microbial reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl)-1H-benzazepin-2,3-dione **103**



(88) to prepare isosteric 1-benzazepin-2-ones; this led to the identification of [(*cis*)-3-(acetoxy)-1-[2-(dimethylamino)ethyl]-1,3,4,5-tetrahydro-4-(4-methoxyphenyl)-6-trifluoromethyl]-2H-1-benzazepin-2-one] **101** as a longer lasting and more potent antihypertensive agent. A key intermediate in the synthesis of this compound was [(3*R-cis*)-1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-6-(trifluoromethyl)-2H-1-benzazepin-2-one] **102**. An enantioselective microbial process [Scheme 20] was developed for the reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl)-1H-1-benzazepin-2,3-dione **103**, which exists predominantly in the achiral enol form in rapid equilibrium with the two enantiomeric keto forms. Reduction of **103** could give rise to formation of four possible alcohol stereoisomers. Remarkably, conditions were found under which only the single alcohol isomer **102** was obtained. Among various cultures evaluated, microorganisms from the genera *Nocardia*, *Rhodococcus*, *Corynebacterium*, and *Arthobacter* reduced compound **103** with 60–70 % conversion. The most effective culture, *Nocardia salmonicolor* SC 6310, catalyzed the bioconversion of **103** to **102** in 96 % reaction yield with 99.8 % e.e. A preparative-scale fermentation process for growth of *N. salmonicolor* and a bioreduction process using cell suspensions of the organism were demonstrated (89).

#### Asymmetric hydrolysis of 1,4-dihydropyridine diesters

*Streptomyces viridosporus* A-914 was screened as a producer of an enzyme that effectively forms chiral intermediates of 1,4-dihydropyridine calcium antagonists (90). The supernatant liquid of the growing culture of this strain exhibited high activity for enantioselective hydrolysis of prochiral 1,4-dihydropyridine diesters to the corresponding (4*R*) half esters. The responsible enzyme (termed DHP-A) was purified to homogeneity and characterized. Cloning and sequence analysis of the gene for DHP-A (*dhpA*) revealed that the enzyme was a serine protease that is highly similar in both structural and enzymatic features to SAM-P45. Homologous or heterologous expression of *dhpA* resulted in overproduction of the enzyme in culture supernatants, with 2.4- to 4.2-fold higher specific activities than in the parent *S. viridosporus* A-914 (90).

#### Enantioselective Enzymatic Cleavage of the Carbobenzyloxy (Cbz) Group

Amino groups often require protection during synthetic transformations elsewhere in the molecule; at some point, the protecting group must be removed. Enzymatic protection and de-protection under mild conditions have been demonstrated previously. Penicillin G amidase and phthalyl amidase have been used for the enzymatic de-protection of the phenylacetyl and phthaloyl groups from the corresponding amido or imido compounds (90,91). Acylases have been used widely in the enantioselective de-protection of N-acetyl-DL-amino acids (92). Enzymatic de-protection of N-carbamoyl L-amino acids and N-carbamoyl D-amino acids has been demonstrated for microbial L-carbamoylases and D-carbamoylases, respectively (93,94).

The carbobenzyloxy (Cbz) group is commonly used to protect amino and hydroxyl groups during organic synthesis. Chemical de-protection is usually achieved by hydrogenation with a palladium catalyst (95,96). However, during chemical de-protection, some groups are reactive under the hydrogenolysis conditions (e.g. carbon-carbon double bonds) or may interfere with hydrogenolysis (e.g. thiols or sulfides). An enantioselective enzymatic de-protection process has been developed that can be performed under mild conditions without damaging any otherwise susceptible groups in the molecule. A microbial culture was isolated from soil and identified as *Sphingomonas paucimobilis* strain SC 16113; this culture catalyzed the enantioselective cleavage of Cbz-groups from various Cbz-protected amino acids (97). Only Cbz-L-amino acids were de-protected, giving complete conversion to the corresponding L-amino acid. Cbz-D-amino acids gave <2 % reaction yield.

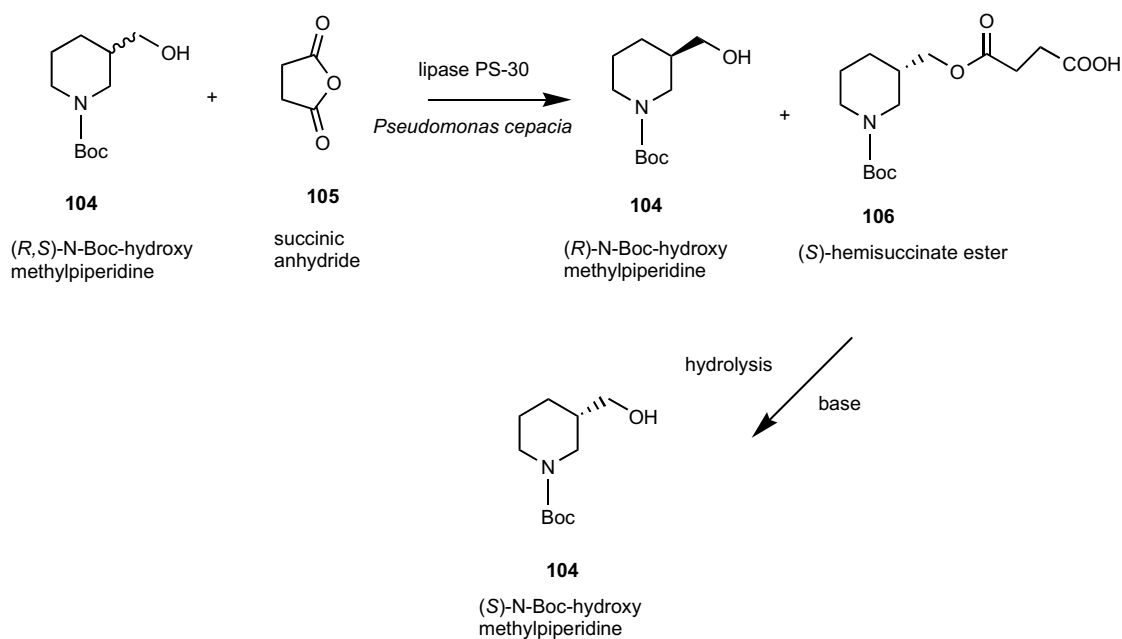
Racemic Cbz-amino acids were also evaluated as substrates for hydrolysis by cell extracts of *S. paucimobilis* SC 16113. As anticipated, only the L-enantiomer was hydrolyzed, giving the L-amino acids in greater than 48 % yields and greater than 99 % e.e.'s. The unreacted Cbz-D-amino acids were recovered in greater than 48 % yield and greater than 98 % e.e. (97). Cbz-cleaving enzyme has been purified and over-expressed in *Escherichia coli* (98).

#### Tryptase Inhibitor

(*R,S*)-N-(*t*-butoxycarbonyl)-3-hydroxymethylpiperidine **104** [Scheme 21] is a key intermediate in the synthesis of a potent tryptase inhibitor (99). (*S*)-**104** was made from (*R,S*)-3-hydroxy methylpiperidine via fractional crystallization of the corresponding L(-)-dibenzoyl tartarate salt followed by hydrolysis and esterification (100). Lipase from *Pseudomonas cepacia* was the best enzyme for the stereospecific resolution of (*R,S*)-N-(*t*-butoxycarbonyl)-3-hydroxymethylpiperidine **104**. (*S*)-**104** was obtained in 16 % yield and greater than 95 % e.e. by hydrolysis of (*R,S*)-acetate by lipase from *P. cepacia*. Lipase PS-30 also catalyzed esterification of the (*R,S*)-N-(*t*-butoxycarbonyl)-3-hydroxymethylpiperidine **104** with succinic anhydride **105** to yield (*R*)-N-(*t*-butoxycarbonyl)-3-hydroxy methylpiperidine **104** and the (*S*)-hemisuccinate ester **106**, which could be easily separated and hydrolyzed by base to the (*S*)-**104**. The yield and e.e. could be improved greatly by repeating the process. Using the repeated esterification procedure, (*S*)-**104** was obtained in 32 % yield (maximum theoretical yield 50 %) and 98.9 % e.e. (101).

#### Acyloin and Aldol Condensation

Asymmetric  $\alpha$ -hydroxyketones (acyloin) are important classes of intermediates in organic synthesis due to their bi-functional aspect, especially having one chiral center amenable to further modification. Enzyme-mediated acyloin formation could provide an advantageous, environmentally-friendly method of preparing optically active asymmetric acyloins (102). Acyloin formations mediated by yeast pyruvate decarboxylase (103) and bacterial benzoylformate decarboxylase (104) have been reported. Though phenylpyruvate decarboxylase (PPD)

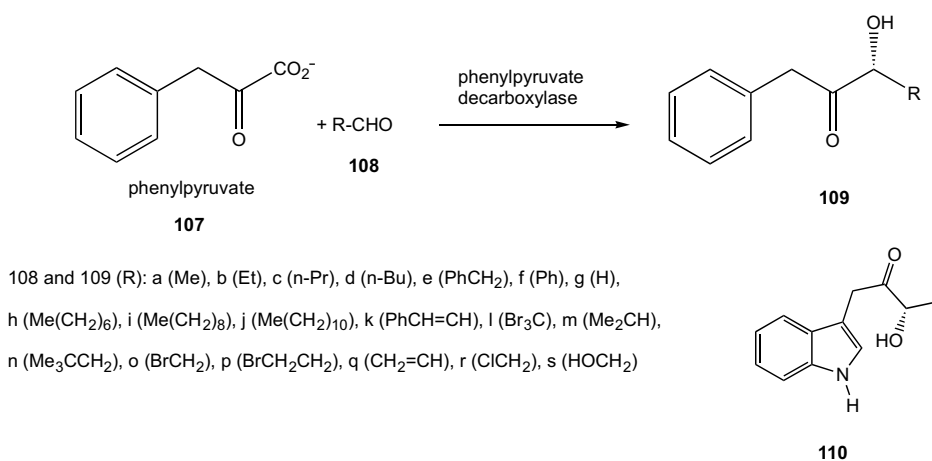


**Scheme 21.** Preparation of a chiral synthon for a tryptase inhibitor: enzymatic resolution of racemic (t-butoxycarbonyl)-3-hydroxymethylpiperidine **104** to (*S*)-**104**

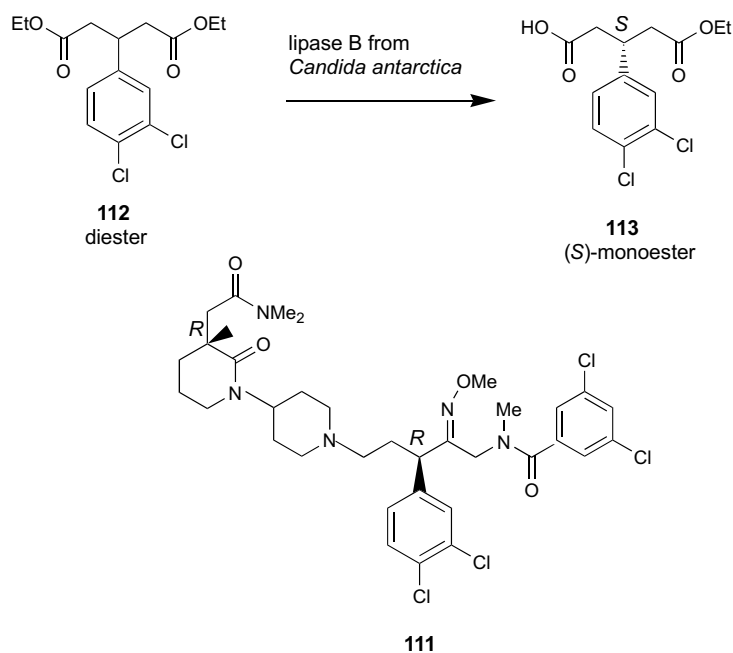
for decarboxylation of phenylpyruvic acid was known for a long time (102), recently we reported the acyloin condensation catalyzed by PPD (105). *Achromobacter eurydice* PPD was used to catalyze the asymmetric acyloin condensation of phenylpyruvate **107** with various aldehydes **108** to produce optically active acyloins PhCH<sub>2</sub>-COCH(OH)R **109** [Scheme 22]. The acyloin condensation yield decreased with increasing chain length for straight chain aliphatic aldehydes from 76 % for acetaldehyde to 24 % for valeraldehyde. The e.e.'s of the acyloin products were 87–98 %. Low yields of acyloin products were obtained with chloro acetaldehyde (13 %) and glyco aldehyde (16 %). Indole-3-pyruvate was a substrate of the enzyme and provided acyloin condensation product 3-hydroxy-1-(3-indolyl)-2-butanone **110** with acetaldehyde in 19 % yield. PPD has been purified from *Achromobacter eurydice*. The purified protein was shown to be of 150 kD molecular weight and to consist of two subunits, one of 90 kD and one of 40 kD (106).

### NK1/NK2 Dual Antagonist

Tachykinins are a group of biologically active neuropeptide hormones that are widely distributed throughout the nervous system. They are implicated in a variety of biological processes such as pain transmission, inflammation, vasodilatation and secretion (107). The effect of Tachykinins is modulated via the specific G-protein coupled receptors like NK1 and NK2. Thus non-peptide NK-receptor antagonists are potentially useful in the treatment of a variety of chronic diseases including asthma, bronchospasm, arthritis, and migraine (108,109). The structure-activity relationship of several non-peptide NK1/NK2 antagonists has led to the discovery of a new class of oxime-based dual antagonist (110,111), namely compound **111** [Scheme 23]. The biological activity of **111** resides mainly in the *R,R*-diastereomers. An enzymatic process for desymmetrization of the prochiral diethyl 3-(3',4'-dichlorophenyl)-glutarate **112** to the cor-



**Scheme 22.** Enzymatic asymmetric acyloin condensation reactions catalyzed by phenylpyruvate decarboxylase



**Scheme 23.** Preparation of a chiral synthon for an NK1/NK2 dual antagonist: enzymatic asymmetric hydrolysis of diethyl 3-(3',4'-dichlorophenyl)-glutarate **112** to the corresponding (*S*)-monoester **113**

responding (*S*)-monoester **113** has been developed using lipase B from *Candida antarctica* in either free or immobilized forms. At 100 g/L of substrate input, a reaction yield of 97 % and e.e. of greater than 99 % were obtained for desired (*S*)-monoester. The process was scaled up to produce 200 kg of product in overall 80 % yield (**112**). DNA family shuffling was used to create chimeric lipase B protein with improved activity towards diethyl-3-(3',4'-dichlorophenyl)-glutarate. Three homologous lipases from *Candida antarctica* ATCC 32657, *Hyphozyma* sp. CBS 648.91 and *Cryptococcus tsukubaensis* ATCC 24555 were cloned and shuffled to generate a diverse gene library and, using a high-throughput screening assay, a chimeric lipase B protein having 20-fold higher activity towards substrate was identified (**113**). The thermostability of lipase was also improved by directed evolution (**114**).

## References

- Food & Drug Administration: FDA's statement for the development of new stereoisomeric drugs, *Chirality*, 4 (1992) 338–340.
- B. Buckland, D. Robinson, M. Chartrain, *Metab. Eng.* 2 (2000) 42–80.
- J. Pesti, R. Dicosimo, *Curr. Opin. Discov. Dev.* 3 (2003) 884–901.
- M. Mahmoudian, *Biocatal. Biotransform.* 18 (2000) 105–116.
- D. Stewart, *Curr. Opin. Chem. Biol.* 5 (2001) 120–129.
- A. Zak, *Curr. Opin. Chem. Biol.* 5 (2001) 130–136.
- R. Patel, *Curr. Opin. Drug Discov. Dev.* 3 (2003) 902–920.
- R. Patel: Stereoselective Biocatalysis for Synthesis of Some Chiral Pharmaceutical Intermediates. In: *Stereoselective Biocatalysis*, R. N. Patel (Ed.), Marcel Dekker, New York (2000) pp. 87–130.
- K. Yoshimura, R. Kato, M. F. Kavlick, A. Nguyen, V. Maroun, K. Maeda, K. Hussain, A. Ghosh, S. Gulnik, J. Erickson, H. Mitsuya, *J. Virol.* 76 (2002) 1349–1358.
- A. Tomasselli, S. Thaisrivongs, R. Henrikson, *Advances in Antiviral Drug Design*, 2 (1996) 173–228.
- G. Bold, A. Fassler, H. Capraro, R. Cozens, T. Klimkait, J. Lazdins, J. Mestan, B. Poncioni, J. Rosel, D. Stover, M. Tintelnot-Blomley, F. Acemoglu, W. Beck, E. Boss, M. Eschbach, T. Hurlimann, E. Masso, S. Roussel, K. Ucci-Stoll, D. Wyss, M. Lang, *J. Med. Chem.* 41 (1998) 3387–3401.
- B. Robinson, K. Riccardi, Y.-F. Gong, Q. Guo, D. Stock, W. Blair, B. Terry, C. Deminie, F. Djang, R. Colonno, P.-F. Lin, *Antimicrob. Agents Chemother.* 44 (2000) 2093–2099.
- A. Fassler, G. Bold, H.-G. Capraro, H. Steiner, *Tetrahedron Lett.* 39 (1998) 4925–4928.
- Z. Xu, J. Singh, M. Schwinden, B. Zheng, T. Kissick, B. Patel, M. Humora, F. Quiroz, L. Dong, D.-M. Hsieh, J. Heikes, M. Pudipeddi, M. Lindrud, S. Srivastava, D. Kronenthal, R. Mueller, *Org. Proc. Res. Develop.* 6 (2002) 323–328.
- R. Patel, L. Chu, R. Mueller, *Tetrahedron: Asymmetry*, 14 (2003) 3105–3109.
- J. Barrish, E. Gordon, M. Alam, P. Lin, G. Bisacchi, P. Cheng, A. Fritz, J. Greytok, M. Hermsmeier, W. Humphreys, K. Lis, M. Marella, Z. Merchant, T. Mitt, R. Morrison, M. Obermeier, J. Pluscec, M. Skoog, W. Slusarchyk, S. Spergel, J. Stevenson, C. Sun, J. Sundeen, P. Taunk, T. Tino, B. Warrack, R. Colono, R. Zahler, *J. Med. Chem.* 37 (1994) 1758–1771.
- R. Patel, A. Banerjee, C. McNamee, D. Brzozowski, L. Szarka, *Tetrahedron: Asymmetry*, 8 (1997) 2547–2552.
- M. Chartrain, B. Jackey, C. Taylor, V. Sandfor, K. Gbewonyo, L. Lister, L. Dimichele, C. Hirsch, B. Heimbuch, C. Maxwell, D. Pascoe, B. Buckland, R. Greasham, *J. Ferment. Bioeng.* 86 (1998) 550–558.
- B. Buckland, D. Robinson, M. Chartrain, *Metab. Eng.* 2 (2000) 42–48.
- M. Chartrain, P. Salmon, D. Robinson, B. Buckland, *Curr. Opin. Biotechnol.* 11 (2000) 209–214.
- N. Zhang, B. Stewart, J. Moore, R. Greasham, D. Robinson, B. Buckland, C. Lee, *Metab. Eng.* 2 (2000) 339–348.
- A. Amanullah, C. Hewitt, A. Nienow, C. Lee, M. Chartrain, B. Buckland, S. Drew, J. Woodley, *Biotechnol. Bioeng.* 80 (2002) 239–249.

23. C. Ireland, P. Leeson, J. Castaner, *Drug. Future*, 22 (1997) 359–370.
24. R. Hanson, Z. Shi, D. Brzozowski, A. Banerjee, T. Kissick, J. Singh, A. Pullockaran, J. North, J. Fan, J. Howell, S. Durand, M. Montana, D. Kronenthal, R. Mueller, R. Patel, *Bioorg. Med. Chem.* 8 (2000) 2681–2687.
25. S. Pianko, J. McHutchison, *J. Gastroent. Hepatol.* 15 (2000) 581–586.
26. P. Ferenci, H. Brunner, K. Nachbaur, C. Datz, M. Gschwantler, H. Hofer, R. Stauber, J. H. Franz, J. Wolfgang, R. Martha, M.-S. Petra, H. Karin, G. Alfred, V. Wolfgang, *Hepatology*, 34 (2001) 1006–1011.
27. S. Sauleda, A. Juarez, J. Esteban, C. Altisent, I. Ruiz, L. Puig, R. Esteban, J. Guardia, *Hepatology*, 34 (2001) 1035–1040.
28. A. Chutaputti, *J. Gastroent. Hepatol.* 15 (2000) E156–E163.
29. H. Bonkovsky, D. Stefancyk, K. McNeal, B. Banner, Q. Liu, G. Zucker, J. Israel, J. Stagiass, J. Colker, *Dig. Dis. Sci.* 46 (2001) 2051–2059.
30. M. Tamarez, B. Morgan, G. Wong, W. Tong, F. Bennett, R. Lovey, J. McCormick, A. Zaks, *Org. Proc. Res. Develop.* 7 (2003) 951–953.
31. G. Hoegenauer, *Antibiotics*, 5 (1979) 344–360.
32. H. Berner, H. Vyplel, G. Schulz, P. Stuchlik, *Tetrahedron*, 39 (1983) 1317–1321.
33. R. Hanson, J. Matson, D. Brzozowski, T. LaPorte, D. Springer, R. Patel, *Org. Proc. Res. Develop.* 6 (2002) 482–487.
34. D. Springer, M. Sorenson, S. Huang, T. Connolly, J. Bronson, J. Matson, R. Hanson, D. Brzozowski, T. LaPorte, R. Patel, *Bioorg. Med. Chem. Lett.* 13 (2003) 1751–1753.
35. M. Suffness, M. Wall: *Taxol: Science and Application*, M. Suffness (Ed.), CRC Press, New York (1995).
36. R. Holton, R. Biediger, P. Joatman: Semisynthesis of Taxol and Taxotere. In: *Taxol: Science and Application*, M. Suffness (Ed.), CRC Press, New York (1995) pp. 97–123.
37. D. Kingston: Natural Taxoids: Structure and Chemistry, In: *Taxol: Science and Application*, M. Suffness (Ed.), CRC Press, New York (1995) pp. 287–317.
38. E. Baldini, T. Prochilo, B. Salvadori, A. Bolognesi, D. Aldrighetti, M. Venturini, R. Rosso, F. Carnino, L. Gallo, P. Giannessi, P. Conte, C. Orlandini, P. Bruzzi, *Br. J. Cancer*, 91 (2004) 45–49.
39. P. Schiff, J. Fant, S. Horwitz, *Nature*, 277 (1979) 665–667.
40. R. Patel, *Ann. Rev. Microbiol.* 98 (1995) 361–395.
41. M. Wani, H. Taylor, M. Wall, P. Coggon, A. McPhail, *J. Am. Oil Chem. Soc.* 93 (1971) 2325–2327.
42. D. G. I. Kingston, A. A. Molinero, J. M. Rimoldi: The Taxane Diterpenoids. In: *Progress in the Chemistry of Organic Natural Products*, Vol. 61, W. Herz, H. Falk, G. W. Kirby (Eds.), Springer Verlag, Wien (1993) pp. 1–206.
43. M. Mattina, G. MacEachern, *J. Chromatogr.* 679 (1994) 269–275.
44. R. Hanson, J. Wasyluk, V. Nanduri, D. Cazzulino, R. Patel, L. Szarka, *J. Biol. Chem.* 269 (1994) 22145–22149.
45. V. Nanduri, R. Hanson, T. LaPorte, R. Ko, R. Patel, L. Szarka, *Biotechnol. Bioeng.* 48 (1995) 547–550.
46. R. Hanson, J. Howell, D. Brzozowski, S. Sullivan, R. Patel, L. Szarka, *Biotechnol. Appl. Biochem.* 26 (1997) 153–158.
47. R. Patel, A. Banerjee, J. Howell, C. McNamee, D. Brzozowski, D. Mirfakhrae, V. Nanduri, J. Thottathil, L. Szarka, *Tetrahedron: Asymmetry*, 4 (1993) 2069–2084.
48. R. Patel, A. Banerjee, R. Ko, J. Howell, W.-S Li, F. Comezoglu, R. Partyka, L. Szarka, *Biotechnol. Appl. Biochem.* 20 (1994) 23–33.
49. E. Baloglu, D. Kingston, *J. Nat. Prod.* 62 (1999) 1068–1071.
50. W. Rose, B. Long, C. Fairchild, F. Lee, J. Kadow, *Clin. Cancer Res.* 7 (2001) 2016–2021.
51. R. Patel, J. Howell, R. Chidambaram, S. Benoit, J. Kant, *Tetrahedron: Asymmetry*, 14 (2003) 3673–3677.
52. R. Hanson, R. Patel, *Process for the Preparation of C-4 Deacetyltaxanes*, WO 2000010989 A1 20000302 CAN 132:194525 AN 2000:144872, *PCT Int. Appl.* (2000) pp. 17.
53. H. Kagechika, E. Kawachi, Y. Hashimoto, K. Shudo, T. Himi, *J. Med. Chem.* 31 (1988) 2182–2192.
54. K. Shudo, H. Kagechika: *Chemistry and Biology of Synthetic Retinoids*, M. Dawson, W. Okamura (Eds.), CRC Press, Boca Raton (1989).
55. G. Morriss-Kay: Retinoids: Their Physiological Function and Therapeutic Potential. In: *Advances in Organ Biology*, Vol. 3, G. V. Sherber (Ed.), Elsevier Science (1997) 79–92.
56. R. Moon, L. Itri, *Retinoids*, 2 (1984) 327–331.
57. H. Kagechika, E. Kawachi, Y. Hashimoto, T. Himi, K. Shudo, *J. Med. Chem.* 31 (1988) 2182–2192.
58. M. Belema, F. Zusi, K. Trampusch, *Active Enantiomer of RAR  $\gamma$ -Specific Agonist*. WO 0016769 CAN 132:246379 AN 2000:209902, *PCT Int. Appl.* (2000) pp. 32.
59. R. Patel, L. Chu, R. Chidambaram, J. Zhu, J. Kant, *Tetrahedron: Asymmetry*, 12 (2002) 349–355.
60. D. Schenk, D. Games, P. Seubert, *J. Mol. Neurosci.* 17 (2001) 259–265.
61. J. Audia, T. Britton, J. Droste, B. Folmer, G. Huffman, V. John, H. Lee, T. Mabry, J. Nissen, WO 9822494 A2 19980528 CAN 129:41414, *PCT Int. Appl.* (1998).
62. R. Patel, A. Banerjee, V. Nanduri, A. Goswami, F. Comezoglu, *J. Am. Oil Chem. Soc.* 77 (2000) 1015–1019.
63. V. Nanduri, A. Banerjee, J. Howell, D. Brzozowski, R. Eiring, R. Patel, *J. Ind. Microbiol. Biotechnol.* 25 (2000) 171–175.
64. V. Nanduri, R. Hanson, A. Goswami, J. Wasyluk, T. LaPorte, K. Katipally, H.-J. Chung, *Enzyme Microb. Technol.* 28 (2001) 632–636.
65. R. Patel, A. Goswami, L. Chu, M.-J. Donovan, V. Nanduri, S. Goldberg, R. Johnston, P. J. Siva, B. Nielsen, F. Junying, W. X. He, Z. P. Shi, K. Wang, R. Eiring, D. Cazzulino, A. Singh, R. Mueller, *Tetrahedron: Asymmetry*, 15 (2004) 1247–1258.
66. A. Archelas, R. Furstoss, *Top. Curr. Chem.* 200 (1999) 159–191.
67. A. Steinreiber, K. Faber, *Curr. Opin. Biotechnol.* 12 (2001) 552–558.
68. J. Catt, G. Johnson, D. Keavy, R. Mattson, M. Parker, K. Takaki, J. Yevich, US 5856529, CAN 130:110151 (1999).
69. A. Goswami, M. Tottleben, A. Singh, R. Patel, *Tetrahedron: Asymmetry*, 10 (1999) 3167–3175.
70. B. Schnell, K. Faber, W. Kroutil, *Adv. Synth. Catal.* 345 (2003) 653–666.
71. H. Stecher, K. Faber, *Synthesis*, 1 (1997) 1–16.
72. A. Goswami, D. Mirfakhrae, R. Patel, *Tetrahedron: Asymmetry*, 10 (1999) 4239–4244.
73. J. Arch, *Pharmacol. Rev. Commun.* 9 (1997) 141–148.
74. J. Bloom, M. Dutia, B. Johnson, A. Wissner, M. Burns, E. Largis, J. Dolan, T. Claus, *J. Med. Chem.* 35 (1992) 3081–3084.
75. L. Fisher, P. Sher, S. Skwish, I. Michael, S. Seiler, K. Dickinson, *Bioorg. Med. Chem. Lett.* 6 (1994) 2253–2258.
76. R. Patel, A. Banerjee, L. Chu, D. Brzozowski, V. Nanduri, L. Szarka, *J. Am. Oil Chem. Soc.* 75 (1998) 1473–1482.
77. R. Patel, C. McNamee, A. Banerjee, J. Howell, R. Robison, L. Szarka, *Enzyme Microb. Technol.* 14 (1992) 731–738.
78. A. Matsuyama, H. Yamamoto, Y. Kobayashi, *Org. Proc. Res. Develop.* 6 (2002) 558–561.

79. S. Davis, G. Christopher, H. John, D. Gray, J. Gruber, G. Huisman, S. Ma, L. Newman, R. Sheldon, *WO 2004015132 A2 20040219 CAN 140:198191 AN 2004:143313, PCT Int. Appl.* (2004) pp. 168.
80. A. Fishman, M. Eroshov, D.-N. Sheffer, V. Jan, U. Cogan, R. Effenberger, *Biotechnol. Bioeng.* 74 (2001) 256–263.
81. R. Patel, A. Banerjee, C. McNamee, D. Brzozowski, R. Hanson, L. Szarka, *Enzyme Microb. Technol.* 15 (1993) 1014–1021.
82. S. Sit, R. Parker, I. Motoe, H. Balsubramanian, C. Catt, P. Brown, W. Harte, M. Thompson, J. Wright, *J. Med. Chem.* 33 (1990) 2982–2999.
83. H. Gijzen, C.-H. Wong, *J. Am. Chem. Soc.* 116 (1994) 8422–8423.
84. W. Greenberg, A. Varvak, S. Hanson, K. Wong, H. Huang, P. Chen, M. Burk, *Proc. Natl. Acad. Sci. USA*, 101 (2004) 5788–5793.
85. M. Chaffman, R. Brogden, *Drugs*, 29 (1985) 387–390.
86. C. Kawai, T. Konishi, E. Matsuyama, H. Okazaki, *Circulation*, 63 (1981) 1035–1038.
87. T. Isshiki, B. Pegram, E. Frohlich, *Cardiovasc. Drug Ther.* 2 (1988) 539–544.
88. D. Floyd, R. Moquin, K. Atwal, S. Ahmed, S. Spergel, J. Gougoutas, M. Malley, *J. Org. Chem.* 55 (1990) 5572–5575.
89. R. Patel, R. Robison, L. Szarka, J. Kloss, J. Thottathil, R. Mueller, *Enzyme Microb. Technol.* 13 (1991) 906–912.
90. A. Arisawa, M. Matsufuji, T. Nakashima, K. Dobashi, K. Isshiki, T. Yoshioka, S. Yamada, H. Momose, S. Taguchi, *Appl. Environ. Microbiol.* 68 (2002) 2716–2725.
91. H. Waldmann, A. Heuser, S. Schulze, *Tetrahedron Lett.* 37 (1996) 8725–8728.
92. C. Costello, A. Kreuzman, M. Zmijewski, *Tetrahedron Lett.* 37 (1996) 7469–7472.
93. A. Bommarius, K. Drauz, K. Gunther, G. Knaup, M. Schwarm, *Tetrahedron: Asymmetry*, 8 (1997) 3197–3200.
94. J. Ogawa, S. Shimizu, *Stereoselective Biocatalysis*, R. Patel (Ed.), Marcel & Dekker, New York (2000) pp. 1–22.
95. H. Sajiki, K. Hattori, K. Hirota, *J. Org. Chem.* 63 (1998) 7990–7992.
96. G. Royer, W. Chow, K. Hatton, *J. Mol. Catal.* 31 (1985) 1–13.
97. R. Patel, V. Nanduri, D. Brzozowski, C. McNamee, A. Banerjee, *Adv. Synth. Catal.* 345 (2003) 830–834.
98. V. Nanduri, S. Goldberg, R. Johnston, R. Patel, *Enzyme Microb. Technol.* 34 (2004) 304–312.
99. G. Bisacchi, W. Slusarchyk, U. Treuner, J. Sutton, R. Zahler, S. Seiler, D. Kronenthal, M. Rondazzo, Z. Xu, Z. Shi, M. Schwinden, *WO 9967215 A1 19991229 CAN 132:64103 AN 1999:819347* (1999).
100. B. Wirz, W. Walther, *Tetrahedron: Asymmetry*, 3 (1992) 1049–1054.
101. A. Goswami, J. Howell, E. Hua, K. Mirfakhrae, M. Soumeillant, S. Swaminathan, X. Qian, F. Quiroz, T. Vu, X. Wang, B. Zheng, D. Kronenthal, R. Patel, *Org. Proc. Res. Develop.* 5 (2001) 415–420.
102. O. Ward, M. Baev: In: *Stereoselective Biocatalysis*, R. Patel (Ed.), Marcel Dekker, New York (2000) 267–287.
103. H. Iding, P. Siegert, K. Mesch, M. Pohl, *Biochim. Biophys. Acta*, 1385 (1998) 307–322.
104. H. Iding, T. Dunnwald, L. Greiner, A. Liese, M. Muller, P. Siegert, J. Grotzinger, A. Demir, M. Pohl, *Chem. Eur. J.* 6 (2000) 1483–1495.
105. Z. Guo, Goswami, D. Mirfakhrae, R. Patel, *Tetrahedron: Asymmetry*, 10 (1999) 4667–4675.
106. Z. Guo, A. Goswami, V. Nanduri, R. Patel, *Tetrahedron: Asymmetry*, 12 (2001) 571–577.
107. E. Burcher, C. Mussap, J. Stephenson: *Tachykinin Receptors*, S. Buck (Ed.), Humana Press, Totowa (1994).
108. B. Veronesi, J. Carter, R. Devlin, S. Simon, M. Oortgiesen, *Neuropeptides*, 33 (1999) 447–456.
109. L. Yuan, E. Burcher, B. Nail, *Pulm. Pharmacol. Ther.* 11 (1998) 31–39.
110. P. Ting, J. Lee, J. Anthes, N.-Y. Shih, J. Piwinski, *Bioorg. Med. Chem. Lett.* 10 (2000) 2333–2335.
111. G. Reichard, Z. Ball, R. Aslanian, J. Anthes, J. N.-Y. Shih, J. Piwinski, *Bioorg. Med. Chem. Lett.* 10 (2000) 2329–2332.
112. M. J. Homann, R. Vail, B. Morgan, V. Sabesan, C. Levy, D. R. Dodds, A. Zaks, *Adv. Synth. Catal.* 343 (2001) 744–749.
113. W.-C. Suen, N. Zhang, L. Xiao, V. Madison, A. Zaks, *Protein Eng. Des. Sel.* 17 (2004) 133–140.
114. N. Zhang, W.-C. Suen, W. Windsor, L. Xiao, V. Madison, A. Zaks, *Protein Eng.* 16 (2003) 599–605.

## Biokatalitička sinteza kiralnih farmaceutskih međuprodukata

### Sažetak

U farmaceutskoj industriji sve je važnija proizvodnja samo jednog enantiomera kao međuprodukta u proizvodnji lijekova. Kiralni međuprodutki i fine kemikalije na visokoj su cijeni u farmaceutskoj i agrokemijskoj industriji za pripremu lijekova i agrikulturnih proizvoda. Prikazan je golemi potencijal mikroorganizama za transformaciju sintetskih kemikalija što imaju kemoselektivnost, regioselektivnost i enantioselektivnost. U radu su opisani biokatalitički procesi za sintezu kiralnih farmaceutskih međuprodukata.