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Review

Synthesis of chiral pharmaceutical intermediates by biocatalysis

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Abstract

Chirality is a key factor in the safety and efficacy of many drug products and thus the production of single enantiomers of drug intermediates has become increasingly important in the pharmaceuticals industry. There has been an increasing awareness of the enormous potential of microorganisms and enzymes derived therefrom for the transformation of synthetic chemicals with high chemo-, regio- and enatio-selectivities. In this article, biocatalytic processes are described for the synthesis of chiral intermediates for pharmaceuticals.

Keywords: Biocatalysis; Enzymatic Processes; Chiral Intermediates; Drugs Synthesis

1. Introduction

The production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceuticals industry [1]. Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. Biocatalysis often offers advantages over chemical synthesis as 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 which could cause problems with isomerization, racemization, epimerization, and rearrangement. Microbial cells and enzymes derived

therefrom can be immobilized and reused for many cycles. In addition, enzymes can be overexpressed to make biocatalytic processes economically efficient, and enzymes with modified activity can be tailor-made. The tools of molecular biology including gene shuffling and directed evolution of biocatalysts can lead to increased enzyme activity, selectivity and stability [2–14]. A number of review articles [14–25] have been published on the use of enzymes in organic synthesis. The field of biocatalysis is very wide and there are number of excellent books have been published on the use of enzymes in organic synthesis and preparation of chiral compounds [26–32]. This review provides some selected examples on the use of enzymes for the synthesis of single enantiomers of key intermediates used in the key pharmaceutical synthesis. This review article excludes the development in metabolic engineering as many number of review articles are published recently [33–36].

2. Antianxiety drug

2.1. Enzymatic preparation of 6-hydroxybuspirone

Buspirone (Buspar®**1**, Fig. 1) is a drug used for treatment of anxiety and depression that is thought to produce its effects by binding to the serotonin 5HT1A receptor [37,38]. Mainly as a result of hydroxylation reactions, it is extensively converted to various metabolites [39] and blood concentrations return to low levels a few hours after dosing [40]. A major metabolite, 6-hydroxybuspirone (**2**, Fig. 1), produced by the action of liver cytochrome P450 CYP3A4, is present at much higher concentrations in human blood than buspirone itself. This metabolite has anxiolytic effects in an anxiety model using rat pups and binds to the human 5-HT1A receptor [40]. Although the metabolite has only about a third of the affinity for the human 5HT1A receptor as buspirone, it is present in human blood at 30–40 times higher concentration than buspirone following a dose of buspirone, and therefore may be responsible for much of the effectiveness of the drug [41]. For development of 6-hydroxybuspirone as a potential antianxiety drug, preparation and testing of the two enantiomers as well as the racemate was of interest. Both the (*R*)- and (*S*)-enantiomers, isolated by chiral HPLC, were effective in tests using a rat model of anxiety [42]. Whereas the (*R*)-enantiomer showed somewhat tighter binding and specificity for the 5HT1A receptor, the (*S*)-enantiomer had the advantage of being cleared more slowly from the blood. An enzymatic process was developed for resolution of 6-acetoxybuspirone **3** (Fig. 1).

L-Amino acid acylase from *Aspergillus melleus* (Amano acylase 30000) was used to hydrolyze racemic 6-acetoxybuspirone to (*S*)-6-hydroxybuspirone **2** in 96% e.e. after 46% conversion. The remaining (*R*)-6-acetoxybuspirone with 84% e.e. was converted to (*R*)-6-hydroxybuspirone **2** by acid hydrolysis [43]. The e.e. of both enantiomers could be improved to >99% by crystallization. Direct hydroxylation of buspirone to (*S*)-6-hydroxbuspirone by *Streptomyces antibioticus* ATCC 14980 has also been described [43].

In an alternate process, enantioselective microbial reduction of 6-oxobuspirone (**4**, Fig. 2) to either (*R*)-and (*S*)-6-hydroxybuspirone was described by Patel et al. [44]. About 150 microorganisms were screened for the enantioselective reduction of **4**. *Rhizopus stolonifer* SC 13898, *R. stolonifer* SC 16199, *Neurospora crassa* SC 13816, *Mucor racemosus* SC 16198, and *Pseudomonas putida* SC 13817 gave >50% reaction yields and >95% e.e.'s of (*S*)-6-hydroxybuspirone. The yeast

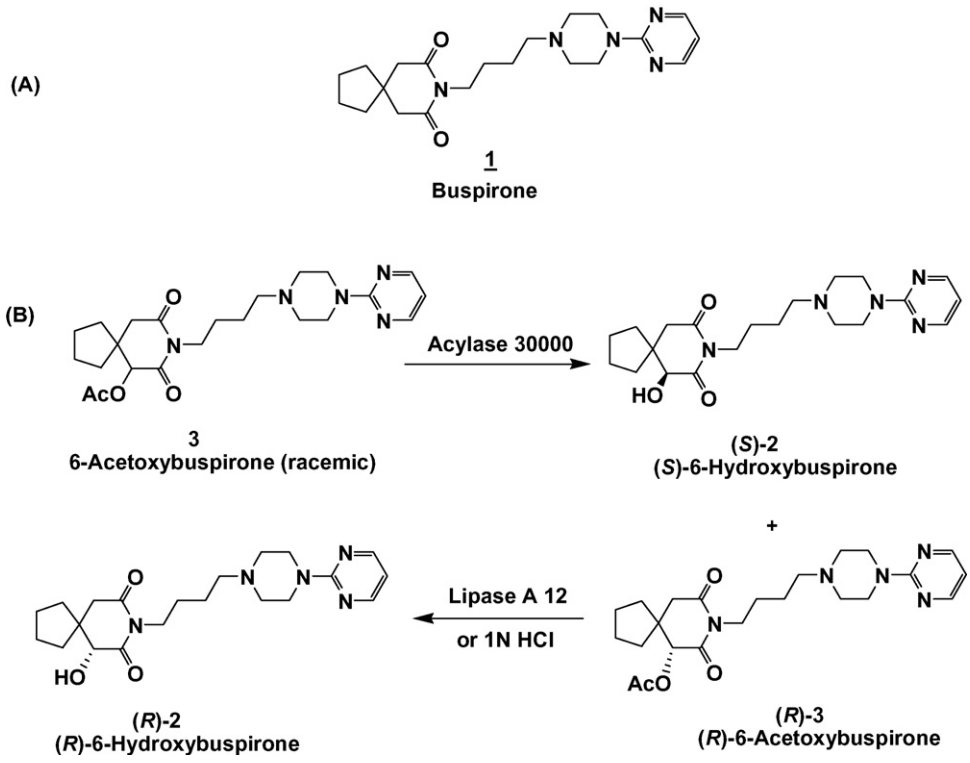

Fig. 1. (A) Antianxiety drug, Busiprone (B) enzymatic resolution of 6-acetoxybuspirone.

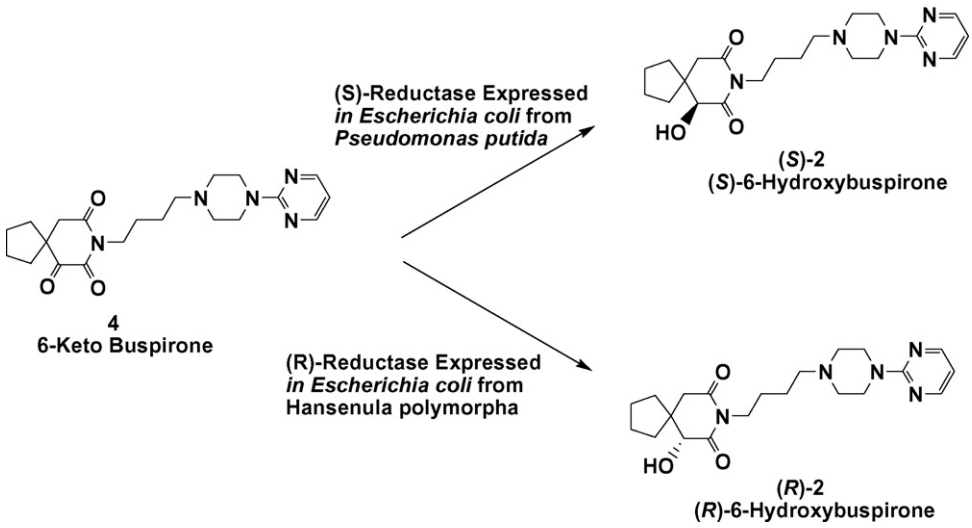

Fig. 2. Enantioselective enzymatic reduction of 6-oxobuspirone.

strains *Hansenula polymorpha* SC 13845 and *Candida maltosa* SC 16112 gave (*R*)-6-hydroxybuspirone **2** in >60% reaction yield and >97% e.e. The NADP-dependent (*R*)-reductase (RHBR) which catalyzes the reduction of 6-oxobuspirone to (*R*)-6-hydroxybuspirone was purified to homogeneity from cell extracts of *H. polymorpha* SC 13845. (*R*)-reductase from *H. polymorpha* SC 13845 was cloned and expressed in *Escharichia coli*. To regenerate the cofactor NADPH required for reduction we have also cloned and expressed the glucose-6-phosphate dehydrogenase gene from *Saccharomyces cerevisiae* in *E. coli*.

The NAD-dependent (*S*)-reductase (SHBR) which catalyzes the reduction of 6-ketobuspirone **4** to (*S*)-6-hydroxybuspirone **2** was also purified to homogeneity from cell extracts of *P. putida* SC 16269. The (*S*)-reductase from *P. putida* SC 16269 was cloned and expressed in *E. coli*. To regenerate the cofactor NADH required for reduction we have also cloned and expressed the formate dehydrogenase gene from *Pichia pastoris* in *E. coli*. Recombinant *E. coli* expressing (*S*)-reductase and (*R*)-reductase catalyzed the reduction of 6-ketobuspirone to (*S*)-6-hydroxybuspirone and (*R*)-6-hydroxybuspirone, respectively, in >98% yield and >99.9% e.e. [45].

3. Antidiabetic drug (DPP-IV inhibitor, Saxagliptin)

3.1. Enzymatic reductive amination of 2-(3-hydroxy-1-adamantyl)-2-oxoethanoic acid

Dipeptidyl peptidase IV (DPP-IV) is a ubiquitous proline-specific serine protease responsible for the rapid inactivation of incretins, including glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide. To alleviate the inactivation of GLP-1, inhibitors of DPP-IV are being evaluated for their ability to provide improved control of blood glucose for diabetics [46–48]. Januvia developed by Merck is a marketed DPP4 Inhibitor [49].

Saxagliptin **5** [48,50] (Fig. 3A), a DPP-IV inhibitor under development by Bristol–Myers Squibb, requires (*S*)-*N*-boc-3-hydroxyadamantylglycine **7** (Fig. 3B) as an intermediate. Previously, several unnatural amino acids [51–53] as intermediates for synthetic routes to various drugs have been prepared by reductive amination of the corresponding keto acids by us. We have developed a process for conversion of the keto acids **6** to the corresponding amino acid **7** using (*S*)-amino acid dehydrogenases. A modified form of a recombinant phenylalanine dehydrogenase cloned from *Thermoactinomyces intermedius* and expressed in *P. pastoris* or *E. coli* was used for this process. NAD produced during the reaction was recycled to NADH using formate dehydrogenase. The modified phenylalanine dehydrogenase contains two amino acid changes at the C-terminus and a 12 amino acid extension of the C-terminus [54,55].

Production of multi-kg batches was originally carried out with extracts of *P. pastoris* expressing the modified phenylalanine dehydrogenase from *T. intermedius* and endogenous formate dehydrogenase. The reductive amination process was further scaled up using a preparation of the two enzymes expressed in single recombinant *E. coli*. The amino acid **7** was directly protected as its boc derivative without isolation to afford intermediate **8**. Yields before isolation were close to 98% with 100% e.e. [54,55].

Reductive amination was also conducted using cell extracts from *E.coli* strain SC16496 expressing PDHmod and cloned FDH from *P. pastoris*. Cell extracts after polyethyleneamine treatment, clarification and concentration were used to complete the reaction in 30 h with >96% yield and >99.9% e.e. of product **7**. This process has now been used to prepare several hundred kg of boc-protected amino acid **8** to support the development of Saxagliptin [54,55].

3.2. Enzymatic ammonolysis of (5S)-4,5-dihydro-1H-pyrrole-1,5-dicarboxylic acid, 1-(1,1-dimethylethyl)-5-ethyl ester

The synthesis of dipeptidyl peptidase-IV inhibitor, Saxagliptin **5** also required (5*S*)-5-aminocarbonyl-4,5-dihydro-1H-pyrrole-1-carboxylic acid, 1-(1,1-dimethylethyl)ester (**10**, Fig. 3C). Direct chemical ammonolyses were hindered by the

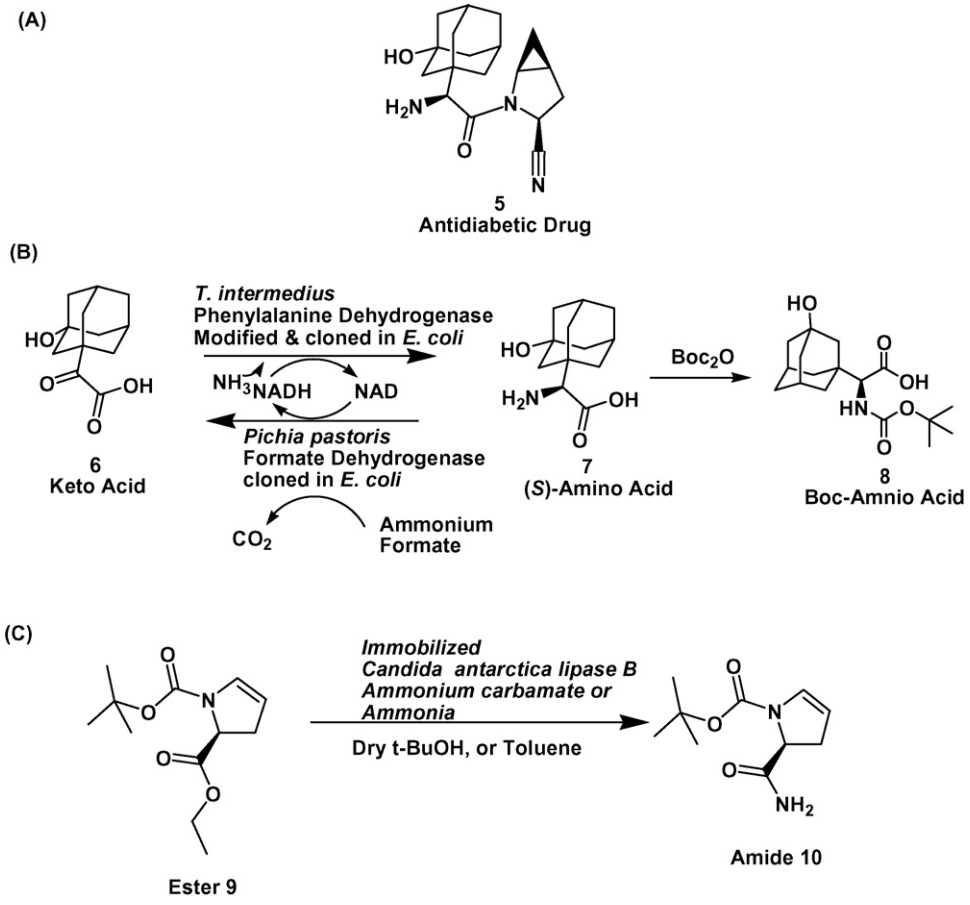

Fig. 3. (A) Antidiabetic drug, Saxagliptin (B) enzymatic reductive amination of 2-(3-hydroxy-1-adamantyl)-2-oxoethanoic acid (C) enzymatic ammonolysis of (5*S*)-4,5-dihydro-1H-pyrrole-1,5-dicarboxylic acid, 1-(1,1-dimethylethyl)-5-ethyl ester.

requirement for aggressive reaction conditions which resulted in unacceptable levels of amide racemization and side-product formation, while milder two-step hydrolysis-condensation protocols using coupling agents such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), [56] initially suffered with low overall yields. To address this issue, a biocatalytic procedure was developed, based upon the *Candida antartica* lipase B (CALB)-mediated ammonolysis of (5*S*)-4,5-dihydro-1H-pyrrole-1,5-dicarboxylic acid, 1-(1,1-dimethylethyl)-5-ethyl ester **9** with ammonium carbamate to furnish **10** without racemization and with low levels of side-product formation [56].

Screening experiments utilized process stream ester feed, which consisted of ca. 22% w/v (0.91 M) of the ester in toluene. Since the latter precluded the use of free ammonia due to its low solubility in toluene, solid ammonium carbamate was employed. Reactions were performed using a mixture of neat process feed, ammonium carbamate (71 g/L, 2 mol equivalent of ammonia) and biocatalyst (25 g/L), shaken at 400 rpm, 50 °C. Under these conditions, CALB and its immobilized forms Novozym 435 and Chirazyme L2 provided racemization-free amide with yields of 69, 43 and 40%, together with 21, 18 and 22% of side-products (by HPLC) respectively, while all other biocatalysts (lipases) furnished less than 5% of the desired product [57]. The ammonolysis reaction with free CALB was then optimized. The inclusion of various additives was investigated with the aim of ameliorating potential inhibitory phenomena, shifting the equilibrium towards amide synthesis and reducing side-product formation. Drying agents such as calcium chloride gave significant improvement (79% amide and 13% side-products). The calcium chloride is known to complex alcohols as well as act as a dessicant, and its presumed binding of ethanol released during the course of amide formation may have served to mitigate any deleterious effects of this alcohol on CALB catalysis. A dramatic increase in amide yield to 84 and 95% was achieved by including Sodalime and Ascarite, respectively, at 200 g/L in the reaction headspace, this presumably by way of adsorption of carbon dioxide liberated from the decomposition of ammonium carbamate. A further increase in yield to 98% was attained via the combined use of 100 g/L of calcium chloride and 200 g/L of Ascarite [57].

A prep-scale reaction with the process ester feed was used. Ester (220 g/L) was reacted with 90 g/L (1.25 mol equivalents) of ammonium carbamate, 33 g/L (15% w/w of ester input) of CALB, 110 g/L calcium chloride and 216 g/L of Ascarite (in the headspace), run at 50 °C, 3 d. Complete conversion of ester was achieved, with the formation of 96% (182 g/L) of amide **10** and 4% of side-products, and after workup 98% potency amide of >99.9% e.e. was isolated in 81% yield [57].

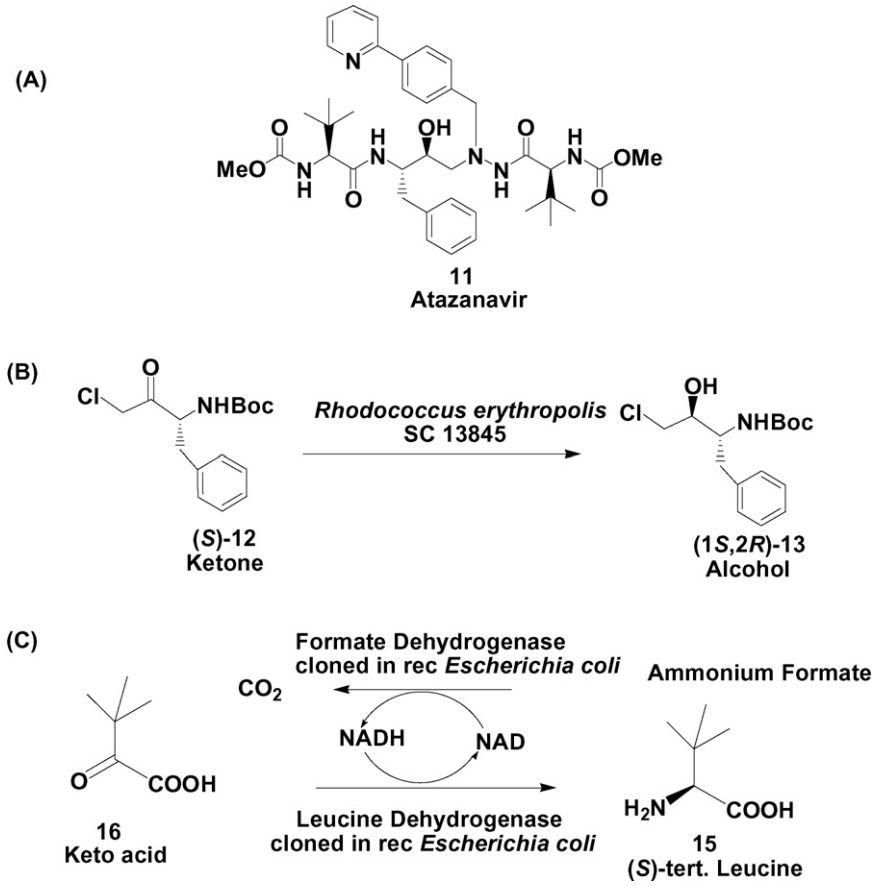

Fig. 4. (A) HIV protease inhibitor, Atazanavir (B) diastereoselective enzymatic reduction of (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethylethyl ester (C) enzymatic synthesis of (*S*)-*tertiary*-leucine.

4. Antiviral drug (HIV protease inhibitor, Reyataz)

4.1. Enzymatic preparation of (1S,2R)-[3-chloro-2-hydroxy-1-(phenylmethyl) propyl]-carbamic acid, 1,1-dimethyl-ethyl ester

Atazanavir (Reyataz, **11** Fig. 4A) is an acyclic aza-peptidomimetic, a potent HIV protease inhibitor [58,59] approved recently by the Food and Drug Administration for treatment of acquired immuno deficiencies syndrome (AIDS). An enzymatic process has been developed for the preparation of (1*S*,2*R*)-[3-chloro-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester (**13**, Fig. 4B), a key chiral intermediate required for the total synthesis of the HIV protease inhibitor atazanavir. The diastereoselective reduction of (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethylethyl ester (**12**) was carried out using *Rhodococcus,Brevibacterium*, and *Hansenula* strains to provide (1*S*,2*R*)-**13**. Three strains of *Rhodococcus* gave >90% yield with a diastereomeric purity of >98% and an e.e. of 99.4% [60]. An efficient single-stage fermentation–biotransformation process was developed for the reduction of ketone **12** with cells of *Rhodococcus erythropolis* SC 13845 to yield **13** in 95% with a diasteromeric purity of 98.2% and an e.e. of 99.4%. Chemical reduction of chloroketone **12** using $NaBH_4$ produces primarily the undesired chlorohydrin diastereomer [61]. (1*S*,2*R*)-**13** was converted to epoxide **14** (Fig. 5) and used in the synthesis of atazanavir [61].

4.2. Enzymatic synthesis of (S)-tertiary-leucine

Synthesis of Atazanavir (**11**) also required (*S*)-*tertiary* leucine (**15**, Fig. 4C). An enzymatic reductive amination of ketoacid **16** to amino acid **15** by recombinant *E. coli* expressing leucine dehydrogenase from *Thermoactinimyces intermedius* has been demonstrated [62]. The reaction required ammonia and NADH as a cofactor. NAD produced during the reaction was converted back to NADH using recombinant *E. coli* expressing formate dehydrogenase from *P. pastoris*. A reaction yield of >95% with an e.e. of >99.5% was obtained for **15** at 100 g/L substrate input. The chiral epoxide **14** prepared from (1*S*,2*R*)-**13** was coupled to compound **17** to obtain compound **18**. *N*-methoxy carbonyl-(*S*)-*tert*-leucine **19** prepared from (*S*)-*tertiary* leucine **15** was coupled to compound **18** (Fig. 5) and after deprotection afforded Atazanavir **11** in the final step of the synthesis [61].

5. Anitiviral drug (Lobucavir)

5.1. Regioselective enzymatic aminoacylation

Lobucavir **20** (Fig. 6) is a cyclobutyl guanine nucleoside analog potentiallu useful as an antiviral agent for the treatment of

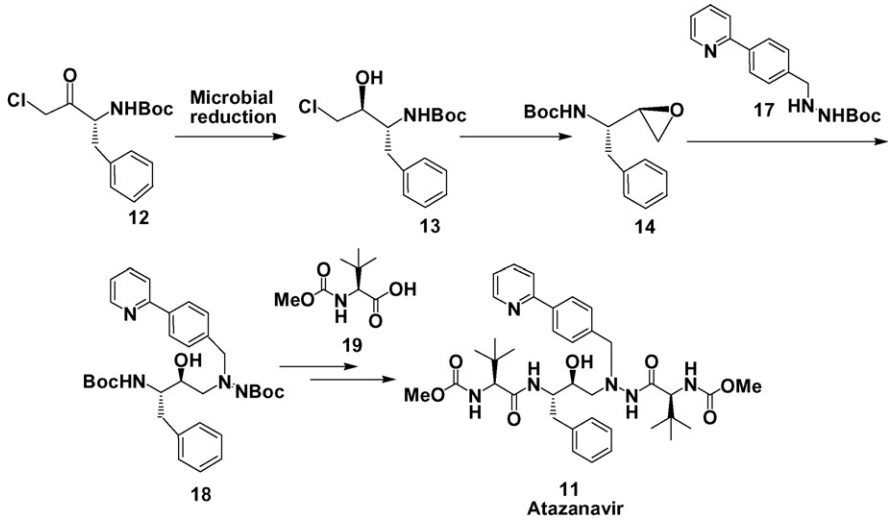

Fig. 5. Chemo-enzymatic synthesis of Atazanavir.

herpes virus and hepatitis B [63]. A prodrug in which one of the two hydroxyls is coupled to valine, **21**, has also been considered for development. Regioselective aminoacylation is difficult to achieve by chemical procedures, but appeared to be suitable for an enzymatic approach [64]. Synthesis of the lobucavir L-valine prodrug **21**, requires regioselective coupling of one of the two hydroxyl groups of lobucavir **20** with valine. Enzymatic processes were developed for aminoacylation of either hydroxyl group of lobucavir [64]. The selective hydrolysis of the di-cbz-valine ester **22** with lipase M gave **24** in 83% yield. When the

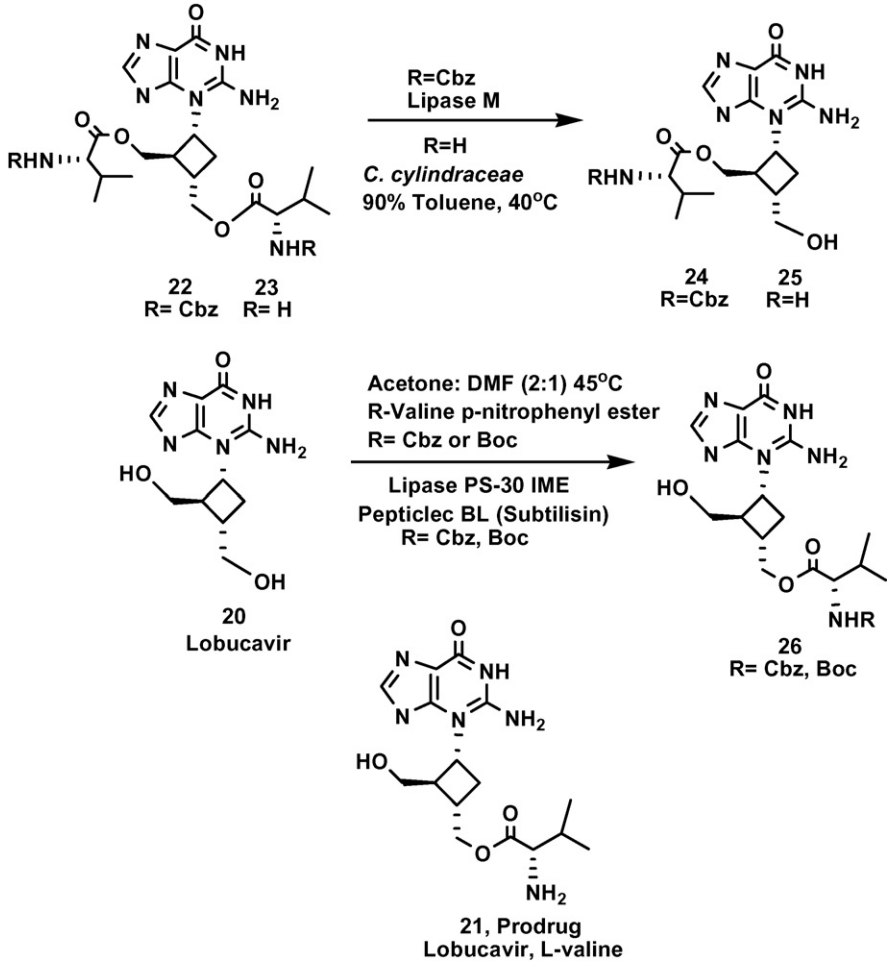

Fig. 6. Anitiviral drug (Lobucavir): regioselective enzymatic aminoacylation.

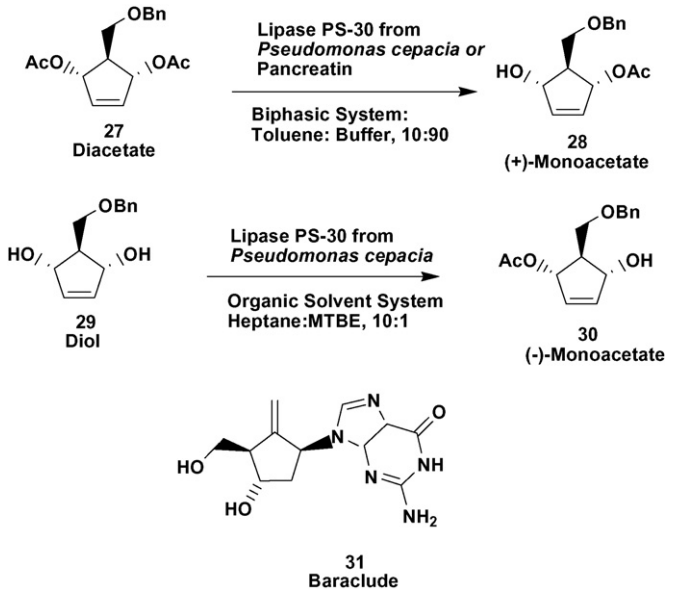

Fig. 7. Antiviral drug, Baraclude: enzymatic asymmetric hydrolysis and acetylation.

divaline ester of **23** dihydrochloride was hydrolyzed with lipase from *Candida cylindraceae*, **25** was obtained in 87% yield. The final intermediates for lobucavir prodrug, the methyl ester of **26** could be obtained by *trans*-esterification of lobucavir using ChiroCLEC^{TM} BL (61% yield) or more selectively by using lipase from *Pseudomonas cepacia* (84% yield) [64].

6. Antiviral drug (hepatitis B viral (HBV) inhibitor)

6.1. Enzymatic asymmetric hydrolysis and acetylation

Chiral monoacetate esters **28** and **30** (Fig. 7) are key intermediates for total chemical synthesis of **31** Baraclude, a potential drug for hepatitis B virus infection [65–67]. Baraclude is a carboxylic analog of 2′-deoxyguanosine in which the furanose oxygen is replaced with an exocyclic double bond has recently approved by FDA for treatment of HBV infection.

Enzymatic hydrolysis of (1α,2β,3α)]-2-[(benzyloxy)methyl]-4-cyclopenten-1,3-diol diacetate has been demonstrated by Griffith and Danishefsky [68] and Danishefsky et al. [69] to afford the corresponding monoester using acetylcholine esterase from electric eel. They have used very expensive enzyme acetylcholine esterase in asymmetric hydrolytic reaction to obtain 98 mole% (M) yield of product with 95% e.e. We have described the enantioselective asymmetric hydrolysis of (1α,2β,3α)-2-[(benzyloxy)methyl]-4-cyclopenten-1,3-diol diacetate **27** (Fig. 7) to the corresponding (+)-monoacetate **28** by cheap enzymes lipase PS-30 from *P. cepacia* and also by Pancreatin. A reaction yield of 85 M% and an e.e. of 98% were obtained using lipase PS-30. Using pancreatin, a reaction yield of 75 M% and an e.e. of 98.5% were obtained. We also demonstrated the enzymatic asymmetric acetylation of (1α,2β,3α)-2-[(benzyloxy) methyl]-4-cyclopenten-1,3-diol **29** to the corresponding (−)-monoacetate **30** in 80 M% yield and 98% e.e. using lipase PS-30 [70].

7. Abacavir (reverse transcriptase inhibitor)

7.1. Enzymatic preparation of γ–lactam 2-azabicyclo[2.2.1]hept-5-en-3-one

Abacavir (Ziagen^{TM}) **32** (Fig. 8), a 2-aminopurine nucleoside analogue, is a selective reverse transcriptase inhibitor for the treatment of human HIV and hepatitis B viruses [71]. The γ-lactam 2-azabicyclo[2.2.1]hept-5-en-3-one (**33**), is a potential intermediate useful in the synthesis of Abacavir. A biocatalytic process was developed for the resolution of racemic γ-lactam **34** (Fig. 8) to yield the desired **33** and amino acid **35** using the

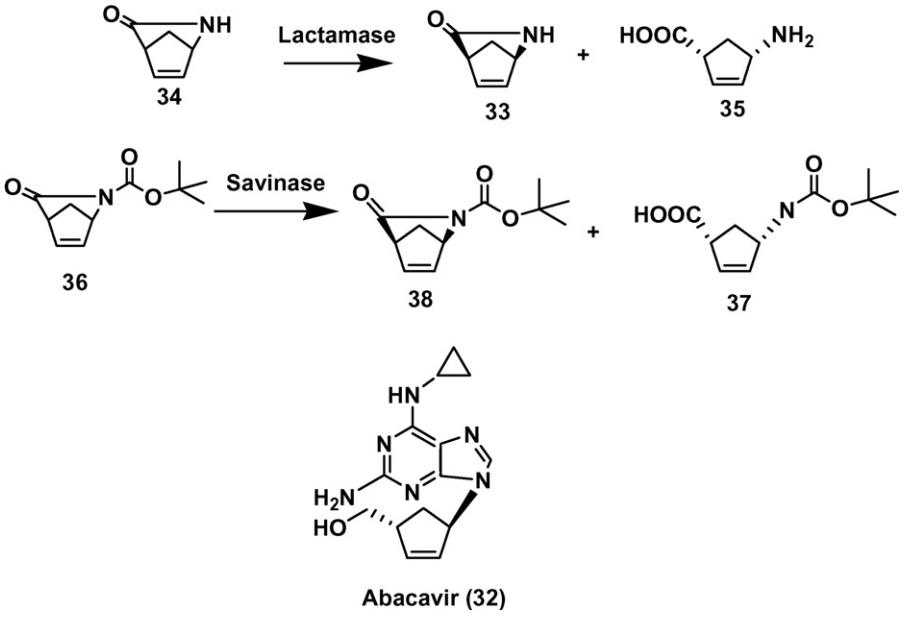

Fig. 8. Reverse transcriptase inhibitor, Abacavir: enzymatic preparation of γ-lactam 2-azabicyclo [2.2.1] hept-5-en-3-one.

γ-lactamase containing organisms *Pseudomonas solonacearum* NCIMB 40249 and *Rhodococcus* NCIMB 40213 [72]. However, due to the lack of a commercially available lactamase, an enzymatic process was developed for the enantioselective hydrolysis (in phosphate buffer, pH 8.0, containing 50% tetrahydrofuran) of racemic *tert*-butyl-3-oxo-2-azabicyclo-(2.2.1)hept-5-ene-2-carboxylate (**36**, Fig. 8). A number of commercially available enzymes hydrolyzed the lactam bond of **36** to yield the corresponding *N*-acyl amino acid **37**, leaving unreacted the desired (1*R*,4*S*)-**38**. A reaction yield of 50% and an e.e. of 99% were obtained when the reaction was carried out at 100 g/L substrate input using savinase [73].

8. Ribavirin prodrug

8.1. Regioselective enzymatic acylation of ribavirin

Ribavirin **39** (Fig. 9) is an antiviral agent used in combination of α–2β interferon to treat hepatitis C [74,75]. Although this therapy is effective against hepatitis C virus, it has several side effects [76,77]. To improve the pharmacokinetics profile and reduce side effects prodrugs of ribavirin were considered for development. In a series of preclinical evaluations, the alanine ester of ribavirin **40** showed improved bioavalibility and reduced side effects [78]. The synthesis of **40** required the acylation of unprotected ribavirin. The chemical acylation gave a mixture of mono-, di-, and triacylated products. An enzymatic process was developed for the regioselective acylation of ribavirin **39** with the oxime ester of (*S*)-carbobenzyloxy-alanine **41** to give the desired **42** using Novozym 435 (*Candida antarctica* lipase B). Chemical deprotection of **42** gave **40**. On preparative scale, the coupling of **43** with acetone oxime **44** in the presence of di-*tert*-butyl dicarbonate in THF was carried out giving **41** in >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 addition of the lipase. Following 24 h reaction at 60 °C, the product **42** was isolated in 85% yield [79].

9. Crixivan™ (HIV protease inhibitor)

9.1. Enzymatic preparation of cis-(1S,2R)-indandiol and trans-(1R,2R)-indandiol

Cis-(1*S*,2*R*)-indandiol **45** or *trans*-(1*R*,2*R*)-indandiol **45** (Fig. 10) are both potential precursors to *cis*-(1*S*,2*R*)-1-aminoindan-2-ol (**46**), a key chiral synthon for Crixivan™ (Indinavir, **47**), an HIV protease inhibitor [80]. Evaluation of microbial cultures for oxygenation reaction yielded two strains, *Rhodococcus* sp. B 264-1 (MB 5655) and I-24 (MA 7205), capable of biotransforming indene **48** to *cis*-(1*S*,2*R*)-indandiol and *trans*-(1*R*,2*R*)- indandiol, respectively [81]. *Rhodococcus* sp. MB 5655 was found to have a toluene dioxygenase, while *Rhodococcus* sp. MA 7205 was found to harbor both toluene and naphthalene dioxygenases as well as a naphthalene monooxygenase which catalyzes the 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 **45** with an e.e. of >99%. *Rhodococcus* sp. MA 7205 cultivated under similar conditions produced up to 1.4 g/L of *trans*-(1*R*,2*R*)-indandiol **45** with an e.e. of >98%. Process development studies yielded titers >4.0 g/L of *trans*-(1*R*,2*R*)-indandiol [82]. A metabolic engineering approach [83] and a directed evolution technique [84] were evaluated to avoid side reactions, block degradative pathways and enhance the key reaction to convert indene to *cis*-1-amino-1-indanol **46** or *cis*-indandiol **45**. The implementation of a single phase indene fed-batch bioconversion was carried out to get *cis*-(1*S*,2*R*)-

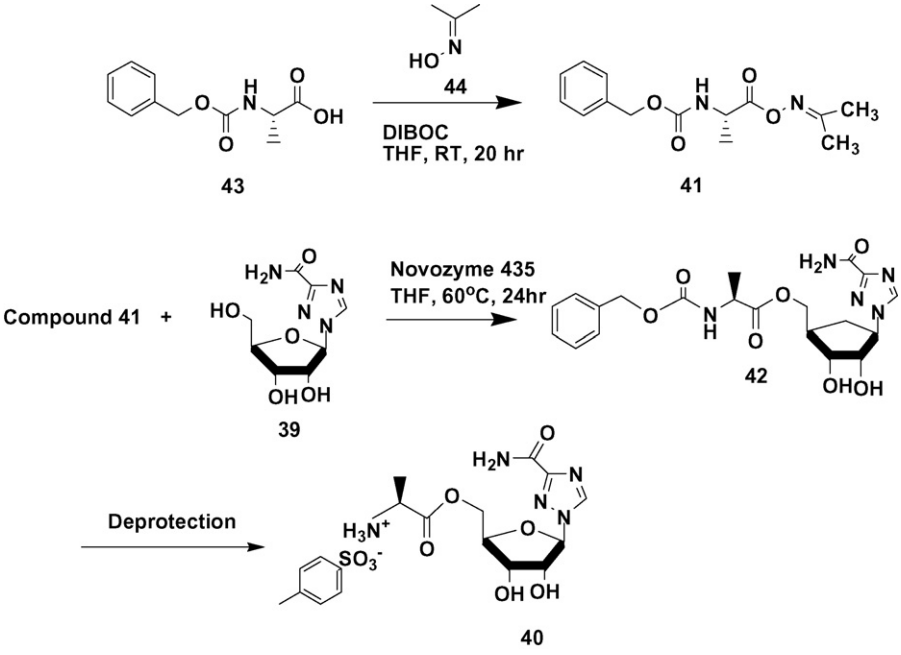

Fig. 9. Ribavirin prodrug: regioselective enzymatic acylation of ribavirin.

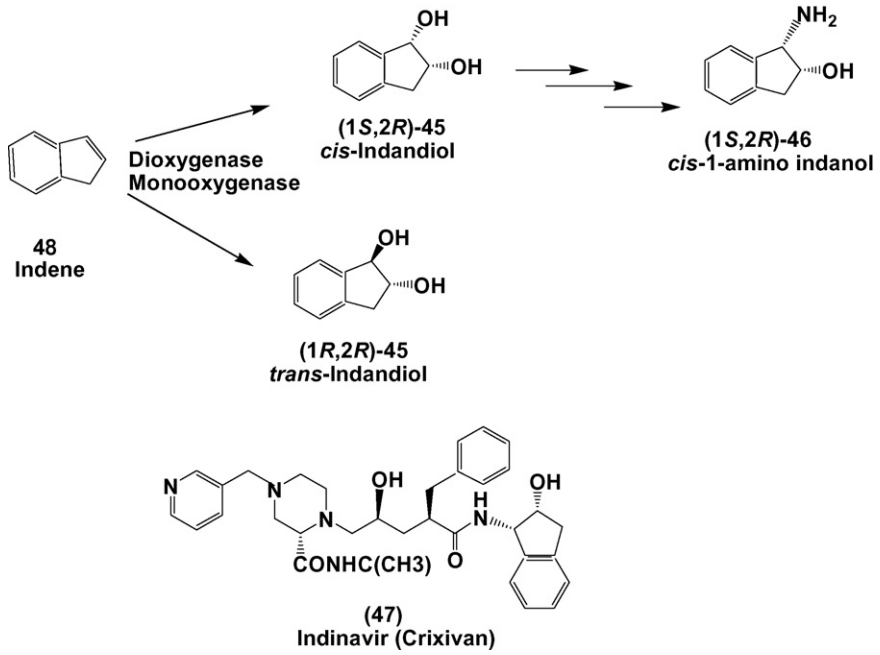

Fig. 10. HIV protease inhibitor, Crixivan: enzymatic preparation of *cis*-(1*S*,2*R*)-indandiol and *trans*-(1*R*,2*R*)-indandiol.

indandiol **45** production rates up to 200 mg/L/h by a combination of suitable indene feeding rates in the stationary phase and operating with a high-biomass concentration [85].

10. Carbovir^{TM} (*c*-d4G)

10.1. Enzymatic deamination process

The 2′,3′-didehydro-2′,3′-dideoxycarbocyclic nucleoside, (±)-carbovir, is a potent and selective inhibitor of HIV *in vitro* [86]. Its hydrolytic stability and ability to inhibit infection and replication of the virus in human T-cell lines has made carbovir a potentially useful anti-retroviral agent. Carbovir (−)-**49** (Fig. 11) is approximately two-fold more active than the corresponding racemate [87]. One of the routes to (−)-**49** was synthesis from the chiral natural fermentation product (−)-aristeromycin **50** readily available as a secondary metabolite of *Streptomyces citricolor*. Synthesis of (−)-**49** from (−)-**50** involved nine steps requiring two challanging transformations; (1) an adenine to guanine base interconversion, and (2) introduction of the 2′,3′-double bond from the 2′,3′-diol [88]. One approach for the base conversion involved the hydrolytic deamination of *cis*-4-[2,6-diamino-9H-purin-9-yl]-2-cyclopentenemethanol dihydrochloride (−)-**51** using adenosine deaminase. Using this approach (−)-**51** was prepared from aristeromycin in 8 steps [89]. The chemoenzymic route was investigated and a process developed to produce Carbovir (−)-**49** on a kilogram scale. Commercial preparations of adenosine deaminase (calf intestinal mucosa and *Aspergillus* sp.), directly adsorbed onto an anion-exchanger provided a rapid method for its recovery (50–340 units/mg protein) from crude preparation. The partially purified recovered enzyme after immobilization on Eupergit-C were used in the bioconversion process (up to 70 L working volume). The immobilized enzyme was reused up to 10 cycles without any significant loss of activity. This work demonstrated the potential of adenosine deaminase as a catalyst for large scale production of optically pure (−)-carbovir **49** [90].

10.2. Enzymatic hydroxylation of 2-cyclopentylbenzoxazole

By employing protein engineering and substrate engineering the biohydroxylation of 2-cyclopentylbenzoxazole **52** (Fig. 12)

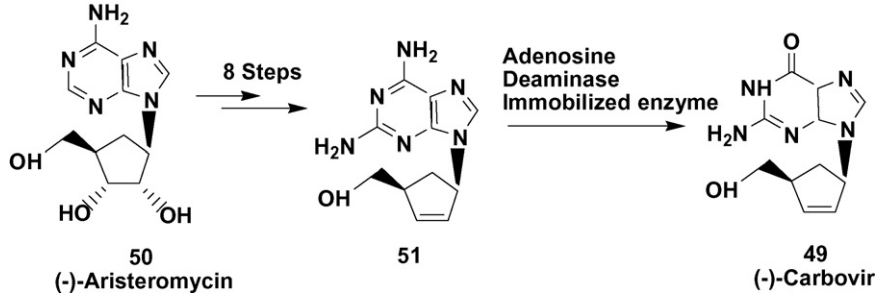

Fig. 11. Carbovir: enzymatic deamination process.

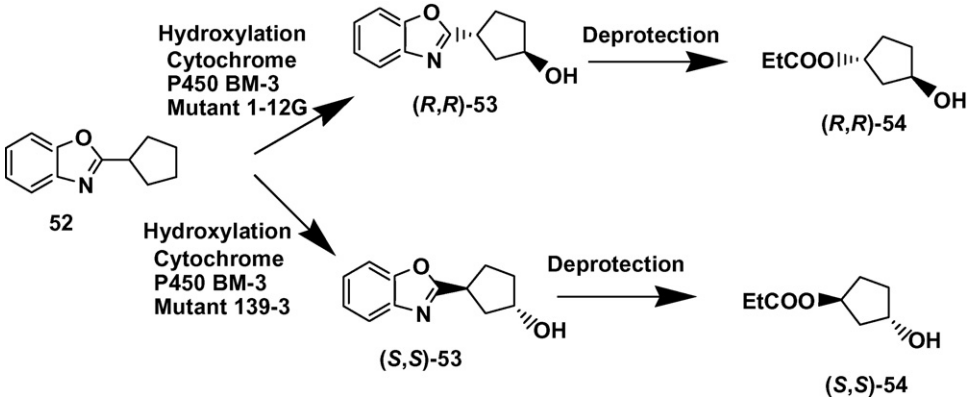

Fig. 12. Carbovir: enzymatic hydroxylation of 2-cyclopentylbenzoxazole.

to compound **53** has been demonstrated by Muenzer et al. [91]. Compound **53** after deprotection afford compound **54**, a potential useful precursor for the synthesis of carbovir. Protecting group removal provided end product **54**. In the initial screen, regardless of the microorganisms and fermentation conditions used, only a single diastereoisomer, (*S*,*S*)-**53**, could be obtained with synthetically useful diastereoselectivities. Side products were also formed during this biotransformation [91]. To circumvent this problem the cytochrome P450 BM-3, a medium chain (C_{12}–C_{18}) fatty acid hydroxylase from *Bacillus megaterium* engineered to accept non-natural substrates with enhanced regioselectivity, enantioselectivity, catalytic rates and total turnover was evaluated [92,93]. Mutations in the active site, for example, enable the enzyme to hydroxylate alicyclic, heterocyclic, aromatic and even polyaromatic compounds [94]. Biohydroxylation using the wild-type enzyme expressed in *E. coli* gave very low enantioselectivity (1.5%, *S*,*S*) and high diastereoselectivity (87%). Mutant 139-3 also afforded (*S*,*S*)-**53**, but in high e.e. (86%) and d.e. (96%). In dramatic contrast, mutant 1-12G was found to produce (*R*,*R*)-**53** in high selectivities (89% e.e., 94% d.e.) [91].

11. Zanamavir (Relenza™)

11.1. Enzymatic preparation of N-acetyl-D-neuraminic acid

Relenza™ **55** (2,3-didehydro-2,4-dideoxy-4-guanidinyl-*N*-acetylneuraminic acid, Fig. 13) is a potent and selective inhibitor of influenza virus sialidase (neuraminidase) and has been approved by the FDA for the treatment of type A and B influenza; the two types most responsible for flu epidemics. *N*-acetyl-D-neuraminic acid **56** (NANA) is the key intermediate for synthesis of Relenza [95]. The chemical synthesis of

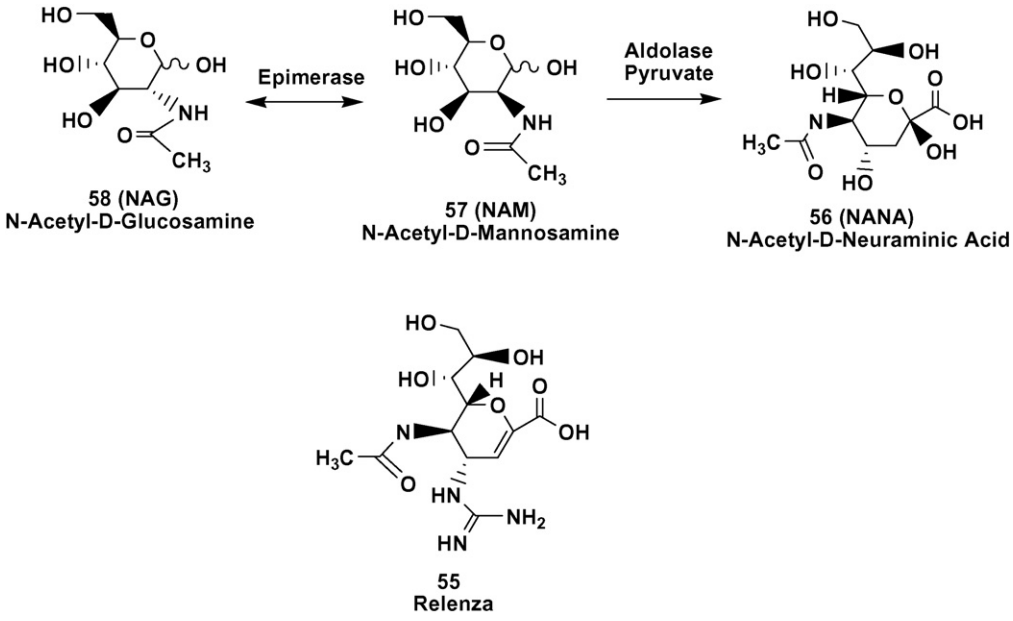

Fig. 13. Zanamavir (Relenza): enzymatic preparation of *N*-acetyl-D-neuraminic acid.

NANA is lengthy, requiring complex protection and deprotection steps [96]. The most promising option was the use of the NANA aldolase. The synthesis of NANA using the aldolase either from *E. coli* or *Clostridium perfringens* has been reported [97,98]. These groups developed batch processes for the production of NANA from *N*-acetyl-D-mannosamine (NAM) **57** and pyruvate using free or immobilized NANA aldolase. In order to drive the equilibrium towards NANA, the pyruvate was used in large excess making the downstream processing rather difficult. A continuous process for NANA synthesis was developed by introducing the NANA-2-epimerase for epimerization of *N*-acetyl-D-glucosamine (NAG) **58** and integrating the epimerization with NANA synthesis in an enzyme-membrane reactor [99]. Subsequently, the enzyme from *E. coli* was overexpressed in an inducible system (tac-promoter) at very high expression levels [100]. The homogenized bacterial cells were directly used to immobilize the enzyme from crude extracts onto Eupergit-C beads without any clarification and the immobilized enzyme was used.

In the biotransformation process, a selective precipitation of NAG using isopropanol was developed to produce a NAM-enriched mixture.This was used in the reaction at a very high NAM concentration (up to 20% w/v) so that NAM itself drives the reaction and it was not necessary to add a large molar excess of pyruvate. At the end of the reaction, NANA (155 g/L) was crystallized directly from the reaction mixture by the addition of acetic acid. In the manufacturing scale, the same batch of enzymes was reused >2000 cycles in batch column reactors, without any significant loss of activity, to produce multi-tonne quantities of NANA [101–103].

12. Epivir (Lamuvidine™)

12.1. Enzymatic deamination process for preparation of epivir

(2′*R-cis*)-2′-deoxy-3-thiacytidine (3TC, Epivir, **59** Fig. 14), has been approved by the FDA, and is marketed for the treatment of HIV (human immunodeficiency virus). Epivir is a potent and selective inhibitor of the reverse transcriptase enzyme which catalyzes the conversion of the HIV RNA to a double stranded DNA. Epivir is also active against HBV (hepatitis B virus) and is sold as Lamivudine [104]. In contrast to the majority of nucleoside analogues that display antiviral activity primarily residing in the 'natural' ß-D-isomer, the enantiomers of ±**59** are equipotent *in vitro* against HIV-1 and HIV-2 but the 'unnatural' ß-L-(−)-**59** isomer (Epivir), Fig. 14, is substantially less cytotoxic than its corresponding 'natural' ß-D-(+)-isomer [105]. One of the initial routes considered for the preparation of the enantiomers of **59** was the enzymic resolution using 5′-nucleotidase and alkaline phosphatase. The chemically synthesized monophosphate derivative (±)-**60** was resolved using 5′-nucleotidase from *Crotalus atrox* venom, and the resulting mixture was separated by chromatography and purified on silica gel, to give (+)-**59** (e.e. >99%, Fig. 14). Hydrolysis of the remaining monophosphate (−)-**60** with alkaline phosphatase from *E. coli* afforded (−)-**59** (Epivir) in an optically pure form [106]. To produce much larger quantities of Epivir, a scaleable end-stage resolution, via enantioselective deamination of (±)-**59** with cytidine deaminase [107] was developed to yield (−)-**59** (Epivir) and (+)-**61**.

To use *E. coli* as a source of large quantities of enzyme, the cloning and overexpression of enzyme, fermentation and

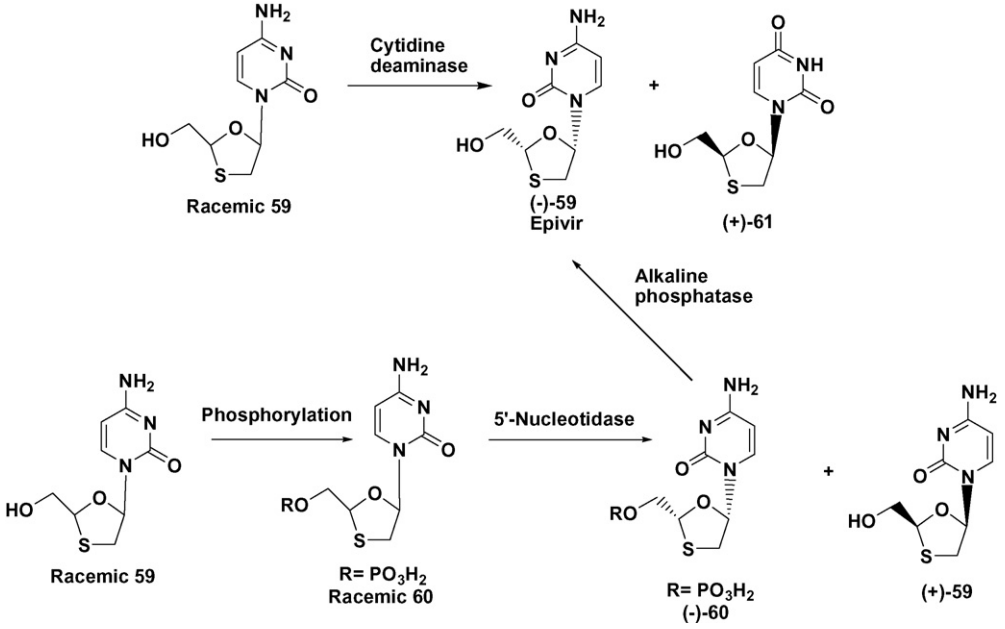

Fig. 14. Epivir (Lamuvidine™): enzymatic deamination process for preparation of epivir.

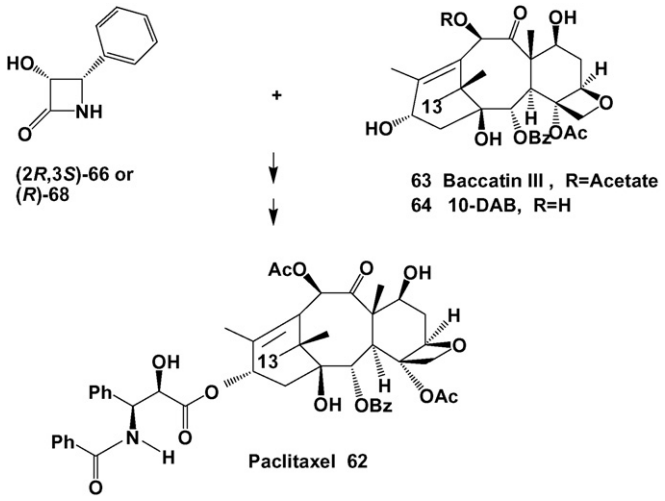

Fig. 15. Anticancer drug, Paclitaxel: paclitaxel semi-synthetic process.

immobilization of cytidine deaminase for reuse, and development of an efficient isolation process was developed for large scale manufacturing of Epivir. Using this approach, 20 kg of optically pure Epivir was isolated. This process was used on a manufactruring scale to prepare tonnes of Epivir, using immobilized cytidine deaminase from the recombinant strain. The same batch of enzyme was used for at least 15 cycles [90,107].

13. Anticancer drugs

13.1. Paclitaxel semi-synthetic process

Among the antimitotic agents, paclitaxel (Taxol®) (**62**, Fig. 15), a complex, polycyclic diterpene, exhibits a unique mode of action on microtubule proteins responsible for the formation of the spindle during cell division. Various types of cancers have been treated with paclitaxel and it was approved for use by the FDA for treatment of ovarian cancer and metastatic breast cancer. Paclitaxel was originally isolated from the bark of the yew *Taxus brevifolia* and has also been found in other *Taxus* species. Paclitaxel was obtained from *T. brevifolia* bark in very low (0.07%) yield, and cumbersome purification from other related taxanes was required. It is estimated that about 20,000 pounds of yew bark (equivalent to about 3000 trees) are needed to produce 1 kg of purified paclitaxel [108]. The development of a semi-synthetic process for the production of paclitaxel from baccatin III **63** or 10-deacetylbaccatin III **64** and C-13 paclitaxel side-chain (2*R*,3*S*)-**66** or acetate (*R*)-**70** was a very promising approach. Taxanes, baccatin III and 10-DAB can be derived from renewable resources such as the needles, shoots and young *Taxus* cultivars [109]. Thus, preparation of paclitaxel by a semi-synthetic process would eliminate the harvesting of yew trees.

Using selective enrichment techniques, two strains of *Nocardioides* were isolated from soil samples that contained the novel enzymes C-13 taxolase and C-10 deacetylase [110–112]. The extracellular C-13 taxolase derived from the filtrate of the fermentation broth of *Nocardioides albus* SC 13911 catalyzed the cleavage of the C-13 side-chain from paclitaxel and related taxanes such as taxol C, cephalomannine, 7-β-xylosyltaxol, 7-β-xylosyl-10-deacetyltaxol, and 10-deacetyltaxol (Fig. 16A). The intracellular C-10 deacetylase derived from fermentation of *Nocardioides luteus* SC 13912 catalyzed the cleavage of the C-10 acetate from paclitaxel, related taxanes and baccatin III to yield 10-DAB (Fig. 16B) The C-7 xylosidase derived from fermentation of *Moraxella* sp. (Fig. 16C) catalyzed the cleavage of the C-7 xylosyl group [113] from various taxanes.

Fermentation processes were developed for growth of *N. albus* SC 13911 and *N. luteus* SC 13912 to produce C-13 taxolase and C-10 deacetylase, respectively, in 5000-L batches. A bioconversion process was demonstrated for the conversion of paclitaxel and related taxanes in extracts of *Taxus* cultivars to the single compound 10-DAB using both enzymes. The concentration of 10-DAB was increased by 5.5 to 24 by treatment with the two enzymes. The bioconversion process was also applied to extracts of the bark of *T. bravifolia* to give a 12-fold increase in 10-DAB concentration. Enhancement of the 10-DAB concentration in yew extracts was useful in increasing the amount and ease of purification of this key precursor for the paclitaxel semi-synthetic process using renewable resources.

Another key precursor for the paclitaxel semi-synthetic process is the chiral C-13 paclitaxel side-chain. Various groups have prepared C-13 side-chain of paclitaxel by different methods [114–119].

The enantioselective microbial reduction of 2-keto-3-(*N*-benzoylamino)-3-phenyl propionic acid ethyl ester **65** (Fig. 17A) to yield (2*R*,3*S*)-*N*-benzoyl-3-phenyl isoserine ethyl ester **66** was demonstrated using two strains of *Hansenula* [114]. Preparative-scale bioreduction of ketone **65** was demonstrated using cell suspensions of *H. polymorpha* SC 13865 and *Hansenula fabianii* SC 13894 in independent experiments. In both batches, a reaction yield of >80% and e.e.'s of >94% were obtained for (2*R*,3*S*)-**66**. In a single-stage bioreduction process, cells of *H. fabianii* were grown in a 15-L fermentor for 48 h, then the bioreduction process was initiated by addition of 30 g of substrate and 250 g of glucose and continued for 72 h. A reaction yield of 88% with an e.e. of 95% was obtained for (2*R*,3*S*)-**66**.

Two enantiocomplementary bakers' yeast enzymes reduced an α-chloro-β-keto ester **67** (Fig. 17B) to yield precursors for both enantiomers of the *N*-benzoyl phenylisoserine **68** and **69**, paclitaxel side chaín synthons [119] was demonstrated on a small scale. After base-mediated ring closure of the chlorohydrin enantiomers, the epoxides were converted directly to the oxazoline form of the target molecules using a Ritter reaction with benzonitrile. These were hydrolyzed to the ethyl ester form of the taxol side-chain enantiomers under acidic conditions.

In an alternate process for the preparation of the C-13 paclitaxel side-chain, the enantioselective enzymatic hydrolysis of racemic acetate *cis*-3-(acetyloxy)-4-phenyl-2-azetidinone (**70**, Fig. 18A) to the corresponding (*S*)-alcohol **71** and the unreacted desired (*R*)-acetate **70** was demonstrated [115] using lipase

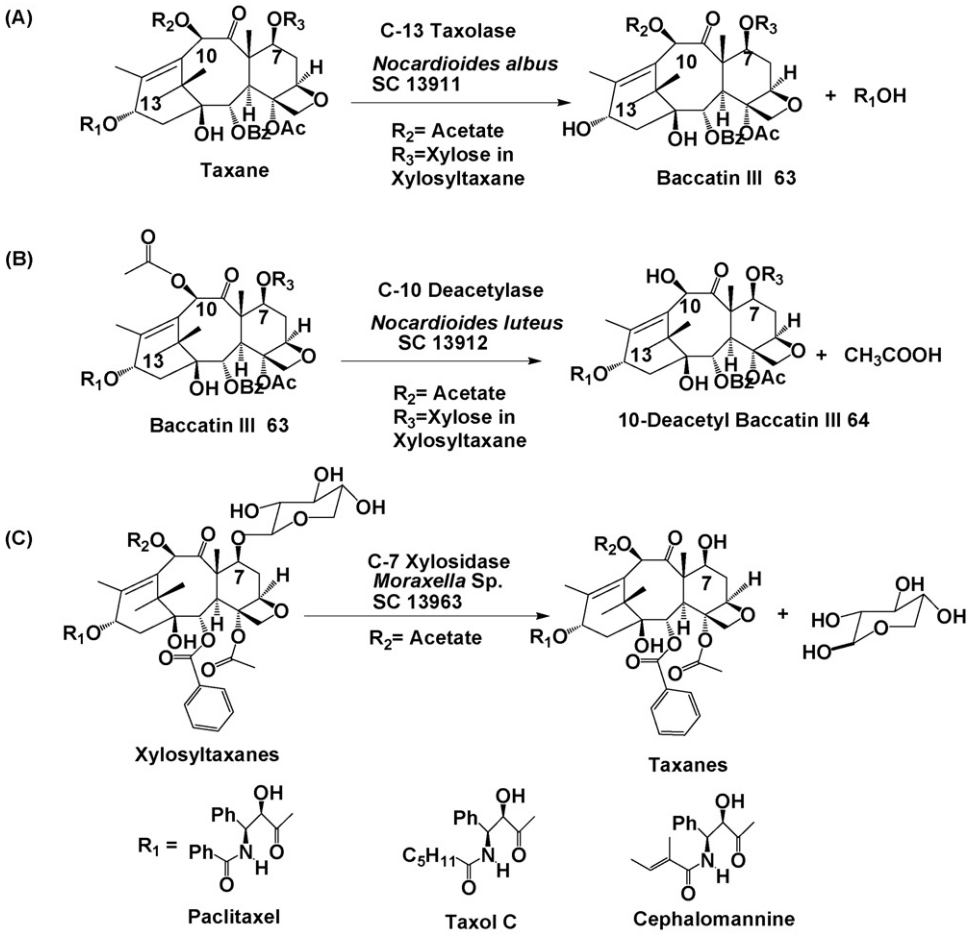

Fig. 16. Anticancer drug, Paclitaxel: (A) enzymatic cleavage of the C-13 side-chain from taxanes (B) enzymatic cleavage of the C-10 acetate from taxanes (C) enzymatic cleavage of the C-7 xylosyl groups from taxanes.

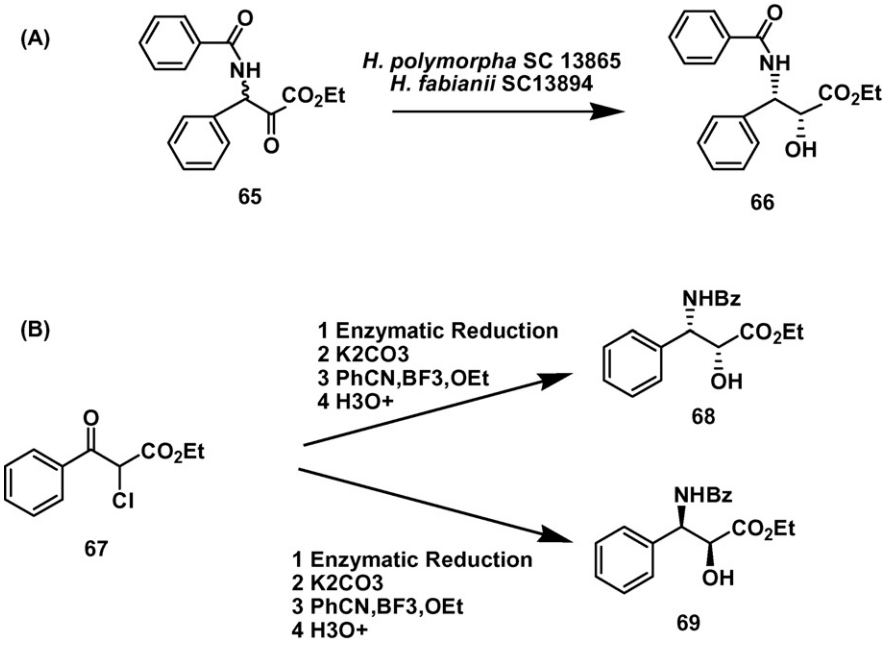

Fig. 17. Preparation of the chiral C-13 paclitaxel side-chain synthon: (A) enantioselective microbial reduction of 2-keto-3-(*N*-benzoylamino)-3-phenyl propionic acid ethyl ester (B) enzymatic reduction of α-chloro-β-keto ester.

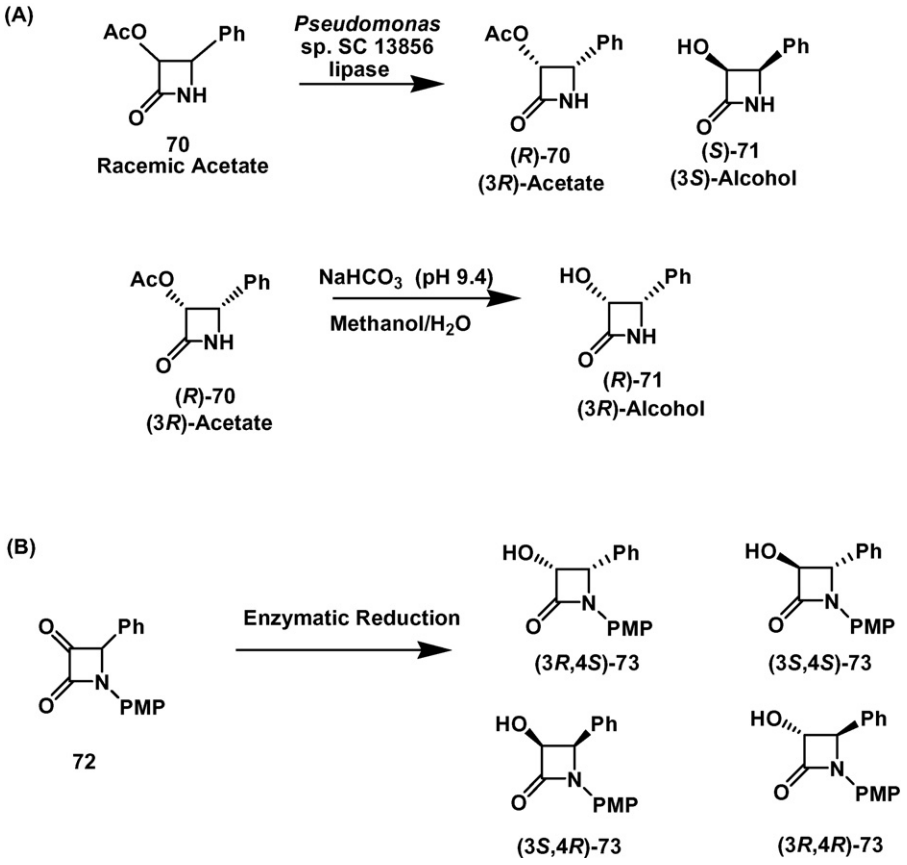

Fig. 18. (A) Enantioselective enzymatic hydrolysis of cis-3-(acetyloxy)-4-phenyl-2-azetidinone (B) enzymatic reduction of 3-oxo-4-phenyl-β-lactam.

PS-30 from *P. cepacia* (Amano International Enzyme Company) and BMS lipase (extracellular lipase derived from the fermentation of *Pseudomonas* sp. SC 13856). Reaction yields of 48% (theoretical maximum yield 50%) with e.e. >99.5% were obtained for the (*R*)-**70**. BMS lipase and lipase PS-30 were immobilized on Accurel polypropylene (PP), and the immobilized lipases were reused (10 cycles) without loss of enzyme activity, productivity or the e.e. of the product (*R*)-**70**. The enzymatic process was scaled up to 250 L (2.5 kg substrate input) using immobilized BMS lipase and lipase PS-30, respectively. From each reaction batch, *R*-acetate **70** was isolated in 45 M% yield (theoretical maximum yield 50%) and 99.5% e.e. The (*R*)-acetate was chemically converted to (*R*)-alcohol **71**. The C-13 paclitaxel side-chain synthon, (2*R*,3*S*)-**66** or (*R*)-**70**) produced either by the reductive or resolution process could be coupled to bacattin III **63** after protection and deprotection to prepare paclitaxel by a semi-synthetic process [112].

Homochiral 3-hydroxy-4-substituted β-lactams **73** serve as precursors to the corresponding α-hydroxy-β-amino acids—key components of many therapeutically important compounds including paclitaxel. A short synthetic sequence for these targets using a biocatalytic reduction employing 3-oxo-4-phenyl-β-lactam **72** as a probe (Fig. 18B), 19 individual yeast reductases were screened for their efficiency and enantioselectivity. Four highly selective candidates all belonging to the aldose reductase (AKR) superfamily were identified. Two are *Re*-face selective and two are *Si*-face selective [118].

13.2. Water soluble taxane derivative

Due to the poor solubility of paclitaxel, various groups are involved in the development of water soluble taxane analogs [120,121]. Taxane **74** (Fig. 19A) is a water soluble taxane derivative which when given orally was as effective as i.v. 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) [122].

The chiral intermediate (3*R*-*cis*)-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **75** (Fig. 19B) was prepared for the semi-synthesis of the orally active taxane **74**. The enantioselective enzymatic hydrolysis of racemic *cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **75** to the corresponding undesired (*S*)-alcohol **76** and unreacted desired (*R*)-acetate **75** was carried out using immobilized lipase PS-30 (Amano International Enzyme Co.) or BMS lipase (extracellular lipase derived from the fermentation of *Pseudomonas* sp. SC 13856). Reaction yields of 48–49% (theoretical maximum yield 50%) with e.e.s of >99% were obtained for the (*R*)-acetate **75**. Acetoxy β-lactam **75** was converted to *R*-hydroxy β-lactam **76** for use in the semisynthesis of **74** [123].

The synthesis of oral taxane **74** also required 4,10-dideacetyl baccatin **77** (Fig. 19C) as starting material for the chemical synthesis of the C-4 methylcarbonate derivative of 10-deacetylbaccatin III **78**. A microbial process was developed for deacetylation of 10-deacetylbaccatin III **64** to 4,10-dideacetylbaccatin

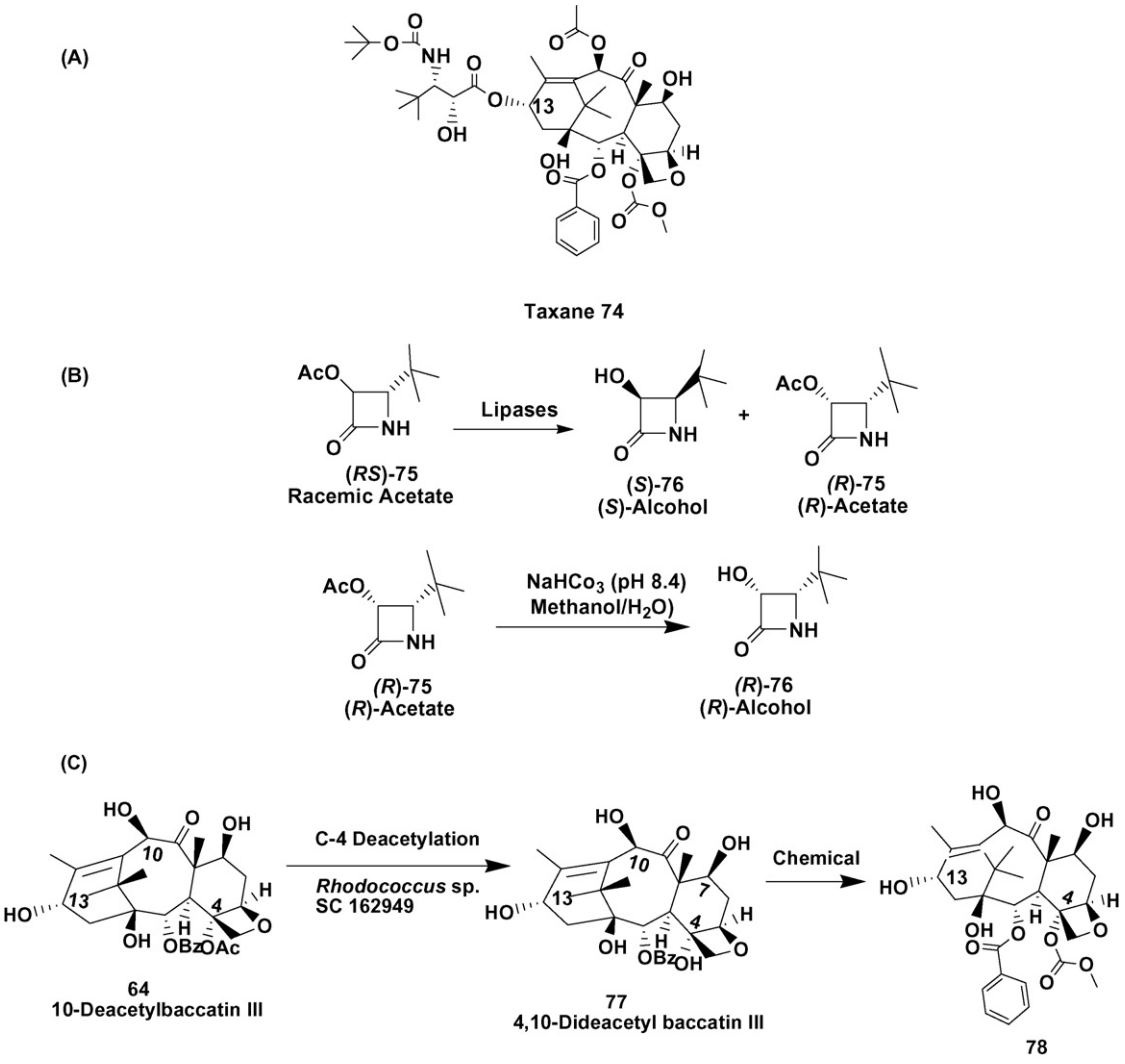

Fig. 19. (A) Water soluble taxane (B) enantioselective enzymatic hydrolysis of *cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone (C) enzymatic C-4 deacetylation of 10-deactylbaccatin III.

III **77** using a *Rhodococcus* sp. SC 162949 isolated from soil using culture enrichment techniques [124].

14. Epothilones

14.1. Microbial hydroxylation of epothiolone B to epothilone F

The clinical success of paclitaxel has stimulated research into compounds with similar modes of activity in an effort to emulate its antineoplastic efficacy while minimizing its less desirable aspects, which include poor-water solubility, difficult synthesis and emerging resistance. The epothilones are a novel class of natural product cytotoxic compounds derived from the fermentation of the myxobacterium *Sorangium cellulosum* that are non-taxane microtubule-stabilizing compounds that trigger apoptosis [125,126]. The natural product epothilone B **79** (Fig. 20) has demonstrated broad spectrum antitumor activity *in vitro* and *in vivo*, including vs tumors with paclitaxel resistance [127]. The role of **79** as a potential paclitaxel successor has initiated interest in its synthesis, resulting in several total syntheses of **79** and various derivatives thereof [128,129]. The epothilone analogs were synthesized in an effort to optimize the water solubility, *in vivo* metabolic stability and antitumor efficacy of this class of antineoplasic agents [130,131].

A fermentation process was developed for the production of epothilone B, and the titer of epothilone B was optimized and increased by a continuous feed of sodium propionate during fermentation. The inclusion of XAD-16 resin during fermentation to adsorb epothilone B and to carry out volume reduction made the recovery of product very simple [126]. A microbial hydroxylation process was developed for conversion of epothilone B **79** to epothilone F **80** by *Amycolatopsis orientalis* SC 15847. A bioconversion yield of 37–47% was obtained when the process was scaled up to 100–250 L. Recently, the epothilone B hydroxylase along with the ferredoxin gene have been cloned and expressed in *Streptomyces rimosus* from *A. orientalis* SC 15847 by our colleagues at Bristol–Myers Squibb. Mutants and variants thereof of this cloned enzyme was used in the hydrox-

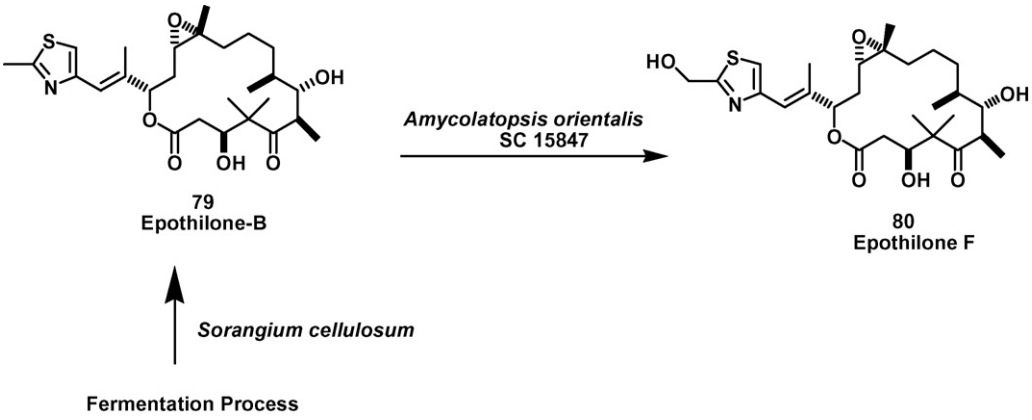

Fig. 20. Microbial hydroxylation of epothiolone B to epothilone F.

ylation of epothilone B to epothilone F to obtain higher yields of product [132].

15. IGF-1 receptor inhibitor

15.1. Enzymatic preparation of (S)-2-chloro-1-(3-chlorophenyl)ethanol

The synthesis of the leading candidate compound **81** [133] in an anticancer program [134,135] required (*S*)-2-chloro-1-(3-chlorophenyl)ethanol (**82**, Fig. 21) as an intermediate. Other possible candidate compounds used (*S*)-alcohol **83**. From microbial screen of the reduction of ketone **84** to (*S*)-alcohol **82**, two cultures namely *H. polymorpha* SC13824 (73.8% e.e.) and *Rhodococcus globerulus* SC SC16305 (71.8% e.e.) were identified that had the highest enantioselectivity. A ketoreductase from *H. polymorpha*, after purification to homogeneity, gave (*S*)-alcohol **82** with 100% e.e. Amino acid sequences from the purified enzyme were used to design PCR primers for cloning the ketoreductase. The ketoreductase was cloned and expressed in *E. coli* together with a glucose-6-phosphate dehydrogenase from *S. cerevisiae* to allow regeneration of the NADPH required for the reduction process. An extract of *E. coli* containing the two recombinant enzymes was used to reduce 2-chloro-1-(3-chloro-4-fluorophenyl)-ethanone **84**. Intact *E. coli* cells provided with glucose were used to prepare (*S*)-alcohols **82** and **83** from the reduction of corresponding ketones **84** and **85**, respectively, in >89% yields with >99.8% e.e. [136].

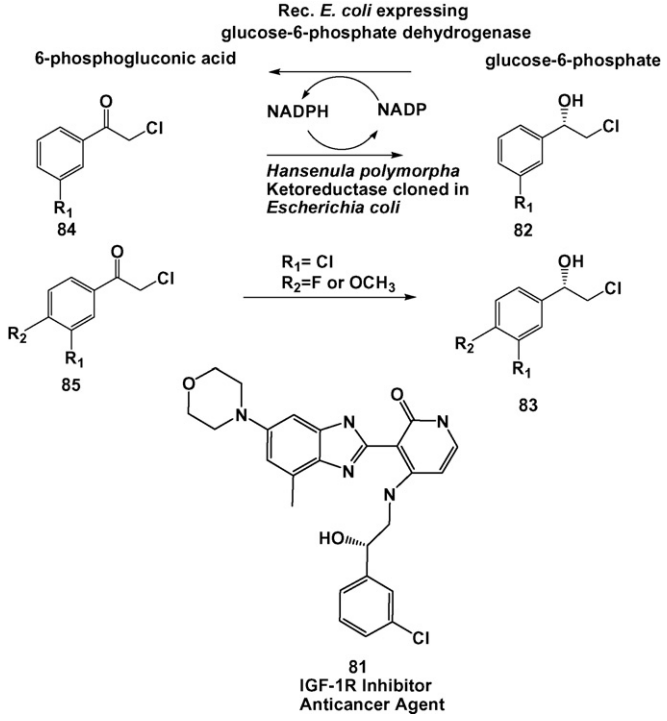

Fig. 21. Enzymatic preparation of (*S*)-2-chloro-1-(3-chlorophenyl)ethanol.

16. Retinoic acid receptor agonist

16.1. Enzymatic preparation of 2-(R)-hydroxy-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthalenyl)acetate

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. They have been shown to effect cellular growth and differentiation and are promising drugs for the treatment of cancers [137,138]. A few retinoids are already in clinical use for the treatment of dermatological diseases such as acne and psoriasis [139]. (*R*)-3-Fluoro-4-[[hydroxy-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-acetyl]amino]benzoic acid **86** (Fig. 22) is a retinoic acid receptor gamma-specific agonist potentially useful as a dermatological and anticancer drug [140].

Ethyl 2-(*R*)-hydroxy-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthalenyl)acetate **87** (Fig. 21) and the corresponding acid **88** were prepared as intermediates in the synthesis of the retinoic acid receptor gamma-specific agonist **86** [120]. Enantioselective microbial reduction of ethyl 2-oxo-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6-naphthalenyl) acetate **89** to alcohol **87** was carried out using *Aureobasidium pullulans* SC 13849 in 98% yield and with an e.e. of 96%. At the end of the reaction, hydroxyester **87** was adsorbed onto XAD-16 resin and, after filtration, recovered in 94% yield from the resin with acetonitrile extraction. The recovered

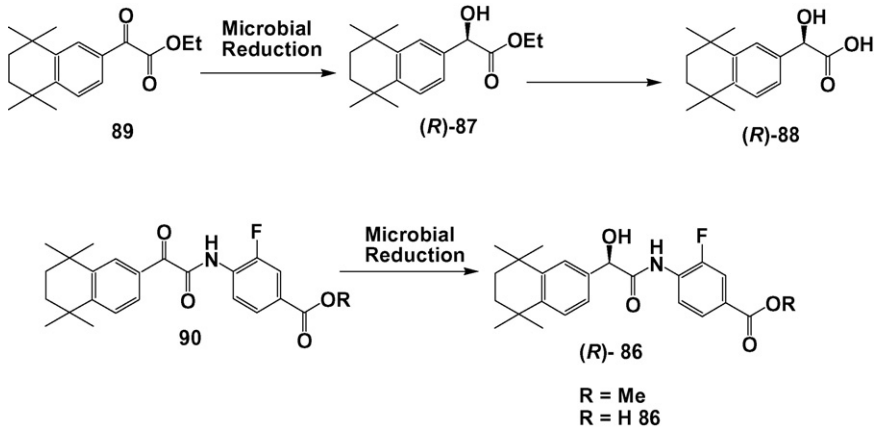

Fig. 22. Enzymatic preparation of 2-(*R*)-hydroxy-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthalenyl)acetate.

(*R*)-hydroxyester **87** was treated with Chirazyme L-2 or pig liver esterase to convert it to the corresponding (*R*)-hydroxyacid **88** in quantitative yield. The enantioselective microbial reduction of ketoamide **90** to the corresponding (*R*)-hydroxyamide **86** by *A. pullulans* SC 13849 was also demonstrated [141].

17. Antileukemic agent

17.1. Enzymatic preparation of a prodrug of 9-β-D-arabinofuranosyl guanine

Compound **91** (Fig. 23), a prodrug of 9-β-D-arabinofuranosyl guanine **92**, was developed for the potential treatment of leukemia. Compound **92** is poorly soluble in water and its synthesis by conventional techniques was difficult. Compound **91** was prepared enzymatically from 6-methoxyguanine **93** and arauracil **94** using uridine phosphorylase and purine nucleotide phosphorylase. Each protein was cloned and overexpressed in independent *E. coli* strains. Fermentation conditions were optimized for production of both enzymes and a co-immobilzed enzyme preparation was used in the biotransformation process at 200 g/L substrate input. Enzyme was recovered at the end of the reaction by filtration and reused in several cycles [142,143]. *In vivo*, compound **91** is rapidly demethoxylated to compound **92** by adenosine deaminase. The more water soluble 5′-acetate ester **95** was subsequently prepared by the enzymatic acylation process using Novozym-435 (immobilized *C. antarctica* lipase) in 1,4-dioxane (100 g/L substrate) with vinyl acetate as the acyldonor [144].

18. Farnesyl transferase inhibitor

18.1. Enzymatic resolution of substituted (6,11-dihydro-5H-benzo-[5,6]cyclohepta-[1,2-β]pyridin-11-yl)piperidines

The resolution of secondary amines via enzyme-catalyzed acylation is a relatively rare process. The kinetic resolution process by enzymatic acylation of the substituted (6,11-dihydro-5H-benzo-[5,6]cyclohepta[1,2-β]pyridin-11-yl)piperidines **96** (Fig. 24) was developed for the synthesis of compound **97**, a selective farnesyl transferase inhibitor. In the case of **96**, the molecule exists as a pair of enantiomers due to atropisomerism

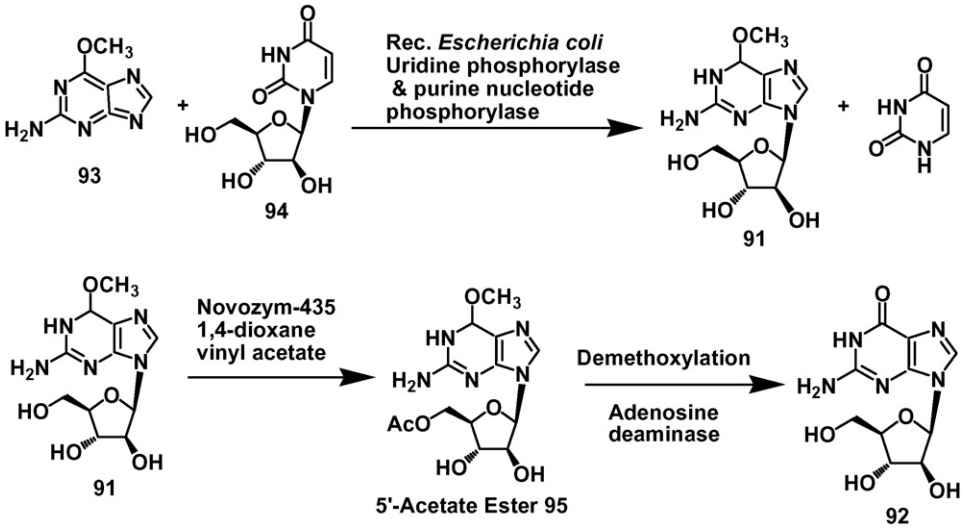

Fig. 23. Enzymatic preparation of a prodrug of 9-β-D-arabinofuranosyl guanine.

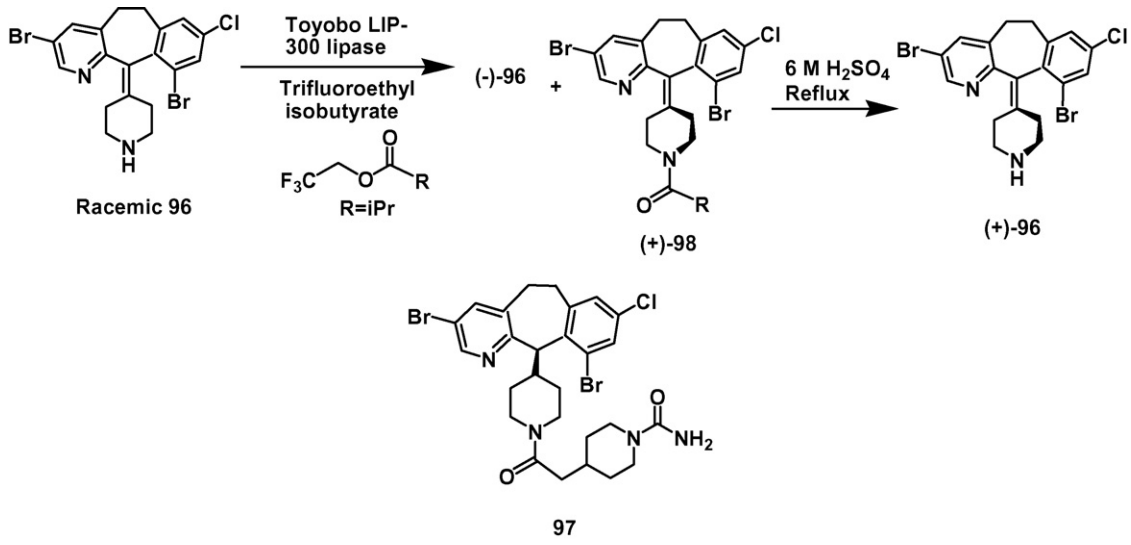

Fig. 24. Farnesyl transferase inhibitors: enzymatic resolution of substituted (6,11-dihydro-5H-benzo-[5,6]cyclohepta-[1,2-β]pyridin-11-yl)piperidines.

about the exocyclic double bond. The enzymatic acylation of (±)-**96** was optimized in terms of acylating agent, solvent, and moisture content. The use of lipase, Toyobo LIP-300, and trifluoroethyl isobutyrate as acylating agent resulted in isobutyrylation of the (+)-enantiomer, which is easily separated from the unwanted (−)-**96**. A reaction yield of 46% with an e.e. of 97% was obtained for the isobutyramide **98**. Hydrolysis of the isobutyramide **98** yielded the desired (+)-**96** in high enantiomeric excess. (−)-**96** may be recovered from the resolution step, racemized, and resubjected to enzymatic acylation to increase material throughput [145].

19. Nucleosides and analogues

19.1. Enzymatic transglycosidation

Nucleoside analogues have been used in the treatment of AIDS, and other viral infections, such as those caused by herpes viruses, influenza A and B viruses [146,147].

In addition to the classical antiviral activity of nucleosides, nucleoside analogues are used in cardio-protection and brain-protection in ischemic heart disease and stroke, respectively, and in treatment of certain human leukemias [148,149]. Nucleoside analogs are key components of antisense oligonucleotides, useful for preparing triple ADN helix, as potential and selective inhibitors of gene expression and antimicrobial drugs, such as oxetanocine (AXT-A) or oxanosine [150].

Therefore, much effort has been expended on the synthesis of nucleoside analogues. Nucleoside analogues can be prepared by base interchange using two different kind of intracellular enzymes, nucleoside phosphorylases (NP) or *N*-2′-deoxyribosyltransferases.

Nucleoside phosphorylases catalyze the reversible phosphorolysis of nucleosides and the transferase reaction involving purine or pyrimidine bases. Purine and pyrimidine nucleoside phosphorylases have been isolated from a large number of bacteria [151,152]. These enzymes display a fairly broad substrate specificity. In contrast, *N*-2′-deoxyribosyltransferases specifi-

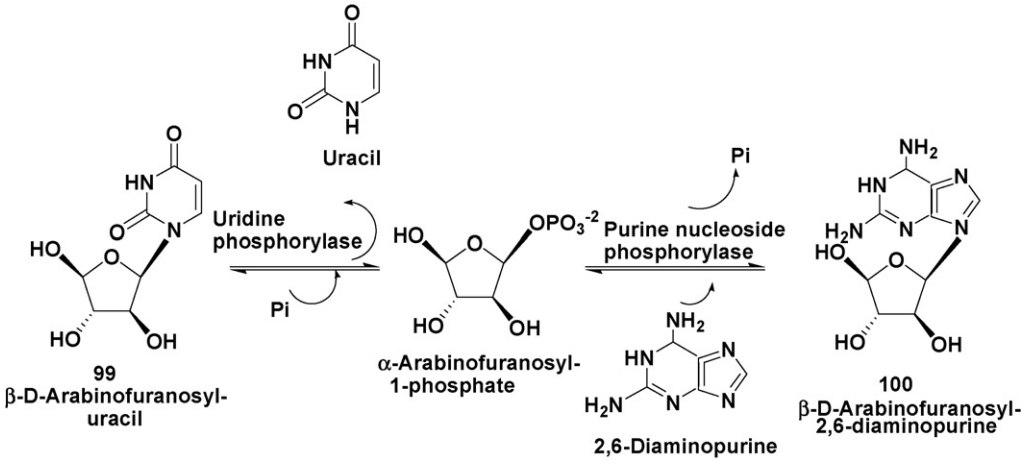

Fig. 25. Nucleosides and its analogues: enzymatic transglycosidation.

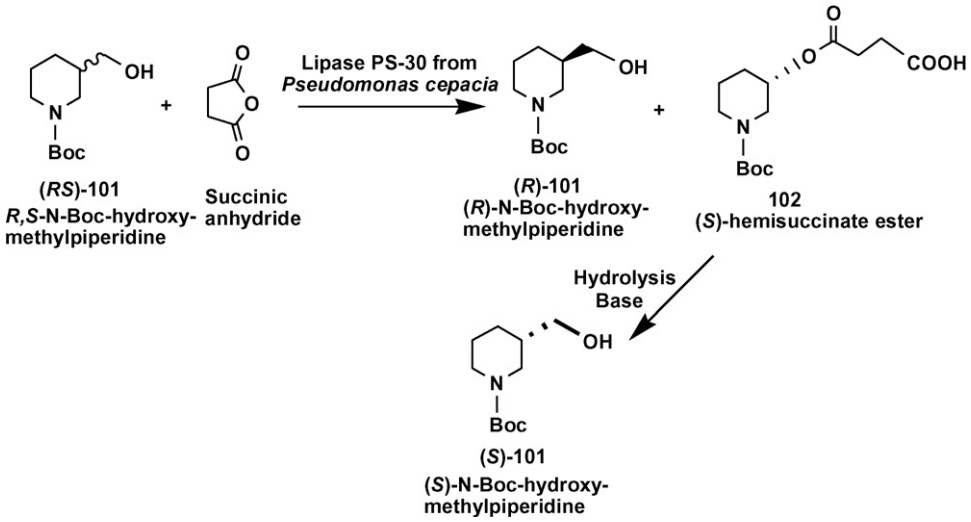

Fig. 26. Tryptase inhibitors: enzymatic preparation of (*S*)-*N* (*tert*-butoxycarbonyl)-3-hydroxymethyl piperidine.

cally catalyze the exchange of the base from a 2′-deoxyribosyl nucleoside with a free purine or pyrimidine [153].

Uridine phosphorylase (UP) and purine nucleoside phosphorylase (PNP) can be combined for the preparation of nucleosides and analogues thereof by transglycosidation reactions. The recombinant enzymes UP and PNP have been expressed in high-biomass bacterial fermentation processes and enzymes are coimmobilized to prepare various nucleosides and analogues thereof [154]. Uridine, 2′-deoxyuridine, 2′-3′-dideoxyuridine or arabinofuranosyl-uracil can serve as sugar donor and a panel of natural and modified purines have been utilized as sugar acceptors [155]. The conversion of β-D-arabinofuranosyl-uracil **99** (Fig. 25) to β-D-arabinofuranosyl 2,6-daminopurine **100** by UP and PNP was demonstrated [156].

20. Tryptase inhibitors

20.1. Enzymatic preparation of (S)-N (tert-butoxycarbonyl)-3-hydroxymethyl piperidine

(*S*)-*N*(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine **101** (Fig. 26) is a key intermediate in the synthesis of a potent tryptase inhibitor [157]. (*S*)-**101** was made by lipase PS-30 (*Pseudomonas cepacia*) catalyzed resolution of (*R,S*)-N-(*tert*-butoxy carbonyl)-3-hydroxymethylpiperidine **101**. (*S*)-**101** was obtained in 16% yield and > 95% e.e. Lipase PS also catalyzed esterification of the (*R,S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxy methylpiperidine **101** with succinic anhydride to yield (*R*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxy methylpiperidine **101** and the (*S*)-hemisuccinate ester **102** which could be easily separated and hydrolyzed by base to afford (*S*)-**101** (yield 47.8 and e.e. 85.7%). The yield and e.e. could be improved greatly by subjecting isolated (*S*)-**101** to secong succinylation. Using the repeated esterification procedure, (*S*)-**101** was obtained in 32% yield and 98.9% e.e [158].

21. Anticholesterol drugs: hydroxymethyl glutaryl CoA (HMG CoA) reductase inhibitors

21.1. Preparation of (S)-4-chloro-3-hydroxybutanoic acid methyl ester

(*S*)-4-chloro-3-hydroxybutanoic acid methyl ester **103** (Fig. 27A) is a key chiral intermediate in the total chemical synthesis of **104**, a inhobitor of HMG CoA reductase [159,160]. The reduction of 4-chloro-3-oxobutanoic acid methyl ester **105** to (*S*)-**103** was demonstrasted using cell suspensions of *Geotrichum candidum* SC 5469. In the biotransformation process, a reaction yield of 95% and e.e. of 96% were obtained for (*S*)-**103** at 10 g/L substrate input. The e.e. of (*S*)-**103** was increased to 98% by heat-treatment of cell-suspensions (55 °C for 30 min) prior to conducting the bioreduction of **105** [160].

21.2. Preparation of (S)-4-chloro-3-hydroxybutonoate

In an alternate approach, the asymmetric reduction of ethyl 4-chloroacetoacetate **106** to (*S*)-4-chloro-3-hydroxybutonoate **107** (Fig. 27B) was demonstrated by a secondary alcohol dehydrogenase (PfODH) from *Pichia finlandica*. The gene encoding PfODH was cloned from *P. finlandica* and overexpressed in *E. coli*. Formate dehydrogenase was used to regenerate the cofactor NADH required for this reaction. Using recombinant *E. coli* coexpressing both PfODH and formate dehydrogenase from *Mycobacetrium* sp., produced (*S*)-**107** in 98.5% yield and 99% e.e. at 32 g/L substrate input [161].

21.3. Preparation of (R)-4-cyano-3-hydroxybutyrate

An enzymatic process was developed for the preparation of ethyl (*R*)-4-cyano-3-hydroxybutyrate **108** (Fig. 28A) from ethyl 4-chloroacetoacetate **109**. The genes encoding ketoreductase from *Candida magnoliae*, glucose dehydrogenase from

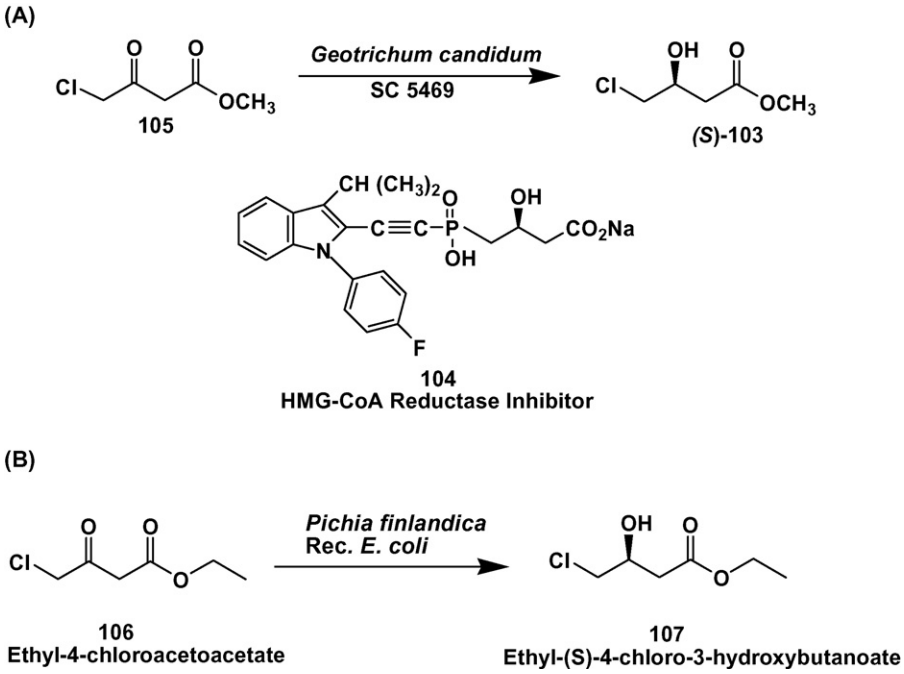

Fig. 27. Anticholesterol drugs: (A) preparation of (*S*)-4-chloro-3-hydroxybutanoic acid methyl ester (B) enzymatic reduction of ethyl-4-chloroacetoacetate.

Bacillus subtilis and formate dehydrogenase from *Candida boidinii* and halohydrin dehydrogenase from *Agrobacterium tumefaciens*, were separately cloned into *E. coli* BL21. Each enzyme was then produced by fermentation. Ethyl (*R*)-4-cyano-3-hydroxybutyrate was prepared by the following procedure. Ethyl 4-chloroacetoacetate **109** was incubated at pH 7.0 with ketoreductase, glucose dehydrogenase and NADP for 40 h to produce ethyl (*S*)-chloro-3-hydroxybutyrate **110**. The ethyl

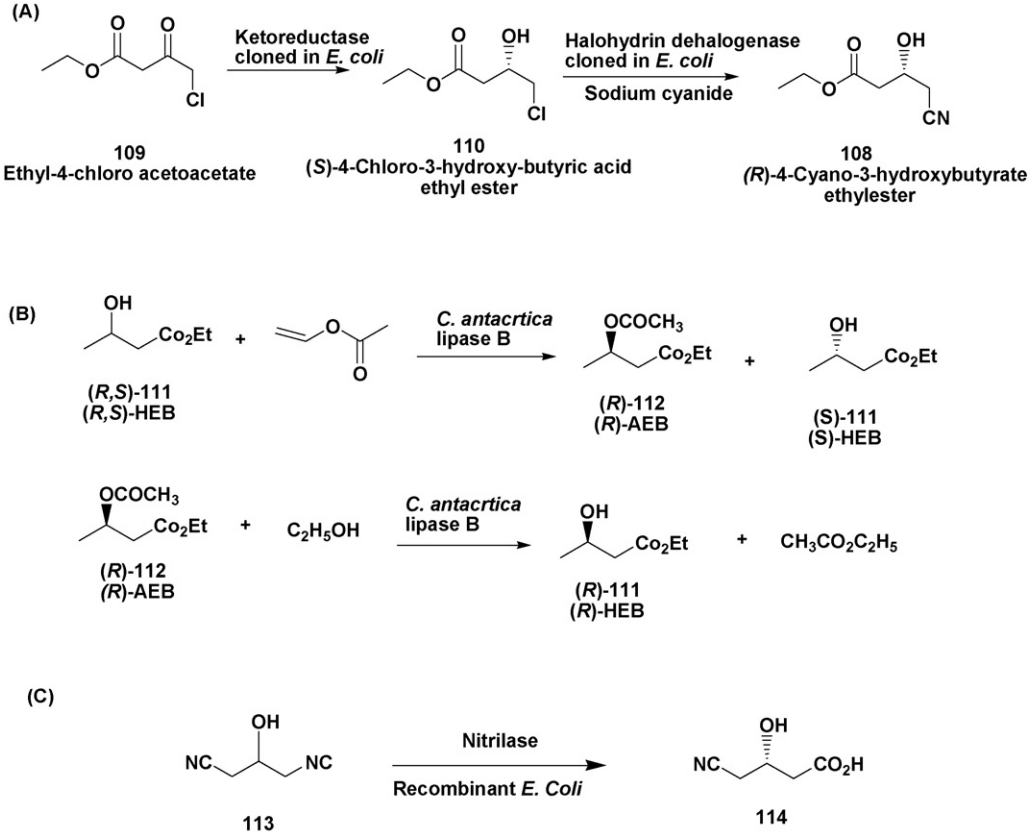

Fig. 28. Anticholesterol drugs: (A) preparation of (*R*)-4-cyano-3-hydroxybutyrate ethyl ester (B) enzymatic preparation of (*R*)-and (*S*)-ethyl-3-hydroxybutyrate (C) enzymatic synthesis of chiral carboxylic acids by nitrilase.

(*S*)-**110** was extracted with ethyl acetate, dried, filtered and concentrated to yield a ~97% pure ester. The dried ethyl (*S*)-**110** 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 **108** was recovered. (*R*)-4-cyano-3-hydroxybutyrate is potentially useful in synthesis of many HMG CoA reductase inhibitors [162,163].

21.4. Preparation of (R)- and (S)-ethyl-3-hydroxybutyrate

An efficient two-step enzymatic process for production of (*R*)-and (*S*)-ethyl-3-hydroxybutyrate (HEB) **111** (Fig. 28B) 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 **111** with vinyl acetate for the production of (*S*)-HEB via the selective acetylation of (*R*)-HEB. In the second reaction, (*R*)-enriched ethyl-3-acetoxybutyrate (AEB) **112** was subjected to alcoholysis with ethanol to derive optically pure (*R*)-HEB. Immobilized CALB was employed in both stages, with high productivity and selectivity. The immobilized enzyme was packed in a column and the reactants were circulated through the enzyme bed sequentially until each of the targeted conversions was reached. The desired products were separated from the reaction mixture after 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 [164].

21.5. Enzymatic synthesis of chiral carboxylic acids by nitrilase

Synthesis of a broad diversity of chiral carboxylic acid by nitrilases has been demonstrated [165,166]. Enzymatic desymmetrization of prochiral 3-hydroxyglutaronitrile **113** using a nitrilase [167,168] was demonstrated (Fig. 28C). Following esterification of the resulting (*R*)-3-hydroxy-4-cyanobutyric acid **114** an intermediate useful for the manufacture of the cholesterol-lowering drug Lipitor (atorvastatin calcium) was produced. Nitrilases were identified in genomic libraries created by extraction of DNA directly from environmental samples and were expressed in *E. coli*. The resulting library was screened for highly enantioselective (*R*)-specific nitrilases [168]. (*R*)-3-Hydroxy-4-cyanobutyric acid was produced using a 100-mM initial nitrile concentration in 98% yield and 94.5% e.e. The enantioselectivity of this wild-type nitrilase decreased with increasing nitrile concentration, where only 87.8% e.e. was obtained at 2.25 M substrate concentration. Mutagenesis of the nitrilase using a technique that combinatorially saturated each amino acid in the protein to each of the other 19 amino acids resulted in an improved variant (Ala190His) that was expressed in *E. coli*. This variant nitrilase gave an enantiomeric excess of 98.5% at 3 M substrate concentration. Nitrilases from this library have been used to produce a range of (*R*)-mandelic acid derivatives and analogs, and (*S*)-phenyllactic acid, with high yields and enantioselectivities [169].

21.6. 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 **115** to (3*S*,5*R*)-dihydroxy-6-(benzyloxy) hexanoic acid, ethyl ester **116** [Fig. 29A] has been demonstrated by cell suspensions of *Acinetobacter calcoaceticus* SC 13876 [170]. Compound **116** is a key chiral intermediate required for the chemical synthesis of [4-[4 a, 6ß(E)]]-6-[4,4-bis [4-fluorophenyl)-3-(1-methyl-1H-tetrazol-5-yl)-1,3-butadienyl]-tetrahydro-4-hydroxy-2H-pyren-2-one **117**, an anticholesterol drug which acts by inhibition of HMG CoA reductase [171]. A reaction yield of 85% and an e.e. of 97% were obtained. Cell extracts of *A. calcoaceticus* SC 13876 in the presence of NAD^+, glucose, and glucose dehydrogenase reduced **115** to the corresponding monohydroxy compounds [3-hydroxy-5-oxo-6-(benzyloxy) hexanoic acid ethyl ester **118** and 5-hydroxy-3-oxo-6-(benzyloxy) hexanoic acid ethyl ester **119**]. Both **118** and **119** were further reduced to the (3*S*,4*R*)-dihydroxy compound **116** in 92% yield and 99% e.e. by cell extracts. (3*S*,5*R*)-**116** was converted to **120**, a key chiral intermediate for the synthesis of **117**. Three different ketoreductases were purified to homogeneity from cell extracts, and their biochemical properties were compared. Reductase I only catalyzes the reduction of ethyl diketoester **115** to its monohydroxy products whereas reductase II catalyzes the formation of dihydroxy products from monohydroxy substrates. A third reductase (III) was identified which catalyzes the reduction of diketoester **115** to *syn*-(3*R*,5*S*)-dihydroxy ester **116** [172], which now has been cloned and expressed in *E. coli*.

Whole baker's yeast cells catalyzed regioselective reduction of *t*-butyl 6-chloro-3,5-dioxohexanoate **121** (Fig. 29B) regioselectively to the *t*-Butyl (*R*)-6-chloro-5-hydroxy-3-oxohexanoate **122** was demonstrated [173]. The enantioselectivity was low (41% e.e.). A variety of process conditions were evaluated in order to improve both the enantioselectivity and yield of this reduction. Including a nonpolar resin in the reaction mixture afforded the (*R*)-alcohol in 94% e.e. and 50% isolated yield. The enantioselectivity was further improved to >99% e.e. by substituting purified YGL157w in place of whole yeast cells. This reductase was identified by screening a collection of yeast enzymes uncovered by genome sequence analysis [173]. (*S*)-**122** was prepared by reduction of **121** with *Lactobacillus brevis* ketoreductase in >99.5% e.e. [174].

21.7. Enzymatic preparation of a 2,4-dideoxyhexose derivative

The chiral 2,4-didoexyhexose derivative required as a potential key intermediate for the HMG CoA reductase inhibitors has also been prepared using 2-deoxyribose-5-phosphate aldolase (DERA). A one-pot tandem aldol reactions catalyzed by a DERA, in which two equivalent of acetaldehyde **123** were added in sequence to 2-carbon aldehyde acceptors to afford

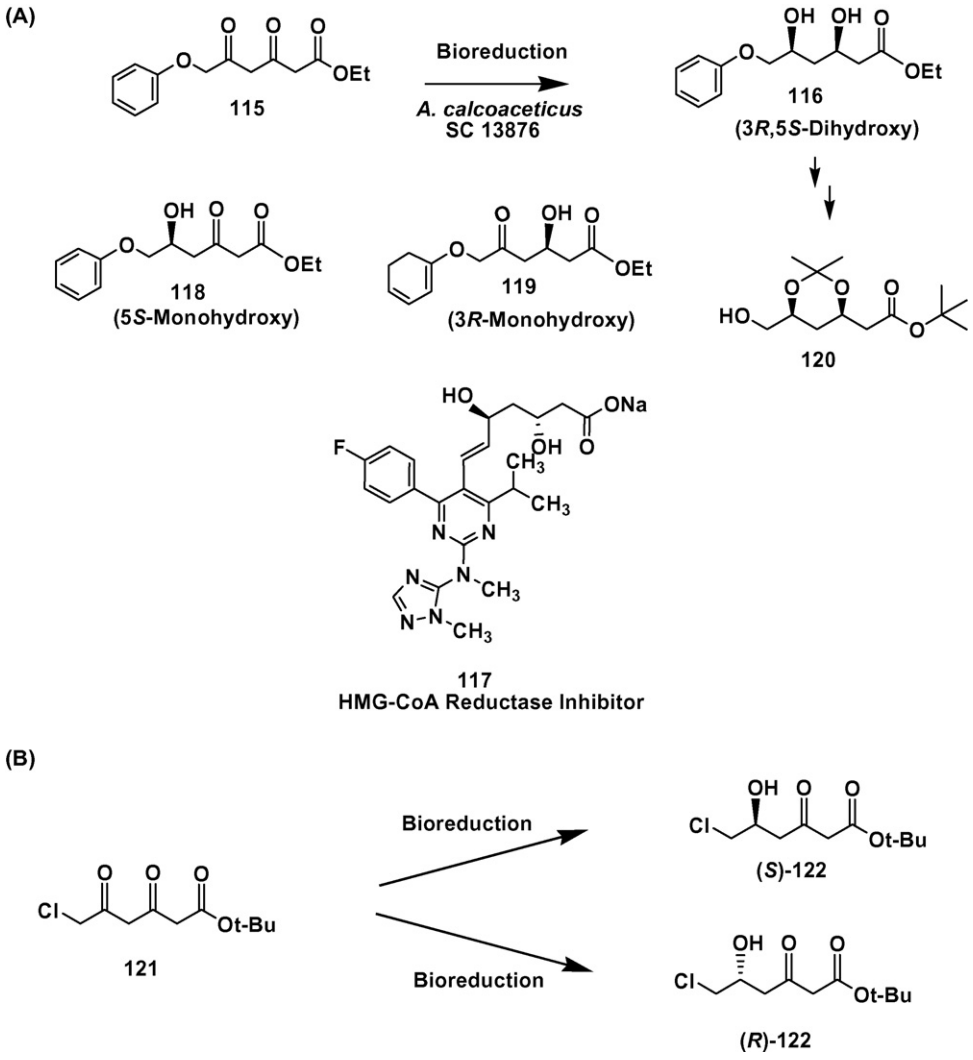

Fig. 29. Anticholesterol drugs: (A) diastereoselective enzymatic reduction of 3,5-dioxo-6-(benzyloxy) hexanoic acid, ethyl ester (B) enzymatic reduction of *t*-butyl 6-chloro-3,5-dioxohexanoate.

six-membered lactol derivatives (Fig. 30). Because the DERA-catalyzed reaction is an equilibrium process, the intermediate 4-carbon adduct **124** is reversibly formed under the reaction conditions. The second condensation between this intermediate **124** and a second equivalent of acetaldehyde drives the equilibrium favorably because of the stability of the cyclized lactol form **125** of the product [175]. The structural similarity of the enzymatic products to the lactone moiety (and its interchangeable 3,5-dihydroxy acid form) of the cholesterol-lowering HMG CoA reductase inhibitors mevastatin and lovastatin was reported [175]. Today, HMG CoA reductase inhibitors (known as statins) have worldwide sales of approximately $20 billion, led by

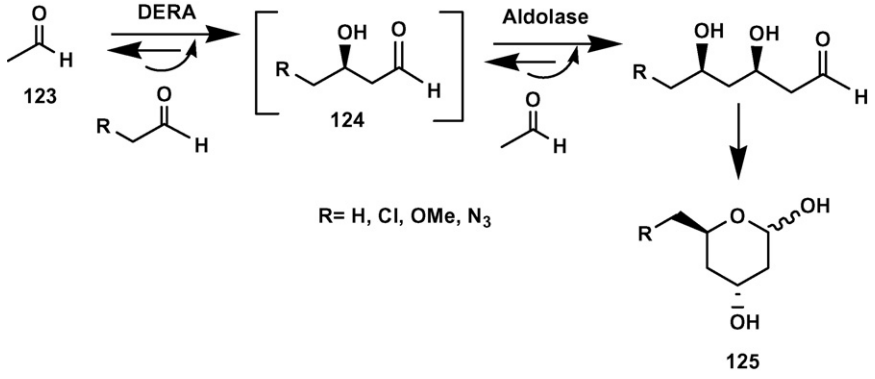

Fig. 30. Anticholesterol drugs: enzymatic preparation of a 2,4-dideoxyhexose derivative.

Pfizer's Lipitor (atorvastatin). Atorvastatin and AstraZeneca's newly approved Crestor (rosuvastatin) are completely synthetic molecules, unlike the earlier generation of statins that were fungal metabolites or semi-synthetic derivatives thereof. The synthetic statins share the chiral 3,5-dihydroxy acid side-chain also found in the natural products.

The above process has been improved and optimized for production of key intermediates for the common chiral side-chain of cholesterol-lowering drugs. An improvement of almost 400-fold in volumetric productivity relative to the published enzymic reaction conditions has been achieved, resulting in an efficient process that has been run at a rate of 30 g/L/h. 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 >99.9% and diastereomeric excess at 96.6%. In addition, down-stream chemical process have been developed to convert the enzymic product efficiently to intermediates applicable to preparation of atorvastatin and rosuvastatin [176]. DERA was improved by directed evolution for the synthesis of a key intermediate (3*R*,5*S*-6-chloro-2,4-trideoxyhexapyranoside [177].

22. Anticholesterol drugs: squalene synthase inhibitor

22.1. Enzymatic synthesis of S-[1-(acetoxyl)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester

Squalene synthase is the first pathway-specific enzyme in the biosynthesis of cholesterol and catalyzes the head-to-head condensation of two molecules of farnesyl pyrophosphate (FPP) to form squalene. It has been implicated in the transformation of FPP into presqualene pyrophosphate (PPP). FPP analogs are a major class of inhibitors of squalene synthase [178]. However, this class of compounds lacks specificity and are potential inhibitors of other FPP consuming transferases such as geranyl pyrophosphate synthase. To increase enzyme specificity, analogs of PPP and other mechanism-based enzyme inhibitors, such as **126** (Fig. 31), have been synthesized [179]. *S*-[1-(Acetoxyl)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester **127** is a key chiral intermediate required for the total chemical synthesis of **126**. The enantioselective acetylation of racemic [1-(hydroxy)-4-(3-phenyl)butyl] phosphonic acid, diethyl **128** has been demonstrated with *G. candidum* lipase in toluene using isopropenyl acetate as the acyl donor [180]. A reaction yield of 38% (theoritical max. 50%) and an e.e. of 95% was obtained for **127**.

23. Anti-Alzheimer's drugs

23.1. Enantioselective enzymatic reduction of 5-oxohexanoate and 5-oxohexanenitrile

Ethyl-(*S*)-5-hydroxyhexanoate **129** and (*S*)-5-hydroxyhexanenitrile **130** (Fig. 32) are key chiral intermediates in the synthesis of anti-Alzheimer's drug **131** [181]. Both chiral compounds have been prepared by enantioselective reduction of ethyl-5-oxohexanoate **132** and 5-oxohexanenitrile **133** by *Pichia methanolica* SC 16116. Reaction yields of 80–90% and >95% e.e.'s were obtained for each chiral compound. In an alternate approach, the enzymatic resolution of racemic 5-hydroxyhexane nitrile **134** by enzymatic succinylation was demonstrated using immobilized lipase PS-30 to obtain (*S*)-5-hydroxyhexanenitrile **130** in 35% yield (maximum yield is 50%). (*S*)-5-Acetoxyhexanenitrile **135** was prepared by enantioselective enzymatic hydrolysis of racemic 5-acetoxyhexanenitrile **136** by *C. antarctica* lipase. A reaction yield of 42% and an e.e. of >99% were obtained [182].

23.2. Enantioselective microbial reduction of substituted acetophenone

The chiral intermediates (*S*)-1-(2′-bromo-4′-fluorophenyl) ethanol **137** and (*S*)-methyl 4-(2′-acetyl-5′-fluorophenyl)-butanol **138** (Fig. 33) are potential intermediates for the synthesis of potential anti-Alzheimer's drug **139** [183,184]. The chiral intermediate (*S*)-1-(2′-bromo-4′-fluoro phenyl)ethanol **137** (Fig. 33A) was prepared by the enantioselective microbial reduction of 2-bromo-4-fluoro acetophenone **140** [185]. Organisms from genus *Candida*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sphingomonas* and Baker's yeast reduced **140** to **137** in >90% yield and 99% enantiomeric excess (e.e.).

In an alternate approach, the enantioselective microbial reduction of methyl-4-(2′-acetyl-5′-fluorophenyl) butanoates

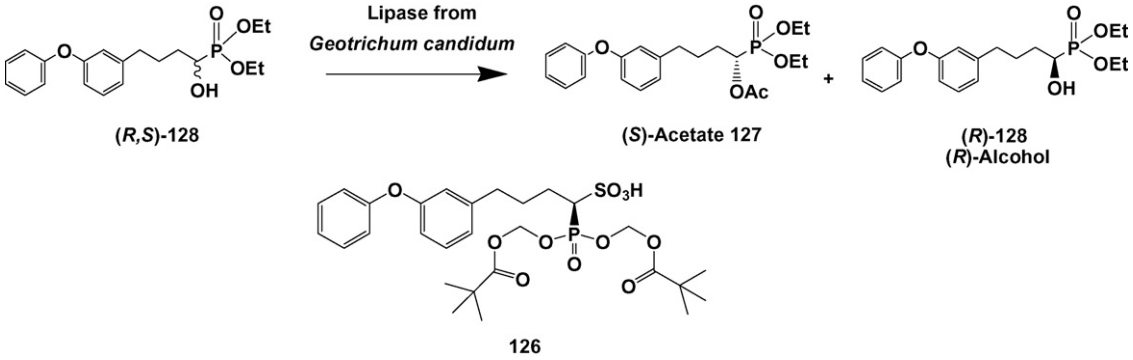

Fig. 31. Anticholesterol drugs: enzymatic synthesis of *S*-[1-(acetoxyl)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester.

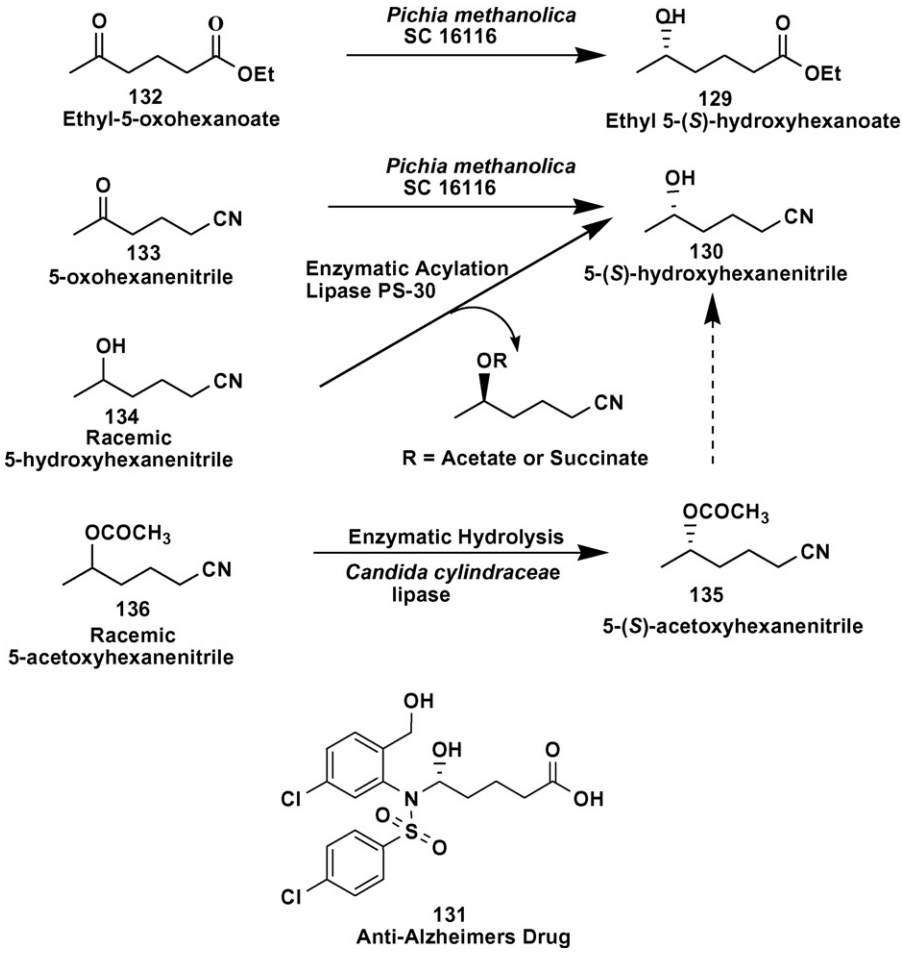

Fig. 32. Anti-Alzheimer's drugs: enantioselective enzymatic reduction of 5-oxohexanoate and 5-oxohexanenitrile.

141 (Fig. 33B) 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 **138**. The reductase which catalyzed the enantioselective reduction of ketoesters was purified to homogeneity from cell extracts of *P. methanolica* SC 13825. It was cloned and expessed in *E. coli* and recombinant cultures were used for the enantioselective reduction of the keto-methyl ester **141** to the corresponding (*S*)-hydroxy methyl ester **138**. On preparative scale, a reaction yield of 98% with an e.e. of 99% was obtained [185].

24. Oxime-based NK1/NK2 dual antagonists

24.1. Enzymatic desymmetrization of diethyl 3-[3′,4′-dichlorophenyl]glutarate

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, vasodialation and secretion [186].

The effect of tachykinins is modulated via the specific G-protein coupled receptors NK1 and NK2. Thus non-peptide NK-receptors antagonists are potentially useful in the treatment of variety of chorinic diseases including asthma, bronchospasm, arthritis, and migraine [187]. The structure–activity relationship of several non-peptide NK1/NK2 antagonists has led to the discovery of a new class of oxime-based NK1/NK2 dual antagonists [188], for example compound **142** (Fig. 34). The biological activity of **142** resides mainly in the *R*,*R*-diastereomer. An enzymatic process for desymmetrization of the prochiral diethyl 3-[3′,4′-dichlorophenyl]glutarate **143** to the corresponding (*S*)-monoester **144** has been developed using lipase B from *C. antarctica* with either the free or the immobilized enzyme. At 100 g/L substrate input, a reaction yield of 97% and an e.e. of >99% were obtained for the desired (*S*)-monoester. The process was scaled up to produce 200 kg of product in 80% overall isolated yield [189]. DNA family shuffling was used to create a chimeric lipase B protein with improved activity towards diethyl 3-[3′,4′-dichlorophenyl]glutarate. Three homologous lipases from *C. 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 the substrate was identified [190]. The thermostability of the lipase was also improved by directed evolution [191].

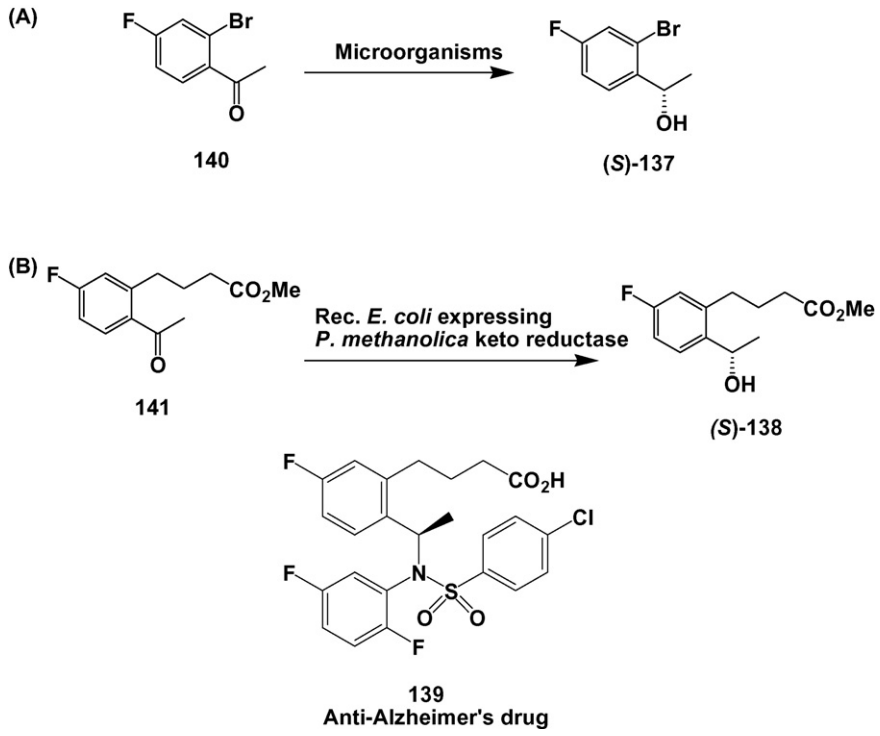

Fig. 33. Anti-Alzheimer's drugs: (A) enantioselective microbial reduction of substituted acetophenone (B) enantioselective microbial reduction of methyl-4-(2′-acetyl-5′-fluorophenyl) butanoates.

25. Antiinfective drugs

25.1. Microbial hydroxylation of pleuromutilin or mutilin

Pleuromutilin **145** (Fig. 35) is an antibiotic from *Pleurotus* or *Clitopilus* basidiomycetes strains which kills mainly gram-positive bacteria and mycoplasms. A more active semi-synthetic analogue, tiamulin, has been developed for the treatment of animals including poultry infection and has been shown to bind to prokaryotic ribosomes and inhibit protein synthesis [192]. Metabolism of pleuromutilin derivatives results in hydroxylation by microsomal cytochrome P-450 at the 2- or 8-position and inactivates the antibiotics [193]. Modification of the 8-position of pleuromutilin and analogues is of interest as a means of preventing the metabolic hydroxylation. Microbial hydroxylation of pleuromutilin **145** or mutilin **146** would provide a functional group at this position to allow further modification. The target analogues would maintain the biological activity of the parent compounds but not be susceptible to metabolic inactivation.

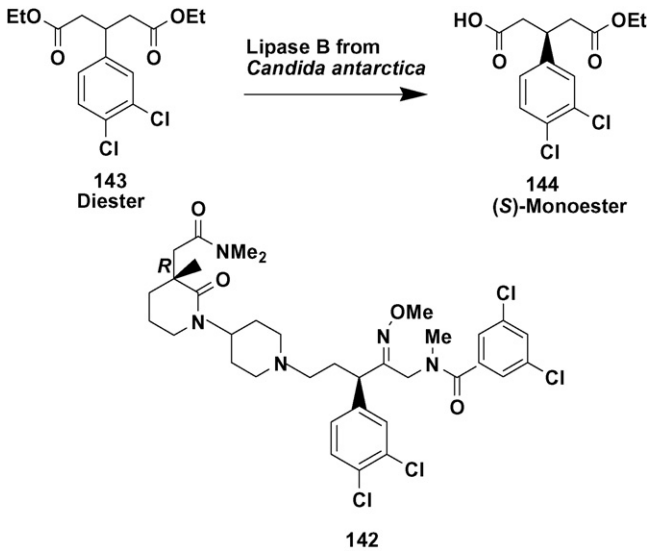

Fig. 34. NK1/NK2 dual antagonists: enzymatic desymmetrization of diethyl 3-[3′,4′-dichlorophenyl]glutarate.

Biotransformation of mutilin and pleuromutilin by microbial cultures has been investigated to provide a source of 8-hydroxymutilin or 8-hydroxypleuromutilin [194]. *Streptomyces griseus* strains SC 1754 and SC 13971 (ATCC 13273) hydroxylated mutilin to (*S*)-8-hydroxymutilin **147**, (*S*)-7-hydroxymutilin **148**, and (*S*)-2-hydroxymutilin **149**. *Cunninghamella echinulata* SC 16162 (NRRL 3655) gave (*S*)-2-hydroxymutilin or (*R*)-2-hydroxypleuromutilin **150** 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 (*S*)-8-hydroxymutilin, 17 g of (7*S*)-hydroxymutilin and 13 g of (*S*)-2-hydroxymutilin from 162 g of mutilin [194].

A C-8 ketopleuromutilin **151** derivative has been synthesized from the biotransformation product 8-hydroxymutilin [195]. 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 precipitated

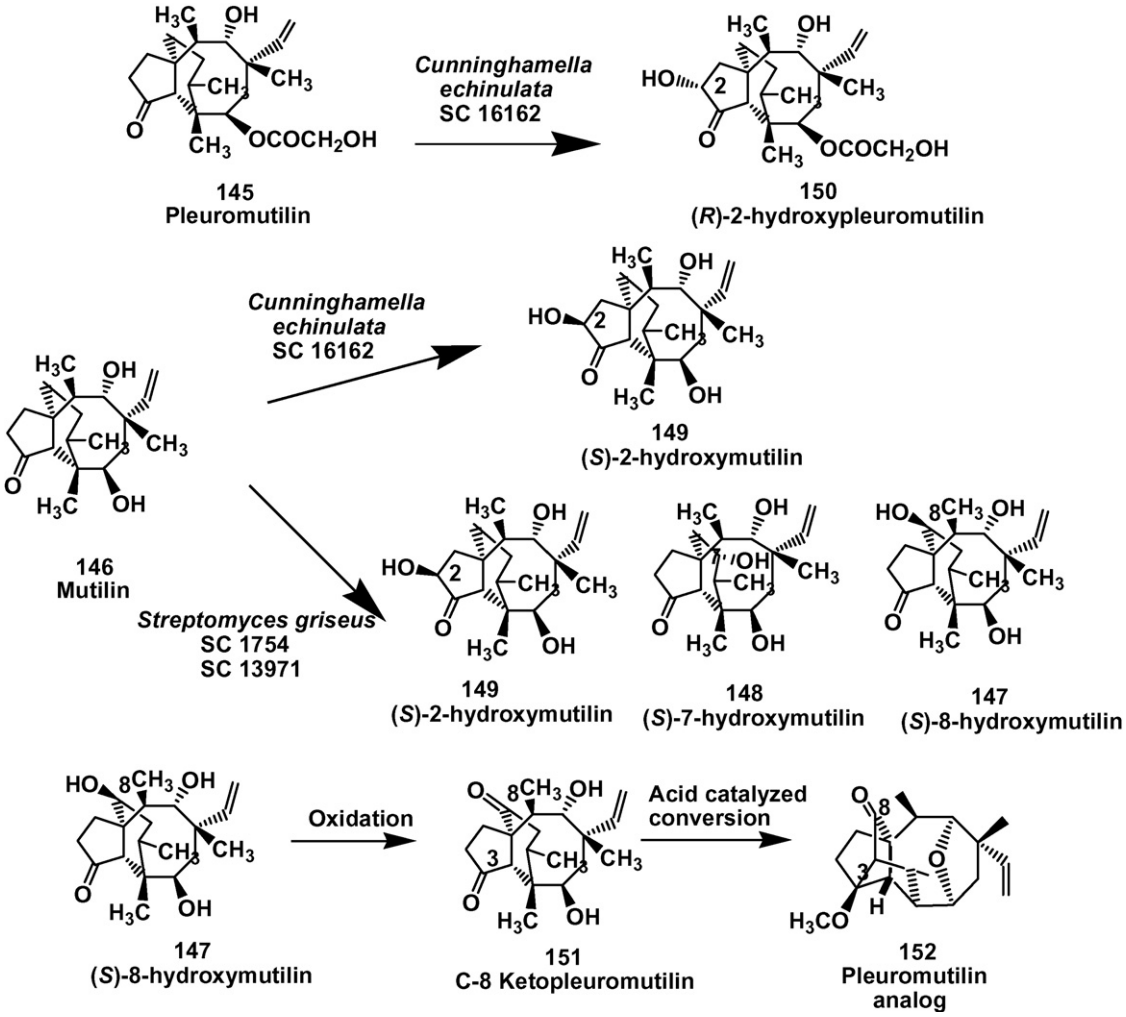

Fig. 35. Antiinfective drugs: microbial hydroxylation of pleuromutilin or mutilin.

interesting intramolecular chemistry to afford **152** with a novel pleuromutilin-derived ring system by acid catalyzed conversion of C-8 ketopleuromutilin.

25.2. Enzymatic synthesis of (S)-β-hydroxyvaline

The asymmetric synthesis of β-hydroxy-α-amino acids by various methods has been demonstrated [196] because of their utility as starting materials for the total synthesis of monobactam antiobiotics.

(*S*)-β-hydroxyvaline **153** (Fig. 36), is a key chiral intermediate required for the total synthesis of orally active monobactam [197], Tigemonam **154** (Fig. 36). The synthesis of (*S*)-β-hydroxyvaline **153** from α-keto-β-hydroxyisovalerate **155** by reductive amination using leucine dehydrogenase from *Bacillus sphaericus* ATCC 4525 has been demonstrated [198]. The NADH required for this reaction was regenerated by either formate dehydrogenase from *C. boidinii* or glucose dehydrogenase from *B. megaterium*. In this process, an overall reaction yield of 98% and an e.e. of 99.8% were obtained for the (*S*)-β-hydroxyvaline **153**.

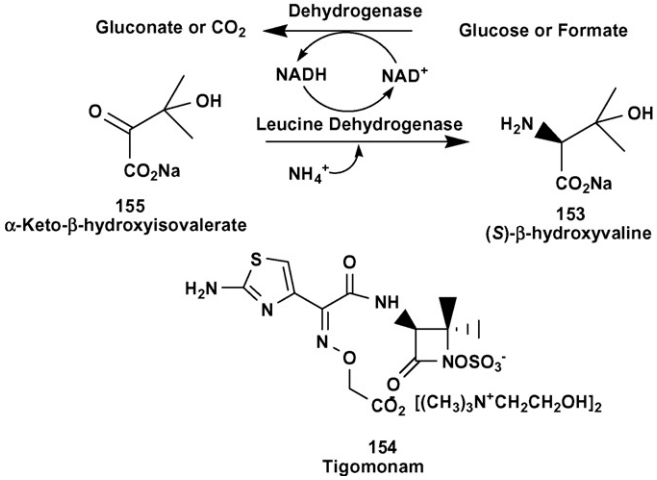

Fig. 36. Antiinfective drugs: enzymatic synthesis of L-β-hydroxyvaline.

26. α1-Adrenoreceptor antagonist

26.1. Enzymatic esterification and ammonolysis

Afuzosin **156** (Fig. 37), a quinozoline derivative, acts as a potent and selective antagonist of α1-adrenoreceptor mediated contaction of the prostate and the prostatic capsule, thereby reducing the symptoms associated with benign prostatic hypertrophy [199]. Several routes have been reported for the chemical synthesis of Afuzosin, with tetrahydro-*N*-[3-(methylamino)-propyl]-2-furancarboxamide **157** as a key intermediate [200]. The reported synthesis from 2-tetrahydrofuroic acid involves toxic reagents and harsh reaction conditions [201]. Lipase-catalyzed ammonolysis reactions using ammonia as the nucleophile have been demonstrated with esters [202]. A lipase-catalyzed process has been described for the one-pot conversion of carboxylic acids into substituted amides via *in situ* formation of the ethyl ester and subsequent ammonolysis [203]. The procedure was optimized for the preparation of **157** and involved the treatment of the corresponding carboxylic acid

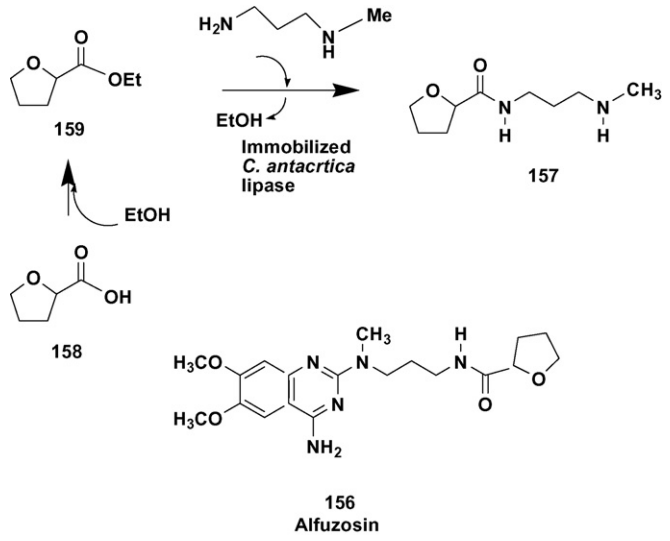

Fig. 37. α1-Adrenoreceptor antagonist: enzymatic esterification and ammonolysis.

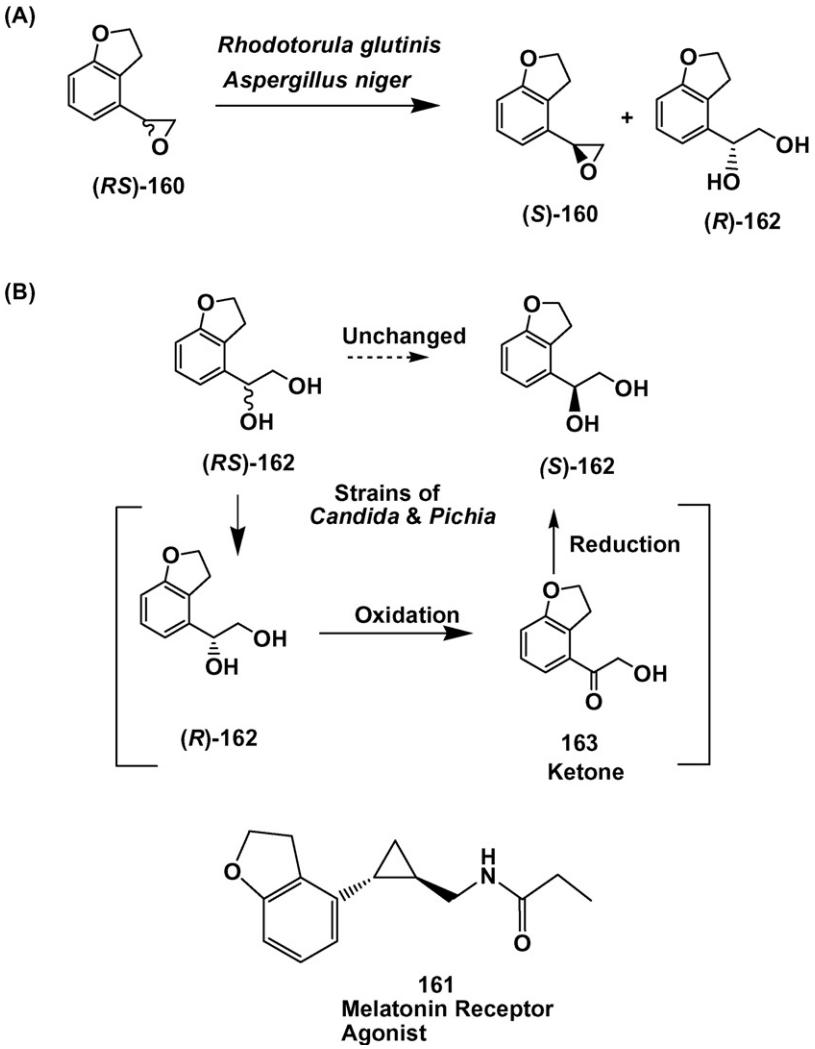

Fig. 38. (A) Melatonin receptor agonist: enantioselective enzymatic hydrolysis of racemic 1-{2′,3′-dihydro benzo[b]furan-4′-yl}-1,2-oxirane (B) dynamic kinetic resolution of (*R,S*)-1-{2′,3′-dihydrobenzo[b]furan-4′-yl}-ethane-1,2-diol.

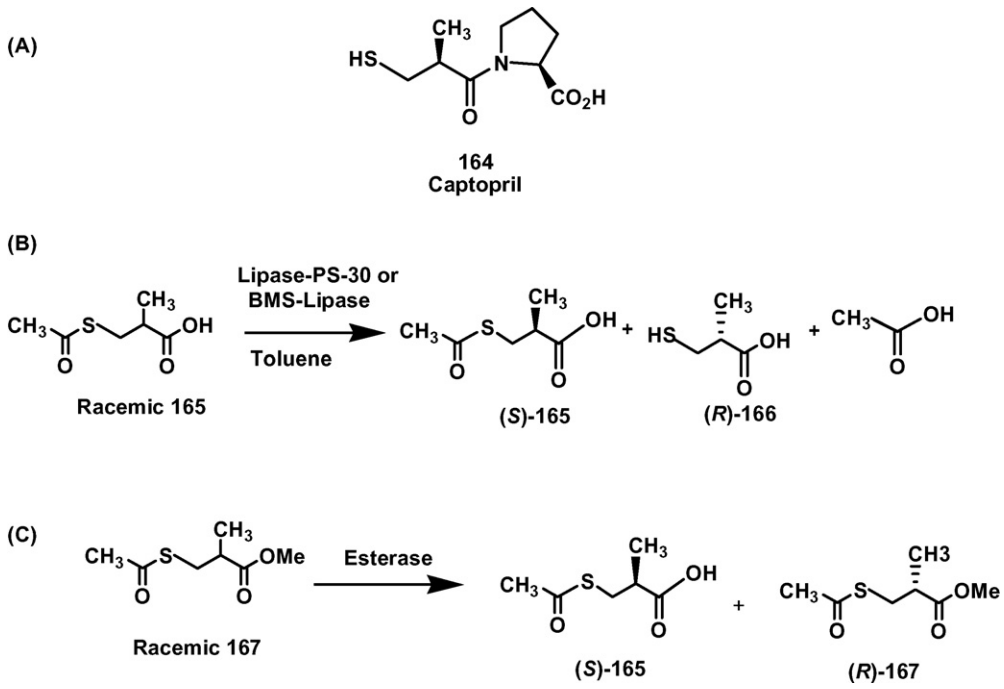

Fig. 39. (A) Antihypertensive drug, Captopril (B) enantioselective hydrolysis of racemic 3-acetylthio-2-methyl propanoic acid by lipase (C) enantioselective hydrolysis of racemic 3-mercapto-2-methylpropionic acid, methyl ester by esterase.

158 with ethyl alcohol to prepare ester **159** in the presence of immobilized *C. antarctica* lipase followed by addition of *N*-methyl-1,3-propanediamine. The amide **157** was obtained in 72% yields. Immobilized enzyme was reused over eight cycles in this process. This process was proven to be general and can be applied to open-chain, cyclic, hydroxy-, amino-, dicarboxylic, and unsaturated acids [203]. The enzyme shows regioselective behavior in relation to primary and secondary amino groups.

27. Melatonin receptor agonist

27.1. Enantioselective enzymatic hydrolysis of racemic 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 [204,205]. The (*S*)-epoxide **160** (Fig. 38A) is a key intermediate in the synthesis of a melatonin receptor agonist **161** [206,207]. The enantiospecific hydrolysis of the racemic 1-{2′,3′-dihydro benzo[b]furan-4′-yl}-1,2-oxirane **160** to the corresponding (*R*)-diol **162** and unreacted *S*-epoxide **160** has been demonstrated [208]. Two *A. niger* strains (SC 16310, SC 16311) and *Rhodotorula glutinis* SC 16293 selectively hydrolyzed the (*R*)-epoxide, leaving behind the (*S*)-epoxide **160** in >95% e.e. and 45% yield (theoretical maximum yield is 50%). Several solvents at 10% vol/vol were evaluated in an attempt to improve the e.e. and yield and were found to have significant effects on both the extent of hydrolysis and the e.e. of unreacted (*S*)-epoxide **160**. Most solvents gave a lower e.e. product and slower reaction rate than that of reactions without any solvent supplement, although MTBE gave a reaction yield of 45% and an e.e. of 99.9% for unreacted (*S*)-epoxide **160**. MTBE increased the *E* value of enzyme from 14 to 67.

27.2. Biocatalytic dynamic kinetic resolution of (R,S)-1-{2′,3′-dihydrobenzo[b]furan-4′-yl}-ethane-1,2-diol

While commonly used biocatalytic kinetic resolution of racemates often provide compounds with high e.e., the maximum theoretical yield of product is only 50%. The reaction mixture contains a ca. 50:50 mixture of reactant and product which 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" process involving a biocatalyst or biocatalyst with metal-catalyzed *in situ* racemization [209–212].

S-1-(2′,3′-Dihydrobenzo[b]furan-4′-yl)ethane-1,2-diol (**162**, Fig. 38B) is a potential precursor of *S*-epoxide **160** [206]. The dynamic kinetic resolution of the racemic diol **162** to the (*S*)-enantiomer **162** has been demonstrated [213]. Seven cultures [*C. boidinii* SC 13821, SC 13822, SC 16115, *P. methanolica* SC 13825, SC 13860 and *H. polymorpha* SC 13895, SC 13896] were found to be promising, providing (*S*)-diol **162** in 87–100% e.e.s and 60–75% yields. A new compound was formed during these biotransformations and was identified as the hydroxy ketone **163** by an LC-MS. The area of the HPLC peak for hydroxy ketone **163** 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 **162** in 3–4 days to (*S*)-diol **162** in >70% yield and 90–100% e.e.

28. Antihypertensive drugs

28.1. Enzymatic preparation of (S)-3-acetylthio-2-methyl propanoic acid

Captopril is designated chemically as 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline **164** (Fig. 39A), it prevents the conversion of angiotensin I to angiotensin II by inhibition of angiotensin converting enzyme (ACE). The potency of captopril as an inhibitor of ACE depends critically on the configuration of the mercaptoalkanoyl moiety; the compound with the *S*-configuration is about 100 times more active than its corresponding *R*-isomer [214,215]. The synthesis of the (*S*)-sidechain of captopril by the lipase-catalyzed enantioselective hydrolysis of racemic 3-acetylthio-2-methyl propanoic acid (**165**, Fig. 38B) to yield desired (*S*)-**165**, (*R*)-3-mercapto-2-methylpropanoic acid **166** and acetic acid has been demonstrated [216]. Lipase from *Rhizopus oryzae* ATCC 24563 (heat-dried cells) and lipase PS-30 from *P. cepacia* in toluene catalyzed the hydrolysis of the thioester bond of the undesired enantiomer to yield the desired (*S*)-1**65** in >28% yield (theoretical max is 50%) and e.e.'s of >95% using each enzyme. In an alternate process, desired 3-mercapto-(2*S*)-methylpropionic acid (*S*)-**165** was prepared by enantioselective hydrolysis of racemic 3-mercapto-2-methylpropionic acid, methyl ester **167** (Fig. 39C) by esterase from *Pseudomonas* sp. MRC strain. A reaction yield of 49% (max yield is 50%) and e.e. of 99.9% was obtained for (*S*)-**165** [217].

28.2. Asymmetric enzymatic hydrolysis of diacetate to (S)-monoacetate

(*S*)-monoacetates (**168**, **169**, Fig. 40) are key chiral intermediates for the chemo-enzymatic synthesis of Monopril **170**, an antihypertensive drug which acts as an ACE inhibitor [218]. The asymmetric hydrolysis of 2-cyclohexyl-1,3-propanediol diacetate **171** and 2-phenyl-1,3-propanediol diacetate **172** to the corresponding (*S*)-monoacetate **168** and (*S*)-monoacetate **169** by porcine pancreatic lipase (PPL) and *Chromobacterium viscosum* lipase was demonstrated [219]. In a biphasic system using 10% toluene, reaction yields of >65% with e.e.s of 99% were obtained for (*S*)-**168** using each enzyme. (*S*)-**169** was obtained in 90% reaction yield with 99.8% e.e. using *C. viscosum* lipase under similar conditions.

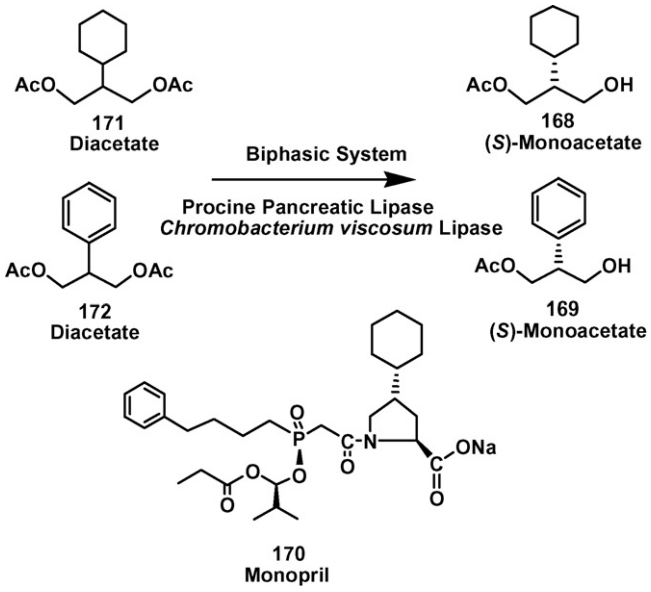

Fig. 40. Antihypertensive drug, Monopril: enzymatic preparation of (*S*)-2-cyclohexyl- and (*S*)-2-phenyl 1,3-propanediol monoacetates.

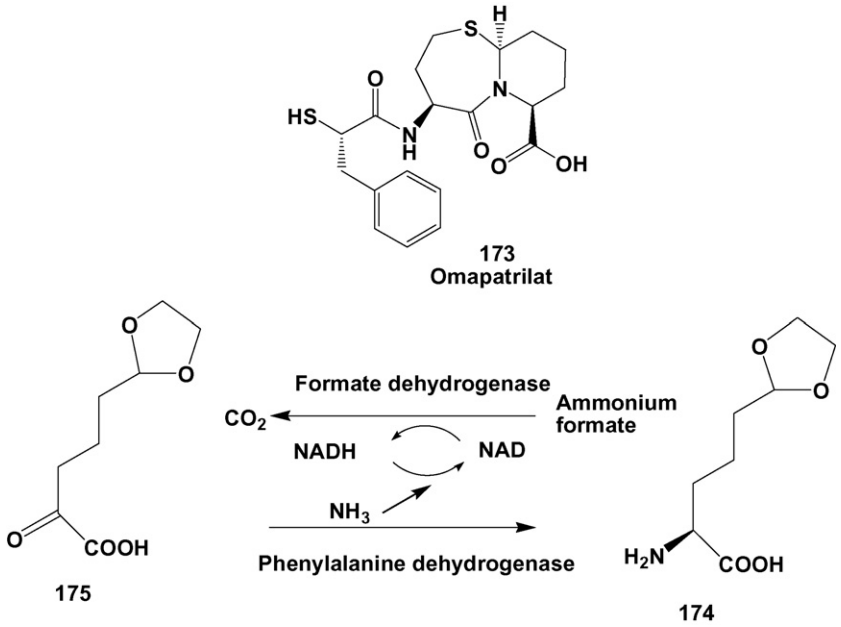

Fig. 41. Antihypertensive drug, Ompatrilat: enzymatic synthesis of allysine ethylene acetal.

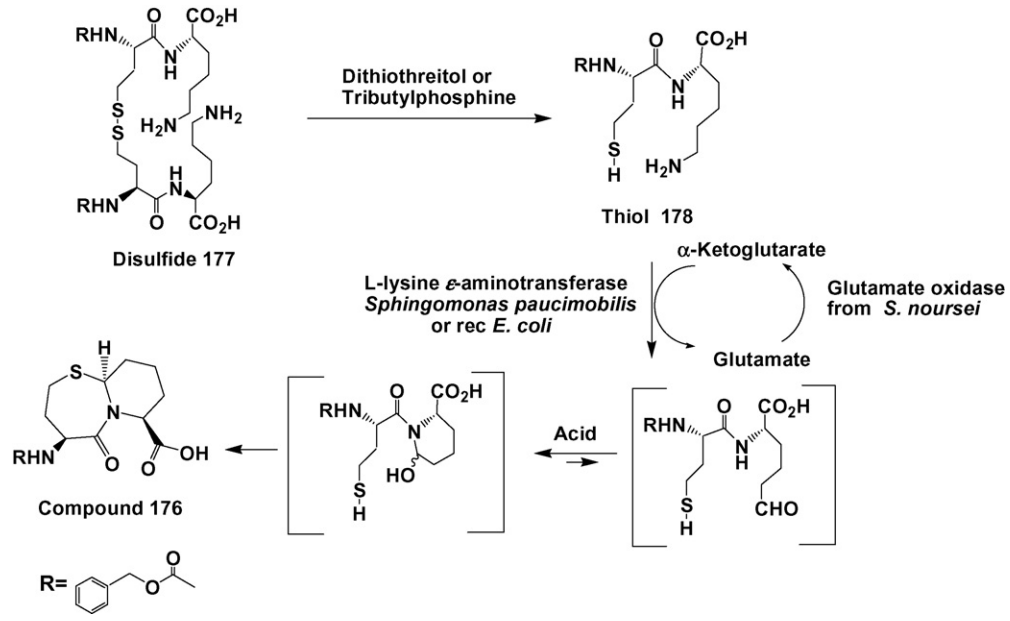

Fig. 42. Antihypertensive drug, Ompatrilat: enzymatic synthesis of thiazepine.

28.3. Enzymatic synthesis of allysine ethylene acetal

Omapatrilat (**173**, Fig. 41) is an antihypertensive drug which acts by inhibiting angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) [220]. (*S*)-2-Amino-5-(1,3-dioxolan-2-yl)-pentanoic acid [(*S*)-allysine ethylene acetal] **174** is one of three building blocks used in an alternative synthesis of Omapatrilat. Synthesis of **174** was demonstrated by reductive amination of ketoacid acetal **175** using phenylalanine dehydrogenase (PDH) from *T. intermedius* [221]. The reaction required ammonia and NADH; NAD produced during the reaction was recyled to NADH by the oxidation of formate to CO_2 using formate dehydrogenase (FDH). *T. intermedius* PDH was cloned and expressed in *E. coli* and recombinant culture was used as a source of PDH. *P. pastoris* [222] grown on methanol are also useful sources of FDH. Expression of *T. intermedius* PDH in *P. pastoris*, inducible by methanol, allowed generation of both enzymes in a single fermentation. A total of 197 kg of **174** was produced in three 1600-L batches using a 5% concentration of substrate **175** with an average yield of 91 M % and e.e. >98% [221]. (*S*)-allysine ethylene acetal was converted to Omapatrilat **173** [222]

28.4. Enzymatic synthesis of thiazepine

[4*S*-(4a,7a,10ab)]-1-Octahydro-5-oxo-4-[[(phenylmethoxy) carbonyl]amino]-7H-pyrido-[2,1-b] [1,3]thiazepine-7-carboxylic acid (**176**, Fig. 42) is a key intermediate in the synthesis of Omapatrilat **173** [220]. An enzymatic process was developed for the preparation of compound **176** from compound **177**. L-lysine-ε-aminotransferase from *S. paucimobilis* SC 16113, was used for process development. Due to the low activity of the enzyme, L-lysine ε-aminotransferase (LAT) was overexpressed in *E. coli* strain GI724(pAL781-LAT) and a biotransformation process was developed [223]. The aminotransferase reaction required α-ketoglutarate as the amine acceptor. Glutamate formed during this reaction was recycled back to α-ketoglutarate by glutamate oxidase (GOX) from

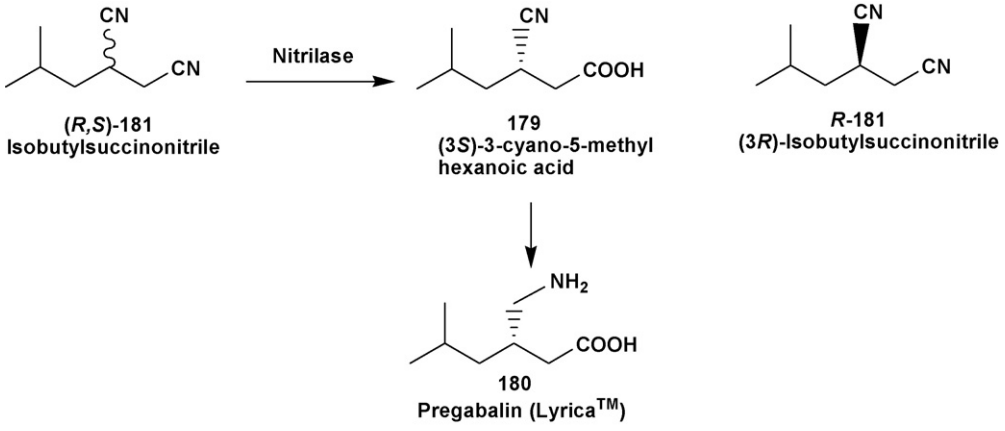

Fig. 43. GABA analogs: enzymatic synthesis of (3*S*)-3-cyano-5-methyl hexanoic acid.

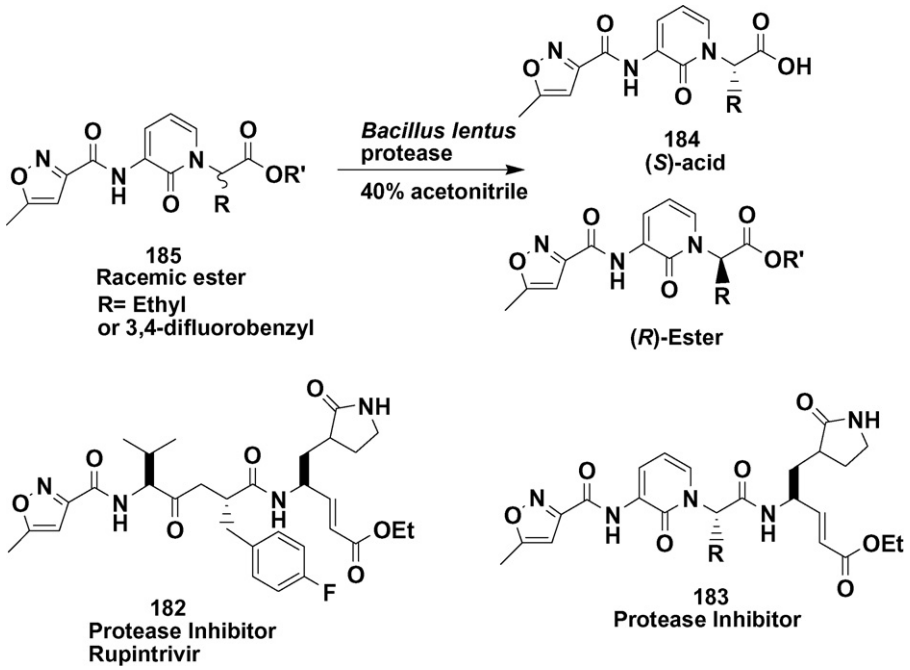

Fig. 44. Rhinovirus Protease Inhibitor: enzymatic preparation of (2*S*)-2-[3-{[(5-methylisoxazol-3-yl)carbonyl]amino}-2-oxopyridin-1(2*H*)-yl]pent-4-ynoic acid.

Streptomyces noursei SC 6007. The extracellular GOX was cloned and expressed in *Streptomyces lividans* [223].

In biotransformation process, the thiol substrate **178** was generated *in situ* from the disulfide N^2-[*N*[[(phenylmethoxy) carbonyl] L-homocysteinyl] L-lysine)-1,1-disulphide **177** by treatment with DTT or tributylphosphine. The conversion of compound **178** to compound **176** was carried out using LAT from *E. coli* GI724[pal781-LAT] in the presence of α-ketoglutarate and dithiothreitol (DTT or tributylphosphine) and glutamate oxidase. Reaction yields of 65–67 M% were obtained [223].

29. GABA analog

29.1. Enzymatic synthesis of (3S)-3-cyano-5-methyl hexanoic acid

(3*S*)-3-cyano-5-methyl hexanoic acid (**179**, Fig. 43) is a key chiral intermediate for the preparation of Pregabalin (Lyrica® API) **180**, a marketed GABA analog for the treatment of neuropathic pain and partial seizures [224]. (3*S*)-3-cyano-5-methyl hexanoic acid **179** was prepared by a regio- and stereo-specific hydrolysis of racemic isobutylsuccinonitrile **181**. Several plant and bacterial nitrilases were cloned from the gene bank and their substrate specificity and enantioselectivity were studied using a fluorescent assay by capturing the NH_3 byproduct. The desired nitrilase AtNit1 from *Arabidopsis thaliana* with high enantioselectivity ($E > 150$) was then optimized to increase expression and engineered to improve activity [225]. At 20 g/L substrate input, the reaction yield of 95% and e.e. of 98.5% was obtained for desired product **179** [226].

30. Rhinovirus protease inhibitor

30.1. Enzymatic preparation of (2S)-2-[3-{[(5-methylisoxazol-3-yl) carbonyl]amino}-2-oxopyridin-1(2H)-yl]pent-4-ynoic acid

Currently, no effective therapy exists to directly treat the common cold. Since the condition is mainly caused by rhinovirus infections, the use of inhibitors that target the 3C protease, a protein required for viral replication, has been reported. The design and development of substrate-derived tripeptidyl protease inhibitors have been investigated [227]. A series of promising compounds including Rupintrivir™ **182** and **183** (Fig. 44) have emerged as lead candidates for this ailment with Rupintrivir being entered into human clinical trials [228]. (2*S*)-2-[3-{[(5-methylisoxazol-3-yl)carbonyl]amino}-2-oxopyridin-1(2*H*)-yl]pent-4-ynoic acid **184** is a key intermediate in the synthesis of a human rhinovirus (HRV) protease inhibitor. An efficient enzymatic process has been developed for the kinetic resolution of racemic ester **185** to afford (*S*)-acid **184** in 49% chemical yield/per cycle with 98% e.e. The enzymatic process was carried out in the presence of 40% acetonitrile using the alkaline protease from *Bacillus lentus* [229].

31. Enzymatic Baeyer–Villiger reactions

Enzyme-mediated Baeyer–Villiger oxidation offers a "green chemistry" approach for the production of chiral lactones [230]. An increasing number of flavin-dependent Baeyer–Villiger monooxygenases (BVMOs) with a remarkably broad profile of non-natural substrates has been identified during recent

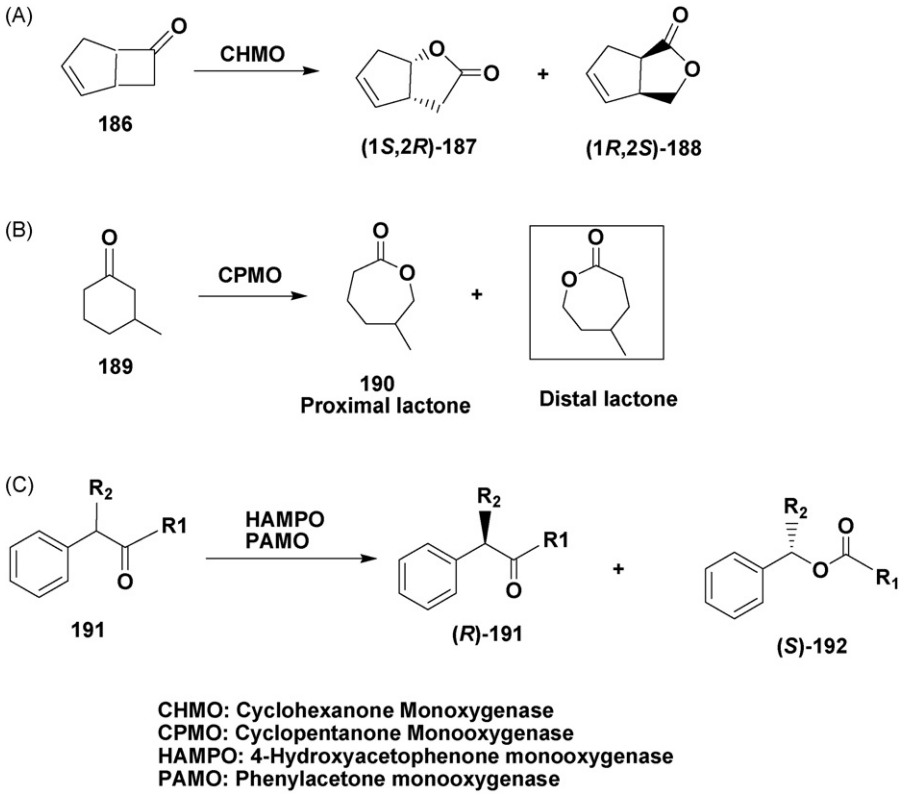

Fig. 45. (A) Baeyer-Villiger oxidation of *rac*-bicyclo[3.2.0]hept-2en-6one (B) Baeyer–Villiger oxidation of *rac*-3-methylcyclohexanone (C) Baeyer–Villiger oxidation of racemic ketones.

years [231–248]. Enantiodivergent whole-cell Baeyer–Villiger oxidation of *rac*-bicyclo[3.2.0]hept-2en-6one **186** (Fig. 45A) by cyclohexanone monooxygenase has been demonstrated on a kilogram scale. A high productivity was obtained combining a resin-based *in situ* substrate feeding and product removal methodol. (*in situ* SFPR), a glycerol feed control, and an improved oxygenation device (using a sintered-metal sparger). As expected both regioisomeric lactones [(−)-(1*S*,5*R*)-**187** and (−)-(1*R*,5*S*)-**188**] were obtained in nearly enantiopure form (e.e. >98%) and good yield [235]. Also regioselective Baeyer–Villiger oxidation of *rac*-3-methylcyclohexanone **189** (Fig. 45B) by cyclopentanone monoxygenase yielding only proximal lactone **190** has been demonstrated [237].

A set of racemic cyclic and linear ketones **191** (Fig. 45C), as well as 2-phenylpropionaldehyde, were tested as substrates in the enzymatic Baeyer–Villiger oxidation catalyzed by two monooxygenases: phenylacetone monooxygenase (PAMO) and 4-hydroxyacetophenone monooxygenase (HAPMO) to yield (*R*)-**191** and (*S*)-lactones **192**. Excellent enantioselectivites ($E > 200$) was obtained in the kinetic resolution processes depending on the substrate structure and the reaction conditions. The parameters affecting the biocatalytic properties of these enzymes were also studied, in order to establish a deeper understanding of these novel biocatalysts [241].

Use of recombinant whole-cell expression systems for Baeyer–Villiger monooxygenases of various bacterial origin were utilized in the regiodivergent biooxidation of cyclic terpenones enabling access to enantio- and regioisomeric lactones on a preparative scale [242].

Recombinant *E. coli* B834 (DE3) pDB5 expressing the Rv3049c gene encoding a Baeyer–Villiger monooxygenase from *Mycobacterium tuberculosis* H37Rv was used for regioselective oxidations of fused bicyclic ketones. This whole-cell system represents the first recombinant Baeyer–Villiger oxidation biocatalyst that effectively resolves the racemic starting materials in this series. Within biotransformations using this organism one substrate enantiomer remains in high optical purity, while the second enantiomer is oxidized to one type of regioisomeric lactone [243].

A heterobicyclic lactone was obtained via stereoselective Baeyer–Villiger biooxidation with recombinant whole-cells expressing cyclopentanone monooxygenase from *Comamonas* sp. NCIMB 9872 was used for the total syntheses of various natural products, such as (+)-showdomycin, (+)-*trans*-kumausyne, goniofufurone analogs [244].

The design and evolution of cyclopentanone monooxygenase (CPMO) was described by a relatively inexpensive and rapid way to obtain mutant enzymes with the desired characteristics. Several successful mutants with enhanced enantioselectivities were identified. For example, mutant-catalyzed oxidation of 4-methoxycyclohexanone gave the corresponding lactone with 92% entantiometric excess compared to the 46% e.e. achieved with wild-type cyclohexanone monoxygenase [245].

E. coli expressing cyclohexanone monooxygenase catalyzed a highly enantioselective Baeyer–Villiger oxidation of α-

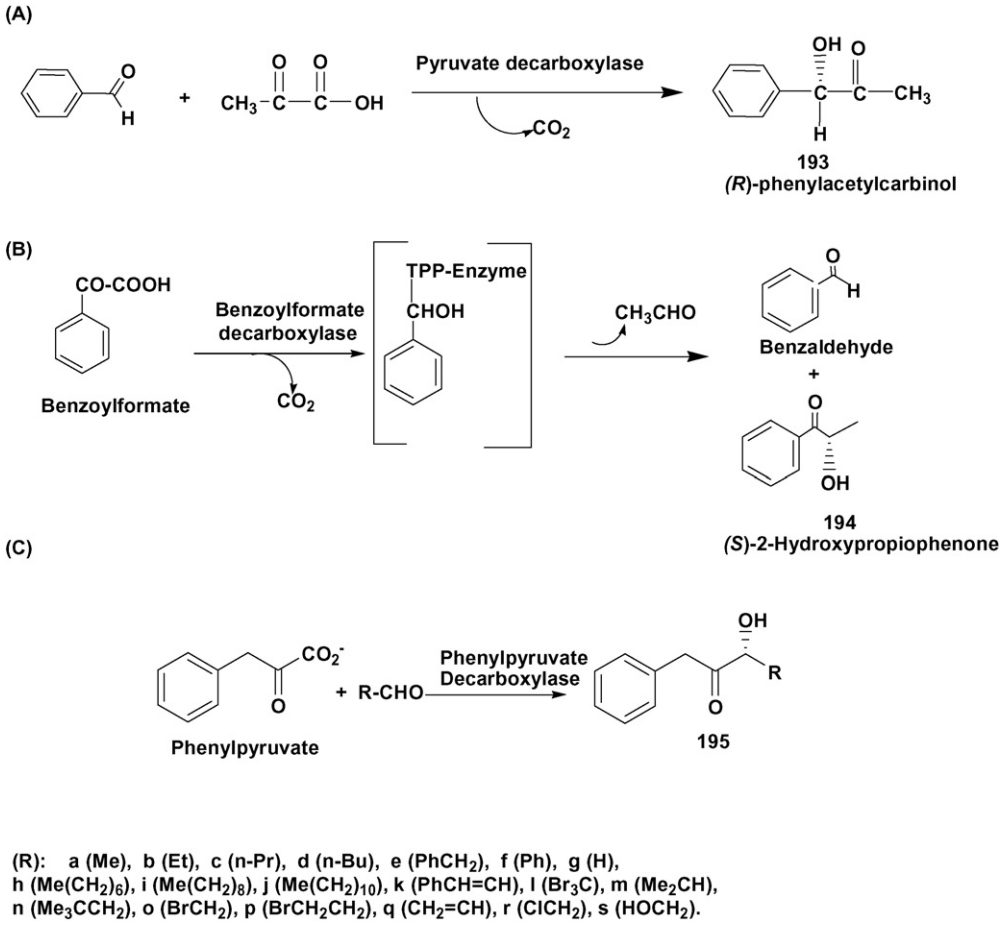

Fig. 46. (A) Enzymatic preparation of production of *(R)*-phenylacetylcarbinol (B) enzymatic preparation of *(S)*-2-hydroxypropiophenone (C) enzymatic asymmetric acyloin condensation of phenylpyruvate.

substituted cyanocyclohexanones, leading to the corresponding enantiopure caprolactones (e.e.'s >97%). Classical kinetic resolution and regiodivergent parallel kinetic resolution patterns were observed depending on the length of the linear chain at the alpha position [246].

Among available Baeyer–Villiger monooxygenases, phenylacetone monooxygenase is an attractive biocatalyst because of its thermostability, however, it is only active with a limited number of substrates. By means of a comparison of the PAMO structure and a modeled structure of the sequence-related cyclopentanone monooxygenase, several active-site residues were selected for a mutagenesis study in order to alter the substrate specificity. The M446G PAMO mutant was found to be active with a number of aromatic ketones, amines and sulfides for which wild-type PAMO shows no activity. In addition to an altered substrate specificity, the enantioselectivity towards several sulfides was dramatically improved. This newly designed Baeyer–Villiger monooxygenase extends the scope of oxidation reactions [247,248].

32. Enzymatic acyloin and aldol condensations

Stereoselective carbon–carbon bond forming reactions are useful synthetic methods in the creation of up to two adjacent stereocenters [249]. Several classes of enzymes are able to catalyze stereoselective C–C bond formation reactions such as aldolases, oxynitrilases and thiamin diphosphate-dependent deacardoxylases [250–262].

Pyruvate decarboxylase was used for the production of *(R)*-phenylacetylcarbinol **193** (Fig. 46A), a precursor to (−)-ephedrine, is still used on an industrial scale [253]. The optimization of a continuous enzymatic reaction yielding (*R*)-phenylacetylcarbinol **193**, was evaluated using three different three pyruvate decarboxylases (PDC), PDC from *S. cerevisiae*, PDC from *Zymomonas mobilis*, and a potent mutant of the latter, PDC*W392M*, using acetaldehyde and benzaldehyde as substrates. Among these, the mutant enzyme was the most active and most stable. The reaction conditions were optimized and the carboligation was performed using a continuous reaction system and feeding both aldehydes in equimolar concentration. Initial studies using a continuously operated enzyme-membrane reactor gave (*R*)-PAC with a space-time yield of 81 g L^{-1} d^{-1} using a substrate concentration of 50 mM of both aldehydes. The yield was increased by cascadation of enzyme-membrane reactors. The new strategy allows the synthesis of (*R*)-PAC from cheap substrates [254].

Bacterial and yeast decarboxylases have been evaluated for production of hydroxyl ketones, α-arylacetate, *R*-amino

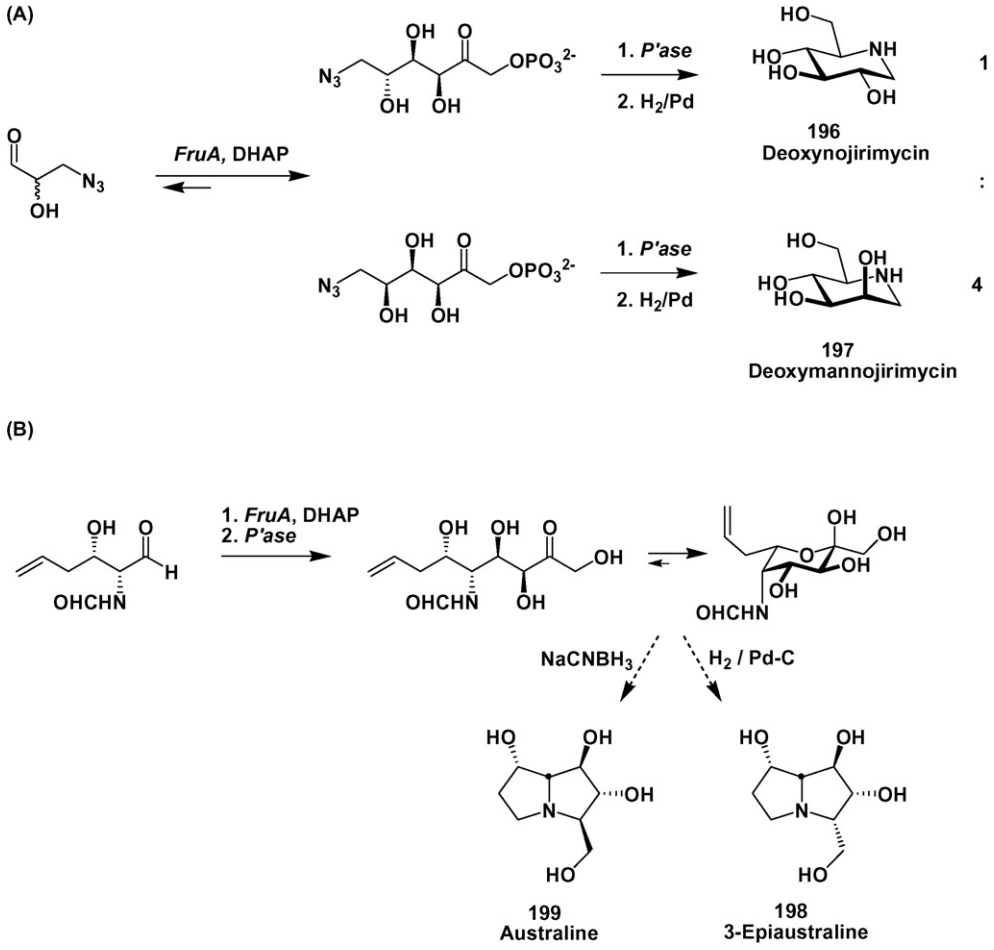

Fig. 47. (A) Enzymatic preparation of deoxynojirimycin and deoxymannojirimycin (B) enzymatic preparation of 3-epiaustraline and australine.

acids, dopamine and organic acids [253–258]. Benzoylformate decarboxylase dependent formation of acyloin compound (*S*)-2-hydroxypropiophenone **194** (Fig. 46B) has been demonstrated [255]. A review article describing various benzylformate catalyzed acyloin condensation reactions have been published [255,256,260,262].

Phenyl pyruvate (PPD)-dependent asymmetric acyloin condensation of phenylpyruvate with various aldehydes to produce optically active acyloins $PhCH_2COCH(OH)R$ **195** (Fig. 46C) has been demonstrated [261].

Today, many useful synthetic applications have been demonstrated for different classes of aldolases, and for related C–C bond forming enzymes [263–271]. The synthesis of rare and novel "aza sugars", which represent powerful glycosidase inhibitors or show potent antiviral activity has been developed using D-fructose 1,6-bisphosphate aldolase [268–270]. Prominent examples concern the FruA catalyzed synthesis of deoxynojirimycin **196** and deoxymannojirimycin **197** (Fig. 47A) from 3-azido-2-hydroxypropanal [270].

Similarly, a D-fructose 1,6-bisphosphate aldolase-mediated enenatioselective dihydroxy acetone phosphate addition to intermediate served as the key step in the chemo-enzymatic synthesis of 3-epiaustraline **198** and australine **199** (Fig. 47B) [271]. Australine and 3-epiaustraline are naturally occurring pyrrolizidine alkaloids, having potent glycosidase inhibitory and antiviral activities.

Lyases have extensively used in the formation of C–N bond [272–275]. Tyrosinphenol lyase-catalyzed synthesis of L-DOPA **200** (Fig. 48A) using *Erwinia herbicola* was developed to yield 110 g/L of product. More then 125 tons of L-DOPA is produced per year using biotransformation process [276,277].

A two-step chemoenzymatic synthesis of (*R*)-2-amino-1-(2-furyl)ethanol **201** (Fig. 48B) was developed by a highly enantioselective cyanohydrin reaction of furan-2-carbaldehyde **202** with hydrocyanic acid catalyzed by the hydroxynitrile lyase from *Hevea brasiliensis*. Subsequent sodium borohydride reduction furnished the desired product with an e.e. of >99.5%. This procedure can be considered a convenient general route for the stereoselective synthesis of ethanol amine derivatives underlining the role of biocatalysis for the generation of stereogenic centres in the synthesis of chiral intermediates [278].

33. Dynamic kinetic resolution (DKR)

The deracemization of a number of pharmaceutically valuable building blocks including epoxides, alcohols, amines and acids have been carried out by biocatalysis [11,18,279–302]. DKR involves the combination of an enantioselective trans-

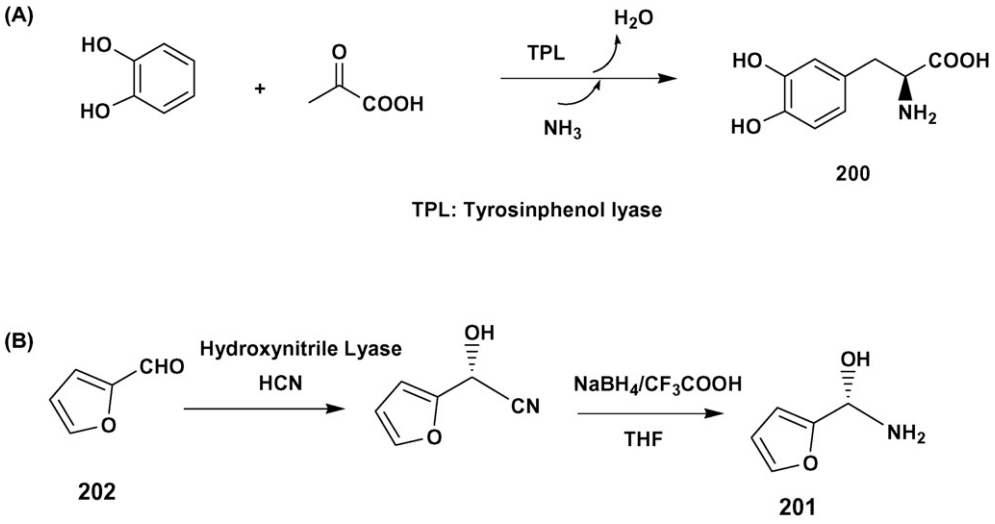

Fig. 48. (A) Lyase-catalyzed synthesis of L-DOPA (B) lyase-catalyzed synthesis of (*R*)-2-amino-1-(2-furyl)ethanol.

formation with an *in situ* racemisation process such that, in principle, both enantiomers of the starting material can be converted to the product in high yield and e.e. The racemization step can be catalyzed either enzymatically by racemases or non-enzymatically by transition metals.

Propanolol (**203**, Fig. 49) belongs to the group of β-adrenergic blocking agents and activity mainly resides in the (*S*)-enantiomers. Synthesis of (*S*)-**203** was achieved by dynamic kinetic resolution (DKR) of (±)-**204** using Novozyme-435 in toluene at 80 °C and *p*-chlorophenyl acetate as acyl donor in the presence of ruthenium complex **205**. The (*R*)-acetate **206** was produced in ee >99 and 86% isolated yield [290]. The enzyme was recycled and used again for another cycle without any loss of activity. Using the same procedure, racemic **207** was subjected to DKR to produce the (*R*)-acetate **208** (e.e. >99, 92% conversion and 84% isolated yield), a precursor of (*R*)-denopamine **209**, a potent orally active β_1 receptor agonist for the treatment of hypertension [290].

The preparation of optically active chiral amines by deracemization of racemic mixtures employs the simultaneous use of a highly selective oxidase biocatalyst and a chemical reducing agent or catalyst, and can be used for the preparation of a wide range of optically pure amines in yields often approaching 100% [11,18,281,282,291]. An example is the conversion of racemic

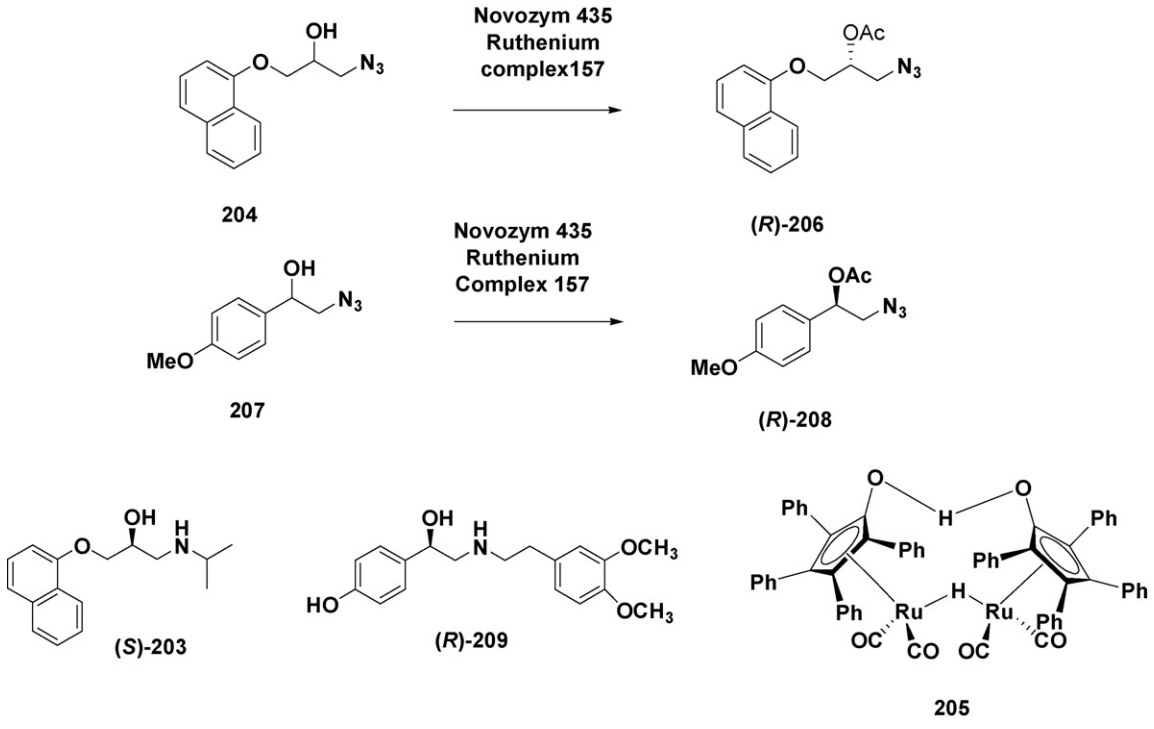

Fig. 49. Dynamic resolution for preparation of propanolol by enzyme metal complex.

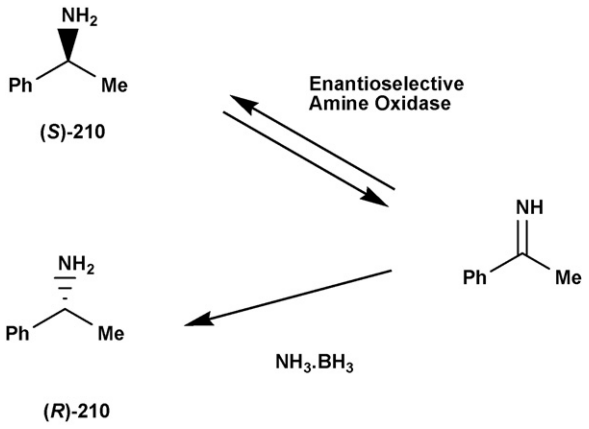

Fig. 50. Preparation of (*R*)-α-methyl benzylamine by enzymatic deracemization.

α-methyl benzylamine **210** (Fig. 50) to (*R*)-α-methyl benzylamine **210** by amine oxidase from *Aspergillus niger* [291].

The combination of *in situ* racemization mediated with thiyl radicals and lipase-catalyzed enzymic resolution enables the dynamic kinetic resolution of nonbenzylic amines. It was applied either to the conversion of racemic mixtures or to the inversion of (*S*)-enantiomers of nonbenzylic amines. Five racemic amines and two (*S*)-amines were transformed into the corresponding (*R*)-amides (in isolated yields ranging from 47% to 81%, with e.e.'s varying from 86% to >99%) in the presence of either ethyl laurate or lauric acid [284].

A practical procedure for the dynamic kinetic resolution of primary amines has been developed employing a palladium nanocatalyst as the racemization catalyst, a Novozym-435 as the resolution catalyst, and ethyl acetate or ethyl methoxyacetate as the acyl donor. Eleven primary amines and one amino acid amide have been efficiently resolved with good yields (85–99%) and high enantiomeric excesses (97–99%) [285].

The dynamic kinetic resolution of secondary amines using a iridium based catalyst coupled with an Candia rugosa lipase-catalyzed step was achieved on a large scale with high yields and e.e. The 1-Methyl-1,2,3,4-tetrahydroisoquinoline was underwent iridium-lipase-catalyzed dynamic kinetic resolution to provide the optically pure product with high enantiomeric excess [286].

The DKR of secondary alcohols (Fig. 51) has been demonstrated by combining enantioselective lipases with transition-metal-based racemisation catalysts by various groups [291–297]. Kim has recently shown that (*S*)- as well as (*R*)-configured alcohols **211** can be prepared by the use of a commercially available (*S*)-selective subtilisin from *Bacillus licheniformis* as the enantioselective acylating catalyst [293].

Dynamic kinetic resolution of a series of secondary alcohols was developed with a novel dinuclear ruthenium complex, bearing tetrafluorosuccinate and rac-BINAP ligands as the racemization catalyst. Novozym 435 has been used as the enzyme, and iso-propyl butyrate as the acyl donor. Typically the reaction reached complete conversion within 1 day with 0.1 mol% of racemization catalyst relative to the substrate. The addition of the ketone corresponding to the substrate stabilizes the active Ru complex and, therefore, increases the rate of the reaction [300].

A novel dynamic kinetic resolution process was developed for racemic allyl alcohol using a combination of lipases and [VO(OSiPh3)3] [i.e., *tris*[(triphenylsilyl)oxy]vanadium oxide]. The 1,3-transposition of allyl alcohols catalyzed by *tris*[(triphenylsilyl)oxy]vanadium oxide gave thermodynamic. Mixtures of two regioisomers, which underwent highly stereoselective and chemoselective esterification with lipases. Optically enriched allyl acetates were isolated in high yields [301].

(*S*)-Selective kinetic resolution was achieved through the use of a commercially available protease after activation with a combination of two different surfactants. The kinetic resolurtion pro-

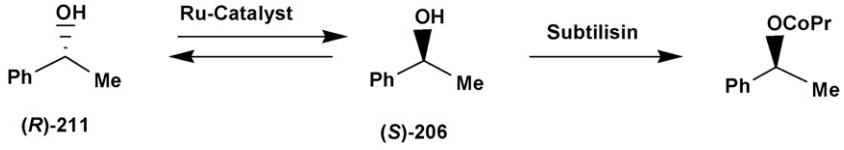

Fig. 51. DKR of secondary alcohols by (*S*)-selective subtilisin and transition metal complex.

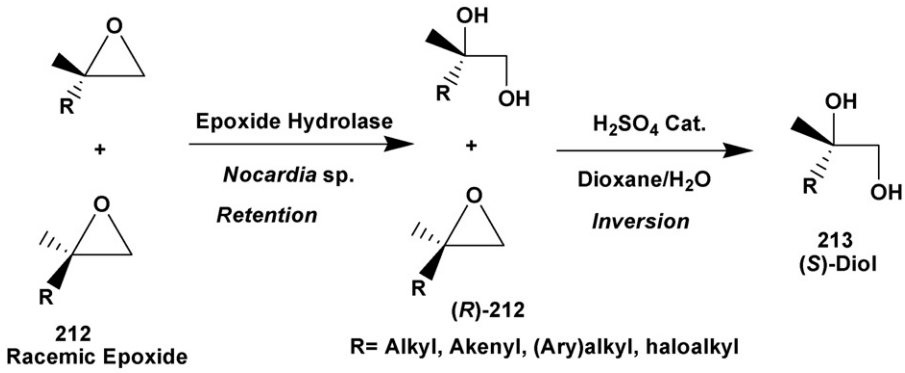

Fig. 52. Enzymatic kinetic resolution of 1-chloro-2-(2,4-difluorophenyl)-2,3-epoxypropane.

cess was optimized with respect to activation of the protease and to the acyl donor. The kinetic resolution process was compatible with a range of functionalized seconday alcohols giving good to high enantiomeric ratio values (up to >200). The enzymic resolution process was combined with a ruthenium-catalyzed racemization to give an (*S*)-selective dynamic kinetic resolution (DKR) of seconday alcohols The DKR process works under very mild reaction conditions to give the corresponding esters in high yields and with excellent enantioselectivities [302].

Using recombinant *A. niger* epoxide hydrolase under optimized conditions, the hydrolytic kinetic resolution of 1-chloro-2-(2,4-difluorophenyl)-2,3-epoxypropane **212** (Fig. 52) was demonstrated at a substrate concentration as high as 500 g/L (i.e., 2.5 M) and afforded the (unreacted) (*S*)-epoxide **212** and the corresponding vicinal diol **213**, both in nearly enantiopure form and quantitative yield [239].

34. Conclusion

The production of single enantiomers of drug intermediates has become increasingly important in the pharmaceutical industry. Organic synthesis is one approach to the synthesis of single enantiomers, and biocatalysis provides an alternate opportunity to prepare pharmaceutically useful chiral compounds. In many cases biocatalysis offers advantages over chemical catalysis due to the diverse stereoselective and regioselective transformations that can be catalyzed by enzymes under mild conditions such as ambient temperature and atmospheric pressure. In this article, the utility of a wide array of different classes of enzymes for the catalysis of different types of chemical reactions has been demonstrated to provide a key single enantiomer intermediates for pharmaceutical drug candidates. This includes the use of hydrolytic enzymes (lipases, esterases, proteases, dehalogenases, acylases, amidases, nitrilases, lyases, epoxide hydrolases) in the resolution of variety of racemic compounds and in the asymmetric synthesis of enantiomerically enriched chiral compounds. Oxido-reductases and aminotransferases have been used in the synthesis of chiral alcohols, aminoalcohols, amino acids and amines. Aldolases and decarboxylases have been effectively used in asymmetric synthesis by aldol condensation and acyloin condensation reactions. Monoxygenases have been used in enantioselective and regioselective hydroxylation, epoxidation, and Baeyer–Villiger reactions. Dioxygenases have been used in the chemo-enzymatic synthesis of chiral diols. Enzymes and enzymes with metal complexes were used in dynamic kinetic resolution to prepare chiral amines, aminoacids, alcohols and epoxides. In the course of the last decade, progress in biochemistry, protein chemistry, molecular cloning, random and site-directed mutagenesis, directed evolution of biocatalysts and fermentation technology has opened up unlimited access to a variety of enzymes and microbial cultures as tools in organic synthesis.

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References

[1] R. Patel, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2006.
[2] M. Oliver, C.A. Voigt, F.H. Arnold, Enzyme Catalysis in Organic Synthesis, second ed., vol. 1, 2002, p. 95.
[3] P.C. Cirino, F.H. Arnold, Curr. Opin. Chem. Biol. 6 (2) (2002) 130.
[4] E.L. Haseltine, F.H. Arnold, Annu. Rev. Biophys. Biomol. Struct. 36 (2007) 1.
[5] M.T. Reetz, M. Hermes, BIOspektrum 11 (5) (2005) 681.
[6] U.T. Bornscheuer, Curr. Opin. Biotechnol. 13 (6) (2002) 543.
[7] T. Eggert, Thorsten, Industrial Biotransformations, second ed., 2006, p. 93.
[8] S.B. Rubin-Pitel, H. Zhao, Comb. Chem. High Throughput Screen. 9 (4) (2006) 247.
[9] R.J. Kazlauskas, Curr. Opin. Chem. Biol. 9 (2) (2005) 195.
[10] M. Schmidt, M. Baumann, E. Henke, M. Konarzycka-Bessler, U.T. Bornscheuer, Methods Enzymol. 388 (2004) 199.
[11] N.J. Turner, R. Carr, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, 2007, p. 743.
[12] M.T. Reetz, C. Torre, A. Eipper, R. Lohmer, M. Hermes, B. Brunner, A. Maichele, M. Bocola, M. Arand, A. Cronin, Y. Genzel, A. Archelas, R. Furstoss, Org. Lett. 6 (2) (2004) 177.
[13] J. Sylvestre, H. Chautard, F. Cedrone, M. Delcourt, Marc. Org. Proc. Res. Devel. 10 (3) (2006) 562.
[14] K.L. Tee, U. Schwaneberg, Comb. Chem. High Throughput Screen. 10 (3) (2007) 197.
[15] J.A. Pesti, R. DiCosimo, Curr. Opin. Drug Discov. Devel. 6 (2003) 884.
[16] R.N. Patel, Curr. Org. Chem. 10 (11) (2006) 1289.
[17] S. Yolanda, W. Kroutil, K. Faber, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 27.
[18] N.J. Turner, Curr. Opin. Chem. Biol. 8 (2) (2004) 114.
[19] V. Alphand, G. Carrea, R. Wohlgemuth, R. Furstoss, J.M. Woodley, Trends Biotechnol. 21 (7) (2003) 318.
[20] A. Hidalgo, U.T. Bornscheuer, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 159.
[21] T. Ishige, K. Honda, S. Shimizu, Curr. Opin. Chem. Biol. 9 (2) (2005) 174.
[22] J.D. Stewart, Curr. Opin. Chem. Biol. 5 (2) (2001) 120.
[23] W.-D. Fessner, S. Jennewein, Stefan, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 363.
[24] D.R. Boyd, N.D. Sharma, C.C.R. Allen, Curr. Opin. Biotechnol. 12 (6) (2001) 564.
[25] V. Gotor-Fernandez, F. Rebolledo, V. Gotor, in: R. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRF Press, Florida, 2007, p. 203.
[26] A.S. Bommarius, B.R. Riebel (Eds.), Biocatalysis, Wiley-VCH, 2004.
[27] A.A.J. Straathof, P. Adlercreutz (Eds.), Applied Biocatalysis, CRC Press, 2000.
[28] K.D. Drauz, H. Waldman (Eds.), Enzyme Catalysis in Organic Synthesis, Wiley-VCH, 1994.
[29] K. Faber (Ed.), Biotransformations in Organic Chemistry, Springer, 2005.

[30] A. Liese, K. Seelbach, C. Wandrey (Eds.), Industrial Biotransformations, Wiley-VCH, 2006.
[31] R.N. Patel (Ed.), Stereoselective Biocatalysis, Marcel-Dekker, 2000.
[32] K. Buchholz, V. Kasche, U.T. Bornscheuer, Biocatalysts and Enzyme Technology, Wiley-VCH, 2005.
[33] J.W. Frost, J. Lievense, New J. Chem. 18 (3) (1994) 341.
[34] K.E. Tyo, H.S. Alper, G.N. Stephanopoulos, Trends Biotechnol. 25 (3) (2007) 132.
[35] V.F. Wendisch, M. Bott, B.J. Eikmanns, Curr. Opin. Microbiol. 9 (3) (2006) 268.
[36] A.J. Kinney, Curr. Opin. Biotechnol. 17 (2) (2006) 130.
[37] B. Fulton, R.N. Brogden, CNS Drugs 7 (1) (1997) 68.
[38] J.F. Heiser, C.S. Wilcox, CNS Drugs 10 (5) (1998) 343.
[39] H.K. Jajoo, R.F. Mayol, J.A. LaBudde, I.A. Blair, Drug Metab. Dispos. 17 (6) (1989) 634.
[40] R.F. Mayol, U.S. (2000) 14 pp., Cont.-in-part of U.S. Ser. No. 484,161 US 6150365 A 20001121 CAN 133:359250 AN 2000:819478.
[41] J.P. Yevich, R.F. Mayol, Pat. Appl. Publ. (2005) 14, Cont.-in-part of U.S. Ser. No. 201,733. US 2005137206 A1 20050623 CAN 143:53538 AN 2005:547263.
[42] J.P. Yevich, R.F. Mayol, J. Li, F. Yocca, PCT Int. Appl. (2003) 19, WO 2003009851 A1 20030206 CAN 138:131155 AN 2003:97305.
[43] R.L. Hanson, W.L. Parker, D.B. Brzozowski, T.P. Tully, A. Kotnis, R.N. Patel, Tetrahedron: Asymmetry 16 (16) (2005) 2711.
[44] R.N. Patel, L. Chu, V.N. Nanduri, L. Jianqing, A. Kotnis, W.L. Parker, M. Liu, R. Mueller, Tetrahedron: Asymmetry 16 (16) (2005) 2778.
[45] S.L. Goldberg, V.B. Nanduri, L. Chu, R.M. Johnston, R.N. Patel, Enzyme Microb. Technol. 39 (2006) 1441.
[46] B. Gallwitz, Treat. Endocrinol. 4 (2005) 361.
[47] L.L. Nielsen, Drug Discov. Today 10 (2005) 703.
[48] E.M. Sinclair, D.J. Drucker, Curr. Opin. Endocrinol. Diabet. 12 (2005) 146.
[49] N.A. Thornberry, A.E. Weber, Curr. Top. Med. Chem. 7 (6) (2007) 557.
[50] D.J. Augeri, J.A. Robl, D.A. Betebenner, D.R. Magnin, A. Khanna, J.G. Robertson, A. Wang, L.M. Simpkins, P. Taunk, Q. Huang, S.-P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G.E. Welzel, D.M. Egan, J. Marcinkeviciene, S.Y. Chang, S.A. Biller, M.S. Kirby, R.A. Parker, L.G. Hamann, J. Med. Chem. 48 (2005) 5025.
[51] R.L. Hanson, J. Singh, T.P. Kissick, R.N. Patel, L.J. Szarka, R.H. Mueller, Bioorg. Chem. 18 (1990) 116.
[52] R.L. Hanson, M.D. Schwinden, A. Banerjee, D.B. Brzozowski, B.-C. Chen, B.P. Patel, C.G. McNamee, G.A. Kodersha, D.R. Kronenthal, R.N. Patel, L.J. Szarka, Bioorg. Med. Chem. 7 (1999) 2247.
[53] R.L. Hanson, J.M. Howell, T.L. LaPorte, M.J. Donovan, D.L. Cazzulino, V. Zannella, M.A. Montana, V.B. Nanduri, S.R. Schwarz, R.F. Eiring, S.C. Durand, J.M. Wasylyk, W.L. Parker, M.S. Liu, F.J. Okuniewicz, B.-C. Chen, J.C. Harris, K.J. Natalie Jr., K. Ramig, S. Swaminathan, V.W. Rosso, S.K. Pack, B.T. Lotz, P. Bernot, A. Rusowicz, D.A. Lust, K.S. Tse, J.J. Venit, L.J. Szarka, R.N. Patel, Enzyme Microb. Technol. 26 (2000) 348.
[54] T.C. Vu, D.B. Brzozowski, R. Fox, J.D. Godfrey, R. L. Hanson, S.V. Kolotuchin, J.A. Mazzullo Jr., R.N. Patel, J. Wang, K. Wong, J. Yu, J. Zhu, D.R. Magnin, D.J. Augeri, L.G. Hamann (Bristol-Myers Squibb Company), PCT Int. Appl., WO 2004/052850 A2, 2004.
[55] R.L. Hanson, S.L. Goldberg, D.B. Brzozowski, T.P. Tully, D. Cazzulino, W.L. Parker, O.K. Lyngberg, T.C. Vu, M.K. Wong, R.N. Patel, Adv. Syn. Cat. 349 (8–9) (2007) 1369–1378.
[56] M. Kunishima, C. Kawachi, K. Hioki, K. Terao, S. Tani, Tetrahedron 57 (8) (2001) 1551–1558.
[57] I. Gill, R.N. Patel, Bioorg. Med. Chem. Lett. 16 (3) (2006) 705.
[58] G. Bold, A. Faessler, H.-G. Capraro, R. Cozens, T. Klimkait, J. Lazdins, J. Mestan, B. Poncioni, J. Roesel, D. Stover, M. Tintelnot-Blomley, F. Acemoglu, W. Beck, E. Boss, M. Eschbach, T. Huerlimann, E. Masso, S. Roussel, K. Ucci-Stoll, D. Wyss, M. Lang, J. Med. Chem. 41 (8) (1998) 3387.
[59] B.S. Robinson, K.A. Riccardi, Y.F. Gong, Q. Guo, D.A. Stock, W.S. Blair, B.J. Terry, C.A. Deminie, F. Djang, R.J. Colonno, P.-F. Lin, Antimicrob. Agents Chemother. 44 (8) (2000) 2093.
[60] R.N. Patel, L. Chu, R.H. Mueller, Tetrahedron: Asymmetry 14 (20) (2003) 3105.
[61] Z. Xu, J. Singh, M.D. Schwinden, B. Zheng, T.P. Kissick, B. Patel, M.J. Humora, F. Quiroz, L. Dong, D.-M. Hsieh, J.E. Heikes, M. Pudipeddi, M.D. Lindrud, S.K. Srivastava, D.R. Kronenthal, R.H. Mueller, Org. Proc. Res. Devel. 6 (3) (2002) 323.
[62] R. Hanson, S. Goldberg, R. Patel, unpublished results.
[63] C. Ireland, J. Leeson, Castaner, Drugs Future 22 (1997) 359.
[64] 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.N. Patel, Bioorg. Med. Chem. 8 (2000) 2681.
[65] S.F. Innaimo, M. Seifer, G.S. Bisacchi, D.N. Standring, R. Zahler, R.J. Colonno, Richard Antimicrob. Agents Chemother. 41 (1997) 1444.
[66] E.V. Genovesi, L. Lamb, I. Medina, D. Taylor, M. Seifer, S. Innaimo, R.J. Colonno, D.N. Standring, J.M. Clark, J. Antimicrob. Agents Chemother. 43 (3) (1999) 726.
[67] Y.R. Pendri, C.-P. Chen, S. Patel, J.M. Evans, J. Liang, D.R. Kronenthal, G.L. Powers, S.J. Prasad, J.T. Bien, Z. Shi, R.N. Patel, Y. Chan, S.K. Rijhwani, A. Singh, S. Wang, M. Stojanovic, R. Polniaszek, C. Lewis, J. Thottathil, D. Krishnamurty, M.X. Zhou, P. Vemishetti, PCT Int. Appl. (2004) 143, WO 2004052310 A2 20040624 CAN 141:38813 AN 2004:513501.
[68] D.A. Griffith, S.J. Danishefsky, J. Am. Chem. Soc. 118 (1996) 9526.
[69] S.J. Danishefsky, M.P. Cabal, K. Chow, J. Am. Chem. Soc. 111 (1989) 3456.
[70] R.N. Patel, A. Banerjee, Y.R. Pendri, Liang, Jing, C.-P. Chen, R. Mueller, Tetrahedron: Asymmetry 17 (2) (2006) 175.
[71] C. Evans, S. Roberts, K. Shoberu, A. Sutherland, J. Chem. Soc. Perkin Trans. 1 (1992) 589.
[72] M. Mahmoudian, A. Lowdon, M. Jones, M. Dawson, C. Wallis, Tetrahedron: Asymmetry 10 (1999) 1201.
[73] M. Mahmoudian, Biocatal. Biotransfor. 18 (2000) 105.
[74] S. Pianko, J. McHutchison, J. Gastroenterol. Hepatol. 15 (2000) 581.
[75] P. Ferenci, H. Brunner, K. Nachbaur, C. Datz, M. Gschwantler, H. Hofer, R. Stauber, J. Franz, J. Wolfgang, R. Martha, M.-S. Petra, H. Karin, V. Alfred, Wolfgang, Hepatology (Philadelphia) 34 (2001) 1006.
[76] S. Sauleda, A. Juarez, J. Esteban, C. Altisent, I. Ruiz, L. Puig, R. Esteban, J. Guardia, Hepatology (Philadelphia) 34 (2001) 1035.
[77] H. Bonkovsky, D. Stefancyk, K. McNeal, B. Banner, Q. Liu, G. Zucker, J. Israel, J. Stagias, Colker. Dig. Dis. Sci. 46 (2001) 2051.
[78] M.J. Homann, W.-C. Suen, N. Zhang, A. Zaks, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 645.
[79] M. Tamarez, B. Morgan, G. Wong, W. Tong, F. Bennett, R. Lovey, J. McCormick, A. Zaks, Org. Proc. Res. Devel. 7 (2003) 951.
[80] P.J. Reider, Adv. AIDS Chemother. Chim. 51 (6) (1997) 306.
[81] 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. Technol. 86 (1998) 550.
[82] B. Buckland, D. Robinson, M. Chartrain, Metab. Eng. 2 (2000) 42.
[83] M. Chartrain, P. Salmon, D. Robinson, B. Buckland, Curr. Opin. Biotechnol. 11 (2000) 209.
[84] N. Zhang, B. Stewart, J. Moore, R. Greasham, D. Robinson, B. Buckland, C. Lee, Metab. Eng. 2 (2000) 339.
[85] A. Amanullah, C. Hewitt, A. Nienow, C. Lee, M. Chartrain, B. Buckland, S. Drew, J. Woodley, Biotechnol. Bioeng. 80 (2002) 239.
[86] E.L. White, W.B. Parker, L.J. Macy, S.C. Shaddix, G.S. McCaleb, A. John, R. Vince, W.M. Shannon, Biochem. Biophys. Res. Commun. 161 (1989) 393.
[87] S.G. Carter, J.A. Kessler, C.D. Rankin, Antimicrob. Agents Chemother. 34 (1990) 1297.
[88] A.M. Exall, M.F. Jones, C. Leung, P.L. Myers, I.L. Paternoster, H. Singh, R. Storer, W. Richard, G.G. Weingarten, C. Williamson, Christopher, et al., J. Chem. Soc. Perkin Trans. 1 (1991) 2467.
[89] K.T. Pun, B.S. Baines, G.C. Lawrence, 5th European congress on biotechnology (Copenhagen) 1990, Absract no. TUP 111, p 292.
[90] M. Mahmoudian, M.J. Dawson, in: W.R. Strohl (Ed.), Biotechnology of antibiotics, second ed., Marcel Dekker Inc., New York, 1997, p. 753.

[91] D.F. Muenzer, P. Meinhold, M.W. Peters, S. Feichtenhofer, H. Griengl, F.H. Arnold, A. Glieder, A. de Raadt, Chem. Commun. (Cambridge, United Kingdom) 20 (2005) 2597.
[92] A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, Tetrahedron: Asymmetry 7 (2) (1996) 491.
[93] V.B. Urlacher, S. Lutz-Wahl, R.D. Schmid, Appl. Microbiol. Biotechnol. 64 (3) (2004) 317.
[94] F.H. Arnold, C.R. Otey, PCT Int. Appl. (2005) 122, WO 2005017106 A2 20050224 CAN 142:256750 AN 2005:161040.
[95] M. von Itzstein, W.Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. Phan, M.L. Smythe, H.F. White, et al., Nature 363 (6428) (1993) 418.
[96] M.P. De Ninno, Synthesis 8 (1991) 583.
[97] M. Mahmoudian, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 53.
[98] C. Auge, S. David, C. Gautheron, Tetrahedron Lett. 25 (25) (1984) 4663.
[99] U. Kragl, M. Kittelmann, O. Ghisalba, C. Wandrey, Annals of the New York Academy of Sciences, 1995, 750 (Enzyme Engineering XII), p. 300.
[100] M.D. Bednarski, H.K. Chenault, E.S. Simon, G.M. Whitesides, J. Am. Chem. Soc. 109 (1987) 1283.
[101] M.J. Kim, W.J. Hennen, H.M. Sweers, C.H. Wong, J. Am. Chem. Soc. 110 (1988) 6481.
[102] M.H. Dawson, D. Noble, M. Mahmoudian, PCT W0 9429476, 1994.
[103] M. Mahmoudian, D. Noble, C.S. Drake, R.F. Middleton, D.S. Montgomery, J.E. Piercey, D. Ramlakhan, M. Todd, M.J. Dawson, Enzyme Microb. Technol. 20 (1997) 393.
[104] A.S. Fauci, H. Masur, E.P. Gelmann, P.D. Markham, B.H. Hahn, H.C. Lane, Ann. Intern. Med. 102 (6) (1985) 800.
[105] J.A.V. Coates, N. Cammack, H.J. Jenkinson, J. Helen, I.M. Mutton, B.A. Pearson, R. Storer, J.M. Cameron, C.R. Penn, C.R. Antimicrob, Agents Chemother. 36 (1992) 202.
[106] R. Storer, I.R. Clemens, B. Lamont, S.A. Noble, C. Williamson, B. Belleau, Nucleosides Nucleotides 12 (1993) 225.
[107] M. Mahmoudian, B.S. Baines, C.S. Drake, R.S. Hale, P. Jones, J.E. Piercey, D.S. Montgomery, I.J. Purvis, R. Storer, et al., Enzyme Microb. Technol. 15 (1993) 749.
[108] R. Holton, R. Biediger, P. Joatman, in: M. Suffness (Ed.), Taxol: Science and Application, CRC press, New York, 1995, p. 97.
[109] D. Kingston, in: M. Suffness (Ed.), Taxol: Science and Application, CRC press, New York, 1995, p. 287.
[110] R. Hanson, J. Wasylyk, V. Nanduri, D. Cazzulino, R. Patel, L. Szarka, J. Biol. Chem. 269 (1994) 22145.
[111] V. Nanduri, R. Hanson, T. LaPorte, R. Ko, R. Patel, L. Szarka, Biotechnol. Bioeng. 48 (1995) 547.
[112] R.N. Patel, Ann. Rev. Microbiol. 98 (1995) 361.
[113] R. Hanson, J. Howell, D. Brzozowski, S. Sullivan, R. Patel, L. Szarka, Biotechnol. Appl. Biochem. 26 (1997) 153.
[114] R. Patel, A. Banerjee, J. Howell, C. McNamee, D. Brozozowski, D. Mirfakhrae, V. Nanduri, J. Thottathil, L. Szarka, Tetrahedron: Asymmetry 4 (1993) 2069.
[115] R. Patel, A. Banerjee, R. Ko, J. Howell, W.-S. Li, F. Comezoglu, R. Partyka, L. Szarka, Biotechnol. Appl. Biochem. 20 (1994) 22.
[116] D.M. Gou, Y.C. Liu, C.S. Chen, J. Org. Chem. 58 (5) (1993) 1287.
[117] C.J. Sih, Eur. Pat. Appl. (1994) 37, EP 582469 A2 19940209 CAN 121:83034 AN 1994:483034.
[118] M.M. Kayser, M. Drolet, J.D. Stewart, Tetrahedron: Asymmetry 16 (24) (2005) 4004.
[119] B.B. Feske, I.A. Kaluzna, J.D. Stewart, J. Org. Chem. 70 (23) (2005) 9654.
[120] E. Baloglu, D. Kingston, J. Nat. Prod. 62 (1999) 1068.
[121] W. Rose, B. Long, C. Fairchild, F. Lee, J. Kadow, Clin. Cancer Res. 7 (2001) 2016.
[122] H. Mastalerz, D. Cook, C.R. Fairchild, S. Hansel, W. Johnson, J.F. Kadow, B.H. Long, W.C. Rose, J. Tarrant, M.-J. Wu, M.Q. Xue, G. Zhang, M. Zoeckler, D.M. Vyas, Bioorg. Med. Chem. 11 (2) (2003) 4315.
[123] R. Patel, J. Howell, R. Chidambaram, S. Benoit, J. Kant, Tetrahedron: Asymmetry 14 (2003) 3673.
[124] R.L. Hanson, W.L. Parker, R.N. Patel, Biotechnol. Appl. Biochem. 45 (2) (2006) 81.
[125] K. Gerth, S. Pradella, O. Perlova, S. Beyer, R. Muller, J. Biotechnol. 106 (2–3) (2003) 233.
[126] D. Benigni, R. Stankavage, S.-J. Chiang, H. Hou, B. Eagan, D. Gu, D. Hou, L. Mintzmyer, T.P. Tully, B.L. Davis, I. Hargro, M. Mascari, G. Galvin, G. Stein, C.W. McConlogue, F.T. Comezoglu, PCT Int. Appl. (2004) 85, WO 2004026254 A2 20040401 CAN 140:286248 AN 2004:270015.
[127] S. Goodin, M.P. Kane, E.H. Rubin, J. Clin. Oncol. 22 (10) (2004) 2015.
[128] K.C. Nicolaou, F. Roschangar, D. Vourloumis, Angew. Chem. Int. Ed. 37 (15) (1998) 2014.
[129] K.-H. Altmann, Org. Biomol. Chem. 2 (15) (2004) 2137.
[130] C.N. Boddy, K. Hotta, M.L. Tse, R.E. Watts, C. Khosla, J. Am. Chem. Soc. 126 (24) (2004) 7436.
[131] N. Lin, K. Brakora, M. Seiden, Curr. Opin. Investig. Drugs 4 (6) (2003) 746.
[132] J.D. Basch, S.-J. Chiang, S.W. Liu, A. Nayeem, Y.-L. Sun, PCT Int. Appl. (2004) 192, WO 2004078978 A1 20040916 CAN 141:255532 AN 2004:756881.
[133] M. Wittman, J.M. Carboni, R. Attar, B. Balasubramanian, P. Balimane, P. Brassil, F. Beaulieu, C. Chang, W. Clarke, J. Dell, J. Eummer, D. Frennesson, M. Gottardis, A. Greer, S. Hansel, W. Hurlburt, B. Jacobson, S. Krishnananthan, F.Y. Lee, A. Li, T.-A. Lin, P. Liu, C. Ouellet, W. Sang, M.G. Saulnier, K. Stoffan, Y. Sun, U. Velaparthi, H. Wong, Z. Yang, K. Zimmermann, M. Zoeckler, D. Vyas, J. Med. Chem. 48 (18) (2005) 5639.
[134] J.M. Carboni, W.W. Hurlburt, M.M. Gottardis, PCT Int. Appl. (2004) 39, WO 2004030625 A2 20040415 CAN 140:315050 AN 2004:308360.
[135] F. Beaulieu, C. Ouellet, K. Zimmermann, U. Velaparthi, M.D. Wittman, PCT Int. Appl. (2004) 65, WO 2004063151 A2 20040729 CAN 141:140461 AN 2004:606437.
[136] R.L. Hanson, S. Goldberg, A. Goswami, T.P. Tully, R.N. Patel, Adv. Synth. Catal. 347 (7–8) (2005) 1073.
[137] H. Kagechika, E. Kawachi, Y. Hashimoto, K. Shudo, T. Himi, J. Med. Chem. 31 (1988) 2182.
[138] K. Shudo, H. Kagechika, in: M.I. Dawson, W.H. Okamura (Eds.), Retinoids, CRC Press, Boca Raton, FL, 1989.
[139] G.M. Morriss-Kay, Adv. Org. Biol. 3 (Retinoids: Their Physiological Function and Therapeutic Potential), 79 (1997).
[140] R.C. Moon, L.M. Itri, Retinoids 2 (1984) 327.
[141] R.N. Patel, L. Chu, R. Chidambaram, J. Zhu, J. Kant, Tetrahedron: Asymmetry 13 (2002) 349.
[142] M. Mahmoudian, J. Eaddy, M. Dawson, Biotechnol. Appl. Biochem. 29 (3) (1999) 229.
[143] M. Mahmoudian, Focus Biotechnol. 1 (2001) 249.
[144] T.A. Krenitsky, D.R. Averett, J.D. Wilson, A.R. Moorman, G.W. Koszalka, S.D. Chamberlain, D. Porter, G. Wolberg, PCT Int. Appl. (1992) 54, WO 9201456 A1 19920206 CAN 116:236100 AN 1992:236100.
[145] B. Morgan, A. Zaks, D.R. Dodds, J. Liu, R. Jain, S. Megati, F.G. Njoroge, V.M. Girijavallabhan, J. Org. Chem. 65 (18) (2000) 5451.
[146] C. Perigaud, G. Gosselin, J.L. Imbach, Nucleosides Nucleotides 11 (2–4) (1992) 903.
[147] R. Garg, S.P. Gupta, H. Gao, M.S. Babu, A.K. Debnath, C. Hansch, Chem. Rev. (Washington, D.C.) 99 (12) (1999) 3525.
[148] S. Ichikawa, A. Matsuda, Expert Opin. Therap. Patents 17 (5) (2007) 487.
[149] J. Zhang, F. Visser, K.M. King, S.A. Baldwin, J.D. Young, C.E. Cass, Cancer Metastasis Rev. 26 (1) (2007) 85.
[150] J.-J. Toulme, Pharmacocinetique et Biodistribution du Medicament: Therapie Genique Anti-sens (1997) 257.
[151] D.W. Hutchinson, Trends Biotechnol. 8 (12) (1990) 348.
[152] M. Ferrero, V. Gotor, Chem. Rev. (Washington, D.C.) 100 (12) (2000) 4319.
[153] E.S. Lewkowicz, A.M. Iribarren, Curr. Org. Chem. 10 (11) (2006) 1197.
[154] E. Spoldi, D. Ghisotti, S. Cali, M. Grisa, G. Orsini, G. Tonon, G. Zuffi, Nucleosides, Nucleotides Nucleic Acids 20 (4–7) (2001) 977.
[155] M.C. Rogert, J.A. Trelles, S. Porro, E.S. Lewkowicz, A.M. Iribarren, Biocatal. Biotransfor. 20 (5) (2002) 347.
[156] G. Zuffi, D. Ghisotti, I. Oliva, E. Capra, G. Frascotti, G. Tonon, G. Orsini, Biocatal. Biotransfor. 22 (1) (2004) 25.

[157] G. Bisacchi, W. Slusarchyk, U. Treuner, J. Sutton, R. Zahler, S. Seiler, D. Kronenthal, M. Rondazzo, Z. Xu, Z. Shi, M. Schwinden (1999), WO 9967215, A1 19991229, CAN 132:64103, AN 1999:819347.
[158] 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.N. Patel, Org. Proc. Res. Devel. 5 (2001) 415.
[159] E. Jagoda, B. Stouffer, M. Ogan, H.M. Tsay, N. Turabi, S. Mantha, F. Yost, J.I. Tu, Therap. Drug Monit. 15 (3) (1993) 213.
[160] R.N. Patel, C. McNamee, A. Banerjee, J. Howell, R. Robison, L. Szarka, Enzyme Microb. Technol. 14 (1992) 731.
[161] A. Matsuyama, H. Yamamoto, Y. Kobayashi, Org. Proc. Res. Devel. 6 (2002) 558.
[162] S.C. Davis, J.H. Grate, D.R. Gray, J.M. Gruber, G.W. Huisman, S.K. Ma, L.M. Newman, R. Sheldon, L.A. Wang, U.S. Pat. Appl. Publ. (2004) 151, Cont.-in-part of U.S. Ser. No. 639,159. US 2004214297 A1 20041028 CAN 141:378916 AN 2004:905518.
[163] S.C. Davis, R.J. Fox, V. Gavrilovic, G.W. Huisman, L.M. Newman, PCT Int. Appl. (2005) 63, WO 2005017141 A1 20050224 CAN 142:256747 AN 2005:158791.
[164] A. Fishman, M. Eroshov, D.N. Sheffer, V. Jan, U. Cogan, R. Effenberger, Biotechnol. Bioeng. 74 (74) (2001) 263.
[165] M. Wieser, T. Marco, Nagasawa, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, 2000, p. 461.
[166] T. Nagasawa, T. Yoshida, A. Matsuyama, Jpn. Kokai Tokkyo Koho (2006) 17, JP 2006223246 A 20060831 CAN 145:247658 AN 2006:884452.
[167] G. DeSantis, K. Wong, B. Farwell, K. Chatman, Z. Zhu, G. Tomlinson, H. Huang, X. Tan, L. Bibbs, P. Chen, K. Kretz, M.J. Burk, J. Am. Chem. Soc. 125 (38) (2003) 11476.
[168] G. DeSantis, Z. Zhu, W.A. Greenberg, K. Wong, J. Chaplin, S.R. Hanson, B. Farwell, L.W. Nicholson, C.L. Rand, D.P. Weiner, D.E. Robertson, M.J. Burk, J. Am. Chem. Soc. 124 (31) (2002) 9024.
[169] D.E. Robertson, J.A. Chaplin, G. DeSantis, M. Podar, M. Madden, E. Chi, T. Richardson, A. Milan, M. Miller, D.P. Weiner, K. Wong, J. McQuaid, B. Farwell, L.A. Preston, X. Tan, M.A. Snead, M. Keller, E. Mathur, P.L. Kretz, M.J. Burk, J.M. Short, Appl. Environ. Microbiol. 70 (4) (2004) 2429.
[170] R.N. Patel, A. Banerjee, C. McNamee, D. Brzozowski, R. Hanson, L. Szarka, Enzyme Microb. Technol. 15 (1993) 1014.
[171] S. Sit, R. Parker, I. Motoe, H. Balsubramanian, C. Cott, P. Brown, W. Harte, M. Thompson, J. Wright, J. Med. Chem. 33 (1990) 2982.
[172] Z. Guo, Y. Chen, A. Goswami, R.L. Hanson, R.N. Patel, Tetrahedron: Asymmetry 17 (10) (2006) 1589.
[173] M. Wolberg, I.A. Kaluzna, M. Mueller, J.D. Stewart, Tetrahedron: Asymmetry 15 (18) (2004) 2825.
[174] M. Wolberg, W. Hummel, C. Wandrey, M. Mueller, Angew. Chem. Int. Ed. 39 (2000) 4306.
[175] H. Gijsen, C.-H. Wong, J. Am. Chem. Soc. 116 (1994) 8422.
[176] W. Greenberg, A. Varvak, S. Hanson, K. Wong, H. Huang, P. Chen, M.J. Burk, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 5788.
[177] S. Jennewein, M. Schuermann, M. Wolberg, I. Hilker, R. Luiten, M. Wubbolts, D. Mink, Biotechnol. J. 1 (5) (2006) 537.
[178] A. Steiger, H. Pyun, R. Coates, J. Org. Chem. 57 (1992) 3444.
[179] M. Lawrence, S. Biller, O. Fryszman US Patent 5447922 (1995).
[180] R.N. Patel, A. Banerjee, L. Szarka, Tetrahedron: Asymmetry 8 (1997) 1055.
[181] C.V.C. Prasad, O.B. Wallace, J.W. Noonan, C.P. Sloan, W. Lau, S. Vig, M.F. Parker, D.W. Smith, S.B. Hansel, C.T. Polson, D.M. Barten, K.M. Felsenstein, S.B. Roberts, Bioorg. Med. Chem. Lett. 14 (12) (2004) 3361.
[182] V.B. Nanduri, A. Banerjee, J. Howell, D. Brzozowski, R. Eiring, R.N. Patel, J. Ind. Microbiol. Biotechnol. 25 (2000) 171.
[183] C.V.C. Prasad, S. Vig, D.W. Smith, Q. Gao, C.T. Polson, J.A. Corsa, V.L. Guss, A. Loo, D.M. Barten, M. Zheng, K.M. Felsenstein, S.B. Roberts, Bioorg. Med. Chem. Lett. 14 (13) (2004) 3535.
[184] D. Schenk, D. Games, P. Seubert, J. Mol. Neurosci. 17 (2001) 259.
[185] R.N. Patel, A. Goswami, L. Chu, M.-J. Donovan, V.B. Nanduri, S. Goldberg, R. Johnston, S. Prasad, B. Nielsen, F. Junying, W. He, S. Zhongping, K. Wang, R. Eiring, D. Cazzulino, A. Singh, R. Mueller, Tetrahedron: Asymmetry 15 (2004) 1247.
[186] E. Burcher, C. Mussap, J. Stephenson, in: S. Buck (Ed.), Tachykinin Receptors, Humana Press, Totowa, NJ, 1994.
[187] B. Veronesi, J. Carter, R. Devlin, S. Simon, M. Oortgiesen, Neuropeptides (Edinburgh) 33 (1999) 447.
[188] L. Yuan, E. Burcher, B. Nail, Pulmonary Pharmacol. Therapeut. 11 (1998) 31.
[189] P. Ting, J. Lee, J. Anthes, N.-Y. Shih, J. Piwinski, Bioorg. Med. Chem. Lett. 10 (2000) 2333.
[190] M. Homann, R. Vail, B. Morgan, V. Sabesan, C. Levy, D. Dodds, A. Zaks, Adv. Synth. Catal. 343 (2001) 744.
[191] W.-C. Suen, N. Zhang, L. Xiao, V. Madison, A. Zaks, Protein Eng. Des. Sel. 17 (2004) 133.
[192] G. Hoegenauer, Antibiotics 5 (1979) 344.
[193] H. Berner, H. Vyplel, G. Schulz, P. Stuchlik, Tetrahedron 39 (1983) 1317.
[194] R. Hanson, J. Matson, D. Brzozowski, T. LaPorte, D. Springer, R.N. Patel, Org. Proc. Res. Devel. 6 (2002) 482.
[195] D. Springer, M. Sorenson, S. Huang, T. Connolly, J. Bronson, J. Matson, R. Hanson, D. Brzozowski, T. LaPorte, R.N. Patel, Bioorg. Med. Chem. Lett. 13 (2003) 1751.
[196] E.M. Gordon, M.A. Ondetti, J. Pluscec, C.M. Cimarusti, D.P. Bonner, R.B. Sykes, J. Am. Chem. Soc. 104 (22) (1982) 6053.
[197] R.B. Sykes, D.P. Bonner, K. Bush, N.H. Georgopapadakou, J.S. Wells, J. Antimicrob. Chemother. 8 (1981) 1.
[198] R.L. Hanson, J. Singh, T.P. Kissick, R.N. Patel, L.J. Szarka, R.H. Mueller, Bioorg. Chem. 18 (2) (1990) 116.
[199] S. Langer, P. Hicks, J. Cardiovasc. Pharm. 6 (1984) 547.
[200] P. Manoury, J. Binet, A. Dumas, F. Lefevre-Borg, I. Cavero, J. Med. Chem. 29 (1986) 19.
[201] P. Manoury, J. Binet, I. Cavero, Eur. Pat. Appl. (1982) 21, EP 61379, A1 19820929, CAN 98:89179, AN 1983:89179.
[202] V. Sanchez, F. Rebolledo, V. Gotor, Tetrahedron: Asymmetry 8 (1997) 37.
[203] A. Baldessari, C. Mangone, J. Mol. Catal. B: Enzym. 11 (2001) 335.
[204] A. Archelas, R. Furstoss, Top. Curr. Chem. 200 (1999) 159.
[205] A. Steinreiber, K. Faber, Curr. Opin. Biotech. 12 (2001) 552.
[206] J. Catt, G. Johnson, D. Keavy, R. Mattson, M. Parker, K. Takaki, J. Yevich (1999), US 5856529, CAN 130:110151.
[207] L.-Q. Sun, K. Takaki, J. Chen, L. Iben, J.O. Knipe, L. Pajor, C.D. Mahle, E. Ryan, C. Xu, Bioorg. Med. Chem. Lett. 14 (20) (2004) 5157.
[208] A. Goswami, M. Totleben, A. Singh, R. Patel, Tetrahedron: Asymmetry 10 (1999) 3167.
[209] B. Schnell, K. Faber, W. Kroutil, Adv. Synth. Catal. 345 (2003) 653.
[210] H. Stecher, K. Faber, Synthesis 1 (1997) 1.
[211] M.-J. Kim, Y. Ahn, J. Park, Bull. Korean Chem. Soc. 26 (4) (2005) 515.
[212] B. Martin-Matute, J.-E. Baeckvall, Curr. Opin. Chem. Biol. 11 (2) (2007) 226.
[213] A. Goswami, D. Mirfakhrae, R. Patel, Tetrahedron: Asymmetry 10 (1999) 4239.
[214] M. Ondetti, D. Cushman, J. Med. Chem. 24 (1981) 355.
[215] M. Ondetti, B. Rubin, D. Cushman, Science 196 (1977) 441.
[216] R.N. Patel, J. Howell, C. McNamee, K. Fortney, L. Szarka, Biotechnol. Appl. Biochem. 16 (1992) 34.
[217] R.N. Patel, unpublished results.
[218] J. Moniot, U.S. Patent Application, CN 88-100862 (1988).
[219] R.N. Patel, R. Robison, L. Szarka, Appl. Microbiol. Biotechnol. 34 (1990) 10.
[220] J. Robl, C. Sun, J. Stevenson, D. Ryono, L. Simpkins, M. Cimarusti, T. Dejneka, W. Slusarchyk, S. Chao, L. Stratton, R. Misra, M. Bednarz, M. Asaad, H. Cheung, B. Aboa Offei, P. Smith, P. Mathers, M. Fox, T. Schaeffer, A. Seymour, N. Trippodo, J. Med. Chem. 40 (1997) 1570.
[221] R. Hanson, J. Howell, T. LaPorte, M. Donovan, D. Cazzulino, V. Zannella, M. Montana, V. Nanduri, S. Schwarz, R. Eiring, S. Durand, J. Wasylyk, L. Parker, M. Liu, F. Okuniewicz, B.-C. Chen, J. Harris, K. Natalie, K. Ramig, S. Swaminathan, V. Rosso, S. Pack, B. Lotz, P. Bernot, A. Rusowicz, D. Lust, K. Tse, J. Venit, L. Szarka, R.N. Patel, Enzyme Microb. Technol. 26 (2000) 348.
[222] R.N. Patel, Biomol. Eng. 17 (2001) 167.

[223] R.N. Patel, A. Banerjee, V. Nanduri, S. Goldberg, R. Johnston, R. Hanson, C. McNamee, D. Brzozowski, T. Tully, R. Ko, T. LaPorte, D. Cazzulino, S. Swaminathan, L. Parker, J. Venit, Enzyme Microb. Technol. 27 (2000) 376.
[224] T.Z. Su, M.R. Feng, M.L. Weber, J. Pharm. Exp. Ther. 313 (2005) 1406.
[225] Z. Xie, J. Feng, E. Garcia, M. Bernett, D. Yazbeck, J. Tao, J. Mol. Catal. B: Enzym. 3–4 (2006) 75.
[226] M.P. Bruns, J.K. Weaver, J.W. Wong, Patent WO2005100580A1, 2005.
[227] S.E. Webber, K. Okano, T.L. Little, S.H. Reich, Y. Xin, S.A. Fuhrman, D.A. Matthews, R.A. Love, T.F. Hendrickson, A.K. Patick, J.W. Meador, R.A. Ferre, E.L. Brown, C.E. Ford, S.L. Binford, S.T. Worland, J. Med. Chem. 41 (1998) 2786.
[228] Q. Tian, N.K. Nayyar, S. Babu, L. Chen, J. Tao, S. Lee, A. Tibbetts, T. Moran, J. Liou, M. Guo, T.P. Kennedy, Tetrahedron Lett. 42 (2001) 6807.
[229] C.A. Martinez, D.R. Yazbeck, J. Tao, Tetrahedron 60 (3) (2004) 759.
[230] V. Alphand, R. Furstoss, J. Org. Chem. 57 (1989) 1306.
[231] N.M. Kamberbeek, D.B. Janssen, W.J.H. van Berkel, M.W. Fraaje, Adv. Synth. Catal. 345 (2003) 667.
[232] M.D. Mihovilovic, B. Mueller, P. Stanetty, Eur. J. Org. Chem. 22 (2002) 3711.
[233] J.D. Stewart, Curr. Org. Chem. 2 (1998) 195.
[234] M.D. Mihovilovic, Curr. Org. Chem. 10 (11) (2006) 1265.
[235] I. Hiker, R. Wohlgemuth, V. Alphand, R. Furstoss, Biotechnol. Bioeng. 92 (6) (2005) 702.
[236] F. Rudroff, V. Alphand, R. Furstoss, M.D. Mihovilovic, Org. Proc. Res. Devel. 10 (3) (2006) 599.
[237] D.E.T. Pazmino, R. Snajdrova, D.V. Rial, M.D. Mihovilovic, M.W. Fraaije, Adv. Synth. Catal. 349 (8+9) (2007) 1361.
[238] A. Kirschner, J. Altenbuchner, U.T. Bornscheuer, Appl. Microbiol. Biotechnol. 73 (5) (2007) 1065.
[239] M.D. Mihovilovic, R. Snajdrova, A. Winninger, F. Rudroff, Synlett 18 (2005) 2751.
[240] V.B. Urlacher, R.D. Schmid, Curr. Opin. Chem. Biol. 10 (2) (2006) 156.
[241] C. Rodriguez, G. De Gonzalo, M.W. Fraaije, V. Gotor, Tetrahedron: Asymmetry 18 (11) (2007) 1338.
[242] P. Cernuchova, M.D. Mihovilovic, Org. Biomol. Chem. 5 (11) (2007) 1715.
[243] R. Snajdrova, G. Grogan, M.D. Mihovilovic, Bioorg. Med. Chem. Lett. 16 (18) (2006) 4813.
[244] H. Iwaki, Y. Hasegawa, S. Wang, M.M. Kayser, P.C.K. Lau, Appl. Environ. Microbiol. 69 (4) (2003) 2414.
[245] C.M. Clouthier, M.M. Kayser, M.T. Reetz, J. Org. Chem. 71 (22) (2006) 8431.
[246] N. Berezina, E. Kozma, R. Furstoss, V. Alphand, Adv. Synth. Catal. 349 (11+12) (2007) 2049.
[247] M. Bocola, F. Schulz, F. Leca, A. Vogel, M.W. Fraaije, M.T. Reetz, Adv. Synth. Catal. 347 (7–8) (2005) 979.
[248] D.E. Pazmino, R. Snajdrova, D.V. Rial, M.D. Mihovilovic, M.W. Fraaije, Adv. Synth. Catal. 349 (8–9) (2007) 1361.
[249] W.-D. Fessner, Modern Aldol React. 1 (2004) 201.
[250] H. Griengl, H. Schwab, M. Fechter, Trends Biotechnol. 18 (6) (2000) 252.
[251] M. Pohl, B. Lingen, M. Muller, Chem.-A Eur. J. 8 (23) (2002) 5288.
[252] W.-D. Fessner, V. Helaine, Curr. Opin. Biotechnol. 12 (6) (2001) 574.
[253] G.A. Sprenger, M. Pohl, J. Mol. Catal. B: Enzym. 6 (3) (1999) 145.
[254] G. Goetz, P. Iwan, B. Hauer, M. Breuer, M. Pohl, Biotechnol. Bioeng. 74 (4) (2001) 317.
[255] O.P. Ward, A. Singh, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and biotechnology industries, CRC Press, Florida, 2007, p. 605.
[256] H. Ohta, T. Sugai, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Marcel Dekker, New York, 2000, p. 487.
[257] M. Yagasaki, A. Ozaki, J. Mol. Catal. B: Enzym. 4 (1–2) (1998) 1.
[258] E. Prosen, O.P. Ward, J. Ind. Microbiol. 13 (5) (1994) 287.
[259] A.S. Demir, T. Dunnwald, H. Iding, M. Pohl, M. Muller, Tetrahedron: Asymmetry 10 (24) (1999) 4769.
[260] M. Pohl, A. Liese, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 661.
[261] Z. Guo, A. Goswami, K.D. Mirfakhrae, R.N. Patel, Tetrahedron: Asymmetry 10 (24) (1999) 4667.
[262] B. Lingen, M. Pohl, A.S. Demir, A. Liese, M. Mueller, Oxidative Stress Dis. 11 (2004) 113.
[263] S.M. Dean, W.A. Greenberg, C.-H. Wong, Adv. Synth. Catal. 248 (8–9) (2007) 1308.
[264] C.-H. Wong, W.A. Greenberg, Asymmetric Synth. (2007) 217.
[265] A.S. Rowan, C.J. Hamilton, Nat. Prod. Reports 23 (3) (2006) 412.
[266] L.J. Whalem, C.-H. Wong, Aldrichim. Acta 39 (3) (2006) 63.
[267] A.S. Samland, G.A. Sprenger, Appl. Microbiol. Biotechnol. 71 (3) (2006) 253.
[268] T. Kajimoto, K.K.C. Liu, R.L. Pederson, Z. Zhong, Y. Ichikawa, J.A. Porco, C.-H. Wong, J. Am. Chem. Soc. 113 (16) (1991) 6187.
[269] G.C. Look, C. Gary, C.H. Fotsch, H. Christopher, C.-H. Wong, Accounts Chem. Res. 26 (1993) 282.
[270] T. Ziegler, O.A. Straub, F. Effenberger, Angew. Chem. 100 (5) (1988) 737.
[271] A. Romero, C.-H. Wong, J. Org. Chem. 65 (24) (2000) 8264.
[272] X. Xing, W. Li, J. Liu, Huagong Keji 14 (2) (2006) 46.
[273] M. MacDonald, G.B. D'Cunha, Biochem. Cell Biol. 85 (3) (2007) 273.
[274] F. Effenberger, S. Foerster, C. Kobler, Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 677.
[275] V. Gotor, J. Biotechnol. 96 (1) (2002) 35.
[276] T. Tsuchida, Y. Nishimoto, T. Kotani, K. Iiizumi, Jpn. Kokai Tokkyo Koho (1993) 7, JP 05123177 A 19930521 Heisei. CAN 119:93708, AN 1993:493708.
[277] H. Yamada, Studien in Organic Chemistry, in: K. Kieslich, C.P. van der Beek, J.A.M. de Bont (Eds.), Screening for Microbial Biocatalysis, vol. 53, Elsevier, Amsterdam, 1998, p. 13.
[278] T. Purkarthofer, T. Pabst, C. Van den Broek, H. Griengl, O. Maurer, W. Skranc, Org. Proc. Res. Devel. 10 (3) (2006) 618.
[279] B. Martin-Matute, J.E. Baeckvall, Curr. Opin. Chem. Biol. 11 (2) (2007) 226.
[280] Y. Simeo, W. Kroutil, K. Faber, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Florida, 2007, p. 27.
[281] I. Archer, R. Carr, I. Fotheringham, R. Speight, N.J. Turner, Chim. l'Industria (Milan, Italy) 87 (6) (2005) 100.
[282] R. Azerad, D. Buisson, Curr. Opin. Biotechnol. 11 (6) (2000) 565.
[283] A.J. Carnell, Adv. Biochem. Eng./Biotechnol. 63 (1999) 57 (Biotransformations).
[284] S. Gastaldi, S. Stephane, S. Escoubet, N. Vanthuyne, G. Gil, M.P. Bertrand, Org. Lett. 9 (5) (2007) 837.
[285] M.-J. Kim, W.-H. Kim, K. Han, Y.K. Choi, J. Park, Org. Lett. 9 (6) (2007) 1157.
[286] M. Stirling, J. Blacker, M.I. Page, Tetrahedron Lett. 48 (7) (2007) 1247.
[287] A. Parvulescu, D. De Vos, P. Jacobs, Chem. Commun. (Cambridge, United Kingdom) 42 (2007) 5307.
[288] M.T. Reetz, K. Schimossek, Chimia 50 (12) (1996) 668.
[289] O. Pamies, J.E. Baeckvall, J. Org. Chem. 66 (11) (2001) 4022.
[290] G.J. Roff, R.C. Lloyd, N.J. Turner, J. Am. Chem. Soc. 126 (13) (2004) 4098.
[291] Y. Zhu, K.-L. Fow, G.-K. Chuah, Chem.-A Eur. J. 13 (2) (2007) 541.
[292] L. Boren, B. Martin-Matute, Y. Xu, Chem.-A Eur. J. 12 (1) (2005) 225.
[293] M.-J. Kim, H.M. Kim, D. Kim, Y. Ahn, J. Park, Green Chem. 6 (9) (2004) 471.
[294] H.K. Lee, D. Kim, J. Park, J. Am. Chem. Soc. 125 (2003) 11494.
[295] G.K.M. Verzijl, J.G. De Vries, Q.B. Broxterman, PCT Int. Appl. (2005) 30, WO 2005014509 A1 20050217 CAN 142:239850 AN 2005:141002.
[296] G.R. Allan, A.J. Carnell, J. Org. Chem. 66 (19) (2001) 6495.
[297] W. Kroutil, K. Faber, Tetrahedron: Asymmetry 9 (16) (1998) 2901.
[298] Y. Zhu, K.-L. Fow, G.-K. Chuah, S. Jaenicke, Chem.-A Eur. J. 13 (2) (2007) 541.

[299] A. Berkessel, M.L. Sebastian-Ibarz, T.N. Mueller, Angew. Chem. Int. Ed. 45 (39) (2006) 6567.
[300] S.F.G.M. van Nispen, J. van Buijtenen, J.A.J.M. Vekemans, J. Meuldijk, L.A. Hulshof, Tetrahedron: Asymmetry 17 (15) (2006) 2299.
[301] S. Akai, K. Tanimoto, Y. Kanao, M. Egi, T. Yamamoto, Y. Kita, Angew. Chem. Int. Ed. 45 (16) (2006) 2592.
[302] L. Boren, B. Martin-Matute, Y. Xu, A. Cordova, J.-E. Baeckvall, Chem.-A Eur. J. 12 (1) (2006) 225.