Biocatalysis is a highly efficient and a powerful tool for organic chemists to prepare optically pure molecules. A broad range of biocatalytic methods has been already in use for large-scale manufacture of drug intermediates. This module covers some of the recent developments in the enzyme catalysis.

Lecture 37: Acylation and Hydrolytic Reactions

11.1 Acylation of Alcohols and Amines

The enzymatic resolution of alcohols and amines affords an effective method to access optically active alcohols and amines from racemic or prochiral substrates.

11.1.1 Reactions with Alcohols

The use of lipase for the resolution of racemic alcohols is a widely known technology. However, this method gives the product with maximum up to 50% yield. This limitation can be overcome by coupling the lipase-catalyzed enantioselective resolution with a racemization of the alcohol substrate, thus obtaining a dynamic kinetic resolution process. The latter process can be pursued employing a nonchiral metal complex as a catalyst. For example, using the combination of Ru complex and CAL-B, the acylation of racemic alcohol can be accomplished with 78-92% yield and 99% ee (Scheme 1).
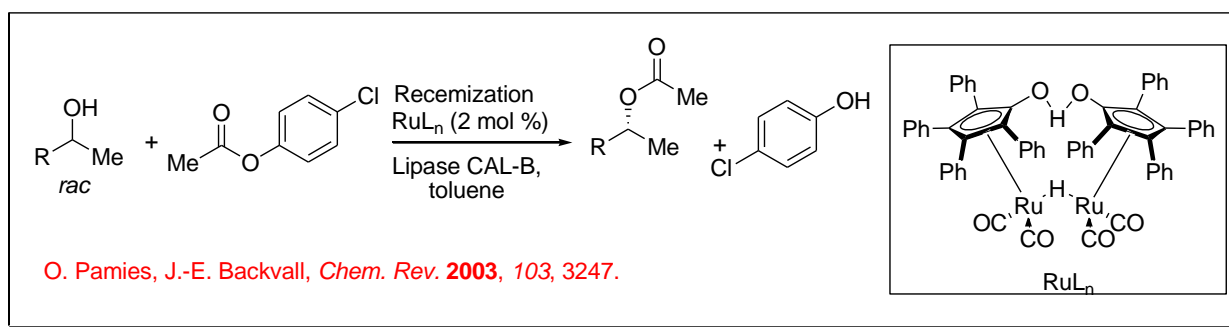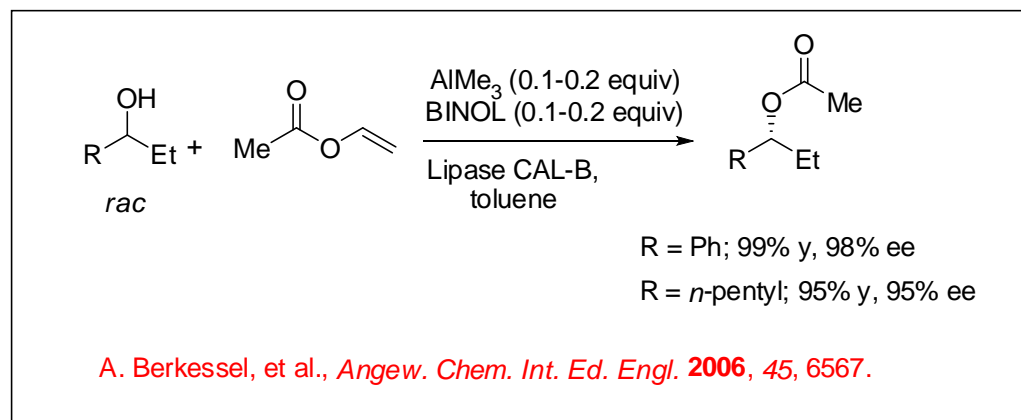

Scheme 1

This methodology has been subsequently utilized for the enantio- and diastereoselective synthesis of chiral polymers. For example, dimethyl adipate reacts with a mixture of racemic and *meso*-alcohols to give chiral polyester (Scheme 2). Ru complex acts as a racemization catalyst in combination with lipase CAL-B as biocatalyst for the resolution.

I. Hilker et al., *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 2130.
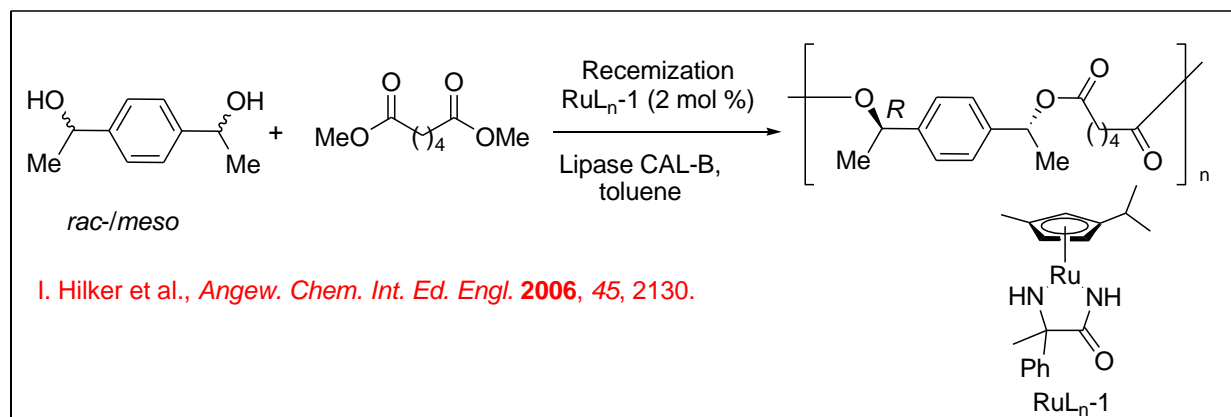
Scheme 2

Furthermore, the transformation has been demonstrated employing a cheap and readily available aluminium complex prepared from AlMe$_3$ and BINOL as the racemization catalyst. For example, racemic 1-phenyl-1-propanol can be acylated with 99% yield and 98% ee (Scheme 3).

R = Ph; 99% y, 98% ee
R = *n*-pentyl; 95% y, 95% ee

A. Berkessel, et al., *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 6567.

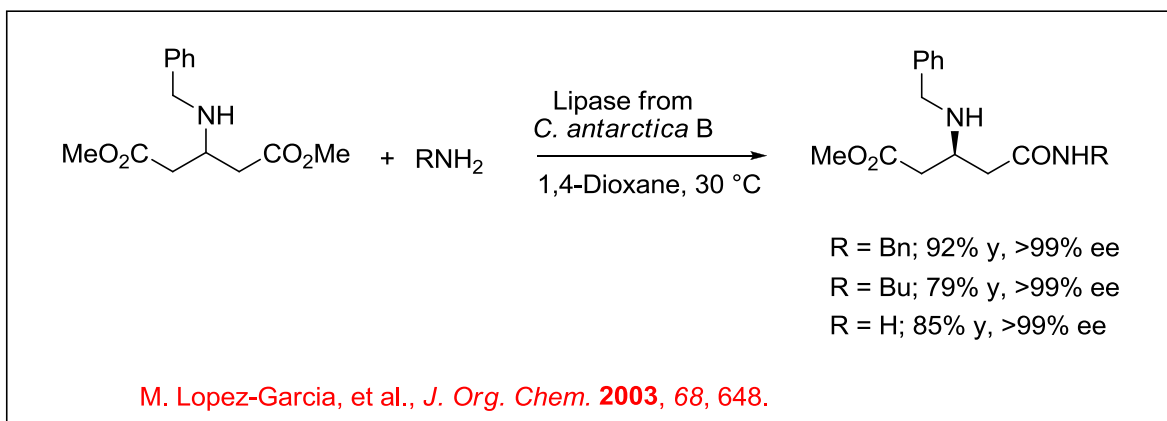**Scheme 3**

11.1.2 Reactions with Amines

Optically pure amines serve as versatile intermediates in the manufacture of pharmaceuticals and agrochemicals. The lipase-catalyzed acylation of amines proceeds efficiently with excellent enantioselectivity (Scheme 4). In this reaction, one of the enantiomer is converted into amide and the remaining amine enantiomer can be obtained in enantiomerically enriched form. The reaction functions in organic medium, MTBE as solvent, and E value exceeds 2000 (E = environmentally impact of the process).
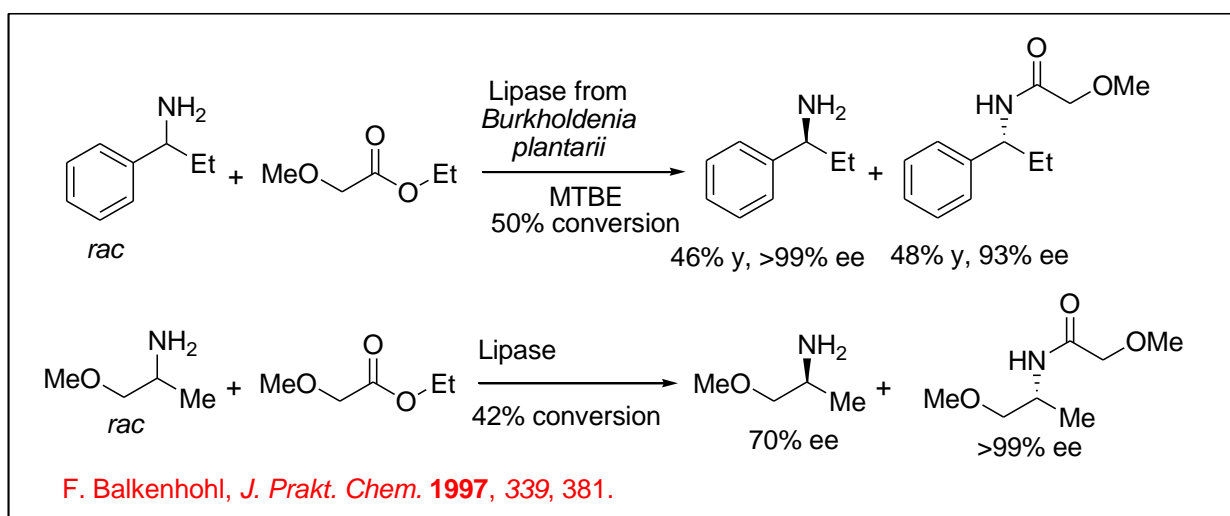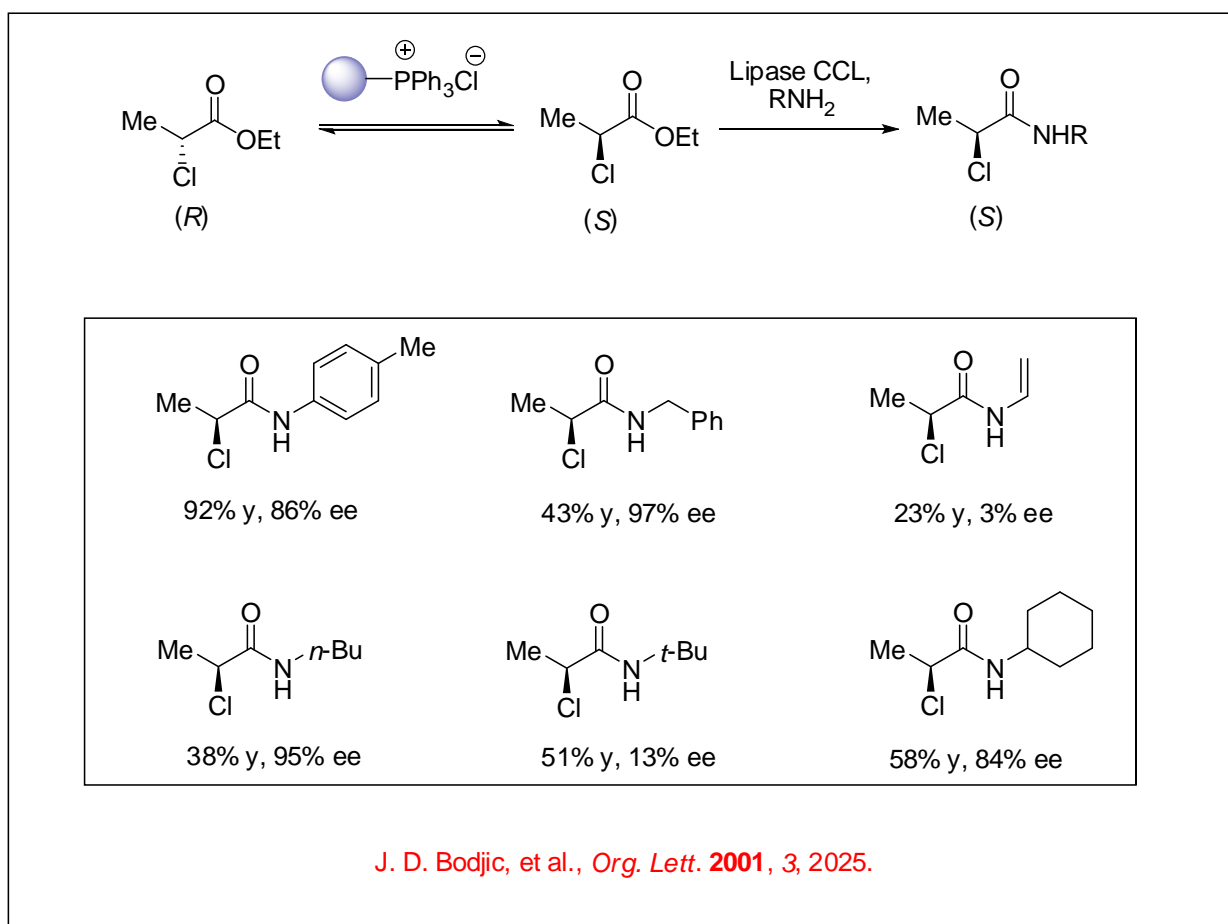

Scheme 4

11.1.3 Other Acylations

Enzymatic catalytic transformation of achiral amines and racemic acid components known as aminolysis affords elegant approach for the synthesis of enantioenriched acids. An interesting example is the reaction of dimethyl 3-(benzylamino)glutarate to give monoamides with excellent enantioselectivity (Scheme 5). The monoamides are intermediates for the synthesis of unnatural β-amino acids.

Scheme 5

A dynamic kinetic resolution with enzymatic aminolysis provides effective route towards the access of enantiomerically enriched acids. For example, in the presence of an immobilized phosphonium chloride for racemization of ethyl 2-chloropropionate and lipase, aminolysis can be carried out to give amides with up to 92% yield and 86% ee (Scheme 6).


92% y, 86% ee

43% y, 97% ee

23% y, 3% ee

38% y, 95% ee

51% y, 13% ee

58% y, 84% ee

J. D. Bodjic, et al., *Org. Lett.* **2001**, *3*, 2025.

Scheme 6

11.2 *Hydrolytic Reactions*

The enzymatic hydrolysis of racemic esters, amides, nitriles and epoxides affords effective methods for the synthesis of optically pure carboxylic acids, amines, amides, esters and alcohols. The reactions of a broad range of substrates have been well explored.

11.2.1 Ester Hydrolysis

Hydrolysis of racemic or prochiral ester using enzymes such as lipase, esterase and protease provides effective method for the resolution of broad range of substrates. Recently, the hydrolysis of indole ethyl ester has been shown using a lipase from Pseudomonas fluoresens (Scheme 7). The process runs at a high substrate concentrate 100g/L and turned out to be technically feasible to perform successfully on a 40-kg scale.
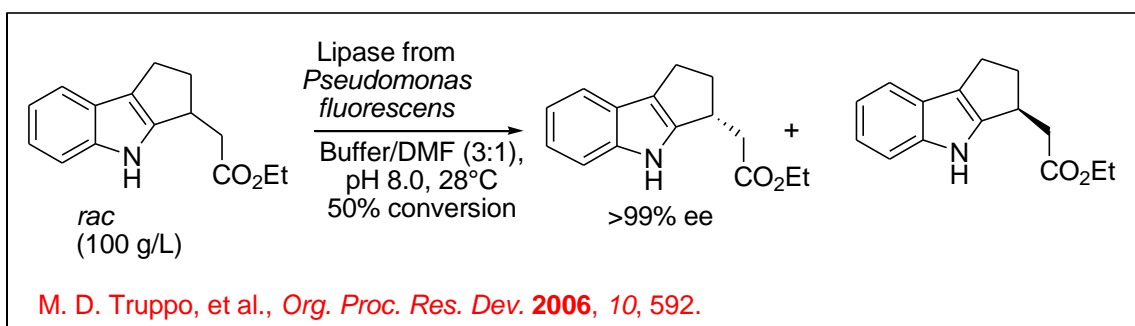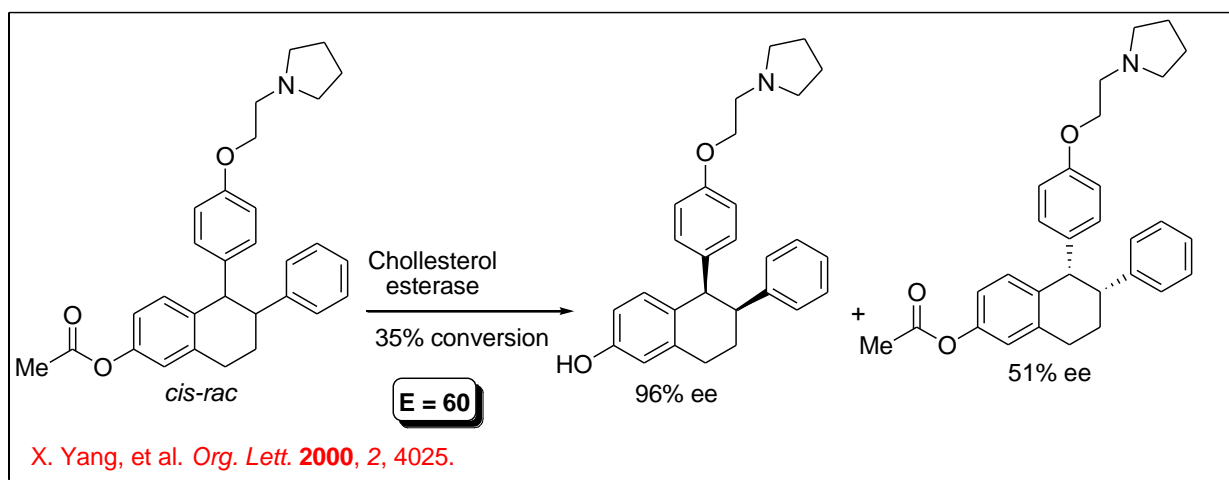

Scheme 7

Lipases are also suitable for the resolution of complex molecules having more than one additional functional group. For example, acyloin acetate can be hydrolyzed with E > 300 leading to diol in excellent enantioselectivity (Scheme 8).
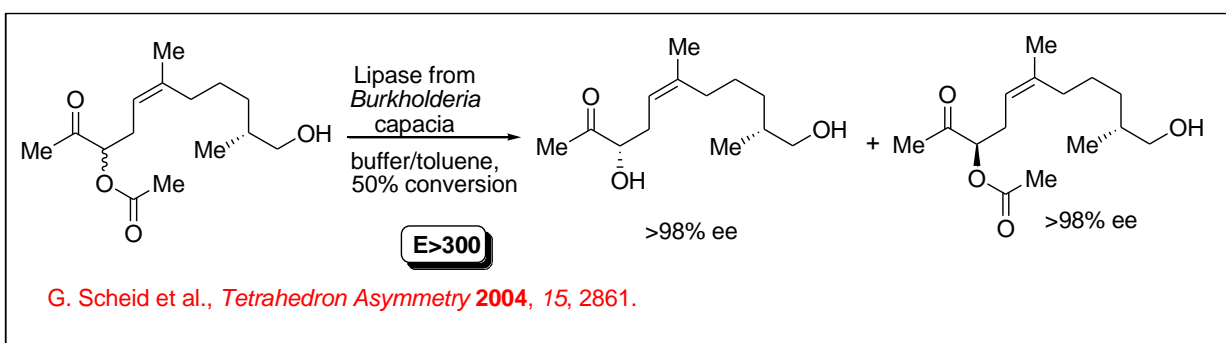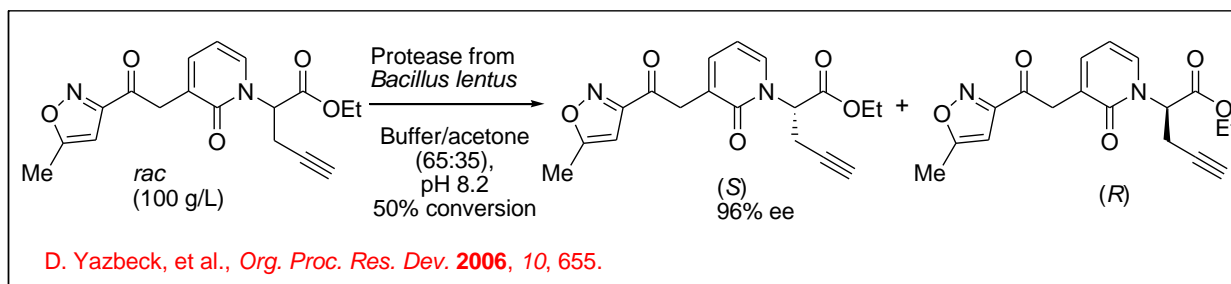

Scheme 8

Hydrolases can also recognize "remote chiral centers". For example, ester group separated from the stereogenic center by an aromatic group proceeds hydrolysis with enantioselectivity having the E value of 60 (Scheme 9). The product, Lasofoxifene (*cis*), is a potent and selective estrogen receptor modulator.

X. Yang, et al. *Org. Lett.* **2000**, *2*, 4025.

Scheme 9

The synthesis of an intermediate for a rhinovirus protease inhibitor has been accomplished by an impressive resolution employing a protease from Bacillus lentus (Scheme 10).

D. Yazbeck, et al., *Org. Proc. Res. Dev.* **2006**, *10*, 655.

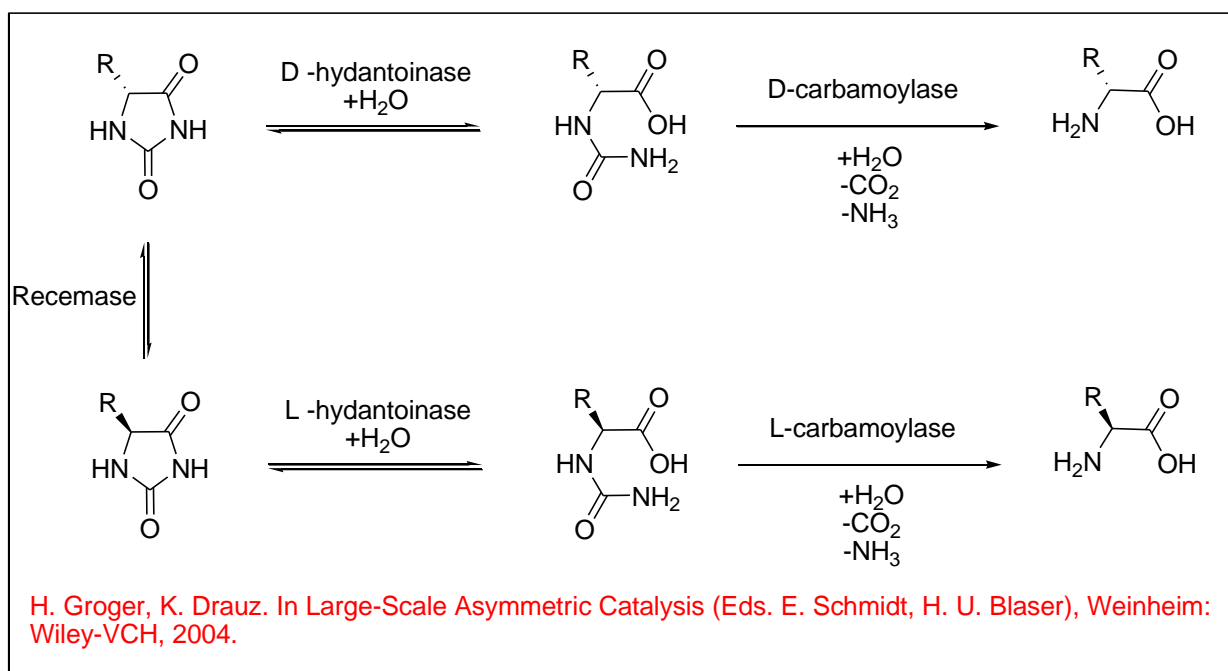**Scheme 10**

11.2.2 Nitrile Hydrolysis

Nitrilases are used for the hydrolysis of racemic or prochiral nitriles to give carboxylic acids. For example, nitrilase from *A. faecalis* catalyzes the hydrolysis of α-hydroxy nitriles to give (*R*)-mandelic acid with excellent enantioselectivity (Scheme 11).

K. Yamamoto, et al., *Appl. Environ. Microbiol.* **1991**, *57*, 3028.
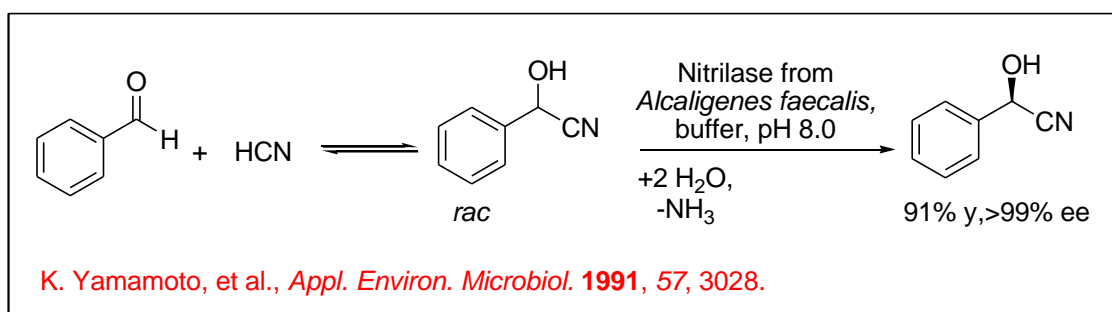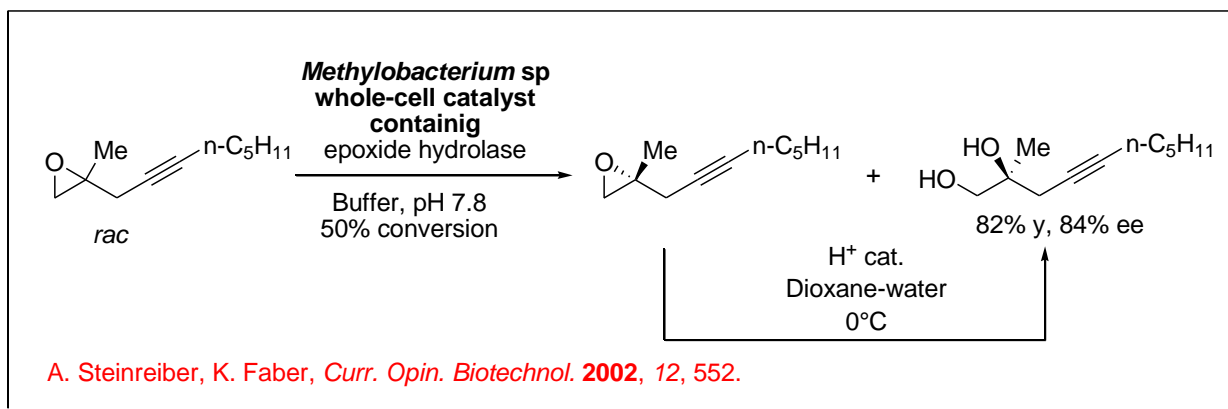
Scheme 11

11.2.3 Hydantoin Hydrolysis

Hydantoinases and carbamoylases hydrolyses racemic hydantoins to give optically pure α-amino acids (Scheme 12). In the beginning, the hydantoinase catalyzes the hydrolytic ring opening of the hydantoin to give an *N*-carbamoyl amino acid that proceeds cleavage to give the desired α-amino acid.

H. Groger, K. Drauz. In Large-Scale Asymmetric Catalysis (Eds. E. Schmidt, H. U. Blaser), Weinheim: Wiley-VCH, 2004.

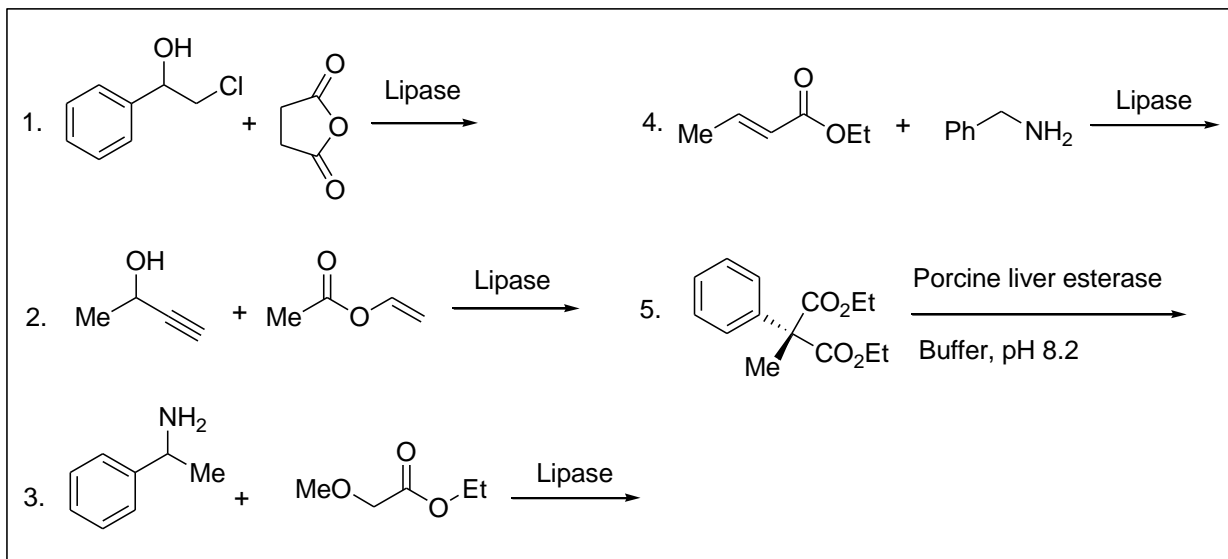**Scheme 12**

11.2.4 Epoxide Hydrolysis

Hydrolysis of racemic epoxide using epoxide hydrolase proceeds with high enantioselectivity. For example, the resolution of aliphatic epoxide having functional group can be accomplished using *Methylobacterium* sp. with good enantioselectivity (Scheme 13).

Scheme 13

Problems

A. Complete the following reactions.

B. Describe enzyme-catalyzed amide hydrolysis.

Reference/Text Book

1. I. Ojima, *Catalytic Asymmetric Synthesis*, 3rd ed., Wiley, New Jersey, 2010.

2. M. B. Smith, *Organic Synthesis*, 2nd edition, McGraw Hill, New Delhi, 2004.

Lecture 38

# Carbon-Carbon Bond Forming and Reduction Reactions

Biocatalysts are turned out to be versatile catalysts for carbon-carbon bond forming and reduction reactions in organic synthesis.

11.3 Formation of Carbon-Carbon Bonds

Carbon-carbon bond formation belongs to the heart of organic synthesis. The biocatalyzed route provides effective tool for the construction of carbon-carbon with excellent enantioselectivity.

11.3.1 Hydrocyanation of Aldehydes

The biocatalytichydrocyanation of aldehydes is one of the oldest methods in organic synthesis. One of the well-established technologies for the large-scale hydrocyanation of aldehydes is the oxynitrilase (Griengl process)catalyzed production of (*S*)-phenoxybenzaldehyde cyanohydrins, which is an important intermediate for the industrial pyrethroid manufacture (Scheme 1). This method is turned out to be useful for the reactions of numerous aldehydes.

A. Liese, K. Seelbach, C. Wandrey, Industrial Biotransformations Weinheim: Wiley-VCH, 2006.
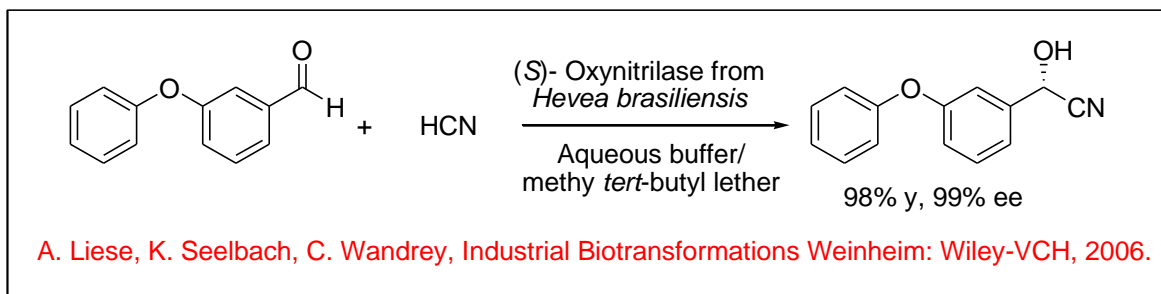
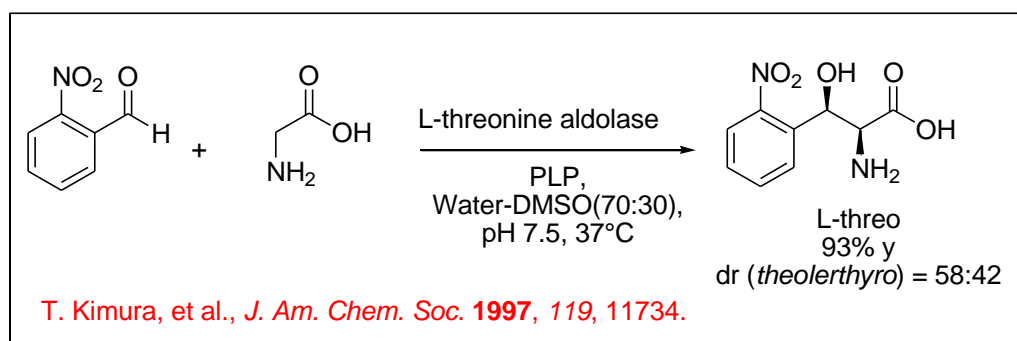**Scheme 1**

11.3.2 Benzoin Condensation

The development of an asymmetric cross-benzoin condensation *via* enzymatic cross-coupling reactions is a synthetically useful process. Highly enantiomerically enriched mixed benzoins can be obtained from two different substituted benzaldehdyes using benzaldehydelyase as a catalyst (Scheme 2). One of the aldehydes acts as acceptor, whereas the other one acts as donor.
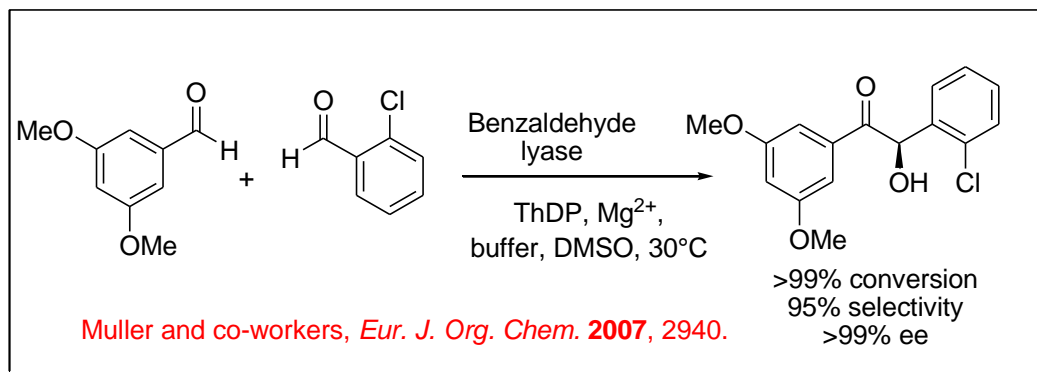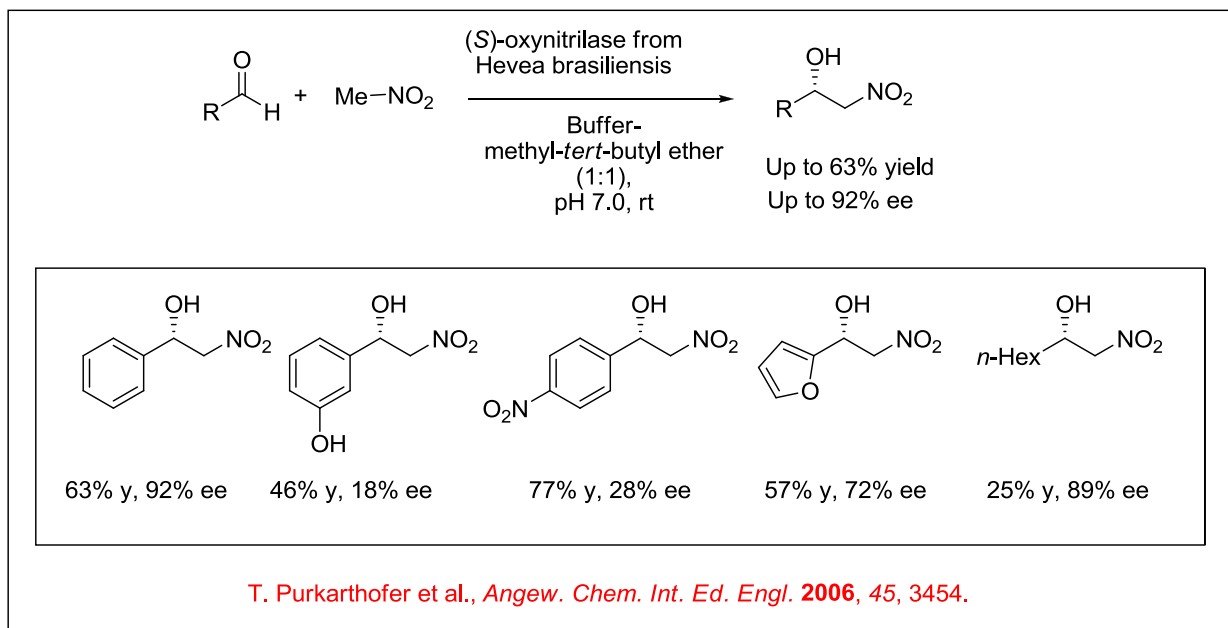

Scheme 2

11.3.3 Aldol Reaction

The biocatalyticaldol reactions are highly specific with respect to donor component, whereas a broad substrate scope is observed for the acceptor molecules. One of the examples is the reaction of glycine (donor) with substituted benzaldehyde (acceptor) employing threonine aldolases to give ☐-amino β-hydroxy acids with excellent enantioselectivity (Scheme 3).

Scheme 3

11.3.4 Nitroaldol Reaction

Enzymes are also useful for the non-natural reactions. For example, using (*S*)-oxynitrilase the reaction of nitromethane with a broad range of aldehydes can be accomplished with excellent
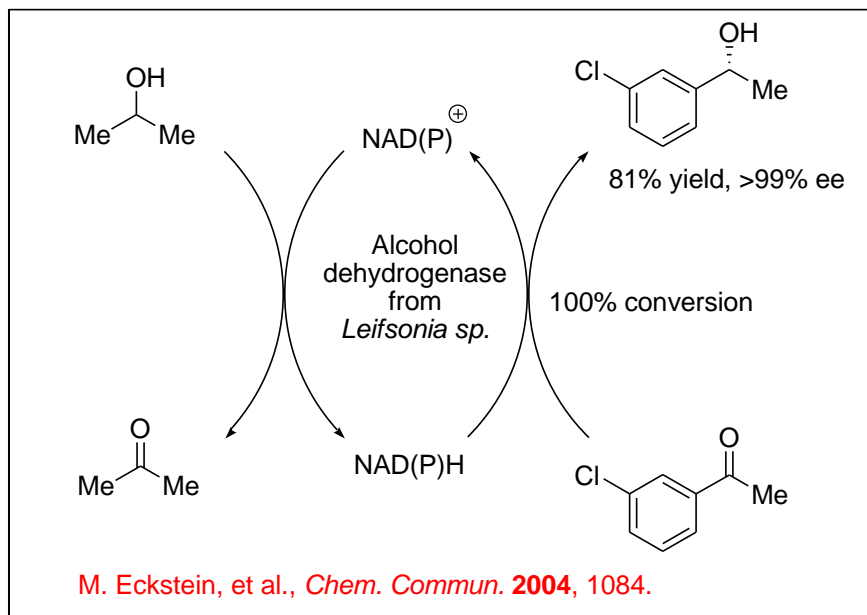
Scheme 4

enantioselectivity (Scheme 4). Nitroalkane acts donor, whereasthe aldehydes are acceptors.

11.4 Reduction Reactions

The enantioselective reduction of C=X double bonds (X = O, NR,C) to C-XH single bonds plays a major role in asymmetric synthesis.
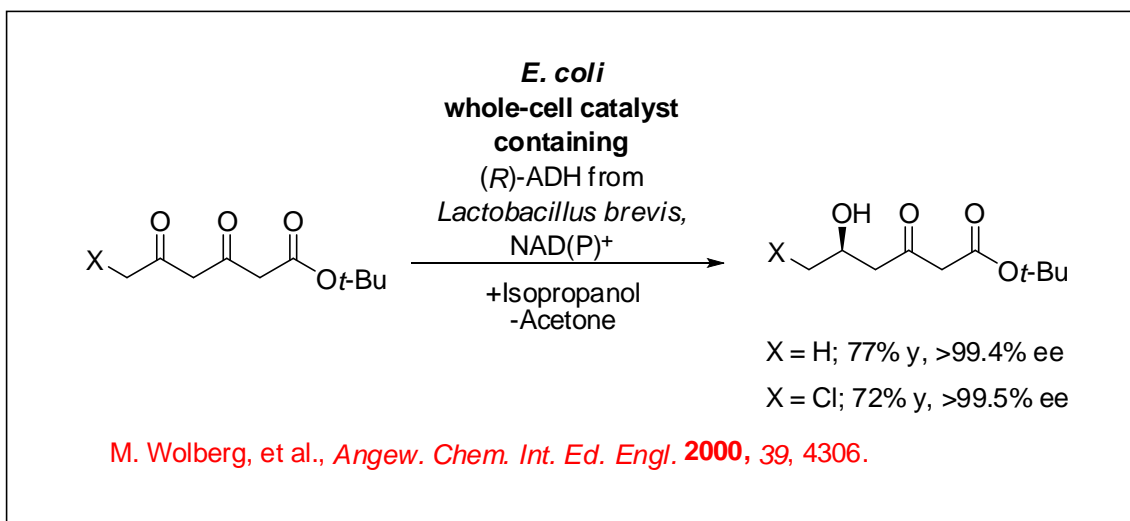
11.4.1 Reduction of Ketones

The enantioselective reduction of ketones represents an atom-economical approach towards optically active alcohols. The biocatalytic reduction of ketones is based on the use of an alcohol dehydrogenase (ADH) as a catalyst, and a cofactor as a reducing agent. For example, ADH from *Leifsonia* sp. catalyses the reduction of substituted acetophenoneto give secondary alcohols with high enantioselectivity(Scheme 5). In this process, 2-propanol acts as a reducing agent oxidizing into acetone.
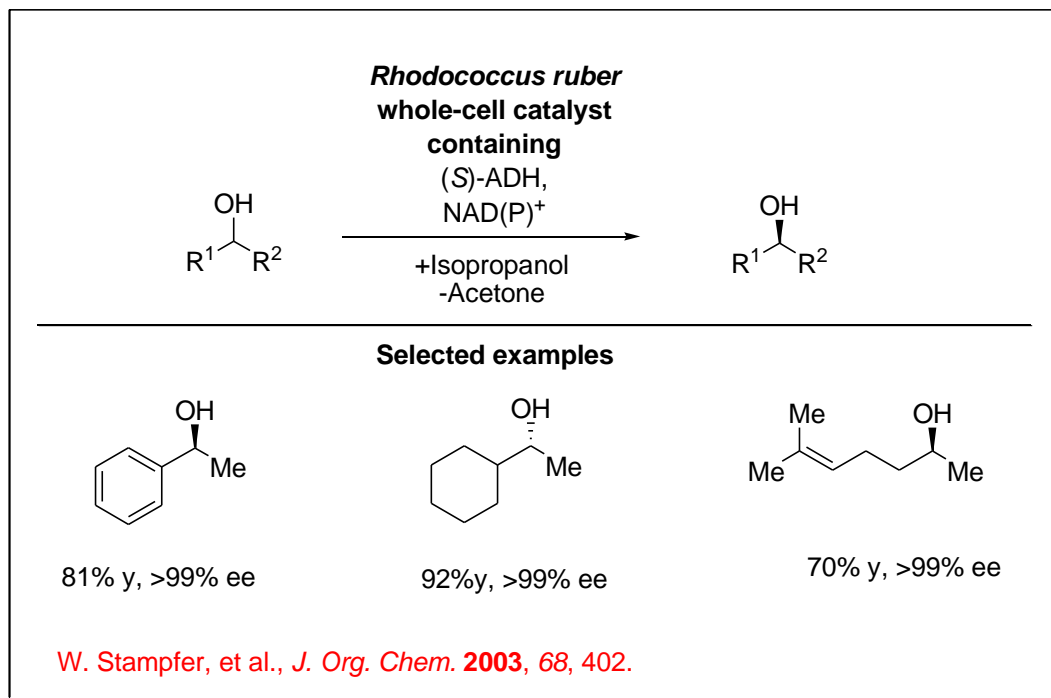

Scheme 5

Theketo group of2,5-diketo ester can be selectively reduced with excellentregio- and enantioselectivity using *E. coli* cells with overexpressed ADH from *Lactobacillus brevis* (Scheme 6). In this process 2-propanol acts as a reducing agent oxidizing into acetone.
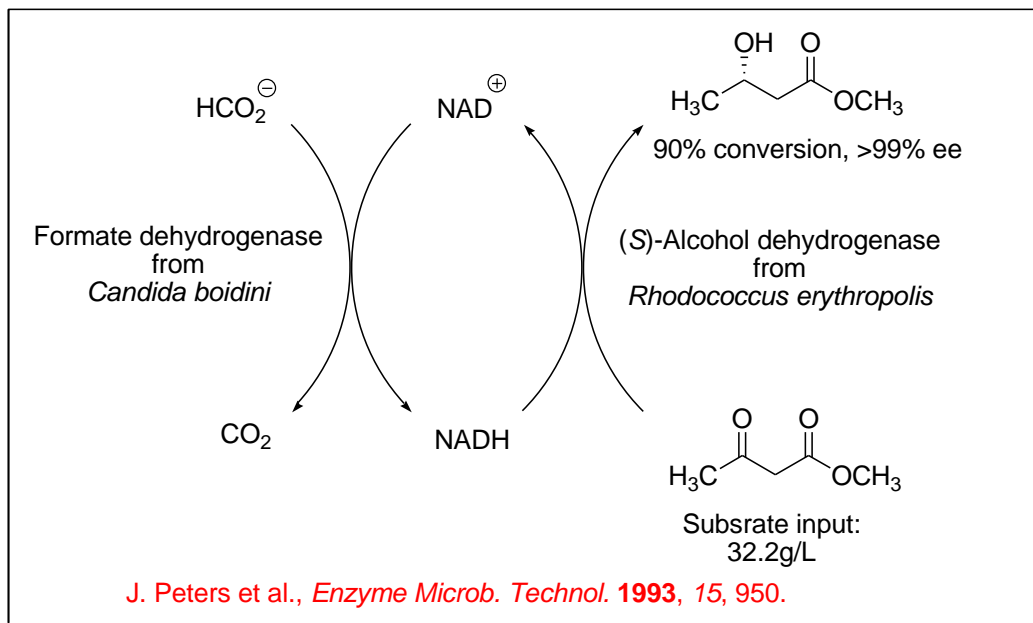

Scheme 6

The reduction of a wide range of aliphatic and aromatic ketones can be accomplished employing *R. ruber* ADH to give the corresponding alcohols with excellent enantioselectivityin 2-propanol (Scheme 7).
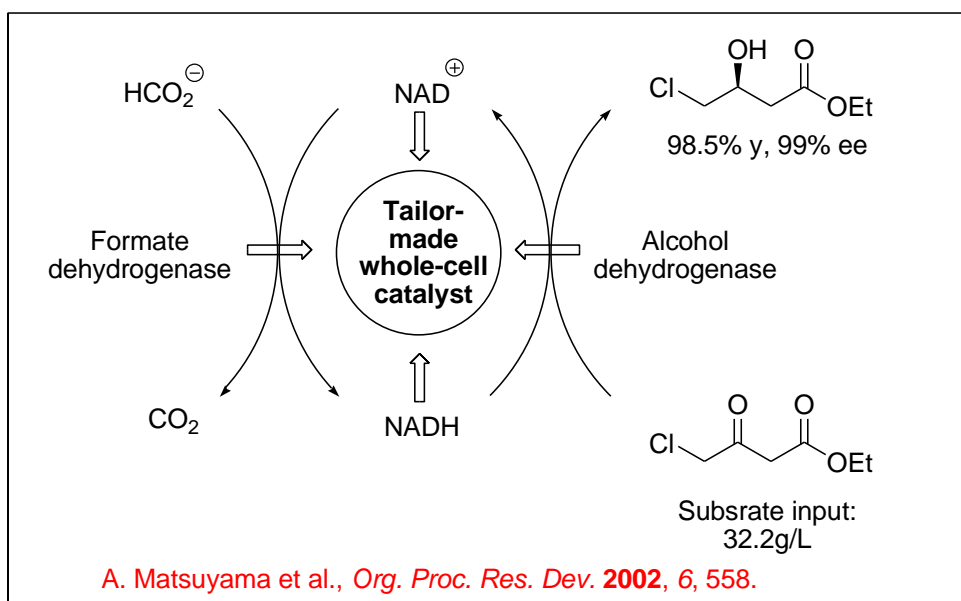

Scheme 7

Whereas formate dehydrogenase (FDH) from *C. boidinii* catalyzes selectively the reduction of keto group of β-keto esters with high enantioselectivity. In this reaction, formate is oxidized into carbon dioxide (Scheme 8).

90% conversion, >99% ee

Formate dehydrogenase from *Candida boidini*

(*S*)-Alcohol dehydrogenase from *Rhodococcus erythropolis*

Subsrate input: 32.2g/L

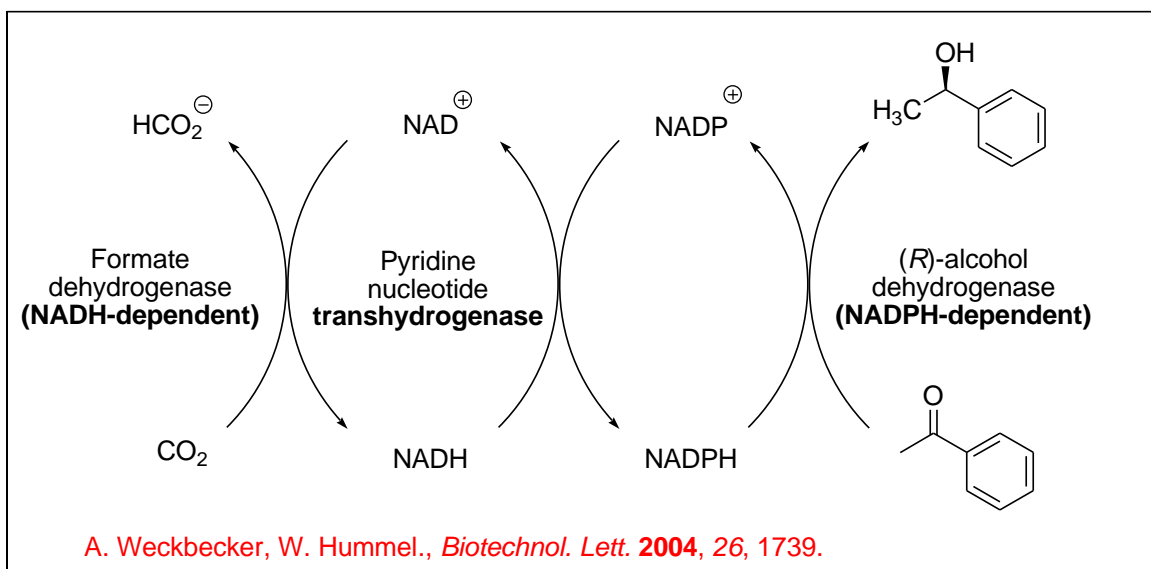J. Peters et al., *Enzyme Microb. Technol.* **1993**, *15*, 950.

Scheme 8

The FDH-based whole-cell can be used for the reduction of ethyl 4-chloro-3-oxobutanoate with 99% ee (Scheme 9).

98.5% y, 99% ee

Formate dehydrogenase

Tailor-made whole-cell catalyst

Alcohol dehydrogenase

Subsrate input: 32.2g/L

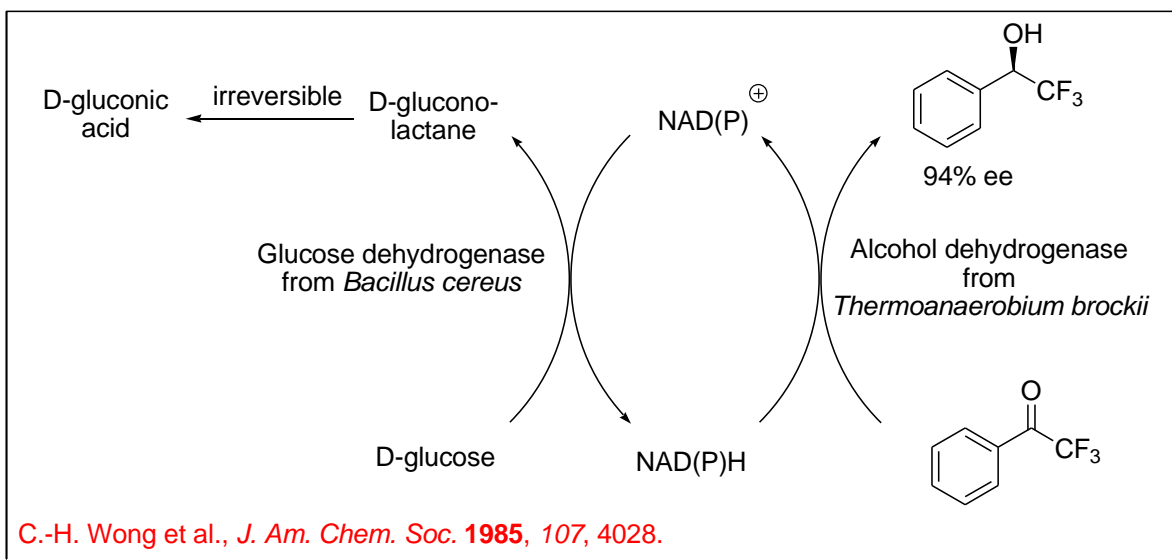A. Matsuyama et al., *Org. Proc. Res. Dev.* **2002**, *6*, 558.

Scheme 9

The use of FDH from *C. boidinii* has limitation due to its inability to regenerate $NADP^+$. This has been overcome by expanding the application range of FDH-based cofactor regeneration to $NADP^+$-dependent ADHs (Scheme 10). This involves the integration of an additional enzymatic step within the cofactor-regeneration cycle that is exemplified in the reduction of acetophenone to (*R*)-phenylethanol. In this process, the pyridine nucleotide transhydrogenase (PNT)-catalyzes regeneration of NADPH from $NADP^+$ under consumption of NADH forming NAD^+.


A. Weckbecker, W. Hummel., *Biotechnol. Lett.* **2004**, *26*, 1739.

Scheme 10

C.-H. Wong et al., *J. Am. Chem. Soc.* **1985**, *107*, 4028.

Scheme 11

Further, for recycling the cofactor NAD(P)H, the use of a glucose dehydrogenase (GDH) has been demonstrated. In this system, D-glucose is oxidized to D-gluconolactone, while the oxidized cofactor NAD(P$^+$) is reduced to NAD(P)H. Since D-gluconolactone is then hydrolyzed into D-gluconic acid, the reaction is irreversible shifting the whole process towards the desired alcohol product formation. This GDH coupled cofactor-regeneration process has been used for the reduction of ketone to alcohol with high enantioselectivity (Scheme 11).
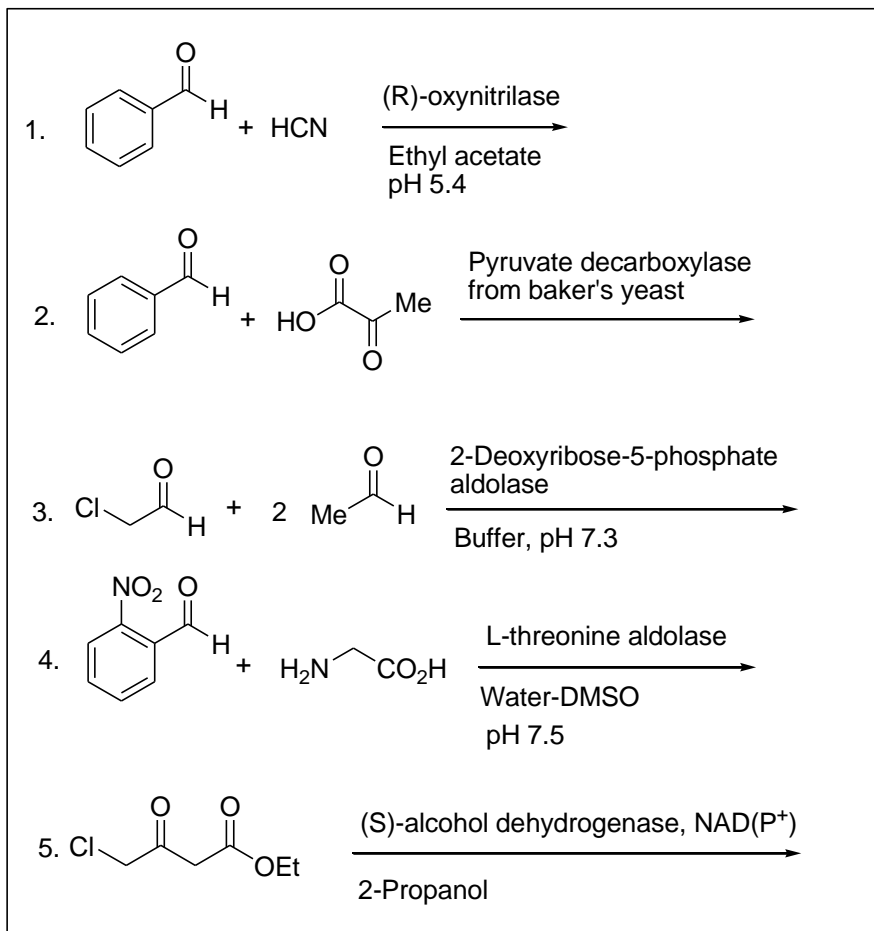
This principle has been recently used for the reduction of ethyl 6-benzyloxy-3,5-dioxohexanoate to afford ethyl (3*R*,5*S*)-6-benzyloxy-3,5-dihydroxyhexanoate with 99% ee employing ADH from *Acinetobactercalcoaceticus* in combination with a GDH and glucose (Scheme 12).
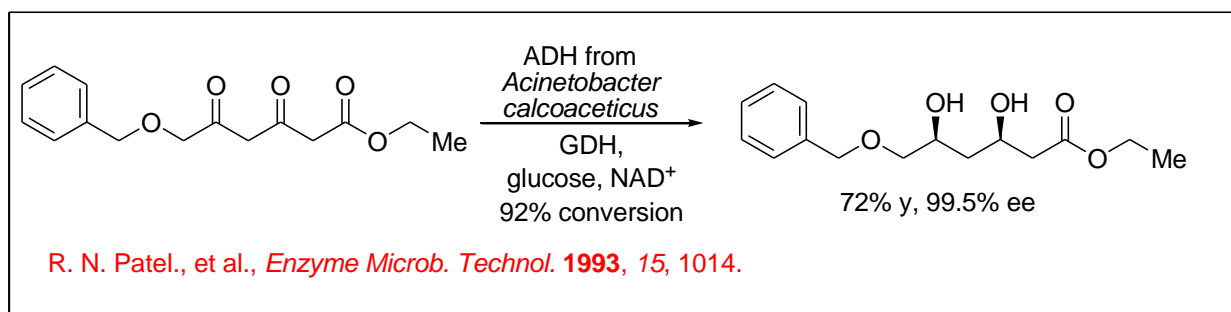
Scheme 12

Problems

C. Complete the following reactions.

Reference/Text Book

3. I. Ojima, *Catalytic Asymmetric Synthesis*, 3rd ed., Wiley, New Jersey, 2010.

4. M. B. Smith, *Organic Synthesis*, 2nd edition, McGraw Hill, New Delhi, 2004.

Lecture 39

Enantioselective Reductions

11.4.2 Reduction of Ketones

Recombinant whole-cell catalytic system having *E.coli*, co-expressing both the ADH from *S. salmonicolor*and the GDH from *B. megaterium*, has been developed for the asymmetric reduction of 4-chloro-3-oxobutanoate in a mixture of*n*-butyl acetate/water (Scheme 1). It is an elegant approach toward tailor-made biocatalysts containing both of the desired enzymes, ADH and GDH, in overexpressed form (Scheme 1).
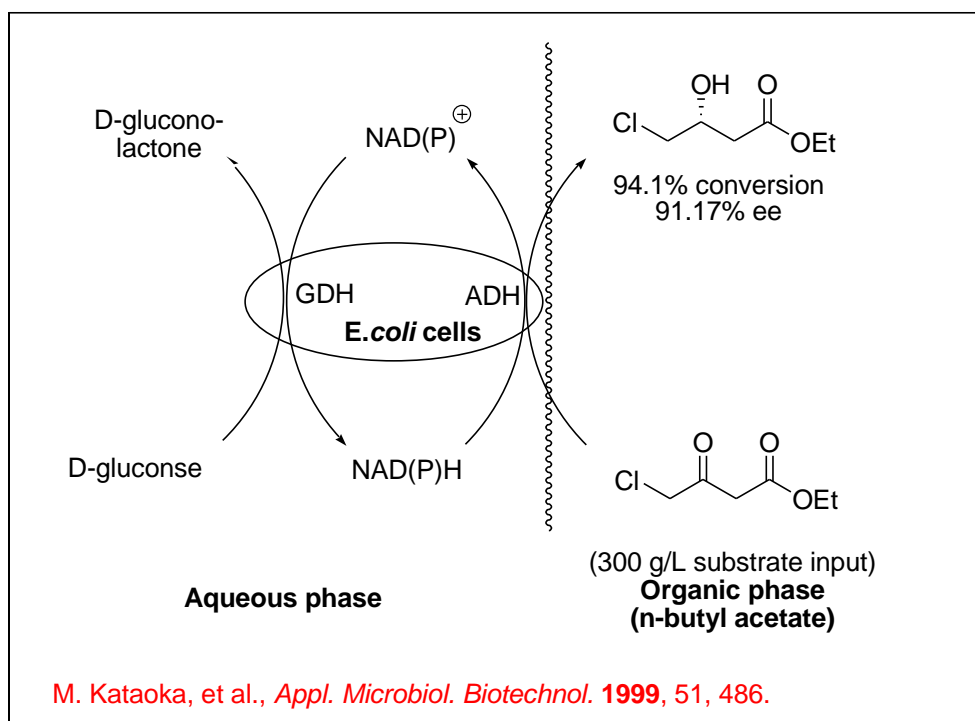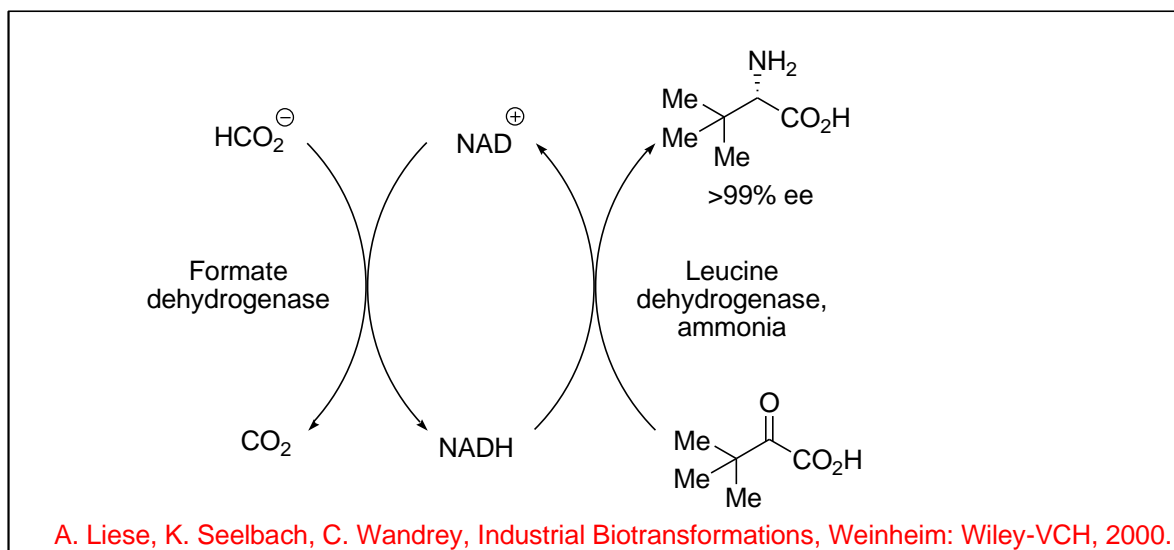

Scheme 1

The application of recombinant whole-cell biocatalytic system has been further demonstrated in pure aqueous media without the need of addition of external amount of cofactor (Scheme 2). This method is economical and simple, and finds applications for the reduction of a wide range of ketones (Scheme 2).

E. coli
whole-cell catalyst
containing
(*S*)- or (*R*)-ADH,
GDH,
NAD(P)$^+$

$R^1 \overset{O}{\underset{}{C}} R^2$ → $R^1 \overset{OH}{\underset{}{C}} R^2$ or $R^1 \overset{OH}{\underset{}{C}} R^2$

D-glucose

Selected examples

94% conversion
>99.8% ee
(156 g/L substrate input)

>95% conversion
>99.4% ee
(212 g/L substrate input)

94% conversion
97% ee
(140 g/L substrate input)

H. Groger et al., *Adv. Synth. Catal.* **2007**, *349*, 709.

Scheme 2

HCO$_2^\ominus$ → NAD$^\oplus$

Formate dehydrogenase

Leucine dehydrogenase, ammonia

>99% ee

CO$_2$ → NADH

A. Liese, K. Seelbach, C. Wandrey, Industrial Biotransformations, Weinheim: Wiley-VCH, 2000.

Scheme 3

11.4.3 Reductive Amination of α-Keto Acids

Enzyme catalyzed asymmetric reductive amination of α-keto acids represents a straightforward method to access optically active α-amino acids. For example, L-*tert*-leucine, which serves as

building block for the pharmaceutical industry, is obtained with high conversion and enantioselectivity using a leucine dehydrogenase for the reductive amination and an FDH from *C.boidinii* (Scheme 3). The latter is required for an *in situ* recycling of the cofactor NADH.
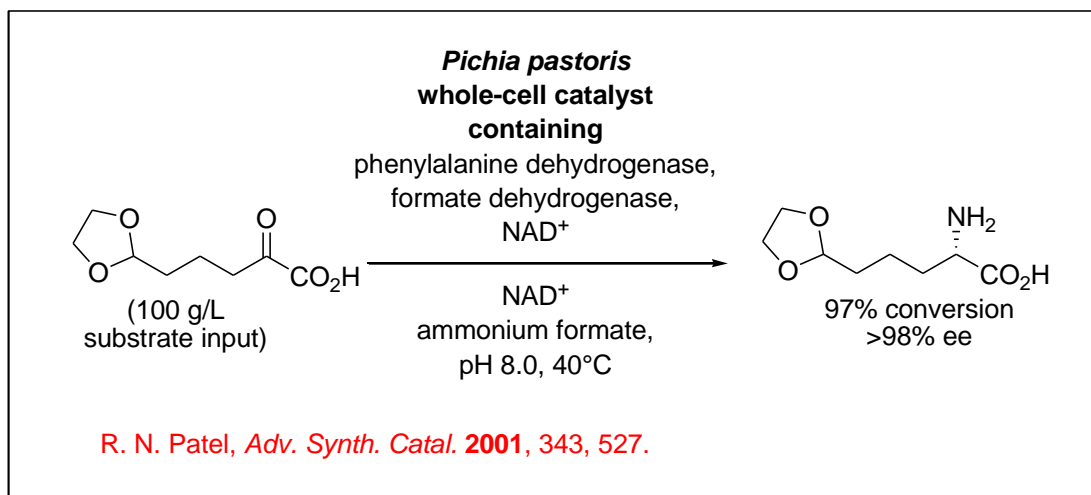
Similarly, the synthesis of L-6-hydroxynorleucine can be accomplished from α-keto acid with complete conversion and >99% enantioselectivity(Scheme 4). In this reaction, a beef liver glutamate dehydrogenasehas been used as L-amino acid dehydrogenase and a GDH from *B. megaterium*has been used for the cofactor regeneration.
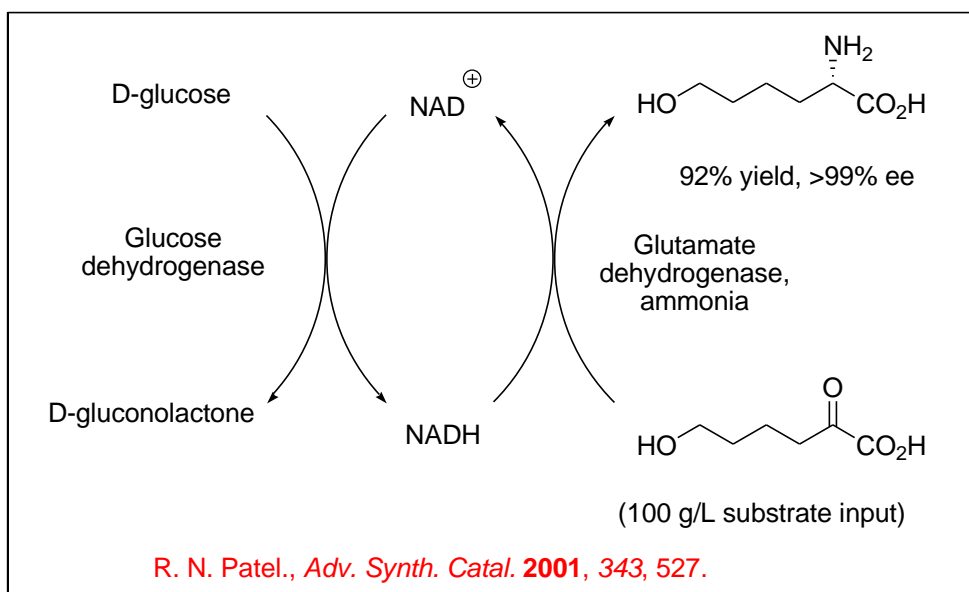
Scheme 4

However, the need for the addition of expensive cofactor NAD^+ as well as the isolation and cost of the enzymes make these approaches are limited. Thus, efforts have been made to address these aspects by employing a whole-cell catalyst, having both an amino acid dehydrogenase and FDH in overexpressed form. For example, the synthesis of L-allysine ethylene acetal has been shown

using a whole-cell catalyst, *Pichiapastoris* cells having a phenylalanine dehydrogenase from *Thermoactinomycesintermedius* and an FDH from *P.pastoris* (Scheme 5).

Pichia pastoris
whole-cell catalyst
containing
phenylalanine dehydrogenase,
formate dehydrogenase,
NAD^+

(100 g/L
substrate input)

NAD^+
ammonium formate,
pH 8.0, 40°C

97% conversion
>98% ee

R. N. Patel, *Adv. Synth. Catal.* **2001**, 343, 527.

Scheme 5

## 11.4.4 Reduction of Activated Carbon-Carbon Double Bonds

The reduction of carbon-carbon double bonds using the biocatalytic systems has high potential in organic chemistry. However, this process is less explored compared to the C=O reduction of ketones and keto esters. The reduction of the carbon-carbon double bond in ketoisophorone has been accomplished using whole-cell catalyst overexpressing an enolatereductase from *Candida macedoniensis* and a GDH (Scheme 6). This study can be regarded as one of the pioneering works in the reduction of carbon-carbon double bonds using biocatalytic systems.
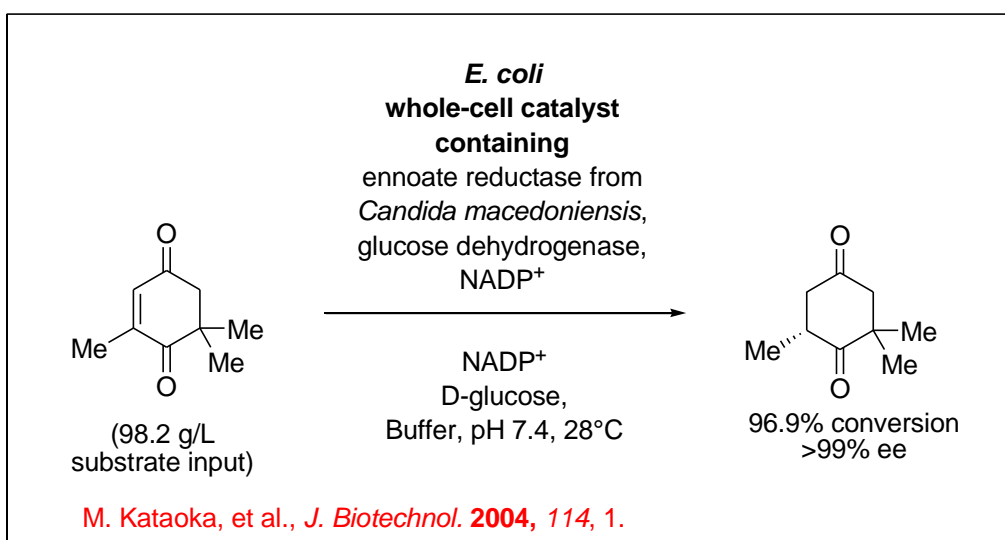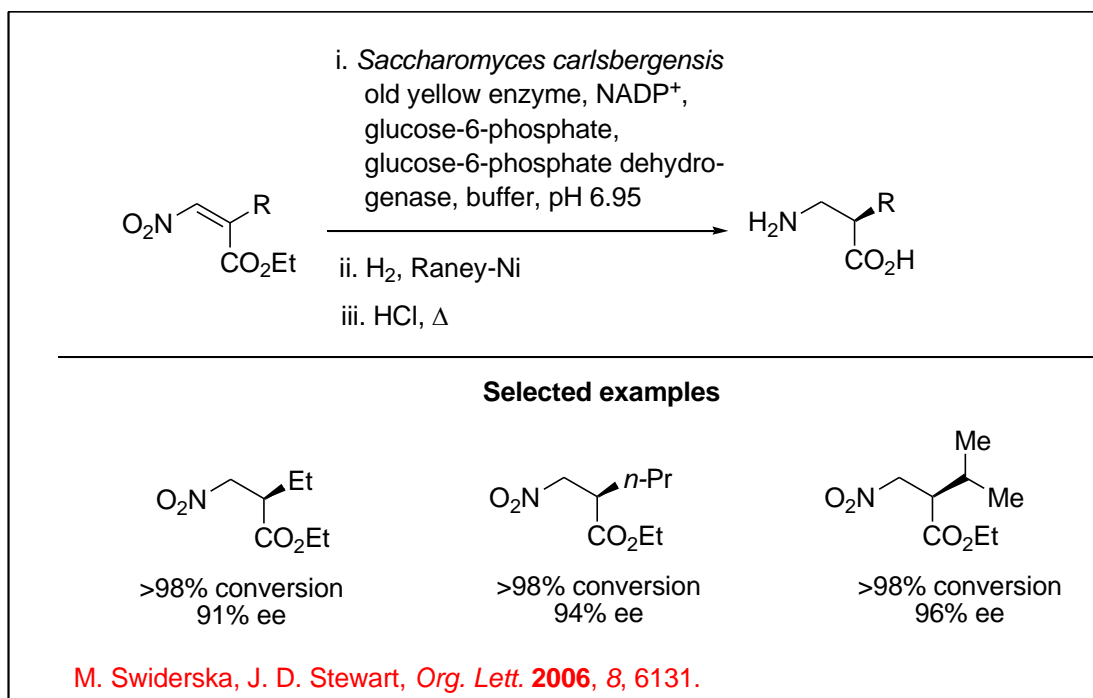

Scheme 6

α,β-Unsaturated carboxylic acids can also be used as substrates. For example, α–chloroacrylic acid can be converted into α-chloropropionate using an enolatereductase from *Burkholderia sp*., in high enantioselectivity (Scheme 7).

Besides, enone and α,β-unsaturated carboxylic acid, nitroalkanes are also suitable substrates for enoatereductase. For example, the reduction of carbon-carbon double bond in *Z*-nitroalkenesproceed reaction to give 2-substituted 3-nitropropanoates with high conversion and in most cases with high enantioselectivity (Scheme 8).
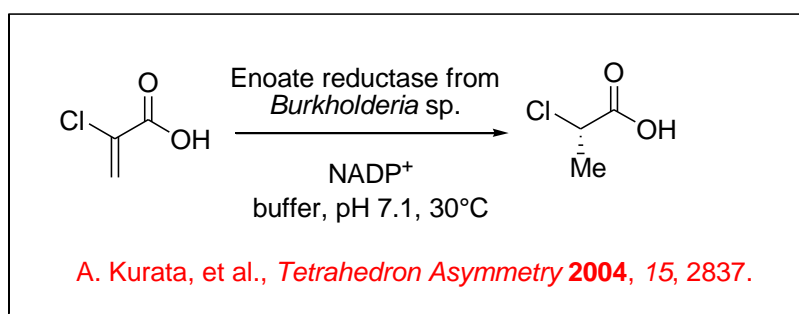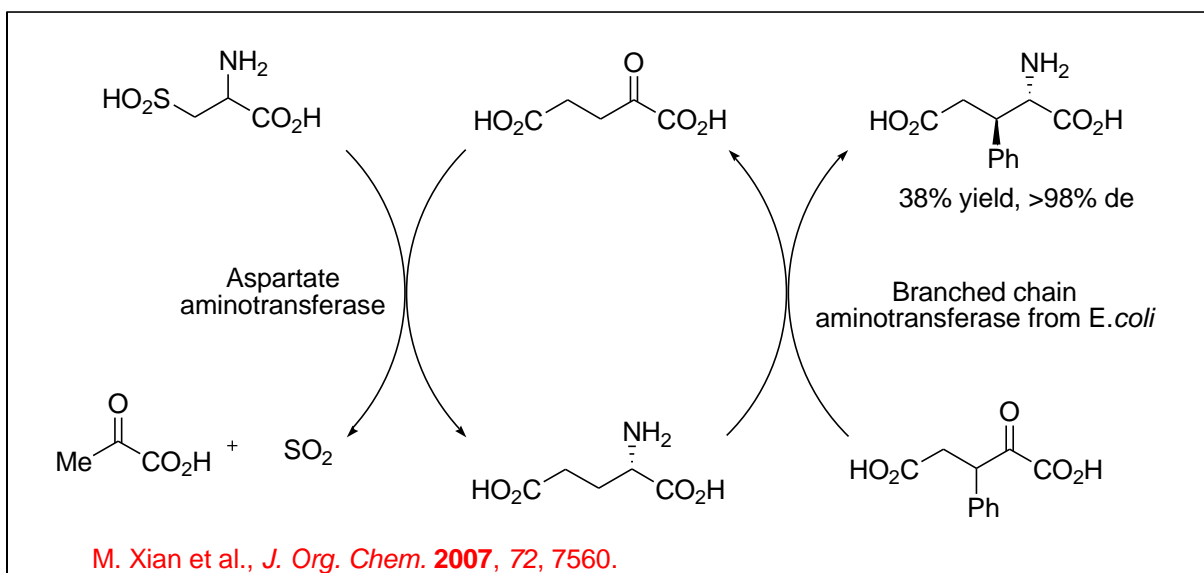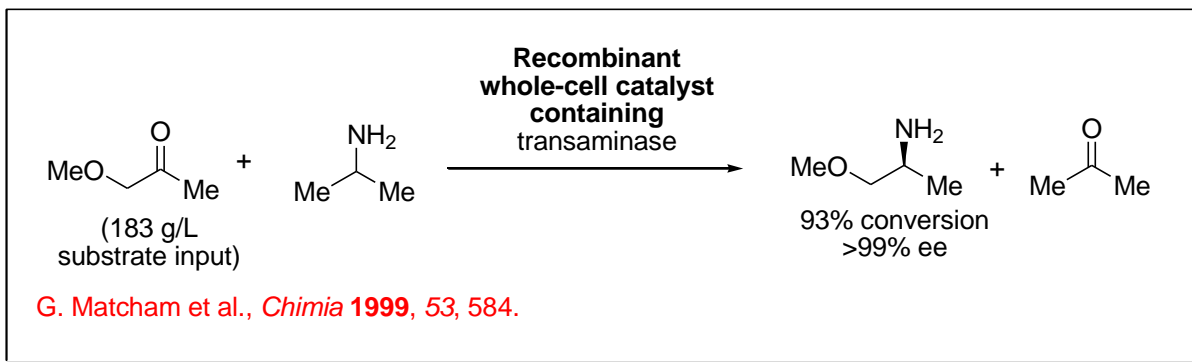

Scheme 7

Scheme 8

11.4.5 Transamination

Depending on the nature of the transaminase, α-keto acids and ketones proceed reaction to give α-amino acids and amines with a stereogenic center in α-position, respectively. For example, a coupling of the transaminase process with an irreversible aspartate aminotransferase-catalyzed transamination process using cysteine sulfinic acid as an amino donor has been used for the synthesis of various types of non-natural 3- or 4-substituted glutamic acid analogues (Scheme 9).


Scheme 9

Furthermore, the highly efficient synthesis (*S*)-methoxyisopropylamine has been accomplished using a recombinant whole-cell catalyst overexpressing a transaminase. A key feature in this process is the high substrate concentration and the desired target molecule can be obtained with excellent enantioselectivity (Scheme 10).

G. Matcham et al., *Chimia* **1999**, *53*, 584.

Scheme 10

Problems

D. Complete the following reactions.
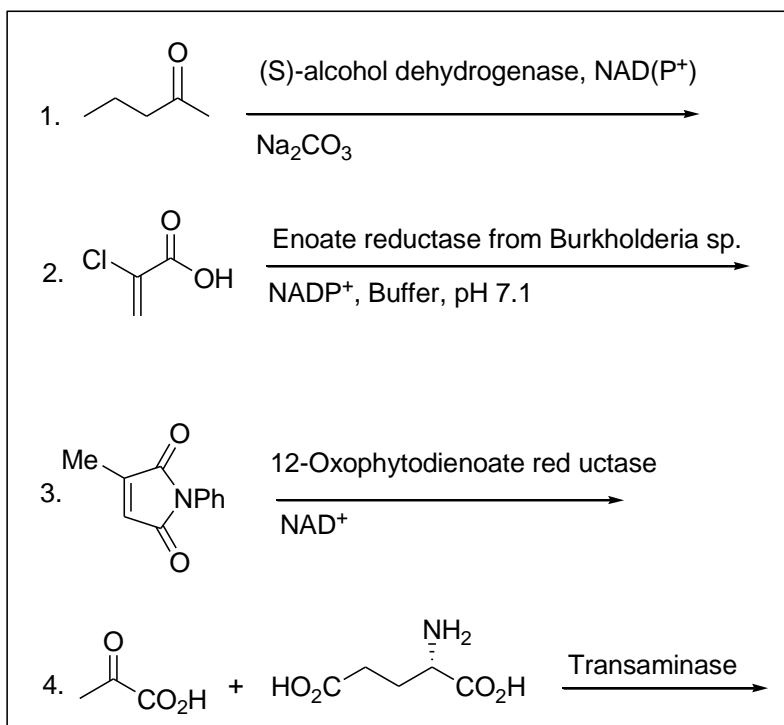
Reference/Text Book

5.   I. Ojima, *Catalytic Asymmetric Synthesis*, 3rd ed., Wiley, New Jersey, 2010.

6.   M. B. Smith, *Organic Synthesis*, 2nd edition, McGraw Hill, New Delhi, 2004.

Lecture 40
11.5 Enantioselective Oxidations

Substrates

Biocatalysts are also turned to be useful for asymmetric oxidations. A wide range of asymmetric oxidations using biocatalytic systems has been explored.

## 11.5.1 Baeyer-Villiger Oxidation

Baeyer-Villiger reaction is known for more than 100 years. However, the asymmetric version of this reaction remains as challenge for organic chemists. Depending on the nature of ketones the reaction can be carried out as a resolution of racemic ketones as well as an asymmetric desymmetrization reaction from prochiral ketones. The enzymes used for this reaction is known


M. J. Taschner, D. J. Black, *J. Am. Chem. Soc.* **1988**, *110*, 6892.
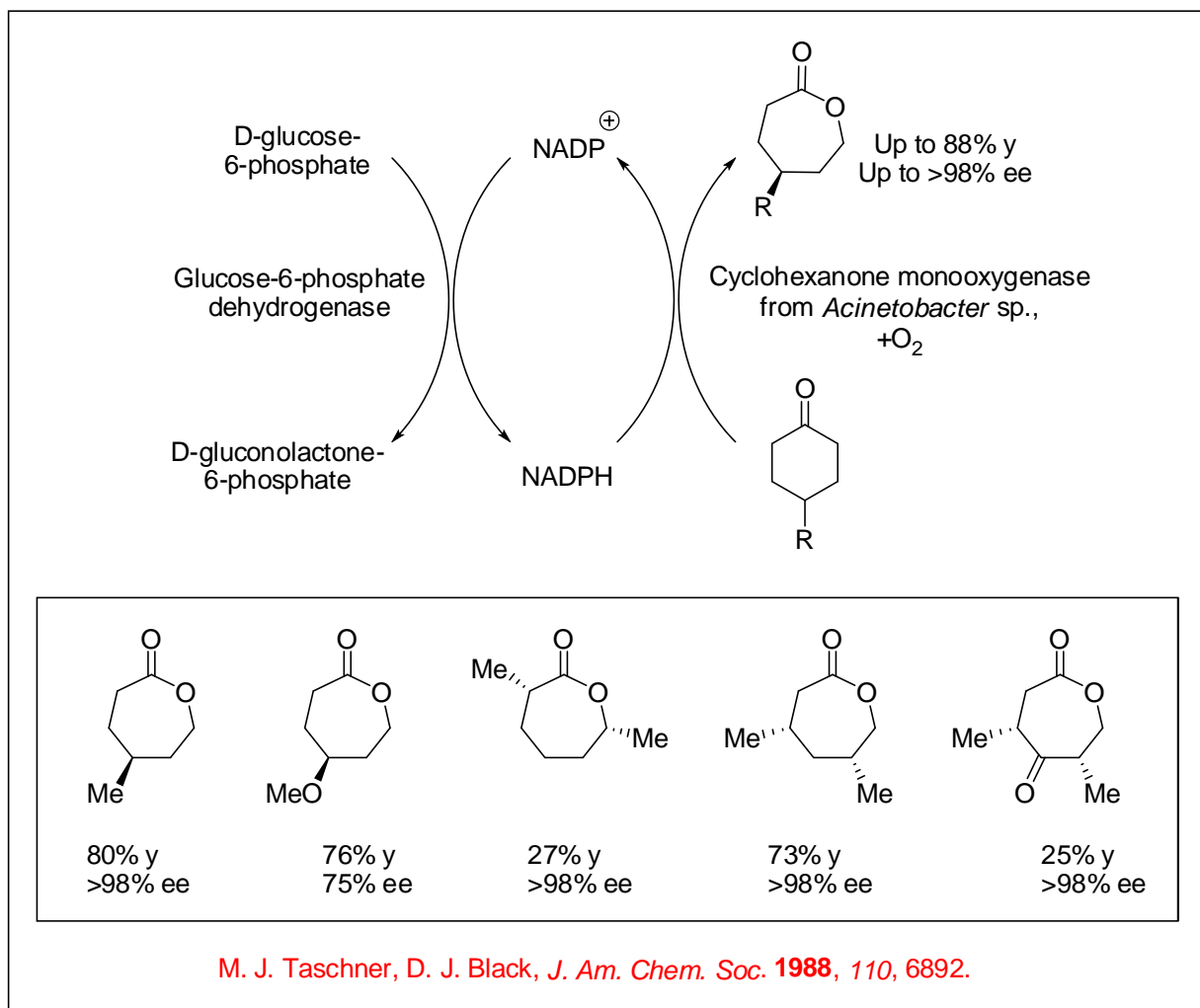
Scheme 1

as Baeyer-Villiger monooxygenases. These enzymes are cofactor dependant and are generally obtained from microbial sources. For example, 4-substituted monocyclic cyclohexanones can be oxidized into the lactones in good yield and with high enantioselectivities (Scheme 1). In this process, the reduced form of the cofactor (NADPH) is needed under the formation of NADP$^+$ that is *in situ* recycled using an enzymatic coupled cofactor reproduction.

The scale up of the process has also been explored. For example, the racemic bicyclo[3.2.0]hept-2-enone with input of 25g/L proceeds oxidation in the presence of a recombinant whole-cell biocatalyst to afford regioisomeric lactones with high enantioselectivity (Scheme 2).
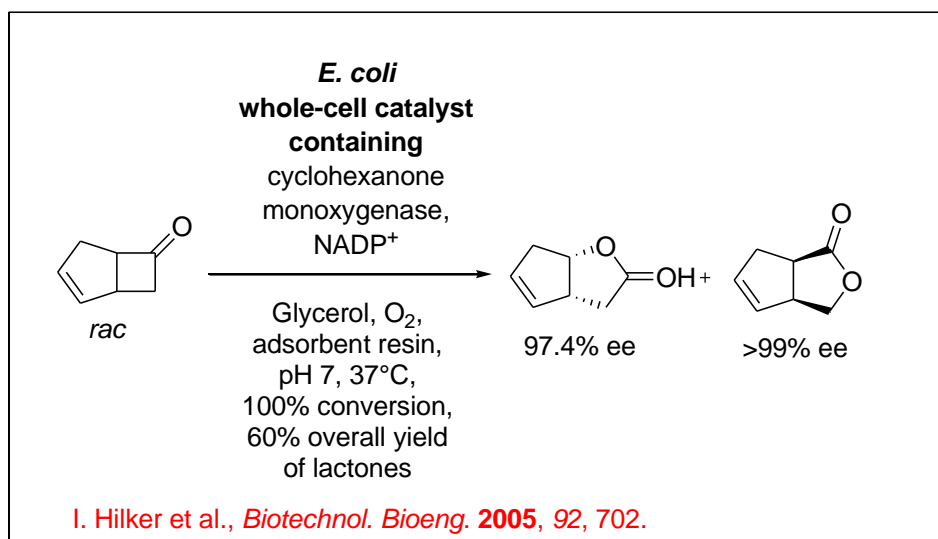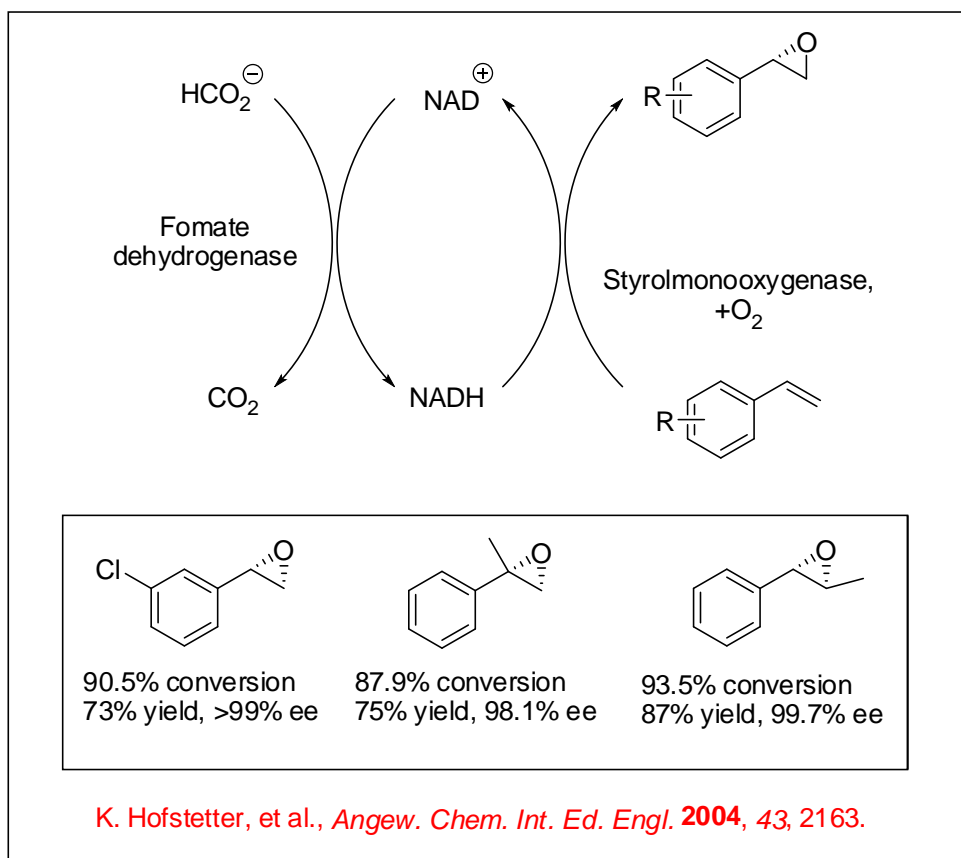

Scheme 2
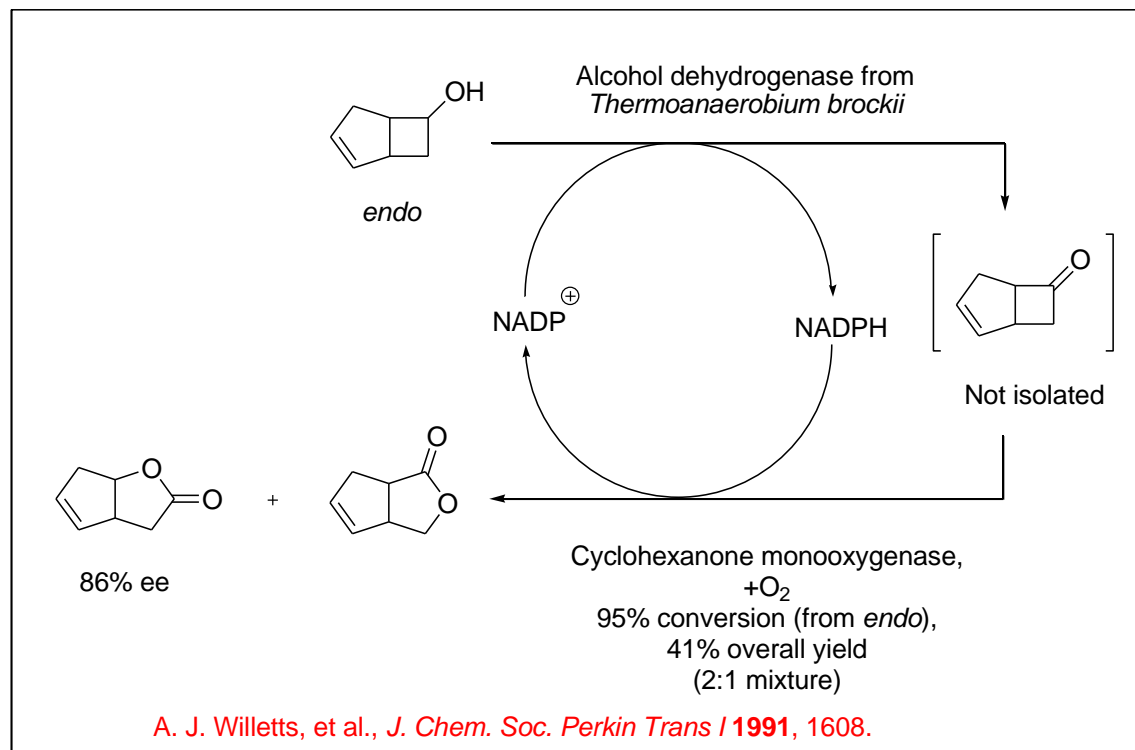
Scheme 3

A further process improvement is the coupling of a cyclohexanone monooxygenase with an ADH from *T. brockii*, a cosubstrate-free "double oxidation" of an alcohol into lactones (Scheme 3). In this system, the oxidized form of the cofactor (NADP$^+$) is consumed in the initial ADH-catalyzed step, while the reduced form of the cofactor (NADPH) is then needed for the second, monooxygenase-catalyzed oxidation step. In the second step, the oxidized form of the cofactor (NADP$^+$), which is then needed for the first step, is produced again.
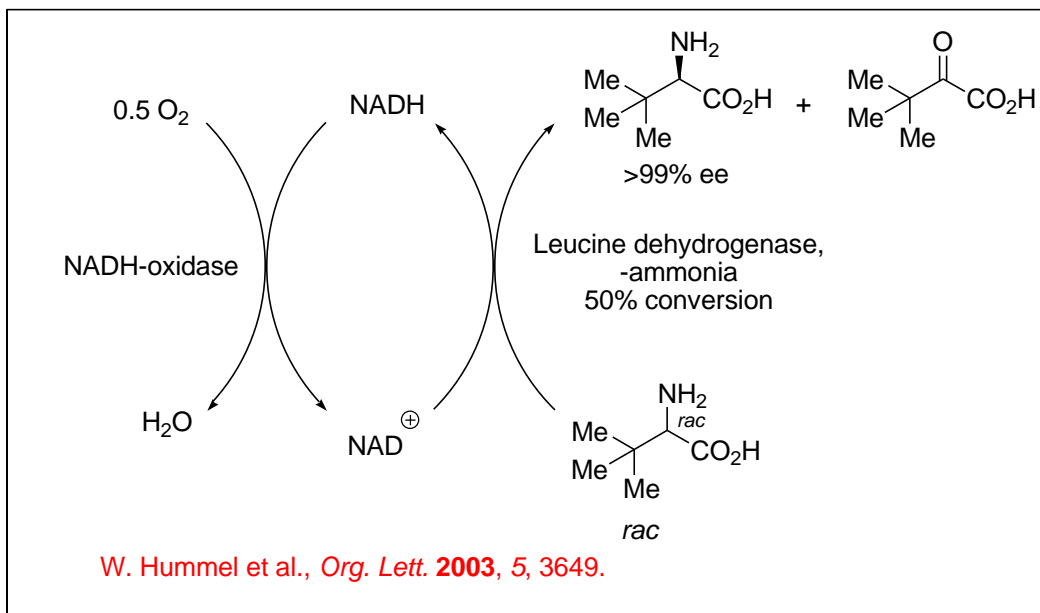
11.5.2 Epoxidation

Optically active epoxides serve as versatile building blocks in organic synthesis. Besides metal and organocatalysts, cofactor dependent monooxygenase turned out to be valuable catalyst for the epoxidation of alkenes. For example, the epoxidation of styrene has been shown using a stable recombinant FAD/NADH-dependent styrene monooxygenase in aqueous-organic emulsions (Scheme 4). The reaction condition is also effective for the oxidation of other styrene derivatives.
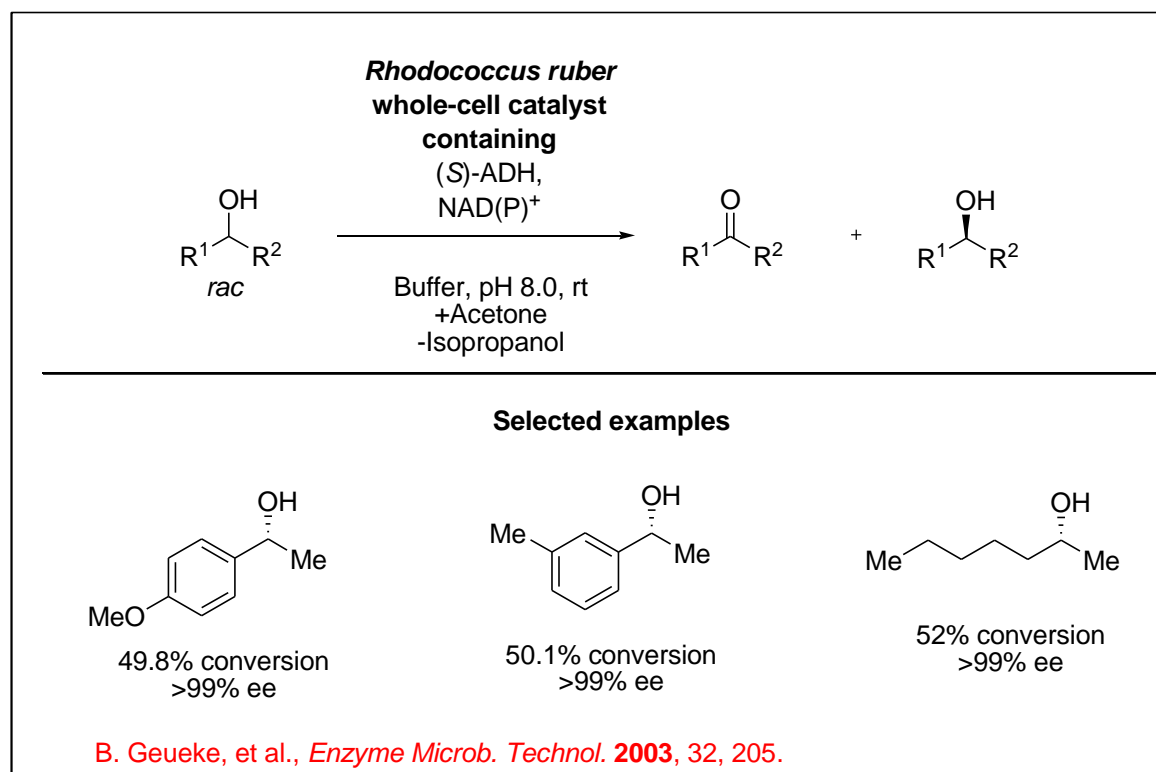

Scheme 4

11.5.3 Oxidation of Amino Acids

The asymmetric oxidation of amine group in amino acids provides effective method for the synthesis unnatural amino acid which is important in drug synthesis. For example, racemic *tert*-leucine can be oxidized to D-*tert*-leucine using a leucine amino dehydrogenase and an NADH-oxidase from *E-coli* with excellent enantioselectivity (Scheme 5).

Scheme 5

Rhodococcus ruber
**whole-cell catalyst
containing
(S)-ADH,
NAD(P)$^+$**

Buffer, pH 8.0, rt
+Acetone
-Isopropanol

Selected examples

49.8% conversion
>99% ee

50.1% conversion
>99% ee

52% conversion
>99% ee

B. Geueke, et al., *Enzyme Microb. Technol.* **2003**, 32, 205.

Scheme 6

11.5.4 Oxidation of Alcohols

The oxidation of secondary alcohols into ketones has also been investigated using biocatalytic systems. For example, the oxidation of racemic secondary alcohols proceeds in the presence of an ADH from *R. ruber* (Scheme 6). The recycling of the cofactor NADPH is carried out *in situ* using acetone, which is reduced into 2-propanol under the formation of NADP$^+$.

11.5.5 Sulfoxidation

Optically active sulfoxides play important role in organic synthesis as chiral auxiliary as well as intermediates for the construction of optically active molecules. Optically active sulfoxide is also present as structural unit in many biologically active compounds. The enzymatic oxidation of sulfides provides an effective method for the synthesis optically active sulfoxides. For example, cyclopentyl methyl sulfide undergoes oxidation in the presence of chloroperoxidase with excellent conversion and enantioselectivity.
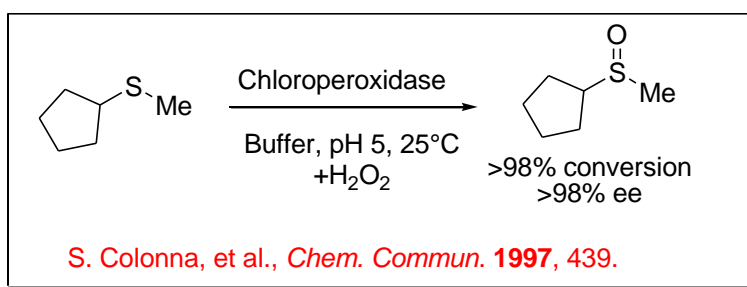
Scheme 7

Problems

E. Complete the following reactions.
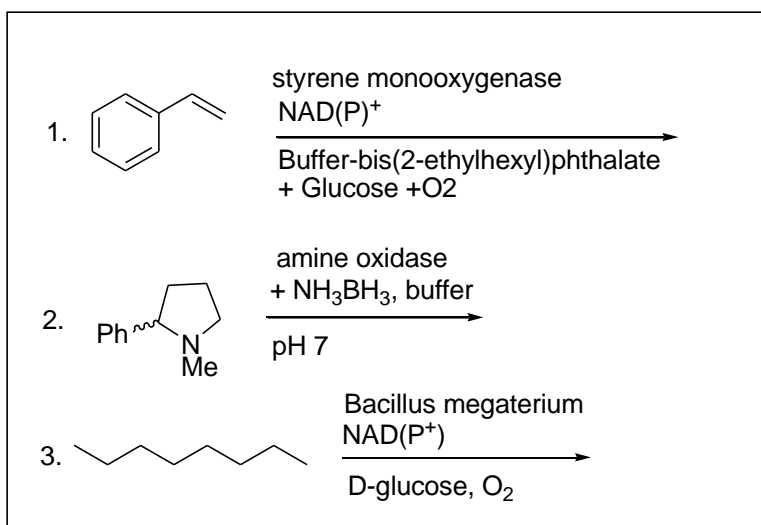
F.   Describe enzyme catalyzed hydroxylation of alkanes and oxidation of amines.

Reference/Text Book

7. I. Ojima, *Catalytic Asymmetric Synthesis*, 3rd ed., Wiley, New Jersey, 2010.

8. M. B. Smith, *Organic Synthesis*, 2nd edition, McGraw Hill, New Delhi, 2004.