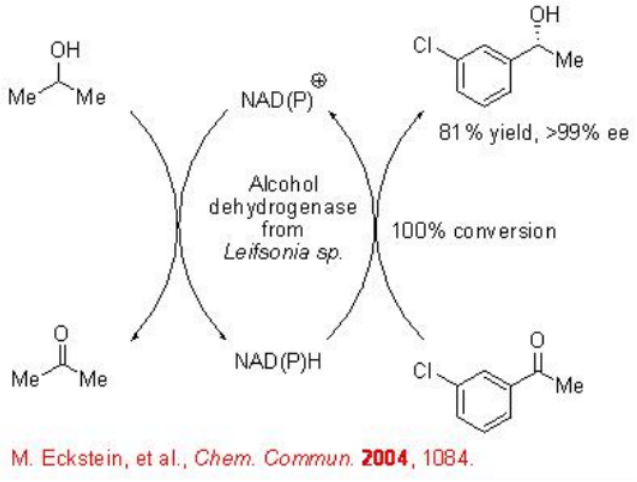
11.3: Reduction Reactions

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

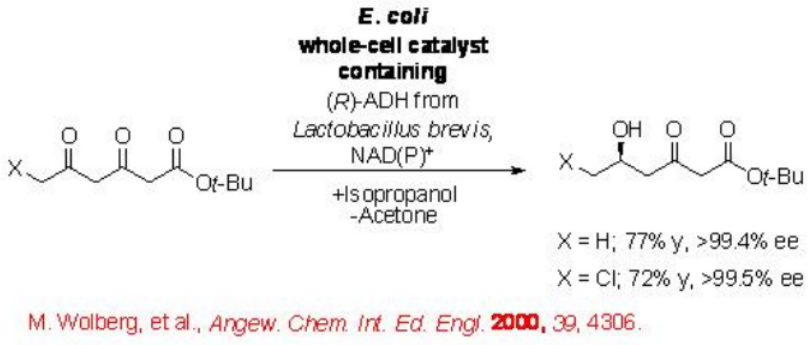
Reduction of Ketones

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 acetophenone to give secondary alcohols with high enantioselectivity (Scheme 11.3.1). In this process, 2-propanol acts as a reducing agent oxidizing into acetone.



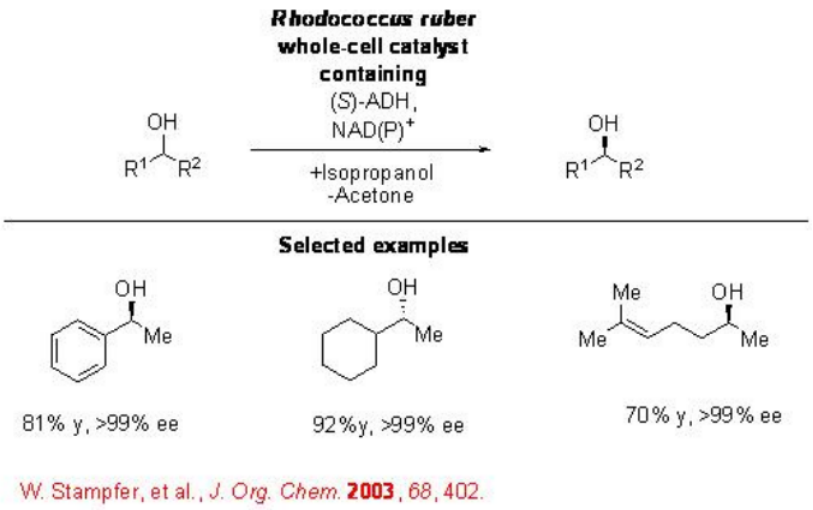
Scheme 11.3.1

The keto group of 2,5-diketo ester can be selectively reduced with excellent regio- and enantioselectivity using *E. coli* cells with overexpressed ADH from *Lactobacillus brevis* (Scheme 11.3.2). In this process 2-propanol acts as a reducing agent oxidizing into acetone.



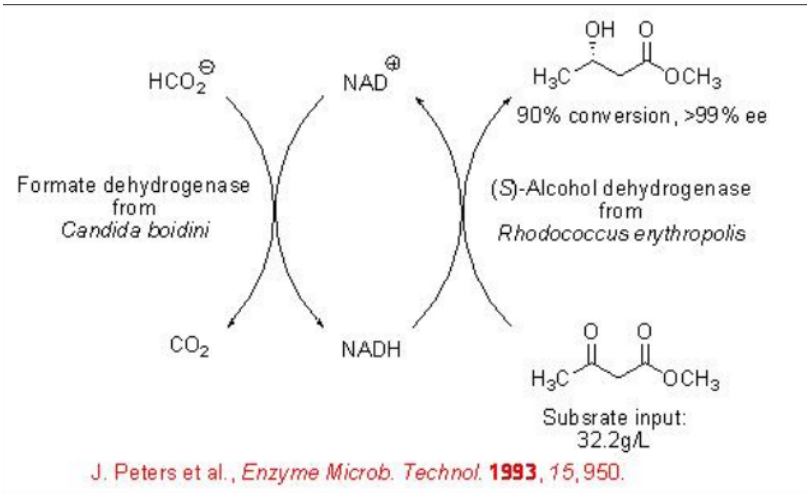
Scheme 11.3.2

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 enantioselectivity in 2-propanol (Scheme 11.3.7).



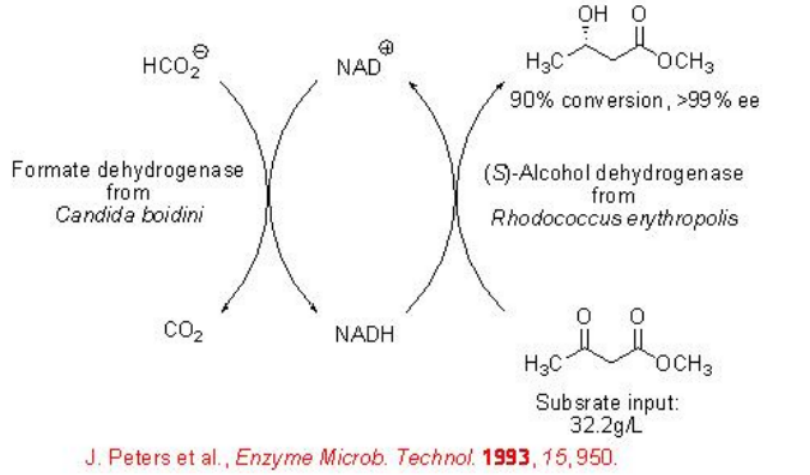
Scheme 11.3.3

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 11.3.4).



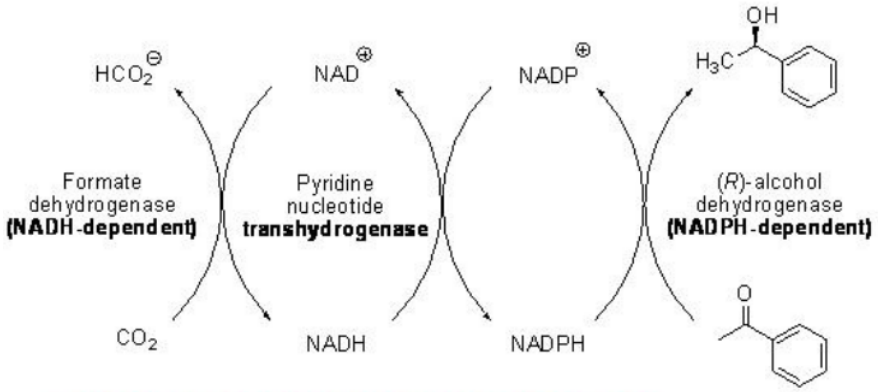
Scheme 11.3.4

The FDH-based whole-cell can be used for the reduction of ethyl 4-chloro-3-oxobutanoate with 99% ee (Scheme 11.3.5).



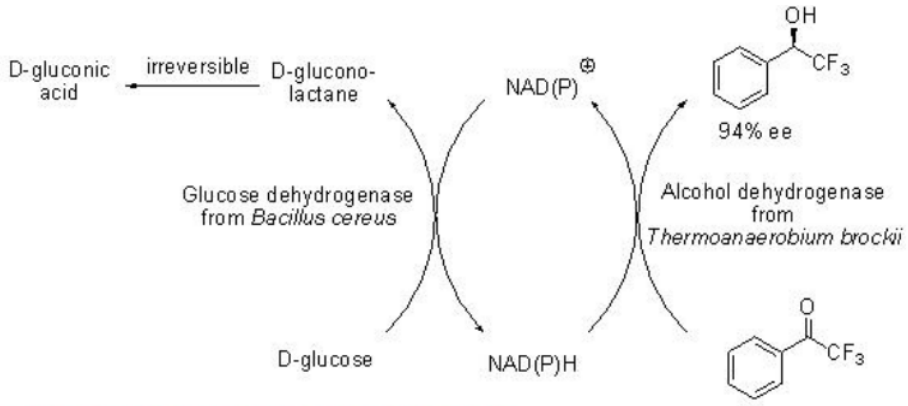
Scheme 11.3.5

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 11.3.6). 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 11.3.6

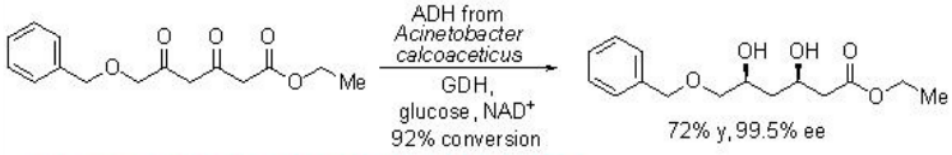


C.-H. Wong et al., *J. Am. Chem. Soc.* **1985**, *107*, 4028.

Scheme 11.3.7

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 11.3.7).

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 *Acinetobacter calcoaceticus* in combination with a GDH and glucose (Scheme 11.3.8).

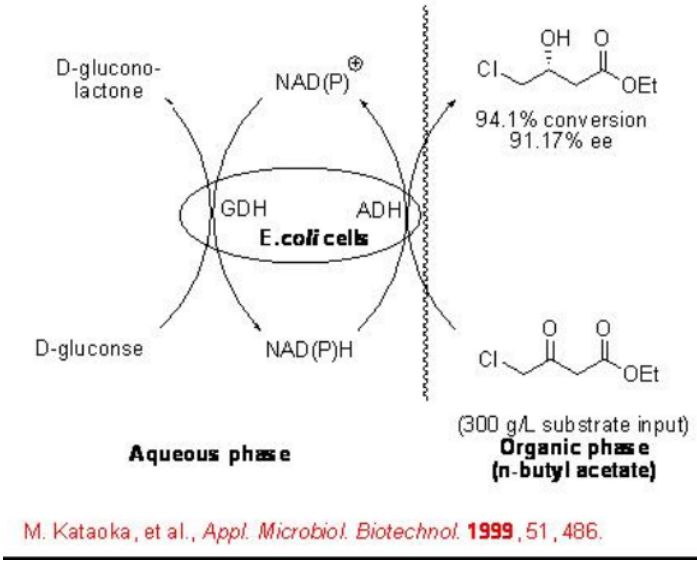


R. N. Patel., et al., *Enzyme Microb. Technol.* **1993**, *15*, 1014.

Scheme 11.3.8

Reduction of Ketones

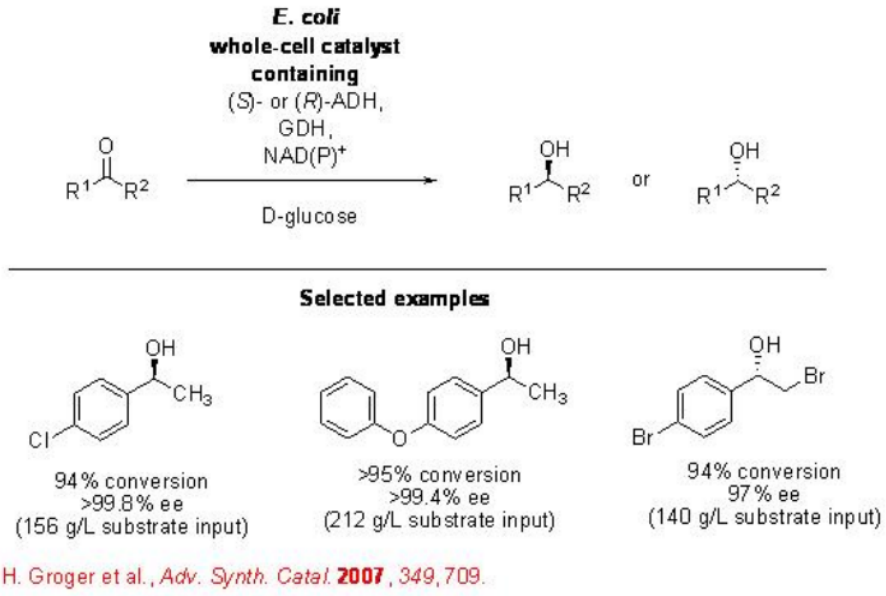
Recombinant whole-cell catalytic system having *E. coli* , co-expressing both the ADH from *S. salmonicolor* and the GDH from *B. megaterium* , has been developed for the asymmetric reduction of 4-chloro-3-oxobutanoate in a mixture of *n* -butyl acetate/water (Scheme 11.3.9). It is an elegant approach toward tailor-made biocatalysts containing both of the desired enzymes, ADH and GDH, in overexpressed form (Scheme 11.3.9).



M. Kataoka, et al., *Appl. Microbiol. Biotechnol.* **1999**, 51, 486.

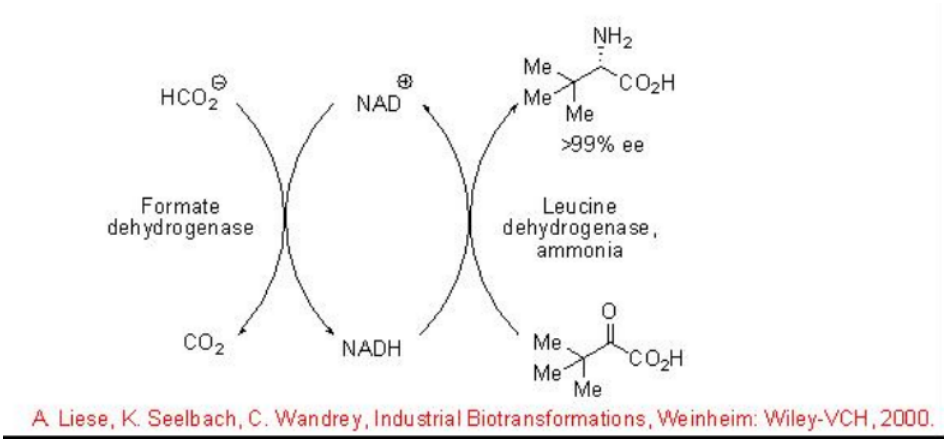
Scheme 11.3.9

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 11.3.10). This method is economical and simple, and finds applications for the reduction of a wide range of ketones (Scheme 11.3.10).



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

Scheme 11.3.10

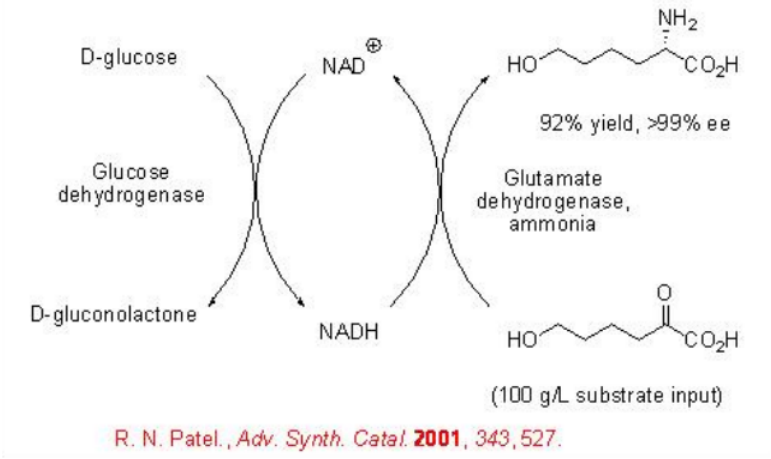


Scheme 11.3.11

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 11.3.11). 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 11.3.12). In this reaction, a beef liver glutamate dehydrogenase has been used as L-amino acid dehydrogenase and a GDH from *B. megaterium* has been used for the cofactor regeneration.

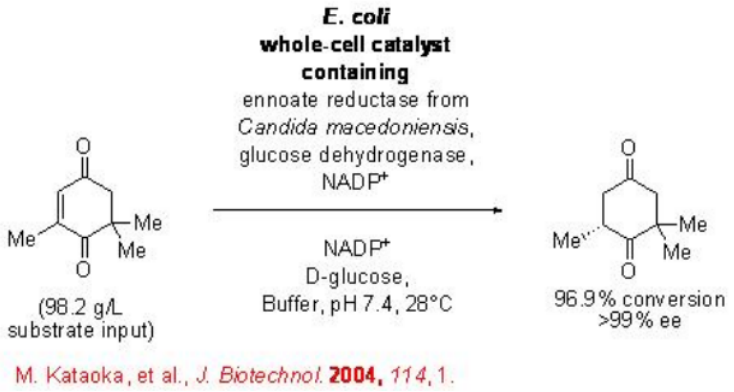


Scheme 11.3.12

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, *Pichia pastoris* cells having a phenylalanine dehydrogenase from *Thermoactinomyces intermedius* and an FDH from *P. pastoris* (Scheme 11.3.13).

Reduction of Activated Carbon-Carbon Double Bonds

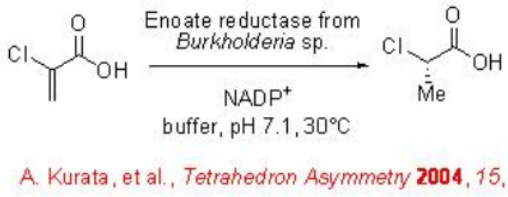
The reduction of carbon-carbon double bonds using the biocatalytic systems has high potential in organic chemistry. However, this process is less explored compared to the C=O reduction of ketones and keto esters. The reduction of the carbon-carbon double bond in ketoisophorone has been accomplished using whole-cell catalyst overexpressing an enolate reductase from *Candida macedoniensis* and a GDH (Scheme 11.3.13). This study can be regarded as one of the pioneering works in the reduction of carbon-carbon double bonds using biocatalytic systems.



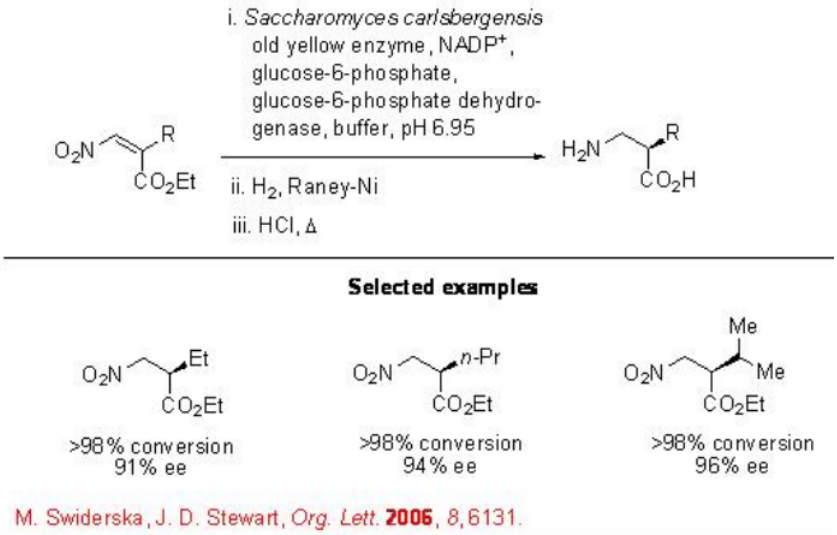
Scheme 11.3.13

α,β -Unsaturated carboxylic acids can also be used as substrates. For example, α -chloroacrylic acid can be converted into α -chloropropionate using an enolate reductase from *Burkholderia sp* ., in high enantioselectivity (Scheme 11.3.14).

Besides, enone and α,β -unsaturated carboxylic acid, nitroalkanes are also suitable substrates for enoate reductase. For example, the reduction of carbon-carbon double bond in *Z* -nitroalkenes proceed reaction to give 2-substituted 3-nitropropanoates with high conversion and in most cases with high enantioselectivity (Scheme 11.3.15).



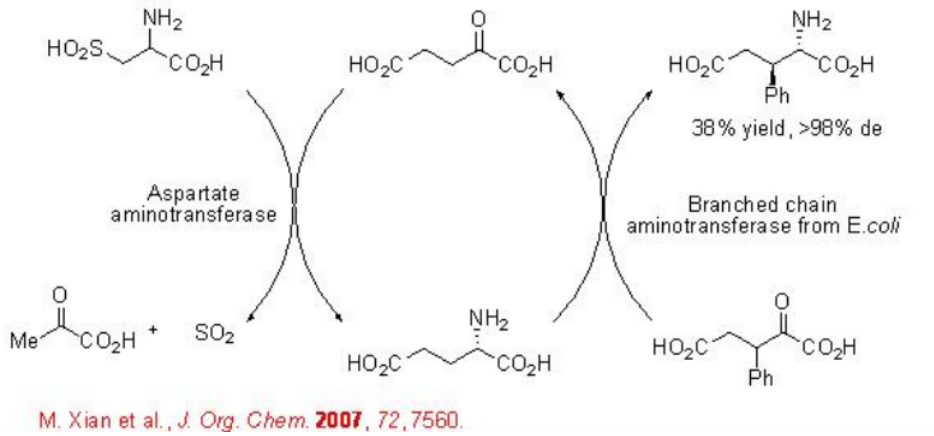
Scheme 11.3.14



Scheme 11.3.15

Transamination11.3.1

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 11.3.16).



Scheme 11.3.16

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 11.3.17).

Scheme 11.3.17