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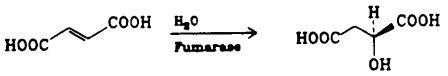
4.9.5. L-Hydroxycarboxylic Acids

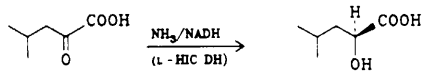
L-Malic Acid from Fumaric Acid. The reversible hydration of fumaric acid to yield L-malic acid [*97-67-6*] is catalyzed by fumarate hydratase (E.C. 4.2.1.2) [*9032-88-6*], also called fumarase [4.304]. The reaction can be performed with isolated enzyme or with cell cultures, e.g., immobilized cells of *Brevibacterium ammoniagenes* [4.303].



L-Malic acid

Fungal cells of various *Aspergillus* species produce concentrations of up to 400 g/L of L-malic acid [4.351].

L-α-Hydroxycarboxylic Acids from α-Oxo Acids. α-Oxo acids can be converted to α-hydroxycarboxylic acids in a pure enantiomeric form by the action of specific dehydrogenases and NADH. In a continuous membrane reactor, regeneration of cofactor in the presence of formate and formate dehydrogenase takes place at the same time [4.352] as the production of hydroxycarboxylic acids.



γ-Oxoisocaproic acid

L-α-Hydroxyisocaproic acid

Examples are the enzymatic production of L-α-hydroxyisocaproic acid [*13748-90-8*] by using L-α-hydroxyisocaproate dehydrogenase (L-HIC DH) [4.353] from *Lactobacillus confusus*. D-α-Hydroxycarboxylic acids are prepared by the action of D-lactate dehydrogenase [4.354] or D-α-hydroxyisocaproate dehydrogenase (D-HIC DH) from *L. casei* [4.355].

4.10. Enzymes in Organic Synthesis

Enzymes are proteins that have catalytic activity. Their potential value as catalysts in organic chemistry has been recognized for many years. Currently more than 2000 enzymes are known [4.356] and several hundred are commercially available. Many others can be obtained through well-developed procedures [4.357]. Enzymes can, in principle, be produced economically in large quantities by recombinant DNA techniques [4.358]. Many of the technical problems that have slowed commercial development of enzyme-catalyzed synthesis — for example, cofactor regeneration, enyzme immobilization, and enzyme stabilization — have been solved. Difficulties in the synthesis of complex biologically important substances represent an increasing problem in many areas of chemistry and pharmacology. For these and other reasons, enzyme-based synthetic chemistry has grown rapidly in recent years [4.359]–[4.364]. This section emphasizes the use of individual enzymes to catalyze reactions useful in synthetic organic chemistry.

4.10.1. General Considerations

Enzymes exhibit three characteristic catalytic activities: (1) remarkable acceleration of reaction rates; (2) highly selective mode of action; and (3) susceptibility to regulation by substrates, products, or other species present in solution. *Selectivity* is the most useful of these characteristics. Regulation is most often a nuisance, because it can result in an inhibition of catalytic activity by products.

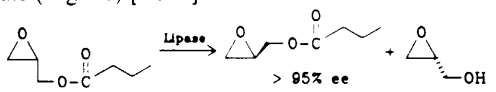
In considering the application of enzymes in organic synthesis, the availability, specific activity, stability, and lifetime of individual enzymes and their accessory cofactors must be considered. Enzymes need not be particularly pure for most applications as catalysts. Enzymes are normally used in aqueous solutions, but some (lipases) require aqueous–organic interfaces for activity, and many tolerate modest concentrations of organic cosolvents. To enhance their stability and allow their recovery from reaction mixtures, enzymes are often used in immobilized form (see Section 3.3). Of the numerous immobilization methods developed [4.365]–[4.367], glutaraldehyde is the most common in industrial applications [4.365], [4.367]. For laboratory-scale syntheses, covalent attachment of the enzyme to a cross-linked polyacrylamide-co-*N*-acryloxysuccinimide (PAN) polymer is the most general immobilization technique [4.368]. During immobilization, the active site of the enzyme is usually protected by adding a substrate or an inhibitor. Addition of thiols and use of an inert atmosphere during manipulation of enzymes prevent their oxidative inactivation [4.368].

4.10.2. Enzymes Not Requiring Coenzymes

Many enzymes do not require cofactors; these enzymes are readily available, inexpensive, stable, and simple to use. They represent the group most widely used industrially in large-scale applications [4.365], [4.367], [4.369] and probably the first group of biocatalysts that will become part of the standard repertoire of organic chemists.

4.10.2.1. Esterases, Lipases, and Amidases

Esterases, lipases, and amidases are widely used in kinetic resolutions of racemic mixtures [4.359]–[4.364]. For example, certain epoxyesters can be resolved with hog pancreatic lipase (E.C. 3.1.1.3) [*9001-62-1*] [4.370]; this procedure provides an alternative to the asymmetric epoxidation of allylic alcohols by using transition metals (Fig. 40) [4.371].



44 45 46

Figure 40. Enantioselective hydrolysis of glycidyl butyrate catalyzed by hog pancreatic lipase

Pig liver esterase (PLE) (E.C. 3.1.1.1) [*9016-18-6*] has been applied in an asymmetric synthesis of chrysanthemic [*10453-89-1*], permethrinic [*55701-05-8*], and caronic [*497-42-7*] acids from the corresponding racemic methyl esters [4.372].

SIH et al. have developed a valuable theoretical treatment of such enzymatic transformations, relating the extent of conversion of racemic substrate to enantiomeric excess of the product and enantiomeric selectivity of the enzyme [4.373].

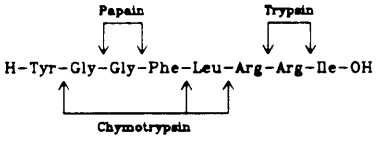
The enzyme-catalyzed asymmetric hydrolysis of meso diesters offers a particularly useful approach to chiral synthons. Synthesis of the antiviral agent showdomycin (**49**), [*16755-07-0*] provides an example (Fig. 41) [4.374]. (+)-Biotin [*58-85-5*] has been prepared from an imidazolone by a similar approach [4.375].

Lipases are especially useful catalysts, because they operate at the water—organic interface [4.376] and can be applied to water—insoluble substrates. Many are inexpensive and stable, and show broad substrate specificity [4.376].

In addition to hydrolysis, esterase- and lipase-catalyzed transesterification has been investigated [4.377], [4.378], and enantiomeric excesses up to 95% have been obtained.

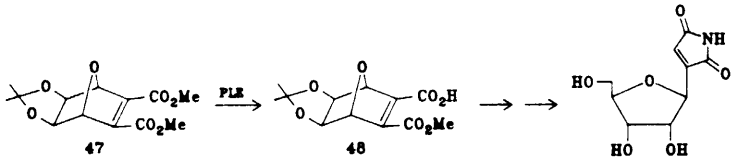
Amidases have been used primarily to hydrolyze *N*-acylamino acids [4.365], [4.379]. Chymotrypsin (E.C. 3.4.21.1) [*9004-07-3*] and acylase (E.C. 3.5.1.14) [*9012-37-7*] are used in the kinetic resolution of amino acids [4.365], [4.379]. A synthetic route to semisynthetic penicillins is based on the production of 6-aminopenicillanic acid [*551-16-6*] with penicillinase (E.C. 3.5.2.6) [*9001-74-5*] [4.380]. Transacylations catalyzed by proteases have found impressive applications in the synthesis of the penicillin derivatives ampicillin [*69-53-4*], and amoxicillin [*26787-78-0*] [4.381], and of the cephalosporin cephalexin [*15686-71-2*] [4.382].

Amidases also catalyze the formation of bonds in polypeptides and proteins [4.383]–[4.386]: the conversion of porcine to human insulin by a trypsin-catalyzed reaction of porcine insulin with threonine methyl ester [4.384], the thermolysin-catalyzed synthesis of an aspartame precursor [4.385], and the total synthesis of oligopeptides such as dynorphin (**50**) [4.383], [4.386] (Fig. 42) are examples. To effect dehydration, experimental conditions must be carefully optimized. Its sensitivity to conditions limits the general utility of this method to cases in which the demand for the product justifies the effort.



50: Dynorphin

Figure 42. Enzymatic synthesis of dynorphin



47 48 **49**: Showdomycin

Figure 41. Enatioselective hydrolysis of a mesodiester catalyzed by pig liver esterase (PLE)

4.10.2.2. Aldolases

Aldolases catalyze the cleavage and formation of carbon–carbon bonds in certain carbohydrates [4.387]. Fructose 1,6-diphosphate aldolase (E.C. 4.1.2.13) [*9024-52-6*] from rabbit muscle condenses dihydroxyacetone phosphate [*57-04-5*] (DHAP; **52**) with a variety of aliphatic, heterosubstituted, and differentially protected aldehydes (Fig. 43; **51**) [4.388], [4.389]. This aldolase has been used in the synthesis of rare, nonnatural, and isotopically labeled carbohydrates [4.388], [4.389]. *N*-Acetylneuraminic acid aldolase (E.C. 4.1.3.3) [*9027-60-5*] has been used to prepare *N*-acetylneuraminic acid [*131-48-6*] [4.390].

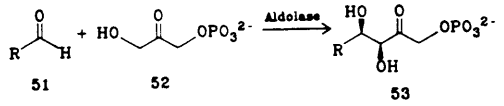


Figure 43. Stereospecific aldol addition catalyzed by fructose 1,6-diphosphate aldolase

4.10.2.3. Lyases, Hydrolases, and Isomerases

Lyases, hydrolases, and isomerases have found broad application in industrial chemistry, e.g., in the conversion of starch to glucose catalyzed by α-amylase (E.C. 3.2.1.1) [*9000-90-2*] and glucamylase (E.C. 3.2.1.3) [*9032-08-0*] (Section 4.2) [4.391], the isomerization of glucose to fructose by glucose isomerase (E.C. 5.3.1.18) [*9055-00-9*] (Section 4.3) [4.392], and the production of aspartic acid (with aspartase (E.C. 4.3.1.1) [*9027-30-9*] [4.365], [4.379]) and of malic acid from fumaric acid (with fumarase (E.C. 4.2.1.2) [*9032-88-6*] [4.365]) (Section 4.9). These enzymes also are used in laboratory synthesis on nonnatural substrates. Galactosidase (α-galactosidase (E.C. 3.2.1.22) [*9025-35-8*]; β-galactosidase (E.C. 3.2.1.23) [*9031-11-2*]), like other glycosidases, has been used in the synthesis of glycosides [4.393]. Epoxide hydrolases can be used to open epoxides regiospecifically [4.394].

4.10.3. Enzymes Requiring Coenzymes, but Not Cofactor Regeneration Systems

In many enzymatic systems, required cofactors bind tightly to their respective enzymes and regenerate automatically during the course of the enzyme-mediated reaction: the most important enzyme–cofactor systems exhibiting this type of behavior are those utilizing flavins, pyridoxal phosphate, thiamine pyrophosphate, lipoamide, and certain metal ions as cofactors. The pyridoxal phosphate-containing transaminases have been used for amino acid synthesis by amine transfer from glutamic or aspartic acid to a 2-oxo acid (Section 4.9) [4.395]. The iron-dependent enzyme horseradish peroxidase (E.C. 1.11.1.7) [*9003-99-0*] catalyzes the selective hydroxylation of organic compounds [4.396], and L-DOPA has been prepared this way. ε-Caprolactam [*105-60-2*] has been synthesized from cyclohexanone by using a monooxygenase-catalyzed Baeyer–Villiger oxidation [4.397].

4.10.4. Enyzmes Requiring Added Coenzymes

Approximately 70% of all enzymes require nucleoside triphosphates, nicotinamide derivatives, or coenzyme A as cofactors [4.356]. These coenzymes are too expensive to be used stoichiometrically, and methods for their in situ regeneration are now available.

4.10.4.1. Enyzmes Requiring Nucleoside Triphosphates

Regeneration of nucleoside triphosphates from the corresponding diphosphates can be achieved by using readily available phosphate donors such as acetyl phosphate [*590-54-5*] [4.398] and phosphoenolpyruvate (**57**) [*73-89-2*]

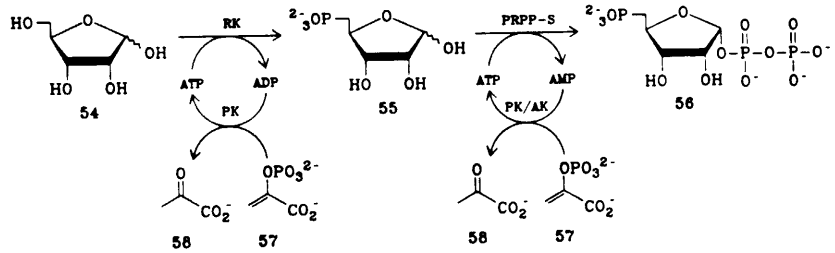


Figure 44. Enzyme-catalyzed synthesis of phosphoribosyl pyrophosphate (PRPP)
PK = pyruvate kinase; AK = adenylate kinase; RK = ribokinase; PRPP-S = PRPP synthase

[4.399]: acetate kinase (E.C. 2.7.2.1) [*9027-42-3*] and pyruvate kinase (E.C. 2.7.1.40) [*9001-59-6*] will accept GTP, UTP, and CTP, as well as the deoxynucleoside triphosphates dATP, dGTP, dUTP, and dCTP [4.398], [4.400]. Adenylate kinase (E.C. 2.7.4.3) [*9013-02-9*] catalyzes the conversion of AMP to ADP [4.401]; unfortunately, this kinase is specific for AMP and does not accept XDP (X = U, G, C). No truly practical regeneration scheme for conversion of these monophosphates to triphosphates is available.

The ATP regeneration schemes have been applied in the synthesis of sugar phosphates [4.402], dihydroxyacetone phosphate [4.388], and *sn*-glycerol 3-phosphate [4.403]. Syntheses of NAD [4.404], ribulose 1,5-diphosphate [4.405], and phosphoribosyl pyrophosphate (PRPP) (Fig. 44; **56**) [4.406], a key intermediate in the biosynthesis of nucleotides, represent more complex examples.

4.10.4.2. Enzymes Requiring Nicotinamide Coenzymes

In contrast to the nucleoside triphosphates, regeneration of nicotinamide cofactors is more difficult, both because these compounds are more expensive and because they are intrinsically unstable in solution [4.364]. For reactions in which NAD(P) is used as an oxidant, product inhibition may present a severe problem [4.407]. For regeneration of NADH from NAD, three systems are practical:

1) Formate–formate dehydrogenase (E.C. 1.2.1.2) [*9028-85-7*] is very efficient, and the only byproduct formed during the reaction is carbon dioxide [4.408], [4.409].
2) Glucose–glucose dehydrogenase (E.C. 1.1.1.47) [*9028-53-9*] is also attractive as a hydride-donating system, although the work-up of reaction mixtures in this system is more complicated [4.410].
3) The same problem arises in the use of glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49) [*9001-40-5*] and glucose 6-phosphate [4.411].

The formate-utilizing enzyme is applicable only to the regeneration of NADH, but carbohydrate-based reductions regenerate both NADH and NADPH. Reductions mediated by NADH have been successfully applied to asymmetric reduction of ketones. Horse liver alcohol

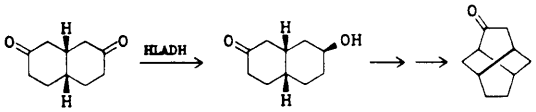
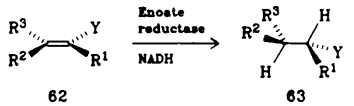


Figure 45. Stereoselective reduction of a ketone with horse liver alcohol dehydrogenase (HLADH)



R^1 = H, Me, Et, OCH_3, F, Cl, Br, NHCHO
R^2 = alkyl, aryl, alkyloxycarbonyl
R^3 = H, Me, Et
Y = CO_2, CHO

Figure 46. Stereoselective reduction of olefins catalyzed by enoate reductase

dehydrogenase (HLADH) (E.C. 1.1.1.1) [*9031-72-5*] accepts a variety of substrates [4.412]. For instance, it reduces *cis*-decaline-2,7-dione (**59**) stereospecifically; the product can be converted to (+)-(4*R*)-twistanone [*13537-95-6*] (Fig. 45; **61**) [4.413]. JONES and co-workers have devised a convenient model that allows assessment of the success of an HLADH-catalyzed reduction [4.414]. Lactate dehydrogenase (L-LDH (E.C. 1.1.1.27) [*9001-60-9*]; D-LDH (E.C. 1.1.1.28) [*9028-36-8*]) is another important catalyst. It reduces α-oxo acids enantiospecifically to α-hydroxy acids [4.415]. Both the D- and the L-selective enzymes are available, and both enantiomers of many α-hydroxy acids can be generated (Section 4.9). Enoate reductase (E.C. 1.3.1.31) reduces α,β-unsaturated carbonyl compounds of type **62** to the saturated derivatives **63** and, thereby, introduces two chiral centers at the same time (Fig. 46) [4.416].

In situ regeneration of NAD has been achieved in most cases with the non-enzyme-catalyzed reoxidation of NADH by oxidized flavin [4.417]. This procedure has been used successfully in preparing chiral lactones from meso diols; a synthesis of grandisol [*26532-22-9*] (**66**) provides an example (Fig. 47) [4.418]. The disadvantages of this system are the large amount of flavin needed, the slow reaction rate, and the requirement for exposure of the enzymes to dioxygen. Probably the best of the proposed alternatives is based on the conversion of ketoglutarate and ammonia to glutamic acid through oxidation of NADH to NAD [4.407].

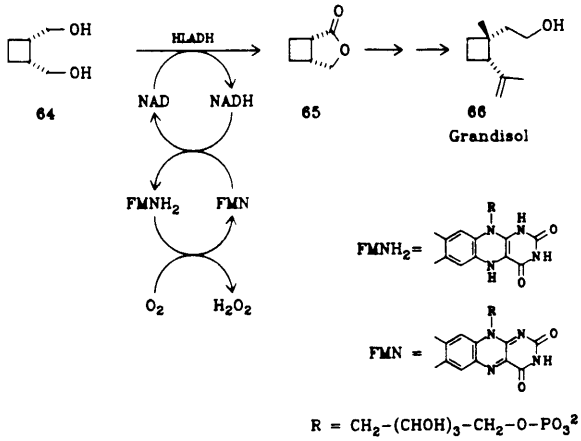


Figure 47. Horse liver alcohol dehydrogenase (HLADH) catalyzed stereoselective oxidation of a meso diol with in situ NAD regeneration.

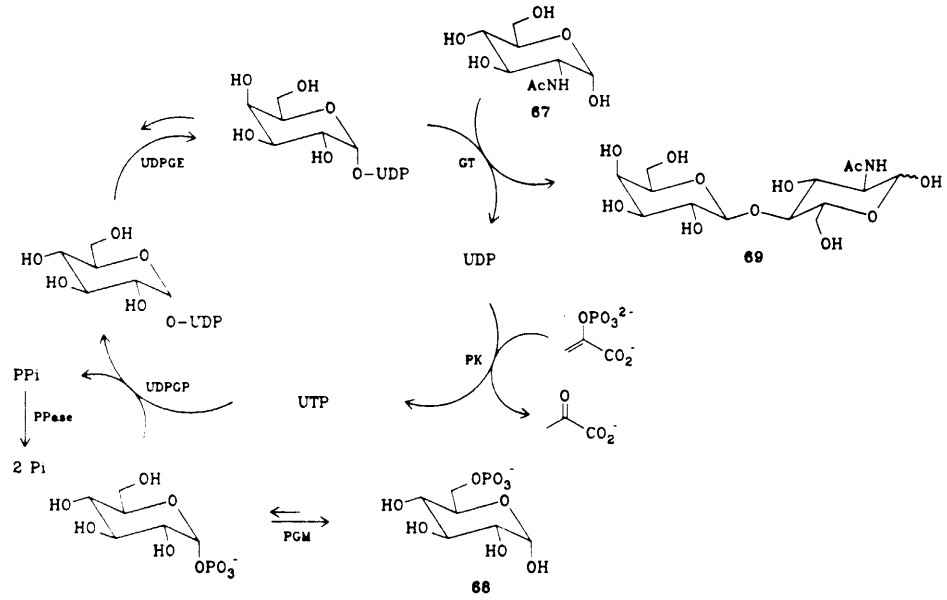


Figure 48. Enzymatic synthesis of lactosamine
GT = galactosyltransferase; PK = pyruvate kinase; PGM = phosphoglucomutase; UDPGP = UDP-glucose pyrophosphorylase; UDPGE = UDP-glucose epimerase; PPase = inorganic pyrophosphatase

4.10.4.3. Enzymes Requiring Other Cofactors

Currently no straightforward method exists for the synthesis and regeneration of 3′-phosphoadenosine 5′-phosphosulfate [*482-67-7*] (PAPS), an important compound for biochemical sulfations [4.419]. *S*-Adenosylmethionine [*485-80-3*] (SAM), a methyl donor in enzyme-catalyzed reactions, can be synthesized from ATP and methionine [4.420], but the SAM synthetase required is difficult to obtain, and no strategy for SAM regeneration has been proposed. Strategies for regeneration of acetyl-CoA are still in the stage of early development.

4.10.5. Synthesis with Multienzyme Systems

A special feature of enzymatic syntheses is the ability to assemble complex systems of cooperating enzymes and carry out a multistep synthesis in a single reaction vessel. Because of their selectivity, enzymes will accept only the desired substrates. Removal of impurities and byproducts may be less important in enzyme-catalyzed syntheses than in classical multistep transformations.

Some examples of such complex systems include the formation of ribulose 1,5-diphosphate [*2002-28-0*], an important substrate in plant biochemistry, from glucose [4.421], and the construction of lactosamine (**69**), a core disaccharide common in glycoprotein glycans, from glucose 6-phosphate (**68**) and *N*-acetylglucosamine (**67**) (Fig. 48) [4.422]. This latter type of procedure has been extended to oligosaccharides [4.405].

Even more difficult enzyme systems are involved in oxygen-incorporating transformations. These enzymes, especially those of cytochrome P450, can carry out very useful reactions, e.g., selective epoxidation of olefins and functionalization of unactivated hydrocarbons [4.423], [4.424]. These enzymes are in many cases membrane-bound, and they are difficult to obtain and handle. Large-scale applications of these purified enzymes are unlikely to become practical in the near future.

4.10.6 Outlook

Enzymatic methods will be used increasingly in research and industry for the preparation of chiral compounds. In addition, they will be usefull in the synthesis of complex molecules needed in immunology, endocrinology, intermediary metabolism, molecular genetics, and plant or insect biology. Water-soluble synthetic targets such as carbohydrates and nucleic acids are now commonly manipulated with enzymes. Enzymology complements both classical synthetic chemistry and biological synthetic techniques. Recombinant DNA and RNA techniques are developing rapidly, and with their development, the design and engineering of synthetic catalysts may become feasible [4.358]. Enyzmes will also be widely used in other synthetic applications [4.425] and will continue to find use in isotopic labeling, analysis, and waste treatment.

5. Enzymes in Analysis and Medicine

5.1. Survey

5.1.1. Enzymes in Clinical Diagnosis and Food Analysis

Enzymes can be used as chemical reagents to (1) determine the concentration of substrates, (2) measure the catalytic activity of enzymes present in biological samples, and (3) serve as labels in enzyme immunoassays to determine the concentrations of enzymatically inert substances.

Within the framework of these topics, enzymes serve the following functions:

1) *Analyte Recognition and Transformation.* This is based on the high degree of specificity of most enzymes and is the major advantage of the analytical application of enzymes.
2) *Signal Generation.* In favorable cases of substrate determination, the signal is produced by the primary enzyme reacting with the analyte itself; however, in many systems, the signal must be generated by coupling with an indicator enzyme. In homogeneous as well as heterogeneous immunoassays, the marker enzyme serves to generate a detectable physical signal in response to the antigen–antibody reaction.
3) *Removal of Interferents.* Substances infering with the assay may selectively be converted by specific enzymes. Examples are the conversion of pyruvate by lactate dehydrogenase in the assay of aspartate aminotransferase via coupling to malate dehydrogenase, and of ascorbic acid by ascorbate oxidase in hydrogen peroxide-dependent assays.

The principles and methods of enzymatic analysis are described in detail in [5.1]; a short survey of enzymes as reagents in clinical chemistry is given in [5.2].

The following quality criteria are relevant in the choice of an enzyme to be used for analytical application in clinical diagnosis or food analysis.

Specificity. Generally, enzymes are more specific than other chemical reagents [5.3]. However, side activities are often present; unlike contaminating activities, these cannot be removed by purification and may prohibit the use of an enzyme for a particular purpose.