5.1 Reduction of Aldehydes and Ketones

Hugo Bertschy, George M. Whitesides

Department of Chemistry, Harvard University, Cambridge, MA 02138

H. Keith Chenault

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

5.1.1 General Remarks

Enzyme catalyzed reductions of carbonyl groups have found important applications in the synthesis of chiral compounds. Today, hydroxy acids, amino acids and alcohols [1-3] are utilized in the food industry and serve as chiral building blocks for the synthesis of pharmaceuticals, flavors and agrochemicals.

Dehydrogenases, classified under EC 1.1., are enzymes that catalyze reduction and oxidation of carbonyl groups and alcohols, respectively [4]. In vivo they are involved in many metabolic pathways and in energy conversion. A large number of dehydrogenases require nicotinamide cofactors as their cosubstrate; others require cofactors such as flavins [5] and pyrroloquinoline quinone (PQQ) [4,6]. The dehydrogenases most commonly used to catalyze preparative-scale reductions of aldehydes and ketones are NAD(P)H dependent.

Dehydrogenase-catalyzed transformations on a practical scale can be performed with purified enzymes or with whole cells. Bakers' yeast is the whole cell system most often used for the reduction of aldehydes and ketones; yeast-catalyzed reduction is the simplest biotransformation for an organic chemist to perform on a lab scale. Unfortunately, processes involving whole cell systems can be complex, since they contain multiple enzymes that may compete for the same substrate. The activities of these oxidoreductases depend on the structure of the substrate and the metabolic state of the cells;

often poor selectivities are obtained. Some applications of yeast as reducing agent and catalyst are discussed in chapter B 5.1.3. The problem of low selectivity can be avoided when isolated enzymes are used; these higher selectivities and simpler product isolations must, however, be paid for with a higher initial effort. The required enzymes have to be purchased or purified and cofactor regeneration systems have to be developed.

The stereospecific enzyme catalyzed reduction of ketones and aldehydes has a mayor advantage over the use of hydrolytic enzymes: by exploiting the prochirality of carbonyl groups, the syntheses of racemic intermediates can be avoided (compare chapter B 1). Today, virtually every aldehyde or ketone can be reduced either enzymatically or microbially [7]. Modern screening methods and genetic engineering techniques are rapidly leading to new enzymes that exhibit broad substrate specificity and high stereoselectivity [8,9]. Compounds such as α,β-unsaturated ketones [10] and alkyne ketones [8] can now be reduced enzymatically with high optical yield. In this chapter we give a comprehensive overview of the most important enzyme catalyzed reductions of aldehydes and ketones now known.

5.1.2 Reductions with Isolated Enzymes

5.1.2.1 Regeneration of Reduced Nicotinamide Cofactors: NAD(P)H from NAD(P)

During the reduction of a carbonyl compound, the reduced nicotinamide cofactor, NAD(P)H, suffers loss of a hydride and emerges from the reaction in oxidized form. Thus the reduced cofactor must either be used stoichiometrically or be regenerated by in situ reduction of NAD(P). The high cost of nicotinamide cofactors, especially in their reduced forms, necessitates that they be regenerated in situ.

During synthetic fermentations, cellular organisms regenerate nicotinamide cofactors as part of their normal metabolism. In processes using isolated enzymes, however, a nicotinamide cofactor must be regenerated explicitly by a second reaction, which is separate from the "product-forming" reaction.

Although one can refer to a "cofactor-regenerating" reaction in isolation, one must remember that cofactor regeneration does not operate in isolation. The evaluation of a particular method of cofactor regeneration must take into account the synthetic system to which the regenerative system is coupled.

Many methods for regenerating nicotinamide cofactors exist. These methods have been reviewed recently [11,12]. The useful methods regenerate enzymatically active cofactor at rates that allow convenient rates of product formation. Economic considerations require that total turnover numbers[1] for NAD(P)H be 10^3-10^6, and so the regenerating reaction must be 99.93-99.99993% efficient for the formation of enzymatically active cofactor. Because only the 1,4-dihydropyridine form of NAD(P)H is enzymatically active, methods for regenerating NAD(P)H from NAD(P) must be highly regioselective in their reduction of the pyridinium ring. At present, only enzymatic methods possess the regioselectivity and kinetic properties necessary to achieve total turnover numbers of $\geq$500 at convenient rates.

In addition, useful methods for cofactor regeneration share several other common features. The reagents and equipment required are readily available, inexpensive, easily manipulated, stable under operating conditions, and compatible with the various other components of enzyme reactors. They allow convenient monitoring of the progress of the synthetic reactions and do not interfere with product isolation or purification.

((figure 1))

Enzymatic methods for regenerating nicotinamide cofactors fall into three categories (figure 1). The simplest, which is often used for reactions catalyzed by alcohol dehydrogenases, is the coupled-substrate approach. In this strategy, one enzyme serves as both the synthetic and regenerating enzyme.

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[1]Total turnover number = moles of product formed/moles of cofactor in the reaction

A substrate other than the synthetic substrate is present, typically in excess, and is oxidized in order to regenerate NAD(P)H (figure 1a). The second strategy is the coupled-enzyme approach, sometimes called the substrate-coupled approach [3,13][2]. It is specific for nicotinamide-dependent reactions run in series and resulting in no net oxidation or reduction of the substrate (figure 1b). The oxidized product and NAD(P)H formed by the first enzymatic step serve as substrates for a second enzymatic reaction. An example of this strategy is the two-step conversion of α-hydroxy carboxylic acids to α-amino acids [14-16] (compare chapter B 5.3). The third strategy for regenerating nicotinamide cofactors couples an auxiliary enzymatic reaction, complete with secondary enzyme and substrate, to the synthetic reaction (figure 1c). This coupled-system approach is the most general. It has as potential disadvantages the added cost of the secondary enzyme and substrate and the possible complication of product isolation by the product of the regenerative reaction. In specific cases, however, the products of both the "synthetic" and "regenerative" reactions may be desirable and readily separable [17,18].

Enzymatic methods for the regeneration of NADH from NAD are well developed. Several excellent coupled-system methods exist from which to choose. In contrast, methods to regenerate NADPH from NADP are somewhat less developed. A need still exists for a general method that is convenient and inexpensive enough for multikilogram-scale regeneration of NADPH from NADP.

5.1.2.1.1 Formate/Formate Dehydrogenase

The formate dehydrogenase (FDH, EC 1.2.1.2) catalyzed oxidation of formic acid to carbon dioxide is the most highly developed enzymatic method for regenerating NADH from NAD [19-21].

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[2]We prefer the designation coupled-enzyme since an enzyme is the additional component coupled to the "synthetic" system in order to regenerate cofactor. Also, this designation avoids confusion with the coupled-substrate terminology.

The method has been applied in the enzymatic reduction of carbonyl compounds to alcohols [22-26] and amines [27-29]. It has been developed commercially for the enzymatic production of L-tert-leucine (L-α-t-butylglycine) [15] and is currently the only method of regenerating a nicotinamide cofactor that operates on a commercial scale. FDH and deuterioformate have been used to regenerate NADH-4-d for the stereoselective preparation of deuterated compounds [30,31]. FDH accepts soluble poly(ethylene glycol)-NAD(H) conjugates as cofactors and is thus suitable for NADH regeneration in continuous-flow membrane reactors [21,25].

Formate/FDH is the most broadly applicable and has produced total turnover numbers for NADH of up to 6 x 10^5 [27]. Formate is inexpensive, stable, innocuous to enzymes, and strongly reducing (E'_o = -0.42 V). At neutral pH, it is a poor general acid catalyst of the decomposition of NAD(P)H [32]. The by-product of the reaction, carbon dioxide, is also innocuous to enzymes and does not complicate product isolation. Although the initial cost of FDH is still higher than that of some other enzymes used to regenerate NADH, it is stable under synthetic operating conditions [19,21,28] and can be reused or used for extended periods of time. The low specific activity of FDH (3 U mg^{-1}) does not appear to reduce the real space-time yields of reactors [28].

Unfortunately, formate/FDH cannot be used for the direct regeneration of NADPH. FDH does not accept NADP as a substrate. Regeneration of NADPH may be possible, however, by using catalytic quantities of NAD and NADP and NAD(P) transhydrogenase (EC 1.6.1.1).

5.1.2.1.2 Glucose/Glucose Dehydrogenase

Glucose dehydrogenase (GDH; EC 1.1.1.47) catalyzes the oxidation of glucose to gluconolactone. The spontaneous hydrolysis of the initial product to form gluconate (figure 2) makes ((figure 2))

the overall reaction strongly exothermic (E'_o = -0.47 V) and thus favorable for the regeneration of NAD(P)H. The reductant, glucose, is readily available, inexpensive, and stable. Glucose is innocuous

to nicotinamide cofactors and actually increases the stability of some enzymes in solution. The by-product, gluconate, is also innocuous to enzymes and cofactor but may, in some cases, interfere with the isolation of product.

Glucose dehydrogenases from Bacillus cereus [33,34] and Bacillus megaterium [35-38] are probably the glucose dehydrogenases best characterized for regenerating NAD(P)H in preparative synthesis. These enzymes have high specific activities (200-250 U mg^{-1}) and operate nearly equally well with NAD or NADP as their cofactor. They are stable to dioxygen and, in the presence of 0.5-1.0 M NaCl, exhibit high thermal stabilities [33,34,36]. Glucose dehydrogenases from Gluconobacter scleroides [39] and beef liver [40] have also been used regenerate NAD(P)H in preparative enzymatic syntheses.

The greatest disadvantage of glucose/GDH is the high initial cost of GDH. As with FDH, however, this disadvantage diminishes as the extent to which the enzyme is to be retained and reused increases. The high stability of GDH may make glucose/GDH the method of choice for regenerating NADH, either repeatedly or continuously, over an extended period of time. Even for one-time use, glucose/GDH is probably the best general method for regenerating NADPH from NADP.

GDH cannot be used in conventional membrane-bound reactors since macromolecular derivatives of NAD(P) are nearly inactive as cofactors for the enzyme [41,42]. The use of anionically charged ultrafiltration membranes, however, allows underivatized NAD(P)(H) to be retained in continuous-flow reactors [38,42].

5.1.2.1.3 Glucose-6-Phosphate/Glucose-6-Phosphate Dehydrogenase

Analogous to glucose/GDH is the regeneration of NAD(P)H by glucose-6-phosphate (G6P) and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) [32,43,44]. Nonenzymatic hydrolysis of

the initial product, 6-phosphoglucono-lactone, to 6-phosphogluconate makes the oxidation of G6P by NAD(P) highly exothermic (E'_o = -0.43 V). This system remains poorly developed, however, because of difficulties related to the substrate. G6P is expensive, and it is modestly difficult to prepare on a large scale [32]. Although innocuous to enzymes, G6P and 6-phosphogluconate are potential general acid catalysts for the decomposition of NAD(P)H. They may complicate the isolation of product. To obviate some of these problems, glucose-6-sulfate has been substituted for G6P in regenerating NAD(P)H with G6PDH [45]. Glucose-6-sulfate is readily prepared from glucose, does not catalyze the decomposition of NAD(P)H, and is a moderately good substrate (15% V_{max}) for G6PDH from Saccharomyces cerevisiae.

The best feature of the G6P/G6PDH regeneration system is the enzyme. G6PDH from Leuconostoc mesenteroides is commercially available, inexpensive, high in specific activity (700 U mg^{-1}), and stable against autooxidation and alkylation [46]. It employs either nicotinamide cofactor and can thus be used to regenerate NADH or NADPH. Yeast G6PDH is similar in many ways to the bacterial enzyme, but accepts only NADP as its cofactor.

The high cost of G6P makes G6P/G6PDH generally less useful than formate/FDH or glucose/GDH for regenerating NAD(P)H. Because the expensive component of G6P/G6PDH is the stoichiometric substrate, repeated or long-term use does not lower its effective cost significantly.

5.1.2.1.4 Alcohols/Alcohol Dehydrogenase

Ethanol, isopropanol, and other alcohols have been used extensively as coupled substrates for the regeneration of NAD(P)H in reductions catalyzed by alcohol dehydrogenases (ADH; EC 1.1.1.) [8,9,47-53]. Alcohol/ADH has also served as a coupled system for NAD(P)H regeneration in reductions catalyzed by other enzymes [17]. The low cost of ethanol and yeast ADH (YADH) and the volatility of ethanol and acetaldehyde make ethanol/YADH an especially attractive system. YADH and

horse liver alcohol dehydrogenase (HLADH) catalyze the reduction of NAD. Alcohol dehydrogenases from Leuconostoc mesenteroides, Thermoanaerobium brockii, and Lactobacillus kefir utilize NADP.

Alcohols are weak reducing agents, and are typically used in excess in order to shift the equilibrium toward reduction. In certain cases, the aldehyde or ketone by-product must be removed or reacted further in order to drive the equilibrium or to avoid product inhibition [3,30,35,54]. A potential problem is the deactivation of enzymes by alcohol or the aldehyde or ketone by-product. While not as generally useful as formate/FDH or glucose/GDH, alcohol/ADH is a good method for regenerating NAD(P)H in a coupled-substrate approach.

5.1.2.1.5 Hydrogen/Hydrogenase

Several anaerobic bacteria produce hydrogenase (EC 1.12.1.2) enzymes that catalyze the reduction of NAD or other redox dyes by dihydrogen. Dihydrogen is attractive as a reductant because it is inexpensive, strongly reducing, and innocuous to enzymes and nicotinamide cofactors. Consumption of dihydrogen provides a convenient measure of the progress of reduction and there is no by-product formation. At present, however, methods based on hydrogenase remain impractical [55-58] as hydrogenase is extremely sensitive to inactivation by dioxygen and other oxidants and is not commercially available.

5.1.2.1.6 Chemical, Electrochemical and Photochemical Methods of Regenerating NAD(P)H

In principle, the use of a chemical reductant to regenerate NAD(P)H spontaneously in situ has the advantage of eliminating the need for a regenerative enzyme. Dithionate is an inexpensive reductant that has been used to regenerate NADH in preparative syntheses [35,59]. Total turnover numbers for NADH have reached 105 [59]. Disadvantages of the use of dithionate include its instability in solution [60] and its potential for reductive inactivation of enzymes or direct reduction of substrates [59]. In addition chemical methods have inherently limited selectivities for the formation of the 1,4-dihydropyridine reduction product.

Electrochemical regeneration of NAD(P)H is attractive because it avoids the use of a secondary enzyme and generates no by-product. Electricity is inexpensive and electrical current provides a convenient measurement of the progress of reaction. Unfortunately, the direct cathodic reduction of NAD(P)H suffers from poor regioselectivity, dimerization of the radical intermediates formed by the initial one-electron reduction of NAD(P), and electrode fouling [60-63]. Yields of enzymatically active NAD(P)H are low. Strategies explored to improve the selective reduction of NAD(P) to enzymatically active NAD(P)H include the use of polymer-supported cofactor [64], coated electrodes [65], electron transfer reagents [66-68], and enzymes to mediate electron transfer between electrode and cofactor [69-71]. All of these systems suffer from poor selectivity, instability, or complexity of operation.

Photochemical methods for the regeneration of NAD(P)H utilize light energy to promote the transfer of an electron from a photosensitizer via and electron transport reagent to NAD(P). Two one-electron transfers are required to form NAD(P)H and the reduced photosensitizer is regenerated in situ by chemical reduction. Photochemical methods for regenerating reduce nicotinamide cofactors have been reviewed briefly [11] and more extensively [72]. To date, the total turnover numbers for NAD(P)H regeneration and chemical conversions of photosensitizer have been low.

5.1.2.2 Reductions Catalyzed by Alcohol Dehydrogenases

5.1.2.2.1 Stereoselectivity and Active Site Models for Alcohol Dehydrogenases

What a synthetic chemist would like to know when he or she uses a particular enzyme to reduce a carbonyl group, is whether the substrate will fit into the binding site, and whether or not it will be reduced or oxidized at a useful rate. Different models exist that explain the structural and stereochemical selectivity of oxidoreductases and allow one to predict whether or not a particular substrate will react with the enzyme in question. These models have been developed for only a small number of enzymes. HLADH with its broad substrate specificity and good stereoselectivity has found

the most interest so far. The two most popular models have been developed by Prelog [73] and Jones [74].

Prelog's Diamond Lattice Section Model

The Prelog model was developed initially for reductions of ketones with cell free alcohol dehydrogenase from Curvularia falcata (CFADH) [73]. It is also called the diamond lattice section model.

There have been several reviews, describing the derivation of this model and its use [49,73,75]. The basic procedure is as follows: For a specific enzyme, (for example HLADH) the initial reaction rates of reduction or oxidation were determined with different substrates under comparable conditions. Different cyclohexane and decaline derivatives of known absolute configuration were used as substrates. The parts of the carbon skeletons of these substrates consisting of chair form cyclohexane rings were considered as portions of a diamond lattice. Substrates with high reduction rates were incorporated in the diamond lattice as having substituents occupying volumes of space that was accepted by the enzyme. Those parts of the lattice, incorporating the structures of slowly reduced or non substrates, were considered to indicate regions where unfavorable interactions occurred. The skeletons of all substrates were superimposed at the reacting carbon-oxygen bond and a diamond lattice section was obtained (figure 3). Since the geometry of the transition state resembles that of the

((figure 3))

alcohol more than that of the carbonyl compound, the reacting carbon oxygen bond is drawn to fit that of the alcohol [73]. The back of the lattice section is thought to be a flat coenzyme binding site of well defined geometry and orientation. Hydrogen transfer occurs from the rear section of the lattice. This model allows a chemist to fit his substrate to the lattice and see whether any unfavorable interactions are present. Only substrates with small groups can be tolerated in the so called "forbidden positions".

Diamond lattice models have been developed for several oxidoreductases including alcohol dehydrogenases from horse liver (HLADH), pig liver (PLADH), Curvularia falcata and Mucor javanicus (MJADH), and 3α-hydroxysteroid dehydrogenase from Pseudomonas testosteroni [49]. Prelog's model was used to rationalize the steric course of HLADH reductions [49]. The diamond lattice model predicts correctly the attack of a pro-R nicotinamide hydride on the re face of the carbonyl group to form an (S)-alcohol. This selectivity is known as Prelog's rule. The synthetically useful and commercially available dehydrogenases from bakers' yeast [76] and Thermoanaerobium brockii [9] also follow this rule (see exceptions chapter B 5.1.2.2.2.3). MJADH [49] and alcohol dehydrogenases from the Pseudomonas sp. strains PED and SBD6 (PADH) [9], and from Lactobacillus kefir (LKADH) [8] transfer the nicotinamide hydride to the si face of the carbonyl group in anti-Prelog manner to give (R)-alcohols. MJADH transfers the pro-S, PADH and LKADH transfer the pro-R nicotinamide hydride.

Prelog's model has been updated [77] more recently and an extended diamond lattice model for HLADH has been proposed based on molecular graphics [78].

Jones' Cubic-Space Section Model

Jones active site model has been developed for HLADH. It was formulated based on specificity, X-ray and kinetic data available for this oxidoreductase [74,79-81]. The picture of the hydrophobic active site pocket of HLADH was represented in a cubic-space section model (figure 4). This model ((figure 4))

specifies very clearly the regions where substituents are and are not tolerated. It allows a chemist to assess more easily (relative to the Prelog model) the likely success of an oxidation or reduction.

Other Models

Rationales for the stereospecificity of microbial [82] and HLADH [83,84] catalyzed reductions based on symmetry properties have been proposed by Nakazaki. His quadrant rule has been applied to several asymmetric bi- and tricyclic ketones [85] and chiral but not asymmetric ketones [82].

5.1.2.2.2 Prelog Specific Alcohol Dehydrogenases

Commercially available alcohol dehydrogenases (ADH, EC 1.1.1.1) from yeast (YADH), horse liver (HLADH), and Thermoanaerobium brockii (TBADH) catalyze the reduction of aldehydes and ketones to the corresponding alcohols. These three dehydrogenases accept a broad range of structure and functionality in potential substrates. Hydride is delivered to only one enantioface of the carbonyl compound, and the reductions are highly enantioselective for the formation of the new carbinol center. In some cases, reductions are also highly enantioselective with respect to chiral centers previously existing in the carbonyl compound. Kinetic resolutions are achieved by the selective reduction of one enantiomer of a chiral ketone or aldehyde.

5.1.2.2.2.1 Yeast Alcohol Dehydrogenase

Yeast alcohol dehydrogenase (YADH) catalyzes the NADH-dependent reduction of aldehydes and methyl ketones to the corresponding primary and methyl carbinols. The Michaelis constants for straight-chain aliphatic and α,β-unsaturated aldehydes containing 2-6 carbon atoms are all near 1 mM [86]. Maximal velocity data for aldehydes are presented in table 1. Methyl ketones are reduced ((table 1))

at approximately 1% of the rate of aldehydes. YADH does not reduce cyclic ketones (cyclopentanone, cyclohexanone and cycloheptanone) or 3-hexanone, a ketone in which both alkyl groups attached to the carbonyl group are larger than a methyl group. The enzyme's narrow range of synthetically useful

substrates is ostensibly a consequence of its role in vivo, which is specifically the reduction of acetaldehyde to ethanol.

The complete enantioselectivity of YADH for the delivery of the pro-R hydride to the re face of aldehydes and methyl ketones (compare B 5.1.2.2.1) is of synthetically valuable. Reductions of 1-deuterioaldehydes [40,90][3] and methyl ketones [34,91] produce the corresponding (S) alcohols. Reductions of unlabelled aldehydes by NAD[^{2}H] produce (R)-1-deuterioalkanols [30,90,92]. One exception to this stereochemical rule is the reduction of pyruvate by YADH to form D-lactate, which is (R) in configuration [92].

A disadvantage of YADH is its instability. Active YADH is tetrameric with eight free cysteine thiol groups per monomer. The enzyme is thus sensitive to heat, urea, high salt concentrations, and organic solvents, all of which presumably cause dissociation of the protein into monomers. Essential thiol groups on the enzyme are sensitive to oxidation, alkylation, and complexation by heavy metals. YADH is unstable at pH values above 8.5 or below 6.0 [93]. Thermally, the enzyme exhibits a sharp loss of activity and change in optical rotation when heated above 36 °C [94]. Its half-life at 44 °C is 20 minutes [95]. Inhibition of YADH by heavy metals decreases in the order $Hg^{2+} > Ag^{2+} > Cd^{2+} > Cu^{2+} > Pb^{2+}$ [93]. Immobilization of YADH on solid supports often results in significant loss of catalytic activity [96].

5.1.2.2.2.2 Horse Liver Alcohol Dehydrogenase

Historically, the alcohol dehydrogenase most highly utilized for organic synthesis is HLADH. Although this enzyme is perhaps most commonly known for its enantioselective oxidations of meso-diols to lactones [97], it has also been widely used for reductions of aldehydes and ketones.

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[3]Originally, the absolute configuration of (-)-ethanol-1-d was incorrectly assumed to be (R) [40].

HLADH is a dimeric protein with one bound zinc ion and fourteen free thiol groups per monomer. It is inhibited by fatty acids, fatty acid amides, imidazole, thiol-blocking reagents, and organic molecules that coordinate zinc. The abiiity of heavy metals to inhibit HLADH decreases in the order $Hg^{2+} > Ag^{2+} > Cd^{2+} > Cu^{2+}$ [93]. HLADH is inhibited by a number of anions: halides, cyanide, oxalate, perchlorate, sulfate, thiosulfate, thiocyanate, nitrate, and sulfite. It is, however, noticeably more stable against thermal inactivation than YADH. HLADH maintains 90% of its original activity after being heated at 44 °C for thirty minutes [94]. HLADH has been successfully immobilized [96,98] and used in organic solvents [99,100].

HLADH is NADH-dependent and delivers hydride to the re face of aldehydes (as discussed in chapter B 5.1.2.2.1). Reductions using 1-deuterioaldehydes as substrates or NAD[^{2}H] as the cofactor yield primary 1-deuterio aliphatic [98,101-104] allylic [102], and benzylic [105-107] alcohols that are essentially enantiomerically pure. Kinetic data for the reaction of HLADH with aldehydes are presented in table 2.

((table 2))

Although only sketchy kinetic data is available, acyclic ketones appear to be poor substrates for HLADH [49]. Even acetone and 2-butanone react slowly [89,93]. The fact that isopropanol, (±)-2-butanol, and 3-pentanol are oxidized by HLADH with maximal velocities that are 10-30% of that of ethanol [110] suggest that the corresponding ketones are also substrates. While K_m for ethanol is around 0.5 mM (it is very dependent on pH), Michaelis constants for isopropanol, (±)-2-butanol, and 3-pentanol are 2.5-12.6 mM [110]. The enantioselectivities for the oxidations of racemic 2-butanol, 3-hexanol, and 2-octanol are moderate to poor [110]: these data suggest that reductions of acyclic ketones by HLADH would also be at most moderately enantioselective. Preparative reductions of α- and β-ketoacetals and β- and γ-ketoesters have, however, given products having good to excellent enantiomeric purities (table 3) [18,34]. The rates of reaction of HLADH with these latter substrates have not been reported.

((table 3))

HLADH shows good reactivity with cyclic and bicyclic ketones in which the carbonyl group to be reduced is in a four or six-membered ring [49,111]. Cyclopentanone, cycloheptanone, cyclooctanone, and cyclononanone are reduced at rates that are at most about two percent of that of cyclohexanone [111,112]. Endocyclic sulfur atoms seem often to enhance the reactivities of cyclic ketones, whereas an endocyclic oxygen atom retards the reduction of a six-membered cyclic ketone (table 4). 3-Thiolanone, 3-thianone, and 3-thiepanone are all reduced with small enantioselectivities. ((table 4))

The (S) alcohols in entry 1 and 2 and the (R) alcohol in entry 10 are produced with 33, 28, and 10% enantiomeric excesses, respectively [112].

Alkyl branches α to the carbonyl groups of six-membered cyclic ketones slow the rates of reduction to 1% or less of the rates of reduction of the parent compounds [49,114,115]. Reductions of these compounds generate trans-substituted alcohols having high (88-92%) values of diastereomeric excess but variable (44-90%) values of enantiomeric excess (figure 5; eq 1) [115,116]. Alkyl branches β or γ to carbonyl groups have much smaller effects on rates of reduction than α-branches [49,114,117]. HLADH reduces (±)-3-methylcyclohexanone, 4-methylcyclohexanone, 4-isopropylcyclo-hexanone, and 4-tert-butylcyclohexanone at 50, 41, 21, and 2.5% the rate of cyclohexanone respectively [114]. Reductions of 2-alkylpyran-4-ones proceed with high enantioselectivities (> 98% ee) and moderate to good diastereoselectivities (63-89% de) (eq 2) [113]. Reductions of 2-alkylthian-4-ones proceed with high enantioselectivities (> 98% ee), but diastereoselectivities are low (20-50% de) and unpredictable as to which diastereoisomer is favored (figure 5; eqs 3 and 4) [117].

((figure 5))

HLADH has been used extensively for the enantioselective reduction of decalones, bicyclo[4.3.0]nonanones, and related bicyclic structures. Prelog's diamond-lattice model [73] and Jones' cubic-space model [74] summarize the structural and stereochemical selectivity of HLADH with respect to these substrates and serve as schematic models of the active site of HLADH. In general, 1-decalone and α-tetralone structures are reduced very slowly (≤ 0.2% of the rate of cyclohexanone) [49]. β-Tetralone is reduced at 1% of the rate of cyclohexanone. 2-Decalone (cis or trans) and its derivatives are reduced rapidly and with high enantio- and diastereoselectivities (table 5) [79,118]. ((table 5))

Although the reductions of bicyclo[4.3.0]nonan-3-ones and their derivatives generally proceed at high rates, they suffer from either poor enantioselectivity or poor diastereoselectivity. Two exceptions to this trend are the reductions of trans-8-oxabicyclo[4.3.0]-nonan-3-one and trans-8-thiabicyclo[4.3.0]nonan-3-one (table 5). Note that only the carbonyl group in the six-membered ring is reduced in bicyclo[4.3.0]nona-3,8-diones.

HLADH catalyzes the enantioselective reduction of other bicyclic and bridged tri- and pentacyclic ketones at rates that are 14-100% of that of cyclohexanone (figure 6) [49,74,83,121,122]. Chiral ((figure 6))

resolutions of C_2-symmetric ketones have been achieved using HLADH-catalyzed reductions [83]. (-)-Camphor is reduced selectively over (+)-camphor but at a rate that is only 0.1% of that of cyclohexanone. Bicyclo[2.2.2]octan-2-one is reduced at 0.9% of the rate of cyclohexanone [49].

5.1.2.2.2.3 Thermoanaerobium brockii Alcohol Dehydrogenase

In 1981, the physical and catalytic properties of an NADPH-dependent alcohol dehydrogenase isolated from the thermophilic bacterium, Thermoanaerobium brockii (TBADH), were reported [123-125]. The most striking characteristic of TBADH is its thermal stability. The enzyme is active at temperatures up to 90 °C. It shows no loss of activity when heated at 65 °C for 70 minutes and shows

a loss of only 25% of its original activity when heated at 86 °C for 70 minutes [123]. TBADH is stable toward chemical protein denaturants. It is stable in 20% (v:v) isopropanol and loses only part of its activity after 24 hours at 50 °C in 30% isopropanol. Isopropanol is commonly used in synthetic procedures as a coupled substrate for the regeneration of NADPH (total turnover numbers of up to 20,000). TBADH has been successfully immobilized (15-30% immobilization ylelds) and used continuously at 37 °C for 30 days with no loss of activity [125]. TBADH is tetrameric, sensitive to p-chloromercuribenzoate (a thiol-blocking reagent), but insensitive to 5 mM EDTA (a metal-chelating agent) [123].

TBADH accepts a wide range of aldehydes and acyclic and cyclic ketones as substrates (table 6). Unlike YADH and HLADH, TBADH exhibits synthetically useful activity with acyclic ((table 6))

ketones in which both alkyl groups attached to the carbonyl group are larger than a methyl group. Ketones with long alkyl chains on one or both sides of the carbonyl group or with sterically demanding groups near the carbonyl group are not substrates (figure 7). TBADH reduces 2,2,2-trifluoroacetophenone, which is not a substrate of YADH or HLADH [34].

((figure 7))

((table 7))

TBADH reduces achiral ketones with good to excellent enantiofacial selectivity (table 7) [18,34,51,122,124,126-133]. Ideal substrates are methyl alkyl ketones or ethyl alkyl ketones in which the alkyl group has four or more carbon atoms. Double and tripie bonds, small alkyl branches, ester or nitrile groups, and oxygen or halogen substituents are all tolerated on the larger alkyl chain. Note that TBADH selectively reduces the terminal carbonyl group of dec-9-ene-2,6-dione in the presence of the internal carbonyl group [132]. Phenyl rings reduce the rates and enantioselectivities of reductions,

especially when the aromatic ring is close to the carbonyl group. Five-membered heteroaromatic rings generally have similar but less adverse effects.

The absolute sense of enantioselectivity of TBADH-catalyzed reductions changes as the size of the alkyl groups attached to the carbonyl group changes. When both alkyl groups are isopropyl or cyclopropyl or smaller, TBADH delivers hydride selectively to the si face of the ketone, opposite to the face reduced by YADH and HLADH. The (R) alcohols produced from smaller substrates typically have low to moderate enantiomeric purities (44-86% ee). As the alkyl groups attached to the carbonyl group grow larger, selective reduction from the re face of the ketone gives (S) alcohol products, often with high enantiomeric purities (>95% ee). The size-dependent inversion of stereoselectivity suggests the existence of two hydrophobic binding domains in the enzyme's active site, one large and one small, with the smaller site having the higher binding affinity [124].

The enantioselectivity of TBADH may vary with environmental factors, such as the concentrations of substrates, time of reaction, temperature, pH, or buffer [125]. The effects of reaction conditions on the enantioselectivity of TBADH are greatest with "poor" substrates, which inherently form products of low enantiomeric purity. Concentrations of substrates or times of reaction that allow reoxidation of the initially formed product result in low optical yields due to racemization. Enantioselectivity decreases with increasing temperature. In reductions of 2-pentanone, the enantiomeric excess of the product, 2-pentanol, decreases linearly from 77% for a reaction performed at 7 °C to 58% for a reaction performed at 50 °C. The enantioselectivity of TBADH decreases as the pH increases above 7.5-8.0. Phosphate buffers, when compared to glycine-NaOH or Tris-HCI buffers, lower both the catalytic activity and the enantioselectivity of TBADH.

With "good" substrates, which inherently form products of high enantiomeric purity, the effects of reaction conditions on the enantioselectivity of TBADH follow the same trends as those observed with poor substrates. Because the energetic distinctions between the two modes of enantiofacial attack are intrinsically great, the magnitude of these effects is, however, much smaller than with poor substrates.

Reductions by TBADH may be enantioselective with respect to chiral centers already present in the substrate. In the presence of racemic 3-methyl-2-pentanone, TBADH reduces the (R) ketone selectively and gives the trans alcohol having a diastereomeric excess of 78% (figure 8; eq 1) [124]. Reduction of bicyclo[3.3.0]oct-2-en-8-one occurs selectively, consuming the (1R,5S) ketone and leaving the (1S,5R) ketone enantiomerically enriched (figure 8; eq 2) [134]. To date, the extent of substrate-level enantioselectivity exhibited by TBADH has been modest.

((figure 8))

5.1.2.2.3 Other Alcohol Dehydrogenases

Alcohol dehydrogenases from pig liver (PLADH), Mucor javanicus (MJADH), and Curvularia falcata (CFADH) have been studied as catalysts for reductions useful in organic synthesis [49]. All are NADPH-dependent. All reduce a variety of derivatives of cyclohexanone and 2-decalone. The three enzymes are notable for their ability to reduce derivatives of cis and trans 1-decalone,[4] decalin-1,4-dione, and α-tetralone, which are not substrates of HLADH or YADH. MJADH does not reduce cyclopentanone. CFADH does not reduce acetone. PLADH does not reduce cyclopentanone, acetone, or acetaldehyde. PLADH and CFADH are selective for the reduction of the re faces of ketones, while MJADH is si face-selective. Both PLADH and MJADH are thermally unstable. None of these three enzymes is commercially available.

Recently, alcohol dehydrogenases from Lactobacillus kefir (LKADH) [8,44,135] and Pseudomonas sp. (PADH) [9,131] have been evaluated as catalysts for synthesis. Both differ from YADH, HLADH, and TBADH in that they generally deliver hydride to the si face of ketones and generate (R) alcohols. LKADH uses NADPH as its cofactor; PADH uses NADH. Neither enzyme is

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[4]MJADH does not reduce cis-1-decalone and its derivatives.

commercially available at present, but their microbial sources have been deposited at the American Type Culture Collection (ATCC). LKADH and PADH accept a wide range of acyclic and cyclic ketones as substrates (table 8). Although methyl alkyl ketones are particularly good substrates for both ((table 8))

enzymes, ketones in which both alkyl groups are significantly larger than a methyl group are tolerated. The ability of the two enzymes to reduce aromatic ketones is capricious. Although both reduce benzocycloheptanone, neither reduces the isosteric benzothian-4-one. Although LKADH reduces 4-chloro-1-(2-thiophenyl)butanone, it does not accept 4-chloro-1-(4-fluorophenyl)-1-butanone or 4-chloro-1-(4-hydroxyphenyl)-1-butanone as substrates. Substituents on phenyl rings seem to decrease substrate activity.

LKADH is notable for its ability to reduce conjugated enones and ynones. For all cases in which both LKADH and PADH have been used to reduce the same substrate, LKADH has provided the product of higher enantiomeric purity. LKADH can be used as a catalyst in large-scale reductions of carbonyl compounds; an example was the synthesis of (R)-phenylethanol in a membrane reactor [135]. The need for the expensive NADPH as cofactor is the main backdraw of LKADH catalyzed reductions.

A thermostable alcohol dehydrogenase from the thermophilic bacterium, Sulfolobus sulfataricus, has been isolated and characterized [136]. Although the NADH-dependent dehydrogenase accepts acetone, butanone, 3-methylbutanone, and cyclopentanone as substrates, it is particularly active with 3-methylcyclohexanone and p-anisaldehyde. The enzyme is active at temperatures up to 95 °C. Its half-lives at 70 and 60 °C are 5 and 20 hours, respectively. After 24 hours at 50 °C, the enzyme retains 70% of its original activity.

5.1.2.3 Reductions Catalyzed by Dehydrogenases Other than ADH

Carbonyl groups have been reduced enzymatically with a number of dehydrogenases other than ADH. Their substrate- and stereospecificities complement the activities of alcohol dehydrogenases.

5.1.2.3.1 Lactate Dehydrogenases, Hydroxyisocaproate Dehydrogenase and Malate Dehydrogenase in the Synthesis of α-Hydroxy Acids

Lactate dehydrogenases (LDH) were successfully used in the synthesis of chiral α-hydroxyacids. In vivo L- and D-lactate dehydrogenases (L-LDH; EC 1.1.1.27 and D-LDH; EC 1.1.1.28) catalyze the NADH dependent conversion of pyruvate and lactate in anabolic and catabolic pathways [137]. L- and D-lactic dehydrogenases from different sources are commercially available and inexpensive [138]. In immobilized form, LDH has satisfactory stability [43]. Lactate dehydrogenases are enzymes which possess simultaneously broad substrate specificity and high enantioselectivity. The substrate specificities of LDHs have been systematically examined [139,140]. Both enzymes, isolated from several sources, reduce a broad variety of aliphatic α-oxoacids. D-LDH shows a narrower substrate specificity than L-LDH. Only a few of the most reactive substrates have been reduced on a preparative scale (see table 9). Best results were achieved when formate/FDH was used as NADH regeneration system. The corresponding 2-hydroxy acids were isolated in yields >94% with high enantiomeric excess (>98%).

((table 9))

An interesting application of LDH is the reduction of chloropyruvic acid. L-Lactic dehydrogenase from rabbit muscle and D-lactic dehydrogenase from Lactobacillus leichmanii were used to synthesize L-and D-β-chlorolactic acid with high enantiomeric excess (>97 %) [43] using glucose-6-phosphate / glucose-6-phosphate dehydrogenase as the NADH regeneration system. The treatment of these compounds with base lead to optically active (D)- and (L)-glycidic acid (ee >97%), two important synthetic intermediates (figure 9).

((figure 9))

D-LDH from Leuconostoc mesenteroides was used to prepare (R)-2-hydroxy-4-phenylbutanoic acid, a building block of angiotensin converting enzyme inhibitors [141]. More recently the reduction of unsaturated 2-oxoacids with L-LDH from Bacillus stearothermophilus was reported [10] (see table 9; entries 9 - 11). R-2-Hydroxyisocaproate dehydrogenase (R-HicDH) was used to synthesize R-2-hydroxy-methylpentanoic acid (table 9; entry 14). This enzyme catalyzes the R-specific reduction of a broader variety of α-oxoacids than does D-LDH. R-HicDH accepts sidechains that are linear, branched alkyl, arylalkyl, hydroxyalkyl and heterocyclic [142]. L-malate was prepared from oxalacetate with malate dehydrogenase (MalDH; EC 1.1.1.37) embedded in a polyacrylamide matrix [143] (table 9; entry 15).

D- and L-LDH catalyzed reduction of pyruvic acid has been used in combination with different NADH regeneration systems in order to demonstrate the efficiency of these methods [68,69,144,145].

Amino acid dehydrogenases are another important class of enzymes that catalyze the reduction of α-oxoacids. The reductive amination is discussed elsewhere (see chapter B 5.3).

5.1.2.3.2 Glycerol Dehydrogenases, Sorbitol Dehydrogenases, Mannitol Dehydrogenase and Aldose Reductases in the Synthesis of Polyols

α-Hydroxyketones were reduced stereoselectively to the corresponding chiral diols with glycerol dehydrogenases (GlyDH; EC 1.1.1.6) from Enterobacter aerogenes and Cellulomonas sp. [31] (see table 10). Both dehydrogenases show relatively broad substrate specificities. Various ((table 10))

aliphatic and acyclic hydroxy aldehydes and ketones are reduced in an R-specific manner with both enzymes. For most synthetic applications, the dehydrogenase from Cellulomonas sp. is superior because of its higher specific activity and lower cost. Sugars are poor substrates and bulky substituents

α to the ketone are not tolerated. GlyDH has also been used in the reductive kinetic resolution of racemic α-hydroxy ketones (figure 10) to generate the alcohol and the unreacted ketone with high enantiomeric excesses [31].

((figure 10))

Sorbitol dehydrogenases (SDH; EC 1.1.1.14) catalyze the diastereoselective reduction of ketoses to the corresponding polyols (table 10; entries 1 - 6). An interesting application of SDH is the reduction of aldolase products in the synthesis of so called "inverted" aldoses [146-149] (compare chapter B 4.1). Mannitol dehydrogenase (ManDH) is another ketose dehydrogenase that has been used in the synthesis of polyols [150]. Aldose reductases (AlR) from different microbial sources have been isolated and used in the reduction of several aldoses such as arabinose, galactose, glucose, maltose, mannose, ribose and xylose [150,151]. β-Lactose and aromatic aldehydes, such as benzaldehyde and anisaldehyde, were also reduced to their corresponding alcohols with AlR.

5.1.2.3.3 Steroid Dehydrogenases

Dehydrosteroids can readily be reduced with hydroxysteroid dehydrogenases (HSDH; EC 1.1.1.). HSDHs are widely distributed and have been detected in a variety of microorganisms and mammals. In contrast to many alcohol dehydrogenases, these enzymes have quite narrow substrate specificities. Their high regio- and stereospecificity was used in the synthesis of neutral steroids [152] and bile acids [153,154]. Deuterated cholic acids **3**, **4** and **5** were prepared on a preparative scale by coupling the HSDH catalyzed reduction of 3,7,12-trioxo-5-β-cholan-24-oic acid (**2**) to the NADP(^{2}H) regeneration system 1-D-glucose/glucose dehydrogenase [154] (figure 11). The reduction reactions showed high regio and stereospecificities (≥98%) and lead to products with high isotopic purity (≥94% ^{2}H).

((figure 11))

The enzyme 3α,20β-hydroxysteroid dehydrogenase (3α,20β-HSDH; EC 1.1.1.53) has successfully been used to reduce bicyclo[3.2.0]hept-2-en-6-ones stereoselectively to their corresponding alcohols [122,155] (compare yeast-mediated reduction chapter B 5.1.3.4). Reduction of racemic 7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one (**6**) by 3α,20β-HSDH led to the alcohol **7** with high enantiomeric excess (95%) [156] (figure 12).

((figure 12))

5.1.3 Yeast Mediated Reductions

5.1.3.1 General

Yeasts are a phylogenetically diverse group of fungi. About 600 different yeast strains are known. The fermentation behavior of yeasts is well documented and different industrial applications such as brewing, wine making, baking, ethanol production, oil and fat synthesis and degradation, and waste water treatment are known [157]. The species generally used in baking and brewing is Saccharomyces cerevisiae.

The use of bakers' yeast as a reagent in organic synthesis has a long tradition and several review articles devoted to bakers' yeast mediated reactions have been published [158-161]. The low cost, good availability and the utility of bakers' yeast in organic synthesis allows synthetic chemist to consider bakers' yeast as a simple reagent. Several Organic Syntheses preparations exist for the reduction of carbonyl compounds with commercially available bakers yeast and table sugar [162-164]. Starting from ethyl acetoacetate (**8**) for example, ethyl (S)-(+)-3-hydroxybutanoate (**9**) was synthesized in good yields (59 - 76 %; ee = 85%) (see figure 13).

((figure 13))

The use of other yeast strains or microorganisms is more demanding and often requires good knowledge in microbiological techniques. In this chapter only the baker's yeast mediated reductions of carbonyl groups will be discussed[5].

Yeast has a broad substrate specificity and in many cases yeast shows high enantio- and diastereoselectivity (see tables 11 - 20). Yeast accepts a broad variety of substrates such as cyclic and acyclic β-ketoesters, α-hydroxyketones, β-diketones, aliphatic and aromatic ketones and aldehydes and cyclic ketones. Even if bakers' yeast is used today like a "normal" chemical reagent, users have to be aware of the following problem: it is important to make sure that the strain in use corresponds exactly to the one described in the literature [165]. Different strains of yeast (and even the same strain grown under different conditions) show different types and levels of enzyme activities. These differences can lead to other specificities[6], and side reactions resulting from enantioselective esterase activity [167] may also occur. Minor variations in the experimental protocol may also lead to profound specificity variations [159].

5.1.3.2 Reductions of α,β- and δ- Oxoesters and Oxoacids

Yeast mediated reductions of β-ketoesters for the formation of chiral intermediates are one of the most extensively studied microbial transformations in asymmetric synthesis. Extensive studies demonstrate the capacity but also the backdraws of yeast mediated reductions of carbonyl groups.

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[5] The term yeast used throughout the next chapter refers to baker's yeast.

[6] A strain of yeast from the Oriental Yeast Co., Ltd., showed in some cases enantioselectivities opposite to the ones observed with commonly used brands of bakers' yeast [166].

Other carbonyl substrates of bakers' yeast will be discussed less extensively in this chapter. For more detailed information we refer to specific review articles [158-161].

Yeast accepts a broad variety of β-ketoesters as substrates. Many of the resulting alcohols, in particular 3-hydroxybutanoates, have been used in the synthesis of natural products or have been converted to chiral synthons [163]. Enantioselectivities of yeast mediated β-ketoester reductions depend strongly on the relative size of the ketone sidechain R^1 and the alkoxy moiety R^2 (table 11). ((table 11))

Prelog's rule [73] has proven to be poorly applicable to these reactions. This failure of Prelog's rule is due to the fact that baker's yeast contains several enzymes that catalyze redox reactions at carbonyl or alcohol groups. In fact three enzymes capable of actively reducing acetoacetic esters have been purified from yeast [180]. Two of them show D- and one shows L-selectivity[7]. YADH is clearly not the most important enzyme in yeast mediated reduction reactions (it is fairly specific for short-chain aldehydes and alcohols; compare B 5.1.2.2.3). The two most active enzymes in yeast have opposite enantioselectivities and respond differently to variations in the size of the alkoxy group. Yeast reductions of β-ketoacids or salts of these acids result in many cases in products having the D-configuration [169,181] (table 11; entries 16 and 22). In general the use of longer chain alkoxy groups increases the preference for L-products [158] (exceptions have been observed (table 11; entries 16 - 18) [169]). Bulkier β-ketoesters are not very good substrates for the D-specific enzyme. As a result the L-specific enzyme catalyzed reduction becomes predominant. Yeast mediated reductions of methyl-

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[7] The stereoselectivities of the dehydrogenases found in baker's yeast depend mainly on the size of substituents attached to the cabonyl group of the substrate. Cahn Ingold Prelog nomenclature leads to confusion in some cases where the smaller substituent has higher priority (compare table 11). D-, L-nomenclature is used to avoid these problems.

and octyl-3-oxopentanoate (**10** and **11**) and methyl- and octyl-4-chloro-3-oxobutanoate (**12** and **13**) (figure 14) are typical examples for how structural modifications of the substrates can be used to control the stereochemical course of yeast reductions [182]. Other examples are given in table 11.

((figure 14))

Methods other than variation of the substrate sidechains exist to activate or deactivate selectively one of the yeast oxidoreductases. The use of specific inhibitors [168], the variation of substrate concentrations (if the K_m -values of the enzymes involved are different) [161,176,180], or simply the use of other strains [166] or of mutants of yeast that lack the unwanted enzyme [180] are in many cases sufficient to control slectivity. The stereoselectivity may also be influenced by changing the fermentation conditions by working under aerobic or anaerobic conditions [183,184], by immobilizing the yeast in a polymer matrix [172,176,185], or by the addition of different salts to the reaction mixture [182].

In many cases, 2-substituted racemic β-ketoesters are reduced with high diastereo- and enantioselectivity because the two enantiomeric forms can equilibrate through the enolic form [159]. L-Enantioselectivity is observed when R^3 is small. The reduction leads to a mixture of the diastereoisomers (2R,3S) and (2S,3S) in various ratios where the size of the substituents in position 2 strongly influences the diastereoselectivity of these reactions (see table 12). Entries 1, 3, 8 and 10 show that the nature of the alkoxy group can also influence the stereoselectivity in yeast mediated reactions of α-substituted β-ketoesters.

((table 12))

Comparable stereoselectivities were observed with cyclic β-ketoesters. Very high diastereo- and enantioselectivities were observed when starving yeast was used [183]. A comparative study on the reduction of 2-oxocyclopentane- and 2-oxocyclohexane carboxylates with yeast has been published [193]. Dia- and enantioselectivities were often higher than with acyclic compounds [158]. Cyclic β-

ketoesters were synthesized temporarily, in order to get β-hydroxy esters with good selectivities after chemical ring opening. Yeast mediated reduction of **18** is a typical example of this synthetic strategy. The β-hydroxy ester **19** was isolated in good yields (86%) and with high stereoselectivity (de = 98%. ee > 85 %) after Raney-Ni-desulfurization [194] (figure 15).

((figure 15))

A particularly interesting example of yeast mediated reactions are the stereospecific reductions of 2-methyl-3-oxopropionates [195]. Chirality is introduced at a different position from the reaction center. The chirality arises on the C-2 position, whereas the reduction takes place at the C-3 position (figure 16). Starting from a racemic mixture of **20** the alcohol **21** was isolated with good optical

((figure 16))

yields (ee = 90 %). The R-enantiomer is reduced faster than the S-enantiomer and the aldehyde **20** enolizes rapidly: interconversion between the two enantiomeric forms is expected to be fast enough to keep the starting material as a racemic mixture during the whole reaction. Best results were obtained when bulky esters were used.

Various lactones have been synthesized by yeast mediated reduction of aliphatic 4- and 5-oxo-carboxylic acids or esters [196-198]. δ-Oxo-carboxylic acid **22** was reduced with bakers' yeast for example, to give the highly functionalized δ-lactones **23** and **24** (figure 17). Compound **23** was used as a precursor in the synthesis of cis-(+)-rose oxide [199].

((figure 17))

α-Ketoesters do not always give optically pure 2-hydroxy esters. However, with some short chain and some aromatic α-ketoesters good enantioselectivities were observed (table 13).

((table 13))

5.1.3.3 Reductions of Diketones

Yeast mediated reductions of 1,3-diketones are well developed as the interest in chiral 1,3-diols and β-hydroxy ketones as chiral synthons has lead to a number of investigations. Some examples of simple acyclic β-diketones are summarized in table 14. The yeast reduction leads in most cases to the (L)-alcohol with high enantiomeric excess.

((table 14))

3-Substituted diketones, such as 3-methyl-2,4-pentadione, are poor substrates [203]. Temporarily cyclized β-diketones, such as tetrahydrothiopyran-4-one **25**, were successfully reduced with high stereoselectivity (figure 18). Chemical desulfurization lead to the insect pheromone (4R, 5S)-sitophilure (**26**) [207].

((figure 18))

Yeast reduction of a wide range of simple 2-substituted cyclic β-diketones have been described [208]. In all cases the alcohol group with (S)-configuration was formed. The configuration at the C2-center was variable. Diastereo- and enantioselectivities were shown to be strongly dependent on the ring size. Cyclopentadiones are reduced predominantly to the (2S, 3S) stereoisomer; in contrast, cyclohexadiones yield preferentially the (2R, 3S) product (tables 15 and 16). When the ring size was increased to seven, eight or nine membered, the yields dropped and stereoselectivity became less predictable [209].

((table 15))

((table 16))

Unlike β-diketones, 1,4-diketones are reduced by yeast to the corresponding diols. 2,5-Hexanedione, for example, was reduced to (2S, 5S)-2,5-hexanediol with high stereoselectivity (50% yield; 96% ee, 25% meso product) [211].

5.1.3.4 Reductions of α-Hydroxy Ketones, Aromatic and Aliphatic ketones and Others

A variety of substituted 1,2-diols have been prepared by yeast-mediated reduction of α-hydroxy ketones (table 17). The anti-Prelog products were formed in many cases with high ((table 17))

enantioselectivities. Bakers' yeast ability to mediate reductions of simple α-hydroxy ketones is comparable to the one displayed by glycerol dehydrogenase from microbial origin [31] (compare B 5.1.2.3.2)

Simple aromatic ketones are poor substrates for yeast-mediated reductions. Electron withdrawing groups adjacent to the ketone moiety or in para position of an aromatic ring increase the reactivity of the ketone (table 18).

((table 18))

Aliphatic acyclic ketones can be reduced with yeast efficiently. A wide range of functional groups adjacent to the ketone function are tolerated. Simple ketones were extensively investigated in the 1960s [219] and in most cases the corresponding (S)-alcohols were obtained. The best enantioselectivities were achieved when R1 and R2 of greatly different size were used. When longer chain ketones were reduced, at least one sidechain had to be methyl. In the case where R1 and R2 were large, no reduction was observed. In table 19, only some of the most recently published yeast mediated reductions of ((table 19))

ketones are listed. For other examples we refer to previously published review papers [158-161].

In some cases cyclic and polycyclic ketones can be reduced with yeast with good stereoselectivities. Prostaglandins [228] and prostaglandin precursors [229] for example, have been synthesized starting from highly functionalized cyclopentanones. Racemic separations were achieved with 2-substituted cyclohexanones [230]. Miscellaneous selectivities were observed when bicyclo[3.2.0]hept-2-en-6-one **27** and other bicyclic compounds such as **28**, **29** and **30** were reduced with bakers' yeast (table 20). Reductions of pyrrolobenzodiazepinetriones [231], benzazepin-2-ones [232] and bicyclo[3.3.1]nonane-2,6-dione [233] have been described as well.

((table 20))

Yeast also accepts a broad variety of substrates other than the ones mentioned above. Some of these less common, but in many cases highly functionalized substrates are shown in figure 19.

((figure 19))

Acetyldimethylphenylsilane (**31**) [238], sulfenyl and sulfonyl ketones (**32**, **33**) [239,240], isoxazolines (**34**) [241], keto dithianyl derivatives (**35**) [191] and even chromium complexes (**36**) [242] were reduced asymmetrically with bakers' yeast.

Abbreviations

ADH: alcohol dehydrogenase; AlR: aldose reductase; ATCC: American Type Culture Collection; CFADH: Curvularia falcata alcohol dehydrogenase; FDH: formate dehydrogenase; G6P: glucose-6-phosphate; G6PDH: glucose-6-phosphate dehydrogenase; GDH: glucose dehydrogenase; GlyDH: glycerol dehydrogenase; R-HicDH: R-2-Hydroxyisocaproate dehydrogenase; HLADH: horse liver alcohol dehydrogenase; HSDH: hydroxysteroid dehydrogenase; LDH: lactate dehydrogenase; LKADH: Lactobacillus kefir alcohol dehydrogenase; MalDH: malate dehydrogenase; ManDH: Mannitol dehydrogenase; MJADH: Mucor javanicus alcohol dehydrogenase; NAD: nicotinamide adenine dinucleotide; NADH: 1,4-dihydronicotinamide adenine dinucleotide; NADP: nicotinamide

adenine dinucleotide phosphate; NADPH: 1,4-dihydronicotinamide adenine dinucleotide phosphate; PADH: Pseudomonas sp. alcohol dehydrogenase; PLADH: pig liver alcohol dehydrogenase; PQQ: pyrroloquinoline quinone; SDH: sorbitol dehydrogenase; TBADH: Thermoanaerobium brockii alcohol dehydrogenase; YADH: yeast alcohol dehydrogenase.

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5.1.4 References

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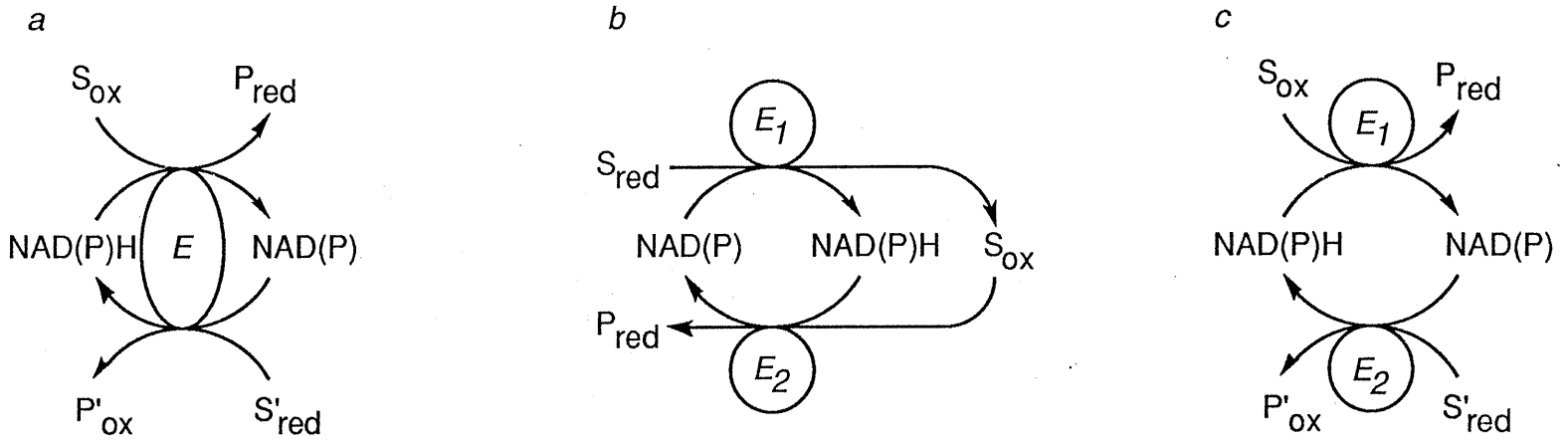


figure 1: Strategies for enzymatic regeneration of nicotinamide cofactors. Illustrated in the direction of reduced product formation are the (a) coupled-substrate, (b) coupled-enzyme, and (c) coupled-system approaches.

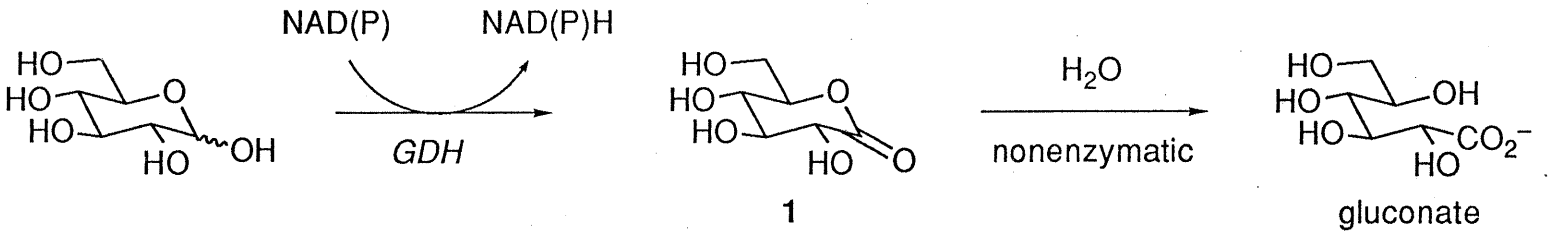


figure 2: Glucose dehydrogenase catalyzed regeneration of NAD(P)H from NAD(P). The spontaneous hydrolysis of gluconolactone (**1**) is strongly exothermic and makes the regeneration of nicotinamid cofactors favorable.

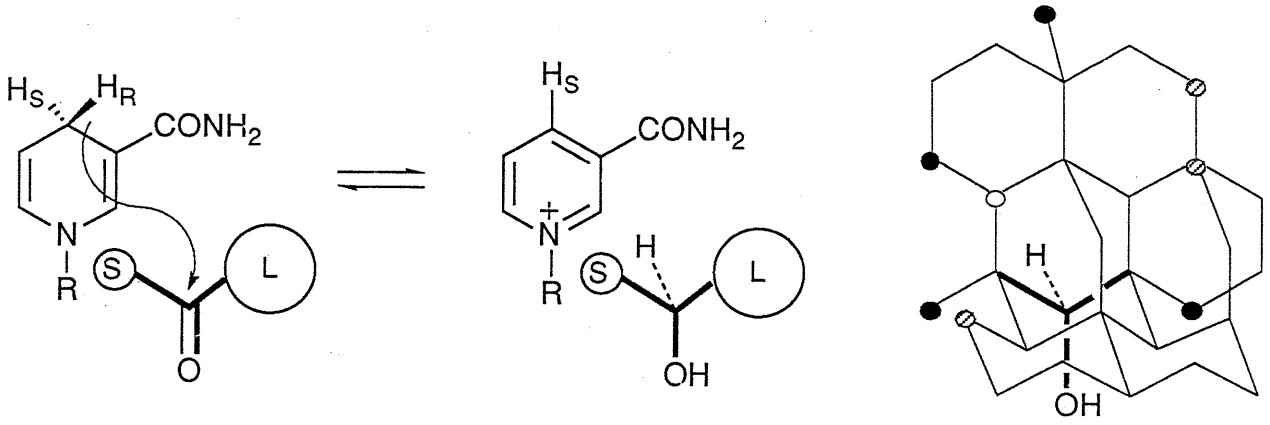


figure 3: Diamond lattice section for HLADH according to [49]. The positions marked with ● are "forbidden positions". Oxidoreduction will not take place if binding of a potential substrate places a group in one of these locations. "Undesirable" positions are marked with ⊘. Occupation of these positions by a part of a substrate does not necessarily preclude the reaction but the rate of reaction will be very slow. The positions under the lattice are also in this catagory. The position marked with ○ is called "unsatisfactory": the rate of reduction with substrates having substituents in this position will be slow. For HLADH the Prelog model predicts the attack of a pro-R NADH-hydride to the re face of the carbonyl groups [73]. R = adenosine diphosphate ribose.

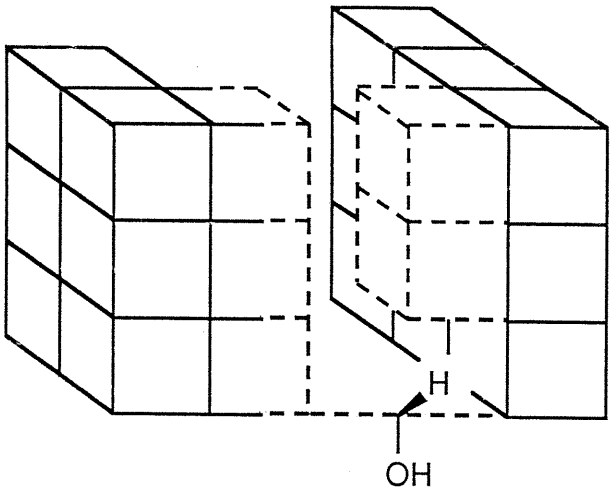
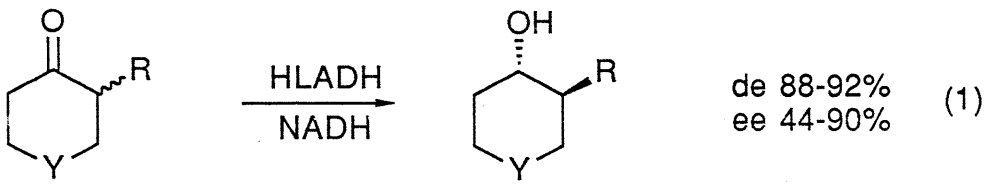
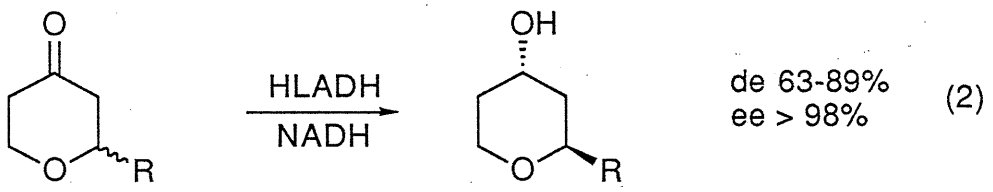


figure 4: Jones cubic-space section model [74]: the forbidden regions are shown by solid lines and the limited regions by dashed lines. The open spaces represent the allowed areas.



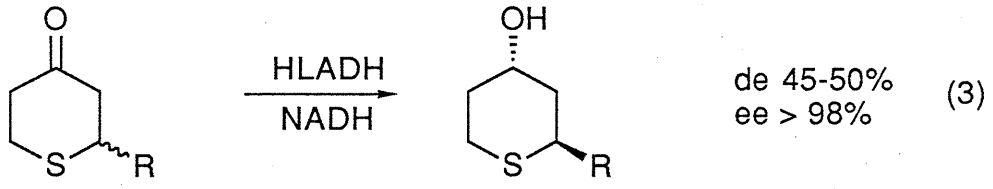
de 88-92%
ee 44-90% (1)

Y = CH_2 or S. R = Me or Et.



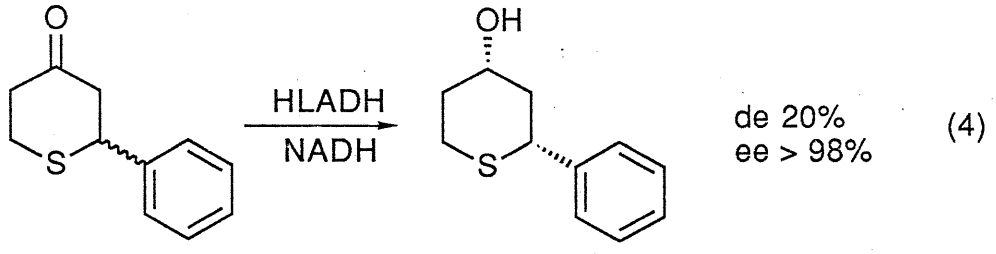
de 63-89%
ee > 98% (2)

R = Me, Et, iPr, Ph.



de 45-50%
ee > 98% (3)

R = Me, Et, or iPr.



de 20%
ee > 98% (4)

figure 5: HLADH catalyzed reductions of substituted six-membered cyclic ketones.

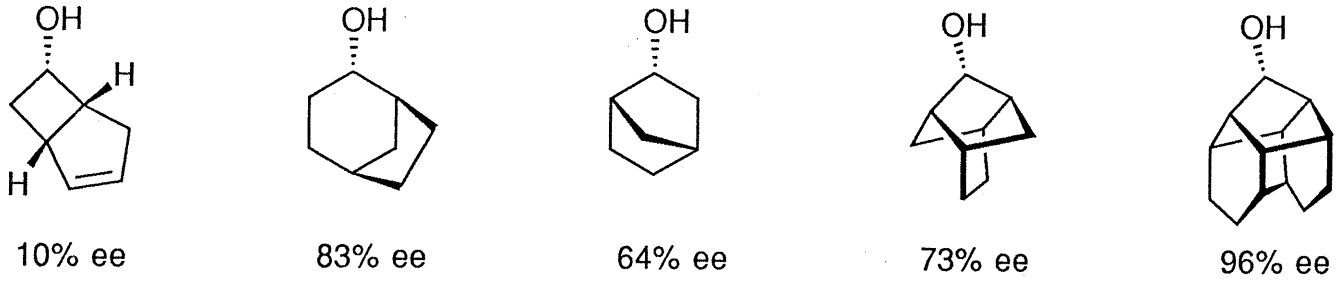


figure 6: Products of enantioselective reductions of bi-, tri-, and pentacyclic ketones catalyzed by HLADH.

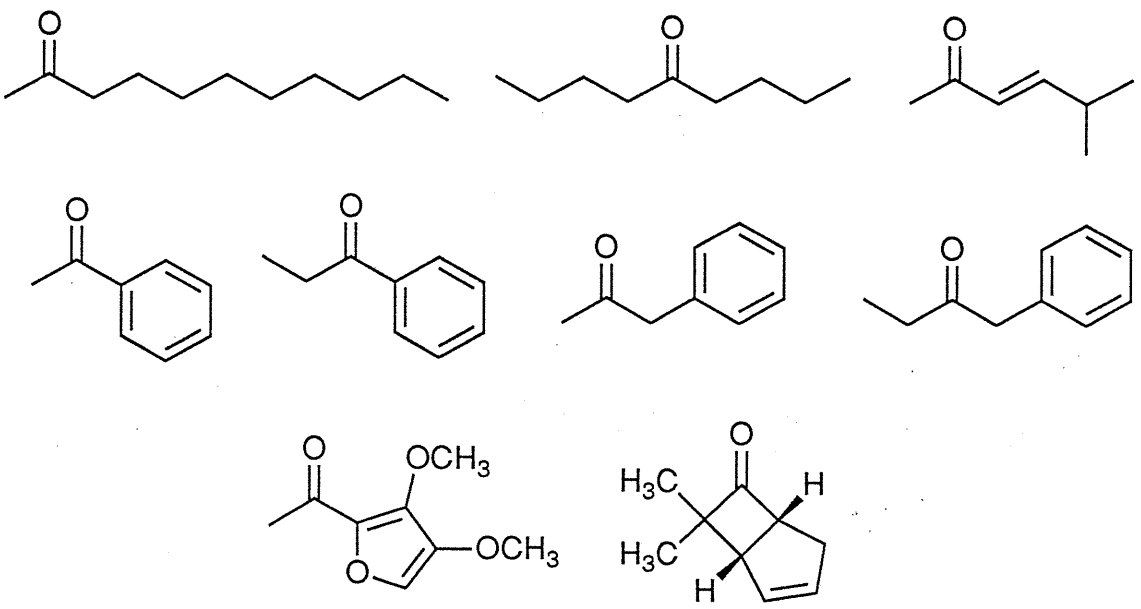


figure 7: Compounds that are not substrates of TBADH.

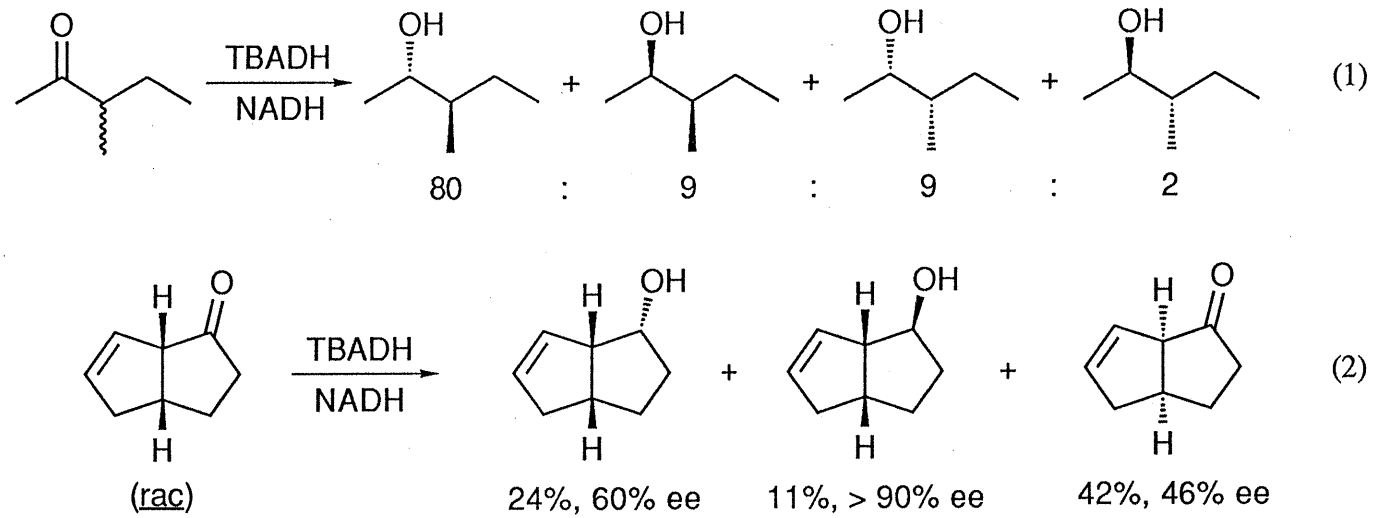


figure 8: TBADH catalyzed reductions of racemic ketones.

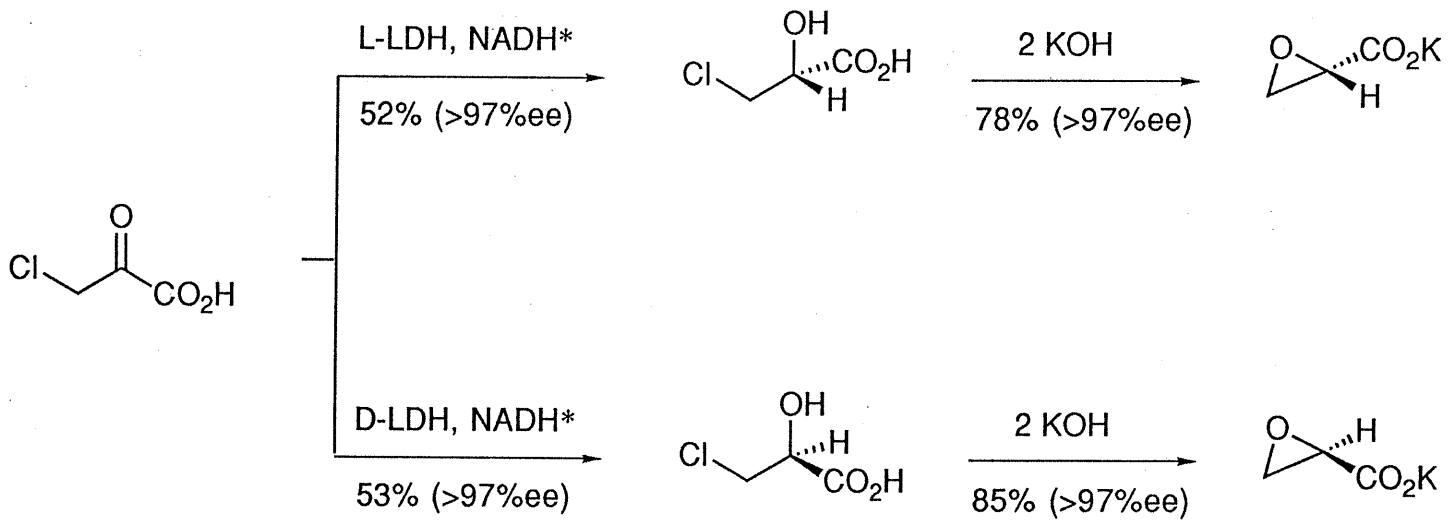


figure 9: Synthesis of D- and L-chlorolactic acid and potassium D- and L-glycidates (LDH = lactate dehydrogenase). *: NADH was regenerated with glucose-6-phosphate and glucose-6-phosphate dehydrogenase [43].

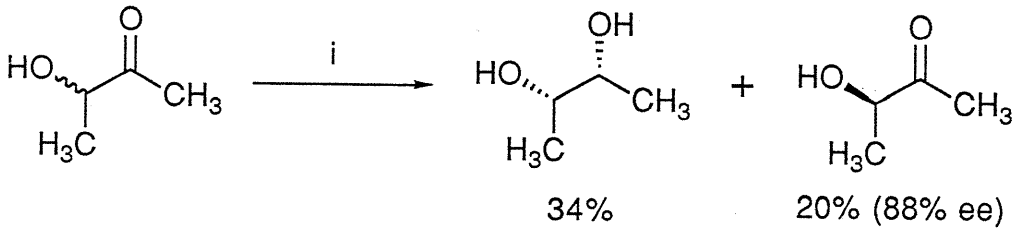


figure 10: Kinetic resolution of racemic α-hydroxyketones. i: glycerol dehydrogenase, glucose dehydrogenase and 0.5 equivalents of glucose [31].

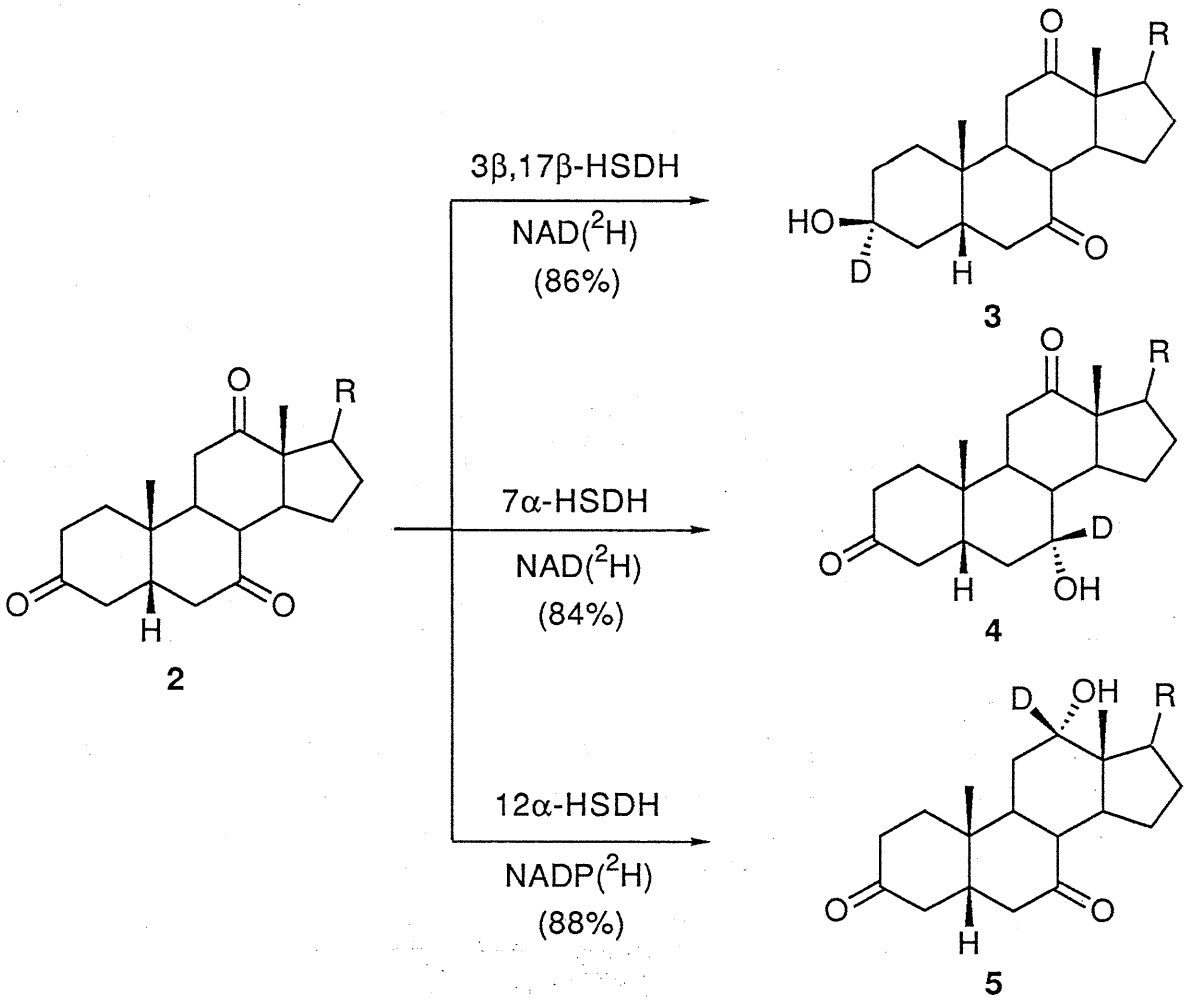
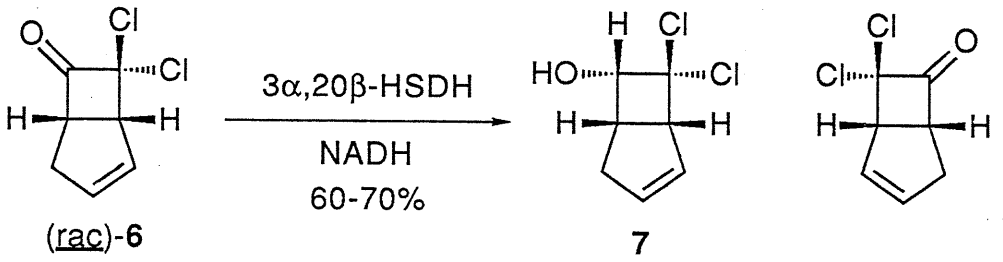


figure 11: Synthesis of selectively deuterated bile acids (R = $CH(CH_3)(CH_2)_2CO_2H$). The coenzymes NAD(^{2}H) and NADP(^{2}H) were regenerated with [1-^{2}H]glucose/glucose dehydrogenase [154]. 3β,17β-HSDH, 7α-HSDH, 12α-HSDH: 3β,17β-, 7α-, 12α-hydroxysteroid dehydrogenases (EC 1.1.1.51, EC 1.1.1.159, EC 1.1.1.176).



(rac)-6 7

figure 12: 3α,20β-HSDH catalyzed reduction of racemic 7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one (**6**). NADH was regenerated with EtOH/YADH [156].

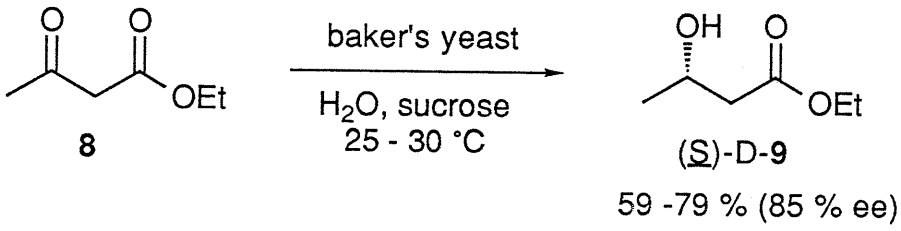


figure 13: Yeast-mediated reduction of ethyl acetoacetate published as Organic Syntheses preparation [163].

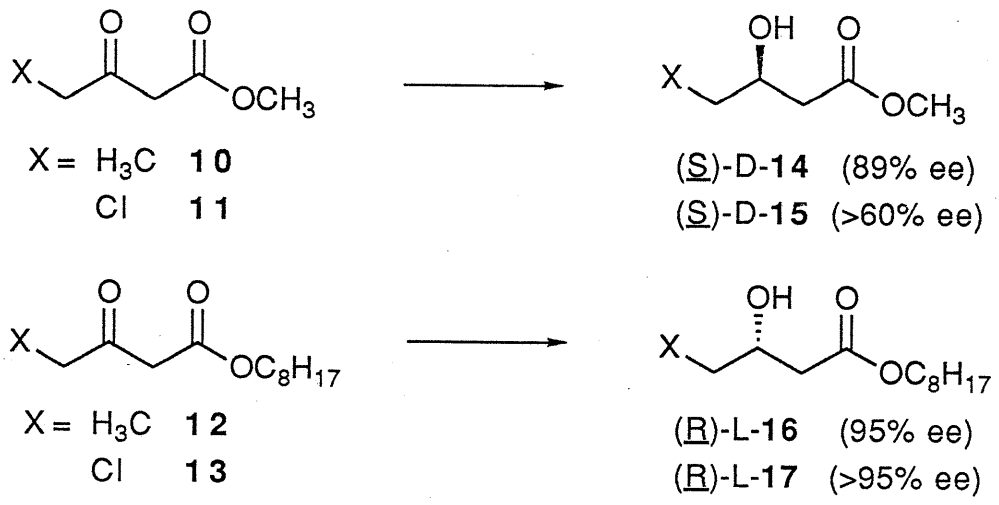


figure 14: Yeast mediated syntheses of ethyl- and octyl-4-chloro-3-hydroxybutanoate (**14** and **15**) [176,182] and methyl- and octyl-3-hydroxypentanoate (**16** and **17**) [173,176]. Structural modifications of the substrates result in opposite stereoselectivities.

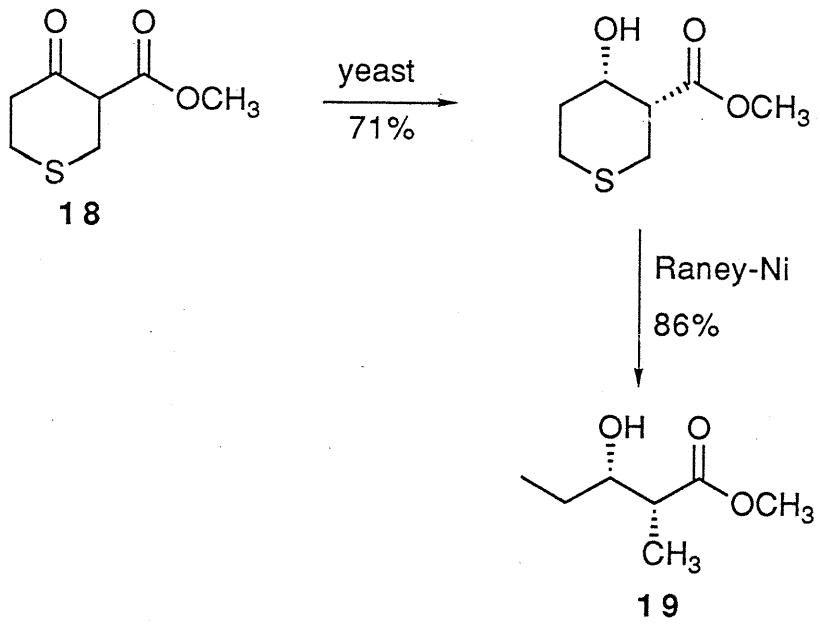
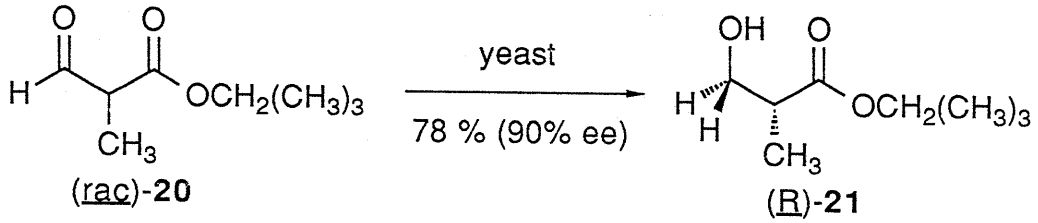


figure 15: Yeast mediated reduction of a temporarily cyclized β-ketoesters [194].



(rac)-**20** (R)-**21**

figure 16: Reductive resolution of racemic 2,2-dimethylpropyl 2-methyl-3-oxopropionate [195].

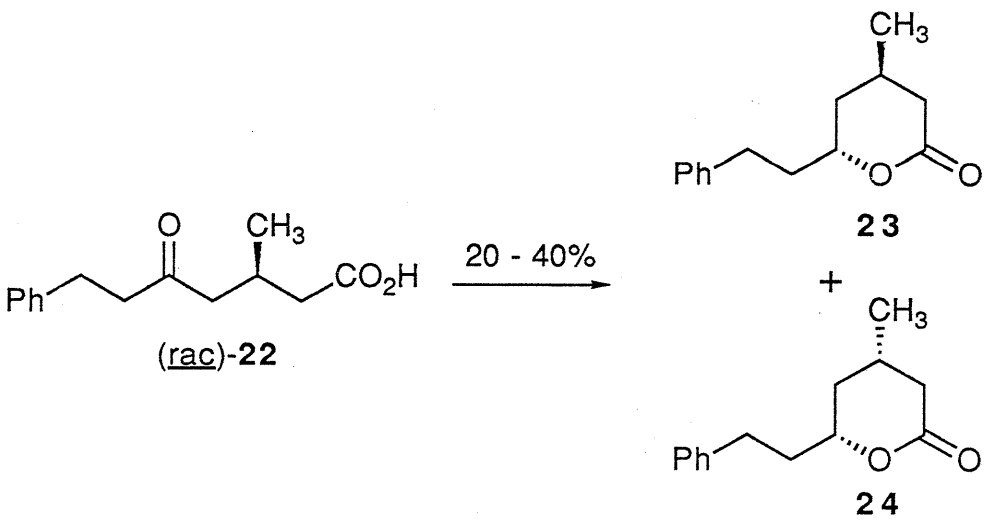


figure 17: Yeast-mediated synthesis of δ-lactones [199]. **23** and **24** were isolated in a ratio of 87 : 13 in optically pure form (100% ee).

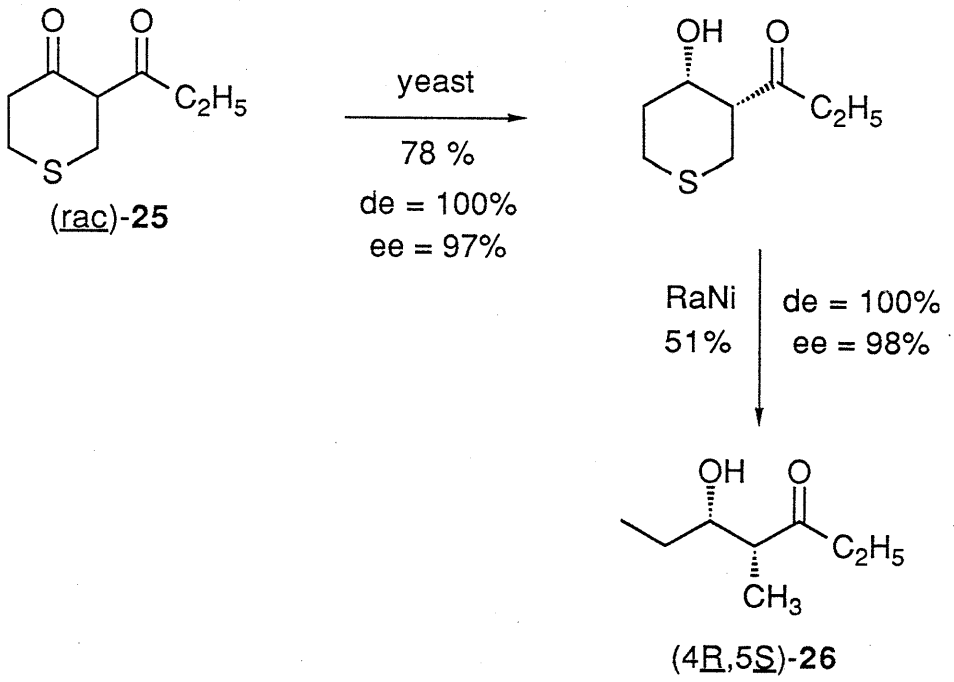
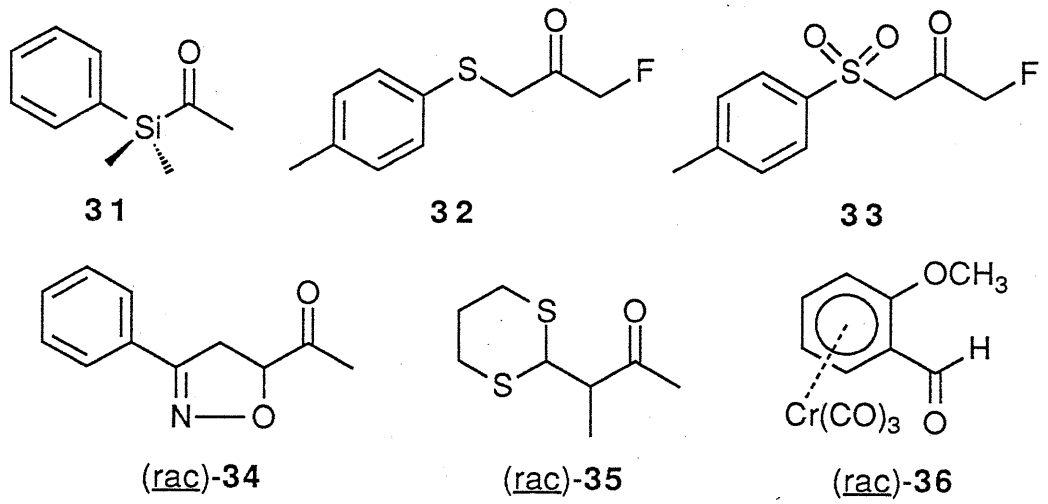


figure 18: Yeast-mediated reduction of the temporarily cyclized β-diketone **25**, followed by chemical desulfurization [207].



31 **32** **33**

(rac)-**34** (rac)-**35** (rac)-**36**

figure 19: Some highly functionalized carbonyl derivatives that are substrates for the reduction with bakers' yeast.

table 1: Relative Rates of Reduction of Aldehydes Catalyzed by YADH.[a]

aldehyde	V_{max} (rel.)
acetaldehyde	100
propanal	51
butanal	10
hexanal	5.0
acrolein	94
but-2-enal	6.2
hex-2-enal	4.4
benzaldehyde	0.01
3-phenylpropanal	8.7
cinnamaldehyde	0.88
glycolaldehyde	5[b]
lactaldehyde	9[b]
furfural	17[b]

[a]Data taken from references [86-89]. [b]These values are relative initial velocities, and do not necessarily correspond to relative maximal velocities.

table 2: **Kinetic Data for Reductions of Aldehydes Catalyzed by HLADH.**[a]

aldehyde	V_{max} (rel.)	K_m, μM
acetaldehyde	100	230
propanal	150	130
butanal	114	25
3-methylbutanal	693[b]	
hexanal	144	74
acrolein	101	280
but-2-enal	58	570
hex-2-enal	37	160
benzaldehyde	70	80
p-chlorobenzaldehyde	47	
p-methoxybenzaldehyde	66	
naphthaldehyde	~10	2
3-phenylpropanal	96	10
cinnamaldehyde	34	9
glycolaldehyde	5[b]	
lactaldehyde	9[b]	

glyceraldehyde	6.7[b]
furfural	787[b]

[a]Data taken from references [86,89,108,109]. [b]These values are relative initial velocities, and do not necessarily correspond to relative maximal velocities.

table 3: Enantioselective Reductions of α- and β-Ketoacetals and β- and γ-Ketoesters Catalyzed by HLADH.

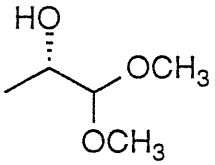
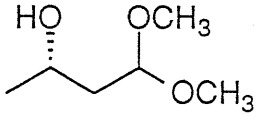
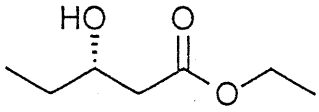
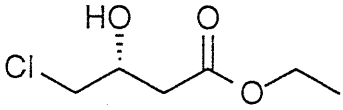
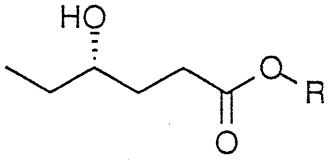
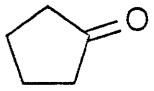
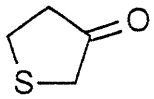
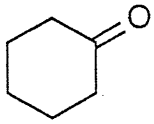
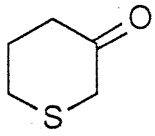
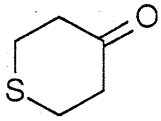
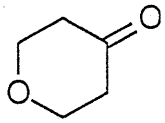
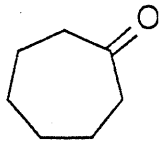
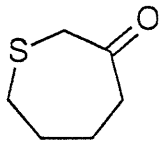
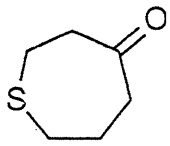
entry	product	ee [%]	yield [%]	reference
1		100	90	[18]
2		89	90	[18]
3		84	90	[18]
4		98	72	[18]
5	 R = CH_3, nC_4H_9	>98	-	[34]

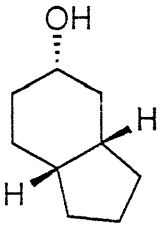
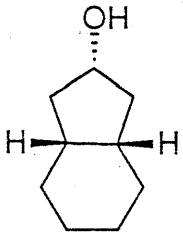
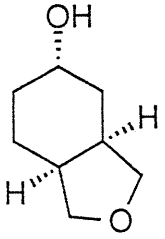
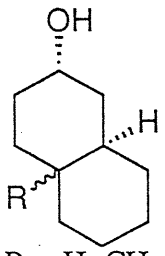
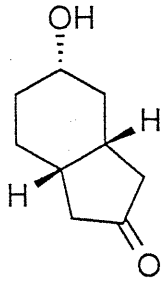
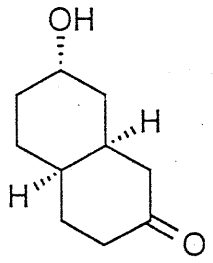
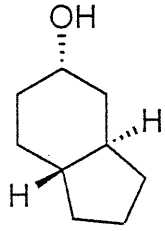
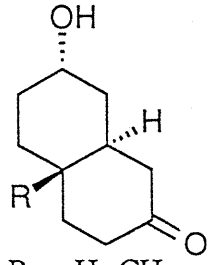
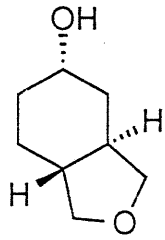
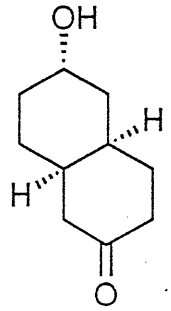
table 4: The Effect of Ring Size and Heteroatoms on the Reductions of Cyclic Ketones Catalyzed by HLADH.[a]

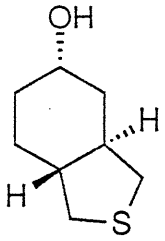
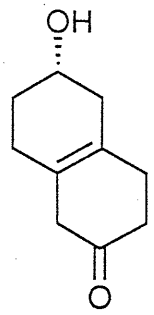
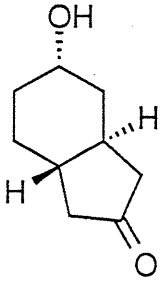
entry	substrate	V_{rel}[b]	K_m, mM	relative V_{max}
1		1	2.83	0.5
2		10	3.07	3.6
3		100	7.69	100
4		218	4.15	116
5		124	12.39	109
6		14	-	-
7		2	1.33	0.4
8		20	8.00	18
10		1	0.46	0.1

[a]Data taken from references [112] and [113]. Yields vary from 26 to 41%; ee vary from 10 to 33%.

[b]Initial velocity at 1 mM substrate concentration.

table 5: **Stereoselective Reductions of Bicyclic Ketones Catalyzed by HLADH.**

entry	product	yield (ee)[%]	V_{rel}[a]	ref.	entry	product	yield (ee)[%]	V_{rel}[a]	ref.
1		37 (12)	227	[119]	8		-	11	[119]
2		17 (93[b])	9	[120]	9	 R = H, CH_3 (cis or trans)	- (>98)	2	[74,114]
3		55 (60)	130	[119]	10		89 (>98)	24	[79,118]
4		17 (51[c])	190	[119]	11	 R = H, CH_3 or CH_2OH	40-79 (>98)	10-20	[79,118]
5		26 (>97)	53	[120]	12		50 (>98)	35	[79,118]

6		33 (>97)	1.4	[120]	13		18 (>98)[e]	14	[79,118]
7		42 (94[d])	120	[119]					

[a]Initial velocities at pH 6.5-7.0, 0.14-20 mM racemic or meso substrate. For cyclohexanone, $V_{rel} = 100$. [b]De = 55%. The (1R, 3R,6S) diastereoisomer had 19% ee. [c]De = 3%. The (1S, 3S,6S) diastereoisomer had 94% ee. [d]De = 79%. The (1S, 3R,6S) diastereoisomer had 95% ee. [e]After removal of isomerized α,β-unsaturated contaminant.

table 6: Relative Rates of Reduction of Aldehydes and Ketones Catalyzed by TBADH.[a]

aldehyde or ketone	V_{max} (rel.)
acetaldehyde	75
acetone	100
butanone	73
2-pentanone	40
2-hexanone	6.1[b]
3-hexanone	5.5[b]
2-heptanone	5.5[b]
3-heptanone	1.2[b]
4-heptanone	0.06[b]
cyclopropyl methyl ketone	4.8
cyclopentanone	58
cyclohexanone	60
cyclohex-2-enone	47
2-methylcyclohexanone	58
3-methylcyclohexanone	19

4-methylcyclohexanone	4.8
cycloheptanone	29
cyclooctanone	< 0.96
norbornanone	47

[a]Data taken from references [123] and [124]. [b]These values are relative initial velocities, and do not necessarily correspond to relative maximal velocities.

table 7: Alcohols Produced by TBADH-Catalyzed Reductions.

Enantiomeric excesses of the products are plotted vs. the relative rate of reduction of the ketone starting materials. Rates are relative to acetone = 100. Data taken from references [18,34,51,122,124] and [126-133].

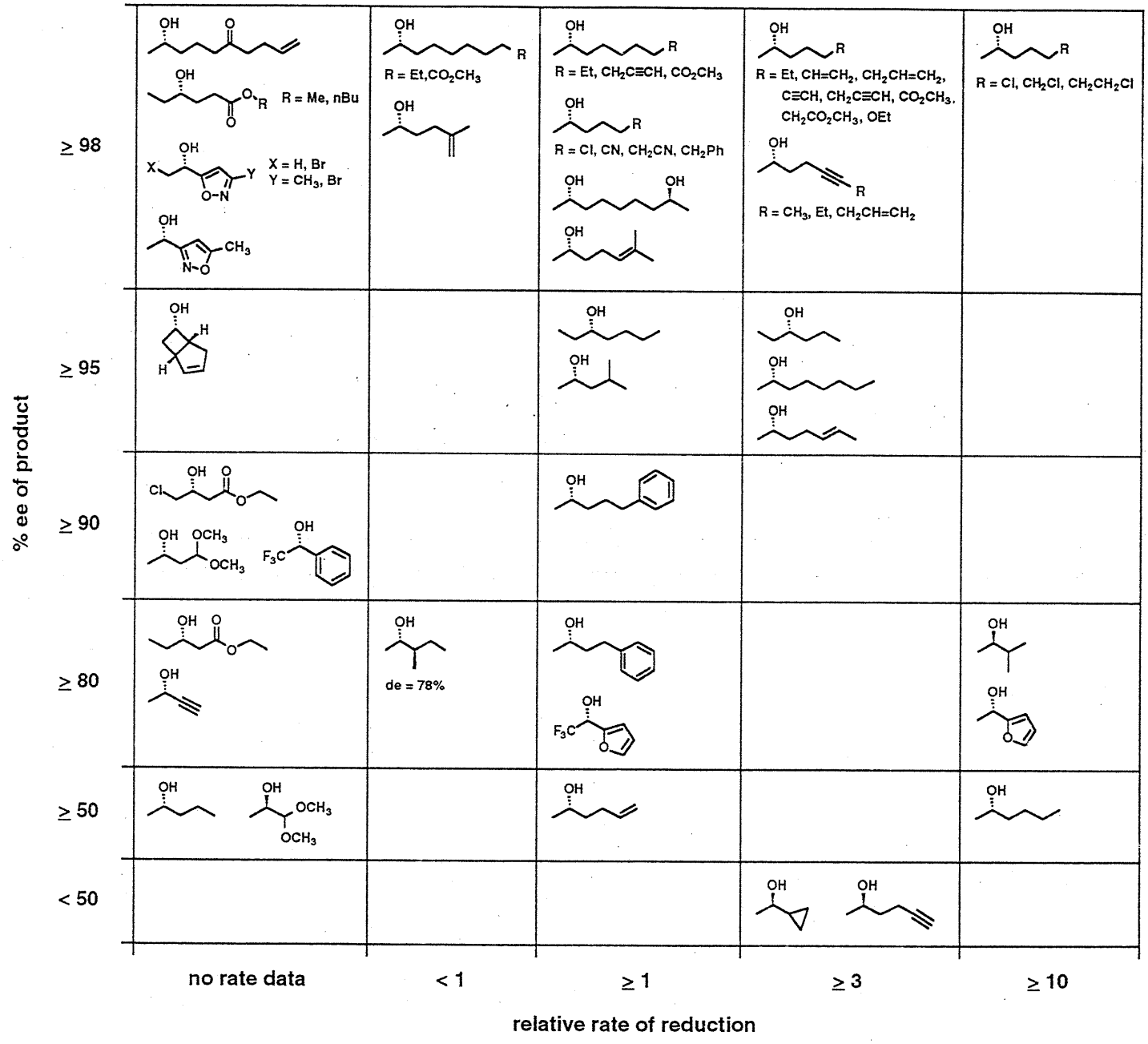

% ee of product
≥ 98
≥ 95
≥ 90
≥ 80
≥ 50
< 50
R = Me, nBu
X = H, Br
Y = CH3, Br
R = Et,CO2CH3
R = Et, CH2C≡CH, CO2CH3
R = Cl, CN, CH2CN, CH2Ph
R = Et, CH=CH2, CH2CH=CH2, C≡CH, CH2C≡CH, CO2CH3, CH2CO2CH3, OEt
R = CH3, Et, CH2CH=CH2
R = Cl, CH2Cl, CH2CH2Cl
de = 78%
no rate data
< 1
≥ 1
≥ 3
≥ 10
relative rate of reduction

table 8: **Rates and Enantioselectivities of Reductions of Carbonyl Compounds Catalyzed by LKADH and PADH.**

Rates are relative to 1-phenoxy-2-propanone = 100 for LKADH and butanone = 100 for PADH. Asterisks indicate the % ee of products of reductions (****, ≥ 98%, ***, ≥ 90%, **, ≥ 50%, and *, < 50%). Asterisks to the left of compounds are for reductions catalyzed by LKADH; asterisks underneath compounds are for reductions catalyzed by PADH. Data taken from references [8,9,44,131] and [135].

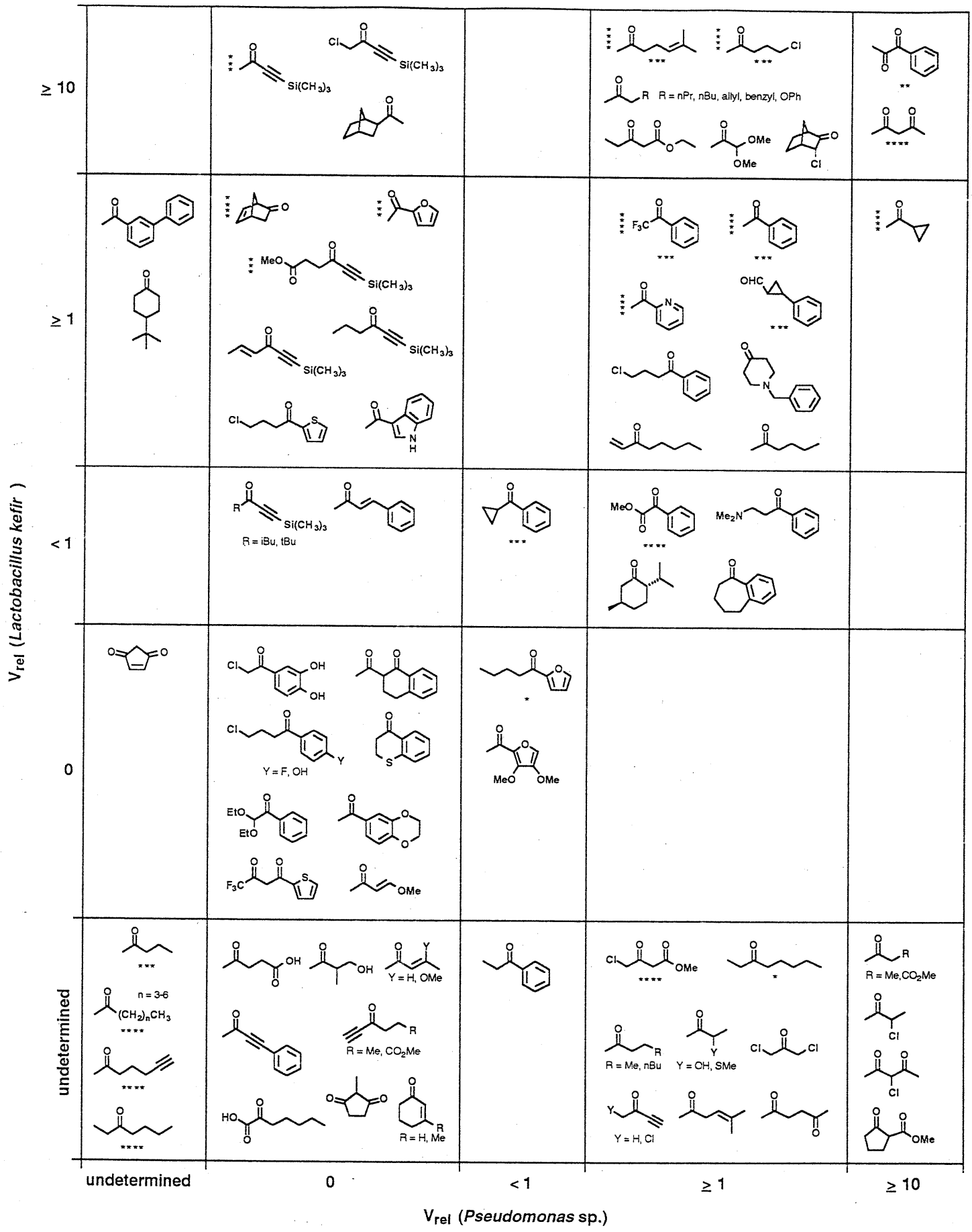
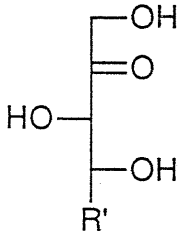
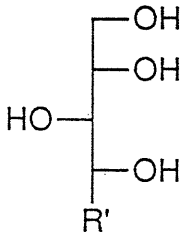
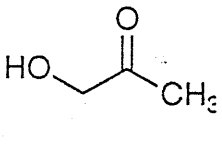
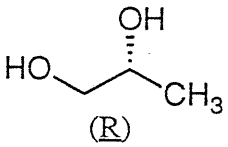
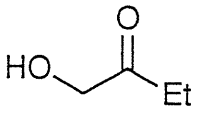
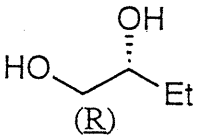
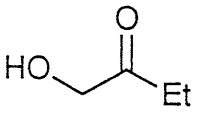
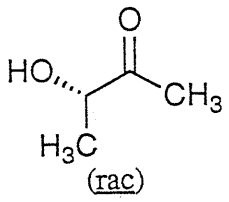
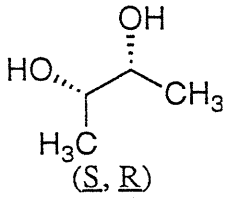
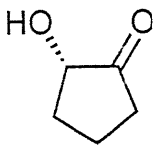
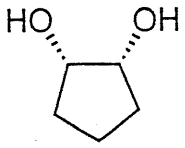

≥ 10
≥ 1
< 1
0
undetermined
V_{rel} (*Lactobacillus kefir*)
undetermined
0
< 1
≥ 1
≥ 10
V_{rel} (*Pseudomonas* sp.)
R = nPr, nBu, allyl, benzyl, OPh
R = iBu, tBu
Y = F, OH
Y = H, OMe
R = Me, CO_2Me
R = H, Me
n = 3-6
R = Me, nBu
Y = OH, SMe
Y = H, Cl
R = Me, CO_2Me

table 9: Reduction of α-Oxoacids

entry	R'	product-configuration	enzyme / cofactor (regeneration System)	yield [%] (ee [%])	ref.
1	$ClCH_2$	(S)-L-	L-LDH / NADH (G6P/G6PDH)	52 (>97)	[42]
2	$ClCH_2$	(R)-D-	D-LDH / NADH (G6P/G6PDH)	53 (>97)	[42]
3	C_2H_5	(S)-L-	L-LDH / NADH (formate / FDH)	99 (>99)	[139]
4	C_2H_5	(R)-D-	D-LDH / NADH (formate / FDH)	95 (>98)	[140]
5	C_3H_7	(S)-L-	L-LDH / NADH (formate / FDH)	97 (>99)	[139]
6	C_6H_5	(S)-L-	L-LDH / NADH (formate / FDH)	96 (>99)	[139]
7	C_6H_5	(R)-D-	D-LDH / NADH (formate / FDH)	98 (>98)	[140]
8	cyclopropyl	(S)-L-	L-LDH / NADH (formate / FDH)	94 (>99)	[139]
9	$CH_2=C(CH_3)$	(S)-L-	L-LDH / NADH (formate / FDH)	66 (99)	[10]
10	$CH_3CH=CH$	(S)-L-	L-LDH / NADH (formate / FDH)	98 (97)	[10]
11	$C_6H_5CH=CH$	(S)-L-	L-LDH / NADH (formate / FDH)	85 (98)	[10]
13	$C_6H_5(CH_2)_2$	(R)-D-	D-LDH / NADH (formate / FDH)	85 (-)	[141]
14	$(CH_3)_2CHCH_2$	(R)-D-	R-HicDH / NADH (formate / FDH)	88 (100)	[142]
15	$^-O_2CCH_2$	(S)-L-	L-MalDH / NADH (formate / FDH)	-	[143]

table 10: **Reduction of Ketoses and α-Hydroxyketones**

entry	substrates	products	enzyme / cofactor (regeneration system)	yield [%] (ee [%])	ref.
					
1	R' = $CH_2SC_6H_5$		SDH / NADH (formate / FDH)	50 [a]	[146]
2	R' = $CH(OCH_2CH_3)_2$		SDH / NADH (formate / FDH)	69	[147]
3	R' = $CH{=}CH_2$		SDH / NADH (formate / FDH)	61	[148]
4	R' = $CH_2CH{=}CH_2$		SDH / NADH (formate / FDH)	77	[148]
5	R' = (D)-(HO)CHCH=CH_2		SDH / NADH (formate / FDH)	68	[148]
6	R' = (L)-(HO)CHCHCH_2		SDH / NADH (formate / FDH)	92	[148]
7		 (R)	GlyDH / NADH (formate / FDH)	50 (98)	[31]
8		 (R)	GlyDH / NADH (formate / FDH)	70 (98)	[31]
9		(R)-2-d_1-1,2-butanediol	GlyDH / NAD(^{2}H) (formate-d_2 / FDH)	42 (98 % d_1)	[31]

10	 (rac)	 (S, R) and (R)-ketone	GlyDH / NADH (G6P / G6PDH) or (glucose / GDH)	34 and 20 (88) or 39 and 21 (<80)	[31] [31]
11	 (rac)	 (S, R) and (R)-ketone	GlyDH / NADH (G6P / G6PDH)	23 and 22 (70-86)	[31]

[a]Isolated as peracetate

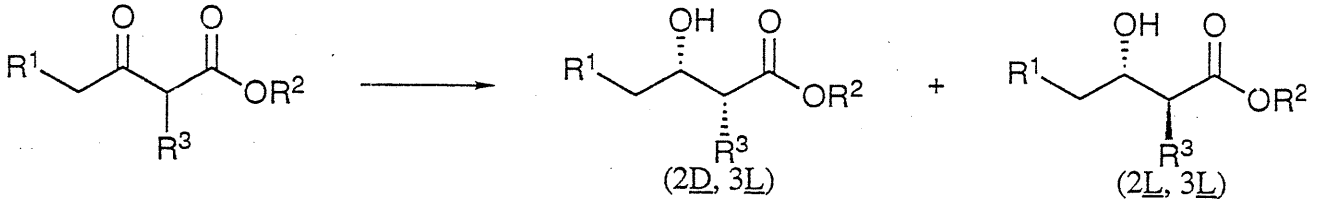
table 11: **Yeast Catalyzed Reductions of β-Keto Acids and β-Keto Carboxylates**

entry	R^1	R^2	product-configuration	yield [%]	ee [%]	ref.
1	CH_3	CH_3	(R)-D-	56	96	[168]
2	CH_3	CH_3	(S)-L-	23	87	[169]
3	CH_3	C_2H_5	(S)-L-	32	96	[169]
4	CF_3	C_2H_5	(R)-L-	75, 70	50, 53	[170,171]
5	CCl_3	C_2H_5	(S)-D-	70	84	[170]
6	C_2H_5	CH_3	(R)-D-	67	40	[172]
7	C_2H_5	C_8H_{17}	(S)-L-	75	>95	[173]
8	t-$BuOCH_2$	CH_3	(R)-L-	70	82	[175]
9	t-$BuOCH_2$	C_2H_5	(R)-L-	72	97	[175]
10	N_3CH_2	C_2H_5	(R)-L-	70-80	80	[176]
11	N_3CH_2	C_8H_{17}	(R)-L-	70-80	100	[176]
12	$ClCH_2$	C_2H_5	(S)-D-	42, -	85, >60	[168,176]
13	$ClCH_2$	C_8H_{17}	(R)-L-	40, 60 , -	75, 70, >95	[165,176,177]
14	$BrCH_2$	C_2H_5	(S)-D-	40-50	100	[175]
15	$H_5C_2SCH_2$	CH_3	(R)-L-	55	70	[165]
16	$H_2C=CH(CH_2)_2$	K	(R)-D-	38	99	[169]
17	$H_2C=CH(CH_2)_2$	CH_3	(R)-D-	30	92	[169]

18	$H_2C=CH(CH_2)_2$	C_4H_9	(R)-D-	66	81	[169]
19	$NC(CH_2)_4$	C_2H_5	(S)-L-	46	56	[178]
20	$NC(CH_2)_4$	t-Bu	(S)-L-	60	64	[178]
21	$NC(CH_2)_4$	C_8H_{17}	(S)-L-	77	82	[178]
22	n-$C_{15}H_{31}$	K	(R)-D-	40[a]	98	[179]

[a]Isolated as methyl ester after treatment with CH_2N_2.

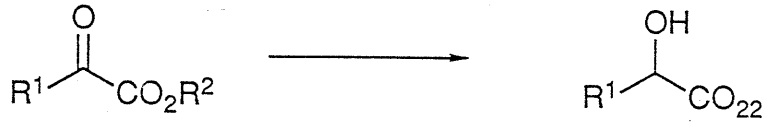
table 12: Yeast Catalyzed Reductions of α-Substituted-β-Keto Esters



entry	R^1	R^2	R^3	(2D, 3L) : (2L, 3L) (ee [%])	yield [%]	ref.
1	CH_3	CH_3	CH_3	81 : 19 (100 , 100)	71	[186,187]
2	CH_3	C_2H_5	CH_3	6.4 : 1 (>95)	59	[188]
3	CH_3	C_8H_{17}	CH_3	95 : 5 (100 , high)	82	[186]
4	CH_2OCH_2Ph	CH_3	CH_3	10 : 90 (68 , 6)	50	[189]
5	CH_2OCH_2Ph	C_2H_5	CH_3	54 : 46 (36 , 24)	46	[189]
6	CH_2OCH_2Ph	i-C_3H_7	CH_3	24 : 76 (96 , 50)	64	[189]
7	CH_3	C_2H_5	$CH_2CH{=}CH_2$	25 : 75 (100 , 100)	84	[187]
8	CH_3	C_2H_5	CH_2Ph	34 : 66 (100 , 100)	21	[187]
9	CH_3	CH_3	SCH_3	72 : 28 (>96 , >96)	72	[190]
10	CH_3	CH_3	SPh	83 : 17 (>96 , >96)	40	[190]
11	CH_3	t-C_4H_9	SCH_3	41 : 59 (>96 , >96)	75	[190]
12	CH_3	C_2H_5	dithian	96 : 14 (>99, >99)	62	[191]
13	CH_3	C_2H_5	OH	28 : 72 (90, >99)	68	[192]
14	C_2H_5	C_2H_5	OH	10 : 90 (69, 97)	72	[192]
15	n-C_4H_9	C_2H_5	OH	20 : 80 (70[a], 97)	67	[192]

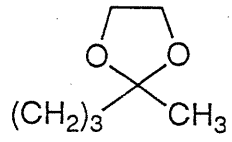
[a]The absolute configuration in this case is (2S, 3R).

table 13: Yeast mediated Reductions of α-Ketoesters



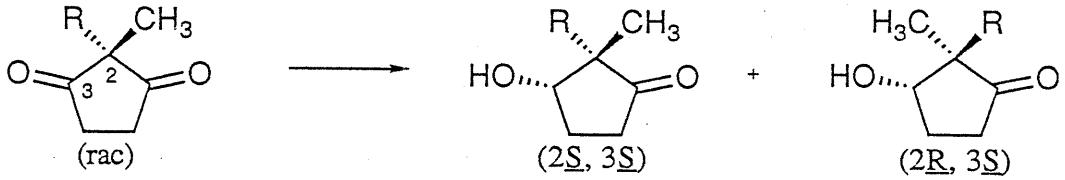
entry	R^1	R^2	product configuration	yield [%]	ee [%]	ref.
1	CH_3	C_2H_5	(R)-D-	49	92	[200]
2	CH_3	C_2H_5	(S)-L-	47	91	[201]
3	C_2H_5	C_2H_5	(S)-L-	42	75	[202]
4	C_6H_5	CH_3	(R)-D-	59	100	[200]
5	2-(5-(CH_3CO_2)furyl)	CH_3	(R)-D-	55	92	[202]

table 14: **Yeast Mediated Reduction of β-Diketones**

entry	R^1	R^2	product-configuration	yield [%]	ee [%]	ref.
1	CH_3	CF_3	(S)-D-	78	70-72	[171]
2	CH_3	CH_3	(S)-L-	90, 18	>99, >99	[203,204]
3	CH_3	C_2H_5	(S)-L-	100, 48	>99, 97	[203,204]
4	CH_3	C_3H_7	(S)-L-	62	94	[204]
5	CH_3	C_4H_9	(S)-L-	42	>99	[204]
6	CH_3	C_5H_{11}	(S)-L-	25	96	[204]
7	CH_3	C_8H_{17}	(S)-L-	23	97	[204]
8	CH_3	$CH(CH_3)_2$	(S)-L-	24	92	[204]
9	CH_3	$(CH_2)_2CH=CH_2$	(S)-L-	100	>99	[203]
10	CH_3	C_6H_5	(S)-L-	21, 100[a]	94, 99	[204,205]
11	CH_3		(S)-L-	65	97.5	[206]
12	C_2H_5	C_2H_5	(R)-D-	100	30	[203]

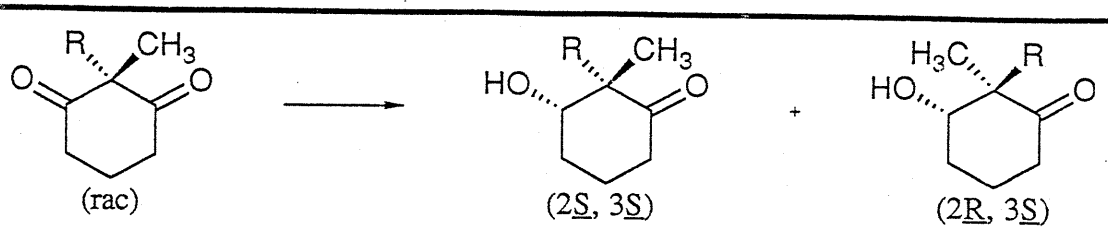
[a]Mixture of 2-hydroxy and 4-hydroxyketones (85 : 15).

table 15: **Yeast Mediated Reduction of 2,2-Disubstituted Cyclopentadiones**



entry	R	(2S, 3S)	:	(2R, 3S)	yield [%]	ee [%]	ref.
1	C_3H_7	100		-	60	>98	[208]
2	$HCC{\equiv}CH_2$	67		33	60	>98	[208,210]
3	$CH_2{=}CHCH_2$	90		10	75	>98	[208,210]
4	$CH_2{=}CHCH_2(CH_3)$	100		-	75	>98	[208,210]
5	$CH_3O_2CCH_2CH_2$	100		-	52	>98	[208]

table 16: **Yeast Mediated Reduction of 2,2-Disubstituted Cyclohexadiones**



entry	R	(2S, 3S)	:	(2R, 3S)	yield [%]	ee [%]	ref.
1	CH_3	100[a]			47 - 52	>96	[164]
2	C_3H_7	22		78	80	>98	[208]
3	$HCC{\equiv}CH_2$	27		73	75	>98	[208]
4	$CH_2{=}CHCH_2$	45		55	80	>98	[208]
5	$CH_2{=}CHCH_2(CH_3)$	40		60	49	>98	[208]
6	$CH_3O_2CCH_2CH_2$	35		65	20	>98	[208]

[a]The starting material is prochiral. The product has (S) configuration.

table 17: **Yeast Mediated Reduction of α-Hydroxy Ketones**

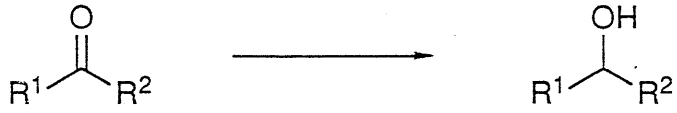
entry	R^1	R^2	product-configuration	yield [%]	ee [%]	ref.
1	CH_3	H	(R)-D-	60	91	[212]
2	C_2H_5	H	(R)-D-	49-58	-	[162]
3	C_6H_5	$COCH_3$	(S)-D-	70	94	[213]
4	$(CH_3)_2CHCH_2$	H	(R)-D-	65	100	[212]
5	$(CH_3)_3C$	H	(R)-D-	66	82	[212]
6	$(CH_3)_2C=CH(CH_2)_2$	H	(R)-D-	68	97	[214]
7	$C_6H_5OCOCH_2$	H	(S)-L-[a]	99	>99	[215]
8	p-$(H_3CO)C_6H_5$	H	(R)-D-	85	98	[216]
9	p-$(H_3CO)C_6H_5$	$COCH_3$	(R)-D-	25	82	[216]
10	$C_6H_5SO_2CH_2$	H	(S)-L-[a]	87	99	[217]

[a]The stereoselectivity of all reductions in this table are the same. In entries 7 and 10 the ester and sulfone respectively, are considered higher priority than the hydroxy function (OR^2).

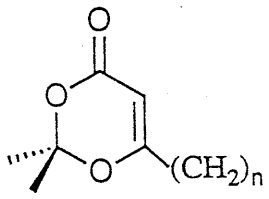
table 18: **Yeast Mediated Reductions of Aromatic Aldehydes and Ketones**

entry	R^1	R^2	product-configuration	yield [%]	ee [%]	ref.
1	C_6H_5	CF_3	(R)	87	>80	[218]
2	naphtyl	CF_3	(R)	61	>80	[218]
3	C_6H_5	CH_3	(S)	45	89	[219]
4	C_6H_5	C_2H_5	(S)	19	72	[219]
5	C_6H_5	n-C_3H_7	(S)	12	81	[219]
6	C_6H_5	n-C_4H_9	(S)	45	89	[219]
7	p-(X)C_6H_4					
	X = CH_3	CH_3	(S)	9	84	[220]
	F	CH_3	(S)	16	88	[220]
	Cl	CH_3	(S)	30	86	[220]
	Br	CH_3	(S)	26	90	[220]
	CO_2CH_3	CH_3	(S)	22	86	[220]
	CN	CH_3	(S)	16	96	[220]

table 19: Yeast Mediated Reductions of Aliphatic Ketones

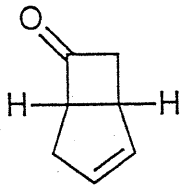
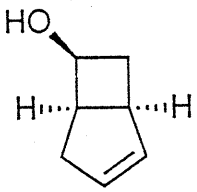
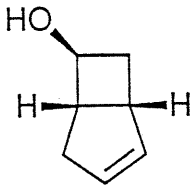
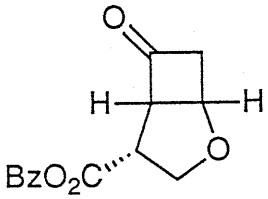
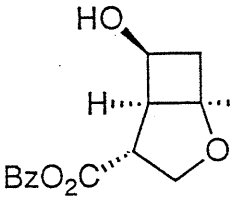
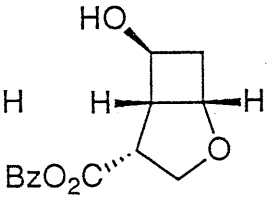
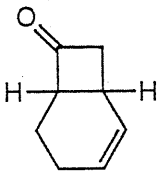
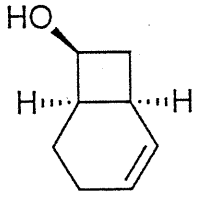
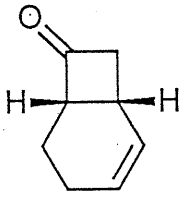
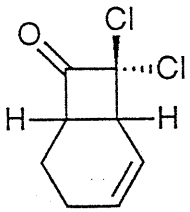
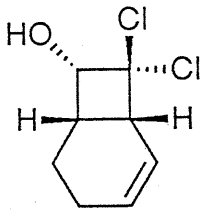
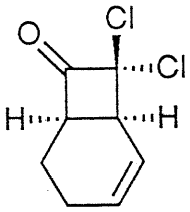


entry	R^1	R^2	product configuration	yield [%]	ee [%]	ref.
1	$C_2H_5O_2C(CH_2)_2$	CH_3	(S)	60	>98	[221]
2	$C_2H_5O_2C(CH_2)_7$	CH_3	(S)	46	96	[222]
3	$HO(CH_2)_n$					
	n = 2	$SO_2C_6H_5$	(S)	42	94	[217]
	3	$SO_2C_6H_5$	(S)	74	93	[217]
	4	$SO_2C_6H_5$	(S)	39	96	[217]
	5	$SO_2C_6H_5$	(S)	84	72	[217]
4	$NCCH_2$	CH_3	(S)	55	>98	[223]
5	$NC(CH_2)_2$	CH_3	(S)	33	97	[223]
6	$NCCH_2$	C_6H_5	(S)	21	85	[222]
7	$NCCH_2$	C_8H_{17}	(R)	66	15	[222]
8	$O_2N(CH_2)_2$	CH_3	(S)	40 - 50	98	[224]
9	$O_2N(CH_2)_3$	CH_3	(S)	60	94	[224]
10	$(CH_3)_2NCS_2CH_2$	CH_3	(S)	91	>96	[225]
11	$(CH_3)_2NCS_2CH_2$	C_5H_{11}	(S)	39	>96	[225]
12	$(CH_3)_2NCS_2CH(CH_3)$	CH_3	(S)	82[a]	>96	[225]

13						
	n = 0	CH_3	(S)	90	91	[226]
	1	CH_3	(S)	44	90	[226]
	2	CH_3	(S)	58	>99	[226]
	3	CH_3	(S)	15	94	[226]
14	p-$(H_3C)C_6H_4SO_2(CH_2)_n$					
	n = 2	CH_3	(S)	68	98	[227]
		C_2H_5	(S)	34	67	[227]
	2	CH_3	(S)	44	98	[227]
	3	CH_3	(S)	6	81	[227]
	4					
15	$H_3COC_6H_4CH_2$	CH_3	(S)	80	95	[216]
16	$C_6H_5(CH_2)_2$	CH_3	(S)	82	90	[216]

[a]Threo : erythro = 28 : 72.

table 20: Yeast Mediated Reduction of Bicyclic Ketones

entry	substrate[a]	products	yields [%] (ee [%])	reference
1	 **27**	 	38 (88), 18 (84)	[234,235]
2	 **28**	 	- (95), - [b]	[236]
3	 **29**	 	25 (92), 32 (40)	[237]
4	 **30**	 	27 (≥99), 14 (88)	[237]

[a]All substrates were racemic mixtures. [b]Yields and ee value of the all cis product were not given.