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ENZYMES IN ORGANIC SYNTHESIS

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INTRODUCTION

While the abilities of enzymes to act as specific and chiral catalysts have been recognized for many years, particularly by the pharmaceutical industry, it is only now that these biochemical procedures are becoming accepted as routine procedures in organic synthesis. This Report provides illustrative examples of the enormous scope and range of synthetic opportunities that currently exist in this rapidly developing field. The literature has been reviewed to the end of 1984.

Although the synthetic use of enzymes is being adopted with increasing and enthusiastic alacrity, some apprehension remains amongst many organic chemists, particularly with respect to the experimental techniques involved. Accordingly, in order to accentuate the ease with which the biological approach can be exploited with everyday organic chemical laboratory equipment, emphasis has been placed in this review on the biochemical catalysts to which chemists have the most ready access, such as commercially available enzymes and baker's yeast.

The experimental methodology for exploiting enzymes is very straightforward. Yeast is also easy to use since, often, all that is required is to throw a handful into tap water, together with the substrate. The yeast itself contains enough nutrients to do the job. Reactions effected by other readily obtained (from culture collections) microorganisms have been included when they provide an especially appropriate illustration of a useful transformation. Routine use of such microorganisms does not present a problem. Guides to procedures for selecting the most suitable bug for a desired conversion, and descriptions of simple fermentation techniques written from organic chemical perspectives, are readily available. Any new skills required are quickly learned by anyone familiar with organic synthesis methodology. Information on these and other general and experimental aspects of enzymic and fermentative reactions, including the effects of organic solvents and inhibitors, are provided in several recent reviews.[1-15]

More than 2000 enzymes are known.[16] Several hundred of these are commercially available, including a significant number in immobilized[17] forms, from biochemical supply houses such as Sigma and Boehringer. In comparison with other catalysts, enzymes are exceptional in three main respects.

(1) They are extremely versatile and catalyze a broad spectrum of reactions. There is an enzyme-catalyzed equivalent for most types of organic reactions. Some major exceptions are the Diels–Alder reaction, and also the Cope rearrangement, although other [3,3]-sigmatropic reactions, such as the

Claisen rearrangement,[18] are known. Enzyme-mediated reactions take place under mild conditions, often at room temperature and close to neutral pH. This minimizes problems of isomerization, racemization, epimerization and rearrangement that often plague traditional methodology.

(2) Enzymes can be highly efficient catalysts. The rates of enzyme-promoted reactions can be faster than those of the corresponding uncatalyzed reactions by factors of up to 10^{12}.

(3) Enzymes are generally very selective in terms of the type of reaction catalyzed and with respect to the structure and stereochemistry of the substrate and product. These properties collectively constitute the specificity of an enzyme and are its most important feature for selective and asymmetric synthetic exploitation.

The International Union of Biochemistry classification divides enzyme-catalyzed reactions into six main groups.[16]

(1) *Oxidoreductases.* Enzymes of this group catalyze oxidation–reduction reactions involving oxygenation, such as C—H → C—OH, or overall removal or addition of hydrogen atom equivalents, as for $\mathrm{CH(OH)} \rightleftharpoons \mathrm{C{=}O}$ and $\mathrm{CH{-}CH} \rightleftharpoons \mathrm{C{=}C}$.

(2) *Transferases.* These enzymes mediate the transfer of groups such as acyl, sugar, phosphoryl, and aldehyde or ketone moieties from one molecule to another.

(3) *Hydrolases.* The range of functional groups hydrolyzed by this group is very broad. It includes glycosides, anhydrides and esters, as well as amides, peptides and other C—N-containing functions.

(4) *Lyases.* These enzymes catalyze additions, usually of HX, to double bonds such as C═C, C═N, and C═O, and the reverse processes.

(5) *Isomerases.* Various isomerizations, including C═C bond migration, *cis–trans* isomerization, and racemization, can be effected.

(6) *Ligases.* These are often called synthetases and mediate the formation of C—O, C—S, C—N, C—C, and phosphate ester bonds.

At the present time, the enzymes of groups 1, 2, 3 and 4 are the most useful in synthesis.

The majority of enzymes catalyzing reactions of organic chemical interest require coenzymes such as NAD(P)/H† or ATP. These cofactors are too expensive to be used in the stoichiometric amounts formally required. Accordingly, when coenzyme-dependent enzymes are employed as catalysts, the expensive cofactors required are used in catalytic amounts in conjunction with an efficient, inexpensive, system for continuous *in situ* regeneration of the active form of the cofactor. Methods are now available that permit the economical research-scale use of NAD(P)/H or ATP in reactions where up to 1 kg of substrate is converted to product.[10]

ENZYME SPECIFICITY

The synthetic opportunities provided by enzymes stem from the specificity with which the catalyses are effected. Several distinct aspects of enzyme specificity are recognized.[19–21] Of these, it is the degree to which enzymes discriminate between structural and stereochemical features of their substrates that determines their synthetic utility.

The most useful enzymes for organic synthesis applications are those which accept a broad structural range of substrates, while retaining the ability to operate stereospecifically on each. Although these requirements are basically antithetical, they are satisfied by many enzymes. Generally, mammalian enzymes fit such criteria best. Microbial enzymes usually have narrower structural specificity tolerances. However, this is compensated for by the larger selection of microorganisms available.

The identification of an enzyme capable of catalyzing a specific reaction on a new substrate

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† Abbreviations used: NAD/H and NADP/H, oxidized and reduced forms of nicotinamide adenine dinucleotide and its phosphate, respectively; ATP, adenosine triphosphate; ADP, adenosine diphosphate; GTP, guanosine triphosphate; GDP, guanosine diphosphate; UTP, uridine triphosphate; SAM, S-adenosylmethionine; CoA, coenzyme A; P, phosphate; Ur, uracil; Hy, hypoxanthine; Cy, cytosine; Ad, adenine; Ads, adenosine; A, adenylic acid; G, guanidylic acid; C, cytidylic acid; T, thymidylic acid; I, inosinic acid; Z, carbobenzyloxy; PRPP, 5-phosphoribosepyrophosphate; NRRL, Northern Regional Research Laboratory, Peoria, Illinois; YADH, yeast alcohol dehydrogenase; HLADH, horse liver alcohol dehydrogenase; CT, chymotrypsin; PLE, pig liver esterase; PLADH, pig liver alcohol dehydrogenase; PPL, porcine pancreatic lipase; MJADH, *Mucor javanicus* alcohol dehydrogenase; CFADH, *Curbularia falcata* alcohol dehydrogenase; DAP, 2,6-diaminopimelic acid; PEP, phosphoenol pyruvic acid; ee, enantiomeric excess; HPETE, hydroperoxyeicosatetraenoic acid.

structure is performed initially as for any other chemical transformation, by seeking literature analogies. For example, the known conversion of cinerone (**1**) to cinerolone (**2**)[22] was used as the literature guide in the selection of ***Aspergillus niger*** as a suitable organism for the stereospecific hydroxylation of **3** to the prostaglandin synthon **4**[23] (Fig. 1).

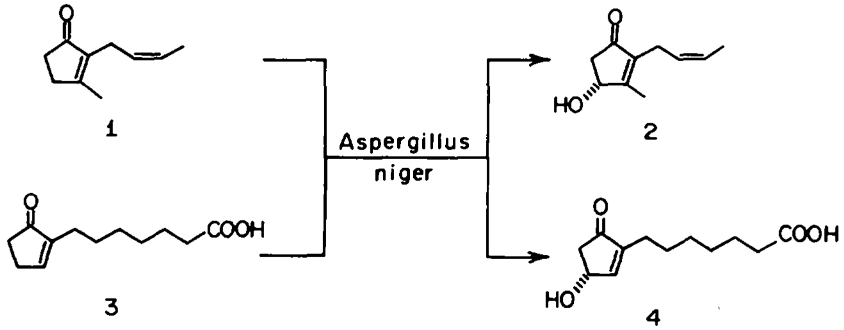

Fig. 1. The same enzyme or microorganism transforms similar substrates with the same regio- and stereospecificity.

Another simplifying practical factor is that a broad structural range of substrates is often accessible using a very limited number of enzymes. This is illustrated in Fig. 2 by the spectrum of $CH(OH) \rightleftharpoons C{=}O$ oxidations that can be achieved on substrates ranging in complexity from simple aliphatic to polycyclic using only three alcohol dehydrogenases of overlapping specificities (Fig. 2). Such combinations are not exclusive. Other appropriate enzymes can be substituted. For example, glycerol dehydrogenase would serve as an excellent replacement for YADH.

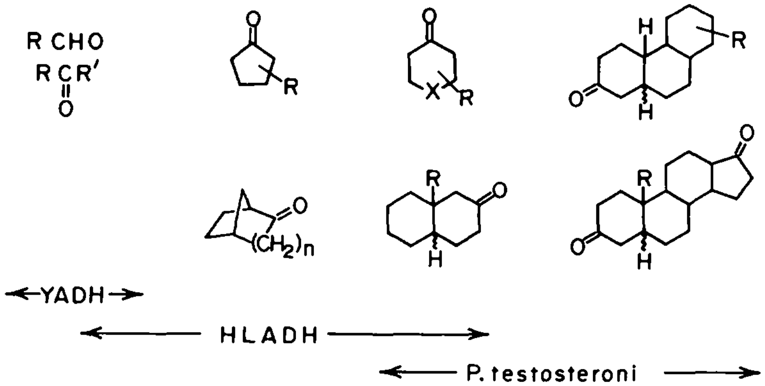

Fig. 2. A broad structural range of aldehyde and ketone, and the corresponding alcohol, substrates is accessible with only three alcohol dehydrogenases of suitably overlapping specificities.

EXPLOITING STRUCTURAL SPECIFICITY FOR SELECTIVE REACTIONS

The structural specificities of enzymes can be exploited to effect selective or regiospecific reactions on only one of two or more chemically similar functions in a molecule. The ease with which this can be done is often extremely difficult to duplicate chemically, especially in a single-step reaction.

Selective hydroxylation of aromatic compounds such as **5** and **7** is possible with horseradish peroxidase. The reactions must be carried out at 0° in order to prevent non-enzymic hydroxylation by the dihydroxyfumaric acid and oxygen cofactors used. D-3,4-Dihydroxyphenylglycine (**6a**), L-DOPA (**6b**), and L-epinephrine (**8**) have been prepared in this way[24] (Fig. 3). Phenolic coupling, as for phenol to **9**, can also be accomplished using horseradish peroxidase.[25]

One of the most dramatic illustrations of the power of the oxidative enzyme approach is the facility of the microbial conversion of benzene to *cis*-1,2-dihydroxycyclohexa-3,5-diene (**10**), a key intermediate in the production of poly(1,4-phenylene)[26] (Fig. 4).

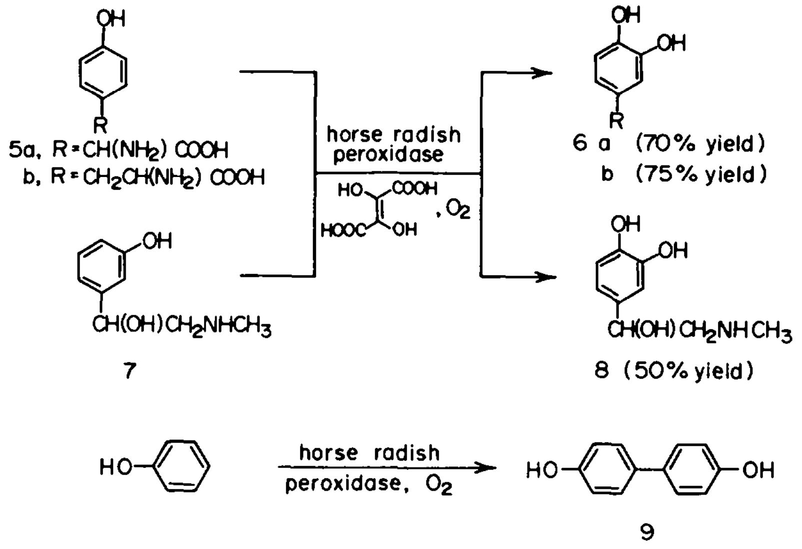

Fig. 3. Selective horseradish peroxidase-catalyzed oxidations.

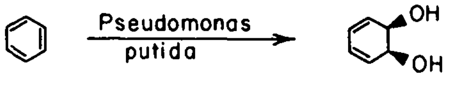

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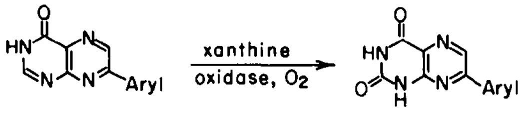
Fig. 4. Specific bis-hydroxylation of benzene.

The metabolic role of xanthine oxidase is to convert hypoxanthine and xanthine to xanthine and uric acid, respectively. It will also selectively hydroxylate unnatural substrates such as the substituted pteridinones **11** to give **12**[27] (Fig. 5). Xanthine oxidase has been used to purify mixtures of *o*-, *m*-, and *p*-substituted benzaldehydes[28] and, together with other enzymes,[29] for separating *cis*- and *trans*-isomers. However, the use of enzymes for the latter purpose does not appear to offer any significant practical advantage over more traditional separation methodology.



11 12 (>90%yields)

Fig. 5. Selective xanthine oxidase-catalyzed oxidations.

Halogenation of vinylic hydrogens is accomplishable using chloroperoxidases, as illustrated in Fig. 6 by the conversion of the analgesic and sedative drug antipyrine (**13**) into either its chloro (**14a**) or bromo (**14b**) derivative.[30] Mono- or bischlorinations of barbiturates **15** to **16** or **17** are also easily effected.[31] In fact, oxidative enzyme-catalyzed drug transformations of the type shown in Figs 3–6 can facilitate enormously the preparations of drug metabolites, full characterization of which is often required in new drug development.[32,33]

O- and N-demethylations can be performed selectively, as illustrated by the controlled conversion of griseofulvin (**18**) to **19–21**, respectively (Fig. 7).[34] New examples of enzymic and microbial selective cleavage reactions of this type continue to be identified.[11,35,36]

Non-enzymic methods for achieving types of oxidations shown in Figs 3–7 are available, but they suffer from disadvantages such as lack of selectivity, the need for vigorous reaction conditions, and low

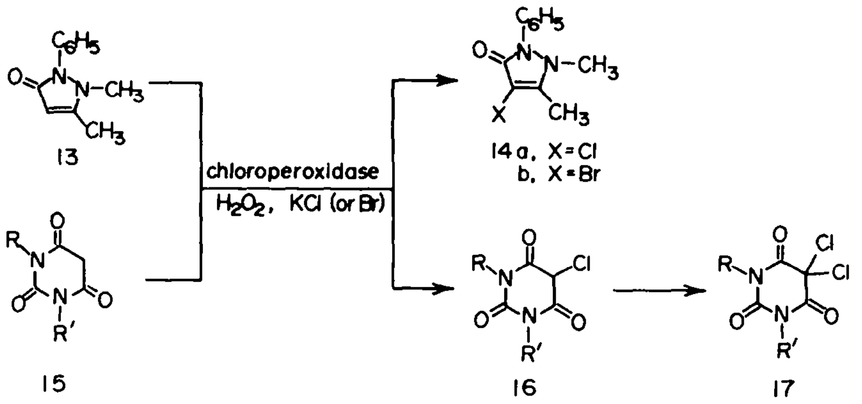


Fig. 6. Selective oxidative halogenations.

yields. While the yield of **9** from phenol is also low, this reaction cannot be effected in a single-step process in any other way at present.

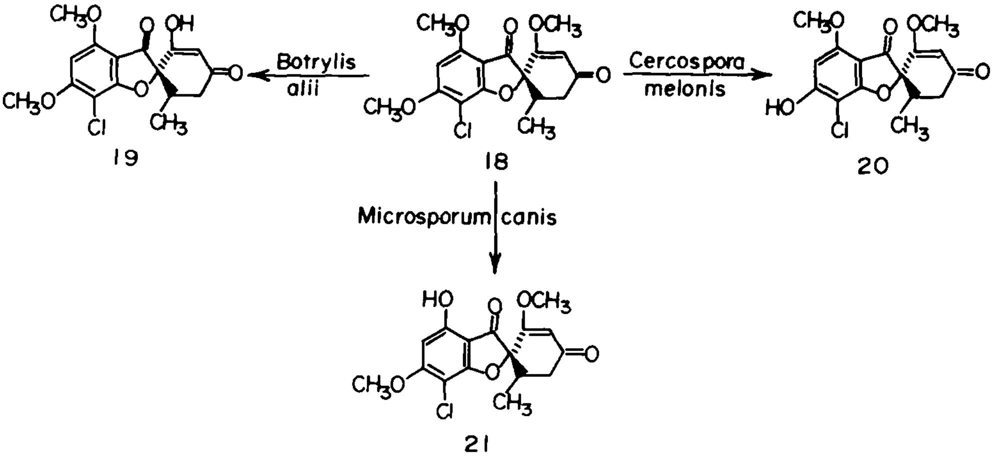


Fig. 7. Selective O-demethylation.

Enzymic Baeyer–Villiger oxidations are relatively common and can sometimes provide access to lactone products that are either sterically or electronically unfavoured with peracid reagents. For example, the microbial conversion of fenchone (**22**) yields a 9 : 1 mixture of **23** and **24** (Fig. 8), in contrast to the 3 : 2 mixture afforded by chemical oxidation.[37] However, this difference between the enzymic and

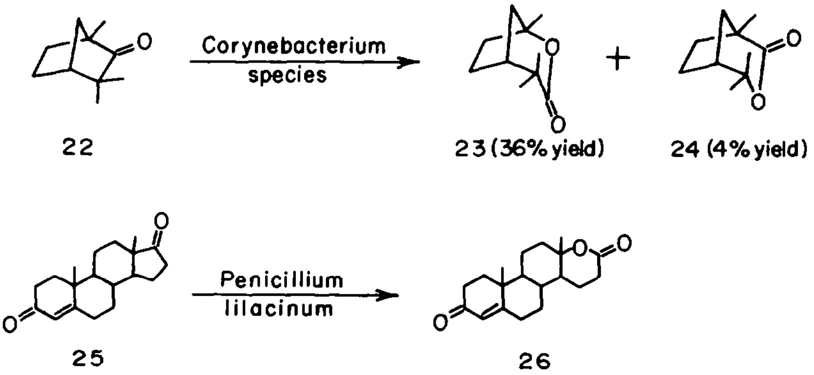


Fig. 8. Microbial Baeyer–Villiger reactions.

chemical methods is not always found and the 3° > 2° > 1° order of group migration preference observed in peroxide-induced Baeyer–Villiger oxidations is generally followed in the enzymic transformations also.[3] Another illustration of a microbial Baeyer–Villiger reaction is the conversion of **25** to **26**.[38]

Other types of selective oxidations have been documented. Two examples of commercial value are depicted in Fig. 9. The direct conversions of **27** to **28**[39] and of **29** to **30**[40] shown are difficult to duplicate chemically. 12-Ketochenodeoxycholic acid (**30**) can also be obtained via selective enzyme-catalyzed reduction of dehydrocholic acid.[41]

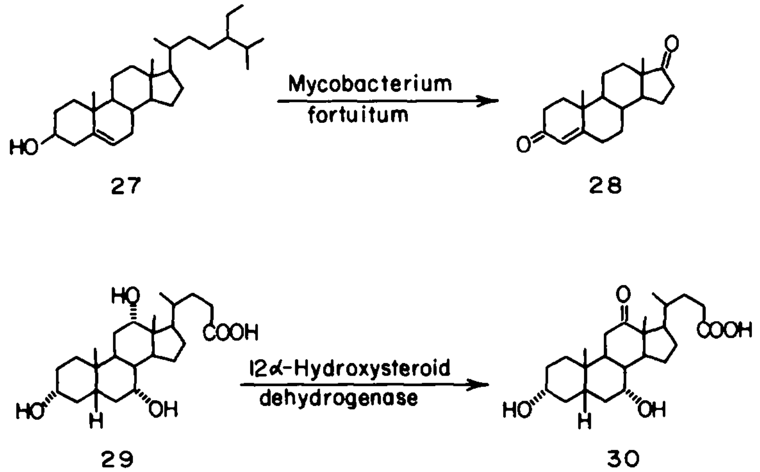
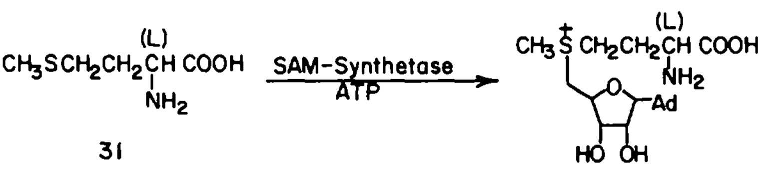


Fig. 9. Various types of selective oxidations of steroids can be achieved.

S-Adenosyl-L-methionine (**32**) is a cofactor in various enzyme-catalyzed reactions, particularly in transmethylation processes. It is expensive to purchase, and its instability complicates its chemical synthesis. However, the enzymic route shown in Fig. 10 now permits the synthesis of **32** from L-methionine (**31**) and ATP.[42]



31

32

Fig. 10. Enzymic preparation of S-adenosylmethionine.

The abilities of hydrolytic enzymes to catalyze selective hydrolyses have been widely exploited. Hydrolysis of nitriles, including acrylonitrile, to amides and subsequently to carboxylic acids, is achieved under very mild conditions[43,44] (Fig. 11). By using yeast[45] or porcine pancreatic lipase[46,47] to

$$\mathrm{R\,C{\equiv}N} \xrightarrow[\mathrm{H_2O}]{\text{nitrile hydratase}} \mathrm{R\,CONH_2} \xrightarrow[\mathrm{H_2O}]{\text{amidase}} \mathrm{RCOOH}$$

Fig. 11. Mild hydrolyses of nitriles and amides.

hydrolyze the methyl ester functions of the highly sensitive prostaglandin precursors **33** and **35a,b** to the corresponding acids **34** and **36a,b**, the undesirable side reactions that plague non-enzymic hydrolyses can be avoided (Fig. 12). Pig liver esterase is also a useful enzyme for prostaglandin methyl ester hydrolyses of this type.[48]

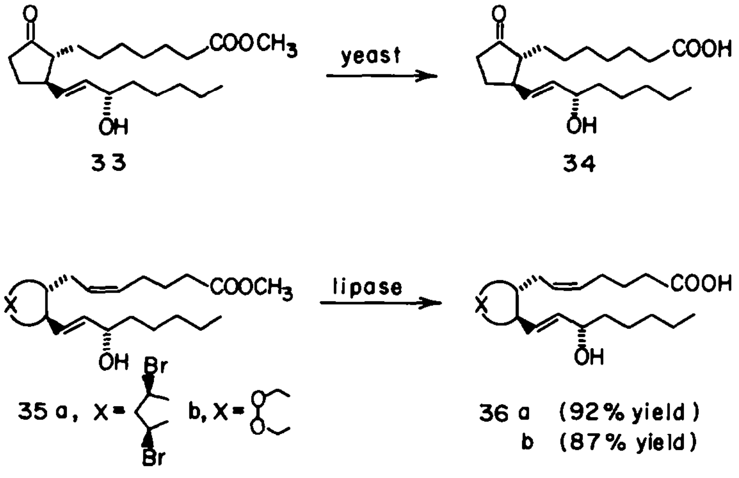


Fig. 12. Mild hydrolysis of ester protecting groups.

In other areas of protecting group chemistry, the use of enzyme-specific groups adds a new dimension of control. For example, either of the protected hydroxyl groups of the tropane diester[37] can be exposed at will (Fig. 13). The 6-α ester, an acetate in this case, is more sensitive to base hydrolysis and gives **38** when treated with 1 equivalent of hydroxide. In contrast, chymotrypsin attacks only the 3-β-dihydrocinnamoyl ester function that its active site binds well, thereby forming **39** exclusively.[49] Analogous protecting group applications have been documented in the oligonucleotide[50] and peptide[51,52] fields.

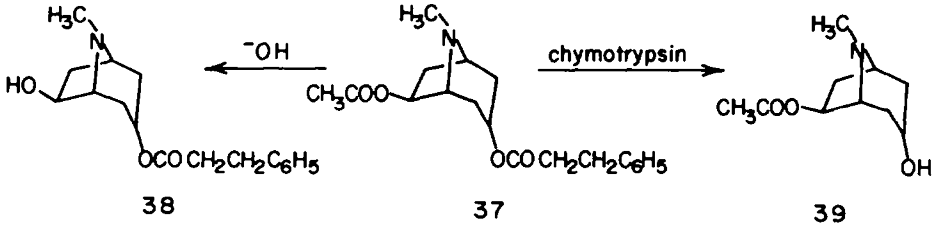
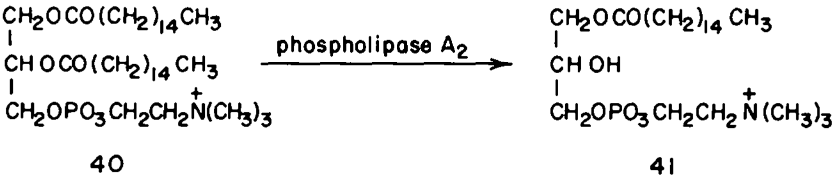


Fig. 13. Selective ester protecting group removal.

Regiospecific hydrolysis of polyesters are achievable, as illustrated by the phospholipase A_2-catalyzed hydrolysis of the phospholipid **40**. Cleavage occurs only at the secondary alcohol centre to give **41** as the exclusive product[53] (Fig. 14).



$$\begin{array}{l} CH_2OCO(CH_2)_{14}CH_3 \\ | \\ CHOCO(CH_2)_{14}CH_3 \\ | \\ CH_2OPO_3CH_2CH_2\overset{+}{N}(CH_3)_3 \end{array} \xrightarrow{\text{phospholipase } A_2} \begin{array}{l} CH_2OCO(CH_2)_{14}CH_3 \\ | \\ CHOH \\ | \\ CH_2OPO_3CH_2CH_2\overset{+}{N}(CH_3)_3 \end{array}$$

Fig. 14. Regiospecific ester hydrolysis.

Penicillin acylase-catalyzed† hydrolysis of the phenylacetamido group of penicillin G (**42**) does not affect the sensitive β-lactam ring.[54] This is now an important procedure for the industrial production of

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† In this reaction, and in some of the subsequent ones, an immobilized enzyme preparation was employed. However, only when immobilization of an enzyme or cell contributes in an important way to the process will this fact be noted since the mechanism of action and specificity of the catalytic process is, with rare and minor exceptions, unaffected.

6-aminopenicillanic acid (**43**)[55] (Fig. 15). Deacetylation of cephalosporin C (**44**) to **45**[56,57] and related structures[58,59] can also be effected, even on a continuous production basis.[57]

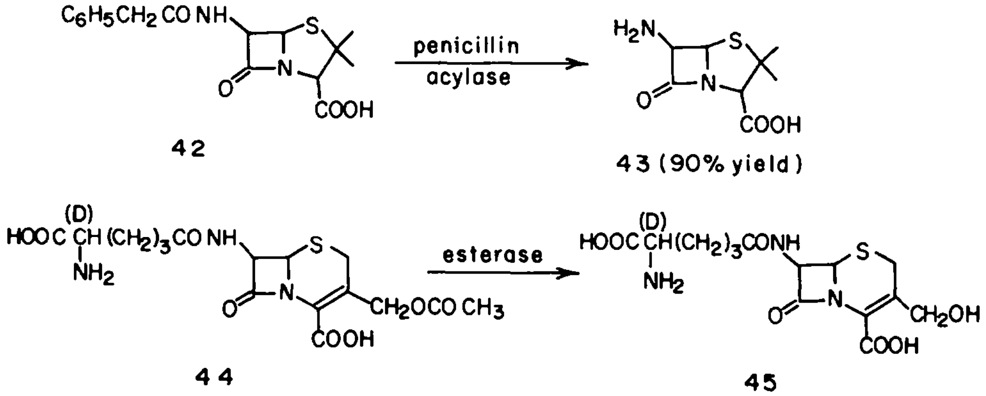

Fig. 15. Selective hydrolysis of β-lactam amides and esters.

Transacylation reactions can be induced. The penicillin and cephalosporin fields again provide representative illustrations (Fig. 16). A major advantage of such methodology is that no protecting groups are required. The protease from *Xanthomonus citri* condenses 6-aminopenicillanic acid (**43**) and D-phenylglycine methyl ester (**46a**) or its *p*-hydroxy derivative **46b** to give high yields of ampicillin (**47a**) and amoxicillin (**47b**), respectively.[60] The cephalosporanic acid **48** is similarly converted into cephalexin (**49a**).[61] In addition, the acylase of *Penicillium chrysogenum* catalyzes the exchange of the D-amino acid acyl side chains of cephalosporin C.[62] Trypsin-mediated exchange of threonine (as its ester derivatives) for the terminal alanyl residue of porcine insulin (**50**) is the basis of a commercial process for the production of human insulin (**51**).[63–65]

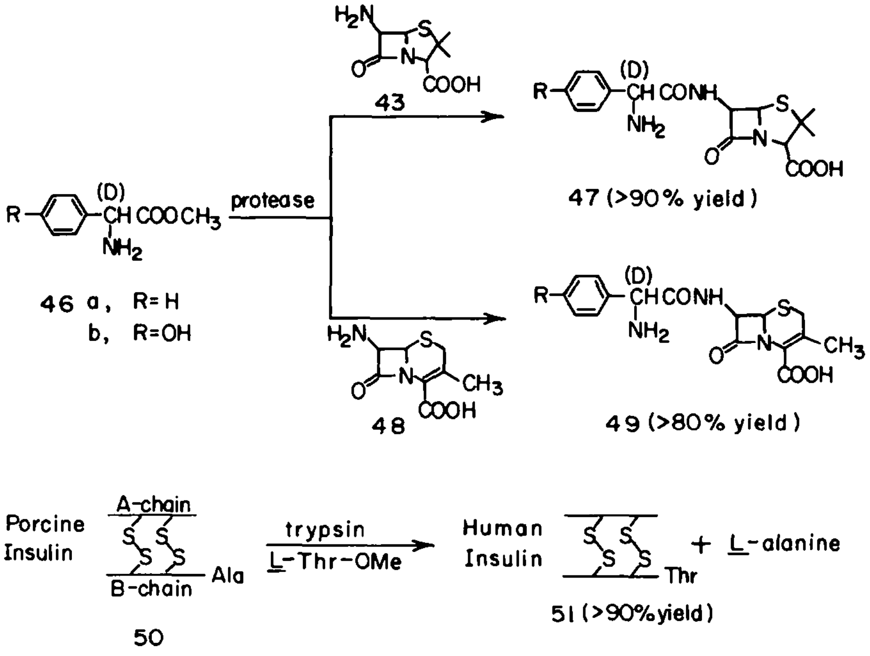

Fig. 16. Selective transacylations.

A quite different exploitation of the selectivity of hydrolytic enzymes is provided in Fig. 17, in which the separation of difficult-to-purify mixtures, such as that of α- and β-naphthol, is achieved via selective hydrolysis of their sulfates. When a mixture of 52 and 53 is subjected to sulfatase-catalyzed hydrolysis, only the β-sulfate is cleaved. The β-naphthol (54) product and unreacted α-sulfate 52 are then easily separable.[66]

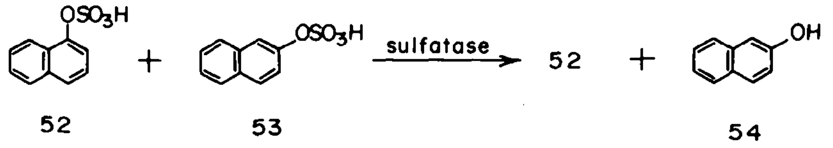

Fig. 17. Purification of mixtures of isomeric sulfates by selective hydrolysis.

As with all reactions, it is the thermodynamically preferred products that accumulate in enzyme-catalyzed processes. All of the transformations discussed so far have been of this type. However, by exploiting the principle of microscopic reversibility, catalyses in normally thermodynamically less preferred directions can be induced by appropriate alteration of the reaction conditions.[67] Such manipulations include rapid removal of unstable product by the use of flow systems with immobilized enzymes,[17] using organic solvents,[68] and by precipitation.[69–71] Control of pH can also be exploited.[72]

It is in the peptide synthesis area that enzyme-catalyzed reversal of the thermodynamically preferred direction has been exploited most extensively.[73] Thermolysin-catalyzed coupling of L-phenylalanine methyl ester (56) with N-carbobenzoxy-L-aspartic acid (55) gives excellent yields of the Aspartame precursor 57[74] (Fig. 18). Racemic 56 can also be used. In the latter process, which is the basis of a commercial production route,[75] the unreactive D-56 is recovered and recycled. The reaction is driven in the peptide bond forming direction by using an immobilized enzyme system and by exploiting the fact that 57 and L- or D-56 form an insoluble addition compound. The specificity of the enzyme precludes any need to selectively protect the side chain carboxyl group of 55.

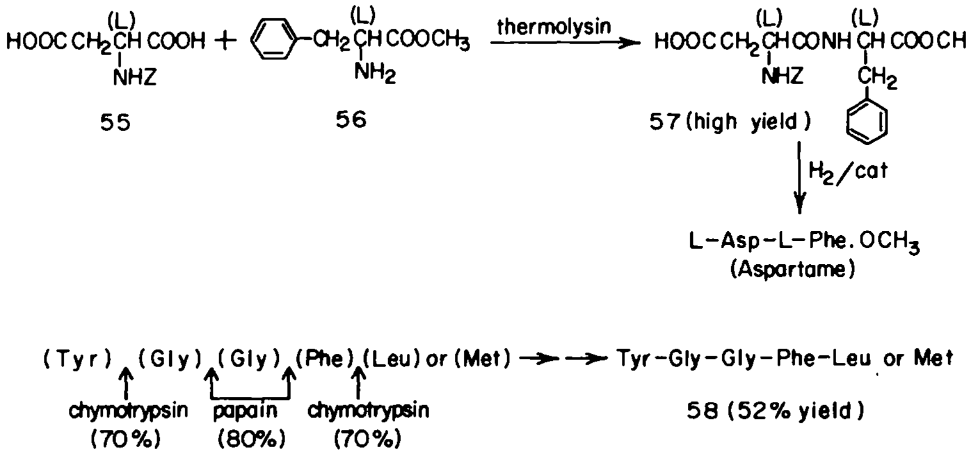

Fig. 18. Selective peptide bond formation. Bracketed amino acids, e.g. (Tyr) incorporate protecting groups.

By exploiting the different specificities of papain and chymotrypsin, convergent, multi peptide-bond, syntheses of both leucyl and methionyl enkaphalins (58) are achieved in good yields from the component amino acids.[76] Angiotensin II,[77] the octapeptides dynorphin[78] and cholecystokinin,[79] substance P pentapeptide,[80] and enniatin[81] have also been prepared by similar approaches. In all of these routes, preference for the peptide bond formation direction is induced by disturbing the equilibria with organic solvents or by precipitation of the products. Enzymes solubilized in reverse micelles can also be used.[82]

The clostripapain-mediated coupling of bovine pancreatic ribonuclease decapeptide (amino acid residues 1–10) and pentapeptide (amino acid residues 11–15) fragments to the pentadecapeptide (amino acid residues 1–15) illustrates another ingenious exploitation of biological specificity.[83] The desired pentadecapeptide binds strongly and specifically to ribonuclease S (amino acid residues 21–124). This is capitalized on by adding ribonuclease S to the reaction medium to act as a molecular trap for the thermodynamically less favoured pentadecapeptide product as soon as it is formed. In this way the normally preferred peptide-hydrolysis reactions are suppressed and a 15% yield of the 15-peptide is obtained. Many other peptide bond formation reactions have been reported for a variety of proteases. The number of current reviews[75,84,85] of the area reflects a continuing interest in the topic by many groups.

Glycosidases have been widely used to liberate aglycones of diverse structures from their corresponding glycosides. This topic has been extensively reviewed.[2,86,87] The reverse reaction can also be achieved, as illustrated by the synthesis of variously substituted and chemically unstable cardiac glycosides **59** using a β-galactosidase in aqueous acetonitrile solution[88] (Fig. 19). Preparations of oligosaccharides by enzymic coupling of monosaccharides have been reported,[89,90] including the first enzymic synthesis of an unnatural disaccharide **61** from the deoxyfluorofructose **60** and UDP-glucose.[91] Furthermore, the controversy over the anti-cancer potential of Laetrile (**64**) was settled only after its unambiguous enzyme-catalyzed synthesis from glucuronic acid (**62**) and mandelonitrile (**63**).[92]

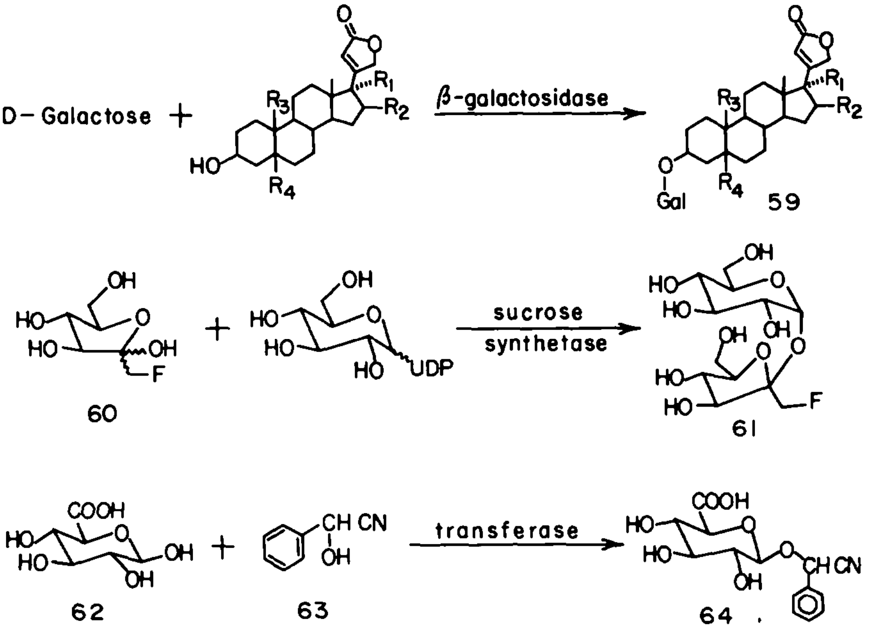

Fig. 19. Selective glycoside syntheses.

Many structurally specific phosphate-hydrolyzing enzymes are known. Some have been exploited by organic chemists, as in the use of acid phosphatase in polyprenyl pyrophosphate structure determination.[93] However, it is their application to selective phosphate bond formation without the need for protecting groups that is currently of the greatest synthetic importance. In contrast to the difficulties involved in its non-enzymic preparation, arginine phosphate (**65**) is conveniently made using arginine kinase[94] (Fig. 20). Selective mono- and pyrophosphorylations of monosaccharide moieties can be effected, as demonstrated by the preparations of **66**,[95] **67**,[96] **68**[97] and **69**.[98]

Enzyme-mediated phosphorylation is also of great value in the nucleic acid field. Ribonuclease-catalyzed coupling of the cyclic phosphate **70** with the mononucleotide uridine gives the UU dinucleotide **71** (Fig. 21).[99] The trinucleotide protein-synthesis termination codons UAA, UAG, and UGA can be prepared in a similar manner.[100]

In polynucleotide synthesis, enzyme-catalyzed phosphate bond formations provide elegant and unique solutions to the problems of controlled couplings of oligonucleotide intermediates (Fig. 22). Gene synthesis relies heavily on this technique. In tRNA gene syntheses, oligonucleotide paired-chain precursors such as **72** and **73** are chemically synthesized. The base sequences in these pairs are complementary so that in solution the two chains associate strongly by hydrogen bonding. However,

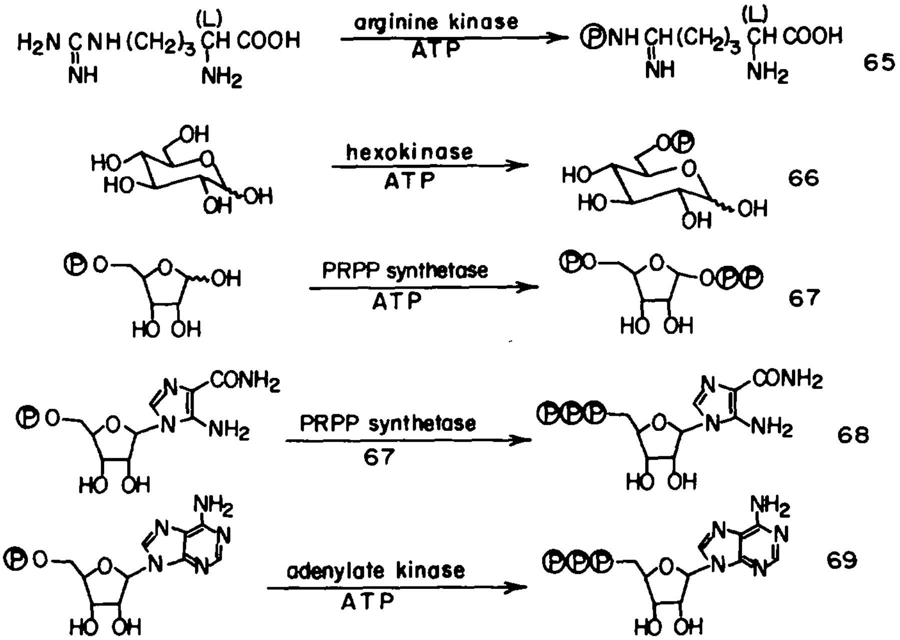

Fig. 20. Selective phosphorylations.

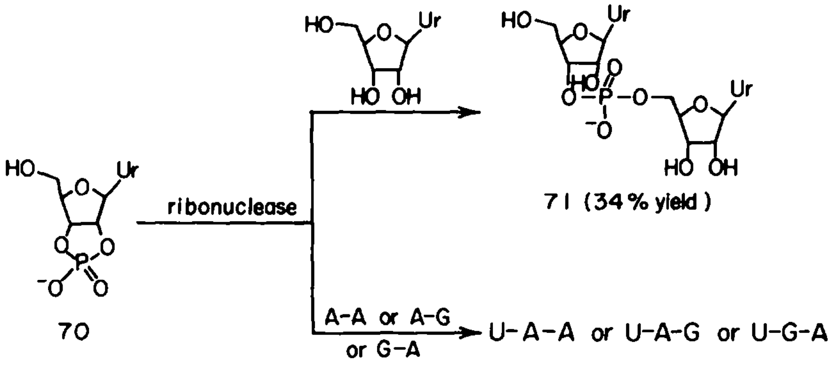

Fig. 21. Selective oligonucleotide syntheses.

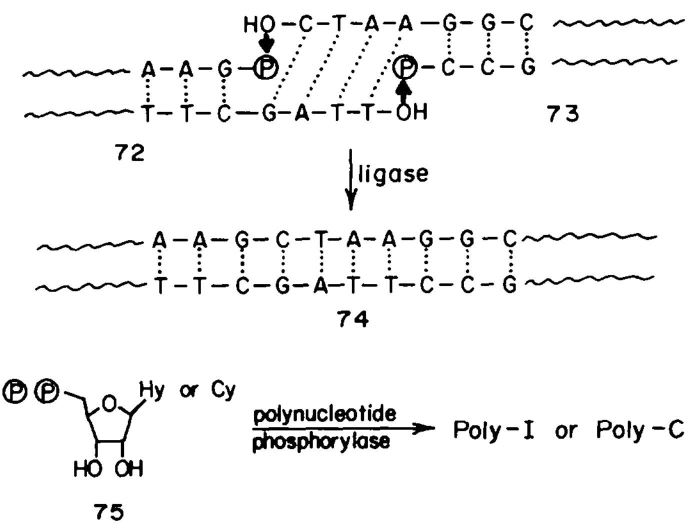

Fig. 22. Controlled polynucleotide syntheses.

for each of **72** and **73**, one chain has four extra nucleotides that "hang over" the ends of the paired sequences. These two pendant sequences, AATC and GATT, respectively, in this example, are themselves complementary. When **72** and **73** are mixed in solution, they therefore associate uniquely through the AATC–GATT hydrogen bonding interactions. With **72** and **73** locked in place in this manner, a ligase is added that snaps two phosphate bonds together to couple the oligonucleotides into the larger nucleic acid fragment **74**. This process is repeated until the desired sequence is made. The structural genes for yeast alanine tRNA (77 nucleotides) and the *E. coli* tyrosine suppressor tRNA (126 nucleotides) have been made in this way.[101] The field continues to be an active one.[102–105] Polynucleotide synthesis is not limited to this approach. The interferon inducer poly-I : poly-C has been prepared by phosphorylase-mediated polymerizations of inosine and cytidine 5′-pyrophosphates (**75**), respectively.[106]

In other nucleotide chemistry, enzyme-catalyzed base exchange has proven of great value. The preparations of NAD analogues such as **76**[107] and the antiviral agent **77**[108] are illustrative of the current activity[109] in this area (Fig. 23).

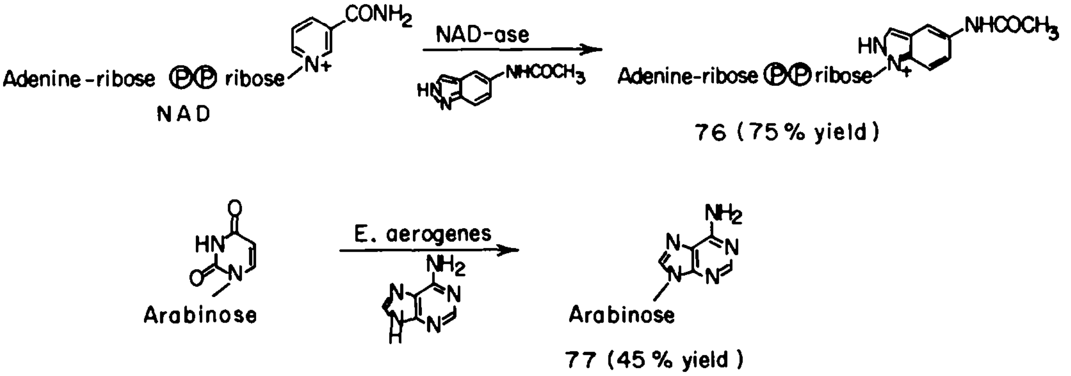

Fig. 23. Enzyme-catalyzed exchange of nucleotide bases.

One of the most remarkable selective enzyme-catalyzed reactions is the exchange of the side chains of L-amino acids. These reactions are pyridoxal phosphate coenzyme-dependent and proceed via a Schiff-base intermediate (**132** of Fig. 41). The conversion of D,L- or L-serine (**78**) to L-DOPA (**79**)[110,111] illustrates the process (Fig. 24). Many side chain groups can be introduced in this way, including unnatural ones such as selenomethyl.[112] Another reaction involving Schiff-base intermediates that is very difficult to achieve nonenzymically is the condensation of δ-aminolevulinic acid to porphobilinogen (**80**).[113]

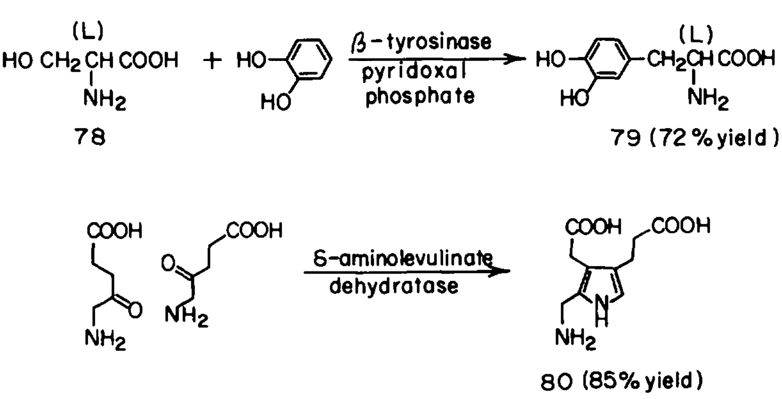

Fig. 24. Enzyme-catalyzed exchange and condensation reactions.

Selective decarboxylations can be effected by pyridoxal phosphate-dependent enzymes, as exemplified by the industrially valuable conversion of L-aspartic acid (**81**) to L-alanine (**82**, Fig. 25).[114]

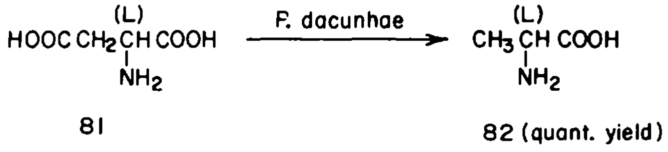

Fig. 25. Selective decarboxylation.

In dollar terms, the glucose isomerase-mediated conversion of glucose to fructose is by far the most valuable of the enzyme-based industrial processes. Nevertheless, even though glucose isomerase itself has been exploited in an improved process for producing D-mannitol from D-glucose,[115] isomerases are not of broad synthetic utility. This is because most enzyme-catalyzed isomerizations, including *cis–trans* interconversions,[116] are usually easily accomplished nonenzymically. However, racemases are used to recycle unwanted enantiomers in resolutions of racemates. Some examples of such applications are contained in the next section.

EXPLOITING ENANTIOMERIC SPECIFICITY†

The abilities of enzymes to discriminate between enantiomers of racemic substrates are well documented.[1–3,6–8,10,117] When an enzyme is enantiomerically specific, transformations of a racemate stop at the 50%-conversion stage when all of the reactive enantiomer has reacted. In many such resolutions, one enantiomer has an unwanted absolute configuration and must be discarded, a practice that limits the maximum yield of usable material to 50%. This is often unacceptable by current asymmetric synthetic standards. Accordingly, only examples of enzymic resolutions that permit the "wrong" enantiomer to be recycled, that serve a unique need, or that illustrate a novel application, are included in this review.

Hog kidney acylase-catalyzed resolutions of N-acyl amino acids were the first major applications of enzymes for enantiomer resolutions.[118] They are still important. Acylases meet the broad structural specificity and narrow stereospecificity criteria required for general applicability.[2,117,118] They are stereospecific for L-enantiomers, with the unhydrolyzed N-acyl D-amino acids being recyclable via chemically induced racemization. Acylase-mediated resolutions of the (±)-**83** to L-**84** type

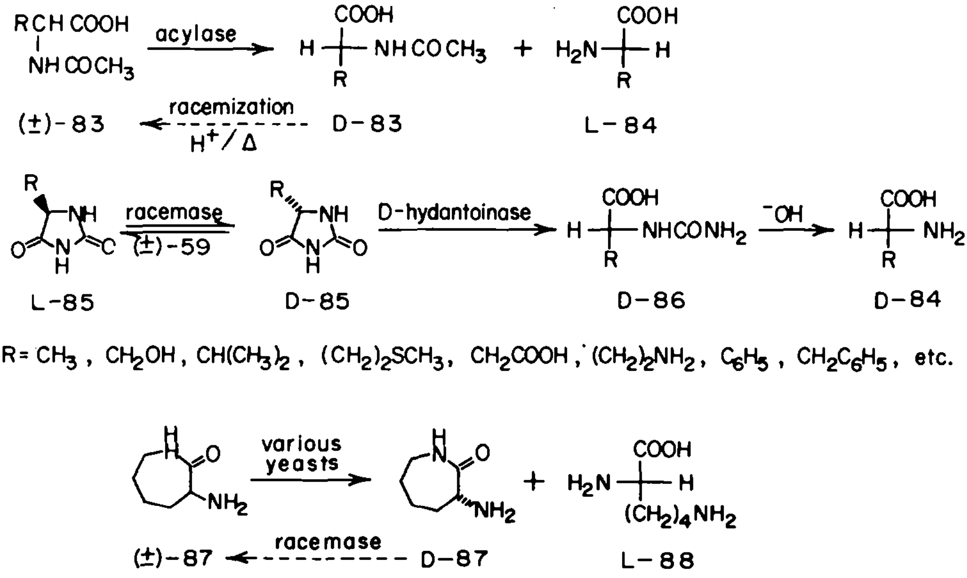

Fig. 26. Resolution of amino acids, with recycling of unreactive enantiomers.

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† In this, and all subsequent sections dealing with chiral molecules, the ee's of all compounds discussed are >90% unless specified otherwise.

(Fig. 26) are industrially important.[119] There have also been many academic applications of acylases. The penicillin synthon L-**84**, R = $—C(CH_3)═CH_2$,[120] and the insect pheromone precursor (2*S*,3*R*)-**84**, R = $—CH(CH_3)C_3H_7$,[121] were obtained via stereospecific hydrolyses of their (±)-N acyl derivatives using commercially available acylases.

Hydantoinase-catalyzed hydrolyses of (±)-**85**-type substrates are other efficient routes to resolved α-amino acids.[122,123] Racemic hydantoins (±)-**85** are readily and inexpensively prepared from aldehydes via the Bucherer reaction. The hydantoinase of Fig. 26 is stereospecific for D-enantiomer hydrolyses and yields D-**86** carbamate products. The unhydrolyzed L-hydantoin enantiomers L-**85** can be recycled by chemical epimerization or, more attractively, by *in situ* racemization of L- to (±)-**85** as the hydrolysis proceeds. This is induced by adding a racemase to the reaction mixture. The D-carbamate amino acids D-**86** are readily converted to the corresponding amino acids D-**84** using mild base. D-Phenylglycine and D-*p*-hydroxyphenylglycine for use in semisynthetic penicillin and cephalosporin syntheses have been produced in this way.[122] This reaction is not restricted to D-amino acid production. Hydantoinases of both D- and L-enantiomeric specificity are widely available from bacterial, yeast, and mammalian sources.[122,124,125] Cyclic amides can be resolved in a similar manner, as illustrated by the stereospecific hydrolysis of (±)-δ-aminocaprolactam ((±)-**87**) to L-lysine (L-**88**) and recyclable D-δ-aminocaprolactam (D-**87**) by various yeasts and bacteria.[126]

Stereospecific hydrolyses of their ester derivatives provide other methods for resolution of amino acids. Many serine proteases, such as chymotrypsin, trypsin and subtilisin, which exhibit L-enantiomer preferences, are particularly valuable in this regard.[2] However, the use of such enzymes is by no means restricted to resolutions of amino acid substrates. Versatile enzymes such as chymotrypsin operate with enantiomeric specificity on a broad structural range of racemic ester substrates. Figure 27 illustrates the spectrum of chiral acids and esters that are accessible in this way.[2] An additional advantage of using chymotrypsin as an enzymic catalyst is that its stereospecificity is easily predictable using a simple model.[2,127] In all the cases in Fig. 27, the reactive ester stereoisomers are those whose chiralities parallel those of the natural L-amino acids. The unreactive enantiomers of Fig. 27 can all be recycled via chemical racemization. Another enzyme whose potential for enantiomer discriminations of broad synthetic applicability is becoming increasingly recognized is pig liver esterase.[128,129]

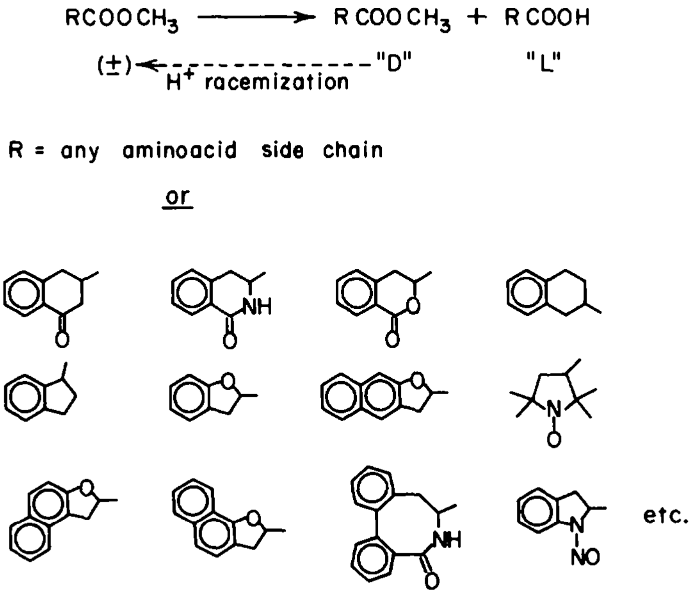


Fig. 27. Illustration of broad structural variations tolerated by versatile enzymes such as chymotrypsin.

While hydrolysis of amides or esters by hydrolytic enzymes is heavily preferred thermodynamically, enantiomeric discriminations in the opposite amide- or ester-bond formation direction are also readily achieved. The equilibrium can be displaced towards the thermodynamically disfavoured products in several ways. The papain-catalyzed coupling of (±)-**89** with *p*-toluidine is easily induced because the toluamide product L-**90** is insoluble in the aqueous reaction medium and precipitates as soon as it is formed[69] (Fig. 28). In addition, hydrazides of various amino acids can be prepared stereospecifically using this method.[130]

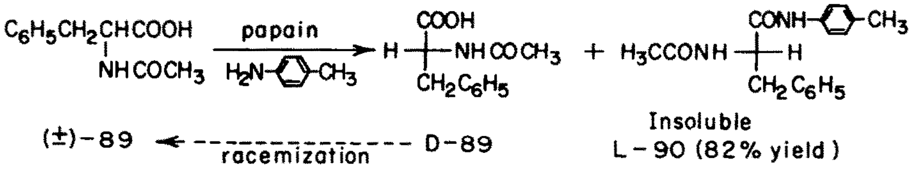


Fig. 28. Enantiomeric specificity in amide bond formation.

Alternatively, ester formation from acids and alcohols can be induced in chymotrypsin-promoted reactions by operating in biphasic aqueous–organic systems, whereby the minute amounts of thermodynamically disfavoured esters present in the aqueous layer at equilibrium are extracted into an organic layer such as chloroform. In this way, the ester products are rendered unavailable to the enzyme and therefore accumulate.[131] Using this basic technique, resolutions of chiral alcohols can be effected by exploiting the abilities of immobilized forms of pig liver esterase and a yeast lipase to promote enantiomerically specific transesterifications for a broad range of acyclic racemic alcohols of which (±)-**91** and (±)-**92** are representative[132] (Fig. 29). Lipases are particularly well suited to operating in high organic solvent environments.[133] In some cases, their stereospecificities can be strongly influenced by the nature of the alcohol moiety of their ester substrates. For example, the lipase of *Candida cylindracea* is more enantiomerically discriminating towards its octyl than its methyl ester substrates.[134]

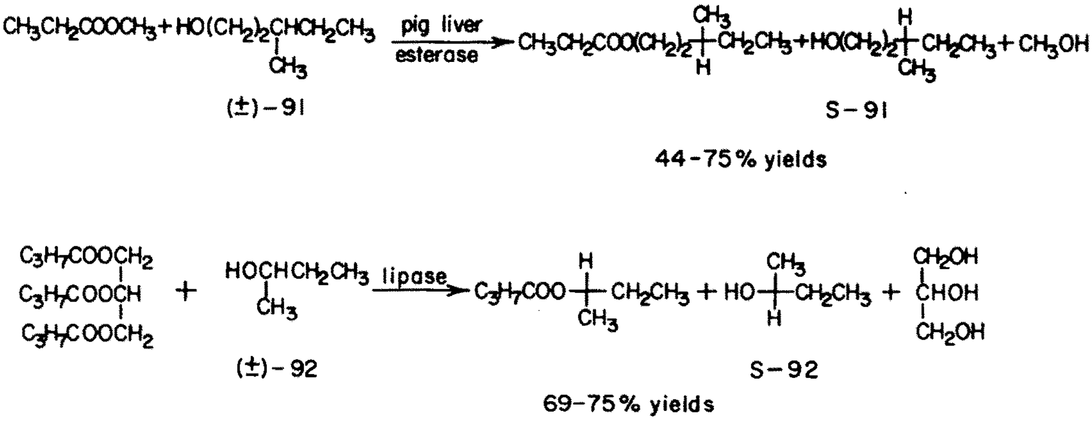


Fig. 29. Enantiomerically specific transesterifications.

For valuable chiral compounds, resolutions in which the unwanted enantiomer has to be discarded can become acceptable. For example, methyl *trans*-(2*R*,4*R*)-2,4-dimethyl glutarate (**94**) is a useful chiral synthon for macrolide and polyether antibiotics. One of the most convenient routes to (2*R*,4*R*)-**94** is by a microbial esterase-catalyzed hydrolysis of (±)-**93**, even though the unreactive diester (2*S*,4*S*)-**93** cannot easily be recycled because its base-mediated epimerization also produces the *meso*-diester diastereomer[135] (Fig. 30).

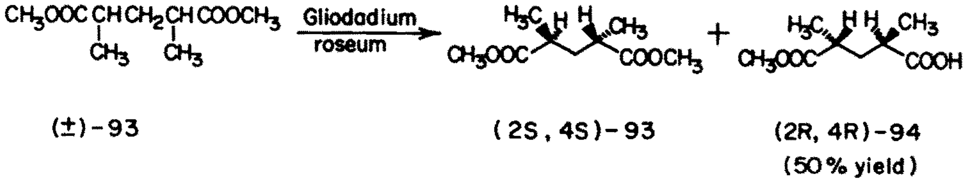


Fig. 30. A resolution of a racemic diester.

Another important illustration of this type is provided in Fig. 31. The value of chiral epoxides as synthetic intermediates is well recognized. However, despite the success of the Sharpless[136] approach for stereospecific *trans* epoxide preparations, epoxy alcohols such as **96** have not been readily available so far. This problem is now solvable using porcine pancreatic lipase-catalyzed hydrolysis of racemic epoxy esters, of which (±)-**95** and (±)-**97** are representative only.[137] The hydrolyses proceed with high enantiomeric specificity for a range of epoxide substitution patterns. For both the epoxy alcohol products **96** and **98** and the recovered epoxy esters ee's in excess of 90% are readily obtained by controlling the extent of hydrolysis and by varying the reaction conditions. Reactions at −10° in 20% dimethylformamide at pH 6 favour high enantiomeric excesses.[138] While neither the unreactive esters (*R*)-**95** nor (2*S*,3*R*)-**97** can be recycled, discarding the unwanted enantiomeric series does not represent a serious loss since the starting materials are inexpensive.

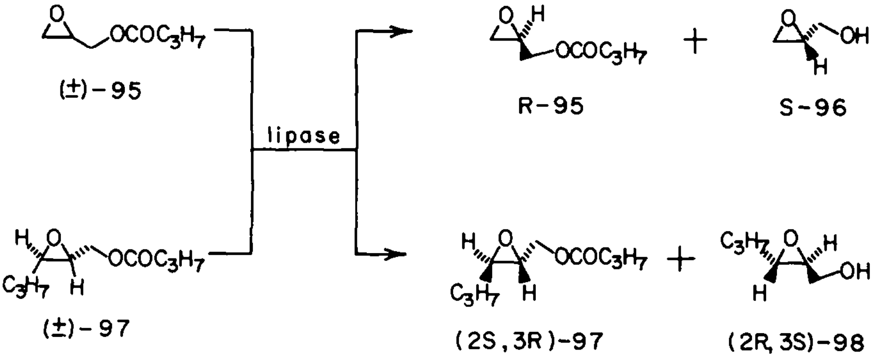


Fig. 31. Resolutions of epoxy esters.

If the chiral synthon desired is sufficiently valuable, it can be obtained from a racemate by selective destruction of its enantiomer. This is depicted in Fig. 32, where a D-amino acid oxidase yielded a bleomycin precursor L-**99** from (±)-*erythro*-**99** by effecting stereospecific oxidation of the D-isomer to the ketoacid **100**.[139] Conversely, if D-**99** had been required, an L-amino acid oxidase could have been used.

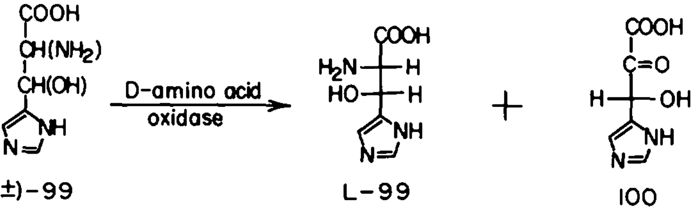


Fig. 32. Formation of pure stereoisomers via enantiomerically specific destruction.

Another remarkable resolution that involves stereospecific oxidative degradation is the conversion of (±)-**101** to the anti-inflammatory drug ibuprofen (*R*-**102**) by a *Rhodococcus* species[140] (Fig. 33).

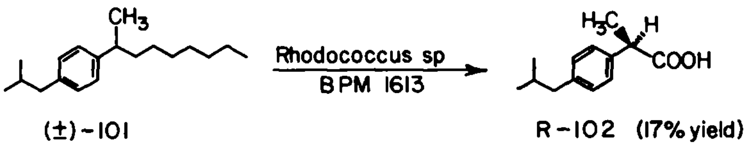


Fig. 33. Resolution via stereospecific oxidation.

In resolutions of esters with hydrolytic enzymes, it is usually the acid moiety that is chiral. However, enantiomeric distinctions are also observed in hydrolyses of esters of chiral alcohols. Some examples are provided in Fig. 34. Resolution of *l*-menthol ((−)-**104**) via stereospecific enzyme-catalyzed hydrolysis of its racemic ester (±)-**103** is of commercial interest.[141] Alkynyl esters and alcohols such as **105** and **106** are broadly useful natural product synthons[142] and *S*-**107** is a precursor of β-adrenergic blocking agents[143] as is *S*-**108**.[144] The stereospecific conversion of (±)-**109** to *S*-**109** and *R*-**110**,[145] of value as precursors of intermediates of the arachidonic acid cascade, provides yet another illustration of the broad potential of enzymic solutions to resolution problems.

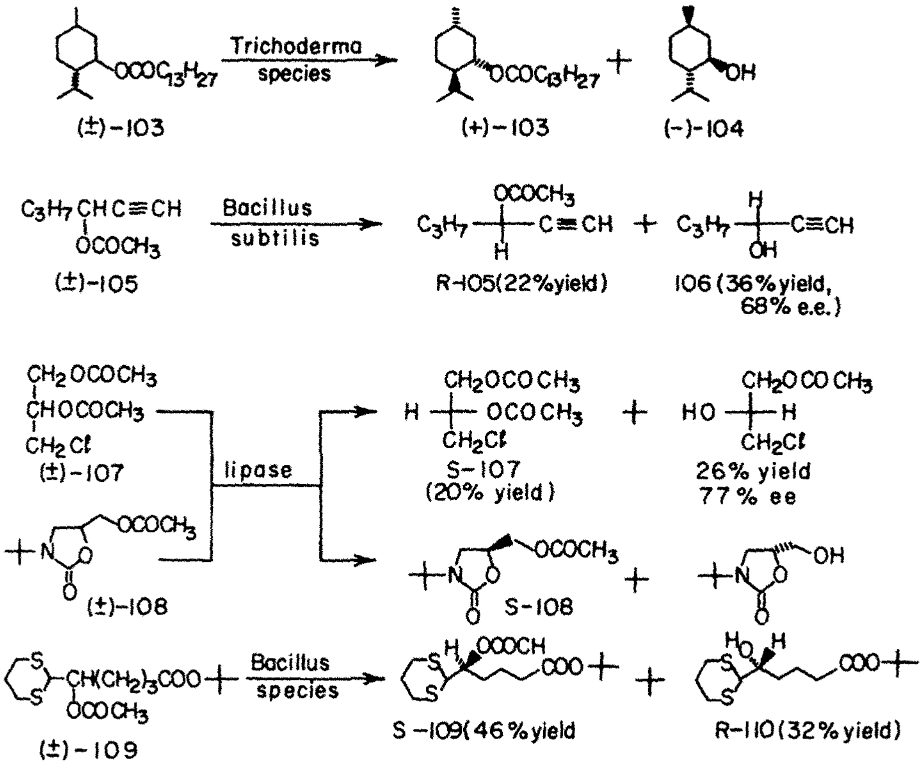


Fig. 34. Resolutions of esters of racemic alcohols.

An interesting route to enantiomerically pure α-hydroxyacids is shown in Fig. 35. While the L- and D-lactic acid products, (*S*)- and (*R*)-**112**, respectively, are readily available, this resolution demonstrates what can be achieved when enzymes with either L- or D-specificity are available. The first enzyme hydrolyses only the L-enantiomer of (±)-**111** to give (*S*)-**112** and (*R*)-**111**. The latter can then be converted to (*R*)-**112** with D,L-dehalogenase.[146]

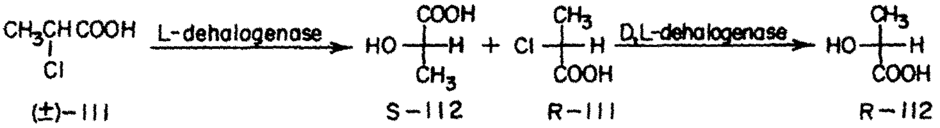


Fig. 35. Preparation of both enantiomers of a chiral hydroxyacid from a racemic chloroprecursor.

Mention has been made of the optimization of ee via control of reaction conditions.[138] Quantitative analytical procedures for identifying the conditions leading to maximum ee's have also been developed.[135,147]

EXPLOITING PROCHIRAL STEREOSPECIFICITY

As has been noted above, the involvement of racemic intermediates in asymmetric synthesis is avoided whenever possible, especially when the unwanted enantiomer cannot be reused. Even when recycling of the "wrong" stereoisomer is feasible, the additional steps that are needed often constitute a major disadvantage. These difficulties can be avoided by exploiting the prochiral stereospecificity capabilities of enzymes, whereby all of a symmetrical substrate can be converted into the desired chiral product.

Stereospecific additions of stereoheterotopic faces

Enzymes can operate stereospecifically on one of the two enantiotopic or diastereotopic faces of planar groups such as C=C, C=N or C=O. Many examples of asymmetric synthetic value have been documented.[2,5,7,8,10,148]

With a few exceptions, such as some conjugated carbonyl functions, stereospecific reduction of an aldehyde or ketone in virtually any molecule can be effected either enzymically or microbially.[3,5,149] Examples of useful acyclic chiral alcohols obtainable in this way are shown in Fig. 36. α-Sulfenyl-β-ketoesters can also be reduced stereospecifically.[150] Alcohols **113a,b** produced using yeast reductions,[151] have been used in syntheses of compactin,[152] griseoviridin,[153] and a bee pheromone.[154] Yeast was also employed in the preparation of aromatic trifluoromethyl alcohols **113c** of value for inducing asymmetric reactions[155,156] and as chiral solvents for ee and absolute configuration determinations.[157] Alcohol **113d**, obtained via *Sporotrichum exile* fermentation, has served as a synthon for yohimbine alkaloids,[158] and the *Kloeckera corticis*-derived **113e** as a chiral hydroxyaldehyde precursor of arachidonic acid metabolites.[145] Alcohols **113f** were obtained using glycerol dehydrogenase.[138] The use of thermostable enzymes promises to extend the viability of this asymmetric reduction approach to many new substrates.[159] The nature of the substituents R and R′ has a considerable influence on the ee levels attainable.[160–162]

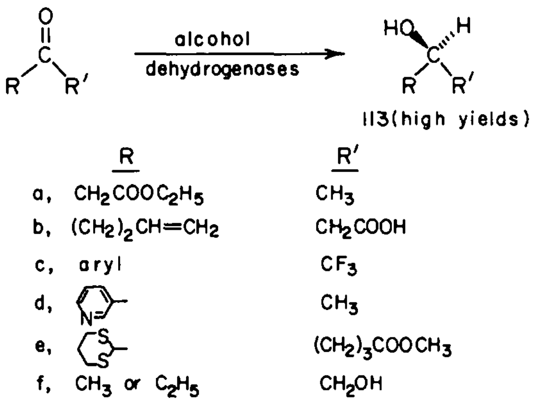
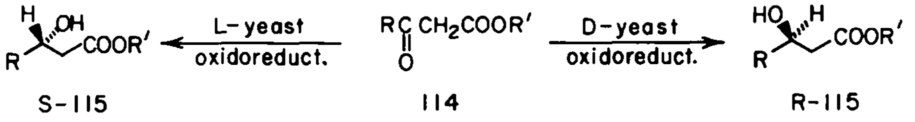


Fig. 36. Stereospecific reductions of a broad structural range of ketones can be achieved.

As indicated already, yeast-mediated transformations are among the easiest for organic chemists to carry out. Furthermore, for yeast and purified yeast alcohol dehydrogenase, the stereospecificities of reductions are easily predicted by the Prelog rule.[163] This states that when groups R and R′ are sterically larger (L) and smaller (s), respectively, as for **113a–e**, the hydride equivalent is delivered to the *Re*-face of the carbonyl group as defined by a Cahn–Ingold–Prelog priority sequence of oxygen > L > s, although it is sometimes difficult to predict what the L > s order of substituents should be.[164] This rule also applies to other oxidoreductases, such as horse liver alcohol dehydrogenase. For cyclic ketones, more sophisticated (but still easy to use) models have been developed for predicting the stereospecificities of alcohol dehydrogenase-catalyzed reductions.[2,163,165–168]

Either enantiomer of a chiral alcohol can be produced at will by selecting enzymes with opposite enantiotopic face specificities for the same carbonyl substrate. Yeast contains two fatty acid synthetases with such properties. For example, β-keto esters **114** are reduced to the *R*-alcohols (*R*)-**115** by the D-enzyme and to the *S*-alcohols (*S*)-**115** by the L-enzyme[149] (Fig. 37). In this case it is important to note that the stereospecificities of the two "enantiotopic" enzymes are not identical and are strongly influenced by the substrate structure. The chiralities of the hydroxy products **115** can be predicted for both enzymes using a rule based on steric size distinctions between R and R′.[149] Fermentations with different organisms of opposite enantiotopic face specificities can also be used to select the enantiomer produced from β-keto ester reductions.[169] L- or D-Lactate dehydrogenases can be similarly exploited. Reduction of chloropyruvic acid to either L- or D-chlorolactic acid (L- or D-**116**) is easily accomplished. These chloroacids are then readily cyclized to the corresponding L- and D-glycidic acids (L- and D-**117**).[170] Quantitative expressions for analyzing the stereospecificities of pairs of enzymes that compete for the same substrate have been developed.[171]



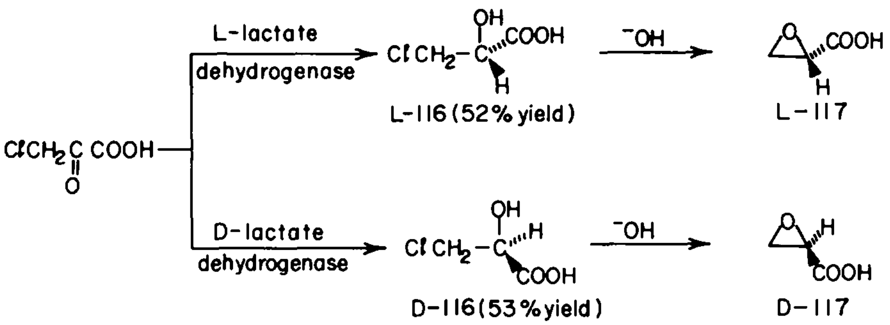


Fig. 37. Production of both enantiomers using enzymes with opposite enantiotopic face specificities.

Another stereospecific reaction of asymmetric synthetic importance is oxynitrilase-mediated addition of HCN to aldehydes. This enzyme, which is readily obtained from almonds, catalyzes exclusive *Si*-face addition of cyanide to a broad structural range of aldehydes (Fig. 38). The enzyme is readily immobilized. Its use in this form as a column in a flow system enables kilogram quantities of *R*-cyanohydrins **118** to be produced. These are easily converted into other useful synthons such as α-hydroxy acids **119**, aminoalcohols **120**, and acyloins **121**.[172] The oxynitrilase is subject to inhibition under the reaction conditions, but these and some purification problems are likely to be overcome in the near future.[58,173]

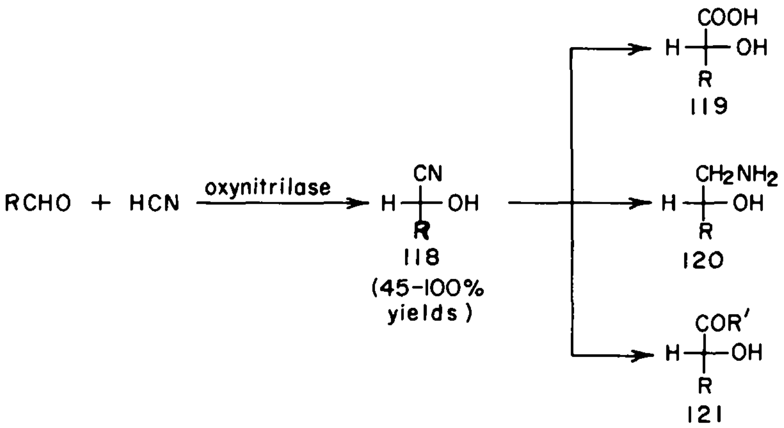


Fig. 38. Stereospecific cyanohydrin formation.

Chiral acyloins can also be obtained directly (Fig. 39). Yeast-induced condensation of benzaldehyde and acetaldehyde is of historic interest since it represents one of the first industrial exploitations of a microbial transformation, with the acyloin **122** being converted chemically into D-ephedrine.[5,174] Recently, even more exciting examples of this synthetically important stereospecific condensation have been reported. In the yeast transformations of aldehydes **123** to the diols **125**, the acetaldehyde required is fermentatively generated *in situ*. The initially formed acyloins **124** are not isolated but are further reduced[175] with *Re*-face specificity to give the pheromone synthon **125a**,[176] the potential pseudomonic acid precursor **125b**,[177] and the α-tocopherol chromanyl intermediate **125c**.[178]

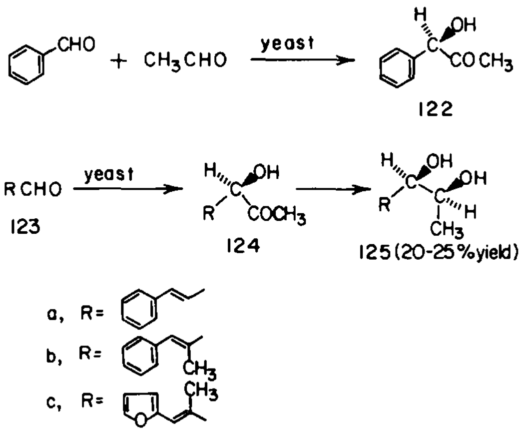


Fig. 39. Stereospecific acyloin condensations.

Controlled aldol condensations can be carried out. Aldolase† needs dihydroxyacetone phosphate (**126**) as one of its substrates, but will accept a broad structural range of aldehydes **127** as cosubstrate. High yields of structurally diverse aldols **128** can be obtained[179–182] (Fig. 40). An aldol condensation followed by hemiacetal formation is involved in the conversion of N-acetylmannosamine (**129**) and pyruvic acid to N-acetylneuraminic acid (**130**).[183]

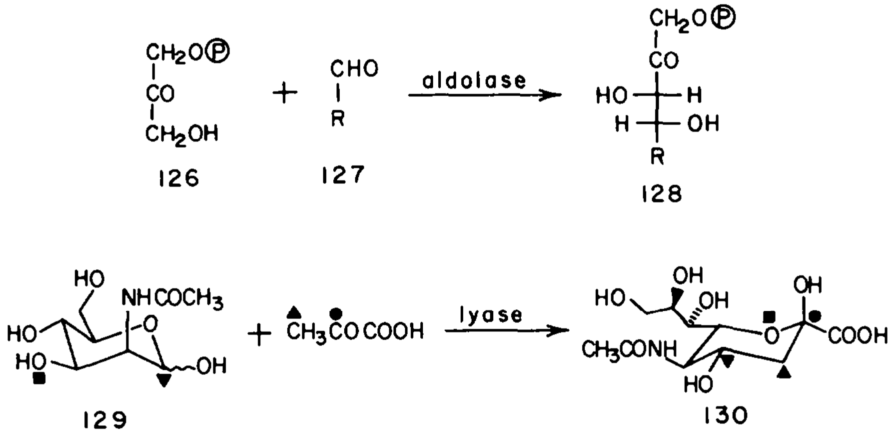


Fig. 40. Stereospecific aldol condensations. The ■▼●▲ symbols in the **129** → **130** conversion are included only to aid in atom identification.

Enzyme-catalyzed additions to C=N bonds are synthetically useful reactions. In particular, pyridoxal phosphate (**131**)-dependent enzymes induce a wide range of amino acid transformations.[184] The Schiff-base intermediate **132** is common to almost all of these interconversions. An inspired recognition of this led to the Fig. 41 route to L-tyrosine (**134a**), L-DOPA (**134b**), L-tryptophan (**134c**) and L-6-hydroxy-tryptophan (**134d**) from the achiral precursors pyruvic acid, ammonia and the respective side chain sources RH.[185] Regrettably, the cost of pyruvic acid and its instability in aqueous solution are among the reasons hindering commercialization of this process. Although the addition of the side

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† Other aldolase-catalyzed condensations are included in the multi-enzyme section.

chain source RH to the aminoacrylate **132** does not introduce a chiral centre, this step provides a dramatic illustration of the ease with which enzymes catalyze processes that would be very difficult to achieve by more traditional means.† The imine intermediate **133** cleaves hydrolytically under the enzyme's influence such that addition of the proton at the α-carbon of the target amino acid occurs with *Re*-face specificity. A broad range of L-amino acids can be synthesized using pyridoxal phosphate-dependent enzymes.[122,123,187]

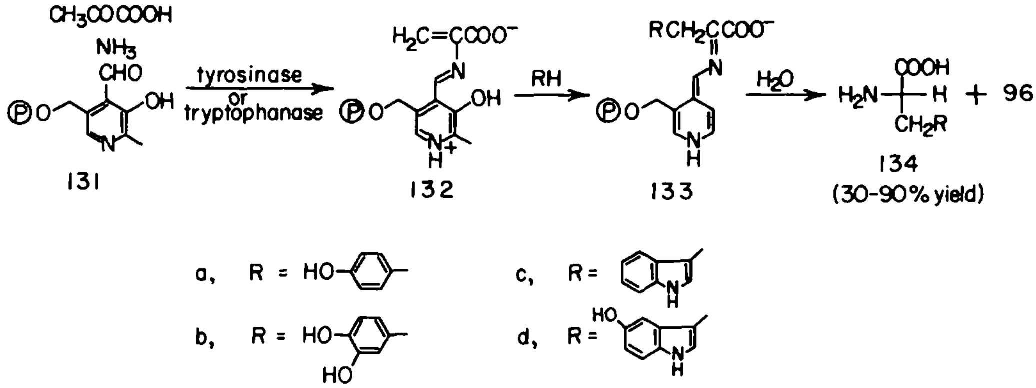

Fig. 41. L-Amino acid synthesis using pyridoxal phosphate (**131**)-dependent enzymes.

Highly stereospecific additions to C=C bonds can be achieved. For additions of HX to C=C functions, mainly of α,β-unsaturated acids, the addition occurs in an *anti*-manner for 17 enzymes studied in detail[188] (Fig. 42). Such lyase-catalyzed reactions have been exploited both industrially and in academic laboratories (Fig. 43). Additions to fumaric acid (**135**) can be performed on a very large scale to give in excess of 40 tons per month of L-aspartic acid (**81**)[189] or L-malic acid (**136**).[190] In addition,

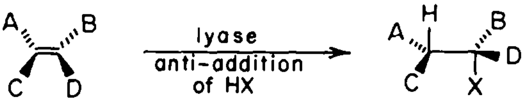

Fig. 42. Lyase-catalyzed additions involve stereospecific *anti*-additions of HX.

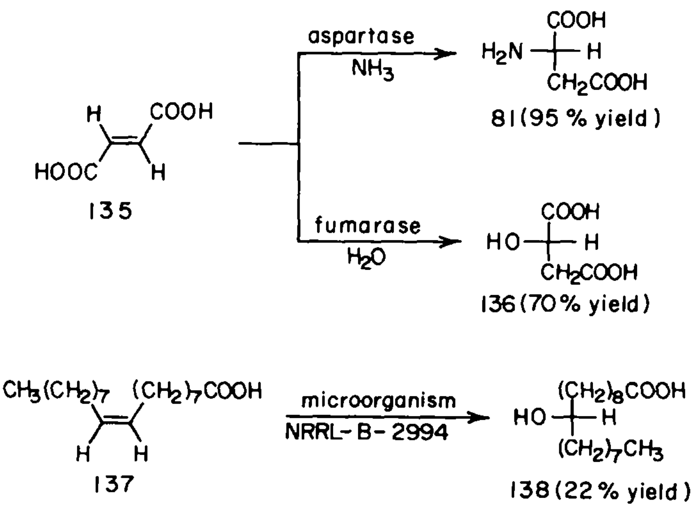

Fig. 43. Stereospecific additions to C=C bonds.

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† The **132** → **133** step is reversible and can be regarded as a formal Friedel–Craft reaction, or incredibly, its reverse in the opposite **133** → **132** direction,[186] that occurs in aqueous solution of pH 7 at room temperature! The **78** → **79** conversion of Fig. 24 is also in this category.

substituted fumarates can serve as substrates.[191] Yeast-mediated Michael additions to α,β-unsaturated ketones have also been reported.[192] Synthetically viable additions to unactivated double bonds are rare, but are known, as illustrated by the formation of (*R*)-10-hydroxystearic acid (**138**) from oleic acid (**137**).[193]

The remarkable C=C additions shown in Fig. 44 provide a further dramatic illustration of the degree of stereochemical control that enzymes can exert. In the stereospecific farnesyl pyrophosphate synthetase-catalyzed addition of geranyl pyrophosphate (**139a**) to 3-methylpent-3-enyl pyrophosphate (**140**), the *E*-isomer leads exclusively to the *S*-enantiomer of **141a**, and *Z*-**140** only to *R*-**141a**. The enzyme is obtained from pig liver and has a broad structural specificity. This structural tolerance has been exploited in the synthesis of the pheromone faranal (*R*-**141b**), using the enzyme to effect stereospecific coupling of homogeranyl pyrophosphate (**139b**) with *Z*-**140**.[194] In each of the reactions in Fig. 44, the additions occur by attack on the *Re*-face of C-4 of both *E*- and *Z*-**140**.

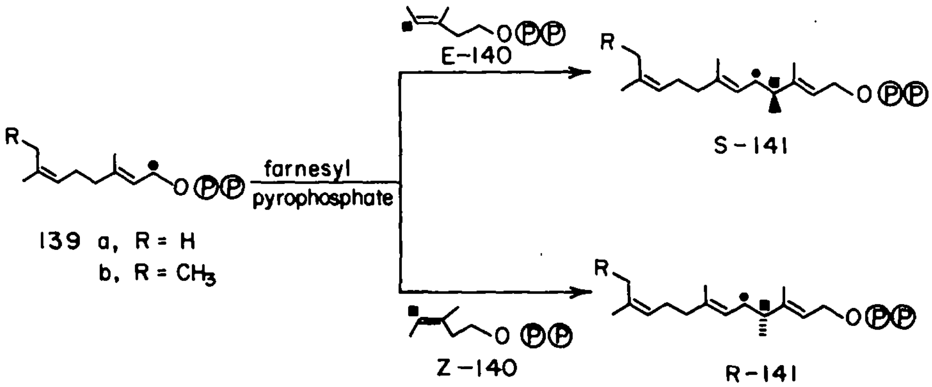


Fig. 44. Stereospecific additions to C=C bonds in the isopyrenoid field. The positions coupled are identified by ● and ■.

In contrast to stereoselective chemical epoxidations, which require allylic alcohol activation,[136] stereospecific enzyme-catalyzed epoxidations are achieved without difficulty, even on unactivated alkenes. While only 1–2% conversions of substrates are reported for the fermentation reactions depicted in Fig. 45, multigram quantities of epoxides **142**–**146** are readily obtained by these procedures.[195–198] Enantioface selective epoxidations of alkenes can also be accomplished using liver microsomal preparations.[199]

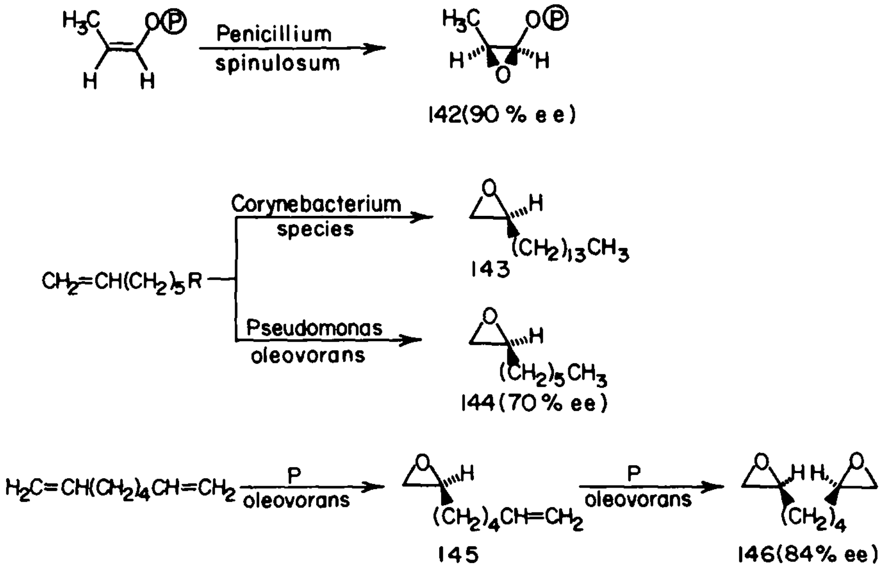


Fig. 45. Stereospecific epoxidations of unactivated C=C bonds.

In some instances, chiral epoxides of predictable absolute configurations can also be obtained from the halohydrins produced by chloroperoxidase-catalyzed addition of hypohalous acids to double bonds. The enzyme will utilize iodide, bromide, and chloride, but not fluoride, ions. The steroidal epoxides **147** and **148** depicted in Fig. 46[200] are just two examples from the range of possible substrate structures.[201] However, it must be cautioned that when the chloroperoxidase serves only to provide a source of hypochlorous acid, no stereospecificity can be expected.

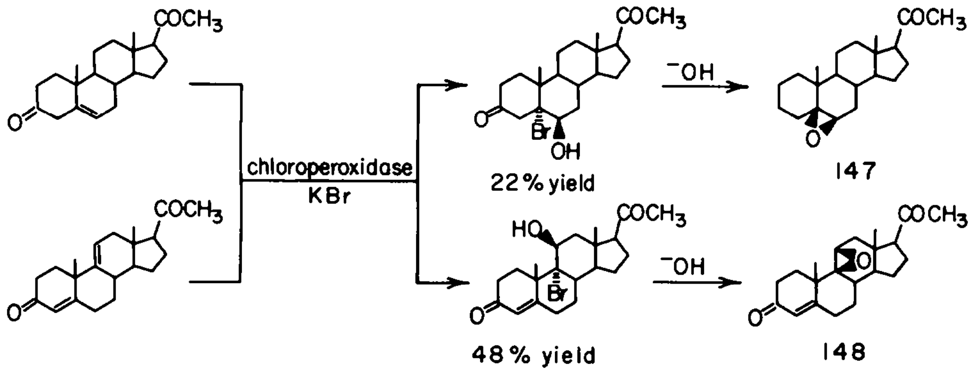


Fig. 46. Epoxide preparations via stereospecific additions of hypohalous acid equivalents to C=C bonds.

Stereoheterotopic face discriminations are exploitable in enzyme-mediated reductions of double bonds. Such reductions are catalyzed by NADH-dependent enoate reductases, which can be present in yeasts as illustrated by the preparations of **149**, **151** and **153**,[202,203] or by hydrogenases using molecular hydrogen as a cosubstrate, as in the stereospecific conversions of the allenes **155** and **157** to **156** and **158**, respectively[204] (Fig. 47). Large-scale reactions are possible. Reduction of **150** to the carotenoid precursor **151** has been performed on 13 kg of the starting enedione. The conversion of **152** to **153** was also carried out on a multigram scale in order to provide lactone **154** as a chiral synthon for the phytyl chain of α-tocopherol.[205]

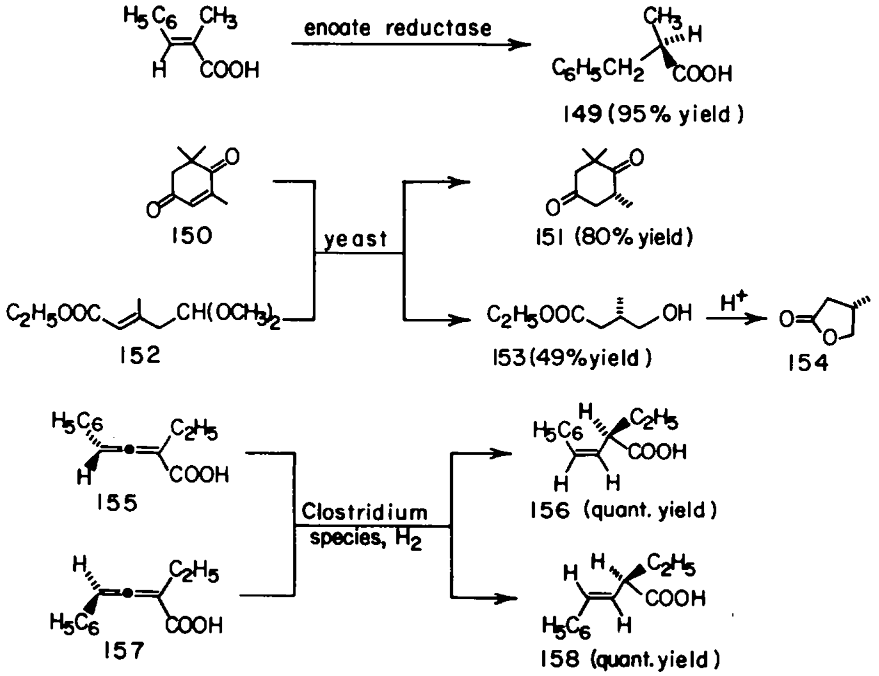


Fig. 47. Stereospecific reductions of C=C bonds.

Enzymic stereoheterotopic face distinctions are not restricted to sp^2-hybridized carbon functions. A number of enantiotopically specific oxidations of sulfides to sulfoxides have been effected.[206–208] Again, by choosing appropriate organisms, controlled formation of either an *R*- or *S*-sulfoxide can be achieved (Fig. 48). The asymmetric synthetic opportunities opened up by the availability of chiral sulfoxides are exemplified by the preparation of *R*-mevalonolactone from enantiomerically pure *R*-**159**.[206]

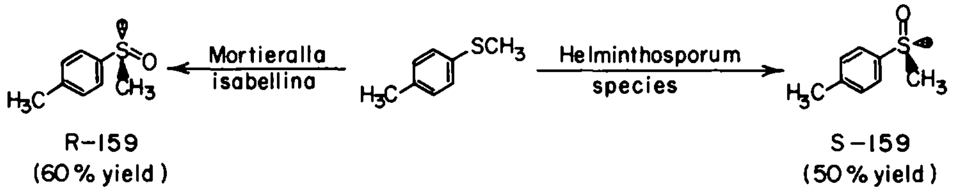


Fig. 48. Enantiotopically specific oxidations of sulfides provide both enantiomeric sulfoxides.

Distinctions between enantiotopic atoms and groups

Discrimination between enantiotopic atoms or groups is another valuable aspect of enzyme specificity that enables asymmetric transformations of symmetrical substrates to be achieved. Enantiotopically specific transformations of compounds possessing prochiral centres exemplify one aspect of this potential.

Horse liver alcohol dehydrogenase-catalyzed oxidations of diols such as **160**, **164** and **166** proceed with pro-*S* selectivity (Fig. 49). An additional advantage in the oxidations of **160** and **164** is that the initially formed hydroxyaldehydes, such as **161**, are themselves substrates and undergo further *in situ* oxidation via their hemiacetal forms **162** to give lactones directly. The preparation of the *S*-lactone **163**, a potential phytyl chain synthon,[8] and *S*-mevalonolactone (**165**)[209,210] exemplify this approach. Stereospecific oxidation of glycerol (**166**) to L-glyceraldehyde (**167**) can be accomplished using either HLADH[211] or galactose oxidase.[212] The latter enzyme is of interest because it is *independent* of nicotinamide coenzymes.

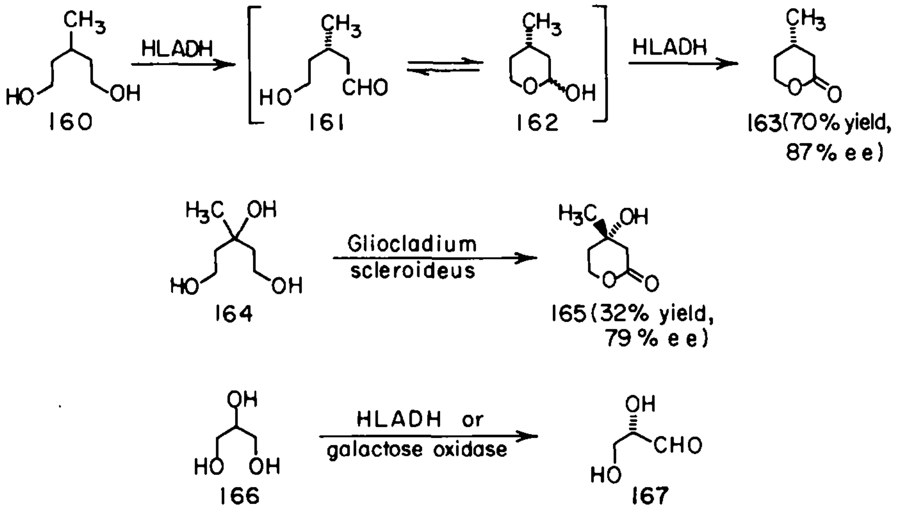


Fig. 49. Enantiotopically specific oxidations of diols.

Enantiotopic group specific reductions are also catalyzable by alcohol dehydrogenases. HLADH-promoted reductions of the decalindiones **168** and **171** are specific for only the pro-*R* keto functions, to yield the enantiomerically pure ketoalcohols **169** and **172**, respectively, of 9*S*-ring junction configurations (Fig. 50).[213] Even with the still more symmetrical diones **173** and **175**, which lack a ring junction prochiral centre, the reductions are completely stereospecific, yielding **174** and **176**, respectively.[213] Synthetically valuable reductions of this kind can also be effected with yeast, as exemplified by the conversion of **177** to **178**. The chiral synthon value of such ketoalcohols has been demonstrated by the conversion of **169** to (+)-4-twistanone (**170**)[213,214] and of **178** to the coriolin precursor **179**.[215] Alcohol dehydrogenases from *Curvularia lunata*, *Mucor javanicus* and pig liver have enantiotopic specificities that complement the examples in Figs 49 and 50.[2]

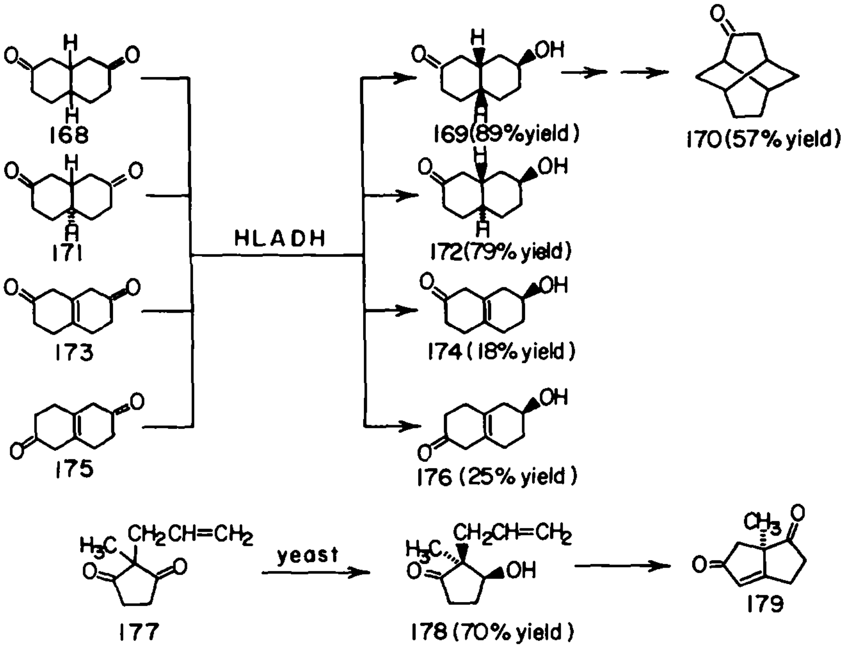


Fig. 50. Enantiotopically specific reductions of diones.

At the present time, hydrolytic enzymes possess the greatest versatility with respect to enantiotopic group discriminations of synthetic value. There are several esterases that have broad applicabilities in this regard. Chymotrypsin- or pig liver esterase-catalyzed hydrolyses of C-3-substituted glutarate diesters **180** are generally pro-*S* ester group selective (Fig. 51). The *S*-half-ester products **181** are attractive chiral intermediates since they can be converted at will into derivatives such as lactones **182** of either *S*- or *R*-enantiomeric series. *S*- and *R*-mevalonolactones (**182a**) have been prepared in this way,[216] and the acid-ester **181b** has been used in megamycin[217] and *β*-lactam[218] syntheses. The

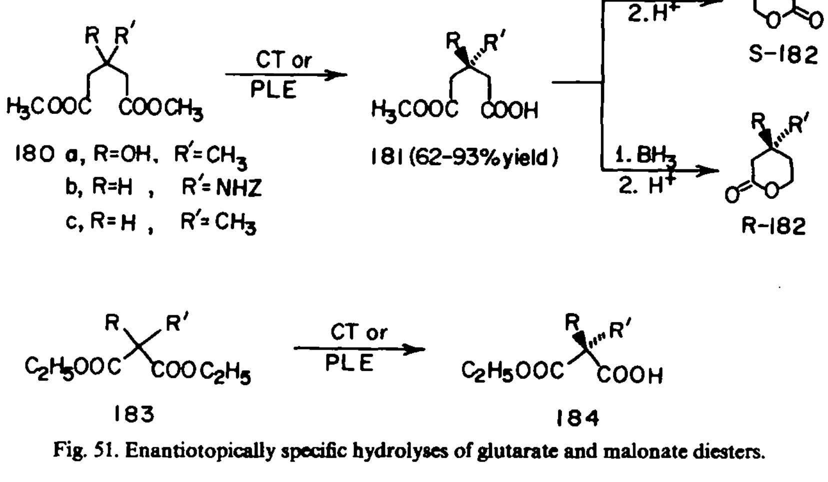
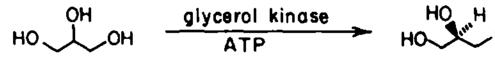


Fig. 51. Enantiotopically specific hydrolyses of glutarate and malonate diesters.

synthon value of **182c**[219] (**163** of Fig. 49)† has been noted previously.[8] It has also served as a verrucarin precursor.[222] Chymotrypsin and pig liver esterase, which generally exhibit opposite enantiotopic specificities, tolerate a broad spectrum of C-3 substituents in their stereospecific catalyses of **180**→**181** reactions.[128,216,220,223,224] Substituted malonate diesters **183** can also be hydrolyzed to the corresponding acid esters **184**, R, R′ = H, alkyl, aryl, acetoxy, or acetamido, with high enantiotopic selectivities by chymotrypsin,[223] pig liver esterase[225] or microbial esterases.[226] However, when C-2 is epimerizable, i.e. when R or R′ = H, the chiral products **184** racemize very easily.[2,223] While a good model for predicting chymotrypsin stereospecificity is available,[127] those for pig liver esterase[227,228] are at a very early stage of development and should be applied cautiously until they are refined.

Stereospecific esterification of enantiotopic hydroxyl groups is also possible. *sn*-Glycerol-3-phosphate (**185**) is readily obtained from glycerol using glycerol kinase[229] (Fig. 52).‡



185

Fig. 52. Enantiotopically specific phosphorylation.

A major lacuna in current synthetic methodology is the inability to effect controlled hydroxylation of unactivated carbon atoms. In contrast, hydroxylases can accomplish this readily and stereospecifically.[148] The enantiotopically specific hydroxylation of isobutyric acid[230,231] (Fig. 53) has been extensively used because the (*S*)-β-hydroxyisobutyric acid (**186**) product is a versatile chiral synthon for many natural products, such as α-tocopherol,[232] *R*- and *S*-muscone,[233] maysine,[234] calcimycin,[235] and polyether and macrolide antibiotics.[236] Two alternative routes to **186**, one involving microbial oxidation of 2-methylpropane-1,3-diol[237] and the other a yeast reduction of ethyl α-formylpropanoate,[238] have been reported. The latter avoids some fermentation problems associated with the *P. putida* method of Fig. 53. Many stereospecific oxidations of enantiotopic methyl groups are known.[5] The hydroxylations of **187** to the anti-inflammatory **188**[239] and, diastereotopically specifically, of **189** to the hydroxypinane **190**[240] are two more examples.

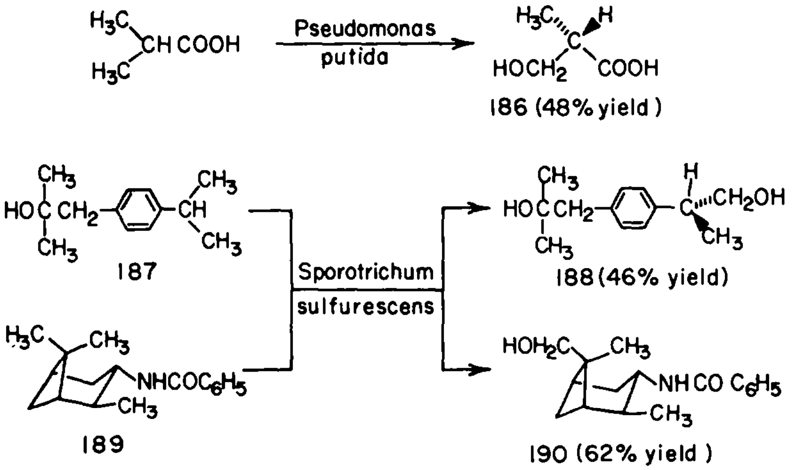


Fig. 53. Enantiotopically specific hydroxylations of methyl groups.

Stereospecific hydroxylations of enantiotopic hydrogens of methylene groups are well documented. One example has already been given (Fig. 1). The stereospecificity of many microbial hydroxylations is predictable. Some models available for this purpose are discussed in the next section.

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† The stereospecificities of different batches of PLE can vary somewhat, presumbly as a result of different isozymal compositions. For example, while our initial purchase of enzyme gave **181c** of > 97% ee for reactions in aqueous solution pH 7 at 20°[220] subsequent batches have afforded only ~78% ee levels. However, using optimized reaction conditions, namely 20% aqueous methanol, pH 7, −10°, restores the ee of **181c** to 97%.[221]

‡ *sn* is a citrate-based nomenclature in common biochemical use (H. Hirschmann, *J. Biol. Chem.* **235**, 2762 (1960)).

Analogous enantiotopically specific peroxidation reactions, which formally appear to be the result of stereoheterotopically specific addition to a double bond, presumably undergo double bond isomerization prior to substitution (Fig. 54). Using a lipooxygenase from potatoes, arachidonic acid (**191**) is converted into (*S*)-5-HPETE (**192**), an intermediate required for the synthesis of the slow reacting substance of anaphylaxis.[241] Use of an immobilized soybean lipooxygenase permits continuous production of (*S*)-15-HPETE (**193**) and the (*S*)-13-hydroperoxy derivative **195** of linoleic acid (**194**).[242]

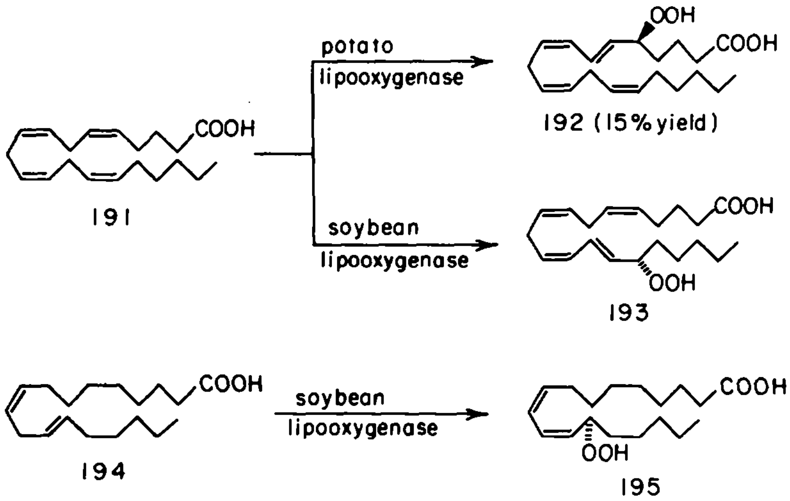

Fig. 54. Enantiotopically specific hydroperoxidations.

Enzymic discriminations of enantiotopic groups of *meso*-compounds have been widely exploited in asymmetric synthesis. An incredibly broad structural range of *meso*-diols can be stereospecifically oxidized using HLADH. The 1,2-disubstituted diol oxidations shown in Fig. 55 are just a few of the examples that have been documented.[243] As for the **160**–**163** conversion (Fig. 49), hemiacetal intermediates are involved in the formation of the lactone products **196**–**200**. The synthetic utility of these lactones has been demonstrated by the conversion of **196** into an iridoid aglycone,[244] of **197** into grandisol,[245] and of **198** into methyl chrysanthemate.[243] Lactones **199** ($n = 1$) and **200**, respectively, are attractive intermediates for macrolide[246] and prostaglandin[243,247] syntheses.

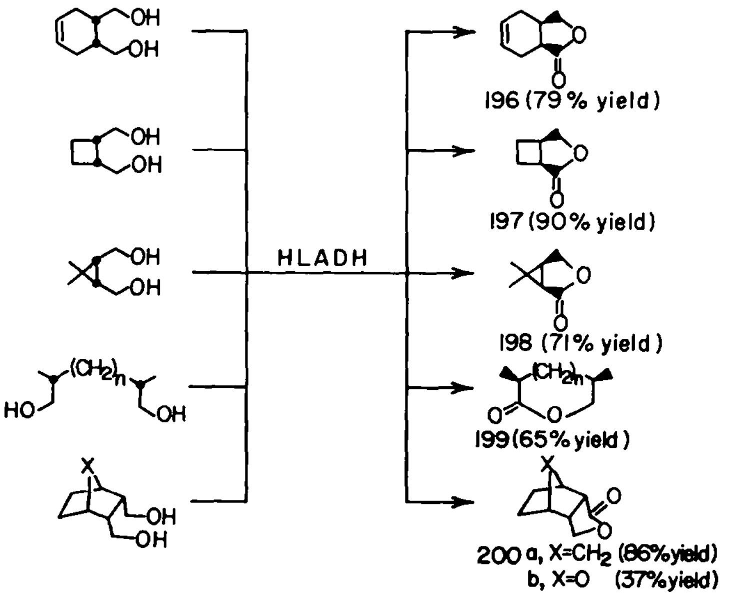

Fig. 55. Stereospecific oxidations of *meso*-diols.

The situation for *meso*-1,3-disubstituted diols is similar (Fig. 56). Diols **201** and **203** are smoothly converted to lactones **202** and **204**, respectively.[248] Severe product inhibition precludes preparative-scale oxidation of **203** (X = S).[249]

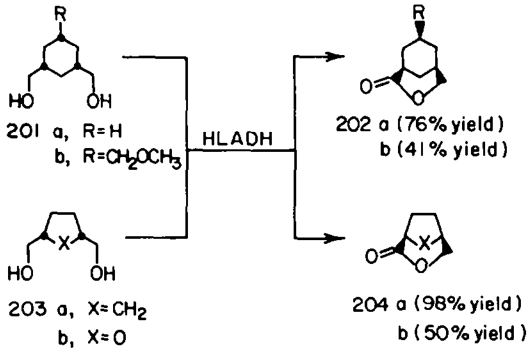

Fig. 56. Stereospecific oxidations of 1,3-*meso*-diols.

Similar enantiotopic group distinctions in *meso*-substrates are possible for secondary alcohol functions. Glycerol dehydrogenase-catalyzed oxidation of *cis*-1,2-dihydroxycyclohexane (**205**) yields (*S*)-**206**[138] (Fig. 57).

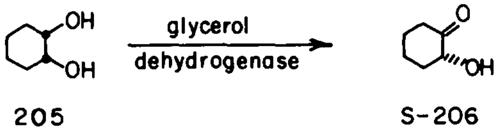

Fig. 57. Stereospecific oxidation of *cis*-cyclohexane-1,2-diol.

Stereospecific reductions of *meso*-diketones can be performed, as demonstrated by the conversion of the decalindione **207** to **208**.[2,250] The analogous transformation of the achiral diketone **209** to **210**[213] is also readily accomplished (Fig. 58).

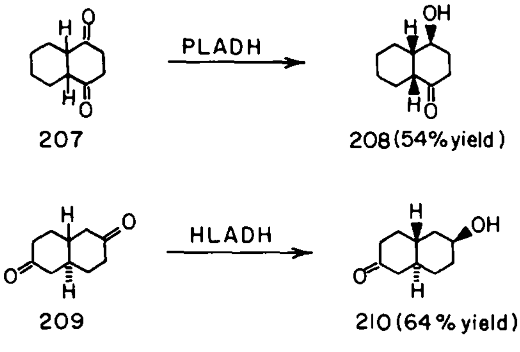

Fig. 58. Enantiotopically specific reductions of *meso* and related achiral diketones.

The abilities of hydrolytic enzymes to operate on *meso*-diesters with enantiotopic specificity are other approaches with broad asymmetric synthetic potential (Fig. 59). Either enantiomer of the acid ester **212**, and hence lactones (2*S*,4*R*)- and (2*R*,4*S*)-**213**, are accessible using esterases of opposite enantiotopic specificities to catalyze the hydrolysis of the *meso*-glutarate diester **211**.[135] The versatility of pig liver esterase is further manifest by its catalysis of the conversion of **214** to the biotin intermediate (4*S*,5*R*)-**215**,[251] of **216** to the C-nucleoside synthons **217**,[252] and of **218** to **219**,[227,253–256] from which the lactone **196** has been prepared for use in the syntheses of brefeldins[253] and prostaglandins.[257]

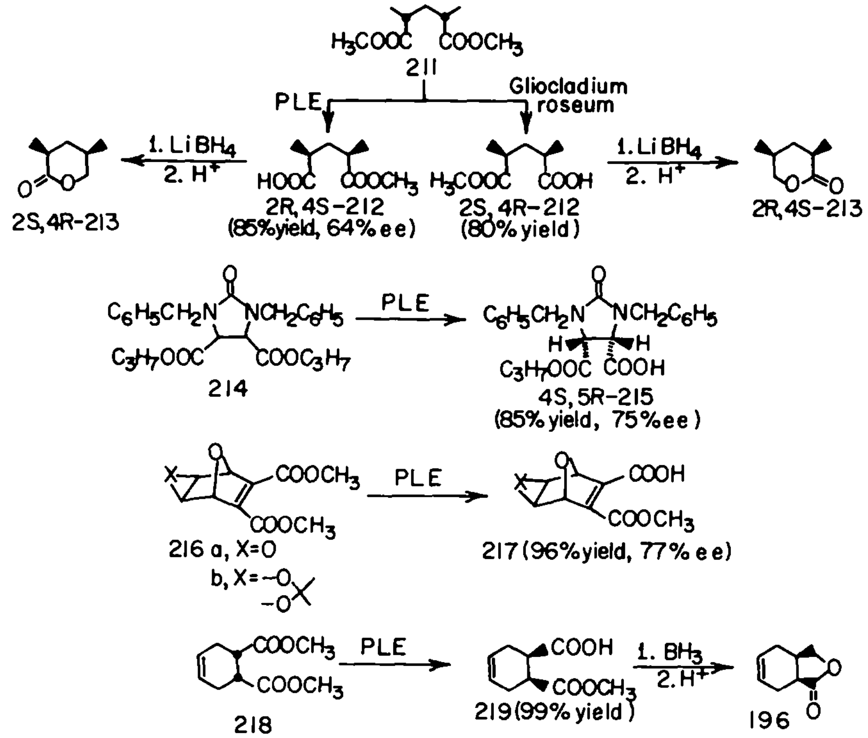

Fig. 59. Enantiotopically specific hydrolyses of *meso*-diesters.

Similar enantiotopic discriminations can be induced for *meso*-diester substrates in which the prochiral components are alcohols (Fig. 60). The transformations of **220** to **221**[258] and of **222** to **223**[137,259] complement those of **211** to **212** and **218** to **219**, respectively, while the **224** to **225** type of conversion[258,260] is currently unique. PLE-catalyzed hydrolysis of the bisacetoxymethyl analogue of **214** provides a desired biotin precursor of 92% ee.[261]

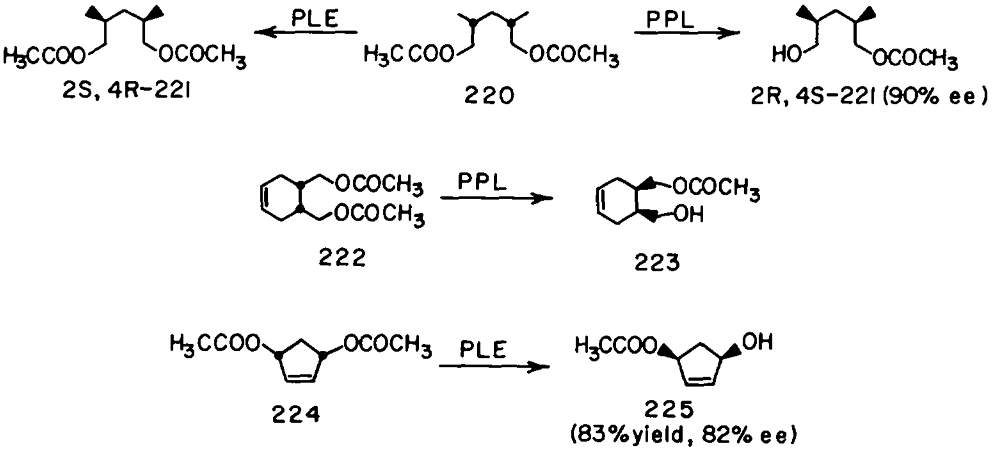

Fig. 60. Stereospecific hydrolyses of *meso*-diesters of prochiral alcohols.

Pig liver esterase exhibits a broad tolerance of structural variations in its *meso*-diester substrates. Furthermore, in its hydrolysis of the monocyclic 1,2-diesters **226**, **228**, **230** and **232**,[227,254,256,262] an unprecedented reversal of stereospecificity[262] is observed on going from the six-membered ring series **226** to the cyclobutyl-(**230**) and cyclopropyl-(**232**) diesters (Fig. 61). The cyclopentyl diester **228** represents the crossover point, with only marginal (17% ee) *R*-centre ester selectivity being manifest.

The half esters **227**, **229**, **231** and **234** are readily converted to lactones of either enantiomeric type, as shown for **197** and **235**.[256]

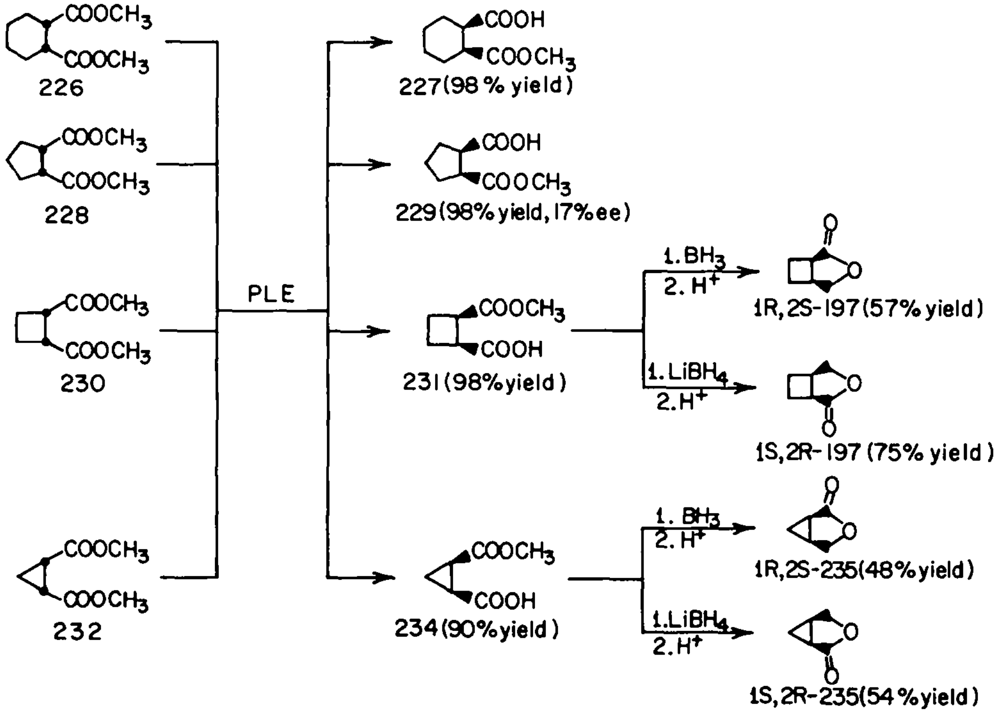

Fig. 61. A reversal of stereospecificity is observed in PLE-catalyzed hydrolyses of monocyclic *meso*-diesters.

Some of the esterase- and alcohol dehydrogenase-derived lactones of Figs 49, 51, 55, 59 and 62 are identical. In such situations, and where enantiomerically pure products are obtained by both methods, the use of the esterase approach, which avoids any coenzyme recycling problems, is highly preferred.

Other stereospecific transformations of *meso* and related substrates are shown in Fig. 62. The epoxide hydrolase from rabbit liver discriminates between the enantiomeric conformers of the *meso*-epoxide **236** to give the *trans*-diol **237**.[263,264] (A similar enantiomeric conformer discrimination is

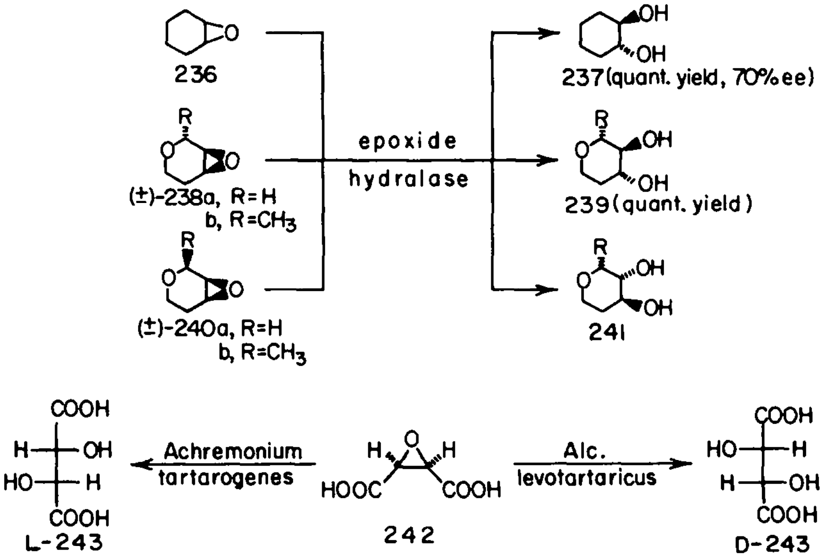

Fig. 62. Stereospecific hydrolyses of *meso* and related epoxides.

considered to operate in the HLADH-catalyzed oxidation of *cis*-1,2-bis(hydromethyl)cyclohexane.[243,265]) Even more remarkable is the epoxide hydrolase catalysis of the hydrolysis of the racemic epoxides **238**. Both enantiomers of **238** are hydrolyzed to the same diol **239**, with the enzyme directing diaxial opening of the epoxide ring via nucleophilic attack at C-3 of (3*S*,4*R*)-**238** and at C-4 of the (3*R*,4*S*)-enantiomer at practically equal rates.[266] The situation for the 2-methyl derivatives is analogous. After 50% of hydrolysis, the *trans*-racemate **238** gives rise only to **239** while the *cis*-compounds (±)-**240** lead exclusively to the **241** diastereomers.[267] Furthermore, by appropriate selection of the source of the hydrolase, attack at either the *S*- or *R*-centre of a *meso*-epoxide can be effected, as demonstrated by the conversion of **242** to L- or D-tartaric acid (**243**).[268]

While attention in this section has focused almost exclusively on alcohol dehydrogenases and hydrolases, other enantiotopically specific enzymic transformations of *meso*-compounds are known. The industrially interesting preparation of L-lysine (**88**) by stereospecific decarboxylation of **244** (Fig. 63) is just one further example.[269]

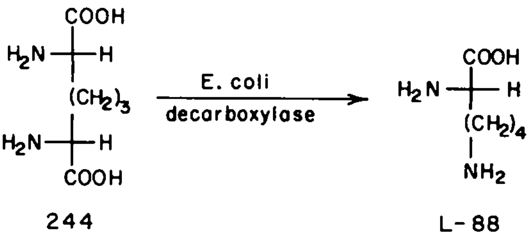

Fig. 63. Stereospecific decarboxylation of a *meso*-diacid.

Distinctions between diastereotopic atoms and groups

Diastereotopically specific substitutions of hydroxyl groups for hydrogen atoms of unactivated methylene groups dominate this aspect of enzyme-mediated reactions. In the cases that have been studied, the hydroxylations take place with retention of configuration.[148] Hundreds of diastereotopically specific hydroxylations of methylene groups are known for an enormous range of substrate structures. The field is well documented in excellent books and reviews.[3–5,11,148,270–275] One can confidently expect to find an organism that will effect almost any desired hydroxylation on virtually any substrate structure. Some illustrative examples are depicted in Figs 64 and 65.

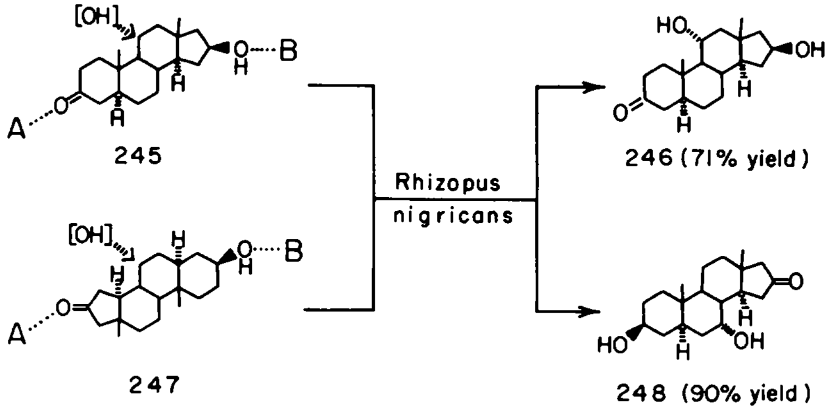

Fig. 64. Controlled hydroxylation of steroids. A and B represent the enzymic binding sites for C=O and OH groups, respectively.

The breakthrough in corticoid synthesis provided in 1952[276] by the 11α-hydroxylation of progesterone by *Rhizopus nigricans*† stimulated an interest in microbiological hydroxylations that

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† The organism names used in this review are those most commonly used in the microbial transformation literature. Some, such as *Rhizopus nigricans* (now designated *Rhizopus stolonifer*) have been renamed as their characterization has become more accurate.

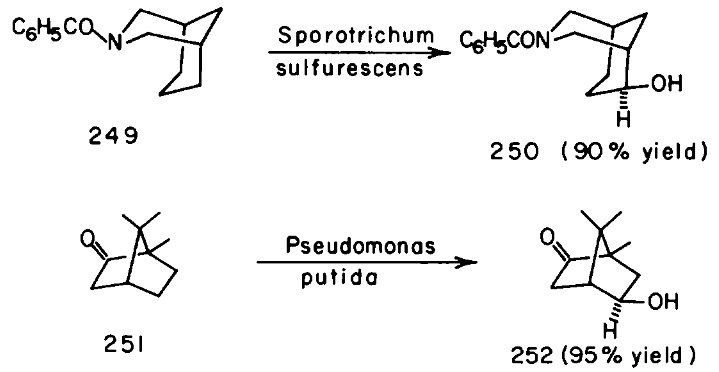


Fig. 65. Diastereotopically specific hydroxylations are catalyzed by cytochrome P-450-containing organisms.

continues unabated to this day. Stereospecific hydroxylation of virtually any steroid carbon atom can now be achieved at will.[148] The same generalization applies to terpenes[5,273] and alkaloids.[274,275] In addition, for many microorganisms the position and stereochemistry of hydroxylation is predictable using simple models.[3,148] For *Rhizopus nigricans*, the cytochrome P-450 responsible for hydroxylation always delivers its hydroxyl equivalent, designated as [OH] in Fig. 64, from a unique active site location and direction. The orientation of a steroid substrate relative to this hydroxylation delivery site is controlled by the binding of its C=O and OH groups to complementary electrophilic centres, designated as A and B, also positioned uniquely within the active site of the hydroxylase. With 3-keto-16β-hydroxy-5α-androstane (**245**) as substrate, this results in delivery of the hydroxyl equivalent to the 11-position to give **246**. Conversely, if the 3-keto and 16-hydroxy functionalities are reversed, as in **247**, the substrate binds the other way around. Delivery of [OH] from the same active site location then yields the 7α-hydroxylated product **248**.[277] *Calonectria decora* is another well-documented organism for which a similar active site model has been derived.[278]

Hydroxylations with *Sporotrichum sulfurescens* (now called *Beauvaria bassiana*) have also been widely applied. This organism prefers amide substrates. A simple active site model of predictive value has been developed which reflects the preference of this hydroxylase to introduce the hydroxyl group ~ 5.5 Å away from an amide N-atom and in the *anti*-orientation with respect to the N-acyl group.[148] The stereochemical consequence of this is reflected in the conversion of **249** to **250** (Fig. 65).[279] The conversion of camphor (**251**) to *exo*-5-hydroxycamphor (**252**) by *Pseudomonas putida* is of interest because the cytochrome P-450 responsible for the hydroxylation is stable and has been isolated in crystalline form.[280] Regrettably, the enzyme has a very narrow specificity and only substrates closely related to camphor are oxidized. Furthermore, its regiospecificity is easily disturbed. 5,5-Difluorocamphor is hydroxylated at the C-9 position.[281] The stability of the *P. putida* hydroxylate is unusual since most cytochrome P-450's are unstable. As a result, stereospecific hydroxylations using purified enzymes are unlikely to be preparatively viable in the foreseeable future. Fermentations will remain unbeatable for hydroxylations for many years yet, although progress is being made with immobilized cells.[282]

A different, and dramatic, diastereotopic hydrogen-specific enzyme-catalyzed cyclization is shown in Fig. 66. Various unnatural and chemically modified peptides can be cyclized to new penicillin and cepham antibiotics using isopenicillin-N-synthetases.[283–287] The conversion of **253** to **254** illustrates this reaction.

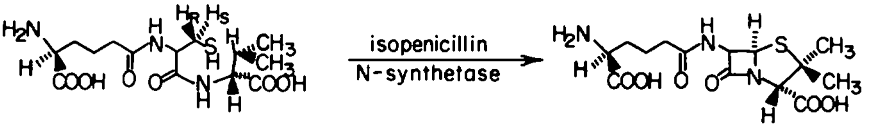


Fig. 66. A diastereotopically specific β-lactam cyclization.

Diastereotopic atom specific distinctions are not restricted to hydrogen atoms. Diastereotopic oxygen atoms on phosphorus are discriminated in the synthesis of the thio-ATP analogue **256** from **255**[288] (Fig. 67).

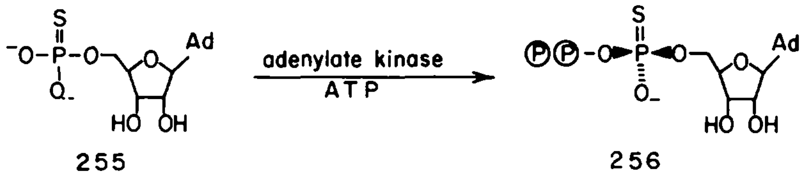


Fig. 67. A diastereotopically specific O-atom distinction.

EXPLOITING COMBINATIONS OF ENZYME SPECIFICITY

The degree of stereochemical control achievable using non-enzymic methods in asymmetric synthesis has seen spectacular improvements in recent years. Nevertheless, no chemical chiral reagent can yet approach the abilities of enzymes to combine several different specificities in a single-step reaction. The concurrent control of different specificities that enzymes can achieve in one catalytic process provides a significant advantage, especially when several tedious or difficult steps would be needed to accomplish the same goal by traditional methods, even if the latter were available. Some combinations of specificity, involving enantiomeric discriminations coupled with selective ester hydrolyses (Fig. 34) and concurrent enantiotopic group and face distinctions (Figs 4 and 58), have been seen already. Various other specificity combinations, using alcohol dehydrogenases as an illustrative enzyme class, are shown in Figs 68–72.

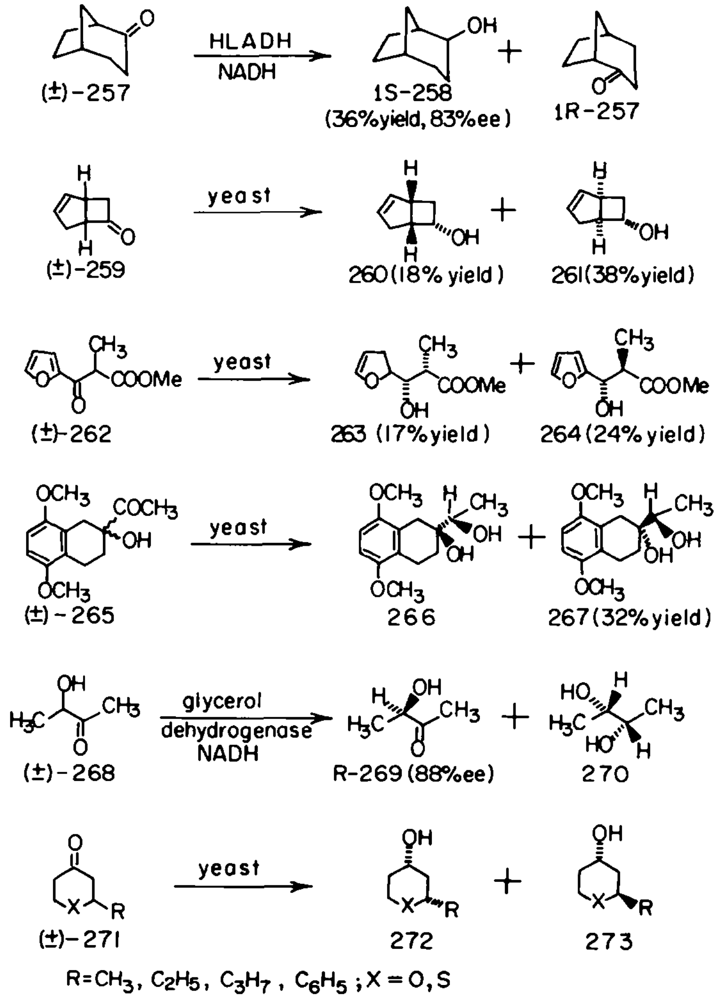


Fig. 68. Combinations of enantiomeric and diastereotopic face specificities can be exploited.

The reductions shown in Fig. 58 are selective for one enantiomer of the racemic ketone substrate and are also diastereotopically specific for one of the faces of the carbonyl groups. The enantiomeric distinctions can involve substrates with common types of chiral centres, such as **257**, **259**, **262**, **265**, **268** and **271** (Fig. 68), or of the more unusual chiralities present in **274** and **276** (Fig. 69). In the reduction of (±)-**268**, the unreactive enantiomer *R*-**268** was the target and no serious effort was made to isolate the highly water soluble diol product **270**. Clearly, this too can be recovered if needed.[138] The HLADH-catalyzed reduction of (±)-**257** is of interest since the alcohol product (1*S*)-**258** is the thermodynamically less-preferred *exo*-epimer.[289] This emphasizes the fact that the geometry of an initial, kinetically controlled, product of an enzyme-catalyzed reaction reflects only the direction of attack of the reacting functions in the ES complex. In this case the hydride equivalent is delivered to the *Re*-face of the carbonyl group of (1*S*)-**257**. The preparations of (*S*)-**275**[290] and (*M*)-**277**[291] were performed in connection with an investigation of molecules of novel chiralities.

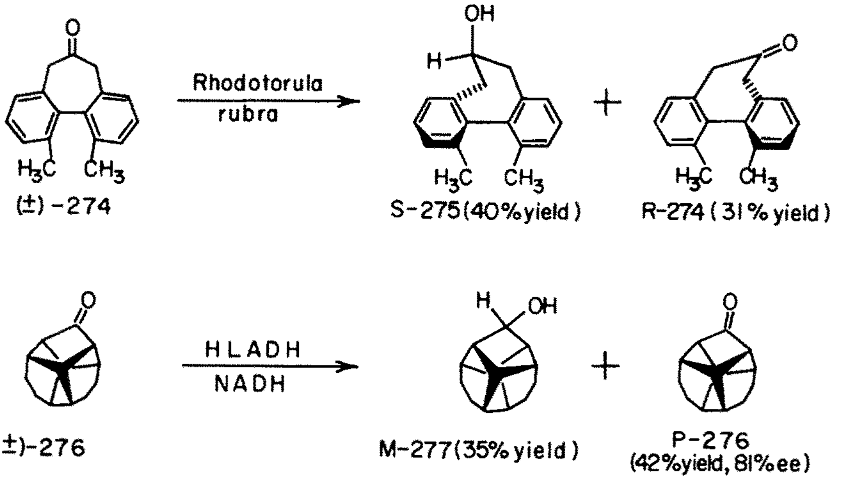


Fig. 69. Reductions of ketones of unusual chirality.

Reductions of (±)-**259** to the prostaglandin precursors **260** and **261**,[292] of (±)-**262** to the polyoxoantibiotic and oudemansin synthons **263** and **264**,[293] of (±)-**265** to **266** and **267** (useful for the synthesis of adriamycin and its analogues[294]), and of (±)-**271** to **272** and **273**,[295,296] are noteworthy because both enantiomers of each racemate are substrates, albeit with one reduced faster than the other. When enantiomeric specificity is lacking in this way, the initial conclusion is often that no resolution is therefore possible by the enzymic procedure. However, this may not be true. In such reactions, the individual reductions of each enantiomer remain completely stereospecific, thus giving rise to pairs of diastereomeric products. These are easily separated by chromatography in stereoisomerically pure form. The starting ketone enantiomers can then be obtained by individual chemical oxidations of these product stereoisomers. Accordingly, when both enantiomers of a racemate are substrates in a stereospecific enzymic process, the reaction is allowed to proceed to completion rather than terminating at the 50% point as is usual for enantiomerically specific transformations. Using this approach, quantitative yields of pure diastereomeric products can be isolated.[295]

Enantiomeric specificity coupled with regio- (or chemo-) specificity is another synthetically useful combination (Fig. 70). For example, very few chemical oxidizing agents are able to operate selectively on either a primary or secondary alcohol group in the same molecule. However, as a consequence of the substrate-fit requirements of its active site, HLADH exerts this control easily and predictably[167] for diols such as (±)-**278** and (±)-**280**. The regiospecificity is not chemically controlled, but reflects the binding orientation of the substrate at the active site. Only the hydroxyl group that can locate itself at the oxidation centre of the active site can be oxidized. For (±)-**278**, it is the primary hydroxyl function of the (1*R*,2*S*)-enantiomer that fits acceptably at the oxidation site and is therefore oxidized. The secondary alcohol group cannot be similarly accommodated and remains unchanged. The (1*R*,2*S*)-aldehyde formed initially then undergoes a second enzyme-catalyzed oxidation to give the prostaglandin synthon (1*R*,2*S*)-**279** directly.[297] Optically pure material can be obtained by recrystallization.[298] Conversely, for (±)-**280**, only the secondary alcohol function can fit the oxidation site of HLADH. This regiospecific distinction is again accompanied by enantiomeric discrimination and the ketoalcohol (3*S*)-**281** is the only product.[297]

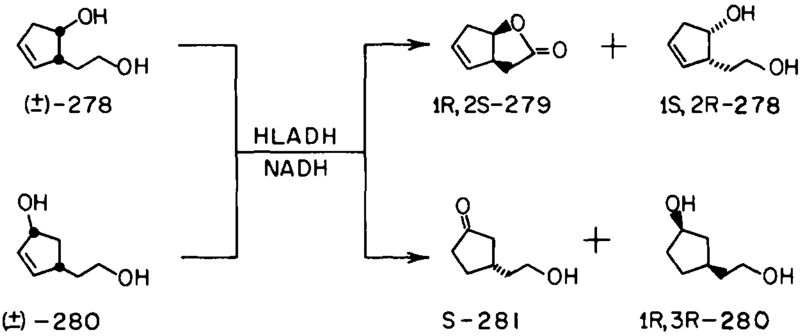


Fig. 70. Combinations of enantio- and regiospecificity.

Regio- and stereoheterotopically specific reductions of one carbonyl group of polyketones is another specificity combination that can be exploited (Fig. 71). *Rhizopus arrhizus*-induced reduction of the trione **282** is regiospecific for a cyclopentane keto group. It is also doubly enantiotopically specific, with hydride addition being directed to the *Si*-face of the pro-*R* carbonyl group. The hydroxydiketone **283** obtained in this way is a key intermediate in a versatile steroid synthesis.[299] Similarly, the regio- and diastereotopically group and face specific reductions of (±)-**284** affords **285** from which PGE_1 can be synthesized in four steps.[300] Fermentative reduction with *Aueobasidium pullulans* is stereospecific for the *Re*-faces of the carbonyl groups attached to both the *R*- and *S*-centres of achiral diketone **286**. The diol product **287**, which is also achiral, was the foundation on which the first synthesis of compactin was built.[301]

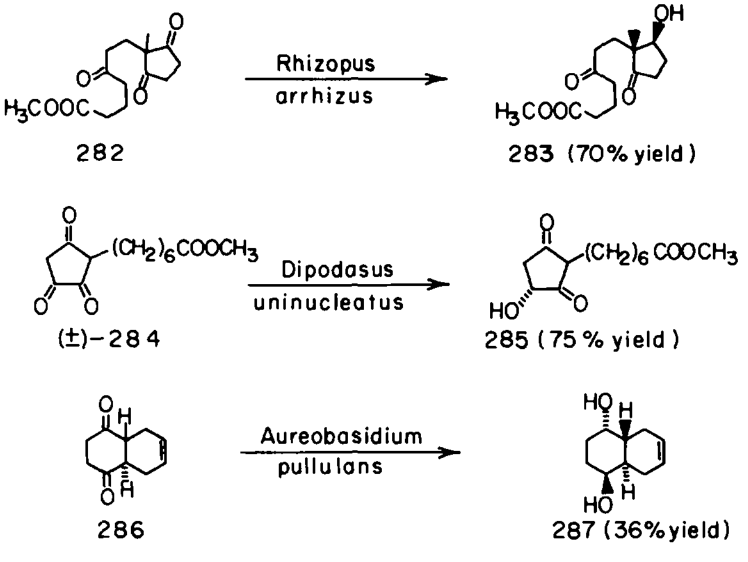
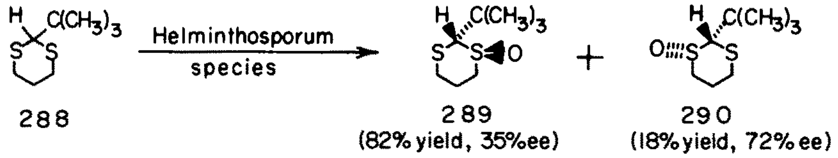


Fig. 71. Combinations of regio- and stereoheterotopic group and face specificities.

While the *Helminthosporium* species-mediated oxidation of disulfide **288** is not completely stereospecific, the enantiomeric purities of the diastereomeric *trans*- and *cis*-sulfoxide products **289** and **290**, respectively, are significant[302] (Fig. 72).

The different specificities of enzymes from divergent sources permit subtle control of the product stereochemistry desired. For example, any of the three diastereomeric alcohols (2*S*,9*R*)-, (2*R*,9*S*)-, or (2*S*,9*S*)-**292** can be obtained at will from racemic *trans*-2-decalone ((±)-**291**) using the alcohol



288

289 (82% yield, 35%ee)

290 (18% yield, 72% ee)

Fig. 72. A combination of enantiotopic group and face specificity.

dehydrogenases of HLADH, MJADH and CFADH, respectively Fig. 73). The enantiomeric and stereoheterotopic specificities of these enzymes are well documented and a simple active site model of predictive value is available for each.[2,167]

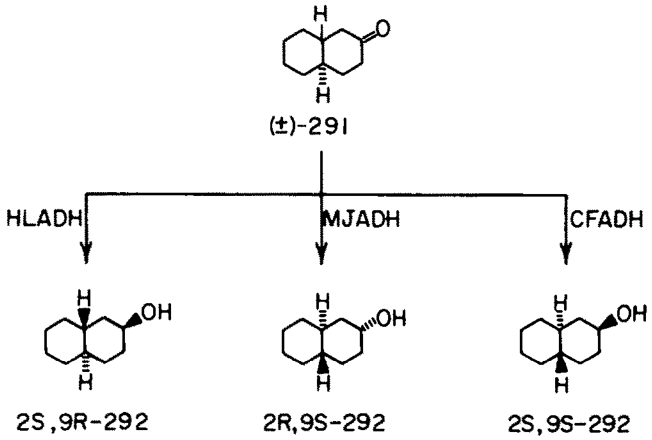


Fig. 73. Different enzymes can have different enantiomeric and diastereotopic face specificities for the same substrate.

MULTIPLE ENZYME REACTIONS

The fact that most enzymes are specific with respect to the type of reaction they catalyze enables them to operate independently on their own substrate in the presence of other enzymes and their substrates. This allows multiple, sequential, synthetic transformations to be carried out in one-pot reactions, or in flow systems using columns of immobilized enzymes or cells. Multi-enzyme systems have already been widely applied for coenzyme recycling.[10]

The preparative viability of multi-enzyme-mediated syntheses was firmly established by the pioneering work on the preparation of gramicidin S from its component amino acids.[303] Recently, interest in multi-enzyme syntheses has intensified and it is already clear that the approach has enormous synthetic potential.

Some two-enzyme transformations are depicted in Fig. 74. There is industrial interest in the conversions of fumaric acid (**135**) to L-alanine (**82**)[304,305] and of Reichstein's compound S (**293**) to prednisolone (**294**).[306,307] The glyoxalase-catalyzed conversion of **295** to **296**[308–310] also works well but remains of academic interest at the present time. The Δ^1-dehydrogenase used for the **293** → **294** oxidation is not required to be stereospecific because the substrates are already of the natural series.[311] However, such dehydrogenases do possess the ability to operate stereospecifically and have been used for resolving racemic steroids and related compounds.[312–314] Amino acids and peptides such as L-citrulline (**297**)[315] and glutathione (**298**)[316] can be prepared using the multi-enzyme approach (Fig. 75).

Carbohydrate metabolism provides a rich pool of enzymes of synthetic value. Some monosaccharide preparations are shown in Fig. 76. The expensive ribulose-1,5-diphosphate (**300**) can now be obtained more economically, and in quantity, from 6-phosphogluconic acid (**299**).[317] Aldolase-catalyzed condensations of dihydroxyacetone phosphate (**301**) with L- or D-lactaldehyde lead to 6-deoxysorbose (**302**) and the blood group-related monosaccharide 6-deoxyfucose (**303**), respectively. Either **302** or **303** is then readily converted into the flavour principle furaneol (**304**).[318]

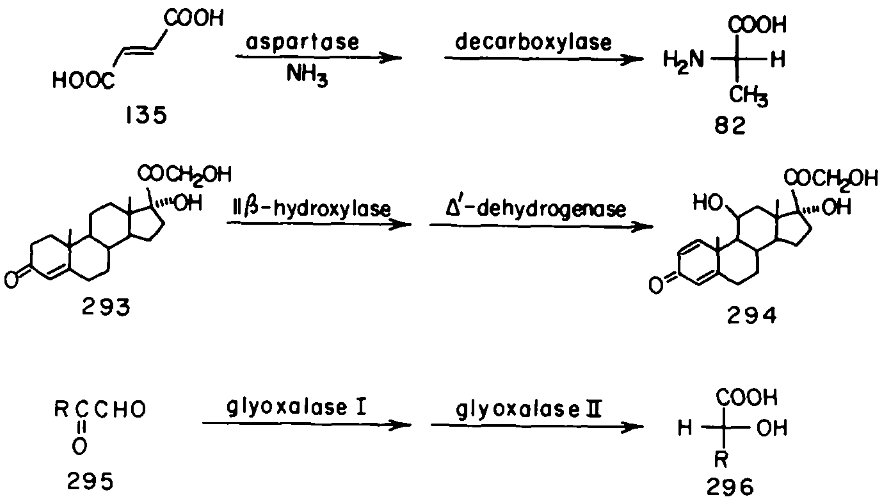


Fig. 74. Some stereospecific two-enzyme transformations.

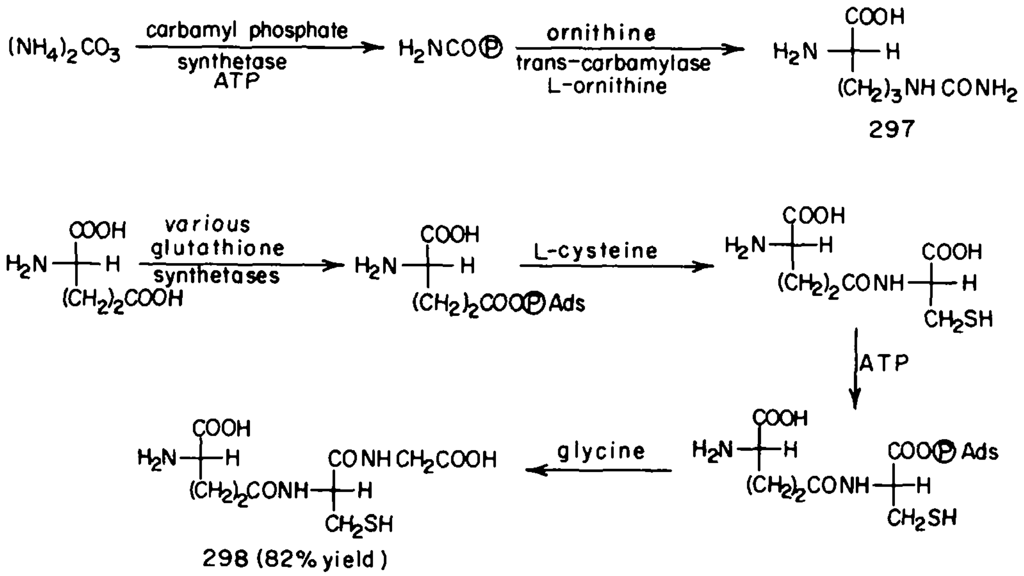


Fig. 75. Some multi-enzyme syntheses of amino acids and peptides.

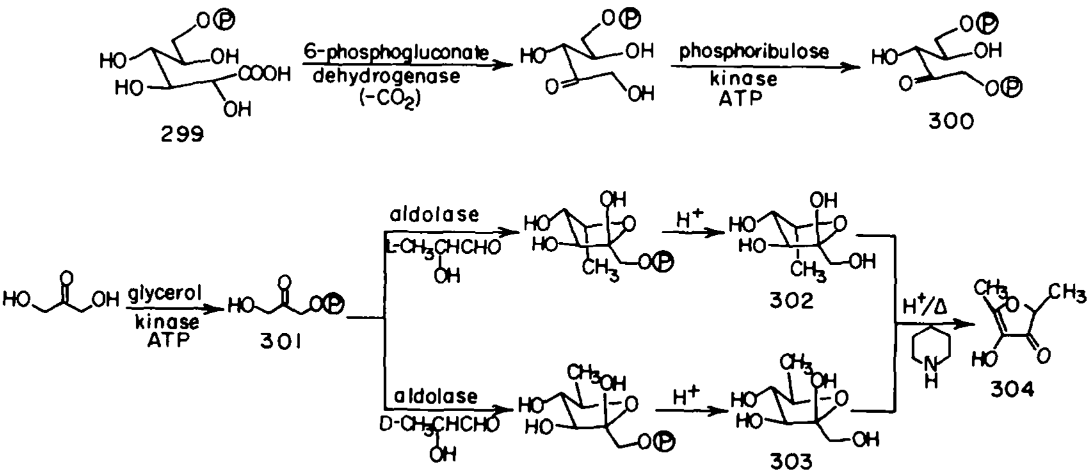


Fig. 76. Some multi-enzyme-catalyzed preparations of monosaccharide derivatives.

Enzyme-based approaches can avoid problems of regioselectivity and the multiple protection and deprotection steps required in chemical syntheses of oligosaccharides. The synthesis of N-acetyllactosamine (**305**) from glucose-6-phosphate (**66**) illustrate the type of multi-enzyme methodology that can be exploited[319] (Fig. 77). Sucrose and trehalose have also been prepared enzymically.[138]

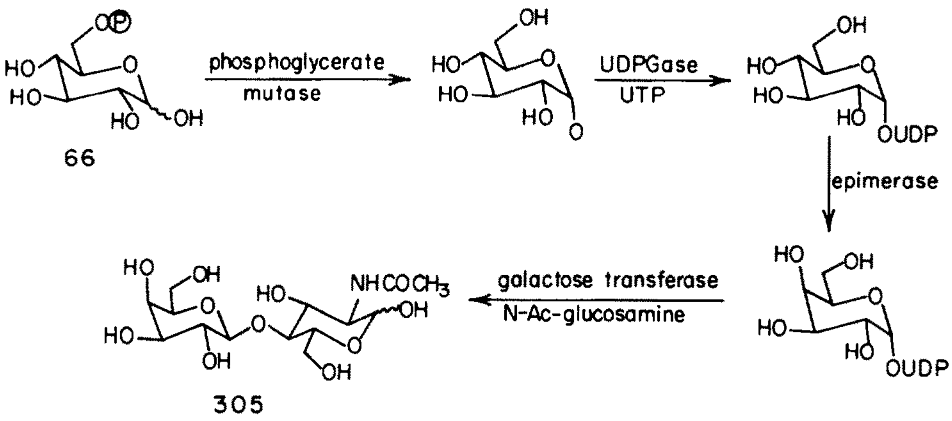


Fig. 77. Oligosaccharide synthesis by a multi-enzyme approach.

Improvements over chemical routes have been demonstrated in nucleoside and nucleotide synthesis (Fig. 78). The difficult-to-make antiviral D-arabinonucleosides **307** are readily prepared enzymically from the cytosine arabinonucleoside **306**.[320] The conversion of orotic acid (**308**) to uridine monophosphate (**309**) was performed in order to demonstrate the general utility of 5-phosphoribose pyrophosphate (**67**) as a nucleotide synthon.[96] The ATP analogue **310** is widely used in mechanistic enzymology but is very expensive due to the difficulties in preparing and purifying it by traditional approaches. Of the enzymic routes to ATP-γ-S (**310**),[321,322] the multi-catalyst synthesis from dihydroxyacetone via its phosphate **301** is the best if large amounts are needed.[322]

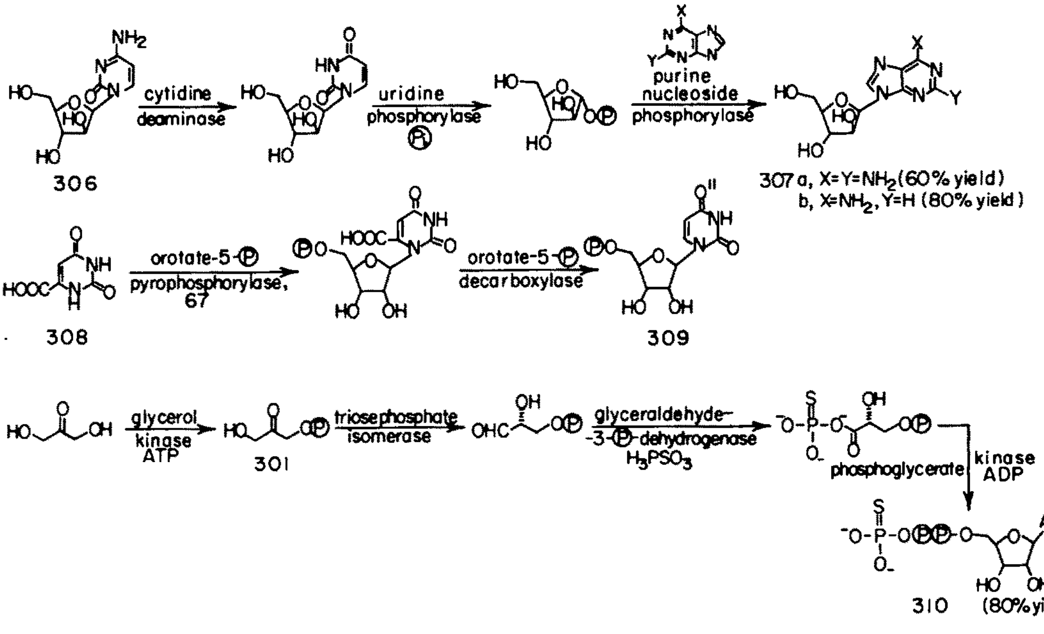


Fig. 78. Multi-enzyme syntheses of nucleosides and nucleotides.

One of the most complex multi-enzyme syntheses reported so far is the use of *Brevibacterium ammonigenes* enzymes to prepare coenzyme A (**312**) from L-cysteine, pantothenic acid (**311**) and

ATP[323,324] (Fig. 79). The couplings proceed via the normal biosynthesis pathway from **311** → 4′-phosphopantothenic acid → 4′-phosphopantothenoylcysteine → 4′-phosphopantotheine → dephosphocoenzyme A → **312**.[325] Multi-enzyme routes to penicillins and cephalosporins that exploit the biosynthetic pathway are also being explored.[286]

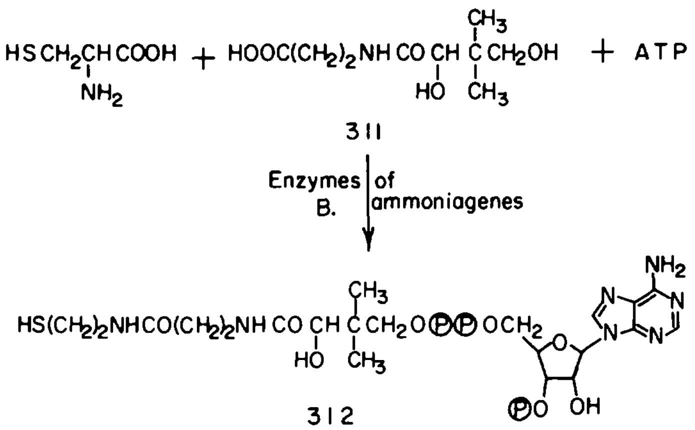


Fig. 79. Enzymic synthesis of coenzyme A.

STEREOSPECIFIC INTRODUCTION OF ISOTOPIC LABELS

From the broad range of stereospecific catalyses described above, it is evident that enzymes provide attractive, and often unique, opportunities for the stereospecific introduction of isotopes at predetermined locations, and with complete control of any chiralities induced. It is therefore not surprising that they have been widely used for these purposes. In fact, many of the reactions already discussed have been used to prepare labelled products.

Stereospecific enzyme-catalyzed introduction of deuterium or tritium can be accomplished in several ways. As noted above, alcohol dehydrogenase-catalyzed reductions of aldehydes occur with predictable stereospecificity.[2,163] This can be exploited to prepare either *R*- or *S*-deuterated (**313**) or tritiated (**314**) alcohols.[326–332] In Fig. 80, the yeast enzyme used always delivers the hydride equivalent

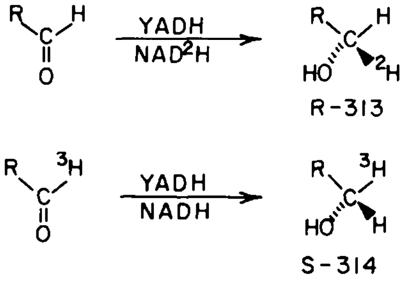


Fig. 80. Controlled preparations of *R*- or *S*-deuterated or tritiated alcohols.

to the *Re*-face of a carbonyl group. Other alcohol dehydrogenases can be selected that operate on *Si*-faces.[2] Deuterium or tritium can be introduced stereospecifically via enzyme-catalyzed exchange reactions (Fig. 81)[333–337] and by using enzymes of opposite-face stereospecificities, as exemplified in the preparations of the *R*- and *S*-enantiomers of 2-tritioglycolic acid (**315**)[338] (Fig. 82). Dehydrogenases, such as succinate dehydrogenase, also catalyze stereospecific exchange of hydrogen isotopes into their substrates.[339] Labelled compounds of the types shown in Figs 80–82 have found applications in many fields, including alkaloid biosynthesis,[328] prostaglandin,[331] steroid[336] and chiral methyl group studies.[327,329,330,338,340]

Decarboxylases provide alternative routes for hydrogen-isotope introduction (Fig. 83). This can be accomplished via stereospecific decarboxylation, as in the preparation of (*R*)-^{2}H-tyramine (**317**) from

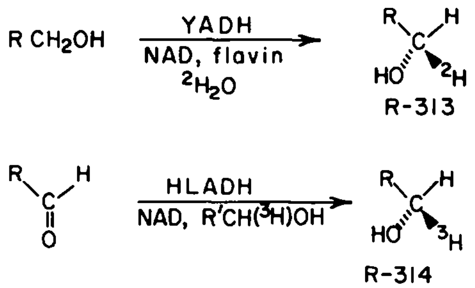


Fig. 81. Stereospecific introduction of ^2H or ^3H via alcohol dehydrogenase-catalyzed exchange reactions.

L-tyrosine (**316**),[341] or by exchange, as for the conversion of 2-tritiocyclohexanone to the chiral methyl precursor **318**.[342]

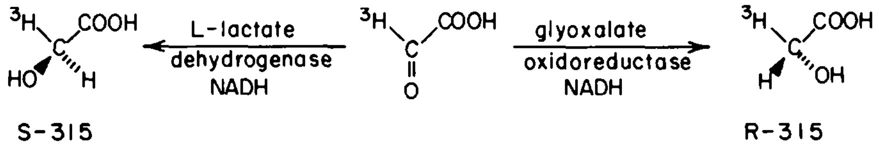


Fig. 82. Preparations of *R*- and *S*-tritioglycollic acids using enzymes of opposite enantiotopic face specificities.

Some labelling applications of lyases are summarized in Fig. 84. A cellular lyase converts oleic acid (**137**) into **319**.[193] In deuterium oxide, the aspartase-mediated addition of ammonia to fumaric acid (**135**) gives (2*S*,3*S*)-[3-^{2}H]-aspartic acid (**320**).[343] The *Clostridum kluyveri* hydrogenase-catalyzed conversion of cinnamic acid (**321**) to the labelled phenylpropanoic acid **322** is stereospecific only for deuteration at the C-3 position.[344] Alternatively, stereospecific deuteration of opposite chirality at the propanoate C-3-position can be achieved using phenylpyruvate tautomerase, which converts phenylpyruvic acid to the (*R*)-[3-^{2}H]-derivative **323**.[345]

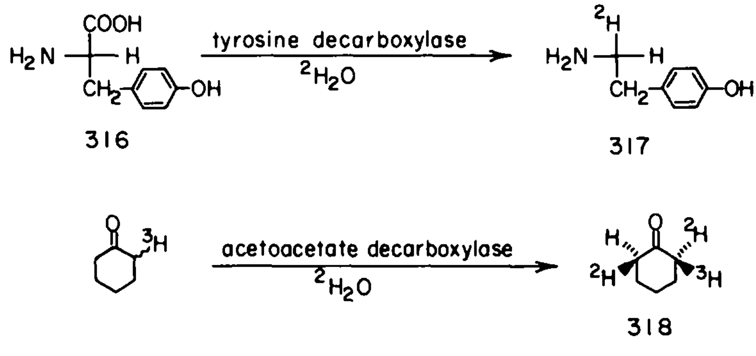


Fig. 83. Stereospecific deuterations using decarboxylases.

While the examples cited so far have focused on stereospecific introduction of a hydrogen isotope, the complementary approach of enzymic resolution of labelled racemates should not be ignored.† Figure 85 illustrates acylase-catalyzed resolution of tritiated amino acids (±)-**324** such as L-histidine ((*S*)-**325a**),[348] L-alanine ((*S*)-**325b**),[349] and L-phenylalanine ((*S*)-**325c**).[350] Enzyme-catalyzed exchange

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† The small steric differences between isotopes are insufficient to enable enzymes to effect resolutions on racemates of enantiomers whose chiralities are due only to isotopic differences in their ligands, e.g. $C(X)(Y)(CH_3)(C^2H_3)$.[346] In fact, the first observation of a steric discrimination between isotopes has just been reported. A 1% discrimination (equivalent to a 20 calorie energy difference) between protium and deuterium binding has been observed for fumarase-catalyzed dehydration of H- and ^{2}H-labelled *S*-malic acid.[347]

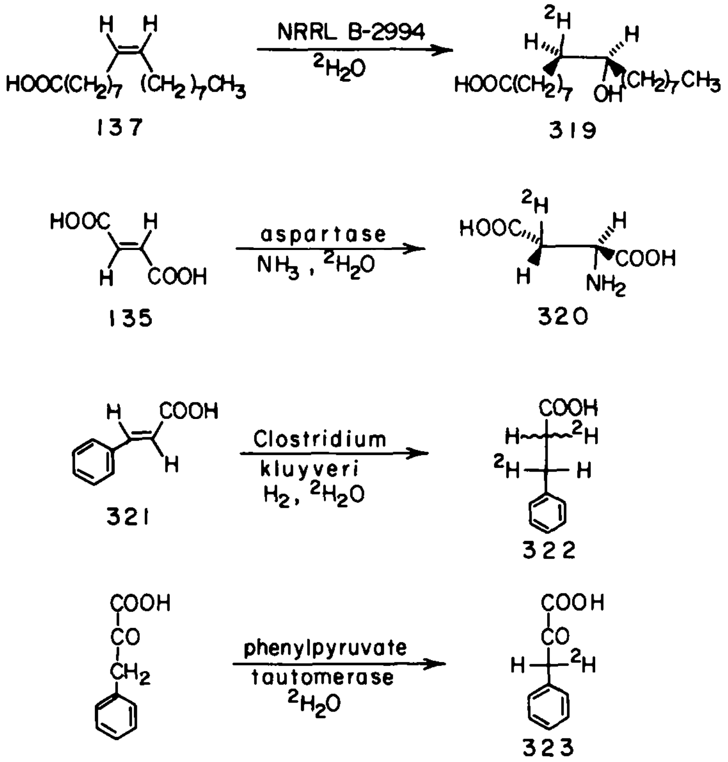

Fig. 84. Stereospecific deuteration with lyases.

of ^1H by ^2H or ^3H has also seen widespread application for stereospecific labelling of amino acids.[351–353]

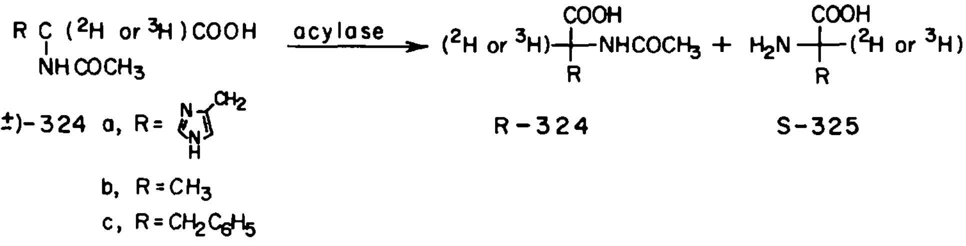

Fig. 85. Acylase-catalyzed resolutions of ^2H- or ^3H-labelled aminoacids.

The preparation of tritiated L-carnitine ((*S*)-**327**) from γ-aminobutyric acid (**326**) involves stereospecific hydroxylation of an achiral labelled precursor[354] (Fig. 86).

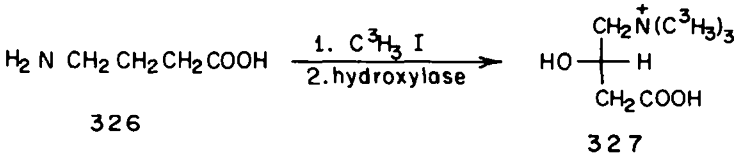

Fig. 86. Preparation of ^3H-labelled L-carnitine.

Multi-enzyme sequences can be exploited to considerable advantage. Isomerization of fructose-6-phosphate (**328**) with glucose isomerase in buffered deuterium oxide gives the labelled glucose-6-phosphate **329** (Fig. 87). This is readily cleaved by alkaline phosphatase to yield [2-^2H]-glucose (**330**).[355]

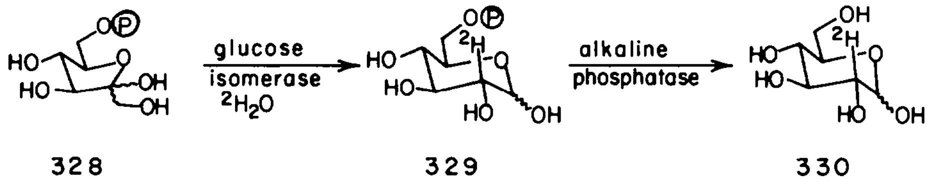

Fig. 87. Stereospecific deuteration of glucose.

Figure 88 provides a further insight into the degree of control possible. Incubation of *meso*-diaminopimelic acid (**244**) with a racemase in deuterium oxide exchanges both α-protons to give **331**. The enzymic decarboxylation proceeds with inversion (in contrast to retention in other pyridoxal phosphate-dependent decarboxylations, such as **316** → **317**) yielding the deuterated L-lysine **332**. Oxidation of **332**, first with L-lysine amino acid oxidase to (*S*)-**333** and then with peroxide, gives *S*-[5-^{2}H]-5-aminovaleric acid ((*S*)-**334**). The *R*-enantiomer of **334** is obtained by the complementary sequences **244** → **335** → *R*-**333** → *R*-**334**. Lysine decarboxylase-catalyzed decomposition of **335** affords [1-^{2}H]-cadaverine (**336**) of *R*-configuration, which in turn can be converted to C-2- and C-5-labelled pelletierine.[356]

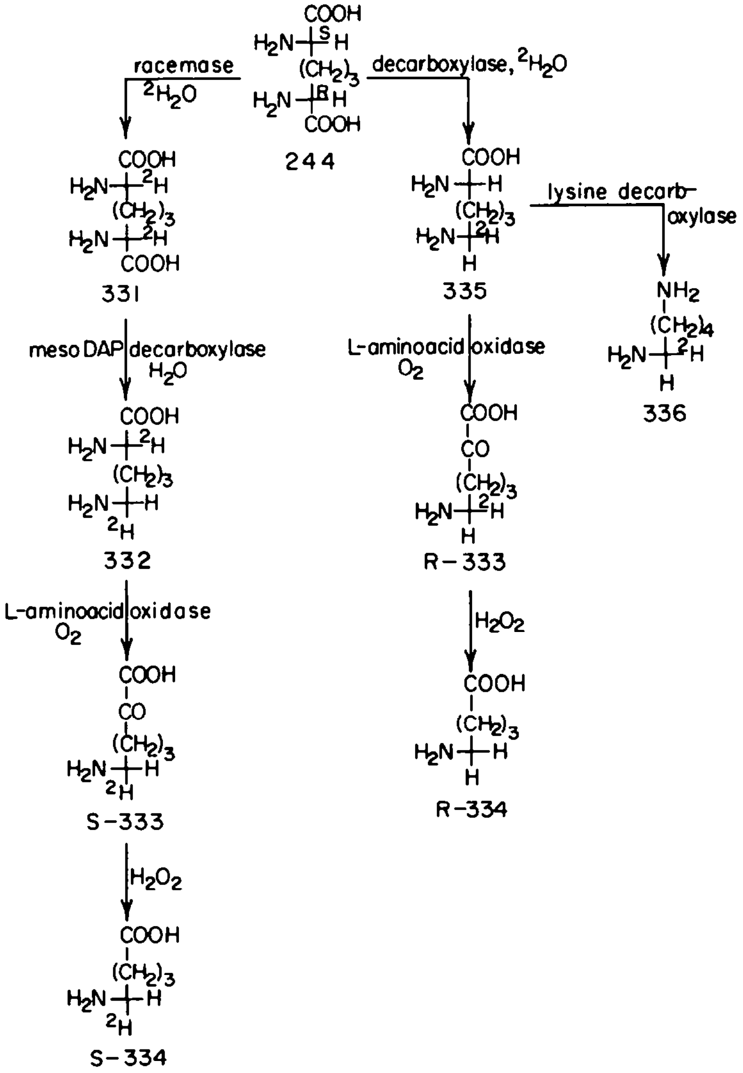

Fig. 88. Multi-enzyme control of the chiralities of deuterated amino acids and derivatives.

Enzymic introduction of carbon isotopes is facile. Short-lived ^{11}C derivatives can be prepared rapidly (Fig. 89). Synthesis time for the purified L-[^{11}C]-lactic acid **338** from (±)-**337** is 45 min.[357] Another reaction starting from $^{11}CH_3I$ is the preparation of ^{11}C-*S*-adenosyl methionine (**340**) from L-homocysteine (**339**). This process takes only 20 min.[358] Subsequent conversion of **340** to labelled (*S*)-(−)-epinephrine (**341**) can be accomplished in 15 min.[359] The preparation of the labelled L-aspartic acid **344** from phosphoenolpyruvic acid (**342**) via oxaloacetic acid (**343**) is another fast procedure that is complete within 15–25 min.[360] Other related ^{11}C-labelling procedures have been reviewed.[361]

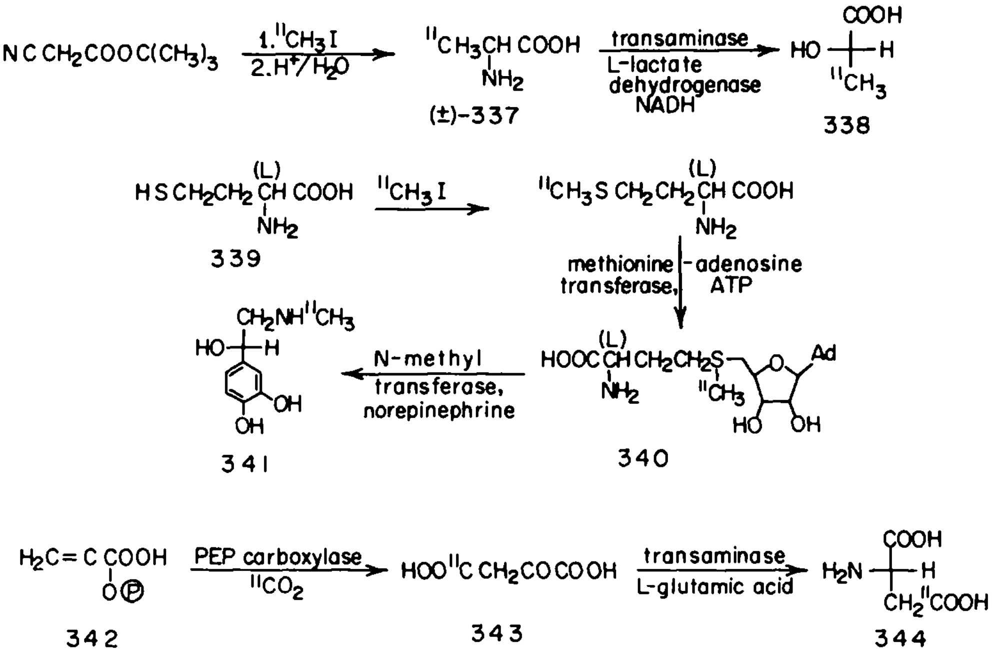

Fig. 89. Enzymic routes to short-lived ^{11}C-labelled compounds can be carried out rapidly.

Some enzyme-catalyzed methods for ^{13}C-labelling are illustrated in Fig. 90. The ^{13}C-labelled β-methylaspartic acid (2*S*,3*R*)-**345** was required for β-lactam biosynthesis studies and is best prepared enzymically. The β-C^3H_3 analogues of **345** can be obtained in a similar manner.[362] The porphyrin precursor, porphobilinogen, is readily prepared (cf. Fig. 24) in the ^{13}C-form **347** by enzyme-catalyzed condensation of two molecules of labelled δ-aminolevulinic acid (**346**).[113,363] The ^{14}C-labelled analogue is similarly obtainable.[364]

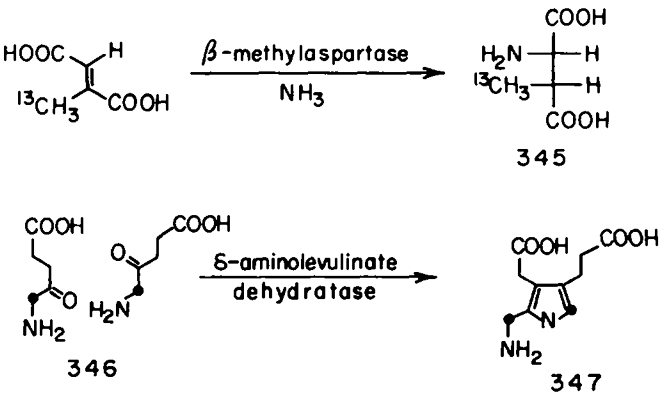

Fig. 90. Some enzyme-catalyzed preparations of ^{13}C-labelled compounds (● = ^{13}C).

Good enzymic routes to ^{13}C-labelled monosaccharides are available.[365] The multi-enzyme route from **348** (obtained from $K^{13}CN$) permits the singly [2-^{13}C] or doubly [2,5-^{13}C]-labelled glucoses **349** and **350**, respectively, to be made in excellent yields[182] (Fig. 91). [5-^{14}C]-Fructose-6-phosphate can be synthesized from [2-^{14}C]-glycerol with a similar multi-enzyme system.[366] ^{14}C-Labelled galactosides have also been prepared enzymically.[367]

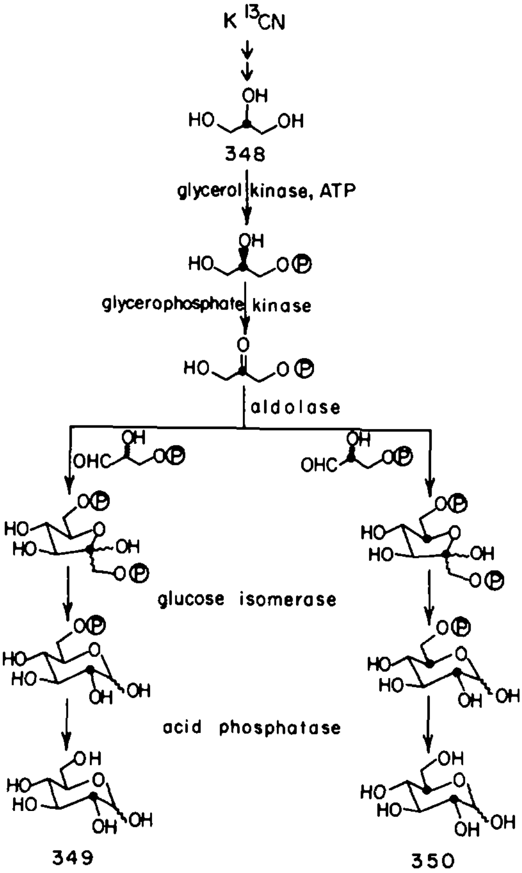

Fig. 91. Enzyme-catalyzed syntheses of ^{13}C-labelled monosaccharides (● = ^{13}C).

An efficient ^{14}C-labelling procedure is summarized in Fig. 92. ^{14}C-Acetyl-L-carnitine (**351**) of 99% purity is produced by the exchange reactions shown.[368] A catalytic amount only of coenzyme A is needed. In addition, resolution of ^{14}C-racemates remains a useful approach, particularly for amino acids.[369] The resolution of (±)-**352** to (*S*)-**353** and (*R*)-**352** is illustrative.[370]

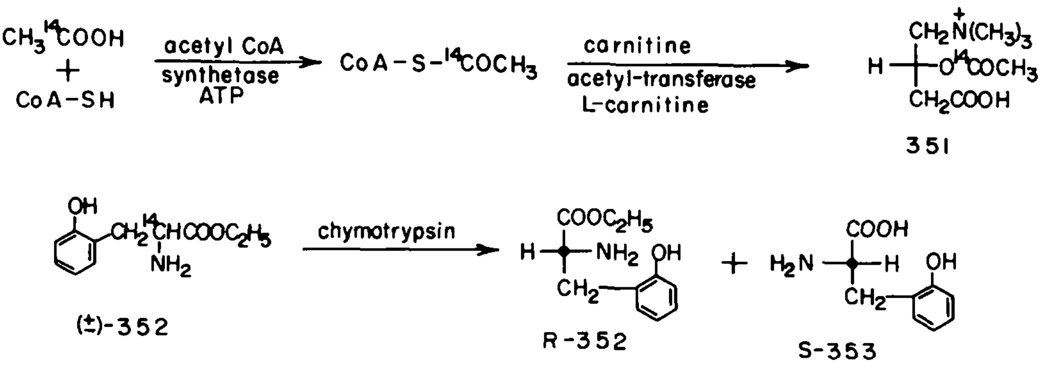

Fig. 92. Some preparations of ^{14}C-labelled amino acids.

^{13}N is another short-lived isotope that benefits from rapid enzymic introduction.[361] The procedure depicted in Fig. 93 is a versatile one that permits labelling of several L-amino acids **356** via ^{13}N-Lglutamic acid (**355**), obtained from α-ketovaleric acid (**354**), as an intermediate.[361,371,372] ^{15}N-Aspartic acid has been prepared by an analogous route.[373] The potential of an aspartase-catalyzed addition of $^{15}NH_3$ to cinnamic acid (**321**) for the preparation of the labelled L-phenylalanine (**357**), and of its enzymic hydroxylation to the corresponding ^{15}N-L-tyrosine (**358**), has been recognized.[374]

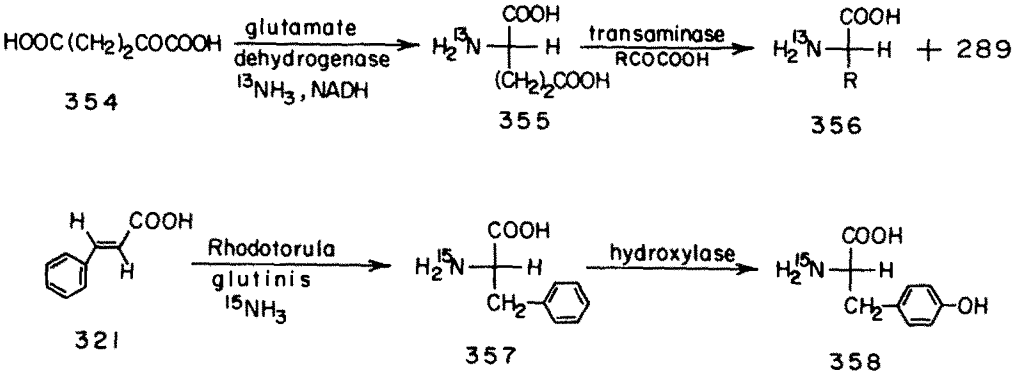

Fig. 93. Stereospecific introduction of ^{13}N and ^{15}N into amino acids.

Enzymes are invaluable in the preparation of chiral [^{16}O,^{17}O,^{18}O] phosphates of known absolute configurations. Some examples are given in Fig. 94. The conversion of **359** to **360** involves a diastereotopically specific phosphorylation.[375,376] Each of the **166** to **361**,[377] **362** to **363**,[378] and **364** to **365**[379] reactions proceeds with inversion, as does the thiophosphate analogue of the latter process.[380]

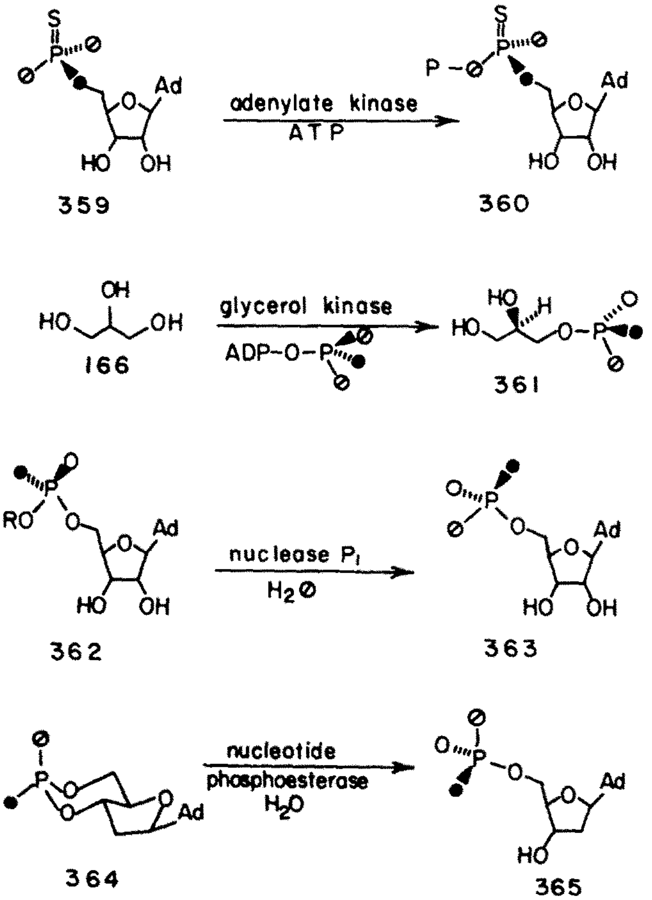

Fig. 94. Stereospecific enzymic routes to chiral [^{16}O, ^{17}O, ^{18}O] phosphates (○ = ^{16}O; ⊘ = ^{17}O; ● = ^{18}O).

Enzymatic introduction of ^{32}P has been reported for both small (**366**, **369**) and large (**367**, **368**) molecules[381–383] (Fig. 95). ^{35}S-Labelling of ATP-γ-S (**310**) to give **370** has been effected using an enzyme-catalyzed exchange reaction[384] (Fig. 96). A preferable preparation is from D-glyceraldehyde-3-phosphate (**371**) via the ^{35}S-labelled 1,3-diphosphoglycerate **372**.[385] Many other examples of the use of enzymes in phosphate stereochemistry studies have been reported.[386]

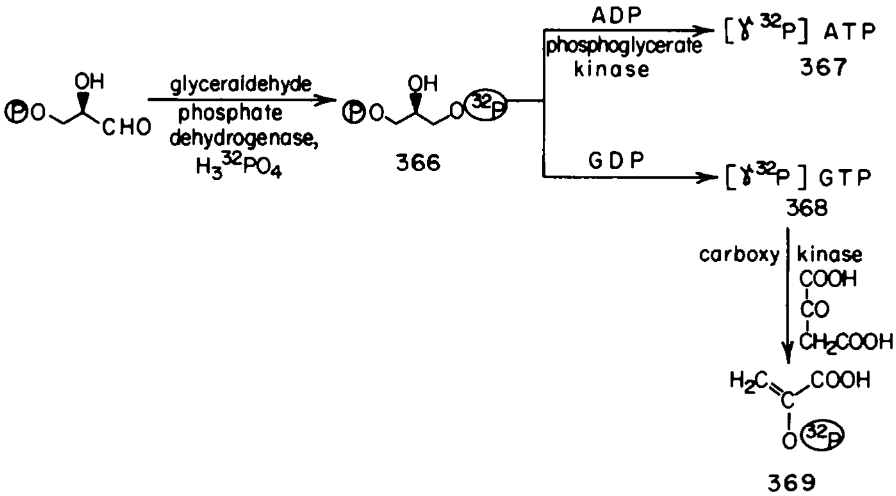

Fig. 95. Enzyme-catalyzed introduction of ^{32}P.

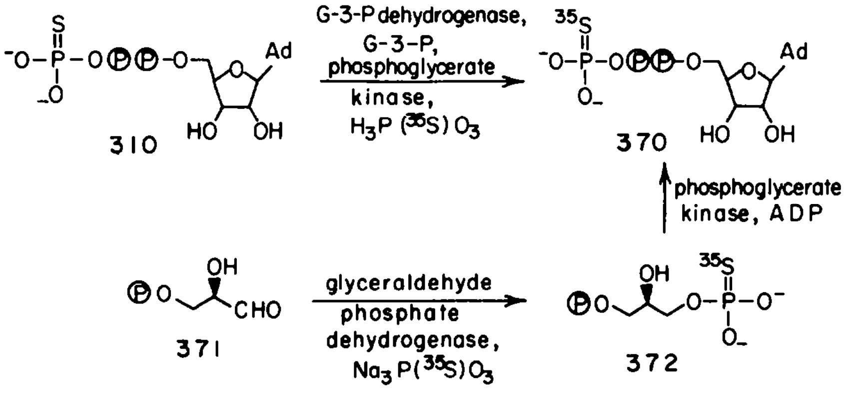

Fig. 96. Preparations of ATP-γ-^{35}S.

PROGNOSIS

The powerful, and often unique, addition to the arsenal of asymmetric synthetic methods available to the organic chemist that enzymes represent is being recognized with increasing frequency. More and more syntheses are now appearing that incorporate enzymic or microbiological steps, usually to set the stereochemistry of a key intermediate. While some resistance to using biological techniques remains, most has evaporated and enzymes are well on the way to being considered as routine reagents. To a rapidly growing extent, enzymic and chemical methods are being evaluated on an objective basis, with the only criterion for the selection being "which is best?". This healthy trend will continue and strengthen and should soon become an integral part of normal retrosynthetic analysis.

While much progress in the synthetic application of enzymes has already been made, the field is still at a very early stage in its evolution and enormous potential for further progress remains. The impact of enzymes on synthesis has only just begun to be felt. The examples recorded so far represent only the tip of the iceberg. The use of enzymes for enantiomeric excess and absolute configuration determinations also presents novel and exceptional opportunities.[7]

In the near future, the main development of the field will continue along the lines covered in this Report, but at an ever accelerating rate. Further ahead, equally exciting new advances are on the horizon as the tailoring of enzymes and microorganisms for specific purposes via chemical or genetic modification gains momentum.[387,388]

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