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Review

Application of biocatalysts in organic synthesis

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Introduction

Several general and extensive reviews [1-7], special issues of periodicals [8-12], or specialized books [13-19] have been published in the last ten years on the subject of biocatalysis in organic synthesis. They express the explosive development and success of this methodology in the area of the synthesis of natural products, pharmaceuticals and agrochemicals. It is certainly out of the scope of this review to try to cover all the facets of enzyme-catalyzed reactions. However it will be the aim of this report to concentrate on the most valuable methodological aspects, already in use in hundreds of chemical laboratories for practical organic synthesis. Ten years ago, classical organic chemists would probably have been more reluctant to consider an enzymic solution for one of their synthetic problems [20]. This was due, in most cases, to the fact that "biological systems" would have to be handled. Where isolation, growth and maintenance of whole microorganisms are concerned, such hesitation is probably still justified. On the other hand, isolated enzymes, which may be obtained more easily and increasingly from commercial sources, in either a crude or partially purified form, can generally be utilized like any other shelf chemical catalyst. The fact that, in some cases, the extensive understanding of certain enzymic reactions is still missing does not preclude their empirical utilization, in a pragmatic and somewhat empirical way, provided their usefulness has been established.

Compared to the chemical catalysts usually employed, enzymes are exceptional in several respects.

1) They are highly efficient catalysts. The rates of enzyme-promoted reactions can be faster than those of the corresponding uncatalyzed reactions by factors of 10^8-10^{12} (sometimes attaining diffusion-controlled

rates) [21], which is far above the usual acceleration observed in chemical catalysis.

2) Enzymes act in aqueous solvent, under mild conditions : pH range 5-8, temperature around 20-40°C. This may minimize side reactions, such as decomposition, isomerization, racemization or rearrangements which often plague chemical methodologies.

3) Enzymes are very versatile reagents. There is an enzyme-catalyzed equivalent for all types of organic reaction, except for some rearrangement reactions (such as the Cope reaction) or the Diels-Alder reaction. Even in this case, some exceptions have been recently reported in the literature [22], indicating that even unknown or unnatural reactions can be forced by enzymic catalysis. In this area, the usual rational approach derived from the biochemist's knowledge of natural enzymic reactions and substrates has been shown to be surpassed by organic chemist's imagination.

4) Enzymes acting on their natural substrates are generally very selective, especially considering all the types of chemical selectivity possible : *i)* a single type of reaction catalyzed (*chemoselectivity*); *ii)* discrimination betweeen identical functional groups located in structurally different positions of the substrate molecule (*regioselectivity and diastereoselectivity*); and *iii)* discrimination betweeen enantiomeric substrates, or enantiotopic groups (or faces) of a prochiral substrate (*enantioselectivity*). Enantioselectivity derives from the fact that enzymes are highly chiral reagents, exclusively constituted of L-amino acids, and that any type of chirality effectively or potentially present in the substrate molecule involves the formation of diastereomeric enzyme-substrate(product) complexes, thus resulting in a highly stereoselective reaction.

As early as 1948, there already existed a convincing rationale to explain, on theoretical grounds, the enantioselectivity of enzyme reactions. Ogston [23] emphasized that only a three-point attachment of the substrate, involving three different binding groups at the active site, considered as a topologically organized surface, was enough to get a high degree of enantioselection. This concept holds (fig 1) not only for an "asymmetric" tetrasubstituted atom *Xabcd*, the enantiomers of which can be discriminated in a resolution reaction, but also for a "prochiral" group or molecule (fig 2), the enantiotopic faces or substituents of which can be discriminated, resulting in the creation of a new asymmetric center. Such a simple model, representing positive or negative interactions (steric, electrostatic, hydrophobic interactions and hydrogen bonds) between functional groups of the substrate and amino-acid residues involved in the active site, and based exclusively on static affinity parameters, has been universally adopted as the foundation for enzyme enantioselection (see [24]), and later adapted to other asymmetric recognition phenomena (such as chiral chromatography).

These enantioselection properties have been probably a determining factor in the exponential development of enzyme use in organic chemistry in the last 15 years, on the one hand because of the increasing demand for the synthesis of asymmetric molecules in the fields of pharmaceuticals and agrochemicals [25], and on the other because of the current limitations arising from the "chiral pool" and the use of chiral chemical

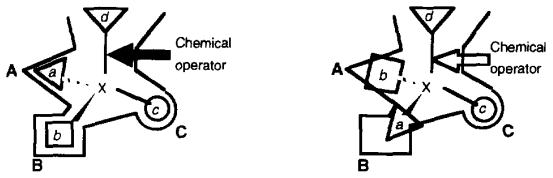


Fig 1. Schematic representation of enantiomer discrimination through the three-point attachment rule (adapted from Jones [312]). Only the left model allows an adequate complementarity of groups *a*, *b* and *c* with the corresponding **A**, **B** and **C** groups of the binding area, ensuring the optimal orientation of the reactive group *d* towards the "chemical operator" required for a successful transformation. A convenient orientation of the reactive group *d*, in the enantiomeric substrate (right), does not allow us to fit more than one interaction of groups *a*, *b* and *c* in any orientation selected due to their reverse spatial orientation.

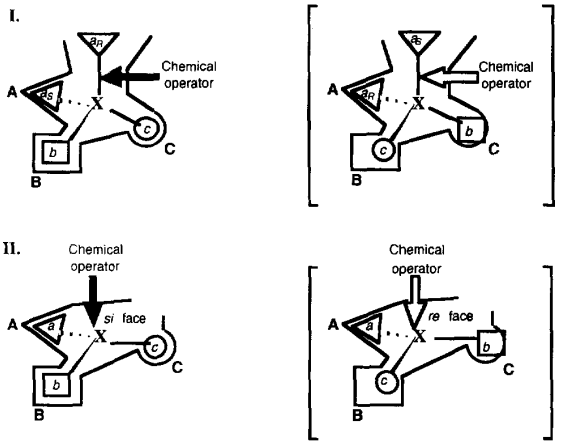


Fig 2. Schematic representation of enantiotopic groups (**I**) or enantiotopic faces (**II**) discrimination through the three-point attachment rule (adapted from Jones [312]). In both cases, only the left models ensure optimal interactions and thus allow us to rationalize the selective formation of a single stereoisomer.

reagents and catalysts in asymmetric synthesis. This report will mostly concern enzymic (microbial) *enantioselective* reaction applications either for the resolution of racemic compounds, exploiting the enantioselectivity of biocatalysts towards the substrate or for asymmetric synthesis of a single stereoisomer, exploiting the enantioselectivity of biocatalysts toward stereoheterotopic (diastereotopic or enantiotopic) faces or groups of a prochiral group or molecule, respectively.

In this context, one of the main questions to be addressed will be how a biocatalyst, designed by nature to effect a (stereo)specific reaction on a specific substrate (or a family of substrates) will accept a new, sometimes structurally very different, unnatural substrate and what will remain of the original stereoselectivity of this biocatalyst.

Of the more than 2500 enzymes classified [26], and the vast unrecognized pool of enzyme activities in nature, only a minor fraction has been investigated and used for chemical applications (fig 3). Hydrolase-catalyzed reactions represent more than half of the

whole, followed by oxidoreduction reactions. A significant amount of the investigations described are concerned with biotransformations effected with whole-cell systems whose endogenous enzymic activities may or may not be well defined.

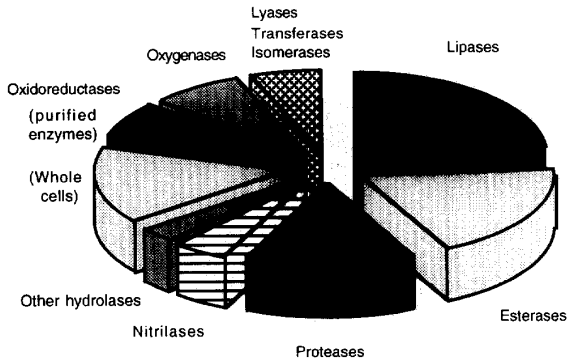


Fig 3. Pie chart showing the frequency of use of biocatalyst classes in biotransformations (from [325]).

Exploiting the enantioselectivity of biocatalysts for the resolution of racemic compounds

The ability of enzymes to discriminate between enantiomers of racemic substrates is probably the best documented chapter in the use of enzymes. In an ideal case, when a high enantioselectivity is exhibited, the transformation of the racemic substrate stops at the 50% conversion stage, and enantiomerically pure residual substrate and transformation product can be obtained.

However, when an enzyme acts on unnatural substrates, sometimes structurally remote from the natural situations, enantioselectivity often deteriorates and only a kinetic resolution may be observed. The optical purity of the product and that of the residual substrate are thus dependent on the progress of the reaction. In the case of an irreversible reaction, a simple calculation, developed by Sih [27, 28] on a model of chemical kinetic resolutions [29, 30] may help to provide the best compromise between yield and optical purity, using a calculated parameter E (*enantiomeric ratio*), which is the ratio of enzyme efficiencies for the transformation of each enantiomer and is independent of reaction time, substrate and enzyme concentration. Determination of E may result from a single measurement of enantiomeric excesses of substrate and/or product, at a given degree of conversion, in the transformation of the racemic substrate, using equations [1], or [2], and/or [3]. The knowledge of E value may prove extremely useful, for example, in simulating resolution experiments (fig 4 and 5) to determine the best compromise between optical activity and yield, or in comparing the enantioselectivities of different enzymes toward a single substrate, or, conversely, single enzyme enantioselectivities toward a family of substrates derived from a similar structure, in order to determine the best experimental resolution conditions.

$$\text{Enz} + \text{A} \leftrightarrows \text{Enz-A} \longrightarrow \text{Enz} - \text{A}'$$

$$\text{Enz} + \text{B} \leftrightarrows \text{Enz-B} \longrightarrow \text{Enz} + \text{B}'$$

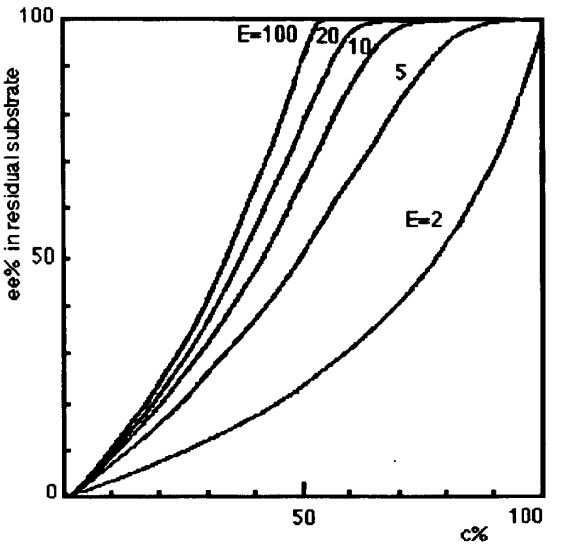


Fig 4. Simulated plots of enantiomeric excess *in residual substrate* versus conversion degree in kinetic resolution experiments, for E values varying from 2 to 100, using equation [1].

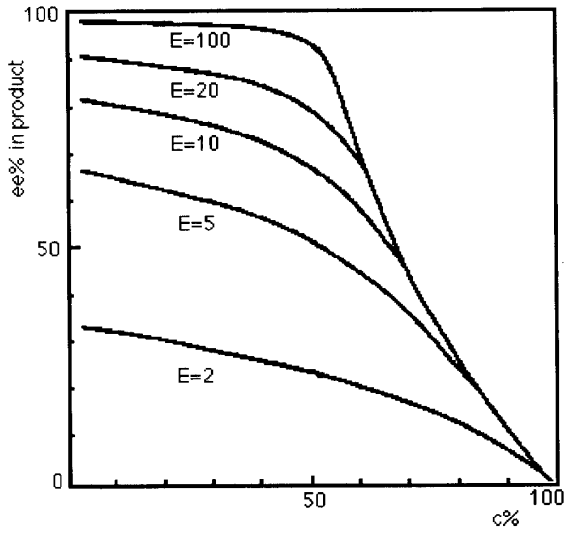


Fig 5. Simulated plots of enantiomeric excess *in product* versus conversion degree in kinetic resolution experiments, for E values varying from 2 to 100, using equation [2].

$$E = \frac{(k_{\text{cat A}}/K_{\text{MA}})}{(k_{\text{cat B}}/K_{\text{MB}})} = \frac{\ln[(1-c)(1-\text{ee}_S)]}{\ln[(1-c)(1+\text{ee}_S)]} \quad [1]$$

$$E = \frac{\ln[1-c(1+\text{ee}_P)]}{\ln[1-c(1-\text{ee}_P)]} \quad [2]$$

where A and B are the fast and slow reacting enantiomers, respectively, of the racemic substrate S, A′ and B′ the enantiomers of the product P, and ee_S and ee_P the enantiomeric excesses of S (= [A − B]/[A + B]) and P (= [A′ − B′]/[A′ + B′]), measured for a conversion

Table I. Hydrolytic enzymes commonly used in the resolution of racemic compounds.

Enzymes	(Prevailing abbreviation) and commercial names	Origin
Lipases		
Candida cylindracea (=rugosa) lipase	(CCL) AY, lipase My	Amano, Meito Sangyo, Sigma
Candida antartica B lipase	SP-435, lipase B	Novo
Pseudomonas sp *(=aeruginosa)* lipase	(PSL) lipase AK, SAM-II	Amano
Pseudomonas fluorescens (=cepacia) lipase	(PFL, PCL) lipase P, PS	Amano, Fluka
Mucor (Rhizomucor) miehei lipase	lipozyme	Novo, Gist-Brocades
Humicola lanuginosa lipase	lipase CE	Novo, Amano
Aspergillus niger lipase	lipase AP6	Amano, Fluka
Geotrichum candidum lipase	lipase GC	Amano, Sigma
Rhizopus delemar lipase	lipase D	Amano, Sigma, Fluka
Porcine pancreatic lipase	(PPL)	Boehringer, Gist-Brocades, Sigma...
Esterases		
Pig-liver esterase	(PLE)	Amano, Sigma, Boehringer...
Horse-liver esterase	(HLE)	Sigma, Fluka, Amano...
Carboxylesterase NP		Gist-Brocades
Acetylesterase (from orange *flavedo*)		Sigma
Proteases		
α-Chymotrypsin	(α-CT)	Sigma, Boehringer...
Papain		Sigma,...
Subtilisin A (from *Bacillus licheniformis*)	alcalase	Novo, Sigma
Aspergillus oryzae protease	protease A	Amano
Thermolysin (from *B thermoproteolyticus*)		Sigma
Other hydrolases		
Amino acylase (from porcine kidney)		Sigma
Amino acylase (from *Aspergillus* sp)		Amano
Penicillin acylase (from *E coli*)	Pen G acylase	Recordati
Amino-acid amidases (aminopeptidases)		Sigma...
D- or L Hydantoinases (dihydropyrimidinases) from *Agrobacterium radiobacter* or *B brevis*		Recordati
Nitrilase, nitrile hydratase (from *Brevibacterium* or *Rhodococcus* sp)	SP361, SP 409	Novo

factor c. When both ee_S and ee_P are available, c may also be calculated from equation [3]

$$c = ee_S/(ee_S + ee_P) \quad [3]$$

It is currently admitted that E values $\leqslant 10$ cannot lead to useful resolutions, while E values $\geqslant 100$ approximate ideal resolution conditions (see fig 4 and 5). However, for moderate E values ($E \approx 20$) it is possible to obtain highly pure substrate *and* product by using a so-called "two-step" enzymic resolution [31], consisting of terminating the reaction at about 40% conversion, where the optimal chemical and optical yields are reached for the product. After separation, the residual substrate is submitted to a second reaction step, until an overall conversion of about 60% is reached, affording an optimal chemical/optical yield for the substrate. Another method consists of obtaining an enriched product in a first enzymic reaction, converting it chemically into substrate, and then repeating the resolution experiment with this enriched substrate, producing an enhancement of the final enantiomeric excess in the product fraction. Such "recycling" experiments can easily be rationalized and simulated by introducing ee_0 values (initial enantiomeric excess) in equations [1] and [2] [27, 32]. Note that the residual substrate is always easier to obtain in a high enantiomeric purity, by delaying the termination of the reaction until all the faster reacting enantiomer has been exhausted.

Reversible reactions introduce more complicated situations, which usually result in a deterioration of resolution characteristics, particularly those concerning the residual substrate. This can be rationalized and calculated by introducing the equilibrium constant K into the above equations [33].

Most enzymic resolution reactions have been realized through the use of a limited number of hydrolases (table I). Theoretically, any type of enzymic transformation that can discriminate enantiomers of the substrate may be used for resolution. However, there are several good reasons for using hydrolytic enzymes [34, 35] : no coenzyme is necessary for their activity and their production in bulk quantities for other industrial uses (detergent and food industry) results in a general availability and moderate prices. Some peculiar features, such as reaction versatility, tolerance to a large number of substrate structures, complementary enantioselectivity, simple acyl-enzyme-based mechanisms resulting in a possible exchange of any of the acyl moieties or nucleophile partners of the reaction, compatibility with organic solvents, etc., are also exhibited by most of these enzymes. Moreover, in hydrolase reactions, sub-

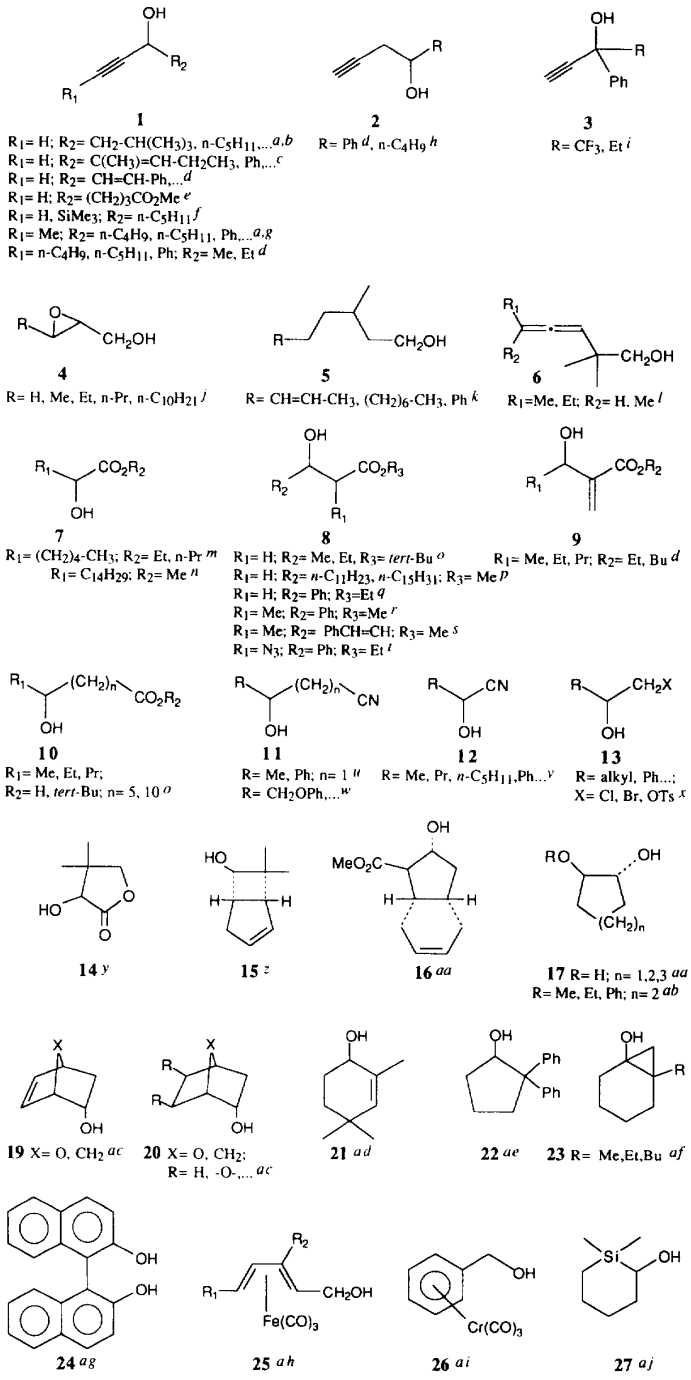


Fig 6. Selected examples of chiral alcohols successfully resolved using lipase-catalyzed esterification, or hydrolysis of the corresponding esters. *a Bacillus subtilis* var *niger* [326,327]. *b* Lyophilized baker's yeast [328,329], or CCL [330]. *c Bacillus subtilis* var *niger* [331], or *Pseudomonas fluorescens* lipase [332]. *d Pseudomonas* sp lipase [333] *e* α-Chymotrypsin [334]. *f* PPL [335]. *g Rhizopus nigricans* [336]. *h Rhizopus nigricans* [337]. *i* CCL [338]. *j* PPL [339-341]. *k Pseudomonas fluorescens* lipase [342]. *l* CCL [343]. *m* Baker's yeast [344]. *n Pseudomonas* sp lipase [345]. *o Pseudomonas* sp lipase [346]. *p Geotrichum candidum* lipase [347]. *q* CCL [348]. *r Aspergillus* sp lipase [349]. *s Aspergillus* sp lipase [350]. *t* CCL, or *Pseudomonas* sp lipase [351]. *u Pseudomonas* sp lipase [352]. *v Candida tropicalis* [535], or Rhodococcus butanica [353]. w Bacillus coagulans whole cells [354, 355]. *x Pseudomonas fluorescens* [356], or *Humicola lanuginosa* lipase [357]. *y* Lyophilized baker's yeast, or *Aspergillus* sp lipase [358], or CCL [359]. *z* hydrolase from crude PPL [360, 361]. *aa Pseudomonas fluorescens* lipase [35, 82]. *ab Pseudomonas* sp lipase [270, 362], or pig-liver acetone powder [363]. *ac* CCL [264,364,365]. *ad* PLE [366]. *ae* horse-liver acetone powder [367]. *af* CCL [368]. *ag Absidia glauca* whole cells [369], or cholesterol esterase [370]. *ah* Baker's yeast [371], or *Pseudomonas cepacia* lipase [372]. *ai Pseudomonas* sp lipase [373,374]. *aj* CCL [375].

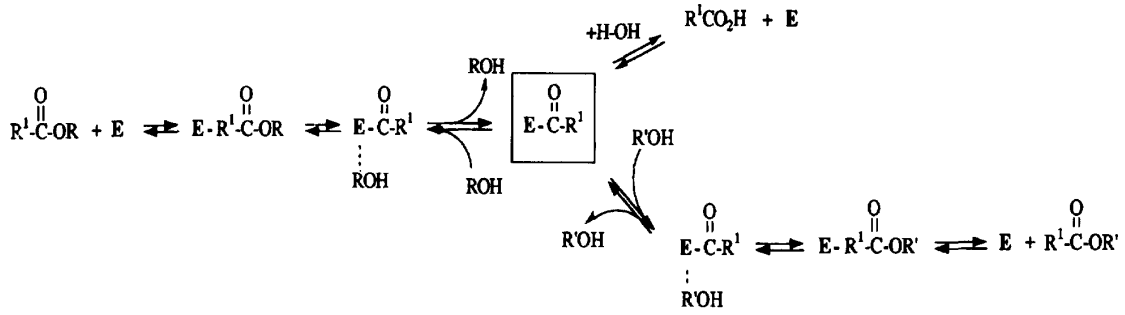


Fig 7. Reversible reaction pathway for a hydrolytic enzyme involving an acyl-enzyme intermediate operating in hydrolysis, esterification and transesterification reactions.

strate and product can be easily interconverted by simple existing chemical methods, allowing the use of most sophisticated tricks of the enzymic resolution methodology (product recycling, for example), and the recovery of both enantiomers of substrate *or* product.

A large number of primary, secondary, and even tertiary alcohols (probably several hundreds) have been successfully resolved (fig 6) using essentially lipase activity [36] of purified enzymes [37] or whole microbial cells. Several of them, such as the propargylic or homopropargylic alcohols **1-3**, the protected functionalized alcohols **4-6**, the hydroxyesters **7-10**, and various other functionalized secondary alcohols (**11-16**) are of general use as chiral building blocks or intermediates in numerous asymmetric syntheses [6]. Others (**17**, **22**, **24**) have been selected as useful new chiral auxiliaries for asymmetric induction or chiral ligands for the elaboration of asymmetric catalysts based on transition-metal complexes. A number of organometallic reagents, exhibiting planar chirality, such as **25** or **26**, have also been resolved by enzymic hydrolysis of their esters [38], allowing, sometimes for the first time, their use as asymmetric reagents. Some examples of enzymic resolution of chiral organometallic compounds, containing a silicon atom as an asymmetric center, such as **27**, have been described.

However, in most cases, conventional hydrolysis methods proved to be of limited value when working with such water-insoluble ester substrates. The addition of water-miscible cosolvents (acetone, acetonitrile, DMSO, dioxane, *tert*-BuOH, etc) in a 10-40% (vol/vol) range, is in some cases sufficient to bring the substrates into solution and promote active hydrolysis by esterases, which are known to operate in true solution. When working with lipases, which are typically enzymes acting at a water/lipid interface, it is generally preferred to use biphasic reactions, by dissolving the ester substrate in water-immiscible solvents (toluene, hexane, diethyl ether, etc) and achieving the spatial separation of the water-dissolved biocatalyst from the organic phase and the bulk of substrate [39, 40].

The preferred methodology is indeed to use the reversibility of lipase reactions, through the formation of a postulated acyl-enzyme intermediate (fig 7), in enantioselective esterification or transesterification reactions [41, 42], using solid enzyme powders (or immobilized enzymes) retaining the necessary residual bound water in order to remain catalytically active, in the presence of various acyl donors (table II) and in a completely water-immiscible organic solvent in order to avoid stripping water from the enzyme [39]. The logarithm of the partition coefficient (log P) of solvents between octanol and water is now universally accepted as an index for hydrophobicity, and therefore compatibility of organic solvents with high enzyme activity [43]. Only solvents with log P > 3.5 (table III) are considered as water-immiscible and show minimal effects on enzyme activity. Polarizability of solvents may also play some role in equilibrium constants and initial reaction rates of acyl transfer reactions [44].

Resolution of alcohols by esterification reaction with medium or long chain carboxylic acids was first used with several lipases. Such reactions, which suffer from the stoichiometric formation of water resulting in equilibrated reaction [33] and poor control of the pH of the microaqueous phase, are now favorably replaced by transacylation reactions which avoid the formation of water. Classical transesterification reactions do not generate water, but another alcohol nucleophile, which can compete for the attack of the acyl-enzyme intermediate with the racemic alcohol to be resolved, thus leading to a reversible slow reaction. On the contrary, activated "quasi-irreversible" acyl donors, which generate weakly nucleophilic alcohol species by transesterification, such as oxime esters, 2-haloethyl esters, or "irreversible" acyl donors such as acid anhydrides (see table II), give faster reactions. Enol esters, such as vinyl or isopropenyl esters [45, 46], which belong to the later category, liberate the corresponding enols as reaction products which instantaneously isomerize to acetaldehyde or acetone, respectively, and are thus most widely used in lipase-catalyzed transesterification reactions [46, 47]. In addition, recovery of enzyme for repeated use is generally possible [48], and may be facilitated through immobilization by simple adsorption on inert carriers (Celite, glass beads, etc). Acid anhydrides have occasionally been employed (see table II), and the use of succinic anhydride has recently been recommended for kilogram scale resolution of secondary alcohols, making use of the ease of separating the acylated product from the unreacted alcohol [49].

Table II. Selected examples of acyl donors used in acyl transfer reactions catalyzed by lipases.

Acyl donors	Lipase	Solvent	Compound resolved
"Reversible"			
Carboxylic acids (propionic, butyric, lauric acid...)	CCL	Heptane	(±)-Menthol [250, 251]
	PSL	Toluene/MeOH	(±)-3-Benzylthio-2-methylpropanoic acid [252]
Glycerol triesters (triacetin, tributyrin..)	CCL	Isooctane	(±)-Menthol [253]
	CCL	Tributyrin	(±)-Butan-2-ol [254]
Cyclohexyl palmitate, 2,2'-biphenyl-dipalmitate	PPL, Phospholipase A_2	Chloroform	(±)-Lysophosphatidylcholine [255]
"quasi irreversible"			
Trichloroethyl esters (butyrate,...)	PPL	Diethyl ether	(±)-Hexadecan-2-ol [256]
Trifluoroethyl esters (laurate, butyrate...)	PPL	"	(±)-6-Methylhept-5-en-2-ol [257, 258]
	PPL	"	(±)-Alkan-2-ols [259]
Cyclohexanone oxime esters (acetate, acrylate,...)	CCL	Toluene	(±)-2-Ethyl hexanol [260-262]
S-Ethyl thioesters (octanoate....)	*C antartica* lipase		(±)-Octan-2-ol [263]
"irreversible"			
Enol esters (vinyl or isopropenyl esters)	PPL	Benzene	(±)-Octan-2-ol [46]
	CCL	Toluene	(±)-2-hydroxy-5,6-epoxynorbornane [264]
Acetic, butyric anhydride	PSL	Benzene	(±)-1-Phenylethanol [111]
Succinic anhydride	PFL	Diethyl ether	(±)-1-Phenylethanol [265]
	PSL	*tert*-BuOMe	(±)-1-Phenylethanol [49]

Table III. Log P values for commonly used organic solvents.

Solvent	log P	Comments
Dimethylsulphoxide	−1.3	
N,*N*-Dimethylformamide	−1.0	Completely water-miscible; may be used to solubilize substrates in concentrations up to 40-50% without affecting significantly enzyme activity
Methanol	−0.76	
Acetonitrile	−0.33	
Ethanol	−0.24	
Acetone	−0.23	
Tetrahydrofuran	0.49	
Ethyl acetate	0.68	partially water-miscible; fast enzyme deactivation
Diethyl ether	0.85	
Butyl acetate	1.7	
Chloroform	2.0	
Benzene	2.0	water-immiscible; suitable for use with dry enzyme powders
Toluene	2.5	
Octanol	2.9	
Dibutyl ether	2.9	
Carbon tetrachloride	3.0	
Cyclohexane	3.2	
Hexane	3.5	
Heptane	4.0	
Dodecane	6.6	

It is somewhat surprising that this reverse methodology may involve such dramatic effects on the enantioselectivity ratio value, most generally increasing it, sometimes by a factor of 10 [28, 47]. Although this might be unexpected for reactions with an identical acyl enzyme intermediate and the same microscopic reaction route, such variations are probably the result of conformational changes or a decrease in the flexibility of the active site in low-water solvent conditions. This offers the possibility of modulating and controlling the substrate specificity and enantioselectivity of the enzyme by variation of the solvent. Even a reversal of enantioselectivity has been described in such conditions with lipases [50, 51] and proteases [52]. However, the prediction of such effects is very difficult, and the relationships between log P values and enantiomeric ratio variations is still a controversial subject [53, 54].

Table IV. Some examples of (*trans*)-2-substituted cyclohexanols resolved by lipase-catalysis and used as chiral auxiliaries in asymmetric synthesis [266-268].

R=	Experimental conditions	References
- Me	Esterification by lauric acid and CCL, in hexane or heptane, 40°C (E= 250)	[250]
- Et	Esterification by lauric acid and CCL, in hexane or heptane, 40°C (E= 120)	[250]
- i-Pr	Esterification by lauric acid and CCL, in hexane or heptane, 40°C (E= 350)	[250]
- *tert*-Bu	Esterification by lauric acid and CCL, in hexane or heptane, 40°C (E= 330)	[250]
	Hydrolysis of (±)-chloroacetyl ester by PLE (as pig liver acetone powder), in phosphate buffer-acetone (9:1), 35°C, pH 7.0	[269]
- Ph	Hydrolysis of (±)-acetyl ester by PLE (as pig liver acetone powder), in 0.5 M phosphate buffer-acetone (9:1), 31°C, pH 7.5	[55]
	Hydrolysis of (±)-acetyl ester by PSL, in 0.1 M phosphate buffer, 20°C, pH 7.0 (E=140)	[270]
	-*id*- from (±)-chloroacetyl ester (E=180)	[270]
	Transesterification by vinyl acetate and PSL in *tert*-BuOMe (E ⩾ 100)	[271]
- CH_2-Ph	Hydrolysis of (±)-acetyl ester by PSL, in 0.1 M phosphate buffer-acetone (9:1), 20°C, pH 7.0 (E=260)	[270]
	-*id*- from (±)-chloroacetyl ester (E > 145)	[270]
	Transesterification by vinyl acetate and PSL in *tert*-BuOMe (E ⩾ 100)	[271]
- $C(CH_3)_2$-Ph	Esterification by lauric acid and CCL in cyclohexane, 40°C; recycling of the lauric ester (E=100)	[272]
- $C(CH_3)_2$-Naph	Esterification by lauric acid and CCL in cyclohexane, 40°C; recycling of the lauric ester (E=125)	[272]

The preparation of new chiral auxiliaries derived from unnatural molecules may in the future constitute a choice area for the use of enzymic resolution methods. For example, sterically hindered cyclohexane-derived alcohols, the esters of which are currently used for asymmetric induction in several types of reactions [55], may be prepared in either enantiomeric form by the use of esterase activities. Representative examples of such asymmetric reagents, prepared by enzymic resolution, are given in table IV. There is no doubt that these methods will develop rapidly, in conjunction with the increasing demand of asymmetric organic synthesis.

Hydrolase-catalyzed reactions have also been used for the regioselective protection of polyhydroxy compounds like sugars [56, 57] or hydroxysteroids [58, 59], and the regioselective deprotection of hydroxy and amino groups in carbohydrate and nucleoside esters [60]. An example of a protease-catalyzed regioselective acylation of the alkaloid castanospermine is given in figure 8 [61].

Similar methods (mostly hydrolysis) have been applied to the resolution of carboxylic acids, as esters, thioesters or lactones, using lipases, esterases and proteases. Pig-liver esterase has been extensively used for

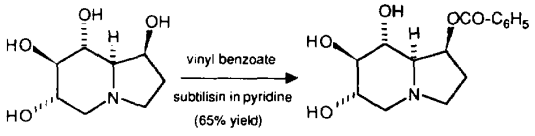


Fig 8. Subtilisin-catalyzed regioselective esterification of castanospermin [61].

the hydrolytic resolution of a number of methyl or ethyl esters of carboxylic acids. An active site model (fig 9) based on accumulated data from very different substrates has been progressively refined [62], and is currently used for the prediction of activity and enantioselectivity of this enzyme.

Several examples of successful resolutions of acids have been applied to the synthesis of important asymmetric pharmaceuticals. One recent route to Diltiazem (fig 10), a calcium antagonist used as an antihypertensive agent, involves the early enzymic resolution of an intermediate phenylglycidate ester [63]. Despite the fact that the maximum theoretical yield of this key step is 50%, because the hydrolysis product, a phenylglycidic acid, decomposes in the reaction conditions and thus cannot be recycled by racemization, this synthetic route

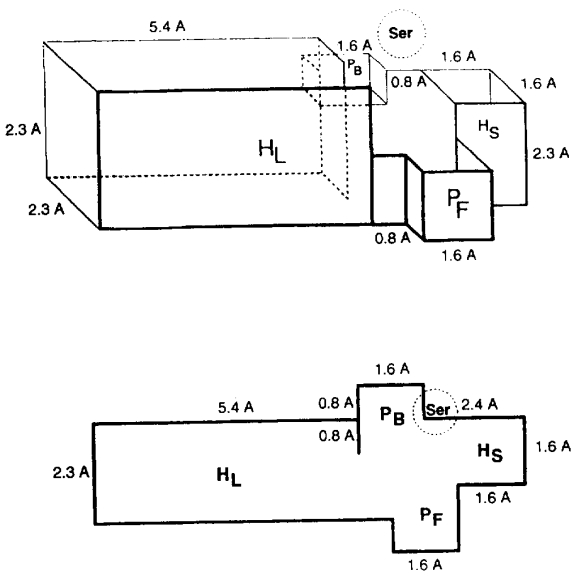


Fig 9. Active-site model of PLE (adapted from [62, 376]); dimensions in angstroms. The boundaries of the model represent the physical constraints placed on the available substrate binding volume by the aminoacid residues of the active site of the enzyme. The important binding regions for specificity determinations are two hydrophobic pockets, $\mathbf{H_L}$ and $\mathbf{H_S}$, and two pockets of more polar character, $\mathbf{P_F}$ and $\mathbf{P_S}$. The best fit of a potential substrate is determined by locating the ester group to be hydrolyzed within the serine sphere and then placing, if possible, the remaining substrate moieties in the **H** and **P** pockets according to a simple set of rules [376]. For clarity, only the top perspective of the model (lower scheme) can be used. In this projection, the restricted "gate" to the $\mathbf{H_L}$ pocket, adjacent to the $\mathbf{P_B}$ and $\mathbf{P_F}$ regions, is clearly seen. A recent readjustment of the model [377], based on enantioselective hydrolyses of new unnatural substrates, has extended the dimensions of the $\mathbf{H_L}$ pocket to 6.2 Å length and 3.9 Å height.

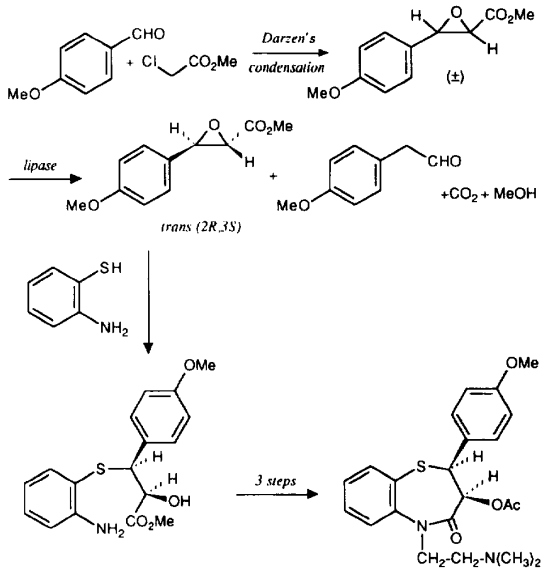


Fig 10. A synthesis of Diltiazem involving resolution of a phenylglycidate ester (adapted from [63]).

is the shortest and has the additional advantage of eliminating the transport of an undesired isomer all along the synthetic steps. Other enzyme-catalyzed hydrolytic resolutions are currently used in the industrial preparation of the (*S*)-enantiomers of α-arylpropionic acids, which are used as nonsteroidal antiinflammatory drugs (table V), and α-aryloxypropionic acids [64], which are common herbicides.

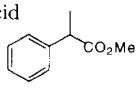
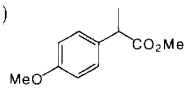
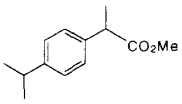
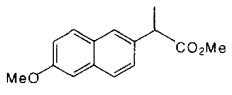
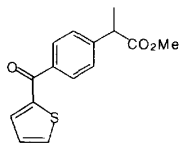
Occasionally, some transesterification reactions have been described with an enzyme powder suspended in the liquid mixture of racemic ester and acyl donor, in the absence of added solvent, exhibiting very high volumic productivities [65]. The reverse methodology in organic solvents has also been used in (macro)lactonization-resolution reactions [66-68] and in symmetric or asymmetric polyester formation [69-72]. Amino nucleophiles, such as amines or hydrazines can also lead to the resolution of acids or esters with lipases in organic solvents, allowing the formation of optically active amides under mild reaction conditions [73, 74].

Beside the classical structural modifications of ester substrates [75-77], various methods for improving the activity and enantioselectivity of hydrolytic enzymes have been used. These include extreme temperatures [78, 79], ultrasound treatment [80], amplification of enantioselectivity by use of sequential kinetic resolution [41, 81, 82] or double kinetic resolution [83], enantioselective inhibition [67], chemical modification of enzyme [84], genetic engineering of enzyme [85, 86], solvent variation [53, 87] and control of water activity [88]; these have been praised and successfully applied in many cases.

The hydrolytic activity of lipases, esterases and certain proteases may be exploited for the resolution of synthetic natural and unnatural racemic amino acids. The approaches most commonly employed are the hydrolysis of esters or amides of N-protected amino acids, or the cleavage of the amide bond of N-acylated amino acids. The first approach is illustrated (fig 11) by the optical resolution of various usual and unusual *N*-benzyloxycarbonyl amino acid esters or amides by lipases or amidases; the second approach is mainly exemplified by the use of aminoacylases (from mammalian or microbial origin) to effect the enantioselective amidolysis of N-acylated derivatives. The latter method, used for the preparation of optically pure *L*-methionine from its *N*-acetyl DL-derivative, was the first industrial application of immobilized enzymes [89].

Another hydrolytic method, used for unnatural D- or L-amino acid syntheses, starts from 5-substituted hydantoins [90], which are easily prepared by a Bucherer-Berg's condensation of an aldehyde, potassium cyanide and ammonium carbonate. Hydantoinases (dihydropyrimidinases) catalyze the enantioselective hydrolytic ring-opening of hydantoins to form the corresponding *N*-carbamoyl-α-amino acids (fig 12). Both D- and L-hydantoinases are known, depending on the microbial source of enzyme. The D- or L-carbamoyl derivatives can easily be converted to the corresponding amino acid by chemical or enzymic hydrolysis. Interestingly, the 5-substituted hydantoins are easily racemized at pH $\geqslant$ 8

Table V. Enzymic resolution of non-steroidal antiinflammatory pharmaceuticals and apparented α-arylpropionic acids.

Structure of the ester substrate	Enzyme	Stereochemical preference	E	Ref
2-Phenylpropionic acid 	CCL	*S*	10	[28, 273]
	Tetranitromethane modified CCL	*S*	100	[84]
	Horse-liver acetone powder (crude HLE)	*R*	40	[274]
2-(4′-Methoxyphenyl) propionic acid 	Horse liver acetone powder (crude HLE)	*R*	50	[274]
Ibuprofen 	Carboxylesterase NP (from *Bacillus subtilis*)	*S*	> 100	[275]
	CCL	*S*	84	[28]
		S	> 100	[276]
	Horse-liver acetone powder (crude HLE)	*S*	27	[274]
Naproxen 	Carboxylesterase NP (from *Bacillus subtilis*)	*S*	> 100	[275]
	CCL	*S*	> 100	[273]
	CCL immobilized on ion-exchange resin (continuous process)	*S*	–	[277]
Suprofen 	CCL	*S*	> 100	[28]

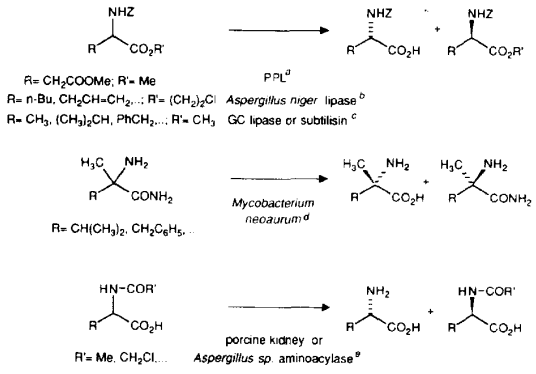


Fig 11. Enzymic resolution of natural and unnatural amino-acid derivatives. *a*, Guibé-Jampel *et al* [65]. *b*, Miyazawa *et al* [378]. *c*, Chiou *et al* [379]; Miyazawa *et al* [380]. *d*, Kruizinga *et al* [381]. *e*, Fu and Birbaum [382]; Chenault *et al* [383].

particularly when R is an aromatic group. It follows that the reaction using an enantioselective hydantoinase can be achieved in a 100% theoretical yield, contrarily to an usual resolution process, owing to the fast equilibration of the substrate enantiomers. This method is industrially operated by Kanegafuchi for the production of D-*p*-hydroxyphenylglycine.

Another example of such a "deracemization" reaction is the production of L-lysine by the Toray procedure (fig 13), which combines an enantioselective hydrolysis of D,L-α-amino ε-caprolactam to L-lysine catalyzed by a yeast (*Cryptococcus laurentii*), with a bacterial racemase-catalyzed epimerization of the remaining D-α-amino caprolactam (*Achromobacter obae*). The

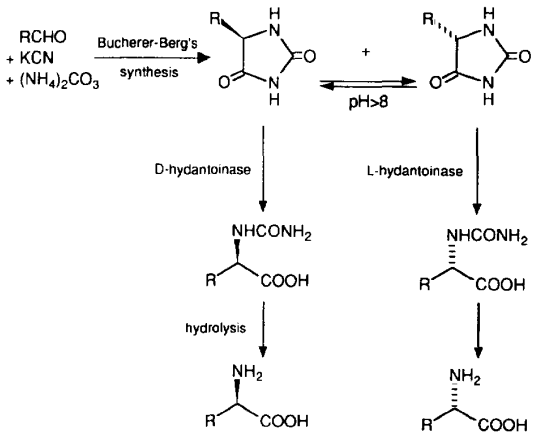


Fig 12. Resolution and deracemization of 5-substituted hydantoins using D- or L-hydantoinases.

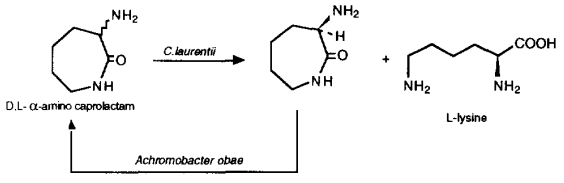


Fig 13. L-Lysine production by enzymic deracemization of α-amino ε-caprolactam.

combined operation of both enzymic activities, in the form of microorganism whole-cells, results in a nearly quantitative yield of L-lysine [91, 92].

Nitrilases and nitrile hydratase activities of whole microorganisms have been used for the stereoselective hydrolysis of α-amino nitriles [93], which are easily obtained by a Strecker synthesis, in order to afford optically active natural and unnatural amino acids (fig 14). In this case, the stereoselectivity of the overall reaction mainly originates from the associated amidase activity.

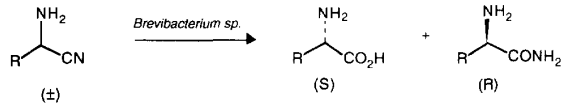


Fig 14. Microbial enantioselective hydrolysis of α-amino-nitriles.

It should be noted that the main application of nitrile hydrolyzing enzymes is not related to stereochemical problems; the chemical hydrolysis of nitriles usually requires drastic (and polluting) conditions, which are generally not compatible with the presence of other functional groups. The use of enzymic methods allows us to escape most of these drawbacks, as was recognized in the early seventies by Galzy *et al* [94]. Since 1987, Nitto Chem Ind has used the quantitative hydrolysis of acrylonitrile (fig 15) to obtain acrylamide, one of the most important commodity chemicals for the synthesis of several polymers. This hydrolysis uses the nitrile hydratase activity of the whole cells of a selected strain of *Pseudomonas chloroaphis* (or *Rhodococcus rhodochrous*) at concentrations exceeding 400 g L^{-1}, producing annual amounts approximating 10 000 tons of acrylamide [4, 95].

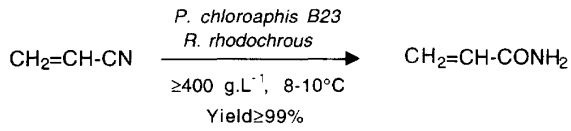


Fig 15. Microbial hydrolysis of acrylonitrile to acrylamide.

Epoxides, which constitute highly valuable and versatile synthetic intermediates, have been resolved using the microsomal or cytosolic hepatic epoxide hydrolases which are responsible for the detoxification of xenobiotics [96-99]. For obvious reasons, these reactions were considered as difficult to scale up. High epoxide hydrolase activities have recently been detected in some microorganisms and used to perform preparative resolutions of racemic epoxides [100-102]. Interestingly, depending on the microorganism, opposite enantioselectivities may be observed, allowing the preparation of both enantiomers of styrene epoxide (fig 16). However, in this case, owing to probably different hydrolytic mechanisms, both types of microorganisms afford the same R-diol product, allowing a deracemizing hydrolysis of the (±)-epoxide through simultaneous incubation with both microorganisms.

Exploiting the enantioselectivity of biocatalysts for the *de novo* creation of asymmetric centers

The enantioselectivity of biocatalysts may also be applied to a prochiral molecule, for discriminating between

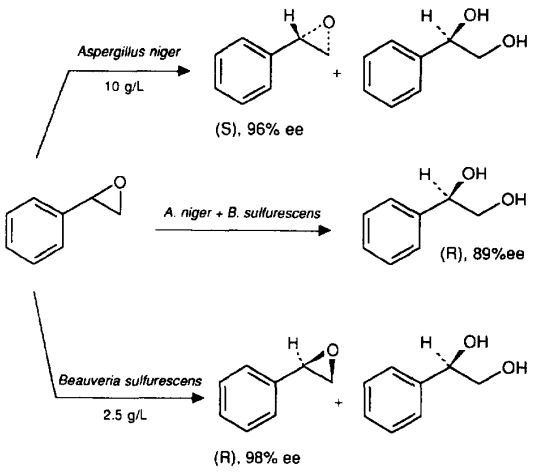


Fig 16. Hydrolysis of racemic styrene oxide with opposite enantioselectivities by microbial epoxide hydrolase activities (adapted from Pedragosa-Moreau *et al* [101]).

enantiopic groups or atoms in a *meso* compound, or to a prochiral group such as a carbonyl group based on a trigonal carbon atom, thus discriminating enantiotopic faces, as illustrated in the Ogston rationale (fig 2). In contrast to the resolution of a racemate, such enantioselective reactions may lead to the creation of new chiral centers, represented by a single enantiomer (homochiral compound), obtained in a theoretical 100% yield.

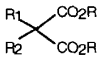
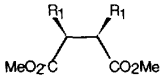
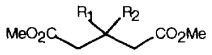
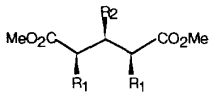
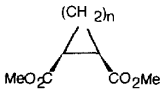
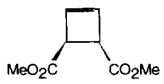
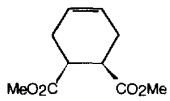
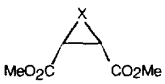
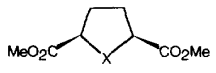
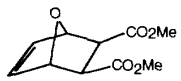
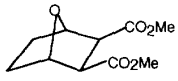
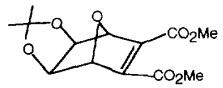
Discrimination of enantiotopic groups or atoms in prochiral substrate

This concept has been realized with a large number of open chain and cyclic *meso cis*-diester substrates, either symmetrical dicarboxylic acids esters or symmetrical diol esters, using hydrolysis or transesterification with PLE [103, 104], PPL or PFL [35, 105]. Using mostly PLE, high enantiomeric excesses are generally obtained for the half-esters of dicarboxylic acids, such as 2-substituted malonates, substituted glutarates [106, 107] or various carbocyclic or heterocyclic *cis*-dicarboxylates (table VI). The hydrolysis reaction usually stops at the monoester stage. If required, the interconversion of one enantiomer to the other can be achieved by a simple protection-deprotection sequence (the "*meso* trick"), making both enantiomers of the product available (fig 17). Depending on the *meso* diester structure, one or several asymmetric centers can be created in one operation. Another currently used method is the selective manipulation of the free or esterified carboxylic acid group through reduction by selective reagents, resulting in the formation of synthetically useful homochiral enantiomeric lactones [103, 104, 108].

Several valuable chiral intermediates for elaborated synthetic strategies have been prepared by such methods, as exemplified in figure 18 for a currently used synthon, which can be obtained easily and economically in hundreds of grams scale and high optical purity by such PLE-mediated hydrolysis of the corresponding symmetrical diester [109].

An extension of this methodology to *meso* cyclic acid anhydrides has recently been described, using an alcohol

Table VI. Enantioselective hydrolysis of open-chain and cyclic *meso* diesters.

Substrate		Enzyme	Yield (%)	ee (%)	absolute configuration	References
	R=R$_1$=Me; R$_2$=OH	PLE	37-82	6-46	*S*	[278, 279]
	R=R$_1$=Me; R$_2$=OBu^t	PLE	90	96	*R*	[279]
	R=Et; R$_1$=Me; R$_2$=Ph	PLE	–	86		[280]
	R=R$_1$=Me; R$_2$=PhCH$_2$	α-CT[a]	> 90%	> 98	*R*	[281, 282]
	R=Et; R$_1$=F; R$_2$=Me	CCL	87	91	–	[283]
	R$_1$=Me	PLE	94	18	2*R*,3*S*	[278]
	R$_1$=OH	PLE	92	48	2*S*,3*R*	[278]
	R$_1$=H; R$_2$=Me	PLE	95	79-90	*R*	[27, 278, 284]
	R$_1$=H; R$_2$=OH	PLE	80-90	12-22	*S*	[278, 285, 286]
		α-CT[a]	80-90	60-68	*R*	[278, 285, 286]
		C equi[b]	70	97	*S*	[287]
	R$_1$=H; R$_2$=OCH$_2$OMe	α-CT[a]	95	93	*R*	[288]
	R$_1$=Me; R$_2$=OH	PLE	62	> 98	*S*	[289]
	R$_1$=H; R$_2$=NHAc	PLE	81	93	*R*	[290]
	R$_1$=H; R$_2$=NHCO$_2$Bu^t	PLE	93	90	*S*	[290]
	R$_1$=Me; R$_2$=H	PLE	85-98	60-64	2*R*,4*S*	[108, 278]
	G roseum[c]	80	> 98	2*S*,4*R*	[108]	
	α-CT[a]	48	100	2*R*,4*S*	[278]	
	R$_1$=Me; R$_2$=OH	PLE	95	98	2*R*,3*S*,4*S*	[278]
	*n* = 1	PLE	90-92	> 98	1*R*,2*S*	[278, 291, 292]
	n = 2	PLE	98-99	86-97	1*R*,2*S*	[278, 291, 292]
	n = 3	PLE	80	9-17	1*S*,2*R*	[278, 291, 292]
	n = 4	PLE	75-98	78 > 97	1*S*,2*R*	[278, 291, 292]
		PLE	96	86	1*R*,2*S*	[293]
		PLE	90-99	85 > 98	1*S*,2*R*	[278, 291, 294, 295]
	X=O	PLE	50-69	20-31	2*S*,3*R*	[296-298]
	X=NH	PLE	–	92	2*S*,3*R*	[299]
	X=CH$_2$-CO-CH$_2$	PLE	83	82	1*S*,2*R*	[300]
	X=CMe$_2$	PLE	23-42	43-80	1*S*,2*R*	[278, 291]
	X=CH$_2$	PLE	82	34	1*S*,3*R*	[301]
		CE[d]	95	90		[302]
	X=O	PLE	98	42	2*S*,5*R*	[301]
	X=S	PLE	83	46	2*S*,5*R*	[301]
	X=N-CH$_2$Ph	PLE	85	80-100	2*S*,5*R*	[303, 304]
		PLE	86	75	1*R*,2*S*,3*R*	[305]
			4*S*			
		PLE	82	> 98	1*R*,2*S*,3*R*,	[305]
				4*S*		
		PLE	96	80	1*S*,4*R*,5*R*	[306]
				6*S*		

[a] α-Chymotrypsin. [b] *Corynebacterium equi* whole cells. [c] *Gliocladium roseum* whole cells. [d] Cholesterol esterase.

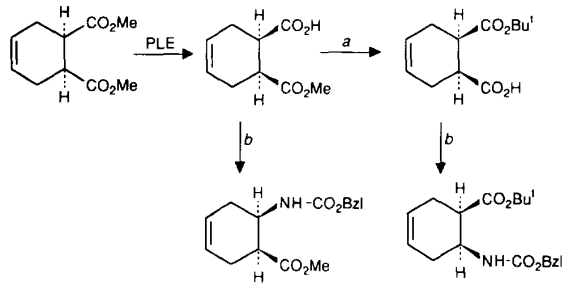


Fig 17. Alternative preparation of enantiomeric building blocks by interconversion of monoesters of cyclohex-4-ene-1,2-dicarboxylic acid, using a simple manipulation of the protective groups : *a*, *t*-BuOH esterification of the free carboxylic group, followed by selective alkaline hydrolysis of the methyl ester; *b*, Curtius reaction, converting the free carboxylic group to a protected amino group, with full retention of the initial configuration (adapted from [295]).

nucleophile in organic media, resulting in asymmetric ring opening, and affording the opposite enantiomer of the hydrolytically obtained hemiester [110-112] (fig 19).

Similar results have been obtained, using mostly PPL or PFL [35], with the corresponding *meso* diol diesters, but the hydrolysis (or the diol esterification) reaction does not generally stop at the intermediate monoester stage. The reaction kinetics and the optical enrichment of the chiral monoester depend on the relative rate constants of the individual steps. Some combinations of enantioselective hydrolysis (first step) and kinetic resolution (second step) ($k_1 > k_2$ and $k_4 > k_3$) can result in an enhancement of optical purity, as illustrated in figure 20, but the maximal enantiomeric excess of the hemiester does not correspond to a maximal hemiester concentration, necessitating a careful calculation of ee-conversion dependence and separation of substrate and products [76, 81, 113, 114]. Some significant examples of the products of enzyme-catalyzed asymmetric hydrolysis of diol diesters (or esterification of diols) are given in figure 21. If necessary, starting from the corresponding prochiral diol, both enantiomeric forms of a hemiester can be obtained by an elegant combination of enzymic hydrolysis and esterification [115] (fig 19).

In a recent application of enol esters hydrolysis, it was shown [116] that a dienol diacetate (fig 22) can be enantiotopically hydrolyzed to give an optically pure monoenol acetate, which immediately tautomerized to the corresponding keto enol acetate.

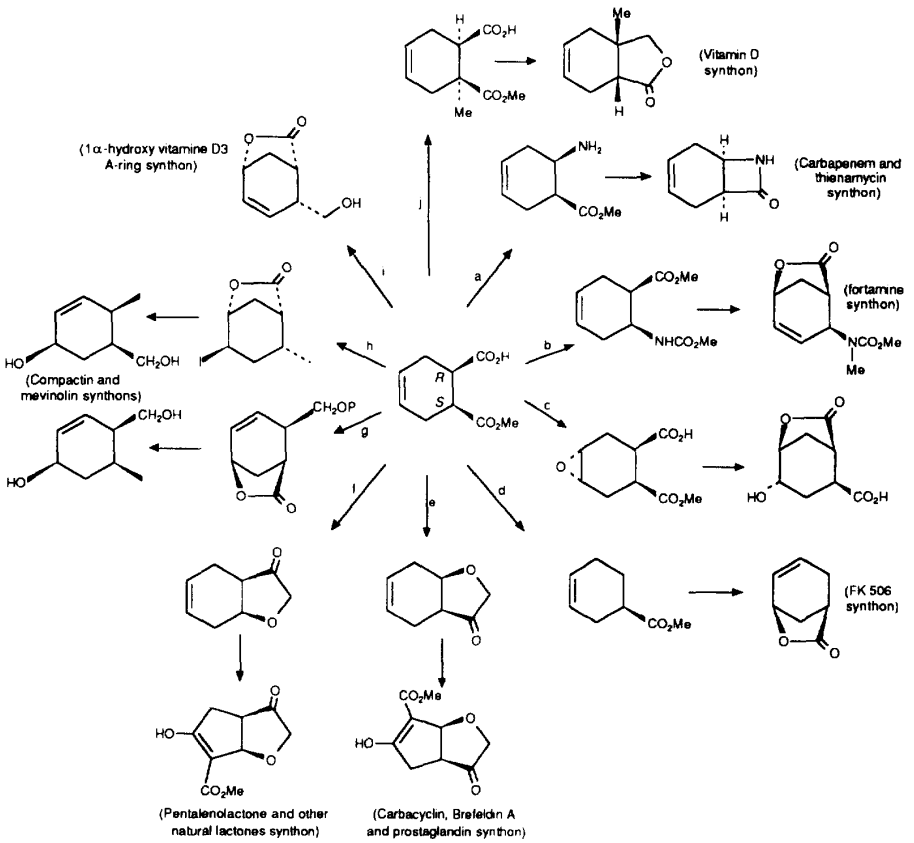


Fig 18. Synthetic strategies derived from the symmetrization-asymmetrization concept ("*meso* trick") [295, 384], applied to the homochiral synthon obtained by enantioposelective hydrolysis of dimethyl *cis*-cyclohex-4-ene-1,2-dicarboxylate with PLE. *a*, Kurihara *et al* [303]; Kaga *et al* [385]; *b*, Kobayashi *et al* [386, 387]; *c*, Kuhn *et al* [388]; *d*, Kocienski *et al* [389]; *e, f*, Gais and Lukas [294]; Gais *et al* [384]; Hemmerle and Gais [390]; Shäfer and Baringhaus [391]; *g, h*, Kobayashi *et al* [386, 392]; *j*, Shimada *et al* [109].

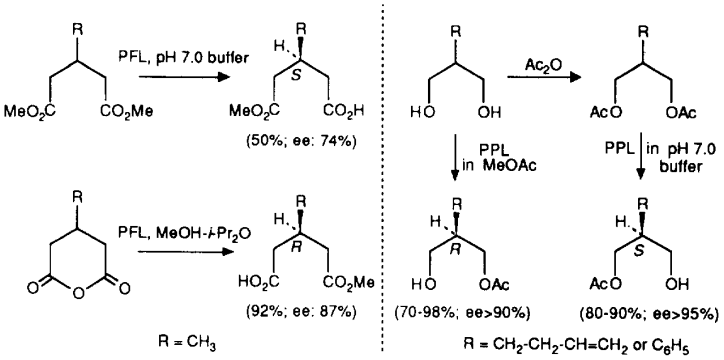
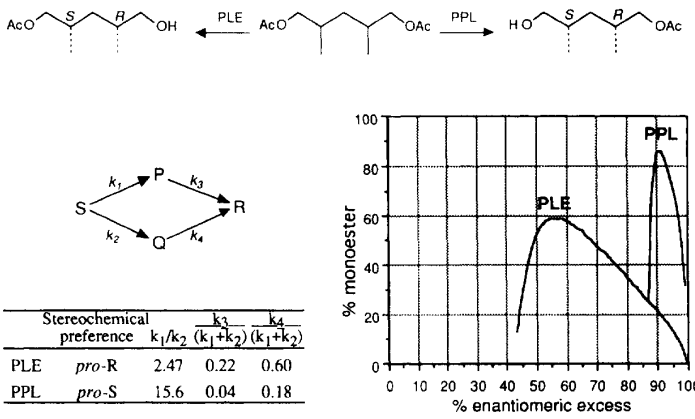


Fig 19. Alternative preparation of enantiomeric monomethyl 3-methylglutarates (*left*), using enantiotopic hydrolysis of the diester, or alcoholysis of the cyclic 3-methylglutaric anhydride by PFL [110, 393]. Alternative preparation of 2-substituted propane-1,3-diol monoacetates (*right*) using enantiotopic hydrolysis versus transesterification by PPL [115].



Stereochemical preference		k_1/k_2	$\frac{k_3}{(k_1+k_2)}$	$\frac{k_4}{(k_1+k_2)}$
PLE	*pro*-R	2.47	0.22	0.60
PPL	*pro*-S	15.6	0.04	0.18

Fig 20. Asymmetrization and enantiomeric excess enhancement *via* sequential hydrolysis of a *meso*-diester (1,5-diacetoxy-*cis*-2,4-dimethylpentane) by PLE or PPL [81, 113]. The kinetics of these "double-step" hydrolyses, determined by the partition coefficients k_1/k_2 and the relative values of k_3 and k_4, can be calculated and plotted to show the ee % versus monoacetate-concentration dependence and to determine the maximal recovery of asymmetric monoester at a given ee % value.

Several other enzymic reactions that act on *meso* substrates such as nitrilase-catalyzed hydrolyses, Baeyer-Villiger-like oxidations, and dehydrogenase-catalyzed oxidations, etc, have also been shown to be able to discriminate between enantiopic groups or atoms and generate enantiomerically pure compounds from symmetrical molecules. For example, horse-liver alcohol dehydrogenase (HLADH, see later) has frequently been used to enantiotopically oxidize *meso* primary diols, using NAD^+ regeneration through a flavin mononucleotide (FMN)-oxygen recycling system. The initial asymmetric oxidation product (a possibly optically active hydroxyaldehyde, fig 23) cyclizes spontaneously to a hemiacetal, which is further oxidized by HLADH to an (*S*)-lactone in an efficient complementary kinetic resolution [117].

Discrimination of enantiotopic faces of substrate

Any enzymic reaction on one of the two faces of a sp_2 (prochiral) carbon atom, such as a carbonyl group or a C=C (C=N) double bond, offers a potential asymmetrization leading to chiral products. Reduction, mainly by NAD or NADP-dependent dehydrogenases, has been widely used for the creation of new asymmetric centers. Other addition reactions of the carbonyl group, such as aldolization, HCN addition, or acyloin formation, represent the enzymic counterparts of classical chemical reactions currently used for the C–C bond formation, and frequently provide practical routes to high enantiomeric purity chiral synthons.

The use of purified nicotinamide cofactor-dependent dehydrogenases in preparative reduction reactions requires an efficient regeneration of the expensive NAD(P)H cosubstrate. A turnover number of 10^3 to 10^5 is generally required for the nicotinamide cofactor in order to obtain a productive and economically feasible reaction. Such cofactor recycling is almost invariably carried out by coupled enzymic reactions (fig 24), by using either an alternative substrate of the same enzyme, which can be converted in the oxidation direction, or a second independent substrate-enzyme combination, functioning simultaneously with the same cofactor in the oxidation direction (table VII). Two of the disadvantages thus encountered are the need for an auxiliary

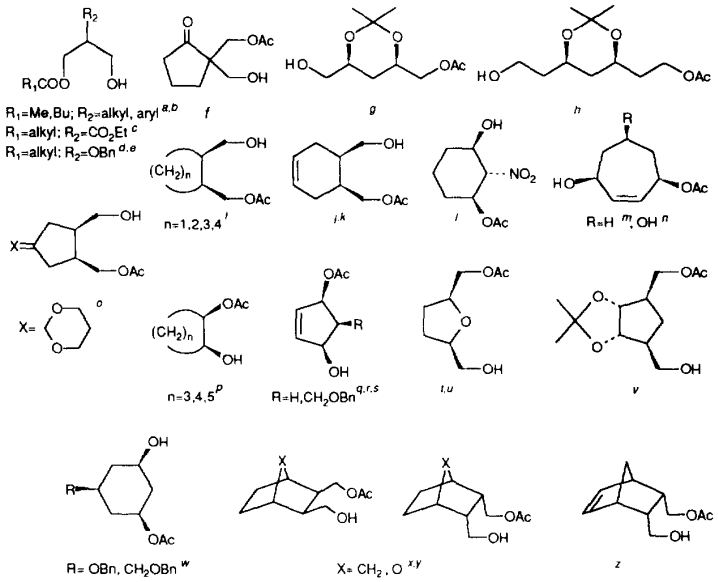


Fig 21. Some asymmetric diol monoesters successfully prepared in one or both homochiral forms by enantiotopic hydrolysis of *meso* diol diesters, or enantiotopic transesterification of the corresponding diols, using various enzymes. *a* PPL [81, 115, 394-396]. *b* PFL [395, 397-400]. *c* PPL [401]. *d* PPL [402-404]. *e* PFL [46, 402, 405]. *f* Electric eel acetyl-cholinesterase [406]. *g* PFL [407-409]. *h* PFL [410]. *i* PPL [411, 412]. *j* PPL [395, 412]. *k* PLE [339]. *l* PLE [413]. *m Candida antartica* lipase [414]. *n* Acetyl cholinesterase [415]. *o* PPL [390]. *p* PFL [397, 416]. *q* PPL [417, 418]. *r* PLE [419]. *s* Electric eel acetyl-cholinesterase [420]. *t* PLE, CCL [421, 422]. *u Mucor javanicus* lipase [423]. *v Rhizopus delemar* lipase [424]. *w* PLE [425]. *x* PPL, CCL [426]. *y* PFL [427]. *z Geotrichum candidum* lipase [428].

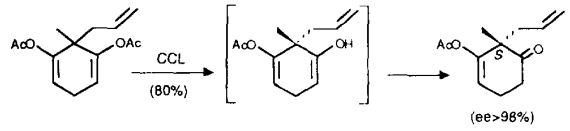


Fig 22. Asymmetric synthesis of an (*S*)-keto enol acetate by enzymic hydrolysis of a prochiral dienol diacetate [116].

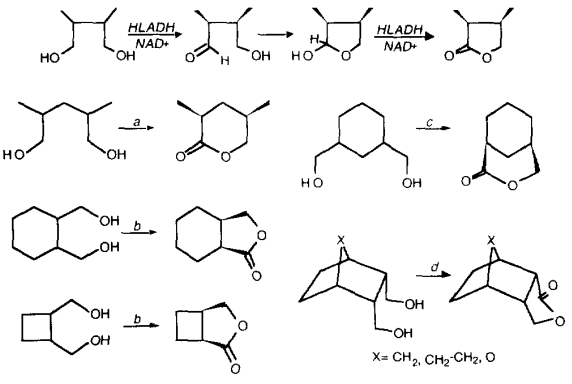


Fig 23. Primary *meso*-diols asymmetrization by oxidation with HLADH (with NAD^+ recycling). *a* Ng *et al* [117]. *b* Jakovac *et al* [429]. *c* Bridges *et al* [430]. *d* Jones and Francis [431]; Lok *et al* [432].

substrate (used in large amounts to drive the reduction reaction), and the possible problems associated with the formation of a secondary oxidized product. Some of these drawbacks have been eliminated, for example in NADH-recycling, by using the formate/formate de-

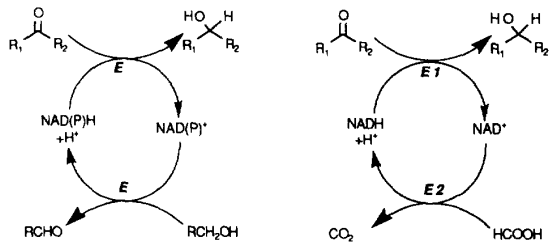


Fig 24. Reduced-nicotinamide cofactor recycling *via* coupled enzymic reactions, using the same enzyme and an auxiliary reduced substrate (*left*), or using a second auxiliary enzyme (formate dehydrogenase, *right*) and its reduced substrate.

hydrogenase system, which combines the use of a cheap substrate with the formation of a volatile co-product (CO_2). Notwithstanding the apparent simplicity of this method, a large part of the research effort into dehydrogenase applications on an industrial scale has been concerned with the problem of recycling. More sophisticated methods, using membrane-compartmented modified high-molecular-weight cofactors have been investigated further and used for large-scale processes [118].

- *Carbonyl reduction*

One of the features of known carbonyl reductases (table VIII), with a few valuable exceptions, is that they generally deliver a hydride from the reduced cofactor to the left face of the carbonyl group, with the large (L) and small (S) substituents arranged as shown in figure 25 (*re* face). This is agreement with Prelog's rule,

Table VII. Recycling enzymic coupled systems for the *in situ* regeneration of reduced nicotinamide cofactors.

Enzyme	Regeneration reaction	References
Glucose dehydrogenase	Glucose + NAD(P) → Gluconate + NAD(P)H + H^+	[307]
Glucose-6-phosphate dehydrogenase	Glucose-6-Ph + NAD(P) → 6-Ph-gluconolactone + NAD(P)H + H^+	[308]
Alcohol + aldehyde dehydrogenases	Ethanol + 2 NAD(P) → CH_3CO_2H + 2 NAD(P)H + 2 H^+	[309]
Hydrogenase (+ one-electron dye)	H_2 + NAD(P) → NAD(P)H + H^+	[124]
Formate dehydrogenase	HCO_2H + NAD → CO_2 + NADH + H^+	[310, 311]

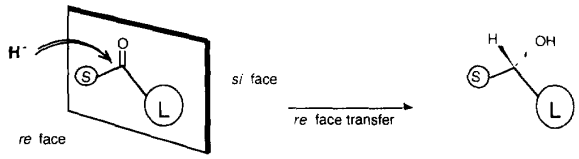


Fig 25. Stereoselective reduction of a carbonyl group by the *re* face, defined by the clockwise arrangement of oxygen, large (L) and small (s) groups (Prelog's rule model).

which was primarily defined for the *Curvularia falcata* alcohol dehydrogenase [119].

To avoid the problems associated with reduced-cofactor regeneration, whole cell-microorganism-mediated reductions have been extensively used. Baker's yeast, for example, is a cheap and easily available reagent which contains a wide range of dehydrogenase activities, all the necessary cofactors, and an integrated regenerating system in the form of the fermentative glucose oxidation pathway (fig 26). It is generally thought that some dehydrogenase reactions may use NADH formed in the 3-phosphoglyceraldehyde oxidation, though most of the carbonyl reductases preferentially use NADPH. It is possible that other reduced coenzyme sources are available in semi-fermenting yeasts and other microorganisms, such as part of the pentose phosphate pathway [120] and/or ethanol and acetate oxidases [121, 122]. Cofactor recycling is thus automatically carried out by the cell so long as an oxidizable substrate (generally sucrose or glucose), is provided.

Baker's yeast and filamentous fungi are currently used to reduce a full range of carbonyl compounds [5, 123-126], from simple aliphatic ketones to complex functionalized carbonyl compounds (fig 27). Some of them already contain a racemic asymmetric center and are resolved (only one enantiomer reacts). In other cases, both enantiomers can be reduced. Most of these reductions generally follow Prelog's rule and are highly stereoselective, except when several oxidoreductases with opposite stereoselectivities are involved. This problem is generally detected by a substrate-concentration effect on the optical purity of the secondary alcohol produced (fig 28), depending on the corresponding K_M of the different dehydrogenases [127]. Some of the more important carbonyl reductases involved in β-ketoester reduction by yeast include L-β-hydroxyacylCoA dehydrogenase and the fatty acid synthetase complex [128]. Glycerol dehydrogenase [129, 130], lactate dehydrogenase, D-3-hydroxybutyrate dehydrogenase, ketopantoyl lactone reductase [4] and other yet unidentified enzymes [131-138] probably play a role in the reduction of β-keto esters and other carbonyl substrates.

Table VIII. Purified dehydrogenases (DH) currently used for carbonyl group reduction.

	Cofactor	Stereochemical course of the reduction: Hydride transfer	Product	Substrate	References
Horse-liver alcohol DH (HLADH)	NAD(P)H	*re* face	*S*	Mostly cyclic ketones	[312]
Yeast alcohol DH (YADH)	NAD	*re* face	*S*	Aliphatic aldehydes	[312]
Pseudomonas sp alcohol DH	NAD	*si* face	*R*	Acyclic ketones	[313]
Candida parapsilosis alcohol DH	NAD	*re* face	*S*	Broad range	[314]
Rhodococcus erythropolis alcohol DH	NAD	*re* face	*S*	Broad range	[314]
Mucor javanicus alcohol DH (MJADH)	NADP	*si* face	*R*	See HLADH	[312]
Curvularia falcata alcohol DH (CFADH)	NADP	*re* face	*S*	See HLADH	[312]
Pig liver alcohol DH (PLADH)	NADP	*re* face	*S*	See HLADH	[315-317]
Thermoanaerobium brockii alcool DH (TBADH)	NADP	*re* face	*S*	Acyclic ketones	[318, 319]
Lactobacillus kefir alcohol DH	NADP	*si* face	*R*	Broad range	[320]
Bacillus stearothermophilus L-lactate DH	NAD	*re* face	L(*S*)	α-Keto acids	[321]
Staphylococcus epidermidis D-lactate DH (SELDH)	NAD	*si* face	D(*R*)	α-Keto acids	[322]
Geotrichum candidum glycerol DH	NAD	*si* face	*R*	α-Hydroxyketones	[129]
Pseudomonas putida carnitine DH	NAD	*si* face	*R*	3-Dehydrocarnitine	[323, 324]

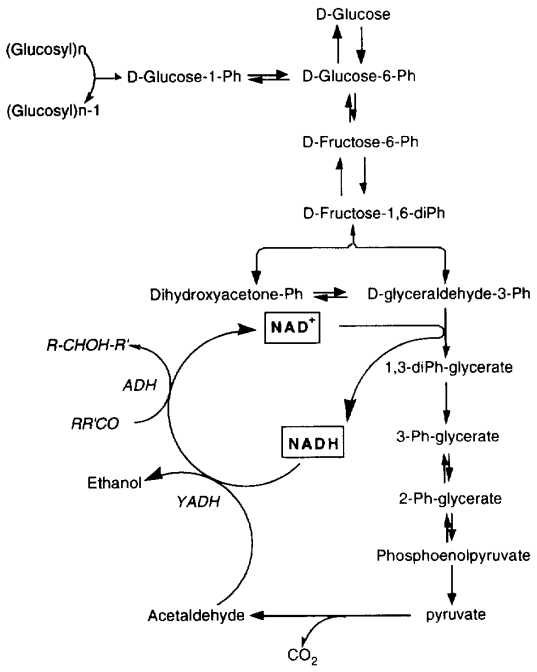


Fig 26. D-Glucose fermentation pathway in yeast. The significant step for oxidized cofactor regeneration, in the absence of an electron-transport system to oxygen, is the reduction of acetaldehyde to ethanol by the classical yeast alcool dehydrogenase (YADH). The NADH pool may be additionally oxidized in the reduction of an exogenous carbonyl compound, using different alcohol dehydrogenase(s). As the NAD-NADH system operates in a catalytic mode, D-glucose constitutes the final reducing equivalent donor.

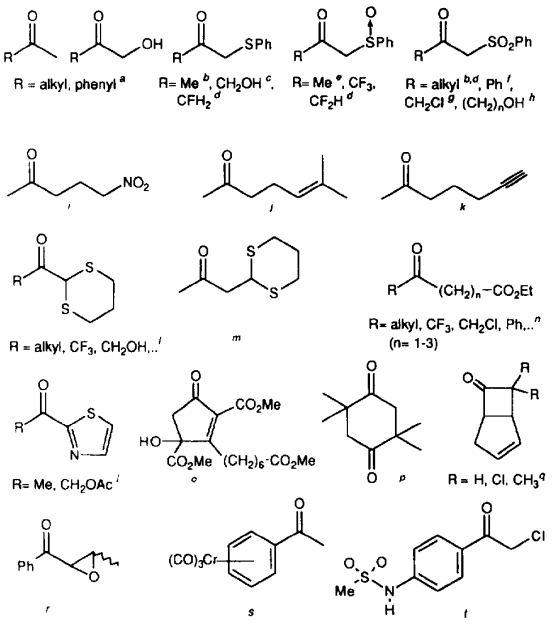


Fig 27. Some examples of keto compounds reduced by baker's yeast or by other microorganisms into the corresponding secondary alcohols with a high enantiomeric excess. *a*, MacLeod *et al* [433]; Cervinka *et al* [434]. *b*, Crumbie *et al* [435]. *c*, Fujisawa *et al* [436]. *d*, Yamasaki *et al* [437]. *e*, Iruchijma and Kojima [438]. *f*, Nakamura *et al* [439]. *g*, Tanikaga *et al* [440]. *h*, Sato *et al* [441]. *i*, Nakamura *et al* [442]. Fantin *et al* [443]. *j*, Belan *et al* [257]. *k*, Le Drian and Greene [444]. *l*, Guanti *et al* [445]. *m*, Bernardi *et al* [446]. *n*, Servi [125]; Buisson *et al* [142]; Santaniello *et al* [6]. *o*, Okano *et al* [447]. *p*, d'Angelo *et al* [448]. *q*, Roberts [449]. *r*, Takeshita *et al* [450]. *s*, Gillois *et al* [451]. *t*, Patel *et al* [452].

As a consequence of such enzyme multiplicity, it is possible to modulate the stereoselectivity of the reduction by modifying the enzymic equipment of the microorganism : (i) controlling the carbon source [139] used for cell growth, the cell-treatment (immobilization [140, 141], ageing [142], heat treatment [143]) and the incubation parameters (addition of specific inhibitors [144, 145], nature of oxidizable cosubstrate, etc); and (ii) using selected or mutant microorganisms. Another extensively used method, which does not really account for the presence of multiple enzymes, is to manipulate the size of the carbonyl substituents by chemical means in order to enhance or reverse the stereoselectivity, as shown in figure 28 with baker's yeast-mediated reduction of 4-chloro-β-keto esters [146].

The presence of several oxidoreductase activities may be beneficial as shown in the one-step deracemization of pantoyl lactone (fig 29) mediated by ketopantolactone and ketopantoic acid reductases [147].

Another interesting situation is found in the reduction of racemic α-monosubstituted β-dicarbonyl compounds (1,3-diketones, 3-oxoesters), which can be easily epimerized in the incubation conditions *via* keto-enol tautomerization (fig 30). Diastereoselective reduction, combining enantiomer selection and stereoselective reaction, frequently results in the high-yield formation of a unique stereoisomer containing two asymmetric centers [126, 148-150]. This concept, which has a chemical counterpart ("dynamic kinetic resolution") using chiral hydrogenation catalysts [151-153], has been illustrated in numerous examples (fig 31), using baker's yeast or other microorganisms to obtain valuable new asymmetric building blocks for the synthesis of natural products. A different situation is found with symmetrical enolizable or non-enolizable α-monosubstituted or α,α'-disubstituted 1,3-diketone substrates (fig 32), which are reduced stereoselectively on one of the enantiotopic carbonyl groups, thus creating at least two potential asymmetric centers. The highly stereoselective reduction of the prochiral α,α'-dimethyl-1,3-cyclohexanedione by baker's yeast has been used by Mori [154, 155] as a source of versatile asymmetric starting material for the synthesis of various natural products (fig 33).

Recently, non-immobilized or immobilized whole-cell microorganisms have been satisfactorily used for keto ester reduction in pure organic solvent (hexane or benzene) with some noticeable changes in the stereoselectivity of the reaction [156-159].

- *C=C reduction*

The stereospecific biohydrogenation of "activated" carbon-carbon double bonds has been also exploited

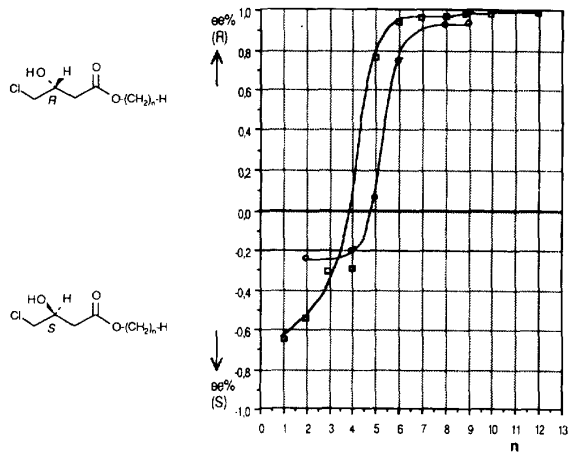


Fig 28. Variation of enantiomeric excess and reversion of stereoselectivity observed for the reduction of 4- chloro-3-keto esters by baker's yeast when the size of ester grouping was increased. Note the different curves obtained for two substrate concentrations (□—□, 0.91 mmol; ○—○, 2.7 mmol), indicating the involvement of at least two oxidoreductases with opposite stereoselectivities (adapted from Sih *et al* [123]).

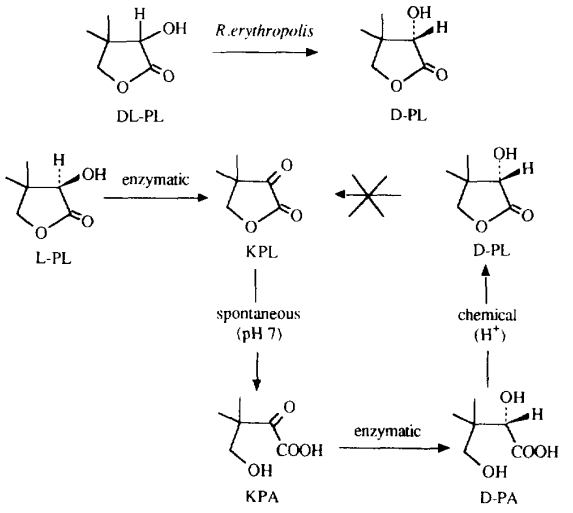


Fig 29. Dehydrogenase reactions in *Rhodococcus erythropolis* leading to the conversion of L-pantolactone (L-PL) into D-pantolactone (D-PL), through stereoselective oxidation to ketopantolactone (KPL), spontaneous hydrolysis to ketopantoic acid (KPA) and stereospecific reduction of KPA to D-pantoic acid (D-PA) (adapted from Shimizu *et al* [147]).

for synthetic purposes. NADH-dependent enoate reductases have been isolated in many microorganisms such as *Clostridium* or *Proteus* sp. [124]. Baker's yeast and related microorganisms can also stereoselectively reduce carbonyl-conjugated double bonds (α,β-unsaturated aldehydes, ketones and esters), and some di- and trisubstituted double bonds activated by electron-withdrawing substituents (α- or β-substituted allylic alcohols, nitro olefins, etc), as shown in figure 34. The reduction rate and the stereochemistry of the reduced products were generally found to be dependent on the *E* or *Z* geometry of the double bond, and sometimes offered the possibility of obtaining both enantiomers.

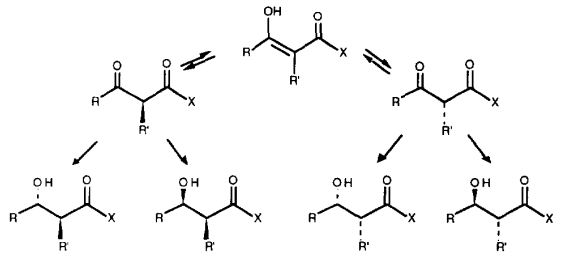


Fig 30. Stereoisomers expected from diastereo- and enantioselective reductions of β-dicarbonyl compounds.

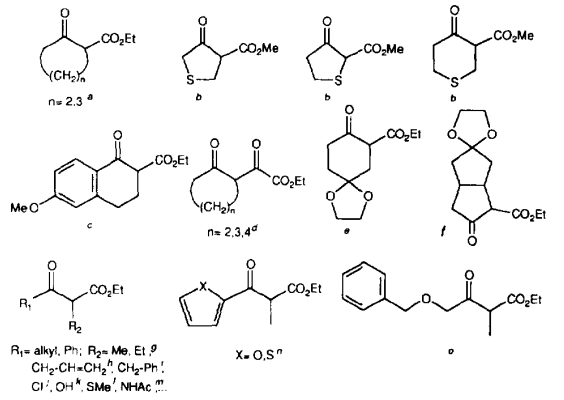


Fig 31. Cyclic or acyclic (±)-α-substituted β-dicarbonyl compounds diasteroselectively and enantioselectively reduced by baker's yeast or other microorganisms. *a*, Deol *et al* [148]; Frater [149]; Buisson and Azerad [453]; Seebach *et al* [454]; Sato *et al* [455]. *b*, Hoffmann *et al* [456]. *c*, Seebach *et al* [454]; Buisson *et al* [457]. *d*, Tsuboi *et al* [457-459]. *e*, Kitahara and Mori [460]. *f*, Brooks *et al* [461]. *g*, Frater [149]; Nakamura *et al* [462]; Buisson *et al* [463]; Azerad and Buisson [126]. *h*, Nakamura *et al* [464]. *i*, Furuichi *et al* [131]. *j*, Cabon *et al* [465]; Akita *et al* [466]. *k*, Sato *et al* [467]. *l*, Fujisawa *et al* [468]. *m*, Soukup *et al* [469]; Kato *et al* [470]. *n*, Akita *et al* [471]; Furuichi *et al* [472]. *o*, Buisson *et al* [473].

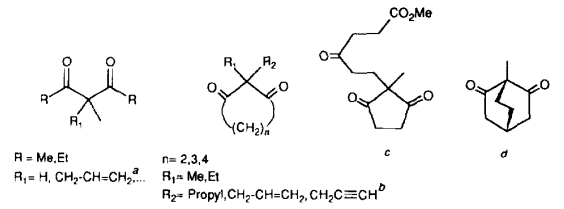


Fig 32. Some examples of 1,3-diketones enantiotopically reduced by microorganisms with creation of an asymmetric tetrasubstituted carbon atom. *a*, Bolte *et al* [474]; Fauve and Veschambre [475, 476]. *b*, Lee *et al* [477]; Lu *et al* [478]; Brooks *et al* [320, 479-482]. *c*, Bellet *et al* [483]; Lanzilotta *et al* [484, 485]. *d*, Mori and Nagano [486].

- *C=N reduction*

Nicotinamide cofactor-dependent L-amino acid dehydrogenases, which catalyze the formation of ketoacids

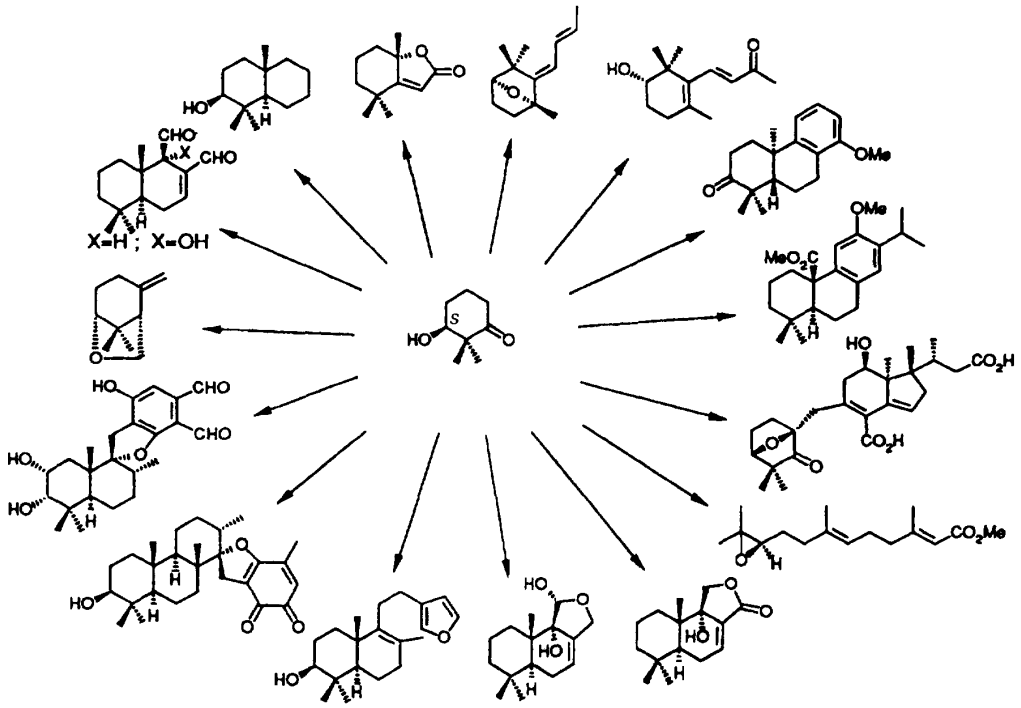


Fig 33. Natural products synthesized using the versatile asymmetric synthon, (*S*)-2,2-dimethyl-3-hydroxycyclohexanone, prepared by microbial reduction of the corresponding prochiral 1,3-diketone (adapted from [155]).

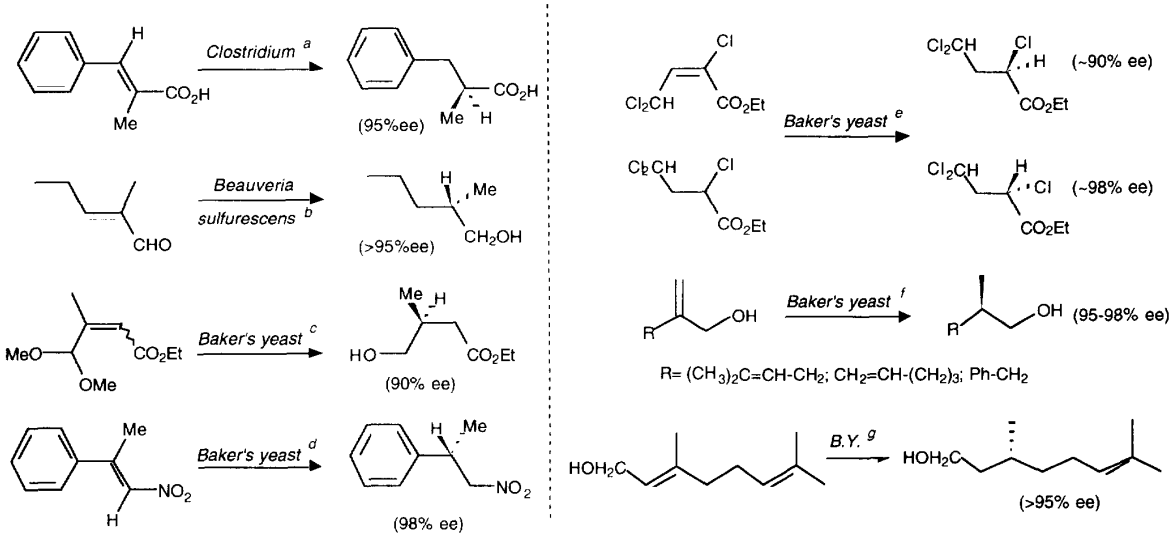


Fig 34. Some stereoselective double bond biohydrogenation reactions using whole-cell microorganisms. *a*, Simon *et al* [124, 487]. *b*, Desrut *et al* [488]; Kergomard *et al* [489]. *c*, Ferraboschi *et al* [342, 490]. *d*, Ohta *et al* [491]. *e*, Utaka *et al* [492]. *f*, Ferraboschi *et al* [493]. *g*, Gramatica *et al* [494].

from chiral α-amino acids, have important synthetic applications when used to obtain the reverse formation of natural or unnatural L-amino acids, in the presence of a ketoacid and ammonium salt (fig 35A) *via* the reduction of the corresponding imino compound [160]. Microbial AlaDH, GluDH, LeuDH and PheDH are currently used for such syntheses and accept a range of 2-oxoacids as substrates, associated with a cofactor-regenerating system. A particularly efficient process for L-amino acid (and analogues) syntheses combines the use of a reduced α-hydroxyacid substrate with NADH regeneration, catalyzed by the corresponding α-hydroxyacid and amino acid dehydrogenases (fig 35B).

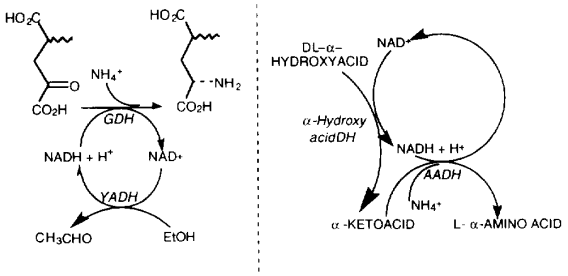


Fig 35. **A** (*left*), synthesis of diastereomeric 4-methyl L-glutamic acids using glutamate dehydrogenase (GDH) and yeast alcohol dehydrogenase-ethanol as NADH regenerating system [495]. **B** (*right*), coupled oxidation (by an α-hydroxyacid dehydrogenase) and imino-reduction (by an amino-acid dehydrogenase, AADH) for the preparation of an L-α-amino acid from the corresponding DL-α-hydroxyacid [496].

• *HCN addition*

The D-oxynitrilase from bitter almonds (*Prunus amygdalus*) [161] which normally promotes the decomposition of amygdalin into glucose, benzaldehyde and cyanide, is capable of catalyzing the asymmetric addition of HCN to a wide range of aromatic and aliphatic aldehydes [162-165] to afford the corresponding *R*-cyanohydrins (fig 36). Another enzyme, isolated from sorghum (*Sorghum bicolor*), has the opposite stereospecificity but accepts a narrower range of substrates and is restricted to aromatic aldehydes [166, 167] . The non-enzymic reaction that leads to a racemic product or to the racemization of the optically active product can be suppressed by using suitable conditions (acidic pH, low water activity). Acetone cyanohydrin can be used as a cyanide donor, which avoids the hazards associated with hydrogen cyanide [168]. Immobilization and use of organic solvents allow us to obtain high yields and higher enantiomeric excess [163]. Optically pure cyanohydrins [169, 170] are very versatile synthetic intermediates and the two functional groups are easily manipulated into homochiral α-hydroxyacids, α-hydroxyaldehydes, α-amino acids, etc.

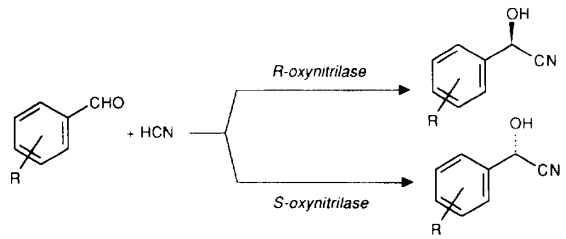


Fig 36. Enantioselective cyanohydrin synthesis, using almond oxynitrilase (*R*), or sorghum oxynitrilase (*S*).

• *Acyloin condensations*

This reaction uses the thiamine pyrophosphate-dependent pyruvate decarboxylase of yeasts [171-173] and produces a reactive acetaldehyde intermediate with reversed polarity. This is similar to the intermediate formed chemically in the so-called "umpolung" reactions, and is thus capable of acting as a nucleophile and adding on the carbonyl group of another aldehyde. With benzaldehyde as a cosubstrate and using yeast whole-cells in the presence of glucose or pyruvate, this constitutes the key step in the industrial process for the manufacture of L-ephedrine [174] (fig 37). Other applications to the synthesis of high optical purity (*R*)-α-ketols (acyloins), derived from aromatic or aliphatic aldehydes have been described [175, 176].

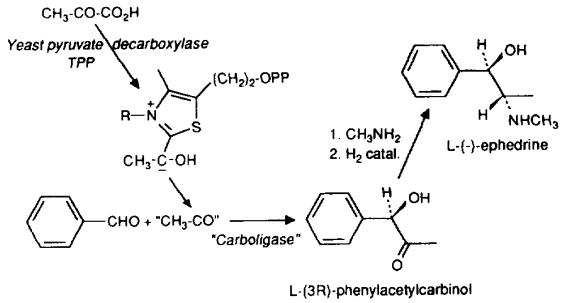


Fig 37. Industrial synthesis of L-(−)-ephedrine based on a microbial mediated acyloin condensation.

• *Aldolisation reactions*

Carbon-carbon-bond-forming enzymes have been used extensively in the synthesis of carbohydrates [19, 177, 178]. Aldolase and to a lesser extent transaldolase activities have been exploited for the synthesis of a large number of natural and unnatural sugar and sugar analogues. Although these enzymes suffer from a relatively narrow substrate specificity, they undergo reactions equivalent to the well-known chemical aldol condensation (addition of an activated methylenic group onto a carbonyl group) and generally have a high stereospecificity. They have allowed the stereospecific synthesis of invaluable sugar-like molecules widely used in biochemical studies.

Over 20 aldolases have been identified so far. These enzymes catalyze the stereospecific condensation of a ketone donor with an aldehyde (fig 38). One group of enzymes, represented by D-fructose-1,6-diphosphate aldolase (FDP aldolase) from rabbit muscle [179, 180], uses dihydroxyacetone phosphate (DHAP) as the single nucleophilic ketone substrate and forms a keto-1-phosphate sugar upon reaction with an aldehyde. A second group contains *N*-acetyl neuraminic acid aldolase (NacNA aldolase) and uses pyruvic acid (or phosphoenol pyruvate) as the nucleophile to form 3-deoxy-2-keto acids. The third type of aldolase is represented by a unique example, 2-deoxyribose-5-phosphate aldolase, which uses acetaldehyde as the nucleophilic substrate to form 2-deoxyaldoses. A common feature of the aldolase reactions is that the stereochemistry of the newly formed C–C bond is completely controlled by the enzyme, irrespective of the structure and stereochemistry of the substrates. FDP aldolase generates exclusively 3*S*,4*R*-diols (D-*threo* stereochemistry), while fuculose-1 phosphate aldolase and rhamnulose 1-phosphate aldolase generate 3*R*-vicinal diols (with major D-*erythro* and L-*threo* configurations respectively). Despite the

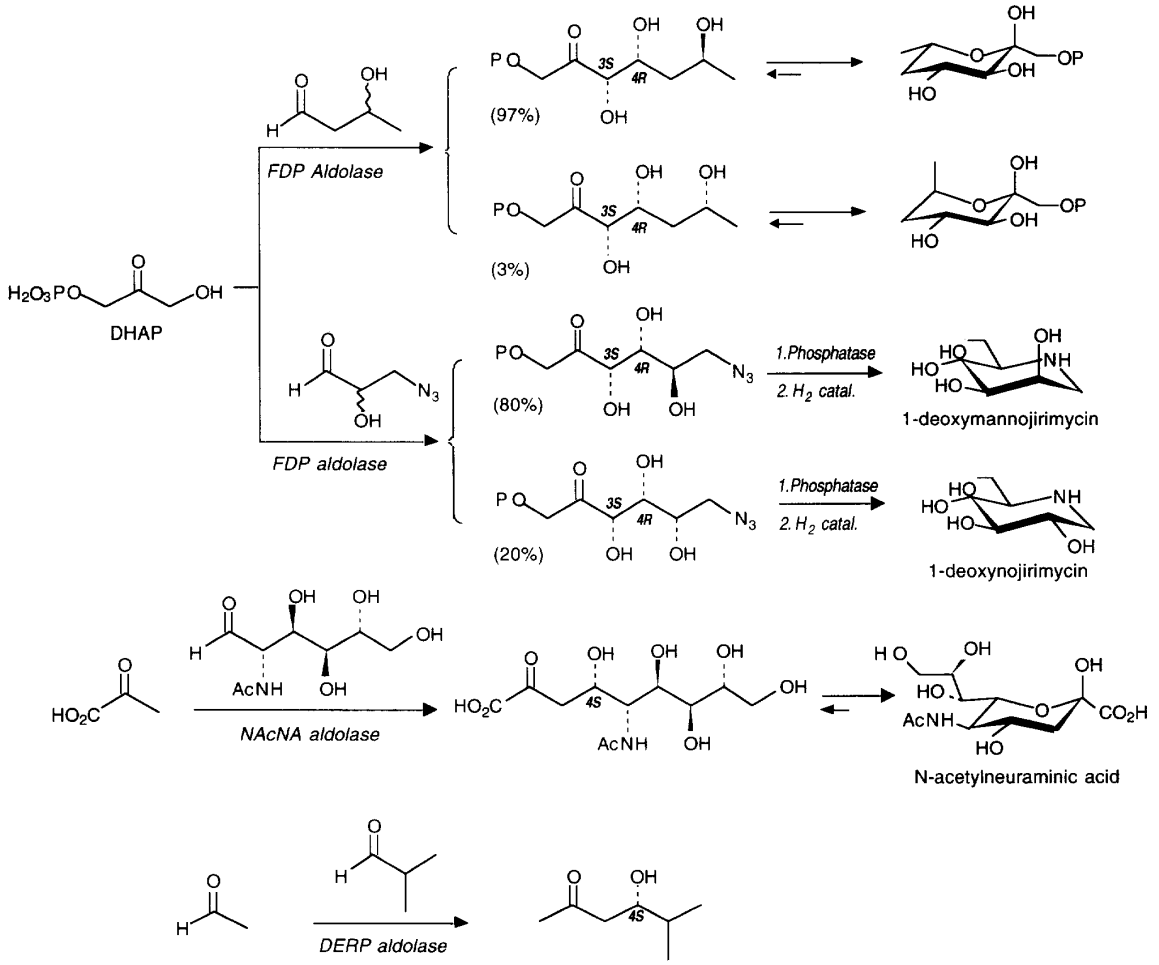


Fig 38. Some aldol addition reactions catalyzed by fructose diphosphate aldolase (FDP aldolase) [181, 497], *N*-acetyl neuraminic acid aldolase (NAcNA aldolase) [498] and 2-deoxyribose-5-phosphate aldolase (DERP aldolase) [499].

fact that the DHAP-utilizing aldolases are quite specific for this substrate, more than 75 aldehydes have been found to replace the natural aldehyde cosubstrate. These range from formaldehyde to C6-aldose sugars with the exception of aromatic aldehydes and allow the synthesis of a variety of deoxy-, methoxy-, fluoro-, C-alkylated or nitrogen-containing sugars analogues [177, 181]. In most cases, the enantioselectivity of the enzyme causes a kinetic resolution of a racemic aldehyde substrate, affording a major (or homochiral) stereoisomeric product containing more than two asymmetric centers. Some attempts to replace DHAP by dihydroxyacetone and added inorganic arsenate or vanadate have been claimed to be successful *via* the spontaneous reversible formation of dihydroxyacetone esters [182].

Pyruvate-utilizing aldolases, such as *N*-acetylneuraminic acid aldolase (NAcNA aldolase) and 3-deoxy-D-manno-2-octulosonate aldolase (KDO aldolase), or phosphoenolpyruvate-utilizing aldolases, such as D-manno-2-octulosonate-8-phosphate synthetase (KDPO synthetase) and 3-deoxy-D-arabino-2-heptulosonate 7-phosphate synthetase (DAHP synthetase), catalyze the condensation of pyruvate (or phosphoenolpyruvate) with various natural and unnatural aldehydic sugars or sugar phosphates (fig 38). NAcNA aldolase from animal or microbial origin has allowed the stereospecific syntheses of a number of sialic acid analogues, which have been used for biological and biochemical studies [183].

2-Deoxyribose-5-phosphate aldolase (DERP aldolase) from bacteria can use acetaldehyde (and to a lesser extent propionaldehyde, for example) as a nucleophile and accepts a wide range of unnatural aldehydic cosubstrates to form 3*S*-alcohols (fig 38).

Another enzyme involved in sugar phosphate metabolism, transketolase, is found in spinach leaves and yeast. This is a thiamine pyrophosphate-dependent aldolase which removes one of the limitations of aldolases in ketose synthesis : it does not need a phosphorylated substrate and accepts hydroxypyruvate as a nucleophile donor. This leads to the transfer of a hydroxyacetyl group on a variety of aldehydic acceptors in a highly enantio- and stereospecific reaction, limited to the formation of 3*S*,4*R* (D-*threo*) products [184, 185].

Other useful reactions

Miscelleanous addition, elimination or transfer reactions

Elimination reactions catalyzed by water or ammonia lyases (fig 39) have been exploited for synthetic processes. L-Malate is obtained by the fumarase-catalyzed addition of water to fumarate, using a microbial whole cell process [186, 187], while the pig-heart enzyme can use chloro- and difluorofumarate as substrates, converting them to L-*threo*-chloromalic acid and 2,3-difluoromalate, respectively [188]. Aspartate-ammonia lyase (aspartase) catalyzes the addition of ammonia to the *si* face of fumarate to form L-aspartate [189]. Other L-amino acid ammonia lyases (L-phenylalanine, L-histidine, L-tyrosine ammonia lyases) have been similarly used to form the corresponding L-amino acids and some analogues from the appropriate unsaturated substrates [190].

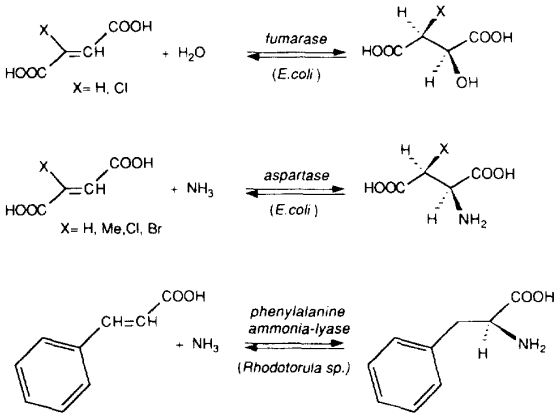


Fig 39. Lyase reactions used for asymmetric synthesis.

Enzymes dependent on pyridoxal phosphate (PLP) have also found some applications in the preparation of natural L-amino acids or analogue molecules [4]. A glutamate-oxaloacetate aminotransferase has been used for the preparation of 4-alkylated L-glutamic acid analogues, using aspartic acid or cysteine sulfinate for the irreversible regeneration of the pyridoxamine form of the coenzyme [191]. Other lyase reactions involving PLP as a coenzyme are also capable of operating in the reverse direction. Some are of industrial interest for the synthesis of L-tryptophan, L-tyrosine and modified analogues. These are catalyzed by tryptophanase or tyrosine phenol lyase (in whole-cell processes), using pyruvate, ammonia and indol or phenol as acceptors (fig 40) [4, 192, 193]. A β-decarboxylase (from *Pseudomonas dacunhae*) has also been successfully used to obtain L-alanine from L-aspartic acid [189].

Other group-transfer reactions have very limited applications because of both the need of expensive coenzymes (CoA-SH or cobamide coenzymes, for example) and the fact that the enzymic reaction products are not extremely called for (compare later to the synthesis of oligosaccharides *via* glycosyl transferase reaction).

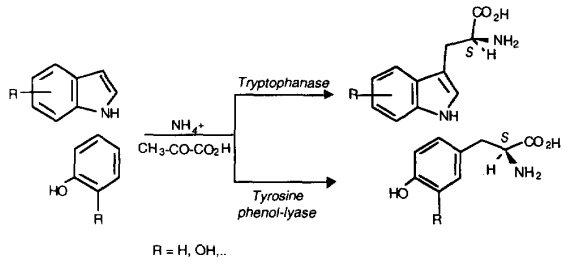


Fig 40. Enzymic syntheses of L-tryptophane or L-tyrosine (and analogues) using the reversal reaction of tryptophanase from *Escherichia coli* [192], or tyrosine-phenol lyase (β-tyrosinase) from *Erwinia herbicola* [4].

Biological macromolecule syntheses

• *Oligosaccharide and glycoside synthesis*

Several enzymic methods are available for the synthesis of glycosides in which glycosidases and glycosyl transferases are used the most frequently [177, 178, 194, 195]. The sugar-nucleotide-dependent glycosyl transferases seem to be more suitable for the selective synthesis of complex oligosaccharides because of their enantio- and regioselectivity for acceptor structures, but the major problems are the poor availability of enzymes and the high price of sugar nucleotides. Furthermore, the nucleotide phosphates released are often inhibitors of the transferase reaction. Cloning and expression of specific glycosyl transferases and regeneration of sugar nucleotides from the released nucleotide phosphate have been developed to solve such problems, and multienzyme systems, sometimes in the immobilized form, have been used successfully in one-pot reactions for the large-scale synthesis of many oligosaccharides (fig 41), which may contain natural or unnatural sugar analogues [196-198].

One alternative is to use the reversal of the hydrolytic action of glycosidases (glycohydrolases) at high concentrations of mono- or oligosaccharide substrates. An equilibrated thermodynamically controlled reaction [199, 200] or, better, a kinetically controlled process using a glycoside as glycosyl donor [201-203] can be used (fig 42). Such reactions, using α- or β-glucosidases, galactosidases, mannosidases, etc, are simple synthetic procedures which have the same advantages as transferase reactions : elimination of the need for sugar protection, and complete control of the stereochemistry at the newly formed anomeric center. However, regiocontrol with respect to the nucleophile acceptor remains a major problem, with the production of varying ratios of isomeric oligosaccharides and the resulting separation problems.

• *Enzymic peptide synthesis*

An important goal for enzyme utilization is to develop practical catalysts for the condensation of amino acids, either for the recurrent step-by-step synthesis of oligopeptides, avoiding the tedious protection-deprotection methods and allowing the incorporation of unnatural or D-amino acids, or for the coupling of large peptidic unprotected fragments, prone to racemization by chemical methods. Native enzymes for such biosynthetic reactions are not suitable for technical use, even

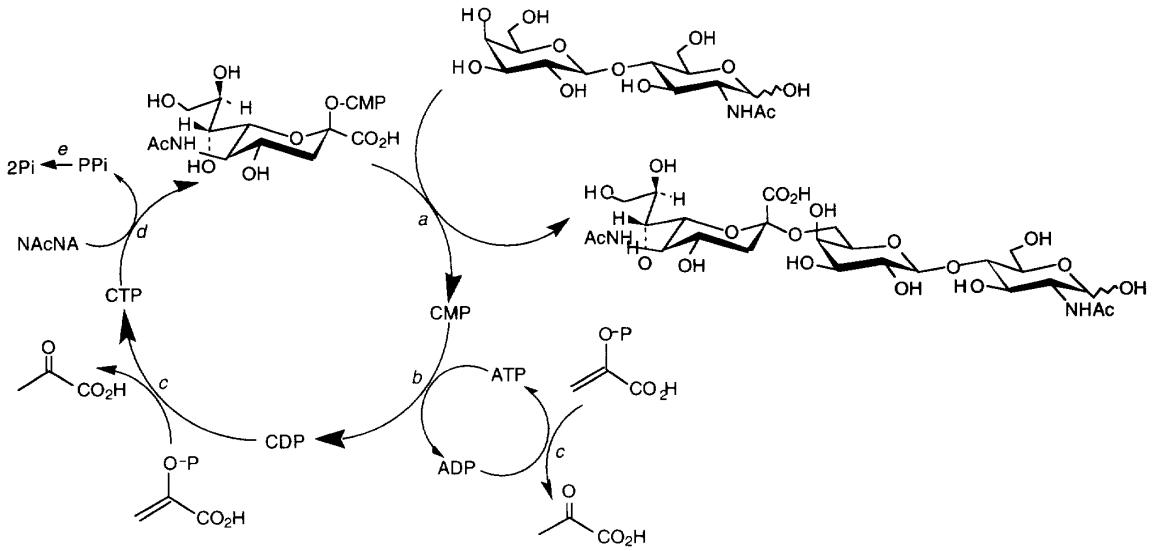
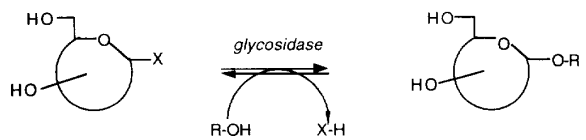


Fig 41. Enzymic sialylation of *N*-acetyl lactosamine with *in situ* regeneration of *N*-acetyl neuraminic acid-cytidine monophosphate (CMP-NAcNa) using five enzymes [198] : *a*, α-2,6-sialyl transferase; *b*, adenylate kinase; *c*, pyruvate kinase; *d*, CMP-NAcNa synthetase; *e*, pyrophosphatase.



ROH = alcohol, sugar nucleophile
Equilibrated glycosylation: X=OH
Transglycosylation: X= F, Glycosyl, Ph, p-NO_2-Ph

Fig 42. Synthesis of oligosaccharides and glycosides using the equilibrium reaction of glycosidases, in thermodynamically or kinetically controlled reactions.

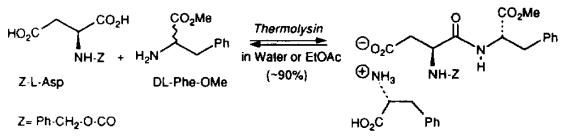


Fig 43. Enzymic synthesis of *Z*-L-Phe-L-Asp-OMe, a precursor of the sweetener peptide aspartame, using *Z*-L-Asp and DL-Phe-OMe as substrates [500-502].

in the case of specific bacterial peptide multienzyme complexes. Proteases remain possible catalysts, despite the problem of preventing the amidasic degradation of the final products, and the unfavorable equilibrium constants. Nevertheless, the reversal of hydrolysis has been used in the direction of ligation, adjusting the physicochemical parameters of the protease activity. However, in the growing field of peptide pharmaceuticals, in all but a few cases enzymic techniques can only be integrated as complementary procedures. This compares poorly with the potent chemical or biological recombinant techniques [204-206].

In the studies on step-by-step syntheses of peptides, two different procedures have been used : i) simply reversing the equilibrium towards synthesis (*thermodynamic control*) by using water-miscible solvents, which increase the pK value of the carboxy component, or water-immiscible solvents (biphasic systems) and then extracting the peptide; or ii) using an aminolysis of *N*-protected amino acid or peptide esters (*kinetic control*), which is limited to proteases which yield acyl-enzyme complexes (serine or cysteine proteases). One successful example of the first approach is the thermolysin-catalyzed aspartame synthesis (fig 43), where the primary product forms an insoluble salt of D-phenylalanine, shifting the equilibrium towards the quantitative formation of the product [207]. Other peptides with biological or pharmacological properties have been synthesized using α-chymotrypsin, subtilisin, papain, or carboxypeptidase Y [206]. The second approach is characterized by a shorter reaction time and provides the opportunity to suppress the competitive hydrolysis reaction (at least a 1000:1 ratio is considered as useful for preparative purposes). The most popular leaving groups for the acyl donor amino acid are ethyl or methyl esters, and in some cases (in organic solvents), even D-amino acid derivatives can be incorporated [208, 209]. In addition, chemically or genetically modified immobilized enzymes (highly active in organic solvents) have been specifically prepared and used as effective catalysts [210-212]. Recently, lipases and esterases have also been used as peptide synthesis catalysts in organic solvents [213, 214].

The kinetically controlled coupling of large peptide fragments and the semi-synthesis of proteins is particularly sensitive to aminolysis reactions, and has been limited to a few examples [215]. The most well-known is the Novo large-scale trypsin catalyzed semi-synthesis of human insulin from porcine insulin [216].

"Bio-oxygenation" reactions

A number of enzymic activities involved in the incorporation of oxygen into organic molecules have been

occasionally used for preparative purposes [217, 218]. Dioxygenases catalyze the introduction of dioxygen into an organic substrate, generating a hydroperoxy or an endoperoxy derivative. Monooxygenases introduce or activate molecular oxygen, but only one oxygen atom is introduced in the product. The reaction requires reducing equivalents, which are generally supplied by NADPH through a complex electron-transport system.

• *Dioxygenase-catalyzed reactions*

Lipoxygenase, a non-heme iron-containing dioxygenase, has been used for the enantio- and regioselective preparation of unsaturated fatty-acid hydroperoxides [219-221]. Reduction of the hydroperoxides may then lead to the corresponding asymmetric alcohols (fig 44).

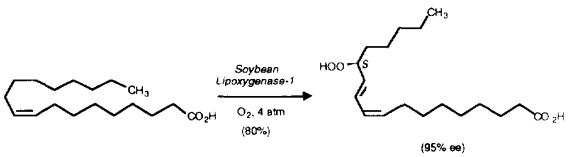


Fig 44. Regio- and enantioselective preparation of (9*Z*, 11*E*, 13*S*) - 13 - hydroperoxyoctadeca - 9,11 - dienoicacid from linoleic acid using a commercial soybean lipoxygenase [220].

Arene dioxygenase activity was first discussed by Gibson [222] and has recently been exploited using whole-cells microorganisms, for the regio- and enantioselective preparation of *cis*-dihydrodiols. The simplest, cyclohexadienediol, has been proposed as a source of polyphenylene, which is difficult to obtain by other methods [223, 224]. Substituted arenes (methyl-, chloro-, fluoro-, etc) have been shown to give the corresponding asymmetric *cis*-diols in high optical purity. These have proved to constitute invaluable precursors for the synthesis of natural and unnatural carbocyclitols [225-227] (fig 45). Similar techniques have been applied to the preparation of cis-diols deriving from polycyclic or heteroaromatic compounds [228, 229].

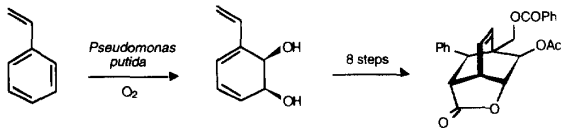


Fig 45. Asymmetric synthesis of a natural product derivative, (−)-zeylena acetate, from styrene *via* a dioxygenase-mediated reaction (from Hudlicky *et al* [503]).

• *Monooxygenase-catalyzed reactions*

The Baeyer-Villiger reaction, a well-established synthetic reaction, is typically effected by the reaction of a peracid reagent on a keto compound, producing an ester group. The enzymic counterpart of this reaction [230] uses a flavoprotein monooxygenase, in the presence of oxygen and NAD(P)H. The "cyclooxygenase" from *Acinetobacter* sp [231] can oxidize a number of cyclic and acyclic ketones to the corresponding lactones with a regio- and a stereoselectivity similar to that of the chemical reaction (retention of configuration). With substituted cyclohexanones containing enantiotopic substituents, the oxygen introduction is highly stereoselective, producing useful asymmetric lactonic compounds (fig 46). With racemic keto-substrates, the enzymic Baeyer-Villiger reaction, which is also catalyzed by whole-cell microorganisms, is often enantioselective, either causing a kinetic resolution of the substrate or, most interestingly compared with the chemical reaction, a regiodivergent oxidation on each enantiomer [232].

Most other monooxygenase-mediated reactions involve a membrane-bound cytochrome P450 enzyme, associated with a complex redox system for electron transport from NADPH, and thus whole-cell microorganisms have been used preferentially for the wide range of reactions catalyzed by these enzymes. For pharmacological and toxicological reasons, these reactions have been extensively investigated in the mammalian microsomal hepatic detoxification systems for xenobiotics. They can be easily mimicked by using simple microorganisms like actinomycetes or filamentous fungi [233]. These microorganisms possess adequate enzymic equipment and may provide an economical alternative for the large-scale production of certain metabolites, which would otherwise be difficult to synthesize chemically. This is the concept of "microbial models of mammalian metabolism", first proposed by Smith and Rosazza [234], and now recognized by a number of comparative studies [235] to be a valid proposal. This is illustrated in figure 47 for alkaloids and alkaloid-like drugs.

Beyond this concept, biohydroxylation reactions may represent a powerful method for the introduction of hydroxyl groups (as an intermediate step for any other specific functionalization) into previously elaborated molecules, with the additional benefit of the usual regio- and stereolectivity of enzymic reactions [218, 236]. Starting from easily accessible natural materials (alkaloids [237, 238], steroids [238], or terpenes [239, 240]), it is possible to obtain new complex molecules which can be tested for biological activity or used as asymmetric synthons or synthetic intermediates. Some of these preparatively useful reactions, sometimes with a high commercial significance, such as the 11α-hydroxylation of progesterone, are illustrated in figure 48. Typically, monooxygenase-catalyzed reactions (table IX) have been used to hydroxylate aromatic rings, "activated" (allylic or benzylic) methylenic carbon atoms, and most interestingly, "unactivated" methyl or methylenic groups.

The field of application of these reactions has been extended to useful transformations of several purely synthetic organic materials of chemical interest. For example, isobutyric acid is hydroxylated by a variety of microorganisms in an enantiogenic manner, producing high yields of either the (*R*)- or the (*S*)-enantiomer of β-hydroxyisobutyric acid [241-243], a versatile chiral synthon already used for the asymmetric syntheses of the side chain of α-tocopherol, carbapenem antibiotics, muscone, etc. Some other regio- and enantioselective hydroxylation reactions used for a complementary functionalization of synthetic materials are shown in figure 49.

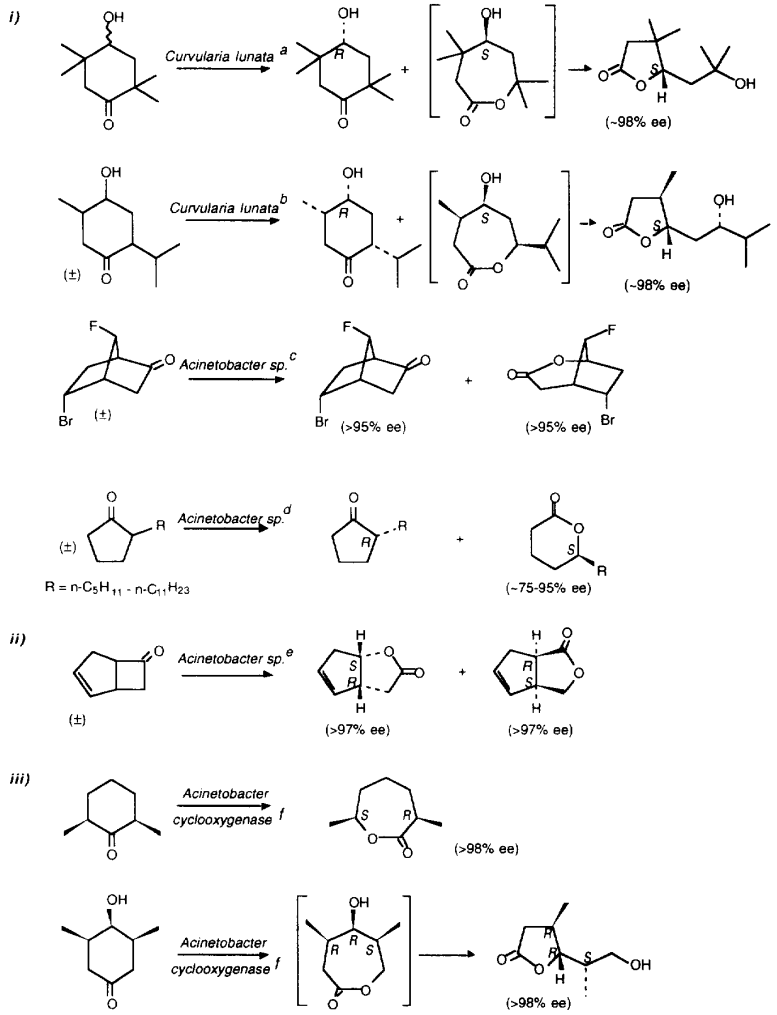


Fig 46. Enantiospecific enzyme-catalyzed Baeyer-Villiger type oxidations illustrated *i)* by the resolution of racemic 4-hydroxycyclohexanones : *a*, Ouazzani-Chahdi *et al* [504], *b*, Azerad *et al* [505] ; substituted racemic bicyclo[2.2.1]heptanones : *c*, Levitt *et al* [506] ; 2-alkyl cyclopentanones : *d*, Alphand *et al* [507, 508]. *ii)* by the enantioselective regiodivergent oxidation of a bicyclo[3.2.0]heptenone : *e*, Alphand *et al* [509, 510]. *iii)* by the enantiotopic oxidation of substituted *meso* cyclohexanones. *f*, Taschner [511, 512].

However, in each case, an empirical specific approach was employed and much remains to be done in order to make the regio- and stereochemical outcome of the hydroxylation reactions general and predictable. For a full utilization of the high potential of such reactions, it will be necessary to further refine existing models [1, 244, 245], taking into account the generally observed flexibility and lack of specificity of detoxification-dedicated P450-monoxygenases [246]. Moreover, the general use of whole bacterial cells instead of pure enzymes introduces intrinsic limitations : the number and specificity of enzymes involved, inductive or constitutive enzymes, toxicity of substrate and product(s), and permeability and sequestration problems.

Other oxidation reactions mediated by cytochrome P450 of whole-cell microorganisms have been occasionally exploited. Terminal olefin oxidation in gas/solid reactors has been developed for the economical production of chiral epoxides [247-249]. Organic sulfide oxidation by microorganisms has been used for the production of optically active sulfoxides, which are widely used in organic asymmetric syntheses. However this biotransformation rarely gives enantiomerically pure products, and the reaction is often plagued by the formation of significant amounts of sulfones [16, 218].

Peroxidases, and especially a chloroperoxidase from *Caldariomyces fumago*, are available as a commercial preparation, and have been employed for a variety of oxidation reactions, such as aromatic hydroxylation, addition of hypohalous acid to olefinic bonds, and nitrogen, phosphorus or sulfur oxidation, using hydrogen peroxide(or an organic peroxide) as an oxygen donor [218].

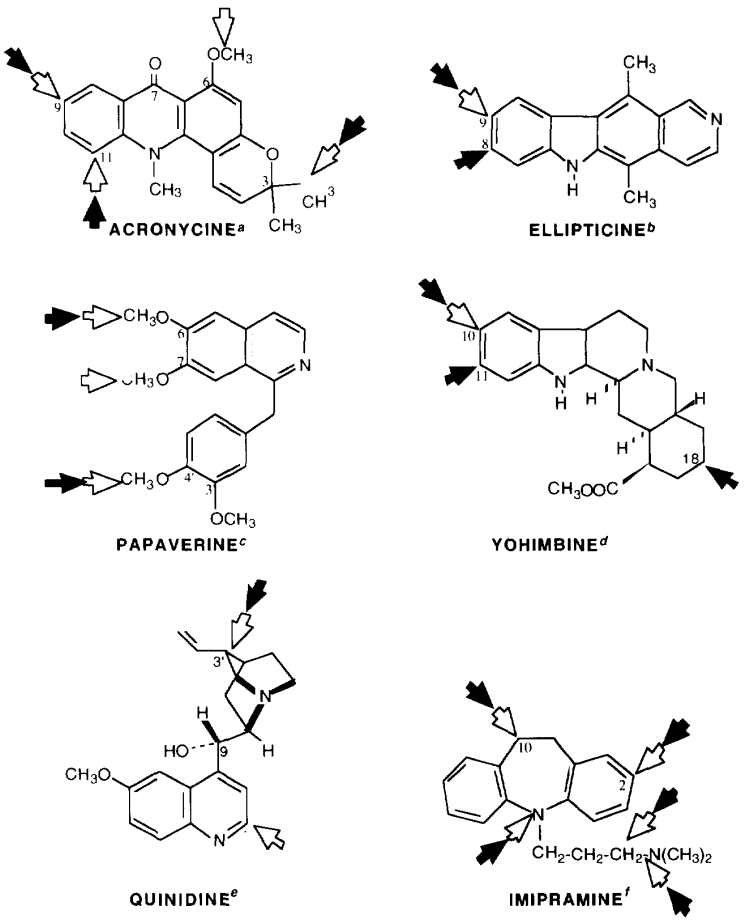


Fig 47. Comparison of the sites of major oxidation reactions (hydroxylation, O- or N-dealkylation, N-oxidation, etc) observed in the biotransformation of natural alkaloids or related drugs in mammals (⇨) or in microorganisms (➡) : *a*, Betts *et al* [513], Brannon *et al* [514], Rosazza [515]; *b*, Chien and Rosazza [516], Sariaslani and Rosazza [1]; *c*, Rosazza *et al* [517]; *d*, Patterson *et al* [518]; *e*, Eckenrode [519], Azerad [236]; *f*, Hufford *et al* [520].

Table IX. Main oxidation reactions catalyzed by cytochrome P450-monooxygenases.

1 – Aromatic hydroxylation :

Ar → arene oxide (→ *trans*-dihydrodiol → phenol)

2 – Hydroxylation of activated carbon atoms :

–CH=CH–CH_2 → –CH=CH–CHOH–

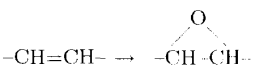
3 – Hydroxylation of non-activated carbon atoms :

R–CH_3 → R–CH_2OH (→ R–H + HCHO)
R–CH_2–R′ → R–CHOH–R′
R–CH(R′)–R″ → R–C(R′,R″)–OH

4 – O- and N-dealkylation :

R–O–CH_3 → R–O–CH_2OH → HCHO + R–OH
R–N–CH_3 → R–N–CH_2OH → HCHO + R–NH

5 – Epoxidation of Olefins :



6 – Oxidation of heteroatoms :

R–NH–R′ → R(R′)NO (N-oxide)
R–S–R′ → R(R′)SO (sulfoxide) → R(R′)SO_2 (sulfone)

Conclusion and prospects

Some of the main bioconversion reactions with enzymes or whole microbial cells have been examined. In view of the impressive amount of published work which has been displayed in the past 15 years in this field and the corresponding high expectations, it is surprising to observe that there are in practice only a few commercial applications of biocatalysts in the fine and speciality chemical industries. Major obstacles appear to be : i) the existence of a well-developed traditional chemical technology and the resulting opposition to a new technology based on biological systems; ii) the inherent disadvantages of biocatalysis techniques; and iii) regulatory constraints.

However, the high potentialities of biocatalysis in organic chemistry, and especially in the developing field of "chirotechnology", together with the expected advances in research, will probably increase in the future the number of applications of biocatalysts in organic transformations. The most promising developments will probably arise from the following.

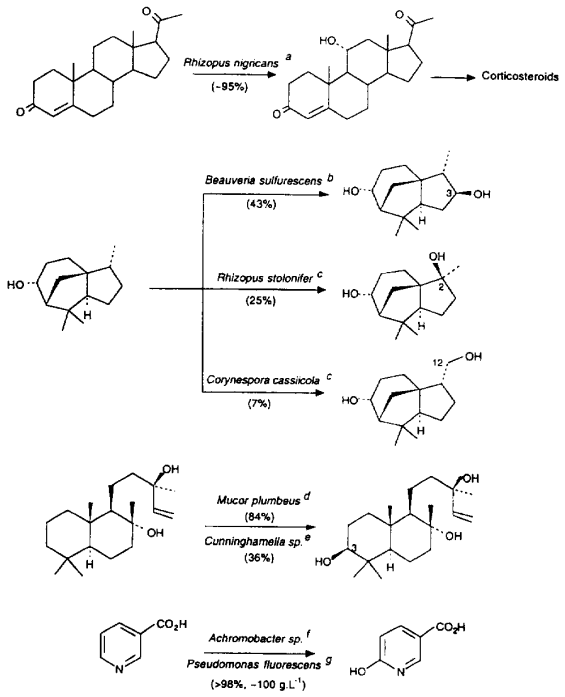


Fig 48. Some preparatively useful monooxygenase-catalyzed hydroxylation reactions, using whole-cell microorganisms : *a*, Peterson and Murray [521]; *b*, Lamare and Furstoss [240]; *c*, Abraham *et al* [522]; *d*, Aranda *et al* [524, 525]; *e*, Kouzi and McChesney, [523]; *f*, Kulla [526]; *g*, Nagasawa *et al* [527].

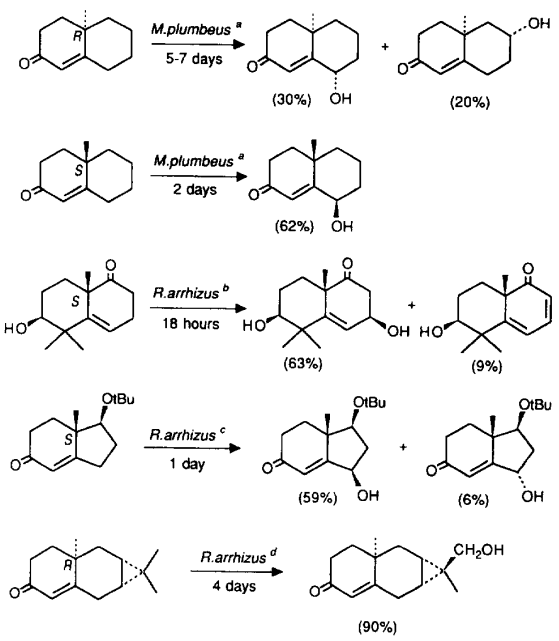


Fig 49. Some preparatively useful monooxygenase-catalyzed hydroxylation reactions on synthetic materials, using whole-cell microorganisms, for use as chiral intermediates for steroid or terpenoid syntheses : *a*, Hammoumi *et al* [528, 529], Azerad, [236]; *b*, Ouazzani *et al* [530, 531]; Arséniyadis *et al* [532]; *c*, Arséniyadis *et al* [532, 533]; *d*, Hebda *et al* [534].

1) The increased availability of purified enzymes from new sources, avoiding the whole cell utilization with its inherent problems, and allowing the organic chemist to use them as ordinary "shelf reagents". This is already the case for most lipasic enzymes.

2) The improvement of biocatalyst properties by the use of chemical modifications or modern genetic techniques (site-directed mutagenesis or biosynthetic incorporation of unnatural amino acids) in order to increase activity, enantioselectivity and resistance to solvents and high temperature.

3) The increased understanding of reaction mechanisms and reactive-site topologies, allowing, through computer-aided modelization, the prediction of enantioselective enzyme-substrate interactions and the substitution of the presently used rather primitive operational models which are based on substrate screening.

4) The discovery of new unexpected enzymic activities in microorganisms from "exotic" environments. At the present, only a fraction of the available microorganisms from nature and collections has been screened for known reactions. Much remains to be done in the direction of non-classical microbial metabolisms to find new enzymic activities. In this regard, the emergence of catalytic antibodies as practical tools, complementary to natural enzymic activities, will have to be considered and will possibly upset the forecast of biocatalytic applications.

References

1 Sariaslani FS, Rosazza JP, *Enzyme Microb Technol* (1984) 6, 242

2 Whitesides GM, Wong C-H, *Angew Chem Int Ed Engl* (1985) 24, 617

3 Jones JB, *Tetrahedron* (1986) 42, 3351

4 Yamada H, Shimizu S, *Angew Chem Int Ed Engl* (1988) 27, 622

5 Csuk R, Glänzer BI, *Chem Rev* (1991) 91, 49

6 Santaniello E, Ferraboschi P, Grisenti P, Manzocchi A, *Chem Rev* (1992) 92, 1071

7 Turner NJ, *Nat Prod Rep* (1994) 11, 1

8 Azerad R, Sih C-J Ed, *Enantioselective Synthesis using Biological Systems, Biocatalysis* special issue, (1990) Vol 3 (1-2)

9 Meijer EM Ed, *Biocatalysis in Organic Chemistry, Recl Trav Chim Pays-Bas* special issue (1991) Vol 110 (5)

10 Crout DHG, Roberts SM, Jones JB Ed, *Enzymes in Organic synthesis, Tetrahedron-Asymmetry* special issue (1993) Vol.4 (5-6)

11 Kieslich K, Leuenberger HGW, Seebach D Ed, *Proceedings of the International Bioorganic Symposium "Biotransformations in Organic Chemistry : Principles and Applications", Interlaken 1993, Chimia* special issue (1993) Vol 47 (4)

12 Crout DHG, Griengl H, Faber K, Roberts SM Ed, *Proceedings of the European Symposium on Biocatalysis, Graz 1993, Biocatalysis* special issue (1994) Vol 9 (1-4)

13 Tramper J, VanderPlas HC, Linko P, *Biocatalysis in Organic Synthesis, Studies in Organic Synthesis*, vol 22, Elsevier, Amsterdam, 1985

14 Porter R, Clark S Ed, *Enzymes in Organic Synthesis. CIBA Foundation Symposium*, 111, Pitman, London, 1985

15 Schneider MP Ed, *Enzymes as catalysts in Organic Synthesis*, NATO ASI Series C, Reidel, Dordrecht, 1986

16 Davies HG, Green RH, Kelly DR, Roberts SM, *Biotransformations in Preparative Organic Chemistry. The Use of Isolated Enzymes and Whole Cells Systems in Synthesis*, Academic, London, 1989

17 Faber K, *Biotransformations in Organic Chemistry*, Springer, Berlin, Heildelberg, 1992

18 Servi S, *Microbial Reagents in Organic Synthesis*, NATO ASI Series C, Kluwer, Dordrecht, 1992

19 Wong C-H, Whitesides GW, *Enzymes in Synthetic Organic Chemistry*, Elsevier, Oxford, 1994

20 Seebach D, *Angew Chem Int Ed Engl* (1990) 29, 1320

21 Walsh C, *Enzymatic Reaction Mechanisms*, Freeman, San-Francisco, 1979

22 Kakulapati RR, Nanduri B, Yadavalli VDN, Trichinapally NS, *Chem Lett* (1992) 2059

23 Ogston AG, *Nature* (1948) 162, 963

24 Jones JB, *In : Applications of Biochemical Systems in Organic Chemistry*, Jones JB, Sih CJ, Perlman D Eds, Wiley, New York, 1976, Vol 1, p 1

25 Margolin AL, *Enzyme Microb Technol* (1993) 15, 266

26 International Union of Biochemistry, *Enzyme Nomenclature*, Academic, New York, 1979

27 Chen C-S, Fujimoto Y, Girdaukas G, Sih CJ, *J Am Chem Soc* (1982) 104, 7294

28 Sih CJ, Wu SH, *Top Stereochem* (1989) 19, 63

29 Balavoine G, Moradpour A, Kagan HB, *J Am Chem Soc* (1974) 96, 5152

30 Martin VS, Woodard SS, Katsuki T, Yamada Y, Ikeda M, Sharpless KB, *J Am Chem Soc* (1981) 103, 6237

31 Oberhauser T, Bodenteich M, Faber K, Penn G, Griengl H, *Tetrahedron* (1987) 43, 3931

32 Guo Z-W, *J Org Chem* (1993) 58, 5748

33 Chen C-H, Wu SH, Girdaukas G, Sih CJ, *J Am Chem Soc* (1987) 109, 2812

34 Boland W, Frössl C, Lorenz M, *Synthesis* (1991) 1049

35 Xie Z-F, *Tetrahedron-Asymmetry* (1991) 2, 733

36 Vorderwülbecke T, Kieslich K, Erdmann H, *Enzyme Microb Technol* (1992) 14, 631

37 Kazlauskas RJ, Weissfloch ANE, Rappaport A, Cuccia LA, *J Org Chem* (1991) 56, 2656

38 Bergbreiter DE, Momongan M, *Appl Biochem Biotechnol* (1992) 32, 55

39 Klibanov AM, *Acc Chem Res* (1990) 23, 120

40 Borzeix F, Monot F, Vandecasteele JP, *Enzyme Microb Technol* (1992) 14, 791

41 Chen C-H, Sih CJ, *Angew Chem Int Ed Engl* (1989) 28, 695

42 Santaniello E, Ferraboschi P, Grisenti P, *Enzyme Microb Technol* (1993) 15, 367

43 Laane C, Boeren S, Vos K, Veeger C, *Biotechnol Bioeng* (1987) 30, 81

44 Valivety RH, Jonhston GA, Suckling CJ, Halling P, *Biotechnol Bioeng* (1991) 38, 1137

45 Degueil-Castaing M, De Jeso B, Drouillard S, Maillard B, *Tetrahedron Lett* (1987) 28, 953

46 Wang Y-F, Lalonde JJ, Momongan M, Bergbreiter DE, Wong C-H, *J Am Chem Soc* (1988) 110, 7200

47 Faber K, Riva S, *Synthesis* (1992) 895

48 Berger B, Faber K, *J Chem Soc Chem Commun* (1991) 1198

49 Gutman AL, Brenner D, Boltanski A, *Tetrahedron-Asymmetry* (1993) 4, 839

50 Wu S-H, Chu F-Y, Wang K-T, *Bioorg Med Chem Lett* (1991) 1, 339

51 Ueji S, Fujino R, Okubo N, Miyazawa T, Kurita S, Kitadani M, Muromatsu A, *Biotechnol Lett* (1992) 14, 163

52 Fitzpatrick PA, Ringe D, Klibanov AM, *Biotechnol Bioeng* (1992) 40, 735

53 Secundo F, Riva S, Carrea G, *Tetrahedron-Asymmetry* (1992) 3, 267

54 Bornscheuer U, Herar A, Kreye L, Wendel V, Capewell A, Meyer HH, Scheper T, Kolisis FN, *Tetrahedron-Asymmetry* (1993) 4, 1007

55 Whitesell JK, Lawrence RM, *Chimia* (1986) 40, 318

56 Therisod M, Klibanov AM, *J Am Chem Soc* (1986) 108, 5638

57 Riva S, Chopineau J, Kieboom APG, Klibanov AM, *J Am Chem Soc* (1988) 584

58 Riva S, Bovara R, Ottolina G, Secundo F, Carrea G, *J Org Chem* (1989) 54, 3161

59 Ottolina G, Carrea G, Riva S, *Biocatalysis* (1991) 5, 131

60 Waldmann H, Heuser A, Reidel A, *Synlett* (1994) 65

61 Delinck DL, Margolin AL, *Tetrahedron Lett* (1990) 31, 3093

62 Toone EJ, Jones JB, *Tetrahedron-Asymmetry* (1993) 2, 1041

63 Sheldon RA, *Chirotechnology. Industrial Synthesis of Optically Active Compounds*, Dekker, New York, 1993

64 Dernoncour R, Azerad R, *Tetrahedron Lett* (1987) 28, 4661

65 Guibé-Jampel E, Rousseau G, *Tetrahedron Lett* (1987) 28, 3563

66 Makita A, Nihira T, Yamada Y, *Tetrahedron Lett* (1987) 28, 805

67 Guo Z-W, Sih CJ, *J Am Chem Soc* (1989) 111, 6836

68 Fouque E, Rousseau G, *Synthesis* (1989) 661

69 Gutman AL, Oren D, Boltanski A, Bravdo T, *Tetrahedron Lett* (1987) 28, 5367

70 Gutman AL, Bravdo T, *J Org Chem* (1989) 54, 5645

71 O'Hagan D, Zaidi NA, *J Chem Soc Perkin Trans 1* (1993) 2389

72 Linko Y-Y, Wang Z, Seppälä J, *Biocatalysis* (1994) 8, 269

73 Gotor V, Covadonga A, Rebolledo F, *Synlett* (1990) 387

74 Gotor V, Brieva R, Gonzàlez C, Rebolledo F, *Tetrahedron* (1991) 47, 9207

75 MacFarlane ELA, Rebolledo F, Roberts SM, Turner NJ, *Biocatalysis* (1991) 5, 13

76 Sih CJ, Gu Q-M, Holdgrün X, Harris K, *Chirality* (1992) 4, 91

77 Gupta AK, Kazlauskas RJ, *Tetrahedron-Asymmetry* (1993) 4, 879

78 Holmberg E, Hult K, *Biotechnol Lett* (1991) 13, 323

79 Lin G, Liu S-H, *Org Prep Proc Int* (1993) 25, 463

80 Vulfson EN, Sarney DB, Law BA, *Enzyme Microb Technol* (1991) 13, 123

81 Wang Y-F, Chen C-H, Girdaukas G, Sih CJ, *J Am Chem Soc* (1984) 106, 3695

82 Caron G, Kazlauskas RJ, *J Org Chem* (1991) 56, 7251

83 Brown SM, Davies SG, DeSousa JAA, *Tetrahedron-Asymmetry* (1993) 4, 813

84 Gu Q-M, Sih CJ, *Biocatalysis* (1992) 6, 115

85 Forney LJ, Wong DCL, Ferber DM, *Appl Environm Microbiol* (1989) 55, 2550

86 Wong C-H, Chen S-T, Hennen WJ, Bibbs JA, Wang Y-F, Liu JL-C, Pantoliano MW, Whitlow M, Bryan PN, *J Am Chem Soc* (1990) 112, 945

87 Parida S, Dorfick JS, *J Org Chem* (1993) 58, 3238

88 Högberg HE, Edlund H, Berglund P, Hedenström E, *Tetrahedron-Asymmetry* (1993) 4, 2123

89 Chibata I, *Immobilized Enzymes - Research and Development*, Kodansha, Tokyo, 1978

90 Bommarius A, Kottenhahn M, Klenk H, Drauz K, *In : Microbial reagents in Organic Synthesis*, Servi S Ed, Kluwer, Dordrecht, 1992, p 161

91 Fukumura T, *Agr Biol Chem* (1976) 40, 1687

92 Fukumura T, *Agr Biol Chem* (1977) 41, 1327

93 Arnaud A, Galzy P, Jallageas J-C, *Bull Soc Chim Fr II* (1980) 87

94 Jallageas JC, Arnaud A, Galzy P, *Adv Biochem Eng* (1980) 14, 1

95 Nagasawa T, Yamada H, *TIBTech* (1989) 7, 153

96 Berti G, *In : Enzymes as Catalysts in Organic Synthesis*, Schneider MP Ed, Reidel, Dordrecht, 1986, p 349

97 Bellucci G, Chiape C, Marioni F, *J Chem Soc Perkin Trans I* (1989) 2369

98 Wistuba D, Schurig V, *Chirality* (1992) 4, 178

99 Wistuba D, Träger O, Schurig V, *Chirality* (1992) 4, 185

100 Chen X-J, Archelas A, Furstoss R, *J Org Chem* (1993) 58, 5528

101 Pedragosa-Moreau S, Archelas A, Furstoss R, *J Org Chem* (1993) 58, 5533

102 Hechtberger P, Wirnsberger G, Mischitz M, Klempier N, Faber K, *Tetrahedron-Asymmetry* (1993) 4, 1161

103 Ohno M, Otsuka M, *In : Organic Reactions*, 1989, Vol 37, p 1

104 Zhu L-M, Tedford MC, *Tetrahedron* (1990) 46, 6587

105 Ader U, Breitgoff D, Laumen KE, Schneider MP, *Tetrahedron Lett* (1989) 30, 1793

106 Francis CJ, Jones JB, *J Chem Soc Chem Commun* (1984) 579

107 Kotani H, Kuze Y, Uchida S, Miyabe T, Iimori T, Okano K, Kobayashi S, Ohno M, *Agr Biol Chem* (1983) 47, 1363

108 Chen C-S, Fujimoto Y, Sih CJ, *J Am Chem Soc* (1981) 103, 3580

109 Shimada M, Kobayashi S, Ohno M, *Tetrahedron Lett* (1988) 29, 6961

110 Yamamoto Y, Yamamoto K, Nishioka T, Oda J, *Agr Biol Chem* (1988) 52, 3087

111 Bianchi D, Cesti P, Battistel E, *J Org Chem* (1988) 53, 5531

112 Ozegowski R, Kunath A, Schick H, *Liebigs Ann Chem* (1993) 805

113 Wang Y-F, Chen C-H, Girdaukas G, Sih CJ, *In : Enzymes in Organic Synthesis, CIBA Foundation Symposium 111*, Porter R, Clark S Ed, Pitman, London, 1985, p 128

114 Guo Z-W, Wu S-H, Chen C-H, Girdaukas G, Sih CJ, *J Am Chem Soc* (1990) 112, 4942

115 Ramos-Tombo GM, Shar H-P, Fernandez I, Busquets X, Ghisalba O, *Tetrahedron Lett* (1986) 27, 5707

116 Duhamel P, Renouf P, Cahard D, Yebga A, Poirier J-M, *Tetrahedron-Asymmetry* (1993) 4, 2447

117 Ng GSY, Yuan L-C, Jakovac IJ, Jones JB, *Tetrahedron* (1984) 40, 1235

118 Wichmann R, Wandrey C, Bückmann AF, Kula M-R, *Biotechnol Bioeng* (1981) 23, 2789

119 Prelog V, *Pure Appl Chem* (1964) 9, 119

120 Kataoka M, Nomura Y, Shimizu S, Yamada H, *Biosci Biotechnol Biochem* (1992) 56, 820

121 Kometani T, Kitatsuji E, Matsuno R, *J Ferm Bioeng* (1991) 71, 197

122 Kometani T, Kitatsuji E, Matsuno R, *Agr Biol Chem* (1991) 55, 867

123 Sih CJ, Chen C-S, *Angew Chem Int Ed Engl* (1984) 23, 570

124 Simon H, Bader J, Günther S, Neumann S, Thanos J, *Angew Chem Int Ed Engl* (1985) 24, 539

125 Servi S, *Synthesis* (1990) 1

126 Azerad R, Buisson D, *In : Microbial Reagents in Organic Synthesis*, Servi S Ed, Kluwer, Dordrecht, 1992, p 421

127 Chen C-S, Zhou BN, Shieh WR, VanMiddlesworth F, Gopalan AS, Girdaukas G, Sih CJ, *Biorg Chem* (1984) 12, 98

128 Shieh W-R, Gopalan AS, Sih CJ, *J Am Chem Soc* (1985) 107, 2993

129 Lee LG, Whitesides GM, *J Org Chem* (1986) 51, 25

130 Nakamura K, Yoneda T, Miyai T, Ushio K, Oka S, Ohno A, *Tetrahedron Lett* (1988) 29, 2453

131 Furuichi A, Akita H, Matsukara H, Oishi T, Horikoshi K, *Agr Biol Chem* (1985) 49, 2563

132 Heidlas J, Engel K-H, Tressl R, *Eur J Biochem* (1988) 172, 633

133 Heidlas J, Tressl R, *Eur J Biochem* (1990) 188, 165

134 Ward OP, Young CS, *Enzyme Microbial Technol* (1990) 12, 482

135 Nakamura K, Kawai Y, Nakajima N, Ohno A, *J Org Chem* (1991) 56, 4778

136 Young CS, Ward OP, *Biotechnol Bioeng* (1992) 39, 457

137 Peters J, Zelinski T, Kula M-R, *Appl Microbiol Biotechnol* (1992) 38, 334

138 Shieh W-R, Sih CJ, *Tetrahedron-Asymmetry* (1993) 4, 1259

139 Ushio K, Inouye K, Nakamura K, Oka S, Ohno A, *Tetrahedron Lett* (1986) 27, 2657

140 Nakamura K, Higaki M, Ushio K, Oka S, Ohno A, *Tetrahedron Lett* (1985) 26, 4213

141 Nakamura K, Kawai Y, Oka S, Ohno A, *Tetrahedron Lett* (1989) 30, 2245

142 Buisson D, Sanner C, Larchevêque M, Azerad R, *Biocatalysis* (1992) 249

143 Nakamura K, Kawai Y, Ohno A, *Tetrahedron Lett* (1991) 32, 2927

144 Nakamura K, Inoue K, Ushio K, Oka S, Ohno A, *Chem Lett* (1987) 679

145 Nakamura K, Kawai Y, Ohno A, *Tetrahedron Lett* (1990) 31, 267

146 Zhou B-N, Gopalan AS, Van Middlesworth F, Shieh W-R, Sih CJ, *J Am Chem Soc* (1983) 105, 5925

147 Shimizu S, Hattori S, Hata H, Yamada H, *Appl Environ Microbiol* (1987) 53, 519

148 Deol BS, Ridley DD, Simpson GW, *Aust J Chem* (1976) 29, 2459

149 Frater G, *Helv Chim Acta* (1979) 62, 2829

150 Van Middlesworth F, Sih CJ, *Biocatalysis* (1987) 1, 117

151 Genêt JP, Pinel C, Mallart S, Juge S, Thorimbert S, Laffitte JA, *Tetrahedron-Asymmetry* (1991) 2, 555

152 Genêt JP, Pfister X, Ratovelomanana-Vidal V, Pinel C, Laffitte JA, *Tetrahedron Lett* (1994) 35, 4559

153 Kitamura M, Tokunaga M, Noyori R, *J Am Chem Soc* (1993) 115, 144

154 Mori K, Mori H, Yanai M, *Tetrahedron* (1986) 42, 291

155 Mori K, *Bull Soc Chim Belg* (1992) 101, 393

156 Nakamura K, Kondo S-I, Kawai Y, Ohno A, *Tetrahedron Lett* (1991) 32, 7075

157 Nakamura K, Takano S, Ohno A, *Tetrahedron Lett* (1993) 34, 6087

158 Naoshima Y, Maeda J, Munakata Y, *J Chem Soc Perkin Trans 1* (1992) 659

159 Jayasinghe LY, Smallridge AJ, Trewhella MA, *Tetrahedron Lett* (1993) 34, 3949

160 Hummel W, Kula M-R, *Eur J Biochem* (1989) 184, 1

161 Becker W, Pfeil E, *J Am Chem Soc* (1966) 88, 4299

162 Becker W, Freund H, Pfeil E, *Angew Chem* (1965) 77, 1139

163 Effenberger F, Ziegler T, Förster S, *Angew Chem Int Ed Engl* (1987) 26, 458

164 Brussee J, Roos EC, Van der Gen A, *Tetrahedron Lett* (1988) 29, 4485

165 Brussee J, Loos WT, Kruse CG, Van der Gen A, *Tetrahedron* (1990) 46, 979

166 Effenberger F, Hörsch B, Förster S, Ziegler T, *Tetrahedron Lett* (1990) 31, 1249

167 Niedermeyer U, Kula M-R, *Angew Chem Int Ed Engl* (1990) 29, 386

168 Ognyanov VI, Datcheva VK, Kyler KS, *J Am Chem Soc* (1991) 113, 6992

169 Effenberger F, *In : Microbial Reagents in Organic Synthesis, NATO ASI Series C, vol 381*, Servi S Ed, Kluwer, Dordrecht, 1992, p 25

170 North M, *Synlett* (1993) 807

171 Bringer-Meyer S, Sahm H, *Biocatalysis* (1988) 1, 321

172 Schmauder H-P, Gröger D, *Pharmazie* (1968) 32, 320

173 Kren W, Crout DHG, Dalton H, Hutchinson DW, König W, Turner MM, Dean G, Thomson N, *J Chem Soc Chem Commun* (1993) 341

174 Budesinsky Z, Protiva M, *In : Synthetische Arzneimittel*, Akademie Verlag, Berlin, 1961, p 24

175 Fuganti C, Grasselli P, *In : Enzymes in Organic Synthesis, CIBA Foundation Symposium 111*, Porter R, Clark S Eds, Pitman, London, 1985, p 112

176 Stumpf B, Kieslich K, *Appl Microbiol Biotechnol* (1991) 34, 598

177 Toone EJ, Simon ES, Bednarski MD, Whitesides GM, *Tetrahedron* (1989) 45, 5365

178 Drueckhammer DG, Hennen WJ, Pederson RL, Barbas CFI, Gautheron CM, Krach T, Wong C-H, *Synthesis* (1991) 499

179 Wong C-H, Whitesides GW, *J Org Chem* (1983) 48, 3199

180 Bednarski MD, Simon ES, Bischofberger N, Fessner W-D, Kim M-J, Lees W, Saito T, Waldmann H, Whitesides GM, *J Am Chem Soc* (1989) 111, 627

181 Durrwachter JR, Wong C-H, *J Org Chem* (1988) 53, 4175

182 Drueckhammer DG, Durrwachter JR, Pederson RL, Crans DC, Daniels L, Wong C-H, *J Am Chem Soc* (1989) 54, 70

183 Kragl U, Godde A, Wandrey C, Lubin N, Auge C, *J Chem Soc Perkin Trans 1* (1994) 119

184 Bolte J, Demuynck C, Samaki H, *Tetrahedron Lett* (1987) 28, 5525

185 Dalmas V, Demuynck C, *Tetrahedron-Asymmetry* (1993) 4, 2383

186 Sato T, Nishida Y, Tosa T, Chibata I, *Biochim Biophys Acta* (1979) 570, 179

187 Chibata I, Tosa T, Takata I, *Trends in Biotechnol* (1983) 1, 9

188 Findeis MA, Whitesides GM, *J Org Chem* (1987) 52, 2838

189 Takamatsu S, Umemura I, Yamamoto K, Sato T, Tosa T, Chibata I, *Eur J Appl Microbiol Biotechnol* (1982) 15, 147

190 Yamada S, Nabe K, Izuo N, Nakamichi I, Chibata I, *Appl Environ Microbiol* (1981) 42, 773

191 Echalier F, Constant O, Bolte J, *J Org Chem* (1993) 58, 2747

192 Decottignies-LeMaréchal P, Calderon-Seguin R, Vandecasteele JP, Azerad R, *Eur J Appl Microbiol* (1979) 7, 33

193 Para G, Baratti J, *Appl Microbiol Biotechnol* (1988) 28, 222

194 David S, Augé C, *Pure Appl Chem* (1987) 59, 1501

195 Wong C-H, *Chimia* (1993) 47, 63

196 Augé C, Gautheron C, Pora H, *Carbohyd Res* (1989) 193, 288

197 Augé C, Fernandez Fernandez R, Gautheron C, *Carbohyd Res* (1990) 200, 257

198 Ichikawa Y, Liu JL-C, Shen G-J, Wong C-H, *J Am Chem Soc* (1991) 113, 6300

199 Ajisaka K, Nishida H, Fujimoto H, *Biotechnol Lett* (1987) 9, 243

200 Ajisaka K, Nishida H, Fujimoto H, *Biotechnol Lett* (1987) 9, 387

201 Nilsson KGI, *TIBTECH* (1988) 6, 256

202 Crout DHG, MacManus DA, Critchley P, *J Chem Soc Perkin Trans 1* (1990) 1865

203 Taubken N, Thiem J, *Synthesis* (1992) 517

204 Jakubke H-D, Kuhl P, Könnecke A, *Angew Chem Int Ed Engl* (1985) 24, 85

205 Jakubke H-D, *In : Microbial reagents in Organic Synthesis*, Servi S Ed, Kluwer, Dordrecht, 1992, p 421

206 Sinisterra JV, Alcantara AR, *J Mol Catal* (1993) 84, 327

207 Oyama K, *In : Biocatalysis in Organic Media, Proceedings of an International Symposium*, Wageningen, The Netherlands, Dec. 1986, Laane C, Tramper J, Lilly MD Eds, Elsevier, Amsterdam, 1986, p 209

208 West JB, Wong C-H, *J Org Chem* (1986) 51, 2728

209 Margolin AL, Tai D-F, Klibanov AM, *J Am Chem Soc* (1987) 109, 7885

210 Babonneau M-T, Jacquier R, Lazaro R, Viallefont P, *Tetrahedron Lett* (1989) 30, 2787

211 Bonneau PR, Graycar TP, Estell DA, Jones JB, *J Am Chem Soc* (1991) 113, 1026

212 Fang J-M, Wong C-H, *Synlett* (1994) 393

213 Margolin AL, Klibanov AM, *J Am Chem Soc* (1987) 109, 3802

214 West JB, Wong C-H, *Tetrahedron Lett* (1987) 28, 1629

215 Schellenberger V, Jakubke H-D, *Angew Chem Int Ed Engl* (1991) 30, 1437

216 Markussen J, Vølund A, *In : Enzymes in Organic Synthesis, CIBA Foundation Symposium 111*, Porter R, Clark S Eds, Pitman, London, 1985, p 188

217 Fonken GS, Johnson RA, *Chemical Oxidations with Microorganisms*, Dekker, New York, 1972

218 Holland HL, *Organic Synthesis with Oxidative Enzymes*, VCH, New York, 1992

219 Zhang P, Kyler KS, *J Am Chem Soc* (1989) 111, 9241

220 Iacazio G, Langrand G, Baratti J, Buono G, Triantaphylides C, *J Org Chem* (1990) 55, 1690

221 Yoneda K, Sasakura K, Tahara S, Iwasa J, Baba N, Kaneko T, Matsuo M, *Angew Chem Int Ed Engl* (1992) 31, 1336

222 Gibson DT, Koch JR, Kallio RE, *Biochemistry* (1968) 7, 2653

223 Ballard DGH, Courtis A, Shirley IM, Taylor SC, *J Chem Soc Chem Commun* (1983) 954

224 Van den Tweel WJJ, Marsman EH, Vorage MJAW, Tramper J, De Bont JAM, *In : Bioreactors and Biotransformations*, Moody GW, Baker PB Eds, Elsevier Applied Science, Barking, UK, 1987, p 231

225 Widdowson DA, Ribbons DW, Thomas SD, *Janssen Chim Acta* (1990) 8, 3

226 Hudlicky T, Price JD, Luna H, Andersen CM, *Synlett* (1990) 309

227 Ley SV, Redgrave AJ, *Synlett* (1990) 393

228 De Luca ME, Hudlicky T, *Tetrahedron Lett* (1990) 31, 13

229 Boyd DR, Sharma ND, Boyle R, McMurray BT, Evans TA, Malone JF, Dalton H, Chima J, Sheldrake GN, *J Chem Soc Chem Commun* (1993) 49

230 Roberts SM, Willetts AJ, *Chirality* (1993) 5, 334

231 Walsh CT, Chen Y-CJ, *Angew Chem Int Ed Engl* (1988) 27, 333

232 Furstoss R, *In : Microbial Reagents in Organic Synthesis*, Servi S Ed, Kluwer, Dordrecht, 1992, p 333

233 Sebek OK, *Mycologia* (1983) 75, 383

234 Smith RV, Rosazza JP, *J Nat Prod* (1983) 46, 79

235 Griffiths DA, Best DJ, Jezequel SG, *Appl Microbiol Biotechnol* (1991) 35, 373

236 Azerad R, *Chimia* (1993) 47, 93

237 Holland HL, *In : The Alkaloids*, Manske RHF, Rodrigo RGA Ed, Academic, New York, 1981, Vol XVIII, p 323

238 Iizuka H, Naito A, *Microbial Conversion of Steroids and Alkaloids*, Springer, Berlin, 1981

239 Krasnobajew V, *In : Biotechnology*, K Kieslich Ed, Verlag Chemie, Weinheim, 1984, Vol 6a (*Biotransformations*), p 31

240 Lamare V, Furstoss R, *Tetrahedron* (1990) 46, 4109

241 Goodhue CT, Schaeffer JR, *Biotechnol Bioeng* (1971) 13, 203

242 Hasegawa J, Hamaguchi S, Ogura M, Watanabe K, *J Ferment Technol* (1981) 59, 257

243 Hasegawa J, Ogura M, Kanema H, Kawaharada H, Watanabe K, *J Ferment Technol* (1983) 61, 37

244 Johnson RA, Herr ME, Murray HC, Fonken GS, *J Org Chem* (1968) 33, 3217

245 Furstoss R, *In : Enzymes as Catalysts in Organic Synthesis*, Schneider MP Ed, Reidel, Dordrecht, 1986, p 361

246 Sariaslani FS, *Adv Appl Microbiol* (1991) 36, 133

247 Hou CT, Patel RN, Laskin AI, Barnabe N, *J Appl Biochem* (1982) 4, 379

248 Habets-Crützen AQH, Carlier SJN, De Bont JAM, Wistuba D, Schurig V, Hartmans S, Tramper J, *Enzyme Microbial Technol* (1985) 7, 17

249 Mahmoudian M, Michael A, *Appl Microbiol Biotechnol* (1992) 37, 23

250 Langrand G, Secchi M, Buono G, Baratti J, Triantaphylides C, *Tetrahedron Lett* (1985) 26, 1857

251 Langrand G, Baratti J, Buono G, Triantaphylides C, *Tetrahedron Lett* (1986) 27, 29

252 Patel RN, Howell JM, Fortney KF, Szarka LJ, *Appl Microbiol Biotechnol* (1991) 36, 29

253 Lokotsch W, Fritsche K, Syldatk C, *Appl Microbiol Biotechnol* (1989) 31, 467

254 Cambou B, Klibanov AM, *J Am Chem Soc* (1984) 106, 2687

255 Lin G, Liu S-H, Wu F-C, Jen W-J, *Synth Commun* (1993) 23, 2135

256 Kirchner G, Scollar MP, Klibanov AM, *J Am Chem Soc* (1985) 107, 7072

257 Belan A, Bolte J, Fauve A, Gourcy JG, Veshambre H, *J Org Chem* (1987) 52, 256

258 Stokes TM, Oehlschlager AC, *Tetrahedron Lett* (1987) 28, 2091

259 Morgan B, Oehschlager AC, Stokes TM, *J Org Chem* (1992) 57, 3231

260 Ghogare A, Kumar GS, *J Chem Soc Chem Commun* (1989) 1533

261 Ghogare A, Kumar GS, *J Chem Soc Chem Commun* (1990) 134

262 Mischitz M, Pöschl U, Faber K, *Biotechnol Lett* (1991) 13, 653

263 Örhrner N, Martinelle M, Mattson A, Norin T, Hult K, *Biocatalysis* (1994) 9, 105

264 Weissfloch A, Azerad R, *Bioorg Med Chem* (1994) 2, 493

265 Terao Y, Tsuji K, Murata M, Achiwa K, Nishio T, Watanabe N, Seto K, *Chem Pharm Bull* (1989) 37, 1653

266 Whitesell JK, Chen H-H, Lawrence RM, *J Org Chem* (1985) 50, 4663

267 Comins DL, Salvador JM, *Tetrahedron Lett* (1993) 34, 801

268 Mezrab B, Dumas F, D'Angelo J, Riche C, *J Org Chem* (1994) 59, 500

269 Esser P, Buschmann H, Meyer-Stork M, Sharf HD, *Angew Chem Int Ed Engl* (1992) 31, 1190

270 Laumen K, Breitgoff D, Seemayer R, Schneider MP, *J Chem Soc Chem Commun* (1989) 148

271 Laumen K, Seemayer R, Schneider MP, *J Chem Soc Chem Commun* (1990) 49

272 Comins DL, Salvador JM, *J Org Chem* (1993) 58, 4656

273 Gu Q-M, Chen C-S, Sih CJ, *Tetrahedron Lett* (1986) 27, 1763

274 Ahmar M, Girard C, Bloch R, *Tetrahedron Lett* (1989) 30, 7053

275 Mutsaers JHGM, Kooreman HJ, *Recl Trav Chim Pays-Bas* (1991) 110, 185

276 Mustranta A, *Appl Microbiol Biotechnol* (1992) 38, 61

277 Battistel E, Bianchi D, Cesti P, Pina C, *Biotechnol Bioeng* (1991) 38, 659

278 Mohr P, Waespe-Sarcevic N, Tamm C, Gawronska K, Gawronski JK, *Helv Chim Acta* (1983) 66, 2501

279 Luyten M, Müller S, Herzog B, Keese R, *Helv Chim Acta* (1987) 70, 1250

280 Schneider M, Engel N, Boensman H, *Angew Chem Int Ed Engl* (1984) 23, 66

281 Björkling F, Boutelje J, Gatenbeck S, Hult K, Norin T, Szmulik P, *Tetrahedron* (1985) 41, 1347

282 Björkling F, Boutelje J, Gatenbeck S, Hult K, Norin T, *Tetrahedron Lett* (1985) 26, 4957

283 Kitazume T, Sato T, Ishikawa N, *Chem Lett* (1984) 1811

284 Lam LKP, Hui RAHF, Jones JB, *J Org Chem* (1986) 51, 2047

285 Brooks DW, Palmer JT, *Tetrahedron Lett* (1983) 24, 3059

286 Mohr P, Rösslein L, Tamm C, *Helv Chim Acta* (1987) 70, 142

287 Gopalan AS, Sih CJ, *Tetrahedron Lett* (1984) 25, 5235

288 Roy R, Rey AW, *Tetrahedron Lett* (1987) 28, 4935

289 Huang FC, Lee LFH, Mittal RSD, Caspi E, Eck CR, *J Am Chem Soc* (1975) 97, 4144

290 Ohno M, *In : Enzymes in Organic Synthesis, CIBA Foundation Symposium 111*, Porter R, Clark S Eds, Pitman, London, 1985, p 171

291 Schneider M, Engel N, Hönicke P, Heinemann G, Gorisch H, *Angew Chem Int Ed Engl* (1984) 23, 67

292 Sabbioni G, Shea ML, Jones JB, *J Chem Soc Chem Commun* (1984) 236

293 Harvey I, Crout DHG, *Tetrahedron-Asymmetry* (1993) 4, 807

294 Gais HJ, Lukas KL, *Angew Chem Int Ed Engl* (1984) 23, 142

295 Kobayashi S, Kamiyama K, Iimori T, Ohno M, *Tetrahedron Lett* (1984) 25, 2557

296 Sabbioni G, Jones JB, *J Org Chem* (1987) 52, 4565

297 Häbich D, Hartwig W, *Tetrahedron Lett* (1987) 28, 781

298 Crout DHG, Gaudet VSB, Hallinan KO, *J Chem Soc Perkin Trans 1* (1993) 805

299 Renold P, Tamm C, *Tetrahedron-Asymmetry* (1993) 4, 2295

300 Gais HJ, Bulow G, Zatorski A, Jentsch M, Maidonis P, Hemmerle H, *J Org Chem* (1989) 54, 5115

301 Jones JB, Hinks JB, Hultin PG, *Can J Chem* (1985) 63, 452

302 Chênevert R, Martin R, *Tetrahedron-Asymmetry* (1992) 3, 199

303 Kurihara M, Kamiyama K, Kobayashi S, Ohno M, *Tetrahedron Lett* (1985) 26, 5831

304 Boutelje J, Hjalmarsson M, Hult K, Lindbäck M, Norin T, *Bioorg Chem* (1988) 16, 364

305 Bloch R, Guibe-Jampel E, Girard C, *Tetrahedron Lett* (1985) 26, 4087

306 Ohno M, Ito Y, Arita M, Shibata T, Adachi K, Sawai H, *Tetrahedron* (1984) 40, 145

307 Wong C-H, Drueckhammer DG, Sweers HM, *J Am Chem Soc* (1985) 107, 4028

308 Wong C-H, Whitesides GM, *J Am Chem Soc* (1981) 103, 4890

309 Wong C-H, Whitesides GM, *J Org Chem* (1982) 47, 2816

310 Shaked Z, Whitesides GM, *J Am Chem Soc* (1980) 102, 7104

311 Wandrey C, Bückmann AF, Kula M-R, *Biotechnol Bioeng* (1981) 23, 1789

312 Jones JB, Beck JF, *In : Applications of Biochemical Systems in Organic Chemistry*, Jones JB, Sih CJ, Perlman D Eds, Wiley, New York, 1976, Vol 1, p 236

313 Bradshaw CW, Fu H, Shen G-J, Wong C-H, *J Org Chem* (1992) 57, 1526

314 Peters J, Zelinski T, Minuth T, Kula M-R, *Tetrahedron-Asymmetry* (1993) 4, 1683

315 Dutler H, Coon MJ, Kull A, Vogel H, Waldvogel G, Prelog V, *Eur J Biochem* (1971) 22, 203

316 Dutler H, Kull A, Mislin R, *Eur J Biochem* (1971) 22, 213

317 Hirose Y, Okutsu M, Anzai M, Naemura K, Chikamatsu H, *J Chem Soc Perkin Trans 1* (1992) 317

318 Keinan E, Hafeli EK, Seth KK, Lamed R, *J Am Chem Soc* (1986) 108, 162

319 Keinan E, Seth KK, Lamed R, Ghirlando R, Singh SP, *Biocatalysis* (1990) 3, 57

320 Bradshaw CW, Hummel W, Wong C-H, *J Org Chem* (1992) 57, 1532

321 Hirschbein BL, Whitesides GM, *J Am Chem Soc* (1982) 104, 4458

322 Kim M-J, Kim JY, *J Chem Soc Chem Commun* (1991) 326

323 Vandecasteele JP, *Appl Environ Microbiol* (1980) 39, 327

324 Goulas P, *Biochim Biophys Acta* (1988) 957, 335

325 Faber K, Franssen MCR, *TIBTECH* (1993) 11, 461

326 Mori K, Akao H, *Tetrahedron* (1980) 36, 91

327 Oritani T, Yamashita K, *Agr Biol Chem* (1980) 44, 2407

328 Glänzer BI, Faber K, Griengl H, *Tetrahedron* (1987) 43, 5791

329 Glänzer BI, Königsberger K, Berger B, Faber K, Griengl H, *Chem Phys Lipids* (1990) 54, 43

330 Kawashima M, Hasegawa T, *Biotechnol Lett* (1992) 14, 1135

331 Sugai T, Kuwahara S, Hoshino C, Matsuo N, Mori K, *Agr Biol Chem* (1982) 46, 2579

332 Mitsuda S, Nabeshima S, *Recl Trav Chim Pays-Bas* (1991) 110, 151

333 Burgess K, Jennings LD, *J Am Chem Soc* (1991) 113, 6129

334 Pontikis R, Randrianasolo LR, LeMerrer Y, Nam NH, Azerad R, Depezay JC, *Can J Chem* (1989) 67, 2240

335 Shimizu M, Kawanami H, Fujisawa T, *Chem Lett* (1992) 107

336 Kawai K-I, Imuta M, Ziffer H, *Tetrahedron Lett* (1981) 22, 2527

337 McGahren WJ, Sax KJ, Kunstmann MP, Ellestad GA, *J Org Chem* (1977) 42, 1659

338 O'Hagan D, Zaidi NA, *J Chem Soc Perkin Trans 1* (1992) 947

339 Ladner WE, Whitesides GM, *J Am Chem Soc* (1984) 106, 7250

340 Bianchi D, Cabri W, Cesti P, Francalanci F, Rama F, *Tetrahedron Lett* (1988) 29, 2455

341 Marples BA, Rogers-Evans M, *Tetrahedron Lett* (1989) 30, 261

342 Ferraboschi P, Grisenti P, Manzocchi A, Santaniello E, *J Chem Soc Perkin Trans 1* (1992) 1159

343 Gil G, Ferre E, Meou A, Triantaphylides C, *Tetrahedron Lett* (1987) 28, 1647

344 Ushio K, Yamauchi S, Masuda K, *Biotechnol Lett* (1991) 13, 495

345 Sugai T, Ohta H, *Agr Biol Chem* (1990) 54, 3337

346 Scilimati A, Ngooi TK, Sih CJ, *Tetrahedron Lett* (1988) 29, 4927

347 Feichter C, Faber K, Griengl H, *Tetrahedron Lett* (1989) 30, 551

348 Dike SY, Ner DH, Kumar A, *Bioorg Med Chem Lett* (1991) 1, 383

349 Itoh T, Kuroda K, Tomosada M, Takagi Y, *J Org Chem* (1991) 56, 797

350 Akita H, Matsukura H, Oishi T, *Tetrahedron Lett* (1986) 27, 5241

351 Gruber-Khadjawi M, Hönig H, Weber H, *Chirality* (1992) 4, 103

352 Itoh T, Takagi Y, Nishiyama S, *J Org Chem* (1991) 56, 1521

353 Kakeya H, Sakai N, Sugai T, Ohta H, *Agr Biol Chem* (1991) 55, 1877

354 Matsuo N, Ohno N, *Tetrahedron Lett* (1985) 26, 5533

355 Ohta H, Miyamae Y, Tsuchihashi G-I, *Agr Biol Chem* (1989) 53, 215

356 Hiratake J, Inagaki M, Nishioka T, Oda J, *J Org Chem* (1988) 53, 6130

357 Chen C-S, Liu Y-C, *Tetrahedron Lett* (1989) 30, 7165

358 Glänzer BI, Faber K, Griengl H, *Enzyme Microb Technol* (1988) 10, 689

359 Bevinakatti HS, Banerji AA, Newadkar RV, *J Org Chem* (1989) 54, 2453

360 Cotterill IC, Finch H, Reynolds DP, Roberts SM, Rzepa HS, Short KM, Slawin AMZ, Wallis CJ, Williams DJ, *J Chem Soc Chem Commun* (1988) 470

361 Cotterill IC, Sutherland AG, Roberts SM, Grobbauer R, Spreitz J, Faber K, *J Chem Soc Perkin Trans 1* (1991) 1365

362 Hönig H, Seufer-Wasserthal P, *Synthesis* (1990) 1137

363 Basavaiah D, RamaKrishna P, Bharathi TK, *Tetrahedron Lett* (1990) 31, 4347

364 Saf R, Faber K, Penn G, Griengl H, *Tetrahedron* (1988) 44, 389

365 Königsberger K, Faber K, Marshner CH, Penn G, Baumgartner P, Griengl H, *Tetrahedron* (1989) 45, 673

366 Mori K, Puapoomchareon P, *Liebigs Ann Chem* (1991) 1053

367 Randrianasolo-Rakotozafy LR, Azerad R, Dumas F, Potin D, D'Angelo J, *Tetrahedron-Asymmetry* (1993) 4, 761

368 Barnier JP, Blanco L, Rousseau G, Guibé-Jampel E, Fresse I, *J Org Chem* (1993) 58, 1570

369 Wu SH, Zhang L-Q, Chen C-S, Girdaukas G, Sih CJ, *Tetrahedron Lett* (1985) 26, 4323

370 Kazlauskas RJ, *J Am Chem Soc* (1989) 111, 4953

371 Howell JAS, Palin MG, Hafa HE, Top S, Jaouen G, *Tetrahedron-Asymmetry* (1992) 3, 1355

372 Uemura M, Nishimura H, Yamada S, Nakamura K, Hayashi Y, *Tetrahedron Lett* (1993) 34, 6581

373 Nakamura K, Ishihara K, Ohno A, Uemura M, Nishimura H, Hayashi Y, *Tetrahedron Lett* (1990) 31, 3603

374 Yamasaki Y, Hosono K, *Tetrahedron Lett* (1990) 31, 3895

375 Fritsche K, Syldatk C, Wagner F, Hengelsberg H, Tacke R, *Appl Microbiol Biotechnol* (1989) 31, 107

376 Toone EJ, Werth MJ, Jones JB, *J Am Chem Soc* (1990) 112, 4946

377 Provencher L, Wynn H, Jones JB, Krawczyk AR, *Tetrahedron-Asymmetry* (1993) 4, 2025

378 Miyazawa T, Takitani T, Ueji S, Yamada T, Kuwata S, *J Chem Soc Chem Commun* (1988) 1214

379 Chiou A-J, Wu S-H, Wang K-T, *Biotechnol Lett* (1992) 14, 461

380 Miyazawa T, Iwanaga H, Yamada T, Kuwata S, *Chirality* (1992) 4, 427

381 Kruizinga W, Bolster J, Kellog RM, Kamphuis J, Boesten WH, Meijer EM, Schoemaker HE, *J Org Chem* (1988) 53, 1826

382 Fu S-CJ, Birbaum SM, *J Am Chem Soc* (1953) 75, 918

383 Chenault HK, Dahmer J, Whitesides GM, *J Am Chem Soc* (1989) 111, 6354

384 Gais HJ, Lukas KL, Ball WA, Braun S, Lindner HJ, *Liebigs Ann Chem* (1986) 687

385 Kaga H, Kobayashi S, Ohno M, *Tetrahedron Lett* (1988) 29, 1057

386 Kobayashi S, Eguchi Y, Shimada M, Ohno M, *Chem Pharm Bull* (1990) 38, 1479

387 Kobayashi S, Kamiyama K, Ohno M, *Chem Pharm Bull* (1990) 38, 350

388 Kuhn T, Tamm C, Riesen A, Zehnder M, *Tetrahedron Lett* (1989) 30, 693

389 Kocienski P, Stocks M, Donald D, Perry M, *Synlett* (1990) 38

390 Hemmerle H, Gais HJ, *Tetrahedron Lett* (1987) 28, 3471

391 Shäfer H-J, Baringhaus K-H, *Liebigs Ann Chem* (1990) 351

392 Kobayashi S, Kamiyama K, Ohno M, *J Org Chem* (1990) 55, 1169

393 Yamamoto K, Nishioka T, Oda J, Yamamoto Y, *Tetrahedron Lett* (1988) 29, 1717

394 Guanti G, Banfi L, Narisano E, *Tetrahedron Lett* (1989) 30, 2697

395 Guanti G, Banfi L, Riva R, *Tetrahedron-Asymmetry* (1994) 5, 9

396 Mori K, Chiba N, *Liebigs Ann Chem* (1989) 957

397 Xie Z-F, Nakamura H, Suemune H, Sakai K, *J Chem Soc Chem Commun* (1988) 966

398 Atsuumi S, Nakano M, Koike Y, Tanaka S, Ohkubo M, Yonezawa T, Funabashi H, Hashimoto J, Morishima H, *Tetrahedron Lett* (1990) 31, 1601

399 Santaniello E, Ferraboschi P, Grisenti P, *Tetrahedron Lett* (1990) 31, 5657

400 Takabe K, Sawada H, Satani T, Yamada T, Katagiri T, Yoda H, *Bioorg Med Chem Lett* (1993) 3, 157

401 Ehrler J, Seebach D, *Liebigs Ann Chem* (1990) 379

402 Breitgoff D, Laumen K, Schneider MP, *J Chem Soc Chem Commun* (1986) 1523

403 Suemune H, Mizuhara Y, Akita H, Sakai K, *Chem Pharm Bull* (1986) 34, 3440

404 Kerscher V, Kreiser W, *Tetrahedron Lett* (1987) 28, 531

405 Murata M, Terao Y, Achiwa K, Nishio T, Seto K, *Chem Pharm Bull* (1989) 37, 2670

406 Suemune H, Harabe T, Xie ZF, Sakai K, *Chem Pharm Bull* (1988) 36, 4337

407 Xie Z-F, Sakai K, *Chem Pharm Bull* (1989) 37, 1650

408 Xie Z-F, Suemune H, Sakai K, *Tetrahedron-Asymmetry* (1993) 4, 973

409 Miyazawa T, Yoshida N, *Chem Lett* (1993) 1529

410 Bonini C, Racioppi R, Viggiani L, Righi G, Rossi L, *Tetrahedron-Asymmetry* (1993) 4, 793

411 Kasel W, Hultin PG, Jones JB, *J Chem Soc Chem Commun* (1985) 1563

412 Laumen K, Schneider MP, *Tetrahedron Lett* (1985) 26, 2073

413 Eberle M, Egli M, Seebach D, *Helv Chim Acta* (1988) 71, 1

414 Johnson CR, Bis SJ, *Tetrahedron Lett* (1992) 33, 7287

415 Johnson CR, Senanayake CH, *J Org Chem* (1989) 54, 735

416 Xie Z-F, Suemune H, Sakai K, *J Chem Soc Chem Commun* (1988) 1638

417 Sugai T, Mori K, *Synthesis* (1988) 19

418 Cotterill IC, Cox PB, Drake AF, Legrand DM, Hutchinson EJ, Latouche R, Pettman RB, Pryce RJ, Roberts SM, Ryback G, Sik V, Williams JOJ, *J Chem Soc Perkin Trans 1* (1991) 3071

419 Laumen K, Schneider MP, *Tetrahedron Lett* (1984) 25, 5875

420 Deardoff DR, Matthews AJ, McMeekin DS, Craney CL, *Tetrahedron Lett* (1986) 27, 1255

421 Naemura K, Takahashi N, Chikamatsu H, *Chem Lett* (1988) 1717

422 Naemura K, Fukuda R, Takahashi N, Konishi M, Hirose Y, Tobe Y, *Tetrahedron-Asymmetry* (1993) 4, 911

423 Estermann H, Prasad K, Shapiro M, Repic O, Hardtmann GE, Bolsterli JJ, Walkinshaw MD, *Tetrahedron Lett* (1990) 31, 445

424 Tanaka M, Yoshioka M, Sakai K, *Tetrahedron-Asymmetry* (1993) 4, 981

425 Carda M, Van Der Eycken J, Vandewalle M, *Tetrahedron-Asymmetry* (1990) 1, 17

426 Asensio G, Andreu C, Marco JA, *Chem Ber* (1992) 125, 2233

427 Patel RN, Liu M, Banerjee A, Szarka LJ, *Appl Microbiol Biotechnol* (1992) 37, 180

428 Murata M, Uchida H, Achiwa K, *Chem Pharm Bull* (1992) 40, 10

429 Jakovac IJ, Goodbrand HB, Lok KP, Jones JB, *J Am Chem Soc* (1982) 104, 4659

430 Bridges AJ, Raman PS, Ng GSY, Jones JB, *J Am Chem Soc* (1984) 106, 1461

431 Jones JB, Francis CJ, *Can J Chem* (1984) 62, 2578

432 Lok KP, Jakovac IJ, Jones JB, *J Am Chem Soc* (1985) 107, 2521

433 MacLeod R, Prosser H, Fikentscher L, Lanyi J, Mosher HS, *Biochemistry* (1964) 3, 838

434 Cervinka O, Hub L, *Coll Czechoslov Chem Commun* (1966) 31, 2615

435 Crumbie R L., Deol BS, Nemorin JE, Ridley DD, *Aust J Chem* (1978) 31, 1965

436 Fujisawa T, Itoh T, Nakai M, Sato T, *Tetrahedron Lett* (1985) 26, 771

437 Yamasaki T, Asai M, Ohnogi T, Lin JT, Kitazume T, *J Fluorine Chem* (1987) 35, 537

438 Iriuchijima S, Kojima N, *Agr Biol Chem* (1978) 42, 451

439 Nakamura K, Ushio K, Oka S, Ohno A, Yasui S, *Tetrahedron Lett* (1984) 25, 3979

440 Tanikaga R, Hosoya K, Kaji A, *J Chem Soc Perkin Trans 1* (1987) 17991803

441 Sato T, Okumura Y, Itai J, Fujisawa T, *Chem Lett* (1988) 1537

442 Nakamura K, Inoue Y, Shibahara J, Oka S, Ohno A, *Tetrahedron Lett* (1988) 29, 4769

443 Fantin G, Fogagnolo M, Guerzoni ME, Marotta E, Medici A, Pedrini P, *Tetrahedron-Asymmetry* (1992) 3, 947

444 Le Drian C, Greene AE, *J Am Chem Soc* (1982) 104, 5473

445 Guanti G, Banfi L, Narisano E, *Tetrahedron Lett* (1986) 27, 3547

446 Bernardi R, Cardillo R, Ghiringhelli D, Vajna de Pava O, *J Chem Soc Perkin Trans 1* (1987) 1607

447 Okano K, Suemune H, Sakai K, *Chem Pharm Bull* (1989) 37, 1995

448 d'Angelo J, Revial G, Azerad R, Buisson D, *J Org Chem* (1986) 51, 40

449 Roberts SM, *In : Enzymes in Organic Synthesis. CIBA Foundation Symposium 111*, Porter R, Clarck S Ed. Pitman, London, 1985, p 31

450 Takeshita M, Miura M, Unuma Y, *J Chem Soc Perkin Trans 1* (1993) 2901

451 Gillois J, Buisson D, Azerad R, Jaouen G, *J Chem Soc Chem Commun* (1988) 1224

452 Patel RN, Banerjee A, McNamee CG, Szarka LJ, *Appl Microbiol Biotechnol* (1993) 40, 241

453 Buisson D, Azerad R, *Tetrahedron Lett* (1986) 27, 2631

454 Seebach D, Roggo S, Maetzke T, Braunschweiger H, Cerkus J, Krieger M, *Helv Chim Acta* (1987) 70, 1605

455 Sato T, Maeno H, Noro T, Fujisawa T, *Chem Lett* (1988) 1739

456 Hoffmann RW, Helbig W, Ladner W, *Tetrahedron Lett* (1982) 23, 3479

457 Buisson D, Cecchi R, Laffitte J-A, Guzzi U, Azerad R, *Tetrahedron Lett* (1994) 35, 3091

458 Tsuboi S, Nishiyama E, Utaka M, Takeda A, *Tetrahedron Lett* (1986) 27, 1915

459 Tsuboi S, Nishiyama E, Furutani H, Utaka M, Takeda A, *J Org Chem* (1987) 52, 1359

460 Kitahara T, Mori K, *Tetrahedron Lett* (1985) 26, 451

461 Brooks DW, Wilson M, Webb M, *J Org Chem* (1987) 52, 2244

462 Nakamura K, Miyai T, Nagar A, Oka S, Ohno A, *Bull Chem Soc Jpn* (1989) 62, 1179

463 Buisson D, Azerad R, Sanner C, Larchevêque M, *Biocatalysis* (1990) 3, 85

464 Nakamura K, Miyai T, Kawai Y, Nakajima N, Ohno A, *Tetrahedron Lett* (1990) 31, 1159

465 Cabon O, Larchevêque M, Buisson D, Azerad R, *Tetrahedron Lett* (1992) 33, 7337

466 Akita H, Todokori R, Endo H, Ikari Y, Oishi T, *Synthesis* (1993) 513

467 Sato T, Tsurumaki M, Fujisawa T, *Chem Lett* (1986) 1367

468 Fujisawa T, Itoh T, Sato T, *Tetrahedron Lett* (1986) 25, 5083

469 Soukup M, Wipf B, Hochuli E, Leuenberger HGW, *Helv Chim Acta* (1987) 70, 232

470 Kato N, Fujie M, Hasegawa M, Shimao M, Kita K, Yanase H, *Biosci Biotech Biochem* (1993) 57, 303

471 Akita H, Furuichi A, Koshiji H, Horikoshi K, Oishi T, *Tetrahedron Lett* (1982) 23, 4051

472 Furuichi A, Akita H, Koshiji H, Horikoshi K, Oishi T, *Chem Pharm Bull* (1984) 32, 1619

473 Buisson D, Henrot S, Larchevêque M, Azerad R, *Tetrahedron Lett* (1987) 28, 5033

474 Bolte J, Gourcy J-G, Veschambre H, *Tetrahedron Lett* (1986) 27, 565

475 Fauve A, Veschambre H, *Tetrahedron Lett* (1987) 28, 5037

476 Fauve A, Veschambre H, *J Org Chem* (1988) 53, 5215

477 Lee SF, Barth G, Djerassi C, *J Am Chem Soc* (1981) 103, 295

478 Lu Y, Barth G, Kieslich K, Strong;P.D., Duax WL, Djerassi C, *J Org Chem* (1983) 48, 4549

479 Brooks DW, Grothaus PG, Irwin WL, *J Org Chem* (1982) 47, 2820

480 Brooks DW, Mazdiyasni H, Chakrabarti S, *Tetrahedron Lett* (1984) 25, 1241

481 Brooks DW, Mazdiyasni H, Sallay PJ, *J Org Chem* (1985) 50, 3411

482 Brooks DW, Woods KW, *J Org Chem* (1987) 52, 2036

483 Bellet P, Nominé G, Mathieu J, *C R Acad Sci Paris* (1966) 263(C), 88

484 Lanzilotta RP, Bradley DG, McDonald KM, *Appl Microbiol* (1974) 27, 130

485 Lanzilotta RP, Bradley DG, Beard CC, *Appl Microbiol* (1975) 29, 427

486 Mori K, Nagano E, *Biocatalysis* (1990) 3, 25

487 Simon H, Günther H, Bader J, Tischer W, *Angew Chem Int Ed Engl* (1981) 20, 861

488 Desrut M, Kergomard A, Renard MF, Veschambre H, *Tetrahedron* (1981) 37, 3825

489 Kergomard A, Renard MF, Veschambre H, *J Org Chem* (1982) 47, 792

490 Ferraboschi P, Grisenti P, Fiecchi A, Santaniello E, *Org Prep Proced Int* (1989) 21, 371

491 Ohta H, Kobayashi N, Ozaki K, *J Org Chem* (1989) 54, 1802

492 Utaka M, Konishi S, Okubo T, Tsuboi S, Takeda A, *Tetrahedron Lett* (1987) 28, 1447

493 Ferraboschi P, Casati S, Santaniello E, *Tetrahedron-Asymmetry* (1994) 5, 19

494 Gramatica P, Manitto P, Ranzi BM, Delbianco A, Francavilla M, *Experientia* (1982) 38, 775

495 Righini-Tapie A, Azerad R, *J Appl Biochem* (1984) 6, 361

496 Wandrey C, Fiolitakis E, Wichmann U, Kula M-R, *Ann New York Acad Sci* (1984) 434, 91

497 Pederson RL, Kim M-J, Wong C-H, *Tetrahedron Lett* (1988) 29, 4645

498 Augé C, Bouxom B, Cavyé B, Gautheron C, *Tetrahedron Lett* (1989) 30, 2217

499 Chen L, Dumas DP, Wong C-H, *J Am Chem Soc* (1992) 114, 741

500 Isowa Y, Ohmori M, Ishikawa T, Mori K, Nonaka Y, Kihara K, Oyama K, Sato H, Nishimura S, *Tetrahedron Lett* (1979) 2611

501 Oyama K, Kihara K, Nonaka Y, *J Chem Soc Perkin Trans 2* (1981) 356

502 Oyama K, Nishimura S, Nonaka Y, Kihara K, Hashimoto T, *J Org Chem* (1981) 46, 5241

503 Hudlicky T, Seoane G, Pettus T, *J Org Chem* (1989) 54, 4239

504 Ouazzani-Chahdi J, Buisson D, Azerad R, *Tetrahedron Lett* (1987) 28, 1109

505 Azerad R, Buisson D, Maillot S, Ouazzani-Chahdi J, *In : Bioorganic Chemistry in Health-Care and Technology*, NATO Symposium Series Vol 97, UK Pandit, FC Alderweireldt Ed, Plenum, New York, 1991, p 233

506 Levitt MS, Newton RF, Roberts SM, Willetts AJ, *J Chem Soc Chem Commun* (1990) 619

507 Alphand V, Archelas A, Furstoss R, *Biocatalysis* (1990) 3, 73

508 Alphand V, Archelas A, Furstoss R, *J Org Chem* (1990) 55, 347

509 Alphand V, Archelas A, Furstoss R, *Tetrahedron Lett* (1989) 30, 3663

510 Alphand V, Furstoss R, *J Org Chem* (1992) 57, 1306

511 Taschner MJ, Black DJ, *J Am Chem Soc* (1988) 110, 6892

512 Taschner MJ, Peddada L, Cyr P, Chen Q-Z, Black DJ, *In : Microbial Reagents in Organic Synthesis*, Servi S Ed, Kluwer, Dordrecht, 1992, p 347

513 Betts RE, Walters DE, Rosazza JP, *J Med Chem* (1974) 17, 599

514 Brannon DR, Horton DR, Svoboda GH, *J Med Chem* (1974) 17, 653

515 Rosazza JP, *LLoydia* (1978) 41, 279

516 Chien MM, Rosazza JP, *Drug Metab Disp* (1979) 7, 211

517 Rosazza JP, Kammer M, Youel L, Smith RV, Erhardt PW, Truong DH, Leslie SW, *Xenobiotica* (1977) 7, 133

518 Patterson EL, Andres WW, Krause EF, Hartman RE, Mitscher LA, *Arch Biochem Biophys* (1963) 103, 117

519 Eckenrode FM, *J Nat Prod* (1984) 47, 882

520 Hufford CD, Capiton GA, Clark AM, Baker JK, *J Pharm Sci* (1981) 70, 151

521 Peterson DH, Murray HC, *J Am Chem Soc* (1952) 74, 1871

522 Abraham W-R, Washausen P, Kieslich K, *Z Naturforsch* (1987) 42c, 414

523 Kouzi SA, McChesney JD, *Helv Chim Acta* (1990) 73, 2157

524 Aranda G, Lallemand J-Y, Hammoumi A, Azerad R, *Tetrahedron Lett* (1991) 32, 1783

525 Aranda G, ElKortbi MS, Lallemand JY, Neuman A, Hammoumi A, Facon I, Azerad R, *Tetrahedron* (1991) 47, 8339

526 Kulla HG, *Chimia* (1991) 45, 81

527 Nagasawa T, Hurh B, Yamane T, *Biosci Biotech Biochem* (1994) 58, 665

528 Hammoumi A, Revial G, D'Angelo J, Girault J-P, Azerad R, *Tetrahedron Lett* (1991) 32, 651

529 Hammoumi A, Girault J-P, Azerad R, Revial G, D'Angelo J, *Tetrahedron-Asymmetry* (1993) 4, 1295

530 Ouazzani J, Arsenayidis S, Alvarez-Manzaneda R, Rumbero A, Ourisson G, *Tetrahedron Lett* (1991) 32, 1983

531 Ouazzani J, Arsenayidis S, Alvarez-Manzaneda R, Cabrera E, Ourisson G, *Tetrahedron Lett* (1991) 32, 647

532 Arséniyadis S, Rodriguez R, Brondi R, Spanevello R, Ouazzani J, Ourisson G, *In : Microbial reagents in Organic Synthesis*, Servi S Ed, NATO ASI Series C Vol 381, Kluwer, Dordrecht, 1992, p 313

533 Arséniyadis S, Ouazzani J, Rodriguez R, Rumbero A, Ourisson G, *Tetrahedron Lett* (1991) 32, 3573

534 Hebda C, Szykula J, Orpiszewski J, Fischer P, *Hoppe-Seyler Biol Chem* (1991) 372, 337

535 Ohta H, Hiraga S, Miyamoto K, Tsuchihashi GI, *Agric Biol Chem* (1988) 52, 3023