The Biocatalytic Approach to the Preparation of Enantiomerically Pure Chiral Building Blocks

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I. Introduction

The construction of organic compounds containing one or more chirality centers utilizing chiral starting materials is certainly one of the most exciting and spectacular chapters of the contemporary organic chemistry. Several extensive reviews are available on the subject, going from early accounts on the use of chemical chiral auxiliary reagents[1] or the synthetic applications of chiral starting materials and reagents[2] to the excellent and extensive report recently published on the use of optically active compounds for the synthesis of natural products, i.e. pheromones.[3] The perspectives of large-scale asymmetric synthesis[4] and industrial developments in biocatalysis[5] have also been covered.

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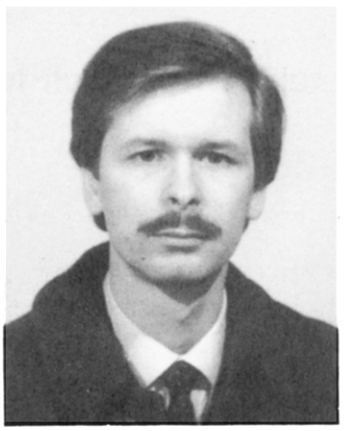
Enzo Santaniello, born in 1944, graduated in chemistry from the University of Milano, Italy, in 1967. He was then associated with the Institute of Organic Chemistry at the University of Milano and, from 1970, with the Department of Chemistry and Biochemistry, Faculty of Medicine, at the same university. He has been a Post-Doctoral Fellow, from 1973 to 1975, at the Worcester Foundation for Experimental Biology (MA) carrying out a research program on the synthesis of C-10 chiral steroids, under the supervision of Dr. Eliahu Caspi. Since 1986 he has been a Full Professor of Chemistry at the Faculty of Medicine, University of Milano. The current research activities of his group, formed by the authors of the review, are mainly focused on the use and study of biological systems suitable to the synthesis of chiral compounds.

Patrizia Ferraboschi graduated in chemistry from the University of Milano, Italy, in 1975. In 1977 she was appointed as Ricercatore at the Institute of Endocrinology, Faculty of Pharmacy at the University of Milano, carrying out a program research on the chemistry of hormonal steroids. At the same time, she had been associated with the group of Prof. E. Santaniello at the Department of Chemistry and Biochemistry, Faculty of Medicine, at the same university. She moved to this group in 1991.



Paride Grisenti, born in 1960, graduated in Chimica e Tecnologie Farmaceutiche from the University of Milano, Italy, in 1985. Since 1986 he has been associated at the Department of Chemistry and Biochemistry, Faculty of Medicine, at the same university, where he is presently completing his Dottorato di Ricerca in Biochemistry, under the supervision of Prof. E. Santaniello.

Ada Manzocchi graduated in chemistry from the University of Milano, Italy, in 1970. From 1970 to 1973 she was associated with the Institute of Biochemistry, Facoltà di Agraria at the University of Milano. In 1974 she moved to the Department of Chemistry and Biochemistry, Faculty of Medicine, at the same university and in 1979 she obtained the Diploma di Perfezionamento in Biochemistry at the University of Pavia. Since 1984 she has been an Associate Professor of Chemistry at the Faculty of Medicine, University of Milano.

The arsenal of the synthetic organic chemists is at present extraordinarily rich in chiral building blocks and methods for their preparation and elaboration. The goal of the preparation of the necessary chiral intermediate can usually be reached in a few ways. One approach can rely upon mother nature, who furnishes a great variety of natural products containing chirality centers, the so-called "chiral pool".[6] The elaboration of these natural products by well-established synthetic methodologies offers the entry to a wide variety of chiral intermediates. A second approach can be generalized as the use of a "nonnatural" chiral pool, made by structurally simple optically active compounds in which the chirality center(s) can be artificially introduced by the aid of chemical or biochemical auxiliaries. These man-made chiral building blocks can be utilized for the elaboration of more complex synthons to be used for the final synthetic target and must, therefore, be multifunctional. A complementary approach, strictly related to the previous one, can build up, either chemically or biochemically, a complex tailored intermediate in which the proper chirality is adequately introduced in the exact structural frame which is needed for the synthetic strategy.

The current literature is rich in examples of applications of the three approaches outlined above, and the present review will offer an overview of the applications of the concept of using biocatalytic systems for the preparation of chiral compounds not available from natural sources in amounts adequate for synthetic applications. It should be considered that the natural chiral pool relies upon the most available stereoisomer, but quite often nature is able to produce also the "unnatural" stereoisomer, even as a curiosity. However, the "wrong" compound will be less available from the natural source and at much higher price than the "natural" one. The nonnatural chiral synthons can be either the stereoisomer opposite to the "natural" one, or polyfunctional chiral compounds of general synthetic applicability with a structure not easily or not at all

found in nature, or tailored intermediates prepared for a precise synthetic target.

Due to the enormous bibliographic material to be examined and the great number of reviews covering almost all aspects of asymmetric synthesis, we will limit our overview to the synthesis of compounds in which only one or a few chirality centers have been introduced by means of biocatalysis and restrict our analysis to the literature that has appeared on this subject from 1988 to the end of 1991. We will cover mainly highly enantioselective biotransformations, considered to be such only if the enantiomeric excess (ee) is at least >95%. This means that the presence of the other stereoisomer is tolerated around 2% or below, so that the synthetic target will be nearly optically pure. Sometimes, also biotransformations with lower ee will be considered, if they are particularly interesting or promising, in view of the several methods already currently available to increase the optical purity of many biocatalytic processes.

II. The Biocatalytic Approach

The application of biocatalysis to organic synthesis is at present a well-defined area of research, to which several books[7-10] and reviews[11-14] have already been devoted. A whole issue of an international journal of organic chemistry is dedicated in 1991 to the same topic,[15] and in a recent book concerning organic synthesis highlights, a chapter is dedicated to bioorienting methodologies.[16] Biocatalysts of synthetic utility to organic chemists for the preparation of chiral synthons can be constituted by whole cells of animals,[17,18] plants,[19-22] or microorganisms.[23-27] In these cases, the complex machinery of the enzymes present in the cells can be utilized without disrupting the integrity of the membrane or as a crude homogenate.[12] A more purified system, the cost of which is raised by the purification process of the single components, is constituted by single enzymes, many of which are now commercially available. A list of the suppliers, still valid today, has been reported in Vol. 1 of ref 7. Since Cornforth's account on "the logic of working with enzymes",[28] several books[29-32] and review articles[33-46] have been devoted to the application of enzymes to organic chemistry. These unique biocatalysts can be further divided in coenzyme-dependent enzymes and enzymes which do not require a cofactor for their catalytic activity. The native enzymes can be modified by well-established immobilization techniques[47-49] in order to recover the biocatalyst and improve its stability toward the unusual conditions in which they are forced to work to be useful for organic chemists.

Additionally, new techniques from molecular biology open new horizons in two main directions. The protein engineering, like the site-directed mutagenesis originally developed in 1982[50] tends to change the core of the amino acids at the active site of the native enzyme,[51] in order to improve the original catalytic turnover or change the enzyme selectivity. The modification of the active site can also be performed by chemical methods.[52] New man-made enzymes can be prepared taking advantage of the opportunities offered by the invention of monoclonal antibodies. If these can be selected for specific reactions and work catalytically, then we will have at our disposal a new array of artificial enzymes (catalytic antibodies or abzymes)[53-60] (Scheme 1).

In order to rationalize the description of the application of biocatalysts to the preparation of polyfunctional chiral compounds, we prefer to use a systematic approach which collects the examples available in the current literature into divided sections corresponding to the reactions which can be catalyzed by enzymes. This applies well either to purified enzymes or more complex biological systems from animals, plants, or microorganisms, used as biocatalysts for the preparation of enantiomerically pure compounds. In fact, also when the whole-cell machinery is utilized, generally only the activity of one of the enzymes present in the natural mixture is exploited. Often, the activity of other enzymes eventually able to work on the foreign substrate can be detrimental to the desired transformation, for unwanted side reactions and the possibility of interference to the clean stereochemical outcome. On the other hand, as in the case of a reaction catalyzed by a cofactor-dependent enzyme realized by a microorganism, one can take the risk of cross-reactions during the process when the benefits coming from the natural regeneration of the cofactors and the low price of the system are considered. The available enzymes are traditionally divided[61] into six classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) according to the specific type of reaction that they are able to catalyze (Scheme 2).

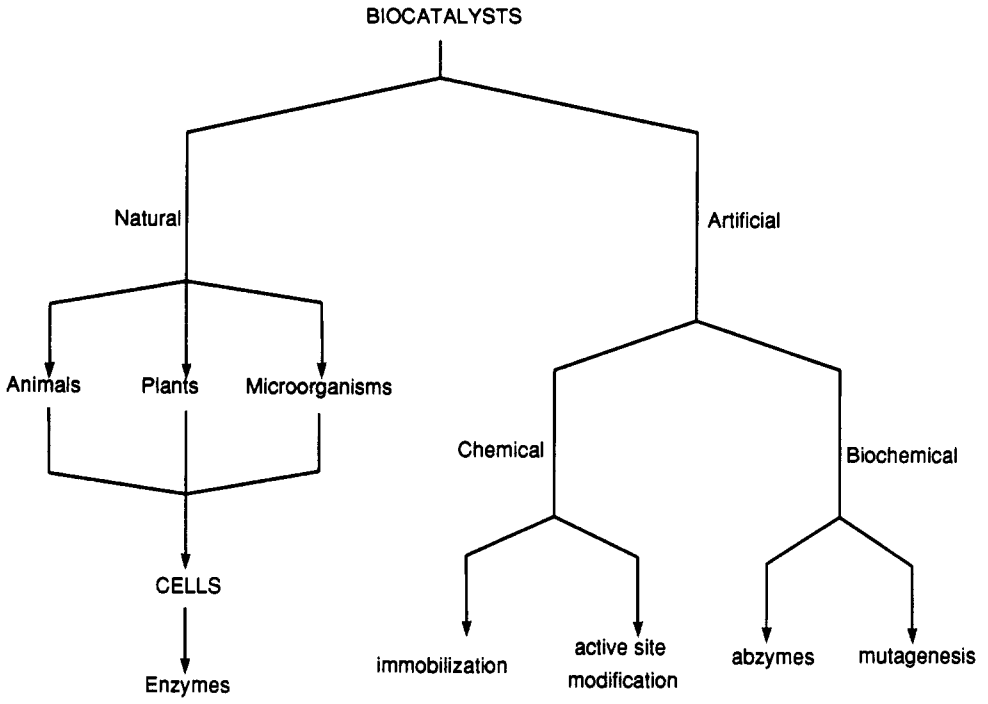
Through the pressures of evolution, the enzymes have developed the ability to catalyze a wide variety of reactions, many of which are useful to organic synthesis. Only a few reactions, like for instance the Diels–Alder condensation, are excluded by this domain. Some exceptions to this statement have appeared in the most recent literature, since often an organic chemist forces the enzyme to realize unknown reactions or known transformations of substrates considered unnatural, hopefully without dramatic changes in its catalytic power.

III. Reductions

A. General Remarks

Biocatalytic reductions are catalyzed by the first class of enzymes, the oxidoreductases, which catalyze reactions of reduction and oxidation, specifically removal or addition of hydrogen. Purified reductases useful for synthetic purposes have the disadvantage that they need expensive cofactors like NADH and NADPH, and although several methods for chemical and biochemical recycling of the coenzymes have been found, this delicate problem still remains the main obstacle to a more general use of this useful category of biocatalysts. Nonetheless, an example of the application of the NADH-dependent oxidoreductase, horse liver alcohol dehydrogenase (HLADH), for the preparation of γ-lactones[62] can be already found in *Organic Syntheses*. This well-known annual publication of satisfactory methods for the preparation of organic chemicals has also included some examples of chiral synthons prepared by bakers' yeast-mediated reductions.[63,64] The

Scheme 1



Scheme 2

ENZYMES	REACTIONS CATALYZED	IUB ENZYME COMMISSION CLASS	COENZYMES REQUIRED
Oxidoreductases	Reductions/Oxidations	1.a.b.c	yes
Transferases	A-(B) + C = A-C + (B)	2.a.b.c	yes
Hydrolases	Hydrolyses/Condensations (A-B + H_2O = AH + BOH) Hydrations (A-B + H_2O = HA-BOH) Transesterifications	3.a.b.c	no
Lyases	Additions/Eliminations	4.a.b.c	no
Isomerases	Isomerizations	5.a.b.c	no
Ligases	Formation of C-X	6.a.b.c	yes

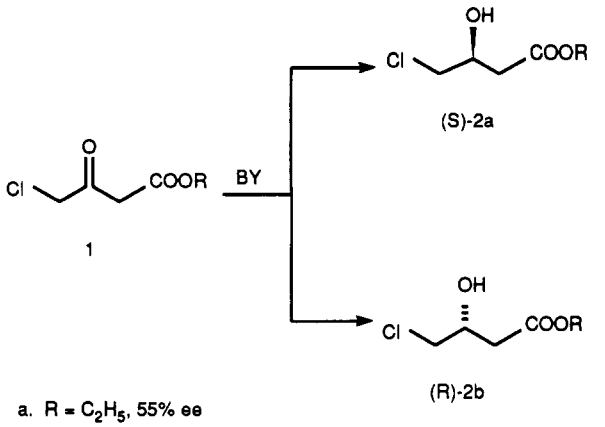
latest examples are representative of the fact that most of the reductions realized with the aid of biocatalysts useful for the preparations of chiral synthons rely upon the common bakers' yeast (*Saccharomyces cerevisiae*, BY), a microorganism which can also be used by people not trained in microbiology and can be considered an useful "reagent", easily accessible in every laboratory of organic chemistry. The use of this yeast has been recently reviewed in two exaustive reports (nearly 200 and 500 references, respectively), where the literature up to 1988 has been fully covered.[65,66] Therefore, we will refer to the use of BY only from 1989 and offer an overview of other biocatalytic reductions from 1988.

B. Bakers' Yeast-Mediated Reductions

1. Introduction

Fermenting BY using glucose as energy source in tap water is generally used for highly enantioselective reductions. However, when the process is not completely enantioselective, simple modifications of the experimental conditions may influence the stereochemistry and the ee of the product. For instance, use of organic media,[67] addition of other compounds,[68-71] or change of energy source[72] can be helpful. Also immobilization techniques[73,74] in water or an organic solvent[75,76] or enclosure in a dialysis tube[77] can reach the

Scheme 3



a. R = C_2H_5, 55% ee

b. R = C_8H_{17}, 97% ee

same goal. Traditionally, BY is able to reduce variously substituted carbonyl groups to the corresponding hydroxy compounds, and the stereochemical outcome of these highly enantioselective reductions depend on the presence of dehydrogenases which generally follow the so called Prelog's rule.[78] Several exceptions have been found, and this can be due to the presence of competing dehydrogenases with different stereochemical requirements. If the rates of the dehydrogenases are relatively different, the ee of the product is still good, although the configuration is opposite to the expected one. In another case, the stereochemistry reversal is accompanied by a lower enantioselectivity. Here, slight modifications of the substrate can sometimes direct the reduction toward the desired stereoisomer with enhanced ee. A classical case has been reported for the reduction of 4-chloroacetoacetates **1a** and **1b** by Sih and co-workers[79] (Scheme 3).

BY is also capable of reducing activated double bonds and a few other functional groups. A short report of the various biotransformations which BY can carry on α,β-unsaturated aldehydes has been recently published.[80]

2. Aromatic Carbonyl Compounds

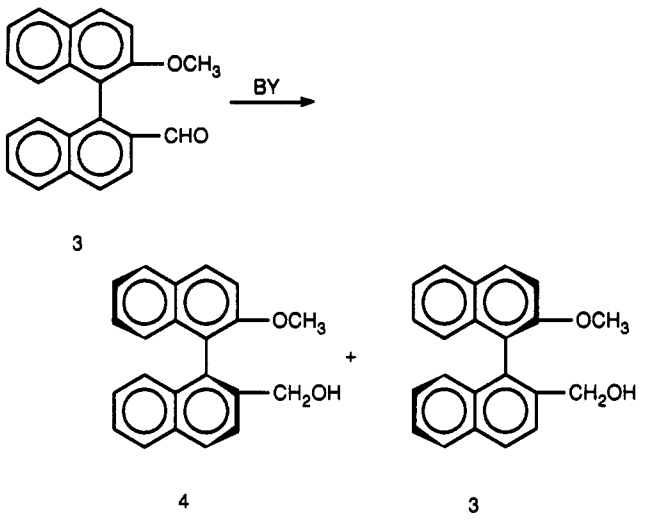
Although a great variety of structural framework can be designed for the substrates, generally the highest ee of the products is obtained for aromatic aldehydes or aryl methyl ketones. Thus, the kinetic resolution of an aldehyde within a binaphthyl structure leads to the discrimination of the axial chirality of biaryls, as reported for 2-formyl-1,1′-binaphthyls **3** (maximum 70% ee for **4**)[81] (Scheme 4).

The reduction of arylpropan- and arylbutan-2-ones **5a** and **5b** affords, under controlled experimental conditions, the corresponding carbinols **6a** and **6b**, which can be oxidized, after acetylation, to the corresponding hydroxy acids **7**[82] (Scheme 5).

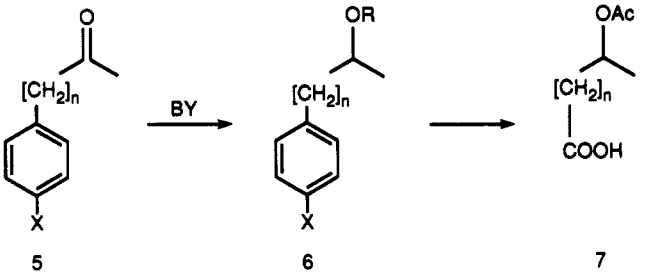
3. α-Substituted Carbonyl Compounds

Following a report on the enantioselective reduction of acetylisoxazolines,[83] the same authors described a kinetic resolution of racemic **8** in a 2-propanol/water mixture. The carbonyl function of optically pure unreacted (5*R*)-**8** was then reduced enantioselectively to configurationally opposite alcohols (5*R*)-**9b,c** with BY or *Aspergillus niger*[84] (Scheme 6). The conversion of

Scheme 4



Scheme 5



a. n = 1, X = OCH_3 b. n = 2, X = H

8 to enantiomerically pure dihydroxy ketones or triols has been described in the previous report.[83]

In racemic aromatic *trans*-2,3-epoxy ketones of structure **10**, the carbonyl group has been reduced and the oxirane ring hydrolyzed by the yeast, so that the final triol **11** has been obtained with >99.5% diastereomeric excess (de).[85] (Scheme 7).

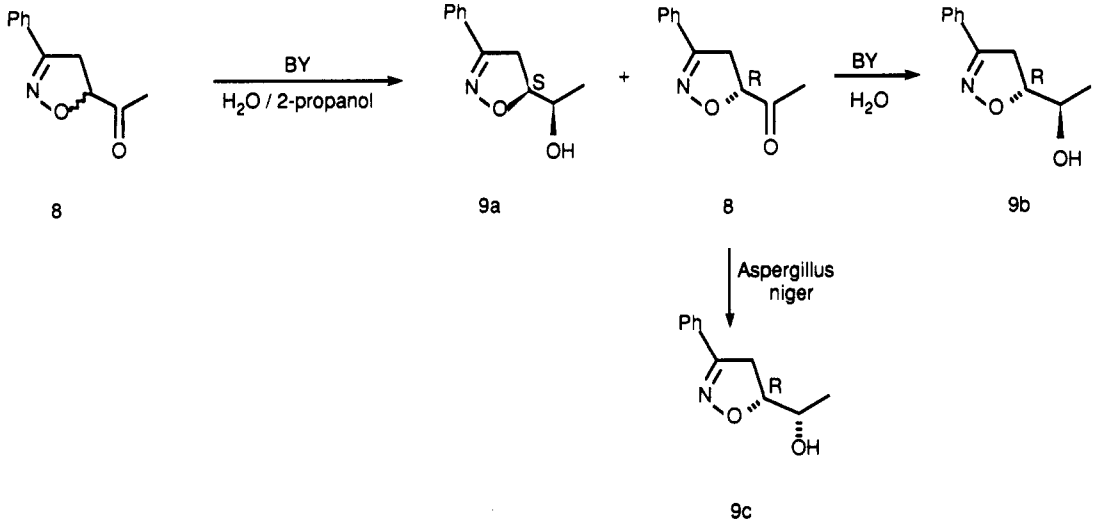
Among various four-carbon chlorinated ketones, the reduction of (*R*,*S*)-3-chlorobutan-2-one (**12**) led to a 1:1 mixture of (2*S*,3*S*)- and (2*S*,3*R*)-**13**, which was converted in a few steps into optically pure (*S*)-(+)-but-3-en-2-ol (**14**)[86] (Scheme 8).

The reduction of α-haloacetophenones was critically studied[87] and the results are shown in the Scheme 9.

Under appropriate conditions, α-fluoro and α-chloro derivatives **15a** and **15b** afforded the (*R*)-halohydrins **16a** and **16b** (67 and 37% yield, 97 and 90% ee, respectively), whereas poor yields of 97% ee (*R*)-bromohydrin **16c** were obtained from **15c**. Also a few fluorinated ketones containing an auxiliary sulfur group were reduced.[88] For example, from the compounds **17a–c** the corresponding optically active fluorohydrins **18a–c** had the best ee (75–85%, 50–60% yield, Scheme 10).[88] A few of the microorganisms tested gave results comparable to the BY biotransformations.

Also 2-alkyl- and 2-aryl-3-oxobutyronitriles are substrates for the yeast bioreduction.[89] Among the reported examples, it is noteworthy that only the *syn*-2-phenyl-3-hydroxybutyronitrile (**20**; >98% de) was obtained from the corresponding ketone **19** (Scheme 11).

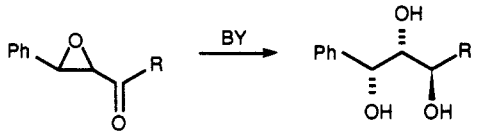
Scheme 6



8 9a 8 9b

9c

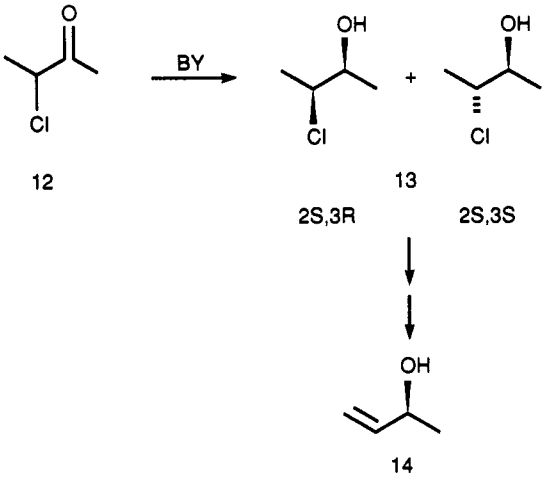
Scheme 7



10 11 d.e.> 99.5%

R = nC_4H_9, nC_3H_7, C_2H_5

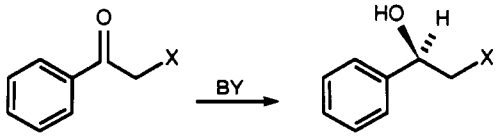
Scheme 8



12 13 2S,3R 2S,3S

14

Scheme 9

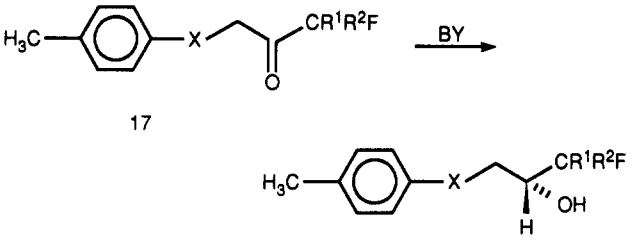


15 16

a. X = F
b. X = Cl
c. X = Br

An example of reduction of sulfur-containing cycloalkanone is the biotransformation of 2-(phenylthio)cyclopentanone (**21**; Scheme 12) which is more cleanly reduced than the corresponding cyclohexane congener and affords the optically pure (1*S*,2*R*)-2-(phenylthio)-cyclopentanol (**22**).[90]

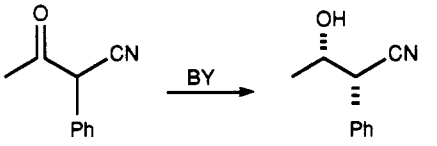
Scheme 10



17 18

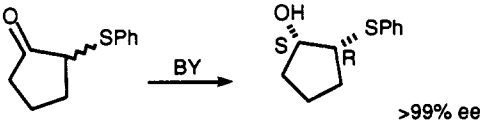
a. $R^1 = R^2 = H$; X = S or SO_2
b. $R^1 = H$; $R^2 = Ph$; X = S or SO_2
c. $R^1 = F$; $R^2 = Cl$; X = SO_2

Scheme 11



19 20

Scheme 12

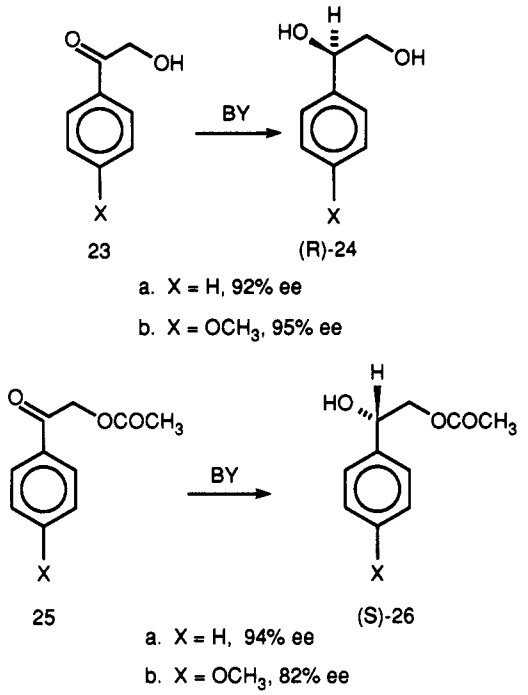


21 22

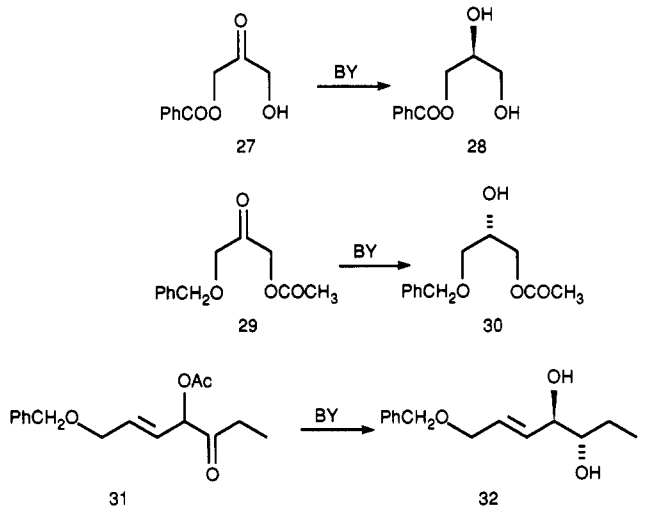
4. α-Hydroxy Ketones

It has been already shown that α-hydroxy ketones are good substrates for BY reductions.[91] In the case of phenacyl alcohols **23a** and **23b**, the corresponding (*R*)-diols **24a** and **24b** are obtained (85 and 25% yield, 92 and 95% ee, respectively). From the acetates **25a** and **25b**, the (*S*)-monoacetates **26a** and **26b** (70 and 25% yield, 94 and 82% ee, respectively) are formed[82,92] (Scheme 13). The diacetate of the (*R*)-diol **24b** was oxidized to the enantiomerically pure diacetate of (*S*)-glyceric acid.[82] Similar results to the above reduction have been obtained for the bioreduction of the monobenzoate of dihydroxyacetone, compound **27**, from

Scheme 13



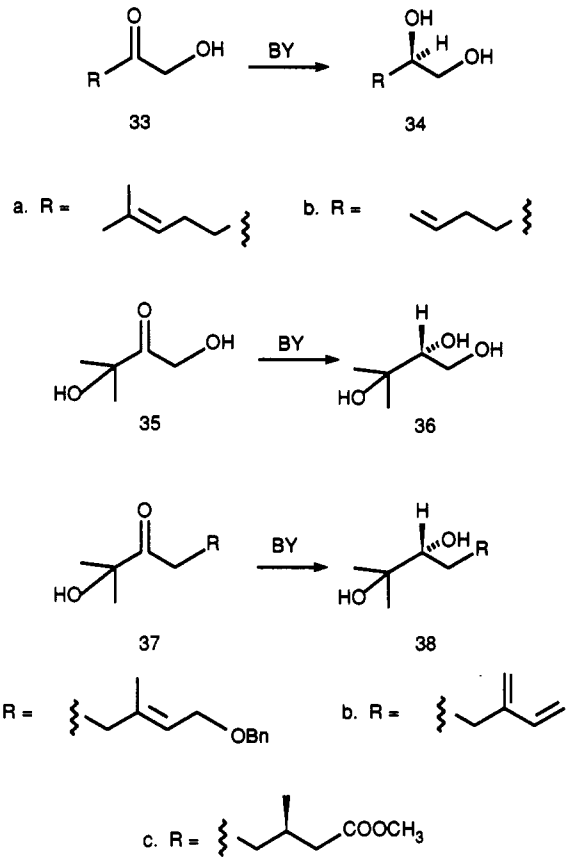
Scheme 14



which the optically pure glycerol derivative **28** was prepared.[93] Also the acetate **29** was a good substrate for BY and the optically pure acetate **30** was obtained (Scheme 14). It is interesting to note that the acetate function in **29** survived to the incubation conditions and the reduction product was recovered quantitatively. The chiral compound **32**, an intermediate for the synthesis of (+)-*endo*-brevicomin can be obtained by reduction and hydrolysis of the acetate **31** (20% yield).[94]

Other hydroxy ketones have been reduced to the corresponding chiral diols with high enantioselectivity (Scheme 15). The two compounds **33a** and **33b** afford nearly optically pure (*R*)-diols **34a** and **34b** (68 and 47% yield).[95] Surprisingly, from the ketone **35**, the (*R*)-(+)-3-methyl-1,2,3-butanetriol (**36**) was produced in 73% yield (90% ee).[96] Interestingly, according to the Prelog's rule,[78] the (*S*)-enantiomer should be obtained. Finally, hydroxy ketones **37a–c** are reduced to the corresponding (*R*)-diols **38a–c**.[97] Independently

Scheme 15



from the nature of the substituent R, the ee was always >95% and yields of isolated diols **38a–c** ranged from 68.6 to 96.5%.

5. β- and γ-Substituted Carbonyl Compounds

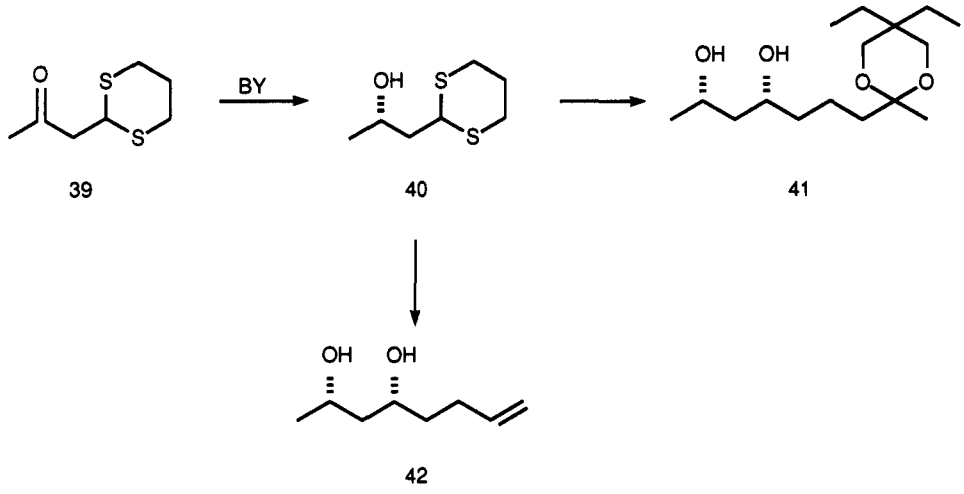
A recent application to the synthesis of optically active 1,3-diols, like **41** and **42**, uses as starting material the well-known (*S*)-1-(1,3-dithian-2-yl)-2-propanol (**40**), which can be prepared by a bioreduction process from the ketone **39** in excellent chemical yield and optically purity[98] (Scheme 16).

The reduction of a ketone bearing a β-acetal moiety in the molecule, compound **43**, can be realized with BY entrapped in calcium alginate beads, efficiently and enantioselectively to the corresponding hydroxy derivative **44**, a useful intermediate for the synthesis of (*R*)-(+)-α-lipoic acid (**45**)[99] (Scheme 17).

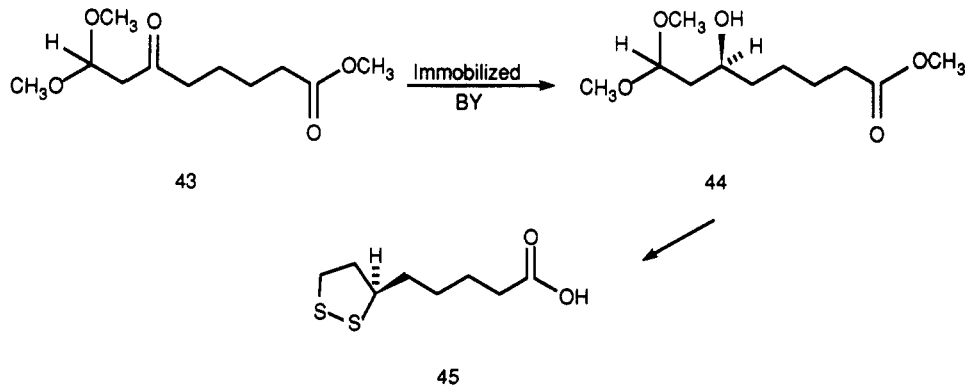
The acetoacetylated Meldrum's acid **46** was reduced at the non-enolyzed carbonyl function to the enantiomerically pure derivative **47** that was efficiently converted into (+)-parasorbic acid (**48**).[100] Prochiral methyl ketones connected with the 6-(4-oxo-1,3-dioxynyl) group directly or through methylene chain, i.e. the ketone **49**, were reduced to the corresponding (*S*)-alcohols.[101] The best ee (>99%) was achieved in the case of compound **50** (Scheme 18).

Nitro ketones like 4-nitro-2-butanone (**51**) and 5-nitro-2-pentanone (**52**) are substrates for BY and are reduced to nearly optically pure (*S*)-(+)-**53** and (*S*)-(+)-**54** (99 and 97% ee, 51 and 40% yield, respectively). These nitro alcohols are transformed in a few steps to the optically pure (*S*)-(+)-sulcatol (**55**)[102] or the pheromone **56**[103] (Scheme 19).

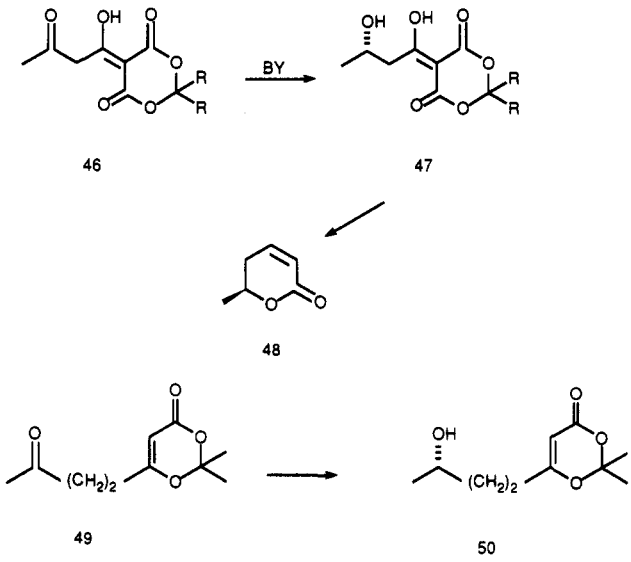
Scheme 16



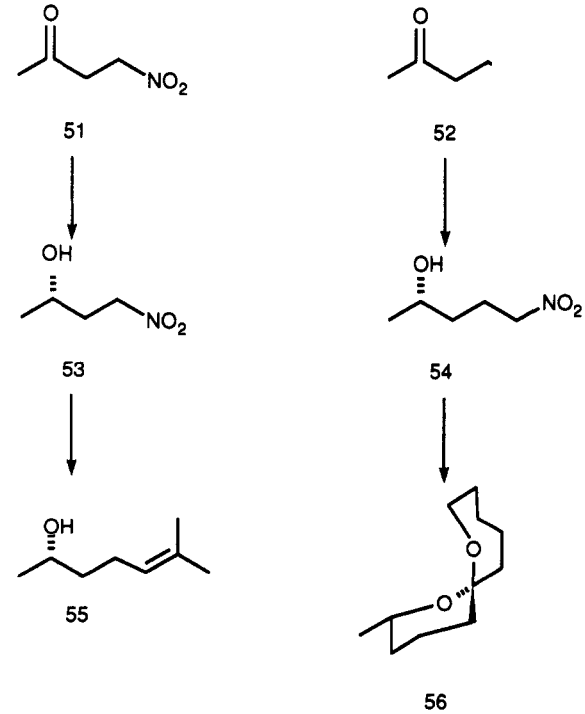
Scheme 17



Scheme 18



Scheme 19

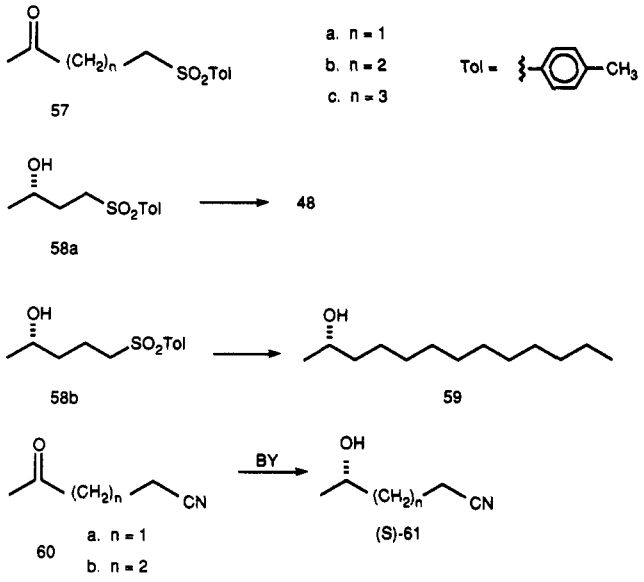


Among the keto sulfones **57a–c**, the compounds **57a,b** are efficiently reduced (68 and 44% yield, respectively)[104] to the (*S*)-hydroxy compounds **58a,b** (98% ee), which are chiral intermediates for the synthesis of enantiomerically pure parasorbic acid **48** and the pheromone (*S*)-(+)-2-tridecanol **59** (Scheme 20). The 4- and 5-keto nitriles **60a,b** can be converted (55 and 33% yield) to the corresponding (*S*)-alcohols **61a,b** (>98 and 97% ee, respectively). The above hydroxy nitriles are useful intermediates for the preparation of (*S*)-(−)-4-methylbutyrolactone and (*S*)-(−)-5-hexanolide.[105]

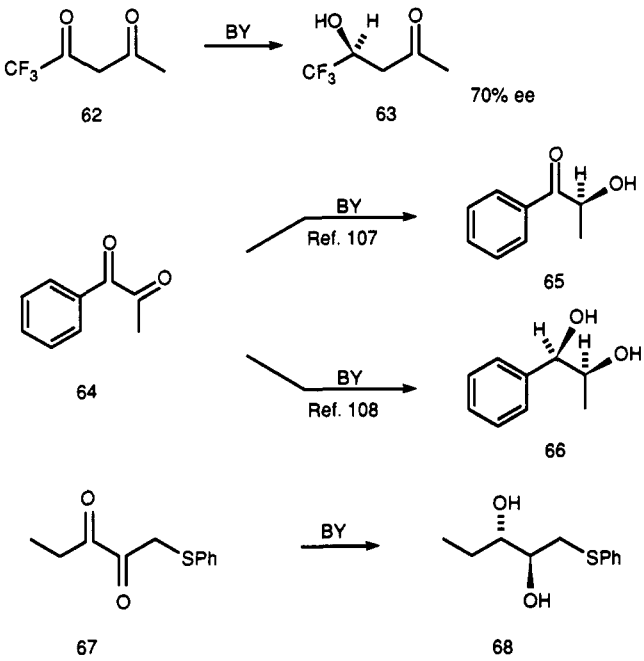
6. Diketones

In the case of β-diketones it is possible to reduce only one carbonyl group, as in the case of 1,1,1-trifluoro-2,4-pentanedione (**62**) which is reduced to (*S*)-(−)-**63** (70% ee).[106] Depending on the experimental conditions,

Scheme 20



Scheme 21



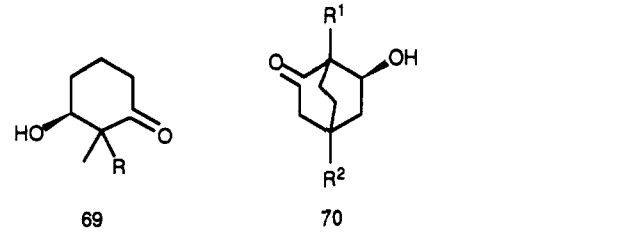
it is possible to reduce the α-diketone **64** to the hydroxy ketone **65** (80% yield, 95% ee)[107] or to the diastereomerically pure diol **66** (67% yield).[108] Recently the reduction of the diketone **67** to the diol **68** (38% yield, only one diastereomer after crystallization), an intermediate for the synthesis of (+)-*endo*-brevicomin, has been reported[109] (Scheme 21).

The experimental protocol for the reduction of 2,2-dimethyl-1,3-cyclohexanedione has been recently published in *Organic Syntheses*.[64] Additional examples of synthetic applications of chiral cyclic hydroxy ketones **69a,b** have been recently published.[110,111] The reduction of other cyclic diketones, bicyclo[2.2.2]octane-2,6-diones, yields the hydroxy ketones **70a–d**[112,113] (Scheme 22).

7. Keto Acids and Esters

Also keto acids and esters are excellent substrates for BY and some oxidoreductases involved in the process

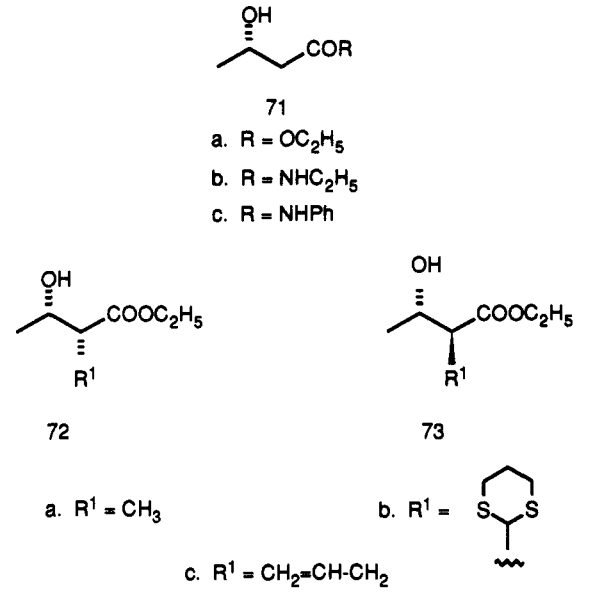
Scheme 22



a. R = CH_3
b. R = CH_2CH_3

a. $R^1 = R^2 = H$, 52%, 95% ee
b. $R^1 = CH_3$; $R^2 = H$, 59%, 99.5% ee
c. $R^1 = H$; $R^2 = CH_3$, 58%, 98% ee
d. $R^1 = CH_2CH_2OAc$; $R^2 = H$, 71%, 98.8% ee

Scheme 23



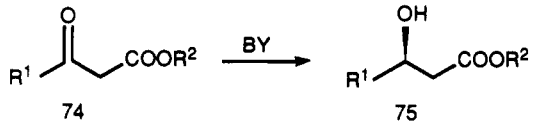
a. R = OC_2H_5
b. R = NHC_2H_5
c. R = NHPh

a. $R^1 = CH_3$
b. R^1 =
c. $R^1 = CH_2{=}CH{-}CH_2$

have been isolated.[114–117] β-Keto esters seem to be the most studied of this class of compounds, and this is the case for the product of bioreduction of ethyl acetoacetate, ethyl (*S*)-3-hydroxybutyrate (**71a**). This valuable chiral synthon can be prepared now by a well-established experimental protocol.[63] This work contains several references to synthetic applications of the compound **71a**, which is still frequently used for the preparation of enantiomerically pure chiral compounds.[118–123] (*S*)-Hydroxybutanamides **71b,c** can be prepared in 95 and >98% ee, respectively, with alginate- and polyurethane-immobilized[74] or fermenting[124] BY. Also the 2-substituted β-keto esters[68,125] or the corresponding acyl enolates[126] are reduced with high diastereoselectivity to the *syn*-hydroxy esters **72a,b**. In the case of the reduction of 2-allyl-3-oxobutanoates, the anti compound **73c** is obtained with BY,[127,128] whereas a reduction mediated by *Mucor javanicus*[129] affords the *syn*-hydroxy ester **72c** (Scheme 23).

A few β-keto acids **74a–d** can be reduced (Scheme 24), and except for 3-oxobutanoic acid (**74a**), they all furnished the (*R*)-hydroxy acids **75b–d**.[130] Similar stereochemistry was found for the reduced product **75e**,[131] whereas reduction of the keto acid **74f** did not afford the (*R*)-hydroxy acid **75f** satisfactorily.[132] Synthetically useful hydroxy esters **75g,h** were prepared from **74g,h** in good yield (69 and 60%) and ee ranging from 78 to

Scheme 24



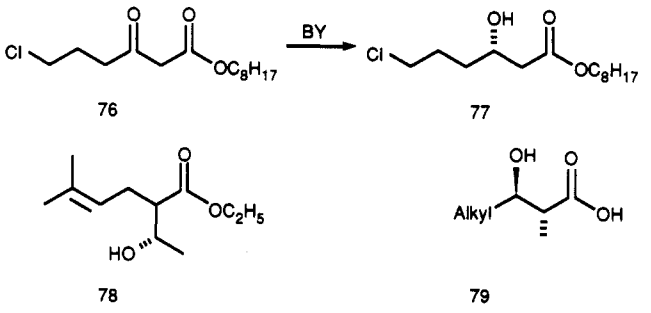
74 75

a. $R^1 = CH_3$; $R^2 = H$
b. $R^1 = C_2H_5$; $R^2 = H$
c. $R^1 = nC_3H_7$; $R^2 = H$
d. $R^1 = C_5H_{11}$; $R^2 = H$
e. $R^1 = CH_2{=}CH\text{-}CH_2CH_2$; $R^2 = H$
f. $R^1 = (CH_3)_2C{=}CH\text{-}CH_2CH_2$; $R^2 = H$
g. $R^1 = CH_2{=}CH\text{-}CH_2$; $R^2 = CH_3$
h. $R^1 = Ph$; $R^2 = C_2H_5$

Table 1. BY Reduction of β-Keto Acids and Esters 74a–h

product	yield, %	ee, %	ref
(*S*)-**75a**	13	86	130
(*R*)-**75b**	21	98	130
(*R*)-**75c**	37	>98	130
(*R*)-**75d**	71	>98	130
(*R*)-**75e**	34	>99	131
(*R*)-**75g**	69	78	133
(*S*)-**75h**	60	90 ● 3	135

Scheme 25



76 77 78 79

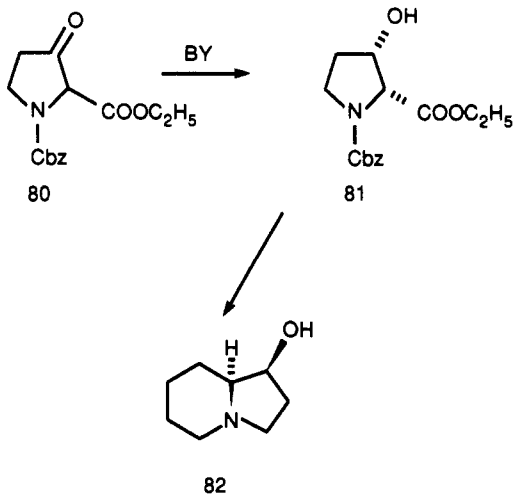
90 ± 3%.[133,134] The results are collected in the Table 1.

The reduction of the octyl ester **76** afforded with a good enantioselectivity (90% ee) the (*S*)-hydroxy ester **77**, an intermediate of the synthesis of (*R*)-α-lipoic acid **45**.[135] In Scheme 25 is also depicted the stereochemistry of the α-substituted β-hydroxy ester **78** (>97% de, 29% yield)[132] and the acid **79** (68% yield, >98% de, >98% ee),[136] which are obtained by the reduction of the corresponding keto ester and potassium salt.

A highly diastereoselective synthesis of the chiral building block *N*-(carbobenzyloxy)-(2*R*,3*S*)-3-hydroxyproline ethyl ester (**81**) can be efficiently realized from *N*-(carbobenzyloxy)-(2*S*)-2-carbethoxypyrrolidin-3-one (**80**). The cyclic chiral hydroxy ester **81** has been used for the enantiocontrolled synthesis of the indolizidine alkaloid **82**[137] (Scheme 26).

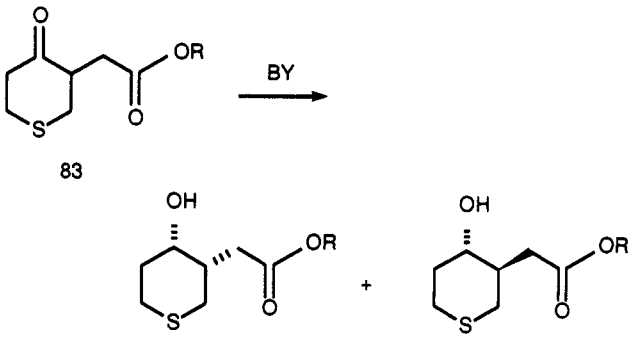
Another interesting case of the reduction of a cyclic keto ester has been recently reported.[138] (4-Oxotetrahydrothiopyran-3-yl)acetates **83a–d** have been used as substrates for BY, and the ratio of the two diastereomers **84** and **85** has been determined as a function of the nature of the ester (Scheme 27). In all cases, collected in the Table 2, the optical purity of the two centers of chirality was >97%.

Scheme 26



80 81 82

Scheme 27



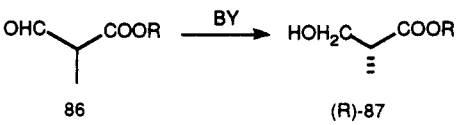
83 84 85

a. R = H b. R = CH_3
c. R = C_2H_5 d. R = C_8H_{17}

Table 2. BY Reduction of (4-Oxotetrahydrothiopyran-3-yl)acetates 83a–d

products	yields, %	cis/trans	ee, % cis	ee, % trans
84a + 85a	28	70/30	>99	98
	36	73/27	>99	97
84b + 85b	68	52/48	>99	>99
	52	49/51	>99	>99
84c + 85c	67	45/55	>99	>99
	86	52/48	>99	>99
84d + 85d	32	10/90	98	97

Scheme 28

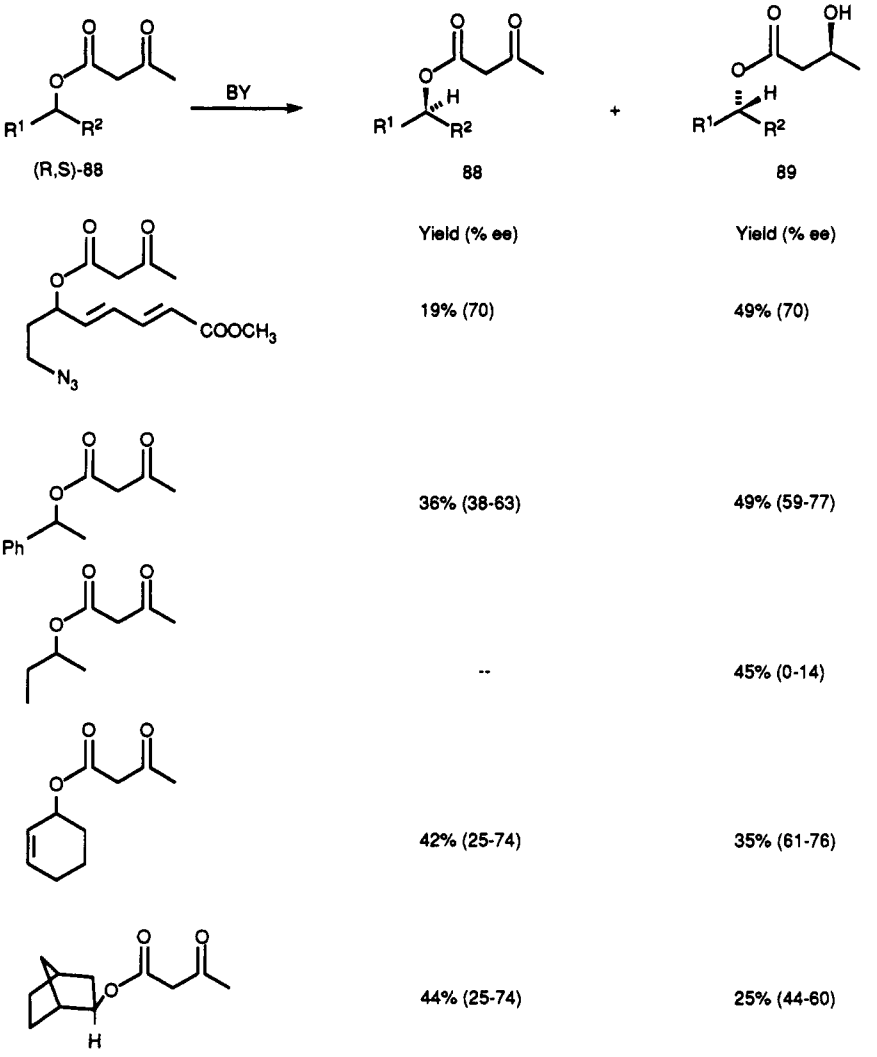


86 (R)-87

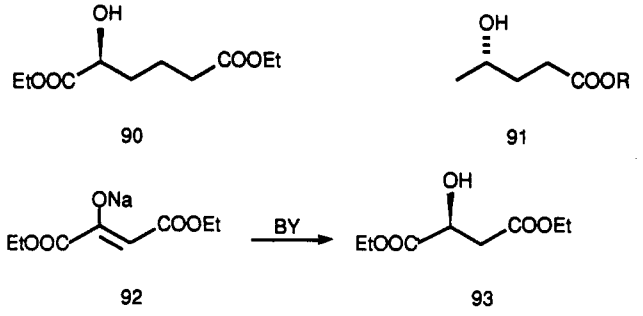
a. R = C_2H_5, 70-80%, 60-65% ee
b. R = $CH_2C(CH_3)_3$, 78%, 90% ee

The BY-mediated reduction of ethyl 2-formylpropionate (**86a**) has been reported to yield (*R*)-hydroxy ester **87a** with moderate ee (60–65%) and satisfactory yields (70–80%).[139] A more recent study has shown that changes in the size of the ester group improve the enantioselectivity. Thus, the reduction of the bulky ester **86b** gives the best result and (*R*)-**87b** was isolated in 78% yield (90% ee) (Scheme 28).[140]

Scheme 29



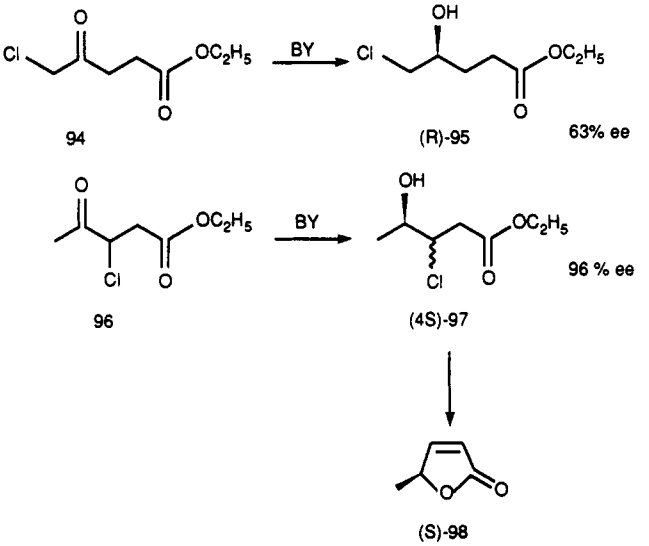
Scheme 30



By relying upon the easy reduction of β-keto esters, a kinetic resolution of racemic alcohols through the reduction of their esters **88a–e** has recently been proposed.[141] The authors have investigated the resolution of a variety of secondary alcohols, separating the hydroxy esters **89a–e** from the unreacted keto esters **88a–e** (Scheme 29). In this preliminary study, the optically active **88a–e** and **89a–e** biocatalytically prepared showed maximum 77% ee. Other keto esters can be reduced by BY, so that optically pure (*S*)-**90**[142] and (*S*)-**91**[143] can be prepared in 22 and 60% yield, respectively. In the Scheme 30 it is also reported the reduction of the diethyl oxalacetate sodium salt (**92**) to afford diethyl (*S*)-malate (**93**, 72%, >98% ee).[144]

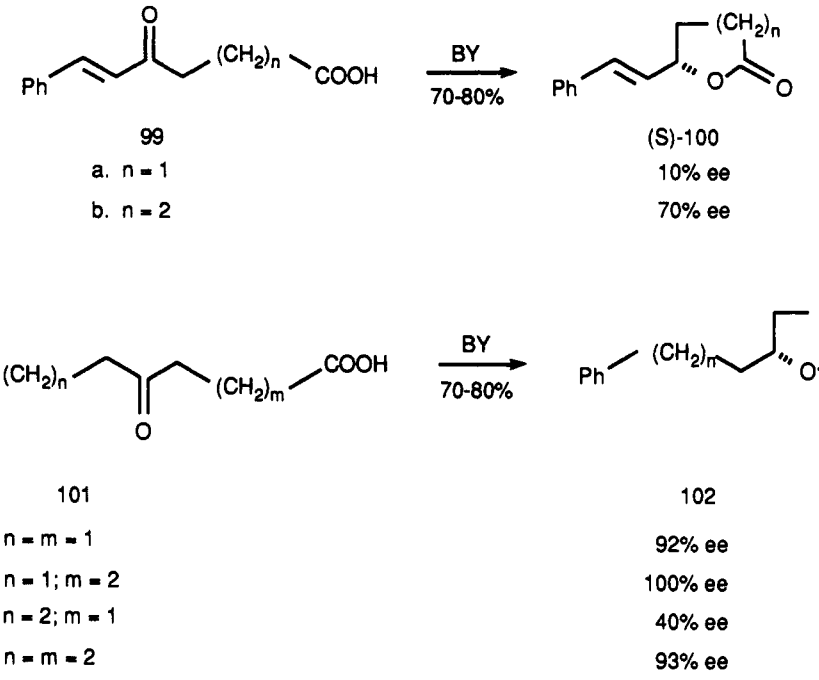
Interesting applications of bioreduction of γ- and δ-keto acids and esters have been recently reported.

Scheme 31



γ-Keto esters containing a chlorine atom, such as ethyl 5-chloro-4-oxopentanoate (**94**) is reduced with moderate enantioselectivity (63% ee) in 43% yield.[145] Ethyl 3-chloro-4-oxopentanoate (**96**) is reduced with excellent enantioselectivity (96% ee) to the 1:1 epimeric mixture of hydroxy esters **97** (75% yield), which was used as such for the synthesis of (*S*)-butenolide (**98**) (Scheme 31).

Scheme 32



Scheme 33

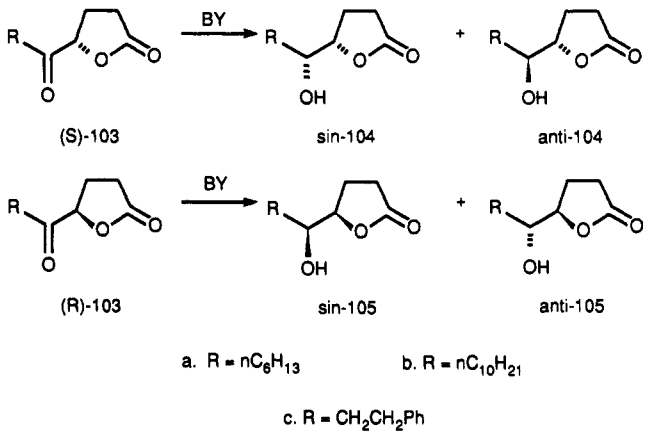


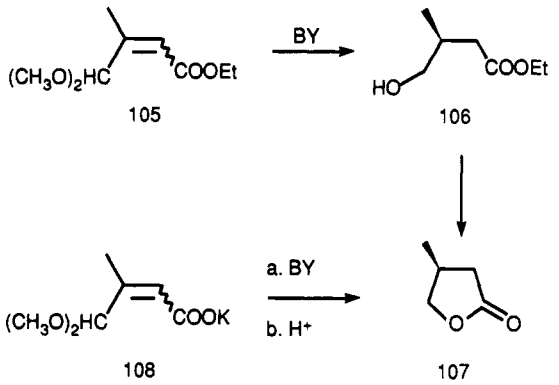
Table 3. BY Reduction of 4-Acylbutanolides 103a–c

substrate	alcohols		yield, %
	syn, %	anti, %	
(*S*)-**103a**	60	40	100
(*S*)-**103b**	56	44	100
(*S*)-**103c**	20	80	25
(*R*)-**103a**	100	0	9
(*R*)-**103b**	100	0	10
(*R*)-**103c**	79	21	88

Longer chain chloro keto esters were not satisfactorily reduced by BY and the ee of the obtained hydroxy esters was improved to >96% by their enzymatic hydrolysis with a lipase.[146] The capability of BY to transform γ- and δ-keto acids directly to optically active γ- and δ-lactones has long been recognized.[147] Recently, a series of arylalkenyl and arylalkyl γ- and δ-keto acids **99** and **101** has been reduced[148] to the corresponding γ- and δ-lactones **100** and **102**, the ee ranging from 70 to 100% for the latest compounds (Scheme 32).

The reduction of 4-acylbutanolides (**103**) to afford the diastereomeric mixture of hydroxy lactones **104** and **105** has been studied.[149] This detailed study has recorded either the reduction of (*R*,*S*)-**103** and the biotransformation of enantiomerically pure (*S*)- and (*R*)-**103**. In the latest case (Scheme 33), the ratio of *sin*/*anti*-**104** from (*S*)-**103** and *sin*/*anti*-**105** from (*R*)-**103** has been determined. The results are collected in Table 3 and indicate that the mode of yeast transformation of substrates with the same functionalities, and only subtle structural modifications, is still unpredictable.

Scheme 34

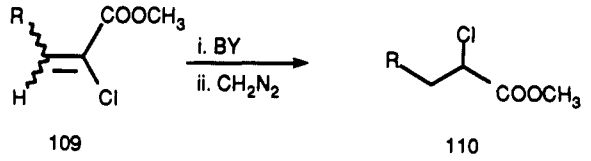


8. Activated Double-Bond Hydrogenation and Masked Carbonyl Compound Reduction

In the α,β-unsaturated ester **105**, the hydrogenation of the double bond, the hydrolysis of the acetal moiety, and the reduction of the intermediate aldehyde generated the chiral hydroxy ester **106**, successively cyclized to the (*S*)-lactone **107**.[150] An enantioselective hydrogenation–hydrolysis–reduction of the potassium salt **108** allowed a direct preparation of the chiral lactone **107** (90% ee)[151] (Scheme 34).

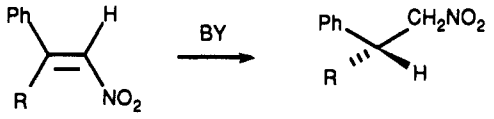
The asymmetric hydrogenation of the carbon–carbon double bond in 2-chloro-2-alkenoates **109a–f**[152] and in nitroalkenes **111a,b**[153] affords the corresponding chiral saturated derivatives **110a–f** and **112a,b** (Scheme 35). Apparently, in the case of compounds **109**, the double-bond configuration strongly influenced the stereochem-

Scheme 35



109 110

a. R = CH_3CH_2 d. R = CH_2Cl
b. R = $(CH_3)_2CH$ e. R = $CHCl_2$
c. R = $CH_3(CH_2)_3$ f. R = CCl_3



111 112

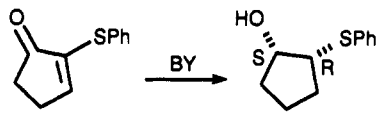
a. R = CH_3 50%, 98% ee
b. R = C_2H_5 64%, 97% ee

Table 4. BY Reduction of 2-Chloro-2-alkenoates 109a–f

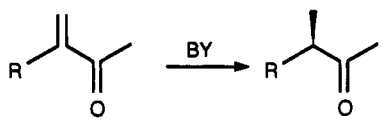
		products 110[a]		
substrate	E/Z	yields, %	ee, %	config
109a	*E*	23–28	47	*R*
	Z	23–35	>98	*S*
109b	*E*	6–10	68	*R*
	Z	16–19	>98	*S*
109c	*E*	30–40	25	*R*
	Z	32–40	>98	*S*
109d	*Z*	0	*b*	*b*
109e	*E*	54–65	92	*R*
	Z	58–71	98	*S*
109f	*Z*	41–69	>98	*S*

[a] The BY hydrogenation products are free acids which were converted to the methyl esters **110a–f**. [b] Not reported.

Scheme 36



113 22



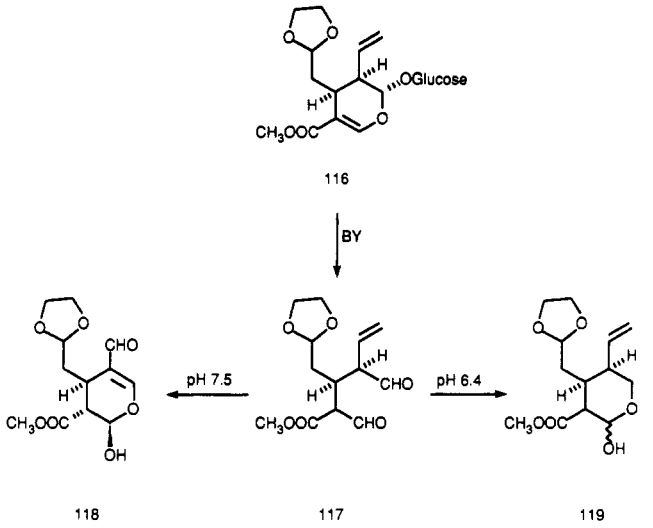
114 (R)-115

a. R = C_6H_{13} c. R = $C_{11}H_{23}$
b. R = C_8H_{17} d. R = Ph

istry of the products **110**. Table 4 collects the results of the BY reduction of 2-chloro-2-alkenoates **109a–f**.

BY is able to carry out the hydrogenation of the double bond and the reduction of the ketone function in the sulfur-containing α,β-unsaturated cyclopentenone derivative **113** to afford a nearly optically pure compound **22**[90] (Scheme 36). In the case of α-methylene ketones **114a–d**, the bioreduction of the methylene group is the major reaction, since the main products are the (*R*)-ketones **115a–d**, accompanied by minor amounts of the corresponding saturated alcohols.[154] The yields range between 22 and 29% and the ee are ≥95%.

Scheme 37



116 118 117 119

In a nonconventional hydrolysis–reduction procedure, the derivative of secologanin (**116**) was subjected to the β-glucosidase activity present in BY.[155] The intermediate dialdehyde **117**, depending on the pH of the incubation medium, could afford the cyclic compound **118** (pH 7.5) or the reduced aglucone epimeric mixture **119** (pH 6.4). The latest chiral intermediate was used for an enantiospecific synthesis of (+)-antirhine in a four-step sequence (Scheme 37).

C. Microorganism-Mediated Reductions

As pointed out earlier, the enormous potentiality of the microorganisms as biocatalysts is far from being completely exploited. The main difficulty for a widespread use of microorganisms among the organic chemists' community, certainly depends on the scarce familiarity with the microbiological techniques. A few people well trained in both disciplines, organic chemistry and microbiology, can take the greatest advantage of the immense opportunities offered by these versatile biocatalysts. Also a good, interdisciplinary cooperation between groups of synthetic organic chemists and microbiologists oriented toward applications in the field of organic chemistry can produce excellent fruits. Here, we will examine the most significant applications of microorganisms to reductive processes for the preparation of enantiomerically pure compounds, starting from 1988. A comparison with BY will be made, when possible, to underline similarities or, better, configurationally opposite results. Besides the general review by Yamada and Shimizu,[12] excellent accounts on microbial asymmetric reductions should be consulted.[156,157]

1. Ketones and Diketones

In order to test the viability of new biocatalytic methods, simple ketones are tested as substrates for microorganisms not previously used for reductions. This is the case of Lactobacilli strains[158] and acetic acid bacteria.[159] A wide screening of several yeasts and mold strains on the same class of ketones, i.e. 2-acylthiazoles, can be very helpful in finding out the best biocatalyst for the most enantioselective preparation of

Scheme 38

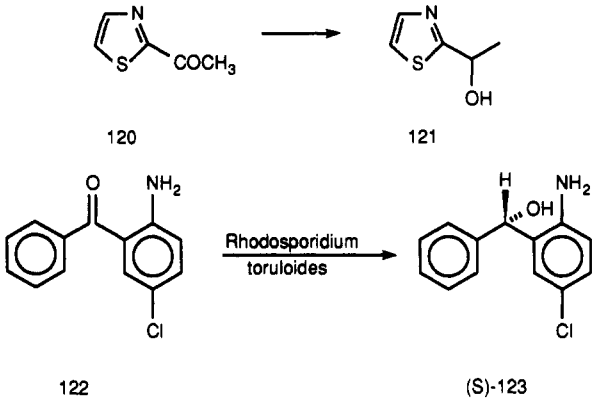
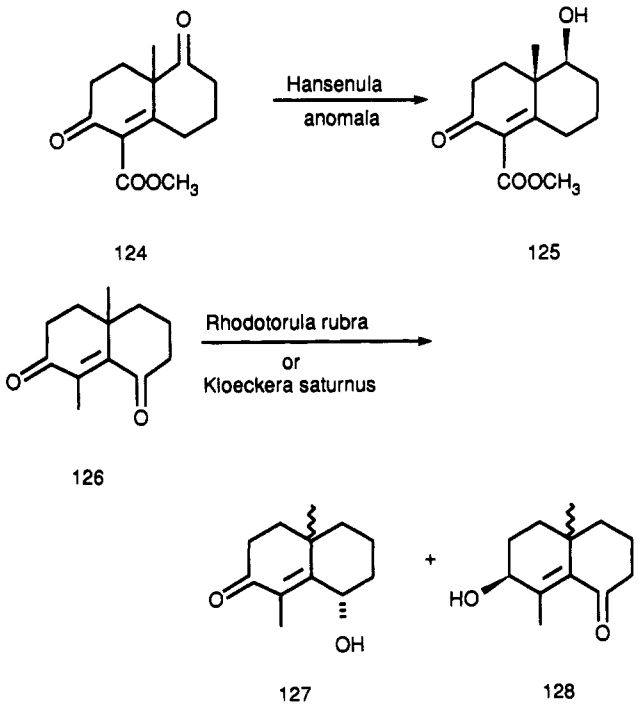


Table 5. Microbial Preparation of the Alcohol 121

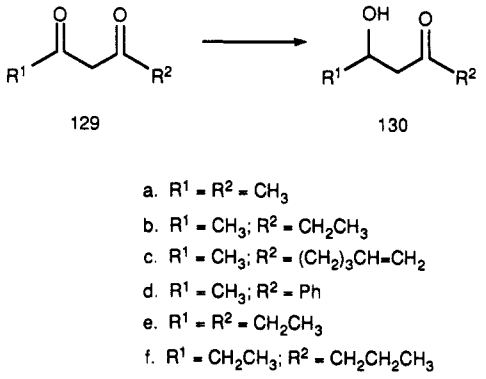
microorganism	yield, %	ee, %	config
Penicillum digitatum	38	>95	*S*
Rhizopus nigricans	88	>95	*S*
Mucor rouxianus	53	>95	*S*
Candida utilis	78	>95	*S*
Saccharomyces cerevisiae ML30 (subsp. chevalieri)	98	>95	*S*
Pichia membranaefaciens	86	>95	*S*
Yarrowia lipolytica F	17	>95	*R*
Yarrowia lipolytica AD	64	>95	*S*
Yarrowia lipolytica G	17	>95	*R*

Scheme 39



products.[160] Table 5 shows the most significant bioreductions of 2-acetylthiazole (**120**) to nearly optically pure (*R*)- or (*S*)-alcohols **121**. In Scheme 38, the reduction of 2-amino-5-chlorobenzophenone (**122**) in the presence of the yeast *Rhodosporidium toruloides* to afford the optically pure (*S*)-**123**, an intermediate for the synthesis of the (*S*)-isomer of *N*-isonicotinoyl-2-amino-5-chlorobenzhydrol, a strong rice plant growth regulator, is also reported.[161] The reduction of 3,8-dioxo-4-(methoxycarbonyl)-9-methyl-$\Delta^{4(10)}$-octalin **124** by *Hansenula anomala* results in the formation of only one isomer, specifically the compound **125** (36%, >99% ee).[162] In this case, the immobilization with acryloylpyrrolidine, acrylamide, and methylenebisacrylamide afforded a less diastereoselective immobilized microorganism.[163] In Scheme 39 is also reported the bioreduction of the 4,9-dimethyl-3,5-dioxo-$\Delta^{4(10)}$-octalin **126** with the yeasts *Rhodotorula rubra* or *Kloekera saturnus*. In this way, the diastereomeric mixture of hydroxy ketones **127** and **128** was obtained and the single isomers isolated (1.6–16.3% yield) with high optical purity (88 to >99% ee).[164]

Scheme 40



a. $R^1 = R^2 = CH_3$
b. $R^1 = CH_3$; $R^2 = CH_2CH_3$
c. $R^1 = CH_3$; $R^2 = (CH_2)_3CH{=}CH_2$
d. $R^1 = CH_3$; $R^2 = Ph$
e. $R^1 = R^2 = CH_2CH_3$
f. $R^1 = CH_2CH_3$; $R^2 = CH_2CH_2CH_3$

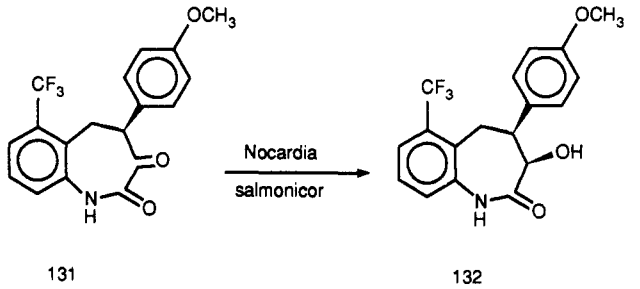
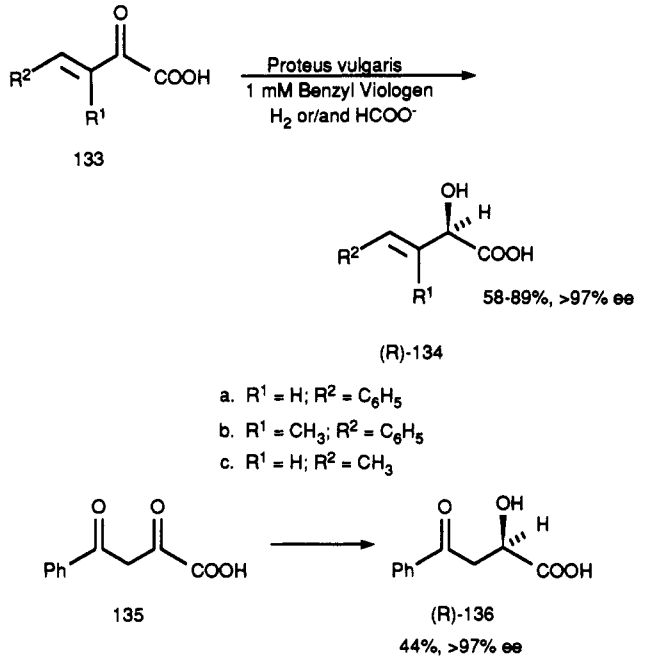


Table 6. Reduction of β-Diketones 129 by Microorganisms

		ketols				
substrate	microorganism	yield, %[a]	convn, %	ee, %	config	ref
129a	*G. candidum*	35	100	74	*S*	165a
	A. niger	30	75	95	*R*	165a
	bakers' yeast	90[b]	90	99	*S*	165a
	Mortierella isabellina	*c*	*c*	99	*S*	165b
129b	*G. candidum*	60[b]	100	99	2*R*	165a
	A. niger	*c*	100	99	2*R*	165a
	bakers' yeast	100[b]	100	99	2*S*	165a
	Mortierella isabellina	*c*	100	99	2*S*	165b
	Kloeckera apiculata	*c*	(50/50)[d]	96	*S*	165b
129c	*G. candidum*	65	100	99	2*R*	165a
	A. niger	*c*	75	95	2*R*	165a
	bakers' yeast	100[b]	100	99	2*S*	165a
129d	*G. candidum*	70	100	90	3*R*	165a
	A. niger	65	100	99	3*R*	165a
	bakers' yeast	80	(85/15)[e]	98	*S*	165a
129e	*G. candidum*	55	100	70	*R*	165a
	A. niger	65[b]	100	95	*R*	165a
	bakers' yeast	100[b]	100	30	*R*	165a
129f	*G. candidum*	70	(65/35)[f]	99	*R*	165a
	A. niger	70	100	99	3*R*	165a
	bakers' yeast	70	(33/67)[f]	98	*R*	165a
	A. foetidus	*c*	(69/31)[f]	99	*R*	165b
	Helminthosporium sp.	*c*	100	90	3*S*	165b
	Candida rugosa	*c*	(30/70)[f]	99	*R*	165b

[a] Isolated yields. [b] GLC yields. [c] Not reported. [d] 2-Hydroxy 4-ketone/4-hydroxy 2-ketone ratio. [e] 3-Hydroxy 1-ketone/4-hydroxy 2-ketone ratio. [f] 3-Hydroxy 5-ketone/5-hydroxy 3-ketone ratio.

Scheme 41



133 (R)-134 135 (R)-136

Several acyclic β-diketones **129** can be selectively reduced to the corresponding hydroxy ketones **130** by several microorganisms.[165a,b] The most significant results, including comparison with BY, are collected in Table 6. For a few diketones, also the diol is recovered optically pure.[165b] This is, for instance, the case of (2*S*,4*S*)-pentane-2,4-diol obtained from **129a** with *Mortierella isabellina* (55% yield, 99% ee).

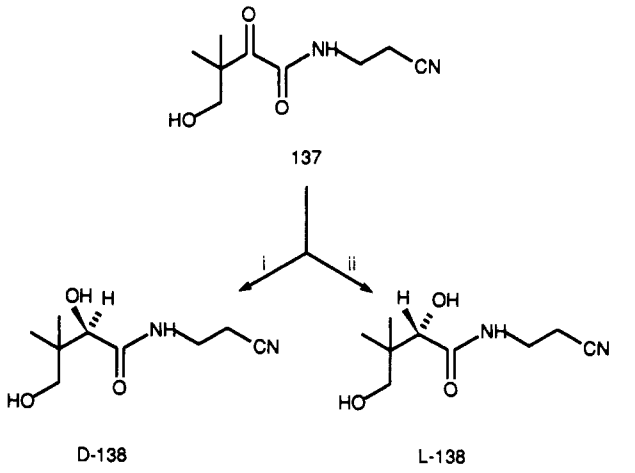
In Scheme 40, the bioreduction of the cyclic ketone **131** is also reported. In order to prepare the required hydroxy compound **132**, several strains of bacterial and yeast cultures were screened, and among these, *Nocardia salmonicolor* SC6310 was able to catalyze effectively the transformation of compound **131** to optically pure **132** (96% conversion yield at 1.5–2.0 g L^{-1} concentration).[166]

In some cases, a microorganism grown on a special medium can become specialized to carry out a single chemical reaction. Cyclohexanol-grown *Acinobacter* specie can promote regio- and stereoselective reduction of a cycloalkanedione like camphorquinone [84% of the 1:1 mixture of (+)-3-*exo*-hydroxybornan-2-one and (+)-2-*exo*-hydroxybornan-3-one, 72 and 85% ee, respectively].[167]

2. Keto Acids and Esters

Resting cells of *Proteus vulgaris* are very efficient in reducing structurally different 2-oxo acids to (*R*)-2-hydroxy acids in high yields and complete stereoselectivity.[168] The biocatalyst consists of wet-packed or freeze-dried cells of *P. vulgaris* which possess, in addition to a 2-hydroxy carboxylate viologen oxidoreductase, a hydrogenase and a viologen-dependent formate dehydrogenase (FDH) which can regenerate the reduced form of artificial electron mediators. A recent paper from the same group[169] shows that, using hydrogen gas or formate as reducing agents, *P. vulgaris* can reduce a variety of oxo acids. For example, the compounds **133a–c** and **135** are reduced to the corresponding (*R*)-hydroxy acids **134a–c** and **136** with ee > 97% (Scheme 41).

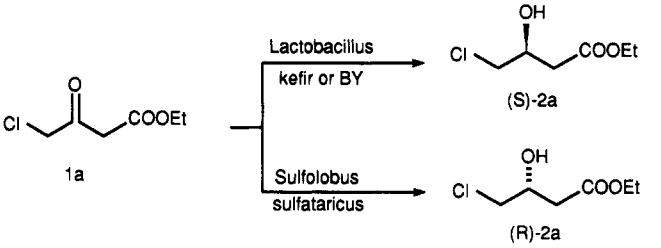
Scheme 42



137 D-138 L-138

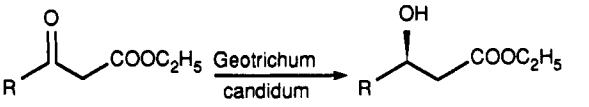
i. Sporidiobolus salmonicolor ii. Rhodotorula glutinis

Scheme 43



1a (S)-2a (R)-2a

Scheme 44



139 (R)-140

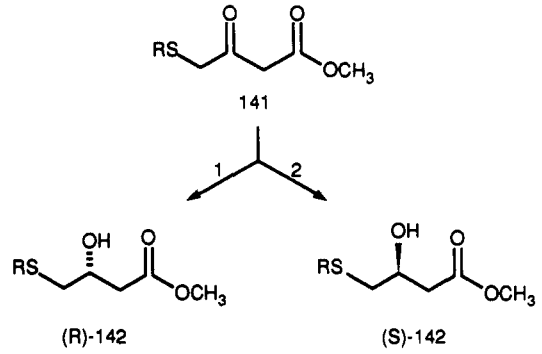
a. $R = CH_3$	75%, 96% ee
b. $R = CH_2CH_3$	52%, 99% ee
c. $R = nC_3H_7$	60%, 98% ee
d. $R = nC_5H_{11}$	21%, 90% ee

After a screening of several microorganisms, it was found that the reduction of 2′-ketopantothenonitrile (**137**) can proceed toward the D-(+)-pantothenonitrile (**138**) with *Sporidiobolus salmonicolor* (93.6% ee).[170] In a similar manner, the L-(−)-isomer **138** was obtained from **137** with *Rhodotorula glutinis* (Scheme 42).

One of the typical bioreductions of a substituted β-keto ester like **1** required the proper substrate manipulation, since BY afforded only partially resolved (*S*)-**2a** or optically pure (*R*)-**2b** (Scheme 3). The production of both ethyl (*R*)- and (*S*)-hydroxy esters **2a** is a problem which can be solved only by the use of different microorganisms. In fact, very recently it has been reported that resting cells of *Sulfolobus sulfataricus* reduce the keto ester **1a** to (*R*)-**2a**[171] and *Lactobacillus* strains can produce the (*S*)-**2a**,[172] both optically pure (Scheme 43).

β-Keto esters **139a–d** can be reduced by *Geotrichum candidum*, which can afford optically active (90–99% ee) hydroxy esters **140a–d**[173] (Scheme 44). In a detailed study on the incubation conditions, the authors state that for **140a,b** the enantioselectivity is markedly

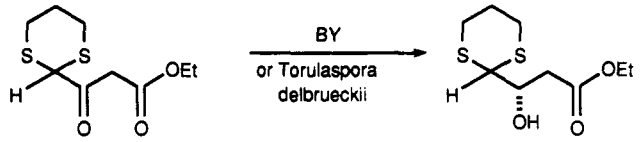
Scheme 45



1. Saccharomyces cerevisiae (30-67%, 50-73% ee)
2. Candida guilliermondii (8-32%, 80-90% ee)

a. R = CH_3CH_2 d. R = $CH_3(CH_2)_4$
b. R = $CH_3CH_2CH_2$ e. R = Ph
c. R = nC_4H_9 f. R = $pClC_6H_4$

Scheme 46



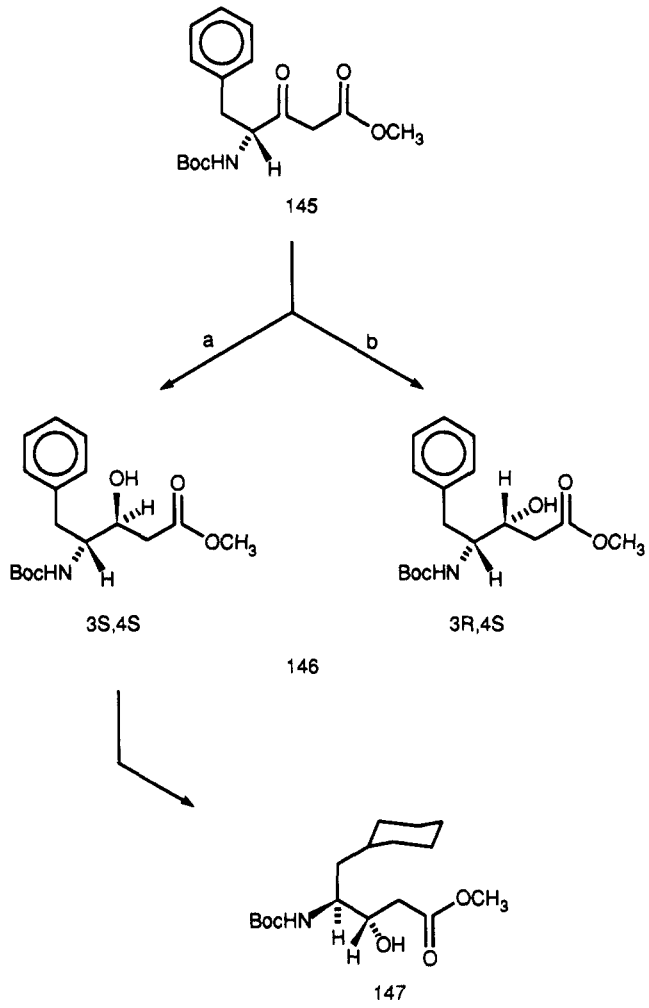
increased by preincubation of the mycelium before addition of the substrate. In the case of the product **140a**, the stereochemical outcome is opposite to the BY-mediated reduction. This is also the case of ethyl 4,4,4-trifluoro-3-oxobutanoate which was reduced with opposite stereochemistry by BY [45% yield of (*R*)-hydroxy ester, 76% maximum ee] and *Candida utilis* [34% yield of (*S*)-hydroxy ester, 59% maximum ee].[174]

Methyl 3-oxobutanoates bearing thioether functions at position 4, compounds **141a–f**, were reduced with different yeast strains, such as *Saccharomyces cerevisiae*, *Candida guilliermondii*, *Hansenula polymorpha*, and *Pichia membranaefaciens*.[175] Among these microorganisms, the best yields and ee for preparative purposes were obtained with pure strains of *S. cerevisiae* and *C. guilliermondii*. Interestingly, *S. cerevisiae* afforded the (*R*)-hydroxy esters **142a–f**, whereas the (*S*)-hydroxy esters **142a–f**[175] were formed with *C. guilliermondii* (Scheme 45).

Reduction with BY and *Torulaspora delbrueckii* of the β-keto ester **143** furnished the same (*S*)-**144** (95% ee in both cases, 78 and 58% yield, respectively)[176] (Scheme 46). Similarly, the enantioselectivity of BY-mediated β-keto esters reductions, i.e., conversion of the enolate **92** to malate **93**, was not improved by changing the microbial biocatalysts.[144]

BY reduction of the β-keto ester **145** gave unsatisfactory results, which were dependent on the origin and strain of the yeast, so that selected strains of yeasts of the genera *Kloeckera*, *Hansenula*, *Candida*, and *Torulopsis* were studied. Among these strains, it was found that the (3*S*,4*S*)-**146** (92% de) was formed by *Hansenula anomala*, whereas *Candida boidinii* reduced **145** to (3*R*,4*S*)-**146** (90% de).[177] The (3*S*,4*S*)-**146** was the right chiral intermediate for the synthesis of the statine analog **147** (Scheme 47).

Scheme 47

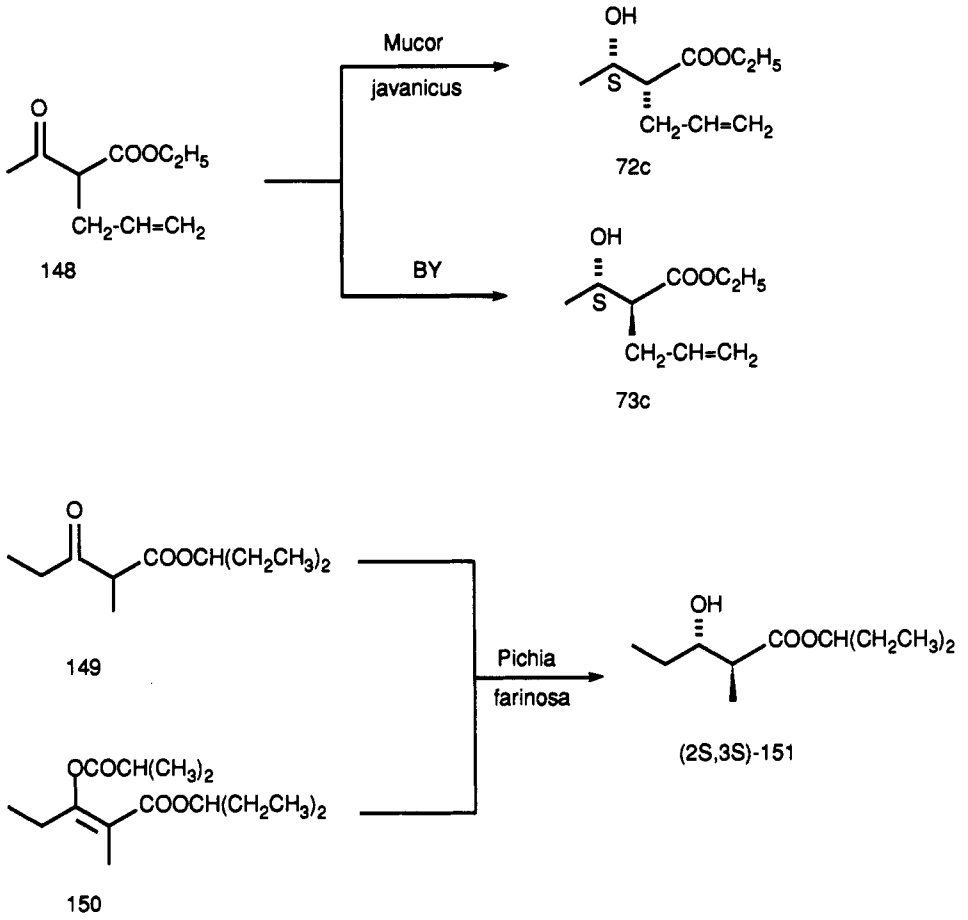


a. Hansenula anomala b. Candida boidinii

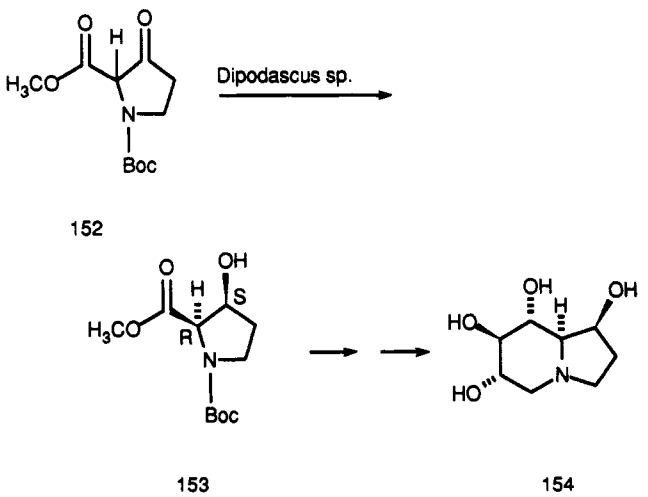
It has been already reported in the BY reductions, that from the α-allyl-β-keto ester **148** the *syn*-hydroxy ester **72c** is preferentially obtained with *Mucor javanicus*[129] and the *anti*-**73c** with BY.[128] The diastereoselectivity was not improved with *Candida tropicalis* or *Aspergillus oryzae*. The stereocontrolled reduction of α-methyl-β-keto esters by *Geotrichum candidum* for the preparation of diastereomerically pure **72a** and **73a** has been reported.[178] In all cited examples, the stereochemistry of the carbon bearing the hydroxy group is *S* (>98% ee). In Scheme 48, an interesting microbial reduction of the keto ester **149** and its enol ester **150** is also reported.[179] The reduction of the latest compounds by *Pichia farinosa* resting cells afforded moderate ee and de of the (2*S*,3*S*)-hydroxy ester **151**, an isomer of the aggregation pheromone (−)-sitophilate [(2*S*,3*R*)-**151**]. When growing cells of *P. farinosa* were used the ee of (2*S*,3*S*)-**151** reached 70 and 92% from **149** and **150**, respectively (65 and 63% yield). Inversion of the (3*S*)-hydroxy group and successive enzymatic hydrolysis (*Pseudomonas* lipase) of the chloroacetate of (2*S*,3*R*)-**151** afforded the natural isomer with enhanced ee (99%) and de (from 43 to 98%).

Also, in the synthesis of castanospermine **154** the required chiral building block was a protected 3-hydroxyproline similar to the already cited **81**.[137] The reduction of the pyrrolidinone **152** to the chiral intermediate **153** proceeded with the yeast *Dipodascus* sp.[180]

Scheme 48



Scheme 49



(Scheme 49) with higher optical yield (>99% de) than a similar reduction in the presence of BY.[181] In the latest case, the ethyl ester was used instead of the methyl ester **152**; the obtained chiral hydroxyproline (80% ee) had the same configuration as **153** and was converted into retronecine and related pyrrolizidine alkaloids.

In a more complex system, like the polyfunctionalized cyclopentane **155**, the microbial reduction to **156** through a resolution process with *Rhodotorula rubra* allows the preparation of the optically pure unreacted (−)-**155**.[182] The intermediate **155** was used as a chiral synthon for the synthesis of prostaglandin E_1 (Scheme 50).

3. Activated Double-Bond Hydrogenation

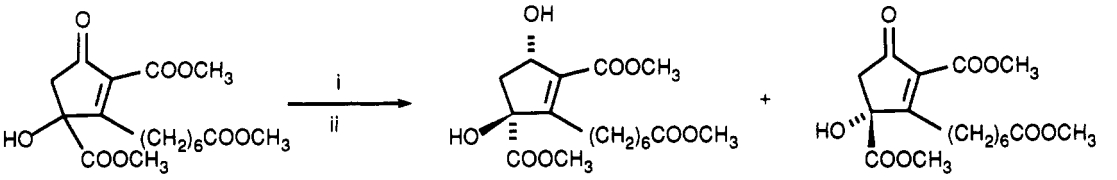
Several microorganisms are able to carry out the addition of hydrogen to unsaturated ketones in cyclic systems. This can happen with the simultaneous reduction of the carbonyl function, as in the case of the cyclohexenone **157** which was reduced with *Geotrichum candidum* leading to a single optically pure (1*S*,3*S*)-**158**.[183] An interesting example of microbial biohydrogenation of an unsaturated ketone system with no reduction of the carbonyl group has been described.[184] In this case, racemic abscisic acid (**159**) is hydrogenated in a highly stereoselective fashion. From the (*S*)-enantiomer, the corresponding chiral 2′,3′-dihydro derivative **160** is formed with cultures of *Aspergillus niger* (72% yield, >95% ee). The above biohydrogenations are described in the Scheme 51.

A few other biohydrogenations can be realized in the presence of microorganisms, but although very interesting in principle, they are not useful for synthetic applications, due to a complex mixture of products[185] or low enantiomeric excess.[186] (*Z*)-3-Fluoro-4-phenyl-1-(*p*-tolylsulfonyl)but-3-en-2-one can be reduced at the carbonyl group enantioselectively with *Geotrichum candidum* and *Phanerochaete chrysosporium*, but the biohydrogenation of the double bond is less facile and the diastereoselectivity low.[187]

D. Reductions Catalyzed by Plant Cell Cultures

The biotransformations catalyzed by plant cell cultures has been recently reviewed.[19-22] Early examples of biotransformations were, for instance, oxidative and reductive transformations of biogenetic-like compounds

Scheme 50



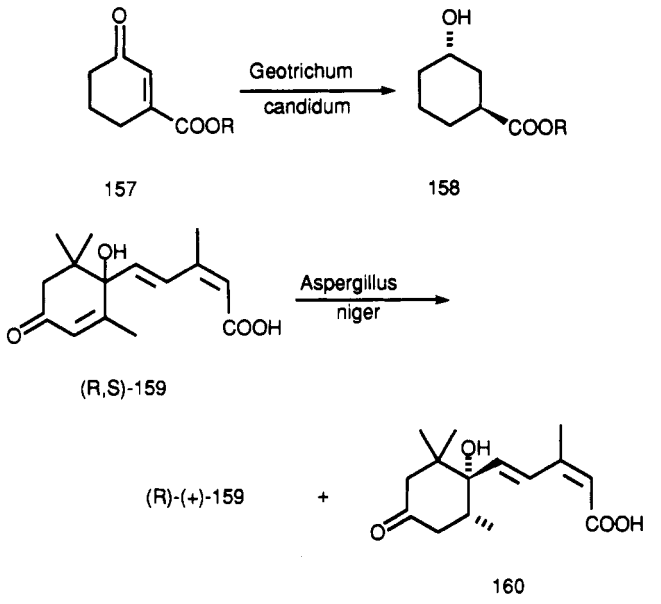
(±)-155 (+)-156 (-)-155

i. Rhodotorula rubra 48%, >99% ee 46%, >99% ee

Schizosaccharomyces octosporus 14%, >99% ee 80%, 8% ee

ii. CH_2N_2

Scheme 51

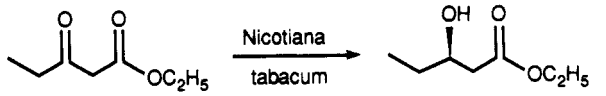


157 158

(R,S)-159

(R)-(+)-159 + 160

Scheme 52



139b (R)-140b

of the terpene series. Thus, these transformations were performed on *p*-menthane derivatives with cultured cells of *Nicotiana tabacum*[188] or bicyclo[2.2.1]- and bicyclo[3.1.1]heptane derivatives with the same biocatalyst.[189] Early applications of plant cells to the biotransformation of synthetically important foreign compounds are for instance the reduction of β-keto esters.[190] In this case, immobilized cells of *Nicotiana tabacum* on calcium alginate beads were used, and the method suffered with low yields of the hydroxy esters. In the case of (*S*)-3-hydroxybutanoates the ee was often high and the stereochemistry was identical to the one realized with BY. Ethyl 3-oxopentanoate (**139b**) affords with the above plant cells (*R*)-**140b** with 94–99% ee (12–15% yield)[192] (Scheme 52). The same substrate has been previously reduced by BY and *Thermoanaerobium brockii* to give respectively *R*- and *S*-isomers (40% and 84% ee, respectively).[191]

E. Dehydrogenase-Catalyzed Reductions

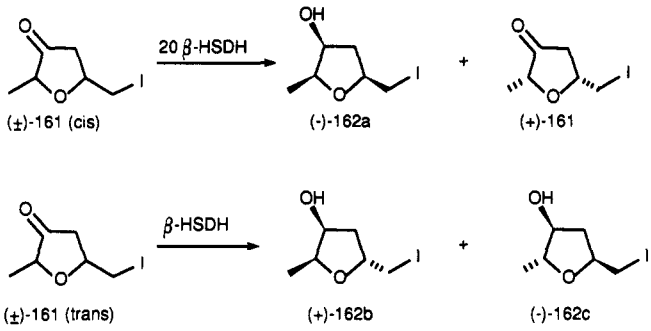
The class of enzymes which can catalyze useful asymmetric reductions is that of oxidoreductases and many purified enzymes are already commercially available. The main disadvantage of these biocatalysts is that they need expensive cofactors, although many methods are currently available for their efficient regeneration. Besides the previously cited general references on the use of enzymes, the application of dehydrogenases for the synthesis of chiral compounds has been recently reviewed,[193,194] and a special report on the dehydrogenase from a thermostable bacterium *Thermoanaerobium brockii* (TBADH) has appeared.[195] It should also be mentioned that, if an alcohol dehydrogenase and its cofactor are coimmobilized on different supports, the reductions catalyzed by these enzymes can be performed in organic solvent and the regeneration of the cofactor is feasible.[196,197] Most of the applications of dehydrogenases are related to the reduction of prochiral carbonyl compounds in order to prepare optically active hydroxy compounds. An interesting report studying the influence of addition of NADH and NADPH to the dehydrogenases present in whole cells and extracts by BY has been published. Parameters such as biotransformation rates and enantioselectivity have been examined.[198]

1. Acyclic and Cyclic Ketones

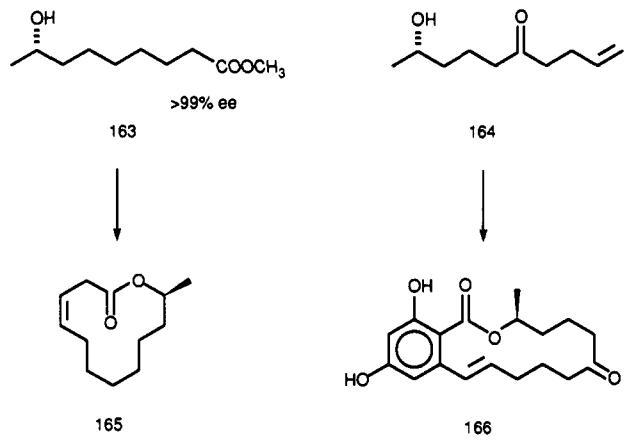
The use of horse liver alcohol dehydrogenase (HLADH) in asymmetric synthesis is identified with the name of J. B. Jones, who has deeply explored the potentiality of this enzyme for the synthesis of a great number of chiral hydroxy compounds.[7] The classical reduction of methyl ketones to chiral secondary alcohols can be efficiently realized with alcohol dehydrogenases. Results have been recently reported on the kinetic evaluation of binding and activation parameters which control the substrate specificity and stereoselectivity of HLADH, using unbranched, acyclic secondary alcohols and ketones.[199] For instance, acylfurans are reduced in the presence of TBADH[200] and acetophenone by an enzyme from *Lactobacillus kefir*.[201] A new NAD-dependent alcohol dehydrogenase from a *Pseudomonas* specie has been recently isolated.[202] This enzyme is capable of catalyzing a highly enantioselective transfer of the *pro*-(*R*)-hydrogen of NADH to the *si* face of the carbonyl group of acyclic ketones, with an "anti-Prelog" stereochemical outcome.

The stereochemical course of HLADH-catalyzed reduction has been studied on substituted cyclohexanones under varying conditions.[203] A few applications of HLADH to the reduction of various cyclic ketones

Scheme 53



Scheme 54



can be found in the recent literature.[204,205] The enzymes steroid dehydrogenases, which catalyze also the reduction of nonsteroidal type of carbonyl compounds,[206,207] have found additional applications in the chemoenzymatic synthesis of all the stereoisomeric muscarines.[208] Diastereoselective reductions of the compound (±)-**161** cis and (±)-**161** trans were achieved using commercially available 3α,20β- or 3β,17β-hydroxysteroid dehydrogenase (20β-HSDH or β-HSDH), which require as cofactor NADH, in situ regenerated from formate in a continuous reaction catalyzed by formate dehydrogenase (FDH). The optically active diastereomers **162a–c** and (+)-**161** were used for the synthesis of the remaining four stereoisomers (Scheme 53).

2. Keto Acids and Esters

A few examples of applications of dehydrogenases ad hoc purified from microorganisms and not commercially available can be found for the reduction of α-keto lactones[209] or an α-substituted β-keto ester, i.e., ethyl 2-allyl-3-hydroxybutanoate (**72c**).[117,210] A glycerol dehydrogenase has been purified from *Geotricum candidum* and can be used for the asymmetric reduction of keto esters.[211] A peculiar application of the above enzyme is that the reduction to optically pure ethyl (*S*)-4-chloro-3-hydroxybutanoate (**2a**) can be realized. It should be mentioned that commercially available glycerol dehydrogenase from *Enterobacter aerogenes* or *Cellulomonas* sp. had been previously used for the reduction of α-hydroxy ketones to optically active diols.[212] In Scheme 54 are reported two applications of reductions catalyzed by TBADH. Methyl 8-oxanonanoate is reduced to methyl (*S*)-(+)-8-hydroxynonanoate (**163**; 80% yield, >99% ee), a chiron for the total synthesis of ferrulactone II (**165**).[213] Dec-9-ene-2,6-dione is reduced to (*S*)-(+)-9-hydroxydec-1-en-5-one (**164**), a trifunctional chiron utilized for the total synthesis of (*S*)-(−)-zearalenone (**166**).[214] The same enzyme has been reported to catalyze the reduction of a bicyclic ketone **227** with low enantioselectivity and no diastereoselectivity.[215]

Various α-keto acids **167a–d** can be efficiently and enantioselectively reduced to either (*S*)- or (*R*)-α-hydroxy acids **168a–d** using commercially available L- and D-lactate dehydrogenases (L- and D-LDH)[216,217] (Scheme 55). Further, the optically pure (*R*)-(+)-hydroxy acid **170** can be prepared from the corresponding α-keto acid **169** (Scheme 56) using D-hydroxyisocaproate dehydrogenase isolated from *Lactobacillus casei* with cofactor regeneration.[218]

3. Amino Acid Synthesis

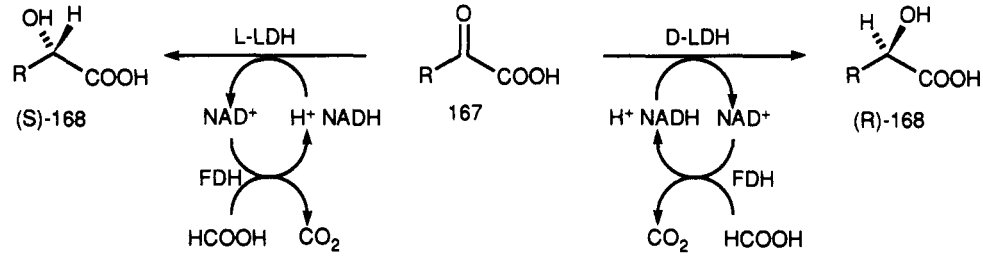
Amino acid dehydrogenases (AADH) catalyze the formation of keto acids from chiral α-amino acids and are cofactor dependent.[193] Synthetic applications of the above enzymes can become important when they catalyze the reverse formation of chiral amino acids **172** from keto acids **171** (Scheme 57). The problem of the regeneration of the cofactor can be solved, for instance, with the use of FDH, in the case of phenylalanine dehydrogenase for the production of optically pure **172a–c**.[219] In this way, the commercially important, nonnatural L-2-amino-4-phenylbutanoic acid (**172b**) can be enzymatically prepared from the corresponding α-keto acid **171b**.[220] For the preparation of optically pure (2*R*,3*R*)- and (2*R*,3*S*)-3-fluoroglutamic acids, glutamate dehydrogenase and yeast alcohol dehydrogenase for the NADH regeneration were used.[221]

IV. Oxidations

A. General Remarks

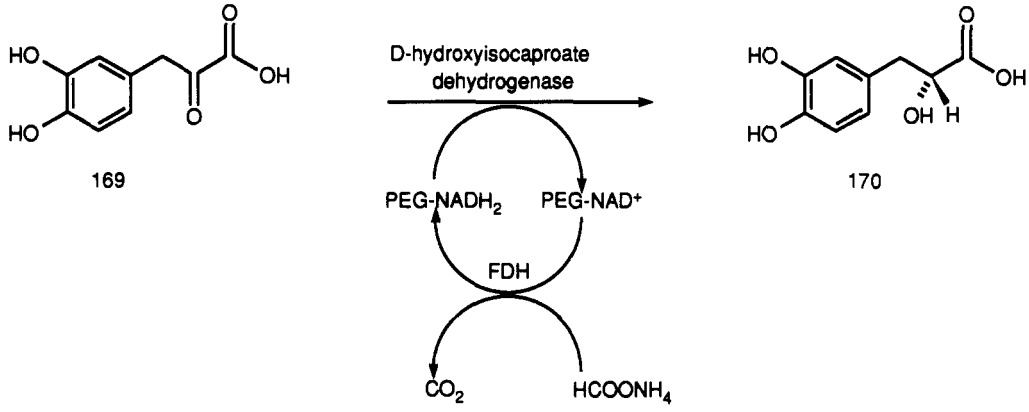
The biological oxidations are generally catalyzed by oxidoreductases, and the purified enzymes quite often need cofactors for their catalytic activity. As for reductions, the biocatalytic approach to the oxidations relies much on the use of microorganisms, since these crude systems are able to make use of the complex machinery of enzymes and coenzymes during their metabolic cycle. Examples of industrial applications have been reported.[222] In some cases, as already cited in the reductions section, commercially available dehydrogenases are also able to carry out the oxidations on a wide variety of substrates and can be considered as a sophisticated "off-the-shelf" reagent.[62] For the enantioselective preparations of chiral synthons, the most interesting oxidations can be the hydroxylations of unactivated saturated carbons or carbon–carbon double bonds in alkene or arene systems, together with the oxidative transformations of various chemical functions. A brief mention will be given here to the biocatalytic oxidative coupling of two molecules, which can give rise to the synthesis of complex bisindole alkaloids, such as anhydrovinblastine.[223] In this case an immobilized enzyme system from cell-free extract of *Catharanthus roseus* was used, while, in another example, from *Berberis stolonifera* the activity of a cytochrome P-450 catalyzed the intermolecular phenol oxidative coupling of two isomeric benzylisoquinoline alkaloids.[224] Purified oxidizing enzymes, such as mush-

Scheme 55

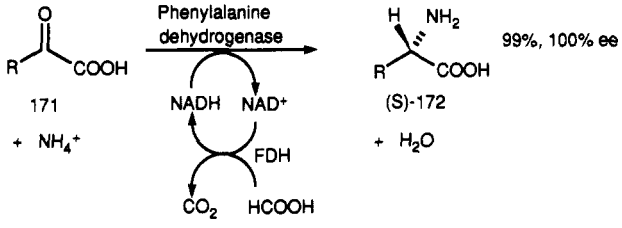


a. R = CH_3CH_2
b. R = $CH_3CH_2CH_2$
c. R = Cyclopropyl
d. R = $PhCH_2$

Scheme 56



Scheme 57



a. R = CH_2Ph
b. R = CH_2CH_2Ph
c. R = C_7H_{15}

room tyrosinase[225] or horseradish peroxidase,[226] are able to catalyze oxidative formation of oligomeric compounds from suitable precursors.

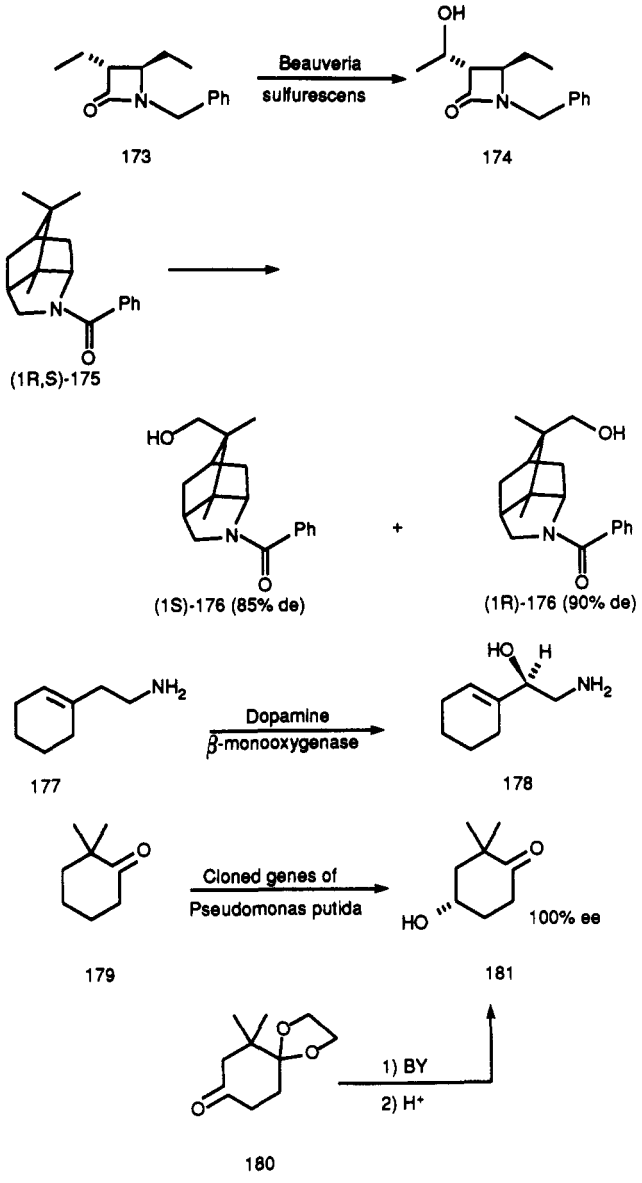
B. Hydroxylation of sp^3 Carbons

There are several reports on the enantioselective introduction of an hydroxyl function by oxidative cleavage of a carbon–hydrogen bond. These oxidations are frequently carried out by means of microorganisms, since the purified enzymes are coenzyme dependent, and therefore, the problem of recycling the expensive cofactor has to be considered in the application of these biocatalysts. Although interesting, the oxidation of α-pinene with *Acetobacter methanolicus* affords oxidized products with maximum 62% ee.[227] In the case of β-lactams, the fungus *Beauveria sulfurescens* is able to perform regioselective and sometimes stereoselective biotransformations.[228] This is especially significant when the process is applied to compounds like the β-lactam **173** shown in Scheme 58. The hydroxylation to the secondary alcohol **174** (10% yield) was in competition with the result of a debenzylation process. In a detailed study, it has been shown that, among other biotransformations, the same fungus furnished also a good example of stereoselective hydroxylation of a methyl group in a cyclic system like compound **175**. The regioselectivity of the process is governed by the absolute configuration of the substrate **175**, and the main products of oxidation were the optically active alcohols (30% yield) (1*S*)- and (1*R*)-**176** (85 and 90% de, respectively).[229]

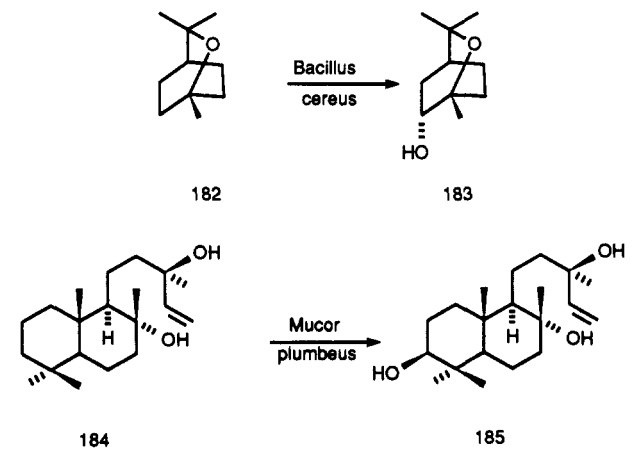
The allylic oxidation can be catalyzed by a purified enzyme, dopamine β-monooxygenase. This enzyme catalyzed the allylic oxygenation of 2-(1-cyclohexenyl)-ethylamine (**177**) to the corresponding alcohol **178** as the only product.[230] The cyclohexanone **179** could be converted to optically pure **181** by a hydroxylation catalyzed by P-450 camphor monooxygenase of the cloned genes of *Pseudomonas putida* PpG1 (100% ee, 34% yield). The same (*S*)-**181** could be prepared optically pure by the much simpler BY reduction of the monoprotected diketone **180**, followed by an acid hydrolysis (100% ee, 59% yield).[231]

The stereospecific hydroxylation by *Bacillus cereus* of 1,8-cineole (**182**) to the hydroxy derivative **183** (Scheme 59) is a good example of oxidation of unactivated carbon in a bicyclic skeleton.[232] Another recent example is the quantitative oxidation of sclareol (labd-14-en-8α,13β-diol, **184**) to a mixture of triols. Among several microorganisms tested, *Mucor plumbeus* afforded the best results (84% yield) of the pure 3β-hydroxy derivative **185**.[233] The allylic oxidation of bicyclic compounds (Scheme 60) like **186**[234] and **188**[235] with selected microorganisms could be performed in a highly regio- and stereoselective manner. The oxidation of **186** with *Rhizopus arrhizus* gave the diol **187** (63%

Scheme 58

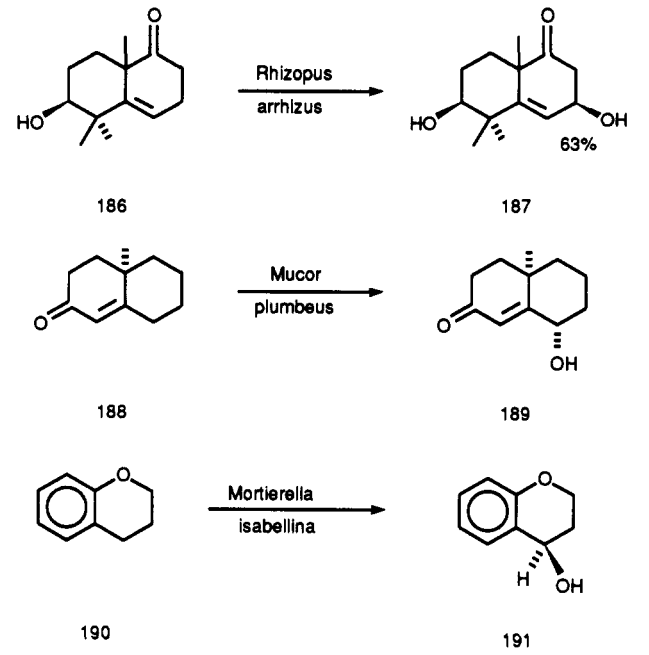


Scheme 59



yield), and the highest yield of the oxidation product **189** was obtained by oxidation of **188** with *Mucor plumbeus*. *Mortierella isabellina* oxidizes at the benzylic position the chroman **190** to (*R*)-chroman-4-ol (**191**) with high ee (>98%) but low yields (10%). The same fungus oxidizes the thiochroman only to the corresponding sulfoxide.[236]

Scheme 60



C. Soybean Lipoxygenase

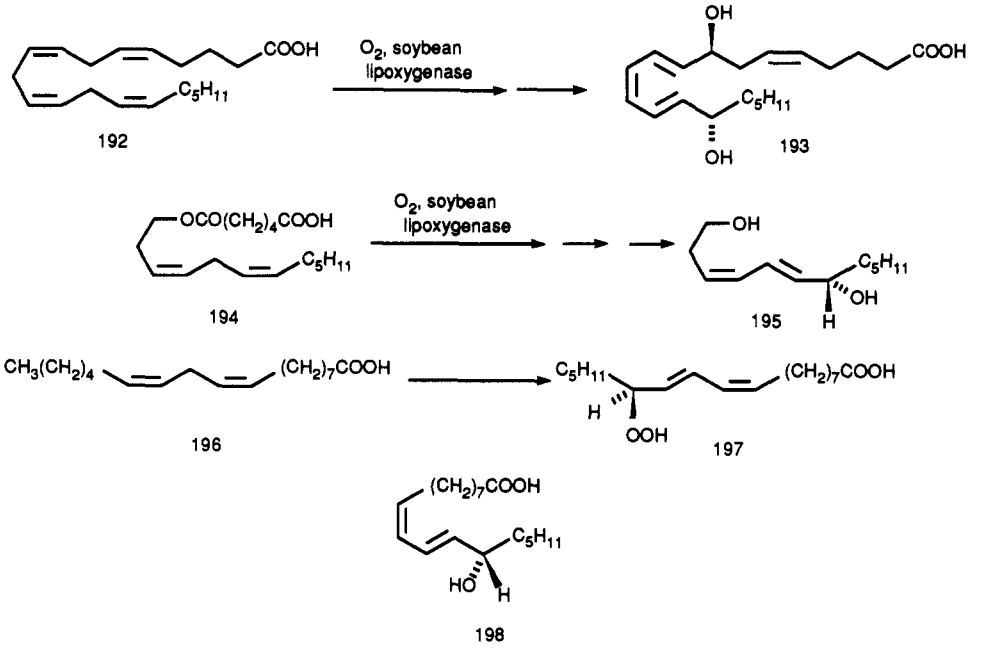
This commercially available oxidoreductase (E.C. 1.13.11.12) does not need a cofactor, and this renders its use very attractive. Its natural substrate, arachidonic acid (**192**) has been already used for a practical synthesis of the diol **193**, a chiral intermediate for the synthesis of (7*E*,9*E*,11*Z*,13*E*)-(5*S*,6*R*,15*S*)-trihydroxyeicosatetraenoic acid (6*R*-lipoxin A).[237] In the Scheme 61 a few examples of applications of the stereoselective oxidation catalyzed by the above enzyme are collected. Thus, the asymmetric hydroxylation of synthetic adipate ester **194** to the diol **195** can be achieved, after in situ reduction of the intermediate hydroperoxide. In this example, the adipoyl prosthesis provides the additional lipophilic scaffold required for the enzyme recognition of the substrate.[238,239] The (13*S*)-hydroperoxide **197** can be prepared from linoleic acid (**196**) by the same enzymatic approach.[240] Oxidation catalyzed by soybean lypoxygenase immobilized with oxirane acrylic beads, followed by sodium borohydride reduction gave (13*S*,9*Z*,11*E*)-13-hydroxyoctodeca-9,11-dienoic acid (**198**), which was used for the synthesis of some macrocyclic C_{13}-lactones.[241]

D. Epoxidation and Dihydroxylation of Alkenes

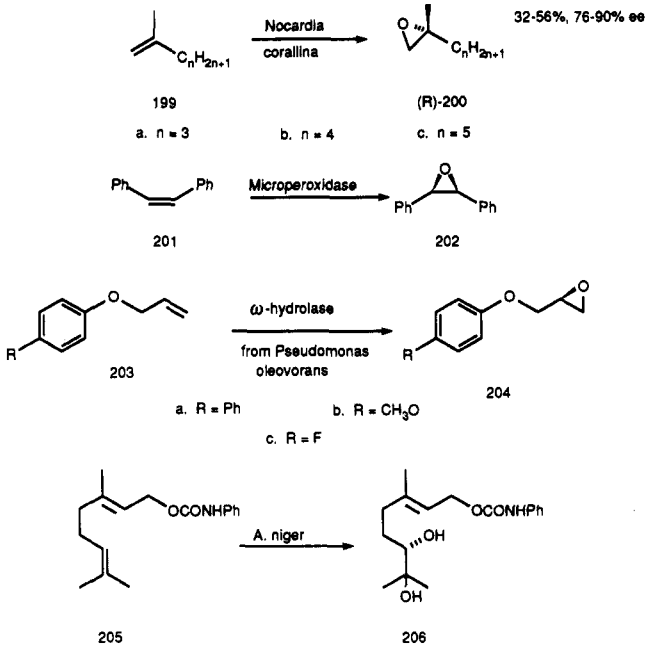
The olefinic double bond can be oxidized biocatalytically also in absence of any special activating group present in the molecule (Scheme 62).

Thus, the microorganism *Nocardia corallina* can oxidize alkenes like **199a–c** to the optically active 1,2-epoxy-2-methylalkanes **200a–c**, useful precursors of tertiary alcohols, which were converted to prostaglandin ω-chain.[242] Microperoxidase-11, an undecapeptide prepared by enzymatic hydrolysis of cytochrome *c*, is an effective biocatalyst for sulfide and olefin oxidation, as well as amine N-demethylation.[243] An example is the action of this peptide on diphenylethylene (**201**) to afford essentially only the *cis*-epoxide **202**. Several microorganisms may perform this kind of oxidative transformation. Allylic alcohol ethers are asymmet-

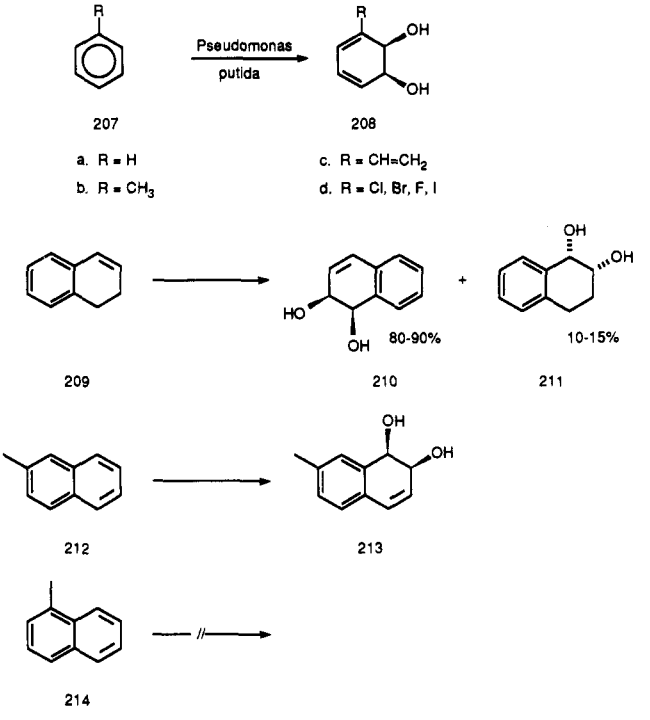
Scheme 61



Scheme 62



Scheme 63

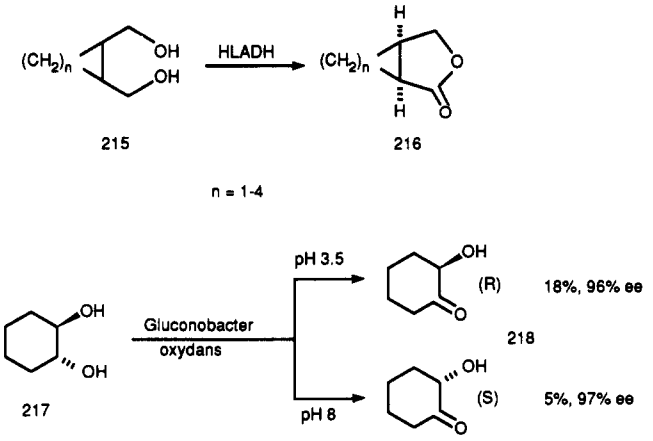


rically epoxidized by ω-hydroxylase from *Pseudomonas oleovorans* to the corresponding epoxides. The highest ee (92–99%) were obtained from **203a–c**, which were oxidized to the (*R*)-epoxides **204a–c** (3–4% yield).[244] The *Aspergillus niger* corn steep–liquor medium gives access to the regiospecific and asymmetric oxidation of the remote double bond of geraniol (**205**) for the preparation of the (*S*)-diol **206** (49% yield, >95% ee).[245]

E. Aromatic Compounds

A mutant strain of *Pseudomonas putida* (Pp 39D) can be used as a biocatalyst to oxidize aromatic compounds into diols on a preparative scale.[246] Although the acting enzyme in the aromatic oxidation is unknown, *Escherichia coli* has been commented on for improvement of the efficiency.[247] For example, the bio-catalytic oxidation of the aromatic ring of benzene derivatives **207a–d** affords the optically pure *cis*-diols **208a–d** and is a viable biotransformation for the synthesis of several chiral compounds (Scheme 63). Thus, the microbial oxidation of benzene **207a** leads to **208a**, which is the intermediate for the synthesis of *myo*-inositol 1,4,5-triphosphate and related derivatives,[248] or the two stereoisomers of pinitol[249] and of conduritol.[250,251] *cis*-Cyclohexadienediol (**208b**) obtained by the microbial degradation of toluene **207b** is also a versatile chiral pool intermediate for the formal total synthesis of an inositol analog[252] and the prostanoid compound, $PGE_2\alpha$.[253] Besides the oxidation of styrene **207c**,[254] which affords a polyfunctional chiral synthon like **208c**, halobenzenes **207d** seem well suited for the oxidation process. The halogenated diols **208d**

Scheme 64



can be further elaborated to other *cis*-dihydrodiol derivatives[255,256] and are excellent chiral synthons for the preparation of carbohydrates,[257,258] pyrrolizidine alkaloids,[259] and cyclitols.[260-264] Interestingly, dihydronaphthalene **209** is also oxidized (80–90% yield) to optically pure diols **210** and **211**.[265] It should be noted that the diol **210** is an unexpected rearrangement product, whereas the *cis*-diol which should occur from the double-bond oxidation, namely **211**, was formed in only 10–15% yield. For substituted naphthalenes, the position of the substituent seems to play an important role.[266] In fact, 2-methylnaphthalene (**212**) is oxidized mainly to the diol **213** and 1-methylnaphthalene (**214**) is recovered untransformed. It has also been reported that methyl anthracenes are oxidized by the fungus *Cunninghamella elegans* to the corresponding *trans*-dihydro diol derivatives with 50–90% ee.[267]

F. Hydroxylated Compounds and Aldehydes

The oxidation of diols like **215** by horse liver alcohol dehydrogenase to the lactones **216** is feasible on a preparative scale[62] (Scheme 64). The method also applies to bicyclic diols, and changes of coenzymes have also been reported for the improvement of oxidations by alcohol dehydrogenase.[268] An interesting example of pH-dependent stereoselective oxidation has been reported for racemic *trans*-1,2-cyclohexanediol (**217**), which could be oxidized by *Gluconobacter oxidans* either to (*R*)- or (*S*)-**218**, depending on acidic or basic conditions.[269]

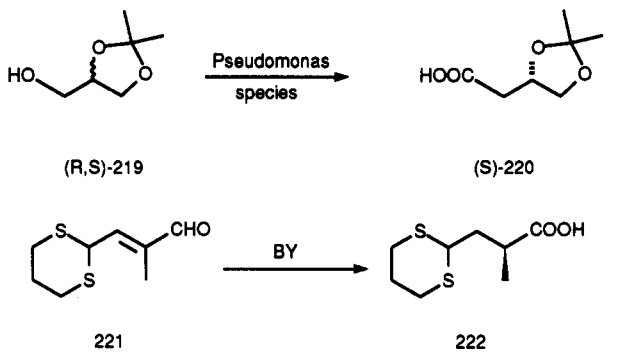
Oxidative degradation of compounds like glucose to chiral 2,3-butanediol can be achieved with several microorganisms, such as *Klebsiella oxytoca*[270] or *Bacillus polymyxa*.[271] An interesting example of resolution of the racemic isopropylidene glycerol **219**, which is not easily accomplished by resolution methods[272] can be realized via the enantioselective oxidation to the corresponding (*S*)-acid **220** with a *Pseudomonas* sp. as biocatalyst[273] (Scheme 65).

Finally, several α,β-unsaturated aldehydes and allylic alcohols or sulfur-functionalized prenyl derivatives type compound **221** are enantioselectively hydrogenated and oxidized with bakers' yeast to give bifunctional chiral C-5 building blocks, like compound **222**.[274]

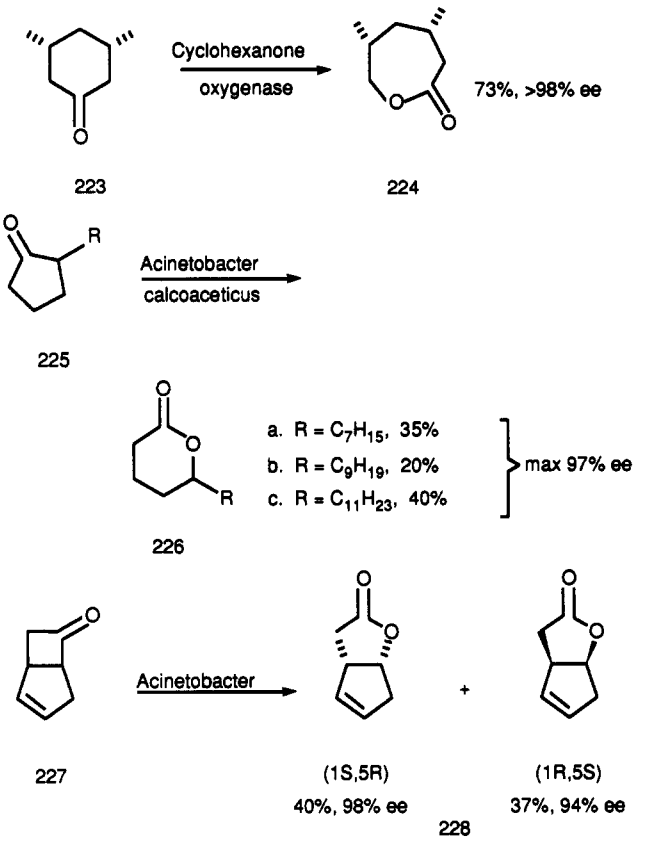
G. Baeyer–Villiger Oxidations

There exist a few microorganisms, capable of growing on aliphatic molecules, which contain enzymes catalyzing Baeyer–Villiger reactions. These monooxygenases are involved in the breakdown of ketones to provide simpler organic molecules and can be used as a biocatalyst for the formation of enantiomerically pure lactones. A review on the subject has been recently published.[275]

Scheme 65

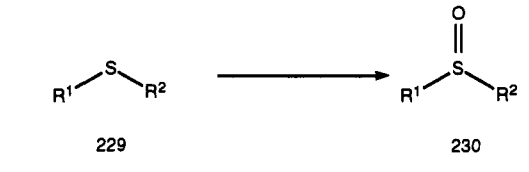


Scheme 66



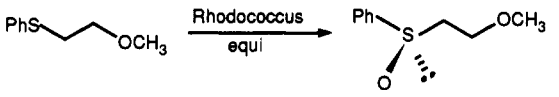
The enzyme most frequently used for these applications seems to be the NADPH-dependent cyclohexanone oxygenase which can oxidize the cyclic ketone **223** to the corresponding lactone **224**[276] (Scheme 66). The same oxidation could also be efficiently performed using the microorganism from which the enzyme is purified, i.e. *Acinetobacter calcoaceticus*.[277] Microbial transformations seem particularly suitable for the enantioselective biological Bayer–Villiger reaction. *Acinetobacter calcoaceticus* is able to oxidize substituted cyclopentanones like **225a–c** to the corresponding chiral lactones **226a–c**.[278] The yields were 20–40% and ee up to 97%. The same microorganism can effect the Baeyer–Villiger reaction of a bicyclic ketone **227**, to afford the lactones **228**.[279] Other bicyclic ketones can

Scheme 67



229 230

a. $R^1 = pCH_3C_6H_4$; $R^2 = tC_4H_9$
b. $R^1 = pBrC_6H_4$; $R^2 = CH_3$
c. $R^1 = Ph$; $R^2 = CH_2CH{=}CH_2$
d. $R^1 = pCH_3OC_6H_4$; $R^2 = CH_3$
e. $R^1 = PhCH_2$; $R^2 = CH_3$
f. $R^1 = Ph$; $R^2 = CH(CH_3)_2$



231 232

Table 7. Asymmetric Oxidation of Sulfides 229

substrate	biocatalyst	sulfoxide yields, %	sulfoxide ee, %	sulfoxide config	ref
229a	*A. niger*	20–25	91–100	*S*	285
229b	*M. isabellina*	66	100	*R*	285
229c	*C. equi*	38	100	*R*	285
229d	chloroperoxidase[a]	70	92	*R*	286
229e	chloroperoxidase[a]	51	91	*R*	287
229f	bovine serum albumine[b]	67	89	*R*	287

[a] In the presence of *t*-BuOOH. [b] Biomimetic reaction, dioxiranes as oxidant.

be oxidized by the same microorganism,[280-283] and a *Pseudomonas* specie grown on cyclopentanol can perform the same reaction on norbornanone.[284]

H. Sulfoxidation of Organic Sulfides

A review has been published, covering the subject of the biotransformation of sulfides to chiral sulfoxides,[285] which enjoy wide applications in organic synthesis as useful intermediates. The enantioselective oxidation of sulfides may be accomplished by means of microorganisms, such as *Aspergillus niger*, *Mortierella isabellina*, or *Corynebacterium equi*, capable of oxidizing, for example, the sulfides **229a–c** to the chiral sulfoxides **230a–c**.[285] By using *tert*-butyl hydroperoxide and chloroperoxidase, the highest ee (91–92%) was obtained for sulfoxides **230d,e** from **229d,e**.[286] An interesting biomimetic system, constituted by in situ generated dioxiranes and bovine serum albumin as a chiral auxiliary, affords with moderate enantioselectivity chiral sulfoxides.[287] The best result was obtained for the sulfoxide **230f** (89% ee) from the sulfide **229f**. The results are collected in the Scheme 67 and Table 7. Some pure enzymatic oxidations have also been recently reported. Thus, the *Pseudomonas oleovorans* monooxygenase, which is known to carry out hydroxylation at the terminal methyl of alkanes as well as epoxidation of terminal olefins, catalyzes the stereoselective sulfoxidation of methyl thioether substrates (88% ee).[288] Apparently, this is the first clear example of oxygenase-produced chiral aliphatic sulfoxides. The enzyme catalyzes this reaction in addition to oxygenative O-demethylation of branched vinyl methyl ethers. *Rhodococcus equi* seems to realize a highly enantioselective transformation of 2-alkoxyethyl sulfides, for example **231**, to the corresponding chiral sulfoxide **232**.[289] An interesting example of chiral sulfoxidation by BY has been reported for *S*-benzyl-8-mercaptooctanoic acid methyl ester, although the ee is about 70%.[290] Finally, a nonchiral interesting sulfur oxidation seems to be the BY-mediated conversion of thiocarbamates and ureas to the corresponding oxoderivatives[291] and the microbial degradation of (*R,S*)-2-oxothiazolidine-4-carboxylic acid allows the preparation of the optically pure (*S*)-isomer.[292]

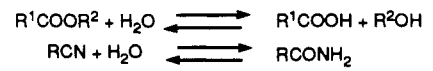
V. Hydrolysis

A. Introduction

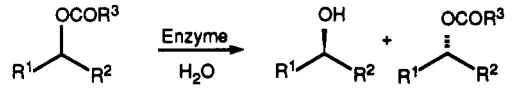
One of the most exploited chapters in biocatalysis certainly is the aqueous enzymatic hydrolysis of a racemate or desymmetrization of meso or prochiral substrates to achieve the enantioselective preparation of chiral compounds. A variety of commercially available enzymes belongs to the class of hydrolases, the third class of enzymes (an esterase is 3.1.1.*n*), which catalyze the reaction of cleavage of a given compound (i.e., an ester) by action of water into two molecules (i.e., an acid and an alcohol). This reaction of hydrolysis will be considered separately from the other hydrolytic reaction, catalyzed by other hydrolases, in which a molecule of water is added to a substrate like, for instance a nitrile, and only one molecule of product is formed (in this case an amide) (Scheme 68).

The hydrolytic enzymes do not need cofactors for their catalytic action and are often produced in bulk, since a few of them, i.e., lipases, proteases, and penicillin acylase,[293] have already found industrial applications. Many examples of their use in organic synthesis have been already reported in most of the cited books and reviews devoted to biocatalysis. The general problem of the resolution of enantiomers via biocatalysis has been recently treated[294] and frequently the enhancement of the enantioselectivity of an enzyme-catalyzed hydrolysis has been the subject of specific papers.[295-298] It should be mentioned also that microorganisms,[299,300] crude animal liver concentrate or acetone powder,[301,303] and monoclonal antibodies[304,305] are capable of catalyzing the stereoselective hydrolysis–resolution process. One of the more used hydrolases in organic synthesis is certainly pig liver esterase (PLE); its use for the preparation of chiral synthons by ester hydrolysis has been fully covered in 1989[306] and 1990.[307,308] Therefore, we will give here only the most recent applications of this very useful enzyme, for the catalytic action of which a space cubic model has been very recently proposed.[309] Finally, a very recent review has appeared dealing with the applications of esterolytic and lipolytic enzymes in organic synthesis.[310] In this work, it is possible to find a list of the most commonly used hydrolytic enzymes.

Scheme 68



Scheme 69



a. $R^1 = CH_3$; $R^2 = CH_2CH_2CH{=}C(CH_3)_2$; $R^3 = CH_2Cl$

b. $R^1 = CCl_3$; $R^2 = CH{=}C(CH_3)_2$; $R^3 = CH_3$

c. $R^1 = CHFCl$; $R^2 = Ph$; $R^3 = CH_3$

d. $R^1 = CHFCl$; $R^2 = Ph$; $R^3 = (CH_3)_2CH$

e. $R^1 = CHFCl$; $R^2 = CH_2Ph$; $R^3 = (CH_3)_2CH$

f. $R^1 = CHFCl$; $R^2 = CH_2CH_2Ph$; $R^3 = (CH_3)_2CH$

g. $R^1 = CH_2Cl$; $R^2 = CH(OC_2H_5)_2$; $R^3 = CH_3$

h. $R^1 = CH_2{=}CHCH_2OCH_2$; $R^2 = CH_2OTs$; $R^3 = CH_3$

Table 8. Enzymatic Hydrolysis of Acyclic Esters 233a–h

substrate	biocatalyst	alcohol yield, %	alcohol ee, %	alcohol config	ref
233a[a]	Lipase P30[c]	53	89	*R*	316
233b[a]	*B. subtilis*	38	≥98	*R*	317
233c[b]	CCL	28[d]	63	*S*	320
233d[b]	Lipase P[c]	32[d]	>98	*S*	320
233e[b]	Lipase P[c]	23[d]	>98	*S*	320
233f[b]	Lipase P[c]	31[d]	>98	*S*	320
	CCL	42[d]	9	*R*	320
233g[a]	Lipase LP-80[c]	46	>98	*S*	321
233h[a]	Lipase LP-80[c]	41	90	*R*	321

[a] The corresponding chiral acetates are obtained as follow: (*S*)-**233a** (45%, 100% ee); (*S*)-**233b** (40%, ≥98% ee); (*R*)-**233g** (51.5%, 98% ee); (*S*)-**233h** (41%, 94% ee). [b] All reported data refer to the major isomer. [c] Amano Pharmaceutical Co., Ltd. [d] Hydrolysis ratio (%).

B. Hydrolysis of Esters

1. From Acyclic Alcohols

There are almost no limits to the possible structures of racemic acyclic alcohols that can be enantioselectively resolved via the enzyme-catalyzed hydrolysis of the corresponding esters **233**. Generally, if the best conditions are met for the substrate, the yields of both enantiomerically pure alcohol and unchanged ester approach 50%. In the Scheme 69, the configurations of unreacted ester **233** and the alcohol produced **234** are arbitrarily chosen only to show that the reaction is a resolution process. In Table 8 the correct configurations are indicated. For these substrates, lipases are among the most used hydrolases and can be purchased from several companies, like Fluka (Switzerland), Sigma (United States) and others, which specialize in enzyme manufacturing like Amano (Japan) or Novo (Denmark). It should also be noticed that frequently the same enzyme has different names when they are purchases from different companies. In the following schemes and tables the names of the enzymes will be reported as found in the original paper. Sometimes a screening has to be performed among various enzymes in order to choose the most convenient procedure. For some esters of secondary alcohols is also possible to predict the stereochemical outcome of the hydrolysis by a rule based on the size of the substituents at the stereocenter.[311] In many cases, when the ee, although good, does not approach the desired limits of 95–98%, recrystallizations or repeated resolutions can lead to more satisfactory results. Sometimes, in the course of the

Scheme 70

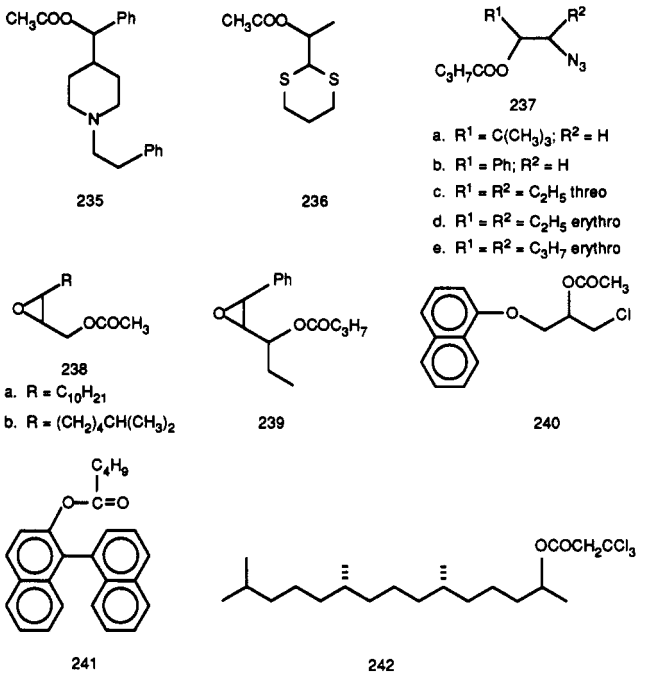


Table 9. Enzymatic Hydrolysis of Esters 235–241

substrate	biocatalyst	alcohol yield, %	alcohol ee, %	alcohol config	ester yield, %	ester ee, %	ester config	ref
235	*A. niger*	32[a]	≥97[a]	*S*	21	≥97	*R*	322
236	lipase	35	99	*R*	40	93	*S*	323
237a	CCL	35	>98	*R*	46	>98	*S*	324
237b	Lipase P[b]	40	>98	*S*	41	>98	*R*	324
237c	Lipase P[b]	37	>98	*R,R*	38	>98	*S,S*	324
237d	Lipase P[b]	39	>98	*R,S*	35	>98	*S,R*	324
237e	Lipase P[b]	28	>98	*R,S*	28	>98	*S,R*	324
238a	PPL	32	>95	2*S*,3*R*	*c*	*c*	2*R*,3*S*	326
238b	PPL	20	>95	2*S*,3*R*	*c*	*c*	2*R*,3*S*	326
240	Lipase P[b]	48	>95	*S*	45	>95	*R*	328
241	Lipase P[b]	40	99	*aS*	49	>99	*aR*	329

[a] Values obtained after a second enzymatic hydrolysis of the acetate obtained from the partially resolved alcohol. [b] *Pseudomonas* sp. (Amano Pharmaceutical Co., Ltd). [c] Not specified.

synthesis of peculiar structures, the use of hydrolases can help to solve problems of chemoselective transformations otherwise difficult to achieve chemically.[312] Acetates of secondary alcohols **233**, where the substituents are simple aromatic and alkyl groups can be resolved by means of lipase from *Pseudomonas* sp.[313,314] or PLE.[315]

Double bonds as in **233a**[316] or **233b**[317] are compatible with the mild hydrolytic conditions and the acetates are hydrolyzed with high enantioselectivity, whereas compounds containing a triple bond were resolved by several lipases with variable ee.[318] In the latest case, BY-mediated hydrolysis was much more enantioselective. Acetates of dihalogenotrifluoroethyl alcohols are resolved with variable ee.[319] The fluorochlorinated analogs **233d–f** are efficiently resolved by Lipase P into the alcohols **234d–f** (>98% ee), whereas lipase from *Candida cylindracea* (CCL) gives poorly resolved alcohols **234c,f**.[320] The trichloromethyl unsaturated ester **233b** is efficiently resolved by *Bacillus subtilis*.[317] Many chemical groups are compatible with the ester moiety that has to be hydrolyzed and therefore resolved, like a chloromethyl and acetal groups as in **233g** or a tosyl

Scheme 71

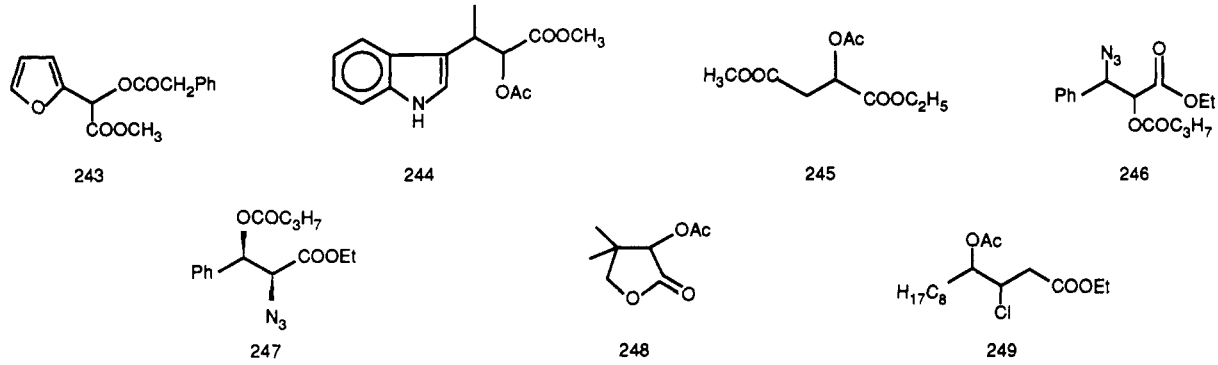


Table 10. Hydrolysis of Esters 243–249

		ester			alcohol			
substrate	biocatalyst	yield, %	ee, %	config	yield, %	ee, %	config	ref
243	penicillin acylase[a]	40	82	*R*	*b*	*b*	*S*	332
244	Lipase OF-360[c]	62	54	2*R*,3*S*	37	93	2*S*,3*R*	333
245	PLE	38	88	*S*	26	84	*R*	144
246 (34:46 *threo/erythro*)	Lipase P[d]	64	*b*	*e*	29	98[f]	2*S*,3*S*	334
247	CCL	26	>98	2*S*,3*R*	23	>98	2*R*,3*S*	335
248	lyophilized yeast	45	95	*R*	40	86	*S*	336
249	Lipase P[d]	*b*	*b*	*b*	31	94	3*R*,4*R*	337

[a] Immobilized on Eupergit C. [b] Not reported. [c] Meito Sangyo Co., Ltd, successively immobilized with ENTP-4000. [d] *Pseudomonas* sp. (Amano Pharmaceutical Co., Ltd). [e] Mixture of (2*R*,3*R*), (2*S*,3*R*), and (2*R*,3*S*)-isomers. [f] 98% de.

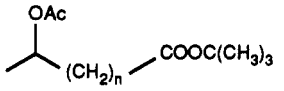
moiety as in **233h**.[321] The previous compounds are collected in Scheme 69. Among the various structures of esters resolved by lipases, one can mention an arylpiperidine derivative **235**,[322] or protected α- and β-hydroxyaldehydes like **236**.[323] Also an azide can be present like in compounds **237a–e**,[324] an epoxy ring[325] as in **238a,b**[326] and **239**,[327] naphthyl **240**,[328] and 1,1′-binaphthyl **241**[329] moieties. The esters of a long-chain saturated alcohol like **242** is a substrate for *Pseudomonas fluorescens* lipase (PFL), but the maximum ee is obtained after two consecutive resolutions.[330] The structures of the esters **235–242** are collected in the Scheme 70, and in Table 9 are reported the data for the resulting alcohols and unreacted esters.

2. From Hydroxy and Enol Esters

The enzyme-catalyzed hydrolysis of the esters of the alcoholic function in hydroxy esters can proceed with high enantioselectivity, but in some cases the competitive hydrolysis between the O ester and the carboxylate group can be a main disadvantage from the point of view of chemical yields and optical purity of the products.[331] The hydrolysis of the phenylacetate of an α-hydroxy ester, compound **243**, can be realized with the aid of penicillin acylase immobilized on Eupergit C.[332] Another α-hydroxy ester which can be resolved by hydrolysis with a lipase (lipase OF-360 immobilized with the photo-cross-linked resin prepolymer ENTP-4000) is the racemic compound **244**.[333] The acetate of (*R*,*S*)-malate, compound **245**, can be hydrolyzed by PLE, and the best results were at 0 °C in 20% methanol.[144] The diastereomeric mixture of the butyrate **246**[334] and the *erythro*- **247**[335] are resolved by hydrolysis of the butanoate ester moiety with lipases. The *O*-acetyl pantoyl lactone **248** can be hydrolyzed with high ee by lyophilized *Saccharomyces cerevisiae* Hansen or a crude lipase from *Aspergillus* sp.[336] Also a hydroxy ester like ethyl 4-acetoxy-3-chlorododecanoate (**249**)[337] is a good substrate for the hydrolytic enzyme Lipase P to afford the corresponding hydroxy ester (31% yield, 94% ee). The structures of the esters **243–249** are reported in Scheme 71. Table 10 collects the data for the resulting alcohols and unreacted esters.

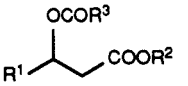
The hydrolysis of (acyloxy)alkanoates is complicated by the lack of chemoselectivity, and *tert*-butyl esters **250a–d** were prepared to block the hydrolytic action at the carbon terminus.[338] Also, the choice of a different lipase (from *Geotrichum candidum*) allowed the selective hydrolysis of secondary acetates or butyrates **251a,b** to prepare (*S*)-3-hydroxyalkanoates (84 and 92% ee).[339] The acetate of ethyl 3-hydroxy-3-phenylpropionate (**251c**) was resolved by a lipase to afford (*S*)-ethyl 3-hydroxy-3-phenylpropionate with 95% ee.[340] This hydroxy ester was the chiral intermediate for the synthesis of the antidepressant (*R*)-(−)-Thiazesim and could be prepared also in 85% ee by BY reduction of ethyl benzoylformate.[134] The BY-mediated hydrolysis of 3-acetoxy esters[299] has been applied to the furane derivative **252a** (82% ee),[176] but lipases of various provenience still remain the preferred biocatalyst for these resolutions. This is not true for the difluoro ester **252b**,[341] and this result is in contrast with the data from other acylated derivatives of hydroxy esters bearing fluorine atoms.[342] The diethylamide **252c** was successfully resolved by the a lipase-catalyzed hydrolysis of the acetate group.[341] Good results have been obtained for the hydrolysis of the acetates of other fluorinated hydroxy esters and hydroxy ketones. The hydrolytic resolution of the racemic *threo*-ester **253** is the key step for a stereocontrolled synthesis of 4,4,4-trifluorothreonine.[343] Similarly, the acetoxy difluoro and trifluoromethyl ketones **254**[344] and **255** afford optically active products.[345]

Scheme 72



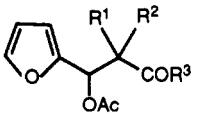
250

a. n = 0 c. n = 5
b. n = 1 d. n = 10



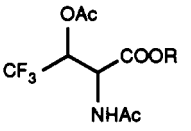
251

a. $R^1 = C_{11}H_{23}$; $R^2 = CH_3$; $R^3 = C_3H_7$
b. $R^1 = (CH_2)_4CH(C_4H_9)_2$; $R^2 = CH_3$; $R^3 = C_3H_7$
c. R^1 = Ph; $R^2 = C_2H_5$; $R^3 = CH_3$

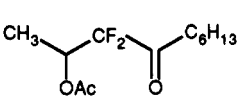


252

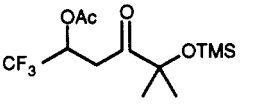
a. $R^1 = R^2$ = H; $R^3 = OC_2H_5$
b. $R^1 = R^2$ = F; $R^3 = OCH_3$
c. $R^1 = R^2$ = F; $R^3 = N(C_2H_5)_2$



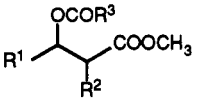
253



254

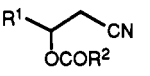


255



256

a. $R^1 = CH_3$; R^2 = Ph; $R^3 = CH_3$
b. R^1 = Ph; $R^2 = SCH_3$; $R^3 = CH_2SCH_3$
c. R^1 = Ph; $R^2 = CH_3$; $R^3 = CH_3$



257

a. $R^1 = CH_3$; $R^2 = CH_2SCH_3$
b. R^1 = Ph; $R^2 = CH_2SPh$
c. $R^1 = PhCH_2CH_2$; $R^2 = CH_2SPh$
d. R^1 = PhCH=CH; $R^2 = CH_2SPh$

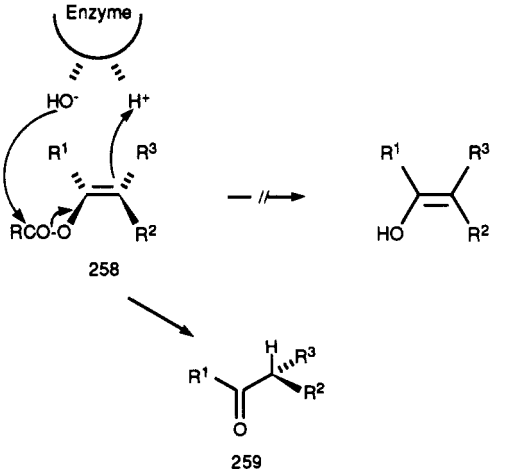
Table 11. Enzymatic Resolution of Secondary Alcohols

substrate	lipase	alcohol yield, %	alcohol ee, %	alcohol config	ref	substrate	lipase	alcohol yield, %	alcohol ee, %	alcohol config	ref
250a	*Pseudomonas* sp. K-10[a]	63[b]	>99	*R*	338	**254**	PFL P[a]	72[b]	39	*c*	344
250b	*Pseudomonas* sp. AK[a]	41[b]	>99	*R*	338	**255**	CCL MY[d]	27	>95	*R*	345
250c	*Pseudomonas* sp. AK[a]	31[b]	>99	*R*	338	**256a** (syn)	*Aspergillus* sp. A6[a]	23	96	3*R*	347
251a	*Geotrichum candidum*[a]	40[b]	84	*S*	339	**256b** (anti)	*Aspergillus* sp. A6[a]	38	>98	3*R*	347
251b	*Geotrichum candidum*[a]	40[b]	92	*S*	339	**256c** (anti)	*Aspergillus* sp. A6[a]	50	86	3*S*	347
251c	A[a]	*c*	95	*S*	340	**257b**	*Pseudomonas* sp. P[a]	43	94	*R*	350
252c	CCL MY[d]	61[b]	58	*R*	341	**257d**	*Pseudomonas* sp. P[a]	39	95	*R*	350
253 (rac. threo)	CCL MY[d]	37[b]	86	2*R*,3*R* (syn)	343						

[a] Amano Pharmaceutical Co., Ltd. [b] Extent of conversion (%). [c] Not reported. [d] Meito Sangyo Co., Ltd.

It has already been shown that optically active *syn*- or *anti*-α-methyl-β-hydroxy esters are released from the racemic *syn*- or *anti*-β-acetoxy esters by hydrolysis with lipases.[346] In addition to this example, the resolution of esters of other α-substituted-β-hydroxy esters **256a–c** has also been reported.[347] In Scheme 72, the structures of compounds **250–256** are reported and Table 11 collects the data for the described hydrolyses. Scheme 72 also contains the structures of several 3-hydroxy nitriles derivatives. In order to obtain optically active 3-hydroxy nitriles, the lipase-catalyzed resolution of 3-acetoxy derivatives did not proceed satisfactorily and the enantioselectivity of this reaction could be enhanced by addition of L-methioninol.[348] Changing the acetyl into a methylthioacetyl moiety, the racemic compound **257a** was resolved into optically pure ester (*S*)-**257a** and 84% ee 3-hydroxy nitrile.[349] Also phenylthioacetyl group was enantioselectively hydrolyzed. Various thioesters were examined and, among the most significant results, the compounds **257b–d** were efficiently hydrolyzed and therefore resolved (>98% ee for unreacted esters, >88% ee for 3-hydroxy nitriles).[350]

Scheme 73



258

259

A new, interesting enzymatic hydrolysis has been reported by Ohta.[351] The enantioface differentiation during the hydrolysis of an enol ester **258** proceeds with

Scheme 74

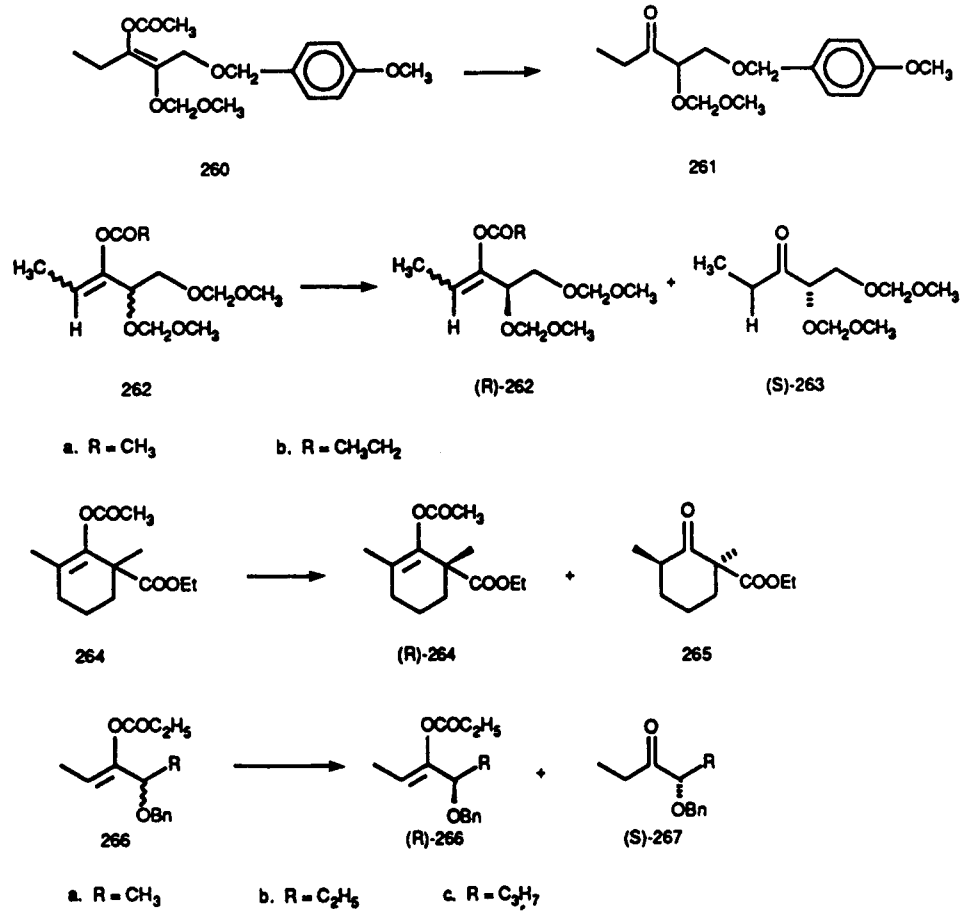


Table 12. Biocatalytic Hydrolysis of Enol Esters

biocatalyst	unreacted enol ester	yield, %	ee, %	config	ketone	yield, %	ee, %	config	ref
P. miso	**260**	*a*	*a*	*a*	**261**	83	85	*R*	353
B. coagulans	**262a**	33[b]	>95	*R*	**263a**	64	42	*S*	354
B. coagulans	**262b**	38	>95	*R*	**263b**	43	53	*S*	354
Lipase OF[c]	**264**	32	>99	*R*	**265**	54	47	2*S*,6*R*	355
Lipase OF[c]	**266a**	12	>99	*R*	**267a**	72	16	*S*	356
Lipase OF[c]	**266b**	27	>99	*R*	**267b**	54	53	*S*	356
Lipase OF[c]	**266c**	27	>99	*R*	**267c**	36	51	*S*	356

[a] Not reported. [b] Determined by GLC. [c] Meito Sangyo Co., Ltd.

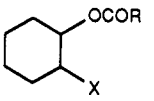
simultaneous proton attack and acyl group elimination (Scheme 73). In an initial approach, the ketone **259** is formed without the intermediacy of the free tautomeric enol, and thus stereospecifically, only when the microorganism *Pichia miso* was used as biocatalyst. Other microorganisms and commercially available enzymes tested afforded only a racemic **259**.[352]

Synthetic applications of the method have been reported by the authors, using either *Pichia miso* for compound **260**[353] or another microorganism, *Bacillus coagulans*, for the enantioface-differentiating hydrolysis of the enol ester **262a,b**.[354] Finally, the *Candida cylindracea* lipase (Lipase OF) was found to be a good catalyst for this reaction, which has been successfully applied to other enol esters. Also the cyclic compound **264**[355] and the enol esters **266a–c**[356] are substrates for the reaction. The chiral α-hydroxy ketone derivatives **261**, **263**, **265**, and **267** have been prepared with ee's ranging from 16 to 85% (36–83% yields). In the biocatalytic process also unreacted enol esters **262**, **264**, and **266** can be recovered enantiomerically pure. All the results are collected in the Scheme 74 and Table 12.

3. From Cyclic Alcohols

Esters of cyclic alcohols bearing other chemical groups are efficiently hydrolyzed by various hydrolases. The class of 2-substituted cyclohexanols presents many examples of applications of this methodology. *trans*-Esters **268a–v** are efficiently hydrolyzed by lipases to afford in most cases the enantiomerically pure stereoisomeric couple of acetate and alcohol.[357–360] In the Scheme 75 and Table 13 are collected the above results. The esters of cyclohexenols **269**,[361] **270**[362] and the 2,3-(isopropylidenedioxy)-1-cyclohexanols **271a–c**[363] can be similarly resolved and the data are shown in the Scheme 76 and Table 14. The acetate of the ketal of 4-hydroxycyclopent-2-en-1-one **272**[364] and the cyclopentenone derivatives **273a,b**[365–368] are good substrates for microbial lipases, as the racemic tetrahydrofuran derivative **274**.[369] Also a few examples of heterocyclic compounds can be found (Scheme 77), like an inter-

Scheme 75



trans-268

a. X = Ph
b. X = $PhCH_2$
c. X = PhO
d. X = $PhCH_2O$
e. X = CH_3O
f. X = N_3
g. X = OCH_2CH_3
h. X = $CH(CH_3)_2$
i. X = $OC_{10}H_{21}$
j. X = Cl
k. X = Br
l. X = I
m. X = SCH_3
n. X = CH_2CH_3
o. X = $CH=CH_2$
p. X = C≡CH
q. X = N≡C
r. X = $N(CH_3)_2$
s. X = $NHCOOCH_2Ph$
t. X = $NHCOC_3H_7$
u. X = NO_2
v. X = CN

Table 13. Enzymatic Hydrolysis of *trans*-2-Substituted-cyclohexanols 268a–v

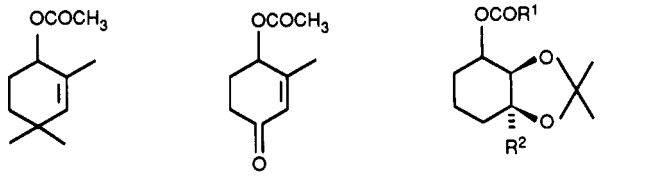
substrate		lipase	alcohol yields, %	alcohol ee, %	alcohol config	ester yields, %	ester ee, %	ester config	ref(s)
268a	R = CH_3	*Pseudomonas* sp.	20	98	*RS*	64	36	*SR*	357
	R = CCl_3		44	95	*RS*	43	97	*SR*	
268b	R = CH_3	*Pseudomonas* sp.	40	98	*RS*	46	83	*SR*	357
	R = CCl_3		46	>95	*RS*	43	>95	*SR*	
268c	R = CH_3	*Pseudomonas* sp.	42	>99	*RR*	45	96	*SS*	357
268d	R = CH_3	*Pseudomonas* sp.	47	>95	*RR*	45	>95	*SS*	357
	R = CCl_3		41	88	*RR*	43	86	*SS*	
268e	R = CH_3	*Pseudomonas* sp.	45	98	*RR*	49	96	*SS*	357
		Pseudomonas sp.	32	>98	*RR*	44	>98	*SS*	359
	R = C_3H_7	*Candida cylindracea*	36	87	*RR*	32	93	*SS*	
268f	R = C_3H_7	*Candida cylindracea*	40	96	*RR*	35	>98	*SS*	360, 358
268g	R = C_3H_7	*Candida cylindracea*	39	23	*RR*	32	40	*SS*	359
		Pseudomonas sp.	34	94	*RR*	46	98	*SS*	
268h	R = C_3H_7	*Candida cylindracea*	37	30	*RR*	37	32	*SS*	359
		Pseudomonas sp.	33	>98	*RR*	40	96	*SS*	
268i	R = C_3H_7	*Candida cylindracea*	38	17	*RR*	36	19	*SS*	359
		Pseudomonas sp.	39	97	*RR*	43	>98	*SS*	
268j	R = C_3H_7	*Candida cylindracea*	36	89	*RR*	32	90	*SS*	359
		Pseudomonas sp.	37	>98	*RR*	46	>98	*SS*	
268k	R = C_3H_7	*Candida cylindracea*	37	83	*RR*	33	85	*SS*	359
		Pseudomonas sp.	27	>98	*RR*	42	>98	*SS*	
268l	R = C_3H_7	*Candida cylindracea*	34	76	*RR*	31	83	*SS*	359
		Pseudomonas sp.	33	>98	*RR*	40	>98	*SS*	
268m	R = C_3H_7	*Candida cylindracea*	36	90	*RR*	31	93	*SS*	359
		Pseudomonas sp.	38	>98	*RR*	42	97	*SS*	
268n	R = C_3H_7	*Candida cylindracea*	36	91	*RR*	38	96	*SS*	359
		Pseudomonas sp.	38	92	*RR*	42	94	*SS*	
268o	R = C_3H_7	*Candida cylindracea*	35	87	*RS*	32	83	*SR*	359
		Pseudomonas sp.	36	>98	*RS*	41	97	*SR*	
268p	R = C_3H_7	*Candida cylindracea*	37	90	*RS*	32	94	*SR*	359
		Psueodmonas sp.	25	>98	*RS*	43	>98	*SR*	
268q	R = C_3H_7	*Candida cylindracea*	40	83	*RR*	28	>98	*SS*	359
		Pseudomonas sp.	39	>98	*RR*	37	95	*SS*	
268r	R = C_3H_7	*Candida cylindracea*	38	5	*RR*	52	<2	*a*	359
		Pseudomonas sp.	35	5	*RR*	55	<2	*a*	
268s	R = C_3H_7	*Candida cylindracea*	2	69	*RR*	92	<2	*a*	359
		Pseudomonas sp.	11	71	*RR*	80	10	*SS*	
268t	R = C_3H_7	*Candida cylindracea*	2	5	*RR*	92	<2	*a*	359
		Pseudomonas sp.	8	90	*RR*	85	15	*SS*	
268u	R = C_3H_7	*Candida cylindracea*	40	>98	*RR*	20	85	*SS*	360
268v	R = C_3H_7	*Candida cylindracea*	40	86	*SR*	40	93	*RS*	360
		Pseudomonas sp.	38	>98	*SR*	28	95	*RS*	360

[a] Not determined.

mediate for the synthesis of castanospermine **154**, which can be obtained by action of lipases on the compound **275**,[180] and the hydrolysis by *Bacillus subtilis* of the azetidinone benzoate **276**.[370] Table 15 collects the results from the above hydrolyses.

A few bicyclic alcohols could not be resolved efficiently by enzymatic methods,[371,372] but a good number of works deal with successful hydrolysis–resolution of bicyclic alcohols, specially applied to bicyclo[3.2.0]-heptenes **277a** and **277c**.[373–376] From *endo*-**277a** the corresponding >94% ee alcohol was obtained using pig pancreas lipase (PPL) or CCL,[373] and PFL gave (1*R*,5*S*,6*R*)-alcohol (98% ee) and the optically pure unreacted (1*S*,5*R*,6*S*)-**277a**.[374,375] For *endo*- and *exo*-**277c**, few biocatalysts were used, i.e., lipases and steapsin, and the corresponding optically pure alcohol can be obtained.[376] Similar results were obtained for bicyclo-[4.2.0]octene **277b**.[373,377] The bicycloheptenes are useful chiral intermediates for the synthesis of pheromones or leukotrienes. Similarly, esters of other bicyclic systems like bicyclo[3.3.0]octenol or -octanol,[378,379] are efficiently and stereoselectively hydrolyzed. The same

Scheme 76



269 270 271

a. $R^1 = CH_3$; $R^2 = H$
b. $R^1 = C_3H_7$; $R^2 = H$
c. $R^1 = C_3H_7$; $R^2 = CH_3$

Table 14. Enzymatic Resolution of Acetates 269 and 270 and Esters 271a–c

		alcohol			ester			
substrate	enzyme	yield, %	ee, %	config	yield, %	ee, %	config	ref
269	PLE	26	100	*R*	21	96[a]	*S*	361
270	PLE	45[b]	90	*R*	32	>99	*S*	362
271a	CCL	46	>95	*R*	49	73	*S*	363
	SAM II[c]	47	>95	*R*	42	>95	*S*	363
271b	CCL	45	72	*R*	46	>95	*S*	363
	SAM II[c]	43	>95	*R*	40	>95	*S*	363
271c	CCL	32	>99	*R*	45	71	*S*	363
	SAM II[c]	40	>99	*R*	44	69	*S*	363

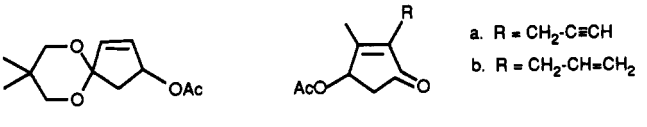
[a] After two enzymatic hydrolyses. [b] Extent of conversion. [c] Fluka Chemie AG (Buchs, Switzerland).

applies to compounds type **278a** and **278b**[380] and the lipase used was CCL (>93% ee for the alcohol and >94% ee for unreacted **278a,b**). The same enzyme affords more variable ee when the substrates are *endo*-bicyclo-[2.2.1]heptanol or -[2.2.2]octanol butyrates.[381] Also the CCL-catalyzed hydrolysis of a tricyclic acetate was achieved for the synthesis of α-cuparenone.[382] For the enantioselective resolution of the acetate **279**, it was necessary to use Lipase OF 360 (Meito Sangyo, Japan) immobilized on Celite and work in an organic solvent saturated with water [(*S*)-alcohol with 94% ee, (*R*)-**279** with 98% ee].[383] The above compounds are collected in Scheme 78.

4. From Cyclic Diols and Triols

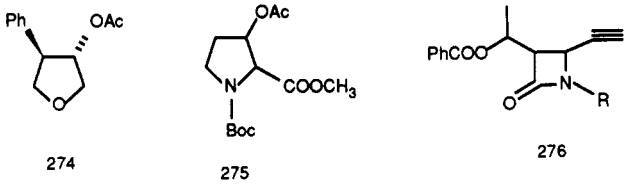
The enzymatic hydrolysis of the diesters of cyclic diols is frequently the method of choice for the preparation of the corresponding optically pure mono esters. In the case of meso diesters, the asymmetrization generally can be brought about with approximately 100% yield of an optically pure monoester. For instance, the cyclopropyl dibutyrate **280** can be hydrolyzed to a chiral monoester by catalysis of PPL with quantitative yield and almost 100% optical purity.[384] It should be mentioned that a recent review is available on the enzymatic and microbial preparation of optically active cyclopropanes.[385] In a similar manner, the hydrolysis of the diesters of a *meso*-epoxy diol, compounds **281a,b** affords up to 90% yield of 95% ee (2*S*,3*R*)-monoesters.[386] Also, the PFL-catalyzed hydrolysis of cyclic diacetates type **282a,b** has been studied[387,388] and some synthetic applications of the chiral cyclic diols published.[389] Acyclic and cyclic 2-nitro 1,3-diols were prepared by action of PLE on the corresponding diacetates, as shown for the diacetate **283**.[390] Another similar category of esters of cyclic diols, compounds **284a–d**[391] were resolved with high ee to the corresponding mono esters. A detailed study on the enzymatic hydrolysis of tetrahydrofuran diesters **285a–c**,[392,393] led

Scheme 77



272 273

a. $R = CH_2\text{-}C{\equiv}CH$
b. $R = CH_2\text{-}CH{=}CH_2$



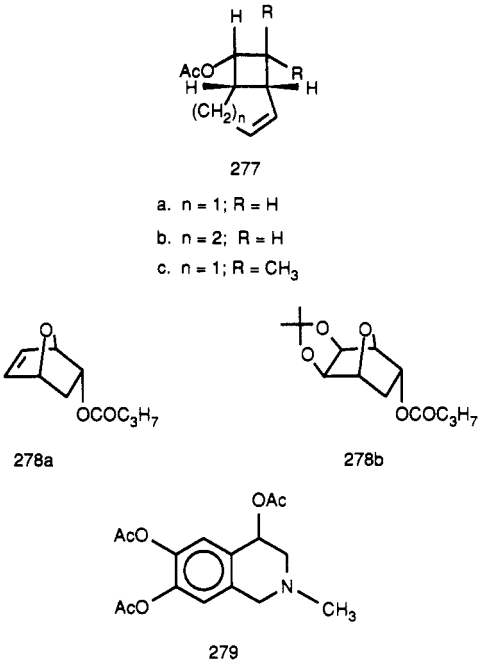
274 275 276

Table 15. Hydrolysis of Esters 272–276

		ester			alcohol			
substrate	biocatalyst	yield, %	ee, %	config	yield, %	ee, %	config	ref(s)
272	Lipase P[a]	86	>95	*R*	42	>95	*S*	364
273a	arthrobacter lipase	46	*b*	*S*	46	100	*R*	365–368
273b	arthrobacter lipase	53[c]	*b*	*S*	43[c]	98	*R*	368
274	SAM II[d]	42	≥98	3*R*,4*S*	45	93	3*S*,4*R*	369
275	CCL	*b*	>99	2*R*,3*S*	*b*	>99	2*S*,3*R*	180
	PPL	*b*	>99	2*R*,3*S*	*b*	64	2*S*,3*R*	180
276	*Bacillus subtilis*	*b*	*b*	*b*	28	95	*R*	370

[a] Amano Pharmaceutical Co., Ltd. [b] Not specified. [c] Determined by GC. [d] Fluka Chemie AG (Buchs, Switzerland).

Scheme 78



277

a. n = 1; R = H
b. n = 2; R = H
c. n = 1; R = CH_3

278a 278b

279

Scheme 79

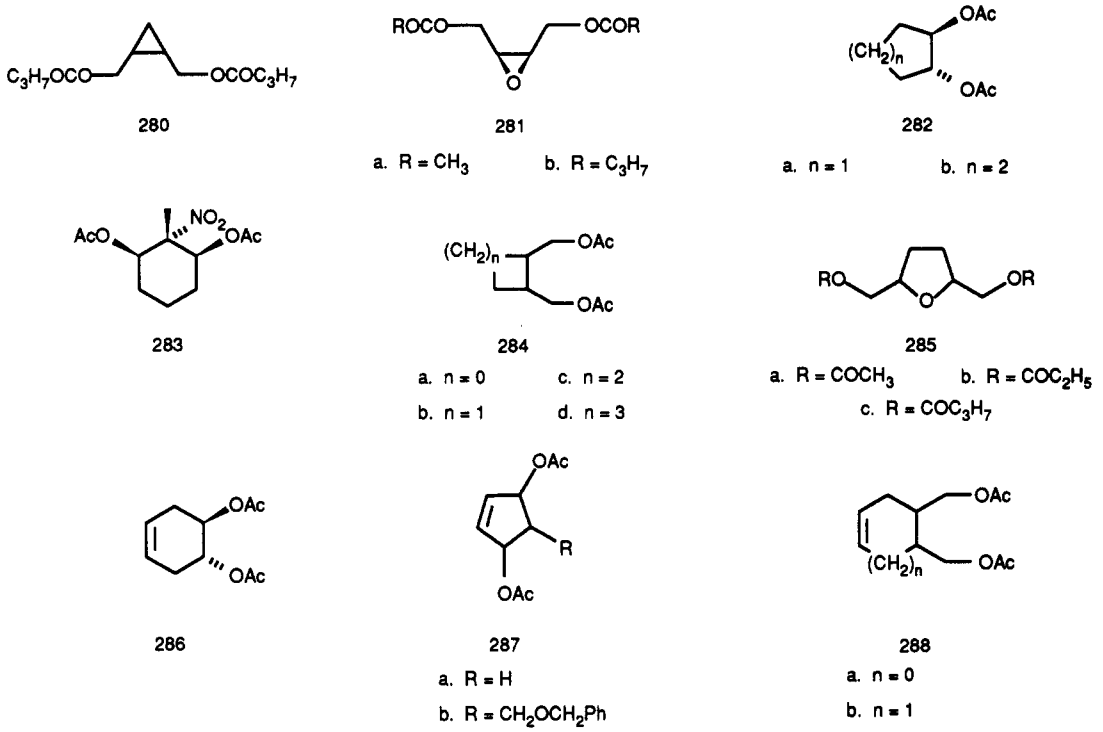


Table 16. Enzymatic Hydrolysis of Diesters 280–288

substrates	enzyme	monoester yield, %	monoester ee, %	monoester config	ref(s)
280 (*cis*)	PPL	99	>99	1*R*,2*S*	384
281a	PPL	54[a]	90	2*S*,3*R*	386
281b	PPL	90	95	2*S*,3*R*	386
282a	PFL	43	>99	1*R*,2*R*	389
282b[b]	PCL[c]	42[b]	>99[b]	*R*[b]	388
282b	PFL	42	>99	1*R*,2*R*	387, 389
283	PLE	80–90	>95	*R*[d]	390
284a	*Pseudomonas* sp. lipase	83	90	1*R*,2*S*	391
284b	*Pseudomonas* sp. lipase	87	>95	1*R*,2*S*	391
284c	*Pseudomonas* sp. lipase	86	>95	1*R*,2*S*	391
284d	*Pseudomonas* sp. lipase	54	50	1*R*,2*S*	391
285a	PLE	86	96	2*S*,5*R*	392
	PPL	53	41	2*S*,5*R*	392
	CCL	20	80	2*R*,5*S*	392
285b	PLE	54	42	2*S*,5*R*	392
	PPL	57	33	2*S*,5*R*	392
	CCL	69	26	2*R*,5*S*	392
285c	PLE	50	33	2*S*,5*R*	393
	Mucor javanicus lipase	75	>99	2*R*,5*S*	393
286	PFL	46.5	99[e]	*R*,*R*	394
287a (*cis* + *trans*)	PPL	47.3 (*cis*)	100	1*R*,4*S*	395
		6.5 (*trans*)		1*R*,4*R*	
287a (*cis*)	acetylcholinesterase	89	100	1*R*,4*S*	396
287b (*cis*)	PPL	96	>95	1*R*,4*S*	397
288b (*cis*)	PPL	95	>99	1*R*,2*S*	399

[a] Extent of conversion (%). [b] Values refer to the diol; (*S*)-diacetate (38%, >99% ee) and (*R*)-monoester (3.7%, 95% ee) were also isolated. [c] *Pseudomonas cepacia* lipase. [d] Configuration of the carbon bearing the CH_2OH group. [e] (*S*,*S*)-Diester with 93% ee was isolated.

to the choice of the best experimental conditions for the preparation of enantiomerically pure monoesters. A few examples of enantioselective hydrolysis of diesters of cyclohexenyl **286**[394] or cyclopentenyl diols **287a,b**[395–397] have been reported. In these compounds, the presence of the double bond often offers the possibility of further chemical transformations toward the synthesis, for instance, of prostaglandins. Other cycloalkenyl diacetates, compounds **288a,b**, have been enzymatically hydrolyzed. Whereas for **288a** the hydrolysis was only regioselective,[398] 4-cyclohexenedimethanol diacetate (**288b**), which had been already hydrolyzed by a *Pseudomonas* sp. lipase,[391] is enantioselectively hydrolyzed from PPL to the corresponding monoacetate for the synthesis of (+)-meroquinene.[399]

All the above results are collected in the Scheme 79 and in Table 16.

The substituted cyclohexanediol monoacetates **289a,b**[400] or monoprotected diol acetates **290a,b**,[401,402] the cyclopentene derivatives **291**[403] and **292**,[404] or the

Scheme 80

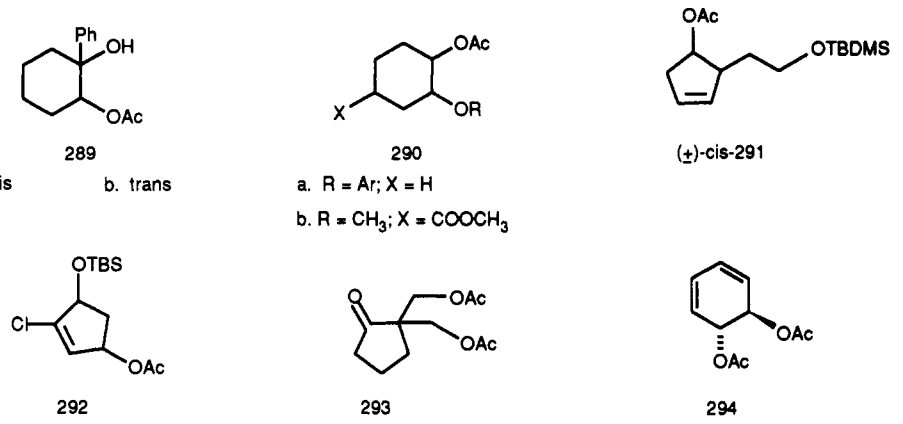
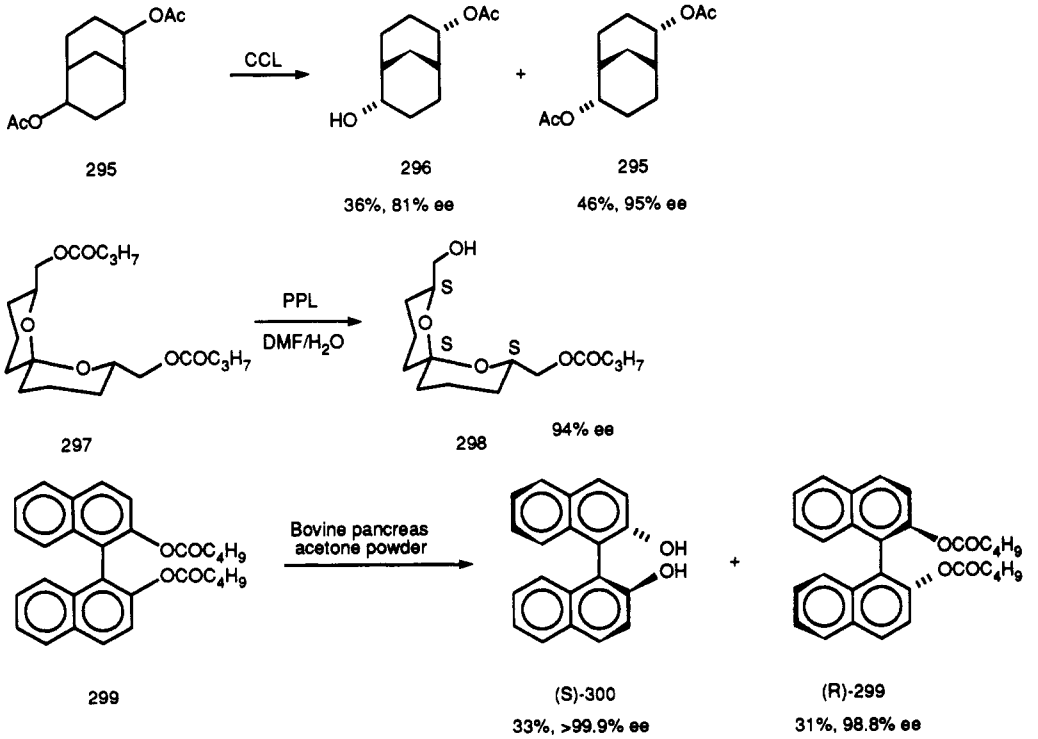


Table 17. Hydrolysis of Cyclic Esters 289–294

		hydrolyzed			unreacted			
substrate	biocatalyst	yield, %	ee, %	config	yield, %	ee, %	config	ref
289a	PLE	46	84	1*R*,2*R*	46	85	1*S*,2*S*	400
	PPL	47	56	1*R*,2*R*	53	57	1*S*,2*S*	400
	CCL	46	27	1*S*,2*S*	46	26	1*R*,2*R*	400
289b	PLE	47	78	1*S*,2*R*	50	82	1*R*,2*S*	400
	PPL	41	78	1*S*,2*R*	49	72	1*R*,2*S*	400
	CCL	54	25	1*S*,2*R*	38	60	1*R*,2*S*	400
290a	pig liver acetone powder	65–76[a]	90–99	*b*	60–75[a]	60–90	*b*	401
290b	Lipase AK[c]	50	*E* > 100	*R*	*b*	*b*	*S*	402
291	Lipase PS[c]	37	>99	1*R*,2*S*	39	92	1*S*,2*R*	403
292	PPL	25	100	1*S*,4*R*	*b*	*b*	*b*	404
293	acetylcholinesterase[d]	36	90	2*R*	*b*	*b*	*b*	405
294	PLE	15	95[e]	1*R*,2*R*	41	96	1*S*,2*S*	406

[a] Based on the percentage of hydrolysis. [b] Not reported. [c] Amano Pharmaceutical Co., Ltd. [d] From electric eel. [e] Two-step hydrolysis (first step 63% ee).

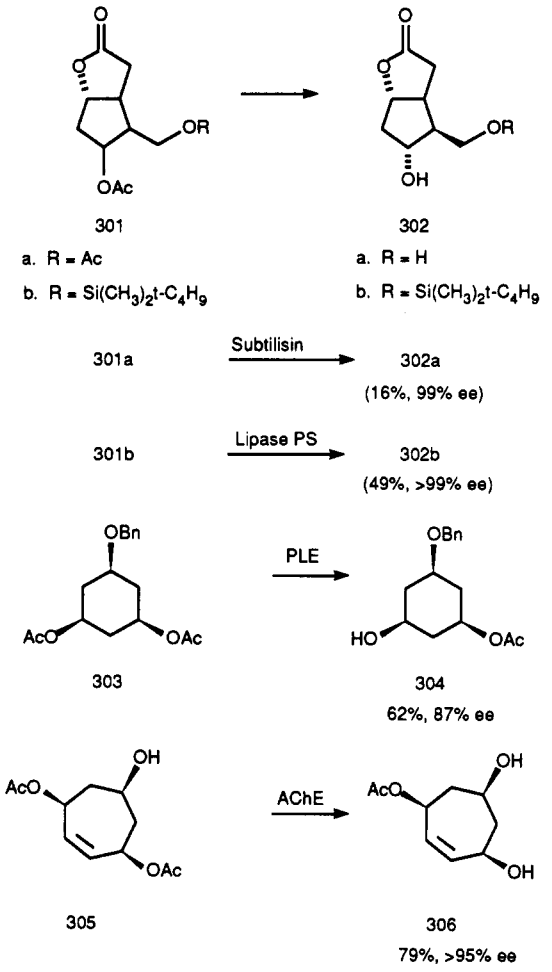
Scheme 81



cyclopentanone diol diacetate **293**,[405] are additional examples of the great versatility of the enzymatic procedure. Also, the resolution of a sensitive diol like *trans*-1,2-dihydroxy-1,2-dihydrobenzene through its diacetate **294**[406] witnesses the mildness and efficacy of the method (Scheme 80, Table 17).

Also the diacetate of the bicyclic diol **295**[407] can be enzymatically resolved to afford the monoacetate **296** (81% ee) and the unreacted diacetate (95% ee), and for the dioxaspiro dibutyrate **297** the best ee of the

Scheme 82



monoester **298** (94%) was achieved in the presence of PPL only if a water/dimethylformamide mixture is the incubation medium.[408] Interestingly, the resolution of the binaphthyl dipentanoate (**299**) was realized with bovine pancreas acetone powder, to afford nearly optically pure diester **299** and diol **300**[409] (Scheme 81).

More hydroxylated cyclic systems can be resolved hydrolytically and an application to suitable substrates is the synthesis of (−)-Corey lactone **302a**, a well-recognized key intermediate in the synthesis of prostaglandins.[410] The hydrolysis of the racemic diacetate **301a** with subtilisin affords the optically pure compound **302a** (16% yield)[411] and from the acetate silyl ether **301b**, the optically pure derivative **302b** (49% yield) was obtained with Lipase PS.[412] The hydrolysis of the *meso*-diacetate **303** with PLE affords the (1*S*,3*R*,5*S*)-monoacetate **304** (62% yield, 87% ee).[413-415]

In the above cases, collected in Scheme 82, lipases or PLE were the hydrolases of choice. A noticeable exception to the almost general attitude of employing the above enzymes is constituted by the use of electric eel acetyl cholinesterase (AChE). Esters of the cycloheptene series like the diacetate **305**[416] seem especially well suited for the hydrolytic action of this enzyme. In fact, the triol monoacetate **306** was obtained (79% yield, >95% ee). Instead, for the asymmetric hydrolysis of a similar compound, the cycloheptene diacetate, *cis*-3,7-diacetoxy-4,6-dimethylcycloheptene, a lipase was the efficient biocatalyst.[417]

Scheme 83

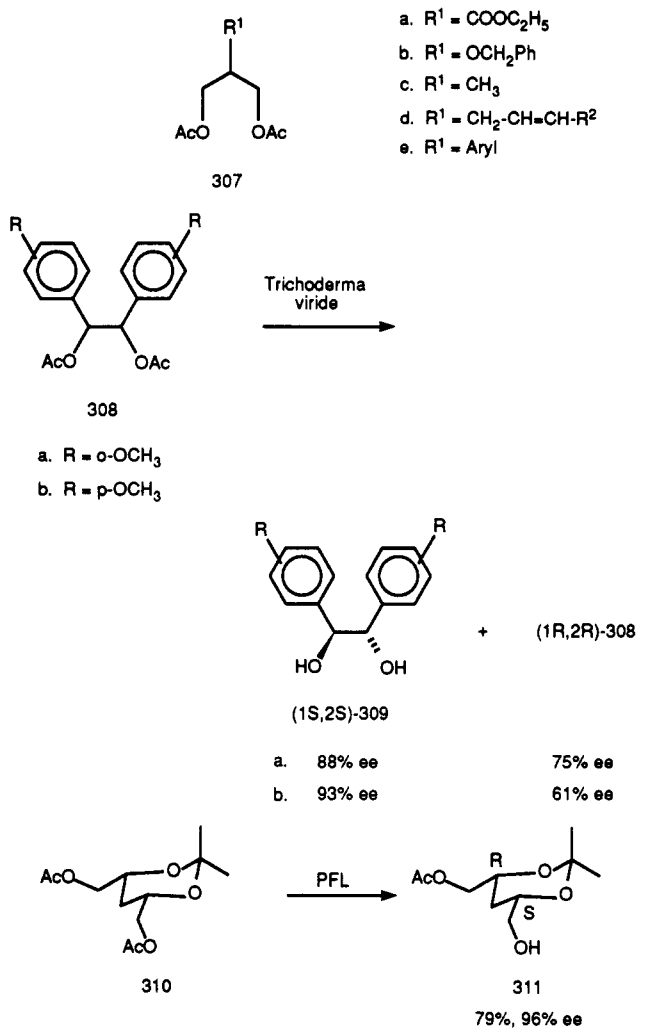


Table 18. Asymmetric Hydrolysis of Prochiral Diacetates 307a–e

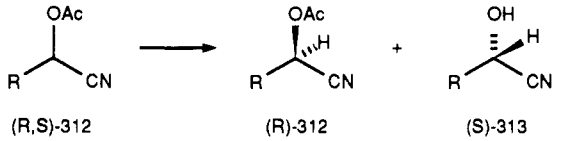
substrate	enzyme	monoester yield, %	monoester ee, %	config	ref(s)
307a	PLE	31	28[a]	*b*	419
307b	*Pseudomonas* sp. lipase	*c*	<75	*R*	422
	LPL	*c*	87	*R*	422
307c	PFL	33	>99	*R*	423
307d R^2 = H	Lipase MY[d]	35	89[e]	*S*	425
	Lipase P[f]	86	90[g]	*R*	426
307d R^2 = C_5H_{11}	PPL	63	96	*S*	427, 428
307d R^2 = $(CH_3)_2CH$	PPL	75	97	*S*	428, 429
307e R^1 = Ph	PLE	63	12	*R*	430
	PPL	80	92	*S*	430
307e R^1 = CH_2Ph	Lipase P[f]	41	>94	*S*	431
307e R^1 = $pCH_3OC_6H_4$	PPL	77	96	*S*	430
307e R^1 = 2-Naphtyl	PPL	30	>96	*S*	430

[a] From dipentanoate, with PPL in 30% MeOH is possible to obtain a monoester with 84% ee. [b] (+)-Isomer. [c] Not reported. [d] Meito Sangyo Co., Ltd. [e] In 30% aqueous acetone. [f] Amano Pharmaceutical Co., Ltd. [g] At 0 °C and pH 5.

5. From Acyclic Diols

The esters of some acyclic diols, like 3-methylhex-5-ene-1,3-diol[418] or **307a**[419] were hydrolyzed with mod-

Scheme 84



(R,S)-312 (R)-312 (S)-313

a. R = CH_3
b. R = C_3H_7
c. R = C_5H_{11}
d. R = C_7H_{15}
e. R = C_9H_{19}
f. R = $(CH_3)_2CHCH_2$
g. R = $PhCH_2$
h. R = $PhCH_2CH_2$
i. R = cyclohexyl
j. R = t-C_4H_9
k. R = $(CH_3)_2CH$

Table 19. Hydrolysis of Aliphatic Cyanohydrin Acetates 312a–k

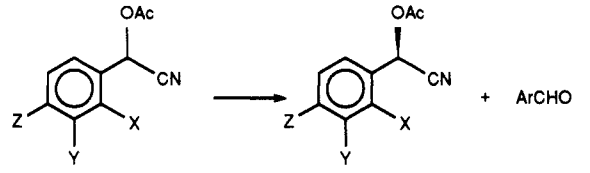
substrate	biocatalyst	(*R*)-acetate[a] yield, %	ee, %	ref
312a	*Candida tropicalis*	28	78	434
312b[b]	*Candida tropicalis*	22	81	434
	PFL	36	15	436
312c	*Candida tropicalis*	14	97	434
312d	*Candida tropicalis*	35	84	434
312e[c]	*Candida tropicalis*	35	70	434
312f	*Candida tropicalis*	30	98	434
312g	PFL	38	≥98	435
312h	PFL	45	≥98	435
312i	PFL	45	≥98	435
312j	PFL	30	95	435
312k	PFL	31	92	435

[a] The corresponding alcohols are not isolated or show low ee. [b] In ref 436 are also reported hydrolyses of other esters with different lipases. [c] In this case at 46% conversion the alcohol with 99% ee can be isolated.

erate enantioselectivity. In the latest case, changing the type of the ester moiety from diacetate to dihexanoate improved the ee of the PLE-mediated hydrolysis from 27 to 70% ee.[419] Additionally, using 30% aqueous methanol, the ee could be raised to 84%. Examples of the enantioselective hydrolysis of prochiral diacetate of 1,3-diols **307b–e** have been already reported a few years ago.[420,421] In the case of 2-*O*-benzylglycerol diacetate **307b**, the problem of acyl migration during the hydrolysis has been studied and a pH control is necessary.[422] The process of intramolecular transesterification was, however, not observed when the esterification of the diol was accomplished in neutral organic solvents. The prochiral diacetate of 2-substituted-1,3-propanediols **307c–e** can be hydrolyzed almost independently from the bulk of the substituent R. In fact, the substrates enantioselectively hydrolyzed bear at the 2-position groups of different size, such as methyl in the diacetate **307c**[423] (similar results were from the dibutyrate as well[424]), allyl as in **307d**,[425,426] and more in general (*E*)-alkenyl **307d**,[427-429] or various aryl **307e**.[430,431] The results are collected in Scheme 83 and Table 18.

In Scheme 83 are also shown other biocatalytic reactions. The microbial resolution by *Trichoderma viride* of diacetate **308** leads to optically active 1,2-bis(methoxyphenyl)ethane-1,2-diols (**309**),[432] and the lipase-catalyzed asymmetrization of the *meso*-1,3-diacetate **310** affords the compound **311**, a chiral building block for the synthesis of a hunger modulator.[433]

Scheme 85



(R,S)-314 (R)-314

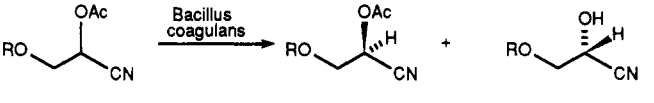
a. X = Y = Z = H
b. X = Y = H; Z = CH_3
c. X = H; Y = Z = OCH_3
d. X = H; Y = OCH_3; Z = OAc
e. X = H; Y,Z = 3,4-O-CH_2-O-
f. X = F; Y = Z = H
g. X = Cl; Y = Z = H
h. X = Br; Y = Z = H
i. X = CH_3; Y = Z = H
j. X = OCH_3; Y = Z = H
k. X = NO_2; Y = Z = H
l. X = Z = OCH_3; Y = H
m. X = H; Y = Z = Cl
n. X = H; Y = Z = OCH_2Ph

Table 20. Hydrolysis of Aromatic Cyanohydrin Acetates 314a–n

substrate	biocatalyst	(*R*)-acetate yield, %	ee, %	ref
314a	*Bacillus coagulans*	37	60	437
	PFL	42	≥98	435
	PFL	*a*	71	436
314b	*Bacillus coagulans*	26	>95	437
314c	*Bacillus coagulans*	43	>95	437
314d	*Bacillus coagulans*	25	>95	437
314e	*Bacillus coagulans*	33	>99.5	437
314f	PFL	43	93	435
314g	PFL	39	82	435
314h	PFL	39	70	435
314i	PFL	40	80	435
314j	PFL	42	95	435
314k	PFL	46	5	435
314l	PFL	47	0	435
314m	PFL	40	93	435
314n	PFL	35	≥98	435

[a] Not reported.

Scheme 86



(R,S)-315 (S)-315 (R)-316

76->95% ee

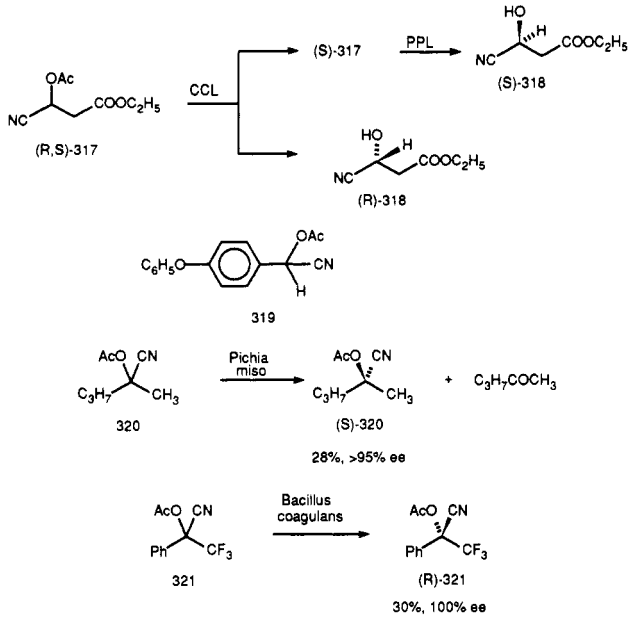
a. R = Ph
b. R = o$CH_3C_6H_4$
c. R = m$CH_3C_6H_4$
d. R = p$CH_3C_6H_4$
e. R = pClC_6H_4
f. R = 4-Cl,3-CH_3-C_6H_3
g. R = 1-Naphtyl

6. Cyanohydrin Acetates

The aqueous enzymatic hydrolysis of the acetates of aliphatic cyanohydrins **312a–k** could be the method of choice for the preparation of optically pure aldehyde cyanohydrins **313a–k**. However, good yields and ee can be obtained for unreacted (*R*)-acetates **312a–k**, whereas the corresponding cyanohydrins could not be isolated or show low ee (Scheme 84 and Table 19). The resolution can be realized either by use of microorganisms like *Candida tropicalis*[434] or lipase (PFL).[435,436]

The hydrolysis of the acetates of aromatic cyanohydrins **314a–n** is effective only for the preparation of unchanged (*R*)-acetates, since the corresponding cy-

Scheme 87



anohydrins are directly hydrolyzed to aromatic aldehydes (Scheme 85). The biocatalytic systems are *Bacillus coagulans*[437] or PFL.[435,436] The results are collected in the Scheme 85 and Table 20.

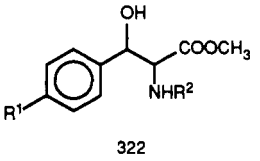
The (aryloxy)acetaldehydes cyanohydrins acetates **315a–g** were hydrolyzed by *Bacillus coagulans*,[438] and in this case, the (*S*)-acetates were obtained with excellent enantioselectivity (75 to >95% ee). Only in a few instances did the hydrolyzed (*R*)-cyanohydrins show appreciable ee (Scheme 86).

The CCL-catalyzed resolution of the acetate **317** gives the access to a highly enantioselective synthesis of both enantiomers of 4-amino-3-hydroxybutanoic acid (GABOB).[439] The (*R*)-cyanohydrin **318** affords the optically pure (*R*)-GABOB, and the (*S*)-acetate **317** requires an additional hydrolysis with PPL to afford optically pure (*S*)-GABOB. Interestingly, the resolution of the cyanohydrin **319** can be realized by two different lipases with opposite stereoselection.[440] The acetate of ketone cyanohydrins can also be resolved by special biocatalysts. For example, *Pichia miso* is able to hydrolyze enantioselectively aliphatic compounds as the acetate **320**[441,442] and the trifluoromethyl cyanohydrin acetate **321**, used for the synthesis of the Mosher acid, could be prepared by a resolution procedure with *Bacillus coagulans* cells.[443] All the above structures are collected in the Scheme 87.

7. Acyclic Monoesters

The hydrolyses examined previously refer to acylated chiral and prochiral alcohols, but also the esters of chiral carboxylic acids with simple alcohols are good substrates for hydrolases. Biocatalytic systems other than enzymes are able to perform interesting hydrolyses. (Aryloxy)propionates have been enantioselectively hydrolyzed also with bovine serum albumin in the presence of β-cyclodextrins[444] and for the same esters the lipase-catalyzed hydrolysis reaches the highest ee in the presence of methorphan.[445] Catalytic antibodies can also promote the stereospecific hydrolysis of alkyl

Scheme 88



a. $R^1 = NO_2$; $R^2 = COCHCl_2$

b. $R^1 = SCH_3$; $R^2 = H$

a. $R^1 = CH_2Ph$; $R^2 = R^3 = CH_3$

b. R^1 =  ; $R^2 = CH_3$; $R^3 = C_2H_5$

c. $R^1 = NO_2CH_2CH_2$; $R^2 = CF_3$; $R^3 = CH_2Ph$

d. $R^1 = CH_3OC_6H_4$; $R^2 = CH(CH_3)_2$; $R^3 = CH_3$

e. $R^1 = PhCH_2OCH_2CH_2$; $R^2 = CH_2OH$; $R^3 = CH_3$

f. $R^1 = C_4H_9$; $R^2 = F,Br,OH$; $R^3 = C_2H_5$

g. $R^1 = PhCH_2CH_2$; $R^2 = OH$; $R^3 = C_2H_5$

h. $R^1 = CH_3SCH_2CH_2$; $R^2 = OH$; $R^3 = C_2H_5$

i. $R^1 = mNO_2C_6H_4$; $R^2 = CH_2CH_3$; $R^3 = CH_3$

j. $R^1 = mNH_2C_6H_4$; $R^2 = CH_2CH_3$; $R^3 = CH_3$

Table 21. Hydrolysis of Acyclic Esters 323a–j

		ester			acid			
substrate	biocatalyst	yield, %	ee, %	config	yield, %	ee, %	config	ref
323a	Lipase P[a]	44	98	*R*	50	95	*S*	454
323b	PPL	*b*	99	*R*	*a*	99	*S*	455
323c	Lipase P[a]	*b*	>98	*S*	49	>98	*R*	456
323d	HLE[c]	42	96	*S*	35	91	*R*	457
323e	CCL	*b*	≥97	*R*	*b*	70	*S*	458
323f	Lipase P-30[a]	34–43	95–100	*R*	40–53	69–79	*S*	459
323g	Lipase P-30[a]	43	99	*R*	33	92	*S*	459
323h	PFL	40	>98	*R*	42	98	*S*	460
323i	chymotrypsin	*b*	*b*	*b*	42	92	*S*	461
	Lipase PS[a]	*b*	*b*	*b*	44	87	*R*	461
323j	chymotrypsin	*b*	99	*R*	45	94	*S*	461

[a] Amano Pharmaceutical Co., Ltd. [b] Not specified. [c] Esterase contained in horse liver acetone powder (Sigma).

esters.[446] Although very interesting, the resolution of amino acids are not treated in the present review. Only a mention will be given to the resolution of less common amino acids esters by proteinases,[447-449] yeast in organic solvents,[450] or the action of lipases on carbobenzyloxy amino esters.[451]

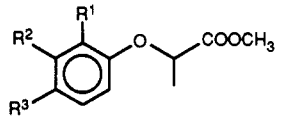
In Scheme 88 and Table 21 are collected a few examples of resolution of racemic α-substituted esters **323a–j**, including the amino esters **322a** [subtilisin as biocatalyst, 48% yield, >97% ee of the corresponding (2*S*,3*R*)-acid] and **322b** [*Streptomyces griseus* immobilized on sepharose, 95% ee of the (2*S*,3*R*)-acid]. The above resolutions afforded the proper intermediates for the synthesis of chloramphenicol[452] and florfenicol.[453]

Usually, the enzyme-catalyzed resolution process gives access to an enantiomerically pure acid **324** and its enantiomeric ester **323**, which is the unreacted product. The structures **323** are flexible, since the enantioselective hydrolysis can be realized on esters bearing at the α-position a group like methyl (**323a,b**),[454,455] trifluoromethyl (**323c**),[456] isopropyl (**323d**),[457] hydroxymethyl (**323e**),[458] fluorine, bromine, or hydroxy (**323f–h**).[459,460] Examples of enantioselective resolution of α-ethyl aromatic esters refer to compounds **323i,j**.[461]

Other functions can be present near to the carboxyl ester moiety and examples are collected in Scheme 89 and Table 22.

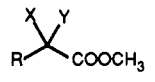
(Aryloxy)propionates **325a–d** can be resolved with α-chymotripsin or a lipase,[462] and the carboxylate ester function bound to a quaternary chirality center are enantioselectively hydrolyzed with lipases as in the case

Scheme 89



325

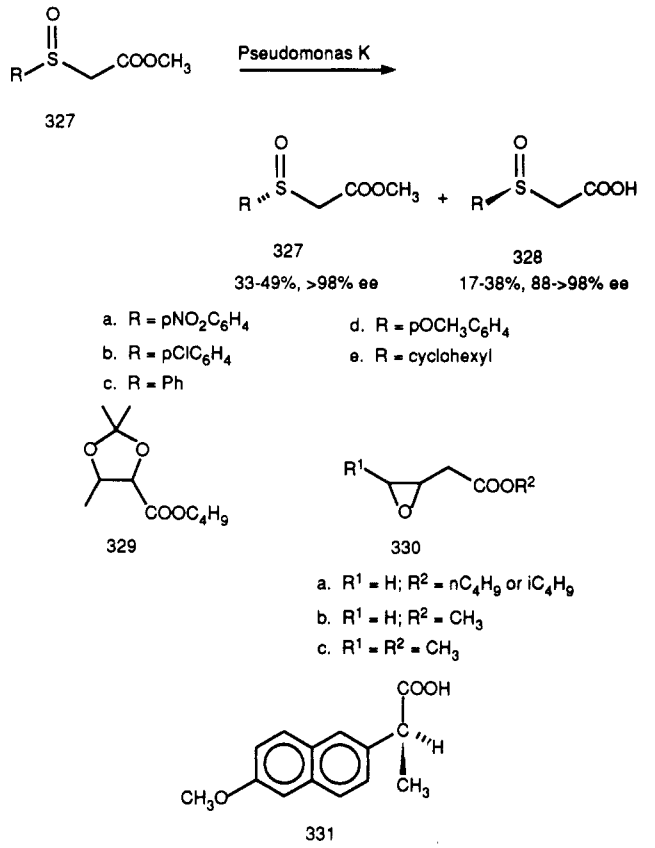
a. $R^1 = R^2 = R^3 = H$
b. $R^1 = Cl$; $R^2 = R^3 = H$
c. $R^1 = H$; $R^2 = Cl$; $R^3 = H$
d. $R^1 = R^2 = H$; $R^3 = Cl$



326

a. $R = CH_2{=}CH{-}CH_2$; $X = CH_3$; $Y = OCH_2Ph$
b. R = Ph; $X = CH_2{-}C(CH_3){=}CH_2$; Y = OH
c. R = Ph; $X = CF_3$ or CH_3; Y = OH
d. $R = NO_2$; $X = CH_3$; $Y = CH_2$-(3-indolyl)

Scheme 90



of **326a**,[463-465] whereas the presence of an hydroxy group as in **326b** or **326c** does not allow a completely stereoselective resolution (88% maximum ee).[466,467] The resolution of α-nitro-α-methyl carboxylic esters **326d** is achieved with α-chymotrypsin as biocatalyst.[468] Other structures are collected in the Scheme 90. (Methylsulfinyl)acetates **327a–e** are resolved by a *Pseudomonas* sp. lipase into optically pure esters **327a–e** and acids **328a–e**.[469] *cis*- and *trans*-dimethyldioxolanecarboxylate **329** can be resolved with the aid of lipases.[470] *trans*-**329** are resolved with opposite stereochemistry with CCL and PPL, while PPL is unable to hydrolize *cis*-**329**. The epoxy esters **330a–c** require as biocata-

Table 22. Hydrolysis of Esters 325 and 326a–d

		ester			acid			
substrate	enzyme	yield, %	ee, %	config	yield, %	ee, %	config	ref(s)
325a	α-chymotrypsin	47	66	*S*	40.5	70	*R*	462
	lipase[a]	39.5	61	*S*	48	48	*R*	462
325b	α-chymotrypsin	46.5	80	*S*	38.5	75	*R*	462
	lipase[a]	38.5	36	*S*	47.5	39	*R*	462
325c	α-chymotrypsin	37.5	100	*S*	39	87	*R*	462
	lipase[a]	41	24	*S*	47.5	27	*R*	462
325d	α-chymotrypsin	42.5	48	*S*	35.5	59	*R*	462
	lipase[a]	49.5	100	*S*	49	89	*R*	462
326a	Lipase OF[b]	40	100	*R*	52	82[c]	*S*	463–465
326b	PLE	44	86	*R*	39	80[d]	*S*	466
326c, X = CH_3	protease from *Aspergillus oryzae*	48[e]	70	*S*	48[e]	75[d]	*R*	467
326c, X = CF_3	protease from *Aspergillus oryzae*	60[e]	88	*S*	40[c]	88[d]	*R*	467
326d	α-chymotrypsin	27	>98	*f*	*g*	*g*	*g*	468

[a] Type VII (Sigma). [b] Meito Sangyo Co., Ltd. [c] The acid enantiomerically pure was obtained after a second hydrolysis. [d] The enantiomerically pure product could be obtained after one recrystallization. [e] % Conversion. [f] L-Configuration. [g] The other product isolated was the decarboxylation product, 2-nitro-3-(3-indolyl)propane (48%).

Scheme 91

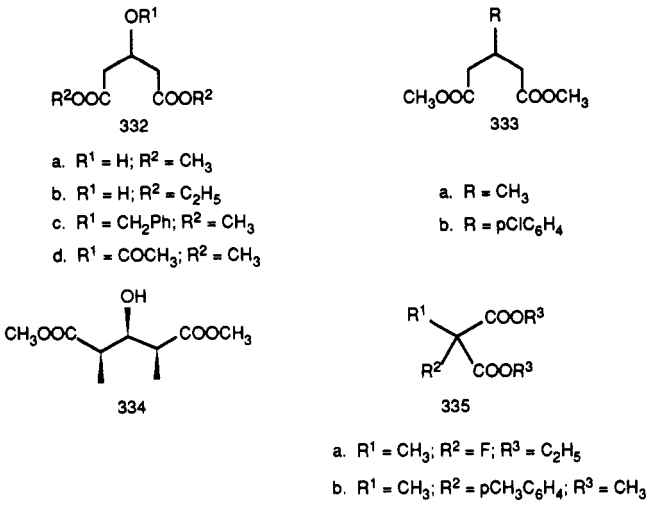


Table 23. Hydrolysis of Acyclic Diesters

		monoester			
substrate	enzyme	yield, %	ee, %	config	ref
332a	PLE	87	16	*S*	477
	PLE	76	30 ± 5	*S*	478
	α-chymotrypsin	78	55 ± 5	*R*	478
332b	Esterase 30,000	94	>98	*S*	480
332c	α-chymotrypsin	86	92	*R*	481
332d	PLE	38	90	*R*	478
	α-chymotrypsin	82	84	*R*	478
333a	PLE	92	100	*R*	482
333b	α-chymotrypsin	85	≥98	*R*	483
335a	Lipase MY[a]	75	86	*S*	492
335b	PLE	*b*	96	*R*	493

[a] Furnished by Meito Sangyo Co., Ltd., successively immobilized with calcium alginate on ceramic honeycomb. [b] Not specified.

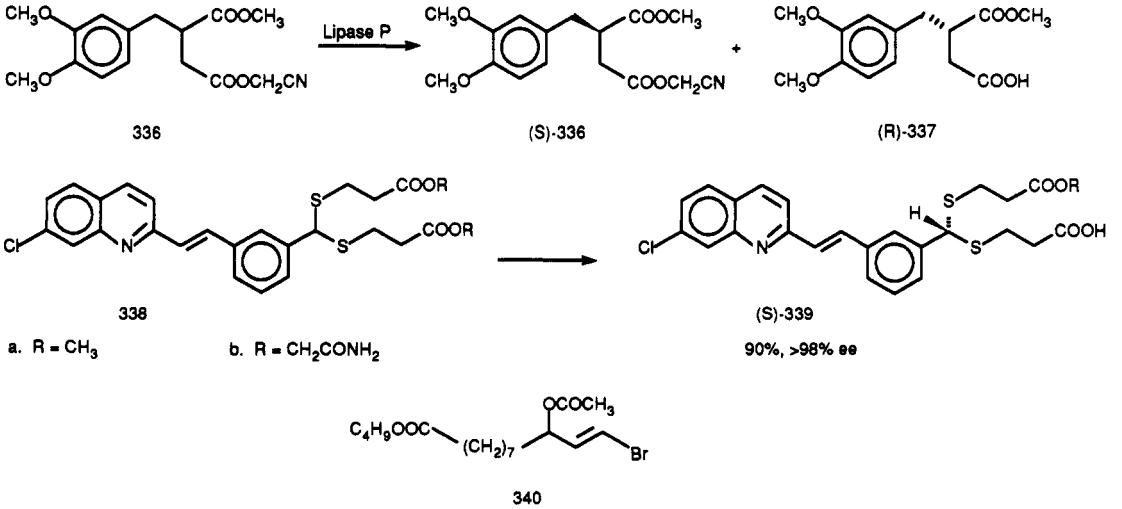
lysts either steapsin[471] or PLE.[472] 2-Aryl (for example (*S*)-(+)-naproxen, **331**) and 2-(aryloxy)propionates can be prepared with >98% ee using a carboxylesterase obtained in high production level, free of the unfavorable presence of a nonspecific lipase, by cloning the enzyme into *Bacillus subtilis* 1-85.[473] Naproxen **331** can also be prepared in a continuous reactor (25% yield, >95% ee) using CCL immobilized on Amberlite XAD-7.[474]

8. Acyclic Diesters

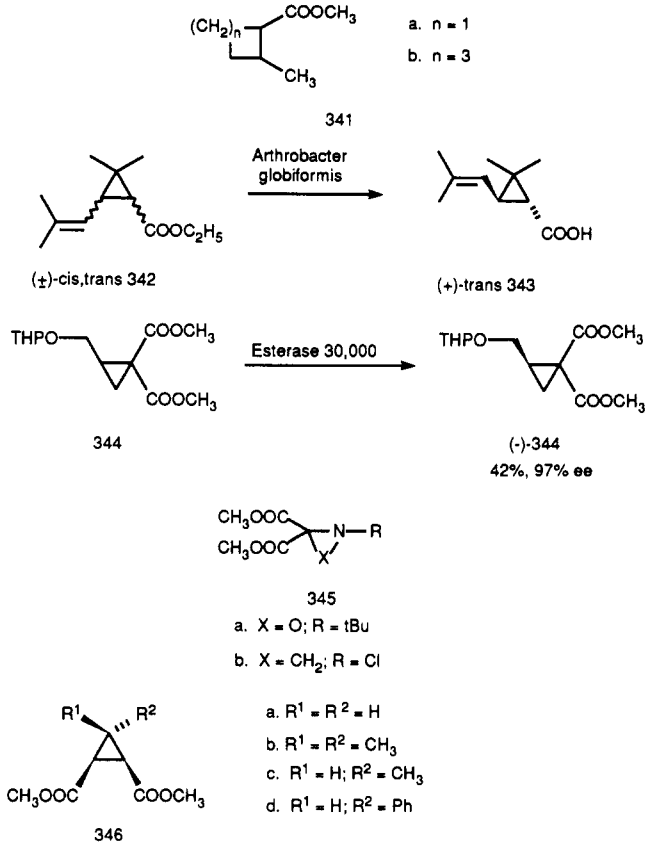
Among the successful examples of PLE-catalyzed hydrolysis of prochiral 3-substituted diesters,[475] the 3-hydroxyglutarate **332a** constitutes the exception, since it is poorly asymmetrized by the above enzyme.[476-478] Using PLE on a dipropyl 3-hydroxyglutarate, the ee of the monoester did not exceed 70%,[479] while a recent report states that with "Esterase 30,000" a highly asymmetric hydrolysis of the diethyl ester **332b** could be achieved.[480] If the substrate is protected, for instance, like the benzyl derivative **332c** the enantioselectivity of the hydrolysis is low with PLE[477,481] but reaches >98% ee with chymotrypsin.[481] The 3-acetate **332d** is hydrolyzed by PLE with higher enantioselectivity than **332a**, but α-chymotrypsin seems specially suited to a highly enantioselective resolution of **332d**.[478] New applications of the enzymatic hydrolysis of previously reported diesters like **333a**[482] for the synthesis of (*R*)- and (*S*)-4-amino-3-methylbutanoic acid, as well as the diester **333b** for the synthesis of both enantiomers of baclofen [4-amino-3-(4-chlorophenyl)-butanoic acid], have been reported.[483] Optically pure diester **334** has been previously prepared,[484] and recent applications to the synthesis of macrolides have been recently reported.[485-487] The PLE-catalyzed hydrolysis of substituted malonates **335**[488] is an established procedure which has been applied to mono- or disubstituted carboxylic acids.[489] Temperature and solvent influence on the enantioselectivity of the above enzymatic hydrolysis has been reported[490] and a few prochiral malonates **335** have been used as model substrates[491] to probe the dimensions of a region of the PLE active site model.[309] Interesting enantioselective hydrolyses of the malonates **335a,b** have been reported.[492,493] Scheme 91 and Table 23 collect the above results. Additional examples of enzymatic hydrolyses of diesters are collected in the Scheme 92.

2-Aryl-succinates as the compound **336** can be resolved by a lipase (PFL) and up to >95% ee has been found for the monoacid **337**.[494] It has been seen from

Scheme 92



Scheme 93



the reported examples, that, in most cases, the compounds which undergo the enzymatic hydrolysis have the chiral or prochiral center one or two bonds away from the reacting carboxylate group. A noticeable exception is given by the diesters **338a,b**, where up to five bonds separate the prochiral center from the ester moiety which undergo the enzymatic hydrolysis to furnish the optically pure (*S*)-**339a,b**.[495,496] More recently, a molecular recognition in the hydrolysis of racemic butyl (*E*)-9-acetoxy-11-bromoundec-10-enoate (**340**) has been reported.[497] In this case, the chirality center is seven carbons away from the active hydrolytic site and the enantioselectivity at the recognition site is complete with CCL.

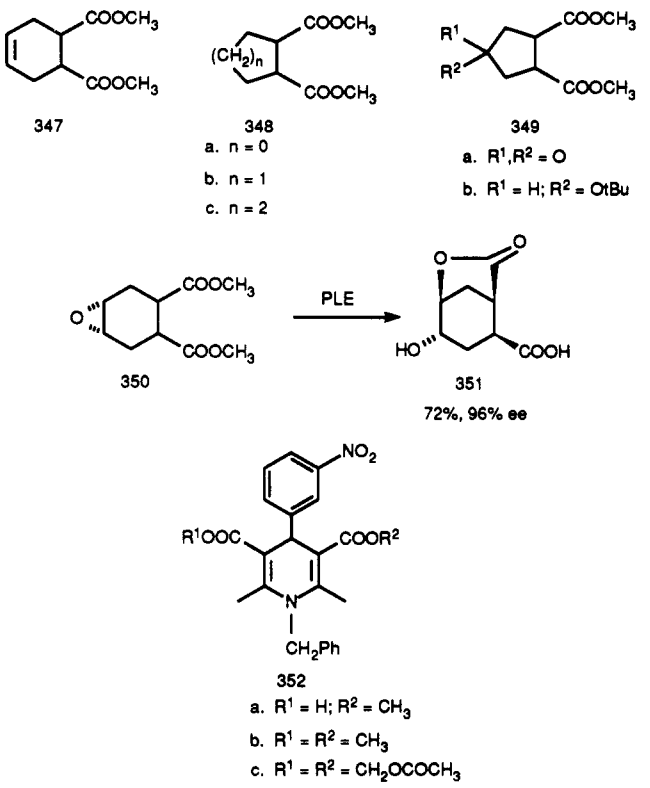
9. Cyclic Mono- and Diesters

Monocyclic monoesters **341a,b** are excellent substrates for PLE,[498] whereas the resolution of ethyl chrysanthemate (**342**) with the same enzyme proceeded with moderate stereoselectivity.[499] Recently, a screening of over 200 strains led to 31 strains capable of hydrolyzing the ester **342**. Among these microorganisms, *Arthrobacter globiformis* was able to furnish optically pure (+)-*trans*-**343** from the mixture of (±)-*cis,trans*-**342**.[500] Scheme 93 also collects the results of enzymatic hydrolysis of cyclopropane diesters.

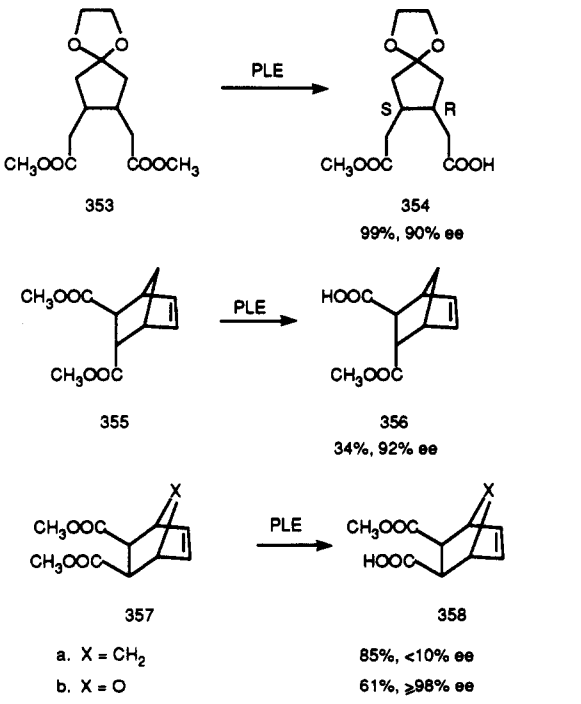
The cyclopropyl malonate **344** has been hydrolyzed with Esterase 30,000, and the unchanged diester was recovered with near optical purity (97% ee).[501] An interesting example is the enzymatic hydrolysis of heterocyclic malonates, namely the *N*-alkyloxaziridine-3,3-dicarboxylic esters **345a** and *N*-chloro-2,2-bis(methoxycarbonyl)aziridine (**345b**). The diester **345a** has been hydrolyzed with PPL (unchanged ester 87% ee),[502] and for **345b** a lipase from *Rhizopus delemar* gave the highest ee of the diester (76%).[503] The two esters **345a,b** are the first examples of an enzymatic preparation of optically active aziridines and oxaziridines, which have potential as chiral synthons in organic chemistry.[504]

Also, the PLE-mediated hydrolysis of cyclopropane-1,2-dicarboxylates **346a–d** proceeds with a high degree of stereoselectivity.[505] It should be noted that for the diester **346d** only the cis-isomer can be asymmetrized, because PLE does not distinguish between the enantiomers of the trans-isomers.

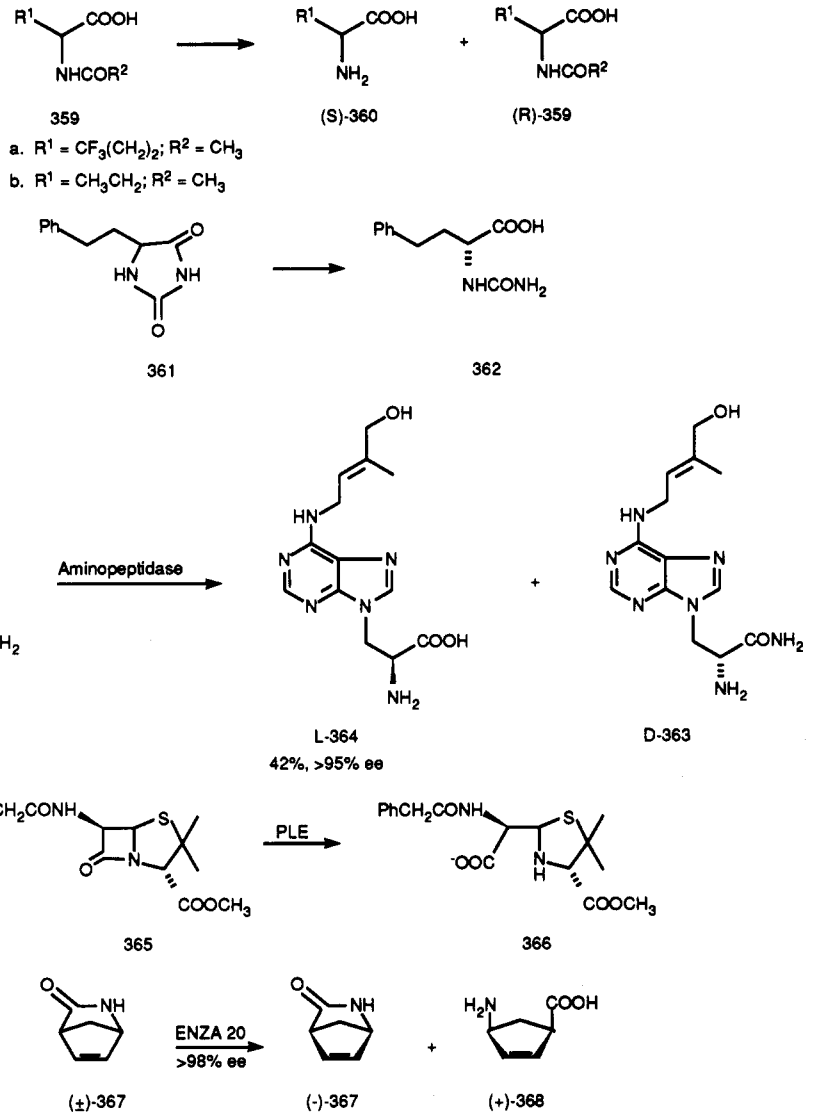
Scheme 94



Scheme 95



Scheme 96



Cyclic diesters like **347** are traditional substrates for the PLE-catalyzed hydrolysis-resolution process. Synthetic applications of this method are still pursued.[506-510] Diesters **348a-c** are resolved with high enantioselectivity by PLE, as reported in a study devoted to clarifying the role of the isoenzymes which constitute the commercial enzyme.[511] PLE seems to be the favorite enzyme for hydrolysis of this class of compounds, as shown for the hydrolysis of a series of 4-substituted-cyclopentanedicarboxylates.[512] The best results in this case are obtained for the compounds **349a,b** (82-84% ee for the monoacids) (Scheme 94).

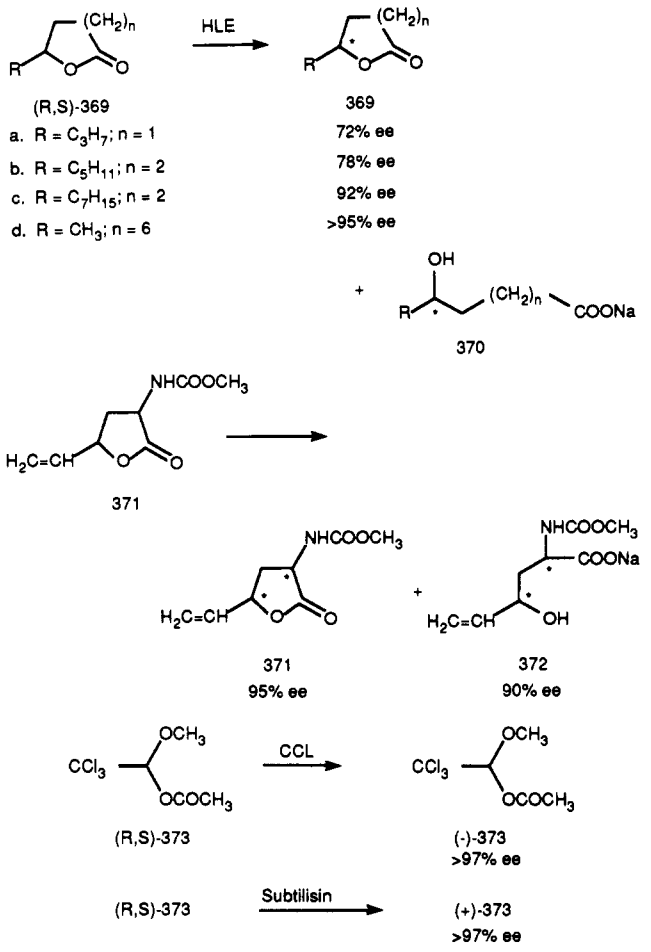
The PLE-catalyzed hydrolysis of the epoxy cycloalkane diester **350** affords the hydroxy acid **351**, which occurs from an intramolecular substitution reaction.[513] A nice application of the conceptual strategy necessary to solve chemoenzymatic problems, is the synthesis of optically active dihydropyridine derivatives, for instance the monoacid **352a**. These compounds are useful intermediates for the synthesis of optically active calcium channel blockers, such as nimodipine and nicardipine.[514] The enzyme-catalyzed hydrolysis of conventional diesters, like **352b** was ineffective, probably due to steric congestion of the ester functions. To relieve this steric hindrance, the diester **352c** was prepared and enzymatically cleaved and the enantioselectivity brought to 97% by a careful choice of the enzyme and experimental conditions.[514] Similarly, a Lipase B (from *Pseudomonas fragi*) realized the same resolution in diisopropyl ether saturated with water, which was carried out successfully (>99% ee in most cases) on a variety of derivatives structurally related to **352c**.[515]

Among several prochiral diesters, the bicyclic diester **353** gave the best results of hydrolysis with PLE in aqueous acetone to the corresponding monoester **354**[516] (Scheme 95).

A recent study on the PLE and PPL hydrolysis of 3,4-(isopropylidenedioxy)-2,5-tetrahydrofuranyl diesters gave up to 72% ee of the corresponding monoesters.[517]

Norbornane-type diesters can be valuable intermediates for the synthesis of natural cyclic compounds and the PLE-catalyzed hydrolysis has been studied in details.[518] Apparently, the *exo*-ester function of the bicyclo[2.2.1]heptene diester **355** (syn to the C-7 methylene group) is hydrolyzed with high preference.[518,519] The PLE enantioselective hydrolysis can be realized when the diesters are trans, and the monoacid **356** (92% ee) can be prepared. Interestingly, when the lipophylic methylene bridge of *cis*-**357a** is replaced by the more polar oxa bridge as in **357b**, the hydrolysis proceeds

Scheme 97



with high chemical and optical yields to the monoester **358b**.[520] Finally, the PLE-catalyzed hydrolysis of spiro-[3.3]heptane dicarboxylates proceeds with only low to moderate enantioselectivity.[372]

C. Amides and Lactams

Most of the reported enzymatic hydrolyses of amides are concerned with amino acids chemistry.[521] The above hydrolytic enzymes have some interest from the preparative point of view, since many nonnatural or less-common amino acids can be synthesized by chemoenzymatic methods.[522-524] Typically, by commercially available porcine kidney acylase the resolution of the acylamides of the amino acids **359a,b** proceeds efficiently to optically pure (*R*)-**359a,b** and (*S*)-**360a,b**[525,526] (Scheme 96).

Microorganisms have the hydrolytic capability of cleaving the hydantoin ring in compounds like **361** so that the carbamoyl amino acid **362** can be prepared optically pure.[527] An aminopeptidase able to hydrolytically cleave amides type **363** can be purified from *Pseudomonas putida* as the enzyme of choice for the preparation of an unusual amino acid such as L-lupinic acid (**364**).[528] In another work, *Mycobacterium neoaurum* was used for the hydrolysis–resolution of other amides.[529] Regarding the hydrolytic opening of lactam rings, it should be reported that, carrying out the reaction on the millimolar scale, PLE is able to open the β-lactam ring in the benzylpenicillin (**365**).[530] Interestingly, it has been observed that the methyl ester function survives to the PLE hydrolysis, and the compound **366** is therefore obtained. Strains of microorganisms from the soil were grown with the capability of cleaving the lactam ring in the cyclic compound **367**.[531] From the racemic lactam, with one biocatalyst (ENZA 20, *Pseudomonas solanacearum* NCIB 40249) it is possible to isolate the enantiomerically pure unreacted (−)-**367** and the hydrolyzed (+)-**368**. With another microorganism (ENZA 1, *Rhodococcus equi* NCIB 40213) and with the same procedure, it is possible to prepare the enantiomers of **367** and **368**, which can be further elaborated for synthetic purposes.[531-533]

D. Various Hydrolyses

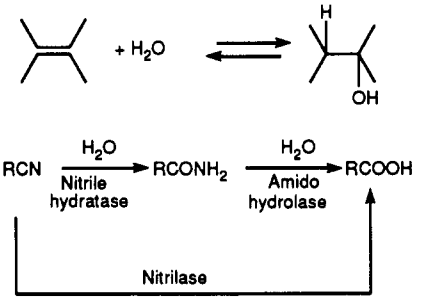
The resolution of racemic compounds via hydrolysis has a broad spectrum of applications, besides the conventional chemical groups examined in the previous paragraphs. Among these less usual groups, it could be mentioned the enantioselective hydrolysis of phenylacetate esters of various compounds catalyzed by penicillinacylase from *Escherichia coli*[534] or the lipase-catalyzed hydrolysis of *N*-(acyloxy)methyl groups for the synthesis of chiral 5,5-disubstituted 1-methylbarbiturates.[535] Racemic lactones can be resolved by the hydrolytic action of enzymes like lipases or esterases. This is the case of lactones of various sizes like **369a–c** which were hydrolyzed to the hydroxy acids **370a–c** with several enzymes.[536] Horse liver acetone powder which contains an esterase (HLE) was more enantioselective of PPL and PLE in the hydrolysis of δ-lactones **369b,c**. The unreacted lactones **369b,c** showed in fact, up to 92% ee when hydrolyzed by HLE. γ-Lactones like **369a** are hydrolyzed with a higher ee by PPL. The resolution of a medium-ring lactone **369d** affords optically pure (*S*)-**369d** either with PLE and HLE.[537] HLE is also able to hydrolyze bicyclic lactones with variable ee of the unreacted substrate.[538] PPL is able to hydrolize N-substituted α-amino-γ-butyrolactones, and the best result (95% ee) has been obtained for the unreacted **371** and the hydroxy acid **372** (90% ee)[539] (Scheme 97).

Unusual hydrolyses have been reported, like the interesting, although not chiral, antibody-catalyzed cleavage of a trityl group[540] or the resolution of a phosphorylated 2′-*ara*-fluoroguanosine catalyzed by the 5′-nucleotidase from *Crotalus atrox* venom.[541] Interestingly, the hydrolysis of the acetate of the acetal **373** gives the >97% ee unreacted ester (−)-**373**, when CCL, PPL, or cholesterol esterase are used, while the action of subtilisin on the same substrate allows the preparation of the (+)-**373** (>97% ee).[542]

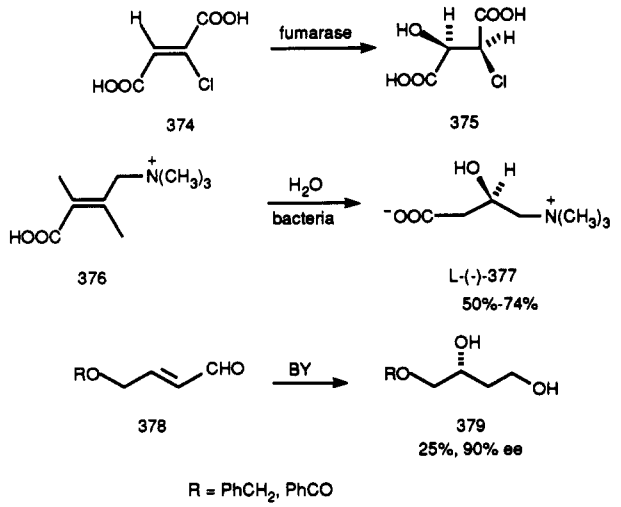
VI. Hydration

This process is treated separately from the hydrolysis reaction, although both require the addition of water to a molecule. As opposed to the classical hydrolysis reaction, which brings to the formation of two products, a hydration consists in the formation of a single product in which the hydrogen atom and the hydroxyl group are finally bound to two contiguous atoms of the same molecule. Examples of hydration reaction are the addition of water to a double bond or the hydrolysis of a nitrile. In the latter case, the first process is a water addition mediated by a nitrile hydratase which affords an amide. The subsequent hy-

Scheme 98



Scheme 99



drolysis catalyzed by an amido hydrolase affords a final carboxylic acid. It is also possible to realize biocatalytically the direct conversion of the nitrile to the corresponding acid with a nitrilase. Depending on the nature of the substrate, it is possible to use both the processes of biotransformation[543,544] (Scheme 98).

Concerning the double bond hydration (Scheme 99), an enzyme which could be used in this respect is pig heart fumarase (E.C. 4.2.1.2). Its requirements about the structure of substrates has been studied and one application has been the chiral synthesis of *threo*-L-chloromalic acid (**375**) from chlorofumaric acid (**374**).[545] An additional example of this biocatalytic process is the hydration of the crotonobetaine (**376**) to L-(−)-carnitine (**377**).

Several bacteria have been screened and among these the most interesting for the extent of the production of optically pure **377** are resting cells of *Escherichia coli* (74% at 5 mmol/L of **376**)[546] or intact cells of *Proteus mirabilis* under partially anaerobic conditions (50% at 62.5 g/L of **376**).[547] An interesting hydration of a double bond, accompanied by the well-known reducing capability of BY, is the recently found conversion of a few 4-oxy-substituted crotonaldehydes like **378** to (2*R*)-1,2,4-butanetriols **379**. Here, the BY-mediated reduction of the aldehydic group and hydrogenation of double bond are the main processes, but ca. 25% of the hydration reaction, i.e. compound **379**, can be also isolated.[548,549]

The potentially useful enzymatic hydrolysis of nitriles has been recently reviewed,[543,544] and preliminary studies on enzymes preparations from *Rhodococcus* sp. have already been published.[550-553] The first example of enantioselective hydrolysis of aromatic nitriles, specifically **380a-c**, has appeared in 1991.[554] The enzymatic system of *Rhodococcus butanica*, a microorganism which grows utilizing 2-cyanoethanol or benzonitrile, hydrolyzes the above **380a-c** to chiral amides **381a-c** or carboxylic acids **382a-c**. A recent publication from the same group shows also that the hydrolysis of optically active cyanohydrins with the same microorganism affords α-hydroxy acids with complete retention of configuration.[554] The products are obtained in ee which can reach >99%, provided that the right time of incubation is chosen (Scheme 100).

Due to the mildness of the experimental conditions of the enzymatic versus the chemical hydrolysis, it can be foreseen that more papers will deal with this interesting biocatalytic approach in the future. A recent example of hydration by *Brevibacterium imperiale* of several 2-(aryloxy)propionitriles, which essentially differ for the aromatic substituents, has been reported.[555] The most representative nitrile, compound **383** apparently is transformed via a fast hydration to the racemic amide **384**, which is resolved in the next hydrolytic step. In fact, at 47% conversion to the acid, >95% ee (*R*)-2-(aryloxy)propionic acid **385** is formed along with the (*S*)-amide **384** (89% ee). In the first example of asymmetrization of prochiral 3-substituted glutaronitriles,[556] optically pure mononitriles **387a,b** are obtained from **386a,b** using *Rhodococcus butanica* as biocatalyst. It should be mentioned that if a benzyl group is present at position 3 the selectivity of the chiral recognition is dramatically lower (29% ee).

The hydrolysis of epoxides to the corresponding diols can be enantioselectively catalyzed by the rabbit liver microsomal epoxide hydrolase (E.C. 3.3.2.3).[557-559] Recent applications have been reported for carbohydrate epoxides[560,561] and other variously substituted cycloalkene oxides.[562-564] Although in many instances good enantioselectivity was observed (up to 95% ee), generally the reactions have been run on micromolar quantities and the usefulness of the method on a larger scale has not yet been demonstrated. Also the stereochemistry of the hydrolysis catalyzed by the cytosolic epoxide hydrolase has been studied,[563,565,566] and alkene oxides too have been examined.[565,566]

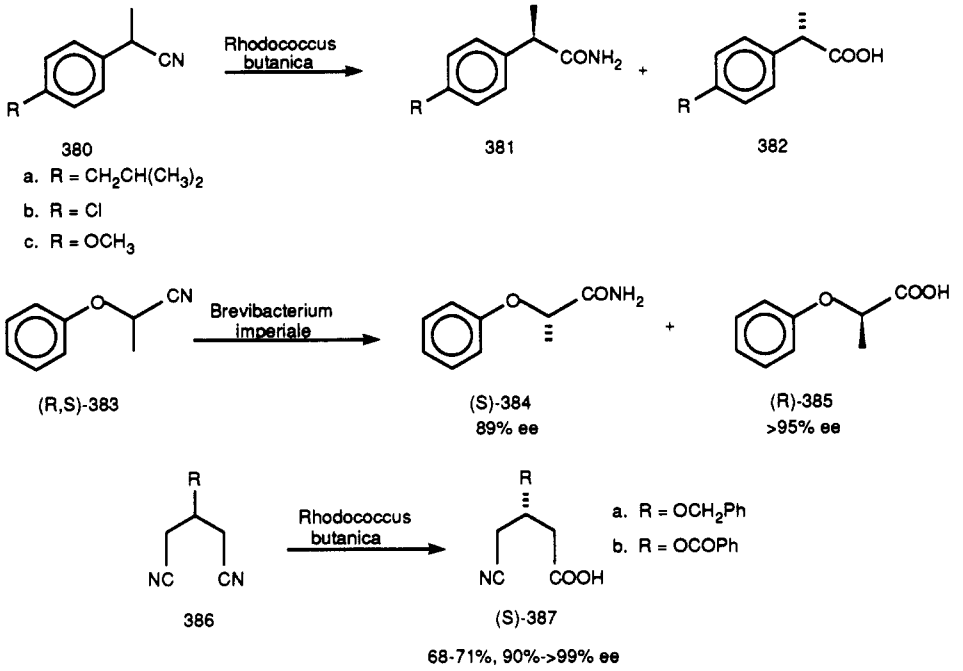
VII. Esterifications

A. General Remarks

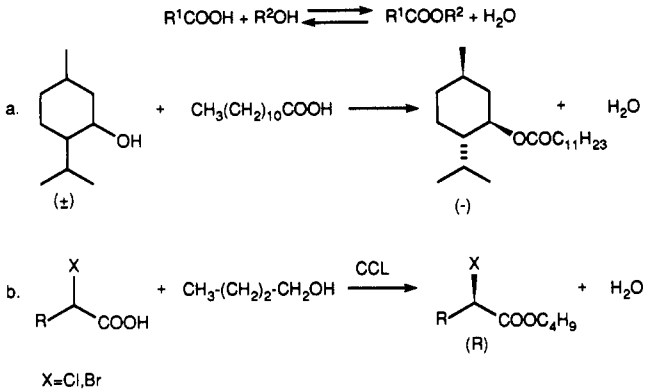
Hydrolytic enzymes such as esterases, lipases, and proteases generally may catalyze also the reverse reaction, i.e. esterification, but this reaction needs a low water concentration to minimize the hydrolysis (Scheme 101). In order to carry out esterification procedures of preparative significance, the unwanted hydrolysis has to be suppressed, and this can be accomplished by a few experimental procedures.

For instance, α-chymotrypsin catalyzes the formation of ethyl esters of aromatic amino acids in ethanol (90% yield) with a water concentration 2.5-10% (v/v).[567] With immobilized chymotrypsin yields of 95% are obtained with ethanol dissolved in chloroform.[568] This introduces the very important theme of the use of organic solvents for enzyme-catalyzed reactions, which have already been reviewed by several authors.[569-580] Among several studies on the solvent effects on enzymes catalysis,[581-586] recent works on the influence of the nature of the solvent

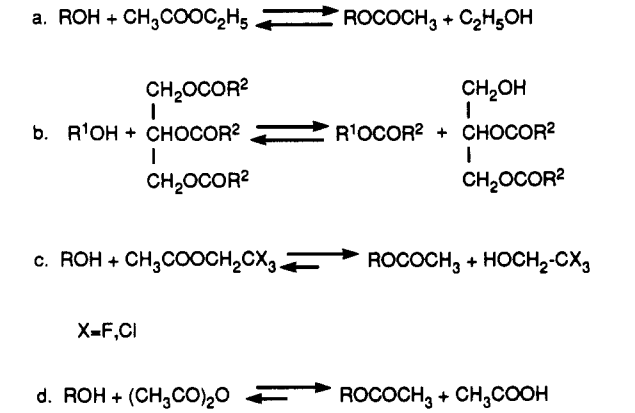
Scheme 100



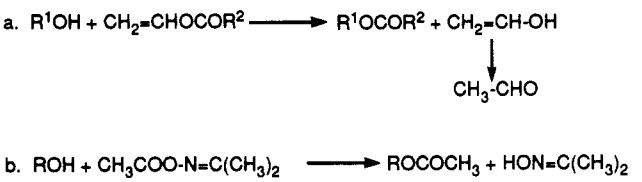
Scheme 101



Scheme 102



Scheme 103



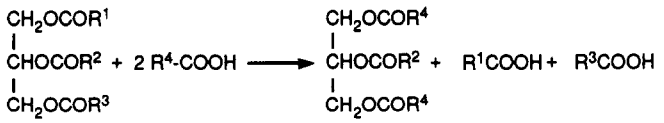
on the enzyme enantioselectivity in esterifications have appeared.[587,588] Among the various reactions in bi- and monofasic systems, the direct esterification and the transesterification procedures have enjoyed a widespread application, especially if the enantioselective resolution of a racemic mixture of alcohols and acids is concerned. For instance, 95% ee of (−)-menthyl laurate can be obtained from the resolution of racemic menthol via the esterification with lauric acid in heptane (Scheme 101, entry a).[589] In an analogous manner, the optical resolution of racemic 2-halo carboxylic acids can be achieved in the same solvent by the direct esterification of the acid with 1-butanol (Scheme 101, entry b).[590]

The enzyme-catalyzed transesterification in organic solvents has proven to be more effective than the conventional esterification procedure (Scheme 102). The acylation of an alcohol ROH could be performed with an ester like ethyl acetate, which can act either as solvent and acylating agent,[591] or a triglyceride, which after the exchange, will be transformed into a diglyceride[592] (Scheme 102, entries a and b).

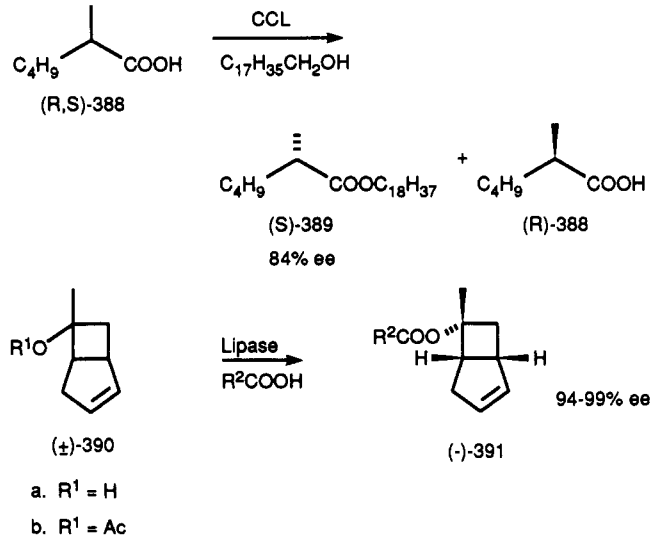
If a good leaving group is present on the acyl donor, the equilibrium is shifted to the right, and for this purpose, trichloroethyl or trifluoro esters[593] or anhydrides[594] can be used as acylating agents (Scheme 102, entries c and d). In the case of anhydrides, a recent study pointed out that the enantioselectivity is enhanced when the acid coproduct is removed.[595] The best experimental protocol, however, seems to be the irreversible transesterification procedure, which can be achieved using, as acylating agent, vinyl carboxylates (Scheme 103, entry a). In this case, the back reaction is prevented because the vinyl alcohol irreversibly tautomerizes to acetaldehyde.[596,597]

More recently, oxime esters have also been proposed as acyl transfer agent (Scheme 103, entry b) for an irreversible process.[598,599] Transesterifications can also be achieved by the interchange of the acid moiety of an ester, like the change of the fatty acid in triglyceride, commonly called interesterification. A classical example (Scheme 104) is the transformation of olive oil

Scheme 104



Scheme 105



to cocoa butter-like fat, where the exchange at the 1,3-position is catalyzed by a specific lipase from *Rhizopus delemar* in a water–hexane mixture.[600]

In the next sections, applications of the various procedures will be reported, excluding the regioselective enzymatic acylation of carbohydrates, which have been made possible after the pioneering work by Klibanov[601] and Wong.[602] A short account on this subject and on hydrolysis of esters of carbohydrates has been recently published.[603] Also, the formation of peptides will not be treated in the present work.

The majority of applications in organic synthesis regards the enzymatic transesterification procedures. Many papers report comparison between enzymatic hydrolysis and esterification, and they will be cited in most significant cases. It should be kept in mind that the advantage of the procedures in organic medium is more relevant when the substrate is insoluble in water or both the substrate and the product are water-sensitive.

A few examples of the direct esterification procedure for the resolution of racemic acids are given in refs 604 and 605. An example of this approach is the resolution of 2-methylhexanoic acid (**388**).[605] In a detailed study, the best ee (84% for the ester **389**) was found for a CCL-catalyzed reaction with octadecanol in heptane. The direct esterification of racemic alcohols in organic solvents can be vigorously catalyzed by a lipid-coated lipase.[606,607] The racemic cyclic alcohol **390a** can be resolved by direct esterification with an acid (94.3% ee of the ester **391**) in the presence of an immobilized lipase from *Mucor miehei* (Lipozyme).[608] Also the acetate **390b**, by a CCL-mediated interesterification with the same acid as above, affords the ester **391** (99.4% ee).[608] If the previous acid used for the esterification contains a chirality center and CCL is used, the diastereomeric excess of the ester **391** is high.[609] The Scheme 105 collects the above examples of the direct esterification procedure.

B. Resolution of Racemic Alcohols by Transesterification

1. Transesterification with Nonactivated Esters

Several examples of resolution of racemic alcohols by transesterification with nonactivated esters in organic solvents can be found in the current literature. For these transesterifications, when simple methyl-alkanoates are used, they serve also as solvents of the enzymatic reaction. This biocatalytic approach has been employed for the enantioselective resolution of various secondary alcohols[610] and diols.[611] In Scheme 106 a few examples of this approach are presented for bicyclic compounds.

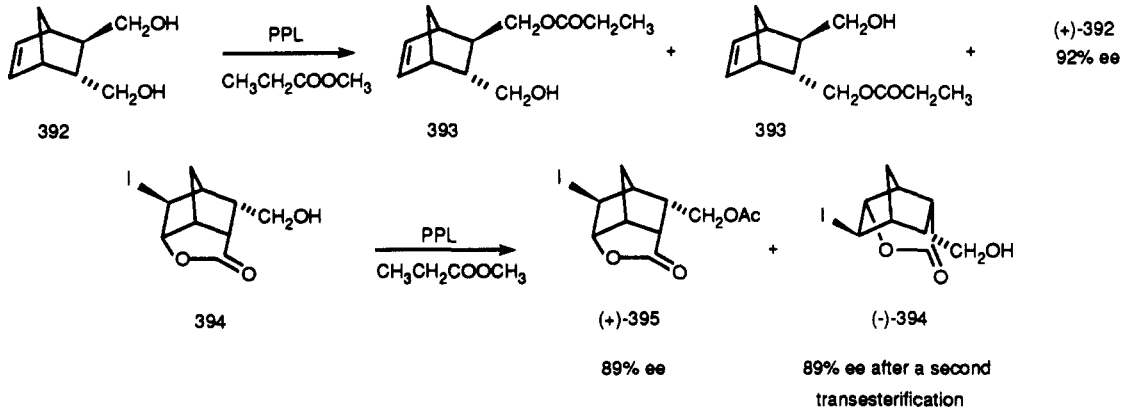
In the case of *trans*-bis(hydroxymethyl)norbornene (**392**) the PPL-catalyzed esterification with methyl propanoate afforded, as the best result, the (+)-unreacted diol (30% yield, 92% ee) and an *endo*-/*exo*-monopropionates **393** mixture (1:2.5).[612] The same procedure, using methyl acetate, applies to the *endo*-norbornene lactone **394**,[612] and in this case the (+)-acetate **395** showed an ee (89%) higher than the unreacted (−)-alcohol **394** (54% ee). As often happens, a second PPL-catalyzed esterification with the same acylating agent allowed a recovery of **394** with an ee value of 89%.

The transesterification of the ester of a racemic alcohol with methanol or 1-butanol in the presence of the yeast lipase CCL in an organic solvent can be used as a synthetic method for the preparation of chiral hydroxy compounds **397a,b** (Scheme 107). Good results were obtained for the product of the reaction in the case of the (*R*)-mandelate **397a** (92% ee)[613] and of the unreacted ester (>99% ee) for the isoserine derivative (*S*)-**396b**.[614] A successful application of the above procedure is the resolution of the racemic thioester **398**.[615] For this substrate, the transesterification with propanol in hexane (PPL as the catalyst) afforded a 95% ee (*S*)-thioester **398** and a 88% ee (*R*)-thiol **399**, the carbomethoxy moiety being unaffected by the enzymatic hydrolysis. The same methodology (Amano P lipase in diisopropyl ether with butanol) applies also to the preparation of nearly optically pure (2*R*,3*S*)-monoacetates **401a,b** from the *meso*-aziridine diacetates **400a,b**.[616] The previously reported acetate **240** is resolved with the same method to afford >95% ee of (*S*)-**402** with the lipase from *Pseudomonas cepacia*.[328] Comparing this procedure with other biocatalytic approaches, the aqueous hydrolysis of **240** has been previously cited.[328] Finally, the esterification with vinyl acetate of racemic alcohol **402** furnishes the (*R*)-alcohol and (*S*)-acetate with >93% ee.

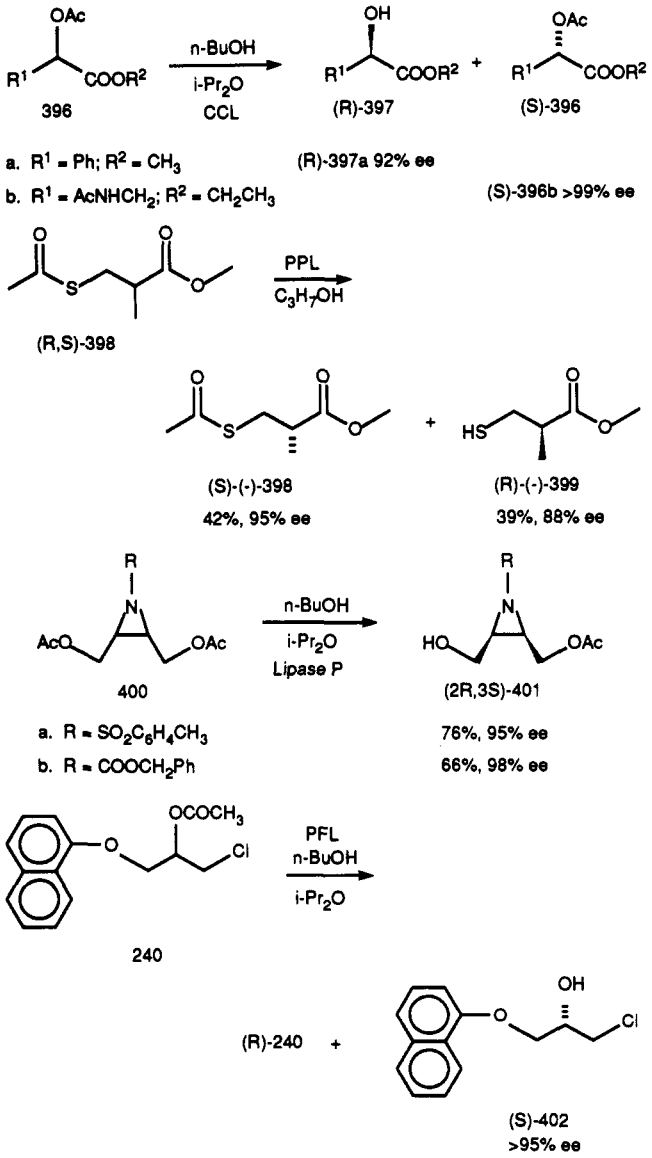
2. Transesterification with Activated Esters

The enzymatic transesterification with activated esters (Scheme 102, entry c) such as trichloro- or trifluoroethyl carboxylates[590] can be the enzymatic procedure to achieve the regioselective acylation of a polyfunctional compound such as chloramphenicol.[617] The method applies satisfactorily to the enantioselective syntheses of chiral esters or alcohols by the resolution of a racemic acyclic alcohol. In Scheme 108, only one example of the resolution of secondary alcohols is reported.[618-620] 6-Heptene-2-ol (**403**) has been resolved with trifluoroethyl butyrate in the presence of PPL into 93% optically pure (*R*)-butyrate **404** at 40%

Scheme 106

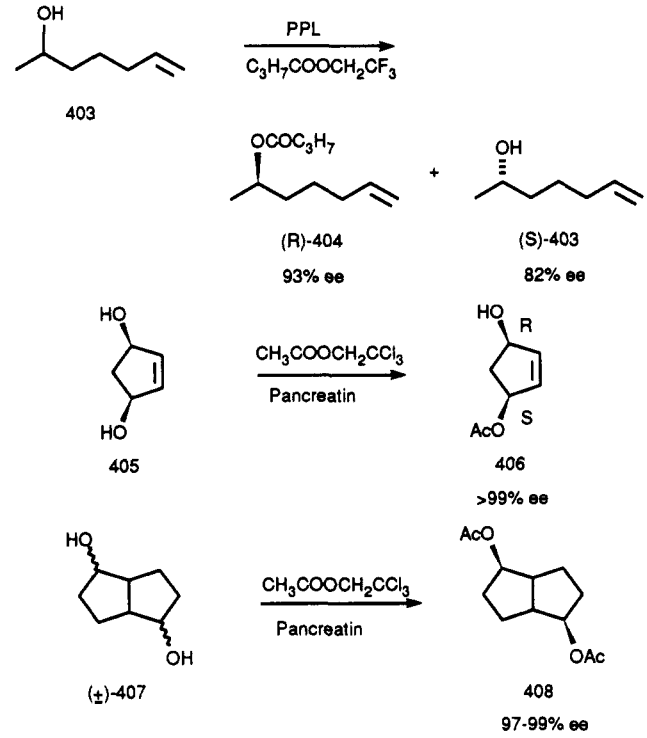


Scheme 107



conversion.[619] The (*S*)-alcohol **403** (82% ee) required an additional esterification to reach 97% ee. Cyclic substrates are 3-methyl-2-cyclohexen-1-ol,[621] or cyclopentene diols.[622-624] *cis*-Diol **405** can be asymmetrized by means of the crude enzyme pancreatin to the optically pure synthon for prostaglandins, i.e. (1*S*,4*R*)-(−)-4-hydroxycyclopent-2-enyl acetate (**406**).[624] This work investigated different procedures with various

Scheme 108

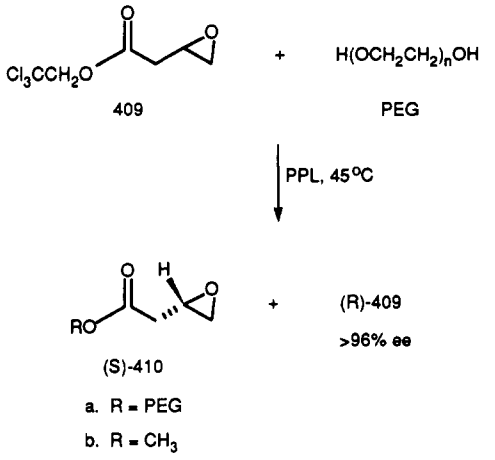


trichloroethyl and vinyl alkanoates as acylating agents, with the aim to obtain the highest ee. Also the racemic bicyclo[3.3.0]octane-2,6-diol (**407**) was resolved by pancreatin.[625] The *ent*-diacetate **408** was obtained in 30–35% yield and 97–99% ee. An interesting resolution of a racemic ester, i.e. 2,2,2-trichloroethyl 3,4-epoxybutanoate (**409**) has been achieved by PPL in warm diisopropyl ether.[626] In this work, the enzyme catalyzed the enantioselective transesterification of compound **409** with polyethylene glycol (PEG, molecular weight 1500). The (*S*)-PEG ester **410a** could be precipitated by cooling and converted to the (*S*)-methyl ester **410b**, using a PPL-catalyzed transesterification of **410a** with methanol (>89% ee). The unreacted (*R*)-trichloroethyl ester **409** was obtained with near optical purity (>96% ee, Scheme 109).

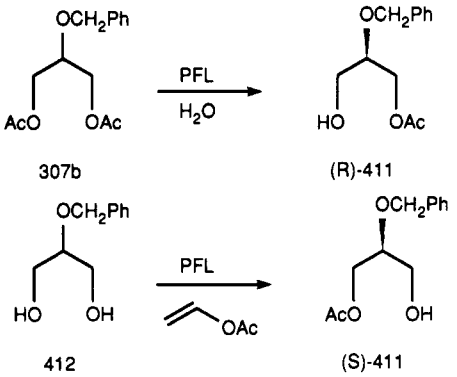
3. *Irreversible Transesterification*

The typical procedure for an enzyme-catalyzed transesterification relies upon the use of vinyl carboxylates as acylating agents.[596,597] As indicated in the Scheme 103, the formed vinyl alcohol completely tautomerizes

Scheme 109



Scheme 110



to acetaldehyde and this makes the process irreversible and efficient. In order to minimize the inactivation of the biocatalyst and therefore enhance the possibilities of its reuse, an immobilized enzyme can be employed.[627] Lipases, among which a special place is occupied by PFL,[628] are the enzymes of choice for synthetic applications. It should kept in mind that, according to a recent indication from Amano (Japan), the earlier denomination of *Pseudomonas fluorescens* should be changed into *Ps. cepacia* and, in fact, very recent papers already show the use of the new name. Recently, various acrylic esters were synthesized by this method, using the transesterification of vinyl acrylate with various alcohols.[629] In a few cases one can compare hydrolysis and transesterification protocols, as, for example, in the preparation of optically active 3-hydroxyalkanoates.[630] Especially significant is the preparation of optically pure glycerol derivatives (Scheme 110). The enzymatic hydrolysis of the prochiral 2-*O*-benzylglycerol diacetate (**307b**) to afford (*R*)-1-*O*-acetyl-2-*O*-benzylglycerol (**411**) has been reported.[631] A later work stated that the aqueous hydrolysis of **307b** could not lead to optically pure **411**, due to pH-dependent acyl migration.[422] This problem could be overcome by the use of the irreversible transesterification of the 2-benzylglycerol **412**.[632] In this way, the same enzymes catalyze the reaction in organic solvents, and yields and ee (>96%) are reproducible and higher than that obtained from the hydrolysis reactions. Additionally, it is interesting to note that the (*S*)-monoacetate **411** is the product of the reaction in organic solvent and is the enantiomer of the product of the aqueous procedure (Scheme 110). This fact seems quite general and additional examples are found in the recent literature.

Scheme 111

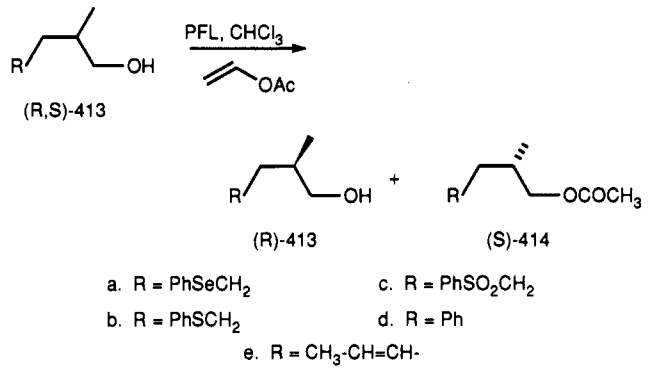


Table 24. PFL-Catalyzed Transesterification of 413a–e

	ester 414			alcohol 413			
substrate	yield, %	ee, %	config	yield, %	ee, %	config	ref
413a	40	>98	*S*	38	>98	*R*	634
413b	38	98	*S*	42	98	*R*	636
413c	36	98	*S*	42	98	*R*	636
413d	45	74	*S*	43	97	*R*	635
413e	36	>98	*S*	32	98	*R*	637

Working on the same problem, it has recently been shown that direct esterification with acetic acid or transesterification with other acylating reagents such as various acetic acid esters afford the (*S*)-**411**.[633]

a. Acyclic Primary and Secondary Alcohols. Using vinyl acetate in organic solvents and the commercially available PFL, the primary alcohols **413a–e** which bears a methyl group at the position 2 are efficiently resolved into nearly optically pure unreacted (*R*)-alcohols **413a–e** and the corresponding (*S*)-acetates **414a–e**[634–637] (Scheme 111 and Table 24).

Also secondary alcohols are efficiently resolved by the same procedure, and generally the same enzyme, a lipase from *Pseudomonas* species, is the right biocatalyst. A few preliminary studies on simple alcohols have been devoted to establishing the best experimental protocols.[638–640] Interestingly, a comparison with other procedures has been made.[638] For instance, the alcohol **415a** can be resolved into optically pure (*S*)-**415a** and the (*R*)-acetate **416a**, and the transesterification with ethyl acetate was ineffective. Alternatively, the aqueous hydrolysis with the same enzyme of the acetate **416a** affords the (*R*)-alcohol **415a** and the unreacted (*S*)-acetate **416a** (Scheme 112). In the Scheme 112 and Table 25 are reported the enantioselective resolutions of a few racemic secondary alcohols bearing other functions. This is the case of halohydrins, like **415b**,[641] or an α-hydroxy ester such like **415c**.[642] The long chain α-hydroxy ester **415d** and the corresponding acid can be resolved, as well as the 2-hydroxytetracosanoic acid (**415e**).[643] The reaction worked also on the monotosylate of diols, like **415f**,[644,645] and nitro alcohols, like **415g**.[646,647] Two well-known chiral compounds such as (*R*)-sulcatol (**55**)[648] and ethyl (*S*)-3-hydroxybutanoate (**71a**)[649] can be prepared by the transesterification procedure. Other interesting chiral compounds can be prepared by the above biocatalytic method.[650–658] The overall substrates are collected in the Scheme 113.

The structural features are various, since the substrates are dihydro-α-ionol (**417**),[650] the α-aryl-4-piperidinemethanol derivative **418**,[651] the chlorohydrin

Scheme 112

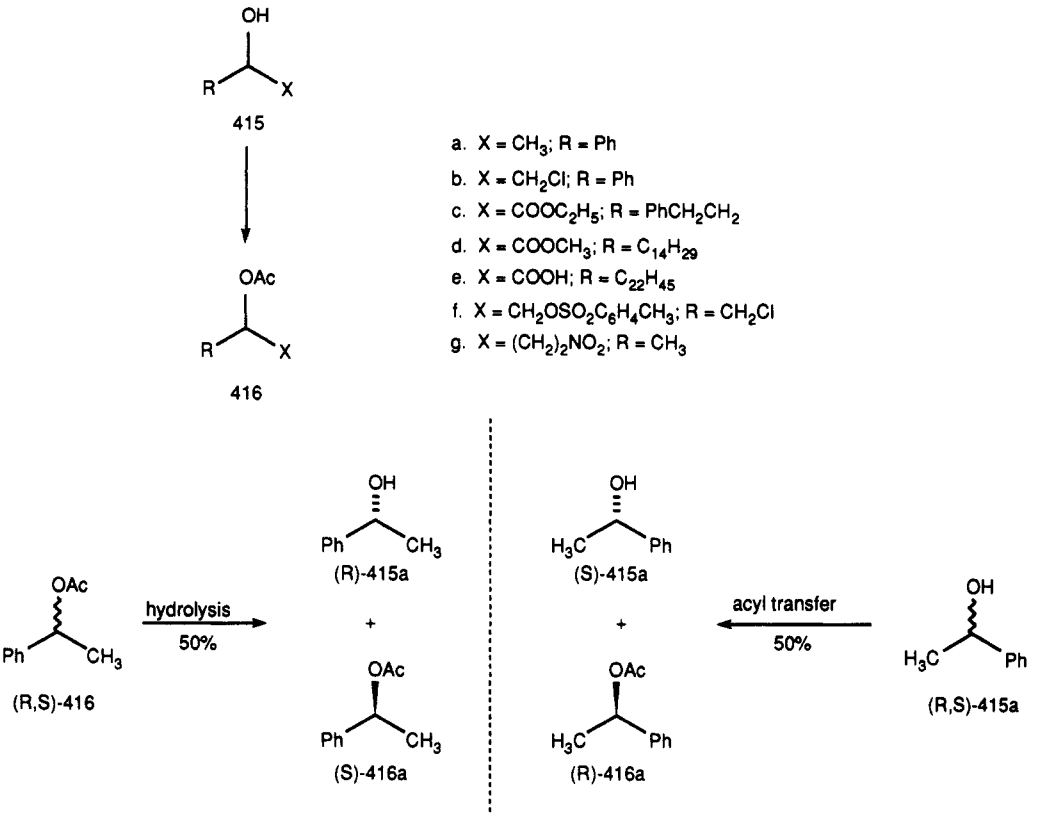
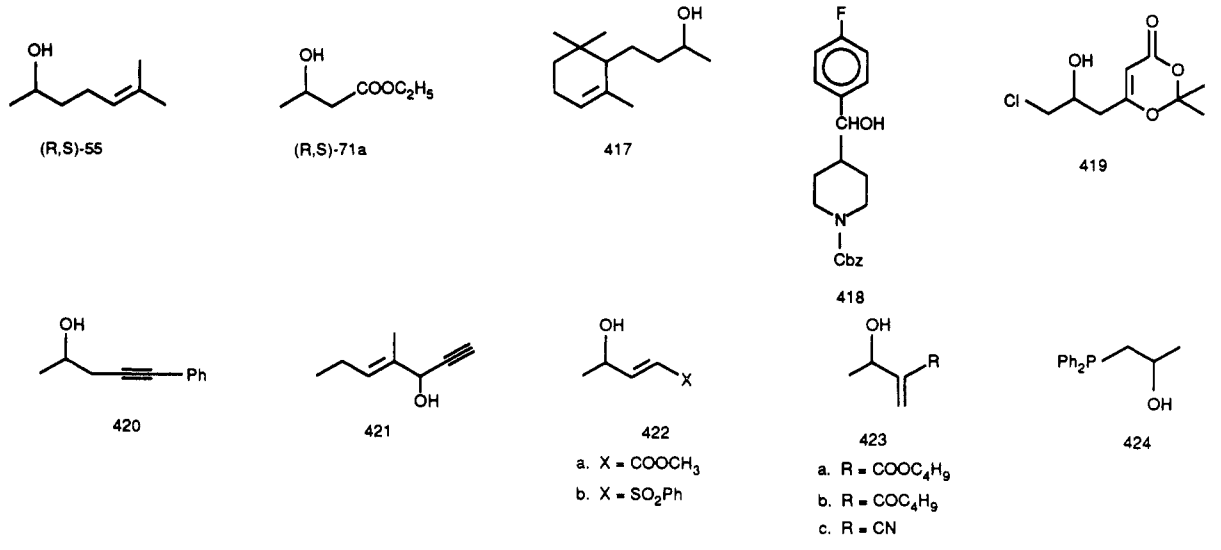


Table 25. Lipase-Catalyzed Transesterification of 415a–g with Vinyl Esters

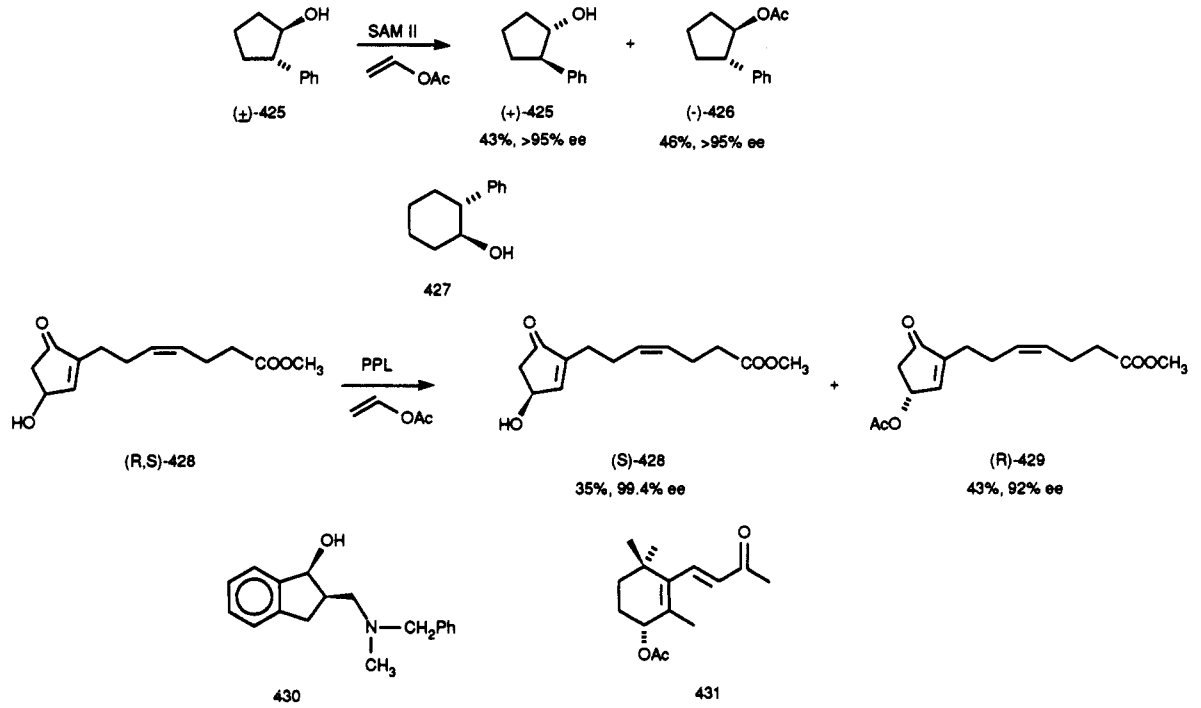
		acetate 416			alcohol 415			
substrate	lipase	yield, %	ee, %	config	yield, %	ee, %	config	ref(s)
415a	*P. fragi*	46	95	*R*	42	92	*S*	639
415a	*P. nitroreducens*	44	94	*R*	39	91	*S*	639
415a	PFL	45[a]	100[a]	*R*	41[a]	97[a]	*S*	638–640
415b	PFL	52	92	*S*	44	97	*R*	641
415c	PFL	48[b]	>98	*S*	51[b]	>98	*R*	642
415d	PFL	55	66	*S*	34	>98	*R*	643
415e[c]	Lipase PS[d]	55	77	*S*	45	>99	*R*	643
415f	PFL	42[a,b]	97[a]	*S*	47[a,b]	75[a]	*R*	644–645
415g	CCL	52	28	*S*	26	95	*R*	646

[a] Best results in the series. [b] Extent of conversion. [c] Data refer to the corresponding methyl ester. [d] Amano Pharmaceutical Co., Ltd.

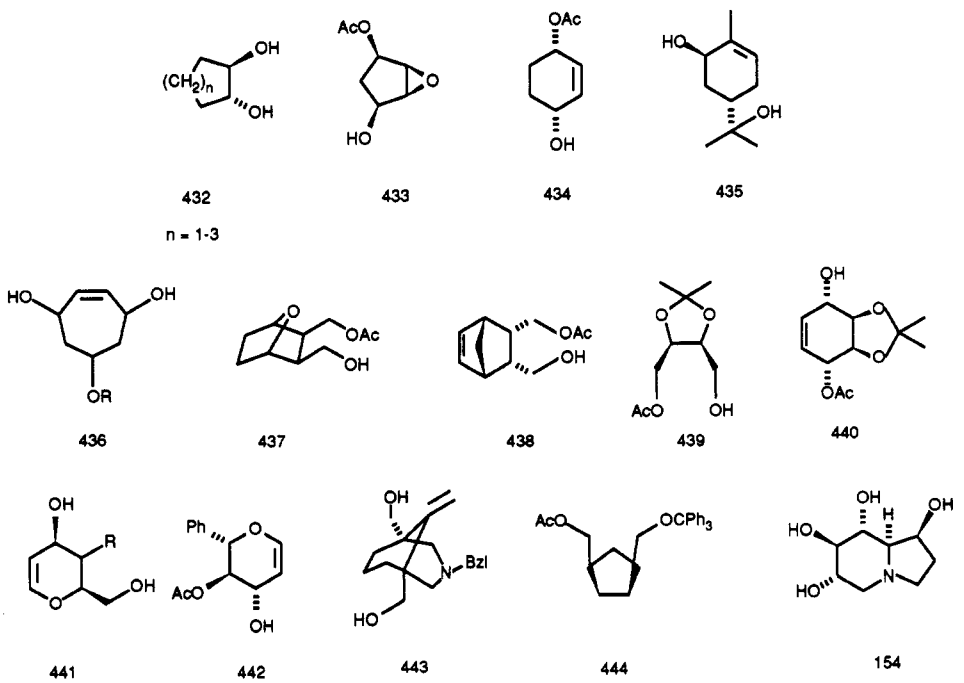
Scheme 113



Scheme 114



Scheme 115



419,[652] and the acetylenic alcohols **420**[653] and **421**.[654] The resolution effectively works on γ-hydroxy-α,β-unsaturated compounds, such as **422a**[655] **422b**,[656] α-methylene-β-hydroxy esters and ketones **423a,b**,[657] or nitriles **423c**.[658] In the latest work, the influences of the reaction conditions have also been studied. Finally, an example of resolution of (2-hydroxyalkyl)diphenylphosphine of type **424** has been described.[659]

b. Cyclic Alcohols and Diols. The enzymatic transesterification of cyclic substrates proceeds with good yields and high enantio- or diastereoselectivity. The reported resolutions of cyclic alcohols are collected in Scheme 114 which deals with cyclopentanols and cyclohexanols like **425**[660] and **427**.[661] In particular, from *trans*-**425** the lipase-catalyzed transesterification with vinyl acetate gave rise to (+)-alcohol **425** and (−)-acetate **426** (≥95% ee). The prostaglandin synthon **428** was resolved by the same method into optically pure (*S*)-**428** and 92% ee (*R*)-**429**.[662] This useful biocatalytic method applies to the preparation of chiral intermediates for the synthesis of drugs or pheromones. For the synthesis of a new serotonin uptake inhibitor, compound **430** was prepared[663] and the (*R*)-acetate **431** was used as intermediate for the synthesis of abscisic acid.[664]

In the case of cyclic diols the method is excellent and the enzymatic transesterification works enantioselectively on different substrates. In the Scheme 115 these results are collected for the most significant examples of the type of substrate described in the literature.

Unless specified, the product of monoacetylation is generally shown and the ee of all the products shown is always >95%. *trans*-1,2-Cycloalkanediols **432** afford in the presence of the lipase SAM II optically pure diacetates, whereas the optical purity of the monoacetates or diols depend on the ring size.[665] The crude enzymatic preparation pancreatin has already been used for the asymmetric esterification of the diol **405**.[624] Similarly, the optically pure monoacetate **433** can be prepared from the corresponding epoxy diol also with other lipases (Amano PS, Yarrowia, Sp. 382).[666] (3*R*,6*S*)-3-Hydroxy-6-acetoxycyclohex-1-ene (**434**, 98% ee) is prepared from the corresponding diol in isopropenyl acetate (solvent and acylating agent) in the presence of Lipase P-30.[667] (+)-(1*R*,5*S*)-Sobrerol **435** is one of the enantiomers of the commercial drug sobrerol, which is marketed as a racemate. The (+)-**435** is prepared optically pure from the racemic *trans*-**435** in the presence of Lipase PS immobilized onto Hyflo Super Cell in *tert*-amyl alcohol.[668] The two C-6 isomeric 6-[(*tert*-butyldimethylsilyl)oxy]-2-cycloheptene-1,4-diol monoacetates (**436**) can be prepared in >95% ee from the corresponding diols.[669]

Efficient asymmetric transesterification applies also to other cyclic diols, so that highly optically pure monoacetates may be prepared, such as the acetates of 7-oxabicyclo[2.2.1]heptanediol (**437**),[670] of *cis-endo*-5-norbornene-2,3-dimethanol (**438**),[671] of the acetonides **439**[672] and **440**,[251] of the glycals **441**[673] and **442**,[674] and of the bicyclic compound **443**, an intermediate for the synthesis of the alkaloid atisine.[675] The subtilisin-catalyzed acylation in pyridine can be the method of choice for the regioselective esterification of castanospermine **154**.[676] (1*R*,4*S*)-Acetate **444** is prepared from the corresponding monotrityl *cis*-diol.[677] Interesting structures which are obtained by resolution of the corresponding cyclic diols are spirocompounds as 2,6-bis(hydroxymethyl)spiro[3.3]heptane[678] and the cyclic precursors of calicheamicinone or *ent*-calicheamicinone.[679]

c. Acyclic Diols. The enzymatic acylation of prochiral 2-substituted 1,3-diols affords enantiomerically pure monoacyl derivatives if the substituent at position 2 is bulky enough to allow the enzymatic discrimination. The enzymes which are able to catalyze the reaction are various lipases. This has already been reported for several prochiral 2-substituted 1,3-propanediols of general structure **445**[385] (Scheme 116 and Table 26).

More recently, the irreversible esterification procedure has been applied to a few prochiral diols, like **445a–c**.[680–683] The asymmetrization of 2-O-substituted glycerol **412** has already been studied in detail.[422,597,631–633] Additional work has been done on the asymmetrization of compound **412** and its applications[684–686] and on the regio- and stereoselective enzymatic esterification of glycerol and its derivatives.[687] The enzymatic resolution of the racemic mephenesin **447** leads to the synthesis of the (*R*)- and (*S*)-**447**, a potent muscle relaxant.[688] The case of 2-methyl-1,3-propanediol (**445d**) is a special one, since it can be enantioselectively esterified to **446d** only under controlled conditions.[689] Resolution of the racemic monoester **446d** is realized by the transesterification method, leading to optically pure unreacted (*S*)-**446d** and the achiral diacetate. Also the silyl monoether **448** can be enantioselectively resolved.[689] Recently, the enantioselective, enzymatic preparation of selectively protected 1,2- and 1,3-diols, compounds **450** and **451**, has been reported.[690] Finally, the biocatalytic asymmetrization of 2,3,4-tri-*O*-benzyladonitol with vinyl acetate in hexane in the presence of CCL leads to (2*S*,3*R*,4*R*)-1-*O*-acetyl derivative **452** (70% yield, >95% ee).[691]

Scheme 116

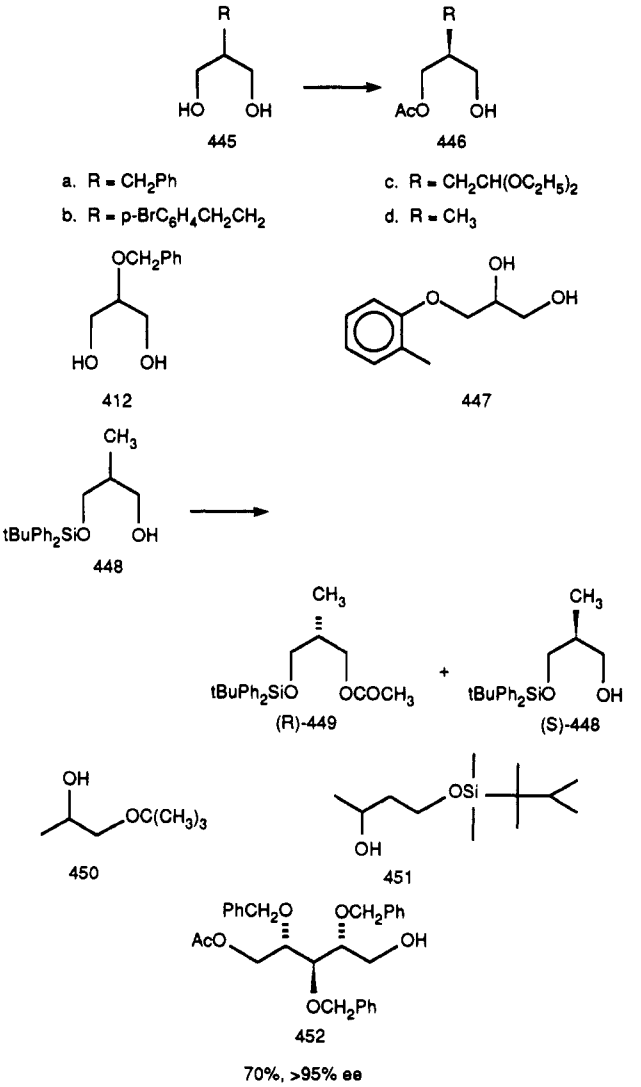


Table 26. Lipase-Catalyzed Transesterification of Acyclic Diols and Monoderivatives

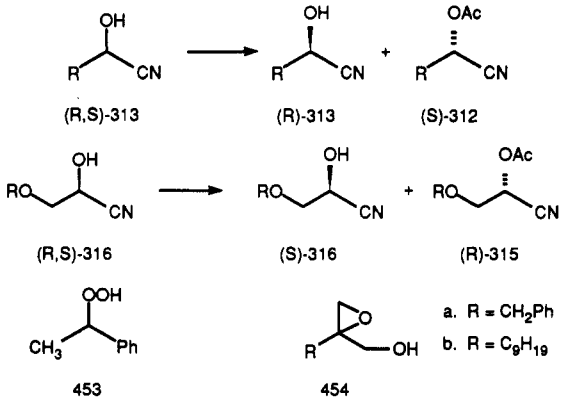
		monoester			
substrate	lipase	yield, %	ee, %	config	ref(s)
445a	PPL	90	13	*S*	385
445a	PFL	96	97	*R*	680, 681
445b	PPL	90	85	*R*	682
445c	PFL	95	98	*R*	683
445d	PFL	70	60	*R*	680
445d	PFL	40[a]	98	*S*	689
447	PFL	48	94	*R*[b]	688
448	PFL	39	>98	*R*[c]	689
450	PFL	*d*	*d*	*R*[e]	690
451	PFL	50	>95	*R*[e]	690

[a] At 60% of transesterification. [b] The (*S*)-diacetate (48%, 81% ee) was recovered by column chromatography. [c] At 60% conversion the unreacted (*S*)-alcohol (38%, 98% ee) was isolated. [d] Not reported. [e] At 50% conversion the (*S*)-alcohol (>98% ee) was isolated.

d. Various Substrates. The resolution of special classes of compounds containing hydroxy groups, which can be irreversibly esterified by the enzymatic procedure in organic solvents, is especially representative of the mildness and efficacy of the method. In Scheme 117 are shown the basic structures of racemic compounds like cyanohydrins, hydroperoxides, and epoxy alcohols, which can be resolved as indicated above. Table 27 collects the results of these reactions.

Optically active cyanohydrins have been prepared using ester hydrolases in the selective hydrolysis of cyanohydrin acetates (see section V.B.6). In most cases, in this process the unreacted ester was recovered with high optical purity, and the cyanohydrin product was disregarded due to its rapid racemization and/or hydrolysis in aqueous solution. The enzymatic reaction in organic solvent effected on racemic cyanohydrins **313** or **316** is not only enantioselective, but also prevents the above side reactions.[436,692] By the irreversible transesterification procedure, the acetates (*S*)-**312** and (*R*)-**315** can be isolated, as well as the optically active cyanohydrins (*R*)-**313** and (*S*)-**316**.[692] As usual, the stereochemical outcome of the enzymatic hydrolysis is the opposite to the transesterification process. Also, the sensitive and relatively unstable racemic hydroperoxide **453** is an excellent example of the best resolution attainable by the mild enzymatic esterification conditions.[693,694] Finally, a peculiar type of epoxy alcohol, 2-substituted oxirane methanols **454a,b**, can be useful synthons bearing a quaternary center of chirality and are resolved efficiently by the same procedure, in the presence of PFL.[695]

Scheme 117

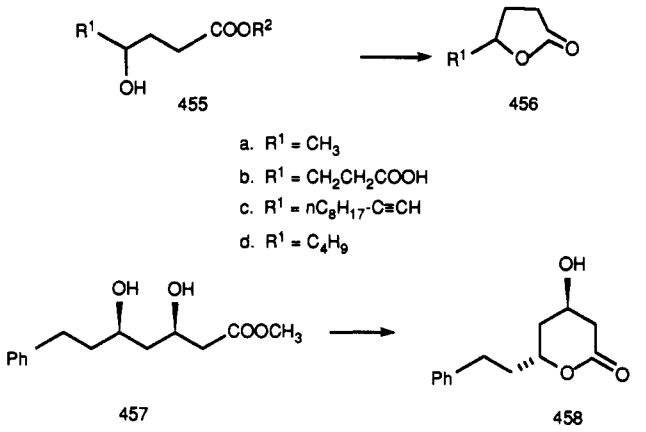


C. Enzymatic Ring Closure and Opening

1. Lactones from Hydroxy Esters

Since the first observation that certain hydroxy acids undergo cyclization to the corresponding lactones in the presence of *Mucor miehei* lipase,[696] enzymes have been used for the stereoselective ring closure of the appropriate hydroxy esters **455** and **457** to the corresponding lactones **456** and **458** (Scheme 118). Also a monoclonal antibody elicited by a transition-state analog catalyzes the stereospecific formation of a δ-lactone from the corresponding hydroxy ester.[697] A few examples have been gathered up to now for this cyclization reaction, which is generally catalyzed by a lipase in organic solvent, and the structures can go from macrocyclic lactones[698,699] to γ-lactones functionalized at the γ-position, compounds **456a–d**.[700–702]

Scheme 118



Alternate to the lactonization of **455c**, the enzymatic esterification of this hydroxy ester with anhydrides afforded the corresponding diester, which was further elaborated to yield the optically pure lactone **456c**.[703] In another work, it has been shown that a hydroxy ester like **455d** is cyclized to the enantiomerically pure lactone **456d**.[704] The same procedure (PPL in diethyl ether) was not enantioselective on the corresponding δ-hydroxy esters. Similar results have been obtained for the cyclization of methyl 5-hydroxy hexadecanoate, although ee reached 80% when Lipase P was immobilized on Florisil in isooctane in the presence of Triton X-100.[705] Nonetheless, the ester **457** affords the corresponding δ-lactone **458** (35% yield, >98% ee), when PPL in diethyl ether was used for the ring closure.[706] Although formally not related to lactones and not leading to chiral product, it should be briefly mentioned in this paragraph that the cyclization of *O*-(allylcarbamoyl)salicylonitrile to the 1,3-benzoxazine-2(3*H*)-one is catalyzed by the enzyme catalase[707] or by ultrasonically stimulated BY.[708]

2. Ring Opening of Anhydrides

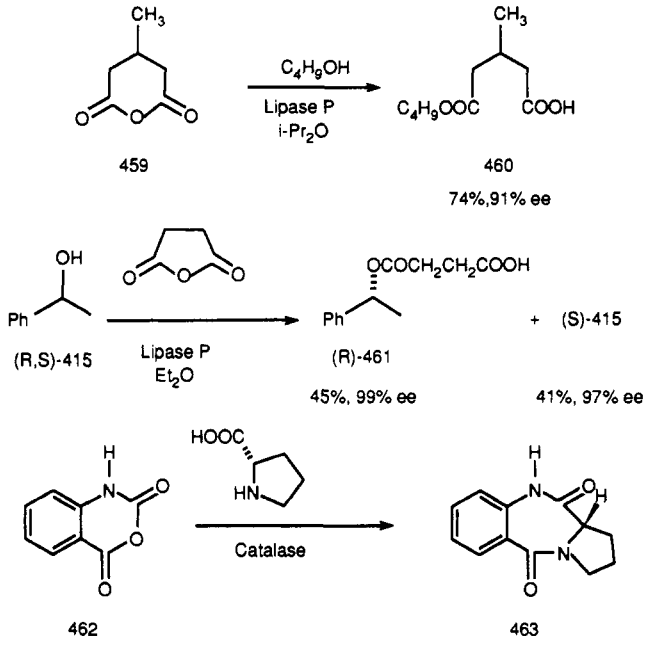
As mentioned earlier, enantioselective or regioselective esterifications using anhydrides as acylating agent

Table 27. Lipase-Catalyzed Transesterification of Cyanohydrins, Hydroperoxides, and Epoxy Alcohols

		ester			alcohol			
substrate	lipase	yield, %	ee, %	config	yield, %	ee, %	config	ref
313h	lipoprotein lipase[a]	22[b]	90	*S*	59[b]	98	*R*	692
316g	lipoprotein lipase[a]	25[b]	91	*R*	56[b]	96	*S*	692
453	lipoprotein lipase[a]	*c*	*c*	*c*	62[b]	100	*S*	693
454a	PFL	32[d]	>98	*S*	34[e]	>98	*S*	695
454b	PFL	38[d]	96	*S*	36[e]	96	*S*	695

[a] From *Pseudomonas* sp. [b] Extent of conversion (%). [c] Not reported. [d] At 40% conversion. [e] At 60% conversion.

Scheme 119



have been reported.[594] Recent applications of this method can be found in the very recent literature.[709-712] The enzyme-catalyzed opening of cyclic anhydrides has been reported[713] and appears to be a method which holds promise for more applications in the preparation of chiral synthons. The ring opening of cyclic anhydrides is catalyzed by a lipase such as PFL and the reaction is carried out in an organic solvent. A similar reaction has been reported for oxazol-5(4*H*)-ones.[714] In Scheme 119, the discrimination between the two enantiotopic carbonyl groups of 3-substituted glutaric anhydrides is reported to afford the corresponding monoester.[713] Several glutaric anhydrides substituted at the position 3 with a few groups have been the substrates for the lipase-catalyzed reaction in organic solvents with 1-butanol.[715,716] Ring opening of the prochiral anhydride **459** in diisopropyl ether is the best example of this reaction and the butyl ester **460** is obtained with 74% yield and 91% ee.[713]

As an application of the above method, studies on the regioselectivity of the ring opening of α-substituted cyclic anhydrides have been published.[717] An example has been reported also for lipase-catalyzed esterification of racemic alcohols with succinic anhydride.[718] This reaction proceeds enantioselectively to give the succinic acid monoester, which can easily be separated from the nonreacting alcohol by washing with alkaline solution. A good example is reported in the Scheme 119, specifically the reaction of the racemic alcohol **415** in diethyl ether in the presence of Lipase P to afford the (*R*)-monoester **461** (45% yield, 99% ee) and (*S*)-alcohol **415** (41% yield, 97% ee). Finally, the condensation of a specific cyclic anhydride, isatoic anhydride **462**, with proline in mild conditions such as a phosphate buffer in the presence of catalase as biocatalyst has been reported.[719] In this way, the pyrrolo[1,4]benzodiazepine **463** is prepared in good yields and high optical purity. Furthermore, in this work other biocatalytic systems have been used in order to perform asymmetric transformations of other groups for synthesis of the heterocyclic compound **463**.

VIII. Asymmetric Glycosylation

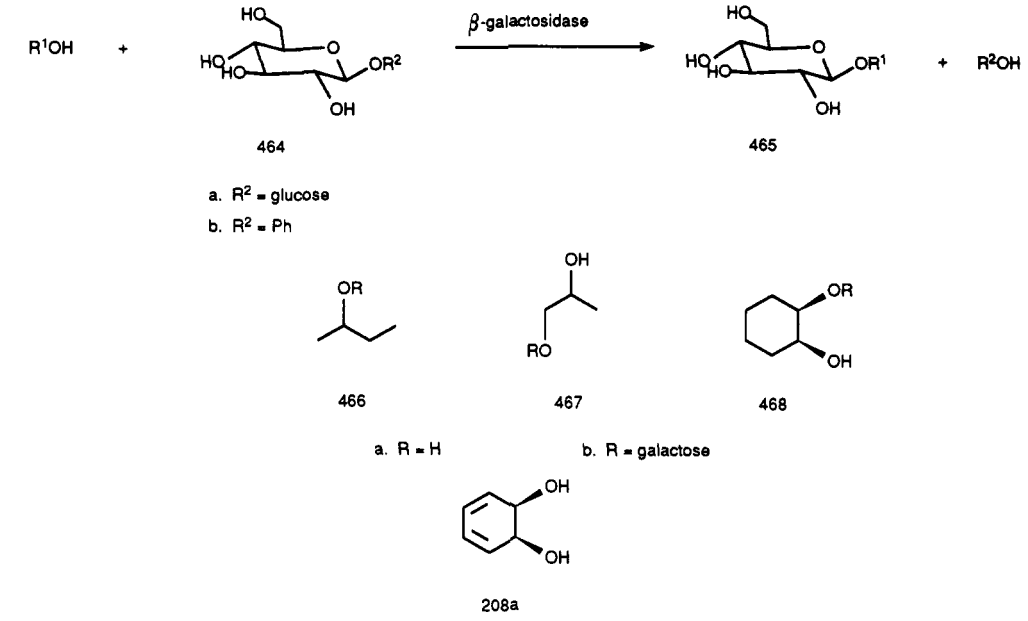
The formation of glycosides of an alcohol seems a reaction well suited for a crude system like plant cell cultures. For example, *Mentha piperita* L. cell cultures is the most active among four species examined, and a few alcohols, among which linalool and phenylethanol, are converted to the corresponding glycosides in 58% and 70% conversion, respectively.[720] Use of specific purified enzymes is restricted to a few biocatalysts. Glucosyl transferases have been used in carbohydrate synthesis,[721-724] or in the nucleoside synthesis, where specific transferases catalyze the reaction.[725,726] Crude liver homogenate containing all necessary enzymes has been recently employed for a multienzymatic one-pot synthesis of β-glucuronides.[727] The applications of accessible and relatively inexpensive glycosidases to asymmetric synthesis should exploit the transferase activity of this hydrolytic enzyme. β-Glucosidases and β-galactosidases from different sources are the enzymes of choice for the preparation of alkyl or hydroxyalkyl gluco- or galactopyranosides.[728-730] An interesting application is the synthesis of β-2-deoxy-D-glycosides, which uses the above enzymes and glycals as substrates.[731] The β-galactosidase (β-D-galactoside galactohydrolase, E.C. 3.2.1.23) from *E. coli* is able to catalyze the β-galactosyl transfer from lactose **464a** or phenyl β-galactopyranoside (**464b**) to alcohols and diols (Scheme 120).

Some enantioselectivity has been observed when a secondary alcohol like **466a** is the substrate for the conversion to the β-galactoside **466b**.[732] This paper presents also a study on the regioselectivity on a substrate as the 1,2-diol **467a** and the β-galactoside **467b** is the main product. Similar results have been obtained using a crude preparation of β-glycosidase from *Sulfolobus solfataricus*.[733] The first example of diastereoselective enzymatic glycosylation is constituted by a report on galactosyl transfer to cyclic *meso*-diols.[734] The most favorable example is the reaction of the *meso*-diol **468a** with lactose **464a** or with phenyl β-galactopyranoside (**464b**) in the presence of commercially available *E. coli* or *Aspergillus oryzae* β-galactosidases yielding the mono-β-D-galactoside **468b** with 90% de. The same reaction, using galactosyl transferase from *E. coli* and lactose **464a** as galactosyl transfer agent, has been used for the synthesis of the diastereomeric β-galactopyranosides of *cis*-cyclohexa-3,5-diene-1,2-diol (**208a**).[735]

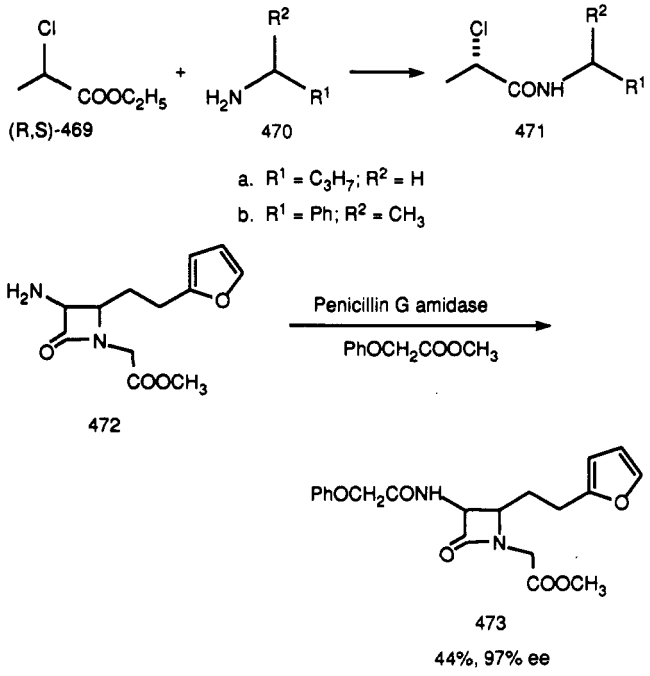
IX. Acylation of Amines

It has already been recognized by Klibanov that lipases and other hydrolases can catalyze the reaction between carboxylic esters and amines in organic solvents.[736] In a more recent study, the role of the solvent for the resolution of racemic amines with trifluoroethyl butyrate in the presence of subtilisin has been clarified.[737] It should be mentioned that also monoclonal antibodies can catalyze the formation of an amide between an ester and an amine.[738] Lipases and esterases catalyze amide synthesis from primary amines and aliphatic esters.[739] The reaction between racemic ethyl 2-chloropropionate (**469**), and nonchiral amines or diamines afford chiral amides and diamides.[740-742] For example, the CCL-catalyzed reaction of the ester **469**

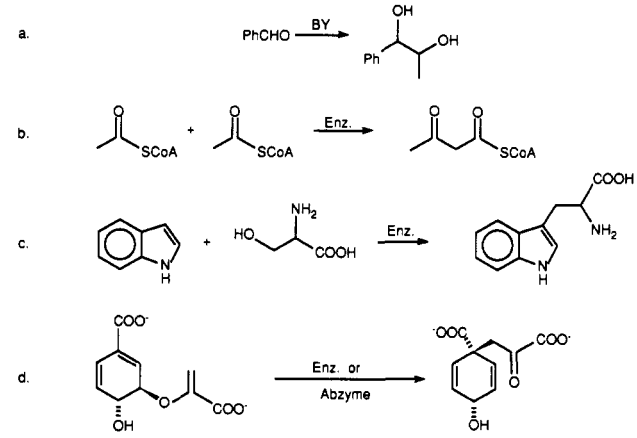
Scheme 120



Scheme 121



Scheme 122



with butylamine (**470a**) in hexane afforded the (*S*)-amide **471a** (62% yield, 95% ee).[740] If the amines are racemic, the aminolysis of the above ester **469** proceeds in an enantioselective manner,[743] as shown in Scheme 121.

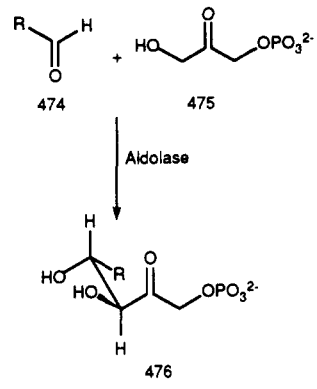
Here, it is shown that from **469** and α-methylbenzylamine **470b** a diastereomeric mixture (1:3) of (2*S*,1′*S*)- and (2*S*,1′*R*)-amides **471b** is formed (46 and 95% ee, respectively). If an amino alcohol is the substrate, the acylation can be directed selectively toward the amino group.[744,745] A recent example of the application of this useful methodology is the enantioselective acylation of the amino group present in the compound **472**, a β-lactam intermediate in the synthesis of loracarbef, a carbacephalosporin antibiotic.[746] In this report, the esters used for the transesterification can be methyl phenylacetate or phenoxy acetate and the aminolysis is catalyzed by penicillin G amidase from *E. coli*. Use of methyl phenoxyacetate and **472** affords the amide **473** (44% yield, 97% ee).

X. Biocatalytic Carbon–Carbon Formation

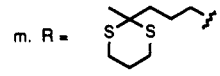
A. General Remarks

Among the great variety of reactions catalyzed by biocatalytic systems, reports on the formation of carbon–carbon bonds seem especially attractive, due to the general interest for this fundamental reaction in organic chemistry. However, compared to the other classes of reactions, the asymmetric C–C bond formation is scarcely represented and seems confined to a few examples. For instance, the BY-mediated acyloin condensation (Scheme 122, entry a), discovered more than 70 years ago,[747] has found many synthetic applications,[80,748] due to the recognized easy availability of this biocatalyst and the operational simplicity of the experimental procedure. This important reaction has already been extensively reviewed.[65,66] Many enzymes can catalyze interesting condensation reactions, which finally lead to the formation of a C–C bond, but some severe limitations preclude a general application of them to a wide variety of substrates. An enzyme like acetoacetyl-CoA thiolase (acetyl-CoA:acetyl-CoA *C*-acetyl-

Scheme 123



a. R = N_3-CH_2-CHOH-
b. R = Cbz-NH-CH_2
c. R = Cbz-NH-CH_2-CH_2
d. R = AcNH-CH($COOCH_3$)-CH_2
e. R = R-CH(N^+)-
f. R = $(CH_3)_2C(OH)$-CH_2
g. R = CH_2OH
h. R = CHOH-CH_2Cl
i. R = $CH(OC_2H_5)_2$
l. R = CH_3-CO-$(CH_2)_3$



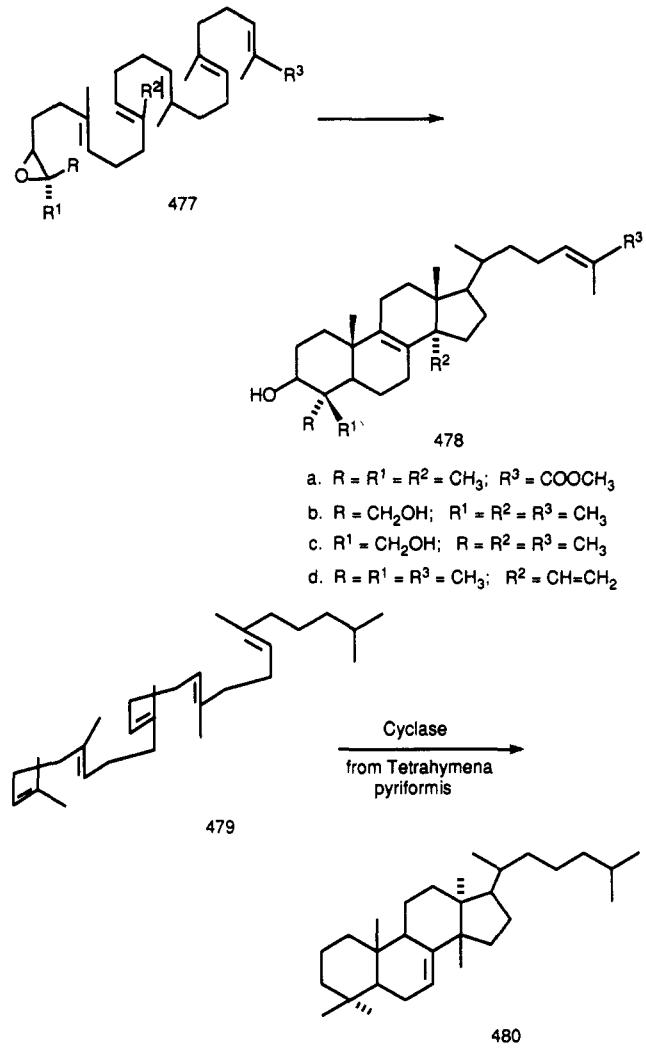
transferase, E.C. 2.3.1.9), an ubiquitous enzyme which catalyzes the condensation of two molecules of acetyl-CoA (Scheme 122, entry b) to yield acetoacetyl-CoA[749] could be interesting for wider application. However, the need of CoA thiol esters intermediate can be a major obstacle to a more general use, although some example of coenzyme A recycling has been already reported.[750]

Tryptophan synthetase which catalyzes the condensation between indole and serine (Scheme 122, entry c), is commercially available, but its applications seem confined to the preparation of tryptophan and related compounds,[751,752] although the use of this enzyme to prepare labeled L-cysteine from L-serine has been recently reported.[753] Prenyltransferase is an enzyme which has to be purified from plants[754] or pig liver[755] and is important for the synthesis of monoterpenes and other isoprenoid compounds. A final example is the Claisen rearrangement of chorismate to prephenate (Scheme 122, entry d), which finds a special place in the biosynthesis of aromatic amino acids via the shikimate pathway.[756] The enzyme chorismate mutase which catalyzes the above rearrangement is rather specific for the substrate.[757] This fascinating C–C bond-forming reaction has also been catalyzed by an antibody.[758,759]

B. Aldolic Condensation

One of the most classical reactions to bring about a carbon to carbon connection is certainly the aldolic condensation. In nature, several of these important condensations are catalyzed by specific enzymes which belong to the class of the lyases. Most of these enzymes can be found in the carbohydrate formation, and the topic has been reviewed by Whitesides[42] and Wong.[760] Aldolases are well suited for preparative purposes of this class of compounds.[761] The reaction catalyzed by an aldolase is depicted in Scheme 123, and only a very few structural variations on compound **475** can be accepted by the enzyme examined, i.e., rabbit muscle aldolase (E.C. 4.1.2.13).[762] Some greater flexibility in the structure of aldehydes **474** can be found, and a few

Scheme 124



a. R = R^1 = R^2 = CH_3; R^3 = $COOCH_3$
b. R = CH_2OH; R^1 = R^2 = R^3 = CH_3
c. R^1 = CH_2OH; R = R^2 = R^3 = CH_3
d. R = R^1 = R^3 = CH_3; R^2 = CH=CH_2

applications of this condensation have been accomplished, keeping constant the dihydroxyacetone counterpart **475**. Substrates and products **476a–m** are collected in the Scheme 123.

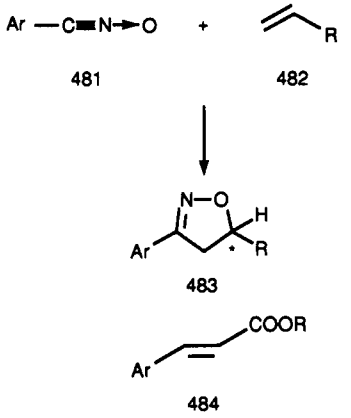
Several nitrogen containing aldehydes like compounds **474a–e** have been utilized for the synthesis of heterocycles and amino sugars.[763–771] Hydroxy aldehydes **474f–h**[772–774] as well as protected dialdehyde **474i**[775] have been used for the synthesis of less common carbohydrates. Various carbohydrates have been recently prepared by this enzymatic reaction.[776–779] Also non-carbohydrate compounds, such as (+)-*exo*-brevicomin, can be enzymatically synthesized by the condensation between the compound **475** and the keto aldehyde **474l** or its thioketal **474m**.[780]

C. Cyclization of Squalene-like Substrates

An interesting example of carbon–carbon bond formation is the cyclization process which involves squalene oxide and squalenoid compounds.[781] In this preliminary report, BY was used as biocatalyst and yields of the process were satisfactory only when the yeast was ultrasonically stimulated. This preparation was a source of sterol cyclase and allowed the cyclization of **477a** to the lanosterol derivative, ganoderic acid Z methyl ester, **478a** (Scheme 124).

In a following paper, it was shown that hydroxylated derivatives **477b,c** were also cyclized to the correspond-

Scheme 125



ing lanosterol derivatives **478b,c**.[782] Finally, when a C-10 vinylic substrate **477d** was cyclized in the same way, it was found that the yeast cyclase was able to rearrange a substituent other than a hydrogen or a methyl group and the 30-vinyllanosterol **478d** was obtained.[783] A recent paper uses the same method to cyclize the 29-hydroxy-2,3-oxidosqualene to 19-hydroxylanosterol.[784] Also a cyclase from the protozoon *Tetrahymena pyriformis* is able to cyclize the 2,3-dihydrosqualene (**479**) to euph-7-ene (**480**),[785] a product not isolated until now from this organism, or from any other natural source.

D. Cycloaddition Reactions

Recent reports witness the enormous capability of BY to catalyze unusual reactions, even the less accessible C–C bond formation. Asymmetric 1,3-dipolar cycloadditions of nitrile oxides **481** to vinylpyridines **482** (Scheme 125, R = pyridine) can be realized in the presence of β-cyclodextrin, yielding up to 64% ee 2-isoxazolines **483**.[786]

Also in the case of BY-mediated cycloaddition of various benzonitrile *N*-oxides **481** to *N*-vinyl carbazole **482** (R = carbazole) the ee was 51%.[787] Also when the dipolarophile was changed from **482** to cinnamic esters **484**, the asymmetric cycloaddition to nitrile oxides **481** was feasible and in this case addition of β-cyclodextrin reversed the regioselectivity observed when BY was used as the sole biocatalyst.[788] Finally, also the classical Diels–Alder reactions were apparently catalyzed either by BY[789] or a suitably prepared antibody.[790]

E. Cyanohydrins Formation

The formation of a carbon–carbon bond via the preparation of cyanohydrins by addition of cyanide to aldehydes, if catalyzed by an enzyme, certainly can constitute an efficient access, for instance, to the preparation of chiral α-hydroxy acids or β-amino alcohols. For this reason, the long-known reaction catalyzed by almond oxynitrilase (E.C. 4.1.2.10)[791] has been rediscovered and expanded to various substrates with preparative purposes[792] (Scheme 126).

Thus, it has been reported that (*R*)-cyanohydrins are obtained by addition of HCN to aldehydes and ketones under catalysis of (*R*)-oxynitrilase in organic solvents, which avoid the danger of racemization for the aqueous reaction.[793,794] Also ground, defatted almonds can be a crude preparation of oxynitrilase, efficient for the

Scheme 126

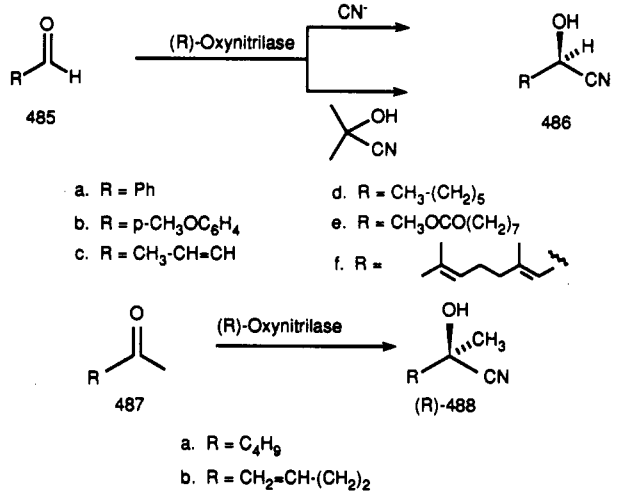


Table 28. Oxynitrilase-Catalyzed Formation of Cyanohydrins

	(*R*)-cyanohydrins		
substrate	yield, %	ee, %	ref
485a	95[a]	≥98	795
485b	65	99[b]	795
485c	99[a,c]	69	796
	94[a,d]	95	796
485d	65	92	801
485e	68	97	801
485f	46	99	801
487a	90	98	794
487b	80	97	794

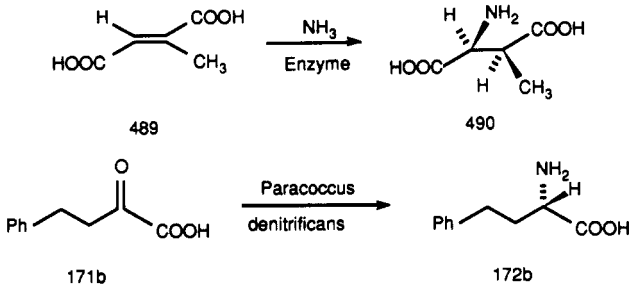
[a] % Conversion. [b] After crystallization. [c] Before optimization. [d] After optimization.

synthesis of gram quantities of chiral cyanohydrins **486**, mostly from aromatic aldehydes, i.e. **485a,b**.[795] The study with the crude system was also devoted to optimizing the reaction conditions to improving the ee, especially on the aldehyde **485c** (from 69 to 95% ee).[796] The use of (*S*)-oxynitrilase from a different source allowed the formation of the corresponding (*S*)-cyanohydrins.[797] Applications are reported on the synthesis of (*R*)-α-hydroxy carboxylic acids and (*R*)-1-amino-2-alkanols,[798] (*R*)- and (*S*)-2-amino alcohols,[799] and (*R*)-α-sulfonyloxynitriles.[800] Furthermore, a detailed study for the preparation of enantiomerically pure cyanohydrins, avoiding the problem of in situ racemization, has been accomplished, realizing the synthesis by an enzymatic transcyanation of several aliphatic and aromatic aldehydes with acetone cyanohydrins in an ether–buffer biphasic solvent system.[801] For example, from the aldehydes **485d–f** it is possible to prepare the cyanohydrins **486d–f** with 92–99% ee. Finally, also from ketones like **487a,b** the corresponding (*R*)-cyanohydrins **488a,b** were prepared with near optical purity.[794] The above results are collected in Table 28.

F. Various Condensations

Examples of enzymatic dimerization can be found, for instance, in the alkaloid biochemistry in plant cell cultures,[802,803] microorganisms,[804] or enzymes like horseradish peroxidase.[805] In fluorine chemistry, a few examples of C–C bond forming can be found, in the presence of urease or catalase,[806] or lipases, which can

Scheme 127



489 490 171b 172b

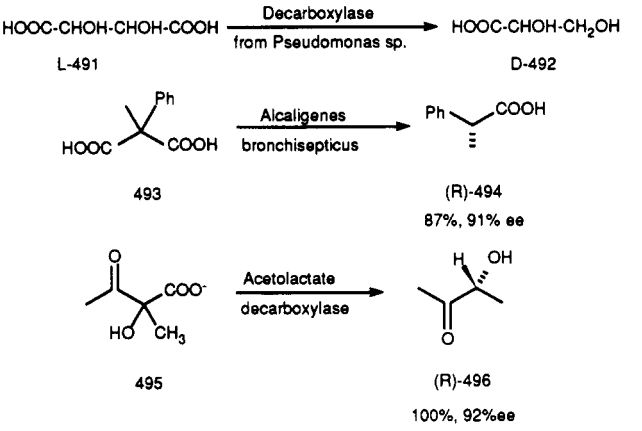
catalyze asymmetric Michael additions for the synthesis of optically active trifluorinated compounds.[807] The BY-mediated C-alkylation, i.e. ethylation at the α-position of cyanoacetone, can be regarded as an interesting carbon–carbon bond formation reaction.[808] The mechanism of this reaction has been proposed and demonstrated in a recent report.[809] The enzyme transketolase catalyzed the stereospecific transfer of the hydroxyacetyl group of hydroxypyruvate to various aldehydes, showing also enantioselectivity in the case of racemic substrates.[810]

XI. Additions and Eliminations

These reactions are generally catalyzed by the fourth class of enzymes, i.e. lyases, and only a few examples of these potentially useful biocatalysts can be found in the literature. Also the enzyme-catalyzed hydration of a double bond[545–549] could pertain to this section. Furthermore, the enantiospecific synthesis of 3-methylaspartic acid (**490**) consists in the amination of methylfumaric acid (**489**) (Scheme 127) and is catalyzed by a specific enzyme.[811] An aminotransferase activity present in the microorganism *Paracoccus denitrificans* has been exploited for the industrial production of L-2-amino-4-phenylbutyric acid (**172b**) from 2-oxophenylbutyric acid (**171b**).[812]

A few lyases, namely decarboxylases, are able to catalyze a carbon–carbon cleavage potentially very useful from a synthetic point of view. Several amino acid decarboxylases are available,[813] and many of them are commercial, but their substrate specificity is a major drawback for a more general use of these biocatalysts. α-Chymotrypsin has been reported to catalyze the decarboxylation of a keto acid[814] or a few α-nitro-α-methyl carboxylic acids.[468] The well-known BY-mediated acyloin condensation[65,66] has been studied with two other yeast strains (*Saccharomyces fermentati* and *S. delbrueckii*) as well[815] and can be accomplished between a few aldehydes and aliphatic keto acids.[816] The acyloin condensation is catalyzed by pyruvate decarboxylase, as recently shown in a detailed study.[817] An enzymatic preparation from a *Pseudomonas* sp. is able to decarboxylate L-tartaric acid (**491**) into D-glyceric acid (**492**)[818] and a microorganism, *Alcaligenes bronchisepticus*, seems to have the useful capability to perform the asymmetric decarboxylation of disubstituted malonic acids. For example, the compound **493** is converted (87% yield, 91% ee) to the (*R*)-acid **494**.[819] A specific enzyme, acetolactate decarboxylase, can transform racemic α-acetolactate (**495**) into (*R*)-acetoin (**496**) of high optical purity (92% ee).[820] The above results are collected in the Scheme 128.

Scheme 128



L-491 D-492 493 (R)-494 87%, 91% ee 495 (R)-496 100%, 92%ee

XII. Biotransformations of Organometallic Compounds

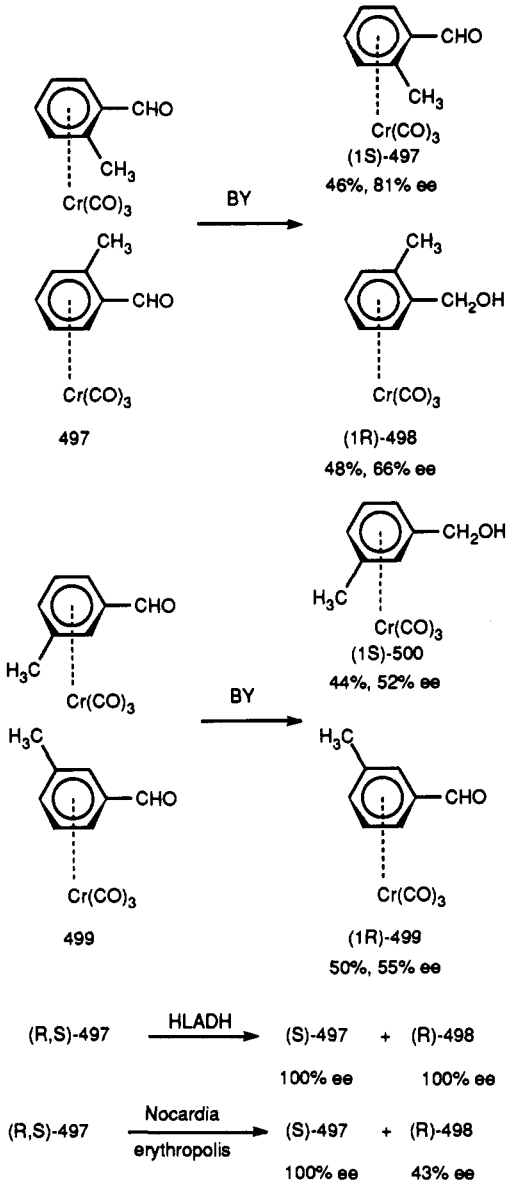
A. Reductions and Oxidations

Chiral organometallic compounds like ferrocenes[821] or carbonylchromium[822] complexes can be used as chiral auxiliaries in asymmetric synthesis, and a few groups have already investigated the biocatalytic approach to the synthesis of enantiomerically pure compounds of this class. Examples of this approach can be already found in previously cited reviews.[65,310] The enantioselective reduction of tricarbonyl(2-methoxybenzaldehyde)chromium by BY was reported in 1988.[823] Recently, the racemic 2-methylbenzaldehyde **497**, when reduced by BY, affords the (*S*)-aldehyde **497** (81% ee, 46% yield) along with the (*R*)-alcohol **498** (66% ee, 48% ee).[824] In the Scheme 129, the reduction of the meta-substituted aldehydes, like **499**, resolved into (*R*)-**499** and (*S*)-**500** with moderate enantioselectivity is reported.[824] The 2-methyl aldehyde **497** is also a substrate for a HLADH-mediated reduction, which affords the same products as BY, but with 100% ee.[825] With *Nocardia erythropolis*, it has been reported that the aldehyde **497** is resolved into to 100% ee (*S*)-**497** and 43% ee (*R*)-**498**.[826]

In Scheme 130 is shown the HLADH-catalyzed enantioselective reduction–resolution of (*R,S*)-tricarbonyl-(η^5-1-formyl-2-methylcyclopentadienyl)manganese (**501**) to optically active **501** and the (–)-alcohol **502**.[825,827] This reduction of **501** can be effected also with *Candida boidinii* to afford 100% ee of (1*S*)-**501** and 44% ee of (1*R*)-**502**.[826]

Many examples already are available of ferrocene derivatives prepared in enantioselective fashion by a biocatalytic approach. Thus, the reduction of ferrocenecarboxaldehyde by BY can constitute an undergraduate laboratory excercise,[828] and the 1-formyl-2-methylferrocene is the exception to the good results previously seen for organochromium and -manganese aldehydes, since it is reduced to the corresponding alcohol by HLADH with only 18% ee.[829] Instead, the reduction of 1,2-diformylferrocene (**503**) to (*S*)-**504** by HLADH[829,830] proceeds with high enantioselectivity, and also various microorganisms such as *Nocardia erythropolis* and *Candida boidinii* can lead to an alcohol **504** with the same configuration as above (95 and 92% ee, respectively).[826] The results are collected in the Scheme 131.

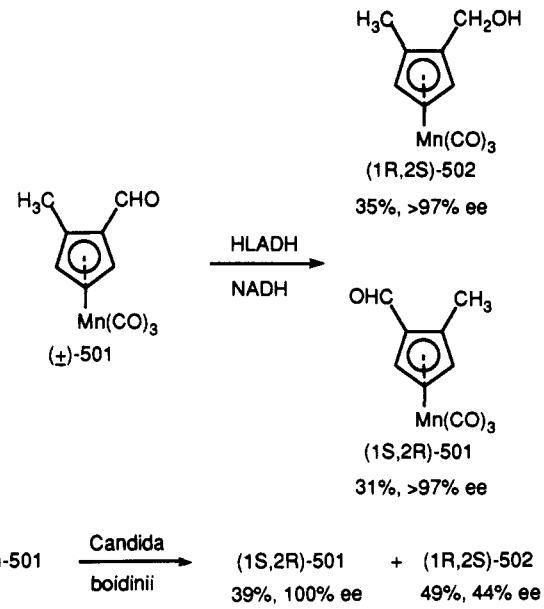
Scheme 129



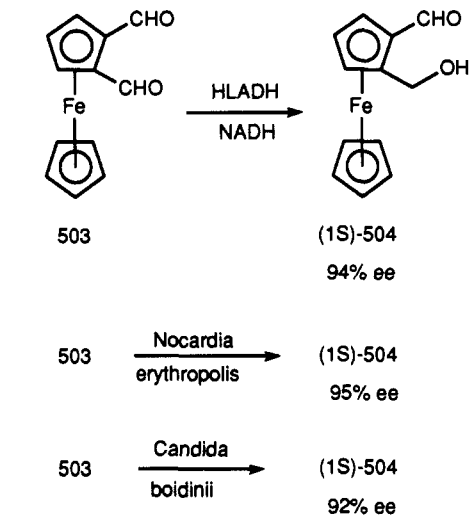
Several organometallic methyl ketones can be enantioselectively reduced to the corresponding alcohols by a variety of methods. BY reduction of the $Cr(CO)_3$-complexed acetophenone **505** quantitatively affords the (*S*)-alcohol **506** and the ee approach 100% (Scheme 132).[831,832] This is the most significant example of many ketones used as substrates and is interesting also because, in the absence of $Cr(CO)_3$, i.e. the reduction of acetophenone is much slower and proceeds with variable yields and ee which does not exceed 90%. Also other microorganisms are able to perform the same bioreduction, but the highest ee are still from BY and *Saccharomyces rosei*.[833] Racemic tricarbonyl(η^5-1-acetyl-2-methylcyclopentadienyl)manganese (**507**) is reduced with highest ee with *Rhodotorula rubra* to the optically pure (1*R*,2*S*,1′*S*)-alcohol **508**.[827] Also the unreacted (1*S*,2*R*)-ketone **507** was recovered optically pure. The ferrocenyl ketone **509** can be resolved through reduction by means of different yeasts to 96% ee (*S*)-alcohol **510**.[833]

The reverse oxidation of the racemic alcohol **510** to the ketone **509**, catalyzed by HLADH, allows the preparation of unreacted optically pure (*R*)-**510**.[825,829]

Scheme 130



Scheme 131

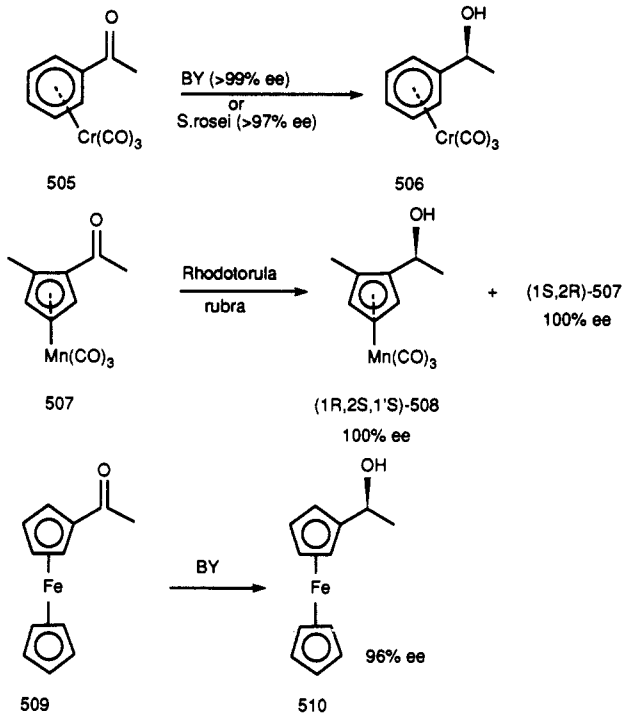


Other metallocenyl alcohols **511a,b** behave similarly when a HLADH-catalyzed oxidation is performed,[825] since 92% ee of the unreacted (*R*)-**511a,b** can be prepared. A ferrocenyl diol such as **512** can be oxidized to the hydroxyaldehyde **504** by HLADH[829,830] or several microorganisms[826] with good enantioselectivity (86% ee for HLADH). An investigation of enzymatically prepared organometallic compounds like ferrocenyl and chromium alcohols on their possible nonlinear optical properties has been recently published.[834]

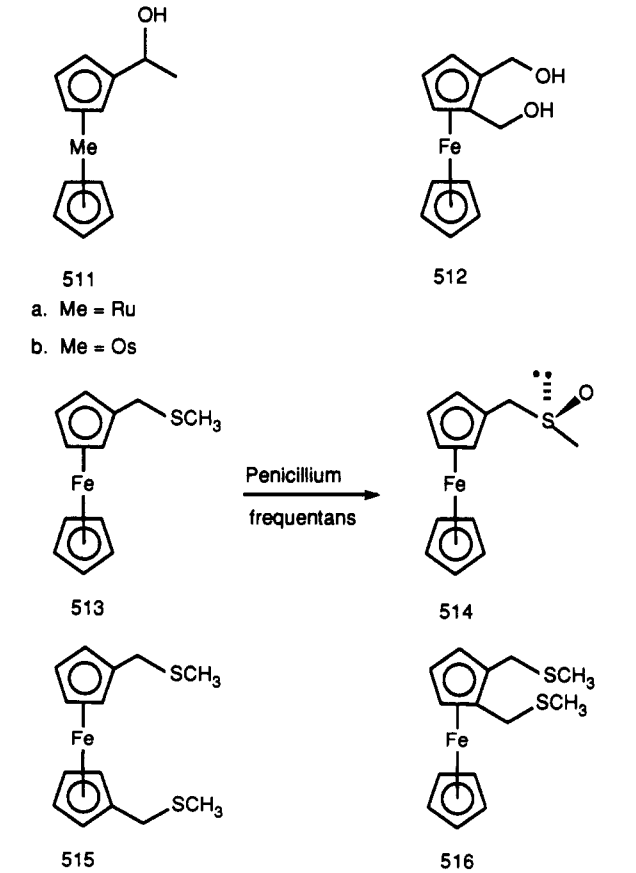
Scheme 133 collects results on the oxidation of organometallic sulfides. Thus, ferrocenyl sulfide **513** is enantioselectively oxidized to optically pure (*R*)-sulfoxide **514** with *Penicillium frequentans*, whereas the (*S*)-isomer of **514** can be prepared with *Corynebacterium equi* with no more than 69% ee.[835] Also, organometallic bissulfides **515** and **516** can be enantioselectively oxidized to mixtures of monosulfoxides using the same microorganisms as above.[836]

Bioconversion of organosilicon compounds is of great interest in the fundamental study of enzymology[837] and for the preparation of optically active organosilicon compounds.[838,839] In the case of silicium containing carbonyl compounds, like 1,1-dimethyl-1-silacyclohexan-

Scheme 132

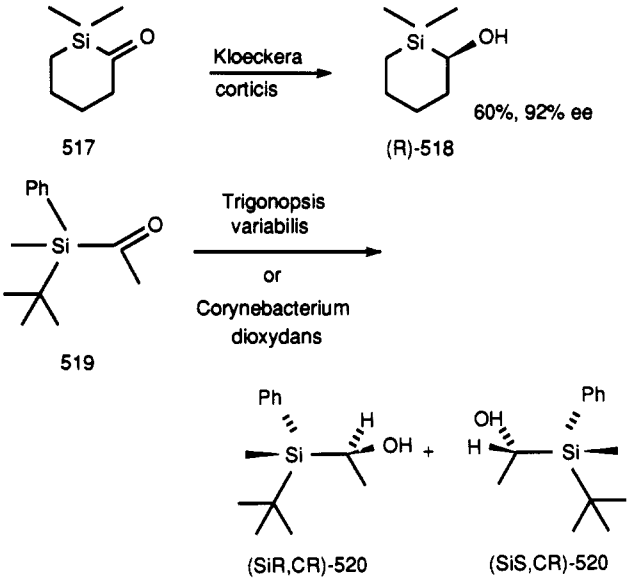


Scheme 133

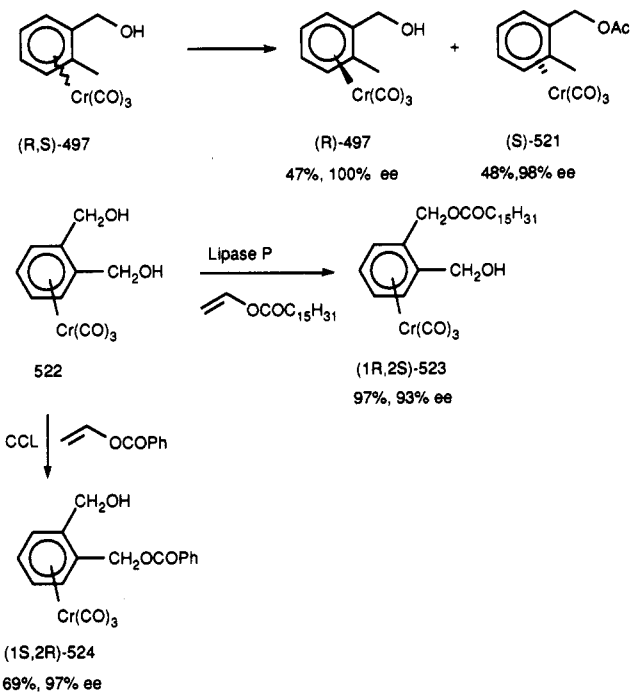


2-one **517**[840] or acetyl(*tert*-butyl)methylphenylsilane (**519**),[841] a few microorganisms can effect enantioselective reductions. In the first case, the yeast *Kloeckera corticis* reduced the ketone **517** to (*R*)-**518** (60% yield, 92% ee) and the (Si*R*,*S*)-**519** was reduced to diastereomeric mixtures of optically pure alcohols **520** with *Trigonopsis variabilis* or *Corynebacterium dioxydans* (Scheme 134).

Scheme 134



Scheme 135

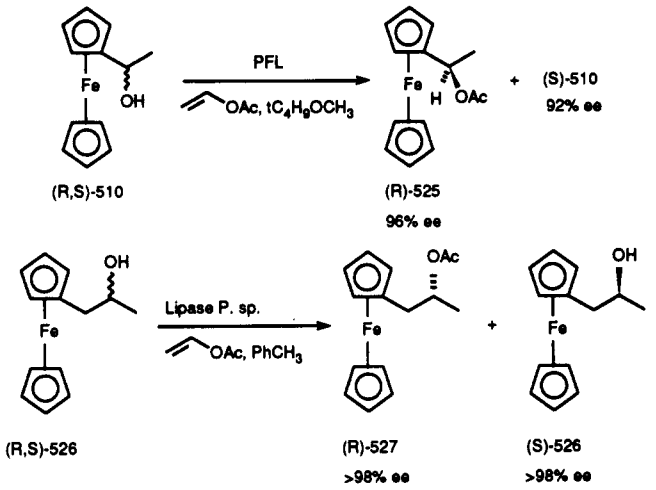


B. Hydrolyses and Esterifications

Many organometallic compounds can be prepared by biocatalytic hydrolysis or esterification. This applies to kinetic resolution of racemic tricarbonylchromium alcohols.[842] Typically, Lipase P (Amano) allows the preparation of optically pure (*R*)-**497** and (*S*)-**521** from racemic **497**. The diol **522** is resolved with opposite stereochemistry to the corresponding monoesters, depending on the lipase used.[843] Interestingly, the Lipase P-catalyzed transesterification with vinyl palmitate in toluene afforded (1*R*,2*S*)-**523** (97% yield, 93% ee), while use of CCL and vinyl benzoate in the same solvent gave the (1*S*,2*R*)-**524** (69% yield, 97% ee). The data are collected in the Scheme 135.

Also racemic ferrocenyl alcohols like **510**[844] and **526**[845] can be efficiently resolved to the (*R*)-acetates **525** and **527** and to the (*S*)-alcohols **510** and **526**, respectively

Scheme 136



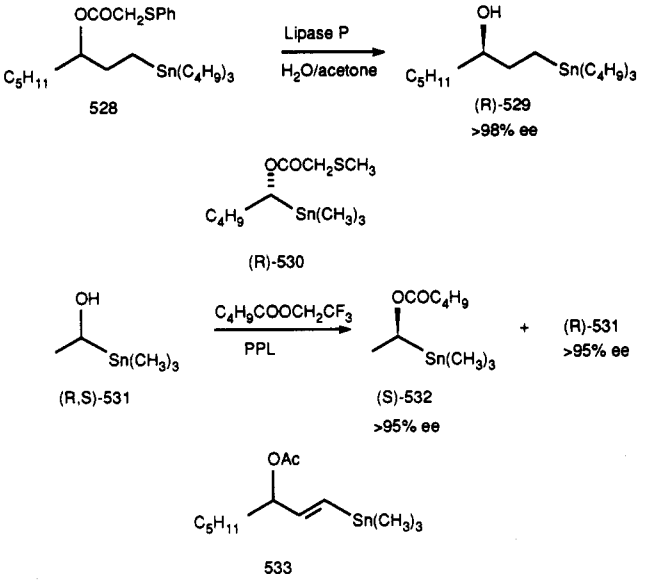
by a lipase-catalyzed transesterification with vinyl acetate in organic solvents (Scheme 136).

Optically active organotin compounds have an enormous potentiality in organic synthesis, like α-hydroxystannanes, for example, which can be the precursors of α-alkoxymetal reagents.[846] Apart from an application of lipase transesterification via *O*-stannyl ethers,[847] the first resolution of organotin compounds, i.e. γ-hydroxystannanes, has been recently reported.[848] As the most significant example, from the racemic ester **528** the optically pure (*R*)-**529** was obtained. The method also applies to esters of α-hydroxystannanes, and the hydrolysis was again catalyzed by a lipase.[849] The best result was for the (*R*)-ester **530** which was recovered with 86% ee from the aqueous medium. The methyl thioester overcomes the problems of the inhibition of the biocatalyst and of the loss of optical purity during the process. As usual, the transesterification in organic solvent solved the problem. Thus the lipase-catalyzed reaction of racemic **531** with 2,2,2-trifluoroethyl valerate afforded the (*S*)-ester **532** and the (*R*)-alcohol **531**, both with >95% ee.[850] Good results were obtained also for the resolution of (γ-hydroxyvinyl)stannanes, like compound **533**.[851] The results on stannyl derivatives are collected in the Scheme 137.

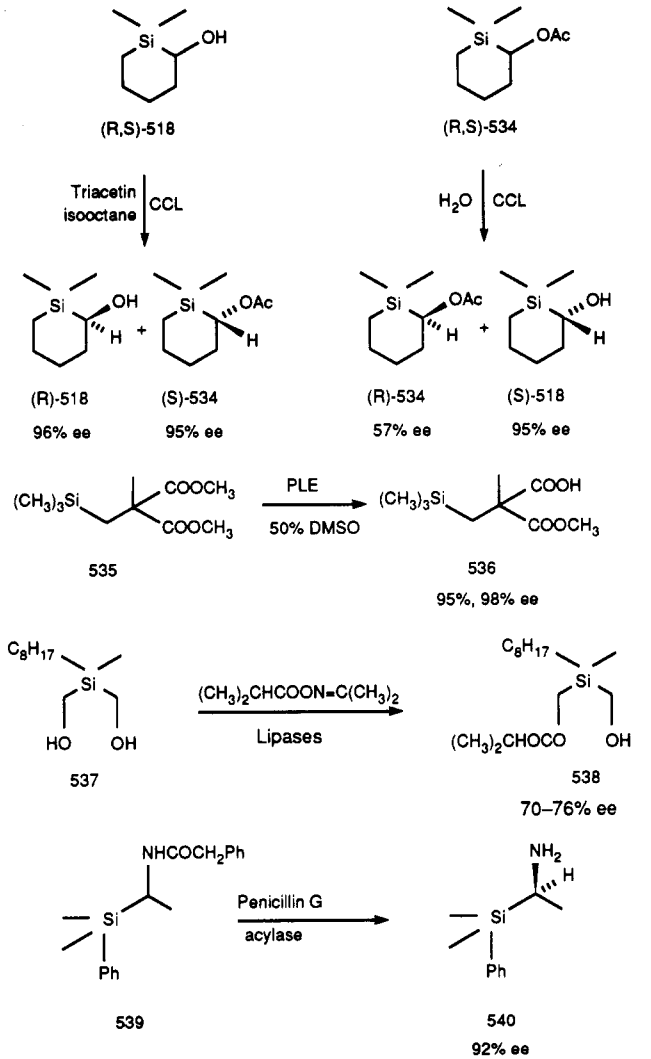
The resolution of racemic 1,1-dimethyl-1-silacyclohexan-2-ol (**518**) is a good example of the application of complementary enzymatic hydrolysis and transesterification for the preparation of both enantiomers of the starting compound.[852] The method also demonstrates that organosilicon compounds can be excellent substrates for biocatalytic methodologies. CCL in the presence of triacetin in isooctane afforded in nearly quantitative yield (*R*)-alcohol **518** and (*S*)-acetate **534** (96 and 95% ee) (Scheme 138). In contrast, the CCL-mediated hydrolysis of the racemic acetate **534** furnished a 95% ee of (*S*)-alcohol **518** and 57% ee of (*R*)-acetate **534**.

The well-documented hydrolysis of prochiral malonates[488] seems to apply also to the resolution of trimethylsilylmalonates like **535**. A few hydrolases were screened, and the best results was obtained with PLE in 50% DMSO (98% ee, 95% yield of **536**, configuration not specified).[853] Although the ee of the product **538** did not exceed 76%, the transesterification of the 2-sila-1,3-propanediol **537** is another good example of the asymmetrization of a prochiral organosilicon compound.[854] Finally the versatility of the biocatalytic approach to this field is shown by the resolution of the phenylacetamide **539** to the 92% ee (*R*)-amine **540** by penicillin G acylase immobilized on Eupergit.[855]

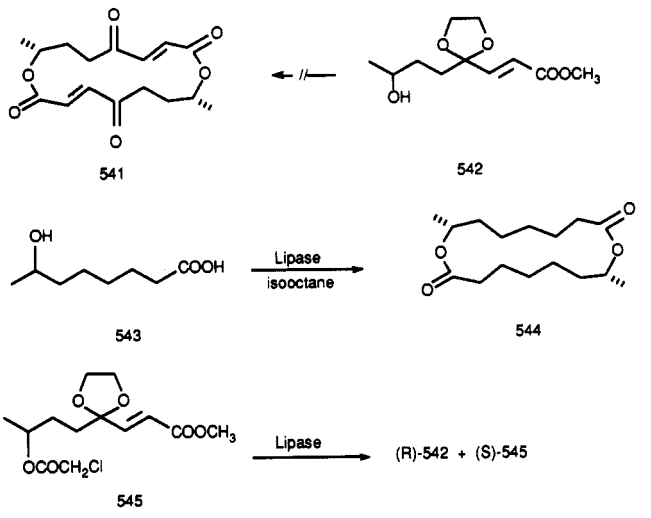
Scheme 137



Scheme 138



Scheme 139



XIII. Multienzymatic Approach

Several applications of the use of more than one biocatalyst for the synthesis of a given compound can be found in the current organic chemistry literature. The examples are different and one can find applications of different enzymes to structurally related compounds, which belong to the same class.[856] An impressive example of multienzymatic biotransformations could be the use of several compatible enzymes in sequence for a multistep synthesis. The realization of this concept is illustrated by the one-step synthesis of a sialyl trisaccharide, where sialic acid and *N*-acetyllactosamine are generated in situ and also the regeneration of UDP-glucose, UDP-galactose, and CMP-sialic acid is reported.[857]

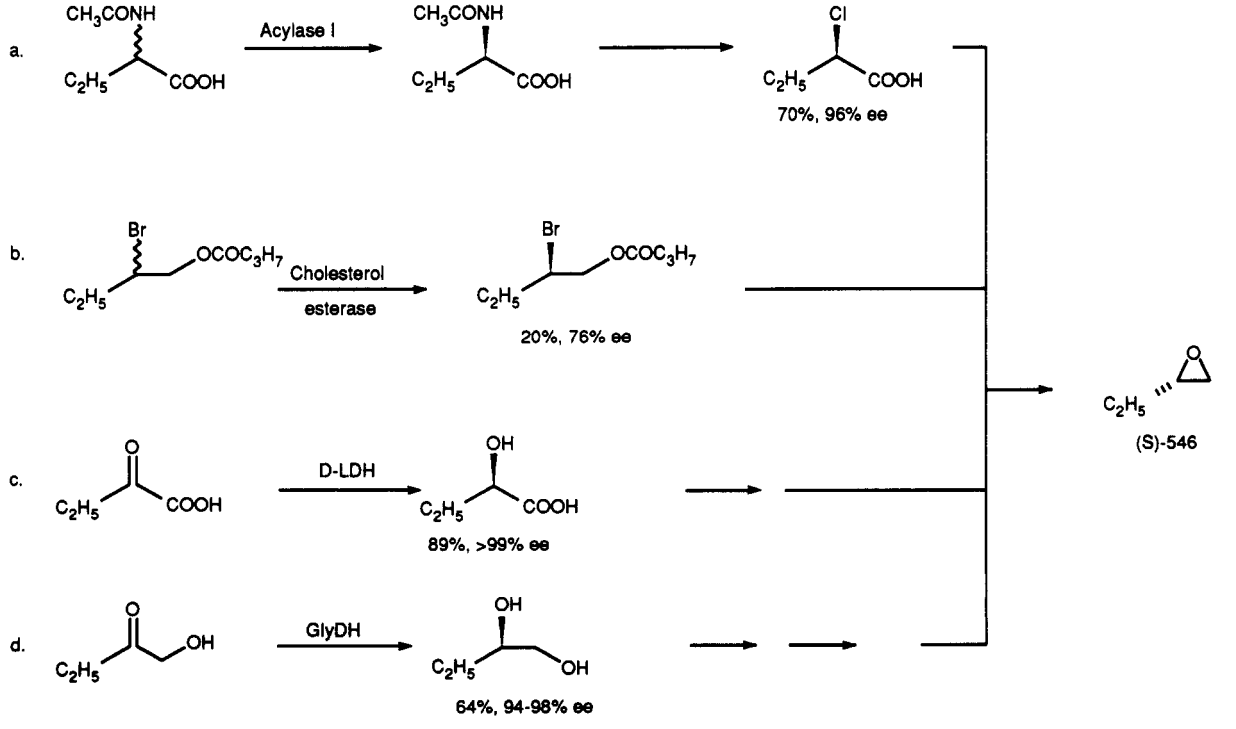
Typically, however, a multienzymatic approach can be the screening of various biocatalysts in order to find the most suitable conditions to perform the transformation with the maximum ee, optimistically 100%. Many of the already reported works in this review contain several successful or less fortunate efforts to prepare optically pure compounds, and in many cases only one, generally the best (or a few successful among the many cases studied), biotransformation has been reported by the reviewers. It is interesting, however, to illustrate in some detail the logic of a chemoenzymatic synthesis, when more than one step is mediated by biocatalysts. The choice of the most representative examples is purely arbitrary, and other equally interesting works in similar areas will be briefly mentioned. This could be the case of the synthesis of prostacyclin analogs, where the homochiral bicyclooctane building units are prepared by the choice of the more advantageous enzymatic and microbiological hydrolysis of suitable esters.[814] In Scheme 139 the main enzymatic steps for the synthesis of the natural antifungal macrodiolide (−)-pyrenophorin (**541**) are reported.[858]

The useful intermediate hydroxy ester **542** could not be enzymatically lactonized to the macrocyclic lactone **541**.

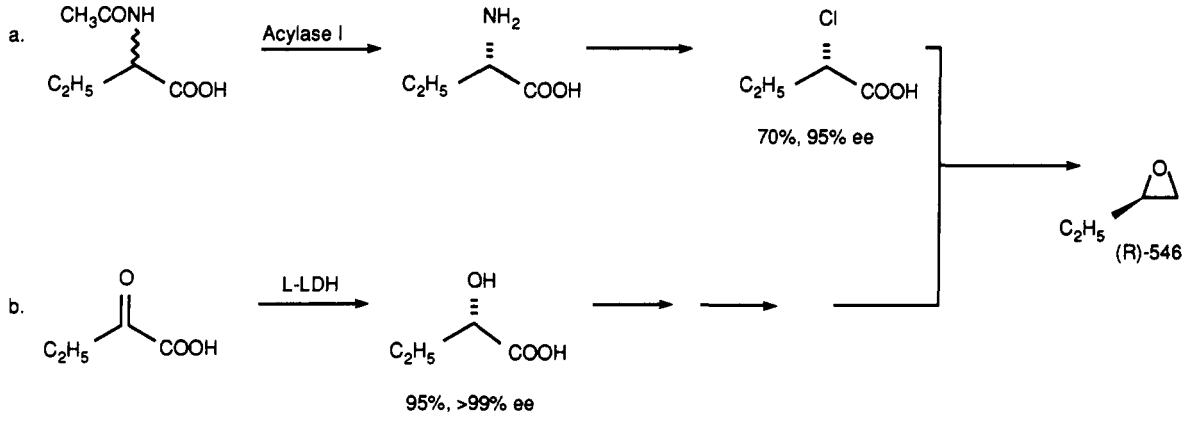
This was contrary to other successful results reported for other substrates, and the authors established that, differently from **542**, the simpler hydroxy acid **543** could be cyclized to **544** by a lipase, albeit in low yields. However, another biocatalytic method was used to solve the problem, namely the enzymatic hydrolysis of the chloroacetate **545** to the enantiomerically pure (*R*)-**542** and (*S*)-**545**, which was chemically converted into the macrolactone **541**.

Another didactic example of the multienzymatic approach can be the use of different enzymes to prepare both the stereoisomers of 1-butene epoxide (**546**)[859] by complementary chemical and enzymatic methods. The direct resolution of the ester of the most suitable bromo alcohol afforded the less satisfactory result (entry b, 76% ee). Reduction of the keto acid and the hydroxy ketone with the aid of dehydrogenases (entries c and

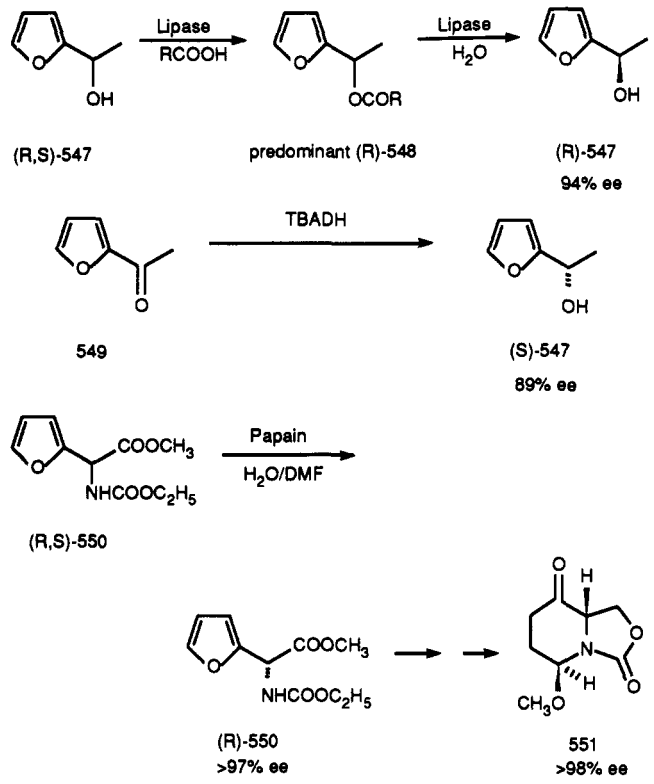
Scheme 140



Scheme 141



Scheme 142



d) afforded enantiomerically pure products, easily transformed into the final (*S*)-**546** (Scheme 140).

In the Scheme 141 the multienzymatic approach to the preparation of (*R*)-**546** is reported. This compound could be prepared with L-lactate dehydrogenase (L-LDH) as catalyst (entry b), instead of the D-LDH, used for the (*S*)-**546** (Scheme 140, entry c). The hydrolysis of the *N*-acyl-2-amino butanoic acid afforded the (*S*)-amino acid necessary for the synthesis of the (*R*)-**546** (entry a). On the other hand, the unreacted (*R*)-amide could be used for the preparation of (*S*)-**546** as already shown in the Scheme 140 (entry a). Families of structurally related chiral synthons may be prepared by different biocatalytic routes, as in the case of the chemoenzymatic synthesis of chiral isoxazoles derivatives.[860] This multienzymatic approach will be described for a series of chiral furan derivatives (Scheme 142), which can be useful building blocks for the synthesis of other optically active structures.[861]

The resolution of the (*R*,*S*)-carbinol **547** was obtained with 94% ee only after a double enzyme-catalyzed resolution. In fact, the esterification of **547** to the (*R*)-furylacetate (**548**) was not completely enantioselective and the hydrolysis of the enriched **548** furnished (*R*)-**547** with 94% ee. The preparation of the (*S*)-**547** (89% ee) required the action of the alcohol dehydrogenase TBADH on the acylfuran **549**. For the synthesis of enantiomerically pure furylglycine derivative **550**, the hydrolysis of the carbomethoxy moiety of the racemic **550** was enantioselective (>97% ee) only when papain was the enzyme and 20% of dimethylformamide was present in the aqueous medium. The synthetic application of (*R*)-**550** is illustrated with the preparation of compound **551** (>98% ee).

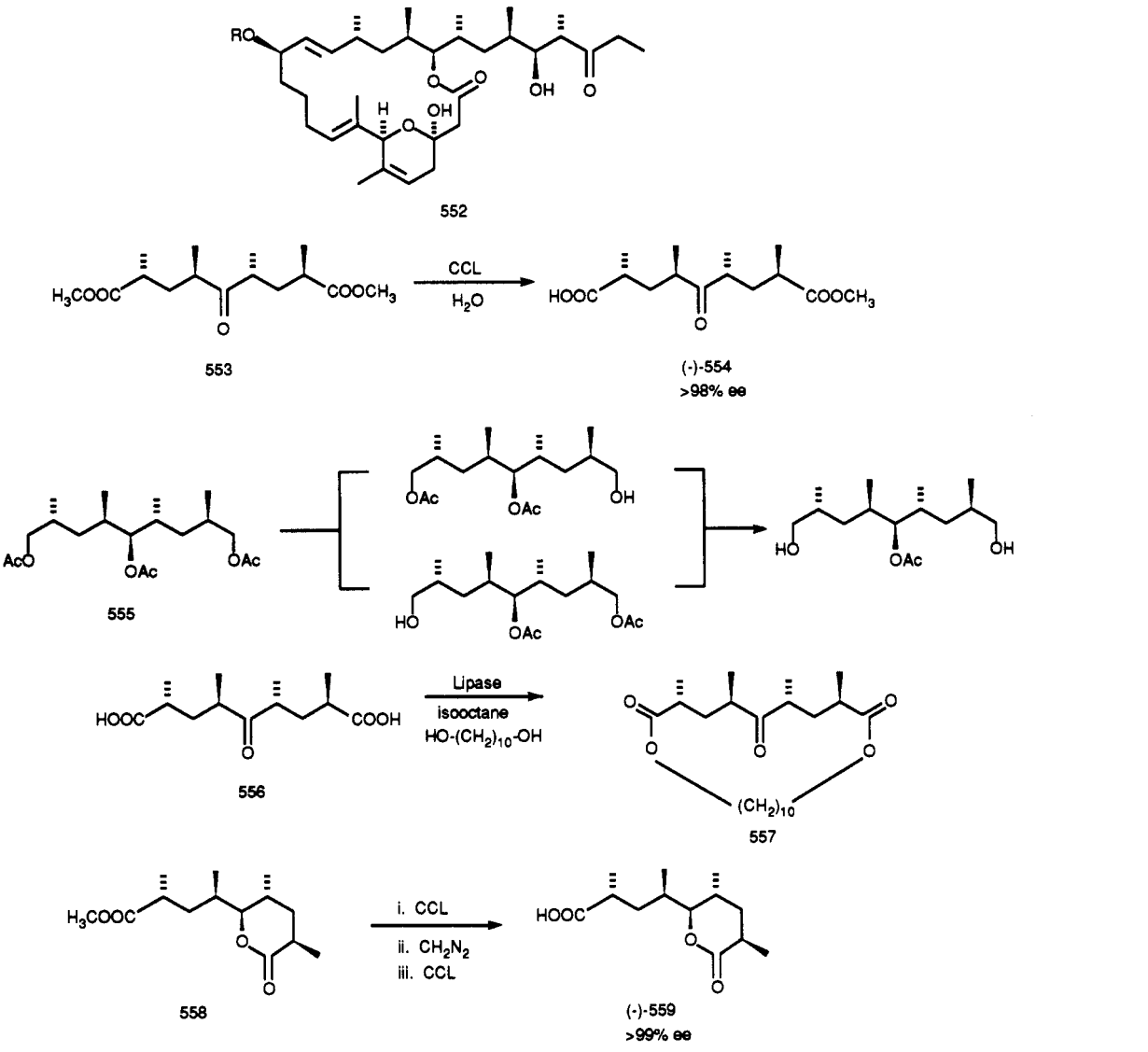
An excellent example of multienzymatic approach is the synthesis of venturicidin (**552**).[862] The main optically active building block on the synthesis of the C_{15}–C_{23} fragment of **552** presented five consecutive centers of chirality and the challenging problem was the diastereoselective resolution of the necessary intermediate. The keto diester **553** with four chirality centers was efficiently resolved to (−)-**554** (>98% ee), whereas the hydrolysis of the triacetate **555** furnished a mixture of products and the optical purity was very low (Scheme 143).

The enzymatic macrolactonization between the keto diacid **556** and a long-chain diol afforded the lactone **557** with good chemical yields, but with no enantioselectivity. Finally, the solution to the chiral synthesis of the optically pure synthon containing five chirality centers, compound **559**, was a double resolution procedure, using as substrate the lactone–acid ester **558**. A first resolution of **558** with CCL afforded 74% ee **559**, which was methylated and hydrolyzed with CCL to afford optically pure **559**. It is worth noting the spectacular stereoselectivity of the enzymatic reaction, which was reached using the most enantioselective lipase after a careful screening of enzymes. Additionally, one should also mention that the enzyme can distinguish chemoselectively between a lactone and an ester moiety.

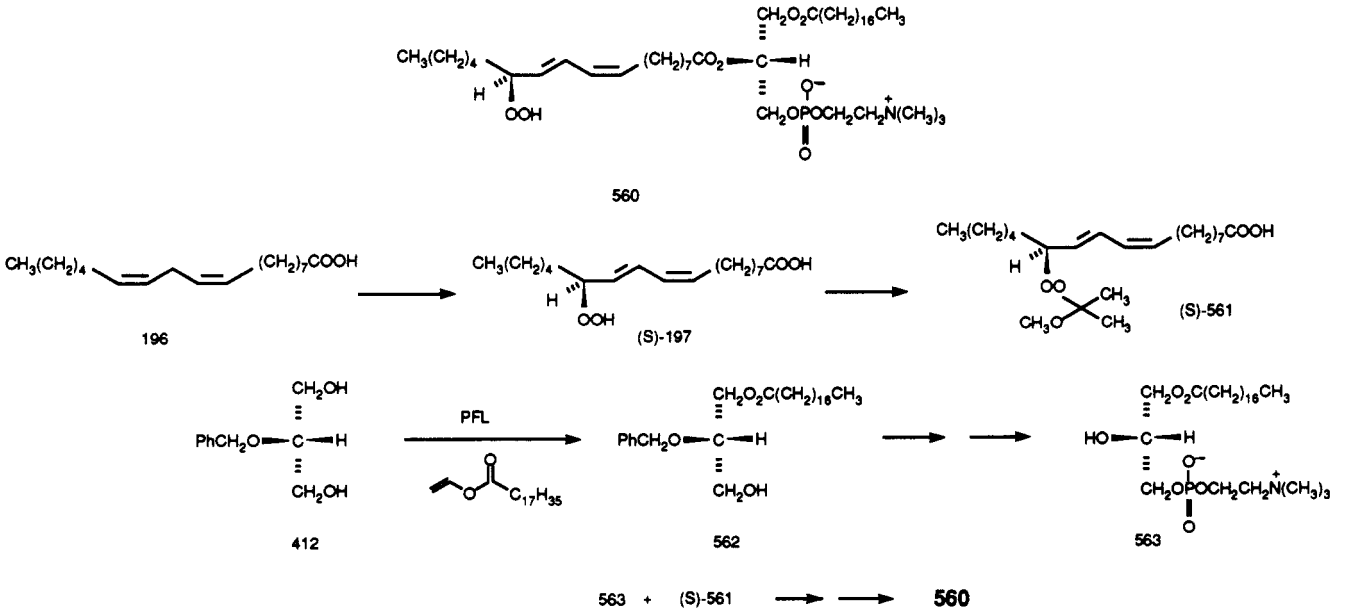
Finally, a very recent synthesis of the diacyl glycerophosphocholine hydroperoxide (**560**) offers an excellent example of the use of enzymes for the solution of stereochemical and chemical problems in the crucial steps of the synthesis of a sensitive and complicated molecule, such as **560**.[863] In Scheme 144 the synthesis of the target molecule is outlined, starting from linoleic acid **196**.

The (*S*)-hydroperoxide **197** can be enzymatically prepared by an oxidation enantioselectively catalyzed

Scheme 143



Scheme 144



by the soybean lipoxygenase and has to be then protected as the derivative **561**. During this three-step protection, the acid function of (*S*)-**197** was transformed in an ester group, which, later on was gently hydrolyzed with PFL to finally afford the desired (*S*)-**561**. Again, a lipase-catalyzed transesterification procedure was

adopted for the mono esterification of the 2-benzyl glycerol **412**. The vinyl stearate was used for a selective esterification with PFL, and compound **562** was chemically converted to the 1-stearoyl-*sn*-glycero-3-phosphocholine (**563**). The latest compound reacted with the (*S*)-acid **561** to afford, after hydrolysis of the hydroperoxide protecting group, the desired compound **560**.

XIV. Conclusions and Perspectives

A very recent report from the IUPAC Division Commission on Biotechnology timely and adequately has indicated the characteristics, methodologies, and applications of biotransformations in organic chemistry and furnishes the entries to many leading references in the field.[864] A survey of the current titles in the chemical literature indicates that the most frequently used biosystems applied to organic synthesis are microorganisms and purified enzymes. These biochemical auxiliaries are applied to the synthesis of a wide variety of chiral synthons, whereas plant cell cultures seem more restricted to a biogenetic type of approach for the synthesis of peculiar structures like alkaloids[19] or to special transformations like glycosylations.[720] The mammalian cells or crude preparations of enzymes from them offer a great potentiality, but their applications are very much restricted, at present. Also among the biocatalysts more used, like microorganisms and enzymes, the preference of synthetic organic chemists usually goes to those which can be easily handled without any special equipment or any knowledge of biochemical or microbiological techniques, and BY (*Saccharomyces cerevisiae*) is the most used microorganism. The application of special classes of microorganisms are sometimes disclosed to organic chemistry, for instance, dehalogenating bacteria which have already been applied to the synthesis of chiral building blocks.[865-868] Purified enzymes which do not require coenzymes, such as hydrolases (lipases, esterases, proteases), are the preferred biocatalysts to be used for the preparation of optically active compounds. The discovery of new reactions for old, but commercially available enzymes, and usual transformations of unusual substrates according to well-established reaction processes, eventually in nonconventional conditions, still attracts many research groups and is certainly a fast growing area. Considering the engineering of new biocatalysts, many exciting perspectives are around the corner. The monoclonal catalytic antibodies generated by chemically constructed haptens based on organic compounds which mimic the transition state of known enzyme-catalyzed reactions are the possible new generation of man-made enzymes. These abzymes have been made possible by the spectacular development of immunological techniques, and the field certainly holds the exciting promise of a new fast developing area.[869-872] The great development of new techniques in molecular biology certainly can open unprecedented doors to new applications. For instance, an enzymatic activity present in a microorganism can be raised by several orders of magnitude and the concentration of the required product can become much higher. This has been recently achieved, for example, by cloning in *Escherichia coli* the gene for D-aminopeptidase from *Ochrobactrum anthropi* and constructing an expression plasmid, which could be used for the *R* stereospecific hydrolysis of racemic amino acids.[873] The construction of new enzymes is an exciting area,[874,875] which may take advantage of site-directed mutagenesis methodology as a powerful tool for the modeling of catalytically active sites. In this way, by modifications of existing ones, artificial enzymes can be built with new characteristics. A highly stable mutant of subtilisin containing six stabilizing site-specific mutations have been prepared for new and old reactions to be carried out in dimethylformamide.[876] A mutagenesis at the binding site of the same enzyme has been directed to the differentiation of the amide versus the ester hydrolysis.[877] Finally, it should not be forgotten that also chemical mutations of enzymes are possible for restructuring existing proteins.[878,879] Chemical transformation of the active site cysteine of the proteinase papain (Cys 25) into serine or glycine has been already demonstrated.[880] More recently, it has been shown that the chemical conversion of the active site serine (Ser 221) of subtilisin into the selenol analog (selenolsubtilisin) makes possible the transformation of the protease into an acyl transferase.[52]

All these premises suggest that different routes toward the synthesis of optically pure compounds can rely upon a variety of biochemical methods as a well-established source of chirality and prove that the skill of organic chemists in most, if not all, interdisciplinary fields of biotechnology is strongly required.

XV. Abbreviations

AADH	α-amino acid dehydrogenase
BY	bakers' yeast
CCL	*Candida cylindracea* lipase
de	diastereomeric excess
ee	enantiomeric excess
FDH	formate dehydrogenase
GlyDH	α-glycerophosphate dehydrogenase
HLADH	horse liver alcohol dehydrogenase
HLE	horse liver esterase
β-HSDH	3β,17β-hydroxysteroid dehydrogenase
20β-HSDH	3α,20β-hydroxysteroid dehydrogenase
LDH	lactate dehydrogenase
PFL	*Pseudomonas fluorescens* lipase
PLE	pig liver esterase
PPL	pig pancrease lipase
TBADH	*Thermoanaerobium brockii* dehydrogenase

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