Kirk-Othmer Encyclopedia of Chemical Technology.

ENZYMES IN ORGANIC SYNTHESIS

The application of enzymes in organic synthesis is a modern and rapidly growing area in synthetic organic chemistry. Although the great potential of enzymatic catalysis had been recognized for many years, its application was rather limited. The rapid expansion of this area in recent years has been brought about by a number of factors, most importantly that a large number of enzymes have become commercially available. Of about 2500 enzymes identified thus far about 300 are available in a partly purified form. Moreover, because of advances in molecular biology, fermentation, and purification techniques, the cost of some enzymes has been reduced to less than $500/kg.

The synthetic utility of enzymes for the preparation of organic molecules is tremendous. Enzymes catalyze virtually all types of chemical reactions with the exception of Diels-Alder condensation. They possess remarkably high catalytic power (up to 10^{12} rate acceleration compared to the nonenzymatic reactions) and unsurpassed stereo- and regioselectivity. From a technological standpoint they also offer a number of advantages: the mild temperatures, neutral pH, and atmospheric pressure under which most enzymes operate result in processes that are environmentally acceptable, low energy consuming, and usually do not require high capital investments.

Biotransformations are carried out by either whole cells (microbial, plant, or animal) or by isolated enzymes. Both methods have advantages and disadvantages. In general, multistep transformations, such as hydroxylations of steroids, or the synthesis of amino acids, riboflavin, vitamins, and alkaloids that require the presence of several enzymes and cofactors are carried out by whole cells. Simple one- or two-step transformations, on the other hand, are usually carried out by isolated enzymes. Compared to fermentations, enzymatic reactions have a number of advantages including simple instrumentation; reduced side reactions, easy control, and product isolation.

Enzymatic transformations have been the subject of the most extensive investigation in recent years, resulting in the publication of thousands of original papers. The principal emphasis of this article is on reactions carried out by isolated enzymes that have the broadest synthetic utility, ie, hydrolases, oxidoreductases, and lyases. Biotransformations catalyzed by living cells are considered to a lesser extent. Enzymes that are used on a large scale such as amylase and glucose isomerase and also endonucleases and ligases that are routinely used for DNA synthesis are extensively covered elsewhere (see Enzyme applications, industrial; Genetic engineering). For more detailed information on biotransformations the reader may consult several books and numerous reviews (1–14).

1. Hydrolases

1.1. Quantitative Analysis of Selectivity

One of the principal synthetic values of enzymes stems from their unique enantioselectivity, ie, ability to discriminate between enantiomers of a racemic pair. Detailed quantitative analysis of kinetic resolutions

of enantiomers relating the extent of conversion of racemic substrate (c), enantiomeric excess (ee), and the enantiomeric ratio (E) has been described in an excellent series of articles (7, 15, 16).

During a resolution process, the R- and S-enantiomers compete for the free enzyme to form the noncovalent enzyme–substrate complexes ES and ER. These proceed to form transition-state intermediates [ES]‡ and [ER]‡:

$$\mathrm{S,R + E} \rightleftharpoons \begin{matrix} \mathrm{ES} \rightleftharpoons [\mathrm{ES}]^{\ddagger} \rightleftharpoons \mathrm{E + P} \\ \mathrm{ER} \rightleftharpoons [\mathrm{ER}]^{\ddagger} \rightleftharpoons \mathrm{E + Q} \end{matrix} \qquad (1)$$

Since the rates for Michaelis-Menten kinetics at the steady state are described by

$$v_S = (k_{\mathrm{cat}}/K_m)_S\,[E]\,[S] \quad \text{and} \quad v_R = (k_{\mathrm{cat}}/K_m)_R\,[E]\,[R]$$

where K_{m} is the Michaelis constant, then

$$v_S/v_R = (k_{\mathrm{cat}}/K_m)_S\,[S] \,/\, (k_{\mathrm{cat}}/K_m)_R\,[R]$$

The ratio of specificity constants $(k_{\mathrm{cat}}/K_{\mathrm{m}})_S$ and $(k_{\mathrm{cat}}/K_{\mathrm{m}})_R$ determines the enantioselectivity of the reaction. Since (see Fig. 1)

$$(k_{\mathrm{cat}}/K_m)_S \sim \exp\left(\Delta G_S/RT\right) \quad \text{and} \quad (k_{\mathrm{cat}}/K_m)_R \sim \exp\left(\Delta G_R/RT\right)$$

where R and T are the gas constant and reaction temperature respectively, then

$$(k_{\mathrm{cat}}/K_m)_S \,/\, (k_{\mathrm{cat}}/K_m)_R = \exp\left(\Delta\Delta G/RT\right) = E \qquad (2)$$

where E is the enantiomeric ratio that depends only on the specificity constant and is independent of time and substrate concentrations. Hence enantioselectivity of an enzyme is brought about by the free-energy difference of the diastereomeric transition states for the two enantiomers. Equation 2 can be converted into 3 that relates E to degree of conversion, c, and enantiomeric excess, ee_{S}:

$$\ln\left(\left[1-c\right]\left[1-ee_S\right]\right)/\ln\left(\left[1-c\right]\left[1+ee_S\right]\right) = E \qquad (3)$$

Theoretical plots of ee_{S} (substrate) and ee_{P} (product) as a function of c are shown in Figure 2**a** and **b.** It can be seen that the ee_{S} increases with the extent of conversion. Consequently the enantiomeric purity of the substrate can be increased by sacrificing the yield and carrying out the reaction to higher degrees of conversion. Conversely, if high purity product is required the conversion should be terminated at early stages.

1.2. Enzyme-Catalyzed Asymmetric Synthesis

The extent of kinetic resolution of racemates is determined by differences in the reaction rates for the two enantiomers. At the end of the reaction the faster reacting enantiomer is transformed, leaving the slower reacting enantiomer unchanged. It is apparent that the maximum product yield of any kinetic resolution cannot exceed 50%.

The situation is different if the substrate is a prochiral or meso compound. Since these molecules have a center or plane of symmetry the binding of pro-S or pro-R forms is equivalent. The chirality appears only as a result of the transformation. Hence, at least theoretically, the compound can be converted to one enantiomer quantitatively.

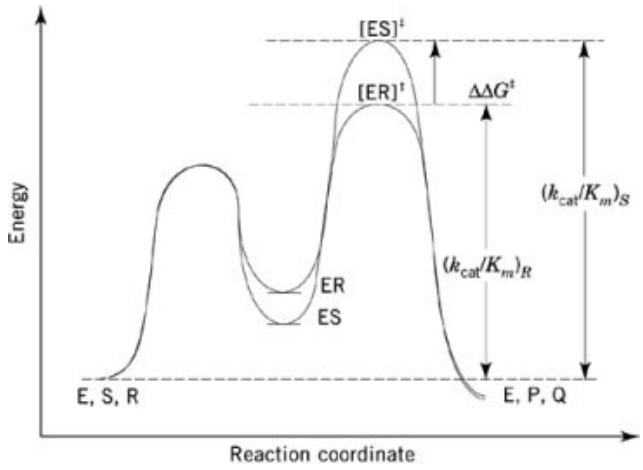


Fig. 1. Free-energy profile for a kinetic resolution depicted by equation **1** that follows Michaelis-Menten kinetics.

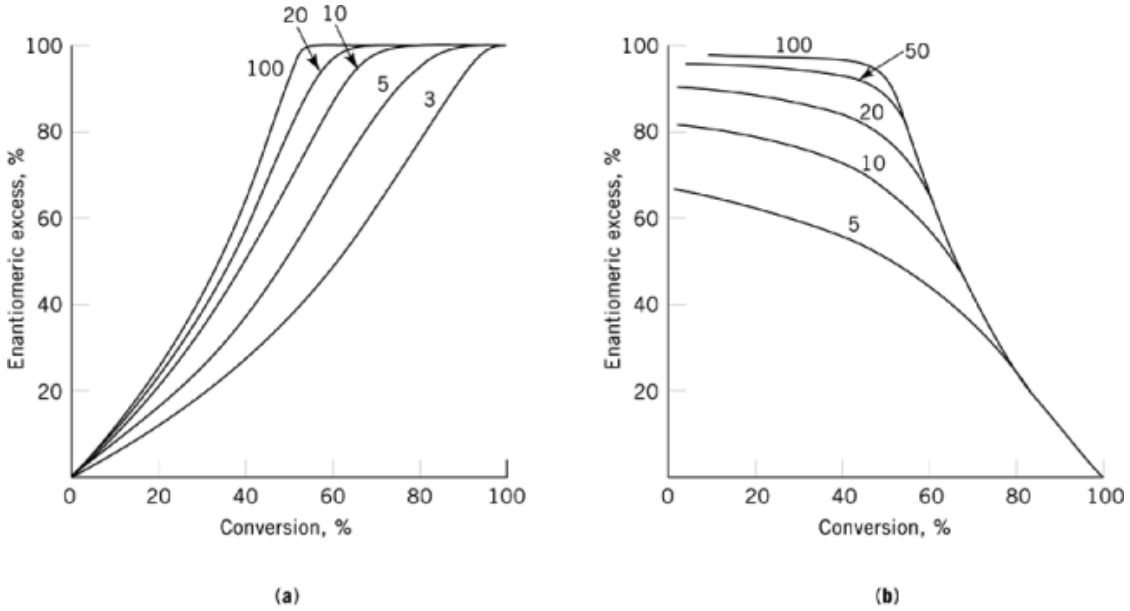


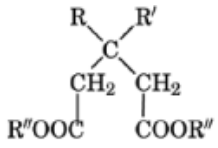
Fig. 2. Enantiomeric excess (*ee*) as a function of the conversion for various enantiomeric ratios (*E*) noted on the curves: (**a**) remaining substrate, $ee_S=(R-S)/(R+S)$; (**b**) product $ee_P=(P-Q)/(P+Q)$. Reprinted with permission (15).(Courtesy of the American Chemical Society.)

Hydrolytic enzymes such as esterases and lipases have proven particularly useful for asymmetric synthesis because of their abilities to discriminate between enantiotopic ester and hydroxyl groups. A large number of esterases and lipases are commercially available in large quantities; many are inexpensive and accept a broad range of substrates.

1.2.1. Dicarboxylic Acid Monoesters

Enzymatic synthesis of monoesters of dicarboxylic acids by hydrolysis of the corresponding diesters is a widely used and thoroughly studied reaction. It is catalyzed by a number of esterases, lipases, and proteases and is usually carried out in an aqueous buffer, pH 6–8 at room temperature. Organic cosolvents may be added to increase solubility of the substrates. The pH is maintained at a constant level by the addition of aqueous hydroxide. After one equivalent of base is consumed the monoesters are isolated by conventional means.

A number of examples of enantioselective hydrolysis of diesters (**1** and **2**) of malonic and glutaric acids are given in Table 1.

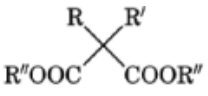


(1)

(a) R = OH; R′ = H; R″ = CH_3

(b) R = CH_3; R′ = H; R″ = CH_3

(c) R = $OCH_2C_6H_5$; R′ = H; R″ = CH_3



(2)

(a) R = CH_3; R′ = F; R″ = C_2H_5

(b) R = CH_3; R′ = *p*-$CH_3C_6H_4$; R″ = CH_3

(c) R = C_2H_5; R′ = CH; R″ = CH_3

(d) R = C_7H_{15}; R′ = CH_3; R″ = CH_3

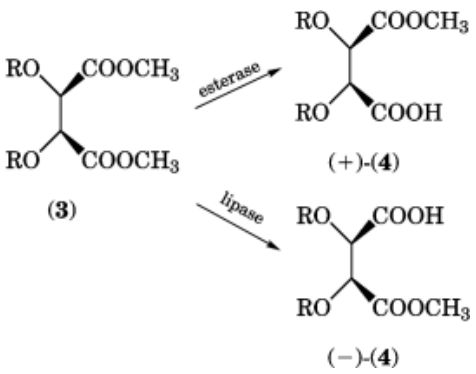
Porcine liver esterase (PLE) gives excellent enantioselectivity with both dimethyl 3-methylglutarate [19013-37-7] **1b** and malonate **2b** diester. It is apparent from Table 1 that the enzyme's selectivity strongly depends on the size of the alkyl group in the 2-position. The hydrolysis of ethyl derivative **2c** gives the S-enantiomer with 75% *ee* whereas the hydrolysis of heptyl derivative **2d** results in the R-monoester with 90% *ee*. Chymotrypsin [9004-07-3] (CT) does not discriminate glutarates that have small substituents in the 3-position well. However, when hydroxyl is replaced by the much bulkier benzyl derivative **1c**, enantioselectivity improves significantly.

Similar to the hydrolysis of malonates and glutarates, conversion of mesoderivatives of tartaric acid can be accomplished with *Candida cylindracea* lipase (CCL) and porcine liver esterase in very good yield (23). Hydrolysis of (**3**) with esterase gives (+)-(**4**) with an *ee* 90.5% in >90% yield. Hydrolysis with lipase results in the formation of (−)-(**4**) (*ee* 92.5%, >90% yield).

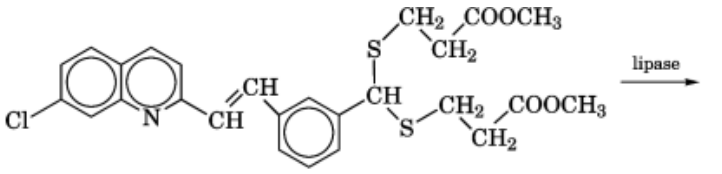
Table 1. Enantioselective Hydrolysis of Malonate[a] and Glutarate[b] Esters

		Monoester			
Substrate	Enzyme	Yield, %	*ee*, %	Configuration	References
(**1a**)	CT	78	55 ± 5	*R*	17
	PLE	76	30 ± 5	*S*	17
(**1b**)	PLE	92	100	*R*	18
(**1c**)	CT	86	92	*R*	19
(**2a**)	lipase MY[c]	75	86	*S*	20
(**2b**)	PLE	[d]	96	*R*	21
(**2c**)	PLE	90–98	75	*S*	22
(**2d**)	PLE	90–98	90	*R*	22

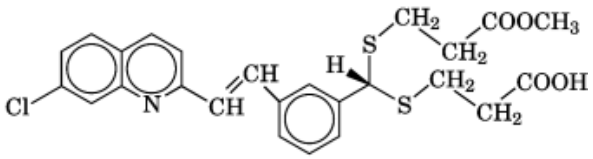
[a]Structure (**2**).
[b]Structure (**1**).
[c]From *Candida cylindracea*.
[d]Not reported.



It is generally believed that selectivity of hydrolytic enzymes strongly depends on the proximity of the chiral center to the reacting carbonyl group, and only a few examples of successful resolutions exist for compounds that have the chiral center removed by more than three bonds. A noticeable exception to this rule is the enantioselective hydrolysis by *Pseudomonas fluorescens* lipase (PFL) of racemic dithioacetal (**5**) that has a prochiral center four bonds away from the reactive carboxylate (24). The monoester (**6**) is obtained in 89% yield and 98% *ee*.



(**5**)



(**6**)

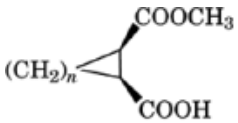
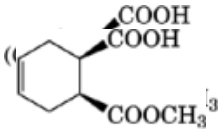
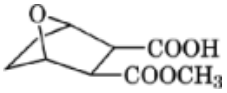
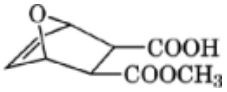
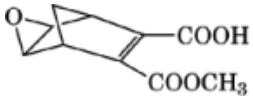
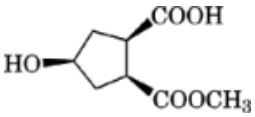
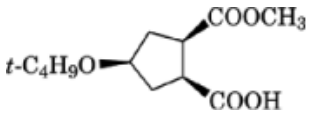
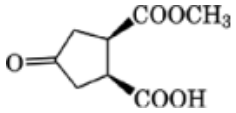
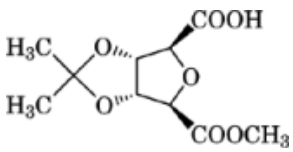
PLE catalyzes the hydrolysis of a wide range of meso-diesters (Table 2). This reaction is interesting from both theoretical and practical standpoints. Indeed, the analysis of a large range of kinetic data provided sufficient information to create a detailed active site model of PLE (31). From a practical standpoint, selective hydrolysis of *meso*-cyclo-1,2-dicarboxylates leads to chiral synthons that are valuable intermediates for the synthesis of a variety of natural products.

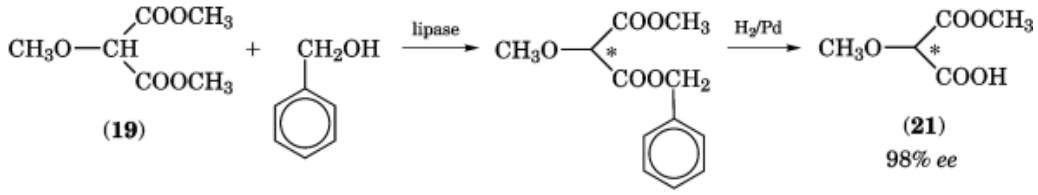
The hydrolysis of three- and four-membered rings (Table 2) is enantiotopically specific for the pro-(*S*)-methoxycarbonyl group leading to the formation of 1(*R*),2(*S*)-**7** and 1(*R*),2(*S*)-**8**; in the case of five- and six-membered rings **9,10**, the pro-(*R*)-group is hydrolyzed (25). This reversal of configurations of products was rationalized by postulating the presence of two binding cavities: large and small (31). Smaller molecules such as cyclopropane- and cyclobutanecarboxylates are accommodated in a smaller cavity leading to the formation of 1(*R*),2(*S*)-compounds. The larger rings can only bind in the larger cavity, thus reversing product configuration.

Hydrolysis of (**11**) that has a structure similar to (**10**) is also enantiotopically specific for the pro-(*S*)-methoxycarbonyl group and proceeds with very high yield (26). Bicyclic exo- **12,13** and endo-diesters that are valuable intermediates for the synthesis of natural cyclic compounds are excellent substrates for porcine pancreatic lipase (PPL) hydrolysis affording the corresponding monoesters in high chemical yield and optical purity (27). Dimethyl esters of meso-1,2-cyclopentanedicarboxylic acids **15–17** are also good substrates for PLE (29). Interestingly, the enzyme selectivity strongly depends on the nature of the substituent in the 4-position; whereas pro-(*R*)-ester group is preferentially hydrolyzed in hydroxyl diester (**15**), it is the pro-(*S*)-ester group that is cleaved in *tert*-butoxy diester (**16**). Hydrolysis of meso diesters of derivatives of 3,4-(isopropylidenedioxy)tetrahydrofuran (**18**) proceeds with only moderate selectivity giving monoesters with only 72% *ee* (32).

The transformations described thus far were catalyzed by enzymes in their traditional hydrolytic mode. More recent developments in the area of enzymatic catalysis in nonaqueous media (11, 16, 33–35) have significantly broadened the repertoire of hydrolytic enzymes. The acyl–enzyme intermediate formed in the first step of the reaction via acylation of the enzyme's active site nucleophile can be deacylated in the absence of water by a number of nucleophiles. Thus, in nonaqueous media, hydrolysis can be substituted by a number of alternative, synthetic reactions that include transesterification, aminolysis, thiotransesterification, oximolysis, etc (36). This principle has been successfully utilized for the synthesis of optically active malonic and glutaric acid monoesters. Chemoenzymatic syntheses of derivatives of malonic acid monoesters (**20**) according to the following scheme have been reported (37):

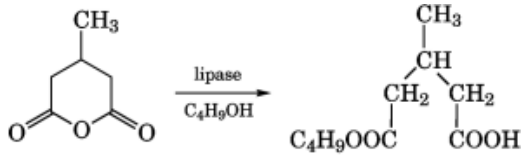
Table 2. PLE-Catalyzed Hydrolysis of Monocyclic meso-Diesters

Product	Yield, %	*ee*, %	References
 $n = 1,2$	87–88	>97	25
	92($n = 1$) 88($n = 2$)	17 97	25
 $n = 1,2$	98	98	26
	82	98	27
	86	75	27
	100	85	28
	76	80	29
	76	84	29
	86	88	29
	86	72	30



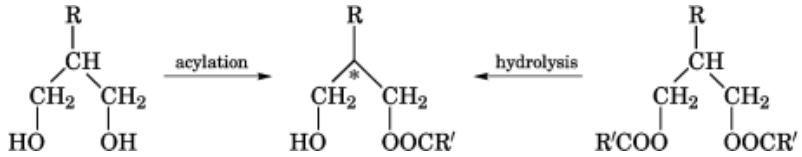
The reaction of dimethyl 2-methoxymalonate [5018-30-4] (**19**) with benzyl alcohol [100-51-6] catalyzed by *Candida cylindracea* lipase results in monoester (**21**) with 98% *ee*.

Chiral 3-substituted glutaric acid monoester (**23**) can be obtained via *Pseudomonas fluorescens* lipase-catalyzed nucleophilic ring opening of anhydride (**22**) by butanol (38).



1.2.2. Monoacyl Diols

Enzymatic synthesis of chiral monoacyl diols can be carried out either by direct enzymatic acylation of prochiral diols or by hydrolysis of chemically synthesized dicarboxylates.



Generally, these two methods complement each other. With some rare exceptions an enzyme that produces an S ester in the hydrolysis reaction produces an R isomer in acylation reaction and vice versa.

A number of examples of monoacylated diols produced by enzymatic hydrolysis of prochiral carboxylates are presented in Table 3. PLE-catalyzed conversions of acyclic diesters strongly depend on the structure of the substituent and are usually poor for alkyl derivatives. Lipases are much less sensitive to the structure of the side chain; the yields and selectivity of the hydrolysis of both alkyl (**26**) and aryl (**24**) derivatives are similar. The enzyme selectivity depends not only on the structure of the alcohol, but also on the nature of the acyl moiety (48).

Cyclic diesters are often even better substrates for lipases and esterases than acyclic derivatives. Small-ring monoacetates (**28**, $n = 1 - 3$) are obtained in higher yield and *ee* than the larger derivatives (for **28**, $n = 4$ *ee* is only 50%) (43). Hydrolysis of tetrahydrofuran diester results in monoester (**29**) of $ee > 99\%$ (44).

The synthetic utility of the above transformations stems from the fact that many monoesters obtained as a result of hydrolysis may be converted to pharmaceutically important intermediates. For example, the optically active glycerol derivative (**27**) is a key intermediate in the production of β-blockers. Allyl derivative (**25**) may be converted into (*S*)-paraconic acid [4694-66-0] ((*S*)-5-oxo-3-tetrahydrofurancarboxylic acid) that is a starting material for the synthesis of (3*R*)-A-factor. The unsaturated chiral cyclic monoacetate (**31**) is an optically active

synthon for prostaglandins, and the monoester (**29**) is used for the synthesis of platelet activating factor (PAF) antagonists.

Table 3. Enzymatic Hydrolysis of Prochiral Carboxylates

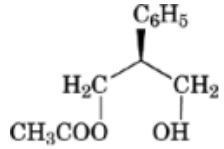
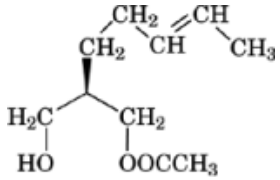
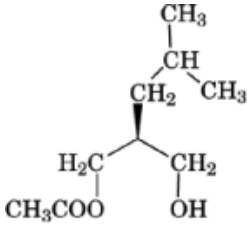
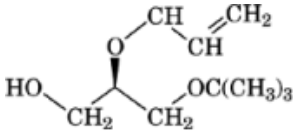
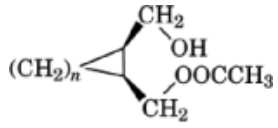
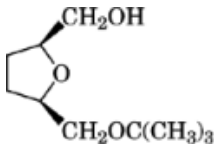
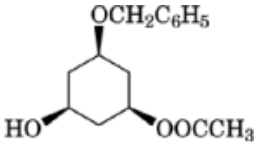
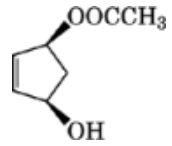
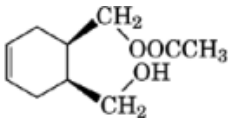
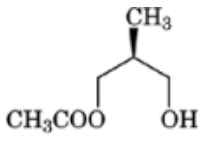
Product	Catalyst[a]	Yield, %	*ee*, %	Refs.
	PPL	80	92	39
	lipase P	86	90	40
	PPL	75	97	41
	lipase *Mucor* sp.	100	95	42
 $n = 1$–4	lipase *Pseudomonas* sp.	54–83	50–95	43

Table 3. ***Continued***

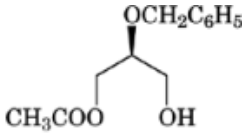
Product	Catalyst[a]	Yield, %	*ee*, %	Refs.
	lipase *Mucor javanicus*	75	>99	44
	PLE	62	87	45
	PPL	89	100	46
	PPL	95	>99	47

[a] PPL = porcine pancreatic lipase; PLE = porcine liver esterase.

In contrast to the hydrolysis of prochiral esters performed in aqueous solutions, the enzymatic acylation of prochiral diols is usually carried out in an inert organic solvent such as hexane, ether, toluene, or ethyl acetate. In order to increase the reaction rate and the degree of conversion, activated esters such as vinyl carboxylates are often used as acylating agents. The vinyl alcohol formed as a result of transesterification tautomerizes to acetaldehyde, making the reaction practically irreversible. The presence of a bulky substituent in the 2-position helps the enzyme to discriminate between enantiotopic faces; as a result the enzymatic acylation of prochiral 2-benzoxy-1,3-propanediol (**34**) proceeds with excellent selectivity ($ee > 96\%$) (49). In the case of the 2-methyl substituted diol (**33**) the selectivity is only moderate (50).



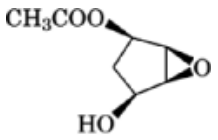
(**33**)



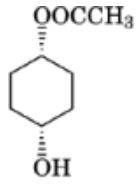
(34)

Enzymatic transesterification of cyclic diols often proceeds with very good yield and selectivity **35–39**.

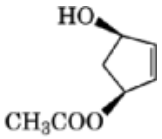
For example, lipase PS-catalyzed acylation of the corresponding epoxy diol results in monoacetate (**35**) with *ee* > 95% (51). The same enzyme suspended in isopropenyl acetate which serves as both the reaction medium and the acylating agent catalyzes the conversion of the corresponding prochiral *cis*-2-cyclohexene-1,4-diol [53762-85-9] into monoacetate (**36**) in 98% *ee* (52). A crude mixture of enzymes obtained from pancreas, pancreatin, acylates 2-cyclopentene-1,4-diol [4157-01-1] to enantiomerically pure 1(*S*),4(*R*)-4-hydroxycyclopent-2-enyl acetate (**37**) (53). Homochiral monoester (**38**), which is a chiral building block for the synthesis of arachidonic acid metabolites, is obtained in 80% yield and 95% *ee* via lipase SAM-II-catalyzed transesterification of the corresponding diol with vinyl acetate (54). Lipase from *Pseudomonas cepacia* enantioselectively acylates (**39**) with acetic anhydride in toluene resulting in the product of R-configuration in 78% yield and >99% *ee* (55).



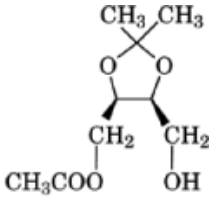
(35)



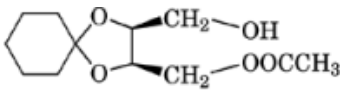
(36)



(37)



(38)



(39)

1.3. Kinetic Resolutions

From a practical standpoint the principal difference between formation of a chiral molecule by kinetic resolution of a racemate and formation by asymmetric synthesis is that in the former case the maximum theoretical yield of the chiral product is 50% based on a racemic starting material. In the latter case a maximum yield of 100% is possible. If the reactivity of two enantiomers is substantially different the reaction virtually stops at 50% conversion, and enantiomerically pure substrate and product may be obtained in close to 50% yield. Conveniently, the enantiomeric purity of the substrate and the product depends strongly on the degree of conversion so that even in those instances where reactivity of enantiomers is not substantially different, a high purity material may be obtained by sacrificing the overall yield.

The variety of enzyme-catalyzed kinetic resolutions of enantiomers reported in recent years is enormous. Similar to asymmetric synthesis, enantioselective resolutions are carried out in either hydrolytic or esterification–transesterification modes. Both modes have advantages and disadvantages. Hydrolytic resolutions that are carried out in a predominantly aqueous medium are usually faster and, as a consequence, require smaller quantities of enzymes. On the other hand, esterifications in organic solvents are experimentally simpler procedures, allowing easy product isolation and reuse of the enzyme without immobilization.

1.3.1. Optically Active Acids and Esters

Enantioselective hydrolysis of esters of simple alcohols is a common method for the production of pure enantiomers of esters or the corresponding acids. Several representative examples are summarized in Table 4. Lipases, esterases, and proteases accept a wide variety of esters and convert them to the corresponding acids, often in a highly enantioselective manner. For example, the hydrolysis of (*R*)-methyl hydratropate [34083-55-1] (**40**) catalyzed by lipase P from Amano results in the corresponding acid in 50% yield and 95% *ee* (56). Various substituents on the α-carbon **41–44** are readily tolerated by both lipases and proteases without reduction in selectivity (57–60). The enantioselectivity of many lipases is not significantly affected by changes in the alcohol component. As a result, activated esters may be used as a means of enhancing the reaction rate.

Lipase-catalyzed kinetic resolutions are often practical for the preparation of optically active pharmaceuticals (61). For example, suprofen [40828-46-4] (**45**), which is a nonsteroidal antiinflamatory drug, can be resolved by *Candida cylindracea* lipase in $>$95% *ee* at 49% conversion (61). Moreover, lipase-based processes for the resolution of naproxen [22204-53-1] and ibuprofen [15687-27-1] (61) have also been developed.

PPL and lipase from *Pseudomonas* sp. catalyze enantioselective hydrolysis of sulfinylalkanoates. For example, methyl sulfinylacetate (**46**) was resolved by *Pseudomonas* sp. lipase in good yield and excellent selectivity (62). This procedure was suitable for the preparation of sulfinylalkanoates where the ester and sulfoxide groups are separated by one or two methylene units. Compounds with three methylene groups were not substrates for the lipase (65).

The resolving of a variety of α-substituted carboxylic acid esters by a previously undescribed enzyme, *Candida lipolytica* esterase, has been reported (64). α-Methyl-α-amino (**49**) and α-methyl-α-hydrazino (**48**) esters, which function as inhibitors of acid decarboxylase enzymes, are obtained on a multigram scale in optically pure form.

Table 4. Optically Active Acids and Esters Produced by Hydrolysis

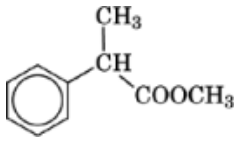
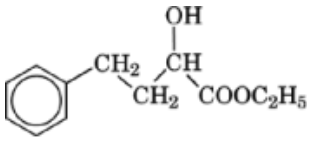
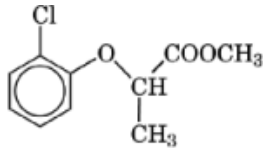
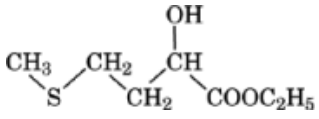
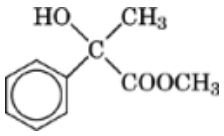
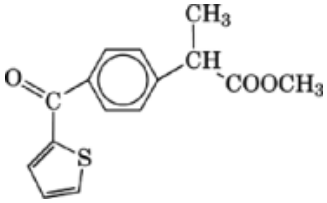
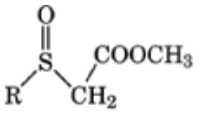
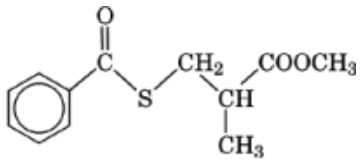
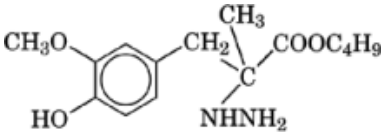
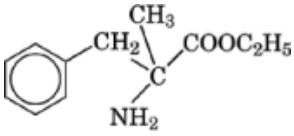
Substrate	Enzyme	Ester			Acid			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
	lipase P	44	98	*R*	50	95	*S*	56
	lipase P-30	43	99	*R*	33	92	*S*	57
	α-chymotrypsin	46.5	80	*S*	38.5	75	*R*	58
	lipase P	40	>98	*R*	42	98	*S*	59
	protease *Asp. oryzae*	48	70	*S*	48	75	*R*	60
	lipase *C. cylindracea*	49[a]	90	*R*	49[a]	95	*S*	61

Table 4. ***Continued***

Substrate	Enzyme	Ester			Acid			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
 R = C_6H_5, *p*-ClC_6H_4, *p*-$CH_3OC_6H_4$	lipase *Pseudomonas* sp.	33–49	98	*S*	17–38	88–98	*R*	62
	lipase *A. niger*	32[a]	45	*S*	45[a]	98	*R*	63
	esterase *C. lipolytica*	50[a]	98	*R*	50[a]	>99	*S*	64
	esterase *C. lipolytica*	50[a]	>99	*R*	[b]	[b]	*S*	64

[a] Conversion.
[b] Not reported.

1.3.2. Optically Active Alcohols and Esters

In addition to the hydrolysis of esters formed by simple alcohols described above, lipases and esterases also catalyze the hydrolysis of a wide range of esters based on more complex and synthetically useful cyclic and acyclic alcohols (Table 5). Although the hydrolysis of acetates often gives the desirable resolution, to achieve maximum selectivity and reaction efficiency, comparison of various esters is recommended.

Both saturated (**50**) and unsaturated derivatives (**51**) are easily accepted by lipases and esterases. Lipase P from Amano resolves azide (**52**) or naphthyl (**53**) derivatives with good yields and excellent selectivity. PPL-catalyzed resolution of glycidyl esters (**54**) is of great synthetic utility because it provides an alternative to the Sharpless epoxidation route for the synthesis of β-blockers. The optical purity of glycidyl esters strongly

depends on the structure of the acyl moiety; the hydrolysis of propyl and butyl derivatives of epoxy alcohols results in esters with $ee > 95\%$ (30).

Table 5. Optically Active Alcohols and Esters Produced by Hydrolysis

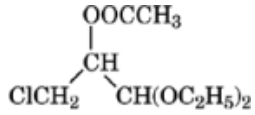
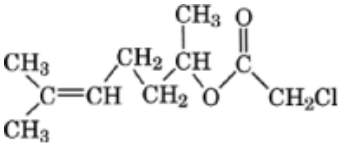
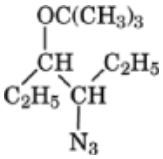
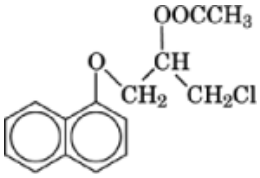
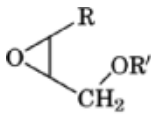
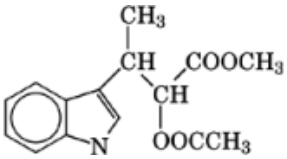
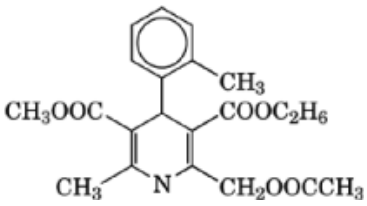
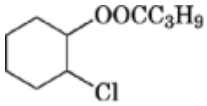
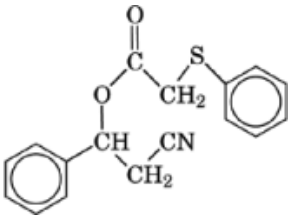
Substrate	Enzyme	Ester			Alcohol			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
	lipase LP-80	46	>98	*S*	51.5	98	*R*	63
	lipase P-30	53	89	*R*	45	100	*S*	66
	lipase P	39	>98	*R,S*	35	>98	*S,R*	67
	lipase P	48	95	*S*	45	>95	*R*	68
 R = alkyl R^0 = acyl	PPL	32–45	90–95	2*S*,3*R*	15–40	30–65	2*R*,3*S*	(69, 30)

Table 5. ***Continued***

Substrate	Enzyme	Ester			Alcohol			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
	lipase OF-360	62	54	2*R*,3*S*	37	93	2*S*,3*R*	70
	lipase *Pseudomonas* sp.	50	91	*R*	50	98	*R*	71
	lipase *Pseudomonas* sp.	37	>98	*R*,*R*	46	>98	*S*,*S*	72
	lipase P	94	43	*R*	98	35	*S*	73

Regioselective hydrolysis of diesters is a challenging problem in synthetic chemistry because the side reactions always reduce the yield of desired product. Some lipases are well suited to perform this task. Lipase OF-360 (Meito Sangyo) hydrolyzes diester (**55**) in 74% theoretical yield and 93% *ee* (70). Lipase from *Pseudomonas cepacia* suspended in diisopropyl ether saturated with water hydrolyzes triester (**56**) with a remarkable efficiency and regio- and stereoselectivity (71).

Two more examples in Table 5 include the hydrolysis of esters of trans-alcohols that proceed with high efficiency practically regardless of the nature of the substituents (72) and resolution of β-hydroxynitriles with lipase from *Pseudomonas* sp. In the latter case the enantioselectivity of the hydrolysis was improved by introducing sulfur into the acyl moiety (73).

Resolution of racemic alcohols by acylation (Table 6) is as popular as that by hydrolysis. Because of the simplicity of reactions in nonaqueous media, acylation routes are often preferred. As in hydrolytic reactions, selectivity of esterification may depend on the structure of the acylating agent. Whereas *Candida cylindracea* lipase-catalyzed acylation of racemic-α-methylbenzyl alcohol [98-85-1] (**59**) with butyric acid has an enantiomeric value E of 20, acylation with dodecanoic acid increases the E value to 46 (16). Not only acids but also anhydrides are used as acylating agents. *Pseudomonas fl.* lipase (PFL), for example, catalyzed acylation of α-phenethanol [98-85-1] (**59**) with acetic anhydride in 42% yield and 92% selectivity (74).

Table 6. Optically Active Alcohols and Esters Produced by Acylation

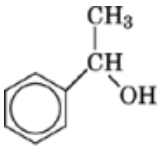
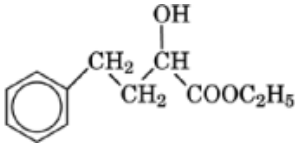
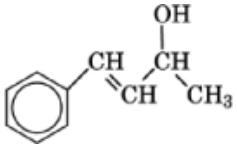
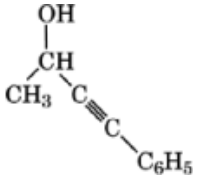
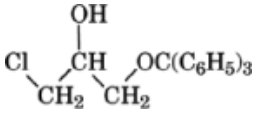
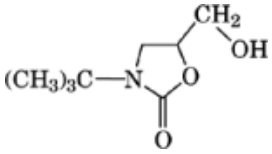
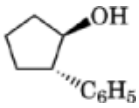
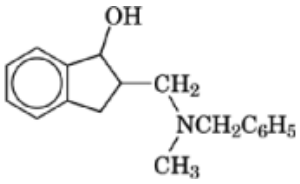
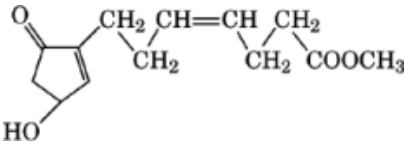
Substrate	Enzyme	Alcohol			Ester			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
	lipase *P. fragi*	46	95	*R*	42	92	*S*	74
	PFL	48	98	*S*	51	98	*R*	75
	PPL	47[a]	96	*S*	47[a]	85	*R*	76
	lipase *Pseudomonas* sp.	47	>95	*S*	48	>95	*R*	77
	lipase PS	54	72	*R*	43	>98	*S*	78

Table 6. ***Continued***

Substrate	Enzyme	Alcohol			Ester			Refs.
		Yield, %	*ee*, %	Configuration	Yield, %	*ee*, %	Configuration	
	lipase *P. fluorescens*	42	95	*S*	40	95	*R*	74
	lipase *P. fluorescens*	43	95	*S,R*	46	95	*R,S*	79
	lipase P	46	98	*S*	43	97	*R*	80
	PPL	35	99.4	*S*	43	92	*R*	81

[a]Conversion.

Various racemic secondary alcohols with different substituents, eg, α-hydroxyester (**60**), are resolved by PFL nearly quantitatively (75). The effect of adjacent unsaturation on enzyme-catalyzed kinetic resolutions was thoroughly studied for a series of allylic (**61**), propargylic (**62**), and phenyl-substituted 2-alkanols (76, 77). Excellent selectivity was observed for (*E*)-allylic alcohols whereas (*Z*)-isomers showed poor selectivity (76).

Lipase-catalyzed enantioselective transesterification of *O*-substituted-1,2-diols is another practical route for the synthesis of β-blockers. Lipase PS suspended in toluene catalyzes the transesterification of (**63**) with vinyl acetate to give the (*S*)-ester in 43% yield and >98% *ee* (78). The desired product, optically pure (*R*)-tritylglycidol, is then easily obtained by treating the ester with alcoholic alkali. Moreover, *Pseudomonas* lipase catalyzes the acylation of oxazolidinone (**64**) with acetic anhydride in very good yield and selectivity (74). PPL-catalyzed transesterification of a number of *trans*-norbornene derivatives proceeds in about 30% yield and 92% *ee* (79, 80).

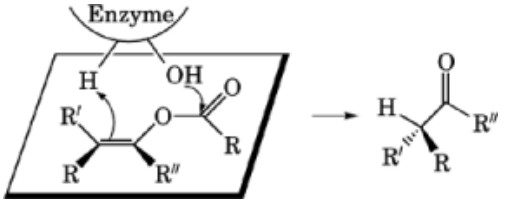
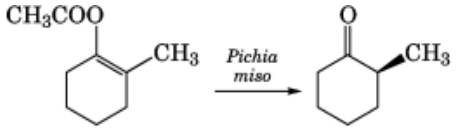


Fig. 3. Enzyme-mediated hydrolysis of an enol ester.

Cyclic alcohols are excellent targets for enantioselective enzymatic acylations. For example, acylation of (**65**) with vinyl acetate catalyzed by lipase SAM-II gives the (*R*),(*S*)-ester with 95% *ee* (81). Similarly (**66**), which is a precursor for seratonin uptake inhibitor, is resolved in a high yield and selectivity with Amano lipase P (82). The prostaglandin synthon (**67**) is resolved by the same method into the optically pure alcohol in 35% yield (83).

1.4. Hydrolysis of Enol Esters

Enzyme-mediated enantioface-differentiating hydrolysis of enol esters is an original method for generating optically active α-substituted ketones (84–86). If the protonation of a double bond occurs from one side with the simultaneous elimination of the acyl group (Fig. 3), then the optically active ketone should be produced. Indeed, the incubation of 1-acetoxy-2-methylcyclohexene [1196-73-2] (**68**) with *Pichia miso* affords (*S*)-2-methylcyclohexanone [22554-27-4] (**69**) in 77% yield and 90% *ee* (84).

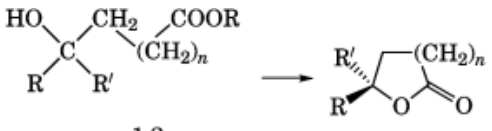


(**68**) (**69**)

Moreover, fermentation of various α-substituted cycloalkanone enol esters results in optically active six-, eight-, ten-, and twelve-membered ring ketones with 70–96% *ee* (84). Isolated enzymes catalyze similar transformations. *Bacillus coagulans* and *Candida cylindracea* lipase OF (Meito Sangyo) hydrolyze a number of cyclic and acyclic enol esters, giving ketones in 40–80% yield and 14–85% *ee* (85, 86).

1.5. Chiral Lactones and Polyesters

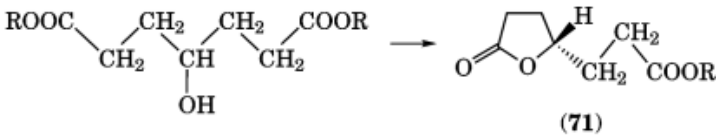
Similar to intermolecular reactions described previously, lipases also catalyze intramolecular acylations of hydroxy acids; the reactionsults in the formation of lactones.



$n = 1,2$

PPL suspended in dry ether catalyzes the lactonization of a number of γ-hydroxy acids. For example, (*S*)-γ-methylbutyrolactone [19041-15-7] (R = CH_3, R′ = H, $n = 1$), and γ-phenylbutyrolactone (R = C_6H_5,

R′ = H, $n = 1$) may be produced in nearly quantitative yields (87–89). When prochiral derivatives (**70**) of γ-hydroxypimelic acid were submitted to the action of PPL and *Pseudomonas fluorescens* lipases, optically active lactones with up to 98% *ee* were produced.



(**70**) (**71**)

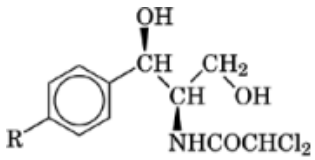
R = H, CH_3, C_2H_5, *i*-C_3H_7, $CH_2C_6H_5$

The nature of the product strongly depends on the length of the hydroxy acid; generally when the hydroxyl group is remote the yield of lactone drops significantly. For example, 10-hydroxydecanoic acid [1679-53-4] does not produce any decanolide; instead, the reaction proceeds by intermolecular oligomerization, and a complex mixture of di-, tri-, tetra-, and pentalactones results (90). However, when *Pseudomonas* sp. or *Candida cylindracea* lipases are incubated with 16-hydroxyhexadecanoic acid [506-13-8], hexadecanolide is the predominant product (91).

Lipase-catalyzed intermolecular condensation of diacids with diols results in a mixture of macrocyclic lactones and linear oligomers. Interestingly, the reaction temperature has a strong effect on the product distribution. The condensation of α,ω-diacids with α,ω-dialcohols catalyzed by *Candida cylindracea* or *Pseudomonas* sp. lipases leads to macrocyclic lactones at temperatures between 55 and 75°C (91), but at lower temperatures (<45°C) the formation of oligomeric esters predominates. Optically active trimers and pentamers can be produced at room temperature by PPL or *Chromobacterium viscosum* lipase-catalyzed condensation of bis(2,2,2-trichloroethyl) (±)-3-methyladipate and 1,6-hexanediol (92).

1.6. Regioselective Acylation of Hydroxy Compounds

Aliphatic diols can be selectively acylated at the primary position by a number of lipases in nonaqueous solvents. For example, PPL suspended in solutions of various diols in ethyl carboxylates catalyzes transesterification in a highly regioselective manner, producing primary monoesters in up to 97% yield (93). Similarly, chloramphenicol [56-75-7] (**72**) (R = NO_2) can be acylated by a number of lipases to produce optically pure, water-insoluble 3-*O*-palmitate in a highly selective manner (94).



R = NO_2, CH_3SO_2

(**72**)

Acid anhydrides are also useful for acylation of primary alcohols in organic solvents. The reaction produces high yields of the primary acylated products with only traces (1–2%) of diesters (95).

This preference for the primary position is preserved during the acylation of polyhydroxy compounds. For example, PPL suspended in pyridine acylates glucose (**73**), galactose, mannose, and fructose with trichloroethyl carboxylates in a regioselective manner with an overwhelming preference toward C-6 primary hydroxyl (96)

(Table 7). Similarly, the primary hydroxyl of various glucopyranosides can be selectively acylated with PPL and *Candida antarctica* lipase (97, 98). It is apparent that the high selectivity of lipases toward primary groups can be attributed, at least partly, to higher chemical reactivity of the primary hydroxy group compared to the secondary hydroxyl.

Much more synthetically useful is the ability of lipases and proteases suspended in nonaqueous medium to discriminate between groups that have similar chemical reactivity. This was originally demonstrated with regioselective acylation of sugar derivatives and steroids in organic solvents (98, 99). For example, lipases exhibit a remarkable regioselectivity by discriminating among the four secondary hydroxyl groups in C-6 protected glucose (**74**), galactose, and mannose. Whereas PPL exclusively acylates the C-2 hydroxyl group, *Chromobacterium viscosum* lipase displays an overwhelming preference for the C-3 hdyroxyl group (Table 7). Following this methodology C-2 and C-3 monoesters of glucose have been obtained on a gram scale (98).

Table 7. Regioselective Acylation of Hydroxycompounds

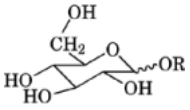
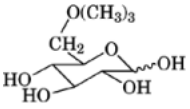
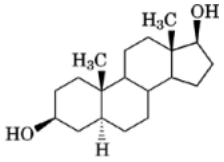
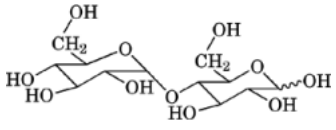
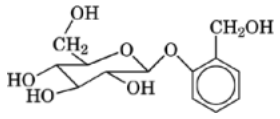
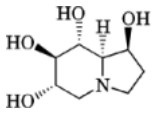
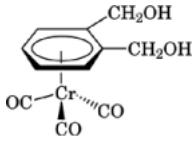
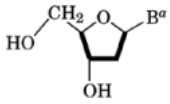
Substrate	Enzyme	Yield, %	Isolated product	Refs.
	PPL	51	6-*O*-acylglucopyranose	96
	lipase *Candida antarctica*	85–95	6-*O*-acylglucopyranoside	(97, 98)
	PPL	51	2,6-di-*O*-butyrylglucose	98
	Chromobacterium viscosum lipase	80	3,6-di-*O*-butyrylglucose	
	*Chromobacterium viscosum* lipase	83	3β-monobutyryl ester	99
	subtilisin	60	17β-monobutyryl ester	99
	subtilisin	45	6′-monobutyrylmaltose	100

Table 7. ***Continued***

Substrate	Enzyme	Yield, %	Isolated product	Refs.
	subtilisin	34	6′-monobutyrylsalicilin	100
	subtilisin	82	1-*O*-acylcastanospermine	101
	PFL	76–97	1(*R*),2(*S*)-monoester	102
	CCL	66–75	1(*S*),2(*R*)-monoester	
	lipase PS	64–82	3′-carbonates	103

[a]

[a] B = thymine, uracil, or adenine.

A number of steroids have been regioselectively acylated in a similar manner (99, 104). *Chromobacterium viscosum* lipase esterifies 5α-androstane-3β,17β-diol [571-20-0] (**75**) with 2,2,2-trifluoroethyl butyrate in acetone with high selectivity. The lipase acylates exclusively the hydroxy group in the 3-position giving the 3β-(monobutyryl ester) of (**75**) in 83% yield. In contrast, *Bacillus subtilis* protease (subtilisin) displays a marked preference for the C-17 hydroxyl. *Candida cylindracea* lipase (CCL) suspended in anhydrous benzene regioselectively acylates the 3α-hydroxyl group of several bile acid derivatives (104).

By taking advantage of the remarkable ability of subtilisin [9014-01-1] to remain catalytically active in anhydrous dimethylformamide, a number of carbohydrates and other sugar-related compounds have been regioselectively acylated with trichloroethyl butyrate (100). In the case of maltose [69-79-4] (**76**) and salicin [138-52-3] (**77**), for example, acylation occurs exclusively at the C-6′ positions.

Castanospermine [79831-76-8] (**78**) contains four secondary hydroxyl groups and therefore represents a significant challenge for regioselective modifications. Subtilisin suspended in pyridine acylates the C-1 hydroxy group with remarkable selectivity resulting in 1-*O*-butyrylcastanospermine in 82% isolated yield (101). The 1-*O*-acyl derivatives can be further acylated at the C-7 hydroxyl with *Chromobacterium viscosum* lipase. Organometallic substrates such as (η^6-1,2-benzene-dimethanol)tricarbonylchromium (**79**) can also be selectively acylated with lipases (102). Lipase from *Pseudomonas fluorescens* acylates (**79**) resulting in the formation of 1(*R*)2(*S*)-monoester with 93% *ee.* Conversely, *Candida cylindracea* lipase exhibits opposite selectivity giving the 1(*S*),2(*R*)-enantiomer.

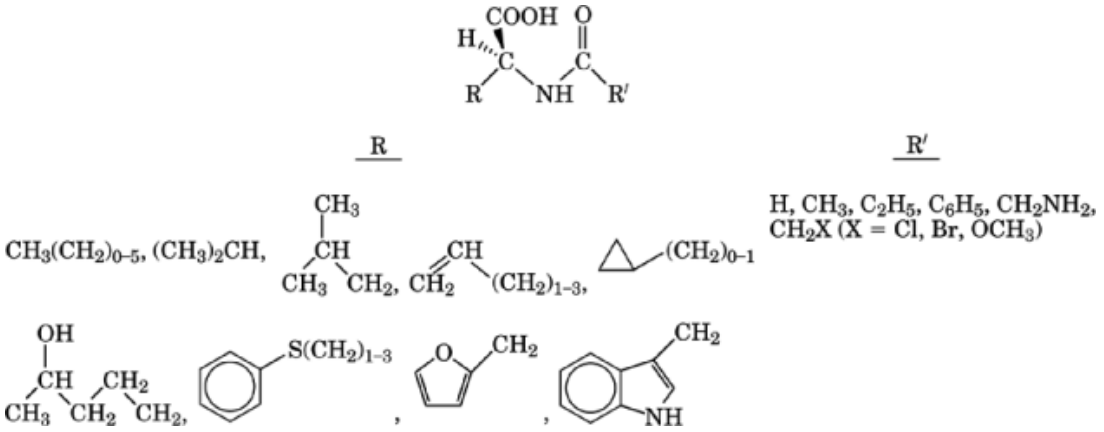


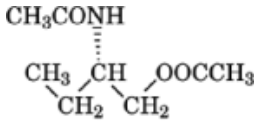
Fig. 4. Examples of enzymatically resolved *N*-acyl amino acids.

Regioselective acylation of the 3′-hydroxyl group of unprotected nucleosides (**80**) deserves special attention. This reaction is highly unusual because lipase PS catalyzes acylation of the secondary hydroxyl group in the presence of primary hydroxyl group in a highly selective manner. Treatment of the nucleosides with a slight excess of *O*-[(alkyloxy)carbonyl]-oximes in the presence of the lipase in THF at 60°C yields 3′-carbonates as the only product in 65–82% yield (103). Interestingly, the lipase also exhibits preference for the secondary hydroxyl group of nucleosides in the hydrolytic mode. The hydrolysis of 2′-deoxy-3′,5′-dihexanoyl pyrimidine nucleosides proceeds exclusively at the secondary hydroxy group resulting in 2′-deoxy-5′-*O*-acylnucleosides (105).

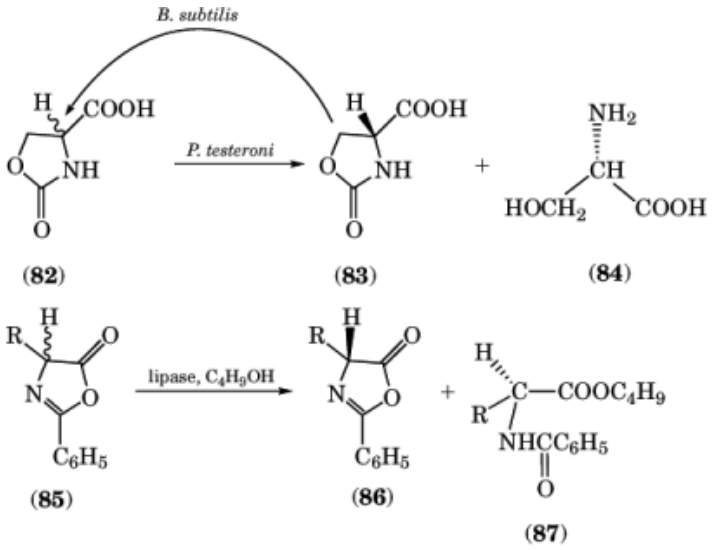
1.7. Resolution of Racemic Amines and Amino Acids

Acylases (EC 3.5.1.14) are the most commonly used enzymes for the resolution of amino acids. Porcine kidney acylase (PKA) and the fungal *Aspergillus* acylase (AA) are commercially available, inexpensive, and stable. They have broad substrate specificity and hydrolyze a wide spectrum of natural and unnatural *N*-acyl amino acids, with exceptionally high enantioselectivity in almost all cases. Moreover, their enantioselectivity is exceptionally good with most substrates. A general paper on this subject has been published (106) in which the resolution of over 50 *N*-acyl amino acids and analogues is described. Also reported are the stabilities of the enzymes and the effect of different acyl groups on the rate and selectivity of enzymatic hydrolysis. Some of the substrates that are easily resolved on 10–100 g scale are presented in Figure 4 (106). Lipases are also used for the resolution of *N*-acylated amino acids but the rates and optical purities are usually low (107).

Amino alcohols can be resolved by a number of pathways including hydrolysis, esterification, and transesterification. For example, hydrolysis of *N,O*-diacetyl-2-amino-1-butanol with PPL followed by recrystallization results in **80a** with 95% *ee* (108). Hydrolysis of racemic acetates or butyrates of 2-[(alkoxycarbonyl) amino]-1-alkanols with PFL gives (*R*)-alcohol (**81**) with 95% *ee* (109). (*S*)-(**81**) can be obtained by transesterification of the racemic (**81**) with ethyl acetate which also serves as the reaction medium (109).

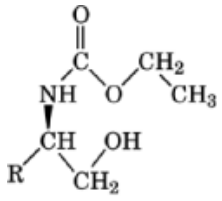


(**80a**)



(82) (83) (84) (85) (86) (87)

Fig. 5. Enzymatic resolution of amino acids by ring-opening reaction.



(81)

Unprotected racemic amines can be resolved by enantioselective acylations with activated esters (110, 111). This approach is based on the discovery that enantioselectivity of some enzymes strongly depends on the nature of the reaction medium. For example, the enantioselectivity factor (defined as the ratio of the initial rates for (*S*)- and (*R*)-isomers) of subtilisin in the acylation of α-methyl-benzylamine with trifluoroethyl butyrate varies from 0.95 in toluene to 7.7 in 3-methyl-3-pentanol (110). The latter solvent has been used for enantioselective resolutions of a number of racemic amines (110).

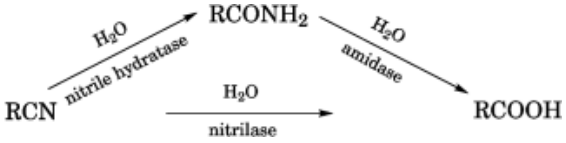
Two interesting approaches for resolution of racemic amino acids have been reported (112, 113). Both are based on enantioselective ring opening with *in situ* racemization of the nonreactive isomer (Fig. 5). The enantioselective hydrolysis of oxazolidinone **82** by cells of *Pseudomonas testeroni*, coupled with *Bacillus subtilis*-catalyzed racemization of unreacted substrate, results in the production of L-serine [56-45-1] **84**. A more versatile and technically simpler system is based on the enantioselective esterification of azlactones **85** that undergo ring opening with various nucleophiles, including alcohols, and racemization of the unreacted isomer. These two procedures allow, at least in principle, a quantitative conversion of a racemic azlactone to a single isomer of the product, thus overcoming the usual problem of 50% maximum yield of kinetic resolutions.

1.8. Hydrolysis of Nitriles

The chemical hydrolysis of nitriles to acids takes place only under strong acidic or basic conditions and may be accompanied by formation of unwanted and sometimes toxic by-products. Enzymatic hydrolysis of nitriles by

nitrile hydratases, nitrilases, and amidases is often advantageous since amides or acids can be produced under very mild conditions and in a stereo- or regioselective manner (114, 115).

There are two distinct classes of enzymes that hydrolyze nitriles. Nitrilases (EC 3.5.5.1) hydrolyze nitriles directly to corresponding acids and ammonia without forming the amide. In fact, amides are not substrates for these enzymes. Nitriles also may be first hydrated by nitrile hydratases to yield amides which are then converted to carboxylic acid with amidases. This is a two-enzyme process, in which enantioselectivity is generally exhibited by the amidase, rather than the hydratase.



The hydrolysis of nitriles can be carried out with either isolated enzymes or immobilized cells. For example, resting cells of *P. chlororaphis* can accumulate up to 400 g/L of acrylamide in 8 h, provided acrylonitrile is added gradually to avoid nitrile hydratase inhibition (116). The degree of acrylonitrile conversion to acrylamide is 99% without any formation of acrylic acid. Because of its high efficiency the process has been commercialized and currently is used by Nitto Chemical Industry Co. on a multithousand ton scale.

A crude mixture of enzymes isolated from *Rhodococcus* sp. is used for selective hydrolysis of aromatic and aliphatic nitriles and dinitriles (117). Nitrilase accepts a wide range of substrates (Table 8). Even though many of them have low solubility in water, such as (**88**), the yields are in the range of 90%. Carboxylic esters are not susceptible to the hydrolysis by the enzyme so that only the cyano group of (**89**) is hydrolyzed. This mode of selectivity is opposite to that observed upon the chemical hydrolysis: at alkaline pH, esters are more labile than nitriles. Dinitriles **90,91** can be hydrolyzed regioselectively resulting in cyanoacids in 71–91% yield. Hydrolysis of (**92**) proceeds via the formation of racemic amide which is then hydrolyzed to the acid in 95% *ee* (118). Prochiral 3-substituted glutaronitriles (**93**) are hydrolyzed by *Rhodococcus butanica* in up to 71% yield with excellent selectivity (119).

1.9. Peptide Synthesis

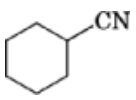
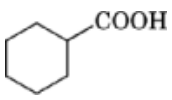
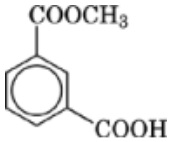
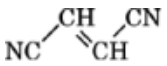
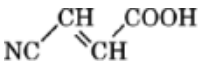
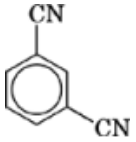
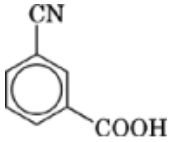
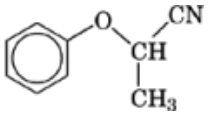
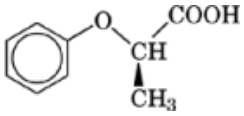
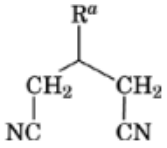
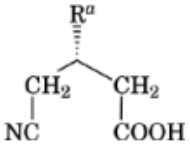
The literature on the enzymatic synthesis of peptides is enormous (120–124). Here the basic principles that govern peptide synthesis are illustrated and recent trends in this area reviewed.

Despite significant progress in the chemical synthesis of peptides and proteins by both liquid- and solid-phase methodologies, a number of shortcomings still exist. The principal limitation of chemical methods stems from the formation of by-products at each individual condensation step that accumulate during the course of the repeated reactions. Moreover, even the small amount of racemization that often occurs in the presence of highly activated coupling reagents reduces the purity of final product and complicates purification dramatically. In this respect the use of enzymes for amino acid or peptide coupling has a number of advantages. Mild reaction conditions and the excellent stereo- and regioselectivity of enzymes require only minimal protection, precludes racemization, and guarantees the structural fidelity of the product.

There are two basic strategies for enzyme-catalyzed peptide synthesis: equilibrium- and kinetically controlled synthesis. The former is the direct reversal of proteolysis and involves the condensation of an amino component with unactivated carboxyl component. The latter proceeds by the aminolysis of an activated peptide ester.

$$\mathrm{R{-}COOH + H_2N{-}R' \rightleftharpoons R{-}CO{-}NH{-}R' + H_2O}$$

Table 8. Enzymatic Hydrolysis of Nitriles

Substrate	Product	Yield, %	*ee*, %	References
		91		117
		92		117
		71		117
		91		117
		47	95	118
		68–71	90–99	119

[a] [a]

[a] R = $OCH_2C_6H_5$, $OCOC_6H_5$.

$$R—COY + H_2N—R' \rightharpoonup R—CO—NH—R' + HY$$

For the equilibrium-controlled enzyme-catalyzed peptide synthesis the equilibrium position lies far over in the direction of the hydrolysis, and under physiological conditions, the product yield is negligible. The equilibrium position is determined exclusively by thermodynamic factors and like any other catalysts the enzymes only accelerate the attainment of the equilibrium.

In order to obtain a reasonable product yield, a number of approaches have been developed to shift the equilibrium toward condensation. The addition of organic cosolvents, for example, lowers the dielectric constant of the reaction medium, thus reducing the pK difference between α-carboxyl and α-amino groups. Since the predominant contribution to the energetic barrier of peptide condensation is the energy required for proton transfer, this decrease in ΔpK lowers the free energy (ΔG_{syn}) of the reaction (125). ΔG_{syn} can also be minimized by carrying out the reaction at a thermodynamically optimal pH value. It can be shown (121) that pH_{opt} depends on pK of the carboxyl (pK_1) and the amino (pK_2) component and obeys the following equation: $pH_{opt} = 0.5(pK_1 + pK_2)$. Ionic equilibrium can also be perturbed in favor of peptide synthesis by elevation of the temperature.

The most frequently used technique to shift the equilibrium toward peptide synthesis is based on differences in solubility of starting materials and products. Introduction of suitable apolar protective groups or increase of ionic strength decreases the product solubility to an extent that often allows nearly quantitative conversions. Another solubility-controlled technique is based on introduction of a water-immiscible solvent to give a two-phase system. Products preferentially partition away from the reaction medium thereby shifting the equilibrium toward peptide synthesis.

Although equilibrium-controlled peptide synthesis has been successfully used on a number of occasions, including thermolysin-catalyzed synthesis of aspartame (126) and semisynthesis of insulin (127), the method has a significant drawback; a water-miscible organic cosolvent added to the reaction medium to suppress the ionization of unactivated carboxy components significantly reduces the reaction rate.

Kinetically controlled enzymatic coupling is considered to be more efficient despite the fact that hydrolysis of the growing polypeptide chain may reduce the yield of desired product. A number of interesting approaches have appeared recently that overcome the problem of secondary hydrolysis and increase product yield and purity. They include the use of high concentrations of specific water-miscible organic cosolvents that inhibit amidase activity to a much higher degree than esterase activity (128), the use of proteases and lipases in nonaqueous solvents (129–132), the use of chemically modified proteases with suppressed amidase activity (133, 134), and the use of site-specific mutagenesis for design of stable protease mutants that are active at high concentrations of organic cosolvents (135). Several examples of these methodologies are presented in Table 9.

Hydrolysis of esters and amides by enzymes that form acyl enzyme intermediates is similar in mechanism but different in rate-limiting steps. Whereas formation of the acyl enzyme intermediate is a rate-limiting step for amide hydrolysis, it is the deacylation step that determines the rate of ester hydrolysis. This difference allows elimination of the undesirable amidase activity that is responsible for secondary hydrolysis without affecting the rate of synthesis. Addition of an appropriate cosolvent such as acetonitrile, DMF, or dioxane can selectively eliminate undesirable amidase activity (128).

The first two entries in Table 9 illustrate the above principle. Both tripeptide Boc—Cys(SBzl)—D–Val—OBzl and dipeptide Z—Tyr—D–Arg—OMe are enkephalin precursors and can be synthesized in 72% yield, with no secondary hydrolysis observed during the course of the reaction. An alternative way to inhibit amidase activity is based on modifications of residues that are directly involved in catalytic steps, such as chymotrypsin methylated at $N^{\epsilon 2}$ of histidine 57, which is inert toward amide substrates. This modified enzyme has been used as a catalyst for the synthesis of a number of peptides, including Z—L–Phe—L–Leu—NH_2 in high yield (133). Similarly, the Ser residue of the active site of subtilisin BNP′ has been converted to dehydroalanine (134). The resulting anhydrosubtilisin was found to be a useful catalyst for the condensation of peptide fragments. Z—L–Phe—Gly—Gly— NH_2 was synthesized from the corresponding fragments (Table 9) in 90% yield.

Another useful strategy that helps to eliminate hydrolysis and also shift the thermodynamic equilibrium toward peptide-bond formation is the use of nonaqueous systems (Table 9, entries 5–10). Remarkably, not only proteases such as thermolysin [9073-78-3], subtilisin, and chymotrypsin but also lipases (porcine pancreatic lipase) are capable of catalyzing the formation of peptide bonds under these conditions. The use of lipases may

Table 9. Enzyme-Catalyzed Synthesis of Peptides[a]

Entry	Carboxy terminal	Amino terminal	Product	Enzyme[b]	Solvent	Yield, %	Refs.
1	Boc—Cys(SBzl)—OMe	D–Val—OBzl	Boc—Cys(SBzl)D–Val – OBzl	CT	50% DMSO/water	72	128
2	Z—Tyr—OMe	D–Arg—OMe	Z—Tyr—D–Arg—OMe	CT	50% CH_3CN/water	72	128
3	Z—L–Phe—OCH_2CN	L–Leu—NH_2	Z—L–Phe—L–Leu—NH_2	Me–CT	50% DMSO/water	88	133
4	Z—L–Phe—C_6H_4Cl	Gly—Gly—NH_2	Z—L–Phe—Gly—Gly—NH_2	anhydrosubtilisin	60% DMF/water	90	134
5	Z—Gly—Pro—Phe—Pro—Leu	Leu—NH_2	Z—Gly—Pro—Phe—Pro—Leu—Leu—NH_2	thermolysin	*tert*-amyl alcohol, 9% formamide	73	130
6	Z—Leu—Leu—OMe	Phe—Leu—$O(CH_3)_3$	Z—Leu—Leu—Phe—Leu—$OC(CH)_3)_3$	subtilisin BNP′	acetonitrile	95	136
7	Bzl—Tyr—OC_2H_5	L–Phe—NH_2	Bzl—Tyr—Phe—NH_2	CT	1,1,1-tri-chloroethane	95–98	132
8	N – Ac—L–Phe—OC_2H_4Cl	L–Leu—NH_2	N – Ac—L–Phe—L–Leu—NH_2	PPL	toluene	83	129
9	N – Ac—L–Tyr—OC_2H_4Cl	L–Leu—NH_2	N – Ac—L–Tyr—L–Leu—NH_2	PPL	tetrahydrofuran	76	129
10	N – F—D–Ala—OC_2H_4Cl	L–Phe—NH_2	N – F—D–Ala—L–Phe—NH_2	subtilisin	*tert*-amyl alcohol	82	131

[a] Boc = *t*–butyloxycarbonyl; Bzl = benzyl; Me = methyl; Z = carbobenzoxy; N–Ac = N–acyl and –F = –formyl.
[b] CT = α–chymotrypsin; PPL = porcine pancreatic lipase; and Me – CT = chymotrypsin methylated at $N^{\epsilon 2}$ of His 57.

be advantageous in some instances since, in contrast to proteases, they are unable to catalyze the secondary hydrolysis of amide bonds. Another payoff of conducting peptide condensation in nonaqueous media stems from the fact that stereospecificity of enzymes in organic solvents can be significantly altered (137). As a result, peptides containing D-amino acids can be synthesized efficiently under nonaqueous environments (131). An often-cited drawback of the use of organic solvents is that they reduce the enzymatic activity.

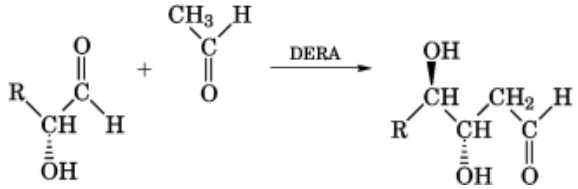
2. Lyases

2.1. Aldol Additions

These reactions catalyzed by lyases are perhaps the most synthetically useful enzymatic reactions for carbon–carbon bond formation. Because of the broad synthetic utility of this method, the enzymatic aldol reactions have received considerable attention in recent years and have been extensively covered in a number of books and reviews (10, 138–140).

There are two distinct groups of aldolases. Type I aldolases, found in higher plants and animals, require no metal cofactor and catalyze aldol addition via Schiff base formation between the lysine ϵ-amino group of the enzyme and a carbonyl group of the substrate. Class II aldolases are found primarily in microorganisms and utilize a divalent zinc to activate the electrophilic component of the reaction. The most studied aldolases are fructose-1,6-diphosphate (FDP) enzymes from rabbit muscle, rabbit muscle adolase (RAMA), and a Zn^{2+}-containing aldolase from *E. coli*. *In vivo* these enzymes catalyze the reversible reaction of D-glyceraldehyde-3-phosphate [591-57-1] (G-3-P) and dihydroxyacetone phosphate [57-04-5] (DHAP). Although their requirement for G-3-P is strict, the enzymes accept a wide range of aldehydes as electrophiles (Fig. 6). The stereospecificity of the reaction is absolute; the configuration of the vicinal diols at the newly formed C-3–C-4 is always D-threo. A great variety of aldol additions have been carried out, allowing the synthesis of numerous nitrogen-containing sugars, deoxysugars, and fluorosugars, based, among others, on D-threose, L-threose, D-ribose, L-arabinose, D-xylose, D-glucose-6-P, D-galactose-6-P, and D-mannose-6-P, where P = phosphate. Some other examples of **94** are (**a–j**).

Deoxyribose-5-phosphate aldolase (DERA) and 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPG) are also useful for asymmetric aldol additions. DERA, the only aldolase that utilizes aldehydes as the nucleophile, is capable of catalyzing the synthesis of a wide range of products. In addition to its natural substrate, acetaldehyde, it accepts a number of analogues. When acetaldehyde [75-07-0], acetone [67-64-1], or fluoroacetone are used as substrates, a new stereogenic center with 3(*S*)-configuration is formed. The reaction with propanal [123-38-6] results in two new stereogenic centers with 2(*R*)-and 3(*S*)-configurations (147).



KDPG is a member of a yet unexplored group of aldolases that utilize pyruvate or phosphoenol pyruvate as the nucleophile in the aldol addition. They are quite tolerant of different electrophilic components and accept a large number of unnatural aldehydes (148). The reaction itself, however, is quite specific, generating a new stereogenic center at the C-4 position.

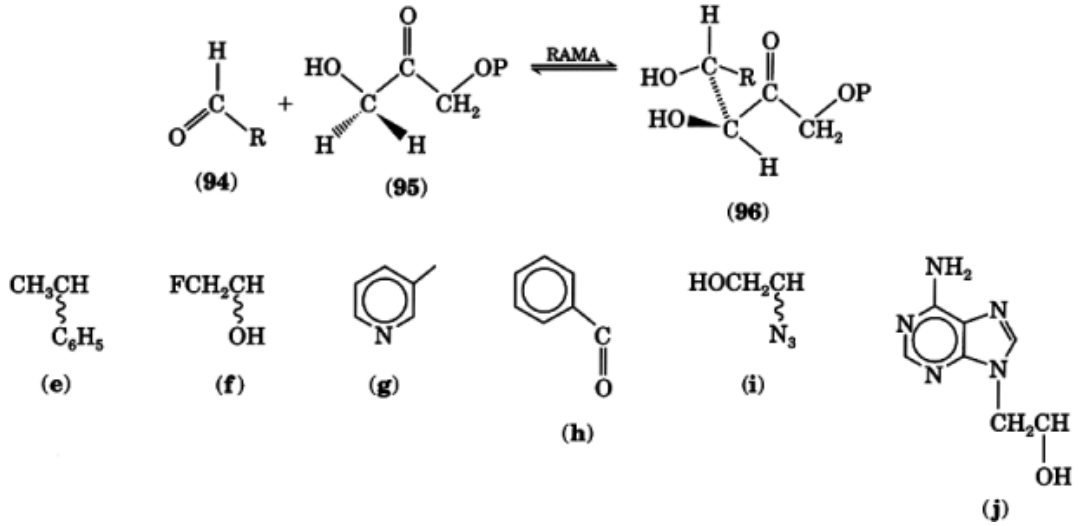


Fig. 6. FDP-aldolase-catalyzed addition of electrophiles (**94**) with DHAP (139–146). Representative R groups in (**94**) are given as (**a–j**): (**a**) methyl, CH_3; (**b**) azidomethyl, N_3CH_2; (**c**) benzyloxymethyl, $C_6H_5CH_2OCH_2$; (**d**) carboxaldehyde, CHO.

Transketolases (TK) are widely used in organic synthesis to extend the chain of an acceptor aldose by two carbon units.

The TK-catalyzed reaction requires the presence of thiamine pyrophosphate and Mg^{2+} as cofactors. Although the substrate specificity of the enzyme has not been thoroughly investigated, it has been shown that the enzyme accepts a wide variety of 2-hydroxyaldehydes including D-glyceraldehyde 3-phosphate [591-57-1], D-glyceraldehyde [453-17-8], D-ribose 5-phosphate [4300-28-1], D-erythrose 4-phosphate [585-18-2], and D-erythrose [583-50-6] (139, 149–151). Most substrates, with the exception of hydroxypyruvate, have a threo configuration of hydroxyl groups at the C-3 and C-4 positions (139). The new stereocenter formed in TK-catalyzed addition is formed in the threo configuration with high diastereo-selectivity (151). Using TK-catalyzed condensations of hydroxypyruvic acid with a number of aldehydes a practical preparative synthesis of L-idose [5934-56-5], L-gulose [6027-89-0], 2-deoxy-L-xylohexose, and L-xylose [609-06-3] has been carried out (151).

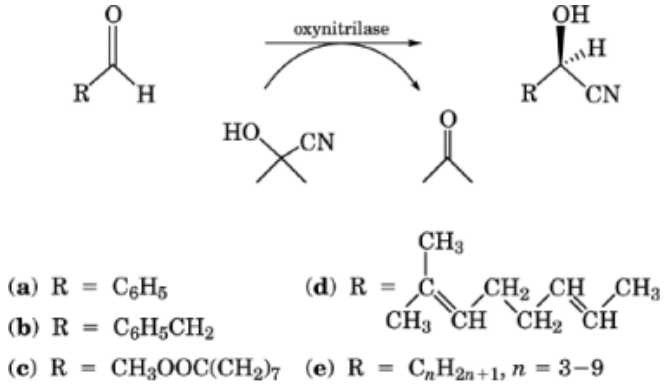


Fig. 7. Cyanohydrin formation.

Table 10. Synthesis of Cyanohydrins by Oxynitrilase-Catalyzed Transacylation

Substrate[a]	Yield, %	*ee*, %	References
(**97a**)	72–95	92–98	(157, 158)
(**97b**)	83	88	158
(**97c**)	68	97	158
(**97d**)	46	99	158
(**97e**)	24–100[b]	63–97	157

[a]See Figure 7.
[b]Percentage conversion.

2.2. Cyanohydrin Synthesis

Another synthetically useful enzyme that catalyzes carbon–carbon bond formation is oxynitrilase (EC 4.1.2.10). This enzyme catalyzes the addition of cyanides to various aldehydes that may come either in the form of hydrogen cyanide or acetone cyanohydrin (152–158) (Fig. 7). The reaction constitutes a convenient route for the preparation of α-hydroxy acids and β-amino alcohols. Acetone cyanohydrin [75-86-5] can also be used as the cyanide carrier, and is considered to be superior since it does not involve hazardous gaseous HCN and also virtually eliminates the spontaneous nonenzymatic reaction. (*R*)-oxynitrilase accepts aromatic **97a,b**, straight-**97c,e**, and branched-chain aliphatic aldehydes, converting them to (*R*)-cyanohydrins in very good yields and high enantiomeric purity (Table 10).

The application of (*S*)-oxynitrilase has been reported only recently (159). The enzyme isolated from shoots of *Sorghum bicolor* catalyzes the condensation between various 3- and 4-substituted benzaldehydes and hydrogen cyanide resulting in (*S*)-cyanohydrins in 80–90% yield and up to 99% *ee*.

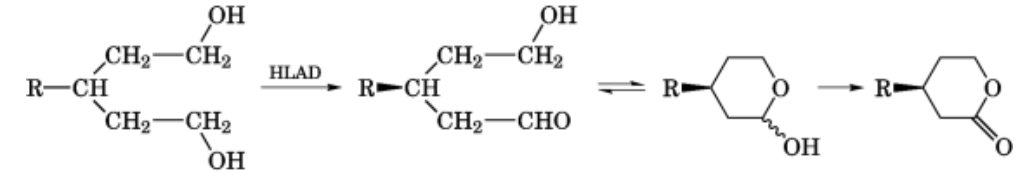
3. Oxidoreductases

Biocatalytic redox reactions offer great synthetic utility to organic chemists. The majority of oxidase-catalyzed preparative bioconversions are still performed using a whole-cell technique, despite the fact that the presence of more than one oxidoreductase in cells often leads to product degradation and lower selectivity. Fortunately, several efficient cofactor regeneration systems have been developed (160), making some cell-free enzymatic bioconversions economically feasible (161, 162).

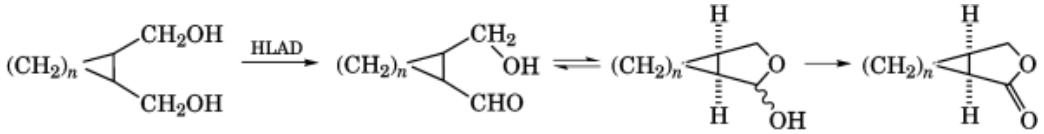
The two oxidoreductase systems most frequently used for preparation of chiral synthons include baker's yeast and horse liver alcohol dehydrogenase (HLAD). The use of baker's yeast has been recently reviewed in great detail (6, 163) and therefore will not be covered here. The emphasis here is on dehydrogenase-catalyzed oxidation and reduction of alcohols, ketones, and keto acid, oxidations at unsaturated carbon, and Bayer-Villiger oxidations.

3.1. Chiral Alcohols and Lactones

HLAD has been widely used for stereoselective oxidations of a variety of prochiral diols to lactones on a preparative scale. In most cases pro-(*S*) hydroxyl is oxidized irrespective of the substituents. The method is applicable among others to *cis*-1,2-bis(hydroxymethyl) derivatives of cyclopropane, cyclobutane, cyclohexane, and cyclohexene. Resulting γ-lactones are isolated in 68–90% yields and of 100% *ee* (164, 165).



(**98**) (**99**)

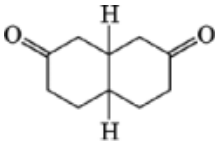
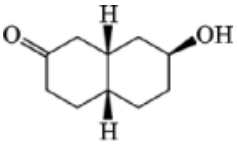
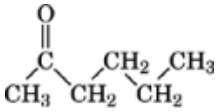
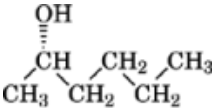
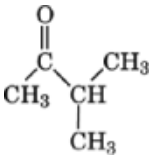
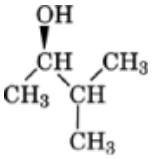
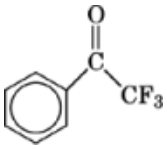
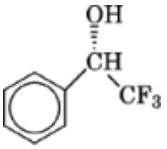
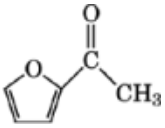
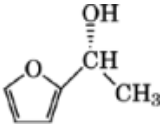
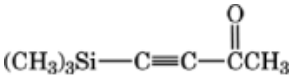
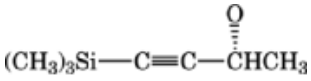
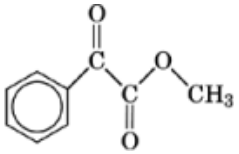
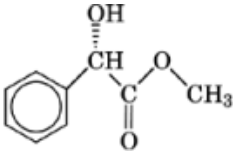
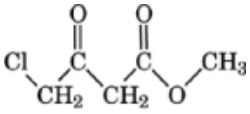
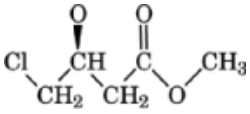


(**100**) $n = 1$–4 (**101**)

Although alcohol dehydrogenases (ADH) also catalyze the oxidation of aldehydes to the corresponding acids, the rate of this reaction is significantly lower. The systems that combine ADH and aldehyde dehydrogenases (EC 1.2.1.5) (AldDH) are much more efficient. For example, HLAD catalyzes the enantioselective oxidation of a number of racemic 1,2-diols to L-α-hydroxy aldehydes which are further converted to L-α-hydroxy acids by AldDH (166).

Alcohol dehydrogenase-catalyzed reduction of ketones is a convenient method for the production of chiral alcohols. HLAD, the most thoroughly studied enzyme, has a broad substrate specificity and accommodates a variety of substrates (Table 11). It efficiently reduces all simple four- to nine-membered cyclic ketones and also symmetrical and racemic cis- and trans-decalindiones (167). Asymmetric reduction of aliphatic acyclic ketones (C-4–C-10) **103,104** can be efficiently achieved by alcohol dehydrogenase isolated from *Thermoanaerobium brockii* (TBADH) (168). The enzyme is remarkably stable at temperatures up to 85°C and exhibits high tolerance toward organic solvents. Alcohol dehydrogenases from horse liver and *T. brockii* transfer pro-(*R*)-hydride to the *re* face of the carbonyl group to give (*S*)-alcohols, a process which can be described by Prelog's rule (171). In contrast alcohol dehydrogenase from *L. kefir* (LKADH) and *Pseudomonas* sp. (PEDADH) exhibit anti-Prelog specificity and transfer pro-(*R*)-hydride to the *si* face of carbonyl compounds to yield (*R*)-alcohols (169, 170). Both enzymes exhibit broad substrate specificity and high enantioselectivity for the synthesis of chiral aromatic **105,108**, cyclic **106**, aliphatic **109**, and trimethylsilyl-protected terminal alkyne **107** ketones.

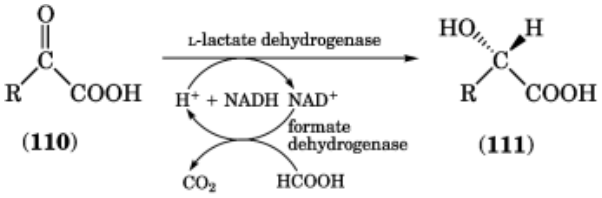
Table 11. Alcohol Dehydrogenase-Catalyzed Reductions on Ketones

Substrate	Product	Enzyme	Yield, %	*ee*, %	Refs.
		HLAD	89	>98	167
		TBADH	50[a]	96	168
		TBADH	50[a]	86	168
		LKADH	71	>99	169
		LKADH	65	65	169
		LKADH	25	94	169
		PSADH	79	98	170
		PSADH	76	98	170

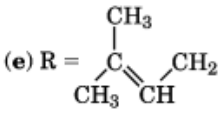
[a]Stopped at 50% conversion.

3.2. Hydroxy and Amino Acids

Reduction of 2-oxoacids with NADH, catalyzed by lactate dehydrogenase [9001-60-9], is an established method for the preparation of homochiral α-hydroxy acids of both S and R configurations (172–174). The enzyme is found in all higher organisms and can be easily isolated from a variety of mammalian (172) and bacterial (173, 10) sources. The reduction of **110a–d**, where **a** = glyoxylic acid [298-12-4] **b** = pyruvic acid [127-17-3], **c** = α-ketobutyric acid [600-18-0], and **d** = 4-hydroxy-2-oxobutanoic acid [22136-38-5]), proceeds with excellent yield (97–99%) and selectively ($ee > 99\%$). Although reduction of β,γ-unsaturated substrates is relatively slow, it provides synthetically useful functionalized allylic alcohols with high optical purity.



(**a**) R = H
(**b**) R = CH_3
(**c**) R = CH_3CH_2
(**d**) R = $HOCH_2CH_2$



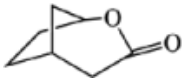
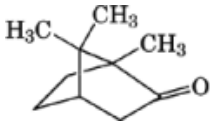
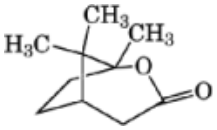
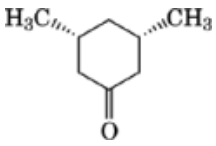
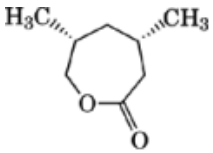
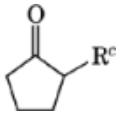
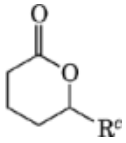
There are several amino acid dehydrogenases that catalyze reductive amination of oxo acids to L-amino acids in nearly quantitative yield (161). The enzymes accept a variety of 2-keto acids and are useful for synthesis of unnatural amino acids. For example, leucine dehydrogenase was used for the synthesis of 2-amino-3,3-dimethylbutanoic acid (175) and phenylalanine dehydrogenase was used for synthesis of L-2-amino-4-phenylbutanoic acid (176), a precursor of angiotensin-converting enzyme inhibitors (qv). In most cases regeneration of NADH was accomplished with formate–formate dehydrogenase or glucose–glucose dehydrogenase systems.

3.3. Baeyer-Villiger Oxidations

The Baeyer-Villiger reaction is a useful transformation in organic synthesis providing a mild technique for converting ketones into esters or lactones. The biological equivalent of this transformation is a common reaction that occurs during degradation of steroids, aldehydes, and cyclic ketones. The enzyme that catalyzes the insertion of an oxygen atom is classified as a monooxygenase and uses flavin to facilitate the passage of electrons (177). A number of these enzymes have been purified to homogeneity and characterized.

Cyclohexanone oxygenase from *Acinetobacteria* converts a variety of alicyclic ketones into lactones in a regio- and enantioselective manner (Table 12). The reaction can be carried out by a whole-cell process **181** or with the isolated enzyme (178). For example, 2-norbornanone [497-38-1] **112** is converted to the corresponding lactone in 81% yield (178). The enzyme, however, is not selective: both enantiomers react equally well. The oxidation rate of camphor [76-22-2] **113**, is about one-third that of **112**; nevertheless, given sufficient amount of time, the product yield reaches 89%. Substituted cylohexanones **114** and cyclopentanones **115** are converted into the corresponding lactones in moderate to good yield and selectivity (179–181).

Table 12. Baeyer-Villiger Oxidation of Ketones

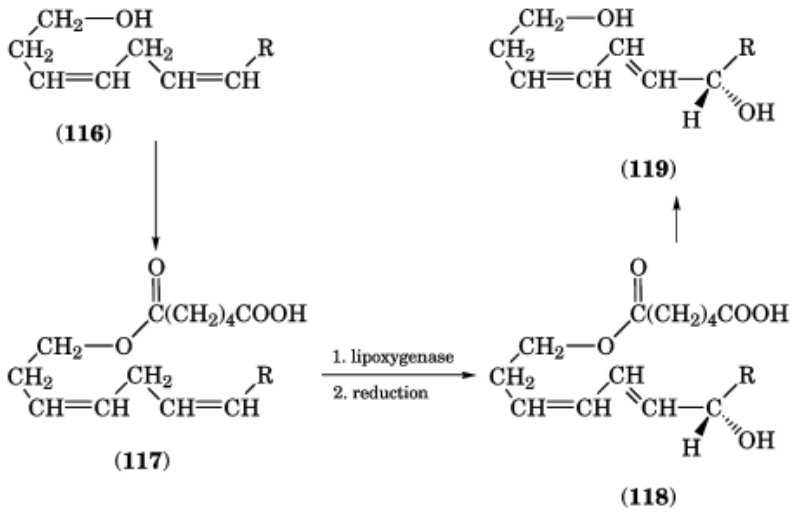
	Substrate	Product	Yield, %	References
(112)			81	178
(113)			89	178
(114)[a]			73–82	(179, 180)
(15)[b]	 [c]	 [c]	20–65 25–95% *ee*	181

[a] *cis*-3,5-Dimethylcyclohexanone [7214-52-0].
[b] 2-Heptylcyclopentanone [137-03-1], 2-nonylcyclopentanone [40566-27-6].
[c] R = C_7H_{15}, C_9H_{19}, and $C_{11}H_{23}$

3.4. Lipoxygenase-Catalyzed Oxidations

Lipoxygenase-1 catalyzes the incorporation of dioxygen into polyunsaturated fatty acids possessing a 1(*Z*),4(*Z*)-pentadienyl moiety to yield (*E*),(*Z*)-conjugated hydroperoxides. A highly active preparation of the enzyme from soybean is commercially available in purified form. From a practical standpoint it is important to mention that the substrate does not need to be in solution to undergo the oxidation. Indeed, the treatment of 28 g/L of linoleic acid [60-33-3] with 2 mg of the enzyme results in (13*S*)-hydroperoxide of linoleic acid in 80% yield and 95% *ee* (182).

Another application of lipoxygenase (183) is the hydroxylation of unsaturated alcohols (**116**) via their adipate monoesters (**117**) where R is $(CH_2)_4CH_3$, $CH_2CH(CH_3)_2$, $CH_2C_6H_5$, $(CH_2)_3C_6H^5$, or $(CH_2)_3C(O)CH_3$.



(116) (117) (118) (119)

Adipoyl moiety is first attached in order to provide an appropriately spaced proximal carbonyl group. The esters are oxidized enzymatically and then deacylated. The procedure results in the synthesis of diols (**119**) with excellent enantiomeric purity (*ee* 96–98%) in 72–92% yield.

4. Summary

The area of biotransformations has experienced a phenomenal growth in recent years as judged by the number of publications. In addition to the fermentation technology that has been utilized for years for the production of molecules varying from ethanol and vitamins to amino acids and steroids, the practical use of isolated enzymes as catalysts in organic synthesis has started to achieve great prominence. This phenomenon has been brought about by a number of factors. In response to an ever-growing demand for new biocatalysts, more enzymes have become available in recent years. Such primary suppliers as Amano, Genencor, Novo, Meito Sangyo, Toyo Jozo, Rhône-Poulenc, Serva, Nagase, Tanabe, Boehringer-Mannheim, and others now trade dozens of enzymes, many in ton quantities. Moreover, advances in molecular biology and genetic engineering have resulted in new enzymes with altered specificity and increased stability. For example, highly stable subtilisin mutants with an increased propensity for peptide condensation (184) have been constructed and successfully used for peptide synthesis (135). A modified lactate dehydrogenase that is specific for new substrates and lacks allosteric regulation (185) has been used for enantioselective reductions (173).

Impressive developments in the area of immunology have culminated in the development of catalytic antibodies (186, 187). These synthetic biocatalysts that have the potential to catalyze virtually any type of reaction with unsurpassed selectivity have great promise in the future.

Perhaps the biggest impact on the practical utilization of enzymes has been the development of nonaqueous enzymology (11, 16, 33, 35). The use of enzymes in nonaqueous media greatly expands the scope of suitable transformations, simplifies their use, and enhances stability. It also provides an easy means of regulation of the substrate specificity and regio- and enantioselectivity of enzymes by changing the reaction medium.

It is apparent that the use of enzymatic catalysis continues to grow Greater availability of enzymes, development of new methodologies for their utilization, investigation of enzymatic behavior in nonconventional environments, and the design and synthesis of new biocatalysts with altered selectivity and increased stability are essential for the successful development of this field. As more is learned about selectivity of enzymes toward

unnatural substrates, the choice of an enzyme for a particular transformation will become easier to predict. It should simplify a search for an appropriate catalyst and help to establish biocatalytic procedures as a useful supplement to classical organic synthesis.

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