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Enzymatic synthesis of organophosphorus compounds

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Abstract. Data on biocatalytic methods for the preparation of chiral organophosphorus compounds are generalized and described systematically. Various examples of enzymatic and microbiological synthesis of hydroxyphosphonates, aminophosphonates, phosphinites, phosphine oxides and tertiary phosphines are discussed. The bibliography includes 154 references.

I. Introduction

Enzyme catalysis (biocatalysis) has found increasing number of applications in organic chemistry including the synthesis of optically active compounds.[1–11] For example, the synthesis in the presence of lipases results in chiral compounds of different structure not only on the laboratory, but also on the industrial scale.[8] A great advantage of biocatalysts is that they can be found for almost all types of organic reactions.

There are two approaches to the use of bio-reagents in organic synthesis. One employs enzymes isolated from living organisms, while the other uses microorganisms that reproduce in the reaction medium and excrete enzymes into it. Recently, the former approach has been more widely used because (i) the isolated enzymes are more convenient for practical applications and (ii) such catalysts have become commercially available.

Whereas 20 – 30 years ago enzymes were used only in the synthesis of C-, H-, N- and O-containing compounds, now they are used to obtain organoelement compounds as well, including organophosphorus compounds (OPC).[12–15] Research in the field of biocatalysis began with the synthesis of optically active hydroxyphosphonates which was followed by the synthesis of α- and β-aminophosphonates, phosphinous acid derivatives, tertiary phosphines and phosphine oxides. Chiral OPC containing the phosphorus atom as a stereogenic centre are used in various fields of science and industry including biology, pharmacology, asymmetric catalysis, *etc*.[12]

Hammerschmidt[16] pioneered in 1988 in the systematic research on the biosynthesis of compounds with C–P bonds. At the same time, Natchev[17] reported the use of biocatalysis in the synthesis of some phosphorus-containing analogues of L-amino acids and peptides (in particular, the enzymatic synthesis of D- and L-phosphinothricin (2-amino-4-hydroxymethylphosphinylbutanoic acid, Pht) and a natural tripeptide antibiotic bialaphos (L-Pht-Ala-Ala). Information on enzymatic and microbiological resolution of racemic phosphonic acids can be found in the patents[18,19] issued in the early 1980s.

Currently, biocatalysis is one of the most convenient methods of synthesis of chiral organophosphorus compounds. Research in this field progresses intensively and a large array of experimental data has been accumulated. The results obtained seem to be quite interesting and require generalization; this is the reason for the present review to appear. It should be noted that, in spite of many reviews and monographs devoted to biocatalysis applications in organic chemistry (see, *e.g.*, Refs 1 – 11), analogous reviews concerning the chemistry of organoelement compounds are absent. In this review we systematize and analyze for the first time the data on biocatalytic methods of synthesis of chiral OPC. Earlier, a number of reviews generalizing the results obtained in original studies were published. Among them, mention may be made of the reviews by Mikołajczyk and Kielbasiński[13,14] as well as by Kafarski and co-workers.[15]

II. Enzymatic synthesis of organophosphorus compounds

A major problem in carrying out enzymatic reactions involving OPC is poor solubility of most of the starting compounds in water. In addition, many OPC are unstable in aqueous solutions. Therefore, chiral OPC are usually synthesized in organic solvents.[7,20,21]

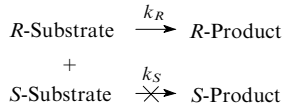
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Enzymes can be introduced into organic media in the freeze-dried form, in the immobilized form on solid supports (ceramics or organic polymers) or by dispersion (*via* the formation of covalent enzyme–polymer complexes or microemulsions with surfactants).[22,23] Many lipases retain their activity in organic solvents, which allows them to be used in the synthesis of chiral OPC.[24]

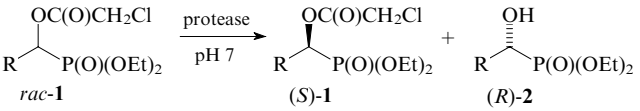
1. Kinetic resolution of hydroxyphosphonates

Usually, the resolution of functionalized racemic phosphonates into enantiomers using biocatalysts is conducted under kinetic control.[24,25] The kinetic resolution is possible if reactions of the *R*- and *S*-enantiomers with chiral reagents proceed at different rates, *e.g.*:



Ideally, if only the *R*-enantiomer enters into reaction ($k_S = 0$), the resulting mixture contains the unreacted starting compound (50%) and the product obtained from the *R*-enantiomer (50%). This method allows both optical isomers to be resolved with ease using a single enzyme.

For example, hydrolysis of acylated racemic hydroxyphosphonate **1** in the presence of Chirazyme® P-2 protease in phosphate buffer (pH 7) mainly leads to hydroxyphosphonate (*R*)-**2**, while acyloxyphosphonate (*S*)-**1** remains unreacted. Then, these compounds are chromatographically separated and subsequent hydrolysis of the acyl group results in both optical isomers of the hydroxyphosphonate, namely, (*R*)-**2** and (*S*)-**2**, in enantiopure form.[22]



Protease is Chirazyme® P-2; R = n-C_5H_{11}, n-C_9H_{19}, cyclo-$C_6H_{11}CH_2CH_2$.

Conventional enantioselectivity parameters fail to fully characterize enzymatic reactions because such processes are often reversible. Specific equations describing the enantioselectivity factor (E) of biocatalytic reactions were proposed.[25] They account for the enantiomeric excesses (*ee*) of the product (ee_p) of the biocatalytic reaction and the unreacted substrate (ee_s), as well as the degree of conversion (c).[25] For a reversible enzymatic reaction (K is the equilibrium constant) one has:

$$E = \frac{\ln[1-(1+K)c(1+ee_p)]}{\ln[1-(1+K)c(1-ee_p)]} = \frac{\ln[1-(1+K)(c+ee_s(1-c))]}{\ln[1-(1+K)(c-ee_s(1-c))]}.$$

If the reaction is irreversible, or the reverse reaction is negligible ($K \cong 0$), the equation is simplified:

$$E = \frac{\ln[1-c(1+ee_p)]}{\ln[1-c(1-ee_p)]} = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]},$$

where

$$c = \frac{ee_s}{ee_s+ee_p}.$$

Thus, to calculate E, two of the three variables ee_s, ee_p and c should be measured. For a non-selective reaction, one has $E = 1$; successful resolution of enantiomers requires meeting the condition $E > 20$.[4]

To find out which of the two enantiomers of a racemate undergoes transesterification faster and to predict the absolute configurations of the products, the Kazlauskas rule is used.[26] This simple empiric model is in fact an extended Prelog rule based on the assumption that enantioselectivity is proportional to the size difference between the large (L) and medium-size (M) substituents in the substrate. According to the Kazlauskas rule, these substituents are located in two different pockets of the active site of an enzyme, in accordance with their sizes; this determines the absolute configuration of the product of the enzymatic reaction (Fig. 1).[24–29]

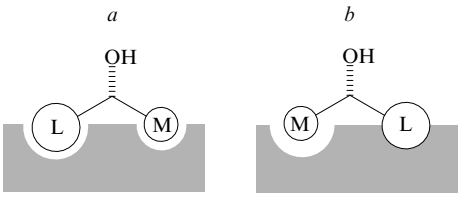


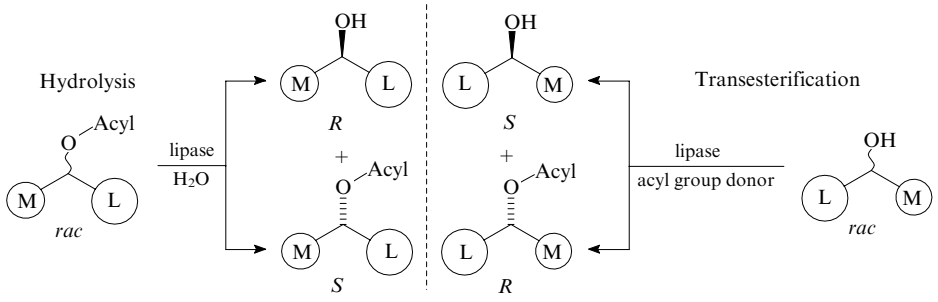
Figure 1. Favourable (*a*) and unfavourable (*b*) arrangement of substituents in enantiomers of hydroxyphosphonates [L = (*R*)-$(RO)_2P(O)(CH_2)_nCH$; $n = 0$], according to the Kazlauskas rule.

In the case of secondary alcohols, enzymatic ester hydrolysis and transesterification reactions can proceed concurrently. Scheme 1 shows the symmetry of both processes in accordance with the Kazlauskas rule.[24]

A similar stereospecifity is also observed for hydroxyphosphonic acid esters. In α-hydroxyphosphonates $(RO)_2P(O)CH(OH)R'$, the phosphonic group is usually taken as the larger substituent, and R′ is the medim-size substituent. In this case, the (*S*)-hydroxyphosphonate undergoes transesterification, whereas (*R*)-hydroxyphosphonate does not enter the reaction. Correspondingly, hydrolysis of the racemic α-acyloxyphosphonate results in (*S*)-hydroxyphosphonate while (*R*)-acyloxophosphonate remains in the mixture.

For β-hydroxyphosphonates $(RO)_2P(O)CH_2CH(OH)R'$, it is taken that L = $(RO)_2P(O)CH_2$ and M = R′. Since in this case the order of priority of substituents at the stereogenic centre changes, the (*R*)-hydroxyphosphonate undergoes transesterification, while the (*S*)-hydroxyphosphonate does not react. Correspondingly, hydrolysis of β-acyloxyphosphonates involves the *R*-enantiomer and gives the (*R*)-hydroxyphosphonate, whereas the (*S*)-acylphosphonate remains unchanged. Such a stereospecifity of the reactions of α- and β-hydroxyphosphonates is observed in most cases, although biocatalytic reactions involving hydroxyphosphonates sometimes proceed contrary to the Kazlauskas rule.[28] Recently, this rule has been widely used as a convenient empirical rule to predict the stereochemistry of the products of enzymatic resolution of secondary alcohols and amines.[27–30]

Scheme 1



a. Resolution of α-hydroxyphosphonates by enzymatic transesterification

Resolution of racemates using lipases is one of the most convenient methods for obtaining enantiopure compounds. In the case of α-hydroxyphosphonates, enzymatic transesterification in organic solvents is used as a rule.[30–39] To attain kinetic resolution, various acyl group donors are introduced into the reaction mixture. These are ethyl acetate, ethyl chloroacetate, ethyl benzoate, *p*-chlorophenyl acetate, vinyl acetate or isopropenyl acetate. Since enzymatic transesterification–hydrolysis reactions are reversible, and the same enzyme can catalyze both the forward and reverse reactions, vinyl acetate and isopropenyl acetate are considered as the best acylating agents. Vinyl acetate is transformed to vinyl alcohol and then to acetaldehyde due to keto–enol tautomerism. Isopropenyl acetate is transformed to acetone and the reaction becomes irreversible.

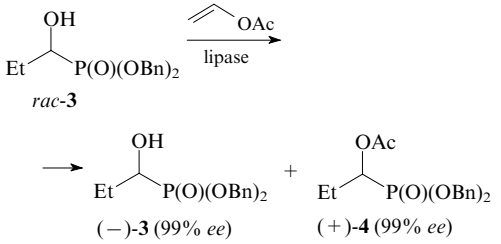
$$R^1OH + MeC(O)OC(R^2){=}CH_2 \xrightarrow[-R^1OC(O)Me]{} [HOC(R^2){=}CH_2] \longrightarrow O{=}C(R^2)Me$$

R^1 = Alk; R^2 = H, Me.

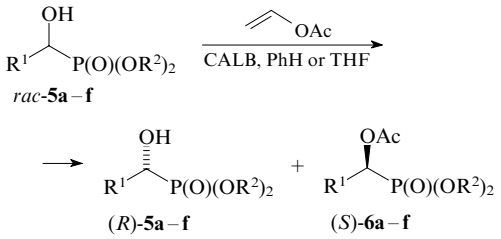
In such cases, the transesterification proceeds much faster compared to the reactions of free caboxylic acids or esters like ethyl acetate.[36]

Unlike asymmetric synthesis, the kinetic separation allows the desired optical isomer to be obtained in 50% yield only because the reaction is conducted until 50% conversion.[21–24] To increase the yield of the target isomer, dynamic kinetic resolution is used (see Section I.3).[30–35]

Kushi *et al.*[37] resolved enantiomers of hydroxyalkylphosphonates in the presence of lipases isolated from the bacteria *Bacillus brevis*, *Pseudomonas fluorescens* and *P. putida*. The reactions were carried out in diisopropyl ether with vinyl acetate as the acyl group donor. Enantioselective transesterification of dibenzyl 1-hydroxypropylphosphonate (**3**) with vinyl acetate catalyzed by *P. fluorescens* resulted in dextrarotatory acyloxophosphonate (+)-**4** and unreacted levorotary hydroxyphosphonate (−)-**3** with 99% *ee* for both products. Enantiopure alcohol (−)-**3** was obtained in 82% yield (calculated with respect to the reacted substrate).



The use of enzyme reactions was greatly simplified when lipase B from *Candida antarctica* (CALB) became commercially available and conditions for reactions involving it were established.[33] For example, acetylation of racemic hydroxyphosphonates **5a**–**f** to corresponding *S*-acetates in the presence of CALB followed by chromatographic separation led to enantiopure alcohols (*R*)-**5a**–**f** and acetates (*S*)-**6a**–**f** in about 50% yields (Table 1).[32] The absolute configurations of the reaction products conformed to the Kazlauskas rule assuming that the phosphonic group plays the role of the substituent L at the asymmetric centre and that the alkyl group plays the role of the substituent M.

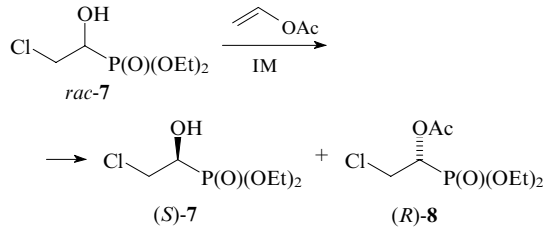


Among the lipases used to resolve diethyl 1-hydroxy-2-chloroethylphosphonate (**7**), the highest stereoselectivity

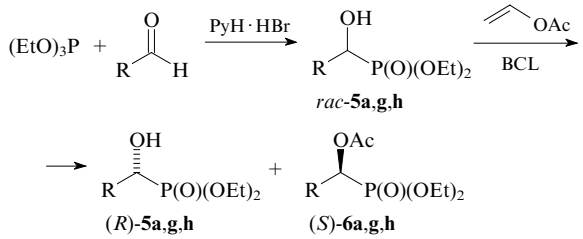
Table 1. Acetylation of hydroxyphosphonates **5a**–**f** in the presence of CALB.[32]

Compound	R	R′	Time /h	(*R*)-**5**		(*S*)-**6**	
				yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	Me	Et	30	43	95	44	95
b	Me	Pr^i	30	42	95	43	95
c	Et	Me	48	50	50.8	31	95
d	Et	Et	50	47	79.7	41	95
e	$CH_2{=}CH$	Et	30	41	95	40	95
f	$CH_2{=}CH$	Pr^i	30	43	95	40	95

was shown by the lipase isolated from *Mucor miehei* (IM), but the transesterification rate was low. In particular, the reaction of hydroxyphosphonate **7** with vinyl acetate in anhydrous diisopropyl ether at 30 °C gives the acylation product (*S*)-**8** in 50% yield after seven days.[40]



Lipase isolated form *Burkholderia cepacia* (BCL) is also an efficient biocatalyst of the resolution of racemic α-hydroxyphosphonate.[38] Thus, in the presence of this lipase, vinyl acetate esterifies only the *S*-enantiomer of hydroxyphosphontes **5a**,**g**,**h**, obtained by reacting triethyl phosphite with aldehydes in the presence of pyridinium bromides.[39] As the result, equimolar mixtures of the corresponding α-acyl phosphonates and hydroxyphosphonates were obtained and then separated by column chromatography.[38]



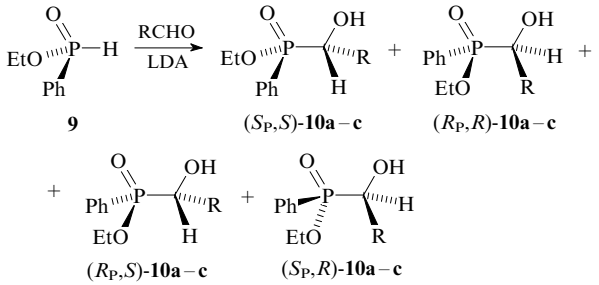
R = Me (**a**), Pr^n (**g**), n-C_5H_{11} (**h**).

It was established that the rate of transesterification and the optical purity of products depend only slightly on the solvent used (THF, toluene, vinyl acetate), but show a strong dependence on the temperature and the amount of the lipase. Raising the temperature to 40 and 60 °C caused the reaction rate to increase by a factor of about 1.5 and 2, respectively. The time taken to achieve a 50% conversion of α-hydroxyphosphonates (*i.e.*, complete esterification of the *S*-enantiomer) decreased with increasing the amount of the catalyst. For example, the time needed for 50% conversion of compound **5a** in THF at 20 °C depended on the hydroxyphosphonate : lipase mass ratio as follows:

Ratio	1 : 0.05	1 : 0.1	1 : 0.2	1 : 0.5	1 : 1
Time /h	48	36	24	16	12

The conversion times of compounds **5a**,**g**,**h** in THF reduced as the alkyl chain length increased, being 60 h at 25 °C for **5a**,**g** and 300 h at 45 °C for hydroxyphosphonate **5h**. The optical purity of the products (*R*)-**5a**,**g**,**h** and (*S*)-**6a**,**g**,**h** was at least 99%, as was established by ^{31}P NMR spectroscopy in a chiral solvating agent Cinchonidine[41] and by derivatization with Mosher's acid [(*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid].[42]

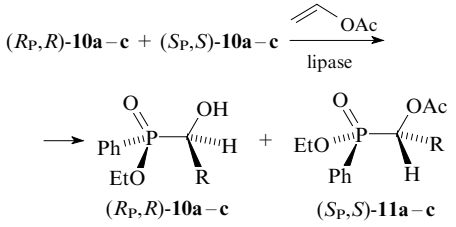
The kinetic resolution of diastereomers in the presence of the lipases CAL (immobilized lipase from *Candida antarctica*, Chirazyme L-2) and Amano AK (immobilized lipase from *Pseudomonas fluorescens*) was reported (Ref. 43).† First, racemic phosphinates **10a**–**c** containing two stereogenic centres were obtained by the reactions of ethylphenylphosphinate **9** with aldehydes and separated by crystallization into the major [diastereomers (S_P,*S*)-**10** and (R_P,*R*)-**10**] and minor products [diastereomers (R_P,*S*)-**10** and (S_P,*R*)-**10**].



R = Me (**a**), Et (**b**), Pr^i (**c**); LDA is lithium diisopropylamide.

Subsequent transesterification of the major product catalyzed by CAL and AK using vinyl acetate as the acyl group donor (Scheme 2) gave enantiopure acyl phosphinates (S_P,*S*)-**11a**–**c** (*ee* >98%) and hydroxyphosphinates (R_P,*R*)-**10a**–**c** at 50% conversion. The influence of the alkyl substituent R in the biocatalyst on the acylation process was more pronounced for CAL compared to AK.

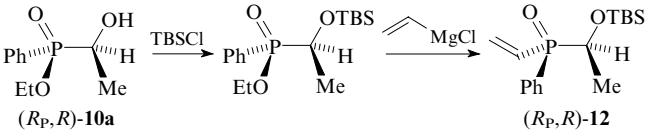
Scheme 2



Lipase	Compound	R	Time /h	Conversion (%)	*ee* for (R_P,*R*)-**10** (%)
CAL	**a**	Me	1	50	>98
	b	Et	32	17	28
	c	Pr^i	see [a]	—	—
AK	**a**	Me	8	50	>98
	b	Et	29	50	>98
	c	Pr^i	75	9	2

[a] No reaction.

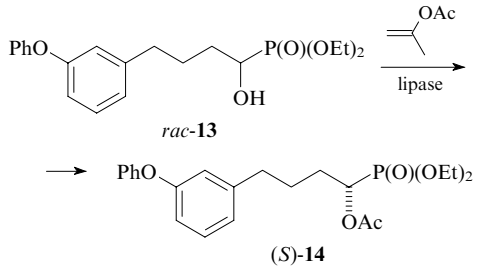
After protection of the OH group, ethyl (1-hydroxyethyl)phenylphophinate (R_P,*R*)-**10a** was transformed to P-chiral vinylphosphine oxide (R_P,*R*)-(**12**), a convenient agent for the synthesis of other optically active phosphine oxides, by the reaction with vinylmagnesium bromide.[43]



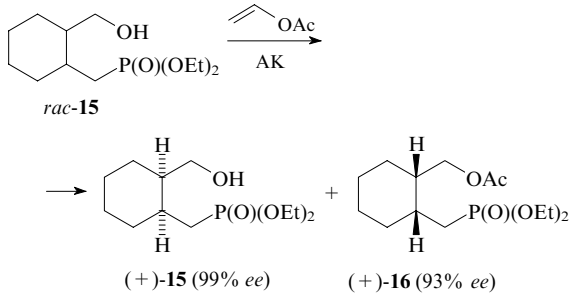
TBS = Bu^{t}Me$_2$Si.

† In this Section, we report only a few examples of using compounds with chiral phosphorus atom because they were used to obtain chiral hydroxyphosphonates; see Section II.4 for more detail.

In a similar way, Pattel *et al.*[44] conducted enantioselective acetylation of racemic [1-hydroxy-4-(3-phenoxyphenyl)butyl]phosphonate (**13**) by isopropenyl acetate in toluene in the presence of lipase from *Geotrichum candidum*. The resulting phosphonate (*S*)-**14** is an intermedate in the total synthesis of the squalene synthase inhibitor BMS-188494. The yield of the major product was 38% and its optical purity was 95%.



Transesterification of *cis*-1-diethylphosphonomethyl-2-hydroxymethylcyclohexane (**15**) with vinyl acetate catalyzed by the AK lipase afforded alcohol (+)-**15** and the corresponding acetate (+)-**16** in good yields and high enantiomeric excess. The reaction was solvent-free, the enantioselectivity factor was 152. The alcohol (+)-**15** was then transformed to an optically active hydantoin, a starting compound for the synthesis of conformationally hindered (2*R*)-amino-5-phosphonopentanoate (AP 5) analogues.[45]

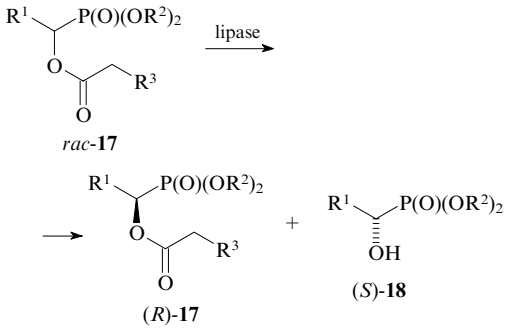


Racemic hydroxymethylphosphonates were resolved into enantiomers using lipase isolated from *Pseudomonas fluorescens*. The products were used to obtain chiral phosphosulfonates possessing high herbicidal activity. Biological tests showed that the activity of (+)-phosphosulfonates is higher than that of the (−)-isomers and the racemate.[46]

b. Resolution of α-hydroxyphosphonates by enzymatic hydrolysis

Enzymatic hydrolysis is a convenient method of obtaining chiral hydroxyphosphonates.[40,47–57] Such reactions are usually carried out in two-phase systems in the presence of a buffer maintaining pH 7 using a mixture of hexane with *tert*-butyl methyl ether as the best solvent. Hydrophobic solvents decrease the activity of enzymes, but the addition of saturated aqueous solutions of salts ($MgCl_2$ or LiCl) considerably increases the enantioselectivity of the processes involving lipases.

Hammerschmidt and co-workers.[47–50] studied hydrolysis of acyloxyphosphonates by different enzymes in two-phase systems in the presence of a buffer. The biocatalysts used were eight lipases and pig liver esterase.[47,48] β-Acyloxyphosphonates **17** were hydrozyled in the system *tert*-butyl methyl ether–hexane–enzyme–phosphate buffer (pH 7) at room temperature. The pH value, which changed in the course of reaction, was maintained constant by an autotitrator. It was found that lipases preferably hydrolyzed the *S*-enantiomers of the esters, while Chirazyme P-2 protease hydrolyzed the *R*-enantiomers.[50] The highest enantioselectivity was found for hydrolysis of (acetoxy)phenylmethylphosphonates by the lipase from *Rhizopus oryzae* (FAP 15) and *Aspergillus niger* (AP 6).



Lipase is FAP 15, AP 6; R^1 = Alk, Ar, 1-Naph, 2-Naph, 2-Th, 3-Th, 2-Fu, 2-Py, 3-Py; R^2 = Me, Et, Pr^i; R^3 = Me, CH_2Cl; Naph is naphthyl, Th is thienyl , Fu is furyl, Py is pyridyl.

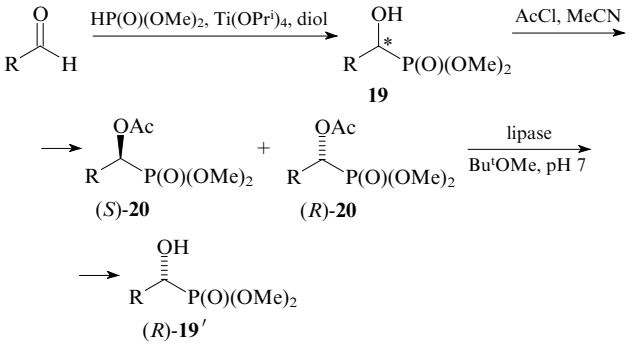
As an example, Table 2 presents data on the hydrolysis of 11 acyloxyphosphonates **17** (R^1 = Alk) in the presence of lipase AP 6. As can be seen, for compounds with branched alkyl chains the process had lower enantioselectivity and led to products with a different configuration. As the length of the alkyl chain increased (R^1 = n-C_nH_{2n+1}, n = 2, 3, 5), the

Table 2. Hydrolysis of β-acyloxyphosphonates **17** (R^1 = Alk) catalysed by lipase AP-6.[47]

R^1	R^2	R^3	Acetate **17**			Hydroxyphosphonate **18**		
			yield (%)	*ee* (%)	configuration	yield (%)	*ee* (%)	configuration
Et	Pr^i	Me	47	51	*R*	37	77	*S*
Pr^n	Pr^i	Me	45	68	*R*	30	83	*S*
Pr^i	Et	Me	30	26	*S*	38	32	*R*
Bu^i	Et	Me	47	3	*S*	39	7.5	*R*
Bu^t	Et	CH_2Cl	47	15	*S*	43	15	*R*
n-C_5H_{11}	Et	Me	49	48	*R*	38	69	*S*
	Pr^i	Me	36	80	*R*	43	87	*S*
	Pr^i	CH_2Cl	56	52	*R*	37	92	*S*
n-C_7H_{15}	Et	CH_2Cl	47	57	*R*	42	63	*S*
	Pr^i	CH_2Cl	43	44	*R*	51	73	*S*
n-C_9H_{19}	Et	CH_2Cl	49	56	*R*	45	62	*S*

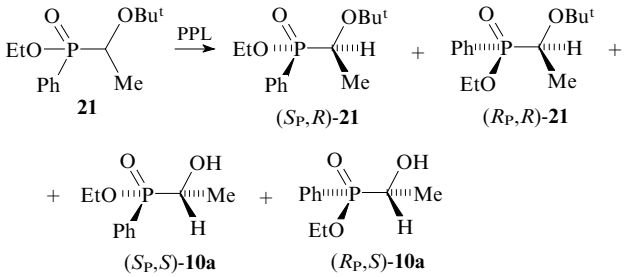
reaction rate decreased, while the enantioselectivity of hydrolysis increased from 77% *ee* to 87% *ee* and the conversion changed only slightly. On going from diethyl to diisopropyl acyloxyphosphonates, the reaction rates decreased with simultaneous increase in the optical yields of the products (*S*)-**18**. Hydrolysis of hydroxyphosphonate chloroacetates proceeded about 50 times faster than that of the corresponding acetates.

To increase the enantioselectivity of the reaction, and at the same time, to decrease the time of complete kinetic resolution, enantioenriched mixtures can be used instead of racemates. Rowe and Spilling [51] employed this method to increase the optical purity of 1-hydroxyphosphonates **19** containing unsaturated groups. The reactions of aldehydes with dialkyl phosphites catalyzed by chiral titanium alcoholates (titanium tetraisopropoxide with a chiral diol as a ligand) led to enantioenriched α-hydroxyphosphonates **19** with 42% – 77% *ee*. The enantioenriched products **19** were then acetylated and the resulting acetates **20** were subjected to lipase-catalyzed hydrolysis (Table 3) to give the alcohols (*R*)-**19′** in good yields and high *ee* (up to 99%). Hydrolysis of acetates **20** was conducted in Bu^{t}OMe at room temperature and pH 7.

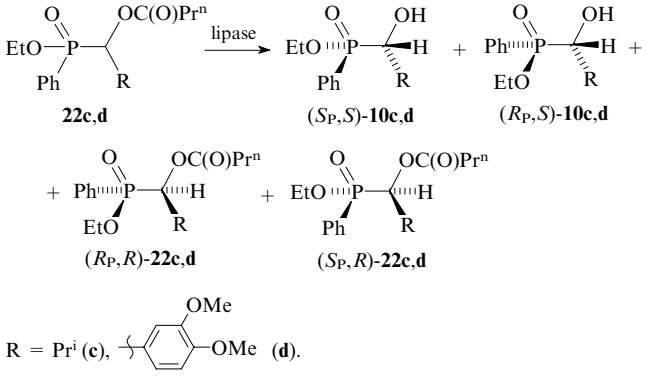


Diol is (1*S*,2*S*)-*trans*-cyclohexanediol or dimethyl L-tarthrate.

Kafarski and co-workers [52 – 54] resolved α-hydroxyphosphinates containing two asymmetric centres in the presence of lipases from various microorganisms including *Candida cylindracea*, *Aspergillus niger*, *Rhizopus niveus*, *Mucor javaniceus*, *Porcine pancreas*, *Pseudomonas cepacia* and *Mucor circinelloides*. The kinetic resolution of ethyl (1-hydroxyethyl)phenylphosphinate **10a** was achieved by enzymatic transesterification with vinyl butyrate or by hydrolysis of ethyl (1-terbutoxyphenyl)phenylphosphinate **21** using lipases.[53] Hydrolysis of compound **21** in the presence of porcine pancreatic lipase (PPL) resulted in two diastereomers **10a** with *ee* > 98% at conversions varying from 10% to 49% depending on the reaction conditions.[54]



Butyryloxyalkylphosphinates **22c**,**d** were hydrolyzed using enzymes from *Candida cylindracea* and *Aspergillus niger*, as well as PPL.[52] Reactions were carried out in two-phase systems at pH 7 with a mixture of diisopropyl ether and hexane as the organic phase. The best results were achieved using the lipase from *Aspergillus niger*, which provided isolation of the diastereomers (S_P,*S*)- and (R_P,*S*)-**10c**,**d** as the major products of the reaction (82% *ee*).



R = Pr^i (**c**), (**d**).

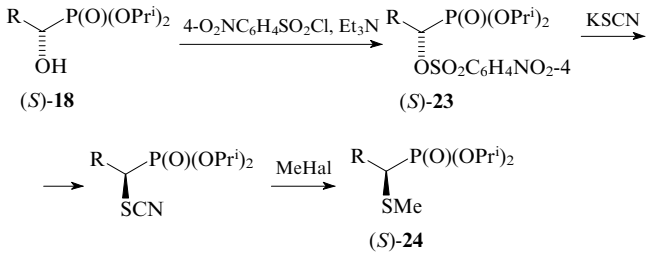
Optically active α-sulfonyl phosphonates **23** and the corresponding methyl sulfides **24** were synthesized from chiral (93% *ee* – 97% *ee*) α-hydroxyphosphonates **18** obtained by enzymatic resolution of compounds **17** (see Table 2).[23, 47] Attempts to obtain chiral phosphonates with divalent sulfur atom at the C_α-atom by enantioselective

Table 3. Enzymatic hydrolysis of enantioenriched 1-acyloxyphosphonates **20**.

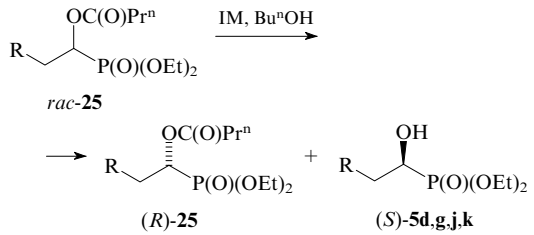
R	Lipase or its source	Starting acetate **20**		Alcohol (*R*)-**19′**	
		ee (%)	configuration	*ee* (%)	configuration
	FAP 15	42	*S*	79	72
Ph	PSL	70	*R*	99	72
MeCH=CH	FAP 15	64	*S*	97	79
PhCH=CH	PSL	73	*R*	99	68
	Rhizopus arrizhus	73	*S*	91	70
PhCH=CMe	PSC	77	*R*	99	75
n-C_5H_{11}C≡C	AY	49	*R*	92	59
	Rhizopus arrizhus	49	*S*	90	74
n-C_5H_{11}C=CH	FAP 15	69	*S*	95	74

Note. PSL is lipase from *Pseudomonas* sp., PSC is lipase from *Pseudomonas cepacia* and AY is lipase from *Candida cylindracea*.

hydrolysis of corresponding racemic α-acetylthiophosphonates failed. Namely, hydrolysis in the presence of the AP 6 and Prozyme 6 lipases and the Chirazyme P-2 protease gave racemic products and the reactions in the presence of the lipases isolated from *Candida rugosa* and *Candida cylindracea,* as well as the FAP 15 lipase did not occur at all.

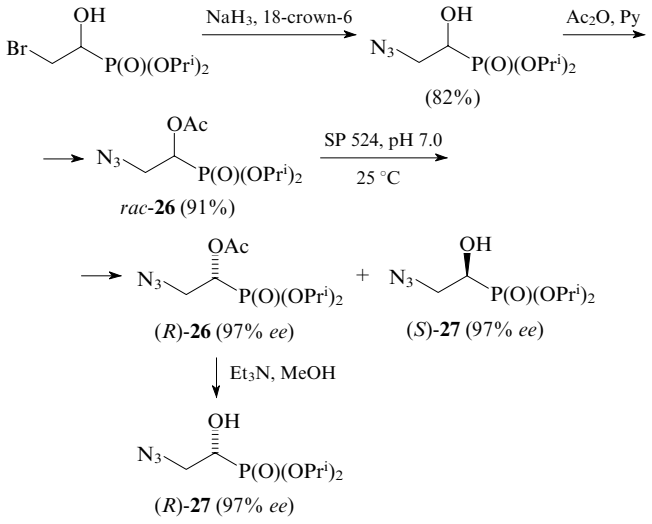


Alcoholysis of butyroxy derivative **25** by n-butyl alcohol in anhydrous benzene led to enantiopure hydroxyphosphonates (*S*)-**5d**,**g**,**j**,**k** in 40% yield.[40]

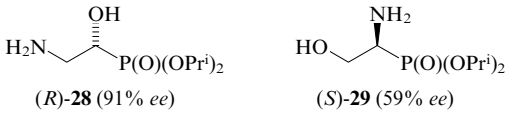


R = Me (**d**), Et (**g**), Pr^i (**j**), CH$_2$=CH (**k**).

Hammarschmidt *et al.*[57] reported the resolution of diisopropyl (2-azido-1-hydroxyethyl)phosphonates to enantiomers using the SP 524 lipase (genetically engineered hybrid of *Mucor* and *Aspergillus oryzae*). The racemic diisopropyl 2-azido-1-acetoxyethylphosphonate (**26**) obtained from the corresponding bromide undergoes hydrolysis in the presence of SP 524 with the formation of α-hydroxyphosphonate (*S*)-(**27**) and ester (*R*)-**26**, which is then hydrolyzed to give stereoisomer (*R*)-**27**.

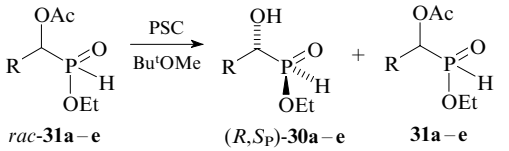


The phosphonates (*R*)-**27** and (*S*)-**27** were then transformed to α-phosphaisoserine [(*R*)-**28**] and α-phosphaserine [(*S*)-**29**].[57]



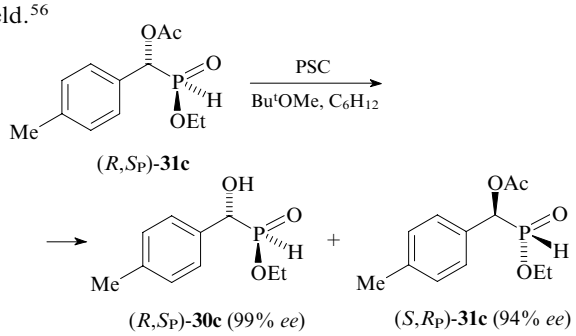
Yuan *et al.*[40] reported CALB to be efficient in resolving azide-containing racemic phosphonates into enantiomers.

Chiral α-hydroxy-H-phosphinates **30a**–**e** were obtained by enzymatic hydrolysis of the corresponding racemic acetates **31a**–**e** in the presence of lipase from *Pseudomonas cepacia*. Enantiopure α-hydroxy-H-phosphinates (*R*,*S*$_P$)-**30a**–**e** were isolated from a mixture of four stereoisomeric α-acetoxy-H-phosphinates.[56] It should be noted that hydroxyphosphonates with aromatic substituents are usually stable against lipase-catalyzed transesterification and hydrolysis, which is explained by both electronic and steric factors. In this case, a possible reason is the substitution of the ethoxy group at the phosphorus atom for a smaller hydrogen atom.



R = n-C$_5$H$_{11}$ (**a**), Bn (**b**), 4-XC$_6$H$_4$ [X = Me (**c**), H (**d**), Cl (**e**)].

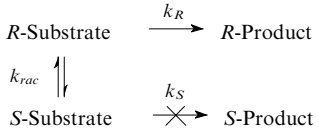
Only one of the four diastereomers **31c** present in the mixture, *viz.*, the acetate (*R*,*S*$_P$)-**31c**, underwent hydrolysis under the action of PSC lipase in a hexane/*tert*-butyl methyl ether mixture in phosphate buffer (pH 7). The reaction led to the corresponding hydroxyphosphonate (*R*,*S*$_P$)-**30c** obtained in 18% yield and to 99% *ee*. At the same time, the acetate (*S*,*R*$_P$)-**31c** regenerated and was isolated in 33% yield.[56]



c. Dynamic kinetic resolution of α-hydroxyphosphonates

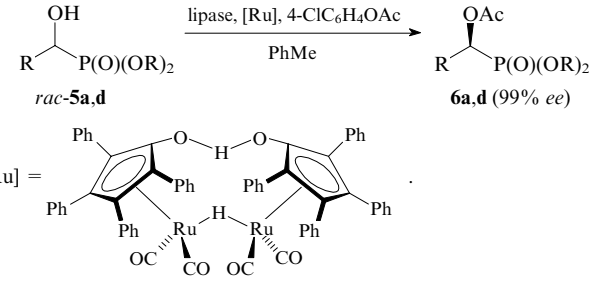
The kinetic resolution of a racemate allows each enantiomer to be isolated in 50% yield. However, this limitation can be overcome using the dynamic kinetic resolution (DKR) technique.[30–34, 58–60]

In DKR, one deals with continuous isomerization of the substrate in the course of resolution, *i.e.*, the *R*- and *S*-enantiomers are in equilibrium. As a consequence, at $k_S = 0$, the starting *R*-enantiomer is completely transformed to the *R*-product.



A combination of metal complex catalysis and biocatalysis was successfully used in the DKR of some hydroxy-

phosphonates. For instance, simultaneous action of an enzyme [lipase from *Pseudomonas cepacia* or CALB (trade name Novozym-435)] and a ruthenium complex that catalyzes the isomerization of alcohols, resulted in the transformation of the racemic hydroxyphosphonates **5a**,**d** to enantiopure acetates **6a**,**d** in high yields.[58]



rac-**5a**,**d** **6a**,**d** (99% *ee*)

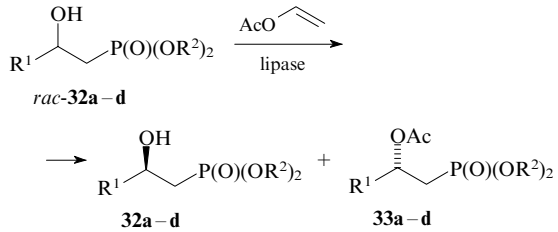
Compound	R	Lipase	T/°C	Yield (%)
a	Me	CALB	60	70
	Me	CALB	70	76
	Me	CALB	80	86
	Me	PSC	60	69
	Me	PSC	80	87
b	Et	PSC	80	85

2. Kinetic resolution of β- and ω-hydroxyphosphonates

Similarly to α-hydroxyphosphonates, biocatalytic resolution of racemic phosphonates containing a hydroxyl group in β-, γ- and δ-positions relative to the phosphorus atom (so-called ω-hyrdoxyphosphonates) is carried out in two ways, by transesterification of carboxylic acid esters and by hydrolysis of the corresponding acetylated hydroxyphosphonates.[61–68]

a. Resolution of β- and ω-hydroxyphosphonates by enzymatic transesterification

Racemic β-hydroxyalkylphosphonates **32a–d** were acylated with vinyl acetate in the presence of lipases from *Pseudomonas cepacia* and *Pseudomonas fluorescens*, as well as the lipase Amano AH-S (LAH-S).‡ The reactions were carried out under kinetic control. The unreacted substrates **32a–d** and the acetylated products **33a–d** were separated by column chromatography.[61]



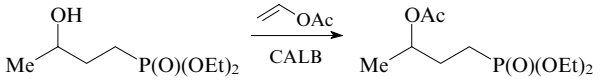
rac-**32a–d** **32a–d** **33a–d**

Compound	R^1	R^2	Lipase	*ee* for **32** (%)	*ee* for **33** (%)
a	Me	Et	AK	93	90
b	Bu^n	Me	AK	61	92
c	$Ph(CH_2)_3$	Me	LAH-S	52	43
d	2-Py	Et	PSC	45	62

‡ Hereafter, only the commercial names of lipases are given if the chemical name is not given in the original publication.

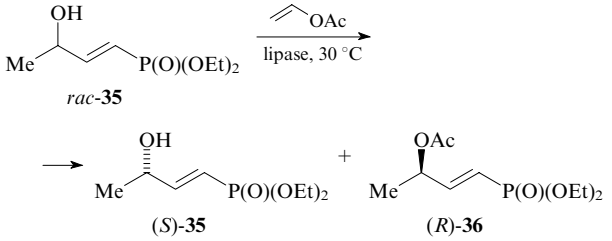
Successful resolution of β-hydroxyalkylphosphonates **32** (R^1 = Me, Et, CH=CH_2, CH_2Cl; R^2 = Me, Et) to *R*- and *S*-enantiomers by CALB-catalyzed transesterification with vinyl acetate was reported.[32] Compounds **32** with methyl, ethyl or vinyl group at the C_β-atom were acetylated quite smoothly with good enantioselectivity (the enantioselectivity factor exceeded a value of 100). At the same time, for compound **32e** (R^1 = CH_2Cl, R^2 = Et) the enantioselectivity was low (50% *ee*, $E < 5$).

The CALB-catalyzed transesterification of diethyl γ-hydroxybutylphosphonate (**34**) with vinyl acetate took 50 h, but its enantioselectivity was also not high.[32]



34

A number of enzymes (Liposyme, Amano AK, Amano PS, acylase from *Aspergillus melleus*, Amano AP 6, Amano AY, CALB, PPL, CRL (lipase from *Candida rugosa*), Amano R10) were tested for the ability to kinetically resolve racemic diethyl 3-hydroxybut-1-enylphosphonate (**35**).[62] The Liposyme lipase (Fluka) appeared to be the most efficient biocatalyst. In the presence of Liposyme, diethyl 3-acetoxybut-1-enylphophonate [(*R*)-**36**] was formed with 96% *ee* and the unreacted (*S*)-hydroxyphosphonate **35** was isolated with 99% *ee*. Similar results were obtained in the preparative-scale experiments with a few grammes of the starting compounds. The yields were 46%–48%.



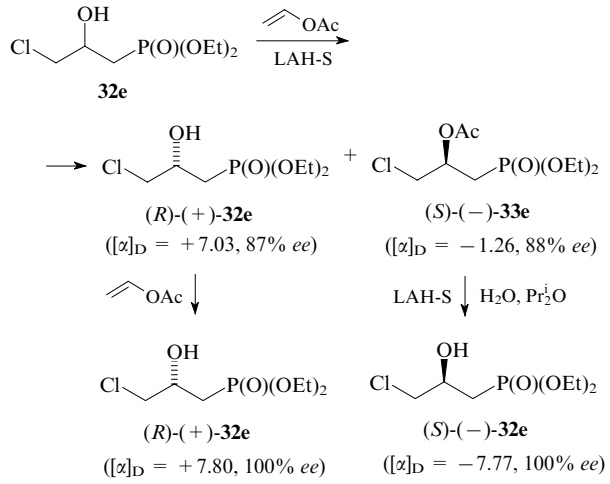
rac-**35** (*S*)-**35** (*R*)-**36**

Lipase	Time /h	Conversion (%)	*ee* for **36** (%)	*ee* for **35** (%)
Liposyme	21	51	99	96
	17	49	95	99
Amano AK	21	50	97	96
Amano PS	17	45	81	98
PPL	49	19	22	99
CRL	10	13	8	54
Amano R-10	10	8	6	69

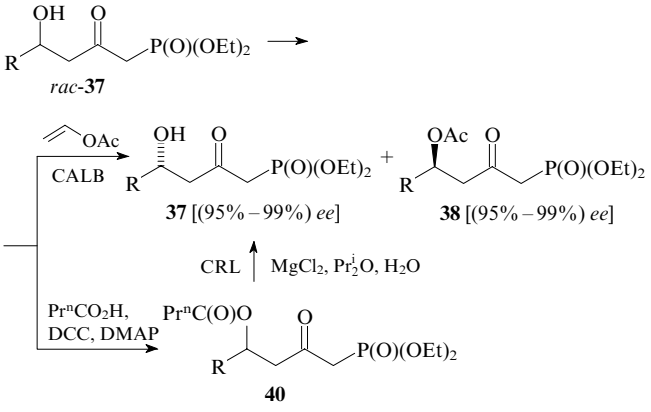
The Amano AK and Amano PS lipases also showed high efficiency in the kinetic resolution of racemic β-hydroxyphosphonate (in particular, a *ee* of 98% was achieved using the latter enzyme). The reaction was relatively fast and the enantioselectivity factor exceeded a value of 200. Good enantiopurity of the acylated product (98% *ee*) was also achieved in the presence of acylase from *Aspergillus melleus*, but the unreacted starting β-hydroxyphosphonate **35** was isolated only with 49% *ee*.[62]

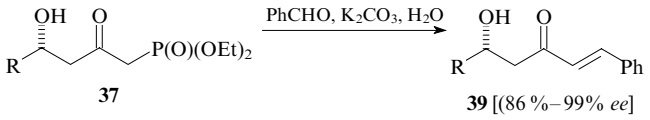
Taking into account interest in P-containing analogues of natural amino acids, such as carnitine and γ-amino-β-hydroxybutyric acid (GABOB), a number of methods for synthesis including the enzymatic resolution procedures

were reported.[40, 63, 69] For instance, an efficient method for synthesis of enantiomerically pure phosphocarnitine starting from readily available 2-hydroxy-3-chloropropyl phosphonate (**32e**) was proposed.[63] Enantioenriched acetate (*S*)-(−)-**33e** with 88% *ee* obtained by enzymatic interesterification with the Amano AH-S lipase or the lipase from *Pseudomonas fluorescens* was then subjected to additional enzymatic hydrolysis in the presence of the same lipase. This allowed one to substantially increase the purity of the hydroxyphosphonate. Hydrolysis of the acetate (*S*)-**33e** was carried out in diisopropyl ether saturated with a buffer (pH 7.2). The solution was stirred over a period of 25 days at 30 °C, the reaction was monitored by ^{31}P NMR spectroscopy. When the alcohol (*R*)-**32e** to acetate (*S*)-**33e** ratio became equal to 2.5 : 1, the reaction was stopped and the products were separated by column chromatography. The resulting β-hydroxyphosphonate (*S*)-(−)-**32e** was obtained in 25% yield with 100% *ee* (the $[\alpha]_D$ values measured in MeOH at a concentration of 2.21% are shown below).



Racemic 4-hydroxy-2-oxophosphonates **37** were resolved into enantiomers by CALB-catalyzed transesterification with vinyl acetate in benzene or by hydrolysis of the corresponding butyrates catalyzed by lipase from *Candida rugosa* in diisopropyl ether containing a saturated magnesium chloride solution.[65] The reaction led to *S*-(or *R*)-ω-hydroxyphosphonates **37** in 35% – 42% yields and acetates **38** in 48% – 51% yields. Reactions of compounds **37** with benzaldehyde in the presence of aqueous potassium carbonate gave chiral enones **39** (optical purity 95% – 99%).



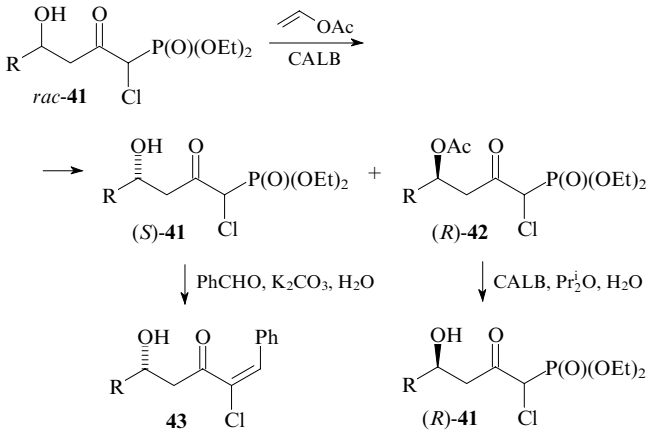


DCC is dicyclohexylcarbodiimide, DMAP is 4-dimethylaminopyridine.

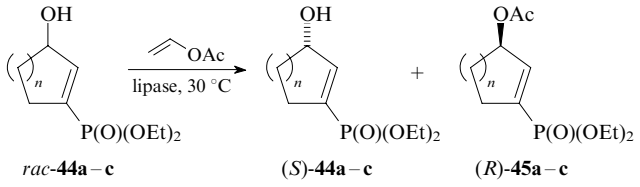
R	Lipase	Yield (%)		*ee* for **39** (%)	Configuration for **39**	*E*
		37	**39**			
Me	CALB	40	48	99.1	*S*	> 25
Et	CALB	42	45	95	*S*	> 19
CH_2=CH	CALB	35	51	95	*R*	> 17
Ph	CRL	35	69	98.7	*R*	> 100
4-FC_6H_4	CRL	38	84	95.9	*R*	> 87
4-MeC_6H_4	CRL	37	81	100	*R*	> 100
4-$MeOC_6H_4$	CRL	32	92	96.8	*R*	> 92
4-ClC_6H_4	CRL	34	78	99.4	R	> 100
2-BrC_6H_4	CRL	36	86	98.0	R	> 100
2-ClC_6H_4	CRL	38	90	97.0	R	> 100
2-Fu	CRL	31	95	85.9	R	> 19

It should be noted that ω-hydroxyphosphonates **37** were also resolved into enantiomers by enzymatic hydrolysis of butyrate **40**.

Racemic α-chloro-β-oxo-γ-(hydroxyalkyl)phosphonates **41** (R = Me, Et, CH_2=CH) were at first resolved by CALB-catalyzed transesterification.[66] This resulted in optically active hydroxyketophosphonates (*S*)-**41** in 42% – 45% yields and acetylated products (*R*)-(**42**) in 45% – 50% yields. The acetates **42** were then additionally hydrolyzed to alcohols (*R*)-**41** in the presence of CALB (or CRL). Subsequent treatment of compounds **41** with benzaldehyde and potassium carbonate gave mixtures of geometric isomers of chiral α,β-unsaturated ketones, from which compounds **43** were isolated with enantiopurity > 98%.



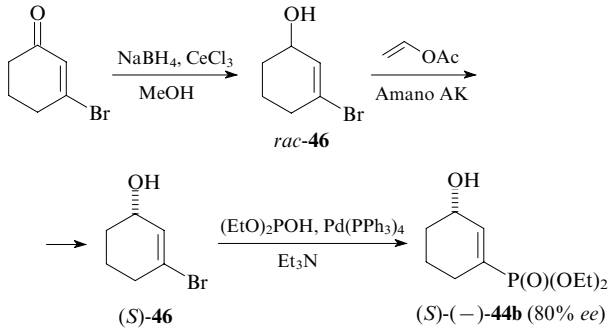
Enantiopure cyclic diethyl (*S*)-3-hydroxycycloalk-1-enylphosphonates **44a** – **c** were obtained by enzymatic resolution of the corresponding racemates.[70, 71] The reaction also afforded the *R*-acetates **45a** – **c**. Among the lipases used (Amano AY, Amano PS, Amano AK, PPL), Amano AK and Amano PS showed the best results, *viz.*, they allowed the enantiopurity of compounds (*S*)-**44a** – **c** to be as high as up to 99%.[71]



rac-**44a–c** (*S*)-**44a–c** (*R*)-**45a–c**

n = 1 (**a**), 2 (**b**), 3 (**c**).

A somewhat different method was used to obtain a chiral six-membered hydroxyphosphonate (*S*)-**44b**. Enzymatic resolution in the presence of the Amano AK lipase was carried out during the formation of bromocyclohexanol **46** and resulted in *S*-enantiomer with 80% *ee*.[71] Subsequent palladium-catalyzed phosphonylation led to hydroxyphosphonate (*S*)-**44b** with the same enantiopurity.



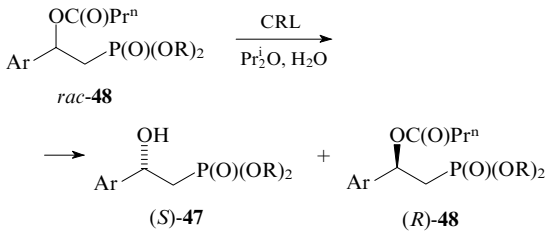
b. Enzymatic hydrolysis of β- and ω-hydroxyphosphonate esters

Enzymatic hydrolysis of β- and ω-hydroxyphosphonate esters is a convenient method for resolution of racemic mixtures of β- and ω-hydroxyphosphonates. In some cases, this method was used to obtain hydroxyphosphonate enantiomers that could not be resolved by enzymatic transesterification.[72–76] For instance, Hammerschmidt and coworkers.[72] reported hydrolysis of some chloroacetoxyphosphonates by the lipase from *Candida cylindracea* and by the protease subtilisin from *Bacillus subtilis* in a two-phase system and obtained (*S*)-hydroxyphosphonates with 51% *ee* to 92% *ee*.

Hydrolysis of racemic chloroacetoxyphosphonates containing cyclic alkyl groups catalyzed by the AP 6 lipase from *Aspergillus niger* resulted in corresponding alcohols and S-enantiomers of the esters, obtained in high yields with satisfactory to high *ee* values.[73–75]

Attolini *et al.*[62] reported enzymatic solvolysis of diethyl 3-hydroxybut-1-enylphosphonate **35** with isopropyl alcohol in diisopropyl ether which acted as the acceptor of the acyl group. The acylated product **36** had an *R* absolute configuration while the unreacted hydroxyphosphonate had an *S* absolute configuration.

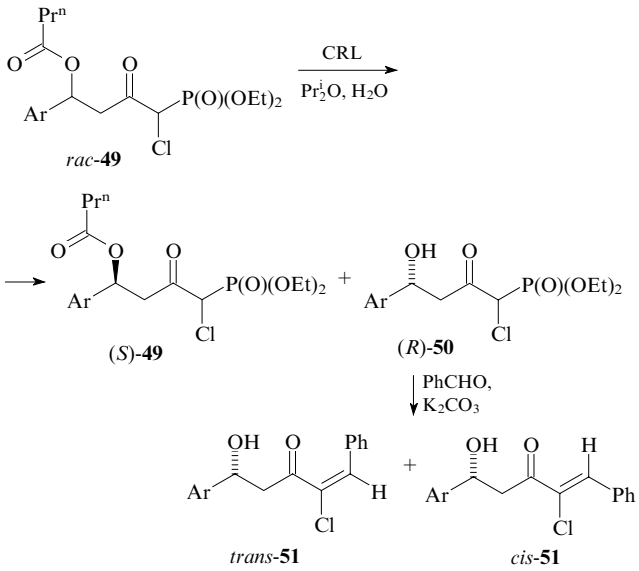
Yuan and co-workers[64] proposed a convenient procedure for the synthesis of optically pure 2-hydroxy-2-arylethylphosphonates **47**. Enantioselective hydrolysis of the corresponding butyrates **48** was conducted in the presence of lipases in organic solvents. For instance, both compounds, (*S*)-**47** and (*R*)-**48**, were obtained in diisopropyl ether saturated with water in high yields with ~95% *ee*. The CRL lipase showed the highest efficiency among the lipases studied, *viz*., the enantioselectivity factor was equal to 65 in the water–buffer system, An alternative procedure involved CRL-catalyzed hydrolysis in diisopropyl ether saturated with 0.5% aqueous magnesium chloride. Unfortunately, the isolation of the products from aqueous solutions was difficult, so the product yields were low.



Ar = $X_nC_6H_{5-n}$ (X_n = H, 4-Me, 4-Et, 4-NO_2, 2-Br, 4-Br, 2-Cl, 3-Cl, 4-F, 2-CF_3, 2,4-Cl_2), 2-Fu, 2-Naph; R = Me, Et, Pr^i.

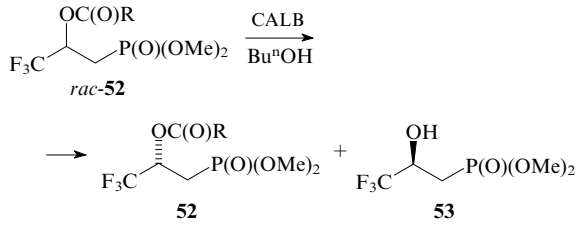
A similar reaction of butyrates containing both aliphatic and aromatic substituents was studied.[65] The absolute configurations of the resulting hydroxyphosphonates were determined by X-ray diffraction analysis. The product yields depended strongly on the nature of substituents in the starting compounds and on the organic solvent used.

Racemic α-chloro-β-oxo-δ-hydroxyalkylphosphonate **49** was resolved into enantiomers by CRL-catalyzed hydrolysis in diisopropyl ether saturated with aqueous $MgCl_2$ solution. The obtained alcohols (*R*)-**50** were used as chiral reagents in the Horner–Wadsworth–Emmons reaction which resulted in chiral α,β-unsaturated ketones **51**.[66]



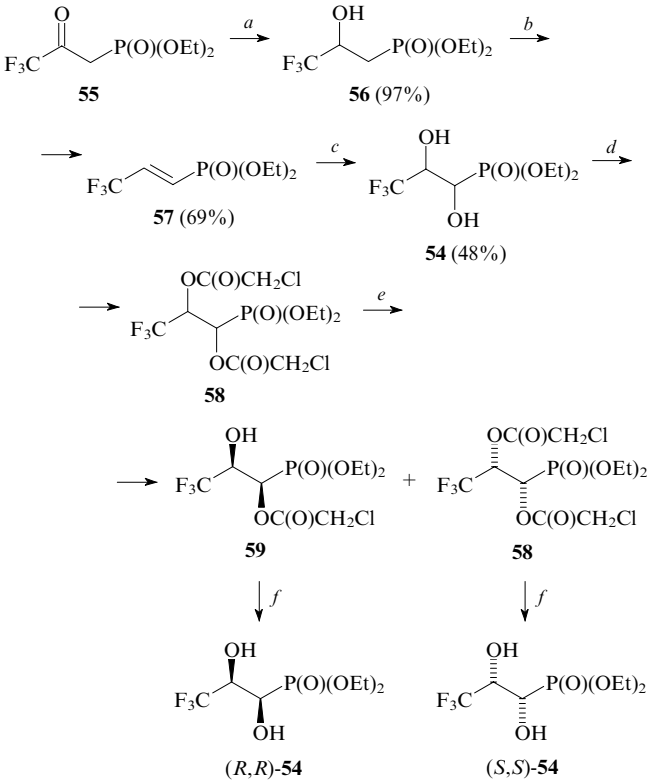
The presence of CF_3, CH_2Cl and CH_2N_3 substituents in the hydroxyphosphonate molecules complicates biocatalytic resolution of these compounds into enantiomers. For example,[32] it was reported that compounds containing the CF_3 group do not enter into the enzymatic acetylation reaction at all due to the electron-withdrawing effect of this group. However, Yuan and co-workers[67] succeeded in choosing suitable enzymes and developed methods for the resolution of racemic α- and β-hydroxyalkylphosphonates bearing the trifluoromethyl group into enantiomers. The best results were obtained when alcoholysis catalyzed by the lipase from *Mucor miehei* and by CALB and hydrolysis catalyzed by the lipase from *Candida rugosa* were carried out in organic solvents. Esters of trifluoromethyl-containing β-hydroxyphosphonates **52** were transformed to alcohols **53** under the action of n-butanol as a nucleophile in the presence of CALB in anhydrous benzene at 30 °C. As a 50% conversion

was reached, the reaction was stopped and the mixture of chiral acyloxyalkylphosphonate **52** and alcohol **53** was separated chromatographically.



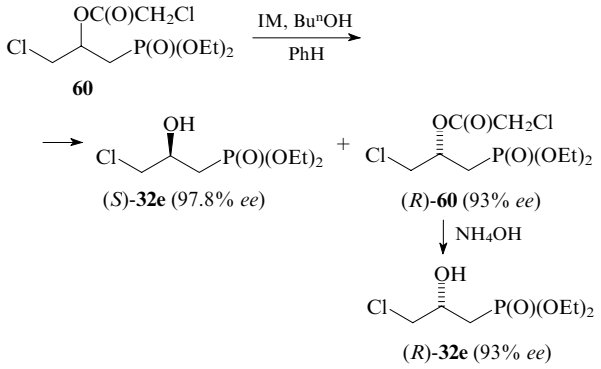
R = $ClCH_2$, Pr^n.

A convenient and efficient method for obtaining chiral 2-trifluoromethyl-1,2-dihydroxypropylphosphonate **54** based on enzymatic catalysis was reported.[68] The reaction of ethyl trifluoroacetate with methylphosphonate anion affords 2-oxo-3,3,3-trifluoromethylpropylphosphonate (**55**) which is then reduced with sodium borohydride to 2-hydroxy-3,3,3-trifluoropropylphosphonate (**56**) and then transformed to vinylphosphonate **57** under the action of trimethylamine. Dihydroxylation of compound **57** with potassium permanganate results in diol **54**. Enzymatic alcoholysis of racemic product **58** catalyzed by CALB or immobilized lipase IM leads to optically active phosphonates **58** and **59** in satisfactory yields with reasonable *ee* values (*ee* ~75%–88%). These products are then hydrolyzed to diols **54**. The absolute configurations of the obtained products follow the Kazlauskas rule if the diethy lphosphonyl group is treated as the large substituent.



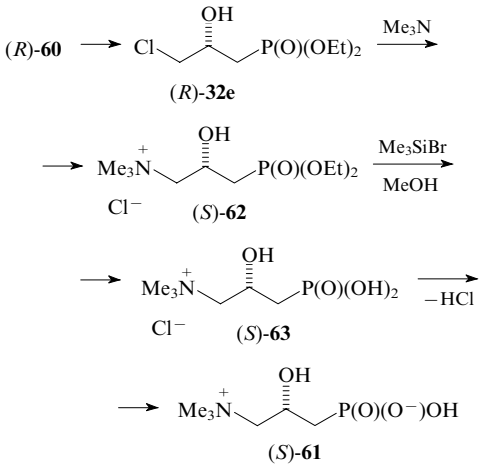
(*a*) $NaBH_4$; (*b*) Me_3N; (*c*) $KMnO_4$; (*d*) $ClCH_2CO_2H$, DCC, DMAP; (*e*) CALB or IM, Bu^nOH, 30 °C, 40 h; (*f*) NH_4OH.

Racemate **32e** was resolved into enantiomers by alcoholysis of chloroacetates **60** with n-butanol in anhydrous benzene in the presence of lipases IM or CALB.[40] Chromatographic purification afforded enantiopure hydroxyphosphonate (*S*)-**32e** and chloroacetate (*R*)-**60**, which was subsequently treated with ammonia.

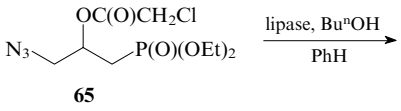


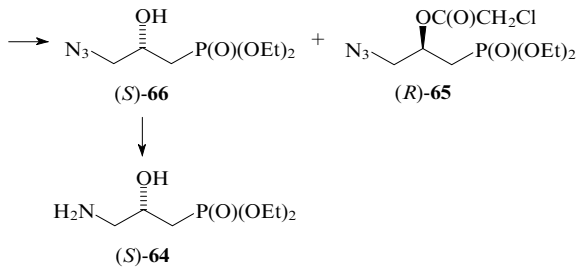
Enantiomers (*S*)- and (*R*)-**32e** were used as the starting compounds in the synthesis of phosphocarnitine (**61**) (Scheme 3). First, compound (*R*)-**32e** derived from chloroacetate (*R*)-**60** was converted to the trimethylammonium salt (*S*)-**62** which was treated with bromotrimethylsilane in methanol. Subsequent dehydrochlorination of the phosphocarnitine salt (*S*)-(**63**) resulted in the optically pure phosphorus analogue of carnitine (*S*)-**61**.

Scheme 3

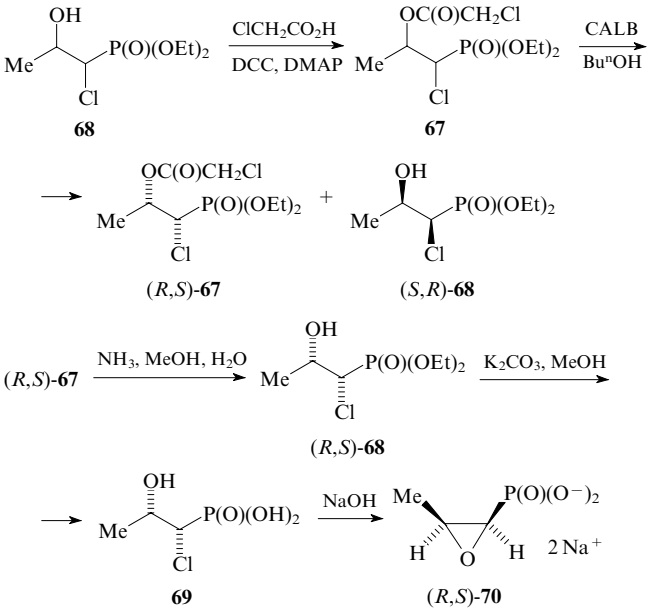


Enantiomers of ethyl ester of phospho-GABOB (**64**) were synthesized[40] according to Hammerschmidt.[57] The key step of the synthesis is enzymatic resolution of racemic chloroacetate **65** into enantiomers in the presence of lipases (CALB or IM) resulting in the *R*-enantiomer of ester **65** and the alcohol (*S*)-**66**, which is then transformed to compound (*S*)-**64**.





CALB-catalyzed alcoholysis of racemic chloroacetate **67** obtained from β-hydroxyphosphonate **68** resulted in compounds (*R*,*S*)-**67** and (*S*,*R*)-**68** with the optical purity >95% (estimated from analysis of the ^{19}F and ^{31}P NMR spectra of the corresponding Mosher esters).[40] Satisfactory results were also obtained using the lipase IM. Hydrolysis of the optically pure chloroacetate (*R*,*S*)-**67** with ammonia in aqueous methanol was not accompanied by racemization and resulted in hydroxyphosphonate (*R*,*S*)-**68** in 85% yield. The latter was hydrolyzed to acid **69**, which undergoes cyclization to a Fosfomycin sodium salt (*R*,*S*)-**70** (Fosfomycin is a natural broad-spectrum antibiotic) in alkaline medium.

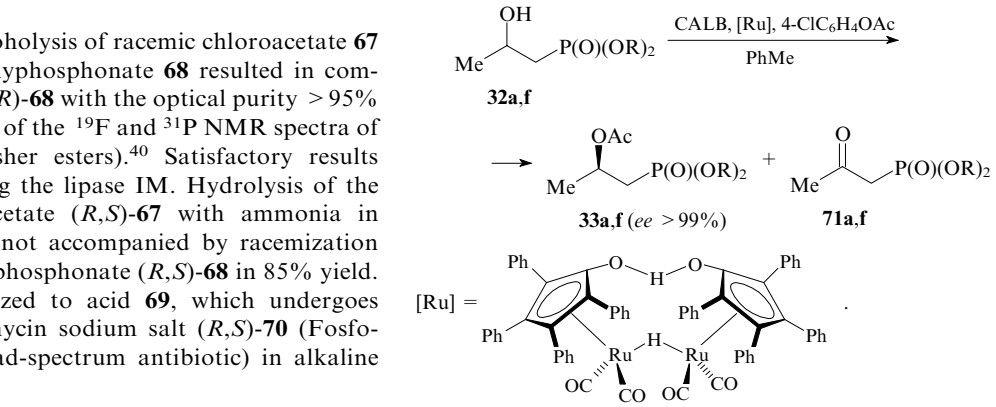


c. Dynamic kinetic resolution of β-hydroxyphosphonates

Dynamic kinetic resolution of racemic β-hydroxyphosphonates **32a**,**f** [R = Et (**a**), Me (**f**)] under the action of lipases was combined with isomerization of the alcohol catalyzed by a ruthenium complex.[31, 58–60]

To this end, racemic dialkyl 2-hydroxypropylphosphonates **32a**,**f** were treated with 3 equiv. of 4-chlorophenylacetate in toluene at 60 °C in the presence of different lipases. In all cases, the enantioselectivity of acylation was high enough, although the yields and enantiopurity of the products varied depending on the type of the lipase used. The lipase CALB (trade name Novozym 435) showed the highest activity, whereas other lipases (CRL and lipases from *Porcine pancreas*, *Candida cylindracea* and *Aspergillus* sp.) were of low activity.

Dynamic kinetic resolution of racemic β-hydroxyphosphonates significantly differs from that of α-hydroxyphosphonates by longer resolution times (48 h *vs.* 24 h, respectively), low yields of the products **33a**,**f** and the formation of the side ketophosphonates **71a**,**f**. Bubbling hydrogen through the reaction mixture (Run 4) or the addition of 2,4-dimethylpentan-3-ol (Run 5) inhibited the formation of ketophosphonates **71a**,**f**; however, no decrease in the efficiency of dynamic kinetic resolution or the enantiopurity of the products **33a**,**f** was observed.[58]

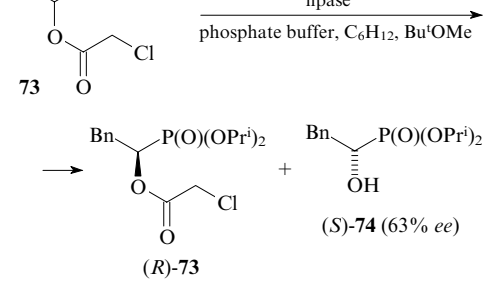


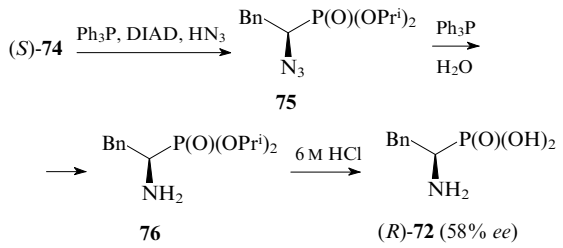
Run, No.	Compound	R	*T*/°C	Yield (%)	
				33	**71**
1	**a**	Et	60	54	18
2	**a**	Et	70	65	10
3	**a**	Et	80	69	19
4	**a**	Et	70	53	0
5	**a**	Et	70	57	0
6	**f**	Me	70	62	16

3. Enzymatic resolution of aminophosphonates

Aminophosphonic acids are of great interest as potential biologically active compounds. Therefore, attempts have been made to use biocatalysis for the synthesis of their enantiomers. Information on the use of chemoenzymatic methods for the synthesis of chiral aminophosphonic acids was first disclosed in patents issued to Hoechst Aktiengesellschaft back in the 1980s (see, *e.g.*, Ref. 18).

Hammerschmidt and co-workers[72–76] succeeded in the chemoenzymatic synthesis of phosphorus-containing analogues of phenylalanine, tyrosine, valine, β-aminoisocaproic acid, isoleucine and α-amino-γ-methylthioacetic acid. The reaction sequence resulting in aminophosphonic acid (*R*)-**72** is shown below as an example. Enzymatic resolution of racemate **73** in the presence of lipase from *Aspergillus niger* gave the optically active phosphonates (*R*)-**73** and (*S*)-**74**.

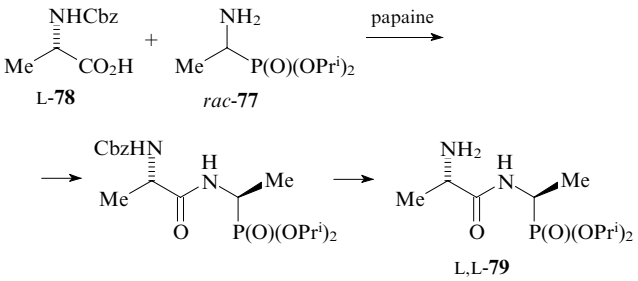




DIAD is diisopropyl acetylenedicarboxylate.

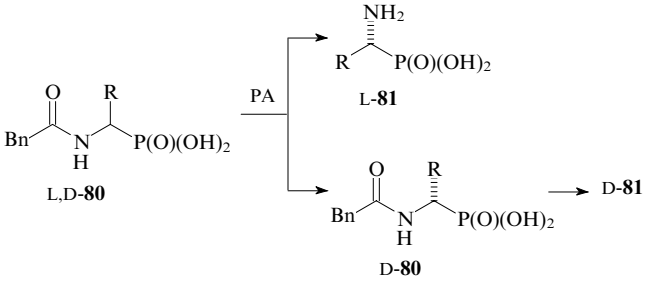
The Mitsunobu reaction involving (*S*)-**74** proceeded *via* the formation of azide **75** and led to amine **76**. Aminophosphonic acid (*R*)-**72** was isolated after subsequent acid hydrolysis.

The protease papaine (enzyme isolated from *Carica papaya* plant, which catalyzes hydrolysis of proteins and peptides) was used to resolve racemic aminophosphonic acid **77**.[77] The reaction of *N*-benzyloxycarbonylalanine L-**78** with racemic aminophosphonate **77** was carried out in acetonitrile in the presence of papaine supported on solid polyamide at ambient temperature.[78] This method was successfully used to obtain optically active antibacterial agent alaphosphalin (**79**).[77]



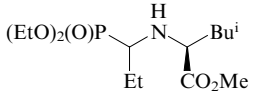
Cbz = BnOC(O).

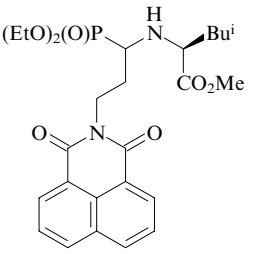
Kuhar and co-workers[79–82] resolved racemic *N*-phenylacetylaminophosphonic acids **80** into compounds D-**80** and L-**81** using penicillin acylase (PA)-catalyzed hydrolysis.



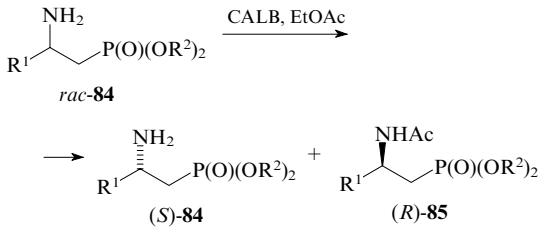
R	Configuration for **81**	Yield (%)	$[\alpha]_D^{20}$
Me	L	80	−17.0
Me	D	80	+16.5
Ph	L	73	+24.0
Ph	D	71	−23.0
Bu^i	L	76	−34.5
Bn	L	77	−50.0

Khushi *et al.*[37] carried out one of the first enzymatic resolutions of aminophosphonates **82** and **83** by hydrolysis of the corresponding esters of phosphono(amino)isocaproic acid by protease subtilisin Carlsberg and lipase from *Aspergillus oryzae*. The kinetically controlled reaction was carried out in aqueous solution (pH 7); the corresponding unreacted ester was separated from the solution at pH 8.5.





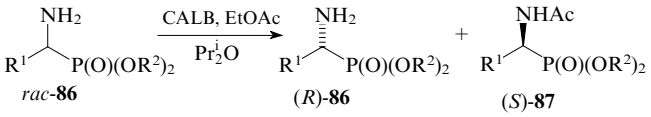
Racemic β-aminoalkylphosphonates **84** were kinetically resolved into enantiomers by CALB-catalyzed N-acylation.[83] Ethyl acetate was used as the acylating agent because vinyl acetate, due to its high nucleophilicity, acylated the aminophosphonates even in the absence of CALB. As a result, acylation of diethyl 2-aminopropylphosphonate **84** (R^1 = Me, R^2 = Et) gave a mixture of corresponding enantioenriched aminophosphonate (*S*)-**84** (99.5% *ee*) and *N*-acylaminophosphonate (*R*)-**85** (78% *ee*), which was then separated by column chromatography.



R^1	R^2	Time /h	**84**		**85**	
			yield (%)	*ee* (%)	yield (%)	*ee* (%)
Me	Et	120	40	99.5	54	78
Me	Pr^n	120	41	100	53	76
Me	Pr^i	148	40	100	55	72
Et	Et	148	44	64	42	79
Et	Pr^n	240	41	56	40	74
Et	Pr^i	240	43	26	41	41
CH$_2$=CH	Et	24	0	0	>90	0

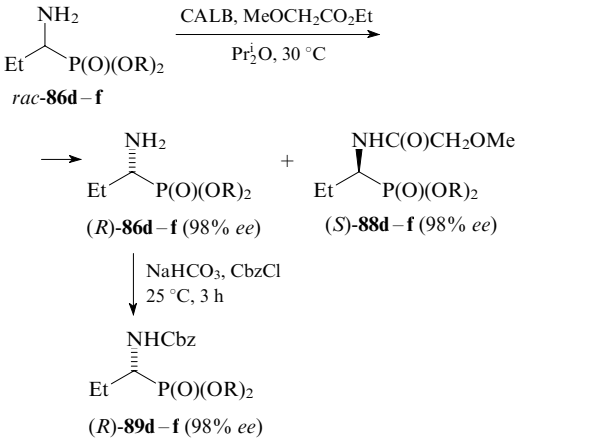
The efficiency of the process was affected by substituents at the phosphorus atom. It was found that the R^2 group should be not too large for the CALB-catalyzed resolution to proceed successfully. For example, the enantioselectivity of resolution of diisopropyl 2-aminophosphonate **84** (R^1 = Me, R^2 = Pr^i) under the same conditions was low (the ee values of the products were about 54% and 64%, respectively). The enantioselectivity was increased to $E > 70\%$ when the reaction of **84** (R^1 = Me, R^2 = Pr^i) was carried out in diisopropyl ether. The enantioselectivity of acylation also decreased when the vinyl group was used as the R^1 substituent.

N-acylation of α-aminophosphonates **86a–d** with ethyl acetate in the presence of CALB was slow, a 50% conversion was achieved within five days, although the enantioselectivity in most cases was reasonably high (*ee* >90%). The reaction resulted in a mixture of compounds (*R*)-**86a–d** and (*S*)-**87a–d**.



Compound	R^1	R^2	(*R*)-**86**		(*S*)-**87**	
			yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	Me	Et	41	99.7	48	90
b	Me	Pr^i	42	90	42	90
c	Et	Me	44	96	43	98
d	Et	Et	73	18	10	100

In some cases, a more reactive ethyl metoxyacetate was used instead of ethyl acetate. This improved, *e.g.*, the efficiency of the resolution of enantiomers of α-aminophosphonates **86d**–**f**.[83] Then, the esters of aminophosphonic acids (*R*)-**86d**–**f** were separated from the acylated derivatives (*S*)-**88d**–**f** and transformed to compounds **89d**–**f** with protected amino group.



R = Et (**d**), Pr^n (**e**), Pr^i (**f**).

The stereocourse of CALB-catalyzed acylation of all α- and β-aminoalkylphosphonates shown above follows the Kazlauskas rule (Fig. 2).

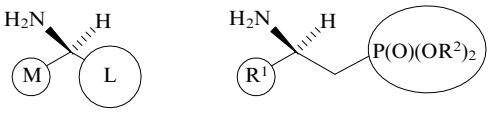
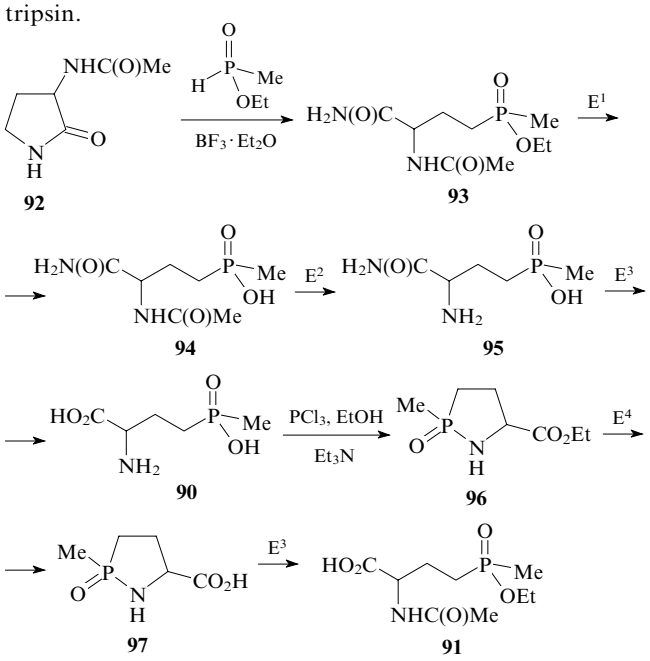


Figure 2. Absolute configurations of *S*-stereoisomers of aminoalkylphosphonates resulting from CALB-catalyzed resolution.[83]

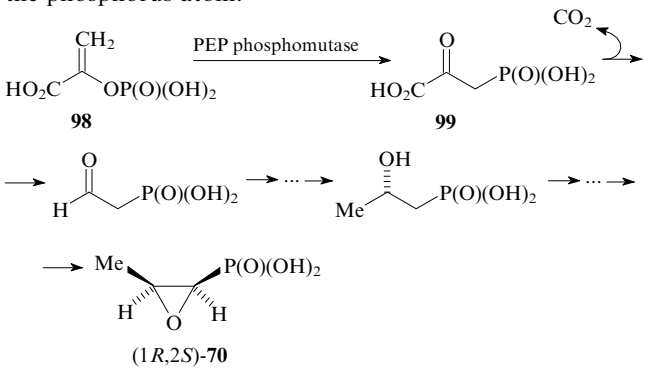
Natchev[17,84] reported enzymatic synthesis of D- and L-enantiomers of phosphinothricin (2-amino-4-hydroxymethylphosphinylbutanoic acid, **90**) and its derivative **91**. L-Phosphinothricin is a known herbicide isolated from *Streptomyces hydroscopicus*. Acetylated D,L-3-aminopyrrolidin-2-one (**92**) was transformed to acyclic phosphinate **93** by the reaction with ethylmethylphosphinate. Successive enzymatic hydrolysis by phosphodiesterase, acylase and glutaminase *via* the formation of intermediates **94** and **95** led to phosphinothricin **90**, which was transformed to azaphospholidynincarboxylic acid ester **96** and then to cyclic phosphonic acid **97** after treatment with glutaminase. The final step also involved the formation of the L-phosphinothricin derivative **91** under the action of α-chemotripsin.



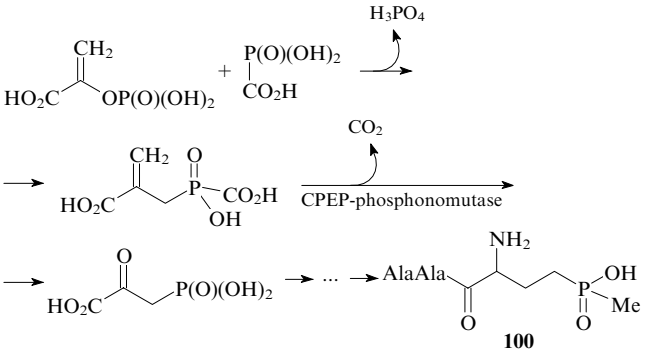
E^1 is phosphodiesterase, E^2 is acylase 1, E^3 is glutaminase, and E^4 is α-chemotripsin.

4. Biosynthesis of compounds with C–P bond

The discovery of natural phosphonates, such as 2-aminoethylphosphonic acid, phosphinothricin, fosfomycin, *etc.* was followed by intensive research on the mechanism of C–P bond formation in biological systems.[85–94] It was established that the key stage of phosphonate formation involves a rearrangement of phosphoenol pyruvate (PEP) to phosphopyruvate[86–88] catalyzed by the enzymes responsible for the P–C bond formation. In particular, fosfomycin (**70**) is formed as a result of biocatalyzed rearrangement of phosphoenol pyruvate (**98**) to phosphopyruvate (**99**) under the action of PEP phosphomutase (EC 5.4.2.9) isolated from *Tetrahymena pyriformis* and *Streptomyces hygroscopicus*.[88] As a result of intramolecular rearrangement, PEP is transformed to phosphopyruvic acid which then undergoes decarboxylation with the formation of phosphonoacetaldehyde and, subsequently, fosfomycin (1*R*,2*S*)-**70**.[86,87] Using ^{18}O labels, it was proved[93] that transfer of phosphate group from oxygen to carbon atom in enol pyruvate **98** catalyzed by *Tetrahymena pyriformis* is an intramolecular process proceeding with retention of the absolute configuration of the phosphorus atom.[91,92]

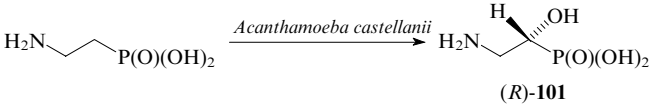


The formation of the herbicide bialaphos (SF-1293, 100) proceeds by a similar mechanism. This tripeptide antibiotic is produced by *Streptomyces veridochromogenes* bacteria and possesses herbicidal and fungicidal activity. Biocatalysis is carried out by CPEP-phosphonomutase (CPEP is carboxyphosphoenol pyruvate) isolated from infusoria *Tetrahymena pyriformis*. This enzyme initiates the condensation of PEP with phosphonoformate and subsequent rearrangement of phosphoenol pyruvate to phosphonopyruvate with the formation of a P–C bond. The reaction catalyzed by CPEP-phosphonomutase also proceeds with retention of the absolute configuration of the phosphorus atom.[94]

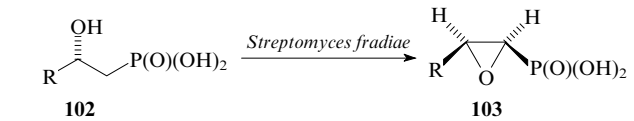


Ala is the alanine residue.

Biosynthesis of 2-amino-1-hydroxyethylphosphinic acid (OH-AEP) (*R*)-101 was reported.[17,89] Enantioselective hydroxylation of 2-aminoethylphosphinic acid (AEP) is carried out by the enzyme isolated from amoeba *Acanthamoeba castellanii*.

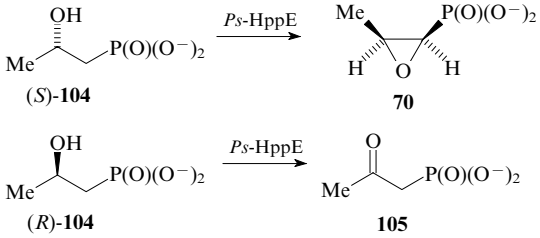


Hammerschmidt and co-workers[95–97] studied biocatalytic epoxidation of (*S*)-2-hydroxyalkylphosphonic acids **102** to epoxides **103** (fosfomycin analogues). The reaction was initiated by the enzyme isolated from a Streptomyces fradiae strain. Replacement of hydrogen at the C_α atom of deuterated 2-hydroxypropylphosphonic acids catalyzed by this enzyme proceeded stereospecifically. It was found that the growth-supporting microenvironment of *Streptomyces fradiae* contains not only fosfomycin (*cis*-epoxide), but also 3% of its co-metabolite (*trans*-epoxide). The formation of *cis*-epoxides involved inversion of the absolute configuration of the carbon atom, whereas *trans*-epoxides were formed with retained configuration. This fact was proved by transforming deuterated hydroxyphosphonates to epoxides. Deuterated (1*S*,2*S*)- and (1*R*,2*S*)-2-hydroxy-1-D-propylphosphonic acids were synthesized from (1*S*,2*S*)-2-benzyloxy-1-D-propanol obtained by catalytic reduction of the corresponding aldehyde in the presence of horse liver alcohol dehydrogenase.[86]

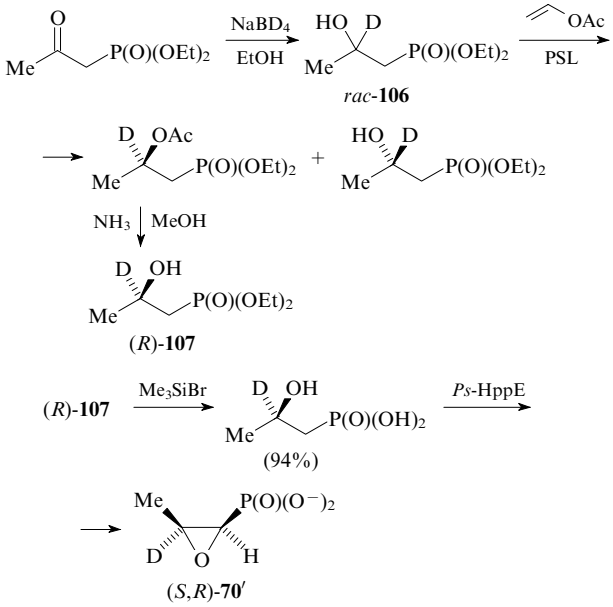


R = H, Et.

A similar biocatalytic transformation of hydroxyphosphonate **104** to fosfomycin **70** in the presence of (*S*)-2-hydroxypropylphosphonic acid epoxidase (*Ps*-HppE) isolated from phytopathogenic bacteria *Pseudomonas syringae* was reported.[98,99] The purified *Ps*-HppE catalyzed the epoxidation of (*S*)-hydroxypropylphosphonic acid **104** with the formation of fosfomycin salt **70**, while the oxidation of hydroxyphenylphosphonic acid (*R*)-**104** under the same conditions gave 2-oxopropylphosphonic acid (**105**).



Acylation of hydroxyphosphonate **106** with vinyl acetate was carried out in isopropyl ether in the presence of lipase from *Pseudomonas syringae*. The reaction was completed in 5 days at 30 °C. Chromatographic separation on silica gel followed by deacetylation afforded optically active hydroxyphosphonate (*R*)-**107** (yield 46%), which was then transformed to a deuterated analogue of fosfomycin (*S*,*R*)-**70′**. The isotope label experiment showed that the epoxide oxygen atom of fosfomycin (*S*,*R*)-**70′** is derived from the hydroxyl group of hydroxyphosphonate **106**.[98]



5. Enzymatic synthesis of compounds with chiral phosphorus atom

The problem of creation of a chiral centre at the phosphorus atom by biocatalytic methods is of great practical and theoretical importance.[100–119] Compounds with chiral phosphorus atom are used as ligands in asymmetric synthesis catalyzed by transition metal complexes.[120,121]

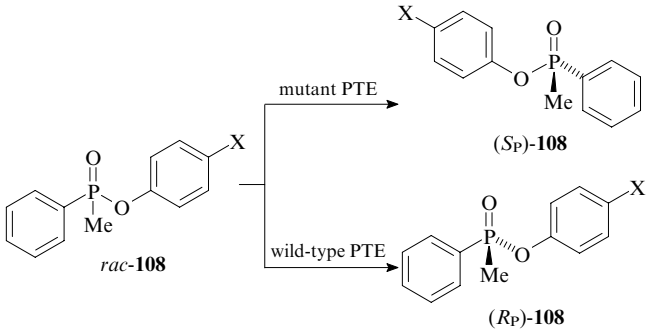
The order of substituent priority in chiral phosphonates and phosphinates as well as the determination of absolute configuration of the phosphorus atom in these compounds deserve particular attention.[122–124] The order of substituent priority in tetrahedral phosphorus compounds differs from

that in carbon compounds with a true C=O multiple bond (Alk < R–O–C < C=O). The P=O bond in phosphates, phosphonates and related compounds is traditionally represented as double bond, although it is more correct to treat it as a single bond with two electron pairs localized on the oxygen atom. That is why substituents at tetrahedral phosphorus have the following priority order: Alk < P=O < R–O–P. For long, classification of stereochemical descriptors for tetrahedral phosphorus compounds was ambiguous. Opinions differed on the priority order of the P=O group and the R–O–P ester group. This led to contradictions in notation of the stereodescriptors R and S in determining the absolute configurations of P atoms in such compounds. But the IUPAC recommendations [124] published in 2004 to a great extent harmonized the situation. In this review, in order to avoid contradictions, we used the IUPAC recommendations even if the authors of original communications used older rules.

a. Kinetic resolution of esters of phosphorus-containing acids

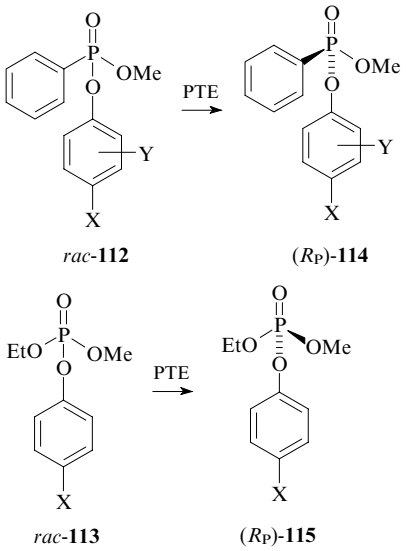
Raushel and co-workers [100–105] resolved racemic phosphinates bearing phenol as the leaving group at the phosphorus atom into enantiomers using phosphotriesterase from *Pseudomonas diminuta* (PTE). An X-ray diffraction study [103] revealed that the active site of PTE contains two Zn^{2+} ions linked by a hydroxyl bridge. The active site of PTE is asymmetrical, which allows one to use the enzyme for kinetic resolution of racemic phosphates and phosphonates with high enantioselectivity. It was found that the rates of enzymatic hydrolysis of stereoisomers of phosphinate **108a** under the action of PTE differ appreciably, *viz*., by more than three orders of magnitude for the reaction in aqueous methanol (pH 8.0) in the presence of HEPES buffer [HEPES is 4-(2-hydroxyethyl)-1-piperazinoethanesulfonic acid]. Hydrolysis led to chiral phosphinate **108a** (Scheme 4) whose optical purity estimated by chiral electrophoresis exceeded 99.8%. The absolute configurations of products were proved by X-ray diffraction analysis of compound (S_P)-**109** obtained by derivatization with the protected amino acid **110**. Based on the results obtained, the authors concluded that PTE-catalyzed hydrolysis of the ester of phosphinate *rac*-**108a** leads to methylphenylphosphinate (S_P)-**111** and that phosphinate (R_P)-**108a** remains unreacted.[100]

It was found that wild-type PTE mainly hydrolyses S_P-stereoisomers of phosphinates **108** while mutant phosphotriesterase I106T/F132A/H254G/H257W (TAGW) mainly hydrolyses (R_P)-phosphinates.[100] In the latter case, the enantioselectivity factor for the formation of compounds (S_P)-**108** was equal to 17.



X = H, NO_2, CHO, CN, Ac, CO_2Me, Cl, F.

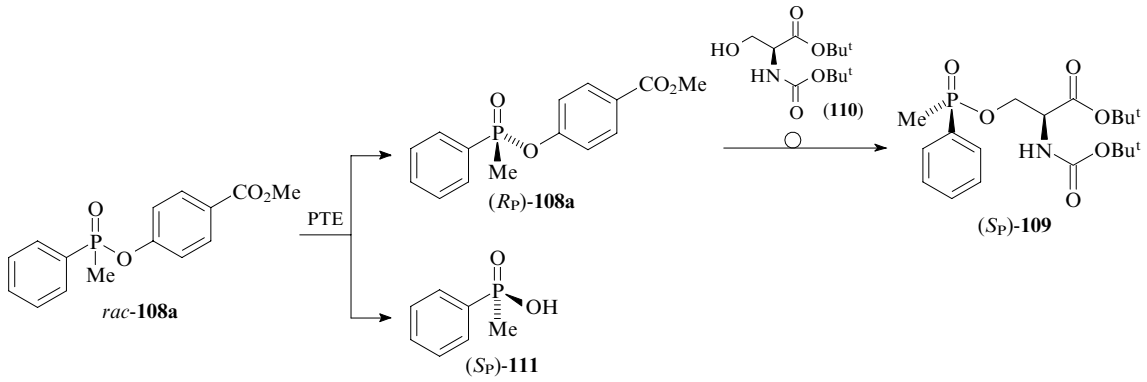
The stereoselectivity of wild-type PTE was affected by the pK_a value of the phenol corresponding to the leaving group.[101–104] Under optimal conditions, the stereoselectivity of hydrolysis of phosphinates **112** and phosphates **113** by PTE was very high.[104] Compounds (R_P)-**114** and (R_P)-**115** were chromatographically purified on silica gel and isolated in 98% yield with 99% *ee*.[101]



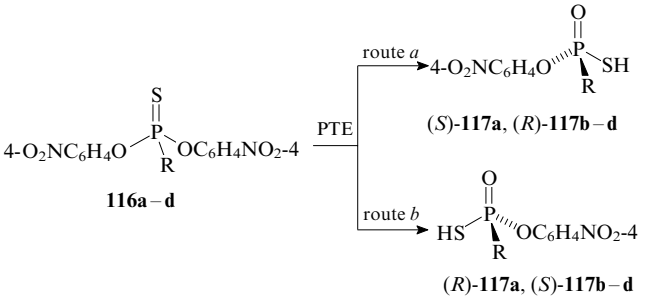
X = NO_2, CHO, CN, Ac, CO_2Me, Cl; Y = 2-F, 3-F, H.

It was shown that hydrolysis of *S*- and *R*-enantiomers of phosphates by mutant or wild-type PTE proceeds at different rates.[102] The authors of that study isolated preparative-

Scheme 4

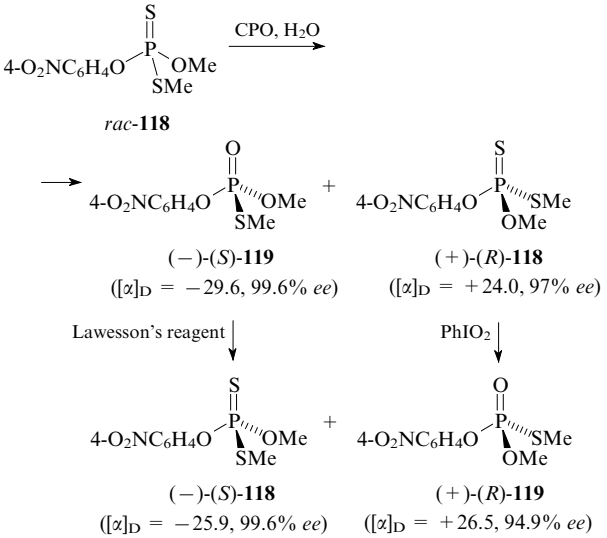


scale amounts of enantiopure R_P- and S_P-enantiomers of organic phosphonates **116** using enzymatic kinetic resolution of the corresponding racemates. The reactions were initiated by introducing wild-type and mutant (G60A, I106G/F132G/H257Y, I106A/F132A/H257W) enzymes into the reaction mixture. As shown below, hydrolysis of prochiral (*S*)-4-nitrophenoxyl group under the action of PTE (route *a*) resulted in the *S*-thioacid while hydrolysis of prochiral (*R*)-4-nitrophenoxyl group (route *b*) led to the *R*-thioacid. The stereochemistry of the products was affected by substituents in compounds **116** and by the nature of PTE. Hydrolysis of phosphonate **116a** (R = Me) by wild-type PTE mainly gave thioacid (*S*)-**117a**, while the reactions carried out in the presence of mutant enzymes resulted in thioacid (*R*)-**117a**. On the contrary, hydrolysis of thioacid esters **116b**–**d** by either wild-type or mutant PTE mainly afforded thioacids (*S*)-**117b**–**d** with 99% *ee*. Chiral thiophosphates **117** thus obtained are thought to be the starting compounds for the synthesis of complex organic and organophosphorus compounds.[102]



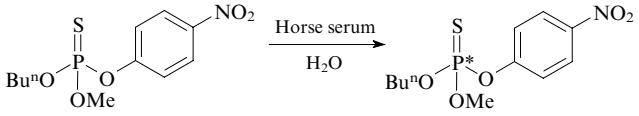
R = Me (**a**), OMe (**b**), OEt (**c**), OPr^i (**d**).

Biocatalytic oxidation of racemic *O*,*S*-dimethyl *O*-4-nitrophenylphosphoroditioate (**118**) by chloroperoxidase from *Caldariomyces fumago* (CPO) resulted in (−)-(*S*)-enantiomer of the corresponding thiophosphate (**119**) and nonoxidized substrate (+)-(*R*)-**118**.[125] Both compounds were isolated with high enantiomeric yield (99.6% and 97%, correspondingly). Thionation of (−)-(*S*)-thiophosphate **119** with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphethane-2,4-disulfide] gave (−)-(*S*)-phosphorodithioate **118** with complete stereospecificity, while oxidation of the unreacted substrate (+)-(*R*)-**118** by iodoxybenzene gave oxone (+)-(*R*)-**119** with 94.9% *ee*.



Raushel and co-workers[126] studied the mechanism of hydrolysis of triphosphates in ^{18}O-labelled water by phosphodiesterase isolated from *Pseudomonas diminuta*. Hydrolysis of (S_P)-*O*-(4-nitrophenyl) *O*-ethylphenyl phosphorothioate by phosphodiesterase resulted in the S_P-enantiomer of *O*-ethylphenyl phosphonothioic acid. The authors concluded that the reaction proceeds with inversion of the absolute configuration of phosphorus atom.

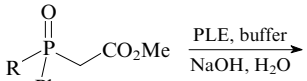
Dudman and Zerner[107] reported enzymatic synthesis of optically active phosphates containing an asymmetric phosphorus atom. Hydrolysis of racemic methyl n-butyl 4-nitrophenyl phosphate **120** catalyzed by horse serum led to optically pure phosphate (+)-**120** with 99% *ee*.



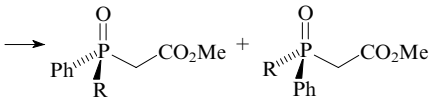
rac-**120** (+)-**120** ($[\alpha]_D^{25}$ = 8.48)

Hydrolytic activity of PTE towards enantiomers of some neuroparalytic substances of the sarin and soman type was studied.[106] It was found that mutant forms of PTE hydrolyze the S_P-enantiomers of these compounds at a higher rate. The mutant forms of PTE were more active in hydrolysis of the S_P-enantiomers in the corresponding racemic mixtures as well; this feature was proposed for the resolution of such mixtures.

Enzymatic hydrolysis of racemic phosphinates **121a**–**e** was carried out in the presence of pig liver esterase (PLE) in a phosphate buffer at 30 °C.[113, 116, 118, 119] An autotitrator was used to maintain a constant pH value. The reaction was stopped when a 50% conversion was attained. The unreacted esters (*S*)-**121a**–**e** and the hydrolysis products, acids (*R*)-**122a**–**e**, were separated chromatographically (see Refs 113, 116, 118, 119).



rac-**121a**–**e**

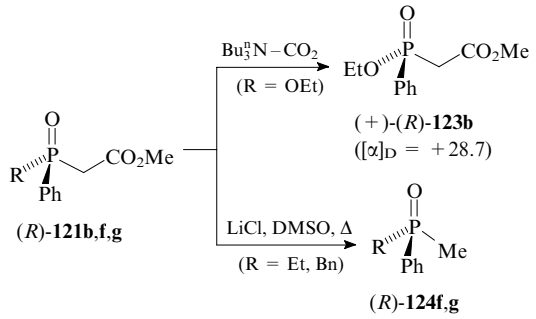


(*S*)-**121a**–**e** (*R*)-**122a**–**e**

Compound	R	**121**		**122**	
		yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	MeO	40	9.5	44	64
b	EtO	46	67	40	71
c	PrO	69	48	30	50
d	Pr^nO	50	26	47	14
e	Bu^nO	54	30	33	40

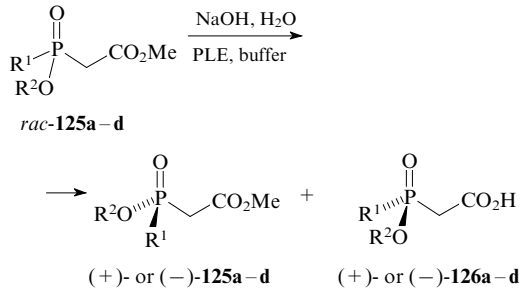
Enantiopure esters **121b**,**f**,**g** were decarboxylated with the formation of optically active phosphinates **123b** and phosphine oxides **124f**,**g**. The absolute configurations were

determined by chemical correlations and by circular dichroism spectroscopy.[116]



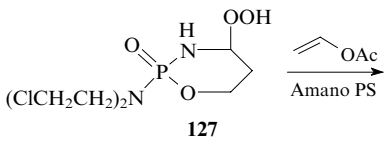
R = EtO (**b**), Et (**f**), Bn (**g**).

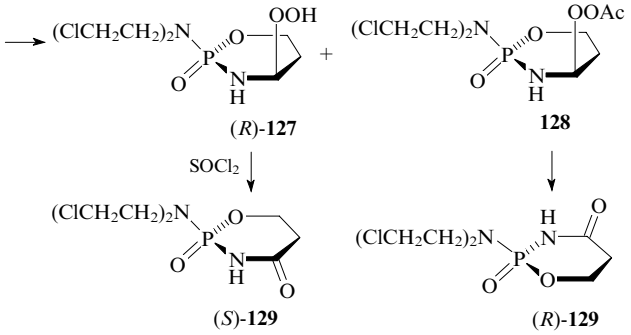
Hydrolysis of phosphonyl acetates **125a**,**b**, phosphoryl acetates **125c** and phosphamide **125d** in the presence of PLE resulted in mixtures of P-chiral phosphorus-containing acetic acids **126a**–**d** and esters **125a**–**d**.[118] Enzymatic hydrolysis under kinetically controlled resolution was carried out until 50% conversion.[113] PLE-catalyzed hydrolysis of phosphonyl acetates proceeded enantioselectively to give optically active products in good chemical yields, but the optical yields were relatively low. In all cases, the reaction mainly involved the *R*-enantiomers of acetates **125a**–**d**.



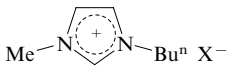
Compound	R^1	R^2	125		126	
			yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	Et	Me	50	38	34	42
b	Et	Et	40	–	60	–
c	PhO	Et	66	20	22	52
d	Et$_2$N	Me	20	90	58	25

Japanese chemists[108] reported kinetic resolution of racemic 4-hydroperoxycyclophosphamide **127** possessing antitumour activity. Acylation in the presence of lipase Amano PS led to compounds (*R*)-**127** and **128**, which were transformed to the corresponding *S*- and *R*-stereoisomers of **129**.





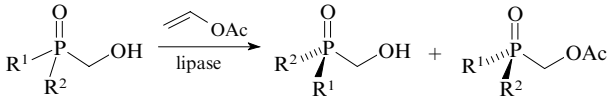
Various organic media including ionic liquids and supercritical CO_2 were used for the resolution of racemic phosphinates.[115] Acetylation of racemic P-chiral hydroxymethylphosphinates and phosphine oxides in the presence of a lipase was carried out under kinetic resolution conditions in ionic liquids BMIM$^+$X$^-$ (BMIM is 1-n-butyl-3-methylimidazolium).[111, 112]



X = PF_6, BF_4.

Both lipases from *Pseudomonas fluorescens*, namely, immobilized Amano AK and non-immobilized PFL, were six times more efficient in BMIM$^+$PF$_6^-$ compared to organic solvents.[110] Contrary to this, reactions in BMIM$^+$BF$_4^-$ showed almost no stereoselectivity.

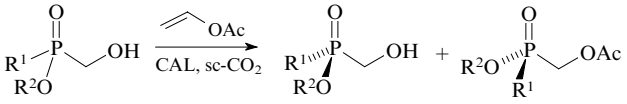
Racemic hydroxymethylphosphinates **130a**–**d** and hydroxymethylphosphine oxide **130e** were acetylated with vinyl acetate in the presence of lipases in ionic liquids. Of two ionic liquids studied, BMIM$^+$PF$_6^-$ showed the best performance, which allowed one to increase the stereoselectivity of enzymatic resolution and to obtain compounds **130*a**–**e** and **131*a**–**e** with satisfactory optical purity. The *E* values were 3–6 times higher when substrates containing bulky organic substituents were used.



Compound	R^1	R^2	Lipase	*ee* for 130* (%)	*ee* for 131* (%)
a	Ph	MeO	PFL	89	89
b	Ph	EtO	AK	79	83
c	Ph	Pr^{i}O	PFL	96	80
d	Et	Pr^{i}O	PFL	95	50
e	Ph	Bu^t	AK	43	53

Kinetic resolution of racemic P-chiral hydroxymethylphosphinates **130a**–**d** by enzymatic transesterification in supercritical carbon dioxide (sc-CO_2) under different conditions was studied.[114, 115] It was found that the excess pressure affects the yields and enantiopurity of the products **130*a**–**d** and **131*a**–**d**. The reaction rate reached its maximum when the pressure approached a critical value of 11 MPa and decreased with further increase in the pressure up to 15 MPa. Optimal results were obtained at a pressure of 13 MPa, namely, a 50% conversion was attained with

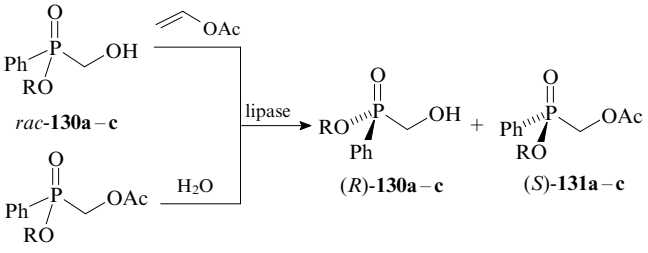
ease and the enantioselectivity was highest. Thus, by varying the pressure, one can change the reactivity of the substrate and the stereoselectivity of the process. The stereoselectivity was also influenced by the solvent used, by the volume of substituents at the phosphorus atom and by the solubility of the substrates in sc-CO_2. The best results were obtained using lipase from *Candida antarctica* (Novozym 435).[114]



rac-**130a–d** **130*a–d** **131*a–d**

Compound	R^1	R^2	Conversion (%)	**130***		**131***	
				yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	Ph	Me	8	81	4	5	7
b	Ph	Et	93	6	88	82	6
c	Ph	Pr^i	46	52	38	46	27
d	Et	Pr^i	100	—	—	90	—

Resolution of racemic hydroxymethylphosphinates **130a–c** by transesterification with vinyl acetate in the presence of lipase from *Pseudomonas cepacia* (Amano PS) proceeded with moderate ee. In some cases, hydrolysis of the corresponding acetates **131a–c** was more efficient and led to an increase in *ee*. It was found that three successive hydrolyses of enantioenriched acetates **131a–c** catalyzed by PFL and Amano PS increased the enantiopurity of the products to more than 92%.[117]



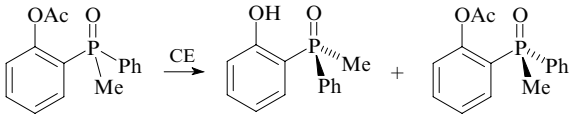
rac-**130a–c** *rac*-**131a–c** (*R*)-**130a–c** (*S*)-**131a–c**

Compound	R	Lipase	**130**		**131**	
			yield (%)	*ee* (%)	yield (%)	*ee* (%)
a	Me	PFL	44	80	39	89
	Me	Amano PS	42	92	44	86
b	Et	PFL	42	54	53	47
	Et	Amano PS	30	54	68	21
c	Pr^i	PFL	52	36	45	24

b. Enzymatic resolution of phosphine oxides and phosphines

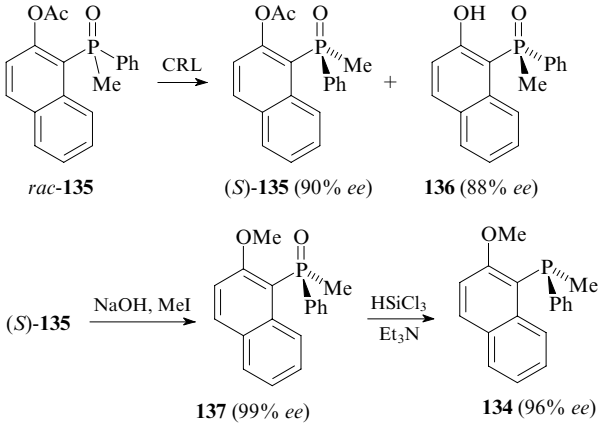
Among different classes of chiral phosphorus-containing compounds, chiral phosphine oxides are of particular importance.[120,121] They are used as chiral inductors in asymmetric synthesis and are precursors of phosphines — chiral ligands in transition metal complexes. Therefore, the development of biocatalytic methods for the synthesis of phosphine oxides containing chiral phosphorus atoms is of considerable interest.

Serreqi and Kazlauskas[127] reported the synthesis of P-chiral hydroxyaryl phosphines and phosphine oxides by enzymatic acetylation. Among the commercially available enzymes tested, the best results were shown by cholinesterase (CE) and CRL. CE-catalyzed hydrolysis of a synthetic substrate (phosphine oxide acetate **132**) proceeded seven times faster than hydrolysis of natural cholesterol acetate under the same conditions. Similar results were obtained using CRL. The enantioselectivity of the resolution varied from low to high, but the optical purity of compound (*S*)-**133** reached a value of 95%.



rac-**132** (*S*)-**133** (*R*)-**132**

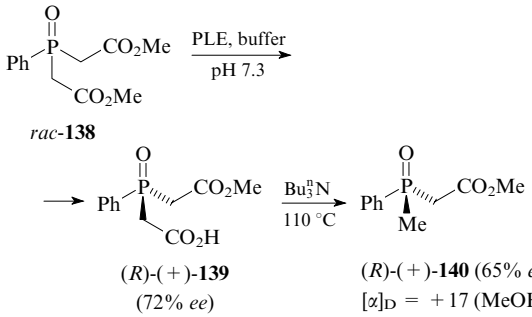
The same strategy was also used to synthesize chiral tertiary naphthylphosphines **134**, the starting compounds for the synthesis of chiral Wittig reagents. Biocatalytic hydrolysis affords a mixture of acetate **135** and alcohol **136**. Subsequent methylation of (*S*)-**135** leads to chiral methoxyphosphine oxide **137** (99% *ee*). The stereospecific reduction of compound **137** gives a chiral tertiary phosphine **134** (96% *ee*). Reactions using CRL and CE exhibited nearly the same enantioselectivity.[127]



rac-**135** (*S*)-**135** (90% *ee*) **136** (88% *ee*)

137 (99% *ee*) **134** (96% *ee*)

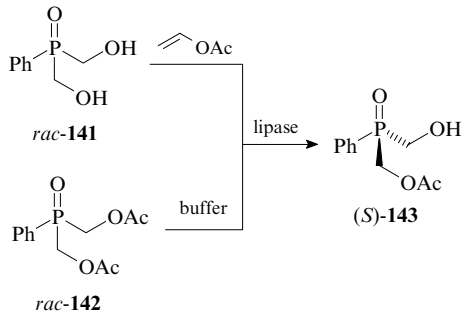
Mikołajczyk and co-workers[109] studied biocatalytic desymmetrization of bifunctional phosphinates and phosphine oxides. Hydrolysis of bis(methoxycarbonylmethyl)-phenylphosphine oxide (**138**) was carried out in a phosphate buffer in the presence of pig liver esterase. Chiral monoacetate (*R*)-**139** was isolated by column chromatography in 92% yield and decarboxylated to give a chiral phosphine



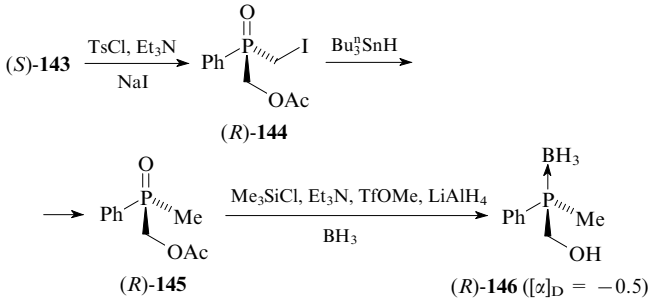
rac-**138** (*R*)-(+)-**139** (72% *ee*) (*R*)-(+)-**140** (65% *ee*) $[\alpha]_D = +17$ (MeOH)

oxide **140**. The absolute configuration of compound **140** was known; this allowed one to determine the *R*-configuration of compound **139**.

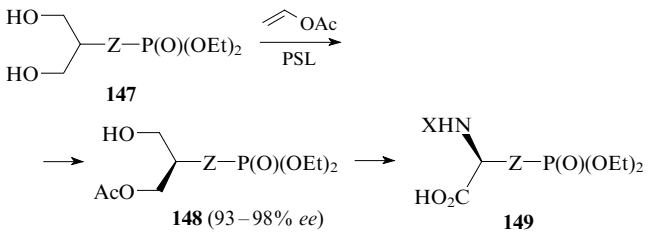
Biocatalytic acetylation of prochiral bis(hydroxymethyl)phenylphosphine oxide (**141**) and biocatalytic hydrolysis of prochiral bis(acetoxymethyl)phenylphosphine oxide (**142**) were carried out in the presence of various lipases including PFL, LAH-S, Amano AK and Amano PS.[109] Among them, the best results were obtained using PFL in chloroform. In this case, chiral compound (*S*)-**143** was formed in 50% yield with 79% *ee*. Replacement of the solvent (including the use of ionic liquid $BMIM^+PF_6^-$) and the introduction of various additives had no effect on the selectivity.



The absolute configuration of compound (*S*)-**143** was determined by chemical correlations. To this end, alcohol (*S*)-**143** was converted to iodide (*R*)-**144** which was then reduced to phosphinate (*R*)-**145** whose transformation resulted in a borane complex of phosphinic acid (*R*)-**146** with the known absolute configuration.[109]



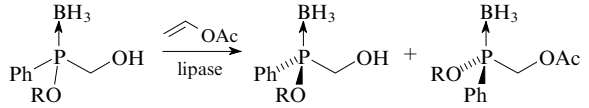
A similar technique was employed for desymmetrization of prochiral 2-(ω-phosphono)alkylpropane-1,3-diols **147**. Acetylation of diol **147** catalyzed by lipase from *Pseudomonas* sp. led to chiral phosphonates **148** (93% *ee*–98% *ee*), which were converted to protected chiral α-amino-ω-phosphonic acids **149**.[128]



Z = CH_2, CH_2CH_2, CH_2CF_2; X = Cbz, Boc.

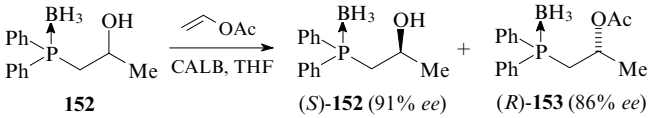
6. Enzymatic resolution of borane complexes with trivalent phosphorus compounds

In contrast to P-chiral hydroxymethylphosphine oxides, analogous P-borane complexes appeared to be poor substrates for lipase-catalyzed reactions.[129–131] The reactions proceeded slowly with low stereoselectivity, probably due to the size difference between the oxygen atom and BH_3 group and to different electronic effects of the P=O and P–BH_3 bonds. Mikołajczyk *et al.*[129] reported biocatalytic resolution of alkoxy-(hydroxymethyl)phenylphosphineboranes **150** using transesterification with vinyl acetate. The reactions were carried out in diisopropyl ether or cyclohexane in the presence of lipases CAL, PSC, Amano AK, Amano PS and Amano AH. The reaction in diisopropyl ether was slow (it took 10 to 40 days to complete) and showed a low stereoselectivity (*ee* of products **150** and **151** were from 2% to 20%). The reaction in cyclohexane proceeded much faster and took 6 to 21 h to complete, but the selectivity remained low (37% *ee*). It should be noted that the reactions in the presence of lipases Amano AK and Amano PS led to optical antipodes of enantioenriched phosphinites.

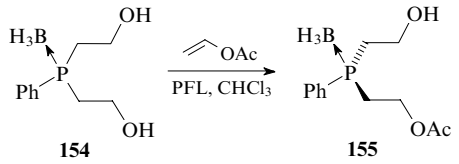


R = Et, Pr^i.

The stereoselectivity of resolution increased in the case of a borane complex with 2-hydroxypropylphosphine **152** containing an asymmetric centre in the side chain. A large number of enzymes was studied, *viz*., Amano R10, Amano AK, Amano AY, CALB, PPL, PFL, lipases from *Mucor miehei*, *Candida* sp., *Aspergillus niger*, *Candida lipolytica*, as well as acylase I and proteinase 6. The highest efficiency of resolution was achieved with CALB. For instance, the reaction of racemic (2-hydroxypropyl)diphenylphosphineborane **152** with vinyl acetate in the presence of CALB gave the (*S*)-enantiomer of alcohol **152** (91% *ee*) and acetate **153**. The enantioselectivity factor was equal to 41, thus being reasonably high.[130]

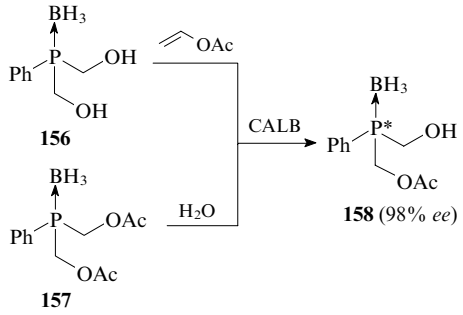


Desymmetrization of prochiral diol **154** was carried out using vinyl acetate as the acylating agent in the presence of various lipases including CAL, PFL, PSC, Amano AK, Amano AH and Amano PS. Among them, only PLF appeared to be efficient. The nature of solvent had strong effect on the stereoselectivity of the process, namely, replacement of chloroform by diisopropyl ether resulted in an optical antipode of product **155**. This is probably due to different polarities of these solvents because the reaction in chloroform (solvent of lower polarity) showed a higher stereoselectivity (*ee* of the product was 90%) compared to the reaction in diisopropyl ether (10% *ee*).[129]



154 **155**

Wiktelius *et al.*[131] used various lipases [Amano A, Amano PS, CALB (Novozym 435), Amano AK, PPL (Fluka)] for desymmetrization of prochiral phosphine complexes **156** and **157** that gave enantiomers **158**. The best results were obtained using CALB, *viz.*, compounds **158** formed with enantiopurity higher than 98%.

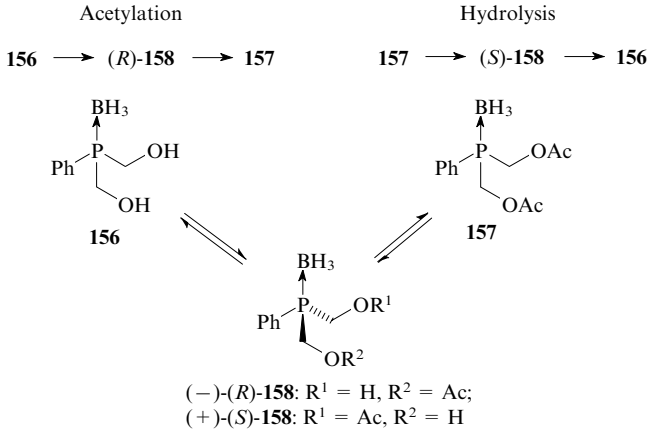


156 **158** (98% *ee*)

157

Scheme 5 demonstrates the stereochemistry of acetylation (vinyl acetate, lipase) and hydrolysis (lipase, THF–Pr^i_2O, pH 7) of compounds **156** and **157**, respectively, under the conditions suitable for regeneration of substrates. Hydrolysis of acetoxyphosphonates proceeded slower and showed a lower stereoselectivity than biocatalytic acetylation of hydroxyphosphonates. This is probably due to a smaller size difference between substituents. Desymmetrization led to different enantiomers of compound **158**.[131]

Scheme 5



Thus, lipases that are relatively cheap and experimentally convenient enzymes (especially when immobilized on solid supports) gradually replace microorganisms in everyday laboratory practice. Unfortunately, in the case of racemic OPCs, lipases sometimes exhibit low activity and one should still use microorganisms in biocatalysis.

III. Microbiological synthesis of chiral organophosphorus compounds

Living microorganisms that excrete enzymes directly into the reaction medium can be used to carry out biocatalytic reactions in which racemic substrates are transformed to enantiopure compounds.[132–136] The main advantage of using microorganisms is high stereoselectivity of reactions they catalyze. The drawbacks include low reaction rates (conversion times from a few days to weeks) and low substrate concentrations in the reaction medium. Usually, microbiological catalysis is carried out in aqueous solutions;[132] therefore, substrates to be used should be water-soluble. Recently, techniques were developed to allow one to carry out microbiological reactions in nonaqueous media.[135] This is essential for OPCs that are often unstable of insoluble in water. Such techniques use two-phase systems, emulsions or anhydrous media. However, in all cases, proper conditions are required for living biocatalysts to be active and vital in organic media. In particular, freeze-drying and immobilization increases the stability of biocatalysts against poisoning by toxic organic solvents. These techniques were employed for, *e.g.*, bioreduction of α-ketophosphonates, which are readily hydrolyzed in water with cleavage of the P–C-bond.[136]

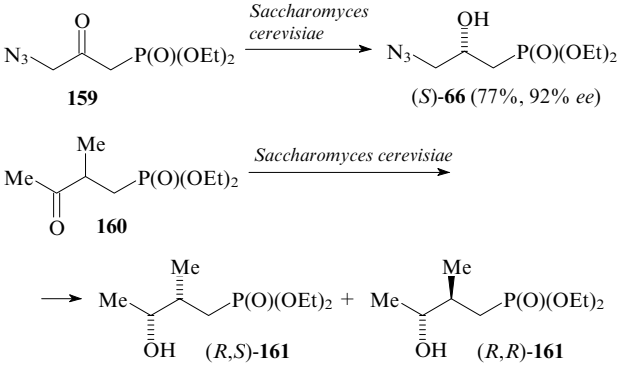
In some cases, the activity of microorganisms can be increased by pre-incubation under heat treatment, anaerobic cultivation or cultivation under starvation conditions. Moreover, various additives affect the activity of microorganisms.[132,133] Recently, two monographs[2,3] and a review[134] concerning the use of microorganisms for stereoselective biotransformations of organic compounds have been published. This Section is, in fact, a supplement to these publications because no data on organophosphorus compounds were reported.

Biosynthesis of OPC is carried out using various yeasts, microscopic fungi and bacteria[135–149] including *Acinetobacter baumanni*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Rhodococcus* sp., *Serratia liquefaciens* bacteria; *Aspergillus niger*, *Beauveria bassiana*, *Beauveria brongniartii*, *Cladosporium* sp. Op328, *Cunninghamella elegans*, *Geotrichum candidum*, *Penicillium citrinum*, *Penicillium oxalicum*, *Verticillium* sp., fungi, as well as yeasts *Saccharomyces cerevisiae*, *Rhodotorula rubra* and *Rhodotorula glutinis*. Some of these organisms are sources of biocatalysts that are isolated and used separately. Freeze-dried microorganisms (this form is most convenient for practical use) are commercially available from chemical and biochemical supply companies (Sigma-Aldrich, Fluka, *etc.*).

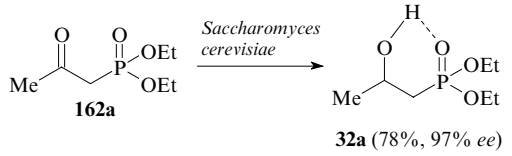
1. Yeast-catalyzed synthesis

Baker's yeast (*Saccharomyces cerevisiae*) is a readily available and versatile catalyst widely used in organic chemistry.[132–143] Ordinary yeast from a grocery store is often well-suited for experiments. The best-studied process which is most often carried out in the presence of this biocatalyst is reduction of ketones including phosphorus-containing ones. For instance, yeast-catalyzed asymmetric reduction of diethyl 2-oxoalkylphoshonates **159** and **160** respectively leads to 2-hydroxyalkylphosphonates **66** and **161** obtained in good yields with enantiopurity as high as about

97% – 100%.[132, 139] The reduction of phosphonate **160** affords a mixture of diastereomers in a total yield of 70% and an (*R*,*S*) : (*R*,*R*) ratio of 2 : 1.

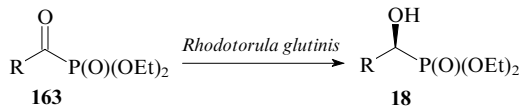


A similar biocatalyzed reduction of diethyl 2-oxopropylphosphonate **162a** resulted in 2-hydroxyalkylphosphonate **32a** with *ee* ~97%.[132] According to spectroscopic studies, this hydroxyphosphonate exists in a 'frozen' conformation due to the formation of an intramolecular hydrogen bond between the phosphonate oxygen atom and the hydroxyl hydrogen atom.



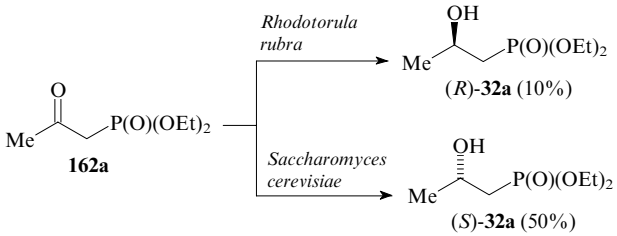
The efficiency of biocatalyzed reduction of β-ketophosphonates **160** and **162a** using *Saccharomyces cerevisiae* depends strongly on the nature of the substrate.[130, 138] For example, in the case of compound **160**, the product yields decrease if methyl group in the immediate vicinity of the carbonyl group creates steric hindrance. Using anaerobic-cultivated yeast, one can to some extent overcome this problem and increase the product yield and the selectivity of reduction.[140] In particular, anaerobic pre-incubation allowed one to carry out biocatalyzed reduction of diethyl-3-oxo-4-methylbutylphosphonate which could not be reduced under normal conditions and to obtain the corresponding hydroxyphosphonate with 85% *ee*.[140]

Microorganisms *Rhodotorula rubra*, *Rhodotorula glutinis*, *Cladosporium* sp., *Verticillium* sp. and *Saccharomyces cerevisiae* were used for enantioselective reduction of diethyl-α-ketophosphonates **163** which are rapidly hydrolyzed in water with cleavage of C—P bond. To suppress hydrolysis, the process was carried out under anhydrous conditions. The reaction catalyzed by freeze-dried cells immobilized on Celite R 630 resulted in α-hydroxyphosphonates **18**.[135, 136, 140] The best results were obtained in the reduction of compounds **163** catalyzed by immobilized *Rhodotorula glutinis* cells in anhydrous hexane, *viz*., the products **18** were obtained with the enantiopurity as high as 99%.[135, 142, 145]



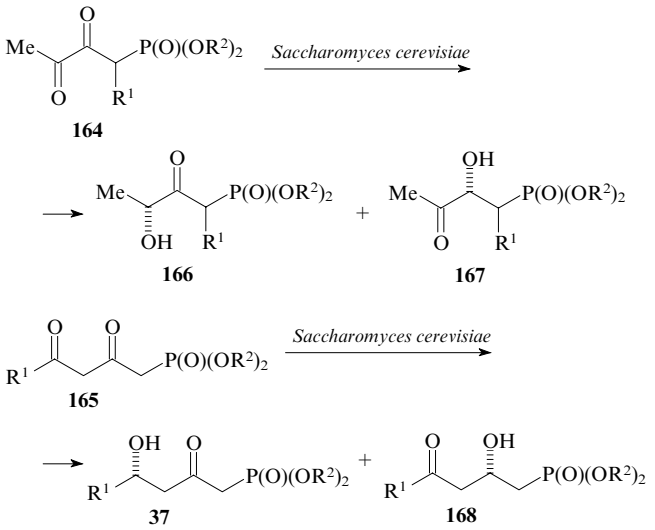
R = H, Me, Pr^i, $PhCH_2CH_2$.

Chiral (*R*)- and (*S*)-diethyl 2-hydroxypropylphosphonates **32a** of high enantiopurity were obtained by biocatalyzed reduction of ketophosphonate **162a** in the presence of the following microorganisms: *Rhodotorula rubra*, *Rhodotorula glutinis*, *Rhodotorula gracilis* and *Saccharomyces cerevisiae*.[143] Reduction in the presence of *Saccharomyces cerevisiae* gave only *S*-enantiomers, while the reaction in the presence of *Rhodotorula rubra* afforded (*R*)-hydroxyphosphonate **32a**. The products were characterized by *ee* > 90%.



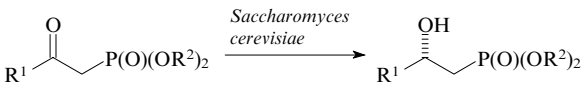
The addition of ethyl chloroacetate or methyl vinyl ketone to the baker's yeast reducing medium also led to changes in the absolute configuration of the product, namely, the *R*-stereoisomer of 2-hydroxyphosphonate formed. With no additives, (*S*)-2-hydroxyphosphonate formed with 99% *ee*.

Biocatalyzed reduction of dioxoalkylphosphonates **164** and **165** containing two ketone groups gives, in the presence of baker's yeast, mixtures of isomers of hydroxy(keto)alkylphosphonates **166**, **167** and **37**, **168**, respectively, in reasonable chemical yields, the optical yields ranging from moderate to good [from 80% *ee* to (92% – 94%) *ee*]. Some of these compounds were isolated as individual substances and used as chiral Horner – Wadsworth – Emmons reagents.[141]



R^1 = Me, CF_3, Pr^n; R^2 = Et, Bu^n.

Asymmetric reduction of halogenated diethyl 2-ketoalkylphosphonates **169e**,**g** – **n** with dry yeast gave the corresponding diethyl 2-hydroxyphosphonates **32e**,**g** – **n** in good yields with satisfactory enantiopurity.[139, 141]



169e,g–n **32e,g–n**

Compound	R^1	R^2	Time /h	Yield (%)	*ee* (%)	Configuration
e	CH_2Cl	Et	12	82	72	*R*
g	CH_2Cl	Me	24	74	70	*R*
h	CH_2Cl	Pr^i	24	57	13	–
i	CH_2Cl	Bu	24	88	70	*R*
j	CH_2Br	Et	24	35	83	*R*
k	CH_2Br	Pr^i	24	41	52	–
l	CH_2Br	Bu^n	24	55	87	*R*
m	CF_3	Et	48	86	52	–
n	C_3F_7	Et	24	55	20	–

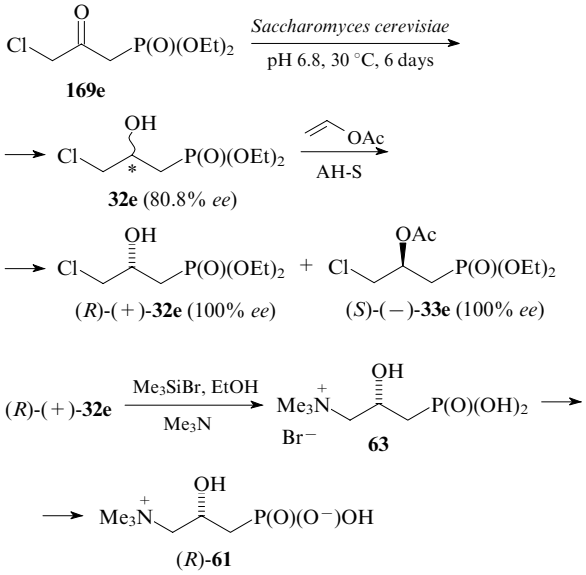
Note. A dash means that the configuration was not determined.

Reduction of ketophosphonates **169** was carried out at 30 °C under aerobic conditions. In the case of chemically nonreactive compounds, anaerobic reduction was used. The stereoselectivity of reduction depended on the nature of 3-substituted-2-oxopropylphosphonates. Electron-withdrawing groups in the side chain caused the optical yields of the products to decrease. For instance, the enantiomeric yields and purities of 1-bromo-2-hydroxypropylphosphonates **32j–l** were higher than those of the corresponding chloro derivatives. Also, biocatalyzed reduction of phosphonates **169m,n** bearing CF_3 or C_3F_7 groups proceeded with low enantiomeric excesses.[141] Bulky isopropyl groups at the phosphorus atom in compounds **32h,k** also hampered the process.

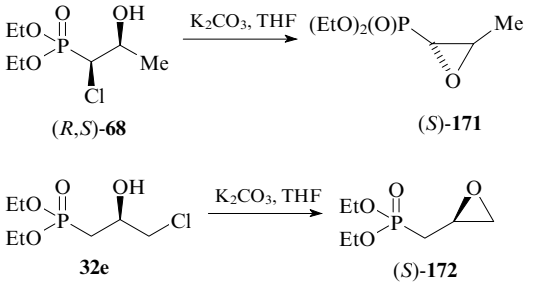
3-Substituted-2-hydroxyalkylphosphonates **32** thus obtained were used as chiral reagents for the synthesis of phosphorus-containing analogues of bioactive molecules, such as carnitine and GABOB.[40, 139, 141] The sequence of reactions is similar to that presented in Scheme 3.

An improved version of enantioselective synthesis of phospho-GABOB (**170**) involves double biocatalysis to obtain enantioenriched hydroxyphosphonate **66**.[40] Bioreduction of ketone **159m** results in hydroxyphosphonate **66** (90% *ee*) which is then subjected to additional enzymatic resolution in the presence of CALB to give enantiopure product (*S*)-**66′**. Subsequent reduction of azide (*S*)-**66′** with hydrogen in the presence of palladium affords 3-amino-2-hydroxypropylphosphonate (*S*)-**64**, which was treated with bromotrimethylsilane and methanol to form phospho-GABOB (*S*)-**170** in high yield with high *ee* (Scheme 6).

Mikołajczyk *et al.*[63] developed a method for chemoenzymatic synthesis of phosphocarnitine enantiomers using microbiological reduction in the key step of the process. Phosphocarnitine (**61**) was synthesized from diethyl 2-oxo-3-chloropropylphosphonate **169e** in three steps including reduction, additional enzymatic purification of enantioenriched phosphonates **32e** and **33e**, transformation of compound **32e** to phosphonic acid and the final reaction with trimethylamine resulting in trimethylammonium salt (*R*)-**61**. This technique allowed one to obtain enantiopure phosphonates (*R*)-(+)-**32e** and (*S*)-(−)-**33e**, which were then converted to phosphocarnitine enantiomers.

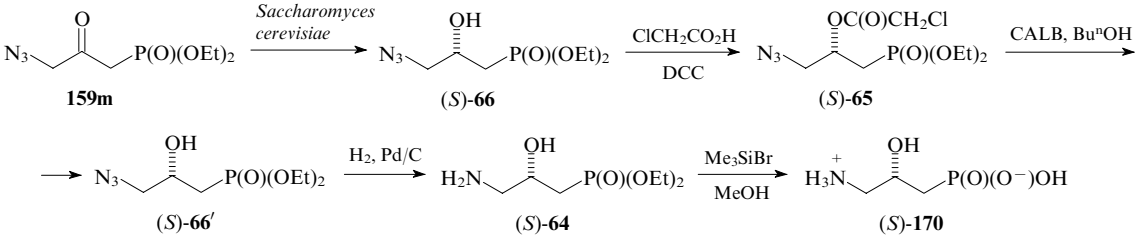


Enantiopure hydroxy(chloro)alkylphosphonates **32e** and **68**[40, 139] obtained by the microbiological method were also used in the synthesis of precursors of fosfomycin. Treatment of chiral compounds (*R*)-**32e** and (*R,S*)-**68** with potassium carbonate in THF led to chiral epoxyphosphonates (*S*)-**171** and (*S*)-**172** in good yields with satisfactory enantiopurity.

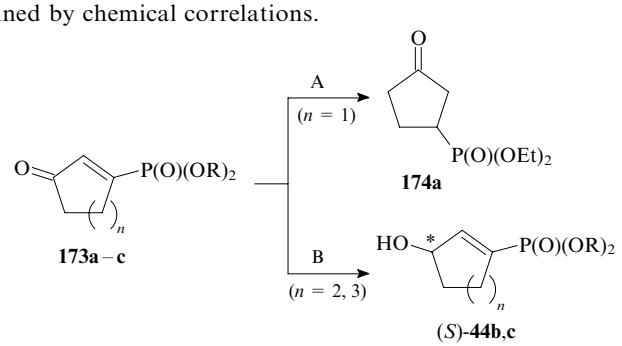


Attolini *et al.*[70, 71] carried out enantioselective reduction of cyclic dialkyl 3-oxoalk-1-enylphosphonates **173a–c** with various types of yeast, *viz.*, fermented yeast, freeze-dried dry yeast, as well as acetone extracts from dry powdered yeast. Reduction of the six- and seven-membered enones **173b,c**

Scheme 6



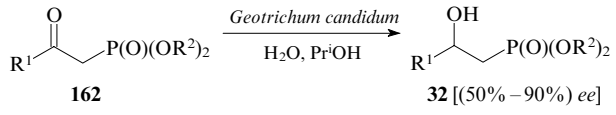
with fermented yeast led to the corresponding phosphonates **44b**,**c** in satisfactory yields with enantiomeric excesses ranging from moderate to good. At the same time, reduction of five-membered enonephosphonate **173a** involved the C═C double bond and gave a cyclic ketone **174a** with low *ee*. Variation of the reaction conditions did not considerably increase the optical purity of reaction products. For the six-membered rings, it was found that bulky substituents at the phosphorus atom increase the enantioselectivity of the reduction to 95% *ee*. Reduction of six-membered enonphosphonates **173b** with acetone yeast extract increased the yields of the reduction products **44b**, although their optical purity remained almost the same. Reduction of cyclic enones with dry yeast in organic solvents did not increase the product yields and enantioselectivity of the reaction. As the volume of the alkyl substituent at the phosphorus atom in the six-membered compounds **173b** increased on going from Me to Et and Pr^i, the optical yields of compounds **44b** increased from 45% to 95%. The *S* absolute configurations of the six-membered hydroxyphosphonates **44b** were determined by chemical correlations.



n = 1 (**a**), 2 (**b**), 3 (**c**); R = Me, Et, Pr^n;
A stands for fermented yeast, B stands for dry yeast.

2. Synthesis using microscopic fungi

Microscopic fungi *Geotrichum candidum* (milk mould) available as two different strains, IFO 4597 and IFO 5767, usually exhibit high stereoselectivity in the reduction of ketones of different structure. However, the reduction of 2-oxoalkylphosphonates with these fungi was efficient only for diethyl 2-oxopropylphosphonate **162** (R^1 = Me, R^2 = Et) which was converted to diethyl (+)-(*R*)-2-hydroxypropylphosphonate (**32a**) under the action of *Geotrichum candidum* with 98% *ee* in satisfactory yield.[61] Other substrates **162** containing larger substituents either were reduced in low yields or did not react at all.



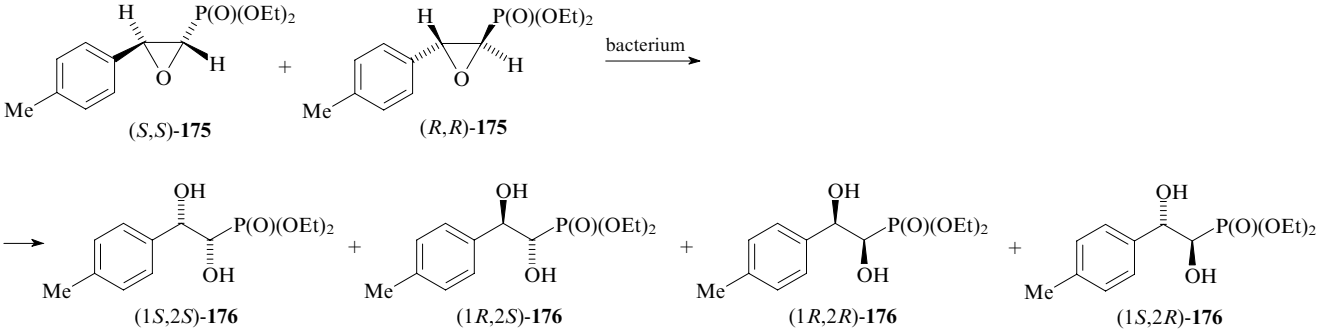
162 **32** [(50% – 90%) *ee*]

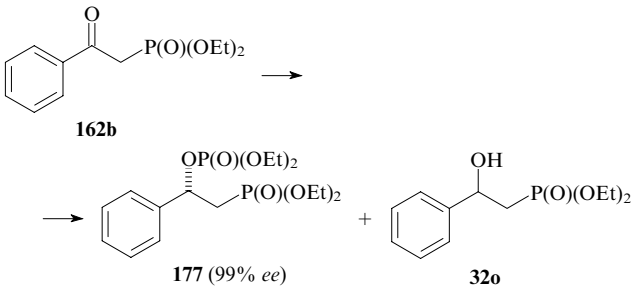
R^1 = Me, Bu^n, Ph, Ph(CH$_2$)$_3$; R^2 = Me, Et.

Hydrolytic oxirane ring opening in substituted 1,2-epoxyphosphonates **175** was carried out under the action of microbial cell cultures.[136, 144, 148] In contrast to chemical hydrolysis, phosphorus-containing oxiranes mainly converted to *erythro*-1,2-dihydroxyphosphonates containing small amounts of *threo*-isomers under biocatalysis conditions.[148] For example, chemical hydrolysis of compound *trans*-**175** led to a mixture of *threo*- and *erythro*-stereoisomers **176** in 79% yield with 85 : 15 ratio. At the same time, microbiological hydrolysis by *Beauveria bassiana* fungi gave these stereomers in a total yield of 59% and a 42 : 58 ratio.[136, 144] The *erythro*-isomer was obtained with 100% *ee* and the *threo* -isomer was obtained with 98% *ee* as 2*S*-enantiomer. It was found that this type of reaction is typical of such microorganisms as *Aspergillus niger*, *Cunninghamella elegans*, *Beauveria bassiana*, *Beauveria brongniartii*, *Rhodotorula glutinis* and *Rhodococcus* sp. (Scheme 7).

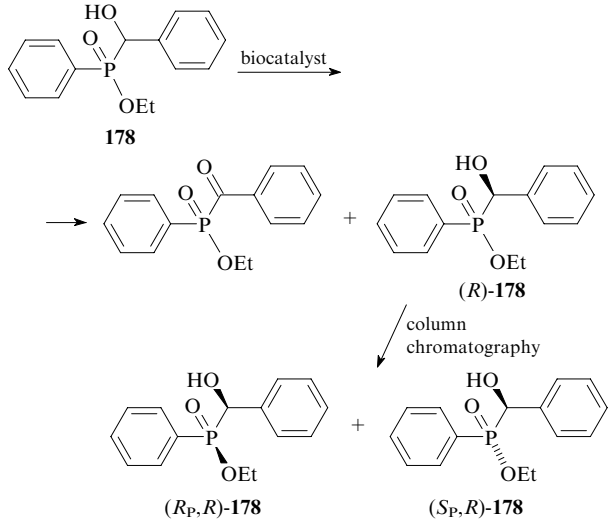
Biotransformation of diethyl 2-oxo-2-phenylethylphosphonate (**162b**) under the action of *Beauveria bassiana* 271B, *Rhodotorula rubra* 70403, *Rhodotorula glutinis* 10134, *Geotrichum candidum* 6593, *Penicillium oxalicum* and *Cladosporium* sp. Op328 strains resulted in the optically active *O*-phosphorylated 2-hydroxy-2-phenylethylphosphonate (**177**) with 99% *ee* and a small amount of the corresponding hydroxyphosphonate **32o**.[54, 144, 145] The biotransformation took 72 h to complete at ambient temperature with vigorous stirring using ethyl acetate as an additive.[145] A similar transformation catalyzed by *Cladosporium* sp. led solely to the *S*-stereoisomer of compound **32o**. The reaction under the action of *Beauveria bassiana* also gave the target compound (total yield was 85%) even with no additives. To increase the biocatalyst activity, fungi *Geotrichum candidum* were grown using chemical additives affecting their activity. For instance, the introduction of ethanol into the reaction mixture increased the yield of compound **177**.[55] Microscopic fungi *Penicillium oxalicum*, *Aspergillus flavus*, *Geotrichum candidum* and *Rhodotorula gracillis* were grown on dextrose potato agar and then on Chapeck growth medium that provided the conditions favorable for the growth of microorganisms.

Scheme 7



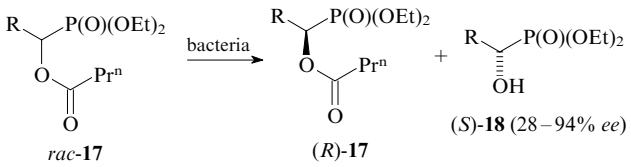


Racemic ethyl 1-hydroxybenzyl(phenyl)phosphinate (**178**) was used as substrate in the fungi-catalyzed process. The racemic mixture contained four stereoisomers, a levorotatory pair (R_P,*R*) + (S_P,*S*) and a dextrorotatory pair (R_P,*S*) + (S_P,*R*). Preincubation of the biological catalyst (pretreated fungi *Geotrichum candidum* with or without chemical additives) in the presence of oxalic acid to improve the efficiency of the biotransformation allowed one to resolve phosphinate **178** into pairs of diastereomers (R_P,*R*)-**178** and (S_P,*R*)-**178** with high *ee* values (> 99%).[146]



3. Synthesis using bacteria

Kafarski *et al.*[136, 137, 149] successfully used lipolytic bacteria to resolve racemic α-hydroxyalkylphosphonates **17** into enantiomers. For example, hydrolysis of racemic diethyl 1-butyryloxyalkylphosphonates **17** by *Pseudomonas fluorescens* and *Penicillium citrinum* bacteria resulted in chiral hydroxyphosphonates (*S*)-**18** with good enantioselectivity.[142] *Penicillium citrinum* bacteria hydrolyzed substrates containing aliphatic substituents in α-position, whereas *Pseudomonas fluorescens* bacteria were more efficient in the hydrolysis of substrates with aromatic substituents. The degree of substrate conversion increased with time, being accompanied by a decrease in the enantiopurity of the products.



Bacteria are *Pseudomonas fluorescens* or *Penicillium citrinum*; R = Et, Pr^n, Bu^n, Bu^i, All, 4-XC_6H_4 (X = H, Cl, MeO), $PhCH_2CH_2$, PhCH(Me), 2-Fu.

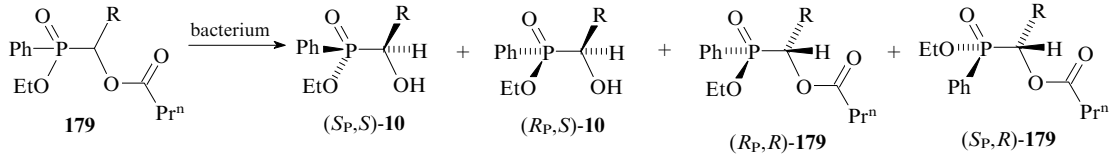
Bacteria appeared to be efficient in the resolution of diastereomeric mixtures of hydroxyphosphinates containing two asymmetric centres. For example, racemic phosphinate esters **179** (mixtures of four diastereomers) were at first hydrolyzed using four types of bacteria, namely, *Bacillus subtilis*, *Acinetobacter baumanni*, *Serratia liquefaciens* and *Pseudomonas aeruginosa*.[53, 137] These bacteria predominantly hydrolyzed diastereomers having *S* absolute configuration of the Cα atom. The best results (90% *ee* for products) were obtained with *Bacillus subtilis*. Thus four diastereomers, *viz*., (S_P,*S*)-**10**, (R_P,*R*)-**179**, (R_P,*S*)-**10** and (S_P,*R*)-**179**, gave two pairs of diastereomers, which were separated by column chromatography. Then, diastereoenriched acyloxyphosphinates **10** were hydrolyzed again with *Bacillus subtilis*. The diastereomeric pairs thus obtained were purified by preparative HPLC. As a result, some diastereomers, *e.g*., hydroxyphosphonate (R_P,*S*)-**10** (R = Ph), were isolated in optically pure form. The absolute configuration of this compound was determined by X-ray analysis (Scheme 8).[137]

Pseudomonas fluorescens bacteria and *Penicillium citrinum* mould fungi were also used for kinetic resolution of 1-butoxyphosphonates to obtain optically active diethyl (*S*)-α-hydroxyalkylphosphonates in moderate to high yields with satisfactory optical purity.[147, 149] Using various strains of bacteria (*Aspergillus fumigatus*, *Proteus vulgaris*) and fungi *Actinomycetes*, *cis*-1,2-epoxypropylphosphonic acid was successfully resolved into *S*- and *R*-enantiomers.[150]

IV. Conclusion

In this review, we considered various procedures for enzymatic kinetic resolution and some other techniques that allow one to obtain individual enantiopure OPCs in high optical yield. It should be noted that, in spite of impressive achievements in the synthesis and research on the properties of chiral OPCs, some problems still persist. The search for enantioselective methods providing easy access to optically active phosphorus-containing acids, tertiary phosphines and phosphine oxides remains topical. In this connection, elaboration of highly efficient methods of enzymatic and

Scheme 8



R = Me, Ph.

microbiological synthesis of chiral OPCs is of particular importance.

Currently, lipases are widely used to obtain chiral compounds for organophosphorus chemistry. The possibility of carrying out enzymatic processes with high selectivity makes lipases very attractive catalysts, especially for transformations that cannot be performed under other conditions. We have demonstrated versatile applications of biocatalysts, *e.g.*, the possibility to obtain various biologically active OPCs in organic solvents. Most biocatalytic reactions can be carried out in health- and environmentally safe conditions. The advantages of biocatalysis over chemical processes are due to its high stereoselectivity and applicability at ambient temperature and pressure. Enzymes can catalyze various types of chemical reactions, thus providing access to a broad spectrum of chiral substances. Therefore, combining biocatalysis and asymmetric synthesis will give an impetus to the design of novel synthetic strategies of practical and theoretical importance.[151–154]

One can expect that biocatalytic methods of synthesis of organophosphorus compounds will remain at the focus of intensive research, especially in the field of research on biologically active compounds. In the future, targeted design of lipase application techniques, their modification and creation of new preparative forms of lipases will serve as a basis for novel broad-spectrum biocatalysts for industry, especially, pharmacy.

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