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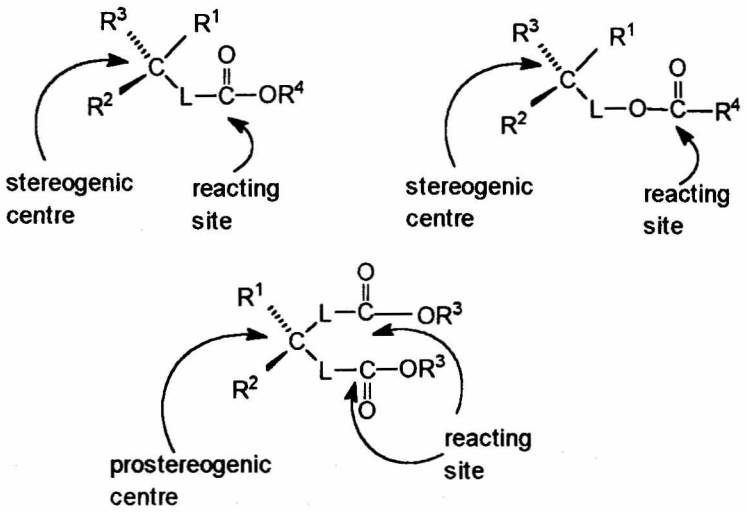
APPLICATION OF HYDROLYTIC ENZYMES IN THE SYNTHESIS OF CHIRAL HETEROORGANIC COMPOUNDS

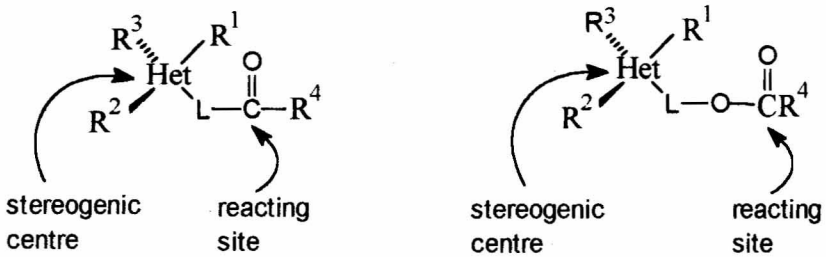
Abstract: Commonly available hydrolytic enzymes are capable of recognizing heteroatom stereogenic centres. A review of the application of these enzymes in the preparation of optically active sulfur and phosphorus compounds, based on the results from the authors' Laboratory, is presented.

The application of enzymes, which are proteins created by Nature to catalyze chemical reactions occurring in living organisms, for the transformation of unnatural organic compounds, has recently become a powerful synthetic methodology [1,2]. This is due to the salient feature of enzymes which, being very specific in their natural functions, can also be used *in vitro* and accept a wide range of man-made substrates. As enzymes are intrinsically chiral, they have been widely used in the synthesis of optically active molecules, both from racemic and prochiral substrates. This is particularly true for ester hydrolases, such as esterases and lipases, since a lot of them combine a high reaction stereoselectivity with a broad substrate tolerance. Scheme 1 shows the most common approach utilizing hydrolytic enzymes. The stereogenic or prostereogenic centre is here recognized and stereoselectively bound in the enzyme active site, but is not directly involved in the chemical transformation (formation or cleavage of the ester bond), which takes place at a remote reacting site. The stereogenic centre may be located either in the acid or in the alcohol part of the ester moiety.



SCHEME 1

The above scheme shows the situation in which the stereogenic or prostereogenic centres are located on a carbon atom. However, the same approach can be applied to heteroorganic substrates, the only difference being the fact that a heteroatom and not carbon will be the stereogenic centre (Scheme 2). It is worth noting that this approach to the synthesis of optically active heteroorganic compounds envisages the use of the same common hydrolytic enzymes which catalyze the formation or cleavage of the ester moiety.



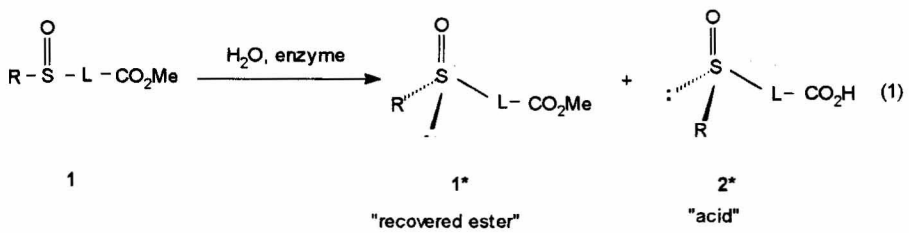
Het = P, S, Si, Ge

SCHEME 2

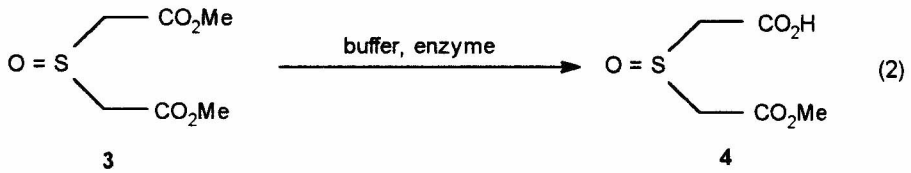
As a continuation of our interest in the synthesis and transformation of optically active sulfur and phosphorus derivatives, we undertook investigations on the possibility of using enzymes for these purposes, utilizing the methodology depicted above. In this context, it should be emphasized that optically active sulfur and phosphorus compounds play an important role in asymmetric and stereoselective synthesis. Of particular importance are chiral sulfoxides [3] and related derivatives used in transmitting chirality from sulfur to other centres, and phosphine oxides [4] which are precursors of chiral phosphines, serving mainly as chiral ligands in homogeneous catalysis. The aim of the present paper is to summarize the results obtained in our laboratory. Some earlier papers of ours as well as of other authors will be discussed only briefly, as they were exhaustively presented elsewhere [5].

Synthesis of optically active sulfoxides

The enzymatic approach to the synthesis of chiral sulfoxides was a subject of several accounts. It was based on the enzyme-promoted hydrolysis of racemic sulfinylcarboxylates performed under kinetic resolution conditions and gave products in good yields and with high ee (Eq. 1) [5,6].



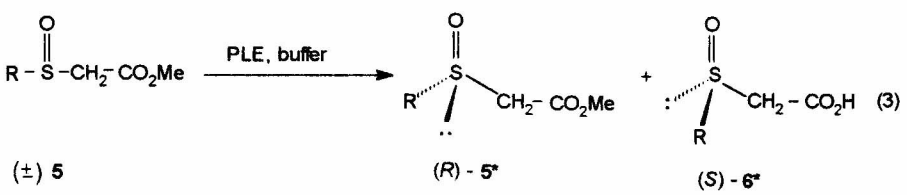
To avoid the disadvantages connected with a kinetic resolution of racemic mixtures (e.g. a theoretical yield of 50% and sometimes the loss of one of the enantiomers) we decided to apply prochiral dimethyl sulfinyldiacetate **3**, which in principle should be an ideal substrate for the enzyme-promoted asymmetric synthesis [7]. Among several enzymes tested, two, *i.e.* porcine liver esterase (PLE) and α-chymotrypsin (α-CT) proved to be useful and gave enantiomerically enriched monoesters **4**. Interestingly, the two enzymes exhibited opposite stereoselectivity. This enabled us to obtain, after repeated crystallization of each enriched sample from acetone, both pure enantiomers and to determine their absolute configuration by an X-ray analysis as (+)-(*S*) and (-)-(*R*) [8] (Eq. 2). Soon thereafter our results were fully confirmed by the independent investigations of a Japanese group [9].



3 4 (2)

Enzyme	Yield of **4** (%)	$[\alpha]_D$ (MeOH)	Ee (%)	Abs. config.
PLE	70	+15.8	79	*S*
α-CT	63	-18.3	92	*R*

As PLE was the main enzyme used by us and the Japanese authors, we attempted to identify the molecular interactions within the enzyme active site that determine enantiorecognition. In the absence of an X-ray structure of PLE we decided to check whether the empirical model of the active site of PLE, developed by Jones [10] for C-chiral and prochiral substrates, can be applied to our S-prochiral substrate **3**. Moreover, to examine its applicability also to S-chiral substrates, we performed the PLE-catalyzed hydrolysis of a series of



(±) **5** (*R*) - **5*** (*S*) - **6*** (3)

R = phenyl, p-tolyl, t-butyl

racemic sulfinylacetates **5** under the kinetic resolution conditions. We determined absolute configurations of both the unreacted esters **5*** and acids **6*** and found out that the enantioselectivity of the hydrolysis was identical within the series of substrates investigated [11] (Eq. 3). In both cases the Jones model proved to be suitable for predicting the stereoselectivity of the PLE-mediated hydrolysis (Figs 1, 2).

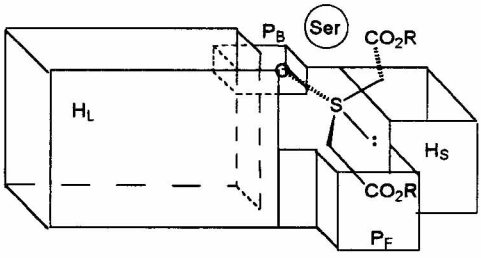


Fig. 1.

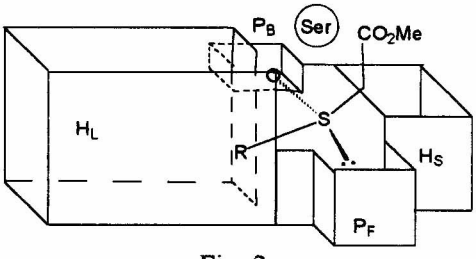


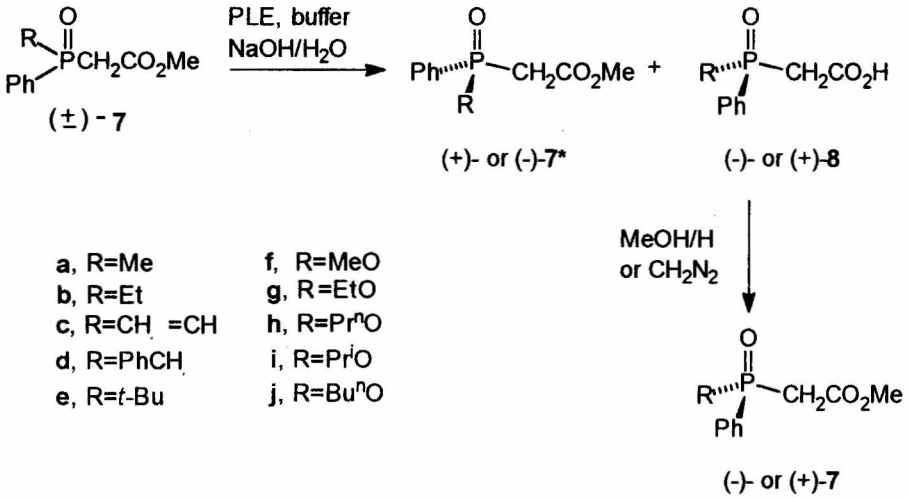
Fig. 2.

According to this model the prochiral substrate **3** should be located in the PLE active site (represented here in the form of five binding loci - four cube-like "pockets" and a serine locus) as follows (Fig. 1). The ester group that undergoes hydrolysis should be placed within the spherical locus of the catalytically active serine function, the second, unreacting ester group - in the front polar pocket (P_F) together with a highly polar lone electron pair, and the sulfinyl oxygen atom in the back polar pocket (P_B). Such a mode of location leads to the conclusion that the (*S*) enantiomer of the monoester **4** should be produced, which is in agreement with the experiment. In turn, the preferentially bound (and thus faster hydrolyzed) enantiomers of **5** require the following location in the PLE active site (Fig. 2). The ester group should be located within the serine locus, the sulfinyl oxygen atom in the back polar pocket (P_B), the large organic substituent in the large hydrophobic pocket (H_L) and the lone electron pair the front polar pocket (P_F). The model predicts here a preferential accommodation of the (*S*) enantiomer of **5**, leading to (*S*) - **6***, which is again in full agreement with the results obtained.

Synthesis of optically active phosphoryl compounds

In the first set of experiments [12,13] we studied the PLE-promoted hydrolysis of racemic phosphinylacetates **7a-e**, which structurally resemble sulfinylacetates **5** presented earlier. As the results were very encouraging (the ee

values exceeded in some cases 95%, except for **7e** which proved to be unreactive under the reaction conditions), we decided to extend our investigations to the phosphonylacetates (**7f-j**) [14] (Scheme 3 and Table 1).



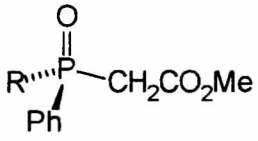
SCHEME 3

Table1
Enzymatic hydrolysis of phosphoroacetates **7**

Substr.	Ester **7***				Acid **8**			
	Yield (%)	$[\alpha]_D$ (MeOH)	ee (%)	Abs. conf.	Yield (%)	$[\alpha]_D$ (MeOH)	ee (%)	Abs. conf.
7a	50	+22.0	82	*R*	42	- 22.2[a]	82	*S*
7b	45	+9.7	>96	*R*	41[a]	- 8.2[a]	81	*S*
7c	40	- 54	~100	*S*	22	+54.5	-	*R*
7d	46	- 23.3	80	*R*	43	+16.9 (+21.7)[a]	~79	*S*
7f	40	-16.1	~95	*S*	44	+9.1 (+10.8)[a]	~64	*R*
7g	46	- 11.3	~67	*S*	40	+13.8 (+11.8)[a]	~71	*R*
7h	69	- 8.7	~48	*S*	30[a]	+13.0 (+9.1)[a]	~50	*R*
7i	50	- 5.8	~26	*S*	47[a]	+3.0[a]	~15	*R*
7j	54	- 3.0	~30	*S*	33[a]	+4.0[a]	~25	*R*

a) After re-esterification

Determination of the absolute configuration of the reaction products (**7,8a-j**), which was based on chemical correlation, circular dichroism (CD) spectra and X-ray analysis revealed that in all cases enantiomers of the same spatial arrangement (shown below) are recognized by the enzyme and hence preferentially hydrolyzed.



7a-j

This general regularity allowed us to apply again the Jones PLE active site model to explain the stereoselectivity observed [13,14]. Thus, the faster hydrolyzed enantiomers of 7 should be accommodated in the enzyme active site as follows (Fig. 3a).

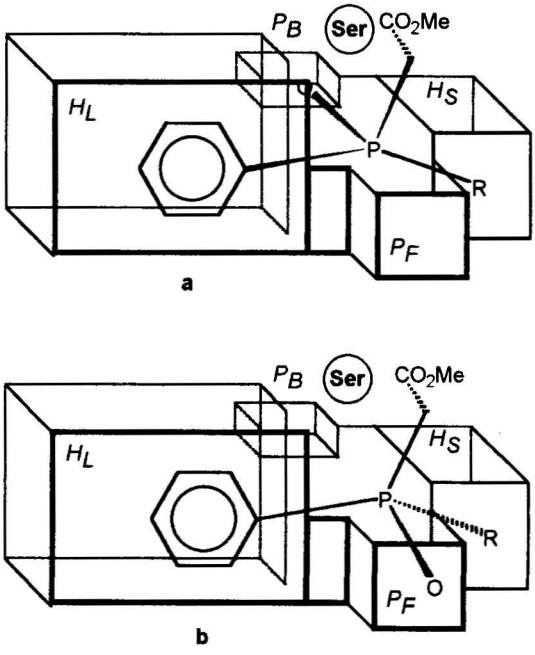
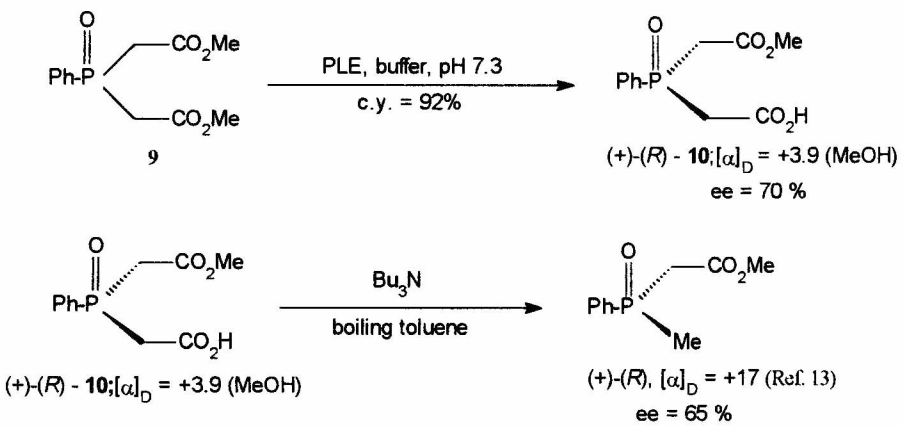


Fig. 3.

The methoxycarbonyl group should be located within the serine locus, the phenyl group in the large hydrophobic pocket (H_L), the alkyl or alkoxyl group in the small hydrophobic pocket (H_S) and the phosphoryl oxygen atom in the back polar pocket (P_B). The location of the three former groups is obvious.

However, some uncertainty arises as to whether the phosphoryl oxygen may alternatively be accommodated in the front polar pocket, P_F (Fig. 3b), which would lead to the preferential hydrolysis of the opposite enantiomer. This is, in our opinion, not the case. According to Jones predictions, the P_F pocket is mostly used to bind the second nonhydrolyzed ester function of diester substrates (cf. Fig. 1 and Fig. 4 below), but it can also accept nonpolar groups. On the other hand, the P_B pocket interacts well, among others, with carbonyl functions, but is too polar to accept hydrophobic moieties. Therefore, the strongly polar P=O group should be preferentially accommodated in the P_B pocket, hence favoring the model shown in Fig.3a, which is in full agreement with the experimental results.

To check whether the Jones model will also be applicable to prochiral phosphoryl substrates (as it is for the sulfinyl analog - cf. **3**, Fig.1), we subjected to the PLE-mediated hydrolysis dimethyl phosphinyldiacetate **9** (for its synthesis see Ref. 15). As a result, the monoester **10** was isolated whose absolute configuration was determined as (+)-(*R*) by chemical correlation (Scheme 4) [16].



SCHEME 4

The diacetate 9 should be located in the PLE active site as follows (Fig. 4).

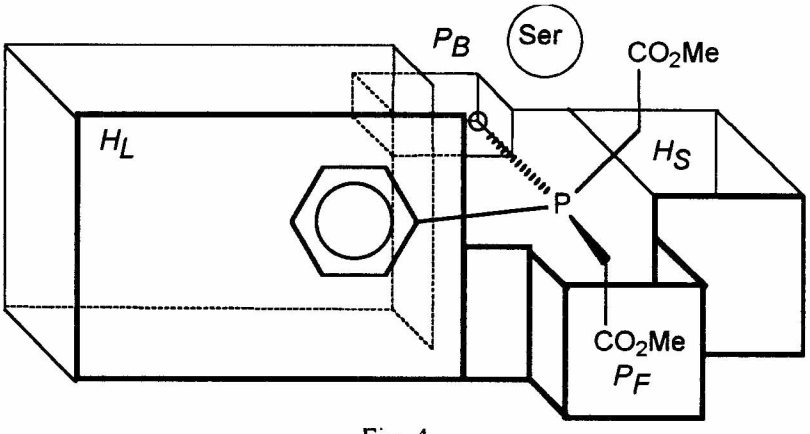
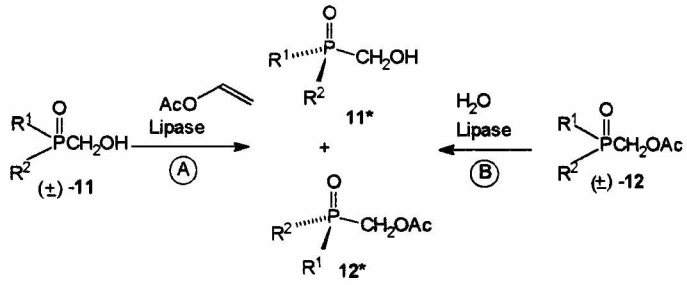


Fig. 4.

The ester group which undergoes hydrolysis should be placed within the locus of serine, the second ester group in the P_F pocket, the phosphoryl oxygen atom in the P_B pocket and finally the large organic substituent (Ph) in the H_L pocket. This mode of location clearly shows that the (*R*) enantiomer of **10** should be formed, which is again in agreement with the experiment.

All the experiments presented so far were performed in aqueous (buffer) solutions, which constitute a natural environment for enzymes. However, hydrolytic enzymes proved to display high catalytic activity also in organic solvents [2]. This concerns mainly lipases which are able to catalyze ester hydrolysis, ester formation, transesterification and other transformations of esters. In this respect, of particular importance are the reactions which allow for the synthesis of optically active primary, secondary and even tertiary alcohols. Encouraged by the successful enzymatic syntheses of chiral phosphorylacetates, we decided to apply the enzymatic methodology to the preparation of α- and β-hydroxyalkanephosphonates, the compounds which have recently gained increasing attention due to their interesting biological activity. To this end we used a lipase-mediated acetylation of P-chiral, racemic hydroxymethanephosphinates and phosphonates **11** (procedure A) or a reverse hydrolysis (procedure B) of the corresponding O-acetyl derivatives **12** (Scheme 5, Table 2) [17]. The reactions were performed in organic solvents under the kinetic resolution conditions and gave both the unreacted substrates and products in good yields and with reasonably high ee.



a, R^1=Ph, R^2=MeO
b, R^1=Ph, R^2=EtO
c, R^1=Ph, R^2= *i*-PrO
d, R^1=*i*-PrO, R^2=Me
e, R^1=*i*-PrO, R^2=Et
f, R^1=*i*-PrO R^2=MeO

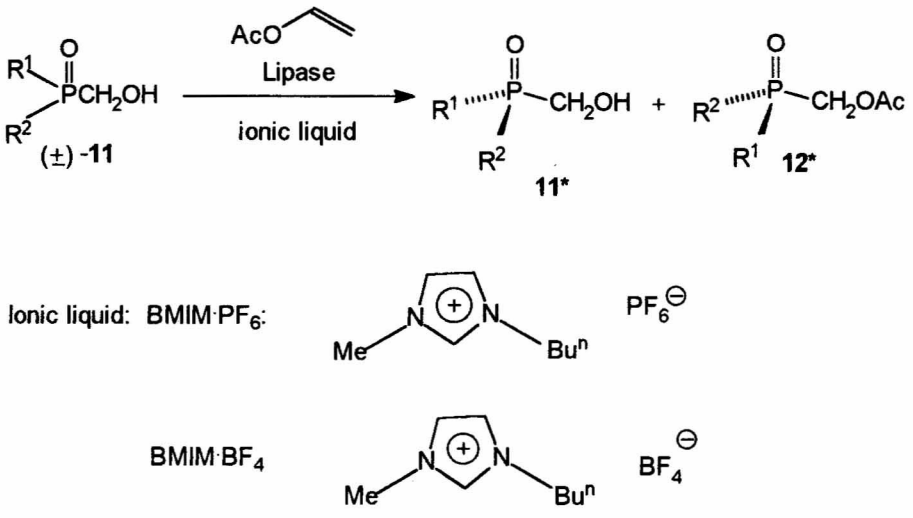
SCHEME 5

Table 2
Lipase-promoted resolution of racemic **11** and **12**

Substr.	Lipase[b)]	Proc.	11*				12*			
			Yield (%)	$[\alpha]_D$ MeOH	Ee (%)	Abs. conf.	Yield (%)	$[\alpha]_D$ MeOH	ee (%)	Abs. conf.
a	PFL	A	44	- 24.7	80	*R*	39	+53.5	89	*S*
a	AM	A	42	- 29.7	92	*R*	44	+51.4	86	*S*
b	PFL	A	42	- 18.5	58	*R*	53	+35.5	47	*S*
b	AM	A	30	- 17.2	54	*R*	68	+14.9	21	*S*
c	PFL	A	37	- 46.6	80	*R*	46	+27.1	21	*S*
d	PFL	A	46	- 15.6 - 18.4[c]	85 >95[c,d]	n.d.[a)]	54	+13.2 +15.5[c)]	85 >95[c,d)]	n.d.[a)]
e	PFL	A	41	- 13.5 - 14.5[c]	86 >95[c,d]	n.d.[a)]	59	+13.3 +18.4	64 >95[c,d)]	n.d.[a)]
f	PFL	B	55	+0.5 +3.0[C)]	16 >92[c,d)]	*S*	45	- 0.93 - 2.3[c,d)]	34 >92[c,d)]	*R*

a) n.d. - not determined
b) PFL: Lipase from *Pseudomonas fluorescens* in *i*-Pr_2O or *t*-BuOMe; AM: AMANO PS Lipase in CH_2Cl_2
c) After repeated hydrolysis of the enantiomerically enriched substrates
d) None of the other enantiomer was detected; e) Neat.

Very recently we have replaced organic solvents with ionic liquids, which are becoming more and more popular as effective solvents for a great variety of organic reactions, among them enzyme-promoted transformations [18]. We have used them as solvents in the reaction of lipase-promoted acetylation of hydroxymethanephosphinates and phosphonates with the hope of enhancing enantioselectivity of the process [19]. To our satisfaction, certain lipases proved to be up to six times more enantioselective in BMIM·PF_6 solutions than in common organic solvents (Scheme 6, Table 3).



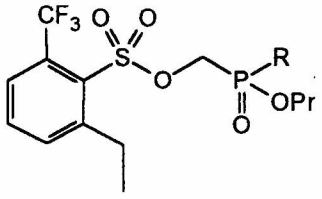
SCHEME 6

Table 3
Kinetic resolution of **11** in ionic liquids

Entry	Substr.	Lipase	Ionic liquid	Recovered alcohol **11***				Acetate **12***				E	E[b]
				Yield[a] [%]	$[\alpha]_D$ ($CHCl_3$)	Ee [%]	Abs. conf	Yield[a] [%]	$[\alpha]_D$ ($CHCl_3$)	Ee [%]	Abs. conf.		
1.	**11a**	AK	PF	33.3	-21.5	89	*R*	37.7	+49.8	89	*S*	51	45
2.	**11a**	PFL	PF	40.0	-20.0	75	*R*	42.0	+41.0	78	*S*	18	
3.	**11a**	AK	BF	44.0	+0.36	1.2	*S*	10.0	-1.2	4.8	*R*	1.1	
4.	**11a**	PFL	BF	14.0	-0.83	1.4	*R*	37.0	+0.5	0.3	*S*	1.0	5
5.	**11b**	AK	PF	36.0	-12.1	79	*R*	36.5	+39.6	83	*S*	26	
6.	**11b**	PFL	PF	44.0	-9.5	63	*R*	30.0	+37.3	76	*S*	14	
7.	**11b**	AK	BF	24.0	-1.1	0.4	*R*	33.0	+0.2	1	*S*	1.0	5
8.	**11c**	AK	PF	36.0	-21.3	95	*R*	48.5	+31.0	80	*S*	32	
9.	**11c**	PFL	PF	39.0	-16.1	86	*R*	49.0	+26.0	68	*S*	15	
10.	**11c**	AK	BF	56.0	+0.4	4.0	*S*	38.0	-0.3	4.5	*R*	1.3	12
11.	**11e**	PFL	PF	32.0	-12.1	95	n.d.	55.0	+10.4	50	n.d.	12	

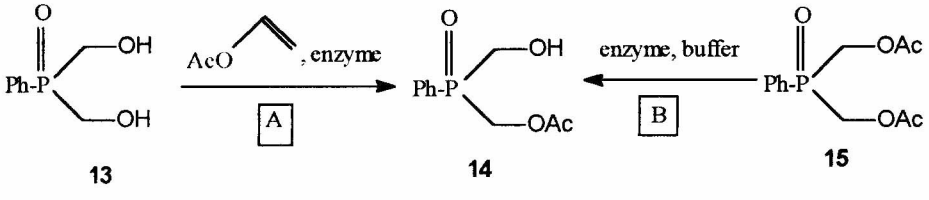
Lipases: AK: Lipase AK (AMANO); PFL: Lipase from *Pseudomonas fluorescens* (FLUKA)
Ionic liquids: PF: BMIM PF_6; BF: BMIM BF_4
n.d. = not determined
a) Yields after separation
b) E-values calculated for the best results obtained in diisopropyl ether using the same enzymes

It should be added that hydroxymethanephosphonates **11d** and **11e** were used for the preparation of enantiomeric phosphosulfonate herbicides. Biological studies revealed that only the compounds obtained from the dextrorotatory enantiomers of **11d** and **11e** exhibited high herbicidal activity, the opposite ones being completely inactive [20].



R=Me, Et

Similar procedures have been applied by us to the enzymatic desymmetrization of prochiral bis(hydroxymethyl)phenylphosphine oxide **13** and its diacetyl derivative **15** (Scheme 7, Table 4). It should be noted that although all the lipases tested exhibited the same sense of stereoselectivity, it was possible to obtain both enantiomerically enriched forms of the monoester **14** by changing the procedure from the acetylation of **13** (A) to the hydrolysis of **15** (B). Investigations to determine the absolute configuration of **14** are under way [16].



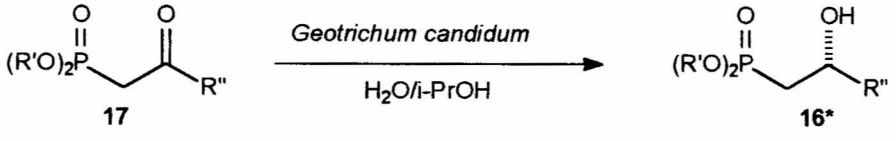
SCHEME 7

Table 4
Enzymatic desymmetrization of **13** and **15**

		Monoacetate **14**		
Enzyme/Solvent	Method	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	e.e. (%)
PFL/$CHCl_3$	A	50	+3.9	79
PFL/DPE	B	50	-3.4	68
AHS/DPE	B	40	-2.4	50
AK/$CHCl_3$	A	76	+0.7	15
AK/THF	A	40	+2.6	53
PFL/BMIM·PF_6	A	35	+0.6	14
PFL/BMIM·PF_6	B	15	-2.0	42

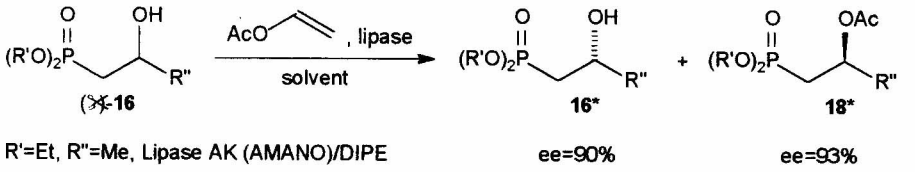
Lipases: PFL: from *Pseudomonas flourescens*; AHS: AMANO - AHS lipase; AK: AMANO AK lipase.
Solvents: DPE: diisopropyl ether; BMIM·PF_6: ionic liquid (see Scheme 6)

2-Hydroxyalkanephosphonates **16** are the only chiral phosphoryl compounds investigated by us, in which the stereogenic centre is located on the carbon and not on the phosphorus atom. Two different procedures were applied to obtain optically active products: a microorganism-mediated asymmetric reduction of the corresponding 2-oxo phosphonates **17** (Scheme 8) and a kinetic resolution of the racemic substrates using lipases (Scheme 9) [21]. In both cases the best results were obtained with the substrates having a small organic substituent in the position 2.



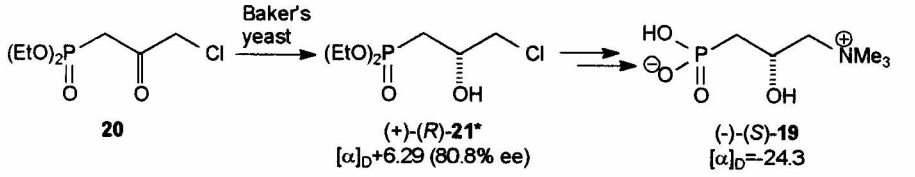
R'=Et, R"=Me, ee=98%

SCHEME 8

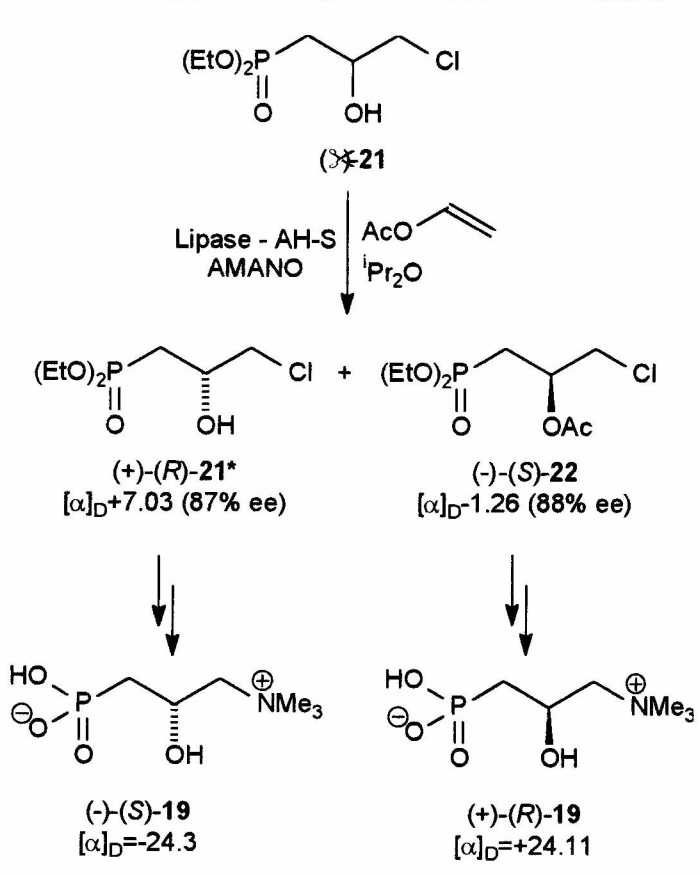


SCHEME 9

Similar procedures have been used by Mikołajczyk *et al.*[22] in the preparation of both enantiomers of phosphocarnitine **19**, in which biocatalytic procedures were applied at the crucial step of the synthesis, *i.e.* the formation of a chiral centre on the β-carbon atom. The reduction of the 2-oxo precursor **20** was in this case performed with baker's yeast (Scheme 10), while the kinetic resolution of the racemic 2-hydroxy phosphonate **21** was achieved with lipase AHS, AMANO (Scheme 11).



SCHEME 10



SCHEME 11

Acknowledgments

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REFERENCES

1. K. Faber, *Biotransfomations in Organic Chemistry*, 3rd Edition, Springer Verlag, Berlin, 1997
2. A.M.P. Koskinnen, A.M. Klibanov, (Eds.) *Enzymatic Reactions in Organic Media*, Blackie Academic and Professional, London, 1996
3. M. Mikołajczyk, J. Drabowicz, P. Kiełbasiński, *Chiral Sulfur Reagents: Applications in Asymmetric and Stereoselective Synthesis*, CRC Press, Boca Raton, 1997
4. K.M. Pietrusiewicz, M. Zabłocka, *Chem. Rev.* **1994**, *94*, 1375-1411
5. P. Kiełbasiński, M. Mikołajczyk, in *Enzymes in Action: Green Solutions for Chemical Problems*, Zwanenburg, B.; Mikołajczyk, M.; Kiełbasiński, P. (Eds.), Kluwer Academic Publishers, Dordrecht, 2000, pp. 161-191
6. *E.g.* K. Burgess, I. Henderson, K.-K. Ho, K.-K. *J. Org. Chem.* **1992**, *57*, 1290-1295
7. M. Mikołajczyk, P. Kiełbasiński, R. Żurawiński, M.W. Wieczorek, J. Błaszczyk, *Synlett* **1994**, 127-129
8. M.W. Wieczorek, J. Błaszczyk, M. Mikołajczyk, P. Kiełbasiński, R. Żurawiński, *Heteroatom Chem.* **1994**, *5*, 167-173
9. S. Tamai, S. Miyauchi, C. Morizane, K. Miyagi, H. Shimizu, M. Kumo, S. Sano, M. Shiro, Y. Nagao, *Chem. Lett.* **1994**, 2381-2384
10. E.J. Toone, M.J. Werth, J.B. Jones, *J. Am. Chem. Soc.* **1990**, *112*, 4946-4952; L. Provencher, J.B. Jones, *J. Org. Chem.* **1994**, *59*, 2729-2732
11. P. Kiełbasiński, *Tetrahedron: Asymmetry* **2000**, *11*, 911-915
12. P. Kiełbasiński, R. Żurawiński, K.M. Pietrusiewicz, M. Zabłocka, M. Mikołajczyk, *Tetrahedron Lett.* **1994**, *35*, 7081-7084
13. P. Kiełbasiński, R. Żurawiński, K.M. Pietrusiewicz, M. Zabłocka, M. Mikołajczyk, *Polish J. Chem.* **1998**, *72*, 564-572
14. P. Kiełbasiński, P. Góralczyk, M. Mikołajczyk, M.W. Wieczorek, W.R. Majzner, *Tetrahedron: Asymmetry* **1998**, *9*, 2641-2650
15. P. Kiełbasiński, M. Mikołajczyk, *Synthesis* **1995**, 144-146
16. P. Kiełbasiński, R. Żurawiński, M. Albrycht, M. Mikołajczyk, to be published
17. P. Kiełbasiński, J. Omelańczuk, M. Mikołajczyk, *Tetrahedron: Asymmetry* **1998**, *9*, 3283-3287
18. For recent reviews see: (a) T. Welton, *Chem. Rev.* **1999**, *99*, 2071-2083; (b) P. Wasserscheid, W. Keim, *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3772-3789
19. P. Kiełbasiński, M. Albrycht, J. Łuczak, M. Mikołajczyk, *Tetrahedron: Asymmetry* **2002**, *13*, 735-738
20. L.A. Spangler, M. Mikołajczyk, E.L. Burdge, P. Kielbasiński, H.C. Smith, P. Łyżwa, D.J. Fisher, J. Omelańczuk, *J. Agr. Food Chem.* **1999**, *47*, 318-321
21. R. Żurawiński, K. Nakamura, J. Drabowicz, P. Kiełbasiński, M. Mikołajczyk, *Tetrahedron: Asymmetry* **2001**, *12*, 3139-3145
22. M. Mikołajczyk, J. Łuczak, P. Kiełbasiński, *J. Org. Chem.* **2002**, *67*, 7872-7875.

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Zastosowanie enzymów hydrolitycznych w syntezie chiralnych związków heteroorganicznych

Streszczenie: Powszechnie dostępne enzymy hydrolityczne są w stanie rozpoznawać stereogenne centra heteroatomowe. Niniejszy przegląd przedstawia zastosowanie tego typu enzymów w syntezie optycznie czynnych połączeń siarki i fosforu, ze szczególnym uwzględnieniem wyników badań pochodzących z Laboratorium autorów.