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Application of bacteria and fungi as biocatalysts for the preparation of optically active hydroxyphosphonates

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Abstract

Review on the use of whole-cell biocatalysts for the preparation of optically active hydroxyalkanephosphonates is presented. There are three general processes applied so far, namely the use of lipolytic organisms either for enantioselective hydrolysis of acyloxyalkanephosphonates or enantioselective acylation of hydroxyalkanephosphonates, the use of baker's yeast and other fungi for bioreduction of ketophosphonates and the use of bacteria and fungi for hydrolytic oxirane ring opening in substituted 1,2-epoxyethanephosphonates.

Keywords: Whole-cell biocatalysis; Biotransformations; Enantioselectivity; Chemo-enzymatic synthesis; Organophosphorus compounds

1. Introduction

Substituted alkanephosphonic acids constitute a class of mimics of natural acids in which a carboxylic group is replaced by phosphonic or related function. Acting as antagonists of these acids, they inhibit enzymes involved in carboxylic acids metabolism and thus affect a variety of physiological processes. Aminoalkanephosphonic acids are the most prominent amongst them since they have already been found to act as antibacterial agents, neuroactive compounds, anticancer drugs and pesticides [1], with some of them being already commercialized. Hydroxyalkanephosphonic acids have received less attention because of their not fully explored, biological activity. However, the isolated examples of their activity consider inhibition of such important medicinal enzymes as renin [2] or human immunodeficiency virus (HIV) protease and polymerase [3]. It is now well established that usually only one of the inhibitor enantiomers exerts physiological activity and therefore the preparation of optically active hydroxyphosphonates is still challenging and desirable.

Biocatalysis represents an effective and sometimes preferable alternative to the standard synthesis of fine chemicals in their optically active forms. The use of biocatalysis in organophosphorus chemistry is surprisingly scarce and generally limited to the synthesis of optically active hydroxyalkanephosphonic acids and their esters. In order to achieve this goal only two general procedures have been used so far. One of them is lipase-mediated kinetic resolution of hydroxyalkanephosphonates, the other—bioreduction of ketophosphonates by using yeast and fungi. We have recently added to this list enantioselective preparation of diethyl 1,2-dihydroxyalkanephosphonates by means of hydrolytic epoxide ring opening.

Biocatalysis may be performed using whole cells or isolated enzymes. The use of isolated enzymes is advantageous because undesirable by-product formation mediated by contaminating enzymes is minimized. However, isolation and purification of enzymes is in many cases expensive and enzymes are frequently less stable under biocatalytic conditions. Thus, in many lab-scale and industrial biotransformation processes, biocatalysts are used in the form of whole cells. This is also the major procedure when considering biocatalytic processes leading to optically active hydroxyphosphonates.

2. Lipase-catalyzed resolution of dialkyl hydroxyalkanephosphonates

Lipases (triacylglycerol lipases, EC 3.1.1.3) constitute the most important group of biocatalysts for biotechnological

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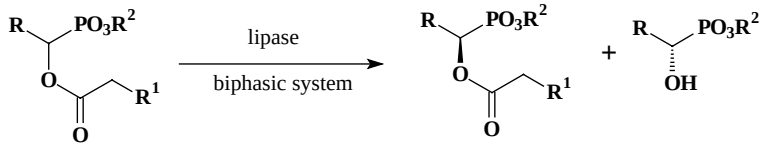


18-51% yield, 8-98% ee

R = substituted phenyl (CH_3, CH_3O)
1-naphtyl-, 2-naphtyl-, 2- and 3-thienyl, 2- and 3-furyl, 2- and 3-piridyl
various linear and branched alkyls (from ethyl to hexyl)
R^1= H, Cl
R^2= CH_3, ethyl, *i*-propyl
biphasic system: phosphate buffer/hexanes/*t*-butyl methyl ether,
lipases from: *Aspergillus niger* (lipase AP 6), *Rhizopus oryzae* (FAP 15),

Scheme 1. Enantioselective hydrolysis of 1-acyloxyalkanephosphonates [4].

applications. Since they display exquisite chemo-selectivity, regioselectivity and stereoselectivity, the enzymes from various sources are highly useful in the chiral resolution of primary and secondary alcohols. Lipases remain enzymatically active in organic solvents, what makes them the ideal tools in organic synthesis. Their ability to catalyze hydrolysis as well as transesterifications is well recognized, which reflects in more than 1000 original articles on lipases that appear each year. Therefore it is not surprising that lipases rank among the first biocatalysts used in organophosphorus chemistry.

First reports on the use of lipases for the preparation of optically active hydroxyalkanephosphonic acids were published a decade ago. They considered both the use of lipases for the esterification of hydroxylakanephosphonates and hydrolysis of acyloxyalkanephosphonates. Both methods are more and more frequently used and developed up to today.

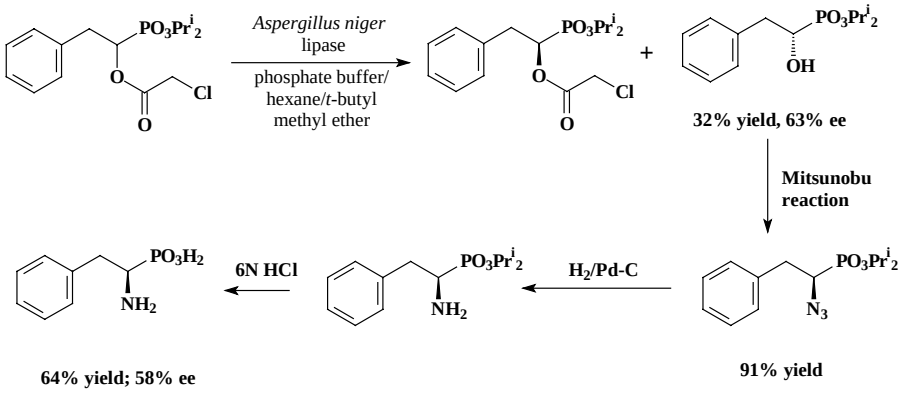
Hammerschmidt and coworkers had exploited the use of lipases for enantioselective hydrolysis of series of 1-acyloxyalkanephosphonates in organic-buffer biphasic system (Scheme 1) [4].

The two products were easily separable by means of flash chromatography and their configurations were deduced basing on derivatization with Mosher acid chloride followed examination of NMR spectra. It is worth to notice that yield and enantiomeric excess of the reaction were strongly dependent on the source of the used lipase as well as on the chemical structure of hydrolyzed acyloxyphosphonate. This method was further applied by Mikolajczyk and coworkers for the preparation of a wide variety of chiral 1-hydroxy- and 2-hydroxyalkanephosphonates [5].

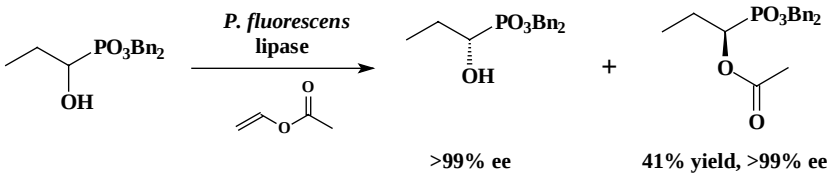
Because aminoalkanephosphonic acids are today far more useful than hydroxyphosphonates this procedure was successfully applied for the chemo-enzymatic synthesis of phosphonic acid analogues of phenylalanine (see Scheme 2), tyrosine, valine, leucine, isoleucine and methionine [4a,6].

The second approach for the preparation of optically active hydroxyphosphonates, namely lipase-mediated transesterifications in organic media, was introduced by Kushi et al. (Scheme 3) [7].

This procedure is now being the most commonly used for the preparation of a wide structural variety of 1- and 2-hydroxylakanephosphonates [8]. The yield of the reaction was found to be strongly dependent on the structure of hydroxyphosphonate (aliphatic, as well as aromatic substrates were used) and acylating agent (with vinyl acetate being of choice), as well as on the source of lipase used (the best results were achieved in the case of *Candida antarctica* and *Pseudomonas fluorescens* lipases) and on selection of suitable organic solvent. This procedure was also used



Scheme 2. Chemoenzymatic synthesis of optically pure phosphonic acid analogue of phenylalanine.

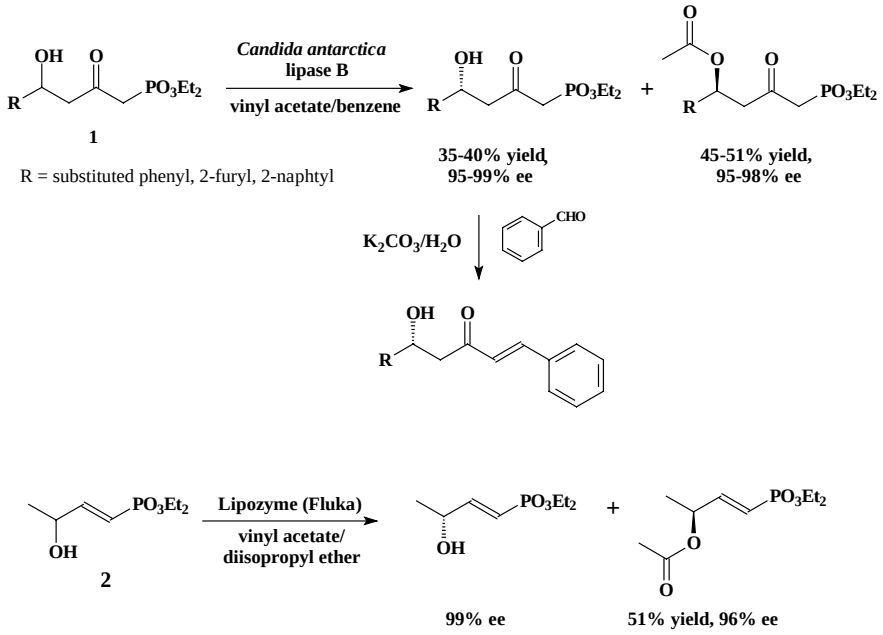


Scheme 3. Lipase-mediated enantioselective acylation of dibenzyl-1-hydroxypropane-phosphonate [7].

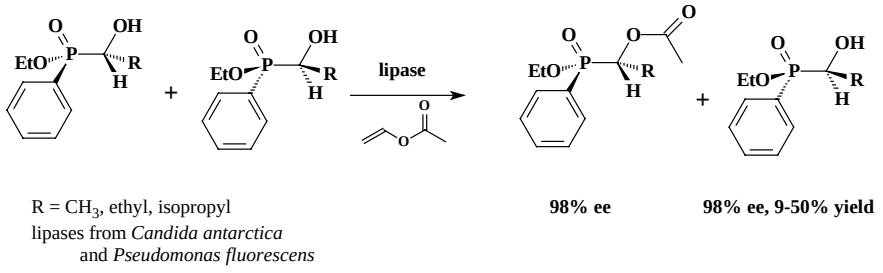
for the resolution of 4-hydroxy-2-oxoalkanephosphonates (compound **1**) and diethyl 3-hydroxy-1-butenylphosphonate (compound **2**), a useful building blocks for the synthesis of biologically active compounds by Horner–Emmons approach (Scheme 4) [9].

Lipase-catalyzed acylation was also used to transfer the chirality from carbon to phosphorus atom [10]. The kinetic separation of two enantiomeric phosphinates (Scheme 5) was carried out by *C. antarctica* and *P. fluorescens* lipases using vinyl acetate acyl donor. The starting hydroxyphosphinates were reacted to give acetyl phosphinates with 50% yield and of high optical purity.

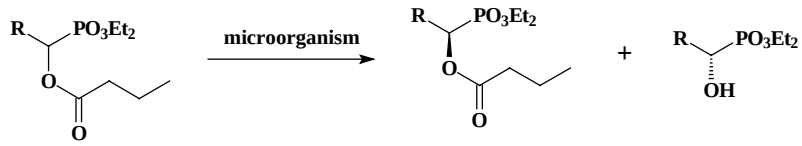
Since lipases rank among the least expensive enzymes the use of whole-cell cultures is less competitive than in other cases. The application of two lipolytic organisms, bacterium (*P. fluorescens*) and fungi (*Penicillium citrinum*), was reported by Lejczak and coworkers [11]. These organisms were successfully used for the enantioselective hydrolysis of structurally variant 1-butyryloxyalkanephosphonates affording corresponding hydroxyphosphonates with moderate to good yields and of satisfactory optical purity (Scheme 6). Both strains exhibited the same enantioselectivity and hydrolyzed preferentially esters of *S* configuration. Certain chemo-selectivity among the used microorganisms towards



Scheme 4. Lipase-mediated synthesis of chiral substrates for Horner–Emmonds reaction.



Scheme 5. Lipase-mediated transfer the chirality from carbon to phosphorus atom.



R = ethyl, *n*-propyl, *i*-butyl, *n*-butyl, CH_2=$CHCH_2$-phenyl, *p*-chlorophenyl, *p*-methoxyphenyl, $C_6H_5CH_2CH_2$-, 2-furyl, $C_6H_5CH(CH_3)$-

Scheme 6. The use of lipolytic microorganisms for the preparation of optically active diethyl 1-hydroxyalkanephosphonates.

phosphonic substrates was also observed. Thus, *P. citrinum* efficiently hydrolyzed substrates containing aliphatic substituent at α-position, whereas *P. fluorescens* was more efficient in the case of aromatic substrates. As might be expected, the conversion of the substrates increased with the time of biotransformation but it was accompanied by decreasing enantiomeric purity of the products, which usually happens with whole-cell system biocatalysis.

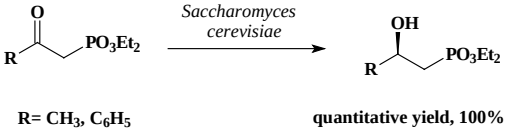
The optical purities of the products were determined by ^{31}P NMR spectroscopy using quinine as chiral solvating agent [12]. This direct, clean and simple method seems to be the one of choice alongside with the method recently elaborated by Blazewska and Gajda [13].

3. Bioreduction of diethyl ketophosphonates

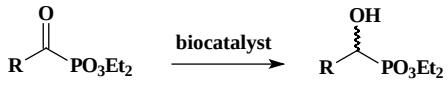
Carbonyl reductions are probably the most thoroughly studied and exploited biotransformations performed by baker's yeast, because the catalyst is cheap and versatile and the process is easy to perform. This biocatalyst is indeed a source of hundreds of intracellular and extracellular enzymes, which are able to display their catalytic activity within the narrow experimental conditions, and thus was found to be extremely useful in organic synthesis. Moreover, the yeast cells have the ability to regenerate their own respective cofactors, which is highly advantageous. The first use of this catalyst in aerobic conditions was reported by Lejczak and coworkers for the preparation of enantiomerically pure β-hydroxyalkanephosphonates (Scheme 7) [14].

Enlargement of the pool of substrates undoubtedly showed that the efficiency of this catalyst for the reduction of β-, γ- and δ-hydroxyphosphonates is strongly dependent on the chemical nature of the substrate [15]. For example, the steric hindrance introduced by substituents which are in close proximity to the carbonyl moiety totally abort or significantly reduced reductability of the substrate. The use of anaerobically grown yeast enabled, at least in part, to overcome this problem. Also the use of *Geotrichum candidum* (IFO 4597 and 5767 strains), a fungi known from the ability to reduce stereoselectively various ketones, resulted in satisfactory reduction of 2-hydroxyalkanephosphonates [8a].

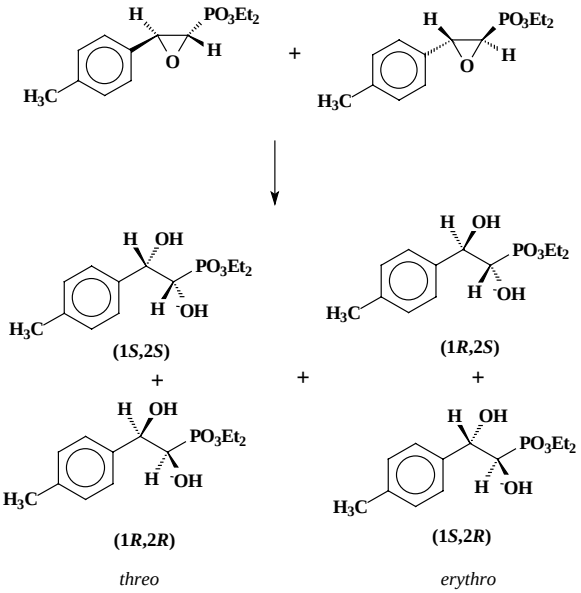
In most cases the whole-cell biocatalysis is carried out in aqueous solutions. There is, however, also a possibility to perform these reactions in media other than water. This is of importance when substrates or products of the biotransformation are unstable or insoluble in water. Several technical solutions of whole-cell catalysis in non-aqueous media have been developed so far. They include the use of two-phase media, application of biocatalysis in emulsion



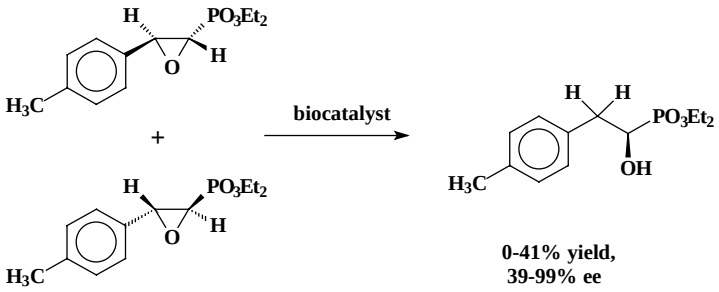
Scheme 7. Reduction of 2-hydroxyalkanephosphonates by means of baker's yeast.



Scheme 8. Reduction of diethyl 1-hydroxyalkanephosphonates by various strains of yeast and fungi.



Scheme 9. Representative example of biocatalytic ring opening in epoxyethylphsophonates.



Scheme 10. Representative example of side-reaction accompanying hydrolytic ring opening in epoxyethanephosphonates.

systems, or the use of reactions in anhydrous media. In all of these cases the biocatalyst needs special preparation to maintain activity in such unnatural conditions and enabling to maintain cell viability in organic environment. Lyophilization and/or immobilization of the cells are often the methods of choice. Such a solution was therefore used for the bioreduction of 1-oxoalkanephosphonates, the compounds which are extremely unstable in water and easily decompose to yield a wide variety of organophosphorus compounds [16]. Two strains of fungi (*Verticillium* sp. and *Cladosporium* sp.), and three strains of yeast (*Saccharomyces cerevisie*, *Rhodotorula rubra* and *Rhodotorula glutinis*) turned out to be useful biocatalysts for enantioselective reduction of a variety of 1-oxoalkanephosphonates (Scheme 8) under anhydrous conditions [17] using lyophilized cells immobilized on Celite R 630.

The observed strong dependence of biocatalyst efficiency on the reaction conditions such as biotransformation time and presence of various additives, chosen either in order to regenerate cofactors, or to selectively block certain dehydrogenases, had proven undoubtedly that there are several different oxireductases involved in the reduction of these substrates [17].

4. Biocatalytic hydrolysis of epoxyethanephosphonates

Hydrolytic opening of oxirane ring using epoxide hydrolases or microbial cell cultures belongs to the most classic biotransformations. In order to apply this system for the hydrolysis of *trans*-1,2-epoxyethanephosphonates substituted in position 2 by various aromatic groups, the chemical course of the reaction had to be studied in some detail. Thus, Sharpless asymmetric dihydroxylation of diethyl *trans*-vinylphosphonates leading to *threo*-1,2-dihydroxyethanephosphonates enabled differentiation between *threo* and *erythro* isomers and provided standards for the determination of the absolute configuration by means of ^{31}P NMR with quinine as chiral discriminator [12]. Contrary to chemical hydrolysis, biocatalysis gave *erythro*-1,2-dihydroxyphosphonates, with *threo* isomers being the minor ones (representative example of this reaction is shown in Scheme 9). Upon chemical hydrolysis the 85:15 mixture of *threo* to *erythro* stereoisomers was obtained in 79% of yield, whereas hydrolysis with *Beauveria bassiana* afforded the 42:58 ratio of these stereoisomers in 59% yield. It is worth to note that isomer *erythro* was obtained with 100% enantiomeric excess (at present it is not possible to determine its exact configuration), whereas isomer *threo* was obtained with 98% ee as 1*S*,2*S*-enantiomer [18].

This pattern of reaction is typical for all the microorganisms used, namely *Aspergillus niger*, *Cunninghamella elegans*, *B. bassiana*, *Beauveria brongnartii*, *R. glutinis* and *Rhodococcus* sp. In all the cases formation of isomer *threo* predominates and it is formed with high enantiomeric excess. Isomer *threo* constitutes 0–42% of the product and is obtained either as pure 1*S*,2*S*-isomer or as a racemic mixture (also depending on the microorganism used). This reaction is accompanied by the formation of 1-hydroxyethanephosphonates (representative example of this process is shown in Scheme 10), most likely the products of biocatalytic hydride transfer (possibly catalyzed by dehydrogenases). Reactions leading to these products appeared to be stereospecific and afforded in most cases (with exception of reactions catalyzed by *R. glutinis*) enantiomers of *S* configuration as the predominant one [19].

Acknowledgements

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