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Simple and effective method for the deracemization of ethyl 1-hydroxyphosphinate using biocatalysts with lipolytic activity

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Abstract—The kinetic resolution of ethyl (1-hydroxyethyl)phenylphosphinate was carried by enzymatic esterification with vinyl butyrate or by hydrolysis of ethyl (1-butyryloxyethyl)phenylphosphinate either by the use of lipases or whole cells of microorganisms (bacteria and fungi). Since no stereodifferentiation of the phosphinate moiety was observed, the biotransformations gave diastereoisomers of ethyl (1-hydroxyethyl)phenylphosphinate with excellent enantiomeric excesses above 98%, while the percentage conversion ranged from 10% to 49% depending upon the procedure and conditions of the biocatalysis.

1. Introduction

Green chemistry is a field of science, which has developed very dynamically over the last few years. One of its major techniques is the application of biocatalysts, namely enzymes or whole cells (mostly microbial ones), to obtain desired products of various interests.[1] A range of methods are based upon the improvement of classic kinetic resolution processes. An example of such a process is deracemization, followed by repeated resolution, dynamic resolution and follow-up stereoinversion reactions.[2]

α-Heteroatom substituted phosphinic acids are of particular interest due to their usefulness in the development of catalytic antibodies[3] and pharmacologically active substances.[4] Therefore, there is a growing interest in the preparation of such phosphonates in enantiomerically pure forms and biotransformations are one of the most promising means to reach that objective.

The purpose of this study was to obtain, biocatalytically, ethyl (1-hydroxyethyl)phenylphosphinate **1** with good enantiomeric excess in the most simple and effective way while offering the possibility of scale up. Two different strategies were examined. Hydrolysis of ethyl (1-butyryloxyethyl)phenylphosphinate **2** and transesterification of **1** using lipases of different origin.

2. Results and discussion

All the three tested methods of the biotransformation of ethyl (1-hydroxyethyl)phenylphosphinate appeared to be a simple and effective means for the resolution of its stereoisomers.

Enzymatic hydrolysis of compound **2** (Scheme 1) gave the major pair of diastereoisomers (S_P,*S*) and (R_P,*S*) of compound **1** with excellent enantiomeric excess >98% and the maximum possible yield of 50% (Table 1).

Experimental data showed that hydrolysis of the discussed substrate stopped when the chemical yield reached a value of 50%, which is typical for a kinetic resolution. The only exception was observed when lipase from *Rhizopus* sp. was used as a biocatalyst. In this case, the reaction progress exceeded 50%, and consequently the enantiomeric purity of the product was eroded (Table 1). However, a satisfactory result was obtained when shortening the reaction time to 18 h.

Hydrolysis of compound **2** (Scheme 1) by whole cells of the chosen microorganisms gave the same (S_P,*S*) and (R_P,*S*) pair of diastereoisomeric ethyl (1-hydroxyethyl)-phenylphosphinate **1** with good enantiomeric excess and

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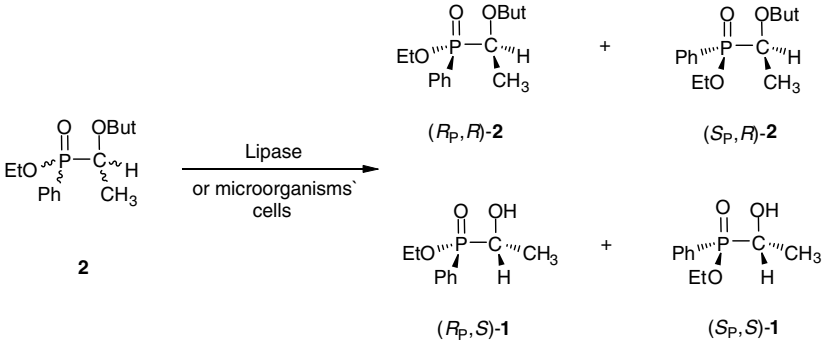


Scheme 1.

Table 1. Results of the enzymatic hydrolysis of ethyl 1-butyryloxyphosphinate

Lipase	Time (h)	Conversion (%)		ee of alcohol (%)	
		(S_P,R) (R_P,S)	(R_P,R) (S_P,S)	(R_P,S)	(S_P,S)
Rhizopus niveus	24	32	32	>98	>98
	95	34	34	>98	>98
Rhizopus sp.	18	46	47	>98	>98
	24	54	54	61	72
	95	65	55	45	74
Mucor javanicus	24	49	49	>98	>98
	95	46	48	>98	>98
Pseudomonas cepacia[a]	95	10	46	>98	>98
Mucor circinelloides[a]	21	40	40	>98	>98

[a] Immobilized lipase.

satisfactory chemical yield. However, the results comparable to the enzymatic hydrolysis were obtained only when *Bacillus subtilis* cells were used as a biocatalyst (Table 2).

The reverse reaction, an enzymatic esterification of compound **1** (Scheme 2), allowed us to obtain the pair of (S_P,S)- and (R_P,S)-isomers of compound **2** with enantiomeric excesses above 98% (Table 3). The stereochemistry of the phosphorus atom had no influence on the course of the biocatalytic process. Summing up, the results presented in this work clearly show that lipases and microorganisms are able to convert substrates bearing an (*S*)-configuration at the carbon atom adjacent to the phosphorus, whereas the configuration of the phosphinate atom is meaningless here.

Table 2. Results of hydrolysis of ethyl 1-butyryloxyphosphinate by microorganisms

Microorganism	Time (h)	Conversion (%)		ee of alcohol (%)	
		(S_P,R) (R_P,S)	(R_P,R) (S_P,S)	(R_P,S)	(S_P,S)
Pseudomonas aeruginosa	22	20	7	>98	>98
	71	32	17	>98	68
Bacillus subtilis	20	30	33	>98	>98
	24	41	41	>98	79
Acinetobacter baumannii	48	38	36	>98	68
	72	60	43	20	66
Serratia liquefaciens	94	17	9	49	39

2.1. Determination of the absolute configuration

Spectroscopic data for the two stereoisomers of compound **1** (Fig. 1) were described previously in the literature.[5] The authors of this paper defined the pair of isomers shown in Figure 1 as (R_P,R)- and (S_P,S)-isomers. However, our studies indicate that this configurational assignment is not correct.

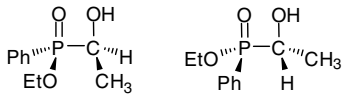
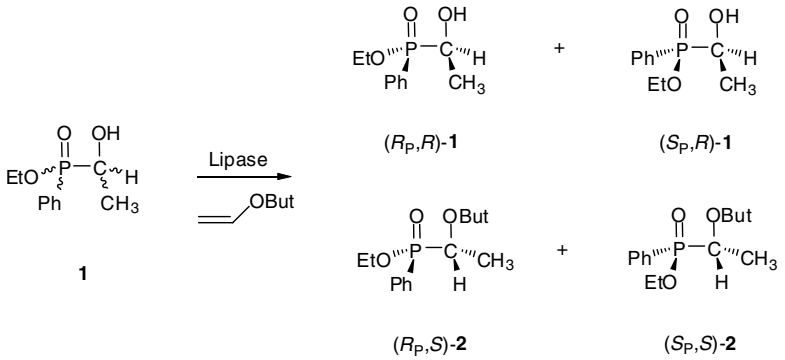


Figure 1. Two isomers of the ethyl (1-hydroxyethyl)phenylphosphinate **1** obtained by Shioji.[5]

It is obvious that the proper evaluation of the absolute configuration at the asymmetric phosphorus atom should consider the double-bonded oxygen as a priority over the ester oxygen atom. If so, the correct configuration of the discussed isomers has to be set as (S_P,R) and (R_P,S), not (R_P,R) and (S_P,S). The pair of the stereoisomers (shown in Fig. 1 after Shioji et al.)[5] of compound **1** was obtained according to the Haynes procedure,[6] which results in the steric course of this reaction. Their configurational assignment additionally strengthens our opinion about incorrect configurational assignment being given in the literature. If this is so, it is possible to link configuration on the basis of ^{31}P NMR spectra of compound **1** as shown in Figure 2. This assignment is also confirmed by the differences in the ^{1}H and ^{13}C NMR spectra observed between two diastereomeric pairs. These spectra for (S_P,R)- and (R_P,S)-isomers (Fig. 2) are identical with those described by Shioji.[5]

The absolute configuration was additionally analyzed by the method reported by Mosher.[7] Thus, a purified mixture



Scheme 2.

Table 3. Results of the enzymatic esterification of ethyl 1-hydroxyphosphinate

Lipase	Enzyme amount (mg)	Time (h)	Conversion (%)		ee of ester (%)	
			(S_P,R) (R_P,S)	(R_P,R) (S_P,S)	(R_P,S)	(S_P,S)
Candida cylindracea	200	72	44	42	91	79
Aspergillus niger	200	168	44	44	91	>98
Rhizopus niveus	200	168	<5	<5	—	—
Mucor javanicus	20	96	48	46	38	51
Porcine pancreas	20	24	40	45	>98	>98
Pseudomonas cepacia[a]	20	24	26	43	>98	>98
Mucor circinelloides[a]	20	24	43	47	>98	>98

[a] Immobilized lipase.

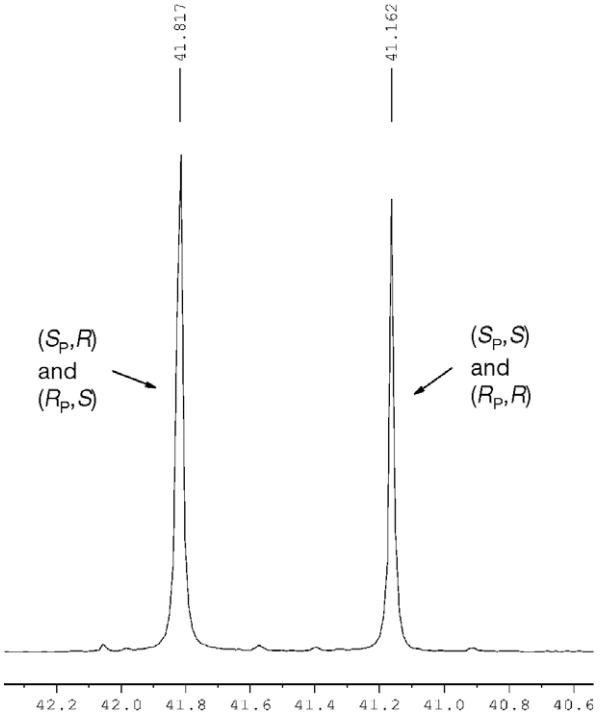


Figure 2. ^{31}P NMR spectra of ethyl 1-hydroxyethylphenylphosphinate diastereomers.

of isomers of compound **1** (molar ratio of: (S_P,R):(R_P,S):(S_P,S):(R_P,R) = 0.35:1.00:0.53:0.18), obtained after hydrolysis by the lipase from *Rhizopus* sp., was acylated using *S*(+)MTPA-Cl (chemical yield of 85%). After the reaction was complete, the mixture of products consisted of four isomers of (*R*)-MTPA ester **3**. ^{1}H NMR chemical shifts of the methyl groups of Mosher ester of hydroxyphosphonate (CH_3–CH–P) of these isomers were assigned as follows: (S_P,S,R)—1.37; (R_P,R,R)—1.43; (R_P,S,R)—1.43; (S_P,R,R)—1.53. In this study, a mixture of isomers of the ratio 0.53:0.18:1.00:0.35 was used. The signals from this methyl group of (*S*)-configuration are situated upfield compared to (*R*)-isomers. This derives from the anisotropic effect exerted by the phenyl group (Fig. 3) and thus derives directly from the Mosher model. Since there are overlapping signals at 1.43 ppm, the additional assignment was made using 2D ^{1}H–^{31}P HMQC as a proof. ^{1}H NMR chemical shifts of phosphinate ester methyl group (CH_3–CH_2–O–P) were as follows: (S_P,S,R)—1.34; (R_P,R,R)—1.29; (R_P,S,R)—1.32; (S_P,R,R)—1.26. Thus, the signals of this group of (*R*)-configuration are situated upfield when compared with the respective (*S*)-isomers (Fig. 3).

The absolute configuration of the asymmetric carbon atom of compound **1** (obtained by hydrolysis or esterification), which was previously defined using the Mosher method, was then assigned in ^{31}P NMR spectra recorded with the addition of quinine as a chiral discriminator (Figs. 4 and 5).

According to both ^{31}P NMR spectra, recorded with the addition of quinine, it can be concluded that the signals deriving from the (*R*)-isomers at the phosphorus atom are situated upfield compared to the corresponding (*S*)-isomers (Figs. 4 and 5).

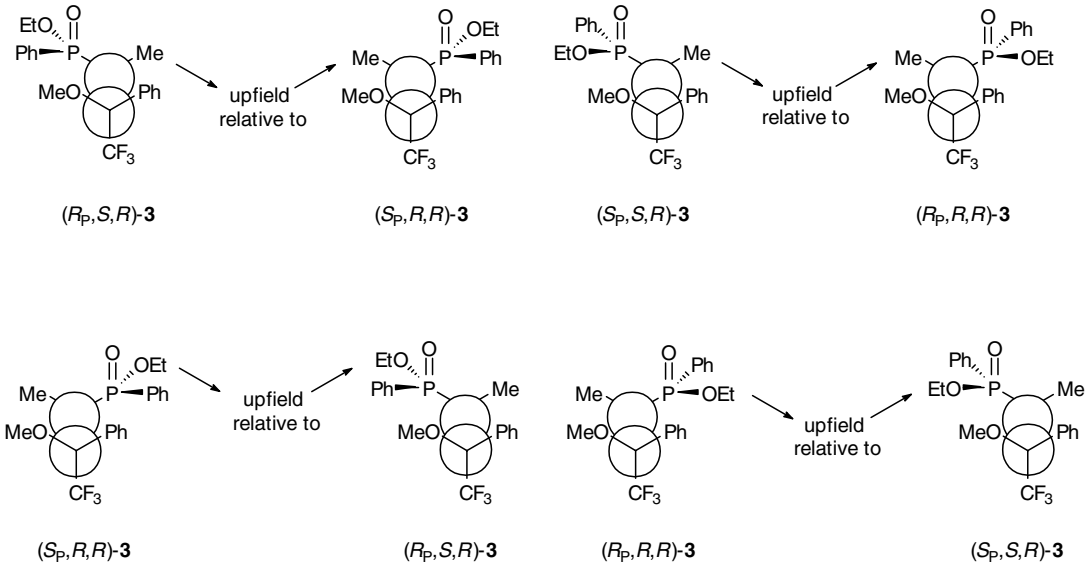


Figure 3. Signals used for the determination of absolute configuration of α-carbon atom of ethyl (1-hydroxyethyl)phenylphosphinate **1** by Mosher's procedure.

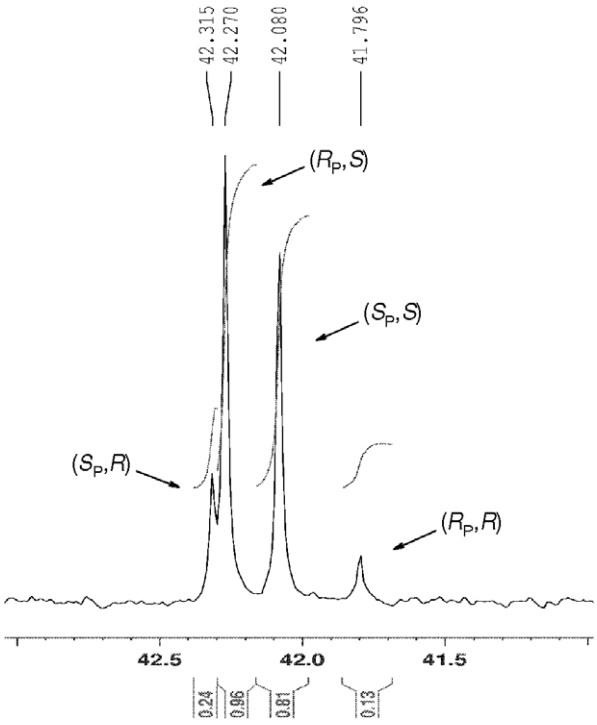


Figure 4. ^{1}H NMR spectrum of **1** with quinine after hydrolysis of **2** by lipase from *Rhizopus* sp.—24 h.

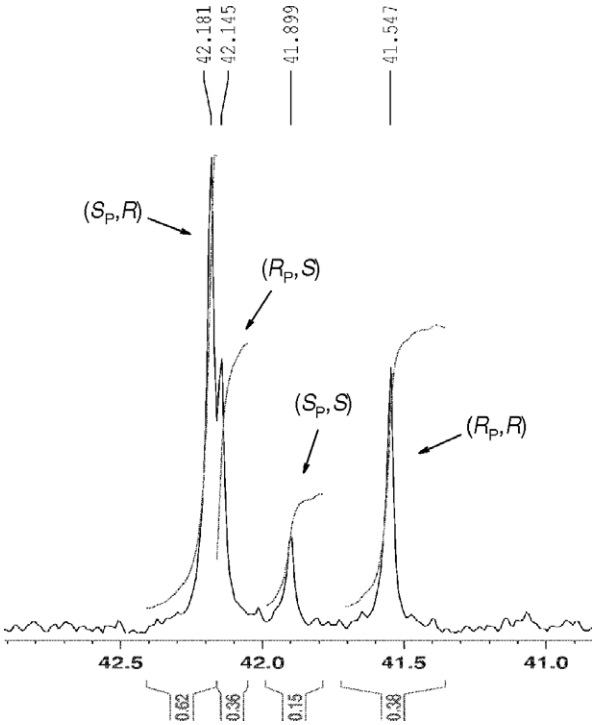


Figure 5. ^{1}H NMR spectrum of **1** with quinine after esterification of **1** by lipase from *Mucor javanicus*—68 h.

3. Experimental

3.1. Materials and methods

All materials were obtained from commercial suppliers: Sigma, Aldrich, Fluka, POCh, Serva, and used without purification. LC was performed on silica gel 60 (60–230 mesh). The sources of lipases were *Candida cylindracea* (Sigma), *Aspergillus niger* (Fluka), *Rhizopus niveus* (Fluka), *Rhizopus* sp. (Serva), *Mucor javanicus* (Fluka), *Penicillium roqueforti* (Fluka) and porcine pancreas (Sigma). Also lipase from *Pseudomonas cepacia* immobilized in Sol–gel-AK (Fluka) and that from *Mucor circinelloides* immobilized in situ. NMR spectra were recorded in $CDCl_3$ using Bruker Avance™ 600 operating at 600.58 MHz for ^{1}H, 243.12 MHz for ^{31}P and 151.02 MHz for ^{13}C or using

Bruker Avance DRX 300 system operating at 300.13 MHz for ^{1}H and 121.50 MHz for ^{31}P. TMS for ^{1}H spectra and $CHCl_3$ for ^{13}C spectra were used as internal standards, while 85% phosphoric acid in H_2O was used as the external standard for ^{31}P measurements.

3.2. Synthetic procedures and spectral data of the chemical compounds

3.2.1. Ethyl (1-hydroxyethyl)phenylphosphinate 1. Compound **1** was synthesized according to the Texier-Boullet method:[8] 5 g of aluminium oxide was ground with 5 g of potassium fluoride. Then 20 mmol of ethylphenylphosphinate and 20 mmol of the acetic aldehyde were added and the reaction mixture left at room temperature for 48 h. The mixture was then extracted with methylene dichloride (4×50 ml). Product **1** was purified by means of silica gel liquid chromatography using methylene dichloride and ethyl acetate (5:3 v/v) as eluent (R_f (**1**) = 0.15). This was obtained with 54% yield as a mixture of diastereomers in a 5:3 molar ratio. For isomers (R_P,*S*) and (S_P,*R*)—^{31}P NMR (243.1 MHz, $CDCl_3$): δ 41.82; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.32 (dd, $J = 6.9$, 17.6 Hz, 3H, $CHCH_3$), 1.34 (t, $J = 7.1$ Hz, 3H, CH_2CH_3), 3.96–4.06 (m, 2H, OCH_2), 4.13–4.20 (m, 1H, CHP), 7.46–7.50 (m, 2H, aromatic), 7.55–7.58 (m, 1H, aromatic), 7.78–7.85 (m, 2H, aromatic); ^{13}C NMR (151.0 MHz, $CDCl_3$): δ 16.57 (d, $J = 5.9$, $CHCH_3$), 17.09 (d, $J = 3.4$, CH_2CH_3), 61.55 (d, $J = 7.5$, OCH_2), 66.12 (d, $J = 115.5$, CHP), 128.48, 128.55, 132.56, 132.63 (aromatic); For isomers (R_P,*R*) and (S_P,*S*)—^{31}P NMR (243.1 MHz, $CDCl_3$): δ 41.16; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (dd, $J = 7.1$ 16.5 Hz, 3H, $CHCH_3$), 1.33 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 4.13–4.20 (m, 3H, CHP and OCH_2), 7.46–7.50 (m, 2H, aromatic), 7.55–7.58 (m, 1H, aromatic), 7.78–7.85 (m, 2H, aromatic); ^{13}C NMR (151.0 MHz, $CDCl_3$): δ 16.43, 16.53 (d, $J = 6.3$ Hz, $CHCH_3$), 17.09, 61.48 (d, $J = 7.3$ Hz, CH_2CH_3), 65.89 (d, $J = 116.5$ Hz, OCH_2), 128.49, 128.57, 132.43, 132.49, 132.56 (aromatic).

3.2.2. Ethyl (1-butyryloxyethyl)phenylphosphinate 2. Compound **1** was converted to **2** according to the following procedure: 10 mmol of **1** was added to 100 ml of the reaction media containing chloroform and triethylamine (10:1 v/v), followed by the addition of 11 mmol of butyryl chloride. The resulting mixture was stirred for 24 h at room temperature. α-Butyryloxyphosphinate was purified by means of column chromatography as described above (R_f (**2**) = 0.51). A mixture of diastereomers in a 5:3 molar ratio was obtained with 45% yield. For (S_P,*R*)- and (R_P,*S*) isomers—^{31}P NMR (243.1 MHz, $CDCl_3$): δ 37.11; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.89 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2CH_3$), 1.36 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 1.42 (dd, $J = 7.1$, 15.5 Hz, 3H, $CHCH_3$), 1.55–1.61 (m, 2H, $CH_2CH_2CH_3$), 2.21–2.31 (m, 2H, $CH_2CH_2CH_3$), 3.99–4.22 (m, 2H, OCH_2CH_3), 5.31–5.35 (m, 1H, CHP), 7.47–7.51 (m, 2H, aromatic), 7.57–7.60 (m, 1H, aromatic), 7.80–7.84 (m, 2H, aromatic); ^{13}C NMR (151.0 MHz, $CDCl_3$): δ 13.53 ($CH_2CH_2CH_3$), 14.21 (OCH_2CH_3), 16.58 (d, $J = 5.9$, $PCHCH_3$), 18.28 ($CH_2CH_2CH_3$), 36.04 ($CH_2CH_2CH_3$), 61.54 (d, $J = 6.6$, $POCH_2$), 66.81 (d, $J = 121.2$, CHP), 128.56, 128.64, 132.40, 132.47 (aromatic), 132.84 (d, $J = 2.2$, aromatic), 172.36 (CO); for (R_P,*R*) and (S_P,*S*) isomers—^{31}P NMR (243.1 MHz, $CDCl_3$): δ 37.53; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.82 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2CH_3$), 1.32 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 1.42 (dd, $J = 7.1$ 15.5 Hz, 3H, $CHCH_3$), 1.47–1.54 (m, 2H, $CH_2CH_2CH_3$), 2.13–2.23 (m, 2H, $CH_2CH_2CH_3$), 3.99–4.22 (m, 2H, OCH_2CH_3), 5.45–5.50 (m, 1H, CHP), 7.47–7.51 (m, 2H, aromatic), 7.57–7.60 (m, 1H, aromatic), 7.80–7.84 (m, 2H, aromatic); ^{13}C NMR (151.0 MHz, $CDCl_3$): δ 13.42 ($CH_2CH_2CH_3$), 14.25 (OCH_2CH_3), 16.47 (d, $J = 6.1$, $PCHCH_3$), 18.28 ($CH_2CH_2CH_3$), 36.01 ($CH_2CH_2CH_3$), 61.40 (d, $J = 6.5$ Hz, $POCH_2$), 65.94 (d, $J = 123.4$ Hz, CHP), 128.45, 128.53, 132.50, 132.56 (aromatic), 132.79 (d, $J = 3.0$, aromatic) 172.36 (CO).

3.3. Enzymatic reactions—general procedures

Enzymatic hydrolysis of ethyl (1-butyryloxyethyl)phenylphosphinate **2** was carried out in a biphasic system (3.8 ml) consisting of 0.05 M phosphate buffer, pH 7 (3.0 ml) and a mixture of diisopropyl ether (0.2 ml) with *n*-hexane (0.6 ml). After the addition of 0.2 mM of substrate and 100 mg of suitable lipase (see Table 1) the reactions were carried out at room temperature with shaking (150 rpm). The reaction was stopped after certain periods of time, and the product was extracted twice with 15 ml of ethyl acetate and the organic phase dried over anhydrous magnesium sulfate. After filtration, the organic solvent was removed by evaporation and the obtained ethyl phosphinate analyzed with ^{31}P NMR using quinine as a chiral discriminator.[9]

Enzymatic transesterification of ethyl (1-hydroxyethyl)phenylphosphinate **1** was carried out in diisopropyl ether (2 ml) with the addition of 20 mg of powdered molecular sieves (3 Å mesh). 0.02 mM of the substrate, 20 or 200 mg of suitable lipase and 0.165 mM of vinyl butyrate were added (see Table 2). The reactions were carried out at 36 °C in a shaker (150 rpm). The reaction was stopped after certain periods of time by filtration followed by evaporation of the organic layer. The resulting product was purified by HPLC (C-18 column, gradient: 40% acetonitrile in water–70% acetonitrile in water, retention time of **2**: 11.2 min) and analyzed by HPLC (CHIRALPAK AD, Diacel, 10% 2-propanol in *n*-hexane, retention time: (S_P,*S*)—7.6 min; (R_P,*R*)—8.2 min; (R_P,*S*)—8.5 min and (S_P,*R*)—9.7 min).

3.4. Microorganisms, growth and whole cell biotransformation conditions

Pseudomonas aeruginosa, *Bacillus subtilis*, *Serratia liquefaciens* and *Acinetobacter baumannii* are from our own collection. These microorganisms were identified by Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany. In order to assure the most vigorous growth and the highest lipolytic activity, several media were tested. The medium of choice consisted of 10 g of starch soluble, 1 g of yeast extract, 5 g of $(NH_4)_2SO_4$, 2 g of K_2HPO_4, 100 μl of tributyrin and 1000 ml of distilled

water. Microorganisms were incubated at 26 °C with shaking at 150 rpm for 3 days after which the cells were centrifuged at 3000 rpm for 10 min and washed twice in 0.017 M phosphate buffer, pH 7.0. Biotransformations were performed in 100 ml solution of 0.017 M phosphate buffer, pH 7.0, after addition of 50 μl of substrate with shaking at 150 rpm at room temperature. After biotransformation, the biomass was centrifuged, the supernatant was extracted twice with ethyl acetate and dried over anhydrous magnesium sulfate. After filtration, the organic solvent was evaporated and the product analyzed by means of ^{31}P NMR spectroscopy using quinine as a chiral discriminator.[9]

Acknowledgement

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