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Enzymes in Organic Chemistry, Part 1: Enantioselective Hydrolysis of α-(Acyloxy)phosphonates by Esterolytic Enzymes

Yong-Fu Li, Friedrich Hammerschmidt*

Institut für Organische Chemie der Universität Wien, Währingerstrasse 38, A-1090 Wien, Austria

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Abstract: α-Hydroxyphosphonates (±)-**3** were prepared and transformed into esters (±)-**5**. Eight lipases as well as pig liver esterase were tested as catalysts for enantioselective hydrolyses of α-(acyloxy)phosphonates in a biphasic system. Two of them proved to be useful. The highest enantioselectivity was obtained with lipase F-AP 15 and α-(acetyloxy)phenylmethylphosphonates (±)-**5a** and (±)-**5b** as substrates. The (S)-enantiomers were exclusively hydrolyzed to give optically pure alcohols (S)-(-)-**3a** and (S)-(-)-**3b**. Lipases AP 6 and F-AP 15 were used to prepare phosphonates (S)-(-)-**3b**, (S)-(+)-**3d** and (S)-(-)-**3e** on a preparative scale with an enantiomeric excess of 81%, 87%, and 89%, respectively. The absolute configurations of the α-hydroxyphosphonates were assigned by Horeau's method and ^{1}H-NMR spectroscopy of Mosher's derivatives.

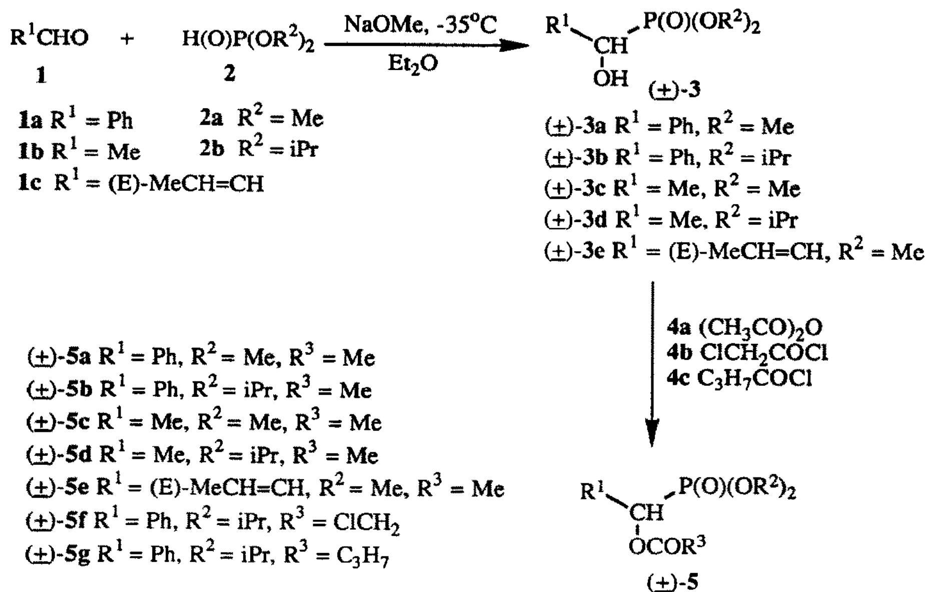
Introduction

Chiral, nonracemic α-hydroxyphosphonates are useful precusors[1,2] for a variety of α-substituted phosphonates and especially for α-aminophosphonic acids[3] which have received considerable attention over the past decade in medical, bioorganic and organic chemistry owing to their proven or potential biological activities as analogues of amino acids. The number of known chiral α-hydroxyphosphonates[1,2] with only one chiral center is very limited and there are simple and generally applicable methods needed for their preparation. The use of various enzymes as catalysts in organic synthesis has been extensively explored in recent years.[4] Optically active alcohols can be obtained by enantioselective hydrolysis of racemic esters using esterases[5] and lipases.[6] Sih and coworkers developed equations for a quantitative treatment of biochemical resolutions of enantiomers.[7] To the best of our knowledge, only one optically active α-hydroxyphosphane oxide[8] and a few 2-hydroxyalkyldiphenyl-phosphines[9], but no dialkyl phosphonates, have been prepared by enzyme mediated resolution. This paper reports our first results on a project initiated to prepare antipodes of α-hydroxyphosphonates, -phosphinates, and -phosphine oxides by enzyme catalyzed kinetic hydrolysis of the corresponding α-acyloxy derivatives.

Results and Discussion

Five representative α-hydroxyphosphonates (±)-**3** were prepared in high yield by reaction of aldehydes **1** with phosphites **2** in the presence of a catalytic amount of $NaOCH_3/CH_3OH$ at -35°C in dry ether by a modified literature procedure[10] (Scheme 1). Dimethyl and diisopropyl phosphonates were chosen as representative esters. The methyl group is more labile towards dealkylation than the isopropyl group, which is of relevance for transformations using nucleophilic substitution reactions to replace the activated hydroxyl group. Acylation of

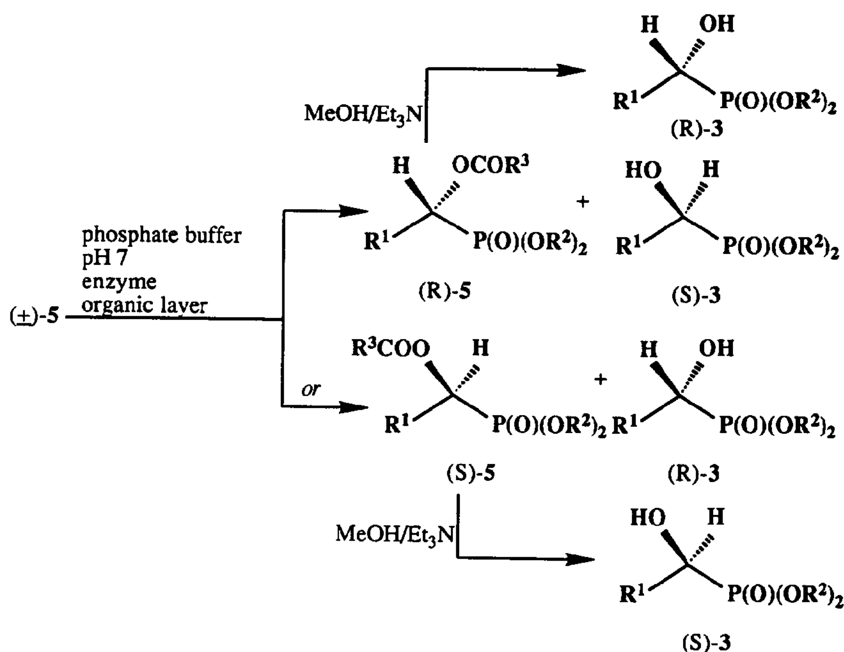
phosphonates (±)-**3** with acetic anhydride, chloroacetyl or butyryl chloride in the presence of triethylamine or pyridine afforded the corresponding α-(acyloxy)phosphonates (±)-**5**.



Scheme 1

Enzymatic hydrolyses of entries 1-17 were carried out on a 1 mmole scale of substrate, using 0.05 M phosphate buffer (15 ml, pH 7), an organic phase (4ml; tert-butyl methyl ether/hexane 1:3, for (±)-**5f**, 2:2), and various enzymes (Scheme 2). The mixture was stirred while keeping the pH constant at 7 by addition of 0.5 N NaOH with an autotitrater. At the end of the reaction, when 0.5 mmole or less of base had been added, enzymatic hydrolysis was stopped by adjusting the pH to 4 with 1 N HCl. Alcohol **3** and unreacted ester **5** were extracted and separated by flash chromatography on silica gel. Recovered ester **5** was hydrolyzed to **3** in dry methanol containing triethylamine. Under these conditions base labile α-hydroxyphosphonates are not cleaved into aldehyde and phosphite. Derivatization with Mosher's reagent[11] (S)-(+)-MTPA-Cl was used to determine the enantiomeric excesses (e.e.) of the alcohols **3** (for details and absolute configuration see later). The results were summarized in Table 1. Six lipases and pig liver esterase (PLE) were tested as catalysts for the enantioselective hydrolysis of α-(acetyloxy)phosphonate (±)-**5a** (entries 1-7). With PLE, porcine pancreas lipase (PPL), lipase N Conc (LNC), SAM-I and SAM-II the reaction rate or the enantioselectivity (or both) were too low. Lipase PS and lipase from *Candida cylindracea* did not hydrolyze α-(acetyloxy)phosphonates (±)-**5b** (not listed in Table 1). The reaction catalyzed by lipase AP 6 was rapid but showed low enantioselectivity. Lipase F-AP 15 proved to be the best of all the enzymes tested. The isolated α-hydroxyphosphonate (-)-**3a** was optically pure and the reaction time was six times longer than that of lipase AP 6 compared on an equal amount basis. Pig liver esterase was also allowed to react with substrates (±)-**5a**, (±)-**5b**, and (±)-**5g** in phosphate buffer pH 7 with 5% of acetone instead of an organic

phase (not listed in Table 1). This enzyme was not further evaluated because of low enantiomeric purity of the alcohols yielded, except for alcohol (+)-**3b** obtained from α-(acyloxy)phosphonate (±)-**5g** (ee 82%, conversion 35%, reaction time 142 h) or long reaction time. Lipases F-AP 15 and AP 6 were used for enantioselective hydrolysis of other α-(acyloxy)phosphonates. The rate of hydrolysis of compound (±)-**5b** with lipase F-AP 15 decreased compared to (±)-**5a**, as the small methyl groups were replaced by bulky isopropyl groups in the phosphonate residue (entry 8). The α-(acyloxy)phosphonate (S)-(-)-**5b** was preferentially saponified with excellent enantioselectivity. The chloroacetate (±)-**5f** showed a reduced reactivity compared to the acetate (±)-**5a**, and the enantiomeric excess of the alcohol (S)-**3b** dropped to 20%. Usually chloroacetates – compared to acetates – accelerate the enzymatic hydrolysis.[12] When the phenyl group of the phosphonates (±)-**5a** and (±)-**5b** was replaced by a methyl group, as in α-(acetyloxy)phosphonates (±)-**5c** and (±)-**5d**, the enantioselectivity decreased and increased again for the (E)-2-butenylphosphonate (±)-**5e**. Independent of the substrate, the alcohols produced had (S)-configuration and the recovered esters (R)-configuration. These results seem to indicate that good substrates for lipase F-AP 15 are α-(acetyloxy)phosphonates having an aromatic or at least a medium size aliphatic substituent and a dimethyl phosphonate moiety.



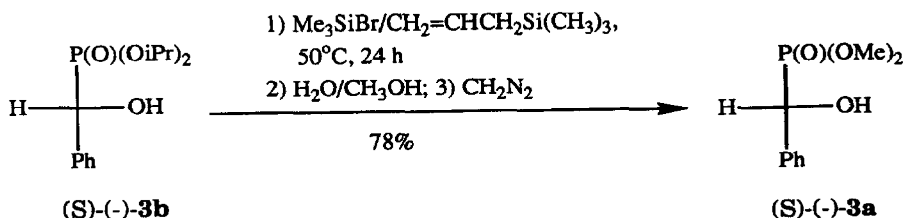
Scheme 2

As a general rule the rate of hydrolysis increased by a factor of 10 to 50 when lipase AP 6 was used as a catalyst instead of F-AP 15 with the same substrate (entries 5, 13-15). Chloroacetate (±)-**5f** (entry 16) reacted more rapidly than the acetate (±)-**5b**, while the butyrate (±)-**5g** was practically stable towards lipase AP 6 (entry 17). The esters of α-(acyloxy)phosphonates were hydrolyzed selectively and the enantiomeric excess increased from dimethyl to

diisopropyl phosphonates (from 6% to 95% and 19% to 89%; for detail see Table1).

Compounds (±)-**5b**, (±)-**5d**, and (±)-**5e** were additionally hydrolyzed on a preparative scale (10 mmol) by lipases AP 6 and F-AP 15 (entries 18-20), affording α-hydroxyphosphonates (S)-**3b**, (S)-**3d**, and (S)-**3e** with e.e. values of 81%, 87%, and 89%, respectively. The reaction with substrate (±)-**5d** was performed at 0°C, the others at 25°C. Crystalline α-hydroxyphosphonate (S)-(-)-**3b** was recrystallized from methylene chloride/hexane to give optically pure phosphonate, which was acetylated to yield optically pure α-(acetyloxy)phosphonate (S)-(-)-**5b**. Thus, homochiral α-hydroxyphosphonates are now available in large quantities for transformations involving the hydroxyl group.

Determination of absolute configurations and enantiomeric excesses of α-hydroxyphosphonates: The absolute configuration of phosphonate (-)-**3a** is (S), as determined by Wynberg et al..[13] Horeau's method[14] for the determination of absolute configuration was applied to alcohols (-)-**3a**, (-)-**3b**, (+)-**3d**, and (-)-**3e** to give 2-phenylbutyric acid with values for $[\alpha]_D^{20}$ of -14.58 (optical yield 45%), -5.19 (16%), +23.6 (73%), and +11.18 (35%), respectively. On the basis of these results (S)-configuration was assigned to phosphonates (+)-**3d** and (-)-**3e**, assuming that the phosphonate group is the "large" substituent in these compounds. These data are in agreement with the findings for two other dimethyl α-hydroxyphosphonates.[1,15] In the case of the phenylmethylphosphonates (-)-**3a** and (-)-**3b** both the dimethyl and the diisopropyl phosphonate group have to be surprisingly the "medium" substituent and phenyl the "large" one to correctly assign (S)-configuration to both compounds. The low optical yield of 16% for the reaction of phosphonate (-)-**3b**, close to the critical limit of 15% for the optical yield for a secure assignment of configuration, indicates that the phenyl group is insignificantly "larger" than the diisopropyl phosphonate group, but definitely "larger" than the dimethylphosphonate group. The (S)-configuration of diisopropyl phosphonate (-)-**3b** was unequivocally proven by its conversion into the dimethyl phosphonate (-)-**3a** by the reaction sequence given in Scheme 3. Bromotrimethylsilane in the presence of allylsilane as scavenger for HBr was used to protect the hydroxyl group and to dealkylate the phosphonate group.[16] Hydrolysis of the silylated compound and esterification of the phosphonic acid formed with a distilled etheral solution of diazomethane afforded the dimethyl phosphonate (-)-**3a** having (S)-configuration. (S)-configuration was assigned to α-hydroxyethylphosphonate (+)-**3c**, assuming that the dimethyl- and diisopropyl ester have the same configuration for the same sign of optical rotation. This conclusion is supported by ^{1}H-NMR spectroscopy of the corresponding Mosher's derivative.



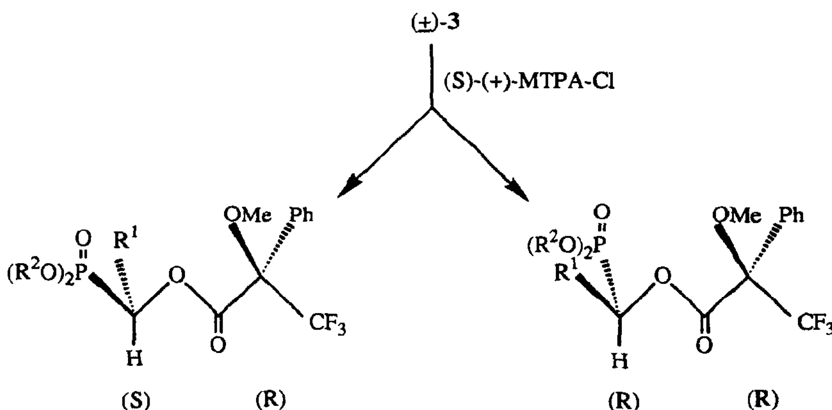
Scheme 3

Table 1. Enzymatic Hydrolysis of α-(acyloxy)phosphonates (±)-5

Entry[a]	Substrate 5	Enzyme, amount	Temp. (°C)	Time (h),	Convsn (%)[b]	Product 3	Produced alcohol 3 yield (%)[c],	ee (%)[d],	Conf.,	$[\alpha]_D$[e]	E[f]	Alcohol 3 from recovered ester 5 yield (%)[c],	ee (%)[d],	Conf.,	$[\alpha]_D$[e]
1	(±)-**5a**	F-AP 15, 100 mg	35	8.5,	49.1	**3a**	31	>99	S	-45.96	>100	35	90	R	+41.22
2	(±)-**5a**	PLE, 200 µl	27	45.0,	46.4	**3a**	32	7	R	+3.61	1.2	37	3	S	-1.96
3	(±)-**5a**	PPL, 99 mg	35	66.0,	49.9	**3a**	28	11	R	+5.40	1.4	37	7	S	-3.01
4	(±)-**5a**	LNC, 104 mg	35	92.0,	50.0	**3a**	29	5	S	-2.07	1.2	41	4	R	+1.62
5	(±)-**5a**	AP 6 21 mg	25	7.0,	47.6	**3a**	40	6	S	-2.61	1.2	24	7	R	+3.19
6	(±)-**5a**	SAM-I, 100 mg	35	139.0,	46.7	**3a**	29	18	R	+5.13	1.7	38	6	S	-2.17
7	(±)-**5a**	SAM-II, 100 mg	35	145.0,	47.6	**3a**	36	8	R	+3.03	1.3	37	2	S	-1.08
8	(±)-**5b**	F-AP 15, 95 mg	30	62.0,	48.6	**3b**	37	>99	S	-28.18	>100	46	86	R	+22.14
9	(±)-**5c**	F-AP 15, 100 mg	35	35.0,	35.0	**3c**	21	50	S	+4.69	3.9	29	9	R	-0.88
10	(±)-**5d**	F-AP 15, 100 mg	35	74.0,	50.0	**3d**	42	20	S	+1.46	1.8	36	19	R	-1.21
11	(±)-**5e**	F-AP 15, 103 mg	35	15.0,	50.0	**3e**	31	82	S	-9.52	26.0	33	81	R	+7.27
12	(±)-**5f**	F-AP 15, 100 mg	35	69.0,	42.8	**3f**	35	20	S	-5.19	1.7	33	12	R	+3.54
13	(±)-**5b**	AP 6, 23 mg	25	22.5,	50.2	**3b**	46	95	S	-25.09	138.4	29	91	R	+24.61
14	(±)-**5c**	AP 6, 25 mg	25	10.0,	46.4	**3c**	31	19	S	+1.72	1.7	23	25	R	-2.05
15	(±)-**5d**	AP 6, 25 mg	25	6.5,	41.0	**3d**	30	89	S	+5.92	31.3	27	90	R	-6.03
16	(±)-**5f**	AP 6, 26 mg	25	0.75,	48.5	**3f**	43	67	S	-18.40	9.6	35	74	R	+21.1
17	(±)-**5g**	AP 6, 53 mg	35	42.0,	0.65	-	-	-	-	-	-	-	-	-	-
18	(±)-**5b**	AP 6, 200 mg	25	19.0,	49.0	**3b**	42	81	S	-22.74	20.2	45[g]		R	+28.43
19	(±)-**5d**	AP 6, 102 mg	0	36.0,	46.2	**3d**	39	87	S	+5.81	27.2	45[g]		R	-15.25
20	(±)-**5e**	F-AP 15, 500 mg	25	22.5,	40.6	**3e**	39	89	S	-9.76	35.5	45[g]		R	+16.48

a) Entries 1-17: substrate (1 mmol), buffer solution pH 7 (15 ml), tert-butyl methyl ether / hexane (1/3; 4 ml; except entries 12 and 16: 2/2; 4 ml); entries 18-20: substrate (10 mmol), buffer solution pH 7 (50ml); tert-butyl methyl ether / hexane (1/3; 20 ml). *b)* conversion was calculated from the volume of 0.5 N NaOH added by autotitrater. *c)* Yields refer to racemate used; pure product after flash chromatography. *d)* Determined by ^{1}H-NMR spectroscopy after derivatization with (S)-(+)-MTPA-Cl. *e)* Recorded in acetone solution at 20°C. *f)* Enantiomeric ratio; calculated from conversion and the e. e. of the product.[7] *g)* Ester isolated by flash chromatography without further chemical hydrolysis.

The enantiomeric excesses of the α-hydroxyphosphonates obtained by enzymatic hydrolysis or chemical hydrolysis of recovered α-acyloxyphosphonates were determined by derivatization with (S)-(+)-MTPA-Cl and ^{1}H-NMR spectroscopy (Scheme 4). On the basis of the preferred conformation of such esters (as given in Scheme 4), the chemical shifts for certain resonances can be used to assign the absolute configuration of the secondary alcohols.[17] In the derivatives of (R)-**3a** and (R)-**3b** the OMe group (δ = 3.50, 3.49) of the MTPA portion is shielded by the phenyl group of the phosphonate compared to the derivatives of (S)-**3a** and (S)-**3b** (δ = 3.56, 3.62). In the derivatives of compounds (S)-**3c**, (S)-**3d**, and (S)-**3e** the methyl groups (δ = 1.45, 1.50 for <u>Me</u>CHP; 1.71 for (E)-<u>Me</u>CH=CH) of the phosphonates are shielded by the phenyl ring of the MTPA-esters relative to the methyl groups (δ = 1.56, 1.60; 1.78) of the Mosher's derivatives of the corresponding (R)-α-hydroxyphosphonates.



Scheme 4

Conclusions

Eight lipases and pig liver esterase were studied in enzyme-mediated hydrolyses with 1 mmole of α-(acyloxy)phosphonates (±)-**5** in 0.05 M phosphate buffer (pH 7) in a biphasic system. Lipases F-AP 15 and AP 6 were found to be the best enzymes regarding reaction rate and enantioselectivity. With these two lipases the (S)-esters were selectively saponified to give the alcohols (S)-(-)-**3a**, (S)-(-)-**3b**, (S)-(+)-**3d**, and (S)-(-)-**3e** with excellent to good enantiomeric excesses. They were also used for the hydrolysis of esters (±)-**5b**, (±)-**5d**, and (±)-**5e** on a preparative scale (10 mmol) to obtain α-hydroxyphosphonates with an enantiomeric excess of 81%, 87%, and 89%, respectively. For lipase AP 6 the reaction rate decreased from dimethyl to diisopropyl α-(acyloxy)phosphonate, while the enantioselectivity increased. The latter substrates were only slowly hydrolyzed by lipase F-AP 15. Absolute configurations were assigned by Horeau's method and the ^{1}H-NMR spectroscopy also used for the determination of enantiomeric excesses of Mosher's derivatives. Investigations towards optically active α-hydroxyphosphonates and the transformations into the corresponding α-amino or α-aminoxy phosphonic acids are under way. The method will also be applied to α-(acyloxy)phosphinates, -phosphine oxides

and acyloxy substituents in β- and γ-position, instead of the α-position, of the same phosphorus containing compounds.

Experimental

Analytical Methods. ^{1}H-NMR spectra were recorded on a Bruker AM 400 WB (400 MHz) spectrometer in $CDCl_3$ solution with chemical shifts reported in ppm relative to internal standard TMS and coupling constants in Hz. IR spectra were run on a Perkin Elmer 1600 FT-IR spectrometer as films obtained by applying a solution from the NMR sample to a silicon plate and allowing the solvent to evaporate.[18] Optical rotations were measured at 20°C on a Perkin Elmer 241 polarimeter in acetone solution in a 1 dm cell. Silica gel 60 Merck (0.040-0.063 mm) was used for flash chromatography. TLC was carried out on 0.2 mm thick Merck plates, silica gel 60 F_{254}. Spots were visualized by UV and/or dipping into a solution of 24g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1g of $Ce(SO_4)_2\cdot 4H_2O$ in 500 ml 10% H_2SO_4 in water, followed by heating on a hot plate at 200 °C. Melting points were determined on a Reichert Thermovar instrument and were uncorrected. A Metrohm 702 SM Titrino instrument was used as an autotitrater. (S)-(+)-α-methoxy-α-trifluoromethylphenyl acetyl chloride [JPS Chimie $[\alpha]_D^{20} = +136.5$ (c = 5.2, CCl_4), ee ≥ 99.5%] was used for derivatization of α-hydroxyphosphonates to determine their enantiomeric excesses by ^{1}H-NMR spectroscopy.

Lipase F-AP 15 (*Rhizopus oryzae*), lipase AP 6 (*Aspergillus niger*), lipase N Conc (LNC) (*Rhizopus*), lipases SAM-I (*Pseudomonas fluorescens*) and SAM-II (*Pseudomonas sp.*) and lipase PS (*Pseudomonas sp.*) were gifts from Amano Enzyme Europe Limited (UK); pig liver esterase (PLE) was bought from Boehringer Mannheim (Vienna), porcine pancreas lipase (PPL) and lipase from *Candida cylindracea* were from pharma biotechnologie hannover (BRD).

General procedure [10] for preparation of dialkyl 1-hydroxyphosphonates: (±)-**3**

To a solution of aldehyde **1** (20 mmol) and phosphite **2** (20 mmol) in dry diethyl ether (20 ml) at -35°C under argon, a saturated solution (0.1 ml) of CH_3ONa in methanol was added dropwise over a period of 5 min. The mixture was stirred for another 10 min and a few drops of sulfuric acid were added. The solvent was removed under reduced pressure. Water was added to the residue and the phosphonate was extracted with ethyl acetate (three times). The organic phases were combined, dried ($MgSO_4$) and the solvent was removed in vacuum to afford compounds (±)-**3** as colorless crystals which were recrystallized with methylene chloride / petroleum ether, or oils which were purified by bulb to bulb distillation under reduced pressure.

Dimethyl (1-hydroxyphenylmethyl)phosphonate: (±)-3a

Yield: 93%; m.p. 100-101°C (lit. [19] 102 °C). IR ν_{max} 3265, 2957, 1238, 1029, 834, 701 cm^{-1}. ^{1}H-NMR: δ3.67, 3.70 (2x3H, 2xd, J = 10.3, $P(OMe)_2$), 4.11 (1H, br s, OH), 5.05 (1H, d, J = 10.8, PhC<u>H</u>), 7.29-7.50 (5H, m, C_6H_5).

Elemental analysis (%): $C_9H_{13}O_4P$ calcd.: C: 50.01, H: 6.06; found: C: 49.77, H: 6.22.

Diisopropyl (1-hydroxyphenylmethyl)phosphonate: (±)- 3b

Yield: 91%; m.p. 91-92°C (lit.[20] 93°C). IR υ_{max} 3263, 2985, 1379, 1226, 992 cm^{-1}. ^{1}H-NMR: δ 1.13, 1.25, 1.27, 1.28 (4x3H, 4xd, J = 6.4, P(OCH<u>Me</u>$_2$)$_2$), 3.16 (1H, br s, OH), 4.62 (2x1H, m, P(OC<u>H</u>Me$_2$)$_2$), 4.96 (1H, d, J = 10.8, PhC<u>H</u>), 7.28-7.50 (5H, m, C_6H_5). Elemental analysis (%): $C_{13}H_{21}O_4P$ calcd.: C: 57.35, H: 7.77; found: C: 57.62, H: 7.59.

Dimethyl (1-hydroxyethyl)phosphonate: (±)- 3c

Yield: 84%; b.p. 90°C/0.001 mmHg (lit.[21] 95°C/0.08 mmHg). IR υ_{max} 3356, 2960, 2856, 1458, 1373, 1222, 1114, 1033, 905, 833, 799 cm^{-1}. ^{1}H-NMR: δ 1.45 (3H, dd, J = 7.4, 17.7, <u>Me</u>CH), 3.80, 3.83 (2x3H, 2xd, J = 10.3, P(OMe)$_2$), 4.08 (1H, dq, J = 3.9, 7.4, MeC<u>H</u>), 4.21 (1H, br s, OH). Elemental analysis (%): $C_4H_{11}O_4P$ calcd.: C: 31.18, H: 7.19; found: C: 31.28, H: 7.21.

Diisopropyl (1-hydroxyethyl)phosphonate: (±)-3d

Yield: 88%; b.p. 100°C/0.001 mmHg. IR υ_{max} 3315, 2979, 2936, 1386, 1220, 1108, 989 cm^{-1}. ^{1}H-NMR: δ 1.34 (4x3H, overlapping d, P(OCH<u>Me</u>$_2$)$_2$), 1.41 (3H, dd, J = 6.9, 17.2, <u>Me</u>CH), 3.92 (1H, br s, OH), 3.96 (1H, dq, J = 3.5, 6.9, MeC<u>H</u>), 4.75 (2H, m, P(OC<u>H</u>Me$_2$)$_2$). Elemental analysis (%): $C_8H_{19}O_4P$ calcd.: C: 45.71, H: 9.11; found: C: 45.34, H: 9.22.

Dimethyl [(E)-1-hydroxy-2-butenyl]phosphonate: (±)- 3e

Yield: 85%; b.p. 130°C/0.001 mmHg (lit.[10] 130°C/0.01 mmHg). ^{1}H-NMR spectrum was identical with that reported for (±)-3e.[10]

General procedure for preparation of dialkyl 1-(acetyloxy)phosphonates: (±)-**5a-e**

Phosphonate (±)-**3** was dissolved in dry pyridine (5 ml for every 10 mmol of substrate) and acetic anhydride **4a** (1.7 ml for every 10 mmol of substrate). The reaction mixture was stirred at room temperature for 18 h. Volatile materials were removed under reduced pressure and the crude product was purified by bulb to bulb distillation at high vacuum to give compounds (±)-**5a-e** as colorless oils.

Dimethyl [1-(acetyloxy)phenylmethyl]phosphonate: (±)-5a

Yield 92%; b.p. 130°C/0.001 mmHg. IR υ_{max} 3032, 2958, 2855, 1757, 1455, 1372, 1264, 1227, 1186, 1032, 837, 700 cm^{-1}. ^{1}H-NMR: δ 2.18 (3H, s, MeCO), 3.65, 3.72 (2x3H, 2xd, J = 10.3, P(OMe)$_2$), 6.17 (1H, d, J = 13.3, PhC<u>H</u>), 7.32-7.50 (5H, m, C_6H_5). Elemental analysis (%): $C_{11}H_{15}O_5P$ calcd.: C: 51.17, H: 5.86; found: C: 51.09, H: 5.88.

Diisopropyl [1-(acetyloxy)phenylmethyl]phosphonate: (±)- 5b

Yield 92.0%; b.p. 110°C/0.001 mmHg. IR υ_{max} 2978, 1752, 1374, 1227, 994 cm^{-1}. ^{1}H-NMR: δ1.08, 1.23, 1.28, 1.29 (4x3H, 4xd, J = 6.4, P(OCH<u>Me</u>$_2$)$_2$), 2.16 (3H, s, MeCO), 4.59, 4.67 (2x1H, 2xm, P(OC<u>H</u>Me$_2$)$_2$), 6.08 (1H, d, J = 14.3, PhC<u>H</u>), 7.29-7.50 (5H, m, C_6H_5). Elemental analysis(%): $C_{15}H_{23}O_5P$ calcd.: C: 57.32, H: 7.38; found: C: 57.58, H: 7.28.

Dimethyl [1-(acetyloxy)ethyl]phosphonate: (±)- 5c

Yield 89%; b.p. 105°C/0.01 mmHg (lit.[22] 85-87 °C/2 mmHg). IR υ_{max} 2960, 2857, 1751, 1448, 1374, 1228, 1032, 834 cm^{-1}. ^{1}H-NMR: δ 1.47 (3H, dd, J = 7.4, 17.2, <u>Me</u>CH), 2.12 (3H, s, MeCO), 3.80, 3.86 (2x3H, 2xd, J = 10.3, P(OMe)$_2$), 5.30 (1H, dq, J = 7.4, 8.9, CHP). Elemental analysis(%): $C_6H_{13}O_5P$ calcd.: C: 36.74, H: 6.68; found: C: 37.02, H: 6.71.

Diisopropyl [1-(acetyloxy)ethyl]phosphonate: (±)- 5d

Yield 90%; b.p. 115°C/0.001 mmHg. IR υ_{max} 2983, 2938, 1752, 1375, 1229, 1107, 1068, 989 cm^{-1}. ^{1}H-NMR: δ 1.33 (4x3H, overlapping d, P(OCH<u>Me</u>$_2$)$_2$), 1.44 (3H, dd, J = 6.9, 16.2, <u>Me</u>CH), 2.11 (3H, s, MeCO), 4.76 (2x1H, m, P(OC<u>H</u>Me)$_2$)$_2$), 5.19 (1H, dq, J = 6.9, 8.9, CHP). Elemental analysis(%): $C_{10}H_{21}O_5P$ calcd.: C: 47.62, H: 8.39; found: C: 47.55, H: 5.92.

Dimethyl [(E)-1-acetyloxy-2-butenyl]phosphonate: (±)-5e

Yield 95%; b.p. 130°C/0.001 mmHg. ^{1}H-NMR spectrum was identical with that reported for (±)-5e.[10]

Preparation of diisopropyl 1-(chloroacetyloxy)phenylmethylphosphonate: (±)-**5f**.

Chloroacetyl chloride **4b** (30 mmol) was added to a solution of α-hydroxyphosphonate (±)-**3b** (20 mmol) in dry methylene chloride (20ml) with triethylamine (35 mmol). The reaction mixture was kept at room temperature for 18h. Water was added. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$, brine, and then dried (Na_2SO_4). The solvent was removed and the residue was purified by flash chromatography (R_f = 0.59, methylene chloride/ ethyl acetate: 5/1) and bulb to bulb distillation (b. p. 160°C/0.001 mmHg) to afford a colorless oil which solidfied on standing, yield 83%; m.p. 62-64°C. IR υ_{max} 2982, 2938, 1772, 1258, 1157, 1104, 999, 698 cm^{-1}. ^{1}H-NMR: δ1.08, 1.24, 1.28, 1.30 (4x3H, 4xd, J = 6.4, P(OCH<u>Me</u>$_2$)$_2$), 4.16 (2H, AB system, J = 15.3, $ClCH_2$), 4.61, 4.68 (2x1H, 2xm, P(OC<u>H</u>Me$_2$)$_2$), 6.12 (1H, d, J = 13.8, CHP), 7.34-7.51 (5H, m, C_6H_5). Elemental analysis(%): $C_{15}H_{22}ClO_5P$ calcd.: C: 51.66, H: 6.36; found: C: 51.88, H: 6.52.

Preparation of diisopropyl 1-(butyryloxy)phenylmethylphosphonate: (±)-**5g**

Butyryl chloride **4c** (30 mmol) was added to a solution of α-hydroxyphosphonate (±)-**3b** (20 mmol) in

dry pyridine (20 ml). The reaction mixture was kept at room temperature for 18 h. Volatile materials were removed under reduced pressure (0.01 mm, up to 40°C). 2 N HCl (30 ml) and CH_2Cl_2 (70 ml) were added to the residue. After stirring for 10 min, the organic phase was separated, dried (Na_2SO_4), concentrated and bulb to bulb distilled to afford compound (±)-**5g** as colorless oil. Yield 80%; b.p. 125-130°C/0.01 mmHg. IR υ_{max} 2978, 1751, 1386, 1259, 1163, 1104, 995 cm^{-1}. ^{1}H-NMR: δ 0.93 (3H, t, J = 7.4, <u>Me</u>CH_2CH_2CO), 1.08, 1.22, 1.27, 1.29 (4x3H, 4xd, J = 6.4, P(OCH<u>Me</u>$_2$)$_2$), 1.67 (2H, m, CH_3<u>CH_2</u>CH_2CO), 2.40 (2H, t, J = 7.4, CH_2CO), 4.62 (2H, m, P(OC<u>H</u>Me$_2$)$_2$), 6.09 (1H, d, J = 14.3, CHP), 7.29-7.49 (5H, m, C_6H_5). Elemental analysis (%): $C_{17}H_{27}O_5P$ calcd.: C: 59.64, H: 7.95; found: C: 59.55, H: 8.08.

General procedure for enzymatic hydrolysis of dialkyl 1-(acyloxyalkyl)phosphonates (±)-**5**

Phosphonate (±)-**5** (1 mmol) was placed into a three-necked (pH-electrode, device for addition of base, argon balloon) flask (25 ml), followed by addition of organic solvents (see Table 1) and sterile 0.05 M phosphate buffer (15ml; prepared by dissolving 25 mmole of KH_2PO_4 in 300 ml of distilled water, adding 1 N NaOH to adjust pH to 7, followed by addition of water to a final volume of 500 ml, and then by autoclaving at 121 °C for 20 min). The mixture was stirred vigorously in a constant temperature water bath (see Table 1) and 0.5N NaOH was added by the autotitrater to bring pH to 7.0. The enzyme was added, the pH again adjusted to 7.0, and kept there by automatic addition of base. When the appropriate amount of base had been added, 1N HCl was added to bring the pH to 4.0. The mixture was filtered through Celite and the filtrate was extracted with ethyl acetate (three times). The organic layers were combined, dried (Na_2SO_4), and concentrated. Unreacted ester **5** and hydroxy-phosphonate **3** were separated by flash chromatography (for yield, optical rotation value and enantiomeric excess see Table 1). Separation of mixtures of **3** and **5** (solvents system; R_f for **3**, R_f for **5**): **3a/5a** [methylene chloride (MC) / ethyl acetate (EA), 5:1; 0.12, 0.43]; **3b/5b** (MC/ EA, 5:1; 0.15, 0.45); **3c/5c** (MC/EA, 5:3; 0.10, 0.28); **3d/5d** (MC/EA, 5:3; 0.14, 0.31); **3e/5e** (MC/EA, 5:1; 0.11, 0.33).

The unreacted ester **5** was dissolved in dry methanol (5 ml) and triethylamine (1 ml) and stirred at room temperature until completion (TLC, ca. 24 h). The solution was concentrated and the crude product was purified by flash chromatography to yield α-hydroxyphosphonate **3** (for yield, optical rotation value and enantiomeric excess see Table 1).

The α-(acetyloxy)phosphonates (±)-**5b**, (±)-**5d**, and (±)-**5e** (10 mmol) were hydrolyzed in a similar way, except that different quantities of 0.05 M phosphate buffer (pH 7, 50 ml) and organic solvents (tert-butyl methyl ether/hexane: 1/3; 20 ml) were added. For details see Table 1. Resolution of (±)-**5b** furnished 1.42g (45%) of (R)-(+)-**5b** and 1.14g (42%) of (S)-(-)-**3b**. Recrystallization of (S)-(-)-**3b** from methylene chloride/hexane afforded optically pure material 0.788g (ee >99%), m.p. 98-100°C, $[\alpha]_D$ = -28.18 (c = 1.7). The ^{1}H-NMR spectrum was identical with that of (±)-**3b**.

Using the general acetylation procedure, (S)-(-)-**3b** with ee >99% was acetylated to yield (S)-(-)-**5b**, $[\alpha]_D$ = -37.51

(c = 1.029). Its IR and ^{1}H-NMR spectra were identical with those of (±)-**5b**.

General procedure for preparation of Mosher's derivatives of phosphonates 3 with (S)-(+)-MTPA-Cl

α-Hydroxyphosphonate **3** (0.037 mmol) was dissolved in a mixture of methylene chloride (1ml) and pyridine (1ml). (S)-(+)-α-methoxy-α-trifluoromethylphenylacetic chloride (MTPA-Cl) (20 mg, 0.079mmol) was added. The solution was allowed to stand at room temperature for 14 h. The solvents were removed at reduced pressure. Water was added and the crude material was extracted with ether. The organic phase, was washed with dilute hydrochloric acid, a saturated aqueous solution of sodium carbonate and water, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (methylene chloride/ethyl acetate 10:1; R_f(**3a**) = 0.64, R_f(**3b**) = 0.64, R_f(**3c**) = 0.55, R_f(**3d**) = 0.56, R_f(**3e**) = 0.59), giving Mosher's derivatives (yield ca. 95%) for ^{1}H-NMR analysis. The following signals were used for the determination of e.e.: for **3a**: δ = 6.35, 6.32; for **3b**: δ = 6.20, 6.18; for **3c**: δ = 1.58, 1.49; for **3d**: δ = 4.73, 4.61; for **3e**: δ = 5.62, 5.51.

Determination of absolute configuration of phosphonates 3 by the Horeau's method

The procedure given in ref.[14] was followed using phosphonates (-)-**3a** (ee >99%), (-)-**3b** (ee >99%), (+)-**3d** (ee 87%) and (-)-**3e** (ee 89%). The values for $[\alpha]_D^{20}$ (optical yield, not corrected for compounds with only ee = 87% for (+)-**3d** and 89% for (-)-**3e**) of the isolated chiral 2-phenylbutyric acid were -14.58 (45%), -5.19 (16%), +23.6 (73%), and -11.18 (35%).

Conversion of (S)-(-)-3b to (S)-(-)-3a

(S)-(-)-**3b** (100 mg, 0.37 mmol, ee >99%) was dissolved in dry CCl_4 (4ml) and bromotrimethylsilane (281mg, 1.84 mmol) and allyltrimethylsilane (126 mg, 1.1 mmol) were added. The reaction mixture was kept at 50°C for 24 h. Volatile materials were removed under reduced pressure (0.5 mmHg), and the residue was stirred with a mixture of water (2ml) and methanol (2ml) at room temperature for 0.5 h. A distilled etheral solution of diazomethane was added until a yellow color persisted. Removal of solvents and flash chromatography (methlyene chloride/ethyl acetate: 5:1; R_f for (S)-(-)-**3a** 0.12, R_f for (S)-(-)-**5a** 0.43) afforded 66 mg (78%) of (S)-(-)-**3a**. m.p. 96-98°C. $[\alpha]_D$ = -45.79 (c = 1.32). The ^{1}H-NMR spectrum was identical with that of (±)-**3a**.

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