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Enzymes in Organic Chemistry, Part 2:[1] Lipase-catalysed Hydrolysis of 1-Acyloxy-2-arylethylphosphonates and Synthesis of Phosphonic Acid Analogues of *L*-Phenylalanine and *L*-Tyrosine

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Abstract: α-Hydroxyphosphonates (±)-**3**, prepared by base catalysed addition of phosphites **2** to aldehydes **1**, were acylated to give esters (±)-**4**. Diethyl 1-acyloxy-2-arylethylphosphonates (±)-**4a**, **4b**, and **4e** were hydrolysed by lipase from *Aspergillus niger* in a biphasic system to afford (*R*)-α-hydroxyphosphonates of low enantiomeric purity. The corresponding diisopropyl phosphonates (±)-**4c**, **4f** and **4g** gave (*S*)-α-hydroxyphosphonates with an ee of up to 78%. The absolute configuration of the α-hydroxyphosphonates was assigned by ^{31}P NMR spectroscopy of their (*R*)-MTPA-esters. (*S*)-**3b** and **3e** were chemically transformed via their azides to phosphonic acid analogues of *L*-phenylalanine and *L*-tyrosine, respectively.

Introduction

The last few years have witnessed an increasing interest in phosphonic acids[2] as stable transition state analogue enzyme inhibitors, as non-hydrolysable antimetabolites of biologically important phosphates and as amino acid antimetabolites. The phosphonic acid and especially the phosphonous acid are considered isosteric replacements for the carboxyl group in amino acids. They may be accepted by enzymes as false substrates and interfere with biological processes.[3]

Many methods exist for the synthesis of racemic α-aminophosphonic acids.[4] They very often involve addition of phosphorus acid esters to imines generated in situ. Optically active α-aminophosphonic acids[5] have been obtained by chemical[6,7] and enzymatic[8] resolution and a variety of asymmetric syntheses.[9,10]

Esterolytic enzymes[11] are frequently used to prepare optically active secondary alcohols by kinetic resolution. We have recently demonstrated that antipodes of α-hydroxyphosphonates are accessible by lipase-catalysed enantioselective hydrolysis of the corresponding α-acyloxy derivatives.[1] The development of methods for the synthesis of optically active α-hydroxyphosphonates is gaining momentum.[12] In this paper we disclose our results on the enantioselective hydrolysis of 1-acyloxy-2-arylethylphosphonates and the use of the optically active α-hydroxyphosphonates obtained to synthesise phosphonic acid analogues of *L*-phenylalanine and *L*-tyrosine.

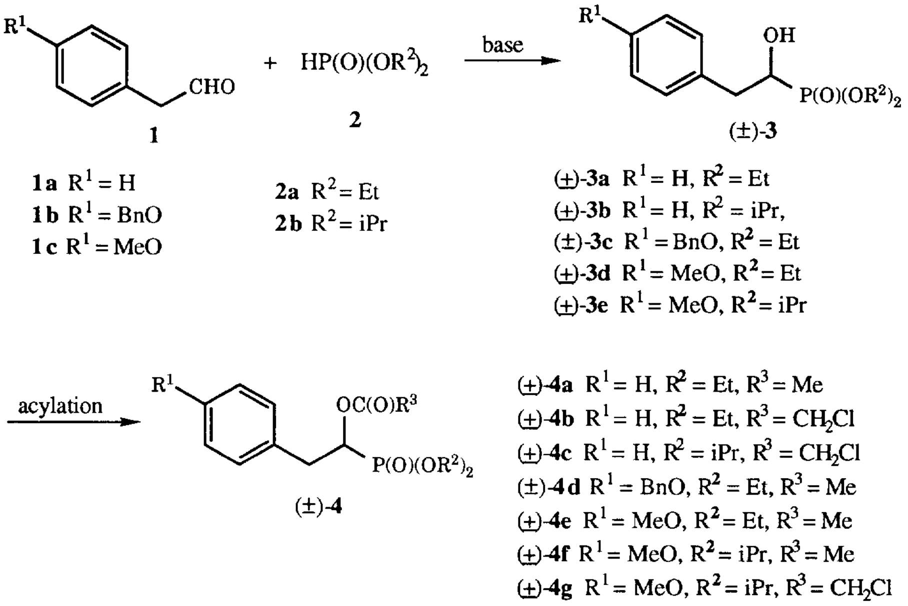
Table 1 Enzymatic Hydrolysis of 1-Acyloxy-2-arylethylphosphonates [(±)-**4**]

Entry	Substrate		AP 6	Buffer	HE:BME^a;		Temp.	Time	Convsn.^{b}	Produced alcohol **3**					Alcohol from recovered **4**				
	(±)-**4**, mmol		(mg)	(ml)	ml		(°C)	(hrs)	(%)	yield(%)	ee(%)	conf.	[α]$_D$	(c.^{d})	yield(%)^c	ee(%)	conf.	[α]$_D$	(c.^{d})
1	**a**	1.0	50.3	17	3:1;	4	22	16.73	45	44	17^e	(*R*)	-6.14	1.84	52		(*S*)	+5.47	2.82^f
2	**a**	1.0	80.2	17	3:1;	4	23	21.45	45	32	92	(*R*)	-34.20	0.69	48	54	(*S*)	+21.63	1.91
3	**b**	1.0	6.6	17	1:1;	4	21	3.63	45	46	8	(*R*)	-4.08	1.50	47	3	(*S*)	+1.32	1.51
4	**b**	1.0	10.0	17	1:1;	4	0	3.70	40	43	8^e	(*R*)	-3.08	1.63	55		(*S*)	+2.24	2.06^f
5	**c**	1.0	31.6	17	1:1;	4	24	18.77	45	41	52	(*S*)	+16.74	1.60	40	43	(*R*)	-14.87	1.56
6	**c**	6.2	92.9	35	3:1;	15	24	15.00	35	31	63	(*S*)	+20.56	1.97	61	32	(*R*)	-10.75	2.00
7	**c**	10.0	599.0	50	1:1;	20	0	7.30	22	21	65	(*S*)	+20.51	2.17	61	12	(*R*)	-4.96	2.02
8	**d**	1.1	98.0	17	3:1;	4	20	100.00	0	no		reaction							
9	**e**	1.1	100.0	15	3:1;	4	25	8.00	40	33	10	(*R*)	n.d.^{g}		n.d.^{g}				
10	**f**	1.0	150.0	15	3:1;	4	30	63.00	47	32	78	(*S*)	+16.65	1.90	59		(*R*)	-11.94	2.20^f
11	**g**	2.9	100.0	30	1:1;	5	20	4.30	44	38	72	(*S*)	+15.40	2.30	45		(*R*)	-12.63	2.50^f

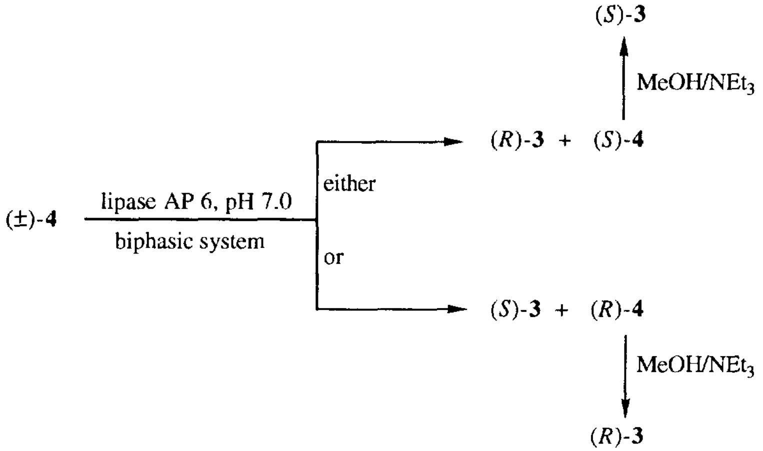
a) HE = hexane; BME = *tert*-butyl methyl ether. b) Conversion was calculated from the volume of 0.5 N NaOH added by the autotitrator. c) Yield of acetate multiplied by yield of chemical hydrolysis. d) Concentration (g/100 ml) of compound in acetone. e) Calculated from value of entry 2. f) Data of recovered ester **4**. g) n. d. = not determined.

Synthesis of 1-acyloxy-2-arylethylphosphonates and their lipase-catalysed resolution

Phenylacetaldehyde (**1a**) and its *para* substituted derivatives **1b** and **1c** were treated with phosphites **2a** and **2b** with base catalysis to yield α-hydroxyphosphonates (±)-**3a** and **3c-e** (Scheme 1).[1] Phosphonate (±)-**3b** was prepared by addition of the lithium salt of diisopropylphosphite to aldehyde **1a** at -78 °C, which gave a much cleaner reaction product than when sodium ethoxide was employed as base. The α-hydroxyphosphonates (±)-**3** were acetylated or chloroacetylated to afford α-acyloxyphosphonates (±)-**4**. α-Acyloxyphosphonates (±)-**4** were hydrolysed enzymatically with lipase AP 6 (lipase from *Aspergillus niger*) in a well stirred biphasic system (phosphate buffer pH 7.0, mixture of hexane / *tert*-butyl methyl ether) at room temperature as reported previously (Scheme 2).[1] The pH was kept constant by automatic addition of 0.5 N NaOH (autotitrator) and the reaction was stopped with 1 N HCl at a conversion of up to 45%. Workup gave mixtures of α-hydroxyphosphonate **3** and unreacted α-acyloxyphosphonate **4** which were separated by flash chromatography. The optically active ester **4** was hydrolysed chemically under mild conditions in MeOH/NEt$_3$ so as not to cause a base induced partial racemisation of the α-hydroxyphosphonate (Table 1).

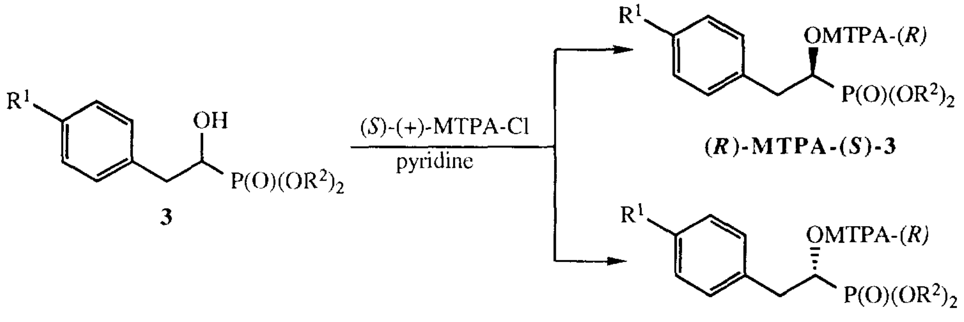


Scheme 1 Preparation of α-hydroxyphosphonates (±)-**3** and α-acyloxyphosphonates (±)-**4**



Scheme 2 Lipase-catalysed hydrolysis of 1-acyloxy-2-arylethylphosphonates (±)-**4**

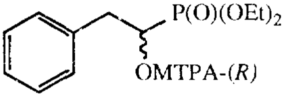
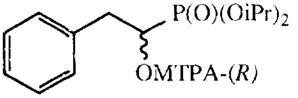
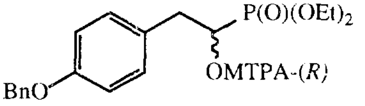
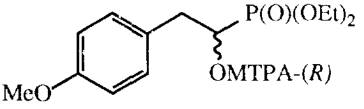
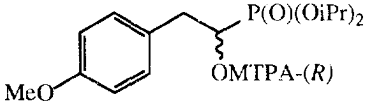
The enantiomeric purity of the α-hydroxyphosphonates **3** were determined by derivatisation[13] with Mosher acid chloride [(*S*)-(+)-MTPA-Cl] and use of ^1H and / or ^{31}P NMR spectroscopy (Scheme 3). The absolute configuration was deduced from the chemical shifts of the resonances of the phosphorus atoms in the two diastereomeric Mosher esters. The phosphorus of the Mosher ester derived from the (*S*)-α-hydroxy-



Scheme 3 Preparation of Mosher esters of α-hydroxyphosphonates **3**

phosphonate resonates at lower field than that of the (*R*)-hydroxyphosphonate.[14] This general rule formed the basis for the assignment of absolute configuration. The chemical shift differences [δ(*S*,*R*) - δ(*R*,*R*)] are about 0.5 ppm (Table 2). In two cases, as will be seen later, the assignment was verified by chemical correlation of the α-hydroxyphosphonates with phosphonic acid analogues of *L*-phenylalanine and *L*-tyrosine of known configuration.

Table 2 Assignment of configuration at C-1 of diastereomeric Mosher esters, prepared from α-hydroxyphosphonates **3** on the basis of ^{31}P NMR chemical shifts

Mosher esters	Chemical shifts δ (ppm)		Δδ
	(*S*)	(*R*) at C-1	
	19.01	18.52	0.49
	16.91	16.38	0.53
	19.25	18.73	0.52
	19.27	18.75	0.52
	17.00	16.45	0.55

When α-acyloxyphosphonate (±)-**4a** was used as substrate for lipase AP 6, the enantiomeric excess of α-hydroxyphosphonate (*R*)-**3a** formed varied from 17 to 92% (Tables 1, entries 1 and 2). These results seemed to depend on the purity of the starting α-hydroxyphosphonate (±)-**3a** used for the preparation of the α-acyloxyphosphonate (±)-**4a**. When chromatographically homogeneous (±)-**3** was used as starting material for the preparation of α-acyloxyphosphonate (±)-**4**, the ee of the α-hydroxyphosphonate formed on enzymatic hydrolysis was just 17%. If the starting material was not pure and thus contained an impurity of unknown structure, the ee increased to up to 92%. The preferentially hydrolysed ester had (*R*)-configuration. The chloroacetate (±)-**4b** was hydrolysed more easily by lipase AP 6 than the corresponding acetate both at ambient and at 0 °C (entries 3 and 4). The (*R*)-ester **4b** was more reactive, but the ee was very low, 8% at 45% conversion. To increase the enantioselectivity of the lipase-catalysed hydrolysis the diethylphosphoryl group was replaced by the more bulky diisopropylphosphoryl group which resulted in a concomitant decrease in the reaction rate. To compensate for this the corresponding chloroacetate (±)-**4c** was used as a substrate for the enzyme (entries 5, 6, and 7). This time the α-hydroxyphosphonate **3b** formed had (*S*)-configuration and an ee of 52% at 40% conversion. The ee went up to 63% when the reaction was carried out at ambient temperature and stopped at a conversion of just 35%. When the reaction was carried out at 0 °C, the enantiomeric purity of the α-hydroxyphosphonate **3b** remained almost unchanged, although the conversion was only 22%.

The acyloxyphosphonates derived from *para* substituted phenylacetaldehydes showed similar behaviour, except for compound (±)-**4d**. Presumably the benzyloxy group, which is readily cleaved by hydrogenolysis, is so big that substrate (±)-**4d** cannot be accepted by the enzyme and is therefore not hydrolysed (entry 8). The α-chloroacetyloxyphosphonate (±)-**4e** yields on enzymatic hydrolysis α-hydroxyphosphonate (*R*)-**3d** with 10% ee at 40% conversion. When the corresponding diisopropyl phosphonate (±)-**4f** was allowed to react with lipase AP 6, the reaction rate decreased by a factor of about 10, but the enantiomeric purity of the α-hydroxyphosphonate **3e** formed on hydrolysis was 78% at 47% conversion and was of (*S*)-configuration. The chloroacetate (±)-**4g** was hydrolysed enzymatically with a similar enantioselectivity as acetate (±)-**4f** and the reaction rate increased by a factor of 20 - 30.

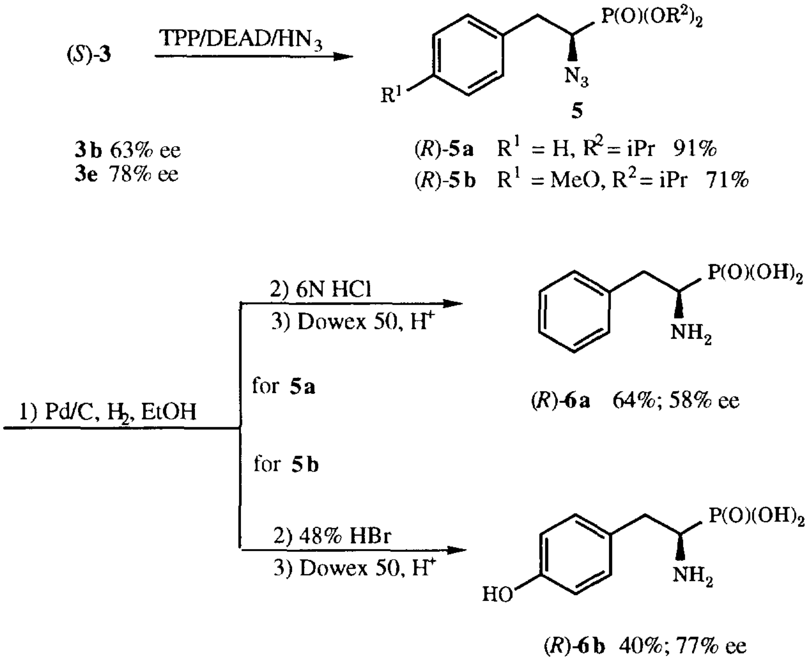
We also tested a second enzyme for its ability to hydrolyse α-acyloxyphosphonates. Lipase F-AP 15 (from *Rhizopus oryzae*) reacted very slowly with substrates (±)-**4a** and **4e** to produce α-hydroxyphosphonates with (*R*)-configuration.

The two sets of α-acyloxyphosphonates derived from unsubstituted and *para* methoxy substituted phenylacetaldehydes are substrates for lipase AP 6. The commercially available product contains only a small amount[15] of lipase as an admixture with other enzymes. We assume that it is only the lipase which reacts with the added substrates. Surprisingly, the same enzyme can give either (*R*)- or (*S*)-α-hydroxyphosphonate preferentially depending on whether the diethyl or diisopropyl phosphonate is used. It is likely that the enzyme has two binding sites, one for the benzyl group and the other for the phosphonate group. The $ArCH_2$ and $P(O)(OEt)_2$ groups can bind to either site resulting in low enantioselectivity. Dimethyl- and diisopropyl α-acyloxyphosphonates derived from benzaldehyde, acetaldehyde, and crotonaldehyde consistantly gave (*S*)-α-hydroxyphosphonates on enzymatic hydrolysis.[1] The cases reported here demonstrate that the configuration of the α-hydroxyphosphonate can in part be determined by the ´protecting´ group. Some of the α-acyloxyphosphonates used here are borderline cases for which the enantioselectivity changes from (*S*) to (*R*) depending on the protecting group used for the phosphonic acid.

Synthesis of (*R*)-1-amino-2-phenylethylphosphonic acid and (*R*)-1-amino-2-(4-hydroxyphenyl)ethylphosphonic acid (phosphonic acid analogues of *L*-phenylalanine and *L*-tyrosine)

In higher plants the biopolymer lignin and various aromatic secondary metabolites, notably many alkaloids and flavonoids are formed from the aromatic amino acids *L*-phenylalanine and / or *L*-tyrosine.[16] The first step in the biosynthesis of these natural products involves phenylalanine ammonia lyase (PAL). It catalyses the elimination of ammonia from phenylalanine, thereby removing the pro-(3*S*) hydrogen to form (*E*)-cinnamic acid. (*R*)-1-Amino-2-phenylethylphosphonic acid (APEP) is an effective inhibitor (K_i = 1.5 μM) of this enzyme *in vivo* .[17] This chiral compound is therefore an attractive synthetic target.

α-Hydroxyphosphonate (*S*)-**3b**, obtained by lipase AP 6 catalysed enantioselective hydrolysis was transformed in 91% yield using $Ph_3P/DEAD/HN_3$[18] into azide (*R*)-**5a** (Scheme 4). This azide was reduced with hydrogen over palladium on charcoal and deprotected with refluxing 6 N HCl. The free aminophosphonic acid analogue of *L*-phenylalanine was purified by ion exchange chromatography on Dowex 50, H^+ and isolated in 64% yield with an ee of 58% as determined by comparison with the optical rotation reported in the literature.[5,19]



Scheme 4 Synthesis of phosphonic acid analogues of *L*-phenylalanine and *L*-tyrosine

(*R*)-1-Amino-2-(4-hydroxyphenyl)ethylphosphonic acid [(*R*)-**6b**], the phosphonic acid analogue of tyrosine, was isolated as a component of two tripeptides from *Actinomyces K 26* and *Actinomadura spiculosospora*.[20] It is the only α-aminoalkylphosphonic acid so far isolated from biological material. Its synthesis is given in Scheme 4 and is very similar to the transformations used for the phosphonic acid analogue of phenylalanine. Originally it was planned to use the benzyl group to protect the phenolic hydroxy group. It was hoped to remove it hydrogenolytically when the azide group was reduced. As the requisite α-acyloxyphosphonate (±)-**4d** was not a substrate for lipase AP 6, the methyl group was substituted as protecting group. It was removed by refluxing aqueous hydrobromic acid. The aminophosphonic acid analogue (R)-**6b** of *L*-tyrosine was isolated in 40% yield and had an ee of 77% (by comparison with optical rotation of literature[6]). These two correlations provide additional evidence for the correct assignment of absolute configurations by NMR spectroscopy of Mosher esters, as both methods are in agreement.

Experimental

^1H NMR spectra were recorded on a Bruker AM 400 WB (400 MHz) spectrometer in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in ppm relative to internal standard TMS or HDO, when D_2O was used as solvent, and coupling constants in Hz. ^{31}P NMR spectra were recorded on the same spectrometer (162 MHz) using 85% H_3PO_4 as external standard. IR spectra were run on a Perkin Elmer 1600 FT-IR spectrometer on films obtained by applying a solution from the NMR sample to a silicon plate and allowing the solvent to evaporate[21] or on a film of liquid compound between NaCl windows. Optical rotations were measured at 20°C on a Perkin Elmer 241 polarimeter in a 1 dm cell in acetone solution or in other solvents as given.

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 24 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ and 1 g of $Ce(SO_4)_2.4H_2O$ 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 autotitrator. (*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 absolute configurations and enantiomeric excesses by NMR spectroscopy.

Lipases AP 6 (*Aspergillus niger*) and F-AP 15 (*Rhizopus oryzae*) were gifts from Amano Enzyme Limited (UK). Abbreviations used: MC = methylene chloride; EA = ethyl acetate.

Diethyl 1-hydroxy-2-phenylethylphosphonate [(±)-**3a**]: Phenylacetaldehyde (**1a**) (0.66 g, 5.5 mmol) and diethyl phosphite (0.69 g, 5 mmol) were dissolved in dry ether (7 ml). The reaction mixture was cooled to -35°C with a dry ice-acetone bath.[1,22] A saturated solution of sodium ethoxide in ethanol (0.03 ml) was added. The mixture was stirred for 10 min and two drops of sulfuric acid were added. The solvents were removed under reduced pressure. Water was added to the residue and the phosphonate was extracted with ethyl acetate (3 x 20 ml). The organic layers were combined, dried with $MgSO_4$ and the solvents were removed in vacuo. Flash chromatography ($R_f = 0.31$, impurity with $R_f = 0.38$; MC:EA = 5:3), starting with MC:EA = 15:1 and ending with 1:1, afforded phosphonate (±)-**3a** (0.933 g, 72%; 0.166 g of mixture). IR: υ_{max} 3300, 2983, 1496, 1454, 1393, 1219, 1027, 970 cm^{-1}. ^{1}H NMR: δ 1.31, 1.33 (2x3H, 2xt, J = 6.9, $P(OCH_2C\underline{H}_3)_2$), 3.03 (2H, AB part of ABXP system, J = 3.5, 6.9, 10.3, 2x14.3, $PhC\underline{H}_2$), 3.25 (1H, br, t, J = 6.4, OH), 4.10 (1H, m, CHP), 4.16 (2x2H, m, $P(OC\underline{H}_2CH_3)_2$), 7.20 (5H, m, C_6H_5). Elemental analysis: $C_{12}H_{19}O_4P$ Calcd.: C: 55.81%, H: 7.42%; Found: C: 55.82%, H: 7.40%.

Diisopropyl 1-hydroxy-2-phenylethylphosphonate [(±)-3b]: n-BuLi (9.4 ml of a 1.4 N solution in hexane, 15 mmol) was added dropwise to a stirred solution of diisopropylphosphite (2.16 g, 13 mmol) in dry THF (34 ml) at -78°C under argon, followed by phenylacetaldehyde (1.8 g, 15 mol) in dry THF (7 ml) after 5 min and acetic acid (0.94 ml, 16 mmol) in dry THF (3 ml) after 10 min. The cooling bath was removed and stirring was continued for 10 min at room temperature. The solvent was removed in vacuo and the residue was diluted with water (20 ml). The product was extracted with methylene chloride (3 x 20 ml). The extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (R_f = 0.33; MC:EA = 5:3) to afford α-hydroxyphosphonate (±)-**3b** as colorless oil (3.10 g, 83%). IR (film): υ_{max} 2978, 1454, 1219, 1106, 982 cm^{-1}. ^{1}H NMR: δ 1.31, 1.33, 1.36 (2x3H, 1x6H, 3xd, J = 6.4, $P(OCH\underline{Me}_2)_2$), 2.65 (1H, dd, J = 2.96, 5.41, OH), 3.00 (2H, AB parts of ABXP system, J = 3.0, 6.4, 2x10.0, 14.3, $PhC\underline{H}_2$), 4.02 (m, 1H, CHP), 4.77 (2x1H, m, $P(OC\underline{H}Me_2)_2$), 7.26 (m, 5H, C_6H_5). Elemental analysis: $C_{14}H_{23}O_4P$ Calcd.: C: 58.73%, H: 8.10%; Found: C: 58.72%, H: 8.27%.

Diethyl 1-hydroxy-2-(4-benzyloxyphenyl)ethylphosphonate [(±)-3c]: Aldehyde[23] **1b** (0.854 g, 3.77 mmol) and diethyl phosphite (0.625 g, 4.53 mmol) were dissolved in dry ether (20 ml). The solution was cooled to -35°C. A saturated solution of sodium ethoxide in ethanol (0.05 ml) was added dropwise. After 10 min the reaction was worked up as given for (±)-**3a**. Purification by flash chromatography (R_f = 0.16; MC:EA = 5:3) afforded the α-hydroxyphosphonate (±)-**3c** (0.915 g, 67%). IR: υ_{max} 3288, 2984, 1611, 1512, 1454, 1390, 1242, 1177, 1026, 970 cm^{-1}. ^{1}H NMR: δ 1.32, 1.34 (2x3H, 2xt, J = 6.9, $P(OCH_2C\underline{H}_3)_2$), 2.81 (1H, dd, J = 5.4, 8.9, OH), 2.99 (2H, AB part of ABXP system, J = 3.5, 6.9, 2x10.3, 14.3, $PhC\underline{H}_2$), 4.04 (1H, m, CHP), 4.17 (2x2H, m, $P(OC\underline{H}_2CH_3)_2$), 5.04 (2H, s, $PhC\underline{H}_2O$), 7.06 (4H, AA′BB′ system, C_6H_4), 7.39 (5H, m, C_6H_5). Elemental analysis: $C_{19}H_{25}O_5P$ Calcd.: C: 62.63%, H: 6.92%; Found: C: 62.78%, H: 6.90%.

Diethyl 1-hydroxy-2-(4-methoxyphenyl)ethylphosphonate [(±)-3d]: Aldehyde[23] **1c** (1.072 g, 7.14 mmol) and diethyl phosphite (1.18 g, 8.57 mmol) were dissolved in dry ether (20 ml). The solution was cooled to -35°C. A saturated solution of sodium ethoxide in ethanol (0.05 ml) was added dropwise. After 10 min the reaction was worked up as given for (±)-**3a**. Purification by flash chromatography (R_f = 0.16; MC:EA = 5:3) afforded the α-hydroxyphosphonate (±)-**3d** (2.029 g, 99%). IR: υ_{max} 3313, 2985, 1612, 1514, 1443, 1248, 1179, 1031, 970 cm^{-1}. ^{1}H NMR: δ 1.33, 1.34 (2x3H, 2xt, J = 7.4, $P(OCH_2C\underline{H}_3)_2$), 2.75 (1H, br, s, OH), 2.99 (2H, AB part of ABXP system, J = 3.5, 6.9, 2x10.3, 14.3, $PhC\underline{H}_2$), 3.79 (3H, s, OMe), 4.05 (1H, m, CHP), 4.17 (2x2H, m, $P(OC\underline{H}_2CH_3)_2$), 7.02 (4H, AA′BB′ system, C_6H_4). Elemental analysis: $C_{13}H_{21}O_5P$ Calcd.: C: 54.16%, H: 7.34%; Found: C: 54.28%, H: 7.24%.

Diisopropyl 1-hydroxy-2-(4-methoxyphenyl)ethylphosphonate [(±)-3e]: Aldehyde **1c** (0.705 g, 4.7 mmol; prepared[23] in analogy to **1b**) and diisopropyl phosphite (0.831 g, 5.0 mmol) were dissolved in dry ether (20 ml). The solution was cooled to -35°C. A saturated solution of sodium methoxide in methanol (0.01 ml) was added. After 10 min the reaction was worked up as given for (±)-**3a**. Purification by flash chromatography (R_f = 0.09; MC:EA = 5:3) afforded the α-hydroxyphosphonate (±)-**3e** (1.17 g, 79%). IR: υ_{max} 3288, 2978, 1612, 1514, 1466, 1386, 1248, 1178, 1108, 989 cm^{-1}. ^{1}H NMR: δ 1.31, 1.33, 1.35x2 (4x3H, 4xd, J = 6.4, $P(OCH\underline{Me_2})_2$), 2.73 (1H, dd, J = 5.4, 8.9, OH), 2.96 (2H, AB part of ABXP system, J = 3.5, 6.4, 2x10.3, 14.3, $PhC\underline{H_2}$), 3.79 (3H, s, OMe), 3.96 (1H, m, CHP), 4.77 (2x1H, m, $P(OC\underline{H}Me_2)_2$), 7.02 (4H, AA'BB' system, C_6H_4). Elemental analysis: $C_{15}H_{25}O_5P$ Calcd.: C: 56.95%, H: 7.97%; Found: C: 57.03%, H: 7.82%.

Diethyl 1-acetyloxy-2-phenylethylphosphonate [(±)-4a]: The acetate was prepared as reported recently[1]; yield: 95%, R_f = 0.63 (MC:EA = 5:3). IR: υ_{max} 2984, 1751, 1456, 1371, 1224, 1028, 972 cm^{-1}. ^{1}H NMR: δ 1.30, 1.33 (2x3H, 2xt, J = 6.9, $P(OCH_2C\underline{H_3})_2$), 1.97 (3H, s, MeCO), 3.14 (2H, AB part of ABXP system, J = 3.9, 5.9, 2x10.8, 14.3, $PhC\underline{H_2}$), 4.15 (2x2H, m, $P(OC\underline{H_2}CH_3)_2$), 5.48 (1H, ddd, J = 3.9, 8.4, 10.8, CHP), 7.24 (5H, m, C_6H_5). Elemental analysis: $C_{14}H_{21}O_5P$ Calcd.: C: 55.60%, H: 7.05%; Found: C: 55.68%, H: 7.06%.

Diethyl 1-chloroacetyloxy-2-phenylethylphosphonate [(±)-4b]: Prepared by the procedure used for the synthesis of (±)-**4c**. The residue was purified by flash chromatography (R_f = 0.67; MC:EA = 5:3) to yield (±)-**4b** as a colorless oil (71%). IR: υ_{max} 2984, 1770, 1456, 1324, 1260, 1163, 1020, 974 cm^{-1}. ^{1}H NMR: δ 1.24, 1.27 (2x3H, 2xt, J = 6.9, $P(OCH_2C\underline{H_3})_2$), 3.09 (2H, AB part of ABXP system, J = 4.0, 5.4, 2x10.5, 14.7, $PhC\underline{H_2}$),3.88 (2H, AB system, J = 15.0, CH_2Cl), 4.09 (2x1H, m, $P(OC\underline{H_2}CH_3)_2$), 5.44 (1H, ddd, J = 4.0, 8.4, 10.5, CHP) 7.18 (5H, m, C_6H_5). Elemental analysis: $C_{14}H_{20}ClO_5P$ Calcd.: C: 50.30%, H: 5.99%; Found: C: 49.94%, H: 5.82%.

Diisopropyl 1-chloroacetyloxy-2-phenylethylphosphonate [(±)-4c]: A solution of chloroacetic acid anhydride (1.88 g, 11 mmol) in dry methylene chloride (6 ml) was added to a solution of phosphonate (±)-**3b** (2.094 g, 7.32 mmol) in dry pyridine (1.77 ml, 22 mmol) and dry methylene chloride (30 ml) at 0°C and stirring was continued for 1 hr. The cooling bath was removed and water (10 ml) was added. After stirring for 10 min at room temperature conc. hydrochloric acid (1 ml) was added. The organic phase was separated and the aqueous was extracted with methylene chloride (3 x 10 ml). The combined organic layers were washed with water (10 ml), a saturated aqueous solution of $NaHCO_3$ (10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography (R_f = 0.72; MC:EA = 5:3) to yield (±)-**4c** as a colorless oil (2.333 g, 88%). IR (film): υ_{max} 2982, 1770, 1605, 1496, 1455, 1413, 1386, 1323, 1257, 1160, 1104, 995 cm^{-1}. ^{1}H NMR: δ 1.32, 1.33, 1.35, 1.36 (4x3H, 4xd, J = 6.4, $P(OCH\underline{Me_2})_2$), 3.13 (2H, AB part of ABXP system, J = 4.0, 5.4, 2x10.5, 14.5, $PhC\underline{H_2}$), 3.94 (2H, AB system, J = 15.3, CH_2Cl), 4.78 (2x1H, m, $P(OC\underline{H}Me_2)_2$), 5.47 (1H, ddd, J = 4.0, 8.9, 10.5, CHP), 7.25 (5H, m, C_6H_5). Elemental analysis: $C_{16}H_{24}ClO_5P$ Calcd.: C: 52.97%, H: 6.67%; Found: C: 52.68%, H: 6.55%.

Diethyl 1-acetyloxy-2-(4-benzyloxyphenyl)ethylphosphonate [(±)-4d]: α-Hydroxyphosphonate (±)-**3c** was acetylated[1]. Pure acetate (±)-**4d** was obtained by flash chromatography (R_f = 0.25; MC:EA = 5:1) in nearly quantitative yield. IR: υ_{max} 2984, 1750, 1611, 1513, 1455, 1370, 1226, 1178, 1026, 971 cm^{-1}. ^{1}H NMR: δ 1.30, 1.33 (2x3H, 2xt, J = 6.9, $P(OCH_2C\underline{H}_3)_2$), 1.98 (3H, s, MeCO), 3.08 (2H, AB part of ABXP system, J = 3.9, 5.9, 2x10.8, 14.8, $PhCH_2$), 4.11 (2x2H, m, $P(OC\underline{H}_2CH_3)_2$), 5.03 (2H, s, $PhC\underline{H}_2O$), 5.43 (1H, ddd, J = 3.9, 8.4, 10.8, CHP), 7.01 (4H, AA′BB′ system, C_6H_4), 7.40 (5H, m, C_6H_5). Elemental analysis: $C_{21}H_{27}O_6P$ Calcd.: C: 62.06%, H: 6.70%; Found: C: 62.12%, H: 6.65%.

Diethyl 1-acetyloxy-2-(4-methoxyphenyl)ethylphosphonate [(±)-4e]: The α-hydroxyphosphonate (±)-**3d** (1.997 g, 6.93 mmol) was acetylated.[1] Pure acetate (±)-**4e** (2.252 g, 98%) was obtained by flash chromatography (R_f = 0.27; MC:EA = 5:1). IR: υ_{max} 2986, 1751, 1612, 1514, 1443, 1371, 1250, 1225, 1179, 1031, 973 cm^{-1}. ^{1}H NMR: δ 1.31, 1.34 (2x3H, 2xd, J = 6.9, $P(OCH_2C\underline{H}_3)_2$), 1.98 (3H, s, MeCO), 3.08 (2H, AB part of ABXP system, J = 3.9, 5.4, 2x10.8, 14.3, $PhC\underline{H}_2$), 3.78 (3H, s, MeO), 4.15 (2x2H, m, $P(OC\underline{H}_2CH_3)_2$), 5.44 (1H, ddd, J = 3.9, 8.4, 10.8, CHP), 6.97 (4H, AA′BB′ system, C_6H_4). Elemental analysis: $C_{15}H_{23}O_6P$ Calcd.: C: 54.54%, H: 7.02%; Found: C: 54.58%, H: 7.00%.

Diisopropyl 1-acetyloxy-2-(4-methoxyphenyl)ethylphosphonate [(±)-4f]: The α-hydroxyphosphonate (±)-**3e** (1.45 g, 4.58 mmol) was acetylated.[1] Acetate (±)-**4f** (1.335 g, 81%) was obtained by flash chromatography (R_f = 0.34; MC:EA = 5:1) and bulb to bulb distillation (155-160°C/0.005 mmHg). IR: υ_{max} 2980, 1751, 1612, 1514, 1466, 1374, 1250, 1179, 1106, 989 cm^{-1}. ^{1}H NMR: δ 1.27, 1.29, 1.32x2 (4x3H, 4xd, J = 5.9, $P(OCH\underline{Me}_2)_2$), 1.93 (3H, s, MeCO), 3.02 (2H, AB part of ABXP system, J = 2x3.9, 2x10.8, 14.6, $PhC\underline{H}_2$), 3.74 (3H, s, OMe), 4.72 (2x1H, m, $P(OC\underline{H}Me_2)_2$), 5.37 (1H, ddd, J = 3.9, 8.9, 10.8, CHP), 6.93 (4H, AA′BB′ system, C_6H_4). Elemental analysis: $C_{17}H_{27}O_6P$ Calcd.: C: 56.98%, H: 7.59%; Found: C: 57.11%, H: 7.45%.

Diisopropyl 1-chloroacetyloxy-2-(4-methoxyphenyl)ethylphosphonate[(±)-4g]: 1,1′-Carbonyldiimidazole (1.2 g, 7.4 mmol) and chloroacetic acid (0.7 g, 7.4 mmol) were dissolved in dry methylene chloride (10 ml). The mixture was stirred at room temperature for 20 min. Phosphonate (±)-**3e** (1.17 g, 3.7 mmol) in dry methylene chloride (10 ml) was then added to the above solution. The mixture was allowed to stand overnight at room temperature. Water (20 ml) was added. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3x10 ml). The combined organic phases were dried with sodium sulfate. After removal of the solvents under reduced pressure, the residue was purified by flash chromatography (R_f = 0.55; MC:EA = 5:1) to afford the product (±)-**4g** (0.956 g, 66%). IR: υ_{max} 2981, 1770, 1612, 1514, 1466, 1387, 1250, 1179, 1105, 992 cm^{-1}. ^{1}H NMR: δ 1.32, 1.33, 1.35x2 (4x3H, 4xd, J = 6.4, $P(OCH\underline{Me}_2)_2$), 3.09 (2H, AB part of ABXP system, J = 3.5, 4.2, 10.3, 10.8, 14.8, $PhC\underline{H}_2$), 3.78 (3H, s, OMe), 3.95 (2H, AB system, J = 14.8, $ClCH_2$), 4.77 (2x1H, m, $P(OC\underline{H}Me_2)_2$), 5.42 (1H, ddd, J = 3.5, 8.4, 10.8, CHP), 6.96 (4H, AA′BB′ system, C_6H_4). Elemental analysis: $C_{17}H_{26}ClO_6P$ Calcd.: C: 51.98%, H: 6.67%; Found: C: 52.10%, H: 6.55%.

(1*R*)-Diisopropyl 1-azido-2-phenylethylphosphonate [(*R*)-5a]: To a stirred mixture of α-hydroxyphosphonate (*S*)-**3b** (0.58 g, 2.03 mmol, ee 63%) and triphenylphosphine (0.805 g, 3.07 mmol) in dry toluene (15 ml) and dry methylene chloride (3 ml) at 0°C under argon were added diethyl azodicarboxylate (0.48 ml, 3.07 mmol) and a solution of HN_3 in toluene (0.5 N, 6 ml).[18] Stirring was continued for 30 min at

0°C and 1 hr at room temperature and then methanol (0.2 ml) was added. After 30 min the solvent was removed in vacuo. Hexane was added to the residue and the mixture was allowed to stand overnight. The crystals were removed and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography ($R_f = 0.34$; petrol ether:acetone = 3:1) to give azide (*R*)-**5a** (0.574 g, 91%); $[\alpha]_D = -40.0$ (c = 2.68). IR (film): υ_{max} 2981, 2121, 1455, 1386, 1258, 1178, 1104, 990 cm^{-1}. ^{1}H NMR: δ 1.33, 1.35 (1x3H, 3x3H, J = 6.4, P(OCH$\underline{Me_2}$)$_2$), 2.89 (2H, AB part of ABXP system, J = 3.0, 5.9, 7.4, 11.8, 14.3, PhC$\underline{H_2}$), 3.55 (dt, J = 3.0, 2x11.8, CHP), 4.79 (2x1H, m, P(OC$\underline{H}$Me$_2$)$_2$), 7.26 (5H, m, C_6H_5). Elemental analysis: $C_{14}H_{22}N_3O_3P$ Calcd.: C: 54.01%, H: 7.12%; N: 13.50; Found: C: 54.04%, H: 7.23%, N: 13.45%.

(1*R*)-1-Amino-2-phenylethylphosphonic acid [(*R*)-6a]: Azide (*R*)-**5a** (0.510 g, 1.64 mmol) was dissolved in ethanol (58 ml) containing concentrated hydrochloric acid (1 ml). Palladium on active carbon (10%, 0.1 g) was added. The reaction mixture was hydrogenated in a Parr apparatus at room temperature for 4 h. After removal of the catalyst and the solvent, the residue was dissolved in 6 N HCl (50 ml) and refluxed for 20 hr. The solvent was evaporated under reduced pressure. The crude material was dried in a dessicator over KOH, dissolved in a solution of NaOH (1N, 4 ml) and applied to a column filled with Dowex 50, H$^+$ and eluted with water. Ninhydrine positive fractions were pooled and concentrated to give a crystalline solid. It was dissolved in hot glacial acetic acid for transfer to another flask. Concentration on a rotary evaporator and drying left white crystals of (*R*)-**6a** (0.265 g, 64%), containing acetic acid (0.83 mol per mol of aminophosphonic acid, by ^{1}H NMR) which was removed by drying for 1 hr at 120°C/0.01 mm; m.p. 263-265°C; {$[\alpha]_{578}$ = -31.34 (c = 1.005 of dried sample, 1N NaOH), 1N NaOH, 58% ee (determined by comparison with reported optical rotation of monohydrate}, {$[\alpha]_{578}$ = +49.9 (c = 2, 1N NaOH) for monohydrate of (*S*)-(+)-enantiomer}.[19] ^{1}H NMR: (D_2O/NaOD): δ 2.46 (1H, ddd, J = 5.9, 12.0, 14.0, PhC$\underline{H_a}$), 2.84 (1H, dt, J = 2.5, 2x12.0, PhC$\underline{H_b}$), 3.18 (1H, ddd, J = 2.5, 4.9, 14.0, CHP), 7.30 (5H, m, C_6H_5). Elemental analysis for sample dried sample: $C_8H_{12}NO_3P$ Calcd.: C: 47.76%, H: 5.97%, N: 6.97%; Found: C: 47.46%, H: 5.80%, N: 6.75%.

(1*R*)-Diisopropyl 1-azido-2-(4-methoxyphenyl)ethylphosphonate [(*R*)-5b]: α-Hydroxyphosphonate (*S*)-**3e** {0.307 g, 0.971 mmol; $[\alpha]_D$ = +16.65 (c = 1.92), 78%ee} was transformed into azide (*R*)-**5b** by the procedure used for the synthesis of (*R*)-**5a**. The oily residue was purified by flash chromatography ($R_f = 0.56$; MC:EA = 5:1) to afford azide (*R*)-**5b** (0.235 g, 71%) {$[\alpha]_D$ = -34.24 (c = 0.70)}. IR: υ_{max} 2981, 2106, 1612, 1514, 1466, 1386, 1251, 1179, 1142, 1105, 990 cm^{-1}. ^{1}H NMR: δ 1.37, 1.39x3 (4x3H, 4xd, J = 6.4, P(OCH$\underline{Me_2}$)$_2$), 2.97 (2H, AB part of ABXP system, J = 3.0, 7.4, 11.8, 2x14.8, PhC$\underline{H_2}$), 3.54 (1H, dt, J = 3.0, 11.8, CHP), 3.80 (3H, s, OMe), 4.82 (2x1H, m, P(OC$\underline{H}$Me$_2$)$_2$), 7.02 (4H, AA′BB′ system, J = 8.9, C_6H_4). Elemental analysis: $C_{15}H_{24}N_3O_4P$ Calcd.: C: 52.78%, H: 7.09%, N: 12.31%; Found: C: 52.83%, H: 7.04%, N: 12.24%.

(1*R*)-Amino-2-(4-hydroxyphenyl)ethylphosphonic acid [(*R*)-6b]: Azide (*R*)-**5b** (0.22 g, 0.64 mmol) was transformed into (*R*)-**6b** by the procedure used for the synthesis of (*R*)-**5a**. After removal of the catalyst and the solvent the residue was dissolved in 48% HBr (10 ml) and refluxed for 2hr. The solvent was evaporated under reduced pressure and the crude material was purified by ion exchange chromatography on Dowex 50, H$^+$ ($R_f = 0.44$; BuOH:EtOH:NH_3:H_2O = 3:3:3:1)[9] to afford crystalline aminophosphonic acid (*R*)-

6b (55 mg, 40%). {$[\alpha]_{578}$ = -40.9 (c = 0.11, 1N HCl), 77%ee}{$[\alpha]_{578}$ = -53.0, (c = 1.5, 1N HCl)}[6]. ^{1}H NMR (D_2O): δ 2.93 (1H, ddd, J = 8.9, 11.8, 14.8, PhC$\underline{H}_a$), 3.39 (1H, dt, J = ca. 4.9, 14.8, PhC$\underline{H}_b$), 3.64 (1H, ddd, J = 3.9, 11.8, 13.3, CHP), 7.15 (2H, AA'BB' system, C_6H_4).

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