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Enzymes in Organic Chemistry - 5:[1] First and Chemo-enzymatic Synthesis of α-Aminooxyphosphonic Acids of High Enantiomeric Excess

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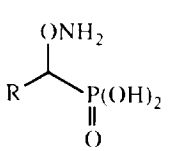
Abstract: α-Acyloxyphosphonates (±)-**5a** and (±)-**5b**, derived from 3-phenylpropionaldehyde and acetaldehyde, were resolved by lipase-catalyzed enantioselective hydrolysis. Three of the four chiral, non-racemic α-hydroxyphosphonates obtained had 99% ee, the fourth 91%. They were transformed chemically into α-aminooxyphosphonic acids. © 1997 Elsevier Science Ltd.

Introduction

α-Amino acids play a key role in the biosphere in metabolic processes and as building blocks of structural proteins and enzymes. Analogs with various modifications have been prepared to study the function of enzymes and to interfere with vital processes of living organisms. An important group of analogs of α-amino acids is obtained by replacing the CO_2H group by the isosteric $P(O)(OH)_2$ or P(O)(H)(OH) group. These α-amino phosphonic[2] and phosphonous acids[3] have been prepared by an array of methods in racemic and optically active form and some of them are enzyme inhibitors. α-Aminooxy carboxylic and phosphonic acids have been studied much less as analogs of amino acids. The latter have so far been obtained only as racemates.[4,5] To demonstrate their importance the more prominent members of the very limited number of representatives and their biological effects are given. Aminooxymethyl- (**1a**) and 1-aminooxyethylphosphonic



1a R = H
b R = Me
c R = $PhCH_2$
d R = $PhCH_2CH_2$

(**1b**) acid[5] are potent slow binding inhibitors of alanine and aspartate aminotransferase by forming oximes with the enzyme-bound pyridoxal 5-phosphate. In contrast to (*S*)-1-aminooxy-3-phenylpropionic acid (K_i = 1.4 nM for buckwheat PAL) the corresponding racemic phosphonic acid analog **1c** (K_i = 1.5 μM) is a less active competitive inhibitor for the phenylalanine ammonia-lyase (PAL).[4,6] The homolog (±)-1-aminooxy-3-phenylpropylphosphonic acid (**1d**) is even less active.[4,7] These compounds have been prepared from racemic

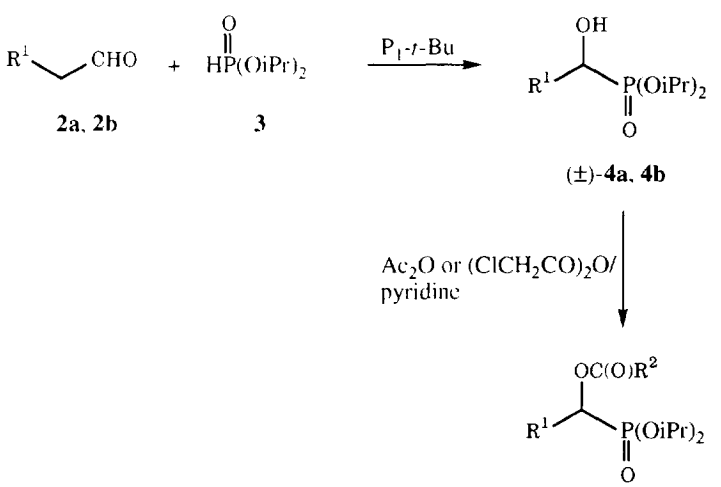
diethyl 1-hydroxyalkylphosphonates using triphenylphosphine/diethyl azodicarboxylate/N-hydroxyphthalimide (Mitsunobu reaction) followed by deprotection.[4,5]

During the last few years we have been studing the lipase-catalyzed[1] enantioselective hydrolysis of α-acetoxy- and α-chloroacetoxyphosphonates to obtain chiral, non-racemic α-hydroxyphosphonates, which have resently attracted much attention.[8,9] They are ideal starting materials for the synthesis of other α-substituted phosphonates using S_N2 reactions as demonstrated by the preparation of α-aminophosphonic acids.[9,10]

As the absolute configurations of the biologically active enantiomers of α-aminooxyphosphonic acids **1b** and **1d** are unknown, we selected all four antipodes as target molecules. We delineate a synthesis starting with known α-hydroxyphosphonates (±)-**4a** and (±)-**4b**, which are acylated, resolved enzymatically, transformed by use of the Mitsunobu reaction to protected α-aminooxyphosphonates which are finally deprotected. The isopropyl group has proven to be the protecting group of choice for phosphorus in the preceding studies and was also used here. It increased the enantioselectivity of the lipase-catalyzed hydrolyses and was more easily removed in refluxing 6 N HCl than the ethyl group.

Lipase-catalyzed kinetic resolution of diisopropyl α-acyloxyphosphonates

α-Hydroxyphosphonate (±)-**4a**[11] was prepared[1] by addition of diisopropylphosphite (**3**) to 3-phenylpropionaldehyde (**2a**) in 70% yield, catalyzed by *tert.*-butylaminotris(dimethylamino)phosphorane (P_1-*t*-Bu) as base (Scheme 1). It was acylated with chloroacetic anhydride/pyridine[10] in dry dichloromethane at



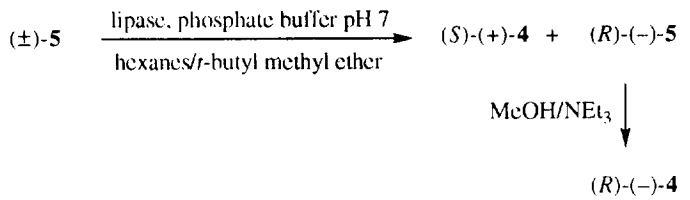
2a, **2b** **3** (±)-**4a**, **4b**

a $R^1 = PhCH_2CH_2$ (±)-**5a** $R^2 = CH_2Cl$

b $R^1 = Me$ (±)-**5b** $R^2 = Me$

Scheme 1 Preparation of α-hydroxyphosphonates (±)-**4** and α-acyloxyphosphonates (±)-**5**

0 °C to afford chloroacetate (±)-**5a** in 92% yield. It was tested as substrate for lipase AP 6 (from *Aspergillus niger*; 6 mg) on a 1 mmol scale for enantioselective hydrolysis in a biphasic system (phosphate buffer pH 7.0/hexanes and *tert.*-butyl methyl ether) as described[1] (Scheme 2). The consumption of base (0.5 N NaOH)



Scheme 2 Lipase-catalyzed hydrolysis of α-acyloxyphosphonates (±)-**5**

was recorded as function of time. The reaction was stopped by addition of 1 N HCl to bring pH to 4, when 45% of the ester had been hydrolyzed. The conversion as determined by ^{1}H NMR spectroscopy of the crude product mixture was 48%. Workup and chromatography yielded α-hydroxyphosphonate (*S*)-(+)-**4a** (yield 44%, 86% ee) and unchanged ester (*R*)-(−)-**5a** (50% yield) which was hydrolyzed chemically to give quanitatively α-hydroxyphosphonate (*R*)-(−)-**4a** with 79% ee. The enantiomeric excesses and absolute configurations were determined by ^{31}P- and ^{1}H NMR spectroscopy of the corresponding Mosher esters.[11,12]

To get adequate amounts of these α-hydroxyphosphonates, the enzymatic hydrolysis was carried out on a 10 mmol scale of substrate with 63 mg of AP 6. As the ee of the isolated α-hydroxyphosphonate is increased with decreasing conversion, the reaction was stopped already at a conversion of 30% as determined by consumption of base (31% by ^{1}H NMR). This time the alcohol (*S*)-(+)-**4a**, which has also been prepared by oxazaborolidine-catalyzed reduction[13] of the corresponding α-ketophosphonate with enantiomeric excesses of 66 and 95%, had an ee of 91% at a yield of 30% and $[\alpha]_D^{20}$ = +22.07. The unchanged ester (*R*)-(−)-**5a** {68% yield, $[\alpha]_D^{20}$ = -7.48} was subjected again to kinetic resolution by 205 mg of AP 6 to give at a conversion of 45% as determined by consumption of base α-hydroxyphosphonate (*S*)-(+)-**4a** (44% yield), which was not further dealt with, and ester (*R*)-(−)-**5a** {49% yield, $[\alpha]_D^{20}$ = -18.92}. It was hydrolyzed in dry methanol/triethylamine to furnish α-hydroxyphosphonate (*R*)-(−)-**4a** { 98% yield, $[\alpha]_D^{20}$ = -24.23; ee 99%}. These two enantiomeric alcohols were then used as starting materials for the chemical manipulation to the target α-aminooxyphosphonic acids **1d**.

1-Acetoxyethylphosphonate (±)-**5b** was prepared similarly without isolation[12] of intermediate α-hydroxyphosphonate (±)-**4b** in 85% yield (Scheme 1). Its lipase-catalyzed hydrolysis with AP 6 has been described previously.[14] The reported value of 89%, and on repetition now of 85% for the ee of the α-hydroxyphosphonate (*S*)-(+)-**4b** at 45% conversion was considered improveable. As this α-hydroxyphosphonate is also planned to be transformed to biologically active α-aminoethylphosphonic acid, available lipases (SP 523, SP 524, 525, and 526) and proteases (B-, M-, N-, S-"Amano", and SP 539) were tested to find a better hydrolytic enzyme than AP 6. Only protease M and lipases SP 524 and SP 525 hydrolyzed substrate (±)-**5b**, the latter being significantly better than AP 6. On a 1 mmol scale of (±)-**5b**, 11 mg of SP 524 were sufficient to effect a conversion of 45% in 70 min. The isolated α-hydroxyphosphonate

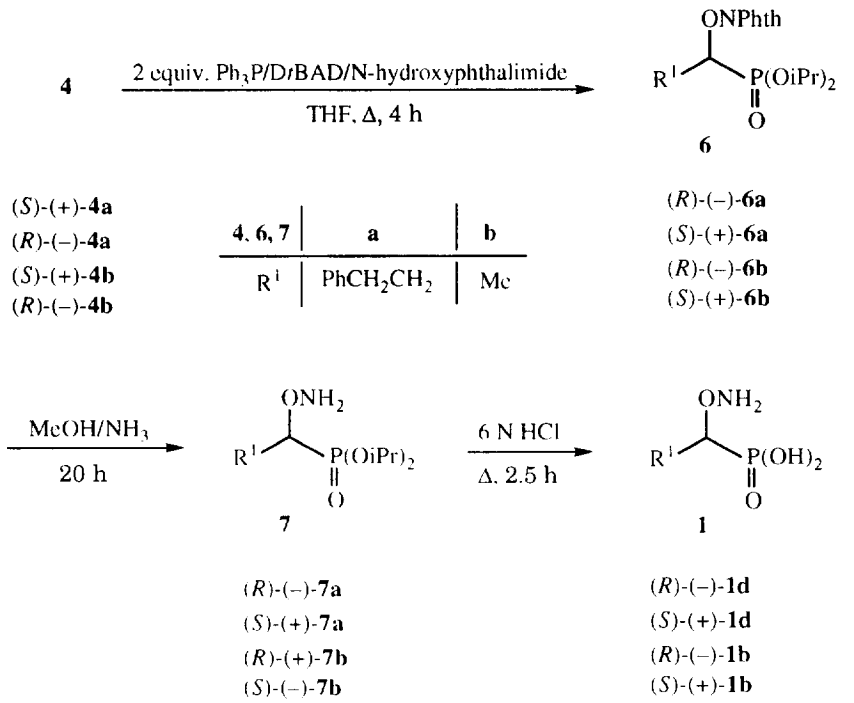
(*S*)-(+)-**4b** had an excellent enantiomeric excess of 97%. Then the resolution was carried out on a 10 mmol scale using 50 mg of enzyme SP 524. The hydrolysis was stopped after 2 h at a conversion of 40%. The α-hydroxyphosphonate had an enantiomeric excess of 99% and the ester (*R*)-(−)-**5b** was subjected to a second hydrolysis with 51 mg of the same enzyme. After a conversion of 14% the consumption of base stopped, indicating that ester (*R*)-(−)-**5b** is not a substrate for the lipase. The α-hydroxyphosphonate (*S*)-(+)-**4b** had an enantiomeric excess of 95% and the ester (*R*)-(−)-**5b** of 99%, determined after chemical hydrolysis. This result indicates that for future experiments a second hydrolysis is not necessary to obtain both enantiomers of α-hydroxyphosphonate **4b** with ee greater than 95%, if the reaction is stopped when the release of acetic acid is finished.

Chemical transformation of α-hydroxyphosphonates to α-aminooxyphosphonic acids

The procedures for the functional group manipulation reported in the literature[4] are based on diethyl α-hydroxyphosphonates and standard Mitsunobu conditions[15] (triphenylphosphine/diethyl azodicarboxylate/N-hydroxyphthalimide) were no successful in our case. It is assumed that the substitution of the hydroxy by the phthalimidooxy group occurs also with inversion of configuration in analogy to other Mitsunobu reactions. No protected α-aminooxyphophonate could be isolated, if α-hydroxyphosphonate **4a** was used as alcohol, possibly for steric reasons. When diethyl azodicarboxylate was replaced by the corresponding di(*tert.*-butyl) ester (D*t*BAD) the yields of the desired products (*R*)-(−)- and (*S*)-(+)-**6a** went up to 75% and 74% for (*S*)-(+)- and (*R*)-(−)-**4a**, respectively, if two equivalents of the reagents were used (Scheme 3). It is assumed that the substitution of the hydroxy by the phthalimidooxy group occurs also with inversion of configuration in analogy to other Mitsunobu reactions.

The deprotection of α-(phthalimidooxy)alkylphosphonates **6** was carried out in two steps. The removal of the phthaloyl group is traditionally effected with hydrazine. It was found that less toxic ammonia in methanol is equally effective. Diisopropyl α-aminooxyphosphonates **7** were purified by flash chromatography using hexanes/ethanol as eluent. Acetone and acetone containing solvent mixtures were deleterious, when brought into contact with the O-substituted hydroxylamines. The well known oximes formed readily. The isopropyl groups were removed by refluxing a solution of α-aminooxyphosphonates **7** in 6 N HCl for only 2.5 h. Evaporation of solvent on a rotary evaporator and drying over KOH in a vacuum dessiccator afforded in high yields crystalline, homogenous (400 MHz ^{1}H NMR) α-aminooxyphosphonic acids **1b** and **1d**, which were crystallized from methanol/ethanol. Longer reaction times lead to the formation of impurities, shorter ones give mixtures of fully and only partly deprotected product. The enantiomeric excesses of the prepared compounds should be the same as those of the α-hydroxyphosphonates used as starting materials, that is 91% for (*R*)-(−)-**1d** and 99% for (*S*)-(+)-**1d**, (*R*)-(−)- and (*S*)-(+)-**1b**.

The chemo-enzymatic synthesis of the two pairs of antipodes demonstrates the utility of lipases for the kinetic resolution of α-acyloxyphosphonates. Contrary to enantioselective chemical reactions, the enantiomeric excess of an enzymatic resolution depends on the degree of conversion. When the hydrolysis is stopped at an appropriate time, even substrates with a moderate enantioselectivity yield α-hydroxyphosphonates of good to excellent ee.



Scheme 3 Synthesis of α-aminooxyphosphonic acids from α-hydroxyphosphonates

EXPERIMENTAL

All starting materials and enzymes were obtained from commercial suppliers and were used without further purification. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise given, using tetramethylsilane as internal standard on a Bruker AM 400 WB at 400.13 and 100.61 MHz, respectively. ^{31}P NMR spectra were recorded on the same spectrometer at 161.97 MHz using H_3PO_4 (85%) as external standard. In order to get undistorted ^{31}P signal intensities for an accurate integration, adequate relaxation times were used without irradiation during this period to avoid NOE enhancements. IR spectra were run on a Perkin Elmer 1600 FT-IR spectrometer as films on a silicon plate.[16] Optical rotations were measured at 20 °C on a Perkin Elmer 241 polarimeter in a 1 dm cell. TLC was carried out on 0.25 mm thick Merck plates, silica gel 60 F_{254}. Flash chromatography was performed with Merck silica gel 60 (230 - 400 mesh). Spots were visualized by 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 of 10% H_2SO_4 in water, followed by heating with a hot gun. A Metrohm 702 SM Titrino instrument was used as an autotitrator. (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride {JPS Chimie; $[\alpha]_D^{20}$ = +136.5 (c = 5.2, CCl_4), ee >99.5% } was used for derivatization of α-hydroxyphosphonates.

Lipases (SP 523, 524, 525, 526 are lipases from selected fungi which were expressed in *Aspergillus oryzae* as host organism, from Novo Nordisk; lipase AP 6 was from Amano Enzyme Limited) and proteases [SP 539 (alkaline endoprotease, from selected microorganism expressed in a *Bacillus* strain), from *Novo Nordisk*; protease B (from strain belonging to *Penicillium sp.*), M (from strain belonging to *Aspergillus oryzae*), N (from strain belonging to *Bacillus subtilis*), and S (from strain belonging to *Bacillus sp.*), from Amano] were gifts from the companies given. Abbreviations used: MC = methylene chloride; EA = ethyl acetate.

(±)-Diisopropyl 1-hydroxy-3-phenylpropylphosphonate [(±)-**4a**]: This α-hydroxyphosphonate[11] was prepared from 3-phenylpropionaldehyde (**2a**) (5.73 g, 5.52 ml, 44 mmol), diisopropylphosphite (**3**) (6.65 g, 6.67 ml, 40 mmol) with phosphazene base P_1-*t*-Bu (0.468 g, 0.51 ml, 2 mmol) in diethyl ether (60 ml) according to a literature procedure.[12] Excess aldehyde was removed after workup by bulb to bulb distillation. Crystallization from hexanes/trace of MC gave 4.67 g of α-hydroxyphosphonate (±)-**4a**. The mother liquor yielded a second crop of 2.04 g. The remaining mother liquor was separated by flash chromatography (MC:EA = 5:1 to 5:3 to 1:1; R_f = 0.29, MC:EA = 5:3) to give another 1.69 g of (±)-**4a**; total yield 70%; m.p. 70-72 °C (hexanes/trace of MC) (lit.[11] 70-72 °C). The ^{1}H NMR spectrum is identical with the one reported. ^{13}C NMR: δ 23.90 (d, *J* = 4.9, $\underline{Me}_2CHO$), 23.96 (d, *J* = 5.0, $\underline{Me}_2CHO$), 24.70 (d, *J* = 2.7, $\underline{Me}_2CHO$), 24.10 (d, *J* = 2.6, $\underline{Me}_2CHO$), 31.77 (d, *J* = 13.9, $\underline{C}H_2Ph$), 33.00 ($\underline{C}H_2CH_2Ph$), 67.10 (d, *J* = 162.0, PCH), 70.98 (d, *J* = 7.4, $Me_2\underline{C}HO$), 71.16 (d, *J* = 7.2, $Me_2\underline{C}HO$), 125.86, 128.33, 128.56, 141.50 (Ph).

(±)-Diisopropyl 1-chloroacetoxy-3-phenylpropylphosphonate [(±)-**5a**]: α-Hydroxyphosphonate (±)-**4a** (2.14 g, 7.15 mmol) was acylated with chloroacetic anhydride/dry pyridine according to the procedure used for the preparation of diisopropyl 1-chloroacetoxy-2-phenylethylphosphonate.[10] Flash chromatography (MC:EA = 10:1; R_f = 0.74, MC:EA = 5:3) of the crude product yielded 2.46 g (92%) of (±)-**5a** as an oil. IR: ν_{max} 3028, 2981, 1769, 1604, 1497, 1455, 1387, 1252, 1163, 1105, 990 cm^{-1}. ^{1}H NMR: δ 1.22, 1.24, 1.26 (3H, 3H, 6H, 3xd, *J* = 6.2, $\underline{Me}_2CHO$), 2.12 (2H, m, $\underline{C}H_2CH_2Ph$), 2.64 (2H, m, $\underline{C}H_2Ph$), 3.95 (2H, AB system, *J* = 14.8, CH_2Cl), 4.66 (2H, m, $Me_2\underline{C}HO$), 5.21 (1H, dt, *J* = 3.9, 8.9, PCH), 7.08-7.24 (5H, m, Ph). ^{13}C NMR: δ 23.81 (d, *J* = 4.9, $\underline{Me}_2CHO$), 23.92 (d, *J* = 5.0, $\underline{Me}_2CHO$), 23.99 (d, *J* = 3.8, $\underline{Me}_2CHO$), 24.11 (d, *J* = 3.6, $\underline{Me}_2CHO$), 31.02 ($\underline{C}H_2CH_2Ph$), 32.01 (d, *J* = 12.9, $\underline{C}H_2Ph$), 40.53 (CH_2Cl), 70.03 (d, *J* = 169.9, PCH), 71.62 (d, *J* = 7.1, $Me_2\underline{C}HO$), 71.83 (d, *J* = 6.7, $Me_2\underline{C}HO$), 126.19, 128.38, 128.48, 140.38 (Ph), 166.44 (d, *J* = 4.9, CO). Elemental analysis: $C_{17}H_{26}ClO_5P$ Calcd.: C: 54.19%, H: 6.95%; Found: C: 54.22%, H: 6.91%.

(±)-Diisopropyl 1-acetoxyethylphosphonate [(±)-**5b**]: α-Hydroxyphosphonate (±)-**4b**[14] was prepared from acetaldehyde (**2b**) (1.54 g, 1.86 ml, 33 mmol), diisopropylphosphite (**3**) (4.98 g, 5.0 ml, 30 mmol) with phosphazene base P_1-*t*-Bu (0.234 g, 0.25 ml, 1 mmol) in dry diethyl ether (30 ml) according to the synthesis of (±)-**4a**. α-Hydroxyphosphonate (±)-**4b** was not isolated.[12] After stirring for 20 h, dry pyridine (15 ml) and acetic anhydride (5 ml) were added and stirring was continued for another 20 h. Then dry toluene was added and the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (MC:EA = 5:1) and bulb to bulb distillation (85-95 °C/0.5 Torr) (lit.[14] 100 °C/0.01 Torr) to give 6.42 g (85%) of (±)-**5b**. Its ^{1}H NMR spectrum agrees with the data reported in the literature.

Enzymatic hydrolysis of α-acyloxyphosphonates (±)-5a and (±)-5b

Hydrolysis of (±)-5a on a 1 mmol scale: α-Chloroacetoxyphosphonates (±)-**5a** (0.376 g, 1 mmol) was hydrolyzed with lipase AP 6 (14 mg) in a biphasic system [phosphate buffer (17 ml, pH 7)/hexanes (2 ml)/*tert.*-butyl methyl ether (2 ml)] as reported.[1] At a conversion of 45% (48% by ^{1}H NMR of crude product) as determined by the consumption of 0.5 N NaOH after 19.75 h, the reaction was stopped. Flash chromatography (MC:EA = 10:1, 1:1) of the crude product gave crystalline (*S*)-(+)-**4a**[13] {0.132 g (44%); $[\alpha]_D^{20}$ = +21.70 (c = 1.71, acetone), ee 86%} and ester (*R*)-(−)-**5a** {0.187 g (50%); $[\alpha]_D^{20}$ = -14.52 (c = 1.21, acetone)}. The ester (0.161 g, 0.428 mmol) was hydrolyzed chemically using Et_3N/MeOH to afford crystalline (*R*)-(−)-**4a** {0.128 g (100%); $[\alpha]_D^{20}$ = - 18.93 (c = 1.60, acetone), ee 79%}.

Enzymatic hydrolysis of (±)-5a on a preparative scale: Ester (±)-**5a** (3.76 g, 10 mmol) was hydrolyzed as before with lipase AP 6 (63 mg) in a biphasic system [phosphate buffer (50 ml, pH 7)/hexanes (10 ml)/*tert.*-butyl methyl ether (10 ml)]. The reaction was stopped after 21 h (conversion 30% by consumption of base; 31% by ^{1}H NMR) and worked up. Flash chromatography (MC:EA = 20:1, 1:1) of the crude product gave crystalline (*S*)-(+)-**4a** {0.891 g (30%); $[\alpha]_D^{20}$ = +22.07 (c = 1.50, acetone), ee 91%} and ester (*R*)-(−)-**5a** {2.57 g (68%); $[\alpha]_D^{20}$ = -7.48 (c = 1.55, acetone)}. The ester was subjected to a second hydrolysis with lipase AP 6 (0.205 g) in a biphasic system [phosphate buffer (50 ml, pH 7)/hexanes (10 ml)/*tert*-butyl methyl ether (10 ml)]. The reaction was stopped after 53.2 h (conversion 45% by consumption of 0.5 N NaOH; 47% by ^{1}H NMR) and worked up. Flash chromatography of the crude product gave crystalline (*S*)-(+)-**4a** {0.883 g (44%); $[\alpha]_D^{20}$ = +2.77 (c = 1.88, acetone), ee 91%} and ester (*R*)-(−)-**5a** {1.23 g (49%); $[\alpha]_D^{20}$ = -18.92 (c = 1.86, acetone)}. The ester (*R*)-(−)-**5a** (1.2 g, 3.19 mmol) was hydrolyzed chemically to yield crystalline (R)-(−)-**4a** {0.940 g (98%); $[\alpha]_D^{20}$ = -24.23 (c = 1.66, acetone), ee 99%}.

Hydrolysis of (±)-5b on a 1 mmol scale: α-Acetoxyphosphonate (±)-**5b** (0.252 g, 1 mmol) was tested with various hydrolytic enzymes. The reactions were carried out analogously to the hydrolysis of (±)-**5a** except that the composition of the biphasic system [phosphate buffer (17 ml, pH 7)/hexanes (3 ml)/*tert.*-butyl methyl ether (1 ml)] was slightly different. Lipase SP 524 (11 mg) effected a conversion of 45% in 70 min. Purification by flash chromatography (MC:EA = 5:1, 0:1) gave (*S*)-(+)-**4b** {0.084 g (41%); $[\alpha]_D^{20}$ = +6.84 (c = 1.33, acetone), ee 97%} and (*R*)-(−)-**5b** {0.124 g, 50%; $[\alpha]_D^{20}$ = -16.76 (c = 1.64, acetone)}.

Hydrolysis of (±)-5b on a preparative scale: Ester (±)-**5b** (2.66 g, 10 mmol, containing 5% acetic acid) was hydrolyzed similarily to (±)-**5a** with lipase SP 524 (50 mg) in a biphasic system [phosphate buffer (50 ml, pH 7)/hexanes (15 ml)/*tert.*-butyl methyl ether (5 ml)]. The reaction was stopped after 2 h (conversion 40% by consumption of base; 41% by ^{1}H NMR) and worked up. Flash chromatography (MC:EA = 5:1, 0:1) of the crude product gave (*S*)-(+)-**4b** {0.789 g (38%); $[\alpha]_D^{20}$ = +7.23 (c = 1.90, acetone), ee 99%} and ester (*R*)-(−)-**5b** {1.47 g (58%); $[\alpha]_D^{20}$ = -14.66 (c = 1.69, acetone)}. The ester (1.41 g, 5.58 mmol) was subjected to a second hydrolysis with lipase SP 524 (51 mg) in a biphasic system [phosphate buffer (50 ml, pH 7)/hexanes (15 ml)/*tert.*-butyl methyl ether (5 ml)]. The reaction was stopped after 4 h (conversion 15% by consumption of base; 14% by ^{1}H NMR) and worked up. Flash chromatography of the crude product gave (*S*)-

(+)-**4b** {0.159 g (14%); $[\alpha]_D^{20}$ = +6.25 (c = 1.60, acetone), ee 95%} and ester (*R*)-(–)-**5b** {1.15 g (82%); $[\alpha]_D^{20}$ = -20.92 (c = 2.17, acetone)}. The ester (*R*)-(–)-**5b** (0.971 g, 3.85 mmol) was hydrolyzed chemically to yield (*R*)-(–)-**4b** {0.80 g (99%); $[\alpha]_D^{20}$ = -6.76 (c = 1.48, acetone), ee 99%}.

(*R*)-(–)- and (*S*)-(+)-Diisopropyl 3-phenyl-1-(phthalimidooxy)propylphosphonate [(*R*)-(–)- and (*S*)-(+)-6a]: A solution of di(*tert.*-butyl) azodicarboxylate (D*t*BAD) (1.29 g, 5.6 mmol) in dry THF (4 ml) was added dropwise to a suspension of (*S*)-(+)-**4a** (0.841 g, 2.8 mmol), N-hydroxyphthalimide (0.915 g, 5.6 mmol), and triphenylphosphane (1.47 g, 5.6 mmol) in dry THF (7 ml), stirred at room temperature under argon. Then the solution was heated at 50 °C until the starting material was nearly consumed (TLC, MC:EA = 5:3; R_f = 0.49; 4 h). Water (2 drops) was added and the solvent was removed in vacuo. Flash chromatography (hexanes/acetone = 8:1) of the residue gave (*R*)-(–)-**6a**, which was dissolved in dry toluene, concentrated in vacuo, and dried at 40 °C/0.1 Torr for 4h to remove traces of acetone to yield a viscous oil {0.936 g (75%); $[\alpha]_D^{20}$ = -13.99 (c = 1.26, acetone)}. - (*R*)-(–)-**4a** (0.894 g, 2.98 mmol) was transformed by the same procedure into (*S*)-(+)-**6a** {0.975 g (74%); $[\alpha]_D^{20}$ = +16.69 (c = 1.97, acetone)}. The spectroscopic data are identical. IR: ν_{max} 2980, 1791, 1732, 1468, 1455, 1375, 1251, 1187, 1105, 1082 cm^{-1}. ^1H NMR: δ 1.24, 1.31, 1.32, 1.38 (3H each, 4xd, *J* = 6.2, $\underline{Me}_2CHO$), 2.25 (2H, m, $C\underline{H}_2CH_2Ph$), 3.05 (2H, AB part of ABXY system, J_{AB} = 13.8, J_{AX}, J_{AY} = 5.9, 9.4, J_{BX}, J_{BY} = 6.9, 9.4, $C\underline{H}_2Ph$), 4.65 (1H, ddd, *J* = 2.0, 3.9, 6.4, PCH), 4.71, 4.97 (1H each, 2xm, $Me_2C\underline{H}O$), 7.14-7.30 (5H, m, Ph), 7.77 (4H, m, C_6H_4). ^{13}C NMR: δ 23.76 (d, *J* = 5.5, $\underline{Me}_2CHO$), 23.88 (d, *J* = 4.7, $\underline{Me}_2CHO$), 23.99 (d, *J* = 3.9, $\underline{Me}_2CHO$), 24.21 (d, *J* = 3.0, $\underline{Me}_2CHO$), 31.44 ($\underline{C}H_2CH_2Ph$), 31.96 (d, *J* = 10.4, $\underline{C}H_2Ph$), 71.47 (d, *J* = 7.1, $Me_2\underline{C}HO$), 72.04 (d, *J* = 6.7, $Me_2\underline{C}HO$), 81.19 (d, *J* = 161.8, PCH), 123.44, 126.00, 128.39, 128.61, 128.96, 134.34, 141.22 (Carom), 163.09 (CO). Elemental analysis: $C_{23}H_{20}NO_6P$ Calcd.: C: 61.88%, H: 6.55%, N: 3.14%; Found: C: 61.62%, H: 6.36%, N: 3.20%.

(*R*)-(–)- and (*S*)-(+)-Diisopropyl 1-(phthalimidooxy)ethylphosphonate [(*R*)-(–)- and (*S*)-(+)-6b]: α-Hydroxyphosphonate (*S*)-(+)-**4b** (0.755 g, 3.60 mmol) gave in analogy to the preparation of (*R*)-(–)-**6a** α-(phthalimidooxy)phosphonate (*R*)-(–)-**6b** {1.03 g (81%); $[\alpha]_D^{20}$ = -20.43 (c = 1.65, acetone)} as a viscous oil. - (*R*)-(–)-**4b** (0.720 g, 3.43 mmol) was transformed by the same procedure into (*S*)-(+)-**6b** {1.19 g (98%); $[\alpha]_D^{20}$ = +21.03 (c = 1.55, acetone)}. The spectroscopic data are identical. IR: ν_{max} 2982, 1791, 1732, 1468, 1376, 1242, 1186, 1143, 1108, 1082, 1054 cm^{-1}. ^1H NMR: δ 1.36, 1.38, 1.43 (3H, 6H, 3H, 3xd, *J* = 6.2, $\underline{Me}_2CHO$), 1.61 (3H, dd, *J* = 6.9, 16.2, CH_3), 4.72 (1H, dq, *J* = 6.9, 7.5, PCH), 4.82, 4.96 (1H each, 2xm, $Me_2C\underline{H}O$), 7.74, 7.83 (4H, 2xm, C_6H_4). ^{13}C NMR: δ 14.20 (d, *J* = 1.2, CH_3), 23.46 (d, *J* = 5.3, $\underline{Me}_2CHO$), 23.66 (d, *J* = 5.9, $\underline{Me}_2CHO$), 23.71 (d, *J* = 4.1, $\underline{Me}_2CHO$), 23.90 (d, *J* = 3.4, $\underline{Me}_2CHO$), 71.51 (d, *J* = 7.1, $Me_2\underline{C}HO$), 71.88 (d, *J* = 6.6, $Me_2\underline{C}HO$), 78.07 (d, *J* = 163.2, PCH), 123.21, 128.56, 134.16 (C_6H_4), 163.00 (CO). Elemental analysis: $C_{16}H_{22}NO_6P$ Calcd.: C: 54.08%, H: 6.24%, N: 3.94%; Found: C: 54.36%, H: 6.38% N: 4.15%.

(*R*)-(–)- and (*S*)-(+)-Diisopropyl 1-aminooxy-3-phenylpropylphosphonate [(*R*)-(–)- and (*S*)-(+)-7a]: Aqueous ammonia (25%, 1.4 ml) was added to a solution of α-(phthalimidooxy)phosphonate

(*R*)-(–)-**6a** (0.909 g, 2.04 mmol) in methanol (14 ml). After stirring for 20 h at room temperature (TLC control, MC:EA = 5:3, R_f = 0.17), the mixture was concentrated in vacuo. Purification of the residue by flash chromatography (hexanes/EtOH = 9:1) gave α-aminooxyphosphonate (*R*)-(–)-**7a** {0.569 g (89%); $[\alpha]_D^{20}$ = -10.87 (c = 1.27, EtOH)} as a viscous oil. - (*S*)-(+)-**6a** (0.951 g, 2.13 mmol) was transformed by the same procedure into (*S*)-(+)-**7a** {0.556 g, (83%); $[\alpha]_D^{20}$ = +11.64 (c = 1.50, EtOH)}. The spectroscopic data are identical. IR: ν_{max} 3310, 3242, 2979, 1729, 1603, 1454, 1385, 1375, 1239, 1178, 1142, 1106, 986 cm^{-1}. ^1H NMR: δ 1.26, 1.31, 1.33 (3H, 3H, 6H, 3xd, *J* = 6.4, $\underline{Me}_2CHO$), 2.00 (2H, m, $C\underline{H}_2CH_2Ph$), 2.73 (1H, dt, *J* = 7.9, 14.0, $C\underline{H}Ph$), 2.89 (1H, ddd, *J* = 6.4, 8.5, 14.0, $C\underline{H}Ph$), 3.79 (1H, m, PCH), 4.74 (2H, m, $Me_2C\underline{H}O$), 5.89 (2H, br s, NH_2), 7.16-7.31 (5H, m, Ph). ^{13}C NMR: δ 23.94, (d, *J* = 4.9, $\underline{Me}_2CHO$), 24.05 (d, *J* = 3.4, $\underline{Me}_2CHO$), 24.19 (d, *J* = 3.6, Me_2CHO), 31.00 (d, *J* = 2.4, $\underline{C}H_2CH_2Ph$), 31.96 (d, *J* = 12.9, $\underline{C}H_2Ph$), 70.59 (d, *J* = 7.2, $Me_2\underline{C}HO$), 71.01 (d, *J* = 6.9, $Me_2\underline{C}HO$), 80.73 (d, *J* = 163.2, PCH), 125.95, 128.38, 128.52, 141.29 (Ph). Elemental analysis: $C_{15}H_{26}NO_4P$ Calcd.: C: 57.13%, H: 8.31%, N: 4.44%; Found: C: 56.89%, H: 8.28%, N: 4.65%.

(*R*)-(+)- and (*S*)-(–)-Diisopropyl 1-aminooxyethylphosphonate [(*R*)-(+)- and (*S*)-(–)-7b]: α-(Phthalimidooxy)phosphonate (*R*)-(–)-**6b** (0.931 g, 2.62 mmol) gave in analogy to the preparation of (*R*)-(–)-**7a** α-aminooxyphosphonate (*R*)-(+)-**7b** { 0.530 g (90%); $[\alpha]_D^{20}$ = +1.48 (c = 1.55, EtOH)} as a viscous oil. - (*S*)-(+)-**6b** (1.16 g, 3.25 mmol) was transformed by the same procedure into (*S*)-(–)-**7b** {0.542 g (74%); $[\alpha]_D^{20}$ = -1.52 (c = 1.78, EtOH)}. The spectroscopic data are identical. IR: ν_{max} 3469, 3309, 2980, 1736, 1593, 1386, 1375, 1283, 1230, 1178, 1142, 1107, 1062, 986 cm^{-1}. ^1H NMR: δ 1.31, 1.32, 1.33 (6H, 3H, 3H, 3xd, *J* = 6.2, $\underline{Me}_2CHO$), 1.38 (3H, dd, *J* = 7.4, 16.2, CH_3), 3.93 (1H, dq, *J* = 7.4, 12.8, PCH), 4.75 (2H, m, $Me_2C\underline{H}O$), 5.75 (2H, br s, NH_2). ^{13}C NMR: δ 14.54 (CH_3), 23.90, 23.97 (2xd, *J* = 4.7, $\underline{Me}_2CHO$), 24.04, 24.16 (2xd, *J* = 3.6, $\underline{Me}_2CHO$), 70.65 (d, *J* = 7.1, $Me_2\underline{C}HO$), 70.96 (d, *J* = 6.8, $Me_2\underline{C}HO$), 77.09 (d, *J* = 164.2, PCH). Elemental analysis: $C_8H_{20}NO_4P$ Calcd.: C: 42.66%, H: 8.95%, N: 6.22%; Found: C: 41.29%, H: 8.86%, N: 5.92%.

(*R*)-(–)- and (*S*)-(+)-1-Aminooxy-3-phenylpropylphosphonic acid [(*R*)-(–)- and (*S*)-(+)-1d]: α-Aminooxyphosphonate (*R*)-(–)-**7a** (0.503 g, 1.567 mmol) was refluxed in a mixture of water (5 ml) and concentrated HCl (5 ml) for 2.5 h. The solution was concentrated on a rotary evaporator and dried in a vacuum dessiccator over KOH to give white, crystalline α-aminooxyphosphonic acid (*R*)-(–)-**1d** {0.340 g (94%); $[\alpha]_D^{20}$ = -16.44 (c = 1.69, 0.5 N NaOH)}, homogenous by ^1H NMR, m.p. 210-213 °C (MeOH/EtOH) (lit.[4] m.p. 180-190 °C for (±)-**1d**). - (*S*)-(+)-**7a** (0.496 g, 1.57 mmol) was transformed by the same procedure into (S)-(+)-**1d** {0.335 g (92%); $[\alpha]_D^{20}$ = +18.65 (c = 1.12, 0.5 N NaOH)}, m.p. 212-214 °C. The ^1H NMR spectra (D_2O/DCl) are in agreement with the reported data.[4] The ^1H NMR spectra recorded in D_2O/NaOD are also identical. ^1H NMR: δ 1.79, 1.94 (2H, 2xm, $C\underline{H}_2CH_2Ph$), 2.63 (1H, ddd, *J* = 7.4, 8.9, 13.5, $C\underline{H}Ph$), 2.77 (1H, ddd, *J* = 4.7, 9.8, 13.5, $C\underline{H}Ph$), 3.36 (1H, ddd, *J* = 3.0, 5.4, 10.3, PCH), 7.13-7.30 (5H, m, Ph).

(*R*)-(–)- and (*S*)-(+)-1-Aminooxyethylphosphonic acid [(*R*)-(–)- and (*S*)-(+)-1b]: α-Aminooxyphosphonate (*R*)-(+)-**7b** (0.480 g, 2.13 mmol) gave in analogy to the preparation of (*R*)-(–)-**1d** crystalline α-aminooxyphosphonic acid containing a small amount of an impurity (^1H NMR, D_2O/DCl)

(*R*)-(–)-**1b** {0.280 g (93%); $[\alpha]_D^{20}$ = -4.90 (c = 1.00; 0.5 N NaOH)}, m.p. 173-175 °C (MeOH/EtOH) (lit.[4] m.p.187-189 °C for (±)-**1b**). - (*S*)-(–)-**7b** (0.507 g, 2.25 mmol) was transformed by the same procedure into crystalline (*S*)-(+)-**1b** {0.300 g (95%); $[\alpha]_D^{20}$ = +6.26 (c = 1.63, 0.5N NaOH)}, m.p. 193-195 °C. The ^{1}H NMR spectra recorded in D_2O/DCl are in agreement with the data reported in the literature.[4] The ^{1}H NMR spectra recorded in D_2O/NaOD are also identical. ^{1}H NMR: δ 1.19 (3H, dd, *J* = 6.9, 13.3, CH_3), 3.54 (1H, quint, *J* = 6.9, PCH).

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REFERENCES

1. Eidenhammer, G.; Hammerschmidt, F. *Synthesis* **1996**, 748-754.
2. Dhawan, B.; Redmore, D. *Phosphorus and Sulfur* **1987**, *32*, 119-144; Kafarski, P.; Lejczak, B. *Phosphorus, Sulfur, and Silicon* **1991**, *63*, 193-215.
3. Dingwall, J. G.; Ehrenfreund, J.; Hall, R. G. *Tetrahedron* **1989**, *45*, 3787-3808; Solodenko, V. A.; Belik, M. Y.; Galushko, S. V.; Kukhar, V. P.; Kozlova, E. V.; Mironenko, D. A.; Svedas, V. K. *Tetrahedron: Asymmetry* **1993**, *4*, 1965-1968.
4. Maier, L.; Spörri, H. *Phosphorus, Sulfur, and Silicon* **1992**, *70*, 39-48.
5. Lacoste, A.M.; Dumora, C.; Zon, J. *J. Enzyme Inhibition* **1993**, *7*, 237-248.
6. Zon, J.; Amrhein, N. *Liebigs Ann. Chem.* **1992**, 625-628.
7. Maier, L.; Diel, P. J. *Phosphorus, Sulfur, and Silicon* **1994**, *90*, 259-279.
8. Yokomatsu, T.; Shibuya, S. *Tetrahedron: Asymmetry* **1992**, *3*, 377-378; Yokomatsu, T.; Yamagishi, T.; Shibuya, S. *Tetrahedron: Asymmetry*, **1993**, *4*, 1401-1404.
9. Gajda, T. *Tetrahedron: Asymmetry* **1994**, *5*, 1965-1972; Meier, C.; Laux, W. H. G. *Tetrahedron: Asymmetry* **1995**, *6*, 1089-1092; Arai, T.; Bougauchi, M.; Sasai, H.; Shibasaki, M. *J. Org. Chem.* **1996**, *61*, 2926-2927; Devitt, P. G.; Mitchell, M. C.; Weetman, J.M.; Taylor, R. J.; Kee, T. P. *Tetrahedron: Asymmetry* **1995**, *6*, 2039-2044; Kozlowski, J. K.; Rath, N. P.; Spilling, C. D. *Tetrahedron* **1995**, *51*, 6385-6396.
10. Drescher, M.; Li, Y.-F.; Hammerschmidt, F. *Tetrahedron* **1995**, *51*, 4933-4946.
11. Hammerschmidt, F.; Li, Y.-F. *Tetrahedron* **1994**, *50*, 10253-10264.
12. Drescher, M.; Hammerschmidt, F.; Kählig, H. *Synthesis* **1995**, 1267-1272.
13. Meier, C.; Laux, W. H. G.; Bats, J. W. *Liebigs Ann.* **1995**, 1963-1979.
14. Li, Y.-F.; Hammerschmidt, F. *Tetrahedron: Asymmetry* **1993**, *4*, 109-120.
15. Hughes, D. L. *Org. Reactions* **1992**, *42*, 335-656.
16. Mikenda, W. *Vibrational Spectroscopy* **1992**, *3*, 327-330.

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