Enzymes in Organic Chemistry; Part 3:[1] Enantioselective Hydrolysis of 1-Acyloxyalkylphosphonates by Lipase from *Aspergillus niger* (Lipase AP 6)

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Dedicated to Prof. Dr. F.O. Olaj on the occassion of his 60th birthday.
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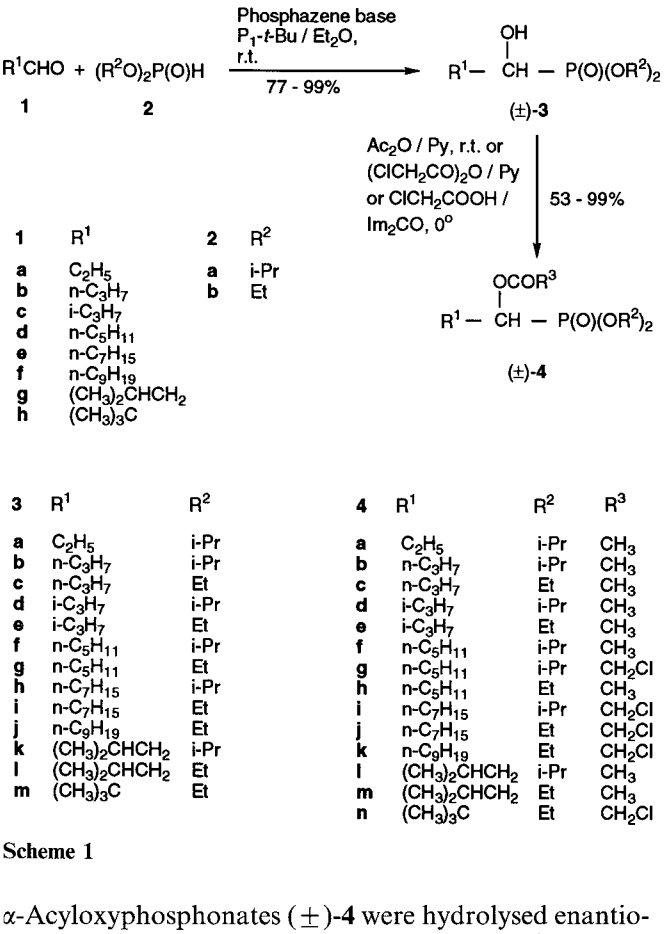
Racemic α-acyloxyalkylphosphonates (±)-**4** are prepared and tested for kinetic resolution by lipases AP 6 and to a minor extent by F-AP 15. The former proves to be a useful enzyme in terms of broadness of application, reaction rate and enantiomeric excess. Lipase F-AP 15 transformed neither of the two substrates checked for hydrolysis. Enzymatic hydrolysis of α-acyloxyphosphonates containing straight chain alkyl groups with lipase AP 6 yields (*S*)-α-hydroxyalkylphosphonates **3** preferentially. Substrates with branched chain alkyl groups are hydrolysed with lower enantioselectivity, the (*R*)-ester being saponified more easily than the (*S*)-ester.

Lipases from various sources are widely used to hydrolyse[2] enantioselectively racemic esters or esterify[3] enantioselectively the corresponding racemic alcohols with acyl donors in organic media. α-Hydroxyphosphonates are a special class of alcohols which are labile under basic conditions. They are of interest as starting materials for other α-substituted phosphonates, especially α-aminophosphonic acids. Some para-substituted α-hydroxyphenylmethylphosphonic acids are inhibitors of inositol monophosphatase.[4]

Optically active α-hydroxyphosphonates have therefore attracted much interest in recent years. They can be prepared by chemical resolution,[5] enantioselective synthesis,[6] and using enzymes.[1,7,8] In our previous papers,[1,8] we first demonstrated that lipases can be used to resolve enzymatically α-acyloxyphosphonates derived from a few representative aldehydes. The α-hydroxyphosphonates obtained can be transformed into optically active α-aminophosphonic acids.[1]

The present investigation was undertaken to study the scope of the method using a variety of α-acyloxyalkylphosphonates with linear and branched chain alkyl groups and lipases from *Aspergillus niger* (lipase AP 6) and in two cases also from *Rhizopus oryzae* (F-AP 15). Lipase AP 6 has been shown to contain just a few percent of lipase in admixture with other hydrolases.[9] At present we assume that only the lipase reacts with the substrates. Aldehydes **1a–h** were reacted with dialkylphosphites **2a–b** at room temperature in anhydrous diethyl ether in the presence of a catalytic amount (0.1 equiv) of the strong phosphazene base P_1-*t*-Bu [*tert*-butylaminotris(dimethylamino)phosphorane][10] to give racemic α-hydroxyphosphonates (±)-**3a–m** (Scheme 1, Table 1).[1,11] Workup and purification afforded products in yields ranging from 77–99%. The aldehyde with the shortest alkyl group for R^1 was propionaldehyde **(1a)**, that with the longest one was decanal **(1f)**. Additionally, aldehydes with branched alkyl groups such as isobutyraldehyde **(1c)**, 3-methylbutyraldehyde **(1g)**, and pivalaldehyde **(1h)** were used as educts as well. For reasons of stability towards nucleophilic dealkylation and higher enantioselectivity on enzymatic hydrolysis diisopropyl phosphite **(2a)** was used preferentially over diethyl phosphite **(2b)**. The α-hydroxyphosphonates (±)-**3** were acetylated using acetic anhydride/pyridine or chloroacetylated using chloroacetic anhydride/pyridine or chloroacetic acid/carbonyldiimidazole (Im_2CO) to give esters (±)-**4a–n** in yields ranging from 53–99% (Scheme 1, Table 2).



Scheme 1

α-Acyloxyphosphonates (±)-**4** were hydrolysed enantioselectively under argon at room temperature in a well stirred biphasic system (hexane/*tert*-butyl methyl ether/buffer pH 7) as previously reported, keeping the pH constant by automatic addition of 0.5 N sodium hydroxide (autotitrator) (Scheme 2, Table 4).[8] The reactions were carried out with 1 mmol of substrate and amounts of lipases as given in Table 4. When 0.9 mL of base had been added, corresponding to a conversion of 45%, the reaction was stopped by addition of 1 N hydrochloric acid to bring pH to 4.0. Extractive workup furnished a mixture of unreacted ester **4** and α-hydroxyphosphonate **3**. The conversion as determined by ^{1}H NMR spectroscopy of the mixture agreed with the value calculated from the amount of base added. The two compounds were easily separated by flash chromatography. Ester **4**

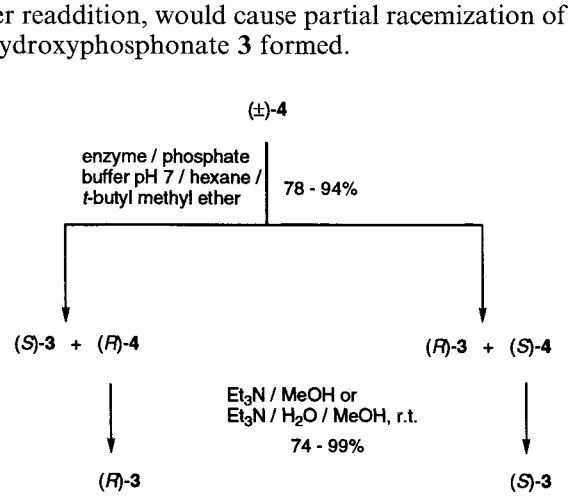
Table 1. α-Hydroxyalkylphosphonates (±)-**3** Prepared

Product[a]	Yield[b] (%)	bp (°C/ 0.01 Torr)	IR (neat) ν (cm^{-1})	^{1}H NMR ($CDCl_3$/TMS) δ, J (Hz)
3a	77	102–110	3314, 1215, 1016, 991	1.01 (t, 3H, J = 7.4, CH_3), 1.275, 1.278, 1.28 [3d, 6H, 3H and 3H, J = 6.4, $(CH_3)_2CHO$], 1.69 (m, 2H, $PCHCH_2$), 3.63 (m, 1H, PCH), 3.77 (dd, 1H, J = 3.9, 5.9, OH), 4.69 [m, 2H, $(CH_3)_2CHO$]
3b	83	90–100	3301, 1226, 989	0.89 (t, 3H, J = 7.2, CH_3), 1.27, 1.28, 1.29 [3d, 6H, 3H and 3H, J = 6.4, $(CH_3)_2CHO$], 1.39 (m, 1H, PCHCHH), 1.62 (m, 3H, $PCHCHHCH_2$), 3.63 (br s, 1H, OH), 3.73 (m, 1H, PCH), 4.69 [m, 2H, $(CH_3)_2CHO$]
3c	85	95–110	3319, 1224, 1031, 969	0.95 (t, 3H, J = 7.2, CH_3), 1.33 (dt, 6H, J = 1.5, 7.2, CH_3CH_2O), 1.44 (m, 1H, PCHCHH), 1.68 (m, 3H, $PCHCHHCH_2$), 2.29 (t, 1H, J = 7.4, OH), 3.87 (m, 1H, PCH), 4.16 (m, 4H, CH_3CH_2O)
3d	95	100–110	3315, 1214, 986	1.00, 1.02 [2d, 3H each, J = 6.9, $(CH_3)_2CH$], 1.29 [d, 6H, J = 5.9, $(CH_3)_2CHO$], 1.30 [d, 6H, J = 6.4, $(CH_3)_2CHO$], 2.03 [m, 1H, $(CH_3)_2CH$], 3.22 (t, 1H, J = 6.4, OH), 3.51 (q, 1H, J = 6.4, PCH), 4.71 [m, 2H, $(CH_3)_2CHO$]
3e	79	75–80	3313, 1216, 1032, 967	1.01, 1.02 [2d, 3H each, J = 6.9, $(CH_3)_2CH$], 1.29 (t, 6H, J = 7.2, CH_3CH_2O), 2.04 [m, 1H, $(CH_3)_2CH$], 3.13 (br s, 1H, OH), 3.60 (dd, 1H, J = 5.9, 6.4, PCH), 4.12 (m, 4H, CH_3CH_2O)
3f	77	95–105	3300, 1229, 986	0.81 (t, 3H, J = 6.6, CH_3), 1.24 [d, 6H, J = 6.4, $(CH_3)_2CHO$], 1.25 [d, 3H, J = 5.9, $(CH_3)_2CHO$], 1.26 [d, 3H, J = 6.4, $(CH_3)_2CHO$], 1.25 (m, 5H, CH_2), 1.57 (m, 3H, CH_2), 3.38 (br s, 1H, OH), 3.67 (dt, 1H, J = 3.9, 9.9, PCH), 4.65 [m, 2H, $(CH_3)_2CHO$]
3h	93	130–140	3300, 1223, 989	0.85 (t, 3H, J = 6.9, CH_3), 1.22–1.30 (m, 12H, CH_2), 1.30 [d, 6H, J = 5.9, $(CH_3)_2CHO$], 1.31 [d, 3H, J = 6.4, $(CH_3)_2CHO$], 1.32 [d, 3H, J = 5.9, $(CH_3)_2CHO$], 1.64 (m, 2H, CH_2), 3.40 (br s, 1H, OH), 3.74 (dt, 1H, J = 3.9, 9.9, PCH), 4.72 [m, 2H, $(CH_3)_2CHO$]
3i	99	110–120	2927, 1222, 1056, 1028, 968	0.83 (t, 3H, J = 6.9, CH_3), 1.20–1.40 (m, 9H, CH_2), 1.29, 1.30 (2t, 3H each, J = 6.9, CH_3CH_2O), 1.65 (m, 3H, CH_2), 3.60 (br s, 1H, OH), 3.80 (ddd, 1H, J = 3.9, 9.4, 9.9, PCH), 4.12 (m, 4H, CH_3CH_2O)
3j	81	115–120	3300, 1228, 1028, 967	0.84 (t, 1H, J = 6.9, CH_3), 1.20–1.32 (m, 13H, CH_2), 1.30, 1.31 (2t, 3H each, J = 7.2, CH_3CH_2O), 1.65 (m, 3H, CH_2), 3.35 (br s, 1H, OH), 3.80 (dt, 1H, J = 3.9, 9.8, PCH), 4.13 (m, 4H, CH_3CH_2O)
3m	97	110–130	3316, 1225, 1028, 966	1.05 [s, 9H, $(CH_3)_3C$], 1.30 (t, 6H, J = 7.2, CH_3CH_2O), 3.10 (br s, 1H, OH), 3.53 (d, 1H, J = 7.4, PCH), 4.13 (m, 4H, CH_3CH_2O)

[a] Satisfactory microanalyses obtained: C ± 0.71, H ± 0.48.

[b] Yields after bulb to bulb distillation.

was hydrolysed under very mild conditions chemically in anhydrous methanol (containing 10% of water for acetates) and triethylamine at room temperature to prevent decomposition to aldehyde and phosphite which, after readdition, would cause partial racemization of the α-hydroxyphosphonate **3** formed.



Scheme 2

To determine the absolute configuration and the enantiomeric purity of the α-hydroxyphosphonates obtained by enzymatic and chemical hydrolysis, α-hydroxyphosphonates **3** were derivatised with (*S*)-(+)-MTPA-Cl [(*S*)-(+)-Mosher acid chloride] in pyridine.[1] As we have shown,[12] the absolute configuration of Mosher esters of α-hydroxyphosphonates can be deduced from the ^{31}P NMR spectra and sometimes also from the ^{1}H NMR spectra.[8] The phosphorus of diastereomers derived from α-hydroxyphosphonates having (*S*)-configuration at the carbon atom resonate at lower field than the phosphorus of Mosher esters derived from α-hydroxyphosphonates having (*R*)-configuration (Table 5). The ^{1}H NMR signals of the OCH_3 group of the Mosher acid part of (*S*)-3-(*R*)-MTPA esters are observed consistently at lower field than those of (*R*)-3-(*R*)-MTPA esters. The enantiomeric excess was routinely determined from the integral of appropriate signals (OCH_3 of MTPA group or other signals not overlapping in the ^{1}H NMR spectrum; ^{31}P NMR signals in ^{31}P NMR spectrum). The enantiomeric excesses determined by ^{1}H and ^{31}P NMR spectroscopy agreed well within experimental error (Table 4).

Lipase AP 6 hydrolyses the (*S*)-enantiomer more easily than the (*R*)-enantiomer of the racemic acetates **4a, b** and **f**. (Table 4, Entries 1, 2, 6, 7). The reaction rate decreased with increasing length of the alkyl chain R^1 from ethyl to pentyl while the e.e. increased from 77 to 87% at a conversion of 43 and 48%, respectively. Reducing the bulkiness of the phosphonate group by replacing *i*-propyl by ethyl increased the reaction rate by a factor of about sixteen (Entries 7 and 10). At the same time the enantiomeric excess dropped significantly from 87 to 69% at a comparable conversion. The chloroacetate (±)-**4g** is hydrolysed about 50 times faster than the corresponding acetate (±)-**4f**, the e.e. being virtually the same (Entries 7 and 8). To keep the reaction rate at an experimentally acceptable level when the length of the alkyl chain R^1 is further increased to heptyl and nonyl the diethyl α-(chloroacetyloxy)alkylphosphonates have

Table 2. α-Acyloxyalkylphosphonates (±)-**4** Prepared

Product[a]	Yield[b] (%)	bp (°C/0.01 Torr)	IR (neat) ν (cm^{-1})	^{1}H NMR ($CDCl_3$/TMS) δ, J (Hz)
4a	65	65–70	1750, 1228, 990	0.92 (t, 3H, J = 7.4, CH_3), 1.27, 1.28 [2d, 3H each, J = 5.9, $(CH_3)_2CHO$], 1.29 [d, 6H, J = 6.4, $(CH_3)_2CHO$], 1.80 (m, 2H, CH_2), 2.05 (s, 3H, $COCH_3$), 4.70 [m, 2H, $(CH_3)_2CHO$], 5.10 (dt, 1H, J = 4.4, 9.8, PCH)
4b	99	70–75	1752, 1228, 989	0.87 (t, 3H, J = 7.4, CH_3), 1.25 [d, 3H, J = 6.4, $(CH_3)_2CHO$], 1.26, 1.28 [2d, 3H each, J = 5.9, $(CH_3)_2CHO$], 1.29 [d, 3H, J = 7.4, $(CH_3)_2CHO$], 1.37 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 2.05 (s, 3H, $COCH_3$), 4.69 [m, 2H, $(CH_3)_2CHO$], 5.18 (dt, 1H, J = 5.4, 9.4, PCH)
4c	90	120–130	1749, 1228, 1028	0.89 (t, 3H, J = 7.2, CH_3), 1.28, 1.29 (2t, 3H each, J = 7.2, CH_3CH_2O), 1.40 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 2.08 (s, 3H, $COCH_3$), 4.10 (m, 4H, CH_3CH_2O), 5.25 (dt, 1H, J = 4.9, 8.4, PCH)
4d	90	100–110	1748, 1228, 1107, 1002	0.97, 0.99 [2d, 3H each, J = 6.9, $(CH_3)_2CH$], 1.26 [d, 3H, J = 6.4, $(CH_3)_2CHO$], 1.27 [d, 3H, J = 5.9, $(CH_3)_2CHO$], 1.29 [d, 6H, J = 6.4, $(CH_3)_2CHO$], 2.08 (s, 3H, $COCH_3$), 2.17 [m, 1H, $(CH_3)_2CH$], 4.69 [m, 2H, $(CH_3)_2CHO$], 5.01 (dd, 1H, J = 6.4, 9.8, PCH)
4e	96	65–70	1748, 1228, 1027, 969	0.80, 1.00 [2d, 3H each, J = 6.9, $(CH_3)_2CH$], 1.27, 1.28 (2t, 3H each, J = 7.2, CH_3CH_2O), 2.09 (s, 3H, $COCH_3$), 2.19 [m, 1H, $(CH_3)_2CH$], 4.10 (m, 4H, CH_3CH_2O), 5.05 (dd, 1H, J = 6.4, 9.4, PCH)
4f	89	90–95	1750, 1228, 988	0.82 (t, 3H, J = 6.9, CH_3), 1.26, 1.27 [2d, 3H each, J = 6.4, $(CH_3)_2CHO$], 1.28, 1.29 [2d, 3H each, J = 5.9, $(CH_3)_2CHO$], 1.29 (m, 6H, CH_2), 1.75 (m, 2H, CH_2), 2.05 (s, 3H, $COCH_3$), 4.69 [m, 2H, $(CH_3)_2CHO$], 5.16 (ddd, 1H, J = 3.9, 8.9, 9.8, PCH)
4g	62	80–95	1768, 1252, 1164, 990	0.87 (t, 3H, J = 6.6, CH_3), 1.30, 1.33 [2d, 6H each, J = 5.9, $(CH_3)_2CHO$], 1.29 (m, 6H, CH_2), 1.83 (m, 2H, CH_2), 4.10 (s, 2H, CH_2Cl), 4.73 [m, 2H, $(CH_3)_2CHO$], 5.24 [ddd, 1H, J = 4.0, 8.9, 9.9, PCH]
4h	85	85–90	1750, 1227, 1026, 970	0.82 (t, 3H, J = 6.6, CH_3), 1.26, 1.28 (2t, 3H each, J = 6.9, CH_3CH_2O), 1.19–1.44 (m, 6H, CH_2), 1.77 (m, 2H, CH_2), 2.06 (s, 3H, $COCH_3$), 4.09 (m, 4H, CH_3CH_2O), 5.20 (ddd, 1H, J = 3.9, 8.4, 9.8, PCH)
4i	81	155–165	1769, 1256, 1164, 990	0.84 (t, 3H, J = 6.6, CH_3), 1.20–1.31 [m, 22H, $(CH_3)_2CHO$, CH_2], 1.81 (m, 2H, CH_2), 4.08 (s, 2H, CH_2Cl), 4.71 [m, 2H, $(CH_3)_2CHO$], 5.22 (dt, 1H, J = 3.9, 9.4, PCH)
4j	62	110–115	1767, 1256, 1164, 1024, 971	0.83 (t, 3H, J = 6.6, CH_3), 1.29–1.40 (m, 10H, CH_2), 1.29, 1.30 (2t, 3H each, J = 7.2, CH_3CH_2O), 1.84 (m, 2H, CH_2), 4.08 (s, 2H, CH_2Cl), 4.12 (m, 4H, CH_3CH_2O), 5.25 (ddd, 1H, J = 4.4, 8.4, 9.8, PCH)
4k	53	120–130	1769, 1251, 1161, 1028, 971	0.83 (t, 3H, J = 6.9, CH_3), 1.28, 1.29 (2t, 3H each, J = 6.9, CH_3CH_2O), 1.29–1.33 (m, 14H, CH_2), 1.82 (m, 2H, CH_2), 4.07 (s, 2H, CH_2Cl), 4.12 (m, 4H, CH_3CH_2O), 5.25 (m, 1H, PCH)
4l	57	75–80	1749, 1228, 989	0.87, 0.90 [2d, 3H each, J = 6.4, $(CH_3)_2CH$], 1.27, 1.28, 1.31 [3d, 3H, 3H, 6H, J = 6.4, $(CH_3)_2CHO$], 1.55 (m, 2H, $PCHCH_2$), 1.75 [m, 1H, $(CH_3)_2CH$], 2.06 (s, 3H, $COCH_3$), 4.70 [m, 2H, $(CH_3)_2CHO$], 5.28 (ddd, 1H, J = 3.0, 9.8, 11.3, PCH)
4m	94	70–75	1748, 1371, 1098, 1023, 970	0.85, 0.88 [2d, 3H each, J = 6.2, $(CH_3)_2CH$], 1.26, 1.27 (2t, 3H each, J = 6.9, CH_3CH_2O), 1.56 (m, 2H, $PCHCH_2$), 1.76 [m, 1H, $(CH_3)_2CH$], 2.05 (s, 3H, $COCH_3$), 4.09 (m, 4H, CH_3CH_2O), 5.31 (ddd, 1H, J = 3.0, 9.4, 11.3, PCH)
4n	94	130–140	1770, 1252, 1163, 1024, 970	1.06 [s, 9H, $(CH_3)_3C$], 1.27, 1.29 (2t, 3H each, J = 6.9, CH_3CH_2O), 4.10 (m, 6H, CH_3CH_2O, CH_2Cl), 5.07 (d, 1H, J = 10.3, PCH)

[a] Satisfactory microanalyses obtained: C ± 0.53, H ± 0.29.

[b] Yields after flash chromatography and bulb to bulb distillation.

to be prepared (Entries 11–13). The e.e. decreases to 62% for the (*S*)-α-hydroxyphosphonate (+)-**3j** obtained from (±)-**4k**. For 1 mmol of (±)-**4k** 200 mg of lipase AP 6 were added to achieve a conversion of 46% in 27 h. This is probably one of the phosphonates with the longest alkyl chain which can be resolved enzymatically at a reasonable rate.

α-Acyloxyphosphonates (±)-**4d, e, l, m**, and **n** bearing a methyl group in β- or γ-position, show a different behaviour compared to their unbranched counterparts of the same carbon atom number. Diisopropyl α-acetyloxyphosphonates (±)-**4d** and **4l** are surprisingly not substrates for lipase AP 6 (Entries 4 and 14). The α-acetyloxyphosphonates derived from butanal and hexanal are easily saponified enzymatically. The corresponding diethyl phosphonates (±)-**4e** and **m** are substrates for this lipase, but the enantioselectivity is reversed and low (the e.e.s are 32 and 7.5%, respectively) compared to phosphonates with straight alkyl chains (Entries 5 and 15). The chloroacetate (±)-**4n** of hydroxyphosphonate **3m** derived from pivalaldehyde behaves similarily (Entry 16).

Lipase F-AP 15 was allowed to react with α-acetyloxyphosphonates (±)-**4c** and **h**, but none of the substrates was hydrolysed (Entries 3 and 9). α-Acetyloxyphosphonates derived from acetaldehyde, crotonaldehyde, and benzaldehyde are accepted as substrates by this enzyme.[8]

In summary, lipase AP 6 can be used for the enantioselective hydrolysis of readily available α-acyloxyalkylphosphonates with *n*-alkyl chains of up to 10 carbon atoms. This method opens an entry to optically active α-hydroxyphosphonates which can serve as starting materials for the preparation of α-aminophosphonic acids and other α-substituted phosphonates. The e.e.s of the α-hydroxyphosphonates can still be easily increased by stopping the reaction at a lower conversion than 45%. The reaction, though carried out on a 1 mmol scale, is surely amenable to scale up.

Table 3. ^{13}C NMR Data of Compounds **3** and **4**

Product	^{13}C NMR ($CDCl_3$/TMS) δ, J (Hz)
3a	10.47 (d, J = 13.7, CH_3), 23.91 [d, J = 4.8, $(\underline{C}H_3)_2CHO$], 24.05 [d, J = 3.2, $(\underline{C}H_3)_2CHO$], 24.09 [d, J = 3.1, $(\underline{C}H_3)_2CHO$], 24.66 (d, J = 0.90, CH_2), 69.50 (d, J = 161.6, PCH), 70.78, 70.95 [2d, J = 7.2, $(CH_3)_2\underline{C}HO$]
3b	13.68 (CH_3), 18.96 (d, J = 13.7, $\underline{C}H_2CH_3$), 23.93 [d, J = 4.9, $(\underline{C}H_3)_2CHO$], 24.06 [d, J = 3.1, $(\underline{C}H_3)_2CHO$], 24.09 [d, J = 2.9, $(\underline{C}H_3)_2CHO$], 33.30 ($PCH\underline{C}H_2$), 67.66 (d, J = 161.8, PCH), 70.79, 70.95 [2d, J = 7.3, $(CH_3)_2\underline{C}HO$]
3c	13.62 (CH_3), 16.42 (d, J = 5.4, $\underline{C}H_3CH_2O$), 18.83 (d, J = 13.7, $\underline{C}H_2CH_3$), 33.27 ($PCH\underline{C}H_2$), 62.44, 62.58 (2d, J = 7.1, $CH_3\underline{C}H_2O$), 67.39 (d, J = 160.3, PCH)
3d	17.86 [d, J = 7.8, $(\underline{C}H_3)_2CH$], 19.92 [d, J = 9.3, $(\underline{C}H_3)_2CH$], 23.92 [d, J = 5.0, $(\underline{C}H_3)_2CHO$], 24.10 [d, J = 3.7, $(\underline{C}H_3)_2CHO$], 30.13 [d, J = 2.1, $(CH_3)_2\underline{C}H$], 70.81 [d, J = 8.2, $(CH_3)_2\underline{C}HO$], 70.89 [d, J = 7.9, $(CH_3)_2\underline{C}HO$], 73.26 (d, J = 157.5, PCH)
3e	16.43 (d, J = 5.6, $\underline{C}H_3CH_2O$), 17.72 (d, J = 7.6, $\underline{C}H_3CH_2O$), 19.81 [d, J = 9.6, $(\underline{C}H_3)_2CH$], 30.11 [d, J = 2.3, $(CH_3)_2\underline{C}H$], 62.32 (d, J = 7.1, $CH_3\underline{C}H_2O$), 72.93 (d, J = 156.4, PCH)
3f	13.97 (CH_3), 22.49 ($\underline{C}H_2CH_3$), 23.95 [d, J = 4.7, $(\underline{C}H_3)_2CHO$], 24.08 [d, J = 3.2, $(\underline{C}H_3)_2CHO$], 24.11 [d, J = 2.7, $(\underline{C}H_3)_2CHO$], 25.47 (d, J = 13.3, $PCHCH_2\underline{C}H_2$), 31.28, 31.48 (CH_2), 68.09 (d, J = 161.1, PCH), 70.89, 71.04 [2d, J = 7.3, $(CH_3)_2\underline{C}HO$]
3h	14.05 (CH_3), 22.61 (CH_2), 23.98 [d, J = 4.7, $(\underline{C}H_3)_2CHO$], 24.10 [d, J = 3.4, $(\underline{C}H_3)_2CHO$], 24.13 [d, J = 3.3, $(\underline{C}H_3)_2CHO$], 25.80 (d, J = 13.0, $PCHCH_2\underline{C}H_2$), 29.12, 29.25, 31.33, 31.77 (CH_2), 68.17 (d, J = 161.1, PCH), 70.87, 71.01 [2d, J = 7.3, $(CH_3)_2\underline{C}HO$]
3i	14.02 (CH_3), 16.46 (d, J = 5.1, $\underline{C}H_3CH_2O$), 22.58 (CH_2), 25.69 (d, J = 13.1, $PCHCH_2\underline{C}H_2$), 29.09, 29.22, 31.31, 31.75 (CH_2), 62.41 (d, J = 7.3, $CH_3\underline{C}H_2O$), 62.54 (d, J = 7.0, $CH_3\underline{C}H_2O$), 67.80 (d, J = 159.9, PCH)
3j	14.06 (CH_3), 16.48 (d, J = 5.8, $\underline{C}H_3CH_2O$), 22.63 (CH_2), 25.70 (d, J = 13.3, $PCHCH_2\underline{C}H_2$), 29.26, 29.45, 29.51, 31.33, 31.85 (CH_2), 62.43 (d, J = 7.3, $CH_3\underline{C}H_2O$), 62.53 (d, J = 7.0, $CH_3\underline{C}H_2O$), 67.85 (d, J = 160.1, PCH)
3m	16.37 (d, J = 5.6, $\underline{C}H_3CH_2O$), 16.43 (d, J = 5.7, $\underline{C}H_3CH_2O$), 26.54 [d, J = 6.2, $(\underline{C}H_3)_3C$], 34.54 [d, J = 3.1, $(CH_3)_3\underline{C}$], 62.12, 62.28 (2d, J = 7.2, $CH_3\underline{C}H_2O$), 76.16 (d, J = 135.5, PCH)
4a	10.23 (d, J = 12.4, CH_3), 20.74 ($CO\underline{C}H_3$), 22.96 ($PCH\underline{C}H_2$), 23.81 [d, J = 4.9, $(\underline{C}H_3)_2CHO$], 23.91 [d, J = 4.8, $(\underline{C}H_3)_2CHO$], 24.02 [d, J = 3.7, $(\underline{C}H_3)_2CHO$], 24.12 [d, J = 3.3, $(\underline{C}H_3)_2CHO$], 69.66 (d, J = 170.7, PCH), 71.23 [d, J = 6.9, $(CH_3)_2\underline{C}HO$], 169.94 (d, J = 6.1, CO)
4b	13.49 (CH_3), 18.85 (d, J = 12.8, $\underline{C}H_2CH_3$), 20.70 ($CO\underline{C}H_3$), 23.76, 23.87 [2d, J = 4.9, $(\underline{C}H_3)_2CHO$], 23.98 [d, J = 3.7, $(\underline{C}H_3)_2CHO$], 24.07 [d, J = 3.3, $(\underline{C}H_3)_2CHO$], 31.49 ($PCH\underline{C}H_2$), 68.02 (d, J = 170.5, PCH), 71.22 [d, J = 7.2, $(CH_3)_2\underline{C}HO$], 71.24 [d, J = 6.7, $(CH_3)_2\underline{C}HO$], 169.84 (CO)
4c	13.43 (CH_3), 16.28 (d, J = 6.0, $\underline{C}H_3CH_2O$), 16.38 (d, J = 5.7, $\underline{C}H_3CH_2O$), 18.81 (d, J = 12.8, $\underline{C}H_2CH_3$), 20.61 ($CO\underline{C}H_3$), 31.27 ($PCH\underline{C}H_2$), 62.56 (d, J = 6.4, $CH_3\underline{C}H_2O$), 62.65 (d, J = 7.2, $CH_3\underline{C}H_2O$), 67.45 (d, J = 167.9, PCH), 169.81 (d, J = 5.0, CO)
4d	18.29 [d, J = 7.7, $(\underline{C}H_3)_2CH$], 19.80 [d, J = 8.7, $(\underline{C}H_3)_2CH$], 20.69 ($CO\underline{C}H_3$), 23.80 [d, J = 5.0, $(\underline{C}H_3)_2CHO$], 23.94 [d, J = 4.9, $(\underline{C}H_3)_2CHO$], 24.05 [d, J = 3.7, $(\underline{C}H_3)_2CHO$], 24.16 [d, J = 3.2, $(\underline{C}H_3)_2CHO$], 29.21 [d, J = 1.4, $(CH_3)_2\underline{C}H$], 71.07 [d, J = 4.4, $(CH_3)_2\underline{C}HO$], 71.14 [d, J = 4.8, $(CH_3)_2\underline{C}HO$], 72.92 (d, J = 168.3, PCH), 169.91 (d, J = 5.4, CO)
4e	16.29 (d, J = 6.0, $\underline{C}H_3CH_2O$), 16.41 (d, J = 5.6, $\underline{C}H_3CH_2O$), 18.26 [d, J = 7.8, $(\underline{C}H_3)_2CH$], 19.62 [d, J = 8.4, $(\underline{C}H_3)_2CH$], 20.58 ($CO\underline{C}H_3$), 29.05 [d, J = 1.4, $(CH_3)_2\underline{C}H$], 62.39 (d, J = 6.3, $CH_3\underline{C}H_2O$), 62.57 (d, J = 7.1, $CH_3\underline{C}H_2O$), 72.31 (d, J = 165.6, PCH), 169.87 (d, J = 4.9, CO)
4f	13.86 (CH_3), 20.75 ($COCH_3$), 22.31 (CH_2), 23.79 [d, J = 5.2, $(\underline{C}H_3)_2CHO$], 23.89 [d, J = 5.1, $(\underline{C}H_3)_2CHO$], 24.00, 24.10 [2d, J = 3.5, $(\underline{C}H_3)_2CHO$], 25.25 (d, J = 12.4, $PCHCH_2\underline{C}H_2$), 29.44, 31.22 (CH_2), 68.34 (d, J = 170.3, PCH), 71.18 [d, J = 1.8, $(CH_3)_2\underline{C}HO$], 71.25 [$(CH_3)_2\underline{C}HO$], 169.84 (d, J = 5.9, CO)
4g	13.86 (CH_3), 22.28 (CH_2), 23.78, 23.93 [2d, J = 5.1, $(\underline{C}H_3)_2CHO$], 24.02 [d, J = 4.1, $(\underline{C}H_3)_2CHO$], 24.11 [d, J = 3.3, $(\underline{C}H_3)_2CHO$], 25.18 (d, J = 12.2, CH_2), 29.29, 31.13 (CH_2), 40.60 (CH_2Cl), 70.33 (d, J = 170.3, PCH), 71.71 [d, J = 7.1, $(CH_3)_2\underline{C}HO$], 71.90 [d, J = 6.8, $(CH_3)_2\underline{C}HO$], 166.50 (d, J = 5.5, CO)
4h	13.83 (CH_3), 16.29 (d, J = 6.0, $\underline{C}H_3CH_2O$), 16.39 (d, J = 5.8, $\underline{C}H_3CH_2O$), 20.64 ($CO\underline{C}H_3$), 22.27 (CH_2), 25.19 (d, J = 12.3, $PCHCH_2\underline{C}H_2$), 29.22, 31.15 (CH_2), 62.52 (d, J = 6.3, $CH_3\underline{C}H_2O$), 62.62 (d, J = 7.1, $CH_3\underline{C}H_2O$), 67.76 (d, J = 167.7, PCH), 169.82 (d, J = 5.3, CO)
4i	13.99 (CH_3), 22.52 (CH_2), 23.79 [d, J = 5.2, $(\underline{C}H_3)_2CHO$], 23.95 [d, J = 5.3, $(\underline{C}H_3)_2CHO$], 23.99 [d, J = 4.0, $(\underline{C}H_3)_2CHO$], 24.13 [d, J = 3.3, $(\underline{C}H_3)_2CHO$], 25.53 (d, J = 12.1, $PCHCH_2\underline{C}H_2$), 28.90, 28.93, 29.34, 31.62 (CH_2), 40.61 (CH_2Cl), 70.41 (d, J = 169.9, PCH), 71.47 [d, J = 7.1, $(CH_3)_2\underline{C}HO$], 71.65 [d, J = 6.7, $(CH_3)_2\underline{C}HO$], 166.49 (d, J = 5.5, CO)
4j	14.00 (CH_3), 16.35 (d, J = 5.9, $\underline{C}H_3CH_2O$), 16.44 (d, J = 5.8, $\underline{C}H_3CH_2O$), 22.53 (CH_2), 25.52 (d, J = 12.0, CH_2), 28.90, 29.18, 31.62 (CH_2), 40.55 (CH_2Cl), 62.84 (d, J = 6.2, $CH_3\underline{C}H_2O$), 69.85 (d, J = 167.2, PCH), 166.53 (d, J = 5.2, CO)
4k	14.03 (CH_3), 16.33 (d, J = 5.7, $\underline{C}H_3CH_2O$), 16.42 (d, J = 5.6, $\underline{C}H_3CH_2O$), 22.60 (CH_2), 25.51 (d, J = 12.2, CH_2), 28.95, 29.17, 29.23, 31.79 (CH_2), 40.54 (CH_2Cl), 62.82 (d, J = 7.3, $CH_3\underline{C}H_2O$), 62.83 (d, J = 5.9, $CH_3\underline{C}H_2O$), 69.84 (d, J = 167.2, PCH), 166.71 (d, J = 5.1, CO)
4l	20.78 ($CO\underline{C}H_3$), 21.26, 23.17 [$(\underline{C}H_3)_2CH$], 23.80 [d, J = 5.2, $(\underline{C}H_3)_2CHO$], 23.90 [d, J = 4.9, $(\underline{C}H_3)_2CHO$], 24.01 [d, J = 3.6, $(\underline{C}H_3)_2CHO$], 24.11 [d, J = 3.2, $(\underline{C}H_3)_2CHO$], 24.45 [d, J = 13.1, $(CH_3)_2\underline{C}H$], 38.16 ($PCH\underline{C}H_2$), 66.71 (d, J = 170.5, PCH), 71.28 [d, J = 6.9, $(CH_3)_2\underline{C}HO$], 71.35 [d, J = 6.5, $(CH_3)_2\underline{C}HO$], 169.77 (d, J = 5.2, CO)
4m	16.28 (d, J = 5.7, $\underline{C}H_3CH_2O$), 16.38 (d, J = 5.6, $\underline{C}H_3CH_2O$), 20.65 ($CO\underline{C}H_3$), 21.15, 23.05 [$(\underline{C}H_3)_2CH$], 24.42 [d, J = 13.1, $(CH_3)_2\underline{C}H$], 37.84 ($PCH\underline{C}H_2$), 62.61 (d, J = 5.9, $CH_3\underline{C}H_2O$), 62.67 (d, J = 6.6, $CH_3\underline{C}H_2O$), 66.10 (d, J = 167.7, PCH), 169.74 (d, J = 4.5, CO)
4n	16.24 (d, J = 6.1, $\underline{C}H_3CH_2O$), 16.38 (d, J = 5.9, $\underline{C}H_3CH_2O$), 26.64 [d, J = 6.2, $(\underline{C}H_3)_3C$], 34.48 [d, J = 2.4, $(CH_3)_3\underline{C}$], 40.51 (CH_2Cl), 62.41 (d, J = 6.4, $CH_3\underline{C}H_2O$), 62.56 (d, J = 7.4, $CH_3\underline{C}H_2O$), 76.80 (d, J = 162.9, PCH), 166.38 (d, J = 4.1, CO)

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 $CDCl_3$ using TMS 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

Table 4. Enzymatic Hydrolysis of (±)-**4**

Entry	Substrate	Enzyme, mg	Temp. (°C)	Time (h); Convsn[a] (%)	Produced Alcohol				Recovered Ester	Alcohol from Recovered Ester			
					Yield (%)	e.e.[b] (%)	Conf.	$[\alpha]_D$(c)[c]	$[\alpha]_D$(c)[c]	Yield[d] (%)	e.e.[b] (%)	Conf.	$[\alpha]_D$(c)[c]
1	**4a**	AP6, 70	24	19.68; 43	37	77 (75)	(*S*)	+12.5 (1.0)	−16.94 (2.4)	47	51	(*R*)	−8.61 (1.3)
2	**4b**	AP6, 124	23	19.18; 45/47	39	83 (81)	(*S*)	+16.16 (1.5)	−22.60 (2.1)	45	68	(*R*)	−12.36 (1.3)
3	**4c**	F-AP15, 100	23	17.45	no reaction		–	–	–	–	–	–	–
4	**4d**	AP6, 120	25	17.78	no reaction		–	–	–	–	–	–	–
5	**4e**	AP6, 100	24	19.23; 45/48	38	32 (33)	(*R*)	−3.48 (1.4)	+7.17 (2.1)	30	26	(*S*)	+2.71 (1.1)
6	**4f**	AP6, 152	40	51.72; 29	27	98	(*S*)	+17.68 (1.3)	−10.74 (1.5)	61	43	(*R*)	−7.00 (1.5)
7	**4f**	AP6, 152	23	66.42; 48/49	43	87	(*S*)	+15.47 (1.5)	−21.80 (1.3)	36	80	(*R*)	−13.38 (1.6)
8	**4g**	AP6, 57	23	3.28; 37/42	37	92	(*S*)	+14.77 (1.2)	−15.21 (1.4)	56	52 (51)	(*R*)	−9.14 (1.4)
9	**4h**	F-AP15, 101	23	25.33	no reaction		–	–	–	–	–	–	–
10	**4h**	AP6, 41	24	15.48; 45/44	38	69	(*S*)	+13.97 (1.2)	−14.78 (1.4)	49	48 (46)	(*R*)	−9.57 (1.5)
11	**4i**	AP6, 103	25	49[e]	51	73 (71)	(*S*)	+11.89 (1.4)	−22.74 (1.8)	43	84	(*R*)	−13.57 (1.4)
12	**4j**	AP6, 152	22	3.07; 45/46	42	63	(*S*)	+11.58 (1.8)	−15.61 (1.9)	47	57 (55)	(*R*)	−9.59 (2.0)
13	**4k**	AP6, 200	26	27.37; 45/46	45	62 (63)	(*S*)	+10.25 (2.4)	−13.21 (1.7)	49	56	(*R*)	−8.97 (1.9)
14	**4l**	AP6, 106	22	19.00	no reaction		–	–	–	–	–	–	–
15	**4m**	AP6, 125	23	24.58; 44/47	39	7.5 (5)	(*R*)	−1.15 (1.4)	+1.60 (1.3)	47	3 (4)	(*S*)	+1.26 (1.3)
16	**4n**	AP6, 105	25	6.17; 45/48	43	15	(*R*)	−0.90 (1.7)	+3.04 (1.7)	47	15	(*S*)	+1.09 (1.6)

[a] Convsn = conversion determined from 0.5 N NaOH consumed/conversion determined by ^{1}H NMR.
[b] e.e. as determined by ^{1}H NMR spectroscopy (by ^{31}P NMR spectroscopy).
[c] In acetone solution at 20 °C; concentration was rounded to the nearest tenth.
[d] Yield of ester after enzymatic hydrolysis multiplied by yield of chemical hydrolysis.
[e] Including 3 h at the beginning when no base was consumed.

Table 5. 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 Ester of	Chemical Shift (δ) of C-1 of **3** (*S*)	(*R*)	Δδ
3a	17.54	17.08	0.46
3b	17.83	17.33	0.50
3d	18.79	18.64	0.15
3e	19.37	18.95	0.42
3f	17.81	17.32	0.49
3g	19.96	19.46	0.50
3h	17.73	17.24	0.49
3i	19.97	19.47	0.50
3j	19.98	19.49	0.49
3l	20.42	19.93	0.49
3m	18.79	18.64	0.15

plate.[13] Optical rotations were measured at 20 °C on a Perkin Elmer 241 polarimeter in acetone solution 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 the TLC plates into a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (23 g) and of $Ce(SO_4)_2 \cdot 4H_2O$ (1 g) in 10% H_2SO_4 (500 mL) in water, followed by heating on a hot plate. 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), e.e. > 99.5%)] was used for derivatization of α-hydroxyalkylphosphonates.

Dialkyl 1-(chloroacetyloxy)alkylphosphonates (±)-**4i** and **n** were prepared according to the literature procedure.[1]

Dialkyl 1-(acetyloxy)alkylphosphonates (±)-**4** were prepared as reported.[8] α-Acetyloxyphosphonates (±)-**4h, l** and **m** were obtained by following the general procedure for the preparation of (±)-**4** except that after 6 h anhdr. pyridine (5 mL) and Ac_2O (1.7 mL) were added and stirring was continued for 20 h. Dry toluene (15 mL) was added and the volatiles were evaporated. The residue was purified by flash chromatography (CH_2Cl_2/EtOAc 5:1) and by bulb to bulb distillation under reduced pressure (Table 2).

Chloroacetates recovered from enzymatic resolution were hydrolysed within 1 d according to the reported procedure.[1] Acetates were hydrolysed within 5–11 d (followed by TLC) by the same procedure except that water (0.5 mL) was added to the methanolic solution.

Dialkyl 1-Hydroxyalkylphosphonates (±)-3; General Procedure:
A solution of aldehyde **1** (11 mmol), phosphite **2** (10 mmol), phosphazene base P_1-*t*-Bu (1 mmol) in anhydr. Et_2O (15 mL) was stirred under Ar at r.t. for 20 h. After the addition of conc. H_2SO_4 (0.053 mL, 1 mmol), the solvent was removed in vacuo, the residue was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). After drying ($MgSO_4$), the organic layer was evaporated and the product was purified by bulb to bulb distillation under reduced pressure (Table 1).

Dialkyl 1-(Chloroacetyloxy)alkylphosphonates (±)-4g, j and k; General Procedure:
Under Ar at 0 °C, chloroacetic acid (0.945 g, 10 mmol) in anhydr. CH_2Cl_2 (5 mL) was added dropwise to a suspension of *N,N*-carbonyldiimidazole (1.620 g, 10 mmol) in anhydr. CH_2Cl_2 (10 mL). Stirring was continued for 30 min at r.t. and dialkyl 1-hydroxyphosphonate (±)-**3** (5 mmol) in anhydr. CH_2Cl_2 (5 mL) was added. After stirring for 20 h, the mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo, and purified by bulb to bulb distillation under reduced pressure (Table 2).

Mosher Esters of 3; General Procedure:
A solution of dialkyl 1-hydroxyalkylphosphonates **3** (0.1 mmol), anhydr. CH_2Cl_2 (0.5 mL), anhydr. pyridine (1 mL) and (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) (0.25 mmol, 0.316 mL of a 0.79 M solution of (*S*)-(+)-MTPA-Cl in CH_2Cl_2) was stirred under Ar for 20 h at r.t. After addition of H_2O (1 drop) the solvent was removed in vacuo. The residue was diluted with HCl (1 N, 5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were washed with H_2O (10 mL), sat. aq $NaHCO_3$ (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/EtOAc, 10:1).

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