Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 3139–3145

TETRAHEDRON: *ASYMMETRY*

Biocatalytic syntheses of chiral non-racemic 2-hydroxyalkanephosphonates

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Received 8 November 2001; accepted 13 December 2001

Abstract—A series of 2-oxoalkanephosphonates **2** were screened for reduction with *Geotrichum candidum*. Only diethyl 2-oxopropanephosphonate **2a** underwent asymmetric reduction to give (+)-(*R*)-diethyl 2-hydroxypropanephosphonate **3a** with 98% e.e. In turn, a series of racemic 2-hydroxyalkanephosphonates **3** were acetylated under kinetic resolution conditions in the presence of various lipases to give the corresponding 2-acetoxyalkanephosphonates **4** and recovered alcohols **3** in good yields and with e.e. up to 93%.

1. Introduction

Chiral 2-hydroxyalkanephosphonates **3** have recently attracted considerable attention due to their potential biological activity and versatility as substrates for the synthesis of a variety of organophosphorus derivatives.[1,2] For example, 2-hydroxyphosphonates **3** have been used as substrates in the synthesis of various 2-aminoalkanephosphonic acids,[3] and 2-amino-1-hydroxyethanephosphonic acids.[4] Therefore, several attempts at their synthesis have been reported, which may be divided into two groups. The first approach involves asymmetric reduction of the corresponding 2-oxoalkanephosphonates **2** in the presence of chiral catalysts, e.g. (*S*)- or (*R*)-BINAP-Ru(II), which gave the products in yields of up to 99% and with e.e. of up to 98%,[2] and the catalyst (*S*)-5,5-diphenyl-2-butyl-3,4-propano-1,3,2-oxazaborolidine which resulted in the formation of the product with 91% e.e.[5] The other approach is based on the use of biocatalysts: selected 2-oxoalkanephosphonates were stereoselectively reduced with baker's yeast[6,7] to give the products with up to 92% e.e.

In continuation of our studies on the use of enzymes for the synthesis of chiral sulfur and phosphorus derivatives,[8] among them *P*-chiral hydroxymethanephosphonates,[9] we have decided to check the applicability of this methodology to the synthesis of the title compounds. The following two strategies have been applied to perform this task:

– Microorganism-mediated reduction of 2-oxoalkanephosphonates
– Enzymatic kinetic resolution of racemic 2-hydroxyalkanephosphonates.

2. Results and discussion

2.1. Synthesis of substrates: 2-oxoalkanephosphonates 2 and racemic 2-hydroxy-alkanephosphonates 3

The required 2-oxoalkanephosphonates **2** were synthesized from the corresponding methanephosphonates **1** via acylation of their carbanions with a variety of carboxylic acid esters. In turn, racemic 2-hydroxyalkanephosphonates **3** were obtained by reduction of the corresponding 2-oxoalkanephosphonates **2** with sodium borohydride (Scheme 1).

2.2. Microorganism-mediated reduction of 2

Four representative 2-oxoalkanephosphonates **2** were screened for asymmetric reduction by live cultures of *Geotrichum candidum*. *Geotrichum candidum* is a fungus which was previously found to be very effective in the stereoselective reduction of a wide variety of

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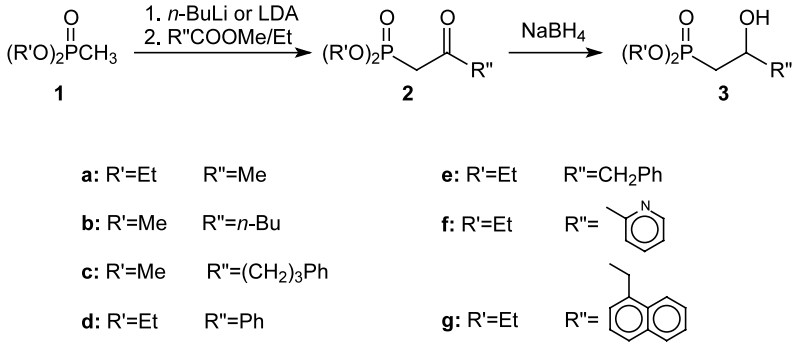
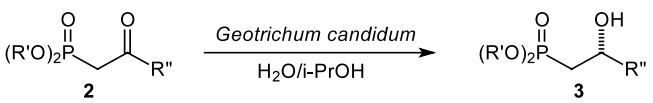
PII: S0957-4166(01)00567-5



Scheme 1.

ketones[10,11] and is available in two different forms, IFO 4597 and IFO 5767. However, our experiments proved that only one substrate, **2a** (R′ = Et, R″ = Me), underwent the desired reduction to give diethyl 2-hydroxypropanephosphonate **3a** in a reasonable yield and with good to high e.e. Other substrates, bearing more sterically demanding substituents either gave the reduction products in very low yields or did not undergo reaction at all. The results are collected in Eq. (1) and Table 1.



(1)

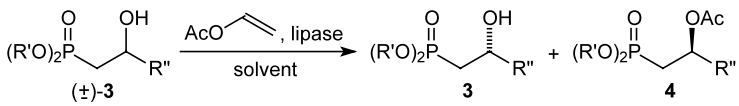
Table 1. Reduction of 2-oxoalkanephosphonates with *Geotrichum candidum* IFO 4597 and 5767

Substrate	Microorganism	2-Hydroxyalkanephosphonate **3**		
		Yield[a] (%)	$[\alpha]_D$ (MeOH)	E.e.[b] (%)
2a	IFO 4597	78 (72)	+7.2	98
2a	IFO 5767	70 (65)	+6.4	86
2b	IFO 4597	5	Product not isolated	
2b	IFO 5767	10	Product not isolated	
2d	IFO 4597	6	Product not isolated	
2d	IFO 5767	0	–	–
2e	IFO 4597	3	Product not isolated	
2e	IFO 5767	0	–	–

[a] Yield determined by ^{31}P NMR; in parentheses: yield of pure isolated products.
[b] Determined on the basis of diastereomer composition of the Mosher esters-vide infra.

2.3. Kinetic resolution of racemic 3

Racemic 2-hydroxyalkanephosphonates **3a–g** were acetylated using vinyl acetate in the presence of a series of lipases (Eq. (2)). The reaction was carried out under kinetic resolution conditions, i.e. it was usually stopped at ca. 50% conversion, which was determined by ^{31}P NMR. The unreacted substrate **3** was then separated from the acetylated product **4** by column chromatography. Some of the optically active hydroxyalkanephosphonates **3** were chemically transformed into the corresponding acetates **4** for analytical purposes.



(2)

Of the seven substrates investigated, three, namely **3d**, **3e** and **3g**, turned out to be unreactive under the conditions applied; the reaction was stopped after 72 h, at which time no or minimal conversions (up to 5%) were observed. In the remaining cases the products were obtained in good yields but with varying e.e. (Table 2). The highest enantioselectivities were obtained with the substrates in which the sizes of the substituents at the stereogenic carbon atom were most differentiated. This is generally in accordance with empirical substrate models developed for lipase-promoted formation or hydrolysis of secondary alcohol esters, which indicate that the stereoselectivity of the enzymatic reaction depends mostly on the spatial requirements of the substituents.[12] Nevertheless, the general statement that 'the rule for secondary alcohols applies to all lipases tested so far'[12] seems doubtful in the light of the fact that in our hands different lipases showed different senses of stereoselectivity with respect to the same substrate (Table 2, entry 6 versus 7–11, entry 12 versus 15–17). Moreover, for **3a** whose absolute configuration is known to be (+)-(*R*),[2,6] the general model[12] predicts the opposite stereochemistry for the reaction than that observed by us. In our opinion, it is the heteroatom moiety whose specific, yet undefined, interactions with the active site of the enzyme may account for the inconsistency observed which, in the end, does not allow the above models to be used to predict the absolute configuration of the products.

2.4. Determination of the enantiomeric excess values and absolute configuration of the products

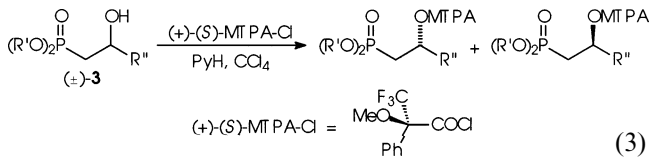
The enantiomeric excess values of optically active acetates **4** were determined by means of ^{1}H NMR

Table 2. Kinetic resolution of **3**

Entry	Substrate	Lipase	Solvent	Time (h)	Temp. (°C)	Recovered alcohol **3**			Acetate **4**			
						Yield (%)	$[\alpha]_D$ (MeOH)	E.e. (%)	Yield (%)	$[\alpha]_D$ (MeOH)	E.e. (%)	*E*
1.	**3a**	LPL	DIPE	24	30	47	+6.1	81	45	+7.2	93	55.2
2.	**3a**	AK	DIPE	48	30	44	+6.7	90	47	+7.2	93	95.1
3.	**3a**	PS	DIPE	194	30	45	+6.4	87	46	+7.1	89	49.2
4.	**3a**	LAH-S	DIPE	20	30	48	+5.7	76	42	+6.4	81	21.7
5.	**3a**	LAH-S	Hex	21	30	45	+6.2	83	45	+6.7	86	35.4
6.	**3b**	LPL	DIPE	72	30	40	+3.0*	25	51	−1.5	12	1.6
7.	**3b**	AK	DIPE	270	45	30	−11.6*	92	61	+5.9	49	8.0
8.	**3b**	AK	DIPE	245	45	41	−7.0*	56	42	+7.3	59	7.0
9.	**3b**	PS	DIPE	260	45	n	–	–	n	–	–	–
10.	**3b**	LAH-S	DIPE	72	30	45	−3.6*	28	48	+3.5	28	2.3
11.	**3b**	LAH-S	Hex	130	30	45	−5.3*	43	48	+4.6	37	3.3
12.	**3c**	LPL	DIPE	82	30	39	−2.1*	20	53	+1.3	13	1.5
13.	**3c**	AK	DIPE	144	45	n	–	–	n	–	–	–
14.	**3c**	PS	DIPE	240	45	n	–	–	n	–	–	–
15.	**3c**	LAH-S	DIPE	70	30	45	+5.0*	46	49	–4.6	44	4.0
16.	**3c**	LAH-S	DIPE	72	30	42	+5.1*	52	52	−4.5	41	3.9
17.	**3c**	LAH-S	Hex	100	30	41	+3.4	33	52	−2.1	19	1.9
18.	**3f**	LPL	DIPE	194	30	46	−18.8	62	45	+31.9	72	11.4
19.	**3f**	AK	DIPE	194	45	n	–	–	n	–	–	–
20.	**3f**	LA–HS	DIPE	194	30	n	–	–	n	–	–	–

Lipases: LPL: Lipoprotein Lipase from *Pseudomonas aeruginosa* (Toyobo Co); PS: Lipase from *Pseudomonas cepacia* (AMANO), crude preparation; AK: Lipase from *Pseudomonas fluorescens* (AMANO), crude preparation; LHS-S: Lipase AH-S (AMANO).
DIPE: diisopropyl ether, Hex: hexane.
n: Conversion <20%, product not isolated.
*: Specific rotation of the corresponding acetate.

spectra of their complexes with (+)-(*R*)-*t*-butylphenylphosphinothioic acid[13] in C_6D_6 which proved to be the reagent of choice from among a variety of chiral solvating agents (CSAs) examined. In these cases the e.e. values were calculated from the relative integrals of the acetyl methyl group signals. In turn, to determine the e.e. values of the recovered alcohols **3** it was necessary to convert them into the Mosher esters by the reaction with (+)-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) in pyridine (Eq. (3)). The e.e. values were routinely calculated from the integrals of the appropriate diastereomer signals observed in ^{31}P, ^{19}F (for the CF_3 group of MTPA) and ^1H NMR (for the methoxy group in MTPA). The results are collected in Table 3.



(3)

An attempt was made at the determination of the absolute configuration of the optically active products **3** and **4** on the basis of **3a**, whose absolute configuration is known to be (+)-(*R*).[2,6] To this end, we have taken into account the report by Noyori et al.[2] who found out that the ^{31}P NMR signals in (*S*)-**3a** and its structural analogs appear upfield in the (−)-(*R*)-MTPA esters relative to the (+)-(*S*)-MTPA ones. As we have used (+)-(*S*)-MTPA esters, it is the (*R*)-enantiomers of **3**

Table 3. Chemical shifts for diastereomeric Mosher esters

3	Sign of rotation of **3**	^{31}P NMR		^{19}F NMR		^1H NMR	
		Chemical shifts (δ)	$\Delta\delta$ (ppm)	Chemical shifts (δ)	$\Delta\delta$ (ppm)	Chemical shifts (δ)	$\Delta\delta$ (ppm)
a	(+)	25.42 <u>25.26</u>	0.16	−71.61 <u>−71.71</u>	0.10	3.48 <u>3.41</u>	0.07
b	(−)	28.40 <u>28.27</u>	0.13	−71.48 <u>−71.64</u>	0.16	3.52 <u>3.43</u>	0.09
c	(−)	28.19 <u>28.08</u>	0.11	−71.40 <u>−71.55</u>	0.15	3.50 <u>3.40</u>	0.10
f	(+)	25.44 <u>25.26</u>	0.18	−71.58 <u>−71.78</u>	0.20	3.61 <u>3.48</u>	0.13

The signals of the predominant diastereomers are underlined.

whose ^{31}P NMR signals should appear upfield, which is the case in **3a**. Moreover, the fact that the sense of magnetic nonequivalence in the ^{1}H and ^{19}F NMR spectra recorded by us is also the same within the series of the products investigated, may provide additional support for our considerations. Thus, if one assumes that within a series of structurally similar Mosher esters the diastereomers of the same absolute configuration at the carbinol moiety show the same sense of magnetic non-equivalence, i.e. that all the (*R*)-**3**-(*S*)-MTPA signals (the underlined values in Table 3) appear upfield relative to the (*S*)-**3**-(*S*)-MTPA signals, the absolute configurations of the particular alcohols should be as follows:

3a: (+)-(*R*)[2,6] **3b:** (−)-(*R*) **3c:** (−)-(*R*) **3f:** (+)-(*R*)

However, this tentative assignment needs further confirmation and must be treated with caution. As a matter of fact, only the substituents R″ in **3b** (R″ = *n*-Bu) and (to a lesser extent) in **3c** (R″ = $CH_2CH_2CH_2Ph$) may be considered as similar to that in **3a** (R″ = Me). The pyridyl moiety in **3f**, attached directly to the stereogenic carbon atom, may exert such an unpredictable influence on the NMR chemical shifts, that the above considerations may prove useless.

3. Conclusion

The methodologies described above have enabled us to obtain chiral non-racemic 2-hydroxyalkanephosphonates in good yields and with reasonable to very good enantiomeric excesses. They may be considered complementary to the methods described in the literature. Moreover, the lipase-promoted kinetic resolutions may serve as new examples for attempts to find a general model for a lipase active site (or for an ideal substrate), which could be applied to heteroorganic secondary alcohols. The models developed so far do not give an unequivocal explanation for the stereoselectivities observed in the cases described above.

4. Experimental

4.1. General

The enzymes were purchased from AMANO or Toyobo Co. NMR spectra were recorded on Bruker instruments at 200 or 500 MHz for ^{1}H, 81 or 121 MHz for ^{31}P, 50 MHz for ^{13}C and 188 MHz for ^{19}F with $CDCl_3$ or C_6D_6 as solvents. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. Column chromatography was carried out using Merck 60 silica gel. TLC was performed on Merck 60 F_{254} silica gel plates.

4.2. Synthesis of substrates 2

All the substrates were prepared via the acylation reaction of the carbanions of methanephosphonates **1**, (which were generated using 2 equiv. of a base: BuLi or LDA) with the appropriate carboxylic acid esters. The reaction was performed in THF at −78°C (carbanion generation and addition of an ester), then allowed to reach room temperature at which it was quenched with aqueous solution of ammonium chloride. After extraction with $CHCl_3$ the products were purified by distillation or column chromatography using hexane–acetone 4:1. The phosphonates **2a**, **2d** and **2f** were characterized earlier.[6]

4.3. Dimethyl 2-oxohexanephosphonate 2b

Obtained from dimethyl methanephosphonate and methyl pentanoate as a colorless liquid after distillation (bp 100–114°C/0.6 mmHg) in 93% yield. ^{31}P NMR ($CDCl_3$): δ 23.34. ^{1}H NMR ($CDCl_3$): δ 3.74 (d, J= 11.0, 6H), 3.04 (d, J=22.6, 2H), 2.57 (t, J=7.2, 2H), 1.65–1.45 (m, 2H), 1.41–1.15 (m, 2H), 0.86 (t J=7.1, 3H). ^{13}C NMR ($CDCl_3$): δ 201.19 (d, J=6.1), 52.27, 52.15, 43.06, 40.47 (d, J=127.8), 24.76, 21.34, 13.09.

4.4. Dimethyl 2-oxo-5-phenylpentanephosphonate 2c

Obtained from dimethyl methanephosphonate and methyl 4-phenylbutanoate as a colorless liquid after column chromatography in 94% yield. ^{31}P NMR ($CDCl_3$): δ 23.40. ^{1}H NMR ($CDCl_3$): δ 7.33–7.10 (m, 5H), 3.75 (d, J=11.4, 6H), 3.05 (d, J=22.8, 2H), 2.61 (t, J=7.4, 4H), 1.91 (quintet, J=7.4, 2H). ^{13}C NMR ($CDCl_3$): δ 201.26 (d, J=6.1), 141.14, 128.12, 128.05, 125.63, 52.72, 52.60, 42.90, 40.89 (d, J=128.2), 34.41, 24.53.

4.5. Diethyl 2-oxo-2-phenylethanephosphonate 2d

Obtained from diethyl methanephosphonate and methyl benzoate as a colorless liquid after distillation (bp 148–150°C/0.5 mmHg) in 80% yield. ^{31}P NMR ($CDCl_3$): δ 20.41. ^{1}H NMR ($CDCl_3$): δ 8.03–7.97 (m, 1H), 7.63–7.42 (m, 4H), 4.13 (dq, J=7.0, J=7.0, 4H), 3.62 (d, J-22.8, 2H), 1.27 (t, J=6.9, 6H). ^{13}C NMR ($CDCl_3$): δ 191.68 (d, J=6.6), 136.30 (d, J=2.2), 133.40, 128.78, 128.35, 65.43, 62.30, 38.23 (d, J=129.8), 16.06, 15.93.

4.6. Diethyl 2-oxo-3-phenylpropanephosphonate 2e

Obtained from diethyl methanephosphonate and methyl phenylacetate as a colorless liquid after distillation (bp 110–120°C/0.4 mmHg) in 80% yield. ^{31}P NMR ($CDCl_3$): δ 20.30. ^{1}H NMR ($CDCl_3$): δ 7.38–7.12 (m, 5H), 4.16 (dq, J=7.1, 4H), 3.09 (d, J=22.8, 2H), 1.33 (t, J=6.8, 6H). ^{13}C NMR ($CDCl_3$): δ 198.76 (d, J= 6.1), 132.96, 128.97, 128.00, 126.47, 61.98, 61.85, 49.92, 40.61 (d, J=127.5), 15.66, 15.54.

4.7. Diethyl 2-oxo-2-(2′-pyridyl)ethanephosphonate 2f

Obtained from diethyl methanephosphonate and methyl picolinate as a colorless liquid after distillation (bp 152–156°C/1 mmHg) in 82% yield. ^{31}P NMR (C_6D_6): δ 20.98. ^{1}H NMR (C_6D_6): δ 8.28 (d, J=4.6, 1H), 7.92 (d, J=7.8, 1H), 7.02–6.90 (m, 1H), 6.63–6.59 (m, 1H), 4.64 (d, J=22.6, 2H), 4.00 (dq, J=7.3, J=7.3,

4H), 1.01 (t, $J=7.2$, 6H). ^{13}C NMR (C_6D_6): δ 193.75 (d, $J=7.8$), 153.15 (d, $J=2.1$), 148.97, 136.97, 127.36, 122.05, 62.11, 62.00, 36.16 (d, $J=127.8$), 16.32, 16.20.

4.8. Diethyl 2-oxo-3-(1-naphthyl)propanephosphonate 2g

Obtained from diethyl methanephosphonate and methyl 1-naphthylacetate as a colorless liquid after distillation (bp 115–120°C/0.01 mmHg) in 73% yield. ^{31}P NMR ($CDCl_3$): δ 20.28. ^{1}H NMR ($CDCl_3$): δ 8.02–7.72 (m, 3H), 7.56–7.35 (m, 4H), 4.35 (s, 2H), 4.15 (quintet, $J=7.0$, 4H), 3.09 (d, $J=22.8$, 2H), 1.33 (t, $J=7.0$, 6H). ^{13}C NMR ($CDCl_3$): δ 191.56 (d, $J=6.1$), 133.70, 132.08, 130.06, 128.57, 128.36, 128.11, 126.34, 125.74, 125.40, 123.79, 62.60, 62.47, 48.77, 40.66 (d, $J=127.28$), 16.19, 16.07.

4.9. Chemical reduction of 2; synthesis of racemic 2-hydroxyalkanephosphonates 3–general procedure

A solution of **2** in methanol or ethanol (depending on the substituent at phosphorus) was treated with an equimolar amount of sodium brohydride at 0°C. The unreacted $NaBH_4$ was decomposed with acetone and the crude product was purified by column chromatography using hexane-acetone as eluent to afford the desired 2-hydroxyalkanephosphonate **3** in 90–95% yield.

4.10. Diethyl 2-hydroxypropanephosphonate 3a

^{31}P NMR ($CDCl_3$): δ 30.61. ^{1}H NMR ($CDCl_3$): δ 4.25–4.01 (m, 4H), 3.51 (s, 1H), 2.03–1.82 (m, 2H), 1.32 (t, $J=7.1$, 6H), 1.26 (dd, $J=6.2$, $J=2.4$, 3H). ^{13}C NMR ($CDCl_3$): δ 62.61 (d, $J=4.9$), 61.62 (d, $J=6.3$), 61.55 (d, $J=6.5$), 35.03 (d, $J=137.4$), 24.13 (d, $J=15.9$), 16.24, 16.13.

4.11. Dimethyl 2-hydroxyhexanephosphonate 3b

^{31}P NMR ($CDCl_3$): δ 33.90. ^{1}H NMR ($CDCl_3$): δ 4.10–3.85 (m, 1H), 3.73 (d, $J=10.8$, 3H), 3.72 (d, $J=11.0$, 3H), 3.38 (d, $J=3.2$, 1H), 2.16–1.74 (m, 2H), 1.60–1.15 (m, 6H), 0.86 (t, $J=6.9$, 3H). ^{13}C NMR ($CDCl_3$): δ 65.99 (d, $J=5.1$), 52.10 (d, $J=6.7$), 51.97 (d, $J=7.2$), 37.73 (d, $J=15.4$), 32.37 (d, $J=137.9$), 27.25, 22.19, 13.65. MS (CI): m/z 211 (M^++1, 100). Anal. calcd for $C_8H_{19}O_4P$: C, 45.71; H, 9.11; O, 30.44; P, 14.73. Found: C, 45.82; H, 9.10; P, 14.60%.

4.12. Dimethyl 2-hydroxy-5-phenylpentanephosphonate 3c

^{31}P NMR ($CDCl_3$): δ 33.54. ^{1}H NMR ($CDCl_3$): δ 7.32–7.10 (m, 5H), 4.12–3.90 (m, 1H), 3.74 (d, $J=11.0$, 3H), 3.71 (d, $J=10.8$, 3H), 3.50–3.30 (br. s, 1H), 2.62 (t, $J=7.0$, 2H), 2.05–1.40 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 141.83, 127.99, 127.86, 125.31, 65.71 (d, $J=4.9$), 52.01 (d, $J=6.5$), 51.84 (d, $J=6.8$), 37.47 (d, $J=15.2$), 35.24, 32.32 (d, $J=137.8$), 26.85. HRMS (CI): m/z 273.1253 ($C_{13}H_{21}O_4P$+H requires 273.1256). Anal. calcd for $C_{13}H_{21}O_4P$: C, 57.35; H, 7.77; O, 23.50; P, 11.38. Found: C, 57.30; H, 7.85; P, 11.45%.

4.13. Diethyl 2-hydroxy-2-phenylethanephosphonate 3d

^{31}P NMR ($CDCl_3$): δ 29.61. ^{1}H NMR ($CDCl_3$): δ 7.43–7.23 (m, 5H), 5.19–5.03 (m, 1H), 4.24–3.98 (m, 4H), 3.88 (s, 1H), 2.27–2.14 (m, 2H), 1.35 (t, $J=7.0$, 3H), 1.29 (t, $J=7.1$, 3H). ^{13}C NMR ($CDCl_3$): δ 143.60 (d, $J=14.9$), 128.28, 127.47, 125.43, 68.60 (d, $J=4.3$), 61.83 (d, $J=7.3$), 61.68 (d, $J=7.4$), 35.80 (d, $J=136.4$), 16.25 (d, $J=2.7$), 16.13 (d, $J=2.9$). HRMS (CI): m/z 258.1020 ($C_{12}H_{19}O_4P$ requires 258.1021).

4.14. Diethyl 2-hydroxy-3-phenylpropanephosphonate 3e

^{31}P NMR ($CDCl_3$): δ 30.80. ^{1}H NMR ($CDCl_3$): δ 7.33–7.12 (m, 5H), 4.31–3.93 (m, 5H), 3.57 (br. s, 1H), 2.95–2.70 (m, 2H), 2.05–1.75 (m, 2H), 1.27 (t, $J=7.1$, 6H). ^{13}C NMR ($CDCl_3$): δ 137.57, 129.28, 128.24, 126.32, 67.41 (d, $J=4.9$), 61.66 (d, $J=6.2$), 61.61 (d, $J=6.5$), 44.26 (d, $J=16.2$), 32.53 (d, $J=138.6$), 16.22, 16.10.

4.15. Diethyl 2-hydroxy-2-(2′-pyridyl)ethanephosphonate 3f

^{31}P NMR (C_6D_6): δ 38.81. ^{1}H NMR (C_6D_6): δ 8.41 (d, $J=4.8$, 1H), 7.75 (d, $J=8.0$, 1H), 7.32–7.17 (m, 1H), 6.75–6.62 (m, 1H), 6.21–6.04 (m, 1H), 5.66–5.47 (m, 1H), 4.03–3.75 (m, 4H), 2.90–2.69 (m, 1H), 2.43–2.13 (m, 1H), 1.02 (t, $J=7.1$, 3H), 1.00 (t, $J=7.1$, 3H). ^{13}C NMR (C_6D_6): δ 164.06 (d, $J=16.2$), 148.67, 136.51, 122.00, 120.32, 69.64 (d, $J=4.6$), 61.83 (d, $J=6.1$), 61.45 (d, $J=6.4$), 34.68 (d, $J=138.2$), 16.40, 16.27. MS (CI): m/z 260 (M^++1, 100). Anal. calcd for $C_{11}H_{18}NO_4P$: C, 50.96; H, 7.00; N, 5.40; O, 24.69; P, 11.95. Found: C, 50.90; H, 7.21; P, 11.83%.

4.16. Diethyl 2-hydroxy-3-(1′-naphthyl)propanephosphonate 3g

^{31}P NMR ($CDCl_3$): δ 30.61. ^{1}H NMR ($CDCl_3$): δ 8.11–8.04 (m, 1H), 7.90–7.73 (m, 2H), 7.58–7.12 (m, 4H), 4.52–4.31 (m, 1H), 4.15–3.92 (m, 4H), 3.47–3.20 (m, 3H), 2.00 (dd, $J=17.2$, $J=6.0$, 2H), 1.28 (t, $J=7.6$, 3H), 1.24 (t, $J=7.5$, 3H). ^{13}C NMR ($CDCl_3$): δ 133.80, 132.02, 128.86, 128.61, 128.05, 127.62, 127.32, 125.90, 125.46, 125.42, 123.73, 66.86 (d, $J=4.4$), 61.75, 61.63, 41.44 (d, $J=15.5$), 33.01 (d, $J=138.5$), 16.23, 16.11. HRMS (CI): m/z 323.1412 ($C_{17}H_{23}O_4P$+H requires 323.1412)

4.17. Dimethyl 2-acetoxypropanephosphonate 4a

^{31}P NMR ($CDCl_3$): δ 27.09. ^{1}H NMR ($CDCl_3$): δ 5.25–5.03 (m, 1H), 4.07 (quintet, $J=7.3$, 4H), 2.32–1.84 (m, 2H), 2.00 (s, 3H), 1.33 (d, $J=5.4$, 3H), 1.29 (t, $J=7.2$, 6H). ^{13}C NMR ($CDCl_3$): δ 169.90, 66.00, 61.64, 61.54, 32.53 (d, $J=140.1$), 21.04, 20.88, 16.27, 16.14. MS (CI): m/z 239 (M^++1, 100). Anal. calcd for $C_9H_{19}O_5P$: C, 45.38; H, 8.04; O, 33.58; P, 13.00. Found: C, 45.21; H, 8.16; P, 12.81%.

4.18. Dimethyl 2-acetoxyhexanephosphonate 4b

^{31}P NMR ($CDCl_3$): δ 30.08. ^{1}H NMR ($CDCl_3$): δ 5.11 (double quintet, $J=12.2$, $J=6.1$, 1H), 3.72 (d, $J=11.0$, 6H), 2.22–1.92 (d, 2H), 2.04 (s, 3H), 1.73–1.58 (m, 2H), 1.40–1.18 (m, 4H), 0.87 (t, $J=6.6$, 3H). ^{13}C NMR ($CDCl_3$): δ 170.10, 68.73 (d, $J=2.3$), 52.27 (d, $J=6.0$), 52.15 (d, $J=6.3$), 34.53 (d, $J=8.8$), 29.76 (d, $J=140.7$), 27.27, 22.14, 20.90, 13.71. MS (CI): m/z 253 (M^++1, 100). Anal. calcd for $C_{10}H_{21}O_5P$: C, 47.62; H, 8.39; O, 31.71; P, 12.28. Found: C, 47.51; H, 8.30; P, 12.40%.

4.19. Dimethyl 2-acetoxy-5-phenylpentanephosphonate 4c

^{31}P NMR ($CDCl_3$): δ 30.20. ^{1}H NMR ($CDCl_3$): δ 7.30–7.07 (m, 5H), 5.22–5.04 (m, 1H), 3.69 (d, $J=11.0$, 3H), 3.68 (d, $J=11.0$, 3H), 2.66–2.53 (m, 2H), 2.22–1.88 (m, 2H), 2.01 (s, 3H), 1.78–1.50 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 170.15, 141.74, 128.27, 128.22, 125.74, 68.59 (d, $J=2.1$), 52.35 (d, $J=6.4$), 52.22 (d, $J=6.5$), 35.29, 34.42 (d, $J=8.6$), 29.85 (d, $J=140.4$), 26.82, 20.97. MS (CI): m/z 315 (M^++1, 100). Anal. calcd for $C_{15}H_{23}O_5P$: C, 57.32; H, 7.38; O, 25.45; P, 9.85. Found: C, 57.38; H, 7.31; P, 10.01%.

4.20. Diethyl 2-acetoxy-2-(2′-pirydyl)ethanephosphonate 4f

^{31}P NMR (C_6D_6): δ 26.75. ^{1}H NMR (C_6D_6): δ 8.41–8.37 (m, 1H), 7.24–6.98 (m, 1H), 6.66–6.50 (m, 2H), 4.00–3.73 (m, 4H), 2.93–2.54 (m, 2H), 1.81 (s, 3H), 1.01 (t, $J=7.0$, 3H), 0.97 (t, $J=7.0$, 3H), 0.99 (dt, $J=7.0$, $J=7.0$, 6H). ^{13}C NMR (C_6D_6): δ 169.48, 158.85, 149.49, 136.29, 122.82, 122.07, 71.50 (d, $J=2.2$), 61.48 (d, $J=6.7$), 61.35 (d, $J=6.7$), 31.36 (d, $J=141.2$), 20.72, 16.38 (d, $J=5.9$), 16.31 (d, $J=2.1$). MS (CI): m/z 302 (M^++1, 100). Anal. calcd for $C_{13}H_{20}NO_5P$: C, 51.83; H, 6.69; N, 4.65; O, 26.55; P, 10.28. Found: C, 51.91; H, 6.77; P, 10.35%.

4.21. *Geotrichum candidum* reduction of 2-general procedure

(a) Reduction with *Geotrichum candidum* IFO 4597: A mixture of the substrate (50 mg), microorganism (5 g) and isopropanol (0.25 g) was shaken in water (50 mL) at 30°C for 4 days; the reaction mixture was extracted with diethyl ether (5×50 mL) and the crude product was purified by column chromatography;
(b) Reduction with *Geotrichum candidum* IFO 5767: A mixture of substrate (50 mg), the microorganism (2.5 g) and *iso*-propanol (0.3 g) was shaken in water (20 mL) at 30°C for 4 days; the reaction mixture was extracted with diethyl ether (5×50 mL) and the crude product was purified by column chromatography using acetone/hexane 1:4 as eluent.

4.22. Kinetic resolution of 3-general procedure

A mixture of the phosphonate (100 mg), an appropriate amount of an enzyme (vide infra), vinyl acetate (0.5 mL) and 4 D molecular sieve (50 mg) was stirred in the solvent (8 mL). The reaction was monitored by ^{31}P NMR to be stopped at ca. 50% conversion. The precipitates were then filtered off and the products were separated by column chromatography using acetone–hexane (in gradient 1:4 to 1:2) as eluent.

Substrate/enzyme ratio:

LPL:	for **3a**	1:0.8
	for others	1:1
AK:	for all substrates	1:2.5
PS:	for all substrates	1:2.5
LAH-S:	for **3a**	1:0.18
	for others	1:1

Acknowledgements

We would like to thank the State Committee for Scientific Research (KBN, PL) and the Ministry of Education, Science, Sports and Culture (Mombusho, Japan) for a support of the bilateral project entitled 'Application of Enzymes and Microorganisms in the Synthesis of Chiral Carbon and Heteroatom Compounds'. Financial support by the State Committee of Scientific Research (KBN), Grant No 3T09A 085 18 for P.K. and R.Ż. is gratefully acknowledged.

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