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Enzymatic resolution of α-hydroxyphosphinates with two stereogenic centres and determination of absolute configuration of stereoisomers obtained

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ABSTRACT

The use of quinine as a chiral solvating agent allows us to determine a tentative absolute configuration at the phosphorus atom of hydroxyphosphinates with two stereogenic centres (at the phosphorus and α-carbon atoms). Two ethyl butyryloxyalkane(*P*-phenyl)phosphinates were hydrolysed using various lipases. In all cases isomers possessing α-carbon atom with an (*S*)-configuration were hydrolysed preferentially. The absolute configuration of both chiral centres of obtained α-hydroxyphosphinates was determined by using (*S*)-(+)-MTPA-Cl and quinine. The mode of chiral discrimination of α-hydroxyphosphinates by quinine was studied by means of computational chemistry, which confirmed the experimental findings that the signals in ^{31}P NMR spectra of compounds with an (R_P)-configuration are situated upfield when compared with the respective (S_P) isomers.

1. Introduction

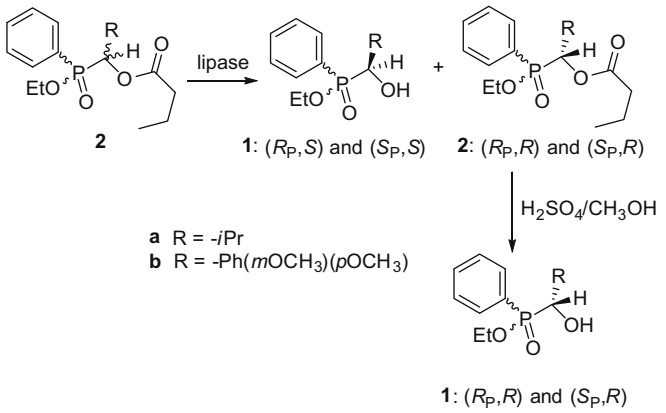
Chiral hydroxyphosphonates are an interesting group of compounds because of their well-recognised biological activity. For example, some of these compounds act as inhibitors of chosen enzymes and exhibit interesting antibacterial, antiviral or anticancer activities.[1] In some cases, their activity depends not only on the configuration of the stereogenic centre at the carbon atom but also on the spatial structure of the phosphorus atom.[2] The simple and effective methods for the determination of the absolute configuration of both centres are still challenging and need to be developed. So far, the application of quinine, a well-acknowledged chiral discriminator,[3] seems to be the method of choice when considering the determination of the enantiomeric excess of hydroxyphosphonates. Thus, quinine has been widely applied as a chiral solvating agent to determine the absolute configuration at the α- or β-carbon atoms of hydroxyphosphonates with one stereogenic centre.[4]

Herein we report preliminary studies on the use of quinine for the determination of the absolute configuration at the phosphorus atom of 1-hydroxy-2-methylpropane(*P*-phenyl)phosphinate **1a** and 1-hydroxy-3,4-methoxyphenylmethane(*P*-phenyl)phosphinate **1b**, compounds possessing two stereogenic centres, one located at the α-carbon atom and the other at the phosphorus atom.

2. Results and discussion

2.1. Enzymatic hydrolysis

Racemic hydroxyphosphinates **1a** and **1b**, obtained by the addition of ethyl phenylphosphinite to the appropriate aldehyde,[5] were converted into butyryloxyphosphinates (compounds **2**) by simple acylation with butyryl chloride. Esters **2** were then hydrolysed using various lipases (Scheme 1).



Scheme 1. Hydrolysis of butyryloxyphosphinates **2** by lipases.

The hydrolysis was stopped when the chemical yield reached a value close to 50% (which is typical for kinetic resolution) or before

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the seventh day of reaction. Hydrolysis of 1-butyryloxy-2-methylpropane(*P*-phenyl)phosphinate **2a** with lipase from porcine pancreas gave the pair of diastereoisomers (S_P,*S*) and (R_P,*S*) of compound **1a** as a major product with excellent enantiomeric excess >98% (Table 1). In the case of compound **1b**, the best results were obtained when *Aspergillus niger* lipase was applied. As can be seen from Table 1, the reaction was stereoselective in most cases; isomers bearing an α-carbon atom of (*S*)-configuration were hydrolysed preferentially, unfortunately with a lack of the stereoselectivity towards the phosphorus atom.

2.2. Determination of the absolute configuration at the α-carbon atom

After the purification of compounds **2**, we unsuccessfully tried to resolve the diastereoisomers using column chromatography via the application of various eluents. Only when the silica gel column and a mixture of methylene chloride/*n*-hexane/ethyl acetate (1:1:0.5 v/v) as eluent were used was some enrichment of isomers obtained. These mixtures were hydrolysed using *Candida cylindracea* lipase (compound **2a**; time of hydrolysis—168 h) and *A. niger* lipase (compound **2b**; time of hydrolysis—48 h) leading to a mixture of compounds in molar ratios of (R_P,*S*):(S_P,*R*):(R_P,*R*):(S_P,*S*) = 2.36:0.44:0.39:0.99 (compound **1a**) and (R_P,*S*):(S_P,*R*):(R_P,*R*):(S_P,*S*) = 1.20:0.48:0.57:1.10 (compound **1b**) as determined by ^{31}P NMR using quinine as a chiral discriminator (Fig. 1). The remaining, non-hydrolysed esters **2** were then chemically hydrolysed (Scheme 1) and purified to yield hydroxyphosphinates **1a** and **1b** with molar ratios of (R_P,*S*):(S_P,*R*):(R_P,*R*):(S_P,*S*) = 0.18:2.45:1.00:0.35 in the case of compound **1a** and (R_P,*S*):(S_P,*R*):(R_P,*R*):(S_P,*S*) = 1.00:3.35:2.30:0.85 for compound **1b**. These mixtures of isomers, after purification, were acylated using (*S*)-(+)-MTPA-Cl. After the reactions were complete, the mixtures of four isomers of (*R*)-MTPA esters **3** were obtained and the absolute configuration of their α-carbon atom additionally analysed by the method proposed by Mosher.[6]

The ^{1}H NMR chemical shifts of the phosphinate ester methyl groups of **3a** and **3b** (CH_3–CH_2–O–P) were assigned as follows: **3a** (S_P,*S*,*R*)—1.29 ppm; (R_P,*R*,*R*)—1.248 ppm; (R_P,*S*,*R*)—1.27 ppm; (S_P,*R*,*R*)—1.245 ppm; **3b** (S_P,*S*,*R*)—1.34 ppm; (R_P,*R*,*R*)—1.31 ppm; (R_P,*S*,*R*)—1.29 ppm and (S_P,*R*,*R*) – 1.27 ppm. The signals derived from the (R_P,*R*,*R*)—and (S_P,*R*,*R*)-isomers of compound **3a** are very close to each other; for this reason we described the chemical shifts with an accuracy to the third place. The signals resulting from a methyl group of (*S*)-configuration are downfield if compared with the (*R*)-isomers (difference of about 0.02–0.04 ppm). The ^{1}H NMR chemical shifts of methyl groups ((CH_3)$_2$CH–CH–P) of compound **3a** were assigned as follows: (CH_3)′ moiety of isomers (S_P,*S*,*R*)—1.00 ppm; (R_P,*R*,*R*)—1.05 ppm; (R_P,*S*,*R*)—0.892 ppm; (S_P,*R*,*R*)—1.04 ppm, whereas for (CH_3)″ moiety of isomers (S_P,*S*,*R*)—0.75 ppm; (R_P,*R*,*R*)—0.85 ppm; (R_P,*S*,*R*)—0.889 ppm and (S_P,*S*,*R*)—0.99 ppm. The ^{1}H NMR chemical shifts of the methoxy groups ((CH_3–O)$_2$–Ph–CH–P) of compound **3b** were assigned as follows: for the (CH_3–O)′ moiety of isomers (S_P,*S*,*R*)—3.854 ppm; (R_P,*R*,*R*)—3.87 ppm; (R_P,*S*,*R*)—3.847 ppm and (S_P,*R*,*R*)—3.854 ppm., whereas for (CH_3–O)″ moiety of isomers (S_P,*S*,*R*)—3.52 ppm; (R_P,*R*,*R*)—3.69 ppm; (R_P,*S*,*R*)—3.53 ppm and (S_P,*R*,*R*)—3.57 ppm.

As can be seen from these data, the signals of the α-hydroxyphosphinate R groups with an (*S*)-configuration are situated upfield when compared with the respective (*R*)-isomers (difference of about 0.05–0.15 ppm for compound **3a** and of 0.007–0.17 ppm for compound **3b**). This derives from the anisotropic effect exerted by the phenyl group (Fig. 2) and thus conforms directly from the Mosher model.

2.3. Determination of the absolute configuration at the phosphorus atom

Spectroscopic data for the two stereoisomers of compound **1a** (Fig. 3) have been described in the literature.[7] The authors therein defined the pair of isomers as (R_P,*R*)- and (S_P,*S*)-, but our studies indicate that this configurational assignment was not correct. We showed this in the case of 1-hydroxyethyl(*P*-phenyl)phosphinate **1c**[8] where the correct configuration of the discussed isomers has to be set as (S_P,*R*) and (R_P,*S*). Moreover, Shioji used (*R*)-MTPA-Cl,[7] which resulted in a change of configuration during derivatisation to an (*S*)-MTPA-ester. Since our spectra for (S_P,*R*)- and (R_P,*S*)-isomers (Fig. 3) are identical with those described by Shioji, this additionally supports our assignment.

Comparison of the ^{31}P NMR spectra of 1-hydroxy-2-methylpropane(*P*-phenyl)phosphinate **1a** obtained after enzymatic hydrolysis, recorded in the presence of quinine (Fig. 1—spectrum A) with the spectra of two other compounds described previously, namely, 1-hydroxyethyl(*P*-phenyl)phosphinate **1c**[9,10] and 1-hydroxy-1-phenylmethane(*P*-phenyl)phosphinate **1d**[8] (Figs. 4 and 5) indicates that the signals of enantiomers with (R_P) configuration are situated upfield versus those of (S_P) configuration. This finding allowed to determine the absolute configuration at the phosphorus atom of compound **1b** (Fig. 1).

In conclusion, the use of a Mosher reagent enables us to establish configuration of the α-carbon atom in the hydroxyphosphonates. The knowledge of this configuration and the use of quinine enable us to determine, tentatively, the configuration at the phosphorus atom in α-hydroxyphosphinates.

2.4. Determination of the absolute configuration at the phosphorus atom using a theoretical method

The theoretical investigation of the change of the chemical shift in ^{31}P NMR spectra as a result of complexation of hydroxyphosphinates by quinine was carried out using **1c** as a model compound by means of DFT methods. Compound **1c** (as well as all hydroxyphosphinates **1a**–**d**) appears as a mixture of four isomers because of the presence of two stereogenic centres, on phosphorus and α–carbon atoms. Additionally all these isomers may have different conformations (synclinals and antiperiplanar). The synclinal conformation is stabilised by the strong intramolecular hydrogen bonds between the hydroxyl group and the P=O group (O–H···O=P) whereas the antiperiplanar conformation forms the

Table 1
Hydrolysis of butyryloxyphosphinates **2** by lipases via Scheme 1.

Lipase	Substrate	Time (h)	Conversion of hydrolysis (%)		Enantiomeric excess of hydroxyphosphinates (%)	
			(R_P,*R*); (S_P,*S*)	(S_P,*R*); (R_P,*S*)	(S_P,*S*)	(R_P,*S*)
Candida cylindracea lipase	**2a**	166	39	53	>98	62
	2b	168	44	41	42	52
Aspergillus niger lipase	**2a**	166	21	41	>98	90
	2b	22	46	36	54	82
Lipase from porcine pancreas	**2a**	166	32	52	>98	>98
	2b	168	25	9	82	<5

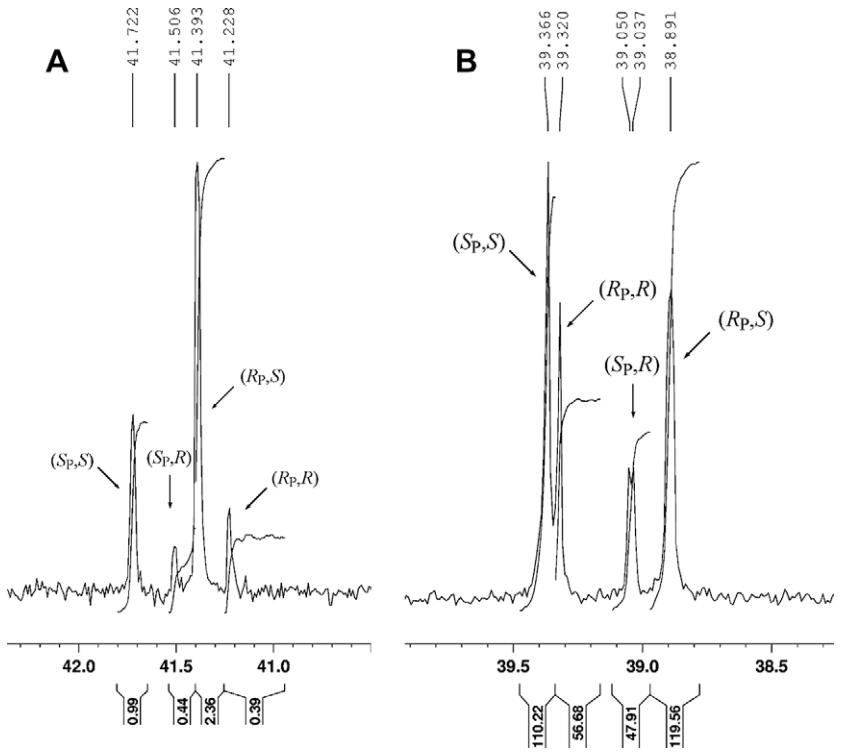

Figure 1. ^{31}P NMR spectra of mixtures of stereoisomers of hydroxyphosphinates **1a** (spectrum A; *Candida cylindracea* lipase—168 h) and **1b** (spectrum B; *Aspergillus niger* lipase—48 h) resolved with quinine.

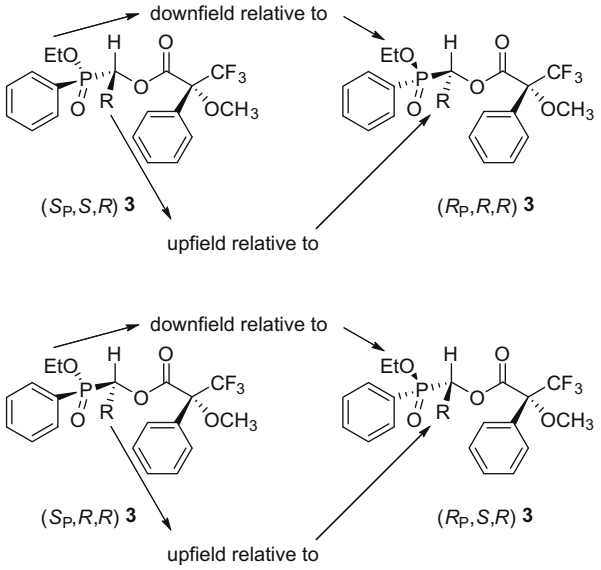

Figure 2. Determination of absolute configuration on the α-carbon atom.

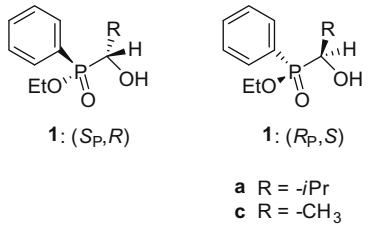

Figure 3. Configuration of the two isomers of ethyl 1-hydroxy-2-methylpropane(*P*-phenyl)phosphinate **1a** and of 1-hydroxyethyl(*P*-phenyl)phosphinate **1c** as obtained by Shioji.[7]

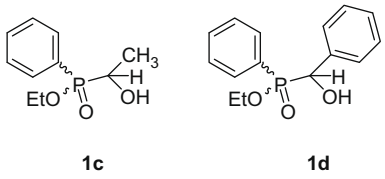

Figure 4. Structures of the two ethyl hydroxyalkane(*P*-phenyl)phosphinates **1** described previously.[8–10]

O–H···O–P hydrogen bond. The difference in energy (ΔG) between synclinal and antiperiplanar conformations amount to 0.2–0.4 kcal/mol with synclinal conformations being dominant in solution.

Quinine is a good chiral solvating agent for hydroxyphosphonates and hydroxyphosphites because it forms diastereoizomeric complexes and causes the possibility to distinguish these isomers by NMR technology. Previous theoretical studies on the structure of the complexes of hydroxyphosphonates with quinine[11] showed that the most probable complexes in solution are formed between quinine molecule and hydroxyphosphonate of a synclinal conformation.[12] The mode of interaction between quinine molecule and one of the **1c** isomers calculated in this work is showed in Figure 6.

Quinine, when interacting with **1c**, forms two strong hydrogen bonds of the type of (QUININE)–O–H···O=P and (**1c**)O–H···O(QUININE)–H. The energy of interactions in this complex is in the range of 10–13 kcal/mol with the base superposition (BSSE) taken into consideration. The investigation of complexes formed with different synclinal conformations of **1c** showed that the energy of these conformations is similar and that quinine forms complexes with all

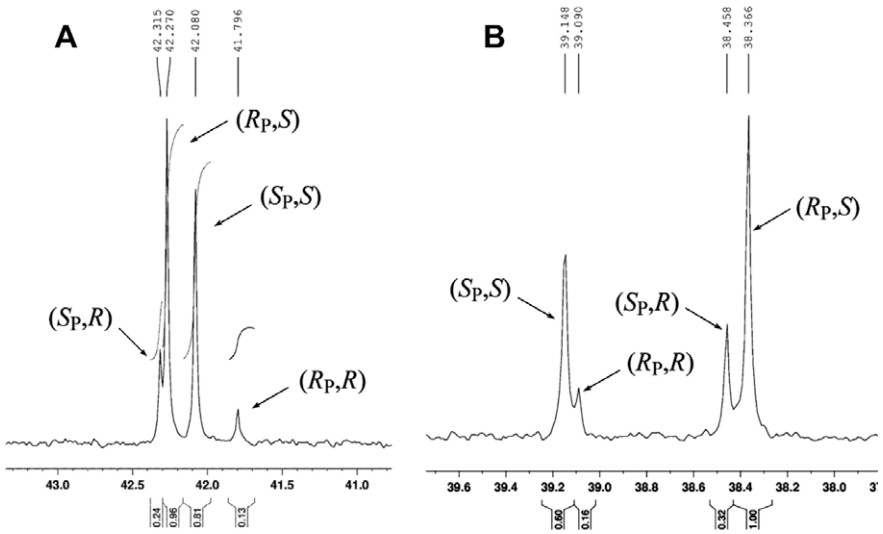

Figure 5. ^{31}P NMR spectra of hydroxyphosphinates **1c** (spectrum A; *Rhizopus* sp. Lipase—24 h) and **1d** (spectrum B; *Candida cylindracea* lipase—46 h) in the presence of quinine.

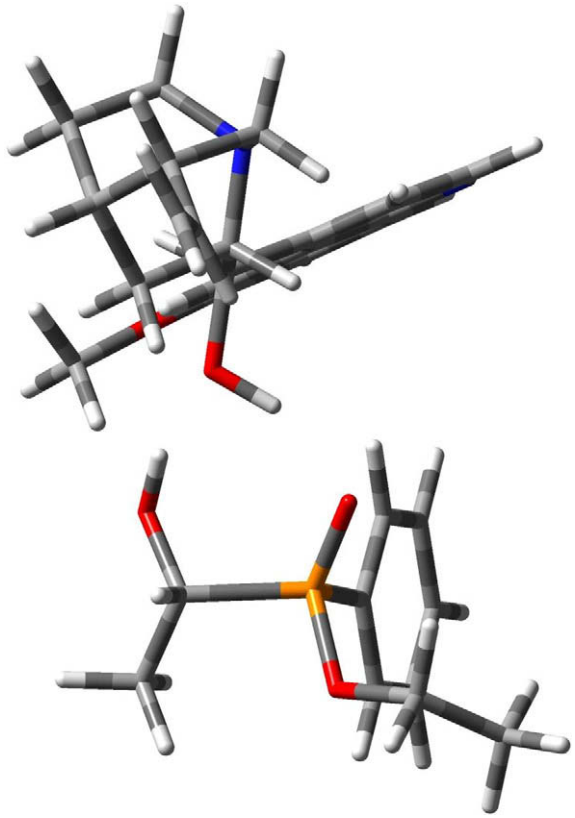

Figure 6. The structures of complex ethyl 1-hydroxyethyl(*P*-phenyl)phosphinate with quinine (+*sc*R_P*R*:Quinine). B3LYP/6-31G(*d*,*p*).

the conformers forming species being in equilibrium in solution. The calculated Boltzmann selection for +*sc*, −*sc* conformations of **1c** complexed with quinine is as follows: S_PR (98.0, 2.0%); R_PS (88.6, 11.4%); S_PS (69.3, 30.7%); R_PR (17.6, 82.4%).

The observed chemical shifts for each atom represent the average conformation of the compound in solution. One method for the precise determination of the chemical shifts is the analysis based on the representative population of conformers.[13] In the calculations of the NMR parameters for three conformers of compound **1c**, +*sc*, −*sc*, *ap* were taken into consideration for each enantiomer. The measured difference between the chemical shifts for the phosphorus atom of diastereoisomers (S_PR,R_PS) and (R_PR,S_PS) for **1c** is equal to 0.66 ppm. The calculated difference when taking into consideration the Boltzmann distribution for the three conformers is 1.59 ppm. Calculations were performed for the population of conformers +*sc*, −*sc*, *ap* for enantiomer (S_PR) being 46.8%, 32.7% and 20.5%, respectively, and 51.0%, 17.5% and 31.5% for the (S_PS)-isomer. The calculated chemical shifts for the phosphorus atom of the (S_PR)-isomer have higher values than those for (S_PS)-isomer which is in agreement with the experimental assignment.

The chemical shift in the ^{31}P NMR spectra of solutions with quinine is the average of three different conformers bonded with a quinine molecule or three non-bonded. When quinine is used in excess it was observed that the calculated chemical shifts of each isomer are as follows: 42.32 ppm for (S_PR); 42.27 ppm for (R_PS); 42.08 ppm for (S_PS) and 41.80 ppm for (R_PR). Calculated chemical shifts obtained from theoretical experiments of + and − synclinal conformers **1c** are as follows: 43.25 ppm for (S_PR); 42.16 ppm for (R_PS); 42.02 ppm for (S_PS) and 41.71 ppm for (R_PR). The results of our theoretical calculation are similar to the results obtained experimentally and the signals from the isomers follow the same order. The high difference between chemical shifts of different conformers of the same complex (e.g., +*sc*R_PR:Quinine 41.10 ppm, −*sc*R_PR:Quinine 44.55 ppm) can induce errors in interpretation when only theoretical experiments are taken into consideration. The theoretical experiments can only be used when they are performed for more conformers with the appropriate calculated method.

The theoretical studies confirmed the experimental determination of the absolute configuration of phosphorus atom in the hydroxyphospinates and the experimental assignment of the signals in ^{31}P NMR for certain isomers. They additionally showed the mode of interaction of the quinine with the hydroxyphosphinate molecules.

3. Experimental

3.1. General

All materials were purchased from commercial suppliers: Sigma, Aldrich, Fluka, POCh and Serva, and were used without

purification. The sources of lipases were *C. cylindracea* (Sigma), *A. niger* (Fluka) and porcine pancreas (Sigma). NMR spectra were measured on a Bruker Avance™ 600 at 600.58 MHz for ^{1}H; 243.12 MHz for ^{31}P and 151.02 MHz for ^{13}C in $CDCl_3$ or on a Bruker Avance DRX 300 instrument operating at 300.13 MHz for ^{1}H and 121.50 MHz for ^{31}P in $CDCl_3$. Chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. ^{1}H NMR are referenced to internal standard TMS ($\delta = 0.00$), the centre line of $CHCl_3$ of the ^{13}C NMR spectra ($\delta = 77.23$) and 85% phosphoric acid in H_2O for ^{31}P NMR spectra were used as an external reference. All compounds were purified by gradient column chromatography using Merck Silica Gel 60 (63–230 mesh) or by HPLC (Varian, Dynamax HPLC Column 250 × 21.4 mm; MICROSORB 300-10 C18).

3.2. Synthesis of ethyl hydroxyalkane-(*P*-phenyl)phosphinates 1

Compounds **1a** and **1b** were synthesised according to the Texier–Boullet method: Aluminium oxide (5 g) was mixed with 5 g of potassium fluoride and powdered in a grinder. Then 20 mmol of ethyl phenylphosphinate and 20 mmol of the required aldehyde were added into this mixture and left at room temperature for 48 h. After this time the mixture was eluted by dichloromethane. Compounds **1a** and **1b** were purified by a gradient column chromatography on silica gel using dichloromethane/ethyl acetate (5:3 v/v) as eluent.

3.2.1. Ethyl 1-hydroxy-2-methylpropane(*P*-phenyl)phosphinate 1a

$R_f = 0.30$, molar ratio 1:1, yield 42%. Mixture of (R_P,*R*) and (S_P,*S*) isomers: ^{31}P NMR δ (ppm): 40.11; ^{1}H NMR: δ (ppm): 1.00 (d, $J = 6.6$ Hz, 3H, CHCHCH_3), 1.01 (d, $J = 6.7$ Hz, 3H, CHCHCH_3), 1.32 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 3.69–3.77 (m, 1H, C*H*P), 3.89–3.95 (m, 1H, PCHC*H*), 4.10–4.19 (m, 2H, OCH_2), 7.45–7.50 (m, 2H, Ph), 7.55–7.58 (m, 1H, Ph), 7.80–7.86 (m, 2H, Ph); ^{13}C NMR δ (ppm): 16.70 (d, $J = 5.3$ Hz, CHCHCH_3), 18.15 (d, $J = 7.6$ Hz, CHCHCH_3), 20.32 (d, $J = 7.8$ Hz, OCH_2CH_3), 29.77 (d, $J = 2.9$ Hz, PCHCH), 61.33 (d, $J = 7.4$ Hz, OCH_2), 75.29 (d, $J = 113.4$ Hz, *C*HP), 128.67, 128.75, 129.51 (d, $J = 118.7$ Hz) 132.63 (2C), 132.57 (Ph).

Mixture of (R_P,*S*)- and (S_P,*R*)-isomers: ^{31}P NMR δ (ppm): 40.03; ^{1}H NMR: δ (ppm): 1.01 (d, $J = 6.7$ Hz, 6H, CHCHCH_3), 1.06 (d, $J = 6.8$ Hz, 6H, CHCHCH_3), 1.34 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 3.69–3.77 (m, 1H, C*H*P), 3.95–4.02 (m, 1H, PCHC*H*), 4.10–4.19 (m, 2H, OCH_2), 7.45–7.50 (m, 2H, Ph), 7.55–7.58 (m, 1H, Ph), 7.80–7.86 (m, 2H, Ph); ^{13}C NMR δ (ppm): 16.74 (d, $J = 5.3$ Hz, CHCHCH_3), 17.47 (d, $J = 5.9$ Hz, CHCHCH$_3$), 20.45 (d, $J = 10.1$ Hz, OCH_2CH_3), 29.59 (d, $J = 4.0$ Hz, PCHCH), 61.46 (d, $J = 7.3$ Hz, OCH_2), 75.40 (d, $J = 109.9$ Hz, *C*HP), 128.72, 128.80, 129.62 (d, $J = 119.0$ Hz) 132.41, 132.47, 132.73 (d, $J = 2.7$ Hz) (Ph).

3.2.2. Ethyl 1-hydroxy-1-(3,4-methoxyphenyl)methane(*P*-phenyl)phosphinate 1b

$R_f = 0.17$, molar ratio 1:1, yield 38%. Mixture of (R_P,*R*) and (S_P,*S*) isomers: ^{31}P NMR δ (ppm): 38.98; ^{1}H NMR: δ (ppm): 1.32 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 3.64 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.94–4.18 (m, 2H, OCH_2), 5.12 (d, $J = 9.8$ Hz, 1H, C*H*P), 6.73–6.82 (m, 3H, Ph), 7.32–7.35 (m, 2H, Ph), 7.44–7.49 (m, 3H, Ph); ^{13}C NMR δ (ppm): 16.43 (CH_2CH_3), 55.67 (OCH_3), 56.04 (OCH_3), 61.99 (OCH_2), 73.09 (d, $J = 113.1$, *C*HP), 110.35 (d, $J = 4.2$ Hz), 110.65 (d, $J = 1.9$ Hz), 119.79 (d, $J = 6.3$ Hz), 127.29 (d, $J = 122.3$ Hz), 128.16, 128.34, 128.66, 132.68 (d, $J = 2.1$ Hz), 132.88, 133.13, 148.61 (d, $J = 2.7$ Hz, *C*OCH$_3$), 148.71 (d, $J = 2.9$ Hz, *C*OCH$_3$) (Ph).

Mixture of (R_P,*S*)- and (S_P,*R*)-isomers: ^{31}P NMR δ (ppm): 37.53; ^{1}H NMR: δ (ppm): 1.29 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 3.68 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.94–4.18 (m, 2H, OCH_2), 5.05 (d, $J = 7.3$ Hz, 1H, C*H*P), 6.73–6.82 (m, 3H, Ph), 7.38–7.42 (m, 2H, Ph), 7.52–7.55 (m, 1H, Ph), 7.63–7.66 (m, 2H, Ph); ^{13}C NMR δ (ppm): 16.39 (CH_2CH_3), 55.79 (OCH_3), 56.04 (OCH_3), 61.94 (OCH_2), 73.43 (d, $J = 112.4$, *C*HP), 110.56 (d, $J = 4.2$ Hz), 110.76 (d, $J = 1.7$ Hz), 120.17 (d, $J = 6.3$ Hz), 128.06 (d, $J = 110.1$ Hz), 128.24, 128.42, 129.00, 132.62 (d, $J = 2.1$ Hz), 132.94, 133.19, 148.71 (d, $J = 2.9$ Hz, *C*OCH$_3$), 148.94 (d, $J = 2.5$ Hz, *C*OCH$_3$) (Ph).

3.3. Synthesis of ethyl butyryloxyalkane(*P*-phenyl)phosphinates 2

Compounds **1a** and **1b** were converted to **2a** and **2b** with butyryl chloride by the following procedure: Compound **1** (10 mmol) was added to 100 ml of the reaction media containing chloroform and triethylamine (10:1 v/v), followed by addition of 11 mmol of butyryl chloride. The resulting solution was stirred for 24 h at room temperature. After this time, the mixture was washed successively by 100 ml of 5% hydrochloric acid, 100 ml distilled water and dried over anhydrous magnesium sulfate. The product was purified by means of silica gel column chromatography using dichloromethane/ethyl acetate (5:3 v/v) as eluent.

3.3.1. Ethyl 1-butyryloxy-2-methylpropane(*P*-phenyl)phosphinate 2a

$R_f = 0.68$, molar ratio 1:1, yield 54%. Mixture of (R_P,*R*)- and (S_P,*S*)-isomers: ^{31}P NMR δ (ppm): 37.24; ^{1}H NMR: δ (ppm): 0.81 (t, $J = 7.4$ Hz, 3H $CH_2CH_2CH_3$), 0.93 (d, $J = 6.7$, 3H, CHCH_3), 1.10 (d, $J = 6.8$, 3H, CHCH_3), 1.29 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.56–1.62 (m, 2H, $CH_2CH_2CH_3$), 2.03–2.32 (m, 3H, $CH_2CH_2CH_3$, *C*HCH$_3$), 3.91–3.98 (m, 2H, OCH_2), 5.28 (dd, $J = 7.2$, 7.2 Hz, 1H, C*H*P), 7.45–7.49 (m, 2H, Ph), 7.54–7.57 (m, 1H, Ph), 7.78–7.82 (m, 2H, Ph); ^{13}C NMR δ (ppm): 13.71 ($CH_2CH_2CH_3$), 16.61 (d, $J = 6.5$ Hz, CHCH_3), 18.38 (OCH_2CH_3), 18.79 (d, $J = 8.2$ Hz, CHCH_3), 20.03 (d, $J = 6.1$ Hz, $CH(CH_3)_2$), 28.99 ($CH_2CH_2CH_3$), 35.92 ($CH_2CH_2CH_3$), 61.34 (d, $J = 6.3$ Hz, OCH_2), 74.11 (d, $J = 121.3$ Hz, *C*HP), 128.54, 128.73, 129.83 (d, $J = 77.7$ Hz), 132.52, 132.66 (Ph), 133.83 (d, $J = 2.9$ Hz, Ph), 172.67 (d, $J = 3.9$ Hz, *C*O).

Mixture of (R_P,*S*)- and (S_P,*R*)-isomers: ^{31}P NMR δ (ppm): 36.72; ^{1}H NMR: δ (ppm): 0.90 (t, $J = 7.4$ Hz, 3H $CH_2CH_2CH_3$), 0.99 (d, $J = 6.8$, 3H, CHCH_3), 1.01 (d, $J = 6.7$, 3H, CHCH_3), 1.33 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.43–1.48 (m, 2H, $CH_2CH_2CH_3$), 2.03–2.32 (m, 3H, $CH_2CH_2CH_3$, *C*HCH$_3$), 4.08–4.16 (m, 2H, OCH_2), 5.18 (dd, $J = 2.4$, 6.4 Hz, 1H, C*H*P), 7.45–7.49 (m, 2H, Ph), 7.54–7.57 (m, 1H, Ph), 7.78–7.82 (m, 2H, Ph); ^{13}C NMR δ (ppm): 13.83 ($CH_2CH_2CH_3$), 16.70 (d, $J = 5.8$ Hz, CHCH_3), 18.49 (OCH_2CH_3), 18.59 (d, $J = 6.8$ Hz, CHCH_3), 20.27 (d, $J = 7.7$ Hz, $CH(CH_3)_2$), 28.83 ($CH_2CH_2CH_3$), 36.08 ($CH_2CH_2CH_3$), 61.30 (d, $J = 5.9$ Hz, OCH_2), 74.63 (d, $J = 117.3$ Hz, *C*HP), 128.62, 128.81, 129.00 (d, $J = 80.1$ Hz), 132.45, 132.60 (Ph), 132.75 (d, $J = 2.2$ Hz, Ph), 172.51 (d, $J = 4.5$ Hz, *C*O).

3.3.2. Ethyl 1-butyryloxy-1-(3,4-methoxyphenyl)methane(*P*-phenyl)phosphinate 2b

$R_f = 0.47$, molar ratio 1:1, yield 69%. Mixture of (R_P,*R*) and (S_P,*S*) isomers: ^{31}P NMR δ (ppm): 34.38; ^{1}H NMR: δ (ppm): 0.90 (t, $J = 7.4$ Hz, 3H $CH_2CH_2CH_3$), 1.32 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.59–1.64 (m, 2H, $CH_2CH_2CH_3$), 2.35 (td, $J = 2.0$, 7.2 Hz, 2H, C(O)CH_2CH$_2$CH$_3$), 3.76 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.01–4.14 (m, 2H, OCH_2), 6.16 (d, $J = 7.9$ Hz, 1H, C*H*P), 6.74–6.99 (m, 3H, Ph), 7.40–7.74 (m, 5H, Ph); ^{13}C NMR δ (ppm): 13.70 ($CH_2CH_2CH_3$), 16.73 (d, $J = 5.6$ Hz, OCH_2CH_3), 18.46 ($CH_2CH_2CH_3$), 36.20 (C(O)CH_2CH$_2$CH$_3$), 55.91 (OCH_3), 56.00 (OCH_3), 62.04 (d, $J = 7.1$ Hz, OCH_2), 72.81 (d, $J = 118.9$ Hz, *C*HP), 111.04 (d, $J = 1.8$ Hz), 111.29 (d, $J = 4.3$ Hz), 120.86 (d, $J = 5.7$ Hz), 125.78, 128.43, 128.52, 128.47 (d, $J = 129.8$ Hz), 132.66, 132.72, 132.85 (d, $J = 3.2$ Hz), 148.87 (d, $J = 2.1$ Hz, *C*OCH$_3$), 149.40 (d, $J = 2.9$ Hz, *C*OCH$_3$) (Ph) 172.09 (d, $J = 7.4$ Hz, *C*O).

Mixture of (R_P,*S*)- and (S_P,*R*)-isomers: ^{31}P NMR δ (ppm): 33.83; ^{1}H NMR: δ (ppm): 0.84 (t, $J = 7.4$ Hz, 3H $CH_2CH_2CH_3$), 1.27 (t,

J = 7.1 Hz, 3H, CH_2CH_3), 1.52–1.58 (m, 2H, $CH_2CH_2CH_3$), 2.28 (td, J = 2.9, 7.4 Hz, 2H, $C(O)CH_2CH_2CH_3$), 3.77 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.01–4.14 (m, 2H, OCH_2), 6.26 (d, J = 10.3 Hz, 1H, CHP), 6.74–6.99 (m, 3H, Ph), 7.40–7.74 (m, 5H, Ph); ^{13}C NMR δ (ppm): 13.61 ($CH_2CH_2CH_3$), 16.60 (d, J = 5.9 Hz, OCH_2CH_3), 18.49 ($CH_2CH_2CH_3$), 36.22 ($C(O)CH_2CH_2CH_3$), 55.93 (OCH_3), 56.00 (OCH_3), 61.88 (d, J = 6.6 Hz, OCH_2), 72.15 (d, J = 121.0 Hz, CHP), 111.08, 111.40 (d, J = 4.4 Hz), 121.11 (d, J = 5.7 Hz), 126.93, 128.43, 128.51, 128.62 (d, J = 128.9 Hz), 132.72, 132.78, 132.83 (d, J = 3.1 Hz), 148.89 (d, J = 1.8 Hz, $COCH_3$), 149.51 (d, J = 2.8 Hz, $COCH_3$) (Ph), 171.95 (d, J = 7.6 Hz, CO).

3.4. Synthesis of Mosher esters 3

A mixture of stereoisomers of compound **1** was derivatised according to a literature method: At first, 0.10 mmol of **1** was dissolved in the mixture composed of dry dichloromethane (300 μl) and dry pyridine (300 μl), followed by the addition of 0.14 mmol of (*S*)-(+)MTPA-Cl. The mixture was left for 3 days at room temperature. After this time 3-dimethylamino-1-propylamine (0.20 mmol) was added into the mixture. After 5 min the mixture was diluted with diethyl ether (10 ml), washed with cold 5% hydrochloric acid (10 ml) and water (10 ml), and dried over anhydrous magnesium sulfate. After filtration of drying agent ether was evaporated and compound **3** was purified by means of HPLC (gradient: from 40% of acetonitrile in water to 100% of acetonitrile, death time 7.5 min).

3.4.1. Mosher ester 3a

Retention time 15.0 min.

(R_P,*R*,*R*): ^{31}P NMR δ (ppm): 34.80; ^{1}H NMR δ (ppm): 0.85 (d, J = 6.8 Hz, 3H, $CHCHCH_3$), 1.05 (d, J = 7.1 Hz, 3H, $CHCHCH_3$), 1.25 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.12–2.37 (m, 1H, PCHCH), 3.45 (s, 3H, OCH_3), 3.91–4.19 (m, 2H, OCH_2), 5.54 (dd, J = 5.2, 10.9 Hz, 1H, CHP), 7.34–7.82 (m, 10H, Ph).

(S_P,*R*,*R*): ^{31}P NMR δ (ppm): 34.36; ^{1}H NMR δ (ppm): 0.99 (d, J = 6.8 Hz, 3H, $CHCHCH_3$), 1.04 (d, J = 6.8 Hz, 3H, $CHCHCH_3$), 1.25 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.12–2.37 (m, 1H, PCHCH), 3.46 (s, 3H, OCH_3), 3.91–4.19 (m, 2H, OCH_2), 5.39 (dd, J = 3.5, 5.0 Hz, 1H, CHP), 7.34–7.82 (m, 10H, Ph).

(R_P,*S*,*R*): ^{31}P NMR δ (ppm): 34.31; ^{1}H NMR δ (ppm): 0.889 (d, J = 6.8 Hz, 3H, $CHCHCH_3$), 0.892 (d, J = 6.8 Hz, 3H, $CHCHCH_3$), 1.27 (t, J = 7.2 Hz, 3H, CH_2CH_3), 2.12–2.37 (m, 1H, PCHCH), 3.63 (s, 3H, OCH_3), 3.91–4.19 (m, 2H, OCH_2), 5.39 (dd, J = 3.5, 5.0 Hz, 1H, CHP), 7.34–7.82 (m, 10H, Ph).

(S_P,*S*,*R*): ^{31}P NMR δ (ppm): 34.73; ^{1}H NMR δ (ppm):0.73 (d, J = 5.6 Hz, 3H, $CHCHCH_3$), 1.00 (d, J = 8.6 Hz, 3H, $CHCHCH_3$), 1.29 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.12–2.37 (m, 1H, PCHCH), 3.42 (s, 3H, OCH_3), 3.91–4.19 (m, 2H, OCH_2), 5.56 (dd, J = 5.4, 10.8 Hz, 1H, CHP), 7.34–7.82 (m, 10H, Ph).

3.4.2. Mosher ester 3b

Retention time 13.9 min.

(R_P,*R*,*R*): ^{31}P NMR δ (ppm): 32.72; ^{1}H NMR δ (ppm): 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.42 (s, 3H, $CHOCH_3$), 3.69 (s, 3H, $PhOCH_3$), 3.87 (s, 3H, $PhOCH_3$), 3.97–4.18 (m, 2H, OCH_2), 6.33 (d, J = 8.7 Hz, 1H, CHP), 6.73–6.85 (m, 3H, Ph), 7.30–7.64 (m, 10H, Ph).

(S_P,*R*,*R*): ^{31}P NMR δ (ppm): 32.92; ^{1}H NMR δ (ppm): 1.29 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.38 (s, 3H, OCH_3), 3.57 (s, 3H, $PhOCH_3$), 3.855 (s, 3H, $PhOCH_3$), 3.97–4.18 (m, 2H, OCH_2), 6.45 (d, J = 13.1 Hz, 1H, CHP), 6.73–6.85 (m, 3H, Ph), 7.30–7.64 (m, 10H, Ph).

(R_P,*S*,*R*): ^{31}P NMR δ (ppm): 32.87; ^{1}H NMR δ (ppm): 1.31 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.44 (s, 3H, OCH_3), 3.53 (s, 3H, $PhOCH_3$), 3.848 (s, 3H, $PhOCH_3$), 3.97–4.18 (m, 2H, OCH_2), 6.35 (d, J = 12.1 Hz, 1H, CHP), 6.73–6.85 (m, 3H, Ph), 7.30–7.64 (m, 10H, Ph).

(S_P,*S*,*R*): ^{31}P NMR δ (ppm): 33.26; ^{1}H NMR δ (ppm): 1.33 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.44 (s, 3H, OCH_3), 3.52 (s, 3H, $PhOCH_3$), 3.855 (s, 3H, $PhOCH_3$), 3.97–4.18 (m, 2H, OCH_2), 6.31 (d, J = 10.0 Hz, 1H, CHP), 6.73–6.85 (m, 3H, Ph), 7.30–7.64 (m, 10H, Ph).

3.5. Enzymatic reactions—general procedures

Enzymatic hydrolysis of ethyl 1-butyryloxyphosphinate **2** was carried out in a biphasic system (3.8 ml) consisting of 0.05 M phosphate buffer, pH 7.0 (3.0 ml), and mixture of diisopropyl ether (0.2 ml) with *n*-hexane (0.6 ml). After the addition of 0.2 mmol of substrate and 100 mg of suitable lipase (see Table 1), the reaction was carried out at room temperature with shaking (150 rpm). The reaction was stopped after certain periods of time, and the product was extracted twice with 15 ml of ethyl acetate and organic phase was dried over anhydrous magnesium sulfate. After filtration, the organic solvent was removed by evaporation and the obtained hydroxyphosphinate was analysed with ^{31}P NMR using quinine as a chiral discriminator.

3.6. Computational details

The structures of 1-hydroxyethyl(*P*-phenyl)phosphite **1c** (Fig. 4) and quinine complexes were optimised using the density functional theory (DFT) with the B3LYP functional[14] and the standard 6-31G(*d*,*p*) basis of atomic orbitals.[15] All enantiomers of **1c** (S_PR, R_PS, S_PS, R_PR) were considered as conformers of three local minima, namely, those with sinclinal+ (*sc*+), sinclinal- (*sc*−) and antiperiplanar (*ap*) position of the phosphonic moiety. The **1c**:quinine complexes were optimised for + and − sinclinal conformers of **1c**. Vibrational frequencies for **1c** were calculated by applying the ideal gas, rigid rotor and harmonic oscillator approximations.[16] All the conformer energy minima of **1c** were confirmed by frequency calculations.

The NMR parameters were calculated using the coupled perturbed density functional theory (CP-DFT) method by including the diamagnetic spin–orbit, paramagnetic spin–orbit, Fermi-contact and spin-dipolar terms.[17] The calculations were performed within NMR dedicated IGLOII basis set for H, C, N and O atoms.[18] Application of this procedure has usually been found to lead to reasonable agreement with experimental data for chemical shifts of diasterotopic protons,[13] chemical shift effects of complexation alizarin in metal[19] and coupling constants across hydrogen bond.[20] The chemical phosphorus shifts were defined in relation to TPP (−17.8 ppm) as standard.

The computations were carried out using the GAUSSIAN 03 suite of codes[21] and results were visualised by applying the GAUSSVIEW[22] package.

Figures with optimised complex ethyl 1-hydroxyethyl(*P*-phenyl)-phosphinate:quinine and Cartesian coordinates for −scR_PR:Quinine and −scR_PR:Quinine are available free of charge via the Internet at http://www.molnet.eu/images/stories/Supp_Majewska_2009.pdf.

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