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Enzymes in organic chemistry. Part 9:[1] Chemo-enzymatic synthesis of phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine and L-α-aminobutyric acid of high enantiomeric excess

Friedrich Hammerschmidt * and Frank Wuggenig

Institut für Organische Chemie der Universität Wien, Währingerstraße 38, A-1090 Wien, Austria

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Abstract

Diisopropyl α-chloroacetoxyphosphonates derived from propanal, isobutanal, 3-methylmercaptopropanal, 3-methylbutanal and (*S*)-2-methylbutanal were resolved by enzyme-catalysed hydrolysis. Lipases preferentially hydrolysed the (*S*)-esters and protease Chirazyme® P-2 the (*R*)-esters. Replacing the isopropyl groups by *t*-butyl groups reduced the reaction rate; replacing them by a 2,2-dimethylpropane-1,3-diyl group increased the reaction rate. (*S*)-α-Hydroxyphosphonates (ee 92–99%), obtained with the protease except one, were transformed into phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine and L-α-aminobutyric acid. Their enantiomeric purity was determined by HPLC on a chiral stationary phase after derivatisation at nitrogen.

1. Introduction

Certain α-amino acids are important intermediates of metabolism and building blocks of biopolymers. The synthesis of phosphonic acid analogues of proteinogenic and non-proteinogenic amino acids — the phosphonic group is considered to be an isosteric replacement of the carboxyl group — has gained momentum during recent years. α-Aminophosphonic acids themselves and oligopeptides containing them display various interesting biological activities.[2] As the absolute configuration at the α-position of the substituted phosphonic acids influences their biological properties, a number of strategies have been developed for the preparation of chiral, non-racemic α-aminophosphonic acids.[3] The more prominent synthetic methods include chemical[3] and enzymatic resolution,[4] enantioselective addition[5] of phosphites to achiral imines, addition of phosphites to homochiral imines,[6] acyliminium ions[7] and nitrones,[8]

* Corresponding author. Fax: 0043-1-31367-2280; e-mail: frha@felix.orc.univie.ac.at

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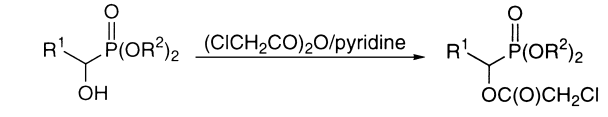
alkylation of α-lithiated homochiral enamines[9,10] derived from α-aminomethylphosphonic acid, and manipulation[11,12] of α-hydroxyphosphonates. The latter approach benefits from attractive methods available for the synthesis of chiral, non-racemic α-hydroxyphosphonates.[13]

We have pioneered lipase-catalysed kinetic resolution of α-acyloxyphosphonates to produce optically active α-hydroxyphosphonates. Five of them were transformed into the α-amino-[14] and α-aminooxyphosphonic[15] acids. Recently, we found that proteases mediate the preferential hydrolysis of (*R*)-α-chloroacetoxyphosphonates, which is in contrast to the enantioselectivity of the lipases tested before.[1] The enantiomeric excesses with Chirazyme® P-2, an alkaline endoprotease of serine type, for various substrates, notably with branched alkyl groups, were significantly better than those with lipase AP 6 (from *Aspergillus niger*).

These results prompted us to prepare phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine, and L-α-aminobutyric acid of high enantiomeric excesses chemoenzymatically. These α-aminophosphonic acids have nonpolar side chains and biological effects. Phosphonic acid analogues of methionine and leucine were used as C-termini of pentapeptides which were tested as enkephalin analogues for analgesic acitivity.[16] Phosphonic acid analogues of (±)-valine, (±)-α-aminobutyric acid and L- and D-leucine were studied as inhibitors of leucine aminopeptidase from porcine kidney and had K_i (μM) of 3.6, 1.2, 0.23 and 220, respectively.[17] Not surprisingly, L-phosphaleucine is about 1000 times as effective as D-phosphaleucine. When a series of five α-aminophosphonic acids was tested towards possible use as seed dressing agents, the α-aminopropylphosphonic acid was found to be the most promising.[18] Chiral, nonracemic phosphaisoleucine has not yet been prepared. Furthermore, the *t*-butyl and 2,2-dimethylpropane-1,3-diyl groups were studied as protecting groups at phosphorus.

2. Results and discussion

The α-chloroacetoxyphosphonates (±)-**2a–f** and (1*RS*,2*S*)-**2g** used as substrates for the enzyme-catalysed resolutions, were prepared by esterification of the corresponding α-hydroxyphosphonates with chloroacetic anhydride/pyridine (Scheme 1).[19]



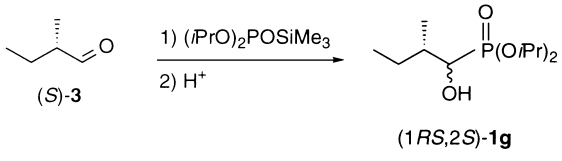
(±)-**1a-f**, (1*RS*,2*S*)-**1g** (±)-**2a-f**, (1*RS*,2*S*)-**2g**

1/2	R^1	R^2
a	Et	*i*Pr
b	*i*Pr	*i*Pr
c	*t*Bu	*i*Pr
d	$MeS(CH_2)_2$	*i*Pr
e	$MeS(CH_2)_2$	*t*Bu
f	*i*Pr	$Me_2C(CH_2)_2$
g	(2*S*)-*s*Bu	*i*Pr

Scheme 1. Chloroacetylation of α-hydroxyphosphonates

Racemic phosphonates (±)-**1a–c**,[20] **f**[21] and (±)-**2a–c**[21] are known compounds. α-Hydroxyphosphonates (±)-**1d**,[20] **e**[12] were synthesised by the Abramov reaction from 3-methylmercaptopropional and diisopropyl and di-*t*-butyl phosphite, respectively. A different approach

was selected for α-hydroxyphosphonate (1*RS*,2*S*)-**1g** (Scheme 2). Diisopropyl trimethylsilyl phosphite was added to base-labile, crude (*S*)-2-methylbutanal prepared by oxidation of (*S*)-2-methylbutanol with TEMPO/NaOCl.[22] The optically active, commercially available alcohol was assessed for its enantiomeric excess by ^{1}H NMR spectroscopy of its (*R*)-MTPA ester and that of the racemic alcohol. A sample from Aldrich had an ee of only 94%. The methylene hydrogens of the CH_2O group of the (*S*)-alcohol give a doublet (δ=4.15 ppm, *J*=5.9 Hz), and the corresponding hydrogens of the (*R*)-alcohol an AB system coupling with 2-H (δ=4.15 ppm, J_{AB}=10.8 Hz, *J*=5.9, 6.4 Hz). The sample obtained from Fluka did not contain a detectable amount of the (*R*)-alcohol (ee >99%) and was used for the preparation of (*S*)-2-methylbutanal. The intermediate α-silyloxyphosphonate was not isolated, but deprotected immediately to give α-hydroxyphosphonates (1*RS*,2*S*)-**1g** as a mixture of two diastereomers in equal amounts which could not be separated by flash chromatography or HPLC (signals were only partially resolved). The diastereomeric α-chloroacetoxyphosphonates (1*RS*,2*S*)-**2g** behaved similarly (one signal by HPLC). The diastereomeric ratios of samples of (1*RS*,2*S*)-**1g** and **2g** or mixtures thereof as obtained by enzymatic hydrolysis could be determined easily by ^{1}H NMR spectroscopy, as the signals of the hydrogens at C-1 were well separated. To detect a possible partial racemisation of (*S*)-2-methylbutanal, occurring during its preparation or reaction with phosphite, the mixture of diasteromeric α-hydroxyphosphonates (1*RS*,2*S*)-**1g** and its racemate, prepared from racemic 2-methylbutanol, were derivatised with (*S*)-MTPACl. The ^{31}P NMR spectrum of the Mosher ester of (1*RS*,2*S*)-**1g** shows two singlets, at 18.06 and 17.21 ppm, respectively. The signal at lower field was assigned to the Mosher ester of the α-hydroxyphosphonate with (*S*)-configuration at C-1 on the basis of precedence.[23] The ^{31}P NMR spectrum of the Mosher ester of all four stereoisomers of (1*RS*,2*RS*)-**1g** shows four singlets of equal intensity; the ones at 18.06 and 17.21 ppm, and two additional ones at 17.71 and 17.60 ppm, respectively. These are assigned to the Mosher esters of the α-hydroxyphosphonates with (*R*)-configuration at C-2 and (*S*) and (*R*) at C-1, respectively. On the basis of this NMR spectroscopic investigation, we can exclude a partial racemisation of (*S*)-2-methylbutanal. Consequently, the two diasteromeric α-hydroxyphosphonates (1*RS*,2*S*)-**1g** are enantiomerically pure (ee >99%).



Scheme 2. Preparation of α-hydroxyphosphonate (1*RS*,2*S*)-**1g**

In preliminary experiments to find the best enzymes for the different substrates, the hydrolyses of the chloroacetates were carried out on a 1 mmol scale in a biphasic system to a conversion of about 45%, using a protease and lipases. The results as obtained by methods reported previously are given in Table 1.[20] Lipase SP 524 (lipase from a selected fungus which was expressed in *Aspergillus oryzae* as a host organism, from Novo Nordisk) which was found to be best suited of all enzymes tested for the kinetic resolution of racemic diisopropyl α-acetoxyethylphosphonate, was also very effective for substrate (±)-**2a** (entry 1).[15] Merely 1.5 mg of enzyme were sufficient to give a conversion of 49% in 1 h. The α-hydroxyphosphonate formed had (*S*)-configuration and an ee of 96%. The protease Chirazyme® P-2 preferentially hydrolysed the (*R*)-enantiomer of α-chloroacetoxyphosphonate (±)-**2a** furnishing α-hydroxyphosphonate (−)-**1a** with an enantiomeric excess of 80% (entry 2). The results of the Chirazyme® P-2 catalysed resolution on an analytical scale of substrates (±)-**2b** and **c** have already been published in the preceding paper. α-Hydroxyphosphonates (*R*)-(−)-**1b** and **c** were obtained with enantiomeric excesses of 85 and 96%, respectively. α-Chloroacetoxyphosphonate (±)-**1d** needed to prepare the starting material for the synthesis of phosphamethionine was tested with lipase AP 6 (lipase

from *Aspergillus niger*) and protease Chirazyme® P-2 (entries 3 and 4). The α-hydroxyphosphonates isolated had virtually the same enantiomeric excesses (77 and 75%), but opposite absolute configurations. The hydrolysis of α-chloroacetoxyphosphonates (1*RS*,2*S*)-**2g** produced α-hydroxyphosphonate (1*R*,2*S*)-**1g** diastereoselectively (de 84%, entries 8 and 9). The additional stereogenic centre seems to influence only the reaction rate, but not the selectivity at C-1. The only difference between the two experiments is the absence of organic solvent during hydrolysis in entry 9, which caused a fourfold increase in reaction rate. We have not tested whether this effect is a general trend for other substrates as well.

In all the substrates studied so far, the protecting group R^2 at phosphorus was isopropyl which proved very successful for enantioselective hydrolysis, transformations at C-1 and removal in the past. To evaluate the influence of other protecting groups we have also used, in two cases, the more bulky *t*-butyl and the less bulky 2,2-dimethylpropane-1,3-diyl group for both R^2 over the isopropyl group. The reaction rate for di-*t*-butyl phosphonate (±)-**2e** was decreased relative to (±)-**2d** for Chirazyme® P-2 and lipase AP 6 by factors of roughly 10 and 20, respectively (entries 3 to 6). The ee was unchanged (75%) in the former case and decreased significantly (55 versus 75%) in the latter (entries 4 and 6). Furthermore, the substitution of the hydroxyl group for azide by the Mitsunobu reaction did not proceed as cleanly with **1e** as with diisopropyl phosphonate **1d** as will be shown later. These drawbacks do not outweigh the anticipated easier removal of the *t*-butyl over the isopropyl group. The use of this protecting group was, therefore, abandoned. Replacing the two isopropyl substituents of (±)-**2b** by a 2,2-dimethylpropane-1,3-diyl group gives (±)-**2f**. This chloroacetate was hydrolysed by Chirazyme® P-2 by a factor of about 30 times faster than diisopropyl chloroacetoxyphosphonate (±)-**2b** (entry 7).[20] Concomitantly, the selectivity changed from (*R*) to (*S*), assuming that the principles underlying the determination of the absolute configuration by NMR spectroscopy of the Mosher ester are not affected by the cyclic structure.[23] The shift difference in the ^{31}P NMR spectrum is only 0.044 ppm (δ 12.59/12.55) as compared to 0.15 ppm for the diastereomeric (*R*)-MTPA-esters of (±)-**2b**, which is already very low.[20] Chloroacetate (±)-**2f** was also hydrolysed by various lipases, but the enantioselectivity was low as judged from the consumption of base as function of time. Lipase AP 6 is a very efficient catalyst for the hydrolysis of different substrates, but did not seem to show any selectivity in this case. The extreme stability of the 2,2-dimethylpropane-1,3-diyl group towards removal (see later) in combination with its negative influence on the enantioselectivity did not justify more experiments.

Finally, α-chloroacetoxyphosphonates (±)-**2a**–**d** and (1*RS*,2*S*)-**2g** were resolved enzymatically on a preparative scale (4–6 mmol), using lipase SP 524 for (±)-**2a** and protease Chirazyme® P-2 for the others (entries 10–14). As we planned to prepare phosphonic acid analogues of L-amino acids, the starting α-hydroxyphosphonates had to have (*S*)-configuration. Because the enantiomeric excess of an enzymatic resolution depends on the conversion, the reactions were stopped at appropriate times. α-Hydroxyphosphonate (*S*)-(+)-**1a** obtained with lipase SP 524 at a conversion of 44% had an enantiomeric excess of 97%. The kinetic resolutions with protease Chirazyme® P-2 were stopped at conversions between 56 and 64% to get the (*S*)-esters of high enantiomeric excess, which were hydrolysed chemically by NEt_3 in dry MeOH to give the corresponding (*S*)-α-hydroxyphosphonates [ee 92–99% for **1b**–**d**, de 98% for (1*S*,2*S*)-**1g**]. The de of chloroacetoxyphosphonate (1*S*,2*S*)-**2g** as determined by ^{1}H NMR spectroscopy was 99.5%, the de of the corresponding α-hydroxyphosphonates (1*S*,2*S*)-**1g** was 97.8%. We attribute the very small decrease in diastereomeric excess to a small amount of racemisation, possibly during chemical ester cleavage with NEt_3 in methanol.

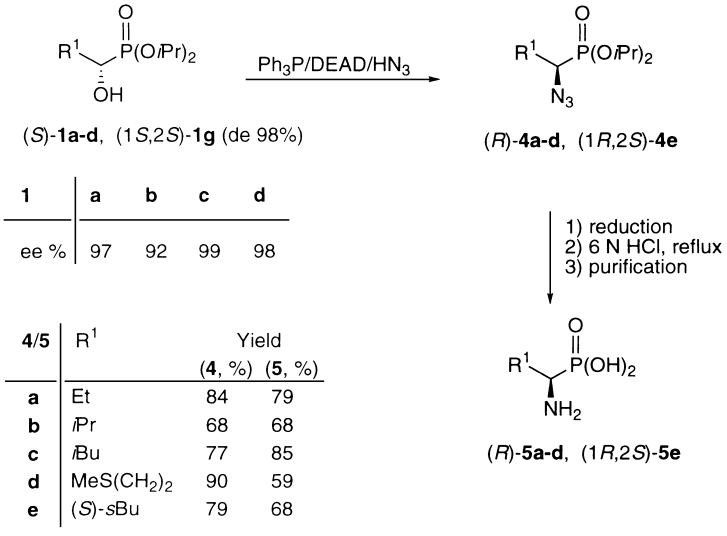
The transformation of α-hydroxyphosphonates (*S*)-**1a**–**d** and (1*S*,2*S*)-**1g** into the aminophosphonic acids was straightforward (Scheme 3). The hydroxyl group was replaced by azide with inversion of configuration using Ph_3P/diethyl azodicarboxylate (DEAD)/HN_3 (Mitsunobu reaction).[14] Azides **4** were reduced catalytically (Pd/C/H_2; 5 h) in the presence of hydrochloric acid,[14] except sulfur containing azide

Table 1. Enzymatic hydrolysis of (±)-**2a–f** and (1*RS*,2*S*)-**2g**

Entry	Substrate	Enzyme; amount[a]	Temp. (°C)	Time (h); Convsn[b] (%)	Produced alcohol **1** Yield (%)	ee[c] (%)	Conf	$[\alpha]_D$[d] (*c*)	Recovered ester **2** $[\alpha]_D$[d] (*c*)	Yield (%)	Alcohol from recovered ester Yield[e] (%)	ee[c] (%)	Conf	$[\alpha]_D$[d] (*c*)
1	**2a**	SP 524; 1.5 mg	27	1.0; 49/49	34	94 (96)	(*S*)	+16.2 (1.0)	-38.0 (1.5)	43				
2	**2a**	P-2; 15 µl	27	3.8; 45/45	33	80 (81)	(*R*)	-13.6 (1.8)	+26.4 (2.3)	51				
3	**2d**	P-2; 8 µl	22	5.0; 45/47	41	77 (77)	(*R*)	-33.2 (1.1)	+26.9 (1.1)	46				
4	**2d**	AP 6; 12.5 mg	25	2.9; 45/49	41	74 (75)	(*S*)	+31.8 (1.0)	-27.0 (1.1)	45	35	71 (70)	(*R*)	-30.9 (1.4)
5[f]	**2e**	P-2; 150 µl	22	2.7; 49/44	35	77 (75)	(*R*)	-30.7 (1.1)	+24.6 (0.9)	46	41	61 (62)	(*S*)	+23.7 (1.1)
6	**2e**	AP 6; 136 mg	24	6.2; 45/27	19	56 (55)	(*S*)	+21.2 (1.0)	-8.6 (1.1)	49				
7	**2f**	P-2; 16 mg	25	1.7; 45	40	60 (61)	(*S*)	+3.3 (1.1)	+1.9 (1.4)	36	33	43 (41)	(*R*)	-2.8 (1.0)
8	**2g**	P-2; 500 µl	21	64.5; 45/49	37	84 de[g]	(*R*)	-13.0 (1.0)	+14.3 (1.0)	43				
9[h]	**2g**	P-2; 500 µl	23	17.3; 45/47	43	84 de[g]	(*R*)	-13.1 (1.1)	+14.1 (1.0)	47	44	66 de[g]	(*S*)	+6.6 (1.0)
10[i]	**2a**	SP 524; 2 mg	23	2.3; 44/44	41	95 (97)	(*S*)	+16.5 (1.2)	-29.0 (1.9)	54				
11[i]	**2b**	P-2; 1000 µl	26	83.1; 60/54	49		(*R*)	-7.3 (1.0)	+27.8 (2.2)	42	40	90 (92)	(*S*)	+9.8 (1.0)
12[i]	**2c**	P-2; 500 µl	24	63.5; 56/53	44		(*R*)	-22.5 (1.1)	+38.5 (1.0)	41	39	98 (99)	(*S*)	+25.4 (1.0)
13[i]	**2d**	P-2; 50 µl	22	46.5; 64/68	54		(*R*)	-24.8 (1.0)	+38.3 (1.0)	28	27	98 (98)	(*S*)	+42.8 (1.0)
14[h,i]	**2g**	P-2; 1000 µl	22	111; 64/63	59		(*R*)	-10.9 (1.0)	+19.9 (1.5)	36	35	98 de[g]	(*S*)	+10.5 (1.2)

[a] For Chirazyme® P-2: lyophilisate or solution of it; [b] Convsn = conversion determined from 0.5 N NaOH consumed / conversion determined by ^{1}H NMR; [c] Ee as determined by ^{1}H NMR spectroscopy (by ^{31}P NMR spectroscopy); [d] In 2 ml of acetone solution at 20 °C; concentration was rounded to the nearest tenth; [e] Yield of ester after enzymatic hydrolysis multiplied by yield of chemical hydrolysis; [f] No 1 M HCl was added at the end of hydrolysis to bring pH to 4; [g] De determined by ^{1}H NMR of mixture after workup or of hydroxyphosphonate obtained by chemical hydrolysis; [h] Hydrolysis was carried out in absence of organic solvent; [i] Amount of substrate used: 6.37 mmol **2a**, 5.65 mmol **2b**, 3.83 mmol **2c**, 5.59 mmol **2d**, 5.50 mmol **2g**.

(*R*)-**4d**, which was reduced with triphenylphosphine (Staudinger reaction).[24] The crude reaction products were deprotected with refluxing 6 N HCl (5 h). Under these conditions the iminophosphorane derived from (*R*)-**4d** was split into amine and triphenylphosphine oxide as well. The α-aminophosphonic acid hydrochlorides, except the one of phosphamethionine, were purified by ion-exchange chromatography on Dowex 50, H^+ with water as eluent to give free aminophosphonic acids (*R*)-**5a–c** and (1*R*,2*S*)-**5e**. Crystalline phosphamethionine (*R*)-**5d** was obtained by treating a solution of the hydrochloride in ethanol with propylene oxide.[24]

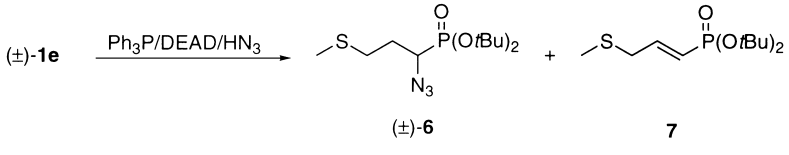


1	a	b	c	d
ee %	97	92	99	98

4/5	R^1	Yield (4, %)	Yield (5, %)
a	Et	84	79
b	*i*Pr	68	68
c	*t*Bu	77	85
d	$MeS(CH_2)_2$	90	59
e	(*S*)-*s*Bu	79	68

Scheme 3. Transformation of α-hydroxyphosphonates into α-aminophosphonic acids

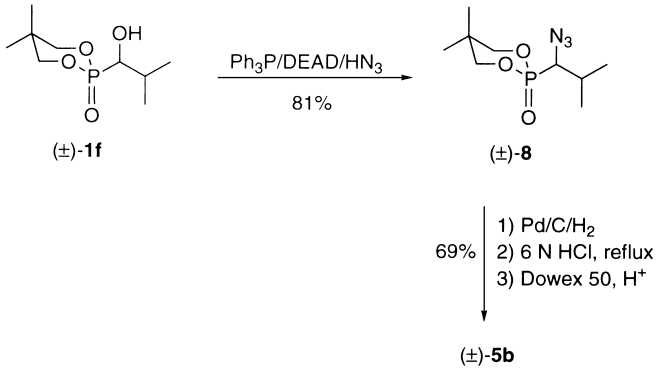
Additionally, two more α-hydroxyphosphonates with different protecting groups at phosphorus were subjected to the Mitsunobu reaction. The reaction rate of di-*t*-butyl phosphonate (±)-**1e** was very low (Scheme 4) and the unsaturated phosphonate **7** was formed as a side product[25] along with the desired azide (±)-**6**. The two bulky *t*-butyl groups shield C-1 against nucleophilic attack by azide and thus elimination leading to **7** becomes an alternative pathway.



Scheme 4. Mitsunobu reaction of α-hydroxyphosphonate (±)-**1e**

Hoping that the chloroacetate of (±)-**1f** could be resolved by hydrolases, a preliminary experiment was carried out with racemic material to determine the stability of the protecting group (Scheme 5). Thus, α-hydroxyphosphonate (±)-**1f** was smoothly transformed by the method of Gajda[12] into crystalline azide (±)-**8** which was reduced catalytically and then refluxed in 6 N HCl. Samples were withdrawn at intervals of 24 to 48 h, concentrated in vacuo and investigated as solutions in D_2O by ^1H NMR spectroscopy. The protecting group at phosphorus was extremely resistant towards hot hydrochloric acid and was removed only after 8 days, which disqualifies it for further application. Workup and purification afforded racemic phosphavaline (±)-**5b** in 69% yield.

The substitution of the hydroxyl group by azide in the Mitsunobu reaction is an S_N2 process and the ee (de) of the α-aminophosphonic acids (*R*)-**4a–d** [(1*R*,2*S*)-**5e**] should be the same as that of the starting α-hydroxyphosphonates. Nevertheless, we wanted to prove it by an independent method, because



Scheme 5. Transformation of α-hydroxyphosphonate (±)-**1f** into α-aminophosphonic acid (±)-**5b**

in the case of the 1-aminopropylphosphonic acid (*R*)-**5a** the specific optical rotation of our product ($[\alpha]_{578}^{20}$=−17.8 (0.9, 1 N NaOH) was lower than that reported in the literature[10] ($[\alpha]_{578}^{20}$=−21.6, 1.0, 1 N NaOH, ee 98%). The α-aminophosphonic acids (*R*)-**5a**–**c** were derivatised at nitrogen and investigated by HPLC on a chiral stationary phase.[26] The enantiomeric excesses were found to be 98% for (*R*)-**5a** (ee of starting α-hydroxyphosphonate 97%), 92% for (*R*)-**5b** (92%), and 99% (*R*)-**5c** (99%), respectively. The transformation of chiral, nonracemic α-hydroxyphosphonates into α-aminophosphonic acids via azides prepared by the Mitsunobu reaction proceeds stereospecifically with inversion of configuration without neighbouring group participation of the P=O group.

3. Conclusions

We have demonstrated that racemic (and one diastereomeric mixture) diisopropyl α-chloroacetoxyphosphonates can be resolved on a preparative scale using protease Chirazyme® P-2 to furnish (*R*)-α-hydroxyphosphonates and (*S*)-α-chloroacetoxyphosphonates of high enantiomeric purity (diastereomeric purity). The latter are hydrolysed chemically and transformed into phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine and L-α-aminobutyric acid of the same ee as the starting material, as proven for three of them by HPLC on a chiral stationary phase.

4. Experimental

4.1. General

All starting materials and enzymes were obtained from commercial suppliers and were generally used without further purification. ^{1}H and ^{13}C NMR (*J* modulated) spectra were recorded in $CDCl_3$, unless otherwise given, using tetramethylsilane as internal standard on a Bruker AM 400 WB spectrometer 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. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in hertz. IR spectra were run on a Perkin–Elmer 1600 FT-IR spectrometer; liquid samples were measured as films between NaCl plates or on a silicon disc,[27] solids as Nujol mulls between NaCl plates. Optical rotations were measured at 20°C on a Perkin–Elmer 341 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 visualised by dipping the plate into a solution of 24 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1 g of $Ce(SO_4)_2\cdot 4H_2O$ 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 derivatisation of α-hydroxyphosphonates. Enzymes [lyophilised preparations of lipases SP 524 and AP 6 and protease Chirazyme® P-2 or a solution of it (5 ml of solution contains 600 mg of lyophilisate with a protein content of 80%)] were stored at +4°C and used as supplied. Abbreviations used: MC=methylene chloride; EA=ethyl acetate, PE=petroleum ether, AC=acetone, ee=enantiomeric excess.

*4.2. (±)-Diisopropyl 1-hydroxy-3-(methylthio)propylphosphonate (±)-**1d***

Yield 89%; oil; R_f=0.13 (MC:EA, 5:3); (*R*)-MTPA-(*S*)-**1d**/(*R*)-MTPA-(*R*)-**1d**: ^{31}P NMR: δ 17.34/16.79; IR (NaCl): ν_{max} cm^{-1}: 3288, 2978, 2920, 1439, 1386, 1375, 1224, 1178, 1142, 1106, 1075, 990; ^{1}H NMR: δ 4.68 (two oct overlap to non, *J*=6.4, 2H, OCH), 3.92 (dt, *J*=4.7, 9.2, 1H, CHP), 3.40 (br.s, 1H, OH), 2.65 (AB system, J_{AB}=13.1, *J*=2×6.5, 2×7.6, 2H, SCH_2), 2.04 (s, 3H, SMe), 1.91 (m, 2H, CH_2), 1.28 (d, *J*=6.4, 3H, Me), 1.26 (d, *J*=6.4, 9H, Me); ^{13}C NMR: δ 71.32 (d, *J*=7.2), 71.14 (d, *J*=7.3), 66.55 (d, *J*=163.5), 30.64 (d, *J*=1.5), 30.38 (d, *J*=15.4), 24.11 (d, *J*=3.2), 24.07 (d, *J*=3.2), 23.95 (d, *J*=4.7, 2C), 15.25. Anal. calcd for $C_{10}H_{23}O_4PS$: C, 44.43; H, 8.58. Found: C, 44.64; H, 8.58.

*4.3. (±)-Di-*t*-butyl 1-hydroxy-3-methylthiopropylphosphonate (±)-**1e***

Yield 48%; colourless crystals; R_f=0.39 (PE:EA, 1:2); mp 68–70°C (PE); (*R*)-MTPA-(*S*)-**1e**/(*R*)-MTPA-(*R*)-**1e**: ^{31}P NMR: δ 10.98/10.59; IR (Si) ν_{max} cm^{-1}: 3299, 2979, 2919, 1435, 1476, 1394, 1370, 1258, 1230, 1168, 1078, 1039, 990; ^{1}H NMR: δ 3.82 (dt, *J*=3.4, 9.8, CHP), 3.40 (br.s, 1H, OH), 2.67 (AB system, J_{AB}=13.3, *J*=5.4, 3×7.9, 2H, SCH_2), 2.07 (s, SMe), 1.93 (m, 2H, CH_2), 1.48 and 1.47 (2s, 2×9H, *t*-Bu); ^{13}C NMR: δ 82.78 and 82.74 (d, *J*=9.1), 67.88 (d, *J*=166.6), 31.11, 30.61 (d, *J*=13.8), 30.52 (d, *J*=4.0, 6C), 15.29. Anal. calcd for $C_{12}H_{27}O_4PS$: C, 48.30; H, 9.12. Found: C, 48.52; H, 9.01.

*4.4. (1*RS*,2*S*)-Diisopropyl 1-hydroxy-2-methylbutylphosphonate (1*RS*,2*S*)-**1g***

(*S*)-(−)-2-methylbutanol (1.76 g, 20 mmol) {$[\alpha]_D^{20}$=−6.3±0.3 (10.0, ethanol) for product from Fluka; $[\alpha]_D^{20}$=−5.8 (neat) for product from Aldrich} was oxidised by a known procedure.[22] Diisopropyl trimethylsilyl phosphite (4.75 g, 20 mmol) was added to the dried solution of the aldehyde in MC. The solution was stirred for 5 h under argon at room temperature. Then 4 ml of methanol and 4 drops of concentrated HCl were added and stirring was continued for 2 h. The organic phase was extracted with saturated $NaHCO_3$, dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography.

Yield 51%; viscous oil; R_f=0.19 (PE:EE, 2:1); IR (NaCl) ν_{max} cm^{-1}: 3301, 2977, 2949, 2935, 1466, 1386, 1187, 1178, 1107, 987; ^{1}H NMR [(1*R*,2*S*)-**1g** in brackets were distinguishable]: δ 4.72 (m, 4H, OCH), 3.72 (dd, *J*=3.8, 8.3, 1H, CHP), [3.56 (t, *J*=6.3, 1H, CHP)], 2.85 (s, 1H, OH), 2.75 (s, 1H, OH), 1.76 (m, 3H), [1.51 (m, 1H)], 1.30 (d, *J*=5.9, 12H, CH_3), 1.298 (d, *J*=6.4, 12H, CH_3), 1.20–1.00 (m, 2H), 1.00 (d, *J*=6.4, 3H, C*H*$_3$CH), [0.99 (d, *J*=5.9, 3H, C*H*$_3$CH)], 0.88 (t, *J*=7.4, 3H, C*H*$_3$CH$_2$), [0.87 (t, *J*=7.4, 3H, C*H*$_3$CH$_2$)]; ^{13}C NMR: δ [72.59 (d, *J*=156.4)], 71.16 (d, *J*=157.9), 70.92 (d, *J*=7.6, 3C), 70.85 (d, *J*=6.1), [36.75 (d, *J*=1.5)], 36.29 (d, *J*=2.3), 26.66 (d, *J*=12.2), [24.50 (d, *J*=8.4)], 24.13 (d, *J*=3.8),

24.09 (d, *J*=3.0), 23.94 (d, *J*=3.8), [15.67 (d, *J*=6.9)], 14.05 (d, *J*=4.6), 11.60, [10.98]. Anal. calcd for $C_{11}H_{25}O_4P$: C, 52.37; H, 9.99. Found: C, 52.26; H, 9.80.

*4.5. (±)-2-(1-Chloroacetoxy-2-methylpropyl)-5,5-dimethyl-1,3,2-dioxaphosphorinan-2-one (±)-**2f***

Compound **1f** (3.15 g, 14.2 mmol) was esterified using chloroacetic anhydride/pyridine and purified by flash chromatography (MC:EA, 1:1, as eluent, R_f=0.55) to give 3.55 g (84%) of (±)-**2f** as a very viscous oil; IR (NaCl) ν_{max} cm^{-1}: 2970, 1762, 1473, 1281, 1164, 1062, 1000; ^{1}H NMR: δ 5.24 (dd, *J*=7.6, 14.5, 1H, CHP), 4.13 (s, 2H, CH_2Cl), 4.13–3.92 (m, 4H, OCH_2), 2.35 (m, 1H, PCCH), 1.14 (s, 3H, Me), 1.06 (d, *J*=6.9, 3H, Me), 1.04 (d, *J*=6.9, 3H, Me), 1.01 (s, 3H, Me); ^{13}C NMR: δ 166.53 (d, *J*=5.3), 76.68 (d, *J*=6.9), 76.33 (d, *J*=6.1), 73.74 (d, *J*=161.0), 40.47, 32.52 (d, *J*=6.9), 29.18 (d, *J*=1.5), 21.65, 21.11, 19.41 (d, *J*=8.4), 18.22 (d, *J*=6.6). Anal. calcd for $C_{11}H_{20}ClO_5P$: C, 44.23; H, 6.75. Found: C, 44.24; H, 6.71.

*4.6. (±)-Diisopropyl 1-chloroacetoxy-3-(methylthio)propylphosphonate (±)-**2d***

Yield 81%; oil; R_f=0.53 (MC:EE, 5:3); IR (NaCl) ν_{max} cm^{-1}: 2980, 2934, 1769, 1438, 1386, 1376, 1248, 1163, 1105, 989; ^{1}H NMR: δ 5.37 (dt, *J*=4.9, 9.3, 1H, CHP), 4.73 (m, 2H, OCH), 4.09 (AB system, J_{AB}=14.8, 2H, CH_2Cl), 2.53 (AB system, J_{AB}=13.3, *J*=5.3, 6.9, 2×8.4, 2H, SCH_2), 2.12 (m, 2H, CH_2), 2.07 (s, SMe), 1.32 (d, *J*=5.9, 6H, Me), 1.31 (d, *J*=6.4, 3H, Me), 1.30 (d, *J*=5.9, 3H, Me); ^{13}C NMR: δ 166.38 (d, *J*=4.5), 71.99 (d, *J*=6.7), 71.80 (d, *J*=7.3), 69.25 (d, *J*=170.4), 30.12 (d, *J*=13.5), 29.14, 24.13 and 24.00 (d, *J*=3.6), 23.97 (d, *J*=4.7), 23.82 (d, *J*=4.9), 15.37. Anal. calcd for $C_{12}H_{24}ClO_5PS$: C, 41.56; H, 6.97. Found: C, 41.67; H, 6.95.

*4.7. (±)-Di-*t*-butyl 1-chloroacetoxy-3-(methylthio)propylphosphonate (±)-**2e***

Yield 72%; viscous oil; R_f=0.67 (PE:EE, 1:2); IR (NaCl) ν_{max} cm^{-1}: 2980, 2934, 1766, 1476, 1429, 1394, 1370, 1327, 1260, 1168, 1040, 986; ^{1}H NMR: δ 5.21 (ddd, *J*=3.8, 7.8, 9.7, 1H, CHP), 4.04 (AB system, J_{AB}=14.8, 2H, CH_2Cl), 2.48 (AB system, J_{AB}=13.3, *J*=5.4, 6.9, 8.4, 8.9, 2H, SCH_2), 2.03 (s, SMe), 2.20–1.91 (m, 2H, CH_2), 1.45 and 1.44 (2s, 2×9H, *t*-Bu); ^{13}C NMR: δ 166.55 (d, *J*=4.8), 83.97 (d, *J*=8.4), 83.82 (d, *J*=9.7), 70.87 (d, *J*=174.0), 40.74, 30.45 (d, *J*=3.9, 3C), 30.28 (d, *J*=3.8, 3C), 30.27 (d, *J*=13.5), 29.53, 15.37. Anal. calcd for $C_{14}H_{28}ClO_5PS$: C, 44.86; H, 7.53. Found: C, 45.15; H, 7.27.

*4.8. (1*R,*2*S*)- and (1*S,*2*S*)-Diisopropyl 1-chloroacetoxy-2-methylbutylphosphonate (1*RS,*2*S*)-**2g***

Yield 94%; viscous oil; R_f=0.76 (PE:EE, 1:4); IR (NaCl) ν_{max} cm^{-1}: 2979, 2952, 2938, 1769, 1466, 1402, 1386, 1254, 1164, 1105, 991; ^{1}H NMR [(1*R*,2*S*)-**2g** in brackets were distinguishable]: δ 5.22 (dd, *J*=4.4, 10.3, 1H, CHP), [5.09 (dd, *J*=7.4, 9.3, 1H, CHP)], 4.72 (m, 4H, OCH), 4.09 (AB system, J_{AB}=15.3, 4H, CH_2Cl), 1.97 (m, 2H, CH_2), [1.64 (m, 1H, CH_2)], 1.45 (m, 1H, CH_2), 1.32–1.26 (overlapping d, *J*=~6.0, 24H, CH_3), 1.20 (m, 2H, CH_2), 1.02 (d, *J*=6.9, 6H, C*H*$_3$CH), 0.90 (t, *J*=7.4, 3H, C*H*$_3$CH$_2$), [0.86 (t, *J*=7.4, 3H, C*H*$_3$CH$_2$)]; ^{13}C NMR: δ 166.54 (d, *J*=7.0), 166.48 (d, *J*=5.3), (d, *J*=4.6), [74.37 (d, *J*=167.8)], 73.07 (d, *J*=168.6), 71.56 (d, *J*=5.7), [71.50 (d, *J*=6.3)], 71.39 (d, *J*=7.4), [71.36 (d, *J*=7.5)], [40.65], 40.60, 35.64, [35.52], 26.69 (d, *J*=11.1), [24.76 (d, *J*=7.9)], 24.18 (d, *J*=2.3), 24.10–23.93 (overlapping d), 23.78 (d, *J*=2.3), [15.56 (d, *J*=6.1)], 14.69 (d, *J*=4.6), 11.42, [10.84]. Anal. calcd for $C_{13}H_{26}ClO_5P$: C, 47.49; H, 7.97. Found: C, 47.22; H, 8.05.

*4.9. (*R*)-(−)-Diisopropyl 1-azidopropylphosphonate (*R*)-(−)-**4a***

Yield 84%; oil; R_f=0.30 (PE:AC, 5:1); $[\alpha]_D^{20}$=−54.0 (1.0, AC); IR (NaCl) ν_{max} cm^{-1}: 2980, 2937, 2103, 1465, 1386, 1381, 1251, 1178, 1142, 1106, 987; ^{1}H NMR: δ 4.74 (m, 2H, OCH), 3.20 (dt, *J*=3.6, 11.4, 1H, CHP), 1.88 (m, 1H, CH_2), 1.63 (m, 1H, CH_2), 1.33 (d, *J*=6.4, 6H, Me), 1.324 and 1.321 (d, *J*=6.4, 3H, Me), 1.06 (t, *J*=7.4, 3H, Me); ^{13}C NMR: δ 71.66 (d, *J*=7.3), 71.59 (d, *J*=8.4), 59.55 (d, *J*=156.8), 24.12 (d, *J*=3.5, 2C), 23.94 (d, *J*=4.7, 2C), 22.12, 11.48 (d, *J*=14.2). Anal. calcd for $C_9H_{20}N_3O_3P$: C, 43.37; H, 8.09; N, 16.86. Found: C, 43.47; H, 7.81; N, 17.07.

*4.10. (*R*)-(−)-Diisopropyl 1-azido-2-methylpropylphosphonate (*R*)-(−)-**4b***

Yield 68%; oil; R_f=0.33 (PE:AC, 5:1); $[\alpha]_D^{20}$=−56.0 (1.1, AC); IR (Si) ν_{max} cm^{-1}: 2980, 2936, 2104, 1467, 1387, 1257, 1178, 1142, 1106, 991; ^{1}H NMR: δ 4.76 (m, 2H, OCH), 3.21 (dd, *J*=5.4, 12.8, 1H, CHP), 2.13 (m, 1H, C*H*Me_2), 1.34 (d, *J*=6.4, 3H, Me), 1.33 (d, *J*=5.9, 6H, Me), 1.32 (d, *J*=5.9, 3H, Me), 1.03 and 1.08 (d, *J*=7.4, 3H, Me); ^{13}C NMR: δ 71.57 (d, *J*=7.6), 71.47 (d, *J*=8.0), 64.59 (d, *J*=156.1), 29.30, 24.20 (d, *J*=3.6), 24.13 (d, *J*=3.4), 23.97 (d, *J*=4.8), 23.92 (d, *J*=5.0), 21.08 (d, *J*=10.5), 18.64 (d, *J*=6.5). Anal. calcd for $C_{10}H_{22}N_3O_3P$: C, 45.62; H, 8.42; N, 15.96. Found: C, 45.58; H, 8.48; N, 16.14.

*4.11. (*R*)-(−)-Diisopropyl 1-azido-3-methylbutylphosphonate (*R*)-(−)-**4c***

Yield 77%; oil; R_f=0.35 (PE:AC, 5:1); $[\alpha]_D^{20}$=−56.6 (1.1, AC); IR (Si) ν_{max} cm^{-1}: 2980, 2112, 1467, 1387, 1261, 1178, 1142, 1106, 989; ^{1}H NMR: δ 4.75 (m, 2H, OCH), 3.34 (ddd, *J*=3.4, 11.8, 12.8, 1H, CHP), 1.82 (m, 1H, C*H*Me_2), 1.60 (m, 2H, CH_2), 1.33 (d, *J*=6.4, 12H, Me), 0.95 (d, *J*=6.7, 3H, Me), 0.90 (d, *J*=6.9, 3H, Me); ^{13}C NMR: δ 71.72 (d, *J*=7.3), 71.59 (d, *J*=7.2), 55.83 (d, *J*=156.7), 36.80, 25.04 (d, *J*=13.6), 24.14 (d, *J*=3.6), 24.11 (d, *J*=3.4), 23.97 and 23.93 (d, *J*=4.2), 23.15, 20.85. Anal. calcd for $C_{11}H_{24}N_3O_3P$: C, 47.64; H, 8.72; N, 15.15. Found: C, 47.91; H, 8.53; N, 14.88.

*4.12. (*R*)-(−)-Diisopropyl 1-azido-3-(methylthio)propylphosphonate (*R*)-(−)-**4d***

Yield 90%; oil; R_f=0.61 (ether); $[\alpha]_D^{20}$=−83.8 (1.1, AC); IR (Si) ν_{max} cm^{-1}: 2981, 2922, 2100, 1453, 1386, 1376, 1263, 1178, 1142, 1106, 987; ^{1}H NMR: δ 4.76 (m, 2H, OCH), 3.64 (dt, *J*=3.3, 11.5, 1H, CHP), 2.64 (AB system, J_{AB}=13.4, *J*=4.4, 7.4, 2×7.9 and long range coupling with phosphorus, *J*=1.0, 2H, SCH_2), 2.07 (s, 3H, SMe), 2.04 and 1.86 (m, 1H, CH_2), 1.33 (d, *J*=5.9, 12H, Me); ^{13}C NMR: δ 71.88 (d, *J*=7.3), 71.85 (d, *J*=7.0), 56.04 (d, *J*=157.9), 30.81 (d, *J*=15.1), 30.38 (d, *J*=15.4), 24.12 and 24.11 (d, *J*=3.8), 23.97 and 23.96 (d, *J*=4.6), 15.18. Anal. calcd for $C_{10}H_{22}N_3O_3PS$: C, 40.67; H, 7.51; N, 14.23. Found: C, 40.43; H, 7.23; N, 13.98.

*4.13. (1*R*,2*S*)-(−)-Diisopropyl 1-azido-2-methylbutylphosphonate (1*R*,2*S*)-(−)-**4e***

To a stirred solution of 252 mg (1.0 mmol) of (+)-**2e** and 524 mg (2.0 mmol) of triphenylphosphine in 5 ml of dry toluene, 0.31 ml (348 mg, 2.0 mmol) of diethyl azodicarboxylate were added under argon and cooling with an ice bath. After addition of 1 ml of a 1.06 M solution of HN_3 in toluene, a precipitate was formed and another 0.9 ml of the HN_3 solution was added at room temperature. Stirring was continued for 4 h. Then 1 ml of methanol was added and after 1 h the solvent was removed in vacuo. Hexane was added to the residue and the mixture was allowed to stand overnight. The crystalline solid was removed and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography.

Yield 79%; oil; R_f=0.25 (PE:AC, 5:1); $[\alpha]_D^{20}$=−58.2 (1.0, AC); IR (Si) ν_{max} cm^{-1}: 2979, 2104, 1453, 1386, 1254, 1178, 1142, 1106, 986; ^{1}H NMR: δ 4.73 (m, 2H, OCH), 3.40 (dd, *J*=4.0, 14.0, CHP of trace of diastereomeric azide), 3.20 (dd, *J*=6.5, 13.0, 1H, CHP), 1.82 and 1.70 (m, 1H, CH_2), 1.30 (d, *J*=6.0, 9H, Me), 1.28 (d, *J*=5.5, 3H, Me), 1.03 (t, *J*=7.0, 3H, Me), 0.84 (d, *J*=7.4, 3H, Me); ^{13}C NMR: δ 71.16 and 70.98 (d, *J*=7.6), 63.36 (d, *J*=155.3), 35.42, 24.95 (d, *J*=7.6), 24.18 (d, *J*=2.3), 24.15 (d, *J*=3.8), 23.95 (d, *J*=5.3), 23.93 (d, *J*=5.3), 16.83 (d, *J*=8.4), 10.58. Anal. calcd for $C_{11}H_{24}N_3O_3P$: C, 47.65; H, 8.72; N, 15.15. Found: C, 47.86; H, 8.52; N, 14.99.

*4.14. (*R*)-(−)-1-Aminopropylphosphonic acid (*R*)-(−)-**5a***

Yield 79%; crystalline solid; mp 263–264°C (decomp.) (lit.[10]: 276°C); $[\alpha]_D^{20}$=−18.0, $[\alpha]_{578}^{20}$=−17.8 (0.9, 1 M NaOH) {lit.[10]: $[\alpha]_{578}^{20}$=−21.6 (1.0, 1 M NaOH), ee 98%}; IR (Nujol mull) ν_{max} cm^{-1}: 1629, 1537, 1155, 1077, 1019, 921; ^{1}H NMR (D_2O, NaOD): δ 2.26 (dt, *J*=3.3, 10.2, 1H, CHP), 1.63 and 1.17 (m, 1H, CH_2), 0.83 (t, *J*=7.4, 3H, Me). Anal. calcd for $C_3H_{10}NO_3P$: C, 25.91; H, 7.25; N, 10.07. Found: C, 26.14; H, 6.95; N, 9.89.

*4.15. (*R*)-(+)-1-Amino-2-methylpropylphosphonic acid (*R*)-(+)-**5b***

Yield 68%; crystalline solid; mp 265–268°C (decomp.) (lit.[28]: 272–273°C); $[\alpha]_D^{20}$=+0.4, $[\alpha]_{578}^{20}$=+0.4 (0.9, 1 M NaOH) {lit.[28]: $[\alpha]_{578}^{20}$=+0.6 (1.0, 1 M NaOH)}; IR (Nujol mull) ν_{max} cm^{-1}: 1624, 1532, 1233, 1167, 1061, 934; ^{1}H NMR (D_2O): δ 3.04 (dd, *J*=6.0, 13.7, 1H, CHP), 2.18 (m, 1H, C*H*Me_2), 1.07 and 1.03 (d, *J*=6.4, 3H, Me).

*4.16. (*R*)-(−)-1-Amino-3-methylbutylphosphonic acid (*R*)-(−)-**5c***

Yield 85%; crystalline solid; mp 273–276°C (decomp.) (lit.[6]: 281–283°C); $[\alpha]_D^{20}$=−31.6 (0.9, 1 M NaOH) {lit.[6]: $[\alpha]_D^{20}$=−25.0 (0.7, 1 M NaOH), ee ≥99%}; IR (Nujol mull) ν_{max} cm^{-1}: 1614, 1203, 1015, 942, 934; ^{1}H NMR (D_2O): δ 3.25 (ddd, *J*=5.0, 9.0, 13.9, 1H, CHP), 1.78–1.52 (m, 3H, CH and CH_2), 0.91 and 0.87 (d, *J*=6.4, 3H, Me).

*4.17. (*R*)-(−)-1-Amino-3-(methylthio)propylphosphonic acid (*R*)-(−)-**5d***

Yield 59%; crystalline solid; mp 263–265°C (decomp.); $[\alpha]_D^{20}$=−26.8 (0.9, H_2O), $[\alpha]_D^{20}$=−43.5 (1.0, 0.25 M NaOH) {lit.[8]: $[\alpha]_D^{20}$=−17.2 (1.1, H_2O), $[\alpha]_D^{20}$=30.5 (1.1, 0.25 M NaOH), ee 76.8%, see also Smith III et al.[6]}; IR (Nujol mull) ν_{max} cm^{-1}: 2282, 1648, 1537, 1183, 1029, 1003; ^{1}H NMR (D_2O, NaOD): δ 3.19 (ddd, *J*=4.4, 8.9, 13.0, 1H, CHP), 2.65 (AB system, J_{AB}=13.9, *J*=5.9, 2×7.3, 8.4, 2H, CH_2), 2.16 (m, 1H, CH_2), 2.08 (s, 3H, Me), 1.90 (m, 1H, CH_2), 0.83 (t, *J*=7.4, 3H, Me).

*4.18. (1*R*,2*S*)-(−)-1-Amino-2-methylbutylphosphonic acid (1*R*,2*S*)-(−)-**5e***

Yield 68%; crystalline solid; mp 256–258°C (decomp.); $[\alpha]_D^{20}$=−8.5 (1.0, 1 M NaOH); IR (Nujol mull) ν_{max} cm^{-1}: 1599, 1531, 1167, 1022, 928; ^{1}H NMR (D_2O, NaOD): δ 2.64 (dd, *J*=2.8, 13.8, CHP of trace of other diastereomer), 2.50 (dd, *J*=4.5, 12.5, 1H, CHP), 1.76 (m, 2H), 1.11 (m, 1H), 1.04 (d, *J*=6.7, Me), 0.92 (t, *J*=7.3, 3H, Me); ^{13}C NMR (D_2O, NaOD): δ 55.73 (d, *J*=136.9), 36.13, 24.28 (d, *J*=4.6), 17.68 (d, *J*=9.2), 11.49; ^{31}P NMR (D_2O, NaOD): δ 22.45. Anal. calcd for $C_5H_{14}NO_3P$: C, 35.93; H, 8.44; N, 8.38. Found: C, 36.12; H, 8.17; N, 8.26.

4.19. (±)-Di-t-butyl 1-azido-3-(methylthio)propylphosphonate (±)-6

Yield of (±)-**6** 59% and of byproduct **7** 15%; R_f=0.37 (MC:EE, 10:1); IR (NaCl) ν_{max} cm^{-1}: 2981, 2934, 2099, 1394, 1371, 1268, 1170, 1038, 983; ^1H NMR: δ 3.44 (dt, *J*=3.1, 10.9, 1H, CHP), 2.64 (AB system, J_{AB}=13.2, *J*=4.4, 7.4, 2×7.9 and long range coupling with phosphorus, *J*=1.0, 2H, SCH_2), 2.07 (s, 3H, SMe), 2.14–2.01 and 1.85 (m, 1H, CH_2), 1.52 and 1.51 (s, 9H, Me); ^{13}C NMR: δ 83.90 (d, *J*=9.9), 83.65 (d, *J*=9.0), 57.39 (d, *J*=159.4), 31.01 (d, *J*=14.7), 30.49 (d, *J*=3.9, 3C), 30.46 (d, *J*=3.8, 3C), 28.25, 15.21. Crude **7** was isolated as byproduct; IR (Si) ν_{max} cm^{-1}: 2980, 2919, 1631, 1394, 1370, 1260, 1173, 1039, 979; ^1H NMR: δ 6.49 (ddt, *J*=7.2, J_{AB}=16.7, J_{PH}=20.7, 1H, C*H*=CHP), 5.74 (ddt, *J*=1.2, J_{AB}=16.7, J_{PH}=18.7, 1H, CHP), 3.13 (dt, *J*=1.2, 7.2, 2H, CH_2S), 2.00 (s, 3H, SCH_3), 1.45 (s, 18H, *t*-Bu). (±)-**6**: Anal. calcd for $C_{12}H_{26}N_3O_3PS$: C, 44.57; H, 8.10. Found: C, 44.83; H, 7.86.

4.20. (±)-2-(1-Azido-2-methylpropyl)-5,5-dimethyl-1,3,2-dioxaphosphorinan-2-one (±)-8

The azide was prepared from 0.99 g (4 mmol) of (±)-**1f** by the method of Gajda.[12] The crude product was purified by flash chromatography using diethyl ether (R_f=0.22) as eluent to give 0.80 g (81%) of crystalline azide; mp 73–74°C (PE/few drops of MC); IR (Nujol mull) ν_{max} cm^{-1}: 2107, 1272, 1055, 1005; ^1H NMR: δ 4.14 (m, 2H, OCH_2), 3.98 (m, 2H, OCH_2), 3.47 (dd, *J*=4.9, 13.3, 1H, CHP), 2.25 (m, 1H, PCCH), 1.08 (d, *J*=7.9, 3H, Me), 1.07 and 1.02 (2 s, each 3H, Me), 1.01 (d, *J*=5.4, 3H, Me); ^{13}C NMR: δ 76.31 (d, *J*=6.9), 76.08 (d, *J*=6.9), 64.43 (d, *J*=152.6), 32.60 (d, *J*=6.9), 29.42, 21.65, 21.33, 20.90 (d, *J*=11.2), 18.41 (d, *J*=6.0). Anal. calcd for $C_9H_{18}N_3O_3P$: C, 43.69; H, 7.33; N, 17.06. Found: C, 43.98; H, 7.14; N, 17.06.

Azide (±)-**8** (0.70 g, 2.83 mmol) was catalytically reduced and then deblocked by refluxing in 30 ml of 6 N HCl for 8 days. Removal of volatiles in vacuo and purification of the residue by ion exchange chromatography (Dowex 50, H^+) with water as eluent afforded 0.3 g (69%) of racemic aminophosphonic acid (±)-**5b**.

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