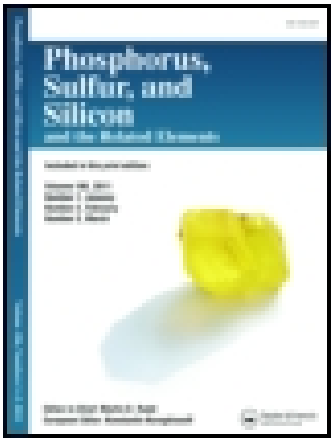


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

THE PHOSPHONIC ANALOGUES OF THREONINE AND β-PHENYLSERINE: PREPARATION AND ANALYSIS OF STEREOISOMERS

Ewa Matczak-jon [a], Józef Barycki [b], Malgorzata Milewska [b] & Wanda Sawka-dobrowolska [c]

[a] Institute of Inorganic Chemistry

[b] Institute of Organic Chemistry, Biochemistry and Biotechnology, Wroclaw University of Technology, 50-370, Wroclaw

[c] Department of Chemistry, University of Wroclaw, 50-383, Wroclaw, Poland

Published online: 24 Sep 2006.

To cite this article: Ewa Matczak-jon, Józef Barycki, Malgorzata Milewska & Wanda Sawka-dobrowolska (1998) THE PHOSPHONIC ANALOGUES OF THREONINE AND β-PHENYLSERINE: PREPARATION AND ANALYSIS OF STEREOISOMERS, Phosphorus, Sulfur, and Silicon and the Related Elements, 142:1, 101-115, DOI: 10.1080/10426509808029670

To link to this article: http://dx.doi.org/10.1080/10426509808029670

Phosphorus, Sulfur and Silicon, 1998, Vol. 142, pp. 101-115
Reprints available directly from the publisher
Photocopying permitted by license only

THE PHOSPHONIC ANALOGUES OF THREONINE AND β-PHENYLSERINE: PREPARATION AND ANALYSIS OF STEREOISOMERS

EWA MATCZAK-JON[a*], JÓZEF BARYCKI[b], MAŁGORZATA MILEWSKA[b] and WANDA SAWKA-DOBROWOLSKA[c]

[a]*Institute of Inorganic Chemistry,* [b]*Institute of Organic Chemistry, Biochemistry and Biotechnology, Wroclaw University of Technology, 50–370, Wroclaw; and* [c]*Department of Chemistry, University of Wroclaw, 50–383 Wroclaw Poland*

(Received 03 August, 1998)

The simple procedures for the preparation of 1-amino-2-hydroxypropanephosphonic (**3**) and 1-amino –2-hydroxy-2-phenylethane phosphonic (**4**) acids in acceptable yields are described. We showed by NMR studies with chiral $Pr(hfc)_3$ shift reagent and by HPLC that the reactions are not stereospecific and both products constitute a mixture of all feasible stereoisomers. The crystal structure of the racemic (**3**) was also determined by X-Ray.

Keywords: Phosphonic acids and derivatives; Stereoisomerism; NMR; X-Ray crystallography; shift reagents

INTRODUCTION

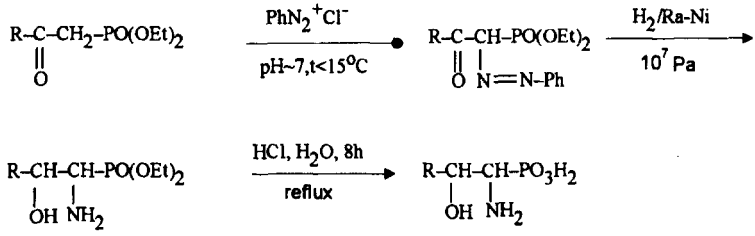
There is a vast literature[1–7] on the synthesis and various properties of the phosphonic analogues of protein and non-protein amino acids but only two β -hydroxy-α-aminoalkanephosphonic acids with two chiral centres have been described[8–12]. In the papers on the preparation of 2-phenyl-2-hydroxy-1-aminoethanephosphonic acid (the analogue of β-phenylserine) there are no references to the stereochemistry of the described products[13]. Stereochemistry is well elaborated in the recent Italian paper

* author to whom correspondence should be adressed

on the phosphonic analogues of threonine but the synthetic procedures are tedious[14].

In this report we describe simple procedures for the preparation of 1-amino-2-hydroxypropanephosphonic **3** and 1-amino-2-hydroxy-2-phenylethanephosphonic acid **4** in acceptable yields. The key step in our syntheses was the reaction of phenyldiazonium chloride with 2-oxophosphonates followed by reduction and hydrolysis.

Although TLC analysis using several developing solvents failed to show the presence of diastereomeres of acids **3** and **4** both ^{13}C and ^{1}H NMR with lanthanide shift reagents demonstrated the presence of four stereoisomers of the esters **1** and **2.** In order to confirm this finding we have synthesized dipeptide **6** and resolved the resulting stereoisomers by means of HPLC. Four peaks observed in elution profile clearly supported the presence of four stereoisomers of the starting phosphonate.

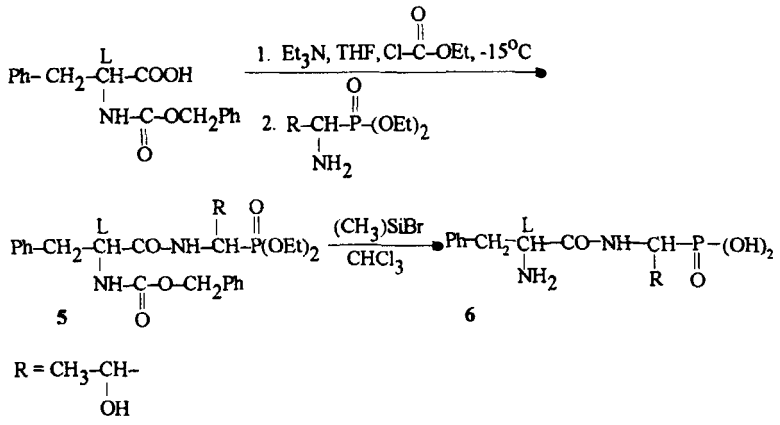


1 R=CH_3 **3** R=CH_3

2 R=C_6H_5 **4** R=C_6H_5

Both methods used for determination of the stereoisomeric composition of crude products **1, 2** and **6** are indirect and depend on the use of chiral derivatizing agents that convert the mixture of stereoisomers into diastereomers prior to further NMR or HPLC analysis[15,16]. Our trials with $Yb(hfc)_3$ and $Pr(hfc)_3$ lanthanide induced shift reagents indicated that only the latter one sufficiently differentiates ^{13}C and ^{1}H NMR spectra when added to **1** and **2.**

Most applications of both chiral and nonchiral shift reagents prefer usually ^{1}H over the more tedious ^{13}C NMR experiments[17–21]. However, as will be demonstrated in this paper ^{13}C NMR may be an amenable method



because it is more sensitive to electronic environment of the nuclei as well as it may be an aid in the assignment of ^{1}H NMR resonances[22-23].

RESULTS AND DISCUSSION

Concentration and stoichiometry of $Pr(hfc)_3$ and substrate which influence both the time of ^{13}C experiment as well as the quality of the spectra were optimised using racemic dimethyl 1-amino-2-methylpropanephosphonate **7**[24] as a model sample. The ester contains two pairs of diastereotopic groups (OCH_3 and CH_3) with ^{13}C resonances due to ^{13}C-^{31}P coupling appearing as a triplet (overlapped doublets) and a pair of doublets, respectively (see TABLE I). Successive addition of increments of the chiral $Pr(hfc)_3$ to a solution containing a racemic mixture of this compound resulted in substantial separation of methoxy triplets (FIGURE 1) and slight derivatization of upfield shifted CH_3 resonances. This, together with a severe broadening followed by disappearance of C_α doublet after relatively small increments of the praseodymium complex (LSR:substrate molar ratio < 0.1) revealed P=O of the dimethyl phosphonate group as a possible LSR binding site.

Both diethyl phosphonate esters **1** and **2** ,the ^{13}C assignments of which are also given in TABLE I, seemed to coordinate $Pr(hfc)_3$ in a similar way.

This resulted in almost immediate disappearance of the C_α resonance followed by gradual broadening and disappearance of the C_β signal of

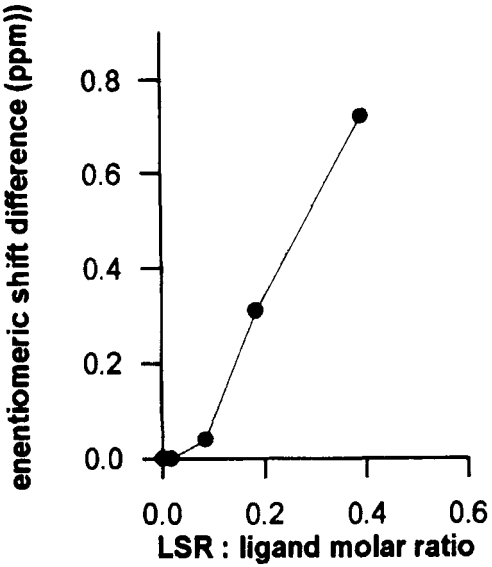


FIGURE 1 Plot of the enantiomeric shift difference $\Delta\delta$ for OCH_3 carbon resonances of ester 7

these compounds. The signals of the phenyl substituent being more distant from the coordination centre were almost intact with the exception of the signals corresponding to the ortho carbons (δ = 127.50 ppm) for which considerable upfield shift (6.5 ppm) and broadening were observed only when LSR:substrate of 0.8 molar ratio was applied. Similarly no useful diastereomeric shift difference was discernible for the methyl resonances of the threonine residue. Thus again the diethyl phosphonate moiety appeared to be the only "reporter group" useful in the stereochemical analysis.

The striking feature of both **1** and **2** was greater ^{13}C NMR non-equivalence observed in the presence of LSR for their OCH_2-$\underline{CH_3}$ versus $O\underline{CH_2}$-CH_3 resonances. Moreover, the presence of the phenyl substituent at C_β stereogenic centre considerably improved diastereoselection (see FIGURE 2). Chiral recognition of stereoisomers which differ in stereocentres being in a close proximity to coordination site (C_α) proceeded easily even at $Pr(hfc)_3$:substrate molar ratio smaller than 0.2. However, satisfactory derivatization which resulted in a separation of the ethoxy methyl resonances belonging to all four diastereomers was possible only for ester **2** at LSR: substrate molar ratio > 0.6.

TABLE I ^{13}C NMR assignments of phosphonate esters 1, 2 and 7[1]

Compound	*1*	*2*	*7*
C_α	54.89(143.9)	55.27(148.2)	54.26(158.6)
C_β	67.99(4.4)	75.41 (0.5)	19.54(2.5)
CH_3	19.65 (6.9)		17.64(4.7)
			20.79(12.7)
Ph		127.50	
		128.66	
		128.87	
O-$\underline{CH_2}$-CH_3	62.86(7.2)	63.09 (7.2)	
	62.68(6.9)		
O-CH_2-$\underline{CH_3}$	16.91(5.4)	16.82 (5.9)	
	16.86(5.1)		
O-CH_3			52.99 (7.1)

[1]Spectra recorded in $CDCl_3$, $J_{C\text{-}P}$values (Hz) given in parentheses.

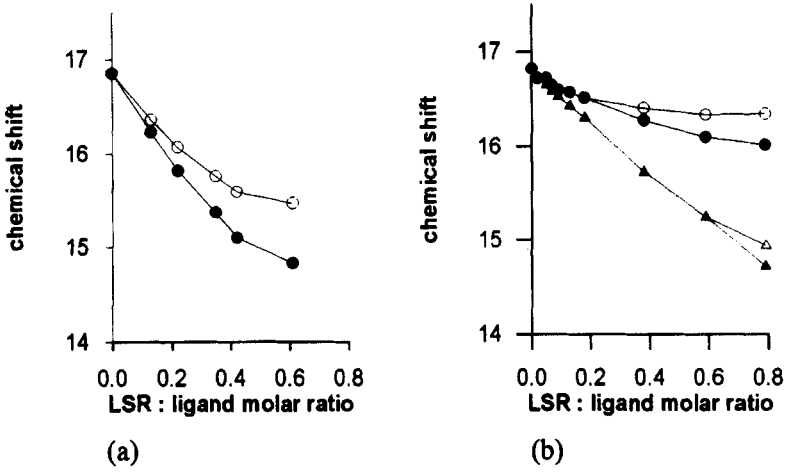


FIGURE 2 Plots of the OCH_2-$\underline{CH_3}$ ^{13}C chemical shifts of ester **1** (a) and ester **2** (b) as a function of $Pr(hfc)_3$ to substrate molar ratio

For the "fully shifted" samples of both **1** and **2** with maximum $Pr(hfc)_3$: substrate molar ratio achieved in ^{13}C NMR experiments ^{1}H-^{13}C HMQC spectra were recorded which allowed to correlate carbon resonances of derivatized diastereomers with their respective proton signals. As shown in FIGURE 3 considerable enhancing of the resolution in the proton dimension allowed to overcome the problem of the peaks overlapping. Thus, HMQC easily resolved the OCH_2-$\underline{CH_3}$ resonances derived from all feasible diastereomers not only in the LSR:ester **2** system but also in the LSR-ester **1** mixture at ~ 0.4 molar ratio for which monodentate $Pr(hfc)_3$ binding and 1:1 stoichiometry of derivatizing shift reagent and substrate is known to be retained[25]. Our efforts to integrate accurately each of the diastereomer peaks in 1D proton as well as in 2D HMQC experiments were ineffective. However, approximate integral values revealed that all four stereoisomers are almost equally populated in the mixtures of both esters **1** and **2.**

In order to verify this finding we attempted to separate chromatographically the diastereomeric mixture of dipeptide **6** synthesised from Z-L-phenylalanine and diethyl 1-amino-2-hydroxypropane phosphonate **1.**

Four peaks eluted at 9.17, 10.42, 11.25, and 12.37 min retention times and identified by ES-MS as $[M+Na]^+$ ions at m/z 324.1 indicated stereomeric composition of the phosphonopeptide and are consistent with the results obtained from NMR studies.

Upon several crystallisations from an aqueous solution single crystals of the racemic mixture of (1R, 2S) and (1S, 2R) of 1-amino-2-hydroxypropanephosphonic acid **3** were isolated. The molecular structure and atom numbering of **3** is illustrated on FIGURE 4. Selected bond distances and angles are given in TABLE II.

As can be seen in FIGURE 4 the phosphonic group of compound **3** is ionised with the proton being transferred to the amino group which results in the formation of the zwitterion. The coordination around the P atom differs from regular tetrahedron. The P-O(3) bond length is longer by 0.06 Å than the other two P-O bonds due to the protonation of O(3) oxygen atom. The bond lengths and angles in the phosphonic acid group and threonine moiety are in good agreement with those found earlier for N-phosphonomethyl-L-threonine[27] and O-phospho-DL-threonine and O-phospho-L-threonine[28]. The side-chain conformation of the molecule given by the torsion angles N-C(1)-C(2)-C(3) and N-C(1)-C(2)-O(4) is *gauche, gauche.* The values of these angles [53.7(5) and 68.4(5)°] are similar to the

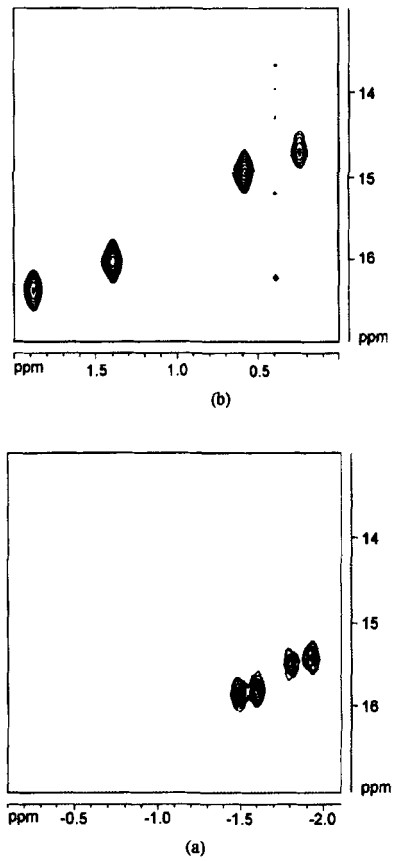


FIGURE 3 Expansion of OCH_2-$\underline{CH_3}$ region of ^1H-^{13}C HMQC spectra at $Pr(hfc)_3$: ester molar ratios of 0.39 (ester **1**) – (a) and 0.79 (ester **2**) – (b)

values found in the O-phospho-DL-threonine [-49.4(3), 73.5(2)] and O-phospho-L-threonine [-53.0(4), 69.5(3)^{O}][28].

There are five hydrogen atoms participating in moderately strong intermolecular hydrogen bonds (TABLE III and FIGURE 5).The nonpolar methyl groups of different molecules occupy isolated interstices in the polar hydrogen bond network forming hydrophobic layers in contrast to the hydrophilic layers formed by the phosphonic acid and amino groups. In the hydrophilic layer the molecules are connected by a complex network of hydrogen bonds.

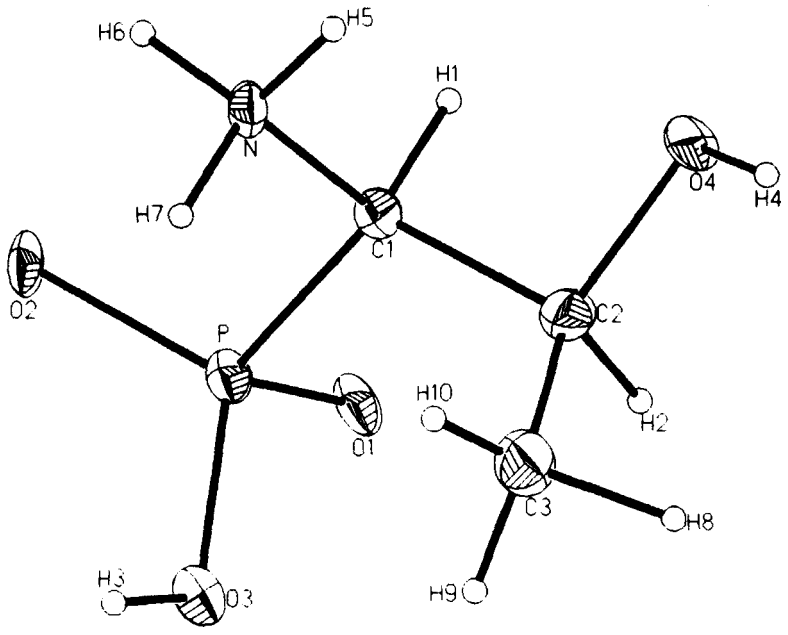


FIGURE 4 ORTEP[26] drawing of the (1R, 2S) diastereomer of 1-amino-2-hydroxypropane phosphonic acid racemate. Displacement ellipsoids are drown at the 35% probability level

Adjacent molecules are held together via O(3)-H(3)...O(1) and N-H(7)...O(2) hydrogen bonds related by b glide plane forming an eight membered ring. Furthermore, the O(4) atoms (as donors) form infinite hydrogen-bond chains with O(1) [- x-0.5, y-0.5, z] parallel to a direction.

TABLE II Bond Lengths (Å), Angles (°) and Torsion Angles (°) for 1-Amino –2-Hydroxypropanephosphonic Acid

P-O(1)	1.495(4)	O(1)-P-O(2)	116.6(2)
P-O(2)	1.509(3)	O(1)-P-O(3)	108.6(2)
P-O(3)	1.566(4)	O(2)-P-O(3)	111.6(2)
P-C(1)	1.816(5)	O(1)-P-C(1)	106.1(2)
O(4)-C(2)	1.442(6)	O(2)-P-C(1)	105.1(2)

N-C(1)	1.499(6)	O(3)-P-C(1)	108.4(2)
C(1)-C(2)	1.547(7)	N-C(1)-C(2)	109.9(4)
C(2)-C(3)	1.507(8)	N-C(1)-P	112.8(3)
		C(2)-C(1)-P	116.6(3)
		O(4)-C(2)-C(3)	110.4(5)
		O(4)-C(2)-C(1)	105.0(4)
		C(3)-C(2)-C(1)	116.1(4)
O(1)-P-C(1)-N		159.6(3)	
O(2)-P-C(1)-N		35.5(4)	
O(3)-P-C(1)-N		-83.9(3)	
O(1)-P-C(1)-C(2)		-71.8(5)	
0(2)-P-C(1)-C(2)		164.1(4)	
O(3)-P-C(1)-C(2)		44.7(5)	
N-C(1)-C(2)-O(4)		-68.4(5)	
P-C(1)-C(2)-O(4)		161.6(4)	
N-C(1)-C(2)-C(3)		53.7(5)	
P-C(1)-C(2)-C(3)		-76.2(6)	

TABLE III Hydrogen-Bonds and Short Contacts (Å and °) for 1-Amino –2-Hydroxypropanephosphonic Acid

D-H...A	*(D...A)*	*(D-H)*	*(H...A)*	*<(DHA)*
O(3)-H(3)... O(1)i	2.602(5)	1.02	1.61	165.0
N-H(6)...O(4)ii	2.819(5)	0.96	1.93	152.5
N-H(7)...O(2)i	2.790(6)	1.03	1.82	157.2
N-H(5)...O(2)iii	2.784(5)	0.96	1.88	156.2
O(4)-H(4)... O(1)vi	2.703(5)	0.86	1.96	144.2
N-H(5)...O(4)	2.896(5)	0.96	2.50	104.6
N-H(6)...O(2)	2.922(5)	0.96	2.50	106.4

Symmetry code: (i) -x+1/2,y-1/2,z: (ii) x+1/2,-y+1/2,-z; (iii) x-1/2,- y+1/2,-z; (vi)-x-1/2,y −1/2,z

In addition, the amino hydrogen atoms H(6) and H(5) participate in intermolecular hydrogen bonds involving O(2) and O(4) atoms of the phosphonic acid and hydroxyl groups. Apart from that, the O(2) of the phosphoryl group and O(4) of the hydroxyl group form relatively short intramolecular contacts with the H(5) and H(6) atoms of the amino group, with N...O(4) and N...O(2) distances of 2.896(5), 2.992(5) Å (H...O 2.50 Å) and angles of 104.6 and 106.4°, respectively.

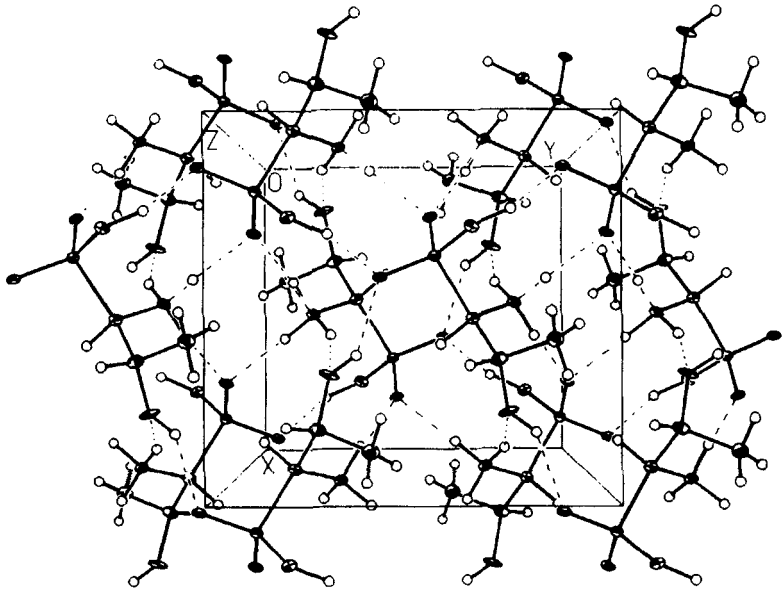


FIGURE 5 Packing diagram of molecules in crystal with hydrogen bonds indicated

CONCLUSIONS

Synthesis of β-hydroxy-α-aminoalkanephosphonates **1** and **2** by means of the reaction of phenyldiazonium chloride with 2-oxophosphonates afforded all four feasible diastereomers. However, as was shown by X-ray, crystallisation from an aqueous solution allows isolation of only one pair of diastereomers (1R, 2S) and (1S, 2R) of the phosphonic acid **3**.

The chiral praseodymium shift reagent may successively derivatize methoxy or ethoxy ^{13}C resonances of phosphonate esters which differ in

the stereogenic C_α centre nearest to the P=O co-ordination site. For those which have an additional C_β stereogenic atom 2D HMQC experiment may be an aid in an assessment of the stereoisomers. Thus, enantiomeric or stereoisomeric composition of a wide range of phosphonate esters appears to be easy to evaluate by practically single HMQC 2D experiment.

EXPERIMENTAL

General

Starting materials for preparations were obtained commercially and were used as obtained from suppliers. The products of syntheses were characterised by ^1H and ^{31}P NMR spectroscopy and microanalyses, melting points are uncorrected. All NMR spectra were recorded on a Bruker DRX spectrometer at 300.13 MHz for ^1H, 121.50 MHz for ^{31}P and 75.47 MHz for ^{13}C. Reported δ values are given in relation to $SiMe_4$ (^1H, ^{13}C) and 85% H_3PO_4 (^{31}P) and all downfield shifts are denoted as positive. Coupling constants are in Hz.

Mass spectra were performed on a Finnigan Mat TSQ 700 triple quadrupole mass spectrometer operated with an ESI source. The spray voltage was 4.5 kV and the heated capillary temperature was maintained at 473 K. The relative intensities are given in relation to the highest intensity peak (100%).

The on-line LC-MS analysis was performed using the HPLC system consistent of a P 4000 inert quaternary gradient pump and a 250 mm ODS RP-C18 column operated at 1 ml/min. The mobile phase was water (A) and 80% acetonitrile (B) and the sample was eluted using a linear gradient of 0–35 % B in 30 min. UV detection at 210 nm was applied.

^{13}C NMR LIS Analysis

Freshly prepared, weighted portions of esters **1** or **2** were added to the known amount of $CDCl_3$ and dissolved by shaking to give a final concentration between $2 .10^{-3} - 4.5. 10^{-3}$ M. Increments of solid $Pr(hfc)_3$ were added directly to the sample to give LSR : substrate molar ratio up to 0.6 – 0.8 and spectra were run immediately. $Pr(hfc)_3$ was obtained from Aldrich.

Chloroform-d was purchased from Cambridge Isotope Laboratories. In order to shorten the time of experiment ^{13}C NMR spectra enhanced by polarization transfer (DEPT-45) were recorded in all cases. The $^{1}H - ^{13}C$ HMQC correlation spectra ofthe "fully shifted samples" were acquired using standard Bruker program. Temperature (300 K) was maintained at the stated level (± 1) in all experiments.

X-ray Structure Determination

The crystals of **3** were grown by slow evaporation from water and due to layered structure were generally of poor quality. Thus, single crystal (aprox. dimensions 0.2 × 0.23 × 0.1 mm) for X-ray data collection was selected of testing a number of several specimens.

Crystal data:

$C3H_{10}NO_4P$, M=155.09, colourless, transparent, orthorhombic, space group Pbca, a= 7.915(2), b= 8.493(4), c= 18.688(2) Å, V= 1256.2(5) $Å^3$ (by least-squares refinement of the diffractometer angles for 25 automatically centered reflections in the range 2θ 18–32 °), Z=8, D_c=1.640 g $.cm^{-1}$, F(000)=656, μ= 3.531 mm^{-1}.

Data collection:

The intensity data were collected on an KM4 four-circle diffractometer [temperature 293(2) K]. 2θ range 5–140°; 0<h<9, 0<k<10, 0<1<22 using graphite monochromated Cu-Kα X-radiation (λ=1.5418 Å) and ω-2θ scanning. Of 1500 unique data, 799 had F<4σ(F). The data were corrected for Lorentz and polarization effect, but no absorption correction.

Structure solution:

The approximate positions of the non-hydrogen atoms were determined by direct methods (SHELXS-86[29]). The structure was refined by full-matrix least-squares methods (SHELXL-93[30]) using F^2 data and anisotropic temperature factors for all the non-hydrogen atoms. All the hydrogen atoms were located on Fourier difference maps and included in the refinement with fixed positions. At convergence, the discrepancy factors R(F) , wR(F^2) and S(F^2) were 0.0509 ,0.156 and 1.103, respectively.

The weighting scheme W =1/[$\delta^2(F_o2)$+(a·P)2 + b .P] where P=[f·Max of (0 or F_o2)+(1-f). F_c2)] (a=0.1451, b= 4.05, f = 1/3)was found to give satisfactory analyses of variance. The final difference Fourier map was essentially featureless, with largest difference peak and hole of 0.51 and –0.56 e $Å^3$, respectively.

Atomic coordinates, thermal parameters and bond lengths and angles have been deposited as supplementary material at the Cambridge Crystallographic Data Centre (CCDC)[31].

Syntheses

Diethyl 1-amino- 2-hydroxypropanephosphonate (1)

A solution of 0.1 mole of benzenediazonium chloride, neutralised with sodium acetate to pH=7, was added at 0°C to 0.1 mole of diethyl 2-oxopropanephosphonate and the reaction mixture was neutralised with $NaHCO_3$ below 15°C. The crude product, isolated by extraction with ethyl ether, drying with $MgSO_4$ and evaporation in vacuo, was hydrogenated at room temp. and 10^7 Pa using a Raney nickel catalyst. The reaction mixture was filtered through celite, and the ester **1** was isolated by column chromatography on silica gel (eluent CH_2Cl_2/ethyl acetate 3:2). Final purification was accomplished by crystallisation of the oxalate salt. Pure acid **3** was obtained by hydrolysis of the residue after hydrogenation with cnc. HCl followed by chromatography on Dowex (H^+) W50-X8.

1: NMR ($CDCl_3$) ^1H : δ 1.26 (t, J_{H-H}=6.3, 3H), δ 1.31 (t, J_{H-H}=7.1, 6H), δ 2.97 (d × d J_{H-H}=5.9, J_{H-P}=13.9, 1H), δ 3.95 (m, 1H), δ 4.13 (m, 4H); ^{31}P : δ 28.22 (s) **3**: Yield (71%); mp. 223–225°C[32]; NMR (D_2O) ^1H : δ 1.23 (d, J=6.7, 3H), δ 3.30 (d × d, J_{H-H} 3.7, J_{H-P} 15.3, 1H) δ 4.22 (m., 1H); ^{31}P : δ 10.18 (s) ^{13}C : δ 17.45 , δ 54.80 (J_{C-P}=137.6), δ 65.09(J_{C-P} =2.3); ES-MS m/z (%) 156.0 ([M+H]$^+$, 16), 311.0 ([M_2+H]$^+$, 100) ;
(Calcd. for $C_3H_{10}NO_4P$: C, 23.25%; H, 6.50%; N, 9.05%; P, 20.00%. Found: C, 23.10%; H, 6.25%; N, 8.85%; P, 20.25%)

Diethyl 1-amino –2-hydroxy-2-phenylethanephosphonate (2)

The procedure through the hydrogenation stage was essentially as described for **1** with the difference that diethyl 2-phenyl-2-oxoethanephosphonate was used as substrate. Final purification was accomplished by crystallisation of the oxalate salt, m.p. 67–68°C; (Calcd. for $C_{14}H_{22}NO_8P$:

N, 3.80%; P, 8.31%. Found: N, 3.68%; P, 8.48%). Hydrolysis of **2** with 48% HBr in acetic acid gave **4.**

2: NMR ($CDCl_3$) ^1H : δ 1.26, 1.31 (d × t J_{H-H} = 7.1, 6H), δ 3.24 (d × d J_{H-H}=8.0, J_{H-P} = 13.2, 1H), δ 4.22 – 4.01 (m., 5H), δ 4.80 (d × d J_{H-H} = 8.0, J_{H-P}= 12.4, 1H), δ 7.43 – 7.30 (m., 4H), ^{31}P : δ 27.50 (s)

4:Yield (47 %); m.p. 191–193°C; NMR (D_2O) ^1H : δ 3.51 (d × d, 1H, J_{H-H}=7.3, J_{H-P}=13.1) δ 5.00 (d × d, J_{H-H}=7.3, J_{H-P}=9.7, 1H) δ 7.43–7.33 (m., 5H); ^{31}P : δ 10.55 (s); ^{13}C δ 54.17 (J_{C-P} = 136.9), δ 71.84, δ 127.52, δ 129.40, δ 129.55 ; ES-MS m/z (%) 218.1($[M+H]^+$, 10), 435.1($[M_2+H]^+$, 100) ; (Calcd. for $C_8H_{12}NO_4P$: N, 6.40%; P, 14.20%. Found: N, 6.31%; P, 13.91%)

Diethyl N-benzyloxycarbonyl-L-phenylalanyl-1-amino -2-hydroxypropane phosphonate (5)

N-carbobenzyloxy-L-phenylalanine (0.5 g, 0.0018 mole) was dissolved in 10 ml of $CHCl_3$ and 0.3 ml of anhydrous NEt_3 was added at 0°C[33]. The solution was then cooled to −15°C and treated with 0.2 ml of ethyl chloroformate, followed with 0.0017 mole of the phosphonate **1** in 10 ml of THF. The mixture was left overnight at room temp., the solvents were evaporated under reduced pressure, the residue was dissolved in ethyl acetate and washed consecutively with aqueous HCl, $NaHCO_3$ and NaCl solutions. The crude product obtained after removal of solvent was crystallised from ether. Yield 53%.

N-(L-phenylalanyl)-2-hydroxy-1-aminopropanephosphonic acid (6)

Diethyl N-benzyloxycarbonyl-L-phenylalanyl-1-amino-2-hydroxy-propanephosphonate (1mmole) was dissolved in anhydrous $CHCl_3$ (3 ml) and treated with 4.5 mmole of Me_3SiBr[34]. After 24 h at room temp. the solvent was evaporated, the residue dissolved in 2 ml of methanol and left for several hours. Addition of 2 ml of ether precipitated the desired product as a white solid. ES-MS m/z 303.1 $[M+H]^+$.

Acknowledgements
We gratefully acknowledge Komitet Bada Naukowych for financial support (grants no. 2P3 0305507 and 3TO9 A 07910)

References

[1] P. Mastalerz, *in Handbook of Organophosphorus Chemistry* (Dekker, New York, 1992) p 277.

[2] P. Kafarski and P. Mastalerz, *Beitr. Wirkst. Forsch.*, **21,** 1 (1984).

[3] L. Maier and, P. J. Diel, *Phosphorus, Sulfur, and Silicon,* **90,** 259 (1994).

[4] L. Maier. *ibid,* **53,** 43 (1990).

[5] E. K. Baylis, C. D. Campbell and J. G. Dingwall, *J. Chem. Soc. Perkin Trans. I,* 2845 (1984).

[6] D. Redmore, *Topics in Phosphorus Chemistry* (Interscience, New York, 1987), Chap. 8, p. 515.

[7] V. P. Kukhar, V. A. Soloshonok and V. A. Solodenko, *Phosphorus, Sulfur, and Silicon,* **92,** 239 (1994).

[8] D. Seebach, R. Charczuk, Ch. Gerber, P. Renaud, H. Berner und H. Schneider, *Helv. Chim. Acta,* **72,** 401 (1989).

[9] A. Togni and L. Pastor, *Tetrahedron Lett.,* **30,** 1071 (1989).

[10] M. Sawamura, Y. Ito and T. Hayashi, *Tetrahedron Lett.,***30,** 2247 (1989).

[11] A. Bongini, R. Camerini, S. Hofman and M. Panunzio, *Tetrahedron Lett.,* **35,** 8045 (1994).

[12] M. Kitamura, M. Tokunaga, T. Pham, W. D. Lubell and R. Noyori, *Tetrahedron Lett.,* **36,** 5769 (1995).

[13] U. Schöllkopf, T. Wintel, *Synthesis,* 1033 (1984).

[14] A. Bongini, R. Camerini and M. Panunzio, *Tetrahedron Asymmetry,* **7,** 1467 (1996).

[15] D. Parker, *Chem.Rev.,* **91,** 1441 (1991).

[16] R. Hulst, R. M. Kellog, B. L .Feringa, *Rec. Trav.Chim.Pays-Bas,* **114,** 115, (1995).

[17] M. .D. McCreary, D. W. Lewis, D. L. Wernick, G. M. Whitesides, *J. Am. Chem. Soc.,* **96,** 1038 (1974).

[18] T. Visvanathan, A.Toland, *J. Chem. Edu.,* **72,** 945 (1995).

[19] P. S. Williams, F. J. Troendle, K. S. Venkatasubban, R.Rothchild, *Spect. Lett.,* **29,** 1229 (1996).

[20] F. J. Troendle, K. S. Venkatasubban, R. Rothchild,ibid, **30,** 435 (1997).

[21] S.E.Weinstein, M.S.Vining, T.J.Wenzel, *Magn.Res.Chem.,* **35,** 273 (1997).

[22] M. Shapiro, M. Lin, R. Versace, R. C. Petter, *Tetrahedron* **Asymmetry, 7,** 2169, (1996).

[23] G. König, A. D.Wright, *Magn.Res.Chem.,* **33,** 178 (1995).

[24] B. Lejczak, P. Kafarski, M. Sztajer, P. Mastalerz, J.Med.Chem., **29,** 2211 (1986).

[25] L. Gray, R. Rothchild, *Spect. Lett.,* **27,** 935 (1994).

[26] C.K. Johnson, *ORTEPII, Report ORNL-5138,* Oak Ridge National Laboratory, Tennessee, 1976.

[27] W. Sawka-Dobrowolska, T. Głowiak, J.Barycki, *J. Crystallogr.Spec. Res.,* **19,** 861 (1989).

[28] W. Maniukiewicz, W. Kwiatkowski,R.H. Blessing, *Acta Cryst. Sec. C,* 1736 (1996).

[29] G. M. Sheldrick, *Acta Crystallogr. Sec A,* **46,** 467 (1990).

[30] G. M. Sheldrick *SHELXL 93,* Program for the Refinement of Crystal Structures,University of Gottingen, 1993.

[31] Atomic coordinates, thermal parameters and bond lenghts and angles have been deposited as suplementary material at the Cambridge Crystallographic Data Centre (CCDC)

[32] M. Jeżowska-Bojczuk, T. Kiss, H. Kożłowski, P. Dewek and J. Barycki, *J. Chem. Soc. Dalton Trans.,* 811 (1994).

[33] P. Kafarski, B. Lejczak, P. Mastalerz, J. Szewczyk, C. Wasielewski, *Can. J. Chem.,* **60,** 3081 (1982)

[34] C. Hubert, B. Oussaid, G. Etemad-Moghadam, M. Koenig, B. Garrigues, *Synthesis,* 51 (1994)