Synthesis of the Phosphonic Acid Analog of Serine

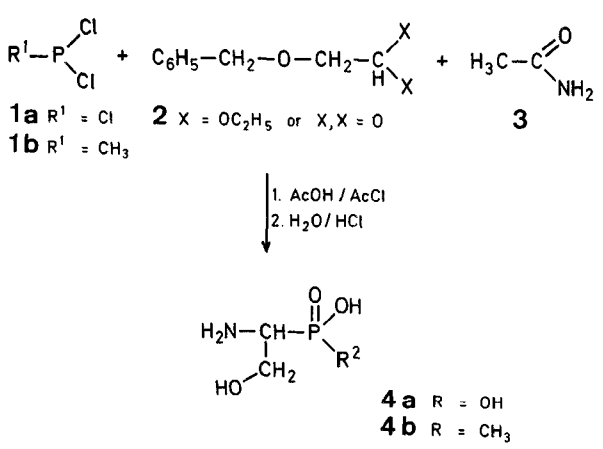
Barbara Lejczak, Paweł Kafarski, Mirosław Soroka, Przemysław Mastalerz

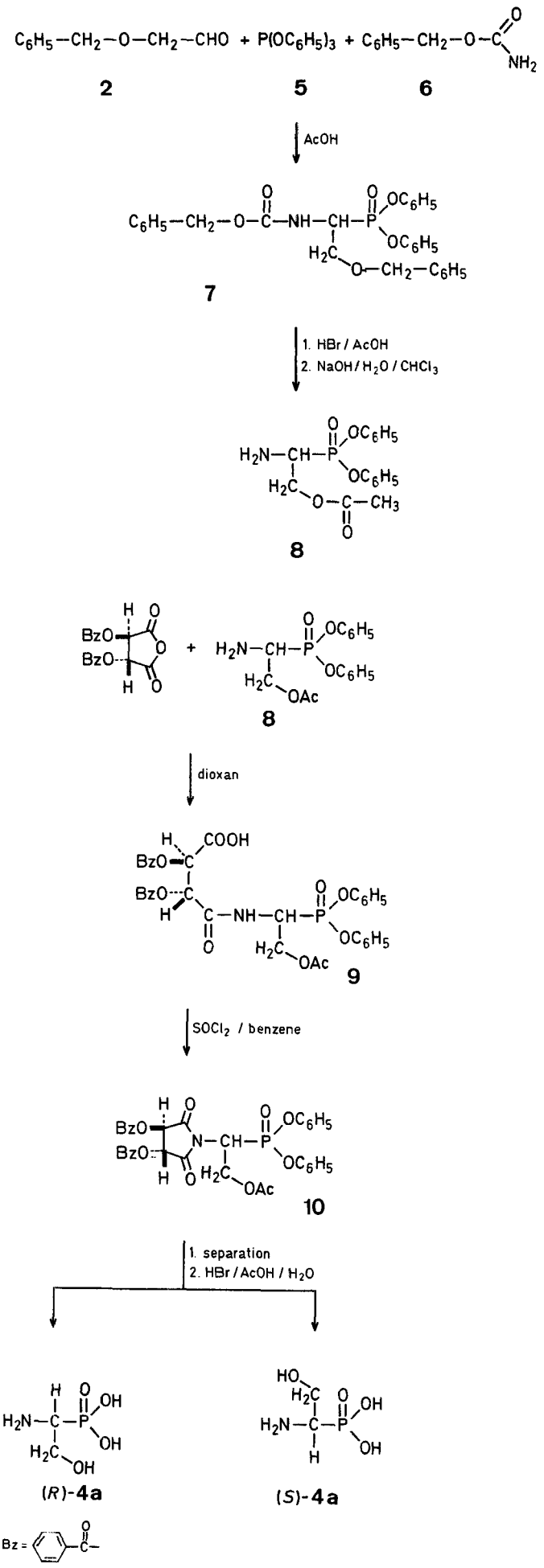
Institute of Organic and Physical Chemistry, Technical University of Wrocław, Wybrzeże Wyspiańskiego 27, Pl-50-370 Wrocław, Poland

The biological activity of aminoalkanephosphonic acid derivatives[1] and of oligopeptides derived from aminoalkanephosphonic acids[2,3,4] stimulates the interest in phosphonic analogs of protein amino acids. We have recently described the preparation of such analogs of glutamic and aspartic acids[5], proline[6], tryptophan[7], cysteine[8] and serine[9,10]. However, the described synthesis of the phosphonic analogue of serine is a multistep process and requires the cumbersome preparation of aziridine-2-phosphonic acid used as a starting material.

We report here a simple method for the synthesis of the phosphonic analogue of serine, the preparation of its enantiomers, and of peptides containing racemic 1-amino-2-hydroxyethanephosphonic acid.

Phosphonic **(4a)** and *P*-methylphosphinic **(4b)** analogues of serine were obtained by amidoalkylation of phosphorus trichloride **(1a)** or methyldichlorophosphine **(1b)** with benzyloxyacetaldehyde **(2)** or its diethyl acetal and acetamide **(3)**. Our procedure represents an improved version of the general method of amidoalkylation described in Ref.[11].

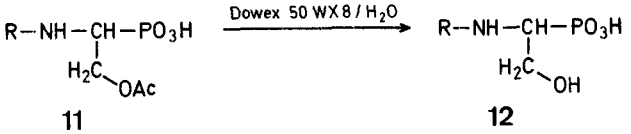




The analogous reaction[12] of triphenyl phosphite (**5**) with benzyloxyacetaldehyde (**2**) and benzyl carbamate (**6**) yielded diphenyl 1-(benzyloxycarbonylamino)-2-benzyloxyethane phosphonate (**7**) which was converted into diphenyl 1-amino-2-acetoxyethanephosphonate (**8**) by reaction with hydrogen bromide in glacial acetic acid followed by treatment with aqueous sodium hydroxide.

Since optically pure aminoalkanephosphonic acids are desirable for biochemical purposes, we undertook efforts to resolve the ester **8**. Thus, ester **8** was reacted with dibenzoyl-L-tartaric anhydride to give the amide **9**. Using the previously described method[13], we were unable to separate the diastereoisomeric amides **9**. They were converted into the imides **10** which were separated by crystallization. Hydrolysis of the resultant diastereoisomeric compounds **10** gave the desired enantiomeric 1-amino-2-hydroxyethanephosphonic acids (*R*)-**4a** and (*S*)-**4a**.

Phosphonodipeptides are known to impede the growth of various bacterial species at low concentration level[1,2,14,15]. Using ester **8** as substrate and the previously described procedure[16] we have therefore synthesized the dipeptides **11** containing a *P*-terminal 1-amino-2-acetoxyethanephosphonic acid. Dipeptides **11** are easily deacetylated to peptides **12** possessing an unblocked hydroxy group by passing them through Dowex 50W X8, 50–100 mesh column and elution with water.



11 **12**

R = Ala, Val, Leu, Pro

The melting points were determined on a Koffler apparatus and are uncorrected. The I.R. spectra were measured on a Perkin Elmer 621 instrument, the ^{1}H-N.M.R. spectra on Tesla BS 467 instrument at 60 MHz.

1-Amino-2-hydroxyethanephosphonic Acid (4a):

Method A: Acetamide (**3**; 11.8 g, 0.2 mol) is mixed with acetic acid (30 ml) and the mixture cooled to ~ 0 °C. To the vigorously stirred mixture, acetyl chloride (7.1 ml, 0.1 mol) is added dropwise (a strongly exothermic effect is observed). After cooling the mixture to 10 °C, a gelous precipitate appears. Then, benzyloxyacetaldehyde (**2**; 20.0 g, 0.12 mol) is added stirring is continued for 2 h. At this point all the solid material dissolves, phosphorus(III) chloride (**1a**; 9.7 ml, 0.1 mol) is added, the solution thus obtained is refluxed for 70 min, and the volatile components are removed under reduced pressure. The resultant oil is dissolved in concentrated hydrochloric acid (300 ml) and the solution refluxed for 8 h. Hydrochloric acid is then evaporated using a rotatory evaporator and the dark residue is dissolved in water (1000 ml). The solution is decolorized with charcoal and concentrated to ~ 20 ml. The resultant solution is applied to a Dowex 50W X8, 50–100 mesh column and product **4a** is eluted with water, collecting ninhydrine-positive fractions. Water is stripped off under reduced pressure and the residue is mixed with absolute ethanol. The crystalline product is isolated by suction and dried in a vacuum exsiccator; yield: 6.6 g (48%). On heating product **4a**, an oil is formed at 80 °C and the product decomposes at 165–168 °C (Ref.[9], m.p. 80 °C).

$C_2H_8NO_4P \cdot 1.5\,H_2O$	calc.	N 8.33	P 18.42
(168.1)	found	8.12	18.62

I.R.(KBr): ν = 3700–1800 ($NH_3^{\oplus}$, OH, $PO_3H^{\ominus}$, CH); 1620 ($NH^{\oplus}$); 1170, 1035 ($PO_3H^{\ominus}$) cm^{-1}.

^{1}H-N.M.R. (D_2O/HMDS$_{ext}$): δ = 3.3–4.7 ppm (m, 3H, CH—CH_2).

Method B: Acetamide (**3**; 11.8 g, 0.2 mol) is mixed with glacial acetic acid (30 ml) and the mixture cooled to ~ 0 °C. To this is added acetyl chloride (7.1 ml, 0.1 mol), dropwise and with stirring. A strongly exothermic effect is observed. The mixture is then cooled to ~ 10 °C, benzyloxyacetaldehyde diethyl acetal (**2**, X = OC_2H_5; 20.6 g, 0.105 mol) is added dropwise, and stirring is continued at room temperature overnight until all solid material has dissolved. Work-up is as in Method A; yield: 4.2 g (30%).

1-Amino-2-hydroxyethane-*P*-methylphosphinic Acid (4b):

Method B is performed with methyldichlorophosphine (**1b**; 9.0 ml, 0.1 mol) in place of **1a**; yield of **4b**: 10.1 g (72%). On heating product **4b**, an oil is formed at ~ 70 °C; the product decomposes at 164–167 °C.

$C_{29}H_{28}NO_6P$	calc.	N 2.71	P 5.99
(517.5)	found	2.38	5.77

I.R.(KBr): ν = 3700–3200 ($NH_3^{\oplus}$, $PO_2^{\ominus}$, OH, CH); 1620 ($NH^{\oplus}$); 1165, 1040 ($PO_2^{\ominus}$) cm^{-1}.

^{1}H-N.M.R. (H_2O/HMDS$_{ext}$): δ = 1.80 (d, 3H, $^2J_{P,H}$ = 15.5 Hz, CH_3); 3.35–4.85 ppm (m, 3H, CH—CH_2).

Diphenyl 1-Benzyloxycarbonylamino-2-benzyloxyethanephosphonate (7):

This compound is prepared according to Ref.[12]; yield: 51%; m.p. 93–96 °C.

$C_{29}H_{28}NO_6P$	calc.	N 2.71	P 5.99
(517.5)	found	2.38	5.77

I.R.(KBr): γ = 3270 (NH); 1705 (CO); 1595, 1535 (NH); 1250 (PO); 945 (POC) cm^{-1}.

^{1}H-N.M.R. ($CDCl_3$/HMDS$_{int}$): δ = 3.2–4.0 (m, 3H, P—CH—CH_2); 4.32 (s, 2H, CH_2—O—CH_2—C_6H_5); 4.98 (s, 2H, C_6H_5—CH_2—O—CO); 5.85 (d, 1H, $^3J_{H,H}$ = 10.5 Hz, NH); 6.98, 7.04 (2s, 5H each, P—O—C_6H_5); 7.10, 7.16 ppm (2s, 5H each, CH_2—C_6H_5).

Diphenyl 2-Acetoxy-1-aminoethanephosphonate Hydrobromide (8 · HBr):

This compound is prepared according to Ref.[12]; yield: 88%; m.p. 137–139 °C.

$C_{16}H_{19}BrNO_5P$	calc.	N 3.37	P 7.44	Br 19.20
(416.2)	found	3.40	7.15	19.30

I.R.(KBr): ν = 3300–2200 ($NH^{\oplus}$); 1745 (CO); 1585 (NH); 1240 (PO); 950 (POC) cm^{-1}.

^{1}H-N.M.R. (D_2O/HMDS$_{ext}$): δ = 2.00 (s, 3H, CH_3); 3.9–4.8 (m, 3H, CH—CH_2); 6.9–7.5 ppm (m, 10H, 2 C_6H_5).

Diphenyl 2-Acetoxy-1-(2,3-dibenzoyloxy-3-carboxypropanoylamino)-ethanephosphonate (9):

A suspension of diphenyl 2-acetoxy-1-aminoethanephosphonate hydrobromide (**8** · HBr; 8.3 g, 0.02 mol) in chloroform (75 ml) is washed with aqueous 1 normal sodium hydroxide (2 × 50 ml) and with saturated sodium chloride solution (2 × 50 ml), dried with sodium sulfate, filtered, and evaporated under reduced pressure to give the free compound **8**; yield: 5.4 g. Compound **8** is dissolved in dioxan (60 ml) and *O,O'*-dibenzoyl-L-tartaric anhydride (5.45 g, 0.016 mol) is added. The mixture is shaken until the anhydride has dissolved and is allowed to stand at room temperature for 3 days. Evaporation of dioxan under reduced pressure affords the amide **9** as a yellow oil; yield: 5.8 g (86%); $[\alpha]_{578}^{20}$: – 69° (*c* 1, acetone).

$C_{34}H_{30}NO_{12}P$	calc.	N 2.07	P 4.58
(675.6)	found	2.18	4.39

^{1}H-N.M.R. (DMSO/HMDS$_{int}$): δ = 2.07 (s, 3H, CH_3); 3.6–5.1 (m, 3H, CH—CH_2); 4.5–5.4 (m, 2H, CH—P, COOH); 6.22 (br.s, 2H, 2 CO—OCH); 6.7–8.4 (m, 20H, 4 C_6H_5); 9.62 ppm (d, 1H, $^3J_{H,H}$ = 9.0 Hz, NH).

Diastereoisomers of Diphenyl 2-Acetoxy-1-(3,4-dibenzoyloxy-2,5-dioxo-1-pyrrolidinyl)-ethanephosphonate (10a and 10b):

Thionyl chloride (3 ml) is added dropwise to a stirred solution of compound **9** (5.8 g, 0.0173 mol) in benzene (100 ml). The mixture is refluxed for 2 h and the benzene then evaporated. The oily residue is

dissolved in warm ether (70 ml) and this solution cooled to ~ −5 °C in the refrigerator. The precipitated crystalline diastereoisomer **10a** is isolated by suction (the filtrate is saved) and recrystallized twice from ether; yield: 3.4 g (60%); m.p. 112–113 °C; $[\alpha]_{578}^{20}$: +95° (*c* 1, acetone). The product is the pure isomer **10a** according to its ^{31}P-N.M.R. spectrum (only one peak).

$C_{34}H_{28}NO_{11}P$	calc.	N 2.13	P 4.71
(657.5)	found	2.28	4.73

I.R.(KBr): *v* = 1755, 1740, 1730, 1710(CO); 1280(PO); 1010, 990(POC) cm^{-1}.

^{1}H-N.M.R. ($CDCl_3$/HMDS$_{int}$): *δ* = 1.94 (s, 3H, CH$_3$); 4.0–5.0 (m, 3H, CH—CH$_2$); 5.72 (s, 2H, 2 COOH); 7.10 [s, 10H, P(OC$_6$H$_5$)$_2$]; 7.0–8.05 ppm (m, 10H $^3J_{H,H}$ = 7.0 Hz, 2 C$_6$H$_5$—CO—O).

^{31}P-N.M.R. ($CDCl_3$/85% $H_3PO_{4\,ext}$): *δ* = 8.12 ppm.

The filtrate from the separation of **10a** is concentrated to a volume of 25 ml. Addition of hexane (80 ml) then leads to the precipitation of an oily product. The oil is separated and dissolved in ether (40 ml). The solution is filtered, hexane (50 ml) is again added, the oily product is isolated, and this operation is repeated 3 times to give the crystalline diastereoisomer **10b**; yield: 2.65 g (47%); m.p. 91–92 °C; $[\alpha]_{578}^{20}$: +72° (*c* 1, acetone).

$C_{34}H_{28}NO_{11}P$	calc.	N 2.13	P 4.71
(657.5)	found	2.07	4.56

I.R.(KBr): *v* = 1745, 1710(CO); 1285, 1265(PO); 1015, 995(POC) cm^{-1}.

^{1}H-N.M.R. ($CDCl_3$/HMDS$_{int}$): *δ* = 1.94 (s, 3H, CH$_3$); 3.65–4.5 (m, 1H, CH—P); 4.5–5.05 (m, 2H, CH$_2$—OAc); 5.76 (s, 2H, 2 CO—O—CH); 7.08 [s, 10H, P(OC$_6$H$_5$)$_2$]; 7.0–8.0 (m, 10H, $^3J_{H,H}$ = 7.0 Hz, 2 C$_6$H$_5$—CO—O).

^{31}P-N.M.R. ($CDCl_3$/85% $H_3PO_{4\,ext}$): *δ* = 8.61 ppm.

(*R*)- and (*S*)-1-Amino-2-hydroxyethanephosphonic Acids [(*R*)-4a and (*S*)-4a]:

The imide **10a** or **10b** (0.9 g, 13.7 mmol) is dissolved in a mixture of 40% hydrobromic acid (20 ml) and acetic acid (20 ml), the solution refluxed for 20 h, and allowed to cool. The precipitated benzoic acid is filtered off and the solvents are removed under reduced pressure. The residue is dissolved in water (30 ml) and the solution filtered and decolorized with charcoal. The solvent is stripped off, the residue dissolved in ethanol (~ 60 ml), and the product **4a** precipitated by the addition of pyridine to pH ~ 6. The precipitate is isolated by suction and dried in vacuum.

(R)-*1-Amino-2-hydroxyethanephosphonic Acid* [(*R*)-**4a**; from **10a**]; yield: 77%; the product becomes oily at ~ 70 °C and decomposes at 198–200 °C; $[\alpha]_{578}^{20}$: −30 °C (*c* 1, 1 normal NaOH).

$C_2H_8NO_4P \cdot H_2O$	calc.	N 8.49	P 18.49
(159.1)	found	8.38	18.52

(S)-*1-Amino-2-hydroxyethanephosphonic Acid* [(*S*)-**4a**; from **10b**]; yield: 77%; the product becomes oily at ~ 70 °C and decomposes at 186–188 °C; $[\alpha]_{578}^{20}$: +35° (*c* 1, 1 normal NaOH).

$C_2H_8NO_4P \cdot 1.5H_2O$	calc.	N 8.33	P 18.42
(168.1)	found	8.28	18.29

Both enantiomers (*R*)-**4a** and (*S*)-**4a** are strongly hygroscopic. Their I.R. and N.M.R. spectra are identical with those of racemic 1-amino-2-hydroxyethanephosphonic acid.

Peptides (11) containing *P*-Terminal 2-Acetoxy-1-aminoethanephosphonic Acid:

These phosphonopeptides are prepared as described in Ref.[16].

1-(L-Alanylamino)-2-acetoxyethanephosphonic Acid (**11**, R = Ala); yield: 39%; m.p. 186–189 °C; $[\alpha]_{578}^{20}$: +91° (*c* 1, H_2O).

$C_7H_{15}N_2O_6P \cdot 2H_2O$	calc.	N 9.66	P 10.67
(290.2)	found	9.70	10.72

I.R.(KBr): *v* = 3700–2000, 3280(NH); 1720, 1660(CO); 1540(NH); 1235, 1150, 1075($PO_3H^{\ominus}$) cm^{-1}.

^{1}H-N.M.R. (D_2O/HMDS$_{ext}$): *δ* = 1.04, 1.09 (d, 3H, $^3J_{H,H}$ = 7.5 Hz, CH$_3$); 1.62 (s, 3H, O—CO—CH$_3$); 3.3–4.3 ppm (m, 4H, 2 CH, CH$_2$—OAc).

1-(L-Valylamino)-2-acetoxyethanephosphonic Acid (**11**, R = Val); yield: 17%; m.p. 209–210 °C (dec.); $[\alpha]_{578}^{20}$: +27° (*c* 1, H_2O).

$C_9H_{19}N_2O_6P \cdot 1.5H_2O$	calc.	N 9.06	P 10.01
(309.3)	found	8.77	10.34

I.R.(KBr): *v* = 3700–2000, 3300(NH); 1730, 1655(CO); 1560, 1520(NH); 1165, 1080, 1035($PO_3H^{\ominus}$) cm^{-1}.

^{1}H-N.M.R. (D_2O + D_2SO_4/HMDS$_{ext}$): *δ* = 1.41 (d, 6H, $^3J_{H,H}$ = 7.0 Hz, 2 CH$_3$); 2.48 (s, 3H, CO—CH$_3$); 2.3–2.8 (m, 1H, $^3J_{H,H}$ = 6.5 Hz, H$_3$C—CH); 4.33 (br.d, 1H, $^3J_{H,H}$ = 6.0 Hz, CH—CH—NH); 4.5–5.3 ppm (m, 3H, CH—CH$_2$—OAc).

1-(L-Leucylamino)-2-acetoxyethanephosphonic Acid (**11**, R = Leu); yield: 35%; m.p. 240–245 °C (dec.); $[\alpha]_{578}^{20}$: +21° (*c* 1, H_2O).

$C_{10}H_{21}N_2O_6P \cdot 3H_2O$	calc.	N 8.00	P 8.84
(350.3)	found	8.15	8.66

I.R.(KBr): *v* = 3700–2000, 3250(NH); 1740, 1655(CO); 1540(NH); 1240, 1160, 1075($PO_3H^{\ominus}$)cm^{-1}.

^{1}H-N.M.R. (D_2O + D_2SO_4/HMDS$_{ext}$): *δ* = 1.05 (d, 6H, $^3J_{H,H}$ = 6.5 Hz, 2 CH$_3$); 1.5–2.05 (m, 3H, H$_3$C—CH—CH$_2$); 2.19 (s, 3H, O—CO—CH$_3$); 4.13 (br.t, 1H, $^3J_{H,H}$ = 6.5 Hz, CH—CO); 4.2–5.0 ppm (m, 3H, CH—CH$_2$—O—CO).

1-(L-Prolylamino)-2-acetoxyethanephosphonic Acid (**11**, R = Pro); yield: 42%; m.p. 240–247° (dec.); $[\alpha]_{578}^{20}$: −43° (*c* 1, H_2O).

$C_9H_{17}N_2O_6P$	calc.	N 10.00	P 11.05
(280.2)	found	9.69	11.31

I.R.(KBr): *v* = 3700–2000, 1735, 1655(CO); 1550(NH); 1230, 1160, 1060($PO_3H^{\ominus}$)cm^{-1}.

^{1}H-N.M.R. (D_2O/HMDS$_{ext}$): *δ* = 2.12 (s, 3H, O—CO—CH$_3$); 1.9–2.7 (m, 4H, CH$_2$—CH$_2$—CH—CO); 3.47 (t, 2H, $^3J_{H,H}$ = 6.5 Hz, CH$_2$—NH); 3.7–4.8 ppm (m, 4H, 2 CH, CH$_2$—OAc).

Peptides (12) containing *P*-Terminal 1-Amino-2-hydroxyethanephosphonic Acid; General Procedure:

The phosphonopeptide **11** (1.0 g) is dissolved in water (10 ml) and this solution is applied to a Dowex 50W × 8 column (200 × 20 mm). The product is eluted with water. The ninhydrin-positive fractions are collected and the solvent is evaporated under reduced pressure. The resultant oily material solidifies upon treatment with ethanol.

1-(L-Leucylamino)-2-hydroxyethanephosphonic Acid (**12**, R = Leu); yield: 80%; m.p. 250–253 °C (dec.); $[\alpha]_{578}^{20}$: +31° (*c* 1, H_2O).

$C_8H_{18}N_2O_5P \cdot H_2O$	calc.	N 10.33	P 11.42
(271.2)	found	10.08	11.44

I.R.(KBr): *v* = 3700–2000, 3450(OH); 3280(NH); 1665 (CO); 1560(NH); 1150, 1035 ($PO_3H^{\ominus}$)cm^{-1}.

^{1}H-N.M.R. (D_2O + D_2SO_4/HMDS$_{ext}$): *δ* = 0.95 (d, 6H, $^3J_{H,H}$ = 6.5 Hz, 2 CH$_3$); 1.3–2.1 (m, 3H, $^3J_{H,H}$ = 6.5 Hz, H$_3$C—CH—CH$_2$); 3.3–4.5 ppm (m, 4H, CH—CH$_2$—OH, CH—CO).

1-(L-Prolylamino)-2-hydroxyethanephosphonic Acid (**12**, R = Pro); yield: 75%; m.p. 266–268 °C (dec.); $[\alpha]_{578}^{20}$: −98° (*c* 1, H_2O).

$C_7H_{14}N_2O_5P \cdot H_2O$	calc.	N 10.57	P 11.68
(265.1)	found	10.76	11.55

I.R.(KBr): *v* = 3700–2000, 3275(NH); 3190(OH); 1650(CO); 1550(NH); 1130, 1050 ($PO_3H^{\ominus}$)cm^{-1}.

^{1}H-N.M.R. (D_2O/HMDS$_{ext}$): *δ* = 1.7–2.65 (m, 4H, $^3J_{H,H}$ = 6.5 Hz, CH$_2$—CH$_2$—CH); 3.33 (t, 2H, $^3J_{H,H}$ = 7.0 Hz, CH$_2$—NH); 3.5–4.65 ppm (m, 4H, 2 CH—N, CH$_2$—OH).

This work was supported by Grant R.1.9.

Received: November 2, 1983

[1] E. Neuzil, A. Cassaigne, *Exp. Ann. Biochim. Med.* **34**, 165 (1980).
[2] A. Cassaigne, A. M. Lacoste, E. Neuzil, *Act. Chim. Therapeut.* **7**, 18 (1980).
[3] F. R. Atherton et al., *Antimicrob. Agents Chemother.* **15**, 677 (1979).
[4] P. Mastalerz, L. Kupczyk-Subotkowska, Z. S. Herman, G. Laskawiec, *Naturwissenschaften* **69**, 46 (1982).

[5] J. Oleksyszyn, E. Gruszecka, P. Kafarski, P. Mastalerz, *Monatsh. Ch.* **113**, 59 (1982).
[6] W. Subotkowski, R. Tyka, P. Mastalerz, *Pol. J. Chem.* **54**, 503 (1980).
[7] W. Subotkowski, J. Kowalik, R. Tyka, P. Mastalerz, *Pol. J. Chem.* **55**, 853 (1981).
[8] J. Kowalik, J. Zygmunt, P. Mastalerz. *Pol. J. Chem.* **55**, 713 (1981).
[9] J. Zygmunt, P. Mastalerz, *Pol. J. Chem.* **52**, 2271 (1978).
[10] J. Zygmunt, P. Mastalerz, *Pol. J. Chem.* **55**, 411 (1981).
[11] J. Oleksyszyn, R. Tyka, P. Mastalerz, *Synthesis* **1978**, 479.
[12] J. Oleksyszyn, L. Subotkowska, P. Mastalerz, *Synthesis* **1979**, 985.
[13] P. Kafarski, B. Lejczak, J. Szewczyk, *Can. J. Chem.*, in press.
[14] T. Kametani et al., *Heterocycles* **18**, 295 (1982).
[15] F. R. Atherton et al., *Antimicrob. Agents Chemother.* **22**, 571 (1982).
[16] B. Lejczak, P. Kafarski, J. Szewczyk, *Synthesis* **1982**, 412.