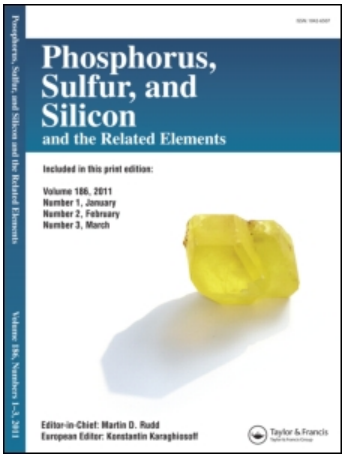

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

SYNTHESIS OF PHOSPHONIC ANALOGUES OF γ-GLUTAMYL TRIPEPTIDES AND DEPSIPEPTIDES

Maria Hoffmann[a]
[a] Department of Organic Chemistry, Technical University of Gdansk, Gdansk, Poland

To cite this Article Hoffmann, Maria(1992) 'SYNTHESIS OF PHOSPHONIC ANALOGUES OF γ-GLUTAMYL TRIPEPTIDES AND DEPSIPEPTIDES', Phosphorus, Sulfur, and Silicon and the Related Elements, 73: 1, 173 — 184
To link to this Article: DOI: 10.1080/10426509208034443
URL: http://dx.doi.org/10.1080/10426509208034443

Phosphorus, Sulfur, and Silicon, 1992, Vol. 73, pp. 173–184
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SYNTHESIS OF PHOSPHONIC ANALOGUES OF γ-GLUTAMYL TRIPEPTIDES AND DEPSIPEPTIDES

MARIA HOFFMANN

Department of Organic Chemistry, Technical University of Gdansk, 80-952 Gdansk, Poland

(Received July 23, 1992; in final form August 17, 1992)

The analogues of biologically active ophthalmic (γ-*L*-glutamyl-α-*L*-aminobutyrylglycine) and norophthalmic (γ-*L*-glutamyl-*L*-alanylglycine) acids containing the phosphonic analogue of glycine or glycolic acid have been synthesized by standard procedure. The key intermediates were *P*-terminal di-*p*-nitrobenzyl phosphonodipeptides and phosphonodidepsipeptides derivatives of amino and hydroxymethylphosphonic acids.

Key words: Aminomethylphosphonate; hydroxymethylphosphonate; γ-glutamylphosphonopeptides, γ-glutamylphosphonodepsipeptides.

INTRODUCTION

Phosphonic analogues of amino acids, either isolated from nature or obtained synthetically display a wide range of interesting properties which may have considerable economic and clinical potential.[1]

In addition to aminophosphonic acids, peptides derived from them also show interesting activity mainly as antibacterial agents and inhibitors of enzymes.[1]

Some phosphonopeptides display significant neurophysiological effects. γ-Glutamylphosphonodipeptides containing phosphonic acid analogues of glycine and alanine are strongly antagonistic to *N*-methyl-*D*-aspartate (NMDA) inhibiting NMDA evoked responses in the rat.[2,3]

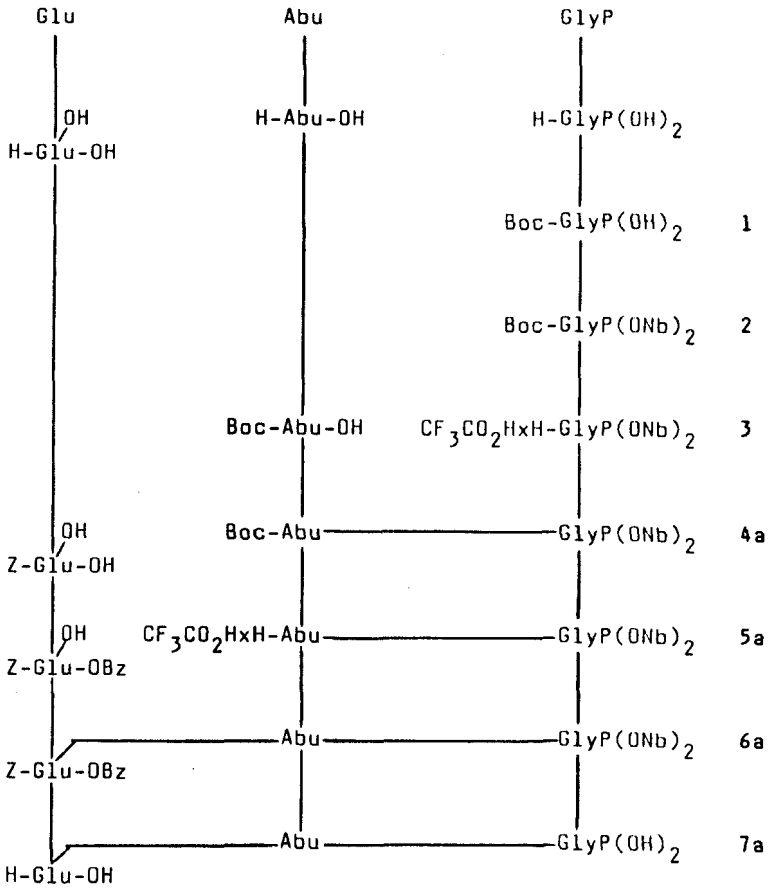
Continuing our study on the synthesis of phosphonopeptides[4] and phosphonodepsipeptides[5–7] we have synthesized several analogues of biologically active natural peptides—namely ophthalmic and norophthalmic acids.

Ophthalmic (γ-*L*-glutamyl-α-*L*-aminobutyrylglycine) and norophthalmic (γ-*L*-glutamyl-*L*-alanylglycine) acids occur in calf lens and they are competitive inhibitors in the reaction catalysed by glyoxalase.[8,9]

RESULTS AND DISCUSSION

The synthesis of all phosphonotripeptides and depsipeptides were accomplished by the 1 + 2 method as detailed in Schemes 1–4.

Di-*p*-nitrobenzyl esters were used to protect phosphonic group in aminomethyl and hydroxymethylphosphonic acids.[10] Di-*p*-nitrobenzyl aminomethylphosphonate trifluoracetat **3** was obtained by three step procedure involving *t*-butoxycarbonyl (Boc) blocking of amino group esterification of *N*-Boc derivative with *O*-(*p*-nitrobenzyl)-*N*,*N*′-dicyclohexylisourea and removal of *N*-Boc with trifluoracetic acid.[11]



Synthesis of Glu—Abu-GlyP 7a

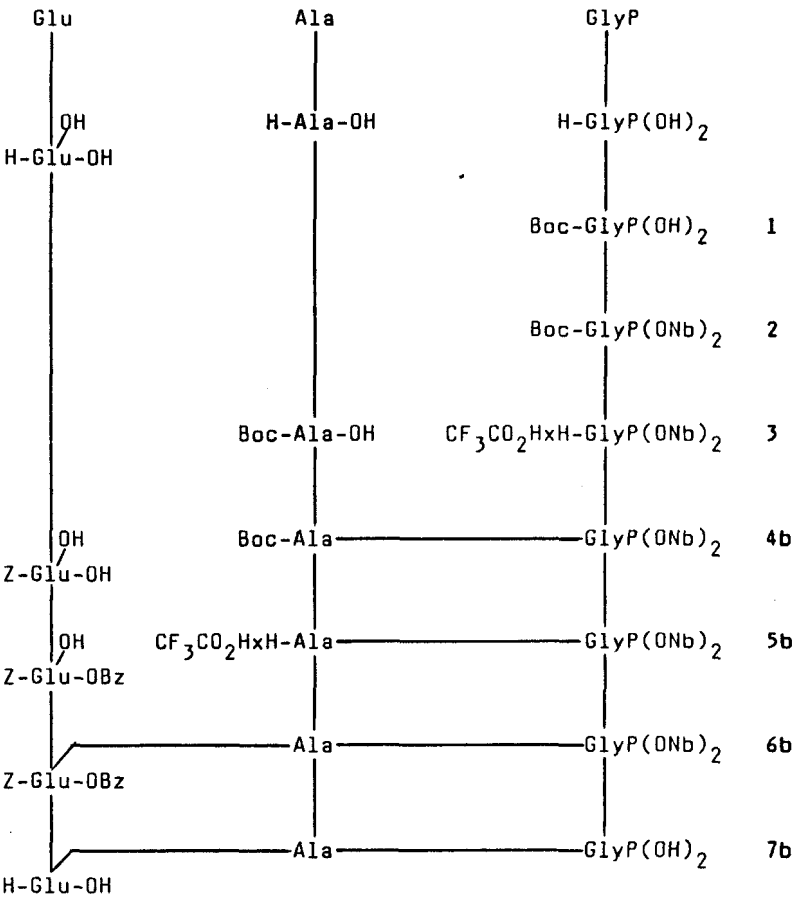
GlyP: $H_2N\text{-}CH_2P(O)(OH)_2$

SCHEME 1

In order to obtain the *P*-terminal dipeptydyl or didepsipeptydyl fragment of phosphonic analogues of ophthalmic and norophthalmic acids bearing the amino and hydroxymethylphosphonic acid residues **4a–d** we used *t*-butoxycarbonyl group as aminobutyryl or alanyl amino group protection. Mixed anhydride activation previously found[12] to give good results in phosphonopeptide synthesis was applied in the synthesis of *P*-terminal phosphonodipeptides **4a** and **4b**.

DCC method in the presence of 4-(*N*,*N*-dimethylamino)pyridine and 1-hydroxybenzotriazole[7] gave good yields of phosphonodidepsipeptides **4c** and **4d**.

The acidolysis of *N*-Boc group in compounds **4a–d** proceeded smoothly to give crystalline trifluoracetates of di-*p*-nitrobenzyl esters of *P*-terminal phosphonodipeptides **5a** and **5b**.



Synthesis of Glu⌐¬—Ala-GlyP 7b

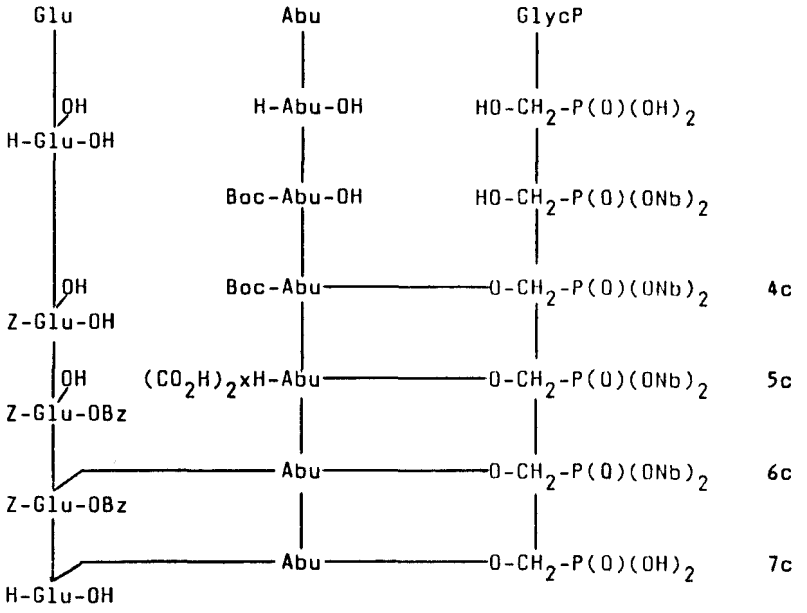
GlyP: $H_2N\text{-}CH_2P(O)(OH)_2$

SCHEME 2

Oily trifluoroacetates of di-*p*-nitrobenzyl esters of phosphonodidepsipeptides were transformed into crystalline oxalates **5c** and **5d**.

In the synthesis of phosphonotripeptide and tridepsipeptide derivatives **6a–d** we employed benzyloxycarbonyl protection of amino group and benzyl protection of α-carboxyl group in Glu since they match phosphonodi-*p*-nitrobenzyl ester susceptibility to the deprotection method, namely catalytic hydrogenolysis.

BOP[13] was successfully applied as peptide coupling reagent in 1 + 2 synthesis of protected γ-glutamylphosphonotripeptides **6a** and **6b**. The synthesis of γ-peptide bond in phosphonotridepsipeptides **6c** and **6d** was accomplished by mixed anhydride method.



Synthesis of Glu—Abu-GlycP 7c

GlycP: $HO\text{-}CH_2P(O)(OH)_2$

SCHEME 3

The simultaneous and nondestructive removal of all protective groups in compounds **6a–d** was achieved by catalytic hydrogenation to give compounds **7a–d**.

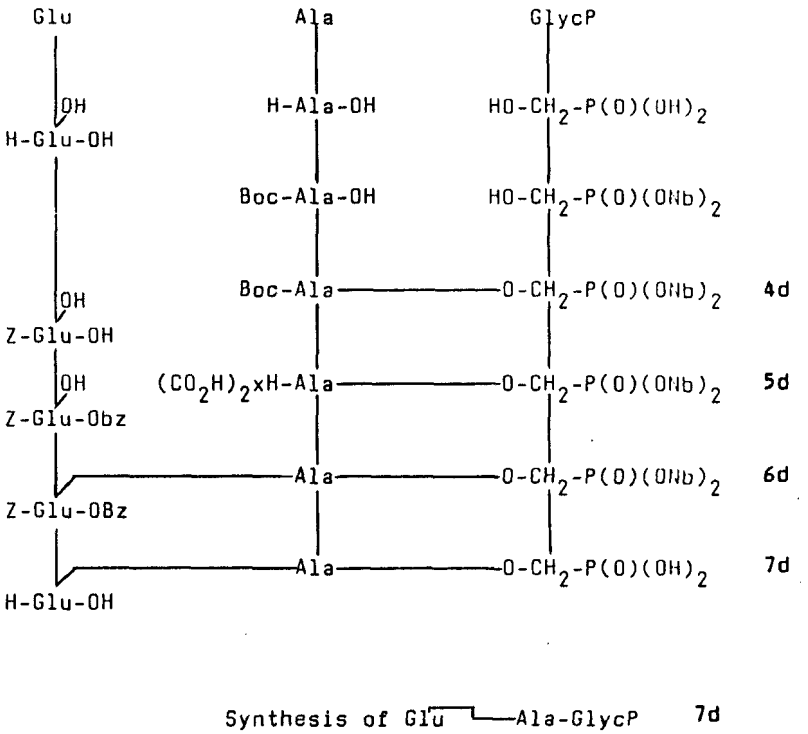
p-Toluidine formed in the hydrogenolysis was removed by ion exchange chromatography.

The newly synthesized compounds **6a–d** and **7a–d** are characterized in Tables I, II and III, respectively.

EXPERIMENTAL

Melting points are not corrected. ^{1}H-NMR spectra were recorded with a Varian EM-360A spectrometer at 60 MHz or a Bruker MSL at 300 MHz. Liquid matrix secondary ions mass spectrometry (LSIMS) was performed on a AMD 604 mass spectrometer. Optical rotations were measured using a Polamat A (Carl-Zeiss, Jena) polarimeter.

N-t-butoxycarbonylaminomethylphosphonic acid, **1**. To a solution of aminomethylphosphonic acid (1.1 g, 10 mmol) and triethylamine (7 ml, 50 mmol) in a mixture of water (10 ml) and tetrahydrofurane (10 ml), di-*tert*-butyldicarbonate (2.4 g, 11 mmol) is added. The mixture is stirred at room temperature for 3 days. The tetrahydrofurane and excess of triethylamine are removed under reduced pressure and the solution is extracted two times with ethyl ether.



SCHEME 4

The water phase is acidified with saturated potassium hydrogen sulfate, extracted 5 times with ethyl acetate and the combined extracts are dried with magnesium sulfate. The solvent is partly removed to give a crystalline product. Yield 1.22 g (61%).

^{1}H-NMR (DMSO) δ: 1.33 (*s*, 9H, $(CH_3)_3C$); 3.17 (dd, 2H, J_{PH} = 10 Hz, J_{HH} = 4 Hz, CH_2P); 6.33–6.82 (*m*, 1H, NH); 9.90 (*s*, 2H, $P(O)(OH)_2$).

$C_6H_{14}NO_5P$ (211.15) calc.: C 34.14 H 6.68 N 6.63%
found: C 34.03 H 7.00 N 6.70%

Di-p-nitrobenzyl ester of N-t-butoxycarbonylaminomethylphosphonic acid, **2**. To a solution of O-(*p*-nitrobenzyl)-*N,N'*-dicyclohexylisourea (7.19 g, 20 mmol) in a mixture of benzene (40 ml) and dimethylformamide (5 ml) *N-t*-butoxycarbonylaminomethylphosphonic acid (2.1 g, 10 mmol) is added. The reaction mixture is refluxed for 8 h. The precipitated urea is filtered off. The filtrate is evaporated under reduced pressure and ethyl acetate (40 ml) is added to the residue. The solution is washed successively with 3% sodium hydrogen carbonate solution (20 ml) and water (20 ml), dried with magnesium sulfate and evaporated. The crude ester is purified by recrystallization from ethyl acetate/*n*-hexane. Yield 3.94 g (82%); m.p. 118–119°C.

^{1}H-NMR ($CDCl_3$) δ: 1.33 (*s*, 9H, $(CH_3)_3C$); 3.70 (dd, 2H, J_{PH} = 10 Hz, J_{HH} = 4 Hz, CH_2P); 4.66–5.00 (*m*, 1H, NH); 5.20 (*d*, 4H, J_{PH} = 8 Hz, 2 $POCH_2$); 7.60, 8.23 (two *d*, 8H arom.).

$C_{20}H_{24}N_3O_9P$ (481.38) calc.: C 49.90 H 5.02 N 8.72%
found: C 50.20 H 5.07 N 8.74%

TABLE I

Synthetic and physical data of protected γ-glutamylphosphonotripeptides **6a**, **6b** and tridepsipeptides **6c**, **6d**

No	Formula	Yield /%/	M.p. /°C/ solvent	$[\alpha]_{546}^{20}$ c, solvent	Molecular formula	Analysis C H N Calc.	/%/ Found
6a	Z-GluOBz-AbuNHCH$_2$P(O)(ONb)$_2$	84	92-5 Et_2O	-22.2 (1.8, $CHCl_3$)	$C_{38}H_{42}N_5O_{13}P$ 807.73	56.52 5.24 8.67	56.78 5.00 8.80
6b	Z-GluOBz-AlaNHCH$_2$P(O)(ONb)$_2$	77	130-132 AcOEt	-26.4 (1.5, $CHCl_3$)	$C_{37}H_{40}N_5O_{13}P$ 793.70	55.98 5.08 8.82	56.30 4.96 8.82
6c	Z-GluOBz-AbuOCH$_2$P(O)(ONb)$_2$	74	102-104 Et_2O	-6.0 (1.5, $CHCl_3$)	$C_{38}H_{41}N_4O_{14}P$ 808.71	56.43 5.11 6.92	56.10 4.90 6.86
6d	Z-GluOBz-AlaOCH$_2$P(O)(ONb)$_2$	60	86-88 Et_2O	-6.7 (1, $CHCl_3$)	$C_{37}H_{39}N_4O_{14}P$ 794.68	55.92 4.94 7.05	56.01 4.72 6.89

Abbreviations used: Z = $C_6H_5CH_2OCO$, Nb = $CH_2C_6H_4$-p-NO_2, Bz = $-CH_2C_6H_5$

TABLE II

^{1}H-NMR Spectra of compounds **6a–d** δ (ppm) in $CDCl_3$

6a	0.86 (t, 3H, J = 7.3 Hz, CH_3); 1.51-2.29 (m, 6H, CH_2CH_2 + CH_2); 3.57-3.72 (m, 1H, CH); 3.81-3.94 (m, 1H, CH); 4.25-4.48 (m, 2H, CH_2P); 5.06-5.15 (m, 8H, $2CH_2C_6H_5$ + $2CH_2C_6H_4$); 6.16 (bd, 1H, NH); 6.32 (bd, 1H, NH); 7.18 (bd, 1H, NH); 7.25-7.32 (m, 10H, $2C_6H_5$); 7.41-7.49 (m, 4H, C_6H_4); 8.11-8.18 (m, 4H, C_6H_4)
6b	1.28 (d, 3H, J = 6.9 Hz, CH_3); 2.04-2.29 (m, 4H, CH_2CH_2); 3.60-3.76 (m, 1H, CH); 3.76-3.92 (m, 1H, CH); 4.35-4.46 (m, 2H, CH_2P); 5.06-5.16 (m, 8H, $2CH_2C_6H_5$ + $2CH_2C_6H_4$); 6.20 (bd, 1H, NH); 6.43 (bd, 1H, NH); 7.22 (bd, 1H, NH); 7.29-7.32 (m, 10H, $2C_6H_5$); 7.41-7.49 (m, 4H, C_6H_4); 8.12-8.17 (m, 4H, C_6H_4)
6c	0.90 (t, 3H, J = 7.3 Hz, CH_3); 1.62-2.24 (m, 6H, CH_2CH_2 + CH_2); 4.39-4.54 (m, 2H, 2CH); 4.52 (d, 2H, J_{PH} = 8.2 Hz, OCH_2P); 5.09-5.21 (m, 8H, $2CH_2C_6H_5$ + $2CH_2C_6H_4$); 5.70 (bd, 1H, NH); 6.32 (bd, 1H, NH); 7.26-7.33 (m, 10H, $2C_6H_5$); 7.48-7.52 (m, 4H, C_6H_4); 8.17-8.23 (m, 4H, C_6H_4)
6d	1.36 (d, 3H, J = 8 Hz, CH_3); 1.52-2.32 (m, 4H, CH_2CH_2); 4.30-4.60 (m, 2H, 2CH); 4.53 (d, 2H, J = 8 Hz, OCH_2P); 5.07-5.25 (m, 8H, $2CH_2C_6H_5$ + $2CH_2C_6H_4$); 5.62 (bd, 1H, NH); 6.28 (bd, 1H, NH); 7.25-7.38 (m, 10H, 2 C_6H_5); 7.45-7.55 (m, 4H C_6H_4); 8.18-8.27 (m, 4H, C_6H_4)

TABLE III

Synthetic, analytical and physical data of phosphonic analogues of ophthalmic and norophthalmic acids **7a–d**

No $(M+H)^+$	Formula ^{1}H-NMR (D_2O) δ (ppm)	Yield /%/ M.p. /°C/	$[\alpha]_{546}^{20}$ c 1, H_2O	Molecular formula	Analysis C H N /%/ Calc.	 Found
7a	HO_2C-CH(NH_2)CH_2CH_2-CONH-CH(CH_2CH_3)-CONH$CH_2PO_3H_2$	75 213-215	-24.0	$C_{10}H_{20}N_3O_7P$ 325.25	36.92 6.19 12.90	36.60 6.39 12.60
326	0.88 (t, 3H, J = 7.4 Hz, CH_3); 1.55-1.86 (m, 2H, CH_2); 2.05-2.20 (m, 2H, CH_2); 2.51 (t, 2H, J = 7.4 Hz, CH_2); 3.40 (d, 2H, J = 12.6 Hz, CH_2P); 3.88 (t, 1H, J = 6.4 Hz, CH); 4.08-4.20 (m, 1H, CH)					
7b	HO_2C-CH(NH_2)CH_2CH_2-CONH-CH(CH_3)-CONH$CH_2PO_3H_2$	78 212-213	-28.0	$C_9H_{18}N_3O_7P$ 311.22	34.73 5.82 13.50	34.55 6.12 13.25
312	1.38 (d, 3H, J = 7.2 Hz, CH_3); 2.11-2.22 (m, 2H, CH_2); 2.51 (t, 2H, J = 7.3 Hz, CH_2); 3.45 (d, 2H, J = 12.5 Hz, CH_2P); 3.88 (t, 1H, J = 6.3 Hz, CH); 4.31 (q, 1H, J = 7.2 Hz, CH)					

7c	$HO_2C-\underset{NH_2}{CH}CH_2CH_2-CONH-\underset{CH_2CH_3}{CH}-COOCH_2PO_3H_2$	60 210-212	-30.0	$C_{10}H_{19}N_2O_8P$ $\times \frac{1}{2} H_2O$ 335.24	35.79 5.96 8.35	35.96 5.88 8.07
327	0.84 (t, 3H, J = 7.4 Hz, CH_3); 1.59-1.89 (m, 2H, CH_2); 2.06-2.16 (m, 2H, CH_2); 2.46 (t, 2H, J = 7.2 Hz, CH_2); 3.91 (t, 1H, J = 6.5 Hz, CH); 4.15 (d, 2H, J = 8.5 Hz, OCH_2P); 4.24-4.31 (m, 1H, CH)					
7d	$HO_2C-\underset{NH_2}{CH}CH_2CH_2-CONH-\underset{CH_3}{CH}-COOCH_2PO_3H_2$	60 204-206	-38.0	$C_9H_{17}N_2O_8P$ $\times \frac{1}{2} H_2O$ 321.22	33.62 5.60 8.71	33.65 5.74 8.57
313	1.33 (d, 3H, J = 7.3 Hz, CH_3); 2.06-2.16 (m, 2H, CH_2); 2.44 (t, 2H, J = 7.2 Hz, CH_2); 3.93 (t, 1H, J = 6.4 Hz, CH); 4.15 (d, 2H, J = 8.5 Hz, OCH_2P); 4.36 (q, 1H, J = 7.2 Hz, CH)					

Di-p-nitrobenzyl aminomethylphosphonate trifluoroacetat, **3**. To Di-*p*-nitrobenzyl *N-t*-butoxycarbonylaminomethylphosphonate (4.81 g, 10 mmol), 6 ml of trifluoroacetic acid is added at 0°C. The mixture is kept for 30 min. at 20°C and the solution is evaporated at room temperature under reduced pressure. The residue crystallizes after addition of ethyl ether. Yield 4.7 g (95%); m.p. 127–128°C.

^{1}H NMR (TFA) δ: 3.00–3.66 (*m*, 2H, CH_2P); 4.80 (*d*, 4H, J_{PH} = 10 Hz, 2 $POCH_2$); 7.00–8.00 (*m*, 11H, NH_3 + 8H arom.).

$C_{17}H_{17}N_3O_9PF_3$ (493.30) calc.: C 41.39 H 3.47 N 8.52%
found: C 41.35 H 3.29 N 8.22%

Trifluoroacetates of P-terminal Di-p-nitrobenzyl Phosphonodipeptides, **5a**, **5b**. *N-t*-butoxycarbonyl-*L*-amino acid (*L*-α-aminobutyric acid or *L*-Ala) (2 mmol) is dissolved in dry $CHCl_3$ (6 ml) containing NEt_3 (0.28 ml, 2 mmol) and the solution is cooled to −5°C. Then ethyl chloroformate (0.2 ml, 0.22 mmol) is added and the mixture is kept at −5°C for 30 min. Next a solution of di-*p*-nitrobenzyl aminomethylphosphonate trifluoroacetat (0.987 g, 2 mmol) in dry $CHCl_3$ (4 ml) containing NEt_3 (3 ml) is added. The mixture is kept at room temperature for 12 h. The resulting solution is washed successively with water (6 ml), 1M $KHSO_4$ (2 × 6 ml), water (6 ml), 5% $NaHCO_3$ solution (2 × 6 ml), water (6 ml) and dried over $MgSO_4$. The solvent is distilled off and the oily residue representing chromatographically pure peptides **4a** (yield 1.1 g, 97%) or **4b** (yield 1.04 g, 95%) is dissolved in 3 ml of trifluoroacetic acid and left to stand at room temperature for 40 min. The reaction mixture is evaporated in vacuo. Trifluoroacetates of *P*-protected phosphonodipeptides **5a** and **5b** precipitate by addition of ethyl ether.

Compound **5a**. Yield 1.1 g (96%); m.p. 145–147°C.

^{1}H-NMR (TFA) δ: 0.50 (*t*, 3H, *J* = 7 Hz, CH_3); 1.17–1.90 (*m*, 2H, CH_2); 3.23–4.10 (*m*, 3H, CH_2P + CH); 4.80 (*d*, 4H, J_{PH} = 10 Hz, 2 $POCH_2$); 6.50–8.00 (*m*, 8H arom. + 3H, NH_3).

$C_{21}H_{24}N_4O_{10}PF_3$ (580.40) calc.: C 43.45 H 4.16 N 9.65%
found: C 43.36 H 4.07 N 9.40%

Compound **5b**. Yield 1.0 g (95%); m.p. 174–177°C.

^{1}H-NMR (TFA) δ: 1.33 (*d*, 3H, *J* = 7 Hz, CH_3); 3.50–3.95 (*m*, 2H, CH_2P); 4.00–4.40 (*m*, 1H, CH); 4.90 (*d*, 4H, J_{PH} = 10 Hz, 2 $POCH_2$); 6.65–8.00 (*m*, 8H arom. + 3H, NH_3).

$C_{20}H_{22}N_4O_{10}PF_3$ (566.38) calc.: C 42.40 H 3.91 N 9.89%
found: C 42.27 H 3.74 N 9.84%

Di-p-nitrobenzyl esters of P-terminal N-t-butoxycarbonylphosphonodidepsipeptides, **4c**, **4d**. To a solution of *N-t*-butoxycarbonyl-*L*-amino acid (*L*-α-aminobutyric or *L*-Ala) (1.1 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.132 g, 1.1 mmol) in 12 ml CH_2Cl_2, di-*p*-nitrobenzyl hydroxymethylphosphonate[10] (0.382 g, 1 mmol), 1-hydroxybenzotriazole (0.140 g, 1 mmol) and DCC (0.226 g, 1.1 mmol) is added. The reaction mixture is kept at 20°C for 18 h. *N*,*N*-dicyclohexylurea (DCU) is filtered off and the filtrate evaporated to dryness. The residue is dissolved in ethyl acetate (20 ml). The solution is washed successively with 1 M $KHSO_4$ (2 × 15 ml), water (1 × 15 ml), 5% $NaHCO_3$ solution (2 × 15 ml), dried with $MgSO_4$ and evaporated to dryness. The residue is purified by flash chromatography on silica gel (eluent-benzene). The solvent is evaporated and the product is crystallized from ethyl ether/hexane.

Compound **4c**. Yield 70%; m.p. 92–94°C.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (*t*, 3H, *J* = 6 Hz, CH_3); 1.40 (*s*, 9H, $(CH_3)_3C$); 1.60–2.20 (*m*, 2H, CH_2); 4.10–4.50 (*m*, 1H, CH); 4.67 (*d*, 2H, J_{PH} = 8 Hz, CH_2P); 4.75–5.20 (*m*, 1H, NH); 5.36 (*d*, 4H, J_{PH} = 8 Hz, 2 $POCH_2$); 7.70, 8.40 (two *d*, 8H arom.).

$C_{24}H_{30}N_3O_{11}P$ (567.47) calc.: C 50.79 H 5.33 N 7.40%
found: C 50.94 H 5.29 N 7.64%

Compound **4d**. Yield 70%; m.p. 70–72°C.

^{1}H-NMR ($CDCl_3$) δ: 1.40 (*d*, 3H, *J* = 7 Hz, CH_3); 1.42 (*s*, 9H, $(CH_3)_3C$); 4.00–4.50 (*m*, 1H, CH); 4.57 (*d*, 2H, J_{PH} = 10 Hz CH_2P); 4.72–5.18 (*m*, 1H, NH); 5.27 (*d*, 4H, 2 $POCH_2$); 7.78, 8.36 (two *d*, 8H arom.).

$C_{23}H_{28}N_3O_{11}P$ (553.44) calc.: C 49.91 H 5.09 N 7.59%
found: C 50.05 H 5.10 N 7.90%

Oxalates of P-terminal di-p-nitrobenzyl phosphonodidepsipeptides, **5c**, **5d**. Compound **4c** or **4d** (2.25 mmol) is treated with 1.4 ml of trifluoroacetic acid at 0°C and allowed to stand for 30 min. at room

temperature. The reaction mixture is evaporated in vacuo and the oily residue is suspended in ethyl acetate (15 ml). The mixture is washed with saturated sodium hydrogen carbonate (2 × 5 ml), saturated aqueous sodium chloride and dried with magnesium sulfate. The resultant solution is poured into a vigorously stirred solution of anhydrous oxalic acid (0.19 g, 2.2 mmol) in ethyl ether (15 ml). The mixture is allowed to stand 2 hours in a refrigerator and the product is collected by filtration.

Compound **5c**. Yield 85%; m.p. 98–100°C.

^{1}H-NMR (TFA) δ: 0.66 (*t*, 3H, J = 7 Hz, CH_3); 1.36–2.10 (*m*, 2H, CH_2); 3.72–4.21 (*m*, 1H, CH); 4.50 (*d*, 2H, J_{PH} = 10 Hz, CH_2P); 5.00 (*d*, 4H, J = 10 Hz, 2 $POCH_2$); 6.70–8.16 (*m*, 11H, NH_3 + 8H arom.).

$C_{21}H_{24}N_3O_{13}P/H_2O$ (575.39)	calc.:	C 43.83	H 4.55	N 7.30%
	found:	C 43.90	H 4.59	N 7.20%

Compound **5d**. Yield 84%; m.p. 93–5°C.

^{1}H-NMR [$(CD_3)_2C{=}O$] δ: 1.66 (*d*, 3H, J = 7 Hz, CH_3); 4.21–5.54 (*m*, 1H, CH); 4.72 (*d*, 2H, J = 8 Hz, CH_2P); 5.24 (*d*, 4H, J = 10 Hz, 2 $POCH_2$); 7.00–8.36 (*m*, 11H, NH_3 + 8H arom.).

$C_{20}H_{22}N_3O_{13}P/H_2O$ (561.37)	calc.:	C 42.79	H 4.30	N 7.48%
	found:	C 42.90	H 4.30	N 7.23%

General procedure for the synthesis of protected γ-glutamylphosphonotripeptides, **6a**, **6b**. Triethylamine (0.28 ml, 2 mmol) is added at 0°C to a suspension of *N*-benzyloxycarbonyl-*L*-glutamic acid α-benzyl ester[14] (408 mg, 1.1 mmol), *P*-terminal di-*p*-nitrobenzyl phosphonodipeptide trifluoroacetate **5a** or **5b** (1 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) (442 mg, 1 mmol) in methylene chloride (8 ml). The mixture is stirred at room temperature for 8 h and evaporated to dryness. Ethyl acetate (15 ml) is added to the residue. The solution is washed successively with saturated potassium hydrogen sulfate (2 × 15 ml) water (1 × 15 ml), 5% sodium hydrogen carbonate (2 × 15 ml), water (15 ml) and dried with magnesium sulfate. The solvent is removed under reduced pressure and the crude product is purified by crystallization (Tables I and II).

General procedure for the synthesis of protected γ-glutamylphosphonotridepsipeptides, **6c**, **6d**. *N*-benzyloxycarbonyl-L-glutamic acid α-benzyl ester (408 mg, 1.1 mmol) is dissolved in dry methylene chloride (4 ml) containing NEt_3 (0.14 ml, 1 mmol) and cooled to −5°C. Ethyl chloroformate (0.1 ml, 0.11 mmol) is added and the mixture is kept at −5°C for 30 min. A solution of di-*p*-nitrobenzyl phosphonodidepsipeptide oxalate **5c** or **5d** (1 mmol) in dry methylene chloride (4 ml) containing NEt_3 (0.28 ml) is added. The mixture is kept at room temperature overnight and then evaporated to dryness. Ethyl acetate (10 ml) is added to the residue. The solution is washed successively with saturated potassium hydrogen sulfate (2 × 15 ml), water (15 ml), 5% sodium hydrogen carbonate (2 × 15 ml), water (15 ml), and dried with magnesium sulfate. The solvent is removed under reduced pressure and the crude product crystallizes after addition of ethyl ether (Tables I and II).

General procedure for the synthesis of γ-glutamylphosphonotripeptides and tridepsipeptides, **7a–d**. To a solution of compound **6b** (0, 5 mmol) in methanol (20 ml) or compounds **6a**, **6c**, **6d** in ethanol (20 ml), 10% palladium on charcoal (150 mg) is added. The mixture is hydrogenated at ambient temperature and pressure for 4 h. The catalyst is filtered off and washed with ethyl alcohol and water. The filtrate is evaporated to dryness. The residue is dissolved in water (5 ml) and passed through Zerolite 225/H^+. The combined fractions are evaporated and the residue crystallizes after addition of ethanol. Yields and properties of free phosphonotripeptides **7a**, **7b** and phosphonotridepsipeptides **7c**, **7d** are presented in Table III.

ACKNOWLEDGEMENT

I wish to thank Dr. O. B. Nagy from Catholic University of Louvain for a gift of *L*-α-aminobutyric acid.

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