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Synthesis and Spectroscopic Characterization of Protected *N*-Phosphonomethylglycine Dipeptides

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Abstract: A series of terminally blocked dipeptides containing *C*-terminal *N*-phosphonomethylglycine (glyphosate, an extremely effective non-selective post-emergence herbicide) have been synthesized by a solution method. The presence of their two conformers, *cis* (*syn*) and *trans* (*anti*), was shown in solutions by NMR spectroscopy. Molecular structures of the peptides were also determined in the solid state by X-ray diffraction. The attempts for the selective and total removal of the groups protecting amino, carboxylic and phosphonate functions were in many cases unsuccessful due to the formation of cyclic structures and breakage of the phosphorus-to-carbon bond. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: conformers; peptide synthesis; *N*-phosphonomethylglycine; phosphono peptides; X-ray diffraction

INTRODUCTION

The disclosure of herbicidal activity of *N*-phosphonomethylglycine (**1**, glyphosate, Scheme 1) in 1971 was a milestone in the biochemistry of aminophosphonic acids [1,2]. This discovery initiated extensive research concerned with the design, synthesis and evaluation of biological properties of new aminophosphonates of commercial interest.

Despite inhibiting 5-enol-pyruvylshikimate-3-phosphonate synthase, an important plant and bacterial enzyme, and thus blocking aromatic amino acids biosynthesis, *N*-phosphonomethylglycine does not exhibit antibacterial activity in concentration suitable for the presumable medical applications [1–4]. This may result from its inability to permeate through bacterial cytoplasmic membranes. Peptide permeases generally exhibit broader specificity than amino acid permeases and this characteristics was often exploited to facilitate entry of unpermeant amino acid analogues into bacterial cells in the form of a peptide which may undergo intracellular hydrolysis [4]. Thus, attachment of additional amino acids to the *N*-phosphonomethylglycine should result in phosphono peptides, which in turn might be promising antibacterials.

Free aminoalkylphosphonic acids are particularly unsuitable as substrates for the synthesis of phosphono peptides because of the nucleophilic character of the phosphonate group that results in the formation of mixed anhydride with acylating *N*-blocked amino acid. This anhydride undergoes hydrolysis yielding non-productive consumption of acylating agent [5]. Thus, the first step of the phosphono peptide synthesis is the preparation of aminophosphonate esters, which are suitable substrates for further syntheses. The conversion of *N*-phosphonomethylglycine into its carboxylic ester, followed by reaction with ethyl orthoformate yielded triethyl (*N*-formyl)glyphosate and has been reported earlier [6] (Scheme 1).

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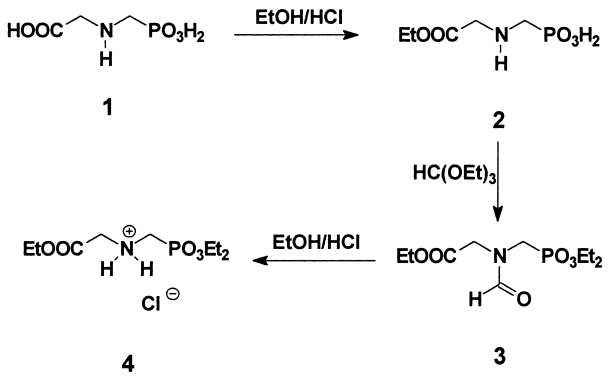


Scheme 1 Synthesis of triethyl (*N*-formyl)phosphonomethylglycinate.

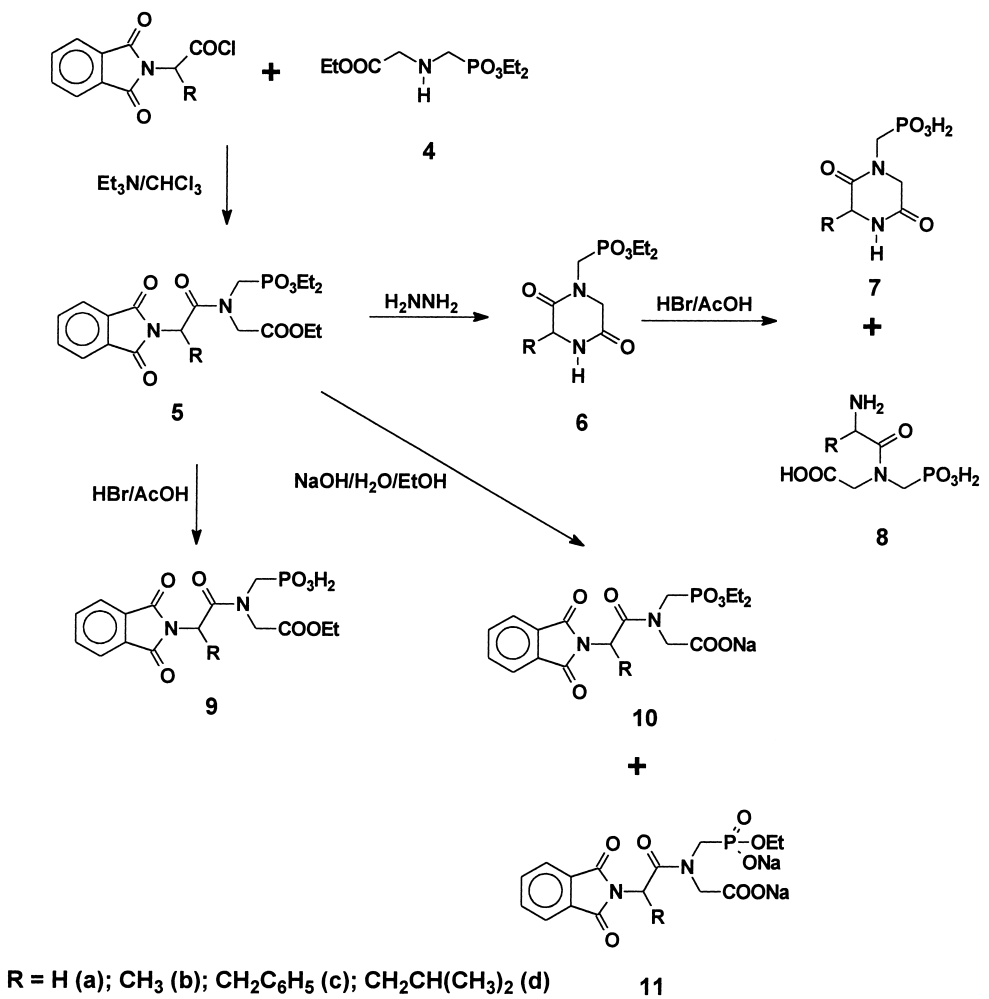
In this paper we describe the studies on the acylation of triethyl phosphonomethylglycinate with *N*-protected amino acids and attempts to remove protecting groups either selectively or totally.

MATERIALS AND METHODS

Syntheses

All the starting materials were purchased from commercial suppliers and were used without further purification. *N*-Phthaloyl-amino acids were prepared according to the literature [7]. Synthetic procedures applied in this work are outlined in Scheme 2.

Triethyl N-phosphonomethylglycinate hydrochloride (4). Triethyl (*N*-formyl)phosphonomethylglycinate (0.01 mol) [6] was dissolved in 10 ml of saturated ethanolic hydrogen chloride solution and left at room temperature for 1 day. Then ethanol was evaporated and a desired compound **4** of analytical purity was obtained as a dense oil. Yield 98%. IR (film) ν (cm^{-1}): 3400 (NH); 1440 (NH_2^+); 1750 (CO);



Scheme 2 Synthesis of dipeptides **5** and removal of their masking groups.

1240 (PO); 1040, 940 (POC). ^{31}P-NMR ($CDCl_3$) δ: 17.6 ppm. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.32 (t, J_{HH} = 7.2 Hz, 3H $COCH_2\mathbf{CH_3}$), 1.34 (t, J_{HH} = 7.1 Hz, 6H, $POCH_2\mathbf{CH_3}$), 3.76 (d, J_{PH} = 13.2 Hz, 2H, $\mathbf{CH_2}$P), 4.19 (s, 2H, $\mathbf{CH_2}$C), 4.20 (q, J_{HH} = 7.2 Hz, 2H, $CO\mathbf{CH_2}CH_3$), 4.30 (q-q, J_{HH} = 7.1 Hz, J_{PH} = 7.6 Hz, 4H, $PO\mathbf{CH_2}CH_3$), 8.0 (s, 2H, $N\mathbf{H_2^+}$). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.4 ($COCH_2\mathbf{C}H_3$), 17.5 (d, J_{PC} = 6.0 Hz, $POCH_2\mathbf{C}H_3$), 42.0 (d, J_{PC} = 151.0 Hz, P$\mathbf{C}H_2$), 48.9 ($\mathbf{C}H_2COO$), 58.8 ($CO\mathbf{C}H_2CH_3$), 63.9 (d, J_{PC} = 7.0 Hz, $PO\mathbf{C}H_2CH_3$), 166.4 ($\mathbf{C}OO$).

Compound 5. *N*-Phthaloyl-amino acid chloride (0.012 mol) was dissolved in dry chloroform (40 ml) and the solution of 0.01 mol of triethyl *N*-phosphonomethylglycinate (**4**) in chloroform (20 ml) was added. The mixture was cooled to 0°C and after 15 min of stirring triethylamine was added dropwise (3 ml). Then the temperature was slowly raised to room temperature and the solution was kept at this temperature overnight. The resulting solution was washed with 5% hydrochloric acid (twice), water, saturated sodium bicarbonate, water and brine. Then it was dried over magnesium sulphate, the drying agent was filtered off and chloroform was evaporated *in vacuo* yielding a desired product of satisfactory purity. Full analytical data are given only for compound **5c** as the representative example.

Triethyl (N-phthalylglycyl)-N-phosphonomethylglycinate (5a). This recrystallized from ethyl acetate–hexane. Yield 51%; m.p. 110–111°C. ^{31}P-NMR ($CDCl_3$) δ: 21.0 (*syn*) and 22.1 (*anti*) ppm in molar ratio 10:9.

Elemental analysis calculated for $C_{20}H_{29}N_2O_8P$ (456.44): 52.63% C, 6.40% H, 6.14% N; found: 52.75% C; 6.35% H; and 5.98% N.

IR (KBr) ν (cm^{-1}): 2985 (NH); 1777, 1772, 1680 (CO); 1245 (PO); 1130, 1024, 957 (POC).

Triethyl (N-phthalyl-L-ananyl)-N-phosphonomethylglycinate (5b). This was recrystallized from ethyl acetate–hexane. Yield 45%, m.p. 77.5–79°C. ^{31}P-NMR ($CDCl_3$) δ: 21.5 (*syn*) and 22.8 (*anti*) ppm in 1:2 molar ratio.

Elemental analysis calculated for $C_{21}H_{31}N_2O_8P$ (470.48): 53.61% C, 6.64% H, 5.95% N; found: 53.40% C; 6.40% H and 5.71% N.

IR (KBr) ν (cm^{-1}): 2990 (NH); 1780, 1774, 1680 (CO); 1240 (PO); 1135, 1025, 959 (POC).

Triethyl (N-phthalyl-L-phenylalanyl)-N-phosphonomethylglycinate (5c). This was recrystallized from ethyl acetate–hexane. Yield 60%, m.p. 107–108°C. ^{31}P-NMR: ($CDCl_3$) δ: 21.6 (*syn*) and 22.8 (*anti*) ppm in 1:2 molar ratio.

Elemental analysis calculated for $C_{27}H_{35}N_2O_8P$ (546.56): 59.33% C, 6.45% H, 5.13% N; found: 59.65% C; 6.39% H; and 5.28% N.

IR (KBr) ν (cm^{-1}): 2987 (NH); 1794, 1717, 1672 (CO); 1202 (PO), 1054, 1024, 957 (POC).

Data for conformer *syn*: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.13 (t, J_{HH} = 7.1 Hz, 3H, $COCH_2C\mathbf{H_3}$), 1.23 (t, J_{HH} = 7.5 Hz, 6H, $POCH_2C\mathbf{H_3}$), 3.65, 3.70, 3.80, 3.91 (d, J_{HP} = 10.7, 11.1, 10.0, 9.0 Hz, respectively, 0.5H each, P$\mathbf{CH_2}$), 3.67 (d-d, J_{HH} = 7.4, 10.4 Hz, 2H, $\mathbf{CH_2}$Ph), 4.06 (q-q, J_{HH} = 7.1 Hz, J_{PH} = 7.2 Hz, 4H, $POC\mathbf{H_2}CH_3$), 4.09 (q, J_{HH} = 7.1 Hz, 2H, $COC\mathbf{H_2}CH_3$), 4.23 (s, 2H, $\mathbf{CH_2}COO$), 5.39 (d-d, J_{HH} = 4.9 and 10.7 Hz, 1H, PhthNC**H**), 7.08–7.12 (m, 5H, Ph), 7.64, 7.72 (d-d, J_{HH} = 3.0 and 6.0 Hz, 2H each, Phth). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.5 ($COCH_2\mathbf{C}H_3$), 16.7 (d, J_{PC} = 6.0 Hz, $POCH_2\mathbf{C}H_3$), 44.4 (d, J_{PC} = 155.8 Hz, P$\mathbf{C}H_2$), 35.1 ($\mathbf{C}H_2$Ph), 48.6 ($\mathbf{C}H_2COO$), 52.2 (PhthN$\mathbf{C}$H), 61.7 ($CO\mathbf{C}H_2CH_3$) 63.3 (d, J_{PC} = 7.0 Hz, $PO\mathbf{C}H_2CH_3$), 127.2, 128.8, 129.6 (Ph), 123.7, 132.1, 134.4, 137.2 (Phth), 167.8 (Phth**C**O), 169.1 (**C**OO), 169.3 (d, J_{PC} = 3 Hz, O=**C**–N).

Data for conformer *anti*: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, J_{HH} = 7.1 Hz, 3H, $COCH_2C\mathbf{H_3}$), 1.25 (t, J_{HH} = 7.5 Hz, 6H, $POCH_2C\mathbf{H_3}$), 3.43, 3.67 (d-d, J_{HH} = 7.0 and 11.0 Hz, 1H each, $\mathbf{CH_2}$Ph), 3.49, 3.54 (d, J_{HP} = 10.0, 10.4 Hz, respectively, 0.5H each, P$\mathbf{CH_2}$), 3.5, 3.67 (q, J_{HH} = 7.2 Hz, 2H, $COC\mathbf{H_2}CH_3$), 4.0, 4.13 (q-q, J_{HH} = 7.1 Hz, J_{PH} = 7.2 Hz, 2H each, $POC\mathbf{H_2}CH_3$), 4.29 (s, 2H, $\mathbf{CH_2}$), 4.33, 4.39 (d, J_{PH} = 11.0, 12.2 Hz, respectively, 0.5H each, P$\mathbf{CH_2}$), 5.15 (d-d, J_{HH} = 5.8 and 9.7 Hz, 1H, PhthNC**H**), 7.08–7.12 (m, 5H, Ph), 7.70, 7.80 (d-d, J_{HH} = 3.0 and 6.0 Hz, 2H each, Phth). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.2 ($COCH_2\mathbf{C}H_3$), 16.8 (d, J_{PC} = 6.0 Hz, $POCH_2\mathbf{C}H_3$), 35.6 ($\mathbf{C}H_2$Ph), 42.2 (d, J_{PC} = 160.4 Hz, P$\mathbf{C}H_2$), 49.4 ($\mathbf{C}H_2COO$), 52.2 (PhthN$\mathbf{C}H_2$), 61.9 ($CO\mathbf{C}H_2CH_3$), 62.9, 63.0 (d, J_{PC} = 6.5, 6.7 Hz, respectively, $PO\mathbf{C}H_2CH_3$), 127.2, 128.8, 129.6 (Ph), 123.7, 131.7, 134.6, 137.1 (Phth), 167.3 (Phth**C**O), 168.2 (d, J_{PC} = 2.3 Hz, O=**C**–N), 168.9 (**C**OO).

Triethyl (N-phthalyl-L-leucyl)-N-phosphonomethylglycinate (5d). Yield 40%, yellowish oil. ^{31}P-NMR ($CDCl_3$) δ: 22.0 (*syn*) and 23.5 (*anti*) ppm in molar ratio of 1:2. Elemental analysis calculated of $C_{24}H_{37}N_2O_8P$ (512.54): 56.24% C, 7.28% H, 5.47% N; found: 56.22% C; 7.35% H; and 5.41% N.

Cycling peptides 6. Peptide **5** (0.01 mol) was dissolved in 95% ethanol (40 ml) and the mixture was refluxed with 5 M hydrazine monohydrate in 95%

EtOH (6 ml, threefold excess) for 1 h and then left at room temperature overnight. The crystallized phthaloyl hydrazide was filtered off and ethanol was evaporated, yielding oil, which contained over 90% of the compounds listed below.

Diethyl cyclo-glycyl-N-phosphonomethylglycine (6a). Yield 40%. ^{31}P-NMR ($CDCl_3$) δ: 24.1 ppm. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.26 (t, $J_{HH} = 7.1$ Hz, 6H, $POCH_2C\mathbf{H_3}$), 3.93 (d, $J_{PH} = 11.2$ Hz, 2H, $C\mathbf{H_2}P$), 4.02 (d, $J_{HH} = 7.1$ Hz, 2H, $HNC\mathbf{H_2}$), 4.14 (q-q, $J_{HH} = 7.2$ Hz, $J_{PH} = 7.2$ Hz, 4H, $POC\mathbf{H_2}CH_3$), 4.15 (s, 2H, $NC\mathbf{H_2}$). ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.55 (d, $J_{PC} = 3$ Hz, $POCH_2\mathbf{C}H_3$), 41.10 (d, $J_{PC} = 156.6$ Hz, $\mathbf{C}H_2P$), 43.96 ($HN\mathbf{C}H_2$), 50.38 ($N\mathbf{C}H_2$), 64.27 (d, $J_{PC} = 6.8$ Hz, $PO\mathbf{C}H_2CH_3$), 166.02 (d, $J_{PC} = 2.3$ Hz, $N\mathbf{C}$=O), 167.5 ($HN\mathbf{C}$=O).

Diethyl cyclo-L-alanyl-N-phosphonomethylglycine (6b) and N-L-alanyl-N-phosphonomethylglycine (7b). Combined yield 35%. ^{31}P-NMR ($CDCl_3$) δ: 24.1 (cyclic product) and 24.2 (desired peptide) ppm in a molar ratio 10:1.

Data for tentative cyclic compound **6b**: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.21 (t, $J_{HH} = 7.0$ Hz, 6H, $POCH_2C\mathbf{H_3}$), 1.34 (d, $J_{HH} = 7.0$ Hz, 3H, $CHC\mathbf{H_3}$), 3.74, 3.79 (d, $J_{PH} = 10.8$ Hz, 0.5H each, $C\mathbf{H_2}P$), 3.96, 4.01 (d, $J_{PH} = 11.5$ Hz, 0.5H each, $C\mathbf{H_2}P$), 4.08 (q-q, $J_{HH} = 6.9$ Hz, $J_{PH} = 7.0$ Hz, 4H, $POC\mathbf{H_2}CH_3$), 4.08 (s, 2H, $NC\mathbf{H_2}$), 4.03–4.16 (m, 1H, $HNC\mathbf{H}$). ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.5 (d, $J_{PC} = 6.0$ Hz, $POCH_2\mathbf{C}H_3$), 18.6 (d, $J_{PC} = 11.3$ Hz, $CH\mathbf{C}H_3$), 41.2 (d, $J_{PC} = 156.1$ Hz, $\mathbf{C}H_2P$), 50.4 ($N\mathbf{C}H_2$), 50.4 ($HN\mathbf{C}H$), 64.27 (d, $J_{PC} = 6.9$ Hz, $PO\mathbf{C}H_2CH_3$), 168.9 (d, $J_{PC} = 2.3$ Hz, $N\mathbf{C}$=0), 167.3 ($HN\mathbf{C}$=O).

Data for tentative peptide **7b**: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.21 (t, $J_{HH} = 7.0$ Hz, 6H, $POCH_2C\mathbf{H_3}$), 1.34 (d, $J_{HH} = 7.0$ Hz, 3H, $CHC\mathbf{H_3}$), 3.65, 3.66 (d, $J_{PH} =$ 11.5 Hz, 1H, 0.5H each, $C\mathbf{H_2}P$), 4.08 (q-q, $J_{HH} = 6.9$ Hz, $J_{PH} = 7.0$ Hz, 4H, $POC\mathbf{H_2}CH_3$), 4.20 (s, 2H, $NC\mathbf{H_2}$), 4.03–4.16 (m, 1H, $HNC\mathbf{H}$). ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.5 (d, $J_{PC} = 6$ Hz, $POCH_2\mathbf{C}H_3$), 18.6 (d, $J_{PC} = 11.3$ Hz, $CH\mathbf{C}H_3$), 43.3 (d, $J_{PC} = 156.1$ Hz, $\mathbf{C}H_2P$), 50.2 ($HN\mathbf{C}H$), 50.4 ($N\mathbf{C}H_2$), 64.2 (d, $J_{PC} = 6.7$ Hz, $PO\mathbf{C}H_2CH_3$), 167.5 ($HN\mathbf{C}$=O), 168.5 (d, $J_{PC} = 2.3$ Hz, $N\mathbf{C}$=O).

Unblocked cyclic peptides 8. Cycling peptide diethyl ester **6** (0.01 mol) was dissolved in 33% HBr in glacial acetic acid and left for 2 days at room temperature. Then, acetic acid was evaporated under reduced pressure and the resulting dense oil was washed twice with ethyl ether and dissolved in ethanol. Crude peptide **7** was precipitated by the addition of propylene oxide. Pure product was obtained by precipitation with acetone from its aqueous solution.

cyclo-Glycyl-N-phosphonomethylglycine (8a). Yield 40%, yellowish oil. ^{13}P-NMR (D_2O) δ: 19.11 (*syn*) and 19.38 (*anti*) ppm in molar proportion of 10:1.

Data for conformer conformer *syn*: ^{1}H-NMR (D_2O) δ (ppm): 3.58 (d, $J_{PH} = 14.8$ Hz, 2H, $C\mathbf{H_2}P$), 3.84 (s, 2H, $NC\mathbf{H_2}$), 4.0 (s, 2H, $HNC\mathbf{H_2}$).

Data for conformer *anti*: ^{1}H-NMR (D_2O) δ: 3.1, 3.5 (d, $J_{PH} = 10.4$; 13.2 Hz, 1H each, $C\mathbf{H_2}P$) 4.1 (s, 2H, $C\mathbf{H_2}$), 3.13 (s, 2H, $HNC\mathbf{H_2}$).

cyclo-L-Alanyl-N-phosphonomethylglycine (8b). Yield 40%, yellowish oil. ^{31}P-NMR (D_2O) δ: 16.5 ppm. ^{1}H-NMR (D_2O) δ (ppm): 1.06 (d, $J_{HH} = 6.0$ Hz, 3H, $CHC\mathbf{H_3}$), 3.35, 3.37, 3.46, 3.47 (d, $J_{PH} = 10.8$ Hz, 0.5H each, $C\mathbf{H_2}P$), 3.61, 3.65, 4.18, 4.22 (s, 0.5H each, $NC\mathbf{H_2}$), 3.75–3.85 (m, 1H, $NHC\mathbf{H}$).

Monoesters 9. Peptide **5** (0.01 mol) was dissolved in 33% HBr in glacial acetic acid and left for 3 days at room temperature. Then, acetic acid was evaporated under reduced pressure and the resulting dense oil was washed twice with ethyl ether and dissolved in ethanol. The product was precipitated by addition of water.

Ethyl (N-phthalylglycyl)-N-phosphonomethylglycine (9a). Yield 51%, m.p. 135–142°C. ^{13}P-NMR (DMSO-d_6) δ: 17.6 (*syn*) and 17.7 (*anti*) ppm in molar ratio of 3:7.

Data for conformer *syn*: ^{1}H-NMR: (DMSO-d_6) δ (ppm): 1.25 (t, $J_{HH} = 7.1$ Hz, 3H, $COCH_2C\mathbf{H_3}$), 3.56 (d, $J_{HP} = 11.4$ Hz, 2H, $PC\mathbf{H_2}$), 4.14 (q, $J_{HH} = 7.1$ Hz, $COC\mathbf{H_2}CH_3$), 4.46 (s, 2H, $C\mathbf{H_2}COO$), 4.54 (s, 2H, $PhthNC\mathbf{H_2}$), 7.80–7.88 (m, 4H, Phth) 7.80 (d-d, $J_{HH} = 3.0$ and 6.0 Hz, 2H, Phth).

Data for conformer *anti*: ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.16 (t, $J_{HH} = 7.1$ Hz, 3H, $COCH_2C\mathbf{H_3}$), 3.79 (d, $J_{HP} = 11.4$ Hz, 2H, $PC\mathbf{H_2}$), 4.03 (q, $J_{HH} = 7.1$ Hz, 2H, $COC\mathbf{H_2}CH_3$), 4.1 (s, 2H, $C\mathbf{H_2}COO$), 4.77 (s, 2H $PhthNC\mathbf{H_2}$), 7.84 (m, 4H, Phth).

Ethyl (N-phthalyl-L-phenylalanyl)-N-phosphonomethylglycine (9c). Yield 80%, m.p. 130–13°C. ^{31}P-NMR (DMSO-d_6) δ: 17.0 (*syn*) and 17.7 (*anti*) ppm in molar ratio 9:10.

Data for conformer *syn*: ^{1}H-NMR: (DMSO-d_6) δ (ppm): 1.09 (t, $J_{HH} = 7.1$ Hz, 3H, $COCH_2C\mathbf{H_3}$), 3.17 (q, $J_{HH} = 7.0$ Hz, 1H, $COCH_2C\mathbf{H_3}$), 3.2 (d-d, $J_{HH} =$ 11.0 and 20.0 Hz, 1H, $C\mathbf{H_2}Ph$), 3.45 (q, $J_{HH} = 7.0$ Hz, 1H, $COC\mathbf{H_2}CH_3$), 3.46, 3.52, 3.70, 3.75 (d,

J_{HP} = 10.0 Hz, 0.5 each, PC**H$_2$**), 3.70 (d-d, J_{HH} = 11.0 and 14.0 Hz, 1H, C**H$_2$**Ph), 4.33, 4.41 (d, J_{HH} = 17.5 Hz, 1H each, **CH$_2$**COO), 5.23 (d-d, J_{HH} = 4.9 and 11.0 Hz, PhthNC**H**), 7.06–7.12 (m, 5H, Ph), 7.73–7.8 (m, 4H, Phth). ^{13}C-NMR: (DMSO-d_6) δ (ppm): 13.9 (COCH$_2$**C**H$_3$), 34.5 (**C**H$_2$Ph), 42.2 (d, J_{PC} = 160.4 Hz, PC**H$_2$**), 47.9 (C**H$_2$**COO), 51.3 (PhthN**C**H$_2$), 61.9 (CO**C**H$_2$CH$_3$), 126.6, 128.2, 129.0 (Ph), 123.2, 130.2, 134.8, 134.9 (Phth), 166.5 (Phth**C**O), 137.1 (**C**OO), 166.8 (d, J_{PC} = 2.3 Hz, O=**C**–N).

Data for conformer *anti*: ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.85 (t, J_{HH} = 7.1 Hz, 3H, COCH$_2$C**H$_3$**), 3.02, 3.07 (d, J_{HP} = 8.6 Hz, 0.5H each, PC**H$_2$**), 3.35 (d-d, J_{HH} = 10.9 and 19.5 Hz, 2H, C**H$_2$**Ph), 4.04, 4.09, 4.10, 4.15 (s, 0.5H each, C**H$_2$**COO), 4.0 (q, J_{HH} = 7.0 Hz, 2H, COC**H$_2$**CH$_3$), 4.19, 4.24 (d, J_{HP} = 15.0 Hz, 0.5H each, P**CH$_2$**), 5.46 (d-d, J_{HH} = 4.5, 11.0 Hz, 1H, PhthN**CH**), 7.06–7.12 (m, 5H, Ph), 7.73–7.8 (m, 4H Phth). ^{13}C-NMR: (DMSO-d_6) δ (ppm): 13.5 (COCH$_2$**C**H$_3$), 33.8 (**C**H$_2$Ph), 44.4 (d, J_{PC} = 155.8 Hz, P**C**H$_2$), 48.2 (**C**H$_2$COO), 61.7 (CO**C**H$_2$CH$_3$), 51.3 (PhthN**C**H), 126.6, 128.2, 129.0 (Ph), 123.2, 130.4, 134.7 (Phth), 167.1 (Phth**C**O), 137.0 (**C**OO), 168.3, 168.5 (d, J_{PC} = 3 Hz, O=**C**–N).

Saponification of peptides 5. Peptide **5** (0.005 mol) was dissolved in absolute ethanol (5 ml) and sodium ethanolate (prepared by dissolving 0.12 g of sodium in 2.5 ml of absolute ethanol) was added dropwise followed by the addition of 0.09 ml (0.005 mol) of water. The obtained solution was then stirred for 3 h at room temperature, the sodium chloride formed was filtered off and washed with ethanol. Ethanolic solutions were combined and the solvent evaporated off. The resulting oil was washed with ether yielding a crude mixture of compounds **10** and **11**. In one case we were able to separate these compounds by dissolving the oil in ethanol and fractional precipitation with ethyl ether. Compound **10c** precipitated first followed by precipitation of compound **11c**.

Sodium diethyl (N-phthalyl-L-phyenylalanyl)-N-phosphonomethylglycinate (10c). Yield 16%, white glassy precipitate. ^{31}P-NMR (D_2O): 18.4 (*syn*) and 19.1 (*anti*) ppm in molar ratio 1:6.

Data for conformer *syn*: ^{1}H-NMR (D_2O) δ (ppm): 1.21 (t, J_{HH} = 7.1 Hz, 6H, POCH$_2$C**H$_3$**), 3.09–3.15 (m, 2H, C**H$_2$**Ph), 3.16, 3.17, (d, J_{HP} = 10.0 Hz, 1H each, PC**H$_2$**), 3.82, 3.88, 3.91, 3.94 (S, 0.5H each, C**H$_2$**COO), 4.06 (q-q, J_{HH} = J_{PH} = 7.0 Hz, 4H POC**H$_2$**CH$_3$), 5.34 (d-d, J_{HH} = 4.9 and 11.0 Hz, 1H PhthNC**H**), 7.02–7.35 (m, 5H Ph), 7.37–7.44 (m, 4H, Phth).

Data for conformer *anti*: ^{1}H-NMR (D_2O) δ (ppm): 1.21 (t, J_{HH} = 7.1 Hz, 6H, POCH$_2$C**H$_3$**), 2.49, 3.00 (d, J_{HP} = 10.0 Hz, 1H each, PC**H$_2$**), 3.01 (d-d, J_{HH} = 10.9 Hz and 19.5 Hz, 2H, C**H$_2$**Ph), 32.0, 3.10 (d, J_{PH} = 15.90 Hz, 1H, PC**H$_2$**), 3.91, (s, 1H, C**H$_2$**COO), 4.06 (q-q, J_{HH} = J_{PH} = 7.0 Hz, 4H POC**H$_2$**CH$_3$), 4.10, 4.12 (s, 0.5H each, C**H$_2$**COO), 5.09 (d-d, J_{HH} = 4.5 and 11.0 Hz, 1H PhthNC**H**), 7.02–7.35 (m, 5H Ph), 7.37–7.44 (m, 4H, Phth).

Disodium ethyl (N-phthalyl-L-phenylalanyl)-N-phosphonomethylglycinate (11c). Yield 30%, yellowish oil. ^{31}P-NMR (D_2O): 17.2 (*syn*) and 18.2 (*anti*) ppm in molar ratio 1:4.

Data for conformer *syn*: ^{1}H-NMR (D_2O) δ (ppm): 1.21, (t, J_{HH} = 7.1 Hz, 3H, POCH$_2$C**H$_3$**), 3.09–3.15 (m, 2H, C**H$_2$**Ph), 3.16, 3.17, (d, J_{HP} = 10.0 Hz, 1H each, PC**H$_2$**), 3.82, 3.88, 3.91, 3.94 (s, 0.5H each, C**H$_2$**COO), 4.06 (q-q, J_{HH} = J_{PH} = 7.0 Hz, 2H POC**H$_2$**CH$_3$), 5.34 (d-d, J_{HH} = 4.9 and 11.0 Hz, 1H PhthNC**H**), 7.02–7.35 (m, 5H Ph), 7.37–7.44 (m, 4H, Phth).

Data for conformer *anti*: ^{1}H-NMR (D_2O) δ (ppm): 1.21 (t, J_{HH} = 7.1 Hz, 3H, POCH$_2$C**H$_3$**), 2.94, 3.00 (d, J_{HP} = 10.0 Hz, 1H each, PC**H$_2$**), 3.01 (d-d, J_{HH} = 10.9 and 19.5 Hz, 2H, C**H$_2$**Ph), 32.0, 3.10 (d, J_{PH} = 15.9 Hz, 1H, PC**H$_2$**), 3.91, (s, 1H, C**H$_2$**COO), 4.06 (q-q, J_{HH} = J_{PH} = 7.0 Hz, 2H, POC**H$_2$**CH$_3$), 4.10, 4.12 (s, 0.5H each, C**H$_2$**COO), 5.09 (d-d, J_{HH} = 4.5 and 11.0 Hz, 1H, PhthNC**H**), 7.02–7.35 (m, 4H, Phth).

NMR Measurements

Proton, phosphorus and carbon NMR spectra were recorded in deuterated DMSO, D_2O and $CDCl_3$ on a Bruker DRX spectrometer operating at 300.13 MHz for ^{1}H, 121.50 MHz for ^{31}P and 75.47 MHz for ^{13}C. Chemical shifts are given in relation to $SiMe_4$, 85% H_3PO_4 and the central peak of the deuterated chloroform triplet, respectively. All downfield shifts are denoted as positive. Structures of the compounds were deduced from the combination of their ^{1}H{^{31}P}-, ^{1}H-^{13}C-HMQC and ^{1}H-^{31}P-HMQC spectra and supported by means of IR spectroscopy and elemental analyses.

Crystallography

The X-ray data were collected at room temperature, on a Kuma diffraction diffractometer equipped with a CCD camera which was positioned at 46 mm from the crystal. Graphite-monochromatized MoKα radiation was employed. The structures were solved by direct methods using the SHELXS86 program [8]

and refined on F^2 values by full-matrix least-squares using the SHELXL program from the SHELXL-97 package [9] with anisotropic displacement parameters for non-hydrogen atoms. Positions of hydrogen atoms were calculated geometrically using the riding model and refined isotropically [10].

RESULTS AND DISCUSSION

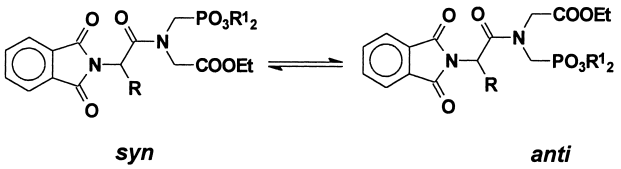
Synthesis of *N*-Phosphonomethylglycine Peptides

Among several standard methods, successfully applied for standard phosphono peptide synthesis, only the method of acid chlorides was found to be useful for the synthesis of dipeptides **5** containing *C*-terminal *N*-phosphonomethylglycine. This standard reaction, as well as efforts to remove blocking groups, is outlined in Scheme 2.

Removal of phthaloyl groups from compounds **5** by hyrazinolysis was accompanied by a cyclization reaction. Formation of cycling peptides **6** predominate and the formation of the desired peptide was observed only in the case of **7b**. Acidolysis of compounds **6** resulted in removal of phosphonate ester groups and the formation of fully deblocked cyclic dipeptides **8** alongside with the formation of products of C–P bond breakage clearly visible in ^{31}P-NMR spectra in the region corresponding to inorganic phosphate (δ around 0 ppm) and in the region corresponding to phosphite (δ around 4–8 ppm). Thus, we did not succeed in preparation of unblocked peptides of *N*-phosphonomethylglycine in this manner. We also undertook efforts to remove phosphonate and carboxylate ester groups in the first steps of the deblocking procedure. Acidolysis of the peptides **5** with a solution of hydrobromide in acetic acid gave selective removal of phosphonate ester moieties leading to compounds **9**, whereas alkaline hydrolysis of **5** in ethanol gave the mixture of compounds **10** and **11**, namely products of total hydrolysis of the carboxylate moiety and partial hydrolysis of the phosphonate group. Further efforts to remove the phthalyl group from compounds **9**, **10** and **11** by hydrazinolysis were unsuccessful and resulted in cyclic peptides and/or in C–P bond breakage under reaction conditions, and thus led to the complex mixtures of products.

Conformations in Solution

The structures of the synthesized peptides were studied by means of NMR spectroscopy (^{31}P, ^{1}H, ^{13}C). We observed the existence of two conformers of



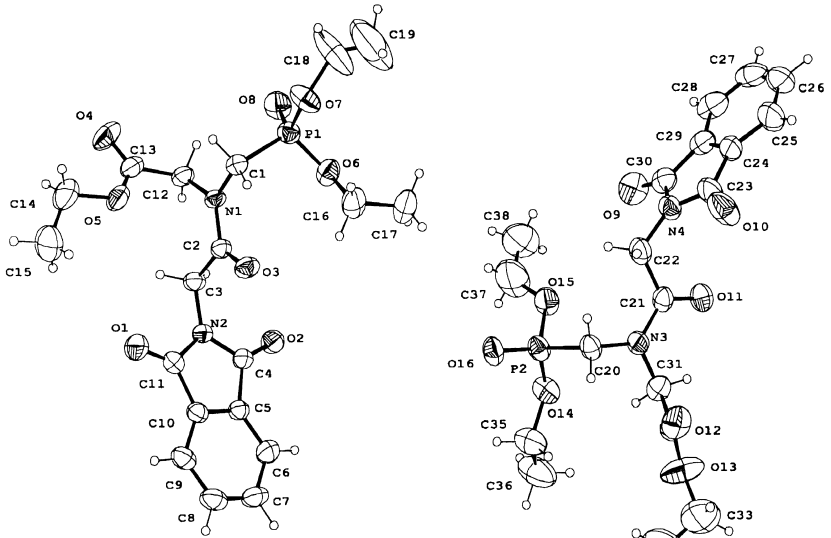
Scheme 3 *Anti* and *syn* conformers of peptide **5**.

peptides **5** and **9** in solutions. This resulted from the slow inversion around partially double peptide bond and led to the existence of *syn* (*cis*) and *anti* (*trans*) conformers (Scheme 3). Their structures were determined by careful examination of coupling constants determined by using a combination of NOE experiments and their ^{1}H{^{31}P}-, 1H-^{13}C-HMQC and ^{1}H-^{31}P-HMQC spectra. The presence of these conformers was additionally supported by the coalescence of doubled signals in ^{1}H- and ^{31}P-NMR spectra in $(CD_3)_2SO$, at temperatures above 370 K. The activation energy of 20.4 kcal/mol found for ethyl (*N*-phthalylglycy)-*N*-phosphonomethylglycinate (**9a**) is similar to standard values reported in the literature for rotation around peptidyl C–N bonds.

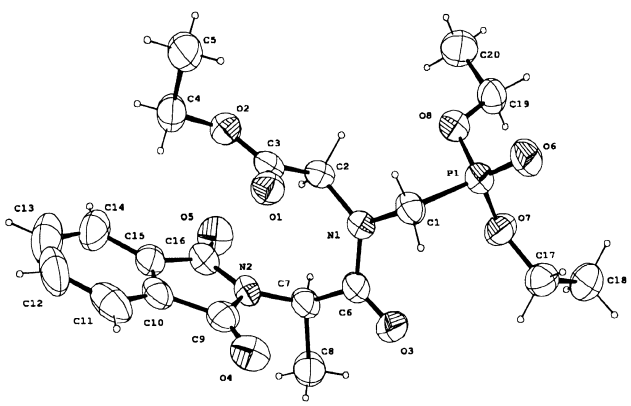
X-ray Crystallography

The three peptides studied differ from each other only by an *N*-terminal amino acid, but their crystal structures are completely different. Molecules **5a**, **5b** and **5c**, with their atom-numbering scheme and atomic displacement ellipsoids, are shown in Figure 1. The analysis of the crystal packing showed the existence of very weak C–H···O intermolecular interactions for all compounds studied. The peptides crystallize in three crystal systems and space groups: $P\bar{1}$ (**5a**), $P2_1/n$ (**5b**) and $P2_12_12_1$ (**5c**). The crystals of all compounds have four molecules in unit cells. In the case of compound **5a** there are two independent molecules in the asymmetric unit while in crystals of **5b** and **5c** only one form of molecule was observed.

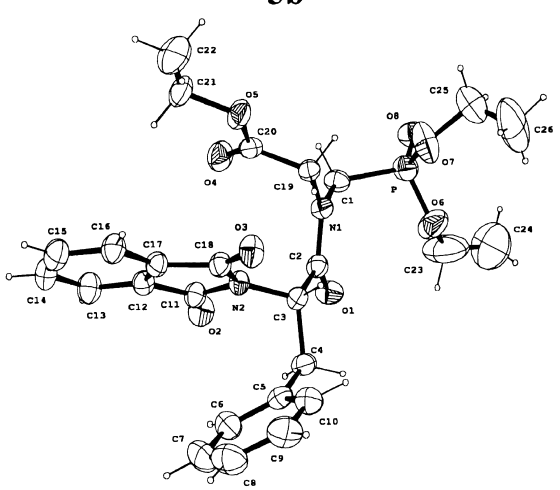
In all cases the observed reflections were weak (especially in the case of **5b)** while the thermal atomic displacements were large, especially within the two ethoxy groups attached to the phosphorus atom. It suggests the considerable atomic thermal vibrations, which might derive from the dynamic character of the ethoxy groups. As shown for the representative example (**5c**), the experiment performed in liquid nitrogen did not result in improved quality of the data. It seemed that lowering of the DSC method showed the occurrence of a weak anomaly on DSC heating and cooling curves at



5a



5b



5c

Figure 1 ORTEP-III [9] view of the molecular structure of the compounds **5a**, **5b** and **5c** with the atom-labeling scheme. The displacement ellipsoids are scaled to include 30% probability. The H atoms are drawn as spheres with a fixed radius.

about 238 K. This anomaly was similar to those observed for glassy transitions [11].

The results obtained for compound **5b** need separate explanation. We performed the diffraction experiment on several crystal specimens obtained by crystallization from various solvents. In spite of all the attempts diffraction patterns of **5b** were unsatisfactory. Thus, the crystal structure of this compound was solved easily (*R* value was about 20%), while the results of refining were unsatisfactory.

CONCLUSIONS

The synthesis of *N*-phosphonomethylglycine peptides is more complicated than any other phosphono peptide. This derives not only from the presence of a secondary amino-group in the molecule, but also from the presence of carboxylate and phosphonate moieties in one molecule. Although we have applied mixed anhydride, active ester and DCC approaches, the only successful procedure for the acylation of triethyl *N*-phosphonomethylglycinate appeared to be the acid chloride method. However, real synthetic problems arose when trying to remove protecting groups from the obtained dipeptides **5**. The removal of any of these groups was always accompanied by undesirable effects with the decomposition of the peptide and *N*-phosphonomethylglycine molecules being most upset.

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