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To the 85th Anniversary of birthday of late Yu.G. Gololobov

Synthesis of Phosphorus Isosters of β-Amyloid Peptides Fragments

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Abstract—We have developed a synthetic route to pseudo dipeptides, analogs of certain fragments of β-amyloid peptides (products of the APP protein hydrolysis). These compounds can be used for preparation of phosphinic acidic oligopeptides representing the peptide sequence of the β-amyloid but containing the phosphorus isoster peptide fragment. Synthesis of pseudo ornityl-glutamate, pseudo arginyl-glutamate, pseudo glycyl-leucine, and pseudo isoleucyl-glycine via amino- and amidoalkylation of phosphonic acids containing the structural isoster of the corresponding amino acid is described.

Keywords: β-amyloid, phosphorus isoster, peptide, phosphonic acid, amino acid, amidoalkylation, hydrophosphoryl compound, methylenebis(benzylcarbamate)

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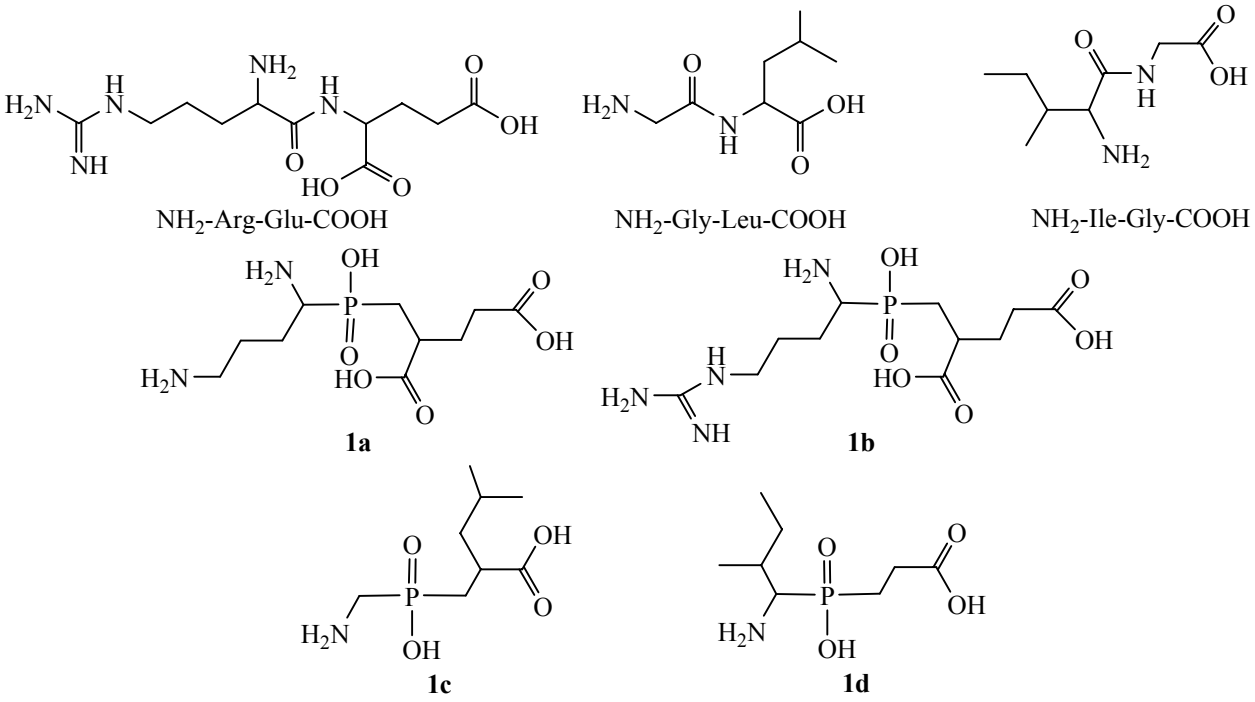
Application of endogenous peptides as well as their fragments and analogs as neuroprotective agents, stimulators of cognitive functions, and memory modulators [1–3] has been recognized among the promising approaches in neuropharmacology. The high specificity of the action has been found for a series of neuropeptides, and their receptor as well as non-receptor intracellular targets have been discovered [4, 5]. The neurodegenerative action of β-amyloid peptides (the products of proteolysis of amyloid precursor protein APP) has been well studied; however, the neuroprotective and cognitive-stimulating peptides isolated from APP have been reported as well [6, 7]. Study of functions and targets of the mentioned compounds is a topical fundamental issue opening the possibilities to develop the neuroactive drugs. Unfortunately, such peptides are metabolically unstable. Design of the peptidomimetics (pseudo peptides, the bioisosters of natural peptides) is an efficient approach towards synthesis of metalloproteinases and aspertyl-proteinases inhibitors [8–10] and can potentially lead to discovery of new neuroprotective agents.

Here we suggest a method to prepare pseudo dipeptides [8–10] via substitution of the amide bond $[C(O)NH_2]$ in the dipeptide molecule with the methylenephosphoryl fragment $[P(O)(OH)CH_2]$, inert towards hydrolysis; this might improve the enzymatic (metabolic) stability of the peptides and even result in appearance of new practically important properties [8–10].

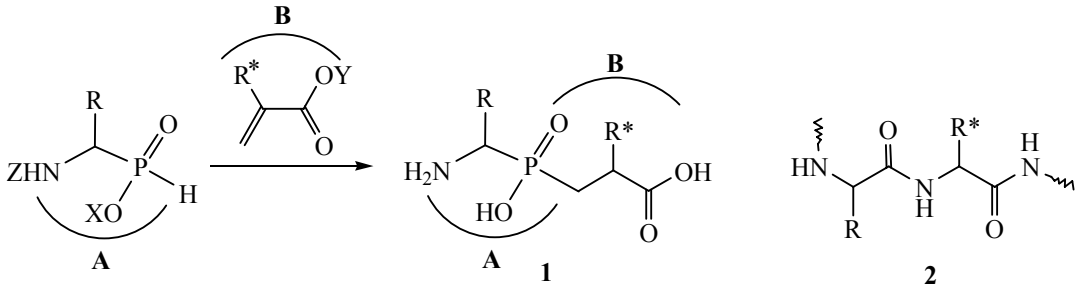
We synthesized the pseudo dipeptides, analogs of short fragments of the β-amyloid peptides Arg-Glu, Gly-Leu, and ILeu-Gly, products of APP hydrolysis. The prepared compounds can be further used in synthesis of more complex peptide fragments, for example, oligopeptides reproducing the sequence of the β-amyloid but containing the phosphorus isoster of the peptide group (Scheme 1).

The known approach to the preparation of the phosphinic analogs **1** of dipeptides **2** [11, 12] is based on the synthesis of the N-protected aminoalkyl-phosphonic component of the pseudo peptide (phosphonic analog of the amino acid) **A** followed by its addition to the α-R*-substituted acrylate (**B**) (Scheme 2). Recently, a synthesis of pseudo peptide fragments has been performed via addition of silyl esters of phosphonic isoster of the amino acid **A** to the cor-

Scheme 1.



Scheme 2.



responding acrylates **B** (the Michael–Pudovik reaction) [10].

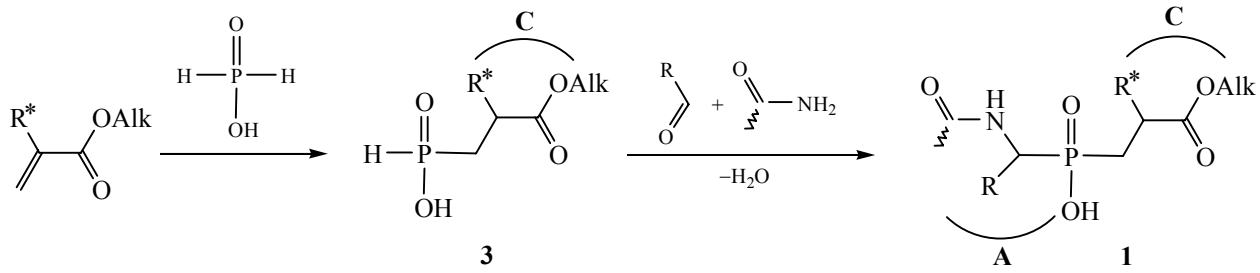
According to that method, design of the α-aminoalkylphosphoryl fragment **A** of the peptide comprises four or more stages including protection and deprotection at nitrogen (N-Cbz) and phosphorus [for example, $PCH(OEt)_2$] atoms [11, 12]. In turn, addition of the silyl ester of the component **A** to acrylates is seriously limited (or fails) as far as the building of complex aminoalkylphosphinic building blocks is concerned [13].

In this work we extended the alternative method of the pseudo peptides synthesis with the reversed order of building the target molecule: initial addition of the hypophosphite to the corresponding acrylates to form the phosphinic acids **3** containing the structural isoster of the amino acid **C**, followed by addition of the amino acid fragment or construction of the α-aminoalkylphosphoryl fragment via the Kabachnik–Fields reaction and the formation of the phosphinic acid isoster of the peptide **1** (Scheme 3) [14, 15].

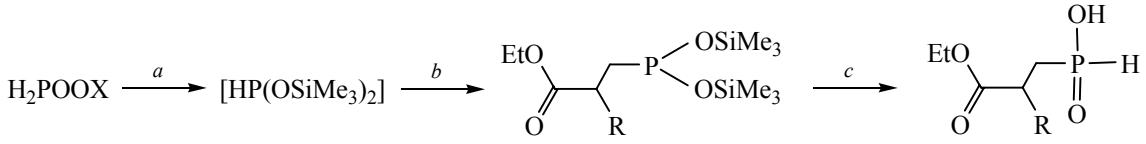
We synthesized pseudo ornityl-glutamate **1a**, pseudo arginyl-glutamate **1b**, pseudo glycyl-leucine **1c**, and pseudo isoleucyl-glycine **1d** via amino- or amidoalkylation of phosphonic acids **3** containing the structural isoster of the corresponding amino acid (Scheme 3).

Basing on the results obtained during the study of addition of bis(trimethylsilyl)hypophosphite **4** (generated in situ from the hypophosphorous acid salts) to

Scheme 3.

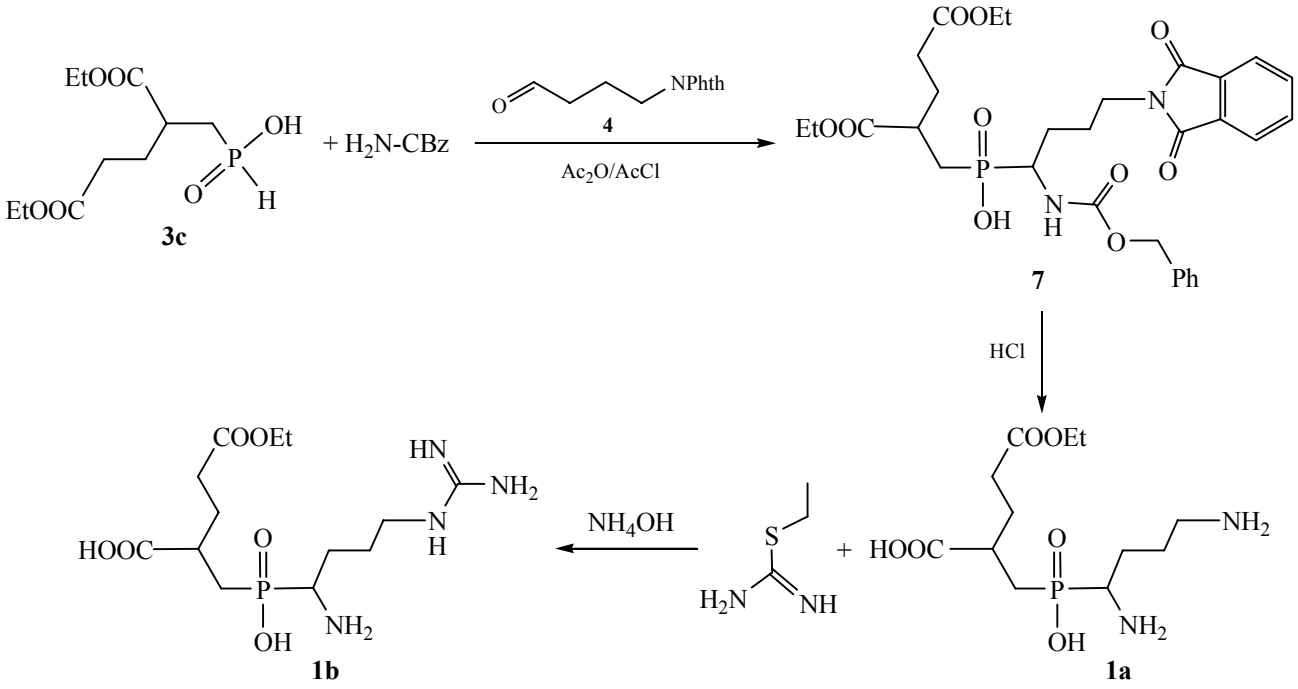


Scheme 4.



a, $(Me_3Si)_2NH$ or $(Me_3Si)_2NH$, NH_4Cl; *b*, EtO(O)C–C(R)=CH_2; *c*, EtOH(H_2O); X = NH_4 or K; R = H (**a**), *i*-Bu (**b**), CH_2CH_2COOEt (**c**).

Scheme 5.

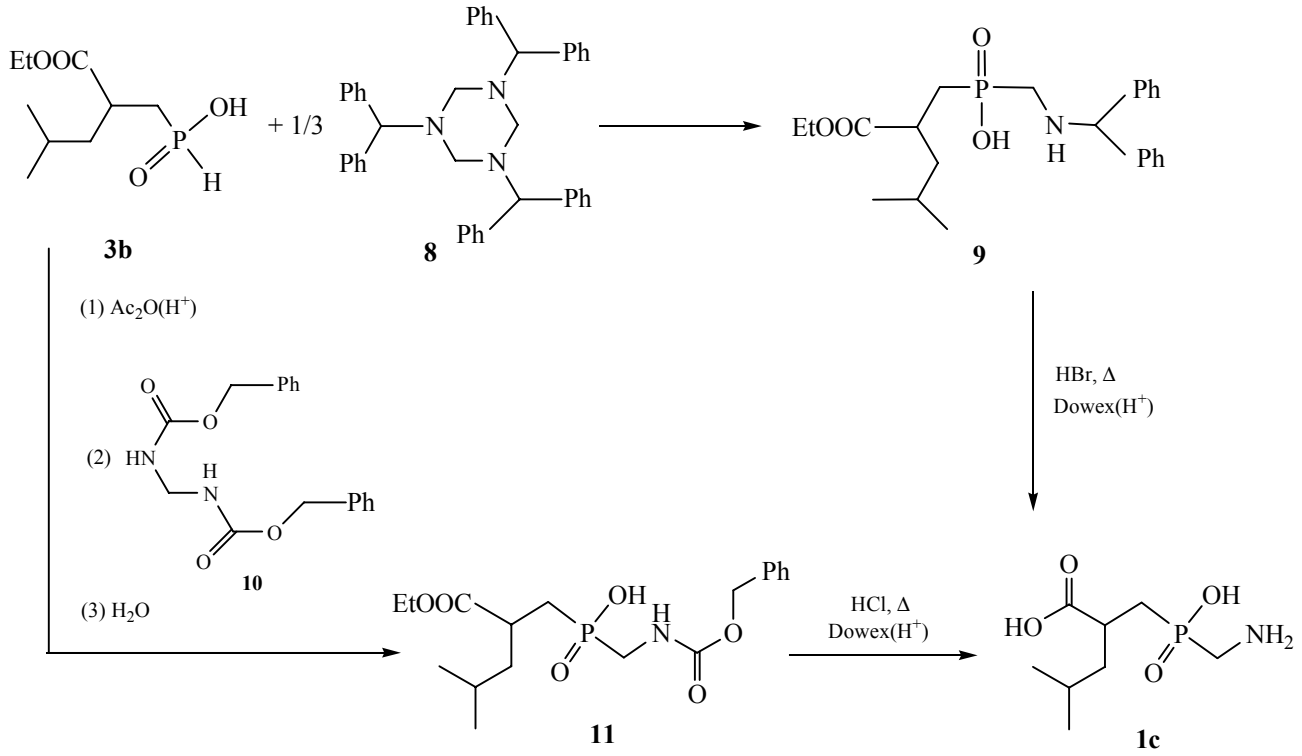


acrylates [16–19], we succeeded in synthesis of phosphonic acids containing structural isosters of glycine **3a**, leucine **3b**, and glutamic acid **3c** via alcoholysis of the addition products (silylphosphonites **5a**–**5c**) (Scheme 4).

The phosphonic acids **3a**–**3c** were used as key intermediates in the synthesis of pseudo peptides **1a**–**1d**. Amidoalkylation of acid **3c** with benzylcarbamate and 4-(phthalyl)aminobutyraldehyde **6** in a mixture of acetic anhydride and acetyl chloride at cooling (cf. Scheme 5 and [20]) afforded the phosphinic acid **7**.

Acid hydrolysis of compound **7** followed by ion-exchange chromatography on a cationite yielded the aminophosphinic acid, pseudo ornityl-glutamate **1a**. In

Scheme 6.



order to obtain the phosphinic acid analog of arginyl-glutamate **1b**, 1,4-diaminobutyl-2,4-di(hydroxycarbonyl)butylphosphinic acid **1a** was treated with S-ethylisothiourea in aqueous ammonia solution (cf. Scheme 5 and [11]).

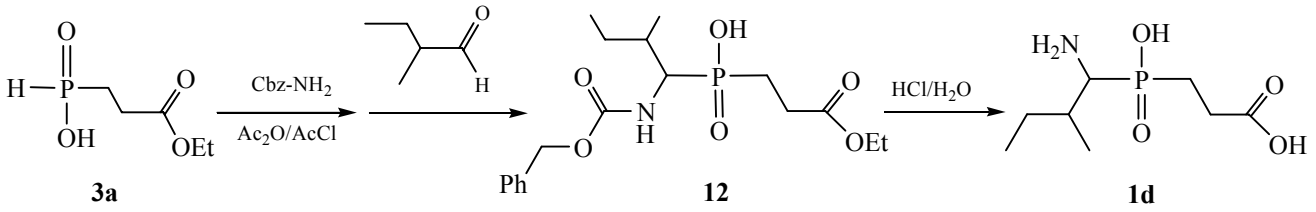
The construction of the aminophosphoryl fragment of pseudo glycyl-peptides and the synthesis of phosphonic isoster of glycine (the pseudo-C-glycyl component) were complicated by the limited applicability of formaldehyde (the latter existed in the polymeric form or in aqueous solution, inappropriate for the usual synthetic procedure). In view of that, we used the purposefully prepared imine trimer, 1,3,5-tris-(diphenylmethyl)-hexahydro-S-triazine **8** [11], its interaction with the phosphonic component **3b** affording the phosphinic acid **9**. Hydrolysis of the latter with hydrobromic acid followed by chromatography on a cationite yielded the phosphinic pseudo glycyl-leucine **1c** (Scheme 6). On top of that, we pioneered at performing the alternative synthesis of the pseudo peptides containing the glycine structural isoster, using methylenebis(benzylcarbamate) **10** (Scheme 6).

According to the earlier suggested mechanism of amidoalkylation of hydrophosphoryl compounds [20–22], *N,N′*-alkylidenebis(alkylcarbamates) in the acetic anhydride medium act as a source of iminium cations generated in situ under conditions of the acid catalysis; these cations are the reactive intermediates in the amide (carbamate) version of the Kabachnik–Fields reaction, capable of the interaction with the nucleophilic $P^{III}OAc$ derivative (generated in situ in the acetic anhydride medium as well) to form the P–C bond via the Arbuzov reaction.

Methylenenbis(benzylcarbamate) **10** was prepared as described elsewhere [23]. The two-component version of amidoalkylation of the phosphonic acid **3b** with compound **10** in acetic anhydride under conditions of acid catalysis [20–23] yielded the target phosphinic acid **11**. Hydrolysis of the latter followed by chromatography on a cationite gave the pseudo glycyl-leucine **1c** (Scheme 6).

The pseudo isoleucyl-glycine **1d** was synthesized via the three-component Kabachnik–Fields reaction (Scheme 7) [20–23]. The interaction of the phosphonic acid **3a** with benzylcarbamate and α-methylbutyraldehyde in a 2 : 1 acetic anhydride–acetyl chloride mixture upon cooling yielded the N-protected aminophosphinic acid **12** combining the structural isosters of isoleucine and glycine. Hydrolysis of compound **12**

Scheme 7.



followed by chromatography on a cationite gave the pseudo isoleucyl-glycine **1d**.

To conclude, we suggested and performed the synthesis of certain phosphorus isosters of four dipeptides. The prepared compounds may be further used as building blocks to produce the pseudo peptides following the β-amyloid sequence.

EXPERIMENTAL

^{1}H, ^{31}P, and ^{13}C NMR spectra were recorded using a Bruker DPX-200 spectrometer using TMS or 85% H_3PO_4 as references. Melting points were determined using a Boetius PHMK device or in an open capillary. TLC analysis was performed using Silufol plates, Merck glass plates (the silica gel UV-254 thickness of 0.2 mm), and Alufol plates (Kavalier) developing with iodine vapor or UV irradiation. Dowex 50WX8-200 (H^+) (Lancaster), KU IKhT (H^+) (Russian Institute of Chemical Technology, Moscow), and Diasorb (Sulfo) (H^+) cationites were used for ion-exchange column chromatography purification. Silpearl and L100/160 (Chemapol) silica gels were used for column chromatography purification.

Benzylcarbamate, formaldehyde diethyl acetal, diphenylaminomethane, phthalic anhydride, *S*-ethylisothiourea hydrobromide, 4-aminobutyraldehyde dimethyl acetal, hexamethyltriamidophosphite, ethyl acrylate, acetic anhydride, acetyl chloride, and hypophosphoric acid were commercial products (Reakor, Alfa Aesar, or Acros Organics). 1,3,5-Tris(diphenylmethyl)hexahydro-*S*-triazine **8** was prepared via the interaction of diphenylaminomethane with aqueous-alcoholic formaldehyde solution as described elsewhere [11]. Diethyl ester of α-methyleneglutaric acid was prepared from ethyl acrylate in the presence of hexamethyltriamidophosphite as catalyst [24]. Ethyl ester of α-isobutylacrylic acid was prepared as described in [25].

4-(Phthalyl)aminobutyraldehyde (4) was prepared via the interaction of 4-aminobutyraldehyde dimethyl acetal with phthalic anhydride in benzene. The formed water was eliminated using a Dean–Stark trap, and the obtained 4-(phthalyl)aminobutyraldehyde dimethyl acetal was treated with 0.5 mol/L solution of hydrochloric acid. Yield 57%. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.55 m (2H, CH_2), 2.08 m (2H, CH_2), 3.26 t (2H, CH_2, $^3J_{HH}$ 7.3 Hz], 7.2–7.40 m (4H, Ar), 9.31 br.t [1H, C(O)H].

***N,N'*-Methylenebis(benzylcarbamate) (10)** was prepared via the interaction of diethoxymethane with 2 eq. of benzylcarbamate in acetic anhydride medium [23]. Yield 43%, mp 142–144°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 4.53 d.d (2H, NCH_2N, $^3J_{HH}$ 6.4, $^3J_{HH}$ 6.9 Hz), 5.07 br.s (4H, CH_2O), 5.77 m (2H, 2NH), 7.33 br.s (10H, Ph). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 48.1 (NCN), 66.9 (CH_2O), 128.1, 128.2, 128.5, 136.0, 156.6 [NC(O)].

Phosphonic acids 3a–3c *(general procedure)*. A mixture of 0.08 mol of ammonium hypophosphite (or 0.08 mol of potassium hypophosphite and 0.08 mol of ammonium chloride) and 0.12 mol of hexamethyldisilazane was stirred at boiling during 3 h. The mixture was cooled to 5–10°C, and 0.02 mol of the corresponding ester (ethyl acrylate, diethyl α-methyleneglutarate, or ethyl α-isobutyracrylate) was added dropwise at that temperature. The mixture was stirred at room temperature during 2–3 h and left overnight under an argon atmosphere. 30 mL of a 1 : 1 water–ethanol mixture was added dropwise upon cooling and vigorous stirring, and the formed mass was evaporated in a vacuum. The residue was dissolved in 50 mL of chloroform and washed with aqueous HCl (0.2 mol/L, 3 × 10 mL). The organic phase was dried over magnesium sulfate, evaporated under reduced pressure, and thoroughly dried in a high vacuum.

2-(Ethoxycarbonyl)ethylphosphonic acid (3a). Yield 57% (with respect to the ester, from a mixture of potassium hypophosphite and ammonium chloride). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.16 t (3H, CH_3, $^3J_{HH}$ 7.3 Hz), 1.90 m (2H, CH_2), 2.48 m (2H, CH_2), 4.28 q

(2H, CH_2O, $^3J_{HH}$ 7.3 Hz), 7.05 d (1H, PH, $^1J_{PH}$ 563.0 Hz), 12.70 br.s (1H, POOH). ^{31}P NMR spectrum (D_2O): δ_P 26.10 ppm. Found, %: C 36.63, 36.72; H 6.75, 6.87. $C_5H_{11}O_4P$. Calculated, % C 36.15; H 6.67.

2,4-Bis(ethoxycarbonyl)butylphosphonic acid (3d). Yield 63% (with respect to the ester, from ammonium hypophosphite). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.17 t (3H, CH_3, $^3J_{HH}$ 7.3 Hz), 1.21 t (3H, CH_3, $^3J_{HH}$ 7.3 Hz), 1.75–2.10 m [4H, $CH_2C(O)$], 1.96 m (2H, CH_2P), 2.68 m [1H, CHC(O)], 3.98 q (2H, CH_2O, $^3J_{HH}$ 7.3 Hz), 4.05 q (2H, CH_2O, $^3J_{HH}$ 7.3 Hz), 6.97 d (1H, PH, $^1J_{PH}$ 560.0 Hz), 12.65 br.s (1H, POOH). ^{31}P NMR spectrum (CCl_4–CD_3OD): δ_P 28.70 ppm. Found P, %: 11.94, 11.90. $C_{10}H_{19}O_6P$. Calculated P, %: 11.63.

2-(Ethoxycarbonyl)-4-methylamylphosphonic acid (3c). Yield 71% (with respect to the ester, from ammonium hypophosphite). ^1H NMR spectrum (CCl_4–CD_3OD), δ, ppm: 0.97–1.05 br.t (3H, CH_3, $^3J_{HH}$ 7.0 Hz), 1.35 d (6H, CH_3, $^3J_{HH}$ 7.0 Hz), 1.48 m (1H, CH), 1.65 m (2H, CH_2CH), 2.10 m (2H, CH_2P), 2.83 m [1H, CHC(O)], 4.17 m (2H, CH_2O), 7.05 d (1H, PH, $^1J_{PH}$ 560.0 Hz), 12.68 br.s (1H, POOH). ^{31}P NMR spectrum (CCl_4–CD_3OD): δ_P 31.10 ppm. Found P, %: 13.63, 13.43. $C_9H_{19}O_4P$. Calculated P, %: 13.94.

2,4-Bis(ethoxycarbonyl)butyl-1-(benzyloxycarbonylamino)-4-(phthalylamino)butylphosphinic acid (7). 5.7 g (26 mmol) of 4-(phthalyl)aminobutyraldehyde **6** was slowly added to a stirred solution of 7.0 g (26 mmol) of compound **3c** and 4.0 g (26 mmol) of benzylcarbamate in 40 mL of a 1: 1 acetic anhydride–acetyl chloride mixture. The reaction mixture was stirred during 30 h, concentrated via co-evaporation with toluene, poured into 50 mL of ice water, and evaporated. The residue was dissolved in 30 mL of chloroform, washed with water (2 × 10 mL), and evaporated in a vacuum. The residue was crystallized from petroleum ether and recrystallized from diethyl ether. Yield 5.3 g (33%), white powder, mp 81–83°C, R_f 0.23 ($CHCl_3$: MeOH : AcOH = 10 : 2 : 1). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.21 m (6H, CH_3), 1.50–2.00 m (1H, PCH_2; 4H, $\underline{CH_2CH_2}CHN$; 2H, $\underline{CH_2}CH_2CO$), 2.00–2.40 m (1H, $P\underline{CH_2}$; 2H, $\underline{CH_2}CO$), 2.80 m (1H, $PCH_2\underline{CH}$), 3.68 m (2H, NCH_2), 3.95 m (1H, $P\underline{CH}N$), 4.08 q (4H, $\underline{CH_2}O$, $^2J_{PH}$ 6.8 Hz), 5.03 m (2H, $O\underline{CH_2}Ph$), 5.82 d.d (1H, NH, $^3J_{PH}$ 9.8, $^3J_{HH}$ 4.7 Hz), 7.2–7.4 m (5H, C_6H_5), 7.55–7.70 m (2H, Phth), 7.70–7.90 m (2H, Phth). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm (hereafter the asterisk marks the signals of the other diastereomer): 14.1, 14.2, 25.1 d ($^3J_{PC}$ 20.5 Hz), 25.2* d ($^3J_{PC}$ 19.8 Hz), 27.8, 28.6, 29.6, 31.3, 37.4, 38.2, 49.4 d ($^1J_{PC}$ 105.0 Hz), 50.0* d ($^1J_{PC}$ 105.0 Hz), 60.5, 61.0, 67.2, 123.3, 128.2, 128.5, 132.0, 134.0, 136.3, 156.4 d ($^3J_{PC}$ 4.4 Hz), 168.5, 172.7, 173.9 ($^3J_{PC}$ 7.7 Hz). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 54.1, 54.4*.

Pseudo ornityl-glutamate (1a). A mixture of 2.0 g (3.2 mmol) of compound **7** and 0.2 g of hydrazine hydrate was refluxed in 5 mL of ethanol during 2 h, cooled and acidified with HCl (6 mol/L) to pH ≈ 1. The precipitate (phthalic anhydride) was separated immediately and then after cooling. The reaction mixture was evaporated almost to dryness, then 18 mL of 8 mol/L HCl was added, and the mixture was refluxed during 17 h. The reaction mixture was cooled, extracted with chloroform (2 × 5 mL), and evaporated. The oily residue was twice co-evaporated with water and purified via chromatography on a KU IKhT (H^+) cationite (eluent: 0.1–0.5 mol/L HCl). The eluate (the positive ninhydrin reaction) was evaporated, the residue slowly solidified at storage. Yield 0.65 g (57%), mp 117–123°C, R_f ~ 0.20 (BuOH : AcOH : H_2O = 4 : 2 : 1). ^1H NMR spectrum (D_2O), δ, ppm: 1.50–2.10 m (8H, $\underline{CH_2CH_2}CHN$, $\underline{CH_2}CH\underline{CH_2}P$), 2.20–2.40 br.t (2H, CH_2CO), 2.64 m (1H, CHCO), 2.94 m (2H, CH_2N), 3.08 m (1H, CHN). ^{31}P NMR spectrum (D_2O): δ_P 33.31 ppm. Found, %: C 33.87, 33.71; H 7.23, 7.30; Cl 9.87, 9.67. $C_{10}H_{21}N_2O_6P{\cdot}HCl{\cdot}H_2O$. Calculated, %: C 34.25; H 6.90; Cl 10.11.

Pseudo arginyl-glutamate (1b). 0.5 g (2.8 mmol) of S-ethylisothiourea hydrobromide was added to a solution of 0.5 g (1.4 mmol) of compound **1a** in 3 mL of 25% aqueous ammonia at pH ≈ 11. The reaction mixture was stirred at room temperature during 7 days, adding aqueous ammonia to maintain pH > 10. After the reaction was complete, the mixture was evaporated, co-evaporated with water, and purified via chromatography on a Dowex 50WX8-200 (H^+) cationite (eluent: water to remove bromine anions, then 0.5% ammonia solution). The eluate was evaporated, the residue was additionally purified via chromatography on a Diasorb (Sulfo) (H^+) cationite (eluent: water); the fractions showing positive ninhydrin reaction were evaporated, and the residue was crystallized from acetone. Yield 0.2 g (37%), mp 114–116°C, R_f ~ 0.23 (BuOH : AcOH : H_2O = 4 : 1 : 1). ^1H NMR spectrum (D_2O), δ, ppm: 1.40–2.00 m (8H, $NHCH_2\underline{CH_2CH_2}$, $P\underline{CH_2}CH\underline{CH_2}$), 2.11 m (2H, CH_2CO), 2.43 m (1H, CHCO), 2.90–3.10 m (1H, CHN), 3.14 m (2H, CH_2N). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 24.0*, 24.5, 25.0, 30.5, 31.7 d ($^1J_{PC}$ 97.3 Hz), 34.0, 38.8*, 40.4, 42.1, 49.7 d ($^1J_{PC}$ 91.1 Hz),

50.1* d ($^1J_{PC}$ 89.7 Hz), 156.7 (NCN), 181.0 (CO), 182.7 d ($^3J_{PC}$ 15.7 Hz, CO). ^{31}P NMR spectrum (D_2O), δ_P, ppm: 35.1*, 35.5. Found: C 36.85, 36.73; H 7.19, 7.23; N 15.57, 15.54. $C_{11}H_{23}N_4O_6P\cdot H_2O$. Calculated, %: C 37.08; H 7.07; N15.72.

Pseudo glycyl-leucine (1c). *a*. A mixture of 2.2 g (10 mmol) of compound **3b** and 2.0 g (3.3 mmol) of compound **7** in 10 mL of toluene was refluxed during 7–10 h and then evaporated almost to dryness. The residue (≈3 g), phosphinic acid **9** (δ_P 43.50 ppm), was refluxed in 10 mL of 45% aqueous HBr during 10 h. The reaction mixture was evaporated in a vacuum, and the residue was purified via chromatography on a KU IKhT (H^+) cationite (eluent: water, then 1 mol/L HCl). The eluate was evaporated, and the oily residue was treated with excess of propylene oxide in aqueous ethanol. Yield 1.1 g (50%), mp 222–224°C (decomp.), R_f 0.12 (*i*-BuOH : AcOH : H_2O = 20 : 5 : 3). ^{1}H NMR spectrum (D_2O), δ, ppm: 0.62 d (3H, CH_3, J_{HH} 6.4 Hz), 0.68 d (3H, CH_3, J_{HH} 6.4 Hz), 1.20 m (1H, CH), 1.35 m (2H, $\underline{CH_2}CH$), 1.68 m (1H, PCH_2), 1.84 m (1H, PCH_2), 2.46 m [1H, CHC(O)], 2.91 d (2H, PCH_2N, J_{PH} 9.4 Hz). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 21.6 (CH_3), 22.1 (CH_3), 25.5 ($\underline{C}HCH_3$), 30.82 d ($P\underline{C}H_2C$, $^1J_{PC}$ 95.6 Hz), 37.5 d (PCH_2N, $^1J_{PC}$ 94.4 Hz), 37.7 d ($PCC\underline{C}H_2$, $^3J_{PC}$ 12.4 Hz), 42.81 d [CHC(O)], $^2J_{PC}$ 4.4 Hz), 176.8 d [C(O), $^3J_{PC}$ 5.0 Hz]. ^{31}P NMR spectrum (D_2O–DCl, pH ≈ 1): δ_P 40.90 ppm. Found: C 42.89, 42.83; H 8.19, 8.23; N 6.17, 6.23. $C_8H_{18}NO_4P$. Calculated, %: C 43.05; H 8.13; N 6.28.

b. 3 mL of acetyl chloride was added to a solution of 2.2 g (10 mmol) of compound **3b** and 3.1 g (10 mmol) of compound **10** in 12 mL of acetic anhydride. The mixture was stirred at room temperature during 17 h, poured into 50 mL of ice water, and evaporated. The residue was treated with 30 mL of saturated solution of sodium hydrogen carbonate and 10 mL of diethyl ether. The crystalline benzylcarbamate was filtered off. The aqueous layer was additionally washed with diethyl ether and acidified to pH ≈ 1–2. The oily product was separated, and the aqueous layer was additionally extracted with ethyl acetate (2 × 10 mL). The extract was combined with the oily product, washed with water, dried over sodium sulfate, and evaporated in a vacuum. The oily residue was the phosphinic acid **11** (R_f ~ 0.30, $CHCl_3$: *i*-PrOH : AcOH = 20 : 4 : 1). ^{1}H NMR spectrum ($CDCl_3$ + a drop of CF_3COOH), δ, ppm: 0.83 m (3H, CH_3), 0.88 m (3H, CH_3), 1.17 br.t (3H, CH_3, J_{HH} ≈ 7.5 Hz), 1.45 m (1H, $C\underline{H}CH_3$), 1.51 m (1H, CH, $CHC\underline{H_2}CH$), 1.58 m (1H, CH, $CHC\underline{H_2}CH$), 1.85 m (1H, CH, PCH_2), 2.17 m (1H, CH, PCH_2), 2.95 d (2H, PCH_2N, $^2J_{PH}$ 10.0 Hz), 3.08 m (1H, CHCO), 3.70 br. q (2H, CH_2O, J_{HH} ≈ 7.5 Hz), 5.10 m (2H, $PhC\underline{H_2}O$), 6.90–7.20 m (5H, Ph). ^{31}P NMR spectrum: δ_P 40.30 ppm.

3 g of the crude acid **11** was hydrolyzed with 20 mL of 8 mol/L of HCl during 15 h. The reaction mixture was evaporated in a vacuum, co-evaporated with water, and the residue was purified via chromatography on a KU IKhT (H^+) cationite (eluent: 1 mol/L HCl). The eluate was evaporated in a vacuum, and the oily residue was treated with excess of propylene oxide in aqueous ethanol. Yield 1.3 g (65%).

Pseudo isoleucyl-glycine (1d). 6 mL of acetyl chloride was added to a solution of 1.7 g (10 mmol) of acid **3a** and 1.5 g (10 mmol) of benzylcarbamate in 12 mL of acetic anhydride, and then 1.0 g (12 mmol) of 2-methylbutyraldehyde was slowly added dropwise. The reaction mixture was stirred at room temperature during 1 d, then poured into 50 mL of ice water, and evaporated in a vacuum. The oily residue (≈ 3 g) was dissolved in 15 mL of chloroform, washed with water (2 × 10 mL), and dried over sodium sulfate. The organic layer was evaporated; the residue was crystallized from petroleum ether and recrystallized from diethyl ether. We isolated 1.8 g (47%) of **1-[(*N*-benzyloxycarbonyl)amino]-2-methylbutyl-2-(ethyloxycarbonyl)ethylphosphinic acid 12**; mp 92–94°C, R_f 0.25 ($CHCl_3$: MeOH : AcOH = 10 : 2 : 1). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.92 br.t (3H, CH_3, $^3J_{HH}$ 7.0 Hz), 0.98 d (3H, CH_3, $^3J_{HH}$ 6.8 Hz), 1.23 br.t (3H, CH_3, $^3J_{HH}$ 7.5 Hz), 1.37 m (1H, $C\underline{H}CH_3$), 1.71 m (1H, $C\underline{H_2}CH$), 2.02 m (2H, PCH_2; 1H, $C\underline{H_2}CH$), 2.55 br.t (2H, CH_2CO, $^3J_{HH}$ 7.5 Hz), 3.95 m (1H, PCHN), 4.12 q (2H, CH_2O, $^3J_{HH}$ 7.5 Hz), 5.11 m (2H, $PhC\underline{H_2}O$), 5.23 m (1H, NH), 7.33 m (5H, Ph), 8.0 br.s (1H, POH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 0.87 d (3H, CH_3, $^3J_{HH}$ 6.5 Hz), 0.97 t (3H, CH_3, $^3J_{HH}$ 7.0 Hz), 1.21 t (3H, CH_3, $^3J_{HH}$ 7.1 Hz), 1.34 m (1H, $C\underline{H}CH_3$), 1.64 m (1H, $C\underline{H_2}CH$), 1.96 m (2H, PCH_2; 1H, $C\underline{H_2}CH$), 2.52 m (2H, CH_2CO), 3.81 d.d (1H, PCHN, $^2J_{PH}$ 10.6, $^3J_{HH}$ 5.3 Hz), 3.97* d.d (1H, PCHN, $^2J_{PH}$ 10.6, $^3J_{HH}$ 2.8 Hz), 4.10 q (2H, CH_2O, $^3J_{HH}$ 7.0 Hz), 5.08 br.s (2H, $PhCH_2O$), 7.30 m (5H, Ph). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 55.8, 55.9*. ^{31}P NMR spectrum (CD_3OD), δ_P, ppm: 50.5, 50.8*.

A solution of 1.6 g (4 mmol) of acid **12** in 15 mL of 6 mol/L of HCl was refluxed during 15 h and then evaporated in a vacuum. The residue was co-

evaporated with water and purified via chromatography on a Dowex 50WX8-200 (H^+) cationite (eluent: 0.5 mol/L HCl). The fractions exhibiting positive ninhydrin reaction were combined and co-evaporated with water, and the residue was treated with propylene oxide in aqueous ethanol. The formed crystalline precipitate was recrystallized from aqueous ethanol. Yield 0.7 g (76%), mp 174–176°C, R_f 0.15 (BuOH : AcOH : H_2O = 20 : 5 : 2). ^{1}H NMR spectrum (D_2O), δ, ppm: 0.86 t (3H, CH_3, $^3J_{HH}$ 7.3 Hz), 0.99 d (3H, CH_3, $^3J_{HH}$ 6.6 Hz), 1.04* d (3H, CH_3, $^3J_{HH}$ 6.0 Hz), 1.39 m (2H, C$\underline{H}_2$CH), 1.60* m (2H, C$\underline{H}_2$CH), 1.87 m (2H, PCH_2), 2.01 m (1H, C$\underline{H}$CHN), 2.55 m (2H, CH_2CO), 3.08* d.d (1H, *PCHN, $^2J_{PH}$ 8.8, $^3J_{HH}$ 5.9 Hz), 3.16 d.d (1H, PCHN, $^2J_{PH}$ 8.4, $^3J_{HH}$ 4.0 Hz). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 10.4*, 10.8 (CH_3), 14.2 d (CH_3, $^3J_{PC}$ 3.3 Hz), 15.8* d (CH_3, $^3J_{PC}$ 5.9 Hz), 24.3 d (PCH_2, $^1J_{PC}$ 95.9 Hz), 24.5* d (PCH_2, $^1J_{PC}$ 95.5 Hz), 24.6 d (CH, $^2J_{PC}$ 4.8 Hz), 26.7 d (CH_2, $^3J_{PC}$ 4.0 Hz), 26.8* d (CH_2, $^3J_{PC}$ 5.9 Hz), 32.9 ($\underline{C}H_2CO$), 33.5* ($\underline{C}H_2CO$), 53.3 d (PCN, $^1J_{PC}$ 91.8 Hz), 54.8* d (PCN, $^1J_{PC}$ 91.1 Hz), 177.4 d (CO, $^3J_{PC}$ 15.0 Hz). ^{31}P NMR spectrum (D_2O): δ_P 44.40 ppm. Found: C 42.76, 42.57; H 8.20, 8.34; N 6.17, 6.23. $C_8H_{18}NO_4P$. Calculated, %: C 43.05; H 8.13; N 6.28.

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