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Synthesis of Phosphinic Acids on the Basis of Hypophosphites: IV.[1] Synthesis of Pseudo-γ-glutamylglycine and Its Enantiomers

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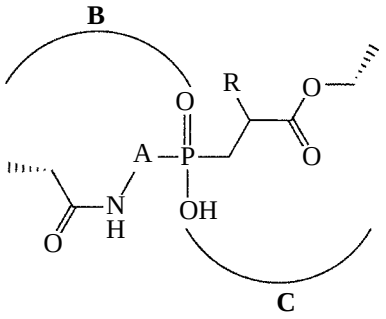
Abstract — A new method for construction of pseudopeptide molecules is proposed, exemplified by the synthesis of [3-amino-3-(hydroxycarbonyl)propyl][2-(hydroxycabonyl)ethyl]phosphinic acid (**I**) (pseudo-γ-glutamylglycine), starting from ammonium hypophosphite. Enzymatic synthesis using immobilized penicillamidase allowed preparation of the enantiomers of acid **I**.

Phosphinic acids are useful as models of transition states of biochemical reactions and can serve as effective inhibitors of natural enzymes [2]. In this connection elaboration of convenient synthetic approaches to phosphine analogs of peptides is a prospective route to potentially physiologically active compounds [3–5]. The key step in the construction of new phosphoryl analogs of natural peptides is substitution of the amide fragment C–C(O)–N of a natural peptide by a phosphinate fragment C–P(OH)(O)–C. The known methods of pseudopeptide synthesis [3–8] involve two steps: synthesis of an aminoalkylphosphonous component **B** (pseudo-N peptide component), a phosphonous analog of a natural amino acid and its subsequent addition to α-substituted acrylates (pseudo-C peptide component) to form a pseudopeptide fragment (**C**) of the desired structure [6–8].



The synthesis of the aminoalkylphosphonous component, that forms the first phosphorus–carbon bond, has been satisfactorily developed [9, 10]. In this case, the synthesis of component B requires introduction of protective groups both to the nitrogen and phosphorus atoms and consists of three or four steps [9, 10]. The synthesis of the pseudopeptide fragment, that forms the second phosphorus–carbon bond, most commonly involves the addition of silyl esters of aminoalkylphosphonous acids to acrylates by the procedure in [11].

In this work we propose a new synthetic approach to pseudopeptides with reversed construction steps: The first phosphorus–carbon bond is formed via addition of a hypophosphite to an acrylate [12] and the second, by construction and addition of the aminoalkyl fragment [13]. This synthetic approach may prove more facile and convenient in one-pot formation of both phosphorus–carbon bonds, and we develop it aiming at preparing various functionally substituted phosphinic acids [14–16].

The proposed pseudo-γ-glutamylpeptide synthesis is simplified by a convenient procedure [13, 17, 18] for preparing vinylphosphoryl compounds. The latter are used as starting materials for adding the amino acid function in the form of diethyl acetamidomalonate and forming a phosphoryl analog of glutaminic acid, viz. fragment **B** of the pseudopeptide molecule.

Here we propose a new method for synthesis of [3-amino-3-(hydroxycarbonyl)propyl][2-(hydroxycabonyl)ethyl]phosphinic acid (**I**) (pseudo-γ-glutamylglycine; γ-Gluψ(PO_2CH_2)Gly) [5, 8] from ammonium hypophosphite (**II**) (Scheme 1) and an enzymatic synthesis of the enantiomers of acid **I** (Scheme 2).

The proposed synthesis of pseudo-γ-glutamylglycine (**I**) is two-step. The first step involves one-pot formation of both phosphorus–carbon bonds and construction of pseudopeptide fragment **C** via the formation of a key intermediate of the synthesis, vinylphosphinate **III**, through a sequence of reactions,

[1] For communication III, see [1].

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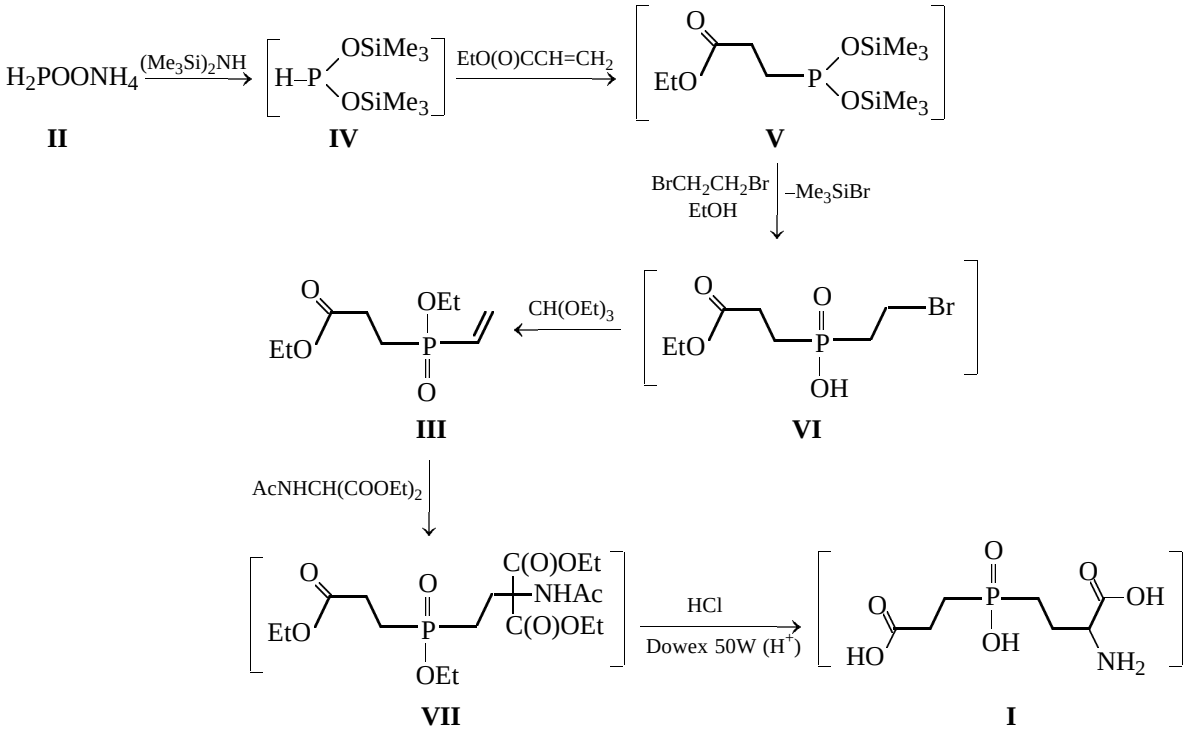
starting from ammonium hypophosphite (**II**), in a total yield of 38–43% (depending on the isolation procedure) without separation of intermediates **IV**–**VI**) (Scheme 1). The second step involves introduction of the amino acid function and isolation of pseudo-γ-glutamylglycine (**I**) (Scheme 1).

Scheme 1.



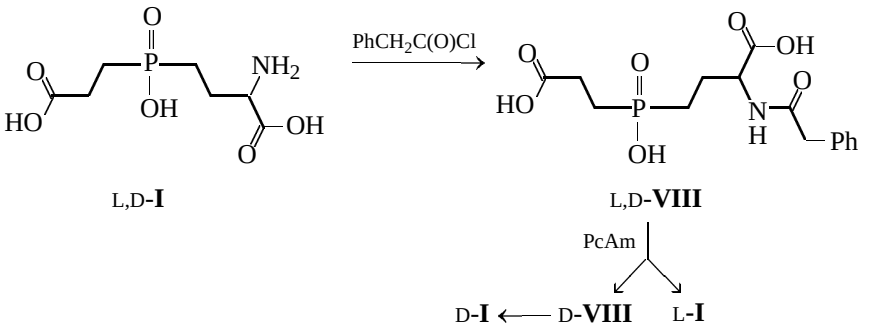
Bis(triaminosilyl) hypophosphite (**IV**) is readily formed from ammonium hypophosphite (**II**) and silazane [19, 20] and added *in situ* to ethyl acrylate to form bis(trimethylsilyl) 3-(ethoxycarbonyl)phopylphosphonite (**V**) [12]. Treatment of the latter *in situ* with excess 1,2-dibromoethane followed by alcoholysis gave (2-bromoethyl)-[2-(ethoxycarbonyl)ethyl]-phosphinic acid (**VI**) which was treated *in situ* with excess triethyl orthoformate. Dehydrobromination of the β-bromoethyl fragment of acid **VI** is accompanied by esterification of the C(O)OH and P(O)OH groups and affords ethyl [2-(ethoxycarbonyl)ethyl]vinylphosphinate (**III**) [13, 17]. Phosphinate **III** as the target product of the first step of the synthesis was isolated, characterized, and used for further transformations in the second step of the synthesis of aminophosphinic acid **I**.

The Michael addition of diethyl acetamidomalonate to vinylphosphinate **III** leads to formation of ethyl [3-(acetylamino)-3,3-bis(ethoxycarbonyl)propyl][2-(ethoxycarbonyl)ethyl]phosphinate (**VII**) which was subjected to acid hydrolysis *in situ* (Scheme 1) to form [3-amino-3-(hydroxycarbonyl)propyl][2-(hydroxycarbonyl)ethyl]phosphinic acid (**I**) in 71% yield (per diethyl acetamidomalonate) after chromatography on Dowex 50W(H^+) as the final step of the synthesis of racemic amino acid **I** [21, 22].

Comprehensive biological investigations often require both enantiomers of a physiologically active molecule. Amino acids are convenient to resolve enzymatically. We propose an enzymatic synthesis of the L and D enantiomers of amino acid **I** with use of immobilized penicillamidase for the stereoselective hydrolysis of N-phenylacetylated acid **VIII** by Scheme 2 [23].

Earlier we took advantage of the high enantioselectivity of penicillamidase in the enzymatic hydrolysis of N-phenylacetylated amino acids in the synthesis of the enantiomers of phosphoruscontaining α-aminocarboxylic acids [24] by analogy with the synthesis of the enantiomers of α-aminophosphonic acids [25]. Amino acid **I** was N-acylated with phenylacetyl chloride by a classical Schotten–Baumann procedure

Scheme 2.



[26]. The hydrolyzed L form of acid **I** was separated from the nonhydrolyzed D form of N-phenylacetylated amino acid **VIII** by ion-exchange chromatography. Acid hydrolysis of D-**VIII** gave the D enantiomer of acid **I** without noticeable racemization [23].

Thus, we propose a new method for construction of pseudo-γ-glutamylglycine [21], which may turn a more convenient synthetic approach to various phosphinic analogs of glutamylpeptides compared with the syntheses proposed in [3, 4]. We also propose an enzymatic synthesis of the enantiomers of pseudo-γ-glutamylglycine [23]. Valyaeva *et al*. [27] recently applied our methodology [13, 21] for the synthesis of a phosphinic analog of γ-glutamylglutamate.

EXPERIMENTAL

The ^{1}H, ^{31}P and ^{13}C NMR spectra were registered on Bruker DPX-200 and Bruker CXP-300 spectrometer against internal TMS and external H3PO4. The optical rotation was measured on Perkin–Elmer 241 and Polamat A polarimeters. The mass spectra were registered on a Finigan 4021 instrument. The melting points were measured on a Boetius hot stage or in an open capillary. All reactions with silyl esters of hypophosphorus and phospinic acids in situ were performed under argon. All solvents used were carefully dried. Ammonium hypophosphite was prepared by a slightly modified published method [11]. Triethyl orthoformate, diethyl acetamidomalonate, propylene oxide, and phenylacetyl chloride were purchased from Khimeks (St. Petersburg).

Thin-layer chromatography of individual compounds and reaction mixtures was performed on Silufol plates (eluent chloroform–acetone, 5:1), Merck glass plates with a 0.2-mm layer of silica gel UV-254, and Kavalier Alufol plates (neutral alumina on an aluminum foil, eluent 1-butanol–acetic acid–water, 5:1:1), developer ninhydrin (110°C). Ion-exchange chromatography was performed on Dowex 50WX8-200 (H+) (Lancaster) and Diasorb-Sulfo (H^+) (BioKhimMak).

Analytical HPLC was performed on a Gilson chromatograph with a US-Vis 118 detector at 254 nm, column Diasorb 130 C16/T (4 × 250 mm), sorbent particle size 5 μm. Separation was performed under conditions of ion-pair chromatography with a specific counter ion, eluent acetonitrile–distilled water (gradient). For control of enzymatic hydrolysis and quantitative analysis the column was calibrated with phenylacetic acid solutions. The enzymatic reaction was performed with immobilized penicillamidase with an activity of 103 U g^{-1} dry biocatalyst [24, 25].

Synthesis of ethyl [2-(ethoxycarbonyl)ethyl]-vinylphosphinate (III). A mixture of 4.0 g of ammonium hypophosphite (**II**) and 15 ml of hexamethyldisilazane was stirred for 2 h at 120–130°C. After cooling to room temperature, 5.3 ml of ethyl acrylate was slowly added dropwise to the mixture, and it was stirred for 2 h at 40°C, and cooled. 1,2-Dibromoethane, 21 ml, was then added in one portion, and stirring was continued for 5 h at 120°C. The trimethylbromosilane formed and excess 1,2-dibromo ethane were removed in vacuo, and 50 ml of aqueous ethanol (1:1) was added to the residue. The mixture was refluxed for 0.5 h and then evaporated in vacuo. The residue was partitioned between 100 ml of ethyl acetate and 50 ml of water, and the aqueous layer was extracted with ethyl acetate (2 × 50 ml). The combined organic layer was evaporated in vacuo. The residue, according to ^{1}H and ^{31}P data ($CDCl_3$), was a 9:1 mixture of phosphinic acid **VI** (δ_P 54.6 ppm) and [2-(ethoxycarbonyl)ethyl]vinylphosphinic acid (δ_P 42.6 ppm). The latter was probably formed by partial dehydrobromination of acid **VI** in the presence of hexamethyldisilazane. To the residue, 40 ml of triethyl orthoformate was added, and the reaction mixture was refluxed in a Dean–Stark trap for 2–3 h with distillation of the ethyl formate and ethanol formed. Unreacted triethyl orthoformate was removed in vacuo, and the residue was distilled. Vinylphosphinate **III**

was isolated as a yellowish oily substance. Yield 3.8 g (38% per ammonium hypophosphite, after 5 steps). bp 125–129°C (3 mm Hg), n_D^{20} 1.4600. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.25 t (3H, CH_3), 1.34 t (3H, CH_3), 2.07 m (2H, PCH_2), 2.60 m [2H, $C(O)CH_2$], 4.03 m (2H, CH_2OC), 4.15 m (2H, CH_2OP), 6.00–6.50 m (3H, $CH{=}CH_2$). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 41.3. Found, %: C 49.14; 49.33; H 7.97, 8.11. $C_9H_{17}O_4P$. Calculated, %: C 49.09; H 7.78. Mass spectrum: found 221, calculated 221. Column chromatography (eluent chloroform–hexane 2 : 1) gave 43% of vinylphosphinate **III**; TLC: R_f 0.4 (chloroform–acetone, 8 : 1).

Synthesis of [3-amino-3-(hydroxycarbonyl)-propyl][2-(hydroxycarbonyl)ethyl]phosphinic acid (I). A mixture of 6.8 g of ethyl acetamidomalonate, 7.5 g of vinylphosphinate **II**, 8.6 g of potassium carbonate, and 0.5 g of tetrabutylammonium bromide in 20 ml of THF was refluxed for 11–13 h with stirring until complete consumption of ethyl acetamidomalonate (control by TLC on Silufol, eluent chloroform–acetone 4–5 : 1, R_f 0.5–0.6). The reaction mixture was partitioned between 50 ml of chloroform and 25 ml of water, and the aqueous layer was neutralized and extracted with chloroform (2 × 25 ml). The combined organic extract was evaporated in vacuo. The residue (ca. 14 g), according ^{1}H and ^{31}P NMR data ($CDCl_3$), was a 10 : 1 mixture of phosphinate **VII** (δ_P 55.8 ppm) and unreacted vinylphosphinate **III** (δ_P 41.3).

To the oily residue, 70 ml of 8N HCl was added, and the mixture was refluxed for 13–15 h, cooled, washed with diethyl ether (3 × 20 ml), and evaporated in vacuo. The residue was chromatographed on a column (200 mm) of Dowex 50W(H^+), eluent 0.5–0.7 N HCl. Fractions with a positive ninhydrin test were concentrated, dissolved in 20 ml of aqueous ethanol (1 : 4), and treated with excess propylene oxide to isolate the target amino acid. Additional recrystallization from aqueous ethanol gave 5.3 g (71% per ethyl acetamidomalonate) of compound **I**. Total yield 28% (per ammonium hypophosphite), mp 205–207°C (decomp.). ^{1}H NMR spectrum (D_2O + DCl), δ, ppm : 1.70 m (2H), 1.88 m (4H), 2.36 d.t (2H, J_{PH} 12 Hz), 3.92 t (1H). ^{13}C NMR spectrum (D_2O), δ_C, ppm.: 26.4 d (PCH_2C, *J* 91.4 Hz), 27.3 d (PCH_2C, *J* 90.2 Hz), 28.9 d (CCH_2CH, *J* 2.4 Hz), 31.4 d [$CCH_2C(O)$, *J* 3.1 Hz], 57.5 d (CH, *J* 15.8 Hz), 183.3 d [$C(O)CH_2$, *J* 17.1 Hz], 183.6 s [C(O)CH]. ^{31}P NMR spectrum, δ_P, ppm.: 50.0 (D_2O), 44.0 (D_2O + NaOD, pH ~10). Found, %: C 34.06, 33.93; H 6.07, 6.08; N 5.89, 5.86. $C_7H_{14}NO_6P \cdot 0.5H_2O$. Calculated, %: C 33.88; H 6.09; N 5.64.

Synthesis of D,L-[2-(Hydroxycarbonyl)ethyl]-[3-(phenylacetyl)amino-3-(hydroxycarbonyl)propyl]phosphinic acid (VIII). A solution of 4.6 ml of phenylacetyl chloride in 5 ml of dioxane was added at 0–2°C to a solution of 6.9 g of racemic amino acid **I** in 60 ml of aqueous dioxane (2 : 1), maintaining the solution pH at about 9.5 with 50% KOH. The reaction mixture was then stirred at room temperature for 10–15 h, acidified to pH 5, and extracted with ether (2 × 30 ml), after which it was acidified to pH 1–2 and extracted with ethyl acetate (3 × 50 ml). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was recrystallized from a mixture of hexane (or pentane) and acetone. The product was pure enough to be brought in further transformations. An analytically pure sample of acid **VIII** was obtained by recrystallization from a mixture of hexane (or pentane) and acetone. Yield 8.2 g (79%), mp 127–129°C. ^{1}H NMR spectrum (CD_3OD), δ, ppm: 1.80 m (2H), 2.00 m (4H), 2.55 m (2H), 3.65 s (2H), 4.46 t (1H), 7.30 m (5H). ^{31}P NMR spectrum (CD_3OD), δ_P, ppm: 53.4. Found, %: C 49.34, 49.33; H 5.64, 5.61; P 8.31, 8.29. $C_{15}H_{20}NO_7P \cdot 0.5H_2O$. Calculated, %: C 49.18; H 5.78; P 8.46. Mass spectrum: calculated for $(C_{15}H_{20}NO_7P \cdot 0.5H_2O + H - CO_2)^+$ 322.4, found 322.5.

Enzymatic synthesis of the enantiomers of amino acid I. Immobilized penicillinamidase, 3–5 g, was added to a solution of 7.1 g of D,L-**VIII** at pH 7.5, maintaining the solution pH at 7.5. The enzymatic hydrolysis was monitored by HPLC by the formation of phenylacetic acid. After 50% hydrolysis, the enzyme was filtered off, and the reaction mixture was acidified to pH 5 and washed with ether (2 × 40 ml). The aqueous layer was acidified to pH 2 with 6N HCl, concentrated to ~20 ml, and passed through a column (3 × 25–30 cm) of a cation exchanger, eluent water. A strongly acidic eluate with a negative ninhydrin test was evaporated in vacuo. To the oily residue whose ^{1}H and ^{31}P spectra were similar to those of **VIII**, 70 ml of 8 N HCl was added, and the mixture was refluxed for 10 h, cooled, washed with ether (2 × 40 ml), and evaporated in vacuo. The residue was dissolved in aqueous ethanol and treated with excess 1,2-propylene oxide. Recrystallization from aqueous ethanol gave 1.7 g [71.0 % per D,L-**VIII**] of D-**I**. mp 194–195°C (decomp.), $[\alpha]_{546}^{25}$ –23.7° (*c* 1.2, 6 N HCl), $[\alpha]_{546}^{25}$ –13.4° (*c* 1.1, H_2O), $[\alpha]_D^{25}$ –20.9° (*c* 1.2, 6 N HCl). The ^{1}H, ^{13}C and ^{31}P NMR spectra were the same as those of the racemate. Found, %: C 34.33, 34.09; H 6.12, 6.08; N 5.86, 5.82. $C_7H_{14}NO_6P \cdot 0.5H_2O$. Calculated, %: C 33.88; H 6.09; N 5.64.

A less acidic eluate with a positive ninhydrin reaction was evaporated in vacuo, and the residue was recrystallized from ethanol. Recrystallization from

aqueous ethanol gave 1.3 g [54.4% per D,L-**VIII**] of L-**I**. mp 186–187°C (decomp.). The ^{1}H, ^{13}C and ^{31}P NMR spectra were the same as those of the racemate. Found, %: C 33.80, 33.91; H 6.04, 5.99, N 5.89, 5.86. $C_7H_{14}NO_6P \cdot 0.5H_2O$. Calculated, %: C 33.88; H 6.09; N 5.64.

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