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LETTERS TO THE EDITOR

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

Synthesis of Pseudo-Methionyl-Glutamate

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One of the most promising approaches to providing physiologically active compounds is the synthesis of phosphinic structural analogs of endogenous polypeptides playing important regulatory role in operation of the human body, including regulation of its cognitive function. One of the known examples in this area is the effect of adrenocorticotropic hormone (ACTH) on the memory [1]; have been found that some hormone fragments possess no hormonal activity, but they can affect cognitive functions, in particular adrenocorticotropic hormone fragment 4–10, on whose base drug Semax has been created [2].

In this work we used the known approach to the synthesis of peptides phosphoisosteres by replacing an amide bond [C(O)NH$_2$] in the dipeptide molecule with non-hydrolyzable methylenephosphoryl moiety [P(O)(OH)CH$_2$]. This approach is effective in the synthesis of inhibitors of metalloproteinases and aspartylproteinases [3, 4], and may lead to increased enzymatic metabolic stability and modification of the properties of short peptides. In this regard the approach is promising for providing effective neuroprotective agents.

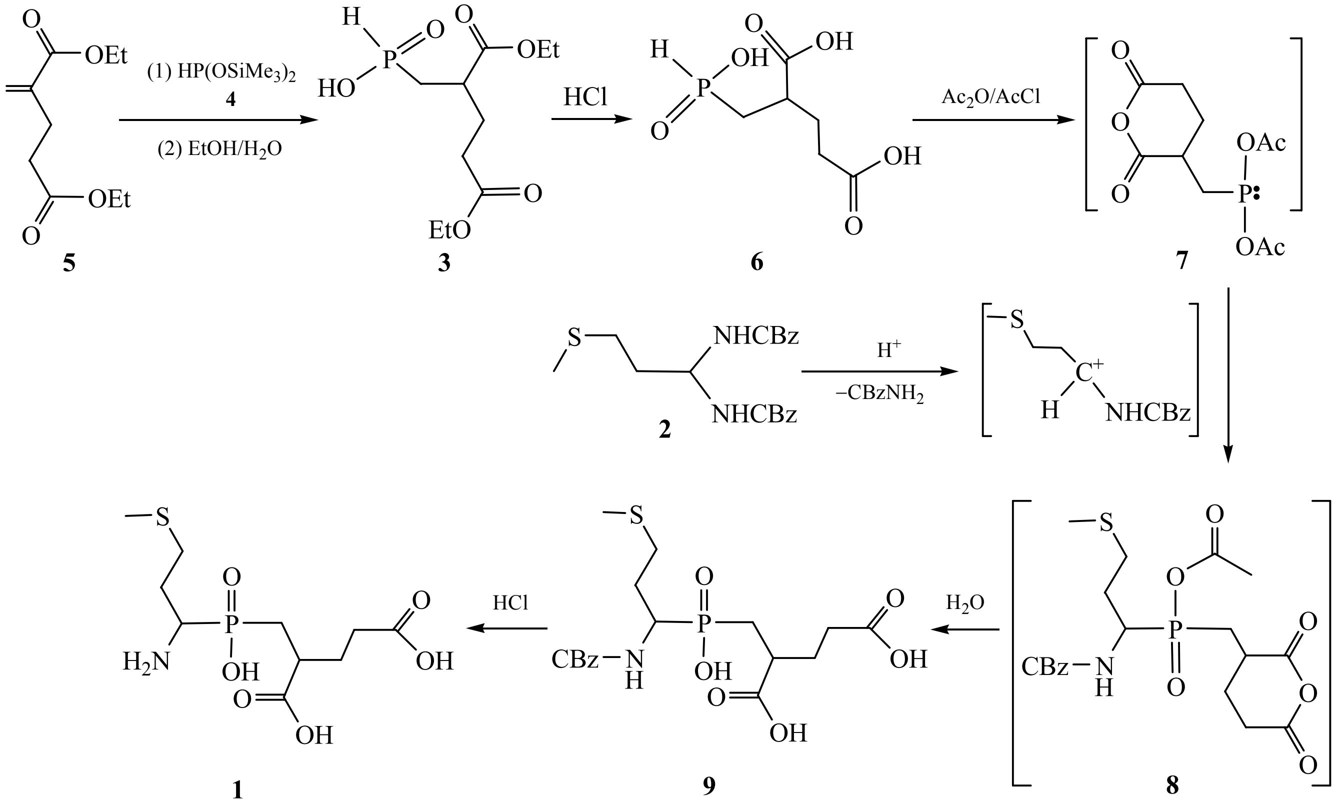
In this work we developed the synthesis of methionyl-glutamate phosphoisoster **1**, which may be of interest for creating oligopeptides in accordance with the sequence present in the adrenocorticotropic hormone, but containing isosteric acidic phosphinic moiety.

The well-known approach to building up the phosphinic peptide molecule is the synthesis of phosphonous isosteres of amino acids [5] followed by their addition to the corresponding α-substituted acrylates [6]. In this report we continued developing an alternative approach [7] to the synthesis of pseudo-peptides with the reverse order of construction of pseudo-peptide molecules. This approach includes preparation of phosphonous acids containing amino acid isoster with the subsequent formation of amino-alkylphosphoryl function [8, 9].

Methods for the synthesis of phosphonic and phosphonous methionine analogues are based on the use of methylthiopropionic aldehyde [5] or its oxime [10, 11]. In this work we used previously synthesized *N*,*N*'-methylthiopropylidenebis(benzylcarbamate) **2** for amidoalkylation of phosphonous acid **3** containing structural isosteres of glutamic acid, since an attempt to carry out amidoalkylation of acid **3** using benzylcarbamate and methylthiopropionic aldehyde in accordance with the three-component amide version of the Kabachnik–Fields reaction failed. In turn, the synthesis of phosphonous acid **3** was readily performed by addition of bis(trimethylsilyl)hypophosphite **4** generated *in situ* from ammonium hypophosphite to diethyl α-methyleneglutarate **5** in accordance with the procedure described earlier in [12] (Scheme 1).

According to the suggested by us mechanism of amidoalkylation of hydrophosphoryl compounds, the

Scheme 1.



formation of phosphorus-carbon bond is the result of the Arbuzov type reaction of $P^{III}OAc$ derivative with acyliminium cations generated *in situ* in acetic anhydride accordingly from hydrophosphorylic component and alkylidenebiscarbamate, which is a sufficiently stable reaction intermediate [13–15].

It was found that the best method of the synthesis of pseudo-methionyl-glutamate **1** is amidoalkylation of phosphonous acid **6** containing no ester groups with methylthiopropylidenebiscarbamate **2** in a mixture of acetic acid and acetyl chloride (1 : 1) in the cold. Probably, under the reaction conditions along with acylation of hydrophosphorylic function P(O)H in $P^{III}OAc$ derivative, the formation occurred of cyclic glutaric anhydride **7** containing exocyclic $P^{III}(OAc)_2$ moiety. According to ^{31}P NMR spectroscopy data, anhydride **7** was much more reactive compared to acylated $P^{III}(OAc)_2$ form of phosphonous acid **3** containing two ester groups. However, isolation of the presumed phosphonous **7** and phosphinate **8** intermediates containing glutaric cyclic moiety was not the purpose of this work.

The treatment of the reaction mixture with water followed by chromatography on silica gel afforded aminophosphinic acid **9**, where *N*-CBz moiety of phosphoryl methionine analogue is linked to the structural glutamic acid isostere. The subsequent acid hydrolysis of acid **9** and chromatography on cationite yielded pseudo-methionyl-glutamate **1**.

In summary, a method for the synthesis of methionyl-glutamate phosphoisostere, interesting for subsequent peptide synthesis in accordance with the sequence inherent to adrenocorticotropic hormone was developed.

Diethyl 2,4-bis(hydroxycarbonyl)butylphosphinate was prepared as described previously [12].

***N,N*'-Methylthiopropylidenebis(benzylcarbamate) (2)** was prepared as described in [13]. Yield 61%, mp 141–142°C. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 2.06 s (3H, SMe), 2.16 m (2H, $\underline{CH_2}CH$), 2.50 t (2H, CH_2S, $^3J_{HH}$ 7.0 Hz), 5.07 br.s (4H, $PhC\underline{H_2}O$), 5.14 m (1H, CHN), 5.76 m (2H, NH), 7.33 br.s (10H, Ph). ^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.82 m (2H, $\underline{CH_2}CH$), 2.00 s (3H, MeS), 2.41 t (2H, CH_2S, $^3J_{HH}$ 7.3 Hz), 5.01

br.s (4H, $PhC\underline{H}_2O$), 5.14 m (1H, CHN), 7.34 m (10H, Ph), 7.67 d (2H, NH, $^3J_{HH}$ 7.3 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 14.6 (MeS), 29.1 (CH_2S), 33.9 ($\underline{C}H_2CH$), 58.8 (NCN), 65.2 (CH_2O), 127.8, 128.3, 137.0, 155.0 (C=O).

2,4-Bis(hydroxycarbonyl)butylphosphonous acid (6) was obtained by hydrolysis of acid **3** with 6 N hydrochloric acid. Yield 91%. ^1H NMR spectrum (D_2O), δ, ppm: 1.62–1.83 m (2H, $C\underline{H}_2CH$; 1H, PCH_2), 1.87–2.10 m (1H, CH_2P), 2.25 t [2H, $CH_2C(O)$, $^3J_{HH}$ 7.4 Hz], 2.68 m [1H, CHC(O)], 6.92 d (1H, PH, $^1J_{PH}$ 560.4 Hz). ^{31}P NMR spectrum (D_2O): δ_P 29.40 ppm.

1-(*N*-Benzyloxycarbonyl)amino-3-methylthiopropyl-2,4-bis(hydroxycarbonyl)butylphosphinic acid (9). To a solution of 2.1 g (10 mmol) of phosphonous acid **6** and 3.9 g (10 mmol) of methylthiopropylidenebiscarbamate **2** in 15 mL of acetic anhydride was added by portions 15 mL of acetyl chloride. The mixture was stirred at room temperature for several hours, then poured into 50 mL of ice water and evaporated in a vacuum. The residue was treated with 30 mL of saturated sodium hydrogen carbonate solution. After removal of benzyl carbamate, aqueous layer was washed with diethyl ether (2 × 10 mL), acidified carefully to pH ~ 1 and extracted with ethyl acetate (3 × 30 mL). The organic layer was evaporated in a vacuum, and the residue was chromatographed on silica gel, eluting with $CHCl_3$–*i*-PrOH mixture (with a gradual increase in the alcohol content). After removing the solvent, the residue was crystallized from petroleum ether. Yield 2.3 g (51%), mp 85–88°C. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.76 m (2H, PCH_2), 1.89 m (2H, $\underline{CH_2}CH_2S$), 2.00 s (3H, SMe), 2.18 m (2H, CH_2), 2.42 m (1H, CHCO), 2.50 m (2H, CH_2S), 2.56 m (2H, CH_2), 3.79 m (1H, PCH), 5.04 m ($PhC\underline{H}_2O$), 7.33 m (5H, Ph), 7.51 m (1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm (the signal denoted with asterisk are corresponded to the minor diastereomer): 14.6 (SMe), 27.2, 27.4* ($\underline{C}H_2CHCO$), 28.5 d ($PCH\underline{C}H_2$, $^2J_{PC}$ 2.2 Hz), 29.0 d (PCH_2, $^1J_{PC}$ 95.1 Hz), 29.3 d (PCH_2, $^1J_{PC}$ 94.8 Hz), 29.8, (SCH_2), 31.2 (CH_2CO), 65.6 ($PhCH_2O$); 127.6, 127.8, 128.4, 137.2 (Ph), 156.3 (CO), 173.9 (CO), 175.7 d (CO, $^3J_{PC}$ 6.6 Hz). ^{13}C NMR spectrum (acetone-d_6), δ_C, ppm: 14.5*, 15.2 (SMe), 38.9 ($\underline{C}HCO$), 50.4 d (PCN, $^1J_{PC}$ 104.7 Hz), 50.7* d (PCN, $^1J_{PC}$ 102.1 Hz), 67.1 ($PhCH_2O$); 128.5, 128.6, 129.2, 138.0 (Ph), 157.4 (CO), 174.8 (CO), 176.4 d (NCO, $^3J_{PC}$ 7.7 Hz). ^{31}P NMR spectrum (DMSO-d_6): δ_P 45.3 ppm.

1-Amino-3-methylthiopropyl-2,4-bis(hydroxycarbonyl)butylphosphinic acid (pseudo-methionyl-glutamate) (1) was obtained by hydrolysis of phosphinic acid **9** with 6 N HCl solution followed by chromatography on cationite. Yield 57%, mp 176–178°C (decomp.). ^1H NMR spectrum (D_2O + 1 drop of DCl), δ, ppm: 1.64–1.92 m (4H, $\underline{CH_2}CH_2CO$, CH_2P), 1.99 s (3H, MeS), 2.02–2.18 m (2H, CH_2S), 2.35 br.t (2H, CH_2CO, $^3J_{HH}$ 7.3 Hz), 2.45–2.80 m (2H, CH_2CO; 1H, CHCO), 3.33 m (1H, PCHN). ^{13}C NMR spectrum (D_2O + 1 drop of DCl), δ_C, ppm: 13.8 (MeS), 26.4 d ($PCH\underline{C}H_2$, $^2J_{PC}$ 6.9 Hz), 28.3 d ($PCH_2CH\underline{C}H_2$, $^3J_{PC}$ 10.7 Hz), 28.5* d ($PCH_2CH\underline{C}H_2$, $^3J_{PC}$ 10.7 Hz), 29.5 d (PCH_2, $^1J_{PC}$ 95.5 Hz), 29.6 d (CH_2S, $^3J_{PC}$ 9.2 Hz), 31.0 (CH_2CO), 31.2* (CH_2CO), 38.5 (CHCO), 39.4* d (CHCO, $^3J_{PC}$ 3.1 Hz), 48.9 br.d (PCN, $^1J_{PC}$ 93.2 Hz), 177.3 ($\underline{C}OCH_2$), 177.4* ($\underline{C}OCH_2$), 178.4 ($\underline{C}OCH$), 179.0* d ($\underline{C}OCH$, $^3J_{PC}$ 3.4 Hz). ^{31}P NMR spectrum (D_2O + 1 drop of DCl), δ_P, ppm: 35.5, 32.6*. Found, %: C 35.90, 35.83; H 6.95, 6.93; S 9.90, 9.93. $C_{10}H_{20}NO_6PS\cdot H_2O$. Calculated, %: C 36.25; H 6.69; S 9.68.

^1H, ^{31}P, and ^{13}C NMR spectra were recorded on a Bruker DPX-200 Fourier spectrometer. Melting points were determined on a Boetius PHMK apparatus or in an open capillary.

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