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LETTERS TO THE EDITOR

Phosphorus-Containing Aminocarboxylic Acids: XV.[1] α,ω-Diamino-ω,ω-diphosphonoalkylcarboxylic Acids

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Abstract—A general method for the synthesis of phosphonic aminocarboxylic acids combining aminodiphosphonic and amino acid functions in one molecule was developed. Amino acids of a new type are AP-acids analogs, which are ligands of glutamate receptors that determine the processes of information transmission and processing in the central nervous system, and can also be promising compounds as new components of radiopharmaceuticals.

Keywords: ω-cyanoalkylacetamidomalonic esters, ω-amino-ω,ω-diphosphonoalkylacetamidomalonic esters, α,ω-diamino-ω,ω-diphosphonic acids, radiopharmaceuticals, glutamate receptors

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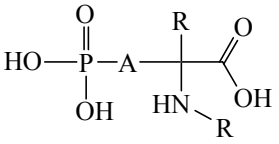
An interest in ω-phosphonic α-aminocarboxylic acids is due to their physiological activity as ligands of glutamate receptors that determine the processes of information transmission and processing in the central nervous system, which is important for the prevention and treatment of Alzheimer, Huntington, Parkinson and other neurodegenerative and psychoneurological diseases, and also for learning and memory processes [2–4].

Pronounced physiological activity and social significance of the diseases associated with it determine a large number of works concerning the synthesis and study of the properties of ω-phosphonic α-aminocarboxylic acids in recent years. The analysis of published data makes it possible to distinguish two basic synthetic approaches including modification of aminocarboxylic function and the hydrocarbon fragment, the spacer **A**, which bounds the phosphonic and aminocarboxylic functions of the canonical molecule [5].

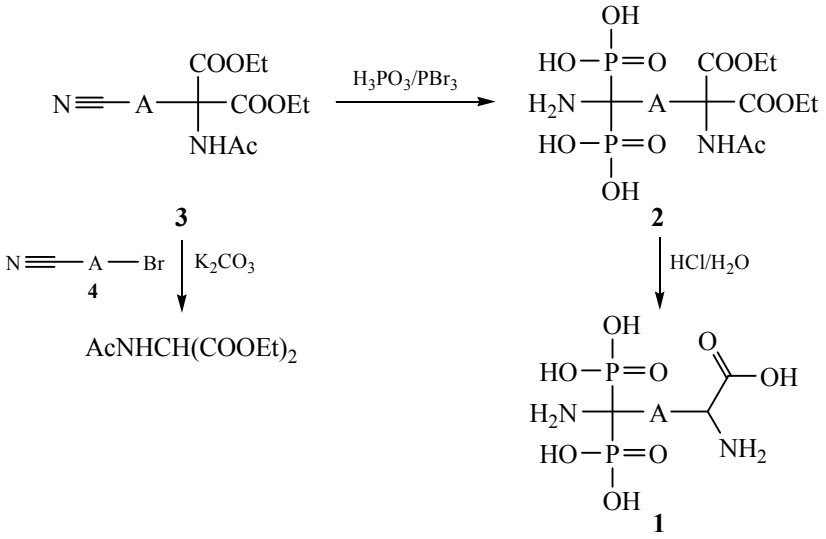


[1] For communication XIV, see [1].

Here, we report on a general synthesis method of ω-phosphonic α-aminocarboxylic acids **1** where the aminodiphosphonic and aminocarboxylic functions are combined in the molecules.

The 1-amino-1,1-diphosphonic fragment can be considered as a structural analog of the amino acid function containing a second phosphonic group, which probably provides not only the complexing, but also the physiological activity of aminodiphosphonic acids [6, 7]. α,ω-Diamino-ω,ω-diphosphonoalkylcarboxylic acids **1** are analogs of AP-acids with enhanced complexing properties, which may lead to modification of the properties of these amino acids as glutamate receptors ligands. In addition, these compounds can be very promising as a ligand component of radiopharmaceuticals, as evidenced by the data on high antitumor activity against osteosarcoma of peptide derivatives of methotrexate and geminal bisphosphonic aminocarboxylic acids [8–10]. In this regard, it should be noted that aminodiphosphonic acids are synthetically more accessible than 1,1-diphosphonic acids. It is due to unique simplicity of one-pot formation of 1-amino-1,1-diphosphonic function by adding two molecules of phosphorous acid to the nitrile group [6, 7]. This approach is developed here for the synthesis of ω-amino-ω,ω-diphosphonoalkylacetamidomalonic esters **2**, whose

Scheme 1.



A = $(CH_2)_n$, n = 2–4.

acid hydrolysis led to the desired aminodiphosphonic aminocarboxylic acids **1**. The corresponding nitriles, ω-cyanoalkylacetamidomalonic esters **3**, were obtained in good yields by alkylating the acetamidomalonic ester with ω-bromoalkylnitriles (Scheme 1).

The structure of all compounds obtained was confirmed by ^{1}H and ^{13}C NMR spectra.

Hence, a general method for the synthesis of a new class of glutamate receptors ligands combining the aminodiphosphonic and aminocarbon functions in the molecule, which may also be of interest as new components of radiopharmaceuticals, was developed.

Acetamidomalonic ester, 3-bromopropionitrile, 4-bromobutyronitrile, 5-bromovaleronitrile were purchased from Reakor company (Alfa Aesar). Phosphorous acid and solvents (dioxane, tetrahydrofuran) were carefully dried before use.

ω-Cyanoalkylacetamidomalonic esters (3). A mixture of 4.3 g (20 mmol) of acetamidomalonic ester, 22 mol of the corresponding ω-bromoalkylnitrile **4**, 8.3 g (60 mmol) of finely dispersed K_2CO_3, and 0.1 g of tetrabutylammonium bromide in 15–20 mL of tetrahydrofuran was stirred under reflux for 13–18 h. The reaction progress was monitored by TLC. After the reaction completed, the mixture was filtered, and the filtrate was evaporated in a vacuum. The residue was poured into a mixture of cold water (30 mL) and ice. The aqueous layer was neutralized with 1 N solution of HCl to pH ~ 7 and extracted with ethyl acetate (3×30 mL). The combined organic extract was dried with magnesium sulfate and evaporated in a vacuum. The residue was crystallized from diethyl or petroleum ether.

Diethyl 2-cyanoethylacetamidomalonate (3a). Yield 83%, mp 92–93°C (mp 94–95°C [11, 12]). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.17 t (3H, CH_3, $^3J_{HH}$ = 6.9 Hz), 1.75 t (2H, CH_2, $^3J_{HH}$ = 7.2 Hz), 2.44 t (2H, CH_2, $^3J_{HH}$ = 7.24 Hz), 4.19 q (4H, $2CH_2O$, $^3J_{HH}$ = 6.9 Hz), 6.77 br.s (1H, NH).

Diethyl 3-cyanopropylacetamidomalonate (3b). Yield 87%, mp 34–35°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.17 t (3H, CH_3, $^3J_{HH}$ = 7.3 Hz), 1.35–1.55 m (2H, CH_2), 1.98 s (3H, Ac), 2.22–2.43 m (4H, $2CH_2$), 4.19 q (4H, $2CH_2O$, $^3J_{HH}$ = 7.3 Hz), 6.81 br.s (1H, NH). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 13.77 ($\underline{C}H_3C$), 16.79, 20.23, 22.79, 31.45 [$\underline{C}H_3C(O)$], 62.63 (CH_2O), 65.75 (CNH), 118.94 (C≡N), 167.42 [2C(O)O], 169.23 [$\underline{C}(O)CH_3$].

Diethyl 4-cyanobutylacetamidomalonate (3c). Yield 71%, oil. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.19 t (3H, CH_3, $^3J_{HH}$ = 7.3 Hz), 1.20–1.35 m (2H, CH_2), 1.52–1.68 m (2H, CH_2), 1.98 s (3H, Ac), 2.22–2.36 m (4H, $2CH_2$), 4.18 q (4H, $2CH_2O$, $^3J_{HH}$ = 7.3 Hz), 6.80 br.s (1H, NH). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 13.80 ($\underline{C}H_3C$), 16.67, 22.37, 22.85, 24.70, 31.03 [$\underline{C}H_3C(O)$], 62.49 (CH_2O), 66.06 (CNH), 119.08 (C≡N), 167.67 [2C(O)O], 169.06 [$\underline{C}(O)CH_3$].

ω-Amino-ω,ω-diphosphonoalkylacetamidomalonic esters (2). To a stirred mixture of 20 mmol of the corresponding nitrile **3** and 40 mmol of phosphorous

acid in 30 mL of anhydrous dioxane were slowly added 20 mmol of phosphorus tribromide at 5–10°C. The resulting mixture was stirred for 5–7 h at room temperature, and then kept without stirring for 1 day. Dioxane was decanted, and 15 mL of acetic acid was added to the residue upon cooling. The resulting mixture was stirred for ~1 h, then poured into ice–water mixture (~20 mL). Next, the mixture was concentrated in a vacuum. To the oily residue were added 20 mL of an aqueous alcohol (1 : 1) and the formed white precipitate was recrystallized from aqueous alcohol (~3 : 1).

Ester **2a** has been previously obtained using phosphorus trichloride, phosphorous acid, and nitrile **3a** [13], but physicochemical and spectral NMR data have not been reported. When isolating ester **2c**, the removal of an excess of phosphorous acid was not possible, so acid **1c** was obtained by acid hydrolysis of a mixture of ester **2** and phosphorous acid followed by evaporation in a vacuum and chromatography of the residue on the cation exchanger.

Diethyl 3-amino-3,3-diphosphonopropylacetamidomalonate (2a). Yield 56%, mp 192–193°C (decomp.). ^{1}H NMR spectrum (DMSO-d_6 + TFA), δ, ppm: 1.13 t (6H, 2CH$_3$, $^3J_{HH}$ = 6.9 Hz), 1.70–1.85 m (2H, CH$_2$), 1.88 s (3H, Ac), 2.35–2.50 m (2H, CH$_2$), 4.09 q (2H, CH$_2$O, $^3J_{HH}$ = 6.9 Hz). ^{31}P NMR spectrum (DMSO-d_6 + TFA): δ_P 14.5 ppm. Found P, %: 13.95, 14.10. $C_{12}H_{24}N_2O_{11}P_2$. Calculated P, %: 14.26.

Diethyl 4-amino-4,4-diphosphonobutylacetamidomalonate (2b). Yield 61%, mp 242–243°C (decomp.). ^{1}H NMR spectrum (D$_2$O + NaOD, pH ~ 4), δ, ppm: 1.14 t (6H, 2CH$_3$, $^3J_{HH}$ = 7.1 Hz), 1.35–1.55 m (2H, CH$_2$), 1.65–1.80 m (2H, CH$_2$), 1.96 s (3H, Ac), 2.05–2.35 m (2H, CH$_2$), 4.17 q (2H, CH$_2$O, $^3J_{HH}$ = 7.1 Hz). ^{1}H NMR spectrum (DMSO-d_6 + TFA), δ, ppm: 1.14 t (6H, 2CH$_3$, $^3J_{HH}$ = 6.9 Hz), 1.33–1.58 m (2H, CH$_2$), 1.72–1.88 m (2H, CH$_2$), 1.91 s (3H, Ac), 2.02–2.18 m (2H, CH$_2$), 4.12 q (2H, CH$_2$O, $^3J_{HH}$ = 6.9 Hz), 7.86 br.s (1H, NH). ^{13}C NMR spectrum (D$_2$O + NaOD, pH ~ 4), δ_C, ppm: 13.23, 18.71 t ($^3J_{PC}$ = 5.4 Hz), 21.66, 31.84, 33.52, 57.13 t ($^1J_{PC}$ = 121.5 Hz), 63.96, 66.87, 169.33, 173.65. ^{31}P NMR spectrum (DMSO-d_6 + TFA): δ_P 15.1 ppm. ^{31}P NMR spectrum (D$_2$O + NaOD, pH ~ 4): δ_P 13.4 ppm. ^{31}P NMR spectrum (D$_2$O + NaOD, pH ~8): δ_P 18.0 ppm. Found P, %: 13.50, 13.68. $C_{13}H_{26}N_2O_{11}P_2$. Calculated P, %: 13.82.

Diethyl 5-amino-5,5-diphosphonopentylacetamidomalonate (2c) was isolated as an oily mixture with phosphorous acid (~4 : 1). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.19 t (6H, 2CH$_3$, $^3J_{HH}$ = 7.3 Hz), 1.20–1.35 m (2H, CH$_2$), 1.50–1.70 m (2H, CH$_2$), 1.98 s (3H, Ac), 2.15–2.40 m (4H, 2CH$_2$), 4.18 q (2H, CH$_2$O, $^3J_{HH}$ = 7.3 Hz), 6.72 d (~0.5H, PH, $^1J_{PH}$ = 644.7 Hz) 6.80 br.s (1H, NH). ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 14.9 (~80%, **2c**), 2.7 (~20%, H_3PO_3).

α,ω-Diamino-ω,ω-diphosphonoalkylcarboxylic acids (1). A mixture of 4 mmol of the corresponding ester **2** and 15 mL of 6 N HCl was refluxed for 13–15 h. The reaction mixture was evaporated in a vacuum, and the residue was co-evaporated with water and treated with 1.2 mL of propylene oxide in 4 mL of aqueous alcohol (1 : 1). The resulting solution was evaporated in a vacuum, and free amino acid was crystallized from water.

2,5-Diamino-5,5-diphosphonovaleric acid (1a, DADP5). Yield 72%, mp 313–314°C. ^{1}H NMR spectrum (D$_2$O), δ, ppm: 2.07–2.52 m (4H, 2CH$_2$), 3.85 t (CH, $^3J_{HH}$ = 8.5 Hz). ^{13}C NMR spectrum (D$_2$O), δ_C, ppm: 22.95 t ($^3J_{PC}$ = 4.1 Hz), 23.33 t ($^2J_{PC}$ = 3.0 Hz), 49.47, 57.33 t ($^1J_{PC}$ = 135.6 Hz), 169.48 t ($^5J_{PC}$ = 4.1 Hz). ^{31}P NMR spectrum (D$_2$O): δ_P 17.31 ppm. Mass spectrum, *m/z:* 293.1 $[M + H]^+$ (calculated for $C_6H_{16}N_2O_8P_2$: 292.1). Found, %: C 20.18, 20.30; H 5.05, 4.96; N 9.67, 9.60; P 21.05, 21.12. $C_5H_{14}N_2O_8P_2$. Calculated, %: C 20.56; H 4.83; N 9.59; P 21.21.

2,6-Diamino-6,6-diphosphonohexanoic acid (1b, DADP6). Yield 78%, mp 270–271°C. ^{1}H NMR spectrum (D$_2$O, pH ~ 1), δ, ppm: 1.25–1.85 m (6H, 3CH$_2$), 3.74 t (1H, CH, $^3J_{HH}$ = 5.9 Hz). ^{13}C NMR spectrum (D$_2$O), δ_C, ppm: 19.47 t ($^3J_{PC}$ = 5.5 Hz), 30.01, 30.67, 53.73, 56.49 t ($^1J_{PC}$ = 118.7 Hz), 174.42. ^{31}P NMR spectrum (D$_2$O, pH ~ 1): δ_P 13.82 ppm. ^{31}P NMR spectrum (D$_2$O, pH ~ 7): δ_P 14.71 ppm. Mass spectrum, *m/z:* 307.1 $[M + H]^+$ (calculated for $C_6H_{16}N_2O_8P_2$: 306.1). Found, %: C 23.20, 23.34; H 5.44, 5.52; N 9.15, 9.20; P 20.08, 20.14. $C_6H_{16}N_2O_8P_2$. Calculated, %: C 23.54; H 5.27; N 9.15; P 20.23.

2,7-Diamino-7,7-diphosphonoheptanoic acid (1c, DADP7). A mixture of **2c**, phosphorous acid, and 6 N hydrochloric acid was refluxed for 13 h, and then evaporated. The residue was chromatographed on the cation exchanger (eluent H$_2$O, 1 N HCl). The ninhydrin positive fractions were evaporated and treated with excess of propylene oxide in aqueous alcohol. Additional crystallization from water allowed the isolation of amino acid **1c**. Yield 58% (relative to nitrile **3c**), mp 264–265°C. ^{1}H NMR spectrum (D$_2$O +

NaOD, pH ~ 8), δ, ppm: 1.23–1.43 m (2H, CH_2), 1.43–1.65 m (2H, CH_2), 1.67–2.02 m (4H, $2CH_2$), 3.62 t (1H, CH, $^3J_{PC}$ = 6.9 Hz). ^{13}C NMR spectrum (D_2O + NaOD, pH ~ 6), δ_C, ppm: 23.54, 24.92, 30.11, 31.67, 54.58, 57.22 t ($^1J_{PC}$ = 119.6 Hz), 175.12. ^{31}P NMR spectrum (D_2O, pH ~ 1): δ_P 13.58 ppm. ^{31}P NMR spectrum (D_2O + NaOD, pH ~ 8): δ_P 16.63 ppm. Mass spectrum, *m*/*z*: 321.2 $[M + H]^+$ (calculated for $C_7H_{18}N_2O_8P_2$: 320.2). Found P, %: 19.44, 19.54. $C_7H_{18}N_2O_8P_2$. Calculated P, %: 19.35.

^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker DPX-200 Fourier spectrometer, TMS (^1H, ^{13}C) and 85% H_3PO_4 references (^{31}P). Melting points were determined on a heating block using open capillaries. TLC analysis was carried out on Silufol plates using plates coated with an aluminum substrate Sil G/UV 254 or glass plates from Merck coated with silica gel UV-254 (eluent chloroform–isopropanol, 3–7%), and also Alufol plates (Kavalier) detecting with iodine vapors or with a ninhydrin solution in the case of amino acids.

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REFERENCES

1. Saratovskikh, I.V. and Ragulin, V.V., *Russ. J. Gen. Chem*., 2005, vol. 75, no. 7, p. 1077. doi 10.1007/s11176-005-0371-2
2. Kafarski, P. and Zon, J., in *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*, Kukhar, V.P. and Hudson, H.R., Eds., Chichester: Wiley & Sons, Ltd., 2000.
3. Collingridge, G.L. and Lester, R.A., *Pharmacol. Rev*., 1989, vol. 41, p. 143. PMID 2558391.
4. Seeburg, P.H., *Trends Neurosci*., 1993, vol. 16, p. 359. doi 10.1016/0166-2236(93)90093-2
5. Ragulin, V.V., *Russ. J. Gen. Chem*., 2018, vol. 88, no. 2, p. 159. doi 10.1134/S1070363218020019.
6. Romanenko, V.D. and Kukhar, V.P., *Arkivoc*, 2012, vol. 2012, no. 4, p. 127. doi 10.3998/ark.5550190.0013.411
7. Chmielewska, E. and Kafarski, P., *Molecules*, 2016, vol. 21, p. 1474. doi 10.3390/molecules21111474
8. Yang, X.N., Zeng, J.C., Song, Y.C., Zhang, H., and Pei, F.X., *Eur. Rev. Med. Pharmacol. Sci*., 2014, vol. 18, no. 15, p. 2116. PMID 25070815
9. Xue, Z.-Y., Li, Q.-H., Tao, H.-Y., and Wang, C.-J., *J. Am. Chem. Soc*., 2011, vol. 133, p. 11757. doi 10.1021/ja2043563
10. Sturtz, G., Appere, G., Breistol, K., Fodstad, O., Schwartsmann, G., and Hendriks, H.R., *Eur. J. Med. Chem*., 1992, vol. 27, p. 825. doi 10.1016/0223-5234(92)90117-J
11. Shimo, K. and Wakamatsu, S., *J. Org. Chem*., 1961, vol. 26, p. 3788. doi 10.1021/jo01068a039
12. Albertson, N.F. and Archer, S., *J. Am. Chem. Soc*., 1945, vol. 67, p. 2043. doi 10.1021/ja01227a501
13. Bandurina, T.A., Kozlov, V.A., Konyukhov, V.N., Pushkareva, Z.V., Kolotygin, Yu.A., Podbereznyi, V.L., and Gavrilov, M.A., USSR Inventors Certificate 798106, 1981.