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RESEARCH ARTICLE

Synthesis of new α-hydroxyphosphonic acids – phosphorus analogues of homoproline

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Abstract: A synthetic approach has been developed for the preparation of new bioisosteres of homoproline, namely α-hydroxyphosphonic acids. At the key stages of the synthesis, the phosphorylation of carbonyl derivatives of (*S*)- and (*R*)-proline via the Abramov reaction was employed. The resulting α-hydroxyphosphonic acids are promising compounds with potential biological activity and may serve as valuable candidates for the development of new pharmaceuticals.

Keywords: α-hydroxyphosphonic acid; phosphorus analogs of amino acids; Abramov reaction; biological activity.

Introduction

Organophosphorus compounds, i.e., compounds containing a C-P bond, are widely used in modern medicine, agriculture, industry, and organic synthesis. Organophosphorus compounds featuring a P-C bond were first isolated from living organisms in 1959 [1, 2]. Shortly thereafter, many related species were identified in hundreds of aquatic and terrestrial animals and microorganisms [3]. α-Hydroxyphosphonic esters, regarded as an important class of biologically active compounds, have attracted attention due to their antibacterial, antiviral, antibiotic, pesticidal, anticancer, and enzyme-inhibitory properties [4, 5]. Several pharmaceutical agents are widely used in contemporary clinical practice, including Cidofovir – a well-known antiviral drug, Risedronic acid – a medication for treating osteoporosis, Fosfomycin – a broad-spectrum antibiotic, and Glyphosate – a systemic herbicide.

Results and Discussion

Organophosphorus compounds containing a P-C bond were first isolated from living organisms in 1959 [1, 2]. Soon afterwards many kinds of related compounds were found in hundreds of aquatic and terrestrial animals and microorganisms [3]. α-Hydroxyphosphonic esters, considered as an important class of biologically active compounds, have attracted attention because of their antibacterial, antiviral, antibiotic, pesticidal, anticancer, and enzyme inhibitor properties [4, 5].

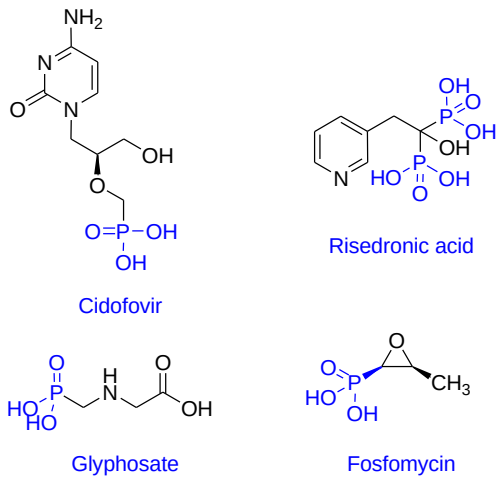

Figure 1. Examples of organophosphorus pharmaceuticals.

Phosphonic analogues of natural compounds, in which the carboxyl group is replaced by a phosphonic group, represent a powerful tool in modern medicinal and

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bioorganic chemistry. Such a substitution imparts unique biochemical and pharmacokinetic properties to the molecules, including resistance to enzymatic hydrolysis and the ability to mimic key transition states in enzymatic reactions. Owing to these distinctive features, phosphonic analogues of natural compounds are widely employed in biological and medical research as enzyme inhibitors, receptor probes, model compounds for studying amino acid transport, biochemical probes, and stable mimetics [6-8]. Therefore, the development and optimization of methods for obtaining phosphonic analogues of natural compounds is an important and timely task in contemporary organic and bioorganic chemistry.

Homoproline - a homolog of the natural amino acid proline – and *α*-hydroxyhomoproline are promising chiral building blocks and biomimetics of proline and hydroxyproline. They possess important properties that have enabled their application in various fields, including use as model compounds in biological studies, as chiral building blocks for the synthesis of enzyme inhibitors, and in the production of pharmaceutical agents and certain catalysts [9-12]. Therefore, we set out to obtain both enantiomers of the phosphonic analogues of (*S*)- and (*R*)-*α*-hydroxyhomoproline. These compounds have not been synthesized previously.

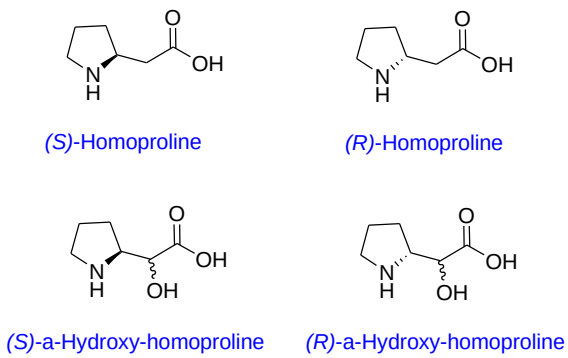

Figure 2. Homoproline and α-hydroxyhomoproline.

In our work, we obtained both enantiomers of the phosphonic analogues of *α*-hydroxyhomoproline. Although similar structures had previously been synthesized by related methods, they had not been studied or described using physicochemical techniques such as NMR, LC-MS, and optical rotation measurements until now [13-16]. In our research, we used the (*S*)- and (*R*)-stereoisomers of *N-Boc*-prolinol (**1**-(*S*), **1**-(*R*)) as starting materials. Thus, (*R*)- and (*S*)-prolinol were converted into the corresponding aldehydes via Swern oxidation in the presence of Et_3N in DMSO. The reaction proceeded with quantitative yield and preserved both the optical purity and the absolute configuration of the asymmetric carbon atom, which was confirmed by comparison of the optical rotation values with literature data. In this way, (*R*)- and (*S*)-*N-Boc*-prolinal (**2**-(*S*), **2**-(*R*)) were obtained.

The resulting (*R*)- and (*S*)-*N-Boc*-prolinal **2** were then subjected to the Abramov reaction. The aldehydes were mixed with diethyl phosphite in the presence of a catalytic amount of diazabicycloundecene (DBU) as a base and stirred at room temperature overnight. The reaction was carried out solvent-free.

Upon completion, the reaction mixture was purified by column chromatography on silica gel and further crystallized from an MTBE-THF mixture. As a result, both stereoisomers of diethyl (*N*-Boc-pyrrolidine)-1-hydroxy-methylphosphonate (**3**-(*S*), **3**-(*R*)) were obtained as a white crystalline powder and were fully characterized using all available physicochemical methods.

Next, the obtained diethyl hydroxyphosphonates **3** were subjected to hydrolysis with trimethylsilyl bromide in dichloromethane. The reaction mixture was stirred overnight at room temperature, protected completely from light. After completion, the mixture was evaporated and kept under high vacuum (0.1 mmHg) to remove residual trimethylsilyl bromide. The residue contained (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids **4**. These were obtained as yellowish crystals in quantitative yields (95%), and were characterized using all available analytical methods. It is also important to note that such pyrrolidine-1-hydroxymethylphosphonic acids had not been previously obtained, neither as racemates nor in optically active forms.

Thus, both enantiomers of (*S*)- and (*R*)-diethyl-(*N-Boc*-pyrrolidine)-1-hydroxymethylphosphonates were characterized for the first time, and both enantiomers of (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids – the phosphonic analogues of homoproline with potential biological activity – were synthesized and described as the first known example.

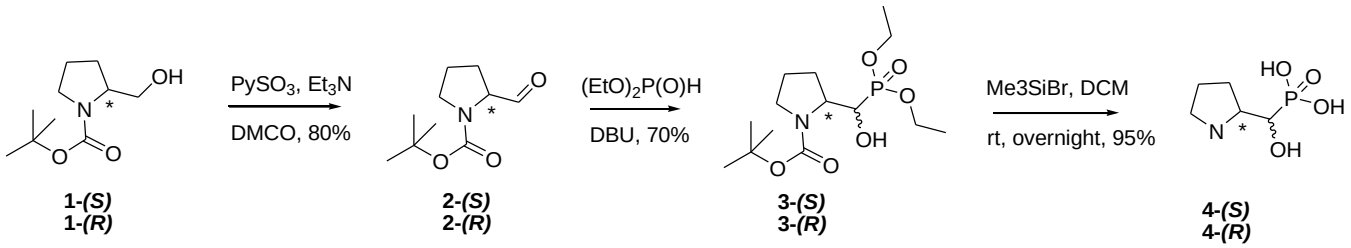

Figure 3. Scheme of the synthesis of (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids.

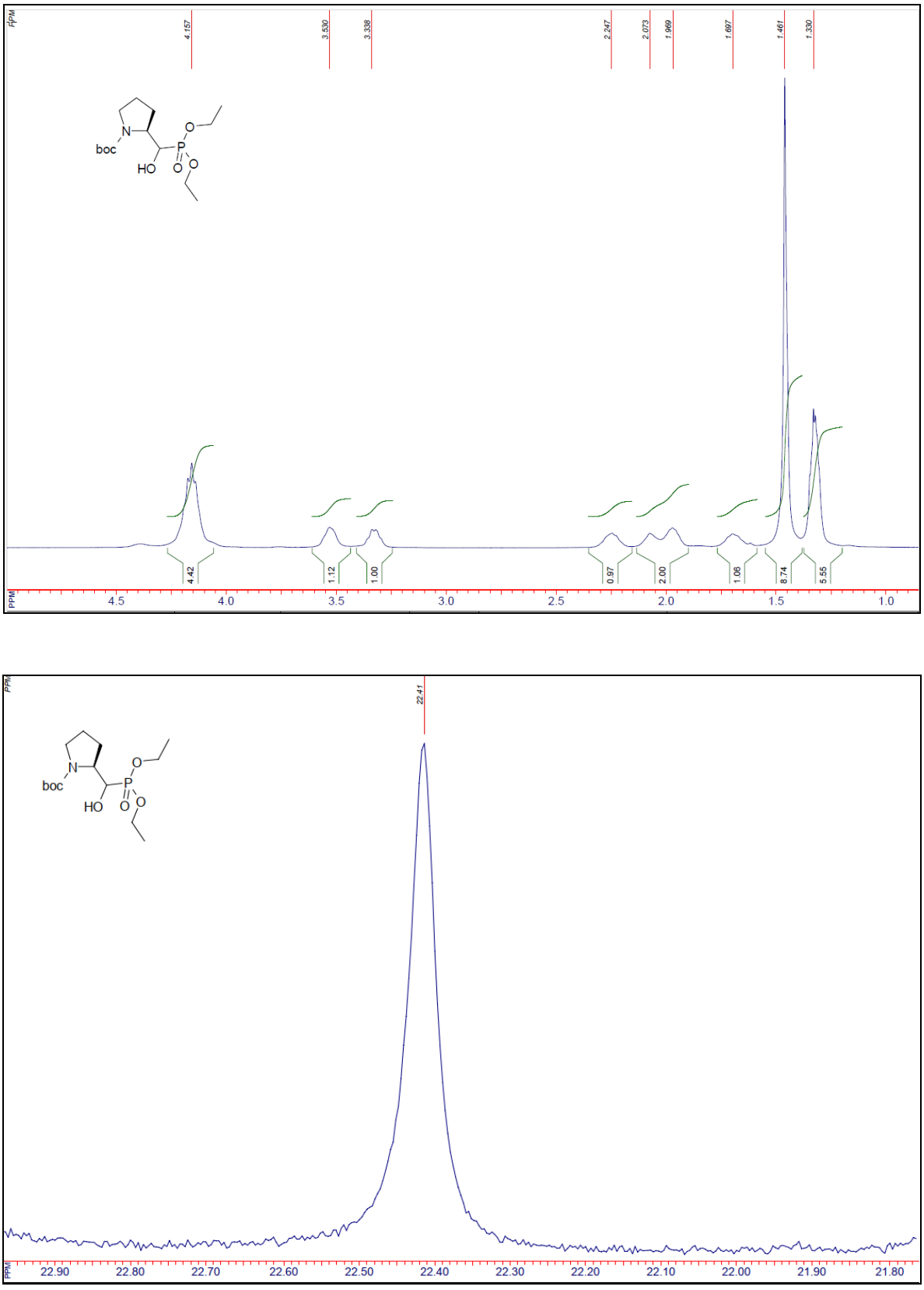

Figure 4. ^{1}H NMR and ^{31}P NMR spectra of (*S*)-diethyl (*N*-Boc-pyrrolidine)-1-hydroxymethylphosphonate **3**.

Conclusions

In this work, we obtained and described both optical isomers, (*S*)- and (*R*)-diethyl-(*N*-Boc-pyrrolidine)-1-hydroxymethylphosphonates, as well as (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids. To achieve this, we employed the phosphorylation of the corresponding carbonyl compounds via the Abramov reaction. All

synthesized diethyl-(*N-Boc*-pyrrolidine)-1-hydroxymethyl-phosphonates were characterized for the first time using all available physicochemical methods. Subsequently, the obtained diethyl-(*N-Boc*-pyrrolidine)-1-hydroxymethyl-phosphonates were converted into optically pure (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids with complete retention of absolute configuration. Both optically active (*S*)- and (*R*)-pyrrolidine-1-hydroxymethylphosphonic acids were produced for the first time in high chemical yields, with optical purity <95%, and were fully described and characterized using modern physicochemical techniques. Thus, both enantiomers of diethyl-(*N-Boc*-pyrrolidine)-1-hydroxymethylphosphonates and pyrrolidine-1-hydroxymethylphosphonic acids were obtained, representing promising building blocks for the synthesis of potential pharmaceuticals and biologically active compounds.

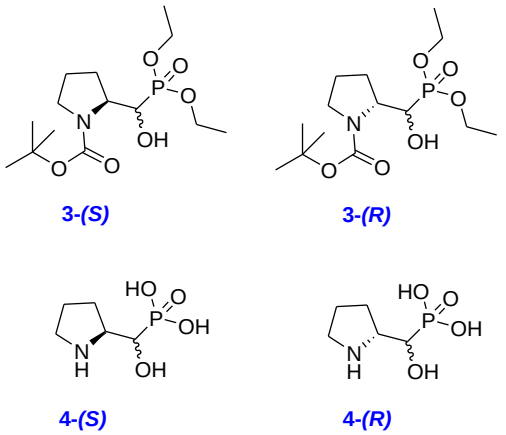

Figure 5. Newly obtained optically active *α*-hydroxy-diethylphosphonates and *α*-hydroxyphosphonic acids.

Experimental section

All solvents were purified according to standard procedures. All starting materials were obtained from Enamine LTD or other commercial suppliers. Melting points were measured using an MPA 100 OptiMelt automated melting point system. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker "Avance III" 500 MHz spectrometer (Germany) at ambient temperature. Chemical shifts (δ) are given in parts per million relative to tetramethylsilane (TMS) as an internal standard. Signal multiplicities are reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet), br (broad), q (quartet). Spin-spin coupling constants (*J*) are given in hertz. Column chromatography was performed on silica gel 60 (70-230 mesh). Optical rotation was measured on a Perkin-Elmer 241 polarimeter (sodium D line, 20 °C). Melting points were uncorrected. All reactions were carried out in glassware dried by flame or in a drying oven. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on silica gel 60 F254 plates (Merck, Germany), and products were visualized using anisaldehyde or UV light. The purity of all compounds was evaluated by TLC and NMR measurements.

Synthesis

General procedure for the synthesis of tert-butyl (2S)-2-formylpyrrolidine-1-carboxylate.

A solution of *N-Boc*-prolinol (10 g, 0.05 mol, 1 eq.) in dry DMSO (150 mL) was treated with Et_3N (20 mL, 0.15 mol, 3 eq.). The mixture was cooled to 0 °C (ice bath) with stirring, and a suspension of Py • SO_3 (23.8 g, 0.15 mol, 3 eq.) in DMSO (50 mL) was added portionwise. The reaction mixture was stirred at room temperature overnight. After completion, the reaction mixture was poured into cold water and extracted with MTBE three times. The combined organic layers were washed with water five times to remove residual DMSO. The organic phase was dried over sodium sulfate and concentrated. The residue contained tert-butyl 2-formylpyrrolidine-1-carboxylate, which was used in subsequent transformations without further purification.

*tert-Butyl (2S)-2-formylpyrrolidine-1-carboxylate (**2-(S)**).*

Yellow oil; Yield 8.8 g, 88.8%. $[a]_D^{20}$ = -100.76 (C = 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 ºC) δ 9.53-9.43 (m, 1H), 4.17-4.02 (m, 1H), 3.58-3.40 (m, 2H), 2.09-1.85 (m, 4H), 1.45-1.40 (m, 9H). GCMS: m/z calcd 170.2 [M^+ -CHO] for $C_{10}H_{17}NO_3$ -CHO (170.1).

NMR spectrum showed a mixture of conformers

*tert-Butyl (2R)-2-formylpyrrolidine-1-carboxylate (**2-(R)**).*

Yellow oil; Yield 8.9 g, 89%. $[a]_D^{20}$ = +101 (C = 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 ºC) δ 9,53-9,43 (m, 1H), 4.17-4.02 (m, 1H), 3.58-3.40 (m, 2H), 2.09-1.85 (m, 4H), 1.45-1.40 (m, 9H). GCMS: m/z calcd 170.2 [M^+ -CHO] for $C_{10}H_{17}NO_3$ -CHO (170.1).

NMR spectrum showed a mixture of conformers

General procedure for the synthesis of diethyl 1-(N-Boc-2-pyrrolidine)-1-hydroxymethylphosphonate.

tert-Butyl-2-formylpyrrolidine-1-carboxylate (8.8 g, 0.044 mol, 1 eq.) was mixed with $(EtO)_2P(O)H$ (6 mL, 0.046 mol, 1.05 eq.) without solvent. DBU (0.2 mL, cat.) was added with stirring. The reaction mixture was stirred at room temperature overnight. The resulting product was purified by column chromatography. As a result, diethyl 1-(*N-Boc*-2-pyrrolidine)-1-hydroxymethylphosphonate was obtained as a white crystalline solid.

*(S)-diethyl 1-(N-Boc-2-pyrrolidine)-1-hydroxymethylphosphonate (**3-(S)**).*

White solid; Yield 8 g, 54.5%. $[a]_D^{20}$ = +57.45 (C = 0.5, MeOH). *R*f = 0.3 (eluent - EtOAc, Alugram Xtra-Sheets SIL G/UV254, stain - anisaldehyde). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 ºC) δ 5.47 (br s 1H), 4.17-4.11 (m, 4H), 3.54-3.48 (m, 1H), 3.35-3.28 (m, 1H), 2.29-2.19 (m, 1H), 2.09-2.02 (m, 2H), 1.73-1.64 (m, 1H), 1.45 (s, 9H), 1.33-1.29 (m, 6H). ^{31}P NMR ($CDCl_3$, 160 МГц, 25 ºC) δ 22.41. ^{13}C NMR

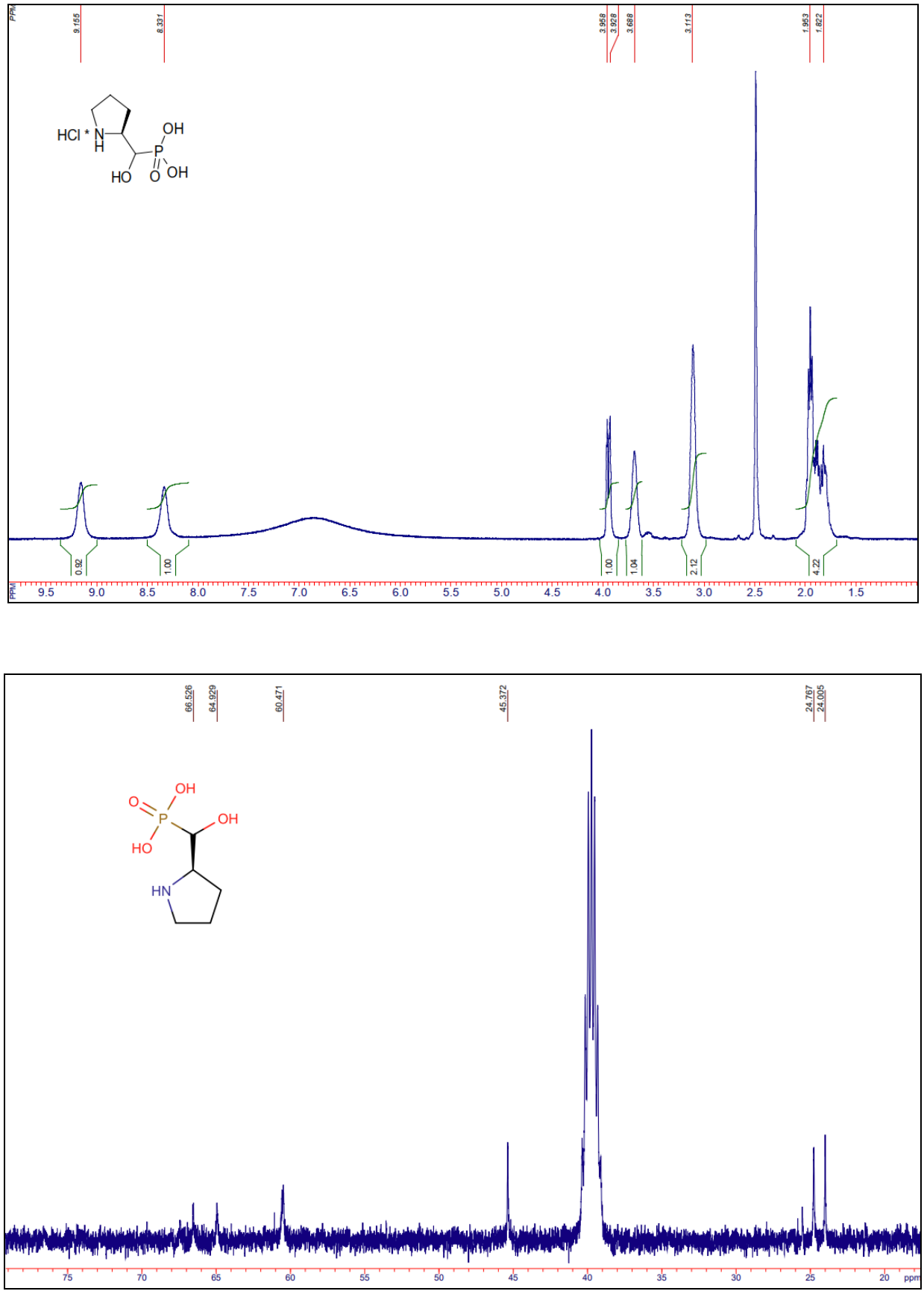

Figure 6. ^{1}H NMR and ^{31}P NMR spectra of (*S*)-pyrrolidine-1-hydroxymethylphosphonic acid **4**.

(125.7 МГц, $CDCl_3$, 25 ºC) δ 154.76, 78.32, 69.47, 68.23, 60.67, 58.56, 56.55, 46.08, 45.19, 26.79, 25.93, 24.72, 22.72, 14.81. LCMS: M 238 ($M+H^+$ -t-BuOCO) (M 337.35) (R).

*(R)-diethyl 1-(N-Boc-2-pyrrolidine)-1-hydroxymethylphosphonate (**3-(R)**).*

White solid; Yield 8.2 g, 55.3%. $[a]_D^{20}$ = -57.34 (C = 0.5, MeOH).*R*f = 0.3 (eluent - EtOAc, Alugram Xtra-Sheets SIL G/UV254, stain - anisaldehyde). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 ºC) δ 5.47 (br s 1H), 4.17-4.11 (m, 4H), 3.54-3.48 (m, 1H), 3.35-3.28 (m, 1H), 2.29-2.19 (m, 1H), 2.09-2.02 (m, 2H), 1.73-1.64 (m, 1H), 1.45 (s, 9H), 1.33-1.29 (m, 6H). ^{31}P NMR ($CDCl_3$, 160 МГц, 25 ºC) δ 22.41. ^{13}C NMR (125.7 МГц, $CDCl_3$, 25 ºC) δ 154.76, 78.32, 69.47, 68.23, 60.67, 58.56, 56.55, 46.08, 45.19, 26.79, 25.93, 24.72, 22.72, 14.81. LCMS: M 238 ($M+H^+$-t-BuOCO) (M 337.35)(R).

General procedure for the synthesis of (2-pyrrolidine)-1-hydroxymethylphosphonic acid hydrochloride.

Diethyl 1-(*N-Boc*-2-pyrrolidine)-1-hydroxymethylphosphonate was dissolved in dry dichloromethane, and Me_3SiBr was added under cooling. The reaction mixture was stirred at room temperature in complete darkness. Upon completion, the solvent was evaporated, and the residue was dissolved in THF, followed by the addition of dioxane·HCl (10 N, 2 eq.). The solvent was evaporated again, and the remaining crystalline material was purified by washing the solid with THF. As a result, (2-pyrrolidine)-1-hydroxymethylphosphonic acid HCl was obtained as a yellowish powder.

*(S)-(2-pyrrolidine)-1-hydroxymethylphosphonic acid hydrochloride (**4-(S)**).*

Yellowish powder; Yield 3.5 g, 67%. $[a]_D^{20}$ = -20.27 (C = 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz, 25 ºC) δ 9.24 (br s, 1H), 8.34 (br s, 1H), 4.05-3.98 (m, 1H), 3.74-3.64 (m, 1H), 3.15-3.05 (m, 2H), 2.01-1.74 (m, 4H). ^{31}P NMR (DMSO-d_6, 160 МГц, 25 ºC) δ 17.27. ^{13}C NMR (125.7 МГц, DMSO-d_6, 25 ºC) δ 66.53, 64.92, 45.37, 24.74, 23.99.

*(R)-(2-pyrrolidine)-1-hydroxymethylphosphonic acid hydrochloride (**4-(R)**).*

Yellowish powder; Yield 3.8 g, 72.8%. $[a]_D^{20}$ = +21.32 (C = 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz, 25 ºC) δ 9.24 (br s, 1H), 8.34 (br s, 1H), 4.04-3.98 (m, 1H), 3.74-3.64 (m, 1H), 3.15-3.05 (m, 2H), 2.01-1.74 (m, 4H). ^{31}P NMR (DMSO-d_6, 160 МГц, 25 ºC) δ 17.28. ^{13}C NMR (125.7 МГц, DMSO-d_6, 25 ºC) δ 66.53, 64.92, 45.37, 24.74, 23.99.

Notes

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The authors declare no conflict of interest.

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Синтез нових α-гідроксифосфонових кислот – фосфорних аналогів гомопроліну

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Резюме: Розроблено синтетичний підхід до синтезу нових біоізостерів гомопроліну – *α*-гідроксифосфонових кислот. На ключових етапах використано реакцію фосфорилювання карбонільних похідних (*S*)- та (*R*)-проліну – реакцію Абрамова. Отримані *α*-гідроксифосфонові кислоти можуть бути потенційно біологічно активними речовинами для розробки нових фармацевтичних препаратів.

Ключові слова: *α*-гідроксифосфонова кислота; фосфорні аналоги амінокислот; реакція Абрамова; біологічна активність.