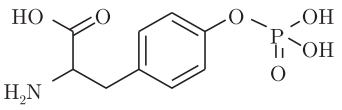
УДК 547.241+577.152.3

D. I. Khavrienko, O. L. Kobzar, M. V. Shevchuk, V. D. Romanenko, S. M. Kobelev, A. D. Averin, Academician of the RAS **I. P. Beletskaya,** Corresponding Member of the NAS of Ukraine **A. I. Vovk,** Academician of the NAS of Ukraine **V. P. Kukhar**

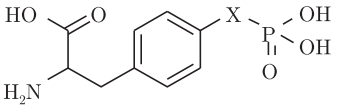
α,α-Difluoro-β-ketophosphonates on a tetraazamacrocyclic platform: Synthesis and inhibitory activity against protein tyrosine phosphatases

The present study offers a new approach for designing inhibitors of protein tyrosine phosphatases. We have synthesized the cyclam derivatives with α,α-difluoro-β-ketophosphonate fragments covalently attached to tetraazamacrocyclic scaffold, which is known to be of medical interest. The obtained functionalized macrocycles were evaluated as inhibitors of PTP1B, TC-PTP, CD45, and other protein tyrosine phosphatases.

The growing awareness of a critical role of protein tyrosine phosphatases (PTPs) in the pathology of a number of disorders such as type 2 diabetes, obesity and cancer has stimulated significant interest in the search for potent and selective PTP inhibitors [1–2]. In the previous studies, one of the directions in designing the inhibitors has focused on the synthesis of enzymatically stable phosphotyrosine mimics (pTyr) which contain the fragments of (phosphonomethyl)phenylalanine (Pmp) **1a** and its fluoro-substituted analogues **1b**, **c** [3–6]. However, there is a limitation in the development of the low molecular PTP inhibitors due to several problems including the selectivity and cell permeability. Therefore, the search for new pTyr mimetics is needed which facilitate the design of drug-like PTP inhibitors as therapeutic agents is needed.



pTyr



1a,X = CH_2(Pmp)
1b,X = CHF(FPmp)
1c,X = CF_2(F_2Pmp)

Efficient PTP inhibitors were shown to possess, besides a phosphate-mimicking component, additional structure motifs that provide interaction with the enzyme surface beyond the catalytic pocket. This implies that the design of PTP inhibitors should be based on the search for the molecules containing not only a phosphotyrosine mimetic moiety but also a suitable scaffold for the binding outside the catalytic site. From this viewpoint, the attachment of the difluoromethylenphosphonate [7, 8] and α,α-difluoro-β-ketophosphonate [9] groups to various scaffolds is of considerable interest to create the novel pTyr surrogates and to evaluate their biological activity.

As a part of our work on identifying the novel PTP inhibitors [10], we now report the synthesis of $(HO)_2P(O)CF_2C(O)$-functionalized tetraazamacrocycles using 1,4,8,11-tetraazacyclotetradecane (cyclam) as a molecular platform. To the best of our knowledge, the cyclic polyamines have

not been used in the development of PTP inhibitors, although they have a great potential in the design of contrast agents for magnetic resonance imaging (MRI) and pharmaceuticals with anti-viral (HIV) and anti-tumor activity [11].

Experimental. ***Methods of the synthesis of cyclam derivatives.*** *N^1, N^4, N^8, N^{11}-tetrakis[1′-oxo-2′,2′-difluoro-2′-(diethylphosphono)ethyl]-1,4,8,11-tetraazacyclotetradecane* (**1a**). Difluoro(diethoxyphosphoryl)acetyl chloride (4.02 g, 16 mMol) was added to a solution of 1,4,8,11-tetraazacyclotetradecane (0.80 g, 4 mMol) and diisopropylethylamine (2.07 g, 16 mMol) in dry CH_2Cl_2 (70 ml) at 0 °C. The resulting solution was stirred at 0 °C (1 h) and then at 20 °C (10 h). The reaction mixture was washed with the aqueous solution of $NaHCO_3$ (20 ml) and then with water (2×20 ml). The organic phase was dried (Na_2SO_4) and concentrated by rotary evaporation to give a white powder. The product was treated with dry acetonitrile, the precipitate was filtered off and placed under high vacuum for 3 h. Yield 3.0 g (71%). ^{1}H NMR ($CDCl_3$): δ = 1.20–1.35 (m, 24H, CH_3), 1.82–1.95 (m, 4H, CCH_2C), 3.45–4.87 (m, 16H, CH_2N), 4.25–4.37 (m, 16H, OCH_2). ^{19}F NMR ($CDCl_3$): δ = −109.6 (m). ^{31}P NMR ($CDCl_3$): δ = 4.1 (m). Anal. Calcd. for $C_{34}H_{60}F_8N_4O_{16}P_4$: N, 5.30%; P, 11.72%. Found: N, 5.56%; P, 11.40%.

N^1, N^4, N^8, N^{11}-tetrakis[1′-oxo-2′,2′-difluoro-2′-(phosphono)ethyl]-1,4,8,11-tetraazacyclotetradecane (**2a**). To a solution of **1a** (0.2 mMol, 1 eq) in MeCN (5 ml) bromotrimethylsilane (3.2 mMol, 16 eq) was added. The resulting solution was stirred at 35 °C overnight. Solvent was evaporated, and the residue was treated with MeOH (3 ml). The mixture was stirred at 20 °C for 15 min, and the product was precipitated by adding acetonitrile (3 ml). Solid was filtered, washed with acetonitrile and dried under vacuum at 20 °C for 3 h to yield **2a** as a colorless solid in 50% yield. ^{1}H NMR ($CDCl_3$): δ = 1.75–2.05 (m, 4H, CCH_2C), 3.58–3.77 (m, 16H, CH_2N). ^{19}F NMR ($CDCl_3$): δ = −109.7 (m, $^2J_{PF} \sim 90$ Hz). ^{31}P NMR ($CDCl_3$): δ = 0.3 (m).

Compounds **1b-d** and **2b-d** were prepared in analogy to **1a** and **2a**. All new compounds have been fully characterized. Synthesis and characterization of N^1, N^8-bis(2-naphthyl)cyclam, used to obtain compounds **1d** and **2d**, have been described earlier [12].

N^1, N^8-Dibenzyl-N^4, N^{11}-bis(3-carboxymethylbenzyl)-1,4,8,11-tetraazacyclo-tetradecane (**3**). Methyl 3-(bromomethyl)benzoate (0.44 g, 1.76 mMol) and 1,8-dibenzylcyclam (0.30 g, 0.8 mMol) were dissolved in dry DMF (5 ml). Finely powdered K_2CO_3 (0.69 g, 5 mMol) and catalytic amounts of dibenzo-18-crown-6 (0.007 g, 0.02 mMol) and KI (0.033 g, 0.2 mMol) were added to this solution. The resulting mixture was stirred at 100 °C for 24 h. After cooling to r. t. the reaction mixture was poured into water (50 ml) and acidified with HCl to pH 3–4. The precipitate was filtered and co-evaporated with EtOH to dryness. After the purification by flash chromatography (EtOAc/hexane 1 : 3 : 0.6), the product was obtained as colorless solid. Yield 0.27 g (49%). ^{1}H NMR ($CDCl_3$): δ = 1.68 (m, 4H, cyclam), 2.38–2.54 (m, 16H, cyclam), 3.31 (s, 8H, NCH_2Ar), 3.79 (s, 6H, CO_2CH_3), 7.08–7.25 (m, 12H, Ar), 7.41 (d, $^3J_{H-H}$= 7.3, 2H, Ar), 7.82 (d, $^3J_{H-H}$ = 7.3, 2H, Ar), 7.95 (s, 2H, Ar) ppm; MS-ESI pos: 678 (10%, M + H^+), 339 (100%, M + $2H^+$).

N^1, N^8-Dibenzyl-N^4, N^{11}-bis{3-[1′-oxo-2′,2′-difluoro-2′-(diethylphosphono)-ethyl]benzyl}-1,4,8,11-tetraazacyclotetradecane (**4**). To a suspension of cerium(III) chloride (0.211 g, 0.89 mMol) in 8 ml of THF diisopropylamine (0.091 g, 0.9 mMol) was added. The mixture was cooled to −78 °C and a solution of 1.6 M *n*-butyllithium (0.54 ml, 0.86 mMol) was added. The mixture was allowed to warm to −40 °C in 40 min. Then it was cooled to −90 °C and a solution of diethyl (difluoromethyl)phosphonate (0.161 g, 0.86 mMol) in 2 ml of THF was added. The mixture was stirred at −90 °C for 1 h, then a solution of **3** (0.264 g, 0.39 mMol) in 10 ml of THF was added. Reaction mixture was then stirred at −80 °C for 1 h and allowed to warm

to −30 °C in 1 h. The reaction was quenched by adding NH_4Cl aqueous solution. The product was extracted with CH_2Cl_2 and dried over Na_2SO_4. The solvent was evaporated *in vacuo*, and the product **4** was purified by flash chromatography (CH_2Cl_2/MeOH from 100 : 4 to 100 : 7). Yield 127 mg (33%). ^{19}F NMR ($CDCl_3$): $\delta = -110.3$ (d, $^2J_{PF} = 95$ Hz). ^{31}P NMR ($CDCl_3$): $\delta = 4.3$ (t, $^2J_{PF} = 95$ Hz).

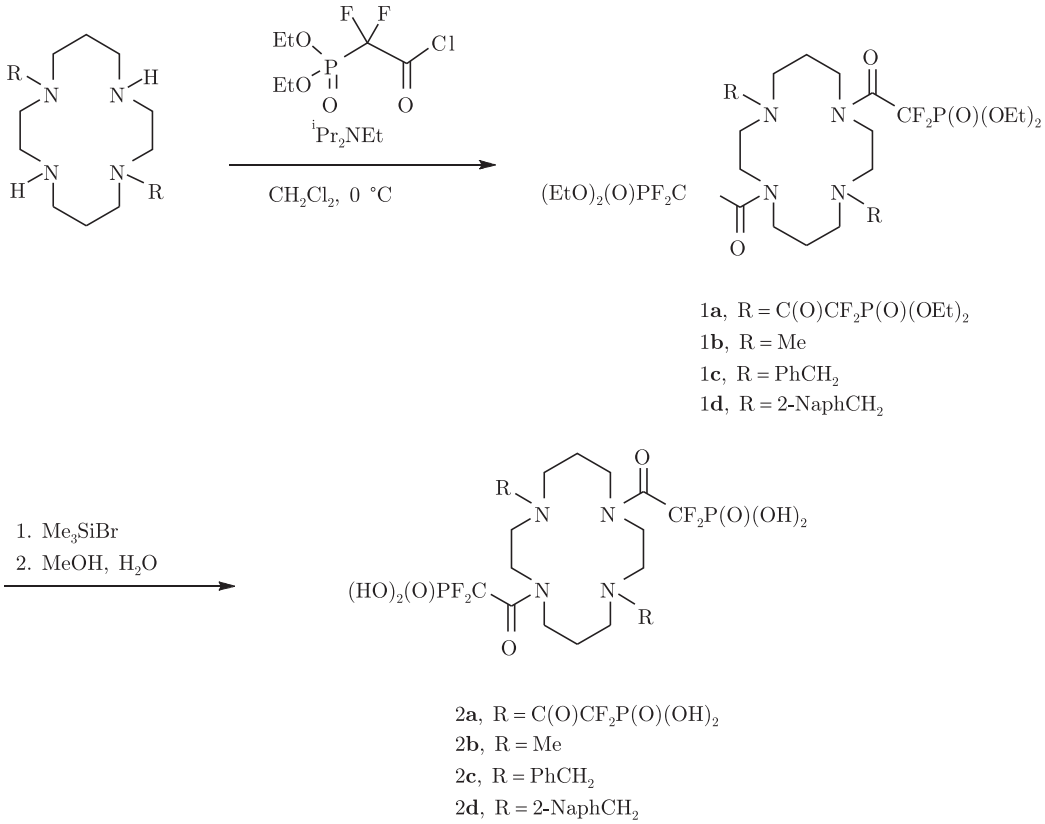
N^1, N^8-Dibenzyl-N^4, N^{11}-bis{3-[1′-oxo-2′,2′-difluoro-2′-(phosphono)ethyl]-benzyl}-1,4,8,11-tetraazacyclotetradecane trisodium salt (**5**). Deprotection of phosphonate ester **4** was accomplished similarly to compound **1a**. Phosphonic acid was converted into its trisodium salt by dissolving the acid in $NaHCO_3$ (3 eq) aqueous solution and evaporating the resulting solution to dryness.

Bioassay for the study of inhibitory activity. Before using in assay, the commercially available preparations of human recombinant PTP-1B and other PTPs were diluted in buffer solution, which contained 50 mM Bis-Tris (pH 7.2), 3 mM EDTA, 2 mM DTT, 75 mM NaCl, 30% glycerol and 0.05% Tween-20, and stored at −70 °C. The system for the inhibitory effect testing consisted of 50 mM Bis-Tris (pH 7.2), 2 mM EDTA, 1 mM DTT, 100 mM NaCl, 1% DMSO and *p*-nitrophenyl phosphate as the enzyme substrate. After 5-minute incubation at 37 °C for PTP1B and 30°C for other enzymes, the reaction was initiated by adding the enzyme in a concentration of 4–10 nM. The enzyme activity was detected at 410 nm by measuring the absorbance of *p*-nitrophenol formed through enzymatic hydrolysis of the substrate.

Discussion. Scheme 1 illustrates the preparation of 1,4,8,11-tetrasubstituted cyclam **1a** containing four α,α-difluoro-β-ketophosphonate groups *via* the direct acylation of the tetraazamacrocycle with difluoro(diethoxyphosphoryl)acetyl chloride. The synthesis was performed by adding acyl chloride to the mixture of cyclam and diisopropylethylamine in dry dichloromethane at 0 °C. Subsequent trans-silylation of the phosphonate ester **1a** with bromotrimethylsilane followed by hydrolysis afforded the desired product **2a**. Its molecular structure was confirmed by analytical and spectral data. Interestingly, ^{19}F NMR spectrum of **1a** exhibited complex multiplet at δ-109.6 ppm and broad triplet in the proton-decoupled ^{31}P NMR spectrum at δ4.1 ppm ($^2J_{PF} \sim 93$ Hz). Similar spectra were observed for **2a**. These spectral features probably can be explained by the existence of the compounds **1a** and **2a** as a mixture of structural conformers. This conclusion is supported by the existence of tri-$CF_3C(O)$-substituted cyclam as a mixture conformers [13] and by the fact that, according to ^{19}F NMR spectra, *N*-acylated piperazine derivative (Ciprofloxacin®) containing β-keto-α, α-difluorophosphonate moiety exists as a mixture of two stereoisomers in a ratio of 70 : 30 [14].

The inhibitory potential of compound **2a** was evaluated *in vitro* using PTP1B, TC-PTP, CD45, SHP2 and PTP β. The experimentally obtained IC_{50} values (the inhibitor concentration necessary to inhibit the enzyme activity by 50%) were in the concentration range of 100–1200 μM for all the PTPs. These results showed low inhibitory potency of compound **2a** on the enzymes tested.

We next investigated the synthesis and PTP inhibiting activity of the α,α-difluoro-β-ketophosphonate-functionalized tetraazamacrocycles derived from N^1, N^8-disubstituted cyclams. The desired phosphonate derivatives **1b-d** were prepared starting from dimethyl-, dibenzyl- and di(2-naphthylmethyl)-substituted cyclams and isolated as phosphonic acids **2b-d**. Molecular structures of these compounds were confirmed by mass spectral (MS), ^{1}H, ^{19}F, ^{31}P NMR, and analytical data. It should be noted that, similarly to compound **1a**, the presence of structural conformers for compounds **1b-d** was detected by ^{19}F and ^{31}P NMR spectra in $CDCl_3$ at room temperature.



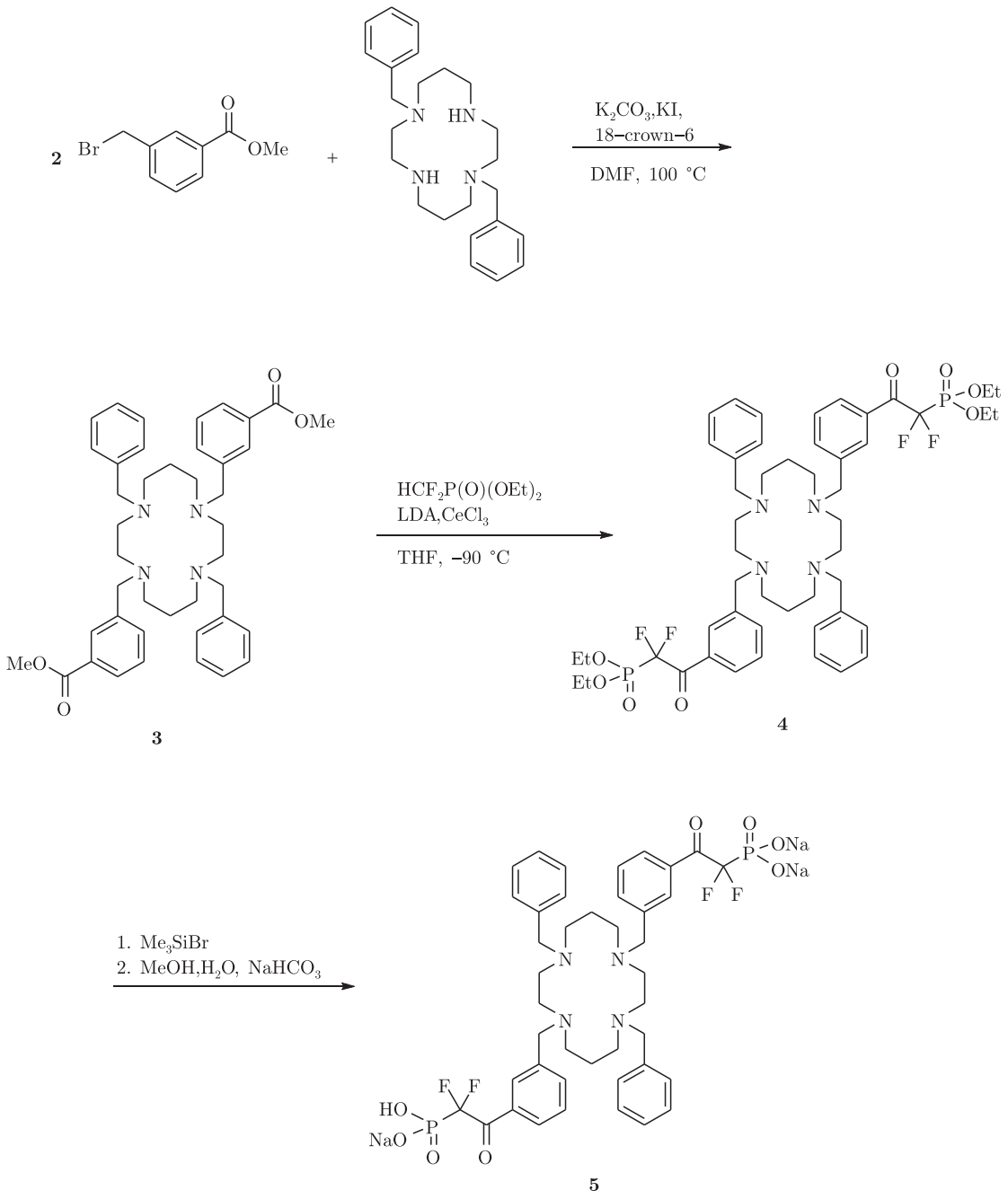
Scheme 1. Synthesis of β-keto-α,α-difluorophosphonate-functionalized cyclams

Compound **2b** was found to be a weak inhibitor of TC-PTP with IC_{50} value approximately 1.1 mM. The replacement of methyl groups in **2b** with benzyl or 2-naphthylmethyl groups gave compounds **2c** and **2d** which showed IC_{50} values of 230 and 110 μM, respectively. In the case of CD45, cyclam derivative **2d** displayed IC_{50} value of 35 μM with a slight reduced inhibitory activity against TC-PTP, SHP2, and PTP β. The presence of 2-naphthylmethyl groups seems to make the derivative **2d** more hydrophobic and leads to increasing the binding of the inhibitor at the region of the active site of CD45 with nonpolar amino acid residues.

The next step in the optimization of cyclam-based PTP inhibitors was the synthesis of the tetraazamacrocycle in which the benzyl groups serve as bridges between N^4, N^{11}-nitrogen sites and α,α-difluoro-β-ketophosphonate moiety. Thus, we examined α,α-difluoro-β-ketophosphonate **5** derived from 1,8-dibenzylcyclam. This compound was prepared by the alkylation of 1,8-dibenzylcyclam with methyl 3-(bromomethyl)benzoate in the presence of K_2CO_3 followed by cerium-mediated reaction of **3** with $LiCF_2P(O)(OEt)_2$ [15]. Deprotection of the phosphonate ester **4** was achieved by transsilylation reaction with excess Me_3SiBr followed by hydrolysis (Scheme 2).

The data of inhibition of PTPs demonstrated that compound **5** is able to inhibit TC-PTP with IC_{50} value of 9.7 μM. This α,α-difluoro-β-ketophosphonate showed about 10-fold selectivity over PTP1B and had practically no effect on CD45, SHP2, and PTP β activity at a concentration of 100 μM.

Thus, the results reported here suggest that macrocyclic polyamines are promising scaffolds for designing effective inhibitors of TC-PTP and other phosphatases, and new synthetic deri-



Scheme 2. Synthesis of α,α-difluoro-β-ketophosphonate **5** derived from 1,8-dibenzylcyclam

vatives of cyclam might be considered as possible regulators of cellular processes controlled by PTPs.

This study was financially supported by the State Fund of Fundamental Research of Ukraine (project F53.3/020) and by the National Academy of Sciences of Ukraine (project 01–2/2014). The authors thank D. Yu. Masich for the participation in the synthesis of some compounds.

1. *Vintonyak V. V., Antonchick A. P., Rauh D., Waldmann H.* The therapeutic potential of phosphatase inhibitors // Curr. Opin. Chem. Biol. – 2009. – **13**. – P. 272–283.
2. *Bialy L., Waldmann H.* Inhibitors of protein tyrosine phosphatases: next-generation drugs? // Angew. Chem. Int. Ed. Engl. – 2005. – **44**. – P. 3814–3839.
3. *Wardle N. J., Hudson H. R., Bligh S. W. A.* O-Phosphotyrosine analogues: Synthesis and therapeutic role in modulation of signal transduction // Curr. Org. Synth. – 2010. – **14**. – P. 426–446.
4. *Burke T. R., Lee K.* Phosphotyrosyl mimetics in the development of signal transduction inhibitors // Acc. Chem. Res. – 2003. – **36**. – P. 426–433.
5. *Chetyrkina S., Estieu-Gionnet K., Lan G. et al.* Synthesis of *N*-Fmoc-4-[(diethylphosphono)-2′,2′-difluoro-1′-hydroxyethyl]phenylalanine, a novel phosphotyrosyl mimic for the preparation of signal transduction inhibitory peptides // Tetrahedron. Lett. – 2000. – **41**. – P. 1923. – 1926.
6. *Solas D., Hale R. L., Patel D. V.* An efficient synthesis of N-α-Fmoc-4-(phosphonodifluoromethyl)-L-phenylalanine // J. Org. Chem. – 1996. – **61**. – P. 1537–1539.
7. *Zhang S., Liu S., Tao R. et al.* A highly selective and potent PTP-MEG2 inhibitor with therapeutic potential for type 2 diabetes // J. Am. Chem. Soc. – 2012. – **134**. – P. 18116–18124.
8. *Meanwell N. A.* Synopsis of some recent tactical application of bioisosteres in drug design // J. Med. Chem. – 2011. – **54**. – P. 2529–2591.
9. *Li X., Bhandari A., Holmes C. P., Szardenings A. K.* α,α-Difluoro-β-ketophosphonates as potent inhibitors of protein tyrosine phosphatase 1B // Bioorg. Med. Chem. Lett. – 2004. – **14**. – P. 4301–4306.
10. *Vovk A. I., Kononets L. A., Tanchuk V. Yu., Cherenok S. O., Drapailo A. B., Kalchenko V. I., Kukhar V. P.* Inhibition of *Yersinia* protein tyrosine phosphatase by phosphonate derivatives of calixarenes // Bioorg. Med. Chem. Lett. – 2010. – **20**. – P. 483–487.
11. *Liang F., Wan S., Li Z. et al.* Medical applications of macrocyclic polyamines // Curr. Med. Chem. – 2006. – **13**. – P. 711–727.
12. *Kobelev S. M, Averin A. D., Maloshitskaya O. A., Denat F., Guilard R., Beletskaya I. P.* Planar-chiral macrobicycles comprising cyclam moiety // Macroheterocycles. – 2012. – **5**. – P. 389–395.
13. *Yang W., Giandomenico C. M., Sartori M., Moore D. A.* Facile N-1 protection of cyclam, cyclen and 1,4,7-triazacyclononane // Tetrahedron Lett. – 2003. – **44**. – P. 2481–2483.
14. *Pajkert R., Milewska M., Röschenthaler G.-V., Koroniak H.* The amination of difluoro(diethoxyphosphoryl)acetyl chloride: Facile synthetic route to novel amides containing difluoromethylenephosphonate moiety // J. Fluorine Chem. – 2009. – **130**. – P. 695–701.
15. *Blades K., Lequeux T. P., Percy J. M.* A reproducible and high-yielding cerium-mediated route to α,α-difluoro-β-ketophosphonates // Tetrahedron. – 1997. – **53**. – P. 10623–10632.

Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine
Lomonosov Moscow State University, Department of Chemistry, Moscow, Russia

Received 12.06.2014

Д. І. Хаврієнко, О. Л. Кобзар, М. В. Шевчук, В. Д. Романенко, С. М. Кобелев, А. Д. Аверін, академік РАН **І. П. Белецька,**
член-кореспондент НАН України **А. І. Вовк,**
академік НАН України **В. П. Кухар**

α,α-Дифторо-β-кетофосфонати на тетраазамакроциклічній платформі: синтез та інгібіторна активність по відношенню до протеїнтирозинфосфатаз

Запропоновано новий підхід до розробки інгібіторів протеїнтирозинфосфатаз. Синтезовано похідні цикламу з α,α-дифторо-β-кетофосфонатними фрагментами, ковалентно зв'язаними з тетраазамакроциклічною платформою. Отримані функціоналізовані макроцикли було досліджено як інгібітори PTP1B, TC-PTP, CD45 та інших протеїнтирозинфосфатаз.

Д. И. Хавриенко, О. Л. Кобзар, М. В. Шевчук, В. Д. Романенко, С. М. Кобелев, А. Д. Аверин, академик РАН **И. П. Белецкая,** член-корреспондент НАН Украины **А. И. Вовк,** академик НАН Украины **В. П. Кухар**

α,α-Дифтор-β-кетофосфонаты на тетраазамакроциклической платформе: синтез и ингибиторная активность по отношению к протеинтирозинфосфатазам

Предложен новый поход к разработке ингибиторов протеинтирозинфосфатаз. Синтезированы производные циклама с α,α-дифтор-β-кетофосфонатными фрагментами, ковалентно связанными с тетраазамакроциклической платформой. Полученные функционализированные макроциклы были изучены в качестве ингибиторов PTP1B, TC-PTP, CD45 и других протеинтирозинфосфатаз.