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Для працівників науково-дослідних установ, вищих навчальних закладів та фахівців хімічного, фармацевтичного, біологічного, медичного і сільськогосподарського профілів.

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Optimization and Scaling up of the Azaindole Derivatives Synthesis

Abstract

In this study, an optimized method for the synthesis of azaindoles was developed and successfully scaled up to a 100 g batch. Improved yields were observed when using electron-deficient azaheterocycles and substrates bearing electron-withdrawing substituents. 6-Chloro-1*H*-pyrrolo[3,2-*c*]pyridine was selected for further functionalization using a carbonylation protocol involving carbon monoxide. As a result, novel and promising building blocks for medicinal chemistry were obtained.

Keywords: azaindoles; Sonogashira coupling; Larock synthesis; carbonylation

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Оптимізація та масштабування методу синтезу похідних азаіндолу

Анотація

У цьому дослідженні було розроблено оптимізований метод синтезу азаіндолів, який успішно масштабовано для одержання до 100 г цільової сполуки. Кращі виходи спостерігали в разі використання електронодефіцитних азагетероциклів та замісників з електронно-акцепторними властивостями. Для подальшої функціоналізації було обрано 6-хлоро-1*H*-піроло[3,2-*c*]піридин шляхом його карбонілювання дією карбон(II) оксиду. Внаслідок цього було одержано нові перспективні будівельні блоки для потреб медичної хімії.

Ключові слова: азаіндоли; реакція Соногашіри; синтез Ларока; карбонілювання

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Supporting information: Copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra of the synthesized compounds.
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■ Introduction

Azaindoles are compounds composed of fused azaheterocyclic and pyrrole rings, forming aromatic frameworks that serve as bioisosteres of indoles and are structurally similar to purine bases. This structural similarity contributes to their broad spectrum of biological activities, making them valuable in pharmaceuticals, industrial applications, and natural product chemistry [1–4].

According to the SciFinder® database, azaindoles have increasingly attracted research attention since 2000 (**Figure 1**). Since 2004, due to their recognized antiviral properties and influenced by global health crises, the number of publications on azaindoles has nearly doubled.

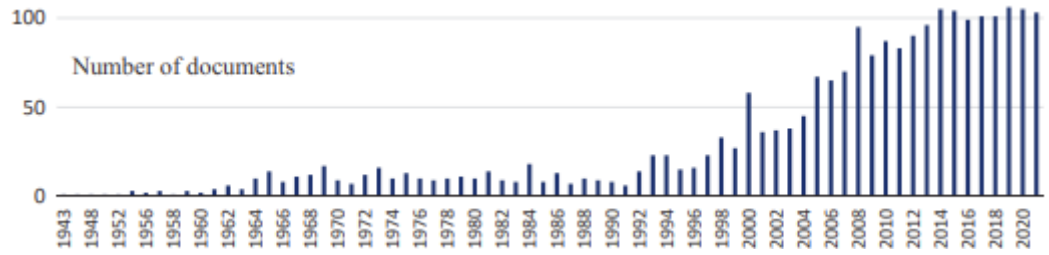


Figure 1. The published papers dedicated to azaindoles

Currently, over 100 articles related to azaindoles are published annually [5].

Numerous synthetic approaches have been developed for azaindole formation, predominantly involving the assembly of a pyrrole ring onto existing azaheterocyclic frameworks. However, methods assembling azaheterocycles onto a pyrrole core have also been documented (**Figure 2**) [5, 6].

■ Results and discussions

Taking into account the significant range of biological activities exhibited by azaindoles discovered over the past two decades, optimizing synthetic routes remains critically important. This study aimed to validate and optimize synthetic methods across various electron-deficient azaheterocycles, such as pyridine, pyrazine, and pyrimidine.

The Larock's synthetic approach was selected to achieve the research purposes due to its versatility and potential for structural diversity. This methodology involves a two-step process: the Sonogashira coupling using TMS-acetylene, followed by the heterocyclization mediated by KO*t*Bu [7–11]. Seven azaindole derivatives were chosen for the synthesis, including compounds (**3a**, **3c**, **3g**) previously described to establish a more convenient and efficient method. For example, the synthesis of 5*H*-pyrrolo[2,3-*b*]pyrazine derivative (**3a**) *via* *N*-mesyl amino pyrazine reported earlier faced difficulties at the initial stage, resulting in low overall yields [12]. Similarly, the synthesis of 6-chloro-1*H*-pyrrolo[3,2-*c*]pyridine (**3c**) involved a complicated multi-step catalytic oxidation [13]. Additionally, the synthesis of 7-methyl-1*H*-pyrrolo[3,2-*b*]pyridine (**3g**) was previously achieved through the Bartoli method with only 18% yield [14] or a multistep Suzuki coupling [15]. Derivatives **3b**, **3d**, **3e**, and **3f** have not been previously reported.

Commercially available amines were utilized for synthesizing **3a**, **3c**, **3f**, and **3g** derivatives, namely 3-chloropyrazin-2-amine (**1a**), 5-bromo-2-chloropyridin-4-amine (**1c**), 2-chloro-5-(trifluoromethyl)pyridin-3-amine (**1f**), and 2-bromo-4-methylpyridin-3-amine (**1g**). 5-Bromo-2-methylpyrimidin-4-amine (**1b**) was obtained with a high yield *via* the halogen reduction using Pd/C followed by the bromination (**Scheme 1**) [16, 17]. 3-Iodo-2-methoxypyridin-4-amine (**1d**) was prepared by the iodination using NIS in acetonitrile at reflux conditions [18]. For 2-bromo-6-(trifluoromethyl)pyridin-3-amine (**1e**), the bromination with NBS was conducted in acetonitrile instead of $CHCl_3$, significantly reducing the formation of regioisomers [19].

The Sonogashira reaction was scaled up to 100 g for each amine, and intermediates **2a–g** were purified by flash chromatography, resulting in excellent yields (**Scheme 2**). The lowest yield (64.3%) was noted for alkyne **2c** due to side reactions employing chlorine atoms. The optimal cyclization conditions involved the use of 1.2 equiv.

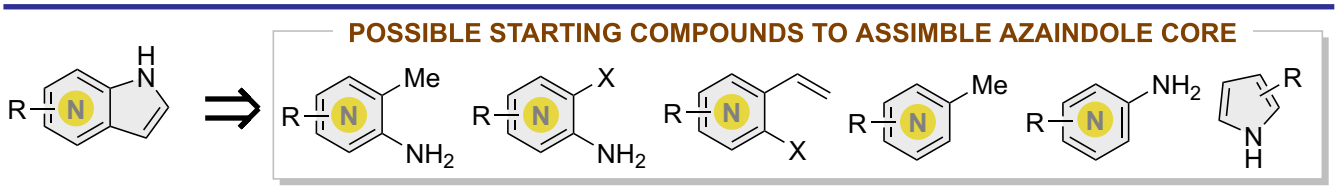
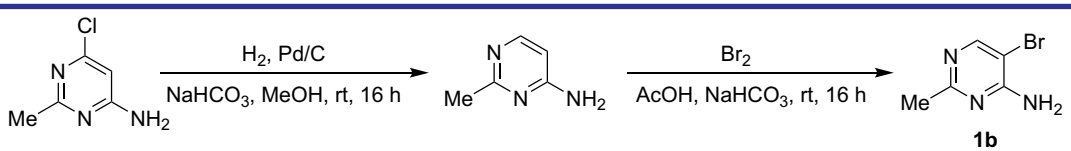


Figure 2. Main strategies toward azaindoles



Scheme 1. The synthesis of starting pyrimidine **1b**

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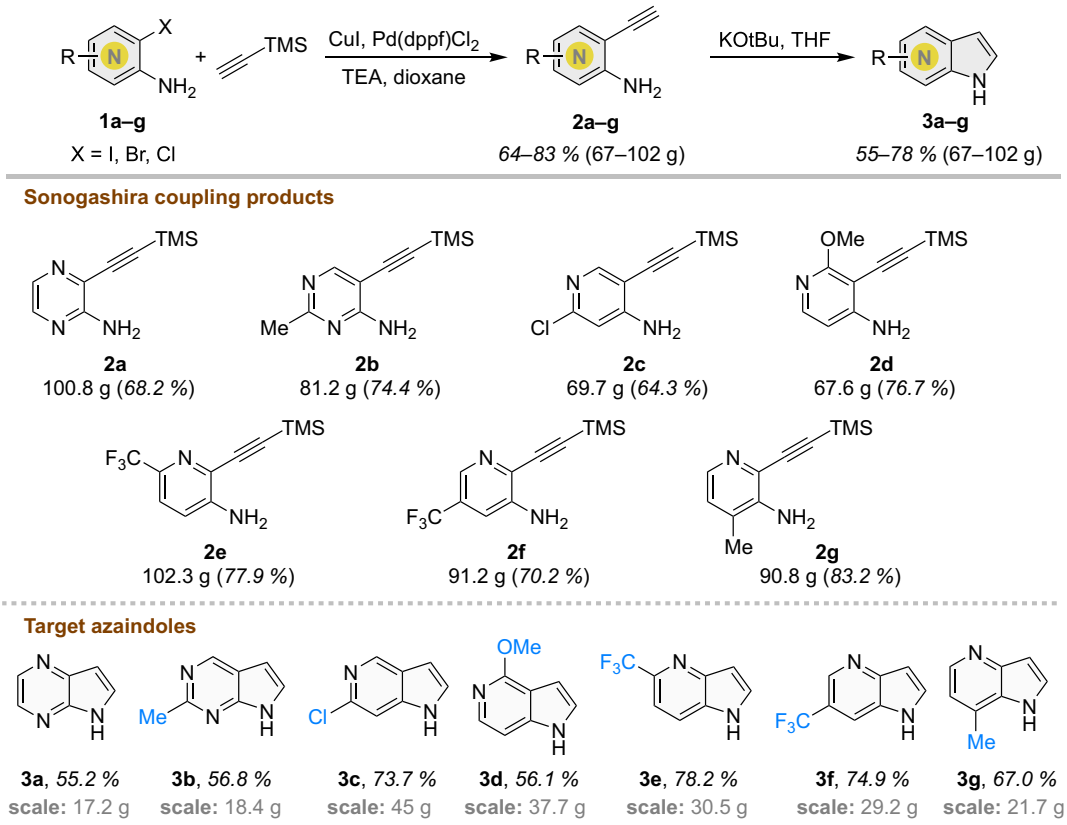


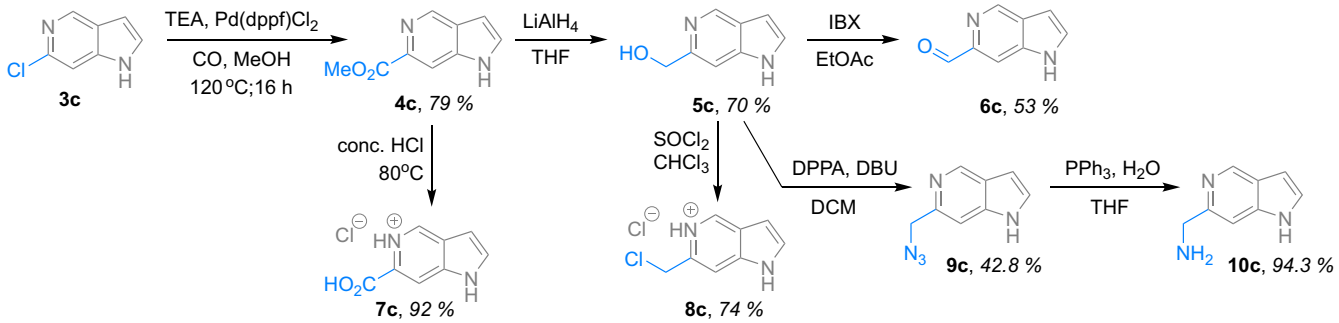
Scheme 2. The synthesis of the target azaindoles: scales and yields

of KO*t*Bu in THF. Compounds **2** with electron-withdrawing substituents, such as CF_3 (**2e**, **2f**) and chlorine (**2c**), along with a weakly electron-donating methyl group (**2g**), secured higher yields of the target azaindoles **3** of about 70%. At the same time, pyrazine and pyrimidine derivatives (**3a**, **3b**) were obtained with 55–57% yields similar to the methoxy derivative **3d** (56.1%).

Derivative **3c**, known for its biological activity [20, 21], offered the additional synthetic flexibility due to its halogen substituent. The catalytic carbonylation with CO in the methanol medium provided the corresponding ester **4c** with a high yield, which after the acidic hydrolysis yielded carboxylic acid **7c** (**Scheme 3**). At the same time, the reduction of the ester with $LiAlH_4$ yielded alcohol **5c**, which was likely to be a valuable intermediate for MedChem research. The reaction of **5c** with $SOCl_2$ produced chloromethyl derivative **8c**, while the oxidation with IBX in EtOAc yielded aldehyde **6c** in a moderate 53% yield. The corresponding amine **10c** was also synthesized *via* azide intermediate **9c**, formed using DPPA-DBU conditions, followed by the Staudinger reduction, without the intermediate purification.

■ Conclusion

In this study, an optimized method for the azaindole synthesis was reviewed and successfully scaled up to a 100 g batch size. Higher yields were achieved with electron-deficient azaheterocycles bearing electron-withdrawing substituents. Additionally, 6-chloro-1H-pyrrolo[3,2-c]pyridine was selected for further functionalization, leading



Scheme 3. The functionalization of product **3c**

to the preparation of novel and promising building blocks suitable for applications in medicinal chemistry.

■ Experimental part

^{1}H and ^{19}F NMR spectra were recorded on a Varian Unity Plus 400 instrument (400 and 376 MHz, respectively), ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 170 Avance 500 instrument (500 and 126 MHz, respectively), ^{13}C NMR spectra were also recorded on an Agilent ProPulse 600 (151 MHz) spectrometer. The NMR chemical shifts were referenced using the solvent signals at 7.26 and 77.1 ppm for ^{1}H and ^{13}C nuclei, respectively, in $CDCl_3$ and 2.48 and 39.5 ppm for ^{1}H and ^{13}C nuclei, respectively, in DMSO-d_6; C_6F_6 was used as the internal standard for ^{19}F NMR spectra. Mass spectra were obtained on an Agilent LC/MSD SL 1100 instrument (the atmospheric pressure electrospray ionization (ES-API)) or an Agilent 5890 Series II 5972 GCMS instrument (the electron impact (EI) ionization (70eV)). HRMS experiments were performed on an Agilent 6224 TOF LC/MS instrument using the electrospray ionization. The composition of hydrochloride salts was determined by the acid-base titration method. Melting points were measured in open capillary tubes and were given uncorrected. All starting compounds and solvents were obtained from Enamine Ltd. and used without additional purification.

The general procedure for the Sonogashira reaction (compounds 2a–g)

To the solution of **1a–g** (100 g) in 1.0 L dioxane, TMS-acetylene (1.2 equiv.) and TEA (4.0 equiv.) were was added. Then the mixture was degassed under argon and stirred for 10 min, CuI (0.05 equiv.) and Pd(dppf)Cl_2 (0.03 equiv.) were added in one portion under the argon atmosphere, the resulting mixture was stirred at 90 °C for 16 h. Upon completion of the reaction, the mixture was filtered through a celite pad and concentrated under vacuum. The crude mixture was purified by flash column chromatography in the corresponding eluent described below to give pure **2a–g**.

3-((Trimethylsilyl)ethynyl)pyrazin-2-amine (**2a**)

Flash chromatography purification using $CHCl_3$–MeCN mixture (9:1) as an eluent.

A yellow solid. Yield – 100.8 g (68.2%). M. p. 115 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.24 (9H, s), 6.48 (2H, s), 7.72 (1H, d, J = 2.5Hz), 7.93 (1H, d, J = 2.2Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: -0.4, 100.1, 100.8, 122.9, 132.5, 142.2, 156.3. LCMS (ES-API), m/z 192 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_9H_{13}N_3Si$ 191.0879, found 191.0875.

2-Methyl-5-((trimethylsilyl)ethynyl)pyrimidin-4-amine (**2b**)

Flash chromatography purification using $CHCl_3$ as an eluent.

A white solid. Yield – 81.2 g (74.4%). M. p. 125 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.21 (9H, s), 2.31 (3H, s), 7.46 (2H, br. s), 8.12 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: -0.2, 25.5, 97.5, 98.0, 102.2, 157.9, 162.9, 166.1. LCMS (ES-API), m/z 206 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_{10}H_{15}N_3Si$ 205.1030, found 205.1035.

2-Chloro-5-((trimethylsilyl)ethynyl)pyridin-4-amine (**2c**)

Flash chromatography purification using $CHCl_3$ as an eluent.

A yellow solid. Yield – 69.7 g (64.3%). M. p. 108 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.93 (1H, s), 6.63 (1H, s), 6.58 (2H, br. s), 0.22 (9H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 0.4, 98.5, 103.1, 103.5, 106.8, 150.6, 152.4, 157.0. LCMS (ES-API), m/z: 225 $[M+H]^+$.

2-Methoxy-3-((trimethylsilyl)ethynyl)pyridin-4-amine (**2d**)

The reaction mixture was stirred at 60° C for 16 h. Flash chromatography purification using $CHCl_3$ as an eluent.

A yellow solid. Yield – 67.6g (76.7%). M. p. 72 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.20 (9H, s), 3.77 (3H, s), 6.09 (2H, br. s), 6.32 (1H, d, J = 6.0 Hz), 7.60 (1H, d, J = 6.0 Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 0.6, 53.6, 88.0, 98.1, 104.0, 104.5, 145.9, 157.8, 164.6. LCMS (ES-API), m/z: 221 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_{11}H_{16}N_2OSi$ 220.1032, found 220.1026.

6-(Trifluoromethyl)-2-((trimethylsilyl)ethynyl)pyridin-3-amine (**2e**)

Flash chromatography purification using $CHCl_3$ as an eluent.

A white solid. Yield – 102.3 g (77.9%). M. p. 110 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.25 (9H, s), 6.16 (2H, s), 7.21 (1H, d, J = 8.5Hz), 7.49 (1H, d, J = 8.5Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: -0.3, 99.8, 100.6, 120.4, 121.4, 125.5, 133.7, 134.0, 148.5. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -65.35. LCMS (ES-API), m/z: 259 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_{11}H_{13}F_3N_2Si$ 258.0798, found 258.0800.

5-(Trifluoromethyl)-2-((trimethylsilyl)ethynyl)pyridin-3-amine **(2f)**

Flash chromatography purification using $CHCl_3$ as an eluent.

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A white solid. Yield – 91.2 g (70.2%). M. p. 132 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.26 (9H, s), 5.94 (2H, s), 7.37 (1H, s), 8.01 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: -0.4, 100.2, 101.7, 116.6, 122.5, 124.7, 129.4, 132.9, 146.0. ^{19}F NMR (376 MHz DMSO-d_6), δ, ppm: -61.99. LCMS (ES-API), *m/z*: 259 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_{11}H_{13}F_3N_2Si$ 258.0804, found 258.0800.

4-Methyl-2-((trimethylsilyl)ethynyl)pyridin-3-amine **(2g)**

The crude mixture was treated with MTBE.

A gray solid. Yield – 90.8 g (83.2%). M. p. 121 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.24 (9H, s), 5.13 (2H, s), 6.97 (1H, d, J = 4.6 Hz), 7.66 (1H, d, J = 4.3 Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: -0.2, 17.0, 98.7, 102.0, 125.3, 125.7, 129.7, 138.0, 144.8. LCMS (ES-API), *m/z*: 205 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_{11}H_{16}N_2Si$ 204.1083, found 204.1089.

The general procedure for heterocyclization 3a–g

KO*t*Bu (1.2 equiv.) was added in one portion to a stirring solution of compound **2a–g** (1 equiv., 50 g) in 1.0 L THF at 0 °C. The temperature was brought to reflux, and the resulting mixture was stirred for 15 h. After that, the solvent was evaporated to dryness, the residue was poured into the mixture of the concentrated HCl and water (100 mL–400 mL), the mixture was stirred for 30 min and filtered through celite; the mother liquid was neutralized with ammonia to pH = 10, stirred for another 30 min. Then the precipitate was collected *via* the vacuum filtration. In case if the precipitate was not formed, the mixture was extracted with DCM (3×200 mL), combined organic layers were dried with the anhydrous Na_2SO_4 and concentrated. Compounds **3a–g** required no additional purification.

5H-Pyrrolo[2,3-b]pyrazine (**3a**)

A white solid. Yield – 17.2 g (55.2%). M. p. 153 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.61 (1H, dd, J = 3.5, 1.7 Hz), 7.85 (1H, t, J = 3.2 Hz), 8.21 (1H, d, J = 2.6 Hz), 8.36 (1H, d, J = 2.3 Hz), 12.03 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 100.8, 131.5, 137.1, 138.5, 139.6, 141.7. LCMS (ES-API), *m/z*: 120 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_6H_5N_3Si$ 119.0492, found 119.0483.

2-Methyl-7H-pyrrolo[2,3-d]pyrimidine (**3b**)

A white solid. Yield – 18.4 g (56.8%). M. p. 179 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.60 (3H, s), 6.49 (1H, d, J = 2.6Hz), 7.42 (1H, s), 8.85 (1H, s), 11.88 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 25.9, 99.6, 116.0, 126.8, 149.2, 152.4, 159.73. LCMS (ES-API), *m/z*: 134 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_7H_7N_3$ 133.0644, found 133.0640.

6-Chloro-1H-pyrrolo[3,2-c]pyridine (**3c**)

A brown solid. Yield – 45 g (73.7%). M. p. 189 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.59 (1H, m), 7.42 (1H, s), 7.48 (1H, m), 8.60 (1H, s), 11.62 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 101.0, 106.4, 125.0, 128.7, 141.7, 141.8, 142.5. LCMS (ES-API), *m/z*: 153 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_7H_5ClN_2$ 152.0139, found 152.0141.

4-Methoxy-1H-pyrrolo[3,2-c]pyridine (**3d**)

A brown solid. Yield – 37.7 g (56.1%). M. p. 139 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 4.10 (3H, s), 6.66 (1H, d, J = 2.3Hz), 6.97 (1H, d, J = 5.7 Hz), 7.13 (1H, m), 7.84 (1H, d, J = 5.7 Hz), 8.59 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 52.9, 99.5, 103.2, 112.1, 124.7, 137.4, 141.5, 157.7. LCMS (ES-API), *m/z*: 149 $[M+H]^+$. HRMS (APCI) *m/z*: calcd for $C_8H_8N_2O$ 148.0637, found 148.0637.

5-(Trifluoromethyl)-1H-pyrrolo[3,2-b]pyridine (**3e**)

A white solid. Yield – 30.5 g (78.2%). M. p. 213 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.7 (1H, s), 7.54 (1H d, J = 8.2 Hz), 7.88 (1H, s), 7.99 (1H, d, J = 8.5 Hz), 11.76 (1H, br). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 102.0, 112.5, 119.4, 121.7–123.8 (q, J = 265 Hz, CF_3), 129.6, 132.3, 139.4, 145.7. ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.47 LCMS (ES-API), *m/z*: 187 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_8H_5F_3N_2$ 186.0402, found 186.0405.

6-(trifluoromethyl)-1H-pyrrolo[3,2-b]pyridine (**3f**)

A white solid. Yield – 29.2 g (74.9%). M. p. 190 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.71 (1H, s), 7.95 (1H, s), 8.16 (1H, s), 8.65 (1H, s), 11.85 (1H, br). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 101.9, 117.3, 117.4, 124.5–126.3 (q, J = 227 Hz, CF_3), 128.1, 134.7, 138.4, 147.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ -58.81. LCMS (ES-API), *m/z*: 187 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_8H_5F_3N_2$ 186.0406, found 186.0405.

Methyl-1H-pyrrolo[3,2-b]pyridine (**3g**)

A brown solid. Yield – 21.7 g (67.0%) M. p. 189 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.48 (3H, s), 6.51 (1H, s), 6.89 (1H, s), 7.57 (1H, s), 8.17 (1H, s), 11.35 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 16.2, 101.8, 117.4, 128.3, 128.9, 142.3, 145.2, 145.6. LCMS (ES-API) *m/z*: 133 $[M+H]^+$. HRMS (APCI), *m/z*: calcd for $C_8H_8N_2$ 132.0685, found 132.0687.

The procedure for the preparation of methyl 1*H*-pyrrolo[3,2-*c*]pyridine-6-carboxylate (4c)

To the solution of compound **3c** (30 g, 0,196 mol) and TEA (32.9 mL, 0.234 mol) in 400 mL MeOH in 500 mL autoclave, $Pd(dppf)Cl_2$ (4.75 g, 0.006 mol) was added. The reaction vessel was flushed three times with CO gas and stirred under 20 atm at 120 °C for 16 h. Upon completion, the reaction mixture was cooled to room temperature, and the precipitate was collected *via* the vacuum filtration, washed with water and MTBE, then dried in air to obtain a pure product **4c** (27.3 g, 78.9%).

A yellow solid. Yield – 27.3 g (78.9%). M. p. 183 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 3.85 (3H, s), 6.70 (1H, d, J = 3.2 Hz), 7.62–7.73 (1H, m), 8.15 (1H, s), 8.89 (1H, s), 11.94 (1H, s). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 52.4, 101.5, 110.0, 127.3, 130.4, 138.6, 139.2, 143.0, 166.8. LCMS (ES), m/z: 177 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_9H_8N_2O_2$ 176.0586, found 176.0581.

The procedure for the preparation of methyl 1*H*-pyrrolo[3,2-*c*]pyridine-6-carboxylate (5c)

To a cooled solution of $LiAlH_4$ (14.25 g, 1.25 equiv.) in THF (1 L) at 0 °C, **4c** (53.0 g, 0.30 mol) was added portionwise, keeping temperature bellow 0 °C. Then mixture was stirred at room temperature for 15 h. Upon completion, the reaction mixture was cooled to 0 °C and carefully neutralized with the water-NaOH solution and water. The mixture was stirred for 1 h at room temperature, and the precipitate was filtered through celite, washed with MTBE, then the mother liquid was evaporated *in vacuo* to give a pure product **5c** (31.3 g, 70.2%)

A yellow solid. Yield 31.3 g (70.2%). M. p. 132 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 4.26 (s, 2H), 5.27 (1H, br. s), 6.51 (1H, s), 7.37 (1H, s), 7.42 (1H, s), 8.69 (1H, s), 11.44 (1H, s). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 65.0, 100.6, 103.1, 123.9, 126.7, 140.7, 142.2, 153.1; LCMS (ES), m/z: 149 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_8H_8N_2O$ 148.0637, found 148.0635.

The procedure for the preparation of 1*H*-pyrrolo[3,2-*c*]pyridin-6-yl)methanol (6c)

To the mixture of **5c** (20.0 g, 0.135 mol) in 400 mL of EtOAc, IBX (1.5 equiv.) was added in one portion. Then the mixture was stirred at reflux for 15 h. Upon completion, the reaction mixture was filtered hot through celite, washed with EtOAc, then the mother liquid was washed with the K_2CO_3 water solution (20 g in 500 mL of water), the organic phase was dried and evaporated *in vacuo* to give a pure product **6c** (10.4 g, 52.7%).

A yellow solid. Yield – 10.4 g (52.7%). M. p. 162 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 6.73 (1H, d, J = 2.5 Hz), 7.75 (1H, d, J = 3Hz), 8.01 (1H, s), 9.01 (1H, s), 10.05 (1H, s), 12.08 (1H, s). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 101.3, 106.6, 127.9, 131.0, 138.6, 143.2, 144.8, 193.6. GCMS, m/z: 146 $[M]^+$; HRMS (APCI), m/z: calcd for $C_8H_6N_2O$ 146.0476, found 146.0480.

The procedure for the preparation of 1*H*-pyrrolo[3,2-*c*]pyridine-6-carboxylic acid hydrochloride (7c)

To the solution of compound **4c** (1.0 g, 0.0057 mol), the conc. HCl (10 mL) was added. The resulting mixture was stirred at 80 °C for 15 h. Upon completion, the reaction mixture was evaporated to dryness to obtain a yellow solid product **7c** in the form of HCl salt (1.04 g, 92.0%).

A yellow solid. Yield – 1.04 g (92%). M. p. 280 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.15 (1H, s), 8.12 (1H, s), 8.49 (1H, s), 9.29 (1H, s), 13.81 (1H, s), 14.80 (1H, br). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 105.9, 111.3, 126.0, 130.9, 135.2, 137.8, 141.6, 162.3. LCMS (ES), m/z: 163 $[M+H]^+$. HRMS (APCI) m/z: calcd for $C_8H_6N_2O_2$ 162.0428, found 162.0429.

The procedure for the preparation of 6-(chloromethyl)-1*H*-pyrrolo[3,2-*c*]pyridine hydrochloride (8c)

To the solution of $SOCl_2$ (1.5 equiv.) in 400 mL of $CHCl_3$, alcohol **5c** (30 g, 0.20 mol) was added dropwise at 0 °C. Then the mixture was stirred at room temperature for 16 h. Upon completion, the precipitate was collected *via* vacuum filtration, washed with $CHCl_3$, then dried to obtain pure product **8c** (30.5 g, 74.2%, hydrochloride salt).

A yellow solid. Yield – 30.5 g (74.2%). M. p. 209 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 5.20 (2H, s), 7.01 (1H, d, J = 0.5 Hz), 7.96 (1H, d, J = 2.2Hz), 8.12 (1H, s), 9.27 (1H, s), 13.37 (1H, s), 15.86 (1H, br. s). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 41.5, 104.8, 110.1, 124.1, 133.6, 136.8, 139.7, 142.4. LCMS (ES), m/z: 167 $[M+H]^+$. HRMS (APCI) m/z: calcd for $C_8H_7ClN_2$ 166.0297, found 166.0298.

The procedure for the preparation of 6-(azidomethyl)-1H-pyrrolo[3,2-c]pyridine (9c)

To a cooled to 0 °C solution of DBU (1.2 equiv.) and **5c** (5.0 g, 0.03 mol) in 200 mL of THF, DPPA (1.2 equiv.) was added portionwise keeping temperature bellow 0 °C. Then the mixture was

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stirred at room temperature for 15 h. Upon completion, the reaction mixture was evaporated to dryness. The crude mixture was purified by column chromatography using the $CHCl_3$-MeCN system as an eluent to give **9c** (2.5 g, 42.8%) as a yellow solid with 80% purity. The product was used in the next step without any purification due to stability issues.

Yield – 2.5 g (42.8%). Purity 80%. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 4.50 (2H, s), 6.58 (1H, d, J = 2.8 Hz), 7.41–7.52 (2H, m), 8.82 (1H, s), 11.66 (1H, s).

The procedure for the preparation of (1*H*-pyrrolo[3,2-*c*]pyridin-6-yl)methanamine (10c)

To the solution of crude **9c** (2.5 g, 0.01 mol) in 50 mL of THF, PPh_3 (1.2 equiv.) was added in one portion and H_2O (1.5 equiv.) in 30 min. The resulting mixture was stirred at room temperature for 15 h. Upon completion, a diluted HCl (2 equiv.) was added, the water solution was extracted twice with $CHCl_3$ (for PPh_3 and Ph_3PO separation), and then the water solution was neutralized with K_2CO_3. The precipitate was collected *via* the vacuum filtration, washed with THF, then dried to obtain a pure product **10c** (2.0 g, 94.3%).

A white solid. Yield – 2.0 g (94.3 %). M. p. 190 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 1.96–2.40 (1H, br. s), 3.85 (2H, s), 6.52 (1H, d, J = 2.3 Hz), 7.37 (1H, d, J = 2.0 Hz), 7.39 (1H, s), 8.72 (1H, s), 11.45 (1H, br. s). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 48.0, 100.5, 103.6, 123.8, 126.6, 140.7, 142.3, 154.0. LCMS (ES), m/z: 148 $[M+H]^+$. HRMS (APCI), m/z: calcd for $C_8H_9N_3$ 147.0794, found 147.0796.

References

1. Zhao, S.-B.; Wang, S. Luminescence and Reactivity of 7-Azaindole Derivatives and Complexes. *Chem. Soc. Rev.* **2010**, *39* (8), 3142. https://doi.org/10.1039/c001897j.
2. Meanwell, N. A.; Krystal, M. R.; Nowicka-Sans, B.; Langley, D. R.; Conlon, D. A.; Eastgate, M. D.; Grasela, D. M.; Timmins, P.; Wang, T.; Kadow, J. F. Inhibitors of HIV-1 Attachment: The Discovery and Development of Temsavir and Its Prodrug Fostemsavir. *J. Med. Chem.* **2018**, *61* (1), 62–80. https://doi.org/10.1021/acs.jmedchem.7b01337.
3. Shirude, P. S.; Shandil, R.; Sadler, C.; Naik, M.; Hosagrahara, V.; Hameed, S.; Shinde, V.; Bathula, C.; Humnabadkar, V.; Kumar, N.; Reddy, J.; Panduga, V.; Sharma, S.; Ambady, A.; Hegde, N.; Whiteaker, J.; McLaughlin, R. E.; Gardner, H.; Madhavapeddi, P.; Ramachandran, V.; Kaur, P.; Narayan, A.; Guptha, S.; Awasthy, D.; Narayan, C.; Mahadevaswamy, J.; Vishwas, K.; Ahuja, V.; Srivastava, A.; Prabhakar, K.; Bharath, S.; Kale, R.; Ramaiah, M.; Choudhury, N. R.; Sambandamurthy, V. K.; Solapure, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold Morphing Efforts, Kill Mycobacterium Tuberculosis and Are Efficacious *in Vivo*. *J. Med. Chem.* **2013**, *56* (23), 9701–9708. https://doi.org/10.1021/jm401382v.
4. Bandarage, U. K.; Cao, J.; Come, J. H.; Court, J. J.; Gao, H.; Jacobs, M. D.; Marhefka, C.; Nanthakumar, S.; Green, J. ROCK Inhibitors 3: Design, Synthesis and Structure-Activity Relationships of 7-Azaindole-Based Rho Kinase (ROCK) Inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2018**, *28* (15), 2622–2626. https://doi.org/10.1016/j.bmcl.2018.06.040.
5. Ballesteros-Garrido, R. Recent Developments in the Synthesis of 4-, 5-, 6- and 7-Azaindoles. In *Advances in Heterocyclic Chemistry*; Elsevier, 2023; Vol. 140, pp 67–123. https://doi.org/10.1016/bs.aihch.2023.01.001.
6. Motati, D. R.; Amaradhi, R.; Ganesh, T. Recent Developments in the Synthesis of Azaindoles from Pyridine and Pyrrole Building Blocks. *Org. Chem. Front.* **2021**, *8* (3), 466–513. https://doi.org/10.1039/D0QO01079K.
7. Leboho, T. C.; Giri, S.; Popova, I.; Cock, I.; Michael, J. P.; De Koning, C. B. Double Sonogashira Reactions on Dihalogenated Aminopyridines for the Assembly of an Array of 7-Azaindoles Bearing Triazole and Quinoxaline Substituents at C-5: Inhibitory Bioactivity against Giardia Duodenalis Trophozoites. *Bioorganic & Medicinal Chemistry* **2015**, *23* (15), 4943–4951. https://doi.org/10.1016/j.bmc.2015.05.024.
8. Xu, L.; Lewis, I. R.; Davidsen, S. K.; Summers, J. B. Transition Metal Catalyzed Synthesis of 5-Azaindoles. *Tetrahedron Letters* **1998**, *39* (29), 5159–5162. https://doi.org/10.1016/S0040-4039(98)00986-1.
9. Koradin, C.; Dohle, W.; Rodriguez, A. L.; Schmid, B.; Knochel, P. Synthesis of Polyfunctional Indoles and Related Heterocycles Mediated by Cesium and Potassium Bases. *Tetrahedron* **2003**, *59* (9), 1571–1587. https://doi.org/10.1016/S0040-4020(03)00073-5.
10. Sun, L.; Wang, J. Facile Synthesis of 2-Substituted 4-Azaindoles. *Synthetic Communications* **2007**, *37* (13), 2187–2193. https://doi.org/10.1080/00397910701397359.
11. Barl, N. M.; Sansiaume-Dagousset, E.; Karaghiosoff, K.; Knochel, P. Full Functionalization of the 7-Azaindole Scaffold by Selective Metalation and Sulfoxide/Magnesium Exchange. *Angew Chem Int Ed* **2013**, *52* (38), 10093–10096. https://doi.org/10.1002/anie.201303490.
12. Hopkins, C. R.; Collar, N. 6-Substituted-5H-Pyrrolo[2,3-b]Pyrazines via Palladium-Catalyzed Heteroannulation from N-(3-Chloropyrazin-2-Yl)-Methanesulfonamide and Alkynes. *Tetrahedron Letters* **2004**, *45* (43), 8087–8090. https://doi.org/10.1016/j.tetlet.2004.08.155.
13. Azimov, V. A.; Bychikhina, N. N.; Polezhaeva, A. I.; Mashkovskii, M. D.; Yakhontov, L. N. Synthesis and Pharmacologic Study of 6-Amino Derivatives of 5-Azaindoline. *Pharm Chem J* **1980**, *14* (5), 308–313. https://doi.org/10.1007/BF00777399.
14. Zhang, Z.; Yang, Z.; Meanwell, N. A.; Kadow, J. F.; Wang, T. A General Method for the Preparation of 4- and 6-Azaindoles. *J. Org. Chem.* **2002**, *67* (7), 2345–2347. https://doi.org/10.1021/jo0111614.
15. Subota, A. I.; Volochnyuk, D. M.; Gorlova, A. O.; Grygorenko, O. O. Scalable Synthesis and Properties of 7-Methyl- 4-Azaindole. *Heterocyclic Communications* **2017**, *23* (6), 449–453. https://doi.org/10.1515/hc-2017-0180.
16. Le, P. T.; Richardson, P. F.; Sach, N. W.; Xin, S.; Ren, S.; Xiao, J.; Xue, L. Development of a Scalable Synthesis of 4-Aminopyrimidin-5-Ol, a Versatile Intermediate. *Org. Process Res. Dev.* **2015**, *19* (6), 639–645. https://doi.org/10.1021/acs.oprd.5b00074.
17. Paudler, W. W.; Jovanovic, M. V. Bromination of Some Pyridine and Diazine N-Oxides. *J. Org. Chem.* **1983**, *48* (7), 1064–1069. https://doi.org/10.1021/jo00155a027.

18. Minzong Li; Konteatis Z.; Chen Y.; Zhou S.; Ma G.; Gross S.; Marjon K.; Hyer M.; Mandley E.; Lein M.; Padyana A.; Jin L.; Tong S.; Peters R.; Murtie J.; Travins J.; Medeiros M.; Liu P.; Victoria-Frank; Judd E.; Biller S.; Marks K.; Sui Z.; Reznik S. Leveraging Structure-Based Drug Design to IdentifyNext-Generation MAT2A Inhibitors, Including Brain-Penetrant and Peripherally Efficacious Leads. *Med. Chem.* **2022**, 65, 6, 4600–4615. https://doi.org/10.1021/acs.jmedchem.1c01595
19. Biot, N.; Romito, D.; Bonifazi, D. Substituent-Controlled Tailoring of Chalcogen-Bonded Supramolecular Nanoribbons in the Solid State. *Crystal Growth & Design* **2021**, *21* (1), 536–543. https://doi.org/10.1021/acs.cgd.0c01318.
20. Chessari, G.; Buck, I. M.; Day, J. E. H.; Day, P. J.; Iqbal, A.; Johnson, C. N.; Lewis, E. J.; Martins, V.; Miller, D.; Reader, M.; Rees, D. C.; Rich, S. J.; Tamanini, E.; Vitorino, M.; Ward, G. A.; Williams, P. A.; Williams, G.; Wilsher, N. E.; Woolford, A. J.-A. Fragment-Based Drug Discovery Targeting Inhibitor of Apoptosis Proteins: Discovery of a Non-Alanine Lead Series with Dual Activity Against cIAP1 and XIAP. *J. Med. Chem.* **2015**, *58* (16), 6574–6588. https://doi.org/10.1021/acs.jmedchem.5b00706.
21. Hanan, E. J.; Eigenbrot, C.; Bryan, M. C.; Burdick, D. J.; Chan, B. K.; Chen, Y.; Dotson, J.; Heald, R. A.; Jackson, P. S.; La, H.; Lainchbury, M. D.; Malek, S.; Purkey, H. E.; Schaefer, G.; Schmidt, S.; Seward, E. M.; Sideris, S.; Tam, C.; Wang, S.; Yeap, S. K.; Yen, I.; Yin, J.; Yu, C.; Zilberleyb, I.; Heffron, T. P. Discovery of Selective and Noncovalent Diaminopyrimidine-Based Inhibitors of Epidermal Growth Factor Receptor Containing the T790M Resistance Mutation. *J. Med. Chem.* **2014**, *57* (23), 10176–10191. https://doi.org/10.1021/jm501578n.

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The Study of the Complexation of Calix[4]arene-Hydroxymethylphosphonic Acid and Calix[4]arene-Hydroxymethyldimethylphosphine Oxide with Antiviral Drugs

Abstract

The host-guest complexation of cone-shaped calix[4]arene-hydroxymethylphosphonic acid (**CPA**) and calix[4]arene-hydroxymethyldimethylphosphine oxide (**CPO**) with active pharmaceutical ingredients of antiviral drugs Remdesivir, Nevirapine, Vesatolimod, and Bictegravir in the aqueous-organic mobile phase on a Zorbax CN column has been studied using RP HPLC method. By analyzing the dependence of the drug capacity values on the concentration of calixarene in the mobile phase, the stability constants (K_A = 3672 – 6884 M^{-1}) of the complexes formed have been determined. Quantum-chemical calculations show that the drugs studied form supramolecular *exo*-complexes with **CPA** and **CPO** molecules. These complexes are stabilized by intermolecular hydrogen bonds of proton donor groups $P(O)(OH)_2$ **CPA** and proton acceptor groups $Me_2P{=}O$ **CPO** with the amino group of Remdesivir, the amide group of Nevirapine, the amino group and amide group of Vesatolimod, and the amide group of Bictegravir.

Keywords: calixarenes; antiviral drugs; supramolecular complexes; chromatography; molecular modeling

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Дослідження комплексоутворення калікс[4]арен-гідроксиметилфосфонової кислоти та калікс[4]арен-гідроксиметилдиметилфосфіноксиду з антивірусними препаратами

Анотація

Методами ОФ ВЕРХ у водно-органічній рухомій фазі на колонці Zorbax CN досліджено комплексоутворення конусоподібних калікс[4]арен-гідроксиметилфосфонової кислоти (**CPA**) та калікс[4]арен-гідроксиметилдиметилфосфіноксиду (**CPO**) з активними фармацевтичними інгредієнтами антивірусних препаратів Ремдесивір, Невірапін, Весатолімод та Біктегравір. Аналізом залежності значень ємності препаратів від концентрації каліксарену в рухомій фазі визначено константи стійкості утворених супрамолекулярних комплексів (K_A = 3672 – 6884 M^{-1}). Квантово-хімічні розрахунки доводять, що досліджені препарати утворюють з молекулами **CPA** та **CPO** супрамолекулярні екзокомплекси. Ці комплекси стабілізовані міжмолекулярними водневими зв'язками протонодонорних груп $P(O)(OH)_2$ **CPA** та протоноакцепторних груп $Me_2P{=}O$ **CPO** з аміногрупою Ремдесивіру, амідною групою Невірапіну, аміногрупою та амідною групою Весатилімоду, амідною групою Біктегравіру.

Ключові слова: каліксарени; антивірусні препарати; супрамолекулярні комплекси; хроматографія; молекулярне моделювання

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■ Introduction

Cup-shaped calixarenes [1, 2] and their self-assembled supramolecular aggregates [3], forming host-guest supramolecular complexes with active pharmaceutical ingredients (APIs) of drugs are considered as promising objects in design of the drug delivery vectors [4–11]. Advantages of the calixarene vectors are low cytotoxicity [12–16] and the absence of immune reactions [17]. Among the variety of calixarenes, water-soluble calixarene sulfonic acids are the best studied vectors [18]. They are capable of forming supramolecular complexes with the known APIs – 3-phenyl-1*H*[1]benzofuro[3,2-*c*]pyrazole (tyrosine kinase III inhibitor) [19], Carvediol (treatment of hypertension) [20], Paclitaxel (ovarian, breast, lung and colon cancer treatment) [21], Tramadol (analgesic) [22], Irinotecan (colon cancer treatment) [23], Nifedipine (calcium channel blocker) [24], Tenofovir (antiretroviral drug) [25].

The water-soluble cup-shaped calixarenes functionalized on the upper or lower rim of the macrocycle with hydrophilic organophosphorus groups are also used in the creation of drug delivery systems. It should be noted that phosphorus is a biologically friendly element, and a number of drugs for medicine have been created on the basis of natural and synthetic organophosphorus compounds [26, 27].

Phosphorus-containing calixarenes are characterized by a high biological activity and low cytotoxicity [28–30]. The calixarene and thiacalixarene-phosphonic acids effectively and selectively inhibit ATP-hydrolase systems of smooth muscle cells [31] and therapeutically important phosphatases of various origins [32–36]. It has been shown that the lower-rim calixarene-diphosphoric acid, which forms supramolecular complexes with water-insoluble APIs in aqueous solutions, is appropriate for drug formulation and delivery [37]. This acid also activates the transfer of polyarginine cell-penetrating peptides through biological membranes [38].

The upper rim modification of the cup-shaped calix[4]arene with hydrophilic phosphine oxide groups and phosphonic or phosphinic acid groups yielded water-soluble derivatives that form supramolecular complexes with APIs of 5-Fluorouracil and 5-Methyluracil anticancer drugs [39] as well as with antiretroviral drugs Tenofovir and Emtricitabin [40]. Stability constants of the complexes were determined by the HPLC method in the aqueous-organic medium. The most favored structures of the calixarene complexes with the APIs were optimized at the DFT level of approximation. In the most favored structures, APIs coordinate *via* hydrogen bonding with the phosphorus groups at the upper rim of the calixarene ligands.

Micelles of amphiphilic alkoxycalixarene-methylphosphonic acid form three-component nanoparticles with the antitumor drugs Carboplatin and Taxol in the aqueous medium [41]. In such a three-component nanocomplex, Carboplatin enters the molecular cavity of calixarene, and Taxol is located among the alkyl substituents of the micellar structure. These nanocomposites showed higher cytotoxicity compared to a simple mixture of the two drugs on HT-29 and Caco-2 colon tumor cells.

Micellar alkoxycalixarene-hydroxymethylene-bisphosphonic acids form nanoscale supramolecular complexes with fluorescently labeled polylysine and HIV-1 nucleocapsid due to electrostatic interactions. Such nanocomplexes cross biological membranes and deliver the therapeutically important proteins into cells [42].

In this article, within the context of further research on the drug formulation and delivery, the host-guest complexation of the cup-shaped calix[4]arene-*bis*-hydroxymethylphosphonic acid **CPA** and calix[4]arene-*bis*-hydroxymethyl-dimethylphosphine oxide **CPO** with the antiviral drugs Nevirapine, Remdesivir, Vesatolimod, and Bictegravir (**Figure 1**) in the aqueous-organic medium was studied using RP HPLC and molecular modeling methods.

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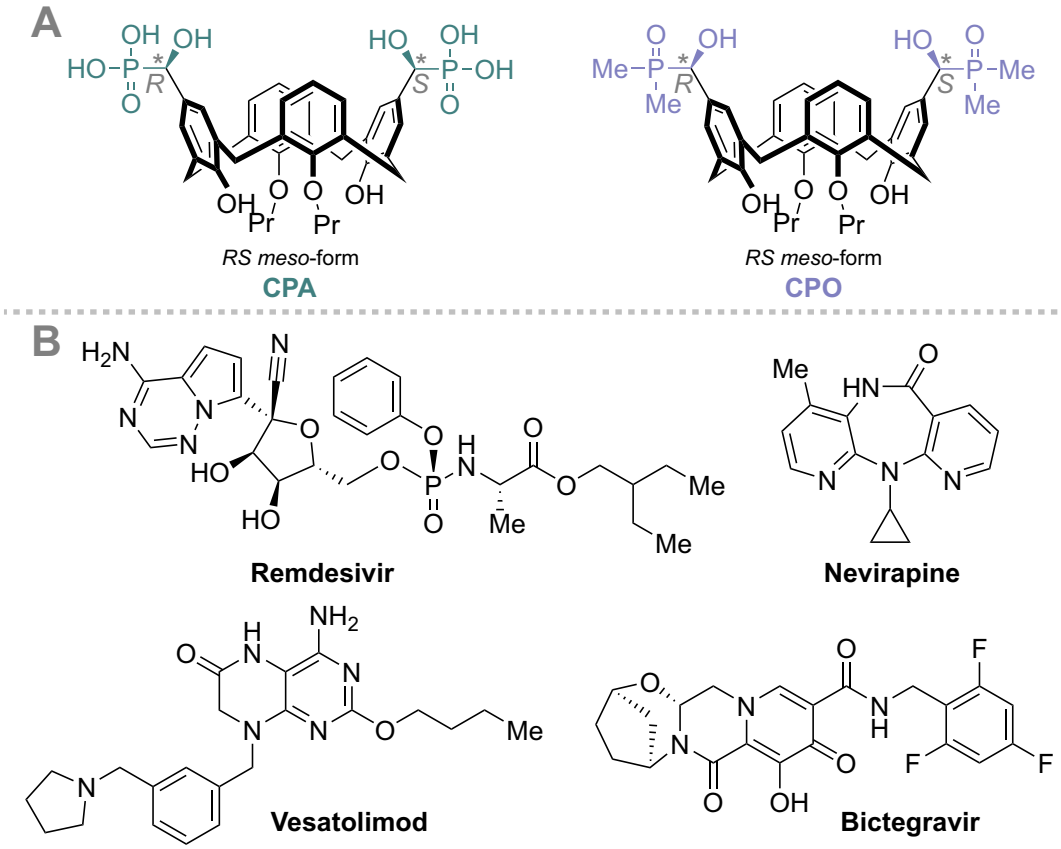

Figure 1. Calixarene-hydroxymethylphosphonic acid **CPA** (*RS meso*-form) and calixarene-hydroxymethyldimethylphosphine oxide **CPO** (*RS meso*-form) (hosts, *A*); and antiviral drugs Remdesivir, Nevirapine, Vesatolimod, Bictegravir (guests, *B*)

■ Materials and methods

Reagents and Materials

Calixarenes **CPA** and **CPO** were synthesized according to the methods [43, 44], respectively. Nevirapine, Vesatolimod and Bictegravir were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Abcam (Cambridge, UK). Remdesivir was obtained from UOSLAB (Kyiv, Ukraine).

HPLC analysis

The RP HPLC analysis of **CPA**, **CPO**, and antiviral drugs was performed on a Hitachi high-pressure liquid chromatography equipment (Hitachi, Ltd., Tokyo, Japan) under isocratic conditions using a Zorbax CN chromatographic column (250 × 4.6 mm) (supplier – Agilent) and the mobile phase of MeCN/H_2O/MeOH (79:20:1 by volume). The choice of a three-component mobile phase was driven by the need to solubilize calixarenes and antiretroviral drugs of different natures simultaneously. The concentration of **CPA** and **CPO** in the mobile phase varied within the range of 0.1×10^{-4}–1.4×10^{-4} M. Samples of the antiviral drugs for analysis were prepared in a solvent identical to the mobile phase ($C = 1\times10^{-5}$ M) and injected in amounts of 20 µL. All chromatograms were obtained at 28 °C. The UV detector wavelength was 254 nm. Each sample was analyzed in triplicate.

Molecular modeling

CPA and **CPO**, as well as their complexes with antiviral drugs, were simulated in vacuum (PM3, software package – evaluation version 8.0.10 of Hyper Chem program) [45]. The RMS gradient was 0.01 kcal mol^{-1}.

■ Results and discussion

Antiviral drugs are widely used to prevent and treat many infectious diseases [46, 47]. However, such drugs may have low bioavailability and cause side effects [48, 49]. Therefore, in some cases they are used in the form of prodrugs or supramolecular complexes with cyclodextrins. To search supramolecular complexing agents for Remdesivir, Nevirapine, Vesatolimod, and Bictegravir, we studied water-soluble calixarenes **CPA** possessing anionic $P(O)(OH)_2$ groups and calixarene **CPO** possessing neutral $Me_2P{=}O$ groups.

It is known that the highly polar $Me_2P{=}O$ group [50] is currently used in medicinal chemistry to improve the water solubility of drug molecules and optimize their pharmacokinetic profile [27, 51]. It was taken into account that the proton-accepting property of the oxygen atom of dimethylphosphine oxide derivatives exceeded the proton-accepting property of the oxygen atoms

of phosphates, phosphonates, sulfones, and carbonyl compounds [52]. A clear example of the effective use of the dimethylphosphine oxide group in medicinal chemistry is the recent creation of the drug Brigatinib [53, 54] and a number of clinical candidates [55]. The proton acceptor group $Me_2P(O)$ determines water solubility and ensures an effective interaction of Brigatinib with the biological target through the formation of strong hydrogen bonds. As a result, the medicinal efficacy of Brigatinib increases by 70 times compared to the corresponding non-phosphorylated analog.

The presence of hydrophilic proton-donating $P(O)(OH)_2$ or proton-accepting $Me_2P{=}O$ groups on the upper rim of **CPA** and **CPO** determines the water solubility of calixarenes. On the other hand, these groups stabilize supramolecular host-guest complexes by forming intermolecular hydrogen bonds P-OH···X or P=O···H-X (X = O, N) with amine, amide, hydroxyl, and other groups of the antiviral drugs.

The main criterion for assessing the complexing properties of a host molecule is the value of the stability constant of the supramolecular complex with a guest molecule. To determine the stability constants of the calixarene complexes, various physical methods are used: microcalorimetry [56], nuclear magnetic resonance [57], UV and fluorescence spectroscopy [58–60], selective transport through liquid membranes [61], mass spectrometry [62, 63], surface plasmon resonance [64], etc. However, the application of these methods may be limited by the unsatisfactory solubility of calixarene receptors or substrate molecules, or the high cost of the methods.

A convenient and rapid method for determining the stability constants of complexes is reversed-phase high-performance liquid chromatography [65, 66]. This method has been used to determine the stability constants of calixarene complexes with organic substrates of various natures in aqueous or aqueous-organic solutions [67–70]. According to this method, stability constants are determined from the dependence of the substrate's retention time or capacity factor on the calixarene concentration in the mobile phase. The addition of calixarenes to the mobile phase reduces the retention time of analytes due to the formation of supramolecular host-guest complexes and the increased polarity of the chromatographic column surface upon calixarene sorption. The inverse sorption of calixarenes by the column surface and the linear dependence of the capacity value $1/k'$ of the analyte on the calixarene concentration indicate 1:1 stoichiometry complexes in the mobile phase flow. This allows to use equation (1) [68, 69] to calculate the stability constants of the host-guest complexes:

$$1 / k' = 1 / k_0' + K_A \times [CA] / k_0' \qquad (1)$$

where k_0' and k' are the capacity factors of the analyte determined before and after the addition of calixarene to the mobile phase; [*CA*] is the concentration of calixarene in the mobile phase.

Under the analysis conditions, antiviral drugs and calixarenes have retention times t_R: 6.735 min (Remdesivir), 6.915 min (Nevirapine), 7.327 min (Vesatolimod), 5.873 (Bictegravir), 7.382 min (**CPA**), and 6.958 min (**CPO**) (**Figure 2**).

Calixarenes **CPA** and **CPO** were characterized by linear adsorption isotherms (**Figures 3** and **4**), which indicated their reverse adsorption on the surface of the Zorbax CN column.

The addition of calixarenes to the mobile phase reduces the retention time t_R of antiviral drugs. The linear nature of the dependences of their parameters $1/k'$ on the concentration of calixarenes in the mobile phase (**Figures 5** and **6**) indicates the formation of supramolecular host-guest complexes with a stoichiometry of 1:1.

The stability constants of the complexes K_A (3672–6884 M^{-1}) calculated by formula (1), and the values of the Gibbs free energies $-\Delta G$ (4.852–5.224 $kJ \cdot mol^{-1}$) calculated by the equation $\Delta G = -RT \times \ln K_A$ are given in **Table 1**. The stability constants K_A are rather close to the stability constant of the 1:1 complex of the calix[4]arene-sulfonic acid with the antiretroviral drug Tenofovir disoproxil fumarate determined by UV/Vis spectroscopy in the DMSO solution [42]. This calix[4]-arene-sulfonic acid complex was further studied for antimicrobial applications against methicillin resistant *Staphylococcus aureus* (MRSA).

The values of the stability constants K_A and Gibbs free energies ΔG depend on the structure of the calixarene host and the antiviral drug guest and increase in the following order

Bictegravir < Vesatolimod <
< Nevirapine < Remdesivir

for complexes with CPA, and

Nevirapine < Vesatolimod <
< Remdesivir < Bictegravir

for complexes with CPO.

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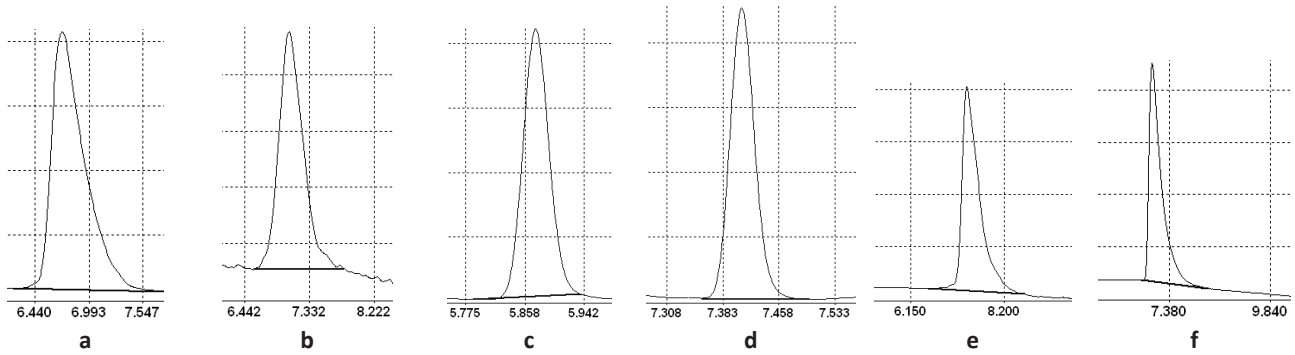

Figure 2. Chromatograms of Remdesivir (a), Nevirapine (b), Bictegravir (c), Vesatolimod (d), **CPA** (e), **CPO** (f)

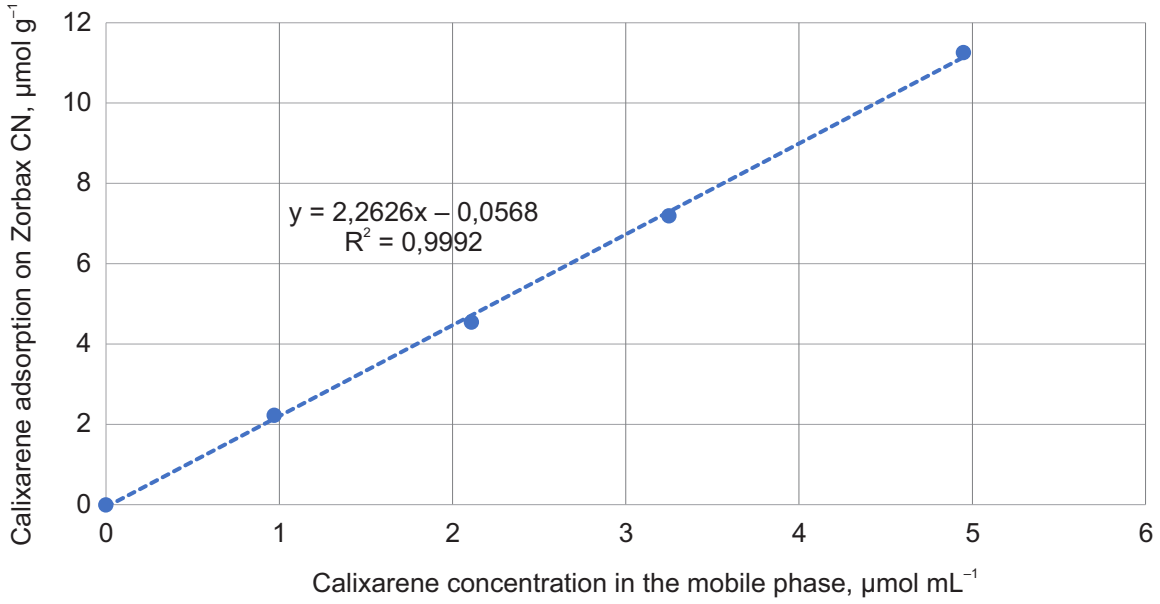

Figure 3. The adsorption isotherm of calixarene **CPA**

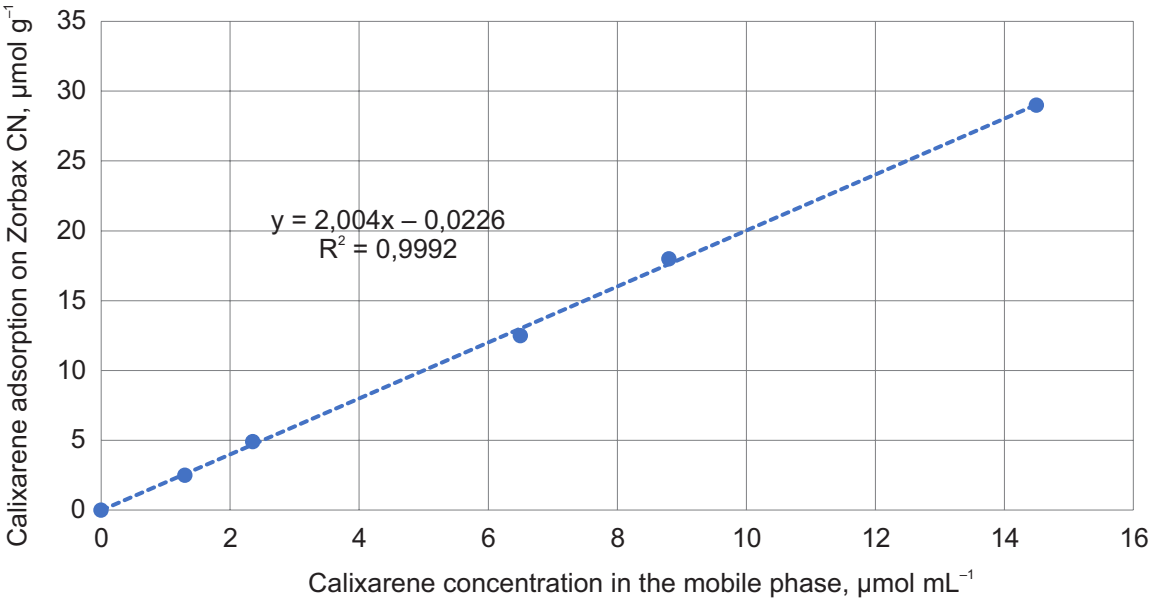

Figure 4. The adsorption isotherm of calixarene **CPO**

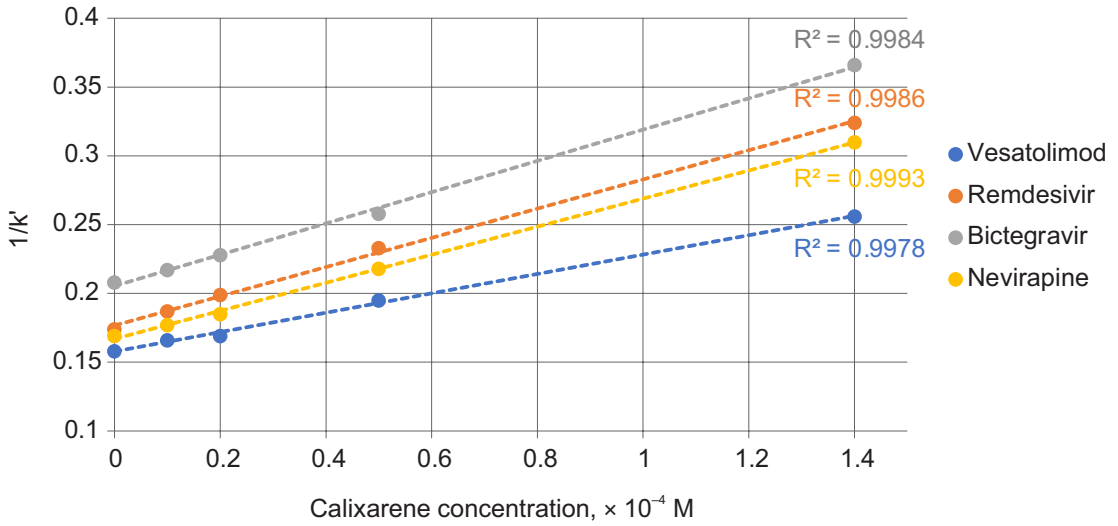

Figure 5. The dependence of 1/*k'* values of the antiviral drugs on the concentration of **CPA** in the mobile phase

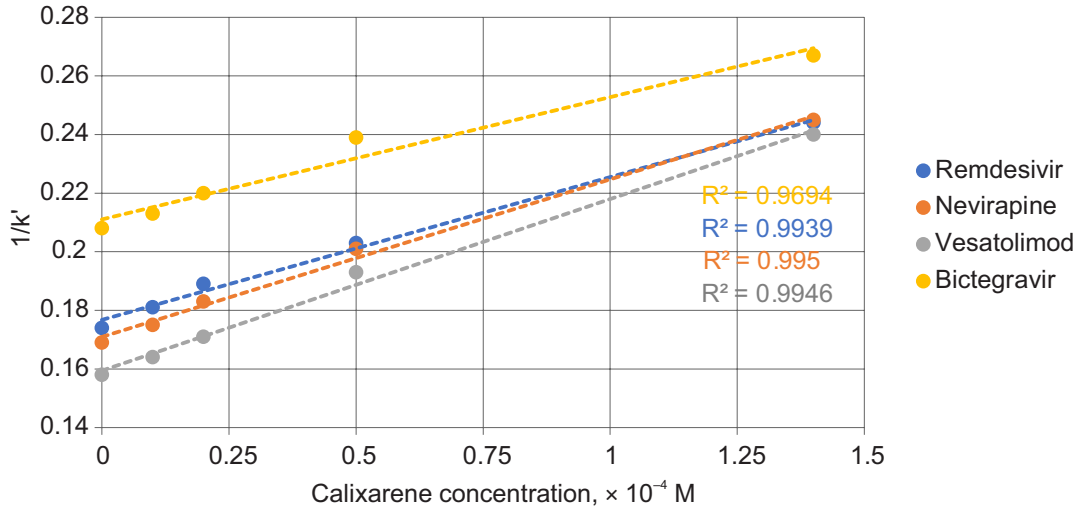

Figure 6. The dependence of 1/*k'* values of the antiviral drugs on the concentration of **CPO** in the mobile phase (R^2=0.99)

Table 1. Stability constants K_A and Gibbs free energies ΔG of the complexes of **CPA** and **CPO** with antiviral drugs

Antiviral drug	CPA		CPO	
	K_A, M^{-1} (RSD, %)	ΔG, kJ·mol^{-1}	K_A, M^{-1} (RSD, %)	ΔG, kJ·mol^{-1}
Remdesivir	6884 (19)	5.224	4695 (15)	4.998
Nevirapine	5305 (27)	5.070	3672 (25)	4.852
Vesatolimod	4875 (21)	5.020	4015 (18)	4.905
Bictegravir	4850 (20)	5.017	5328 (27)	5.072

The dependence of the stability constants on the structure of the calixarene and the antiviral agent is complicated and can be determined by hydrogen bonds, van der Waals forces, solvatophobic and other non-covalent interactions.

To understand the nature of complex formation, the structures of calixarenes and their complexes with molecules of antiviral agents were energetically minimized. According to energy minimization data, **CPA** and **CPO** molecules exist in a *flattened cone* conformation, in which the phosphorylated benzene rings are oriented "coplanarly" and the unsubstituted rings are oriented "perpendicularly" to the main plane of the molecule formed by the methylene groups of the macrocyclic backbone. This conformation is stabilized by two intramolecular hydrogen bonds Ar-OH···O-Alk on the lower rim of the macrocycle (**Figure 7**).

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Figure 7. Energy-minimized molecular structures of **CPA** (a) and **CPO** (b)

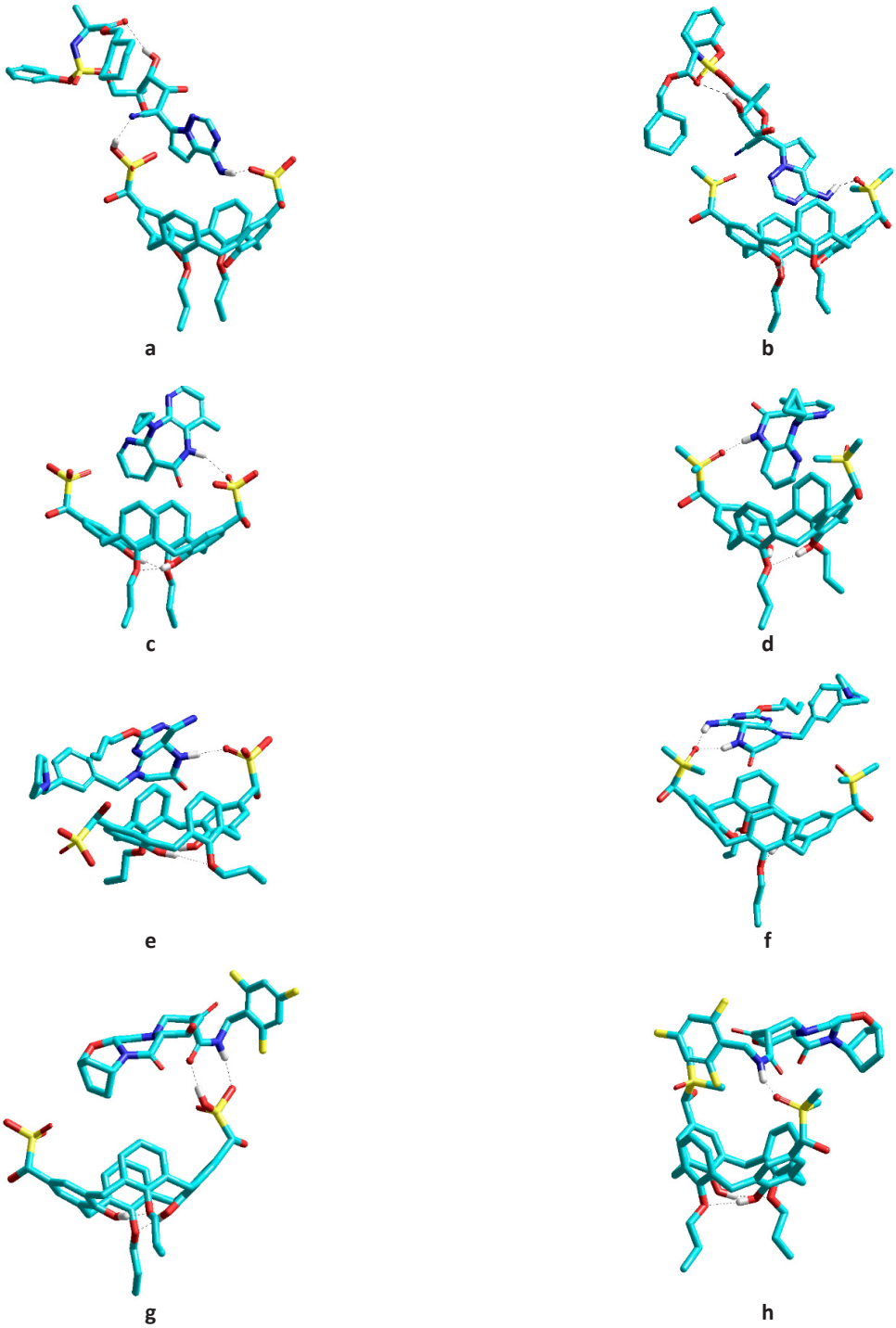

Figure 8. Energy-minimized molecular structures of supramolecular exo-complexes CPA@Remdesivir (a), CPO@Remdesivir (b), CPA@Nevirapine (c), CPO@Nevirapine (d), CPA@Vesatolimod (e), CPO@Vesatolimod (f) CPA@Bictegravir (g), CPO@Bictegravir (h)

According to the calculations, calixarenes form *exo*-complexes with large and branched molecules Remdesivir, Nevirapine, Vesatolimod, and Bictegravir, which "hang" over the upper rim of the macrocycle (**Figure 8**). The complexes are stabilized by intermolecular hydrogen bonds of proton donor groups $P(O)(OH)_2$ of **CPA** and proton acceptor groups $Me_2P{=}O$ of **CPO** with the amino group and nitrile group of Remdesivir, the amide group of Nevirapine, the amino group and amide group of Vesatilimod, and the amide group of Bictegravir.

It should be noted that among the drugs studied, the most compact Nevirapine molecule is directed by a nonmethylated pyridine fragment into the lipophilic macrocyclic cavity of calixarenes (**Figures 8**c,d). In this case, the complexes, in addition to hydrogen bonds of the amide group of Nevirapine with the phosphorus groups of **CPA** and **CPO**, can also be stabilized by van der Waals forces, or solvatophobic interactions.

■ Conclusions

The value of stability constants K_A (3672–6884 M^{-1}) of supramolecular host-guest complexes between water-soluble calix[4]arenehydroxymethylphosphonic acid **CPA** and calix[4]arenehydroxymethyldimethylphosphine oxide **CPO** with active pharmaceutical ingredients of antiviral drugs Remdesivir, Nevirapine, Vesatolimod and Bictegravir depends on the structures of the calixarene and the antiviral drug and increases in the following order

Bictegravir < Vesatolimod <
< Nevirapine < Remdesivir

for complexes with CPA and

Nevirapine < Vesatolimod <
< Remdesivir < Bictegravir

for complexes with CPO.

According to energy minimized data, the **CPA** and **CPO** molecules exist in a *flattened cone* conformation, which is suitable for the host-guest complexation and in which the phosphorylated benzene rings are oriented "coplanarly", while the unsubstituted rings are oriented "perpendicularly" to the main plane of the molecule formed by the methylene groups of the macrocyclic backbone. According to the molecular modeling, calixarenes form *exo*-complexes with large and branched molecules of antiviral drugs, which "hang" over the upper rim of the macrocycle. The complexes are stabilized by intermolecular hydrogen bonds of proton donor groups $P(O)(OH)_2$ of **CPA** and proton acceptor groups $Me_2P{=}O$ of **CPO** with the amino group of Remdesivir, the amide group of Nevirapine, the amino group and amide group of Vesatilimod, and the amide group of Bictegravir.

Thus, the synthetically available water-soluble anionic or neutral calixarenes **CPA** and **CPO** possessing wide possibilities of chemical modification have the prospect of application in formulations of the antiviral drugs and the creation of vectors for their delivery systems.

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■ References

1. *Calixarenes and Beyond*; Neri, P.; Sessler, J. L.; Wang, M.-X., Eds.; Springer International Publishing: Cham, 2016. https://doi.org/10.1007/978-3-319-31867-7.
2. *Calixarenes in the Nanoworld*; Vicens, J.; Harrowfield, J.; Baklouti, L., Eds.; Springer Netherlands, 2007. https://doi.org/10.1007/978-1-4020-5022-4.
3. Liu, Z.; Dai, X.; Sun, Y.; Liu, Y. Organic Supramolecular Aggregates Based on Water-soluble Cyclodextrins and Calixarenes. *Aggregate* **2020**, *1* (1), 31–44. https://doi.org/10.1002/agt2.3.
4. Español, E. S.; Villamil, M. M. Calixarenes: Generalities and Their Role in Improving the Solubility, Biocompatibility, Stability, Bioavailability, Detection, and Transport of Biomolecules. *Biomolecules* **2019**, *9* (3), 90. https://doi.org/10.3390/biom9030090.
5. Rodik, R. V.; Boyko, V. I.; Kalchenko, V. I. Calixarenes in Biotechnology and Bio-Medical Research. In *Frontiers in Medicinal Chemistry*; Atta-ur-Rahman; Choudhary, M. I.; Reitz, A. B., Eds.; Bentham Science Publishers, 2016; pp 206–301. https://doi.org/10.2174/9781681081755116080008.
6. Pan, Y.; Hu, X.; Guo, D. Biomedical Applications of Calixarenes: State of the Art and Perspectives. *Angew. Chem. Int. Ed.* **2021**, *60* (6), 2768–2794. https://doi.org/10.1002/anie.201916380.
7. Fan, X.; Guo, X. Development of Calixarene-Based Drug Nanocarriers. *Journal of Molecular Liquids* **2021**, *325*, 115246. https://doi.org/10.1016/j.molliq.2020.115246.
8. Bai, H.; Wang, J.; Li, Z.; Tang, G. Macrocyclic Compounds for Drug and Gene Delivery in Immune-Modulating Therapy. *IJMS* **2019**, *20* (9), 2097. https://doi.org/10.3390/ijms20092097.
9. Baldini, L.; Casnati, A.; Sansone, F. Multivalent and Multifunctional Calixarenes in Bionanotechnology. *Eur. J. Org. Chem.* **2020**, *2020* (32), 5056–5069. https://doi.org/10.1002/ejoc.202000255.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

10. Ostos, F. J.; Lebrón, J. A.; López-Cornejo, P.; López-López, M.; García-Calderón, M.; García-Calderón, C. B.; Rosado, I. V.; Kalchenko, V. I.; Rodik, R. V.; Moyá, M. L. Self-Aggregation in Aqueous Solution of Amphiphilic Cationic Calix[4]Arenes. Potential Use as Vectors and Nanocarriers. *Journal of Molecular Liquids* **2020**, *304*, 112724. https://doi.org/10.1016/j.molliq.2020.112724.
11. Lebrón, J. A.; López-López, M.; García-Calderón, C. B.; V. Rosado, I.; Balestra, F. R.; Huertas, P.; Rodik, R. V.; Kalchenko, V. I.; Bernal, E.; Moyá, M. L.; López-Cornejo, P.; Ostos, F. J. Multivalent Calixarene-Based Liposomes as Platforms for Gene and Drug Delivery. *Pharmaceutics* **2021**, *13* (8), 1250. https://doi.org/10.3390/pharmaceutics13081250.
12. Perret, F.; Mazzorana, M.; Shahgaldian, P.; Coleman, A. W. First step in the study of the cellular toxicity of the calixarene. International Symposium on Supramolecular Chemistry XI, Fukuoka, 2000; PB-26, 387.
13. Da Silva, E.; Shahgaldian, P.; Coleman, A. W. Haemolytic Properties of Some Water-Soluble Para-Sulphonato-Calix-[n]-Arenes. *International Journal of Pharmaceutics* **2004**, *273* (1–2), 57–62. https://doi.org/10.1016/j.ijpharm.2003.12.008.
14. Coleman, A. W.; Jebors, S.; Cecillon, S.; Perret, P.; Garin, D.; Marti-Battle, D.; Moulin, M. Toxicity and Biodistribution of Para-Sulfonato-Calix[4]Arene in Mice. *New J. Chem.* **2008**, *32* (5), 780. https://doi.org/10.1039/b718962a.
15. Chernyshenko, V. O.; Korolova, D. S.; Nikolaienko, T. V.; Dosenko, V. E.; Pashevin, D. O.; Kalchenko, V. I.; Cherenok, S. O.; Khranovska, N. N.; Garmanchuk, L. V.; Lugovskoy, E. V.; Komisarenko, S. V. Calix[4]arene C-145 Effects on Cellular Haemostasis. *Biotechnologia acta* **2016**, *9* (3), 37–43. https://doi.org/10.15407/biotech9.03.037.
16. Shahgaldian, P.; Da Silva, E.; Coleman, A. W. A First Approach to the Study of Calixarene Solid Lipid Nanoparticle (SLN) Toxicity. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2003**, *46* (3/4), 175–177. https://doi.org/10.1023/A:1026301906487.
17. Dings, R. P. M.; Chen, X.; Hellebrekers, D. M. E. I.; Van Eijk, L. I.; Zhang, Y.; Hoye, T. R.; Griffioen, A. W.; Mayo, K. H. Design of Nonpeptidic Topomimetics of Antiangiogenic Proteins with Antitumor Activities. *JNCI: Journal of the National Cancer Institute* **2006**, *98* (13), 932–936. https://doi.org/10.1093/jnci/djj247.
18. Perret, F.; Lazar, A. N.; Coleman, A. W. Biochemistry of the Para-Sulfonato-Calix[n]Arenes. *Chem. Commun.* **2006**, *2006* (23), 2425–2438. https://doi.org/10.1039/b600720c.
19. Galindo-Murillo, R.; Olmedo-Romero, A.; Cruz-Flores, E.; Petrar, P. M.; Kunsagi-Mate, S.; Barroso-Flores, J. Calix[n]Arene-Based Drug Carriers: A DFT Study of Their Electronic Interactions with a Chemotherapeutic Agent Used against Leukemia. *Computational and Theoretical Chemistry* **2014**, *1035*, 84–91. https://doi.org/10.1016/j.comptc.2014.03.001.
20. Menon, S. K.; Mistry, B. R.; Joshi, K. V.; Modi, N. R.; Shashtri, D. Evaluation and Solubility Improvement of Carvedilol: PSC[n]Arene Inclusion Complexes with Acute Oral Toxicity Studies. *J. Incl. Phenom. Macrocycl. Chem.* **2012**, *73* (1–4), 295–303. https://doi.org/10.1007/s10847-011-0056-x.
21. Chen, M.-X.; Li, T.; Peng, S.; Tao, D. Supramolecular Nanocapsules from the Self-Assembly of Amphiphilic Calixarene as a Carrier for Paclitaxel. *New J. Chem.* **2016**, *40* (12), 9923–9929. https://doi.org/10.1039/C6NJ01986B.
22. Zhao, Q.-H.; Chen, C.-P.; Pang, T.-T.; Du, L.-M.; Li, Y.; Xie, J.-H.; Fu, Y.-L. Investigated the Supramolecular Interaction of Tramadol Hydrochloride with P-Sulfonated Calix[4,6,8]Arene. *J. Chil. Chem. Soc.* **2015**, *60* (4), 2659–2663. https://doi.org/10.4067/S0717-97072015000400007.
23. Wang, Y.-X.; Guo, D.-S.; Duan, Y.-C.; Wang, Y.-J.; Liu, Y. Amphiphilic P-Sulfonatocalix[4]Arene as "Drug Chaperone" for Escorting Anticancer Drugs. *Sci. Rep.* **2015**, *5* (1), 9019. https://doi.org/10.1038/srep09019.
24. Yang, W.; De Villiers, M. M. The Solubilization of the Poorly Water Soluble Drug Nifedipine by Water Soluble 4-Sulphonic Calix[n]Arenes. *European Journal of Pharmaceutics and Biopharmaceutics* **2004**, *58* (3), 629–636. https://doi.org/10.1016/j.ejpb.2004.04.010.
25. Jarange, A. B.; Patil, S. V.; Malkhede, D. D.; Deodhar, S. M.; Nandre, V. S.; Athare, S. V.; Kodam, K. M.; Gejji, S. P. P-Sulfonatocalixarene versus p-Thiasulfonatocalixarene: Encapsulation of Tenofovir Disoproxil Fumarate and Implications to ESI-MS, HPLC, NMR, DFT and Anti-MRSA Activities. *J. Incl. Phenom. Macrocycl. Chem.* **2021**, *99* (1-2), 43-59. https://doi.org/10.1007/s10847-020-01022-w.
26. *Organophosphorus Chemistry: Novel Developments*; Keglevich, G., Ed.; De Gruyter, 2018. https://doi.org/10.1515/9783110535839.
27. Finkbeiner, P.; Hehn, J. P.; Gnamm, C. Phosphine Oxides from a Medicinal Chemist's Perspective: Physicochemical and *in Vitro* Parameters Relevant for Drug Discovery. *J. Med. Chem.* **2020**, *63* (13), 7081–7107. https://doi.org/10.1021/acs.jmedchem.0c00407.
28. Komisarenko, S. V.; Kosterin, S. O.; Lugovskoy, E. V.; Cherenok, S. O.; Tanchuk, V. Yu.; Vovk, A. I.; Kalchenko, V. I. Synthesis and characterization of calixarene methylene bisphosphonic acids as effectors of biochemical processes. In *Ligands: synthesis, characterization and role in biotechnology*; Gawryszewska, P.; Smolenski, P., Eds.; Nova Science Publishers, Inc: New York, 2014; Chapter 3, 67-116.
29. Labyntseva, R. D.; Yavorovska, V. I.; Bevza, O. V.; Kalchenko, V. I.; Kosterin, S. O. Calix[4]arenes as the effectors of smooth muscle myosin ATPase. In *Myosin: biosynthesis, classes and function*; Broadbent, D., Ed. Nova Science Publishers, Inc.: New York, 2018; Chapter 2, 89-135.
30. Tauran, Y.; Coleman, A.; Perret, F.; Kim, B. Cellular and in Vivo Biological Activities of the Calix[n]Arenes. *Current Organic Chemistry* **2015**, *19* (23), 2250–2270. https://doi.org/10.2174/1385272819666150608222114.
31. Kosterin, S. O.; Kalchenko, V. I.; Veklich, T. O.; Babich, L. G.; Shlikov, S. G. *Calix[4]arenes as modulators of ATP-hydrolase systems of smooth muscle cells*. Naukova Dumka: Kyiv, 2019.
32. Trush, V. V.; Cherenok, S. O.; Tanchuk, V. Yu.; Kukhar, V. P.; Kalchenko, V. I.; Vovk, A. I. Calix[4]Arene Methylenebisphosphonic Acids as Inhibitors of Protein Tyrosine Phosphatase 1B. *Bioorganic & Medicinal Chemistry Letters* **2013**, *23* (20), 5619–5623. https://doi.org/10.1016/j.bmcl.2013.08.040.
33. Vovk, A. I.; Tanchuk, V. Yu.; Kononets, L. A.; Cherenok, S. O.; Drapailo, A. B.; Kalchenko, V. I.; Kukhar, V. P. A Novel Approach to the Design of Phosphonate Inhibitors of Protein Tyrosine Phosphatase. *Phosphorus, Sulfur, and Silicon and the Related Elements* **2011**, *186* (4), 958–960. https://doi.org/10.1080/10426507.2010.521213.
34. 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. *Bioorganic & Medicinal Chemistry Letters* **2010**, *20* (2), 483–487. https://doi.org/10.1016/j.bmcl.2009.11.126.
35. Vovk, A. I.; Mischenko, I. M.; Cherenok, S. O.; Tanchuk, V. Yu.; Kalchenko, V. I.; Kukhar, V. P. Phosphorylated Calix[4]Arenes as Inhibitors of Glutathione S-Transferase. *Phosphorus, Sulfur, and Silicon and the Related Elements* **2011**, *186* (4), 961–963. https://doi.org/10.1080/10426507.2010.514313.
36. Trush, V. V.; Kharchenko, S. G.; Tanchuk, V. Yu.; Kalchenko, V. I.; Vovk, A. I. Phosphonate Monoesters on a Thiacalix[4]Arene Framework as Potential Inhibitors of Protein Tyrosine Phosphatase 1B. *Org. Biomol. Chem.* **2015**, *13* (33), 8803–8806. https://doi.org/10.1039/C5OB01247C.
37. Bayrakci, M.; Ertul, Ş.; Yilmaz, M. Phase Solubility Studies of Poorly Soluble Drug Molecules by Using *O*-Phosphorylated Calixarenes as Drug-Solubilizing Agents. *J. Chem. Eng. Data* **2012**, *57* (1), 233–239. https://doi.org/10.1021/je200992c.
38. Perret, F.; Nishihara, M.; Takeuchi, T.; Futaki, S.; Lazar, A. N.; Coleman, A. W.; Sakai, N.; Matile, S. Anionic Fullerenes, Calixarenes, Coronenes, and Pyrenes as Activators of Oligo/Polyarginines in Model Membranes and Live Cells. *JACS* **2005**, *127* (4), 1114–1115. https://doi.org/10.1021/ja043633c.

39. Kalchenko, O. I.; Rozhenko, A. B.; Cherenok, S. O.; Selikhova, A. I.; Suikov, S. Yu.; Kyrylchuk, A. A.; Kalchenko, V. I. Complexation of Water-Soluble Phosphorylated Calixarenes with Uracils. Stability Constants and DFT Study of the Supramolecular Complexes. *J. Incl. Phenom. Macrocycl. Chem.* **2023**, *103* (9–10), 369–383. https://doi.org/10.1007/s10847-023-01198-x.
40. Kalchenko, O. I.; Rozhenko, A. B.; Cherenok, S. O.; Selikhova, A. I.; Drapailo, A. B.; López-Cornejo, P.; Kalchenko, V. I. Stability Constants and DFT Calculations of Anionic Calixarene Complexes with Antiretroviral Drugs Tenofovir and Emtricitabine. *J. Incl. Phenom. Macrocycl. Chem.* **2025**, *105* (5–6), 297–310. https://doi.org/10.1007/s10847-024-01273-x.
41. Li, M.; Mao, L.; Chen, M.; Li, M.; Wang, K.; Mo, J. Characterization of an Amphiphilic Phosphonated Calixarene Carrier Loaded with Carboplatin and Paclitaxel: A Preliminary Study to Treat Colon Cancer in Vitro and in Vivo. *Front. Bioeng. Biotechnol.* **2019**, *7*, 238. https://doi.org/10.3389/fbioe.2019.00238.
42. Rodik, R. V.; Cherenok, S. O.; Postupalenko, V. Y.; Oncul, S.; Brusianska, V.; Borysko, P.; Kalchenko, V. I.; Mely, Y.; Klymchenko, A. S. Anionic Amphiphilic Calixarenes for Peptide Assembly and Delivery. *Journal of Colloid and Interface Science* **2022**, *624*, 270–278. https://doi.org/10.1016/j.jcis.2022.05.124.
43. Cherenok, S. O.; Yushchenko, O. A.; Tanchuk, V. Yu.; Mischenko, I. M.; Samus, N. V.; Ruban, O. V.; Matvieiev, Y. I.; Karpenko, J. A.; Kukhar, V. P.; Vovk, A. I.; Kalchenko, V. I. Calix[4]Arene-α-Hydroxyphosphonic Acids. Synthesis, Stereochemistry, and Inhibition of Glutathione S-Transferase. *Arkivoc* **2012**, *2012* (4), 278–298. https://doi.org/10.3998/ark.5550190.0013.421.
44. Cherenok, S. O.; Shishkina, S. V.; Selikhova. A. I.; Drapailo, A. B.; Kalchenko, V. I. Synthesis and self-assembling of calix[4]arene-hydroxymethyl-dimethylphosphine oxides. Book of Abstracts, XXVI Ukrainian Conference on Organic and Bioorganic Chemistry, Uzhhorod, September 16–20, 2024, 41.
45. Hypercube Downloads http://www.hypercubeusa.com/Download/tabid/357/Default.aspx (accessed May 11, 2025).
46. Gupta, R. K.; Van De Vijver, D. A. M. C.; Manicklal, S.; Wainberg, M. A. Evolving Uses of Oral Reverse Transcriptase Inhibitors in the HIV-1 Epidemic: From Treatment to Prevention. *Retrovirology* **2013**, *10* (1), 82. https://doi.org/10.1186/1742-4690-10-82.
47. Arts, E. J.; Hazuda, D. J. HIV-1 Antiretroviral Drug Therapy. *Cold Spring Harbor Perspectives in Medicine* **2012**, *2* (4), a007161–a007161. https://doi.org/10.1101/cshperspect.a007161.
48. Margolis, A. M.; Heverling, H.; Pham, P. A.; Stolbach, A. A Review of the Toxicity of HIV Medications. *J. Med. Toxicol.* **2014**, *10* (1), 26–39. https://doi.org/10.1007/s13181-013-0325-8.
49. Paik, S. Fatal Nevirapine-Induced Toxic Epidermal Necrolysis in a HIV Infected Patient. *JCDR* **2016**. https://doi.org/10.7860/JCDR/2016/16360.7415.
50. Fedyk, A.; Slobodyanyuk, E. Y.; Stotska, O.; Vashchenko, B. V.; Volochnyuk, D. M.; Sibgatulin, D. A.; Tolmachev, A. A.; Grygorenko, O. O. Heteroaliphatic Dimethylphosphine Oxide Building Blocks: Synthesis and Physico-Chemical Properties. *Eur. J. Org. Chem.* **2021**, *2021* (47), 6591–6603. https://doi.org/10.1002/ejoc.202100581.
51. Fedyk, A. V.; Chalyk, B. A. Synthesis of Functionalized 4,5-Dihydroisoxazoles Decorated with the Dimethylphosphinoyl Group. *J. Org. Pharm. Chem.* **2023**, *21* (2), 41–52. https://doi.org/10.24959/ophcj.23.283988.
52. Laurence, C.; Brameld, K. A.; Graton, J.; Le Questel, J.-Y.; Renault, E. The p K_{BHX} Database: Toward a Better Understanding of Hydrogen-Bond Basicity for Medicinal Chemists. *J. Med. Chem.* **2009**, *52* (14), 4073–4086. https://doi.org/10.1021/jm801331y.
53. National Cancer Institute. FDA grants Brigatinib accelerated approval for metastatic non-small cell lung cancer. https://www.cancer.gov/news-events/cancer-currents-blog/2017/brigatinib-fda-lung-cancer (accessed Jun 23, 2025).
54. Uchibori, K.; Inase, N.; Araki, M.; Kamada, M.; Sato, S.; Okuno, Y.; Fujita, N.; Katayama, R. Brigatinib Combined with Anti-EGFR Antibody Overcomes Osimertinib Resistance in EGFR-Mutated Non-Small-Cell Lung Cancer. *Nat. Commun.* **2017**, *8* (1), 14768. https://doi.org/10.1038/ncomms14768.
55. Yu, H.; Yang, H.; Shi, E.; Tang, W. Development and Clinical Application of Phosphorus-Containing Drugs. *Medicine in Drug Discovery* **2020**, *8*, 100063. https://doi.org/10.1016/j.medidd.2020.100063.
56. Zielenkiewicz, W.; Marcinowicz, A.; Poznański, J.; Cherenok, S.; Kalchenko, V. Complexation of Isoleucine by Phosphorylated Calix[4]Arene in Methanol Followed by Calorimetry, NMR and UV–VIS Spectroscopies, and Molecular Modeling Methods. *Journal of Molecular Liquids* **2005**, *121* (1), 8–14. https://doi.org/10.1016/j.molliq.2004.08.031.
57. Yakovenko, A. V.; Boyko, V. I.; Kalchenko, V. I.; Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *N*- Linked Peptidocalix[4]Arene Bisureas as Enantioselective Receptors for Amino Acid Derivatives. *J. Org. Chem.* **2007**, *72* (9), 3223–3231. https://doi.org/10.1021/jo062410x.
58. Sansone, F.; Barboso, S.; Casnati, A.; Sciotto, D.; Ungaro, R. A New Chiral Rigid Cone Water Soluble Peptidocalix[4]Arene and Its Inclusion Complexes with α-Amino Acids and Aromatic Ammonium Cations. *Tetrahedron Letters* **1999**, *40* (25), 4741–4744. https://doi.org/10.1016/S0040-4039(99)00838-2.
59. Acharya, A.; Ramanujam, B.; Chinta, J. P.; Rao, C. P. 1,3-Diamido-Calix[4]Arene Conjugates of Amino Acids: Recognition of −COOH Side Chain Present in Amino Acids, Peptides, and Proteins by Experimental and Computational Studies. *J. Org. Chem.* **2011**, *76* (1), 127–137. https://doi.org/10.1021/jo101759f.
60. Li, W. Y.; Li, H.; Zhang, G. M.; Chao, J. B.; Ling, L. X.; Shuang, S. M.; Dong, C. Interaction of Water-Soluble Calix[4]Arene with l-Tryptophan Studied by Fluorescence Spectroscopy. *Journal of Photochemistry and Photobiology A: Chemistry* **2008**, *197* (2–3), 389–393. https://doi.org/10.1016/j.jphotochem.2008.02.002.
61. Kim, L.; Hamdi, A.; Stancu, A. D.; Souane, R.; Mutihac, L.; Vicens, J. Selective Membrane Transport of Amino Acids by Functionalised Calix[4]Arenes. *J. Incl. Phenom. Macrocycl. Chem.* **2010**, *66* (1–2), 55–59. https://doi.org/10.1007/s10847-009-9622-x.
62. Durmaz, M.; Alpaydin, S.; Sirit, A.; Yilmaz, M. Enantiomeric Recognition of Amino Acid Derivatives by Chiral Schiff Bases of Calix[4]Arene. *Tetrahedron: Asymmetry* **2007**, *18* (7), 900–905. https://doi.org/10.1016/j.tetasy.2007.04.001.
63. Stone, M. M.; Franz, A. H.; Lebrilla, C. B. Non-Covalent Calixarene-Amino Acid Complexes Formed by MALDI-MS. *J. Am. Soc. Mass Spectrom.* **2002**, *13* (8), 964–974. https://doi.org/10.1016/S1044-0305(02)00417-8.
64. Chen, H.; Gu, L.; Yin, Y.; Koh, K.; Lee, J. Molecular Recognition of Arginine by Supramolecular Complexation with Calixarene Crown Ether Based on Surface Plasmon Resonance. *IJMS* **2011**, *12* (4), 2315–2324. https://doi.org/10.3390/ijms12042315.
65. Lipkowski, J.; Kalchenko, O. I.; Slowikowska, J.; Kalchenko, V. I.; Lukin, O. V.; Markovsky, L. N.; Nowakowski, R. Host-Guest Interactions of Calix[4]Resorcinarenes with Benzene Derivatives in Conditions of Reversed-Phase High-Performance Liquid Chromatography. Determination of Stability Constants. *J. Phys. Org. Chem.* **1998**, *11* (6), 426–437. https://doi.org/10.1002/(SICI)1099-1395(199806)11:6%253C426::AID-POC963%253E3.0.CO;2-R.
66. Kalchenko, O. I.; Lipkowski, J.; Kalchenko, V. I.; Vysotsky, M. A.; Markovsky, L. N. Effect of Octakis(Diethoxyphosphoryloxy)-Tert-Butyl-Calix[8]Arene in Mobile Phase on the Reversed-Phase Retention Behavior of Aromatic Compounds: Host-Guest Complex Formation and Stability Constants Determination. *Journal of Chromatographic Science* **1998**, *36* (5), 269–273. https://doi.org/10.1093/chromsci/36.5.269.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

67. Kalchenko, O. I.; Da Silva, E.; Coleman, A. W. Determination of the Stability Constants of Inclusion Complexes of P-H-37-(Carboxy-Methyloxy)-Calix-[6]-Arene and p-Sulphonato-37-(2-Carboxy-Methyloxy)Calix[6]Arene with 15 Amino Acids by RP HPLC. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2002**, *43* (3/4), 305–310. https://doi.org/10.1023/A:1021203505307.
68. Kalchenko, O.; Cherenok, S.; Suikov, S.; Kalchenko, V. Study of Calixarene Complexation with Biologically Active Carboxylic Acids by RP HPLC Method. *Fr. Ukr. J. Chem.* **2017**, *5* (2), 49–55. https://doi.org/10.17721/fujcV5I2P49-55.
69. Solovyov, A. V.; Cherenok, S. O.; Kalchenko, O. I.; Atamas, L. I.; Kazantseva, Z. I.; Koshets, I. A.; Tsymbal, I. F.; Kalchenko, V. I. Synthesis and Complexation of Amphiphilic Calix[4]Arene Phosphonates with Organic Molecules in Solutions and Langmuir-Blodgett Films. *Journal of Molecular Liquids* **2011**, *159* (2), 117–123. https://doi.org/10.1016/j.molliq.2010.12.007.
70. Kalchenko, O. I.; Cherenok, S. O.; Solovyov, A. V.; Kalchenko, V. I. Influence of Calixarenes on Chromatographic Separation of Benzene or Uracil Derivatives. *Chromatographia* **2009**, *70* (5–6), 717–721. https://doi.org/10.1365/s10337-009-1229-2.

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Annelation of the 1,2,4-Triazine Core to 2,3-Benzodiazepine

Abstract

A one-pot, stepwise method for the annelation of the 1,2,4-triazine core to the seven-membered 2,3-benzodiazepine ring *via* the interaction of the corresponding 2,3-benzodiazepin-1-yl- or 2,3-benzodiazepin-4-ylhydrazines with α-ketoesters has been developed. It has been found that a stepwise formation of an azomethine intermediate followed by solvent replacement and subsequent cyclization enables the desired compounds to be obtained in high yields. Derivatives of a new heterocyclic system of [1,2,4]triazino[3,4-*a*][2,3]benzodiazepine have been synthesized.

Keywords: 2,3-benzodiazepine; α-ketoester; 1,2,4-triazine; annelation

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Анелювання 1,2,4-триазинового ядра до 2,3-бензодіазепіну

Анотація

Розроблено однореакторний метод анелювання 1,2,4-триазинового ядра до семичленного циклу 2,3-бензодіазепіну шляхом взаємодії відповідних 2,3-бензодіазепін-1-іл- або 2,3-бензодіазепін-4-ілгідразинів і α-кетоестерів. З'ясовано, що поетапне утворення азометинового інтермедіату та подальша циклізація після заміни розчинника дозволяють отримувати бажані сполуки з високими виходами. Синтезовано похідні нової гетероциклічної системи [1,2,4]триазино[3,4-*a*]-[2,3]бензодіазепіну.

Ключові слова: 2,3-бензодіазепін; α-кетоестер; 1,2,4-триазин; анелювання

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■ Introduction

2,3-Benzodiazepines have been in the focus of organic and medicinal chemistry for over 50 years since the advent of the anxiolytic Grandaxin. Extensive studies of 2,3-benzodiazepine derivatives have revealed therapeutic applications for the central nervous system, pain, metabolic syndrome, urology, gastrointestinal, and cardiovascular systems (Talampanel) [1], cancer treatment [2], and as AMPAA and AMPAR antagonists [3]. Despite the absence of recent novel medical drugs based on 2,3-benzodiazepine, one of the latest reviews states that "the benzodiazepine saga continues to develop as the number and diversity of agents that modulate GABAA receptors allosterically increases seemingly exponentially. Such modulators are much sought after for their potential subtype selectivity as a result of the greater structural diversity of allosteric sites as distinct from the orthosteric GABA binding sites" [4]. 2,3-Benzodiazepine derivatives are attractive due to their large number of biological activities and their capacity to produce a more subtle effect on various receptors than 1,4- and 1,5-benzodiazepine derivatives [5]. Thus, Grandaxin exerts anxiolytic activity but lacks sedative, amnestic, anticonvulsant, or muscle-relaxant

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properties, and does not bind to the benzodiazepine binding site [6]. At present, there is a number of effective synthetic strategies for the construction, functionalization, and heteroannelation of the 1,2-diazepine core. New condensed heterocyclic systems with a 1,2-diazepine core have been synthesized, and their structures, stabilities, and biological activities have been studied. Most biomedical studies of 2,3-benzodiazepines have been performed on binuclear derivatives, but some studies have shown that the annelation of an additional heterocyclic ring to the seven-membered ring could enhance activity or exhibit a new property. It has been demonstrated that the addition of an azole ring to the seven-membered 2,3-benzodiazepine moiety at N3-C4 atoms provides a high activity of the molecule as an AMPA antagonist and imparts a whole spectrum of activity to the molecule in relation to the central nervous system [7]. The annelation of the imidazole ring retains a high level of the neurotropic activity, emphasizing the anticonvulsant and neuroprotective components in the pharmacological profile [8].

In most fused heterocyclic systems with the 2,3-benzodiazepine fragment, the additional heterocycle is formed at N3-C4 atoms using available 2,3-benzodiazepine-4-thiones or the corresponding hydrazines. Before our studies, only one instance of a fused 2,3-benzodiazepine system with a heterocycle at C(1)-N(2) atoms was reported [9].

For novel C(1)-N(2) heterocyclic fused derivatives of 2,3-benzodiazepine, we used 2,3-benzodiazepinehydrazines-1 converted in moderate to high yields to [1,2,4]triazolo[3,4-*a*][2,3]benzodiazepines by the action of carboxylic acids, acid chlorides, and triethyl orthoformate [10] (**Figure 1**). The nitrosation in acetic acid forms the tetrazolo[5,1-*a*]-[2,3]benzodiazepine heterocyclic system [11]. In addition to five-membered heterocycles, we were also interested in the annelation of six-member heterocycles, including 1,2,4-triazine. Heterocyclic systems with the 1,2,4-triazine core exhibit a high anticancer activity [12, 13]. Thus, we investigated the possibility of adding a 1,2,4-triazine ring to 2,3-benzodiazepines by reacting the 2,3-benzodiazepine hydrazines with 2-ketoesters.

■ Results and discussion

As reported earlier, reactions of 1,3-dicarbonyl compounds with 4-aryl-2,3-benzodiazepin-1-ylhydrazines did not yield annelation products [11]. In contrast, Rosaria Gitto's group obtained products of the triazine ring annelation at N(3)-C(4) atoms by the action of oxalyl chloride on 1-aryl-4-hydrazino-2,3-benzodiazepine [7]. Therefore, we studied the interaction of 2,3-benzodiazepine-1-hydrazines with α-ketoesters (**Scheme 1**). As a result of the interaction of hydrazine derivative **1** with methyl phenylglyoxylate (**2a**) in boiling 2-ethoxyethanol, the expected 7-(4-methoxyphenyl)-3-phenyl[1,2,4]triazino[3,4-*a*][2,3]benzodiazepin-4(8*H*)-one (**3a**) was obtained in 28% yield.

Increasing the cyclization temperature by heating hydrazine **1** and ketoester **2a** in 2-butoxyethanol decreased the yield of cyclization product **3a** to 20%. To improve the yields of cyclization products, we studied the reaction stages of the azomethine **A** formation and the cyclization involving the ester group and the N(2) atom of

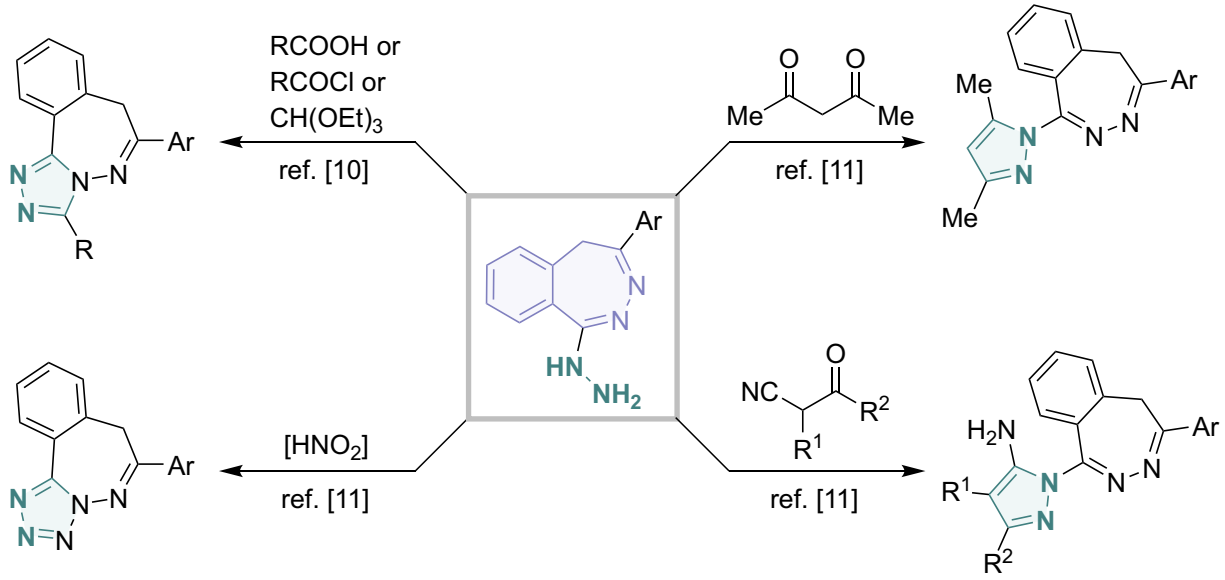
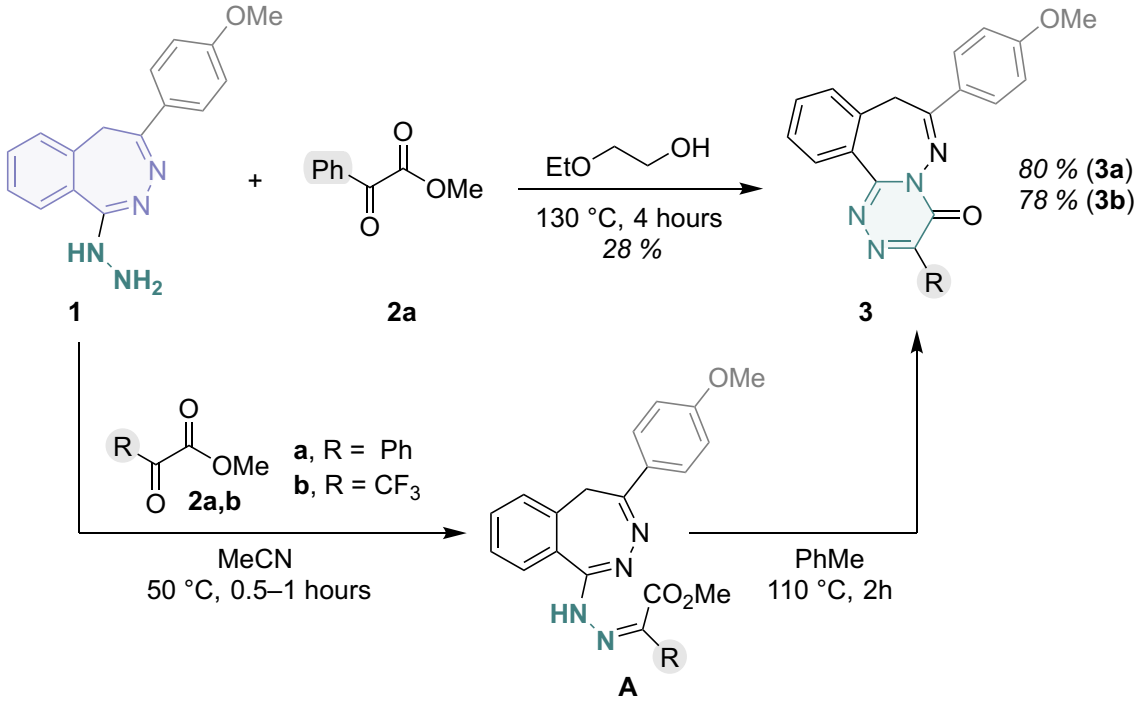


Figure 1. Selected studies in the formation of heterocyclic systems based on 2,3-benzodiazepin-1-yl-hydrazine



Scheme 1. The synthesis of 7-(4-methoxyphenyl)-3-R-[1,2,4]triazino[3,4-*a*][2,3]benzodiazepin-4-ones **3a,b**

the seven-membered ring. In the ^{1}H NMR experiment using hydrazine **1** and methyl trifluoropyruvate (**2b**) as an example, it was found that azomethine A formed quantitatively in acetonitrile at 30 °C within 30 min (**Figure 2***B*). The cyclization of azomethine **A** in boiling acetonitrile was extremely slow (**Figure 2***C*). After removing acetonitrile from the reaction mixture, we successfully carried out the cyclization step in boiling toluene. Under preparative conditions, we performed a stepwise synthesis of azomethine **A** and cyclized it *via* solvent exchange. As a result, 3-(trifluoromethyl)-7-(4-methoxyphenyl)-8*H*-[1,2,4]-triazino[3,4-*a*][2,3]benzodiazepin-4-one (**3b**) was isolated in 78% yield after the crystallization step (**Figure 2***D*).

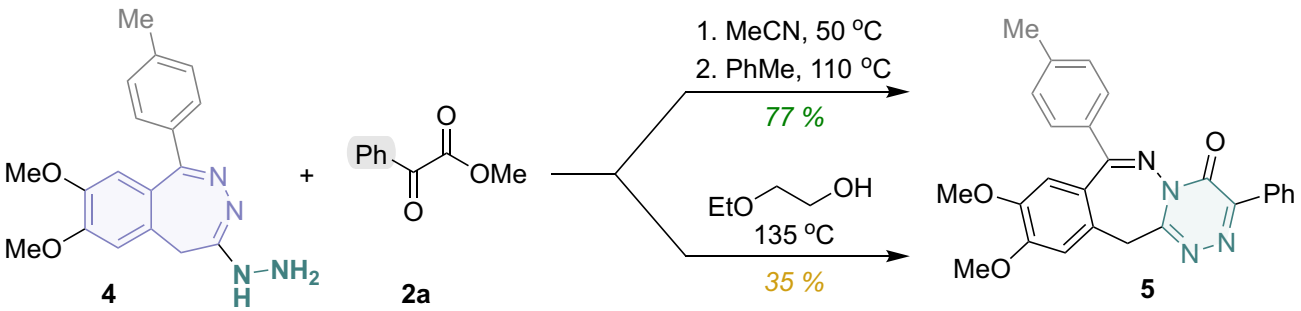
The increase in yield is probably also due to the removal of methanol released during the cyclization from the reaction zone. Triazinodiazepine **3a** was obtained by this method in 80% yield.

Table 1. The optimization of reaction conditions for the cyclocondensation of hydrazines **1** and **4** with ketoesters **2a,b**

Reagents	Solvent	T, °C	Product	Yield, %
1 + **2a**	2-Ethoxyethanol	135	**3a**	28
1 + **2a**	2-Butoxyethanol	170	**3a**	20
1 + **2a**	MeCN → PhMe	50 → 110	**3a**	80
4 + **2a**	2-Ethoxyethanol	135	**5**	35
1 + **2b**	MeCN → PhMe	50 → 110	**3b**	78
4 + **2a**	MeCN → PhMe	50 → 110	**5**	77

The reaction scheme does not require the isolation of the azomethine intermediate (**Table 1**).

This approach is also effective for the 1,2,4-triazine annelation at the N(3)-C(4) 2,3-benzodiazepine bond. The reaction of 1-aryl-2,3-benzodiazepin-4-ylhydrazine (**4**) with ketoester **2a** led to the isomeric heterocyclic structure of 9,10-dimethoxy-7-(4-methylphenyl)-3-phenyl[1,2,4]triazino[4,3-*c*][2,3]benzodiazepin-4(12*H*)-one with 77% yield (**Scheme 2**).



Scheme 2. The synthesis of [1,2,4]triazino[4,3-*c*][2,3]benzodiazepin-4-one **5**

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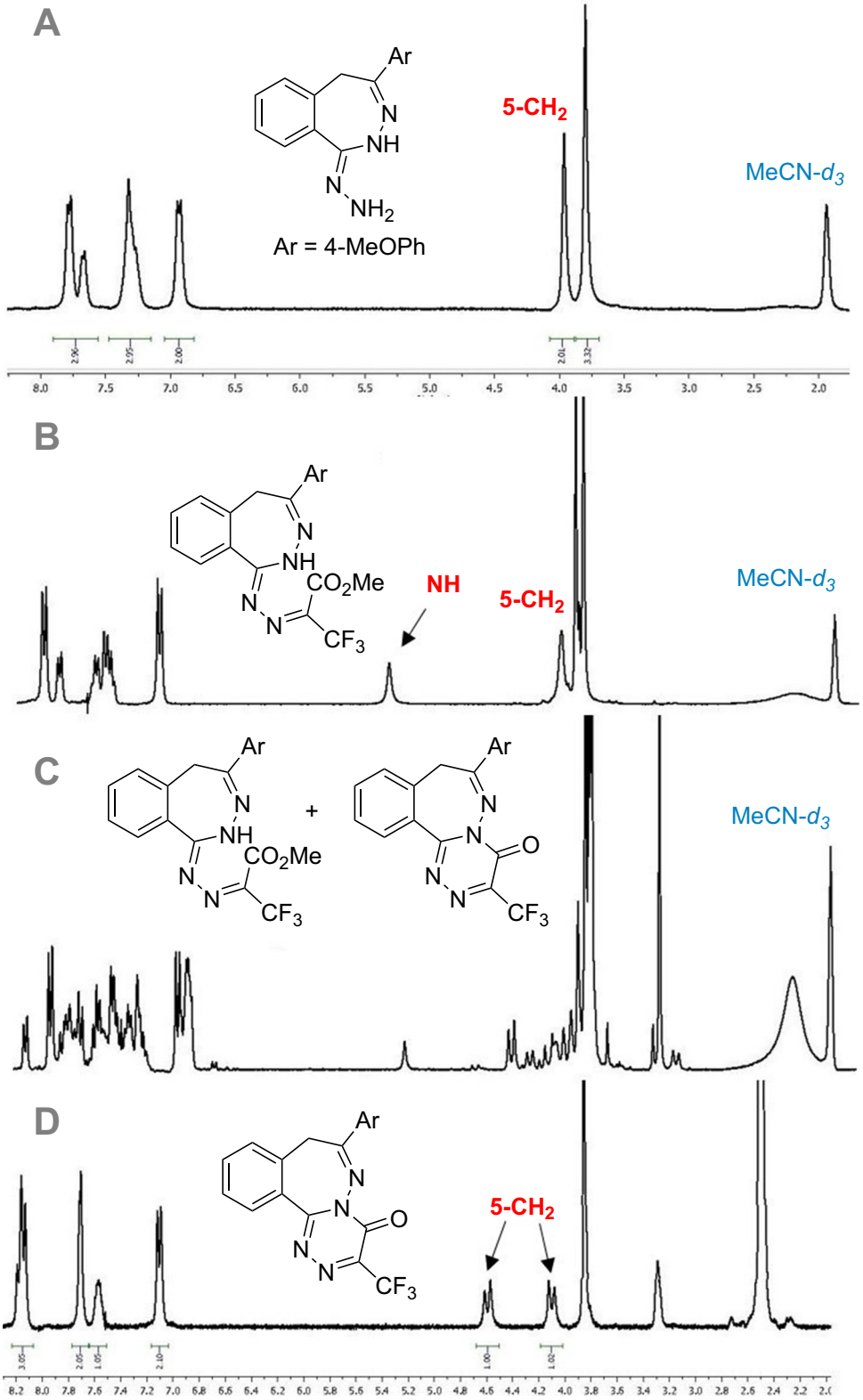

Figure 2. The ^{1}H NMR study of the reaction of hydrazine **1** and **2a**. ^{1}H NMR spectra of **1** in MeCN-d_3 (**A**); ^{1}H NMR spectra of **1** and **2b** in MeCN-d_3, in 30 min at 30 °C (**B**); ^{1}H NMR spectra of **1** and **2b** in MeCN-d_3, 4 h at 80 °C (**C**); ^{1}H NMR spectra of **3b** in DMSO-d_6, after 2 h in toluene refluxing (**D**)

■ Conclusions

In summary, we report an easy approach to the 1,2,4-triazine ring annelation to the "A" or "C" bond of 2,3-benzodiazepine. Unlike the known method [7], the method described allows for the introduction of various substituents into the triazine ring. Considering the availability of the starting reagents and high yields of the final compounds, we propose this simple and convenient method for the 1,2,4-triazine ring annelation to 2,3-benzodiazepines and relative compounds.

■ Experimental part

The solvents were purified according to the standard procedures. The initial hydrazines **1** and **4** were synthetized as described in [10, 11], α-ketoesters **2a,b** were received from commercial sources. The melting points were determined on a Fisher-Johns apparatus. ^{1}H, ^{13}C and ^{19}F NMR spectra were measured in the DMSO-d_6 solution on a Bruker Avance II 400 (400 MHz for protons, 100 MHz for carbon, and 376 MHz for fluorine atoms, respectively) and a Varian Mercury+ (300 MHz on protons and 76 MHz on carbon atoms, respectively) at 25 °C. Tetramethylsilane for ^{1}H and ^{13}C and hexafluorobenzene for ^{19}F were used as internal standards [14]. HPLC-MS spectra were recorded using the chromatography/mass-spectrometric system consisting of an Agilent 1100 high-performance liquid chromatograph equipped with a diode-matrix and mass-selective detector. The parameters of the chromatography-mass analysis were the column SUPELCO Ascentis Express C18, 2.7 μm 4.6 mm×15 cm. The elemental analysis was carried out in the Analytical Laboratory of the Institute of Organic Chemistry, NAS of Ukraine.

The cyclization of 2,3-benzodiazepin-1-yl- (1) and 2,3-benzodiazepin-4-yl-hydrazine (4) with ethyl phenylglyoxylate (*Method A*)

Hydrazine **1** or **4** (3 mmol) and ethyl phenylglyoxylate (3.3 mmol) were refluxed in 10 mL of 2-ethoxyethanol for 4 h. After cooling the mixture, water was added, the precipitated product was filtered, washed with water and recrystallized.

The yield of triazinodiazepine **3a** was 28%, the yield of triazinodiazepine **5** was 35%.

The [1,2,4]triazine ring annelation to 2,3-benzodiazepin-1-yl- and 2,3-benzodiazepin-4-yl-hydrazines (*Method B*)

To hydrazine **1** or **4** (3 mmol) in 50 mL of acetonitrile, α-ketoester **2** (3.15 mmol) was added, and the solution was stirred at 40–50 °C until complete conversion in 0.5–1 h (^{1}H NMR control). The solvent was removed under reduced pressure to dryness, 20 mL of toluene was added, and the mixture was refluxed for 2 h. The solvent was removed under reduced pressure to dryness, and the residue was crystallized.

7-(4-Methoxyphenyl)-3-phenyl[1,2,4]triazino[3,4-*a*][2,3]benzodiazepin-4(8*H*)-one (3a)

Small light-yellow crystals. Yield 0.94 g (80%). M. p. 209–211 °C (methanol). Anal. Calcd for $C_{24}H_{18}N_4O_2$, %: C 73.08; H 4.60; N 14.20. Found, %: C 73.2; H 4.6; N 14.3. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.88 (3H, s, CH_3O); 4.00 (1H, d, J = 13.2 Hz), 4.52 (1H, d, J = 13.2 Hz), 7.02 (2H, d, J = 8.8 Hz), 7.53–7.50 (4H, m), 7.65–7.55 (2H, m), 8.14 (2H, d, J = 8.8 Hz), 8,21 (1H, d, J = 7.76 Hz), 8.36–8.30 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 33.7, 55.3, 114.1, 125.1, 126.6, 127.5, 127.6, 127.8, 128.7, 129.0, 129.9, 130.1, 130.2, 132.3, 133.5, 138.9, 149.1, 151.3, 152.8, 172.0.

7-(4-Methoxyphenyl)-3-trifluoromethyl-[1,2,4]triazino[3,4-*a*][2,3]benzodiazepin-4(8*H*)-one (3b)

Small white crystals. Yield 0.9 g (78%). M. p. 183–185 °C (propanol-2). Anal. Calcd for $C_{19}H_{13}F_3N_4O_2$, %: C 59.07; H 3.39; F 14.75; N 14.50. Found, %: C 58.95; H 3.35; F 14.84; N 14.67. ^{1}H NMR (300 MHz, DMSO-d_6), δ, ppm: 3.86 (3H, s, CH_3O), 4.10 (1H, d, J = 12 Hz, 5-CH_2), 4.59 (1H, d, J = 12 Hz, 5-CH_2), 7.10 (2H, d, J = 9 Hz), 7.52–7.63 (1H, m), 7.71 (2H, d, J = 9 Hz), 8.13–8.19 (3H, m). ^{13}C NMR (76 MHz, DMSO-d_6), δ, ppm: 33.31, 55.66, 114.66, 124.52, 127.83, 128.13, 130.63, 130.89, 133.90, 139.88, 155.76, 163.10, 172.96. ^{19}F{^{1}H} NMR (376 MHz, DMSO-d_6), δ, ppm: -66.88. LC-MS (EI), m/z: 387 $[M+H]^+$.

9,10-Dimethoxy-3-phenyl-7-(p-tolyl)-12H-[1,2,4]triazino[4,3-*c*][2,3]benzodiazepin-4-one (5)

Small light-yellow crystals. Yield 1.0 g (77%). M. p. 245–247 °C (propanol-2). Anal. Calcd for $C_{26}H_{22}N_4O_3$, %: C 71.3; H 5,1; N 12.7. Found, %: C 71.22; H 5.06; N 12.78. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.41 (3H, s, CH_3), 3.63 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.06 (2H, dd, 5-CH_2, J = 14, 6.5 Hz), 6.75 (1H, s), 7.37 (3H, m), 7.49 (3H, m), 7.69 (2H, d, J = 6.8 Hz), 8.05 (2H, d, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 23.1, 38.1, 57.7, 58.0, 113.2, 114.7, 114.8, 120.7, 122.6, 130.0, 130.7, 130.8, 131.3, 132.2, 132.4, 134.3, 135.1, 135.8, 144.3, 150.0, 151.2, 154.8, 156.6, 157.5, 170.0.

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References

1. Knutsen, L. J. S.; Williams, M. Epilepsy. In *Comprehensive Medicinal Chemistry II*; Elsevier, 2007; pp 279–296. https://doi.org/10.1016/B0-08-045044-X/00171-1.
2. Postel-Vinay, S.; Herbschleb, K.; Massard, C.; Woodcock, V.; Soria, J.-C.; Walter, A. O.; Ewerton, F.; Poelman, M.; Benson, N.; Ocker, M.; Wilkinson, G.; Middleton, M. First-in-Human Phase I Study of the Bromodomain and Extraterminal Motif Inhibitor BAY 1238097: Emerging Pharmacokinetic/Pharmacodynamic Relationship and Early Termination Due to Unexpected Toxicity. *European Journal of Cancer* **2019**, *109*, 103–110. https://doi.org/10.1016/j.ejca.2018.12.020.
3. Qneibi, M.; Jaradat, N.; Hawash, M.; Olgac, A.; Emwas, N. Ortho versus Meta Chlorophenyl-2,3-Benzodiazepine Analogues: Synthesis, Molecular Modeling, and Biological Activity as AMPAR Antagonists. *ACS Omega* **2020**, *5* (7), 3588–3595. https://doi.org/10.1021/acsomega.9b04000.
4. Johnston, G. A. R. Commentary on Alleviation of Anxiety: The Benzodiazepine Saga by Willy Haefely. In *Discoveries in Pharmacology*; Elsevier, 2023; pp 197–204. https://doi.org/10.1016/B978-0-323-85519-8.00012-2.
5. Solyom, S.; Tarnawa, I. Non-Competitive AMPA Antagonists of 2,3-Benzodiazepine Type. *CPD* **2002**, *8* (10), 913–939. https://doi.org/10.2174/1381612024607081.
6. Horváth, E. J.; Horváth, K.; Hámori, T.; Fekete, M. I. K.; Sólyom, S.; Palkovits, M. Anxiolytic 2,3-Benzodiazepines, Their Specific Binding to the Basal Ganglia. *Progress in Neurobiology* **2000**, *60* (4), 309–342. https://doi.org/10.1016/S0301-0082(99)00020-9.
7. Gitto, R.; Orlando, V.; Quartarone, S.; De Sarro, G.; De Sarro, A.; Russo, E.; Ferreri, G.; Chimirri, A. Synthesis and Evaluation of Pharmacological Properties of Novel Annelated 2,3-Benzodiazepine Derivatives. *J. Med. Chem.* **2003**, *46* (17), 3758–3761. https://doi.org/10.1021/jm030821p.
8. Elger, B.; Huth, A.; Neuhaus, R.; Ottow, E.; Schneider, H.; Seilheimer, B.; Turski, L. Novel α-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionate (AMPA) Receptor Antagonists of 2,3-Benzodiazepine Type: Chemical Synthesis, in Vitro Characterization, and in Vivo Prevention of Acute Neurodegeneration. *J. Med. Chem.* **2005**, *48* (14), 4618–4627. https://doi.org/10.1021/jm0580003.
9. De Sarro, G.; Chimirri, A.; De Sarro, A.; Gitto, R.; Grasso, S.; Giusti, P.; Chapman, A. G. GYKI 52466 and Related 2,3-Benzodiazepines as Anticonvulsant Agents in DBA/2 Mice. *European Journal of Pharmacology* **1995**, *294* (2–3), 411–422. https://doi.org/10.1016/0014-2999(95)00561-7.
10. Kharaneko, O. I.; Popov, V. Yu.; Bogza, S. L. 4-Aryl-1-Hydrazino-5H-2,3-Benzodiazepine and 1-Aryl-4-Hydrazino-5H-2,3-Benzodiazepine in the Synthesis of Condensed [1,2]Diazepines. *Chem. Heterocycl. Comp.* **2013**, *49* (2), 317–324. https://doi.org/10.1007/s10593-013-1249-6.
11. Khabarov, K. M.; Kharaneko, O. I.; Bogza, S. L. 2,3-Benzodiazepine-1-Thione in the Synthesis of Substituted and Hetero-Annelated 2,3-Benzodiazepines. *Chem. Heterocycl. Comp.* **2009**, *45* (4), 468–474. https://doi.org/10.1007/s10593-009-0280-0.
12. Sztanke, K.; Pasternak, K.; Rzymowska, J.; Sztanke, M.; Kandefer-Szerszeń, M. Synthesis, Structure Elucidation and Identification of Antitumoural Properties of Novel Fused 1,2,4-Triazine Aryl Derivatives. *European Journal of Medicinal Chemistry* **2008**, *43* (5), 1085–1094. https://doi.org/10.1016/j.ejmech.2007.07.009.
13. Sztanke, M.; Rzymowska, J.; Sztanke, K. Synthesis, Structure Elucidation and Identification of Antiproliferative Activities of a Novel Class of Thiophene Bioisosteres Bearing the Privileged 7,8-Dihydroimidazo[2,1-*c*][1,2,4]Triazin-4(6*H*)-One Scaffold. *Bioorganic & Medicinal Chemistry* **2015**, *23* (13), 3448–3456. https://doi.org/10.1016/j.bmc.2015.04.037.
14. Harris, R. K.; Becker, E. D.; Cabral De Menezes, S. M.; Granger, P.; Hoffman, R. E.; Zilm, K. W. Further Conventions for NMR Shielding and Chemical Shifts (IUPAC Recommendations 2008). *Pure and Applied Chemistry* **2008**, *80* (1), 59–84. https://doi.org/10.1351/pac200880010059.

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The Experimental Study of the Quality and Safety of Injectable Implant Medical Devices Based on Hyaluronic Acid in Accordance with the Requirements of the EU Regulation

Abstract
The aim of the article is to present the results of the experimental study of leachables used as primary packaging for medical devices, namely injectable implants based on hyaluronic acid. For the study, a line of injectable implants with identical qualitative composition and differing quantitative hyaluronic acid content was used. When developing the research conditions, the main characteristics of the implant gel were taken into account, and the conditions for using the appropriate medical device were modeled to obtain the most informative results and confirm the safety of the primary packaging selected. The analysis of extracts was carried out using the following methods: GC/MS, HPLC/UV/MS, ICP/MS, and IC. No substances listed as Chemicals of Potential Concern were detected in the extracts obtained, thereby confirming the safety of using the medical device for the patient under the conditions specified by the manufacturer.
Keywords: quality control; safety; hyaluronic acid; chromatography

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Експериментальне дослідження якості та безпечності ін'єкційних імплантатів на основі гіалуронової кислоти відповідно до вимог Регламенту ЄС

Анотація
Мета статті – викласти результати експериментального дослідження вилуговуваних речовин із первинного паковання медичних виробів, а саме ін'єкційних імплантатів на основі гіалуронової кислоти (ГК). Об'єктом дослідження була лінійка ін'єкційних імплантатів, які мають ідентичний якісний, але відмінний кількісний склад за вмістом ГК. Щоб отримати високу інформативність результатів і підтвердити безпечність використання обраного первинного паковання, під час розроблення умов експерименту враховували основні характеристики гелю імплантатів, а також моделювали умови застосування відповідного медичного виробу. В отриманих зразках не виявили речовин, що належать до потенційно небезпечних. Отже, було підтверджено безпеку використання медичного виробу для пацієнта за умови дотримання інструкцій, зазначених виробником.
Ключові слова: контроль якості; безпечність; гіалуронова кислота; хроматографія

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Introduction

Injection implants have become widely used in current medical practice. Regardless of the purpose – medical or cosmetic – the manufacturer is responsible for the product's quality and safety.

The quality of any medical devices is ensured at the stage of their production. Previously, we validated the technological process for manufacturing injectable implants based on hyaluronic acid [1, 2]. It included a theoretical risk assessment at the first stage and direct validation at identified critical points at the second stage.

The most significant safety risks in the use of medicines and medical devices are impurities. These impurities can have different origins; therefore, during implementation and development, it is mandatory to take into account all factors that may contribute to the formation of associated and extraneous impurities and to implement appropriate measures to regulate them. The highest risk is also noted for parenterally administered medical devices [3]. One of the focuses of the recently entered into force European Union Regulation (EU) 2017/745 (MDR) is compliance with the safety standards for medical devices to minimize harm to patients when used for their intended purpose [4]. The biocompatibility assessment is a key element in confirming the safety of products for the human body [5]. In accordance with the requirements of the MDR [4], manufacturers of medical products must conduct studies to confirm the medical device's ability to interact with the patient without causing harm.

One source of hazardous impurities in the use of medical devices is the containers used, such as syringes. Therefore, one element of biocompatibility research is the assessment of the physical and chemical properties of materials, namely the determination of extractable and leachable substances from the primary packaging of a medical device during its shelf life [6–9].

Research on leachable substances is always a scientific challenge, since the extraction of substances from packaging by common solvents of various natures can be predicted. Still, the extraction of substances under the influence of the product itself cannot. For some medical devices, this information is extremely limited [10].

This article presents the results of the study of leachable substances, i.e., those that can be released from the primary packaging of a medical device during its use under the influence of temperature, environment, and other operating conditions, and enter directly into the composition of the medical device itself. Such substances can enter the patient's body and potentially cause undesirable reactions or toxic effects.

For injectable implants, this is critically important, given that such devices are introduced into the human body and remain there until they are completely biodegraded [11]. The safety threshold is 0.15 µg per day for genotoxic or carcinogenic compounds and 1.5 µg per day for others [4]. Depending on the nature of such substances, an adequate method for their detection should be chosen.

The use of hyaluronic acid in various medical devices has continued to expand in recent years, as evidenced by numerous publications on its efficacy and safety [12–15]. At the same time, there are few experimental studies in the literature on the detection of substances extracted or leached from the primary packaging of medical devices. Studies of leachable substances from primary packaging should be conducted by medical device manufacturers at the design and development stage of the product, as well as when making critical changes that could affect the product's physicochemical parameters. Manufacturers are likely to be reluctant to disclose this information. This article presents a description of the methods and results of studies on leachable substances from the primary packaging of medical devices – injectable implants based on cross-linked hyaluronic acid.

Thus, the aim of this article was to demonstrate the research results on leaching chemicals that may pose a potential hazard to the patient in medical devices – injectable implants in pre-filled syringes. The studies were conducted on a finished medical device (a pre-filled syringe with gel).

Materials and methods

Equipment and reagents

To determine the conditions of the study, the following characteristics of the medical device were taken into account:

- *the pH value* – the limits of the line of injectable implants studied – 7.2–7.4 (physiological pH);
- *duration of contact of the implants with the human body* – long term (> 30 days);
- *the type of medical device* – risk class III, implantable product;
- *the shelf life* – 2 years;
- *storage conditions* – from +2 °C to +30 °C.

The algorithm for conducting the study of leachable substances was as follows [16]:

1. Selection of the research material – assessment of primary packaging materials and the medical product composition.

2. Determination of research conditions and methodology – development of an analysis method, research conditions, and determination of the required list of equipment.

3. Conducting the research:

3.1. Modulation of the conditions of use of the finished medical device – to simulate the worst case, a sample of the medical device that was in climatic chambers and underwent product stability studies was used. Thus, samples simulating the medical device at the end of its shelf life (2 years) and with a storage temperature at the upper limit (+30 °C), i.e., the longest contact with the primary packaging expected by the manufacturer, were used;

3.2. Analysis of the components released – the quantitative and qualitative analysis in the samples of substances that could have leached from the primary packaging into the gel of the medical device. The following methods were used for the analytical study:

- head-space gas chromatography for the determination of volatile substances (an Agilent RTX-624 capillary column – 30 m × 0.25 mm, the aliquot volume – 1.0 mL, the ejector temperature – 200 °C, the flow rate – 1 mL min^{-1}, helium as a carrier gas, the GC temperature program – from 40 °C to 240 °C at a rate of 8 °C min^{-1});
- gas chromatography coupled to a mass spectrometric detector (GC/MS) for the determination of semi volatile organic compounds (an Agilent HP-5MS capillary column – 30 m × 0.25 mm, the aliquot volume – 1.0 mL, the injector temperature – 280 °C, the flow rate – 1.0 mL min^{-1}, helium as a carrier gas, the GC temperature program – from 40 °C to 280 °C at a rate of 10 °C min^{-1}, then to 310 °C at a rate of 15 °C min^{-1});
- liquid chromatography coupled with UV and mass spectrometric detectors (HPLC/UV/MS) for the determination of non-volatile organic compounds (an Agilent Zorbax Eclipse XDB-C18 column – 2.1 mm × 50 mm, the aliquot volume – 5 µL, 5 mM ammonium acetate in water and 50:50 MeCN/MeOH (*v*/*v*) were used as the mobile phase, the mobile phase speed – 0.4 mL min^{-1}, the temperature – 380 °C, an UV detector – 210 nm; MS ESI+ and ESI–);
- inductively coupled plasma mass spectrometry (ICP/MS) for metal determination (Data acquisition parameters: the data acquisition mode – spectrum; peak pattern – 3 points; repetition – 3; repetition – 100; the stabilization time – 20 sec; the resolution – standard).

The results of the study were calculated as µg of the extracted product in 1 mL of the product.

4. Evaluation of the results obtained – assessment of the toxicological impact of the components found in the solutions and determination of the safety of the selected materials for the medical device.

To conduct a chemical characterization study, the threshold – the Analytical Evaluation Threshold (AET) – was calculated below.

Determination of AET for the GC/MS and HPLC/UV/MS methods:

$$\mathrm{AET}\left(\frac{\mu g}{mL}\right) = \frac{20\left(\frac{\mu g}{\mathrm{day}}\right) \times \frac{1}{2\ \mathrm{mL} \times 1 \times 1}}{2} = 5\ \mu g\ mL^{-1}$$

Each of the above methods was analyzed with the reference solution, test sample, and blank solution.

The limits of quantification for the metals studied, expressed in µg L^{-1}, are given in **Table 1**.

Sample preparation

Research on volatile organic compounds

To prepare the reference solution, an intermediate solution was prepared by diluting 0.2 mL of the toluene solution to 20 mL with water. Then, 0.5 mL of the intermediate solution was diluted to 50 mL with water. An aliquot of 5 mL of this solution was placed in a vial with a sealed test tube for analysis.

To prepare the test sample, an aliquot of the gel from the medical device, obtained after studying its stability under the conditions specified by the manufacturer in the primary packaging, with a volume of 1 mL, was mixed with 4 mL of water (dilution 1:5).

The resulting solution was placed in a 20 mL sealed test tube and analyzed without further processing.

The sample was prepared in duplicate.

Water was used as a blank solution.

Table 1. Limits of the metal quantification

Metal	Limits of quantification, µg L^{-1}
Li, V, Co, As, Mo, Ru, Rh, Cd, Os, Ir, Pt, Tl	0.10
Ni, Cu, Se, Sn, Sb, Ba, Pb	0.50
Ag, Hg	1.00
Cr, Pd, Au*	5.00

Note: *the sample solution after 50-fold dilution

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The study of semi-volatile organic compounds

To prepare the reference solution, an intermediate solution was prepared by diluting 0.5 mL of the phenanthrene-d_{10} solution to 5 mL with dichloromethane (DCM). 0.1 mL of the intermediate solution was diluted to 10 mL with DCM. This solution was used as the AET of the analytical evaluation.

To prepare the test sample, two 5 mL aliquots of the gel samples from the medical device, obtained after stability testing under the conditions specified by the manufacturer in the original packaging, were mixed with 5 mL of DCM using a laboratory shaker (shaking time – 1 min). One mL of the organic phase was mixed with 4 mL of DCM (5-fold dilution). The resulting solutions were analyzed without further treatment.

An aliquot of DCM was used as a blank solution.

The study of non-volatile organic compounds

A mixture of the standard reference solution Irganox® 1098 and Reserpine (1 μg mL^{-1}) was used as a reference solution.

To prepare the test sample, 1 mL of the medical device gel, obtained after stability testing under the manufacturer's specified conditions in the original packaging, was diluted 5 times with water to a final volume of 5 mL and analyzed without further treatment.

An aliquot of H_2O was used as a blank solution.

The metal content study

As reference solutions, 0.5 mL of the certified standard solution (1000 mg L^{-1}) for each metal and 1.0 mL of concentrated HNO_3 were diluted to 50.0 mL with water.

To prepare the test sample, 1 mL of the medical device gel, obtained after stability testing under the manufacturer's specified conditions in the primary packaging, was mixed with 3 mL of HNO_3 and digested using the microwave procedure. The samples obtained were diluted to 50 mL with water (dilution 1:100).

To prepare the blank solution, 1 mL of H_2O was mixed with 5 mL of HNO_3 and digested using the microwave procedure. The resulting solution was diluted to 50 mL of H_2O (dilution 1:100).

■ Results and discussion

This article presents a study of substances leached from the primary packaging for a group of injectable implants (**Table 2**), using one product line as an example, which was determined to be the worst case (highest sodium hyaluronate content and highest daily dose).

The object of the study was an injectable implant based on cross-linked hyaluronic acid containing 20 mg mL^{-1} sodium hyaluronate, delivered in a prefilled syringe. To assess potentially hazardous substances, the composition and properties of the materials used in all components of the syringe should be considered. The syringe manufactured by Becton (Dickinson & Company) consists of a borosilicate glass cylinder, a bromobutyl rubber seal, and a polyisoprene rubber cylinder tip. The silicone covering the inner space of the cylinder, to facilitate smooth movement of the seal and piston, was also considered. Thus, all components of the primary packaging that come into contact with the medical device during its storage under the conditions specified by the manufacturer are subject to investigation. The manufacturer of hyaluronic acid is confidential information of the medical device manufacturer. However, the manufacturer has completed the internal qualification procedure and meets all the requirements.

It is known that hyaluronic acid can promote the release of substances, such as mangiferin, from polymers [16]. Such information creates a prerequisite for releasing other organic substances from packaging.

These studies aim to identify and control chemicals that may pose a hazard. These chemicals may have harmful effects on the patient's health and the environment. Their list is compiled by organizations, such as the World Health Organization (WHO), the International Agency for Research on Cancer (IARC), the Organization for

Table 2. The group of medical devices under study: injectable implants based on hyaluronic acid

Product name	Sodium hyaluronate, mg mL^{-1}	Volume of primary packaging, mL	Sodium hyaluronate, mg
Injectable implant based on hyaluronic acid	15	1	15
	17.5	1	17.5
	20	1	20
	20	2	40

Economic Co-operation and Development (OECD), the European Chemicals Agency (ECHA), the United States Environmental Protection Agency (EPA), etc., and includes substances that meet at least one of the following criteria:

- carcinogenicity, mutagenicity or toxicity to the reproductive system;
- endocrine-disrupting properties that may have a negative impact on the hormonal system;
- persistence, bioaccumulation and toxicity;
- hazardous properties for ecosystems.

A theoretical assessment of the probability of leaching of harmful substances preceded the study. Based on the results of the forecast, we selected groups of substances that could be leached from the packaging according to the groups: semi-volatile, volatile substances, and metal impurities. The potential impact of substances on the patient was predicted using various mathematical models [17].

Research on volatile substances

In medical devices containing hyaluronic acid, as a rule, volatile organic compounds used in the production of raw materials, the finished product, or during its storage may be present. In particular, these may include alcohols, aldehydes, ketones, and essential oils used to flavor cosmetics. In this case, such substances were not used in the production of the injectable implant. However, volatile solvents, such as ethylene glycol, methylene chloride, and perchloroethylene [18, 19], may be used during the production of hyaluronic acid raw materials. Such substances may remain in the final raw material product and thus enter the finished medical product.

For volatile substances that are leached, the headspace gas chromatography-mass spectrometry method is traditionally used [20]. The leaching of volatile substances was evaluated compared to toluene at a concentration of 5 μg mL^{-1}. During chromatography of the test solution, no unidentified substance with a concentration greater than the standard was detected (**Figure 1**).

Research on semi-volatile organic compounds

The content of extraneous semi-volatile organic compounds was estimated by GC/MS. The substances were extracted from the hyaluronic acid solution with dichloromethane. The results were evaluated against the reference standard of phenanthrene added at a concentration of 5 μg/mL. Thus, any impurities with an area under the peak smaller than the reference are not subject to determination, while impurities with a higher content should be identified and determined.

As a result of the GC/MS study, no compounds with the content above AET were identified (**Figure 2**).

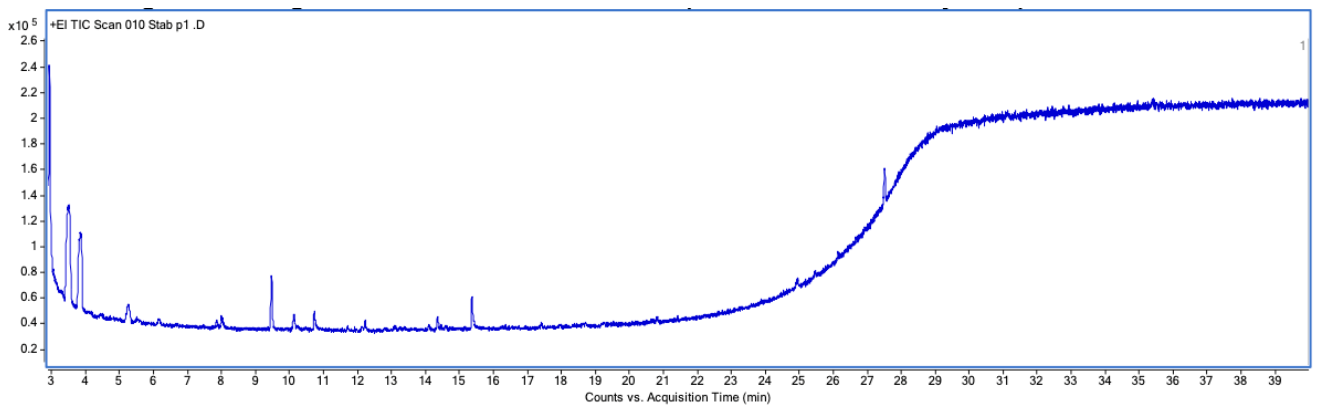

Figure 1. The chromatogram of volatile substances

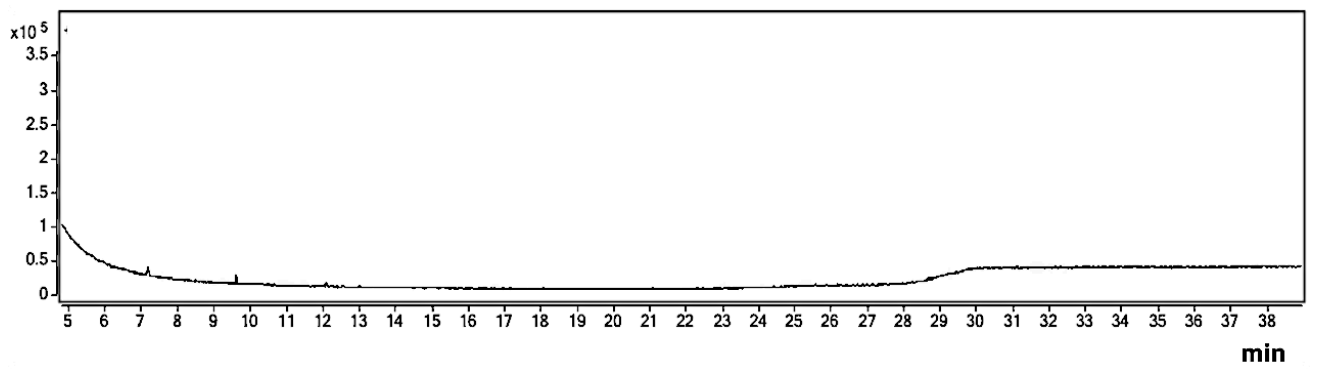

Figure 2. The chromatogram of semi-volatile substances

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Non-volatile organic compounds leached from the primary packaging were determined by HPLC using two detectors – UV and a mass-selective detector. This method is generally accepted for such studies [2]. The results obtained by HPLC/UV/MS were evaluated using two reference standards depending on the ionization mode (positive or negative).

When examining the sample by HPLC/UV/MS at the 0.42 min point, a compound with a concentration of 42.4 μg mL^{-1} was detected, which was higher than the AET value of 5 μg/mL we calculated (**Figure 3***C*).

According to the mass spectrum in the positive ESI mode, the compound was identified as a derivative of the API – hyaluronic acid, so the source of its entry into the implant is not leaching. Therefore, this impurity was not considered by us among the substances leached/extracted from the primary packaging.

Metal impurities

Among the metals potentially leachable from the primary packaging, all metals listed in **Table 1** above were evaluated. The results obtained using the ISP/MS method were evaluated against reference standards for the tested metals. The MS peaks were compared with the MS library for GC and with the known MS templates for LC to identify the detected compounds. Only one metal, lithium (Li), was detected by

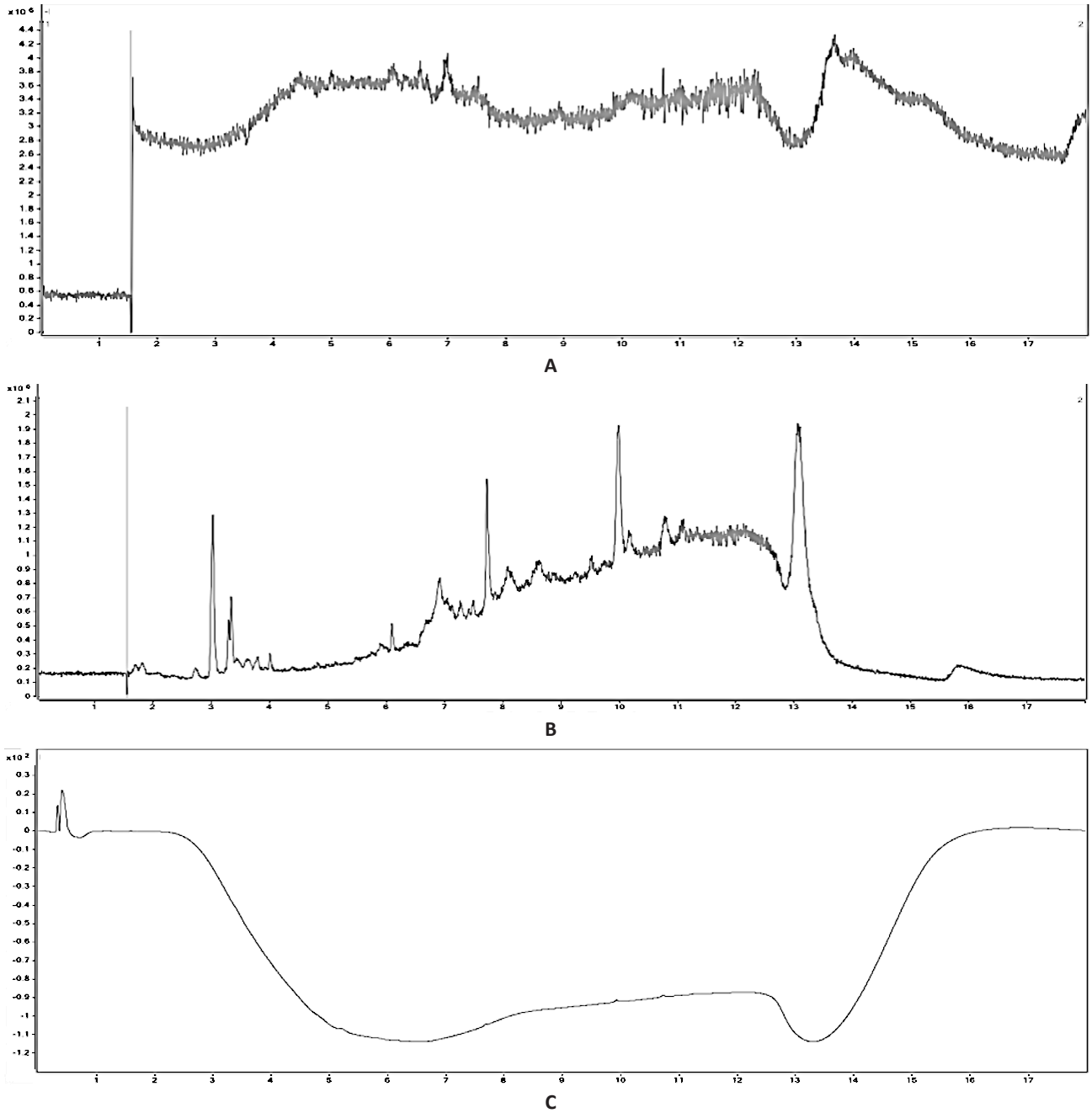

Figure 3. The HPLC chromatogram of the solution studied: **A** – HPLC/MS, negative ionization, **B** – HPLC/MS, positive ionization, **C** – HPLC/UV

the ISP/MS method at a level exceeding the expected acceptable level (0.005 μg L^{-1} after 50-fold dilution). However, Li at such trace levels has no toxicological or biological effect.

The borosilicate glass used to manufacture the syringe cylinders for gel storage contains not only silicon and boron but also alkali metals, such as sodium, potassium, and lithium, at low concentrations. Lithium oxide (Li_2O) is added to the glass to increase thermal resistance, reduce the coefficient of thermal expansion, and improve chemical durability. A portion of these ions may leach into the solution during prolonged contact, especially if the medium has a particular ionic strength, pH, or viscosity that promotes diffusion.

The presence of low concentrations of unknown extractables was considered acceptable.

■ Conclusions

This article presents the algorithm and results of a study on substances leached from the primary packaging of injectable implants containing stabilized hyaluronic acid. Only one unknown organic compound was detected by the HPLC/UV/MS analytical method. However, the peak was studied in the ESI-positive mode and was identified as a compound related to hyaluronic acid; therefore, this substance was not considered as a substance leached from the primary packaging. Other organic compounds in quantities above the AET were found in the gel samples of the medical device, obtained after evaluating its stability under the conditions specified by the manufacturer in the primary packaging. The ICP/MS method detected a small amount of lithium; however, this substance does not pose a concern due to its insignificant risk to human safety and its low concentration in the sample.

The results of the study demonstrate the confirmation of the quality and safety of the use of medical devices – injectable hyaluronic acid-based implants for the patient under the conditions specified by the manufacturer in the selected primary packaging.

■ References

1. Bondarets, I.; Sidorenko, L.; Antonenko, O.; Lebed, S.; Georgiyants, V. An approach to the technological process validation of manufacturing medical devices using the example of injectable implants based on hyaluronic acid. *Sci. Rise Pharm. Sci.* **2024**, *6* (52), 111–123. https://doi.org/10.15587/2519-4852.2024.319456.
2. Li, J.; Sobańtka, A. A systematic analysis of the effect of extraction solvents on the chemical composition of extraction solutions and the analytical implications in extractables and leachables studies. *J. Pharm. Biomed. Anal.* **2023**, *222*, 115081. https://doi.org/10.1016/j.jpba.2022.115081.
3. Bondarets, I.; Sidorenko, L.; Georgiyants, V.; Mishchenko, V. Regulatory and risk-oriented approach to the design and development of medical devices in accordance with Ukraine regulations. *Pharmacia* **2022**, *69* (2), 493–500. https://doi.org/10.3897/pharmacia.69.e82316.
4. Regulation (EU) 2017/745 of the European Parliament and of the Council. 2017. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02017R0745-20250110 (accessed June 8, 2025).
5. Kumaravel, T. S.; Sathya, T. N.; Balaje, R.; Pradeepa, P.; Yogaraj, D.; Murali, M. R.; Navaneethakrishnan, K. R.; Murugan, S.; Jha, A. N. Genotoxicity evaluation of medical devices: A regulatory perspective. *Mutation Research/Reviews in Mutation Research* **2022,** *789*, 108407. https://doi.org/10.1016/j.mrrev.2021.108407.
6. Bello, W.; Pezzatti, J.; Rudaz, S.; Sadeghipour, F. Study of leachable compounds in hospital pharmacy-compounded prefilled syringes, infusion bags and vials. *J. Pharm. Sci.* **2024,** *113* (11), 3227–3237. https://doi.org/10.1016/j.xphs.2024.08.004.
7. Gusain, A. S.; Chandra, S.; Araújo, I. M.; de Lima, J. P. M.; Coutinho, H. D. M. Scientifically Supported Best Practices in Leachable Screening Studies for Pharmaceutical and Parenteral Drug Products. *Future Pharmacol.* **2025**, *5*, 18. https://doi.org/10.3390/futurepharmacol5020018.
8. Satori, C. P.; Christensen, C. D.; Street, S. M.; Giffin, M.; Pohl, C. M.; Christian, W. V. The analytical evaluation threshold for inorganic metal extractables and leachables analysis of medical devices. *Regul. Toxicol. Pharm.* **2024,** *154*, 105725. https://doi.org/10.1016/j.yrtph.2024.105725.
9. Sündermann, J.; Bitsch, A.; Kellner, R.; Doll, T. Is read-across for chemicals comparable to medical device equivalence and where to use it for conformity assessment? *Regul. Toxicol. Pharm.* **2024,** *149*, 105622. https://doi.org/10.1016/j.yrtph.2024.105622.
10. Armstrong, B. L.; Senyurt, A. F.; Narayan, V.; Wang, X.; Alquier, L.; Vas, G. Stir bar sorptive extraction combined with GC–MS/MS for determination of low level leachable components from implantable medical devices. *J. Pharm. Biomed. Anal.* **2013,** *74*, 162–170. https://doi.org/10.1016/j.jpba.2012.10.019.
11. Goud, N. S., Chapter 34 – Biocompatibility Evaluation of Medical Devices. In *A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Third Edition)*, Faqi, A. S., Ed. Academic Press: 2024; pp 957–973. https://doi.org/10.1016/B978-0-323-85704-8.00030-X.
12. Acosta, M.; Bizzoco, L.; Boiocchi, C.; Gobbi, A., Use of New Formulation in Hyaluronic Acid in Regenerative Medicine. In *Regenerative Medicine in Sports and Orthopaedics: A New Approach*, Gobbi, A.; Nakamura, N.; Lane, J. G.; Dallo, I., Eds. Springer Nature Switzerland: Cham, 2025; pp 315–324. https://doi.org/10.1007/978-3-031-84693-9_23.
13. Gao, E. Y.; Tan, B. K. J.; Teo, J. Y. Y.; He, G. S.; Tan, C. J.-W.; Yeo, B. S. Y.; Chua, A. J. K. Hyaluronic Acid for Sinonasal Surgery: A Systematic Review and Meta-Analysis. *International Forum of Allergy & Rhinology* **2025,** *15* (6), 616–625. https://doi.org/10.1002/alr.23537.
14. Muhammad, S.; Syed Wasif Ali, S.; Muhammad Abdul, S. Efficacy of Intraarticular Hyaluronic acid and Cortico- Steroid Co-Injection versus Hyaluronic acid in Knee Osteoarthritis. *Proceedings S.Z.M.C* **2024,** *38* (4), 285–289. https://doi.org/10.47489/szmc.v38i4.638.
15. Torkaman, G.; Rostami-Mehr, E.; Bayat, N.; Mofid, M. Comparing the impact of hyaluronic acid injections and exercise therapy alone and combined on the postural sway and base of support in individuals with knee osteoarthritis: a quasi-experimental study. *International Journal of Therapy and Rehabilitation* **2025,** *32* (4), 1–17. https://doi.org/10.12968/ijtr.2024.0086.
16. United States Pharmacopeia. General Chapter ⟨1664⟩ Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems; *United States Pharmacopeia (USP–NF)*: Rockville, MD, **2022**. https://www.usp.org.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

17. Tanaka, M. L.; Saylor, D. M.; Elder, R. M. Polymer-interface-tissue model to estimate leachable release from medical devices. *Mathematical Medicine and Biology: A Journal of the IMA* **2024,** *41* (4), 382–403. https://doi.org/10.1093/imammb/dqae020.
18. Saadati, F.; Bahrulolum, H.; Talebi, M.; Karimi, M.; Bozorgchami, N.; Ghale, R. A.; Zafar, S.; Aghighi, Y.; Asiaei, E.; Tabandeh, F. Advances and principles of hyaluronic acid production, extraction, purification, and its applications: A review. *Int. J. Biol. Macromol.* **2025,** *312*, 143839. https://doi.org/10.1016/j.ijbiomac.2025.143839.
19. Kostadinova, T.; Trittler, R.; Hoppe, R.; Hug, M. J. 3PC-026 Leachables in syringes containing ethanol, propofol or mRNA vaccine. *European Journal of Hospital Pharmacy* **2025,** *32* (Suppl 1), A29. https://doi.org/10.1136/ejhpharm-2025-eahp.58.
20. Chandra, G. V.; Bavisetti, L.; Anantam, K. Method Development and Method Validation of Volatile Leachables Amount for Oncology Drug Product Injection by Headspace Gas Chromatography Mass Spectrometry Technique. *Res. J. Chem. Environ.* **2025**, *29* (5), 118–127. https://doi.org/10.25303/295rjce1180127.

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Oleksandr Ishchenko: And Gladly Would He Learn, and Gladly Teach

Abstract
The Ukrainian school of organic dye chemistry has long received worldwide recognition. Among the scientists whose achievements embody this success is Academician Oleksandr Oleksandrovych Ishchenko. Over more than half a century of fruitful research at the Department of Color and Structure of Organic Compounds of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, he laid the foundations for the rational design of functional dyes for light-energy conversion, particularly for various laser technologies; became one of the pioneers of polymethine ion-pair photonics; developed methodological approaches to the study of electronic absorption and fluorescence spectra using the method of moments, which provided new insights into the electronic structure of organic chromophores; and made a significant contribution to the systematic study and interpretation of the solvatochromism of polymethines of different types. The driving force behind all these achievements was his enduring passion for science – a deep desire to learn and share knowledge with future generations of researchers.
Keywords: polymethine dyes; color theory; electronic structure; material science; history of chemistry

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Олександр Іщенко: Натхненно пізнавав – натхненно навчав

Анотація
Українська школа органічних барвників давно здобула світове визнання. Серед учених, чиї досягнення уособлюють цей успіх, – академік НАН України Олександр Олександрович Іщенко. За понад півстоліття плідної наукової праці у відділі кольору та будови органічних сполук Інституту органічної хімії НАН України він заклав основи цілеспрямованого дизайну функціональних барвників для перетворювачів світлової енергії, насамперед для різноманітних лазерних технологій; став одним із піонерів фотоніки іонних пар поліметинів; розробив методологічні підходи до дослідження електронних спектрів поглинання та флуоресценції із застосуванням методу моментів, що дозволило отримати нову інформацію про електронну будову органічних хромофорів; зробив вагомий внесок у раціональне дослідження та інтерпретацію сольватохромії поліметинів різної природи. Ключовим рушієм цього успіху було його незмінне натхнення наукою, прагнення пізнавати нове й передавати знання майбутнім поколінням науковців.
Ключові слова: поліметинові барвники; теорія кольору; електронна будова; матеріалознавство; історія хімії

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■ Early Life and Education

Oleksandr Oleksandrovych Ishchenko was born on August 26, 1950, in Piskivka, Kyiv Oblast, Ukraine. Soon afterward, the family of the future scientist moved to Kyiv, which from then on became his lifelong home.

His father, Oleksandr Trokhymovych, had just retired from military service and was working as an engineer while pursuing higher education through evening studies. His mother, Larysa Hryhorivna, was a philologist and university lecturer.

In the courtyards of his Kyiv childhood, one of Oleksandr Ishchenko's first lifelong passions began to take shape – he became an ardent supporter of the Kyiv football club Dynamo. Later, during his student years (1968–1973) at the Faculty of Chemistry of Taras Shevchenko Kyiv State University, he developed other, more active and creative interests – travel and photography, and later, filming his family – his wife Valentyna, also a chemist, and son Yevgen – with a home movie camera. Together with friends, Oleksandr set out on hiking trips to the Carpathians, journeys across the lakes of Karelia, and rafting expeditions along the Chusovaya River in the Urals. Yet all these pursuits filled only the time left free from the main passion of his life – science.

Oleksandr Ishchenko was among the best students of his year. From the very beginning of his studies, he displayed a strong enthusiasm for scientific and creative work. It was this passion that led him to approach Mykhailo Kornilov and ask him to become his research supervisor. At that time, Prof. Kornilov was deeply engaged with a rapidly developing method of physicochemical analysis – NMR spectroscopy. He entrusted the young Ishchenko and his classmate and friend, Oleksandr Turov, with studying the relevant literature, particularly on the principles of the NMR spectrometer operation and the use of lanthanide shift reagents to resolve overlapping signals in ^{1}H NMR spectra – a technique of great importance in the era before high-field superconducting spectrometers became widely available. The open lecture on NMR spectroscopy prepared by the two Oleksandrs attracted undergraduate and graduate students, as well as faculty members who had completed their education before the advent of NMR spectroscopy and wished to acquaint themselves with the capabilities of this rapidly developing field. The lecture was received with great interest, and the two were even invited to present it at other venues in Kyiv.

Prof. Oleksandr Oleksandrovych Ishchenko

■ Journey into Science

After graduating from the University with distinction, Oleksandr Ishchenko – on the advice of Prof. Mykhailo Kornilov, who recognized that his student's "greatest" opinion talent lay in the field of theoretical chemistry – chose Heorhii Dyadyusha as his scientific advisor. The latter was an outstanding theoretician and one of the pioneers of quantum chemistry in Ukraine; at that time, he worked in the Department of Color and Structure of Organic Compounds at the Institute of Organic Chemistry of NAS of Ukraine.

The problems, assigned by Dr. Dyadyusha to his graduate students – Oleksandr Ishchenko, Oleksii Rykov, and Oleksii Kachkovsky – required them to acquire new knowledge in physical and quantum chemistry, as well as in computer programming. The young researchers found a creative solution: they prepared scientific presentations on the topics they needed and discussed them vigorously together.

The topic of Oleksandr Ishchenko's first dissertation research was the mathematical analysis of band shapes in the absorption and fluorescence spectra of organic dyes. By that time, the scientific community already understood that the shape of a band in an electronic spectrum provides much more information about the electronic structure of the corresponding chromophore than do the traditional parameters of the band maximum and intensity. However, no methodology yet existed that would allow a meaningful comparison of the shapes of absorption and fluorescence bands of different

dyes and the formulation of reliable conclusions based on such analysis.

The only parameter then commonly used to describe band shapes was the full width at half maximum (FWHM), which, as Oleksandr Ishchenko demonstrated in his PhD (Candidate of Sciences) thesis *"The chemical structure and absorption band width of cyanine dyes"* (1980), is not sufficiently reliable for comparing bands of markedly different shapes, especially strongly structured ones. Instead, he proposed applying mathematical analysis of spectral bands using the method of moments, defining the criteria necessary for the use of this approach, such as the required signal depth at the band edges, the separation of higher electronic transitions (in absorption spectra), and recommendations for the approximation of band edges of different shapes [1, 2].

It should be noted that the young researcher was fortunate to have convenient model systems at hand, since for cyanines (cationic polymethines) the long-wavelength electronic transition is typically well separated from the higher ones, while the shape of these long-wavelength bands can vary significantly depending on the structure of the terminal heterocyclic fragments and the difference in their electron-donating abilities. Yet this "good fortune" was multiplied by enormous effort: an extensive analysis of the literature and selection of the optimal way to represent spectral bands (a question that, even today, lacks a universal consensus for fluorescence spectra [3]); a careful choice of compounds from among thousands of dyes available in the Department of Color and Structure; hundreds of measured and digitized spectra; writing and debugging a dedicated program in the era of punch cards; exhausting "night shifts" at the Glushkov Computational Center to obtain calculation results more quickly – making corrections to the tasks and resubmitting them for processing while the queue was shorter; and, finally, the analysis of the data obtained.

The result of this tremendous work was not merely a dissertation, but a genuinely advanced tool for the analysis of molecular spectra, as well as a significant broadening of his scientific outlook and deep familiarity with the literature in his chosen field – the electronic structure, spectral properties, and applications of polymethine dyes.

At that time, one of the most active and promising areas of application for functional dyes was laser optics. The young scientist continued to learn intensively, deepening his knowledge in this field and establishing broad scientific contacts with laser physicists in Lithuania, Belarus, Russia, and Kazakhstan. He soon realized that the requirements for dyes in this area extended far beyond the classical structure-color relationships. Accordingly, it became clear that new regularities had to be uncovered – in particular, those linking the molecular structure with the lifetime of its excited state. Here, in addition to the analysis of spectral bands by the method of moments, his background in theoretical and quantum chemistry proved invaluable. A striking example of the synergy between these approaches was Oleksandr Ishchenko's classical interpretation of the differences in band shapes and excited-state lifetimes of isomeric pyrilocyanines and related compounds [4], which opened the way to the rational design of dyes with record-short lifetimes for laser applications [5, 6].

Thanks to the initiative and efforts of the young scientist, the Department of the Color and Structure of Organic Compounds acquired the best spectrofluorometer available at the time (in 1984), which enabled the launch of active studies of the fluorescence properties of polymethines – a direction he himself led. Among the first notable results of this work was the discovery that the fluorescence bands of strongly asymmetric cyanines (with respect to the electron-donating strengths of their terminal groups), unlike their absorption bands, resemble in their shape, Brooker's deviations, and vinylene shifts the fluorescence bands of the corresponding symmetric parent dyes [7], which opened the way to the design of cyanines with record-high Stokes shifts. Moreover, the fluorescence solvatochromism of both asymmetric and symmetric cyanines was found to be determined almost entirely by the solvents polarizability (n_D) and to be independent of their general (ε_D) and specific (nucleophilicity, electrophilicity) polarities [7, 8]. Oleksandr Ishchenko concluded that this result implies both an increase in the electronic symmetry of polymethines in the fluorescent S_1 state compared with the ground S_0 state and a decrease in charge alternation within the chromophore. This interpretation was corroborated by quantum-chemical calculations. It is worth noting that the fluorescent properties of polymethines, and more broadly, fluorescence spectroscopy as a powerful tool for studying organic compounds, became the subject of a brilliant plenary lecture delivered by Oleksandr Ishchenko at the All-Ukrainian Conference in Uzhhorod in 1986, dedicated to

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Department of Color and Structure of Organic Compounds, Institute of Organic Chemistry, NAS of Ukraine (2008)

the 90th anniversary of the birth of Academician Andrii Ivanovych Kiprianov.

In Oleksandr Ishchenko's studies on the solvatochromism of polymethines, his broad scientific outlook and excellent theoretical background – as well as his deep understanding of solvation at the molecular level – were clearly manifested. These qualities formed the basis for his interpretation of the atypical spectral behavior of cyanines in weakly polar solvents [9]. In fact, he was one of the founders of a distinct line of research – the photonics of polymethine ion pairs and their associates.

Here, the knowledge he had gained during his student research projects proved particularly useful. Using NMR spectroscopy, he was able to experimentally determine the localization of counterions in cyanine ion pairs [10, 11] and, by analyzing aromatic solvent-induced shifts (ASIS), to show that in the first solvation shell of polymethines, the molecules of aromatic solvents are oriented predominantly parallel to the plane of the polymethine chromophore [8]. This effect proved to be so pronounced that in strongly nucleophilic pyridine, the long-wavelength absorption bands of cyanines, which undergo strong nucleophilic solvation and hence hypsochromic shifts and broadening, for example in DMF, appear similar to those observed in less polar solvents. These results served as the basis for both practical recommendations on solvent selection for the laser applications of polymethines and for subsequent studies on dye behavior in polymer matrices (composites), as well as for the use of dye-doped functional materials in light-energy conversion and quantum-electronic applications. In this latter field, Oleksandr Oleksandrovych became an internationally recognized expert; he was repeatedly invited to deliver plenary lectures at international conferences, to give lecture courses at Ben-Gurion University (Israel) and Karaganda State University (Kazakhstan), and to contribute review articles on the subject [5, 12].

The defense of his doctoral thesis in 1991, titled "*The Structure and Spectral-Luminescent Properties of Polymethine Dyes*", which later formed the basis of a monograph of the same name [13], just officially confirmed Oleksandr Ishchenko's scientific maturity and outstanding achievements in his chosen field of research. Another early marker of his success as a scientist was the awarding of the A.I. Kiprianov Prize of the NAS of Ukraine in 1997.

This essay cannot accommodate a full account of Oleksandr Ishchenko's numerous subsequent studies, reflected in more than 400 publications in scientific journals, dozens of patents, several book chapters, and four monographs. I will therefore mention only a few more of the most remarkable directions of his work.

The first of these is the study of photoconductive polymer composites, carried out in collaboration

O. O. Ishchenko (left) and M. O. Davydenko (right) showcase their laser defectoscopy device at the exhibition

with Dr.Sci. Mykola Oleksandrovych Davydenko. Among the topics of this extensive research were the influence of external electric fields on the spectral properties and electronic structure of symmetric and asymmetric polymethines in polymer matrices; the effect of the structure of dye dopants on the photoconductivity and photovoltaic characteristics of dyed polymer composites; and the development of highly efficient holographic recording media that require no protection from scattered light, among others [14, 15].

The second one, conducted in collaboration with several research groups – most notably with Volodymyr Ivanovych Bezrodnyi at the Institute of Physics of the NAS of Ukraine – was a development of Oleksandr Ishchenko's ideas on laser materials based on organic dyes [16–18]. The first successful demonstration worldwide of passive mode-locking of Erbium-doped laser at 1340 nm represented a particularly notable achievement in this area [19].

The third – one in which I was fortunate to participate – involved fundamental studies on the solvatochromism, electronic structure, and photophysical properties of merocyanines [20, 21]. The success of this pursuit owed much to Oleksandr Oleksandrovych's deep understanding of the electronic structure of conjugated systems, extensive use of the method of moments for spectral data analysis (using the program he had developed), and the broad collaboration with physicist-spectroscopists that he established. This collaboration made it possible, in particular, to obtain essential spectral data at cryogenic temperatures [22, 23], to record for the first time the absorption spectra of merocyanines in the gas phase [24], and to trace how the molecular structure of merocyanines affects their (photo)isomerization behavior and excited-state lifetimes [25, 26].

■ Imparting Knowledge

Yet throughout his long and fruitful scientific career, Oleksandr Oleksandrovych's defining passion was his tireless desire to share knowledge – to ignite curiosity and a love of science in people's hearts, regardless of their age. Even as a university student, he organized and conducted classes and lectures for schoolchildren and helped prepare prospective university applicants. And he never abandoned this mission after graduation.

Since the early 1990s, already a young Doctor of Sciences, he developed and taught a lecture course on electronic spectroscopy at his alma mater, the Faculty of Chemistry of Taras Shevchenko National University of Kyiv. Remarkably, he initially did so voluntarily and was formally appointed to a professorship only in 1998. His lectures – clear and accessible, yet truly fundamental and richly illustrated with examples both from his own research and from the most up-to-date scientific achievements – attracted not only

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students but also graduate students and even faculty members.

This popularity stemmed not only from his natural teaching talent and ability to connect with an audience but also from his meticulous preparation of both content and visual materials. Both components were continuously refined, improved, and expanded from year to year. Later, to the course on electronic spectroscopy, he added an independently designed special course on materials science and on physical methods for the investigation of chemical compounds. In addition to the Faculty of Chemistry, Oleksandr Ishchenko also taught several courses at the Educational and Research Institute of High Technologies of the same university from its founding in 2009.

■ Concluding Remarks

To encompass an entire human life within the bounds of a memorial article is a task that goes far beyond what is possible. A globe may serve as an adequate model of the Earth, yet it still conceals more than it reveals. Our imagination and experience remind us that behind every genuine success, every achievement that remains in people's memory, lie immense effort – above all, the labor of self-discipline and self-development – as well as a deep love: love for one's chosen path, for knowledge, for people, and the courage to make difficult choices in difficult times.

The scientific career of Professor Oleksandr Ishchenko was far from an "easy walk." Within the Department of Color and Structure of Organic Compounds, he advanced from engineer to the head of the Department – a position he held from 2006 until his sudden passing on July 31, 2024. In 2015, he was elected a Corresponding Member, and in 2021, a Full Member of the National Academy of Sciences of Ukraine. Yet these marks of recognition and professional success also meant that he had less time for what he loved most – the search for new knowledge and the joy of sharing it.

He had many ideas and plans for future studies and projects. He lived, worked, and thought creatively to the very end.

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■ References

1. Ishchenko, A. A.; Derevyanko, N. A.; Kudinova, M. A.; Dyadyusha, G. G.; Tolmachev, A. I. Chemical Structure and Shape of the Absorption Bands of Pyrylocyanine Dyes. *Theoretical and Experimental Chemistry* **1978**, *13* (6), 628–630. https://doi.org/10.1007/BF00520821.
2. Dyadyusha, G. G.; Ishchenko, A. A. Application of the Method of Moments to the Study of the Electronic Spectra of Organic Dyes. *Journal of Applied Spectroscopy* **1979**, *30* (6), 746–750. https://doi.org/10.1007/BF00615763.
3. Catalán, J. On the Mirror Symmetry between the Absorption and Emission of Complex Molecules in Solution. *Journal of Physical Organic Chemistry* **2020**, *33* (3), e4034. https://doi.org/10.1002/poc.4034.
4. Dyadyusha, G. G.; Ishchenko, A. A.; Derevyanko, N. A.; Tolmachev, A. I. Absorption Bandwidth of Isomeric Polymethine Dyes. *Journal of Applied Spectroscopy* **1984**, *41* (6), 1400–1404. https://doi.org/10.1007/BF00659321.
5. Ishchenko, A. A. Laser Media Based on Polymethine Dyes. *Quantum Electronics* **1994**, *24* (6), 471–492. https://doi.org/10.1070/qe1994v024n06abeh000122.
6. Ishchenko, A. A. Physicochemical Aspects of the Creation of Modern Light-Sensitive Materials Based on Polymethine Dyes. *Theoretical and Experimental Chemistry* **1998**, *34* (4), 191–210. https://doi.org/10.1007/BF02523249.
7. Ishchenko, A. A.; Derevyanko, N. A.; Svidro, V. A. Constitution and Fluorescence Spectra of Unsymmetrical Polymethine Dyes. *Dyes and Pigments* **1992**, *19* (3), 169–177. https://doi.org/10.1016/0143-7208(92)80023-G.
8. Ishchenko, A. A.; Svidro, V. A.; Derevyanko, N. A. Solvatofluorochromy of Cationic Cyanine Dyes. *Dyes and Pigments* **1989**, *10* (2), 85–96. https://doi.org/10.1016/0143-7208(89)85001-6.
9. Dyadyusha, G. G.; Ishchenko, A. A.; Derevyanko, N. A.; Tolmachev, A. I. Study of Ionic Equilibria of Indotricarbocyanines in Aromatic Hydrocarbons. *Doklady Physical Chemistry* **1982**, *264* (2), 351–355.
10. Komarov, I. V.; Turov, A. V.; Kornilov, M. Yu.; Derevyanko, N. A.; Ishchenko, A. A. Study of the Chemical Structure of Ion Pairs of Cationic Cyanine Dyes in Weakly Polar Solvents by the Method of PMR Spectroscopy with the Use of Lanthanide Shift Reagents. *Journal of General Chemistry of the USSR* **1989**, *59* (10), 2110–2115.
11. Komarov, I. V.; Turov, A. V.; Ishchenko, A. A.; Derevyanko, N. A.; Kornilov, M. Yu. Study of the Structure of Ion Pairs of Cationic Cyanine Dyes Using Lanthanide Chelates and NMR Quadrupole Nuclei. *Doklady. Chemistry* **1989**, *306* (5), 178–181.
12. Ishchenko, A. A. Photonics and Molecular Design of Dye-Doped Polymers for Modern Light-Sensitive Materials. *Pure and Applied Chemistry* **2008**, *80* (7), 1525–1538. https://doi.org/10.1351/pac200880071525.
13. Ishchenko, A. A. *Structure and Spectral-Luminescent Properties of Polymethine Dyes [in Russian]*; Naukova Dumka: Kyiv, 1994.
14. Davidenko, N. A.; Ishchenko, A. A.; Kuvshinsky, N. G. *Photonics of Molecular Semiconductor Composites Based on Organic Dyes [in Russian]*; Naukova Dumka: Kyiv, 2005.
15. Bulavko, G. V.; Ishchenko, A. A. *Organic Photovoltaic Structures [in Ukrainian]*; Naukova Dumka: Kyiv, 2023. https://doi.org/10.15407/978-966-00-1839-6.

16. Bezrodnyĭ, V. I.; Ishchenko, A. A.; Karabanova, L. V; Slominskiĭ, Y. L. Highly Stable Polymethine-Dye-Based Polymer Switches for Passive Mode Locking in Neodymium Lasers. *Quantum Electronics* **1995**, *25* (8), 819–822. https://doi.org/10.1070/QE1995v025n08ABEH000477.
17. Bezrodnyi, V. I.; Ishchenko, A. A. High Efficiency Lasing of a Dye-Doped Polymer Laser with 1.06 Mm Pumping. *Applied Physics B: Lasers and Optics* **2001**, *73* (3), 283–285. https://doi.org/10.1007/s003400100646.
18. Bezrodna, T.; Bezrodnyi, V.; Ishchenko, A.; Slominskii, Y.; Sharanov, I. New Polymethine Dyes for Liquid and Polymer Passive Q-Switches of Neodymium Lasers. *Optik* **2022**, *267*, 169725. https://doi.org/10.1016/j.ijleo.2022.169725.
19. Bezrodnyĭ, V. I.; Derevyanko, N. A.; Ishchenko, A. A.; Slominskiĭ, Y. L. Polymer Passive Laser Switches for Stimulated Emission in the Region of 1.3 Mm. *Quantum Electronics* **1995**, *25* (8), 823–825. https://doi.org/10.1070/QE1995v025n08ABEH000478.
20. Kulinich, A. V.; Ishchenko, A. A. *Electronic Structure and Spectral and Fluorescence Properties of Merocyanines [in Ukrainian]*; Naukova Dumka: Kyiv, 2022.
21. Kulinich, A. V.; Ishchenko, A. A. Merocyanines: Electronic Structure and Spectroscopy in Solutions, Solid State, and Gas Phase. *Chemical Reviews* **2024**, *124* (21), 12086–12144. https://doi.org/10.1021/acs.chemrev.4c00317.
22. Kulinich, A. V.; Ishchenko, A. A.; Bondarev, S. L.; Knyukshto, V. N. Low-Temperature Effect on the Electronic Structure and Spectral-Fluorescent Properties of Highly Dipolar Merocyanines. *Journal of Physical Chemistry A* **2018**, *122* (50), 9645–9652. https://doi.org/10.1021/acs.jpca.8b09522.
23. Kulinich, A. V.; Ishchenko, A. A.; Bondarev, S. L.; Knyukshto, V. N. Effect of Donor and Acceptor End-Groups on Electronic Structure and Spectral-Fluorescent Properties of Merocyanines in Frozen Ethanol. *Journal of Photochemistry and Photobiology A: Chemistry* **2021**, *405*, 112932. https://doi.org/10.1016/j.jphotochem.2020.112932.
24. Ishchenko, A. A.; Kulinich, A. V.; Bondarev, S. L.; Raichenok, T. F. UV–Vis Absorption Spectra and Electronic Structure of Merocyanines in the Gas Phase. *Spectrochimica Acta – Part A: Molecular and Biomolecular Spectroscopy* **2018**, *190*, 332–335. https://doi.org/10.1016/j.saa.2017.09.054.
25. Kulinich, A. V.; Ishchenko, A. A.; Chibisov, A. K.; Zakharova, G. V. Effect of Electronic Asymmetry and the Polymethine Chain Length on Photoprocesses in Merocyanine Dyes. *Journal of Photochemistry and Photobiology A: Chemistry* **2014**, *274*, 91–97. https://doi.org/10.1016/j.jphotochem.2013.09.016.
26. Voiciuk, V.; Redeckas, K.; Derevyanko, N. A.; Kulinich, A. V.; Barkauskas, M.; Vengris, M.; Sirutkaitis, V.; Ishchenko, A. A. Study of Photophysical Properties of a Series of Polymethine Dyes by Femtosecond Laser Photolysis. *Dyes and Pigments* **2014**, *109*, 120–126. https://doi.org/10.1016/j.dyepig.2014.05.012.

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The Interaction of *N*-(*tert*-butylsulfinyl)imine of Trifluoropyruvate with Diazomethane as a Convenient Synthetic Approach to Enantiomeric Trifluoromethylamino Acids

Abstract
The interaction of enantiomerically pure *N*-*tert*-butylsulfinyl imines of trifluoropyruvate with diazomethane has been studied. It has been shown that there is the [3+2]-cycloaddition at the initial step with the formation of diastereomeric trifluoromethyltriazoline carboxylates in the ratio of 5.6:1. Treating the triazoline carboxylates with trifluoroacetic acid yielded optically pure aziridine carboxylates, which were subsequently converted into their corresponding acids. When subjected to hydrochloric acid in an ethereal solution, trifluoromethylaziridines underwent ring-opening and the sulfinyl group removal, producing α-chloromethylamino acids. The study also demonstrates the potential use of these aziridinecarboxylic acids in the peptide synthesis.
***Keywords*:** amino acids; trifluoromethyl; diazomethane; enantiomeric; *tert*-butylsulfinyl; imine

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Взаємодія *N*-(трет-бутилсульфініл)іміну трифторопірувату з діазометаном як зручний синтетичний підхід до енантіомерних трифторометиламінокислот

Анотація
Досліджено взаємодію енантіомерно чистих *N*-трет-бутилсульфініліміні трифторопірувату з діазометаном. З'ясовано, що на першому етапі відбувається [3+2]-циклоприєднання з утворенням діастереомерних трифторометилтриазолінкарбоксилатів у співвідношенні 5.6:1. Енантіомерно чисті триазоліни було виділено в індивідуальному стані методом перекристалізації. Дія трифтороцтової кислоти на триазолінкарбоксилати приводить до утворення оптично чистих азиридинкарбоксилатів, які були перетворені на відповідні кислоти. В ефірному розчині хлоридної кислоти трифторометилазиридини зазнають розкриття циклу, що супроводжується видаленням сульфінільної групи та приводить до утворення α-хлорометиламінокислот. Продемонстровано можливість застосування азиридинкарбонових кислот у пептидному синтезі.
Ключові слова: амінокислоти; трифторометил; діазометан; енантіомерний; трет-бутилсульфініл; імін

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■ Introduction

α-Amino acids are fundamental to life, not only serving as the structural components of proteins, but also as crucial precursors for other biologically important molecules, including hormones, neurotransmitters, and purines. Consequently, numerous methods for the synthesis of both natural and unnatural α-amino acids have been developed. They allow the creation of new drugs, functional materials, and protein analogs for scientific research. The site-selective introduction of fluorine-containing groups into amino acids allows for the targeted modification of their structure, thereby altering specific physicochemical and/or biological properties [1–7]. Therefore, introducing fluorine into an amino acid is an effective strategy for protecting peptide bonds from enzymatic cleavage, which significantly increases the stability of peptides and proteins in biological systems. Trifluoropyruvate imines contain an oxidized trifluoroalanine fragment, which allows for their reductive functionalization at the azomethine bond. This provides an efficient route to acyclic and heterocyclic derivatives of trifluoromethyl substituted amino acids (CF_3-α-AAs) [8–12].

A prime example of heterocyclization of trifluoropyruvate imines **I** is their interaction with diazomethane, which proceeds *via* the [3+2]-cycloaddition scheme and leads to triazolines **II**, which further transformation provided the first known examples of water-soluble 2-(trifluoromethyl)aziridine-2-carboxylic acids **III** [13–15] (**Figure 1**). This transformation is important since triazoline and aziridine rings can be considered as "built-in" heterocyclic prodrugs due to their chemical reactivity and propensity for ring opening [16].

At the same time, stereochemical aspects are an integral part of the chemistry of α-amino acids since their optical antipodes interact differently with the active sites of receptors, leading to different, and sometimes even opposite, biological activities towards target molecules. Therefore, the synthesis of compounds containing a chiral stereocenter in optically pure form remains an important task. So, imines **I** with a stereo-directing *N*-phenylethyl group were among those investigated in their reaction with diazomethane (**Figure 1**). They were shown to form a mixture of two diastereomeric triazolines **II** in the ratio of 4.5:1, which could be separated chromatographically [15].

Recently, a preparative method for the synthesis of *N*-*tert*-butylsulfinyl imines of trifluoropyruvate **1** has been developed, and it has been shown that this type of imines are convenient substrates for the preparation of enantiomerically pure CF_3-α-AAs derivatives [10]. However, the reaction between *N*-sulfinyl imine **1** and diazomethane has not been previously studied. At the same time, they have significant advantages over *N*-phenylethyl analogs [15], namely a relatively higher reactivity towards nucleophilic agents and the possibility of removing the sulfinyl group from the nitrogen atom after carrying out the necessary transformations. The latter factor is particularly important as it will allow the production of compounds with a *free* amino group, which will significantly expand the scope of the products in synthetic practice.

This work reports the results of the study on the interaction between *N*-*tert*-butylsulfinyl imines of trifluoropyruvate **1** and diazomethane. The subsequent transformations of the resulting products are explored as a pathway to enantiomerically pure CF_3-α-AA derivatives.

■ Results and discussion

It has been found that (S_S)-*N*-*tert*-butylsulfinyl imine of trifluoropyruvate (S_S)-**1** reacts with diazomethane similarly to other fluorinated imines, giving at the first stage the products of the [3+2]-cycloaddition – triazolines (S,S_S)-**2**/(R,S_S)-**2** isolated as a mixture of diastereomers in the ratio of 5.6:1 with the total yield of 99% (**Scheme 1**). Note that the cycloaddition for *N*-sulfinyl imine **1** occurs much faster, completing in minutes, compared to *N*-phenylethyl imine **II** under similar conditions, which takes five days [15]. The reaction progress is easily monitored by ^{19}F NMR spectroscopy, the $sp^2 \rightarrow sp^3$ rehybridization of the imino carbon atom causes a shift in the trifluoromethyl group signal from -71 ppm to -73.5 ppm (minor diastereomer) and -74.3 ppm (major diastereomer). While the diastereoselectivity of the

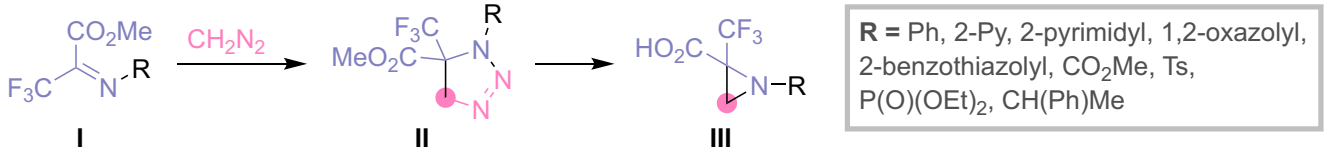

Figure 1. The interaction of trifluoropyruvate imines **I** with diazomethane

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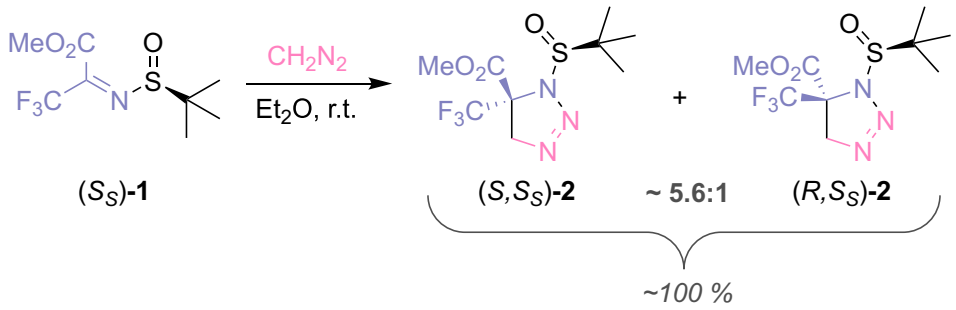


Scheme 1. The cycloaddition of diazomethane to the enantiomeric trifluoropyruvate imine (S_S)-**1**

process typically depends on temperature, the ratio of diastereomers (S,S_S)-**2**/(R,S_S)-**2** in this reaction remains unchanged, even when the temperature is lowered to -78°C.

Triazolines **2** are stable compounds that can be stored long-term without losing their chemical or optical purity. The major diastereomer (S,S_S)-**2** was easily isolated in the yield of 40% in an optically pure form by the trituration with the MTBE/hexane mixture (1:1).

The X-ray diffraction analysis of the single crystal confirmed the (*S*)-configuration of the newly created stereocenter in the major triazoline (S,S_S)-**2**, which was formed from the (S_S)-imine **1** (**Figure 2**). The partially unsaturated nature of the triazole ring caused a slightly pyramidal configuration of the N1 atom (the sum of bond angles centered at this atom is 352.1°). The *tert*-butylsulfinyl substituent was found in the *cis*-conformation to the carboxylate group (the C4–C2–N1–S1 torsion angle is -43.3(8)°) and is turned in such a way that the S1=O1 bond is orthogonal to the C2–N1 endocyclic bond (the C2–N1–S1–O1 torsion angle is 91.6(6)°). The planar carboxylate group is rotated in relation to the triazole ring by -67.9(9)° (the N1–C2–C4–O2 torsion angle).

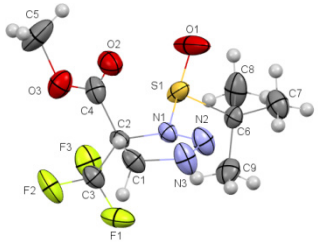
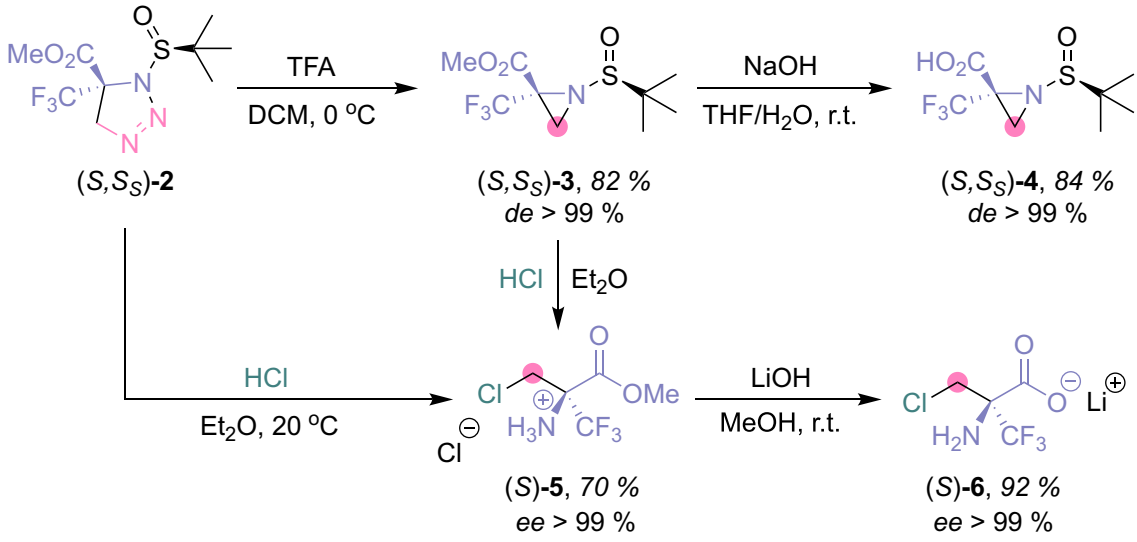


Figure 2. The molecular structure of triazolinecarboxylate (S,S_S)-**2**. Thermal ellipsoids of non-hydrogen atoms are shown at 50 % probability level

Using reaction conditions identical to **Scheme 1**, we obtained diastereomers (R,R_S)-**2** and (S,R_S)-**2** (5.6:1) by reacting diazomethane with the imine of trifluoropyruvate **1** in the (R_S)-configuration.

The acid catalysis with trifluoroacetic acid (0.1 equiv., 0 °C, DCM) caused triazoline (S,S_S)-**2** to undergo the denitrogenation, leading to the quantitative formation of aziridine carboxylate (S,S_S)-**3**, isolated in the yield of 82% (**Scheme 2**). It is worth noting that the ethyl ester of aziridinecarboxylic acid **3** was previously prepared from the corresponding trifluoropyruvate imine using the aza-Corey-Chaykovsky reaction [17]. In contrast to our findings, this approach produced



Scheme 2. The synthesis of aziridine carboxylic acid (S,S_S)-**4** and polyfluorinated chloroethylamino acid derivatives (*S*)-**5**, (*S*)-**6**

the compound in only milligram quantities and with a modest 24% yield.

The sequential treatment of aziridine carboxylate (S,S_S)-**3** with NaOH and diluted acid converted it to aziridine carboxylic acid (S,S_S)-**4** with the yield of 84%. Mild conditions for converting triazoline **2** → aziridine carboxylate **3** → aziridine carboxylic acid **4** allow to avoid opening of the three-membered ring and preserve the protecting sulfinyl group on the nitrogen atom. Additionally, NMR data shows the presence of only one stereoisomer of the aziridines **3**, **4** formed, indicating that the optical purity of the reaction products is preserved. Since the chiral carbon atom is not involved in the **2** → **3** transformation, the stereocenter's configuration in aziridines (S,S_S)-**3**, **4** is maintained.

In the ethereal solution of hydrochloric acid at room temperature, aziridine (S,S_S)-**3** undergoes ring opening, accompanied by removal of the sulfinyl group. As a result, a chloromethyl derivative of trifluoroalanine (*S*)-**5** is formed, isolated in the optically pure form as hydrochloride. Compound (*S*)-**5** can be also obtained directly from triazoline (S,S_S)-**3** by the action of hydrochloric acid on the latter.

It should be noted that the hydrolysis of esters containing several electron-withdrawing substituents near the quaternary carbon atom is often accompanied by the decarboxylation. This process can be either secondary or dominant, depending on the environment around the carbon atom. The decarboxylation can be avoided by obtaining amino acid (*S*)-**6** as a water-soluble lithium salt.

The trifluoromethyl-substituted aziridine-2-carboxylic acid (S,S_S)-**4** (CF_3-Azy) synthesized can be used in the peptide synthesis primarily as a unique electrophilic building block for the late-stage, site-selective chemical modification of peptides. Its highly strained three-membered aziridine ring provides an active anchor for the attachment of various complex molecules, while the trifluoromethyl group can be served as a specific ^{19}F NMR label. The possibility of their use in the peptide synthesis was demonstrated in the **Scheme 3**. For this purpose, compound (S,S_S)-**4** was converted *in situ* to the corresponding anhydride using oxalyl chloride, and the resulting intermediate was subsequently reacted with the (*R*)-phenylglycine methyl ester to give dipeptide (R,S,S_S)-**7**. Notably, the aziridine ring remains intact under these conditions, but undergoes opening with the removal of the sulfinyl group upon treatment with hydrochloric acid in dioxane, forming dipeptide (*R,S*)-**8**, which contains a free amino group and can thus be involved in the further peptide chain extension.

Conclusions

The reaction of *N-tert*-butylsulfinyl imines of trifluoropyruvate and diazomethane leads to the quantitative formation of [3+2]-cycloaddition products, triazolines, in the ratio of 5.6:1. Upon the acid catalysis, the major (S,S_S)-triazoline is converted to enantiomeric aziridine carboxylate (S,S_S)-**3**, which after the reaction with NaOH gives optically pure (S,S_S)- aziridine carboxylic acid. The latter is involved to the peptide synthesis.

Acknowledgments

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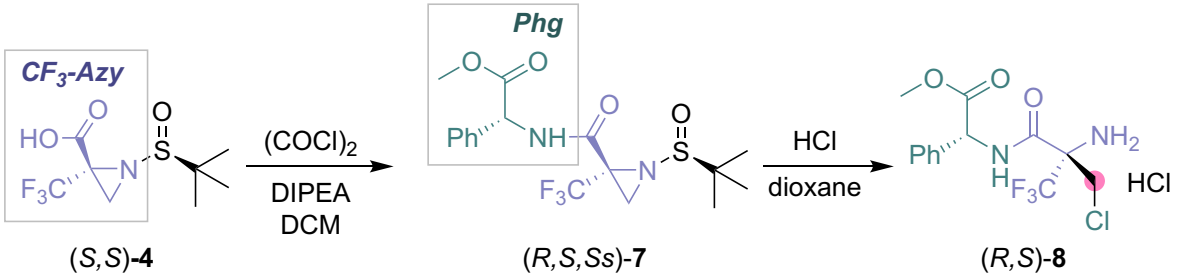
Experimental part

NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer with operating frequencies of 600 MHz (^1H), 150.8 MHz (^{13}C), and 470 MHz (^{19}F); a Bruker Avance DRX 500 spectrometer with operating frequencies of 499.9 MHz (^1H), 125.6 MHz (^{13}C), and 376.5 MHz (^{19}F); a Varian Unity Plus 400 instrument with operating frequencies of 400 MHz (^1H), 100 MHz (^{13}C) and 376.5 MHz (^{19}F); a Mercury Varian Unity Plus 300



Scheme 3. An example of the use of aziridine carboxylic acid (S,S_S)-**4** in the peptide synthesis

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instrument with operating frequencies of 300 MHz (^{1}H) and 76 MHz (^{13}C); a Mercury VX 200 Varian instrument with operating frequency of 188 MHz (^{19}F). Chemical shifts were reported relative to the internal TMS (^{1}H, ^{13}C) or $CFCl_3$ (^{19}F) standards. The optical rotation was measured on an Anton Paar MCP 300 polarimeter (the sample cell path length – 100 mm, the wavelength – 589 nm). The solvents were dried according to the standard procedures. The starting materials were purchased from Enamine Ltd. Melting points were uncorrected. TLC was performed using Kieselgel Merck 60 silica gel (400–630 mesh) as the stationary phase. The elemental analysis was performed in the analytical laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine.

Methyl *(S)*-1-((*S*)-tert-butylsulfinyl)-5-(trifluoromethyl)-4,5-dihydro-1H-1,2,3-triazole-5-carboxylate ((S,S_S)-**2**)

A cooled solution of diazomethane (41.8 mmol) in diethyl ether (250 mL) was added dropwise to the solution of (*S*)-*N-tert*-butylsulfinyl imine of trifluoropyruvate (*S*)-**1** (5.4 g, 20.9 mmol) in diethyl ether (50 mL) at room temperature. The reaction mixture was kept at r.t. for 2 h. ^{19}F NMR spectra showed the formation of the diastereomeric mixture of triazolinecarboxylates (S,S_S)-**2**/(R,S_S)-**2** (5.6:1). The solvent was evaporated to give product (S,S_S)-**2**/(R,S_S)-**2** (6.2 g, ~100%), which was triturated with the mixture of MTBE/hexane (1:1), a white solid was filtrated to obtain the major diastereomer (S,S_S)-**2**.

A white solid. Yield – 2.52 g (40%). M. p. = 90–100°C (dec.). $[\alpha]_D^{20}$ = +533.59 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_9H_{14}F_3N_3O_3S$, %: C 35.87, H 4.84, N 13.94. Found, %: C 36.05, H 4.79, N 14.10. ^{1}H NMR (301.5 MHz, $CDCl_3$), δ, ppm: 1.47 (9H, s, *t*Bu), 3.93 (3H, s, CH_3O), 4.80 (1H, d, $^2J_{HH}$ = 18 Hz, CH_2), 5.13 (1H, d, $^2J_{HH}$ = 18 Hz, CH_2). ^{13}C NMR (125.6 MHz, $CDCl_3$), δ, ppm: 23.49 ($C(\underline{C}H_3)_3$), 53.94 (CH_2), 61.76 ($\underline{C}(CH_3)_3$), 67.00 (q, $^2J_{CF}$ = 30 Hz, $\underline{C}CF_3$), 75.58 (CH_3O), 122.2 (q, $^1J_{CF}$ = 282 Hz, CF_3), 163.7 (C=O). ^{19}F NMR (188.1 MHz, $CDCl_3$), δ, ppm: -72.9.

The X-ray experimental part

The colorless crystals of compound (S,S_S)-**2** ($C_9H_{14}N_3O_3S$) are trigonal. At 173 K $a = b$ = 10.1183(3), c = 11.3463(4) Å, V = 1006.00(7) Å^3, M_r = 301.29, Z = 3, space group $P3_2$, d_{calc}= 1.492 g/cm^3, m(MoK_a) = 0.285 mm^{-1}, F(000) = 468. Intensities of 10656 reflections (2351 independent, R_{int}=0.0360) were measured on a Bruker APEX II diffractometer (graphite monochromated MoK_a radiation, a CCD detector, φ- and ω-scaning, $2\Theta_{max}$ = 50°). The structure was solved by the direct method using the OLEX2 [18] package with SHELXT [19] and SHELXL modules [20]. Positions of the hydrogen atoms were located from electron density difference maps and refined using the "riding" model with U_{iso} = nU_{eq} (n = 1.5 for methyl groups and n = 1.2 for other hydrogen atoms) of the carrier atom. Full-matrix least-squares refinement against F^2 in the anisotropic approximation for non-hydrogen atoms using 2351 reflections was converged to wR_2 = 0.1459 (R_1 = 0.0580 for 2099 reflections with $F > 4\sigma(F)$, S = 1.046). The final atomic coordinates, and crystallographic data for molecule (S,S_S)-**2** were deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 2497351).

Methyl (*S*)-1-((S)-tert-butylsulfinyl)-2-(trifluoromethyl)aziridine-2-carboxylate ((S,S_S)-**3**)

Trifluoroacetic acid (0.094 g, 0.063 mL, 0.83 mmol) was added to the solution of triazolinecarboxylate (S,S_S)-**2** (2.5 g, 8.3 mmol) in DCM (20 mL) at 0 °C. The reaction mixture was kept at r.t. for 4 h, washed with the saturated aqueous solution of $NaHCO_3$, the organic layer was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give a pure aziridine carboxylate (S,S_S)-**3**.

A white solid. Yield – 1.87 g (82%). M. p. = 65–67 °C. $[\alpha]_D^{20}$= -238.62 (*c* 1, $CHCl_3$). Anal. Calcd for $C_9H_{14}F_3NO_3S$, %: C 39.55, H 5.16, N 5.12. Found, %: C 39.37, H 5.20, N 4.88. ^{1}H NMR (301.5 MHz, $CDCl_3$), δ, ppm: 1.24 (9H, s, *t*Bu), 2.44 (1H, s, CH_2), 3.14 (1H, s, CH_2), 3.85 (3H, s, CH_3O). ^{13}C NMR (150.8 MHz, $CDCl_3$), δ, ppm: 22.17 ($C(\underline{C}H_3)_3$), 26.93 (CH_2), 44.0 (q, $^2J_{CF}$ = 38 Hz, $\underline{C}CF_3$), 53.69 (CH_3O), 58.16 ($C(\underline{C}H_3)_3$), 121.6 (q, $^1J_{CF}$ = 276 Hz, $\underline{C}F_3$), 162.8 (C=O). ^{19}F NMR (188.1 MHz, $CDCl_3$), δ, ppm: -71.0.

(*S*)-1-((*S*)-tert-butylsulfinyl)-2-(trifluoromethyl)aziridine-2-carboxylic acid ((S,S_S)-**4**)

Aziridine carboxylate (S,S_S)-**3** (1.8 g, 6.6 mmol) was added to a stirred solution of NaOH (0.52 g, 13 mmol) in THF (10 mL) and water (10 mL). The resulting reaction mixture was left at r.t. overnight. The organic solvent was removed in *vacuo*, the resulting water solution was washed with dichloromethane (5 mL) and acidified to pH 2-3 with the saturated sodium hydrogen sulfate solution. The solution was extracted with DCM (3×15 mL), dried over Na_2SO_4, the solvent was evaporated under reduced pressure to give a pure aziridine carboxylic acid (S,S_S)-**4**.

A white solid. Yield – 1.5 g (84%). M. p. = 115–120 °C (dec.). $[\alpha]_D^{20}$ = -192.51 (*c* 1, $CHCl_3$). Anal. Calcd. for $C_8H_{12}F_3NO_3S$, %: C 37.06, H 4.66, N 5.40. Found, %: C 37.38, H 4.59, N 5.49. ^{1}H NMR (301.5 MHz, $CDCl_3$), *δ*, ppm: 1.30 (9H, s, *t*Bu), 2.48 (1H, s, CH_2), 3.22 (1H, s, CH_2), 8.79 (1H, s, COOH). ^{13}C NMR (75.8 MHz, $CDCl_3$), *δ*, ppm: 22.33 ($C(\underline{C}H_3)_3$), 27.75 (CH_2), 44.39 (q, $^2J_{CF}$ = 37 Hz, $\underline{C}CF_3$), 58.94 ($C(\underline{C}H_3)_3$), 121.69 (q, $^1J_{CF}$ = 277 Hz, $\underline{C}F_3$), 162.84 (C=O). ^{19}F NMR (188.1 MHz, $CDCl_3$), *δ*, ppm: -72.0.

Methyl (*S*)-2-amino-2-(chloromethyl)-3,3,3-trifluoropropanoate hydrochloride ((*S*)-**5**)

A dioxane solution of hydrogen chloride (10 mL) was added to the stirred solution of (*S*,S_S)-**3** (1.86 g, 6.8 mmol) in MeOH (100 mL) at 0 °C. The reaction mixture was allowed to warm up to r.t. and was kept for 6 h. The solvent was removed under reduced pressure; the residue was triturated with hexane and filtered to give compound (*S*)-**5**.

A white solid. Yield – 1.15 g (70%). M. p. = 154–156 °C. $[\alpha]_D^{20}$ = -0.37 (*c* 0.5, MeOH). Anal. Calcd for $C_5H_8Cl_2F_3NO_2$, %: C 24.81, H 3.33, Cl 29.29, N 5.78. Found, %: C 24.72, H 3.47, Cl 29.77, N 5.67. ^{1}H NMR (301.5 MHz, DMSO-d_6), *δ*, ppm: 3.85 (3H, s, CH_3O), 4.0 (1H, d, $^2J_{HH}$ = 12.1 Hz, CH_2), 4.2 (1H, d, $^2J_{HH}$ = 12.1 Hz, CH_2), 5.87 (2H, s, NH_2). ^{13}C NMR (100.6 MHz, DMSO-d_6), *δ*, ppm: 42.73 ($(\underline{C}H)_2$), 55.26 (CH_3O), 65.62 (q, $^2J_{CF}$=28 Hz, $\underline{C}CF_3$), 122.68 (q, $^1J_{CF}$ = 286 Hz, $\underline{C}F_3$), 164.01 (C=O). ^{19}F NMR (188.1 MHz, DMSO-d_6), *δ*, ppm -72.4.

Lithium (*S*)-2-amino-2-(chloromethyl)-3,3,3-trifluoropropanoate ((*S*)-**6**)

Lithium hydroxide (0.04 g, 1.6 mmol) was added to the solution of compound (*S*)-**5** (0.2 g, 0.8 mmol) in methanol (10 mL). The mixture was stirred at r.t. for 16 h. The precipitate formed was filtered off, the solution was evaporated under reduced pressure to obtain a white crystalline residue (S)-**6**.

White crystals. Yield – 0.15 g (92%). M. p. = 160 °C (dec.). Anal. Calcd for $C_4H_4ClF_3LiNO_2$, %: C 24.32, H 2.04, Cl 17.95, N 7.09. Found, %: C 24.06, H 2.23, Cl 18.33, N 6.78. ^{1}H NMR (301.5 MHz, DMSO-d_6), *δ*, ppm: 4.11–4.24 (2H, m, CH_2), 5.12 (2H, s, NH_2). ^{13}C NMR (100.6 MHz, DMSO-d_6), *δ*, ppm: 44.56 (CH_2Cl), 66.46 (q, $^2J_{CF}$ = 30 Hz, $\underline{C}CF_3$), 124.83 (q, $^1J_{CF}$ = 280 Hz, $\underline{C}F_3$), 165.83 (C=O). ^{19}F NMR (188.1 MHz, DMSO-d_6), *δ*, ppm: -69.84.

Methyl (*R*)-2-((*S*)-1-((*S*)-tert-butylsulfinyl)-2-(trifluoromethyl)aziridine-2-carboxamido)-2-phenylacetate ((*R*,*S*,S_S)-**7**)

Oxalyl chloride (0.42 g, 3.2 mmol) was added to the stirring solution of aziridine carboxylic acid (*S*,S_S)-**4** (0.28 g, 1.08 mmol) and DIPEA (0.7 g, 5.4 mmol) in DCM (5 mL) at 0 °C. The reaction mixture was allowed to warm up to r.t. and stirred for 1 h. The solvent was removed under reduced pressure. The residue was dissolved in DCM (5 mL) and added dropwise to the stirring mixture of (*R*)-phenylglycine methylester hydrochloride (0.217 g, 1.08 mmol) and DIPEA (0.42 g, 3.2 mmol) in DCM (5 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 24 h. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexane:EtOAc, 1:1, Rf = 0.6) to give a compound (*R*,*S*,S_S)-**7**.

A white solid. Yield – 135 mg (30%). M. p. = 138–140 °C. $[\alpha]_D^{20}$ = -292.3 (*c* 0.25, DCM). Anal. Calcd for $C_{17}H_{21}F_3N_2O_4S$, %: C 50.24, H 5.21, N 6.89. Found, %: C 50.32, H 5.17, N 6.83. ^{1}H NMR (301.5 MHz, $CDCl_3$), *δ*, ppm: 1.23 (9H, s, *t*Bu), 2.40 (1H, s, CH_2), 3.12 (1H, s, CH_2), 3.72 (3H, s, CH_3O), 5.55 (1H, d, $^2J_{HH}$ = 6.8 Hz, CH), 7.29–7.41 (6H, m, Ph+NH). ^{13}C NMR (150.8 MHz, $CDCl_3$), *δ*, ppm: 22.27 ($C(\underline{C}H_3)_3$), 30.48 (CH_2), 44.55 (q, $^2J_{CF}$ = 30 Hz, $\underline{C}CF_3$), 52.87 (CH_3O), 56.10 (CHPh) 59.38 ($\underline{C}(CH_3)_3$), 121.72 (q, $^1J_{CF}$ = 281 Hz, CF_3), 127.53 (C_{Ar}), 128.56 (C_{Ar}), 128.85 (C_{Ar}), 135.87 (C_{Ar}), 163.81 ((C=O)NH), 171.64 (COOMe). ^{19}F NMR (188.1 MHz, $CDCl_3$), *δ*, ppm: -71.5 ppm.

Methyl (*R*)-2-((*S*)-2-amino-2-(chloromethyl)-3,3,3-trifluoropropanamido)-2-phenylacetate hydrochloride ((*R*,*S*)-**8**)

A dioxane solution of hydrogen chloride (2 mL) was added to the dipeptide (*R*,*S*,S_S)-**7** (85 mg, 0.21 mmol) in dioxane (2 mL) at 0 °C. The reaction mixture was allowed to warm up to r.t. and kept for 2 h. The solvent was removed under reduced pressure. The residue was triturated with hexane and filtered to give compound (*R*,*S*)-**8**.

A white solid. Yield – 60 mg (85%). M. p. = 160 °C. Anal. Calcd for $C_{13}H_{15}Cl_2F_3N_2O_3$, %: C 41.61, H 4.02, Cl 18.90, N 7.46. Found, %: C 41.45, H 4.15, Cl 18.72, N 7.59. ^{1}H NMR (301.5 MHz, DMSO-d_6), *δ*, ppm: 3.91 (1H, d, $^2J_{HH}$ = 11.8 Hz, CH_2), 4.20 (1H, d, $^2J_{HH}$ = 11.8 Hz, CH_2), 5.48 (1H, d, $^2J_{HH}$ = 6.7 Hz, CH), 7.29–7.46 (5H, m, Ph), 8.87 (2H, br s, NH_2). ^{13}C NMR (150.8 MHz, DMSO-d_6), *δ*, ppm: 47.48 (CH_2), 52.81 (CH_3O), 56.10 (CHPh), 68.43 (q, $^2J_{CF}$ = 32 Hz, $\underline{C}CF_3$), 123.72 (q, $^1J_{CF}$ = 279 Hz, $\underline{C}F_3$), 128.53 (C_{Ar}), 129.56 (C_{Ar}), 129.85 (C_{Ar}), 136.38 (C_{Ar}), 167.45 ((C=O)NH), 171.45 (COOMe). ^{19}F NMR (188.1 MHz, DMSO-d_6), *δ*, ppm: -73.0.

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References

1. Bégué, J.; Bonnet-Delpon, D. *Bioorganic and Medicinal Chemistry of Fluorine*, 1st ed.; Wiley, 2008. https://doi.org/10.1002/9780470281895.
2. Zanda, M. Trifluoromethyl Group: An Effective Xenobiotic Function for Peptide Backbone Modification. *New J. Chem.* **2004**, *28* (12), 1401. https://doi.org/10.1039/b405955g.
3. Black, W. C.; Bayly, C. I.; Davis, D. E.; Desmarais, S.; Falgueyret, J.-P.; Léger, S.; Li, C. S.; Massé, F.; McKay, D. J.; Palmer, J. T.; Percival, M. D.; Robichaud, J.; Tsou, N.; Zamboni, R. Trifluoroethylamines as Amide Isosteres in Inhibitors of Cathepsin K. *Bioorg. Med. Chem. Lett.* **2005**, *15* (21), 4741–4744. https://doi.org/10.1016/j.bmcl.2005.07.071.
4. Gauthier, J. Y.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J.-P.; Kimmel, D. B.; Lamontagne, S.; Léger, S.; LeRiche, T.; Li, C. S.; Massé, F.; McKay, D. J.; Nicoll-Griffith, D. A.; Oballa, R. M.; Palmer, J. T.; Percival, M. D.; Riendeau, D.; Robichaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Thérien, M.; Truong, V.-L.; Venuti, M. C.; Wesolowski, G.; Young, R. N.; Zamboni, R.; Black, W. C. The Discovery of Odanacatib (MK-0822), a Selective Inhibitor of Cathepsin K. *Bioorg. Med. Chem. Lett.* **2008**, *18* (3), 923–928. https://doi.org/10.1016/j.bmcl.2007.12.047.
5. Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015**, *58* (21), 8315–8359. https://doi.org/10.1021/acs.jmedchem.5b00258.
6. Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Aceña, J. L.; Soloshonok, V. A.; Izawa, K.; Liu, H. Next Generation of Fluorine-Containing Pharmaceuticals, Compounds Currently in Phase II–III Clinical Trials of Major Pharmaceutical Companies: New Structural Trends and Therapeutic Areas. *Chem. Rev.* **2016**, *116* (2), 422–518. https://doi.org/10.1021/acs.chemrev.5b00392.
7. Han, J.; Remete, A. M.; Dobson, L. S.; Kiss, L.; Izawa, K.; Moriwaki, H.; Soloshonok, V. A.; O'Hagan, D. Next Generation Organofluorine Containing Blockbuster Drugs. *J. Fluorine Chem.* **2020**, *239*, 109639. https://doi.org/10.1016/j.jfluchem.2020.109639.
8. Sakai, T.; Yan, F.; Kashino, S.; Uneyama, K. Asymmetric Reduction of 2-(N-Arylimino)-3,3,3-Trifluoropropanoic Acid Esters Leading to Enantiomerically Enriched 3,3,3-Trifluoroalanine. *Tetrahedron* **1996**, *52* (1), 233–244. https://doi.org/10.1016/0040-4020(95)00866-7.
9. Pajkert, R.; Röschenthaler, G.-V. Synthesis of Novel α-CF3-Trifluoroalanine Derivatives Containing N-(Diethoxyphosphoryl)Difluoroacetyl Group. *J. Fluorine Chem.* **2010**, *131* (12), 1362–1367. https://doi.org/10.1016/j.jfluchem.2010.09.010.
10. Cherednichenko, A. S.; Bezgubenko, L. V.; Rusanov, E. B.; Onys'ko, P. P.; Rassukana, Y. V. Enantiomeric *N* - *Tert* -Butylsulfinyl Imines of Methyl Trifluoropyruvate: Promising Building Blocks in Asymmetric Synthesis of α-Trifluoromethylated Amino Acids and Derivatives. *ChemistrySelect* **2020**, *5* (43), 13569–13574. https://doi.org/10.1002/slct.202003500.
11. Rassukana, Y. Methyl α-Iminotrifluoropropionate: A Novel Convenient Building Block for the Preparation of Functionalized Derivatives Bearing a Trifluoroalanine Residue. *Synthesis* **2011**, *2011* (21), 3426–3428. https://doi.org/10.1055/s-0030-1260249.
12. Rassukana, Y. V.; Yelenich, I. P.; Synytsya, A. D.; Onys'ko, P. P. Fluorinated NH-Iminophosphonates and Iminocarboxylates: Novel Synthons for the Preparation of Biorelevant α-Aminophosphonates and Carboxylates. *Tetrahedron* **2014**, *70* (18), 2928–2937. https://doi.org/10.1016/j.tet.2014.03.030.
13. Rassukana, Y. V.; Bezgubenko, L. V.; Onys'ko, P. P.; Synytsya, A. D. Cycloaddition of N-Substituted Imines of Trifluoropyruvate with Diazomethane: Efficient Synthesis of 2-(Trifluoromethyl)Aziridine-2-Carboxylates. *J. Fluorine Chem.* **2013**, *148*, 14–18. https://doi.org/10.1016/j.jfluchem.2013.01.019.
14. Osipov, S. N.; Kolomiets, A. F.; Fokin, A. V. Cycloaddition Reactions of Methyl 2-N-Trifluoroacetyliminotrifluoropropionate. *Russ. Chem. Bull.* **1988**, *37* (1), 122–126. https://doi.org/10.1007/BF00962670.
15. Rassukana, Y. V.; Bezgubenko, L. V.; Stanko, O. V.; Rusanov, E. B.; Kulik, I. B.; Onys'ko, P. P. Diastereoselective Cycloaddition of (S)-N-(1-Phenylethylimino)Trifluoropropionate and Trifluoroethylphosphonate with Diazomethane. *Tetrahedron: Asymmetry* **2017**, *28* (4), 555–560. https://doi.org/10.1016/j.tetasy.2017.03.009.
16. Kadaba, P. K. Rational Drug Design and the Discovery of the Δ2-1,2,3-Triazolines, A Unique Class of Anticonvulsant and Antiischemic Agents. *Curr. Med. Chem.* **2003**, *10* (20), 2081–2108. https://doi.org/10.2174/0929867033456765.
17. Marsini, M. A.; Reeves, J. T.; Desrosiers, J.-N.; Herbage, M. A.; Savoie, J.; Li, Z.; Fandrick, K. R.; Sader, C. A.; McKibben, B.; Gao, D. A.; Cui, J.; Gonnella, N. C.; Lee, H.; Wei, X.; Roschangar, F.; Lu, B. Z.; Senanayake, C. H. Diastereoselective Synthesis of α-Quaternary Aziridine-2-Carboxylates via Aza-Corey–Chaykovsky Aziridination of *N* - *Tert* -Butanesulfinyl Ketimino Esters. *Org. Lett.* **2015**, *17* (22), 5614–5617. https://doi.org/10.1021/acs.orglett.5b02838.
18. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *OLEX2* : A Complete Structure Solution, Refinement and Analysis Program. *J. Appl. Crystallogr.* **2009**, *42* (2), 339–341. https://doi.org/10.1107/S0021889808042726.
19. Sheldrick, G. M. *SHELXT* – Integrated Space-Group and Crystal-Structure Determination. *Acta Crystallogr., Sect. A:Found. Adv.* **2015**, *71* (1), 3–8. https://doi.org/10.1107/S2053273314026370.
20. Sheldrick, G. M. Crystal Structure Refinement with *SHELXL*. *Acta Crystallogr., Sect. C:Struct. Chem.* **2015**, *71* (1), 3–8. https://doi.org/10.1107/S2053229614024218.

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