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

Cyclen-Containing Phosphonic Acids as Components of Osteotropic ^{68}Ga Radiopharmaceuticals

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Abstract—The known methods of preparation of cyclen-containing phosphonic acids, promising components of radiopharmaceuticals, have been improved, and new approaches have been developed. Application of ^{31}P and ^{13}C NMR spectroscopy for initial screening of complex formation between cyclen-containing phosphonic acids and Ga^{3+} in D_2O has been demonstrated. The conditions of ^{68}Ga complex formation with cyclen-containing phosphonic acids and estimation of their radiochemical yield in water by means of thin-layer chromatography have been elaborated. A correlation between the NMR and TLC data has been found. Biological distribution of the selected ^{68}Ga radiopharmaceuticals has been determined in vivo from the positron emission tomography data. The obtained standardized uptake values point at the osteotropicity of the ^{68}Ga compounds.

Keywords: 1,4,7,10-tetraazacyclododecane, cyclen-containing phosphonic acid, NMR, thin-layer chromatography, positron emission tomography, osteotropic radiopharmaceutical

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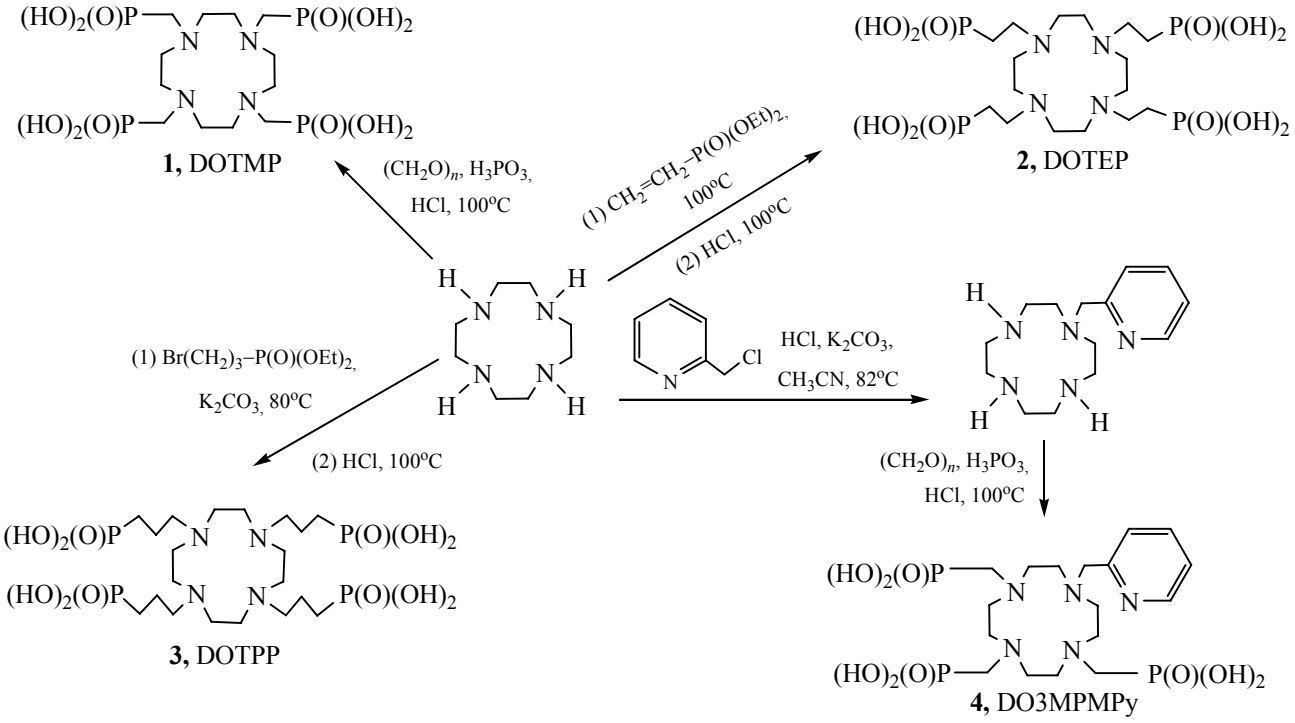
Formation of bone metastases, the most frequent manifestation of oncological diseases, dramatically affects the patients quality of life. Coordination compounds of radionuclides with organic ligands most often act as modern osteotropic radiopharmaceuticals for diagnostics and treatment of such disorders. The properly chosen ligand allows for fast and efficient binding of the radionuclide and its targeted accumulation in the bone tissue. In view of rapid development of the positron emission tomography (PET) providing for the high resolution of the visualization and the emerging role of the ^{68}Ga isotope in nuclear medicine [1–3], development of new organic ligands for osteotropic ^{68}Ga radiopharmaceuticals has become a topical issue.

Phosphonic acids complexes with various radionuclides have been applied for early diagnostics of bone metastasis by means of single-photon emission computer tomography [4–6]. On the other hand, the 1,4,7,10-tetraazacyclododecane (cyclen) group, efficiently binding cations of *d* and *f* elements, has been conventionally used in design of organic ligands as components of radiopharmaceuticals [7–10]. Hence, a combination of cyclen and phosphonic fragments in a molecule of organic ligand is a promising approach to develop osteotropic radiopharmaceuticals. Some compounds of this class have been described in the literature; however, detailed procedures of their preparation and isolation have been often omitted, and the issues of optimization of the number of phosphonic fragments as well as length and structure of the spacer between the cyclen macrocycle and the phosphonic groups have remained open so far. In this work we prepared the symmetric (**1**–**3**) and unsymmetrical (**4**) cyclen-containing phosphonic acids with various side substituents.

Compound **1** (DOTMP) was synthesized in 1984 for the first time [11] and has been well studied since then [12, 13]. Certain lanthanide complexes with

Scheme 1.



DOTMP have been applied as NMR-shifting agents for proteins [14, 15] and biologically important cations [16–18] or as radiopharmaceutical components [10, 19, 20]. In this work compound **1** was prepared via the interaction of 1,4,7,10-tetraazacyclododecane hydrochloride, paraformaldehyde, and phosphonic acid in the presence of concentrated HCl following the procedure adopted from [21] (Scheme 1).

Even though the reaction was performed in the presence of concentrated hydrochloric acid, no formation of hydrochlorides of cyclen **1** containing four fragments of aminomethylenephosphonic acid was detected according to the elemental analysis data [11, 21]. In order to completely avoid the presence of quaternary ammonium chlorides compound **1** was treated with a solution of propylene oxide in aqueous ethanol (1 : 5) in the final stage of the synthesis. Melting point of the so prepared sample was identical to that reported elsewhere [11].

Compound **2** (DOTEP) in the form of the hydrochloride was obtained via hydrolysis of the corresponding octaethyl ester by refluxing in 6 mol/L HCl. Compound **2** containing the fragments of free aminoethylphosphonic acid was prepared via treatment of the hydrochlorides with propylene oxide in aqueous ethanol.

Initially we attempted to prepare compound **2** via the alkylation of cyclen or its tetrahydrochloride with a vinylphosphonate in boiling anhydrous tetrahydrofuran in the presence of fine-ground potassium carbonate (method *a*). However, the low product yield of 27–31% and the requirement of prolonged stirring at boiling called for optimization of the reaction conditions. The presence of potassium cation could deactivate the cyclen nitrogen atoms: the complex formation involving the lone electron pair of nitrogen reduced its nucleophilicity and slowed down the alkylation with electrophilic vinyl- and ω-haloalkylphosphonates. The stability constant of the alkali cations complexes with organic ligands is known to be sufficiently lower in water than that in organic solvents [22], therefore, we carried out cyclen alkylation with 2-bromoethylphosphonate in the presence of potassium carbonate in aqueous medium (method *b*). Indeed, the reaction was accelerated and the product yield was improved. ^{1}H, ^{31}P, and ^{13}C NMR spectra of compound **2** prepared via those two methods were identical.

Compound **3** (DOTPP) was prepared for the first time in the form of dihydrochloride, via acid hydrolysis of the corresponding octaethyl ester obtained in turn via the interaction of cyclen hydrochloride and

diethyl-3-bromopropylphosphonate in the presence of excess potassium carbonate.

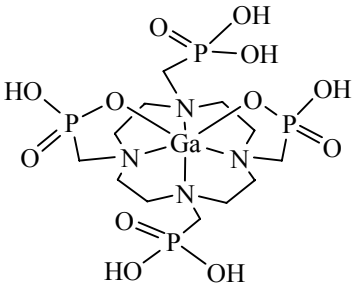
The unsymmetrical ligand **4** (DO3MPMPy) containing the cyclen fragment, a methylenepyridine group, and three methylenephosphonic moieties was mentioned in [23]; however, the synthetic procedure was not reported there. We used 1-(2-pyridylmethylene)-4,7,10-tetraazacyclododecane (prepared via the known procedure [24]) as the starting compound to synthesize compound **4**. The phosphonic groups were introduced via the reaction with paraformaldehyde and phosphonic acid in the presence of concentrated HCl, similarly to compound **1**.

The following parameters are crucial for the choice of promising ligands for radiopharmaceuticals: fast, selective, and efficient binding of the radionuclide at low concentration of the ligand and thermodynamic as well as kinetic stability of the formed complexes [25]. TLC analysis is generally used to determine the yield of the complex formation between a radionuclide and organic compounds [26].

In this work we applied ^{31}P and ^{13}C NMR spectroscopy to study the dynamics of the interaction between the stable cation Ga^{3+} with compounds **1**–**4** and to estimate the binding strength of the potential radionuclide cation with cyclen-containing ligands. The crystalline $Ga(NO_3)_3$ was added by portions to the ligand solution in D_2O at pH of 7.5–8.0.

^{31}P NMR spectrum of compound **1** contained a broadened singlet signal at 11.83 ppm of the width ≈33 Hz (Fig. 1a). The interaction of DOTMP with Ga^{3+} cation resulted in weakening of the signal of the free ligand, its position and width remaining unchanged (within the limits of the experiment accuracy). Simultaneously, two new much narrower (≈4 Hz) and equally strong signals appeared in the spectrum at 12.63 and 10.68 ppm, apparently corresponding to the gallium complex. Increasing the Ga^{3+} concentration in the sample resulted in the progressive increase of integral intensity of the signals assigned to the complex, whereas the signal of the free ligand was weakened and completely disappeared at the equimolar ratio of the ligand and Ga^{3+} (Figs. 1b–1e).

The possible structure of the formed complex is shown below. The Ga^{3+} cation was likely implanted in the cyclen frame and caged between the two oxygen atoms of the opposite phosphoryl fragments. As a result, the ligand ↔ complex exchange was practically avoided, and the signals of both the free ligand and the complex were detected in the spectra.



Apparently, the complex particle contained the gallium-bound as well as the free phosphoryl fragments, giving rise to a pair of the signals in the ^{31}P NMR spectra. The decreased width of the signals as compared to that of the free ligand was attributed to the binding of nitrogen and (partially) phosphoryl oxygen atoms with gallium cation, significantly limiting proton exchange occurring via formation of the intra- and intermolecular associates. Interestingly, the two signals of the complex were shifted towards the opposite spectral regions as compared to the signal of the free ligand. The signal of the phosphorus atoms bound to the cation was shifted downfield due to the deshielding effect, whereas magnetic shielding of the phosphoryl groups not involved in binding with Ga^{3+} favored the upfield shift of corresponding signal. The somewhat broader signal in the strong field confirmed that assignment as well.

^{13}C NMR signal of the free ligand **1** contained a weakly split doublet at 50.27 ppm assigned to carbon atoms of the cyclen fragment and a doublet with $^1J_{CP}$ 132.8 Hz assigned to carbon atoms of the exocyclic methylene groups (Fig. 2a). Addition of the gallium salt weakened the spectral signals of the free ligand and induced the appearance of two additional groups of signals (of both the exocyclic and the cyclen CH_2 groups) pointing at the non-equivalence of the carbon atoms in the complex (Figs. 2b, 2c). The signals of the complex were noticeably shifted downfield; the $^1J_{CP}$ values were slightly changed.

The changes in the ^1H NMR spectra recorded under the same conditions did not contradict the suggested complex structure. The system was equilibrated in less than 5 min after addition of the new portion of the gallium salt; the metal complex yield was close to quantitative.

Interaction of gallium nitrate with compound **2** resulted in the downfield shift of the ligand ^{31}P NMR

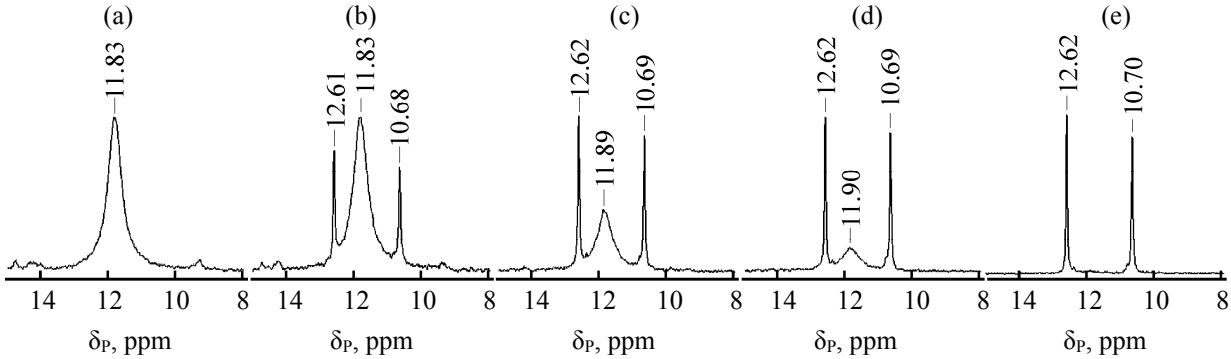


Fig. 1. ^{31}P NMR spectra of free ligand (a) and samples containing DOTMP and $Ga(NO_3)_3$ at the molar ratio of 1 : 0.1 (b), 1 : 0.2 (c), 1 : 0.5 (d), and 1 : 1 (e).

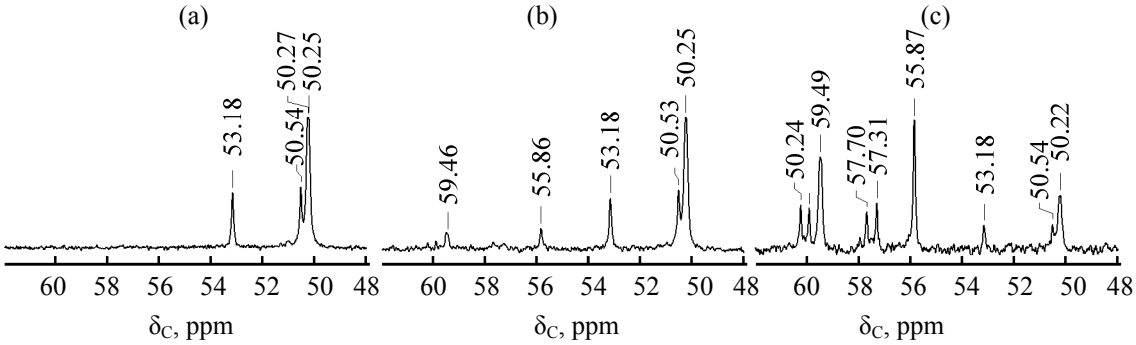


Fig. 2. ^{13}C NMR spectra of free DOTMP (a) and the samples containing DOTMP and $Ga(NO_3)_3$ at the molar ratio of 1 : 0.2 (b) and 1 : 0.5 (c).

signal from 18.54 to 20.64 ppm and its noticeable broadening from ≈60 to ≈120 Hz (Fig. 3). In the case of compound **3**, the ligand ^{31}P NMR signal was broadened as well (from ≈160 to ≈340 Hz) due to the interaction with the salt, but the signal was shifted upfield (from 28.5 to 23.66 ppm). The changes in the complex formation behavior of compound **1** as compared to that of compounds **2** and **3** were attributed to the formation of less stable complexes in the latter cases, owing to the longer side substituents and hence the faster ligand ↔ complex exchange.

^{31}P NMR spectrum of compound **4** contained two signals with the 1 : 2 ratio of the integral intensities. Addition of gallium nitrate led to the appearance of two additional signals assigned to the complex (Fig. 4). The stronger signal corresponding to the phosphoryl groups directly involved in the complex formation with Ga^{3+} was shifted downfield as compared to the signal of the free ligand, thus evidencing its efficient deshielding; the weaker signal was shifted upfield. The marked spectral changes pointed at the direct interaction of Ga^{3+} cation with the phosphoryl groups at the cyclic nitrogen atoms in positions 4 and 10 of compound **4**.

To conclude, NMR study demonstrated a fast and almost quantitative formation of Ga^{3+} complex with compounds **1** and **4**; therefore, those compounds were suitable for further detailed investigation.

Thin-layer chromatography (TLC) method was applied to determine the radiochemical yield of the $^{68}Ga^{3+}$ complexes with the prepared phosphonic acids [27, 28]. In particular, we elucidated the effects of the medium acidity, the ligand concentration, temperature, and reaction duration on the yield of the ^{68}Ga complexes. The optimal conditions of the complex formation process were as follows. The lowest ligand concentration in the reaction medium suitable for the labeling reaction was 1 mg/mL. The reaction yield exceeding 90% was attained after the mixture maintaining during 15 min at 25°C and pH 6±1. In all cases the medium pH was adjusted with 0.2 mol/L solution of sodium acetate and 0.1 mol/L solution of sodium hydroxide. The yield of the complex formation between $^{68}Ga^{3+}$ and DOTMP as a function of the

ligand concentration is shown in Fig. 5. The results showed that the temperature (20–95°C) and the reaction duration (15–45 min) did not significantly affect the radiochemical yield of the ^{68}Ga complexes with compounds **1** and **4**. Yield of the complexes with compounds **2** and **3** did not exceed 20%, coinciding with the NMR study results.

Hence, we demonstrated that the data obtained by means of NMR and TLC could serve for primary screening of promising organophosphorus compounds to be applied as components of radiopharmaceuticals.

Finally, we applied the positron emission tomography to estimate the biological distribution of the ^{68}Ga–DOTMP complex in vivo. In particular, we determined the standardized uptake values (SUV) that revealed accumulation of the ^{68}Ga–DOTMP complex in the normal bone tissue and at the site of bone fracture, thus proving the complex osteotropicity. The accumulation at the site of bone fracture exceeded that in the normal bone tissue. Figure 6 represents the SUV values for organs and tissues exhibiting the strongest radioactivity accumulation (each point shows the average of two animals). The SUV value in the normal bone tissue was of 0.6±0.2 g/mL 90 min after the injection, whereas the SUV value at the site of bone fracture was 1.3±0.2 g/mL. The SUV value for liver was 1±0.2 g/mL; accumulation of the complex in blood was relatively high as well, however, below the listed values. According to the SUV kinetics, 60–90 min after the injection was the optimal time for the bone tumor imaging.

The ^{68}Ga–DO3MPMPy complex exhibited even stronger osteotropicity according to the in vivo experiments (Fig. 7): the SUV values for the normal bone tissue and at the site of bone fracture were

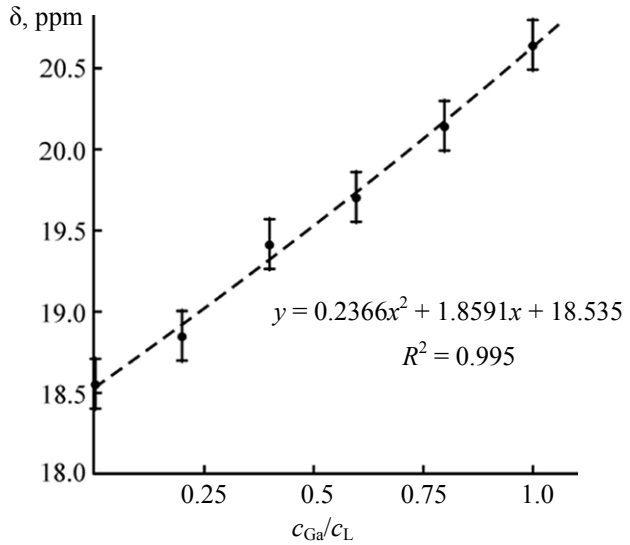


Fig. 3. ^{31}P NMR chemical shift of compound **2** as a function of the added gallium nitrate concentration.

1.5±0.2 and 4.3±0.9 g/mL, respectively, 90 min after the injection. Its accumulation in blood was high as well, and that in liver was much lower than in the case of ^{68}Ga–DOTMP (SUV < 0.4 g/mL).

EXPERIMENTAL

^{1}H, ^{31}P, and ^{13}C NMR spectra were recorded using a Bruker DPX 200 spectrometer at 200, 81.0, and 50.04 MHz, respectively.

Compounds **1**–**4** were synthesized starting from 1,4,7,10-tetraazacyclododecane (cyclen) or its tetrahydrochloride prepared as described elsewhere [29].

1,4,7,10-Tetraazacyclododecan-1,4,7,10-tetrakis-(methylenephosphonic acid) (1, DOTMP) was prepared as described in [21].

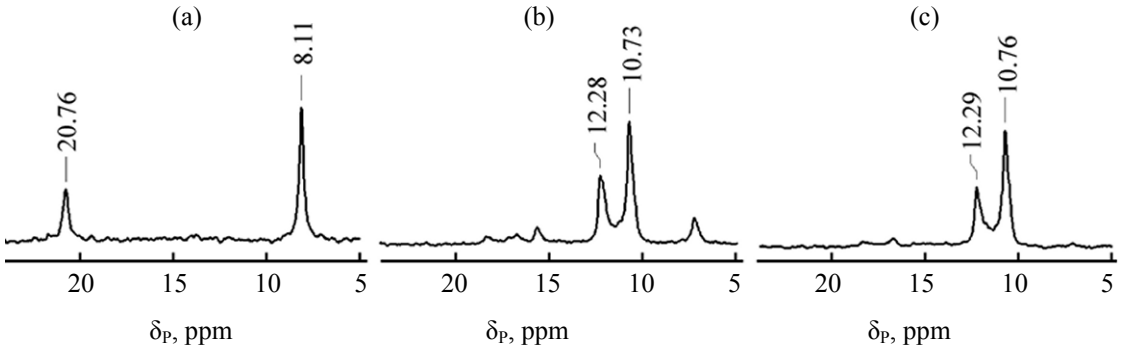


Fig. 4. ^{31}P NMR spectra of free ligand **4** (a) and the samples containing ligand **4** and $Ga(NO_3)_3$ at the molar ratio of 1 : 0.5 (b) and 1 : 1 (c).

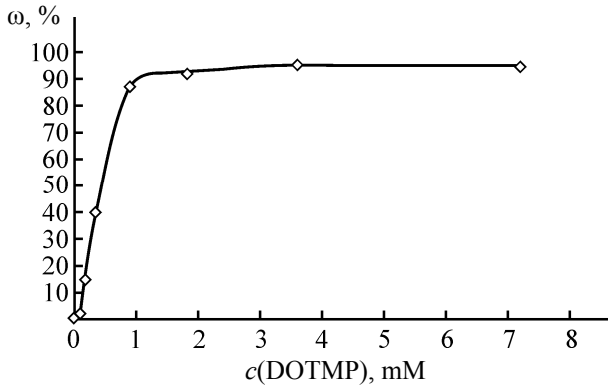


Fig. 5. Yield of the complex formation reaction between $^{68}Ga^{3+}$ and DOTMP as a function of the ligand concentration.

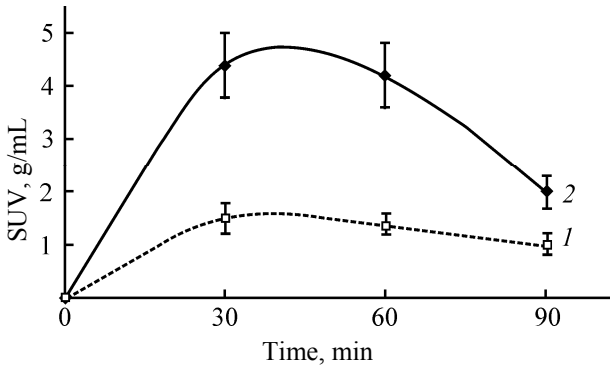


Fig. 6. Biodistribution of ^{68}Ga–DOTMP in vivo. (*1*) bone, intact; (*2*) bone, fracture; (*3*) liver.

1,4,7,10-Tetraazacyclododecan-1,4,7,10-tetrakis-(ethylenephosphonic acid) (2, DOTEP). *a*. A mixture of 0.48 g (2.8 mmol) of cyclen, 1.36 g (13.6 mmol) of diethyl vinylphosphonate, and 3.08 g (22.3 mmol) of thoroughly ground potassium carbonate was refluxed in 8 mL of anhydrous tetrahydrofuran at vigorous stirring in the presence of 0.20 g of tetrabutylammonium bromide. The reaction course was monitored via ^{31}P NMR spectroscopy. After the reaction was complete, the mixture was filtered to remove the main fraction of inorganic salts; the filtrate was evaporated, and the residue was treated with a mixture of 15 mL of chloroform and 10 mL of water. The organic layer was washed with water (with simultaneous neutralization to pH ≈ 7), dried over sodium or magnesium sulfate, and evaporated in a vacuum. The residue was treated with petroleum ether and cooled, and the solution over the formed oily layer was decanted. The procedure was repeated till complete removal of the vinylphosphonate (^1H and ^{31}P NMR monitoring).

The oily residue was octaethyl ester of the tetraalkylated cyclen: **1,4,7,10-tetra-[2-(diethyloxyphosphoryl)ethyl]-1,4,7,10-tetraazacyclododecane**. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.26 t (24H, CH_3, $^3J_{HH}$ 7.3 Hz), 1.70–1.95 m (8H, PCH_2), 2.51 br.s (16H, CH_2N_{cycle}), 2.62–2.78 m (8H, CH_2N_{exo}), 4.03 d.q (16H, CH_2O, J_{HP} 7.3 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 16.1 d ($^3J_{CP}$ 5.9 Hz), 22.4 d ($^1J_{CP}$ 136.5 Hz), 47.9, 51.3, 61.1 d ($^2J_{CP}$ 6.2 Hz). ^{31}P NMR spectrum ($CDCl_3$): δ_P 32.2 ppm.

The oily 1,4,7,10-tetra-[2-(diethyloxyphosphoryl)-ethyl]-1,4,7,10-tetraazacyclododecane was refluxed in 10 mL of 6 mol/L aqueous HCl in the presence of 0.5 g of activated carbon during 15 h, the mixture was filtered, and the mother liquor was evaporated. The residue was twice co-evaporated with water and treated with excess of propylene oxide in aqueous ethanol (1 : 5). Additional crystallization from aqueous ethanol yielded 0.34 g (27%) of white crystalline product; mp 297–301°C (decomp.) (mp 268°C [30]). ^1H NMR spectrum (D_2O), δ, ppm: 1.85–2.10 m (8H, PCH_2), 2.95–3.25 br.s (24H, CH_2N). ^{13}C NMR spectrum (D_2O, pH ≈ 10), δ_C, ppm: 21.5 d ($^1J_{CP}$ 131.4 Hz), 48.1. ^{31}P NMR spectrum (D_2O, pH ≈ 10): δ_P 20.7 ppm. Found, %: C 30.50, 30.11; H 6.86, 7.07. $C_{16}H_{40}N_4O_{12}P_4 \cdot 2H_2O$. Calculated, %: C 30.01; H 6.93.

b. A mixture of 0.35 g (1.1 mmol) of cyclen tetrachloride, 1.29 g (5.2 mmol) of diethyl 2-bromoethylphosphonate, and 2.30 g (16.6 mmol) of potassium carbonate was stirred in 2.5 mL of boiling water during 10 h. The reaction course was monitored by means of ^{31}P NMR spectroscopy. After the reaction was complete, the mixture was extracted with chloroform (3×7.5 mL), and the organic layer was washed with water (2×5 mL) neutralizing the aqueous layer (pH ≈ 7) via dropwise addition of 1 mol/L HCl solution. The combined organic extract was dried over

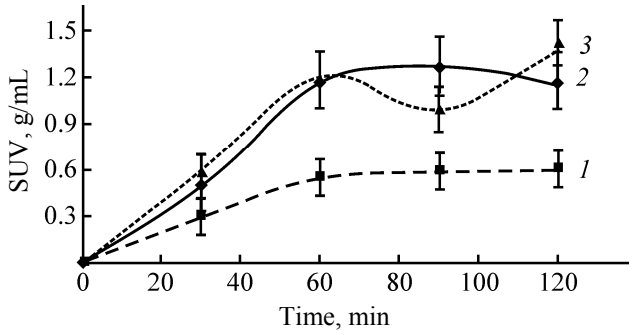


Fig. 7. Biodistribution of ^{68}Ga–DO3MPMPy in vivo. (*1*) bone, intact; (*2*) bone, fracture.

sodium sulfate and evaporated, the residue was treated with petroleum ether and cooled to 0°C, and the solvent was decanted. The procedure was repeated twice. The oily residue was octaethyl ester of the tetraalkylated cyclen: 1,4,7,10-tetra[2-(diethyloxyphosphoryl)ethyl]-1,4,7,10-tetraazacyclododecane. Its spectral properties were identical to those of the specimen prepared via method *a*. Hydrolysis, isolation, and purification of compound **2** were performed as described above in method *a*. Yield 0.37 g (53%). The elemental analysis allowed identification of the substance as the $C_{16}H_{40}N_4O_{12}P_4 \cdot 2H_2O$ dihydrate.

For the sake of comparison with the reference data, we attempted isolation of the acid **2** after hydrolysis of the corresponding octaethyl ester as described in [30], without treatment with propylene oxide. In the latter case, the residue obtained after evaporation of the hydrochloric acid solution was crystallized from ethanol or water. In both cases, dihydrochloride of compound **2** was isolated; white powder, mp 265–267°C (mp 268°C [30]). The elemental analysis data (found, %: C 28.50; H 6.35; Cl 7.60) allowed the substance identification as a 1 : 1 mixture of the mono- ($C_{16}H_{40}N_4O_{12}P_4 \cdot HCl$) and dihydrochloride ($C_{16}H_{40}N_4O_{12}P_4 \cdot 2HCl$) of the tetraphosphorylated cyclen.

1,4,7,10-Tetraazacyclododecan-1,4,7,10-tetrakis-(propylenephosphonic acid) (3, DOTPP). A mixture of 0.22 g (0.69 mmol) of cyclen tetrahydrochloride, 0.90 g (3.5 mmol) of diethyl 3-bromopropylphosphonate, and 1.14 g (8.5 mmol) of thoroughly ground potassium carbonate was refluxed in 5 mL of anhydrous tetrahydrofuran at stirring in the presence of 0.15 g of tetrabutylammonium bromide. The reaction mixture was further treated as described above for compound **2**. We obtained ≈0.45 g of the oily substance, mainly 1,4,7,10-tetra[3-(diethyloxyphosphoryl)propyl]-1,4,7,10-tetraazacyclododecane. The oily product was refluxed in 7 mL of 6 mol/L HCl and evaporated. The residue was purified via chromatography on a cationite (eluent: 2 mol/L HCl) to isolate 0.16 g (31%) of the acid **3** in the form of the dihydrochloride monohydrate; mp 311–314°C (decomp.). ^{1}H NMR spectrum (D_2O), δ, ppm: 1.54 m (8H, PCH_2), 1.76 m (8H, $\underline{CH_2}CH_2P$), 3.00 m (8H, CH_2N_{exo}), 3.18 m (16H, CH_2N_{cycle}). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 16.8, 24.7 d ($^1J_{CP}$ 129.5 Hz), 48.1, 54.1. ^{31}P NMR spectrum (D_2O): δ_P 26.4 ppm. Found, %: C 32.11, 32.33; H 7.26, 7.07; P 16.27. $C_{20}H_{48}N_4O_{12}P_4 \cdot 2HCl \cdot H_2O$. Calculated, %: C 31.97; H 6.98; P 16.49.

1,4,7,10-Tetraazacyclododecane-1-(2-pyridylmethylene)-4,7,10-tris(methylenephosphonic acid) (4, DO3MPMPy). A mixture of 0.37 g (2.2 mmol) of 1-(2-pyridylmethylene)-4,7,10-tetraazacyclododecane prepared as described in [24], 1.07 g (13.0 mmol) of phosphonic acid, 3 mL of water, and 1 mL of concentrated hydrochloric acid was heated to boiling. Then 0.29 g of paraformaldehyde (calculated: 9.8 mmol of formaldehyde) was added in small portions over 1 h. The mixture was refluxed during 1 h and evaporated. The residue was co-evaporated three times with water and treated with acetone. The solution over the formed oily layer was decanted. The residue was dried in a vacuum (10 mmHg, 50°C, 1 h). Yield 0.28 g (31%), foamed glassy substance. ^{1}H NMR spectrum (D_2O), δ, ppm: 2.66–3.88 m (16H, CH_2N_{cycle}), 2.88 d (2H, PCH_2, $^2J_{PH}$ 10.8 Hz), 3.24 d (4H, PCH_2, $^2J_{PH}$ 12.7 Hz), 4.16 s (2H, CH_2Py), 7.54–7.66 m ($1H_{Py}$), 7.95–8.09 m ($1H_{Py}$), 8.49–8.58 m ($1H_{Py}$), 8.78–8.80 m ($1H_{Py}$). ^{13}C NMR spectrum (D_2O), δ_C, ppm: 48.15, 48.71, 49.60, 49.94, 51.94 d [$P\underline{C}H_2N(CH_2)_2NCH_2Py$, $^1J_{CP}$ 142.9 Hz], 51.77, 53.62 d ($^1J_{CP}$ 135.02 Hz), 125.96, 126.81, 128.73, 142.78, 147.09. ^{31}P NMR spectrum (D_2O), δ_P, ppm: 8.11 [2P, $PCH_2N(CH_2)_2NCH_2Py$], 20.76 (1P). Found, %: C 35.83, 35.18; H 6.76, 6.93; N 11.37, 11.38. $C_{17}H_{34}N_5O_9P_3 \cdot 2H_2O$. Calculated, %: C 35.11; H 6.54; N 11.80.

Radiochemical experiments were carried out using a solution of $[^{68}Ga]Cl_3$ obtained using a $^{68}Ge/^{68}Ga$ generator (Cyclotron Co. Ltd., Obninsk, Russia). The labeling reaction was performed via sequential addition of the buffer solution and the $^{68}Ge/^{68}Ga$ generator eluate to a solution of the ligand. The ratio of the buffer solution volume to the ^{68}Ga eluate one was varied to afford pH of 1–6. The ligand concentration in the final solution was constant.

Yield of the reaction between ^{68}Ga and the synthesized ligands was determined by TLC. A sample (5–10 μL) of the ^{68}Ga eluate or the reaction mixture containing ^{68}Ga was applied onto a start line of the chromatography plate. The plates were put into a chromatography chamber containing the eluent solution, and the separation was performed via the ascending eluent method during the time required for the eluent to reach the finish line. The eluent level was determined visually. The so obtained chromatogram was analyzed using a miniGita Star thin-layer scanning radiometer-spectrometer (Raytest Isotopenmeβgerate GmbH, Germany). The radiochemical yield was expressed as follows:

Composition of chromatography systems

Stationary phase	Mobile phase	R_f of free Ga^{3+}	R_f of Ga^{3+} complexes
Cellulose on aluminum sheets (Merck)	water : ethanol : pyridine = 4.0 : 2.0 : 1.0	0	0.9±0.1
	2.4 wt % aqueous solution of HCl : acetone : acetylacetone = 1.6 : 15.0 : 8.3	1	0.1±0.1

$$\% = A_b/(A_b + A_r),$$

with A_b, activity of the part of the chromatogram containing the labeling product and A_r, activity of the rest part of the chromatogram.

The chromatography systems used in this work are described in the table; they allowed for good reproducibility as well as efficient separation of the free ^{68}Ga and its complexes.

Biological behavior of the specimens was studied using a Wizard 2480 gamma-counter and a Genisys4 PET/X-RAY SofieBiosciences positron emission tomograph; 0.5, 1, 2, and 3 h after the injection of labeled compound into the tail vein of white outbred mice. Normal animals as well as those exhibiting osteotylus after bone fracture were used for the experiments. The standardized uptake values (SUV) were determined for the ^{68}Ga–DOTMP and ^{68}Ga–DO3MPMPy specimens. The SUV value reflected the abnormal accumulation of the drug at the pathology locus or in the selected tissue with respect to the overall distribution in the body. The SUV values (g/mL) were determined as follows:

$$\text{SUV} = c_{org}/c_t = (A_{org}/IA)\cdot(m_t/V_{org}) = (\%IA/100\%)(m_t/V_{org}),$$

with c_{org}, concentration of the accumulated activity A_{org} in the volume V_{org} of the organ (calculated using the visualization software of PET instrument), MBq/mL; c_t, concentration of the introduced activity IA with respect to the total body mass m_{TB}, MBq/g; $\%IA$, the fraction of the introduced activity accumulated in the organ.

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