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ARTICLE

Monophosphonate/phosphinate DOTA analogues as ligands for trivalent scandium: thermodynamic study and radiolabelling with cyclotron-produced ^{44m}Sc/^{44}Sc and ^{44}Sc from ^{44}Ti/^{44}Sc generator

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Complexation ability of DOTA analogs bearing one methylenephosphonic (DO3AP) or methylenephosphinic (DO3AP^{PrA} and DO3AP^{ABn}) acid pendant arm toward scandium were evaluated. Stability constants of their scandium(III) complexes were determined by potentiometry combined with ^{45}Sc NMR spectroscopy. The stability constants of the monophosphinate analogues are somewhat lower than that of the Sc-DOTA complex. The phosphorus acid moiety interacts with trivalent scandium even in very acidic solutions forming *out-of-cage* complexes; the strong affinity of the phosphonate group to Sc(III) preclude stability constant determination of the Sc-DO3AP complex. These results were compared with those obtained by free-ion selective radiotracer extraction (FISRE) method which is suitable for trace concentrations. FISRE underestimated the stability constants but their relative order was preserved. Nonetheless, as this method is experimentally simple, it is suitable for quick relative comparison of stability constants values under trace concentrations. Radiolabelling of the ligands with ^{44}Sc was performed using the radioisotope from two sources, ^{44}Ti/^{44}Sc generator and ^{44m}Sc/^{44}Sc from cyclotron. The best radiolabelling conditions for the ligands were pH = 4, 70 °C and 20 min which were, however, not superior to those of the parent DOTA. Nonetheless, *in vitro* behaviour of the Sc(III) complexes in the presence of hydroxyapatite and rat serum showed sufficient stability of ^{44}Sc complexes of these ligands for *in vivo* applications. PET images and *ex vivo* biodistribution of the ^{44}Sc-DO3AP complex performed on healthy Wistar male rats showed no specific uptake on bone and a rapid clearance through urine.

Introduction

On the way to a personalized medicine, nuclear medicine offers both diagnostic tools and therapeutic drugs utilizing various radioisotopes. Until recently, most radiopharmaceuticals were designed to be used solely for either diagnostics or therapeutics. Radionuclides currently used for imaging, such as ^{68}Ga or ^{111}In, are different from those used for therapy, such as ^{90}Y or ^{177}Lu. One radionuclide would be used to image individual patient disease states and evaluate their receptor expression, metabolic rate, clearance and handling, and a second radionuclide would be used for therapy. Problems with this approach arose due to differences in the chemistry of the radionuclides themselves which have been shown to affect the overall pharmacology, in particular accumulation of the radiopharmaceuticals at target and non-target sites, resulting in an over- or underestimation of dose to critical tissues and the dose being outside the optimum range of efficacy. In contrast, using the same metal to perform both diagnosis and therapy would result in a better determination of the absorbed dose and would give a better indication of the therapeutic activity necessary to administer. Such radiopharmaceutical pair utilizing diagnostic and therapeutic radioisotopes is called "theranostics".[1]

Among the radionuclides available, there is significant interest in the therapeutic radioisotope ^{47}Sc (β^-, $\tau_{½}$ 3.35 d, E_β 0.143 (68 %) and 0.204 MeV (32 %); γ, E_γ 159.4 keV, 68 %) as it matches with positron-emitting ^{44}Sc (β^+, $\tau_{½}$ 3.97 h, E_β 0.63 MeV, 94.3 %) or ^{43}Sc (β^+, $\tau_{½}$ 3.89 h, E_β 0.344 MeV (17.2 %) and 0.508 MeV (70.9 %)) and, thus, they form an ideal theranostic pairs. Potential of ^{47}Sc for nuclear medicine has been already investigated.[2–4] The possibility of ^{47}Sc production by neutron irradiation of ^{47}Ti and consecutive solid-phase extraction chromatography has been evaluated.[5] Very recently, the feasibility of photonuclear production of ^{47}Sc from ^{48}Ca or ^{47}Ca/^{47}Sc generators has been studied.[5,6] Due to its dominant positron emission, ^{44}Sc is very suitable for PET imaging. Its half-life is perfectly matching pharmacokinetics of small

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Electronic Supplementary Information (ESI) available: Experimental details on potentiometry, FISRE and NMR measurements, figures with NMR and titration data, FISRE isotherms, experimental details and figure for challenge studies, table of specific activitites of labelled ligands See DOI: 10.1039/x0xx00000x

molecules or (oligo)peptides. In addition to two collinear γ rays produced by annihilation, ^{44}Sc offers a third γ ray suitable for three-photon coincidence imaging which may further increase resolution of the current PET imaging.[7] In addition, the radioisotope can be produced together with its long-lived isomeric excited nucleus, ^{44m}Sc (γ, $\tau_{½}$ 2.44 d, 98.8 %, E_γ 270.9 keV), decaying to ^{44}Sc with soft γ emission. The half-life of ^{44m}Sc is matching *in vivo* pharmacokinetics of antibodies and, due to its low-energy transition (recoil energy only 0.89 eV), it can serve as *in vivo* generator of ^{44}Sc as the daughter ^{44}Sc stays inside chelator after decay of the parent ^{44m}Sc nucleus.[8] The ^{44m}Sc/^{47}Sc theranostic pair with both radionuclides having similar half-lives is very suitable for radiopharmaceuticals utilizing antibodies or their fragments and, thus, they form a unique and very promising theranostic pair for cancer treatments. Utilizations of the Sc-based theranostic pairs can be spread from antibody radioimmunotherapy (^{44m}Sc/^{47}Sc pair) to treatments with labeled oligopeptides or small molecules (^{44}Sc/^{47}Sc pair).

The ^{44}Sc can be produced by generator employing ^{44}Ti as a long-lived parent radioisotope.[9,10] The radioisotope can be also produced in most of medical cyclotrons designed for ^{18}F production.[11] The ARRONAX cyclotron produces the ^{44m}Sc/^{44}Sc pair from enriched $^{44}CaCO_3$ target via the deuteron production route[12] which seems to be a promising to get non-carrier-added (NCA) ^{44}Sc with optimized ^{44m}Sc/^{44}Sc ratio. Others production routes allow a better ^{44m}Sc/^{44}Sc ratio by bombarding a ^{45}Sc target[13–15] leading to carrier-added product and it is a major drawback for production of radiopharmaceuticals.

Metallic radioisotopes utilized in nuclear medicine must be tightly bound in a complex to avoid non-specific deposition in tissues. These complexes must exhibit a high thermodynamic stability, a high selectivity for the particular metal ion, a fast complexation of the metallic radioisotopes, kinetic inertness as well as an ability to be conjugated to a biological vector molecule (bifunctional ligands). The design of new radiopharmaceticals is viable multidisciplinary field involving physics, chemistry, biology and medicine.[16–22]

Scandium is a cousin of lanthanides but with some differences. The Sc(III) is smaller than Ln(III) (thus, being harder and with higher preference for hard oxygen donor ligands) and it prefers donor numbers from six to eight. However, the chemistry of trivalent scandium is much less developed than that of trivalent lanthanides.[23] Mostly, multidentate ligands already used in Gd(III)-based MRI contrast agents as well as for radiolanthanides, i.e. derivatives of DTPA (DTPA = diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid) or DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), are the first choice to bind the ion. It has been shown that DOTA derivatives are suitable ligands for scandium radioisotopes.[24] Their oligopeptide,[10,25–29] antibody[30,31] or other[32,33] conjugates have been investigated for complexation of the scandium radionuclides.

Recently, we have investigated the chemistry of Sc(III)–DTPA and Sc(III)-DOTA complexes in details.[34] The study confirms that DOTA is very suitable chelator for trivalent scandium. Efficient radiolabeling of DOTA with $^{44m/44}$Sc requires elevated temperature (>70 °C).[8] For oligopeptides, such a high temperature is not a critical parameter. However, antibodies or their fragments need much lower labelling temperature (mostly below 37 °C) to preserve their immunoreactivity. Slow formation kinetics of DOTA-like chelators remains an important obstacle limiting their use in some radiopharmaceuticals. Therefore, ligands permitting formation of complexes at much lower temperatures to label antibody conjugates are sought.

Scandium(III) is "harder" metal ion than trivalent lanthanides and oxygen atoms in derivatives of phosphoric acid have "harder" character than those in carboxylate group. Thus, phosphonic ($R–PO_3H_2$) or phosphinic (R_2PO_2H) acid pendants may alter ligand behaviour in the desired direction.[35] Such DOTA derivatives form thermodynamically stable and kinetically inert complexes with somewhat enhanced complexation kinetics.[36–38] Their complexes/conjugates are stable *in vivo* and show good pharmacokinetic properties due to their high hydrophilicity.[39–41] Trivalent gallium is a similar very hard metal ion and phosphinic acid derivatives of NOTA (NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid) showed much faster labeling with ^{68}Ga than the parent ligand.[42,43]

Therefore, monophosphorus acid DOTA analogs, DO3AP, DO3AP^{ABn} and DO3AP^{PrA} (Fig. 1) were considered as better ligands than DOTA. Solution investigations of their complexes was complemented by radiochemical studies with non-carrier-added (NCA) ^{44}Sc from two sources, ^{44}Ti/^{44}Sc generator and from ARRONAX cyclotron, and by *in vivo* / *in vitro* evaluation of the radiolabelled complexes.

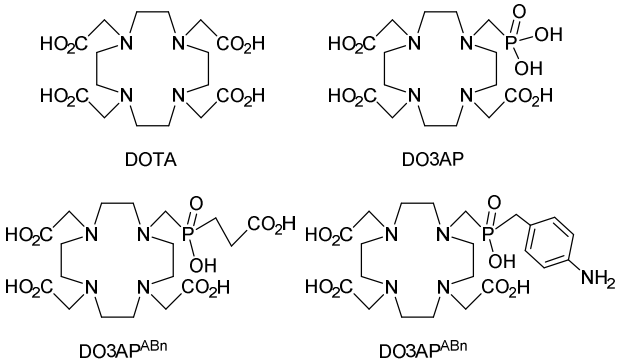

Figure 1. Structures of ligands discussed in this work.

Experimental

General

The phosphorus acid DOTA analogues, DO3AP^{PrA}, DO3AP and DO3AP^{ABn} (prepared as hydrates according to elemental analysis) were synthesized according to literature.[37,44,45] DOTA (reagent grade) was obtained from Macrocyclics. Analytical grade (conc. aq. HCl, conc. aq. ammonia, MeOH, EtOH, HNO_3, ammonium acetate) or pure reagent grade (all other chemicals) reagents were obtained commercially and were used as received unless otherwise specified. Deionized water (18.2 MΩ cm^{-1}; Milli-Q, Millipore) was used throughout the work. Solid-phase extraction resin based on DGA (*N,N,N′,N′*-tetra(*n*-

octyl)diglycolamide; Triskem), cation AG1-X8 (200–400 mesh, Cl^--form; Bio-Rad), anion AG50W-X8 (200–400 mesh, H^+-form; Sigma) exchangers, and Chelex-100 (imino-diacetate functionalized resin; Sigma) were purchased. The concentrations of stable scandium together with potential metals present in the solutions were analysed by ICP-AES (ThermoFischer ICAP 6500 DUO ICP-AES). The wavelengths for each element were selected in order to give the best relative emissions and to prevent interferences. To calibrate the ICP-AES, commercially available single- and multi-elements standards (~10 ppm, SCP Science) were used in dilute nitric acid (1 % w/w). The acidic solutions of the radioisotope batches were collected and were diluted in aq. HNO_3 (1 % w/w) prior to the analysis.

Production of ^{44}Sc

Cyclotron-produced $^{44m/44}$Sc. The procedure of ^{44m}Sc/^{44}Sc production at Arronax cyclotron has been described elsewhere.[12] Briefly, irradiated natural $CaCO_3$ (500 mg) target was dissolved in aq. HCl (4 M, 10 mL) and the resulting solution was loaded onto column filled with DGA resin. Then, the column was washed with aq. HNO_3 (1 M, 10 mL) to remove all other metal ions. Finally, ^{44}Sc was eluted by aq. HCl (0.1 M, 400 μL) and solution was evaporated to dryness. Aq. HCl (0.1 M) was added to get ^{44}Sc(III)-chloride solution with a resulting activity >100 MBq. The solution of $^{44m/44}$Sc was counted on a γ-spectrometer (Ortec-Ametek, France). The energy of the rays were 271.1 keV and 1157 keV for ^{44m}Sc and ^{44}Sc, respectively, with a branching ratio of 98.8 % and 99.9 %. Radionuclidic analysis through gamma spectrometry (HPGe, Ortec) showed that the final solutions contained only $^{44/44m}$Sc. No other significant radionuclides could be detected, even after a decay time corresponding to several half-lives of radioscandium. ICP-AES analyses of the eluate gave a value which is below the detection limit for stable scandium resulting in a specific activity higher than 50 MBq/μmol. The other major metallic contaminants were Fe and Al. In the ^{44}Sc batches, the total concentration of both elements Fe/Al was always 1.14 ± 0.66 ppm.

Generator-produced ^{44}Sc. The procedure how to get ^{44}Sc by this route has been described elsewhere.[10] Briefly, from ^{44}Ti adsorbed onto column filled with AG1-X8 anion exchange resin,, ^{44}Sc was eluted with aq. 0.005 M $H_2C_2O_4$ in aq. 0.07 M HCl (20 mL). The eluate was directly post-processed on miniaturized column filled with AG50W-X8 cation exchange resin (~50 mg) where ^{44}Sc was quantitatively adsorbed on-line and then successively eluted using aq. 0.25 M ammonium acetate buffer (pH 4, 2–3 mL). This ^{44}Sc solution has a small volume and is free of competing oxalates. Radioactivity was determined with a dose calibrator Aktivitätsmessgerät M2316 (Messelektronik, Dresden GmbH). The absolute radioactivity and purity of ^{44}Sc was measured by γ-ray spectrometry with a high-purity germanium (HPGe) well counter detector using both 1157.2 and 1499.4 keV γ-lines.

Potentiometry

Potentiometric titrations were performed by a general method described elsewhere:[34,36] 25 °C, I = 0.1 M $(NMe_4)Cl$, starting volume 5 mL (ligands) or 1 mL (complexes), ligand concentrations 0.004 M, ligand:Sc molar ratio 1:0.95, PHM 240 pH-meter, 2-mL ABU901 autoburette and GKB2401B combined electrode (glass/Ag-AgCl, all Radiometer), pK_w =13.81. Electrode calibration by strong acid-strong base titration was performed before each titration to give a pair calibration/titration which was used for calculations of the constants; the procedure gives reliable pH even at pH below 2 and above 12. To get very acidic protonation constants of $DO3AP^{PrA}$ and $DO3AP^{ABn}$ which were necessary for correct determination of Sc(III) complex stability constants, the ligands were titrated in pH range 1.4–11.8 with electrode calibration done in pH range 1.5–11.8 (~50 points per titration, four titrations per sample). As complexation equilibria in Sc(III)-ligands systems were slow (time to reach full equilibrium was six days; checked by NMR), the titrations were carried out by *out-of-cell* method;[34,36] pH range 1.4–5.5, 23–25 points per titration, two parallel titrations. To help with determination of the first protonation constant of the complexes and enable to correct fit of all equilibrium data for the Sc(III)-$DO3AP^{ABn}$ and -$DO3AP^{PrA}$ systems, the acid-base titrations of the pre-formed complexes were performed.[46] Stock solutions of the ligands (0.02 M) and $ScCl_3$ (0.05 M) were mixed in ampoules (L:Sc molar ratio 1:0.95, c_L = 0.006 M in the final samples) and stock solution of $(NMe_4)OH$ was added (98 % necessary for complete neutralization of the added ligand amount). The ampoule was flame-sealed, heated at 90 °C in an oven for 24 h to ensure complete complexation of Sc(III) ion, cooled to room temperature and opened. The solution was pipetted into the titration vessel, water and excess of $HCl/(NMe_4)Cl$ solution were added (to ensure acidic starting pH and 0.1 M ionic strength), and the solution (starting volume 5 mL) was immediately titrated by $(NMe_4)OH$ stock solution (pH range 1.7–12, ~40 points per titration, 4 parallel titrations, the complex concentration ~0.003 M). Mathematical treatment of all equilibrium data (together with the NMR titration data, see below) was done with OPIUM program package.[47] The constants are concentration constants and were calculated as overall protonation/stability constants β_{hlm} defined generally as $\beta_{hlm} = [H_hLM]/([H]^h \cdot [L] \cdot [M])$; charges are omitted. The calculated overall protonation/stability constants (with standard deviations given directly by the program) are presented in SI (Tables S1 and S2). Throughout the text, pH means $-\log[H^+]$.

NMR measurements

The ^{45}Sc (97.15 MHz) and $^{31}P\{^1H\}$ (161.91 MHz) NMR spectra were recorded on Varian UNITY Inova 400. All experiments, unless stated otherwise, were carried out in 5-mm NMR tubes at 25.0 °C. The ^{31}P NMR chemical shifts were referenced to 85 % aq. H_3PO_4 (δ_P = 0.0 ppm).. The ^{45}Sc NMR chemical shifts were measured with respect to 0.1 M $Sc(ClO_4)_3$ in 1 M aq. $HClO_4$ (δ_{Sc} = 0.0 ppm), 0.01 M $[Sc(ox)_4]^{5-}$ (δ_{Sc} = 8.31 ppm)[34] or 0.01 M $[Sc(tmu)_6](ClO_4)_3$ in CH_3NO_2 (tmu = *N,N,N′,N′*-tetramethylurea; δ_{Sc} = –53.3 ppm)[48] as external

standards in the insert tube. Equation pD = pH + 0.4 (pH is a standard pH-meter reading) was used for D_2O solutions.

Samples for NMR titrations were prepared similarly to the *out-of-cell* titration as given above and analogously to the previous paper.[34] Stock solutions of the ligands, $ScCl_3$, HCl, KCl (if necessary), and water were mixed into tubes (final c_L = 0.004 M, L:Sc molar ratio 1:0.95, V_0 = 1 mL; I = 0.1 M (H,K)Cl, with no control of ionic strength at pH <1.1) to get pH in range 0.5–1.3 and the tubes were tightly closed (12–15 titration points); equilibration time seven days at room temperature. Species abundance in each tube (i.e. the titration point) was determined by ^{45}Sc NMR measurements. Two parallel titrations were carried out. To determine the equilibration time, another samples were prepared as above at three pH's, the samples were left at room temperature and ^{45}Sc NMR spectral changes with time were followed.

Samples for equilibrium studies with the Sc(III)-DO3AP system were prepared by mixing stock solutions of DO3AP, $ScCl_3$, 17 % or 5 % aq. HCl, 1.1 M or 0.2 M HCl, 0.2 M KCl (if necessary), and water into tubes (conditions as above). The solutions were left to equilibrate for at least seven days at room temperature and pH values were determined as above. Job's method was employed to determine stoichiometry of the *out-of-cage* species in the Sc(III)-DO3AP system. Each sample was prepared in a vial by mixing 0.1 M aq. $ScCl_3$ and 0.1 M aq. DO3AP stock solutions with 1 M aq. HCl (final volume 1 mL, pH 0.22, c_{Sc} = 4–20 mM, c_{Sc+L} = 40 mM, 7 points, equilibration time seven days). To determine protonation constants of $[Sc(DO3AP)]^{2-}$ isomeric species, a pre-prepared complex was dissolved in H_2O in 10-mm NMR tube and desired pH was adjusted with aq. HCl or aq. $(NMe_4)OH$ (c_{ScL} ~0.01 M, pH range 4.15–7.67, 26 points,). The pH was determined by a freshly calibrated (three buffers) combined pH electrode fitting the NMR tube.

To characterize the $[Sc(DO3AP^{PrA})]^{2-}$, $[Sc(DO3AP^{ABn})]^{-}$ and $[Sc(DO3AP)]^{2-}$ complexes, their solutions were prepared by dissolving the appropriate ligand (0.05 mmol), $ScCl_3 \cdot 6H_2O$ (0.055 mmol) in water (1 mL) and adjusting the solution pH to ~7 with solid Li_2CO_3. Then, the solutions were stirred in sealed vials at 90 °C for 2 h. Excess of Sc(III) precipitated as white Sc(III)-carbonate/hydroxide was filtered off with a syringe filter and the filtrate was evaporated to dryness. The solid was dissolved in D_2O (0.5 mL) to get solution with the complex concentration ~0.1 M.

Free-ion selective radiotracer extraction (FISRE) method

An aliquot of the Chelex-100 chelating resin was mixed with bulk aq. solution containing ^{45}Sc to give a final concentration of the isotope 10^{-5} M. To prevent the potential interference of nitrate ions on distribution behaviour of the metallic content, aliquots of standard solutions were transferred to a dry teflon beaker pre-cleaned with ultrapure aq. HNO_3 (2 % w/w). Then, they were evaporated to dryness and recovered in the suitable solution three times. The ligands stock solution was added to reach final ligand concentration ranging from 10^{-7} to 10^{-3} M. All measurements were performed at ionic strength I = 0.1 M NaCl. The pH of the suspension was adjusted to pH= 2.6; 2.5 and 2.3 according to the ligand (DO3AP, $DO3AP^{PrA}$ or $DO3AP^{ABn}$, respectively). These pH values were estimated taking into account stability constants values ($\log K_{ScL}$) obtained from the equilibrium data. As the distribution coefficients were calculated as a function of dried resin mass, humidity content was determined by drying five samples of each pre-conditioned resin in oven at 105 °C. The final distribution coefficients were calculated on basis of the arithmetic averages of replicate analysis (at least triplicate analysis). The resin concentration (in g/mL) was chosen for each batch to minimize the global uncertainty of partition coefficient. The reproducibility of $\log K_D$ was better than 5 %.

The resulting suspension was equilibrated for 24 h and the pH re-adjusted, if necessary. In preliminary experiments, sufficient equilibration time was found to be six days for all the systems. Solid and liquid phases were separated by sedimentation. Aliquots (1 mL) of the supernatant were taken for ICP-AES analysis. Scandium concentrations were determined and experimental K_d values were plotted as a function of the total ligand concentration in the solutions. These dependences were used for stability constant determinations. To check a potential presence of protonated complexes, other sets of experiments were performed at fixed ligand concentration (10^{-3} M) in the pH range 2–7 utilizing the same protocol as described above.

Radiolabelling with ^{44}Sc

^{44}Sc from ^{44}Ti/^{44}Sc generator. Labeling of monophosphorus acid DOTA analogues and DOTA with ^{44}Sc was performed by mixing of ligand stock solution with the post-processed ^{44}Sc eluate in 0.25 M ammonium acetate buffer (pH 4.0) and heating the mixture in an oil bath. Several parameters were followed in repeated experiments (3–4 times): temperature, pH of the reaction mixtures and the ligand concentration (precisely, ligand molar excess over radioisotope molar amount). Influence of temperature and incubation time on reaction yield was investigated by heating solutions containing ^{44}Sc (16.2 MBq, $[Sc^{3+}]$ ~$5.8 \cdot 10^{-13}$ M) and 20 nmol of the ligand (DOTA, DO3AP or $DO3AP^{ABn}$) at 40 °C, 70 °C and 90 °C for up to 30 min. To determine effect of pH, 4 M aq. HCl or 4 M aq. NaOH was added dropwise to mixture (3 ml) of ligand (20 nmol) and ^{44}Sc (1.3 MBq, $[Sc^{3+}]$ ~ $4.5 \cdot 10^{-14}$ M) in 0.25 M aq. ammonium acetate buffer to get desired pH (2–6) and the solutions were then heated at 90 °C for 30 min.; pH was measured before and after the heating and no significant change observed. Effect of ligand concentration/excess was evaluated with quantity of ligand being varied from 1 to 30 nmol and the solutions were heated (70 °C, pH = 4, 30 min). Radiolabeling was checked by thin-layer chromatography (TLC Silica-gel 60 plates, Merck). The plates were developed by 0.24 M aq. sodium citrate buffer (pH = 4). Quantitative distribution of radioactivity on TLC plates was measured using an electronic autoradiography system (Instant Imager, Packard Canberra®). The uncomplexed ^{44}Sc moved with R_f = 0.8, whereas the ^{44}Sc complexes stayed on start with R_f = 0.

^{44m}Sc/^{44}Sc from cyclotron (Arronax). For the $^{44m/44}$Sc mixture, the influence of temperature and pH on labelling yields was investigated as described above. To follow influence of the

ligand concentration/excess, lower ligand amounts had to be used (if compared with the ^{44}Sc from generator); they varied from 24 to 240 pmol (pH 4, 70 °C, 30 min). Radiolabeling was followed by spotting 3×1 µL onto a TLC Flex Plate (silica gel 60A, lF-254, 200 µm, Merck) followed by elution with conc. aq. NH_3/H_2O/MeOH 2/1/1 (v/v/v). The resulting TLC plate was counted for 10 min on an autoradiographic system (Cyclone, Perkin Elmer). Under these conditions, the ^{44}Sc complexes moved with R_f = 0.9 whereas the unchelated ^{44}Sc had R_f = 0.

In vitro and *in vivo* experiments

The *in vitro* challenge studies (stability in serum, hydroxoapatite adsorption) were done by standard procedures and details are given in Supporting Information.

Animal studies were carried out in accordance with the guidelines of the French law on Animal Studies. Biodistribution of the ^{44}Sc-DO3AP complex, was followed in four healthy Wistar rats injected intravenously with ^{44}Sc-DO3AP solution (100 µL, 1 MBq). The animals were imaged at selected time intervals, then sacrificed and dissected. Rats were sacrificed at 30 min and 1 h, two rats per time point. Organs were collected, weighed, counted on a gamma counter, and the percentage of injected dose per gram of tissue (% ID/g) was calculated. PET images were acquired in a Siemens Inveon micro PET/CT instrument.

Results and discussion

Characterization of the Sc(III) complexes in solution

Structure of the *in-cage* complexes in solution was determined by ^{1}H, ^{31}P{^{1}H} and ^{45}Sc NMR measurements. In all cases, ^{1}H NMR spectra were very complicated due to the non-equivalence of all protons in a rigid structure. The ^{31}P{^{1}H} NMR spectra showed presence of two signals (Figure 2). On basis of a number of data for lanthanide(III) complexes of DOTA-like ligands, such observation can be explained by presence of two isomeric complexes having square antiprismatic (SA) and twisted square antiprismatic (TSA) arrangements. These isomers have been observed for lanthanide(III) complexes of the title ligands.[44,45] It is surprising as only one isomer, SA, was observed in solution for the $[Sc(DOTA)]^-$ complex,[50] although both isomers have been found in the solid state.[34,50] It was observed that ^{31}P NMR chemical shift and relative abundance of the TSA/SA isomers of the Sc(III)-DO3AP complex is changed in the pH range 4–7. Such behaviour has been observed for lanthanide(III) complexes of DO3AP[44] and is connected with protonation of the coordinated phosphonate group. Thus, ^{31}P{^{1}H} NMR spectra were measured over the pH range (Figure S4) and used for calculation of protonation constants for each complex isomer. The results, logK_a 5.33 and 5.53, corresponds well to the potentiometric data (see below) and the values are similar to those found for the Ln(III) analogues.[44] The isomer ratio was not changed with pH for the complexes of the phosphinate ligands.

The ^{45}Sc NMR spectra (Figure 2) measured in 0.1 M solutions of pre-prepared complexes showed very broad peaks 87 ppm ($\varpi_{½}$ ~5200 Hz), 87 ppm ($\varpi_{½}$ ~5700 Hz) and 90 ppm ($\varpi_{½}$ ~7200 Hz) for the $[Sc(DO3AP)]^{2-}$, $[Sc(DO3AP^{PrA})]^{2-}$ and $[Sc(DO3AP^{ABn})]^-$ complexes, respectively. The values are similar to those observed for the $[Sc(DOTA)]^-$ (δ_{Sc} ~100 ppm, $\varpi_{½}$ ~4300 Hz)[34] also confirming formation of the *in-cage* complex for all ligands.

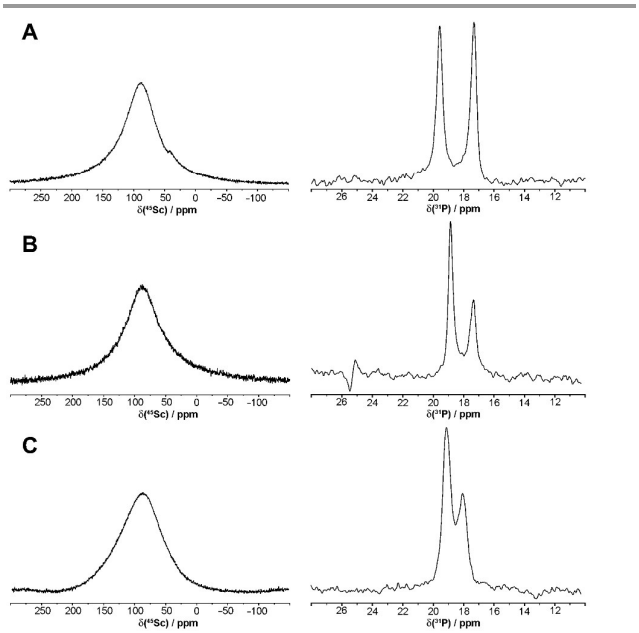

Figure 2 ^{45}Sc and ^{31}P{^{1}H} NMR spectra of $[Sc(DO3AP)]^{2-}$ (**A**), $[Sc(DO3AP^{PrA})]^{2-}$ (**B**) and $[Sc(DO3AP^{ABn})]^-$ (**C**) in D_2O (0.1 M, pD = 7.1).

Equilibrium studies

For the Sc(III)-DO3AP^{ABn} and -DO3AP^{PrA} systems, it was found that equilibrium at pH >1.8 (*i.e.* formation of *in-cage* complexes, see below) is established during several days and the rate of complexation is faster with higher pH (Figure S1). Equilibria in very acidic solutions (formation of *out-of-cage* complexes) were established almost immediately. Similarly to the Sc(III)-DOTA system, Sc(III)-aqua complex disappeared above pH ~1.8. However, some complex formation started even at pH 0 – 0.5 (Figure S2) as indicated with a broad ^{45}Sc NMR peak (~35 ppm). This chemical shift suggests purely oxygen coordination sphere. It can be facilitated by the presence of phosphinic acid group able to bind metal ions even in very acidic solutions (see also below). Therefore, the species can be assigned as *out-of-cage* complexes where Sc(III) ion is coordinated by oxygen atom(s) from one or more pendant arms of the ligands. Once the *in-cage* complexes (Sc(III) ion is sandwiched between N4 and O4 planes as in the $[Sc(DOTA)]^-$ complex[50]) are formed, ^{45}Sc NMR signal of the *out-of-cage* complexes starts to broaden and decreases in intensity and, finally, no ^{45}Sc NMR signal is observed (due to a low concentration of the complexes in these titration experiments). In parallel, two broad closely located ^{31}P{^{1}H} NMR signals assignable to the Sc(III)-bound phosphinate group in the isomeric *in-cage* complexes start to be observed. To quantify abundance of Sc(III)-aqua ion for stability constant determination (see below), $[Sc(tmu)_6]^{3+}$ had to be used as secondary standard[48] as other standards[34] overlapped with the signal of the *out-of-cage* species.

Behaviour of the Sc(III)-DO3AP system was analogous to that observed for the Sc(III)-DO3AP^{ABn} and -DO3AP^{PrA} systems only at pH above ~3.8; no ^{45}Sc and two ^{31}P{^{1}H} NMR peaks were observed confirming formation of the *in-cage* complex (Figure 3). From potentiometric titrations in this pH range, only the constant corresponding to protonation of coordinated phosphonate group in the *in-cage* complex, logK_a 5.29, could be determined (Table S2). However, unexpected results were obtained for the Sc(III)-DO3AP system in more acidic solutions. Here, another peaks appeared in both NMR spectra: δ_{Sc} ~23 ppm, and δ_P –8.3 ppm (Figure 3). The ^{45}Sc NMR signal of Sc(III)-aqua complex start to be observable below pH ~0.8. Surprisingly, the signal at δ_{Sc} ~23 ppm was present even in extremely acidic solutions (up to 1:1 aq. HCl). Most probably, the NMR signal can be assigned to an *out-of-cage* complex species where at least the protonated phosphonate group (and, possibly, the acetate group(s) as well) is bound to scandium(III) ion. Such coordination mode of phosphonate group has been observed with metal ions (e.g. trivalent lanthanides) even under highly acidic conditions.[51,52] Overall stoichiometry of the Sc(III)-DO3AP species present in the acidic solutions can be roughly estimated as Sc : L = 1 : 2 from a Job-like plot of NMR signal intensities (Figure S3) and, overall, the speciation is rather complicated. Unfortunately, such solution behaviour (*i.e.* not determinable correct Sc/DO3AP stoichiometry as well as a number of protons in the species) precluded determination the Sc(III)-DO3AP complex stability constants as the system is too complex to be modeled by equilibrium constant calculations.

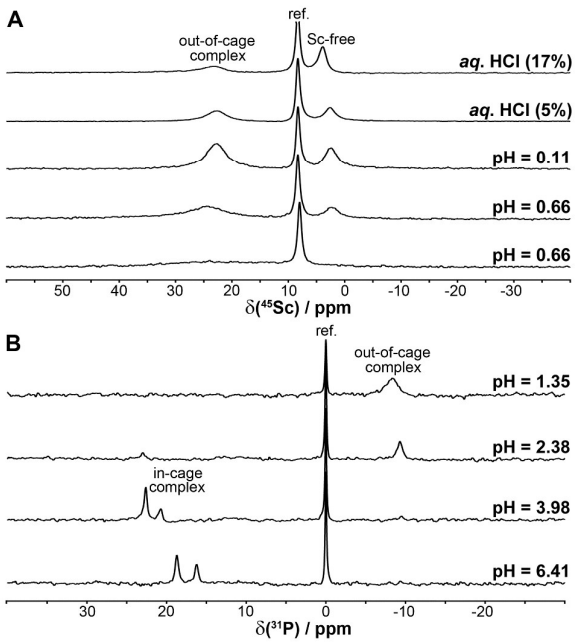

Figure 3 ^{45}Sc (**A**) and ^{31}P{^{1}H} (**B**) NMR spectra of equilibrated solutions prepared by mixing $ScCl_3$ and DO3AP (c_{Sc} = c_L = 0.004 M). The given pH values are those of the equilibrated solutions.

Table 1 Equilibrium stability constants of scandium(III) complexes of DO3AP, DO3AP^{ABn} and DO3AP^{PrA} and their comparison with those of DTPA and DOTA[34] (25 °C, I = 0.1 M (NMe_4)Cl).

Equilibrium[a]	DO3AP^{ABn}	DO3AP^{PrA} [b]	DO3AP[c]	DOTA	DTPA
L + Sc ↔ [Sc(L)]	27.03	28.31		30.79	27.43
[Sc(L)] +H ↔ [Sc(HL)]	5.17	4.18	5.29	1.0	1.36
[Sc(HL)] + 2H ↔ [Sc(H_3L)]	3.96	4.35			
[Sc(OH)(L)] + H ↔ [Sc(L)] + H_2O	12.83	12.03			12.44

[a]Charges are omitted for clarity. [b]The most acidic protonation constants of DO3AP^{PrA} were re-determined: logK_5 2.94 and logK_6 1.54 (Table S1). [c]Other constants could not be determined (see text).

The stability constants were determined by combination of three techniques, ^{45}Sc NMR, out-of-cell and direct (in-cell) potentiometry (for more detailed discussion, see SI). The stability constants are presented in Table 1 and the experimentally determined overall stability constants are given in Table S2. The corresponding distribution diagrams are shown in Figure 4. The Sc(III) complexes of DO3AP^{ABn} and DO3AP^{PrA} are more stable than their lanthanide(III) complexes (logK_{LuL} 24.0 and 25.5, respectively)[37] but less stable than the [Sc(DOTA)]$^-$ complex (logK_{ScL} 30.79).[34] The difference in stability constants between the lutetium(III) and scandium(III) complexes is similar for all ligands. The first Sc(III) complex protonation constants, corresponding to protonation of amino or carboxylate groups in the phosphorus side chain, are almost identical as those of lutetium(III) complexes.[37] Next two protons are attached to the ring nitrogen atoms with formation of *out-of-cage* complexes where scandium(III) ion is bound only to pendant arm oxygen atom(s). Such chemical model has already been suggested for lanthanide(III)-DO3AP systems[36] and such *out-of-cage* species diprotonated on ring amines has been observed for lanthanide(III) complexes in the solid state as well as in solution.[41,53] The very high abundance of the triprotonated species (Figure 4) can be caused by a strong preference of trivalent scandium for hard phosphinate oxygen donors in the *out-of-cage* species.

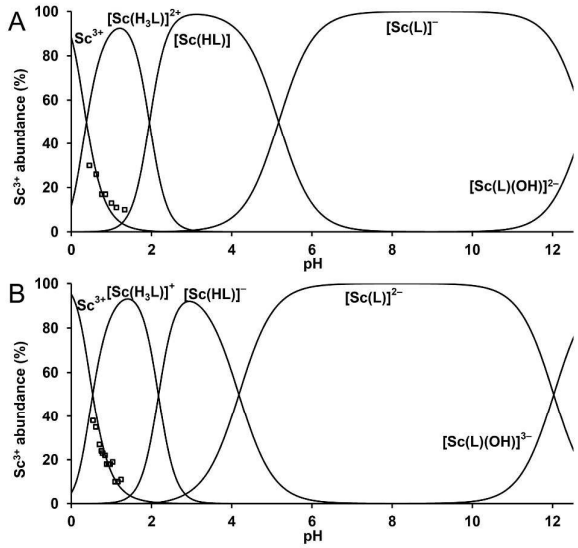

Figure 4 Distribution diagram for Sc^{3+}-DO3AP^{ABn} system (**A**) and Sc^{3+}-DO3AP^{PrA} system (**B**);. $c_{Sc} = c_L = 0.004$ M. Abundance of free Sc^{3+} ion (□) was determined by ^{45}Sc NMR spectroscopy.

As stability constants of the Sc(III)-DO3AP complex cannot be determined by the above method, a competition method with trivalent ytterbium (as metal ion with very high stability constant with DO3AP, $\log K_{YbL}$ ~28.5)[36] as well as transchelation with DTPA were tested. In both cases, the determination failed due to problems with too slow kinetics of the transmetallation or transchelation.

Determination of stability constants under trace concentrations.

Another technique, Free Ion Selective Radiotracer Extraction (FISRE) method[54] was employed to determine stability constants in trace (micromolar) concentrations of the ligands. The technique uses chelating ion exchanger (here, Chelex-100 containing imino-diacetate groups was used) as a competing "ligand". The chelating resin competes with the ligands for Sc^{3+} ion and the speciation is determined by solid/liquid separation. Recently, the method has been used for determination of thermodynamic stability of metallo-radiopharmaceuticals containing Y(III).[55] The technique has been also used to estimate stability constants of scandium(III) complexes with EDTA, DTPA, NOTA and DOTA.[24]

Adsorption of the free Sc^{3+}-aqua ion on imino-diacetate chelating groups ($\overline{X-H}$) can be described by the following equilibrium (Equation 1).

$$Sc^{3+} + 3\overline{X-H} \longleftrightarrow \overline{X_3-Sc} + 3H^+ \qquad (1)$$

The overlined species refer to the species present on the resin (adsorbed species). The electroneutrality of each phase is required. Stability constants could be estimated by fitting dependence of the distribution coefficient (K_d) of Sc(III) between the resin and supernatant on the total ligand concentration in the supernatant. If both ligand and exchange resin are used in a large excess in comparison to the initial Sc concentration, K_d values could be expressed as Equation 2

$$K_d = \frac{K_{ads}\left[\overline{X-H}\right]^3}{\left[H^+\right]^3 \alpha_{Sc(L,OH)}} \qquad (2)$$

where K_{abs} is equilibrium constant for binding scandium(III) to the resin. The $\alpha_{Sc(L,OH)}$ is the complexation coefficient of Sc^{3+} that is defined as ratio between the total aqueous scandium concentration $[Sc(III)]_{sol}$ and the Sc^{3+}-aqua ion concentration $[Sc^{3+}]$ by Equation 3

$$\alpha_{Sc(L,OH)} = \frac{[Sc^{3+}]_{sol}}{[Sc^{3+}]} = 1 + \sum_{h,l} \frac{[ScH_hL_l]}{[Sc^{3+}]} = 1 + \sum_{h,l} \beta_{h,l}\,[L]^l\,[H^+]^h \qquad (3)$$

where $\beta_{h,l}$ is overall stability constant of the complexes in question, $[ScH_hL_l]$, as defined by Equation 4.

$$\beta_{h,l} = \frac{[Sc\ L_l H_h]}{[Sc]\cdot[L]^l\cdot[H]^h} \qquad (4)$$

As $\alpha_{Sc(L,OH)}$ is function of the ligand protonation constants and stability constants of the corresponding Sc(III) complexes, the stability constants could be calculated by fitting of the K_d values determined at various ligand excess and at various solution pH according to Equation 2. For the full set of equations and more explicit explanation of the method, see SI.

Transchelation kinetics of macrocyclic ligands is generally slow. Therefore, equilibration time was at least six days. The experimental data are depicted in Figures 5 and S5. To get a good fit of the data obtained for various pH, not only [Sc(L)] but also [Sc(HL)] species had to be included in all systems (for comparison of different models, see Figure S5). The results are summarized in Table 2. The values obtained by the FIRSE method are in a reasonable qualitative agreement with the values obtained by potentiometry/NMR (Table 1) if errors naturally accompanying utilization of trace concentrations and very high absolute values of the constants are taken into account.

The results show that the method can be used for fast screening of complex stabilities as it works with significantly lower amounts of compounds in comparison with common techniques such are potentiometry or NMR. Further, it could be used also for studying "problematic" metal ions (e.g. trivalent or tetravalent metal ions) or ligands. It can be used for a qualitative evaluation of metal ion-ligand interactions at trace level. In addition, the FISRE method is more easy to carry out, faster and operationally cheaper than the "standard" methods (here, stability constant of the [Sc(DO3AP)]2– complex cannot be determined by the conventional methods) and gives results which can be used for evaluation of complexation ability of new ligands toward metal ions intended to be utilized in radiopharmaceuticals.

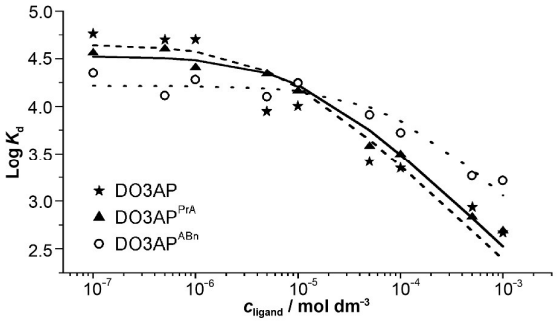

Figure 5 The Sc(III)-ligand isotherms obtained by the FISRE method: DO3AP (full line); DO3AP^{PrA} (dashed line) and DO3AP^{ABn} (dotted line). The lines correspond to the fitting as explained in SI. Solution pH and concentration of the Chelex resin were adjusted for each batch to minimize the global uncertainty of partition coefficient (I = 0.1 M NaCl).

Table 2 Equilibrium constants (log$K$) of scandium(III)-DO3AP, -DO3AP^{ABn} and -DO3AP^{PrA} complexes as determined by the FISRE method.

Equilibrium[a]	DO3AP[c]	DO3AP^{ABn}	DO3AP^{PrA} [b]
L + Sc ↔ [Sc(L)]	27.75(8)	25.51(4)	25.75(8)
H + L + Sc ↔ [Sc(HL)]	33.20(3)	29.20(6)	30.02(8)
[Sc(L)] +H ↔ [Sc(HL)]	5.45	3.69	4.27

[a]Charges are omitted for clarity.

Labelling of the title ligands with generator- (^{44}Ti/^{44}Sc) and cyclotron-produced ($^{44m/44}$Sc) radioscandium

Labelling efficiency of the title ligands was tested at different solution pH's, temperatures and ligand concentrations (or more correctly, at different radiometal-to-ligand molar ratios). The labelling was done with no-carrier-added (NCA) radioscandium from two sources: ^{44}Sc eluated from ^{44}Ti/^{44}Sc generator (finally obtained in 0.25 M aq. ammonium acetate buffer, pH 4)[9,10] and mixture of ^{44m}Sc and ^{44}Sc radioisotopes produced by accelerator (finally obtained in 0.1 M aq. HCl)[12] as both sources can be considered for future regular production of ^{44}Sc. The radioscandium from each source differs in specific activity commonly obtained and/or in cold metal ion impurity content. The labelling results are summarized in Table 3 and Figures 6 and 7.

Table 3 The ^{44}Sc labeling yields (in %) for DOTA and its monophosphorus acid analogues at different metal:ligand ratios. Experimental conditions: t = 70 °C, 30 min, pH = 4. All yields are given within the experimental uncertainties of the cyclone device of ±5%.

Radioisotope source	n_{ligand} (nmol)	Ligand			
		DOTA	DO3AP	DO3AP^{PrA}	DO3AP^{ABn}
Generator ^{44}Sc	1	10.1	10.0	8.6	1.9
	3	96.5	91.6	88.5	4.3
	5	96.7	93.8	95.7	20.8
	10	96.8	95.3	97.0	91.1
	20	97.3	95.7	97.1	94.7
	25	97.1	96.6	96.5	95.1
	30	97.6	97.6	97.7	96.6
Cyclotron ^{44m}Sc/^{44}Sc	0.02	31.1	36.1	28.4	32.5
	0.07	97.8	97.4	88.3	90.6
	0.12	98.9	98.8	94.2	92.7
	0.17	99.4	98.8	98,9	94.7
	0.24	99.6	99.1	99,1	96.4

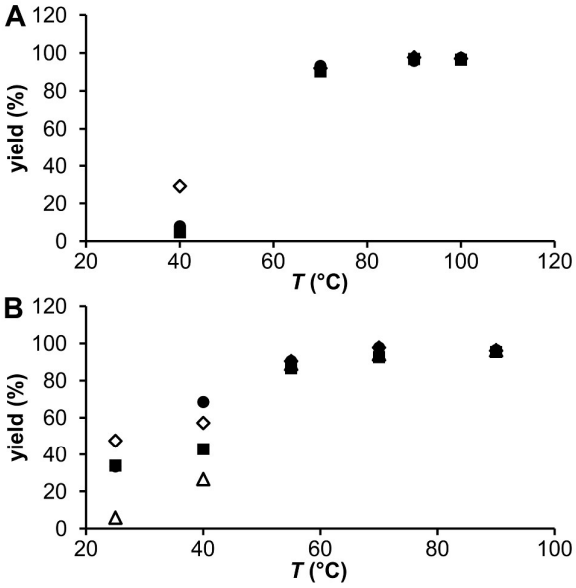

Figure 6 Radiolabeling of DOTA (•) and its monophosphorus acid analogues (◊ DO3AP ▪ DO3AP^{ABn} Δ DO3AP^{PrA}) at different temperatures (pH 4.0, labelling time 30 min): (**A**) generator ^{44}Sc, n(ligand) = 20 nmol; n(Sc) = 4.5·10^{-5} to 1·10^{-4} nmol; A(^{44}Sc) = 3 kBq. (**B**) cyclotron ^{44m}Sc/^{44}Sc, n(ligand) = 0.2 nmol; n(Sc) = 1.5·10^{-6} nmol; A(^{44}Sc) = 3 kBq.

As excess of a chelator over a metal radioisotope determines accessible specific activity, influence of chelator/radioscandium molar ratio on radiochemical yield was tested under conditions previous successfully used for DOTA derivatives: labelling time 30 min, pH 4 and temperature 70 °C. Results are presented in Table 3. With generator ^{44}Sc, overall radiolabelling yields for ^{44}Sc-DOTA and ^{44}Sc-DO3AP were quite similar ranging from 10 % to 95 % if 1 or 5 nmol of the ligand, respectively, were added. In contrast, radiolabeling yield for ^{44}Sc-DO3AP^{ABn} was much lower (approx. 25 % if <10 nmols of DO3AP^{ABn} was used) and 20 nmol of the ligand was required to reach 95 % radiolabeling. With cyclotron $^{44m/44}$Sc, amount of the ligands required to reach the same radiolabelling

yields was significantly lower, although the same general trend was observed. Thus, 0.2 nmol of the ligands is enough for cyclotron $^{44m/44}$Sc to get a minimum of 95 % radiolabeling *versus* more than 5 nmol of the ligands for the ^{44}Sc from ^{44}Ti/^{44}Sc generator. The differences between the radioscandium from the two sources could be explained by a different content of cold metallic impurities competing with the ligands for the scandium radioisotope.

Specific activity (SA), a measure of the radioactivity per unit mass of the compound, is one of the major criteria for radiopharmaceuticals and should be as high as possible. Specific activities of the radioscandium from each source and for each ligand were calculated and the values are summarized in Table S3. The calculated specific activity of the cyclotron $^{44m/44}$Sc is always higher than 10 MBq/nmol (4 h after end of beam). However for the generator ^{44}Sc, specific activity was estimated to be max. ~2 MBq/nmol (for DOTA; 4 h after end of elution).

Influence of temperature and solution pH was tested under conditions suggested by the experiments above: 20 nmol and 0.2 nmol for the generator ^{44}Sc and the cyclotron ^{44m}/^{44}Sc, respectively, and at reaction time 30 min. The results are plotted in Figure 6. At 40 °C, the generator ^{44}Sc revealed 30 % labeling for DO3AP whereas 5–8 % yield was achieved for the other ligands. The yields increased with temperature as expected, to more than 90 % and 95 % at 70 °C and 90 °C, respectively, but with no difference among the ligands. Surprisingly at low temperature, labelling yield with DOTA was significantly lower than that with DO3AP. On the other hand with the cyclotron $^{44m/44}$Sc, even if concentrations of the ligands were much lower compared to those for generator ^{44}Sc, higher radiolabeling yields, mainly at lower temperatures, were obtained. At 40 °C (a temperature still suitable for antibody labelling), radiolabelling yields >40 % (^{44}Sc-DO3AP^{ABn}), >55 % (^{44}Sc-DO3AP) and ~70 % (^{44}Sc-DOTA) were observed; the values for DOTA agreed well with those previously published.[8] Complete binding (>95 %) was achieved at 70 °C for all ligands. Thus, replacement of an acetate group on DOTA skeleton does not lead to noticeable improved radiolabelling at lower temperature if compared with parent DOTA. Monophosphinate ligands were labelled worse than DOTA or DO3AP at lower temperatures.

Influence of solution pH was investigated in the range 2–6 and results are shown in Figure 7. For radioscandium from both sources, the best labelling was observed at pH 4–5 with some decrease at higher pH, probably due to formation of colloidal scandium(III)-hydroxide species, as found in the previous works.[9,10] When solution pH was reduced to 2, labelling efficiency significantly decreased for all the ligands but more for the phosphorus acid DOTA analogues. It is in agreement with the equilibrium data (see above) as monophosphorus acid DOTA analogs form the *out-of-cage* complexes with higher abundances than DOTA does. So, the optimal radiolabeling yields were achieved using facile conditions - pH 4, reaction time 30 min, and incubation temperature 70 °C.

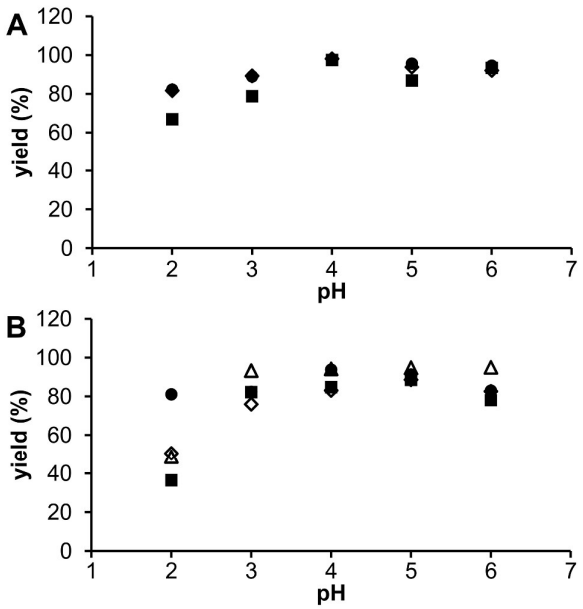

Figure 7 Radiolabelling of DOTA (•) and its monophosphorus acid analogues (◊ DO3AP ▪ DO3AP^{ABn} Δ DO3AP^{PrA}) at different pH (t = 70 °C, labeling time 30 min): (**A**) generator ^{44}Sc, n(ligand) = 20 nmol; n(Sc) = 4.5·10^{-5} to 1·10^{-4} nmol; A(^{44}Sc) = 3 kBq. (**B**) cyclotron ^{44m}Sc/^{44}Sc, n(ligand) = 0.2 nmol; n(Sc) = 1.5·10^{-6} nmol; A(^{44}Sc) = 3 kBq.

Unlike for Ln(III)_ions,[37–40] labelling with ^{44}Sc was not improved. As discussed above, formation of metal ion complexes of DOTA-like ligands is a two-step process,[41,53] *i.e.* formation of the *out-of-cage* complex is followed by proton removal from, and metal ion transfer into, the ligand cavity as rate-determining step. Both steps should be optimized to improve labelling efficiency. Here, basicity of ring nitrogen atoms is similar in all investigated ligands leading to the similar labelling efficiency. In addition, small Sc(III) ion may not fit well into cavity of the title ligands. Somewhat better labelling with DO3AP might be connected with rather hard character of phosphonate group leading to a good interaction with small and hard Sc(III) ion. The effect of right combination of cavity size, basicity of ring amines and suitable properties of pendant donor atoms for efficient formation of the *out-of-cage* complex has been shown for phosphinic acid NOTA analogues. Very small trivalent gallium is perfectly fitting small cavity of NOTA-like ligands, hard phosphinates are very selective for hard Ga(III) ion and they also decrease ring amine basicity. It all leads to a very efficient labelling of these ligands with ^{68}Ga.[42]

In vitro and *in vivo* studies

Even the labelling studies with the monophosphorus acid DOTA analogs did not show improved properties, *in vitro* / *in vivo* properties of their $^{44m/44}$Sc complexes were evaluated. The serum stability can be seen as benchmark of behaviour of compounds in extracellular environment and provide information on possible pathways how the radiopharmaceutical in question can be demetallated.[49] It is well known that phosphonate-containing compounds may have a high affinity to bone and hydroxoapatite (HA), as an *in vitro* model material of

bone, sorption was also investigated. Challenging studies against either hydroxyapatite, or rat serum were monitored as a function of time and are presented in Figure S6. It was observed that ^{44}Sc-DO3AP, ^{44}Sc-DO3AP^{ABn} and ^{44}Sc-DO3AP^{PrA} complexes were stable over several hours in serum, similarly to ^{44}Sc-DOTA. It is also in accordance with previous studies on radiolanthanide complexes of DO3AP^{ABn} and DO3AP^{PrA}.[39,40] Therefore, ligands with DOTA-like structure seem to be good chelators for Sc(III) forming stable complexes. Negligible sorption of all complexes, even that of ^{44}Sc-DO3AP, on HA shows that no bone uptake should be expected after intravenous applications.

To better prove that ^{44}Sc-DO3AP (as the best ligand in the investigated series) has no uptake on bone, its biodistribution was investigated in the healthy rats and the results are shown in Figure 8 and Table S4. These data show no specific uptake and a rapid clearance through urine. The PET image confirmed this observation. Thus, the ^{44}Sc remains in the chelate *in vivo* and the ^{44}Sc-DO3AP complex is not adsorbed on bone.

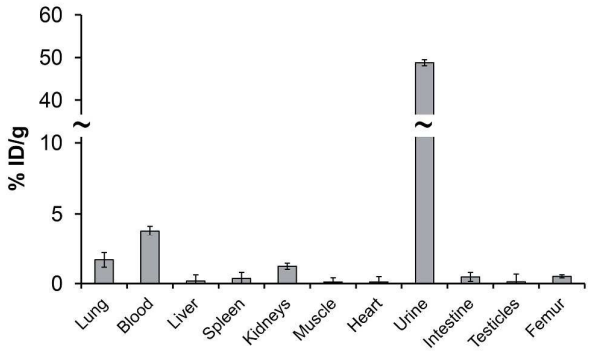

Figure 8. Biodistribution of ^{44}Sc-DO3AP in healthy Wistor rats (n = 4, 1 h p.i.)

Conclusions

The DOTA derivatives bearing one methylphosphonic/phosphinic acid pendant arm, DO3AP, DO3AP^{PrA} and DO3AP^{ABn}, were evaluated as chelators for trivalent scandium. As expected, stability constants with Sc(III) are several orders of magnitude higher than those for trivalent lanthanides. Thermodynamic stability constants were also examined by FISRE method. The method gave qualitatively similar results but it has an advantage over the common method that, as it is based on trace amount of metal ions, can be used for several radiometal ions where conventional methods for stability constant determination can be hardly implemented. Such metal ions of potential radiopharmaceutical interest include e.g. easily hydrolyzing metal ions as Zr(IV), Bi(III), Ac(III) or Th(IV). In addition, experimental conditions of the method are more close to the real used for preparation of radiopharmaceuticals.

Labelling efficiency of DOTA and its analogues was, for the first time, investigated on radioscandium from two sources, generator- or cyclotron-produced ^{44}Sc. Chelator excess over radioscandium necessary for efficient labelling was higher for the generator ^{44}Sc. The difference might be attributed to various amounts of cold metal impurities in radioscandium from each source. The best labelling conditions for DO3AP, DO3AP^{ABn} and DO3AP^{PrA} were the same as for DOTA. The phosphonate ligand, DO3AP, showed somewhat better labelling efficiency at low temperature and it is a hint for possible future ligand design. Specific activity after labelling was higher for the cyclotron produced $^{44m/44}$Sc (~10 MBq/nmol) than for the generator produced ^{44}Sc (~2 MBq/nmol). *In vitro* stability of the Sc(III) complexes is very high as expected for complexes of macrocyclic ligands. No specific uptake and a rapid urine clearance of ^{44}Sc-DO3AP was observed in healthy rats. Slow formation kinetics remains the main challenge in design of chelators for scandium and lanthanide radioisotopes.

Acknowledgements

We thank the French-Czech travel grant (Barrande PHC 21934B) and PICS-CNRS 5196 (to R.K., C.A., S.H.), the Grant Agency of the Czech Republic (13-08336S; to M.P., J.H., V.K., P.H.) for financial support. The work was done in the frame of TD1004 COST Action. The ARRONAX cyclotron is a project promoted by the Regional Council of Pays de la Loire financed by local authorities, the French government and the European Union. This work was also supported by grants from the Pays de la Loire Council "Nucléaire pour la Santé" (NucSan) and from the French National Agency for Research called "Investissements d'Avenir" no. ANR-11-LABX-0018-01.

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Table of contents entry:

Text:
Influence of phosphonic/phosphinic acid pendant arm in DOTA derivatives on properties of their Sc^{3+} complexes and efficiency of their ^{44}Sc labelling were investigated.

Figure: