Supporting Information

for

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

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1. Equilibrium studies

To determine stability constants of Sc(III) complexes with the monophosphinate analogs of DOTA, combination of NMR and potentiometric titrations had to be used, similarly to the Sc(III)-DOTA system.[1] As the complex species are formed even below pH 1.5, protonation constants of the ligands important for the pH range have to be known. As published protonation constants were obtained from titrations in pH range from 1.6–1.9 to 12.0–12.2,[2] both ligands were re-titrated starting from pH 1.4 up to 11.8 (electrode calibration in the same pH range) with emphasis to acidic part of the titrations. The constants determined here are in excellent agreement with those previously published except for the most acidic ones for $DO3AP^{PrA}$, logK_5 2.94 and logK_6 1.54. The results are given in Table S1; any even more acidic protonation constants should have logK_a values well below 1. The protonation constants are in a good agreement with those published, differing only in two the most acidic constants of $DO3AP^{PrA}$. Therefore, the published constants [Chyba! Záložka není definována.] and logK_5 2.94 and logK_6 1.54 of $DO3AP^{PrA}$ determined here were used in metal complex stability constant calculations.

As the complex formation starts below a range suitable for potentiometry and, therefore, NMR spectrometry (as in the case of Sc(III)-DOTA system) [Chyba! Záložka není definována.] had to be used to acquire equilibrium data in acid solutions necessary for evaluation of stability of the ***out-of-cage*** complex. Potentiometry is the most suitable method to cover pH range above 1.5 where ***in-cage*** / ***out-of-cage*** complex equilibrium is expected. As complex formation rate is slow in this less acidic pH region, out-of-cell titration method has to be used at pH > 5. However, this method cannot be used at pH > 4 due to some precipitation (probably $Sc(OH)_3$ during addition of $(NMe_4)OH$ solution (the precipitation is consequence of the slow complex formation kinetics). However, this pH region is important for evaluation of equilibrium between protonated and fully deprotonated ***in-cage*** complexes and, so, normal titration of the pre-formed complexes was carried out. In this experiment, one protonation constant corresponding to protonation of the amino/carboxy group in the side chain was considered below pH 7; in addition, the titration of the pre-formed complex ending above pH 12 enabled determination of stability constant of a hydroxide-complex. Only combination of the data from all three experiments allowed correct description of the Sc(III)-$DO3AP^{ABn}$ and -$DO3AP^{PrA}$ systems and determination of the stability constants (Table S2).

Table S1 Overall[a] (β_h) and stepwise ($\log K_h$) protonatin constants of DO3AP^{ABn} and DO3AP^{PrA} determined by titration starting at pH 1.4 (I = 0.1 M $(NMe_4)Cl$, 25 °C; $\beta_h = [H_hL]/[H^+]^h \cdot [L]$; $K_h = [H_hL]/[H^+] \cdot [H_{h-1}L]$).

Constant	DO3AP^{ABn}	DO3AP^{PrA}	Constant	DO3AP^{ABn}		DO3AP^{PrA}	
$\log\beta_1$	12.26(1)[a]	12.70(3)[a]	$\log K_1$	**12.26**[a]	12.55[b]	**12.70**[a]	12.68[b]
$\log\beta_2$	21.84(1)[a]	22.10(3)[a]	$\log K_2$	**9.58**[a]	9.60[b]	**9.40**[a]	9.44[b]
$\log\beta_3$	26.95(2)[a]	27.04(3)[a]	$\log K_3$	**5.11**[a]	5.11[b]	**4.94**[a]	5.04[b]
$\log\beta_4$	30.99(2)[a]	31.22(3)[a]	$\log K_4$	**4.04**[a]	4.11[b]	**4.18**[a]	4.34[b]
$\log\beta_5$	33.62(2)[a]	34.16(3)[a]	$\log K_5$	**2.63**[a]	2.71[b]	**2.94**[a]	3.10[b]
$\log\beta_6$	35.17(2)[a]	35.70(3)[a]	$\log K_6$	**1.55**[a]	1.54[b]	**1.54**[a]	1.93[b]

[a]Determined in the present work. [b]Reference [**Chyba! Záložka není definována.**].

Table S2 Overall stability constants, β_{hlm}, of DO3AP, DO3AP^{ABn} and DO3AP^{PrA} determined by equilibrium studies (for more details, see text; 25 °C, $\beta_{h11} = [H_hLSc]/[H^+]^h \cdot [L] \cdot [Sc]$, $l = m = 1$).

Equilibrium[a]	Constant	DO3AP	DO3AP^{ABn}	DO3AP^{PrA}
$L + Sc \leftrightarrow [Sc(L)]$	β_{011}		27.07(3)	28.31(10)
$H + L + Sc \leftrightarrow [Sc(HL)]$	β_{111}	5.29(3)[b]	32.24(3)	32.49(9)
$3H + L + Sc \leftrightarrow [Sc(H_3L)]$	β_{311}		36.1(2)	36.84(7)
$H_2O + L + Sc \leftrightarrow [Sc(OH)(L)] + H$	β_{-111}		14.24(5)	16.28(11)

[a]Charges are omitted for clarity. [b]Protonation constant ($\log\beta_1 = \log K_{a1}$) for equilibrium $[Sc(L)] + H \leftrightarrow [Sc(HL)]$.

2. Equation used in the free-ion selective radiotracer extraction (FISRE) method

The distribution coefficient can be defined as Equation (1)

$$K_d = \frac{[Sc(III)]_{ads}}{[Sc(III)]_{sol}} = \frac{[Sc(III)]_{tot} - [Sc(III)]_{sol}}{[Sc(III)]_{sol}} \frac{V}{S} \quad (1)$$

where $[Sc(III)]_{tot}$ is the total concentration of Sc(III) introduced into the solution, $[Sc(III)]_{ads}$ is the concentration of Sc(III) bound on the resin, $[Sc(III)]_{sol}$ is concentration of Sc(III) remaining in the solution after interaction with the resin, V is volume of the solution and S is amount of the resin.

The thermodynamic stability constants of the Sc-ligand complex were determined by analyzing the dependence of K_d values on ligand concentration and on pH. The adsorption of the free Sc^{3+}-aqua ion by imino-diacetate chelating groups ($\overline{X-H}$) can be described by the following equilibrium Equation (2).

$$Sc^{3+} + 3\overline{X-H} \longleftrightarrow \overline{X_3-Sc} + 3H^+ \quad (2)$$

The overlined species refer to the adsorbed species. The electroneutrality of each phase is required. The thermodynamic constant for Equilibrium (2) can be written as follows.

$$K_{ads} = \frac{[H^+]^3[\overline{X_3-Sc}]}{[Sc^{3+}][\overline{X-H}]^3} \quad (3)$$

The distribution coefficient K_d of the Sc(III) cation corresponds to the ratio between the total concentration of adsorbed species and the total concentration of aqueous species

$$K_d = \frac{[Sc(III)]_{ads}}{[Sc(III)]_{sol}} = \frac{[\overline{X_3-Sc}]}{\sum_{i,h,l}[Sc_m(OH)_i H_h L_l]} = \frac{[\overline{X_3-Sc}]}{[Sc^{3+}]a_{Sc(L,OH)}} \quad (4)$$

where $\alpha_{Sc(L,OH)}$ is the complexation coefficient of $Sc^{3+}$and it is defined as the ratio between the total aqueous scandium concentration and the Sc^{3+}-aqua ion concentration. As all studies were performed with low concentrations of scandium, polynuclear species can be neglected (m = 1). For experiments performed in acidic media (pH lower than 3) Sc(III) hydroxide complexes can be also neglected (i = 0) and $\alpha_{Sc(L,OH)}$ can be expressed as Equation (5)

$$a_{Sc(L,OH)} = \frac{[Sc(III)]_{sol}}{[Sc^{3+}]} = 1 + \sum_{h,l}\frac{[ScH_hL_l]}{[Sc^{3+}]} = 1 + \sum_{h,l} b_{h,l}[L]^l[H^+]^h \quad (5)$$

where [L] is the concentration of fully deprotonated ligand. For solutions at pH > 3, Sc(III)-hydroxide complexes must be taken into account. If the ligand can behave as acid or base, this concentration has to be corrected as a function of total ligand concentration and pH of supernatant. For known total ligand concentration and pH, this complexation coefficient depends only on thermodynamic constants of complexation.

By combining Equations (3) and (4), Equation (6) is obtained.

$$K_d = \frac{K_{ads}[\overline{X-H}]^3}{[H^+]^3 a_{Sc(L,OH)}} \quad (6)$$

As concentration of scandium is negligible in comparison with concentration of the resin binding sites, $\overline{X_3 - Sc}$ could be neglected and [$\overline{X - H}$] can be considered as constant. So, Equation (6) can be re-written as

$$\log K_d = \log\left(K_{ads}\left[\overline{X - H}\right]^3\right) - 3\log\left[H^+\right] - \log a_{dSc(L,OH)} \quad (7)$$

Considering the variation of K_d as a function of ligand concentration, and by keeping constant pH, the experimental results could be fitted and, thus, $a_{Sc(L,OH)}$, the speciation of scandium as a function of ligand concentration could be determined. To evaluate the existence of a potential protonated complex [$Sc(H_hL)$], another experiment was performed at a fixed ligand concentration by varying solution pH.

3. NMR Studies

The $[Sc(tmu)_6](ClO_4)_3$ complex used as ^{45}Sc NMR standard was prepared by modified procedure described in the literature [3]. Briefly, the prepared scandium(III) perchlorate hydrate [4] (500 mg, 1.1 mmol) and trimethyl orthoformate (3.17 g, 21.4 mmol, 20 equiv.) were heated in a glass vial at 60 °C under argon atmosphere for 1 h. Then, dried (4-Å molecular sieve, 1 day) tmu (770 mg, 6.63 mmol, 6.2 equiv.) was added. The immediately formed white crystalline precipitate was filtered off, washed with anhydrous ether and subsequently vacuum-dried to give a solid (975 mg, 86 %). To prepare standard solution for the NMR measurements, the solid complex was always dissolved just before the experiments.

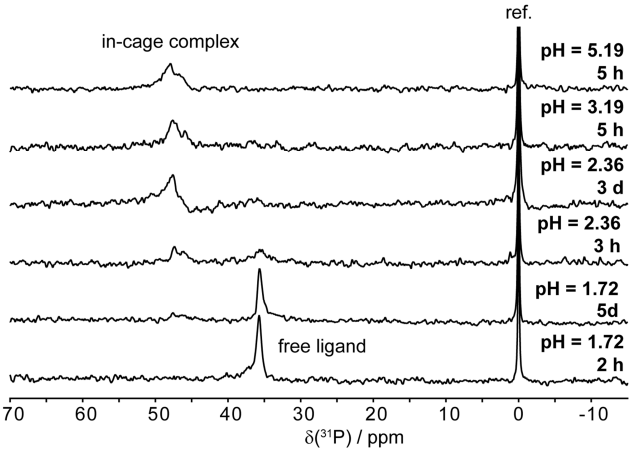


Figure S1. $^{31}P\{^1H\}$ NMR spectra for the formation reaction of the $[Sc(DO3AP^{PrA})]^{2-}$ complex at various pH (c_L = 0.004 M, L:Sc molar ratio 1:0.95).

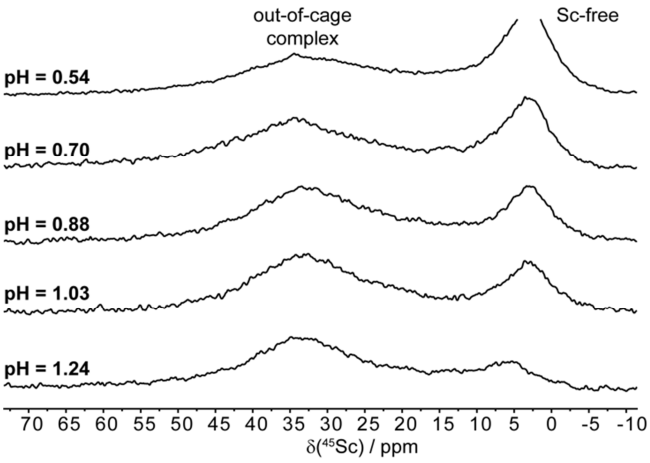


Figure S2. ^{45}Sc NMR spectra of fully equilibrated solutions prepared by mixing $ScCl_3$ and $DO3AP^{PrA}$ (c_{Sc} = c_L = 0.004 M). The given pH values are those for the equilibrated solutions.

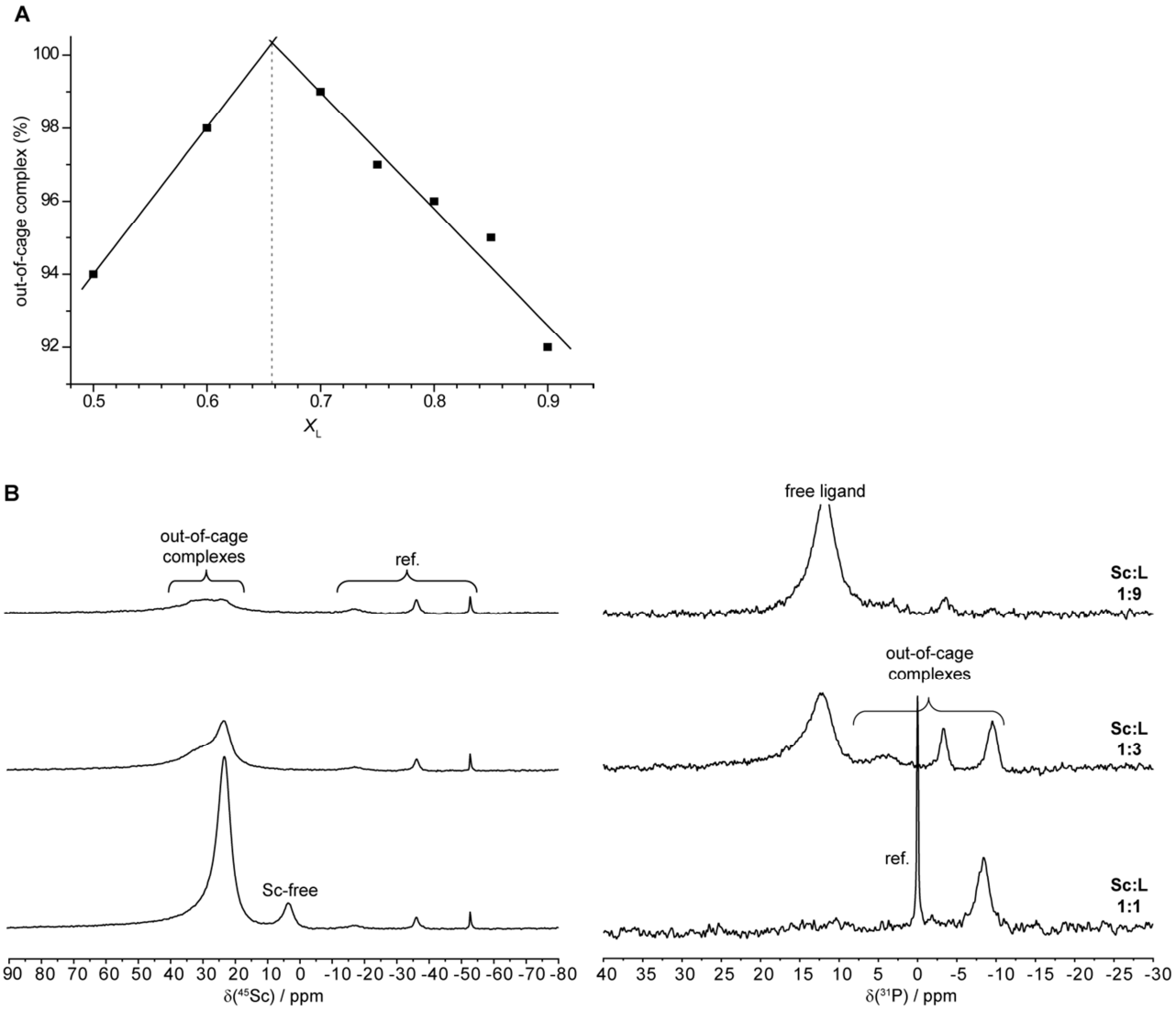


Figure S3. Job's plot (**A**) for Sc^{3+}-DO3AP system (c_{Sc} = 4–20 mM, $c_{Sc} + c_L$ = 40 mM, pH 0.2). An abundance of the ***out-of-cage*** complex was determined by ^{45}Sc NMR spectroscopy. Example of ^{45}Sc and $^{31}P\{^1H\}$ NMR spectra (**B**) of fully equilibrated solutions at different overall Sc(III) : DO3AP molar ratios.

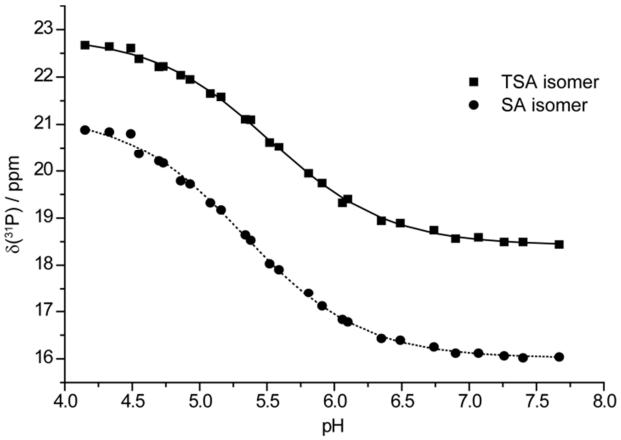
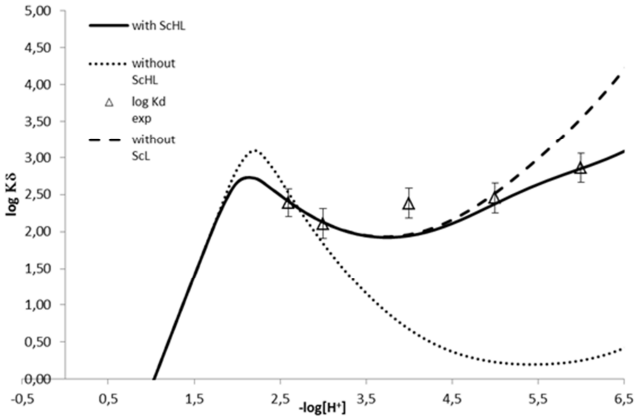


Figure S4. Dependence of δ_P of isomers of the $[Sc(DO3AP)]^{2-}$ complex on pH ($c_{complex}$ = 0.01 M, t = 25 °C; no control of ionic strength).

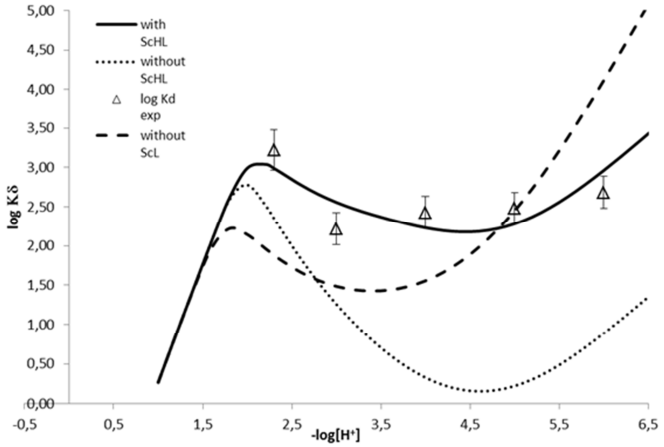
4. Radiochemical Studies

Figure S5 The Sc(III)-ligand isotherms obtained by the FISRE method as function of solution pH: Sc(III)-DO3AP (**A**), Sc(III)-DO3AP^{ABn} (**B**) and Sc(III)-DO3AP^{PrA} (**C**). Experiments were performed in 0.1 M NaCl; pH and the concentration of the Chelex 100 resin were adjusted for each batch to minimize the global uncertainty of partition coefficient.

A



B



C

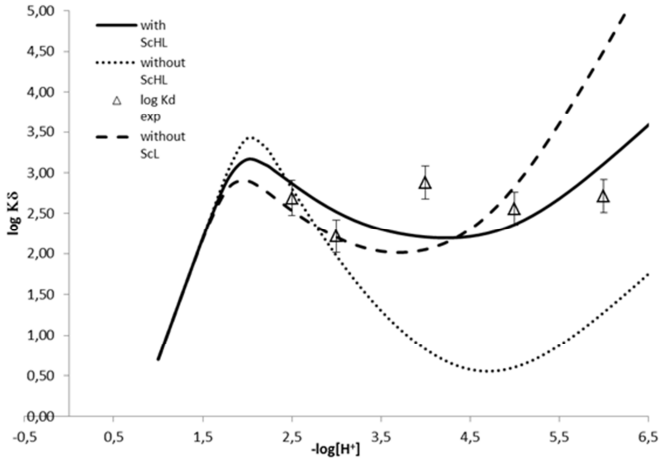


Table S3 Specific activities (SA) of ^{44}Sc-labelled DOTA and its monophosphorus acid analogues. Experimental conditions: t = 70 °C, 30 min, pH = 4.

Source	Parameter	Ligand			
		DOTA	DO3AP	DO3AP^{PrA}	DO3AP^{ABn}
Generator ^{44}Sc	n_{ligand} (nmols)	3.00	10.00	n.d	20.00
	Radiochemical yield (%)	96.5	95.3	n.d	94.7
	Total activity (MBq)[a]	5.5	3.7	n.d	2.5
	Specific activity (MBq/nmol)[a]	1.88	0.37		0.13
Cyclotron ^{44m}/^{44}Sc	n_{ligand} (nmols)	0.07	0.07	0.12	0.17
	Radiochemical yield (%)	97.8	97.4	94.2	94.7
	Total activity (MBq)[b]	1.82	1.82	1.82	1.82
	Specific activity (MBq/nmol)[b]	26	26	15.17	10.70

[a]At 4 h after the end of the elution. [b]At 4 h after End of Beam (EOB).

Challenge studies

For the *in-vitro* stability assays, hydroxyapatite Ultrogel suspension (40 % w/w, cross-linked 4 % beaded agarose, Sigma, U.S.A.) was used as received. The experiment followed previously described procedure for lanthanides(III) or scandium(III) chelates.[49] Rat srum was provided by Sigma (France) and its aliquots (450 µL) were prepared from this stock solution in screw-cap V-bottom vials. The aliquots were kept frozen until use. Before the addition of the Sc-ligand aliquot, the serum aliquot was incubated at 37 °C.

Serum stability

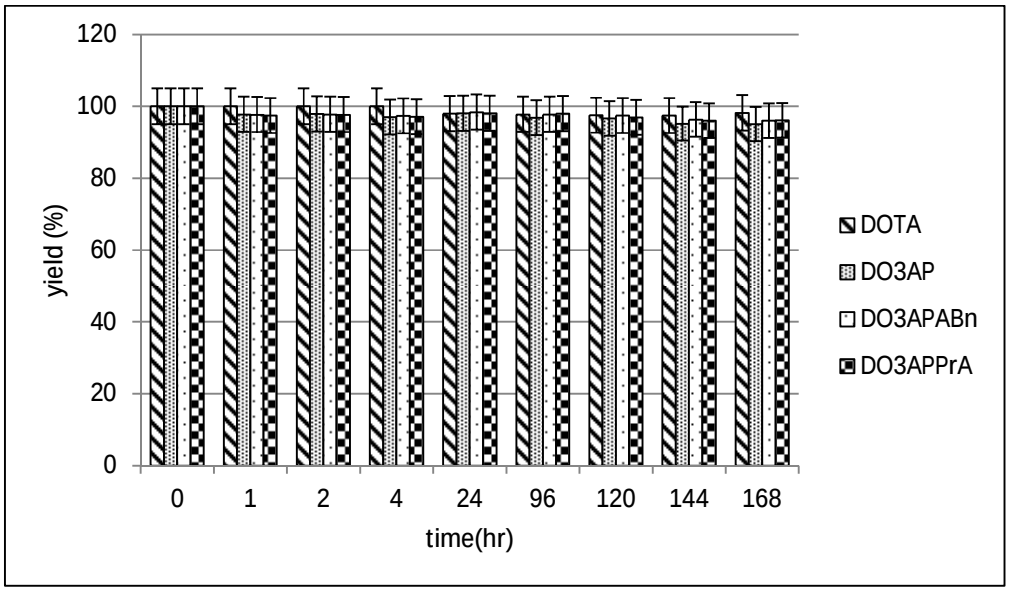
An aliquot (50 µL) of the $^{44m/44}$Sc complex solution was added to serum aliquot (450 µL) incubated just before the experiment at 37 °C for 1 h. Then, the samples were incubated at 37 °C and, at various time points, the reaction mixture (3 µL) was spotted onto a TLC flex plate, and developed and measured as above. The resulting TLC plate was counted for 20 min on a Cyclone counter (Perkin Elmer).

Hydroxyapatite challenge study

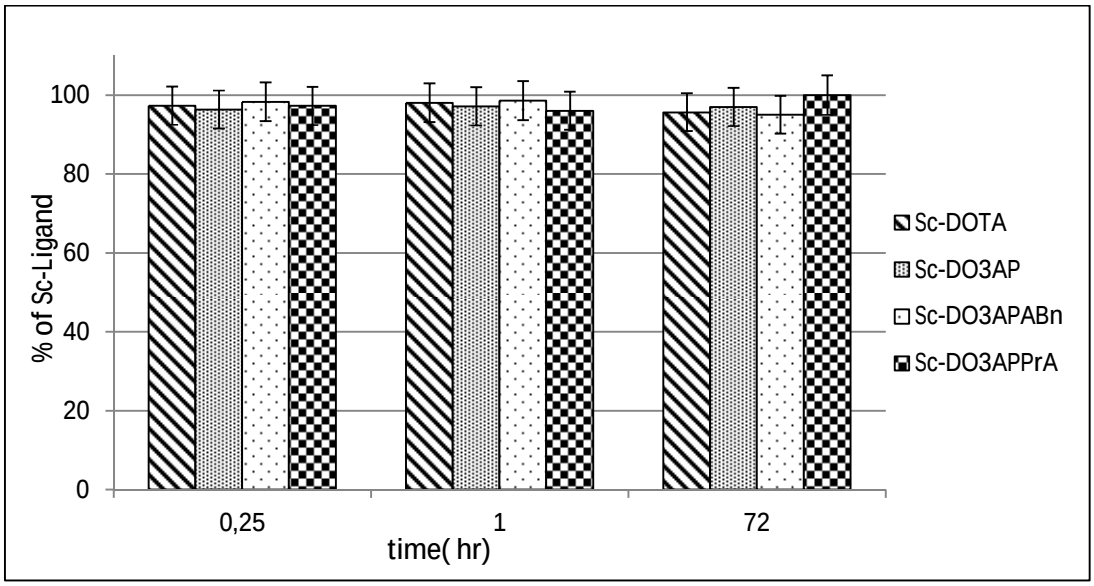
18 vials each containing 0.125 g of hydroxyapatite (HA Ultrogel®) suspension were prepared in a phosphate buffer (10 ml, pH 7.5, with 10 mM $CaCl_2$). Then, the pre-formed $^{44m/44}$Sc-L (L = DOTA, DO3AP, DO3AP^{ABn} or DO3AP^{PrA}) complex solution (50 µL) was added into the HA suspension, suspension was mixed and incubated at 37 °C. The stability/adsorbtion of the Sc-L complexes was monitored at 1 h, 24 h and 3 d after the sample mixing; the suspension was filtered through an acrodisc filter (porosity of 0.8 µm, Corning, USA). The filtrate was washed with an additional ultrapure water (8 mL) to reach a final volume of 10 mL. This fraction corresponding to the supernatant was counted on the gamma counter. Then, the filter was washed with 10 mL of 1.3 M aq. HNO_3 to dissolve the solid HA (this fraction represents the solid phase), and the solution was counted on a germanium gamma counter to determine the $^{44m/44}$Sc fraction adsorbed on the solid phase.

Figure S6 Stability of $^{44m/44}$Sc-radiolabeled complexes in rat serum (**A**) and in the presence of hydroxyapatite in phosphate buffer at pH 7.5 (**B**).

A



B



References:

[1] M. Pniok, V. Kubíček, J. Havlíčková, J. Kotek, A. Sabatie-Gogová, J. Plutnar, S. Huclier-Markai and P. Hermann, *Chem. Eur. J.*, 2014, **20**, 7944–7955.

[2] M. Försterová, I. Svobodová, P. Lubal, P. Táborský, J. Kotek, P. Hermann and I. Lukeš, *Dalton Trans.*, **2007**, 535–549.

[3] D. L. Pisaniello, S. F. Lincoln and E. H. Williams, *J. Chem. Soc., Dalton. Trans.*, **1979**, 1473–1476.

[4] F. Petrů and F. Kůtek, *Z. Chem.*, 1963, **12**, 473–475.