Summary

The presented work continuous the research, which has been started by Kubíček *et al.*[1] on H_7bpamd (Fig. 1), and its Gd(III) complex proposed as a potential bone-seeking contrast agents (CAs) for Magnetic Resonance Imaging (MRI). This work was aimed at synthesis of two other macrocyclic H_4dota-type ligands containing bis(phosphonic acid) pendant arm, H_7bpapd and H_8do3ap^{BP} (Fig. 1), and comparative physicochemical evaluation of their lanthanide(III) complexes as bone-seeking agents for potential application in MRI, radiodiagnosis, radiotherapy and bone pain palliation.

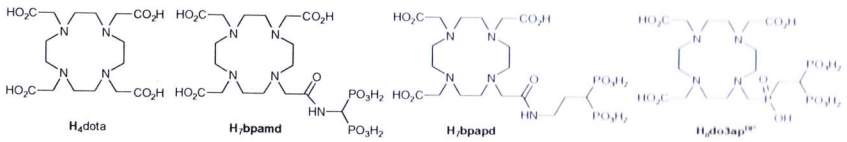
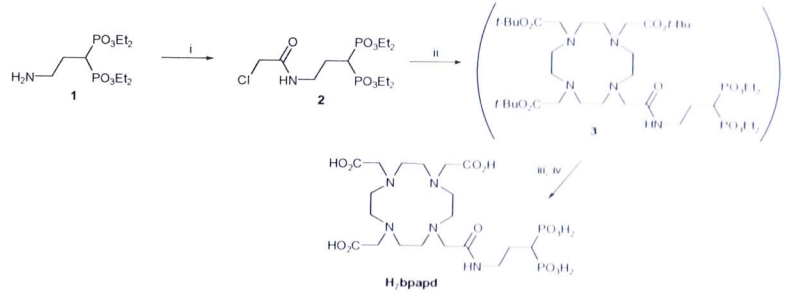
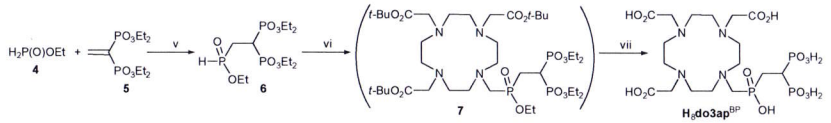


Fig. 1 Discussed ligands.

Desired ligands were prepared according to reaction Schemas 1 and 2. The ligands under study form eight-coordinated lanthanide(III) complexes, with the ninth coordination site occupied by a water molecule. The bis(phosphonate) moiety is not coordinated to the lanthanide(III) ion and remains free for other kind of interaction (*e.g.* for anchoring to hydroxyapatite (HA) or interaction with other metal ions). Upon this interaction the coordinated water molecule is not expelled.



Scheme 1 Synthesis of the ligand H_7bpapd (Yield 30 % based on *t*-Bu$_3$do3a·HBr). i) $ClCH_2C(O)Cl$, acetonitrile, K_2CO_3, –50 °C to RT, 24 h; ii) *t*-Bu$_3$do3a·HBr, acetonitrile, K_2CO_3, RT, 4 d; iii) CF_3COOH/CH_2Cl_2 (1:1), reflux, 12 h; iv) 30% HBr in dry AcOH, RT, 24 h.



Scheme 2 Synthesis of the ligand H_8do3ap^{BP} (Yield 75 % based on *t*-Bu$_3$do3a·HBr). (v) tetrahydrofuran, DIPEA, 25 °C , 12 h; (vi) *t*-Bu$_3$do3a·HBr, $(CH_2O)_x$, toluene, 90–100 °C, 15–24 h; (vii) 6 M HCl, reflux, 12 h.

Lanthanide(III) complexes of H_4dota and its derivatives are known to form two diastereomers in solution: square antiprismatic (SA) and twisted square antiprismatic (TSA).[2] For H_8do3ap^{BP}, the prochiral phosphorus atom of the phosphinate function in the pendant arm creates, upon complexation, another chirality centre; so, that for lanthanide(III) complexes of H_8do3ap^{BP} in principle four enantiomeric pairs of diastereomers may exist. However, selective formation of only two diastereomers suggests that the coordinated phosphinate phosphorus atom occurs exclusively in one of the enantiomeric forms (*R*/*S*). Unfortunately, we were not able to distinguish them. At the end of lanthanide series a tiny amount of the third diastereomer with other phosphorus configuration appears.

The pK_a values of the bis(phosphonate) moiety were determined for the major diastereomers of Yb(III) complexes of H_7bpamd, H_7bpapd and H_8do3ap^{BP} (Table 1). The obtained pK_a values of the Yb-bpapd, Yb-do3ap^{BP} complexes and the first two values of the Yb-bpamd complex are in good agreement with data reported commonly for bis(phosphonate) moieties.[3] The values of pK_{a3} and pK_{a4} for the Yb-bpamd complex are substantially higher, which may be attributed to the stabilization of the mono- and diprotonated species by rather strong hydrogen bonds between the phosphonate functions and the macrocycle.

Table 1 pK_a values of the bis(phosphonate) moieties of major diastereomers (SA, TSA) of the Yb-bpamd, Yb-bpapd and Yb-do3ap^{BP} complexes, respectively, in water (25 °C). The molar fractions of SA, TSA species are given in brackets. 'A' denotes the fully deprotonated complexes. Charges are omitted for the sake of clarity. The first two pK_a values of Yb-do3ap^{BP} complex could not be determined.

Equilibrium	Yb-bpamd	Yb-bpapd	Yb-do3ap^{BP}	
	SA (100 %)[1]	SA (93 %)	SA (56 %)	TSA (38 %)
	pK_a	pK_a	pK_a	pK_a
$H + H_3A \leftrightarrow H_4A$	1.8	1.6	–	–
$H + H_2A \leftrightarrow H_3A$	4.9	4.1	–	–
$H + HA \leftrightarrow H_2A$	9.0	6.7	7.0	7.0
$H + A \leftrightarrow HA$	> 13	10.9	11.2	11.2

An efficacy of potential CA for MRI is usually expressed as relaxivity r_1, this is a proton relaxation rate in 1 mM solution of the corresponding Gd(III) complex. The Gd(III)

complex of the monophosphinate ligand H_8do3ap^{BP} has significantly higher ^{1}H relaxivity in solution ($^{298}r_1$ = 7.4 s^{-1} mM^{-1}) than Gd(III) complexes of the both monoamides H_7bpamd ($^{298}r_1$ = 5.3 s^{-1} mM^{-1})[1] and H_7bpapd ($^{298}r_1$ = 5.0 s^{-1} mM^{-1}) at 20 MHz. This is thanks to a more favourable residence time τ_M of the coordinated water molecule of the Gd-do3ap^{BP} complex, to a higher value of rotational correlation time τ_R, and to a significant contribution to the relaxivity by second-sphere water molecules.

The relaxometric studies on Gd(III) complexes adsorbed onto the HA (model of bone) showed about three fold enhancement of r_1, which is a result of reduced mobility of the complex upon adsorption on the HA surface. Therefore, these compounds might be applicable as positive MRI contrast agents for calcified tissues.

The adsorption efficacy of lanthanide(III) complexes of H_7bpapd and H_8do3ap^{BP} was studied using a model Tb(III) complex containing radionuclide ^{160}Tb. Both complexes exhibited a swift and reversible adsorption onto HA following either Langmuir-binding behaviour, found for complexes of H_7bpapd, or Langmuir-Freundlich ones for complexes of H_8do3ap^{BP} (Fig. 2). The adsorption parameters are summarized in Table 2. Maximum adsorption capacities X_m, of all complexes correspond to monomolecular coverage according to molecular modelling. Affinity constants K of the Tb-bpamd and Tb-do3ap^{BP} complexes are exceptionally high.

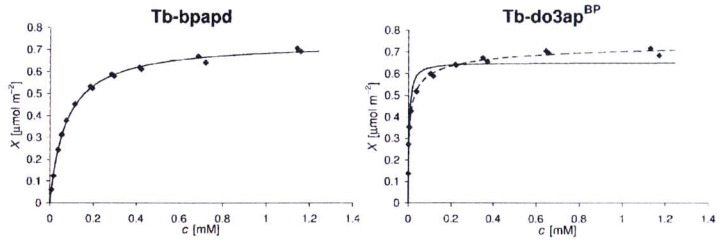


Fig. 2 Adsorption isotherms of the Tb-bpapd, Tb-do3ap^{BP} complexes on HA at 25 °C. The curves represent the results of fittings of the experimental data with the Langmuir model (solid line). The dashed curve gives the result of a fitting with the Langmuir-Freundlich model.

Table 2 Adsorption parameters of the Tb(III) complexes of the ligands under study. Unless otherwise stated the parameters were obtained using a Langmuir model. Fixed parameters are given in parenthesis.

Adsorption parameter	Tb-bpamd[1]	Tb-bpapd	Tb-do3ap^{BP}	Tb-do3ap^{BP}[†]
$X_m/10^{-6}$ [mol m^{-2}]	0.621±6	0.722±10	0.652±18	0.778±18
$K/10^3$ [L mol^{-1}]	196±11	14.1±2	249±44	129±21
n	(1)	(1)	(1)	0.46±3

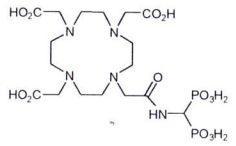
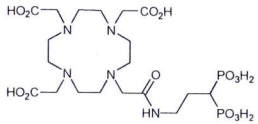
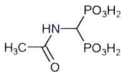
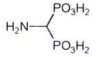
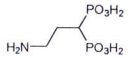
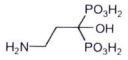
[†] Langmuir-Freundlich model.

In vivo adsorption abilities, specificity for calcified tissues and body retention were evaluated by Single Photon Emission Tomography (SPECT) of Lewis rats upon intravenous injection of the ^{177}Lu-containing complexes of H_7bpamd and H_7bpapd (Fig. 3). These experiments confirmed that both complexes exhibit a very high selectivity for calcified tissues accompanied by an early retention in the skeleton, particularly in parts of newly formed bones. The clearance of ^{177}Lu-complexes from the body is mainly *via* the kidneys. However, the clearance from the skeleton seems to be too slow for potential application in MRI. On the other hand, this might be an advantage for bone palliation.

Fig. 3 γ-scintigraphic image of a rat 76 h after injection of ^{177}Lu-containing Lu-bpamd complex.

Beside studies dealing with bone-targeting and potential application of studied compounds in medicine (which are mentioned above), we were also focused on models describing simultaneous adsorption of two adsorbates theoretically. Some models describing simultaneous adsorption (*i.e.* co-adsorption) of two compounds were published.[4] However, all these models require knowledge of the adsorption parameters of the individual compounds in the mixture and do not provide the possibility to use one of compounds as a probe for the study of the second one. Therefore, we proposed a new physicochemical model, based on Langmuir adsorption isotherm, suitable for indirect determination of the adsortpion efficacy of a co-adsorbing bis(phosphonate) using the ^{160}Tb-containing Tb-bpapd complex as a probe. The results might be evaluated either qualitatively (by simple comparison of the experimental data of several competing systems), as well as quantitatively (values of X_m and K are determined by simultaneous fitting of the experimental data). This model has been successfully applied to several co-adsorbing bis(phosphonates) and their maximum adsorption capacities X_m and affinity constants K were determined (Table 3). Furthermore, we can assume that any other radiolabelled (using for instance ^{14}C or ^{32}P) 'simple' bis(phosphonate), showing Langmuir adsorption behaviour, may act similarly as a probe. In that case, hard availability of the ligand H_7bpapd would not be a limitation.

Table 3 The best fit adsorption parameters of several bis(phosphonates) determined from co-adsorption experiments using the ^{160}Tb-bpapd complex as a probe. Langmuir-based competitive model (n = 1).

Competing bis(phosphonate)		Adsorption parameter	
		$X_m/10^{-6}$ [mol m^{-2}]	$K/10^3$ [L mol^{-1}]
	H_7bpamd	0.609 ±8	236±39
	H_7bpapd	0.687±63	29±7
	H_4acmp2	1.19±3	46±5
	H_4amp2	2.00±7	59±13
	H_4app2	1.50±6	16±2
	H_4pam (pamidronate)	1.82±7	44±10
	H_4hedp (etidronate)	1.58±5	53±9

As an additional contribution to the topic of this thesis, we studied changes in relaxivity r_1 of the Gd-do3ap^{BP} complex as a response to the presence of metal ions. It is known that bis(phosphonates) are strongly chelating agents for divalent and trivalent metal ions. Bis(phosphonate) unit coordinates often two or more metal ions forming coordination oligomers and polymers (COP) in solution as well as in the solid state.[5] The COP containing various metal/ligand ratios could be found. The formation of COP is determined mainly by coordinated metal ions and by the stability constants of their complexes with the

bis(phosphonate) moiety.[3] Recently, we have found that the Gd-do3ap^{BP} complex forms COP in solution upon addition of divalent metal ions (*e.g.* Zn^{2+}, Ca^{2+}, Mg^{2+}). The formation of COP is accompanied by a significant increase in r_1 (more than 400 % enhancement at 20 MHz and 37 °C) due to the decrease of the molecular tumbling rate (Table 4). Changes in the ^{1}H relaxivity were found to be dependent on concentration of added ion and also on solution pH. This phenomenon may be applied in the development of *in vitro* and *in vivo* responsive contrast agents. Thus, the bis(phosphonate) complexes are very promising for further study as potential MRI contrast agents responsive to pH and metal ion concentration.

Table 4 The ^{1}H relaxivities r_1 of the Gd-do3ap^{BP} complex measured upon addition of some endogenous divalent metal ions. All samples were measured in aqueous solutions (2.0 mM Gd-do3ap^{BP}, 37 °C, pH = 7.1–7.4). For comparison the r_1 value of Gd-do3ap^{BP} is shown.

Counter ion M^{2+}	M^{2+}/Gd-do3ap^{BP} ratio	r_1 [s^{-1} mM^{-1}]	
		20 MHz	60 MHz
–	–	5.6	5.1
Mg	3	13.6	12.6
	10	17.9	17.0
Ca	3	20.6	18.6
	10	25.7	22.9
Zn	1	11.1	12.1
	3	22.4	21.3

References

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