Abstract

Lanthanide(III) complexes of DOTA derivatives are utilized in the medical imaging techniques such as magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), and magnetic resonance spectroscopy (MRS), nuclear imaging (PET and SPECT), or optical methods (luminescence).

It has been shown that relaxometric parameters of the Gd(III) complexes of DOTA derivatives with a phosphinic acid pendant arm (Gd–DO3AP^{R}) can reach optimal values (*e.g.* water residence time, τ_M, being close to ~10 ns). The relaxometric parameters can be further modified through the phosphorus substituents. It is also known that the complexes possess a high thermodynamic stability and they are kinetically inert. The main goal of this Thesis is an investigation of the effect of pendant amino group protonation in substituents bound to the phosphorus atom on properties of the complexes. Thus in this Thesis, DOTA derivatives with the phosphinic acid pendant arm with an amino group and their complexes were prepared and characterized. The complexes are intended as contrast agents for molecular imaging techniques (mainly for MRI and ^{31}P MRS).

The first part of the Thesis introduces two new versatile "phospha-Mannich" protocols performed under mild conditions. Amino-*H*-phosphinic acids (AHPAs) were synthesized with excess of the appropriate aldehyde and equimolar amounts of secondary amine and hypophosphorous acid utilizing acetic acid as a solvent (Figure A1). Secondary amines with pK_A > ~8 showed very high conversions to AHPAs which were mostly purified only by a simple ion-exchange chromatography and were isolated in high yields. A small library (~40 compounds) of *α*-aminoalkyl-*H*-phosphinic acids was prepared. For a less nucleophilic secondary amines (pK_A < ~7) and polyamines with the ethylene-diamine fragment, reductive *N*-methylation coupled with hypophosphorus acid oxidation were observed as the main reactions. A mechanism of the AHPA synthesis was proposed. It involves initial *N*-hydroxymethylation of the secondary amine and esterification of the hydroxogroup with acetic acid. It is probably followed by trans-esterification with H_3PO_2 and re-arrangement of the hypophosphorous acid ester to the final product with P–C bond, AHPA.

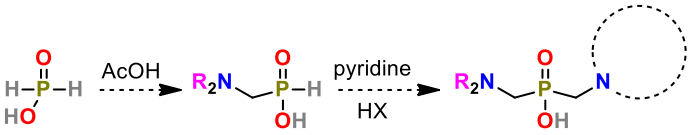

Figure A1

The target cyclen-based ligands with phospho(i)nate pendant arms were prepared by a new variant of Kabachnik–Fields reaction of a cyclen derivative, an

alkyl *H*-phosphinate / H–P(O)(OEt)$_2$ and formaldehyde in anhydrous pyridine as a solvent (Figure A1). The reaction requires a strong acid (*e.g.* HBr) as a catalyst and proceeds with almost quantitative conversion. Reaction was successfully tested on several model compounds. Reaction mechanistic investigations suggested an initial formation of an aminal (>N–CH$_2$–N<) and its fast acid-catalyzed decomposition to iminium species ((>N=CH$_2$)$^+$ X$^-$) which reacted with the phosphorus acid ester with the P–H bond, likely in its tautomeric trivalent form.

Lanthanide(III) complexes of the macrocyclic ligands of the DO3AP^R type with phosphorus-bound group containing a hydrophobic amine were investigated as contrast agents (CAs) for MRA whose properties are altered with (de)protonation of the pendant amino group (Figure A2). Based on TSA / SA isomer abundance in the Ln(III) complexes, the Gd(III) complexes coordinate one water molecule regardless of pH. The Gd(III) complexes have a high TSA isomer abundance (~50–70 %) which is dependent on protonation of the pendant amino group (pK_A 5–7). The Gd(III) complexes with the deprotonated pendant amino group (*i.e.* at pH > 7–8) have an extraordinary short water residence time (τ_M down to ~5 ns at 25 °C) because of it easily accessible octa-coordinated transient state. In the presence of human serum albumin (HSA), relaxivity of the Gd(III) complexes is significantly increased (5–10-times, the highest observed relaxivity was r_{1p} ~55 mM^{-1} s^{-1}; 20 MHz, 37 °C, pH = 7.4) due to the formation of a supramolecular adducts of the complexes with HSA. Strength of the complex–HSA interaction depends on pH and the complexes with the protonated pendant amino group have 1–2 orders of magnitude lower affinity to HSA than that of the deprotonated complexes. Surprisingly, relaxivity of the protonated and deprotonated complexes if theoretically fully bound to HSA (*i.e.* relaxivity of the "pure" CA–HSA conjugate) is almost identical. The concept of the binding / release of molecules to / from its conjugate with HSA triggered by their protonation is an interesting new approach to altering drug pharmacokinetics.

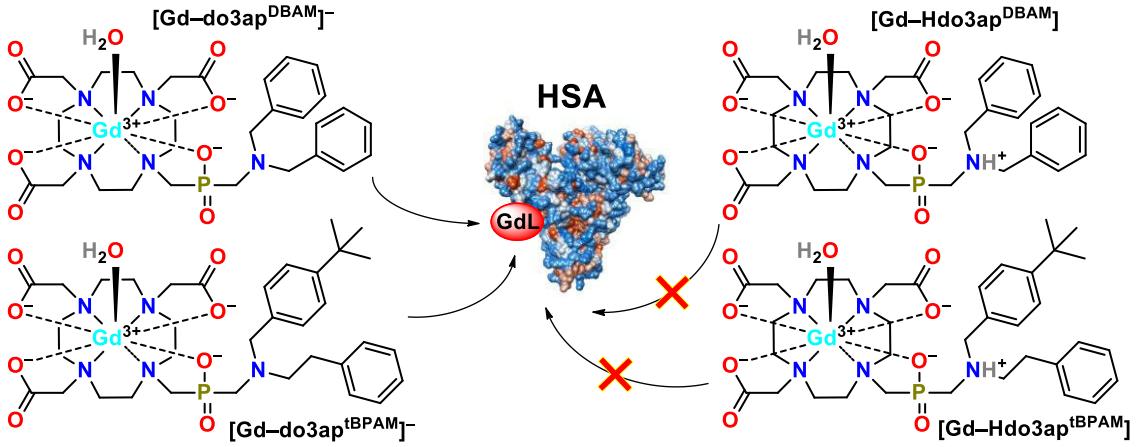

Figure A2

The Ln(III) complexes of the ligands were also investigated by single-crystal X-ray diffraction (17 structures). Results confirmed increase of Ln(III)–OH_2 bond length which is coupled with shorter Ln(III)–QN_4 distance with smaller Ln(III) ions. This large set of structures was compared with structures of Ln(III) complexes of the other DOTA-like ligands. Opening angles ω of Gd–DO3AP^R complexes are smaller than those of Gd–DOTA but still large enough to allow coordination of one water molecule. Hence, the "anhydrous" octa-coordinated transient state of Gd(III) ion during the water exchange is more accessible for the Gd–DO3AP^R complexes than for the Gd–DOTA complex and, thus, water exchange on Gd–DO3AP^R is faster.

The Ln(III) complexes of ligand with primary amino group, DO3AP^{AM} (Figure A3), are the simplest Ln–DO3AP^R whose properties are affected by the pendant amine (de)protonation. The properties of the Ln–DO3AP^{AM} complexes were compared with that of the Ln(III) complexes of the ligand DO3AP^{AcAM} (Figure A3) whose properties cannot be changed with pH. The TSA / SA isomers abundance of their Ln(III) complexes and determination of hydration number (Dy(III)-induced water ^{17}O chemical shift and Eu(III) luminescence) clearly showed presence of monoaqua Gd(III) species regardless of pH. Water residence times are not as extremely short (τ_M ~40 and ~32 ns for Gd–DO3AP^{AM} and Gd–DO3AP^{AcAM}, respectively; at 25 °C, pH ~7) as for the complexes discussed above but still significantly shorter than that for Gd–DOTA. It is likely caused by a more distant hydration break to Gd(III) within the Ln(III) series for Ln–DO3AP^{AM} / Ln–DO3AP^{AcAM}. Surprisingly, relaxivity of Gd–DO3AP^{AM} is not affected by pH and, thus, by protonation state of the amino group (pK_A ~8.3). Relaxivity is lower than expected – it can be caused by a low "effective hydration number q_{eff}" of ~0.8 (at pH ~7) determined by fitting of the measured data.

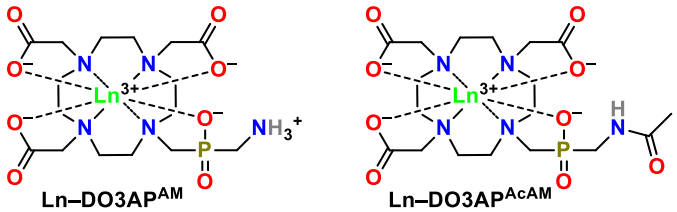

Figure A3

The Ln–DO3AP^{AM} complexes have protonable pendant amine in the proximity to phosphorus atom and, thus, ^{31}P NMR properties were dependent on pH. The coordinated paramagnetic metal ions induced short ^{31}P NMR relaxation times (down to small "ms" scale) as well as expansion of difference (up to tens of ppm) between ^{31}P NMR resonance frequencies of the (de)protonated forms of the complexes. The ^{31}P NMR chemical shifts of the complexes are shifted by tens-to-hundreds ppm away from those of endogenous phosphorus-containing compounds. However, the

pendant amino group pK_A ~8.3 is well above the *in vivo* pH range and the (T_2*/T_1) ratio of ^{31}P nucleus is the best, 0.4–0.7, for complexes with very short T_1 (1–7 ms). Thus, the research is a *proof-of-concept* study which confirmed that the protonable Ln–DO3AP^R complexes can be used as novel CAs for ^{31}P MRS imaging to describe pH *in vivo*; however, the basicity of the complexes has to be further tuned.

The last part of the Thesis introduces a ditopic DO3A–P–DO3A ligand (Figure A4) which has become available by the new Kabachnik–Fields reaction. The ligand structure allows preparation of thermodynamically stable and kinetically inert mono- and either homo- or heteronuclear dimetallic complexes (Figure A4). Metal ions in the dimetallic complexes are connected *via* bridging phosphinate group. It was confirmed in the solid state by single-crystal X-ray diffraction (six structures). The dimetallic complexes showed a complicated isomerism where the TSA / SA isomerism of the macrocyclic chelates is combined with chiral centre located on the phosphorus atom. Up to eight different diastereoisomers were observed in the solution but three-to-four diastereoisomers are the major isomers (overall sum > 85 %). The relaxometric evaluation of the Gd$_2$(III) complex revealed an unexpectedly short water residence time (τ_M ~7 ns at 25 °C) probably connected with the hydration break just behind Gd(III) in the Ln(III) series (determined by Eu(III) and Tb(III) luminescence). It leads to easily accessible octa-coordinated state in the Gd$_2$(III) complex. In the dinuclear complexes, the proximity of two paramagnetic ions connected *via* the phosphinate bridge seemingly does not significantly alter their electronic relaxation times. Hetero-dimetallic complexes GdM–(DO3A–P–DO3A) (Figure A4), where M is a trivalent metal ion (*e.g.* Sc(III), La(III), Bi(III)), were investigated to assess a possible relaxivity dependence of relaxivity on the total weight of molecules. However, the complexes showed no relaxivity dependence on molecular mass of the second coordinated ion and it was instead determined by the TSA / SA isomerism.

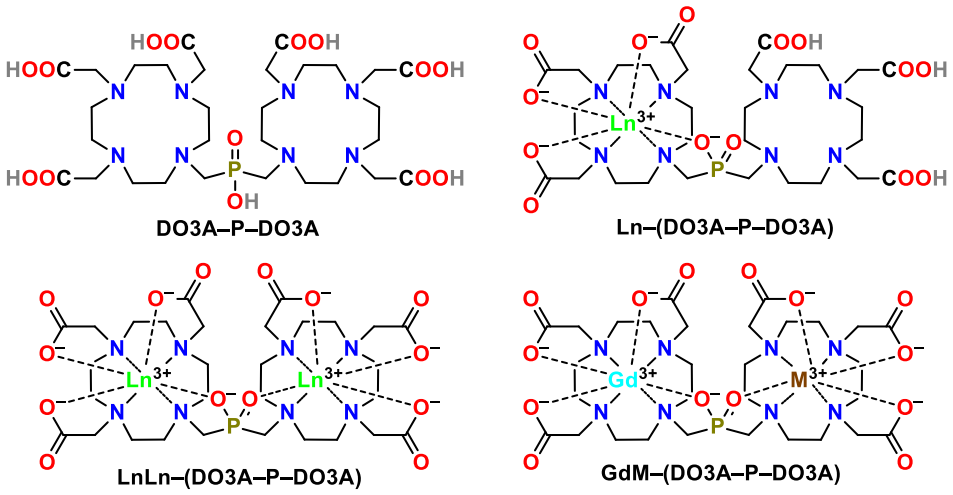

Figure A4