Abstract

Macrocyclic compounds are widely used in a number of biological and medical applications due to their effective and strong chelating abilities. If aptly designed, they bind metal ions without risk of releasing these ions into organism. Thus, they can be used to bind metal radionuclides ions for endoradiotherapy or clinical imaging methods, namely positron emission tomography (PET) and single photon emission computed tomography (SPECT). Gd^{3+} complexes may be utilized in magnetic resonance imaging (MRI). Simultaneously, these ligands can provide way to attach a linker to another structure or moiety designed, for example, for binding onto target protein present on the cell surface, as a fluorescent marker, or for binding different complex for complementary imaging method. Both the ligand and the linker must be properly designed to serve their purposes, *i.e.* the ligand mainly according to metal ion to be bound, and the linker according to conjugated substrate, conditions of conjugation and final application.

This thesis deals with synthesis and study of properties of mainly macrocyclic ligands and their complexes with several types of linkers. These compounds are potentially applicable in diagnostic imaging and biomedicine.

First of the studied ligands was 1,4,7-triazacyclononane (TACN) derivative with three phosphinic acid pendant arms (NOPO) designed primary for complexation of PET nuclide ^{68}Ga (Figure 1).

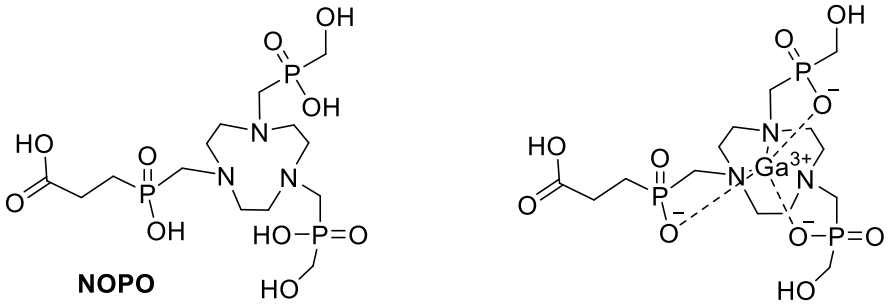

Figure 1. NOPO Ligand and NOPO complex with Ga^{3+}.

Fast complexation of Ga^{3+} ions was proved even in the presence of other potentially concurrent metal ions.[1] The formed gallium complex also boasted a high stability. The binding of targeting peptides through amide bond was achieved with the only carboxylic acid present on pendant arms of macrocycle. The hydroxomethylenes on the other two arms facilitate complexation and render the complex more hydrophilic. Activation of carboxylic acid during peptide conjugation was accompanied with formation of phosphilactone (Figure 2).

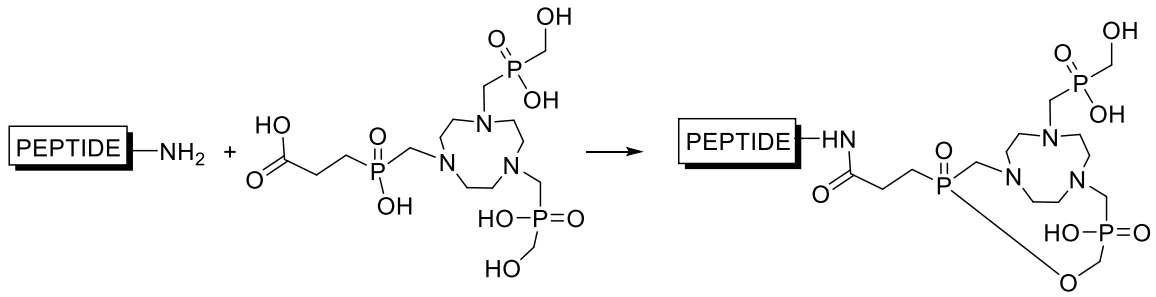

Figure 2. NOPO ligand conjugation with peptide accompanied with phosphilactone formation.

As the lactone was spontaneously cleaved during Ga^{3+} complexation, the intended application was not impeded.[2] Potential for PET diagnostic was demonstrated on living animal models with implanted tumours[1,3] (Figure 3).

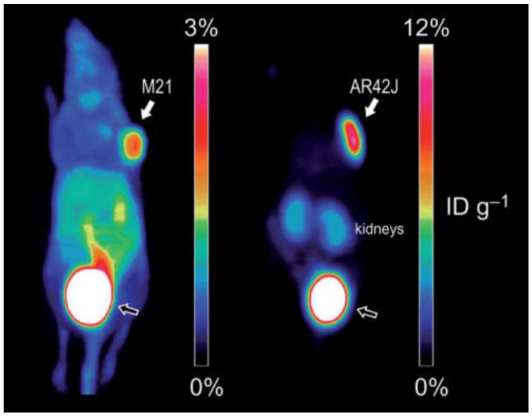

Figure 3. PET images (MIP, 75 min p.i.) of nude mouse tumour xenografts. Left: 68Ga-NOPO-RGDfK (M21 human melanoma); right: 68Ga-NOPO-NOC (AR42J rat pancreas carcinoma). Accumulation of activity is observed in the urinary bladder (indicated by outline arrows) due to renal excretion. From ref.[1]

Next project dealt with synthesis of potential MRI contrast agent based on Gd^{3+} complex with newly designed ligand (Figure 4). It consisted of 1,4,7,10-tetraazacyclododecane (cyclen) with one pendant arm allowing for peptide conjugation *via* carboxylic functional group and another with phosphinic acid. This moiety speeds up exchange of water molecules coordinated to the central Gd^{3+} ion and thus enhances effectiveness of the contrast agent.

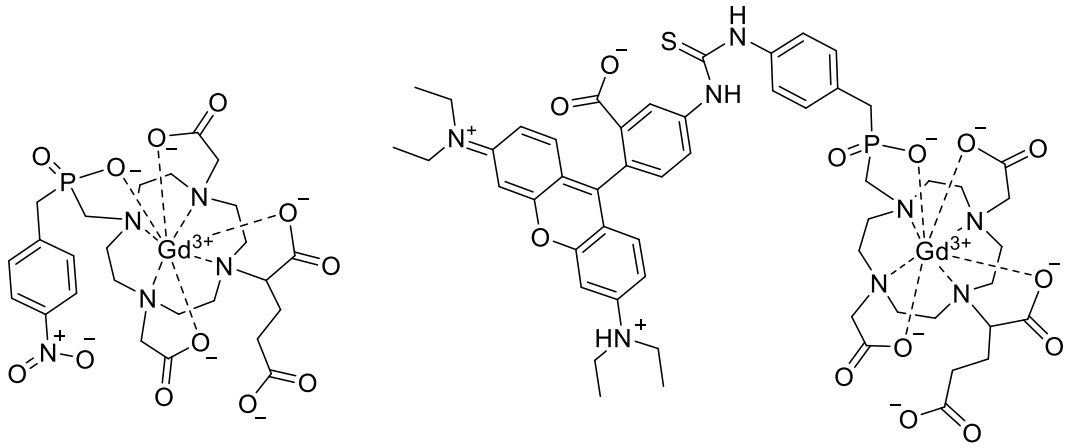

Figure 4. Left: newly synthetized Gd^{3+} based MRI contrast agent; right: Gd^{3+} complex conjugated with fluorescent dye (rhodamine).

Fluorescent dye (rhodamine) was also attached to aminobenzyl functional group on phosphinic pendant arm *via* thiourea linker. Resulting complex could be used as dual MRI/fluorescent contrast agent. Complex was also conjugated to targeting peptide and MRI contrast agent capability was demonstrated on an animal model.[4]

In the next project three Gd^{3+} complexes based on cyclen (Figure 5) were prepared. On their phosphinic pendant arm one of them bears low pH sensitive (hydrazide–hydrazone) hydrolysable

linker, another one reductively cleavable (disulphidic) linker and the last one stable linker, serving as reference.

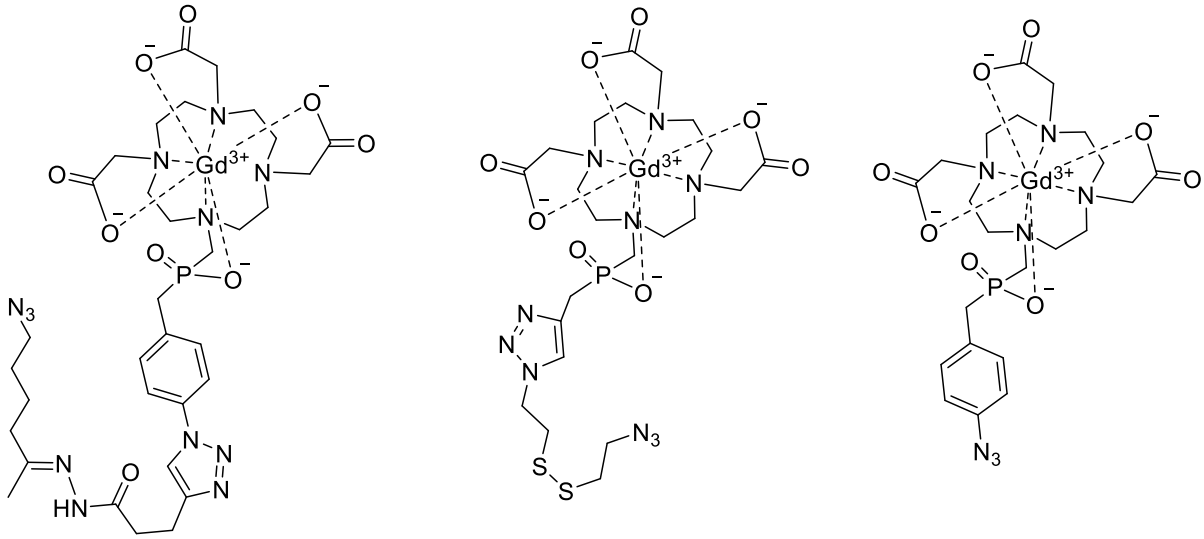

Figure 5. Gd^{3+} complexes used for decoration of nanodiamonds surfaces; left: with pH sensitive hydrazide–hydrazone linker; middle: with reductively cleavable disulphidic linker; right: with stable linker for reference.

These complexes were bound with Cu^{+} catalysed alkyne-azide cycloaddition to polymer coating on nanodiamonds (ND) fluorescent thanks to presence of nitrogen–vacancy (NV) defect in their crystal lattice. Magnetic moment of Gd^{3+} in proximity of ND caused decrease of relaxation time T_1 of NV centres. This can be measured with special technique as change in fluorescence intensity. If the ND environment (low pH or reductive potential, respectively) causes cleavage of the linkers and Gd^{3+} complexes to diffuse from its surface the fluorescence intensity rises. Measuring the fluorescence allows for assessment of change in pH or in reduction potential, respectively, in ND vicinity. Nanosensor responsive to chemical properties of environment with submicrometre resolution were thus constructed. This serves as proof of concept that can be in the future used, for example, in measurements of chemical parameters inside cellular organelles.[5]

In the last project, ligand with structure designed for complexation of PET nuclide ^{68}Ga was synthetized. This ligand has strained cycloalkyne attached (**Figure 6**), allowing for fast and bioorthogonal conjugation with azide or nitrone functional group. This ligand is not based on macrocycle, but the rigidized structure should provide sufficient complex stability for *in vivo* application. This compound has potential to be used as *in vivo* PET marker, but its real applicability has not been tested yet.

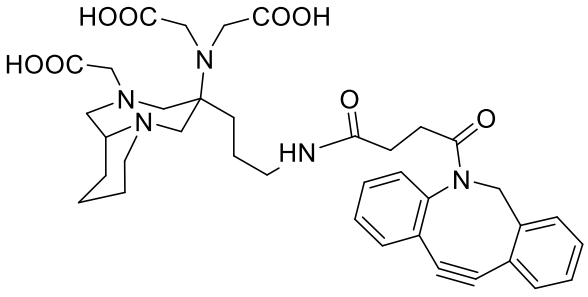

Figure 6. Bifunctional ligand designed for ^{68}Ga complexation and bioorthogonal conjugation with strained cycloalkyne.

References

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