

Fluorinated contrast agents with cation depending T₁ sensitivity

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Introduction

The synthesis of metal ion sensitive MR-contrast agents is important for many medical studies, i.e. neuronal processes. A lot of Gd-complexes were synthesized in the last few years and almost all of them show a decrease of the T₁-time in the presence of Ca²⁺ or other binary cations, like Cu²⁺, Zn²⁺ or Mg²⁺. The molecular structure of the side chain is very important for the sensitivity. Mostly, seven or eight atoms are located between the N-atom of DO3A and the carbon acid or other functional groups, which are capable to interact with cations.^[1-3]

A new class of contrast agents with aminobis(methylenephosphonates) in the side chain show the contrary effect. The presence of Ca²⁺-ions increase the T₁-times.^[4] Eight fluorinated Gd³⁺-complexes were synthesized to study the effect of different metal ions to the relaxation times (see figure 1). The complexes differ in the side chain length (5, 6, 8 or 9 atoms to the carbon acid) and also in the connecting bond to the aromatic system.

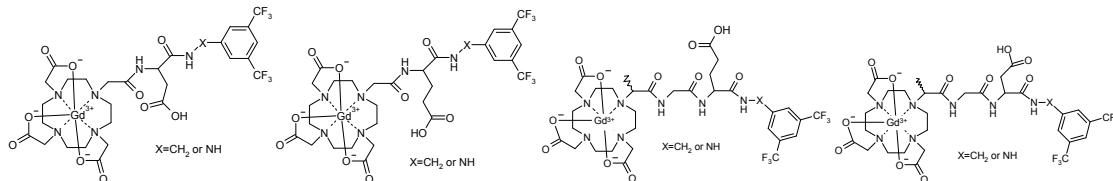


Figure 1: Molecular structures of the synthesized Gd³⁺-complexes.

Methods

The synthesis of two Asp-DOTA, Glu-DOTA, AspGlyMe-DOTA and GluGlyMe-DOTA ligands started with the Fmoc-L-aspartic acid 4-tert-butyl ester or Fmoc-L-glutamic acid 5-tert-butyl ester, respectively. Upon activation with TBTU and DIPEA, they reacted with the fluorinated benzyl derivatives at room temperature for 15h. After elimination of the Fmoc group with 20% piperidine in DMF, the amino acid derivatives reacted on the one hand with bromo acetic acid bromide and on the other hand with activated tris-t-butyl-GlyMe-DOTA. Afterwards, the first compound reacted with DO3A to the corresponding ligand and the tert-butyl esters of all molecules were cleaved with TFA. The Gd³⁺-complexes were obtained upon the addition of GdCl₃ x 6 H₂O in an aqueous solution of the ligand and subsequent neutralisation with 0.1M NaOH.

All complexes were purified by HPLC and chemical structures of the ligands were characterized by 1D-, 2D-NMR and ESI-MS experiments. ¹H- and ¹⁹F-T₁ inversion recovery measurements of the pure Gd³⁺-complexes were performed at 8.4T and 293K sample temperature in 1mmol/L aqueous solution. The dependence of ¹H-T₁ in the presence of metal cations (Na⁺, Ca²⁺) was analyzed at different temperatures in the range of 293K to 313K. MRI experiments of new complexes with different Mⁿ⁺ concentrations were acquired on a Bruker 7T BIOSPEC 70/20.

Results

The relaxivity of the synthesized complexes strongly depends on presence of diamagnetic metal ions (i.e., Na⁺, K⁺, Ca²⁺, Mg²⁺) and additionally Mⁿ⁺-concentration as shown in the ¹H-T₁ measurements. The pure complexes show an r₁ value between 6 and 7 (see table 1). The addition of Na⁺ cations increases the relaxivity. Divalent cations like Ca²⁺ or Mg²⁺ increase the relaxivity less in comparison to the monovalent cations. In addition, it could be observed that divalent cations displace monovalent cations. The T₁-value of an 1mM solution of 3,5-Bis(trifluoromethyl)-benzylamine-Gd³⁺-GluGlyMeDOTA in H₂O spiked with 1eq Na⁺ ions (red data points in figure 2) increases by addition of 1eq Ca²⁺ ions (green data points). Figure 2 also shows, that the T₁-value decreases with decreasing temperature.

All synthesized complexes depend upon the ambient metal ion concentrations, pH-value and temperature. Complexes with longer side chain show the strongest sensitivity towards the ambient conditions.

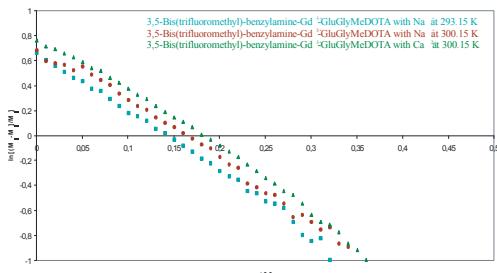


Figure 2: T₁ measurements of 3,5-Bis(trifluoromethyl)-benzylamine-Gd³⁺-GluGlyMeDOTA show a dependence on temperature and metal ion.

Discussion

It could be shown that the relaxivity of all synthesized complexes is sensitive to metal ions. The effect on the relaxivity depends on the kind and concentration of the cation, temperature and pH-value. The different influence between monovalent and divalent cations is due to the aggregation of the Gd³⁺-complexes in presence of divalent cations (figure 3). Complexes with a shorter side chain show smaller sensitivity to metal ions compared to molecules with longer side chains. The behaviour of the synthesized complexes with respect to the relaxation times allows an examination of changes within the chemical and physical environment.

References

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Substances	Gd-Asp-DOTA	Gd-Glu-DOTA	Gd-AspGlyMe-DOTA	Gd-GluGlyMe-DOTA
3,5-Bis(trifluoromethyl)-benzylamine	r_1 (¹ H) 7.84	7.33	6.31	6.17
	r_1 (¹⁹ F) 15.78	14.80	13.34	12.65
3,5-Bis(trifluoromethyl)-phenylhydrazine	r_1 (¹ H) 8.18	7.92	6.83	6.77
	r_1 (¹⁹ F) 15.83	15.04	13.56	12.71

Table 1: Comparison of the measured T₁-values of pure Gd³⁺-complexes.

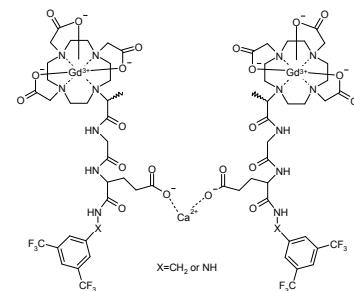


Figure 3: Aggregation in presence of Ca²⁺-ions.