New fluorinated Gd-AspGlyMe-DOTA complexes for ^{19}F-MRI

M. Plaumann[1], E. Küstermann[2], and D. Leibfritz[1]

[1]Institute of Organic Chemistry, University of Bremen, Bremen, Bremen, Germany, [2]CAI, MRI/MRS, University of Bremen, Bremen, Bremen, Germany

Introduction

^{19}F-MRI gets an interesting method for molecular imaging. The high MR sensitivity and very low natural abundance in living organisms makes fluorine interesting in medical diagnostic. Mostly perfluorinated contrast agents like perfluorocarbons will be used. Synthesis of fluorinated Gd-contrast agents allow to record ^{19}F-images without any background signals and additional classical ^{1}H-MR images at the same time. The labeling of C6-cells with Gd^{3+}-DTPA- and -DOTA-complexes of 3,5-Bis(trifluoromethyl)benzylderivates is established in our group.

3,5-Bis(trifluoromethyl)benzylamine and 3,5-Bis(trifluoromethyl)phenylhydrazine were selected as model compounds to synthesize four new Gd-AspGlyMe-DOTA complexes (Fig. 1: complex II) and compare them to Gd-DTPA-, Gd-GlyMe-DOTA- and Gd-AspGlyMe-DOTA-complexes (shown also in Fig. 1) with respect to their lipophilicity and T_1-times.

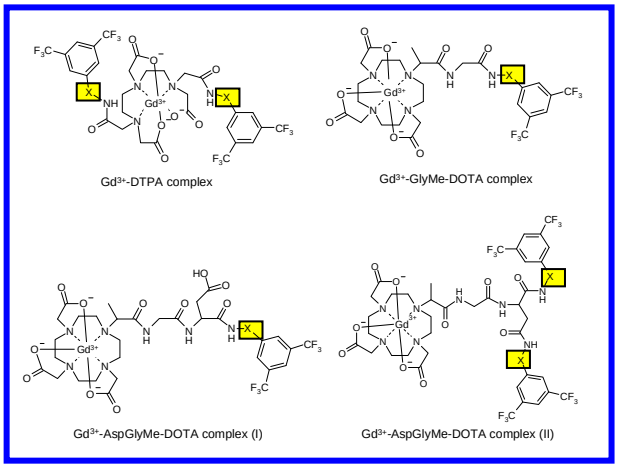

Fig. 1: Synthesized and characterized complexes with X = CH_2 or NH.

Methods

Two different DTPA-ligands were synthesized by reaction of DTPA-bis-anhydride at 333K in dry DMF with 3,5-Bis(trifluoromethyl)benzylamine and 3,5-Bis(trifluoromethyl)phenylhydrazine. The Gd^{3+}-complexes are obtained upon the addition of $GdCl_3$ x 6 H_2O in an aqueous/ethanol solution of the ligand and subsequent neutralisation with 0.1M NaOH.

The synthesis of two GlyMe-DOTA and six AspGlyMe-DOTA ligands starts from tris-t-butyl-GlyMe-DOTA. Fmoc-L-aspartic acid 4-tert-butylester was used for the synthesis of the AspGlyMe-DOTA-complexes. After activation with TBTU and DIPEA, it reacted with the fluorinated benzylderivatives at room temperature for 24h. The t-butyl esters were cleaved with trifluoroacetic acid (TFA) and for complex II the second benzylderivative reacted as in the first step. After elimination of the Fmoc group with $KHCO_3$ solution, the Asp-derivative reacted with activated tris-t-butyl-GlyMe-DOTA. TFA cleaved the t-butyl esters and the ligands were complexed in the same manner as the DTPA derivatives.

All complexes were purified by HPLC and the chemical structures of the ligands were characterized by 1D-, 2D-NMR and ESI-MS experiments. ^{1}H- and ^{19}F-T_1-measurements of the Gd^{3+}-complexes were performed at 8.4T and 293K sample temperature in 1mmol/L aqueous, in the case of DTPA- and AspGlyMe-DOTA-complexes in aqueous/DMSO solutions.

The lipophilicity is deduced from methanol-water partition coefficient (log k'w) of each complex by reversed phase chromatography at 313K column temperature. The methanol content is adapting in different runs with respect to the hydrophobic interaction of the analytes with the RP packing. The logarithm of retention is plotted versus the methanol content and extrapolated to 0% MeOH to give the log k'- value.

Results

Comparison of the ten fluorinated Gd^{3+}-complexes show shorter ^{1}H-T_1- times compared to Gd-DTPA-BMA (T_1=0.244 mmol·s).[1] The ^{1}H relaxivities are between 6.5 and 10 $mM^{-1}s^{-1}$ whereas the GlyMe-DOTA-complexes show slightly higher r_1-values. ^{19}F-NMR spectra of the complexes exhibit a sharp signal around $\delta \approx -63$ ppm. Inversion recovery experiments show ^{19}F-relaxivities between 10 and 14 $mM^{-1}s^{-1}$.

Reversed phase chromatography showed that the lipophilicity of the bisaromatic AspGlyMe-DOTA complexes are higher (log k'w = 6.04) in comparison to the DTPA-complexes (log k'w ≈ 5.6). In the case of the monoaromatic Gd^{3+}-GlyMe-DOTA and Gd^{3+}-AspGlyMe-DOTA complexes, the partition coefficient is much lower. Furthermore, it can be shown, that the lipophilicity of the monoaromatic 3,5-Bis(trifluoromethyl)benzylamide complexes is lower compared to the 3,5-Bis(trifluoromethyl)phenylhydrazine complexes (log k'w = 3.06 in the case of 3,5-Bis(trifluoromethyl)phenylhydrazine-Gd^{3+}-GlyMe-DOTA and log k'w = 2.86 3,5-Bis(trifluoromethyl)benzylamide-Gd^{3+}-GlyMe-DOTA).

The free carbon acid in the monoaromatic Gd^{3+}-AspGlyMe-DOTA complexes reduces the lipophilicity of these molecules (log k'w = 2.02 in the case of 3,5-Bis(trifluoromethyl)phenylhydrazine-Gd^{3+}-AspGlyMe-DOTA and log k'w = 1.55 3,5-Bis(trifluoromethyl)benzylamide-Gd^{3+}-AspGlyMe-DOTA).

MRI experiments and ICP-MS measurements prove the possibility to label C6 cells with different 3,5-Bis(trifluoromethyl)phenylhydrazine-Gd^{3+}- and 3,5-Bis(trifluoromethyl)benzylamide-Gd^{3+}-complexes. Furthermore, the ^{19}F-MRI signal of the Gd-complex in labeled C6 cells is sensitive enough to be detected on a Biospec 4.7T MR scanner.

Discussion

All synthesized lipophilic fluorinated Gd-complexes show very short ^{19}F-T_1- times. The lipophilicity of the monoaromatic Gd^{3+}-complexes has been varied by changing the functional amino group (–NH_2- or NH_2-NH-) or by adding another spacer molecule (Asp). It was shown that ^{19}F-MRI can be an alternative to the conventional ^{1}H-MRI. Due to the higher signal intensity (4 trifluoromethylgroups in DTPA-complexes and bisaromatic AspGlyMe-DOTA complexes) the contrast agent concentration can be kept lower. ICP-MS measurements are also used to confirm cell labelling.

[1] Fossheim, S. (et al.) JMRI 1997, 7: 251