Design, synthesis and characterization of new smart MR contrast agents sensitive to pH

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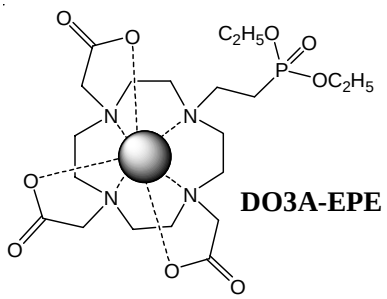
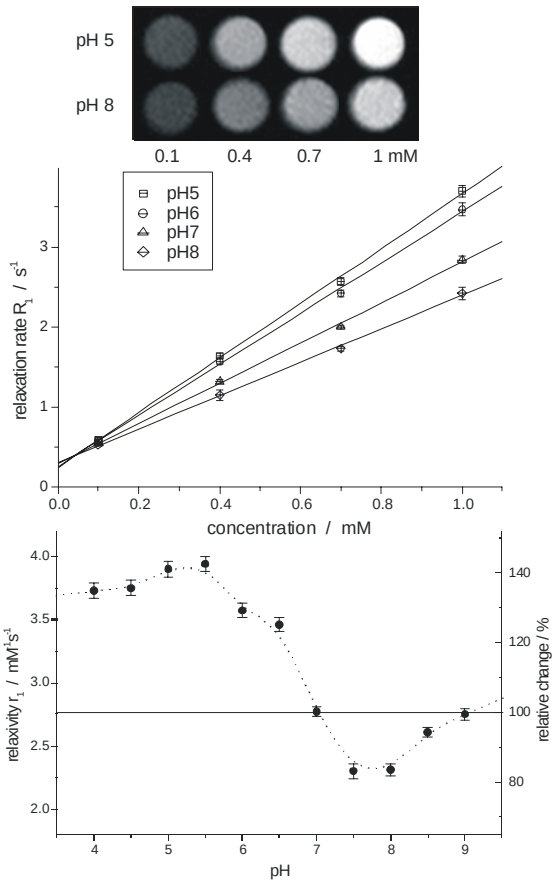
Introduction New gadolinium-based MR contrast agents were designed and synthesized to trace physiological changes of extracellular pH and calcium concentration. DO3A-EP/ DO3A-EPE was appended with -tris-carboxymethyl and -**phosphono-ethyl (-EP) / diethyl-phosphono-ethyl (-EPE)** moieties on tetraazacyclododecane and complexated with Gd. Phosphonate derivatives are known to have a very high affinity towards calcium (MDP, methylene diphosphonate) and can function as reporters of pH or calcium ions. Ethyl phosphonate was appended covalently on the macrocycle without any *amide linkage*, which binds *reversibly* as a function of pH/calcium. Gd-DO3A-EP/-EPE therefore can be used as an extracellular contrast agents reacting to pH/calcium-related changes on cellular activity and neurotransmission. In this paper we demonstrate *in-vitro* MR relaxation changes induced by changes in pH.

Synthesis 1,4,7 tris(carboxymethyl)-10-(2-phosphono-ethyl)-1,4,7,10-tetraazacyclododecane was synthesized from 1,4,7,10-tetraazacyclododecane (cyclen) by reaction with tert-butyl bromoacetate and diethyl 2-bromoethyl phosphonate, giving an excellent yield of 80%. The corresponding carboxylate derivative DO3A-EP was obtained by cleaving the tert-butyl group by trifluoroacetic acid and anisole at 0ºC. **DO3A-EPE** was synthesized from cyclen by the reaction of ethyl-2-bromoacetate to get the mono-substituted product. It was further appended with tert-butylbromoacetate to get 1,4,7-tris(carbobutoxymethyl)-10-[N-2(diethyl-phosphono-ethyl)]-1,4,7,10-tetraazacyclododecane. The corresponding carboxylate derivative DO3A-EPE, was obtained by cleaving the tert-butyl groups and treatment of TFA at RT. Yield was 82%.



Methods The gadolinium complexes of DO3A-EP / -EPE were prepared by using $GdCl_3.6H_2O$ at pH 7-8 on 60-70°C for 18h. For measurements of the relaxivities r_1, four different concentrations 0.1, 0.4, 0.7, and 1 mM of the CA were prepared in tubes. For the series with –EP, pH from 4-9 in 0.5 pH steps was adjusted using glacial acetic acid 17 M and Na_2CO_3, respectively. For the series with –EPE, pH was adjusted from stock solution at pH 8 adding solid p-toluenesulphonicacid and LiOH without changing the concentration of the stock solution separately.

Up to 52 tubes were measured simultaneously using a spin echo sequence with TE 13 ms / TR 0.05 - 8 s or TE 13 - 1248 ms / TR 14 s (21˚C, 200 / 300 MHz). Fitting of $T_{1,2}$ values was done voxelwise on selected ROIs using MATLAB. Relaxivity $r_{1,2}$ was calculated from the slope of $R_{1,2}(c)$ versus the CA concentration by an error-weighted linear regression.



Results and Discussion In T_1-weighted images (Fig. 2 top), different MR image intensities at different pH levels were observed due to changes in the relaxation rates. R_1 values from samples with four different pH values are plotted below. With increasing pH, the relaxivity, i.e. the slope of the relaxation rates R_1 over concentration, decreased. An overview of all results from tubes with different pH values is given in Fig. 2 bottom. The inflexion point occurs at a pH of approximately 6.5. r_1 relaxivity of **Gd-DO3A-EP** increased by 70% from 2.3 to 3.9 $s^{-1}mM^{-1}$ (pH 7.5 to pH 5.5); r_2 relaxivity increased by 57% from 2.8 to 4.4 $s^{-1}mM^{-1}$ (pH 7.5 to pH 5.5). For **Gd-DO3A-EPE**, r_1 increased by 50% with (5.3 / 6.2 / 7.9 ± 0.1) $s^{-1}mM^{-1}$ at pH 8 / 6 / 4, respectively.

Gd-DO3A-EP and Gd-DO3A-EPE show similar relative changes of the relaxivity in the pH range from 8 to 4. In -EPE it is hypothesized that the relaxivity decrease at pH 8 to 4 is due to the formation of ternary adducts with anionic species like carbonate (CO_3^{2-}) and hydroxide anions (OH^-) which displace the inner sphere water molecule(s) as described by Aime [1]..

In conclusion, these data demonstrate the potential of the newly synthesized ligands as novel smart contrast agents responsive to pH. Since Gd-DO3A-EPE is an **uncharged** molecule, it may be preferable to Gd-DO3A-EP in some applications, for example when crossing of lipid membranes is desired *in vivo*.

References **1.** S. Aime et al. Eur. J. Inorg. Chem. 2003, 3530-3533; Chem. Commun. 1999, 1047-1048.

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