Multifunctional gadolinium-based ligand DO3A-EA as novel precursor for conjugation with bioorganic molecules to develop smart contrast agents for MR imaging

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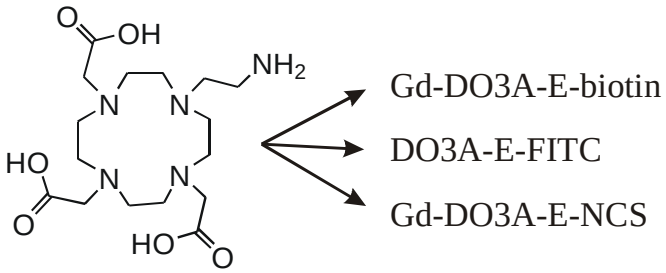
Introduction To image directly neuronal activity by means of magnetic resonance, the physiological changes occurring during neural activation must be converted into detectable MR image contrast. Smart MR contrast agents (CA) exhibit modulation of their relaxivity by specific physiological or biochemical trigger-events such as changes in pH, calcium ion, neurotransmitter concentration, enzymatic activity, or the binding of an intracellular messenger [1]. In an effort to develop novel *smart* MR contrast agents, the multifunctional chelating agent DO3A-EA has been designed and synthesized. It serves as a valuable **multipurpose precursor** for smart contrast agents based on Gadolinium chelates in the design of relaxometric MR probes. Several applications are reported after conjugation with diverse bioorganic molecules. This demonstrates the importance and potential of the precursor not only in the design of *'smart'* CA, but also for multifunctional *'targeted'* CA.

Synthesis 1,4,7-tris(carboxymethyl)10-(aminoethyl)-1,4,7,10-tetraazacyclododecane (**DO3A-EA**) was synthesized from 1,4,7,10 tetraazacyclododecane (cyclen) by the reaction of N-BOC-2-bromoethylamine to get the mono-substituted product. It was further reacted with tert-butylbromoacetate to get 1,4,7-tris(carbobutoxymethyl)-10-(BOC-aminoethyl)-1,4,7,10 tetraazacyclododecane. The corresponding carboxylate derivative DO3A-EA was obtained by cleaving the tert-butyl groups by the treatment of DCM::TFA at RT. Yield was 85%.



DO3A-EA

Methods Gd and Eu complexes were prepared by mixing a 5-10% excess of ligand with hexahydrated Gd^{3+}-and Eu^{3+}-hydrochloride. The pH was adjusted to 6-7. Unbound ligands were removed by using Sep-Pak cartridge C-18 (Agilent). For measurement of the relaxivity r_1 and r_2 for Gd complexes, four different concentrations 0.1, 0.4, 0.7, and 1 mM of the contrast agent were prepared in tubes. 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 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 With DO3A-EA as precursor, the following contrast agents were synthesized and tested:

Gd-DO3A-E-NCS: The nucleophilic amine group of DO3A-EA was converted to DO3A-E-NCS (with thiophosgene at pH 8) containing the highly reactive electrophilic –NCS group. This product forms a stable macrocyclic complex with Gd(III), while the electrophile group remains free and does not participate in complexation. Thus, DO3A-E-NCS provides the additional advantage to exploit the binding of other nucleophilic groups in further synthesis and possibly also use a Gd-preloading approach to avoid the bonding of gadolinium with calcium binding chelates. This gadolinium-based precursor can be used to couple with a calcium binding chelator/dye to prepare calcium-sensitive contrast agents. MR relaxivity of Gd-DO3A-E-NCS at pH 8 was $r_1 = (3.29 \pm 0.08)\ s^{-1}mM^{-1}$.

Gd-DO3A-E-biotin: Biotin-conjugated DO3A can be used for targeted imaging in an antibody-avidin system. Gd-DO3A-E-biotin binds to avidin through the biotin moieties with high affinity in a 4:1 ratio. Binding to the protein avidin will enhance the relaxivity by altering the rotational correlation time τ_R. MR relaxivity of Gd-DO3A-E-biotin at pH 8.5 was found to be $r_1 = (4.85 \pm 0.08)\ s^{-1}mM^{-1}$. The mixture of Gd-DO3A-E-biotin and avidin (6:1) showed an enhancement in relaxivity of 49% for r_1 and 311% for r_2 relative to the unbound biotinylated Gd(III) complex.

DO3A-E-FITC: The conjugation with the fluorescent FITC group can be used for targeted contrast agents tracking cellular binding and internalization. Additional loading with Gd^{3+} can provide MR contrast. Preliminary *in vitro* studies on DO3A-E-FITC were done with NIH-3T3 mouse fibroblasts cell cultures to demonstrate its potential for multifunctional properties. DO3A-E-FITC and Eu-DO3A-E-FITC up to 100 µM did not show significant cytotoxicity after 24 hours inoculation (propidium iodide assay). Fluorescence microscopy of living cells displayed proper co-localization.

Conclusions The presented precursors DO3A-EA and DO3A-E-NCS cover the entire range of reactivity for the coupling to electrophilic and nucleophilic groups, respectively. Therefore, all different kinds of *smart* and *targeted* contrast agents can be easily synthesized by coupling with biological molecules, Ca^{2+} chelators, proteins and antibodies etc.

References 1. Meade et al, Inorg. Chemistry 2002,41,15; Sherry et al, J. Am. Chem. Soc. 2004, 126, 9248-56; Aime et al, J. Am. Chem. Soc. 2001, 123, 7601-9.

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