Multipurpose gadolinium-based ligand DO3A-EA as precursor for conjugation with organic molecules to develop smart and targeted contrast agents for MR and optical imaging

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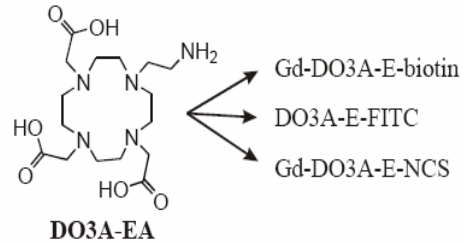
Introduction Smart MR contrast agents (CA) exhibit modulation of their relaxivity by specific physiological or biochemical trigger-events. In an effort to develop novel *smart* MR contrast agents, the multifunctional chelating agent DO3A-EA has been synthesized. It serves as a valuable **multipurpose precursor** for smart contrast agents based on Gadolinium chelates in the design of relaxometric MR probes.



Synthesis 1,4,7-tris(carboxymethyl)10-(aminoethyl)-1,4,7,10-tetraazacyclododecane (**DO3A-EA**) was synthesized from cyclen by the reaction of *tert*-butylbromoacetate to get the tri-substituted product. It was further reacted with N-Boc-2-bromoethylamine 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%.

Results With DO3A-EA as precursor, following CAs were synthesized and tested:

Gd-DO3A-E-NCS: It forms stable macrocyclic complex with Gd(III) and can be used in Gd-preloading approach to avoid the binding of gadolinium with calcium binding chelates. MR relaxivity of Gd-DO3A-E-NCS (pH 7.5) was $r_1 = (3.29 \pm 0.08)\ s^{-1}mM^{-1}$.

Gd-DO3A-E-biotin: It can be used for targeted imaging in an antibody-avidin system. MR relaxivity of Gd-DO3A-E-biotin (pH 7.5) was $r_1 = (4.85 \pm 0.08)\ s^{-1}mM^{-1}$. Mixture of Gd-DO3A-E-biotin and avidin (4:1) showed 30% relaxivity enhancement for r_1 and 311% for r_2 relative to the unbound biotinylated Gd(III) complex.

DO3A-E-FITC: It can be used to track cellular binding and internalization. Additional loading with Gd^{3+} can provide MR contrast. DO3A-E-FITC and Eu-DO3A-E-FITC up to 50 µM did not show cytotoxicity after treatment for 24 hrs (NIH-3T3 cells, PI assay). Fluorescence microscopy of living cells displayed proper co-localization.

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