Novel Azamacrocyclic Conjugates in the Development of "Smart" and Targeted Contrast Agents for MR Imaging

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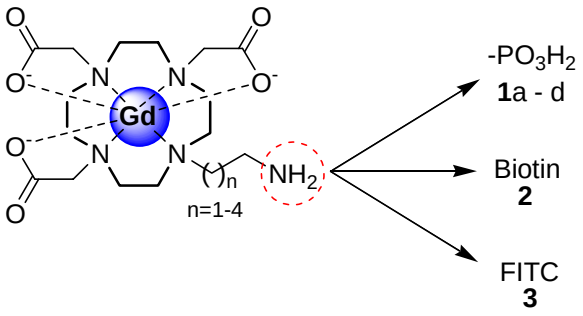
Smart MR contrast agents exhibit modulation of their relaxivity by specific physiological or biochemical trigger-events, while targeted MR contrast agents are envisioned to deliver the large gadolinium chelates into the target tissue. In an effort to develop novel smart and targeted MR contrast agents, the series of the DO3A based multifunctional chelating agents with the variable length of the side chain has been synthesized. They serve as valuable **multipurpose precursors** for contrast agents based on gadolinium chelates in the design of relaxometric MR probes.
The presence of the amino group in the side chain of the macrocycle allows for conversion into various functional groups (aminophosponates, aminocarboxylates, etc.) or for conjugation with different biomolecules, dyes, and polymers. Choice of the functional groups depends on the further application of the compounds.

A series of gadolinium chelate complexes based on the compounds **1a-d** were developed, in order to change relaxivity in magnetic resonance experiments dynamically with Ca^{2+} concentration. The potential of Ca^{2+} MR imaging in neuroscience is evident in the intensive efforts to design gadolinium complexes that can act as calcium-dependent MRI contrast agents. Different lengths of the phosphonate side chains are expected to lead to different binding constants of the phosphonate - gadolinium bonds. The latter property can be exploited for fine-tuning the sensitivity of the agent to calcium ion concentration. The sensitivity of the contrast agents for changes in Ca^{2+} concentrations increased with the chain length of the phosphonate functions, specifically for compound **1d** (n=4) the range of Ca^{2+} concentration is compatible with extracellular physiological conditions.



For the purpose of targeted MRI, two macrocyclic bioconjugates were synthesized. A conjugate **2** of Gd-DO3A-EA with biotin was synthesized for targeted imaging in an antibody-avidin system. Mixture of **2** and avidin (4:1) showed 54% relaxivity enhancement for r_1 and 311% for r_2 relative to the unbound **2**.

A macrocyclic conjugate **3** of Gd-DO3A-EA with FITC was designed to track cellular binding and internalization by both fluorescence detection and MR imaging. The compound did not show cytotoxicity after treatment up to 50 µM for 24 hrs (NIH-3T3 cells, PI assay). Fluorescence microscopy of living cells displayed detectable internalisation.