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Synthesis and characterization of a new lanthanide based MRI contrast agent, potential and versatile tracer for multimodal imaging

Satya Narayana Murthy Chilla [a], Sophie Laurent [a,*], Luce Vander Elst [a,b], Robert N. Muller [a,b]

[a] Department of General, Organic and Biomedical Chemistry NMR and Molecular Imaging Laboratory, University of Mons, Avenue Maistriau, 19, Mendeleiev Building, 7000 Mons, Belgium
[b] Centre for Microscopy and Molecular Imaging (CMMI), Rue Adrienne Bolland 8, 6041 Charleroi-Gosselies, Belgium

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ABSTRACT

In the present work a modular pathway towards the synthesis of a new versatile MRI contrast agent is reported and its physico-chemical properties are described. Two different functional groups were attached on two arms of the gadolinium 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA) in order to get a platform able to bind one probe designed to target specific biological marker and a fluorescent molecule likely to be used for optical imaging. The nuclear magnetic relaxation dispersion (NMRD) profile, the oxygen-17 relaxometric NMR study and stability assessment versus transmetalation of the Gd-complex show that this new contrast agent has a relaxivity and transmetalation stability similar to Gd–DOTA.

1. Introduction

In past two decades, clinical Magnetic Resonance Imaging (MRI) has been developing very rapidly. However unambiguous medical diagnosis often requires the help of MRI contrast agents (CAs). These contrast agents are mainly paramagnetic complexes of Gd(III) or superparamagnetic nanoparticles. The chemistry of numerous paramagnetic complexes has been described in several reviews and books[1,2] and derivatives designed to optimize chemical and magnetic properties have been proposed.[3] The efficacy of a contrast agent is measured by its relaxivity (r_i with i=1,2), the paramagnetic relaxation rate of water proton normalized to a 1 mM concentration of Gd^{3+}. Several tactics were devised to increase the relaxivity of the paramagnetic metal complexes typically by increasing: (i) the molecular size of the paramagnetic system by covalent or non-covalent interaction with macromolecules to slow down the rotational motion in the medium,[4] (ii) the number of coordinated water molecules (q),[5] (iii) the number of paramagnetic metal centres, linking the single complexes in multimeric systems. The significant parameters determining the relaxivity of the complexes are the rotational correlation time (τ_R), the water residence time (τ_M) and the electron spin relaxation times ($\tau_{S1,2}$).

Ligand design has poor influence on the electron spin relaxation, whereas τ_M can be tuned over several orders of magnitude by imposing steric hindrance around the water-binding site in Gd^{3+} complexes of both linear (DTPA-type) and macrocyclic (DOTA-type) ligands.[1] Introduction of an extra methylene group on the backbone of the ligands DOTA and DTPA leads to chelators TRITA and EPTPA, respectively, whose Gd^{3+} chelates display a water exchange around two orders of magnitude faster than the parent Gd–DOTA and Gd–DTPA.[6–8] The development of multifunctional ligands for Gd^{3+} complexation has largely contributed to the advances of magnetic resonance imaging (MRI) in biomedical research.[2,3] These ligands, which could be more versatile, allow for conjugation of the Gd-chelate with specific biological vectors or for the optimization of their efficacy. Ideally, a multifunctional chelator should integrate optimal properties for metal complexation with easy and versatile synthetic possibility for conjugation. Grafting vectors on Gd–DOTA, as mono or diamide chelates has been reported. However, it is well known that complexes bearing amide-chelating units are less stable and exhibit slower water exchange rates that are detrimental to relaxivity.[9–11] Taking into account all these features of Gd-chelates (stability and physico-chemical properties), we preferably design to prepare DOTA derivatives due to their well-recognized kinetic inertness and thermodynamic stability, even if the synthesis of macrocyclic chelates is more difficult and more time consuming than the preparation of non-cyclic analogues. We synthesized and

* Corresponding author. Tel.: +32 65373525; fax: +32 65373533; e-mail address: sophie.laurent@umons.ac.be (S. Laurent).

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characterized a new trendy and versatile agent Gd–DOTA–[Amino Pentyl-Succinic Acid] APSA (Gd–DOTA–APSA), which provides pendant functional groups for conjugation to selected probes. These conjugations are preferably carried out via selective chemistry among amine and acid functionalities, which provide stable compounds. This new derivative is obtained by selective alkylation of two of the nitrogens of the macrocycle by acetate arms bearing different functional groups allowing conjugation through amide or urea linkage.

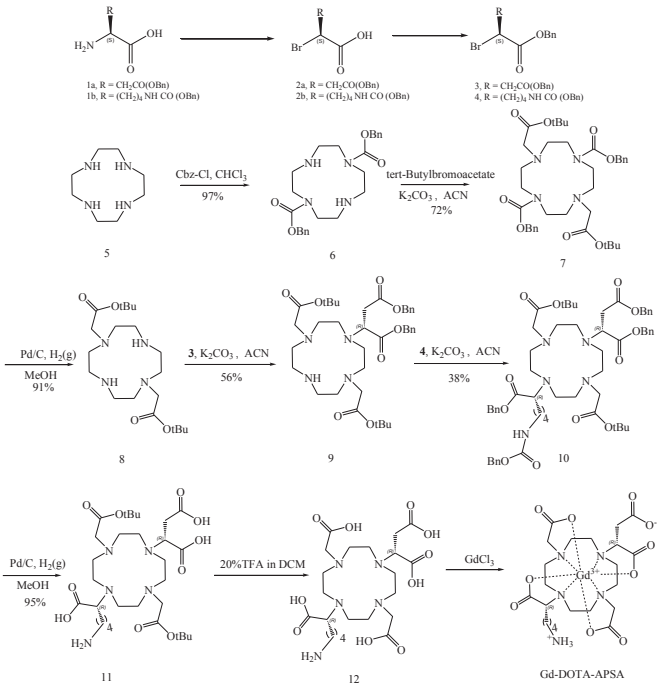
2. Results and discussion

The *trans*-DO2A **8** was synthesized by selective protection of *N*-1 and *N*-7 nitrogens of cyclen by benzylchloroformate and further alkylation and deprotection as previously described[12,13]. The bromo derivatives **2a** and **2b** were synthesized from the benzyl esters of L-aspartic acid and benzylurea derivative of L-lysine by a method analogous to Holmberg followed by Steglich esterification, which provided *S*-dibenzyl 2-bromosuccinate **3** and benzyl (2*S*)-6-{[(benzyloxy) carbonyl]amino}-2-bromohexanoate **4**.[14] The synthetic building blocks **3** and **4**, proved to be efficient alternatives to (unstable) reagents, such as alkene or tosylate-derivatives. Building blocks **3** and **4** were obtained in high yield. They react easily with the *trans*-DO2A **8** via S_N2-type alkylation but the second alkylation needs a little longer reaction time due to steric hindrance of the fourth amine. The mono alkylation of **8** has been optimized by varying the equivalents of bromide **3** in the presence of different bases. Although using K_2CO_3 resulted in the worst selectivity of mono versus bis alkylation, it resulted in the best overall yields for the monoprotection reaction to yield **9**. The final alkylation of **9** was performed alike but by using excess of the bromo analogue under refluxing condition and yielded **10**. Compounds **9** and **10** were obtained with an excellent optical purity of 100% (determined by chiral HPLC). The debenzylation of **10** was performed under hydrogenolysis yielding **11** quantitatively. The final deprotection procedure with TFA afforded the DOTA-(APSA) chelator **12**. Purification of **12** performed by RP-chromatography yielded the trifluoroacetate salt (analytical purity) with a reasonable yield. It is to be noted that the ethyl esters of the bromo analogues of **3** and **4** were synthesized and tested but during the final basic hydrolysis, a semi-hydrolyzed product precipitation occurred, leading to incompletion of reaction.

Finally complexation was performed with $GdCl_3 \cdot 6H_2O$ by maintaining pH between 6.2 and 6.7. The excess of gadolinium ions was removed by using chelex. The absence of excess ions was confirmed by xylenol orange test. The complex was purified by reverse phase chromatography.

The functional groups (e.g., $-NH_2$, COOH) of our complex allow selective conjugation to a wide variety of organic moieties or (bio) macromolecules, via amine as well as carboxylic acid functions (Scheme 1), for purposes of targeting and/or optimization of τ_R. It is to be noted that a metal chelator with one amine group, DO3A-*N*-α-aminopropionate (α-amino-DOTA), has already been reported.[15] The straightforward synthesis and the versatility of further conjugation of our new chelator make this system an excellent multifunctional ligand for the development of imaging agents. The full synthesis and characterization of the ligand are described in the Experimental section. We should note, however, that the non-complexed ligand is not compatible with an extended use in conjugation reactions like peptide couplings.

Proton relaxivity measurements in water revealed that the Gd–DOTA–APSA chelate is stable in the pH range extending from 3 to 8. The magnetic field dependence of the proton longitudinal water proton relaxivities (r_1 NMRD profile) measured at 310 K shows that the relaxivity of our new complex is slightly higher than that of the parent compound Gd–DOTA (Fig. 1).



Scheme 1. Synthesis of Gd–DOTA–APSA.

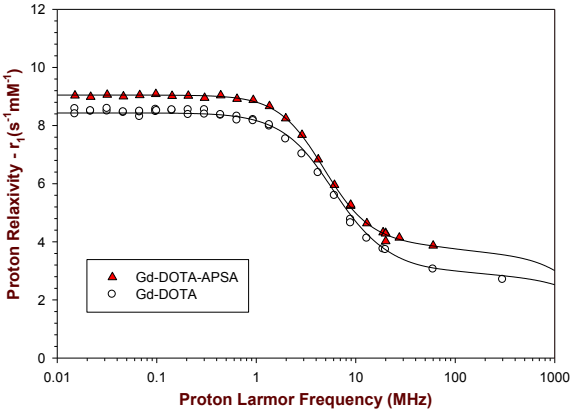


Fig. 1. ^{1}H NMRD profiles of Gd–DOTA–APSA (triangles) & Gd–DOTA (white circles). The lines represent simulations using the best-fit parameters (see Table 1).

The water residence time was determined from variable-temperature ^{17}O NMR studies. The temperature dependence of the reduced ^{17}O transverse relaxation rates ($1/T_{2r}$) (Fig. 2) is typical of a water residence time of the order of 100 ns at 310 K. The analysis of the data using the usual equations[16] gives a τ_M value of 73 ns, a value lower than that of Gd–DOTA (Table 1) but larger than that reported for Gd–DOTMA, a more crowded macrocyclic Gd complex.[17] The NMRD profile was fitted using the inner sphere (Solomon–Bloembergen–Morgan) and outer sphere (Freed) theories. As expected considering the molecular weight of Gd–DOTA–APSA, its value of τ_R is increased as compared to Gd–DOTA (Table 2). Finally the value of τ_{SO} is decreased and similar to that of Gd–$HPDO_3A$.

2.1. Transmetalation

The Gd^{3+} chelates can be sensitive to transmetalation by endogenous ions, such as Cu^{2+}, Ca^{2+} and Zn^{2+}. Among the three metals mentioned, Zn^{2+} has a high affinity for the Gd complexes.