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

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A B S T R A C T

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.

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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 books1,2 and derivatives designed to optimize chemical and magnetic properties have been proposed.3 The efficacy of a contrast agent is measured by its relaxivity (R1 with i=1,2), the paramagnetic relaxation rate of water proton normalized to a 1 mM concentration of Gd3+. 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 (τM) and the electron spin relaxation times (τ1/2).

Ligand design has poor influence on the electron spin relaxation, whereas τM can be tuned over several orders of magnitude by imposing steric hindrance around the water-binding site in Gd3+ complexes of both linear (DTPA-type) and macrocyclic (DOTA-type) ligands.4 Introduction of an extra methylene group on the backbone of the ligands DOTA and DTPA leads to chelators TRITA and EPTPA, respectively, whose Gd3+ chelates display a water exchange around two orders of magnitude faster than the parent Gd–DOTA and Gd–DTPA.6,7 The development of multifunctional ligands for Gd3+ complexation has largely contributed to the advances of magnetic resonance imaging (MRI) in biomedical research.8,9 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.10,11 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...
characterized a new trendy and versatile agent Gd–DOTA–[Amino
Pentyl-Succinic Acid] APSA (Gd–DOTA–APSA), which provides
pattend functional groups for conjugation to selected probes.
These conjugations are preferably carried out via selective chem-
istry 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-DOTA 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 5 dibenzyl 2-bromosuccinate 3 and benzyl (2S)-6-
[(benzoxoxy)carbonyl]amino]-2-bromohexanoate 4.14 The syn-
thetic building blocks 3 and 4, proved to be efficient alternatives to
(unsaturated) reagents, such as alkene or tosylate-derivatives. Building
blocks 3 and 4 were obtained in high yield. They react easily with
the trans-DOTA 8 via Sn2-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 K2CO3 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. Purifi-
cation of 12 performed by RP-chromatography yielded the tri-
fluoracetate 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 during the final basic hydrolysis,
forming a semi-hydrolyzed product precipitation occurred, leading
to incompletion of reaction.

Finally, complexation was performed with GdCl3 6H2O 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 xylanol orange test. The complex was purified by
reverse phase chromatography.

The functional groups (e.g., –NH2, 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 t1.
It is to be noted that a metal chelator with one amine group, DO3A–N–
aminopropanoate (2-aminodota) has already been reported.15 The
straightforward synthesis and the versatility of further conjugation
of our new chelator make this system an excellent multi-
fuctional 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 con-
jugation 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 (r1, 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).

The water residence time was determined from variable-
temperature 17O NMR studies. The temperature dependence of the
reduced 17O transverse relaxation rates (1/T2o) (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 gives a t2 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
compound.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 t2 is increased as compared to
Gd–DOTA (Table 2). Finally, the value of tSo is decreased and similar
to that of Gd–HPDODA.

2.1. Transmetalation

The Gd3+ chelates can be sensitive to transmetalation by end-
dogenous ions, such as Cu2+, Ca2+ and Zn2+. Among the three
metals mentioned, Zn2+ has a high affinity for the Gd complexes.
Fig. 2. Temperature dependence of the reduced $^{17}$O transverse relaxation rate of Gd–DOTA–APSA (26.156 mM).

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gd–DOTA–APSA</th>
<th>Gd–DOTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H^\circ$ [kJ mol$^{-1}$]</td>
<td>52.7±0.23</td>
<td>50.1±0.2</td>
</tr>
<tr>
<td>$S^\circ$ [kJ mol$^{-1}$]</td>
<td>64.1±0.46</td>
<td>48.7±0.2</td>
</tr>
<tr>
<td>$A_\beta$ ($10^9$ rad s$^{-1}$)</td>
<td>$-3.64±0.13$</td>
<td>$-3.42±0.03$</td>
</tr>
<tr>
<td>$B$ [10$^{20}$ s$^{-2}$]</td>
<td>1.79±0.12</td>
<td>1.94±0.09</td>
</tr>
<tr>
<td>$\tau_1$ [ps]</td>
<td>2.7±0.2</td>
<td>11.4±0.5</td>
</tr>
<tr>
<td>$E_\nu$ [kJ mol$^{-1}$]</td>
<td>20.0±3.1</td>
<td>4.0±4.4</td>
</tr>
</tbody>
</table>

$^a$ Ref. 18.

Therefore, this metal ion is able to replace a significant amount of Gd$^{3+}$, which may result in release of the toxic Gd$^{3+}$ aqua ion in the body. To investigate the kinetic stability of Gd–DOTA–APSA, the proton longitudinal relaxation rate of a mixture of Gd$^{3+}$ complex and an equal amount of Zn$^{2+}$ ion in phosphate buffer was as usual monitored at 20 MHz and 37 °C. Upon transmetalation by a diamagnetic Zn$^{2+}$ ion in phosphate buffer, the released Gd$^{3+}$ precipitates as GdPO$_4$, which does not contribute to the relaxivity. Therefore, the overall relaxivity of the solution decreases over time with a rate depending on the rate of transmetalation. This decrease in relaxivity is a good estimation of the kinetic lability of the Gd$^{3+}$ complexes (Fig. 3). For example, Magnevist shows significant decomplexation during a time period of 5 days. On the contrary, Gd–DOTA–APSA is virtually inert towards transmetalation with the Zn$^{2+}$ ion at pH 7.0 for extended periods of time, appearing similar to Gd$^{3+}$ complexes like Gd–DOTA and Gd–HPDOB$^{A,11}$ (Fig. 3). These data show that the replacement of two acetate by aminobutylacetate and succinate arms does not lower significantly neither the thermodynamic nor the kinetic stabilities as compared to Gd–DOTA.$^{19}$

![Graph](image_url)

Fig. 3. Relaxation rate $R_1(t)$ versus time of 2.5 mM Zn$^{2+}$ aqueous solution for Gd–DOTA–APSA (2.5 mM), Gd–DOTA (2.5 mM) and Magnevist (2.5 mM).

3. Conclusion

A new contrast agent was synthesized and characterized. This contrast agent shows promising relaxivity, which could further be optimized by reducing $\tau_m$ and provides versatile functional groups for getting multimodal contrast agent by grafting targets and chromophores. The modular synthetic pathway described can be further implemented in the synthesis of wide variety of specific MRI contrast agents.

4. Experimental section

4.1. General

Cyclen was purchased from Chematech (France) while all chemicals were purchased from Sigma–Aldrich (Belgium), Across (Belgium). All chemicals were used without further purification. All $^1$H and $^{13}$C NMR spectra were recorded on Bruker Avance 500 MHz spectrometer at 25 °C using 5 mm sample tubes. The chemical shifts are given in (δ) parts per million. The pH values of the solution were adjusted using aqueous solution 1 M NaOH and HCl. Mass spectra were obtained on Q-TOF Ultima mass-spectrometer (Micromass, Manchester UK). Samples were dissolved in H$_2$O/MeOH and injected at flow rate 5 μl min$^{-1}$. The cone voltage was 40 V ($T$=90 °C). The compounds $^{2a, 2b, 6, 7}$ and $^8$ were synthesized as reported in Ref. 13. Chiral HPLC was performed on Alliance Waters 2695, ChiralPak® AD-RH column 5 μm (4.6×150 mm), mobile phase: acetonitrile/water (40/60), flow rate: 0.5 ml/min, detection by Waters PDA 486.

4.2. Experimental procedures

4.2.1. Synthesis of (S)-dibenzyl 2-bromosuccinate (3). A solution of $^{2a}$ (9.3 g, 32.1 mol), benzylic alcohol (5 mL, 48.2 mol), N,N-dicyclohexylcarbodiimide (DCC) (10.0 g, 48.5 mol) and 4-dimethylaminoypyridine (DMAP) (catalytic) in CH$_2$Cl$_2$ (25 mL) was stirred at room temperature for 3 h. The precipitated dicyclohexylurea was filtered off and the filtrate was washed with water, dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography to give $^{3}$ (7.29 g) as colourless oil yield 60%. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.35–7.28 (m, 4H, ...
4.2. Synthesis of benzyl (2S)-6-[[benzylxoy] carbonyl[amino]-2-bromohexanoate (4). A solution of compound 2b (1.03 g, 2 mmol), benzyl alcohol (0.33 mL, 3 mmol), N,N'-dicyclohexylcarbodiimide (DCC) (0.678 g, 3.2 mmol) and 4-dimethylaminopyridine (0.03 g, 0.3 mmol) in CH2Cl2 (10 mL) was stirred at room temperature for 3 h. The precipitated dicyclohexylurea was filtered off and the filtrate was washed with 5% acetic acid (10 mL) and water (3×10 mL), dried over anhydrous Na2SO4 and evaporated under vacuum. The crude product was purified by column chromatography to give 4 (0.77 g, 60%) as pale yellow oil.1H NMR (500 MHz): δ: 7.51–7.27 (m, 10H), 5.26 (d, 1H), 5.23 (d, 1H), 3.00 (dd, 4J = 52.03, 51.69, 50.00, 49.86, 46.81, 28.20. HRMS (ESI): calcd for C22H24BrNO4 [M+H]⁺: m/z: 378 [M+H]⁺.

4.2.3. Synthesis of dibenzyl (2R)-2-[4,10-bis(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]succinate (10). A solution of compound 4 (0.465 g, 1.08 mmol) and K2CO3 (0.2 g, 1.4 mmol) in (60 mL) MeOH and the resulting suspension is stirred at room temperature. The reaction mixture was concentrated and washed with Et2O (2×25 mL) and the residue was dried under reduced pressure and yielded 12 quantitatively.1H NMR (500 MHz, D2O) δ: 4.32–4.21 (m, 1H), 4.1–3.42 (m, 8H), 3.09–2.9 (m, 15H), 2.89–2.61 (m, 5H), 1.65–1.52 (m, 4H), 1.38 (s br, 2H).13C (500 MHz, D2O) δ: 176.07, 170.43, 169.23, 160.43, 157.49, 157.04, 156.56, 156.25, 52.41, 51.15, 50.56, 49.84, 46.61, 45.46, 45.37, 44.12, 43.21, 38.68, 38.42, 28.19, 25.94, HRMS (ESI): calcd for C22H46N2O10 [M+H]⁺: 534.2775; found 534.2799.

4.2.4. Synthesis of dibenzyl (2R)-2-[7-[(1R)-5-amino-1-carboxypentyl]-4,10-bis(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]succinate (11). A solution of compound 4 (0.465 g, 1.08 mmol) and K2CO3 (0.2 g, 1.4 mmol) in (60 mL) ACN, after which the mixture is heated under reflux C for 8 h. The solids are filtered off over a pad of Celite and the solvent is removed in vacuo. Chromatographic purification (silica gel, 98/15/0.5 DCM/MeOH/TEA) yielded 55 mg of 9 as pale yellow oil. Yield 56% with 100% optical purity. 1H NMR (500 MHz, CDCl3) δ: 7.48–7.12 (m, 8H), 4.04 (dd, δ = 10.0, 4.0 Hz, 1H), 3.41–3.11 (m, 4H), 2.92–2.42 (m, 14H), 2.49 (dd, δ = 23.6, 9.9 Hz, 4H), 1.46 (s br, 1H); 13C (500 MHz, CDCl3) δ: 171.89, 171.41, 170.91, 170.47, 175.38, 175.73, 128.52, 128.48, 128.33, 128.28, 128.26, 128.17, 127.12, 126.75, 80.88, 66.47, 66.42, 57.13, 56.08, 53.41, 52.03, 51.69, 50.00, 49.86, 46.81, 28.20. HRMS (ESI): calcd for C38H38N6O6 [M+H]⁺: 679.4176; found 679.4150.

4.2.5. Synthesis of (2R)-2-[7-[(1R)-5-amino-1-carboxypentyl]-4,10-bis(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]succinate (12). A solution of compound 4 (0.465 g, 1.08 mmol) and K2CO3 (0.2 g, 1.4 mmol) in (60 mL) MeOH and the resulting suspension is stirred at room temperature. The reaction mixture was concentrated and washed with Et2O (2×25 mL) and the residue was dried under reduced pressure and yielded 12 quantitatively.1H NMR (500 MHz, D2O) δ: 4.32–4.21 (m, 1H), 4.1–3.42 (m, 8H), 3.09–2.9 (m, 15H), 2.89–2.61 (m, 5H), 1.65–1.52 (m, 4H), 1.38 (s br, 2H).13C (500 MHz, D2O) δ: 176.07, 170.43, 169.23, 160.43, 157.49, 157.04, 156.56, 156.25, 52.41, 51.15, 50.56, 49.84, 46.61, 45.46, 45.37, 44.12, 43.21, 38.68, 38.42, 28.19, 25.94, HRMS (ESI): calcd for C22H46N2O10 [M+H]⁺: 534.2775; found 534.2799.

4.2.6. Preparation of Gd3⁺ complex of DOTA-[APSA]. The complex was obtained by adding portion wise GdCl3·6H2O to a solution of 0.1 g, 0.18 mmol) in (5 mL) H2O, maintaining the pH between 6.2 and 6.7 by addition of 1 M NaOH. The final pH of the solution after stirring 52 h was 6.3. The excess of the free lanthanide was removed as Gd(OH)3 precipitate, which appeared at pH 9 after addition of 1 M NaOH. The resulting solution was treated with chlex-100 to remove free Gd3⁺ ions. The absence of free Gd3⁺ ions was confirmed by a xylene orange test. The pH of the supernatant was decreased to 7 and the solution was freeze-dried. The complex was dissolved in water, purified by reverse phase flash chromatography and freeze-dried, yielding 80 mg of Gd–DOTA–APSA as a white powder. HRMS (ESI): calcd for C22H32GdN2O10Na [M+Na⁺]: 707.1569; found 707.1545.

4.2.7. Transmetalation. The stability of the Gd3⁺ complexes was determined by a transmetalation method monitoring the 1H longitudinal relaxation rates of water during 5 days at 37 °C. The measurements were performed on a Bruker Minispec mq-20 spin analyzer at 20 MHz (Bruker, Karlsruhe, Germany) using 7 mm sample tubes containing 2.5 mM of Gd3⁺ complexes and 2.5 mM of ZnCl2 in 300 µl of phosphate buffer solution (26 mM KH2PO4, 41 mM Na2HPO4, pH = 7).

4.2.8. 17O relaxometric measurements. 17O NMR measurements were performed at 11.75 T on 350 µL samples contained in 5 mm o.d. tubes on a Bruker Avance 500 spectrometer (Karlsruhe, Germany). Temperature was regulated by air or nitrogen flow controlled by a Bruker BVT 3200 unit.17O transverse relaxation times of distilled water (pH 6.5–7) were measured using a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 27.5 and 55 µs, respectively. The 17O T2 of water in complex solution was obtained from line width measurements. All spectra were proton decoupled. The data were presented as the reduced transverse relaxation rate (1/T2) of [Gd complex × q × T2] was the molar concentration of the complex, q is the number of coordinated water molecules and T2 is the paramagnetic transverse relaxation rate). The fitting of the experimental data was performed as previously described.10 The sample concentration was determined by ICP-AES on a Jobin Yvon JY 70° instrument (Longjumeau, France) and was further confirmed by 1H relaxometry of a decomplexed sample.

4.2.9. Proton NMRD. Proton nuclear magnetic relaxation dispersion (NMRD) profiles were measured on a Stelar Spinmaster FFC (Mede, Italy) fast field cycling NMR relaxometer over a magnetic field range from 0.24 mT to 1.0 T. Measurements were performed on 0.6 mL samples contained in 10 mm o.d. Pyrex tubes. Additional relaxation rates at 20 and 60 MHz were obtained on a Minispec mq20 and a Minispec mq60, respectively.

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References and notes


