A new bifunctional Gd^{III} complex of enhanced efficacy for MR-molecular imaging applications†

Lorenzo Tei,[a] Giuseppe Gugliotta,[a] Zsolt Baranyai[b] and Mauro Botta*[a]

Received 19th June 2009, Accepted 4th September 2009
First published as an Advance Article on the web 15th September 2009
DOI: 10.1039/b917566k

Increasing the length of the carboxyamide arm of a GdDOTA monoamide (DOTA = 1,4,7,10-tetraaza-1,4,7,10-tetrakis(carboxymethyl)cyclododecane) complex from acetic to propionic accelerates the water exchange rate (k_{ex}) by nearly two orders of magnitude; the ^{1}H relaxivity of the corresponding macromolecular derivatives may then be remarkably enhanced in MRI-based molecular imaging applications, as exemplified in the case of micellar systems.

Magnetic resonance imaging (MRI) represents a particularly important and advantageous modality in the emerging field of molecular imaging, *i.e.* the non-invasive visual representation, characterization and quantification of fundamental biological processes in intact living organisms. The molecular imaging approach offers a great potential for earlier detection and characterization of diseases, and evaluation of treatment.[1] Any molecular imaging procedure requires an imaging probe that is specific for a given molecular event. For this purpose, in recent decades the improvement of the capability of Gd-based contrast agents to target certain organs and tissues at molecular level has been pursued by different approaches. A possible strategy is the synthesis of the chelating unit linked to a suitable function through which the Gd^{III} complex can be covalently bound to the specific biological carrier leading to the so-called bifunctional chelates (BFCs).[2–4] A large group of BFCs reported so far in the literature are based on DOTA monoamide (DOTAMA) derivatives for several reasons: they are relatively easy to synthesise, they can be prepared starting from the commercially available DOTA(O*t*Bu)$_3$ precursor and they form neutral and both thermodynamically and kinetically stable complexes with Gd^{3+} cations.[3,4]

Due to the low sensitivity of the MRI technique, amplification procedures have been designed both to increase the payloads of active paramagnetic centres and to obtain high molar relaxivity per Gd in order to allow the visualization at molecular level. Among the various approaches followed is the formation of multimeric Gd^{III} complexes for cellular labelling,[5] the covalent attachment of the Gd^{III} chelate to biopolymers for binding to activated human platelets[6] or for contrast-enhanced MRI-guided photodynamic cancer therapy,[7] to dendrimers as blood pool agents,[8] to multivalent glycoconjugates for selective binding to lectins,[9] to enzymes for responsive on-off activation,[10] and to virus capsids,[11] liposomes[12] or silica or TiO_2 nanoparticles[13] in order to simultaneously incorporate targeting and/or gene delivery functions. All these systems are based on monoaquo Gd–DOTAMA derivatives and the observed relaxivities, r_{1p}, are typically lower than 20 mM^{-1} s^{-1} (20–60 MHz range and 298 K) when, for rotationally rigid macromolecular conjugates, relaxivities well above 50 mM^{-1} s^{-1} are expected according to the theory of paramagnetic relaxation.[14] The reason stems from the slow rate of exchange, k_{ex}, of the coordinated water molecule in these neutral monoamide derivatives which, at 298 K, is of the order of 1×10^6 s^{-1}, three to four times slower than for the anionic parent complex $[Gd(DOTA)(H_2O)]^-$.[15] The relaxivity of slowly tumbling systems is dominated by the inner sphere term, given by the following equation:

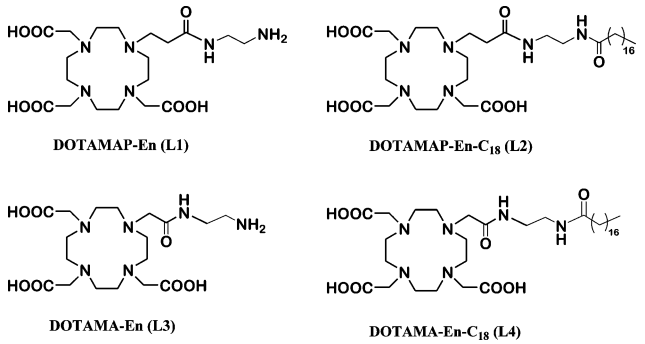
$$R_{1p}^{is} = p_M/(T_{1M} + \tau_M)$$

where p_M is the molar fraction of the bound water molecule, $\tau_M = 1/k_{ex}$ its mean residence lifetime on the coordination site and T_{1M} the longitudinal relaxation time of the bound water protons. When the rotational dynamics of the paramagnetic system is slowed down T_{1M} decreases and it may become comparable to or shorter than τ_M (intermediate/slow exchange regime) and the relaxivity levels off. To avoid this limiting effect on the relaxivity k_{ex} should assume a value around 5×10^7 s^{-1}, *i.e.* nearly two orders of magnitude higher.[14]

In recent years, a number of DTPA (DTPA = diethylenetriamine pentaacetic acid) and DOTA derivatives have been prepared with the objective of obtaining Gd^{III} complexes characterized by a fast rate of water exchange and suitable to be conjugated to macromolecular substrates.[16,17] The most common strategy employed is based on the induction of a steric compression near the water coordination site which results in a significant acceleration of the dissociatively activated exchange process for the monoaquo, nine-coordinate Gd^{III} complexes. In DOTA-like complexes this can be achieved by the insertion of an additional methylenic group in one of the acetic arm of the ligand, which induces an increase of k_{ex} by an order of magnitude.[17] However, the DOTA-propionate derivative has never been conjugated to biological carriers or macromolecules, possibly because a triprotected analogue of DOTA(O*t*Bu)$_3$ was not prepared or because the propionic arm is prone to elimination during the acid activation. Therefore, in order to obtain an easily conjugable, stable Gd^{III} complex with fast water exchange rate we synthesised a Gd–DOTAMA derivative with the amide pendant arm length increased from acetic to propionic in which the functional group for conjugation is a primary amine (**L1**, Scheme 1). In addition, in order to provide the complex with

[a]*Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Viale T. Michel 11, I-15121, Alessandria, Italy. E-mail: mauro.botta@mfn.unipmn.it*
[b]*Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010, Debrecen, P.O. Box 21, Hungary*

† Electronic supplementary information (ESI) available: Synthesis and characterization of ligands **L1**, **L2**, **L3** and **L4**; ^{1}H relaxation rates as a function of temperature for the corresponding Gd complexes at 30 MHz. See DOI: 10.1039/b917566k



Scheme 1 Ligands discussed in this paper.

the possibility of forming large aggregates (micelles) a derivative bearing a stearoyl chain was also synthesized.

The synthesis was accomplished *via* Michael addition of methylacrylate onto DO3A(O*t*Bu)$_3$ followed by aminolysis in ethylenediamine. This ligand was further functionalized with a long C_{18} alkyl chain by reaction with stearoyl chloride in CH_2Cl_2. The deprotected ligands **L1** and **L2** were obtained as analytically pure white solids by precipitation with diethyl ether after hydrolysis of the *t*-butyl esters with TFA (ESI). In order to compare the Gd complexes of propionamide-containing ligands **L1** and **L2** with the corresponding acetamide derivatives we also prepared DOTAMA-En (**L3**)[3] and DOTAMA-En-C_{18} (**L4**). Although the substitution of an acetic by a propionic group might be accompanied by unwanted reduction of the thermodynamic stability,[17] the stability constants of Gd**L1** and Gd**L3** ($\log K_{GdL1} = 20.22$, $\log K_{GdL3} = 20.86$)[18] are quite similar, indicating that the new Gd complex has a sufficiently good thermodynamic stability for biomedical applications. In addition, also the kinetic stabilities are not expected to differ markedly owing to the very similar chemical structure of the complexes.

The water exchange rates and the magnetic field dependence of the relaxivity were obtained by measuring the transverse ^{17}O relaxation rates, R_2, of a solution of the gadolinium complexes as a function of temperature and the proton $1/T_1$ NMRD profiles of Gd**L1** and Gd**L3** (Fig. 1). The strict similarity in the shape and amplitude of the NMRD profiles is a clear indication that both complexes are characterised by the same number ($q = 1$) of inner sphere water molecules. The ^{17}O NMR data show clearly an opposite behaviour for the complexes: R_2 increases with temperature in the case of the slow-exchanging Gd**L3** whereas the occurrence of a fast exchange of the coordinated water molecule in the case of Gd**L1** is unambiguously indicated by

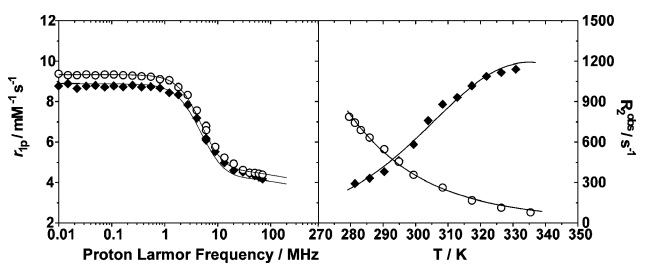


Fig. 1 Left: $1/T_1$ NMRD profiles at 298 K for Gd**L1** (open circles) and Gd**L3** (diamonds). Right: Temperature dependence of the paramagnetic contribution to the water ^{17}O NMR (9.4 T) transverse relaxation rate R_2 for 35 mM solutions of Gd**L1** (open circles) and Gd**L3** (diamonds).

Table 1 Selected parameters obtained from the analysis of the $1/T_1$ NMRD profiles (298 K) and ^{17}O NMR (9.4 T) data for Gd**L1–L4**

Parameter	Gd**L1**	Gd**L2**	Gd**L3**	Gd**L4**
$^{298}r_{1p}$ [a]/mM^{-1} s^{-1}	4.9	24.2	4.6	15.0
$^{298}k_{ex}/\times10^6$ s^{-1}	81.2 ± 1.1	81.2[b]	1.1 ± 0.2	1.1[b]
ΔH_M/kJ mol^{-1}	29.7 ± 1.8	—	34.0 ± 1.8	—
$\Delta^2/\times10^{19}$ s^{-2}	3.9 ± 0.2	0.70 ± 0.03	3.8 ± 0.2	0.65 ± 0.04
$^{298}\tau_V$/ps	15 ± 3	52 ± 4	11 ± 3	31 ± 3
$^{298}\tau_{Rg}$/ps	79 ± 3	2696 ± 213	79 ± 2	2680 ± 232
$^{298}\tau_{RI}$/ns	—	271 ± 14	—	270 ± 28
S^2	—	0.21 ± 0.02	—	0.22 ± 0.03

[a] 20 MHz. [b] Fixed during the fitting.

the exponential decrease of R_2 as a function of temperature.[14,15] Similar information is provided by the temperature dependence of the proton relaxivity (see ESI†). Analysis of the data (Table 1) provides the following values of k_{ex}: 1.1 (±0.2) × 10^6 and 8.1 (±1.1) × 10^7 s^{-1} for Gd**L3** and Gd**L1**, respectively (298 K). In spite of the 80-fold difference in the exchange rate the NMRD profiles differ only slightly, as a consequence of the small size and thus fast rotation of both complexes. The marked influence of k_{ex} on r_{1p} is well evidenced in the case of slowly tumbling systems, as for Gd**L2** and Gd**L4** that aggregate to form micelles at very low concentration (below 0.1 mM). The proton $1/T_1$ NMRD profiles as measured at 278, 298 and 310 K in the frequency range 1 to 80 MHz are reported in Fig. 2.

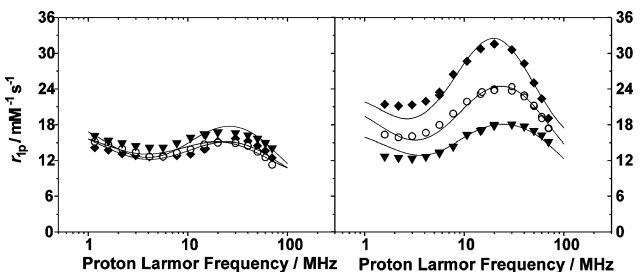


Fig. 2 $1/T_1$ NMRD profiles for Gd**L4** (left) and Gd**L2** (right) at 278 K (diamonds), 298 K (open circles) and 310 K (down triangles).

Over the entire frequency range the relaxivity of Gd**L2** is markedly higher than that of Gd**L4**, with differences that at 20 MHz span from +130% at 278 K to +22% at 310 K. Moreover, it is worth noting the opposite temperature dependence of the NMRD profiles: for Gd**L2** the highest r_{1p} values are observed at 278 K, whereas for Gd**L4** r_{1p} increases with temperature. The data were analyzed using the Lipari–Szabo approach that accounts for the presence of a localized motion (described by the parameter τ_{RI}) superimposed to the overall rotation (described by the parameter τ_{Rg}) of the system. The degree of correlation between the two types of motion is given by the order parameter S^2.[19] The best fit parameters, reported in Table 1, indicate a strictly similar rotational dynamics of the two micellar systems: a large degree of flexibility due to a fast rotation of the metal chelate about the long aliphatic chain superimposed on a slow reorientation of the micelle. The large difference in the relaxivity of the aggregated complexes is entirely attributable to the large difference of the water exchange rate. Although the effect tends to attenuate at high temperatures (Fig. S2, ESI†) for these (and similar) rotationally flexible systems, it is quite remarkable and

expected to produce significant relaxation enhancement in MRI applications of molecular imaging. In fact, at the physiological temperature of 310 K a relaxivity enhancement of more than 20% at 0.5 T and ~10% at 1.5 T are observed.

In conclusion, Gd**L1** shows an optimal high value of the water exchange rate with respect to the corresponding monoacetoamide DOTA derivative Gd**L3**, without compromising the good thermodynamic stability. The presence of a pendant amino group allows an easy conjugation of this new probe to a variety of macromolecular platforms that are expected to show a marked enhancement of the relaxivity, no longer limited by a slow k_{ex}. The conjugation to biological vectors or macromolecular substrates may be carried out using either the *t*-butyl protected ligand precursor of **L1** (ESI†) or the complex Gd**L1**. Finally, as several lipophilic Gd(III) chelates have been used in MR-molecular imaging studies, including the insertion into liposomes[12] and the interaction with lipoproteins for targeting tumour cells,[20] Gd**L2**, with its improved relaxometric properties, may represent a good imaging probe for such diagnostic protocols.

We thank Regione Piemonte (Converging Technologies 2007–NanoIGT project) and Hungarian Science Foundation (Z.B., OTKA K-69098) for financial support. This work was performed within the frame of EU COST Action D-38.

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