

# Efficient relaxivity enhancement in dendritic gadolinium complexes: effective motional coupling in medium molecular weight conjugates†

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Enhancement of the relaxivities of Gd-based MRI contrast agents is achieved by placing the metal ion at the barycentre of the molecular complex in order to improve motional coupling.

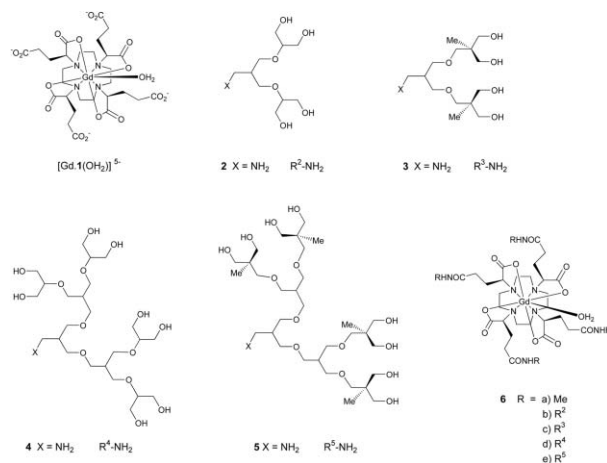
A key objective in MRI contrast agent research is to devise covalently-linked or non-covalently bound conjugates that efficiently catalyse the rate of relaxation of the bulk water proton signal.<sup>1</sup> It is generally appreciated that in order to devise such a high relaxivity MRI contrast agent based on gadolinium, it is necessary to couple effectively the local motion of the Gd–OH<sub>2</sub> vector with the rotational motion of the whole complex, whilst maintaining fast water exchange on and off the Gd ion.<sup>2</sup> Theoretical predictions suggest that maximal relaxivity enhancements will occur when the water exchange rate is in the range 10 to 50 × 10<sup>6</sup> s<sup>−1</sup> (310 K) and when the rotational correlation time,  $\tau_r$ , defining the motion of the Gd–OH<sub>2</sub> vector, is of the order of a few nanoseconds.<sup>3</sup>

Previous attempts to devise such systems have met with limited success. The most useful relaxivity enhancements have been reported in non-covalently bound systems,<sup>4</sup> such as those based on binding of the Gd complex to serum albumin (MW ~ 67 kDa  $\tau_r$  ~ 10 ns). Under physiological conditions, the protein-bound complex of Epix's MS-325 system possesses a limiting relaxivity of the order of 25 mM<sup>−1</sup> s<sup>−1</sup> (65 MHz, 310 K, 0.1 mM complex) at the current clinically used imaging field.<sup>5</sup> Every attempt to devise a high relaxivity covalently-linked high MW conjugate has failed either because of inefficient motional coupling or a slow water exchange rate that quenches the theoretical relaxivity gain. For example, addition of linear polymeric chains (e.g. poly(ethylene oxide) (PEO)) to Gd complexes gives virtually no increase in relaxivity as the rapid segmental motion of a given chain precludes any concerted macromolecular tumbling.<sup>6</sup> Similarly, various Gd complexes have been covalently attached to putative biocompatible polymers (e.g. polylysine, polyethyleneimine) or around the periphery of a spherical object such as a higher order dendrimer, but in each case relaxivity gains were either limited by a slow water exchange rate or by independent motion of the Gd complex moiety, with respect to the overall tumbling motion of the macromolecule.<sup>1,2</sup>

Our approach was based upon the premise that when a Gd ion lies at the barycentre of a macromolecular structure, it will lie upon the axis of any re-orientational motion. A simple example of a

spherical complex possessing an overall density maximum at the centre, is provided by a hydrophilic dendritic structure,<sup>7</sup> packed with second sphere water molecules, with the Gd ion at the focal point. Globular dendrimers based on polyethers,<sup>8</sup> PAMAMS<sup>9</sup> and hyper-branched polymeric analogues<sup>10</sup> conform to this model and possess a maximal intrinsic viscosity at intermediate MW, i.e. in the range 3 to 6 kDa.

The RRRR (or SSSS) [GdDOTA(H<sub>2</sub>O)]<sup>5−</sup> complex, [Gd·1(OH<sub>2</sub>)]<sup>5−</sup> possesses a fast water exchange rate (15 × 10<sup>6</sup> s<sup>−1</sup>, 298 K) and therefore serves as a useful core for dendrimer synthesis by functionalising the C<sub>4</sub>-related peripheral carboxylate groups.<sup>11</sup> By analogy with Frechet's development of hydrophilic PEO dendrimer analogues,<sup>12</sup> we set out to link [Gd·1(OH<sub>2</sub>)]<sup>5−</sup> to the new amino-substituted dendrons 2–5, thereby generating a set of four gadolinium complexes of intermediate molecular weight i.e. 1804, 3100, 2028 and 3548. Complexes formed by amide bond formation of 5 with [Gd·1(OH<sub>2</sub>)]<sup>5−</sup>, possess 32 more methylene groups than the analogue derived from 4, and the difference between tetraamide complexes derived from 2 vs. 3 is 16 methylene groups.



The amines 2–5 were prepared from the reported<sup>12</sup> ketal or *O*-benzyl protected alcohols either *via* a Mitsunobu reaction followed by ketal (H<sub>2</sub>O/dioxan/0.1 M HCl) or *O*-benzyl (Pd(OH)<sub>2</sub>/C : H<sub>2</sub>O–dioxan) deprotection, or *via* the derived azide followed by hydrogenation. The azides were prepared *via* standard mesylation (MsCl/Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>) and azide formation steps (DMF/NaN<sub>3</sub>).† The formation of the tetraamide complexes was undertaken using standard coupling methods, e.g. EDC/HOBt/H<sub>2</sub>O–THF for linking 3 and 5 and HBTU/DMF for linking 2 and 4. Complexes were purified using gel permeation chromatography

† Electronic supplementary information (ESI) available: representative syntheses are given in the ESI, which also details complex characterisation, selected VT <sup>17</sup>O NMR analyses and certain NMRD profiles. See <http://www.rsc.org/suppdata/cc/b413536a/>

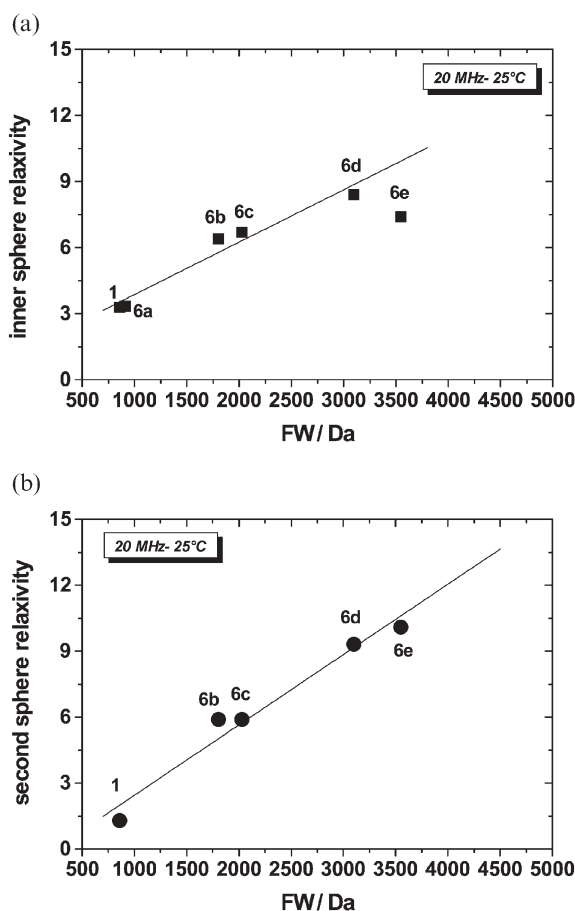
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**Table 1** Relaxivities (298 K, 20 MHz) and relaxation times derived by analysis of VT  $^{17}\text{O}$  NMR analyses and by the fitting of NMRD profiles at 288, 298 and 310 K

	[Gd·1(H <sub>2</sub> O)]	6a	6b	6c	6d	6e
<i>FW</i>	860	912	1804	2028	3100	3548
$r_{1P}/\text{mM}^{-1}\text{s}^{-1a}$	7.1	5.8	13.9	15.0	19.6	19.7
$\tau_c/\text{ps}^a$	10.0	10.0	14.9	14.3	15.8	14.9
$\tau_r/\text{ps}$	94	100	220	240	330	357
$\Delta^2/10^{19}\text{s}^{-2a}$	3.1	5.6	4.3	4.2	4.4	4.3
$\tau_m/\text{ns}^a$	68	42	45	88	85	570
<i>q</i>	1	1	1	1	1	1
$r/\text{\AA}$	3.00	3.00	3.00	3.00	3.00	3.00
$q'^b$	2	—	4	4	8	8
$r'/\text{\AA}^c$	3.80	—	3.80	3.80	4.00	4.00

<sup>a</sup>  $r_{1P}$  is the paramagnetic relaxivity,  $\tau_m$  the water exchange lifetime,  $\tau_c$  is the correlation time of the modulation of the transient zero-field splitting, expressed by the square of its trace value ( $\Delta^2$ ). <sup>b</sup> Number of second sphere water molecules. <sup>c</sup> Mean Gd–H(water) distance of the second sphere waters.

and characterised by negative ion ESMS (ESI<sup>+</sup>) or MALDI-TOFMS (matrix: trihydroxyacetophenone/ammonium citrate). The percentage Gd in sample solutions was verified using atomic emission spectroscopy (ICP-OES), using analytically pure  $\text{H}_3\text{O}^+[\text{Gd}(\text{OH}_2)] \cdot 2\text{H}_2\text{O}$  as the standard.



**Fig. 1** Variation with molecular weight of the inner sphere (upper) and second sphere (lower) contributions to the overall relaxivity of gadolinium complexes (298 K, 20 MHz).

The rate of water exchange at the Gd centre was measured by a VT  $^{17}\text{O}$  NMR study, measuring the rate of transverse relaxation of each complex at 2.1 or 11.7 T. Complex proton relaxivities (NMRD profiles) were determined at 288, 298 and 310 K over the field range 0.001 to 20 MHz, with additional measurements at 60, 65 and 200 MHz. Data were treated using standard fitting procedures and 'best-fit' analyses are reported in Table 1, with results compared to the methylamide parent **6a**, MW 912, for which  $\tau_m = 42$  ns (298 K), and  $r_{1P} = 5.8 \text{ mM}^{-1} \text{ s}^{-1}$  (298 K, 20 MHz). For those cases where the inner sphere water exchange rate remains fast, i.e. **6a–6d**, the inner sphere contribution scales with the increase in molecular volume (Fig. 1: upper). The less hydrophilic complex **6e** with the largest number of methyl and methylene groups, gave a relaxivity that was limited by a slow water exchange rate,  $\tau_m = 0.57 \mu\text{s}$  (298 K). Indeed the observed relaxivity was dominated by a large second sphere contribution that continues to scale with molecular volume (Fig. 1: lower). The linearity of relaxivity vs. MW (molecular volume) for those complexes maintaining a fast inner sphere water exchange rate, coupled with the bonus of a rising second sphere contribution that may be independent of the inner sphere exchange dynamics, suggests that such hydrophilic dendritic conjugates of intermediate MW may afford an efficient and pragmatic means to devise relatively high relaxivity contrast agents in the field range 1.5 to 3 T, that is used in 21<sup>st</sup> Century MRI scanners.

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## Notes and references

- P. Caravan, J. J. Ellison, T. J. McMurphy and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293; R. B. Lauffer, *Chem. Rev.*, 1987, **87**, 901.
- The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. A. E. Merbach and E. Toth, Wiley, Chichester, 2001.
- S. Aime, M. Botta, M. Fasano and E. Terreno, *Chem. Soc. Rev.*, 1998, **27**, 19.
- S. Aime, A. Barge, M. Botta and E. Terreno, in *Metal Ions in Biological Systems*, ed. H. Sigel and A. Sigel, Marcel-Dekker, New York, 2003, vol. 40, pp. 643–682.
- P. Caravan, N. J. Cloutier, M. T. Greenfield, S. A. McDermid, S. U. Dunham, J. W. M. Bulte, J. C. Amedeo, R. J. Looby, R. M. Supkowski, W. D. Horrocks, T. J. McMurphy and R. B. Lauffer, *J. Am. Chem. Soc.*, 2002, **124**, 3152.
- D. M. J. Doble, M. Botta, J. Wang, S. Aime, A. Barge and K. N. Raymond, *J. Am. Chem. Soc.*, 2001, **123**, 10758.
- R. L. Lescanec and M. Muthukumar, *Macromolecules*, 1990, **23**, 2280.
- T. H. Mourey, S. R. Turner, M. Rubinstein, J. M. J. Frechet, C. J. Hawker and K. L. Wooley, *Macromolecules*, 1992, **25**, 2401; C. J. Hawker, K. L. Wooley and J. M. J. Frechet, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1287.
- D. A. Tomalia, *Adv. Mater.*, 1994, **6**, 529.
- L. J. Hobson and W. J. Feast, *Chem. Commun.*, 1997, 2067; L. J. Hobson and W. J. Feast, *Polymer*, 1999, **40**, 1279.
- (a) M. Woods, S. Aime, M. Botta, J. A. K. Howard, J. M. Moloney, M. Navet, D. Parker, M. Port and O. Rousseaux, *J. Am. Chem. Soc.*, 2000, **122**, 9781; (b) Guerbet SA have developed innovative blood-pool MRI contrast agents based on a stereoisomeric mixture of

$[\text{Gd} \cdot \mathbf{1}(\text{OH}_2)]^{5-}$ , with relaxivities of  $24.7 \text{ mM}^{-1} \text{ s}^{-1}$  (P760, MW 5.6 kDa, 20 MHz, 310 K) and  $29 \text{ mM}^{-1} \text{ s}^{-1}$  (P792, MW 6.47 kDa, 60 MHz, 37 °C), limited by sub-optimal water exchange rates: L. Van der Elst, M. Port, I. Raynal, C. Simonot and R. N. Muller, *Eur. J. Inorg. Chem.*, 2003, 2495; M. Port, C. Corot, O. Rousseaux, I. Raynal, L. Devoldere,

- J. M. Idee, A. Bencansse, S. Le Greneur, C. Simonot and D. Meyer, *Magn. Reson. Mater. Phys. Biol. Med.*, 2001, **12**, 121.  
12 S. M. Grayson, M. Jayaraman and J. M. J. Frechet, *Chem. Commun.*, 1999, 1329; S. M. Grayson and J. M. J. Frechet, *J. Am. Chem. Soc.*, 2000, **122**, 10835.