Glycoconjugates of gadolinium complexes for MRI applications†

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Examples of C-4 symmetric, medium MW conjugates incorporating 12 glucose or galactose groups linked *via* four dendritic wedges to a central Gd complex have been characterised; their enhanced relaxivity is interpreted in terms of effective motional coupling and large contributions from second sphere water molecules.

One of the main challenges in contemporary MRI contrast agent[1,2] design is to develop new systems possessing high intrinsic relaxivity in the 3 Tesla field range that is used in new clinical MRI instruments. This requires the definition of gadolinium complexes that combine a fast inner-sphere water exchange rate with effective coupling between the Gd–water vector and the tumbling motion of the whole complex. Moreover, an enhanced contribution from water molecules in the second coordination sphere could also be highly beneficial. In order for the complex to be renally excreted and cleared from the blood pool quickly after being injected, the complex should also be hydrophilic with a molecular mass less than about 3.5 kD.

One means of enhancing motional coupling is to engineer a complex in which the Gd ion lies at the barycentre of the macromolecular structure, so that it resides upon any axis of reorientational motion.[3] Dendritic systems approaching a spherical shape fall into this category, although other complexes with compact structures[4] and well-defined second-spheres of hydration can also be envisaged, provided that they retain fast water exchange at the Gd centre.[5]

The introduction of several sugar groups into Gd contrast agent structures to enhance their water solubility has been considered previously.[1,2,5] Indeed, structures that incorporate glucose and galactose/mannose moieties have been developed, the latter with the intent either to enhance targeting *in vivo*[6] or to serve as a substrate for a native enzyme.[7] Inspired by the introduction of dendritic wedges in carbohydrate dendrimers, based on the work of Stoddart *et al.*,[8] we have set out to examine the behaviour of glycoconjugates of gadolinium complexes based on (*R*,*R*,*R*,*R*/*S*,*S*,*S*,*S*)-[GdgDOTA(OH_2)]$^{5-}$ ([Gd·**1**(H_2O)]$^{5-}$) (Scheme 1)—a system with an established fast water exchange rate.[9] The starting point for this work has been the established trisaccharide wedge **2**[8] and the glycine (Gly)-spaced analogue **3**.† In **2**, the amine is a relatively poor nucleophile as a consequence of the σ-polarisation effect of three β-oxygens and the sterically crowded environment. The Gly-spaced example **3** is less compact, but is a prototype for a variety of amino-acid spacers, allowing the introduction of additional groups that may serve to define the second sphere of hydration.

Amine **3** was synthesised from tris(hydroxymethyl)aminomethane by reaction with Gly-OBn (DCC, HOBt, DMF), followed by stereoselective glycosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide to give the β-glycoside. Stepwise removal of the ester (catalytic OMe^-/MeOH) and *Z*-groups ($Pd(OH)_2$/C, H_2, THF/EtOH) afforded the amine **3** in 57% overall yield. The coupling reaction of **1** with **2** proved to be rather slow and low yielding. The best results were obtained using HBTU (BF_4^- salt) in DMF in the presence of Et_3N, and the product tetra-amide **4** (MW 3202) was isolated in 26% yield following purification by gel permeation chromatography (GPC) (Biogel-6, H_2O). Analysis of other GPC fractions by MALDI-TOF-MS and ES-MS (7T FT-ICR) revealed the presence of considerable quantities of material containing three dendritic wedges and a lactone derived from the undesired competitive lactonization of a sugar hydroxyl by the fourth carboxyl group of the Gd complex. This reaction did not occur to a significant extent in the coupling reaction of **1** and **3**. The GPC-purified tetra-amide product [GdgDOTA-$Glu_{12}Gly_4(OH_2)$]$^-$ (**5**) (MW 3448) gave precise accurate mass data for the doubly-charged (calc: 1715.072; found 1715.0716) and triply-charged (found: 1143.0428; calc: 1143.036) molecular ions (less water) in its electrospray mass spectrum. For purposes of comparison, the analogue with no terminal pyranose groups, **6** (MW 1276), was also prepared.

The water exchange rate at the Gd centre was measured by a VT ^{17}O NMR study, examining the variation of T_2 with temperature at 2.1 T. Very similar rates were obtained for **4** and **5** (the water exchange lifetime, τ_M = 198 and 221 ns, respectively, at 298 K) although exchange was about twice as fast with **6** (τ_M = 93 ns). Using these values, data obtained from field-dependent relaxivities (NMRD profiles) at 25 °C were analysed. Broadly similar profiles were obtained for **4** and **5**, with relaxivities of 23.5 and 19.6 mM^{-1} s^{-1} (298 K, pH 7, 20 MHz), respectively. The former is a high relaxivity value (per unit mass) for a Gd complex.[1,2]

Fitting of the NMRD profile was undertaken to assess the relative inner and second-sphere contributions to the overall relaxivity (Fig. 1 and ESI†). Values for the rotational correlation time, τ_R, were estimated to be 390, 318 and 173 ns for **4**, **5** and **6**,

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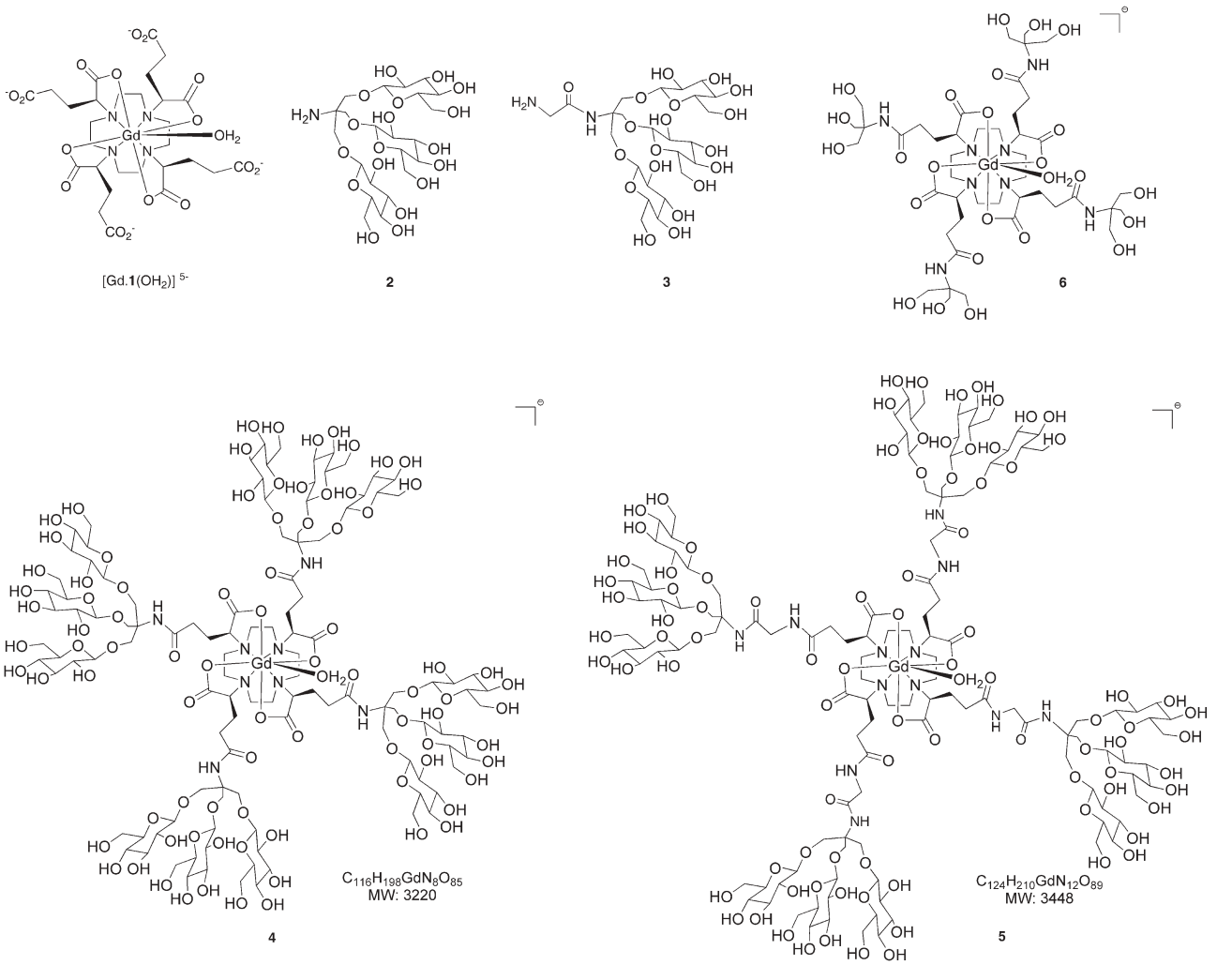
† Electronic supplementary information (ESI) available: The synthesis of **2** and **3**, and characterisation data for the complexes **4** and **5**; the galactosyl analogues have been made by a parallel methodology. Selected VT-^{17}O NMR analyses and NMRD profiles are also given. See DOI: 10.1039/b517997a



Scheme 1

respectively. The lower value for **5** compared to **4**, notwithstanding its higher molecular weight, suggests that the Gly spacer enhances the conformational freedom to such an extent that it begins to inhibit effective motional coupling. The relaxivity may consist of a very significant second sphere contribution, most apparent for complex **4**, over the frequency range 20 to 130 MHz (Fig. 1). Similar behaviour (slightly lower relaxivities) was exhibited by dendritic systems of MW up to 3100 based on [GdgDOTA], which possess branched polyethyleneoxide analogues.[3] The presence of a well-defined network of second-sphere water molecules localised between the glucose groups and the Gd might also be at the origin of the particularly compact solution structure of **4**, consistent with the large value of the reorientational correlation time, τ_R.

The behaviour of **4** as a contrast agent was compared to Pro-Hance in preliminary imaging experiments with mice at 2 T. Complex **4** was administered (0.1 μmol g^{-1}) to BALB-neuT female mice over-expressing the transforming activated rat HER-2/neu oncogene under the control of the mouse mammary tumour virus promoter. Compared to Pro-Hance at the same dose, complex **4** gave rise to a longer-lived signal enhancement, maintaining a 50–60% overall increase over the period 5 to 45 min post-injection. In comparison, the signal enhancement of Pro-Hance had decayed to below 20% by 35 min. Each complex was excreted primarily *via* the renal system, with little evidence of any retention in the liver. A parallel study was undertaken with the galactosyl analogue of **5**, but no significant signal enhancement in the liver was noted, not withstanding the presence of the 12 peripheral galactose groups. Such sugar residues have been introduced into various conjugates that seek liver targeting, based on the recognition of one or multiple β-galactosyl residues by the asialoglycoprotein receptor.[10,11]

This work defines a high relaxivity, medium MW contrast agent that is renally cleared. It opens the way for the synthesis of analogues of **4** and **5** by permutation of the Gly spacer. Introduction of such an α-substituent should enhance the rigidity of the spacing chain as well as offer a way of further increasing the contribution of the second-sphere of hydration, for example, by introducing four proximate charge centres such as carboxylate and amino groups.

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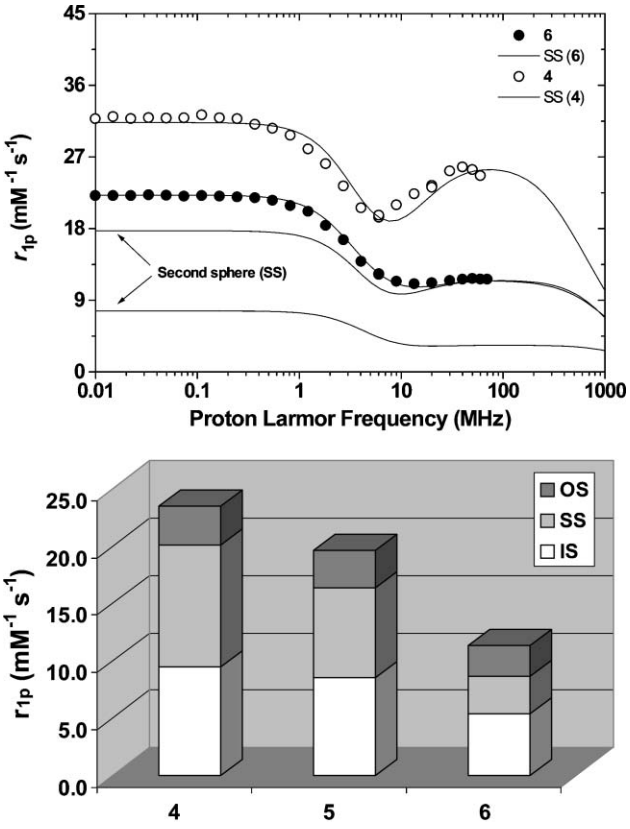

Fig. 1 NMRD profiles for **4** and **6** showing the fit to the experimental data (upper) and highlighting the relative contributions of the second sphere of coordination (see ESI for further details†).

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Notes and references

1 (*a*) P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293; (*b*) D. Parker, R. S. Dickins, H. Puschmann, C. Crossland and J. A. K. Howard, *Chem. Rev.*, 2002, **102**, 1977.

2 *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. A. E. Merbach and E. Toth, Wiley, New York, 2001.

3 D. A. Fulton, M. O'Halloran, D. Parker, K. Senanayake, M. Botta and S. Aime, *Chem. Commun.*, 2005, 474.

4 Here a compact, intramolecularly H-bonded diaqua complex (MW 1576, relaxivity, r_{1p} = 14.3 mM^{-1} s^{-1}, 20 MHz, 298 K) is defined with eight peripheral tris(hydroxymethyl)aminomethane groups on one of its sides: V. L. Pierre, M. Botta and K. N. Raymond, *J. Am. Chem. Soc.*, 2005, **127**, 504.

5 A partially-glycosylated Gd–DTPA–diamide derivative has been reported (slow water exchange will occur in this complex) but no relaxivity data was given: M. Takahaski, Y. Hara, K. Aoshima, H. Kurihara, T. Oshikava and H. Yamashita, *Tetrahedron Lett.*, 2000, **41**, 8485.

6 DOTA–monoamide or DTPA–diamide Gd complexes (slow water exchange quenches any putative relaxivity gains) have been attached to up to four peripheral galactose moieties via a very flexible and extended thioglycosidic link that does not allow motional coupling: (*a*) J. P. André, C. F. G. C. Geraldes, J. A. Martins, A. E. Merbach, M. I. M. Prata, A. C. Santos, J. J. P. de Lima and E. Toth, *Chem.–Eur. J.*, 2004, **10**, 5804; (*b*) P. Baia, J. P. Andre, C. F. G. C. Geraldes, J. A. Martins, A. E. Merbach and E. Toth, *Eur. J. Inorg. Chem.*, 2005, 2110.

7 M. M. Alauddin, A. Y. Louie, A. Shahinian, T. J. Meade and P. S. Conti, *Nucl. Med. Biol.*, 2003, **30**, 261.

8 P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem.–Eur. J.*, 1996, **2**, 1115.

9 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.

10 (*a*) S. Ishibashi, R. E. Hammer and J. Herz, *J. Biol. Chem.*, 1994, **269**, 27803; (*b*) G. Gregoriadis, *Lancet*, 1981, **2**, 241; (*c*) P. H. Weigel and J. H. N. Yik, *Biochim. Biophys. Acta*, 2002, **1572**, 341.

11 (*a*) Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Lonngren, J. Arnarp, M. Haraldsson and H. Lonn, *J. Biol. Chem.*, 1983, **258**, 199; (*b*) E. A. L. Biessen, H. Broxterman, J. H. van Boom and T. J. van Berkel, *J. Med. Chem.*, 1995, **38**, 1846.

