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Lanthanide(III) chelates of DTPA-bisamide glycoconjugates: potential imaging agents targeted for the asyaloglycoprotein receptor

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Abstract

The synthesis and characterization of a new class of DTPA bisamide-linked glycoconjugates of different sugars (lactose, Lac, and galactose, Gal) and valencies (di and tetra) and their Ln(III) complexes is reported. The ^{1}H NMR spectra of the Sm(III) and Eu(III) complexes of $DTPAGal_2$, $DTPAGal_4$, and $DTPALac_2$ obtained between 7 and 80 ºC, indicate that most (if not all) of the four possible diastereoisomeric pairs of structures, resulting from the chirality of the three bound DTPA nitrogen atoms, are present in solution, with different relative populations. The dynamic effects of racemization of the central nitrogen on the NMR spectra show that this process is in fast exchange at 60 ºC for the Sm(III) complexes and in slow exchange at 7 ºC for the Eu(III) complexes. The *in vitro* r_1 nuclear magnetic relaxation dispersion (NMRD) of the water protons of the Gd(III)-DTPA bis-amide glycoconjugate containing two lactosyl moieties, Gd(III)-$DTPALac_2$, was studied, yielding the molecular parameters that govern its relaxivity. Its r_1 value, at 25 ^{o}C and 20 MHz, is 13% higher than that reported for Gd(III) chelates of lower molecular weight DTPA-bisamides, such as DTPA-BMA, consistent with a five times longer τ_R value. The water exchange rate, k_{ex}, and the electron spin relaxation parameters of the Gd(III)-$DTPALac_2$ complex are within the usual range for similar Gd(III)-DTPA bisamide chelates.

Introduction

Magnetic resonance imaging (MRI) is one of the fastest growing techniques in medical diagnosis, due to the excellent spacial resolution and contrast, in particular for soft tissues. The image contrast is based mainly on the differences of water proton longitudinal ($1/T_1$) and transversal ($1/T_2$) relaxation rates in different tissues. This contrast can be enhanced with the administration, prior to the scan, of paramagnetic contrast agents (CAs), usually Gd(III) ($4f^7$) complexes,[1,2] which accelerate the proton relaxation processes in the surrounding water through dipolar interactions between the unpaired electron spin of the metal ion and the proton nuclei of the water molecules. The Gd(III) chelate of DTPA (DTPA = 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecan-1,11-dioic acid)), $[Gd(DTPA)(H_2O)]^{2-}$, was the first contrast agent (CA) used for human MRI, under the name of Magnevist®.[3] Its success stimulated further studies on modifications of its structure, leading amongst others to the neutral derivative $[Gd(DTPA\text{-}BMA)(H_2O)]$ (Omniscan®) (DTPA-BMA = diethylenetriamine-pentaacetic acid-N,N"-bismethylamide), BMA= bismethylamide), in which two carboxylates were converted into amide functions. [4] These, as well as other hydrophilic linear or macrocyclic Gd(III) chelates of similar dimensions and simplicity, once injected, rapidly diffuse from the intravascular space into the interstitial space, but do not enter the intracellular space. Their rapid renal elimination produces a rapid decrease in tissue Gd concentration. [5]

Although much used, eg. in neuro-pathological conditions, which are often associated with disruption of the blood brain barrier (BBB) or altered capillary permeability, these extracellular fluid (ECF) CAs also have inherent disadvantages due to their lack of biospecificity, low relaxivities and little uptake elsewhere in the body. Their rapid diffusion from the vasculature limits their uses as blood pool agents, *eg*. in estimates of blood flow and perfusion. For such applications, several formulations of DTPA conjugates have been tested, both through covalent binding of the Gd(III) chelate to suitable macromolecules (albumin,[6] dextran [7], polylysine [8], dendrimers [9]) or non-covalent binding to HSA (human serum albumin) [10], *eg*. of the Gd-

DTPA derivative MS-325.[11] The search for new ligands with high tissue and/or organ specificity started with hepatobiliary CAs, eg. Gd(III) chelates which are hepatocyte-specific and are excreted through the hepatobiliary system. Amongst these are Gd(III) chelates of DTPA-derived ligands bearing various lipophilic substituent groups which promote specific carrier-mediated uptake into the hepatocytes, such as benzyloxymethyl in $[Gd(BOPTA)(H_2O)]^{2-}$ (BOPTA = 4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11 -triazatridecan-13-oic acid) [12] and ethoxybenzyl in $[Gd(EOB\text{-}DPTA)(H_2O)]^{2-}$ (EOB-DTPA = (S)-N-[2-[bis(carboxymethyl)amino]-3-(4-ethoxyphenyl)propyl]-N-[2-[bis(carboxy methyl)amino]ethyl]glycine).[13] Liposomes can also be efficient carriers to deliver Gd(III)-based CAs to the liver and spleen and hence enhance their MRI image contrast, *eg.* the amphiphilic Gd(DTPA)-stearylamide was incorporated into the phospholipid lamella of egg lecitin and cholesterol liposomes.[14]

The hepatocyte cells on liver express a tissue specific lectin (hepatic lectin) that recognizes terminal β-galactosyl residues on desialylated glycoproteins - the asialoglycoprotein receptor (ASGPR).[15] Liver targeting has been successfully achieved through conjugation of pharmaceutical agents to galactose/lactose.[16] Moreover, a multivalence effect has been demonstrated on the liver uptake of glycoconjugates (tetra>tri>di>mono).[17] Several agents, relying on macromolecular bioconjugates and on polymer scaffolds, have been described for hepatic imaging through the targeting of the ASGPR.[18-25] These agents are inherently polydisperse and ill characterised which constitutes a drawback.

In a previous paper**,** we reported the synthesis and physico-chemical characterization of a new class of multivalent glycoconjugates, Ln(III) chelates of the tetraazatetracarboxylate chelator DOTA conjugated to glycodendrimeric moieties (DOTA = 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane).[26] Previous to our work, only a class of low molecular weight and well characterised galacto/mannopyranosyl conjugates of DOTA-like chelators has been described for potential MRI and scintigraphic applications.[27] These monovalent Gd(III)-

glycoconjugates are responsive contrast agents activated by galactosidase/mannosidase-mediated hydrolysis.[28] In the present paper we report the synthesis and characterization of (thio)glycoconjugates of the linear chelator DTPA, namely the DTPA bisamides **1** and **2** with different terminal sugar residues: galactose (Gal) nd lactose (Lac), and different valences (2 and 4) (Figure 1). The thioglycosides are galactosidase-resistant ligands, probing a conformational space and displaying biological activities, very similar to that of their *O*-linked natural counterparts, ensuring thus an increased metabolic stability *in vivo*.[29] The physico-chemical characterization of some of their Ln(III) complexes in aqueous solution by ^{1}H NMR and water ^{1}H NMRD studies is also described. The proton relaxivity of the Gd(III) chelates describes the efficiency of the magnetic dipolar coupling between the water proton nuclei and the paramagnetic metal ion, therefore it is a direct measure of the efficacy of the chelate as a CA.

Results and Discussion

Synthesis of the ligands: The synthesis of the DTPA-bisamies **1** and **2** (Figure 1) was undertaken through a well established route consisting on the derivatisation of the commercially available DTPA bisanhydride with amine-functionalised blocks. Two different types of amine-functionalised sugar blocks were prepared: a monovalent block **5** (Scheme1) and a divalent glycodendrimer block **9** (Scheme 2).[26,31] The standard DCC/HOBT coupling procedure revealed successful for preparing the fully protected amino-functionalised sugar blocks. These compounds were deprotected with TFA/CH_2Cl_2 to afford the terminal amines **6** and **10** as their TFA salts.

In order to ensure the formation of the required bisamides, the amino-functionalised blocks **6** and **10** were used in a slight excess, over two molar equivalents in relation to the DTPA bisanhydride block (Scheme 3). The intermediate sugar-protected bisamides were carried through and deprotected with KOMe to give the final compounds **1** and **2** in reasonable yields.

NMR studies of the Ln(III)-glycoconjugates: The ^{1}H NMR spectra of the 1:1 diamagnetic (La(III)) and paramagnetic (Sm(III) and Eu(III)) complexes with the ligands $DTPAGal_2$, $DTPALac_2$ and $DTPAGal_4$ were obtained in D_2O, pH 7.5 as a function of temperature (7, 25, 40, 60 and 80 ºC) (see Figure 2 for some typical spectra). The spectra of the La(III) complexes are identical to those of the corresponding free ligand at the same pH, except for the protons of the DTPA moiety, which, as a result of ion coordination, give more complex resonances: multiplets at 2.92, 2.78, 2.68 and 2.45 ppm for the backbone NCH_2CH_2N protons, and a series of partially overlapping AB patterns in the 3.2-3.5 ppm region for the CH_2 protons of the acetate and amide arms, quite similar to what has been described for $[La(DTPA)]^{2-}$ and other La(DTPA-bisamides). [32-36] For the Sm(III) and Eu(III) complexes, the protons of the sugar moieties and the ones at the bridging arms further away from the DTPA moiety are hardly perturbed by the paramagnetic centre, with very small broadenings and paramagnetic shifts smaller than 0.05 ppm, due to the r^{-6} and r^{-3} dependency, respectively, of the dominant dipolar contributions to the paramagnetic relaxation and shift induced by the Ln(III) ion, where r is the Ln(III)-proton distance in the complex.[36] However, the side chain protons closest to the DTPA amide moiety (eg. CH_2(f) and CH_2(g)) are increasingly shifted and broadened, in particular the CH_2 resonances of the DTPA moiety, although these strongly shifted resonances are difficult to assign. However, their features show some similarities with the corresponding paramagnetic complexes of the parent DTPA and some DTPA-bisamide ligands .[36-42]

For the Sm(III) complexes, eg. Sm(III)-$DTPAGal_2$, the strongly shifted resonances are very broad at 25 ºC but become sharper as temperature increases. At 60 ºC some of these resonances are seen in the diamagnetic region of the spectrum (3.32, 2.22, 2.10, 1.69, 0.68 ppm), but seven distinct strongly shifted resonances appear at the low frequency + 0.1 ppm to –1.4 ppm region (0.06, -0.29, - 0.52, - 0.82, - 0.88, -0.98, - 1.39 ppm) (Fig. 2B). In contrast, in the case of the Eu(III) complexes, eg. Eu(III)-$DTPAGal_2$, the strongly shifted resonances are quite sharp at low temperature (7 ºC, see Fig. 2C): five more intense (33.40, 29.48, 27.41, 22.92, 16.75 ppm)

and five less intense (30.90, 38.05, 26.80, 24.38, 20.53 ppm) resonances are observed in the high frequency +35 ppm to +15 ppm region, about eight in the +10 ppm to + 5 ppm region and about twenty in the – 3 ppm to – 18 ppm region. These resonances broaden completely as the temperature increases to 25 ºC, but at 60 ºC some sharp, but somewhat less shifted resonances start to reappear, eg. at + 6.2, + 3.2, + 2.2, + 0.9, -0.1, -4.5, -8.5 and –10.2 ppm (data not shown). Comparison with ^{1}H NMR spectra of corresponding DTPA complexes [33,39] suggests that the strongly shifted resonances at low frequencies for the Sm(III) complex and at high frequencies for the Eu(III) complex correspond to two CH_2 protons of the DTPA ethylenediamine backbone, which have the largest dipolar induced shifts due to their very large and positive axial geometric factor $G = (3\cos^2\theta - 1)/r^3$,[39] where θ is the angle between the Ln(III)-proton vector and the main symmetry axis of the magnetic susceptibility of the complex. [36]

In Ln(III) complexes of DTPA-bis amides with non-chiral centers in the side chains, all three bound nitrogen atoms are chiral and four diastereoisomeric pairs of enantiomers are possible, leading to a maximum of eight NMR signals for each group of magnetically equivalent protons. [36,40] Two isomerization processes have been described in solution for this type of complexes. While the racemization of the terminal N atoms, which involves decoordination-inversion-coordination of the N atoms and the neighbouring acetate groups, has a high energy barrier, a lower energy process involves racemization of the central nitrogen, via interconversion between the two possible conformations of the ethylene bridges, which results in the magnetic averaging of the two halves of the complex around the central glycinate group of DTPA, reducing by half the number of observed resonances. [36,40] This is the dynamic process observed in the complexes studied here, which is in fast exchange at 60 ºC for the Sm(III) complexes and in slow exchange at 7 ºC for the Eu(III), due to the much larger dipolar shifts induced by Eu(III) relative to Sm(III). The minimum of seven low frequency shifted resonances, observed for the Sm(III) complex at high temperature (Fig. 2B), resulting from two

ethylenic protons, indicates that most (if not all) of the four possible isomers are present in solution, with different relative populations. This is confirmed by the observation that the same two ethylenic protons give a minimum of ten (out of a maximum of sixteen) high frequency shifted resonances in the Eu(III) complex at high temperature (Fig. 2C). The Ln(III) complexes of other DTPA-bis amides, such as DTPA-BPA (BPA = bispropylamide) and DTPA-BENGALAA (BENGALAA = N,N"-bis[N-(aza-D-galacto-5,6,7,8,9-pentahydroxynonyl)carba moylmethyl]amide) [35,41] have shown the presence of the four diastereoisomeric pairs, while for bisamides containing long (C14 to C18) aliphatic chains only two pairs have been detected in solution. [42]

Water Proton Relaxation (NMRD) studies of Gd(III)-DTPALac$_2$: The efficiency of a contrast agent is given by its proton relaxivity, defined as the paramagnetic enhancement of the longitudinal water proton relaxation rate referred to 1 mM concentration (r_1, in s^{-1} mM^{-1}). Proton relaxivity has contributions from interactions of the Gd(III) ion with the inner sphere water protons (inner sphere relaxivity) as well as with the bulk water protons (outer sphere relaxivity). The inner sphere term is determined by the exchange rate of the inner sphere water protons (usually equal to the water exchange rate, k_{ex}), the rotational correlation time of the complex (τ_R), and the longitudinal and transverse electronic relaxation rates of the Gd(III) ($1/T_{1e}$ and $1/T_{2e}$). The outer sphere contribution to the overall proton relaxivity depends on the electron spin relaxation rates and the diffusion coefficient for the diffusion of a water proton away from a Gd(III) chelate (see Appendix). [43]

The water proton longitudinal relaxivity of the Gd(III)-DTPALac$_2$ chelate was measured in aqueous solution at 25 and 60 °C at proton Larmor frequencies between 0.2 and 20 MHz. The NMRD profiles obtained (Figure 3) are typical of low molecular weight Gd(III) chelates. They were fitted to the usual Solomon-Bloembergen-Morgan theory that relates the paramagnetic relaxation rates to the microscopic parameters of the Gd(III) chelates (see equations in Supporting Information). In the analysis of the NMRD profiles we fixed the water exchange

rate and its activation enthalpy to values that were previously determined for similar DTPA-bisamide complexes (k_{ex}^{298} = 0.40×10^6 s^{-1} and $\Delta H^{\ddagger}$ = 40.0 kJ mol^{-1}).[43] The diffusion coefficient and its activation energy were also fixed to common values (D_{GdH}^{298} = 24×10^{-10} m^2 s^{-1}; E_{DGdH} = 20 kJ mol^{-1}), as these two parameters are not much dependent on the nature of low molecular weight complexes.[43,44] Thus, in the analysis of the proton relaxivities we fitted the rotational correlation time, τ_R, its activation energy, E_R, and the parameters describing the electron spin relaxation, i.e. the trace of the square of the transient zero-field-splitting (ZFS) tensor, Δ^2, and the correlation time for the modulation of the ZFS, τ_V. The parameters obtained for the Gd(III)-DTPALac$_2$ conjugate are presented in Table 1 and compared with those available for other relevant small Gd(III) complexes. The fits of the NMRD profiles obtained are shown in Figure 3.

The relaxivity of Gd(III)-DTPALac$_2$ (MW = 1569) at 25 ^{o}C and 20 MHz is 5.72 mM^{-1}s^{-1}, corresponding to an increase of 13 % compared to that for the commercial contrast agent Gd(III)-DTPA-BMA, which is a lower molecular weight DTPA-bisamide complex (MW = 574).[44] This relaxivity difference is consistent with the five-fold increase in the rotational correlation time, as it is τ_R that dominates the high-field NMRD values. Another DTPA-bisamide chelate, the Gd(III)-DTPA-BENGALAA, with an intermediate molecular weight (MW = 963), also has an intermediate τ_R value.[41] The temperature dependence of the NMRD profiles clearly shows that the proton relaxivity of these small molecular weight chelates is limited by fast rotation: proton relaxivities increase when the temperature decreases, thus the rotation slows down. The parameters obtained for the electron spin relaxation of the Gd(III) complex are also within the usual range for similar Gd(III)-DTPA bisamide chelates. Although the simplified model of electron spin relaxation used here is not fully adequate to describe Gd(III) chelates,[45] the application of the novel theories requires EPR data in a large field range which was beyond the scope of the present study.

Experimental Section

Materials and equipment. Chemicals were purchased from Sigma-Aldrich and used without further purification. Solvents used were of reagent grade and purified by usual methods. Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck) on aluminium support. Detection was by examination under UV light (254 nm), by adsorption of iodine vapour and by charring with 10% sulphuric acid in ethanol. Flash chromatography was performed on Kieselgel 60 (Merck, mesh 230-400). The relevant fractions from flash chromatography were pooled and concentrated under reduced pressure, at temperature below 40 ^{o}C. FAB mass spectra (positive mode) were recorded using a VG Autospec mass spectrometer with 3-nitrobenzyl alcohol (*NBA*) as matrix. Electrospray ionization (ESI) mass spectra were obtained for compounds with molecular weight above 2000. ^{1}H NMR (1D and 2D) and ^{13}C NMR spectra were run on a Varian Unity Plus 300 NMR spectrometer, operating at 299.938 MHz and 75.428 MHz, for ^{1}H and ^{13}C, respectively. Chemical shifts (δ) are given in ppm relative to the $CDCl_3$ solvent (^{1}H, δ 7.27; ^{13}C 77.36) as internal standard. For ^{1}H and ^{13}C NMR spectra recorded in D_2O, chemical shifts (δ) are given in ppm, respectively, relative to TSP as internal reference (^{1}H, δ 0.0) and *tert*-butanol as external reference (^{13}C, CH_3 δ 30.29). ^{13}C NMR spectra were proton broad-band decoupled using a GARP-1 modulated decoupling scheme. Assignments of the ^{1}H and ^{13}C NMR spectra were aided by two-dimensional gradient based double quantum filtered shift correlated spectra (DQF-COSY) and heteronuclear multiple quantum correlated spectra (HMQC). The pD of the D_2O solutions was adjusted with DCl or CO_2-free NaOD and converted to pH values using the isotopic correction pH = pD - 0.4. The pD values were measured on a HANNA pH-meter with a HI1310 combined electrode (HANNA instruments, Italy).

The $1/T_1$ nuclear magnetic relaxation dispersion (NMRD) profiles of the water protons at 25 and 60 ^{o}C were obtained on a Spinmaster FFC fast cycling NMR relaxometer (Stelar), covering

a continuum of magnetic fields from $5x10^{-4}$ to 0.47 T (corresponding to a proton Larmor frequency range of 0.022-20 MHz). The Gd(III) concentration was verified by ICP measurement (Perkin-Elmer Instruments, Optim 2000 DV).

Synthesis and characterisation

Synthesis of fully protected hexanediamine-functionalised monovalent thioglycosides **(5a**, and **5b).** Typical procedure illustrated for (**5a**): A solution of peracetylated (galactosylthio)propionic acid **3a**[29a] (0.375 g, 0.859 mmol), 1,6-hexanediamine monoBoc (**4**) (0.169 g, 0.781 mmol) and HBT (1-hydroxybenzotriazol) (0.140 g, 0.940 mmol) in dichloromethane (10 cm^3) was ice-cooled. To this solution was added drop wise a solution DCC (dicyclohexylcarbodiimide) (0.194 g, 9.40 mmol) in dichloromethane (5 cm^3). After 15 minutes the reaction mixture was removed from the ice bath and allowed to reach room temperature. The reaction mixture was further stirred at room temperature overnight. The DCU (dicyclohexylurea) precipitate was removed by filtration and washed with dichloromethane. The filtrate was concentrated under reduced pressure to give tick syrup. This material was taken into ethyl acetate (100 cm^3) and sequentially washed with $KHSO_4$ (aq. Sol. 1 M.; 3 x 50 cm^3), $NaHCO_3$ (sat. sol.; 3 x 50 cm^3) and brine (50 cm^3). The organic phase was concentrated under reduced pressure to give a white foam. Purification by dry flash chromatography (CH_2Cl_2 /MeOH; 100% CH_2Cl_2 -> 50% MeOH) afforded the title compound as a white foam (0.480 g, 97 % yield). ^{1}H, δ (300 MHz, $CDCl_3$) 1.34 (4H, m, $NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2NH$), 1.43 (9H, s, ^{tert}Bu), 1.50 (4H, m, $NHCH_2\mathit{CH_2}(CH_2)_2\mathit{CH_2}CH_2NH$), 1.99, 2.05, 2.06 and 2.16 (12H, s, 4xOAc), 2.50 (2H, m, $SCH_2\mathit{CH_2}$), 2.88-3.06 (2H, m, $S\mathit{CH_2}$), 3.09 (2H, m, $NH(CH_2)_5\mathit{CH_2}NHBoc$), 3.24 (2H, m, $NH\mathit{CH_2}(CH_2)_5NHBoc$), 3.48 (1H, m(*br*)), 3.95 (1H, td, *J*= 7.2, 5.7 and 0.9 Hz, H-5), 4.11 (1H, dd, *J*= 11.2 and 5.7 Hz, H-6a), 4.19 (1H, dd, *J*= 11.2 and 7.2 Hz, H-6b), 4.54 (1H, d, *J*= 9.9 Hz, H-1), 5.04 (1H, dd, *J*= 10.2 and 3.3 Hz, H-3), 5.23 (1H, appt, *J*= 9.9 Hz, H-2), 5.43 (1H, dd, *J*= 3.3 and 0.9 Hz, H-4), 5.98 (1H, t(*br*), N*H*); *HRM*S (FAB$^+$, NBA) *Calc. for* $C_{28}H_{47}N_2O_{12}S$ $(M+H)^+$ 635.2844. Found 635.2856.

(**5b**)**:** Starting from peracetylated (lactosylthio)propionic acid **3b**[29a] (1.70 g, 2.35 mmol) and 1,6-hexanediamine monoBoc (**4**) (0.507 g, 2.35 mmol), the title compound (**5b**) was obtained as a white foam (2.01 g, 93%). ^{1}H, δ (300 MHz, $CDCl_3$) 1.33 (4H, m, $NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2NH$), 1.42 (9H, s, $^{\mathit{tert}}$Bu), 1.70 (4H, m, $NHCH_2\mathit{CH_2}(CH_2)_2\mathit{CH_2}CH_2NH$), 1.97, 2.04, 2.05, 2.07, 2.13 and 2.16 (21H, s, 7xO*Ac*), 2.46 (2H, t, *J*= 6.6 Hz, $SCH_2\mathit{CH_2}$), 2.81 (1H, m, $SC\mathit{H_a}H_b$), 3.03 (1H, m, $SCH_a\mathit{H_b}$), 3.08 (2H, m, $NH(CH_2)_5\mathit{CH_2}NHBoc$), 3.21 (2H, m, $NH\mathit{CH_2}(CH_2)_5NHBoc$), 3.45 (1H, m) 3.59 (1H, m, H-5), 3.78 (1H, appt, *J*= 9.6 Hz, H-4), 3.89 (1H, appt, *J*=7.0 Hz, H-5´), 4.03-4.16 (3H, m), 4.28 (1H, m), 4.52 (1H, d, *J*= 7.8 Hz, H-1´), 4.65 (1H, d, *J*= 10.5 Hz, H-1), 4.90 (1H, appt, *J*= 9.6 Hz, H-2), 4.96 (1H, dd, *J*= 10.2 and 3.3 Hz, H-3´), 5.09 (1H, dd, *J*= 10.5 and 7.8 Hz, H-2´), 5.19 (1H, appt *J*= 9.3 Hz, H-3), 5.34 (1H, d, *J*= 2.4 Hz, H-4´), 6.30 (2H, t(*br*), *NH*C(O)); *m/z* (FAB^+, NBA) 923 $(M+H)^+$, 3); *HRMS* (FAB^+, NBA) *Calc. for* $C_{40}H_{63}N_2O_{20}S$ $(M+H)^+$ 923.3695. Found 923.3683.

Synthesis of fully protected amino-functionalised divalent (9)

A solution of divalent thiogalactoside **7**[26] (0.968 g, 0.895 mmol) was stirred overnight with CH_2Cl_2/TFA (3/1, 10 cm^3). The solvent was removed under reduced pressure to give a light yellow foam, which was redissolved in dichloromethane (DCM) (10 cm^3) and the solvent was removed under reduced pressure. This procedure was repeated several times and the material was further dried under vacuum to give the carboxylic acid deprotected compound (**8**) as a thick light yellow foam. ^{1}H NMR analysis revealed the disappearance of the signal at δ 1.4 assigned to the *tert*-butyl group. No further purification or characterisation was carried on this material. All the material obtained (we assumed a 100 % yield on the deprotection reaction) was dissolved in ice-cooled DCM (10 cm^3) and titrated (pH paper) to pH 9-10 with DIPEA (diisopropylethylamine). To this solution was added a solution of 1,6-hexadiamine monoBoc (**4**) (0.230 g, 1.07 mmol) in dichloromethane (5 cm^3) and HBT (0.140 g, 0.940 mmol). To this solution was added drop wise a solution of DCC (0.230 g, 1.10 mmol) in dichloromethane (5

cm^3). After 15 minutes the reaction mixture was removed from the ice bath and allowed to reach room temperature. The reaction mixture was further stirred at room temperature overnight. The DCU precipitate was removed by filtration and washed with dichloromethane. The filtrate was concentrated under reduced pressure to give tick syrup. This material was taken into ethyl acetate (150 cm^3) and sequentially washed with, $NaHCO_3$ (sat. sol.; 3 x 100 cm^3) and brine (100 cm^3). The organic phase was concentrated under reduced pressure to give a light yellow foam. Purification by dry flash chromatography (CH_2Cl_2 /MeOH; 100% CH_2Cl_2 -> 50% MeOH) afforded the title compound as a white foam (1.01 g, 92 % yield). ^{1}H, δ (300 MHz, $CDCl_3$) 1.32 (4H, m, $NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2NH$), 1.43 (9H, s, $^{\mathit{tert}}$Bu), 1.51 (4H, m, $NHCH_2\mathit{CH_2}(CH_2)_2\mathit{CH_2}CH_2NH$), 1.72 (4H, m, $NCH_2\mathit{CH_2}$) 1.99, 2.05, 2.06 and 2.16 (24H, s, 8xOAc), 2.52 (8H, m, overllaping signals from $SCH_2\mathit{CH_2}$ and $N\mathit{CH_2}$), 2.88-3.14 (8H, m, overllaping signals from $S\mathit{CH_2}$, $N\mathit{CH_2}C(O)$ (singulet at 3.02) and $NH(CH_2)_5\mathit{CH_2}NHBoc$), 3.29 (6H, m, $NCH_2CH_2\mathit{CH_2}$) and $NH\mathit{CH_2}(CH_2)_5NHBoc$), 3.40-3.56 (1H, m), 3.96 (2H, td, *J*= 6.4 and 0.9 Hz, H-5), 4.09 (2H, dd, *J*= 11.4 and 6.3 Hz, H-6a), 4.20 (2H, dd, *J*= 11.2 and 6.4 Hz, H-6b), 4.56 (2H, d, *J*= 10.2, H-1), 4.74 (1H, t(*br*), N*H*), 5.05 (2H, dd, *J*= 10.0 and 3.3 Hz, H-3), 5.25 (2H, appt, *J*= 9.9 Hz, H-2), 5.44 (2H, dd, *J*= 3.3 and 0.9 Hz, H-4), 6.74 (2H, t(*br*), N*H*), 7.10 (2H, t(*br*); *m/z* (FAB^+, NBA) 1223 $(M-H)^+$, 100); *HRMS* (FAB^+, NBA) *Calc. for* $C_{53}H_{86}N_5O_{23}S_2$ $(M+H)^+$ 1224.5155. Found 1224.5119.

Synthesis of DTPA-glycoconjugate bisamides 1 and 2

Typical procedure illustrated for **DTPAGal$_2$ (1a)**: A solution of fully protected amino-functionalised monovalent thiogalactoside **5a** (0.618 g, 0.973 mmol) was stirred overnight with CH_2Cl_2/TFA (3/1, 10 cm^3). The solvent was removed under reduced pressure to give a light yellow foam, which was redissolved in DCM (10 cm^3) and the solvent was removed under reduced pressure. This procedure was repeated several times and the material was further dried under vacuum to give a thick light yellow foam (**6a**). ^{1}H NMR analysis revealed the

disappearance of the signal at δ 1.47 assigned to the *tert*-butyl group. No further purification or characterisation was carried on this material. All the material obtained (we assumed a 100 % yield on the deprotection reaction) was dissolved in ice-cooled DCM (5 cm^3) and titrated (pH paper) to pH 9-10 with DIPEA. This solution was added to a solution of DTPA-bis anhydride (0.158 g, 0.442 mmol) in DMF (40 cm^3) and pyridine (1 cm^3). The reaction mixture was stirred at room temperature overnight and concentrated at reduced pressure to give a colourless oil. This material was carried through without further purification or characterisation. The residue was redissolved in a mixture ethanol (10 cm^3) and KOH (aq. sol. 1M, 10 cm^3) and stirred at room temperature overnight. The reaction mixture was adjusted to pH~1 with Amberlist 15. The resin was transferred into a column, thoroughly washed with water and eluted with aq. NH_3 0.5 M. The relevant fractions were pooled and concentrated under reduced pressure (temperature below 40 ^{0}C) to give the fully deprotected glycoconjugate as a off white solid (0.342 g, 71% yield over two steps). ^{1}H, δ (300 MHz, D_2O, pH 7.0): 4.48 (2H, d, *J* = 9.0 Hz, H-1), 3.54 (2H, app t, *J* = 9.0 Hz, H-2), 3.64 (2H, dd, *J* = 9.0 and 3.3 Hz, H-3), 3.70 (2H, m, H-4), 3.96 (2H, app d, *J* = 3.3 Hz, H-5), 3.73 (4H, m, H-6a + H-6b), 1.32 (8H, m, 2 x $NH(CH_2)_2(CH_2)_2(CH_2)_2NH$), 1.51 (8H, m, 2 x $NHCH_2CH_2(CH_2)_2CH_2CH_2NH$), 3.20 (8H, m, 2 x $NHCH_2(CH_2)_4CH_2NH$), 2.60 (4H, m, SCH_2CH_2), 2.95-3.01 (4H, m, SCH_2), 3.24 (4H, s, DTPA amide $NCH_2C(O)$), 3.76 (2H, s, DTPA central acetate $NCH_2CO_2^-$), 3.40 (4H, s, DTPA terminal acetate $NCH_2CO_2^-$), 3.34, 3.08 (8H, two m, DTPA skeleton NCH_2); ^{13}C, δ (75.6 MHz, D_2O): 86.18 (C-1), 69.67 (C-2), 74.05 (C-3), 79.07 (C-4), 68.94 (C-5), 61.30 (C-6), 25.90, 25.83 ($NH(CH_2)_2(CH_2)_2(CH_2)_2NH$), 28.29, 28.42 ($NHCH_2CH_2(CH_2)_2CH_2CH_2NH$), 39.23, 39.54 ($NHCH_2(CH_2)_4CH_2NH$), 26.49 ($SCH_2$), 36.57 ($SCH_2CH_2$), 58.86 (DTPA amide $NCH_2C(O)$), 54.58 (DTPA central acetate $NCH_2CO_2^-$), 58.95 (DTPA terminal acetate $NCH_2CO_2^-$), 50.59, 52.79 (DTPA skeleton NCH_2), 170.77, 173.02, 174.28, 178.40 (DTPA acetate $NCH_2CO_2^-$ and amide $NCH_2C(O)$, other $CH_2C(O)NH$); *m/z* (FAB^+, NBA) 1091

(M+H$^+$, 23); *HRMS* (FAB$^+$, NBA) *Calc. for* $C_{44}H_{80}N_7O_{20}S_2$ $(M+H)^+$ 1090.4899. Found 1090. 4859.

DTPALac$_2$ - (**1b**): Fully protected monovalent thiolactoside (**5b**) (0.997 g, 1.08 mmol) was deprotected as described for compound (**1a**). Reaction with DTPA-bis anhydride (0.183 g, 0.512 mmol), followed by deprotection and purification afforded glycoconjugate **1b** as a off white solid (0.345 g, 48% yield over two steps). ^{1}H, δ (300 MHz, D_2O, pH 7.0): 4.46 (2H, d, *J* = 7.8 Hz, H-1), 3.54 (2H, dd, *J* = 10.2 and 7.8 Hz, H-2), 3.63 (2H, dd, *J* = 10.2 and 3.3 Hz, H-3), 3.66 (2H, dd, H-4), 3.94 (2H, app d, *J* = 3.3 Hz, H-5), 3.70 (4H, m, H-6a + H-6b), 4.58 (2H, d, *J*= 10.2 Hz, H-1'), 3.36 (2H, app t, H-2'), 3.64 (2H, app t, H-3'), 3.68 (2H, dd, H-4'), 3.99 (2H, dd, H-5'), 3.72 (4H, m, H-6a' + H-6b') (Gal unit: -H-1-H-6b; Gluc unit: -H-1'-H-6b'), 1.32 (8H, m, 2 x NH$(CH_2)_2$(*CH_2*)$_2$$(CH_2)_2$NH), 1.52 (8H, m, 2 x NHCH$_2$*CH_2*$(CH_2)_2$*CH_2*CH_2NH), 3.20 (8H, m, 2 x NH*CH_2*$(CH_2)_4$*CH_2*NH), 2.61 (4H, m, SCH$_2$*CH_2*), 2.98-3.01 (4H, m, S*CH_2*), 3.23 (4H, s, DTPA amide N*CH_2*C(O)), 3.76 (2H, s, DTPA central acetate N*CH_2*CO_2^-), 3.38 (4H, s, DTPA terminal acetate N*CH_2*CO_2^-), 3.37, 3.08 (8H, two m, DTPA skeleton N*CH_2*); ^{13}C, δ (75.6 MHz, D_2O): 103.08 (C-1), 71.15 (C-2), 78.42 (C-3), 72.71 (C-4), 60.49 (C-5), 75.55 (C-6), 85.59 (C-1'), 72.11 (C-2'), 78.82 (C-3'), 75.92 (C-4'), 68.76 (C-5'), 61.22 (C-6') (Gal unit: C-1-C-6; Gluc unit: C-1'-C-6'), 25.86, 25.94 (NH$(CH_2)_2$(*CH_2*)$_2$$(CH_2)_2$NH), 28.33, 28.47 (NHCH$_2$*$CH_2$*$(CH_2)_2$*$CH_2$*$CH_2$NH), 39.26, 39.58 (NH*$CH_2$*$(CH_2)_4$*$CH_2$*NH), 26.45 (S$CH_2$), 36.61 (SCH$_2$*C*H$_2$), 58.86 (DTPA amide N*$CH_2$*C(O)), 54.33 (DTPA central acetate N*CH_2*CO_2^-), 59.03, 59.11 (DTPA terminal acetate N*CH_2*CO_2^-), 50.53, 52.88 (DTPA skeleton N*CH_2*), 170.60, 173.41, 174.24, 178.82 (DTPA acetate NCH$_2$*CO_2^-* and amide NCH$_2$*C(O)*, other CH$_2$*C(O)*NH)); *HRMS* (ESI) *Calc. for* $C_{56}H_{100}N_7O_{30}S_2$ $(M+H)^+$ 1414.5950. Found 1414.5971.

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DTPAGal$_4$ – (**2**): Fully protected aminofunctionalised divalent thiogalactoside (**9**) (0.464 g, 0.379 mmol) was deprotected as described for compound (**1a**). Reaction with DTPA-bis anhydride (0.064 g, 0.179 mmol), followed by deprotection and purification afforded

glycoconjugate **2** as an off white solid (0.240 g, 69% yield over two steps). ^{1}H, δ (300 MHz, D_2O, pH 7.0): 4.46 (4H, d, *J* = 9.0 Hz, H-1), 3.52 (4H, app t, *J* = 9.0 Hz, H-2), 3.64 (4H, dd, dd, *J* = 9.0 and 3.3 Hz, H-3), 3.68 (4H, m, H-4), 3.95 (4H, app d, *J* = 3.3 Hz, H-5), 3.71 (8H, m, H-6a + H-6b), 1.28 (8H, m, 2 x $NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2NH$), 1.49 (8H, m, 2 x $NHCH_2\mathit{CH_2}(CH_2)_2\mathit{CH_2}CH_2NH$), 3.18 (8H, m, 2 x $NH\mathit{CH_2}(CH_2)_4\mathit{CH_2}NH$), 3.48 (4H, m, $N\mathit{CH_2}C(O)$), 2.75 (8H, m, $N\mathit{CH_2}$), 1.83 (8H, m, $NCH_2\mathit{CH_2}$), 3.20 (8H, m, $NCH_2CH_2\mathit{CH_2}$), 2.59 (8H, m, $SCH_2\mathit{CH_2}$), 2.97 (8H, m, $S\mathit{CH_2}$), 3.20 (4H, s, DTPA amide $N\mathit{CH_2}C(O)$), 3.71 (2H, s, DTPA central acetate $N\mathit{CH_2}CO_2^-$), 3.33 (4H, s, DTPA terminal acetate $N\mathit{CH_2}CO_2^-$), 3.34, 3.18 (8H, two m, DTPA skeleton $N\mathit{CH_2}$); ^{13}C, δ (75.6 MHz, D_2O): 86.29 (C-1), 69.66 (C-2), 74.09 (C-3), 79.08 (C-4), 68.95 (C-5), 61.41 (C-6), 25.33 ($NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2NH$), 28.53 ($NHCH_2\mathit{CH_2}(CH_2)_2\mathit{CH_2}CH_2NH$), 39.32, 39.18 ($NH\mathit{CH_2}(CH_2)_4\mathit{CH_2}NH$), 56.57 ($N\mathit{CH_2}C(O)$), 52.51 ($N\mathit{CH_2}$), 25.38 ($NCH_2\mathit{CH_2}$), 37.11 ($NCH_2CH_2\mathit{CH_2}$), 26.70 ($SCH_2$), 36.52 ($SCH_2\mathit{CH_2}$), 59.20 (DTPA amide $N\mathit{CH_2}C(O)$), 54.50 (DTPA central acetate $N\mathit{CH_2}CO_2^-$), 59.11 (DTPA terminal acetate $N\mathit{CH_2}CO_2^-$), 50.51, 52.89 (DTPA skeleton $N\mathit{CH_2}$), 170.51, 173.40, 174.48, 178.85 (DTPA acetate $NCH_2\mathit{CO_2}^-$ and amide $NCH_2\mathit{C(O)}NH$, other $CH_2\mathit{C(O)}NH$); *m/z* (FAB^+, NBA) 1932 (M^+, 20), 1055 (100); *HRMS* (FAB^+, NBA) *Calc. for* $C_{78}H_{142}N_{13}O_{34}S_4$ $(M+H)^+$ 1932.8665. Found 1932.8576.

Preparation of Ln(III)-glycoconjugates for NMR studies

The Ln(III)-glycoconjugates were prepared by adding a slight excess (1.1 eq.) of $LnCl_3$ aqueous solution to an aqueous solution of the glycoconjugate. The pH of the solution was slowly adjusted to 5 with KOH (aq), stirred at 70 ^{o}C for eight hours and adjusted to pH 7 with KOH (aq). Any precipitate was filtered off. The solution was concentrated and purified by gel filtration with Sephadex G10, eluting with water. The relevant fractions were pooled and freeze dried to afford the Ln(III) complexes.

Preparation of Gd(III)-DTPALac$_2$ for NMRD measurements

Initially, the $DTPALac_2$ conjugate was left to react with an excess of $Gd(ClO_4)_3$ stock solution and the excess of metal ion was backtitrated with Na_2H_2EDTA solution, allowing the calculation of the exact concentration of glycoconjugate.

The Gd(III) chelate of $DTPALac_2$ was prepared by adding an appropriate quantity of the glycoconjugate to aqueous solution of gadolinium perchlorate (3-5% glycoconjugate excess). The solution pH was slowly adjusted to 7 with KOH (aq). The Gd(III)-glycoconjugate solution was freeze-dried and diluted with 25 mM phosphate buffer (pH 7.4). The absence of free Gd(III) in the solution was verified by using xylenol orange indicator.[30] The Gd(III) concentration (4.63 mM) was verified by ICP measurement.

Conclusions

We have devised the synthesis of a new class of hydrophylic glycoconjugate DTPA-bisamides. Their dendrimeric architecture is especially suited for the variation of the valence of the glycoconjugates from a reduced number of building blocks in an interactive fashion.

The Ln(III) ions in the chelates of these glyconjugates are nine coordinated, as expected for DTPA bisamides, with one inner-sphere water molecule and eight positions occupied by three nitrogens and five oxygens of the DTPA moiety. In aqueous solution, these complexes display the four possible diastereoisomeric pairs, resulting from the chirality of the three bound DTPA nitrogen atoms, like the Ln(III) complexes of other DTPA-bisamides with smaller substituents, such as DTPA-BMA and DTPA-BENGALAA, [35,41] while bisamides containing long, micelle-forming, aliphatic chains, only two pairs have been detected in solution, [42] possibly stabilized by intermolecular interactions.

The value found for the r_1 relaxivity of Gd(III)-$DTPALac_2$ at 25 ^{o}C and 20 MHz is 13% higher than that reported for Gd(III) chelates of lower molecular weight DTPA-bisamides, such as DTPA-BMA, consistent with a longer τ_R value. The τ_R value of these Gd(III) chelates is expected to increase linearly with molecular weight, as long as the internal mobility of the side

chains does not change significantly, leading to a proportional increase of the 20 MHz relaxivity. This result suggests that the relaxivity of the tetravalent Gd(III)-DTPAGal$_4$ glycoconjugate (MW= 2088) would be substantially higher. This finding, taken together with a potential to target the ASGPR (studies under way), makes these compounds promising for the design of medical imaging agents (MRI and gamma scintigraphy).

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Supporting Information:

Table S1: Proton relaxivities of Gd(III)- DTPALac$_2$

Equations for the determination of the relaxivity parameters

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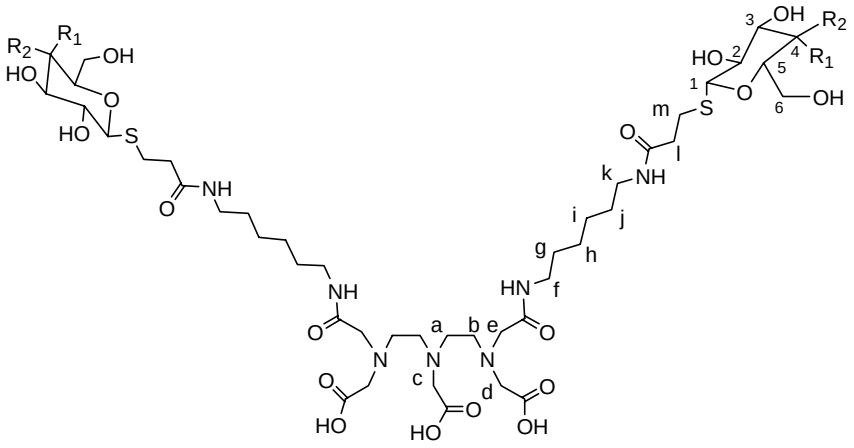
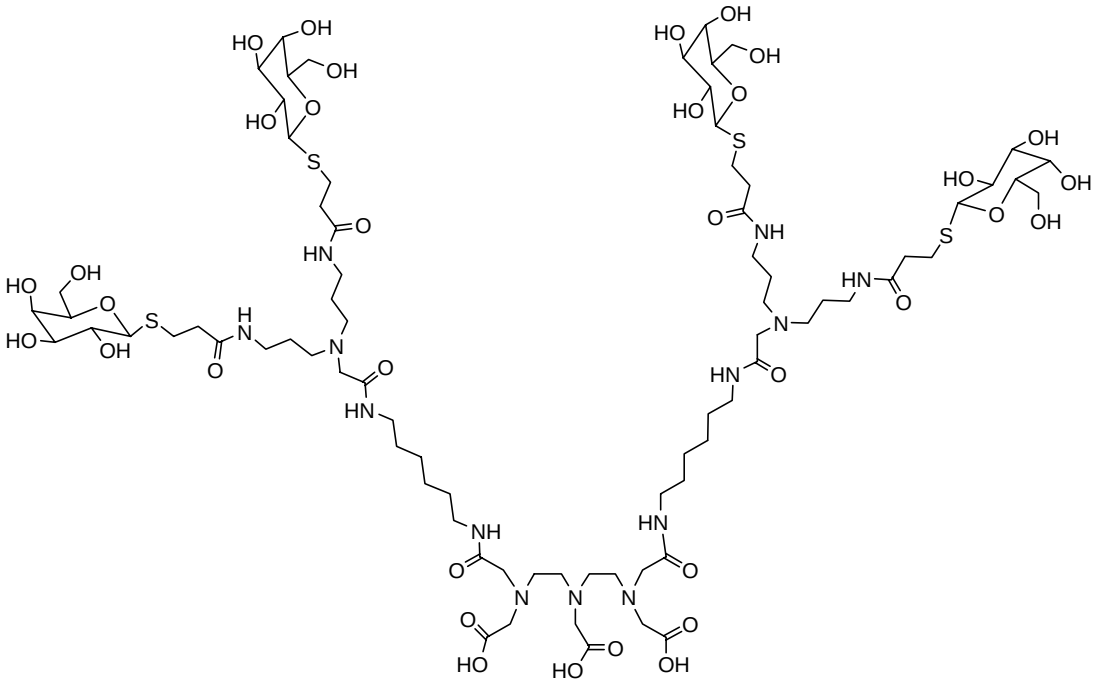
Table 1 - Parameters obtained from the analysis of the NMRD profiles for Gd(III)-DTPALac$_2$ in comparison with other Gd(III) complexes.

	DTPA[44]	DTPA-BMA[44] (bisamide)	DTPA-BENGALAA[41] (bisamide)	DTPALac$_2$ (bisamide)	DOTALac$_2$[26] (monoamide)
k_{ex}^{298}/ 10^6 s^{-1}	3.3	0.45	0.22	0.40[a]	1.2[a]
$\Delta H^{\ddagger}$ / kJ mol^{-1}	51.6	47.6	42.5	40.0[a]	30.0[a]
τ_{rH}^{298} / ps	58	66	265	332±10	306
E_{RH} / kJ mol^{-1}	17.3	21.9	19.7	36.3±0.2	29.9
τ_v^{298} / ps	25	25	16	10±2	33
E_v / kJ mol^{-1}	1.6	3.9	5.5	1[a]	1[a]
Δ^2 / 10^{20} s^{-2}	0.46	0.41	0.53	0.63±0.02	0.12

[a.] Underlined parameters have been fixed in the fit.



1a- R_1= OH and R_2= H - **DTPA(Gal)$_2$**
1b- R_1=H and R_2= β–D-galactopyranosyl - **DTPA(Lac)$_2$**



2-DTPA(Gal)$_4$

Figure 1 - Structures of the DPTA-bisamides glycoconjugates (the labeling of the protons for spectral assignements is shown).

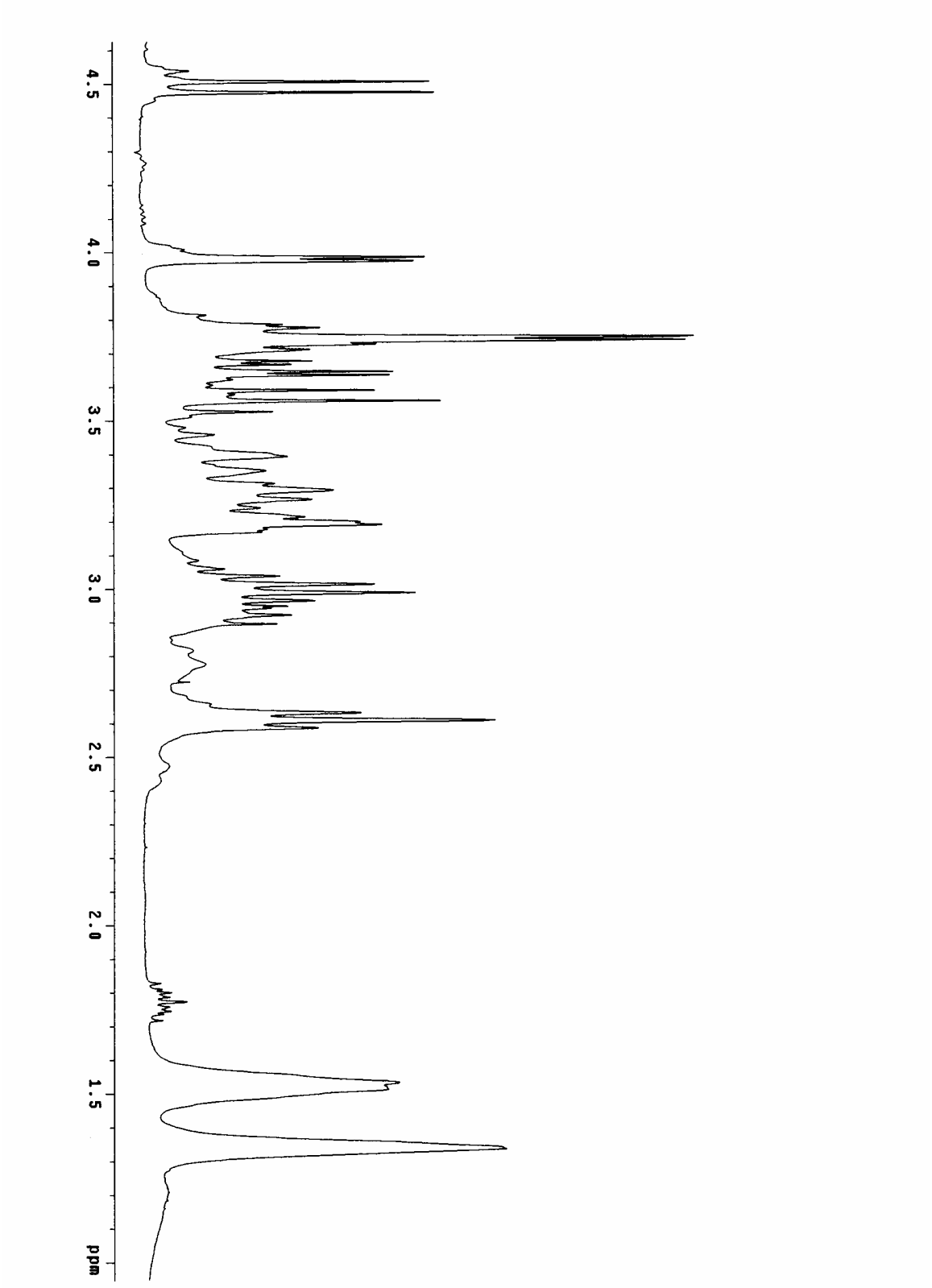


Figure 2A - ^{1}H NMR spectrum of La(III)-DTPAGal$_2$ glycoconjugate in D_2O, pH 7.0, T = 25 ºC.

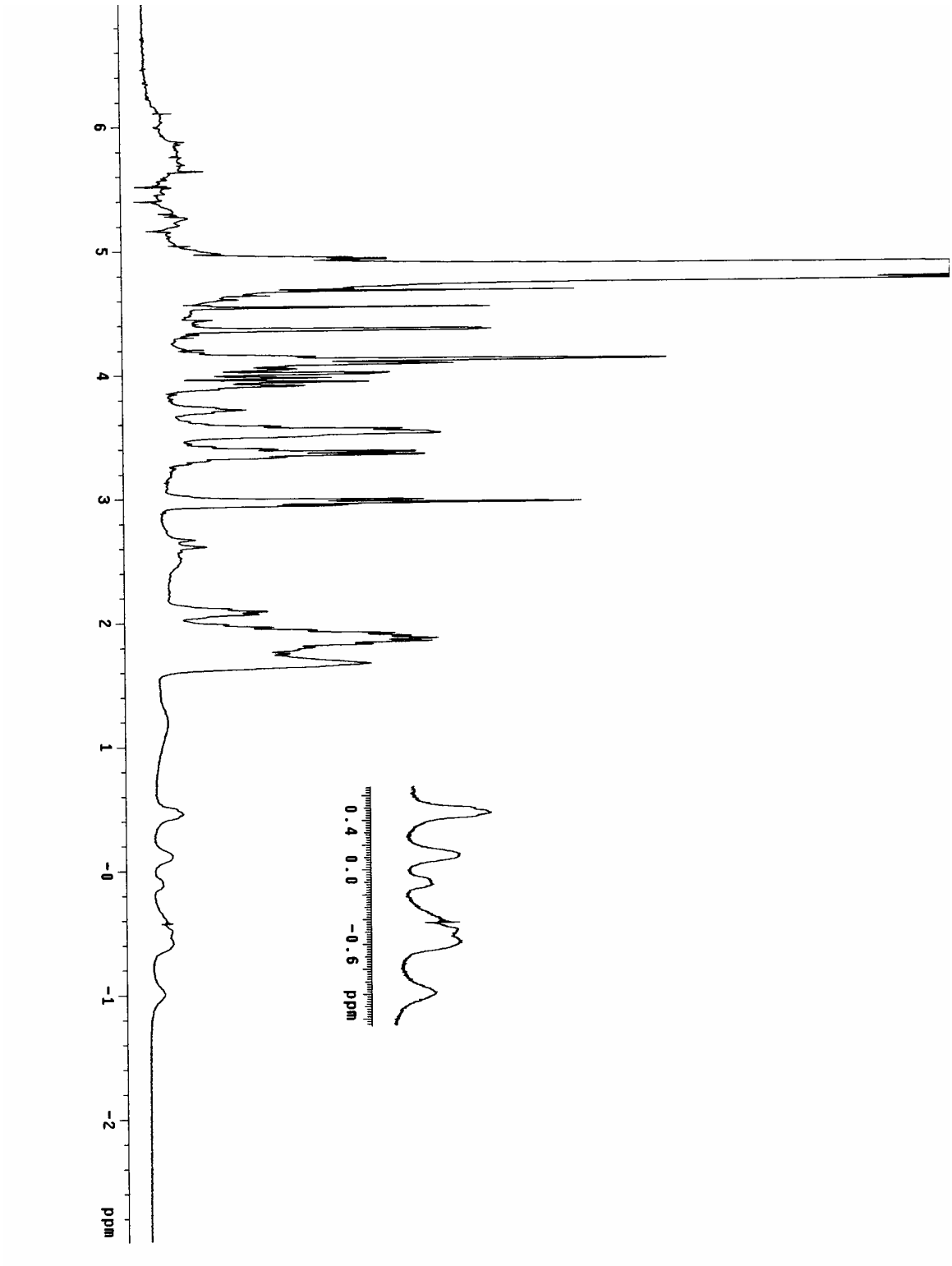


Figure 2B - ^{1}H NMR spectrum of Sm(III)-DTPAGal$_2$ glycoconjugate in D_2O, pH 7.0, T = 60 ºC.

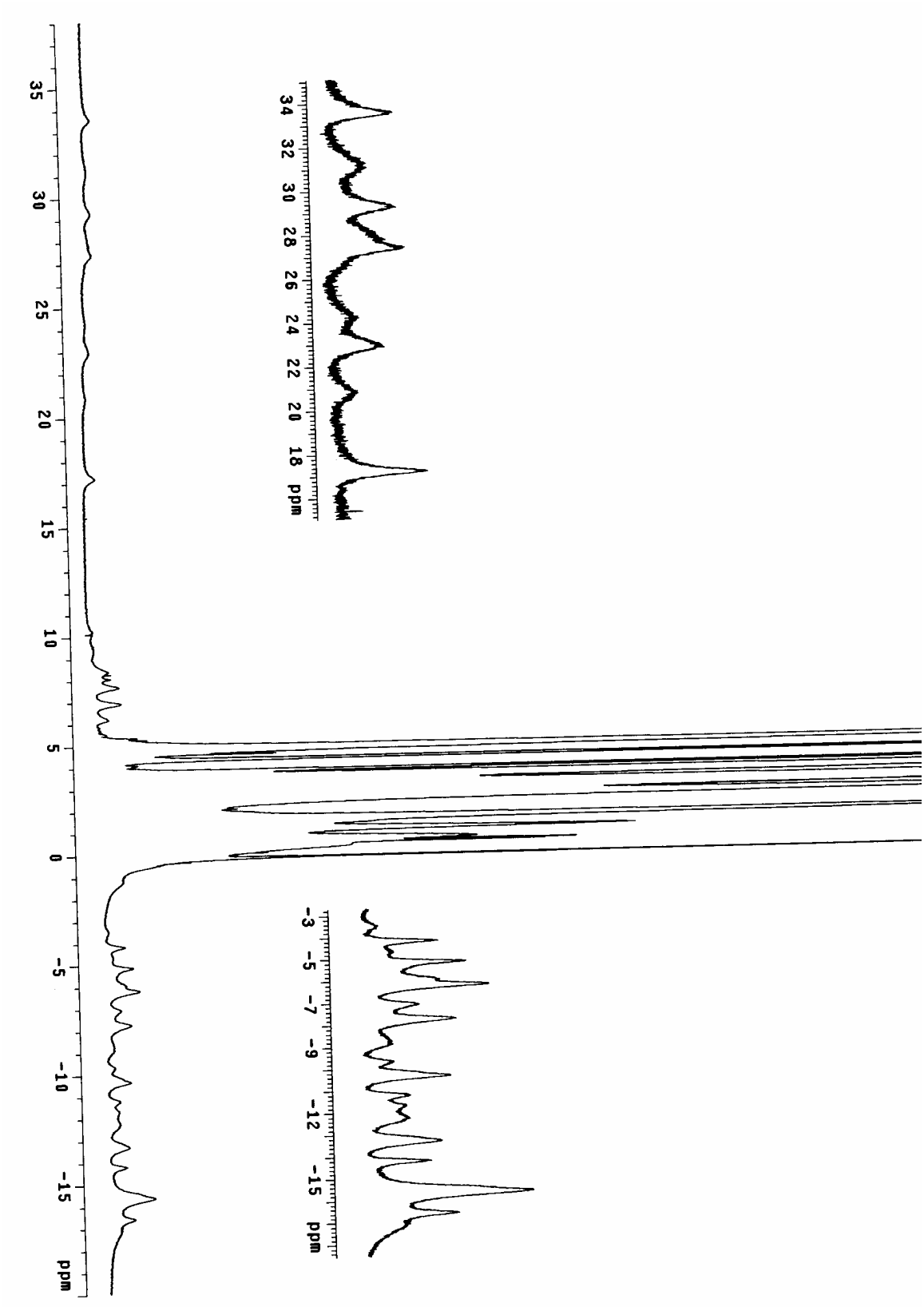


Figure 2C - ^{1}H NMR spectrum of Eu(III)-DTPAGal$_2$ glycoconjugate in D_2O, pH 7.0, T = 7 ºC

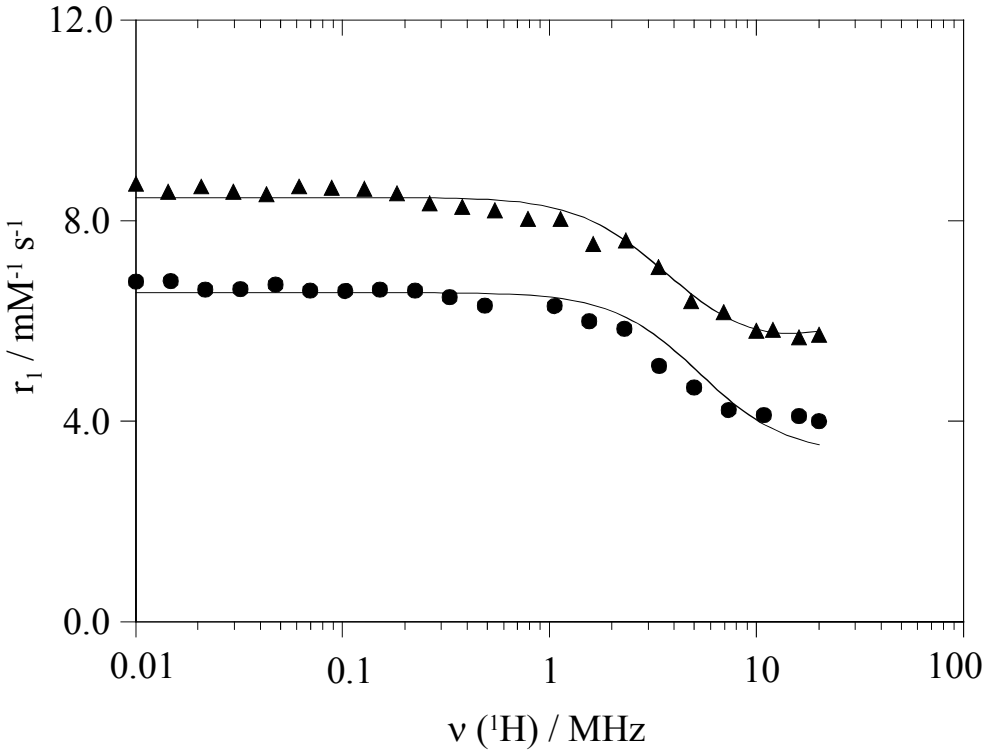
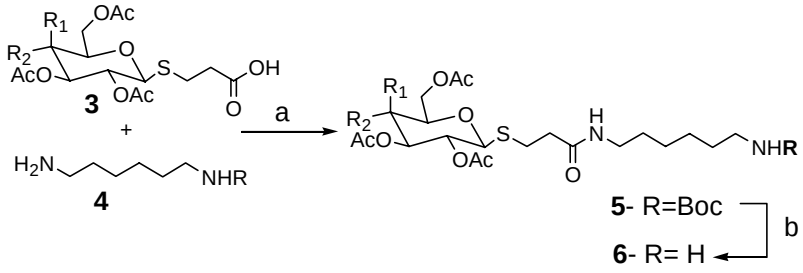
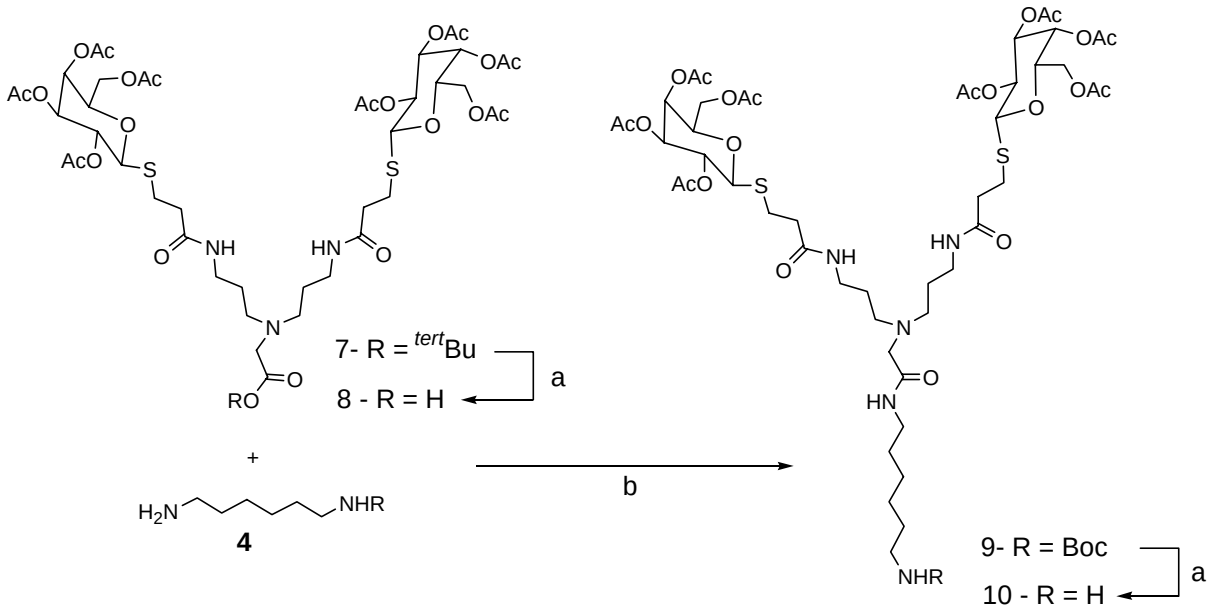


Figure 3 - Variable temperature NMRD profiles for Gd(III)-DTPALac$_2$; T = 25 °C (triangles); and 60 °C (circles). The lines represent the least squares fit to the experimental data points as described in the text.

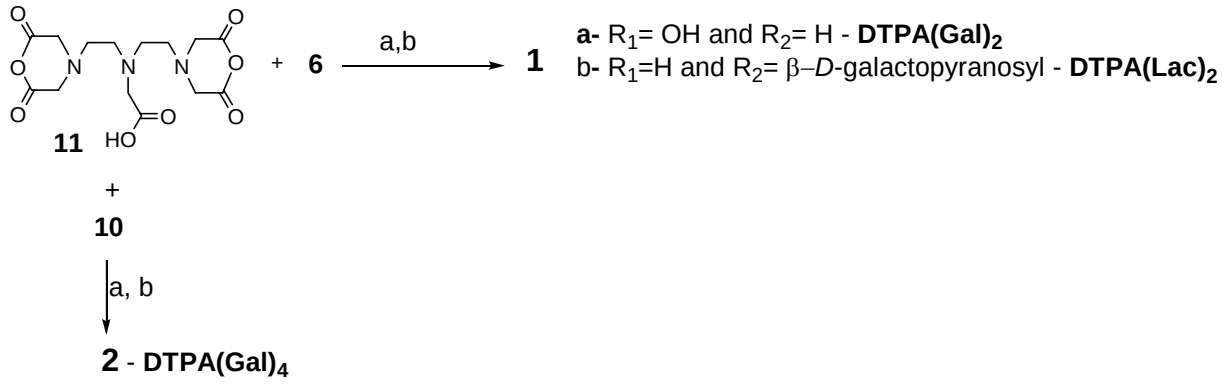


a- R_1= OAc and R_2= H
b- R_1=H and R_2= peracetylated–β–*D*-galactopyranosyl

Scheme 1 : a) DCC/HBT, DCM; b) TFA/DCM (1/3).



Scheme 2 : a) TFA/DCM (1/3); b) i. DIPEA /DCM ii. DCC/HBT, DCM.



Scheme 3 : a) i. DIPEA/DCM ii. DMF / Py; b) i. KOH / EtOH ii. Amberlist 15, elution with NH_3.

Supporting Information

Table S1. Proton relaxivities of Gd(III)-DTPA-Lac$_2$.

Frequency (MHz)	25 °C	Frequency (MHz)	60 °C
10.001	5.811	15.998	4.1
6.9516	6.175	10.853	4.123
4.8342	6.398	7.36	4.229
3.3593	7.080	4.9912	4.672
2.335	7.601	3.384	5.109
1.6231	7.540	2.296	5.843
1.1293	8.033	1.5569	5.993
0.78425	8.037	1.0565	6.301
0.54522	8.204	0.48561	6.311
0.379	8.281	0.32959	6.476
0.26382	8.347	0.22346	6.602
0.1833	8.551	0.15136	6.626
0.12722	8.644	0.10281	6.599
0.088466	8.656	0.069612	6.607
0.061485	8.682	0.047272	6.72
0.042866	8.526	0.032022	6.635
0.029663	8.581	0.021664	6.628
0.020774	8.681	0.014771	6.799
0.01433	8.578	0.010031	6.788
0.010031	8.734	20	4.011
12	5.823		
16	5.671		
20	5.722		

Equations for the determination of the relaxivity parameters

NMRD. The measured proton relaxivities (normalized to 1 mM Gd(III) concentration) contain both inner and outer sphere contributions:

$$r_1 = r_{1is} + r_{1os} \qquad (1)$$

The inner sphere term is given by Equation (2), where q is the number of inner sphere water molecules.

$$r_{1is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^{H} + \tau_m} \qquad (2)$$

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}{}^{H}$ is expressed:

$$\frac{1}{T_{1m}{}^{H}} = \frac{2}{15}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\hbar^2\gamma_S^2\gamma_I^2}{r_{GdH}^6} S(S+1)\left[\frac{3\tau_{d1H}}{1+\omega_I^2\tau_{d1H}^2} + \frac{7\tau_{d2H}}{1+\omega_S^2\tau_{d2H}^2}\right] \qquad (3)$$

Here r_{GdH} is the effective distance between the Gd(III) electron spin and the water protons, ω_I is the proton resonance frequency and τ_{diH} is given by Equation (4), where τ_R is the rotational correlation time of the Gd(III)-H_{water} vector:

$$\frac{1}{\tau_{diH}} = \frac{1}{\tau_m} + \frac{1}{\tau_R} + \frac{1}{T_{ie}} \qquad i = 1\ ,\ 2 \qquad (4)$$

The τ_R rotational correlation time is assumed to have simple exponential temperature dependence with an E_R activation energy:

$$\tau_R = \tau_R^{298} \exp\left\{\frac{E_R}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right\} \qquad (5)$$

The electron spin relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ for metal ions in solution with S > 1/2 are mainly governed by a transient zero-field-splitting mechanism (ZFS).[46] In Equations (6-7) Δ^2 is the trace of the square of the transient zero-field-splitting tensor, τ_v is the correlation time for the modulation of the ZFS with the activation energy E_v, and ω_s is the electron spin Larmor frequency:

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_V\left\{4S(S+1)-3\right\}\left(\frac{1}{1+\omega_S^2\tau_V^2} + \frac{4}{1+4\omega_S^2\tau_V^2}\right) \qquad (6)$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2\tau_V\left[\frac{5.26}{1+0.372\omega_S^2\tau_V^2} + \frac{7.18}{1+1.24\omega_S\tau_V}\right] \qquad (7)$$

$$\tau_V = \tau_V^{298} \exp\left\{\frac{E_V}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right\} \qquad (8)$$

The outer sphere contribution is described by Equation (9), where N_A is the Avogadro constant, and J_{os} is a spectral density function.

$$r_{1os} = \frac{32N_A\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma_S^2\gamma_I^2}{a_{GdH}D_{GdH}} S(S+1)\left[3J_{os}(\omega_I, T_{1e}) + 7J_{os}(\omega_S, T_{2e})\right] \qquad (9)$$

$$J_{\mathrm{os}}\left(\omega,T_{je}\right)=\mathrm{Re}\left[\frac{1+\frac{1}{4}\left(i\omega\tau_{\mathrm{GdH}}+\frac{\tau_{\mathrm{GdH}}}{T_{je}}\right)^{1/2}}{1+\left(i\omega\tau_{\mathrm{GdH}}+\frac{\tau_{\mathrm{GdH}}}{T_{je}}\right)^{1/2}+\frac{4}{9}\left(i\omega\tau_{\mathrm{GdH}}+\frac{\tau_{\mathrm{GdH}}}{T_{je}}\right)+\frac{1}{9}\left(i\omega\tau_{\mathrm{GdH}}+\frac{\tau_{\mathrm{GdH}}}{T_{je}}\right)^{3/2}}\right]$$

$$\mathrm{j}=1,2 \qquad (10)$$

A value of 3.6 Å was used for a_{GdH}. For the temperature dependence of the diffusion coefficient for the diffusion of a water proton away from a Gd(III) complex, D_{GdH}, we assume a exponential temperature dependence, with an activation energy E_{DGdH}:

$$D_{\mathrm{GdH}}=D_{\mathrm{GdH}}^{298}\exp\left\{\frac{E_{\mathrm{DGdH}}}{R}\left(\frac{1}{298.15}-\frac{1}{T}\right)\right\} \qquad (11)$$