Lanthanide(III) Chelates of DTPA Bis(amide) Glycoconjugates: Potential Imaging Agents Targeted at the Asyaloglycoprotein Receptor

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The synthesis and characterisation of a new class of DTPA bis(amide) linked glycoconjugates of different sugars [lactose (Lac) and galactose (Gal)] and valencies (di and tetra) and their Ln^{III} complexes is reported. The ^1H NMR spectra of the Sm^{III} and Eu^{III} complexes of $DTPAGal_2$, $DTPAGal_4$, and $DTPALac_2$, obtained between 7 and 80 °C, indicate that several 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 racemisation of the central nitrogen atom in the NMR spectra show that this process is fast at 60 °C for the Sm^{III} complexes and slow at 7 °C 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 °C and 20 MHz, is 13 % higher than that reported for Gd^{III} chelates of lower molecular weight DTPA bis(amides), such as DTPA-BMA, consistent with a five times larger 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 bis(amide) chelates.

Introduction

Magnetic resonance imaging (MRI) is one of the fastest growing techniques in medical diagnosis due to the excellent spatial 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-triazaundecane-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-BMA)(H_2O)] [Omniscan®; DTPA-BMA = diethylenetriaminepentaacetic acid *N*,*N*′′-bis(methylamide)], in which two carboxylate groups have been 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, for example in neuropathological 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 poor uptake elsewhere in the body. Their rapid diffusion from the vasculature limits their uses as blood pool agents, for example 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 noncovalent binding to HSA (human serum albumin),[10] for example the Gd–DTPA derivative MS-325.[11] The search for new ligands with high tissue and/or organ specificity

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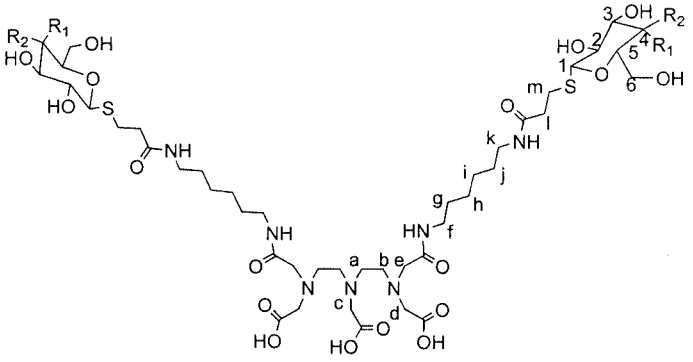
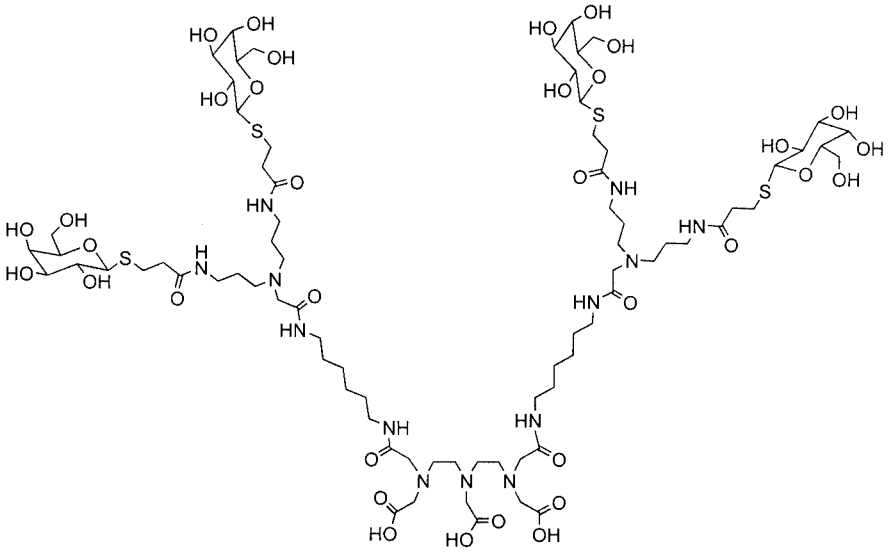
started with hepatobiliary CAs, such as Gd^{III} chelates, which are hepatocyte-specific and are excreted through the hepatobiliary system. Amongst these are Gd^{III} chelates of DTPA-derived ligands bearing various lipophilic substituent groups which promote specific carrier-mediated uptake into the 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(carboxymethyl)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. The amphiphilic Gd(DTPA)–stearylamide, for example, is incorporated into the phospholipid lamella of egg lecithin 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 that rely on macromolecular bioconjugates and on polymer scaffolds have been described for hepatic imaging through the targeting of the ASGPR.[18–25] These agents have the drawback of being inherently polydisperse and ill characterised.

In a previous paper we reported the synthesis and physicochemical characterisation 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)-



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)$_4$

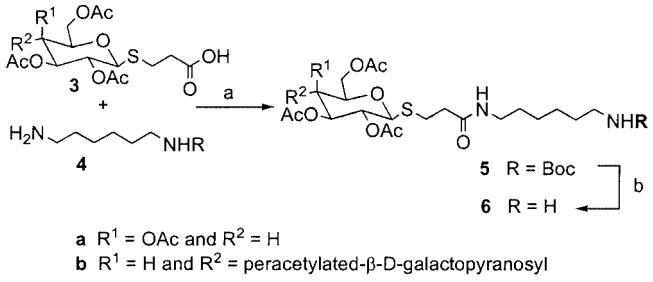
Figure 1. Structures of the DPTA bis(amide) glycoconjugates (the labelling of the protons for spectral assignments is shown).

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 had 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 this paper we report the synthesis and characterisation of (thio)glycoconjugates of the linear chelator DTPA, namely the DTPA bis(amides) **1** and **2** with different terminal sugar residues − galactose (Gal) and lactose (Lac) − and different valences (2 and 4; Figure 1). The thioglycosides are galactosidase-resistant ligands that probe a conformational space and display biological activities very similar to that of their *O*-linked natural counterparts, thus ensuring an increased metabolic stability in vivo.[29] The physicochemical characterisation of some of their Ln^{III} complexes in aqueous solution by ^1H NMR and water ^1H 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 bis(amides) **1** and **2** (Figure 1) was undertaken through a well-established route consisting of the derivatisation of the commercially available DTPA bis(anhydride) with amine-functionalised blocks. Two different types of amine-functionalised sugar blocks were prepared: a monovalent block **5** (Scheme 1) and a divalent glycodendrimer block **9** (Scheme 2).[26,31] The standard DCC/HOBT coupling procedure was found to be 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.

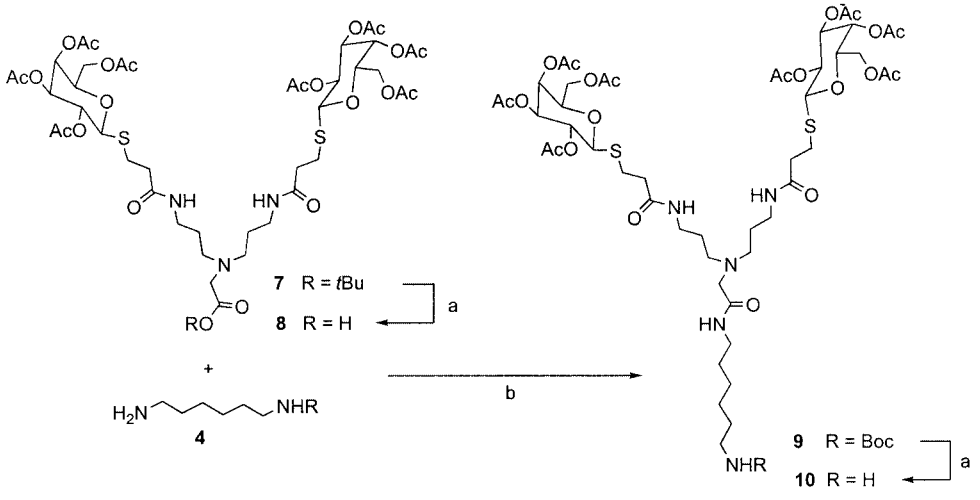


Scheme 1. a) DCC/HBT, DCM; b) TFA/DCM (1:3).

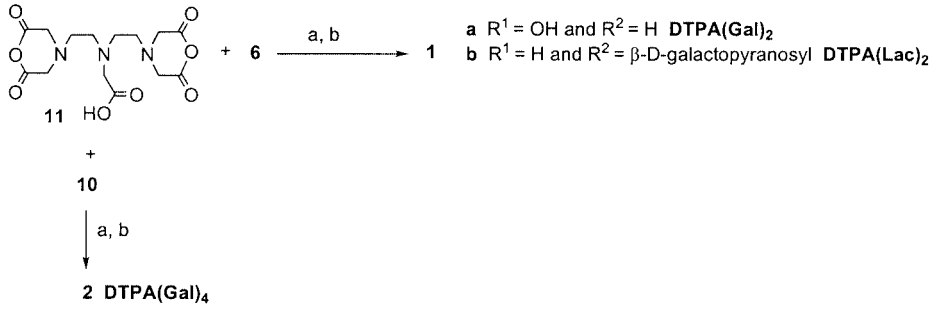
In order to ensure the formation of the required bis(amides), the amino-functionalised blocks **6** and **10** were used in a slight excess, over two mol-equiv. in relation to the DTPA bis(anhydride) block (Scheme 3). The intermediate sugar-protected bis(amides) were carried through and deprotected with KOH in methanol/water to give the final compounds **1** and **2** in reasonable yields.

NMR Studies of the Ln^{III} Glycoconjugates

The ^1H 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 at pH = 7.5 as a function of temperature (7, 25, 40, 60 and 80 °C; see Figures 2–4 for some typical spectra). The spectra of the La^{III} complexes show many identical resonances to those of the corresponding free ligand at the same pH for the protons of the sugar rings and bridging arms (Figure 2). However, the protons of the DTPA moiety, or next to it, such as CH_2(f), give more complex resonances as a result of ion coordination: a multiplet at δ = 2.92 ppm for CH_2(f), multiplets at δ = 2.95, 2.78, 2.68 and 2.45 ppm for the backbone NCH_2CH_2N protons, and a series of partially



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. Amberlyst 15, elution with NH_3.

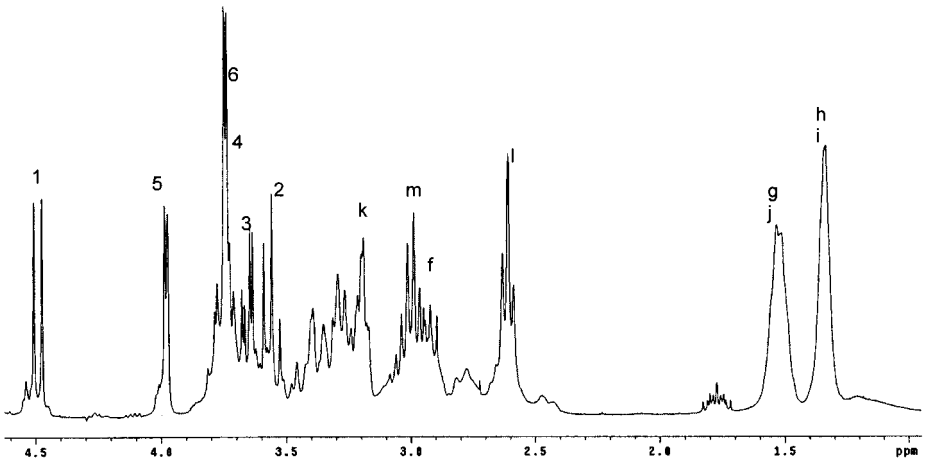


Figure 2. ^{1}H NMR spectrum of La^{III}–DTPAGal$_2$ glycoconjugate in D_2O, pH = 7.0, T = 25 °C.

overlapping AB patterns in the δ = 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 bis(amides)].[32–36] For the Sm^{III} and Eu^{III} complexes, the ligand protons far from the paramagnetic centre, at the sugar rings (H-1–H-6) and at bridging arms [CH_2(g–m)], show very small paramagnetic broadenings and their shifts are easily assigned (see Figure 3).[36] The strongly shifted and broadened proton resonances of the side chain CH_2(f) and the CH_2 of the DTPA amide moiety could not be specifically assigned, despite showing some features similar to the corresponding paramagnetic complexes of the parent DTPA and some DTPA bis(amide) ligands.[36–42]

For the Sm^{III} complexes, most of these strongly shifted resonances are too broad to be detected at 25 °C but sharpen at higher temperatures. At 60 °C some appear in the diamagnetic region (δ = 5.8–4.8 and 3.3–0.6 ppm) and seven are shifted to the low frequency δ = 0.1 to –1.4 ppm region (Figure 3). In contrast, in the Eu^{III} complexes the shifted resonances are quite sharp at low temperature (7 °C, see Figure 4): ten resonances are observed in the high-frequency δ = 35–15 ppm region, about eight in the δ = 10–5 ppm region and about twenty in the δ = –3 to –18 ppm region. These are totally broadened when the temperature is increased to 25 °C, but at 60 °C some sharp, less shifted resonances reappear, for example in the δ = 6.2 to –10.2 ppm region (data not shown).

In Ln^{III} complexes of DTPA bis(amides) with non-chiral centres in the side chains, with a cation nine-coordinate by one inner-sphere water molecule and eight ligand donor atoms, 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] This type of complex can undergo two distinct isomerisation processes in solution. The racemisation of the terminal N atoms, involving decoordination-inversion-coordination of the N atoms and the neighbouring acetate groups, has a high energy barrier. A lower energy process involves racemisation of the central nitrogen atom by 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 and reduces by half the number of observed resonances.[36,40] This is the dynamic process observed in the present study, which is fast at 60 °C for the Sm^{III} complexes and slow at 7 °C for the Eu^{III} complexes due to the much larger dipolar shifts induced by Eu^{III} relative to Sm^{III}.

The total number of strongly shifted proton resonances observed for the Sm^{III} and Eu^{III} complexes studied here, although they could not be assigned, leads to the unambiguous conclusion that they occur as more than one isomer in solution. A comparison of the ^{1}H NMR spectra with those of corresponding DTPA complexes[33,39] suggests that

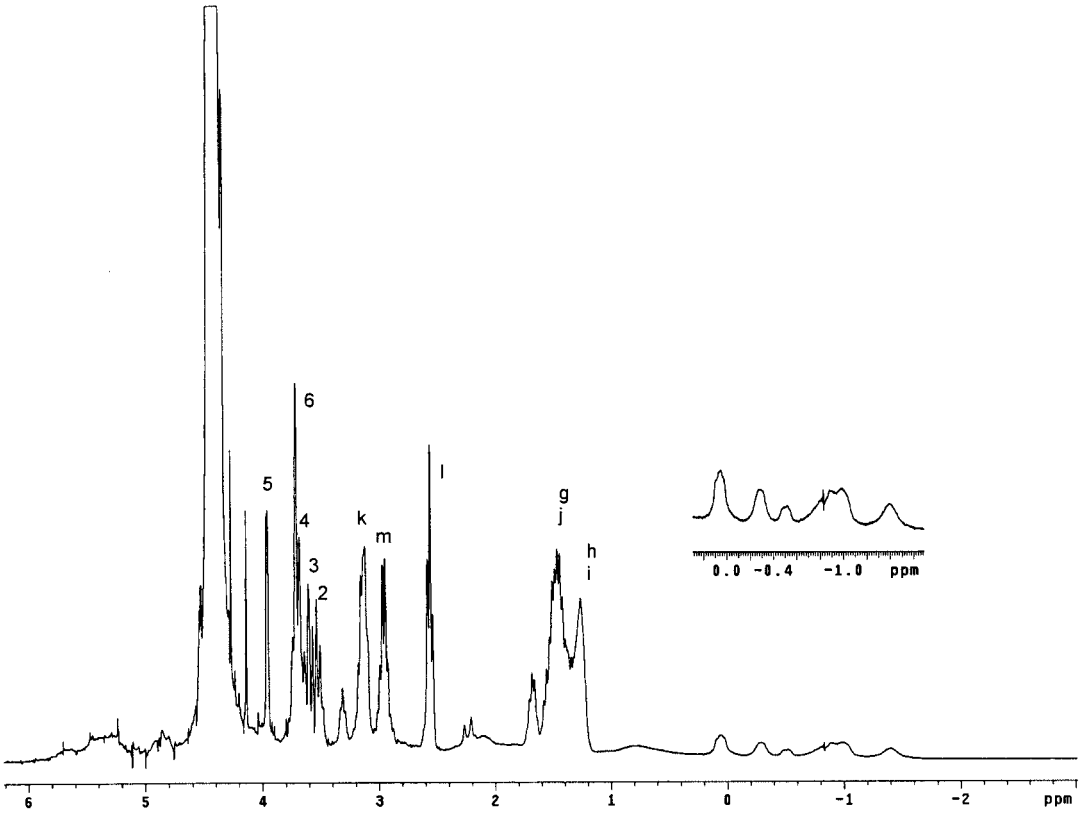


Figure 3. ^{1}H NMR spectrum of Sm^{III}–DTPAGal$_2$ glycoconjugate in D_2O, pH = 7.0, T = 60 °C.

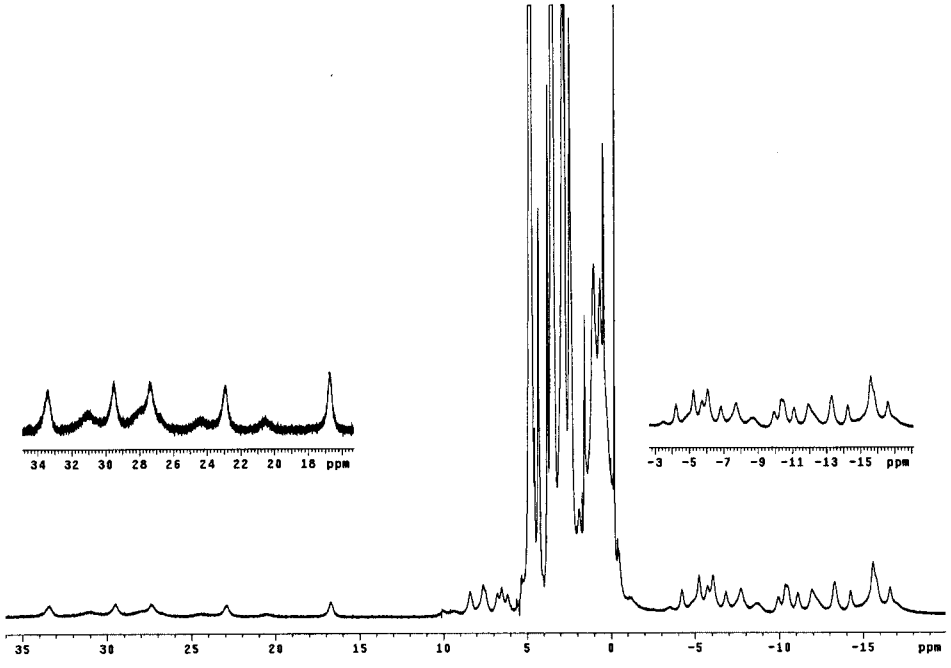


Figure 4. ^{1}H NMR spectrum of Eu^{III}–DTPAGal$_2$ glycoconjugate in D_2O, pH = 7.0, T = 7 °C.

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, in the DTPA complexes, have the largest induced dipolar shifts.[36,39] With this assumption, the minimum of seven low-frequency-shifted resonances observed for the Sm^{III} complex at high temperature (Figure 3), resulting from two ethylenic protons, confirms that several of the four possible isomers are present in solution. This is also in agreement with the observation of a mini-

mum of ten high-frequency-shifted resonances in the Eu^{III} complex at high temperature (out of a maximum of sixteen) for these two ethylenic protons (Figure 4). The Ln^{III} complexes of other DTPA bis(amides), such as DTPA-BPA [BPA = bis(propylamide)] and DTPA-BENGALAA {BENGALAA = *N*,*N*′′-bis[*N*-(aza-D-galacto-5,6,7,8,9-pentahydroxynonyl)carbamoylmethyl]amide},[35,41] show the presence of four diastereoisomeric pairs, while for bis(amides) containing long (C_{14} to C_{18}) 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 at a concentration of 1 mM (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 Supporting Information).[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 5) are typical of low molecular weight Gd^{III} chelates. They were fitted to the usual Solomon–Bloembergen–Morgan relationship 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 number of inner-sphere water molecules to one, and the water exchange rate and its activation enthalpy to values that were previously determined for similar DTPA bis(amide) complexes ($k_{ex}^{298} = 0.40 \times 10^6\ s^{-1}$ and $\Delta H^{\ddagger} = 40.0\ kJ\,mol^{-1}$).[43] The diffusion coefficient and its activation energy were also fixed to common values ($D_{GdH}^{298} = 24 \times 10^{-10}\ m^2\,s^{-1}$; $E_{DGdH} = 20\ kJ\,mol^{-1}$), as these two parameters are not particularly 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.[45] 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 5.

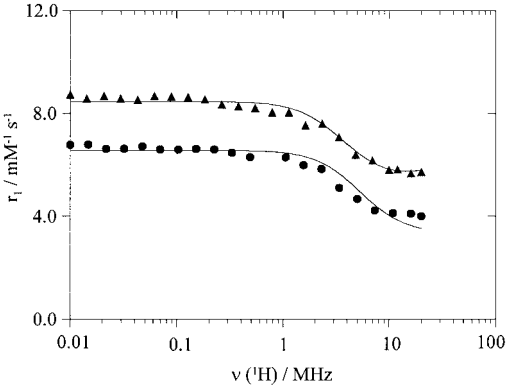


Figure 5. Variable-temperature NMRD profiles for Gd^{III}–DTPALac$_2$; T = 25 °C (triangles); and 60 °C (circles). The lines represent the least-squares fit to the experimental data points as described in the text.

The relaxivity of Gd^{III}–DTPALac$_2$ (mol. wt. = 1569) at 25 °C and 20 MHz is $5.72\ mM^{-1}\,s^{-1}$, which corresponds to an increase of 13% compared to that for the commercial contrast agent Gd^{III}–DTPA-BMA, which is a lower molecular weight DTPA bis(amide) complex (mol. wt. = 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 bis(amide) chelate, Gd^{III}–DTPA-BENGALAA, with an intermediate molecular weight (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

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] [bis(amide)]	DTPA-BENGALAA[41] [bis(amide)]	DTPALac$_2$ [bis(amide)]	DOTALac$_2$[26] [mono(amide)]
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] Parameters in italics have been fixed in the fit.

are also within the usual range for similar Gd^{III}–DTPA bis(amide) chelates. Although the simplified model of electron spin relaxation used here is not fully adequate to describe Gd^{III} chelates,[46] the application of the novel theories requires EPR data in a large field range, which is beyond the scope of the present study.

Conclusions

We have devised the synthesis of a new class of hydrophilic 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 ^1H NMR studies of the Sm^{III} and Eu^{III} chelates of these glycoconjugates in aqueous solution, despite being too complex to be fully assigned, unambiguously show that these complexes occur as more than one of the isomers that result from the chirality of the three bound ligand nitrogen atoms. This is to be compared with the presence of the four possible diastereoisomeric pairs of enantiomers for the Ln^{III} complexes of other DTPA bis(amides) with smaller substituents, such as DTPA-BMA and DTPA-BENGALAA,[35,41] while for bis(amides) containing long, micelle-forming, aliphatic chains, only two pairs have been detected in solution,[42] possibly stabilized by intermolecular interactions.

The NMRD studies of the Gd^{III}–$DTPALac_2$ glycoconjugate chelate are compatible with the presence of one inner-sphere water molecule. The value found for its r_1 relaxivity at 25 °C and 20 MHz is 13% higher than that reported for Gd^{III} chelates of lower molecular weight DTPA bis(amides), such as DTPA-BMA, consistent with a larger 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, thereby leading to a proportional increase of the 20 MHz relaxivity. This result suggests that the relaxivity of the tetravalent Gd^{III}–$DTPAGal_4$ glycoconjugate (mol. wt. = 2088) would be substantially higher. Their potential to target the ASGPR (studies under way), makes these compounds potentially useful for medical imaging agents using gamma scintigraphy, while they also constitute a promising first step towards the design of ASGPR-targeted MRI contrast agents, which will depend on the optimisation of their relaxivity.

Experimental Section

Materials and Equipment: Chemicals were purchased from Sigma–Aldrich and used without further purification. Solvents used were of reagent grade and were 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 or by charring with 10% sulfuric 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 a temperature below 40 °C. FAB mass spectra (positive mode) were recorded with a VG Autospec mass spectrometer with 3-nitrobenzyl alcohol (NBA) as matrix. Electrospray ionisation (ESI) mass spectra were obtained for compounds with molecular weights above 2000. ^1H (1D and 2D) and ^{13}C NMR spectra were recorded with a Varian Unity Plus 300 NMR spectrometer operating at 299.938 MHz and 75.428 MHz, for ^1H and ^{13}C, respectively. Chemical shifts (δ) are given in ppm relative to the $CDCl_3$ solvent (^1H: δ = 7.27 ppm; ^{13}C: δ = 77.36 ppm) as internal standard. For ^1H and ^{13}C NMR spectra recorded in D_2O, chemical shifts (δ) are given in ppm relative to TSP as internal reference (^1H: δ = 0.0 ppm) and *tert*-butyl alcohol as external reference (^{13}C: CH_3 δ = 30.29 ppm). ^{13}C NMR spectra were proton broad-band decoupled using a GARP-1 modulated decoupling scheme. Assignments of the ^1H and ^{13}C NMR spectra were aided by recording two-dimensional DQF-COSY and HMQC spectra. 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 with 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 °C were obtained with a Spinmaster FFC fast cycling NMR relaxometer (Stelar), covering a continuum of magnetic fields from 5×10^{-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 of Fully Protected Hexanediamine-Functionalised Monovalent Thioglycosides 5a and 5b. Typical Procedure 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 mL) was ice-cooled. A solution of DCC (dicyclohexylcarbodiimide; 0.194 g, 9.40 mmol) in dichloromethane (5 mL) was added dropwise to this solution. After 15 min, 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 a viscous syrup. This material was taken up in ethyl acetate (100 mL) and sequentially washed with $KHSO_4$ (aq. 1 M; 3×50 mL), $NaHCO_3$ (satd. sol.; 3×50 mL) and brine (50 mL). The organic phase was concentrated under reduced pressure to give a white foam. Purification by 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). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.34 (m, 4 H, $NHCH_2CH_2CH_2$), 1.43 (s, 9 H, *t*Bu), 1.50 (m, 4 H, $NHCH_2CH_2$), 1.99, 2.05, 2.06 and 2.16 (s, 12 H, $4\times$OAc), 2.50 (m, 2 H, SCH_2CH_2), 2.88–3.06 (m, 2 H, SCH_2), 3.09 [m, 2 H, $CH_2NHC(O)OtBu$], 3.24 [m, 2 H, $C(O)NHCH_2$], 3.48 (br. m, 1 H), 3.95 (ddd, J = 7.2, 5.7 and 0.9 Hz, 1 H, H-5), 4.11 (dd, J = 11.2 and 5.7 Hz, 1 H, H-6a), 4.19 (dd, J = 11.2 and 7.2 Hz, 1 H, H-6b), 4.54 (d, J = 9.9 Hz, 1 H, H-1), 5.04 (dd, J = 10.2 and 3.3 Hz, 1 H, H-3), 5.23 (app t, J = 9.9 Hz, 1 H, H-2), 5.43 (dd, J = 3.3 and 0.9 Hz, 1 H, H-4), 5.98 (br. t, 1 H, N*H*) ppm. HRMS (FAB^+, NBA): calcd. 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%). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.33 (m, 4 H, $NHCH_2CH_2CH_2$), 1.42 (s, 9 H, *t*Bu), 1.70 (m, 4 H, $NHCH_2CH_2$), 1.97, 2.04, 2.05, 2.07, 2.13 and 2.16 (s, 21 H, $7\times$OAc), 2.46 (t, J = 6.6 Hz, 2 H, SCH_2CH_2), 2.81 (m, 1 H,

SCH_aH_b), 3.03 (m, 1 H, SCH_aH_b), 3.08 [m, 2 H, CH_2NHC(O)-O*t*Bu], 3.21 (m, 2 H, $NHCH_2$), 3.45 (m, 1 H) 3.59 (m, 1 H, H-5), 3.78 (app t J = 9.6 Hz, 1 H, H-4), 3.89 (app t, J = 7.0 Hz, 1 H, H-5′), 4.03–4.16 (m, 3 H), 4.28 (m, 1 H), 4.52 (d, J = 7.8 Hz, 1 H, H-1′), 4.65 (d, J = 10.5 Hz, 1 H, H-1), 4.90 (app t, J = 9.6 Hz, 1 H, H-2), 4.96 (dd, J = 10.2 and 3.3 Hz, 1 H, H-3′), 5.09 (dd, J = 10.5 and 7.8 Hz, 1 H, H-2′), 5.19 (app t, J = 9.3 Hz, 1 H, H-3), 5.34 (d, J = 2.4 Hz, 1 H, H-4′), 6.30 (br. t, 2 H, *NH*CO) ppm. MS (FAB^+, NBA): *m*/*z* (%) = 923 (3) $[M + H]^+$. HRMS (FAB^+, NBA): calcd. 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 in CH_2Cl_2/TFA (3:1, 10 mL). The solvent was removed under reduced pressure to give a light yellow foam, which was redissolved in dichloromethane (DCM; 10 mL). The solvent was then 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 viscous, light-yellow foam. ^{1}H NMR analysis revealed the disappearance of the signal at δ = 1.4 ppm 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 for the deprotection reaction) was dissolved in ice-cooled DCM (10 mL) and titrated (pH paper) to pH = 9–10 with DIPEA (diisopropylethylamine). To this solution was added a solution of 1,6-hexanediamine monoBoc (**4**; 0.230 g, 1.07 mmol) in dichloromethane (5 mL) and HBT (0.140 g, 0.940 mmol). A solution of DCC (0.230 g, 1.10 mmol) in dichloromethane (5 mL) was then added dropwise. After 15 min, 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 a viscous syrup. This material was taken up in ethyl acetate (150 mL) and sequentially washed with $NaHCO_3$ (satd. sol.; 3 × 100 mL) and brine (100 mL). The organic phase was concentrated under reduced pressure to give a light-yellow foam. Purification by 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 NMR (300 MHz, $CDCl_3$): δ = 1.32 [m, 4 H, C(O)$NHCH_2CH_2CH_2$], 1.43 (s, 9 H, *t*Bu), 1.51 [m, 4 H, C(O)$NHCH_2CH_2$], 1.72 (m, 4 H, NCH_2CH_2), 1.99, 2.05, 2.06 and 2.16 (s, 24 H, 8 × OAc), 2.52 (m, 8 H, overlapping signals from SCH_2CH_2 and NCH_2), 2.88–3.14 [m, 8 H, overlapping signals from SCH_2, NCH_2C(O) (singlet at 3.02) and CH_2NHC(O)O*t*Bu], 3.29 [m, 6 H, $NCH_2CH_2CH_2$ and $NCH_2C(O)NHCH_2$], 3.40–3.56 (m, 1 H), 3.96 (td, J = 6.4 and 0.9 Hz, 2 H, H-5), 4.09 (dd, J = 11.4 and 6.3 Hz, 2 H, H-6a), 4.20 (dd, J = 11.2 and 6.4 Hz, 2 H, H-6b), 4.56 (d, J = 10.2 Hz, 2 H, H-1), 4.74 (br. t, 1 H, N*H*), 5.05 (dd, J = 10.0 and 3.3 Hz, 2 H, H-3), 5.25 (app t, J = 9.9 Hz, 2 H, H-2), 5.44 (dd, J = 3.3 and 0.9 Hz, 2 H, H-4), 6.74 (br. t, 2 H, N*H*), 7.10 (br. t, 2 H) ppm. MS (FAB^+, NBA): *m*/*z* (%) = 1223 (100) $[M - H]^+$. HRMS (FAB^+, NBA): calcd. for $C_{53}H_{86}N_5O_{23}S_2$ $[M + H]^+$ 1224.5155; found 1224.5119.

Synthesis of DTPA Glycoconjugate Bis(amides9 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 in CH_2Cl_2/TFA (3:1, 10 mL). The solvent was removed under reduced pressure to give a light-yellow foam, which was redissolved in DCM (10 mL); the solvent was then removed under reduced pressure. This procedure was repeated several times and the material was further dried under vacuum to give a viscous, light-yellow foam of **6a**. ^{1}H NMR analysis revealed the disappearance of the signal at δ = 1.47 ppm assigned to the *tert*-butyl group. No further purification or characterisation was carried out on this material. All the material obtained (we assumed a 100% yield for the deprotection reaction) was dissolved in ice-cooled DCM (5 mL) 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 mL) and pyridine (1 mL). The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure to give a colourless oil. This material was carried through without further purification or characterisation. The residue was redissolved in a mixture of ethanol (10 mL) and KOH (aq. sol. 1 M, 10 mL) and stirred at room temperature overnight. The reaction mixture was adjusted to pH ≈ 1 with Amberlyst 15. The resin was transferred into a column, thoroughly washed with water and eluted with aq. 0.5 M NH_3. The relevant fractions were pooled and concentrated under reduced pressure (temperature < 40 °C) to give the fully deprotected glycoconjugate as an off-white solid (0.342 g, 71% yield over two steps). ^{1}H NMR (300 MHz, D_2O, pH = 7.0): δ = 4.48 (d, J = 9.0 Hz, 2 H, H-1), 3.54 (app t, J = 9.0 Hz, 2 H, H-2), 3.64 (dd, J = 9.0 and 3.3 Hz, 2 H, H-3), 3.70 (m, 2 H, H-4), 3.96 (app d, J = 3.3 Hz, 2 H, H-5), 3.73 (m, 4 H, H-6a + H-6b), 1.32 [m, 8 H, C(O)$NHCH_2CH_2CH_2$], 1.51 [m, 8 H, C(O)$NHCH_2CH_2$], 3.20 [m, 8 H, C(O)$NHCH_2$], 2.60 (m, 4 H, SCH_2CH_2), 2.95–3.01 (m, 4 H, SCH_2), 3.24 [s, 4 H, DTPA amide $NCH_2C(O)$], 3.76 (s, 2 H, DTPA central acetate $NCH_2CO_2^-$), 3.40 (s, 4 H, DTPA terminal acetate $NCH_2CO_2^-$), 3.34, 3.08 (two m, 8 H, DTPA skeleton NCH_2) ppm. ^{13}C NMR (75.6 MHz, D_2O): δ = 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 [C(O)$NHCH_2CH_2CH_2$], 28.29, 28.42 [C(O)$NHCH_2CH_2$], 39.23, 39.54 [C(O)$NHCH_2$], 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 $CH_2C(O)NH$] ppm. MS (FAB^+, NBA): *m*/*z* (%) = 1091 (23) $[M + H^+]$. HRMS (FAB^+, NBA) calcd. 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 an off-white solid (0.345 g, 48% yield over two steps). ^{1}H NMR (300 MHz, D_2O, pH = 7.0): δ = 4.46 (d, J = 7.8 Hz, 2 H, H-1), 3.54 (dd, J = 10.2 and 7.8 Hz, 2 H, H-2), 3.63 (dd, J = 10.2 and 3.3 Hz, 2 H, H-3), 3.66 (m, 2 H, H-4), 3.94 (app d, J = 3.3 Hz, 2 H, H-5), 3.70 (m, 4 H, H-6a + H-6b), 4.58 (d, J = 10.2 Hz, 2 H, H-1′), 3.36 (app t, 2 H, H-2′), 3.64 (app t, 2 H, H-3′), 3.68 (dd, 2 H, H-4′), 3.99 (m, 2 H, H-5′), 3.72 (m, 4 H, H-6a′ + H-6b′; Gal unit: H-1–H-6b; Gluc unit: H-1′–H-6b′), 1.32 [m, 8 H, C(O)-$NHCH_2CH_2CH_2$], 1.52 [m, 8 H, C(O)$NHCH_2CH_2$], 3.20 [m, 8 H, C(O)$NHCH_2$], 2.61 (m, 4 H, SCH_2CH_2), 2.98–3.01 (m, 4 H, SCH_2), 3.23 [s, 4 H, DTPA amide $NCH_2C(O)$], 3.76 (s, 2 H, DTPA central acetate $NCH_2CO_2^-$), 3.38 (s, 4 H, DTPA terminal acetate $NCH_2CO_2^-$), 3.37, 3.08 (8 H, two m, DTPA skeleton NCH_2) ppm. ^{13}C NMR (75.6 MHz, D_2O): δ = 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 [C(O)$NHCH_2CH_2CH_2$], 28.33, 28.47 [C(O)$NHCH_2CH_2$], 39.26, 39.58 [C(O)$NHCH_2$], 26.45 (SCH_2), 36.61 (SCH_2CH_2), 58.86 [DTPA amide $NCH_2C(O)$], 54.33 (DTPA central acetate $NCH_2CO_2^-$), 59.03, 59.11 (DTPA terminal acetate $NCH_2CO_2^-$), 50.53, 52.88

(DTPA skeleton NCH_2), 170.60, 173.41, 174.24, 178.82 [DTPA acetate $NCH_2CO_2^-$ and amide NCH_2C(O), other CH_2C(O)NH] ppm. HRMS (ESI): calcd. for $C_{56}H_{100}N_7O_{30}S_2$ $[M + H]^+$ 1414.5950; found 1414.5971.

$DTPAGal_4$ (2): Fully protected amino-functionalised 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 NMR (300 MHz, D_2O, pH = 7.0): δ = 4.46 (d, J = 9.0 Hz, 4 H, H-1), 3.52 (app t, J = 9.0 Hz, 4 H, H-2), 3.64 (dd, J = 9.0 and 3.3 Hz, 4 H, H-3), 3.68 (m, 4 H, H-4), 3.95 (app d, J = 3.3 Hz, 4 H, H-5), 3.71 (m, 8 H, H-6a + H-6b), 1.28 [m, 8 H, C(O)NHCH$_2$CH$_2$*CH$_2$*], 1.49 [m, 8 H, C(O)NHCH$_2$*CH$_2$*], 3.18 [m, 8 H, C(O)NH*CH$_2$*], 3.48 [m, 4 H, N*CH$_2$*C(O)], 2.75 (m, 8 H, N*CH$_2$*), 1.83 (m, 8 H, NCH$_2$*CH$_2$*), 3.20 (m, 8 H, NCH$_2$CH$_2$*CH$_2$*), 2.59 (m, 8 H, SCH$_2$*CH$_2$*), 2.97 (m, 8 H, S*CH$_2$*), 3.20 [s, 4 H, DTPA amide N*CH$_2$*C(O)], 3.71 (s, 2 H, DTPA central acetate N*CH$_2$*CO_2^-), 3.33 (s, 4 H, DTPA terminal acetate N*CH$_2$*CO_2^-), 3.34, 3.18 (two m, 8 H, DTPA skeleton N*CH$_2$*) ppm.^{13}C NMR (75.6 MHz, D_2O): δ = 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 [C(O)NHCH$_2$CH$_2$*CH$_2$*], 28.53 [C(O)NHCH$_2$*CH$_2$*], 39.32, 39.18 [C(O)NH*CH$_2$*], 56.57 [N*CH$_2$*C(O)], 52.51 (N*CH$_2$*), 25.38 (NCH$_2$*CH$_2$*), 37.11 (NCH$_2$CH$_2$*CH$_2$*), 26.70 (S*CH$_2$*), 36.52 (SCH$_2$*CH$_2$*), 59.20 [DTPA amide N*CH$_2$*C(O)], 54.50 (DTPA central acetate N*CH$_2$*CO_2^-), 59.11 (DTPA terminal acetate N*CH$_2$*CO_2^-), 50.51, 52.89 (DTPA skeleton N*CH$_2$*), 170.51, 173.40, 174.48, 178.85 [DTPA acetate $NCH_2CO_2^-$ and amide NCH_2C(O) NH, other CH_2C(O)NH] ppm. MS (FAB^+, NBA): *m/z* (%) = 1932 (20) $[M^+]$, 1055 (100). HRMS (FAB^+, NBA): calcd. 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 equiv.) 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 °C for 8 h 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 back-titrated 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 an 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 addition of xylenol orange indicator.[30] The Gd^{III} concentration (4.63 mM) was verified by ICP measurement.

Supporting Information (see also the footnote on the first page of this article): Table containing the proton relaxivities of Gd^{III}–$DTPALac_2$, and equations for the determination of the relaxivity parameters.

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