Development of New Glucosylated Derivatives of Gadolinium Diethylenetriaminepentaacetic for Magnetic Resonance Angiography

Carmen Burtea, PhD, Sophie Laurent, PhD, Jean-Marie Colet, PhD, Luce Vander Elst, PhD, and Robert N. Muller, PhD

Rationale and objectives: A possible approach for the extension of the vascular residence time of contrast agents relies on the renal reabsorption mechanisms of some molecules such as glucose. In this study, various small-molecular-weight glucosyl derivatives of gadolinium diethylenetriaminepentaacetic (Gd-DTPA) were synthesized and their vascular half-life was studied.

Methods: Several Gd-DTPA-bisamides carrying glucosyl groups bound by different linkers were prepared. The pharmacokinetics and biodistribution of these compounds were determined on Wistar rats.

Results: The sugar moieties linked to Gd-DTPA efficiently reduce the renal excretion of some derivatives. The interaction with renal carrier has not been clearly demonstrated, nor was any interaction observed with blood components.

Conclusions: Two of the new glucosylated derivatives of Gd-DTPA (Gd-DTPA-BC2-β-cellobionA **2** and Gd-DTPA-BC4-β-glucosylA **7**) can be proposed as blood-pool MR contrast agents, considering their vascular remanence.

Key Words: MRI contrast agents, gadolinium, sugars, angiography

(*Invest Radiol* 2003;38: 320–333)

Noninvasive imaging of vascular structures is of paramount relevance since many pathologies, that is, traumatic injuries, ulcers, infectious diseases, tumors, embolism, and atherosclerosis, are characterized by vascular injury and flow reduction. The imaging techniques used to detect and characterize the vascular abnormalities include magnetic resonance imaging, ultrasound, x-ray angiography, helical computed tomography, etc.[1] Several types of contrast agents are currently used for a proper evaluation of the vascular system with these techniques. The prerequisite attributes of these compounds should be the diminished extravasation, prolonged vascular residence time, low toxicity, biologic inertness, excretability from the bloodstream, low accumulation in the reticuloendothelial system, absence of metabolic conversion, and low immunogenicity.[1,2]

Several strategies were developed for the prolongation of the vascular residence time. Some of them mimic the circulating blood cells (liposomes or micelles),[3,4] while others mimic plasma proteins (macromolecules and colloids)[5–7] or reversibly bind plasma proteins.[8,9] However, these approaches encompass different disadvantages, which limit their application for angiography. For example, liposomes leave the circulation rapidly and accumulate in liver and spleen; the concerns regarding patient safety and manufacturing costs restrict their utilization for blood pool agent development.[1]

Different macromolecular substrates consisting of polydisperse linear molecular structures (polylysines, polysaccharides) or monodisperse bulky structures obtained from natural or synthetic sources have been used.[5] The main drawback of plasma protein mimetics is their opsonization and recognition by the reticuloendothelial system, which leads to fast plasma depletion and decrease of the blood pool signal. Some immune response and a transfer from the blood pool to the interstitium have been reported for polymeric blood pool agents. Cardiac toxicity, immunogenicity, and prolonged retention in liver and bone characterize albumin–chelate conjugates, whereas poly(L-lysine) is retained in kidneys and adrenal glands.[1] The foremost disadvantage of dextrans is their polydispersity, which is responsible for the fast elimination from the blood.

Low-molecular-weight contrast agents that bind noncovalently to plasma albumin produce an efficacious alternative

Received for publication November 11, 2002; accepted February 19, 2003.
From the NMR Laboratory, Department of Organic Chemistry, University of Mons-Hainaut, Mons, Belgium.
Supported by the ARC Program 00/05-258 of the French Community of Belgium and COST action D18.
Reprints: Prof. Robert N. Muller, NMR Laboratory, Department of Organic Chemistry, University of Mons-Hainaut, B-7000 Mons, Belgium. E-mail: robert.muller@umh.ac.be

1521-737X/03/3806-0320
DOI: 10.1097/01.RLI.0000066251.65982.e6

to macromolecule-based magnetic resonance blood pool agents, which solve the clearance problems.[8,9]

Another approach, which has not yet been explored, relies on the renal reabsorption mechanisms through which some molecules, freely filtered by the glomeruli, are reabsorbed along the renal tubules and recycled into the bloodstream. Typical of this scheme is glucose, which is completely reabsorbed (about 0.03 mmol min^{-1} kg^{-1}) by an active transport involving a carrier system.[10] This last one belongs to a family of membrane transporters of the facilitative diffusion type, which are expressed in a tissue-specific fashion, and which can transport hexoses down or against a concentration gradient. Each of these transporters has different affinities for glucose and the other hexoses, for example, K_m for glucose ranges from 2 to 5 mmol/L (GLUT1, 3, and 4) to 15 mmol/L (GLUT2).[11–13]

Various glucose derivatives of the extracellular contrast agent Gd-DTPA (Magnevist, Schering, Berlin, Germany) were synthesized in the present study with the aim to prolong their vascular residence through the mechanism of tubular reabsorption.[14,15] In this context, we have studied the influence of the length of the linker and of the isomeric aspects on the pharmacokinetics of the glucose derivatives, taking into account that glucose is naturally present under a mixed form of α and β epimers. The derivatives were evaluated for their relaxivity, pharmacokinetics, biodistribution, and possible interactions with biologic systems.

MATERIALS AND METHODS

Synthesis of the Glucosylated Derivatives of DTPA

Eight gadolinium complexes were synthesized. Three amide derivatives (**1**–**3**) of Gd-DTPA carrying glucosyl groups bound by different linkers (no, ethyl, or butyl) were prepared (Fig. 1). Cellobionic acid activated by *N*-hydroxysuccinimide was used for the synthesis of the new bisamide ligands 1 and 2. The connection was performed by reaction of ethylenediamine or 1,4-diaminobutane with DTPA-bisanhydride (Fig. 2). The Gd-DTPA-B-α,β-glucosAmide **3** (Fig. 1) was obtained by reacting DTPA-bisanhydride with glucosamine.

Five other new derivatives **4**–**8** were synthesized from methyl-α- or β-D-glucose (Fig. 3). The α- or β- blocked glucose structures react with epichlorhydrin, then with aqueous ammonia and finally with DTPA-bisanhydride to give compounds **4** and **5**. The 2 other compounds (**6** and **7**) were also obtained from methyl-α or β-D-glucose, but linked on DTPA via a longer butyl linker (Fig. 4). Derivative **8** was synthesized as described for ligands 6 and 7 from natural D-glucose. Gadolinium complexes have been obtained by

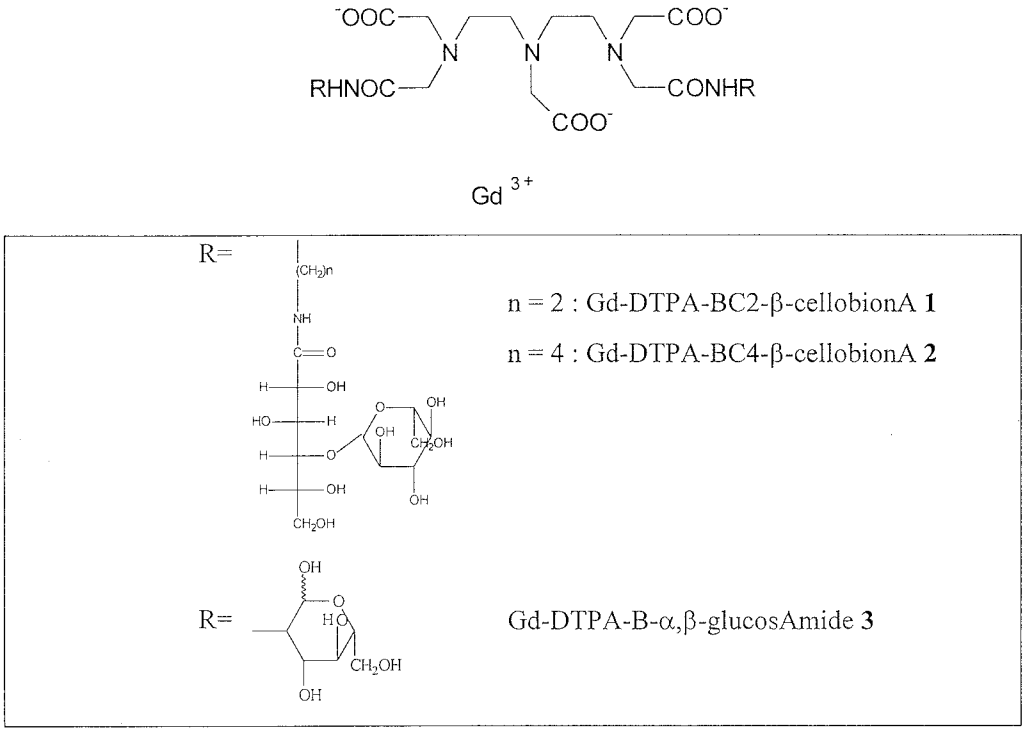


FIGURE 1. Structure of the compounds Gd-DTPA-BC2-β-cellobionA **1** (n = 2), Gd-DTPA-BC4-β-cellobionA **2** (n = 4), and Gd-DTPA-B-α,β-glucosAmide **3**.

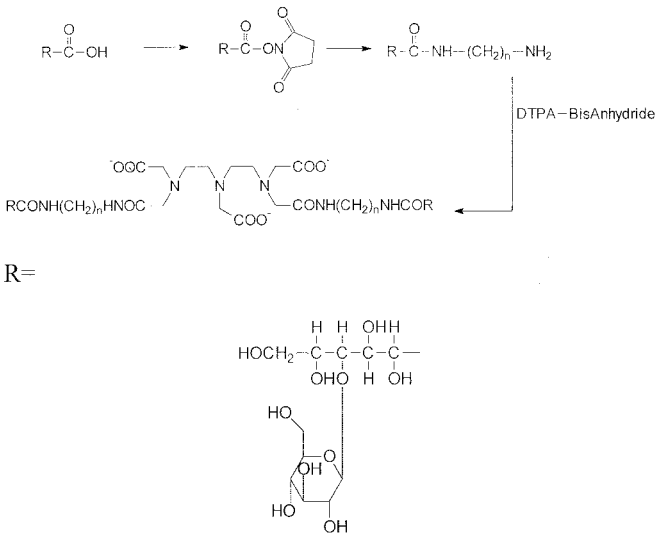


FIGURE 2. Synthesis of derivatives **1** and **2**.

reacting equimolar amounts of hydrated $GdCl_3$ and ligands in water at room temperature.

All organic products (solvents, amines, sugars, DTPA etc.) were purchased from Aldrich (Bornem, Belgium).

Synthesis of DTPA-Bis-cellobionamidylalkylamide 1, 2

Cellobionic Acid

The preparation was carried out according to the method described in the literature[16] with minor modifications. D-Cellobiose (11.3 g) was dissolved in a minimum quantity of water and then diluted with 25 mL methanol. This solution was added to an iodine solution (17.1 g in 240 mL of methanol) at 40°C. At this temperature, 400 mL of 4% KOH solution in methanol was added dropwise with stirring until the color of iodine disappeared completely. The mixture was cooled to 0°C and a crystalline product was obtained, which was filtered and washed first with cold methanol and then with ether. This crude product was purified on cationic exchange resin (Dowex, Acros, Geel, Belgium H^+ type) using 0.5 mol/L ammonium hydroxide as eluent. The fractions were concentrated by evaporation under reduced pressure. The solid was then recrystallized from ethanol–water to afford a crystalline product. Yield: 83%. ^{1}H NMR [D_2O, δ (ppm)]: 4.5-4.4 (1H, m, CH); 4.3–4.2 (5H, m, CH_2, 3x CH); 4.1–3.6 (7H, m, CH_2, 5x CH). ^{13}C NMR [D_2O, δ (ppm)]: 179.2; 104.3; 77.3; 77.0; 75.8; 74.6; 74.5; 72.8; 70.9; 69.4; 63.1; 62.1.

Mono-cellobionamide of Diamines

Cellobionic acid (4.85 g, 13.5 mmol) was dissolved in 15 mL dimethyl formamide (DMF) and 5 mL of triethylamine and cooled to −15°C.[16,17] Dicyclohexylcarbodiimide (2.79 g, 13.5 mmol) dissolved in 5 mL DMF was added with stirring. The reaction was left 20 minutes at −15°C and 15 minutes at −5°C. *N*-hydroxysuccinimide (1.6 g, 13.5 mmol) in 5 mL of DMF was then added and the solution was stirred for 1 hour at -5°C and then 18 hours at room temperature. The formed urea was removed by filtration and washed with DMF. The DMF solution of the activated ester (cell-NHS) can be directly used in the following reactions. The solid cell-NHS can be easily obtained by addition of ethanol to DMF solution. The diamine (20 mmol) was dissolved in 60 mmol DMF; 15 mL of activated ester (4.4 mmol) DMF solution was added dropwise with vigorous stirring at 60°C. The reaction was continued for 3 hours at this temperature. The solvent and excess diamine were distilled under reduced pressure. The resulting syrup was dissolved in 10 mL of methanol, and then the solution was poured slowly into 100 mL of chloroform under stirring. The solid was redissolved in methanol, then precipitated again using chloroform.

N-aminoethyl cellobionamide (n = 2): 87% percent yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.5–4.4 (1H, m, CH); 4.3–4.15 (4H, m, 2x CH, CH_2); 4.1–3.4 (10H, m, 6x CH, 2x CH_2); 3 (2H, t, CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 169.4; 102.9; 83.2; 77.3; 77.0; 76.9; 74.6; 74.1; 72.1; 71.0; 63.1; 62.2; 42.7; 36.4.

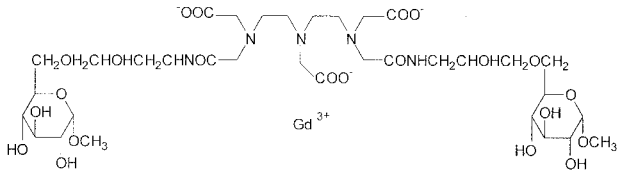
N-aminobutyl cellobionamide (n = 4): 85% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.5–4.4 (1H, m, CH); 4.3–4.1 (4H, m, 2x CH, CH_2); 4.1–3.4 (10H, m, 6x CH, 2x CH_2); 2.55 (2H, t, CH_2); 1.6 (2H, q, CH_2); 1.4 (2H, q, CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 173; 103.1; 81.4; 77.3; 77; 76.9; 74.6; 74.1; 72.4; 71; 63.1; 62.1; 40.4; 35.1; 28.5; 26.4.

DTPA- Linker-Sugar 1-2

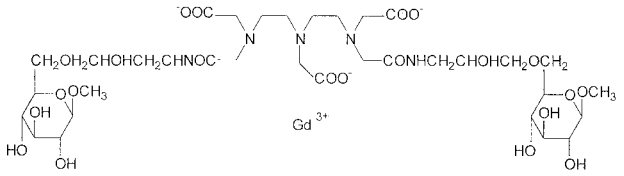
Mono-cellobionamide (2 mmol) of diamine was dissolved in 20 mL of DMF. Freshly prepared DTPA bisanhydride (0.36 g, 1 mmol) was added in small portions at room temperature. The mixture was stirred for 6 hours, DMF was evaporated, and the residue was treated with 100 mL of ethanol. The precipitate was filtered, washed with ethanol then ether, and dried in vacuum.

1,11-bis(cellobionamidylethylamino)-1,11-dioxo-3,6,9-triaza-3,6,9-tri(carboxymethyl)undecane, DTPA-B(cellobionamidylethyl)amide (n = 2) 1: 75% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.5–4.4 (2H, m, 2x CH); 4.4–3.4 (30 H, m, CH_2, CH); 3.3-2.7 (20 H, m, CH_2, CH). ^{13}C NMR [D_2O, δ (ppm)]: 178.2; 171.6; 170.7; 169.3; 102.9; 83.1; 77.1; 74.6; 73.4; 72.1; 71; 63.1; 62.1; 60.6; 59.6; 57.9; 56.4; 52.9; 52.7; 40.7; 39.8; 32.3. Liquid–secondary ions mass spectrometry (L-SIMS): $[M+H]^+$: 1158^+.

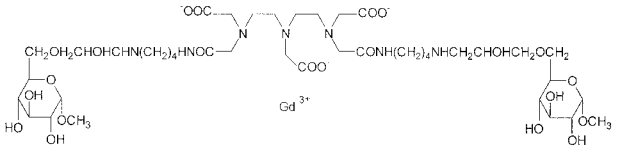
1,11-bis(cellobionamidylbutylamino)-1,11-dioxo-3,6,9-triaza-3,6,9-tri(carboxymethyl)undecane, DTPA-B(cellobionamidylbutyl)amide (n = 4) 2: 74% ^{1}H NMR [D_2O, δ (ppm)]: 4.5–4.4 (2H, m, 2x CH); 4.4–3.4 (30H, m, CH, CH_2); 3.2-2.7



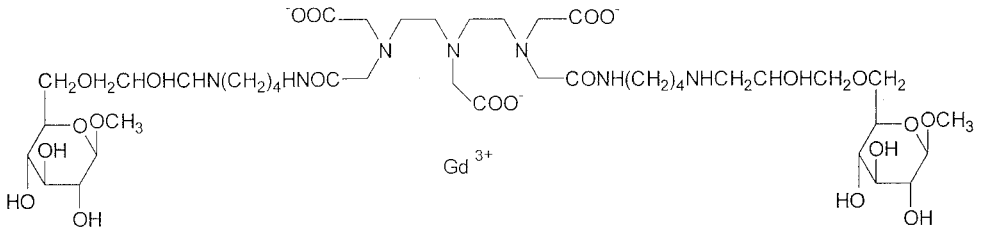
Gd-DTPA-B-α-glucosylA 4



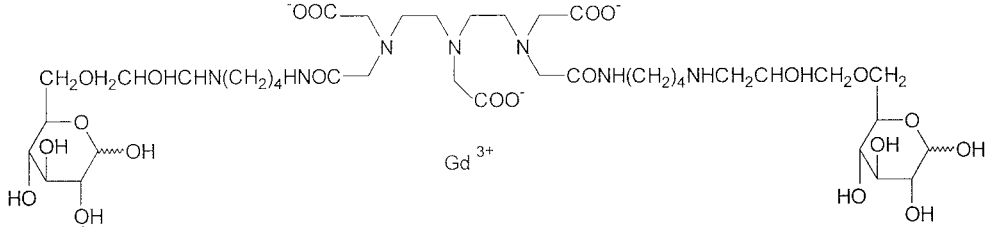
Gd-DTPA-B-β-glucosylA 5



Gd-DTPA-BC4-α-glucosylA 6



Gd-DTPA-BC4-β-glucosylA 7



Gd-DTPA-BC4-α,β-glucosylA **8**

FIGURE 3. Structure of glucosylated derivatives 4—8

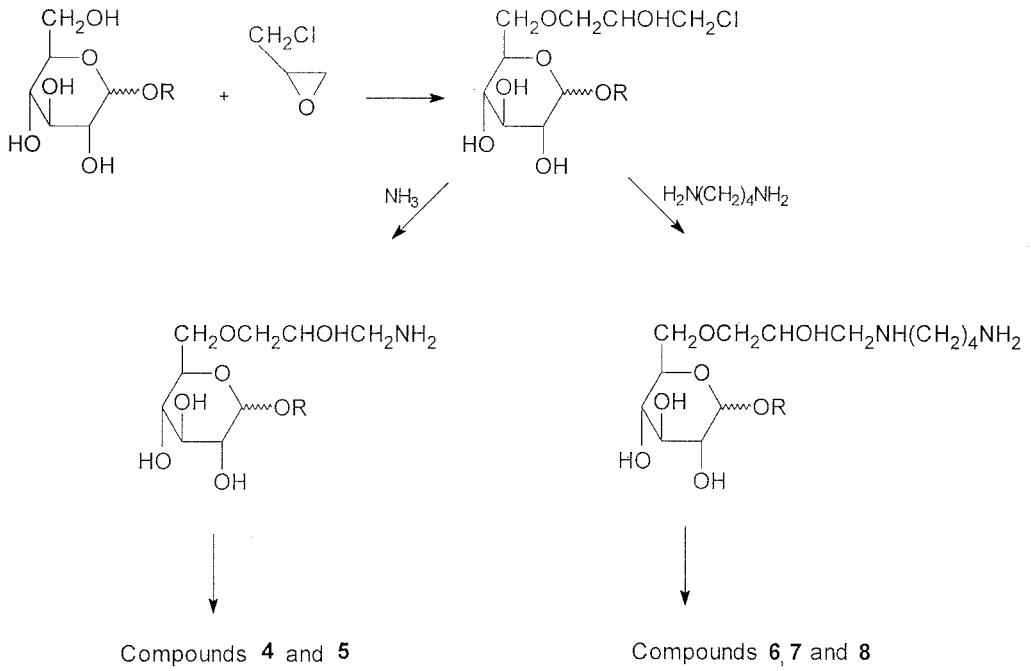


FIGURE 4. Synthesis of compounds **4–7** (R = CH_3) and **8** (R = H).

(20H, m, CH, CH_2); 1.4–1.25 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 178.2; 175.1; 174.2; 172.9; 102.9; 81.3; 77; 74.6; 74.1; 72.1; 71.6; 63.1; 62.1; 60.8; 59.9; 58; 56.5; 52.9; 52.7; 39.5; 38.6; 35.2; 27.4; 25. L-SIMS: $[M+H]^+$: 1214^+.

Synthesis of 1, 11–bisglucosyl-1, 11-dioxo-3, 6, 9-triaza-3, 6, 9-tri (carboxymethyl)undecane, DTPA-Bis(glucosamide), DTPA-BglucosAmide, 3

The bisamide was obtained as described by Gibby and Puttagunta.[18] Glucosamine hydrochloride (4.3 g) was dissolved in 75 mL dimethyl sulfoxide, and 2.8 mL triethylamine was added. The mixture was stirred for 15 minutes. DTPA bisanhydride (3.55 g) was added in small portions. After stirring for 4 hours at room temperature, the mixture was heated to 50°C for 30 minutes. The solution was cooled and filtered. The bisamide was precipitated by adding 250 mL isopropanol. The solid was filtered and washed with ether. The product was then dissolved in 25 mL water and stirred 10 minutes with activated charcoal then filtered. Ethanol (125 mL) was added and the precipitate was filtered and washed with ether.

Yield: 78%; RMN ^1H: [D_2O, δ(ppm)]: 4.5–4.4 (2H, m, 2x CH); 4.1–3.3 (22H, m, 7x CH_2, 8x CH); 3.2–2.8 (8H, m, 4x CH_2). RMN ^{13}C: [D_2O, δ(ppm)]: 177.1; 176.3; 172.4; 94.9; 76.8; 72.6; 71.4; 61.5; 59.8; 57.9; 56.4; 56.2; 52.8; 52.6. L-SIMS $[M+H]^+$: 716^+.

Synthesis of 1, 11–bis[(3(2-methoxyglucosyl)-2-hydroxypropyl)-4-aminobutylamino]-1, 11-dioxo-3, 6, 9-triaza-3, 6 ,9-tri(carboxymethyl)undecane, DTPA-B[(3(2-methoxyglucosyl)-2-hydroxy propyl)-4-aminobutyl] amide (α and β isomers 4, 5, 6, 7, unblocked form 8) methyl-6 (3-amino-2-hydroxypropyl) glucose (α and β isomers)

Methyl-α or β-D-glucose (5 g) was dissolved in 80 mL of 2.5 N NaOH. Epichlorhydrin (2.4 g) was added and the mixture was heated at 40°C under stirring for 24 hours. The solution was evaporated and 60 mL of concentrated ammonia was added. The mixture was heated at 40°C overnight. The solution was distilled under reduced pressure, and 50 mL of methanol were added and evaporated. These 2 last steps were repeated 3 times.

α-Isomer: 82% yield. ^1H NMR [D_2O, δ (ppm)]: 4.8 (1H, d, CH); 4.1–3.9 (3H, m, 3x CH); 3.7 (1H, m, CH); 3.6 (3H, s, CH_3); 3.4–3.2 (5H, m, 2x CH_2, CH); 2.7–2.6(2H, m, CH_2)

β-Isomer: 80% yield. ^1H NMR [D_2O, δ (ppm)]: 4.4 (1H, d, CH); 4.0–3.85 (3H, m, 3x CH); 3.7 (1H, m, CH); 3.6 (3H, s, CH_3); 3.4–3.1 (5H, m, 2x CH_2, CH); 2.7–2.6(2H, m, CH_2)

6 (3-Amino-2-hydroxypropyl) Glucose, Unblocked Form: 83% yield: ^1H NMR [D_2O, δ (ppm)]: 4.4 (1H, d, CH);

4.0–3.85 (3H, m, 3x CH); 3.7 (1H, m, CH); 3.4–3.1 (5H, m, 2x CH_2, CH); 2.7–2.6(2H, m, CH_2)

1,11–bis[(2-Methoxyglucosyl)-2-hydroxypropylamino]-1,11-dioxo-3,6,9-triaza-3,6,9-tri(carboxymethyl)undecane, DTPA-B((2-methoxyglucosyl)-2-hydroxypropyl) amide (α and β isomers) 4-5

The methyl-6 (3-amino-2-hydroxypropyl) glucose (α or β isomers) were dissolved in 100 mL of water and 4.6 g of DTPA-bisanhydride. The pH was adjusted to 9 with NaOH and the mixture was stirred for 6 hours at room temperature. After distillation under reduced pressure and precipitation with acetone, the bisamide was filtered, dissolved in 20 mL water, and dialyzed (500 cutoff, Spectra/Por Polylab, Antwerpen, Belgium). The dialyzed compound was recovered by lyophilization.

DTPA-B-α-glucosylA 4: 66% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.8 (2H, m, 2x CH); 3.8–3.4 (20H, m, 10x CH, 5x CH_2); 3.3 –3.2 (8H, m, 2x CH_3, CH_2); 3 (8H, s, 4x CH_2); 2.95 (2H, s, CH_2); 2.6–2.4 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 178.2; 177.1; 173.9; 99.7; 73.6; 72.0; 71.6; 71; 70.4; 70.0; 62.9; 61.5; 59.2; 58.7; 55.3; 52.1; 49.2; 46.5. L-SIMS: $[M+H]^+$: 908^+, $[M+Na^+]$: 930^+.

DTPA-B-β-glucosylA 5: 70% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.4 (2H, m, 2x CH); 3.7-3.4 (20H, m, 10x CH, 5x CH_2); 3.35–3.2 (8H, m, 2x CH_3, CH_2); 3 (8H, s, 4x CH_2); 2.95 (2H, s, CH_2); 2.6–2.4 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 178.0; 177.4; 173.7; 95.8; 73.5; 72.1; 71.6; 71; 70.4; 70.1; 62.8; 61.5; 59.2; 58.7; 55.3; 52.1; 49.2; 46.5. L-SIMS: $[M+H]^+$: 908^+, $[M+Na^+]$: 930^+.

Methyl-6 (3 (4-aminobutyl)amino-2-hydroxypropyl) glucose (α and β isomers)

Methyl-α or β-D-glucose (5 g) were dissolved in 80 mL of 2.5 N NaOH. Epichlorhydrin (2.4 g) was added and the mixture was heated at 40°C under stirring for 24 hours. The solution was evaporated, 22 g of diaminobutane was added, and the mixture was heated at 40°C overnight. The solution was distilled under reduced pressure, and 50 mL of methanol was added and evaporated. These 2 last steps were repeated 3 times. The compound was extracted with $CHCl_3$ to eliminate the excess of diaminobutane. The aqueous phase was recuperated and evaporated.

α-Isomer: 83% yield: ^{1}H NMR [D_2O, δ (ppm)]: 4.8 (H, d, CH); 4.1–3.9 (3H, m, 3x CH); 3.7 (1H, m, CH); 3.5–3.2 (8H, m, CH_3, 2x CH_2, CH); 3 (2H, t, CH_2); 2.7–2.6 (4H, m, 2x CH_2); 1.7 (2H, quin, CH_2); 1.3 (2H, quin, CH_2).

β-isomer: 79% yield: ^{1}H NMR [D_2O, δ (ppm)]: 4.4 (H, d, CH); 4.0–3.8 (3H, m, 3x CH); 3.6 (1H, m, CH); 3.4–3.1 (8H, m, CH_3, 2x CH_2, CH); 3 (2H, t, CH_2); 2.7–2.6 (4H, m, 2x CH_2); 1.7 (2H, quin, CH_2); 1.3 (2H, quin, CH_2).

6 [3 (4-Aminobutyl)amino-2-hydroxypropyl] Glucose (Unblocked Form): 77% yield: ^{1}H NMR [D_2O, δ (ppm)]: 4.4 (H, d, CH); 4.0–3.8 (3H, m, 3x CH); 3.6 (1H, m, CH); 3.3–3.1 (5H, m, 2x CH_2, CH); 3 (2H, t, CH_2); 2.7–2.6 (4H, m, 2x CH_2); 1.7 (2H, quin, CH_2); 1.3 (2H, quin, CH_2)

1,11–bis[(3(2-Methoxyglucosyl)-2-hydroxypropyl)-4-aminobutylamino]-1, 11-dioxo-3,6,9-triaza-3,6,9-tri(carboxymethyl) undecane, DTPA-B[(3(2-methoxyglucosyl)-2-hydroxypropyl)-4-aminobutyl]amide (α and β isomers 6, 7, Unblocked Form 8)

The methyl-6 [3 (4-aminobutyl)amino-2-hydroxypropyl] glucose (α or β isomers) were dissolved in 100 mL of water and 4.6 g of DTPA-bisanhydride. The pH was adjusted to 9 with NaOH and the mixture was stirred for 6 hours at room temperature. The mixture was distilled under reduced pressure and precipitated with acetone. The bisamide was filtered, dissolved in 20 mL water, and dialyzed (500 cutoff, Spectra/Por). The dialyzed compound was lyophilized.

DTPA-BC4 α-glucosylA 6: 76% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.8 (2H, m, 2x CH); 3.8–3.4 (20H, m, 10x CH, 5x CH_2); 3.3–3.2 (8H, m, 2x CH_3, CH_2); 3 (8H, s, 4x CH_2); 2.95 (2H, s, CH_2); 2.8–2.5 (16H, m, 8x CH_2); 1.5–1.3 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 178.1; 177.2; 174.3; 99.7; 76.4; 76.0; 73.4; 72.6; 70.1; 69.5; 66.3; 59.3; 57.8; 56.5; 54.7; 52.9; 52.6; 52.4; 49.4; 38.6; 26.8; 25.9. L-SIMS: $[M+H]^+$: 1022^+, $[M+Na^+]$: 1044^+.

DTPA-BC4 β-glucosylA 7: 75% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.4 (2H, m, 2x CH); 3.8–3.4 (20H, m, 10x CH, 5x CH_2); 3.3–3.2 (8H, m, 2x CH_3, CH_2); 3 (8H, s, 4x CH_2); 2.95 (2H, s, CH_2); 2.8–2.5 (16H, m, 8x CH_2); 1.5–1.3 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: 178.2; 177.0; 174.3; 95.7; 76.4; 76.1; 73; 72.3; 70.1; 69.4; 66.3; 59.2; 57.8; 56.4; 54.7; 52.9; 52.6; 52.4; 49.4; 38.6; 26.8; 25.9. L-SIMS: $[M+H]^+$: 1022^+, $[M+Na^+]$: 1044^+.

*DTPA-BC4 α,β-glucosylA **8** (unblocked form):* 71% yield. ^{1}H NMR [D_2O, δ (ppm)]: 4.4 (2H, m, 2x CH); 3.8–3.4 (20H, m, 10x CH, 5x CH_2); 3.3–3.2 (2H, m, CH_2); 3 (8H, s, 4x CH_2); 2.95 (2H, s, CH_2); 2.8–2.5 (16H, m, 8x CH_2); 1.5–1.3 (8H, m, 4x CH_2). ^{13}C NMR [D_2O, δ (ppm)]: L-SIMS: $[M+H]^+$: 994^+, $[M+Na^+]$: 1016^+.

Complexation

The Gd(III) complexes were prepared by mixing aqueous solutions of equimolar amounts of hydrated $GdCl_3$ and ligand. The pH was adjusted to 6.5–7. The absence of free Gd(III) was checked with xylenol orange or Arzenazo III (Fluka, Bornem, Belgium) indicator.[19] The complexes were passed through a Sep-Pak column (Accell Plus QMA Cartridges; Waters, Milford, MA) and freeze-dried. The purity of the chelates was verified by HPLC performed on a Waters 600 multisolvent delivery system controlled by the Millenium

software (Waters). Novapak C18 column (4.56 mm × 150 mm) was used. Elution was performed with a linear gradient of pure 0.5 mol/L triethylammonium acetate (pH = 6) to 100% methanol at a flow rate of 1 mL/min for 20 minutes, and a fluorescence detector was used to monitor the elution of the complex. The retention times were as follows: compound **1**: 5.3 minutes, compound **2**: 6.8 minutes, compound **3**: 5.0 minutes, compound **4**: 4.6 minutes, compound **5**: 4.6 minutes, compound **6**: 6.5 minutes, compound **7**: 6.5 minutes, and compound **8**: 6.3 minutes. Their structure was confirmed by L-SIMS.

Spectroscopy

^{1}H and ^{13}C NMR were recorded on a Bruker AMX-300 spectrometer (solvent: deuterium oxide, pD > 9 or $CDCl_3$). For ^{13}C NMR, *t*-butanol was used as internal standard (methyl signal at 31.2 ppm). Mass spectra (L-SIMS) were obtained on a VG AUTOSPEC mass spectrometer (VG Analytical, Manchester, UK).

Relaxometry

The compounds were characterized in vitro by relaxometry in aqueous solutions, in 4% human albumin (Sigma, Borneum, Belgium), and in rat blood. Proton longitudinal relaxation rates were measured at 60 MHz and 37°C on a Bruker Minispec (1.5 T, Bruker, Karlsruhe, Germany). Longitudinal relaxation dispersion profiles (r_1 Nuclear Magnetic Relaxation Dispersion (NMRD) profiles) were recorded in rat blood and blood plasma at 37°C from 0.02 to 40 MHz (proton frequency) on a field cycling relaxometer (Field Cycling Systems, IBM Research, Yorktown Heights, NY). The measurements were performed at different time intervals after preparation (up to 28 hours) for 1 mmol/L Gd-DTPA-BC4-β-cellobionA 2, prepared in rat blood and blood plasma, to identify its possible interactions with blood components.

Animal Studies

All the animal experiments fulfill the requirements of the Ethical Committee of our institution.

Blood Pharmacokinetics

Blood pharmacokinetics were assessed on male Wistar rats (weight 280 ± 50 g, Iffa Credo, Brussels, Belgium) anesthetized with sodium pentobarbital (Nembutal) (Sanofi, Brussels, Belgium) 50 mg/kg b.w., i.p. The rats were tracheotomized, and the left carotid artery was catheterized (Becton Dickinson Angiocath 0.7 × 19 mm) for blood collection. The contrast agent was injected as a bolus through the femoral vein at a dose of 0.1 mmol/kg b.w. Blood samples (0.3 mL) were collected before and at 1, 2.5, 5, 15, 30, 45, and 60 minutes after the injection. The gadolinium content of the blood samples was determined by relaxometry at 37°C and 0.47 T on a Bruker Minispec and by inductively coupled plasma (ICP)–atomic emission spectroscopy (Jobin Yvon JY70+, Longjumeau, France). For ICP, the blood samples (0.3 mL) were mineralized by microwaves (Milestone MSL-1200, Sorisole, Italy) after the addition of 0.6 mL HNO_3 and 0.3 mL H_2O_2.

A 2-compartment distribution model was used to calculate the pharmacokinetic parameters such as the distribution and elimination half-lives ($T_{d1/2}$, $T_{e1/2}$), the volume of distribution (VD_β), total clearance (Cl_{tot}), and the initial blood concentration C_0.[8] The gadolinium concentrations in blood were converted to plasma concentrations by assuming a hematocrit value of 0.53 (blood volume: 58 mL/kg, plasma volume: 31 mL/kg).[7]

To assess the possible tubular reabsorption of Gd-DTPA glucose derivatives, the pharmacokinetic parameters of Gd-DTPA-BC4-β-cellobionA **2** were determined in the presence of phlorizin (Sigma, St. Louis, MO), an inhibitor of glucose carrier.[20,21] For this purpose, the animals were infused (200 μL/min) via the carotid artery with a saline solution (control group) or an aqueous solution of 0.25 mmol/L phlorizin 2 minutes before and during the circulation of Gd-DTPA-BC4cellobionA **2**.

Biodistribution

At the end of the pharmacokinetics experiment, the animals were killed and samples of liver, kidneys, heart, lungs, spleen, muscle, blood, and urine were collected to evaluate their gadolinium content. The total urine volume has been measured. The volume of blood and urine samples submitted to chemical analysis was 0.3 mL. The samples of solid tissues were weighed, dried overnight at 60°C, and subsequently were digested (up to 0.4 g each sample) in acidic conditions (3 mL HNO_3, 1 mL H_2O_2) by microwaves as described above. The gadolinium content was determined by ICP–atomic emission spectroscopy. To calculate the percentage of the injected dose per organ (%ID/organ), the muscle mass was assumed to be 14% of the body weight.[2] The gadolinium content was measured in erythrocytes isolated from blood aiming to evaluate the uptake of contrast agents by their own sugar carriers. The urine has been collected postmortem and the urine volume has been measured to determine the glucose contribution to water reabsorption and urine output. This last parameter has been chosen as an indirect test for the interaction of glucose derivatives with glucose transporter in renal tubules.

Glucose Concentration in Blood and Urine

Glucose concentration has been measured in blood (Gluco Touch, Lifescan, Johnson & Johnson, Beerse, Belgium) and urine (Glukotest, Roche Diagnostics, Bruxelles, Belgium) at the end of the pharmacokinetics experiment to test the stability of the compounds against hydrolysis.

RESULTS AND DISCUSSION

Studies of Relaxivity

The relaxivity of glucosylated derivatives of Gd-DTPA has been determined in blood, human albumin, and in aqueous solutions, at 60 MHz and 37°C (Table 1). The results range between 3.14 seconds^{-1} mmol/L^{-1} and 5.10 seconds^{-1} mmol/L^{-1} in distilled water, between 4.03 seconds^{-1} mmol/L^{-1} and 6.05 seconds^{-1} mmol/L^{-1} in blood (containing 1 mmol/L of contrast agent) and between 4.07 seconds^{-1} mmol/L^{-1} and 5.62 seconds^{-1} mmol/L^{-1} in 4% human albumin solution (containing 1 mmol/L of contrast agent). Those values are comparable with that of Gd-DTPA (3.36 seconds^{-1} mmol/L^{-1} in distilled water, 4.35 seconds^{-1} mmol/L^{-1} in blood, and 4.18 seconds^{-1} mmol/L^{-1} in albumin). The compounds 3, 6, 7, and 8 are characterized by higher r_1 values attributable to larger molecular volumes and subsequently to longer rotational correlation time τ_R, but the ratios r_1 blood/r_1 H_2O and r_1 albumin/r_1 H_2O for solutions containing 1 mmol/L of contrast agent are close to those of Gd-DTPA. These results suggest an insignificant interaction of the new glucosylated derivatives with plasma proteins.

The r_1 NMRD profiles of Gd-DTPA-BC4-β-cellobionA **2** were recorded at 37°C in blood and plasma at different incubation times (Fig. 5). The r_1 NMRD profiles do not show any high field enhancement of relaxivity, confirming the absence of binding to blood components, and exclude the uptake of the compound by erythrocytes, since the r_1 NMRD profile in blood does not change significantly in time. The nonenzymatic glucosylation of albumin is a long time demanding process (1 mol of albumin needs 8 days to incorporate 0.5–0.7 mol of glucose[22,23]), and is irrelevant in the present study.

Pharmacokinetics

The data in Figure 6 were represented as percentages of the initial blood concentration C_0. The pharmacokinetic parameters (Table 2) were calculated by fitting the curves of blood concentration as a function of time after a single bolus i.v. injection.

The pharmacokinetic data demonstrate that glucose prolongs the vascular residence times of glucosylated derivatives as compared with the parent compound, Gd-DTPA (elimination half-life, $T_{e1/2}$ = 14.9 minutes), with the exception of glucosyl derivative **4** (Gd-DTPA-B-α-glucosylA, $T_{e1/2}$ = 13.7 minutes).

The longest $T_{e1/2}$ were found for the compounds **2** (Gd-DTPA-BC4-β-cellobionA, 351.9 minutes) and **7** (Gd-DTPA-BC4-β-glucosylA, 157.5 minutes), for which the total clearance (Cl_{tot}) is the lowest among the investigated compounds (0.4 mL/kg/min for compound **2**, and 1.41 mL/kg/min for compound **7**). This delayed blood clearance is even longer than for other known blood pool contrast agents in rats.[1,8] The small volumes of distribution (VD_β = 70 mL/kg for derivative **2**, $P < 0.05$; VD_β = 164 mL/kg for derivative **7**) indicate a restricted distribution in the extravascular space.[8] Concerning the chemical structure, these compounds contain β-glucosyl moieties and long linkers between Gd-DTPA and glucosyl group. Besides, the chemical structure of derivative **2** presents several other characteristics, such as: higher hydrophilicity due to -OH groups, binding of the glucosyl residue through the -OH group at C1, the position of glucosyl in the linker's backbone is intermediate (ie, not terminal). Certain properties may contribute to its longer $T_{e1/2}$, that is, a particular geometry of the molecule that may allow the interaction with glucose carrier, the hydrophilicity and solubility in blood plasma.

TABLE 1. Relaxivity of Glucosylated Derivatives of Gd-DTPA Measured in Blood, 4% Human Serum Albumin, and Distilled Water, at 60 MHz and 37°C.

Compound	Molecular weight	r_1 in blood (s^{-1} mmol/L^{-1})	r_1 in 4% albumin (s^{-1} mmol/L^{-1})	r_1 in H_2O (s^{-1} mmol/L^{-1})	r_1 blood/ r_1 H_2O	r_1 albumin/ r_1 H_2O
Gd-DTPA	547	4.35	4.18	3.36	1.29	1.24
1	1157	4.64	4.74	3.85	1.20	1.23
2	1157	4.80	4.81	3.86	1.24	1.25
3	715	5.87	5.38	5.10	1.15	1.05
4	930	4.03	4.37	3.44	1.17	1.27
5	930	4.09	4.07	3.14	1.30	1.29
6	1022	5.66	5.36	4.66	1.21	1.15
7	1022	6.05	5.62	4.77	1.27	1.18
8	1016	5.58	5.21	4.60	1.21	1.13

The concentration of the contrast agents in blood and albumin solution was equal to 1 mmol/L.

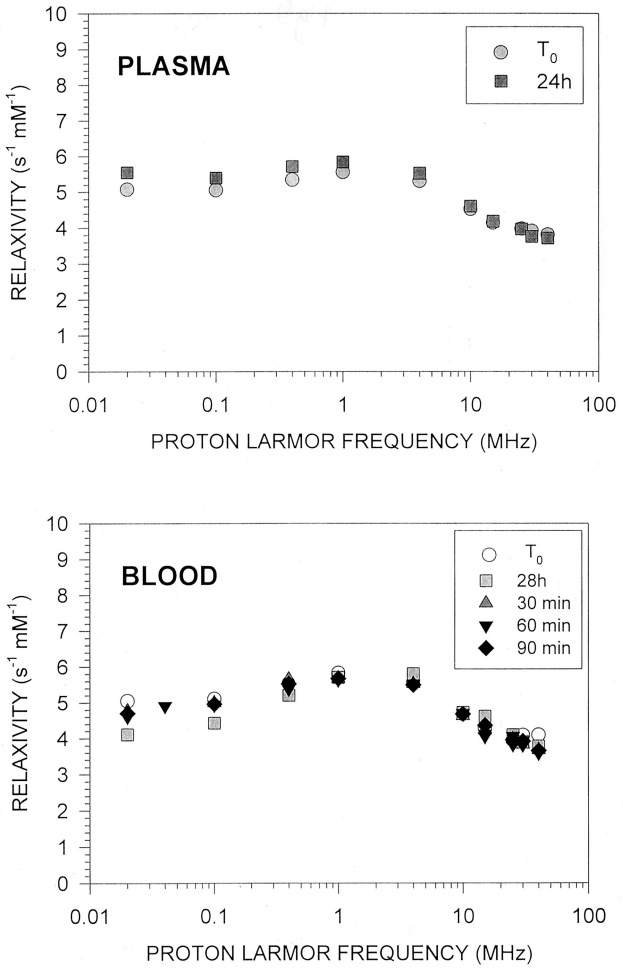


FIGURE 5. r_1 NMRD profiles at 37°C for Gd-DTPA-BC4cellobionA 2 in blood and plasma at T_0 and at different time intervals after preparation [N.B.: The apparent slight low field decrease of the relaxivity is attributed to a small decrease of the actual diamagnetic contribution of the solution with respect to the reference profiles (fresh blood or HSA solution) that are subtracted].

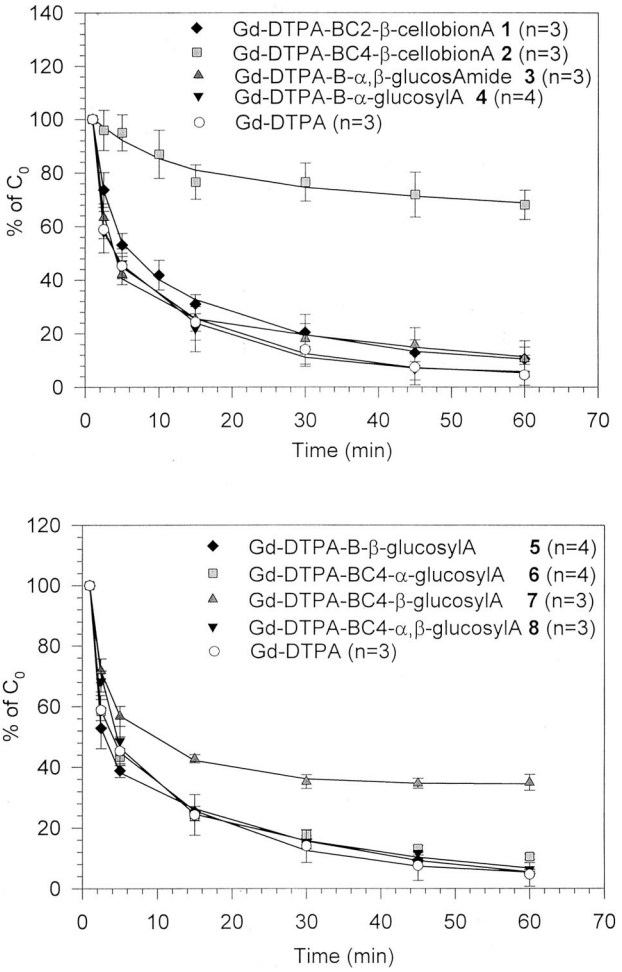


FIGURE 6. Blood pharmacokinetic profiles of glucosylated derivatives of Gd-DTPA versus DTPA in rats. The data are represented as percentages of C_0. The solid line represents the fit of data to a biexponential profile.

Intermediate blood clearance was observed for derivatives **6** (Gd-DTPA-BC4-α-glucosylA, $T_{e1/2}$ = 40.8 minutes; Cl_{tot} = 11.0 mL/kg/min) and **3** (Gd-DTPA-B-α,β-glucosAmide, $T_{e1/2}$ = 38.2 minutes: Cl_{tot} = 9.6 mL/kg/min). Higher volumes of distribution were found for both derivatives (VD_β = 316 mL/kg for compound **3**, VD_β = 373 mL/kg for compound **6**; $P < 0.05$ as compared with Gd-DTPA), which reflect an extended diffusion in the extravascular space. Both of them have different properties as compared with the chemical structure of compounds **2** and **7**: α-glucose (derivative **6**) or a mixture of α- and β-glucose (derivative **3**) in their structure; shorter linker backbone (mainly for the compound **3**); lower hydrophilicity (mainly compound **3**); glucosyl bound through C2 for compound **3**.

Derivatives **1** (Gd-DTPA-BC2-β-cellobionA), **5** (Gd-DTPA-B-β-glucosylA), and **8** (Gd-DTPA-BC4-α,β-glucosylA) present faster clearance. The large VD_β of derivatives **5** (272 mL/kg, $P < 0.05$) and **8** (325 mL/kg, $P < 0.05$) indicate a diffusion in the interstitial space.

Relative values of $T_{e1/2}$ of α and β analogues (compounds **4** and **5**, and compounds **6** and **7**) seem to indicate that β-glucose units are beneficial for the prolongation of the blood half-life.

The increased urine excretion induced by some of the glucosylated derivatives (Fig. 7) could reflect an interaction with glucose transporter in renal tubules. Polyuria due to glucose-induced osmotic diuresis is common in patients with hyperglycemia.[24] Unfortunately, no correlation was found between the blood clearance of contrast agents and the urine volume. The contribution of an elevated free glucose concen-

TABLE 2. The pharmacokinetic parameters of glucosylated derivatives of Gd-DTPA determined for rat

Contrast agent	$T_{d1/2}$ (min)	$T_{e1/2}$ (min)	Cl_{tot} (mL/kg/min)	VD_β (mL/kg)
Gd-DTPA	0.7 ± 0.04	14.9 ± 1.2	16.2 ± 2.2	205 ± 30
Gd-DTPA-BC2	1.6 ± 0.1	23.2 ± 1.3	8.4 ± 0.6	165 ± 6
β-cellobionA **1**	$P < 0.01$	$P < 0.01$	$P < 0.05$	
Gd-DTPA-BC4	6.7 ± 0.2	351.9 ± 40.6	0.4 ± 0.05	70 ± 6
β-cellobionA **2**	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.05$
Gd-DTPA-B	1.5 ± 0.2	38.2 ± 1.9	9.6 ± 0.3	316 ± 18
α,β-glucosAmide **3**	$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.05$
Gd-DTPA-B	0.6 ± 0.06	13.7 ± 2.3	17.2 ± 1.3	226 ± 10
α-glucosylA **4**				
Gd-DTPA-B	0.8 ± 0.1	20.5 ± 1.2	15.4 ± 1.5	272 ± 17
β-glucosylA **5**		$P < 0.05$		$P < 0.05$
Gd-DTPA-BC4	1.4 ± 0.2	40.8 ± 2.3	11.0 ± 0.5	373 ± 66
α-glucosylA **6**	$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.05$
Gd-DTPA-BC4	1.8 ± 0.1	157.5 ± 12.7	1.41 ± 0.2	164 ± 42
β-glucosylA **7**	$P < 0.01$	$P < 0.01$	$P < 0.01$	
Gd-DTPA-BC4	1.9 ± 0.1	24.6 ± 0.6	14.3 ± 2.1	325 ± 53
α,β-glucosylA **8**	$P < 0.01$	$P < 0.01$		$P < 0.05$

$T_{d1/2}$ indicates distribution half-life; $T_{e1/2}$ indicates elimination half-life; Cl_{tot} indicates total clearance; VD_β indicates volume of distribution; the results are represented as averages ± standard error of the mean; the Student *t* test has been calculated for each glucosylated derivative versus Gd-DTPA.

tration in blood and urine as a result of the hydrolysis is excluded, since this parameter remained in normal ranges in blood (80–120 mg/dL, Fig. 8) and urine (less than 40 mg/dL).

The specific interaction with glucose carrier in kidneys has been tested by the administration of phlorizin, an inhibitor of the tubular glucose reabsorption. The pharmacokinetic parameters of glucosyl derivative **2** (Gd-DTPA-BC4-β-cellobionA), which presents the slower blood clearance, were thus determined in animals treated with phlorizin. The blood pharmacokinetics of this derivative did not change in these conditions, which suggests that, if any, an interaction with glucose transporter in the renal tubules is not important for this glucosylated derivative. However, it is important to mention that phlorizin is a specific inhibitor of sodium-dependent glucose transporters (SGLT) in kidneys,[25] but not of other hexose transporters, for example, the neuronal GLUT3.[26] Also, the kidney brush-border membranes contain 2 types of SGLT, that is, the high-glucose/low-phlorizin-affinity SGLT1 and the low-glucose/high-phlorizin-affinity SGLT2.[25] If our glucosylated derivatives interact with various types of glucose transporters expressed in different organs, a competitor like phlorizin could not have a significant influence on their blood clearance.

The prolonged blood residence of glucosylated derivatives is not a consequence of their uptake by erythrocytes, as shown by the proton NMRD profiles and the insignificant gadolinium concentration found in these cells 1 hour after the administration of contrast agents.

Whatever the mechanism of action, the data show that glucose grafting to Gd-DTPA can prolong the blood half-life of the contrast agents, and this effect could be related to several particularities of the chemical structure, such as the length of the linker, the presence of β-glucose, hydrophilicity, and the intermediate position of glucosyl radical in the linker's backbone (ie, not external). It can also be noticed that the freedom of -OH at C6 of glucosyl moiety could be favorable (see compound **2** versus compound **7**) for the prolongation of the blood half-life.

Biodistribution

The biodistribution has been determined in rats, 1 hour after a single i.v. injection of 0.1 mmol Gd/kg. Gd-DTPA, as a parent compound, has been used as a control. The results were calculated as percentages of the injected dose (%ID) and are represented in Figure 9 for solid tissues and in Figure 10 for urine.

The biodistribution studies confirm some of the pharmacokinetic data and allow us to draw some conclusions concerning the possible mechanism of action of the glucosylated compounds. A low gadolinium concentration in urine has been found for the compounds **2** (12.5% of ID) and **7** (25.9% of ID), which fits with the delayed blood clearance.

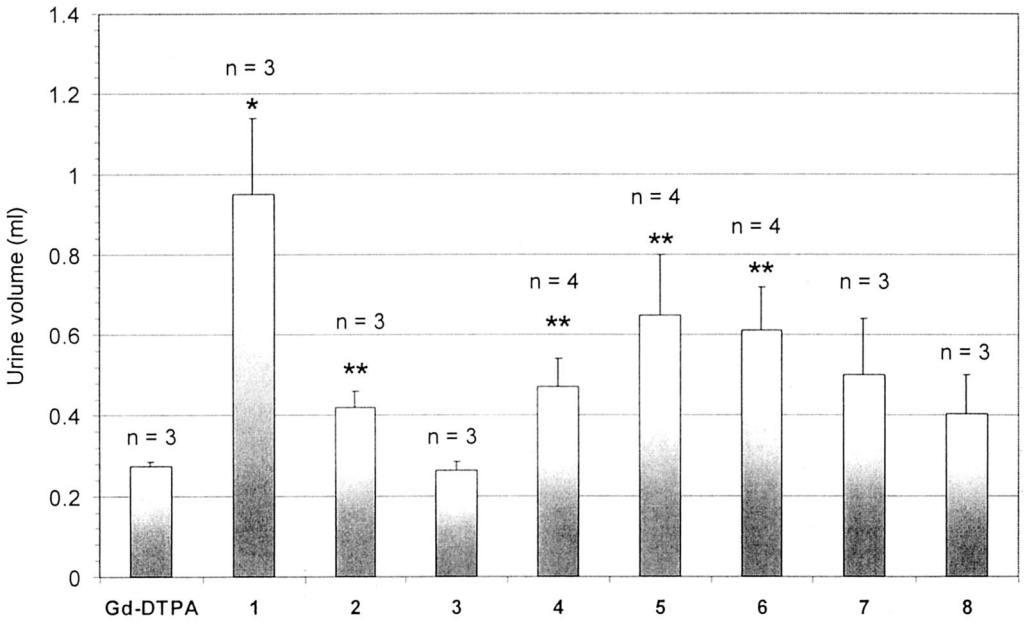


FIGURE 7. Urine volume 1 hour after the administration of glucosylated derivatives of Gd-DTPA in Wistar rats. The results are represented as averages ± standard error of the mean; the Student *t* test has been calculated for each glucosylated derivative versus Gd-DTPA; *$P < 0.01$, **$P < 0.05$.

Interestingly, relatively high concentrations of the glucosylated derivative **2** (2.5% of ID; $P < 0.05$ as compared with Gd-DTPA) and **7** (7.9% of ID; $P < 0.05$ as compared with Gd-DTPA) have been detected in liver. In fact, a reversible binding to the liver glucose carrier could occur without internalization, taking into account that the volumes of distribution do not suggest such an event, a possibility furthermore excluded by the long blood residence time. A recircu-

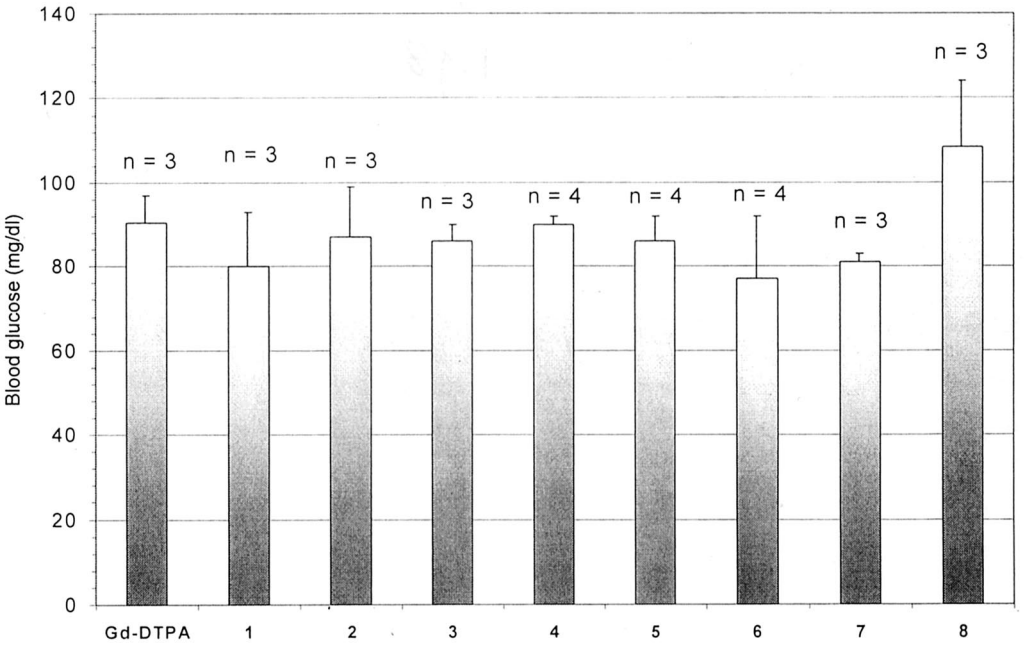


FIGURE 8. Glucose concentration in blood 1 hour after the injection of glucosylated derivatives of Gd-DTPA in Wistar rats. The results are represented as averages ± standard error of the mean.

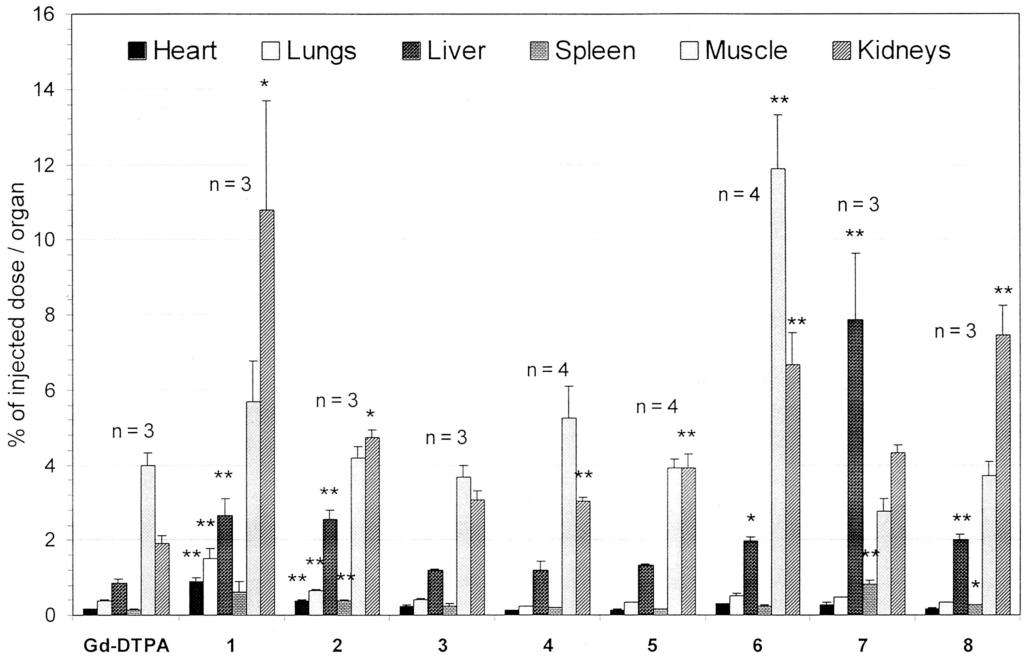


FIGURE 9. The biodistribution of glucosylated derivatives of Gd-DTPA in Wistar rats 1 hour after single i.v. administration of 0.1 mmol Gd/kg. The results are represented as percentages of the injected dose. The results are represented as averages ± standard error of the mean; the Student *t* test has been calculated for each glucosylated derivative versus Gd-DTPA; **P* < 0.01, ***P* < 0.05.

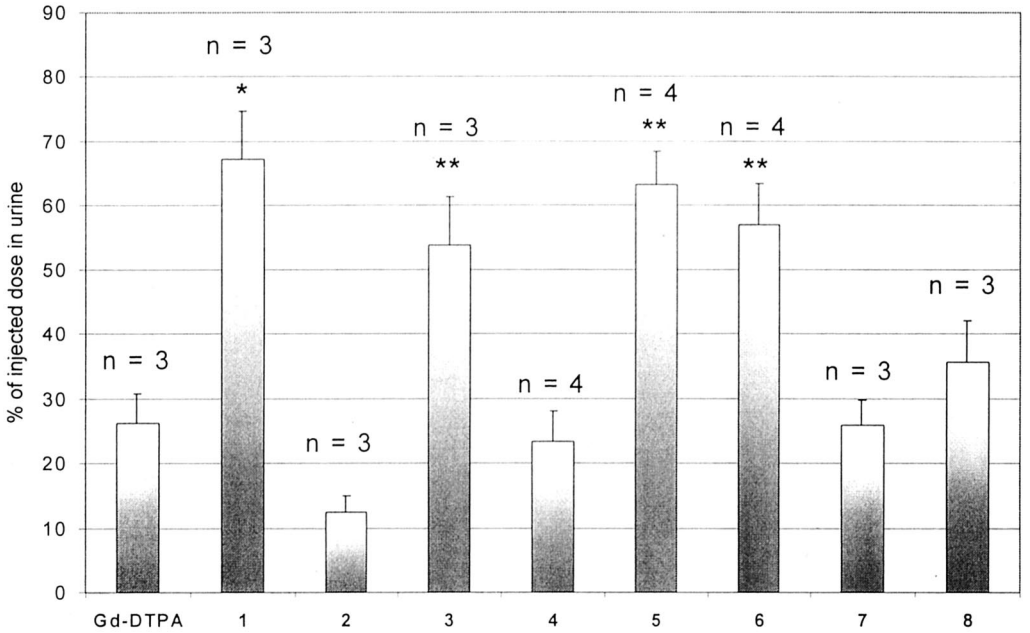


FIGURE 10. Urine concentration of glucosylated derivatives of Gd-DTPA in Wistar rats 1 hour after single i.v. administration of 0.1 mmol Gd/kg. The results are represented as percentages of the injected dose. The results are represented as averages ± standard error of the mean; the Student *t* test has been calculated for each glucosylated derivative versus Gd-DTPA; **P* < 0.01, ***P* < 0.05.

lation between cells and blood could therefore be invoked to account for the delayed blood clearance. At a lower extent, a similar mechanism could have occurred for the compounds **1**, **6**, and **8**, which have been detected in liver (2.7% of ID, $P < 0.05$ for derivative **1**; 2% of ID, $P < 0.01$, $P < 0.05$ for derivatives **6** and **8**, respectively), or in muscle (11.9%, $P < 0.05$ for derivative **6**). However, their higher level of excretion is confirmed by the gadolinium content in kidneys (10.8% of ID, $P < 0.01$ for derivative **1**; 6.7% of ID, $P < 0.05$ for derivative **6**; 7.45% of ID, $P < 0.05$ for derivative **8**) and urine (67.4% of ID, $P < 0.01$ for derivative **1**; 57.1% of ID, $P < 0.05$ for derivative **6**; 36.5% of ID for derivative **8**).

CONCLUSIONS

Our data evidenced that 2 of the molecules studied (Gd-DTPA-BC2-β-cellobionA **2** and Gd-DTPA-BC4-β-glucosylA **7**) could be good candidates as markers of the vascular space. The addition of sugar moieties to the extracellular contrast agent Gd-DTPA can thus efficiently reduce its renal excretion. This property has been demonstrated by the longer elimination phases, the slower total clearance, and the small volumes of distribution of compounds **2** and **7**. Nevertheless, the interaction with renal carrier has not been demonstrated with certitude. The failure of phlorizin to enhance the blood clearance precludes the interaction with glucose carrier.

The interaction with blood plasma proteins and the uptake by erythrocytes have also been excluded as responsible for the delayed blood clearance.

The stability of the compounds in blood has been controlled and excluded the participation of free gadolinium ions or of the free glucose.

For several glucosylated derivatives, a large urine volume has been found 1 hour after their administration in rat as compared with the Gd-DTPA-treated animals. The absence of any correlation between the blood clearance and urine volume cannot sustain the tubular reabsorption as a mechanism of the prolonged blood residence. The saturation of the kidney SGLT could limit its transport capacity, and restrain its contribution to the urine excretion of the glucosylated derivatives.

The biodistribution studies have shown relatively high gadolinium concentrations in liver for the glucosylated derivatives **2**, and **7**, which correlates with the delayed blood clearance. The small volumes of distribution reflect a diminished extravasation of these compounds. Although the specific interaction with different GLUT in these organs remains to be proved, we can presume that a reversible binding to the glucose carrier in liver could occur without internalization. The recirculation between cells and blood could contribute to the delayed blood clearance. GLUT isoforms differ in their tissue expression, substrate specificity, and kinetic characteristics. Hence, GLUT1 is expressed in brain and erythrocytes.[27–29] Furthermore, the brain expresses GLUT3,[28] while GLUT2 is expressed in liver;[30] the muscle, and adipose cells express GLUT4.[31,32] GLUT2, the major facilitative glucose transporter isoform expressed in hepatocytes, has a low affinity and broad substrate specificity.[30] GLUT1 is a multifunctional protein that transports hexoses and interacts with several molecules structurally unrelated to the transported substrates.[33]

A correlation between pharmacokinetic parameters and the chemical structure seems to suggest the importance of glucose presentation. Among the chemical features that seem to contribute to the longer blood half-lives, we can notice the β-glucose, the length of the linker, and possibly the freedom of –OH group at C6 of the glucosyl radical.

The Gd-DTPA derivatives, substituted by glucose units, possibly interact with a large spectrum of cells and could therefore find a broad field of applications in magnetic resonance imaging, not only for angiography, but also for the specific targeting of certain organs (ie, liver, brain) or diseases. For example, the enhanced uptake of radiolabeled glucose by infiltrated granulocytes and tissue macrophages has been already considered among the strategies in infection imaging with specific tracers.[34]

ACKNOWLEDGMENTS

The authors thank Mrs. Patricia de Francisco for her help in preparing the manuscript.

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