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RESEARCH ARTICLE

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Optimising the design of paramagnetic MRI contrast agents: influence of backbone substitution on the water exchange rate of Gd-DTPA derivatives

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Abstract Among other factors influencing the residence time of the coordinated water (τ_M) of paramagnetic contrast agents, the steric hindrance around the gadolinium ion seems to play a beneficial role. Such a crowding can be achieved by substituting the Gd-DTPA backbone on the C4 position. Several Gd-DTPA complexes carrying diverse groups at this position have thus been synthesised and characterised: Gd(S)-C_4-Me-DTPA, Gd(S)-C_4-n-Bu-DTPA, Gd(S)-C_4-iBu-DTPA, Gd(S)-C_4-iPr-DTPA, and Gd-C_4-diMe-DTPA. τ_M has been measured through the evolution of the water oxygen-17 transverse relaxation rate as a function of the temperature. The data show a reduction of τ_M of Gd(S)-C_4-Me-DTPA, Gd(S)-C_4-n-Bu-DTPA, Gd(S)-C_4-iBu-DTPA, Gd(S)-C_4-iPr-DTPA, and Gd-C_4-diMe-DTPA (τ_M^{310} = 91, 82, 108, 98, and 57 ns respectively, as compared to Gd-DTPA (τ_M^{310} = 143 ns)). At 310 K, the nuclear magnetic dispersion relaxation profiles of water protons are very similar for the five complexes which present longitudinal relaxivities slightly higher than those of Gd-DTPA. Regarding zinc transmetallation, C4-monosubstituted derivatives are more stable than Gd-DTPA. These results confirm that a judicious substitution of the DTPA skeleton allows for an acceleration of the coordinated water exchange rate. This observation can be useful for the design of vectorised contrast agents for molecular imaging.

Keywords Gd-DTPA · Gadolinium complexes · MRI · Contrast agents

Introduction

The first paramagnetic complexes of gadolinium(III) used clinically as contrast agents for (MRI) (Magnevist®, Dotarem®, ProHance®, and Omniscan®) have approximately the same efficacy at medium and high magnetic fields. They are nonspecific, distributed in the extravascular space, and quickly excreted through the kidneys. To obtain MRI paramagnetic contrast agents of better efficacy, several strategies aiming at decreasing the rotational mobility have been adopted, such as covalent [1–3] or non-covalent [4,5] grafting of small chelates to a macromolecule. However, the gain obtained through the subsequent increase of the rotational correlation time, τ_R, is often much smaller than expected because of a slow exchange rate of the water molecule(s) coordinated to the paramagnetic ion, which quenches the relaxivity enhancement. Efforts are thus devoted to optimise the water residence time, τ_M, and therefore to alleviate the limitation of the complex's relaxivity. Amide linkages have to be avoided since they are known to prolong the water residence time [6–14]. On the contrary, a C4-substitution of the ethylenic bridge of DTPA by an ethoxybenzyl group, a benzyl, or a

(4,4-diphenylcyclohexyl)phosphanooxymethyl substituent has been shown to decrease the water exchange by 25–42% [15–17]. In this work, we extend the study to other structures in order to investigate the possible effects of a steric constraint on the coordinated water exchange. A series of complexes increasingly crowded by mono- or bisubstitution on the C4 has been synthesised and characterised (Fig. 1). Various alkyl substituents (methyl, *n*-butyl, *i*-butyl, *i*-propyl) have been selected. The corresponding gadolinium complexes have been characterised by proton relaxometry at various magnetic fields and temperatures as well as by oxygen-17 relaxometry.

Materials and methods

Instrumentation

The products were identified by proton and carbon-13 NMR on a Bruker-AMX-300 (Bruker, Karlsruhe, Germany) instrument in D_2O or in $CDCl_3$. For ^{13}C NMR, *t*-butanol was used as the internal standard (methyl signal at 31.2 ppm). The abbreviations used were: "s" for singlet, "d" for doublet, "t" for triplet, "q" for quadruplet, "quin" for quintuplet, "sex" for sextuplet, and "m" multiplet. Mass spectra were obtained on a Q-tof 2 mass spectrometer (Micromass, Manchester, UK). Samples were dissolved in an MeOH/H_2O mixture (50/50) and injected at a rate of 5 µl/min. The reported mass corresponds to the most abundant isotopic peak.

^{17}O NMR measurements of solutions were performed on 2 ml samples contained in 10 mm external diameter tubes on a Bruker-AMX-300 spectrometer (Bruker, Karlsruhe, Germany). The temperature was regulated by air or nitrogen flow controlled by a BVT 2000 unit. ^{17}O transverse relaxation times of distilled water (pH = 6.5–7) were measured using a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 25 and 50 µs, respectively. ^{17}O T_2 of water in complex solution was obtained from line-width measurement. Broadband proton decoupling was applied during the acquisition of all ^{17}O NMR spectra. Concentration of the samples was lower than 25 mM.

Proton nuclear magnetic relaxation dispersion (NMRD) profiles were measured on a Stelar Spinmaster FFC, fast field cycling NMR relaxometer (Stelar, Mede (PV), Italy) over a range of magnetic fields extending from 0.24 mT to 0.35 T and corresponding to proton Larmor frequencies from 0.01 to 15 MHz. Measurements were performed on samples of 0.6 ml in 10 mm OD tubes. Additional relaxation rates at 20 (B_0 = 0.47 T), 60 (B_0 = 1.41 T) and 300 MHz (B_0 = 7.05 T) were respectively obtained on Bruker Minispec PC-20 (Bruker, Karlsruhe, Germany), Minispec mq-60 (Bruker, Karlsruhe, Germany), and the AMX-300 spectrometer. Fitting of the ^1H NMRD was adjusted with data-processing software that uses different theoretical models describing observed nuclear relaxation phenomena (Minuit, CERN Library) [18,19].

Transmetallation by zinc(II) ions was evaluated by the decrease of the water longitudinal relaxation rate at 310 K and 20 MHz (Bruker Minispec PC 20) of buffered phosphate solutions (pH = 7, $[KH_2PO_4]$ = 26 mM, $[Na_2HPO_4]$ = 41 mM) containing 2.5 mM of the gadolinium complex and 2.5 mM of Zn [20].

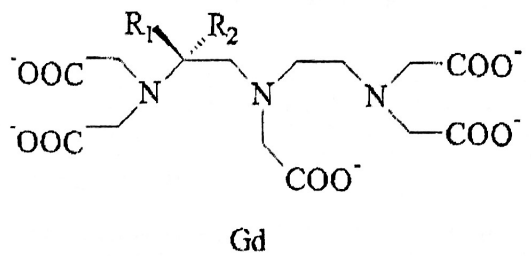

Fig. 1 Structures of complexes: Gd(S)-C_4-Me-DTPA **1**: $R_1 = CH_3$, $R_2 = H$; Gd(S)-C_4-n-Bu-DTPA **2**: $R_1 = (CH_2)_3CH_3$, $R_2 = H$; Gd(S)-C_4-iBu-DTPA **3**: $R_1 = CH_2CH(CH_3)_2$, $R_2 = H$; Gd(S)-C_4-iPr-DTPA **4**: $R_1 = CH(CH_3)_2$, $R_2 = H$; GdC_4-diMe-DTPA **5**: $R_1 = R_2 = CH_3$; Gd-DTPA **6**: $R_1 = R_2 = H$

Synthesis of the ligands

All chemicals were purchased from Sigma-Aldrich (Bornem, Belgium).

(S)-4-methyl-3,9-bis(carboxymethyl)-6-carboxymethyl- 3,6,9-triazaundecanedioic acid, (S)-C_4-Me-DTPA

Methyl L-alanine hydrochloride. Ten grams of L-alanine (122.2 mmol) were suspended in dry methanol (170 ml) under inert atmosphere at −5°C. Twelve millilitres of $SOCl_2$ (170 mmol) were added drop-wise under stirring. The solution was stirred overnight and heated for 4h. Methanol and $SOCl_2$ were evaporated under reduced pressure and the product was lyophilised.

Yd: 92%; ^1H NMR: (D_2O, δ (ppm)): 4.4 (1H, quad, CH), 4.0 (3H, s, OCH_3), 1.75 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 174.1 (CO), 52.2 (CH), 51.7 (OCH_3), 19.9 (CH_3).

(S)-2-methyl-3-oxodiethylenetriamine. 15.71 g of L-alanine methyl ester hydrochloride (112.4 mmol) were dissolved in 20 ml of dry methanol. The solution was treated with an equimolar amount of triethylamine (15.6 ml). 300 ml of ether was added under stirring to precipitate the triethylamine salt. The solution was cooled down to 0°C and stirred during 1 h. The salt was eliminated by filtration and the filtrate was evaporated under reduced pressure to obtain an oil. Under inert gas, 115 ml of ethylenediamine (freshly distilled) was added drop-wise and the solution was stirred during 18 h. After evaporation of the excess of ethylenediamine (azeotrope by addition of toluene), amide was isolated as a yellow oil which solidifies by cooling.

Yd: 61%; ^1H NMR: (D_2O, δ (ppm)): 3.35 (14H, quad, CH), 3.15 (2H, t, CH_2), 2.65 (2H, t, CH_2), 1.1 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 170.7 (CO), 54.1 (CH), 42.4 ($NHCH_2$), 41.9 (CH_2NH_2), 19.8 (CH_3).

(S)-2-methyl-diethylenetriamine trihydrochloride. 9.02g (68.8 mmol) of the (S)-2-methyl-3-oxodiethylenetriamine was suspended in 100 ml of dry THF. 260 ml of a borane solution (1 M in THF) were added drop-wise at −10°C. The mixture was stirred during 1 h at −10°C under inert atmosphere and was then left at room temperature. The solution was heated overnight and then cooled at 5°C. 80 ml of dry methanol was added slowly to destroy the borane excess. The solution was evaporated under reduced pressure and the residue was again treated with 80 ml of methanol. The solvent was evaporated and the residual oil was diluted in 100 ml of dry ethanol. The ethanolic solution was cooled in an ice bath, saturated with hydrochloric acid, and stirred for 2 h. The trihydrochloride precipitated and was collected by filtration and dried at 50°C.

Yd: 53%; ^1H NMR: (D_2O, δ (ppm)): 3.75 (1H, sex, CH), 3.25–3.55 (6H, m, 3x CH_2), 1.4 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 48 (CH), 44.2, 43.5, 34.4 (CH_2), 15.1 (CH_3).

t-Butyl (S)-4-methyl-DTPA-pentaester, t-butyl 3,9-bis[(tert-butyloxycarbonyl)methyl]-(S)-4-methyl-6-[(tert- butyloxycarbonyl)-methyl]-3,6,9-triazaundecanedioate. 36.6 mmol of (S)-2-methyldiethylenetriamine trihydrochloride (8.3 g) was suspended in 65 ml of N-ethyldiisopropylamine, 87 ml of dry DMF, and 50 ml of dry acetonitrile. Under inert atmosphere and at 5°C, 36.6 ml of *t*-butyl bromoacetate were added drop-wise. The mixture was maintained under stirring at 5°C during 1 h and at 50°C for 48 h. The filtrate was evaporated under reduced pressure until obtaining a brown oil. This oil was dissolved in 100 ml of ethyl acetate and 100 ml of water. The aqueous phase was extracted with 3 × 50 ml of ethyl acetate. Organic phases were extracted with 50 ml of water and 50 ml of a $NaHCO_3$ saturated solution. The organic phase was dried over $MgSO_4$ and evaporated. The mixture was purified by chromatography on silica (Merck 60). The column was eluted with heptane, and then with a mixture of heptane/ethyl acetate (2 : 3). Fractions containing the *t*-butyl (S)-4- methyl-DTPA-pentaester were evaporated, an oil was obtained.

Yd: 10%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.3–3.7 (11H, m, CH, 5x CH_2), 2.7–3.0 (6H, m, 3x CH_2), 1.4–1.45 (45H, m, 15x CH_3), 1.05 (3H, d, CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 173.1, 172.9, 172.8 (CO), 81.5 ($C(CH_3)_3$), 61.6 (CH-CH_2–N), 57.5, 57.1, 57.0 (CH_2COO), 57.4, 54.0, 49.9 (CH_2N), 28.3 ($C(CH_3)_3$), 14.1 (CH_3).

(S)-4-methyl-3,9-bis(carboxymethyl)-6-carboxymethyl- 3,6,9-triazaundecanedioic acid, (S)-C_4-Me-DTPA. 2.33 g (3.78 mmol) of *t*-butyl (S)-4-methyl-DTPA-pentaester were treated with 50 ml of concentrated HCl. The solution was stirred during 24 h, evaporated, dissolved in 50 ml of water, and extracted three times with 50 ml of ether. The aqueous phase was evaporated, the residue was dissolved in a minimum of water, and the pH was adjusted to 2. The sample was deposited on a cation exchange resin Dowex AG 50W-X8 (H+, 15 × 2.4 cm). The column was washed with water until a pH close to 6. The product was then eluted with 500 ml of 2 M aqueous NH_3. The ammoniac solution was evaporated and the pH adjusted to 8. The sample was deposited on an anion exchange resin Dowex AG 1X8-400 ($ClCH_2COO$-, 15 × 2.4 cm). The column was washed with water until a pH close to 6 and then eluted with 500 ml of 0.1 M $ClCH_2COOH$. The (S)-C_4-Me-DTPA was eluted with 500 ml of 0.5 M $ClCH_2COOH$. After partial evaporation and continuous extraction during 24 h with ether, the aqueous phase was evaporated and lyophilised.

Yd: 95%; ^{1}H NMR: (D_2O, δ (ppm)): 3.95 (4H, s, 2x CH_2), 3.8 (4H, s, 2x CH_2), 3.6–3.2 (5H, m, CH, 2x CH_2), 3.1–2.7 (4H, m, 2x CH_2), 1.1 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 176.8, 173.0, 172.2 (CO), 61.8, 59.1, 58.7, 56.8, 56.3, 53.5, 51.6 (CH_2 COO, CH_2N, CH), 13.1 (CH_3); ES-MS: 408 $[M+H]^+$, 430 $[M+Na]^+$.

(S)-4-i-butyl-3,9-bis(carboxymethyl)-6-carboxymethyl- 3,6,9-triazaundecanedioic acid, (S)-C_4-iBu-DTPA and (S)-4-n-butyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9- triazaundecanedioic acid, (S)-C_4-n-Bu-DTPA. L-leucine and nor-leucine were treated as described previously [16,21] for the preparation of C_4-Bn-DTPA derivatives.

Methyl L-leucine hydrochloride. Yd: 96%; ^{1}H NMR: (D_2O, δ (ppm)): 4.05 (1H, t, CH); 3.7 (3H, s, OCH_3); 1.75 (1H, m, CH); 1.65 (2H, t, CH_2); 0.85 (6H, d, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 175.0 (CO), 54.5 ($CHCO$), 53.3 (OCH_3), 43.0 (CH_2), 23.1 ($CH(CH_3)_2$), 21.9 (CH_3).

Methyl L-norleucine hydrochloride. Yd: 94%; ^{1}H NMR: (D_2O, δ (ppm)): 3.9 (1H, t, CH); 3.7 (3H, s, OCH_3); 1.6–1.45 (4H, m, 2x CH_2); 1.4(2H, quin, CH_2), 0.9 (3H, t, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 176.5 (CO), 54.4 (CH), 53.5 (OCH_3), 28.5 (CH_2-CH_2–CH_2), 27.2 (CH-CH_2), 23.2 (CH_2–CH_3), 13.9 (CH_2CH_3).

(S)-2-i-butyl-3-oxodiethylenetriamine. Yd: 61%; ^{1}H NMR: (D_2O, δ (ppm)): 3.7 (1H, t, CH); 2.8 (2H, t, CH_2); 2.6 (2H, t, CH_2); 1.7-1.5 (1H, m, CH); 1.6 (2H, t, CH_2); 0.85 (6H, d, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 170.4 (CO), 50.1 ($CHCO$), 42.4 ($NHCH_2$), 42.0 (CH_2NH_2), 41.7 (CH_2), 35.1 ($CH(CH_3)_2$), 26.6 ($CH(CH_3)_2$), 22.6 (CH_3).

(S)-2-n-butyl-3-oxodiethylenetriamine. Yd: 73%; ^{1}H NMR: (D_2O, δ (ppm)): 4.2 (1H, m, CH); 3.4 (2H, t, CH_2); 3 (2H, t, CH_2); 1.4- 1.2 (6H, m, 3x CH_2); 0.9 (3H, t, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 169.9 (CO), 51.8 ($CHCO$), 42.5 (CH_2NH), 41.9 (CH_2NH_2), 33.5 (CH-CH_2), 27.5 (CH_2–CH_2–CH_2), 22.8 (CH_2–CH_3), 13.7 (CH_3).

(S)-2-i-butyl-diethylenetriamine · trihydrochloride Yd: 60%; ^{1}H NMR: (D_2O, δ (ppm)): 3.9–3.8 (1H, m, CH); 3.6–2.9 (6H, m, 3x CH_2); 1.7–1.5 (1H, m, CH); 1.5–1.2 (2H, m, CH_2); 0.9–0.8 (6H, d, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 52.5 ($NHCH_2$), 51.5 (CH-CH_2NH), 48.1 (NH_2CHCH_2), 43.4 ($CHCH_2CH$), 30 ($(CH_3)_2CH$), 24.4 ($CH(CH_3)_2$).

(S)-2-n-butyl-diethylenetriamine · trihydrochloride Yd: 53%; ^{1}H NMR: (D_2O, δ (ppm)): 3.3 (1H, quin, CH); 2.7–2.6 (6H, m, 3x CH_2); 2.1 (2H, d, CH_2); 1.5–1.2 (6H, m, 3x CH_2); 0.9 (3H, t, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 52.5 ($NHCH_2$), 51.1 (CH-CH_2NH), 50.9 (NH_2CHCH_2), 41.4 ($CH_2CH_2NH_2$), 35.2 ($CH_2CH_2CH_2$), 26.1 (CH_2- CH_2CH_3), 22.9 (CH_2–CH_3), 14.0 (CH_3).

t-Butyl (S)-4-isobutyl-DTPA-pentaester, t-butyl 3,9-bis[(tert-butyloxycarbonyl)methyl]-(S)-4-methyl-6-[(tert-butyloxycarbonyl)methyl]- 3,6,9-triazaundecanedioate. Yd: 15%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.6–3.2 (11H, m, CH, 5x CH_2); 2.9–2.6 (6H, m, 3x CH_2); 1.55–1.5 (1H, m, CH); 1.4 (45H, s, 15x CH_3), 1.2–1.1 (2H, m, CH_2); 0.9 (6H, d, 2x CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 173.1, 172.8, 172.7 (CO), 81.4 (C-$(CH_3)_3$), 60.5 ($CHCH_2$), 58.1, 57.1, 57.0 (CH_2COO), 55.6 (N-CH-CH_2), 54.0, 49.9 (NCH_2), 45.3 (CH-CH_2–CH), 28.1 ($C(CH_3)_3$), 26.3 ($CH(CH_3)_2$), 22.1 ($CH(CH_3)_2$).

t-Butyl (S)-4-n-butyl-DTPA-pentaester, t-butyl 3,9-bis[(tert-butyloxycarbonyl)methyl]-(S)-4-methyl-6-[(tert-butyloxycarbonyl)methyl]- 3,6,9-triazaundecanedioate. Yd: 18%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.6–3.2 (11H, m, CH, 5x CH_2); 2.9 (2H, t, CH_2); 2.8- 2.6 (4H, m, 2x CH_2); 1.7–1.65 (2H, m, CH_2); 1.5–1.4 (49H, m, 2x CH_2, 15x CH_3), 0.9 (3H, t, CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 173.1, 172.8, 172.7 (CO), 81.4 (C-$(CH_3)_3$), 60.2 ($CHCH_2$), 58.6 (CH), 58.1, 57.1, 57.0 (CH_2COO), 54.0, 49.9 (NCH_2), 34.4 (CH-CH_2–CH_2), 28.3 (CH_2–CH_2–CH_3), 28.1 ($C(CH_3)_3$), 22.4 (CH_2–CH_3), 14.0 (CH_3).

(S)-4-i-butyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, (S)-C_4-iBu-DTPA. Yd: 95%; ^{1}H NMR: (D_2O, δ (ppm)): 3.9–3 (17H, m, 8x CH_2, CH); 1.5–1.2 (2H, m, CH_2); 0.8 (6H, d, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 177.5; 175.5; 170.6 (CO); 61.1; 60.1; 57.1; 56.5; 53.4; 49.6 (CH_2COO, CH_2, CH); 44.7 ($CHCH_2CH$); 26.3 ($CH(CH_3)_2$); 22.0 ($CH(CH_3)_2$). ES-MS: 450 $[M+H]^+$, 472 $[M+Na]^+$.

(S)-4-n-butyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, (S)-C_4-n-Bu-DTPA. Yd: 92%; ^{1}H NMR: (D_2O, δ (ppm)): 4.0–3.4 (11H, m, 5x CH_2, CH); 2.9–2.6 (6H, m, 3x CH_2); 1.7–1.5 (2H, m, CH_2); 1.3 (2H, quin, CH_2); 1.2 (2H, sex, CH_2); 0.9 (3H, t, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 177.5, 175.6, 170.6 (CO); 61.2, 59.7, 57.1, 56.7, 53.4, 49.6 (CH_2COO, CH_2, CH), 33.8 ($CHCH_2CH_2$), 28.4 ($CH_2CH_2CH_3$), 22.4 (CH_2CH_3), 13.9 (CH_3), ES-MS: 450 $[M+H]^+$, 472 $[M+Na]^+$.

(S)-4-isopropyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, (S)-C_4-iPr-DTPA. L-valine was treated as described previously [16,21] for the preparation of C_4-Bn-DTPA derivatives.

Methyl L-valine hydrochloride. Yd: 95%; ^{1}H NMR: (D_2O, δ (ppm)): 4.0 (1H, d, CH), 3.8 (3H, s, CH_3), 2.3 (1H, m, CH), 1.0 (3H, d, CH_3), 0.95 (3H, d, CH_3); ^{13}C NMR (D_2O, δ (ppm)): 170.3 (CO), 58.4 (CH), 53.5 (OCH_3), 29.4 ($CH(CH_3)_2$), 17.8, 17.5 (CH_3).

(S)-2-Isopropyl-3-oxodiethylenetriamine. Yd: 77%; ^{1}H NMR: (D_2O, δ (ppm)): 3.2 (2H, t, CH_2), 3.1 (1H, d, CH), 2.7 (2H, t, CH_2), 1.75 (1H, m, CH), 0.8 (3H, d, CH_3), 0.75 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 176.8 (CO), 43.4 (CH–CO), 40.2, 38.2 (N–CH_2), 31.4 ($CH(CH_3)_2$), 18.2, 17.7 (CH_3).

(S)-2-Isopropyl-diethylenetriamine trihydrochloride. Yd: 74%; ^{1}H NMR: (D_2O, δ (ppm)): 3.7–3.4 (7H, m, CH, 3x CH_2), 2.1 (1H, m, CH), 1.1 (3H, d, CH_3), 1.0 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 54.1 (CH–CH_2), 48.8, 36.9, 35.8 (CH_2), 29.2 ($CH(CH_3)_2$), 17.7, 17.6 (CH_3).

t-Butyl (S)-4-isopropyl-DTPA-pentaester, t-butyl 3,9-bis((t-butyloxycarbonyl)methyl)-(S)-4-isopropyl-6-[(t-butyloxycarbonyl)methyl]-3,6,9-triazaundecanedioate. Yd: 42%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.8 (1H, m, CH), 3.2–3.6 (12H, m, 6x CH_2), 2.7–2.9 (4H, m, 2x CH_2), 2.05 (1H, m, CH), 1.40–1.45 (45H, m, 15x CH_3), 1.1 (3H, d, CH_3), 0.95 (3H, d, CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 171.8, 170.9, 170.5 (CO), 80.9, 80.7, 80.4 ($C(CH_3)_3$), 63.4 (CH-CH_2), 56.1, 55.8, 53.2, 52.4, 44.5, 37.1 (CH_2), 31.8 ($CH(CH_3)_2$), 28.1 ($C(CH_3)_3$), 19.9, 19.3 ($CH(CH_3)_2$); MS-ES: 716 $[M+H]^+$, 738 $[M+Na]^+$.

(S)-4-Isopropyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, (S)-C4-iPr-DTPA. Yd: 56%; ^{1}H NMR: (D_2O, δ (ppm)): 3.55–4.2 (9H, m, CH, 4x CH_2), 3.1–3.5 (8H, m, 2x CH_2), 1.7 (1H, m, CH), 0.9 (3H, d, CH_3), 0.8 (3H, d, CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 179.8, 177.1, 164.0 (CO), 63.7 (CH-CH_2), 55.1, 52.7, 52.4, 51.6, 46.8, 49.1 (CH_2), 29.2 ($CH(CH_3)_2$), 16.4, 15.5 (CH_3); ES-MS: 436 $[M+H]^+$, 458 $[M+Na]^+$.

4-Dimethyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, C_4-diMe-DTPA

Methyl 2-aminoisobutyrate hydrochloride. 10 g of 2-aminoisobutyric acid (97 mmol) was suspended in 170 ml of dry methanol under inert atmosphere at −5°C. 12 ml (170 mmol) of $SOCl_2$ was added drop-wise under stirring. The solution was stirred overnight and heated during 4 h. The methanol was evaporated under reduced pressure and the product was lyophilised.

Yd: 95%; ^{1}H NMR: (D_2O, δ (ppm)): 3.85 (3H, s, OCH_3), 1.6 (6H, s, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 172.9 (CO), 57.0 (OCH_3), 53.9 ($C(CH_3)_2$), 23.2 (CH_3).

2-Dimethyl-3-oxodiethylenetriamine. 14.13 g of methyl 2-aminoisobutyrate hydrochloride (92 mmol) was dissolved in a minimum of dry methanol. The solution was processed with an equimolar amount of triethylamine (11.4 ml). 300 ml of ether was added under stirring in order to precipitate the triethylamine salt. The solution was then cooled to 0°C and stirred during 1 hour. The salt was eliminated by filtration and the filtrate was evaporated under reduced pressure to obtain an oil. 125 ml of ethylenediamine (freshly distilled) was added drop-wise under inert gas and the solution was stirred during 18 h. The excess of ethylenediamine was evaporated under reduced pressure as an azeotrope by addition of toluene. The amide was isolated as a yellow oil.

Yd: 67%; ^{1}H NMR: (D_2O, δ (ppm)): 3.25 (2H, t, CH_2), 2.75 (2H, t, CH_2), 1.3 (6H, s, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 180.8 (CO), 54.8 ($C(CH_3)_2$), 41.5, 40.1 (CH_2), 27.4 (CH_3).

N,N′-bis(t-butoxycarbonyl)-2-dimethyl-3-oxodiethylenetriamine. 7.46 g (29.3 mmol) of 2-dimethyl-3-oxodiethylenetriamine was dissolved in 70 ml of dry methanol and cooled in an ice bath. 12.22 ml (87.9 mmol) of triethylamine was added drop-wise. A solution of di-t-butyldicarbonate (14.5 g, 64.46 mmol) in dry THF (70 ml) was added drop-wise at 5°C under inert atmosphere. The mixture was stirred for 42h at room temperature and then evaporated. The residue was suspended under stirring during 30 minutes in 100 ml of water and 100 ml of ether. A white solid was recovered. The organic phase was extracted with 3 × 50 ml of an NaCl saturated solution and cooled. After one night, a precipitate appeared and was filtered. The two precipitates were suspended in ether and heated during 10 min. The solution was cooled at ambient temperature and put into the freezer overnight. The N,N′-bis(t-butoxycarbonyl)-2-dimethyl-3-oxodiethylenetriamine was filtered and washed in ether.

Yd: 44%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 7.2 (1H, broad, NH), 5.7 (1H, broad, NH), 3.35 (2H, t, CH_2), 3.25 (2H, t, CH_2), 5.1 (1H, broad, NH), 1.5 (6H, s, 2x CH_3), 1.45 (9H, s, 3x CH_3), 1.4 (9H, s, 3x CH_3); ^{13}C NMR ($CDCl_3$, δ (ppm)): 176.1 (CO), 157.4, 155.4 (COO), 80.2, 79.5 ($C(CH_3)_3$), 56.9 ($C(CH_3)_2$), 40.9, 40.3 (CH_2), 28.8 ($C(CH_3)_3$), 25.9 ($C(CH_3)_2$); MS-ES: 346 $[M+H]^+$, 368 $[M+Na]^+$.

2-Dimethyl-diethylenetriamine trihydrochloride. 4.4g (12.74 mmol) of the N-(t-butoxycarbonyl)-2-dimethyl-3-oxodiethylenetriamine was dissolved in 50 ml of dry THF under inert atmosphere. 50.96 ml of a borane solution (1 M in THF) was added drop-wise at −10°C. The mixture was stirred for 1 h at −10°C, then 16 h at ambient temperature, and finally heated during 3 h to reflux. 60 ml of dry methanol was slowly added at 5°C to destroy the excess of borane. The solution was evaporated under reduced pressure and the residue treated with 75 ml of dry ethanol. The solution was then cooled in an ice bath, saturated with hydrochloric acid, agitated for 2 h, and cooled in the freezer overnight. The trihydrochloride precipitates were collected by filtration and dried with a drying pistol at 50°C.

Yd: 41%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.4 (4H, m, 2 × CH_2), 3.3 (2H, s, CH_2), 1.4 (6H, s, 2x CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 54.9 ($C(CH_3)_2$), 52.9, 35.7, 33.5 (CH_2), 23.6 (CH_3).

t-Butyl 4-dimethyl-DTPA-pentaester, t-butyl 3,9-bis((tert-butyloxycarbonyl)methyl)-4-dimethyl-6-[(t-butyloxycarbonyl)methyl]-3,6,9-triazaundecanedioate. 5.2 mmol of 2-dimethyl-diethylenetriamine trihydrochloride (7.75 g) was solubilised in 8.7 ml of N-ethyldiisopropylamine (52 mmol) and 30 ml of dry DMF. 5.37 ml of t-butyl bromoacetate (36.4 mmol) dissolved in 20 ml of dry DMF was added drop-wise under inert atmosphere and at 5°C. The mixture was maintained under stirring at 5°C for 1 h and at 50°C during 48 h. The solution was evaporated under reduced pressure until obtaining an oil. This oil was dissolved in 50 ml of ethyl acetate and 50 ml of water. The aqueous phase was extracted with 3 × 30 ml of ethyl acetate. Organic phases were extracted with 50 ml of water and 50 ml of an $NaHCO_3$ saturated solution. The organic phase was dried over $MgSO_4$ and evaporated. The mixture was purified by chromatography on silica, deposited on a column, and eluted with a gradient ether–hexane. Fractions containing t-butyl 4-dimethyl- DTPA-pentaester were evaporated and an oil was obtained.

Yd: 36%; ^{1}H NMR: ($CDCl_3$, δ (ppm)): 3.55 (2H, s, CH_2), 3.5 (4H, s, 2x CH_2), 3.45 (4H, s, 2x CH_2), 2.6–2.5 (6H, m, 3x CH_2), 1.45 (45H, s, 15x CH_3), 1.1 (6H, s, 2x CH_3); ^{13}C NMR: ($CDCl_3$, δ (ppm)): 172.5, 172.1, 170.9 (CO), 81.0, 80.6, 80.4 ($C(CH_3)_3$), 59.2 (CCH_2), 57.2 ($C(CH_3)_2$), 56.4, 56.0, 54.9 (CH_2COO), 53.0, 51.8 (NCH_2), 28.5, 28.4, 28.3 ($C(CH_3)_3$), 24.2 (CH_3); MS-ES: 702 $[M+H]^+$, 724 $[M+Na]^+$.

4-Dimethyl-3,9-bis(carboxymethyl)-6-carboxymethyl-3,6,9-triazaundecanedioic acid, C_4-diMe-DTPA. 1.31 g (2.09 mmol) of the 4-dimethyl-DTPA-pentaester was treated with 45 ml of concentrated HCl. The solution was stirred during 24 h at ambient temperature, filtered, and evaporated. The residue was dissolved in a minimum of water and the pH was adjusted to 10 with a $NaHCO_3$

saturated solution. The aqueous phase was extracted with 5 × 30 ml of ethyl acetate and concentrated by evaporation. The pH of the solution was adjusted to 2 with HCl 3 M. The sample was deposited on a cation exchange resin Dowex AG 50W-X8 (H +, 15 × 2.4 cm). The column was washed with water until obtaining a pH close to 6. The C_4-diMe- DTPA was eluted with 500 ml of 2 M aqueous NH_3. The ammonia solution was evaporated, dissolved in a minimum of methanol, filtered, and poured on cold acetone. C_4-diMe-DTPA precipitated and was filtered.

Yd: 86%; ^1H NMR: (D_2O, δ (ppm)): 3.8 (4H, s, 2x CH_2), 3.6 (4H, m, 2x CH_2), 3.2–3.4 (4H, s, 2x CH_2), 2.7–3.0 (4H, m, 2x CH_2), 1.3 (6H, s, 2x CH_3); ^{13}C NMR: (D_2O, δ (ppm)): 177.5; 174.5; 170.6 (CO); 66.3; 62.1; 57.2; 57.1; 54.4; 53.9; 49.6 (CH_2, $C(CH_3)_2$); 24.3 (CH_3); ES-MS: 488 $[M+3Na]^+$.

Synthesis of the corresponding Gd-complexes

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 gadolinium ions was checked with arsenazo (III) indicator. The mass of the complexes was confirmed by ES-MS.

Gd(S)-C_4-Me-DTPA **1**: 562 $[M+H]^+$, 584 $[M+Na]^+$; Gd(S)-C_4-n-Bu-DTPA **2**: 604 $[M+H]^+$, 626 $[M+Na]^+$, Gd(S)-C_4-iBu-DTPA **3**: 604 $[M+H]^+$, 626 $[M+Na]^+$; Gd(S)-C_4-iPr-DTPA **4**: 590 $[M+H]^+$, 612 $[M+Na]^+$; Gd C_4-diMe-DTPA **5**: 576 $[M+H]^+$, 598 $[M+Na]^+$.

Results

Synthesis of the ligands

C_4 monoalkyl derivatives. The 13-step synthesis of (S)-C_4-Me-DTPA has been described by Grote et al. [22] but, considering the length of this procedure, we have preferred the 5-step experimental protocol of Brechbiel [23] using alanine as the starting compound (Fig. 2, R = methyl). The same pathway was used to obtain (S)-C_4-n-Bu-DTPA, (S)-C_4-iBu-DTPA, and (S)-C_4-iPr-DTPA (Fig. 2).

C_4 dimethyl derivative. C_4-diMe-DTPA was synthesised from 2-aminoisobutyric acid (Fig. 3). The 2,2-dimethyl-diethylenetriamine could not be obtained directly by reduction of 2,2-dimethyl-3-oxodiethylenetriamine because of its poor solubility in aprotic solvents. To improve its solubility in an apolar medium, two *t*-butoxycarbonyl groups (BOC) were therefore added to the structure of this intermediate. This modification has allowed us to reduce the BOC compound and, after cleavage of BOC groups, to obtain the diethylenetriamine derivative, precursor of the C_4-DiMe-DTPA obtained by alkylation.

Physicochemical characterisation

Assessment of the residence time of the coordinated water. At 0.47 T, the proton relaxivities of the five new Gd-DTPA derivatives increase when the temperature is lowered from 318 to 278 K indicating that at low temperatures there are no limitations by the water residence time (Fig. 4.)

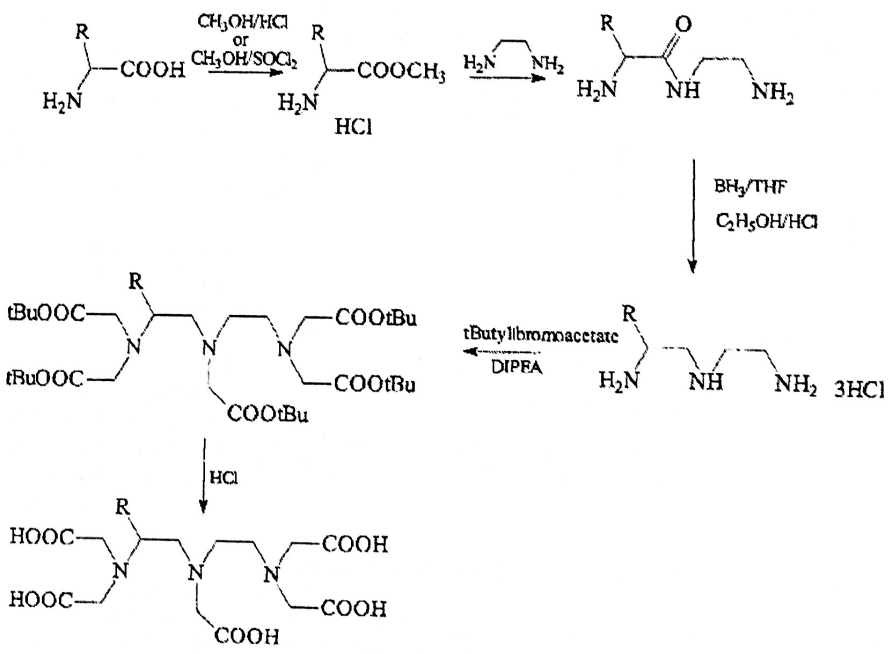

Fig. 2 Synthesis of (S)-C_4-Me-DTPA (R = methyl), (S)-C_4-n-Bu-DTPA (R = *n*-butyl), (S)-C_4-iBu-DTPA (R = isobutyl), and (S)-C_4-iPr-DTPA (R = isopropyl)

Fig. 3 Synthetic route of the C_4-diMe-DTPA

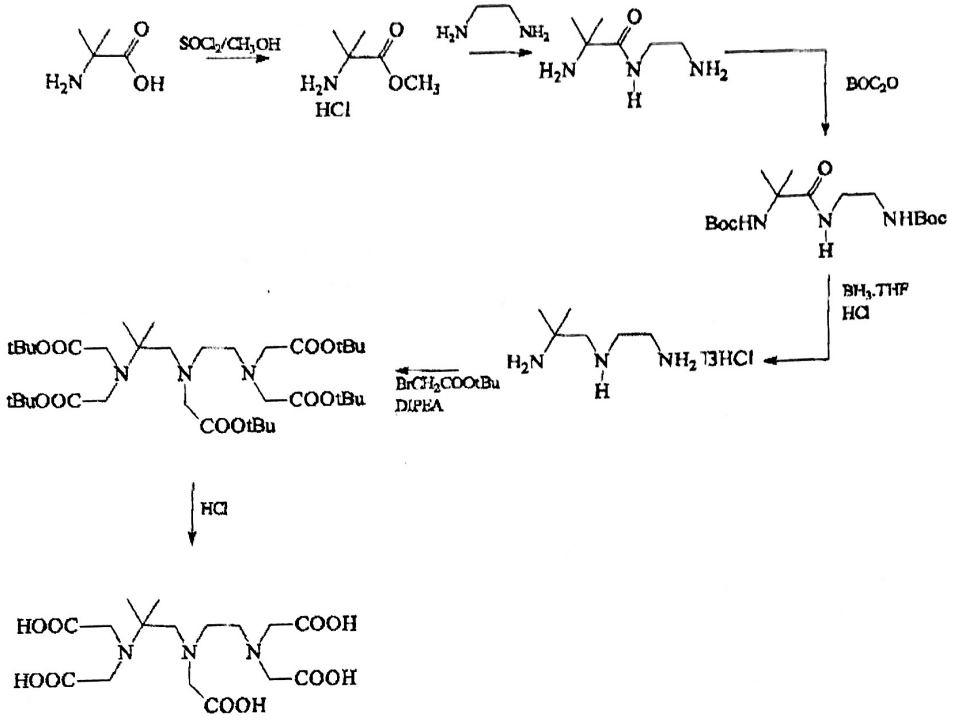

Figure 5 shows the evolution with temperature of the transverse relaxation rate of the water oxygen-17 nucleus in aqueous solutions of the five substituted Gd-complexes and of the parent complex. Whereas the maximum of the curve for Gd-DTPA **6** corresponds to a temperature of approx. 310 K, it is significantly shifted towards lower temperatures for the C-substituted structures, qualitatively indicating a reduction of the residence time. The theoretical adjustment of the data performed as previously described [15–17] allows for the determination of various parameters: $A/\hbar$, the hyperfine coupling constant between the oxygen nucleus of bound water molecules and the Gd^{3+} ion; τ_V, the correlation time modulating the electronic relaxation of Gd^{3+}; E_v, the activation energy related to τ_V; B, related to the mean-square of the zero field splitting energy Δ ($B = 2.42$); and $\Delta H^{\#}$ and $\Delta S^{\#}$, the enthalpy and entropy of activation of the water exchange process. By comparison with the values obtained for other Gd-DTPA derivatives, the number of coordinated water molecules was assumed to be equal to one. The calculated parameters are shown in Table 1.

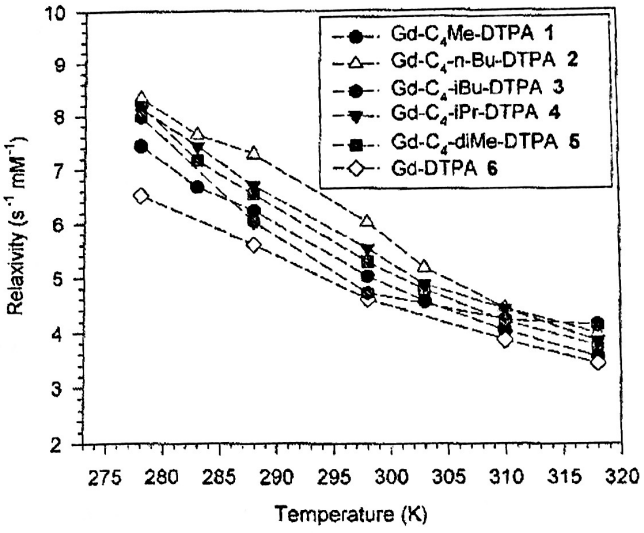

Fig. 4 Evolution of the proton relaxivity vs. temperature for the C_4-substituted Gd-DTPA **1–5** and for the parent compound **6**

Proton NMRD profiles. The proton relaxivity profiles of the complexes are rather similar and the values are slightly larger than those of the parent complex (Fig. 6). The overall variations of the relaxivities around the mean values are equal to ±7% and ±4% at low ($B_0 = 1$ mT, $\nu_0 = 0.04$ MHz) and high ($B_0 = 1.41$ T, $\nu_0 = 60$ MHz) magnetic fields respectively. The experimental data were fitted to the classical models of innersphere [24,25] and outersphere [26] interactions using standard procedures

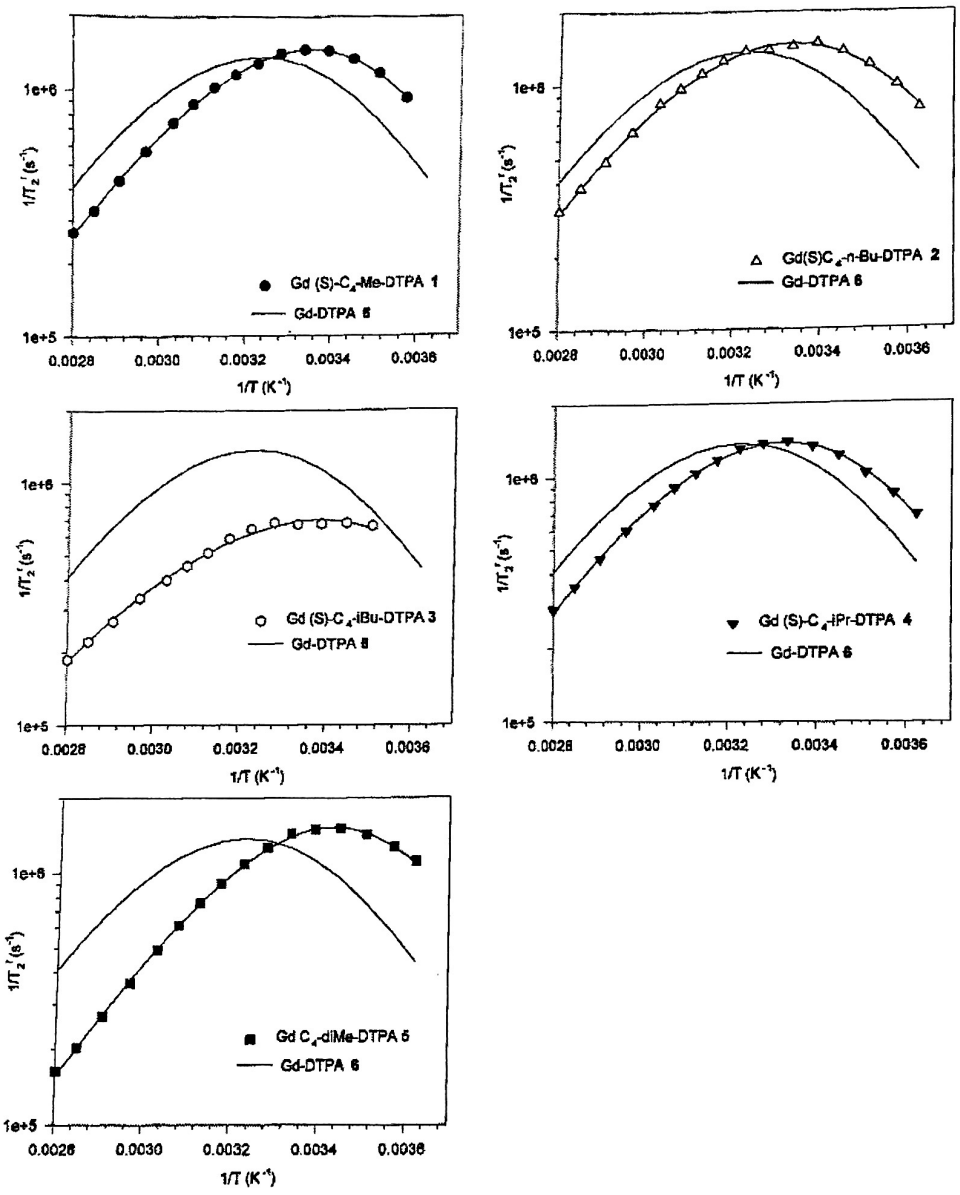

Fig. 5 Evolution of the reduced transverse relaxation rate of the water ^{17}O vs. temperature of the Gd complexes ($1/T_2^r = 55.55/(T_2^o * [\text{Gd-complex}])$)

[18,19]. The number of coordinated water molecules was fixed to 1, the distance of closest approach was set to 0.36 nm, the relative diffusion constant was 3.3×10^{-9} m^2/s, τ_M^{310} was fixed to the value determined by ^{17}O relaxometry, and the distance r for the innersphere interaction was set to the usual value of 0.31 nm. The parameters describing the electronic relaxation rates of Gd^{3+} (τ_V and the electronic relaxation rate at very low fields, τ_{SO}), as well as the rotational correlation time τ_R, were adjusted. The results of the fitting procedures are shown on Fig. 6 and Table 2.

Transmetallation by zinc(II) ions. As previously shown [20], transmetallation of Gd complexes by zinc(II) ions in a buffered solution (pH 7) containing phosphate ions, induces a decrease of the proton relaxation rates due to the precipitation of the released gadolinium ions. The time required to reach a ratio $R_1^p(t)/R_1^p(t=0)$ equal to 80% is similar or larger for the C_4 monosubstituted complexes ($t \approx 200$, 200, 450, 500, 1100 min for Gd-DTPA **6**, Gd(S)-C_4-iBu-DTPA **3**, Gd(S)-C_4-Me-DTPA **1**, Gd(S)-C_4-n-Bu-DTPA **2**, and Gd(S)-C_4-iPr-DTPA **4** respectively) and markedly

Table 1 Parameters of the theoretical adjustment of the ^{17}O relaxometric data

Complexes	τ_M^{298} (ns)	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (J mol^{-1} K^{-1})	A/$\hbar$ (10^6 rad s^{-1})	B (10^{20} s^{-2})	τ_c^{298} (ps)	E_V (kJ mol^{-1})
Gd-DTPA **6**	331 ± 60	51.5 ± 0.3	52.1 ± 0.6	−3.4 ± 0.1	2.6 ± 0.1	12.3 ± 0.3	4.5 ± 4.2
Gd (S)-C_4-Me-DTPA **1**	201 ± 24	48.7 ± 0.2	46.8 ± 0.3	−3.1 ± 0.1	2.1 ± 0.1	13.7 ± 0.8	12.0 ± 3.7
Gd(S)-C_4-n-Bu-DTPA **2**	179 ± 16	47.1 ± 0.2	42.5 ± 0.3	−3.3 ± 0.1	2.8 ± 0.2	15.3 ± 0.9	5.9 ± 0.9
Gd(S)-C_4-iBu-DTPA **3**	247 ± 20	50.0 ± 0.1	49.4 ± 0.3	−2.8 ± 0.1	4.0 ± 0.1	10.7 ± 0.2	9.9 ± 0.6
Gd(S)-C_4-iPr-DTPA **4**	219 ± 22	49.0 ± 0.1	47.1 ± 0.6	−3.1 ± 0.1	1.7 ± 0.1	10.1 ± 0.3	9.0 ± 4.8
GdC_4-diMe-DTPA **5**	126 ± 13	47.6 ± 0.2	47.1 ± 0.4	−2.8 ± 0.2	1.6 ± 0.5	13.5 ± 0.4	12.2 ± 5.7

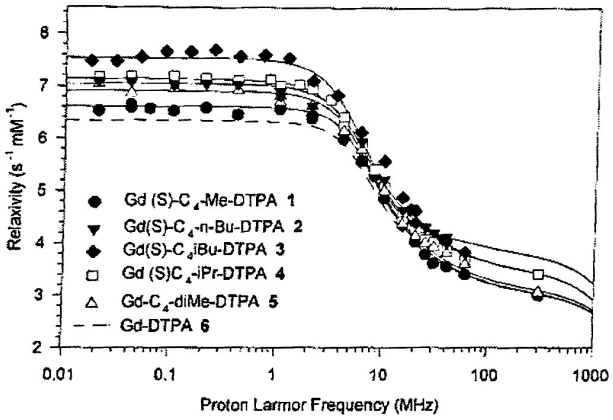

Fig. 6 Proton NMRD profiles of the C_4-substituted complexes (solvent: water, temperature: 310 K)

decreased for Gd-C_4-diMe-DTPA **5** ($t \approx 20$ min) (Fig. 7). The values of the ratio $R_1^p(t)/R_1^p(t=0)$ after 5500 min are larger for all the monosubstituted complexes as compared to Gd-DTPA ($R_1^p(t = 5500$ min$)/R_1^p(t=0) = 48$, 55, 55, 56, and 68% for Gd-DTPA **6**, Gd(S)-C_4-Me-DTPA **1**, Gd(S)-C_4-n-Bu-DTPA **2**, Gd(S)-C_4-iBu-DTPA **3** and Gd(S)-C_4-iPr-DTPA **4** respectively) whereas it is very low for the disubstituted complex Gd-C_4-diMe- DTPA **5** ($R_1^p(t = 5500$ min$)/R_1^p(t=0) < 4\%$).

Discussion

A simple approach to detect a possible limitation of the proton relaxivity by the water residence time is to study the effect of temperature on the proton relaxivity. If the innersphere [24,25] and outersphere [26] interactions contribute to the observed relaxivity, an increase of the relaxivity when temperature is lowered indicates that the water exchange is not limiting. On the contrary when the relaxivity is limited by the water exchange, a plateau or a decrease of the relaxivity is observed at low temperatures. The temperature dependence observed for the proton relaxivity of all the complexes studied in this work indicates thus that, in the temperature range investigated, the exchange rate of the coordinated water molecule does not limit the relaxivity (or does so very little at low temperatures).

The water residence time was quantitatively assessed through the well-established analysis of the temperature dependence of the transverse relaxation rates of the oxygen-17 nucleus of water [7, 8, 10–17, 27]. The calculated values are in agreement with a reduction of the water residence times as shown in Table 1. The results indicate a clear difference between the unsubstituted **6** ($\tau_M^{310} = 143$ ns), monosubstituted **1, 2, 3, 4** ($\tau_M^{310} = 91$, 82, 108, and 98 ns respectively) and bisubstituted **5** ($\tau_M^{310} = 57$ ns) compounds. The values obtained for the monosubstituted complexes are in fairly good agreement with the values previously reported for

Table 2 Proton relaxivity at 20 MHz and values of τ_M, τ_R, τ_{SO}, and τ_V obtained from the theoretical adjustment of the proton NMRD profiles

Complexes	r_1^{310} (s^{-1} mM^{-1}, 20 MHz)	τ_M^{310a} (ns)	τ_R^{310} (ps)	τ_{SO}^{310} (ps)	τ_V^{310} (ps)
Gd-DTPA 6	3.8	143	59	82 (67)	23
Gd(S)-C4-Me-DTPA 1	4.1	91	57	88 (84)	20
Gd(S)-C4-n-Bu-DTPA 2	4.5	82	71	87 (51)	22
Gd(S)-C4-iBu-DTPA 3	4.4	108	70	111 (55)	26
Gd(S)-C4-iPr-DTPA 4	4.4	98	68	96 (131)	20
GdC4-diMe-DTPA 5	4.2	57	60	97 (111)	20

[a] τ_M was fixed to the values obtained by ^{17}O NMR
The values in parentheses are calculated from the B and τ_V values obtained from the analysis of the O-17 T_2 data

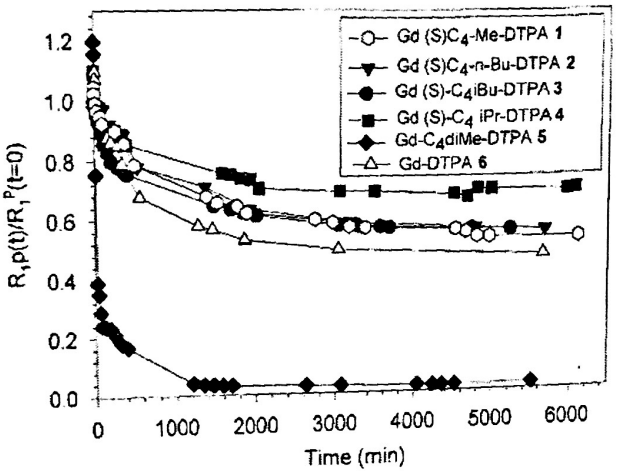

Fig. 7 Evolution of the ratio $R_1^p(t)/R_1^p(t=0)$ as a function of time during transmetallation process for the complexes **1–6**

Gd(S)EOB-DTPA ($\tau_M^{310} = 82$ ns) [15], MS-325 ($\tau_M^{310} = 83$ ns) [17], Gd(S)Bn-DTPA ($\tau_M^{310} = 87$ ns) [16], and Gd(R)Bn-DTPA ($\tau_M^{310} = 108$ ns) [16].

As observed for bisamide complexes [13], a crowding around the first coordination sphere seems to be favourable to the exchange. Obviously, such an effect is produced by the presence of alkyl groups in the C-4 position of the skeleton. The effect of the substituents on the water exchange rate of Gd-DTPA derivatives could also be attributed to differences in the concentrations of their stereoisomers. It has indeed been reported that both stereoisomers of Eu(DOTAM) are characterised by markedly different exchange rates [28,29]. A recent work has however ruled out a major influence of the coexistence of stereoisomers in the case of bisamide derivatives of Gd-DTPA [30].

The proton relaxivities observed at all magnetic fields and at 310 K are quite similar and only slightly higher than to those of Gd-DTPA. This slight difference results mainly from the increase of the rotational correlation times in agreement with the molecular weights of the compounds. The variation of the τ_M values has no effect on the relaxivity observed at 310 K for such small complexes. However, if included in slowly rotating structures, the smaller value of τ_M could result in a significant increase of relaxivity.

The agreement between the fitted parameters characterising the electronic relaxation rates obtained either from the O-17 data (B and τ_V) or from the proton NMRD data (τ_{SO} and τ_V, with $\tau_{SO} = (5B\tau_V)^{-1}$) is quite good for complexes **1**, **4**, **5** and **6** but is not satisfactory for the two other complexes (**2** and **3**). The differences between the values obtained by each experimental approach can be related firstly, to the fact that the influence of τ_{SO} is predominant at low fields in the proton NMRD curves of small complexes whereas the O-17 data are obtained at much higher field and secondly, to the simplified model used for the analysis of our O-17 T_2 data. More sophisticated models involving other contributions, and thus additional parameters for the description of the the electronic relaxation rates like that reported by Powell et al. [8], could probably improve the agreement.

Regarding the stability versus transmetallation by zinc(II) ions, the kinetic index (time required to reach the ratio $R_1^p(t)/R_1^p(t=0) = 80\%$) and the thermodynamic index (value of the ratio $R_1^p(t)/R_1^p(t=0)$ at 5500 min) show, as previously reported [17,20], that a monosubstitution on the C_4 of the ethylenic bridge is beneficial for the kinetic and the thermodynamic stability. On the contrary, a disubstitution on the same carbon (compound 5) has a clearly unfavourable effect.

In summary, five new ligands of the DTPA family have been synthesised with a yield of 3–13%. As compared to Gd-DTPA, the coordinated water exchange rate of the gadolinium complexes increases at 310 K by 30–90% on monosubstitution and by 150% on bisubstitution and therefore offers the possibility to move the limits of relaxivity of macromolecular systems towards higher values. However, if all the monosubstituted derivatives show an increased stability as compared to Gd-DTPA with respect to transmetallation, the disubstituted compound **5** is clearly very sensitive to this process. In spite of the apparent increase of effort required by the syntheses, this substitution strategy can be useful in the quest for paramagnetic MRI contrast agents of higher relaxivities.

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