

Stability of MRI Paramagnetic Contrast Media

A Proton Relaxometric Protocol for Transmetallation Assessment

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RATIONALE AND OBJECTIVES. The suitability of paramagnetic complexes as magnetic resonance contrast agents depends on various factors such as their relaxivity, stability, selectivity, and the inertness toward transmetallation by endogenous ions. The transmetallation of a series of 18 gadolinium complexes by the Zn^{2+} ion was studied in vitro by proton relaxometry.

METHODS. Transmetallation was analyzed through the evolution of the paramagnetic longitudinal relaxation rate of water protons at 37° C in pH = 7 phosphate buffer solutions containing 2.5 mmol/L of the gadolinium complexes and 2.5 mmol/L zinc chloride. The measurements were performed at 0.47 T over a period of at least 3 days.

RESULTS. The results confirm the high stability of macrocyclic systems and a high sensitivity of Gd-diethylenetriaminepentaacetic acid (DTPA) derivatives to transmetallation by Zn^{2+} ions. The decreasing order of stability with respect to metal exchange is as follows: Gd-macrocyclics > Gd-C-functionalized DTPA > Gd-DTPA > primary and secondary Gd-DTPA bisamides. The ternary bisamide analyzed in this study is more stable than the parent compound Gd-DTPA.

CONCLUSIONS. A simple relaxometric protocol has been successfully developed to study the in vitro transmetallation process of gadolinium complexes. The importance of the functionalization and substitution of the DTPA-like complexes is clearly shown.

KEY WORDS. Gd-DTPA; gadolinium complexes; transmetallation; stability; MR contrast agents.

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
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PARAMAGNETIC GADOLINIUM complexes are nowadays widely used as contrast agents in clinical MRI. At clinical doses these molecules are nontoxic, although individually, their components—the metal ion and the free organic ligand—usually are. Gadolinium can inhibit neuromuscular transmission by blocking calcium channels^{1,2} and may be deposited as insoluble salts in bones, liver, and spleen.³ Animal studies have indeed demonstrated a long-term retention of gadolinium in the body.⁴⁻⁶ The kinetics and thermodynamics of decomplexation and transmetallation are therefore matters of great concern. In a previous study,^{7,8} phosphorus nuclear magnetic resonance (NMR) measurements showed that, contrary to the macrocyclic systems, linear chelates of gadolinium partially release the metal in the presence of ATP. Compared with the parent compound Gd-diethylenetriaminepentaacetic acid (DTPA), the process is slower for C₄-substituted systems like Gd-EOB-DTPA but faster and more extensive for Gd-DTPA-BMA.

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It has also been demonstrated that gadolinium chelates could be sensitive to transmetallation by endogenous ions⁹⁻¹²; in such a process, the paramagnetic ion is expelled from the complex. Endogenous ions likely to compete with Gd^{3+} are Cu^{2+} , Ca^{2+} , and Zn^{2+} . Among these, Cu^{2+} is present in very small concentrations in the blood (1–10 $\mu\text{mol/L}$),¹³ whereas Ca^{2+} has relatively low affinity for organic ligands like DTPA and DTPA-BMA (roughly 10 orders of magnitude lower than Gd^{3+}).¹⁴ Only Zn^{2+} can displace a significant amount of gadolinium because the concentration of the former in blood is relatively high (55–125 $\mu\text{mol/L}$), and its association constant toward DTPA and DOTA is only about four orders of magnitude lower than for Gd^{3+} .¹⁴ The stability of the gadolinium complexes in the presence of Zn^{2+} is thus an important issue, because, as mentioned above,

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transmetallation will induce a release of Gd^{3+} into the body and possible depletion of the endogenous ion subsequent to its elimination as a hydrophilic complex by the kidneys.

Various methods have been used to assay the extent of transmetallation of MRI contrast agents in vitro and in vivo: high-performance liquid chromatography (HPLC)⁹; inductively coupled plasma atomic emission spectrometry¹⁰; blood pressure measurements after injection of Gd-DTPA and Gd-DTPA-BMA, with and without addition of Ca^{2+} ¹⁵; measurements of Ca^{2+} by bioluminescence¹⁶; and colorimetry.^{17,18}

In the current report, we propose an easy, fast, and reliable in vitro relaxometric method that allows quantitative evaluation of the transmetallation of gadolinium complexes. In this study, the protocol is applied to the transmetallation by zinc of a large series of macrocyclic and open-chain complexes of gadolinium (Table 1).

Materials and Methods

Chemicals

The phosphate buffer ($[KH_2PO_4] = 0.026$ mol/L, $[Na_2HPO_4] = 0.041$ mol/L) was purchased from Merck (Overijse, Belgium), the $ZnCl_2$ from Fluka (Bornem, Belgium), and the organic products (solvents, amines, DTPA, etc) from Aldrich (Bornem, Belgium). The complexes Gd-DOTA 1, Gd-HP-DO3A 2, and Gd-DTPA-BMA 5, respectively, were supplied by Guerbet (Aulnay-sous-Bois, France), Bracco (Milan, Italy), and Nycomed (Oslo, Norway). Gd-DTPA 3, (S)-Gd-EOB-DTPA 15, and MS-325 17 were provided by Schering (Berlin, Germany).

Analytic Instrumentation

High-performance liquid chromatography was performed on a Waters 600 multisolvent delivery system equipped with a Rheodyne injection valve (20- μ L loop) and a Novaapak C18 column (4.56 mm \times 150 mm) and controlled by MILLENNIUM software (all from Waters, Milford, MA). Elution was performed with a linear gradient of pure 0.05 mol/L triethylammonium acetate (pH = 6) to 100% methanol at a flow rate of 1 mL/min for 20 minutes. An ultraviolet/diode array or a fluorescence detector was used to monitor elution of the ligand or complex. Mass spectra (LSIMS) were recorded on a VG Autospec mass spectrometer (VG Analytical, Manchester, UK). Proton (1H) and ^{13}C high-resolution NMR spectra were recorded on a Bruker AMX-300 instrument with a solvent of deuterium oxide (D_2O). For ^{13}C NMR, *tert*-butanol was used as an internal standard (methyl signal at 31.2 ppm). Water proton relaxation rates were measured at 310° K and 0.47 T on a Bruker Minispec PC-20 (Bruker, Karlsruhe, Germany).

Synthesis of the Ligands

The (S)-Gd- C_4 -benzyloxy (Bz)-DTPA 16 was obtained from commercially available amino acid as described elsewhere.¹⁹ The bisbenzylamide 13 was obtained by reaction of DTPA bisanhydride with benzylamine.¹⁹ The DTPA-

bisamides 4, 6, 7, 8, 9, 10, 11, and 12 were prepared as described by Geraldes et al.²⁰ In brief, DTPA-bisanhydride was added in small portions to a solution of the appropriate amine dissolved in dimethylformamide. The pH was adjusted to 9 with sodium hydroxide, and the mixture was stirred at ambient temperature for 6 hours and filtered to remove insoluble impurities. The filtrate was evaporated under reduced pressure, the residue was treated with acetone, and the viscous solution was kept at 0° C for 12 hours to induce crystallization. The resulting solid was filtered, recrystallized from a mixture of ethanol/isopropanol, and dried to constant weight. Purity was established by HPLC. The ligands were identified by mass spectrometry (LSIMS) and by ^{13}C and 1H high-resolution NMR. The structures of the bisamide derivatives of DTPA studied in this work are given in Table 1.

1,11-Bisamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BA, 4. ²⁰Yield: 73.5%. Mass spectra (LSIMS): $[(M+1)]^+ = 392^+$; 1H NMR (D_2O , δ , in ppm) = 3.2 (4H, singlet, $2 \times CH_2$), 3.1 (4H, singlet, $2 \times CH_2$); 3 (2H, singlet, CH_2), 2.9 to 2.7 (8H, multiplet, $4 \times CH_2$); ^{13}C NMR (D_2O , δ , in ppm) 178.7, 178.1, 171.5, 61.8, 57.6, 56.6, 54.7, 51.3. HPLC = 1.5 minutes.

1,11-Bisethylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BEA, 6. ²⁰Yield: 76%. Mass spectra (LSIMS): $[(M+1)]^+ = 448^+$; 1H NMR (D_2O , δ , in ppm) = 3.1 to 2.9 (14H, multiplet, $7 \times CH_2$), 2.6 to 2.45 (8H, multiplet, $4 \times CH_2$), 0.9 (6H, triplet, $2 \times CH_3$); ^{13}C NMR (D_2O , δ , in ppm) = 178.3, 178.1, 172.1, 61.1, 57.1, 56.9, 52.8, 51.2, 34.8, 13.6. HPLC = 1.9 minutes.

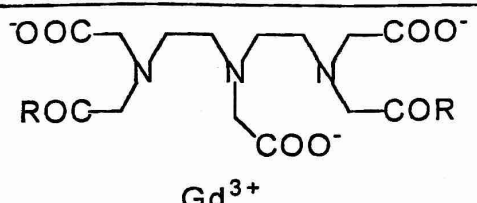
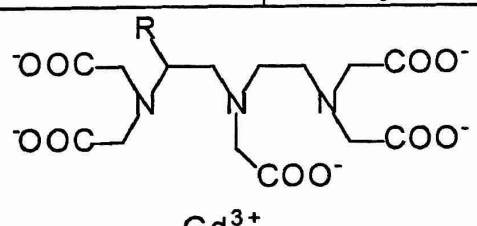
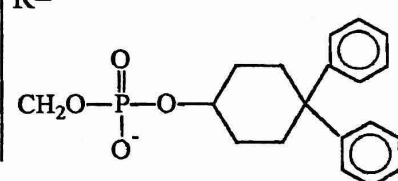
1,11-Bisbutylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BnBA, 7. ²⁰Yield: 71%. Mass spectra (LSIMS): $[(M+1)]^+ = 504^+$; 1H NMR (D_2O , δ , in ppm) = 3.3 to 3 (14H, multiplet, $7 \times CH_2$), 2.7 to 2.5 (8H, multiplet, $4 \times CH_2$), 1.6 to 1.4 (4H, multiplet, $2 \times CH_2$), 1.35 to 1.2 (4H, multiplet, $2 \times CH_2$), 0.9 (6H, triplet, $2 \times CH_3$); ^{13}C NMR (D_2O , δ , in ppm) = 178.0, 177.6, 170.2, 61.6, 57.5, 56.8, 52.5, 52.1, 41.5, 33.8, 20.2, 13.7. HPLC = 8.5 minutes.

1,11-Bisisobutylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BiBA, 8. ²¹Yield: 76%. Mass spectra (LSIMS): $[(M+1)]^+ = 504^+$; 1H NMR (D_2O , δ , in ppm) = 3.1 (4H, singlet, $2 \times CH_2$), 3.0 (4H, singlet, $2 \times CH_2$), 2.9 (2H, singlet, CH_2), 2.8 (4H, singlet, $2 \times CH_2$), 2.5 to 2.35 (8H, multiplet, $4 \times CH_2$), 1.6 (2H, multiplet, $2 \times CH$), 0.8 (12H, doublet, $4 \times CH_3$); ^{13}C NMR (D_2O , δ , in ppm) = 178.5, 178.2, 170.0, 61.4, 57.1, 57.0, 52.8, 51.9, 48.5, 28.9, 18.7. HPLC = 12.2 minutes.

1,11-Bis-*tert*-iobutylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BtBA, 9. Yield: 35.6%. Mass spectra (LSIMS): $[(M+1)]^+ = 504^+$; 1H NMR (D_2O , δ , in ppm) = 3.6 (4H, singlet, $2 \times CH_2$), 3.5 (4H,


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TABLE 1. Structure of DTPA-Bisamides 4-13, Bisester 14, and C-Functionalized Compounds 15-18

	
Gd DTPA-BA 4 Gd-DTPA-BMA 5 Gd-DTPA-BEA 6 Gd-DTPA-BnBA 7 Gd-DTPA-BiBA 8 Gd-DTPA-BtBA 9 Gd-DTPA-BHA 10 Gd-DTPA-BcHA 11 Gd-DTPA-BBMA 12 Gd-DTPA-BBzA 13 Gd-DTPA-BME 14	R=NH ₂ R=NHCH ₃ R=NHCH ₂ CH ₃ R=NH(CH ₂) ₃ CH ₃ R=NHCH ₂ CH(CH ₃) ₂ R=NHC(CH ₃) ₃ R=NH(CH ₂) ₅ CH ₃ R=NHcyclohexyl R=N(CH ₃) ₂ R=NHCH ₂ φ R=OCH ₃
	
(S) Gd-EOB-DTPA 15 (S) Gd-C ₄ Bz-DTPA 16 MS-325 17	R=CH ₂ φOC ₂ H ₅ R=CH ₂ φ R= 
(R,S) Gd-HM-DTPA 18	R=CH ₂ OH

singlet, 2× CH₂), 3.4 (2H, singlet, CH₂), 3.3 to 3.15 (8H, multiplet, 4× CH₂), 1.2 (18H, singlet, 6× CH₃); ¹³C NMR (D₂O, δ, in ppm) = 178.3, 178.1, 170.3, 61.8, 58.1, 57.0, 52.3, 51.8, 41.3, 30.8. HPLC = 6.2 minutes.

1,11-Bis(hexylamino)-1,11-dioxo-3,6,9-triaza-3,6,9-tris(carboxymethyl)undecane: DTPA-BHA, 10. ²⁰Yield: 89%. Mass spectra (LSIMS): [(M+1)]⁺ = 560⁺; ¹H NMR (D₂O, δ, in ppm) = 3.2 to 2.9 (14H, multiplet, 7× CH₂), 2.6 to 2.45 (8H,

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multiplet, $4 \times \text{CH}_2$), 1.6 (4H, quintuplet, $2 \times \text{CH}_2$), 1.4 to 1.3 (12H, multiplet, $6 \times \text{CH}_2$), 0.9 (6H, triplet, $2 \times \text{CH}_3$); ^{13}C NMR (D_2O , δ , in ppm) = 179.2, 178.7, 171.1, 61.8, 58.5, 55.5, 53.4, 51.4, 42.8, 31.7, 29.6, 27.1, 23.1, 14.2. HPLC = 13.2 minutes.

1,11-Biscyclohexylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris-(carboxymethyl)undecane: DTPA-BcHA, 11. $^{21}\text{Yield}$: 80%. Mass spectra (LSIMS): $[(M+1)]^+ = 556^+$; ^1H NMR (D_2O , δ , in ppm) = 3.4 (2H, multiplet, $2 \times \text{CH}$), 3.1 (4H, singlet, $2 \times \text{CH}_2$), 3.0 (4H, singlet, $2 \times \text{CH}_2$), 2.9 (2H, singlet, CH_2), 2.6 to 2.4 (8H, multiplet, $4 \times \text{CH}_2$), 1.6 to 1.5 (8H, multiplet, $4 \times \text{CH}_2$), 1.1 to 0.9 (12H, multiplet, $6 \times \text{CH}_2$); ^{13}C NMR (D_2O , δ , in ppm) = 178.7, 178.4, 171.6, 61.3, 59.0, 54.1, 53.1, 50.9, 37.2, 32.5, 25.5, 24.9. HPLC = 15.6 minutes.

1,11-Bisbismethylamino-1,11-dioxo-3,6,9-triaza-3,6,9-tris-(carboxymethyl)undecane: DTPA-BBMA, 12. Yield : 64%. Mass spectra (LSIMS): $[(M+1)]^+ = 448^+$; ^1H NMR (D_2O , δ , in ppm) = 3.2 (4H, singlet, $2 \times \text{CH}_2$), 3.1 (4H, singlet, $2 \times \text{CH}_2$), 3.0 (2H, singlet, CH_2), 2.7 to 2.5 (8H, multiplet, $4 \times \text{CH}_2$), 2.6 (12H, singlet, $4 \times \text{CH}_3$); ^{13}C NMR (D_2O , δ , in ppm) = 178.5, 178.2, 169.7, 61.7, 58.1, 56.8, 52.8, 51.7, 34.8. HPLC = 5.6 minutes.

***N,N'*-bis[(methyloxycarbonyl)methyl]diethylenetriamine-*N,N',N'*-triacetic acid: DTPA-BME, 14.** The DTPA-bisanhydride was added to a solution of methanol in dimethylformamide by using the procedure described for the bismethylamide. Yield : 82%. Mass spectra (LSIMS): $[(M+1)]^+ = 422^+$; ^1H NMR (D_2O , δ , in ppm) = 3.4 (6H, singlet, OCH_3), 3.1 (8H, singlet, $4 \times \text{CH}_2$), 3 (2H, singlet, CH_2), 2.6 to 2.4 (8H, multiplet, $4 \times \text{CH}_2$); ^{13}C NMR (D_2O , δ , in ppm) = 178.2, 173, 171.1, 59.8, 57.6, 56.5, 53.6, 53.2, 49.3. HPLC = 10.3 minutes.

(*R,S*)-4-hydroxymethyl-3,6,9-tri(carboxymethyl)-3,6,9-triazaundecane-1,11-dicarboxylic acid: (*R,S*)-*C*₄-HM-DTPA, 18. The HM-DTPA was obtained as described by McMurry et al²² from commercially available D,L-serine methyl ester hydrochloride. The ester was treated with ethylenediamine to give the resulting amide, which was isolated as an oil. The amide was reduced with an excess of borane. The alkylation step with *tert*-butyl bromoacetate and the hydrolysis of the pentaester provided the HM-DTPA compound. Yield : 58.7%. Mass spectra (LSIMS): $[(M+1)]^+ = 424^+$; ^1H NMR (D_2O , δ , in ppm) = 3.5 (2H, doublet, CH_2), 3.2 (4H, singlet, $2 \times \text{CH}_2$), 3.1 (4H, singlet, $2 \times \text{CH}_2$), 3.0 to 2.9 (3H, multiplet, CH_2 , CH), 2.6 to 2.4 (6H, multiplet, $3 \times \text{CH}_2$); ^{13}C NMR (D_2O , δ , in ppm) = 177.5, 174.4, 171.2, 171.1, 64.5, 63.4, 62.1, 61.8, 59.6, 58.8, 53.4, 53.1, 53.0. HPLC = 9.5 minutes.

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 to 7. The absence of free

Gd(III) was checked with arsenazo(III) indicator. The neutral complexes were passed through a Sep-Pak column (Accell Plus QMA cartridges, Waters) to eliminate any charged species and freeze-dried. The purity of the chelates was verified by LSIMS and HPLC.

Transmetallation Kinetics

The technique is based on measurement of the evolution of the water proton paramagnetic longitudinal relaxation rate (R_1^P) of a buffered solution (phosphate buffer, pH 7) containing 2.5 mmol/L gadolinium complex and 2.5 mmol/L ZnCl_2 . Ten microliters of a 250 mmol/L solution of ZnCl_2 are added to 1 mL of a buffered solution of the paramagnetic complex. The mixture is vigorously stirred, and 300 μL are taken up for the relaxometric study. A control study, run on Gd-DTPA with zinc acetate, has given results identical to those obtained in the presence of ZnCl_2 . It is, however, worth mentioning that adding the contrast agent to a phosphate-buffered solution of ZnCl_2 prepared and stored, even for a short period of time, should be strictly avoided, because it induces errors owing to the precipitation of zinc phosphate. The R_1^P relaxation rate is obtained after subtraction of the diamagnetic contribution of the proton water relaxation (0.283 s^{-1}) from the observed relaxation rate $R_1 = (1/T_1)$. The measurements were performed on a Minispec PC-20 spin analyzer (Bruker, Karlsruhe, Germany) at 20 MHz and 37° C. The temperature was kept constant through a perchlorinated liquid flow. The samples (0.3 mL) were contained in 7-mm (outer diameter) borosilicate glass (Pyrex) tubes and kept at 37° C in a dry block between measurements (up to 4320 minutes).

Results and Discussion

The paramagnetic ion of the gadolinium complexes studied in this work is coordinated to oxygen and nitrogen atoms of the ligand and to one water molecule exchanging with the bulk (inner-sphere mechanism). In addition, longer distance interactions between the Gd^{3+} ion and the surrounding water molecules contribute to the paramagnetic relaxation enhancement through the outer-sphere mechanism. The resulting proton longitudinal relaxation rate enhancement R_1^P thus essentially arises from the existence of solubilized and hydrated Gd^{3+} ions.²³

If transmetallation of a soluble, paramagnetic gadolinium complex by diamagnetic Zn^{2+} ions were to occur in a phosphate-buffered solution, then the released Gd^{3+} would react to form GdPO_4 , the solubility of which is very low ($K_{sp} = 10^{-22.26} \text{ mol/L}^2$)¹⁴ and whose influence on the longitudinal relaxation rate of water is null or negligible. A decrease of the proton relaxation rate would be observed, the extent of which would be related to the proportion of those "silent" Gd^{3+} ions.



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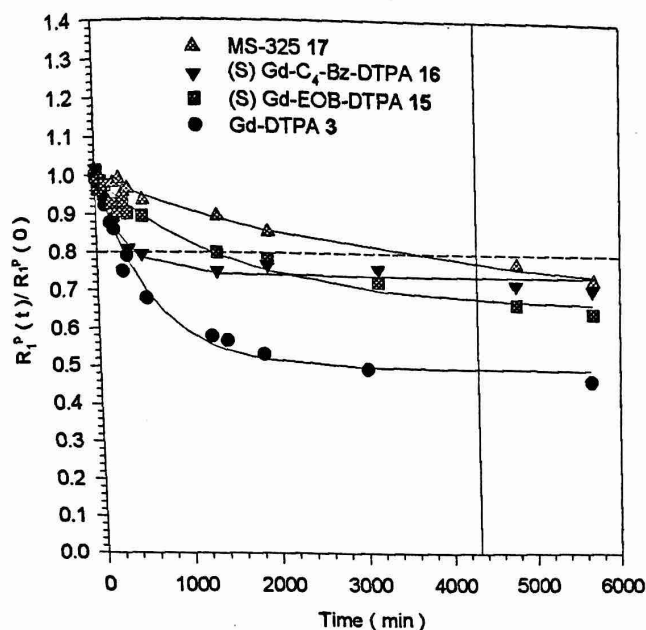


Figure 2. Experimental data and data fitting with the use of Equation 4 for Gd-DTPA 3, (S)-Gd-EOB-DTPA 15, MS-325 17, and (S)-Gd-C₄-Bz-DTPA 16. The fitted values are $[\text{Lig Gd}]_{\infty} = 1.24 \times 10^{-3} \text{ mol/L}$ and $k_1 = 1.6 \times 10^{-3} \text{ min}^{-1}$ for Gd-DTPA; $[\text{Lig Gd}]_{\infty} = 1.66 \times 10^{-3} \text{ mol/L}$ and $k_1 = 6.9 \times 10^{-4} \text{ min}^{-1}$ for (S)-Gd-EOB-DTPA; $[\text{Lig Gd}]_{\infty} = 1.74 \times 10^{-3} \text{ mol/L}$ and $k_1 = 3.3 \times 10^{-4} \text{ min}^{-1}$ for MS-325; and $[\text{Lig Gd}]_{\infty} = 1.86 \times 10^{-3} \text{ mol/L}$ and $k_1 = 3.4 \times 10^{-3} \text{ min}^{-1}$ for (S)-Gd-C₄-Bz-DTPA. The vertical line corresponds to time = 4320 minutes; the horizontal line corresponds to a relative water proton paramagnetic longitudinal relaxation rate $R_1^p(t)/R_1^p(0)$ value of 0.8. See the text for explanation of chemical names.

where k_f and k_r are the kinetic constants for the forward and reverse reactions, respectively. Under the experimental conditions used in this work (phosphate buffer), the concentrations of "free" Zn(II) and Gd(III) are very low owing to their low solubilities at pH 7 $K_{sp} = 10^{-22.26} \text{ mol/L}^2$ and $10^{-35.3} \text{ mol/L}^5$ for GdPO_4 and $\text{Zn}_3(\text{PO}_4)_2$, respectively and were assumed to be approximately constant. With this assumption, the rate of transmetalation can thus be written as

$$\frac{d[\text{Lig Gd}]}{dt} = -k_f[\text{Lig Gd}] + k_r[\text{Lig Zn}] \quad [2]$$

where $[\text{Lig Zn}] = [\text{Lig Gd}]_0 - [\text{Lig Gd}]$, and $[\text{Lig Gd}]_0$ is the initial concentration of the gadolinium complex. The concentration of $[\text{Lig Gd}]$ at a time t is thus

$$[\text{Lig Gd}] = [\text{Lig Gd}]_{\infty} + ([\text{Lig Gd}]_0 - [\text{Lig Gd}]_{\infty})e^{-k_1 t} \quad [3]$$

where $k_1 = k_f + k_r$ and $[\text{Lig Gd}]_{\infty}$ is the concentration of $[\text{Lig Gd}]$ at equilibrium. The kinetic constant for the forward reaction k_f can thus be calculated as

$$k_f = k_1 \frac{[\text{Lig Gd}]_0 - [\text{Lig Gd}]_{\infty}}{[\text{Lig Gd}]_0} \quad [4]$$

The k_f values obtained by fitting the experimental data are equal to $8.4 \times 10^{-4} \text{ min}^{-1}$, $7.6 \times 10^{-4} \text{ min}^{-1}$, $8.7 \times 10^{-4} \text{ min}^{-1}$, $2.3 \times 10^{-4} \text{ min}^{-1}$, and 10^{-4} min^{-1} for Gd-DTPA 3, (R,S)-Gd-C₄-HM-DTPA 18, (S)-Gd-C₄-Bz-DTPA 16, (S)-Gd-EOB-DTPA 15, and MS-325 17, respectively (Fig. 2). Compared with the parent compound, the kinetic constant for the forward reaction is thus similar for (R,S)-Gd-C₄-HM-DTPA 18 and (S)-Gd-C₄-Bz-DTPA 16 and markedly smaller for (S)-Gd-EOB-DTPA 15 and MS-325 17, in good agreement with the semiquantitative interpretation (vide supra). Within the C₄-substituted series, MS-325 17 is then the kinetically most inert compound.

At the end of the observation period (≈ 3 days; 4320 minutes), the ratio of R_1^p was about 75% for MS-325 17, 73% for (S)-Gd-C₄-Bz-DTPA 16, 69% for (S)-Gd-EOB-DTPA 15, and 53% for (R,S)-Gd-C₄-HM-DTPA 18 compared with 49% for the parent complex 3 (Table 2). Therefore, it seems that for this series of C₄-substituted open-chain chelates, the larger the volume of the substituent, the more stable is the compound with respect to the transmetalation process. The steric hindrance induced by the group located on the ethylenic bridge thus has a favorable effect by reducing the accessibility of Zn^{2+} and/or by rigidifying the structure of the complex.

The results obtained for the Gd-DTPA bisamide series (Fig. 3 and Table 2) show that the transmetalation process is markedly faster for all bisamide derivatives than for the parent compound, except Gd-DTPA-BtBA 9 and Gd-DTPA-BBMA 12, for which the kinetics seem similar. The theoretical fitting process with the use of Equation 4 was attempted, but for complexes undergoing extensive and fast transmetalation, only the early data points could be reasonably fitted with the monoexponential equation. Additionally, the calculated value of $[\text{Gd Lig}]_{\infty}$ is always significantly larger than that estimated from the experimental data collected at 4320 minutes. For these complexes, indeed, the decay of R_1^p seems to be biexponential rather than monoexponential. The values of k_f reported below were obtained from the initial part of the curve and are thus related to the fast decay of the R_1^p . They are as follows: $9.3 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BA 4), $6.1 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BMA 5), $4.6 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BEA 6), $2.3 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BnBA 7), $2.5 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BHA 10), $3.7 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BiBA 8), $2.5 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BcHA 11), $3.8 \times 10^{-3} \text{ min}^{-1}$ (Gd-DTPA-BBzA 13), $6.9 \times 10^{-4} \text{ min}^{-1}$ (Gd-DTPA-BtBA 9), and $9.4 \times 10^{-4} \text{ min}^{-1}$ (Gd-DTPA-BBMA 12). As stated above, the tertiary bisamide complex Gd-DTPA-BBMA 12 and the secondary amide Gd-DTPA-BtBA 9 undergo a transmetalation process comparable to that of Gd-DTPA from the kinetic point of view. Within the bisamide series, the phenomenon is thus fastest for the primary bisamide Gd-DTPA-BA 4, whereas for the secondary and ternary bisamides, transmetalation

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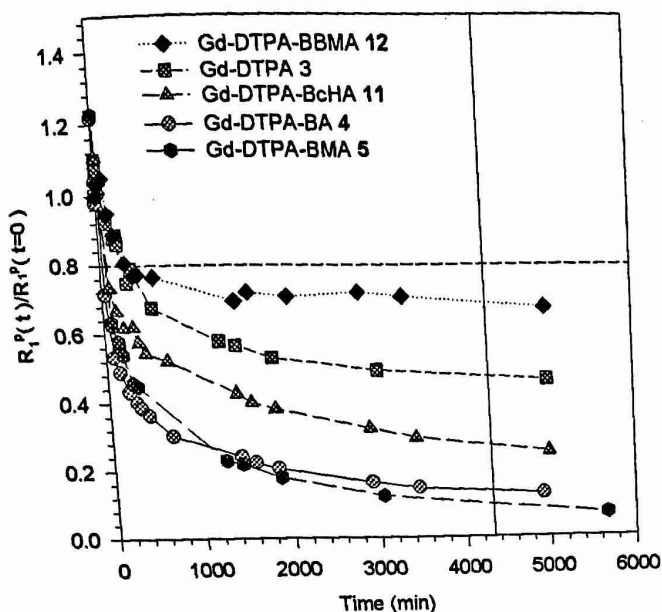


Figure 3. Evolution of the relative water proton paramagnetic longitudinal relaxation rate $R_1^P(t)/R_1^P(t=0)$ vs. time for Gd-DTPA-BA 4, Gd-DTPA-BMA 5, Gd-DTPA-BEA 6, Gd-DTPA-BHA 10, Gd-DTPA-BcHA 11, Gd-DTPA-BBMA 12, and Gd-DTPA 3 (initial concentrations of gadolinium complexes and $ZnCl_2$ were 2.5 mmol/L in phosphate buffer at pH 7, $T = 37^\circ C$, $B = 0.47 T$). The vertical line corresponds to time = 4320 minutes; the horizontal line corresponds to an $R_1^P(t)/R_1^P(0)$ value of 0.8. See the text for explanation of chemical names.

kinetics tends to be reduced by the presence of bulky substituents.

With the exception of Gd-DTPA-BBMA 12, all bisamide derivatives thus show more extensive transmetallation than does the parent compound Gd-DTPA 3. The substitution of two carboxylate functional groups by two amide groups in the first coordination sphere of Gd^{3+} seems to facilitate the exchange of the Gd^{3+} ion by the Zn^{2+} ion. As mentioned above, within the bisamide series, less extensive transmetallation occurs when the substituting groups are bulkier, indicating a favorable effect of steric hindrance against the transmetallation process. This is illustrated in Table 2, wherein isomeric DTPA-bisbutylamide derivatives are compared. The thermodynamic stability index is 0.19, 0.26, and 0.39 for Gd-DTPA-BnBA 7, Gd-DTPA-BiBA 8, and Gd-DTPA-BtBA 9, respectively.

A Gd-DTPA bisester 14 was also evaluated. This compound behaves like its parent structure Gd-DTPA: similar time to reach normalized $R_1^P = 0.80$, similar normalized R_1^P after 3 days (0.43 vs. 0.47), and comparable k_f values (7.8×10^{-4} vs. $8.4 \times 10^{-4} \text{ min}^{-1}$). When the kinetics and extent of transmetallation are compared for bisester and bisamide complexes substituted by groups of the same size (Table 2), it is clear that the bisester compound is less sensitive to transmetallation by zinc than are the bisamides.

This characteristic could be related to the different electro-negativities of the amide and ester groups.

In conclusion, the proposed relaxometric protocol is simple and has been successfully applied to the study of the transmetallation of a series of linear and macrocyclic gadolinium complexes by $Zn(II)$. This work has shown that (1) gadolinium complexes of open-chain chelates like those of DTPA exhibit a higher susceptibility to transmetallation than do macrocyclic complexes, (2) C-functionalized DTPA derivatives are less sensitive to the process than is the parent compound, and (3) the bisamide derivatives exhibit the highest extent of transmetallation, except when bulky substituents are present on the amide groups.


Acknowledgments

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