# Physicochemical Characterization of MS-325, a New Gadolinium Complex, by Multinuclear Relaxometry[+]

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The physicochemical characterization of MS-325 [trisodium {4-(*R*)-[(4,4-diphenylcyclohexyl)phosphanooxymethyl]-3,6,9-triaza-3,6,9-tris(methoxycarbonyl)undecanedioato}gadolinium-(III)], a new derivative of Gd-DTPA {Magnevist®: dimeglumin [{3,6,9-triaza3,6,9-tris(methoxycarbonyl)undecanedioato}gadolinium(III)], presented as a potentially useful angiographic contrast agent, was carried out in various media. Water solution, protein-containing solution, phosphorylated metabolites solution, and $Zn^{2+}$-containing solution were investigated using different NMR techniques such as water $^{1}H$ nuclear magnetic relaxation rates, water $^{17}O$ transverse relaxation rates, and $^{31}P$ longitudinal relaxation rates of phosphorylated metabolites. The proton relaxivity of MS-325 in water was found to be higher than that of the parent compound Gd-DTPA; this can be attributed to the longer rotational correlation time ($\tau_R$) of the hydrated complex, and possibly to an apparently shorter mean distance ($r$) between the protons of the coordinated water molecule and the gadolinium ion. The kinetic and thermodynamic stability of MS-325 in solutions containing phosphorylated metabolites (ATP, phosphocreatine and inorganic phosphate) were measured by $^{31}P$ relaxation rate analysis and found to be higher than for Gd-DTPA. Similarly, the $Zn^{2+}$ transmetallation process studied by proton relaxometry is slower than for the same reference compound. Finally, an analysis of the noncovalent binding of MS-325 to serum proteins by proton relaxometry showed that MS-325 interacts with human serum albumin (HSA) and that the association constant of this interaction is equal to $6100 \pm 2130$ $M^{-1}$. A peak relaxivity of approx. 50 $s^{-1}mM^{-1}$ was determined at 25 MHz for the protein-bound paramagnetic complex. This value is lower than the maximal relaxivity predicted for a paramagnetic center totally immobilized at the surface of the protein.

## Introduction

Small gadolinium complexes, which can interact with high molecular weight molecules through covalent or noncovalent bonds, are potential intravascular Magnetic Resonance Imaging (MRI) contrast agents. A noncovalent binding to endogenous proteins can exist in vivo; this results in a reduced extravasation of the paramagnetic complex, and therefore in a subsequent increase of its persistence in blood, but does not preclude a fairly rapid and selective renal excretion. On the other hand, binding to macromolecules decreases the molecular mobility and thus enhances the relaxivity of the paramagnetic center, i.e. its ability to increase the relaxation rate of the protons of water molecules. Contrast agents that are likely to interact with serum proteins, are therefore attracting a great deal of interest.

MS-325 [trisodium {4-(*R*)-[(4,4-diphenylcyclohexyl)phosphonooxymethyl]-3,6,9-triaza-3,6,9-tris(methoxycarbonyl)-undecanedioato}gadolinium(III)] (Figure 1) is a new MRI contrast agent which has been reported to exhibit a remarkable affinity for serum proteins.[1][2] This derivative of Gd-DTPA [Magnevist®: dimeglumin {3,6,9-triaza-3,6,9-tris-(methoxycarbonyl)undecanedioato}gadolinium(III)] has a diphenylcyclohexyl group attached to one of the ethylene bridges of the backbone by means of a phosphodiester linkage.[1][2] The presence of this substituent is responsible for the noncovalent binding to plasma proteins which, as expected, leads to a reduction in the rate of molecular tumbling and, consequently, to an enhanced efficacy as a relaxing agent in blood.[1] In addition, the interaction with blood macromolecules produces a marked increase in the plasma half-life relative to hydrophilic contrast agents such as Gd-DTPA, the parent compound, or Gd-DOTA {Dotarem®: meglumin [1,4,7,10-tetrakis(methoxycarbonyl)-1,4,7,10-tetraazacyclododecane]gadolinium(III)}.[3] MS-325 has been shown to provide very good and persistent enhancement of blood vessels on MRI angiographic evaluations.[3–8]

So far, few physicochemical data are available concerning a comprehensive description and understanding of the properties of this molecule. The aim of this work is to extend the in vitro physicochemical characterization of MS-325 by quantitatively evaluating and interpreting: 1) the proton relaxivity ($r_1$) in water at various magnetic fields and temperatures; 2) the exchange time of the coordinated water molecule ($\tau_M$) estimated through the analysis of the temperature-dependence of the $^{17}O$ transverse relaxation rate of water in MS-325 aqueous solution; 3) the rotational correlation time ($\tau_R$) calculated from $^{2}H$ longitudinal relaxation rate of the deuterated ligand complexed to lantha-

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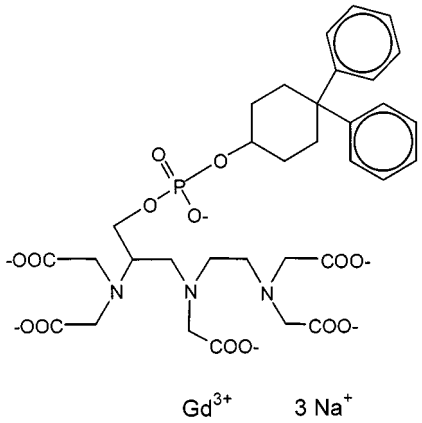


Figure 1. Structure of MS-325 [trisodium {4-(*R*)-[(4,4-diphenylcyclohexyl) phosphonooxymethyl]-3,6,9-triaza-3,6,9-tris(methoxycarbonyl)undecanedioato}gadolinium(III)]

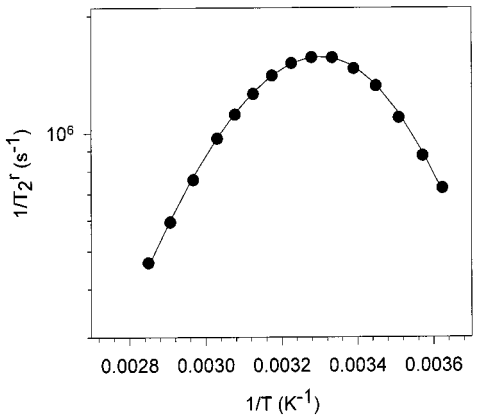


Figure 2. Reduced transverse relaxation rate of oxygen-17 ($1/T_2^r$) as a function of the reciprocal of the temperature for an aqueous solution containing 12.5 mM of MS-325 ($B_o$ = 7.05 T)

num, a diamagnetic lanthanide ion; 4) the noncovalent interaction with human serum albumin studied by proton and deuterium relaxometry; 5) the transmetallation by $Zn^{2+}$ ions using proton relaxometry; and 6) the kinetic and thermodynamic stability of the gadolinium complex in solutions containing phosphorylated metabolites which are potential competitors for the complexation of the lanthanide ions {ATP, phosphocreatine (PCr), and inorganic phosphate (Pi)}. This study will be carried out through the evolution of the $^{31}P$ longitudinal relaxation rates.

Considering the clinical context of this kind of study, most of the data are reported at 310 K, the physiological body temperature, as well as at the standard temperature of 298 K.

## Results and Discussion

### Water Solution

The residence time of water molecules in the first coordination sphere of the complex ($\tau_M$) was obtained from the analysis of the temperature-dependence of the $^{17}O$ transverse relaxation rate of water in MS-325 solution. This procedure allows for the determination of: i) the hyperfine coupling constant ($A/\hbar$) between the $^{17}O$ nucleus and the electron spin; ii) the parameters defining the rate of exchange of the coordinated water molecule {enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) of activation}; and iii) the parameters related to the electronic relaxation time [the correlation time of the modulation of the zero-field splitting $\tau_V$, the activation energy for this process $E_V$, and $B$ which depends on the trace of the square of the zero-field splitting tensor ($\Delta^2$) and on the electron spin $S$ ($B = \Delta^2\{4S(S + 1) - 3\}/25$)].[9][10]

The theoretical fitting of the reduced transverse relaxation rates ($1/T_2^r = [H_2O]/\{[\text{Gd-complex}] \cdot T_2^P\}$) obtained over a temperature range extending from 276 to 351 K (Figure 2) gives a $\tau_M^{298}$ value of 195 ± 32 ns similar to that of 200 ± 51 ns reported for another C-substituted Gd-DTPA derivative (*S*)-Gd-EOB-DTPA [disodium {4-(*S*)-3,6,9-tris(methoxycarbonyl)-4-(4-ethoxybenzyl)-3,6,9-triazaundecanedioato}gadolinium(III)].[9] These $\tau_M$ values are however shorter than those observed for Gd-DTPA ($\tau_M^{298}$ = 331 ± 60 ns,[11] 303 ns[12]). In our fitting, the number of coordinated water molecules (q) was assumed to be equal to 1 as in Gd-DTPA and (*S*)-Gd-EOB-DTPA complexes. The other parameters of the fitting are given in Table 1.

Table 1. Parameters obtained from the fitting of the reduced transverse relaxation rate of oxygen-17 in MS-325 solution ([MS-325] = 12.5 mM)

| | MS-325 | Gd-DTPA[a] | (*S*) Gd-EOB-DTPA[b] |
|---|---|---|---|
| $\tau_M^{298}$ [ns] | 195±32 | 331±60 | 200±51 (201) |
| $\tau_V^{298}$ [ps] | 18.7 ±0.9 | 12.3±0.3 | 15.3±1.94 (2.4) |
| $B$ [$10^{20}$ $s^{-2}$] | 5.22 ±0.24 | 2.60±0.30 | 2.51±0.28 (0.49) |
| $E_V$ [kJ/mol] | 6.4±2.7 | 4.5±4.2 | 7.1±5.9 (12.0) |
| $A/\hbar$ [$10^6$ rad $s^{-1}$] | −4.1±0.06 | −3.41±0.11 | −4.07±0.45 (−4.24) |
| $\Delta H^{\neq}$ [kJ/mol] | 51.3 ±0.3 | 51.5±0.3 | 53.5±0.3 (54.3) |
| $\Delta S^{\neq}$ [J/mol K] | 55.8±0.4 | 52.1±0.6 | 63.0±12.9 (65.7) |

[a] From ref.[11] – [b] the values reported in this table were obtained by improving the fitting of the data of ref.[9]; the values in parentheses are from ref.[9]

The proton relaxivity ($r_1$), defined as the increase of the solvent relaxation rate induced by 1 mmol·$l^{-1}$ of the paramagnetic complex, is the sum of two contributions arising from short-distance interactions (innersphere mechanism) and from longer-distance interactions (outersphere mechanism). The influence of $\tau_M$ on proton relaxivity is well understood. When this exchange time is short relative to the relaxation time of the protons of the coordinated water molecules, the overall proton relaxivity is enhanced by a temperature decrease. On the contrary, when the exchange time is long, the global relaxivity is levelled off or even decreased by a reduction of the temperature. This latter behavior has been observed for bisamide derivatives of Gd-DTPA.[11,13–15] The $\tau_M$ values found for MS-325 by $^{17}O$-NMR spectroscopy over the temperature range explored are characteristic of a nonlimiting exchange. This is con-

firmed by the increase of the relaxivities when the temperature is decreased (Figure 3).

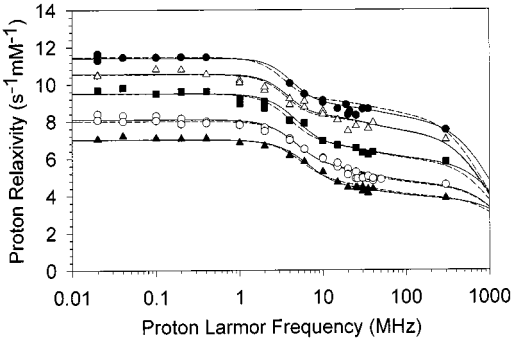


Figure 3. $^1$H NMRD relaxivity profiles of MS-325 in water at different temperatures ([MS-325] = 1 mM): 278 K: closed circles, 288 K: open triangles, 298 K: closed squares, 310 K: open circles, 318 K: closed triangles. The lines correspond to the theoretical fittings of the data points (solid lines: $r$ = 0.295 nm, dashed lines: $r$ = 0.31 nm)

As compared to Gd-DTPA,[9][16] the proton relaxivity of MS-325 at 310 K is higher over the whole proton Larmor frequency range and, in this respect, behaves like that of (*S*)-Gd-EOB-DTPA. For this last compound, the increased relaxivity was mainly attributed to a shorter mean distance of interaction between the protons of the coordinated water molecule and the gadolinium ion (0.281 nm for (*S*)-Gd-EOB-DTPA and 0.31 nm for Gd-DTPA).[9]

The fittings of the proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were performed as described in the experimental section according to the classical paramagnetic relaxation formalisms.[17−19] The following strategy was adopted: after subtraction of the diamagnetic relaxation rate of the solvent, the outersphere and the innersphere contributions were simultaneously fitted after attribution of fixed or calculated values to the various parameters. The relative diffusion constant $D$ was fixed to the diffusion constant of water,[20] $d$, the distance of closest approach for the outer sphere contribution was set at 0.36 nm,[14][21] $\tau_M$ values were those obtained by $^{17}$O NMR, and $q$, the number of water molecules in the first coordination sphere of $Gd^{3+}$, was given a value of one. The parameters describing the electronic relaxation times, $\tau_V$ and $\tau_{SO}$ (the electronic relaxation time at zero field), were optimized for the outersphere and the innersphere contributions simultaneously. It is well-known that parallel and accurate determination of both $\tau_R$, the rotational correlation time of the hydrated complex, and $r$, the distance between the protons of the coordinated water molecules and the gadolinium ion, can be ambiguous, unless independent information about $\tau_R$ and/or $r$ is available. For instance, good fits of the 310 K experimental data can be achieved with pairs of values of $r$ and $\tau_R$ ranging from 0.285 to 0.31 nm for the former and from 68 to 110 ps for the latter (Table 2).

Assuming that $\tau_R$ can be estimated by the Stokes−Einstein law and that the molecular volume is roughly proportional to the molecular weight, the $\tau_R$ of MS-325 should be approximately 1.6 times larger than that of Gd-DTPA.

Table 2. Values of $\tau_R$ obtained from the fitting of the proton NMRD profiles at 310 K using different $r$ values

| $r$ [nm] | $\tau_R$ [ps] |
|---|---|
| 0.285 | 68 |
| 0.290 | 76 |
| 0.295 | 84 |
| 0.3 | 91 |
| 0.31 | 110 |

At 310 K, $\tau_R$ values of MS-325 should thus be around 90−95 ps and correspond to a distance $r$ of 0.3 nm. If (*S*)-Gd-EOB-DTPA is taken as a reference, $\tau_R$ should be around 80−85 ps at 310 K and $r$ should be 0.295 nm. The $\tau_R$ value obtained by the analysis of the deuterium longitudinal relaxation rate of the deuterated ligand complexed to the diamagnetic $La^{3+}$ ion is however markedly longer ($\tau_R$ = 117±12 ps at 310 K) and corresponds to a distance of 0.31 nm. Two values of $r$ (0.295 and 0.31 nm) were thus used for the fittings performed at 278, 288, 298, 310, and 318 K (Figure 3 and Table 3).

As for other C-substituted derivatives of Gd-DTPA,[9,11,21] the proton relaxivity enhancement observed for MS-325, as compared to the unsubstituted Gd-DTPA, can thus be related to a longer rotational correlation time $\tau_R$ and possibly to a shorter apparent H−Gd distance $r$.

## Solutions Containing Phosphorylated Metabolites

In an earlier study, adenosine triphosphate (ATP) was shown to efficiently compete with open-chain chelates for the complexation of gadolinium ions.[22] This metabolite thus appears to be a good probe to assess the kinetics as well as the thermodynamic stability of the complex. The decrease of the $T_1$ of the phosphorus nuclei of ATP in solutions containing Gd-DTPA has indeed been reported to result from the formation of a complex between ATP and $Gd^{3+}$ whose characteristics have been described elsewhere.[23] The same protocol was applied to MS-325 in ATP-containing solutions and the results were compared to those previously obtained for Gd-DTPA[22] and (*S*)-Gd-EOB-DTPA[9] (Figure 4).

Immediately after addition of the gadolinium complex, $^{31}$P relaxation rates increase and reach a plateau after a delay which depends on the structure of the Gd complex. The equilibrium value of the P-31 relaxation rates is reached more quickly for Gd-DTPA (ca. 30 min) than for (*S*)-Gd-EOB-DTPA (ca. 6 h) or for MS-325 (ca. 24 h). The kinetic stability of MS-325 is thus increased as compared to (*S*)-Gd-EOB-DTPA which itself is more stable than Gd-DTPA. The thermodynamic stability was then tested at equilibrium on solutions containing various amounts of contrast agents. The measurements were performed after the steady state was reached for each solution (24 h, 8 h and 1 h after mixing the phosphorylated metabolite solution and MS-325, (*S*)-Gd-EOB-DTPA and Gd-DTPA, respectively) (Figure 5).

Table 3. Values of $\tau_R$, $\tau_{SO}$, $\tau_V$, and $\tau_M$ obtained from the fitting of the proton NMRD profiles at different temperatures ($d$ = 0.36 nm)

| $T$ [K] | $D$ [$10^{-5}$ cm$^2$s$^{-1}$] | $\tau_{so}$ [ps][a] | $\tau_v$ [ps][a] | $\tau_R$ [ps][a] | $\tau_M$ [ns][b] |
|---|---|---|---|---|---|
| 278 | 1.42 | 91 or 110 | 30 or 39 | 165 or 241 | 930 |
| 288 | 1.96 | 86 or 101 | 30 or 38 | 140 or 189 | 415 |
| 298 | 2.62 | 78 or 101 | 22 or 33 | 110 or 142 | 195 |
| 310 | 3.5 | 72 or 93 | 18 or 32 | 84 or 108 | 83 |
| 318 | 4.1 | 66 or 83 | 13 or 25 | 69 or 92 | 50 |

[a] The first values correspond to the results of the fitting performed with $r$ set to 0.295 nm, the second ones correspond to $r$ = 0.31 nm. – [b] These values were obtained from $^{17}$O NMR spectroscopy and fixed during the proton NMRD fitting.

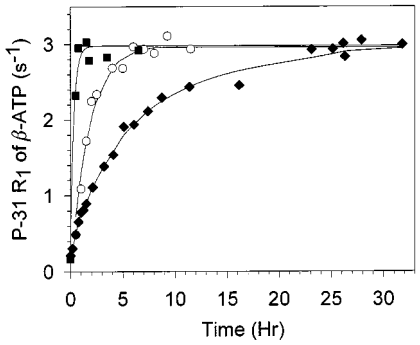


Figure 4. $^{31}$P longitudinal relaxation rates of β-ATP peak in a buffered solution (Krebs–Henseleit, pH = 7.2 to 7.4) containing 20 mM of ATP, PCr, and Pi and 0.124 mM of MS-325 (closed diamonds), (*S*)-Gd-EOB-DTPA (open circles) or Gd-DTPA (closed squares) as a function of the time elapsed after addition of the contrast agent

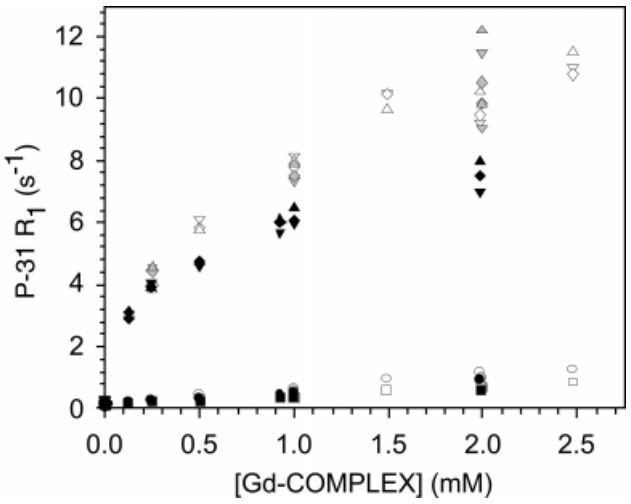


Figure 5. $^{31}$P longitudinal relaxation rates of ATP, PCr, and Pi peaks in a buffered solution (Krebs-Henseleit, pH = 7.2 to 7.4) containing 20 mM of ATP, PCr, and Pi and increasing amounts of MS-325, (*S*)-Gd-EOB-DTPA or Gd-DTPA; the measurements were performed at the equilibrium of the reaction between the Gd complex and phosphorylated metabolites (24 h, 8 h or 1 h, respectively after mixing of the phosphorylated metabolites and the complex); MS-325 (black closed symbols), (*S*)-Gd-EOB-DTPA (open symbols), Gd-DTPA (gray closed symbols), Pi (circles), PCr (squares), α-ATP (reversed triangles), β-ATP (diamonds), γ-ATP (triangles)

The slightly lower P-31 relaxation rate enhancements obtained with MS-325 indicate that the concentration of the complex formed between Gd and ATP is lower. Consequently, the thermodynamic stability of MS-325 appears to be high as compared to Gd-DTPA or (*S*)-Gd-EOB-DTPA.

## Transmetallation by $Zn^{2+}$ Ions

Transmetallation of the complex by $Zn^{2+}$ results in the release of gadolinium ions in solutions. In the presence of phosphate ions, with which they form an insoluble phosphate complex, these released lanthanide ions precipitate and no longer contribute to the proton paramagnetic relaxation rate of the solution. Consequently, the water proton relaxation rate decreases during the transmetallation process and its evolution can be used to quantitatively monitor the evolution of the system. In identical experimental conditions ($B_0$ = 0.47 T, $T$ = 310 K, 2.5 mM of gadolinium complex, 2.5 mM of $Zn^{2+}$ ions and phosphate buffer pH 7) no significant change of the paramagnetic relaxation rate was noticed for the macrocyclic gadolinium complexes Gd-DOTA and Gd-HPDO3A [{10-(2-hydroxypropyl)-1,4,7-tris(methoxycarbonyl)-1,4,7,10-tetraazacyclododecane}-gadolinium(III)] (Figure 6). This observation is in good agreement with the well-known very high kinetic and thermodynamic stabilities of these complexes.[24][25] On the contrary, $R_1^P$ decreased to about 50% of its initial value for Gd-DTPA after 5000 minutes, showing that a significant transmetallation takes place for this open-chain gadolinium complex (Figure 6). Comparatively, the results obtained

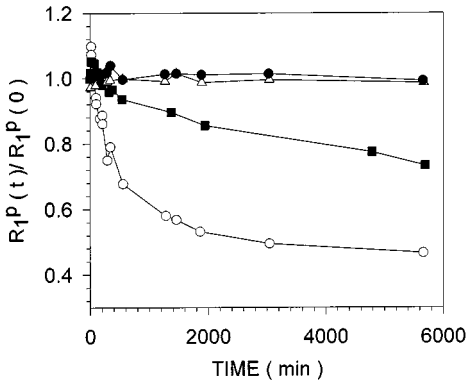


Figure 6. Time evolution of the normalized water proton paramagnetic relaxation rate ($T$ = 310 K, $\nu_o$ = 20 MHz) in solution containing 2.5 mM of gadolinium complex and 2.5 mM of $Zn^{2+}$ (MS-325: closed squares, Gd-DTPA: open circles, Gd-HPDO3A: closed circles, Gd-DOTA: open triangles)

with MS-325 indicate a relatively slower and limited evolution of the proton paramagnetic longitudinal relaxation rate (about 75% of its initial value after 5000 minutes).

These results demonstrate that MS-325 is more stable towards transmetallation by $Zn^{II}$ than Gd-DTPA.

## Serum and Other Protein-Containing Solutions

The high "apparent" proton relaxivity of MS-325 in serum has been related to its binding to serum proteins as studied by ultrafiltration methods.[3][6] Binding to macromolecules results in a significant $\tau_R$ increase which in turn usually induces a hump in the high field part of the $^1H$ NMRD profile. The NMRD profiles recorded in a reconstituted lyophylized serum (Kontrollogen) or in human serum albumin (HSA) are characteristic of such interactions with macromolecules (Figure 7).

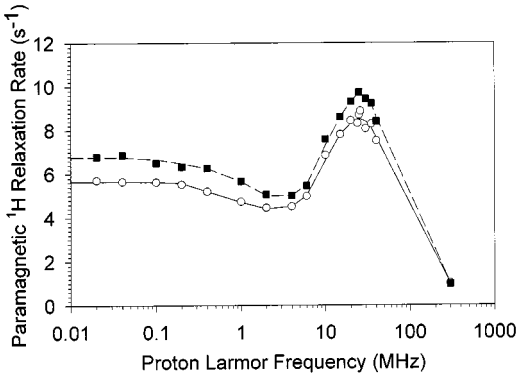


Figure 7. Proton paramagnetic relaxation rate of solutions containing 0.25 mM of MS-325 and 4% of HSA (open circles) or serum Kontrollogen (closed squares ) at 310 K

The non-linear increase of the proton paramagnetic relaxation rate measured at 20 MHz on solutions containing 4% of HSA ([HSA] = $p°$ = 0.6 mM) and various concentrations of MS-325 ($s°$ = 0−2.08 mM) (Figure 8) agrees with a strong binding. At this magnetic field, the paramagnetic relaxation rate of a solution containing 1 mM of MS-325 and 4% of HSA is 4.6 times larger than in pure water.

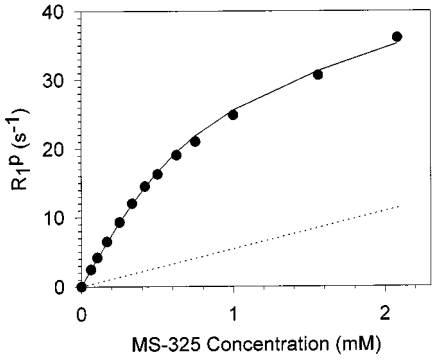


Figure 8. Proton longitudinal paramagnetic relaxation rate in solutions containing 4% of HSA and increasing amounts of MS-325 (closed circles) ($\nu_o$ = 20 MHz, $T$ = 310 K); the plain line corresponds to the fitting of the data and the dashed line represents $R_1^P$ in a water solution free of albumin

The "apparent" relaxivity calculated for each MS-325 concentration decreases from about 40 $s^{-1}mM^{-1}$ at low concentrations to 17 $s^{-1}mM^{-1}$ for the maximum concentration of 2.08 mM. Fitting of the data according to Equation 1 provides, on the one hand, an estimation of the association constant ($K$a) characterizing the interaction between HSA and MS-325 and, on the other hand, a value of the relaxivity of the noncovalently bound complex ($r_1^c$). The relaxivity of the free contrast agent is $r_1^f$. The fitted $K$a and $r_1^c$ values are 6100±2130 $M^{-1}$ and 48.9±3.5 $s^{-1}mM^{-1}$, respectively [the number of equivalent and independent interaction sites ($N$) was set to 1]. Consequently, in solutions containing 4% of HSA and less than 0.3 mM of MS-325, more than 70% of MS-325 interacts with the protein. These results are in good agreement with the ultrafiltration data of Lauffer et al. who reported that the association constant of the strongest site(s) is in the order of $10^4$ $M^{-1}$.[6]

In the experimental conditions of the NMRD study (Figure 7, 0.25 mm of MS-325 and 4% of HSA), 0.18 mM of MS-325 are bound to the protein. The proton paramagnetic relaxation rate profiles (Figure 7) thus represent the combined contributions of the free and the bound Gd complex. Knowing the proton NMRD profile of the free MS-325 as well as the concentration of free and bound Gd complex obtained from the equilibrium study (see above), the proton NMRD profile of the HSA-MS-325 complex can be calculated (Figure 9).

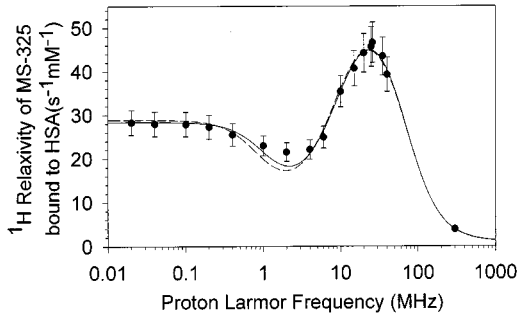


Figure 9. Calculated theoretical $^1H$ NMRD profile of the complex HSA-MS-325 at 310 K (closed circles) and its fittings (plain line: $r$ = 0.295 nm, dashed line: $r$ = 0.31 nm)

The fitting of this profile was then performed using the equations usually adopted for the description of small gadolinium complexes in aqueous solutions for the two distances $r$ used previously (see Experimental Section). These equations, however, are known to be unappropriate to describe the low field range of the profile for slow-rotating systems, especially regarding the electron spin system.[26] Depending on the distance $r$ used for the fittings, the values of $\tau_V$ and $\tau_{SO}$ are 38 or 41 ps and 287 or 351 ps. These values are thus recognized as inaccurate but the values of $\tau_R$ (3.3 or 4.4 ns), which are mainly determined from the high field range, are acceptable. In separate experiments run on the deuterated lanthanum analog of MS-325, the increase of the deuterium linewidths measured in solutions containing 4% of HSA and decreasing concentrations of the complex was analyzed as described in reference 9. The fitted value of $K_a$ and $R_2$ of the complex ($R_2^c$) were 5590

$M^{-1}$ and 1210 $s^{-1}$ respectively. This $R_2^c$ value corresponds to a $\tau_R$ of the order of 6 to 7 ns. All these values of $\tau_R$ are lower than the value of 14 ns calculated for HSA at 310 K using Stokes's law.[27] This difference indicates that the mobility of the paramagnetic center is higher than expected for a complex fully immobilized on the surface of the protein and agrees with the picture of the ligand interacting with the protein through the [(4,4-diphenylcyclohexyl)phosphonooxymethyl] chain and leaving some mobility freedom to the rest of the bound molecule. The longer $\tau_M$ obtained by the fitting (171 or 252 ns as compared to 83±13 ns in aqueous solution free of protein) might result from interactions at the surface of the protein, although this hypothesis seems to be contradicted by the previous discussion about molecular mobility.

In serum Kontrollogen, the paramagnetic relaxation rates are slightly higher than in albumin solution (Figure 7). For other paramagnetic complexes like Gd-DTPA, Gd-DOTA, or Gd-HPDO3A which do not interact with blood macromolecules, 10 to 25% higher water proton relaxation rates were also observed in serum as compared to HSA solutions (unpublished data). This can be explained by the higher viscosity of serum as compared to a 4% HSA solution (at 310 K, the viscosity of serum Kontrollogen is equal to 1.18 cp as compared to 0.84 cp for a 4% HSA solution). From the above, it is thus clear that the increase of the paramagnetic relaxation rate in serum mostly results from the noncovalent interaction of MS-325 with albumin. These results are in good agreement with the high performance liquid chromatography experiments of Lauffer et al.[6]

## Conclusion

The multinuclear magnetic resonance approach used in this work shows that, compared to its parent compound Gd-DTPA, MS-325 exhibits a higher proton relaxivity which can be attributed to a slower tumbling resulting from a higher molecular weight and possibly to an apparently shorter mean distance between the hydrogen atoms of the water molecules and the gadolinium ions. The exchange time of the coordinated water molecule $\tau_M$ does not limit the proton relaxivity as demonstrated by the $^{17}O$ and proton relaxometric studies performed between 278 to 318 K.

In solutions containing ATP, a partial decomplexation of MS-325 occurs but the rate and the extent of this process are smaller than for the parent compound. The kinetic and the thermodynamic stabilities of this C-substituted derivative are thus increased. Similarly, the transmetallation process by $Zn^{2+}$ ions is reduced as compared to Gd-DTPA. These results confirm a previously reported hypothesis regarding the favorable role of C-substitution.[9]

The proton relaxometric study of the noncovalent interaction with serum proteins, and more particularly with HSA, shows that the association constant characterizing the equilibrium between HSA and MS-325 is about 6100±2130 $M^{-1}$ for a number of interaction sites equal to 1. As shown by the parameters calculated from the $^1H$ and $^2H$ relaxometry data, the paramagnetic center of the noncovalently bound complex is not fully immobilized at the surface of the protein and consequently does not develop the maximum relaxivity allowed by the rate of the water exchange. If the paramagnetic complex would experience the rotational correlation time of albumin ($\tau_R$ = 14 ns[27]), its relaxivity calculated with the parameters reported above should be maximal around 20−25 MHz and reach 59 $s^{-1}mM^{-1}$.

## Experimental Section

**General:** MS-325 was synthesized as described by McMurry et al.[2] and Sajiki et al.[28] − Gd-DTPA (Magnevist®) was provided by Schering AG (Berlin, Germany), Gd-DOTA (Dotarem®) by Guerbet (Aulnay-sous-Bois, France), Gd-HPDO3A (ProHance®) by Bracco (Milano, Italy), and (*S*)-Gd-EOB-DTPA (Eovist®) by Schering AG (Berlin, Germany). $D_2O$ (99.9%) was obtained from Aldrich (Bornem, Belgium), serum (Kontrollogen L) from Behring (Marburg, Germany), and nondefatted human albumin (fraction V) from Sigma (Bornem, Belgium). ATP disodium salt (Janssen Chimica, Geel, Belgium), phosphocreatine disodium salt (Sigma, Bornem, Belgium), $Na_2HPO_4$ (Merck, Overijse, Belgium), and $NaN_3$ (Aldrich, Bornem, Belgium) were used for $^{31}P$ NMR measurements. Phosphate buffer pH 7 (Merck, Overijse, Belgium) and $ZnCl_2$ (Fluka, Bornem, Belgium) were used for the transmetallation studies. − The lanthanum complex was synthesized by reaction of $La_2O_3$ with the ligand in water and was precipitated by addition of acetone. Deuteration of the lanthanum complex at the α position of the carboxylic groups was performed by the procedure described by Wheeler and Legg:[29] 200 mg of lanthanum complex were dissolved in 20 mL of $D_2O$, the pD was adjusted to 10.6 by addition of $K_2CO_3$ and the mixture was heated at reflux with stirring for 24 h. The pD was then adjusted to 7 with concentrated hydrochloric acid, the solution was evaporated to a final volume of 10 mL and the solid KCl was removed by filtration. Acetone was added to induce precipitation of the deuterated compound, which was isolated by filtration, dissolved in 10 mL of $H_2O$, dialyzed, and isolated after lyophilization. The deuteration was confirmed by $^1H$-NMR spectroscopy. The sodium and potassium contents were controlled by ion-selective electrodes. The final product was labelled with 10 deuterium atoms and contained 2 $Na^+$ and 1 $K^+$ counterions. − $^1H$ NMR ($D_2O$): La-complex: δ = 7.5−7.15 (10 H); 4.3−1.7 (29 H); La-deuterated complex: δ = 7.5−7.15 (10 H); 4.3−1.7 (19 H)

**NMR Spectroscopy:** Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded on a Field Cycling Relaxometer (Field Cycling Systems, Oradell, New Jersey, USA) working between 0.24 mT and 1.2 T. The samples (0.6 mL) were contained in 10 mm o.d. tubes. Proton relaxation rates were also measured at 0.47 T on a Minispec PC-20 (Bruker, Karlsruhe, Germany). The temperature was controlled by a perchlorinated liquid flow. $T_1$ shorter than 50 ms were measured on this system using a specially-designed pulse sequence to overcome the limitations of the standard Minispec microprocessor. The data points were then fitted with a three-parameter minimization routine. The additional relaxation rates at 7.05 T were obtained on a Bruker AMX-300 (Bruker, Karlsruhe, Germany) spectrometer. − $^1H$ NMRD curves were fitted according to the theoretical innersphere model described by Solomon[17] and Bloembergen[18] and to the outersphere contribution described by Freed.[19] Calculations were performed with previously described software.[30][31] − The proton data ob-

$$R_1^{p\,obs} = 1000 \times \left\{ \left( r_1^{f} \times s^\circ \right) + \frac{1}{2}\left( r_1^{c} - r_1^{f} \right) \left( \left( N \times p^\circ \right) + s^\circ + Ka^{-1} - \sqrt{\left( \left( N \times p^\circ \right) + s^\circ + Ka^{-1} \right)^2 - 4 \times N \times s^\circ \times p^\circ} \right) \right\} \quad (1)$$

tained in HSA solution were fitted using Equation 1,[32] where *Ka* is the association constant, $p^\circ$ is the protein concentration, $s^\circ$ is the concentration of the paramagnetic complex, *N* is the number of independent interaction sites, and $r_1^c$ and $r_1^f$ are the relaxivities of the complex HSA-contrast agent and of the free contrast agent, respectively.

$^{31}$P-NMR $T_1$ measurements were performed on 2 mL samples contained in 10 mm o.d. tubes on a Bruker MSL-200-15 spectrometer (Bruker, Karlsruhe, Germany) fitted with a broadband probe. The temperature was regulated by air or nitrogen flow controlled by a BVT 1000 unit. The solutions contained 20 mM of ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) dissolved in Krebs Henseleit buffer (pH = 7.2−7.4) with 2 mM of $NaN_3$ added to prevent degradation of ATP and PCr by bacteria. The 90° and 180° pulse lengths were 7 μs and 14 μs, respectively. − Transmetallation by $Zn^{II}$ ions was evaluated by the decrease of the water proton longitudinal relaxation rate at 310 K and 20 MHz (Bruker Minispec PC 20) of buffered solutions (pH 7, phosphate buffer) containing 2.5 mM of the gadolinium complex and 2.5 mM of $Zn^{II}$. − $^{17}$O- and $^2$H-NMR spectra were recorded on 2 mL samples (10 mm o.d. tubes) on a Bruker AMX-300 spectrometer (Bruker, Karlsruhe, Germany) using a broadband probe and a Bruker BVT-2000 unit for temperature control. $^{17}$O transverse relaxation times of distilled water (pH = 6.5) were measured using a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 25 μs and 50 μs, respectively. $^{17}$O $T_2$ of water in MS-325 solution ([MS-325] = 12.5 mM, pH = 6.0−7.0) was obtained from linewidth measurement. Checks were performed so that for this paramagnetic solution, the $T_2$ obtained from linewidth measurement and from CPMG sequence were similar. All $^{17}$O spectra were proton decoupled. The concentration of MS-325 was 12.5 mM. The analysis of the $^{17}$O and $^2$H data is described elsewhere.[9] − All $T_1$ measurements at 4.7 T and 7.05 T ($^1$H, $^2$H, $^{31}$P) were performed with the inversion recovery or fast inversion recovery Fourier transform technique and a subsequent three-parameter fit of the peak heights.

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