(m), 1028 (w), 918 (w), 848 (s), 804 (w), 758 (s), 700 (s), 620 (s), 498 (w), 480 (w), 416 (w).

Crystallographic Studies. Details of the data collection procedures and structure refinement methods have been given previously.[12a] Table III contains some pertinent crystallographic data. The crystals were examined under dry, deoxygenated Nujol and mounted in an appropriately sized capillary with epoxy resin. The hydrogen atom positions were calculated after several cycles of anisotropic refinement assuming idealized geometries and a bond distance of 0.95 Å. The monitoring of three intensity standards every 5000 s of beam time indicated no decay. No correction for extinction was performed.

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Supplementary Material Available: Tables of crystal data and data collection parameters, non-hydrogen and hydrogen positional and thermal parameters, anisotropic temperature factors, and full bond distances and angles (9 pages); tables of observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

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Synthesis and Characterization of the Gadolinium(3+) Complex of DOTA-Propylamide: A Model DOTA-Protein Conjugate

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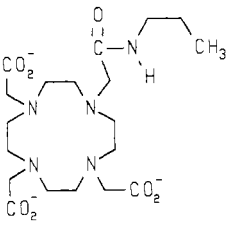
Several types of paramagnetic metal ion–chelate complexes have been proposed for use as contrast-enhancing agents in magnetic resonance imaging (MRI). The chemical properties and the distinct advantages of these different, largely inorganic, species have been extensively discussed in a recent review.[1] $Gd(DTPA)^{2-}$ is the first of these agents to be approved for use in humans and the standard to which newer agents are compared. The macrocyclic complex $Gd(DOTA)^-$ has several properties that make it a better candidate than $Gd(DTPA)^{2-}$, including a more favorable thermodynamic stability constant (log K = 24.7 for $Gd(DOTA)^-$ versus 22.3 for $Gd(DTPA)^{2-}$),[2] a significantly greater in vitro stability in blood serum,[3] which apparently translates into a lower toxicity in animals,[4] and a significantly greater solvent (water) proton relaxivity at low fields.[5] Both complexes contain one inner-sphere water molecule that exchanges rapidly with solvent water, thereby providing an efficient mechanism for relaxing water protons with a relatively small concentration of agent.

The utility of these paramagnetic agents would be greatly expanded if they could be covalently attached to macromolecules that could target or concentrate the agent to specific tissues, lesions, or cells. Several DTPA-conjugated macromolecules have been reported[6-10] and their advantages outlined.[1] In most cases, the chelate was conjugated to the macromolecule via amide or ester linkages by simply mixing the readily available DTPA-dianhydride with the macromolecule of interest. However, this reaction is known to result in a mixture of mono- and diconjugated chelates[11] that have considerably reduced affinities[12] for Gd^{3+}. Interestingly, we have also found that the conversion of one or even two terminal carboxyl groups of DTPA into a propylamine or ester gives ligands which form complexes with Gd^{3+} that have on average fewer inner-sphere water molecules than $Gd(DTPA)^{2-}$ at room temperature and below.[13] This suggests that the amide or ester moieties in these complexes, although weakly bound,[12] interact well enough to preclude the entrance of additional water molecules into the inner coordination sphere of Gd^{3+}.

Chart I



We now report the synthesis, rate of formation, thermodynamic stability, and field dependence of the longitudinal relaxation rate ($1/T_1$) of solvent water protons in solutions of Gd(DOTA-PA), which serves as a model of a monoconjugated DOTA macromolecule. The data indicate that monoconjugated DOTA macromolecules could offer certain advantages over the corresponding DTPA-conjugated systems in the design of in vivo targeted MRI contrast agents.

Materials and Methods

DOTA-PA. The monopropylamide of DOTA was synthesized by using methods reported by Krejcarek and Tucker,[14] with minor modifications. In a typical synthesis, the free-acid form of DOTA (0.17 g, 0.29 mmol) and triethylamine (1.15 mmol) were dissolved in dry DMSO (15 mL) by gentle warming. The resulting clear solution was cooled to room temperature, and isobutyl chloroformate (0.29 mmol) was added dropwise, followed by addition of an excess (2.07 mmol) of dry *n*-propylamine. The resulting mixture was stirred for 30 min and filtered and the DMSO distilled off under vacuum. The residue was dissolved into water, and the solution was loaded onto a 1.5 × 20 cm Dowex-1 anion-exchange column (acetate form), eluted first with water to remove excess amines, followed by a linear 0–0.5 M acetic acid gradient (300 mL total) as previously described.[12] The fractions corresponding to the first peak were combined, the pH was adjusted to 2 with HCl to protonate the acetate, and the sample was freeze-dried and identified by ^1H and ^{13}C NMR and elemental analysis as the monopropylamide of DOTA (see Chart I). The overall yield was about 30%. Anal. Calcd for $C_{19}H_{31}N_5O_7Cl\cdot 5H_2O$ (white solid): C, 40.2; H, 7.3; N, 12.4; Cl, 6.6; O, 33.9. Found: C, 40.2;

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Table I. Ligand Protonation Constants and Gd–Chelate Stability Constants

protonation const	DOTA[a]	DOTA-PA[b]
log K_1	11.2 (11.1)	9.6
log K_2	9.8 (9.2)	9.2
log K_3	4.4 (4.2)	4.4
log K_4	4.4 (4.2)	1.7
stability const	**Gd(DOTA)$^-$**	**Gd(DOTA-PA)**
log K^c	24.6 (23.6)	20.1
log K^{cd}	18.4 (18.1)	16.2

[a] Determined in 0.1 M KNO_3 at 25 °C (from ref 18). Values in parentheses were measured in 1 M NaCL at 25 °C and corrected for Na^+ binding competition (from ref 17). [b] This work; in 0.1 M NaCl at 25 °C. [c] Thermodynamic constants for Gd(DOTA)$^-$ are from ref 2. The first value is obtained by using the protonation constants evaluated in 0.1 M KNO_3 and the value in parentheses by using those found in 1 M NaCl. [d] Conditional stability constants; pH 7.4.

H, 7.3; N, 12.5; Cl, 6.3; O, 33.7 (by difference).

Potentiometry and Stability Constant Determinations. DOTA-PA was titrated at 25 °C against NBS standard 0.1 N NaOH in aqueous solution containing 0.1 M NaCl to maintain constant ionic strength. The solution activity was monitored with an Orion Model 701A digital meter equipped with a combination pH electrode containing a Ag/AgCl reference. The Gd(DOTA-PA) stability constant was determined by using a spectrophotometric procedure detailed in earlier reports.[2,12] The ligand protonation constants and Gd^{3+} stability constants were derived from the raw data by using nonlinear simplex algorithms written by Cacheris.[12]

NMRD Measurements. The field-cycling relaxometer measures the solvent water proton relaxation rate ($1/T_1$) over a continuum of magnetic fields from 0.00025 to 1.4 T (0.01–50-MHz proton Larmor frequency) under computer control with an absolute uncertainty in $1/T_1$ of 1%.[15,16] The resulting set of data is called an NMRD profile. A 2 mM Gd(DOTA-PA) solution containing 5% excess chelate was prepared from standardized stock solutions of Gd^{3+} and chelate; the pH was adjusted to 7.4. A similar solution of Gd(DOTA)$^-$ was prepared for reference NMRD measurements. After the NMRD measurements, each solution was analyzed for metal content by ICP emission spectroscopy to verify the Gd^{3+} content.

Results and Discussion

In Table I, the potentiometrically determined protonation constants of DOTA-PA are compared with values reported earlier for DOTA.[17,18] The first protonation constant in DOTA-PA is about 2 orders of magnitude smaller than the respective constant in DOTA, which corresponds to protonation of a ring nitrogen.[18] A similar decrease was observed between the first protonation constants of DTPA and DTPA-PA.[12]

The stability constant of Gd(DOTA-PA) was determined by competitive binding with arsenazo III as previously described[2] for Gd(DOTA)$^-$. All solutions containing Gd^{3+}, DOTA-PA, and arsenazo III were allowed to equilibrate for a minimum of 50 h at 60 °C before taking the absorbance measurements. DOTA-PA proved to remain intact under these conditions (the absorbances would have been considerably lower had DOTA-PA hydrolyzed to DOTA). The thermodynamic stability constant and the pH 7.4 conditional constant of Gd(DOTA-PA) are compared with the corresponding Gd(DOTA)$^-$ values in Table I. The decreased basicity of at least one nitrogen in DOTA-PA relative to that of DOTA results in about a 10^4 lower stability constant for its complex with Gd^{3+}. It should be noted that the reported thermodynamic constant for Gd(DOTA-PA) is a lower limit since we have assumed no significant interaction between DOTA-PA and Na^+ ions, as has been reported for DOTA.[18] Nevertheless, should significant interaction occur, the thermodynamic constant would likely not increase by more than 5–10%, as illustrated by the two thermodynamic constants listed for Gd(DOTA)$^-$ calculated by using protonation constants determined in 0.1 M KNO_3 versus 1 M NaCl (corrected for Na^+ binding).[17,18] The pH 7.4 conditional constant would be little affected by Na^+ competition.

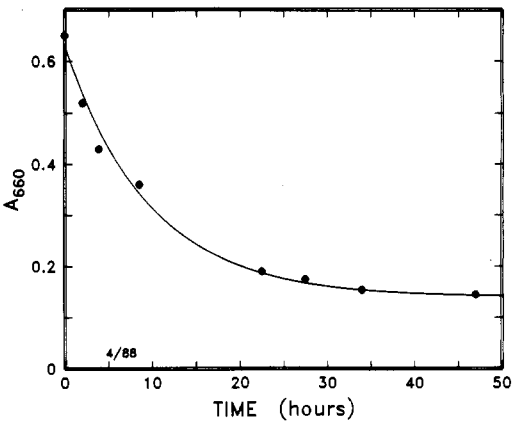

Figure 1. Kinetic data showing the relatively slow complexation of Gd^{3+} by DOTA-PA at pH 4 and 60 °C. The decrease in absorbance at 660 nm corresponds to removal of Gd^{3+} from its arsenazo III complex by DOTA-PA, as indicated by the smooth curve, which is an exponential decay with a rate constant of 0.073 h^{-1}.

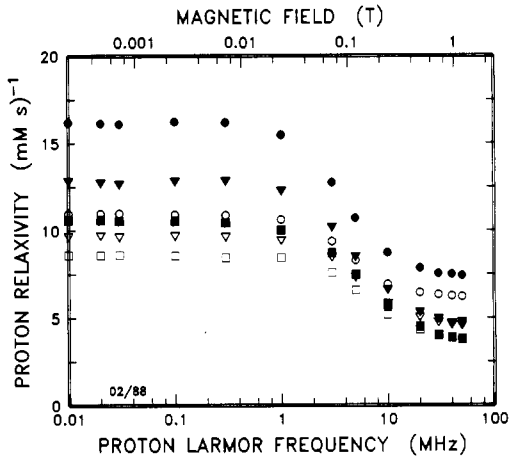

Figure 2. $1/T_1$ NMRD profiles for aqueous solutions of Gd(DOTA-PA) (open symbols) and Gd(DOTA)$^-$ (filled symbols) at pH 7. For each complex, the temperatures are 5, 25, and 35 °C, as one reads from top to bottom.

Both DOTA and DOTA-PA form chelates with Gd^{3+} much more slowly than linear polyamino polycarboxylates such as DTPA. Figure 1 shows a plot of A_{660} versus time for an aqueous solution at pH 4 and 60 °C containing 9.4×10^{-6} M Gd^{3+}, 7.6×10^{-5} M arsenazo III, and 3.7×10^{-4} M DOTA-PA. The decrease in absorbance at 660 nm corresponds to removal of Gd^{3+} from the Gd^{3+}–arsenazo complex[12] by the excess DOTA-PA. This process follows pseudo-first-order kinetics, as indicated by the good fit (solid curve) of an exponential decay with a rate constant of 0.073 h^{-1} (2×10^{-5} s^{-1}). Similar kinetics are observed for DOTA, whereas linear polyaza polycarboxylates like DTPA usually equilibrate within minutes under comparable conditions. Merciny et al.[19] have reported a second-order rate constant of 10^{-2} M^{-1} s^{-1} for Gd(DOTA)$^-$ complex formation at pH 2.85, comparable to the rate of Gd(DOTA-PA) complex formation observed here. Although we have not investigated the rates of Gd^{3+} dissociation from its DOTA-PA complex, the similar formation rates observed for the two complexes indicate that Gd^{3+} could dissociate from DOTA-PA about 100 times more rapidly than from DOTA at physiological pH, i.e., the difference between their pH 7.4 conditional stability constants. Nevertheless, this could still be sufficiently slow to give DOTA-conjugated proteins a significant

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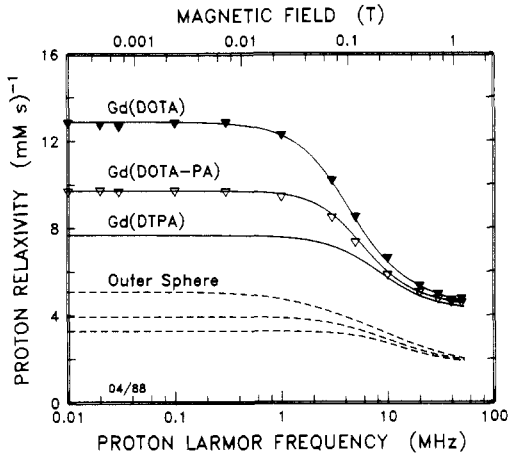

Figure 3. $1/T_1$ NMRD profiles. The two curves through the 25 °C data for $Gd(DOTA)^-$ (closed symbols) and Gd(DOTA-PA) (open symbols) were derived as in ref 13: the outer-sphere contributions (dotted lines) were subtracted from the data, the theory for inner-sphere relaxation was applied,[16] and the outer-sphere contributions were added back to generate the fits (solid lines). See text for fitting parameters. The fits for $Gd(DOTA)^-$ and Gd(DOTA-PA) are derived from those[22] of $Gd(DTPA)^{2-}$ by varying only τ_{SO} in both the inner- and outer-sphere contributions to the relaxivity.

advantage over the respective DTPA-conjugated proteins if indeed release of free Gd^{3+} is an important contribution to the toxicity of these systems.

NMRD profiles of the longitudinal relaxation rate ($1/T_1$) for solutions of $Gd(DOTA)^-$ (filled symbols) and Gd(DOTA-PA) (open symbols) at three temperatures are shown in Figure 2. The results are expressed as relativity, the increment in relaxation rate per millimolar Gd^{3+}, as a function of proton Larmor frequency. Figure 3 shows a quantitative comparison of the 25 °C data of Figure 2 with earlier results for $Gd(DTPA)^{2-}$, both experimental[20] and theoretical.[13,16,21] The dashed lines represent the outer-sphere contributions to the relaxivity of $Gd(DOTA)^-$, Gd(DOTA-PA), and $Gd(DTPA)^{2-}$, as one reads from the top down. (Note, however, that the outer-sphere contribution calculated here for $Gd(DTPA)^{2-}$ is somewhat lower than in ref 13, where an upper limit was calculated and averaged with the relaxivity of $Gd(TETA)^-$, presumed to have a fully coordinated inner coordination sphere.) As before,[13] we fit the difference between the data and the outer-sphere contribution to the theory of inner-sphere relaxation, assuming a single exchangeable water (i.e., $q = 1$) and a fixed rotational relaxation time $\tau_R = 64$ ps,[21] that of $Gd(DTPA)^{2-}$, at 25 °C, for all three small chelate complexes. τ_{SO} was determined for $Gd(DTPA)^{2-}$ to be consistent with both outer- and inner-sphere relaxation, $\tau_{SO} = 66$ ps giving the best fit to the data. For $Gd(DOTA)^-$, $\tau_{SO} > 660$ ps. This long τ_{SO} was noted previously and attributed to the high symmetry of the $Gd(DOTA)^-$ molecule.[5,21] When this symmetry is broken by forming the amide, τ_{SO} is shortened; the fit gives $\tau_{SO} = 132$ ps for Gd(DOTA-PA). The variation of τ_{SO} among these three Gd complexes accounts for the differences in their relaxivity at low fields as well as for the differences in their outer-sphere contributions (Figure 3). At high fields, the field dependence of the electronic relaxation time is such[16] that only τ_R determines the relaxivities, readily accounting for the fact that the three small chelate complexes have nearly the same relaxivities at high fields.

As noted previously[13] for $Gd(DTPA-PA)^-$, the conversion of a single DOTA carboxyl group into an amide does not result in an increase in the number of inner-sphere waters in Gd(DOTA-PA) versus that in $Gd(DOTA)^-$. The NMRD results indicate that both complexes have one exchanging inner-sphere water molecule over the temperature range examined, 5–35 °C. This suggests that the amide functionality, likely the carbonyl oxygen, occupies a site in the Gd^{3+} coordination sphere, thereby preventing an increase in inner-sphere waters. The $Gd(DTPA-PA)^-$ complex shows an unusual temperature-dependent relaxivity, which arises from a change in q from a high of 1 near 37 °C to a low of 0.2 at 5 °C,[13] a phenomenon that is not found for Gd(DOTA-PA). This indicates the entire coordination sphere of Gd(DOTA-PA) is more rigid than that of $Gd(DTPA-PA)^-$, consistent with the anticipated slower release of free Gd^{3+} from the macrocyclic complex.

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