A Novel pH Sensitive MRI Contrast Agent

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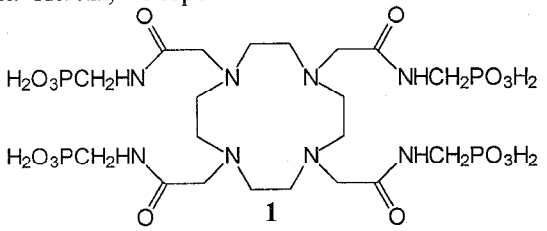
Introduction. Recent kinetic studies of Gd^{3+} complexes have shown that the lifetime of an inner-sphere water molecule (τ_M) varies from 0.84 ns for Gd_{aq}^{3+}, 208 ns for $GdDOTA^-$, to 303 ns for $GdDTPA^{2-}$.[1] Interestingly, τ_M is even longer in Gd^{3+} complexes of DTPA-bis(methylamide) and DOTA-tetrakis(methylamide), about 2 μs and 19 μs,[2] respectively. This feature is normally considered a disadvantage for enhancing the relaxation rate of bulk water. We have been exploring tetraamide-based cyclen derivatives having extended phosphonate or carboxylate non-coordinating side-chains with the intent of generating new systems with specific ion-pairing capabilities. Interestingly, the Gd^{3+} complex of one ligand in this series (structure **1**) was found to have an unusual pH dependent relaxivity (R_1) that may ultimately prove useful as a pH sensitive contrast agent. Herein, we report those initial observations.



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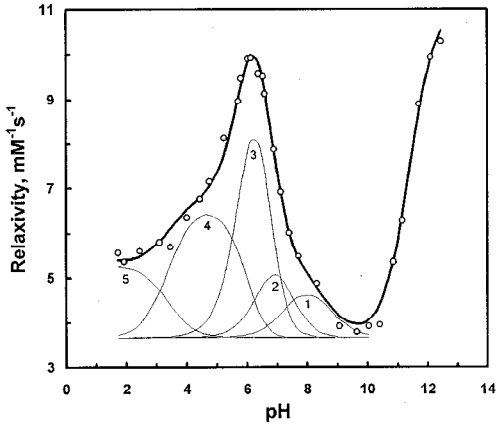
Methods. Ligand **1** was prepared in a multstep synthesis. First, bromoacetyl bromide was reacted with diethyl aminomethylphosphonate in benzene to yield diethyl bromoacetamidomethylphosphonate. Reaction of this alkylating agent with cyclen in acetonitrile at 60°C for 6 hours yielded the ethylester of ligand **1**. The phosphonates were deprotected by stirring the compound in a 30% solution of HBr in glacial acetic acid overnight at room temperature. **1** was isolated in acid form and characterized by high resolution NMR and elemental analysis. Complexes were prepared by mixing **1** with a lanthanide salt (1:1) in aqueous solution followed by pH adjustment to 9. All high resolution ^1H, ^{13}C, ^{31}P and ^{17}O NMR spectra were recorded using a Varian INOVA 500. Water proton relaxation measurements were performed at 20 MHz under strict temperature control using a MRS-6 NMR Analyzer (Institut Jozef Stefan, Ljubljana, Slovenija).

Results. ^{31}P spectra of all Ln(**1**) complexes (except Gd^{3+}) showed single resonances with chemical shifts not dramatically different from that of the free ligand. In comparison with the highly shifted ^{31}P resonances in the analogous $LnDOTP^{5-}$ complexes, this shows that the four phosphonate groups of Ln(**1**) are situated relatively far from the paramagnetic center and not coordinated to the central ion. All ^1H and ^{13}C NMR spectra of Ln(**1**) were consistent with single molecular species having high stereochemical rigidity, with hyperfine shifts similar in magnitude to those reported for the $LnDOTP^{5-}$ and $LnDOTA^-$ complexes. This verified that the Ln^{3+} cations occupy the macrocyclic cavity and are not bound to the appended side-chain functional groups. ^{17}O NMR chemical shifts of water in the presence of variable amounts of Dy(**1**) confirmed that a single water molecule is directly coordinated to Dy^{3+}, while variable temperature ^{17}O linewidth measurements reported an average τ_M of 20.8±1.2 μs (at pH 5.9, 6.6, 7.6 and 9.5), nearly identical to that reported for the simpler DOTA-tetrakis(methylamide) complex.[2]

The pH dependence of the water proton relaxivity of Gd(**1**) proved to be most interesting (Figure). The increase in relaxvity above pH 10, similar to that described earlier for the tetra-methylamide analog, has been ascribed to OH^- catalyzed prototropic exchange of the bound water protons.[2]



Unlike in the tetra-methylamide derivative where the water relaxivity is essentially independent of pH between 8 and 2,[2] the increase in relaxivity of Gd(**1**) below pH 10 appears to parallel protonation of the extended phosphonate groups (log K_n = 8.70, 7.28, 6.55, 6.02, and 3.38). A fit of these data to a model involving five protonated species gave R_1 values of 5.3, 6.7, 13.3, 6.3, 5.1 and 3.7 $mM^{-1}s^{-1}$ for $Gd(\mathbf{1})H_5$, $Gd(\mathbf{1})H_4$, $Gd(\mathbf{1})H_3$, $Gd(\mathbf{1})H_2$, $Gd(\mathbf{1})H_1$ and Gd(**1**), respectively. Interestingly, the calculated relaxivity of $Gd(\mathbf{1})H_3$ is notably higher than the other species, and indeed this species provides the main contribution to the maximum in the relaxivity curve near pH 6 (Figure).

Conclusions. The unusual pH dependency of the bulk water relaxivity of Gd(**1**) makes it a potentially useful pH sensitive MRI contrast agent. Although other approaches to preparing gadolinium complexes with relaxivities that are sensitive to pH over the physiological range have been proposed,[3,4] the present results demonstrate that it may be possible to modulate prototropic exchange by extended pendant arms in ligands such as **1** to design a series of pH sensitive contrast agents with differing tissue distributions and pH sensitivities.

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References:

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