Rational Design of a High Relaxivity MR probe

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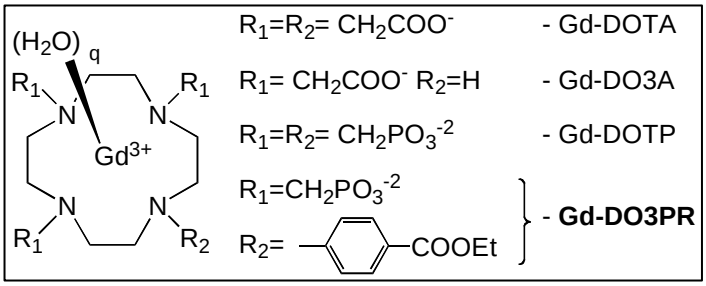
Introduction. The need for high relaxivity MR probes stems from the intrinsic insensitivity of MRI and from the sparsity of biologically relevant molecular targets. Current approved Gd-based agents e.g. Gd-DOTA comprise a multidentate ligand to sequester the Gd and one water molecule (q=1) directly coordinated to the metal ion. Relaxivity can be increased by reducing the denticity of the ligand to allow for coordination of extra water molecules in the inner coordination sphere, e.g Gd-DO3A, q=2.[1] One drawback is that the Gd-DO3A core can bind endogenous anions like phosphate which displace the inner-sphere water and reduce relaxivity.[2] Another approach to high relaxivity is to increase the number of exchangeable protons in close proximity to the paramagnetic center. For instance if the carboxylates of DOTA are replaced by larger phosphonate groups to give DOTP, the complex Gd-DOTP is q=0, but has similar relaxivity to q=1 Gd-DOTA. This is due to exchangeable hydrogens that can bind to the phosphonates, i.e. generate a 2nd hydration sphere.[3,4] We hypothesized that removing one phosphonate arm from DOTP would increase q but still maintain the 2nd-sphere relaxivity resulting in improved relaxivity. The well established macrocyclic cyclen-based core provides stability/inertness and the negatively charged phosphonates may be expected to repel anions and prevent inner-sphere water displacement. Moreover, one phosphonate arm can be replaced with a group that allows easy conjugation to a targeting vector.



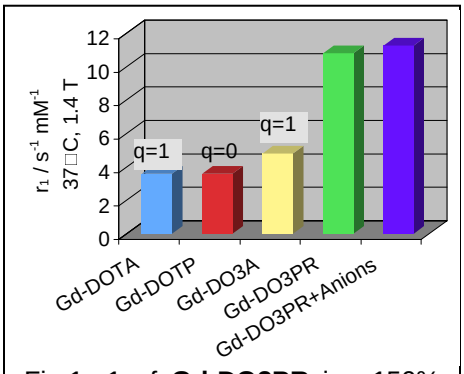


Fig.1 r1 of **Gd-DO3PR** is >150% higher than Gd-DOTA, and is unaffected by coordinating anions.

Methods. Ligand **DO3PR** was prepared starting with tris-BOC protected cyclen, which was subsequently alkylated with ethyl 4-(bromomethyl)benzoate. This intermediate was quantitatively deprotected with TFA and then the phosphonic groups were introduced by a Mannich-type reaction with tris-*tert*-butyl phosphite followed by an *in situ* deprotection of the *tert*-butyl groups. The gadolinium complex, **Gd-DO3PR** was prepared by reaction of the ligand with gadolinium chloride in water at pH 6.5. Relaxivity (r_1) was measured at four fields (0.47,1.4, 4.7, 9.4 T) at pH 7.4 TES buffer (50mM) in the absence and presence of high concentrations (up to 10X the physiological average) of biologically relevant anions (phosphate, carbonate, citrate).

Results. Ligand **DO3PR** was synthesized over three steps in good overall yield (65%). Relaxivity of **Gd-DO3PR** is significantly higher than Gd-DOTA, GdDOTP, and Gd-DO3A, and other similar sized agents. **Gd-DO3PR** relaxivity is unaffected by the presence of biologically relevant anions like citrate, phosphate and carbonate, suggesting little to no binding. Relaxivity increases with decreasing temperature indicating a fast water exchange regime, and implies that much higher relaxivity may be attained upon interaction with slow tumbling macromolecular substrates. Relaxivity was high at all fields studied, r_1 = 12.2, 14.9, 12.1, 9.6 s^{-1} mM^{-1} at 0.47, 1.4, 4.7, and 9.4T respectively at 21°C.

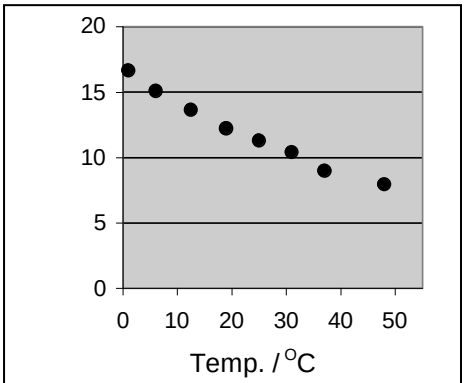


Fig. 2 Relaxivity of **Gd-DO3PR** increases with decreasing temparature, implying that much higher relaxivities may be attained by protein targeting.

Conclusions. Gd-DO3PR is a high relaxivity MR probe prepared using an efficient synthetic strategy in a good yield over four steps. It shows excellent relaxometric properties in the presence and absence of coordinating anions and over a broad field range when compared with agents of similar molecular size. These results underscore the concerted rational design strategy of increasing both inner- (q) and second-sphere hydration, while limiting competitive anion binding.

References: (1) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293-2352. (2) Dickins, R. S.; Aime, S.; Batsanov, A. S.; Beeby, A.; Botta, M.; Bruce, J. I.; Howard, J. A. K.; Love, C. S.; Parker, D.; Peacock, R. D.; Puschmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 12697-12705. (3) Botta, M. *Eur. J. Inorg. Chem.* **2000**, *2000*, 399-407. (4) Burai, L.; Ren, J.; Kovacs, Z.; Bruecher, E.; Sherry, A. D. *Inorg. Chem.* **1998**, *37*, 69-75.