Gadolinium(III) Texaphyrin: A Novel MRI Contrast Agent

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Magnetic resonance imaging (MRI) is now well established as an important tool for the diagnosis and evaluation of a variety of diseases.[1] Nonetheless, considerable effort continues to be devoted to the development and study of potential MRI contrast agents.[2-5] Such agents, which are generally small blood-borne paramagnetic chelate complexes, can allow an increase in image conspicuity but only in cases that are associated with changes in blood flow.[1] On the other hand, agents which have an ability to target internal organs or diseased tissue directly could serve to expand significantly the role of MRI in medicine. In this communication, we present the first example of such an improved contrast agent, namely the water soluble Gd(III) texaphyrin **1**,[6,7] which, based on studies in normal and tumor-bearing animals, shows significant promise as a novel MRI agent capable of both targeting the liver and detecting cancers.

X-ray diffraction analysis of complex **1**, the synthesis of which was recently reported,[6] reveals that the Gd(III) cation is nine coordinate and approximately 0.6 Å from the macrocyclic N5 texaphyrin plane (see Figure 1).[6] The Gd(III) cation is coordinated to all five core nitrogen atoms in a 1:1 fashion and ligated by one bidentate nitrate counterion and two molecules of methanol. Complex **1** thus displays a far more "normal" near-in-plane "porphyrin-like" binding behavior than is observed for the true, structurally characterized lanthanide(III) porphyrin complexes, for which "sitting atop", 2:1 "sandwich", or 3:2 "triple decker sandwich" coordination is routinely observed.[8] Given this better fit of the cation, it was considered that the water-soluble Gd(III) texaphyrin would prove more stable *in vivo* than its corresponding Gd(III) porphyrin analogues for which less than ideal hydrolytic stability has been reported.[9] It was also expected, on the basis of this structure, that between four and five water molecules would be able to interact with the metal center in aqueous solution. The relaxivity, R_1, of complex **1** (16.9 ± 1.5 mM^{-1} at 50 MHz)[10]

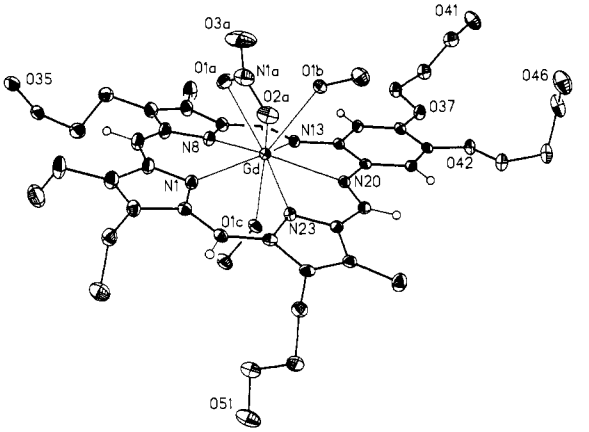
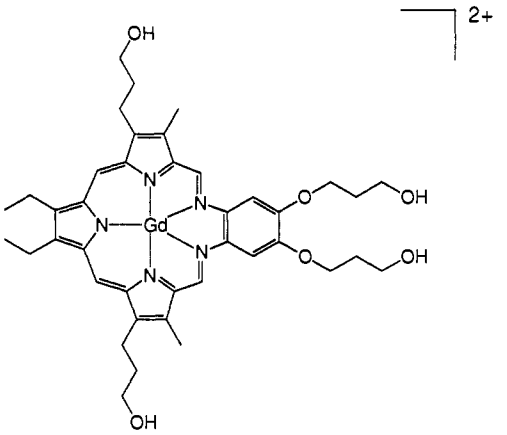


Figure 1. View of **1** showing a partial labeling scheme. The Gd(III) ion is 9-coordinate and lies 0.60 Å out of the plane through the five nitrogen atoms of the macrocycle. Relevant bond lengths (Å) for the Gd(III) ion are N1 2.494(4), N8 2.383(4), N13 2.536(3), N20 2.517(4), N23 2.388(4), O1A 2.489(4), O2A 2.484(3), O1B 2.504(4), O1C 2.491(4). Most hydrogen atoms have been omitted for clarity. Thermal ellipsoids have been scaled to the 30% probability level. For further details, see ref 6.



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(1) For reviews of MRI, see: (a) Edelman, R.; Warach, S. *New Eng. J. Med.* **1993**, *328*, 708-716. (b) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901-927. (c) Tweedle, M. F.; Brittain, H. G.; Eckelman, W. C.; Gaughan, G. T.; Hagan, J. J.; Wedeking, P. W.; Runge, V. M. In *Magnetic Resonance Imaging*, 2nd ed.; Partain, C. L., Ed.; W. B. Saunders: Philadelphia, PA, 1988; Vol. 1, pp 793-809. (d) Moonen, C. T.; van-Zijil, P. C.; Frank, J. A.; Le-Bihan, D.; Becker, E. D. *Science* **1990**, *250*, 53-61. (e) Young, S. W. *Magnetic Resonance Imaging: Basic Principles*; Raven Press, New York, 1988; pp 1-282.

(2) Currently, the bis-*N*-methylglucamine salt of Gd(III) diethylenetriaminepentaacetic acid (DTPA) (Magnevist), the bis-*N*-methylamide of Gd(III) DTPA (Omniscan), and the Gd(III) 10-(2-hydroxypropyl) derivative of 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-1,4,7-triacetic acid (DO3A) (Prohance) are being used clinically in certain enhanced tumor detection protocols. See also refs 1, 4, 5.

(3) For examples of MRI studies using porphyrins, see: (a) Mosseri, S.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 4659-4663 and references therein. (b) Bohdiewicz, P. J.; Lavallee, D. K.; Fawwaz, R. A.; Newhouse, J. H.; Oluwole, S. F.; Alderson, P. O. *Invest. Radiol.* **1990**, *25*, 765-770 and references therein. (c) Patronas, N. J.; Cohen, J. S.; Knop, R. H.; Dwyer, A. J.; Colcher, D.; Lundy, J.; Mornex, F.; Hambright, P.; Sohn, M.; Myers, C. E. *Cancer Treat. Rep.* **1986**, *70*, 391-395.

(4) For relevant DTPA-related references, see: (a) Tweedle, M. F.; Gaughan, G. T.; Hagan, J.; Wedeking, P. W.; Sibley, P.; Wilson, L. J.; Lee, D. W. *Nucl. Med. Biol.* **1988**, *15*, 31. (b) Konings, M. S.; Dow, W. C.; Love, D. B.; Raymond, K.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.* **1990**, *29*, 1488-1491. (c) Armitage, F. E.; Richardson, D. E.; Li, K. C. P. *Bioconjugate Chem.* **1990**, *1*, 365-374. (d) Geraldes, C. F. G. C.; Urbano, A. M.; Alpiom, M. C.; Hoefnagel, M. A.; Peters, J. A. *J. Chem. Soc., Chem. Commun.* **1991**, 656-658.

(5) For leading references to DO3A complexes, see: (a) Zhang, X.; Chang, C. A.; Brittain, H. G.; Garrison, J. M.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* **1992**, *31*, 5597-5600 and references therein. (b) Dischino, D. D.; Delaney, E. J.; Emswiler, J. E.; Gaughan, G. T.; Prasad, J. S.; Srivastava, S. K.; Tweedle, M. F. *Inorg. Chem.* **1991**, *30*, 1265-1269.

(6) Sessler, J. L.; Mody, T. D., Hemmi, G. W.; Lynch, V. *Inorg. Chem.* **1993**, *32*, 3175-3187.

(7) Complex **1** is soluble to ca. 2 mM in pure H_2O and to ca. 8 mM in 5% aqueous mannitol.

(8) (a) Buchler, J. W.; De Cian, A.; Fischer, J.; Kihn-Botulinski, M.; Paulus, H.; Weiss, R. *J. Am. Chem. Soc.* **1986**, 108, 3652-3659. (b) Schaverien, C. J.; Orpen, A. G. *Inorg. Chem.* **1991**, *30*, 4968-4978. (c) Buchler, J. W.; Löffler, J.; Wicholas, M. *Inorg. Chem.* **1992**, *31*, 524-526 and references cited therein. (d) Buchler, J. W.; Scharbert, B. *J. Am. Chem. Soc.* **1988**, *110*, 4272-4276.

(9) (a) Lyon, R. C.; Faustino, P. J.; Cohen, J. S.; Katz, A.; Mornex, F.; Colcher, D.; Baglin, C.; Koenig, S. H.; Hambright, P. *Magn. Reson. Med.* **1987**, *4*, 24-33. (b) Hambright, P.; Adams, C.; Vernon, K. *Inorg. Chem.* **1988**, *27*, 1660-1662. (c) Haye, S.; Hambright, P. *J. Chem. Soc., Chem. Commun.* **1988**, 666-668.

(10) Sherry, A. D.; Geraldes, C. F. G. C., unpublished results. See also ref 6 for an independent determination of this value.

(11) The high relaxivity observed for **1** is rationalized both in terms of the X-ray structure of **1** (see text) and in terms of spin-orbit interactions between the texaphyrin π system and the chelated metal center. See ref 6.

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was, therefore, expected to be high, and, indeed, it was found to be ca. 3–4 times greater than that of the various MRI contrast agents currently being used clinically.[11]

Initial evidence for possible *in vivo* stability came from studies carried out in freshly drawn blood plasma. Specifically, it was found that incubation of complex **1** at physiological temperature and at presumed physiological concentrations (1.4–39 μM/mL) gave rise to no measurable degradation of the complex over a 5-h period. This was determined by monitoring the position and amplitude of the characteristic Q-like absorbance of the Gd(III) texaphyrin complex at 740 nm and observing no change as a function of time. This band, which provides a sensitive signature for metalated texaphyrins,[12] could be monitored both qualitatively and quantitatively since plasma has no absorbance in this wavelength range. Thus, these observations, along with supporting *in vitro* experiments,[13] lead us to suggest that complex **1** should be sufficiently stable *in vivo* to allow its use in MRI contrast enhancement protocols.

Further evidence for *in vivo* stability came from preliminary biolocalization studies carried out using a set of three male and three female Sprague–Dawley rats and a ^{153}Gd-radiolabeled analogue of complex **1** (specific activity of 4×10^6 counts/min/mg). In these experiments, it was found, for instance, that 1 week after IV inoculation at the 4 μmol/kg dose level, 77 and 87% of the radioactivity had cleared from the male and female animals, respectively, with less than 10% of the residual reactivity being found in the bone. On the other hand, 24 h subsequent to IV inoculation at the 4 μmol/kg dose level, 24 ± 3% and 13 ± 2% of the radioactive counts were found in the liver for the male and female animals, respectively, with less than 3% of the radioactivity being found in any other soft organs (e.g., lung, spleen, intestine, kidney) monitored.[14]

Evidence of actual *in vivo* efficacy is presented in Figure 2, which serves to illustrate that the signal intensity of a transplantable tumor may be enhanced using complex **1**. The tumor model used in this study was a ca. 2.5-cm-diameter V2 carcinoma in the thigh muscle of a rabbit. It was imaged 2 weeks following implantation and at this time had a small amount of central necrosis. Contrast enhancement was determined by comparing precontrast to postcontrast signal intensity (the precontrast tumor serving as the control). As illustrated in Figured 2, the signal intensity of this tumor model can be increased dramatically by the IV administration of **1** at the 5 μmol/kg dose level. For instance, 3.5 h postadministration one sees diffusion of the agent throughout the tumor such that it is possible to discern clearly where the malignant tissue ends and the normal tissue begins.

To the best of our knowledge, such long-time enhancement of tumors is without precedent. Indeed, clinically approved, diffusable tracers such as, e.g., GdDTPA,[1,4] are useful for body imaging only for the first few minutes after injection and do not persist in other organs (with the exception of the kidney, from whence they are excreted) for periods as long as 3.5 h. Thus, the persistent tumor enhancement in body imaging suggested by the image in Figure 2b has not been observed with these extant agents.

In other related *in vivo* experiments,[15] complex **1** was found to effect substantial MRI contrast enhancement of both kidney and liver tissue in normal rats at this or lower dosage levels. On the other hand, no signs of toxicity or morbidity were observed in rats given 20 μmol/kg daily for 21 days, as judged by twice daily gross exam, weekly blood chemistries, and organ histology following end-of-study necropsy. Thus, the doses used appear to be safe in both an acute and a subchronic sense.[16] This, in turn, supports the notion that this particular gadolinium(III) texaphyrin complex (i.e., **1**) could emerge as a safe and effective MRI contrast agent for the imaging of kidney, liver, and neoplastic tissues. Current work is focused on exploring this possibility.

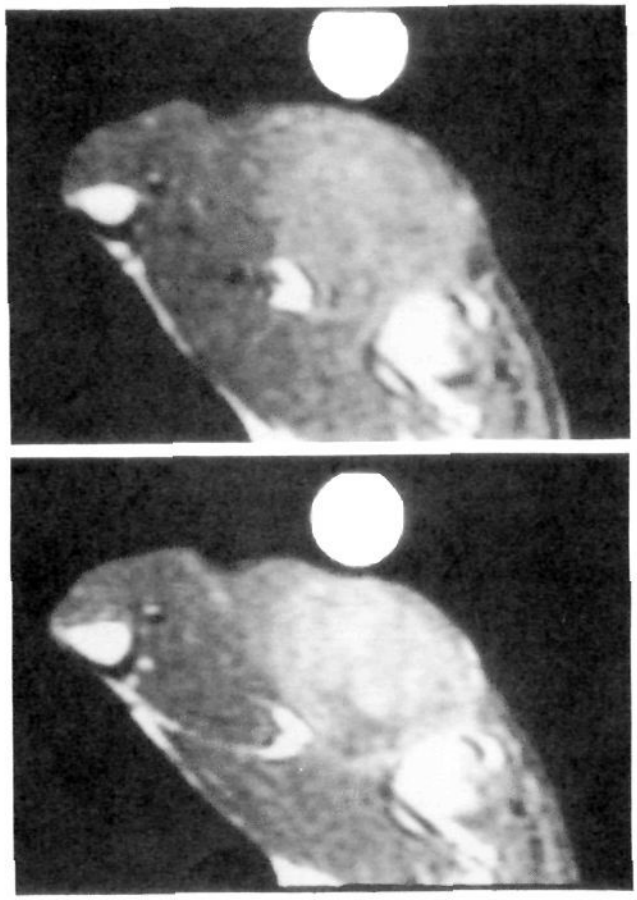

Figure 2. Axial MRI scans of a rabbit bearing a transplanted V2 carcinoma in thigh muscle before (top) and 3 h after (bottom) the administration of 5 μmol Gd(III) texaphyrin **1** per kilogram of body weight. The tumor, ca. 2.5 cm in diameter, is, as confirmed by independent histologic analysis, the mass defined by the now-whitened area of lower frame. The small white spots below the tumor are bones, as is that to the far left of the image; there are no discernible blood vessels in this image. The pulsing sequences used were conventional spin echo sequences TR/TE = 300/15, 16 KHz, 3-mm slice thickness and 256 × 192 matrix, 2 NEX (number of excitations), saturation inferiorly and superiorly with no phase rap.

Acknowledgment. This work was supported by grants from the Foundation for Biotechnology Research (to S.W.Y.), NIH (AI 28845 to J.L.S.), NSF (CHE 9122161 to J.L.S.), and Pharmacyclics Inc. We wish to thank Professor A. Dean Sherry and Dr. Carlos F. G. C. Geraldes for help with MRI relaxivity measurements.

(12) The optical spectrum of the free-base texaphyrin has been identified; the Q-type band of the free-baxe is shifted by ca. 10–15 nm to the blue relative to the metal complex; see: Sessler, J. L.; Murai, T.; Lynch, V. *Inorg. Chem.* **1989**, *28*, 1333–1341.

(13) The relaxivity of **1**, for instance, when monitored in aqueous solution at 25 °C, was found to be invariant in magnitude over a period of 4 days.[10] Also, incubation of **1** in the presence of a ca. 10^4 molar excess of EDTA failed to effect demetalation over a period of at least several weeks.[6]

(14) At the present time it is not known whether the ≤10% radioactivity found after 1 week is due to degradation of the texaphyrin or to encorporation of the intact ^{153}Gd complex into the bone. It is known, however, from studies of monkeys treated with daily 4–8 μM/kg doses of **1** that this macrocyclic complex can remain intact throughout its passage through the liver and into the bile, as judged by both quantitative spectrophotometric and HPLC analyses. This added indication of *in vivo* stability is important since such passage represents the main pathway for its excretion.

(15) Sidhu, M. K.; Muller, H.; Miller, R. A.; Mody, T. D.; Hemmi, G.; Sessler, J. L.; Young, S. W. *Abstr.* **1991** *Natl. Mtg. Am. Assoc. Univ. Radiol.*; March 1991, Orlando, Fl.

(16) The dose level used to effect the model tumor enhancements for Figure 2 (5 μmol/kg) is ca. 100 times lower than the LD_{50} of simple gadolinium salts (ca. 500–700 μmol/kg).[17] We are grateful to a reviewer for kindly making this point.

(17) *The Merck Index*, 11th ed.; Budavari, S., Ed.; Merck & Co.: Rahway, NJ, 1989; p 4237.