

# Synthesis and Application of New Gadolinium-Porphyrins as Potential MR Imaging Contrast Agents for Cancer Detection in Nude Mice

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## ABSTRACT

Two new potential magnetic resonance imaging contrast agents, Gd-hematoporphyrin (Gd-H) and Gd-tetra-carboranymethoxyphenyl-porphyrin (Gd-TCP), were synthesized and applied to nude mice with human melanoma (MM-138) xenografts. These agents showed a high relaxivity because of their greater potential to coordinate water molecules. The reduction of  $T_1$  relaxation times of 16 and 21% was observed in human melanoma tumors grafted in the nude mice 24 h after injection of Gd-TCP and Gd-H, respectively. The percent of injected Gd, that localized to the tumor and measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES), was approximately 21% for Gd-TCP and 28% for Gd-H. A higher concentration of Gd was achieved compared with control indicating selective delivery of Gd-porphyrins to the melanoma xenografts. The data indicate that Gd-TCP can be used as a dual probe for diagnosis in MR imaging and for therapy in boron neutron capture therapy (BNCT). *Iran. Biomed. J. 5 (2): x-x, 2001*

**Keywords:** MR imaging, Contrast agent, Porphyrins, Melanoma, Cancer

## INTRODUCTION

The use of contrast agents to shortening relaxation times following enhanced signal intensity may extend the potential of MR imaging to diagnosis of tumors in the early stages. Paramagnetic chelates using the endogenous porphyrin ring as the chelating agents are a promising and interesting family group of potential MR imaging contrast agents [1]. Gd-porphyrins have been synthesized and are currently being investigated [2-4] and shown selective affinity for a variety of tumors [5]. By choosing gadolinium as the metal for incorporation into the TCP and hematoporphyrin, they can be used simultaneously as MR imaging contrast agents. For this reason, two new gadolinium complexes of porphyrins were synthesized. The synthetic porphyrin, 1, 6, 11, 16 tetra (3-*o*-carboranymethoxy) phenyl-porphyrin was produced by modification of the method of Miura *et al.* [6] and was inserted with Gd to yield Gd-tetra-carboranymethoxyphenyl-porphyrin (Gd-TCP). The naturally occurring porphyrin, hematoporphyrin IX was also inserted with Gd to yield GD-hematoporphyrin (Gd-H).

The porphyrins offer a stable chelate for the transportation of paramagnetic metals into the tumors and by attachment they could destroy the cancer cells. The synthetic porphyrin TCP is an example of this concept where boron atoms have been attached chemically to the porphyrin, thus offering the potential for boron neutron capture therapy (BNCT). The gadolinium complex of these two porphyrins is unknown and in this work the synthesis of these MR imaging contrast agents are investigated.

An animal study was performed for developing radiopharmaceuticals and pharmacokinetics of these contrast agents. In the present study, Gd-hematoporphyrin (Gd- [18, 13-bis (hydroxyethyl)-3, 7, 12, 17-tetramethyl-2H, 23 H porphin-2, 18-dipropionic acid]) and Gd-TCP (Gadolinium-tetra-carboranymethoxyphenyl-porphyrin) were targeted into the nude mice model with a human melanoma (MM-

138) xenograft [7]. The bio-distribution, the  $T_1$  relaxation times, and the signal enhancement of the contrast agents are presented and the results are compared. The gadolinium concentration of tissues was determined using an acid digestion method [8] by ICP-AES.

## MATERIALS AND METHODS

**Gd-H.** Gadolinium (III) nitrate hexa-hydrate (0.30 g, 0.66 mmol) was dissolved in 2 ml of distilled water. Hematoporphyrin ([8, 13-bis (1-hydroxy-ethyl)-3, 7, 12, 17-tetramethyl-2H, 23H-porphine-2, 18-dipropionic acid]) (Sigma, Aldrich) powder (0.40 g, 0.66 mmol) was suspended in 2 ml of distilled water, added to the gadolinium solution and refluxed until the solution become homogeneous. The solution was allowed to cool at room temperature and then was concentrated to 1 ml under reduced pressure by heating. The resulting white solid was filtered, washed carefully with ice-cold water ( $2 \times 0.5$  ml) and dried in the oven at  $80^\circ\text{C}$ . The yield was 0.11 g (21%).

**Gd-TCP.** The experimental procedure for the synthesis of this new MR imaging contrast agent is as follows:

The synthesis of this compound involved the preparation of the known porphyrin, tetra-carboranylmethoxyphenyl-porphyrin (TCP- $\text{H}_2$ ) followed by insertion of the gadolinium ion into this porphyrin. The first part of this synthesis involved the formation of an aldehyde containing the *o*-carborane group. This aldehyde was reacted with pyrrole to form the porphyrin, TCP- $\text{H}_2$ . The gadolinium ion was inserted into TCP- $\text{H}_2$  by adaptation of Miura's method [6] for the nickel complex. The synthetic route for the production of Gd-TCP is shown in Figure 1. The purity and identity of each compound was confirmed by low-resolution mass spectroscopy, LRMS (Shimatzu QP5000/GC17A), and NMR analysis, both of them are previously discussed.

**(3-(*o*-carboranylmethoxy)phenyl)methanol.** (3-(*o*-carboranylmethoxy)phenyl)-methyl ethanoate (5.2 g, 24.5 mmol) was refluxed in a 1:80 (v/v) conc. HCl: MeOH (80 ml) at  $60^\circ\text{C}$  for 1 hour. The solvent was removed under reduced pressure yielding a crimson solid. The yield was 5.8 g (8%).

MS (molecular ion, m/z (peak intensity))

**Observed.** 276.30 (2.3); 277.30 (9.1); 278.30 (28.9); 279.30 (65.8); 280.30 (100.0); 281.30 (93.8); 282.30 (43.4); 283.30 (4.8).

**Calculated.** 276.26 (1.6); 277.26 (8.0); 278.25 (27.3); 279.25 (64.2); 280.25 (100.0); 281.24 (94.3); 282.24 (43.3); 283.24 (3.7).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 6.90 (d, 1H,  $J_{(\text{HH})}$  2.1 Hz, ArH); 7.00 (d, 1H,  $J_{(\text{HH})}$  7.4 Hz, ArH); 7.30 (t, 1H,  $J_{(\text{HH})}$  7.4 Hz, ArH); 6.75 (dd, 1H,  $J_{(\text{HH})}$  7.4, 2.1 Hz, ArH); 4.70 (s, 2H,  $\text{B}_{10}\text{H}_{11}\text{C}_2\text{CH}_2\text{O-Ar}$ ); 4.40 (s, 2H,  $\text{Ar-CH}_2\text{OH}$ ); 4.10 (br s, 1H, carborane CH); 1.5-3.3 (br m, 10H,  $\text{B}_{10}\text{H}_{10}$ ).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 64.7 ( $\text{Ar-CH}_2\text{OH}$ ); 142.9, 157.3 ( $\text{ArCR}$ ); 129.9, 120.8, 113.8, 112.9 ( $\text{ArCH}$ ); 69.1 ( $\text{B}_{10}\text{H}_{11}\text{C}_2\text{CH}_2\text{O-Ar}$ ); 57.8 ( $\text{RCH}$ , carborane); 71.3 ( $\text{CR}$ , carborane).

**$^{11}\text{B}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 51.7, 49.9, 45.5, 42.9, 41.6.

**3-*o*-carboranylmethoxybenzaldehyde.** (3-(*o*-carboranylmethoxy)phenyl)-methanol (7.8 g, 27.7 mmol) was added to pyridinium chlorochromate (9.0 g, 41.6 mmol) in dichloromethane (62 ml) at  $0^\circ\text{C}$  and was stirred for 2.5 hours. The combined solution was washed with water (50 ml  $\times$  3) and then extracted with dichloromethane (50 ml  $\times$  3). The combined extracts were dried with anhydrous sodium sulfate and the solvent was removed under reduced pressure. The resulting compound was purified using flash chromatography (silica, gel  $\text{CH}_2\text{Cl}_2$  eluent) to give 6.2 g (80%) of a crimson crystalline solid.

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MS (molecular ion, m/z (peak intensity))

**Observed.** 275.25 (3.1); 276.30 (10.7); 277.30 (21.1); 278.30 (30.4); 279.30 (29.0); 280.30 (13.6); 281.40 (1.7).

**Calculated.** 275.24 (2.4); 276.24 (8.3); 277.23 (19.5); 278.23 (30.4); 279.23 (28.7); 280.22 (13.2); 281.22 (1.1).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 9.90 (s, 1H, CHO); 7.35 (dd, 1H,  $J_{(\text{HH})}$  1.1, 2.5 Hz, ArH); 7.14 (dt, 1H,  $J_{(\text{HH})}$  7.7, 2.6 Hz, ArH); 7.48 (t, 1H,  $J_{(\text{HH})}$  7.7 Hz, ArH); 7.53 (ddd, 1H,  $J_{(\text{HH})}$  7.7, 2.6, 1.1 Hz, ArH); 4.50 (s, 2H,  $\text{B}_{10}\text{H}_{11}\text{C}_2\text{CH}_2\text{O-Ar}$ ); 4.10 (s, 1H, carborane CH); 1.5-3.3 (br m, 10H,  $\text{B}_{10}\text{H}_{10}$ ).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 191.4 (Ar-CHO); 137.9, 157.5 (ArC-); 130.6, 125.5, 121.8, 112.5 (ArC-H); 69.3 ( $\text{B}_{10}\text{H}_{11}\text{C}_2\text{CH}_2\text{O-Ar}$ ); 57.8 (RCH, carborane); 71.3 (CR, carborane).

**$^{11}\text{B}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 51.9, 50.0, 45.6, 42.9, 41.5.

**1, 6, 11, 16-tetra-[3-(carboranylmethoxy) phenyl] -porphyrin.** 3-*o*-carboranyl- methoxy-benzaldehyde (4.2 g, 15 mmol) and pyrrole (1.04 ml, 15.0 mmol) were added to 1 L of distilled  $\text{CH}_2\text{Cl}_2$  in a 2 L, three-neck, round-bottomed flask fitted with a reflux condenser and nitrogen inlet port, and stirred magnetically at room temperature. After 15 minutes,  $\text{BF}_3$  etherate (0.4 ml of a 2.5 M, 37.5 mmol solution in  $\text{CH}_2\text{Cl}_2$ ,  $10^{-3}$  M) was added and the reaction vessel was shielded from ambient lighting. After 1 hour, p-chloranil (2.8 g, 11.0 mmol) was added. The flask was immersed in a water bath and the solution was refluxed for 1 hour. The solution was then concentrated to approximately 50 ml under reduced pressure, and 10-12 g of Florisil was added. The slurry was further dried under reduced pressure to afford a damp dark powder, which was poured onto the top of a chromatography column (2.5 cm diameter) filled with Florisil (38 cm in height). The column was eluted with  $\text{CH}_2\text{Cl}_2$ /petroleum ether (3:1, 500 ml). The product was then eluted from the column with  $\text{CH}_2\text{Cl}_2$  (300-400 ml). The solvent was removed under reduced pressure and a crystalline purple solid was obtained. The yield was 2.1 g (43%).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 2.85 (br s, 2H, NH); 1.4-3.2 (br s, 40H,  $\text{B}_{10}\text{H}_{10}$ ); 4.12 (s, 4H, carborane CH); 4.48 (s, 8H,  $\text{CH}_2$ ); 7.26 (m, 4H, benz. ArH); 7.76 (m, 8H, benz. ArH); 7.93 (m, 4H, benz. ArH); 8.93 (s, 8H, pyr. ArH).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 131.0 (*meso*-carbons); 143.1, 156.1 (*benz.* ArC-); 129.2, 128.1, 121.0, 114.2 (*benz.* ArCH); 69.6 ( $\text{CH}_2\text{O-Ar}$ ); 57.9 (RCH, carborane); 71.4 (CR, carborane); 128.5 (pyr. ArC-); 121.3 (*pyr.* ArCH).

**$^{11}\text{B}$  ( $\text{CDCl}_3$ ).**  $\delta$  ppm 51.9, 50.0, 45.5, 43.0, 41.9.

UV-Vis. ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}}$  nm: 417, 513, 548, 590, 649.

**Gadolinium-1, 6, 11, 16-tetra-[3-carboranyl-methoxyphenyl]-porphyrin acetate.** A 50-ml, three-neck, round-bottomed flask fitted with a reflux condenser and nitrogen inlet port was filled with 17 ml of distilled  $\text{CH}_2\text{Cl}_2$ . Tetra-carboranylmethoxy-phenyl-porphyrin (0.20 g, 0.16 mmol) was added and the solution was stirred magnetically for 10 minutes. To this solution, a Gadolinium (III) acetate hydrate (0.050 g, 0.16 mmol) in methanol (2 ml) was added and the reaction vessel was shielded from ambient lighting. The flask was immersed in a water bath and the solution was allowed to reflux overnight. Insertion of gadolinium into the porphyrin was monitored by UV-visible (Shimatzu, UV-1601 PC) for completion. The solution was evaporated to dryness under reduced pressure to yield a purple crystalline solid, which was washed with water ( $2 \times 2$  ml). The yield was 0.23 g (100 %).

Observed % Gd 10.6 Calculated % Gd (based on  $\text{Gd}(\text{C}_{56}\text{H}_{76}\text{B}_{40}\text{N}_4\text{O}_4)(\text{CH}_3\text{CO}_2^-)$ ) 10.4.

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UV-Vis. (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}$  nm: 424, 548, 588.

**Sample preparation.** Solutions of different MR imaging contrast agents was prepared in the following procedures:

Solutions of GdCl<sub>3</sub>, Gd-DTPA, and Gd-H were prepared by accurately dissolving the required amount in 0.9% saline solution.

Gd-TCP (15 mg, 0.010 mmol) was dissolved in 1 ml of cremophor EL (CRM) and 2 ml of 1,2-propanediol. This solution was transferred into a 10 ml volumetric flask, and a 0.9% saline solution was added to the mark. This gave a final concentration of 1.0 mM.

**Animal selection.** The animal studies were performed with nude mice (nú nu, BALB/c) of 6-8 week old with a mean weight of 20g (Animal Resources, Western Australia). Animals were randomly divided into five groups of six. Each group was housed per cage in humidity and temperature controlled, isolated animal house at St. George Hospital, Sydney.

**Tumor xenograft model.** The human melanoma cells, MM-138 (St. George Hospital, Sydney), grown in tissue culture and originally derived from human malignant melanoma, were injected ( $2.5 \times 10^6$  cells) subcutaneously in the both flanks of nude mice.

**Injected dose.** Three to four weeks after tumor implantation, when the tumor diameter was 3-5 mm (mean weight of tumors was 200 mg), mice were injected with different contrast agent conjugates. All contrast agents were diluted in physiological saline to a final concentration as injected in bolus doses (10 mmol /kg of body weight). Two groups of six mice were injected each intraperitoneally (i.p.) with Gd-H and Gd-TCP. One group received Gd-DTPA and the fourth group received GdCl<sub>3</sub>. The last group was a control group. The total injected volume was 200  $\mu$ L. The animals were sacrificed by an over-dose of pentobarbital sodium 24 h post i.p. injection, followed by removal of critical organs (tumor, kidney, liver, spleen). These organs were minced for MR imaging and ICP-AES experiments [7]. The gadolinium concentration in tumor and various removed organs in *in vivo* measured using acid digestion method by ICP-AES [8].

**In vivo Proton relaxation times (T<sub>1</sub>) determination.** All MR imaging measurements and spectra were obtained on a 300 MHz, 7.0 Tesla, Varian UNITY Plus (Varian Associated, Inc., CA) with a vertical Oxford Instruments magnet of bore size 89mm using the 15 mm saddle coil (DOTY Scientific Instruments) resonator. The effect of contrast agents on proton relaxation times was measured in tumors and other organs using an inversion recovery (IR) pulse sequence technique.

The T<sub>1</sub> values and Gd concentration data for different contrast agent solutions were used to generate relaxivity rate constants, r<sub>1</sub> in reciprocal mmol seconds. This was accomplished via linear regression analyses of 1/T<sub>1</sub> versus Gd concentration with r<sub>1</sub> calculated as the slope of the fitted lines for data collected different concentrations.

**MR image signal intensity.** The enhancement effect of these agents on MR imaging signal was investigated. All images were obtained using the T<sub>1</sub>-weighted imaging method using IR pulse sequence technique, with T<sub>E</sub> = 15 msec, T<sub>R</sub> = 300 msec, T<sub>1</sub> = 200 msec, 5 mm slice thickness, 3  $\times$  3 cm<sup>2</sup> field of view, and matrix size of 256  $\times$  128. The MR image signal intensity was performed by selecting voxels in the image transfer display. Five signal intensity measurements were randomly obtained for each voxel and an average of those was calculated as the signal intensity.

**Gd concentration measurements.** All samples (tissues) were frozen until used for ICP-AES measurements. The gadolinium content was measured based on an acid digestion procedure using ICP-AES (Applied Research Laboratory, UK) instrument according to the method of Tamat *et al.* [8]. The 342.249 nm atomic emission line of Gd was chosen for the ICP-AES analysis. The tissue uptake of the Gd was calculated as a percentage of the initial injected dose of contrast agent (% i.d.).

## RESULTS AND DISCUSSION

**Relaxivity.** Specific targeting of MR imaging contrast agents demands a detailed knowledge of properties

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of the agent used. These details including the feasibility and the required dose of injection as well as uptake by the selected ligands. The efficacy of porphyrin based contrast agents was calculated by measuring their effect on proton relaxation times. Therefore, a relaxivity measurement was performed to detail investigation of the tissue-specific contrast agents as shown in Table 1. Relaxivity values of Gd-porphyrins are approximately 4 or 5 times higher than Gd-DTPA. This increase is due to its greater potential to coordinate water molecules. These results are consistent with reported relaxivity of porphyrin based contrast agents [2].

**Table 1.** Relaxivity values of gadolinium in aqueous solutions of MR imaging at room temperature (23°C).

Contrast agents	$r_1$ (mM <sup>-1</sup> s <sup>-1</sup> )
GdCl <sub>3</sub>	12.3 ± 0.7
Gd-DTPA	3.7 ± 0.1
Gd-H	16.3 ± 1.4
Gd-TCP	31.7 ± 0.3

**Gadolinium concentration.** The Gd tissue uptake was calculated as a percentage of the injected contrast agent by organs and results are shown in Table 2 and Figure 2. As can be seen from Figure 2, for GdCl<sub>3</sub> and Gd-DTPA the Gd uptake by the tumor was 13% and 18%, respectively.

Tumor uptake of 21% and 28% of the injected gadolinium was recorded for Gd-TCP and Gd-H, respectively. This amount identified the potential of the porphyrin-based compounds as tumor-specific detection agents. Calculations of the concentration of

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