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# The Hydroboration Reaction as a Key for a Straightforward Synthesis of New MRI-NCT agents

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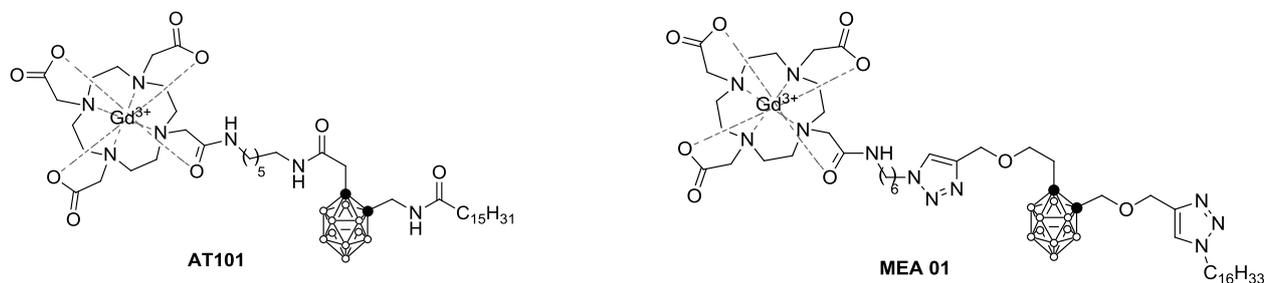
## Abstract

In this study the hydroboration reaction has been exploited to produce in only four steps a new lipophilic GdBNCT/MRI agent (**PB01**). As a matter of fact, the formation of a new B-C bond to link the decaborane with the lipophilic moiety greatly simplifies the synthesis of **PB01** respect to the previously reported dual agents. The complexes obtained (**PB01a** and **PB01b**) have been fully characterised from the relaxometric point of view and, after disaggregation with HP $\beta$ CD, both isomers display high affinity for Low Density Lipoproteins (LDL) that can be exploited as specific carrier of these therapeutic and diagnostic agents for tumour cells. The LDL loading capacity is different for the two isomers. In fact, LDL can be loaded with 75 and 300 units of **PB01a** and **PB01b** respectively and, for this reason, the isomer **PB01b** results the best candidate to perform MRI guided BNCT.

## Introduction

Boron Neutron Capture Therapy (BNCT) is a binary treatment that combines low energy neutron irradiation with the presence of boron-containing compounds in the target cells. This methodology has been proposed for different pathologies but most of the research focuses on cancer.<sup>1-3</sup> Although BNCT has been under investigation for many years, it has not entered in routine clinical setting yet as it has not been shown to be superior to other current therapies. Since this treatment can potentially damage only cells internalising large amounts of boron (at least 20-30 ppm), the inability to accumulate it in a sufficient amount into the tumour is the principal cause of the limited success of this therapy. One of the problems confronting the successful clinical implementation of BNCT is the difficulty of quantitatively mapping the distribution of the boron containing molecules in patients during the treatment. To date there is no non invasive way to evaluate boron concentration in diseased and healthy tissues of the subject undergoing the irradiation. Thus, dose calculations are based on boron content values in blood, tumour, and normal tissue obtained from biodistribution studies performed beforehand.<sup>4,5</sup> Blood samples can be taken just before and even during irradiation to measure the tissue boron concentration, assuming the tumour/blood ratios established in previous biodistribution studies. One of the problems is that the boron distribution varies among patients and that large uncertainties exist in the tumour-to-blood boron concentration ratio. The recent development of more sensitive imaging techniques and molecular medicine protocols based on the use of site specific agents able to carry huge amounts of both therapeutic and diagnostic agents can help BNCT to improve its efficacy in order to be competitive with the other routine tumour treatment protocols.<sup>6</sup> MRI images are characterised by a superb anatomical resolution and simultaneously map the structure and function of soft tissues *in vivo*.<sup>7-9</sup> The quantification of boron biodistribution can be carried out by measuring the MRI signal intensity enhancement of the boron containing compound that has been properly functionalised with a Gd-complex. Moreover, a dual Gd/B compound will show an improved NCT efficiency in respect to a system containing boron alone. In fact, Gadolinium contains at least two stable isotopes (<sup>155</sup>Gd and <sup>157</sup>Gd) that have high thermal neutron cross-sections. In particular, the <sup>157</sup>Gd thermal cross section provides a roughly 65-fold improvement upon <sup>10</sup>B.<sup>10-16</sup> Among boron derivatives, carboranes occupy a special position both for their high boron content and their chemical versatility coupled with a high *in vivo* stability.<sup>17</sup> Several readily functionalised carboranes have been employed to construct boron delivery vehicles for BNCT.<sup>18-21</sup>

The synthesis of new dual agents for applications in MRI/BNCT has been recently developed in our laboratory, where a carborane unit is linked to a lipophilic chain and to a Gd-DOTA complex through amidic bonds (AT101, Figure 1),<sup>22</sup> or triazole units (MEA01, Figure 1).<sup>23</sup>



**Figure 1** Structures of dual agents AT101 and MEA01

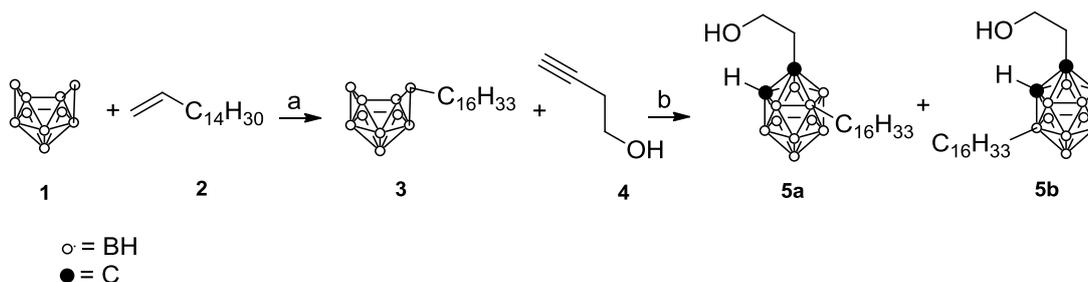
Since both these derivatives were able to form stable adducts with low density lipoproteins (LDLs), LDLs have been exploited as nanosized carriers for highly proliferating tumour cells that overexpress LDL receptors. MRI was performed on tumour melanoma cells incubated in the presence of AT101/ and MEA01/LDL adducts, showing that the high amount of intracellular boron necessary to perform BNCT experiments could be reached even incubating cells in the presence of a relatively low LDL concentration. Furthermore, in the case of AT101, *in vivo* MR images acquisition showed that the amount of B taken up in the tumour region was above the threshold for a successful NCT treatment. After neutron irradiation, the treated mice group showed a markedly lower tumour growth in respect to the control group.<sup>24</sup> More recently we reported the synthesis of a carborane- containing cholesterol derivative (AC01) that was evaluated as potential dual agent for MRI/BNCT applications.<sup>25</sup> Liposome embedded with AC01 were formulated with 1% of a pegylated phospholipid containing a folic acid residue at the end of the PEG chain. Folate receptors are overexpressed in many tumours and, in particular, on human ovarian cancer cells (IGROV-01). *In vitro* tests on IGROV-01 cells demonstrated that AC01 loaded liposomes are efficient carriers for the delivery of the MRI/BNCT dual agents to tumour cells. Moreover the BNCT treatment showed a decrease of surviving cells when the irradiation was carried out after internalisation of the folate-targeted AC01/liposomes.

The preparation of all these dual agents needs between nine and fourteen synthetic steps, giving a low overall synthetic yield, exploiting the formation of new C-C bonds. Herein, we envisaged to exploit a new B-C bond formation in order to functionalise the decaborane cage. As a boron hydride cluster, and differently from other boron derivatives such as diborane and 9-BBN, decaborane shows a unique and low reactivity towards olefins.<sup>26</sup> Both early and late transition metal catalysts catalyse the hydroboration of olefins affording 6-alkyl(alkenyl) and 6,9-

dialkyldecaborane respectively.<sup>27-30</sup> Very recently a platinum sequential hydroboration of decaborane to obtain poly(6-alkenyldecaborane) has been published.<sup>31</sup> Sneddon et al proposed a new methodology where the formation of the B-C bond was promoted by an ionic liquid in the absence of any metal catalyst, best results were observed for reactions with bmimX (X = Cl<sup>-</sup>, BF<sub>4</sub><sup>-</sup>). The ionic-liquid-mediated formation of the B<sub>10</sub>H<sub>13</sub><sup>-</sup> anion seems to be the essential first step of this reaction.<sup>32</sup> Another strategy for the alkylation of the carborane cage by a B-C bond formation exploits the protonation of the polyhedral cage under superacidic conditions<sup>33</sup> in order to generate an electrophilic intermediate that forms a 6-R-*nido*-B<sub>10</sub>H<sub>13</sub> derivatives by electrophilic aromatic substitution. The same group has recently proposed the synthesis of B-vinylcarboranes via the Pd catalysed cross coupling of iodocarboranes.<sup>34</sup> The aim of this study is to develop a new synthetic strategy which permits a new dual agent to be obtained by the use of the hydroboration reaction. This will be the key step to functionalise the carborane cage by a new B-C bond and to check its ability to form stable adducts with LDL, in order to assess if the different functionalisation of the carborane cage can influence the interaction of the aliphatic chain with the biological nanocarrier.

## Results and Discussion

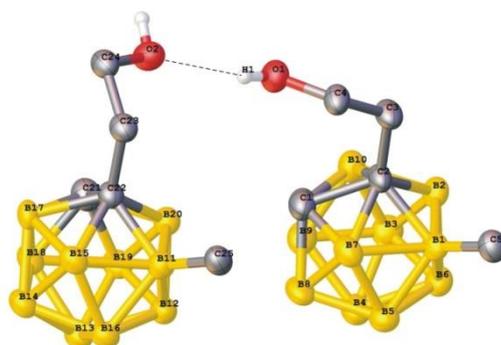
The first step of our synthetic strategy was the ionic liquid promoted hydroboration reaction of decaborane with a linear olefin following the procedure reported by Sneddon,<sup>32</sup> in the toluene/bmim<sup>+</sup>Cl<sup>-</sup> byphasic system at 125 °C, using hexadecene (**2**) as the olefin. Hexadecyldecaborane (**3**) was obtained in 16 h in a 43% yield. The analysis of the decoupled <sup>11</sup>B NMR spectrum showing seven peaks which witness the formation of B-C bond indicated the formation of the desired product. Then, a dehydrogenative insertion using 3-butyne-1-ol was carried out, leading to an equimolar mixture of diastereoisomers **5a** and **5b** with a 20% overall yield. Also this step was carried out in the byphasic system toluene/bmim<sup>+</sup>Cl<sup>-</sup> at 100°C. The isomeric ratio was determined by the analysis of the integration ratio of the carborane CH signal in the <sup>1</sup>H NMR spectra at 3.8 ppm and 4.1 ppm for the two isomers, respectively. The insertion was accomplished at 100 °C using a 4 eq excess of alkyne respect to the hexadecyldecaborane **3**, until the disappearance of the hexadecyldecaborane spot.



**Scheme 1** Synthesis of *C*-(2-hydroxy)-ethyl-*C'*-H-4-(hexadecyl)-*ortho*-carborane (**5a**) and *C*-(2-hydroxy)-ethyl-*C'*-H-9-(hexadecyl)-*ortho*-carborane (**5b**). Reaction conditions and yields: a)  $\text{B}_{10}\text{H}_{14}$ , hexadecene, (bmim)<sup>+</sup>Cl<sup>-</sup>, toluene, 125 °C (43%); b) 6-hexadecyldecahydro-*closo*- $\text{C}_{10}\text{H}_{12}\text{B}_{10}$ , 3-butyn-1-ol, (bmim)<sup>+</sup>Cl<sup>-</sup>, toluene, 100 °C (20%).

The structure of **5a** was determined via single crystal X-ray diffraction. As can be observed in Figure 2, in its asymmetric unit there are two molecules, that are enantiomers and differ prevalently for the disposition of the alcoholic groups which are strictly connected through a strong hydrogen bond involving the OH groups ( $\text{O}(1)\text{-H}(1)\cdots\text{O}(2) = 2.05$ ,  $\text{O}(1)\cdots\text{O}(2) = 2.815(3)$ ).

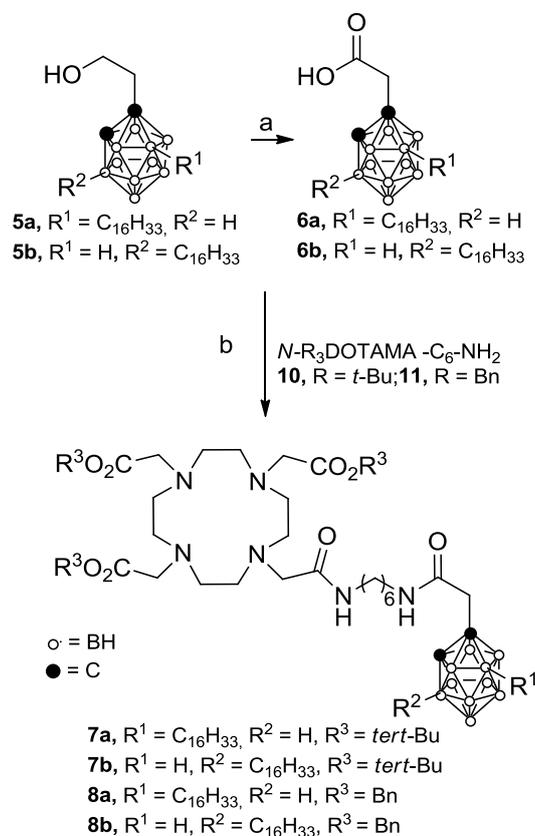
The geometry of the *closo*-carborane cluster is a distorted icosahedron with B-B distances ranging from 1.746(4) to 1.789(4), C-B distances from 1.688(4) to 1.737(4) and C-C distances from 1.657(3) to 1.658(3), that are within the expected values.



**Figure 2** Ortep plot of the two molecules in the asymmetric unit of compound **5a**. Thermal ellipsoids of non-hydrogen atoms are represented at 50% probability. The long alkyne chains and the most of hydrogens are omitted for clarity.

At this point, as described in Scheme 2, *C*-(2-hydroxy)-ethyl-*C'*-H-(4/9)-(hexadecyl)-*ortho*-carborane (**5a/b**) were readily oxidised to the corresponding carboxylic acids (77 and 69% respectively for **6a** and **6b**) by  $\text{CrO}_3$  in acetone/sulfuric acid solution. The structure of derivatives **6a** and **6b** was supported by the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data. In particular, the disappearance of the multiplet at 3.8 ppm assigned to the  $\text{HOCH}_2$ -group for **6a** and **6b**, with the concomitant growth in the  $^{13}\text{C}$  NMR spectrum of the signal at 171.82 and 171.91 ppm corresponding to the carbonyl group was noticed. In order to obtain the multiple agents containing both the BNCT and MRI moieties, derivatives **6a** and **6b** were reacted with tris-*tert*-butyl- or tris-benzyl DOTAMA- $\text{C}_6\text{-NH}_2$  (**10** and

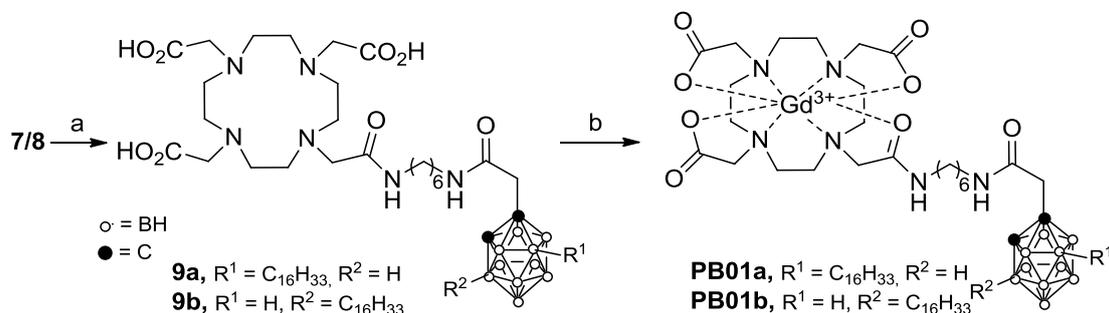
**11** respectively) following a two-steps procedure which exploits *N*-hydroxysuccinimide (NHS) as activating agent of the carboxylic group in the presence of DCC in anhydrous CH<sub>2</sub>Cl<sub>2</sub>. Better results were obtained when tris-*tert*-butyl-DOTAMA-C<sub>6</sub>-NH<sub>2</sub> (**10**) was used, in fact **7a** and **7b** were produced in a 42 and 35% yield respectively, whereas when the benzyl derivatives **11** was utilised, **8a** and **8b** were obtained with lower yields (13% for both), probably for the major steric hindrance attributable to this group. The structures of all these isomers were completely characterised and confirmed by high resolution ESI mass spectrometry were the ions were detected at 1105.9276 [M +H]<sup>+</sup> and 1105.9803 [M +H]<sup>+</sup> for derivatives **7a** and **7b**, and 1207.8837 [M +H]<sup>+</sup> and 1207.8805 [M +H]<sup>+</sup> for **8a** and **8b**.



**Scheme 2** Synthesis of C-[R<sub>3</sub>-DOTAMA-C<sub>6</sub>]-acetamido-C'-H-4-(hexadecyl)-*ortho*-carborane (**7a/8a**) and C-[R<sub>3</sub>-DOTAMA-C<sub>6</sub>]-acetamido-C'-H-9-(hexadecyl)-*ortho*-carborane (**7b/8b**). *Reaction conditions and yields*: a) 3M CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub>, acetone, r. t. (77% for **6a**, 69% for **6b**); b) NHS, DCC, anhydr. CH<sub>2</sub>Cl<sub>2</sub>, rt then iPr<sub>2</sub>EtN, anhydr. CH<sub>2</sub>Cl<sub>2</sub> (42% for **7a**, 35% for **7b**, 13% for both **8a** and **8b**).

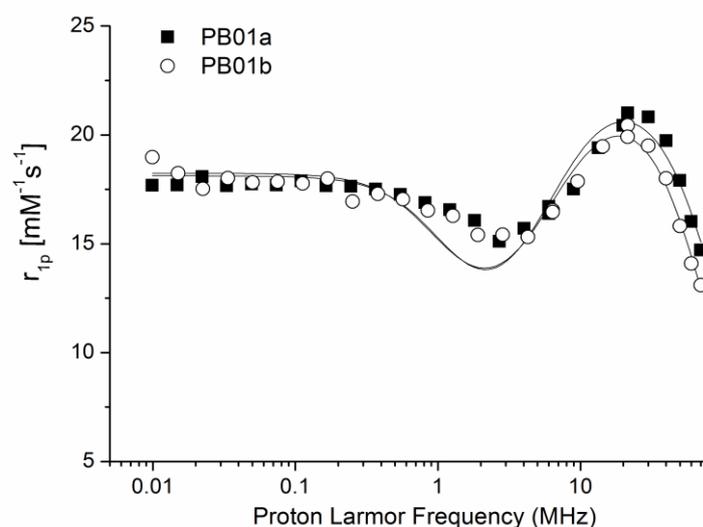
After removal of *tert*-butyl (CF<sub>3</sub>COOH/CH<sub>2</sub>Cl<sub>2</sub>) or benzyl ester groups (Pd/C, H<sub>2</sub>, EtOH/ CH<sub>2</sub>Cl<sub>2</sub>), derivatives **9a** and **9b** were complexed with a stoichiometric amount of GdCl<sub>3</sub> in an aqueous solution of the ligand at pH = 6.5, affording the multimodal MRI-GdBNCT agents **PB01a** and **PB01b** with an overall yield of 3% comparable to that of **AT101**, but lower in respect to **MEA01** (8%) previously reported. This result is, in our opinion, acceptable if we consider that four synthetic steps allow a remarkable saving in terms of time, solvents and reagents to be obtained. Isomers **9a** and **9b** were clearly distinguished by NMR, in particular in the <sup>1</sup>H NMR spectra, the signals

pertinent to the *CH* of the carborane cage resonate at 4.40 and 4.80 ppm for **9a** and **9b** respectively. Structures were confirmed by high resolution ESI mass spectrometry, with the detection of the characteristic ions at 915.8035 [M +H]<sup>+</sup> and 915.8054 [M +H]<sup>+</sup> for **9a** and **9b**.



**Scheme 3** Synthesis of  $\text{Gd}^{3+}$ - $\text{C}$ -[( $\text{COOH}$ )<sub>3</sub>DOTAMA- $\text{C}_6$ ]-acetamido- $\text{C}'$ -H-4-(hexadecyl)-*ortho*-carborane (**PB01a**) and  $\text{Gd}^{3+}$ - $\text{C}$ -[( $\text{COOH}$ )<sub>3</sub>DOTAMA- $\text{C}_6$ ]-acetamido- $\text{C}'$ -H-9-(hexadecyl)-*ortho*-carborane (**PB01b**). *Reaction conditions and yields*: a)  $\text{CF}_3\text{COOH}$ ,  $\text{CH}_2\text{Cl}_2$ , r. t., (>99% for both **9a** and **9b**); or  $\text{H}_2$ , Pd/C, EtOH,  $\text{CH}_2\text{Cl}_2$ , rt (>99% for both **9a** and **9b**); b)  $\text{GdCl}_3$ ,  $\text{H}_2\text{O}$  (>99% for both **PB01a** and **PB01b**).

Both Gd complexes **PB01a** and **PB01b** are highly hydrophobic and form very stable micelles in aqueous solution with average hydrodynamic diameters, measured by Dynamic Light Scattering (DLS), of  $12 \pm 1$  and  $21 \pm 3$  nm, respectively. As a consequence of the long tumbling time of these micellar systems, millimolar relaxivities ( $r_{1p}$ ) measured at 0.5 T and 25°C were of 21 and 20  $\text{mM}^{-1} \text{s}^{-1}$  for **PB01a** and **PB01b**, respectively. The obtained relaxivities are comparable with those obtained with other similar monoamidic Gd-based amphiphilic systems already reported in the literature.<sup>23,24</sup> The relaxivity peak observed in the Nuclear Magnetic Relaxometric Dispersion (NMRD) profile between 4 and 70 MHz (Figure 3) confirmed the aggregation of the complexes in water solution forming micelles with such long tumbling time ( $\tau_r$ ).  $\tau_r$  and the others parameters affecting the observed proton relaxivity have been assessed by fitting the data to the standard Solomon Bloembergen–Morgan (for the inner-sphere contribution) and Freed’s (for the outer-sphere contribution) equations<sup>35,36</sup>.



**Figure 3** Nuclear Magnetic Relaxometric Dispersion (NMRD) profiles (0.01–70 MHz; pH 7, 25°C) of **PB01a** and **PB01b** derivatives. The solid curves through the data points are calculated with the parameters reported in the Table 1.

The fitting was carried out by fixing the values of the following parameters: the hydration number ( $q=1$ ), the Gd–H<sub>2</sub>O distance ( $r = 3.1 \text{ \AA}$ ), the distance of closest approach of the bulk H<sub>2</sub>O molecules ( $a = 3.8 \text{ \AA}$ ) to the metal ion, and the relative diffusion coefficient ( $D=2.0 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ) for solute and solvent. The best fit parameters are listed in Table 1 and clearly confirm the occurrence of remarkably long  $\tau_r$  of 5 and 7 ns for **PB01a** and **PB01b**, respectively. These values are ca.50 times longer than that reported for monomeric complexes with similar molecular weight<sup>37</sup>.

**Table 1.** Best-fitting parameters obtained from the analysis of the NMRD profile recorded at 25°C.

Parameter	PB01a	PB01b
$\Delta^2 [10^{18}; \text{s}^{-2}]$	$6.3 \pm 0.6$	$6.3 \pm 0.6$
$^{298}\tau_v [\text{ps}]$	$42 \pm 4$	$39 \pm 4$
$^{298}\tau_R [\text{ns}]$	$5 \pm 0.8$	$7 \pm 0.9$
$^{298}\tau_M [\mu\text{s}]$	$0.8 \pm 0.3$	$0.87 \pm 0.5$

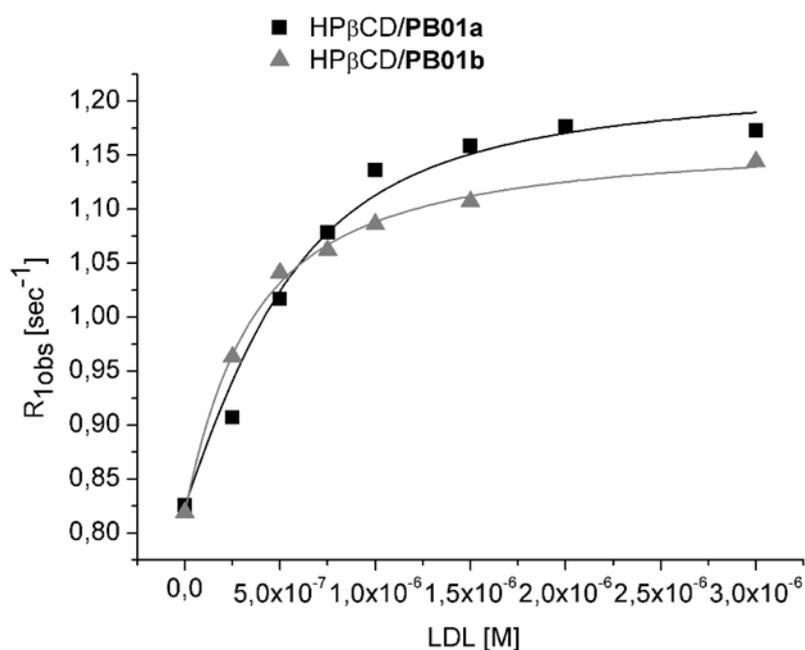
The Critical Micellar Concentration (CMC) was estimated to be  $< 10 \mu\text{M}$ . This value was obtained by measuring the longitudinal water proton relaxation rates of solutions containing decreasing amounts of **PB01a** and **PB01b** (see Figure 13 reported in supporting information). Since the relaxation rates of both **PB01a** and **PB01b** were linearly dependent from the complex concentration and were significantly higher than those expected for their monomeric forms, we can conclude that the CMC was lower than the detection limit established by considering significant only a relaxivity enhancements greater than 10% with respect to the water diamagnetic contribution. In fact, a

change of slope is expected when the Gd complex concentration is lower than the CMC as a consequence of the shorter tumbling time of its monomeric form.

In order to pursue the formation of supramolecular adducts with LDLs, micelles were disaggregated by using an excess amount of (2-hydroxy)propyl- $\beta$ -cyclodextrin (HP $\beta$ CD). HP $\beta$ CD has been used as a consequence of its higher solubility in water (45 g/100 mL) with respect  $\beta$ -cyclodextrin (1.85 g/100 mL) and allowed a complete adducts solubilisation due to the high affinity of the amphiphilic complex lipophilic chains for the hydrophobic cavity of HP $\beta$ CD. The transfer of the **PB01a** and **PB01b** units from micelles to the supramolecular “host/guest” complex with HP $\beta$ CD was followed by measuring the relaxation rate of the solvent water protons (see supporting information). The experimental work up for the preparation of stable LDL adducts was based on an already reported procedure<sup>24</sup> that consisted of the step-wise addition of aliquots of LDL particles to the aqueous solution of the HP $\beta$ CD /**PB01a** and **PB01b** adducts (37°C, 2h) in order to transfer the complexes to LDLs. The formation of the second adduct was favoured because the affinity of both **PB01a** and **PB01b** for LDL is higher than that for HP $\beta$ CD cavity. The binding parameters (thermodynamic association constant  $K_a$ , relaxivity, binding site number) for the equilibrium reported as eq. 1 were determined via the Proton Relaxation Enhancement (PRE) method<sup>38</sup>.



A fixed amount of HP $\beta$ -CD/PB01a and PB01b solution (0.04 mM) was titrated with LDL in order to hinder the micelle formation and promote the incorporation of the Gd complexes into LDL (Figure 4). Since the relaxivity enhancement showed in Figure 4 depends on both the molar fraction of the complex bound to the LDL and the millimolar relaxivity ( $r_b$ ) of the new formed adduct Gd-complex/LDL, by this titration it is possible to obtain the binding parameters reported in Table 2.



**Figure 4** Titration of native LDL into the HPβCD/**PB01a** and **PB01b** adducts (40 μM). The samples were incubated for 2 h at 37°C prior to the R1 measurements.

**Table 2** Binding parameters determined by measuring the relaxation rates of solutions containing a fixed amount of HPβCD /**PB01a** and **PB01b** adducts and increasing LDL concentration. Hydrodynamic particle diameters have been determined by DLS.

Gd-complex	$K_a M^{-1}$	n (binding sites number)	$r_b (mM^{-1}s^{-1})$	particle size (nm)
<b>PB01a</b>	$5.5 \pm 1.7 \times 10^4$	75	$22 \pm 0.6$	$21 \pm 1$
<b>PB01b</b>	$1.2 \pm 0.6 \times 10^4$	300	$19.0 \pm 0.2$	$22 \pm 1$

Both Gd-complexes showed high affinity for LDL thus permitting their use in physiological conditions as already reported for similar compounds.<sup>23,24</sup> Since the value that gives the affinity of an amphiphilic probe for a protein is given by the number of binding sites multiplied by the  $K_a$ , we can conclude that the adduct formed by **PB01a** and **PB01b** with LDL are not significantly different. However, the loading LDL capacity is about 4 times higher in the case of the isomer **PB01b** giving an advantage in its use for the preparation of such LDL based drug delivery agents.

## Conclusions

In summary, a new lipophilic GdBNCT/MRI agent (**PB01**) has been obtained in only four steps exploiting the hydroboration reaction of the carborane cage as a key step. Since the following dehydrogenative insertion is carried out on a substituted decaborane, a mixture of isomers has been obtained and separated. The structure of one of two isomers was determined via single crystal X-ray

diffraction, showing the presence of two enantiomers. The complexes obtained with the two isomers (**PB01a** and **PB01b**) showed high affinity for LDL and can be proposed as specific agents for MRI guided BNCT.

## Experimental

### General

Flasks and all equipments used for the generation and reaction of moisture-sensitive compounds were dried by electric heater under Ar. THF was distilled from benzophenone ketyl, anhydrous Et<sub>2</sub>O was distilled from LiAlH<sub>4</sub> and anhydrous CH<sub>2</sub>Cl<sub>2</sub> from CaH<sub>2</sub> prior to use. BuLi (1.6 M in hexanes) was obtained from Aldrich. (Bmim)<sup>+</sup>Cl<sup>-</sup> was purchased from Solvent Innovation GmbH. Decaborane was bought from KATCHEM spol. s r. o. All commercially obtained reagents and solvents were used as received. Product were purified by preparative column chromatography on Macherey Nagel silica gel for flash chromatography, 0,04-0,063 mm/ 230-400 mesh.

Reactions were monitored by TLC using Silica gel on TLC-PET foils Fluka, 2-25 mm, layer thickness 0.2 mm, medium pore diameter 60 Å. Carboranes and their derivatives were visualized on TLC plates using a 5% PdCl<sub>2</sub> aqueous solution in HCl. <sup>1</sup>H NMR spectra were recorded at 200 MHz, <sup>13</sup>C NMR spectra at 50.2 MHz, <sup>11</sup>B NMR spectra at 64.1 MHz. Data were reported as follows: chemical shifts in ppm from tetramethylsilane as internal standard, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = double-doublet, m = multiplet, br = broad), coupling constants (Hz), and assignment. <sup>13</sup>C and <sup>11</sup>B NMR spectra were measured with complete proton decoupling. Chemical shifts were reported in ppm from the residual solvent as an internal standard. GC-MS spectra were obtained on a mass selective detector HP 5970 B instrument operating at an ionizing voltage of 70 eV connected to a HP 5890 GC with a cross linked methyl silicone capillary column (25 m X 0.2 mm X 0.33 mm film tickness). ESI MS spectra were obtained on a high resolving power mass spectrometer LTQ Orbitrap (Thermo Scientific, Rodano, Italy), equipped with an atmospheric pressure interface and an ESI ion source. Samples were analyzed by flow injection at a 10 µL/min flow rate. The tuning parameters adopted for the ESI source were: source voltage 4.5 kV, capillary voltage 12.00 V, and tube lens voltage 55 V. The heated capillary temperature was maintained at 265°C. The mass accuracy of the recorded ions (vs the calculated ones) was ± 5 mmu (milli-mass units). Analyses were run using both full MS (50-2000 *m/z* range) and MS/MS acquisition in the positive and negative ion mode. IR spectra were recorded on a Perkin Elmer BX FT-IR. Tris-tert-butyl- and tris-benzylDOTAMA-C<sub>6</sub>-NH<sub>2</sub> were synthesised as described in literature

and their spectroscopic data corresponded with those reported.<sup>22, 39, 40</sup>

The  $^1\text{H-T}_1$  Nuclear Magnetic Relaxation Dispersion (NMRD) profiles of water protons were measured over a continuum of magnetic field strengths from 0.00024 to 0.5 T (corresponding to 0.01–21.5 MHz proton Larmor frequencies) on a fast field-cycling Stelar Spinmaster FFC 2000 relaxometer [Stelar S.n.c., Mede (PV), Italy] equipped with a silver magnet. The relaxometer operated under complete computer control with an absolute uncertainty in the  $R_1$  values of  $\pm 1\%$ . Water proton  $T_1$  measurements at fixed frequency were carried out on a Stelar SpinMaster spectrometer operating in the range from 21.5 to 70 MHz, by means of the inversion–recovery method (16 experiments, two scans). The reproducibility of the  $T_1$  data was  $\pm 0.5\%$ .

Gd concentration was determined by using inductively coupled plasma mass spectrometry (ICP-MS; element-2; Thermo-Finnigan, Rodano (MI), Italy). Sample digestion was performed with concentrated  $\text{HNO}_3$  (70%, 2 mL) under microwave heating (Milestone MicroSYNTH Microwave labstation). Orange Xylenol UV spectrophotometry was used to check for the absence of free Gd(III) ions<sup>41</sup>.

### **X-ray analysis.**

Crystal suitable for X-ray diffraction analysis were obtained by evaporation of a solution in  $\text{CH}_2\text{Cl}_2$ . X-ray data were collected on an Oxford Diffraction Gemini R-Ultra diffractometer equipped with nitrogen low temperature device and Enhanced Ultra (Cu) X-ray Source (mirror monochromatised  $\text{Cu-K}\alpha$  radiation,  $\lambda = 1.5418 \text{ \AA}$ ). The  $\omega$  scan was performed with frame width of  $1.0^\circ$ . The intensities were corrected for absorption with the numerical correction based on gaussian integration over a multifaceted crystal model. Softwares used: CrysAlisPro (Agilent Technologies, Version 1.171.36.28) for data collection, data reduction and absorption correction; OLEX2 (version 1.2.5)<sup>42</sup> data solution, structure analysis and drawing preparation; SHELXL-2013/3<sup>43</sup> for refinement. All non-hydrogen atoms were anisotropically refined. Hydrogen atoms were calculated and refined riding with  $U_{\text{iso}} = 1.2$  or  $1.5 U_{\text{eq}}$  of the atom connected. The carbon atoms in the cage were identified analysing their atomic thermal factors, and were clearly positioned.

$\text{C}_{20}\text{H}_{48}\text{B}_{10}\text{O}$ ,  $M = 412.68$ , triclinic,  $a = 7.9458(3)$ ,  $b = 12.8313(5)$ ,  $c = 27.356(1) \text{ \AA}$ ,  $\alpha = 101.325(4)$ ,  $\beta = 93.680(3)$ ,  $\gamma = 101.932(3)^\circ$ ,  $V = 2659.7(2) \text{ \AA}^3$ ,  $T = 132 \text{ K}$ , space group  $P\bar{1}$  (no.2),  $Z = 2$ ,  $D_{\text{calc}} = 1.031 \text{ g cm}^{-3}$ ,  $\mu(\text{CuK}\alpha) = 0.38 \text{ cm}^{-1}$ ,  $F(000) = 904$ . 11360 reflections collected to  $\theta_{\text{max}} = 51.487^\circ$ , 5518 unique ( $R_{\text{int}} = 0.0209$ ) and 4059 observed [ $F^2 > 2\sigma(F^2)$ ]. The final  $R_1 = 0.0534$ ,  $wR_2 = 0.1322$ ,  $S = 1.026$  and CCDC 1030047.

### **Preparation of LDL adducts**

PB01a and PB01b micelles disaggregation was carried out by mixing an excess of HP $\beta$ CD (CycloLab Ltd, Budapest, Hungary) to a 0.09 mM complex solution (3,5h, room temperature; 100:1). PB01a and PB01b-loaded LDL particles were prepared by incubating increasing amount of LDL (0.25-3  $\mu$ M; Biomedical Technologies Inc., Stoughton, MA, USA) and PB01a and PB01b – HP $\beta$ CD adducts (0.04 mM) for 2 h at 37° C. The longitudinal water proton relaxation rate ( $1/T_1$ ) of each solution was monitored on a relaxometer (Stelar, Mede, Italy) operating at 21.5 MHz at 25 °C. The hydrodynamic diameter of LDL adducts were analysed using a Malvern Zetasizer SZ apparatus (Malvern, U.K.) at 25°C in filtered (cutoff, 200 nm) PBS buffer (pH 7).

**6-Hexadecyldecaborane 3** In a dried heavy wall tube containing a stirring bar, 21 mmol of hexadec-1-ene 2 (4.71 g) and decaborane (6 mmol, 0.73 g), were reacted under Ar in a biphasic mixture of 0.30 g (bmim)<sup>+</sup>Cl<sup>-</sup> and 10 mL of anhydrous toluene with vigorous stirring at 125 °C overnight. After cooling to rt the reaction mixture was transferred in a round bottom flask and the toluene was evaporated. The crude was purified by column chromatography (eluant: petroleum ether) affording 0.90 g of white solid (43%). Mp. 44-46 °C.  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3389, 2925, 2854, 2575, 1714, 1459, 1378;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): -1.9 (4H, s, BH), -0.90-5.0 (9H, m, BH), 0.90 (3H, t,  $J$  = 5.5 Hz, CH<sub>3</sub>), 1.30 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.60 (2H, m, CH<sub>2</sub>);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 14.0 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 32.0 (CH<sub>2</sub>).  $\delta_{11\text{B}}$  (64.1 MHz; CDCl<sub>3</sub>): -37.4, -34.0, -0.04, 10.3, 27.0.  $m/z$  (HR, ESI) for C<sub>16</sub>H<sub>46</sub>B<sub>10</sub> Calcd. 348.4530 [M + H]<sup>+</sup>. Found: 348.4518. [M + H]<sup>+</sup>.

**C-(2-Hydroxy)-ethyl-C'-H-4-(hexadecyl)-o-carborane 5a and 5b** In a dried heavy wall tube containing a stirring bar, 10.4 mmol of 3-butyn-1-ol 4 (0.73 g) and 6-hexadecyldecaborane 3 (2.6 mmol, 0.89 g), were reacted under Ar in a biphasic mixture of 0.46 g (bmim)<sup>+</sup>Cl<sup>-</sup> and 5 mL of anhydrous toluene with vigorous stirring at 100 °C for 5 h. After cooling to rt the reaction mixture was transferred in a round bottom flask and the toluene was evaporated. The crude was purified by column chromatography (eluant: CH<sub>2</sub>Cl<sub>2</sub>) affording 76 mg of **5a** as a pale yellow liquid (9%) and 88.5 mg of **5b** as white waxy solid (11%). **5a**  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3423, 2924, 1467, 1334, 1074;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.89 (3H, t,  $J$  = 6.2 Hz, CH<sub>3</sub>), 1.27 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.55 (2H, m, CH<sub>2</sub>(CH<sub>2</sub>)<sub>14</sub>), 2.50 (3H, m, CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>, OH), 3.82 (2H, m, CH<sub>2</sub>OH), 4.10 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.5 (CH<sub>2</sub>), 36.8 (CH<sub>2</sub>), 60.1 (CH), 60.3 (CH<sub>2</sub>), 72.3 (C<sub>q</sub>).  $\delta_{11\text{B}}$  (64.1 MHz; CDCl<sub>3</sub>): -21.9, -13.8, -10.6, -5.1, -1.7, 10.3.  $m/z$  (HR, ESI) for C<sub>20</sub>H<sub>48</sub>B<sub>10</sub>O Calcd. 450.4326 [M + Cl]<sup>-</sup>. Found: 450.4126 . [M + Cl]<sup>-</sup>. **5b**  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3436, 2924, 2853, 2582, 1465, 1336, 1048;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.87 (3H, t,  $J$  = 6.6 Hz, CH<sub>3</sub>), 1.27 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.55 (2H, bs, CH<sub>2</sub>(CH<sub>2</sub>)<sub>14</sub>), 2.50 (3H, m, CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>,

OH), 3.82 (3H, m, CH<sub>2</sub>OH, B<sub>10</sub>H<sub>10</sub>CCH); δ<sub>C</sub> (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 14.0 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 32.4 (CH<sub>2</sub>), 39.7 (CH<sub>2</sub>), 60.5 (CH<sub>2</sub>), 61.5 (CH), 72.7 (C<sub>q</sub>). δ<sub>11B</sub> (64.1 MHz; CDCl<sub>3</sub>): -24.4, -11.7, -10.0, -4.8, -2.5, 10.6. m/z (HR, ESI) for C<sub>20</sub>H<sub>48</sub>B<sub>10</sub>O Calcd. 451.4404 [M + Cl]<sup>-</sup>. Found: 451.4133 . [M + Cl]<sup>-</sup>.

**C-(carboxymethyl)-C'-H-4-(hexadecyl)-o-carborane 6a:** C-(2-Hydroxy)-ethyl-C'-H-4-(hexadecyl)-o-carborane **5a** (52 mg, 0.13 mmol) was dissolved in 5 mL of acetone, then a 3 M solution of CrO<sub>3</sub> (4 equiv, 0.52 mmol, 52 mg) in H<sub>2</sub>SO<sub>4</sub> (1 mL) was added dropwise at 0°C. The reaction mixture was stirred overnight at rt, then quenched with H<sub>2</sub>O. The solvent was evaporated under reduced pressure and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 10 mL), the organic layers were washed once with brine (1 X 10 mL), dried and evaporated giving 42 mg (77 %) of white solid. Mp. 65-69 °C. ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3200, 2924, 2854, 2580, 1722, 1467, 1282, 1009; δ<sub>H</sub> (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.91 (3H, t, J = 6.6 Hz, CH<sub>3</sub>), 1.27 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 2.40 (2H, t, J = 6.7 Hz, CH<sub>2</sub>(CH<sub>2</sub>)<sub>14</sub>), 3.25 (2H, s, CH<sub>2</sub>COOH), 4.45 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH); δ<sub>C</sub> (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 38.8 (CH<sub>2</sub>), 58.7 (CH), 66.6 (C<sub>q</sub>), 171.9 (C<sub>q</sub>). m/z (HR, ESI) for C<sub>20</sub>H<sub>46</sub>B<sub>10</sub>O<sub>2</sub> Calcd. 855.8843 [2M + H]<sup>+</sup>. Found: 855.8904 [2M + H]<sup>+</sup>.

**C-(carboxymethyl)-C'-H-9-(hexadecyl)-o-carborane 6b:** As reported for the preparation of **6a** C-(2-Hydroxy)-ethyl-C'-H-9-(hexadecyl)-o-carborane **5b** (53 mg, 0.13 mmol) in 5 mL of acetone was mixed with 52 mg of CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub> affording 38 mg (69%) of white solid. Mp. 78-82 °C. ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3100, 2924, 2854, 2580, 1722, 1467, 1282, 1009; δ<sub>H</sub> (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.90 (3H, m, CH<sub>3</sub>), 1.27 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 2.40 (2H, t, J = 6.7 Hz, CH<sub>2</sub>(CH<sub>2</sub>)<sub>14</sub>), 3.30 (2H, s, CH<sub>2</sub>COOH), 4.25 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH); δ<sub>C</sub> (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 41.3 (CH<sub>2</sub>), 59.7 (CH), 67.1 (C<sub>q</sub>), 171.8 (C<sub>q</sub>). m/z (HR, ESI) for C<sub>20</sub>H<sub>46</sub>B<sub>10</sub>O<sub>2</sub> Calcd. 856.8876 [2M + H]<sup>+</sup>. Found: 856.8903 [2M + H]<sup>+</sup>.

**General procedure for the coupling between C-(carboxymethyl)-C'-H-(4/9)-(hexadecyl)-o-carborane (6a/6b) and DOTA-C6-derivatives.** In a 50 mL three necked round bottom flask, under inert atmosphere, C-(carboxymethyl)-C'-H-4/9-(hexadecyl)-o-carborane (**6a/6b**) was dissolved in 10 mL of dry CH<sub>2</sub>Cl<sub>2</sub> then 1.2 eq. of dicyclohexylcarbodiimide (DCC) and 1.15 eq of N-hydroxysuccinimide (NHS) were added at room temperature. The resulting mixture was stirred at room temperature overnight and the formation of a white solid was observed. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The solid was then dissolved in 10 mL of dry CH<sub>2</sub>Cl<sub>2</sub> in a 50 mL three necked round bottom flask, under inert atmosphere, then 0.95 eq of trisubstituted DOTAMA-C<sub>6</sub>-NH<sub>2</sub> and 0.95 eq of diisopropylethylamine (DIEA) were added. The solution was stirred for three hours, then the solvent evaporated under reduced pressure. The crude

was purified on silica gel (eluant:gradient CH<sub>2</sub>Cl<sub>2</sub>, 100 mL, then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5 100 mL, then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10 100 mL).

**C-[*ter*-butylDOTAMA-C<sub>6</sub>]-acetamido-C'-H-4-(hexadecyl)-*o*-carborane 7a:** according to the described general procedure, C-(carboxymethyl)-C'-H-4-(hexadecyl)-*o*-carborane **6a** (42 mg, 0.10 mmol) was reacted with 25 mg of DCC (0.12 mmol) and 14 mg of NHS (0.12 mmol) overnight. After filtration and solvent evaporation, 83 mg of solid were dissolved in CH<sub>2</sub>Cl<sub>2</sub> and reacted with 69 mg of tris-*N-tert*-butylDOTAMA-C<sub>6</sub>-NH<sub>2</sub> (0.01 mmol) in the presence of 16 μL of DIEA (0.01 mmol). The crude was purified affording 43 mg of a pale yellow oil (42%).  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3207, 2926, 2855, 2573, 1732, 1668, 1558;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.85 (3H, t,  $J = 6.8$  Hz, CH<sub>3</sub>), 1.26 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.46 (27H, s, 3 X C(CH<sub>3</sub>)<sub>3</sub>), 1.50-1.80 (10H, m, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO, CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.90 (18H, bs, CH<sub>2</sub>NCH<sub>2</sub>COOtBu, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.40 (12H, m, NCH<sub>2</sub>COOtBu, 2 X CH<sub>2</sub>NHCO), 5.10 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 8.80 (1H, bt, NH), 9.10 (1H, bt, NH);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 27.8 (CH<sub>3</sub>), 28.0 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.5 (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 38.3 (CH<sub>2</sub>), 40.3 (CH<sub>2</sub>), 55.4 (CH<sub>2</sub>), 55.6 (CH<sub>2</sub>), 55.9 (CH<sub>2</sub>), 60.8 (CH), 70.7 (C<sub>q</sub>), 81.7 (C<sub>q</sub>), 167.2 (C<sub>q</sub>), 171.2 (C<sub>q</sub>) 172.1 (C<sub>q</sub>).  $m/z$  (HR, ESI) for C<sub>54</sub>H<sub>110</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 1105.9275 [M + Na]<sup>+</sup>. Found: 1105.9276 [M + Na]<sup>+</sup>.

**C-[*ter*-butylDOTAMA-C<sub>6</sub>]-acetamido-C'-H-9-(hexadecyl)-*o*-carborane 7b:** according to the described general procedure, C-(carboxymethyl)-C'-H-9-(hexadecyl)-*o*-carborane **6b** (38 mg, 0.09 mmol) was reacted with 23 mg of DCC (0.11 mmol) and 11 mg of NHS (0.10 mmol) overnight. After filtration and solvent evaporation, 67 mg of solid were dissolved in CH<sub>2</sub>Cl<sub>2</sub> and reacted with 63 mg of tris-*N-tert*-butylDOTAMA-C<sub>6</sub>-NH<sub>2</sub> (0.01 mmol) in the presence of 15 μL of DIEA (0.01 mmol). The crude was purified affording 36 mg of a pale yellow oil (35%).  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3208, 2926, 2855, 2576, 1732, 1668, 1558;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.86 (3H, t,  $J = 6.6$  Hz, CH<sub>3</sub>), 1.26 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.46 (27H, s, 3 X C(CH<sub>3</sub>)<sub>3</sub>), 1.50-1.80 (10H, m, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO, CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.90 (18H, bs, CH<sub>2</sub>NCH<sub>2</sub>COOtBu, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.40 (12H, m, NCH<sub>2</sub>COOtBu, 2 X CH<sub>2</sub>NHCO), 5.95 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 8.80 (1H, bt, NH), 9.10 (1H, bt, NH);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 14.0 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 25.3 (CH<sub>2</sub>), 27.8 (CH<sub>3</sub>), 28.1 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 32.4 (CH<sub>2</sub>), 37.7 (CH<sub>2</sub>), 38.2 (CH<sub>2</sub>), 42.6 (CH<sub>2</sub>), 55.4 (CH<sub>2</sub>), 55.6 (CH<sub>2</sub>), 56.0 (CH<sub>2</sub>), 61.3 (CH), 70.9 (C<sub>q</sub>), 81.7 (C<sub>q</sub>), 167.2 (C<sub>q</sub>), 171.2 (C<sub>q</sub>) 172.1 (C<sub>q</sub>).  $m/z$  (HR, ESI) for C<sub>54</sub>H<sub>110</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 1104.9241 [M + Na]<sup>+</sup>. Found: 1104.9816 [M + Na]<sup>+</sup>.

**C-[benzylDOTAMA-C<sub>6</sub>]-acetamido-C'-H-4-(hexadecyl)-*o*-carborane 8a:** according to the described general procedure, C-(carboxymethyl)-C'-H-4-(hexadecyl)-*o*-carborane **7a** (32 mg, 0.08

mmol) was reacted with 20 mg of DCC (0.10 mmol) and 10 mg of NHS (0.09 mmol) overnight. After filtration and solvent evaporation, 70 mg of solid were dissolved in CH<sub>2</sub>Cl<sub>2</sub> and reacted with 59 mg of tris-*N*-benzylDOTAMA-C<sub>6</sub>-NH<sub>2</sub> (0.08 mmol) in the presence of 13 μL of DIEA (0.08 mmol). The crude was purified affording 12 mg of a pale yellow oil (13%).  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3067, 2925, 2854, 2579, 1738, 1667, 1609, 1557, 1200;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.89 (3H, t,  $J = 6.8$  Hz, CH<sub>3</sub>), 1.26 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.20-1.50 (10H, m, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO,CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.90 (18H, bs, CH<sub>2</sub>NCH<sub>2</sub>COOBn, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.40 (12H, m, NCH<sub>2</sub>COOBn, 2 X CH<sub>2</sub>NHCO), 4.90 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 5.2 (6H, m, COOCH<sub>2</sub>Ph), 7.36 (15H, m, Ph), 8.45 (1H, bs, NH), 8.55 (1H, bt, NH);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 25.3 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.6 (CH<sub>2</sub>), 50.6 (CH<sub>2</sub>), 54.9 (CH), 67.0 (CH<sub>2</sub>), 70.1 (C<sub>q</sub>), 128.4 (CH), 135.0 (C<sub>q</sub>), 167.1 (C<sub>q</sub>), 171.4 (C<sub>q</sub>), 172.9 (C<sub>q</sub>).  $m/z$  (HR, ESI) for C<sub>63</sub>H<sub>104</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 1207.8838 [M + Na]<sup>+</sup>. Found: 1207.8837 [M + Na]<sup>+</sup>.

**C-[benzylDOTAMA-C<sub>6</sub>]-acetamido-C'-H-9-(hexadecyl)-*o*-carborane 8b:** according to the described general procedure, *C*-(carboxymethyl)-*C'*-H-9-(hexadecyl)-*o*-carborane **7b** (57 mg, 0.13 mmol) was reacted with 33 mg of DCC (0.16 mmol) and 16 mg of NHS (0.15 mmol) overnight. After filtration and solvent evaporation, 90 mg of solid were dissolved in CH<sub>2</sub>Cl<sub>2</sub> and reacted with 93 mg of tris-*N*-benzylDOTAMA-C<sub>6</sub>-NH<sub>2</sub> (0.12 mmol) in the presence of 21 μL of DIEA (0.12 mmol). The crude was purified affording 20 mg of a pale yellow oil (13%).  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3067, 2924, 2854, 2580, 1738, 1668, 1557;  $\delta_{\text{H}}$  (200 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.86 (3H, m, CH<sub>3</sub>), 1.26 (28H, s, (CH<sub>2</sub>)<sub>14</sub>), 1.50-1.80 (10H, m, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO,CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.90 (18H, bs, CH<sub>2</sub>NCH<sub>2</sub>COOBn, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.80 (12H, m, NCH<sub>2</sub>COOBn, 2 X CH<sub>2</sub>NHCO), 4.70 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 5.2 (6H, s, COOCH<sub>2</sub>Ph), 7.36 (15H, m, Ph), 8.60 (1H, bs, NH), 9.20 (1H, bt, NH);  $\delta_{\text{C}}$  (50.2 MHz; CDCl<sub>3</sub>, Me<sub>4</sub>Si): 13.9 (CH<sub>3</sub>), 22.5 (CH<sub>2</sub>), 25.4 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 32.4 (CH<sub>2</sub>), 48.1 (CH<sub>2</sub>), 49.9 (CH<sub>2</sub>), 53.2 (CH<sub>2</sub>), 53.3 (CH<sub>2</sub>), 55.5 (CH<sub>2</sub>), 60.4 (CH), 66.7 (CH<sub>2</sub>), 70.1 (C<sub>q</sub>), 128.2 (CH), 128.5 (CH), 134.8 (C<sub>q</sub>), 170.3 (C<sub>q</sub>).  $m/z$  (HR, ESI) for C<sub>63</sub>H<sub>104</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 1207.8805 [M + Na]<sup>+</sup>. Found: 1207.8823 [M + Na]<sup>+</sup>.

**C-[DOTAMA-C<sub>6</sub>]-acetamido-C'-H-6-(hexadecyl)-*o*-carborane (9a and 9b). Procedure A:** in a 50 mL round bottom flask, *C*-[*ter*-butylDOTAMA-C<sub>6</sub>]-acetamido-*C'*-H-6-(hexadecyl)-*o*-carborane (**7**) was cooled to 0 °C, dissolved in 5 mL of a mixture of CF<sub>3</sub>COOH-CH<sub>2</sub>Cl<sub>2</sub> (50-50) in the presence of *i*Pr<sub>3</sub>SiH and stirred for 2 h at rt. After evaporation of CF<sub>3</sub>COOH-CH<sub>2</sub>Cl<sub>2</sub> the crude was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and K<sub>2</sub>CO<sub>3</sub> was added. The solution was filtered and the solvent evaporated.

**Isomer 9a:** following the procedure reported below 15 mg (0.014 mmol) of **7a** were dissolved in 4 mL of CF<sub>3</sub>COOH-CH<sub>2</sub>Cl<sub>2</sub> in the presence of 14 μL of *i*Pr<sub>3</sub>SiH affording 12.7 mg of **9a** (99%).

**Isomer 9b:** following the procedure reported below 17 mg (0.016 mmol) of **7b** were dissolved in 4 mL of CF<sub>3</sub>COOH-CH<sub>2</sub>Cl<sub>2</sub> in the presence of 15 μL of *i*Pr<sub>3</sub>SiH affording 14.4 mg of **9b** (99%).

**Procedure B:** in a 50 mL round bottom flask, *C*-[benzylDOTAMA-C<sub>6</sub>]-acetamido-*C'*-H-6-(hexadecyl)-*o*-carborane (**8**) was dissolved in a mixture of CH<sub>3</sub>OH-CH<sub>2</sub>Cl<sub>2</sub> (50-50), then 20% mol of Pd/C was added, the solution was stirred overnight at rt and checked by TLC. After the disappearance of the reagent spot the solution was filtered and the solvent evaporated. **Isomer 9a:** following the procedure reported below 19 mg (0.016 mmol) of **8a** were dissolved in 2 mL of CH<sub>3</sub>OH-CH<sub>2</sub>Cl<sub>2</sub> in the presence of 4 mg of Pd/C affording 15.0 mg of **9a** (99%). **Isomer 9b:** following the procedure reported below 35 mg (0.029 mmol) of **8b** were dissolved in 2 mL of CH<sub>3</sub>OH-CH<sub>2</sub>Cl<sub>2</sub> in the presence of 7 mg of Pd/C affording 27 mg of **9b** (99%).

**Isomer 9a**  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3434, 3302, 2580, 1682, 1652, 1203;  $\delta_{\text{H}}$  (600 MHz; CD<sub>3</sub>OD, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.93 (3H, t, *J* = 7.9 Hz, CH<sub>3</sub>), 1.32 (26H, s, (CH<sub>2</sub>)<sub>13</sub>), 1.39 (10H, bs, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO, CH<sub>2</sub>CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 1.55 (2H, bs, CH<sub>2</sub>CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.50 (2H, bs, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.50 (26H, m, NCH<sub>2</sub>COOH, CH<sub>2</sub>NHCO, CH<sub>2</sub>NCH<sub>2</sub>COOH), 3.90 (2H, bs, NCH<sub>2</sub>CONH), 4.80 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 7.20 (1H, bs, NH), 7.40 (1H, bs, NH);  $\delta_{\text{C}}$  (150.9 MHz; CD<sub>3</sub>OD, Me<sub>4</sub>Si): 12.4 (CH<sub>3</sub>), 21.6 (CH<sub>2</sub>), 25.4 (2 X CH<sub>2</sub>), 27.9 (CH<sub>2</sub>), 28.1 (3 X CH<sub>2</sub>), 28.4 (CH<sub>2</sub>), 28.5 (2 X CH<sub>2</sub>), 28.7 (8 X CH<sub>2</sub>), 31.0 (2 X CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 38.5 (2 X CH<sub>2</sub>), 39.5 (CH<sub>2</sub>), 48.5 - 52.4 (8 X CH<sub>2</sub>), 53.9 (CH<sub>2</sub>), 55.8 (CH<sub>2</sub>), 59.3 (CH), 69.0 (C<sub>q</sub>), 166.5 (3 X C<sub>q</sub>), 166.6 (2 X C<sub>q</sub>). *m/z* (HR, ESI) for C<sub>42</sub>H<sub>86</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 915.7578 [M + H]<sup>+</sup>. Found: 915.8054 [M + H]<sup>+</sup>.

**Isomer 9b**  $\nu_{\max}$  (neat)/cm<sup>-1</sup> 3440, 3303, 2581, 1682, 1652, 1203;  $\delta_{\text{H}}$  (600 MHz; CD<sub>3</sub>OD, Me<sub>4</sub>Si): 0.4-4.4 (9H, m, BH), 0.92 (3H, t, *J* = 7.9 Hz, CH<sub>3</sub>), 1.30 (26H, s, (CH<sub>2</sub>)<sub>13</sub>), 1.39 (10H, bs, NHCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>NHCO, CH<sub>2</sub>CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 1.55 (2H, bs, CH<sub>2</sub>CH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 2.10-2.50 (2H, bs, COCH<sub>2</sub>B<sub>10</sub>H<sub>10</sub>), 3.10-3.50 (26H, m, NCH<sub>2</sub>COOH, CH<sub>2</sub>NHCO, CH<sub>2</sub>NCH<sub>2</sub>COOH), 3.90 (2H, bs, NCH<sub>2</sub>CONH), 4.62 (1H, bs, B<sub>10</sub>H<sub>10</sub>CCH), 8.15 (1H, bs, NH), 8.40 (1H, bs, NH);  $\delta_{\text{C}}$  (150.9 MHz; CD<sub>3</sub>OD, Me<sub>4</sub>Si): 12.3 (CH<sub>3</sub>), 21.7 (CH<sub>2</sub>), 25.5 (2 X CH<sub>2</sub>), 27.9 (CH<sub>2</sub>), 28.1 (3 X CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 28.4 (2 X CH<sub>2</sub>), 28.6 (2 X CH<sub>2</sub>), 28.7 (8 X CH<sub>2</sub>), 31.0 (2 X CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 38.3 (2 X CH<sub>2</sub>), 42.2 (CH<sub>2</sub>), 48.3 - 52.3 (8 X CH<sub>2</sub>), 60.8 (CH), 69.7 (C<sub>q</sub>), 166.5 (3 x C<sub>q</sub>), 166.6 (2 x C<sub>q</sub>). *m/z* (HR, ESI) for C<sub>42</sub>H<sub>86</sub>B<sub>10</sub>N<sub>6</sub>O<sub>8</sub> Calcd. 915.7533 [M + H]<sup>+</sup>. Found: 915.8035 [M + H]<sup>+</sup>.

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**Supporting Information description,** <sup>1</sup>H NMR, <sup>11</sup>B and <sup>13</sup>C NMR and DEPT spectra of products 5a-5b, <sup>1</sup>H NMR, <sup>13</sup>C NMR and DEPT spectra of products and 6-9 and effect of HPβCD concentration on the R<sub>1obs</sub> values (21,5 MHz) for PB01a and PB01b.

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1. R. F. Barth, *J. Neuroonc.*, 2003, 62, 1-5.
2. R. F. Barth, J. A. Coderre, M. G. H. Vicente and T. E. Blue, *Clin. Canc. Res.*, 2005, 11, 3987-4002.
3. M. F. Hawthorne and M. W. Lee, *J. Neuroonc.*, 2003, 62, 33-45.
4. J. Laakso, M. Kulvik, I. Ruokonen, J. Vahatalo, R. Zilliacus, M. Farkkila and M. Kallio, *Clin. Chem.*, 2001, 47, 1796-1803.
5. M. Kortesiemi, T. Seppala, I. Auterinen and S. Savolainen, *Appl. Rad. Isot.*, 2004, 61, 823-827.
6. S. Geninatti-Crich, A. Deagostino, A. Toppino, D. Alberti, P. Venturello and S. Aime, *Anti-Cancer Agents Med. Chem.*, 2012, 12, 543-553.
7. K. C. Briley-Saebo, S. Geninatti-Crich, D. P. Cormode, A. Barazza, W. J. M. Mulder, W. Chen, G. B. Giovenzana, E. A. Fisher, S. Aime and Z. A. Fayad, *The Journal of Physical Chemistry B*, 2009, 113, 6283-6289.
8. R. E. Mewis and S. J. Archibald, *Coordination Chemistry Reviews*, 2010, 254, 1686-1712.
9. L. Tei, A. Barge, S. G. Crich, R. Pagliarin, V. Negri, D. Ramella, G. Cravotto and S. Aime, *Chemistry*, 2010, 16, 8080-8087.
10. N. Cerullo, D. Bufalino and G. Daquino, *Appl. Rad. Isot.*, 2009, 67, S157-S160.
11. G. De Stasio, B. H. Frazer, B. Gilbert, B. Sobderegger, K. Richter, C. Salt, P. Casalbore, S. Howard, D. Rajesh, J. F. Fowler, M. P. Mehta, R. Pallini and D. Mercanti, *New criteria for GdNCT agents*, 2002.
12. G. De Stasio, D. Rajesh, P. Casalbore, M. J. Daniels, R. J. Erhardt, B. H. Frazer, L. M. Wiese, K. L. Richter, B. R. Sonderegger, B. Gilbert, S. Schaub, R. J. Cannara, J. F. Crawford, M. K. Gilles, T. Tyliczszak, J. F. Fowler, L. M. Larocca, S. P. Howard, D. Mercanti, M. P. Mehta and R. Pallini, *Neurol. Res.*, 2005, 27, 387-398.
13. R. Lawaczeck, C. O. Fischer, U. Kruger, W. Leuther and J. Menrad, *Gadolinium neutron capture therapy (GdNCT) with MRI contrast media. In vivo studies*, 1996.
14. J. T. Masiakowski, J. L. Horton and L. J. Peters, *Med. Phys.*, 1992, 19, 1277-1284.
15. T. Matsumoto, *Depth-dose evaluation for liver cancer treatment by BNCT and GDNCT using an epithermal neutron beam*, 2001.
16. V. N. Mitin, V. N. Kulakov, V. F. Khokhlov, I. N. Sheino, A. M. Arnopolskaya, N. G. Kozlovskaya, K. N. Zaitsev and A. A. Portnov, *Appl. Rad. Isot.*, 2009, 67, S299-S301.
17. R. N. Grimes, *Carboranes, 2nd Edition*.
18. A. H. Soloway, W. Tjarks, B. A. Barnum, F. G. Rong, R. F. Barth, I. M. Codogni and J. G. Wilson, *Chem. Rev.*, 1998, 98, 1515-1562.

19. J. F. Valliant, K. J. Guenther, A. S. King, P. Morel, P. Schaffer, O. O. Sogbein and K. A. Stephenson, *Coord. Chem. Rev.*, 2002, 232, 173-230.
20. G. Wu, R. F. Barth, W. Yang, R. J. Lee, W. Tjarks, M. V. Backer and J. M. Backer, *Anti-Cancer Agents Med. Chem.*, 2006, 6, 167-184.
21. E. L. Crossley, E. J. Ziolkowski, J. A. Coderre and L. M. Rendina, *Mini-Reviews Med. Chem.*, 2007, 7, 303-313.
22. S. Aime, A. Barge, A. Crivello, A. Deagostino, R. Gobetto, C. Nervi, C. Prandi, A. Toppino and P. Venturello, *Org. Biomol. Chem.*, 2008, 6, 4460-4466.
23. A. Toppino, M. E. Bova, S. G. Crich, D. Alberti, E. Diana, A. Barge, S. Aime, P. Venturello and A. Deagostino, *Chem. Eur. J.*, 2013, 19, 720-727.
24. S. Geninatti-Crich, D. Alberti, I. Szabo, A. Deagostino, A. Toppino, A. Barge, F. Ballarini, S. Bortolussi, P. Bruschi, N. Protti, S. Stella, S. Altieri, P. Venturello and S. Aime, *Chem. Eur. J.*, 2011, 17, 8479-8486.
25. D. Alberti, A. Toppino, S. G. Crich, C. Meraldi, C. Prandi, N. Protti, S. Bortolussi, S. Altieri, S. Aime and A. Deagostino, *Org. Biomol. Chem.*, 2014, 12, 2457-2467.
26. H. C. Brown, *Organic Synthesis via Borane*, Wiley-Interscience, New York, 1975.
27. K. Mazighi, P. J. Carroll and L. G. Sneddon, *Inorg. Chem.*, 1993, 32, 1963-1969.
28. D. F. Gaines and A. N. Bridges, *Organometallics*, 1993, 12, 2015-2016.
29. A. N. Bridges, D. R. Powell, J. A. Dopke, J. M. Desper and D. F. Gaines, *Inorg. Chem.*, 1998, 37, 503-509.
30. S. Chatterjee, P. J. Carroll and L. G. Sneddon, *Inorg. Chem.*, 2013, 52, 9119-9130.
31. X. H. Yu, K. Cao, Y. W. Huang, J. X. Yang, J. Li and G. J. Chang, *Chem. Comm.*, 2014, 50, 4585-4587.
32. U. Kusari, P. J. Carroll and L. G. Sneddon, *Inorg. Chem.*, 2008, 47, 9203-9215.
33. O. Bondarev, Y. V. Sevryugina, S. S. Jalisatgi and M. F. Hawthorne, *Inorg. Chem.*, 2012, 51, 9935-9942.
34. K. Z. Kabytayev, A. V. Safronov, S. S. Jalisatgi and M. F. Hawthorne, *J. Organomet. Chem.*, 2014, 749, 106-108.
35. E. Toth and A. E. Merbach, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Wiley, New York, 2001.
36. P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, 99, 2293-2352.
37. G. M. Nicolle, E. Toth, K. P. Eisenwiener, H. R. Macke and A. E. Merbach, *J. Biol. Inorg. Chem.*, 2002, 7, 757-769.
38. S. Aime, M. Chiaussa, G. Digilio, E. Gianolio and E. Terreno, *J. Biol. Inorg. Chem.*, 1999, 4, 766-774.
39. A. Barge, L. Tei, D. Upadhyaya, F. Fedeli, L. Beltrami, R. Stefania, S. Aime and G. Cravotto, *Org. Biomol. Chem.*, 2008, 6, 1176-1184.
40. P. L. Anelli, L. Lattuada, M. Gabellini and P. Recanati, *Bioconjugate Chem.*, 2001, 12, 1081-1084.
41. A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol I*, 2006, 1, 184-188.
42. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Crystallogr.*, 2009, 42, 339-341.
43. G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Adv.*, 2008, 64, 112-122.