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High Relaxivity Trimetallic Nitride (Gd_3N) Metallofullerene MRI Contrast Agents with Optimized Functionality

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Abstract

The water soluble poly(ethylene glycol) (PEG) functionalized and hydroxylated endohedral trimetallic nitride metallofullerene derivatives, $Gd_3N@C_{80}[DiPEG(OH)_x]$, have been synthesized and characterized. The ^1H MRI relaxivities in aqueous solution were measured for the derivatives with four different molecular weights of PEG (350–5000 Da) at 0.35 T, 2.4 T and 9.4 T. The 350/750 Da PEGs derivatives were found to have the highest relaxivities values among the derivatives, 237/232 $mM^{-1}s^{-1}$ for r_1 and 460/398 $mM^{-1}s^{-1}$ for r_2 (79/77 $mM^{-1}s^{-1}$ and 153/133 $mM^{-1}s^{-1}$ based on Gd^{3+} ion) respectively, at a clinical-range magnetic field of 2.4 T. These represent some of the highest relaxivities reported in the literatures for any commercial or investigational MRI contrast agent. Dynamic light scattering results confirm a larger size distribution for 350/750 Da PEGs derivatives (95/96 nm) relative to longer chain length derivatives, 5000 Da PEG derivatives (37nm). Direct infusion of the optimized 350 Da PEG derivatives into live tumor-bearing rat brains demonstrated an initial uniform distribution and hence, the potential for effective brachytherapy applications when the encapsulated Gd^{3+} ions are replaced with radioactive ^{177}Lu.

Keywords

Gadofullerenes; Relaxivity; Contrast Agents

Introduction

Magnetic resonance imaging (MRI) is widely used in the clinical diagnosis of cancer and other diseases. Improved contrast can be obtained by the use of intravenously-injected paramagnetic contrast agents due to their extravasation across leaking vasculature and subsequent accumulation in lesions. Since contrast agents are widely employed in clinical MRI studies, these agents must be biologically safe and have a high relaxivity in order to minimize the amount delivered. Currently, the most widely used MRI contrast agents are gadolinium chelates: the large number of unpaired electrons (seven) per Gd^{3+} ion and favorably long electron spin-lattice relaxation time significantly increase the relaxation rate of water hydrogens.(1,2) However, it has been recently reported that nephrogenic systemic fibrosis

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(NSF), a painful and debilitating disorder of the skin and systemic tissues, has been associated with exposure to Gd^{3+} ions based contrast agents in patients with renal insufficiency.(3,4) New contrast agents with higher relaxivity and lower toxicity are an important target of current investigations.

Because of their shape, capacity for multiple *endo* encapsulants and isolation of the Gd^{3+} ions from the bio-environment, endohedral metallofullerenes (EMFs) represent ideal next generation nanoparticles as diagnostic biomedical nanoprobes. Several new classes of MRI contrast agents, water-soluble endohedral metallofullerenes, have been reported.(5–17) EMFs have been functionalized with hydrophilic groups, e.g., -OH or –COOH.(8–12) These water soluble EMF derivatives show significantly greater relaxivities than the chelated Gd^{3+} compounds in current clinical use.

We have previously reported the synthesis of trimetallic nitride templated (TNT) EMFs, $A_xB_{3-x}N@C_{80}$ (A,B=metal, x=0–3).(18) The TNT EMFs have an additional advantage: they can encapsulate up to three Gd^{3+} ions inside the cage, resulting in higher relaxivity. If different metal atoms/ions are encapsulated, e.g., Lu, Tb, Ho, etc., TNT EMFs can also perform as multimodal (MRI and radiotherapy) platforms with the same biodistribution. We previously reported the synthesis of $Gd_3N@C_{80}[DiPEG5000(OH)_x]$ (PEG=poly(ethylene glycol)),(7) which is water soluble and exhibits high relaxivity. *In vitro* and *in vivo* studies demonstrated the potential applicability of this MRI contrast agent. More recent studies have also demonstrated high relaxivity for related TNT EMFs with different functionality.(13,15,16) The high relaxivity of the TNT EMFs arises from the following factors: (1) the large number of exchangeable water molecules coordinated to the hydroxyl groups which are attached to the carbon cage (Fig. 1) and are simultaneously relaxed by the $(Gd_3N)^{6+}$ endohedral cluster, (2) the slow rotational correlation time caused by the long PEG chain and aggregation effects.

In the current study, we have synthesized pegylated and hydroxylated $Gd_3N@C_{80}$ with different PEG chain lengths (350–5000MW) and the relaxivities of these derivatives were measured at three different magnetic field strengths. The optimum PEG chain of these pegylated and hydroxylated $Gd_3N@C_{80}$ derivativesis is an important structure property for potential MRI studies at different magnetic field strengths. To demonstrate the *in vivo* applicability of these high-relaxivity contrast agents, a convection-enhanced delivery (CED) model was used to infuse $Gd_3N@C_{80}[DiPEG350(OH)_x]$ directly into a brain-tumor-bearing rat.

Materials and Methods

Synthesis of $Gd_3N@C_{80}[DiPEG(OH)_x]$

$Gd_3N@C_{80}$ was produced by the standard Krätschmer-Huffman (K–H) method with the introduction of nitrogen gas into the K–H generator.(18) The product was purified by HPLC as previously reported.(19) 1, 8-Diazabicyclo(5.4.0)undec-7-ene (DBU, FW 152.24), 98%, was purchased from Sigma-Aldrich; 99% CBr_4, 99% 18-crown-6, 50% sodium hydroxide solution and 30 wt.% hydrogen peroxide were used as obtained from Aldrich Chem. Co.. Di {ω-methyl-poly(ethylene glycol)} malonate (DiPEG) 350, 750, 2000 and 5000 (MW. 768, 1568, 4068, 10068, respectively) were prepared from ω-methyl-poly(ethylene glycol)s (PEGs) as following: To a solution of PEG and triethylamine (TEA) in CH_2Cl_2, a solution of malonyl dichloride in CH_2Cl_2 was added dropwise. The molar ratio of PEG:TEA:malonyl dichloride was kept as 2:2:1. The solution was stirred at room temperature under N_2 protection for 3 h. The final mixture was concentrated and dropped into large amount of ethyl ether, and then fractionated by a silica gel column.

$Gd_3N@C_{80}$, DiPEG, DBU, and CBr_4 were dissolved in chlorobenzene in molar ratio 1:20:20:67. After deaeration with bubbling argon for 30 minutes, the solution was stirred overnight at room temperature. The resultant mixture was evaporated and the residue was dissolved in toluene. To this solution the 18-crown-6 and 50% sodium hydroxide solution was added and the resultant solution was stirred for 4 hours. 1.5mL hydrogen peroxide was added drop by drop and more water was added. The mixture was stirred overnight at room temperature. The resultant solution was concentrated and separated by Sephadex G25 (Pharmacia) size-exclusion gel column with distilled water as eluent to afford a narrow golden band (first fraction, pH≤7). (See Supporting Information Figure 1.) The yield was up to 95%.

Relaxivity Measurements

The concentrations of Gd^{3+} ions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin-Elmer Optima 5300DV) at 342.247 nm. The instrument was calibrated by a 10 ppm ICP standard solution. Each molecule was assumed to encapsulate three Gd^{3+} ions, so the concentration of $Gd_3N@C_{80}[DiPEG(OH)_x]$ was assumed to be one third of the Gd^{3+} ion concentration.

The proton relaxation times were measured at three different magnetic field strengths: 0.35 T (TEACH SPIN PS1-B), 2.4 T (Bruker/Biospec) and 9.4 T (Varian Inova 400). The inversion-recovery method was used to measure T_1 and Carr-Pucell-Meiboom-Gill method was used for measurement of T_2. The relaxivities were extracted from linear fits of plots of relaxation rates ($1/T_1$ and $1/T_2$) versus the concentrations of the paramagnetic species.

Dynamic light scattering

Dynamic light scattering (DLS) measurements were carried out with an ALV/CGS-3 compact goniometer system and ALV/LSE-5003 multi-τ digital correlator at 25 °C. He-Ne laser producing vertically polarized light of $\lambda_0 = 632.8$ nm was used as a light source. Each sample was filtered with a 0.5 μm cellulose acetate membrane filter.

Animal Studies

We used a convection-enhanced delivery method (7) to infuse 0.0235 mM $Gd_3N@C_{80}[DiPEG350(OH)_x]$ into a live rat brain tumor and followed the functionalized EMF redistribution over the course of several days. Infusion was applied for 180 min at a rate of 0.2 μL/min with a total infusion volume of 36 μL.

Results and Discussion

^{1}H NMR

^{1}H NMR of $Gd_3N@C_{80}[DiPEG(OH)_x]$ was performed on an Inova 400. Figure 2a displays the NMR spectra of $Gd_3N@C_{80}[DiPEG350(OH)_x]$ and malonyl DiPEG350 in D_2O. In $Gd_3N@C_{80}[DiPEG350(OH)_x]$ the $-OCH_3$ and $–OCH_2CH_2-$ signals appeared at δ3.35 ppm (s, 6H) and 3.67 ppm (m, 52H), respectively; the signals for $–COOCH_2-$ and $COOCH_2CH_2-$ centered at 4.32 ppm (t, 4H) and 3.78 ppm (t, 4H) in the malonate disappeared in the EMF apparently due to the strong paramagnetic effect of the Gd^{3+} ions. The NMR spectra of other PEG derivatives give similar results.

UV-Vis

The UV-Vis spectra of $Gd_3N@C_{80}$ and $Gd_3N@C_{80}[DiPEG2000(OH)_x]$ are shown in Figure 2b. For $Gd_3N@C_{80}$, there is an absorption peak at about 710nm and a second absorption occurs at 410 nm.(20) These absorptions are attributed to the $\Pi \rightarrow \Pi^*$ transitions.(21) For the functionalized metallofullerenes, however, these characteristic features are significantly reduced. This indicates that the conjugated π structure of the carbon cage has been significantly altered. Similar results were obtained from other samples with different molecular weight ($Gd_3N@C_{80}$[DiPEG5000, 750, 350$(OH)_x$]).

Mass Spectra

To further confirm the successful functionalization, MALDI-TOF mass spectra were obtained for DiPEG750, $Gd_3N@C_{80}$[DiPEG750] and $Gd_3N@C_{80}$[DiPEG750$(OH)_x$]. (See supporting information Figure 2) The spectrum for DiPEG 750 was a series of peaks centered at about m/z 1388. The peaks were separated by m/z 44, which is the mass of (CH_2CH_2O) unit. The spectrum for $Gd_3N@C_{80}$[DiPEG750] was a series peaks centered at about m/z 2907. The separation of m/z 1 is attributed to the isotope effect of $Gd_3N@C_{80}$. We can clearly see that $Gd_3N@C_{80}$ was successfully functionalized with DiPEG750. However, in the mass spectrum of $Gd_3N@C_{80}$[DiPEG750$(OH)_x$] we could only observe the peaks for the metallofullerene (m/z 1447) and PEG fragments (centered at m/z 759 and separated by 44). Even though the molecular ion peakwas not observed, the metallofullerene and PEG fragments indicate the successful functionalization.

MRI Relaxivity

The ability of paramagnetic compounds to enhance the relaxation rate can be described by the following equation:(1, 2)

$$\frac{1}{T_{i,obs}}=\frac{1}{T_{i,H_2O}}+\frac{1}{T_{i,para}}=\frac{1}{T_{i,H_2O}}+r_i[M]\quad i=1,2 \tag{1}$$

$1/T_1$ and $1/T_2$ are the longitudinal and transverse relaxation rates. $1/T_{i,obs}$ is the observed value of the solution and $1/T_{i,para}$ is the contribution of the paramagnetic compound. [M] is the concentration of the paramagnetic species. r_1 and r_2 are defined as longitudinal and transverse relaxivities respectively, which is expressed in $(mM \cdot s)^{-1}$.

A major contribution to $1/T_{i,para}$ arises from the simultaneous relaxation of multiple water molecules. This is the result of the dipolar electron-nuclear spin interaction between the large fluctuating Gd^{3+} electron magnetic moment and the water hydrogen nucleus. These relaxed water molecules undergo subsequently rapid exchange with bulk water. Dipole-dipole interactions are described by the Solomon-Bloembergen-Morgan (SBM) equations. In the high-field region (above 10 MHz) these equations reduce to: (1,2)

$$\frac{1}{T_{1M}}=\frac{6}{15}\frac{\gamma_I^2 g^2 \mu_B^2 S(S+1)}{r_H^6}\left(\frac{\tau_C}{1+\omega_H^2\tau_c^2}\right) \tag{2}$$

$$\frac{1}{T_{2M}}=\frac{1}{15}\frac{\gamma_I^2 g^2 \mu_B^2 S(S+1)}{r_H^6}\left(4\tau_c+\frac{3\tau_C}{1+\omega_H^2\tau_c^2}\right) \tag{3}$$

$$\tau_C^{-1}=\tau_M^{-1}+\tau_R^{-1}+\tau_{1e}^{-1} \tag{4}$$

If water exchange process exists, then the relaxation rates are:(1,2)

$$\frac{1}{T_{1,para}}=\frac{q[M]/55600}{T_{1M}+\tau_M} \tag{5}$$

$$\frac{1}{T_{2,para}}=\frac{q[M]/55600}{T_{2M}+\tau_M} \tag{6}$$

Where [M] is in unit of mM, τ_R is the rotational correlation time, τ_M is the residence time of the coordinated water molecules, ω_H is the Larmor Frequency of proton, τ_{le} is the longitudinal relaxation time of electron and q is the number of water molecules that coordinate to the paramagnetic molecules. τ_C is dominated by the shortest correlation time among τ_R, τ_M and τ_{le}. If τ_R is the dominant time, the relaxivity as a function of frequency shows a broad maximum peak that increases with increasing τ_R.(1)

In the present investigation, the relaxivity data of all the $Gd_3N@C_{80}[DiPEG(OH)_x]$ samples were measured. The results are summarized in Table 1. Our previously reported relaxivity data of $Gd_3N@C_{80}[DiPEG5000(OH)_x]$(7) were also listed and they agree with the current data. All the $Gd_3N@C_{80}[DiPEG(OH)_x]$ samples have high r_1 and r_2 values at all three magnetic field strengths. The r_1 values range from 139 mM^{-1} s^{-1} (Gd^{3+} based relaxivity 46.3 mM^{-1} s^{-1}) to 237 mM^{-1} s^{-1} (Gd^{3+} based relaxivity 79.0 mM^{-1} s^{-1}) for the different PEG chains at 2.4 T to be compared to the value of about 4 $mM^{-1}s^{-1}$ for commercially available Gd^{3+} ion chelated contrast agents. Several other groups have also reported r_1 values of mono gadofullerene derivatives, that is, one Gd^{3+} ion per fullerene cage. These r_1 values are typically higher than those of extracellular commercial MRI contrast agents. In our work, the relaxivities of all the $Gd_3N@C_{80}[DiPEG(OH)_x]$ samples are significantly higher on a per molecule basis. A summary of these other studies and the relaxivitiy of $Gd_3N@C_{80}[DiPEG350(OH)_x]$ in the current study are presented in Table 2. (6,8,9,11,12,22)

As previously indicated, a primary reason for the high molar relaxivity of the $Gd_3N@C_{80}[DiPEG(OH)_x]$ samples in comparison with other endohedral metallofullerenes derivatives originate from the presence of three Gd^{3+} ions within the cage: the charge transfer between the $(Gd_3N)^{6+}$ cluster and the $(C_{80})^{6-}$ cage leads to a very stable nanoparticle. Previous studies have confirmed ferromagnetic coupling in this cluster gives rise to a large magnetic moment, 21 μB.(23) The dipolar electron-nuclear spin interaction between the fluctuating Gd^{3+} electron magnetic moment and the large number of exchangeable water hydrogen atoms induces efficient proton relaxation. This is in contrast with the conventional Gd-DTPA complex, which has a single water molecule bound in the first coordination sphere of the metal ion, exchanges rather slowly with the bulk water and the entire complex tumbles very rapidly.

Further factors contributing to the relaxation process relate to the unique property of the fullerenes to aggregate and form large nanoclusters. Aggregation occurs frequently for water soluble metallofullerenes and affects the relaxivity significantly as previous studies have shown.(5,9) For the low molar mass $Gd_3N@C_{80}[DiPEG(OH)_x]$ samples, the aggregates are formed by hydrogen bonding between –OH groups; however, the hydrophobic fullerene-fullerene interactions are also strong. Hence, fullerene aggregates can be formed. According to the Debye-Stokes equation for rigid spherical particles:

$$\tau_R=4\pi\eta r_{eff}^3/3k_BT$$

the rotational correlation time τ_R increases as the particle radius increases which is an important parameter influencing relaxivity as a function of field strength. It has also been postulated that the presence of these aggregates might lead to a ‘confined’ pool of water molecules in the interstitial spaces between individual gadofullerenes which exchanges rapidly with the bulk water increasing again the relaxivity. (24) Depending on the PEG length and aggregate sizes, different restricted pools of water would be created as mentioned above influencing the measured overall relaxivities.

In the limit of $\tau_M \ll T_{1M}, T_{2M}$ for equations (2) and (3), we obtain

$$r_2/r_1=[3+4(1+\omega_H{}^2\tau_C{}^2)]/6 \tag{7}$$

At low field, $\omega_H{}^2\tau_C{}^2 \ll 1$ and the ratio approaches the value 7/6=1.17. . Our measured ratios of r_2/r_1 are listed in Table 3. Based on equation 7, the ratios at 0.35T are consistent with the predicted value. Another result of the SBM equations is $\omega_H\tau_C \approx 1$ at maximum r_1. According to equation (7) this leads to $r_2/r_1 \approx 11/6=1.83$ for the peak. The ratios for the 750 and 350 PEGs at 2.4 T are close to 11/6 = 1.83 and hence the maximum r_1 values should occur near 100 MHz (2.4 T). We can also see that at 2.4 T the 750 Da species has the maximum r_1 of all the samples. Hence at 2.4 T we obtain approximately for the 750/350 sample: $\tau_C=1/\Omega_H=1.59$ns, which is significantly longer than the commercial contrast agent, e.g. $\tau_R=66$ ps for the [Gd(DTPA-BMA)] (1). This assumes that τ_C is dominated by a rotational component.

The aggregation behavior was studied by DLS experiment. The DLS results of all $Gd_3N@C_{80}$[DiPEG(OH)$_x$] samples are shown in Figure 3. The mean peak positions are 75nm, 76nm, 58nm and 37nm for $Gd_3N@C_{80}$[DiPEG350(OH)$_x$], $Gd_3N@C_{80}$[DiPEG750(OH)$_x$], $Gd_3N@C_{80}$[DiPEG2000(OH)$_x$] and $Gd_3N@C_{80}$[DiPEG5000(OH)$_x$] respectively. The 350PEG and 750PEG samples have the largest average aggregation size (~75nm) while the 5000PEG sample is much smaller (~37nm). The trend of decreasing aggregation size with increasing molecular weight is consistent with the relaxivities trend. It should also be noted that these pegylated particles clearly deviate from spherical shape and the ones with longer PEG chains might exhibit internal flexibility. This could be an important additional factor for the observed reduced relaxivities for these larger molecules due to increased anisotropic rotation described by a fast local motion component that controls relaxivity and a slow global motion component. (25)

Animal Infusion Study

Pure T_1 computed images of three consecutive brain slices of a live rat brain following infusion of 0.0235 mM $Gd_3N@C_{80}$[DiPEG350(OH)$_x$] into the brain tumor are shown in Figure 4. The middle column indicates the slice with the tumor, while the left and the right columns are slices without tumor used as a control. Immediately after the infusion is terminated (“3.5 hrs”), the $Gd_3N@C_{80}$[DiPEG350(OH)$_x$] nanoparticles have been distributed throughout the tumor, reducing the tissue T_1 values. The tumor appears as a dark area as the white circle indicates. As time progresses, the brain tumor grows and the $Gd_3N@C_{80}$[DiPEG350(OH)$_x$] nanoparticles appear to be pushed out into the tumor periphery. This is illustrated at day 7 in the T_1 map which shows aggregates on the surface and the T_1-weighted image that shows an enhancement ring around the tumor. These results illustrate the enhanced *in vivo* relaxivity of this optimized $Gd_3N@C_{80}$[DiPEG350(OH)$_x$] system at low molar concentrations. It should also be noted that after 7 days the nanoparticles can still enhance the MRI contrast which indicates that these metallofullerenes agents can be used for long-term imaging diagnostic applications. In addition, these metallofullerenes agents provide a platform for effective brachytherapy applications when Gd is replaced and/or combined with other radioactive agents (^{177}Lu).

Conclusions

We synthesized hydroxylated and pegylated Gd-containing TNT EMFs, $Gd_3N@C_{80}$[DiPEG $(OH)_x$], with different chain lengths of PEG. The relaxivities were measured at three magnetic field strengths. All the samples show significantly higher r_1 and r_2 compared to commercially available Gd-chelates and show considerable promise as a new class of contrast agents. They represent some of the highest relaxivities reported in the literature for any commercial or investigational MRI contrast agents. Analyzing the field dependence of r_1, r_2 and r_2/r_1 indicates reasonable agreement with the predictions of the SBM theory under the assumption that the rotational correlation time τ_R dominates the relaxation process. Direct infusion of these particles into the live tumor-bearing rat brain demonstrates an initial uniform distribution and hence the potential for effective brachytherapy applications when Gd is replaced with radioactive ^{177}Lu.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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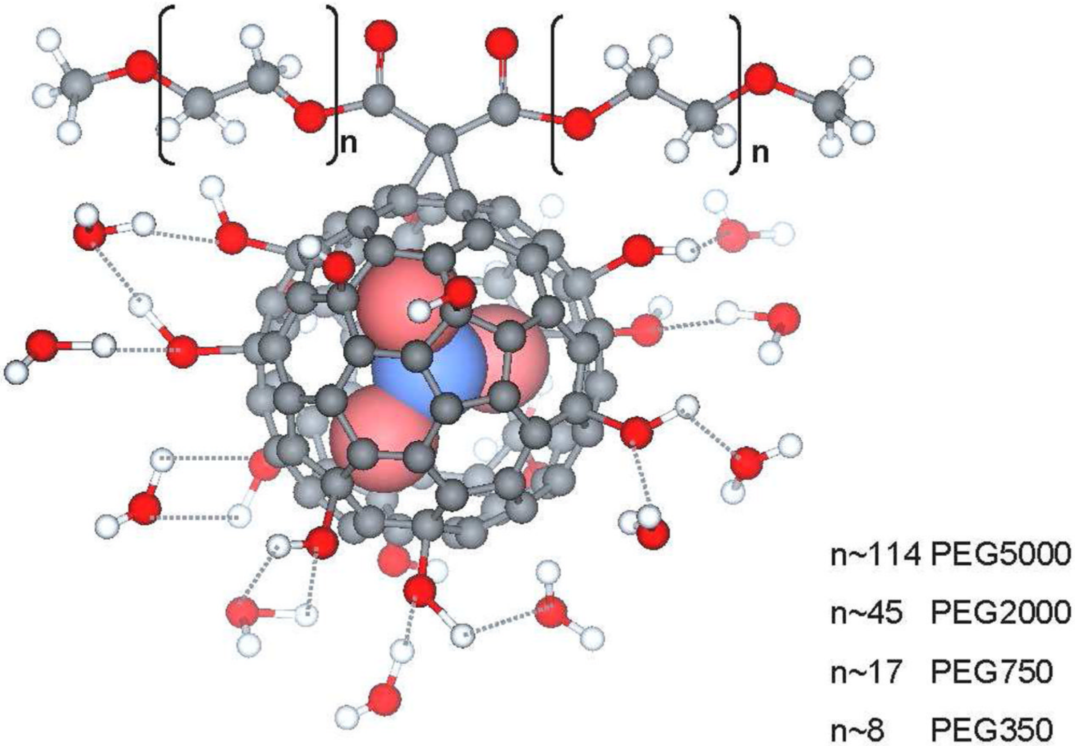
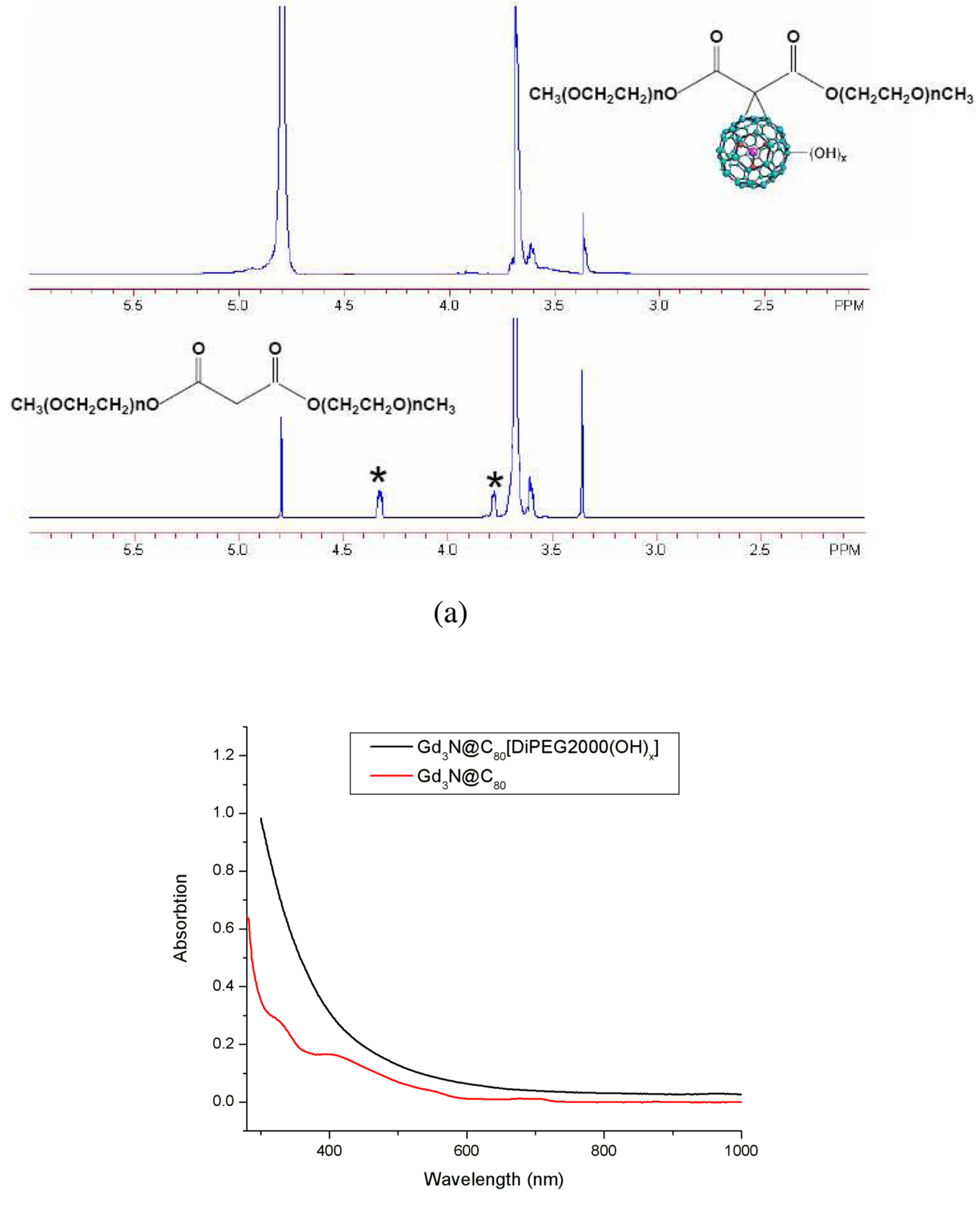


Figure 1.
The $Gd_3N@C_{80}[DiPEG(OH)_x]$ nanoparticle with several hydrogen-bonded water molecules shown.



(a)

(b)

Figure 2.

(a) 400MHz ^{1}H NMR spectra of $Gd_3N@C_{80}$[DiPEG350$(OH)_x$] (top) in comparison with DiPEGM350 malonate (bottom) in D_2O; (b) UV-Vis spectra of $Gd_3N@C_{80}$ and $Gd_3N@C_{80}$[DiPEG2000$(OH)_x$]. Note the disappearance of the characteristic peaks in the derivative at 330 and 410 nm.

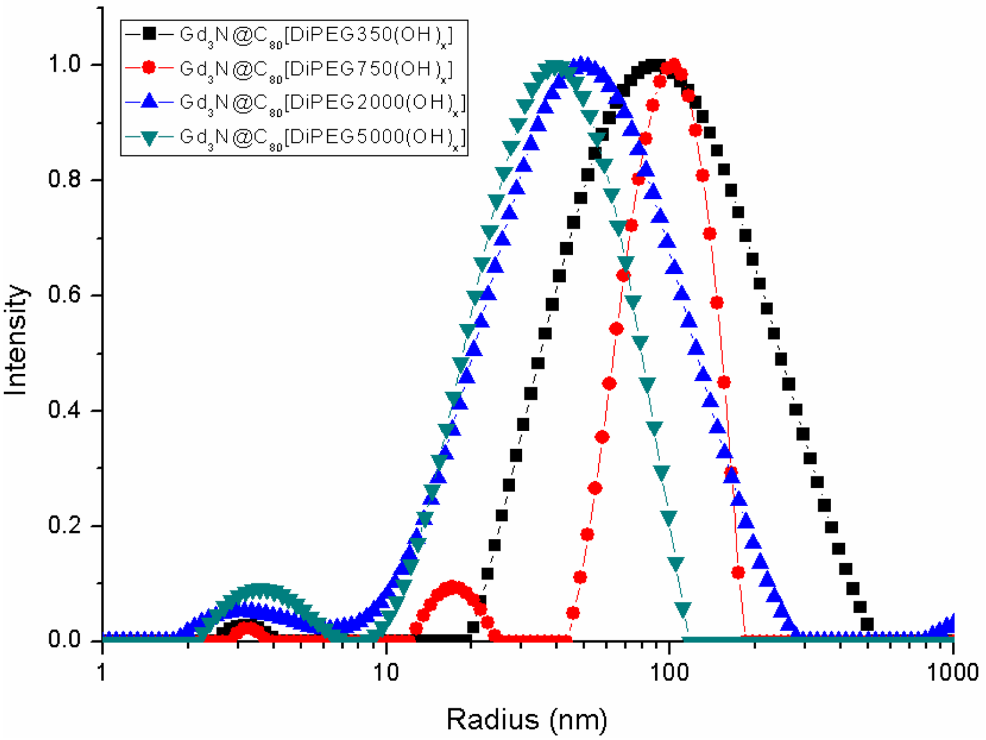


Figure 3.

Size distribution function of $Gd_3N@C_{80}[DiPEG(OH)_x]$ from DLS experiment. The mean peak position is 75nm, 76nm, 58nm and 37nm for $Gd_3N@C_{80}[DiPEG350(OH)_x]$, $Gd_3N@C_{80}[DiPEG750(OH)_x]$, $Gd_3N@C_{80}[DiPEG2000(OH)_x]$ and $Gd_3N@C_{80}[DiPEG5000(OH)_x]$ respectively.

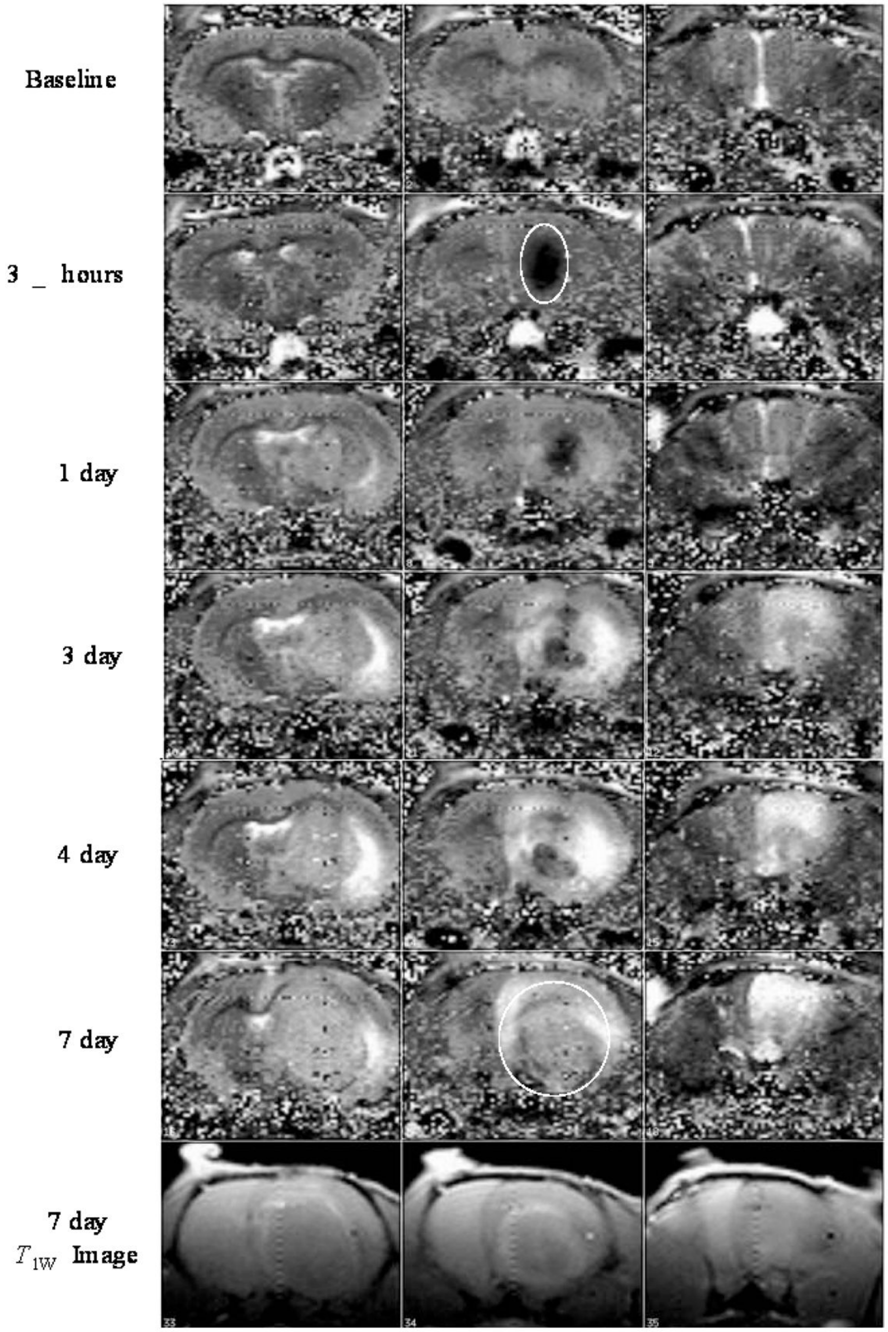


Figure 4.
T_1 computed images and T_{1w} image of a live rat brain after direct infusion into the tumor of 0.0235 mM $Gd_3N@C_{80}[DiPEG350(OH)_x]$.

TABLE 1

Relaxivity data of $Gd_3N@C_{80}[DiPEG(OH)_x]$ series. The relaxivities were measured at room temperature in water.

Mol. Weight of PEG	**Conc. Range (μM)**	**r_1 (0.35T) ($mM^{-1}s^{-1}$)**	**r_2 (0.35T) ($mM^{-1}s^{-1}$)**	**r_1 (2.4T) ($mM^{-1}s^{-1}$)**	**r_2 (2.4T) ($mM^{-1}s^{-1}$)**	**r_1 (9.4T) ($mM^{-1}s^{-1}$)**	**r_2 (9.4T) ($mM^{-1}s^{-1}$)**
5000	0.4–6.5	107±8	127±36	139±6	221±11	52.5±2.4	186±12
5000(7)	1.6–12.6	102	144	143	222	32	137
2000	1.0–15.2	130±4	148±8	158±6	249±12	41.9±3.0	218±11
750	1.1–17.4	152±5	169±20	232±10	398±22	63.3±1.8	274±9
350	1.5–23.5	227±31	268±19	237±9	460±23	68.2±3.3	438±5

TABLE 2

Relaxivity data of $Gd_3N@C_{80}[DiPEG(OH)_x]$ series. The relaxivities were measured at room temperature in water.

Metallofullerene Derivatives	**r_1 ($mM^{-1}s^{-1}$)**	**Magnetic Field Strength (T)**
$Gd@C_{82}O_6(OH)_{16}(NH_2CH_2CH_2COOH)_8$ (6)	9.1	1.5
Gadofullerenol(22)	47	9.4
$Gd@C_{82}(OH)_{40}$ (12)	67	0.47
	81	1.0
	31	4.7
$Gd@C_{60}(OH)_x$ (26)	83.2	1.4
$Gd@C_{60}[C(COOH)_2]_{10}$ (11,26)	24.0	1.4
	4.6	0.47
$Gd_3N@C_{80}[DiPEG750(OH)_x]$	150 (50.0 Gd^{3+} based)	0.35
	246 (82.0 Gd^{3+} based)	2.4
	58.1 (19.7 Gd^{3+} based)	9.4

TABLE 3

The r_2/r_1 ratios of the pegylated/hydroxylated samples

Sample	r_2/r_1 (0.35T)	r_2/r_1 (2.4T)	r_2/r_1 (9.4T)
5000	1.19	1.59	3.54
2000	1.14	1.58	5.20
750	1.11	1.72	4.33
350	1.18	1.94	6.42