Molecular Platform for Design and Synthesis of Targeted Dual-Modality Imaging Probes

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Supporting Information

ABSTRACT: We report a versatile dendritic structure based platform for construction of targeted dual-modality imaging probes. The platform contains multiple copies of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) branching out from a 1,4,7-triazacyclononane-N,N′,N″-triacetic acid (NOTA) core. The specific coordination chemistries of the NOTA and DOTA moieties offer specific loading of 68/67Ga3+ and Gd3+, respectively, into a common molecular scaffold. The platform also contains three amino groups which can potentiate targeted dual-modality imaging of PET/MRI or SPECT/MRI (PET: positron emission tomography; SPECT: single photon emission computed tomography; MRI: magnetic resonance imaging) when further functionalized by targeting vectors of interest. To validate this design concept, a bimetallic complex was synthesized with six peripheral Gd-DOTA units and one Ga-NOTA core at the center, whose ion T1 relaxivity per gadolinium atom was measured to be 15.99 mM−1 s−1 at 20 MHz. Further, the bimetallic agent demonstrated its anticipated in vivo stability, tissue distribution, and pharmacokinetic profile when labeled with 68Ga. When conjugated with a model targeting peptide sequence, the trivalent construct was able to visualize tumors in a mouse xenograft model by both PET and MRI via a single dose injection.

INTRODUCTION

Molecular imaging is gaining importance for noninvasive assessment of biological events in animal and human subjects. Through in vivo real-time visualization and imaging quantification of biological targets of interest, a better understanding of the biological processes underlying the initiation and progression of diseases can be obtained for more efficacious diagnosis and treatment.1,2 However, a single imaging technique is often unable to deliver all the necessary information required for a definitive clinical decision. To date, successful attempts have been seen to combine two or more imaging modalities in order to overcome the shortcomings of single modality systems for optimal visualization and better quantitative delineation of targets of interest. Consequently, multimodal imaging techniques have become the norm in both clinical practice and preclinical research as evidenced by the implementation of PET/CT (PET: positron emission tomography; CT: computed tomography) and PET/MRI (MRI: magnetic resonance imaging) procedures to patient care and the increasing use of a variety of combinations of the currently available imaging techniques in translational or basic biomedical research, such as MRI/optical and PET/NIRF (NIRF: near-infrared fluorescence).3–8 The fusion of PET and MRI is especially desirable because technically their features are complementary. While radionuclide-based imaging techniques, either PET or SPECT (single photon emission computed tomography), are extremely sensitive and quantitative, which allows the studies of biological events or processes at the molecular and cellular level, their spatial resolution is limited (>1 cm for current clinical scanners).9–11 On the other hand, while MRI is able to provide high spatial resolution (<0.1 cm) with exquisite soft tissue contrast even without exogenous contrast agents, contrast agents are often required to highlight specific biological or physiological processes in an amount a few orders of magnitude higher than PET or SPECT.12–14

Given that more and more PET/MRI systems will likely be used in the practice of diagnostic radiology, it becomes important to develop dual modality imaging probes that can realize the full potential of both modalities.

To take advantage of the strengths of PET and MRI, our approach is to design and synthesize a dual-modality agent scaffold offering a “single pharmacological behavior” for both imaging acquisitions. The merging of PET and MRI probe moieties to a single molecular platform would facilitate colocation and cross-validation of each modality in targeted regions of interest (two measures of one event). While MRI can provide the exact location of the probe, motion artifact correction, and PET partial volume correction, PET can afford better imaging quantification for higher detection sensitivity. Further, given that the proton MRI contrast actually reflects the...
map of the proton density, a colocalized PET signal distinct from the proton background could make the MRI contrast more identifiable, which further improves the MRI sensitivity.

Indeed several nanoparticle-based PET/MRI agents have been reported with enhanced magnetic relaxivities and considerable promise as dual-modality imaging agents. However, the Gd-PAMAM complexes rarely aff ord MRI contrast; only a handful are impeded their translation toward clinical applications. Third, the majority of nanoparticle-based PET/MRI agents provide T1 contrast; only a handful are T1-based agents, which are preferred in the cases of low proton density in the target tissues. To date, a wide range of macromolecules, such as perfluorocarbon emulsions, silica nanoparticles, liposomal vesicles, dendrimers, and polymers have been explored for the development of gadolinium-based T1 contrast agents. Of the macromolecular systems, dendrimers, which possess definitive molecular structures and formula, have shown a promising role. For instance, the dendrimer systems of poly(amido amine) (PAMAM) have been used as blood pool, liver, renal, lymphatic, and tumor-specific molecular probes, thereby providing multivalent effect to the construct for better specific binding to the biological target of interest. Of note, the strong interaction between the targeting molecule tethered probe and the corresponding target, triggered by multivalent effect, is expected to further enhance the relaxivity of the probe. For proof of concept, we conjugated each amine with a model integrin αvβ3 targeting peptide, c(RGDyK). The main features of this molecular dual-modality imaging probe design include the apparent in vivo stability (due to the inertness of the Gd-DOTA and Ga-NOTA complexes) and the defined molecular formula with selective loading of two metal ions.

Synthesis. The desired ligand H$_2$L was synthesized in 7 steps with an overall yield of approximately 15%. The structure of each intermediate was verified by $^1$H NMR and $^{13}$C NMR as well as mass spectroscopy. The synthetic route to compound H$_2$L is outlined in Scheme 1 and its functionalization is depicted in Scheme 2. Compound 2 was obtained from bis(3-aminopropyl)amine and carbobenzyloxy (Cbz) protected lysine in three steps (see Supporting Information). The free primary amine of 2 was coupled to the carboxylic acid bearing side arms of NOTA-(OH)$_3$ (Scheme 1) via carbodiimide chemistry to form 3. The N-phthaloyl protected amino groups of 3 were freed by deprotection via hydrazine to afford 4, which contains six amino groups serving as points of attachment for the DOTA units.

The N-hydroxy succinamide activated DOTA ester, 5, was reacted to the six amino groups forming 6. Attempts to deprotect the Boc groups of compound 6 led to partial deprotection of the Cbz groups. To overcome this problem, the ligand was fully deprotected by 30% HBr in an acetic acid solution. The synthesized ligand, H$_2$L, was fully characterized. The ligand carries six DOTA units intended for chelating Gd$^{3+}$

![Figure 1. Dual-modality molecular probe design.](image)
and one NOTA unit intended for chelating Ga\(^{3+}\). Since Gd\(^{3+}\) and Ga\(^{3+}\) can be specifically loaded into the DOTA and NOTA moieties, respectively, based on their ion-size preference, the bimetallic molecular complex is expected to carry the two metal ions in a specified ratio so as to facilitate PET imaging based quantification.

Functionalization of ligand H\(_2\)L was performed by utilizing the previously mentioned amino groups. Maleimido groups were tethered to amines using the commercially available reagent of N-succinimidyl 3-maleimidopropionate (Scheme 2). The model targeting peptide, c(RGDyK), was tethered to the maleimido carrying ligand H\(_2\)L via thiol−maleimide coupling. Given that targeting molecules attached to the bimetallic complex may not be able to stretch out for specific binding because of the steric hindrance from the construct, additionally we introduced a PEG12 linker between the complex and targeting vector.

The thiol group without a spacer (zero) or with a PEG12 linker was introduced to c(RGDyK) using the commercially available reagent: 2-pyridyldithiol-tetraoxaoctatriacontane-N-hydroxysuccinimide (SPDP). The thiol terminated c(RGDyK) peptides with zero and PEG12 linker were subsequently added to H\(_2\)L(Mal)\(_3\) to afford Gd\(_6\)H\(_3\)L[c(RGDyK)]\(_3\) and Gd\(_6\)H\(_3\)L[PEG12c(RGDyK)]\(_3\), respectively.

**Coordination Chemistry of the NOTA Core of H\(_2\)L.** To prove the efficient removal of Gd\(^{3+}\) from the NOTA core during the complexation procedure, the same complexation procedure was followed using Eu\(^{3+}\) instead of Gd\(^{3+}\). Chemically, Eu\(^{3+}\) and Gd\(^{3+}\) have similar reactivities toward NOTA and DOTA. Since Eu\(^{3+}\) is only weakly paramagnetic, the \(^1\)H NMR signals of its complexes are relatively sharp and also paramagnetically shifted. This facilitates relatively straightforward structural characterization by NMR spectroscopy. To accomplish this, the Eu\(^{3+}\) complex of H\(_2\)L, were synthesized via two procedures, i.e., with and without a challenge by excess DTPA, followed by HPLC purification. Both complexes were characterized by \(^1\)H NMR spectroscopy (400 MHz). Figure 2a,b shows the \(^1\)H NMR spectra of Eu\(^{3+}\) complexes of H\(_2\)L with and without the DTPA challenge, respectively. The DTPA treated Eu\(^{3+}\) complex (Figure 2a) showed sharp peaks in the NOTA region (around 3−4 ppm) suggesting a metal free NOTA core as opposed to the untreated complex (Figure 2b), which showed broad peaks indicating the interaction of metal with the NOTA core. This characterization along with the mass

![Figure 2](image_url)
spectroscopy results of Gd₆L and Gd₆H₃L indicates the exclusive Gd³⁺ coordination with the six DOTA moieties of H₂L. Further, the formation of Gd₆LGa was confirmed by the molar ratio of Gd:Ga determined by ICP-mass (Supporting Information). These results demonstrate that Gd³⁺ and Ga³⁺ have been exclusively loaded to the DOTA and NOTA moieties as designed, respectively, for the preparation of the desired dual-modality molecular imaging probe.

**Relaxometric Measurements.** The T₁ relaxation of Gd₆LGa was measured at 20 MHz, 25 °C, to be 95.98 mM⁻¹ s⁻¹ with R² value greater than 0.99 (each Gd³⁺ in the complex accounted for 15.99 mM⁻¹ s⁻¹ (ion relaxivity)). Under the same conditions, the relaxivity of Magnavist was found to be 4.1 mM⁻¹ s⁻¹. Impressively, the T₁ relaxivity of Gd₆H₃L was approximately 4 times higher than that of the Gd-DOTA complex and comparable to the Gd-PAMAM complexes, which have been reported with the ion relaxivity up to 11 mM⁻¹ s⁻¹. It is noteworthy that a similar relaxivity was also reported for a polylysine-based contrast agent (MW 17 453) with 24 Gd-DOTA units. At the physiological temperature of 37 °C, the relaxivity of Gd₆LGa was unchanged while its relaxivity in rat serum at 25 °C increased to 153.1 mM⁻¹ s⁻¹. These observations suggest two things: first, the lack of temperature dependence between 25 and 37 °C suggests that the water exchange rate in the appended Gd-DOTA- monoamide complexes limits the relaxivity somewhat at 25 °C. Given that water exchange is faster at 37 °C, the expected decline in relaxivity with increasing temperatures is offset by faster water exchange and a more favorable relaxivity at 37 °C. This was an unanticipated yet welcomed added advantage for this probe platform design. The higher relaxivity in serum indicates that Gd₆LGa does interact with one or more serum proteins.

**MR Imaging and Relaxivity Measurements of Gd₆LGa at 1.0 T.** The MR imaging potential of Gd₆LGa was evaluated at magnetic field of 1.0 T. Figure 3 shows the T₁ weighted imaging of Gd₆LGa in comparison with Magnevist at a series of decreasing concentrations (0.4 mM, 0.2 mM, 0.1 mM, 0.04 mM, to 0.02 mM).

![](image)

**Figure 3.** T₁-weighted MR images of samples recorded at 1.0 T at 25 °C. Samples of Magnevist (a → e) and Gd₆H₃L (v → z) containing a series of decreasing concentrations from 0.4 mM, 0.2 mM, 0.1 mM, 0.04 mM, to 0.02 mM.

In Vivo Tissue Distribution, Pharmacokinetics, and Stability of Gd₆LGa. As compared to other nanoparticle-based dual modality imaging platforms, our molecular probe design is expected to have a reasonable blood circulation half-life and an efficient clearance profile from the organs. To evaluate the in vivo behavior of this probe design, Gd₆H₃L was radiolabeled with ⁶⁷Ga (t½β = 3.26 d) so that the tissue biodistribution could be evaluated over an extended period. As shown in Table 1, the bimetallic complex, Gd₆LG₆Ga, indeed displayed a rapid and efficient clearance profile from all major organs except kidneys in normal mice. The high renal uptake can be attributed to the net positive charge on the probe due to the presence of amine groups. However, an efficient renal clearance is obvious after the initial high uptake as seen from the decrease of the kidney uptake level from 101.0 ± 4.3%ID/g at 1 h p.i. to 43.0 ± 5.1%ID/g at 24 h p.i. One would anticipate that kidney clearance would be even faster once the positively charged amino groups are removed from the agent through conjugation with a targeting vector. Indeed, when the amine groups of the agent were capped with c(RGDyK), the kidney uptake was impressively reduced to ca. 3%ID/g at 24 h p.i. One would anticipate that kidney clearance would be even faster once the positively charged amino groups are removed from the agent through conjugation with a targeting vector. Indeed, when the amine groups of the agent were capped with c(RGDyK), the kidney uptake was impressively reduced to ca. 3%ID/g at 24 h p.i.

The pharmacokinetics of Gd₆LG₆Ga showed a two-compartment profile with t½α = 11.3 min and t½β = 7.3 h, quite different compared to nanoparticle-based imaging agents which can be described by one-compartment models. The t½ of the probe reveals that the tissue distribution of Gd₆LG₆Ga was relatively rapid while the reasonably long t½β enables the probe to accumulate in its targeted tissues without incurring high background uptake. This indicates that the probe behaves similarly to other clinically available small molecule contrast agents. The presence of amine groups on the ligand provides the ease of incorporation of polyethylene glycol (PEG) chains into the probe for optimization of the in vivo kinetics if necessary.

The in vitro and in vivo stabilities of Gd₆LG₆Ga were evaluated through incubation in rat serum and by mouse urine metabolite analysis, respectively. The bimetallic complex remained intact during the entire course of study, which further validates this molecular probe platform design.

**Receptor-Binding Assay of Targeted Gd₆LGa Complexes.** Two integrin α₅β₃ targeted Gd₆LGa complexes Gd₆H₃L[c(RGDyK)]₃ and Gd₆H₃L[PEG₁₂c(RGDyK)]₃ were prepared to validate that our molecular construct design can be...
utilized for specific imaging of cancer biomarkers. The $\alpha\beta_3$ binding affinities were determined by a competitive cell-binding assay using $^{125}$I-echistatin (PerkinElmer) as the $\alpha\beta_3$-specific radioligand. The best-fit IC$_{50}$ values (inhibitory concentration where 50% of the $^{125}$I-echistatin bound to U87MG cells are displaced) of c(RGDyK), Gd$_6$H$_3$L[c(RGDyK)]$_{3\alpha}$, and Gd$_6$H$_3$L[PEG$_{12\beta}$c(RGDyK)]$_{3\beta}$ were measured to be 199 nM, 489 nM, and 37 nM (Supporting Information), respectively. The significantly improved $\alpha\beta_3$ binding affinity of Gd$_6$H$_3$L[PEG$_{12\beta}$c(RGDyK)]$_{3\beta}$ clearly indicates the critical role of the PEG$_{12\beta}$ linker in the desired targeting property of our molecular scaffold design.

**PET/MR Imaging Using Gd$_6$L[PEG$_{12\beta}$c(RGDyK)]$_{6\beta}$Ga.** To overcome the sensitivity difference between PET and MR, the radioactivity of $^{68}$Ga was controlled in the labeling of Gd$_6$H$_3$L[PEG$_{12\beta}$c(RGDyK)]$_{3\beta}$. The final dose injected into each animal carried about 6.0 mg of the final compound with an activity of 100–150 μCi (Gd: ca. 0.027 mmol/kg; c(RGDyK): 2.0 μmol). Figure 4 shows in vivo dual PET/MR images of integrin $\alpha\beta_3$ positive U87MG tumor enabled by a single dose injection of Gd$_6$L[PEG$_{12\beta}$c(RGDyK)]$_{6\beta}$Ga. The white arrow indicates the tumor site.

![Figure 4. Representative MRI (upper panel), PET (lower panel: left), and fused PET/MR (lower panel: right) images of U87MG tumor xenograft in SCID mice at 1 h post injection of Gd$_6$L[PEG$_{12\beta}$c(RGDyK)]$_{6\beta}$Ga. The white arrow indicates the tumor site.](image)

**EXPERIMENTAL PROCEDURES**

**General Materials and Methods.** All reactions were carried out under N$_2$ atmosphere in degassed dried solvents. Commercially available starting materials were purchased from commercial vendors and used directly without further purification unless otherwise stated. All aqueous solutions were prepared with Milli-Q water. Bulk solvents were removed using rotary evaporator under reduced pressure at 40 °C. Trace solvents were removed under high vacuum. Matrix-assisted laser desorption/ionization (MALDI) mass spectra were acquired on an Applied Biosystems Voyager-DeB1S1S mass spectrometer. Radiolabeled conjugates were purified by Light C-18 Sep-Pak cartridges (Waters, Milford, MA). High performance liquid chromatography (HPLC) was performed on a Waters Xterra Shield RP 18 semiprep column (250 × 10 mm, 10 μm) and by a Waters 2996 photodiode array detector and an in-line Shell Jr. 2000 radio-detector. The mobile phase was H$_2$O with 0.1% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.1% TFA (solvent B). The gradient consisted of 0% B to 80% B in 0–40 min at 4.0 mL/min flow rate. The radioactivity of excised tissue samples and radioactive standards were counted by a Wizard2 300 automatic γ-counter (PerkinElmer).

**Synthesis Procedures. Compound 3.** To a solution of the protected acid 1 (0.10 g, 0.15 mmol) in CH$_2$CN (1.0 mL) were added the deprotected amine 2 (0.40 g, 0.60 mmol), dicyclohexylcarbodiimide (0.15 g, 0.83 mmol), and triethylamine (0.30 g, 0.27 mmol). The resultant solution was stirred for 12 h, filtered, and the solvent evaporated. The crude product was purified by flash chromatography (ethyl acetate to give 3, a NOTA derivative, as a white solid (0.26 g, 0.10 mmol, 67%).$^1$H NMR (400 MHz, CDCl$_3$): δ 8.73 (bs, 6H), 7.71 (m, 12H), 7.61 (m, 12H), 7.23 (m, 15H), 5.56 (bs, 2H), 4.99 (m, 5H), 4.74 (m, 3H), 3.79–3.27 (m, 24H), 3.25–2.77 (m, 13H), 2.60–2.17 (m, 4H), 2.15–1.71 (m, 12H), 160 (m, 4H), 1.38 (m, 43H).$^{13}$C NMR (100 MHz, CDCl$_3$): δ 172.5, 163.8, 156.8, 136.5, 134.1, 133.9, 131.8, 128.4, 127.9, 123.3, 123.2, 79.0, 69.8, 66.5, 64.8, 49.3, 45.9, 44.3, 40.5, 35.5, 35.3, 32.5, 32.2, 29.3, 28.3, 20.51 However, they were not designed to serve the goal of providing targeted contrast to both imaging techniques. To provide proof of concept, the probe design in this work consists of only six gadolinium chelatable DOTA units. While the anticipated $T_1$ relaxivity enhancement was achieved, we recognize that further amplification of MRI sensitivity is necessary in order to realize the practical application of the molecular probe design as dual-modality imaging agents. Given the versatility of the design concept, we believe that it can be readily modified or adapted to reach the desired MRI sensitivity.

In conclusion, we have successfully demonstrated a molecular platform intended for targeted dual-modality imaging of PET/MRI or SPECT/MRI, which carries quantifiable numbers of $^{67/68}$Ga$^{3+}$ and Gd$^{3+}$ ions in their specific chelation moieties. This probe design displays a very favorable high $T_1$ relaxivity for MRI contrast enhancement. With the current rapid growth of hybrid PET/MRI systems in diagnostic radiology, our molecular design of dual-modality agents possessing a “single pharmacological behavior” offers a versatile platform for the development of multimodality imaging agents with potential applications for noninvasive molecular profiling of various diseases.
Compound 4. To a solution of 3 (0.26 g, 0.011 mmol) in ethanol (1.0 mL) was added hydrazine monohydrate (0.1 mL, 2.0 mM) and the mixture was stirred for 12 h at room temperature. After the reaction, the precipitate was removed by filtration. The filtrate was evaporated and extracted with 

\[ \text{CHCl}_3 (3 \times 10 \text{ mL}). \]

The combined organic layers were evaporated to give 4 as light yellow oil (0.15 g, 0.009 mmol, 80%). This compound was used for the next step without further purification. 

Compound H$_2$L(c(RGDyK)). To the maleimide carrying ligand, H$_2$LMAL$_2$ (0.020 g, 0.005 mmol) dissolved in PBS (1×) was added the thiol carrying c(RGDyK)-SH (0.014 g, 0.029 mmol), and the solution was allowed to stir for 18 h. The solution was purified by reverse phase HPLC using water and acetonitrile solvent mixture to give H$_2$L[c(c(RGDyK))$_3$]$_2$ as a white solid (0.010 g, 0.002 mmol, 33.3%). MS (MALDI) m/z calcd for C$_{264}$H$_{462}$N$_9$O$_{57}$S: 6136.9; found: 6139.1 ([M + H]+).

Compound c(RGDyK)-PEG$_2$SH. To the DMP solution (1 mL) of the integrin αβ$_3$ targeting peptide c(RGDyK) (0.040 g, 0.0064 mmol) (Peptides International Inc., Kentucky) dissolved in DMF (1.0 mL) was added N-2-pyridylthiol-tetraoxaoctatriaccontane-N-hydroxysuccinimide (0.160 g, 0.065 mmol) (Thermo Scientific, IL) and the solution was allowed to stir for 6 h. The solvent was evaporated and the product neutralized and purified by reverse phase HPLC using water and acetonitrile solvent mixture and lyophilized. The resultant white solid was dissolved in DMF (1 mL) and dithiothreitol (0.010 g, 0.065 mmol) was then added and the solution was allowed to stir for 3 h. The solvent was evaporated and the product neutralized and purified by reverse phase HPLC using water and acetonitrile solvent mixture and lyophilized. The resultant white solid was dissolved in DMF (1.0 mL) and dithiothreitol (0.010 g, 0.0065 mmol) was then added and the solution was allowed to stir for 3 h. The solvent was evaporated and the product neutralized and purified by reverse phase HPLC using water and acetonitrile solvent mixture and lyophilized. The resultant white solid was dissolved in DMF (1 mL) and dithiothreitol (0.010 g, 0.0065 mmol) was then added and the solution was allowed to stir for 3 h.

Compound 3.5 targeting peptide c(RGDyK) (0.040 g, 0.013 mmol) was dissolved in PBS (1×) and the solution was allowed to stir for 18 h. The solvent was evaporated and the product neutralized and purified by reverse phase HPLC using water and acetonitrile solvent mixture to give H$_2$L[c(c(RGDyK))$_3$]$_2$ as a colorless viscous liquid. (0.025 g, 0.019 mmol, 30.2%). MS (MALDI) m/z found: 2595.9 ([M + H]+).

Compound H$_2$L(c(RGDyK))$_3$. To the maleimide carrying ligand, H$_2$L(MAL)$_2$ (0.030 g, 0.007 mmol) dissolved in PBS (1×) was added the thiol carrying peptide c(RGDyK)-SH (0.00 g, 0.005 mmol), and the solution was allowed to stir for 18 h. The solution was purified by reverse phase HPLC using water and acetonitrile solvent mixture to give H$_2$L[c(c(RGDyK))$_3$]$_2$ as a white solid (0.010 g, 0.002 mmol, 33.3%). MS (MALDI) m/z calcd for C$_{145}$H$_{250}$N$_9$O$_{57}$S: 5584.2; found: 5584.35 ([M + H]+).

Compound H$_2$L(c(RGDyK))$_3$. To the maleimide carrying ligand, H$_2$L(MAL)$_2$ (0.030 g, 0.007 mmol) dissolved in PBS (1×) was added the thiol carrying c(RGDyK)-SH (0.014 g, 0.029 mmol), and the solution was allowed to stir for 18 h. The solution was purified by reverse phase HPLC using water and acetonitrile solvent mixture to give H$_2$L[c(c(RGDyK))$_3$]$_2$ as a white solid (0.010 g, 0.002 mmol, 33.3%). MS (MALDI) m/z calcd for C$_{145}$H$_{250}$N$_9$O$_{57}$S: 5584.2; found: 5584.35 ([M + H]+).

Compound Gd$_6$H$_3$L([c(RGDyK)]$_3$. To the maleimide carrying ligand, Gd$_6$H$_3$L(MAL)$_2$ (0.030 g, 0.008 mmol) dissolved in PBS (1×) was added the thiol carrying c(RGDyK)-SH (0.014 g, 0.029 mmol), and the solution was allowed to stir for 18 h. The solution was purified by reverse phase HPLC using water and acetonitrile solvent mixture to give Gd$_6$H$_3$L[c(c(RGDyK))$_3$] as a white solid (0.010 g, 0.002 mmol, 33.3%). MS (MALDI) m/z calcd for C$_{145}$H$_{250}$N$_9$O$_{57}$S: 5584.2; found: 5584.35 ([M + H]+).
excess amount of EuCl₃·6H₂O and the pH was readjusted to 6.5 and allowed to stir at room temperature overnight. The pH was raised above 8.0 using 1.0 N aqueous NaOH, which caused the excess Eu³⁺ to precipitate as Eu(OH)₃. The solution was filtered and the pH was readjusted to 7.0 using 1.0 N HCl. To the resulting solution, DTPA (0.1 mM, 1 mL) was added and the solution was purified using HPLC to give the desired complex. The desired fractions were pooled together and lyophilized to give Eu₄LH₃ as a white solid. (0.042 g, 0.009 mmol, 54%). MS (MALDI) m/z calc for C₁₃₅H₂₆₂Eu₄N₄₂O₅₄: 4453.4; found: 4454.5 ([M + H]+).

**Compound Cold Gd₆L₆Ga.** The gadolinium complex, Gd₆H₃L₆, was dissolved in a solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH = 6.5, 1.0 M, 1.0 mL). To the resulting solution was added a solution of GaCl₃ (0.0001 g, 0.567 μmol) in 0.6 N HCl (0.3 mL) and the resulting solution was stirred for 1 h. To the mixture was added 500 μL of 5.0 mM ethylenediaminetetraacetic acid (EDTA) and the mixture was stirred for another 5 min at room temperature (EDTA was used to remove nonspecifically bound or free GaCl₃ from the complex). The purification of the bimetallic complex was carried out by passing the mixture through a preconditioned Sep-Pak C-18 heavy cartridge. After thorough rinsing (3 × 5 mL, water) of the cartridge, the bimetallic complex, Gd₆L₆Ga, was eluted by an ethanol—water mixture (70:30). The product was purified with HPLC and characterized by mass spec. MS (MALDI) m/z calc for C₁₃₅H₂₆₂Ga₆N₄₂O₅₄: 4555.28; found: 4556.9 ([M + H]+).

**Compound Gd₆L₆⁶⁸Ga.** To a 1.5 mL eppendorf tube containing 30 μg Gd₆H₃L₆ complex in 1.0 mL of HEPES (pH = 6.5) solution was added a solution of 8.0 mM of GaCl₃ in 0.6 N HCl. The reaction mixture was incubated at 75 °C for 0.5 h on a shaker. To this solution was added DTPA (5.0 mM, 5.0 μL) and the reaction mixture was incubated for 5 min at room temperature. The ⁶⁸Ga-labeled compound was determined to have more than 95% purity. The compound was then analyzed by radio-HPLC to determine the radiochemical purity of the product. The radiochemical purity of the compound was determined to be higher than 95%.

**Compound Gd₆H₃[LPEG₁₂c(RGDyK)]₆⁶⁸Ga.** To a 1.5 mL eppendorf tube containing 80 μg Gd₆H₃L₆[c(RGDyK)]₆ complex in 1 mL of HEPES (pH = 6.5) solution was added a solution of 15.0 μCi of ⁶⁸GaCl₃ in 0.6 N HCl. The reaction mixture was incubated at 75 °C for 0.5 h on a shaker. To this solution was added DTPA (5.0 mM, 5.0 μL) and the reaction mixture was incubated for 5 min at room temperature. The ⁶⁸Ga-labeled conjugate, Gd₆L₆[c(RGDyK)]₆⁶⁸Ga, was purified by passing the mixture through a preconditioned Sep-Pak C-18 light cartridge. After thorough rinsing (3 × 5 mL, water) of the cartridge, Gd₆L₆[c(RGDyK)]₆⁶⁸Ga was eluted by an ethanol—water mixture (70:30) to give 9.0 μCi of the labeled compound. The product was analyzed by radio-HPLC to determine the radiochemical purity of the product. The radiochemical purity of the compound was determined to be higher than 95%.

**Compound Gd₆H₃L₆[PEG₁₂c(RGDyK)]₆⁶⁸Ga.** The c(RGDyK) modified ligand H₂₁L₆[c(RGDyK)]₆ (0.010 g, 0.002 mmol) was dissolved in water (1 mL) and the pH was adjusted to 7 with NaOH (0.1 M). To this solution was added an excess of Gd₆H₃L₆⁶⁸Ga and the pH was again adjusted to 6.5 and allowed to stir at room temperature overnight. The pH was raised above 8.0 using 1.0 N aqueous NaOH, which caused the excess Gd³⁺ to precipitate as Gd(OH)₃. The solution was filtered and the pH was readjusted to 7.0 using 1.0 N HCl. To the resulting solution, DTPA (0.1 mM, 1 mL) was added and the solution was purified using HPLC to give the desired complex. The desired fractions were pooled together and lyophilized to give Gd₆H₃L₆[c(RGDyK)]₆ as a white solid. (0.005 g, 0.001 mmol, 48%). MS (MALDI) m/z calc for C₁₄₆H₂₆₂Ga₆N₄₁O₅₄S₃: 8864.3; found: 8864.7 ([M + H]+).
(pH = 6.5) solution was added a solution of 300 μCi of 68GaCl₃ in 0.6 N HCl. The reaction mixture was incubated at 75 °C for 10 min on a shaker. To this solution was added DTPA (5.0 mM, 5.0 μL) and the reaction mixture was incubated for 1 min at room temperature. The 68Ga-labeled conjugate was purified by passing the mixture through a preconditioned Sep-Pak C-18 plus cartridge. After thorough rinsing (3 x 5 mL, water) of the cartridge, Gd₆H₃[PEG₁₂c(RGDyK)]₆₈Ga was eluted by an ethanol–water mixture (70:30) to give 180–200 μCi of the labeled compound. The product was analyzed by radio-HPLC to determine the radiochemical purity of the product. The radiochemical purity of the compound was determined to be higher than 95%.

**Biodistribution and Pharmacokinetics Studies of Gd₆L⁶⁷Ga.** Male BALB/C mice were injected with 300 μCi of Gd₆L⁶⁷Ga complex to evaluate the tissue distribution of the tracer in mice. Mice were sacrificed 1, 4, and 24 h post injection (p.i.). The organs of interest (blood, heart, lung, liver, spleen, kidney, stomach, muscle, fat, small intestine, large intestine, and brain) were harvested and weighed, and radioactivity was quantified using a γ-counter. Standards were prepared and counted along with the tissue samples to calculate the percentage-injected dose per gram (%ID/g). To determine the pharmacokinetic parameters, mice injected with the tracer were blood sampled from the retro-orbital sinus at 2, 5, 10, and 30 min, and 1, 2, 24, and 48 h p.i. and quantified using a γ-counter. The pharmacokinetic parameters were calculated based on a two-compartment open model.

**In Vitro and Ex Vivo Stability of Gd₆L⁶⁷Ga.** The in vitro stability test was performed in rat serum. Briefly, Gd₆L⁶⁷Ga complex (100 μCi, 5 μL) was added into 100 μL of rat serum (n = 3). The solution was incubated for 1, 4, 24, and 48 h at 37 °C, respectively. The solution was vortexed and centrifuged for 5 min at the speed of 21,000 g. The supernatant was then analyzed by HPLC. For the in vivo stability evaluation, each male mouse was injected with 600 μCi of Gd₆L⁶⁷Ga complex in 100 μL of saline via the tail vein. The urine samples were collected at 1, 4, 24, and 48 h p.i. and then analyzed by HPLC.

**Integrin αvβ₃ Receptor-Binding Assay.** The integrin αvβ₃ binding affinities of c(RGDyK), Gd₆H₃L[PEG₁₂c(RGDyK)]₆₈Ga were determined by a competitive cell-binding assay using 125I-echistatin (PerkinElmer) as the αvβ₃-specific radioligand. The experiments were performed on U87MG human glioblastoma cells following a previously reported method. Briefly, U87MG cells were grown in RPMI 1640 medium supplemented with penicillin, streptomycin, and 10% (v/v) fetal bovine serum (FBS) at 37 °C under 5% CO₂. Suspended U87MG cells in the binding buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂, 1.0 mM MnCl₂, 0.1% bovine serum albumin) were seeded on multiwell DV plates (Millipore) with 5 x 10⁶ cells per well and then incubated with 125I-echistatin (10 000 cpm/well) in the presence of increasing concentrations (0–5000 nM) of each c(RGDyK) peptide conjugate for 2 h. The final volume in each well was maintained at 200 μL. At the end of incubation, unbound ¹²⁵I-echistatin was removed by filtration followed by three rinses with cold binding buffer. The retentate was collected and the radioactivity was measured using a γ-counter. The best-fit IC₅₀ values (inhibitory concentration where 50% of the ¹²⁵I-echistatin bound on U87MG cells are displaced) of c(RGDyK), Gd₆H₃L[PEG₁₂c(RGDyK)]₆₈Ga were calculated by fitting the data with nonlinear regression using GraphPad Prism (GraphPadSoftware, Inc.). Experiments were repeated with quintuplicate samples.

**Relaxivity Measurements at 0.5 T (T₁).** The T₁ values were recorded at 23 MHz (0.5 T) at 25 °C by using a Maran Ultra relaxometer (Oxford Instruments, UK). Longitudinal relaxation times were measured by using the inversion–recovery pulse sequence (180°-t-90°). The T₁ relaxivities were determined by the linear regression analysis of the water proton relaxation rates in solutions ranging in concentration from 0.005 to 12.0 mM, in Millipore water in triplicate.

**MRI Imaging and Relaxometry Measurements at 1.0 T.** The T₁-weighted MR images of samples in 0.5 mL microfuge tubes were collected using Aspect Imaging M2TM 1.0 T (43.5–45 MHz, 60 mm diameter volume coil) Gradient Echo Spoiled External Averaging (GRE-EXT) sequence. For imaging, the following parameters were used: TR = 14.2 ms; effective echo time (TE) = 2.8 ms; FOV 64 × 100 mm², data matrix = 256 × 256, averaging = 4, slice = 1 mm. The T₁-maps of the samples at 1.0 T were determined from a series of multi-T₁-(0.01 ms to 5 s) inversion recovery spin echo sequence (IR-SE with minimum TE = 7.0 ms); TR = 10 s; FOV = 64 × 100 mm²; matrix = 128 × 128; averaging = 1, steady state scans = 10), fitted and calculated using Matlab code. All the fits for T₁ values used to calculate the longitudinal relaxivity, r₁, had fitting coefficients, R² ≥ 0.99. Three trials were performed. The Gd and Ga metal concentrations of samples were determined by inductively coupled plasma mass spectrometry (ICP-MS).

**PET/MR Imaging Using Gd₆L[PEG₁₂c(RGDyK)]₆₈Ga.** The PET and MR imaging studies were performed on a 1.0 T MR scanner (Aspect Imaging, Shoham, Israel) using a 35 mm mouse body coil, and a Siemens Inveon Multimodality PET/CT system (Siemens Medical Solutions Inc., Knoxville, TN, USA), respectively. Ten minutes prior to imaging, the animals were anesthetized using 3% isoflurane at room temperature until stable vitalis were established. Once the animals were sedated, they were placed onto a custom-made interchangeable bed between the MR and PET scanners under 2% isoflurane anesthesia for the duration of the imaging. A T₁-weighted gradient echo, spoiled sequence (echo time/ repetition time [TE/TR] 3/15 ms; field of view [FOV] 80 × 100 mm²; slice thickness 0.8 mm; matrix 256 × 256; number of excitations [NEX] = 3; flip angle = 25°) was performed for each mouse. Each mouse was injected with 100–150 μCi (Gd: ca. 0.027 mmol/kg) via the tail-vein. MR scans were obtained precontrast and 1 h after contrast medium injection.

PET imaging was acquired with the mouse mounted on the interchangeable bed directly following the MR acquisition. A 15 min PET static scan was performed and reconstructed using Fourier rebinning and ordered subsets expectation maximization 3D (OSEM3D) algorithm. Reconstructed MR and PET images were fused and analyzed using the VivoQuant software (Invicro LLC, Boston, USA). For quantification, regions of interest were placed on the tumor and muscle. The latter was used for normalization. The resulting quantitative data were expressed in intensity and %ID/g for MR and PET images.

**S Supporting Information**

Supporting schemes, synthesis of compounds, ICP quantification. This material is available free of charge via the Internet at http://pubs.acs.org.
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**Author Contributions**
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**Notes**
The authors declare no competing financial interest.

**REFERENCES**

(1) Weissleder, R. (2006) Molecular imaging in cancer. *Science* 312, 1168–1171.
(2) Cheon, J., and Lee, J.-H. (2008) Synergistically integrated nanoparticles as multimodal probes for nanobiotechnology. *Acc. Chem. Res.* 41, 1630–1640.
(3) Jennings, L. E., and Long, N. J. (2009) 'Two is better than one'-probes for dual-modality molecular imaging. *Chem. Commun.* 28, 3511–3524.
(4) Ntziachristos, V., Yodh, A. G., Schnall, M., and Chance, B. (2000) Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2767–2772.
(5) Beyer, T., Townsend, D. W., Brun, T., Kinahan, P. E., Charon, M., Rody, R., Jerin, J., Young, J., Byars, L., and Nutt, R. (2000) A combined PET/CT scanner for clinical oncology. *J. Nucl. Med.* 41, 1369–1379.
(6) Murray, C. B., Norris, D. J., and Bawendi, M. G. (1993) Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites. *J. Am. Chem. Soc.* 115, 8706–8715.
(7) Link, S., and El-Sayed, M. A. (1999) Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. *J. Phys. Chem. B* 103, 8410–8426.
(8) Alivisatos, A. P. (1996) Semiconductor clusters, nanocrystals, and quantum dots. *Science* 271, 933–937.
(9) Catana, C., Wu, Y., Judenhofer, M. S., Qi, J., Pichler, B. J., and Cherry, S. R. (2006) Simultaneous acquisition of multislice PET and MR images: initial results with a MR-compatible PET scanner. *J. Nucl. Med.* 47, 1968–1976.
(10) Cherry, S. R. (2006) The 2006 Henry N. Wagner lecture: of mice and men (and positrons)–advances in PET imaging technology. *J. Nucl. Med.* 47, 1735–1745.
(11) Cherry, S. (2001) Fundamentals of positron emission tomography and applications in preclinical drug development. *J. Clin. Pharmacol.* 41, 482–491.
(12) Caravan, P., Ellison, J. J., McMurry, T. J., and Lauffer, R. B. (1999) Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chem. Rev.* 99, 2293–2352.
(13) Raymond, K. N., and Pierre, V. r. C. (2004) Next generation, high relaxivity gadolinium MRI agents. *Bioconjugate Chem.* 15, 3–8.
(14) Seo, W. S., Lee, J. H., Sun, X., Suzuki, Y., Mann, D., Liu, Z., Terahashi, M., Yang, P. C., McConnell, M. V., Nishimura, D. G., et al. (2006) FeCo/graphitic-shell nanocrystals as advanced magnetic resonance-imaging and near-infrared agents. *Nat. Mater.* 5, 971–976.
(15) Morawski, A. M., Winter, P. M., Crowder, K. C., Caruthers, S. D., Fuhrhop, R. W., Scott, M. J., Robertson, J. D., Abendstein, D. R., Lanza, G. M., and Wickline, S. A. (2004) Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI. *Magn. Reson. Med.* 51, 480–486.
(16) Santra, S., Bagwe, R. P., Dutta, D., Stanley, J. T., Walter, G. A., Tan, W., Moudgil, B. M., and Mericle, R. A. (2005) Synthesis and characterization of fluorescent, radio-opaque, and paramagnetic silica nanoparticles for multimodal bioimaging applications. *Adv. Mater.* 17, 2165–2169.
(17) Cheng, Z., and Tsourkas, A. (2008) Paramagnetic porous polymersomes. *Langmuir* 24, 8169–8173.
(18) Cheng, L., Yang, K., Li, Y., Chen, J., Wang, C., Shao, M., Lee, S.-T., and Liu, Z. (2011) Facile preparation of multifunctional upconversion nanoprobes for multimodal imaging and dual-targeted photothermal therapy. *Angew. Chem., Int. Ed.* 123, 7523–7528.
(19) Chen, J., Wiley, B., Li, Z. Y., Campbell, D., Saeki, F., Cang, H., Au, L., Lee, J., Li, X., and Xia, Y. (2005) Gold nanocages: engineering their structure for biomedical applications. *Adv. Mater.* 17, 2255–2261.
(20) Gao, X., Cui, Y., Levenson, R. M., Chung, L. W. K., and Nie, S. (2004) In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 22, 969–976.
(21) Lin, Y.-S., Hung, Y., Su, J.-K., Lee, R., Chang, C., Lin, M.-L., and Mou, C.-Y. (2004) Gadolinium(III)-incorporated mesoporous silica as potential magnetic resonance imaging contrast agents. *J. Phys. Chem. B* 108, 15608–15611.
(22) Rieter, W. J., Kim, J. S., Taylor, K. M. L., An, H., Lin, W., Tarrant, T., and Lin, W. (2007) Hybrid silica nanoparticles for multimodal imaging. *Angew. Chem., Int. Ed.* 119, 3754–3756.
(23) Hak, S., Sanders, H. M. H. F., Agrawal, P., Langereis, S., Grull, H., Keizer, H. M., Arena, F., Terreno, E., Strijkers, G. J., and Nicolay, K. (2009) A high relaxivity Gd(III)DOTA-DSPE-based liposomal contrast agent for magnetic resonance imaging. *Eur. J. Pharm. Biopharm.* 72, 397–404.
(24) Terreno, E., Delli Castelli, D., Cabella, C., Dastru, W., Sanino, A., Stancanello, J., Tei, L., and Aime, S. (2008) Paramagnetic liposomes as innovative contrast agents for magnetic resonance (MR) imaging applications. *Chem. Biodivers.* 5, 1901–1912.
(25) Unger, E. C., MacDougall, P., Cullis, P., and Tilcock, C. (1989) Liposomal Gd-DTPA: Effect of encapsulation on enhancement of hepatoma model by MRI. *Magn. Reson. Imag.* 7, 417–423.
(26) Boswell, C. A., Eck, P. K., Regino, C. A. S., Bernardo, M., Wong, K. J., Milenic, D. E., Choyke, P. L., and Brechbiel, M. W. (2008) Synthesis, characterization, and biological evaluation of integrin αvβ3-targeted PAMAM dendrimers. *Mol. Pharmacol.* 5, 527–539.
(27) Kobayashi, H., and Brechbiel, M. W. (2005) Nano-sized MRI contrast agents with dendrimer cores. *Adv. Drug Delivery Rev.* 57, 2271–2286.
(28) Langereis, S., de Lussanet, Q. G., van Genderen, M. H. P., Meijer, E. W., Beets-Tan, R. G. H., Griffioen, A. W., van Engelsloven, J. M. A., and Backes, W. H. (2006) Evaluation of Gd(III)DPAA-terminated poly(propylene imine) dendrimers as contrast agents for MR imaging. *NMR Biomed.* 19, 133–141.
(29) Langereis, S., Dirksen, A., Hakken, T. M., van Genderen, M. H. P., and Meijer, E. W. (2007) Dendrimers and magnetic resonance imaging. *New J. Chem.* 31, 1152–1160.
(30) Rudovský, J., Botta, M., Hermann, P., Hardcastle, K. I., Lukeš, I., and Aime, S. (2006) PAMAM dendrimeric conjugates with a Gd–DOTA phosphate derivative and their adducts with polyaminocidos: the interplay of global motion, internal rotation, and fast water exchange. *Bioconjugate Chem.* 17, 975–987.
(31) Bolskar, R. D. (2008) Gadofullerene MRI contrast agents. *Nanomedicine* 3, 201–213.
(32) Zhu, W., Okollie, B., Bhujwalla, Z. M., and Artemov, D. (2008) PAMAM dendrimer-based contrast agents for MR imaging of Her-2/neu receptors by a three-step pretargeting approach. *Magn. Reson. Med.* 59, 679–685.
(33) Duarte, M. G., Gil, M. H., Peters, J. A., Colet, J. M., Elst, L. V., Muller, R. N., and Geraldes, C. F. G. C. (2001) Synthesis, characterization, and relaxivity of two linear Gd(DTPA)–polymer conjugates. *Bioconjugate Chem.* 12, 170–177.
(34) Brash, R. C. (1991) Rationale and applications for macro-molecular Gd-based contrast agents. *Magn. Reson. Med.* 22, 282–287.
(35) Kobayashi, H., Kawamoto, S., Saga, T., Sato, N., Hiraga, A., Ishimori, T., Akita, Y., Mamede, M. H., Konishi, J., Togashi, K., et al. (2001) Novel liver macromolecular MR contrast agent with a polypropylenimine diaminobutyl dendrimer core: Comparison to the vascular MR contrast agent with the polyamidoamine dendrimer core. Magn. Reson. Med. 46, 795–802.

(36) Kobayashi, H., Kawamoto, S., Jo, S. K., Sato, N., Saga, T., Hiraga, A., Konishi, J., Hu, S., Togashi, K., Brechbiel, M. W., et al. (2002) Renal tubular damage detected by dynamic micro-MRI with a dendrimer-based magnetic resonance contrast agent. Kidney Int. 61, 1980–1985.

(37) Sugaya, M., Watanabe, T., Yang, A., Starost, M. F., Kobayashi, H., Atkins, A. M., Borris, D. L., Hanan, E. A., Schimel, D., Bryant, M. A., et al. (2005) Lymphatic dysfunction in transgenic mice expressing KSHV k-cyclin under the control of the VEGFR-3 promoter. Blood 105, 2356–2363.

(38) Wiener, E. C., Konda, S., Shadron, A., Brechbiel, M., and Gansow, O. (1997) Targeting dendrimer-chelates to tumors and tumor cells expressing the high-affinity folate receptor. Invest. Radiol. 32, 748–754.

(39) Kobayashi, H., and Brechbiel, M. W. (2003) Dendrimer-based macromolecular MRI contrast agents: characteristics and application. Mol. Imaging 2, 1–10.

(40) Venditto, V. J., Regino, C. A. S., and Brechbiel, M. W. (2005) PAMAM dendrimer based macromolecules as improved contrast agents. Mol. Pharmaceutics 2, 302–311.

(41) Kumar, K., Chang, C. A., Francesconi, L. C., Dischino, D. D., Malley, M. F., Gougoutas, J. Z., and Tweedle, M. F. (1994) Synthesis, stability, and structure of gadolinium(III) and yttrium(III) macrocyclic poly(amino carboxylates). Inorg. Chem. 33, 3567–3575.

(42) Reichert, D. E., Hancock, R. D., and Welch, M. J. (1996) Molecular mechanics investigation of gadolinium(III) complexes. Inorg. Chem. 35, 7013–7020.

(43) Dong, Q., Hurst, D. R., Weinmann, H. J., Cheneverut, T. L., Londy, F. J., and Prince, M. R. (1998) Magnetic resonance angiography with gadomer-17: an animal study. Invest. Radiol. 33, 699–708.

(44) Nicolle, G. M., Töth, É., Schmitt-Willich, H., Radüchel, B., and Merbach, A. E. (2002) The impact of rigidity and water exchange on the relaxivity of a dendritic MRI contrast agent. Chem.—Eur. J. 8, 1040–1048.

(45) Behr, T. M., Goldenberg, D. M., and Becker, W. (1998) Reducing the renal uptake of radiolabeled antibody fragments and peptides for diagnosis and therapy: present status, future prospects and limitations. Eur. J. Nucl. Med. 25, 201–212.

(46) Ma, H. L., Xu, Y. F., Qi, X. R., Mattani, Y., and Nagai, T. (2008) Superparamagnetic iron oxide nanoparticles stabilized by alginate: Pharmacokinetics, tissue distribution, and applications in detecting liver cancers. Int. J. Pharm. 354, 217–226.

(47) Lux, F., Mignot, A., Mowat, P., Louis, C., Dufort, S., Bernhard, C., Denat, F., Boschetti, F., Brunet, C., Antoine, R., et al. (2011) Ultrasmall rigid particles as multimodal probes for medical applications. Angew. Chem., Int. Ed. 123, 12507–12511.

(48) Iznaga-Escobar, N., Arocha, L. A. T., Morales, A. M., Suzarte, M. R., Mesa, N. R., and Rodríguez, R. P. (1998) Technetium-99m-antiepidermal growth factor-receptor antibody in patients with tumors of epithelial origin: part II. pharmacokinetics and clearances. J. Nucl. Med. 39, 1918–1927.

(49) Vologdin, N., Rolla, G. A., Botta, M., and Tei, L. (2013) Orthogonal synthesis of a heterodimeric ligand for the development of the Gd-III-Ga-III ditopic complex as a potential pH-sensitive MRI/PET probe. Org. Biomol. Chem. 11, 1683–1690.

(50) Notni, J., Hermann, P., Dregely, I., and Wester, H.-J. (2013) Simultaneous in vivo PET/MRI imaging with a bi-metal bimodal tracer: Towards Ga-68-labeled responsive gadolinium contrast agents. J. Labelled Comp. Radiopharm. 56, S62–S62.

(51) Suchy, M., Bartha, R., and Hudson, R. H. E. (2013) “Click” chemistry toward bis(DOTA-derived) heterometallic complexes: potential bimodal MRI/PET(SPECT) molecular imaging probes. RSC Adv. 3, 3249–3259.