Amphiphilic Nanoaggregates with Bimodal MRI and Optical Properties Exhibiting Magnetic Field Dependent Switching from Positive to Negative Contrast Enhancement

Michael Harris,[†] Danai Laskaratos,[‡] Luce Vander Elst,[§] Hideaki Mizuno,[‡] and Tatjana N. Parac-Vogt^{*,†,§}

[†]Department of Chemistry, KU Leuven, 3001 Leuven, Belgium

[‡]Department of Chemistry, Biochemistry, Molecular and Structural Biology Section, Laboratory of Biomolecular Network Dynamics, KU Leuven, 3001 Leuven, Belgium

[§]Department of General, Organic, and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, 7000 Mons, Belgium

Supporting Information

ABSTRACT: Mixed micelles based on amphiphilic gadolinium(III)-DOTA and europium(III)-DTPA complexes were synthesized and evaluated for their paramagnetic and optical properties as potential bimodal contrast agents. Amphiphilic folate molecule for targeting the folate receptor protein, which is commonly expressed on the surface of many human cancer cells, was used in the self-assembly process in order to create nanoaggregates with targeting properties. Both targeted and nontargeted nanoaggregates formed monodisperse micelles having distribution maxima of 10 nm. The micelles show characteristic europium(III) emission with quantum yields of 2% and 1.1% for the nontargeted and targeted micelles, respectively. Fluorescence microscopy using excitation at 405 nm and emission at 575–675 nm was employed to visualize nanoaggregates in cultured HeLa cells. The uptake of folate-targeted and nontargeted micelles is already visible after 5 h of incubation and was characterized with the europium(III) emission, which is clearly observable in the cytoplasm of the cells. The very fast longitudinal relaxivity r_1 of ca. $26 \text{ s}^{-1} \text{ mM}^{-1}$ per gadolinium(III) ion was observed for both micelles at 60 MHz and 310 K. Upon increasing the magnetic field to 300 MHz, the nanoaggregates exhibited a large switching to transversal relaxivity with r_2 value of ca. $52 \text{ s}^{-1} \text{ mM}^{-1}$ at 310 K. Theoretical fitting of the ^1H NMRD profiles indicate that the efficient T_1 and T_2 relaxations are sustained by the favorable magnetic and electron-configuration properties of the gadolinium(III) ion, rotational correlation time, and coordinated water molecule. These nanoaggregates could have versatile application as a positive contrast agent at the currently used magnetic imaging field strengths and a negative contrast agent in higher field applications, while at the same time offering the possibility for the loading of hydrophobic therapeutics or targeting molecules.

KEYWORDS: high field MRI, gadolinium, bimodal contrast agents, optical imaging, micelle, nanoaggregates

INTRODUCTION

Over the previous three decades, clinical magnetic resonance imaging (MRI) has been under rapid development. As MRI intrinsically suffers from low sensitivity, contrast agents (CAs) are frequently used in order to improve the performance and quality of the image. Contrast enhancement occurs due to increasing the longitudinal (T_1) or the transverse (T_2) relaxation of water-proton spins, induced by the local presence of paramagnetic materials.¹ These materials acting as CAs influence both the longitudinal and transverse relaxation. If the ratio of induced relaxation times T_1/T_2 is close to 1, the CA is considered to be a T_1 agent, leading to a positive contrast. For T_2 agents, such as iron oxide nanoparticles, this ratio is typically around 6–8 but can reach much higher values.²

Positive contrast enhancement results in a brightening of the MR image, and negative results in a darkening, where the CA is locally influencing the relaxation. Enhancing contrast agent T_1 relaxation performance by maximizing the rotational correlation time (τ_R) and/or minimizing the residence time (τ_M) are well-reported strategies.³ Gadolinium(III) chelates are the most widely used contrast agents for MRI applications because of their remarkable enhancement of the longitudinal relaxation rate of water protons.¹ Increasing the rotational correlation time can be achieved by covalent attachment of the

Received: October 24, 2018

Accepted: January 14, 2019

Published: January 14, 2019

Scheme 1. Synthesis of Eu-DTPA-BC₁₀Coumarin^a

^a(i) Solvent free, YbOTf (5 mol %), 80 °C, 1 h. (ii) Dry DMF, DIPEA, HATU, overnight. (iii) H₂, Pd/C (5 wt %), overnight. (iv) Dry DMF, 50 °C, overnight. (v) Pyridine, 70 °C, 3 h. R = *n*-C₁₀H₂₁.

gadolinium(III) chelates to macromolecules such as linear polymers and dendrimers.^{4–7} Additionally, noncovalent interaction with the most abundant blood pool protein, human serum albumin, has been reported to increase relaxation performance.⁸ Supramolecular assembly can be employed to increase sensitivity by localizing a high concentration of gadolinium(III) ions into amphiphilic complexes assembled with phospholipids into micelles or liposomes.^{9–11} Size and structural properties of such nano-assemblies can be tuned using different phospholipids and surfactants. Unfortunately the theoretical maximum efficiency is yet to be achieved as the fast local motions of the gadolinium complexes typically accompanies the slow diffusion of the larger macromolecule.¹² A few examples to overcome this were reported by Fulton et al. which include Gd-glycoconjugate and Gd-metallosar systems of medium molar weight where efficient motional coupling occurs due to the gadolinium(III) ion lying at the barycenter of any reorientation motion.^{13,14}

Gadolinium(III) chelates are by far the most popular CAs in clinical use; however, their contrast enhancement is only suited for current MRI instrumentation operating at 60 MHz.¹ Efficiency of these CAs decreases significantly from 1 to 100 MHz, only reporting an r_1 of $\sim 3\text{--}4\text{ s}^{-1}\text{ mM}^{-1}$ at 60 MHz and 37 °C.¹ The search for higher spatial resolution and increased sensitivity has led to the development of high-field (3 T) and ultrahigh field MRI (>3 T) applications; however, clinical application is hindered by the increased cost and noise of MR imaging at high-fields and is mainly limited to preclinical applications. Increasing magnetic field would greatly improve the sensitivity of the MRI technique; however, this would render the currently used gadolinium(III) chelates obsolete, unless their contrasting properties can be repurposed or increased at high magnetic fields.¹⁵ Since iron oxide NPs have

fallen out of clinical favor in the past decade, various strategies for designing lanthanide complexes based on DTPA and DOTA ligands as T_2 agents have been examined.¹⁶ By increasing the rotational correlation time of lanthanide complexes via incorporation into micelles, a large increase of longitudinal or transverse relaxation of water protons has been observed.^{8–11,17–20} Interestingly, transverse relaxation enhancement has been rarely reported for gadolinium(III) complexes even when studying their incorporation into micelles and liposomes.^{12,21,22} Transverse relaxation properties have been reported by Richard et al. for gadolinium(III) complexes conjugated to carbon nanotubes and showed that transverse relaxivity in this case was independent of both frequency and gadolinium(III) concentration.²³ Consequently, the carbon nanotubes could be viewed as giant conjugated molecular wires that create strong magnetic inhomogeneity, and the transverse relaxivity was not related to the conjugated gadolinium(III) complexes.

Imaging bimodality has been gaining increasing attention recently as it combines MRI with other diagnostic techniques offering high sensitivity.^{24–27} Clinical application of PET/MRI has come to fruition; however, MRI/optical imaging is lagging behind due to limitations regarding the optical transparency of biological tissues.²⁸ Bimodal MRI/optical imaging has been used in small animal models because organs are in close proximity to the surface of the body.²⁹ In optical imaging, research is mainly focusing on the near-infrared (NIR) window (650 to 1350 nm) for emissive probes, as maximum depth penetration is observed in this region.³⁰ Nanoparticles (NPs) and molecular complexes have been reported as bimodal MRI/optical probes.^{17,18,31–33} Conjugation of iron oxide NPs to fluorescent probes has been reported as a strategy toward creating negative MRI CAs with additional optical proper-

Scheme 2. Synthesis of Gd-DOTA-BC₁₀Coumarin^a

^a(i) Chloroform, K₂CO₃, 50 °C, 3 h. (ii) Acetonitrile, K₂CO₃, reflux, 24 h. (iii) TFA/DCM (50:50), overnight. (iv) Pyridine, 70 °C, 3 h. R = *n*-C₁₀H₂₁.

ties.^{31–36} Further development has included upconverting/paramagnetic lanthanide NPs which can be excited with laser wavelengths in the NIR window and exhibit negative MRI contrast enhancement.^{37,38} Various organic probes have been conjugated to paramagnetic materials to investigate their usefulness in bimodal applications.^{39,40} Similarly, luminescent transition metals and lanthanides have been linked to DTPA or DOTA or have been aggregated into magnetofluorescent liposomes and nanoparticles.^{41–47} Organic fluorescent dyes are limited by very short fluorescence lifetimes (several ns at most), small Stokes' shifts, and poor resistance to photobleaching. On the other hand, lanthanide-based systems combining magnetic and optical properties do not suffer from these drawbacks and represent attractive alternative. Lanthanide luminescence is characterized by sharp emission lines, excited state lifetimes in the millisecond range, and very large pseudo-Stokes' shifts. Because of the low molar absorption coefficients of the *f*–*f* transitions, which are Laporte forbidden, a chromophore in close proximity (~5

Å) to the lanthanide serves as an “antenna” for the phenomenon known as sensitized lanthanide luminescence.

Terbium(III) and dysprosium(III) chelates acting as bimodal CAs for MRI and optical imaging have been reported by our group previously.^{9–11,19} These CAs exhibit *T*₂ contrast enhancement at high magnetic fields; however, these systems require a short excitation wavelength (<300 nm) for observing the luminescence signal, which restricts their biological applications.^{9–11,19} We recently reported that coumarin functionalized Eu(III)-DTPA optical probe shows a multiphoton excited emission under 800 nm excitation, making it appropriate for this application.²⁰ Incorporation of such optical probe into a supramolecular structure together with an MRI probe leads to the same biodistribution of the probes for both techniques, which is advantageous for biological investigations.²⁰ Moreover, we have shown that nanoaggregates based on self-assembled amphiphilic Gd-DOTA and Eu-DTPA bismamide complexes⁴⁸ remain stable in fetal bovine serum for at least 4 h. The shortcoming of this system is relatively

large size of aggregates (around 60 nm) which was presumably caused by the low phospholipid ratio used to form micelles. In this research, we demonstrate the potential of gadolinium(III) as a negative CA in ultrahigh field MRI by proposing a strategy for increasing the rotational correlation time and enhancing the transverse relaxivity. Furthermore, the facile incorporation of a targeting molecule and addition of an optical probe to visualize the nanoaggregates by fluorescence microscopy allows introduction of additional targeting and bimodal properties into the designed CA.

RESULTS AND DISCUSSION

Synthesis of Ligands, Complexes, and Micelle Formation.

Scheme 1 depicts the facile synthetic approach to the synthesis of the DTPA ligand using previously reported procedures.^{20,48–50} Complexation to europium(III) is performed in pyridine according to a modified procedure.¹¹ **Scheme 2** depicts the use of this functionalized amino-coumarin where chloroacetyl chloride was used as a link between the coumarin and DOTA. The symmetrically functionalized DOTA is synthesized as reported,^{9,11} and deprotection of the carboxylate groups was performed using TFA. Complexation was performed in pyridine due to the free ligands being poorly soluble in water. The absence of free lanthanide ions after complexation was verified using an arsenazo(III) indicator solution.⁵¹ The modified folic acid was synthesized according to our previously reported procedure, in which the 2 carboxylate groups were amidated with an alkyl amine and HATU as coupling agent.⁴⁸

The amphiphilic complexes and modified folic acid are effectively incorporated into mixed micelles of approximately 10 nm as determined by DLS (**Figure 1** and **2**). Micelles are formed using 1 equiv of each MRI and optical probe, 12 equiv of DPPC, at least 6.5 equiv of Tween 80, and for the targeted micelles, 0.5 equiv of modified folic acid. To achieve the small size of the micelles, the DPPC needs to be in excess with respect to the lanthanide chelates, and at least 50 mol % of surfactant should be used as described by Lim et al.⁵² In our previous study into the stability of nanoaggregates, to achieve intermolecular energy transfer, the DPPC ratio needed to be low with respect to the lanthanide chelates (3:1) to ensure efficient transfer, resulting in a larger size of 60 nm. The paramagnetic, luminescent, and targeting functionalities become an integral part of the supramolecular structure with decreased rotational motion, improving the relaxometric properties.

To ensure efficient europium(III) emission, the coumarin lanthanide sensitizer is incorporated on the europium(III) acceptor chelate to overcome the limitations on donor/acceptor proximity. Using the cyclic DOTA rather than acyclic DTPA to chelate the gadolinium(III) affords a higher kinetic stability. Additionally, a large increase in relaxation performance is observed due to the immobilization of the gadolinium(III) into the micellar membrane.²¹ The coumarin derivative used allows efficient sensitization of the closely coordinated europium(III) ion giving an intense red emission. The ligands and corresponding gadolinium(III) and europium(III) complexes have been characterized using mass spectrometry, nuclear magnetic resonance spectrometry, and Fourier transform infrared spectroscopy. Furthermore, micelle size has been determined by dynamic light scattering (**Figure 2**).

Photophysical Properties. Upon excitation of the coumarin-based ligand at 360 nm, characteristic red europium-

Figure 1. Schematic representation of the mixed micelles. DPPC (white) and surfactant Tween 80 is omitted.

Figure 2. Dynamic light scattering profiles. Micelles without folate-receptor targeting (black), micelles with folate-receptor targeting (red) (water, 1 wt %, 298 K).

(III) luminescence is exhibited due to the $^5D_0 \rightarrow ^7F_J$ ($J = 0-4$) transitions, and at 450 nm, there is fluorescence emission from the singlet excited state of the organic sensitizer and folate (Figure 3). A time-gated phosphorescence emission spectral measurement of the folate-receptor-targeted micelles removed the coumarin and folate emission, leaving only the europium(III) emission (Supporting Information, Figure S3). As discussed in our previous paper, poor energy transfer in micelles was observed when europium(III) was chelated to DOTA system, and therefore, the DTPA chelator has been

Figure 3. Normalized excitation and emission spectra of micelles without folate-receptor targeting (black) and with folate-receptor targeting (red). Excitation wavelength of 360 nm and excitation spectra taken while monitoring 615 nm (water, 10^{-4} M, 298 K). Cuvette showing red luminescence of micelles without folate added (inset).

Equations developed for cyclen⁵³ and aminocarboxylate⁵⁴ derivatives allow the calculation of the number of water molecules (q) with an error of ± 0.2 – 0.3 for eq 1 and ± 0.1 for eq 2, respectively:

$$q_{\text{Eu}}(\text{H}_2\text{O}) = 1.2(\Delta k_{\text{obs}} - 0.25 - 1.2q^{\text{NH}} - 0.075q^{\text{CONH}}) \quad (1)$$

$$q_{\text{Eu}}(\text{H}_2\text{O}) = 1.11(\Delta k_{\text{obs}} - 0.31 - 0.44q^{\text{OH}} - 0.99q^{\text{NH}} - 0.075q^{\text{CONH}}) \quad (2)$$

Δk_{obs} represents $\Delta k_{\text{H}_2\text{O}}(1/\tau_{\text{H}_2\text{O}}) - \Delta k_{\text{D}_2\text{O}}(1/\tau_{\text{D}_2\text{O}})$ and q^{x} represents the number of OH, NH, or CONH groups coordinated to the lanthanide(III) ion. In this europium(III) complex, only amide groups are considered, $q^{\text{CONH}} = 2$. q_{Eu} values of 0.3–0.5. Despite the expectation that an 8-fold coordinated europium(III) complex should have 1 coordinated water molecule occupying the ninth coordination site, finding a lower value than 1 has been observed in micellar systems where the hard-donor atoms of surfactant Tween 80 compete for lanthanide coordination, and this result fits with previous similar studies.^{9–11,19,21} The quantum yields of 2 and 1.1% for nontargeted and targeted micelles respectively is in the range which is commonly expected for europium(III) complexes. The lower number of water observed in the targeted micelles can be attributed to the addition of folate which also absorbs in the same region as the coumarin derivative but does not transfer energy to the metal center, rather to the changes in the first coordination sphere of europium(III).

Relaxometric Studies. Proton NMRD profiles represent the effect of a 1 mM solution of gadolinium(III) contrast agent on the shortening of the longitudinal (T_1) or transverse (T_2) relaxation times. This is achieved by measuring the (r_1) or (r_2) relaxivity while changing the magnetic field strength. Enhancement of the relaxation rate occurs through dipolar interactions between the gadolinium(III) ion and closely located water molecules exchanging with bulk water. Interactions occur between directly coordinated water molecules, inner-sphere, and longer distance, second-sphere and outer-sphere. Inner-

Table 1. Photophysical Data for the Micelles (0.1 wt % in Water) Recorded at 298 K

	$\tau_{\text{H}_2\text{O}}$ [ms] ^a	$\tau_{\text{D}_2\text{O}}$ [ms] ^a	$q_{\text{H}_2\text{O}}$	$Q_{\text{Eu}}^{\text{Ln}}$ [%]
nontargeted micelles	0.71	1.56	0.44 ^b /0.34 ^c	2.0
targeted micelles	0.73	1.68	0.46 ^b /0.36 ^c	1.1

^aAverage of 3 measurements that vary by no more than 0.01 ms. ^beq 1. ^ceq 2.

Figure 4. Proton longitudinal and transverse NMRD profiles of micelles without folate (black) and with folate (red) at 310 K. Triangles represent r_1 and squares represent r_2 . The plain lines represent the fitted data with τ_{R} equal to 1.9 and 2.8 ns while the dotted lines represent the fitted data with τ_{R} equal to 3 ns.

sphere (IS) interactions are defined by the water coordination number of the first coordination shell (q), (r) is the distance between the paramagnetic center and the water proton nuclei, the water residence time is (τ_M), and (τ_R) is the rotational correlation time of the gadolinium(III) center, (τ_{S0}) is the electronic relaxation of gadolinium(III) at zero field, correlation time related to the electronic relaxation (τ_V). Outer-sphere (OS) interactions include the distance of closest approach (d) and the relative diffusion coefficient (D) of water molecules close to the ion. Second-sphere (SS) includes three additional parameters: the number of water molecules which make up the second hydration sphere (q_{SS}), the distance between these water protons and the paramagnetic center (r_{SS}) and the correlation time influencing this interaction (τ_{SS}).

The proton NMRD profiles of the micelles have been fitted considering IS, SS, OS contributions (Figure 4) (Solomon–Bloembergen–Morgan theory).^{55–57} The parameters r , d , and r_{SS} are fixed to 0.31, 0.36, and 0.36 nm, respectively; D is set to $3.0 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$; and τ_M is adjusted to 500 ns consistent with other Gd-DOTA bisamide complexes.^{8,58–60} For fitting, the hydration number q has been fixed to 1 which corresponds to the ninth coordination position being vacant for a single water molecule and q_{SS} is allowed to vary between 0 and 10. The NMRD profiles of the two micellar systems show characteristic maxima around 40 MHz, which confirms the aggregation into supramolecular objects. For both micelle samples, the profiles are very similar which results in comparable parameters obtained by theoretical fitting (Table 2). The maximum

Table 2. Values of the Parameters Obtained by IS/SS/OS Theoretical Fitting of the ^1H NMRD Profiles of the Micelles in Water at 37 °C^a

	nontargeted	targeted
τ_R (ns)	1.9 ± 0.1 (3)	2.8 ± 0.3 (3)
q	1	1
q_{SS}	7.3 ± 0.6 (9.7 ± 0.6)	6.12 ± 0.4 (6.4 ± 0.5)
τ_{SS} (ps)	49.3 ± 4.3 (40.5 ± 3.0)	54.4 ± 2.6 (53.5 ± 4.6)
τ_{S0} (ps)	121 ± 5 (99 ± 3)	118 ± 4 (114 ± 4)
τ_V (ps)	28.1 ± 0.9 (23.7 ± 0.5)	29.2 ± 0.8 (28.7 ± 0.6)

^aValues outside parentheses is when τ_R is variable, in parentheses is when τ_R is fixed to 3 ns.

relaxivity, r_1 , observed at 60 MHz, 310 K is 25.9 and $26.2 \text{ s}^{-1} \text{ mM}^{-1}$ for nontargeted or targeted micelles, respectively. τ_R values of 1.9 and 2.8 ns (nontargeted or targeted respectively) are lower when comparing with previously reported values for larger micelles of 33 – 40 nm which is good agreement for the smaller sized micelles. It is to be pointed out that good quality fittings are also obtained with higher values of τ_R ($\tau_R = 3 \text{ ns}$) as shown in Figure 4 and Table 2. The fitting also indicates that more than six water molecules are present in the second coordination sphere which is contributing to the relaxation. The r_2 relaxation values of micelles at 20 , 60 , 300 , and 500 MHz are given in Figure 4. The transverse relaxation efficiency r_2 is close to r_1 values at 20 MHz (less than $30 \text{ s}^{-1} \text{ mM}^{-1}$) and increases to values larger than $45 \text{ s}^{-1} \text{ mM}^{-1}$ at high fields (300 and 500 MHz). This is typical of slowly tumbling gadolinium(III) complexes and makes these micelles efficient T_2 contrast agents at high magnetic fields. It is notable that MRI instruments are progressing to higher magnetic field strengths to improve imaging resolution, and these results position this contrast agent system for versatile usage. Reporting the

transverse relaxation enhancement, particularly the r_2/r_1 (Figure 5), show that these formulations perform similarly as

Figure 5. Ratio of proton transverse versus longitudinal relaxivity at 310 K.

negative contrast agents at higher field and positive contrast agents at lower field. A value of ~ 6 at 300 and 500 MHz observed for r_2/r_1 is comparable to iron oxide NPs.²

HeLa Cell Culture Imaging and Viability. For fluorescence microscopy, excitation in the tail region of the coumarin at 405 nm and emission at 575 – 675 nm was used for visualization in cultured HeLa cells (Figure 6). The uptake of folate-targeted and nontargeted micelles is already visible after 5 h incubation (Figure 6, A' and B'). After overnight incubation, the micelles are still present in the cells (Figure 6, C' and D'). In both cases of targeting and nontargeting, the europium(III) emission is clearly observable in the cytoplasm of the cells, particularly located in lipid droplets or endosomes, which are a result of the endocytosis of the micelles. However, there is no significant difference observable between the targeted and nontargeted micelles.

We then evaluated the viability of HeLa cells in the presence of folate-targeted and nontargeted micelles in a range of concentrations. The evaluation is done with an MTT assay, on the basis of perturbation of mitochondrial redox activity (Figure 7). Cell proliferation was completely inhibited in the presence of $50 \mu\text{M}$ folate-targeted micelles, while cells incubated with same concentration of nontargeted micelles only show poor survival. The cytotoxicity is likely a result of the Tween 80 used to support the micelles, which is commonly used for lysing cells by interaction with the cellular bilayer. Overall, cells treated with nonfolate micelles show a higher proliferation trend compared to samples with folate receptor targeting (Figure 7). It is important to note that in this proof-of-concept study, the cytotoxicity has not been optimized and modifying the surfactant concentration or type, would show improved cellular survival.

CONCLUSIONS

This work reports on mixed micelles based on amphiphilic DOTA and DTPA complexes with gadolinium(III) and europium(III), respectively, that were synthesized and evaluated for their paramagnetic and optical properties. Sharp europium(III) emission lines are observed in the visible region upon 360 nm excitation. Fluorescence microscopy shows an intense signal coming from significant nanoaggregate

Figure 6. DIC images (gray) and fluorescence images of the micelles (magenta). Folate targeted micelles (A) and nontargeted (B) with 5 h incubation, folate targeted (C) and nontargeted (D) with 15 h incubation after excitation with a 405 nm laser diode measured with confocal laser scanning microscopy; scale bars indicate 10 μm ; concentration was 10 μM .

Figure 7. Normalized optical density for the MTT assay after addition of micelles and 15 h incubation. Concentrations equal 1, 1.7, 3, 5.5, 10, 16.5, 28.5, and 50 μM for both folate receptor targeted (red) and nontargeted micelles (black). The O.D. is normalized over control cells (without micelles) minus the O.D. of media.

micelles when loaded with hydrophobic therapeutics, like doxorubicin, and the system being used as a theranostic agent.

EXPERIMENTAL SECTION

Chemicals. Reagents and solvents were obtained from the following sources: Acros Organics (Geel, Belgium), Matrix Scientific (Columbia, U.S.A.), ChemLab (Zedelgem, Belgium), Sigma–Aldrich (Bornem, Belgium), and BDH Prolabo (Leuven, Belgium). All chemicals were used without further purification.

Instrumentation and Methods. Bruker Avance 300 and 400 spectrometers (Bruker, Karlsruhe, Germany), operating at 300 or 400 MHz were used for recording ^1H and ^{13}C NMR spectra. Thermo Finnigan LCQ Advantage mass spectrometer was used for obtaining mass spectra, following the same procedure as previously reported.²⁰ TXRF measurements were performed with Bruker S2 Picofox (Bruker, Berlin, Germany) by applying similar procedure as recently reported.²⁰ Absorption spectra, emission spectra and luminescence decays of Ln^{III} micellar complexes were recorded using same instrumentations and procedures as previously reported.²⁰

NMRD Profiles. Stellar Spinmaster FFC, a fast field cycling NMR relaxometer (Stellar, Mede (PV), Italy) was used to measure proton nuclear magnetic relaxation dispersion (NMRD) profiles over a magnetic field strength range extending from 0.24 mT to 0.7 T. Measurements were performed using 0.5 mL of samples in 10 mm o.d. pyrex tubes. Additional relaxation rates at 20, 60, 300, and 500 MHz were obtained by using Minispec mq20, a Minispec mq60, and Bruker Avance 300 and 500 spectrometers (Bruker, Karlsruhe, Germany), respectively. All experiments were performed at 310 K.

DLS Measurements. DLS measurements were performed on a BIC multiangle laser light scattering system with a 90° scattering angle (Brookhaven Instruments Corporation, Holtsville, U.S.A.) by using a similar procedure as recently reported.²⁰

Synthesis and Characterization of Ligands and Complexes. Folate derivative (S)-2-(4-(((2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl)amino)benzamido)-N1,N5-didodecylpentanediamide was synthesized as previously reported.⁴⁸ DTPA-bisanhydride was synthesized according to previous procedure.⁶¹ DOTA-O^tBu-bisamide (di-tert-butyl 2,2'-(1,4,7,10-tetraazacyclododecane-1,7-diyl)-diacetate) was synthesized using previously reported method.¹¹ DTPA-BC₁₀Coumarinamide, and DOTA-BC₁₀Coumarinamide were synthesized as previously reported.²⁰ Lanthanide(III) complexes were synthesized as reported previously.⁹

Gd(III)-DOTA-BC₁₀Coumarinamide. Yield: 74%; IR: $\tilde{\nu}_{\text{max}}$ = 1609 (COO[−] asym. stretch), 1508 (amide II), 1396 cm^{−1} (COOsym. stretch); ESI-MS (−ve mode): m/z : calcd 1211.5 [M-H][−], 1247.5 [M + Cl][−], found 1209.8 [M-H][−], 1245.5 [M + Cl][−]; HRMS (+ve mode): calcd 1213.5043 [M]⁺, found 1212.5056 [M]⁺. The complex decomposes above 300 °C.

Preparation of Micelles. A previously reported method from Harris et al. was used to prepare micelles.⁹

Confocal Imaging and MTT Assay in HeLa Cells. HeLa cells were maintained in Dulbecco's modified Eagle's medium without phenol red (Life Technologies, Carlsbad, CA, U.S.A.) and supplemented with 10% fetal bovine serum (Life Technologies) at 37 °C under a humidified 5% CO₂ atmosphere. For imaging, cells were seeded in 29 mm glass-bottom dishes with a 14 mm microwell #1.5 cover glass (CellVis, Mountain View, CA, U.S.A.), at density $\sim 10^4$ cells per dish. Micelles of 10 μM concentration were added after overnight incubation and imaged after 5 h or the next day. Cells were washed twice and imaged with Hank's Balanced Salt Solution (HBSS, Life Technologies) at room temperature. Imaging was performed on inverted laser scanning microscope FV1000 (Olympus, Tokyo, Japan) with objective lens UPlanSapo60xO (NA 1.35). A 405 nm laser diode was used for excitation. A DM 405/488 was selected as the main dichroic mirror and emission was guided into a grating channel set to 575–675 nm.

For the MTT assay (Vybrant MTT Cell Proliferation Assay Kit, ThermoFisher Scientific, Waltham, MA U.S.A.), HeLa cells were seeded 48–72 h prior to measurement in 96-well plates. Targeted and nontargeted micelles were added the next day after seeding at concentrations 1, 1.7, 3, 5.5, 10, 16.5, 28.5, and 50 μM . The MTT assay was performed according to the manufacturer's instructions. The absorbance from each well was read by a plate reader (Safire,² Tecan Group, Männedorf, Switzerland) at 570 nm.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b18456.

ESI-MS, HRMS, and phosphorescence emission spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: tatjana.vogt@kuleuven.be.

ORCID

Tatjana N. Parac-Vogt: 0000-0002-6188-3957

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

The M.H. would like to acknowledge financial support from the Research Foundation-Flanders (FWO Flanders, Belgium) for a doctoral fellowship. D.L. would like to acknowledge KU Leuven Facultaire Luik Onderzoeksfonds (FLOF) for financial support.

Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Wahsner, J.; Gale, E. M.; Rodriguez-Rodriguez, A.; Caravan, P. Chemistry of MRI Contrast Agents: Current Challenges and New Frontiers. *Chem. Rev.* **2018**, DOI: 10.1021/acs.chemrev.8b00363.
- (2) Qin, J.; Laurent, S.; Jo, Y. S.; Roch, A.; Mikhaylova, M.; Bhujwalla, Z. M.; Muller, R. N.; Muhammed, M. A High-Performance

Magnetic Resonance Imaging T_2 Contrast Agent. *Adv. Mater.* **2007**, 19 (14), 1874–1878.

(3) Helm, L. Optimization of Gadolinium-Based MRI Contrast Agents for High Magnetic-Field Applications. *Future Med. Chem.* **2010**, 2 (3), 385–396.

(4) Villaraza, A. J. L.; Bumb, A.; Brechbiel, M. W. Macromolecules, Dendrimers, and Nanomaterials in Magnetic Resonance Imaging: The Interplay Between Size, Function, and Pharmacokinetics. *Chem. Rev.* **2010**, 110, 2921–2959.

(5) Lebedušková, P.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Lukeš, I.; Peters, J. A. A Gadolinium (III) Complex of a Carboxylic-Phosphorus Acid Derivative of Diethylenetriamine Covalently Bound to Inulin, a Potential Macromolecular MRI Contrast Agent. *Bioconjugate Chem.* **2004**, 15, 881–889.

(6) Huang, C.-H.; Nwe, K.; Al Zaki, A.; Brechbiel, M. W.; Tsourkas, A. Biodegradable Polydisulfide Dendrimer Nanoclusters as MRI Contrast Agents. *ACS Nano* **2012**, 6, 9416–9424.

(7) Li, Y.; Beija, M.; Laurent, S.; Elst, L. V.; Muller, R. N.; Duong, H. T. T.; Lowe, A. B.; Davis, T. P.; Boyer, C. Macromolecular Ligands for Gadolinium MRI Contrast Agents. *Macromolecules* **2012**, 45, 4196–4204.

(8) Debroye, E.; Eliseeva, S. V.; Laurent, S.; Vander Elst, L.; Petoud, S.; Muller, R. N.; Parac-Vogt, T. N. Lanthanide(III) Complexes of Diethylenetriaminepentaacetic Acid (DTPA)–Bisamide Derivatives as Potential Agents for Bimodal (Optical/Magnetic Resonance) Imaging. *Eur. J. Inorg. Chem.* **2013**, 2013, 2629–2639.

(9) Harris, M.; Carron, S.; Vander Elst, L.; Laurent, S.; Muller, R. N.; Parac-Vogt, T. N. Magnetofluorescent Micellar Complexes of Terbium (III) as Potential Bimodal Contrast Agents for Magnetic Resonance and Optical Imaging. *Chem. Commun.* **2015**, 51, 2984–2986.

(10) Harris, M.; Carron, S.; Vander Elst, L.; Laurent, S.; Parac-Vogt, T. N. Magnetofluorescent Nanoaggregates Incorporating Terbium (III) Complexes as Potential Bimodal Agents for Magnetic Resonance and Optical Imaging. *Eur. J. Inorg. Chem.* **2015**, 2015, 4572–4578.

(11) Harris, M.; Vander Elst, L.; Laurent, S.; Parac-Vogt, T. N. Magnetofluorescent Micelles Incorporating Dy III–DOTA as Potential Bimodal Agents for Optical and High Field Magnetic Resonance Imaging. *Dalton Trans* **2016**, 45, 4791–4801.

(12) Accardo, A.; Tesauro, D.; Aloj, L.; Pedone, C.; Morelli, G. Supramolecular Aggregates Containing Lipophilic Gd (III) Complexes as Contrast Agents in MRI. *Coord. Chem. Rev.* **2009**, 253, 2193–2213.

(13) Fulton, D. A.; Elemento, E. M.; Aime, S.; Chaabane, L.; Botta, M.; Parker, D. Glycoconjugates of gadolinium complexes for MRI applications. *Chem. Commun.* **2006**, 0, 1064–1066.

(14) Fulton, D. A.; O'Halloran, M.; Parker, D.; Senanayake, K.; Botta, M.; Aime, S. Efficient relaxivity enhancement in dendritic gadolinium complexes: effective motional coupling in medium molecular weight conjugates. *Chem. Commun.* **2005**, 0, 474–476.

(15) Caravan, P. Strategies for Increasing the Sensitivity of Gadolinium Based MRI Contrast Agents. *Chem. Soc. Rev.* **2006**, 35, 512–523.

(16) Aime, S.; Castelli, D. D.; Crich, S. G.; Gianolio, E.; Terreno, E. Pushing the Sensitivity Envelope of Lanthanide-Based Magnetic Resonance Imaging (MRI) Contrast Agents for Molecular Imaging Applications. *Acc. Chem. Res.* **2009**, 42 (7), 822–831.

(17) Debroye, E.; Dehaen, G.; Eliseeva, S. V.; Laurent, S.; Vander Elst, L.; Muller, R. N.; Binnemans, K.; Parac-Vogt, T. N. A New Metallostar Complex Based on an Aluminum (III) 8-Hydroxyquinoline Core as a Potential Bimodal Contrast Agent. *Dalton Trans* **2012**, 41, 10549–10556.

(18) Dehaen, G.; Eliseeva, S. V.; Kimpe, K.; Laurent, S.; Vander Elst, L.; Muller, R. N.; Dehaen, W.; Binnemans, K.; Parac-Vogt, T. N. A Self-Assembled Complex with a Titanium (IV) Catecholate Core as a Potential Bimodal Contrast Agent. *Chem. - Eur. J.* **2012**, 18, 293–302.

(19) Debroye, E.; Laurent, S.; Vander Elst, L.; Muller, R. N.; Parac-Vogt, T. N. Dysprosium Complexes and Their Micelles as Potential

- 558 Bimodal Agents for Magnetic Resonance and Optical Imaging. *Chem.*
559 - *Eur. J.* **2013**, *19*, 16019–16028.
- 560 (20) Harris, M.; Henoumont, C.; Peeters, W.; Toyouchi, S.; Vander
561 Elst, L.; Parac-Vogt, T. N. Amphiphilic Complexes of Ho (iii), Dy
562 (iii), Tb (iii) and Eu (iii) for Optical and High Field Magnetic
563 Resonance Imaging. *Dalton Trans* **2018**, *47*, 10646–10653.
- 564 (21) Debroye, E.; Eliseeva, S. V.; Laurent, S.; Vander Elst, L.; Muller,
565 R. N.; Parac-Vogt, T. N. Micellar Self-Assemblies of Gadolinium
566 (III)/Europium (III) Amphiphilic Complexes as Model Contrast
567 Agents for Bimodal Imaging. *Dalton Trans* **2014**, *43*, 3589–3600.
- 568 (22) Negri, R.; Carniato, F.; Botta, M.; Giovanzana, G. B.; Tei, L.
569 Synthesis of an Amphiphilic Bis-Aqua Gd (OBETA) Complex for the
570 Preparation of High-Relaxivity Supramolecular Magnetic Resonance
571 Imaging Probes. *ChemPlusChem* **2016**, *81* (2), 235–241.
- 572 (23) Richard, C.; Doan, B.-T.; Beloeil, J.-C.; Bessodes, M.; Tóth, É.;
573 Scherman, D. Noncovalent Functionalization of Carbon Nanotubes
574 with Amphiphilic Gd³⁺Chelates: Toward Powerful T₁ and T₂ MRI
575 Contrast Agents. *Nano Lett.* **2008**, *8* (1), 232–236.
- 576 (24) Debroye, E.; Parac-Vogt, T. N. Towards Polymetallic
577 Lanthanide Complexes as Dual Contrast Agents for Magnetic
578 Resonance and Optical Imaging. *Chem. Soc. Rev.* **2014**, *43*, 8178–
579 8192.
- 580 (25) Louie, A. Multimodality Imaging Probes: Design and
581 Challenges. *Chem. Rev.* **2010**, *110*, 3146–3195.
- 582 (26) Frullano, L.; Meade, T. J. Multimodal MRI Contrast Agents.
583 *JBIC, J. Biol. Inorg. Chem.* **2007**, *12*, 939–949.
- 584 (27) Jančzewski, D.; Zhang, Y.; Das, G. K.; Yi, D. K.; Padmanabhan,
585 P.; Bhakoo, K. K.; Tan, T. T. Y.; Selvan, S. T. Bimodal Magnetic–
586 Fluorescent Probes for Bioimaging. *Microsc. Res. Tech.* **2011**, *74*, 563–
587 576.
- 588 (28) Hebden, J. C.; Arridge, S. R.; Delpy, D. T. Optical imaging in
589 Medicine: I. Experimental Techniques. *Phys. Med. Biol.* **1997**, *42* (5),
590 825–840.
- 591 (29) Balaban, R. S.; Hampshire, V. A. Challenges in Small Animal
592 Noninvasive Imaging. *ILAR J.* **2001**, *42* (3), 248–262.
- 593 (30) Bünzli, J.-C. G. Lanthanide Light for Biology and Medical
594 Diagnosis. *J. Lumin.* **2016**, *170* (Part 3), 866–878.
- 595 (31) Carron, S.; Bloemen, M.; Vander Elst, L.; Laurent, S.; Verbiest,
596 T.; Parac-Vogt, T. N. Ultrasmall Superparamagnetic Iron Oxide
597 Nanoparticles with Europium (III) DO3A as a Bimodal Imaging
598 Probe. *Chem. - Eur. J.* **2016**, *22*, 4521–4527.
- 599 (32) Kandasamy, G.; Surendran, S.; Chakrabarty, A.; Kale, S. N.;
600 Maity, D. Facile Synthesis of Novel Hydrophilic and Carboxyl-Amine
601 Functionalized Superparamagnetic Iron Oxide Nanoparticles for
602 Biomedical Applications. *RSC Adv.* **2016**, *6*, 99948.
- 603 (33) Moorthy, M. S.; Oh, Y.; Bharathiraja, S.; Manivasagan, P.;
604 Rajarathinam, T.; Jang, B.; Vy Phan, T. T.; Jang, H.; Oh, J. Synthesis
605 of Amine-Polyglycidol Functionalised Fe₃O₄@SiO₂ Nanocomposites
606 for Magnetic Hyperthermia, pH-Responsive Drug Delivery, and
607 Bioimaging Applications. *RSC Adv.* **2016**, *6*, 110444–110453.
- 608 (34) Wang, Q.; Zhang, C.; Guo, F.; Li, Z.; Liu, Y.; Su, Z. Novel
609 Bioconjugation Strategy Using Elevated Hydrostatic Pressure: A Case
610 Study for the Site-Specific Attachment of Polyethylene Glycol
611 (PEGylation) of Recombinant Human Ciliary Neurotrophic Factor.
612 *Bioconjugate Chem.* **2017**, *28*, 2841–2848.
- 613 (35) Cano, M.; Núñez-Lozano, R.; Lumberras, R.; González-
614 Rodríguez, V.; Delgado-García, A.; Jiménez-Hoyuela, J. M.; de la
615 Cueva-Méndez, G. Partial PEGylation of Superparamagnetic Iron
616 Oxide Nanoparticles Thinly Coated with Amine-Silane as a Source of
617 Ultrastable Tunable Nanosystems for Biomedical Applications.
618 *Nanoscale* **2017**, *9* (812), 812.
- 619 (36) Gautam, A.; van Veggel, F. C. J. M. Synthesis of Nanoparticles,
620 Their Biocompatibility, and Toxicity Behavior for Biomedical
621 Applications. *J. Mater. Chem. B* **2013**, *1*, 5186.
- 622 (37) Carron, S.; Li, Q. Y.; Vander Elst, L.; Muller, R. N.; Parac-Vogt,
623 T. N.; Capobianco, J. A. Assembly of Near Infra-Red Emitting
624 Upconverting Nanoparticles and Multiple Gd (III)-Chelates as a
625 Potential Bimodal Contrast aAgent for MRI and Optical Imaging.
626 *Dalton Trans* **2015**, *44*, 11331.
- (38) Biju, S.; Harris, M.; Elst, L. V.; Wolberg, M.; Kirschhock, C.;
Parac-Vogt, T. N. Multifunctional β -NaGdF₄:Ln³⁺ (Ln = Yb, Er, Dy)
Nanoparticles with NIR to Visible Upconversion and High Transverse
Relaxivity: a Potential Bimodal Contrast aAgent for High-Field MRI
and Optical Imaging. *RSC Adv.* **2016**, *6* (66), 61443–61448.
- (39) Bernhard, C.; Goze, C.; Rousselin, Y.; Denat, F. First bodypy–
DOTA Derivatives as Probes for Bimodal Imaging. *Chem. Commun.*
2009, *46*, 8267–8269.
- (40) Keliris, A.; Ziegler, T.; Mishra, R.; Pohmann, R.; Sauer, M. G.;
Ugurbil, K.; Engelmann, J. Synthesis and Characterization of a Cell-
Permeable Bimodal Contrast Agent Targeting β -Galactosidase. *Bioorg.*
Med. Chem. **2011**, *19*, 2529–2540.
- (41) Kamaly, N.; Kalber, T.; Kenny, G.; Bell, J.; Jorgensen, M.;
Miller, A. A Novel Bimodal Lipidic Contrast Agent for Cellular
Labelling and Tumour MRI. *Org. Biomol. Chem.* **2010**, *8*, 201–211.
- (42) Soenen, S. J.; Velde, G. V.; Ketkar-Atre, A.; Himmelreich, U.;
De Cuyper, M. Magnetoliposomes as Magnetic Resonance Imaging
Contrast Agents. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*
2011, *3*, 197–211.
- (43) Rieter, W. J.; Kim, J. S.; Taylor, K. M. L.; An, H.; Lin, W.;
Tarrant, T.; Lin, W. Hybrid Silica Nanoparticles for Multimodal
Imaging. *Angew. Chem., Int. Ed.* **2007**, *46*, 3680–3682.
- (44) Howes, P.; Green, M.; Bowers, A.; Parker, D.; Varma, G.;
Kallumadil, M.; Hughes, M.; Warley, A.; Brain, A.; Botnar, R.
Magnetic Conjugated Polymer Nanoparticles as Bimodal Imaging
Agents. *J. Am. Chem. Soc.* **2010**, *132*, 9833–9842.
- (45) Ronchi, S.; Colombo, M.; Verderio, P.; Mazzucchelli, S.; Corsi,
F.; De Palma, C.; Allevi, R.; Clementi, E.; Prosperi, D.; Borsella, E.
Magnetofluorescent Nanoparticles for Bimodal Detection of Breast
Cancer Cells. *AIP Conf. Proc.* **2010**, *1275*, 102–105.
- (46) Bhattacharya, D.; Das, M.; Mishra, D.; Banerjee, I.; Sahu, S. K.;
Maiti, T. K.; Pramanik, P. Folate Receptor Targeted, Carboxymethyl
Chitosan Functionalized Iron Oxide Nanoparticles: A Novel Ultra-
dispersed Nanoconjugates for Bimodal Imaging. *Nanoscale* **2011**, *3*,
1653–1662.
- (47) Chen, Y.; Chen, H.; Zhang, S.; Chen, F.; Zhang, L.; Zhang, J.;
Zhu, M.; Wu, H.; Guo, L.; Feng, J.; Shi, J. Multifunctional
Mesoporous Nanoellipsoids for Biological Bimodal Imaging and
Magnetically Targeted Delivery of Anticancer Drugs. *Adv. Funct.*
Mater. **2011**, *21*, 270–278.
- (48) Harris, M.; De Keersmaecker, H.; Vander Elst, L.; Debroye, E.;
Fujita, Y.; Mizuno, H.; Parac-Vogt, T. N. Following the Stability of
Amphiphilic Nanoaggregates by Using Intermolecular Energy Trans-
fer. *Chem. Commun.* **2016**, *52*, 13385.
- (49) Karami, B.; Farahi, M.; Khodabakhshi, S. Rapid Synthesis of
Novel and Known Coumarin-3-carboxylic Acids Using Stannous
Chloride Dihydrate under Solvent-Free Conditions. *Helv. Chim. Acta*
2012, *95*, 455–460.
- (50) Fiorito, S.; Genovese, S.; Taddeo, V. A.; Epifano, F. Microwave-
assisted Synthesis of Coumarin-3-carboxylic Acids Under Ytterbium
Triflate Catalysis. *Tetrahedron Lett.* **2015**, *56*, 2434–2436.
- (51) Onishi, H.; Sekine, K. Spectrophotometric Determination of
Zirconium, Uranium, Thorium and Rare Earths with Arsenazo III
After Extractions with Thenoyltrifluoroacetone and Tri-n-octylamine.
Talanta **1972**, *19*, 473–478.
- (52) Lim, W. H.; Lawrence, M. Aggregation Behaviour of Mixtures
of Phosphatidylcholine and Polyoxyethylene Sorbitan Monoesters in
Aqueous Solution. *Phys. Chem. Chem. Phys.* **2004**, *6*, 1380–1387.
- (53) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker,
D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. Non-
Radiative Deactivation of the Excited States of Europium, Terbium
and Ytterbium Complexes by Proximate Energy-Matched OH, NH
and CH Oscillators: An Improved Luminescence Method for
Establishing Solution hHydration States. *J. Chem. Soc., Perkin Trans.*
2 1999, *2*, 493–504.
- (54) Supkowski, R. M.; Horrocks, W. D., Jr. On the Determination
of the Number of Water Molecules, q, Coordinated to Europium (III)
Ions in Solution From Luminescence Decay Lifetimes. *Inorg. Chim.*
Acta **2002**, *340*, 44–48.

- 696 (55) Solomon, I. Relaxation processes in a system of two spins. *Phys.*
697 *Rev.* **1955**, 99, 559–565.
- 698 (56) Bloembergen, N. Proton Relaxation Times in Paramagnetic
699 Solutions. *J. Chem. Phys.* **1957**, 27, 572–573.
- 700 (57) Freed, J. H. Dynamic Effects of Pair Correlation Functions on
701 Spin Relaxation by Translational Diffusion in Liquids. II. Finite Jumps
702 and Independent T_1 Processes. *J. Chem. Phys.* **1978**, 68, 4034–4037.
- 703 (58) Dehaen, G.; Eliseeva, S. V.; Verwilt, P.; Laurent, S.; Vander
704 Elst, L.; Muller, R. N.; Deborggraeve, W.; Binnemans, K.; Parac-Vogt,
705 T. N. Tetranuclear d^f Metallostars: Synthesis, Relaxometric, and
706 Luminescent Properties. *Inorg. Chem.* **2012**, 51, 8775–8783.
- 707 (59) Laurent, S.; Vander Elst, L.; Botteman, F.; Muller, R. An
708 Assessment of the Potential Relationship Between the Charge of Gd–
709 DTPA Complexes and the Exchange Rate of the Water Coordinated
710 to the Metal. *Eur. J. Inorg. Chem.* **2008**, 2008, 4369–4379.
- 711 (60) Vander Elst, L.; Sessoye, A.; Laurent, S.; Muller, R. N. Can the
712 Theoretical Fitting of the Proton-Nuclear-Magnetic-Relaxation-
713 Dispersion (Proton NMRD) Curves of Paramagnetic Complexes Be
714 Improved by Independent Measurement of Their Self-Diffusion
715 Coefficients? *Helv. Chim. Acta* **2005**, 88, 574–587.
- 716 (61) Montembault, V.; Soutif, J.-C.; Brosse, J.-C. Synthesis of
717 Chelating Molecules as Agents for Magnetic Resonance Imaging, 3¹.
718 Polycondensation of Diethylenetriaminepentaacetic Acid Bisanhy-
719 dride with Diols and Diamines. *React. Funct. Polym.* **1996**, 29, 29–39.