RESEARCH ARTICLE

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Efficient Synthesis of Macromolecular DO3A@Gn Derivatives for Potential Application in MRI Diagnostics: From Polymer Conjugates to Polymer Nanoparticles

*Anne-Larissa Kampmann, Wolf Hiller, and Ralf Weberskirch**

Herein, the synthesis of three different macromolecular DO3A@Gn conjugates based on poly(2-oxazoline)s is presented. Therefore, poly(2-methyl-2-oxazoline) is synthesized by a ring-opening, cationic polymerization and the polymerization is terminated with DO3A(tBu)$_3$. The best results are obtained after 48 h at 120 °C with degree of termination of 86%. After deprotection of the DO3A ligand and complexation with Gn^{3+}, relaxivity as measured with a magnetic field strength of 9.4 T (400 MHz) reveals values for r_1 of up to 2.32 mM^{-1} s^{-1}. The concept is extended to a block copolymer based on 2-heptyl-2-oxazoline and 2-methyl-2-oxazoline that is again terminated with DO3A(tBu)$_3$ to form micelles with a size of 12.6 ± 0.7 nm after DO3A(tBu)$_3$ termination and deprotection of the 1,4,7,10-tetraazacyclododecane-*N*,*N*,*N*,*N*-tetraacetic acid ligand. After complexation with Gn^{3+}, relaxivity r_1 is 10.1 mM^{-1} s^{-1} as determined from the slope of the plot of $1/T_1$ against the gadolinium(III) concentration at 9.4 T. Finally, crosslinked nanoparticles are prepared from amphiphilic macro-monomers that form micelles in water and are crosslinked throughout the core in the presence of azoisobutyronitrile (AIBN). The nanoparticle is 32.9 ± 7.8 nm in size after Gn^{3+} complexation and reveals a relaxivity r_1 of 6.77 mM^{-1} s^{-1}.

1. Introduction

Magnetic resonance imaging (MRI) is one of the most important noninvasive clinical imaging techniques used for the early detection of diseases.[1–3] There are many different applications of the MRI technique due to the excellent spatial and temporal results, which include clinical neurology, cardiology, cancer, and soft tissue damage.[4,5] However, the sensitivity of MRI to differences in tissue types is relatively low, which makes early diagnosis and treatment planning as the primary goal of personalized medicine more challenging. To overcome this lack of sensitivity contrast agents are usually introduced to shorten the longitudinal (T_1) or transversal (T_2) relaxation times of surrounding water protons. The most important T_1-agents are based on chelating agents such as diethylenetriaminepentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecane-*N*,*N*,*N*,*N*-tetraacetic acid (DOTA). While these contrast agents are typically considered low molecular with molar masses of 600–700 Da, there has been considerable research in the past years to develop macromolecular gadolinium(III) chelates.

Different types of macromolecules have been developed over the past two decades as a carrier material for chelators such as DOTA or DTPA, which include linear homopolymers such polyethylene glycol[6] or polyaspartic acid conjugates,[7] but also dendrimers,[8] polymer micelles[9,10] or crosslinked nanoparticles.[11] Apart from enhancing relaxivity and thus improving image resolution polymeric contrast agents show longer circulation times in blood and are excluded from rapid blood pool clearance by the kidney when their size is above 10 nm and thus makes them applicable for intravascular contrast agents[12] to relative blood volume of tissues, relative blood flow, and endothelial permeability. Furthermore, for solid tumors macromolecular, nanostructured materials may accumulate in the tumor tissue due to the enhanced permeability and retention (EPR) effect related to the leaky vasculature.[13] Particle size has been found to be one of the most critical factors since it determines circulation time and blood clearance and the efficiency of tumor accumulation penetration and accumulation.[14,15] Although tumor accumulation of polymer nanoparticles revealed excellent results for 100 to 150 nm particles,[16] tumor penetration was more effective for smaller particles in the 30–50 nm range.[17,18] The majority of polymer micelles and nanoparticles use polyethylene glycol (PEG) as the water-soluble component. While PEGylation has shown to improve hydrophilicity, in vivo stability and pharmacokinetics, there is an ongoing search for PEG alternatives owing to some of the remaining limitations of PEG, e.g., concerns over antigenic and immunogenic properties and mechanical stability.[19,20] A potential alternative are

A.-L. Kampmann, W. Hiller, R. Weberskirch
Fakultät für Chemie und Chemische Biologie
Otto-Hahn Str. 6, TU Dortmund, Otto-Hahn Str. 6, D-44227 Dortmund, Germany
E-mail: ralf.weberskirch@tu-dortmund.de

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poly(2-alkyl-2-oxazoline)s, with alkyl = methyl or ethyl.[21] These polymers are known to be highly hydrophilic and display excellent biocompatibility and are now being investigated as therapeutics in a wide range of medical applications.[22,23] Interestingly, few reports have dealt with the synthesis of poly(2-oxazoline)s for diagnostic applications. One of the early examples from 1997 described the synthesis of a poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) terminally functionalized with DOTA chelator for radionuclide labeling with ^{111}Indium. These hydrophilic polymers were rapidly cleared from the blood pool, predominantly by glomerular filtration in the kidneys.[24] In a more recent study, NHS-Ester functionalized poyl(2-oxazoline) (POZ) copolymers were functionalized with chelators such as DTPA or DOTA and were labeled with radioactive ^{111}Indium or ^{68}Gallium to investigate the excretion pathway and potential accumulation of the polymers in Wistar rats. PET/CT measurements on ^{68}Gallium-labeled polymer revealed that the majority of the polymer was cleared within 10 min. No accumulation of the degradation products was observed in other organs than the kidneys.[25] Moreover, Mukai and co-workers used partly hydrolyzed poly(2-ethyl-oxazoline) to immobilize DOTA as chelator for ^{111}Indium and indocyanine green (ICG) as a dual imaging probe for single photon emission computed tomography and fluorescence imaging. The results show that these polymers were stable in vivo without dissociation of ^{111}In or ICG from POZ and accumulate in tumor tissue via the EPR effect.[26] An extension of the latter approach was recently reported by Hruby and co-workers where they described the synthesis of a polysaccharide, mannan, grafted with poly(2-methyl-2-oxazoline)s (POx, 100 g mol^{-1}) as a carrier for DOTA/Gn(III) and a fluorescence probe (IR800CW) for in vitro and in vivo by magnetic resonance and fluorescence imaging. The intrinsic targeting properties of the polysaccharide-graft-POx copolymer originate from the binding of the polysaccharide to the mannose receptors and therefore responsible that these copolymers preferably accumulate in immune cells overexpressing the DC-SIGN receptors.[27] However as can be seen from these examples, micellar aggregates or nanoparticles have not been the subject of the investigation with poly(2-oxazoline)s. This motivated us to develop strategies for the preparation of various amphiphilic poly(2-oxazoline)s functionalized with DO3A that can be used as water-soluble DO3A–Gn conjugates with a focus on micelles and core crosslinked nanoparticles.

Here, we present the poly(2-oxazoline) derived DO3A–Gn conjugates by using monofunctionalized DO3A ligands as termination reagents for poly(2-oxazoline)s. This approach was extended to amphiphilic block copolymers to prepare polymer micelles and subsequently polymeric nanoparticles by using the microemulsion approach. Especially, the simply method to fabricate DO3A–Gn-based nanoparticles with tunable size and functional groups on their surface will be of high interest when investigating the effect of particle size, shape or degree of surface functionality.

2. Results and Discussion

2.1. Synthesis and Characterization of DOT3[Gn(III)]–Poly(2-methyl-2-oxazoline) Conjugates

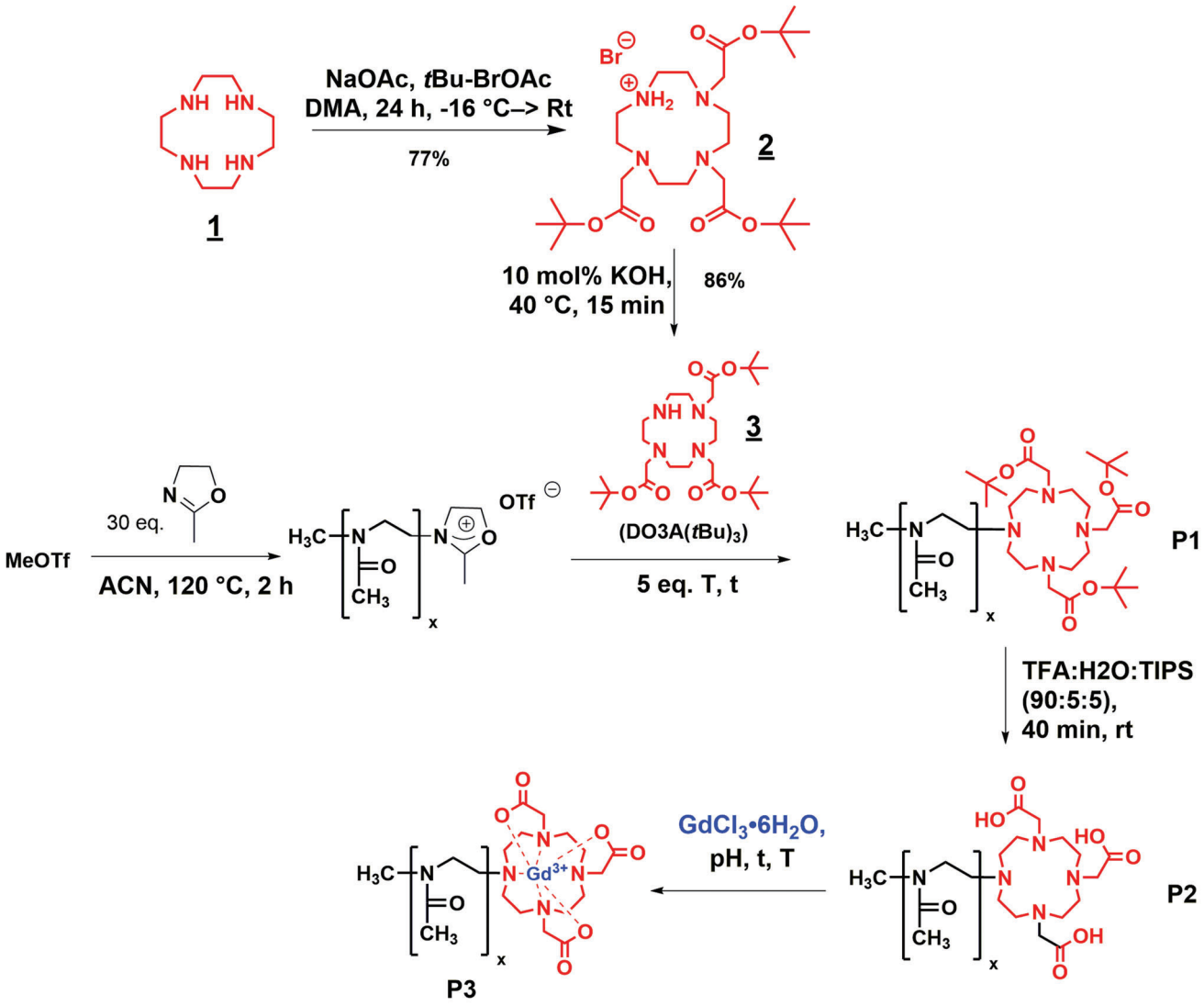
We decided to introduce the **DO3A-**ligand into the poly(2-methyl-2-oxazoline) via the termination reaction. Therefore, **DO3A(*t*Bu)$_3$** ligand **3** was prepared in a two-step synthesis starting from 1,4,7,10-tetraazacyclododecane **1** according to as literature procedure from Jagadish et al.[28] in an overall yield of 66% (**Scheme 1**). Preparation of the poly(2-methyl-2-oxazoline)s with **DO3A**[**Gn(III)**] moieties was conducted according to Scheme 1.

To optimize the termination reaction with **DO3A(*t*Bu)$_3$** four reaction times of 12 to 48 h and reaction temperature of 50 and 120 °C were investigated. Therefore, four poly(2-methyl-2-oxazoline)s were prepared with around 30 repeating units in acetonitrile at 120 °C for 2 h and five eq **DO3A(*t*Bu)$_3$** were added to each polymerization mixture (**Table 1**). The length of the polymers with ≈30 repeating units was chosen because good termination reactions could still be expected with this length based on previous experience.[29]

Afterward the polymer was precipitated in cold diethyl ether four times to separate the **DO3A(*t*Bu)$_3$** excess from the polymer. The degree of termination reaction was analyzed by ^{1}H NMR based on the integrals of the *tert*-butyl groups at 1.44 –1.47 ppm and the methyl group of the initiator at 2.9–3.0 ppm (**Figure 1**). Best results were obtained after 48 h at 120 °C with degree of termination of 86% of poly(2-methyl-2-oxazoline) with **DO3A(*t*Bu)$_3$**.

In the next step, **P1b** and **P1d** were deprotected with a mixture of TFA:H_2O:TIPS (v/v 90:5:5) for 40 min at room temperature. Complete deprotection of the carboxylic acid groups was confirmed by ^{1}H NMR and disappearance of the signals at 1.44–1.47 ppm (see Figures S8 and S9, Supporting Information). The analytical data of the deprotected polymers show low dispersities Đ of 1.08 and 1.10 for **P2b** and **P2d**, respectively (**Table 2**). The small decrease in repeat units after deprotection could be related to the fact that the strongly acidic TFA solution cleaves off some of the side chains which is known also for other acidic reagents.[26]

In the last step, **P2b** and **P2d** were complexed with $Gn(III)Cl_3{\cdot}6H_2O$. Therefore, **P2b** was dissolved in water and the pH adjusted to 6 before 1,5 eq $GdCl_3{\cdot}6H_2O$ were added to the aqueous polymer solution to prevent precipitation of $Gn(III)Cl_3{\cdot}6H_2O$ as stable hydroxyl salt.[30] Then, the pH-value was raised to 8 and the reaction time was varied from 24 to72 h and reaction temperature form room temperature to 50 °C. Noncomplexed gadolinium(III) ions were separated by dialysis against water for 48 h (MWCO = 1000). Afterward, the aqueous polymer solutions were freeze-dried and the Gd-content was quantified by ICP-OES. **P2b** was used to optimize again conditions for complexation with gadolinium(III) ions. Initial experiments at room temperature for 24 h revealed that only 13% complexation of DO3A-ligands (6 from 45 available DO3A-ligands) with gadolinium(III) ions. After increasing the reaction time to 72 h at room temperature the value could be increased to ≈40%. Best results were obtained at 50 °C after 24 h reaction time indicating 84% of the DO3A-ligands was complexed with gadolinium(III) ion. These optimized reaction conditions were transferred to the loading of **P2d** with Gn(III) ions and revealed quantitative complexations results (**P3d**, **Table 3**). In the last step, the longitudinal relaxation time T_1 of **P3b** and **P3d** was investigated. Gn(III) complexes reduce the relaxation time T_1 of the surrounding water molecules as a prerequisite for higher contrast in MRI measurements. A characteristic value to determine the quality of a contrast agent is the relaxivity r_1 which is determined by the slope of $1/T_1$ against Gn(III) concentration. The relaxivity has been measured with a magnetic field strength of 9.4 T (400 MHz)



Scheme 1. Synthesis of the DO3A[Gd(III)]–PMOx conjugates.

Table 1. Analytical data of the termination experiments with **DO3A(*t*Bu)$_3$** for polymers **P1(a–d)**.

Polymer	*t* [h]	*T* [°C]	MOx [a]	M_n [a] [g mol^{-1}]	mod_{DO3A} [b] [%]
P1a	12	50	25 (30)	2660 (3083)	28
P1b	24	50	29 (30)	3000 (3083)	45
P1c	24	120	21 (30)	2320 (3083)	47
P1d	48	120	28 (30)	2910 (3083)	86

[a] Determined by ^{1}H NMR spectroscopy; theoretical degree of polymerization and molar mass in brackets [b] Degree of **DO3A(*t*Bu)$_3$** functionalization via the signal at 1.44–1.47 ppm = 27 H.

for **P3b[Gd]** (A) and **P3d[Gd]** (B) and revealed values of r_1 = 1.12 and 2.32 mM^{-1} s^{-1} for **P3b[Gd]** and **P3d[Gd]**, respectively (see **Figure 2**).

Table 3 summarizes the relaxivity results for poly(2-methyl-2-oxazoline)–DOTA conjugates with Gn(III)] complexes. In addition, a so-called phantom magnetic resonance image (MR image) was recorded of the homopolymer **P3d[Gd]** (**Figure 3**), which is used to visualize the performance of synthesized contrast agents in MRI. Different concentrations of the macromolecular contrast agent are measured simultaneously against air and a black-and-white contrast image is generated. As expected, the contrast to the background becomes brighter with increasing concentration. Since DOTA[Gn] conjugated to linear homopolymers or copolymers reveal lower relaxivities compared to micelles or nanoparticles we intended to expand our approach further to poly(2-oxazoline)-based micelle and nanoparticles.

2.2. Synthesis and Characterization of DOT3[Gn(III)]–PHOx-*block*-PMeOx Conjugates

The cationic ring-opening polymerization of 2-oxazolines provides a versatile monomer system, which allows the synthesis of amphiphilic polymers with different architecture and

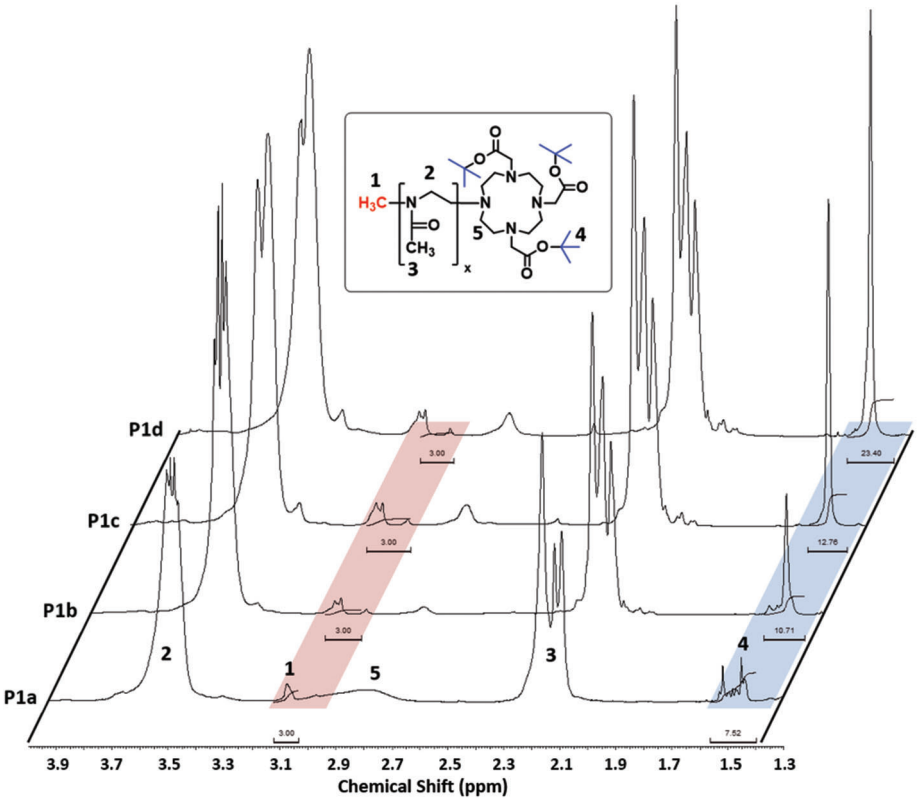


Figure 1. ^{1}H NMR spectra (in $CDCl_3$) of the termination reaction with **P1a** (50 °C, 12 h), **P1b** (50 °C, 24 h), **P1c** (120 °C, 24 h), and **P1d** (120 °C, 48 h). Initiator signal (red), *tert*-butyl group from **DO3A(*t*Bu)$_3$** (blue) with peaks assigned exemplary for **P1a**.

Table 2. Analytical data of the deprotected polymers **P2b** and **P2d**.

Polymer	**DO3A(*t*Bu)$_3$**$_{mod}$ [%]	MOx	M_n [a] [g mol^{-1}]	$Đ$ [b]
P2b	45	23	2110	1.08
P2d	84	25	2410	1.10

[a] Molar mass as determined by ^{1}H NMR spectroscopy [b] Polymer dispersity $Đ$ determined by SEC measurements with PMMA standards in DMF/5 mg mL^{-1} LiBr.

composition, such as block copolymers, graft copolymers, or dendrimers.[31,32]

Based on the successful preparation of poly(2-methy-2-oxazolines)–DOTA conjugates via the termination reaction, we extended this approach toward amphiphilic block copolymers.[33,34] The synthetic strategy involved the preparation of block copolymer based on 2-methyl-2-oxazoline (MeOx) to form the hydrophilic block and 2-heptyl-2-oxazoline (HeOx) to form the hydrophobic block. Termination was again carried out with five eq. DOTA-ligand **3** by using the optimized conditions of 120 °C for 48 h. (**Scheme 2**)

The final block copolymer **P4a** revealed a copolymer composition of 5 eq **HOx** and 29 eq **MeOx** with a degree of termination of 97% as determined by ^{1}H NMR (Figure S10, Supporting Information). Moreover, the dispersity $Đ$ for **P4a** was 1.18 which is in good agreement for poly(2-oxazoline)s.[33,34] Afterward, the *tert*-butyl groups were successfully removed with a mixture of TFA:H_2O:TIPS (v/v 90:5:5) to give **P4** according to as can be seen

Table 3. Analytical data of the block copolymer **P4a** (after termination), **P4** (after deprotection) and the macromonomer **P6** (after termination) and **P7c** (after deprotection).

#	HOx, MOx[a]	mod$_{DO3A}$[a] [%]	M_n[a] [g mol^{-1}]	M_n[b] [g mol^{-1}]	$Đ$[b]	d_h[c] [nm]
P4a	5(8), 29(30)	97	3840	4280	1.18	7.3 ± 0.6
P4	5(5), 23(29)	97	3150	3690	1.18	12.3 ± 0.5
P6	4(4), 28(30)[d]	63	4201	4357	1.34	15.7 ± 1.4
P7c	4(4), 37(28)[d]	63	4798	4931	1.30	26.7 ± 1.7

[a] Determined by ^{1}H NMR spectroscopy, degree of **DO3A(*t*Bu)$_3$**-functionalization via the signal at 1.43–1.47 ppm ≡ 27 H; [b] Molar mass and dispersity $Đ$ were determined by SEC (PS standards, DMF/5 mg mL^{-1} LiBr); [c] Hydrodynamic diameter (d_h) was analyzed by DLS measurements of a 1 mM aqueous polymer solution; [d] Contain the additional monomer M1, 2(4) for **P6** and 2(2) for **P7c**, respectively.

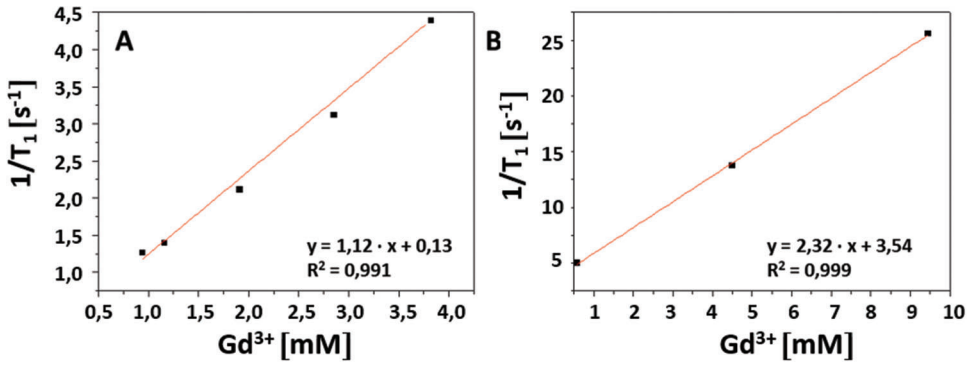


Figure 2. $1/T_1$ versus gadolinium(III) concentration at 9.4 T (400 MHz) of the homopolymers A) **P3b[Gd]** and B) **P3d[Gd]**.

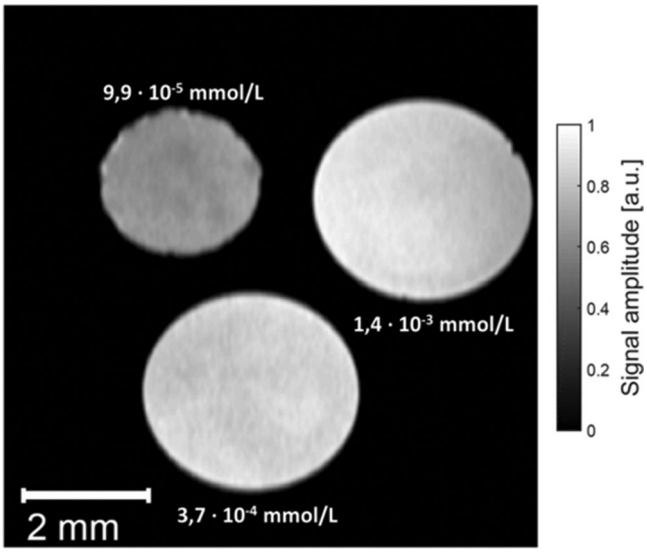


Figure 3. T_1-weighted phantom MR image of **P3d[Gd]** analyzed at three different concentration.

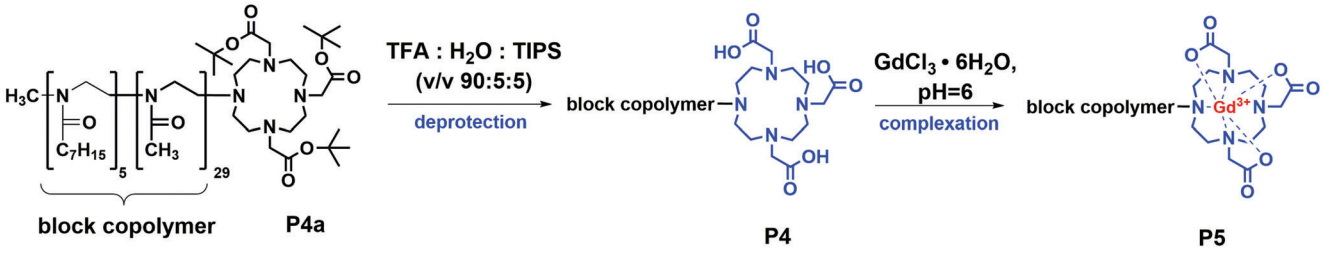
by ^{1}H NMR spectroscopy with the disappearance of the signal at 1.44 ppm (see Figure S11, Supporting Information). The analytical data of **P4a** and **P4** are summarized in Table 3.

The measurement of the hydrodynamic diameter after gadolinium(III) complexation of the block copolymer showed a slight increase from 12 nm for **P4** to 15 nm for **P5**, which may be related to the repulsion of the charged chelate ligands (**Table 4**). A gadolinium(III) number of 0.70 per polymer chain could be calculated, which corresponds to a 65% gadolinium(III) modification of the possible DO3A molecules. Subsequently, the relaxivity r_1 was 10.1 mM^{-1} s^{-1} as determined from the slope of $1/T_1$ against the gadolinium(III) concentration at 9.4 T (see **Figure 4**). The improvement of the r_1-value from the homopolymer to block copolymer is related to the increased rotation time of the gadolinium(III) complex in the micellar structure. Similar values in terms of gadolinium(III) content and relaxivity r_1 could be found in the literature. In 2016, Tong et al.[35] showed a polymer-analogous endgroup functionalization of amphiphilic PEG-*b*-P(AAm-*co*-AN) via a Michael addition using DOTA-NHS and a terminal thiol group on the hydrophilic block of the amphiphile drug loading with doxorubicin and the complexation of the macrocycle DOTA in one step. With a relatively low gadolinium(III) content of 2.8 μg mg^{-1} these polymers formed micelles in water with a size of 130 nm after complexation and a relaxivity r_1 was measured to be 25.88 mM^{-1} s^{-1} at 3 T. Zhang et al. synthesized biodegradable micelles based on poly(L-glutamic acid)-*b*-polylactic acid (PG-*b*-PLA) with the chelating ligand p-aminobenzyl DTPA, which was complexed with gadolinium(III) ions. The micelles had spherical sizes of 230 nm and a relatively high gadolinium(III) content of 50 μg mg^{-1}. This resulted in a relaxivity r_1 of 7.90 mM^{-1} s^{-1} at 4.7 T.[36] In the case of the block copolymer **P5[Gd]**, a visualization of the performance was also carried out via a phantom magnetic resonance image (Figure 4) revealing again an improvement in contrast with increasing gadolinium concentration can be observed.

2.3. Synthesis and Characterization of DOT3[Gn(III)]-Functionalized Core–Shell Nanoparticles

One of the main limitations of micellar aggregates is their inherent instability. Therefore, there is an increasing interest to prepare stable nanoparticles for medical application. Typical approaches are based on shell/core crosslinking of preformed micellar aggregates[37] or by heterogeneous polymerization techniques, which include emulsion,[38] miniemulsion[39] or microemulsion polymerization.[40] The later approach is of particular interest as it allows to prepare well-defined nanoparticles with tunable size in the sub-100 nm range. Recently, we have employed the microemulsion approach to prepare well-defined nanoparticles by using bifunctional, amphiphilic poly(2-oxazoline) macromonomers with multiple methacrylate or styrene groups in their hydrophobic block. After micelle formation in aqueous media the crosslinkable moieties were used to covalently crosslink the particle core.[41,42] Based on this approach, we synthesized a bifunctional macromonomer **M1** with methacrylate moieties in the side chain based on a previous report[41] and copolymerized **M1** with 2-hepytl-2-oxaoline to form the hydrophobic block before the hydrophilic block was added with 2-methyl-2-oxazoline and the block copolymer was terminated with **DO3A(*t*Bu)$_3$**. The synthesis is depicted in **Scheme 3**.

Therefore, an amphiphilic poly(2-oxazoline) was prepared by cationic ringopening polymerization of 2-methyl-2-oxazoline to form the hydrophilic block and a mixture of 2-heptyl-2-oxazoline and 2-(5-pentyl-[(1,2,3-triazol)-4-yl-methacrylat)]-oxazoline **M1** to



Scheme 2. Synthesis of DO3A[Gd] poly(2-heptyl-2-oxazoline)-*block*-poly(2-methyl-2-oxazoline) conjugates.

Table 4. Analytical data of homopolymers **P3a–P3d**, block copolymer **P5**, and core-crosslinked nanoparticle **NP** after complexation with Gn(III) ions.

#	mod_{DO3A} [a] [%]	*T* [h]	*T* [°C]	[Gd] [b] µg	N_{Gd} [c]	Mod_{Gd} [d]	d_h [e] [nm]
P3a	45	24	r.t.	18	0.06	13	n.d.
P3b	45	72	r.t.	32	0.18	40	n.d.
P3c	45	24	50	59	0.38	84	n.d.
P3d	86	24	50	70	0.90	>99	n.d.
P5	97	24	50	26	0.70	65	15.1 ± 1.6
NP	63	72	50	16	0.48	76	32.9 ± 7.8

[a] Determined by ^{1}H NMR spectroscopy [b] Determined by ICP-OES, Gd content from 1 mg polymer [c] Determined with $\mu_Z(t) = \mu_0 [1 - 2e^{-t/T_1}]$; number of Gd atoms per polymer [d] Determined from the ratio of DO3A molecules and gadolinium ions per polymer chain [e] Hydrodynamic diameter (d_h) was analyzed by DLS measurements of a 1 mM aqueous polymer solution.

form the hydrophobic block. The latter 2-oxazoline monomer **M1** with methacrylate moiety was prepared according to a recent literature procedure.[41,42] Termination was again carried out with DO3A-ligand **3** at 50 °C for 48 h. Copolymer **P6** analyzed by ^{1}H NMR spectroscopy showed a polymer composition of twenty eight MeOx units, two units of the 2-oxazoline monomer with methacrylate moiety, and four HMOx units with a degree of termination of 63% with **DO3A(*t*Bu)$_3$**. The lower than expected degree if termination was most likely due to the sterically more hindered block copolymers versus the poly(2-methyl-2-oxazoline) homopolymers. Removal of the *tert*-butyl groups with the mixture TFA:H_2O:TIPS (v/v 90:5:5) at room temperature for 40 min led not only to the deprotection of the carboxylic acid groups but also to partial cleavage of the methacrylate group with a decrease of the vinyl protons at δ = 5.59 and 6.13 ppm. Therefore, the *tert*-butylester was removed with the more selective $CeCl_3 \cdot 7H_2O$/NaI system according to Marcantoni et al.[43]

To optimize the deprotection reaction, **P6** was dissolved in dry acetonitril before addition of $CeCl_3 \cdot 7H_2O$ and NaI to the solution and refluxing at 120 °C for 12, 24, and 48 h. Afterward, three samples **P7(a–c)** were collected after 12, 24, and 48 h and were dialyzed against water (MWCO = 1000) to remove the salt. After freeze-drying and precipitation in cold diethyl ether the ^{1}H NMR spectra were obtained. As can be seen from **Figure 5**, the signal intensity of the *tert*-butyl group at 1.44 ppm (shown in blue) decreases with increasing reaction times and reached 42% deprotection after 12 h and full deprotection after 48 h while maintaining the methacrylate side group. The analytical data from ^{1}H NMR spectroscopy and size exclusion chromatography (SEC) measurements for **P6** and **P7c** are summarized in Table 3.

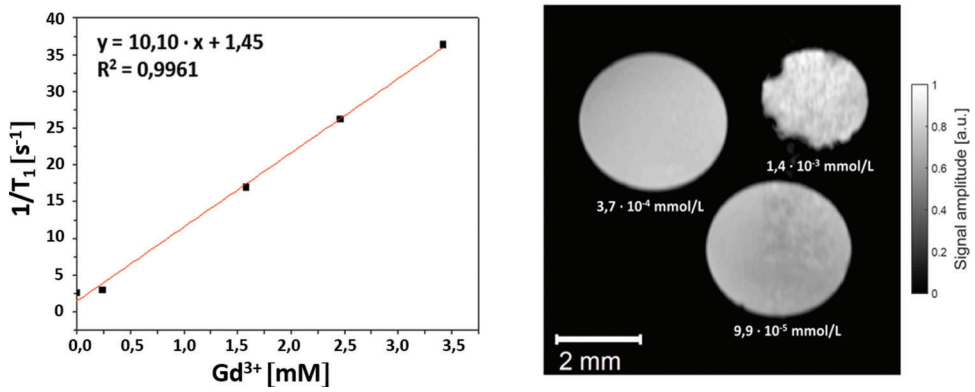
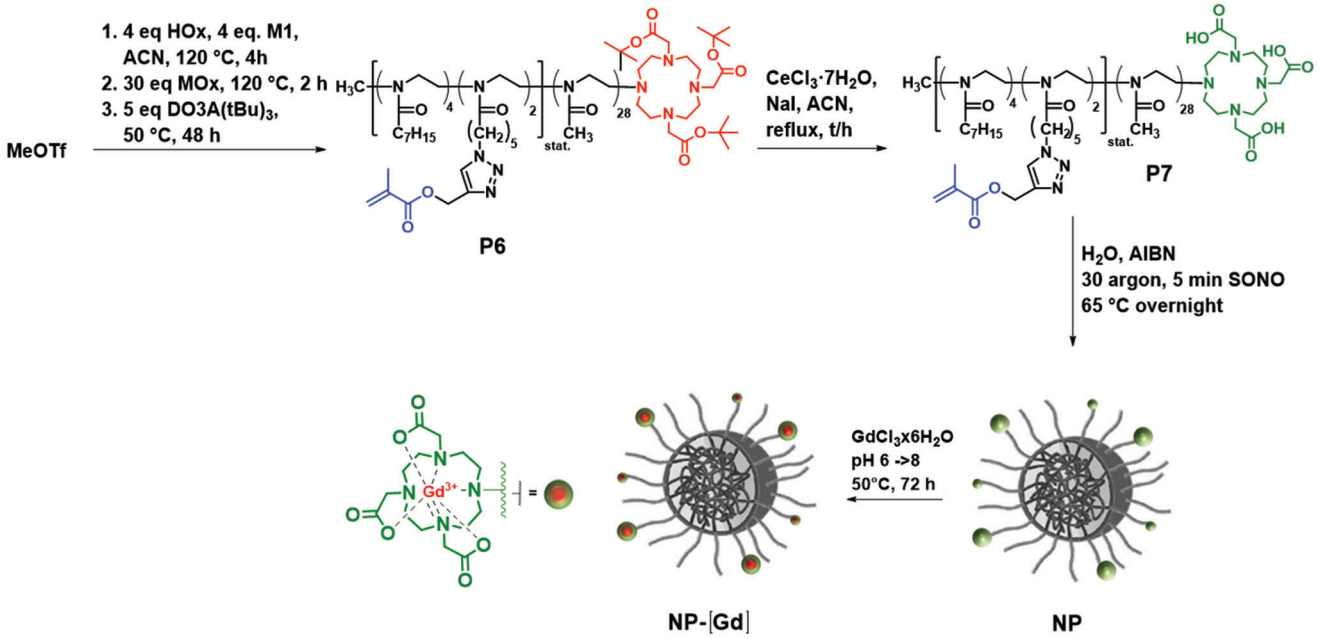


Figure 4. $1/T_1$ versus gadolinium(III) concentration at 9.4 T (400 MHz) of the block copolymer **P5[Gd]** (left). T_1-weighted phantom MR image of **P5[Gd]** analyzed at three different concentration.



Scheme 3. Synthesis of bifunctional poly(2-oxazoline) macromonomer **P7** with **DO3A(*t*Bu)$_3$** endgroups and subsequent nanoparticle formation via free radical polymerization and crosslinking of the nanoparticle core.

In the next step, the DO3A molecules of the nanoparticle **NP** were complexed with gadolinium(III) ions, analogous to the homopolymers and the block copolymers. Therefore, **NP** was predissolved in slightly acidic water (pH = 6) and 1.5 equivalents of $GdCl_3$–$6H_2O$ was added and the pH was slowly increased to 8. The reaction was heated to 50 °C and stirred for 72 h at this temperature. To remove the free gadolinium(III) ions, the nanoparticle **NP-[Gd]** was dialyzed against water for 72 h (MWCO = 5000). The dialysis time was increased to remove possible salts inside the particle. After removing the water by freeze-drying, the gadolinium(III) content of the resulting nanoparticle **NP-[Gd]** was determined to be 16 µg mg^{-1} nanoparticle by ICP-OES. With the determined amount of gadolinium(III) ions of 16 µg mg^{-1} it could be calculated that almost every second polymer chain of **NP-[Gd]** carries a gadolinium(III) ion. Furthermore, the measurement of the hydrodynamic diameter in water (c = 1 mg mL^{-1}) showed an increase in size after complexation to 32.9 ± 7.8 nm. **Figure 6** compares the DLS graphs of the noncomplexed nanoparticle **NP** (black) and the complexed **NP-[Gd]** (red). The same effect was already seen in the complexation of the block copolymer **P4** to **P5-[Gd]**. Here, the micelle diameter increased by a few nanometers. The core crosslinking prevents the dynamic exchange of individual polymer chains. Furthermore, the TEM image in Figure 6B confirm the spherical shape of the polymer nanoparticles **NP**.

Finally, the relaxivity r_1 as determined via the slope of the plot of $1/T_1$ against the gadolinium(III) concentration at 9.4 T gave a value for r_1 of 6.77 mM^{-1} s^{-1}. This value is minimally lower than that of the block copolymer **P5**. Due to the limited mobility of the chains in the nanoparticle, a higher r_1 value was expected. But due to the fact that only every second polymer chain carries a gadolinium(III) ion, the lower concentration was the decisive factor here, leading to a slightly lower relaxivity r_1. In the phantom magnetic resonance image of the nanoparticle **NP-[Gd]**, only two different gadolinium(III) concentrations could be measured due to the small sample quantity. The image in **Figure 7** shows, as expected, an improvement in contrast with increasing gadolinium(III) concentration.

3. Conclusions

In conclusion, we presented a synthetic approach to use poly(2-oxazoline) as a synthetic platform to prepare polymer conjugates with a **DO3A(*t*Bu)$_3$** ligands as endgroup that was introduced during the termination reaction. The degree of endgroup modification ranged from 86% for the homopolymers to 63% for the macromonomers. After complexation with gadolinium(III) ions, these polymers were used to compare the relaxivity r_1 at 9.4 T. The r_1 values ranged from 2.32 mM^{-1} s^{-1} for homopolymers to 10.1 mM^{-1} s^{-1} for block copolymer micelles and 6.77 mM^{-1} s^{-1} for core-crosslinked nanoparticles. This study highlights the potential of the poly(2-oxazoline) chemistry to design effective and stable contrast nanomaterials for potential application in medical diagnostics.

4. Experimental Section

Materials and Measurements: Materials: Methyl triflate (MeOTf), 2-methyl-2-oxazoline (MOx), 2-heptyl-2-oxazoline (synthesized according to Witte and Seelinger[44]), and acetonitrile for polymer preparation were dried by refluxing over CaH_2 under a dry argon atmosphere. The dialysis membranes were composed of regenerated cellulose from Zellu-Trans/Roth V-Series with an MWCO = 1000 or 5000.

All other chemicals were purchased commercially and used, unless otherwise noted, without further purification. Water-free dichloromethane and dimethylformamide were purchased from Acros Organics and dried

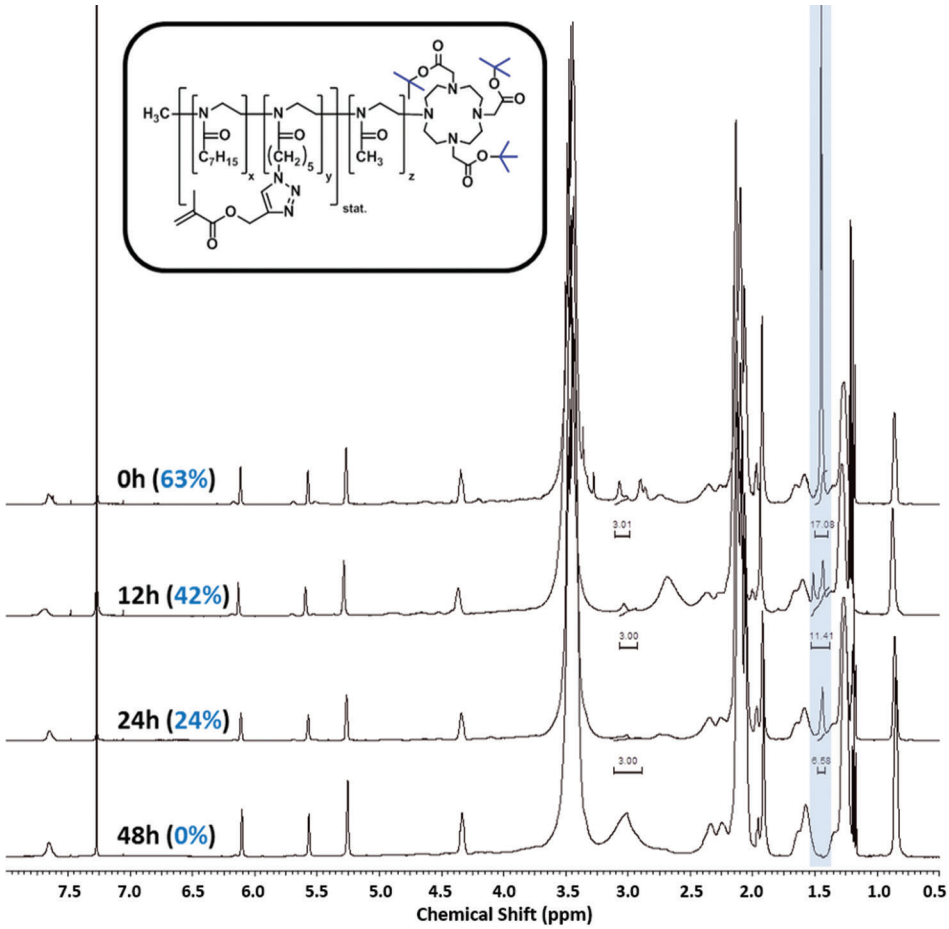


Figure 5. ^{1}H NMR spectra of the deprotection reactions of the triblock polymer after 12 h (**P7a**-12h), 24 h (**P7b**-24h), and 48 h (**P7c**-48h), chemical shift of the *tert*-butyl group marked in blue.

over Al_2O_3 using an M. Braun GmbH MB SPS 800. Other water-free solvents were dried under standard procedures (acetonitrile, $CaCl_2$; isopropanol, Mg) and stored over molecular sieve 3 Å and argon atmosphere.

Measurements: The NMR spectra were recorded on 500 MHz spectrometer AVANCE-III HDX-500 with 5 mm nitrogen cooled Prodigy H(C,N) probe (Bruker BioSpin GmbH) or on a 400 MHz NMR spectrometer Nanobay AVANCE-III HD-400 with 5 mm BBFOsmart probe (Bruker BioSpin GmbH). The spectra were calibrated using the signals of the deuterated solvent $CDCl_3$ at 7.26 ppm. The FT-IR spectra were recorded on a Bruker Tensor 27 Platinum ATR equipped with OPUS software. For collecting spectra, a total number of 32 scans were used. The SEC was performed on a Smartline 2300 (KNAUER) equipped with a refractive index detector (tempered to 60 °C) using a PSS GRAM analytical column set (1 × precolumn + 1 × 1000 A + 1 × 30A). *N*,*N*-dimethylformamide (HPLC-grade) was used as eluent (+5 g L^{-1} LiBr) at a flow rate of 1 mL min^{-1} at 60 °C. SEC columns were calibrated with PMMA standards (from PSS). Prior to each measurement, the polymer samples were purified by using a 0.2 µm Teflon filter (VWR) to remove larger particles. Dynamic light scattering experiments were performed using a Malvern Zetasizer Nano S (ZEN 1600). A 4 mW He-Ne laser (633 nm wavelength) with a fixed detector angle of 173° was used. About 1 mL of dust-free sample was transferred to a special light scattering cell without filtration. The polymer samples were dissolved in methanol or water equilibrated at 25 °C for 1 min before the data acquisition started. The measurements were repeated five times with 10 runs. For further interpretation, the peak average of histograms from the number distributions of 50 accumulations was reported as the average diameter of the particles.

Synthesis of 1,4,7-Tris(tert-butoxycarbonylmethyl)-1,4,7,10-Tetraazacyclodo-Decane·Hydro BROMIDE ((tBu)$_3$-DO3A·HBr): To a solution of cyclene (2.0 g, 11.6 mmol, 1 eq.) and sodium acetate (3.1 g, 38.3 mmol, 3.3 eq.) in 24 mL DMA was added at −16 °C a solution of *t*-butyl bromoacetate (7.5 g, 38.3 mmol, 3.3 eq.) in 8 mL DMA over 0.5 h. The reaction mixture was stirred for 20 h at room temperature. After addition, the reaction mixture was stirred at room temperature for 20 h and added to 120 mL water. Solid $KHCO_3$ (6.0 g, 59.9 mmol) was added in portions to the clear solution until a white solid was formed. This was separated by filtration, dissolved in 100 mL $CHCl_3$ and washed with 40 mL water, dried over $MgSO_4$, filtered, and concentrated. After addition of 100 mL diethyl ether the product crystallized as a white solid (4.4 g, 7.4 mmol, 64%). It was filtered off and dried under high vacuum.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.47 (s, 27H), 2.92 (br s, 12H), 3.12 (s, 4H), 3.31 (s, 2H), 3.39 (s, 4H), 10.05 (s, 2H). **^{13}C NMR** (500 MHz, $CDCl_3$): δ (ppm) = 28.2 (9C), 47.5 (2C), 49.3 (2C), 51.4 (4C), 58.3 (3C), 81.7 (3C), 169.6, 170.5 (2C).

Synthesis of 1,4,7-Tris(tert-butoxycarbonylmethyl)-1,4,7,10-Tetraazacyclodo-Decane ((tBu)$_3$-DO3A): 500 mg of substance 14 was suspended in 20 mL of water and heated to 70 °C. After stirring at this temperature for 1 h, the solution was cooled to 40 °C and 1 mL of a 10% KOH solution was added and stirred at 40 °C for another 15 min. After cooling the reaction, the aqueous phase was extracted three times with cyclohexane (3 × 100 mL). The combined organic phases were then

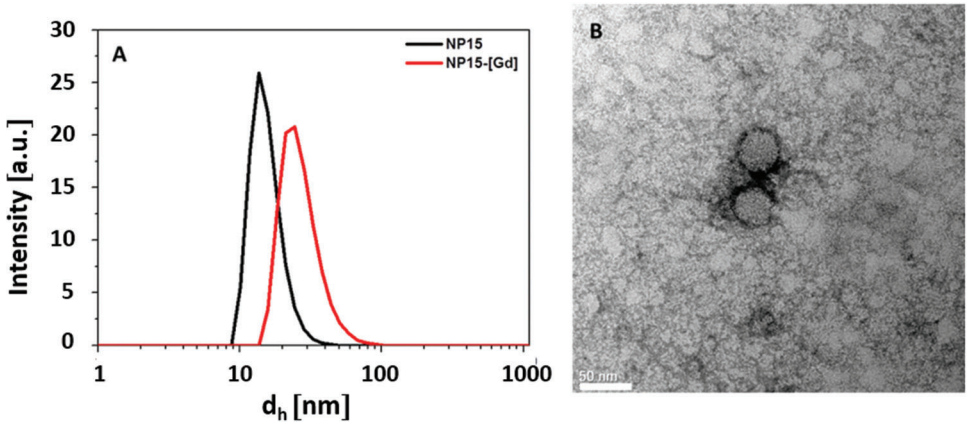


Figure 6. A) DLS graphs of the nanoparticles NP (black) and NP-[Gd] (red) measured in water with a concentration $c = 1$ mg mL^{-1} and B) TEM images of the nanoparticle NP-[Gd] with a concentration $c = 0.01$ mg mL^{-1} in water, colored with uranyl acetate, scale: 50 nm.

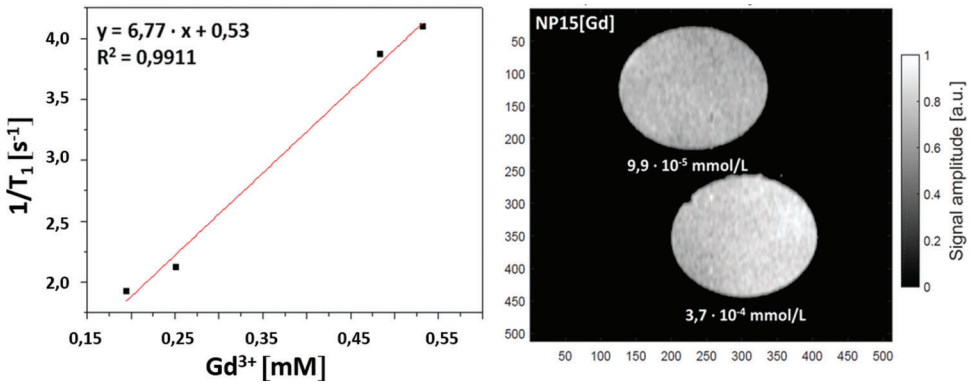


Figure 7. Left: Plot of $1/T_1$ against the gadolinium concentration in a magnetic field strength of 9.4 T (400 MHz) of the nanoparticle **NP-[Gd]**. Right: Phantom magnetic resonance image of the nanoparticle **NP-[Gd]** at different concentrations measured against air.

washed three times with water (3 × 100 mL) and dried over magnesium sulphate. The organic solvent was removed under reduced pressure and the product was dried at high vacuum. A highly viscous oil was obtained (380 mg, 0.74 mmol, 88%).

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.47 (s, 27H), 2.92 (br s, 12H), 3.12 (s, 4H), 3.31 (s, 2H), 3.39 (s, 4H), 10.05 (s, 2H). **^{13}C NMR** (500 MHz, $CDCl_3$): δ (ppm) = 28.2 (9C), 47.5 (2C), 49.3 (2C), 51.4 (4C), 58.3 (3C), 81.7 (3C), 169.6, 170.5 (2C).

LCMS (ESI) m/z berechnet für $C_{26}H_{51}N_4O_6$ ($[H]^+$) 515.3809 gefunden 515.3806.

Synthesis and characterization of **M1** have been described recently.[40]

*Polymerization: Synthesis of Poly[(2-methyl-2-oxazoline)$_{29}$]-**DO3A(tBu)$_3$** (P2b)*: Methyl triflate (44,0 µL, 1 eq.) was added to a cooled solution of 2-methyl-2-oxazoline (1.00 mL, 30 eq., cooled to 0 °C) in 5–10 mL dry acetonitrile and mixed and heated to 110 °C for 2 h. The polymerization was then stopped by adding the terminating reagent **DO3A(*t*Bu)$_3$** (1.08 g, 5 eq.) and the reaction mixture was stirred at 50 °C for 24 h. The solvent was then removed under reduced pressure, the residue was taken up in $CHCl_3$ and K_2CO_3 was added to the solution. The suspension was stirred for 2 h at room temperature and then filtered. The polymer was precipitated in diethyl ether cooled to ≈5 °C via a syringe. The polymer was centrifuged off the precipitation reagent, dissolved in a little $CHCl_3$, and precipitated again. This washing procedure was repeated several times until a white solid was obtained. This was then dried under high vacuum. Yield is 59%.

^{1}H NMR (499,88 MHz, $CDCl_3$): δ (ppm) = 1.43 (s, 11H, *t*Bu), 2.13 (m, 78H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.44 (m, 119H, CH_2–$CH_{2,backbone}$).

*Synthesis of Poly[(2-methyl-2-oxazoline)$_{29}$]-**DO3A(tBu)$_3$** (P2d)*: The same protocol was used as described for P2b, the only change was the termination procedure and the polymerization was stopped by adding the terminating reagent **DO3A(*t*Bu)$_3$** (1.08 g, 5 eq.) and the reaction mixture was stirred at 120 °C for 48 h. After purification of the polymer, the yield was 73%.

^{1}H NMR (499,88 MHz, $CDCl_3$): δ (ppm) = 1.45 (s, 23H, *t*Bu), 2.14 (m, 90H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.45 (m, 118H, CH_2–$CH_{2,backbone}$).

*Synthesis of Poly[(2-heptyl-2-oxazoline)$_5$-block-(2-methyl-2-oxazoline)$_{29}$]-**DO3A(tBu)$_3$** (P4)*: Methyl triflate (44.3 µL, 1 eq.) was added to a cooled solution of 2-heptyl-2-oxazoline (530 µL, 8 eq. cooled to 0 °C) in 5–10 mL dry acetonitrile and mixed and heated to 120 °C for 4.5 h. After the specified reaction time for the first monomer, the reaction mixture was again cooled to 0 °C, and 2-methyl-2-oxazoline (1 mL, 30 eq.) was added and the mixture was heated to 120 °C for another 3 h. The polymerization was then stopped by adding the terminating reagent **DO3A(*t*Bu)$_3$** (1.08 g, 5 eq.) and the reaction mixture was stirred at 120 °C for another 48 h. The solvent was then removed under reduced pressure, the residue was taken up in $CHCl_3$ and K_2CO_3 was added to the solution. The suspension was stirred for 2 h at room temperature and then filtered. The polymer was precipitated in diethyl ether cooled to ≈5 °C via a syringe. The polymer was centrifuged off the precipitation reagent, dissolved in a little $CHCl_3$, and precipitated again. This washing procedure was repeated several times until a white solid was obtained. This was then dried under high vacuum. Yield is 66%.

^{1}H NMR (400.25 MHz, $CDCl_3$): δ (ppm) = 0.88 (brs, 16H, $CH_{3,HOx}$), 1.29 (brs, 44H, 4 × $CH_{2,HOx}$), 1.45 (s, 26H, *t*Bu), 1.60 (brs, 12H, $CH_{2,HOx}$),

2.11–2.16 (m, 117H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.45 (m, 164H, CH_2–$CH_{2,backbone}$).

*Synthesis of Poly[{(2-heptyl-2-oxazoline)$_4$-co-(2-(5-pentyl-[(1,2,3-triazol)-4-yl-meth-acrylat)$_2$]oxazoline)$_3$}$_{stat}$-block-(2-methyl-2-oxazoline)$_{28}$]-**DO3A(tBu)$_3$** (P6)*: Methyl triflate (44.3 µL, 1 eq.) was added to a cooled solution of 2-heptyl-2-oxazoline (599 µL, 4 eq.) and **M1** (332 mg, 4 eq.) in 5–10 mL dry acetonitrile and mixed and heated to 120 °C for 5 h. Then the solution was cooled down to 0 °C, and 2-methyl-2-oxazoline (1 mL, 30 eq.) was added and the mixture was heated again to 110 °C for another 3 h. The polymerization was then stopped by adding the terminating reagent **DO3A(*t*Bu)$_3$** (1.08 g, 5 eq.) and the reaction mixture was stirred at 50 °C for another 48 h. The solvent was then removed under reduced pressure, the residue was taken up in $CHCl_3$, and K_2CO_3 was added to the solution. The suspension was stirred for 2 h at room temperature and then filtered. The polymer was precipitated in diethyl ether cooled to ≈5 °C. The polymer was centrifuged off the precipitation reagent, dissolved in a little $CHCl_3$, and precipitated again. This washing procedure was repeated several times until a white solid was obtained. This was then dried under high vacuum. Yield is 58%.

^{1}H NMR (500.08 MHz, $CDCl_3$): δ (ppm) = 0.86 (s, 13H, $CH_{3,HOx}$), 1.27 (brs, 30H, 4 × $CH_{2,HOx,}$ $CH_{2,12}$), 1.44 (s, 16H, *t*Bu), 1.58–1.66 (brs, 12H, $CH_{2,HOx}$, $CH_{2,12}$), 1.92 (s, 14H, $CH_{3,AOx}$, $CH_{2,12}$), 2.06–2.13 (m, 86H, $CH_{3,MOx}$), 2.19–2.35 (m, 12H), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.46 (m, 137H, CH_2-$CH_{2,backbone}$), 4.34 (brs, 4H, $CH_{2,12}$), 5.26 (s, 3H, $OCH_{2,12}$), 5.58/6.12 (s, 4H, C=CH_2), 7.65 (s, 2H, C=C*H*N).

***DO3A(tBu)$_3$** Deprotection of P1b, P1d, and P4*: 1 g of the respective polymer was stirred in a total of 10 mL of a mixture of TFA:H_2O:TIPS (90:5:5) for 40 min at room temperature. The solvent was then removed under reduced pressure and the solid obtained was dissolved in 5 mL of $CHCl_3$/MeOH (3:1) and precipitated in cold diethyl ether. This procedure was repeated twice. The polymer can be obtained via centrifugation and dried under high vacuum.

P2b: ^{1}H NMR (500,08 MHz, $CDCl_3$): δ (ppm) = 2.15 (m, 87H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.45 (m, 130H, CH_2–$CH_{2,backbone}$).

P2d: ^{1}H NMR (500,08 MHz, $CDCl_3$): δ (ppm) = 2.12 (m, 56H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.43 (m, 86H, CH_2–$CH_{2,backbone}$).

P5: ^{1}H NMR (400,25 MHz, $CDCl_3$): δ (ppm) = 0.88 (brs, 17H, $CH_{3,HOx}$), 1.29 (brs, 39H, 4 × $CH_{2,HOx}$), 1.59 (brs, 9H, $CH_{2,HOx}$), 2.16 (m, 76H, $CH_{3,MOx}$), 3.00/2.93 (m, 3H, $CH_{3,I}$), 3.46 (m, 110H, CH_2–$CH_{2,backbone}$).

***DO3A(tBu)$_3$**-Deprotection of P6*: 34 mg $CeCl_3$–$7H_2O$ (1.5 eq.) and 12 mg NaI (1.3 eq.) were dissolved in 5 mL dry acetonitrile and stirred for 1 h at room temperature. Then 250 mg **P6** (1 eq.) was added and heated to 100 °C for 48 h. The reaction was stopped. After cooling the reaction, the salts were removed by filtration and the organic solvent under reduced pressure. The solid obtained was dissolved in water and dialyzed for 48 h. The water was then filtered through freezing. The water was then removed via freeze-drying and polymer **P7** was obtained via precipitation in cold diethyl ether.

P7-48h: ^{1}H NMR (500.08 MHz, $CDCl_3$): δ (ppm) = 0.85 (s, 13H, $CH_{3,HOx}$), 1.26 (brs, 39H, 4 × $CH_{2,HOx,}$ $CH_{2,12}$), 1.57 (brs, 11H, $CH_{2,HOx}$, $CH_{2,12}$), 1.91 (s, 11H, $CH_{3,12}$, $CH_{2,12}$), 2.06–2.13 (m, 70H, $CH_{3,MOx}$), 2.19–2.35 (m, 13H), 3.43 (m, 118H, CH_2–$CH_{2,backbone}$), 4.33 (brs, 4H, $CH_{2,12}$), 5.25 (s, 3H, $OCH_{2,12}$), 5.56/6.10 (s, 4H, C=CH_2), 7.65 (s, 2H, C=C*H*N).

General Synthesis Procedure for the Gadolinium(III) Complexation of the Polymers P3b, P3d, P5 and the Nanoparticle NP: 1.1 eq. polymer or nanoparticle was dissolved well in 5 mL of water using ultrasound. The pH value was adjusted to 6. Then 1.5 eq. $GdCl_3$-$6H_2O$ was added to the solution and the pH was carefully increased to 8. Then the reaction solution was heated for a time *t* at temperature *T*. Different reaction times and temperatures were tested for the polymers **P2b** and **P2d**. The polymer **P5** was stirred for 24 h at 50 °C and the nanoparticle **NP** for 72 h at 50 °C. The excess salts were removed by dialysis against water. The desired products were obtained via freeze-drying and precipitation in diethyl ether.

Nanoparticle Formation: 40 mg of **P7c** was dissolved in 3 mL of water, corresponding to a polymer concentration of 3 mmol L^{-1}. Then 0.1 wt% AIBN was added and an oxygen-free polymer solution was prepared with the help of argon. The system was then treated in an ultrasonic bath for 5 min to homogenize the solution. The radical crosslinking took place at 65 °C overnight. The water was removed by freeze-drying and the solid obtained was taken up in chloroform. The dissolved nanoparticle **NP** was then precipitated in cold diethyl ether and dried at high vacuum. Successful crosslinking was determined by DLS measurements of the nanoparticle in the nonselective solvent methanol. With a diameter of 15.23 ± 3.40 nm in methanol, the particle showed little swelling compared to the measurement in water (d_h = 13.71 ± 1.39 nm in water), indicating successful core crosslinking.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords

amphiphilic poly(2-oxazoline), bifunctional macromonomers surfactants, microemulsion polymerization, polymeric nanoparticles

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