Articles

Multivalent Contrast Agents Based on Gadolinium−Diethylenetriaminepentaacetic Acid-Terminated Poly(propylene imine) Dendrimers for Magnetic Resonance Imaging

Sander Langereis,[†] Quido G. de Lussanet,[‡] Marcel H. P. van Genderen,[†] Walter H. Backes,[‡] and E. W. Meijer*[,†]

Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, and Department of Radiology, Maastricht University Hospital, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands

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ABSTRACT: A convenient methodology has been developed for the synthesis of gadolinium−diethylenetriaminepentaacetic acid (Gd−DTPA)-terminated poly(propylene imine) dendrimers as contrast agents for magnetic resonance imaging (MRI). In our strategy, isocyanate-activated, *tert*-butyl-protected DTPA analogues were coupled to different generations of poly(propylene imine) dendrimers. Deprotection of the *tert*-butyl esters with trifluoroacetic acid in dichloromethane and extensive dialysis afforded gadolinium-chelating poly(propylene imine) dendrimers. The corresponding Gd−DTPA-based dendritic contrast agents were prepared from $GdCl_3$ in either water or citrate buffer. Atomic force microscopy and cryogenic transmission electron microscopy experiments of the fifth-generation Gd−DTPA based dendritic contrast agent in citrate buffer demonstrated the presence of well-defined spherical particles with nanoscopic dimensions (5−6 nm), and no self-aggregation of dendrimers was observed. The efficiencies of these dendritic contrast agents in MRI, expressed in terms of longitudinal (r_1) and transverse (r_2) relaxivities, were determined at 1.5 T at 20 °C. The r_1 and r_2 values increase considerably with increasing generation of Gd−DTPA-terminated dendrimer. The fifth-generation dendritic contrast agent displays the highest ionic relaxivities (per gadolinium), $r_1 = 19.7$ mM^{-1} s^{-1} and $r_2 = 27.8$ mM^{-1} s^{-1}, which are substantially higher than the ionic relaxivities of parent Gd−DTPA. Moreover, a series of combined gadolinium and yttrium complexes of the fifth-generation dendrimer are prepared, resulting in well-defined dendritic contrast agents with tunable molecular relaxivities.

Introduction

Magnetic resonance imaging (MRI)[1] is one of the most important noninvasive diagnostic techniques used to visualize soft tissue anatomy and disease, such as tumors, which in part can be attributed to the rapid development of MRI contrast agents. Among paramagnetic contrast agents, water-soluble gadolinium chelates, e.g., gadolinium−diethylenetriaminepentaacetic acid (Gd−DTPA) complexes, display intriguing properties in terms of high thermodynamic stability and kinetic inertness.[2] Gd−DTPA complexes reduce longitudinal and transverse relaxation times (T_1 and T_2, respectively) of protons of water molecules, resulting in a pronounced contrast enhancement in an MR image.[3] The ability of a contrast agent to reduce the relaxation times is often expressed in terms of relaxivity ($r_{i=1,2}$), i.e., the change of the relaxation rate ($1/T_{i=1,2}$) divided by the concentration of contrast agent. Drawbacks of low molecular weight MR contrast agents, such as Gd−DTPA (MW = 592), however, are their nonspecificity, their rapid renal excretion and extravasation, and their relatively low relaxivity.[4] In recent years, various encouraging strategies have been explored to improve the effectiveness, i.e., relaxivities, of MRI contrast agents.[4,5] It was early recognized that the proton relaxivity of low molecular weight MR contrast agents is primarily limited by the fast rotation of the gadolinium complex.[2] Consequently, low molecular weight MR contrast agents were attached to various macromolecules, either covalently[6] or noncovalently,[7,8] to reduce the molecular tumbling rate of the complex and to increase the proton relaxivity. However, the gain in proton relaxivity was often much lower than anticipated. This was presumed to be due to high conformational flexibility of the backbone. For instance, relaxivities of macromolecular contrast agents composed of linear flexible polymers, such as poly(lysine)[9] and poly(ethylene glycol),[10] were reported to be independent of their molecular weight and only slightly higher than the relaxivity of the parent gadolinium complex. Lately, several studies have shown that dendrimers are excellent multivalent scaffolds for attaching contrast agents. These highly branched macromolecular architectures have well-defined molecular weights and a precise number of end groups, which reside in virtually identical environments.[11] Wiener et al.[12] and others[13,14] reported

* To whom correspondence should be addressed. E-mail: E.W.Meijer@tue.nl.
[†] Eindhoven University of Technology.
[‡] Maastricht University Hospital.

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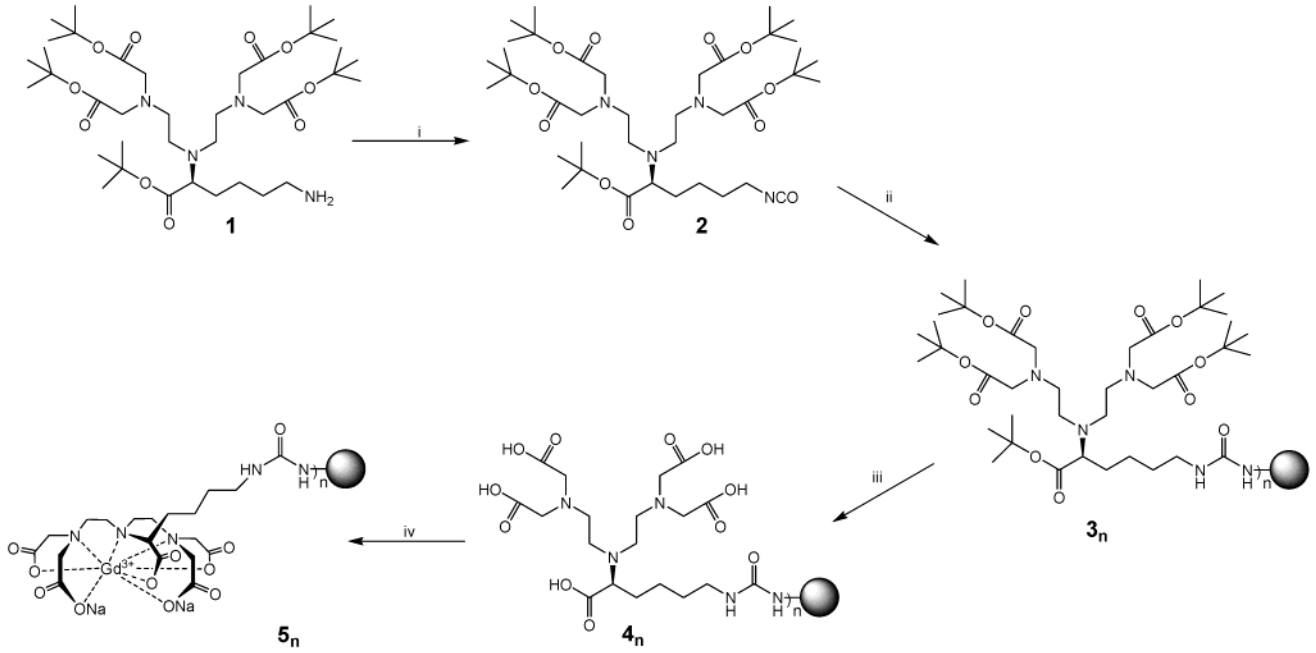
Scheme 1. Gd−DTPA-Terminated Poly(propylene imine) Dendrimers 5_n[a]



[a] n = 4 (**G1**), 16 (**G3**), and 64 (**G5**). n denotes the number of end groups and **G** represents the generation of the dendrimer. Monofunctional gadolinium complex $\mathbf{5}_{n=1}$ was prepared in a similar fashion, starting from propylamine and the isocyanate-activated DTPA synthon **2**. Reagents: (i) di-*tert*-butyl tricarbonate, DCM; (ii) DAB-*dendr*-$(NH_2)_n$, DCM; (iii) TFA, DCM; (iv) $GdCl_3 \cdot 6H_2O$, water or 0.3 N citrate buffer.

on the conjugation of isothiocyanate-activated, gadolinium-chelating groups to different generations of poly(amidoamide) (PAMAM) dendrimers. Their sixth-generation Gd−DTPA dendrimer[12] displays a strong increase in molecular relaxivity, which was attributed not only to the large number of Gd−DTPA complexes attached to a single molecule but also to a higher ionic relaxivity, i.e., relaxivity per gadolinium. This strong relative increase in ionic relaxivity (up to 36 $mM^{-1}\ s^{-1}$ at 20 MHz, 23 °C) with increasing generation of the dendrimer (higher molecular weights) was ascribed to the slower molecular tumbling of the gadolinium complex, i.e., higher rotational correlation times.[15] However, it was demonstrated that the ionic relaxivity reaches a plateau value for the higher generations of the dendrimer as a result of a limited water exchange rate.[13,16] Researchers at Schering AG (Berlin, Germany) developed another class of dendritic contrast agents: Gadomer-17, a polylysine-based contrast agent (MW = 17453) with 24 gadolinium−1,4,7,10-tetrakis(carboxymethyl)cyclododecane (Gd−DOTA) complexes.[17,18] Gadomer-17 has an ionic relaxivity of 17.3 $mM^{-1}\ s^{-1}$ (20 MHz, 39 °C). Recently, Kobayashi and co-workers[14,19] reported the synthesis of dendritic contrast agents composed of poly(propylene imine) dendrimers. Remarkably, their results showed no "saturation" of the ionic relaxivity upon increasing generation of the poly(propylene imine) dendrimer.

These literature data prompted us to report our results on the relaxivities of different generations of Gd−DTPA-based poly(propylene imine) dendrimers. In our study, a convenient and versatile methodology for the synthesis of Gd−DTPA-terminated poly(propylene imine) dendrimers as MR contrast agents is presented. Additionally, a series of combined gadolinium and yttrium complexes of the fifth-generation DTPA-terminated dendrimer was prepared, and the influence of loading gadolinium on the corresponding ionic relaxivities was investigated. From our experience with highly charged, multivalent architectures[20,21] it is known that aggregation of the dendrimers can occur easily under certain conditions. Since aggregation of the contrast agent can have profound implications on its effective molecular weight, and hence on its behavior as contrast agent, its behavior in solution is studied using atomic force microscopy (AFM) and cryogenic transmission electron microscopy (cryo-TEM).

Results and Discussion

Gd−DTPA-Terminated Poly(propylene imine) Dendrimers. Gadolinium-chelating poly(propylene imine) dendrimers of the first, the third, and the fifth generations (with, respectively, 4, 16, and 64 end groups) were synthesized via the reaction with the isocyanate-functionalized DTPA ligand **2** (Scheme 1). The monofunctional model compound $\mathbf{3}_{n=1}$ was prepared by the reaction of **2** with propylamine.

Therefore, *tert*-butyl ester-protected DTPA building block **1** was prepared via an easily accessible four-step literature procedure described by Anelli et al. and Williams et al.[22,23] Conversion of the primary amine to the corresponding isocyanate with di-*tert*-butyl tricarbonate in freshly distilled dichloromethane (DCM) furnished the isocyanate-activated DTPA analogue **2**. The reaction of the *tert*-butyl ester-protected DTPA synthon **2** with propylamine or either one of the generations of poly(propylene imine) dendrimers with propylamine was conducted in DCM. Purification with size exclusion chromatography yielded the corresponding *tert*-butyl ester-protected DTPA-terminated poly(propylene imine) dendrimers $\mathbf{3}_n$. Structural characterization of $\mathbf{3}_{n=4}$ with ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and ESI-QTOF spectrometry showed complete modification of the primary amines of the dendrimer. The higher generation dendrimers $\mathbf{3}_{n=16,64}$ were characterized by means of ^{1}H NMR and IR spectroscopy. Mass spectrometry was not successful for $\mathbf{3}_{n=16,64}$,

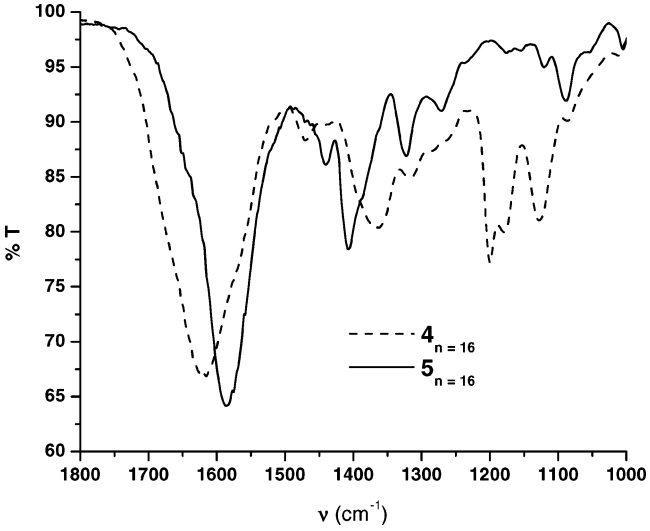


Figure 1. FT-IR (ATR) spectrum of the third-generation DTPA-based dendrimer $\mathbf{4}_{n=16}$ and the corresponding gadolinium complex $\mathbf{5}_{n=16}$.

probably owing to the low degree of ionization of these high molecular weight materials. Cleavage of the *tert*-butyl esters with trifluoroacetic acid (TFA) in DCM afforded the water-soluble gadolinium-chelating dendrimers $\mathbf{4}_n$. TFA adducts were successfully removed via extensive dialysis, and the absence of TFA salts was confirmed with ^{19}F NMR. The synthesis of the corresponding gadolinium complexes $\mathbf{5}_n$ was performed either in demineralized water or in a 0.3 N citrate buffer. Excess (toxic) gadolinium chloride was removed via exhaustive dialysis. Structure elucidation of the gadolinium complexes by means of ^{1}H and ^{13}C NMR spectroscopy was complicated due to the paramagnetic nature of gadolinium. Nevertheless, the formation of the corresponding gadolinium complexes could be confirmed with IR spectroscopy. IR spectroscopy demonstrated that more than 90% of the DTPA ligands were complexated with gadolinium, by the disappearance of the C−O stretching vibration at 1200 cm^{-1} and the shift of the carbonyl stretch from 1622 to 1586 cm^{-1} (Figure 1).

In addition, the gadolinium content was determined with inductively coupled plasma (ICP) analysis. The values for the observed gadolinium content for $\mathbf{5}_{n=1}$ and all generations of dendritic contrast agent were lower than the theoretical values, typically between 70% and 90%. Since IR spectroscopy showed that more than 90% of the DTPA ligands are complexed, and since the ESI-MS data of the amine-terminated poly(propylene imine) dendrimers, used as the starting material, show very few defects,[24] the deviations in the value for the gadolinium content can be attributed to the presence of water or residual salts. Therefore, we used the ideal structure for the dendrimers; i.e., the number of end groups equals 4, 16, and 64 for the first, third, and fifth generations of the poly(propylene imine) dendrimer, respectively.

Cryo-TEM and AFM Studies on the Fifth-Generation Gd−DTPA-Based Dendrimers. ^{1}H NMR spectra of the third and the fifth generations of the DTPA-based dendrimers $\mathbf{4}_{n=16,64}$ in D_2O showed a considerable broadening of signals, due to the aggregation of dendrimers. This is attributed to their multiple zwitterionic character, since both acidic groups and basic tertiary amines are present. This aggregation was strongly pH-dependent; $\mathbf{4}_{n=16}$ was only soluble below pH 2 and above pH 5.5, indicating the presence of an isoelectric point.

Complexation of the dendritic ligands with $GdCl_3$ could lead to aggregation as well, depending on the generation of the dendrimer and the medium. The first-generation dendrimer $\mathbf{4}_{n=4}$ could be complexed with $GdCl_3$ in water, whereas $\mathbf{4}_{n=16}$ and $\mathbf{4}_{n=64}$ could only be successfully complexed in buffered solutions, e.g., 0.3 N citrate buffer at pH 5.8. To exclude self-aggregation of the fifth-generation Gd−DTPA-terminated dendrimer under the experimental conditions used for the relaxivity measurements, cryo-TEM and AFM studies were performed in citrate buffer (Figure 2).

Cryo-TEM is a powerful technique for the elucidation of structures in their natural hydrated state. Cryo-TEM studies on $\mathbf{5}_{n=64}$ in 0.3 N citrate buffer (pH 5.8) confirmed the presence of well-defined isolated dendrimers with an approximate diameter of 5−6 nm, and equally important, no aggregation of dendrimer was observed. The value for the observed diameter is consistent with the calculated value and supported by other literature studies on dendrimers.[25] The contrast of the cryo-TEM image (A) was high (without staining), due to the presence of gadolinium. Topographic AFM images of $\mathbf{5}_{n=64}$ on a silicon wafer are depicted in Figure 2B−E. The gadolinium-loaded dendrimer $\mathbf{5}_{n=64}$ forms well-defined spherical structures on a silicon substrate. Although a constant width of about 32 ± 1 nm for the dendritic structures was found, the measured lateral dimensions are easily overestimated due to tip-convolution effects. Therefore, the values of the measured heights of the structures are a more accurate way to determine the diameter of the spherical structures, especially since soft tapping conditions were applied. The height of these dendritic structures was deduced from the topographic image and was found to be 1.3 ± 0.2 nm, implying flattening of individual dendrimers on a silicon surface.[26,27]

Longitudinal and Transverse Relaxivities of Dendritic Contrast Agents. The ability of Gd−DTPA-based contrast agents to lower the longitudinal and transverse relaxation times (T_1 and T_2, respectively) is commonly expressed in terms of longitudinal and transverse relaxivity (r_1 and r_2, respectively), denoted as $(1/T_{1,2})_{obsd} = (1/T_{1,2})_d + r_{1,2}[Gd]$, where $(1/T_{1,2})_{obsd}$ is the observed relaxation rate in the presence of contrast agent, [Gd] is the concentration of gadolinium, and $(1/T_{1,2})_d$ is the diamagnetic relaxation rate (in the absence of paramagnetic species). Longitudinal and transverse relaxivities were determined for all generations of the Gd−DTPA-based dendritic contrast agents by concentration-dependent measurements of the relaxation times, which gave good linear fits ($R^2 > 0.999$) to the equation $(1/T_{1,2})_{obsd} = (1/T_{1,2})_d + r_{1,2}[Gd]$. All relaxivities were calculated in terms of the actual gadolinium content as determined by ICP measurements.

Higher generations of Gd−DTPA-terminated dendrimers display strongly increased relaxivities (both r_1 and r_2) compared to Gd−DTPA, even when expressed per gadolinium. The fifth-generation dendritic contrast agent shows the highest ionic relaxivities, r_1 = 19.7 mM^{-1} s^{-1} and r_2 = 27.8 mM^{-1} s^{-1}, which are substantially higher than the relaxivities of the parent Gd−DTPA (Table 1, Figure 3). The relaxivity and effective loading of gadolinium of the fifth-generation dendritic contrast agent $\mathbf{5}_{n=64}$ are considerably higher in comparison with those of linear macromolecular analogues.[9]

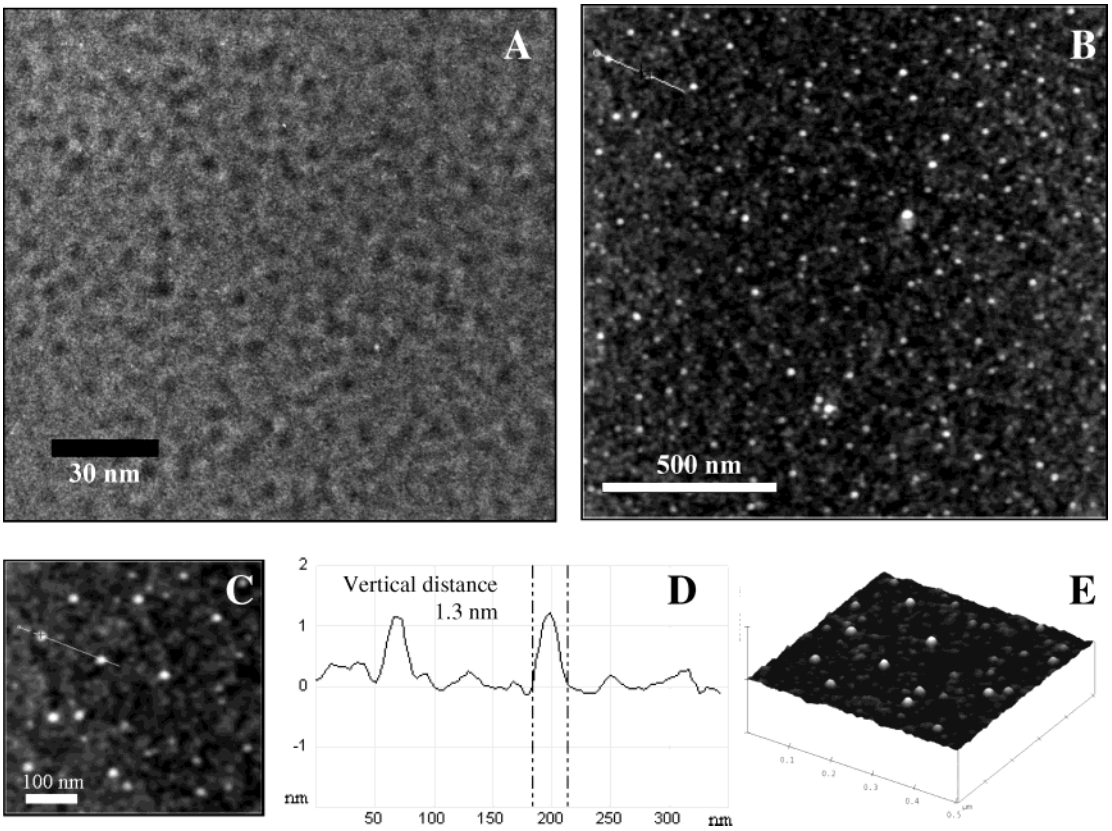


Figure 2. Cryo-TEM image of a 10^{-4} M solution of the fifth-generation dendritic contrast agent $\mathbf{5}_{n=64}$ in 0.3 N citrate buffer, pH 5.8 (magnification 329000×, without staining) (A). Topographic AFM images of the fifth-generation dendritic contrast agent on a silicon wafer deposited from a 0.2 μM solution of $\mathbf{5}_{n=64}$ in citrate buffer (B−E).

Table 1. Longitudinal and Transverse Relaxivities (r_1 and r_2) of Gd−DTPA-Based Dendrimers 5_n[a]

	ionic r_1[b] (mM^{-1} s^{-1})	ionic r_2[b] (mM^{-1} s^{-1})	r_2/r_1 ratio	theoretical no. of Gd ions	molecular r_1[b] (mM^{-1} s^{-1})	molecular r_2[b] (mM^{-1} s^{-1})	MW
Gd−DTPA	4.2 ± 0.1	4.7 ± 0.1	1.1	1	4.2	4.7	592
$\mathbf{5}_{n=1}$ **(G0)**	8.2 ± 0.2	9.6 ± 0.2	1.2	1	8.2	9.6	748
$\mathbf{5}_{n=4}$ **(G1)**	11.7 ± 0.2	13.0 ± 0.2	1.1	4	46.8	52.0	3071
$\mathbf{5}_{n=16}$ **(G3)**	16.0 ± 0.2	19.7 ± 0.2	1.2	16	256	315	12705
$\mathbf{5}_{n=64}$ **(G5)**	19.7 ± 0.3	27.8 ± 0.3	1.4	64	1261	1780	51242

[a] Measured at 20 °C and 1.5 T in either citrate buffer or water (2D-mixed dual-echo sequence). [b] The ionic relaxivity is defined as the relaxivity per gadolinium. The maximum molecular relaxivity is defined as the ionic relaxivity multiplied by the theoretical number of gadolinium moieties attached to a single dendrimer.

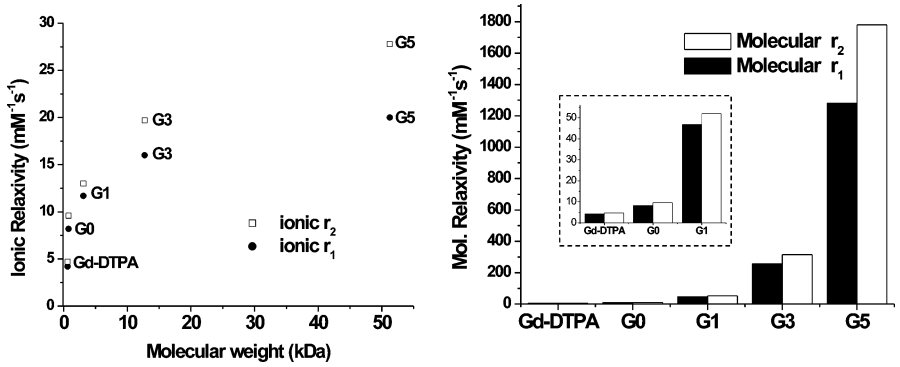


Figure 3. Relaxivities of different generations of Gd−DTPA-based dendrimers $\mathbf{5}_n$: ionic relaxivities versus molecular weight (left) and molecular relaxivities versus the generation of the dendrimer (right). The maximum molecular relaxivity is defined as the ionic relaxivity multiplied by the theoretical number of gadolinium moieties attached to a single dendrimer.

The observed gain of the ionic relaxivity for the higher generations of dendrimers is in agreement with reported literature data,[12,17] implying there is an influence of the molecular weight and/or the local mobility of the gadolinium complexes. Furthermore, "saturation" of the ionic relaxivity upon increasing generation of Gd−DTPA-based poly(propylene imine) dendrimer is observed in contrast to the reported study of Kobayashi et al.[14] In their work, the gadolinium-chelating moiety is attached to the dendrimer through a short, rigid linker. The longitudinal ionic relaxivity of their fifth-generation dendritic contrast agent (r_1 = 29 mM^{-1} s^{-1} at 1.5 T) is

Table 2. Longitudinal Relaxivities (r_1) of Combined Yttrium− and Gadolinium−DTPA-Based Dendritic Contrast Agents of the Fifth-Generation Dendrimer (G5-mixed)[a,b]

	fraction of Gd (x_{Gd})	ionic r_1 (mM^{-1} s^{-1})	av no. of Gd ions[b]	av no. of Y ions[b]	molecular r_1[b] (mM^{-1} s^{-1})	MW
G5-mixed-A	0.34	16.6 ± 0.2	21.5	42.5	357	48348
G5-mixed-B	0.68	16.4 ± 0.2	43.2	20.8	708	49826
G5-mixed-C	0.92	17.6 ± 0.4	59.0	5.0	1038	50902
$\mathbf{5}_{n=64}$ (**G5**)	1.00	19.5 ± 0.2	64.0	0	1248	51242

[a] Measured in citrate buffer, pH 5.8, at 1.5 T and 20 °C (inversion−recovery sequence). [b] From the fraction of gadolinium (x_{Gd}) and the theoretical number of end groups for the fifth-generation DTPA-based dendrimer ($n = 64$), the average number of gadolinium moieties per dendrimer and the average number of yttrium moieties per dendrimer were calculated. Multiplication of the average number of gadolinium moieties by the longitudinal ionic relaxivity (r_1) gives the maximum molecular relaxivity.

higher than the longitudinal ionic relaxivity for $\mathbf{5}_{n=64}$ found in this study ($r_1 = 19.7$ mM^{-1} s^{-1}). In the system presented here the Gd−DTPA complex is covalently linked to the poly(propylene imine) dendrimer through a more flexible linker. Consequently, the local mobility of the gadolinium complex in our dendritic system is relatively high.

However, an additional contribution to the relaxivity may arise from paramagnetic interactions between gadolinium moieties, due to the high local concentration of paramagnetic species at the periphery. Recently, Merbach et al. have seen an enhanced electronic relaxation for gadolinium in a binuclear complex, which may prevent the increase of the longitudinal relaxivity for macromolecular systems with fast water exchange.[28] Therefore, a series of combined gadolinium and yttrium complexes of the fifth-generation DTPA-based dendrimer were prepared, varying the ratio between Gd− and Y−DTPA complexes along the periphery. Yttrium was used as a diamagnetic probe with a size similar to that of gadolinium, thus keeping the molecular weight and local mobility comparable. IR spectroscopy data confirmed the formation of the corresponding complexes. The gadolinium and yttrium contents were independently determined by means of ICP analysis. From these values, the fraction of gadolinium (x_{Gd}) was calculated. It was found that gadolinium was preferentially incorporated. The ionic relaxivity was calculated in terms of gadolinium concentration.

In a new set of experiments, the longitudinal ionic relaxivity was determined with MR spectroscopy using an inversion−recovery pulse sequence (Table 2), which gave a value for the ionic relaxivity of $\mathbf{5}_{n=64}$ similar to that determined previously (Table 1). As shown in Table 2, the ionic longitudinal relaxivity increases with increasing fraction of gadolinium (x_{Gd}), accompanied by a minor increase in the molecular weight. Furthermore, the molecular relaxivity increases from 357 to 1248 mM^{-1} s^{-1}. The observed gain in ionic relaxivity is more distinct at higher loadings of gadolinium and can be explained neither in terms of differences in molecular weight nor in terms of packing density. This effect is attributed to magnetic interaction between gadolinium moieties and is directly the result of the high local concentration of Gd−DTPA end groups in a single dendrimer, and can therefore be called a dendritic effect.

Conclusions

A convenient methodology has been presented for the synthesis of Gd−DTPA-terminated poly(propylene imine) dendrimers as contrast agents for MR imaging. AFM and cryo-TEM images of the fifth-generation Gd−DTPA-functionalized dendrimer in citrate buffer showed the presence of well-defined spherical particles with nanoscopic dimensions. Both the longitudinal (r_1) and the transverse (r_2) relaxivities increase considerably on going to higher generations of the Gd−DTPA-based dendrimers. Additionally, a series of combined gadolinium and yttrium complexes of the fifth-generation DTPA-based dendrimers showed a significant increase of the ionic longitudinal relaxivity on going to higher fractions of gadolinium, suggesting a real dendritic effect.

Experimental Section

General Procedures. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification. Water was demineralized prior to use. Dichloromethane was obtained by distillation from P_2O_5. Amine-terminated poly(propylene imine) dendrimers (DAB-*dendr*-$(NH_2)_n$, $n = 4$, 16, and 64) were kindly provided by DSM (Geleen, The Netherlands) and dried prior to use. Di-*tert*-butyl tricarbonate was prepared according to the procedure described by Peerlings et al.[29] Preparative size exclusion chromatography was performed on BIORAD Bio-Beads S-X1 swollen in dichloromethane. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini-2000 300 MHz spectrometer, a Varian Mercury Vx 400 MHz spectrometer, and a Varian Unity Inova 500 MHz spectrometer at 298 K. Chemical shifts are given in parts per million (δ) relative to the peak for tetramethylsilane (TMS). Infrared spectra were recorded at 298 K on a Perkin-Elmer 1605 FT-IR spectrophotometer. Cryo-TEM measurements were performed on vitrified films of the fifth-generation dendrimer in 0.3 N citric acid buffer at pH 5.8. The vitrified films were transferred to a cryoholder and observed in a Philips CM12 microscope at −170 °C (operating at 120 kV). MALDI-TOF spectra were obtained on a Perspective Biosystems Voyager DE-Pro MALDI-TOF spectrometer using α-cyano-4-hydroxycinnamic acid as matrix (University of California, Berkeley). Samples for the AFM study were prepared by drop casting 0.2 μM $\mathbf{5}_{n=64}$ in 0.3 N citrate buffer onto silicon wafers. Control experiments in the absence of the dendrimer were performed as well. AFM images were recorded under ambient conditions using a Digital Instrument Multimode Nanoscope IIIA operating in the tapping mode regime. Microfabricated silicon cantilever tips (NSG01) with a resonance frequency of approximately 150 kHz and a spring constant of about 5.5 N m^{-1} were used. The scan rate varied from 0.5 to 1.5 Hz. All AFM images shown here were subjected to a first-order plane-fitting procedure to compensate for sample tilt. AFM cross-section analysis was done off-line. ES-QTOF-MS experiments were recorded on a Q-TOF Ultima GLOBAL mass spectrometer (Micromass, Manchester, U.K.). MR measurements were performed for each generation of the Gd−DTPA-terminated dendrimers ($n = 4$, 16, and 64), Gd−DTPA reference complex ($\mathbf{5}_{n=1}$), and Gd−DTPA using a clinical head coil and a 1.5 T MRI system (Philips Intera, release 9, Philips Medical Systems, Best, The Netherlands). Five glass tubes containing different concentrations of dendrimer contrast agent (range 0.05−1.0 mM Gd) were prepared (volume 2 mL). To determine T_1 and T_2 relaxation times, a 2D-mixed (single slice TSE, TR_{SE} 1000 ms, TR_{IR} 2260 ms, IR delay 500 ms, TE_1 15 ms, TE_2 100 ms) dual-echo sequence was performed, matrix dimensions 256 × 256 and voxel size 1.0 × 1.0 × 8 mm^3. All relaxation measurements were performed for circular regions

of interest of area 150 ± 10 mm^2 positioned centrally in each sample. The slopes of $1/T_1$ and $1/T_2$ versus the gadolinium concentration were calculated (r_1, r_2), and the ratios r_2/r_1 were determined for Gd−DTPA-functionalized dendrimers. The gadolinium concentration of the solutions used for the relaxivity measurements was determined by means of ICP analysis (Leeman Labs Echelle spectrometer).

Methods. *tert*-Butyl 2-{{Bis{2-[bis(*tert*-butoxycarbonylmethyl)amino]ethyl}amino}}-6-(3-propylaminocarbonylamino)hexanoate ($\mathbf{3}_{n=1}$). The *tert*-butyl ester-protected DTPA synthon **1** was prepared via a covenient four-step synthesis literature procedure of Anelli et al.[22] and Williams et al.[23] The freely accessible primary amine was converted into an isocyanate, **2**, with di-*tert*-butyl tricarbonate. Into a stirred solution of di-*tert*-butyl tricarbonate[29] (0.124 g, 0.47 mmol) in freshly distilled dichloromethane (2.5 mL) was injected a solution of **1** (0.32 g, 0.43 mmol) in dry dichloromethane (2 mL) under an argon atmosphere. The colorless solution was vigorously stirred for 25 min at room temperature, and IR spectroscopy revealed the presence of the characteristic isocyanate absorption at 2272 cm^{-1}. The excess of di-*tert*-butyl tricarbonate was quenched by the addition of two droplets of dry pyridine. Subsequently, a solution of propylamine (77 mg, 1.30 mmol) in dichloromethane (2 mL) was added to a solution of compound **2**, and the solution was stirred for another 10 min. The reaction was monitored with IR spectroscopy and revealed the complete disappearance of the isocyanate signal. The solvent was evaporated to give in 90% yield monofunctional model compound $\mathbf{3}_{n=1}$ as a colorless oil (0.32 g, 0.39 mmol). ^{1}H NMR ($CDCl_3$, 298 K): δ 4.87 (br t, 1H, N*H*), 4.80 (br t, 1H, N*H*), 3.43 (s, 8H, NCH_2COOC$(CH_3)_3$, 3.26 (m, 1H, CONH$(CH_2)_4$C*H*N), 3.14−3.06 (m, 4H, CONHC$H_2(CH_2)_3$ {2H} + $CH_3CH_2CH_2$N*H*CO {2H}), 2.86−2.62 (m, 8H, NCH_2CH_2N {4H} + NCH_2CH_2N {4H}), 1.66−1.23 (m, 8H, CONHCH_2CH_2-(C$H_2)_2$CHN {6H} + CH_3CH_2CH_2NHCO {2H}; s, 45H, COOC-(C$H_3)_3$), 0.84 (t, 3H, CH_3CH_2). ^{13}C NMR ($CDCl_3$, 298 K): δ 173.0 (1C, C═O), 171.0 (4C, C═O), 158.7 (1C, NH*C*ONH), 81.1 (4C, *C*$(CH_3)_3$), 80.9 (1C, *C*$(CH_3)_3$), 64.3 (1C, CONH$(CH_2)_4$*C*HN), 56.2 (4C, N*C*H_2COOC$(CH_3)_3$), 53.8 (2C, N*C*H_2CH_2N), 50.4 (2C, NCH$_2$*C*H$_2$N), 42.3 (1C, CH_3CH_2*C*H_2NHCO), 40.2 (1C, CONH*C*$H_2(CH_2)_3$), 29.7 (1C, CONHCH$_2$*C*H$_2$), 28.5−28.3 (16C, C(*C*$H_3)_3$ {15C} + CONH$(CH_2)_3$*C*H_2CHN {1C}), 23.8−23.7 (2C, CH_3CH_2*C*H_2NHCO {1C} + CONH$(CH_2)_2$*C*H$_2$ {1C}), 11.6 (*C*H_3-CH_2). FT-IR (ATR): ν (cm^{-1}) 3364 (NH stretch), 2977, 2933, 1724 (C═O stretch), 1639 (C═O amide I stretch), 1567 (C═O amide II stretch), 1457, 1367, 1249, 1220, 1144 (C−O stretch). MALDI-TOF: *m*/*z* [M + H]$^+$ calcd 830.58, obsd 830.60; [M + Na]$^+$ calcd 852.57, obsd 852.58.

2-{{Bis{2-[bis(carboxymethyl)amino]ethyl}amino}}-6-(3-propylaminocarbonylamino)hexanoic acid ($\mathbf{4}_{n=1}$). To a stirred solution of $\mathbf{3}_{n=1}$ (0.19 g, 0.23 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred overnight at room temperature. After evaporation of the solvent a second portion of TFA (2 mL) and dry dichloromethane (4 mL) were added, and stirring was continued overnight. The solution was concentrated in vacuo, resulting in a brown salt. The crude product was dissolved in demineralized water and was extensively dialyzed with a 100 MWCO membrane. The solution was lyophilized, and a white solid, $\mathbf{4}_{n=1}$ (58.5 mg, 0.107 mmol), was obtained in 46% yield. ^{19}F NMR spectroscopy confirmed the successful removal of TFA adducts. ^{1}H NMR (D_2O, 298 K): δ 4.20 (s, 8H, NCH_2COOH), 3.65−3.40 (m, 5H, CONH$(CH_2)_4$C*H*N {1H} + NCH_2CH_2NCH$_2$COOH {4H}), 3.21 (t, 4H, NCH_2-CH_2N), 3.10 (t, 2H, CONHC$H_2(CH_2)_3$), 3.03 (t, 2H, CH_2NHCO), 1.80−1.60 (m, 8H, CONH$(CH_2)_3$CH_2 {2H} + CONHCH$_2$(C$H_2)_2$ {4H} + CH_3CH_2 {2H}), 0.85 (t, 3H, CH_3CH_2). ^{13}C NMR (D_2O, 298 K): δ 174.9 (1C, C═O), 169.1 (4C, C═O), 160.6 (1C, NH*C*ONH), 63.4 (1C, CONH$(CH_2)_4$*C*HN), 55.2 (4C, N*C*H$_2$-COOH), 53.2 (2C, N*C*$H_2CH_2NCH_2$COOH), 46.7 (2C, NCH$_2$-*C*H$_2$N), 42.0 (1C, CH_3CH_2*C*H_2NHCO), 39.7 (1C, CONH*C*H$_2$-(C$H_2)_3$), 29.0 (1C, CONHCH$_2$*C*H$_2$), 27.7 (1C, CONH$(CH_2)_3$*C*H$_2$-CHN), 23.4 (1C, CH_3CH_2*C*H_2NHCO), 22.6 (1C, CONH-(CH$_2)_2$*C*H$_2$), 10.6 (1C, *C*H_3CH_2). FT-IR (ATR): ν (cm^{-1}) 3334 (NH stretch), 2963, 2521 (COOH stretch), 1714 (C═O stretch), 1622 (C═O stretch), 1572 (C═O stretch), 1383, 1217 (C−O stretch), 1148. MALDI-TOF: *m*/*z* [M + H]$^+$ calcd 550.26, obsd 550.32; [M + Na]$^+$ calcd 572.25, obsd 572.30.

Gadolinium Complex $\mathbf{5}_{n=1}$ (G0). The gadolinium complex of $\mathbf{4}_{n=1}$ was prepared by adding a stoichiometric amount of gadolinium chloride hexahydrate (34.2 mg, 0.092 mmol) to a solution of ligand $\mathbf{4}_{n=1}$ (50.4 mg, 0.092 mmol) in water (7 mL). The solution was vigorously stirred for 3 h at room temperature. The pH was continuously monitored and maintained at 7 with 0.1 N NaOH solution. The formation of the complex was monitored with ESI-QTOF-MS. The gadolinium complex $\mathbf{5}_{n=1}$ was dialyzed with a 100 MWCO membrane and lyophilized, resulting in a hygroscopic white powder (quantitative yield). FT-IR (ATR): ν (cm^{-1}) 3350 (NH stretch), 2977, 2933, 1585 (C═O stretch), 1440, 1407, 1324, 1267. ES-QTOF-MS: *m*/*z* [M + H]$^+$ calcd 705.19, obsd 705.17, isotope pattern of gadolinium; [M + Na]$^+$ calcd 727.17, obsd 727.17; [M + 2Na]$^+$ calcd 749.17, obsd 749.16. ICP (Gd): aqueous solution of $\mathbf{5}_{n=1}$, calcd 50.0 μM, obsd 45.4 μM.

Modification of Poly(propylene imine) Dendrimers with DTPA Building Blocks (*tert*-Butyl Ester-Protected) ($\mathbf{3}_n$). $\mathbf{3}_{n=4}$ (G1). An ice-cooled solution of di-*tert*-butyl tricarbonate (0.116 g, 0.44 mmol) in dichloromethane (3.0 mL) was injected into a solution of **1** (0.29 g, 0.40 mmol) in dry dichloromethane (4 mL) under an argon atmosphere. The solution was stirred for 40 min and continuously purged with argon. IR spectroscopy of the colorless solution showed the appearance of the characteristic isocyanate vibration at 2272 cm^{-1}. Residual di-*tert*-butyl tricarbonate was removed by adding two droplets of dry pyridine. The solution of **2** in dichloromethane (4 mL) was slowly added to DAB-*dendr*-$(NH_2)_4$ (29.4 mg, 0.093 mmol) in dry dichloromethane (3 mL). The course of the reaction was monitored with IR spectroscopy, and the solution was vigorously stirred for 16 h. The solution was concentrated in vacuo, and the residue was purified with preparative size exclusion chromatography (Biobeads S-X1 column). The fractions were collected and concentrated in vacuo to give $\mathbf{3}_{n=4}$ (0.208 g, 0.061 mmol, 66%) as a very viscous yellow oil. Thin-layer chromatography showed one spot (MeOH/DCM, 1/9, v/v). ^{1}H NMR ($CDCl_3$, 298 K): δ 5.72 (br, 4H, N*H*), 5.37 (br, 4H, N*H*), 3.42 (s, 32H, NCH_2COOC$(CH_3)_3$), 3.36−3.06 (m, 20H, CONH$(CH_2)_4$C*H*N {4H} + CH_2CH_2NHCO {8H} + CONHCH_2 {8H}), 2.86−2.50 (m, 32H, NCH_2CH_2N {16H} + NCH_2CH_2N {16H}), 2.39 (br, 8H, NC$H_2(CH_2)_2$N), 2.30 (br, 4H, NC$H_2(CH_2)_2$CH_2N), 1.66−1.24 (m, 36 H, CONHCH$_2$C$H_2(CH_2)_2$-CHN {24H}+ NCH_2CH_2CH_2N {8H} + $NCH_2(CH_2)_2CH_2$N {4H}; s, 180H, COOC(C$H_3)_3$). ^{13}C NMR ($CDCl_3$, 298 K): δ 172.9 (4C, C═O), 170.9 (16C, C═O), 159.3 (4C, NH*C*ONH), 81.0 (20C, *C*$(CH_3)_3$), 64.7 (4C, CONH$(CH_2)_4$*C*HN), 56.2 (16C, N*C*H$_2$-COOC$(CH_3)_3$), 54.0 (8C, N*C*H_2CH_2N), 53.6 (2C, N*C*H$_2$-$(CH_2)_2$*C*H_2N), 51.2 (4C, N*C*$H_2(CH_2)_2$N), 50.5 (8C, NCH$_2$*C*H$_2$N), 40.2 (4C, CONH*C*$H_2(CH_2)_3$), 38.5 (4C, N$(CH_2)_2$*C*H$_2$N), 30.2−28.7 (10C, NHCOCH$_2$*C*H$_2$CH$_2$*C*H$_2$ {8C} + NCH_2(*C*H$_2)_2$CH$_2$N {2C}), 28.4 (60C, C(*C*$H_3)_3$), 24.0 (4C, CONH$(CH_2)_2$*C*H$_2$). FT-IR (ATR): ν (cm^{-1}) 3360 (NH stretch), 2977, 2933, 1722 (C═O stretch), 1641 (C═O amide I stretch), 1563 (C═O amide II stretch), 1457, 1367, 1250, 1219, 1144 (C−O stretch). ES-QTOF-MS: *m*/*z* [M + H]$^+$ calcd 3400.5, obsd 3400.0; [M + Na]$^+$ calcd 3422.34, obsd 3422.0; [M + 2Na]$^+$ calcd 3444.3, obsd 3444.0.

$\mathbf{3}_{n=16}$ (G3). An ice-cooled solution of di-*tert*-butyl tricarbonate (0.301 g, 1.15 mmol) in dichloromethane (6.0 mL) was injected into a solution of **1** (0.776 g, 1.04 mmol) in dry dichloromethane (8 mL) under an argon atmosphere. The solution was stirred for 60 min and continuously purged with argon. IR spectroscopy of the colorless solution showed the appearance of the characteristic isocyanate vibration at 2272 cm^{-1}. Residual di-*tert*-butyl tricarbonate was removed by adding two droplets of dry pyridine. The solution of **2** in dichloromethane (14 mL) was slowly added to DAB-*dendr*-$(NH_2)_{16}$ (89.5 mg, 0.053 mmol) in dry dichloromethane (3 mL). The course of the reaction was monitored with IR spectroscopy, and the solution was vigorously stirred for 16 h. The solution was concentrated in vacuo, and the residue was purified with preparative size exclusion chromatography (Biobeads S-X1 column in DCM). The frac-

tions were collected and concentrated in vacuo to give $\mathbf{3}_{n=16}$ (55%, 0.407 g, 0.029 mmoL) as a very viscous yellow oil. ^{1}H NMR ($CDCl_3$, 298 K): δ 6.0−5.3 (br m, 32H, N*H*CON*H*), 3.38 (s, 128H, $NCH_2COOC(CH_3)_3$), 3.16−3.05 (m, 80H, $CONH(CH_2)_4CHN$ {16H} + CH_2CH_2NHCO {32H} + $CONHCH_2$ {32H}), 2.80−2.55 (m, 128H, NCH_2CH_2N {64H} + NCH_2CH_2N {64H}), 2.35−2.10 (br, 84H, $NCH_2(CH_2)CH_2N$ {80H} + $NCH_2(CH_2)_2CH_2N$ {4H}), 1.7−1.1 (m, 100 H, $CONHCH_2CH_2(CH_2)_2CHN$ {72H} + $NCH_2CH_2CH_2N$ {24H} + $NCH_2(CH_2)_2CH_2N$ {4H}; s, 720 H, $COOC(CH_3)_3$). FT-IR (ATR): ν (cm^{-1}) 3364 (NH stretch), 2977, 2934, 1726 (C=O stretch), 1647 (C=O amide I stretch), 1553 (C=O amide II stretch), 1457, 1367, 1253, 1220, 1150 (C−O stretch).

$\mathbf{3}_{n=64}$ (G5). An ice-cooled solution of di-*tert*-butyl tricarbonate (0.330 g, 1.26 mmol) in dichloromethane (6.5 mL) was injected into a solution of **1** (0.783 g, 1.05 mmol) in dry dichloromethane (6 mL) under an argon atmosphere. The solution was stirred for 60 min and continuously purged with argon. IR spectroscopy of the colorless solution showed the appearance of the characteristic isocyanate vibration at 2272 cm^{-1}. Residual di-*tert*-butyl tricarbonate was removed by adding three droplets of dry pyridine. The solution of **2** in dichloromethane (13 mL) was slowly added to DAB-*dendr*-$(NH_2)_{64}$ (94.3 mg, 0.013 mmol) in dry dichloromethane (5 mL). The course of the reaction was monitored with IR spectroscopy, and the solution was vigorously stirred for 16 h. The solution was concentrated in vacuo, and the residue was purified with preparative size exclusion chromatography (Biobeads S-X1 column in DCM). The fractions were collected and concentrated in vacuo to give $\mathbf{3}_{n=64}$ (61%, 0.446 g, 7.89 μmoL) as a very viscous yellow oil. The ^{1}H NMR spectrum showed considerable broadening of peaks. Typical absorptions in the ^{1}H NMR spectrum are listed below. ^{1}H NMR ($CDCl_3$, 298 K): δ 6.4−5.4 (N*H*CON*H*), 3.6−3.3 ($NCH_2COOC(CH_3)_3$), 3.3−2.9 ($CONH(CH_2)_4CHN$ + CH_2CH_2NHCO + $CONHCH_2$), 2.9−2.6 (NCH_2CH_2N), 2.6−2.2 ($NCH_2(CH_2)CH_2N$ + $NCH_2(CH_2)_2CH_2N$), 1.7−1.1 ($COOC(CH_3)_3$ + $CONHCH_2CH_2(CH_2)_2CHN$ + $NCH_2CH_2CH_2N$ + $NCH_2(CH_2)_2CH_2N$). FT-IR (ATR): ν (cm^{-1}) 3357 (NH stretch), 2978, 2934, 1725 (C=O stretch), 1642 (C=O amide I stretch), 1564 (C=O amide II stretch), 1457, 1367, 1250, 1220, 1147 (C−O stretch).

Deprotection of Poly(propylene imine) Dendrimers with DTPA Building Blocks, Giving $\mathbf{4}_n$. $\mathbf{4}_{n=4}$ (G1). To a solution of $\mathbf{3}_{n=4}$ (0.144 g, 0.042 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred overnight at room temperature. After evaporation of the solvent a second portion of TFA (2 mL) and dry dichloromethane (4 mL) were added, and stirring was continued overnight. The solution was concentrated in vacuo, resulting in the TFA salt of $\mathbf{4}_{n=4}$. The crude product was dissolved in demineralized water and was extensively dialyzed with a 1000 MWCO membrane. The solution was lyophilized, and a white solid (0.052 g, 22.8 μmol, 54%) was obtained. ^{19}F NMR spectroscopy confirmed the absence of TFA salts. ^{1}H NMR (D_2O, 298 K): δ 3.99 (s, 32H, NCH_2COOH, 3.40−3.29 (m, 20H, $CONH(CH_2)_4CHN$ {4H} + NCH_2CH_2N {16H}), 3.02−2.80 (m, 44H, NCH_2CH_2N {16H} + $CONHCH_2(CH_2)_3$ {8H} + $N(CH_2)_2CH_2N$ {8H} + $NCH_2(CH_2)_2N$ {8H} + $NCH_2(CH_2)_2CH_2N$ {4H}), 1.80−1.20 (m, 36 H, $CONHCH_2CH_2(CH_2)_2CHN$ {24H} + $NCH_2CH_2CH_2N$ {8H} + $NCH_2(CH_2)_2CH_2N$ {4H}). ^{13}C NMR (D_2O, 298 K): δ 175.1 (C=O), 169.8 (C=O), 169.1 (C=O), 160.6 (NHCONH), 63.5 ($CONH(CH_2)_4CHN$), 55.28 (NCH_2COOH), 53.3 ($NCH_2CH_2NCH_2COOH$), 52.1 ($NCH_2(CH_2)_2CH_2N$), 50.5 ($NCH_2(CH_2)_2N$), 46.7 (NCH_2CH_2N), 39.7 ($CONHCH_2(CH_2)_3$), 36.8 ($N(CH_2)_2CH_2N$), 29.2 ($NHCOCH_2CH_2CH_2$), 27.8 ($NHCO(CH_2)_3CH_2$), 24.3, ($NCH_2CH_2CH_2N$ 23.5 ($NHCO(CH_2)_2CH_2$), 20.7 ($NCH_2(CH_2)_2CH_2N$). FT-IR (ATR): ν (cm^{-1}) 3385 (NH stretch), 2961, 2546 (COOH stretch), 1727 (C=O stretch), 1660 (C=O stretch), 1582 (C=O stretch), 1427, 1147 (C−O stretch), 1135. ES-QTOF-MS: m/z $[M + H]^+$ calcd 2278.1, obsd 2278.0.

$\mathbf{4}_{n=16}$ (G3) was prepared in a fashion similar to the preparation of $\mathbf{4}_{n=4}$. ^{1}H NMR (D_2O): the spectrum exhibits extreme broadening of all signals, which can be ascribed to aggregation of dendrimers. FT-IR (ATR): ν (cm^{-1}) 3364 (NH stretch), 2960, 1622 (C=O), 1470, 1367, 1200, 1127.

$\mathbf{4}_{n=64}$ (G5) was prepared in a fashion similar to the preparation of $\mathbf{4}_{n=4}$. ^{1}H NMR (D_2O, 298 K): the spectrum exhibits extreme broadening off all signals, which might be attributed to aggregation of dendrimers. FT-IR (ATR): ν (cm^{-1}) 3361 (NH stretch), 2956, 2516 (COOH stretch), 1669 (C=O stretch), 1625 (C=O), 1472, 1370, 1179, 1125.

Synthesis of the First-Generation Gd−DTPA-Terminated Dendrimer ($\mathbf{5}_{n=4}$). DTPA-terminated poly(propylene imine) dendrimer $\mathbf{4}_{n=4}$ (20.8 mg, 9.21 μmol) was dissolved in water (7 mL), and the pH was adjusted to 7 with 0.1 N NaOH solution. The solution was vigorously stirred, and a stoichiometric amount of gadolinium chloride hexahydrate (13.7 mg, 36.9 μmol) dissolved in 4 mL of water was added. The pH was maintained at 7 with 0.1 N NaOH. The reaction was stirred for 2 h at room temperature. Subsequently the solution was extensively dialyzed with a 1000 MWCO membrane in water (1 L). The water was refreshed (3×), and the dialyzed solution was lyophilized. A white powder of $\mathbf{5}_{n=4}$ (22.1 mg, 7.20 μmol) was obtained in 78% yield. FT-IR (ATR): ν (cm^{-1}) 3332, 2946, 2934, 1583.1 (C=O stretch), 1389, 1218. ^{1}H NMR (D_2O, 298 K): the spectrum could not be recorded, due to the paramagnetic properties of gadolinium. ICP (Gd): aqueous solution of $\mathbf{5}_{n=4}$, calcd 50.0 μM, obsd 35.5 μM.

General Procedure for the Synthesis of Higher Generations of Gd−DTPA-Terminated Dendrimers ($\mathbf{5}_{n=16,64}$). A solution of DTPA-terminated fifth-generation dendrimer $\mathbf{4}_{n=64}$ (80.0 mg, 2.09 μmol) in 0.3 N citrate buffer, pH 5.8 (12.5 mL), was added dropwise to a solution of gadolinium chloride hexahydrate (74.0 mg, 0.199 mmol) in 0.3 N citrate buffer (25 mL). The solution was stirred for 3 h at room temperature and was extensively dialyzed with a 1000 MWCO membrane in water (5 × 1 L). The suspension was lyophilized, and a yellow powder, $\mathbf{5}_{n=64}$, was obtained.

Data for $\mathbf{5}_{n=16}$ (G3). FT-IR (ATR): ν (cm^{-1}) 3363, 2931, 1586 (C=O stretch), 1440, 1407, 1322, 1088. ICP (Gd): buffered solution of $\mathbf{5}_{n=16}$, calcd 50.0 μM, obsd 37.0 μM.

Data for $\mathbf{5}_{n=64}$ (G5). FT-IR (ATR): ν (cm^{-1}) 3348, 2925, 1582 (C=O stretch), 1405, 1324, 1264, 1087. ICP (Gd): buffered solution of $\mathbf{5}_{n=64}$, calcd 50.0 μM, obsd 36.2 μM.

General Procedure for the Synthesis of Combined Gadolinium− and Yttrium−DTPA-Based Dendrimers. To a solution of DTPA-terminated fifth-generation dendrimer $\mathbf{4}_{n=64}$ in 0.3 N citrate buffer was added dropwise a solution of gadolinium chloride and yttrium chloride in citrate buffer (in total 1.7 equiv of metal per DTPA end group for **G5**-mixed-A and 1.5 equiv of metal per DTPA end group for **G5**-mixed-B and **G5**-mixed-C). The buffered solution was stirred, and stirring was continued overnight at room temperature. The excess (toxic) metal was removed via extensive dialysis with a 1000 MWCO membrane in water (3 × 1 L). The suspension was lyophilized, and a yellow powder was obtained. The ratio between gadolinium and yttrium was determined with ICP analysis.

Data for G5-mixed-A. FT-IR (ATR): ν (cm^{-1}) 3342, 2946, 1583 (C=O stretch), 1409, 1324, 1262, 1087. ICP: Y/Gd ratio calcd 3.98, obsd 1.98.

Data for G5-mixed-B. FT-IR (ATR): ν (cm^{-1}) 3342, 2946, 1582 (C=O stretch), 1407, 1324, 1264, 1086. ICP: Y/Gd ratio calcd 0.99, obsd 0.48.

Data for G5-mixed-C. FT-IR (ATR): ν (cm^{-1}) 3343, 2946, 1583 (C=O stretch), 1408, 1325, 1265, 1087. ICP: Y/Gd ratio calcd 0.25, obsd 0.083.

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