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Improving Proton Relaxivity of Dendritic MRI Contrast Agents by Rigid Silsesquioxane Core

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We report that the cubic octameric polyhedral oligomeric silsesquioxanes (POSS)-core dendrimers, novel gadolinium (Gd) chelators, enhanced the proton relaxivity to Gd^{3+}. The stability of the Gd complex with POSS(G1.5) was similar to those of DOTA and DTPA. From the MTT assay with the primary hepatocytes, the cytotoxicity of $[Gd_2POSS(G1.5)]^{2-}$ showed lower than that of the $[Gd(DOTA)]^{-}$ complex. The detection limits were approximately 100-fold improved than those of DOTA and DTPA-Gd complexes in the MR images.

KEY WORDS: MRI / Contrast Agent / Silsesquioxane / Dendrimer / Gadolinium / POSS /

Magnetic resonance imaging (MRI) is one of powerful diagnostic tools in modern clinical medicine, and paramagnetic complexes, which have the ability to enhance the proton relaxation rate of water tissue, are used as contrast agents for improving sensitivity and specificity. The most commonly used positive contrast agents nowadays are thermodynamically and kinetically stable low molecular weight gadolinium compounds based on a polyaminocarboxylate motif (linear DTPA and cyclic DOTA respectively) for suppressing the toxicity of Gd^{3+} and accelerating the proton relaxation rate of water tissue coordinated to Gd^{3+}.[1] Since contrast agents with high relaxivity can be detected at lower doses, the relaxation mechanism and a number of predominant parameters to achieve sufficient T_1 change have been studied.[1]

Macromolecules are considerable useful for constructing highly-sensitive and functional contrast agents due to the enhancement of relaxivity by the effect of the increased molecular weight which would reduce the rotational tumbling time of the molecule. Numerous high molecular weight complexes have been developed, and many researchers have extensively studied about not only their relaxation mechanisms but also toxicity and clearance system in living bodies for practical usage.[2] In contrast, there is a few reports that mentioned the effect of the core on relaxivity in dendritic chelators.[3] To evaluate the contribution of the structural characteristics on the relaxivity, it is expected to provide new strategy for molecular design of highly-sensitive contrast agents as well as the sophistication of previous products.

Herein, we report cubic octameric polyhedral oligomeric silsesquioxanes (POSS)-core dendrimers[4] used as chelators with Gd^{3+} ions for highly-sensitive positive MRI contrast agents. The relaxivity measurements revealed that the POSS core can enhance the proton longitudinal relaxation of water tissue in the dendrimers. In addition, compared to the Gd complexes with DTPA and DOTA, the observed detection limits of POSS-core dendrimers can be improved approximately 100-fold in the MR image.

Experimental

General

^{1}H NMR and ^{13}C NMR spectra were measured with JEOL EX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. ^{29}Si NMR spectra were measured with JEOL JNM-A400 (80 MHz) spectrometer. Coupling constants (J value) are reported in hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in ^{1}H NMR, $\delta = 77.0$ in ^{13}C NMR) as an internal standard. Masses of dendrimers were determined with an ESI mass spectroscopy or a MALDI-TOF mass spectroscopy (acceleration voltage 21 kV, negative mode) with DHB (2,5-dihydroxybenzoic acid) as a matrix.

POSS(G1.0) (1)[5]

(3-Aminopropyl)triethoxysilane (100 mL, 0.427 mol) and 35–37% HCl (135 mL) in MeOH (800 mL) produced **1** as a white precipitate after 2 d at room temperature. The crude product was obtained after filtration, washing with cold MeOH, and drying. The product was spectroscopically pure in 30% yield (18.8 g). Recrystallization from hot MeOH afforded **1** (4.29 g, 3.66 mmol, 7%) as a white solid. ^{1}H NMR ($(CD_3)_2SO$, 25 °C): δ 8.23 (s, 24H), 2.76 (t, 16H), 1.71 (m, 16H), 0.72 (t, 16H). ^{13}C NMR ($(CD_3)_2SO$, 25 °C): δ 40.53, 20.13, and 7.96. ^{29}Si NMR ($(CD_3)_2SO$, 25 °C): δ −66.4 (s).

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POSS(G1.5) (2)

To a suspension of POSS **1** (1 g, 0.852 mmol) and diisopropylamine (15 mL, 86.1 mmol) in DMF (100 mL), *tert*-butyl bromoacetate (15 mL, 102 mmol) was added, and the reaction mixture was stirred at 60 °C for 16 h. The resulting mixture was concentrated *in vacuo*, and then, added to 100 mL of formic acid, followed by reflux for 24 h. The reaction mixture was concentrated *in vacuo*, and poured into 200 mL of methanol. The compound **2** (240 mg, 0.136 mmol, 16%) was precipitated as a white solid; ^{1}H NMR (D_2O, 400 MHz) δ 3.92 (s, 32H), 3.33 (brs, 16H), 1.83 (brs, 16H), 0.80 (brs, 16H): ^{13}C NMR (D_2O, 100 MHz) δ 161.3, 50.4, 48.2, 26.4, 9.9: ^{29}Si NMR (D_2O, 80 MHz) δ −67.2: MALDI–TOF [$(M + H)^+$] calcd. 1811.08, found 1812.01.

POSS(G2.0) (3)

To a suspension of POSS **1** (1 g, 0.852 mmol) and diisopropylamine (15 mL, 86.1 mmol) in DMF (50 mL) ethyl bromoacetate (9.5 mL, 86.1 mmol) was added, and the reaction mixture was stirred at 60 °C for 16 h. The resulting mixture was concentrated *in vacuo*, and extracted with ethyl acetate. The organic phase was washed with brine and dried over $MgSO_4$. The crude product of ethyl ester (1.07 g) as a yellow oil was obtained after evaporation. Then, the ethyl ester was dissolved in 200 mL of ethylenediamine, and incubated at 60 °C for 24 h. The reaction mixture was concentrated *in vacuo*, and washed with diethyl ether. The white solid **3** (1.30 g, 0.523 mmol, 61%) was precipitated in methanol (100 mL) by adding 35–37% HCl (10 mL); ^{1}H NMR (D_2O, 400 MHz) δ 4.18 (br, 32H), 3.53 (brs, 32H), 3.33 (brs, 16H), 3.14 (brs, 32H), 1.75 (brs, 16H), 0.68 (brs, 16H): ^{13}C NMR (D_2O, 100 MHz) δ 166.7, 59.4, 56.1, 38.9, 36.5, 18.1, 9.9: ^{29}Si NMR (D_2O, 80 MHz) δ −67.4: MALDI–TOF [$(M + H)^+$] calcd. 2484.41, found 2484.97.

POSS(G2.5) (4)

To a suspension of **3** (1 g, 0.403 mmol) and diisopropylamine (15 mL, 86.1 mmol) in DMF (100 mL), *tert*-butyl bromoacetate (15 mL, 102 mmol) was added, and the reaction mixture was stirred at 60 °C for 16 h. The resulting mixture was concentrated *in vacuo*, and then, added to 100 mL of formic acid followed by reflux for 24 h. The reaction mixture was concentrated *in vacuo*, and poured into 200 mL of methanol. The compound **4** (1.05 g, 0.242 mmol, 60%) was precipitated as a white solid; ^{1}H NMR (D_2O, 400 MHz) δ 3.72 (br, 96H), 3.56 (brs, 32H), 3.30 (brs, 32H), 3.20 (brs, 16H), 1.71 (brs, 16H), 0.67 (brs, 16H): ^{13}C NMR (D_2O, 100 MHz) δ 175.1, 172.1, 61.7, 61.2, 59.2, 51.5, 37.5, 20.8, 10.2: ^{29}Si NMR (D_2O, 80 MHz) δ −67.4: ESI–TOF [$(M + 3H)^{3+}$] calcd. 1454.8, found 1454.8.

POSS(G3.0) (5)

To a suspension of **3** (1 g, 0.403 mmol) and diisopropylamine (15 mL, 86.1 mmol) in DMF (50 mL) ethyl bromoacetate (9.5 mL, 86.1 mmol) was added, and the reaction mixture was stirred at 60 °C for 16 h. The resulting mixture was concentrated *in vacuo*, and extracted with ethyl acetate. The organic phase was washed with brine and dried over $MgSO_4$. The crude product of ethyl ester (548 mg) as a yellow oil was obtained after evaporation. Then, the ethyl ester was dissolved in 200 mL of ethylenediamine, and incubated at 60 °C for 24 h. The reaction mixture was concentrated *in vacuo*, and washed with diethyl ether. The white solid **5** (589 mg, 0.103 mmol, 25%) was precipitated in methanol (100 mL) by adding 35–37% HCl (10 mL): ^{1}H NMR (D_2O, 400 MHz) δ 3.46–3.08 (brs, 288H), 2.52 (brs, 16H), 1.43 (brs, 16H), 0.51 (brs, 16H): ^{13}C NMR (D_2O) δ 174.3, 173.3, 58.9, 58.3, 41.9, 39.2, 39.0, 36.8, 36.6, 19.6, 10.0: ^{29}Si NMR (D_2O, 80 MHz) δ −65.7: ESI-TOF-MS calcd. for [$(M + 6H)^{6+}$] 946.8, found 945.4.

POSS(G3.5) (6)

To a suspension of **5** (589 mg, 0.103 mmol) and diisopropylamine (15 mL, 86.1 mmol) in DMF (100 mL), *tert*-butyl bromoacetate (15 mL, 102 mmol) was added, and the reaction mixture was stirred at 60 °C for 16 h. The resulting mixture was concentrated *in vacuo*, and then, added to 100 mL of formic acid followed by reflux for 24 h. The reaction mixture was concentrated *in vacuo*, and poured into 200 mL of methanol. The compound **6** (169 mg, 0.016 mmol, 16%) was precipitated as a white solid; ^{1}H NMR (D_2O, 400 MHz) δ 3.96 (s, 224H), 3.82 (brs, 96H), 3.35 (brs, 96H), 3.23 (brs, 16H), 1.72 (brs, 16H), 0.65 (brs, 16H): ^{13}C NMR (D_2O, 100 MHz) δ 165.5, 164.1, 164.0, 51.9, 51.2, 50.9, 48.7, 46.6, 45.7, 42.7, 36.9, 28.9, 10.7: ^{29}Si NMR (D_2O, 80 MHz) δ −67.3: ESI–TOF [$(M + 7H)^{7+}$] calcd. 1342.1, found 1342.2.

Complexation with Gd^{3+}

An excess volume of $GdCl_3$ was added to a solution of POSS-core dendrimers. After 1 h stirring at room temperature, the mixture was dialyzed with Slide-A-Lyzer® Dialysis Cassettes in water. The Gd content of the complex was determined by extrapolating the obtained fluorescence intensity of Gd^{3+} in the sample to the standard curve with X-ray fluorescence spectroscopy (XRF).

Determination of the Complex Stability Constant $K_{GdL(Thermo)}$

All experiments for determining the complex stability of POSS(G1.5) with Gd^{3+} were according to the previous work.[6] From the spectrophotometric titration data, the equilibrium constant of POSS(G1.5) was determined by the reaction of **1** for Gd^{3+}. Using the stability constants of the Arsenazo-III complexes, the calculations of the conditional stability constants ($K_{GdL(Cond)}$) of the complexes at pH 4.0 were made. By using eq 2, the thermodynamic stability constants ($K_{GdL(Thermo)}$) were calculated from $K_{GdL(Cond)}$ and the stepwise ligand protonation constants (K_n). The K_n values were employed from those of EDTA.[6]

$$GdAz_n + L \Leftrightarrow GdL + nAz \quad (1)$$

$$K_{GdL(Thermo)} = K_{ML(Cond)}(1 + K_1[H^+] + K_1K_2[H^+]^2 + \cdots + K_n[H^+]^n) \quad (2)$$

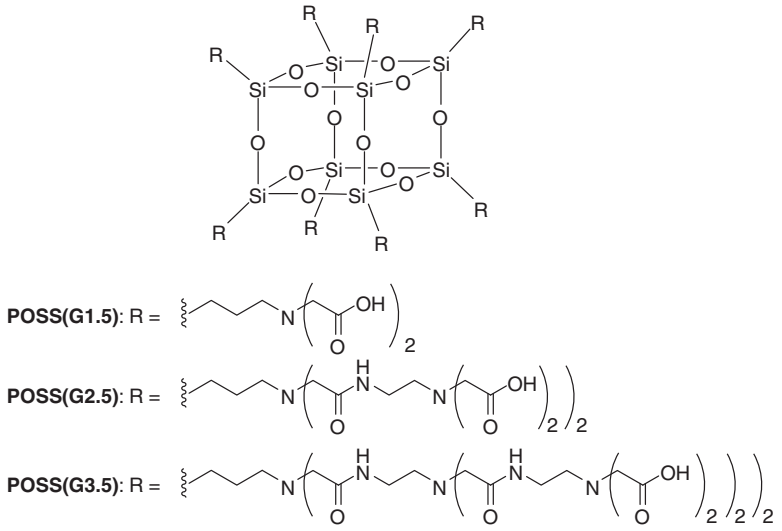


Figure 1. The chemical structures of POSS-core dendrimers.

Isothermal Titration Calorimetry (ITC)

ITC measurements were performed on a Microcal Omega titration calorimeter. An aqueous solution of $GdCl_3$ (1 mM) was injected stepwise (10 μL × 24) into a 50 μM aqueous solution of the dendrimer. All measurements were conducted at 25 °C. The measured heat flow was recorded as a function of time and converted into enthalpy by integration of the appropriate reaction peaks. The number of Gd ions binding to POSS(G1.5) was determined by applying a sequential-site model using Origin software (MicroCal Inc.).

MR Imaging

MR imaging of the samples was carried out using a 7 Tesla Unity Inova MR Scanner (Varian, Palo Alto, CA). Coronal images of the samples were obtained with a T_1-weighted spin-echo sequence. (Repetition time (T_R) 25 ms, echo time (T_E) 12 ms, the field of view was 40 mm, with an image matrix of 256 × 256. Slice thickness was 3 mm.)

Relaxivity

The proton relaxivities of each Gd complex were calculated from the solvent longitudinal relaxation time (T_1) obtained from the inversion recovery method for the Gd complexes in distilled water.[7]

Cell Viability Assay

Primary mouse hepatocytes were used to test the toxic effects of various samples as assessed in the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and incubated at 37 °C in humidified 5% CO_2. 1 d before Gd complex treatment, mouse primary hepatocytes were prepared by the method of collagenase perfusion[8] and were seeded at a 15,000 cells/100 μl/well in 96 well microtiter plates. 3 d after the cells were incubated with Gd complexes, 10 μl of 5 mg/mL MTT in phosphate buffered saline was added to each well, and the plates were kept in a CO_2 incubator for an additional 4 h. After MTT solution was removed, the cells were lysed by adding 100 μl of 10% SDS, 0.01 M NH_4Cl and were incubated overnight. The degree of MTT reduction (*i.e.*, cell viability) in each sample was subsequently assessed by measuring absorption at 600 nm at 37 °C using a plate reader. The absorbances measured from the three wells were averaged, and the percentage MTT reduction was calculated by dividing this average by the absorbance measured from a control sample lacking Gd complexes.

Measurements of τ_R Values with ^2H NMR

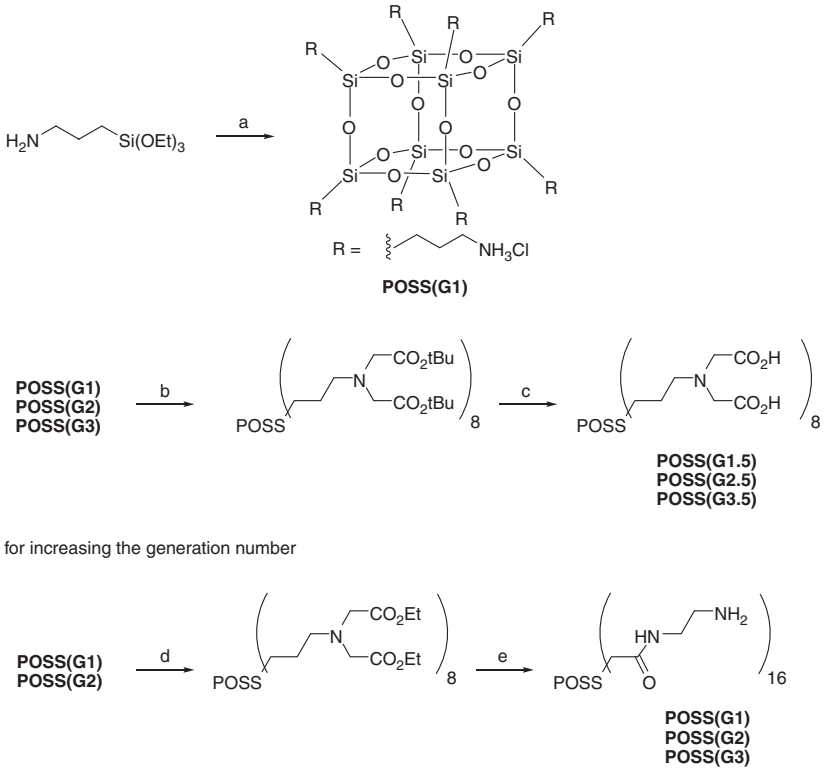
^2H NMR spectra were obtained on JEOL ECX400P NMR spectroscopy operating at 60 MHz. All of the longitudinal relaxation time (T_1) measurements were performed using the standard inversion-recovery method. The temperature inside the probe was maintained at 25 °C ± 0.2 °C by passing a steady flow of air over the sample. The concentrations of each Gd complex were 10 mM. Actual T_1 calculations were performed using the null-point method. The τ_R values were calculated.[9]

The relaxation rate is directly related to the rotational correlation time (eq 3). The quadrupolar coupling constant ($e^2qQ/\hbar$) depends on the hybridization state of the C-atom carrying the ^2H-atom; its value is *ca.* 170 kHz in the case of an sp^3 C-atom.[10]

$$\frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_R \qquad (3)$$

RESULTS AND DISCUSSION

The chemical structures and synthetic scheme of the POSS-core dendrimers (POSS(Gn), n = 1.5, 2.5, or 3.5) are shown in Figure 1 and Scheme 1. According to DTPA, the POSS-core dendrimers were designed for capturing lanthanide ions with



Scheme 1. [a]Reagents: (a) 3-Aminopropyltriethoxysilane, methanol, 35–37% hydrochloric acid, 30%; (b) *tert*-butyl bromoacetate, diisopropylethylamine, dimethylformamide; (c) formic acid, 16% for POSS(G1.5), 60% for POSS(G2.5), and 16% for POSS(G3.5) in two steps, respectively; (d) ethyl bromoacetate, diisopropylethylamine, dimethylformamide; (e) ethylenediamine 61% for POSS(G2.0) and 25% for POSS(G3.0) in two steps, respectively.

high affinity. We synthesized the POSS-core dendrimers of generation 1.5, 2.5, and 3.5 possessing carboxylic acid ends in good yields. Each POSS-core dendrimer was identified by NMR and MS spectrometries, and defect of the dendrons estimated from the ^{1}H NMR spectrum was less than 5%. From the X-ray fluorescence measurements with the residue after the complexation followed by dialysis in water, it was revealed that POSS(Gn) (n = 1.5, 2.5, and 3.5) formed complexes with two, four, and eight Gd^{3+} ions, respectively. The number of Gd ions in the complex with POSS(G1.5) was also determined as two from the curve fitting of the isothermal titration calorimetry spectra.[11] The stability constant of the Gd complex with POSS(G1.5) was evaluated to be 20.0 with the spectrophotometric method using the Arsenazo-III complex.[12] From the MTT assay with the primary hepatocytes, the cytotoxicity of $[Gd_2POSS(G1.5)]^{2-}$ was similar to that of the $[Gd(DOTA)]^-$ complex (Figure 2). Although the stability of the Gd complex with POSS(G1.5) is relatively lower than that of DOTA (25.3) and DTPA (22.2),[6] these results suggest that less significant discharging of Gd^{3+} from the Gd-POSS-core dendrimer complex could occur under biological conditions.

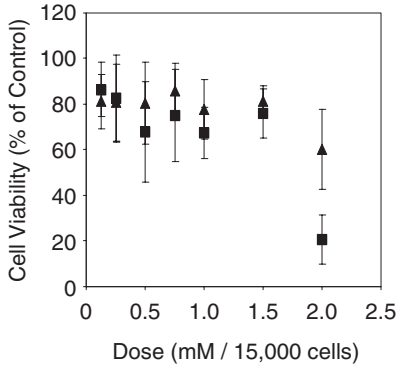


Figure 2. Effect of $[Gd2POSS(G1.5)]^{2-}$ (triangular dots) and $[Gd(DOTA)]^-$ (square dots) on the viability of primary hepatocytes. Cells were incubated with various concentrations of the Gd complexes for 72 h. Results are expressed as viability (% viable cells in comparison with the control) versus complex concentration. Each experiment was performed in triplicate twice and the error bars represent SD values.

Figure 3 shows the MR images of the solutions containing each Gd complex of POSS(G1.5) ($[Gd_2POSS(G1.5)]^{2-}$), POSS(G2.5) ($[Gd_4POSS(G2.5)]^{4-}$), POSS(G3.5) ($[Gd_8POSS(G3.5)]^{8-}$), DOTA ($[Gd(DOTA)]^-$), and DTPA ($[Gd(DTPA)]^{2-}$) at 7 T. From the T_1-weighted images, detection limits of the Gd complexes were evaluated. The proton relaxivity (r_1) of each Gd complex was determined from the T_1 measurements (Figure 4).[13] The contrasts obtained from $[Gd(DOTA)]^-$ and $[Gd(DTPA)]^{2-}$, which are common clinically used contrast agents, can not be discriminated from water below 250 μM concentrations. On the other hand, the Gd complexes of the POSS-core dendrimers gave much clearer contrast even at 30 μM POSS(G1.5) or at 4 μM POSS(G2.5) and POSS(G3.5). It means that the sensitivity of the POSS-core

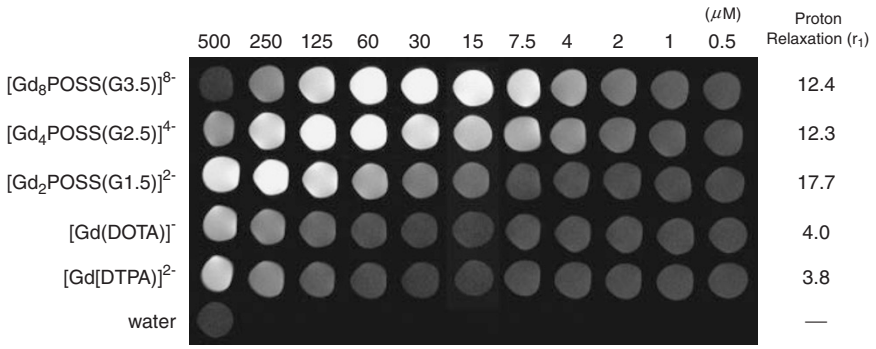


Figure 3. MR imaging of various concentrated Gd complexes and their proton relaxivity. All samples were sealed into 5 mm of glass tubes, and T_1-weighted phantom image was taken at 7 T at 25 °C. The relaxivity r_1 was calculated from the slope of T_1 dependency on the concentration of the paramagnetic species at 7 T.

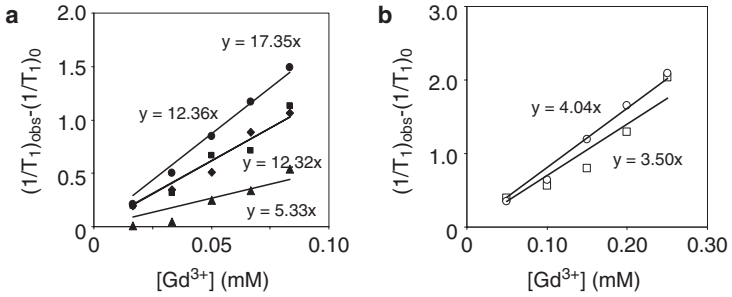


Figure 4. T_1 dependency on Gd^{3+} concentration, (a) $[Gd_2POSS(G1.5)]^{2-}$ (circular dots), $[Gd_4POSS(G2.5)]^{4-}$ (rhombic dots), $[Gd_8POSS(G3.5)]^{8-}$ (square dots), and $[Gd_2PAMAM(G1.5)]^{2-}$ (triangular dots), (b) $[Gd(DOTA)]^{-}$ (circular dots) and $[Gd(DTPA)]^{2-}$ (square dots). The Gd complexes were dissolved in deionized water, and T_1 values were measured at 25 °C. The proton relaxivity r_1 was determined by the slope of the linear approximation.

dendrimer to obtain positive contrasts was improved approximately 100-times larger than those of DOTA and DTPA at 7 T.

The addition of generations, followed by the increase of molecular weights, to the dendrimer-based Gd complexes showed the strong enhancement to the relaxivity.[2a,14] Interestingly, the r_1 value of the $[Gd_2POSS(G1.5)]^{2-}$ ($M_w = 2117$) showed 3-times larger than that of the G1.5 polyamidoamine dendrimer ($M_w = 2889$, $r_1 = 5.3\,mM^{-1}\,s^{-1}$). From previous works, the rigid structure in the Gd complexes can affect the rotational mobility, and the relaxivity was improved without significant increase of the molecular weights.[3] Our results imply that the internal rigidity of the POSS core could provide similar effect to the Gd complexes of the POSS-core dendrimers. This assumption was supported by the decrease of the r_1 values of $[Gd_4POSS(G2.5)]^{4-}$ ($M_w = 4953$) and $[Gd_8POSS(G3.5)]^{8-}$ ($M_w = 10627$), in which the internal rigidity of the POSS core might be less influenced due to the long chain of the dendrons.

In order to evaluate the molecular rotation of the Gd complexes, we compared the rotational correlation time τ_R of the $[Gd_2POSS(G1.5)]^{2-}$ with those of $[Gd(DOTA)]^{-}$ and $[Gd(DTPA)]^{2-}$. The τ_R values of the Gd complexes were determined by ^2H NMR spectroscopy using the Gd complexes deuterated in the α-position to the carboxylate groups.[9,15] From our experiments, the τ_R value of $[Gd_2POSS(G1.5)]^{2-}$ ($\tau_R =$ 2251 ps) was approximately 11-times higher than that of $[Gd(DTPA)]^{2-}$ ($\tau_R = 199$ ps). Compared to those of $[Gd(DTPA)]^{2-}$ and $[Gd(DOTA)]^{-}$ obtained from other workers (58 and 77 ps, respectively), the τ_R value for $[Gd_2POSS(G1.5)]^{2-}$ showed at least 20-fold larger.[9,16] These results suggest that Gd^{3+} in $[Gd_2POSS(G1.5)]^{2-}$ could be highly restricted to rotate. It can be summarized that the POSS core could contribute to the lower tumbling mode of the Gd complexes of the POSS-core dendrimers, and consequently the efficient proton relaxation of water tissue could take place.

CONCLUSION

In conclusion, we report the effect of the core on the proton longitudinal relaxation of water tissue using the POSS-core dendrimers as novel Gd chelators. The relaxivity measurements revealed that the POSS core played a crucial role in the enhancement of the proton longitudinal relaxation of water tissue in the dendrimers. In addition, compared to the Gd complexes with DTPA and DOTA, the observed detection limits of POSS-core dendrimers for the positive contrast can be improved approximately 100-fold in the MR image at the strong magnetic field. Although the structural information should be continued to study, our findings described here should be available for designing novel Gd-based contrast agents, as well as for enhancing the relaxivity of previous ones. The POSS-core dendrimers are promised to be a platform for constructing new MR probes.

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REFERENCES

1. a) K. W.-Y. Chan and W.-T. Wong, *Coord. Chem. Rev.*, **251**, 2428 (2007).
   b) P. Caravan, *Chem. Soc. Rev.*, **35**, 512 (2006).
   c) M. Bottrill, L. Kwok, and N. J. Long, *Chem. Soc. Rev.*, **35**, 557 (2006).
   d) P. Caravan, J. J. Ellison, T. J. McMurry, and R. B. Lauffer, *Chem. Rev.*, **99**, 2293 (1999).
2. a) S. Langereis, A. Dirksen, T. M. Hacheng, M. H. P. van Genderen, and E. W. Meijer, *New J. Chem.*, **31**, 1152 (2007).
   b) D. L. Ladd, R. Hollister, X. Peng, D. Wei, G. Wu, D. Delecki, R. A. Snow, J. L. Toner, K. Kellar, J. Eck, V. C. Desai, G. Raymond, L. B. Kinter, T. S. Desser, and D. L. Rubin, *Bioconjugate Chem.*, **10**, 361 (1999).
   c) M. G. Duarte, M. H. Gil, J. A. Peters, J. M. Colet, L. Vander Elst, R. N. Muller, and C. F. G. C. Geraldes, *Bioconjugate Chem.*, **12**, 170 (2001).
   d) M. J. Allen, R. T. Raines, and L. L. Kiessling, *J. Am. Chem. Soc.*, **128**, 6534 (2006).
   e) Z. Jászberényi, L. Moriggi, P. Schmidt, C. Weidensteiner, R. Kneuer, A. E. Merbach, L. Helm, and É. Tóth, *J. Biol. Inorg. Chem.*, **12**, 406 (2007).
   f) D. A. Falton, E. M. Elemento, S. Aime, L. Chaabane, M. Botta, and D. Parker, *Chem. Commun.*, 1064 (2006).
   g) L. H. Bryant, M. W. Jr, Brechbiel, C. Wu, J. W. M. Bulte, V. Herynek, and J. A. Frank, *J. Magn. Reson. Imaging*, **9**, 348 (1999).
3. a) J. Paris, C. Gameiro, V. Humblet, P. K. Mohapatra, V. Jacques, and J. F. Desreux, *Inorg. Chem.*, **45**, 5092 (2006).
   b) J. Rudovský, P. Hermann, M. Botta, S. Aime, and I. Lukeš, *Chem. Commun.*, 2390 (2005).
   c) G. M. Nicolle, É. Tóth, H. Schmitt-Willich, B. Radüchel, and A. E. Merbach, *Chem. Eur. J.*, **8**, 1040 (2002).
   d) Z. Zhang, M. T. Greenfield, M. Spiller, T. J. Mcmurry, R. B. Lauffer, and P. Caravan, *Angew. Chem., Int. Ed.*, **44**, 6799 (2005).
4. a) R. M. Laine, J. Choi, and I. Lee, *Adv. Mater.*, **13**, 800 (2001).
   b) M. G. Voronkov and V. I. Lavrent'yev, *Top. Curr. Chem.*, 102 (1982).
   c) K. Tanaka, N. Kitamura, K. Naka, and Y. Chujo, *Chem. Commun.*, in press (2008).
   d) K. Naka, M. Fujita, K. Tanaka, and Y. Chujo, *Langmuir*, **23**, 9057 (2007).
   e) K. Tanaka, K. Inafuku, K. Naka, and Y. Chujo, *Org. Biomol. Chem.*, **6**, 3899 (2008).
   f) K. Tanaka, K. Inafuku, and Y. Chujo, *Bioorg. Med. Chem.*, in press (2008).
5. a) M.-C. Gravel and R. M. Laine, *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)*, **38**, 155 (1997).
   b) F. J. Feher and K. D. Wyndham, *Chem. Commun.*, 323 (1998).
   c) M.-C. Gravel, C. Zhang, M. Dinderman, and R. M. Laine, *Appl. Organometal. Chem.*, **13**, 329 (1999).
6. K. Kumar, C. A. Chang, L. C. Francesconi, D. D. Dischino, M. F. Malley, J. Z. Gougoutas, and M. F. Tweedle, *Inorg. Chem.*, **33**, 3567 (1994).
7. R. B. Lauffer, *Chem. Rev.*, **87**, 901 (1987).
8. P. O. Seglen, in "Methods in Cell Biology," 2nd ed., D. M. Preseon, Ed., vol. 13, Academic Press, New York, 1976, chap. 4, p 29.
9. S. Laurent, L. Vander Elst, S. Houźe, N. Guérit, and R. N. Muller, *Helv. Chim. Acta*, **83**, 394 (2000).
10. H. H. Mantsch, H. Saito, and I. C. Smith, *Prog. Nucl. Magn. Reson. Spectrosc.*, **11**, 211 (1977).
11. K. Naka, M. Fujita, K. Tanaka, and Y. Chujo, *Langmuir*, **23**, 9057 (2007).
12. a) M. F. Loncin, J. F. Desreux, and E. Merciny, *Inorg. Chem.*, **25**, 2646 (1986).
   b) M. Kodama, T. Koike, A. B. Mahatma, and E. Kimura, *Inorg. Chem.*, **30**, 1270 (1991).
   c) C. G. Pippin, T. A. Parker, T. J. McMurry, and M. W. Brechbiel, *Bioconjugate Chem.*, **3**, 342 (1992).
13. a) B. P. Hay, E. J. Werner, and K. N. Raymond, *Bioconjugate Chem.*, **15**, 1496 (2004).
   b) J. L. Pascual-Ahuir, I. Tunon, and E. Silla, *J. Comput. Chem.*, **15**, 1127 (1994).
   c) L. A. Castonguay, A. M. Treasurywala, T. J. Caulfield, E. P. Jaeger, and K. E. Kellar, *Bioconjugate Chem.*, **10**, 958 (1999).
14. E. C. Wiener, M. W. Brechbiel, H. Brothers, R. L. Magin, O. A. Gansow, D. A. Tomalia, and P. C. Lauterbur, *Magn. Reson. Med.*, **31**, 1 (1994).
15. A. D. Meltzer and D. A. Tirrell, *Macromolecules*, **25**, 4549 (1992).
16. T.-M. Lee, T.-H. Cheng, M.-H. Ou, C. A. Chang, G.-C. Liu, and Y.-M. Wang, *Magn. Reson. Chem.*, **42**, 329 (2004).

