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(71) Applicants *(for all designated States except US)*: **KYOTO UNIVERSITY** [JP/JP]; 36-1, Yoshida-honmachi, Sakyo-ku, Kyoto-shi, Kyoto, 6068501 (JP). **CANON KABUSHIKI KAISHA** [JP/JP]; 30-2, Shimomaruko 3-chome, Ohta-ku, Tokyo, 1468501 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **YOSHIMURA, Kimihiro** [JP/JP]; c/o CANON KABUSHIKI KAISHA, 30-2, Shimomaruko 3-chome, Ohta-ku, Tokyo, 1468501 (JP). **TSUZUKI, Hidetoshi** [JP/JP]; c/o CANON KABUSHIKI KAISHA, 30-2, Shimomaruko 3-chome, Ohta-ku, Tokyo, 1468501 (JP). **YANO, Tetsuya** [JP/JP]; c/o CANON KABUSHIKI KAISHA, 30-2, Shimomaruko 3-chome, Ohta-ku, Tokyo, 1468501 (JP). **TOMIDA, Yoshinori** [JP/JP]; c/o CANON KABUSHIKI KAISHA, 30-2, Shimomaruko 3-chome, Ohta-ku, Tokyo, 1468501 (JP). **ITO, Shinzaburo** [JP/JP]; c/o Graduate School of Engineering, Kyoto University, Kyoto Daigaku Katsura, Nishikyo-ku, Kyoto-shi Kyoto, 6158510 (JP). **NITAHARA, Satoshi** [JP/JP]; c/o Graduate School of Engineering, Kyoto University, Kyoto Daigaku Katsura, Nishikyo-ku, Kyoto-shi Kyoto, 6158510 (JP). **AOKI, Hiroyuki** [JP/JP]; c/o Graduate School of Engineering, Kyoto University, Kyoto Daigaku Katsura, Nishikyo-ku, Kyoto-shi Kyoto, 6158510 (JP).

(74) Agents: **OKABE, Masao** et al.; No.602, Fiji Building, 2-3, Marunouchi 3-chome, Chiyoda-ku, Tokyo 100-0005 (JP).

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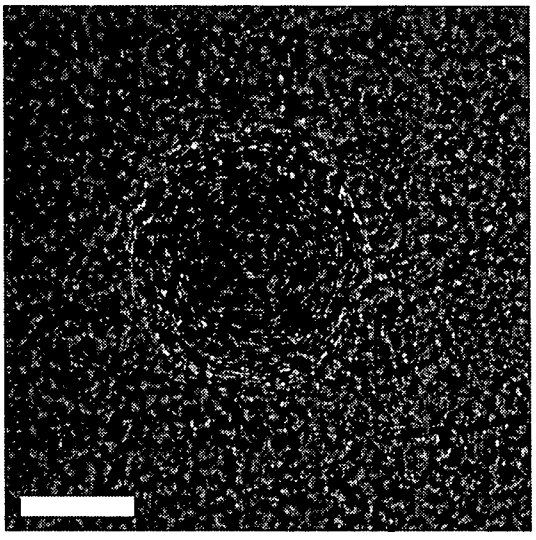
(54) Title: DENDRIMER PARTICLE, MRI CONTRAST MEDIUM, AND METHOD OF MANUFACTURING A DENDRIMER PARTICLE

FIG. 6A



(57) Abstract: Provided is a dendrimer particle having a unit that contains a fluorine atom at a plurality of branch ends of an aliphatic branched polymer (dendrimer). Further, the dendrimer particle is produced using an atom transfer radical polymerization method or the like. Thus, an F-MRI contrast medium, dendrimer particles with high contrast sensitivity and a rigorously controlled size and a method of manufacturing the same are provided.

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DESCRIPTION

DENDRIMER PARTICLE, MRI CONTRAST MEDIUM, AND METHOD OF MANUFACTURING A DENDRIMER PARTICLE

TECHNICAL FIELD

The present invention relates to a fluorine-containing dendrimer particle with high contrast ability, which can be used in a magnetic resonance imaging (MRI) using fluorine as a detection nucleus (hereinafter, referred to as F-MRI), a contrast medium using the same, and a method of manufacturing a dendrimer particle.

BACKGROUND ART

Bio-imaging methods with magnetic nuclear resonance (hereinafter, abbreviated as MRI) have been remarkably developed and widely used in both basic research and clinical application in the medical field as a diagnostic imaging method such as X-ray diagnosis and ultrasonic diagnosis (US).

Currently, MRI generally used for medical purposes is H-MRI that uses proton (^1H) as a detection nucleus and captures a magnetic environment of water molecules in the living body to produce an image. Water molecules are present in almost all regions in the living body. H-MRI can susceptibly capture a difference in magnetic property of protons due to a change in environment around water molecules.

Therefore, the H-MRI is suitable for whole-body imaging. In particular, if there is a difference in magnetic environment between a lesion tissue and a normal tissue, the H-MRI is used as a diagnosis method with more information contents because the difference appears as a change in image to provide disease information.

Here, detectable nuclides in MRI, which are elements with an atomic nuclear spin quantum number of I=1/2, include ^{19}F, ^{23}Na, ^{31}P, ^{15}N, ^{13}C, and the like in addition to ^{1}H, and hence each of them provides information different from one obtained by H-MRI. Of those, ^{19}F has a characteristic of being a stable nucleide which can be detected by NMR-spectroscopy has a detection sensitivity as high as 83% of ^{1}H is a stable element with a natural abundance ratio of 100%, and is applicable to imaging with a conventional H-MRI apparatus. Therefore, F-MRI has been expected to be a next-generation diagnostic method following H-MRI. The most characteristic usage of F-MRI is application to a diagnostic imaging for obtaining tracer information, which utilizes a feature that fluorine atoms are not present in the living body. For instance, positional information about an affected area can be obtained by recognizing an endogenous change attributable to a disease and using a fluorine compound accumulated thereto as a contrast medium. The method can be advantageously used in diagnosis of a morphologically unchanged lesion part which has not been detected by the conventional diagnostic imaging method.

For obtaining the image information peculiar to lesion parts, use of nuclear medical techniques with radioisotopes, such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT), is required. However, there are problems in that many of radioisotopes used in the nuclear medical techniques have short life times and devices for synthesizing the radioisotopes themselves are large-scaled.

Elements that constitute materials used in F-MRI are low-cost, stable isotopes, and hence have no problems in the nuclear medicine as described above. The use of F-MRI can also bring out various kinds of information including chemical shift, diffusion, and relaxation time in addition to positional information, providing an expectation that a large amount of diagnostic information can be obtained.

Here, the term "chemical shift" means a phenomenon that an external magnetic field perceived by an atomic nucleus varies depending on the distribution of electrons around the atomic nucleus to cause a slight shift in resonance frequency. If the manner of chemical bonding is different for the same ^{19}F nucleus (for example, if the ^{19}F nucleus bonds to different atoms or atomic groups), the resonant frequency shifts and the chemical shift can be a physical property to be used as a probe for finding out the structure of a molecule containing the ^{19}F.

In addition, the nuclear spin of an atomic nucleus excited by irradiation of RF pulses in MRI measurement shows

uniform phases at the beginning. However, the phases become nonuniform rapidly by relaxation and the vector sizes decrease rapidly. This is called the T2 relaxation. Simultaneously, some of the downward spins return to upward spins and the vector size in the vertical direction recovers slowly. This is called the T1 relaxation. The T1 relaxation is a process by which a magnetization vector in the vertical direction recovers exponentially with time and the T1 value (T1 relaxation time) is defined as a time (time constant) to return to 1-1/e (63.2%) of the original value. On the other hand, the T2 relaxation is a process by which a magnetization vector in the horizontal direction decreases exponentially with time. The T2 value (T2 relaxation time) is defined as a time (time constant) until a signal attenuates from the maximum (initial value) to 1/e (36.8%).

In addition, the diffusion of object molecules for measurement affects on the MRI signal intensity. For instance, strong diffusion leads to nonuniform phases, causing a decrease in MRI signal intensity. Thus, information about the Brownian movement of molecules in the tissue can be obtained based on the decrease in MRI signal intensity.

Further, useful diagnostic information in which anatomical information (information about the coordinate axis in the living body) coexists with functional information (information about the lesion part) may be

obtained by simultaneously taking F-MRI and H-MRI images at a single diagnosis and then superimposing one on another.

As for studies on F-MRI contrast mediums, studies using an anticancer agent or antibiotic containing a fluorine atom or a fluorinated saccharide are disclosed in Mag. Res. Med. 17, 189 (1991) and the like. Those studies have high clinical significances because of reflection of the information about in-vivo drug effect and metabolism, but the compounds are insufficient in contrast sensitivity. Visualization of those compounds requires massive dosage and long-time imaging. Thus, the compounds have not been yet applied to real clinical fields.

In this way, at present, F-MRI has not been applied to any clinical application. This is partly because, as mentioned above, there is no contrast medium with sufficient sensitivity and performance. Therefore, the development of a new contrast medium, particularly one with improved sensitivity, has been desired for clinical application.

In this regard, Japanese Patent Application Laid-open No. H07-097340 discloses molecular structures in which a fluorine atom and a paramagnetic metal are included in the same molecule in order to obtain high contrasting ability. In addition, US Patent No. 5318770 discloses a plurality of benzene derivatives with trifluoro groups and US Patent No. 5385724 describes the use of the benzene derivatives with trifluoro groups together with paramagnetic metal compounds at the time of imaging. These compounds have no

specificity to behavior in the living body, and hence the clinical efficiencies of these compounds are unclear.

Other exemplary studies of F-MRI contrast media include those using perfluorocarbon (PFC) emulsions as described in H. E. Longmaid et al., Invest. Radiol. 20, 141 (1985). The PFC emulsion has little safety concern because there is a case of administering the PFC emulsion to the living body as artificial blood. In addition, the PFC emulsion has a large number of fluorine atoms, and hence it can be the most advantageous compound among the present fluoro compounds with respect to contrast sensitivity. Contrasting experiments on the blood system, the reticuloendothelial system, and the like using the commercial PFCs have been reported.

Also, neoangiogenesis is actively occurred in a tumor cell site. The newborn blood vessel does not have a fine structure compared with the vessel of the normal tissue blood vessel. Thus, a phenomenon of allowing even fine particles with certain sizes to permeate the blood vessel to the outside (Enhanced Permeability and Retention: EPR effect) has been known. Exemplary studies in which the EPR effect is used for allowing the PFC emulsion particles to be passively incorporated into the tumor site, and the tumor is then subjected to the contrast imaging (R. P. Mason et al., Mag. Res. Imag. 7,475 (1989)).

In the case of F-MRI with PFC emulsions, the quality of the imaging thereof changes basically depending on the

pharmacokinetics of fine particles on the bloodstream. Various kinds of barriers have been found in the body, even in the blood capillary and the retina. As their barrier permeabilities are different depending on their sizes, and naturally, changes in pharmacokinetics occur. In addition, even though the EPR effect is used similarly, the fine particles cannot permeate the newborn blood vessel of the tumor portion if the particle size is too large. Conversely, if the particle size is too small, the fine particles can also permeate through the normal blood vessel walls other than the newborn blood vessel of the tumor portion, so that selective imaging only for the tumor portion cannot be performed. In particular, the emulsion is a molecular self assembly, and the numbers of accumulated molecules among the particles cannot be exactly equalized. Thus, it is difficult to adjust the particle sizes precisely.

DISCLOSURE OF THE INVENTION

In view of the above background art, an object of the present invention is to provide dendrimer particle containing fluorine atoms with precisely controlled sizes.

In addition, an object of the present invention is to provide an F-MRI contrast medium with improved contrast sensitivity and an improved uniformity of size.

Further, an object of the present invention is to provide a method of manufacturing dendrimer particles containing fluorine atoms having precisely controlled

sizes.

The first aspect of the present invention provides a dendrimer particle having a unit that contains a fluorine atom at a plurality of branch ends of an aliphatic highly regular branched polymer.

In addition, the second aspect of the present invention provides an MRI contrast medium containing the dendrimer particle.

Further, the third aspect of the present invention is a method of manufacturing a dendrimer particle including providing a unit that contains a fluorine atom to a plurality of branch ends of an aliphatic highly regular branched polymer with.

Further features of the present invention will become apparent from the following description of exemplary embodiments with reference to the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B each illustrate an H-NMR spectrum of a polyamide amine dendrimer, in which FIG. 1A illustrates that of a raw material (before reaction), PAMAM-NH_2; and FIG. 1B illustrates that of one subjected to acetone re-precipitation and washing with hexane after BMPB bonded thereto.

FIG. 2 illustrates an H-NMR spectrum of PAMAM-g-PTFEMA.

FIG. 3 is a graph illustrating a relationship among

polymerization time, molecular weight (Mn), and molecular weight distribution (Mw/Mn) in polymerization of PAMAM-g-PTFEMA.

FIG. 4 illustrates an F-NMR spectrum of PAMAM-g-PTFEMA.

FIG. 5 is a graph illustrating a relationship between reaction time and molecular weight (Mn) in polymerization reinitiation reaction of PAMAM-g-PTFEMA.

FIGS. 6A and 6B each illustrate a TEM image of the PAMAM-g-PTFEMA, in which FIG. 6A is of a 25.5-hour polymerization sample and FIG. 6B is of a 4-hour polymerization sample (each of the scale bars represents 10 nm).

FIG. 7 is a graph illustrating a relationship among polymerization time, molecular weight (Mn), and molecular weight distribution (Mw/Mn) in the polymerization of PAMAM-g-PTFPMA.

FIGS. 8A1, 8A2, 8A3, 8A4, 8A5, 8A6, 8B1, 8B2, 8B3, 8B4, 8B5, and 8B6 each illustrate an F-MRI image of PAMAM-g-PTFEMA of $Mn=54\times10^4$ $gmol^{-1}$ obtained in Example 5 and a reference material TFT (for evaluating echo time (TE) dependency).

FIGS. 9A, 9B1, and 9B2 each illustrate an F-MRI image of PAMAM-g-PTFEMA of $Mn=54\times10^4$ $gmol^{-1}$ obtained in Example 5 and a reference material TFT (for evaluating of concentration dependency).

FIGS. 10A and 10B each illustrate an F-NMR spectrum obtained in measuring T1-relaxation times by an inversion

recovery method, in which FIG. 10A is of TFT in chloroform solution and FIG. 10B is of PAMAM-g-PTFEMA in chloroform solution.

BEST MODE FOR CARRYING OUT THE INVENTION

In consideration of the aforementioned problems of the F-MRI contrast medium, the inventors of the present invention have arrived at the contrast medium of the present invention, which is capable of controlling the contrast sensitivity and sizes as a result of intensive studies on use of dendrimers.

First, for enhancing contrast sensitivity in F-MRI, a compound with an increased content of fluorine atoms should be used. An increase in content of fluorine atoms may be attained by use of micelles, vesicles, or emulsion obtained by self-assembly of compounds with comparatively lower molecular weights. In this case, however, it is difficult to exactly control the size of the compound. The inventors of the present invention have paid attention to dendrimers to solve the problem, which are polymers with a structure of a branched molecular chain which is branched with high regularity (highly regular branched polymer).

Here, the dendrimer is a general term for the branched polymer with regular dendric branches as described in Hawker, et al., J. Chem. Soc., Chem. Commun. 1990, (15), 1010-1013; D. A. Tomalia, et. al., Angew. Chem. Int. Ed. Engl., 29, 138-175 (1990); J. M. J. Fréchet, Science, 263, 1710 (1994);

Masaaki Kakimoto, Chemistry, vol. 50, page 608 (1995); and the like. Such a molecule has a polymer structure with regular branches extending from the center of the molecule. Thus, as described in, for example, D. A. Tomalia, et. al., Angew. Chem. Int. Ed. Engl., 29, 138-175 (1990), it becomes a globular molecular form because of extremely sterically crowded branch ends generated with increasing molecular weight.

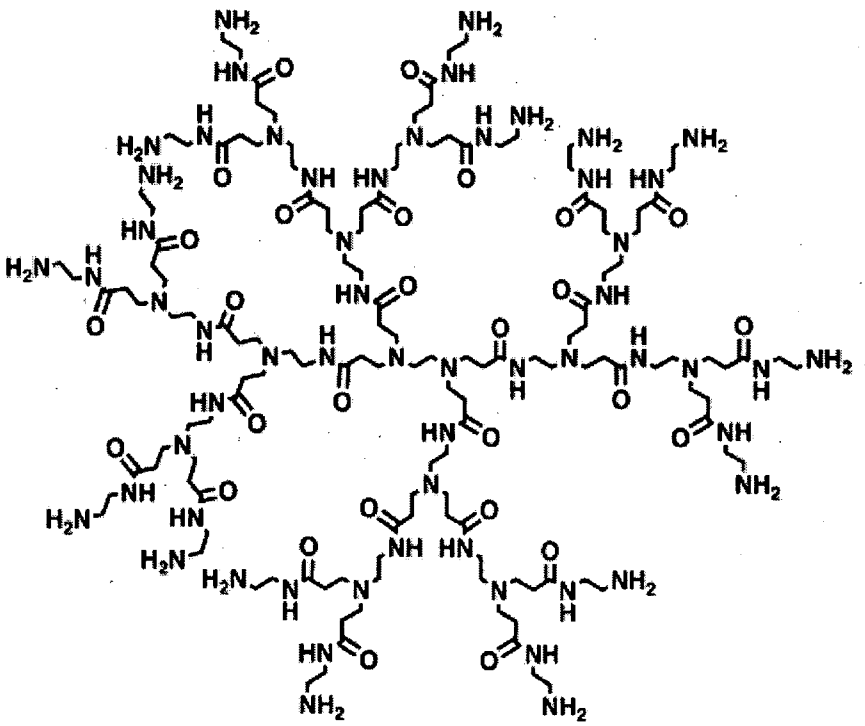
The dendrimer has an advantage in that the size thereof can be exactly controlled by its generation and has a characteristic in that the number of the outermost structures can be regularly changed. Here, the inventors of the present invention have found that the use of many partial structures arranged on the outermost of the dendrimer can effectively increase the content of fluorine and rigorously controls the size thereof.

Here, dendrimers with fluorine atoms include one having a substituent group with a fluorine atom in its benzene ring as disclosed in Japanese Patent Application Laid-Open No. 2002-220468 and one prepared by bonding a fluorine-atom-containing compound to a siloxane dendrimer as disclosed in Japanese Patent Application Laid-Open No. 2003-226611.

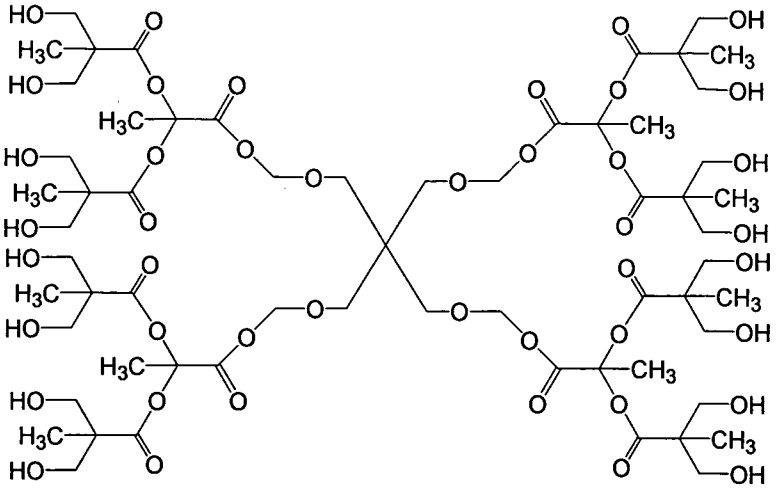
The dendrimers used in the present invention should be of aliphatic compounds but not aromatic or siloxane compounds in terms of biocompatibility and solubility. Therefore, the dendrimer particle of the present invention

has a structure in which a fluorine-containing unit is bound to a plurality of branch ends of an aliphatic dendrimer (highly regular branched polymer).

More specifically, the aliphatic dendrimer which can be used in the present invention preferably includes one having a PAMAM skeleton (polyamideamine) or one having a MPA skeleton. The followings are examples of the molecular structures of such dendrimers: PAMAM dendrimer (generation=2) in formula (I) and MPA dendrimer (generation=2) in formula (II).



(I)



(II)

In particular, the PAMAM with various generations has been already marketed. Both the number of surface functional groups (amino groups) and the particle size can be suitably chosen as listed in Table 1 below.

Table 1

	Number of functional groups	Molecular formula				Particle size
Generation	Number of amino groups	C	H	N	O	(nm)
0	4	22	48	10	4	1.4
1	8	62	128	26	12	1.9
2	16	142	288	58	28	2.6
3	32	302	608	122	60	3.6
4	64	622	1248	250	124	4.4
5	128	1282	2528	508	252	5.7
6	256	2542	5088	1018	508	7.2
7	512	5102	10208	2042	1020	8.8
8	1024	10222	20448	4090	2044	9.8
9	2048	20462	40928	8188	4092	11.4

Further, Lagmuir, 23, 8299-8303, 2007 discloses a study case in which the terminal end of dendrimers is chemically attached to a substrate temporarily and a

fluorine-containing compound is chemically bonded to the dendrimer terminal end using the supercritical condition of carbon dioxide. The state shown in this study case is such that it is fixed on the substrate by chemical bonding, and hence this case cannot be used for a contrast medium required to be administered to the living body. With a method in which a dendrimer on a substrate prepared according to the above study case is microparticulated by cleaving a chemical bond between the substrate and the dendrimer, to the end portion of the dendrimer that had been bonded to the substrate, no fluorine-atom-containing unit is bonded after the cleavage. Accordingly, decrease in contrast sensitivity and the shape out of a globular shape are expected. Further, there is another problem in that the chemical bond between the fluorine-atom-containing unit and the dendrimer is also cleaved when the chemical bond between the substrate and the dendrimer is cleaved, because both the chemical bond between the substrate and the dendrimer and that of the chemical bond between the fluorine-atom-containing unit and the dendrimer are of amide bonds.

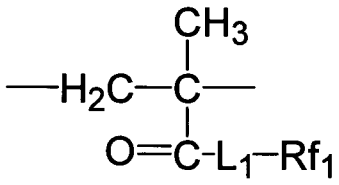
On the other hand, Japanese Patent Publication Nos. H07-057736 and H07-057735 describes the modification of functionalities of the surfaces of PAMAM dendrimers and the like, but do not indicate the use thereof as F-MRI contrast media and their molecular designs therefor.

(Dendrimer particles of the present invention)

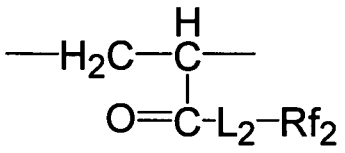
The dendrimer particle of the present invention is characterized by including a fluorine-atom-containing unit at a plurality of branch ends of an aliphatic highly regular branched polymer.

Here, polymers with repeating units that contain fluorine atoms may be used as the fluorine-atom-containing unit.

Examples of the repeating unit may include those represented by the following general formulae (1) and (2):



(1)



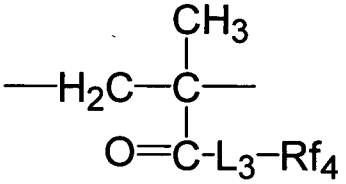
(2)

where Rf_1 and Rf_2 each represent a monomer or an oligomer of a linear or branched alkyl group that contains a fluorine atom, a linear or branched oxyalkyl group that contains a fluorine atom, or a linear or branched oxyalkylene group that contains a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-. The polymerization degree of the oligomer of the linear or branched oxyalkylene group that contains a fluorine atom is preferably 10 or less.

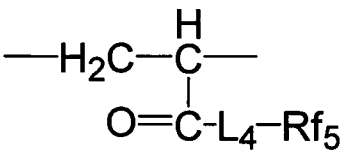
Also in the general formulae (1) and (2), L_1 and L_2 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group

having a hydroxyl group, an oxyalkylene group, and $-NR_1R_2-$, in which R_1 represents hydrogen or an alkyl group and R_2 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

Examples of fluorine-free repeating units may include those represented by the following general formulae (1a) and (2a):



(1a)



(2a)

where Rf_4 and Rf_5 each represent a monomer or an oligomer of a linear or branched alkyl group that does not contain a fluorine atom, a linear or branched oxyalkyl group that does not contain a fluorine atom, or a linear or branched oxyalkylene group that does not contain a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-. The polymerization degree of the oligomer of the linear or branched oxyalkylene group that does not contain a fluorine atom is preferably 10 or less.

Also in the general formulae (1a) and (2a), L_3 and L_4 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_3R_4-$,

in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

On the other hand, in the case of using a compound other than a polymer as a fluorine-containing unit, examples thereof may include one represented by the following general formula (3):

$$Rf_3—L— \quad (3)$$

where Rf3 represents an alkyl group that contains a fluorine atom and may be linear or branched or a linear or branched oxyalkyl group that contains a fluorine atom, in which hydrogen in the alkyl group and the oxyalkyl group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-.

Also in the general formula (3), L represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, a phenylene group, an oxyphenylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

The use of a perfluoroalkyl group or perfluoroalkylene group with many fluorine atoms as Rf_3 of the above formula

(3) can increase the content of fluorine atoms in a dendrimer particle. On the other hand, the use of a perfluoroalkyl group or perfluoroalkylene group with many fluorine atoms causes a decrease in molecular mobility of a fluorine-containing portion, and may lead to a decrease in detection sensitivity in F-MRI. Therefore, insofar as considering the use of the perfluoroalkyl group or perfluoroalkylene group as an F-MRI contrast medium, it is preferable to appropriately control the content of fluorine atoms in the group represented by Rf_3. More specifically, the content of fluorine atoms per unit in the perfluoroalkyl group or perfluoroalkylene group is preferably 1 to 50, more preferably 3 to 30.

Insofar as considering the use of the compound of the present invention as an F-MRI constant medium, the compound is preferably modified to increase its water solubility (more precisely, solubility in body fluids such as the blood).

Such a modification may be the introduction of a hydrophilic group into a dendrimer particle (preferably into the vicinity of the surface thereof). For instance, a hydrophilic group such as -OH, -COOH, $-NH_2$, -O-, or -NH- may be introduced. If the fluorine-containing unit is a polymer, those hydrophilic groups may be introduced into the main chain thereof or may be introduced into the side chain thereof. In addition, with respect to the examples represented by the above general formulae (1) to (3), a

hydrophilic group can be introduced into any of L_1, Rf_1, L_2, Rf_2, L, and Rf_3. More preferably, the hydrophilic group is added to the end of the fluorine-containing unit. In addition, a fluorine-free unit may be also arranged on the outside of the fluorine-containing unit and a hydrophilic group may be then introduced into the fluorine-free unit.

In considering use for a F-MRI contrast medium, the molecular designing is desired to be carried out in consideration of the degree of solvation of the fluorine atoms themselves.

In addition, when the compound of the present invention is used as a tumor-specific F-MRI contrast medium or an inflamed-site-specific F-MRI contrast medium, it is desirable to adjust the particle sizes of the compound within an appropriate range.

Specifically, because large pores with sizes of approximately ten nanometers to several hundreds of nanometers are formed in the blood vessels of the tumor or inflamed site, the particle size is preferably in the range of 10 nm or more and 200 nm or less because the compound of the present invention can enter the tissue of the tumor or inflamed site.

The particles with sizes of 10 nm or more and 200 nm or less can facilitate the entry to the tissue of the tumor or inflamed site from the blood vessel while making it difficult to enter to the normal tissue from the normal capillary vessel.

Further, in view of prolonging the residence time in the tumor tissue, the particle size is preferably 20 nm or more and 200 nm or less, more preferably 50 nm or more and 100 nm or less.

In contrast, in the light of simplicity of the synthesis of dendrimers, the particle size is preferably 2 nm or more and 100 nm or less.

A method of adjusting the particle size may be appropriately selecting the generation of dendrimers to be provided as a core. In addition, in the case of preparing a dendrimer particle using a living radical polymerization method as described later, the particle size can be easily adjusted by controlling the polymerization reaction time.

(Method of manufacturing dendrimer particles)

As a method of providing a fluorine-containing unit at the terminal end of a dendrimer, there are two methods.

One is a method of covalently bonding a fluorine-atom-containing molecule with a comparatively low molecular weight to the terminal end of a dendrimer. The other one is a method of polymerizing fluorine-atom-containing molecules as monomers from the terminal end of a dendrimer.

For distinguishing materials to be used in these two methods, in the following description, the term "fluorine-atom-containing low-molecular-weight compound" means a fluorine-atom-containing molecule as a material to be bonded to the terminal end of a dendrimer without using

a polymerization method. The term "fluorine-atom-containing monomer" means a fluorine-atom-containing molecule as a material to be bonded to the terminal end of a dendrimer using the polymerization method.

(Method of covalently bonding fluorine-atom-containing low-molecular-weight compound)

In this method, an aliphatic dendrimer reacts with a molecule that contains a fluorine atom (fluorine-atom-containing low-molecular-weight compound) represented by the following general formula (6):

$$Rf_3—L—X_1 \quad (6)$$

where Rf_3 represents an alkyl group that contains a fluorine atom and may be linear or branched or a linear or branched oxyalkyl group that contains a fluorine atom, in which hydrogen in the alkyl group and the oxyalkyl group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-.

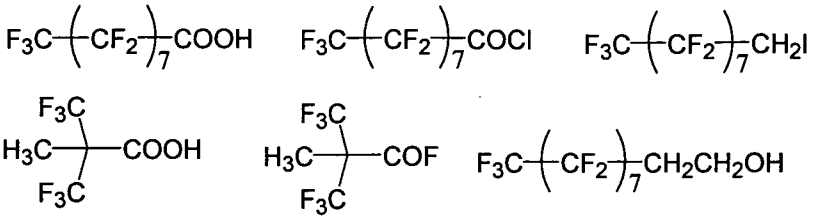
As described above, preferably, Rf_3 may include a group for enhancing its hydrophilic property. For example, an OH group or a COOH group can be added to the terminal end opposite to L. Here, when the fluorine-atom-containing low-molecular-weight compound is caused to bond to the terminal end of the dendrimer, it is preferable that such a group as an OH group and a COOH group be protected by a

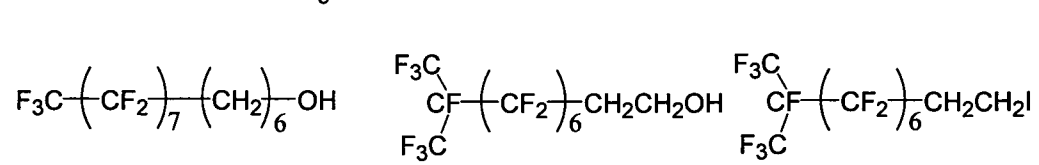
protective group.

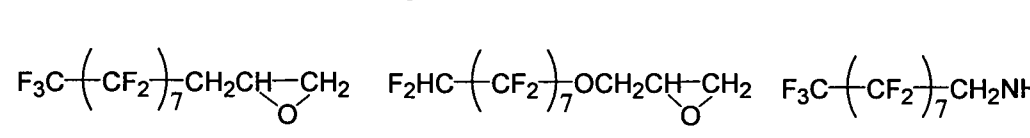
In the general formula (6), X_1 is a functional group to form a covalent bond with a functional group at the terminal end of the dendrimer. Examples of X_1 include an amino group, a hydroxyl group, a carboxyl group, a carboxylic chloride, a carboxylic fluoride, a halogen atom, an epoxy group, an isocyanate group, $-CH=CH_2$, $-C\equiv CH$, and a thiol group. Of those, an amino group, a carboxyl group, a hydroxyl group, a halogen, carboxylic chloride, and carboxylic fluoride are favorably used.

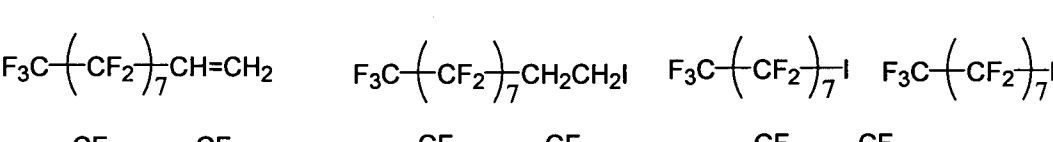
In the general formula (6), L is a linker to bond the functional group X_1 and the group Rf_3 containing a fluorine atom. Examples of L include a single bond (the linker is not substantially present), an alkylene group, an alkylene group having a hydroxyl group, a phenylene group, and an oxyphenylene group. Of those, a single bond, an alkylene group, and an alkylene group substituted with a hydroxy group can be favorably used. Particularly preferred is a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

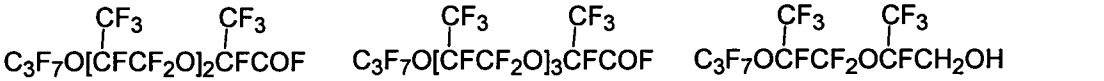
Specific examples of the fluorine-atom-containing low-molecular-weight compound represented by the general formula (6) are illustrated below. However, the present invention is not limited to these exemplified compounds.

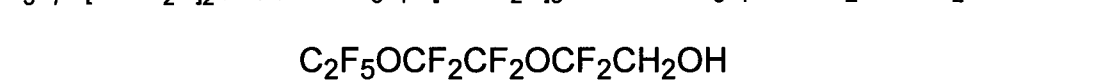


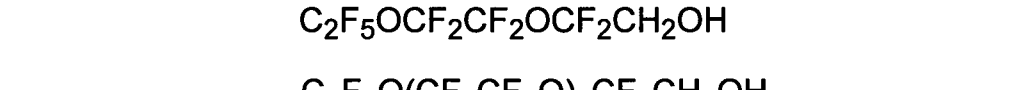










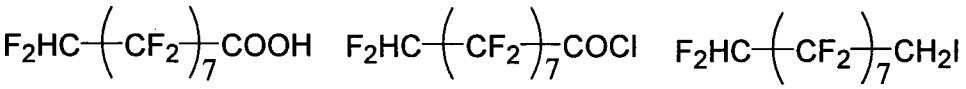


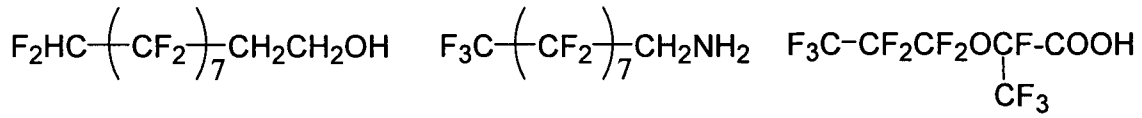
$C_2F_5OCF_2CF_2OCF_2CH_2OH$

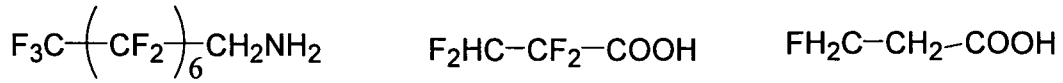
$C_2F_5O(CF_2CF_2O)_2CF_2CH_2OH$

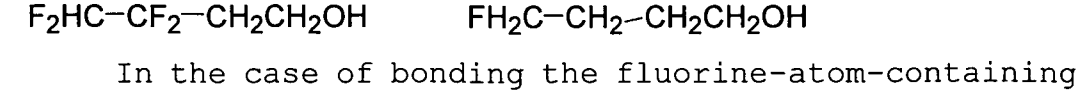
$CF_3O(CF_2CF_2O)_2CF_2COOCH_3$

$C_2F_5(CF_2CF_2O)_2CF_2COOCH_3$







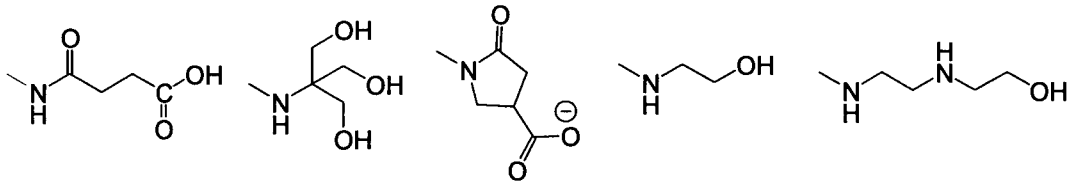


In the case of bonding the fluorine-atom-containing low-molecular-weight compound, because of the aforementioned reasons, it is preferable that the number

of fluorine atoms in the fluorine-atom-containing low-molecular-weight compound be comparatively low. Thus, for attaining an improvement in contrast sensitivity or an increase in fluorine content per particle, a higher generation of a dendrimer to be provided as a core and an increased number of bonded fluorine-atom-containing low-molecular-weight compounds are effective.

For bonding the fluorine-atom-containing low-molecular-weight compound to the terminal end of a dendrimer, the terminal end of the dendrimer should have a reactive functional group. Examples of the functional group include an amino group, a hydroxyl group, a carboxyl group, a halogen atom, an epoxy group, an isocyanate group, $-CH=CH_2$, $-CH\equiv CH$, and a thiol group. Of those, in terms of biocompatibility and easiness of the synthesis of dendrimers, an amino group, a carboxyl group, and a hydroxyl group are preferable.

In addition, spacers with a constant low molecular weight may be used for effectively bonding fluorine-atom-containing low-molecular-weight compounds to dendrimers. For instance, the following functional group may be newly introduced into an amino group terminal end of a dendrimer, whereby the terminal end of the dendrimers is provided with a functional group other than an amino group.



(Method of polymerizing fluorine-atom-containing monomers from the terminal end of a dendrimer)

The polymerization of fluorine-atom-containing monomers from a terminal end of a dendrimer can provide the terminal end of the dendrimer with a polymer including a fluorine-atom-containing repeating unit.

The use of this method can easily increase the fluorine content in the particle.

For polymerizing fluorine-atom-containing reactive monomers, a functional group suitable for the starting point of a polymerization reaction may be bonded to the terminal end of dendrimer in advance.

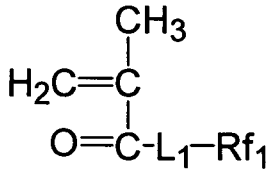
A preferable polymerization method is a living radical polymerization method as described later, particularly an atom transfer radical polymerization method, because this method can control the size of a dendrimer particle precisely, because of a wide range of usable raw materials and easiness of molecular weight control.

The use of this technique can increase the content of fluorine atoms in the finally obtained dendrimer particle.

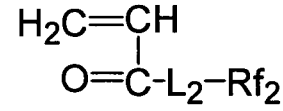
Reactive monomers which can be used herein include those with double bonds and triple bonds; substituents such as a carboxyl group, an amino group, and a hydroxyl group, which are condensation polymerizable; an epoxy group which

is ring-opening polymerizable; and an isocyanate group and a thioisocyanate group, which are addition polymerizable. The sizes of the dendrimer particle may vary as a result of a broaden distribution of molecular weights depending on the polymerization method. In terms of preventing the generation of this problem, any monomer with a double bond, particularly a monomer with an acryl group or a methacryl group is preferable.

For example, acrylic monomers represented by the following general formulae (4) and (5) can be used preferably.



(4)

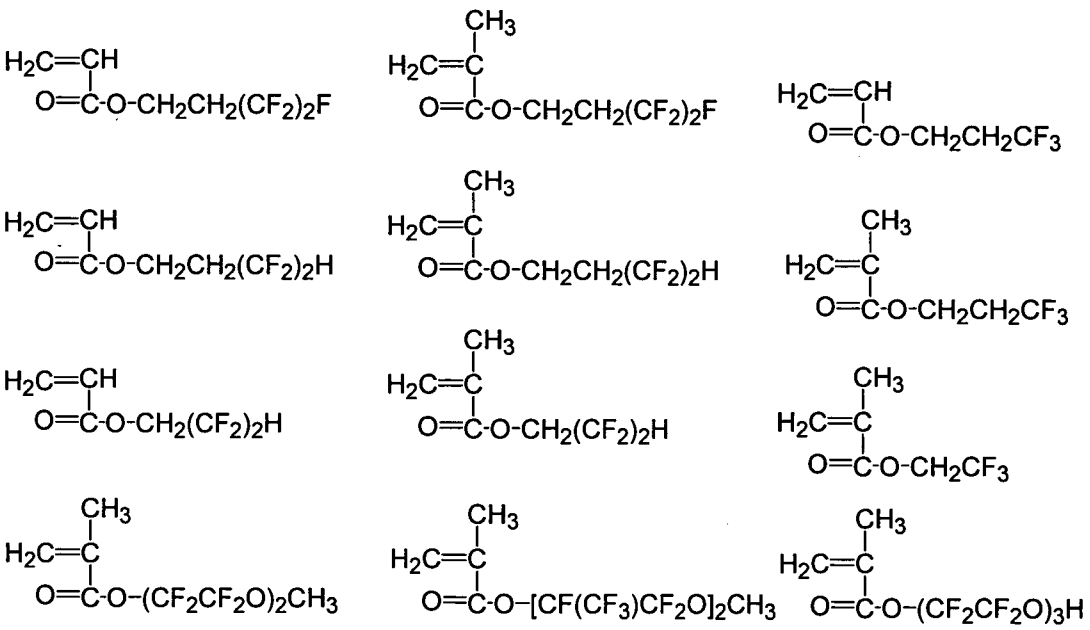


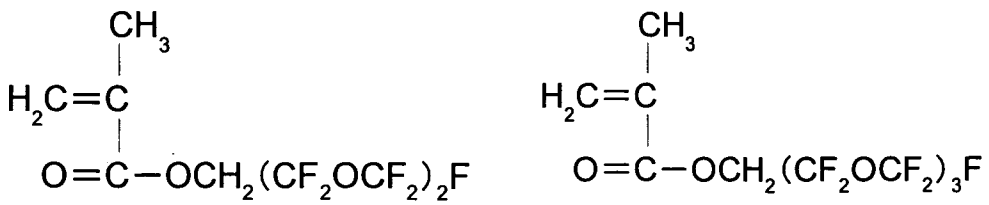
(5)

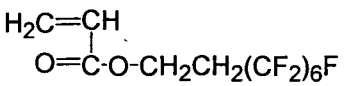
here Rf_1 and Rf_2 each represent a monomer or an oligomer of a linear or branched alkyl group that contains a fluorine atom, a linear or branched oxyalkyl group that contains a fluorine atom, or a linear or branched oxyalkylene group that contains a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-. The polymerization degree of the oligomer of the linear or branched oxyalkylene group that contains a fluorine atom is preferably 10 or less.

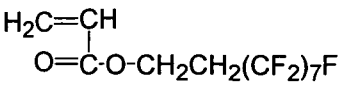
Also in general formulae (4) and (5), L_1 and L_2 each

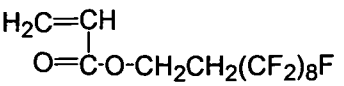
represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_1R_2-$, in which R_1 represents hydrogen or an alkyl group and R_2 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group. Specific examples of fluorine-atom-containing monomers are illustrated below. However, the present invention is not limited to these exemplified compounds. For instance, some of H atoms present on the side chain of the exemplified compound below may be further replaced with F atoms.

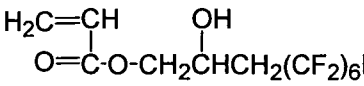


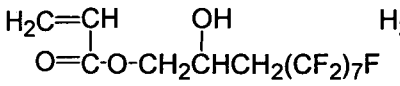


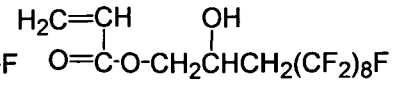


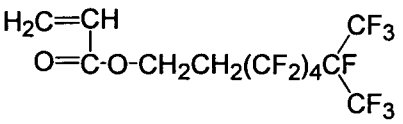


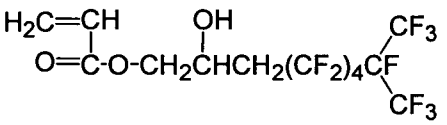


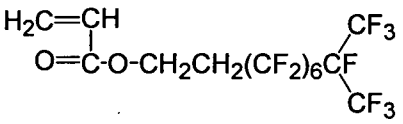


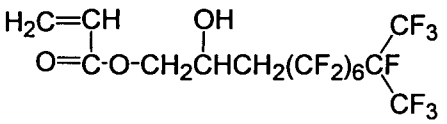


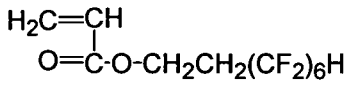


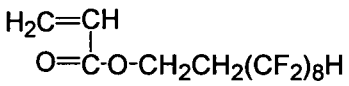


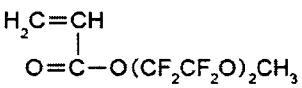


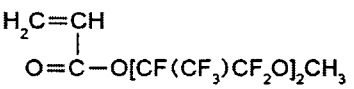


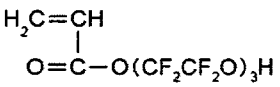










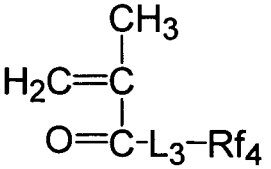


Hydrophilic groups can be introduced into the side chains of these monomers. Some of the examples thereof are exemplified above. The other monomers may be subjected to the replacement of CF_3 or CHF_2 on the side chain with CF_2OH or the like. Alternatively, the side chain may be changed to a polyethylene glycol derivative such as $COO(C_mF_{2m}O)_nR_5$ (m and n each independently represent an integer of 1 or more, preferably 10 or less, and R_5 represents H or an alkyl group).

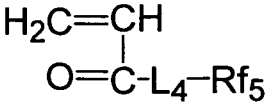
In the polymerization of these monomers, a single kind of monomers or two or more kinds of monomers may be introduced. The introduction of two or more monomers may be carried out by random copolymerization where two or more monomers are polymerized randomly, or may be carried out by block copolymerization where each monomer forms a domain. Further, for a method using two or more monomers, a combination of fluorine-atom-containing monomers and fluorine-atom-free monomers can be used. Thus, additional functions such as solubility and stability can be provided by the fluorine-atom-free monomers.

In terms of providing the solubility, the fluorine-free monomers which can provide the solubility function is desirably located on the outer side than the fluorine-atom-containing monomers. For this purpose, fluorine-atom-free monomers (preferably those with hydrophilic groups) are preferably polymerized after the polymerization of fluorine-atom-containing monomers. In this way, the hydrophilic groups can be arranged near the surface of the particles.

For example, an acrylic monomer represented by one of the following formulae (4a) and (5a) can be preferably used as the fluorine-atom-free monomers:



(4a)



(5a)

where Rf_4 and Rf_5 each represent a monomer or an oligomer

of a linear or branched alkyl group that does not contain a fluorine atom, a linear or branched oxyalkyl group that does not contain a fluorine atom, or a linear or branched oxyalkylene group that does not contain a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-. The polymerization degree of the oligomer of the linear or branched oxyalkylene group that does not contain a fluorine atom is preferably 10 or less.

Also in the formulae (4a) and (5a), L_3 and L_4 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

The conventional polymerization method known in the art can be used as a method of polymerizing these monomers from the terminal end of dendrimer. In general, for obtaining a polymer with a narrow molecular weight distribution, a preferable type of polymerization is one using anionic polymerization, cationic polymerization, or a living radical polymerization method.

The reason of selecting the former anionic

polymerization is to facilitate the generation of molecular chains with a uniform length because the reaction rate at the time of growth is shorter than one at the time of initiating the reaction.

In addition, the latter living radical polymerization has been actively investigated in recent years. Compared with the anionic polymerization, the living radical polymerization is a more preferable polymerization method because of a broader choice of monomers and easiness of setting reaction conditions. The living radical polymerization is based on the establishment of a quick equilibrium between a small amount of growing radical (free radicals) species and a large amount of resting radicals (dormants) species in the growth reaction. Various types of the living radical polymerization have been proposed according to the resting (dormant) chains.

For example, the ATRP (atom transfer radical polymerization) method using a halogenated alkyl as a dormant, the RAFT (reversible addition fragmentation chain transfer) method using dithioesters, the NMP (nitroxide mediated polymerization) method using alkoxyamines, and the optical iniferter method using a dithiocarbamate compound have been proposed.

The ATRP method is a method of polymerizing vinyl monomers using both a polymerization initiator with a highly reactive carbon-halogen bond and a transition metal complex serving as a polymerization catalyst.

In addition, the RAFT method is a method of polymerizing vinyl monomers by adding a chain transfer agent (so-called RAFT agent) with a high chain transfer constant made of dithioesters to a normal radical polymerization system.

In addition, the NMP method is a method including thermally cleaving the carbon-oxygen bond of an alkoxy amine, generating stable nitroxyl radicals and polymer radicals, and polymerizing vinyl monomers to polymer radicals. Under the cleavage, the nitroxyl radicals do not initiate polymerization, but react with only carbon-centered free radicals. The polymer radicals react with the monomers to extend the molecular chain and are then recombined by a coupling reaction with nitroxyl radicals, thereby being present stably as dormant species. However, the use of methacrylate derivatives as monomers has a disadvantage in that a side reaction where nitroxyl radicals abstract hydrogen at the β-position of radical carbons generated in the terminal end of polymerized polymers tends to occur.

The optical iniferter method uses an optical iniferter group such as an N,N-diethyldithiocarbamate group as an initiator and a polymerization reaction is then initiated by ultraviolet radiation.

The living radical polymerization in the present invention may employ any of the above methods. Of those, the ATRP method is preferably used in terms of, for example, a wide range of usable raw materials but the living radical

polymerization is not specifically limited thereto.

Next, the outline of the ATRP method is described.

A polymerization initiator in the ATRP method is not specifically limited as far as it is a compound having at least one of a chlorine atom, a bromine atom, and an iodine atom to be provided as a polymerization initiation point. In general, however, a compound having one or two of chlorine, bromine, or iodine atoms to be provided as polymerization initiation points is used.

Specifically, α-haloesters, α-haloalkylamides, benzyl halides, halogenated alkanes, α-haloketones, α-halonitriles, sulfonyl halides, and the like are used. Of those, α-haloesters are preferred from the viewpoint of easy availability of the raw material.

As the α-haloesters, ethyl 2-bromoisobutyrate or ethyl 2-bromopropionate is exemplified.

As the α-haloalkylamides, 2-chloropropione amide or 2-bromopropione amide is exemplified.

As the benzyl halides, 1-phenylethyl chloride or 1-bromoethyl benzene is exemplified.

As the halogenated alkanes, chloroform or carbon tetrachloride is exemplified.

As the α-haloketones, α-bromoacetone or α-bromoacetophenone is exemplified.

As the α-halonitriles, 2-bromopropionitorile is exemplified.

As the sulfonyl halides, p-toluene sulfonyl bromide

is exemplified.

In order to attain the aspect of the present invention, the polymer formed from monomers should be bonded to each terminal end of a dendrimer. As a method of satisfying such a requirement, two methods are conceivable: one involving carrying out monomer polymerization from each terminal end of a dendrimer as an initiation point of the polymerization reaction and the other one involving polymerizing monomers to have a desired length to be bonded to each terminal end of a dendrimer. However, in consideration of reaction controllability, the use of the former technique is preferable. Therefore, for carrying out the polymerization using the ATRP method, the polymerization reaction with monomers of interest is preferably carried out after bonding a polymerization initiator to be provided as a polymerization initiation point as described above to the terminal end of a dendrimer.

The transition metal complex serving as a polymerization catalyst is not particularly limited, and a metal complex containing, as a central metal, a transition metal (M) selected from the metals belonging to the Group 7 to Group 11 in the periodic table is exemplified. Specific examples thereof include copper compounds having a monovalent copper metal such as cuprous chloride, cuprous bromide, cuprous iodide, and cuprous cyanide; nickel compounds having divalent nickel such as nickel dichloride, nickel dibromide, and nickel diiodide; iron compounds

having a divalent iron such as iron dichloride, iron dibromide, and iron diiodide; and ruthenium compounds having a divalent ruthenium such as ruthenium dichloride, ruthenium dibromide, and ruthenium diiodide.

In addition, the ligand of the transition metal complex is not particularly limited. Examples thereof include 2,2'-bipyridine and derivatives thereof (for example, 4,4'-dinonyl-2,2'-bipyridine and 4,4'-di(5-nonyl)-2,2'-bipyridine), 1,10-phenanthroline and derivatives thereof (4,7-diphenyl-1,10-phenanthroline and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline), tetramethylethylenediamine, pentamethyldiethylene triamine, and hexamethyl(2-aminoethyl)amine.

Any of the dendrimer particles with the aforementioned configurations can be used as a material for an MRI contrast medium.

Hereinafter, the synthesis of fluorine-atom-containing dendrimer and evaluation examples thereof are described, but the present invention is not limited thereto.

(Examples)

(Example 1)

Synthesis of poly(2,2,2-trifluoroethyl methacrylate)polyamideamine dendrimer (PAMAM-g-PTFEMA)

(Reaction 1)

Synthesis of 2-bromo-2-methyl propionyl bromidated polyamide amine dendrimer

Five milliliters of a 20 wt% amino group terminated polyamideamine dendrimer (manufactured by Aldrich Co., G=2) methanol solution was weighed and then subjected to evaporation at 40°C under reduced pressure to distill the methanol off. To the resultant, 5 ml of N,N-dimethylformamide (manufactured by Wako Pure Chemical Industries) was added followed by evaporation at 90°C under reduced pressure to completely distill the solvent off, resulting in 791 mg (0.24 mmol) of a polyamideamine dendrimer as a pale yellow, viscous product.

H-NMR result of the pale yellow, viscous product: H-NMR (d ppm, in D_2O) 2.3 ($-CH_2CH_2CONH-$), 2.5 ($-CH_2CH_2N<$), 2.6 ($-CH_2CH_2NH_2$), 2.7 ($-NCH_2CH_2CO-$), 3.1-3.2 ($-CONHCH_2CH_2-$) (refer to FIG. 1A).

The thus obtained polyamideamine dendrimer was dissolved in 10.7 ml of N,N-dimethylformamide. To This mixture, 550 µl (4.0 mmol) of triethyl amine (manufactured by Wako Pure Chemical Industries) and 324 µl (4.0 mmol) of pyridine (manufactured by Aldrich Co.) was added, followed by stirring at 0°C under nitrogen atmosphere. To this solution, 2.47 ml (0.020 mol) of 2-bromo-2-methylpropionyl bromide (BMPB) (manufactured by Aldrich Co.) was added dropwise and 27 mL of N,N-dimethylformide was then added, followed by stirring for 45 minutes. The reaction temperature was returned to room temperature and the mixture was then stirred for 2 hours, followed by further reaction in a hot bath at 60°C for 48 hours. After terminating the

reaction, the resultant was subjected to evaporation at 90°C and then dried under vacuum, resulting in a brown viscous product. This product was dissolved in methanol and then repeatedly precipitated three times in acetone as a solvent. Further, the resultant was washed with hexane, resulting in a white powder. H-NMR result of the white powder: H-NMR (d ppm, in D_2O) 1.8 (-COC(CH_3)2Br), 2.7 (-CH_2CH_2CONH-), 2.8 (-NCH_2CH_2CO-), 3.0 (-CH_2CH_2N<), 3.2-3.3 (-$CH_2CH_2NH_2$), 3.5 (-CONHCH_2CH_2-) (refer to FIG. 1B).

In the H-NMR spectrum, a new signal derived from a methyl proton in BMPB was observed, revealing that polymerization initiation groups were introduced into fourteen of sixteen NH_2 ends of the polyamideamine dendrimer.

(Reaction 2)

Synthesis of poly(2,2,2-trifluoroethyl methacrylate)polyamideamine dendrimer

Living radical polymerization of 3.53 g (21 mmol) of 2,2,2-trifluoroethyl methacrylate (manufactured by Aldrich Co.) was carried out using 2.67 mg (5.0×10^{-4} mmol) of the product obtained in Reaction 1 as a polymerization initiator, 49.9 mg (0.12 mmol) of 4,4'-di(5-nonyl)-2,2'-bipyridine (manufactured by Aldrich Co.) as a ligand, 6.53 mg (0.070 mmol) of copper chloride (I) (manufactured by Wako Pure Chemical Industries) as a catalyst, and 508 µl of N,N-dimethylformamide as a solvent. A polymerization solution was prepared by subjecting these agents to three

cycles of freeze-pump-thaw degassing with each of a rotary pump and a diffusion pump and then mixing the agents. The solution was loaded into a degassed polymerization tube, followed by sealing. Then, the polymerization reaction was carried out in an oil bath at 90°C for 25 minutes. After terminating the polymerization, the obtained product was re-precipitated in methanol as a solvent, resulting in a white solid product. H-NMR result of the white solid product: H-NMR (Δppm, in $CDCl_3$) 4.3-4.4 ($-COOCH_2CF_3$), 1.9-2.1 ($-CH_2C(CH_3)-$), 0.9-1.1 ($-CH_2C(CH_3)-$) (refer to FIG. 2)

In addition, the molecular weight of the polymer was confirmed by GPC (gel permeation chromatography) to be a number average molecular weight (Mn) of 120,700 g/mol and a weight average molecular weight (Mw) of 153,500 g/mol.

(Example 2)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 1 except that the polymerization reaction time of 2,2,2-trifluoroethyl methacrylate was set to 1 hour. The molecular weight of the polymer was confirmed by GPC to be Mn=168,600 g/mol and Mw=250,400 g/mol.

(Example 3)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 1 except that the polymerization reaction time of 2,2,2-trifluoroethyl methacrylate was set to 4 hours. The molecular weight of

the polymer was confirmed by GPC to be Mn=421,800 g/mol and Mw=673,300 g/mol.

(Example 4)

A fluorine-containing dendrimer was synthesized in a manner similar to Example 1 except that the polymerization reaction time of 2,2,2-trifluoroethyl methacrylate was set to 7 hours. The molecular weight of the polymer was confirmed by GPC to be Mn=511,500 g/mol and Mw=673,300 g/mol.

(Example 5)

A fluorine-atom-containing was synthesized in a manner similar to Example 1 except that the polymerization reaction time of 2,2,2-trifluoroethyl methacrylate was set to 25.5 hours. The molecular weight of the polymer was confirmed by GPC to be Mn=542,600 g/mol and Mw=994,900 g/mol. Hereinafter, this is referred to as PAMAM-g-PTFEMA of $Mn=54\times10^4$ g mol^{-1}.

The GPC measurement of PAMAM-g-PTFEMA obtained in each of Examples 1 to 5 described above was carried out. In a region with a short polymerization time, an increase in molecular weight in proportion to the polymerization time was confirmed, and molecular weight saturation (Mn=550,000 g mol^{-1}) at a polymerization time of 5 hours was shown (FIG. 3). Here, in FIG. 3, the solid circle represents Mw and the open circle represents Mw/Mn.

(Example 6: Evaluation of F-NMR)

The obtained white solid product (PAMAM-g-PTFEMA, 25.5-hour polymerization: Example 5) was subjected to

measurement with a 600-MHz NMR device (manufactured by JEOL, JNM-ECA 600) to obtain an F-NMR spectrum thereof. The used solvent was $CDCl_3$ and the used reference substance was $C_6H_5CF_3$. The result was shown in FIG. 4. As shown in FIG. 4, a single peak was observed at approximately -74.5 ppm.

(Example 7: Evaluation of particle configuration)

The product obtained by the 25.5-hour polymerization (PAMAM-g-PTFEMA) (Example 5) and the product obtained by the 4-hour polymerization (PAMAM-g-PTFEMA) (Example 3) were observed with a transmission electron microscope (manufactured by JEOL, JEM-2100F). The results were shown in FIG. 6A and FIG. 6B. The PAMAM-g-PTFEMA obtained by each of the 25.5-hour polymerization (FIG. 6A) and the 4-hour polymerization (FIG. 6B) was found to have an almost globular shape with a particle size of approximately 20 nm.

(Example 8) Reinitiation reaction of poly(2,2,2-trifluoroethyl methacrylate)polyamide amine dendrimer

Living radical polymerization of 0.60 mL (4.2 mmol) of 2,2,2-trifluoroethyl methacrylate (manufactured by Aldrich Co.) was carried out using PAMAM-g-PTFEMA (Mn=511,500) obtained in Example 4 as a polymerization initiator, 1.24 mg (7.9×10^{-3} mmol) of bipyridine (manufactured by Aldrich Co.) as a ligand, 0.39 mg (3.9×10^{-3} mmol) of copper chloride (I) (manufactured by Wako Pure Chemical Industries) as a catalyst, and 1.2 mL of N,N-dimethylformamide as a solvent. A polymerization

solution was prepared by subjecting these agents to three cycles of freeze-pump-thaw degassing with a rotary pump and a diffusion pump and then mixing the agents. The solution was loaded into a degassed polymerization tube, followed by sealing. Then, the polymerization reaction was carried out in an oil bath at 90°C for 4 hours (11 hours in total with the reaction time of 2,2,2-trifluoroethyl methacrylate in Example 4). After terminating the polymerization, the obtained product was re-precipitated in methanol as a solvent, resulting in a white solid product.

In addition, the molecular weight of the polymer was confirmed by GPC (gel permeation chromatography) to be a number average molecular weight (Mn) of 602,000 g/mol and a weight average molecular weight (Mw) of 1,050,000 g/mol.

(Example 9)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 8 except that the polymerization reaction time of 2,2,2-trifluoroethyl methacrylate was set to 5 hours (12 hours in total with the reaction time of 2,2,2-trifluoroethyl methacrylate in Example 4). The molecular weight of the polymer was confirmed by GPC to be Mn=781,000 g/mol and Mw=1,230,000 g/mol.

(Example 10)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 8 except that the polymerization reaction time of 2,2,2-trifluoroethyl

methacrylate was set to 7 hours (14 hours in total with the reaction time of 2,2,2-trifluoroethyl methacrylate in Example 4). The molecular weight of the polymer was confirmed by GPC to be Mn=797,000 g/mol and Mw=1,270,000 g/mol.

The GPC measurement of the product obtained by the reinitiation reaction of TFEMA using PAMAM-g-PTFEMA as a polymerization initiator in each of Examples 8 to 10 described above was carried out. It was confirmed that the reaction further proceeded (FIG. 5).

Further, in FIG. 5, the solid circle represents Mn of the polymer obtained in each of Examples 1 to 5 and the solid triangle represents Mn of the polymer obtained by the reinitiation reaction in each of Examples 8, 9, and 10. In FIG. 5, the abscissa axis represents the reaction time of the reaction with 2,2,2-trifluoroethyl methacrylate. In the plot of the solid triangles, the sum of the reaction time in Example 4 and the reaction time of 2,2,2-trifluoroethyl methacrylate in each of Examples 8, 9, and 10 is used.

(Example 11)

Synthesis of poly(2,2,3,3-tetrafluoropropyl methacrylate)polyamideamine dendrimer (PAMAM-g-PTFPMA)

(Reaction)

Living radical polymerization of 4.73 g (24 mmol) of 2,2,2-tetrafluoropropyl methacrylate (manufactured by Aldrich Co.) was carried out using 3.01 mg (5.6×10^{-4} mmol)

of the product obtained in Reaction 1 as a polymerization initiator, 63.6 mg (0.16 mmol) of 4,4'-di(5-nonyl)-2,2'-bipyridine (manufactured by Aldrich Co.) as a ligand, 7.74 mg (0.078 mmol) of copper chloride (I) (manufactured by Wako Pure Chemical Industries) as a catalyst, and 756 μl of N,N-dimethylformamide as a solvent. A polymerization solution was prepared by subjecting these agents to three cycles of freeze-pump-thaw degassing with a rotary pump and a diffusion pump, followed by mixing. The solution was loaded into a degassed polymerization tube, followed by sealing. Then, the polymerization reaction was carried out in an oil bath at 90°C for 10 minutes. After terminating the polymerization, the obtained product was re-precipitated in methanol as a solvent, resulting in a white solid product.

The molecular weight of the polymer was confirmed by GPC to be $Mn=14.6\times10^4$ g mol^{-1} and $Mw=18.5\times10^4$ g mol^{-1}.

(Example 12)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 11 except that the polymerization reaction time of 2,2,3,3-tetrafluoropropyl methacrylate was set to 20 minutes. The molecular weight of the polymer was confirmed by GPC to be $Mn=23.3\times10^4$ g mol^{-1} and $Mw=33.8\times10^4$ g mol^{-1}.

(Example 13)

A fluorine-atom-containing dendrimer was synthesized in a manner similar to Example 11 except that the

polymerization reaction time of 2,2,3,3-tetrafluoropropyl methacrylate was set to 30 minutes. The molecular weight of the polymer was confirmed by GPC to be $Mn=35.4\times10^4$ g mol^{-1} and $Mw=50.6\times10^4$ g mol^{-1}.

(Example 14)

A fluorine-atom-containing was synthesized in a manner similar to Example 11 except that the polymerization reaction time of 2,2,3,3-tetrafluoropropyl methacrylate was set to 1 hour. The molecular weight of the polymer was confirmed by GPC to be $Mn=90.9\times10^4$ g mol^{-1} and $Mw=136\times10^4$ g mol^{-1}.

(Example 15)

A fluorine-atom-containing was synthesized in a manner similar to Example 11 except that the polymerization reaction time of 2,2,3,3-tetrafluoropropyl methacrylate was set to 2 hours. The molecular weight of the polymer was confirmed by GPC to be $Mn=126\times10^4$ g mol^{-1} and $Mw=185\times10^4$ g mol^{-1}.

(Example 16)

A fluorine-atom-containing was synthesized in a manner similar to Example 11 except that the polymerization reaction time of 2,2,3,3-tetrafluoropropyl methacrylate was set to 6 hours. The molecular weight of the polymer was confirmed by GPC to be $Mn=143\times10^4$ g mol^{-1} and $Mw=187\times10^4$ g mol^{-1}.

The GPC measurement of PAMAM-g-PTFPMA obtained in each of Examples 11 to 16 described above was carried out. In

a region with a short polymerization time, an increase in molecular weight in proportion to the polymerization time was confirmed, and molecular weight saturation ($Mn=125\times10^4$ g mol^{-1}) at a polymerization time of 2 hours was observed (FIG. 7). Here, in FIG. 7, the solid circle represents Mw and the open circle represents Mw/Mn.

(Example 17)

F-MRI images of PAMAM-PTFEMA with $Mn=54\times10^4$ g mol^{-1} obtained in Example 5 were obtained. The results are represented in FIG. 8A1 to FIG. 8A6 and FIG. 8B1 to FIG. 8B6.

FIG. 8A1 to FIG. 8A6 illustrate F-MRI images of trifluorotoluene (TFT: reference substance, F atom concentration: 50 mM) as a reference. FIG. 8B1 to FIG. 8B6 illustrate F-MRI images of 54×10^4 g mol^{-1} of PAMAM-g-PTFEMA (F atom concentration: 50 mM). Chloroform is used as a solvent.

Here, in the measurement of each sample, TR (repetition time) and the number of accumulations were kept constant as indicated in the following table, while TE (echo time) was changed.

The brightness of the respective images of FIG. 8A1 to FIG. 8A6 were normalize with the brightness of FIG. 8A1 and that of FIG. 8B1 to FIG. 8B6 were normalized with the brightness of FIG. 8B1, thereby adjusting the brightness of FIG. 8A1 and the brightness of FIG. 8B1.

Table 2: Measurement conditions of F-MRI images

Sample a, b.	1	2	3	4	5	6
TR/ms	3000	3000	3000	3000	3000	3000
TE/ms	6	198	390	582	774	996
Number of accumulations	200	200	200	200	200	200

(Example 18)

F-MRI images of PAMAM-g-PTFEMA with Mn=54×10^4 g mol^{-1} obtained in Example 5 were investigated with respect to the concentration dependency. The results are shown in FIG. 9A, FIG. 9B1, and FIG. 9B2.

FIG. 9A illustrates a ^{1}H-MRI image. A schematic diagram illustrated on the right side of FIG. 9A illustrates the arrangement of samples. In addition, FIG. 9B1 illustrates a ^{19}F-MRI image of PAMAM-g-PTFEMA. FIG. 9B2 illustrates a ^{19}F-MRI image of TFT. In the schematic diagram on the right side of FIG. 9A and in FIGS. 9B1 and 9B2, the ^{19}F-MRI images of PAMAM-g-PTFEMA are surrounded with broken-line circles, the ^{19}F-MRI images of TFT provided as a reference substance are surrounded with solid-line circles. Numbers in the circles represent concentrations (unit: mM).

More specifically, with respect to the arrangement of samples in each of FIG. 9A, FIG. 9B1, and FIG. 9B2, the center one is TFT with an F atom concentration of 0.01 mM, and the others, in the counterclockwise direction from the upper left, are TFT with an F atom concentration of 0.1 mM, PAMAM-g-PTFEMA with a F atom concentration of 0.01 mM, TFT

with an F atom concentration of 1 mM, PAMAM-g-PTFEMA with an F atom concentration of 1 mM, and PAMAM-g-PTFEMA with an F atom concentration of 0.1 mM. In each case, chloroform was used as a solvent.

Here, the ^{19}F-MRI image represented in FIG. 9B1 and the ^{19}F-MRI image represented in FIG. 9B2 are those obtained by two-signal simultaneous measurement utilizing a difference in resonant frequencies of PAMAM-g-PTFEMA and TFT. The measurement conditions are TR=1,000 ms, TE=3.8 ms, and number of accumulations=600.

As is evident from FIG. 9A, FIG. 9B1, and FIG. 9B2, the ^{19}F-MRI image of TFT cannot be obtained if the F atom concentration of TFT is not 1 mM. In contrast, the ^{19}F-MRI image of PAMAM-g-PTFEMA can be clearly obtained even if the F atom concentration thereof is 0.1 mM. This indicates that PAMAM-g-PTFEMA has an excellent sensitivity for ^{19}F-MRI contrast mediums.

(Example 19)

PAMAM-g-PTFEMA with Mn=54×10^4 g mol^{-1} obtained in Example 5 was subjected to the measurement of T1 and T2 relaxation times.

In the measurement, each of PAMAM-g-PTFEMA with Mn=54×10^4 g mol^{-1} and TFT as a reference substance was prepared in a 50 mM chloroform solution in terms of an F atom concentration, and then filled in a 2-ml screw tube as a sample. The T1 measurement was carried out by the inversion recovery method and the T2 measurement was carried

out by the spin echo method. Each of FIG. 10A and FIG. 10B illustrates an F-NMR spectrum obtained when the T1 relaxation time measurement was carried out by the inversion recovery method. FIG. 10A illustrates the spectrum of TFT and FIG. 10B illustrates the spectrum of PAMAM-g-PTFEMA with $Mn=54\times10^4$ g mol^{-1}. The inversion times are 0.1, 0.25, 0.5, 0.8, 1.4, 2.0, 3.0, 4.0, 8.0, and 15.0 seconds from the front side. The results of the relaxation time measurement are listed in Table 3 below.

Table 3

Measurement sample	T1/sec.	T2/sec.
TFT	2	2
PAMAM-g-PTFEMA	0.6	0.4

MRI has advantages in that the shorter the T1 relaxation time is, the more the number of accumulations can be increased for a measurement with a certain measurement time, and the longer the T2 relaxation time is, the more the peak of a signal becomes sharp, resulting in a clear image. The PAMAM-g-PTFEMA as prepared had the shorter T1 relaxation time and the shorter T2 relaxation time, compared with those of TFT as a reference. Signals were slightly broadened by polymerization, and the relaxation times were shortened. However, as is evident from the F-MRI images of FIG. 9B1, there was no problem in imaging of signals at all.

According to preferred embodiments of the present invention as described above, the use of the dendrimer particle with a fluorine-atom-containing unit on the

terminal end of the dendrimer can provide an F-MRI contrast medium with high contrast sensitivity and an exactly controlled size.

While the present invention has been described with reference to exemplary embodiments, it is to be understood that the invention is not limited to the disclosed exemplary embodiments. The scope of the following claims is to be accorded the broadest interpretation so as to encompass all such modifications and equivalent structures and functions.

This application claims the benefit of Japanese Patent Application Nos. 2008-052408, filed March 3, 2008 and 2008-230241, filed September 8, 2008, which are hereby incorporated by reference in their entirety.

CLAIMS

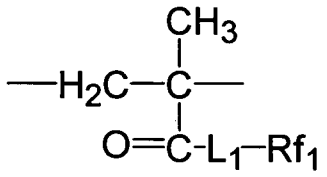
1. A dendrimer particle comprising a unit that contains a fluorine atom at a plurality of branch ends of an aliphatic branched polymer.

2. A dendrimer particle according to claim 1, wherein the unit is a polymer having a repeating unit that contains a fluorine atom.

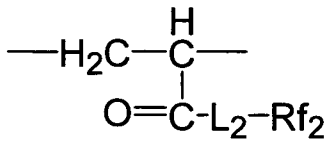
3. A dendrimer particle according to claim 2, wherein the unit is a polymer comprised of a repeating unit that contains a fluorine atom and a repeating unit that does not contain a fluorine atom.

4. A dendrimer particle according to claim 3, wherein the repeating unit that does not contain a fluorine atom is located on the outer side than the repeating unit that contains a fluorine atom.

5. A dendrimer particle according to any one of claims 2 to 4, wherein the repeating unit that contains a fluorine atom has a structure represented by the following general formula (1) or (2):



(1)

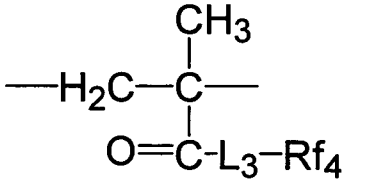


(2)

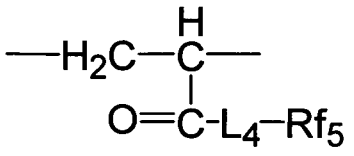
where Rf_1 and Rf_2 each represent a monomer or an oligomer of a linear or branched alkyl group that contains a fluorine atom, a linear or branched oxyalkyl group that contains a fluorine atom, or a linear or branched oxyalkylene group that contains a fluorine atom, in which hydrogen in the alkyl

group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-; and L_1 and L_2 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_1R_2-$, in which R_1 represents hydrogen or an alkyl group and R_2 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

6. A dendrimer particle according to claim 3 or 4, wherein the repeating unit that does not contain a fluorine atom has a structure represented by the following general formula (1a) or (2a):



(1a)



(2a)

where Rf_4 and Rf_5 each represent a monomer or an oligomer of a linear or branched alkyl group that does not contain a fluorine atom, a linear or branched oxyalkyl group that does not contain a fluorine atom, or a linear or branched oxyalkylene group that does not contain a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-,

-CO-, -NH-, or -COO-; and L_3 and L_4 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

7. A dendrimer particle according to claim 1, wherein the unit that contains a fluorine atom is represented by the following general formula (3):

$$Rf_3—L— \quad (3)$$

where Rf_3 represents an alkyl group that contains a fluorine atom and may be linear or branched or a linear or branched oxyalkyl group that contains a fluorine atom, in which hydrogen in the alkyl group and the oxyalkyl group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-; and L represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, a phenylene group, an oxyphenylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene

group.

8. A dendrimer particle according to any one of claims 1 to 7, further comprising a hydrophilic group.

9. A dendrimer particle according to claim 8, wherein the hydrophilic group is at least one of -OH, -COOH, $-NH_2$, -O-, and -NH-.

10. A dendrimer particle according to any one of claims 1 to 9, which has a particle size of 10 nm or more and 200 nm or less.

11. An MRI contrast medium containing the dendrimer particle according to any one of claims 1 to 10.

12. A method of manufacturing a dendrimer particle, comprising the step of providing a unit that contains a fluorine atom to a plurality of branch ends of an aliphatic branched polymer.

13. A method of manufacturing a dendrimer particle according to claim 12, wherein the step of providing the unit that contains a fluorine atom is the step of providing a polymer comprised of monomers that contain fluorine atoms to a plurality of branch ends of an aliphatic branched polymer.

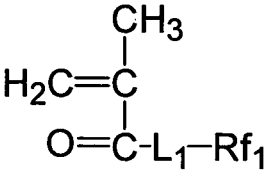
14. A method of manufacturing a dendrimer particle according to claim 13, wherein the step of providing the polymer uses a living radical polymerization method.

15. A method of manufacturing a dendrimer particle according to claim 14, wherein the living radical polymerization method is an atom transfer radical

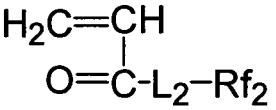
polymerization method.

16. A method of manufacturing a dendrimer particle according to any one of claims 13 to 15, further comprising providing a polymer comprised of monomers that do not contain a fluorine atom to a terminal end of the polymer comprised of monomers that contain fluorine atoms after the step of providing the polymer comprised of monomers that contain fluorine atoms.

17. A method of manufacturing a dendrimer particle according to any of one claims 13 to 16, wherein the monomers that contain fluorine atoms have a structure represented by the following general formula (4) or (5):



(4)

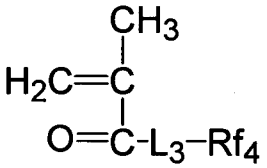


(5)

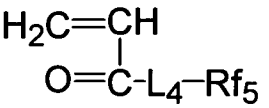
where Rf_1 and Rf_2 each represent a monomer or an oligomer of a linear or branched alkyl group that contains a fluorine atom, a linear or branched oxyalkyl group that contains a fluorine atom, or a linear or branched oxyalkylene group that contains a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-; and L_1 and L_2 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_1R_2-$,

in which R_1 represents hydrogen or an alkyl group and R_2 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group.

18. A method of manufacturing a dendrimer particle according to claim 16, wherein the monomers that do not contain fluorine atoms have a structure represented by the following general formula (4a) or (5a):



(4a)



(5a)

here Rf_4 and Rf_5 each represent a monomer or an oligomer of a linear or branched alkyl group that does not contain a fluorine atom, a linear or branched oxyalkyl group that does not contain a fluorine atom, or a linear or branched oxyalkylene group that does not contain a fluorine atom, in which hydrogen in the alkyl group, the oxyalkyl group, and the oxyalkylene group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-; and L_3 and L_4 each represent a single bond or a divalent linking group selected from -O-, an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene

w

group.

19. A method of manufacturing a dendrimer particle according to claim 12, wherein the step of providing the unit that contains a fluorine atom is the step of reacting an aliphatic branched polymer with a molecule represented by the following general formula (6):

$$Rf_3—L—X_1 \quad (6)$$

where Rf_3 represents an alkyl group that contains a fluorine atom and may be linear or branched or a linear or branched oxyalkyl group that contains a fluorine atom, in which hydrogen in the alkyl group and the oxyalkyl group may be substituted with an atom or an atomic group other than hydrogen, and $-CH_2-$ in the alkyl group and the oxyalkyl group may be substituted with -O-, -CO-, -NH-, or -COO-; L represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, an oxyalkylene group, a phenylene group, an oxyphenylene group, and $-NR_3R_4-$, in which R_3 represents hydrogen or an alkyl group and R_4 represents a single bond or a divalent linking group selected from an alkylene group, an alkylene group having a hydroxyl group, and an oxyalkylene group; and X_1 represents a group selected from an amino group, a hydroxyl group, a carboxyl group, carboxylic chloride, carboxylic fluoride, a halogen atom, an epoxy group, an isocyanate group, $-CH=CH_2$, $-C\equiv CH$, and a thiol group.

FIG. 1A

FIG. 1B

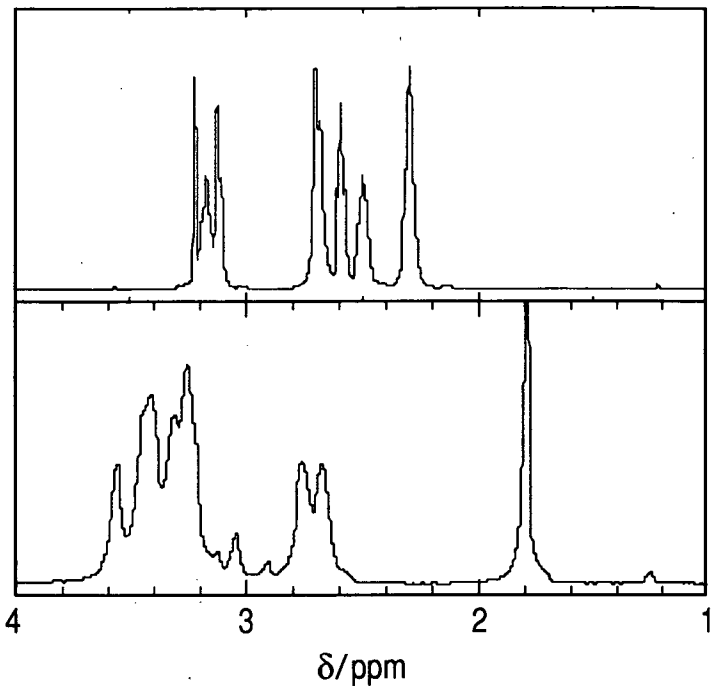


FIG. 2

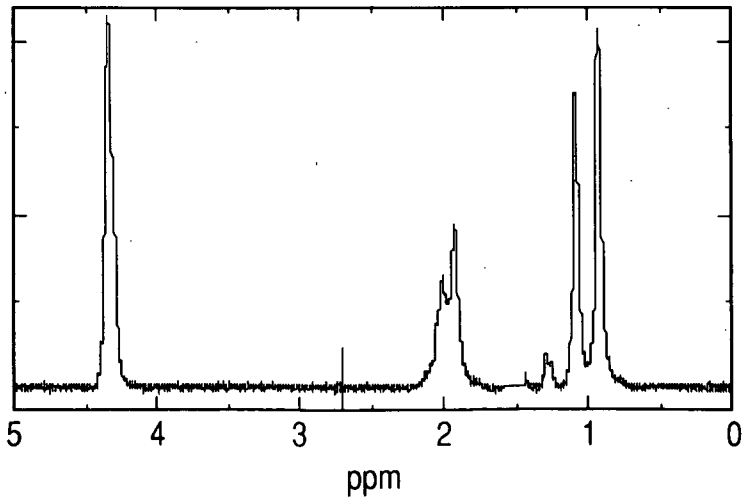


FIG. 3

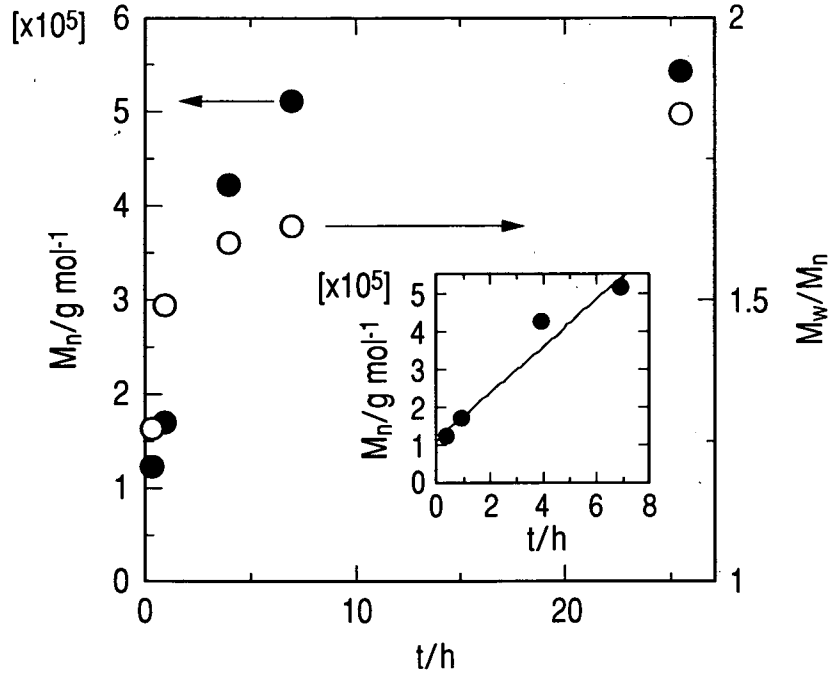

[x10^5]
6
5
4
3
2
1
0
Mn/g mol-1
0
10
20
t/h
2
1.5
1
Mw/Mn
[x10^5]
5
4
3
2
1
0
Mn/g mol-1
0
2
4
6
8
t/h

FIG. 4

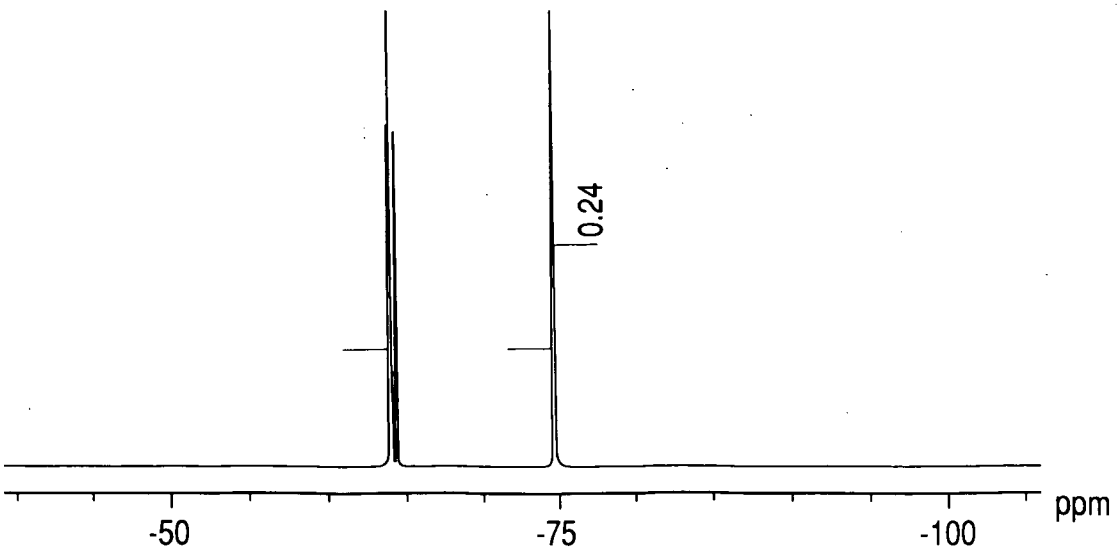

0.24
-50
-75
-100
ppm

FIG. 5

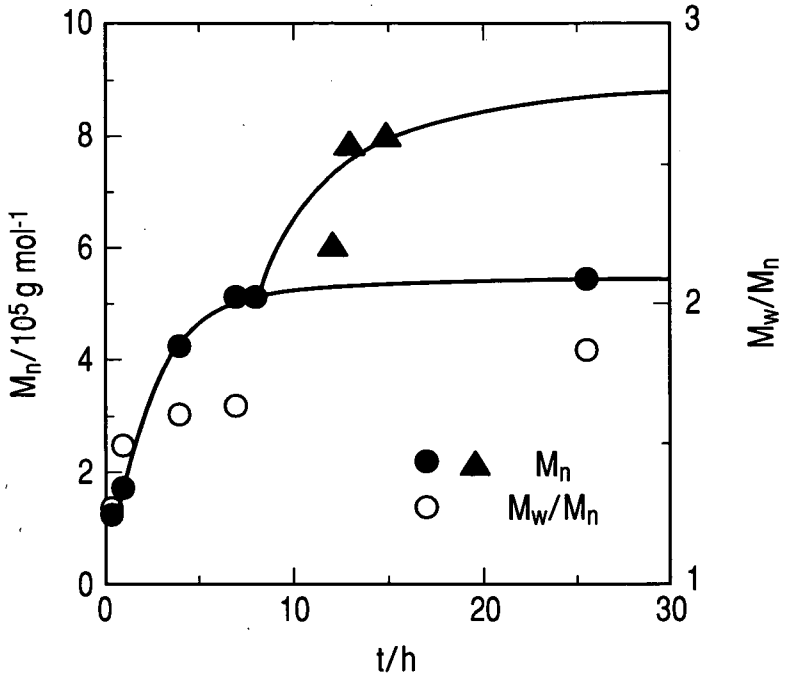


FIG. 6A

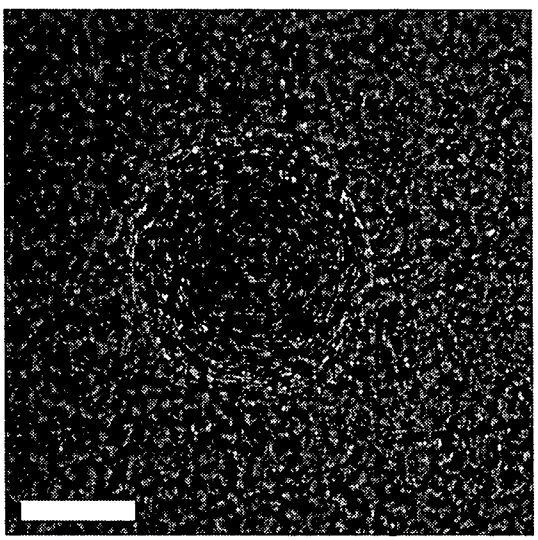


FIG. 6B

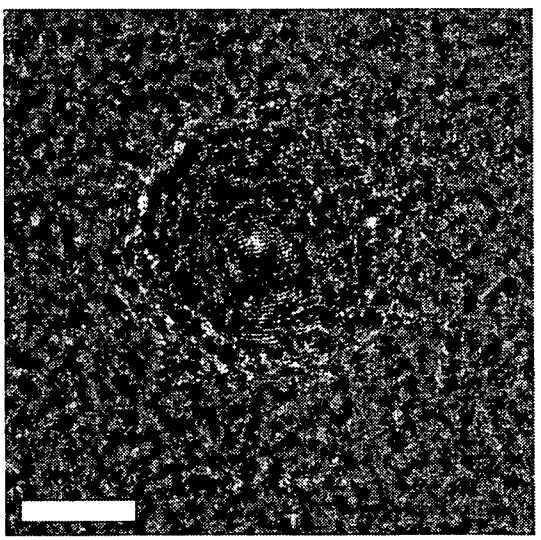


FIG. 7

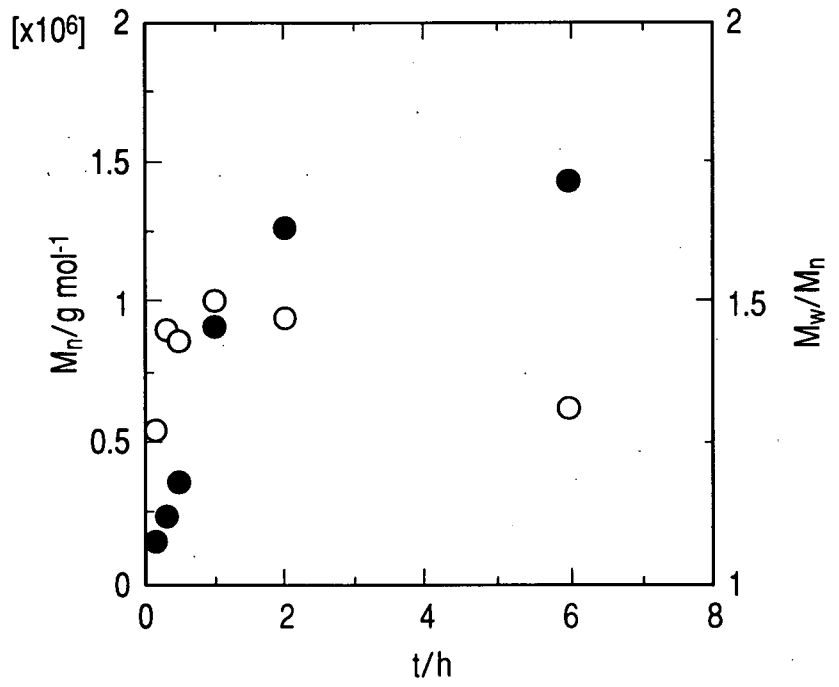


FIG. 8A1

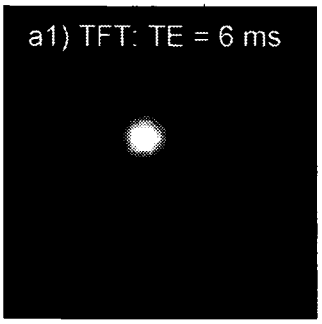


FIG. 8A2

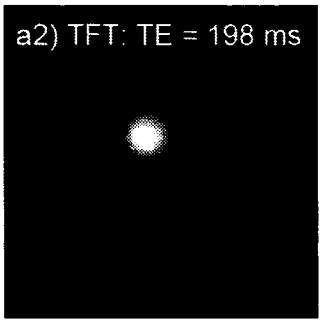


FIG. 8A3

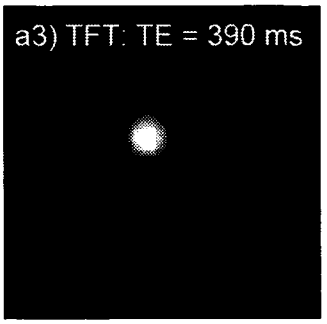


FIG. 8A4

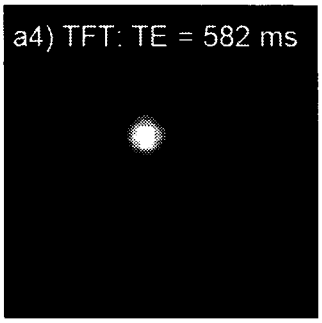


FIG. 8A5

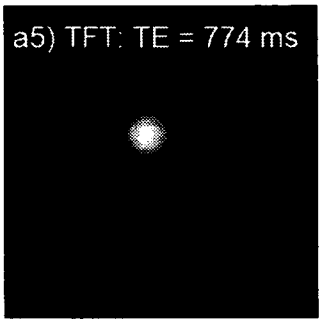


FIG. 8A6

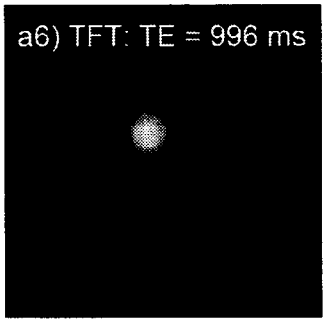


FIG. 8B1

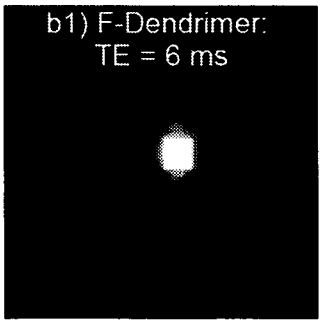


FIG. 8B2

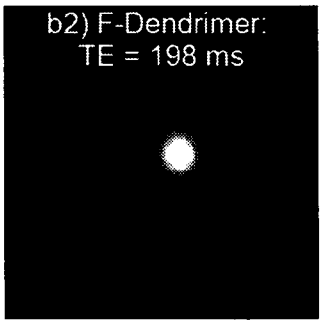


FIG. 8B3

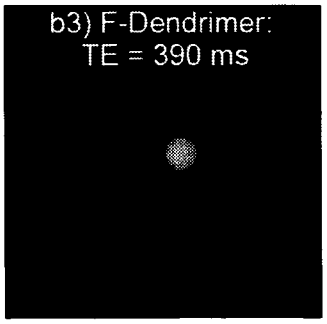


FIG. 8B4

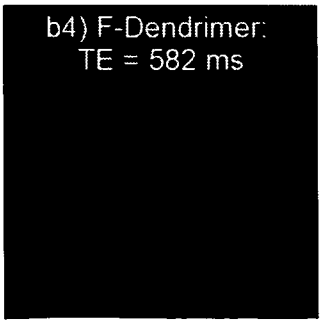


FIG. 8B5

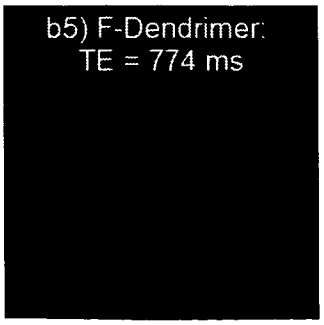


FIG. 8B6

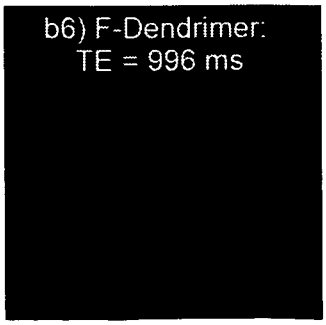


FIG. 9A

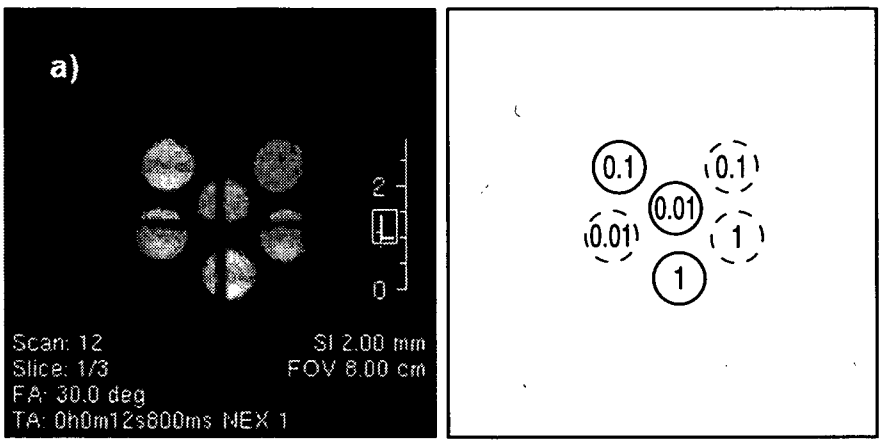


FIG. 9B1 FIG. 9B2

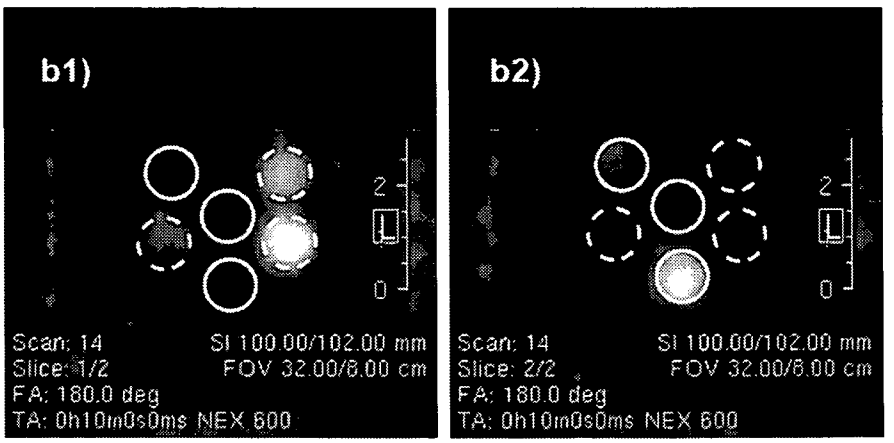


FIG. 10A

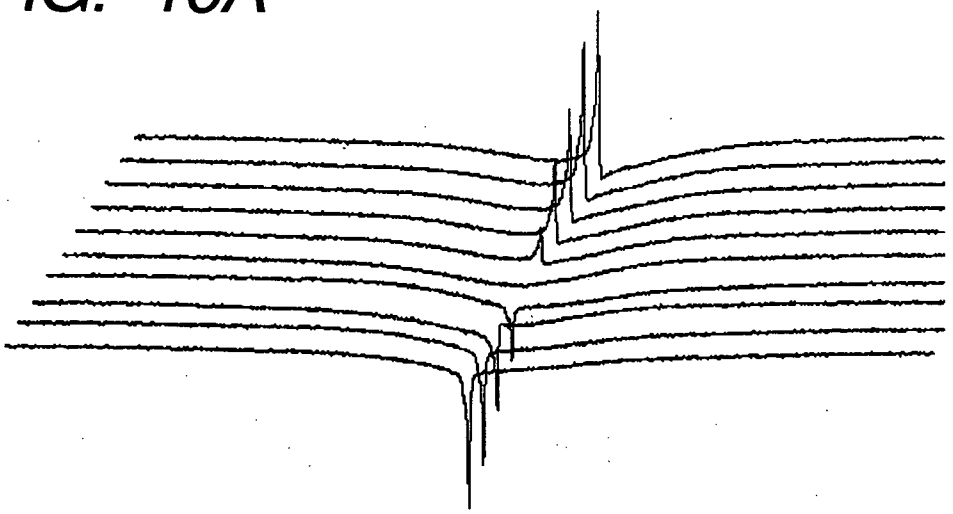


FIG. 10B