

SEGMENTED CHELATING POLYMERS AS IMAGING AND  
THERAPEUTIC AGENTS

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This invention relates to segmented chelating polymers which are useful as imaging agents and as cytotoxic agents and to their compositions and methods of use.

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Magnetic resonance (MR) is a widely used technique for generating spatial images of multi-cellular organisms or bodies for clinical diagnosis. A review of this technique and its clinical applications is provided by D.P Swanson et al, in Pharmaceuticals in Medical Imaging, 1990, Macmillan Publishing Company, pages 645-681.

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Magnetic resonance images are derived instrumentally as a composite of the effects of a number of parameters which are analyzed and combined by computer. Instrument parameter controls, such as radio frequency (Rf), pulsation and timing can be utilized to enhance or attenuate the signals of any of the image-producing parameters to thereby improve image quality and provide better anatomical and functional information. In many cases, magnetic resonance imaging has proven to be a valuable diagnostic tool, inasmuch as normal and diseased tissue, by virtue of their possessing different responses to parameter values, can be differentiated in the image.

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In magnetic resonance imaging of the body of a mammal such as a human, an in vivo image of an organ or tissue is obtained by placing at least the portion of the body to be imaged in a strong external magnetic field, pulsing with radiofrequency energy, and observing the effect of the pulses on the magnetic properties of the protons contained in and surrounding the organ or tissue. This is especially useful in imaging the

circulatory vasculature of the body (i.e. the blood pool). A number of magnetic parameters can be measured. In particular, the proton relaxation times,  $T_1$  and  $T_2$ , are of primary importance.  $T_1$ , also called the spin-lattice or longitudinal relaxation time, and  $T_2$ , also called the spin-spin or transverse relaxation time, are functions of the chemical and physical environment of the organ or tissue water and are measured using well-established Rf pulsing techniques. This information is analyzed as a function of spatial location and transformed by computer into an image.

Often the MR image produced lacks appropriate contrast, e.g., between normal and diseased tissue, reducing diagnostic effectiveness. However, the contrast in the image generated may be enhanced by introducing into the zone being imaged an agent (a "contrast agent") which affects the spin reequilibration characteristics of nuclei (the "imaging nuclei" which generally are protons and more especially are water protons) which are responsible for the resonance signals from which the images are generated. The enhanced contrast thus obtained enables particular organs or tissues to be visualised more clearly by increasing or decreasing the signal level of the particular organ or tissue relative to its surroundings. In other words, a contrast agent, if taken up preferentially by a certain portion of an organ or a certain type of tissue, e.g., diseased tissue, can provide a change in contrast or enhancement in the resultant images of that tissue.

Inasmuch as magnetic resonance images are strongly affected by variations in  $T_1$  and  $T_2$ , it is desirable to have a contrast agent which affects either or both of these parameters. The majority of materials now being proposed as MR imaging contrast agents achieve a contrast effect because they contain paramagnetic materials, which generally act to decrease  $T_1$  and  $T_2$ , or superparamagnetic materials, which generally act to

decrease  $T_2$ . At low concentrations, paramagnetic materials effect  $T_1$  more than  $T_2$ . The use of such materials as MR contrast agents has been widely advocated and broad ranges of suitable materials have  
5 been suggested in the literature.

Thus, for example Lauterbur and others have suggested the use of manganese salts and other paramagnetic inorganic salts and complexes (see Lauterbur et al. in "Frontiers of Biological  
10 Energetics", volume 1, pages 752-759, Academic Press (1978), Lauterbur in Phil. Trans. R. Soc. Lond. B289: 483-487 (1980) and Doyle et al. in J. Comput. Assist. Tomogr. 5(2): 295-296 (1981)), Runge et al. have suggested the use of particulate gadolinium oxalate (see  
15 for example US-A-4615879 and Radiology 147(3): 789-791 (1983)), Schering AG have suggested the use of paramagnetic metal chelates, for example of aminopolycarboxylic acids such as nitrilotriacetic acid (NTA), N,N,N',N'-ethylenediaminetetraacetic acid (EDTA),  
20 N-hydroxyethyl-N,N',N'-ethylenediaminetriacetic acid (HEDTA), N,N,N',N''-diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA) (see for example EP-A-71564, EP-A-130934, DE-A-3401052 and US-A-4639365), and Nycomed Imaging AS  
25 and Nycomed Salutar Inc. have suggested the use of paramagnetic metal chelates of iminodiacetic acids and other aminopolycarboxylic acids such as DTPA-BMA and DPDP (see EP-A-165728, WO-A-86/02841, EP-A-299795, EP-A-290047 and WO-A-90/08138). Besides paramagnetic metals,  
30 paramagnetic stable free radicals have also been suggested for use as positive MR imaging contrast agents (see for example EP-A-133674).

Other paramagnetic MR contrast agents are suggested or reviewed in, for example, EP-A-136812, EP-A-185899,  
35 EP-A-186947, EP-A-292689, EP-A-230893, EP-A-232751, EP-A-255471, WO-A-85/05554, WO-A-86/01112, WO-A-87/01594, WO-A-87/02893, US-A-4639365, US-A-4687659, US-A-4687658,

AJR 141: 1209-1215 (1983), Sem. Nucl. Med. 13: 364 (1983), Radiology 147: 781 (1983), J. Nucl. Med. 25: 506 (1984) and WO89/00557.

5 Superparamagnetic MR contrast agents were disclosed by Schröder and Salford in WO-A-85/02772, by Nycomed AS in WO-A-85/04330, by Widder in US-A-4675173, by Schering AG in DE-A-3443252 and by Advanced Magnetics Inc. in WO-A-88/00060.

10 A major drawback with the use of contrast agents for magnetic resonance imaging is that at effective dosages many paramagnetic materials exert toxic effects on biological systems making them inappropriate for in vivo use. For example, the free solubilized form of  $Gd^{3+}$  salts are quite toxic. To make the gadolinium ion more  
15 suitable for in vivo use, researchers have chelated it using diethylenetriaminepentaacetic acid (DTPA). This agent, GdDTPA, has been successful in enhancing magnetic resonance images of human brain and renal tumors.

20 Despite its satisfactory relaxivity and safety, this formulation has several disadvantages. For example, due to its low molecular weight, Gd-DTPA is cleared very rapidly from the blood stream and tissue lesions (tumors). This limits the imaging window, the number of optimally enhanced images that can be obtained after  
25 each injection, and increases the agent's required dose and its toxic effects. In addition, the biodistribution of Gd-DTPA is suboptimal for imaging body tumors and infections due to its low molecular weight.

30 Several approaches have been taken in attempts to overcome these disadvantages. For example,  $Gd^{3+}$  and  $Gd^{3+}$  chelates have been chemically conjugated to proteins such as albumin, polylysines and immunoglobulins. Drawbacks of conjugating for example DTPA to protein carriers include inappropriate biodistribution and  
35 toxicity. In addition, available proteins are not subject to wide synthetic variation. Additionally, thermal sterilization of protein conjugates tends to be

problematic, especially in the case of albumin conjugates.

Furthermore proteins are metabolized by the body, providing an imaging agent whose molecular weight  
5 changes uncontrollably. This causes blood pool half-life tissue specificity of the imaging agent to change continuously. Proteins are immunogenic substances, which have several known drawbacks for therapeutic or diagnostic use. Solutions to these recognized problems  
10 are clearly of importance generally in the imaging field.

There is a need to provide new classes of magnetic resonance contrast enhancing agents, which are tissue specific, and which remain in the blood pool for long  
15 periods of time.

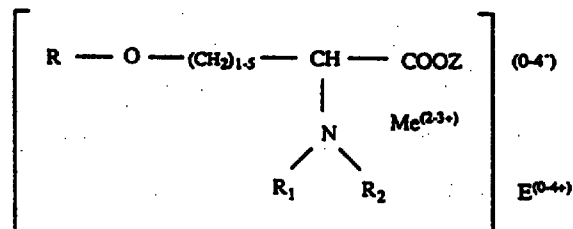
The importance of drug targeting has been recognized in recent years, especially for anticancer drugs, inasmuch as the toxicity of these drugs to normal cells is often dosage limiting. Drug targeting has been  
20 employed in this area, using antibodies to tumor associated antigens or using other proteins and saccharides to avoid the random destruction of healthy tissue. Antibodies and portions or fragments thereof have been most widely used due to their specificity. The  
25 current approaches, however, are expensive and pose immunogenicity problems. Furthermore, not all cancers are susceptible to drug therapy.

Certain tumors are especially susceptible to treatment by radiation, such as those of hematopoietic  
30 origin, for example, leukemias and lymphomas; others are less susceptible to such treatment such as adenocarcinomas of the head and neck, or breast, ovarian, cervical or rectal adenocarcinomas. However, certain of these cancers cannot be practically treated  
35 by externally derived beam radiation because of the location of the tumor and the effect of such radiation on surrounding healthy tissue. Hence, it is advantageous

to deliver a source of radiation such as a radioactive isotope to the tumor, whilst minimizing damage to surrounding healthy tissues which in conventional treatment may lie in the path of the beam. It would be advantageous to deliver the appropriate radiation dosage directly to the tumor with significantly less harm to the surrounding tissue thereby increasing the therapeutic ratio (the ratio of damage to the tumor divided by damage to the most sensitive healthy tissue).

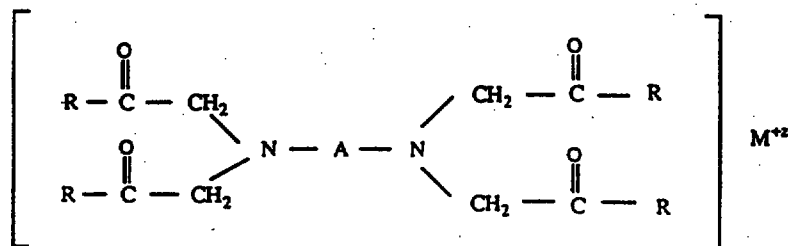
In summary, it would be desirable to prepare a material that would overcome the following disadvantages; 1) toxicity, 2) short blood pool residence time, 3) lack of specificity for targeted tissues, 4) immunogenicity, and 5) uncontrolled metabolism of the polymer. Several attempts have been made to provide such a polymer.

Felder et al in U.S. Patent No. 4,916,246 disclose low molecular weight paramagnetic chelates useful for NMR imaging having the formula:



wherein Me is Fe, Mn, Dy or Gd, Z is H or a negative charge, R<sub>1</sub> and R<sub>2</sub> are substituted alkyl and R can be poly(oxy-alkyl) with 1 to 50 oxygen atoms and from 3 to 150 carbon atoms.

U.S. Patent No. 5,137,711 describes low molecular weight complexes of paramagnetic ions with derivatives of DTPA or EDTA represented by the formula:



wherein A is selected from the group consisting of  
 $-\text{CH}_2\text{CH}_2-$  and:



10 and  $\text{M}^{2+}$  is a paramagnetic ion of an element with an atomic number of 21-29, 42-44 or 58-70, and a valence, Z, of +2 or +3; the  $\text{R}^1$  and R groups may be the same or different and are selected from the group consisting of  $-\text{O}^-$  and



20 wherein  $\text{R}^2$  is  $(\text{CH}_2\text{CH}_2\text{O})_n-\text{R}^4$ , n is 1-10 and  $\text{R}^4$  is H, alkyl having 1 to 8 carbon atoms (i.e.  $\text{C}_{1-8}$ ) or aryl, unsubstituted or substituted with hydroxy, and  $\text{R}^3$  is H,  $\text{R}^2$ ,  $\text{C}_{1-8}$ -alkyl, hydroxy,  $\text{C}_{1-8}$ -alkoxy,  $\text{C}_{1-10}$ -cycloalkyl or an aryl group which is optionally substituted with hydroxy,  
 25 carboxy, halide,  $\text{C}_{1-8}$ -alkoxy or  $\text{C}_{1-8}$ -alkyl, wherein 2 of the  $\text{R}^1$  groups are  $-\text{O}^-$  and the remainder of the  $\text{R}^1$  groups are groups



35 International Patent Publication No. WO 91-18630 discloses substances for treating or diagnosing tumors, having at least one aliphatic amino group, or at least one phenolic hydroxyl and/or amino group and at least

one aliphatic amino group, all substituted with polyethylene glycol chains, whose degree of polymerization,  $n$ , is 5 to 250. The terminal hydroxyl group of the chain is substituted by  $C_{1-12}$  alkyl ester.

5 Herz, et al. in U.S. Patent No. 3,859,337 disclose low molecular weight ethylenediamine tetraacetic acid anhydride derivatives useful as chelating agents.

10 It has now been found that certain metallizable segmented polymers when associated with metal ions provide higher molecular weight chelates of surprising utility as blood pool contrast agents for diagnostic imaging and/or as cytotoxic agents.

Thus viewed from one aspect, the present invention provides a segmented polymer having a molecular weight of at least about 4,500 comprising at least one  
15 poly(alkylene oxidylene) moiety linked via an amide linkage to a chelating agent residue.

Especially preferred segmented polymers according to the invention are those wherein said chelating agent residue in metallated, preferably with a paramagnetic  
20 metal species or with a radionuclide species.

Particularly preferred segmented polymers according to the invention are those of Formula I:



30 (wherein:

Z is a chelating agent residue;

Q is a divalent poly(alkylene oxidylene) moiety having a carbon terminus at R and at L;

L represents an amide linkage;

35  $E^{(b)}$  is one or more counterions each having a charge of  $b$ ;

$b$  is an integer from 1, 2 and 3;

- 9 -

- n is an integer from 1, 2, 3 and 4;  
w is zero or an integer from 1, 2, 3, 4 and 5;  
 $M^{(+a)}$  is a cation, having a charge of +a;  
a is an integer from 1, 2, 3 and 4;  
5 r is 0 or an integer from 1, 2 and 3, with the proviso that that when r is 2 or 3, each  $M^{(+a)}$  can be the same or different;  
d is the total charge on the chelating agent residue and is an integer from 0 to 10;  
10  $d + \sum(b.w) + \sum(a.r) = 0$ ; and  
R is a capping moiety selected from the group consisting of hydrogen, hydroxyl,  $C_{1-4}$ -alkyl,  $C_{6-24}$ -aryl,  $C_{2-5}$ -alkanoyloxyl and  $C_{1-4}$ -alkoxy, or R is an immunoreactive group or cytotoxic drug linked to group Q by a chemical  
15 bond or a linking group).

The segmented polymers of the invention have a variety of end uses. In particular they may be used as diagnostic agents, eg. image contrast enhancing agents in diagnostic imaging techniques such as MRI, X-ray and  
20 scintigraphy, as therapeutic agents, for example in radiotherapy or drug delivery, or as targetting agents, for example in cytotoxic or imaging procedures or in fluorescence imaging. The chelating residues may be metallated with metal species useful diagnostically or  
25 therapeutically, such as paramagnetic or radioactive metal ions. The targetting delivery system effected by the segmented polymers of the invention is of course also especially useful for delivery of metal species useful in diagnostic imaging of body organs or tissues  
30 or as cytotoxic agents.

Accordingly, viewed from a further aspect the present invention provides a pharmaceutical composition comprising a segmented polymer according to the invention together with at least one physiologically  
35 acceptable carrier or excipient.

Moreover, the segmented polymers according to the invention as well as being particularly useful as image

enhancing agents, are particularly useful in chelation therapy for metal poisoning, especially heavy metal poisoning and in detecting such ions, and have the added advantage of being able to remain in the vascular system for remarkably long periods of time. Certain segmented polymers have tissue specificity, e.g. tumour or liver specificity. Indeed, the molecular weight of the segmented polymers may be tailored to produce an agent of a desired overall molecular weight, size, blood pool residence time and tissue specificity. Furthermore, the segmented polymers according to the invention may be useful in detection of flows and leaks in materials that contain liquids, and in apparatus used with liquids for heating, cooling, lubrication and the like.

The segmented polymers of the invention may be prepared by a particularly elegant and simple reaction of a poly(alkylene oxide) with a chelating agent or precursor thereof.

Thus viewed from a different aspect the invention provides a process for the preparation of a segmented polymer, said process comprising reacting a poly(alkylene oxide) with a chelating agent or precursor thereof.

As used herein "segmented" polymer means a polymer having one component or "segment" comprising a chelating agent residue Z and from one to four regions or "segments" each comprising a capped or uncapped poly(alkylene oxidylene) moiety.

As used herein the term "body" refers to a bounded aggregate of matter, preferably the entire material substance or organism, especially an animal, most preferably an animal or human subject.

As used herein "cytotoxic agent" refers to any agent able to kill cells, including a radionuclide, whose emissions lead to cell death including a chelate of a radionuclide that emits cytotoxic radiation, chemotherapeutic agents such as cytotoxic drugs and

cytotoxic antibiotics, toxins or any agent which initiates or which leads to cell death. In a preferred embodiment, the cytotoxic agent is a metal ion associated with the chelating agent residue.

5           The segmented polymer according to the invention can have an imaging ion associated therewith. By "imaging ion", it is meant any metal ion useful for enhancing contrast for example during x-ray, radioscintigraphic, fluorescence or magnetic resonance  
10       imaging. The imaging agent may conveniently be ionically associated with the chelating agent residue. Different types of metal ions may be employed in the same polymer so as to be useful for different types of imaging simultaneously; (for example, the ions of different  
15       elements, or different isotopes of the same element) or the same ion (e.g. ions of the same element or isotopes of the same elements) may be imaged by different methods.

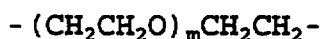
          As used herein aryl refers to monocyclyl to  
20       hexacyclyl aryl, having from 6 to 24 carboxy atoms and may be additionally substituted with one or more C<sub>1-4</sub>-alkoxy, and/or C<sub>1-4</sub>-alkyl groups. Such aryl groups, when used in the polymer according to the invention are useful for fluorescence detection.

25           In formula I above, Q represents a poly(alkylene oxidylene) moiety having a carbon terminus at L and a carbon terminus at R and containing m alkylene oxide units. Exemplary poly(alkylene oxidylene) moieties comprise, for example, poly(ethylene oxides), and/or  
30       poly(propylene oxides) and/or poly(butylene oxides) and the like. Preferred poly(alkylene oxidylene) moieties include poly(ethylene oxidylene) moieties (PEO), poly(propylene oxidylene) (PPO) and random and block copolymers of PEO and PPO. PEO containing polymers are  
35       particularly preferred when it is desirable for the segmented polymer to be soluble in water. It is also contemplated that the poly(alkylene oxidylene) moiety

- 12 -

can comprise glycerol poly(alkylene oxide) triethers, polyglycidols, linear, block and graft copolymers of alkylene oxides with compatible comonomers such as poly(ethyleneimine-co-ethylene oxide), or  
 5 poly(oxazoline-co-alkyleneoxide) and grafted block copolymers such as poly(methyl vinyl ether-co-ethylene oxide).

For magnetic resonance imaging applications, segmented polymers according to the invention preferably  
 10 have an average molecular weight greater than about 4,500, more preferably about 4,500 to about 40,000. These polymers can be derived from poly(alkylene<sup>n</sup> oxidylene) moieties which are commercially available in the corresponding alcohol form as a diol, or as a  
 15 monoalkyl ether alcohol, or monoalkyl ether monoamine form, or alternatively they can be prepared by techniques well known to those skilled in the art. The number of alkylene oxide subunits in Q depends upon 1) n, the number of L-Q-R segments extending from Z; 2)  
 20 upon the "molecular" weight of each alkylene oxide subunit moiety; 3) the desired molecular weight of the polymer; 4) the molecular weight of R and Z, as well as  $M^{(a+)}$ . If n is greater than 1, each poly(alkylene oxide) moiety may be the same or different. Expressed  
 25 mathematically this is:  $n \times [(MW \text{ of } L \text{ \& } R) + (MW \text{ of alkylene oxide subunit } \times m)] + (r \times \text{atomic weight of chelated metal(s)}) + MW \text{ of chelating residue} + \text{molecular weight of } Z = MW \text{ of the polymer}$ . Thus m is a function of n for a given molecular weight of the polymer. A  
 30 particularly preferred class of poly(alkylene oxide) moieties derived from poly(ethylene oxides) can be represented by the structure:



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If four PEO chains are present (n=4) then the upper limit of the most preferred molecular weight of 40,000

will be obtained with  $m$  = about 220. If a molecular weight of 5,000 is desired in the same type of polymer ( $n=4$ ) then  $m$  will be closer to 30.

These poly(alkylene oxide) moieties and their  
5 reactive derivatives, useful in preparing the segmented polymer of the invention, are known in the art. For example, bis(methyl amino) polyethylene glycol and its use as an intermediate is known in the art, for example; Mutter, Tetrahedron Letters, 31, 2839-2842 (1978)  
10 describes a procedure to convert the terminal hydroxyl groups of poly(ethylene oxide) to reactive primary amino groups as well as the preparation of a number of reagents bound to poly(ethylene oxide) amines; Harris et al., J. Polymer Science, 22, 341-352 (1984) describe  
15 various PAG derivatives including for example, amino poly(ethylene oxide). Other poly(alkylene oxide) derivatives are prepared by known chemistries, examples of which are described previously.

As is well known, a chelating molecule is a  
20 compound containing electron donor atoms that can combine by coordinate bonding with a cation to form a coordination complex or chelate. This class of compounds is described in the Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 5, 339-368. By analogy, in the  
25 segmented polymer according to the invention the chelating agent residue is typically a chelating moiety which is a radical or a multivalent radical, rather than a molecule or a compound in itself. The polymer incorporating the chelating agent residue could thus be  
30 described as a chelating polymer.

In structure 1 above, Z represents a chelating agent residue. The chelating agent residue can be derived and/or selected from moieties which contain electron donor atoms. These moieties can be selected  
35 from, for example; polyphosphates, such as sodium tripolyphosphate and hexametaphosphoric acid;

linear, branched or cyclic aminocarboxylic acids,

such as ethylenediaminetetraacetic acid, N(2-hydroxyethyl)ethylenediaminetriacetic acid, nitrilotriacetic acid, N,N-di(2-hydroxyethyl)glycine, ethylenebis(hydroxyphenylglycine) and diethylenetriamine pentaacetic acid and the N-carboxymethylated macrocyclic polyazacycloalkanes such as DOTA and DO3A and the phosphonomethylated analogues;

1,3-diketones, such as acetylacetone, trifluoroacetylacetone, and thienoyltrifluoroacetone;

and hydroxycarboxylic acids, such as tartaric acid, mucic acid, citric acid, gluconic acid, and 5-sulfosalicylic acid;

polyamines, such as ethylenediamine, diethylenetriamine, triethylenetetramine, and triaminotriethylamine;

aminoalcohols, such as triethanolamine and N-(2-hydroxyethyl) ethylenediamine;

aromatic heterocyclic bases, such as 2,2'-dipyridyl, 2,2'-diimidazole, dipicoline amine and 1,10-phenanthroline;

phenols, such as salicylaldehyde, disulfopyrocatechol, and chromotropic acid;

aminophenols, such as 8-hydroxyquinoline and oxinesulfonic acid;

oximes, such as dimethylglyoxime and salicylaldoxime;

peptides containing proximal chelating functionality such as polycysteine, polyhistidine, polyaspartic acid, polyglutamic acid, or combinations of such amino acids, each polyamino acid containing from 2 to about 20 amino acids in the polymer;

Schiff bases, such as disalicylaldehyde 1,2-propylenediimine;

tetrapyrroles, such as tetraphenylporphin and phthalocyanine;

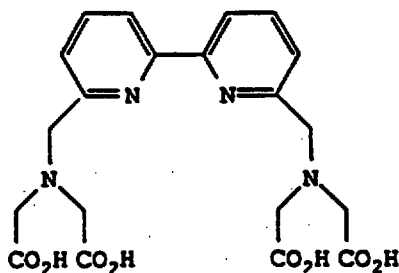
sulfur compounds, such as toluenedithiol, meso-2,3-dimercaptosuccinic acid, dimercaptopropanol, thioglycolic acid, potassium ethyl xanthate, sodium diethyldithiocarbamate, dithizone, diethyl

dithiophosphoric acid and thiourea;  
 synthetic macrocyclic compounds, such as  
 dibenzo[18]crown-6,  $(\text{CH}_2)_6$ -[14]-4,11-diene- $\text{N}_4$ , and  
 (2.2.2)-cryptate; and

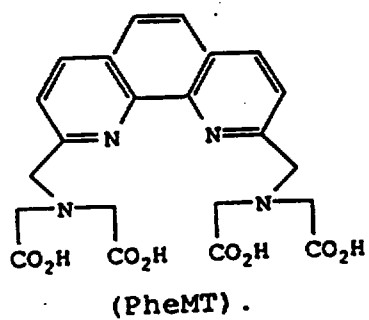
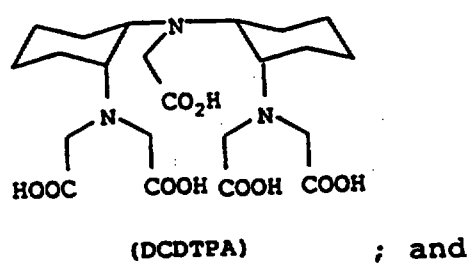
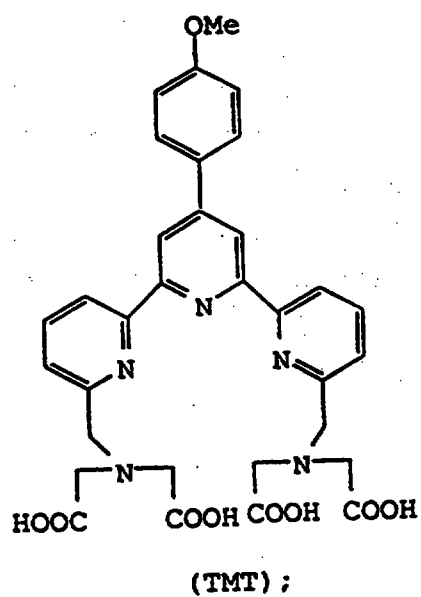
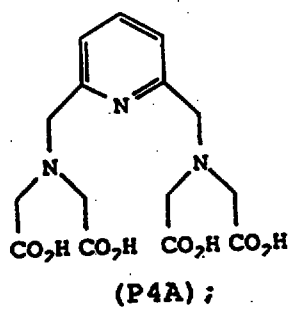
- 5 phosphonic acids, such as nitrilotrimethylenephosphonic acid, ethylenediaminetetra(methylenephosphonic acid), and hydroxyethylidenediphosphonic acid, or combinations of two or more of the above agents.

Preferred chelating agent residues contain one or  
 10 more carboxylic acid or carboxylate groups and include elements present in: ethylenediamine- $\text{N}$ ,  $\text{N}$ ,  $\text{N}'$ ,  $\text{N}'$ -tetraacetic acid (EDTA);  $\text{N}$ ,  $\text{N}$ ,  $\text{N}'$ ,  $\text{N}''$ ,  $\text{N}''$ -diethylenetriaminepentaacetic acid (DTPA); 1,4,7,10-tetraazacyclododecane- $\text{N}$ ,  $\text{N}'$ ,  $\text{N}''$ ,  $\text{N}'''$ -tetraacetic acid  
 15 (DOTA); 1,4,7-10-tetraazacyclododecane- $\text{N}$ ,  $\text{N}'$ ,  $\text{N}''$ -triacetic acid (DO3A); 1-oxa-4,7,10-triazacyclododecane- $\text{N}$ ,  $\text{N}'$ ,  $\text{N}''$ -triacetic acid (OTTA);  
 trans(1,2)cyclohexanodiethylenetriamine pentaacetic acid (CDTPA);

20



(B4A);



Such chelating agent compounds, including their preparation and chemical manipulation are well known in the art. For example, the acid and anhydride forms of EDTA and DTPA are commercially available. Methods for preparing B4A, P4A and TMT are described in U.S. Patent 4,859,777. Other suitable chelating groups are known in the art, and are described for example in PCT/US91/08253.

If Z is a chelating agent residue made of multiple chelating moieties or subunits, each of the subunits can be linked together by a linking group. Thus, more than one chelating moiety can be used to make up the chelating agent residue. If more than one chelating moiety is present in the chelating agent residue, these may be the same or different. Chelating moieties can be linked together using known chemistries and materials. Thus the chelating agent residue can be one moiety or a "core" of chelating moieties. For example, a core of DTPA residues may be prepared by reacting DTPA dianhydride with a diamine, such as ethylene diamine, to form a "core" of DTPA chelators. The anhydrides react with the amine to form amide bonds. Two DTPA moieties linked by one ethylene diamine and three DTPA moieties linked by 2 ethylene diamines are examples of preferred "cores" containing multiple DTPA residues, others are contemplated. Other chelating agent residues, made up of multiple chelating moieties are well known in the art and are prepared by known chemistries.

The segmented polymer according to the invention can be capped at the termini with groups represented by R in formula I linked to the termini of the poly(alkylene oxide) alkylene groups by a chemical bond or a linking group. These can be independently selected from hydrogen, hydroxy, C<sub>1-4</sub>-alkyl, aryl as hereinbefore defined, carboxy, C<sub>2-3</sub>-alkanoyloxy, and C<sub>1-4</sub>-alkoxy. In preferred embodiments, the polymer can be capped by reagents that produce acyl derivatives, for example, by

monoanhydrides, e.g., acetic anhydride or succinic anhydride and iodoacetic acid anhydride which forms an iodomethyl carbonyloxy intermediate that can be further reacted with an R precursor such as a protein or  
5 cytotoxic agent.

R in formula I can also be a cytotoxic drug, for example, a cytotoxic drug useful in the treatment of cancer. The cytotoxic drug will be selected with reference to factors such as, for example, the type of  
10 cancer tumor and the efficacy of a certain chemotherapy agent for treating the cancer tumor involved. The chemotherapy agent may be selected from alkylating agents, antimetabolites, natural products useful as cytotoxic drugs, hormones and antagonists and other  
15 types of cytotoxic compounds.

Examples of alkylating agents include the nitrogen mustards (i.e. the 2-chloroethylamines) such as, for example, chloromethine, chlorambucil, melphalan, uramustine, mannomustine, extramustine phosphate,  
20 mechlor-thaminoxide, cyclophosphamide, ifosamide and trifosfamide; alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone and mitomycin; alkylating agents of the alkyl sulfonate type; such as, for example,  
25 busulfan, hepsulfam, and piposulfan; alkylating N-alkyl-N-nitrosourea derivatives such as, for example, carmustine, lomustine, semustine or streptozotocine; alkylating agents of the mitobronitole, dacarbazine and procarbazine type; and platinum complexes such as, for  
30 example, cisplatin and carboplatin and others.

Examples of antimetabolites include folic acid derivatives such as, for example, methotrexate, aminopterin and 3'-dichloromethotrexate; pyrimidine derivatives such as, for example, 5-fluorouracil,  
35 floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiampirine,

vidarabine, pentostatin and puromycin and others.

Examples of natural products useful as cytotoxic agents include for example vinca alkaloids, such as vinblastine and vincristine; epipodophylotoxins such as, for example, etoposide, and teniposide; antibiotics such as, for example, adrimycin, daunomycin, dactinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin and mitomycin; enzymes such as, for example, L-asparaginase; biological response modifiers such as, for example, alpha-interferon; camptothecin; taxol; and retinoids such as retinoic acid and the like.

Examples of hormones and antagonists include adrenocorticoids, such as, for example, prednisone; progestins, such as, for example, hydroxyprogesterone acetate, medroxyprogesterone acetate and megestrol acetate; estrogens such as, for example, diethylstilbestrol and ethinyl estradiol; antiestrogens such as for example, tamoxifen; androgens such as, for example, testosterone propionate and fluoxymestrone; antiandrogens such as, for example, flutamide; and gonadotropin-releasing hormone analogs such as, for example, leuprolide.

Examples of miscellaneous agents include anthracenediones such as for example, mitoxantrone; substituted ureas such as, for example, hydroxyureas; and adrenocortical suppressants such as, for example, mitotane and aminoglutethimide.

In some embodiments, R is an immunoreactive group covalently bonded to Q through a linking group. As used herein, the term "immunoreactive group" is meant to include an organic compound which is capable of being covalently bound to the polymer and which is found in a living organism or is useful in the diagnosis, treatment or genetic engineering of cellular material or living organisms, and which has a capacity for interaction with another component which may be found in biological fluids or which may be associated with cells to be

- 20 -

treated such as tumor cells.

Depending upon the intended use, the immunoreactive group can be selected from a wide variety of naturally occurring or synthetically prepared materials, including, but not limited to enzymes, amino acids, peptides, polypeptides, proteins, lipoproteins, glycoproteins, hormones, drugs (for example digoxin, phenytoin, phenobarbital, thyrozone, triiodothyronine, gentamicin, carbamazepine, and theophylline), steroids, vitamins, polysaccharides, viruses, protozoa, fungi, parasites, rickettsia, molds, and components thereof, blood components, tissue and organ components, pharmaceuticals, haptens, lectins, toxins, nucleic acids (including oligonucleotides), antibodies, antigenic materials (including proteins and carbohydrates), avidin and derivatives thereof, biotin and derivatives thereof, histones and others known to one skilled in the art.

Preferred immunoreactive groups (sometimes referred to in the art as ligands) are those which have a receptor molecule specific to the ligand of interest. Thus, a specific binding reaction involving ligand, R, and receptor to form a ligand-receptor complex can be used for targeting, and treatment, and for imaging a target site. Examples of such ligand-receptor complexes include, but are not limited to antibody-antigen, avidin-biotin, histone-DNA, repressor (inducer), promoter of operons and sugar-lectin complexes. Additionally, complementary nucleic acids, both natural and antisense are also considered specific binding materials as the term is used herein. Either component of the above listed pairs can be useful as a ligand if the other component is found or attached to the target site.

Useful immunoreactive groups include (1) any substance which, when presented to an immunocompetent host, will result in the production of a specific antibody capable of binding with that substance, or (2)

the antibody so produced, which participates in an antigen-antibody reaction. Thus, the immunoreactive group can be an antigenic material, an antibody, or an anti-antibody. Both monoclonal and polyclonal antibodies  
5 are useful. The antibodies can be whole molecules or various fragments thereof, as long as they contain at least one reactive site for reaction with the reactive groups on the complexing agent or with linking groups as described herein.

10 In certain embodiments, the immunoreactive group can be an enzyme which has a reactive group for attachment to the complexing agent. Representative enzymes include, but are not limited to, aspartate aminotransaminase, alanine aminotransaminase, lactate  
15 dehydrogenase, creatine phosphokinase, gamma glutamyl transferase, alkaline acid phosphatase, prostatic acid phosphatase, tissue plasminogen activator, horseradish peroxidase and various esterases.

If desired, the immunoreactive group can be  
20 modified or chemically altered to provide reactive groups for linking the immunoreactive group to the polymer. These methods are well known in the art. Such techniques include the art recognized use of linking moieties and chemical modification such as described in  
25 WO-A-89/02931 and WO-A-89/2932, which are directed to modification of oligonucleotides, and the disclosure of U.S. Patent 4,719,182. Where the reactive group for linking the immunoreactive group to the polymer is attached to a protein, antibody and the like, it is  
30 called a "protein reactive group"

Two highly preferred uses for the compositions of this invention where R is an immunoreactive group are for the diagnostic imaging of, tumors and the radiological treatment of tumors. Preferred  
35 immunoreactive groups therefore include antibodies, or immunoreactive fragments thereof, to tumor-associated antigens. Specific examples include B72.3

- 22 -

antibodies (described in U.S. Patent Nos. 4,522,918 and 4,612,282) which recognize colorectal tumors, 9.2.27 antimelanoma antibodies, D612 antibodies which recognize colorectal tumors, UJ13A antibodies which recognize  
5 small cell lung carcinomas, NRLU-10 antibodies which recognize small cell lung carcinomas and coalorectal tumors (Pan-carcinoma), 7E11C5 antibodies which recognize prostate tumors, CC49 antibodies which recognize colorectal tumors, TNT antibodies which  
10 recognize necrotic tissue, PR1A3 antibodies, which recognize colon carcinoma, ING-1 antibodies, which are described in International Patent Publication WO-A-90/02569, B174 antibodies which recognize squamous cell carcinomas, B43 antibodies which are reactive with  
15 certain lymphomas and leukemias and others which may be of particular interest.

Such antibodies and other useful immunological groups described above are large, complex protein molecules having multiple sites for appendage of the  
20 segmented polymer complexing. Consequently, the immunoreactive group can have appended to it additional complexing agents each via one of the protein reactive groups. Thus, the term immunoreactive group is intended to include immunological groups having at least one  
25 segmented polymer molecule bonded thereto through at least one protein reactive group.

Additionally, the immunoreactive group can be an antibody or fragment thereof containing a carbohydrate region which can be attached to the polymer through the  
30 carbohydrate region such as described in U.S. Patent 4,937,183. Useful methods for attaching an antibody are also described in U.S. Patent Nos. 4,671,958; 4,699,784; 4,741,900; and 4,867,973.

Additionally, immunoreactive groups that interact  
35 with intracellular components are also specifically contemplated; for example, actin, myosin, histone, DNA, DNAase and the like. These molecules, while useful in

all contemplated applications, are preferred for targeting solid tumors and necrotic cells. Specifically contemplated in this embodiment is the use of a histone linked to the polymer of the invention for imaging solid tumors. Histones have low immunogenicity, bind tightly to nucleic acids, and have difficulty passing through cell and nuclear membranes in intact living cells. However, in necrotic tissue, the cell and nuclear membranes no longer function and are often perforate, allowing entry of foreign materials, including histones. This diffusion into necrotic tissue can be used to accumulate an imaging and/or cytotoxic agent of This invention in the vicinity of cancer cells, especially in solid tumors. Necrosis can occur naturally due to a limited blood supply, or can be induced by cytotoxic agents or irradiation. Thus, this method is useful in imaging and/or treatment of tumors. As a further example, DNAase when attached to the polymer of the invention can be used to target for detection and destruction, free DNA.

Free DNA accumulates in certain diseases, such as systemic lupns where it ultimately causes kidney failure. Thus this polymer can be used to detect DNA accumulation in kidney glomeruli and destroy it.

The immunoreactive group can be attached to the polymer by methods known in the art for derivatizing any functional groups typically found on proteins, or other immunoreactive groups. However, it is specifically contemplated that the immunoreactive group can be a nonprotein biomolecule. Thus, a linking group precursor useful in preparing the polymer of the invention will include those groups which can react with any biological molecule containing an immunoreactive group, whether Or not the biological molecule is a protein, to form a linking group between the polymer agent and the immunoreactive group.

Preferred linking group precursors can be selected

from but are not limited to:

(1) A group that will react directly with the amine or sulfhydryl groups on the protein or biological molecule containing the immunoreactive group, for example, active halogen containing groups including, for example, chloromethylphenyl groups and chloroacetyl [Cl-CH<sub>2</sub>CO-] groups, activated 2-leaving group substituted ethylsulfonyl and ethylcarbonyl groups such as 2-chloroethylsulfonyl and 2-chloroethylcarbonyl; vinylsulfonyl; vinylcarbonyl; epoxy; isocyanato; isothiocyanato; aldehyde; aziridine; succinimidoxycarbonyl; activated acyl groups such as carboxylic acid halides; mixed anhydrides and the like; and other groups known to be useful in conventional photographic gelatin hardening agents.

(2) A group that can react readily with modified proteins or biological molecules containing the immunoreactive group, i.e., proteins or biological molecules containing the immunoreactive group modified to contain reactive groups such as those mentioned in (1) above, for example, by oxidation of the protein to an aldehyde or a carboxylic acid, in which case the "protein reactive group" can be selected from amino, alkylamino, arylamino, hydrazino, alkylhydrazino, arylhydrazino, carbazido, semicarbazido, thiocarbazido, thiosemicarbazido, sulfhydryl, sulfhydrylalkyl, sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and carboxyaryl. The alkyl portions of the protein reactive group can contain from 1 to about 18 carbon atoms. The aryl portions of the protein reactive group can contain from about 6 to about 20 carbon atoms.

(3) A group that can be linked to the protein or biological molecule containing the immunoreactive group, or to the modified protein as noted in (1) and (2) above by use of a crosslinking agent. Certain useful crosslinking agents, such as, for example, difunctional gelatin hardeners, bisepoxides and bisisocyanates become

a part of, i.e., a linking group in, the protein-segmented polymer complexing agent conjugate during the crosslinking reaction. Other useful crosslinking agents, however, facilitate the crosslinking, for example, as consumable catalysts, and are not present in the final conjugate. Examples of such crosslinking agents are carbodiimide and carbamoylonyl crosslinking agents as disclosed in U.S. Patent 4,421,847 and the dication ethers of U.S. Patent 4,877,724. With these crosslinking agents, one of the reactants must have a carboxyl group and the other an amine, alcohol, or sulfhydryl group. The crosslinking agent first reacts selectively with the carboxyl group, then is split out during reaction of the "activated" carboxyl group with, for example, an amine to form an amide linkage between the protein or cytotoxic agent and the segmented polymeric chelating agent, this covalently bonding the two moieties. An advantage of this approach is that crosslinking of like molecules, e.g., proteins with proteins or complexing agents with complexing agents is substantially avoided where m is one, whereas the reaction of difunctional crosslinking agents is less selective and unwanted crosslinked molecules can be obtained. Especially preferred protein reactive groups include amino and-isothiocyanato.

While the segmented polymer according to the invention is described herein primarily in connection with its preferred utilities, i.e., as a contrast agent for use in imaging compositions and methods, it also finds utility in other applications and fields, e.g., as a therapeutic agent, an ion scavenging agent, an ion detecting agent and the like.

The segmented polymer according to the invention may comprise a therapeutic moiety and/or a moiety, preferably a metal, for enhancing contrast during imaging, and thus serve two functions simultaneously. This attribute is particularly useful in the detection

- 26 -

and treatment of tumors.

The segmented polymers according to the invention comprise a chelating agent residue linked to at least one poly(alkylene oxidylene) moiety. Higher molecular weight polymers comprising 100 to 750 alkylene oxide units are preferred in blood pool imaging as they have longer blood pool residence times.

The polymer may be prepared so as to be useful in imaging diseased tissue or state, i.e. useful in identifying or diagnosing the diseased area, and also useful in treating the area optionally in the same dose. This is accomplished by judicious choice of R which can be cytotoxic, and/or useful in imaging (e.g. fluorescence imaging if R=aryl) and of M, the chelated metal, which can be a cytotoxic radionuclide and/or an imaging agent useful in radioscintigraphy, x-ray, fluorescence ore imaging.

Viewed from a further aspect, the present invention provides a method of generating an enhanced image of the human or non-human animal body, said method comprising administering to said body a contrast enhancing amount of a contrast enhancing polymer according to the invention and generating an X-ray, MR, ultrasound or scintigraphic image of at least a part of said body into which said polymer distributes.

Viewed from a still further aspect, the present invention provides a method of therapy practised on the human or non-human animal body, which method comprises administering to said body a therapeutically effective polymer according to the invention.

Viewed from a yet further aspect, the present invention provides the use of the segmented polymers according to the invention for the manufacture of diagnostic or therapeutic agents for use in methods of image generation or therapy practised on the human or non-human animal body.

For magnetic resonance imaging applications, the

chelated metal ion  $M^{(+a)}$  represents a paramagnetic metal ion such as for example an ion of atomic number 21 to 29, 42, 44 and 58 to 70. Ions of the following metals are preferred: Cr, V, Mn, Fe, Co, Ni, Cu, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm and Yb. Especially preferred are  $Cr^{3+}$ ,  $Cr^{2+}$ ,  $V^{2+}$ ,  $Mn^{3+}$ ,  $Mn^{2+}$ ,  $Fe^{3+}$ ,  $Fe^{2+}$ ,  $Co^{2+}$ ,  $Gd^{3+}$  and  $Dy^{3+}$ .  $Gd^{3+}$  and  $Dy^{3+}$  are most preferred.

For fluorescent imaging applications,  $M^{+a}$  represents a fluorescent metal ion, preferably a metal of atomic number 57 to 71, most preferably a  $Eu^{3+}$  ion.

$M^{(+a)}$  can be a radioactive metal ion isotope. As such, it can be a cytotoxic agent and/or an agent useful in diagnostic imaging such as in radiosciintigraphy.

The radioactive metal isotope can be an ion of an isotope of a metal selected, for example, from the group consisting of Sc, Fe, Pb, Ga, Y, Bi, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru, In, Sn, Re, Sr, Sm, Lu, Dy, Sb, W, Re, Po, Ta and Tl ions. Preferred isotopes of radioactive metal ions include  $^{44}Sc$ ,  $^{64,67}Cu$ ,  $^{111}In$ ,  $^{212}Pb$ ,  $^{68}Ga$ ,  $^{90}Y$ ,  $^{153}Sm$ ,  $^{212}Bi$ ,  $^{99m}Tc$  and  $^{186,188}Re$  for therapeutic and diagnostic imaging applications.

Specifically contemplated are segmented polymers where  $r$  (the number of metals in the chelating residue) is greater than 1, and in those cases each  $M^{(+a)}$  can represent the same or a different cation in the polymer; for example a polymer having  $[Mn^{2+}]_2[Gd^{3+}]$  is specifically contemplated; as is  $[Gd^{3+}]_2[^{67}Cu^{2+}]_2$  or any other combinations within or between the classes of cations used in the polymer. Such polymers are thus useful for more than one type of imaging or cytotoxic therapy. It is understood that the sum of positive charges ( $\Sigma r.a$ ) is the sum of net positive charge for all cations. Thus for the listed examples above,  $\Sigma(r.a)$  is 7 and 10, respectively. For example, if  $Z$ , the chelating agent residue, has 5 free carboxylates, ( $d = -5$ ) and one  $Gd^{3+}$  chelated therein ( $M^{(+a)} = Gd^{3+}$ ;  $a = +3$ ), then the total charges of counter ion ( $w \times b$ ) must be +2, thus if  $w$  is

- 28 -

1, b is +2 as in calcium or magnesium; if w is 2, b is +1 as in a proton (assumed ionic for our purposes in formula I), sodium or potassium. If Z has d = -3 and chelates  $Gd^{3+}$  (a = +3), no counter ion is required, but they may exist if  $\Sigma(w \times b) = 0$ .

When E is present, i.e., when w is not zero, b most preferably is 1 or 2. E is especially preferably a pharmaceutically acceptable cation or anion. The skilled artisan will have no difficulty in selecting appropriate counterions. The total positive charge on the cations  $\Sigma(r.a)$  equals the sum of the total charge on the chelating agent residue (d), plus the total charge on any counterions E present (b). When r=0, then w=0. When r=1, w can be 0, 1, 2, 3, 4 or 5. In the most preferred embodiment, r is 1 and w is 0, a=d+b.

E in formula I can be one or more counterions. For example, E can be one or more anions, such as a halide; sulfate; phosphate; nitrate; and acetate and the like. E can be one or more cations such as  $Na^+$ ,  $K^+$ , meglumine, and the like. For in vivo applications, particularly for diagnostic imaging applications, nontoxic physiologically tolerable anions and cations are, of course, desirable.

Where r is 0 the segmented polymers according to the invention can be used in chelation therapy to treat heavy metal poisoning, e.g. lead poisoning and the like. For treatment of heavy metal poisoning, the polymers are administered alone without chelated ions, or in some applications of metal poisoning treatment, some other metal ions such as calcium are chelated by the polymer prior to administration. It is contemplated that such polymers as non chelated materials, as completely chelated or as mixtures of non chelated and chelated polymers can be used to treat poisoning from such metal ions as Sc, Ti, V, Cr, Mn, Fe, Eu, Er, Pb, Co, Ni, Cu, Ga, Sr, Y, Zr, U, Pu, Tc, Ru, In, Hf, W, Re, Os, Dy, Gd, Hf, La, Yb, Tc, As and the like.

In a mixture of chelated and non chelated polymers of this invention, wherein a metal is associated with the chelating agent residue in some of the segmented polymers of the mixture, the metal content in the mixture can vary from about 0.1 up to about 12% based on the total weight of the polymer in the mixture. The mixture of segmented polymers preferably contains the metal in an amount of from 1% to 5%, more preferably 1-4% by weight. Such mixtures can be formed by treating unchelated polymer with enough metal ion to chelate to from 1% to 15% or preferably from 1% to 10% by weight of the mixture.

The concept of drug targeting has gained importance in recent years, especially for anticancer drugs, inasmuch as toxic side effects of anticancer drugs to normal cells are a primary obstacle in cancer chemotherapy due to lack of selectivity to cancer cells. However, the desirability of targeting a certain tissue type finds application in other areas as well. Drug targeting can be accomplished by conjugation of the drug with, or encapsulation in, a specific transporter to the target. Immunoreactive groups or materials such as proteins, saccharides, lipids and synthetic polymers have been used for such transporters. Antibodies have been most widely used of these perhaps due to their target specificity and wide applicability.

It is known in the art that the size and charge of the polymer, as well as potential interactions with blood components, can influence the biodistribution of the polymer. Thus, judicious polymer synthesis can result in passive tissue targeting by the polymer.

For example, the hydrodynamic radius of albumin is approximately 37 Å, its molecular weight is 67 Kd, and its charge is known. It is known that the average half life for albumin circulation through tissue is approximately 24 hours, but this half life is longer in some tissues and shorter in others. It is also known

- 30 -

that the lymphatic system recirculates albumin back into the circulation system. Moreover, the concentration of albumin in certain tissues is appreciable and in other tissues albumin is nearly absent altogether. The skilled  
5 artisan can prepare a polymer of approximately for same size (or preferably the same hydrodynamic radius) and charge, and expect a similar half life and concentration in tissues.

The skilled artisan will recognize that  
10 inflammation of tissues will perturb the normal physiology of that tissue and thus perturb the half life and concentration of macromolecules (such as proteins or the polymer of the invention) in the inflamed tissue or inflamed tissue site. Thus the polymer finds utility in  
15 imaging and/or treating such inflamed tissues or inflamed tissue sites.

The skilled artisan will also appreciate that the absence of a lymphatic system in a tissue will perturb the concentration and increase the half life of  
20 macromolecules in a tissue because no convenient mechanism (e.g. the lymphatic system) is provided for the scavenging of such macromolecules. Such is the case in growing tumors. One can deliver the polymer as a cytotoxic agent, and/or an imaging agent to the growing  
25 tumor surface based on size of the polymer and on vasculature of the surrounding or targeted tissue. Thus, dosing with the appropriate molecular weight polymer will result in accumulation of such polymer in the growing surface of the tumor.

30 The composition according to the invention can be prepared in water-soluble or water dispersable forms depending upon the intended application more preferably in an injectable form for diagnostic imaging or as a composition intended to be administered intravenously.  
35 Such compositions preferably have a molecular weight of at least 4,500, more preferably 4,500 to 40,000. The preparation of water-soluble compositions of at least

4,500 in molecular weight can be accomplished by conventional means by one skilled in the art.

Actual dosage levels of the active ingredient in the compositions according to the present invention may be varied so as to obtain an amount of active ingredient that is effective to obtain a desired therapeutic or diagnostic response. The selected dosage level therefore depends on the desired therapeutic or diagnostic effect, on the route of administration, on the desired duration of treatment and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided dose may be in amounts, for example, of from about 1 picomole to about 10 millimoles of cytotoxic agent per kilogram of body weight. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

The dosages of the contrast agent used according to the method of the present invention will vary according to the precise nature of the contrast agent used. Preferably however, the dosage should be kept as low as is consistent with achieving contrast enhanced imaging and volumes minimized for IV drip or bolus injection. In this way, the toxicity potential is minimized. For most magnetic resonance contrast agents the current appropriate dosage will generally range from 0.02 to 3 mmol paramagnetic metal/kg body weight, especially 0.05 to 1.5 mmol/kg, particularly 0.08 to 0.5, more especially 0.1 to 0.4 mmol/kg. However, it is anticipated that the amount of contrast agent needed

will decrease as detection sensitivity in imaging machines increases. It is well within the skill of the average practitioner in this field to determine the optimum dosage for any particular magnetic resonance contrast agent by relatively routine experimentation, for both in vivo or in vitro applications.

Contrast agents may be formulated with conventional pharmaceutical or veterinary aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc., and may be in a form suitable for injection or infusion directly or after dispersion in or dilution with a physiologically acceptable carrier medium, e.g., water for injection. Thus the contrast agents may be formulated in conventional administration forms such as powders, solutions, suspensions, dispersions, etc. However, solutions, suspensions and dispersions in physiologically acceptable carrier media will generally be preferred.

The contrast agents may be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized.

Parenterally administrable forms, e.g., intravenous solutions, should of course be sterile and free from physiologically unacceptable agents, and should have low osmolality to minimize irritation or other adverse effects upon administration. Thus, the contrast medium should preferably be isotonic or slightly hypertonic. Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other solutions such as