(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/08712 A2

(51) International Patent Classification⁷: A61K 49/00

(21) International Application Number: PCT/US00/20536

(22) International Filing Date: 28 July 2000 (28.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/146,414 29 July 1999 (29.07.1999) US
60/163,650 4 November 1999 (04.11.1999) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

2003 x [ ] = 1 P 830

(54) Title: TARGETING MULTIMERIC IMAGING AGENTS THROUGH MULTILOCUS BINDING

(57) Abstract: The present invention relates to contrast agents for diagnostic imaging. In particular, this invention relates to novel multimeric compounds which exhibit improved relaxivity properties upon binding to endogenous proteins or other physiologically relevant sites. The compounds consist of: a) two or more Image Enhancing Moieties (IEMs) (or signal-generating moiety) comprising multiple subunits; b) two or more Target Binding Moieties (TBMs), providing for in vivo localization and multimer rigidification; c) a scaffold framework for attachment of the above moieties; and d) optional linkers for attachment of the IEMs to scaffold. This invention also relates to pharmaceutical compositions comprising these compounds and to methods of using the compounds and compositions for contrast enhancement of diagnostic imaging.

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TARGETING MULTIMERIC IMAGING AGENTS THROUGH MULTILOCUS BINDING

TECHNICAL FIELD OF THE INVENTION

The present invention relates to contrast agents for diagnostic imaging. In particular, this invention relates to novel multimeric compounds which exhibit improved affinity for physiologically relevant targets, such as proteins, and surprisingly improved relaxivity properties upon binding. The compounds comprise:

a) two or more Image Enhancing Moieties ("IEMs");

b) two or more Target Binding Moieties ("TBMs"), providing for in vivo localization and multimer rigidification;

c) a scaffold framework for attachment of the above moieties ("scaffold");

d) optional linkers for attachment of IEMs to the scaffold ("linker").

This invention also relates to pharmaceutical compositions comprising these compounds and to methods of using the compounds and compositions for contrast enhancement during imaging.

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BACKGROUND OF THE INVENTION

Diagnostic imaging techniques, such as magnetic resonance imaging (MRI), X-ray, nuclear radiopharmaceutical imaging, ultraviolet-visible-infrared light imaging, and ultrasound, have been used in medical diagnosis for a number of years. Contrast media additionally have been used to improve or increase the resolution of the image or to provide specific diagnostic information. In some cases, such as imaging with ultrasound, the introduction of contrast media has been recent.

To be effective, the contrast media must interfere with the wavelength of electromagnetic radiation used in the imaging technique, alter the physical properties of tissue to yield an altered signal, or, as in the case of radiopharmaceuticals, provide the source of radiation itself. MRI and optical imaging methods are unique among imaging modalities in that they yield complex signals that are sensitive to the chemical environment. While the signal from X-ray or radionuclide agents remains the same whether the agents are free in plasma, bound to proteins or other targets, or trapped inside bone, certain contrast agents for MRI and optical imaging will have different signal characteristics in differing physiological environments. An optical dye may exhibit changes in its absorbance, reflectance, fluorescence, phosphorescence, chemiluminescence, scattering, or other spectral properties upon binding. It is important that the contrast agent be sufficiently sensitive and present at high enough concentration so that signal changes can be observed.

Attempts to improve contrast by increasing the number of IEMs

Targeted agents should deliver meaningful concentrations of the imaging moiety to the target so that sufficient improvement in the signal is observed

during the course of imaging. Achieving sufficient sensitivity is a significant problem for MRI in particular, where concentrations in the range of 10 - 1000 micromolar (μM) of the image enhancing moiety are required to produce an adequate signal. The problem can be further complicated for targeted agents if the desired target is present at low concentrations. For example, in order to image biological receptor targets that are present at less than μM concentrations, greater signal enhancement is required at the target site to provide sufficient image contrast. Increased contrast has been approached by using (1) drug delivery vehicles to provide high local concentrations of the contrast agent, (2) multiple IEMs in a single contrast agent, [see, for example, Martin V. V., et al., Bioconjug. Chem., 6: pp. 616-23 (1995); Shukla, R. et al., Mag. Reson. Med., 35: pp. 928-931 (1996); Ranganathan, R. S., et al., Invest. Radiol., 33: pp. 779-797 (1998)], or (3) particular IEMs of defined structure with improved signal enhancement properties. The ideal targeted contrast agent should efficiently combine IEMs and improved signal enhancement properties.

To incorporate a high number of image enhancing moieties into a contrast agent, large concentrations of low molecular weight contrast agents have been packaged within suitable drug delivery vehicles, such as polymerized vehicles or liposomes [Bulte J. W., et al., J. Magn. Reson. Imaging, 9: pp. 329-335 (1999)]. Unfortunately, these materials are difficult to direct to a target.

To increase the number of the image enhancing moieties, investigators have, for example, created polymers, dendrimers, and organic compounds in association with multiple IEMs. High numbers of IEMs, such as Gd(III) chelates for MRI, can be covalently attached to polymers [Schuhmann-Giampieri, G. et al. J.

Invest. Rad., 26: pp. 969-974 (1991); Corot, C. et al. Acta Rad., 38:S412 pp. 91-99 (1997)] and dendrimers [Jacques, V., et al., J. Alloys Cmpd., 249: pp. 173-177 (1997); Margerum, L. D., et al., J. Alloys Compd., 249: pp. 185-190 (1997); Toth, E., et al., Chem. Eur. J., 2: pp. 1607-1615 (1996)]. Polymeric agents typically comprise a mixture of species with a broad and complex molecular weight distribution. These heterogeneous properties adversely affect agent performance and make characterization difficult. Furthermore, it is synthetically difficult to selectively introduce TBMs along with multiple IEMs. Therefore there exists a need for well-defined, homogeneous molecules for use as contrast agents that can provide adequate image enhancement at a target.

Dendrimers (such as "Starburst dendrimers", or "cascade polymers") theoretically offer a single high molecular weight species onto which many IEMs can be covalently attached. [Fischer, M. et al. Angew. Chem., Int. Ed. Eng., 38/7: pp. 884-905 (1999); Weiner, E.C. et al., Mag. Reson. Med., 31: pp. 1-8 (1994)]. However, dendrimers, like polymeric agents, present significant synthetic problems, especially when selectively introducing tissue-specific targeting groups.

Organic molecules have been synthesized with multiple image enhancing moieties. MRI contrast agents of this type are referred to herein as "multimeric chelates" or "multimers" and typically comprise 2-12 IEMs. [Shukla, R. et al., Mag. Reson. Med., 35: pp. 928-931 (1996); Shukla, R. B., et al., Acta Radiol., 412: pp. 121-123 (1997); Ranganathan, R. S., et al., Invest. Radiol., 33: pp. 779-797 (1998)]. Advantages of multimeric chelates include: (1) they are homogeneous molecules in that they have a single size and structure, unlike polymers and dendrimers, (2) they can be readily synthesized and purified, and (3) targeting groups can be

readily incorporated. Unfortunately, the ability of multimeric chelates to improve the MRI signal intensity has been disappointingly low. This is because the proton relaxation rate enhancement (or "relaxivity"), which correlates with signal enhancement, has decreased as the number of IEMs was increased. Therefore, contrast agents wherein the relaxivity does not decrease when the number of IEMs increases are needed to achieve greater signal enhancement at a target.

Attempts to improve contrast by decreasing the rotational of the contrast agent

Attempts have been made to increase the relaxivity of non-targeted multimeric MRI contrast agents by restricting rotational motion. Attempts to restrict rotational motion have focused on (1) decreasing the flexibility of the molecule or (2) restricting rotational motion through binding to a target.

For example, non-targeted agents have been synthesized with rigid frameworks to which multiple Gd(III) chelates are attached [Shukla, R. et al., Mag. Reson. Med., 35: pp. 928-931 (1996); Shukla, R. B., et al., Acta Radiol., 412: pp. 121-123 (1997); Ranganathan, R. S., et al., Invest. Radiol., 33: pp. 779-797 (1998); Jacques, V., et al., J. Alloys Cmpd., 249: pp. 173-177 (1997)]. However, these structures have several drawbacks. First, the relaxivities per Gd(III) ion that have been achieved for agents containing more than two chelates has been less than that observed for single chelates, such as MS-325. Therefore, local chelate motion could still be further reduced. Second, these agent are not targeted. More importantly, even if they were targeted, rigid multimer frameworks would greatly increase the unwanted background signal because the signal enhancement is significant regardless of whether the contrast agent is bound to a target or not.

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Therefore, there exists a need for contrast agents that enhance an image of a target only when bound to the target.

Rotational motion of a single IEM can be effectively limited upon non-covalent target binding, resulting in a relaxivity increase for the target-bound forms of as much as 5-10 fold [U.S. Patent No. 4,880,008]. This relaxivity increase is as good as or better than that observed for IEMs that are covalently linked to the target [Schmiedl, U., Ogan, M., Paajanen, H., Marotti, M., Crooks, L.E., Brito, A.C., and Brasch, R.C. Radiology (1987) 162: pp.205-210; Ogan, M.D., Schmiedl, U., Moseley, M.E., Grodd, W., Paajanen, H., and Brasch, R.C. Invest. Radiol. (1987) 22: pp. 665-71]. Examples of agents which exploit this effect are the liver protein-targeted contrast agents Gd-EOB DTPA [Runge V. M. Crit. Rev. Diagn. Imaging 38: pp. 207-30 (1997)] and Gd-BOPTA [Kirchin.M. A., et al., Invest. Radiol., 33: pp. 798-809 (1998)] or the albumin-targeted agents MS-325 [Lauffer, R. B., et al., Radiology, 207: pp. 529-538 (1998)] and MP-2269 [Hofman Mark B.M. et al. Acadademic Radiology, 5(suppl 1): S206-S209 (1998)]. Relaxivity increases of approximately 7-fold were reported for MS-325 (47 $mM^{-1}s^{-1}$) as a result of non-covalent binding to serum albumin [Lauffer, R. B., et al., Radiology, 207: pp. 529-538 (1998)].

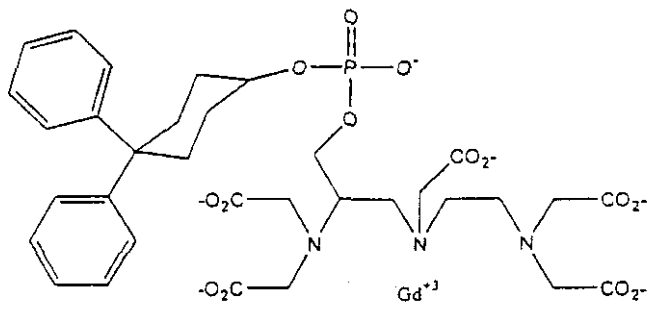

Figure 1: Chemical Structure of the MRI Contrast Agent MS-325

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Upon binding to albumin, the monomeric contrast agent MS-325, having the chemical structure shown in Figure 1, exhibits an increase in signal enhancement. When bound, the complex tumbles at a slower rate than in the unbound state which results in greater relaxivity. Surprisingly, however, the addition of multiple IEMs to targeted contrast agents, such as MS-325, has failed to enhance contrast further because relaxivity decreases at the individual gadolinium centers in the multimeric structure. For example, an albumin-targeted multimer with four Gd(III) ions exhibited molecular relaxivities per Gd(III) of only 9-13 $mM^{-1}s^{-1}$ compared to a relaxivity of 47 $mM^{-1}s^{-1}$ for MS-325, which contains a single Gd(III) [Martin V. V., et al., Bioconjug. Chem., 6: pp. 616-23 (1995)]. Thus, the relaxivity of a targeted multimeric chelate is typically much less *per Gd(III)* than that observed for the analogous targeted single chelate.

Rationale

Table 1 demonstrates that merely increasing the number of IEMs is insufficient to improve total relaxivity because the relaxivity per IEM decreases as the number of IEMs increases despite the presence of the target binding group comprising two phenyl rings. To understand Table 1, it is important to define the extent to which a target-binding MRI contrast agent can achieve its maximum possible relaxivity. This maximum relaxivity for a particular contrast agent is approximately equal to the relaxivity of the molecule when bound to a target ($R1_{bound}$), such as Human Serum Albumin (HSA). The average RI_{bound} is a normalized measure of the average relaxivity for all bound species under a standard set of conditions (such as a specific target or protein concentration, drug concentration, temperature, etc.) that is weighted by the bound population of each species. Therefore, since the value of RI_{bound} is a normalized quantity, comparisons of

relaxivities can be made among different molecules in the bound state by comparing values for $\mathbb{RI}_{bound}$. Comparison of the calculated $\mathbb{RI}_{bound}$ values provides a convenient method for comparing compounds irrespective of their affinities for a target.

Calculating the average ($\mathbb{RI}_{bound}$) requires measuring the relaxivity of the free chelate ($R1_{free}$) as well as the observed relaxivity ($R1_{obs}$) and percent binding of the agent to a target solution typically containing 4.5% of the target, e.g., HSA. The $R1_{obs}$ is a mole fraction (x) weighted average of $R1_{free}$ and $R1_{bound}$:

$$R1_{obs} = x_{free}R1_{free} + \sum_i x_i R1_{bound,i}$$

where $x_{free} + \sum_i x_i = 1$

and $\sum_i x_i = x_{bound}$

Thus:

$$\mathbb{RI}_{bound} = \frac{R1_{obs} - x_{free}R1_{free}}{x_{bound}}$$

The chemical structures and bound relaxivities of a series of albumin-targeted contrast agents are shown in Table 1. In this set of compounds, a compound with a single IEM (i.e., MS-325) is compared with a series of multimers comprising multiple IEMs, but the same diphenylcyclohexyl albumin TBM and the methylene phosphate group are present in all compounds.

Table 1. Chemical structures and bound relaxivities of a series of albumin-targeted contrast agents. The diphenylcyclohexyl target binding moiety (TBM) remains constant.

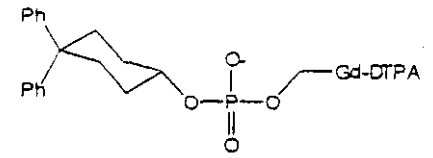
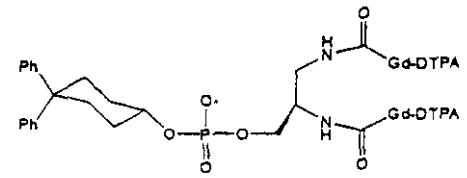
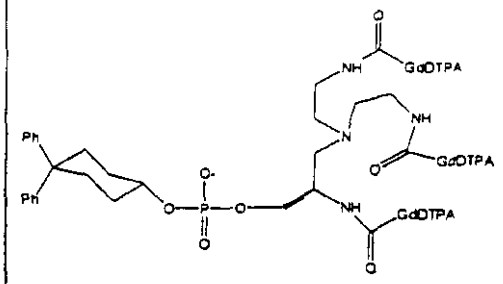
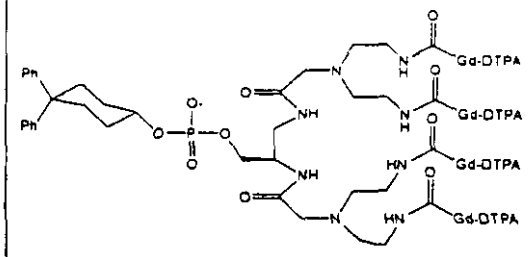
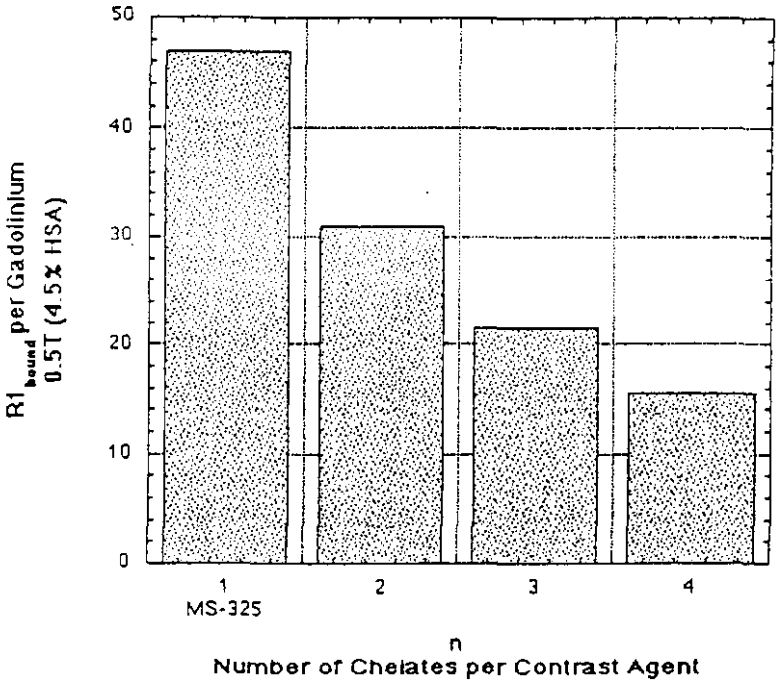
Compound #	Chemical Structure	Ave. RI_{bound} per Gd(III) (20 MHz)	Total RI_{bound} (20 MHz)
MS-325		47	47
M8-01		38.1	76.2
M8-02		23.6	70.7
M8-03		14.9	59.6

Figure 2 is a graphical representation of the same data shown in Table 1. The average RI_{bound} per IEM, in this case a Gd(III) chelate, at 20 MHz is plotted against the number of IEMs.

Figure 2. Plot of bound relaxivity (per gadolinium) for a series of multimeric contrast agents containing the a single diphenylcyclohexyl protein binding group. As the multimer size increases, the data indicate that the relaxivity per gadolinium decreases.



In the molecules of Table 1 and Figure 2, both the structure of the gadolinium chelate (IEM), the methylene phosphate group, and the diphenylcyclohexyl group (TBM) remain constant. The data in Table 1 and Figure 2 show that as the number of chelated paramagnetic metal ions increases, the relaxivity per metal ion is reduced. The number of Gd(III) chelates varies from one (MS-325) to four, but despite this four-fold increase in the number of IEMs, the total relaxivity increases by only about 50%. This modest increase in total relaxivity is a consequence of the decreasing relaxivity per Gd(III) ion. Note that the

average $R1_{bound}$ per Gd(III) decreases from 47 $mM^{-1}s^{-1}$ to 14.9 $mM^{-1}s^{-1}$ despite the contrast agent being bound. This decrease is due to local chelate motion which surprisingly increases with the number of IEMs despite multiple aromatic rings in the single TBM.

Apparently, increasing the number of chelating moieties also increases the rotational freedom of the molecule, at least near the sites of gadolinium chelation. The decrease in relaxivity is especially notable as the size increases beyond two chelated gadolinium ions per multimer molecule. For example, in the case of M8-03 the total relaxivity per gadolinium is only about 15 $mM^{-1}s^{-1}$, approximately one third that observed for MS-325. The total relaxivity for the compound M8-03 is therefore only 60 $mM^{-1}s^{-1}$, just 1.3 times that of MS-325 although four times as many IEMs are present. Obviously, such a modest increase in relaxivity does not justify the added synthetic complexity and cost to develop such agents for in vivo MR imaging. Thus, the simple combination of multiple image enhancing moieties with a single target binding moiety does not generate a commensurate increase in relaxivity. Thus, there exists a need to synthesize multimeric MRI contrast agents wherein the relaxivity at each chelate is maintained even as the number of IEMs increases.

Overall, immobilization of a target-bound contrast agent can be remarkably effective at increasing the relaxivity for a single chelate (e.g. MS-325) but is rather ineffective for multimeric chelates. That is, in order to increase the relaxivity at each chelate site, it is necessary to both reduce the overall rotational correlation time for the molecule to reduce the local chelate motion at each chelation site. There remains a need for a mechanism to efficiently immobilize target-binding multimeric

contrast agents so that more effective signal enhancement is produced during imaging.

A method is needed to improve signal contrast at specific targets. The problem has been approached by (1) increasing the number of IEMs, or (2) decreasing the flexibility of the molecule. Increasing the number of IEMs has been unsuccessful because the contrast agents are not of homogeneous size and structure, pose synthetic difficulties, are difficult to target, or fail to increase contrast proportionately with the increase in IEM number. Decreasing the flexibility has been unsuccessful because rigid contrast agents create high background when unbound. Binding of a multimer to a target through a single TBM is not sufficient to both decrease flexibility and increase relaxivity significantly. Therefore, a need exists to improve contrast at specific targets by increasing the number of IEMs while simultaneously decreasing the flexibility of the molecule only when bound to the target.

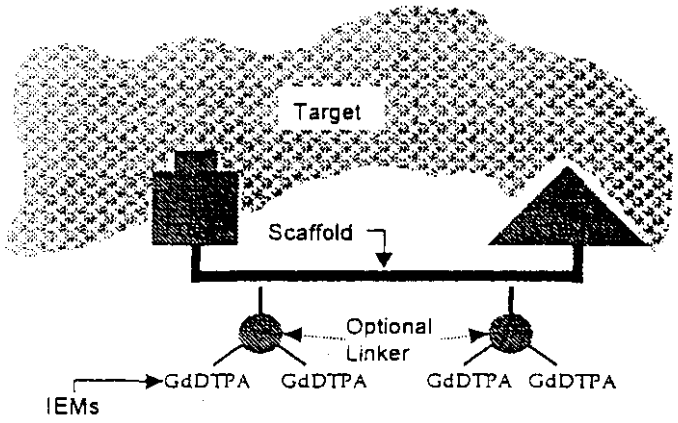
SUMMARY OF THE INVENTION

The current invention provides a mechanism to greatly improve the efficacy of in vivo contrast agents. Great improvements in contrast to noise (signal) at the target are possible if multimeric contrast agents are flexible in the unbound state (resulting in low relaxivity and a weak signal) and less flexible in the bound state (resulting in high relaxivity and a strong signal). That is, it is more important to rigidify the multimeric contrast agent in the bound state than in the unbound state since this minimizes background in the unbound state while high relaxivity is maintained in the bound state. Such agents are bound to proteins or other specific targets by non-covalent interactions at two or more separate

loci. Multilocus binding is achieved by incorporating two or more TBMs into the agent, each of which has some affinity for one or more sites on the target.

More specifically, the invention relates to the use of "multilocus," non-covalent interactions between a contrast agent with multiple IEMs (a "multimer") and a target to simultaneously 1) induce binding to the target (thus giving specificity), 2) anchor several IEMs to the target and 3) thereby rigidify the multiple IEM structure. A key aspect of the invention is that the contrast agent is less flexible in the bound state than in the unbound state. Binding of the contrast agent to the target increases the relaxivity and signal intensity of a metal chelate IEM by increasing the overall rotational correlation time of the metal ion - imaging atom vector, i.e., by limiting rotational motion. Multilocus binding enables further relaxivity enhancement decreasing the flexibility of the multiple chelate structure in the bound state both in general and at the local sites where chelate motion occurs. The flexibility of the molecule in the unbound state provides particular advantages over previously described multimeric MRI agents with rigid structures linking the chelates and with no difference in rigidity between a bound and unbound state. [Ranganathan, R. S., et al., Invest. Radiol., 33: pp. 779-797 (1998)]. The multilocus binding concept for multimeric chelates is shown schematically in Figure 3.

Figure 3. Schematic drawing showing the key components of an example multimeric contrast agent bound to a target through multilocus interactions



1) **Multiple separate TBMs promote binding to the target (thus giving specificity and improved affinity). The TBMs may be the same or different.**

2) **When bound to the target, TBMs anchor the multimer structure at several positions along the scaffold, thus rigidifying the multiple chelate structure.**

3) **Relaxivity is enhanced to a greater extent when bound than when free in solution, thus improving imaging contrast at a specific target.**

In addition to the improvement in image contrast, this invention offers synthetic advantages. A synthetically rigidified chemical framework (such as a fused ring or complex macrocycle) is not necessary since immobilization and rigidification occur upon binding by multilocus attachments to the target. Therefore, there are fewer limitations on the chemical framework structure. Additional benefits include:

a) Multilocus binding increases protein affinity and provides greater target specificity compared to a single interaction [Kramer, R. H. and Karpen, J. W., Nature, 395: pp. 710-713 (1998); Clackson, T. et al., Proc. Natl. Acad. Sci., 95: pp. 10437-10442 (1998); Rao, J. et al., Science, 280: pp. 708-711

(1998); Mann, D. A., et al., J. Am. Chem. Soc., 120: pp. 10,575-10,582 (1998); Spevak, W. et al., J. Med. Chem., 39: pp. 1018-1020.(1996); Lee, R. T. et al., Arch. Biochem. Biophys., 299: pp. 129-136 (1992)].

b) Multilocus binding slows the rate at which the agent dissociates from the target. Increasing the time that the agent remains bound results in an increased diagnostic utilization period.

c) Multilocus binding decreases the flexibility of the multiple chelate structure, reduces the local chelate motion, and thus improves the relaxivity at each metal center. Rigidification of the contrast agent in the bound state compared with the free molecule occurs only upon binding to produce greater imaging contrast. The free molecule induces a relatively small signal change compared with the bound form; consequently a surprisingly greater difference between the signal induced by the bound form relative to the signal induced by the free molecule can be attained. Contrast agents that are rigid in both the bound and unbound states lack this property.

d) The binding-dependent change in signal intensity is also applicable to other imaging modalities where a change in signal intensity may accompany binding, such as optical imaging. The signal intensity may increase or decrease upon binding. In some cases, decreased signal has been shown to correlate with the rigidity of the molecule [Rimet, O., Chauvet, M., Dell'Amico, M., Noat, G., and Bourdeaux, M. Eur. J. Biochem. (1995) 228: pp. 55-59]. In other cases, signal increases upon binding [Sudlow G., Birkett D. J., and Wade D. N. Mol. Pharmacol. 12: pp. 1052-61 (1976); Sudlow G., Birkett D. J., and Wade D. N. Mol. Pharmacol. 11: pp. 824-32 (1975); Kane C. D. and Bernlohr, D. A. Anal. Biochem. 233: pp. 197-204;

Lakowica, J. R. Principles of Fluorescence Spectroscopy Plenum Press, New York, NY pp. 211-213 (1983)].

Multilocus binding could provide either greatly decreased signal intensity (and therefore greatly increased signal contrast) or greatly increased intensity compared to an optical contrast agent with only a single TBM. In either case, the change in signal intensity at the target site will result in improved signal contrast as a result of contrast agent binding.

The multilocus-binding contrast agents of the invention comprise IEMs, a scaffold to which multiple IEMs are attached directly or through optional linkers, and at least two separate TBMs. The TBMs may be the same or different. In some cases, the scaffold may actually comprise the IEM or a part of the IEM, for example, some chelating moieties may also serve as the scaffold or a part of the scaffold.

These multimeric/multilocus-binding compounds are unique in that the local motion of the IEMs is restricted and bound relaxivity is greatly enhanced by non-covalent binding of at least two TBMs to the target at several separate loci along the multimer structure. These interactions allow the multimer to bind the target protein in a "pseudo-cyclic" or a "zipper-like" fashion. This type of binding surprisingly decreases flexibility throughout the multimer, including the TBMs, scaffold, and individual IEMs. Thus, for IEMs that include chelates, local chelate motion is reduced and remarkably enhanced MRI signals are observed with multimers since the relaxivity is increased at each IEM. This increase distinguishes the contrast agents of the present invention from those of the prior art that bind through a single TBM and thus are not "pseudo-cyclized" or "zippered" to the targeted site. Contrast is further enhanced with multimeric/multilocus

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binding structures since they also produce a relatively low signal in the unbound state.

The invention has tremendous utility for all targeted MRI and optical applications, including the targeting of image-enhancing agents to biological structures, such as serum albumin and other diagnostically relevant targets, such as blood clots, particularly in those applications where multiple binding sites for the multimeric/multilocus binding contrast agent exist. These binding sites need not be identical, just in close enough proximity to be simultaneously bound by the TBMs on the contrast agent.

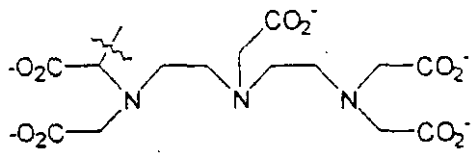
DETAILED DESCRIPTION OF THE INVENTION

In order that the invention herein described may be more fully understood, the following detailed description is set forth.

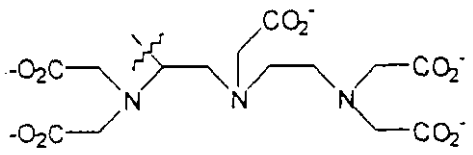
Commonly used chemical abbreviations that are not explicitly defined in this application may be found in The American Chemical Society Style Guide; Second Edition; American Chemical Society, Washington, D.C. (1997) or Journal of Organic Chemistry; Guidelines to Authors (Revised May 2000), Copyright © 2000 American Chemical Society also available at http://pubs.acs.org/instruct/ioceah.pdf.

The publications cited herein are incorporated by reference.

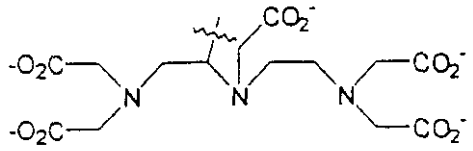
For the purposes of this application, DTPA refers to a structure of any one of Formulae (I)-(IV):



(I)



(II)



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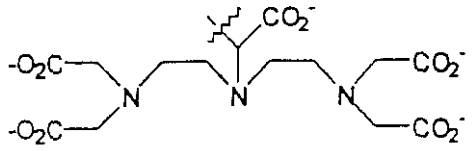

(IV)

The term "specific affinity" as used herein, refers to the capability of the contrast agent to be taken up by, retained by, or bound to a particular biological component to a substantially greater degree than other components. Contrast agents which have this property are said to be "targeted" to the "target" component. Contrast agents that lack this property are said to be "non-specific" agents.

The term "relaxivity" as used herein, refers to the increase in either of the quantities $1/T_1$ or $1/T_2$ per millimolar (mM) concentration of paramagnetic ion, wherein T_1 is the longitudinal, or spin-lattice, relaxation time and T_2 is the transverse, or spin-spin, relaxation time of water protons or other imaging or spectroscopic nuclei, including protons found in molecules other than water. Relaxivity units are $mM^{-1}s^{-1}$.

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The term "open coordination site" as used herein, refers to a site on a metal chelate that is generally occupied by water or solvent molecules.

The term "formation constant" for purposes herein is defined as the equilibrium constant for the reaction describing the formation of that compound.

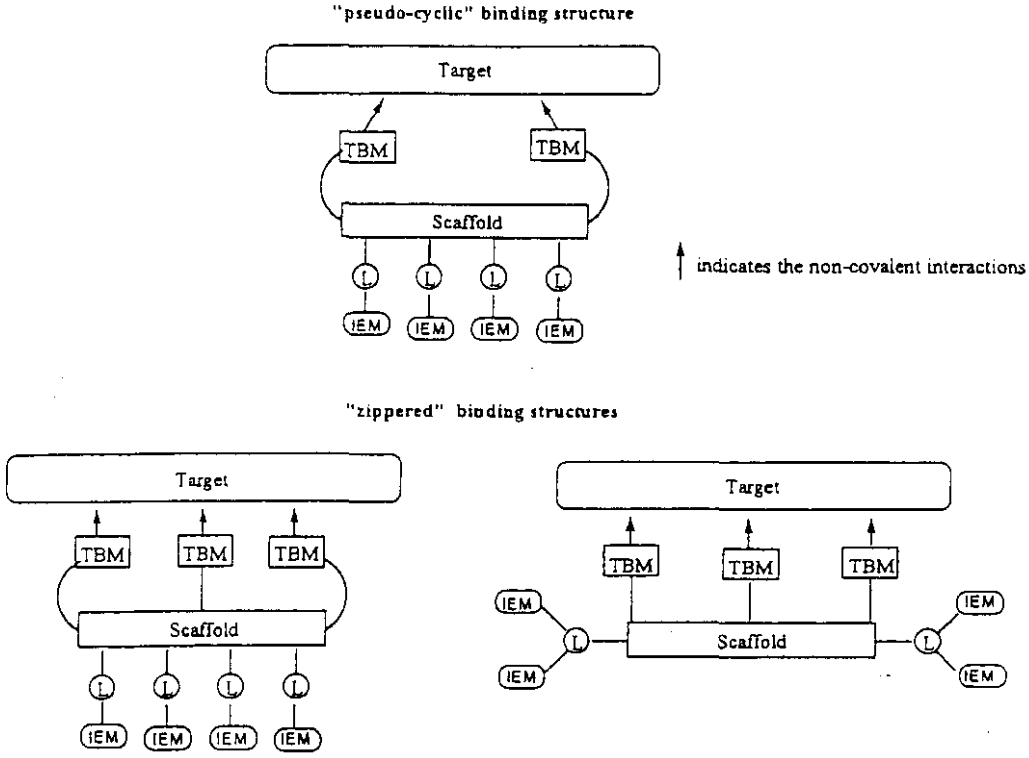
The term "multimer" for purposes herein is defined as a contrast agent or a subunit thereof comprising two or more IEMs.

The term "multilocus" for purposes herein refers to two or more positions of covalent TBM attachment to the "scaffold" (defined below) of a contrast agent.

The term "multilocus binding" for purposes herein refers to non-covalent interactions of the two or more different TBMs with a target. These non-covalent interactions are independent from one another and may be, *inter alia*, hydrophobic, hydrophilic, dipole-dipole, pi-stacking, or Lewis acid-base interactions.

The term "pseudo-cyclic structure" for purposes herein refers to a contrast agent bound through non-covalent interactions to a target at two different loci through two TBMs. (see Figure 4).

Figure 4. Representative examples of multilocus binding structures



The term "zipper structure" for purposes herein refers to a contrast agent bound through non-covalent interactions to a target at three or more different loci through three or more TBMs (see Figure 4).

The present invention relates to novel compounds which enhance the contrast in diagnostic imaging following a decrease in flexibility upon binding to a target. These compounds comprise:

a) two or more Image Enhancing Moieties("IEMs");

b) two or more Target Binding Moieties ("TBMs"), providing for in vivo localization and multimer rigidification;

c) a scaffold framework for attachment of the above ("scaffold");

d) optional linkers for attachment of the IEMs to the scaffold ("linker").

Diagnostic imaging techniques contemplated for use with this invention include, but are not limited to, MRI and ultraviolet-visible-infrared light imaging.

Image Enhancing Moiety ("IEM")

According to the present invention, the IEM can be a chemical or substance which is used to provide the signal or to improve contrast during imaging. In addition, the IEM or a part of an IEM may be optionally used as a scaffold or a part of the scaffold and may possess a minor targeting function.

The IEM may comprise an organic molecule, metal ion or chelate. Many examples of IEMs have been described [Bonnemain, B. J. Drug Target., 6: pp. 167-74 (1998); Swanson, D. P., et al., Pharmaceuticals in Medical Imaging: Radiopaque Contrast Media, Radiopharmaceuticals, Enhancement Agents for Magnetic Resonance Imaging and Ultrasound, McGraw Hill, Inc.,(1990); Johnson, I. Histochem. J., 30: pp. 123-40 (1998)].

A particularly useful IEM is a physiologically compatible metal chelate with one or more cyclic or acyclic organic chelating agents complexed to one or more metal ions. Metal ions preferred for optical imaging include those with atomic numbers 13, 21-31, 39-42, 44-50, or 57-83. Metal ions preferred for MRI include those with atomic numbers 21-29, 42, 44, or 57-83, and more preferably a paramagnetic form of a metal ion with atomic numbers

21-29, 42, 44, or 57-83. Particularly preferred paramagnetic metal ions are selected from the group consisting of Gd(III), Fe(III), Mn(II and III), Cr(III), Cu(II), Dy(III), Tb(III and IV), Ho(III), Er(III), Pr(III) and Eu(II and III). The most preferred is Gd(III).

If the IEM is a metal chelate, it must not dissociate to any significant degree during the imaging agent's passage through the body, including while bound to the target tissue. Significant release of free metal ions can result in toxicity, which would generally not be acceptable.

In general, the degree of toxicity of a metal chelate is related to its degree of dissociation in vivo before excretion. Toxicity generally increases with the amount of free metal ion, that is, a high formation constant is preferred to prevent toxic concentrations of free metal ions. Particularly preferred are formation constants of at least 10^{15} M^{-1}, or at least 10^{16} M^{-1}, or at least 10^{17} M^{-1}, or at least 10^{19} M^{-1},or at least 10^{19} M^{-1}, or at least 10^{20} M^{-1}, or at least 10^{22} M^{-1}, or at least 10^{24} M^{-1} or higher. If the kinetics of metal ion dissociation are very slow, then a complex having a lower formation constant, i.e. of at least 10^{10} M^{-1}, may be sufficient.

Toxicity is also a function of the number of open coordination sites in the complex. In general, fewer water coordination sites lowers the tendency for the chelating agent to release the paramagnetic metal. Preferably, therefore, the complex contains two, one, or zero open coordination sites. The presence of more than two open sites in general will unacceptably increase toxicity by release of the metal ion in vivo.

Relaxivities R_1 and R_2, defined as the increase in $1/T_1$ or $1/T_2$, respectively, per mM of metal

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ion, measure the ability of a contrast agent to enhance the relaxation rate of spectroscopic or imaging nuclei. Relaxivity units are $mM^{-1}s^{-1}$. For the most common form of clinical MRI, water proton MRI, relaxivity is higher when the paramagnetic ion bound to the chelating ligand still has one or more open coordination sites for water exchange (R. B. Lauffer, Chemical Reviews, 87: pp. 901-927 (1987)). However, this must be balanced with the stability of the metal chelate, which generally decreases with increasing numbers of open coordination sites, and the toxicity as mentioned above. Preferably, therefore, except for iron chelates, Fe(II) or Fe(III), the complex contains only one or two open coordination sites. For Gd(III) one or two open coordination sites is most preferred.

In order to effectively enhance MRI images, the complex must be capable of increasing the relaxation rates, or relaxivities, $1/T_1$ (longitudinal, or spin-lattice) and/or $1/T_2$ (transverse, or spin-spin) of the spectroscopic nucleus. The spectroscopic nucleus is preferably a water proton, but other common spectroscopic nuclei include ^{31}P, ^{13}C, ^{23}Na, ^{19}F and protons found in molecules other than water. The spectroscopic nucleus may comprise the IEM, TBM, other biomolecules or injected biomarkers.

In the case of MRI contrast agents, increases in the relaxivities ($1/T_1$ or $1/T_2$) generally occur through dipole-dipole interactions between the paramagnetic ion of the contrast agent and the nuclei undergoing relaxation (e.g. hydrogen atoms in water molecules). It is known that the efficiency of this dipolar interaction (i.e. the relaxivity) is improved if the rate at which the vector defined by the two dipoles (i.e. the vector defined by the paramagnetic ion and a water hydrogen atom) rotates is slowed (R. B. Lauffer, Chemical Reviews, 87: pp. 901-927 (1987)).

The time taken for the vector to rotationally diffuse one radian is referred to as the "rotational correlation time"; the inverse of the rotational correlation time is the "rotational rate." In general, large molecules rotate more slowly in solution than smaller molecules. One method of increasing relaxivity is to form a non-covalent adduct between a small molecule contrast agent and a macromolecule. By forming an adduct, the rotational correlation time of the dipolar vector will likely be the same as that of the macromolecule. However the small molecule may still be able to rotate about one or more axes (so-called "local chelate motion"). The rotational correlation time of the dipolar vector is then a function of this local chelate motion and of the global motion of the macromolecule adduct. Non-covalent adducts of macromolecules will have rotational correlation times that are less than or equal to that of the macromolecule itself; the less local chelate motion there is, the closer the rotational correlation time of the non-covalent adduct approaches that of the macromolecule.

In the case of MRI contrast agents, increases in relaxivity generally occur through dipole-dipole interactions between the metal ion of the contrast agent and the nuclei undergoing relaxation (e.g. the water hydrogen atoms). In addition to increasing the $1/T_1$ or $1/T_2$ values of tissue nuclei via dipole-dipole interactions, MRI agents can affect two other magnetic properties that increase their use and value for clinical purposes:

1) an IEM containing a metal chelate of high magnetic susceptibility, particularly chelates of Dy, Gd, Tb, or Ho, can alter the MRI signal intensity of tissue by creating microscopic magnetic susceptibility gradients (A. Villringer et al, Magn. Reson. Med., 6:

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pp. 164-174 (1988)). No open coordination sites on a chelate are required for this application.

2) an IEM containing a metal (e.g. Tm or Dy) chelate can also be used to shift the resonance frequency of the spectroscopic nucleus. The spectroscopic nucleus is preferably a water proton, but other common spectroscopic nuclei include ^{31}P, ^{13}C, ^{23}Na, ^{19}F and protons found in molecules other than water. The spectroscopic nucleus may comprise the contrast agent, the target, or water. Here, depending on the nucleus and strategy used, zero to three open coordination sites may be employed.

A variety of chelating ligands may be used as IEM moieties in various embodiments of the invention. Such chelating ligands include but are not limited to derivatives of diethylenetriamine pentaacetic acid (DTPA) and derivatives thereof; 1,4,7-triazacyclononane; 1,4,7,10-tetraazacyclododecane (Cyclen) and derivatives thereof; 1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid tert-bu-ester) (DO2A-t-bu-ester); 1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid, t-bu-ester) (DO3A-t-bu-ester); 1,4,7-tris(tert-butoxycarbonyl)-1,4,7-tetraazacyclododecane (DO3-t-BOC); 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and derivatives thereof; 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid) (DOTP); 1,4,7,10-tetraazacyclododecane-1,4,7,10-a, a', a",a'"-tetrakis(methylacetic acid) (DOTMA); ethylenediamine-tetra-acetic acid (EDTA); 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA); ethylenebis-(2-hydroxy-phenylglycine)(EHPG) and derivatives thereof, including 5-Cl-EHPG, 5-Br-EHPG, 5-Me-EHPG, 5-t-Bu-EHPG, and 5-sec-Bu-EHPG;

benzodiethylenetriamine pentaacetic acid (benzo-DTPA) and derivatives thereof, including dibenzo-DTPA, phenyl-DTPA, diphenyl-DTPA, benzyl-DTPA, and dibenzyl DTPA; bis-2(hydroxybenzyl)-ethylene-diaminediacetic acid (HBED) and derivatives thereof; the class of macrocylcic compounds which comprise at least 3 carbon atoms, more preferably at least 6, and comprise at least two heteroatoms (O and/or N), said macrocylcic compounds may comprise one ring, or two or three rings joined together at the heteroatom ring elements, e.g., benzo-DOTA, dibenzo-DOTA and benzo-NOTA, where NOTA is 1,4-triazacyclononane-N,N',N"-triacetic acid, benzo-TETA, benzo-DOTMA, benzo-TETMA, where TETMA is 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-(methyl tetraacetic acid); derivatives of 1,3-propylenediaminetetraacetic acid (PDTA) and triethylenetetraaminehexaacetic acid (TTHA); and derivatives of 1,5,10-N,N',N"-tris(2,3-dihydroxybenzoyl)aminomethylbenzene (MECAM).

Many suitable chelating ligands for MRI agents are known in the art. These metal chelates can also be used for other forms of biological imaging (e.g., optical imaging). In fact, a series of fluorescently detectable MRI contrast agents have recently been described [Hüber, M. M. et al., Bioconjugate Chem., 9: pp. 242-249 (1998)]. For MRI imaging, preferred IEMs include paramagnetic gadolinium chelates such as gadolinium diethylenetriaminepentaacetic acid (GdDTPA), gadolinium tetraamine 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (GdDOTA) and gadolinium 1,4,7,10-tetraazacyclododecane-1, 4, 7-triacetic acid (GdDO3A). It is known in the art that other metals may be substituted for Gd(III) in certain applications. A preferred chelator for use in the invention is DTPA. Examples of representative chelators and chelating

groups contemplated by the present invention are described in WO 98/18496, WO 86/06605, WO 91/03200, WO 95/28179, WO 96/23526, WO 97/36619, PCT/US98/01473, PCT/US98/20182, and U.S. Patent No. 4,899,755, all of which are herein incorporated by reference.

Target binding moiety ("TBM")

According to the present invention, the second component of the contrast agent is two or more Target Binding Moieties (TBMs). The TBMs of the compound (1) allow the contrast agent to bind to proteins or other targets and (2) decrease the flexibility of the molecule in the bound state. This creates an increased concentration of the imaging agent at the site to be imaged and increases the relaxivity in the bound state. For vascular blood pool imaging, serum albumin is a preferred target. Other protein targets include, but are not limited to, alpha acid glycoprotein, fibrinogen, fibrin, and collagen. For imaging clots, fibrin is a preferred target. The TBM therefore must be selected to achieve specificity and high binding affinity for the appropriate protein. Since HSA is present at high concentration in serum (approximately 0.6 mM) and binds a wide array of molecules with reasonably high affinity, it is the preferred target plasma protein for blood pool contrast agents. HSA is a particularly preferred target for cardiovascular imaging.

A wide range of lipophilic or amphiphilic TBMs will efficiently bind to various targets, including Human Serum Albumin (HSA). These include but are not limited to aromatic, and saturated or unsaturated aliphatic groups with 4-200 carbons wherein each carbon is optionally substituted with or replaced by oxygen, nitrogen, halogen, sulfur, or other atoms

that can covalently bind carbon. For binding to other protein targets with high specificity, special targeting groups are often required. Targeting groups of sufficiently high affinity and specificity may be identified using modern techniques, such as combinatorial chemistry, high throughput screening, phage display, systemic evolution of ligands by exponential enrichment (SELEX) and other methods as described, for example, in U.S. Patent Nos. 5,475,096, 5,595,877, and 5,270,163 [see Gold et al. Ann. Rev. of Biochem., 64: pp. 763-797 (1995)], herein incorporated by reference.

The extent of binding of a TBM to a target, such as HSA or fibrin, can be assessed by a variety of equilibrium binding methods. For example, binding to HSA can be measured by ultrafiltration. In a typical binding measurement using ultrafiltration, the targeting group is mixed with 4.5% weight/volume HSA in a pH 7.4 buffer. The sample is loaded into a commercially available centrifugation apparatus equipped with a 30 KDa molecular weight cutoff filter (Millipore Ultrafree MC Low Binding Regenerated Cellulose 30 KDa mol. wt. cutoff catalog # UFC3LTK00), permeable to the targeting group, but not HSA. A small portion (5 - 10%) of the sample volume is filtered by centrifugation at 2000 x g for 20 min through the cutoff filter, and the concentration of unbound targeting group in the sample is measured in the filtrate.

For measuring binding to fibrin, a fibrin clot may be formed in a well of a microtiter plate and contacted with the targeting group. After an incubation time sufficient to establish equilibrium, the supernatant is removed by aspiration (the insoluble fibrin remains bound as a gelled clot to the bottom of

the well). The concentration of unbound targeting group in the supernatant is then measured.

In both methodologies, the concentration of bound targeting group is determined as the difference between the total targeting group concentration initially present and the unbound targeting group concentration following the binding assay. The bound fraction is the concentration of bound targeting group divided by the concentration of total targeting group. Preferably at least 10%, more preferably at least 50%, still more preferably at least 80%, still more preferably at least 90% of the contrast agent is bound to the desired target at physiologically relevant concentrations of drug and target. More preferably at least 92%, even more preferably at least 94%, and most preferably 96% or more of the contrast agent is bound to the target according to the ultrafiltration or microtiter plate methods.

For additional details concerning target binding moieties which comprise fibrin-binding peptides, see U.S. Provisional Patent Application No. 60/146,425 entitled BINDING MOIETIES FOR FIBRIN; identified as DYX-010.0Prv and U.S. Provisional Patent Application No. 60/146,414 filed concurrently on the same day (July 29, 1999) from which the present utility application claims priority; and continuations thereof all of which are incorporated herein by reference in their entirety.

The TBMs may be very diverse depending on the nature of the target and the specific requirements of the binding. Examples of useful TBMs include drugs, lipophilic or amphiphilic organic molecules, porphyrins, receptor ligands, steroids, lipids, hormones, peptides, oligonucleotides (DNA, RNA or chemically modified versions thereof), carbohydrates or other biomolecules or substances that are known to bind

with sufficiently high affinity to one or more components in the specific tissue desired to be imaged. In certain embodiments, one TBM may have higher affinity for the target than the other(s), in which case the higher affinity TBM is designated the "primary" TBM. Thus, all other TBMs with binding affinities lower than that of the primary TBM are designated "secondary."

More preferred TBMs are those that bind reversibly to proteins in plasma, interstitial space (the fluid between cells), or intracellular space. While many biomolecules or substances that bind to a specified target could be used, most useful are those that bind to proteins.

The secondary TBMs may be the same or different than the primary TBM. The number of secondary TBMs may vary from one to ten or more. The exact number of secondary TBMs required will depend on the specificity of the TBMs for the target and the affinity of the TBMs for the target. The additional binding interactions provided by the secondary TBMs must be sufficient to tether the complex and decrease the rotational correlation time at each chelation site. The resulting increased relaxivity provides adequate contrast enhancement of the image. Secondary TBM binding interactions and affinity for the target may be less specific than demanded by the primary targeting TBM, since the initial binding of the primary TBM to the target may provide the necessary specificity of target recognition. In some cases, the target may comprise dimeric binding sites, in which case two identical TBMs would be preferred.

Targets for the contrast agents described in this application are extensive and varied. The target can be any body compartment, cell, organ, or tissue or component thereof. Preferred targets are those that

are of diagnostic and therapeutic relevance, i.e. those that are associated with disease states. Particularly preferred targets are those in association with body fluids, and particularly those in association with blood, plasma, lymph and fluids of the central nervous system. Other preferred targets are proteins that either exist in high concentration or have a large number of binding sites for certain ligands. Multiple binding sites provide contact for one or more secondary TBMs. Included among such target proteins are enzymes and glycoproteins.

The Scaffold for Attachment of IEMs and TBMs

The present invention provides for a third component to the multimer contrast agents, namely a chemical framework or "scaffold" structure to which the IEMs and TBMs may be attached, as depicted in Figure 5. The scaffold is the chemical framework between two or more TBMs to which two or more IEMs may be attached at different positions directly or through the linkers to form the multimeric/multilocus binding compounds. The novel compounds comprising these scaffold structures restrict the local chelate motion by non-covalent binding of the TBMs to a target at several (at least two) separate loci. The multimer contrast agent binds a target in a "pseudo-cyclic" or a "zipper-like" fashion, creating an interaction at two or more loci. This type of binding creates rigidity throughout the multimeric contrast agent structure, including the binding group, scaffold, and individual IEMs.

In general, scaffold structures will be highly diverse organic molecules that may include from one to ten of repeated monomeric subunits. The scaffold can be either open chained or cyclic. TBMs are covalently attached to the interior or termini of

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the structure. In every case, there must be at least two separate TBMs to tether the molecule to the target. The TBMs may either be the same (homogeneous) or different (heterogeneous). Heterogenous TBMs may exhibit either different or similar binding affinities for the target.

Both open chained and cyclic scaffolds also provide a supporting structure for the attachment of IEMs. The number of IEMs may vary from two to about 12. The IEMs may be interspersed with TBMs, and the IEMs may be attached either to the interior part of a linear scaffold structure or to the ends of the structure, optionally via a linker, but to at least two different positions of the structure. The IEMs may either be the same (homogeneous) or different (heterogeneous). Heterogenous IEMs may exhibit either different or similar contrast generating ability.

Within the set of open chained scaffolds, many types of structures can be synthesized using standard techniques. Particularly advantageous are scaffolds that have regularly repeated heteroatoms throughout the structure. Heteroatoms generally allow for easy attachment of IEMs or TBMs. Particularly preferred embodiments are oligomeric alkylene amine scaffolds. These oligomeric alkylene amine scaffolds may be either branched or linear. That is, the structures may have either a linear framework with regularly spaced IEMs and TBMs, or the chemical framework may branch so that groups of IEMs and/or TBMs may emanate from a single site on the scaffold. The scaffolds may also terminate in heteroatoms such as oxygen (alcohols) or nitrogen (amines). Particularly preferred embodiments of open chained scaffolds, both branched and linear, have terminal alcohols or terminal amines.

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Figure 5 shows an example of the multilocus binding of a multimer where the scaffold portion, IEM, TBMs, and (optional) linkers are clearly delineated. Another example of a multimer with a branched linker is illustrated in Figure 6. Again, the scaffold portion, IEM, TBMs, and (optional) linkers are clearly delineated. In Figure 6, the two TBMs are peptides attached to a polyamine scaffold, although these components in no way limit the scope of the invention.

Figure 5. An example of the multilocus binding multimer with a linear linker

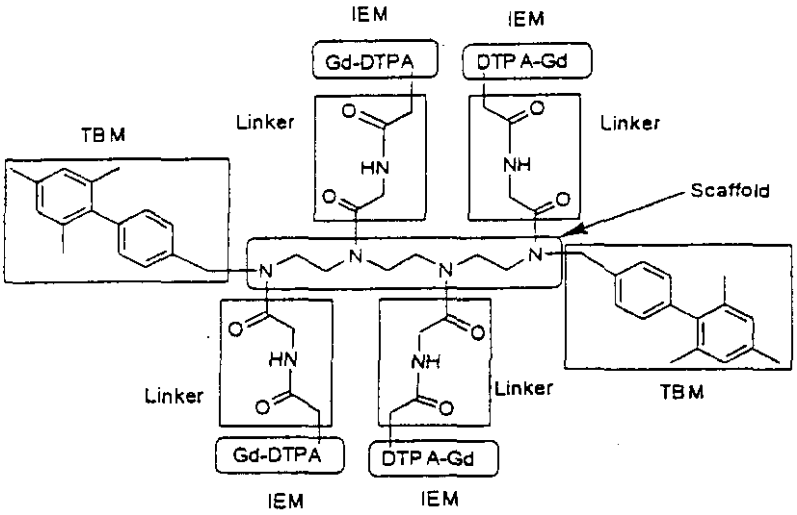

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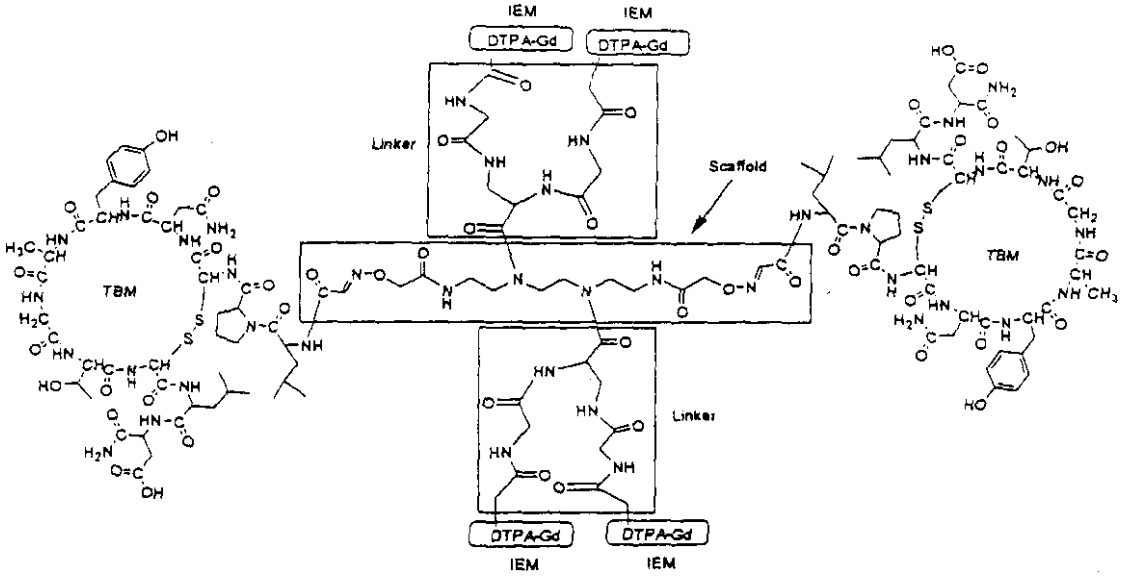
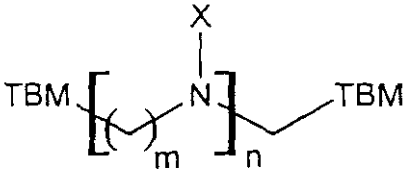
Figure 6. An example of the multilocus binding multimer with a branched linker



A generic structure for a linear scaffold with a repeating amine sub-structure is given by Formula (V),



(V)

X = L-IEM, TBM, or IEM

wherein m can vary from 1 to 10, and n can vary from 2 to 10. Preferably, m is 1 to 2, 2 to 4, 4 to 6, 6 to 8, or 8 to about 10. Preferably, n is 2 to 4, 4 to 6, 6 to 8, or 8 to about 10.

A generic structure for a linear scaffold with a repeating amine sub-structure and terminal

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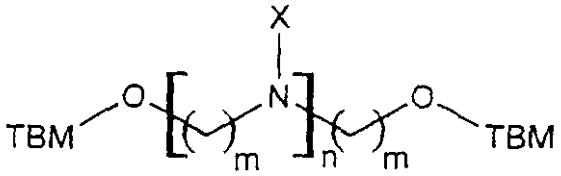
oxygens that allow the formation of ether-linked TBMs is given by Formula (VI),



(VI)

X = L-IEM, TBM, or IEM

wherein m can vary from 1 to about 10, and n can vary from 2 to 10. Preferably, m is 1 to 2, 2 to 4, 4 to 6, 6 to 8, or 8 to about 10. Preferably, n is 2 to 4, 4 to 6, 6 to 8, or 8 to about 10.

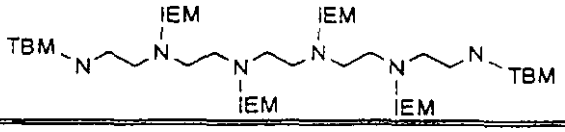
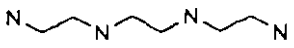
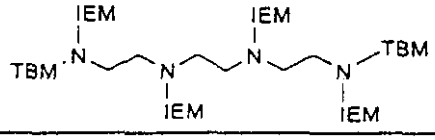
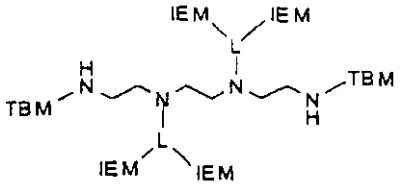
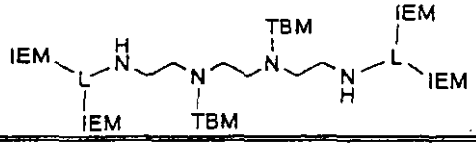
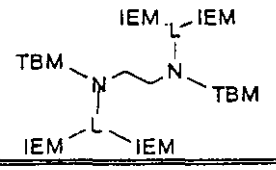
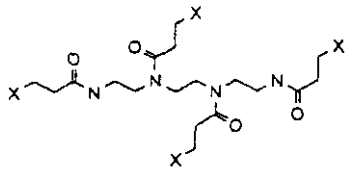
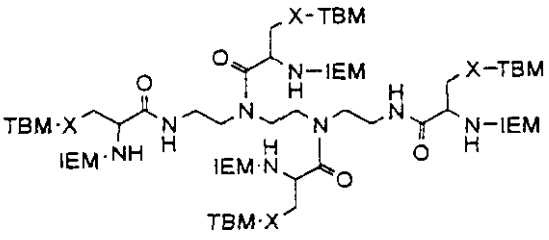
Scaffold structures are not limited to repeating substructures with amines, nor are they limited to structures containing terminal oxygens or nitrogens. The scaffold may contain any repeating substructure that allows attachment of IEMs, optionally via linkers, and TBMs. The carbon atoms comprising the scaffold may be optionally substituted with or replaced by heteroatoms selected from oxygen, nitrogen, sulfur, phosphorus, and halogens. The scaffold may also comprise substituents such as short chain hydrocarbons (1-10 carbon atoms). These hydrocarbon side chains may be optionally substituted with or replaced by heteroatoms selected from oxygen, nitrogen, sulfur, phosphorus, and halogens. Thus the scaffolds may comprise many common organic groups, for example, phosphodiesters, carbamates, sulfates, and sulfonyls. Likewise, substituents that are attached to the scaffold structure may also contain many common organic groups, for example, phosphodiesters, carbamates, sulfates, sulfonyls, and amino acids.

Figure 7 shows some illustrative examples of linear and branched polyamine scaffolds along with

corresponding examples of contrast agents comprising these scaffolds. Figure 8 provides examples of oligomeric scaffolds along with corresponding examples of contrast agents comprising these scaffolds.

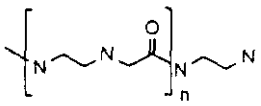
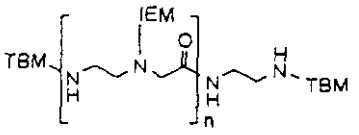
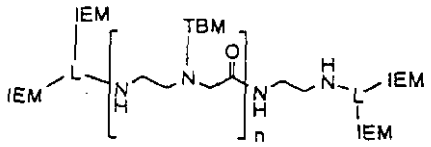
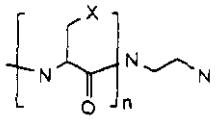
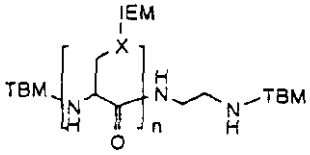
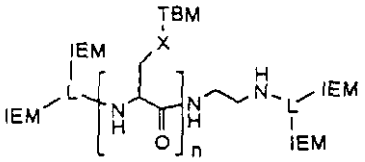
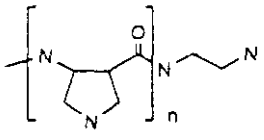
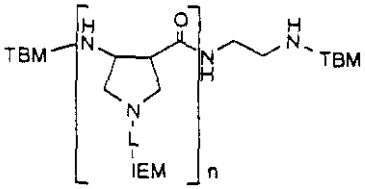
Scaffolds comprising heteroatoms are one preferred embodiment of the present invention since heteroatoms allow easy attachment of IEMs and TBMs, optionally via linkers.

Figure 7. Examples of Linear and Branched Polyamine Scaffolds

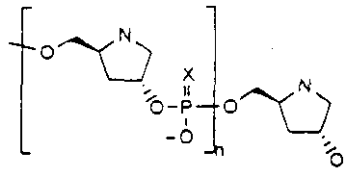
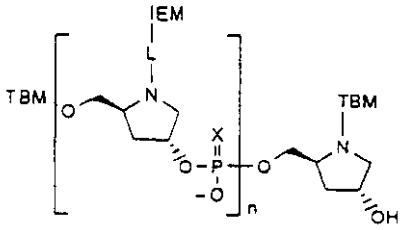
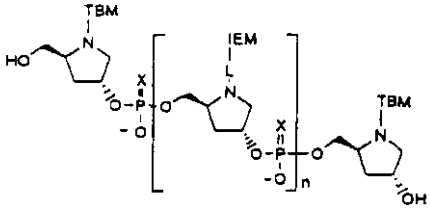
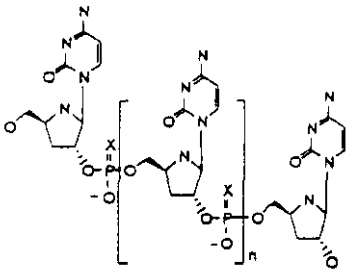
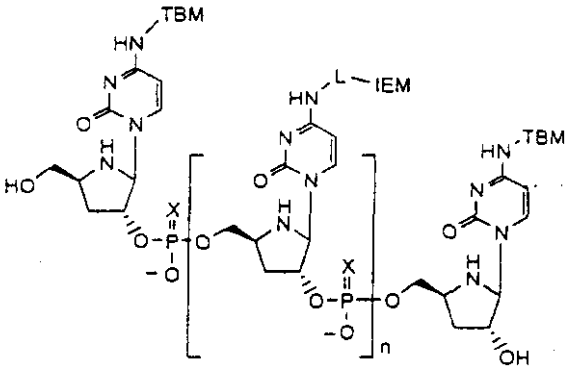
Examples of Scaffolds	Corresponding multimeric contrast agents
	
	
	
	
	
	

X = N, O, S L = Linker

Figure 8. Examples of Oligomeric Scaffolds

Examples of scaffolds	Corresponding multimeric contrast agents
	
	
	
	
	

X = N, O, S L = Linker

Examples of scaffolds	Corresponding multimeric contrast agents
	
	
	

X = N, O, S L = Linker

Another preferred embodiment for the scaffold is one in which the backbone of the scaffold is readily degraded in vivo. Biodegradable contrast agents have scaffold structures that can be degraded by, for example, enzymes that occur in the body of a mammal. Preferably the scaffold contains one or more biodegradable groups that can be specifically degraded by an enzyme. Particularly preferred biodegradable scaffolds are those that may be degraded by human enzymes. Because of the wide range of enzymatic

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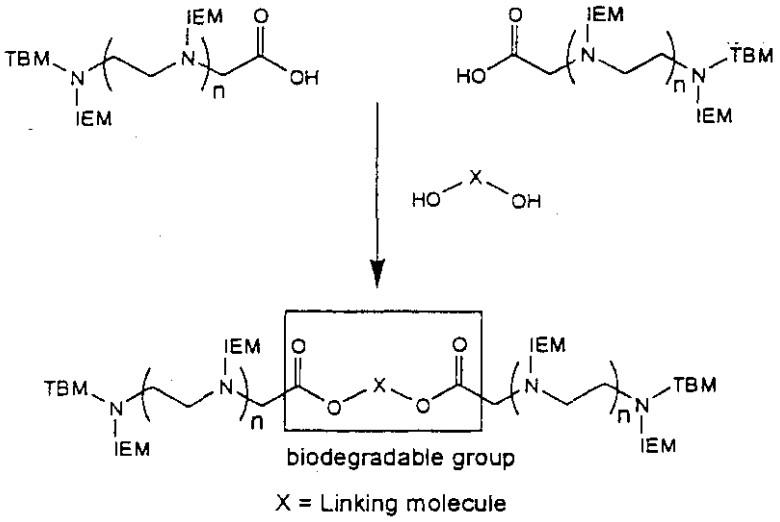
activities known to exist, the exact structure of the biodegradable groups is variable. Particular preferred embodiments of the biodegradable group include, but are not limited to carbonyls, esters, diesters, phosphates, diphosphates, phosphodiesters, anhydrides, sulfonyl groups, sulfates, and carbamates. Such biodegradable scaffolds allow rapid metabolism of the multimeric contrast agent and lessens the chance of toxicity. One example of a biodegradable scaffold for a multimer is shown in Figure 9. In this instance, the scaffold is formed from the condensation of two carboxylic acids in the presence of a molecule containing two terminal alcohols. The specific reaction conditions for such a condensation reaction are well known in the art.

Figure 9. Example of a Biodegradable Scaffold

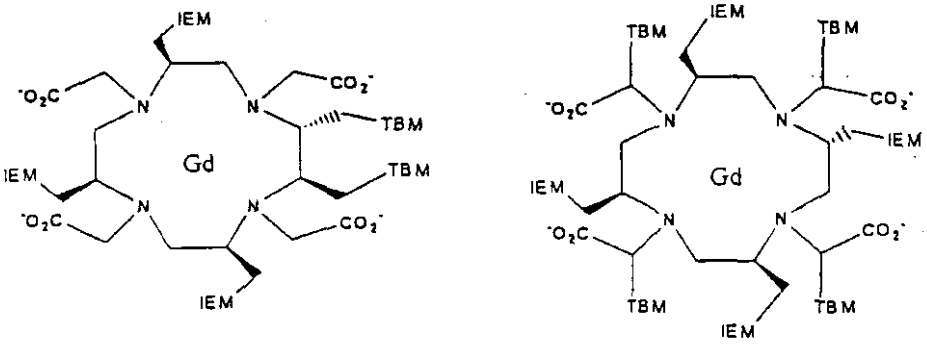


Additionally, the biodegradable group may cleave upon a change in pH or temperature, or upon application of ultrasound or light energy. Such groups are well known in the prodrug literature. [Kratz, F., Beyer, U., and Schutte M.T., Crit. Rev. Ther. Drug Carrier Syst. 16: pp. 245-88 (1999); Dougherty, T.J.,

Gomer, C.J., Henderson, B.W., Jori, G., Kessel, D., Korbelik, M., Moan, J., and Peng, Q. J. Natl. Cancer Inst. 90: pp. 889-905 (1998); Wang, W., Jiang, J., Ballard, C.E., and Wang, B. Curr. Pharm. Des. 5: pp. 265-87 (1999)].

Scaffolds that comprise cyclic elements that chelate metal ions are another set of preferred embodiments of the present invention. More preferred are IEM-containing scaffolds that chelate gadolinium or provide for chelation of gadolinium within the scaffold as well as in separate IEMs that are attached to the scaffold. Examples of such scaffolds are shown in Figure 10.

Figure 10. Examples of Multimers Using a Part of Gadolinium Complexes as Scaffolds



The classification of scaffolds into open chained and cyclic groups does not imply mutual exclusivity between the groups. A wide variety of open chained scaffolds that incorporate motion-constraining cyclic elements is contemplated. The cyclic portion of the scaffold may be a homocyclic or heterocyclic ring or rings. Thus, the rings may be highly constrained like, for example, a cyclopropyl ring, or may be a less conformationally constrained ring such as a cyclohexyl ring.

Both the open chained and cyclic portions of the scaffold may be optionally substituted with or replaced by heteroatoms selected from oxygen, nitrogen, sulfur, phosphorus, and halogens. The scaffold may also comprise substituents such as short chain hydrocarbons (1-10 carbon atoms). These hydrocarbon side chains may be optionally substituted with or replaced by heteroatoms selected from oxygen, nitrogen, sulfur, phosphorus, and halogens. Thus the scaffolds may comprise many common organic groups both in the open chained and cyclic portions of the scaffold. Some examples include, but are not limited to carbonyls, esters, diesters, phosphates, phosphodiesters, anhydrides, sulfonyl groups, sulfates, and carbamates.

The rings may also include derivatives of common biological cyclic compounds such as carbohydrates. Preferred embodiments of the cyclic elements include multiple rings systems, such as heterocyclic derivatives of decalin. Other examples include, but are not limited to, carbocyclic rings comprising from 3 to 7 atoms, wherein up to 4 atoms are optionally substituted with moieties selected from the group consisting of O, S, C(O), S(O), $S(O)_2$, and NH. The rings are optionally substituted with methyl groups and derivatives thereof, alkyl, alkenyl, or alkynyl groups comprising from 2-100 carbons, wherein up to 10 carbons are optionally substituted with heteroatoms selected from oxygen, nitrogen, sulfur, phosphorus, and halogens or are replaced by moieties selected from O, S, C(O), S(O), $S(O)_2$, and NH. Just as some scaffolds, such as amino acid-based scaffolds, may comprise TBMs, the scaffolds may likewise comprise IEMs. The IEMs can be any organic molecule, metal ion or chelate. Preferred embodiments are those with cyclic IEMs, even more preferable are cyclic IEMs that are metal chelates. Particular examples of such linear and cyclic

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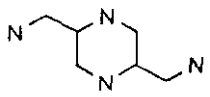
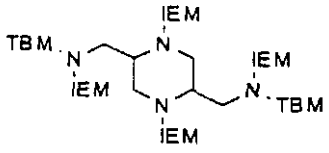
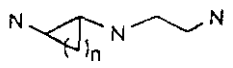
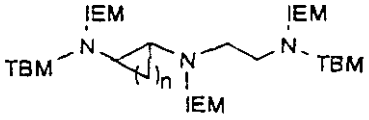
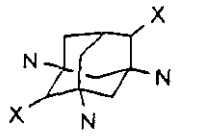
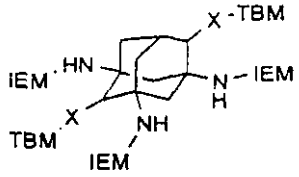
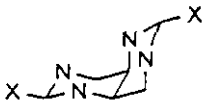
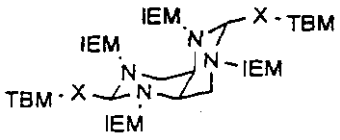
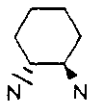
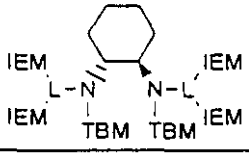
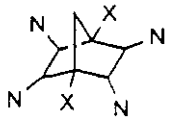
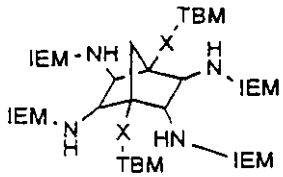
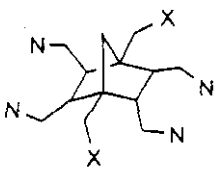
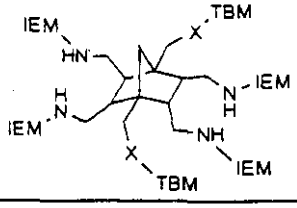
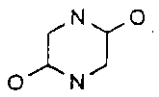
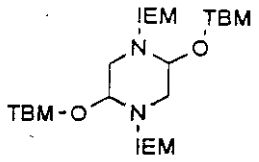
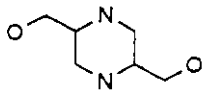
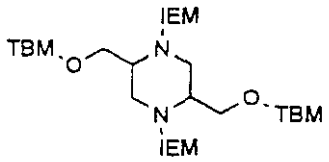
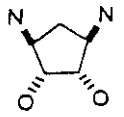
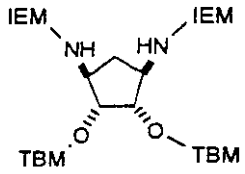
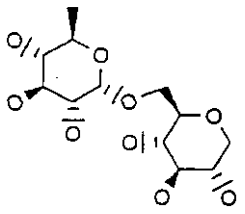
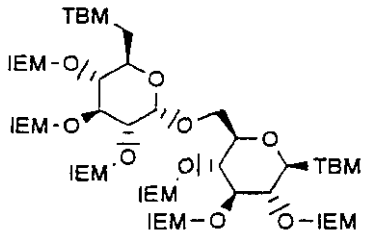
combination scaffolds described above are shown in Figure 11 along with corresponding examples of contrast agents comprising these scaffolds.

Figure 11. Examples of Scaffolds with Cyclic Elements

Examples of Scaffolds - Polyamines	Corresponding multimeric contrast agents
	
	
	
	
	
	
	

Examples of Scaffolds - Polyaminoalcohol or ether derivatives	Corresponding multimeric contrast agents
	
	
	
Examples of Scaffolds - Carbohydrate derivatives	**Corresponding multimeric contrast agents**
	

X = N, O, S, CH_2

n = 1-8

Optional Linkers

The contrast agents of certain embodiments of the present invention are characterized by an optional linker through which IEMs are attached to a scaffold. The image enhancement moiety (IEM) and the target binding moiety (TBM) are described elsewhere herein. The linker moiety (L) can be any small subunit comprising 1 to 30 carbon atoms covalently connected by single or multiple bonds wherein up to 10 of the carbon atoms may be substituted with O, N, P, S, F, Cl, Br, H or I. The linker functions to connect the IEMs to the scaffold. Examples of linkers include linear or branched alkanes, alkenes, or alkynes optionally substituted with functional groups such as, carbonyl, ether, amide, amine, urea, thioether, aryl, phosphate, sulfonamide and the like. The preferred linkers of certain embodiments embody two or more functional chemical groups, one of which is attached to the scaffold and the others of which are attached to the IEMs.

The functional chemical groups may be the same or may be different. Examples of said functional groups include but are not limited to ketones, esters, amides, ethers, carbonates, sufonamides, alkanes, alkenes, alkynes, and carbamates. Examples of some preferred reagents to prepare linkers are amino acids, especially glycine, alanine, serine, homoserine, threonine, tyrosine, cysteine, aminophenylalanine, lysine, ornithine, 2,4-diaminobutyric acid, diaminopropionic acid, hydroxyproline, aspartic acid, and glutamic acid, diols, especially ethylene glycol, dihalides, especially ethylene dichloride, 2-mercaptoethanol, 2-aminoethanol, 1,2-diaminoethanol, dicarboxylic acids, especially oxalic acid, malonic acid, malic acid, succinic acid, fumaric acid, glutaric acid, and adipic acid, and other

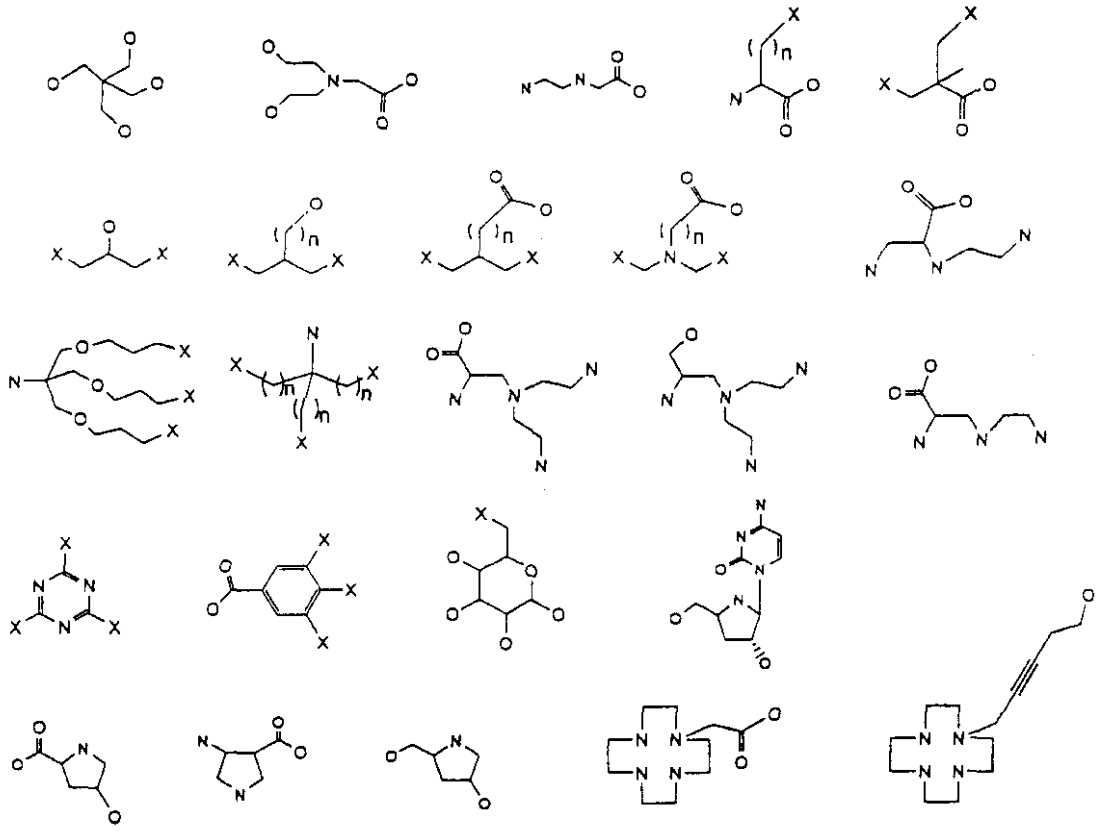
bifunctional, trifunctional and multifunctional small molecules.

Still other linkers without limitation, may be urea, acetal, ketal, double ester, carbonyl, thiourea, sulfone, thioester, ester, ether, disulfide, lactone, imine, phosphoryl, or phosphodiester linkages; substituted or unsubstituted saturated or unsaturated alkyl chains; linear, branched, or cyclic amino acid chains of a single amino acid or different amino acids (e.g., extensions of the N- or C- terminus of the fibrin binding moiety); malonic, succinic, glutaric, adipic and pimelic acids; caproic acid; simple diamines and dialcohols.

Preferably the molecular weight of the linker is well defined. The molecular weights can range in size from less than 100 to greater than 1000. Preferably the molecular weight of the linker is less than 200 and even more preferably is less than 100. In addition, it may be desirable to utilize a linker that is biodegradable in vivo to provide efficient routes of excretion for the imaging agents of the present invention. Depending on their location within the linker, such biodegradable functionalities can include ester, diester, amide, phosphoester, ether, acetal, and ketal functionalities.

In general, known methods can be used to couple the metal chelate or other IEMs to the linker and the linker to the TBM. See, e.g., WO 95/28967, WO 98/18496, WO 98/18497. The present invention contemplates linking of the chelate at any position, provided the metal chelate retains the ability to bind the metal tightly in order to minimize toxicity. Examples of some linkers are shown in Figure 12 with hydrogen atoms omitted for simplicity.

Figure 12. Examples of Linkers with Multiple Connection Points



Contrast Enhancement in Conjunction with Multilocus Binding Interactions

The present invention in its different embodiments improves the average relaxivity of all the paramagnetic metal, e.g. gadolinium, centers in a target bound, multimeric chelate structure. The examples shown in Table 1 and Table 2 illustrate that target binding of a multiple IEM structure through a single TBM alone is

not sufficient to improve the average bound relaxivity per Gd(III) to the same extent as observed for a comparable single IEM structure. Although binding through a single TBM slows the overall rotational correlation time of the multimer, the individual metal, e.g. gadolinium, chelates are apparently still free to rotate in a rather unencumbered fashion. This excess motion reduces the relaxivity at each metal center. Surprisingly, this is found to be true even when the multimer contains a TBM with two aromatic rings. In PCT WO 96/23526, McMurry et al. teach that the albumin-bound relaxivity of contrast agents with a single IEM is improved by the use of a plasma protein binding moiety (PPBM) comprising two or more aromatic rings in a non-planar orientation. As illustrated in Table 1, the use of such a TBM (diphenylcyclohexyl) provides excellent relaxivity enhancement for the single IEM case (MS-325), but surprisingly low albumin-bound relaxivity per IEM for the multimer analogs. In order to further improve the relaxivity of targeted multimers, the local motion of the chelating groups and chelated ion must be decreased.

This invention describes targeted multimers that exhibit high relaxivity as a result of multilocus binding interactions and the accompanying decrease in flexibility. The introduction of two or more TBMs positioned such that there is no significant decrease in relaxivity per IEM upon binding to a target has been found to remarkably improve the relaxivity so that in many cases, the relaxivity per IEM for the contrast agent in the bound state is similar to that previously observed with single IEMs (7-8 fold enhancement). The improvement in relaxivity per IEM results from the decreased flexibility of the target-bound multimer structure, including the attached IEMs. The increase in relaxivity upon binding is typically 1.5-fold or more.

Preferred image resolution is the result of a relaxivity increase of at least 2 or 3 fold. More preferred increases in relaxivity are 4-fold, 5-fold, and 6-fold. Even more preferred increases in relaxivity are 7-8 fold, 9-10 fold or greater than 10 fold increases. The preferred relaxivity at 20 MHz and 37 °C is at least 10 $mM^{-1}s^{-1}$ per IEM, more preferably at least 15 $mM^{-1}s^{-1}$ per IEM, more preferably at least 20 $mM^{-1}s^{-1}$ per IEM, more preferably at least 25 $mM^{-1}s^{-1}$ per IEM, more preferably at least 30 $mM^{-1}s^{-1}$ per IEM, more preferably at least 35 $mM^{-1}s^{-1}$ per IEM, and most preferably at least 40 $mM^{-1}s^{-1}$ per IEM. Preferably the relaxivity of the contrast agent as a whole is greater than 60 $mM^{-1}s^{-1}$ at 20 MHz and 37 °C.

The following data serve to illustrate the contrast enhancement that results from multilocus binding. The benefits of multiple TBMs are evident in three specific comparisons shown in Table 2. In the table, compounds of the invention comprise at least two IEMs and at least two TBMs. Compounds of the invention are compared to compounds that have only a single IEM or a single TBM. The comparison demonstrates the increased relaxivity per IEM for the compounds of the invention. For the measurements in this table, the target is Human Serum Albumin (HSA). First, the comparison of the M8-04 and M8-05 molecules demonstrates that increasing the number of IEMs while the number of TBMs remains constant results in a corresponding increase in total relaxivity. Specifically, this comparison demonstrates that IEMs can be added without a commensurate loss in relaxivity per Gd(III) ion provided that at least two TBMs are present and adequately spaced in the molecule.

Both the M8-04 and M8-05 compounds contain two TBMs, each of which contains a benzofused cyclopentyl group, a phenyl group, and an alkyl substituted phenyl group. These two TBMs are apparently sufficient to tether the molecule so that when the number of IEMs (in

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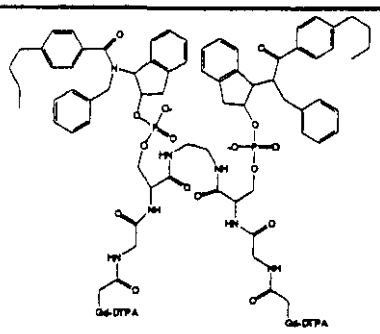
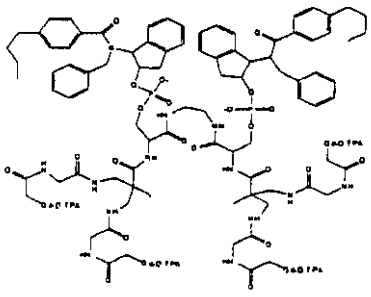
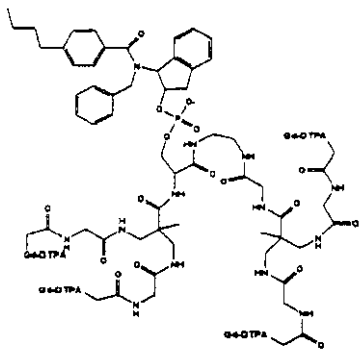
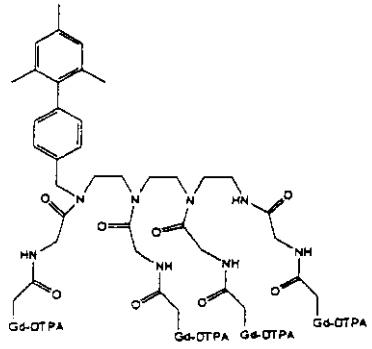
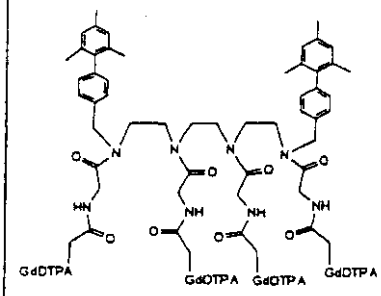
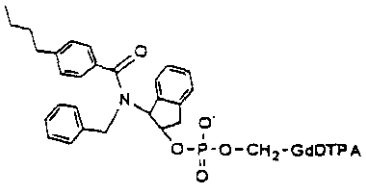
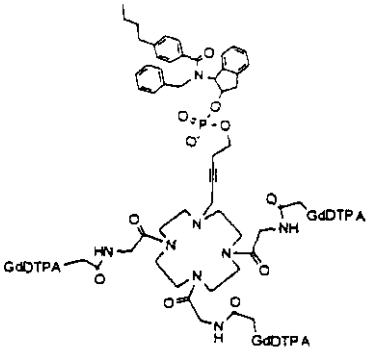
this case DTPA moieties) is increased from two to four, the relaxivity per Gd(III) at each IEM site remains the same (32 versus 32.7 $mM^{-1}s^{-1}$ at 20 MHz). Consequently, the total relaxivity for the molecule doubles in concert with the doubling of the number of IEMs (64 $mM^{-1}s^{-1}$ for two IEMs versus 131 $mM^{-1}s^{-1}$ for four IEMs).

Table 2. The relaxivity increase through multilocus binding: Single TBM vs. Two TBMs

Compound #	# of TBMs	# of IEMs	Chemical Structure	% Bound	$R1_{bound}/Gd^{+3}$ $mM^{-1}s^{-1}$(20 MHz)	Total $R1_{bound}$ $mM^{-1}s^{-1}$ (20 MHz)
M8-04	102	2		99	32	64
M8-05	2	4		97	32.7	131

M8-06	1	4		91.3	27	108

Compound #	*# of* TBMs	*# of* IEMs	*Chemical Structure*	*% Bound*	$R1_{bound}/Gd^{+3}$ $mM^{-1}s^{-1}$(20 MHz)	Total $R1_{bound}$ $mM^{-1}s^{-1}$ (20 MHz)
M8-07	1	4		64.1	16	64
M8-08	2	4		93.7	44.1	176.5

Compound #	# of TBMs	# of IEMs	Chemical Structure	% Bound	$R1_{bound}/Gd^{+3}$ $mM^{-1}s^{-1}$(20 MHz)	Total $R1_{bound}$ $mM^{-1}s^{-1}$ (20 MHz)
M8-09	1	1		99.5	38.7	38.7
M8-10	1	3		66.1	25.7	77.1

Second, the comparison of the M8-06 and M8-05 molecules demonstrates that increasing the number of TBMs while keeping the number of IEMs constant results in an increase in relaxivity per IEM, providing that the TBMs are adequately spaced. In both molecules, the TBMs have the same structure and the IEM structure is the same gadolinium-chelated DTPA moiety. The M8-06 compound, however, contains only a single TBM ~~and~~ while the M8-05 compound contains two TBMs. The two TBMs in the M8-05 compound more effectively tether the molecule because the relaxivity per gadolinium increases from 27.0 $mM^{-1}s^{-1}$ to 32.7 $mM^{-1}s^{-1}$ at 20 MHz. Thus, increasing the number of TBMs increases the relaxivity per IEM providing that the TBMs are adequately spaced.

Third, comparison of the M8-07 and the M8-08 molecules (see Table 2) again demonstrates that increasing the number of adequately spaced TBMs while keeping the number of IEMs constant results in an increase in relaxivity per IEM. In both molecules, the TBMs have the same alkyl substituted biphenyl structure, and both compounds have the same number of IEMs, and the IEM structure is the same gadolinium-DTPA moiety. The core structures (scaffolds) are also the same tetraamines for both the M8-07 and M8-08 molecules. The M8-07 compound contains a single TBM and four IEMs while the M8-08 compound contains two TBMs and four IEMs. Again, the two TBMs in the M8-08 compound more effectively tether the molecule because the bound relaxivity per gadolinium increases from 16.0 $mM^{-1}s^{-1}$ to 44.1 $mM^{-1}s^{-1}$ at 20 MHz in the presence of HSA. Consequently, the calculation reveals that the total relaxivity for the molecule more than doubles in concert with the doubling of the number of TBMs (a 2.75-fold increase from 64.0 $mM^{-1}s^{-1}$ for one TBM to 176.5 $mM^{-1}s^{-1}$ for two TBMs). On the other hand, the free relaxivities for the compounds M8-07 and M8-08 are comparable, namely 9.2

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and 10.3 $mM^{-1}s^{-1}$ at 20 MHz, respectively. The ratios of $R1_{bound}$ to $R1_{free}$ for the compound M8-07 and M8-08 are 1.7 and 4.3, respectively, which indicates that the M8-08 compound will provide better contrast enhancement between target and background. Since the compound M8-08 also has increased HSA affinity and therefore greater target specificity compared to the M8-07, the contrast enhancement from this multilocus binding multimer should be even more superior to the multimer with one TBM.

Finally, the table also includes data for compounds M8-09 and M8-10. These data are included to provide typical relaxivities for molecules that contain a single TBM and multiple IEMs. Comparison of the M8-09 and M8-10 molecules also demonstrates that simple addition of IEMs to a molecule with a certain TBM does not result in a proportionate increase in total relaxivity since a three-fold increase in the number of IEMs only results in a two-fold increase in relaxivity.

Thus, tethering the molecule at two separate sites serves to increase the relaxivity (i.e., decrease the rotational correlation time) for both the molecule as a whole and for the local chelation regions within the contrast agent. The TBMs should be separated by a sufficient distance, however, so that they effectively decrease the flexibility of the entire molecule when the molecule binds to the target. This results in values for the average $\mathbf{RI}_{bound}$ per IEM that do not substantially decrease upon the addition of up to four IEMs. The multilocus binding surprisingly limits flexibility throughout the entire multimeric chelate structure. Thus, for IEMs that include chelates, local chelate motion is reduced following multilocus binding. As a result, remarkably enhanced MRI signals are observed compared to the enhancement for analogous multimeric chelate compounds that bind through only a single TBM and thus are not "pseudo-cyclized" or "zippered" to the

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targeted site. Contrast enhancement is further increased with these multimeric/multilocus binding structures since they also produce a signal in the unbound state that is less than contrast agents that have rigid molecular structures and they have improved binding affinity for the target. In short, the presently described invention provides compounds, compositions, and their methods of use wherein the relaxivity per IEM does not decrease upon the addition of IEMs as a result of multilocus binding interactions (multiple TBMs), and consequently improves contrast.

USES

Blood pool imaging has many potential diagnostic and therapeutic benefits. Detailed images of the circulatory system can provide information that will allow early detection of, for example, aneurisms, embolisms or thromboses and other clots, and areas of restricted blood flow such as those that exist in the coronary arteries in arteriosclerosis. Other common circulatory diseases that may be more accurately diagnosed with high resolution blood pool imaging are circulatory deficiencies associated with diabetes, heart diseases, lymphedema, peripheral vascular disease, Raynaud's Phenomenon, phlebitis and other injuries to the blood vessel lining, heart murmurs, varicose veins and other diseases that result from valve disorders, and vasculitis. Also, contrast agents directed against specific targets could improve the diagnosis of diseases such as neutropenia which results from a neutrophil deficiency. In addition, MR, optical, and other forms of imaging are less invasive than current techniques that require surgical incisions and catheter insertion under general anesthesia.

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MRI and optical contrast agents prepared according to the disclosures herein may be used in the same manner as conventional MRI and optical contrast agents. When imaging a thrombus, certain MR techniques and pulse sequences may be preferred to enhance the contrast of the thrombus compared to the background blood and tissues. These techniques include, but are not limited to, black blood angiography sequences that seek to make blood dark, such as fast spin echo sequences and flow-spoiled gradient echo sequences. These methods also include flow independent techniques that enhance the difference in contrast due to the T_1 difference between contrast-enhanced thrombus and blood and tissue, such as inversion-recovery prepared or saturation-recovery prepared sequences that will increase the contrast between thrombus and background tissues. Methods of preparation for T_2 techniques may also prove useful. Finally, preparations for magnetization transfer techniques may also improve contrast with agents of the invention.

The compositions may be formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings or in animal model systems. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anaesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate. The composition may be stored in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent in activity units. Where the composition is administered

by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade "water for injection" or saline. Where the composition is to be administered by injection, an ampule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

Pharmaceutical compositions of this invention comprise the compounds of the present invention and pharmaceutically acceptable salts thereof, with any pharmaceutically acceptable ingredient, excipient, carrier, adjuvant or vehicle.

The high relaxivity multilocus contrast agent is preferably administered to the patient in the form of an injectable composition. The method of administering the MRI contrast agent is preferably parenterally, meaning intravenously, intraarterially, intrathecally, interstitially or intracavitarilly. Pharmaceutical compositions of this invention can be administered to mammals including humans in a manner similar to other diagnostic or therapeutic agents. The dosage to be administered, and the mode of administration will depend on a variety of factors including age, weight, sex, condition of the patient and genetic factors, and will ultimately be decided by medical personnel subsequent to experimental determinations of varying dosage followed by imaging as described herein. In general, dosage required for diagnostic sensitivity or therapeutic efficacy will range from about 0.001 to 50,000 µg/kg, preferably between 0.01 to 25.0 µg/kg of host body mass. The optimal dose will be determined empirically following the disclosure herein.

Synthesis and use of several high relaxivity multilocus compositions in one embodiment of the invention will be further illustrated in the following examples. The specific parameters included in the following examples are intended to illustrate the

practice of the invention, and they do not in any way limit the scope of the invention. Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from either the spirit of the invention or the scope of the appended claims. Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from either the spirit of the invention or the scope of the appended claims.

EXAMPLES

Example 1. Method for Determining Relaxivity.

The compounds of the present invention were evaluated for relaxivity using a Bruker NMS-120 Minispec NMR spectrometer operating at 0.47 Tesla (20 MHz H-1 Larmor frequency) and 37 °C. T_1 of water protons was determined by an inversion recovery pulse sequence using the instrument's software. Relaxivity was determined in the presence of the target (typically 4.5% HSA) by preparing 4 individual samples. The first contained only 4.5% HSA in phosphate buffered saline (PBS), and the other three contained ca. 20, 30, and 40 μM Gd(III), respectively, in addition to 4.5% HSA in PBS. The samples are incubated at 37 degrees C for at least 15 minutes to ensure temperature equilibration before the T_1 measurement is performed. The Gd(III) content of the samples is determined by inductively coupled plasma - mass spectrometry (ICP-MS). The relaxivity (per Gd(III) ion) is determined by plotting the relaxation rate

$(1/T_1)$ in s^{-1} versus the Gd(III) concentration in mM. The slope of a linear fit to the data gives the relaxivity. The relaxivity of the compounds in the absence of target is also determined in an analogous manner, except there is no target present.

The concentration of species bound to the target under these conditions is determined in a separate experiment using e.g. ultrafiltration or equilibrium dialysis. Knowledge of the amount of species bound to the target, the relaxivity in the presence of the target, and the relaxivity in the absence of the target allows the calculation of the average bound relaxivity as described herein.

Example 2. Experimental Model for Testing Fibrin Binding Contrast Agents of the Present Invention.

A contrast agent with high relaxivity and specificity for clot (thrombus) imaging as described in these examples can distinguish between fibrin in a clot and circulating fibrinogen. It can provide a sensitive, effective detection of thromboses at various stages of development. It can be used to diagnose the presence or absence of early and late thrombi.

One animal model that may be used is the rabbit jugular vein clot model in which the jugular vein is clamped. The vein is clamped at two locations, and the rabbit blood between the clamps is removed. Human fibrinogen, rabbit red blood cells and thrombin are added to the vein between the clamps to generate a clot containing human fibrin. The clot is typically aged for 30 minutes.

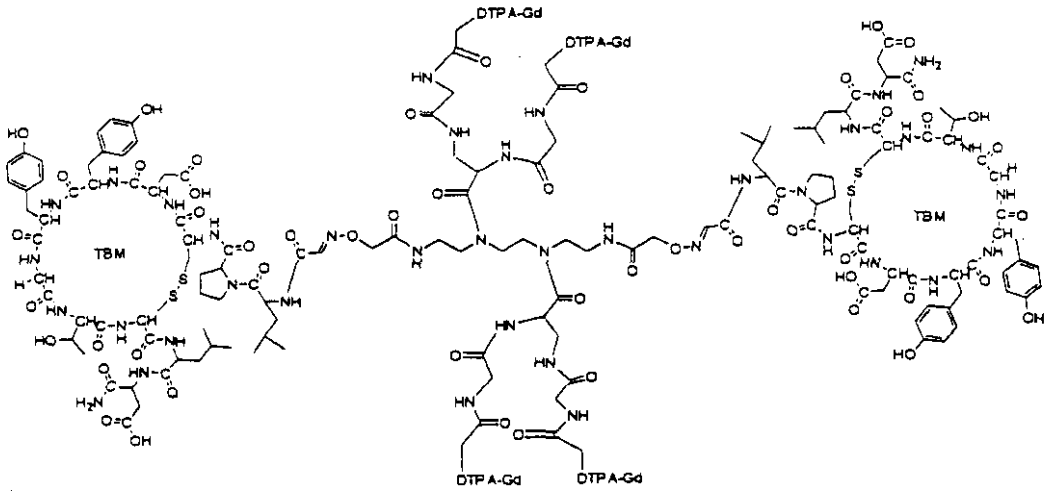
In the rabbit jugular model, a typical machine is a 1.5 Tesla using a spoiled gradient (SPGR) MRI at 36/5/30 deg). Alternatively, a 3D GRE 44/10/30 deg. method of imaging may be used with a 1.5 Tesla machine.

Alternatively, IR 2000/10/700 methods may be employed with a 1.5 Tesla.

Example 3. Example Compound: Synthesis

A preferred embodiment of the invention is a contrast agent for MRI imaging having a chemical structure shown in Figure 13.

Figure 13. Compound M8-11



The contrast agent in Figure 13 comprises four DTPA-Gd molecules that serve as the IEMs. The IEMs are attached to an ethylene diamine scaffold through linkers that comprise a series of repeating amides. The TBMs are two peptides that exhibit high affinity for fibrin. The peptides may cyclize through formation of a disulfide bond between two cysteine residues. The TBMs are attached to the scaffold through an oxime and amide linkage.

Binding of the prototype thrombus agent to the DD(E) amino acid fragment of fibrin is 51% bound at 37°C, 10 μM contrast agent, 2.5 mg/mL fibrin, 50 mM Tris, 150 mM NaCl, 2 mM Ca^{2+}, pH 7.4. Relaxivity for the bound compound is 101.4 $mM^{-1}s^{-1}$ measured at 20 MHz and

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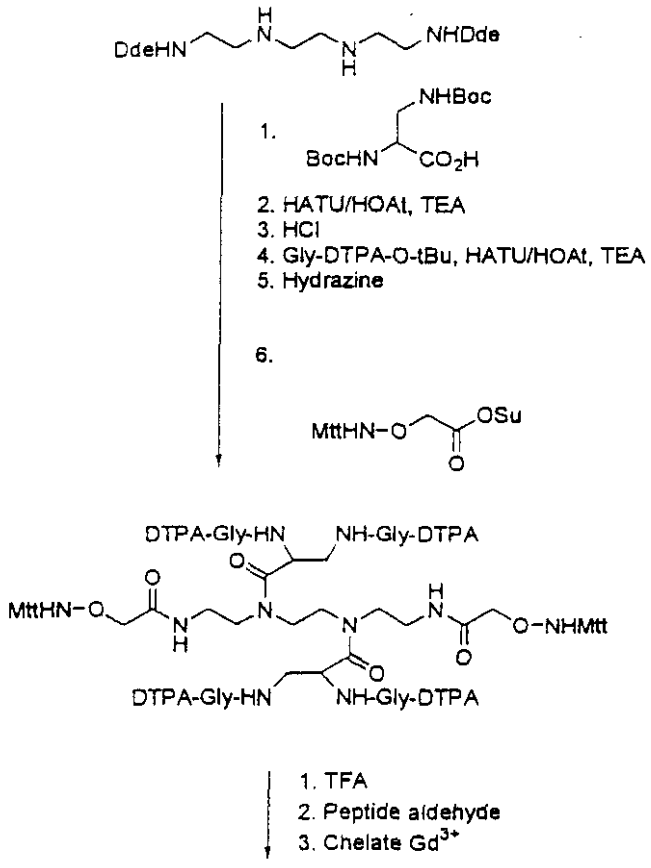
37°C which is 25.4 $mM^{-1}s^{-1}$ per Gd chelate. The free contrast agent has a relaxivity of 67.7 $mM^{-1}s^{-1}$ which is 16.9 $mM^{-1}s^{-1}$ per Gd chelate. The relaxivity of the agent increases greatly upon binding. The synthesis of a thrombus peptide multimer as a prototype MR agent is described in Figure 14. Although a particular peptide and contrast agent are shown (M8-11), one of skill will appreciate that other peptides may be substituted in the compound and that the peptides used in the compound need not be the same.

Figure 14. Synthesis of a multimer contrast agent targeted to human fibrin



M8-11

Experimental Section

Synthesis of DiDde-tetraamine

Tetraamine (Fluka) (1.50mL) was reacted with Dde-OH (NovaBiochem) (4.0g) in 30 mL EtOH. The clear pale yellow solution was refluxed for 16 h. After the completion of the reaction, the reaction mixture was concentrated *in vacuo* to a dry residue. The residue was dissolved in 250mL ether and 2 N HCL solution. The pale yellow acidic aqueous layer was separated and a 50% NaOH solution was until to a pH of 12 was reached and then back extracted with EtOAc. The EtOAc layers were washed with brine, dried (Na_2SO_4), and filtered. The filtrate was evaporated *in vacuo* to yield a pale yellow lumpy solid (2.9 g) which was purified on a Flash silica column using EtOAc/MeOH (2:3)and EtOAc/(MeOH with 1% NH_4OH)(2:3)to yield pure pale yellow solid as the desired product (2.3 g).

^{1}H-NMR(300MHz,$CDCl_3$): δ1.02(s,6H,), 2.34(s,4H,2), 2.56(s,), 2.79(s,2H), 2.92-2.94(t,2H), 3.48-3.54(q,2H), 198.2.

MS: m/z 475.2 $(M+H)^+$

Synthesis of DiDde-tetraamine di-Dpr(Boc)$_2$

Boc-Dpr(Boc)-OH.DCHA (4.85 g) was suspended in 60 mL EtOAc along with 12.0 mL of 2M H_2SO_4. The flask was shaken and the EtOAc layer was separated. The aqueous layer was back extracted with EtOAc. The EtOAc layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated *in vacuo* and the solid obtained was dried to yield 3.23 g of the desired Boc-Dpr(Boc)-OH free acid, as white crystalline solid.

^{1}H-NMR(300 MHz, DMSO-d_6):δ 1.35 (s,18H,), 3.30 (s,2H), 3.96-3.98 (m,1H), 6.87-6.90 (d,1H).

Boc-Dpr(Boc)-OH as free acid (1.52 g) was dissolved in 15 mL DCM at 0°C. To it was added HOAt (0.68 g) and DIEA (0.35 mL) and the clear solution was allowed to stir at 0°C. The DiDde-tetraamine from above was then added to the solution, followed by the addition of HATU (1.90 g)and 2 mmol of TEA. Anhydrous DMF (5 ml) was added and the reaction was stirred for 36 h. The solvents were evaporated *in vacuo* and the residue was taken up in 150 mL EtOAc and washed with 1 N HCl, saturated $NaHCO_3$, brine, then dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to give a white solid (1.92 g). This solid was purified on Flash silica column chromatography using EtOAc (5% MeOH) to give the desired product as a white solid (1.3 g).

^{1}H-NMR (300 MHz, $CDCl_3$):δ 1.01(s,6H),1.35 (s,18H,), 2.34 (s,4H,),2.58 (s,3H), 3.30(s,2H),3.44-3.67(bm,6H), 3.85-4.10 (m,1H),5.44-5.52 (d,2H),5.74(bs,1H).

MS: m/z 1047.7 $(M+H)^+$

Synthesis of Di-Dde-tetraamine-diDpr.HCl salt

DiDde-tetraamine-diDpr $(BOC)_2$ was dissolved in 40 mL 4 N HCl/Dioxane and stirred for 10h. The suspension was triturated with cold ether and evaporated *in vacuo* to obtain copious amounts of white solid. The solid was dried under high-vac pump to yield the desired product in HCl salt form (1.09 g).

MS: m/z 647.3$(M+H)^+$

Synthesis of DiDde-diDpr-tetra-GlyDTPA-OtBu

Gly-DTPA penta-t-Butylester (Gly-DTPA-O-tBu)(2.37 g) was taken up in 10mL DMF at 0°C. HOAt (0.41 g) and DIEA (0.52 mL) were added and the solution was stirred at 0°C. The Di-Dde-tetraamine-diDpr salt

from above (0.39 g) was dissolved in 3 mL DMF and DIEA (0.13 mL) was added. HATU (1.14 g) was added to the mixture along with the additional DIEA (0.09mL). The yellow-colored solution was stirred 36h. The solvents were removed *in vacuo* and the residue taken up in EtOAc. The organic layer was washed with 1 N HCl, saturated $NAHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give a pale yellow colored solid (3.15 g). The crude product was further purified on the Prep RP-HPLC [C-4, ACN/H_2O]. The fractions containing the desired compound were pooled and lyophilized to give a white solid (1.01g).

MS: m/z 1244.4 $(M+3H)^{3+}$; 933.9 $(M+4H)^{4+}$

Synthesis of Tetraamine-tetra-CN-GlyDTPA-O-tBu

DiDde-tetraamine-diDpr-tetra-GlyDTPA-O-tBu (0.38 g) was dissolved in 8 mL 2% v/v hydrazine in DMF and stirred for 10 min at room temp. The reaction mixture was concentrated *in vacuo* and the residue taken up in CH_3CN and purified on Prep-RP-HPLC [C-4,ACN/H_2O]. The fractions containing the desired compound were pooled and lyophilized to give a white solid (0.25 g).

MS: m/z 1702.1 $(M+2H)^{2+}$; 1135.1 $(M+3H)^{3+}$

Synthesis of Di-MeO-trityl-AoA-tetra-Gly-DTPA-O-tBu

Tetraamine-tetra-Gly-DPTA-O-tBu (98 mg) was dissolved in 3 mL DMF at 0°C. Methoxytritylaminooxyacetic acid succinimide ester (MeO-Trt-AoA-OSu) (29 mg) was added along with TEA (9 µL) and stirred overnight, MeOH was added, the solvents were evaporated, and the residue extracted with DCM, washed with 10% aq. citric acid, brine and dried over anhydrous $MgSO_4$. The product was further purified on a Prep RP-HPLC [C-4,ACN/H_2O]. The fractions having the desired

product, were pooled and lyophilized to give a white solid (87 mg).

MS: m/z 2047.5 $(M+2H)^{2+}$; 1365.3 $(M+3H)^{3+}$

Synthesis of AoA-tetra-GlyDTPA-OH

Di-MeOtrityl-AoA-tetra-GlyDTPA-O-tBu (85 mg) was dissolved in 10 mL CH_3Cl/thioanisole/DCM/TIS (64/16/16/4) and stirred at 0°C for 3 h. TIS is $(iPr)_3SiH$ The reaction mixture was diluted with 20mL water and extracted with ether. The aqueous layer was further purified on a Prep RP-HPLC [C-18, ACN/NH_4OAc]. The product was isolated and was lyophilized to a white solid (29 mg).

MS: m/z 1214.4 $(M+2H)^{2+}$; 809.5 $(M+3H)^{3+}$

Oxidation of SLPCDYYGTCLD-NH_2

The peptide, c[SLPCDYYGTCLD-NH_2], 10 mM in NaPi buffer, pH=6.8) was reacted with $NaIO_4$ (20 mM). The oxidation was quenched with ethylene glycol. The reaction purified on a C-18 Sep-Pak cartridge. The product was eluted with 80% CH_3CN containing 0.1% TFA. The solvents were removed in vacuum centrifuge and the desired product alpha-N-glyoxylyl-c[LPCDYYGTCLD-NH_2] was lyophilized to a white powder.

MS: m/z 1315.5 $(M+H)^+$

Chemoselective Ligation-Final assembly: Synthesis of M8-11

Alpha-N-glyoxylyl-c[LPCDYYGTCLD-NH_2] (13.2 mg) and AoA-tetraDPTA-OH (12.3 mg) were reacted in 20 mM sodium acetate buffer, pH4.6 at 22°C. The reaction was purified on a Semi-Prep RP-HPLC [C-18, CH_4CH/5mm NH_4OAc]. This material was converted to the gadolinium complex

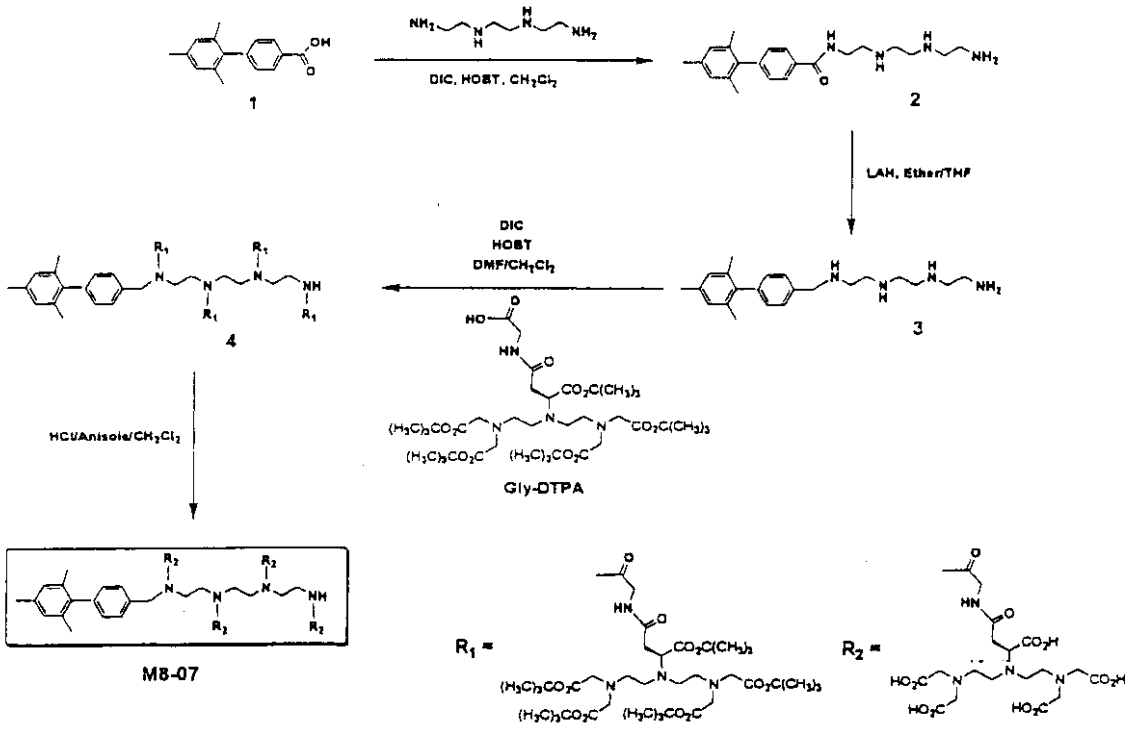
according to standard techniques [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

MS: m/z 1674.6 $(M+3H)^{3+}$; 1256.3 $(m+4H)^{4+}$.

Example 4: Synthesis of Contrast Agents with a Triethylenetetramine Scaffold

Using Di-Dde-tetramine-diDpr and procedures described above along with substitutions for the diamino-BOC substituted ethane linker and the Gly-DTPA-OtBu IEM, various chelates as described above may be attached to the triethylenetetramine scaffold. The chelates may be the same or different to produce a homogenous chelate contrast agent or a contrast agent with heterogenous chelates.

Example 5: Synthesis of M8-07



To a solution of 4-Mesitylbenzoic acid (0.7 g) and triethylenetetramine (4.26 g) in CH_2Cl_2 (200 mL) were added HOBt (0.89 g, 5.83 mmol) and DIC (0.74 g). The mixture was stirred at room temperature overnight. The resultant precipitate was filtered and the solvent was removed under reduced pressure to give a yellow oil. The reaction mixture was submitted to Prep-HPLC on C4 column (eluant: 0.1% TFA/H_2O/CH_3CN) to give the TFA salts of the monoamide as a white foam (0.63 g).

LC-MS: (m/e) 369.1 (M^+).

To solution of the above monoamide (0.63 g) in ether (100 mL) and THF (100 mL) was added LAH (1.30 g) slowly at room temperature. The mixture was refluxed for 2 h and then stirred at room temperature for

overnight. To the mixture was added water dropwise to quench LAH. The resultant precipitate was removed by filtration and the solvent was removed at reduced pressure to give a colorless oil. The reaction mixture was submitted to Prep-HPLC on C4 column (eluant: 0.1% TFA/H_2O/CH_3CN) to give a TFA amine salt as a white foam (180 mg).

LC-MS: (m/e) 354.4 (M^+).

To a solution of the TFA amine salt (180 mg) and diisopropylethylamine (287 mg) in DMF (50 mL) were added a solution of Gly-DTPA-OtBu (1.88 g) in CH_2Cl_2 (50 mL), HOBt (370 mg) and DIC (301 mg). The mixture was stirred at room temperature for overnight. The solvent was removed at reduced pressure to give a yellow oil. The reaction mixture was submitted to Prep-HPLC on C4 column (eluant: 0.1% TFA/H_2O/CH_3CN) to give a crude product as a white solid (0.36 g).

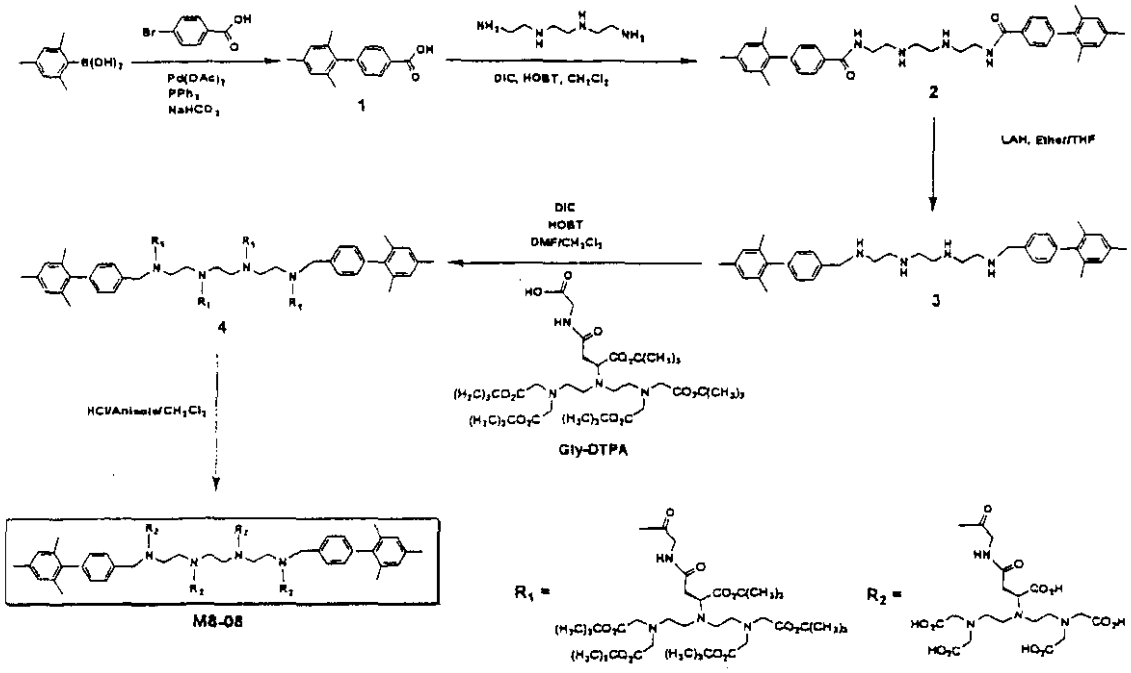
LC-MS: (m/e) 1719.4 (M^{2+}), 1147.2 (M^{3+}).

To a solution of this white solid (0.19 g) in CH_2Cl_2 (4.5 mL) and anisole (4.5 mL) was added dropwise 4.5 mL of 12 N HCl. The mixture was stirred at room temperature for 3 h. To the mixture was added 40 mL of water and washed three times with ether. The aqueous solution was lyophilized to give the crude product which was submitted to Prep-HPLC on C18 column (eluant: 100 mM $AcONH_4$/CH_3CN) to give a white solid (50 mg).

LC-MS: (m/e)1158.6 (M^{2+}), 772.8 (M^{3+}).

This material was converted to the gadolinium complex according to standard techniques [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 6: Synthesis of M8-08



4-Mesitylbenzoic acid. To a solution of mesitylboronic acid (10 g) and 4-bromobenzoic acid (12.9 g) in 1-propanol (150 mL) and DME (200 mL) were added triphenylphosphine (0.128 g), 2M sodium carbonate solution (37 mL) and water (30 mL). To the mixture was added palladium acetate (82 mg) under nitrogen atmosphere. The mixture was heated to reflux overnight. After the heat source was removed, 100 mL of water was added and stirred for 2.5 h while cooling to room temperature. The darkened mixture was diluted with 150 mL of ethyl acetate and the two phases were separated. The organic layer was washed several times with saturated sodium bicarbonate solution until TLC indicated that 4-bromobenzoic acid (R_f = 0.55, eluant: CH_2Cl_2/CH_3OH = 5) was completely removed. The solution was extracted three times with 200 mL of 1N NaOH

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solution. To the combined aqueous layers was added about 50 mL of 12 N HCl to pH 3. The resultant precipitate were filtered, washed with water, and dried to give 4-Mesitylbenzoic acid as a white solid (8.81g).

R_f = 0.75 (eluant: CH_2Cl_2/CH_3OH = 5)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.997 (s,6 H), 2.340 (s,3 H), 6.961 (s,2 H), 7.274 (d,J=8.1Hz,2 H), 8.177 (d,J=8.1Hz,2 H).

To a solution of 4-Mesitylbenzoic acid (1.5 g) and triethylenetetramine (0.43 g) in CH_2Cl_2 (60 mL) were added HOBt (0.96 g) and DIC (0.79 g). The mixture was stirred at room temperature overnight. The resultant precipitate was filtered and dried to give a white solid (1.45 g).

LC-MS: (m/e) 591.3 (M^+).

To solution of the white solid (0.45 g) in ether (20 mL) and THF (80 mL) was added LAH (0.33 g) slowly at room temperature. The mixture was refluxed for 2 h and then stirred at room temperature overnight. To the mixture water was added dropwise to quench LAH. The resultant precipitate was removed by filtration, and the solvent was removed at reduced pressure to give a pale yellow oil. The reaction mixture was submitted to Prep-HPLC on C4 column (eluant: 0.1% TFA/H_2O/CH_3CN) to give the TFA salt as a white solid (140 mg).

LC-MS: (m/e) 564.6 (M^+).

To a solution of the TFA salt (50 mg) and diisopropylethylamine (38 mg) in DMF (30 mL) were added a solution of Gly-DTPA-OtBu (193 mg) in CH_2Cl_2 (30 mL), HOBt (37.5 mg) and DIC (31 mg). The mixture was stirred at room temperature overnight. The solvent was removed

at reduced pressure to give a brown oil. The reaction mixture was submitted to Prep-HPLC on C4 column (eluant: 0.1% TFA/H_2O/CH_3CN) to give a crude product as a pale yellow solid.

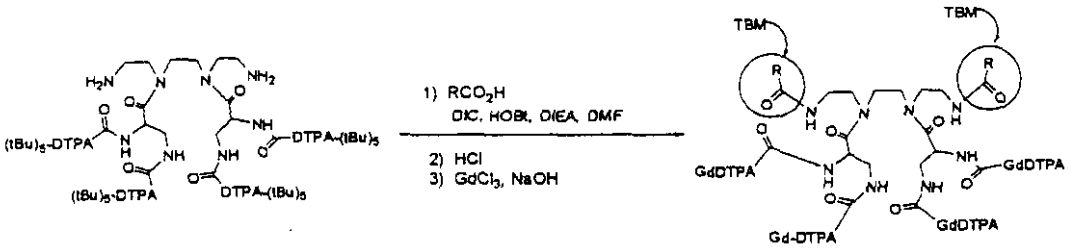
LC-MS: (m/e) 1824.2 (M^{2+}), 1216.3 (M^{3+}), 912.5 (M^{4+}).

To a solution of the pale yellow solid (0.58 g) in CH_2Cl_2 (5 mL) and anisole (5 mL) 10 mL of 12 N HCl was added dropwise. The mixture was stirred at room temperature for 3 h. To the mixture 40 mL of water was added, and the resultant mixture was washed three times with ether. The aqueous solution was lyophilized to give a crude product which was submitted to Prep-HPLC on C18 column (eluant: 100 mM $AcONH_4$/CH_3CN) to give a white solid (11 mg).

LC-MS: (m/e)1263.2 (M^{2+}), 842.4 (M^{3+}), 632.2 (M^{4+}).

This material was converted to the gadolinium complex according to standard techniques [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 7: General synthesis of HSA binding multimers comprising two identical TBMs

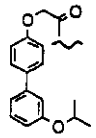


The diamine comprising four t-butyl ester protected DTPAs was dissovled in dimethylformamide (0.75 mL). RCO_2H (3 eq), which represents a variety of carboxylic acids comprising TBMs, DIC (0.052mL, 3.3 eq)

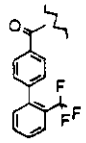
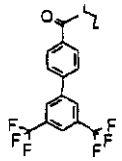
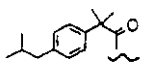
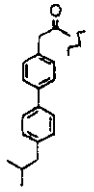
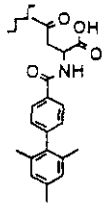
and HOBt (0.051g, 3.3 eq) were added. The reaction mixture was cooled to 0°C prior to the dropwise addition of DIEA (0.105 mL, 6 eq). The reaction mixture was stirred for a total of 8.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and was washed with 0.1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and the residual solvent was removed *in vacuo* to give the t-butylester protected intermediate. The product was taken up in DCM, HCl was added at 0°C, and was stirred for 3 hours. Evaporation of the solvent gave a white solid which was reacted with $GdCl_3$ (4 equiv) and NaOH (12 equiv) to give the final product.

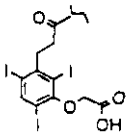
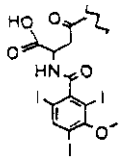
A list of compounds having two identical TBMs that were synthesized according to this method along with the structures of the TBMs and the mass spec data are compiled in Table 3.

Table 3. Multilocus Contrast Agents with two identical TBMs

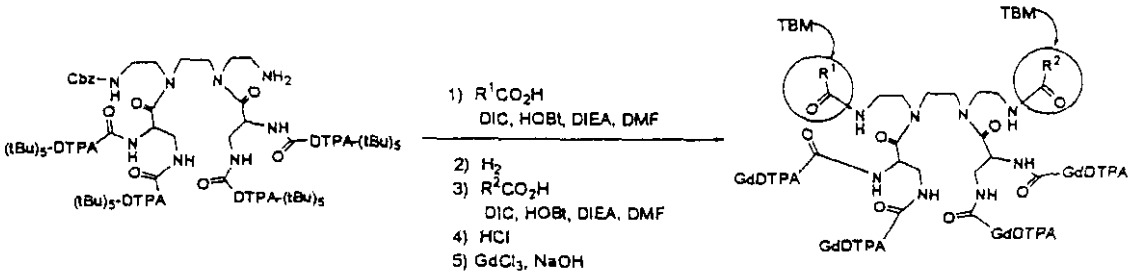
Compound #	Chemical Structure of TBM "R" in General Synthetic Scheme	Mass Spec (m/z, $[M]^{3+}$)
M8-12		1048.5

16

Compound #	*Chemical Structure of TBM* "R" in General Synthetic Scheme	*Mass Spec* (m/z, $[M]^{3+}$)
M8-13		1036.9
M8-14		1080.9
M8-15		1005.0
M8-16		1036.8
M8-17		1094.1

Compound #	Chemical Structure of TBM "R" in General Synthetic Scheme	Mass Spec (m/z, $[M]^{3+}$)
M8-18		1259.1
M8-19		1288.51

Example 8: General synthesis of HSA binding multimers comprising two different TBMs



The CBz-protected monoamine comprising four t-butyl ester protected DTPAs was dissovled in dimethylformamide (0.75 mL). R^1CO_2H (1.5 eq), which represents a variety of carboxylic acids comprising TBM1, DIC (0.052mL, 1.5 eq) and HOBt (0.051g, 1.5 eq) were added. The reaction mixture was cooled to 0°C prior to the dropwise addition of DIEA (0.105 mL, 6 eq). The reaction mixture was stirred for a total of 8.5 h at room temperature.

The reaction mixture was diluted with CH_2Cl_2 and washed with 0.1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and the residual solvent removed *in vacuo*.

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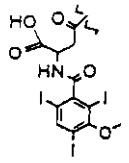
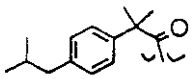
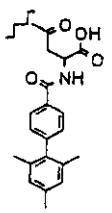
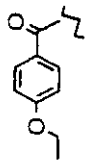
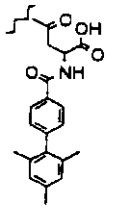
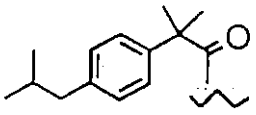
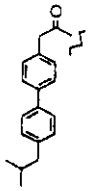
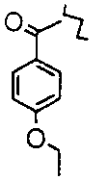
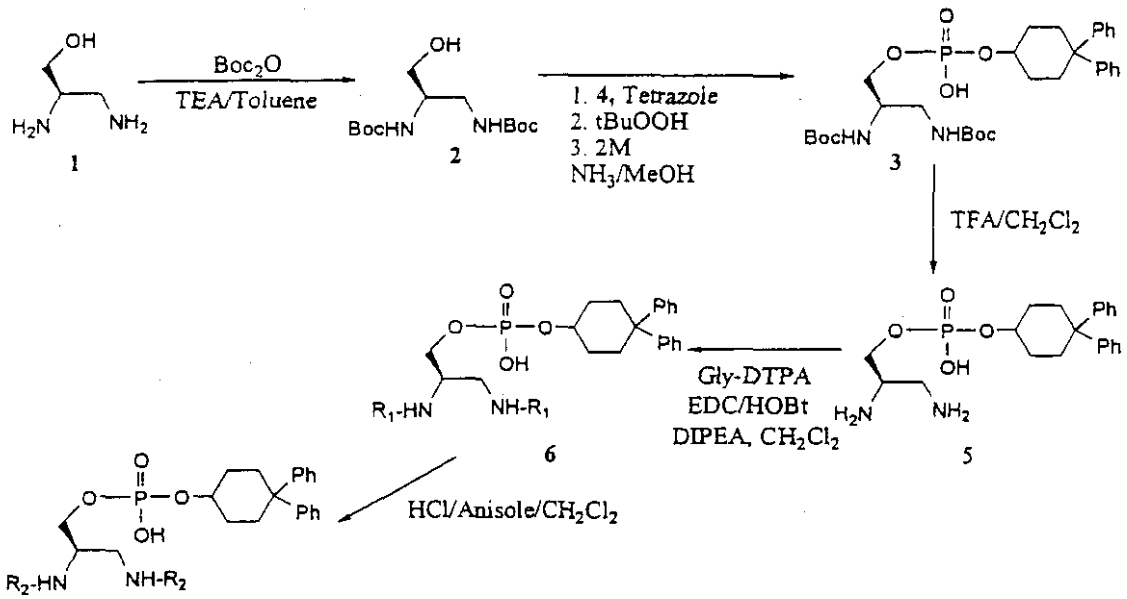
The compound was dissolved in EtOAc and hydrogenated (5% Pd-C). Following filtration of the catalyst, the product was reacted with the second carboxylic acid (R^2CO_2H, 1.5 equiv), which represents a variety of carboxylic acids comprising TBM2, DIC (0.052mL, 1.5 eq) and HOBt (0.051g, 1.5 eq). The reaction mixture was stirred for a total of 8.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with 0.1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and the residual solvent is removed *in vacuo*. The product was resuspended in DCM, HCl was added at 0°C, and the reaction was stirred for 3 hours. Evaporation of the solvent gave a white solid which was reacted with $GdCl_3$ (4 equiv) and NaOH (12 equivalents) to give the final product.

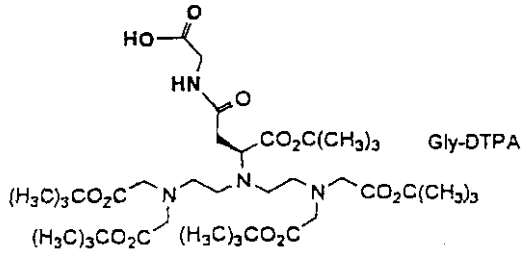
A list of compounds synthesized according to this method along with the structures of TBM1 and TBM2 are compiled in Table 4. Note that "R^1" in the general synthetic scheme corresponds to TBM1 and "R^2" in the general synthetic scheme corresponds to TBM2.

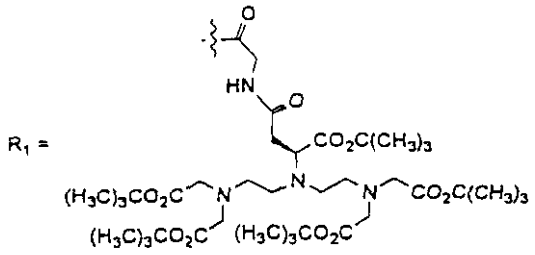
Table 4. Multilocus Contrast Agents with two different TBMs

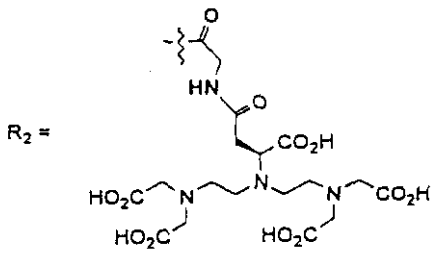
Compound #	Chemical Structure of TBM1 "R^1" in General Synthetic Scheme	Chemical Structure of TBM2 "R^2" in General Synthetic Scheme
M8-20		
M8-21		
M8-22		
M8-23		

Example 9: Synthesis of M8-01





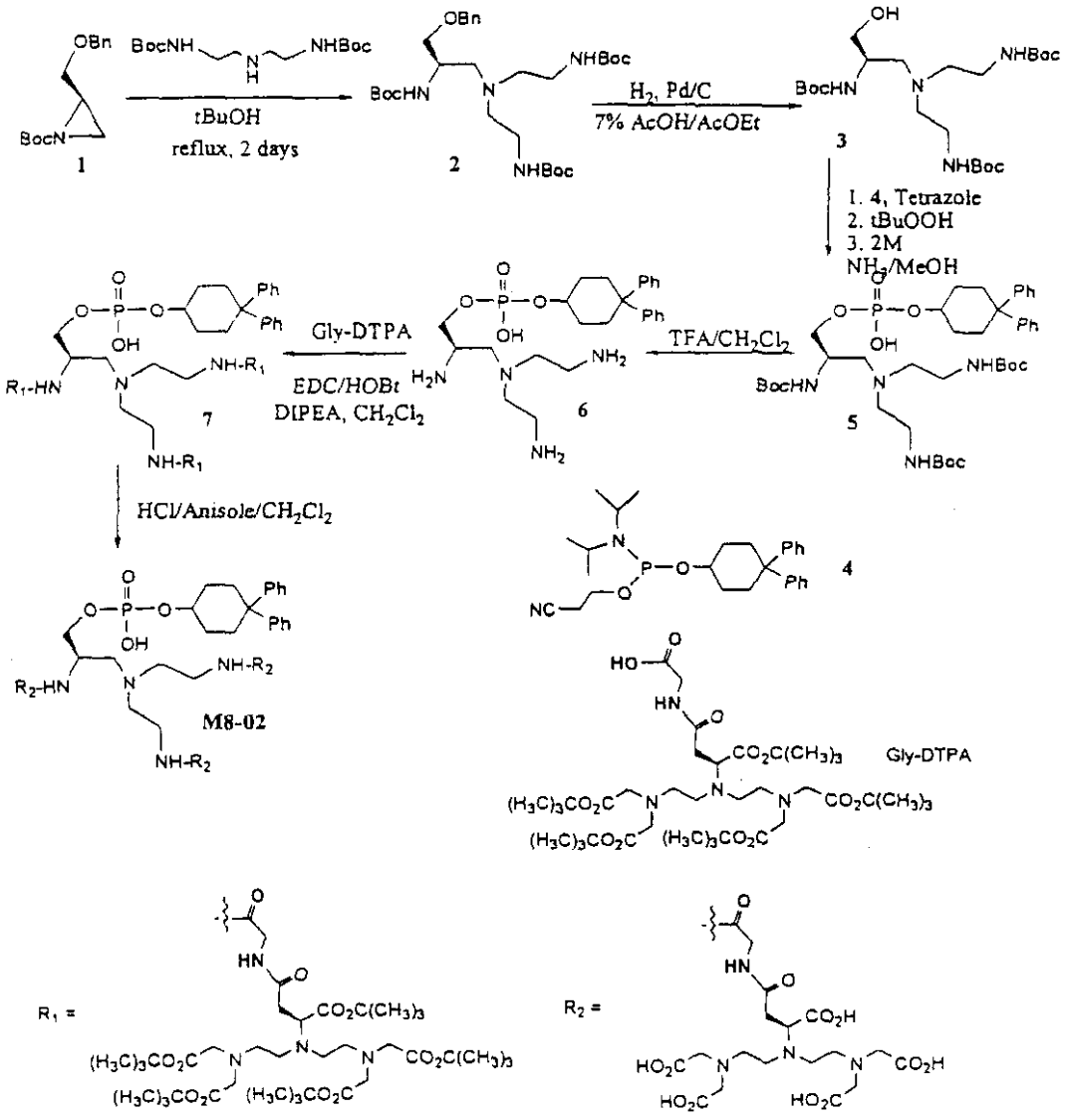




1,2-di(Boc-amino)-3-hydroxypropane (1.0 eq) and diphenylcyclohexyl phosphoramidite (1.1 eq) were dissolved in tetrahydrofuran (1.5 mL/mmol phosphoramidite) and stirred with molecular sieves for 30 min. To the mixture was added tetrazole (1.2 eq) at room temperature. The mixture was stirred for 45 min and ^{31}P NMR indicated completion of the reaction. To the mixture was added *tert*-butylhydroperoxide (1.2 eq). The reaction mixture was stirred for about 1 h until ^{31}P NMR indicated completion of the reaction. The resultant precipitate and molecular sieves were removed by filtration and then methylene chloride was added into the filtrate. The solution was washed sequentially with sodium thiosulfate solution, sodium bicarbonate solution and saturated sodium chloride, and dried over sodium sulfate, filtered and concentrated under vacuum. To the resulting pale yellow oil was added 2M ammonia in methanol. The mixture was stirred overnight and then the solvent was removed under vacuum. The reaction mixture was purified by silica-gel column chromatography (ethylacetate/methanol eluant) to give the phosphodiester.

This phosphodiester was deprotected by stirring in trifluoroacetic acid and methylene chloride after which the mixture was lyophilized yielding the diamine-diphenylcyclohexyl-phosphodiester. This diamine was stirred overnight with 4 equivalents each of Gly-DTPA-O-tBu, EDC, and HOBt in methylene chloride to which diisopropylethylamine had been added to increase the pH. The concentrated reaction mixture was purified by preparative reverse-phase HPLC. The *tert*-butyl esters of the two DTPA units were cleaved by stirring in 4:1:1 hydrochloric acid:anisole:methylene chloride, and subsequently the DTPA-gadolinium chelate was formed with $GdCl_3$ in the usual way [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 10: Synthesis of M8-02



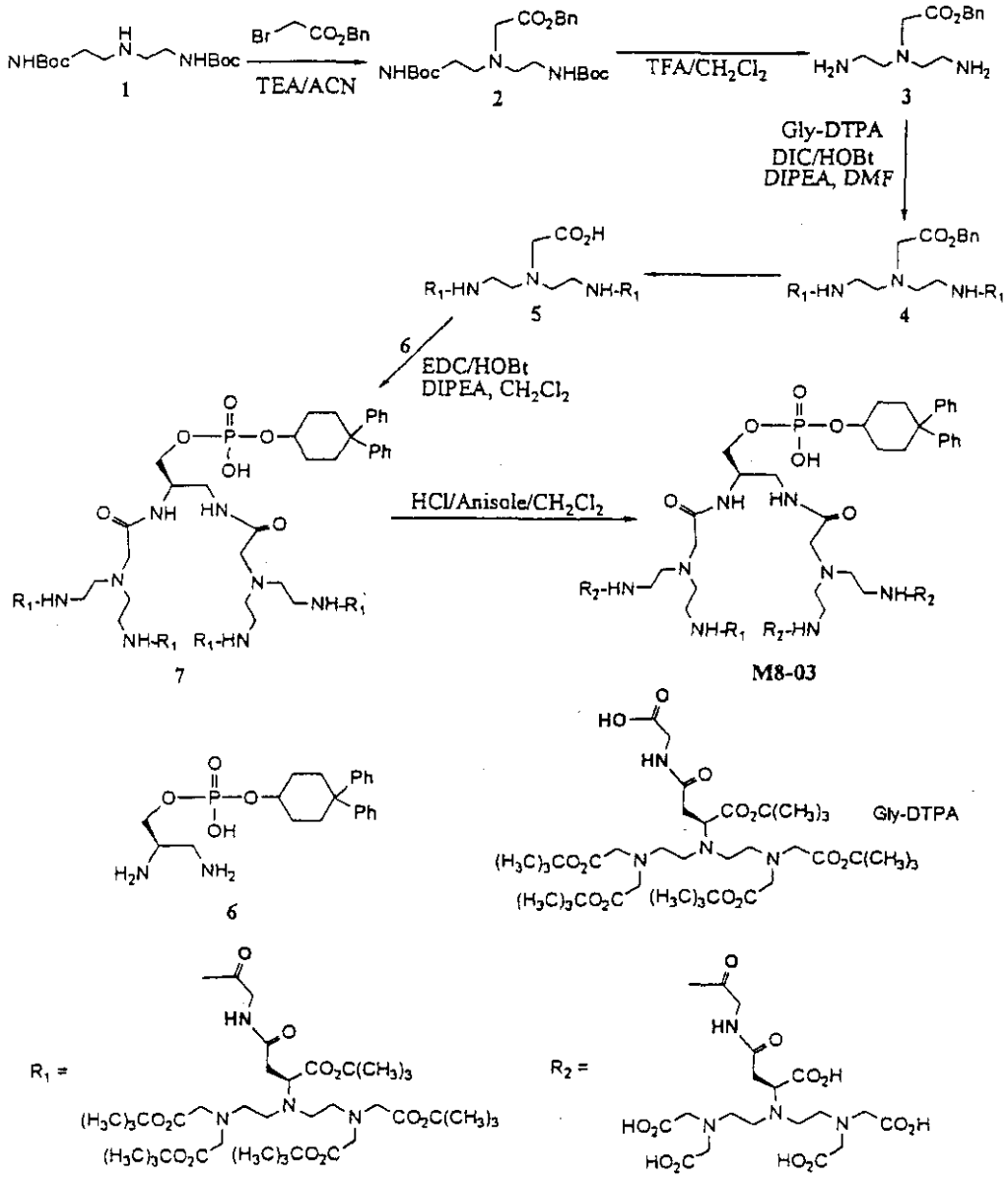
Benzyloxymethylene-*N*-Boc-aziridine (2.82 g), prepared from *N*-Boc-*O*-benzyl-serine, and *N,N''*-diBoc-

diethylenetriamine (3.9 g) were stirred in refluxing *tert*-butanol for 2 d after which solvent was removed under vacuum, and the residue was purified by flash chromatography (methanol and methylene chloride eluant) yielding 4.54 g of material. The ratio of Boc hydrogens to aromatic hydrogens was verified by integration of the ^{1}H NMR spectrum. This triBoc-protected tetraamine benzyl ether (3.8 g) was dissolved in 70 mL 7% acetic acid in ethyl acetate with 1.0 g 10% palladium on carbon. The reaction mixture was placed under an atmosphere of hydrogen at 48 psi for 16 h. Solvent was stripped from the reaction mixture under vacuum and the residue was purified by flash chromatography (methanol and methylene chloride eluant) to yield 3.6 g. The resulting alcohol and diphenylcyclohexyl phosphoramidite were reacted using the conditions stated above (tetrazole, *tert*-butylhydroperoxide, ammonia in methanol) to give the corresponding phosphodiester.

The three Boc groups of this phosphodiester (50 mg) were removed by stirring in 1.0 mL TFA and 1.0 mL DCM for 3 h after which the mixture was lyophilized yielding 54 mL of the triamine. This triamine was dissolved in 1.0 mL methylene chloride and was added dropwise to a solution containing 6 equivalents each of Gly-DTPA-O-tBu, EDC, and HOBt in 2.0 mL methylene chloride to which DIEA had been added to adjust the pH to 9.0. The concentrated reaction mixture was purified by preparative HPLC (C-4 column, 20 mL/min, 30:70 acetonitrile:water to 100:0 gradient over 25 min, then hold for 10 min). The molecular weight of the compound was verified by mass spectrometry. The *tert*-butyl esters of the DTPA subunits were cleaved to reveal the carboxylic acids by stirring in 4:1:1 hydrochloric acid:anisole:methylene chloride for 5 h after which the solution was stripped of solvent under vacuum, dissolved in water and lyophilized. The DTPA-gadolinium chelates were readily formed with $GdCl_3$ in the usual way (see for

example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 11: Synthesis of M8-03



Benzylbromoacetate (11 mL) in 50 mL acetonitrile was added to *N,N''*-diBoc-diethylenetriamine (14 g) in 50 mL acetonitrile and 19 mL triethylamine and stirred for 2 h, after which solvent was removed under vacuum, and the residue was purified by flash chromatography (hexane/ethyl acetate eluant) to yield 11 g of material. The two Boc protecting groups of this compound were removed by stirring all of the material in a 1:1 mixture of trifluoroacetic acid and methylene chloride for 3 h, after which the solution was stripped of solvent under vacuum, partitioned between water and ether, and lyophilized to yield 9.2 g of material. Gly-DTPA-O-tBu, DIC, and HOBt (2.2 equivalents each) were stirred in *N,N*-dimethylformamide for 45 min and then this triamine (1.0 g), as the bis(ammonium trifluoroacetate), was dissolved in *N,N*-dimethylformamide and added to the reaction vessel, and the pH was adjusted to 9.0 by the addition of diisopropylethylamine.

After stirring for 12 hours, the solution was diluted with water and extracted with ethyl acetate which was sequentially washed with aqueous solutions of citric acid, saturated sodium bicarbonate, and saturates sodium chloride. The ethyl acetate solution was concentrated under vacuum and subject to flash chromatography (hexane/ethyl acetate eluant) to yield 845 mg of material confirmed to be the tetramer by mass spectrometry. The benzyl group was removed by hydrogenation of 800 mg of this material dissolved in 10 mL hexane, 9 mL methanol, and 1 mL triethylamine with 20% palladium on carbon under an atmosphere of hydrogen for 3 h after which the solution was filtered through Celite and concentrated under vacuum. This carboxylic acid (750 mg) was stirred in methylene chloride with EDC and HOBt (1.2 equivalents each) for 30 min and the diamine-diphenylcyclohexyl-phosphodiester, described above in the synthesis of M8-01, (80 mg) was dissolved

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in methylene chloride and added to the carboxylic acid solution and the pH was adjusted to 9.0 by the addition of diisopropylethyl amine. After several hours, the mixture was concentrated and purified by preparative HPLC (C-4 column, 20 mL/min, 30:70 acetonitrile:water to 100:0 gradient over 25 min, then hold for 10 min) to yield 150 mg of material, the molecular weight of which was verified by mass spectrometry. The *tert*-butyl esters of the DTPA subunits were cleaved to reveal the carboxylic acids by stirring in 4:1:1 hydrochloric acid:anisole:methylene chloride for 5 h after which the solution was stripped of solvent under vacuum, dissolved in water and lyophilized (yielding 90 mg). The DTPA-gadolinium chelates were readily formed with $GdCl_3$ in the usual way [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

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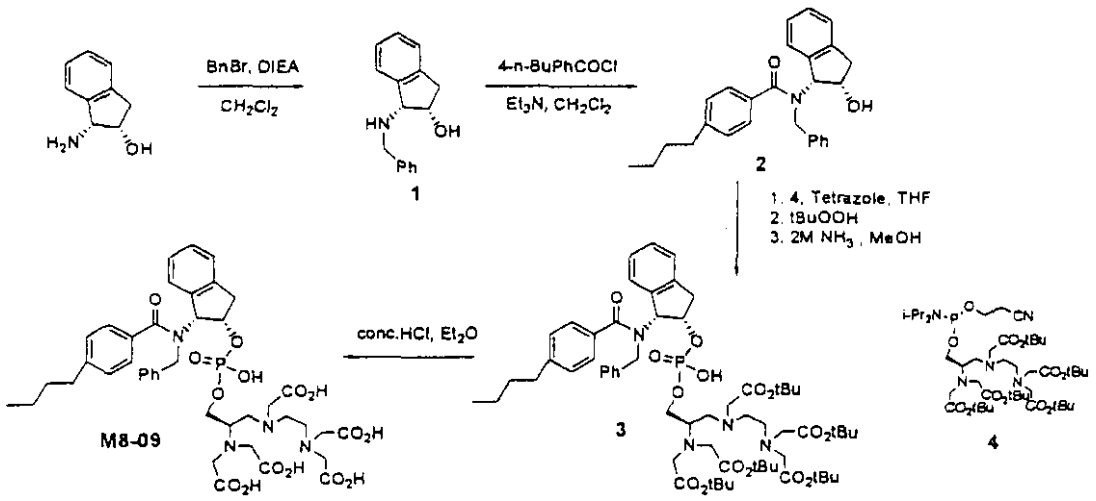
Example 12: Synthesis of M8-09



To a suspension of (1R-2S)-(+)-cis-1-Amino-2-indanol (15 g) in methylene chloride (90 mL) were added diisopropylethylamine (35 mL) and then benzylbromide (17.2 g). The mixture was stirred overnight. The solution was washed with water and extracted twice with a 0.1 N HCl solution, and the pH of the combined aqueous layer was raised to 8 and the aqueous layer was extracted 4 times with CH_2Cl_2. The combined organic layer was washed with saturated sodium chloride, dried over sodium sulfate, filtered and concentrated to give 1-benzylamino-2-indanol (15.62 g), the molecular weight of which was confirmed by mass spectrometry (m/e=239.95 for M$^+$). To a solution of 1-benzylamino-2-indanol (10.5 g) and triethylamine (9.2 mL) in methylene chloride (250 mL) was added dropwise 4-butylbenzoyl chloride (9.1 mL). The mixture was stirred 4 h and then concentrated under vacuum.

The residue was dissolved in ethyl acetate and was washed with water, dried over sodium sulfate, filtered, concentrated under vacuum, and purified by a flash chromatography (hexane /ethyl acetate eluant) to

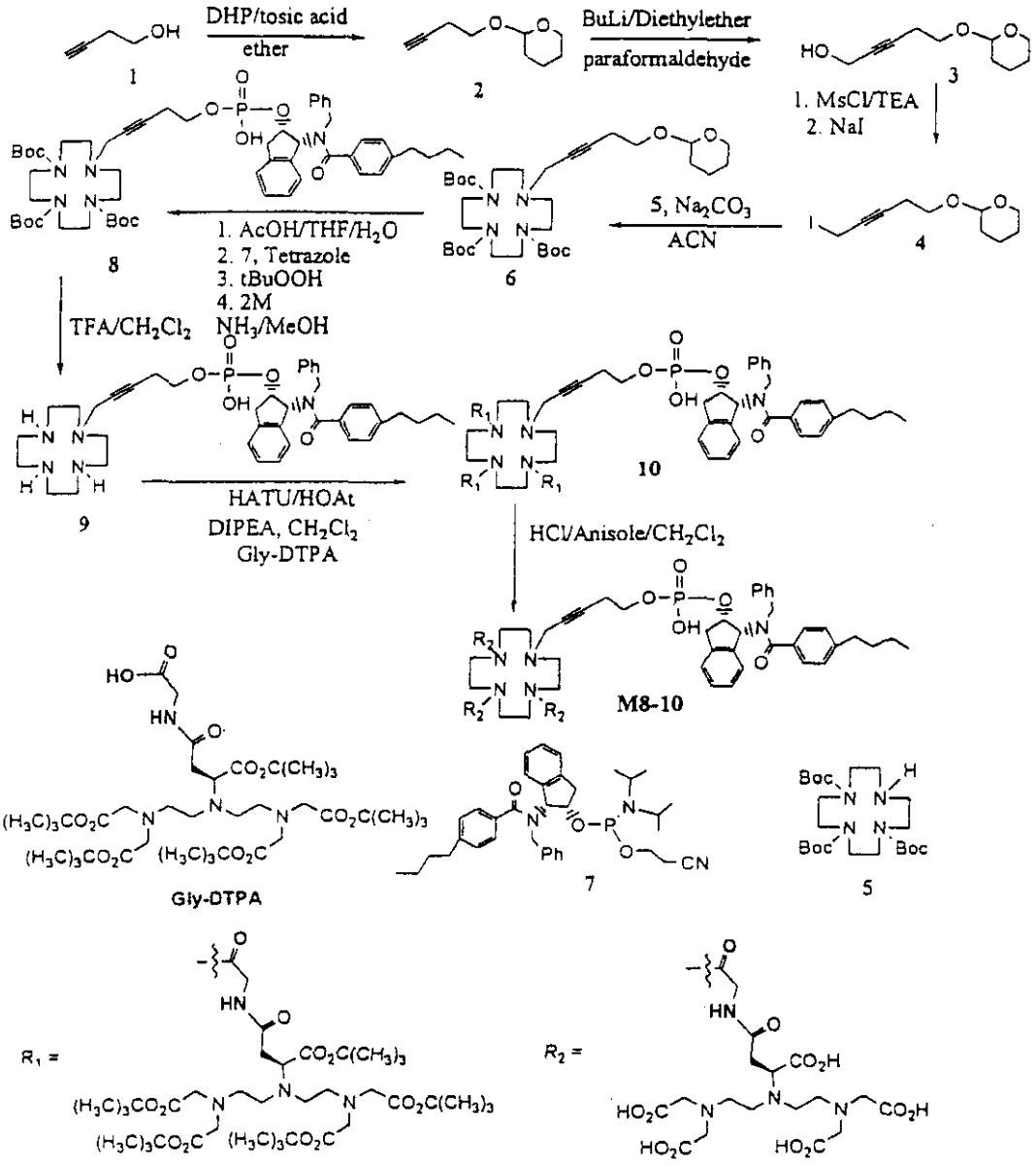
give 1-*para*-butylbenzoyl-1-benzylamino-2-indanol (12.7 g): ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3 H), 1.24-1.4 (m, 2 H), 1.5-1.65 (m, 2 H), 2.6 (t, 2 H), 2.8 (broad d, 1 H), 3.07 (dd, 1 H), 4.52 (m, 1 H), 4.65 (m, 2 H), 5.13 (m, 1 H), 7.09-7.32 (m, 11 H), 7.38-7.62 (m, 2 H). 1-*para*-Butylbenzoyl-1-benzylamino-2-indanol (1.26 g) and DTPA phosphoramidite (2.99 g) were dissolved in tetrahydrofuran (5 mL) and stirred with molecular sieves for 30 min, after which was added tetrazole (265 mg), and the mixture was stirred for 45 min.

^{31}P NMR indicated completion of the reaction, and to the mixture was added *tert*-butylhydroperoxide (0.433 mL), and the reaction mixture was stirred for 1 h until ^{31}P NMR indicated completion of the reaction. The resultant precipitate and molecular sieves were removed by filtration and then methylene chloride was added into the filtrate. The solution was washed sequentially with sodium thiosulfate solution, sodium bicarbonate solution and saturated sodium chloride, and dried over sodium sulfate, filtered and concentrated under vacuum. To the resulting pale yellow oil was added 2M ammonia in methanol. The mixture was stirred overnight and then the solvent was removed at reduced pressure. The reaction mixture was purified by a flash chromatography (ethyl acetate/methanol eluant) to give the phosphodiester adduct as white solid : ^{31}P NMR (THF-d^8) δ -0.28; LC-MS (m/e) 1165.75 (M^+).

The *tert*-butyl esters of the DTPA subunits were cleaved to reveal the carboxylic acids by dissolving in methylene chloride and treating with 12 *N* hydrochloric acid. After several hours, the pH was adjusted to 1.5 by addition of 5*N* sodium hydroxide aqueous solution, and the resulting white precipitate was filtered and was washed twice with hydrochloric acid solution (pH = 1.5). The precipitate was lyophilized for 48 h to give the product as a fine white powder: LC-

MS (m/e) 885.15 (M^+). The DTPA-gadolinium chelates were readily formed with $GdCl_3$ in the usual way [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 13: Synthesis of M8-10



3-Butyne-1-ol (10 g) was stirred in ether (250 mL) with 3,4-dihydropyran (12 g) and tosic acid (20 mg) for 12 h, after which the solution was concentrated under vacuum and the residue was partitioned between ethyl acetate and water. The organic solution was washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated to a the DHP-protected alcohol (18.7 g) which was used without further characterization or purification. The DHP-alcohol (18.7 g) was dissolved in ether (65 mL) and cooled to -75°C after which butyl lithium (50.5 mL of a 2.0 M solution) was added dropwise while maintaining the temperature after which paraformaldehyde (3.5 g) was added. After 4 h, the solution was allowed to warm to room temperature and water (100 mL) was added. The aqueous layer was discarded and the organic layer was concentrated under vacuum to an oil which was purified by flash chromatography (ethyl acetate/hexane eluant) to yield 5-THP-2-pentyn-1,5-ol (13 g) the structure of which was confirmed by ^{1}H NMR.

The alkynol (2.0 g) was converted first to the mesylate (882 mg mesyl chloride, 1.6 mL triethylamine, methylene chloride) and then to the iodide (6.2 g sodium iodide, anhydrous acetone) by the conventional method. This iodoalkyne (1.2 g) was stirred with triBoc-cyclen (200 mg) and sodium carbonate (200 mg) in 8 mL acetonitrile for 1.5 h after which the mixture was stripped of solvent by concentration under vacuum and purified by flash chromatography (ethyl acetate/hexane eluant) and the identity of the adduct (240 mg) was verified by ^{1}H NMR and LC-MS. The adduct (240 mg) was subsequently THP-deprotected by stirring in 4:2:1 acetic acid:tetrahydrofuran:water (12 mL) at 40°C for 6 h after which the reaction was diluted with water and ethyl acetate and the organic layer was extracted sequentially with saturated aqueous sodium bicarbonate and sodium

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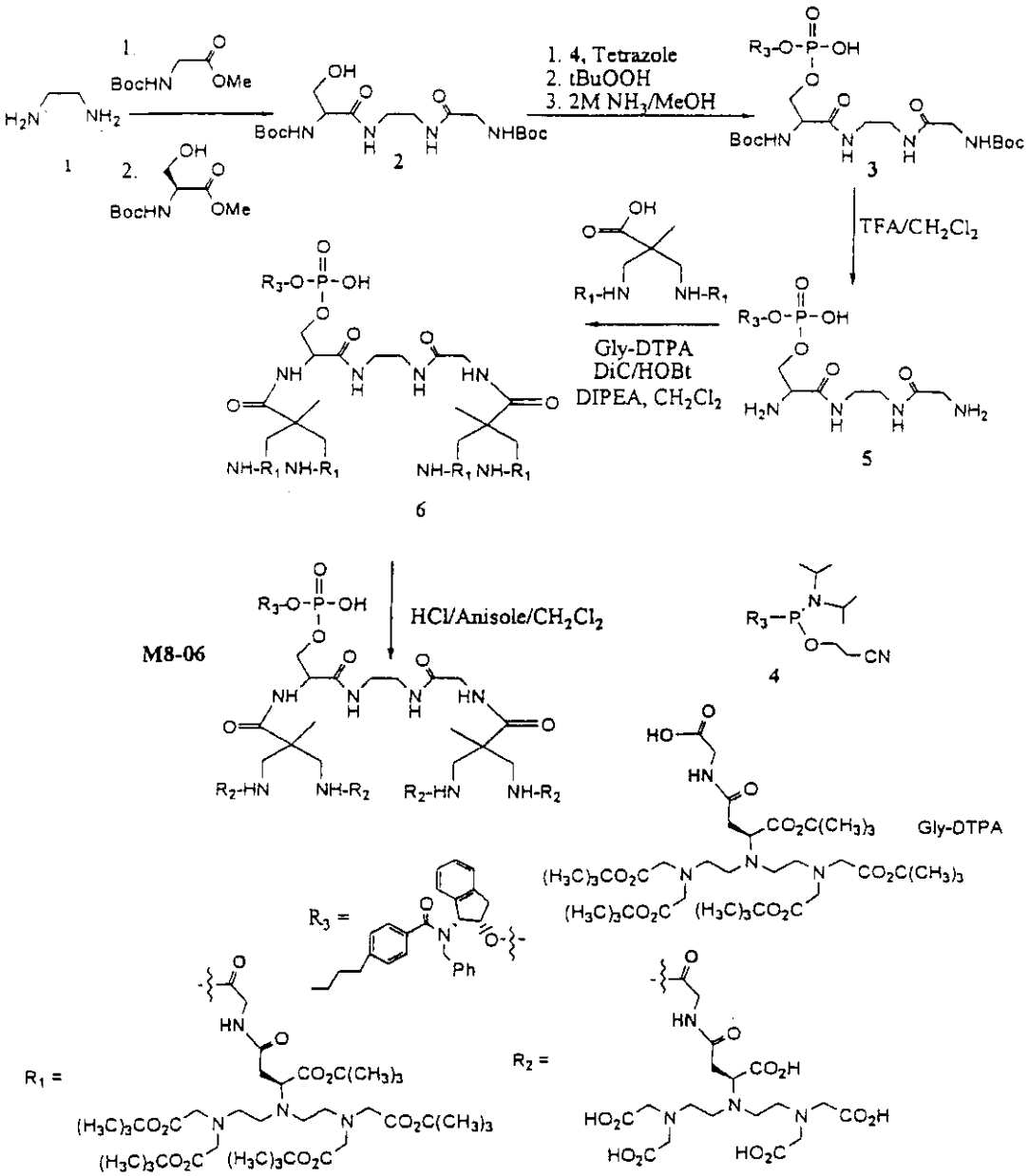
chloride, dried over sodium sulfate, and concentrated to the alcohol (250 mg). A phosphodiester of this alcohol and 1-*para*-butylbenzoyl-1-benzylamino-2-indanol was synthesized by the standard phosphoramidite chemistry described in the synthesis of M8-01 above (tetrazole, *tert*-butylhyrdoperoxide, ammonia/methanol).

The three Boc protecting groups on the cyclen were removed by treatment with acid (trifluoroacetic acid in methylene chloride), and the three resulting amines were converted to the amides with Gly-DTPA-O-tBu using standard methods (HATU/HOAt, diisopropylethyl amine, methylene chloride) as described above. The *tert*-butyl esters of the DTPA subunits were cleaved to reveal the carboxylic acids by stirring in 4:1:1 hydrochloric acid:anisole:methylene chloride for 5 h after which the solution was stripped of solvent under vacuum, dissolved in water and lyophilized. The DTPA-gadolinium chelates were readily formed with $GdCl_3$ in the usual way [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

Example 14: Synthesis of M8-06



Ethylene diamine was reacted sequentially with *N*-Boc-glycine methyl ester and *N*-Boc-serine methyl ester

to form the diamide. The free alcohol and 1-*para*-butylbenzoyl-1-benzylamino-2-indanol were converted to the phosphodiester by the method (tetrazole, *tert*-butylhydroperoxide, ammonia/methanol) discussed above. Benzyl-(3-amino-2-aminomethyl-2-methyl)propanoate and two equivalents of Gly-DTPA-O-tBu were reacted using the conditions described above (DIC/HOBt, diisopropylethylamine, methylene chloride) to form the corresponding diamide. Two equivalents of this diamide were reacted with the ethylene diamine derivative to form a tetramer. The *tert*-butyl esters of the DTPA subunits were cleaved to reveal the carboxylic acids by stirring in 4:1:1 hydrochloric acid:anisole:methylene chloride for 5 h after which the solution was stripped of solvent under vacuum, dissolved in water and lyophilized. The DTPA-gadolinium chelates were readily formed with $GdCl_3$ in the usual way [see for example, Lauffer R. B., et al. Radiology 207: pp. 529-38 (1998)].

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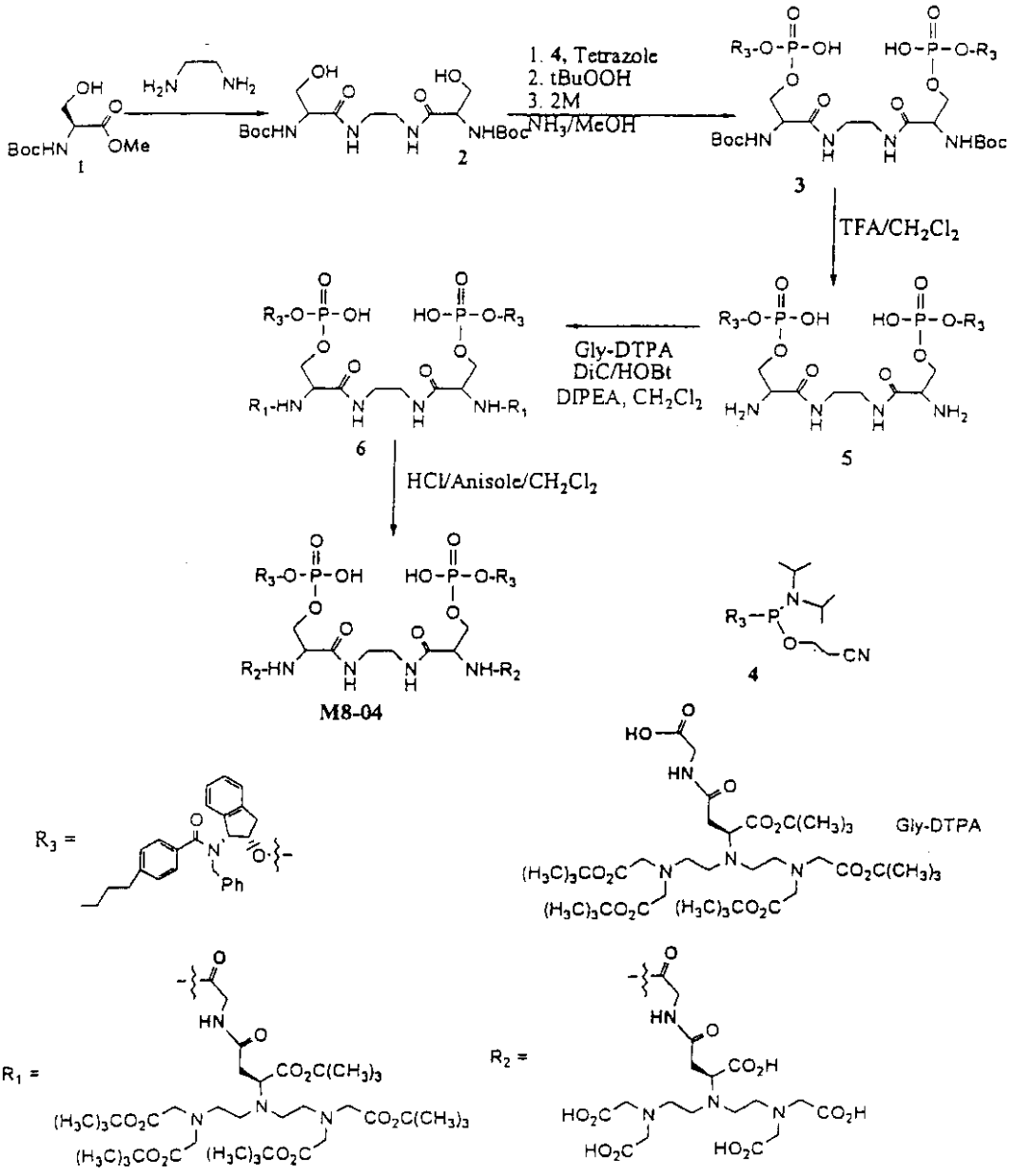
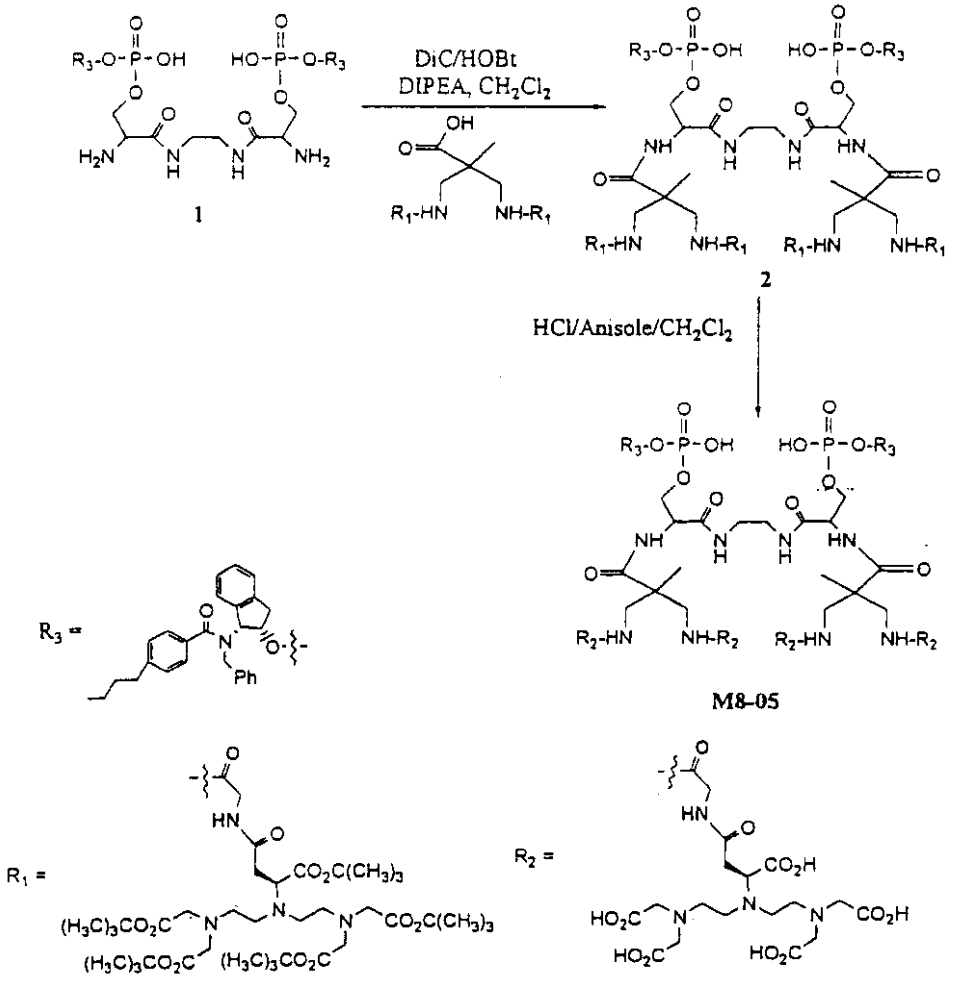
Example 15: Synthesis of M8-04



Ethylene diamine and 2 equivalents of *N*-Boc-serine methyl ester were reacted to form the diamide. The diamide was further reacted to form the diphosphate

derivative as depicted above. The TBMs were attached and deprotected according to the synthetic scheme shown and using the methods discussed above in Example 14.

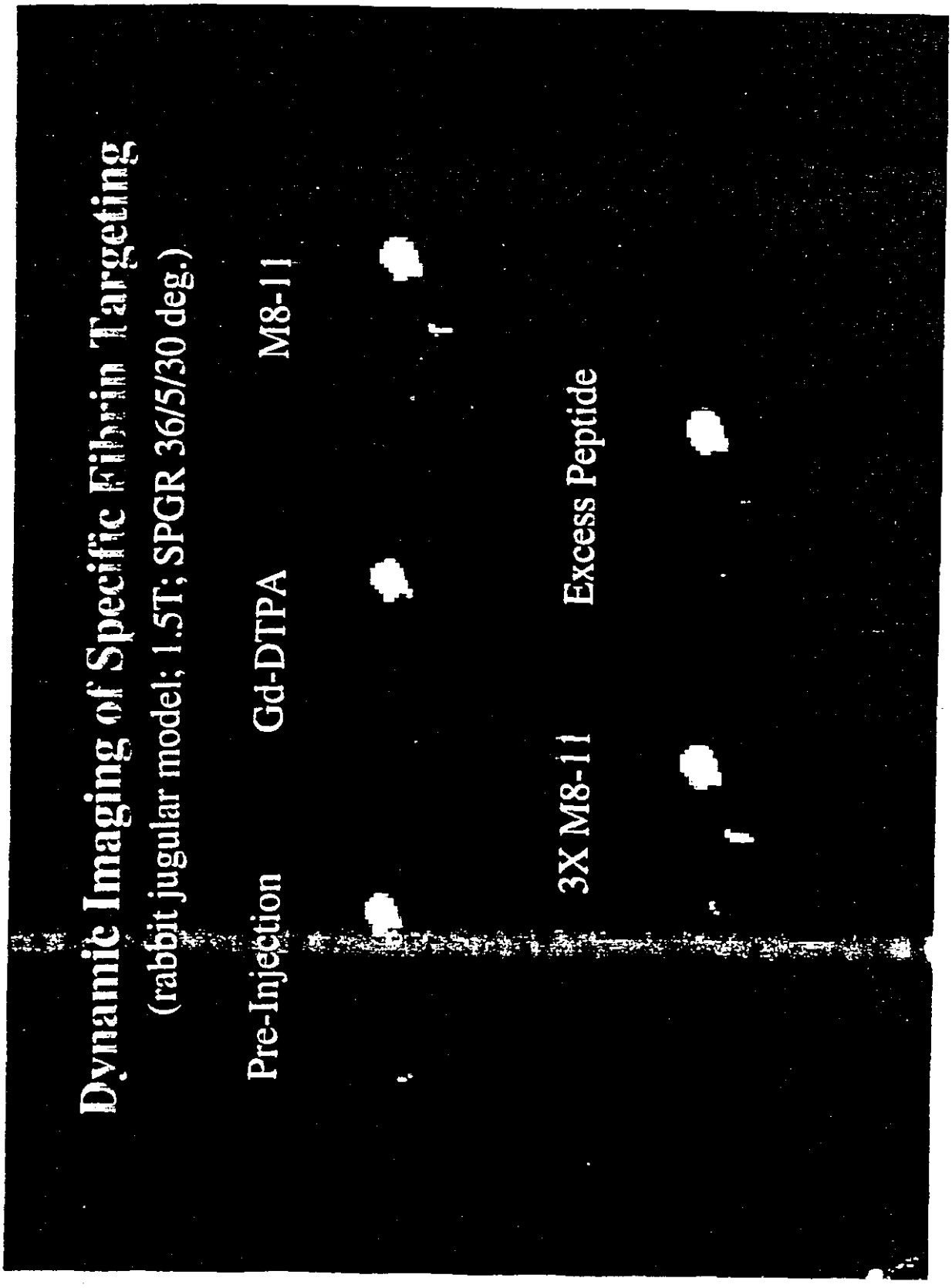
Example 16: Synthesis of M8-05



The diphosphate derivative of the diamide used as the starting material in this synthesis is identical to an intermediate in the synthesis of M8-04 shown in Example 15. The TBMs were attached and deprotected according to the synthetic scheme shown and using the methods discussed above in Example 14.

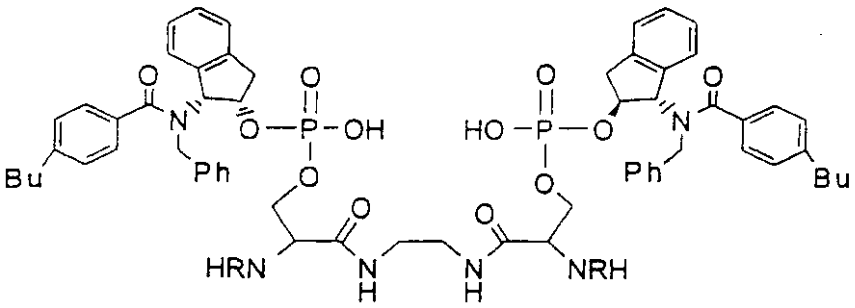
Example 16 - Binding of M8-11 as measured by the Rabbit Jugular Model

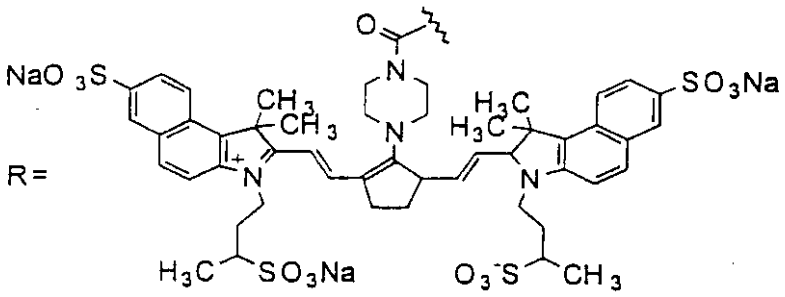
Figure 15 is a color photograph of the images obtained in an experiment using the assay given in Example 1. The experiment shows the images at preinjection, injection with a control untargeted Gd-DTPA compound, injection with M8-11 given in Example 3, injection with three times the dose of M8-11, and injection with excess peptide (in single letter amino acid format) of sequence LPCDYYGTCLD, which competes for the target with the TBM of the contrast agent. The clot is specifically imaged by M8-11, since a contrast agent lacking the TBM does not bind and since the binding is reversed in the presence of excess peptide.


Dynamic Imaging of Specific Fibrin Targeting
(rabbit jugular model; 1.5T; SPGR 36/5/30 deg.)
Pre-Injection
Gd-DTPA
M8-11
3X M8-11
Excess Peptide

Example 16 - Near infrared optical imaging agent

M8-24





The near infrared fluorescent imaging agent M8-24 contains an IEM suitable for optical imaging ("R"). The agent is prepared from the corresponding carboxylic acid derivative of the fluorescent dye (as disclosed in WO 2000/16810). The carboxylic acid derivative is attached to the diphosphate derivative of the diamide according to the conditions for the synthetic step shown in Example 13. In Example 13, the analogous step is the attachment of the Gly-DTPA TBM to the diphosphate derivative of the diamide to form M8-04. This albumin-targeted infrared contrast agent is used, for example, in ophthalmologic angiography and in the diagnosis of cancers of the skin. The optical contrast agent shown above may be used for any of the uses listed for MRI blood pool agents. Furthermore, one of skill will appreciate that the IEMs of such an optical imaging

agent may be varied by substituting a different carboxylic acid derivative of a dye. The agent may therefore be tailored to conform to specific experimental criteria, such as a particular excitation wavelength.

CLAIMS

We claim:

1. A method for increasing contrast using MRI or optical imaging wherein:

(a) a multilocus contrast agent binds to a target,

(b) contrast at the target is enhanced following binding of the contrast agent to the target,

(c) the contrast enhancement improves with either the number of IEMs, the rigidity of the molecule, or both.

2. A method for increasing the relaxivity of a contrast agent wherein:

(a) a multilocus contrast agent binds to a target,

(b) the relaxivity of the contrast agent increases upon binding to the target,

(c) the relaxivity per IEM of the contrast agent does not decrease upon binding, and wherein:

the contrast agent comprises

a) two or more Image Enhancing Moieties (IEMs);

b) two or more Target Binding Moieties (TBMs);

c) a scaffold to which the TBMs and IEMs are attached; and

d) optional linkers for attachment of the IEMs to the scaffold.

3. A method for increasing the relaxivity according to claim 2, wherein the relaxivity is at least 10 $mM^{-1}s^{-1}$ per IEM.

4. A method for increasing the relaxivity according to claim 2, wherein the relaxivity is at least 15 $mM^{-1}s^{-1}$ per IEM.

5. A method for increasing the relaxivity according to claim 2, wherein the relaxivity is at least 20 $mM^{-1}s^{-1}$ per IEM.

6. A method for increasing the relaxivity according to claim 2, wherein the relaxivity is at least 25 $mM^{-1}s^{-1}$ per IEM.

7. A method for increasing the relaxivity according to claim 2, wherein the relaxivity is at least 30 $mM^{-1}s^{-1}$ per IEM.

8. A method for MR imaging of a target in an animal or human subject comprising the steps of administering an MRI contrast agent, allowing the contrast agent to bind to the target, and imaging a region of the subject's body in which the target is located wherein:

the MRI contrast agent comprises a scaffold, at least two IEMs each comprising a chelate of a paramagnetic metal ion and covalently bound to the scaffold either directly or by one or more intervening linkers, and at least two TBMs that have an affinity for the target and are covalently bound to atoms of the scaffold that are different from the atoms at which the IEMs are bound;

wherein the relaxivity of the contrast agent increases upon binding;

and wherein the target is a protein, polysaccharide, cell, fluid, glycoprotein, or a thrombus.

9. A targeted MRI contrast agent comprising a scaffold, 2 to 4 TBMs covalently bound to said scaffold, 2 to 4 IEM's wherein said IEMs are

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either covalently bound to said scaffold or covalently bound to one or more linkers, wherein said linkers, if present, are covalently bound to said scaffold and wherein:

each IEM comprises a chelate of a paramagnetic metal ion,

each TBM is covalently bound to atoms of the scaffold not covalently bound to an IEM,

each TBM has an affiinity for a target, and

the relaxivity of the contrast agent when bound to said target is at least two-fold greater than the relaxivity in the unbound state.

10. A method for increasing the affinity of a contrast agent for its target comprising multilocus binding of the contrast agent to a target, wherein the contrast agent comprises

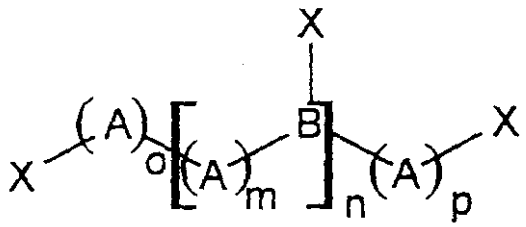
a) and two or more Image Enhancing Moieties (IEM);

b) two or more Target Binding Moieties(TBMs);

c) a scaffold to which the TBMs and IEMs are attached;

d) optional linkers for attachment of the IEMs to scaffold.

11. A multilocus contrast agent having the structure (IX):



(IX)

X = L-IEM, TBM, or IEM

wherein m is an integer from 1 and 10 inclusive;

n is an integer from 2 to 10 inclusive;

o is 0 to 1;

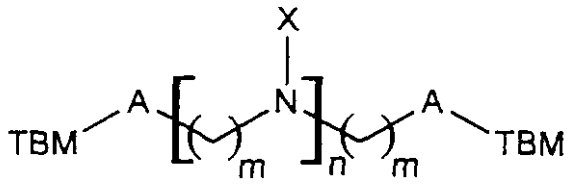
p is 0 to 1;

each A is independently selected from the group consisting of O, CH_2, C=O, C=NH, NH, NR, or CHR; and

B is selected from the group consisting of CH and N; and

R is C_1-C_{10} straight or branched chain alkyl, C_2-C_{10} straight or branched chain alkenyl or alkynyl, and wherein up to four carbon atoms are optionally substituted with halogen, O, N or S.

12. A multilocus contrast agent having the structure (V):



(V)

X = L-IEM, TBM, or IEM

wherein each m is independently an integer from 1 and 8 inclusive;

each A is independently selected from the group consisting of O, CH_2, C=O, C=NH, NH, NR, or CHR;

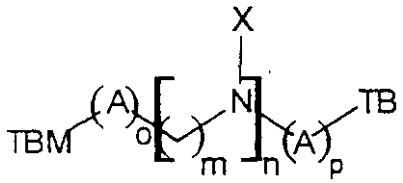
R is C_1-C_{10} straight or branched chain alkyl, C_2-C_{10} straight or branched chain alkenyl or alkynyl, and wherein up to four carbon atoms are optionally substituted with halogen, O, N or S; and

n is an integer from 2 to 10 inclusive.

13. The contrast agent according to claim 12 wherein A is C=O.

14. The contrast agent according to claim 12 wherein A is O.

15. A multilocus contrast agent having the structure of Formula (VI):



(VI)

X = L-IEM, TBM, or IEM

wherein m is an integer from 1 and 10 inclusive;

n is an integer from 2 to 10 inclusive;

o is 0 to 1;

p is 0 to 1;

each A is independently selected from the group consisting of O, CH_2, C=O, C=NH, NH, NR, or CHR; and

B is selected from the group consisting of CH and N; and

R is C_1-C_{10} straight or branched chain alkyl, C_2-C_{10} straight or branched chain alkenyl or alkynyl, and wherein up to four carbon atoms are optionally substituted with halogen, O, N or S.

16. A multilocus contrast agent having the structure (VII):

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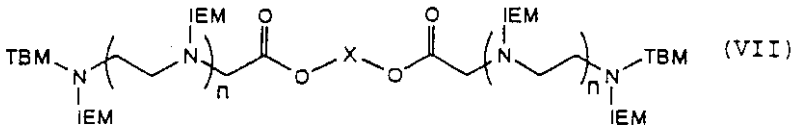


17. The multilocus contrast agent according to any one of claims 11 to 16 wherein the TBM comprises a substituted aryl group.

18. The multilocus contrast agent according to claim 17 wherein the IEM is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA) and derivatives thereof;
1,4,7-triazacyclononane;
1,4,7,10-tetraazacyclododecane (Cyclen) and derivatives thereof;
1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid tert-bu-ester) (DO2A-t-bu-ester);
1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid, t-bu-ester) (DO3A-t-bu-ester);
1,4,7-tris(tert-butoxycarbonyl)-1,4,7-tetraazacyclodo decane (DO3-t-BOC);
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and derivatives thereof;
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(meth ylene phosphonic acid) (DOTP).

19. A contrast agent according to claim 18 wherein the IEM is diethylenetriamine pentaacetic acid (DTPA).

20. A contrast agent according to any of claims 11 to 16 wherein the TBM is a peptide.

21. A contrast agent according to claim 20 wherein the TBM is a fibrin binding peptide.

22. A contrast agent according to the formula:

$$(TBM)_q\text{-}S\text{-}[L_m\text{-}IEM_n]_p$$

wherein:

TBM is a target binding moiety, S is a scaffold, L is a linker, IEM is an image enhancing moiety, and

q, m, n, and p are all independent integers wherein:

q is an integer from 2 to 6 inclusive,

each m is independently 0 or 1,

each n is independently an integer from 2 to 4 inclusive,

p is an integer from 2 to 4 inclusive, and wherein:

each TBM is covalently bound to S by a chemical bonds between two atoms,

each L, if present, is covalently bound to an IEM by a chemical bond between two atoms,

each L, if present, is covalently bound to S by a chemical bond between two atoms, and

the at least two TBMs are attached to different atoms of the scaffold.

23. A contrast agent for optical or MR imaging comprising:

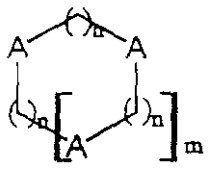
a) two or more Image Enhancing Moieties (IEMs);

b) two or more Target Binding Moieties (TBMs);

c) a scaffold to which the TBMs and IEMs are attached; and

d) optional linkers for attachment of the IEMs to the scaffold,

wherein the scaffold comprises a structure of formula (VIII):



(VIII)

and m and n are independent integers wherein:

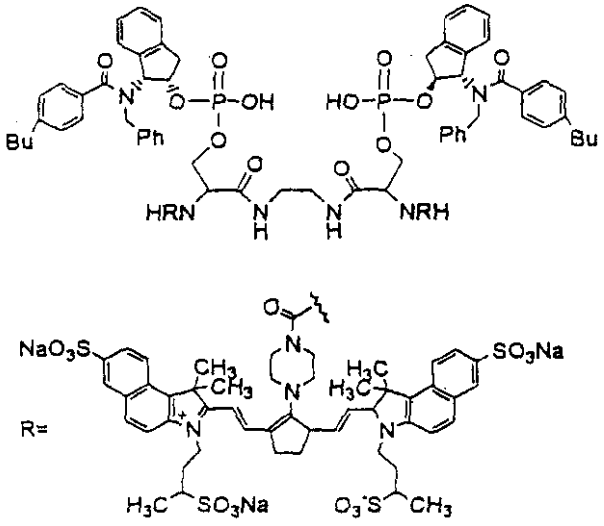
m is an integer from 0 to 3 inclusive,

n is an integer from 0 to 2 inclusive,

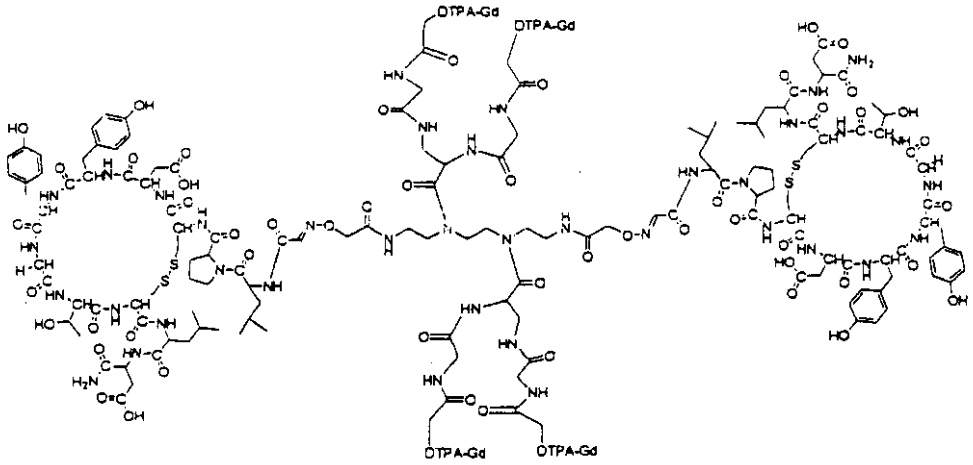
each A is independently selected from the group consisting of NH, NR, O, S, CH_2, C=O, C=NH, C=NR, and CHR, wherein:

R is C_1-C_{10} straight or branched chain alkyl, C_2-C_{10} straight or branched chain alkenyl or alkynyl, and wherein up to four carbon atoms are optionally substituted with halogen, O, N or S.

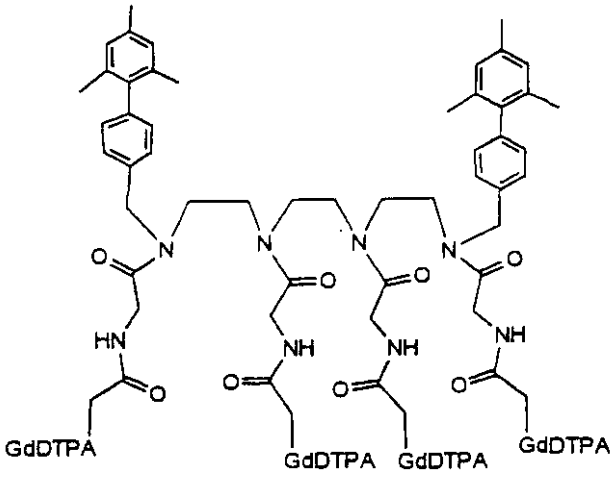
24. The compound M8-24.



25. The compound M8-11.



26. The compound M8-08.



[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
A61K 49/00
A61K 49/10 A61K 49/14

[12]发明专利申请公开说明书

[21]申请号 00813045.0

[43]公开日 2002年11月27日

[11]公开号 CN 1382062A

[22]申请日 2000.7.28 [21]申请号 00813045.0
[30]优先权
[32]1999.7.29 [33]US [31]60/146,414
[32]1999.11.4 [33]US [31]60/163,650
[86]国际申请 PCT/US00/20536 2000.7.28
[87]国际公布 WO01/08712 英 2001.2.8
[85]进入国家阶段日期 2002.3.18
[71]申请人 埃匹克斯医学股份有限公司
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[54]发明名称 通过多位点结合的寻靶多体造影剂

[57]摘要

本发明涉及用于诊断成像的造影剂。具体说,本发明涉及新型的多体化合物,其表现出在结合内源蛋白质或其它生理相关位点时改善的弛豫率。这种化合物包括:a)两个或多个包括多体亚单位的影像增强部分("IEMs")(或信号产生部分);b)两个或多个靶标结合部分("TBMs"),提供体内定位和多体刚性化;c)用于上述部分连接的构架("骨架");和d)将IEMs连接于构架的任选的连接基。本发明还涉及含有这些化合物的药物组合物,和用这些化合物和组合物提高诊断成像对比度的方法。

ISSN1008-4274

权　利　要　求　书

1．一种用磁共振成像 MRI 或光学成像提高对比度的方法，其特征在于，所述的方法包括：

(a)多位点造影剂结合于靶标，

(b)在造影剂结合于靶标后提高靶标上的对比度，

(c)用 IEM 的数量、分子的刚性或这两者改善对比度的提高。

2．一种增加造影剂弛豫率的方法，其特征在于，所述的方法包括：

(a)多位点造影剂结合于靶，

(b)结合于靶标时造影剂的弛豫率增加，

(c)结合后造影剂的每 IEM 的弛豫率没有减少，且其中造影剂包括

a)两个或多个影像增强部分(“IEMs”)；

b)两个或多个靶标结合部分(“TBMs”)；

c)连接 TBM 和 IEM 的构架；和

d)将 IEMs 连接于构架的任选的连接基。

3．如权利要求 2 所述的增加弛豫率的方法，其特征在于，所述的弛豫率至少为每个 IEM $10mM^{-1}s^{-1}$。

4．如权利要求 2 所述的增加弛豫率的方法，其特征在于，所述的弛豫率至少为每个 IEM $15mM^{-1}s^{-1}$。

5．如权利要求 2 所述的增加弛豫率的方法，其特征在于，所述的弛豫率至少为每个 IEM $20mM^{-1}s^{-1}$。

6．如权利要求 2 所述的增加弛豫率的方法，其特征在于，所述的弛豫率至少为每个 IEM $25mM^{-1}s^{-1}$。

7．如权利要求 2 所述的增加弛豫率的方法，其特征在于，所述的弛豫率至少为每个 IEM $30mM^{-1}s^{-1}$。

8．一种在动物或人对象中对靶标进行 MR 成像的方法，包括如下步骤：给予 MRI 造影剂；使造影剂结合于靶标；将身体靶标所在处的对象部位进行成像；其特征在于，所述的 MRI 造影剂包括构架；至少两个 IEM，各 TBM 含有顺磁金属离子的螯合物，且直接或通过一个或多个中间的连接基共价结合于构架；和至少两

个 TBM，各 TBM 具有与靶标的亲和力，而且共价结合于构架上与 IEM 结合的原子不同的原子上；

其中结合后造影剂的弛豫率增加；

且所述靶标是蛋白质、多糖、细胞、液体、糖蛋白或血栓。

9．一种 MRI 造影剂，其特征在于，包含构架、2-4 个共价结合于所述构架的 TBM、2-4 个 IEM 的寻靶所述的 IEM 共价结合于所述的构架或共价结合于一个或多个连接基，其中如果存在所述的连接基，则所述的连接基共价结合于所述的构架，且其中：

各 IEM 含有顺磁金属离子的螯合物，

各 TBM 共价结合于构架的不共价结合 IEM 的原子，

各 TBM 具有对靶标的亲和力，和

当与所述的靶标结合时造影剂的弛豫率至少比未结合状态时的弛豫率高 2 倍。

10．一种用于增加造影剂对其靶标亲和力的方法，包括使造影剂多位点结合于靶标，其特征在于，所述的造影剂包含

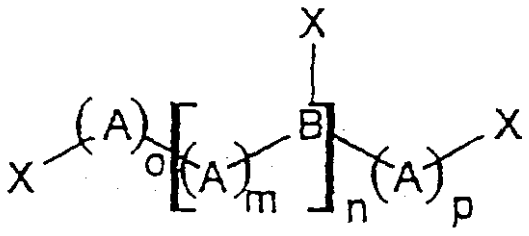
a)两个或多个影像增强的部分(“IEMs”)；

b)两个或多个靶标结合部分(“TBMs”)；

c)连接 TBM 和 IEM 的构架；和

d)将 IEMs 连接于构架的任选的连接基。

11．一种具有如下结构(IX)的多位点造影剂：



(IX)

X=L-IEM、TBM 或 IEM

其中 m 是 1-10 以内的整数；

n 是 2-10 以内的整数；

o 是 0-1；

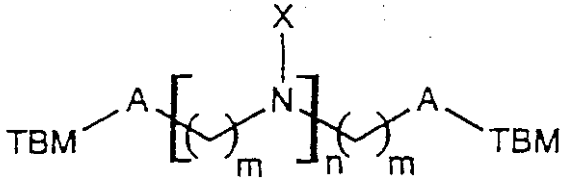
p 是 0-1；

各 A 分别选自基团 O、CH_2、C=O、C=NH、NH、NR 或 CHR；和

B 选自基团 CH 和 N；和

R 是 C_1-C_{10} 直链或支链烷基、C_2-C_{10} 直链或支链链烯基或炔基，且其中至多 4 个碳原子任选地被卤素、O、N 或 S 取代。

12．一种具有如下结构(V)的多位点造影剂：



(V)

X=L-IEM、TBM 或 IEM

其特征在于，所述的各 m 是 1-8 以内的整数；

各 A 分别选自基团 O、CH_2、C=O、C=NH、NH、NR 或 CHR；和

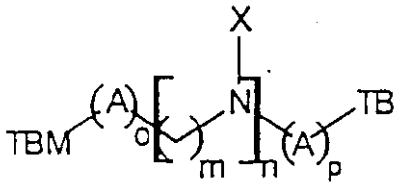
R 是 C_1-C_{10} 直链或支链烷基、C_2-C_{10} 直链或支链链烯基或炔基，且其中至多 4 个碳原子任选地被卤素、O、N 或 S 取代；和

n 是 2-10 以内的整数。

13．如权利要求 12 所述的造影剂，其特征在于，所述的 A 是 C=O。

14．如权利要求 12 所述的造影剂，其特征在于，所述的 A 是 O。

15．一种具有如下结构(VI)的多位点造影剂：



(VI)

X=L-IEM、TBM 或 IEM

其特征在于，所述的各 m 是 1-10 的整数(包含 1 和 10)；

n 是 2-10 以内的整数；

o 是 0-1；

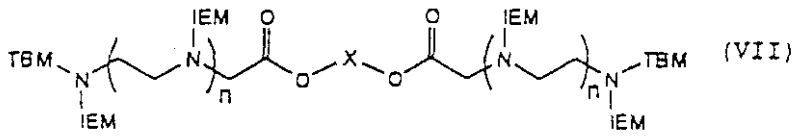
p 是 0-1；

各 A 分别选自基团 O、CH_2、C=O、C=NH、NH、NR 或 CHR；和

B 选自 CH 和 N；

R 是 C_1-C_{10} 直链或支链烷基、C_2-C_{10} 直链或支链链烯基或炔基，且其中至多 4 个碳原子任选地被卤素、O、N 或 S 取代。

16. 一种具有如下结构(VII)的多位点造影剂：



17. 如权利要求 11-16 任一所述的多位点造影剂，其特征在于，所述的 TBM 包括取代的芳基。

18. 如权利要求 17 所述的多位点造影剂，其特征在于，所述的 IEM 选自以下基团：二亚乙基三胺五乙酸(DTPA)和其衍生物；1,4,7-三氮杂环壬烷；1,4,7,10-四氮杂环十二烷(cyclen)及其衍生物；1,4,7,10-四氮杂环十二烷-1,7-二(乙酸叔-丁-酯)(DO2A-t-丁-酯)；1,4,7,10-四氮杂环十二烷-1,4,7-三(乙酸，叔-丁-酯)(DO3A-t-丁-酯)；1,4,7-三(叔-丁氧基羰基)-1,4,7-四氮杂环十二烷(DO3-t-BOC)；1,4,7,10-四氮杂环十二烷-1,4,7,10-四乙酸(DOTA)及其衍生物；1,4,7,10-四氮杂环十二烷-1,4,7,10-四(亚甲基膦酸)(DOTP)。

19. 如权利要求 18 所述的造影剂，其特征在于，所述的 IEM 是二亚乙基三胺五乙酸(DTPA)。

20. 如权利要求 11-16 任一所述的造影剂，其特征在于，所述的 TBM 是肽。

21. 如权利要求 20 所述的造影剂，其特征在于，所述的 TBM 是血纤蛋白结合肽。

22. 一种下式的造影剂

$$(TBM)_q\text{-}S\text{-}[L_m\text{-}IEM_n]_p$$

其特征在于：

TBM 是靶标结合部分，S 是构架，L 是连接基，IEM 是影像增强的部分，和

q、m、n 和 p 分别都是整数，其中：

q 是 2-6 以内的整数，

各 m 为 0 或 1，

各 n 分别是 2-4 以内的整数，

p 是 2-4 以内的整数，而且其中：

各 TBM 通过两个原子间的化学键共价连接于 S，

如果存在，各 L 通过两个原子间的化学键共价连接于 IEM，

如果存在，各 L 通过两个原子间的化学键共价连接于 S，

至少两个 TBM 连接于构架上不同的原子上。

23. 一种用于光学或 MR 成像的造影剂，其特征在于，含有：

a)两个或多个影像增强的部分(“IEMs”)；

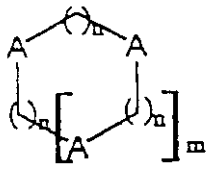
b)两个或多个靶标结合部分(“TBMs”)；

c)连接 TBM 和 IEM 的构架；和

d)将 IEMs 连接于构架的任选的连接基。

其特征在于，

其中所述的构架包括式(VIII)：



(VIII)

且 m 和 n 各是整数，其中：

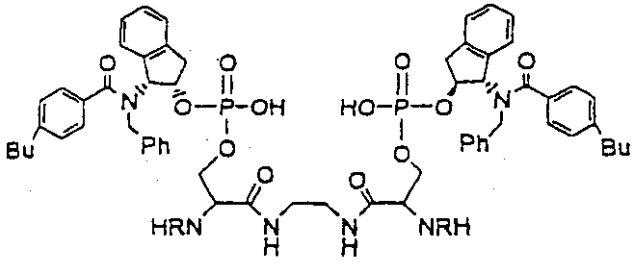
m 是 0-3 以内的整数，

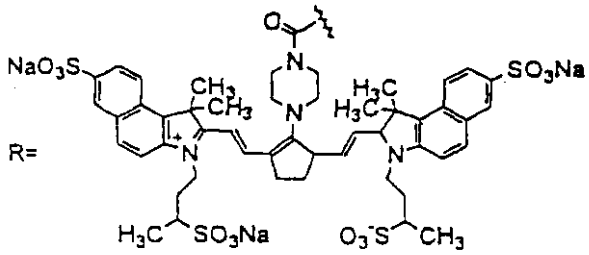
n 是 0-2 以内的整数，

各 A 是分别选自如下基团 NH、NR、O、S、CH_2、C=O、C=NH、C=NR 和 CHR，其中：

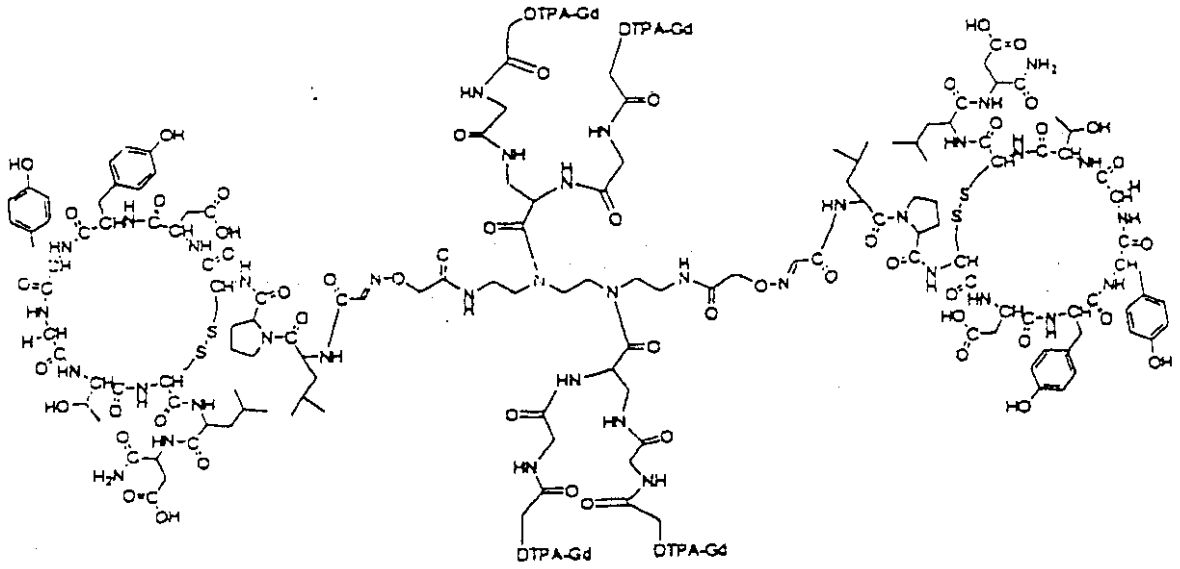
R 是 C_1-C_{10} 直链或支链烷基、C_2-C_{10} 直链或支链链烯基或炔基，且其中至多 4 个碳原子任选地被卤素、O、N、或 S 取代。

24. 化合物 M8-24

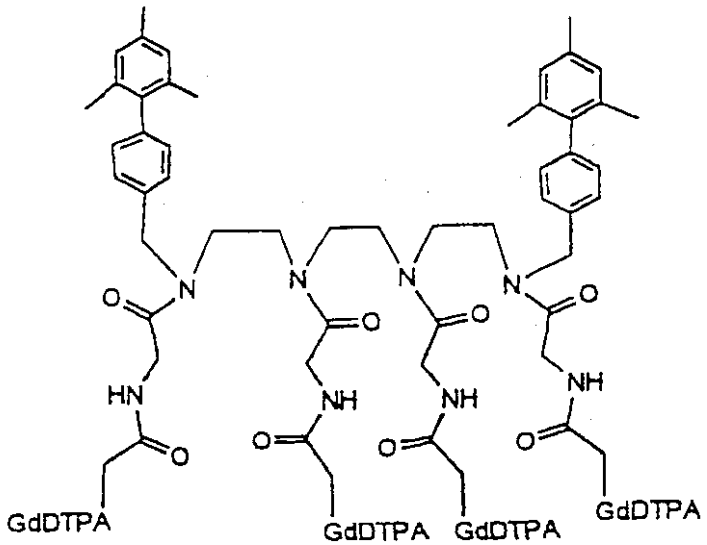




25. 化合物 M8-11



26. 化合物 M8-08



说　　明　　书

通过多位点结合的寻靶多体造影剂

发明领域

本发明涉及用于诊断成像的造影剂。具体说，本发明涉及新型多体化合物，其在结合时表现出改善的与生理相关的靶标如蛋白质的亲和力和惊人地改善的的弛豫率(relaxivity)特性。这种化合物包括：

a)两个或多个影像增强的部分(“IEMs”)；

b)两个或多个靶标结合的部分(“TBMs”)，提供体内定位和多体刚性化；

c)用于上述部分连接的构架(“骨架”)；

d)将 IEMs 连接于构架的任选的连接基(“连接基”)。

本发明还涉及包含这些化合物的药物组合物，和在成像过程中用这些化合物和组合物于反差增强的用途。

发明背景

诊断成像方法如磁共振成像(MRI)、X-射线、核放射性药物成像、紫外线-可见光-红外线光成像和超声，已多年用于医学诊断。人们用添加的造影剂来改善或增加成像的分辨率或提供特定的诊断信息。在一些情况中如用超声波进行成像，造影剂的引入还是最近的事。

为了有效，造影剂必须与成像方法中所用的电磁辐射的波长相干涉，改变组织的物理特性来得到改变的信号，或在放射性药物的情况中自身提供放射源。MRI 和光学成像方法是独特的成像技术，因为它们产生对化学环境敏感的复杂信号。虽然无论这些试剂在血浆中是游离的、或是结合于蛋白质或其它靶标、或被捕获于骨骼中，从 X-射线或放射性核素试剂得到的信号都是保持相同的，但一些 MRI 和光学成像的造影剂在不同的生理环境中有不同的信号特性。一种光学染料在结合时可表现出其吸光度、反射率、荧光、磷光、化学发光、散射或其它光学特性的变化。重要的是，造影剂必须充分灵敏且存在的浓度足够高，从而能够观察到信号的变化。

通过增加 IEM 的数量来改善对比度的尝试

寻靶试剂应送递有意义的浓度的成像部分到靶标，从而在成像过程中能观察到信号的充分改善。获得充足的灵敏度是一重要的问题，尤其对 MRI 而言，其需要浓度范围为 10-1000 微摩尔(μM)的影像增强的部分来产生足够的信号。如果要寻找的靶标以低浓度存在时，对寻靶试剂而言这一问题又进一步复杂化。例如，为了将以低于μM 浓度存在的生物受体靶标成像，在靶标部位需要更大地提高信号来提供充足的成像对比度。业已通过用(1)药物送递载体，提供高局部浓度的造影剂，(2)单个造影剂中含有多个 IEM[参见，Martin V.V.,等人 Bioconjug.Chem.,6:第 616-23 页(1995)；Shukla,R.等人.,Mag.Reson.Med.,35:第 928-931(1996)；Ranganathan,R.S.,等人，Invest.Radiol.,33:第 779-797 页(1998)]，或(3)具有改善的信号增强特性的特定结构的 IEM，来达到增加对比度的目的。理想的寻靶造影剂应有效地将 IEM 和改善的信号增强特性结合在一起。

为了将大量影像增强的部分结合到造影剂中，已将高浓度低分子量的造影剂装在合适的药物送递载体中，如聚合载体或脂质体[Bulte J.W.,等人，J.Magn.Reson.Imaging,9:第 329-335 页(1999)]。不幸的是，这些材料很难指向靶标。

为了增加影像增强部分的数量，研究者已经创造了例如与多个 IEM 相缔合的聚合物、树枝状聚合物(dendrimer)、和有机化合物。大量 IEM 如用于 MRI 的 Gd(III)螯合物可以共价连接于聚合物上[Schunmann-Giapieri,G.等人，J.Invest.Rad.,26:第 969 -974 页(1991)；Corot,C.等人 Acta Rad.,38:S412 第 91-99 页(1997)]和树枝状聚合物上[Jacques,V.,等人，J.Alloys Cmpd.,249:第 173-177 页(1997)；Margerum,L.D.,等人，J.Alloys Compd.,249:第 185-190(1997)；Toth,E.,等人 Chem.Eur.J.2:第 1607 -1615 页(1996)]。聚合试剂通常包括分子量分布很宽并很复杂的品种的混合物。这些异质的特性对该试剂的性能有不良影响，且使表征变得困难。另外，在合成上很难选择性地将 TBM 和多种 IEM 一起引入。因此，需要一种良好确定的同质分子用作能对靶标提供充足成像增强作用的造影剂。

理论上树枝状聚合物(如“星芒树枝状聚合物”或“瀑布状树枝状聚合物)提供了一种单高分子量的能让许多 IEM 共价连接的品种[Fischer,M.等人，

Angew,Chem.,Int. Ed.Eng.,38/7:第 884-905 页(1999)； Weiner,E.C.等人，Mag.Reson.Med.,31:第 1-8 页(1994)]。然而，与聚合物试剂一样，树枝状聚合物存在明显的合成上的问题，尤其当选择性地引入组织特异性寻靶基团时。

已合成了带有多个影像增强部分的有机分子。本文将这种类型的 MRI 造影剂称为“多体螯合物”或“多体”，通常含有 2-12 个 IEM[Shukla, R.等人，Mag.Reson. MEd.,35:第 928-931 页(1996)；Shukla, R.B.,等人，Acta Radiol.412:第 121-123 页(1997)；Ranganathan, R.S.,等人，Invest.Radiol.,33:第 779-797 页(1998)]。多体螯合物的优点包括：(1)它们是均质的分子，因此与聚合物和树枝状聚合物不同，它们具有单一的大小和结构，(2)它们易合成和纯化，和(3)容易结合入寻靶基团。不幸的是，多体螯合物改善 MRI 信号强度的能力令人失望地低。这是因为由于 IEM 数量的增加，与增强信号相关的质子弛豫速率的增强降低了。因此，需要一种增加 IEM 数量时弛豫率不减少的造影剂来完成对靶的更高的信号增强。

通过减少造影剂的转动来改善对比度的尝试

已尝试了通过限制转动运动来增加非-寻靶多体 MRI 造影剂的弛豫。限制转动运动的尝试着重于(1)减少分子的柔韧性或(2)通过与靶标结合限制转动运动。

例如，已合成了带有连接多个 Gd(III)螯合物的刚性构架的非-寻靶试剂[Shukla,R.等人，Mag.Reson.Med.35:第 928-931 页(1996)；Shukla,R.B.,等人 Acta Radiol.412:第 121-123 页(1997)；Ranganathan,R.S.,等人，Invest.Radiol.,33:第 779-797 页(1998)；Jacques,V.等人，J.Allovs Cmpd.,249:第 173-177 页(1997)]。然而，这些结构存在一些缺陷。首先，对于含有两个以上螯合物的试剂，每个 Gd(III)离子所达到的弛豫性比在单个螯合物(如 MS-325)中所观察到的要小。因此，局部螯合物的运动还可进一步减少。第二，这些试剂不是定向的。更重要的是，即使它们是定向的，刚性多体构架也将大大增加不需要的背景信号，因为无论造影剂是否结合于靶标上，都将明显增加信号。因此，需要一种仅在结合于靶标时才增强信号的造影剂。

在非共价靶标信号时，信号 IEM 的转动运动可以有效地被限制，从而将靶

标-结合形态的弛豫率增加 5-10 倍之多[美国专利 No.4,880,008]。这种弛豫率的增加与在共价连接于靶标的 IEM 中所观察到的一样良好或更好[Schmiedl,U.,Ogan,M.,Paajanen,H.,Martti,M., Crooke,L.E.,Brito,A.C.和 Brash, R.C. Invest.Radio.(1987)22:第 665-71 页]。利用这种作用的试剂的例子是肝蛋白-靶向造影剂 Gd-EOB DTPA(Runge V.M.Crit. Rev.Diagn.Imaging 38:第 207-30 页(1997)]和 Gd-BOPTA[Kirchin M.A.,等人，Invest.Radiol.,33:第 798-809 页(1998)]或白蛋白-靶向试剂 MS-325[Lauffer,R.B.,等人，Radiology,207:第 529-538 页(1998)]和 MP2269[Hofman Mark B.M.等人，Acadademic Radiology, 5(suppl1):S206-S209(1998)]。据报道，作为与血清白蛋白的非共价结合的结果，对于 MS325($47mM^{-1}s^{-1}$)弛豫率增加了约 7 倍[Lauffer,R.B.等人，Radiology,207:第 529-538(1998)]。

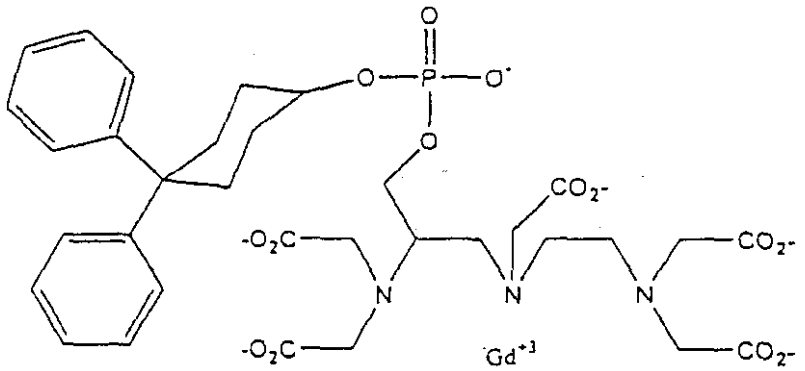


图 1：MRI 造影剂 MS-325 的化学结构

与白蛋白结合时，具有图 1 化学结构的单体造影剂 MS-325 表现出信号增强的增加。当结合时，复合物以比未结合时慢的速率翻滚。然而奇怪的是，将多个 IEM 加到靶向造影剂(如 MS-325)不能进一步提高对比度，因为在多体结构中单个钆中心的弛豫率降低了。例如，带有 4 个 Gd(III)离子的白蛋白靶向多体与含有一个 Gd(III)的 MS-325($47mM^{-1}s^{-1}$)相比，每 Gd（III)的分子弛豫率仅为 9-13($mM^{-1}s^{-1}$)[Martin V.V.,等人，Bioconjug.Chem.,6:第 616-23 页(1995)]。因此，靶向多体螯合物的每个 Gd(III)的弛豫率通常比类似的靶向单个螯合物中所观察到的要小得多。

原理

表 1 表明，仅仅增加 IEM 的数量不足以改善总弛豫率，因为尽管存在含两

个苯环的靶向结合基团，随着 IEM 数量的增加每个 IEM 的弛豫率减少了。为了理解表 1，重要的是确定寻靶-结合 MRI 造影剂能达到其可能最大弛豫率的程度。特定造影剂的这种最大弛豫率约等于结合于靶标(如人血清白蛋白(HSA))时的分子弛豫率($R1_{结合}$)。$RI_{结合}$的平均值是在标准条件组(如特定靶标或蛋白质的浓度、药物浓度、温度等)下所有结合分子的平均弛豫率的归一化的量度，它是由各分子的结合群量的加权。因此，由于 $RI_{结合}$是归一化的量，通过比较 $RI_{结合}$值可进行不同分子在结合状态时弛豫率的比较。计算的 $RI_{结合}$值的比较能提供一种方便的方法来比较化合物而不考虑它们对靶标的亲和力。

平均($RI_{结合}$)的计算需要测定游离螯合物的弛豫率($R1_{游离}$)和弛豫率的观察值($R1_{obs}$)和试剂与通常含有 4.5%靶标(如 HSA)的靶标溶液的结合百分比。$R1_{obs}$是 $R1_{游离}$和 $R1_{结合}$的摩尔分数(x)的加权平均值：

$$R1_{obs} = x_{游离}R1_{游离} + \sum_i x_i R1_{结合,i}$$

其中 $x_{游离} + \sum_i x_i = 1$

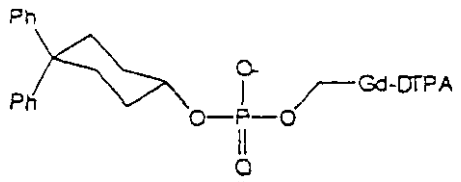
和 $\sum_i x_i = x_{结合}$

因此：

$$RI_{结合} = \frac{R1_{obs} - x_{游离}R1_{游离}}{x_{结合}}$$

表 1 示出一系列白蛋白-定靶造影剂的化学结构和结合弛豫率。在该系列的化合物中，将带单个 IEM 的化合物(即 MS-325)与一系列含有多个 IEM 的多体比较，但在所有化合物中存在相同的二苯基环己基白蛋白 TBM 和亚甲基磷酸基团。

表 1 一系列白蛋白靶向造影剂的化学结构和结合弛豫率。二苯基环己基靶向结合部分(TBM)保持恒定。

化合物#	化学结构	每个 Gd(III)的平均 $R1_{结合}$(20MHz)	总 $R1_{结合}$ (20MHz)
MS-325		47	47

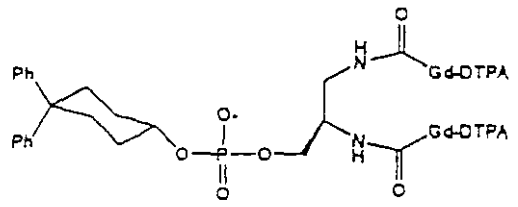
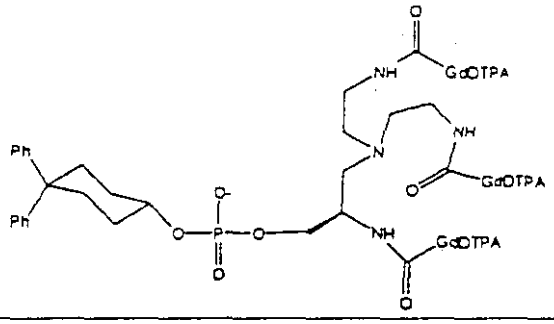
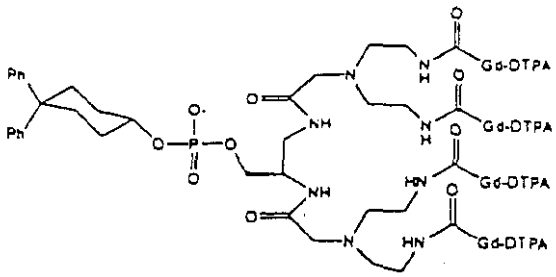
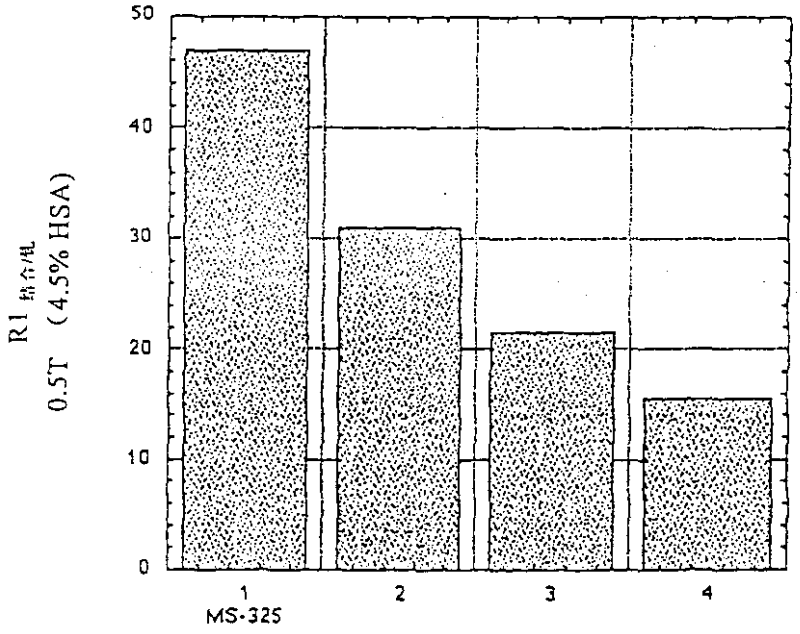
M8-01		38. 1	76. 2
M8-02		23. 6	70. 7
M8-03		14. 9	59. 6

图 2 是表 1 所示的相同数据的图解。每个 IEM 的平均 $RI_{结合}$对 IEM 的数量（在此为 Gd（III)螯便物)在 20MHz 作图。

图 2 含有单个二苯基环己基蛋白质结合基团的一系列多体造影剂的结合弛豫率性(每个钆)图。



在表 1 和图 2 的分子中，钆螯合物(IEM)、亚甲基磷酸基团和二苯基环己基基团(TBM)的结构保持不变。表 1 和图 2 中的数据显示随着螯合的顺磁金属离子数量的增加，每个金属离子的弛豫率就降低。虽然 Gd(IIII)螯合物的数量从 1(MS-325)到 4 变化，IEM 的数量增加了 4 倍，但总弛豫率仅增加了 50%左右。这种总弛豫率的有限增加是每个 Gd(III)离子弛豫率降低的结果。请注意，尽管造影剂是结合的，但每个 Gd(III)的平均 $RI_{结合}$从 $47mM^{-1}s^{-1}$ 下降至 $14.9mM^{-1}s^{-1}$。这种降低是由于螯合物的局部运动，其随着 IEM 数量的增加也惊人地增加(尽管在单个 TBM 中有多个芳环)。

显然，螯合部分数量的增加也增加分子的转动自由度，至少在钆螯合物的位点附近。由于大小的增加超过了每个多体分子两个螯合的钆离子，弛豫率的降低特别明显。例如，对 M8-03 而言，每个钆的总弛豫率仅约为 $15mM^{-1}s^{-1}$，将近是 MS-325 中所观察到的三分之一。因此化合物 M8-03 的总弛豫率仅为 $60mM^{-1}s$-1，是 MS-325 的 1.3 倍(虽然存在 4 倍数量的 IEM)。显然，弛豫率的这种有限增加与开发体内 MR 成像试剂的所增加的合成复杂性和成本不一致。因此，简单地将多个成像增强部分与单靶向结合部分的组合不能产生弛豫率相当的增加。所以，存在对合成多体 MRI 造影剂的需求，其中虽然增加了 IEM 的数量但却能保持各个螯合物的弛豫率。

总而言之，虽然靶标-结合的造影剂的固定化在增加单个螯合物(如 MS-325)的弛豫率上是明显有效的，但对多体螯合物而言是无效的。因此，为了增加各螯合物位点的弛豫率，需要降低分子总转动相关时间从而降低各螯合物位点的局部螯合物运动。还需要一种机制来有效地固定靶标-结合的多体造影剂，从而能在成像时更有效地强化信号。

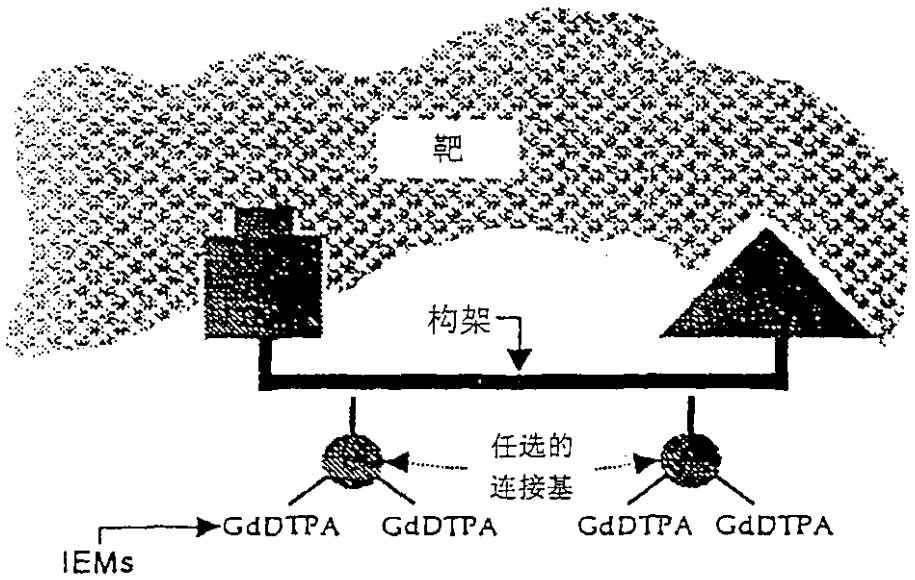
需要一种方法来改善特定靶标的信号对比度。这个问题已通过一些方法来处理(a)增加 IEM 的数量或(2)减少分子的柔性。增加 IEM 的数量已证明是不成功的，因为造影剂的大小和结构不均匀，造成合成困难、难以靶向或不能随着 IEM 数量的增加而成比例地增加对比度。降低柔性也是不成功的，因为当未结合时刚性造影剂造成很大的背景。通过单 TBM 将多体与靶标结合不足以显著降低柔性和增加弛豫率。因此，需要通过增加 IEM 的数量而不同时降低分子的弛豫率(仅当结合于靶时)来改善在特定靶标的对比度。

发明概述

本发明提供极大改善体内造影剂效力的机制。如果多体造影剂在未结合状态是柔性的(导致低弛豫率和弱信号)和在结合状态柔软较低(导致高弛豫率和强信号)，则就可能极大改善靶标上对噪声(信号)的对比度。即，重要的是使结合状态的多体造影剂比未结合状态的更刚性化，因为这使未结合状态的背景最小化，而在结合状态保持高弛豫率。这些试剂通过在两个或多个分开位点的非共价相互作用，结合于蛋白质或其它特异性靶标。通过将两个或多个 TBM(各对靶标的一个或多个位点有亲和力)掺入到试剂中实现多位点结合。

具体说，本发明涉及用造影剂与多个 IEM(“多体”)和靶标的“多位点”、非共价相互作用，来同时发生 1)引发与靶标的结合(从而产生特异性)，2)将若干 IEM 锚定于靶标和 3)从而使此多 IEM 结构刚性化。本发明的关键是造影剂在结合状态比未结合状态的柔性小。造影剂与靶标的结合通过增加金属离子-成像原子载体的总转动相关时间，即通过限制转动运动。增加了金属螯合物 IEM 的弛豫率和信号强度。多位点结合减少了在总体上和发生螯合物运动的局部位点的结合状态的多螯合物结构的柔性，使弛豫率进一步提高，未结合状态分子的柔性提供了优于上述带有连接螯合物刚性结构的多体 MRI 试剂(结合和未结合状态的刚度没有差异)的优点。[Ranganathan,R.S.,等人，Invest.Radiol.,33:第779-797(1998)]。图 3 示意地显示了多体螯合物的多位点结合理论。

图 3 显示通过多位点相互作用结合于靶的多体造影剂例子的关键成分的图



1)多个分开的 TBM 促进与靶标的结合(从而提供特异性和改善的亲和力)。

TBM 可以是相同的或不同的。

2)当结合到靶标时，TBM 在构架的几个位置上锚定多体结构，从而使多体螯合物结构刚性化。

3)当结合时弛豫率提高到比在溶液中游离时更大的程度，从而改善在特异性靶标上的成像对比度。

除了对成像对比度的改善外，本发明还提供合成优势。由于通过多位点连接于靶标的结合时发生固定化和刚性化，由合成产生的刚性化的化学构架(如稠合环或复杂大环)不是必须的。因此，对化学构架结构的限制较少。其它优点包括：

a)与单一相互作用相比，多位点结合增加了蛋白质的亲和力并提供更高的靶标特异性[Kramer,R.H.和 Karpen,J.W.,Nature,395:第 710-713(1998)；Clackson,T.等人，Proc,Natl.Acad.Sci.,95:第 10437-10442(1998)；Rao,J.等人，Science,280:第 708-711 页(1998)；Mann,D.A.,等人，J.Am.Chem.Soc.,120;第 10，575-10，582 页(1998)；Spevak,W.等人，J.Med.Chem.,39：第 1018-1020 页(1996)；Lee,R.T.等人，Arch.Biochem.Biophys.,299:第 129-136 页(1992)]。

b)多位点结合减低了试剂从靶标分离的速度。增加了试剂保持结合的时间，从而增加诊断利用时间。

c)多位点结合减少了多螯合物结构的柔性，降低了局部螯合物的运动，并由此改善了各金属中心的弛豫率。与游离分子相比，仅在结合时发生结合状态造影剂的刚性产生更强的成像对比度。与结合形式相比，游离分子诱导相对小的信号变化；因此在由结合形式引起的信号与游离分子产生的信号之间可以得到令人惊讶地大的差异。无论是结合还是非结合状态都是刚性的造影剂没有这种特性。

d)这种信号强度的结合依赖性变化也适用于其它成像模态，在此信号强度的变化可能伴随着这种结合，如光学成像。当结合时信号强度可以增加或减少。有时候，显示信号的减少与分子的刚性相关[Rimer,O.，Chauver,M.,Dell'Amico,M.,Noat, G.,和 Bourdeaux,M.Eur.J.Biochem.(1995)228:第 55-59 页]。其它时候，结合时信号增加[Sudlow G.,Birket D.J.,和 Wade D.N.Mol.Pharmacol.12:第 1052-61 页(1976)；Sudlow G.,Birkett D.J.,和 Wade D.N.Mol.Pharmacol.11:第

824-32 页(1975)；Kane C.D.和 Bernlohr, D.A.Anal.Biochem.233:第 197-204 页；Lakowica,J.R.“荧光光谱法的原理”Plenum Press,New York,NY 第 211-213 页(1983)]。多位点结合与仅有单个 TBM 的光学造影剂相比或是大大减少了信号强度(从而大大增加了信号对比度)或是大大增加了强度。在这情况下，作为造影剂结合的结果，靶标位点信号强度的变化将导致改善信号对比度。

本发明的多位点结合造影剂包含 IEM、直接或通过任选的连接基连接 IEM 的构架和至少两个分开的 TBM。TBM 可以是相同的或不同的。有时候，构架实际上可以包含 IEM 或 IEM 的一部分，例如一些螯合部分也可作为构架或构架的一部分。

这些多体/多位点结合化合物是独特的，因为 IEM 的局部运动受限，并且通过至少两个 TBM 在多体结构中多个分开位点上与靶标的非共价结合大大提高了结合弛豫率。这些相互作用让多体以“假-环状”或“拉链-状”方式结合于靶标蛋白质。这种类型的结合惊人地减少了整个多体(包括 TBM、构架和各 IEM)的柔性。因此，对包括螯合物的 IEM 而言，减少了局部螯合物运动，而且因为各 IEM 的松率豫增加，由多体可观察到显著增加的 MRI 信号。这增加了本发明造影剂与已有技术的那些通过单个 TBM 结合而不是“假-环状”或“拉链状”结合于靶标位点的造影剂的差异。用多体/多位点结合结构进一步提高对比度，因为它们在非结合状态产生相对低的信号。

本发明可广泛应用于所有靶向 MRI 和光学的应用，包括靶向生物结构(如血清白蛋白)和其它诊断相关靶标(如血凝块)的图像增强剂，尤其是存在用于多体/多位点结合造影剂的多个结合位点的那些应用。这些结合位点不必相同，只要足够临近能让造影剂上的 TBM 同时结合即可。

发明详述

为了更全面的理解本发明，提供以下具体描述。

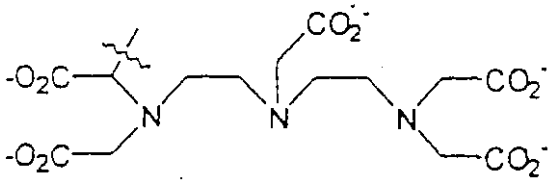
在本文中没有明显定义的常用化学缩写可以在“美国化学协会文体指南(The American Chemical Society Style Guide)”第二版；American Chemical Society,Washington, D.C.(1997)或有机化学杂志中找到；“Guidelines to Authors”（作者指南）(2000 年 5 月修订)，

也可在以下网址中查到

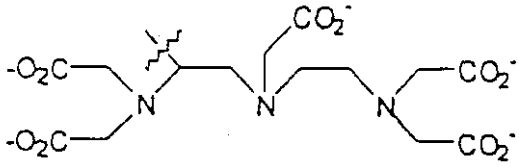
Http://pubs.acs.org/instruct/joceah.pdf.

本文所引用的出版物全部纳入作为参考。

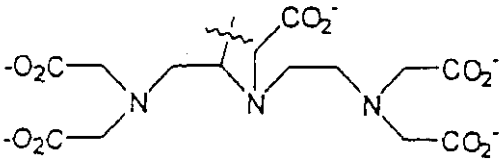
在本申请中，DTPA 指呈任一式(I)-(IV)的结构：



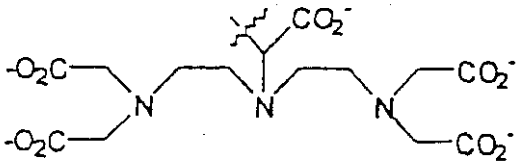
(I)



(II)



(III)



(IV)

本文的术语“特异性亲和力”指造影剂被某一特定生物成分比被其它成分的吸收、保持或结合程度显著更高的能力。将具有这种特性的造影剂被称为对“靶标”成分是“靶向”的。缺少这种特性的造影剂被称为“非特异性”试剂。

本文所用的术语“弛豫率”指每毫摩尔(mM)浓度的顺磁离子的 $1/T_1$ 或 $1/T_2$ 量的增加，其中 T_1 是水质子或其它成像或光谱核(包括在除水以外的分子中所发现的质子)的纵向或自旋-晶格的弛豫时间，T_2 是它们的横向或自旋-自旋弛豫时间。弛豫率单位为 $mM^{-1}s^{-1}$。

本文所用的术语“开放配位位点”指通常被水或溶剂分子占据的金属螯合物上的位点。

本文所用的术语“形成常数”指描述形成该化合物的反应的平衡常数。

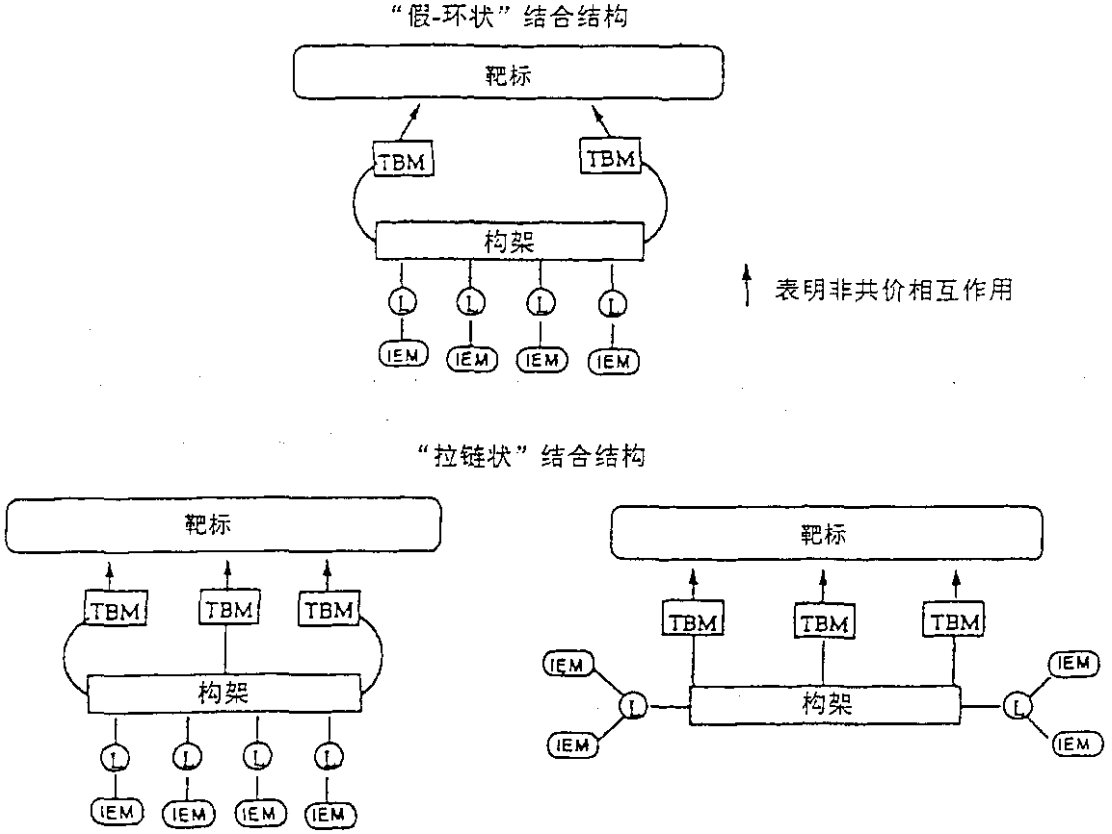
本文所用的术语“多体”指含有两个或多个 IEM 的造影剂或其亚基。

本文所用的术语“多位点”指连接于造影剂“构架”(如下定义)的共价 TBM 的两个或多个位点。

本文所用的术语“多位点结合”指两个或多个不同的 TBM 与靶标的非共价相互作用。这些非共价相互作用彼此无关，且尤其可能是疏水、亲水、偶极-偶极、pi-堆积或路易斯酸-碱的相互作用。

本文所用的术语“假-环状结构”指通过 2 个 TBM 在 2 个不同位点通过非共价相互作用连合于靶标的造影剂。

图 4 显示多位点结合结构的典型例子



本文所用的术语“拉链状结构”指通过 3 个或多个 TBM 在 3 个或多个不同位点上通过非共价相互作用结合于靶标的造影剂(参见图 4)。

本发明涉及新型的随着结合靶标时柔性降低而提供诊断成像对比度的化合物。这些化合物包含：

a)两个或多个影像增强部分(“IEMs”)；

b)两个或多个靶标结合的部分(“TBMs”)，提供用于体内定位和多体刚性化；

c)用于上述部分连接的构架(“骨架”)；

d)将 IEMs 连接于构架的任选的连接基(“连接基”)。

用于本发明的诊断成像技术的用途包括(但不限制于)MRI 和紫外线-可见光-红外线成像。

影像增强部分(“IEMs”)

在本发明中，IEM 可以是用于在成像过程中提供信号或改善对比度的化学制剂或物质。另外，可任选地将 IEM 或 IEM 的一部分用作构架或构架的一部分，且可以具有次要的靶向功能。

IEM 可包含有机分子、金属离子或螯合物。[Bonnemain,B.J.Drug Target.,6:第 167-74 页(1998)；Swanson,D.P.,等人，“医学造影用药：不透放射性的造影剂、放射性药物、磁共振成像和超声波增强剂”,McGraw Hill, Inc.,(1990);Johnson, I. Histochem. J.30:第 123-40 页(1998)]中叙述了 IEM 的许多例子。

一种特别有用的 IEM 是带有一个或多个环状或非环状有机螯合剂(与有一个或多个金属离子络合)的生理相容性的金属螯合物。用于光学成像的优选的金属离子包括那些原子序数为 13，21-31、39-42、44-50 或 57-83 的离子。用于 MRI 的选优的金属离子包括那些原子序数为 21-29，42，44 或 57-83，更优选的是原子序数为 21-29、42、44、或 57-83 的金属离子的顺磁形式。特别优选的顺磁金属离子选自：Gd(III)、Fe(III)、Mn(II 和 III)、Cr(III)、Cu(II)、Dy(III)、Tb(III 和 IV)、Ho(III)、Er(III)、Pr(III)和 Eu(II 和 III)。最优选的是 Gd(III)。

如果 IEM 是金属螯合物，在成像剂通过人体时(包括结合于靶组织时)，它必须不能有任何明显程度的解离。游离金属离子的显著释放将导致毒性，这通常是不能接受的。

通常，金属螯合物的毒性程度与排泄前体内它的解离程度相关。毒性通常随着游离金属离子量的增加而增加，因此为了防止毒性浓度的游离金属离子，高形成常数的是优选的。具体说，优选的是形成常数至少为 $10^{15}M^{-1}$，或至少为 $10^{16}M^{-1}$，或至少为 $10^{17}M^{-1}$、或至少为 $10^{19}M^{-1}$、或至少为 $10^{20}M^{-1}$，或至少为 $10^{22}M^{-1}$，或至少为 $10^{24}M^{-1}$ 或更高。如果金属离子解离的动力学很慢，则具有较低的形成常数(即至少为 $10^{10}M^{-1}$)的复合物也是足够的。

毒性也是复合物中开放配位位点数的函数。通常，水配位位点较少就降低

了螯合剂释放顺磁金属的趋势。因此，较佳地复合物含有 2 个、1 个或 0 个开放配位位点。通常 2 个以上开放位点的存在，通过在体内金属离子的释放将不可接受地增加毒性。

弛豫率 R_1 和 R_2(分别定义为每 mM 金属离子 $1/T_1$ 或 $1/T_2$ 的增加)衡量造影剂提高光谱或成像核弛豫速率的能力。弛豫率单位为 $mM^{-1}s^{-1}$。对临床 MRI、水质子 MRI 最常用的形式而言，当结合于螯合配位体的顺磁离子依旧带有一个或多个水交换的开放配位位点时，弛豫率较高(R.B.Lauffer,Chemical Reviews,87:第 901-927 页(1987)]。然而，这必须与金属螯合物的稳定性平衡，后者通常随着开放配位位点数增加而降低，毒性如上述。因此，较佳地除了铁螯合物 Fe(II)或 Fe(III)以外，复合物仅含有一个或两个开放配位位点。对 Gd(III)而言，一个或两个开放配位位点是最佳的。

为了有效地提高 MRI 影像，复合物必须能够增加光谱核的弛豫速率、或弛豫率、1/T1(纵向或自旋-晶格)和/或 1/T2(横向或自旋-自旋)。较佳地，光谱核是水质子，但其它常见的光谱核包括 ^{31}P、^{13}C、^{23}Na、^{19}F 和除水以外的分子中的质子。光谱核可构成 IEM、TBM、其它生物分子或注射的生物标记物。

对 MRI 造影剂而言，一般通过造影剂顺磁离子和受弛豫的核(如水分子中的氢原子)之间的偶极-偶极相互作用来增加弛豫率($1/T_1$ 或 $1/T_2$)。已知,如果由两个偶极子所确定的矢量(即由顺磁离子和水的氢质子所确定的矢量)的旋转速率降低，则会改善这种偶极相互作用的效率(即驰豫率)(R.B.Lauffer,Chemical Review,87:第 901-927 页(1987))。

矢量旋转扩散一个弧度的所需的时间称为“旋转相关时间”；旋转相关时间的倒数为“旋转速率”。通常，大分子比小分子在溶液中旋转得较慢。一种增加弛豫率的方法是在小分子造影剂和大分子间形成非共价加合物。通过形成加合物，偶极矢量的旋转相关时间将与大分子的相同。然而小分子可能依旧围绕一个或多个轴旋转(所谓“局部螯合物动动”)。偶极矢量的转动相关时间是此局部螯合物运动和大分子复合物的整体运动的函数。大分子非共价加合物的旋转相关时间将小于或等于该大分子本身的；局部螯合物运动越少，非共价加合物的旋转相关时间越接近该大分子的。

对 MRI 造影剂而言，通过造影剂金属离子和受弛豫的核(如水的氢原子)间

的偶极-偶极相互作用增加弛豫率。除了通过偶极-偶极相互反应增加组织的 $1/T_1$ 或 $1/T_2$ 值以外，MRI 剂还影响增加它们用途和临床价值的两个其它磁性特性：

1)含有高磁化率的金属螯合物(尤其是 Dy、Gd、Tb 或 Ho 的螯合物)的 IEM 通过产生微观的磁化率梯度能改变组织的 MRI 信号强度(A.Villringer 等人，Magn.Reson.Med.,6:第 164-174 页(1988))。在此应用中螯合物无需具有开放配位位点。

2)含有金属(如 Tm 或 Dy)螯合物的 IEM 可用于改变光谱核的共振频率。较佳地该光谱核是水质子，但其它常见的光谱核包括 ^{31}P、^{13}C、^{23}Na、^{19}F 和在水以外其它分子中发现的质子。光谱核可以构成造影剂、靶标或水。在此按所用的核和策略，可以使用 1-3 个开放配位位点。

在本发明的各个实施例中可以使用各种螯合的配位体作为 IEM 部分。这些螯合的配位体包括(但不限制于)二亚乙基三胺五乙酸(DTPA)的衍生物和其衍生物；1,4,7-三氮杂环壬烷；1,4,7,10-四氮杂环十二烷(环烯)及其衍生物；1,4,7,10-四氮杂环十二烷-1,7-二(乙酸，叔-丁-酯)(DO2A-t-丁-酯)；1,4,7,10-四氮杂环十二烷-1,4,7-三(乙酸，叔-丁-酯)(DO3A-t-丁-酯)；1,4,7-三(叔-丁氧基羰基)-1,4,7-四氮杂环十二烷(DO3-t-BOC)；1,4,7,10-四氮杂环十二烷-1,4,7,10-四乙酸(DOTA)及其衍生物；1,4,7,10-四氮杂环十二烷-1,4,7,10-四(亚甲基膦酸)(DOTP)；1,4,7,10-四氮杂环十二烷-1,4,7,10-a,a',a'',a'''-四(甲基乙酸)(DOTMA)；乙二胺-四-乙酸(EDTA)；1,4,8,11-四氮杂环十四烷-1,4,8,11-四乙酸(TETA)；亚乙基二-(2-羟基-苯基甘氨酸)(EHPG)及其衍生物，包括 5-氯-EHPG、5-Br-EHPG、5-Me-EHGP、5-t-丁-EHPG 和 5-sec-丁-EHPG；苯并二亚乙基三胺五乙酸(苯并-DTPA)及其衍生物，包括二苯并-DTPA、苯基-DTPA、二苯基-DTPA、苄基-DTPA 和二苄基-DTPA；二-(2-羟基苄基)-亚乙基-二胺二乙酸(HBED)及其衍生物；大环类化合物包括至少 3 个碳原子(较佳地为 6 个)和至少 2 个杂原子(O 和/或 N)，所述的大环化合物可以包括一个环或两个或三个在杂原子环元素处连接的环，如苯并-DOTA、二苯并-DOTA 和苯并-NOTA，其中 NOTA 为 1,4-三氮杂环壬烷-N,N',N''-三乙酸，苯并-TETA、苯并-DOTMA、苯并-TETMA，其中 TETMA 是 1,4,8,11-四氮杂环十四烷-1,4,8,11-(甲基四乙酸)；1,3-丙二胺四乙酸(PDTA)

和三亚乙基四胺六乙酸(TTHA)的衍生物；和 1,5,10-N,N',N"-三(2,3-二羟基苯甲酰基)氨基甲苯(MECAM)的衍生物。

许多适用于 MRI 剂的合适的螯合剂是本领域已知的。也可将这些金属螯合物用于其它形式的生物成像(如光成像)。事实上，最近描述了一系列荧光可检测的 MRI 造影剂(Huber,M.M.等人，Bioconjugate Chem.,9:第 242-249 页(1998)]。对 MRI 成像而言，优选的 IEM 包括顺磁的钆螯合物如二乙三胺五乙酸合钆(GdDTPA)、四胺 1,4,7,10-四氮杂环十二烷-N,N',N",N"'-四乙酸合钆(GdDOTA)和 1,4,7,10-四氮杂环十二烷-1,4,7-三乙酸合钆(GdDO3A)。本领域已知在一些应用中其它金属可以取代 Gd(III)。优选应用于本发明的螯合剂是 DTPA。WO98/18496、WO86/06605、WO91/03200、WO95/28179、WO96/23526、WO97/36619、PCT/US98/01473、PCT/US98/20182 和美国专利 No.4,899,755 描述了用本发明生成的典型螯合剂和螯合基团的例子，本文将这些参考文献全部纳入。

靶标结合部分(“TBM”)

在本发明中，造影剂的另一部分是两个或多个靶标结合部分(TBM)。化合物的 TBM(1)使造影剂结合于蛋白质或其它靶标上和(2)降低结合状态分子的柔性。这将使待成像位点上造影剂浓度增加，并增加结合状态的弛豫率。对血管血池成像而言，血清白蛋白是优选的靶标。其它蛋白质靶包括(但不限制于)α酸性糖蛋白、纤维蛋白原、血纤蛋白和胶原。对成像血栓而言，血纤蛋白是优选的靶标。因此可以选择 TBM 来实现对合适蛋白质的特异性和高度结合亲和力。由于血清中存在高浓度的 HSA(约 0.6mM)，且 HSA 以相对高的亲和力结合大范围的分子，因此它是血池造影剂的优选靶标血浆蛋白质。HSA 是心血管成像特别优选的靶标。

许多亲脂性或两亲的 TBM 能有效地结合各种靶标，包括人血清白蛋白(HSA)。它们包括(但不限制于)芳族和饱和的或不饱和的具有 4-200 个碳原子的脂族基团，其中各碳原子任选地被氧、氮、卤素、硫或其它能共价结合碳的原子取代或替代。为了以高度特异性结合其它蛋白质靶标，通常需要特定的寻靶基团。可用现代技术如组合化学、高通量筛选、噬菌体展示、指数式富集法配

体进化(SELEX)和其它已描述过的方法如美国专利 No.5,475,096、5,595;877 和 5,270,163[参见 Gold 等人，Ann.Rev.of Biochem.,64:第 763-797 页(1995)]中所述的(本文将它们全部纳入作为参考)，可以鉴定具有足够高亲和力和特异性的寻靶基团。

可以用各种平衡结合方法评估 TBM 与靶标(如 HSA 或血纤蛋白)结合的程度。例如，可以用超滤测定与 HSA 的结合。在一典型的用超滤对结合的测定中，将寻靶基团与 4.5%重量/体积 HSA(在 pH 7.4 缓冲液中)混合。将样品加样于可购得的配备有 30KDa 分子量的截断值滤器的离心装置(Millipore Ultrafree MC Low Binding Regenerated Cellulose 30KDa mol.wt.截断值目录#UFC3LTK00)中，该滤器可渗透寻靶基团但不能渗透 HSA。用此截断滤器以 2000×g 离心 20 分钟可以过滤小部分体积(5-10%)样品，测定滤液样品中未结合的寻靶基团的浓度。

为了测定与血纤蛋白的结合，可在微量滴定板的孔中形成血纤蛋白块，并将其与寻靶基团接触。培养足以建立平衡的时间后，吸取上清液(难溶的血纤蛋白在孔的底部保持凝胶块状结合)。然后测定上清液中未结合的寻靶基团的浓度。

在这两种方法中，测定结合的寻靶基团的浓度，作为初始存在的总寻靶基团的浓度与结合试验后未结合的寻靶基团浓度的差。结合分数是将结合的寻靶基团浓度除以总寻靶基团的浓度。较佳地至少 10%，更佳地至少 50%，优选至少 80%，最优选至少 90%造影剂在药物和靶标的生理相关浓度结合于所需的靶标。较佳地至少 92%，更佳地至少 94%，优选 96%或更多的造影剂结合于超滤或微量滴定板方法中的靶标上。

关于含有血纤蛋白结合肽的寻靶部分的详细描述参见题为“血纤蛋白的寻靶部分”的美国临时专利申请 No.60/146,425；DYX-010.0Prv 和同日(1999 年 7 月 29 日)申请的美国临时专利申请 No.60/146,414(本申请享有的优先权)；且本文将它们全部纳入作为参考。

依据靶标的特性和结合的特殊需要 TBM 可以多种多样。有用的 TBM 的例子包括药物、亲脂性或两亲有机分子、卟啉、受体配体、类固醇、脂类、激素、肽、寡核苷酸(DNA、RNA 及它们化学修饰的形式)、碳水化合物或其它已知能

以充分高亲和力结合于待成像的特异性组织中的一种或多种成分的生物分子或物质。在一些实施例中，相对其它 TBM 某种 TBM 对靶标的亲和力比较高，此时将这种较高亲和力的 TMB 称为“主要”TBM。因此，将亲和力比主要 TBM 低的其它 TBM 称为“次要”TBM。

特别优选的 TBM 是那些可逆结合于血浆、间质空隙(细胞间的流体)或细胞内空隙中蛋白质的 TBM。虽然可以使用许多生物分子或物质来结合特异性的靶标，但那些结合蛋白质的是最有用的。

次要 TBM 可以与主要 TBM 相同或不同。次要 TBM 的数目可以是一个到十个或更多。所需次要 TBM 的确切数目取决于 TBM 对靶标的特异性和 TBM 对靶标的亲和力。由次要 TBM 提供的附加结合相互作用应足以束缚复合物并减少各螯合位点的旋转相关时间。由此弛豫率的增加为影像提供了足够的对比度增加。次要 TBM 与靶标的结合相互作用和亲和力可以比主要寻靶 TBM 所需的低，因为主要 TBM 与靶标最初的结合提供了寻靶识别所需的特异性。在一些情况中，靶标可以包括二体结合位点(在此情况下优选两个相同的 TBM)。

本申请所述的造影剂的靶标是广泛和多样的。该靶标可以是任何体区室、细胞、器官或组织或其成分。优选的靶标是那些与诊断和治疗相关的靶标，即那些与疾病症状相关的靶标。特别优选的靶标是那些与体液相关的靶，尤其是那些与血液、血浆、淋巴液和中枢神经系统的液体相关的。其它优选的靶标是以高浓度存在的蛋白质或是对特定配体具有大量结合位点的蛋白质。多个结合位点提供了与一种或多种次要 TBM 的接触。这种靶蛋白质中包括酶和糖蛋白。

连接 IEM 和 TBM 的构架

本发明为多体造影剂提供的第三个成分称为化学构架或“骨架”结构来结合 IEM 和 TBM，如图 5 所示。这种构架是在两个或多个 TBM 之间的化学构架，对此在不同位点直接或通过连接基结合两个或多个 IEM，形成多体/多位点结合化合物。这种新型的含有这些构架结构的化合物通过 TBM 在几个（至少 2 个）分开的位点上对靶标的非共价结合限制螯合物的局部运动。这种多体造影剂以“假-环状”或“拉链状”形式结合于靶标，在两个或多个位点形成相互作用。这种类型的结合在整个多体造影剂结构中(包括结合基团、构架和各 IEM)造成

刚性。

通常，构架结构可以是差异很大的有机分子，包括 1 到 10 个重复的单体亚单元。构架可以是开链或环状的。TBM 共价结合于该结构内部或末端。任任何情况下，都必须有至少两个分开的 TBM 来将该分子结合于靶。TBM 可以是相同的(均质的)或不同的(异质的)。异质的 TBM 对靶标可以表现出不同的或类似的结合亲和力。

开链和环状构架还为 IEM 的连接提供了支持结构。IEM 的数目可以在 2-12 间不等。IEM 可以与 TBM 交替，IEM 可以结合于线性构架结构的内部或该结构的末端(可任选通过连接基)，但必须至少连接于该结构上的 2 个不同位点上。IEM 可以是相同的(均质的)或不同的(异质的)。异质的 IEM 可表现出不同的或类似的产生对比度的能力。

就开链构架而言，可以由标准方法合成许多类型的结构。特别优选的是在整个结构中含有规则重复的杂原子的构架。杂原子通常使 IEM 或 TBM 更易连接。特别优选的例子是低聚亚烷基胺构架。这些低聚亚烷基胺构架可以是支链的或线状的。即，这种构架可以是含有规则地分隔开的 IEM 和 TBM 的线状构架，也可以是分支的化学构架，使得 IEM 和/或 TBM 的基团自构架上的单个位点发散开。这种构架可以杂原子如氧(醇)或氮(胺)封端。开链构架特别优选的例子是以醇或胺为末端的支链或线状构架。

图 5 显示了多体的多位点结合的一个例子，其中清晰地勾画出了构架部分、IEM、TBM 和(任选)的连接基。图 6 显示了另一带有支链连接基的多体的例子。同样清晰地勾画出了构架部分、IEM、TBM 和(任选的)连接基。在图 6 中，两个 TBM 是结合于聚胺构架的肽，但这些内容并不限制本发明的范围。

图 5 带有线性连接基的多位点结合多体的例子

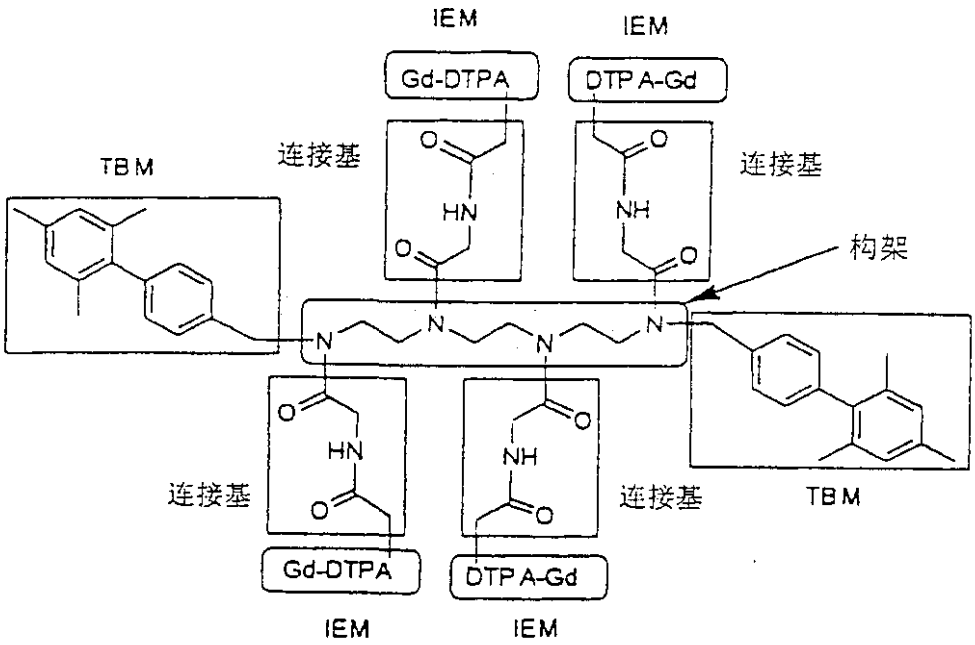
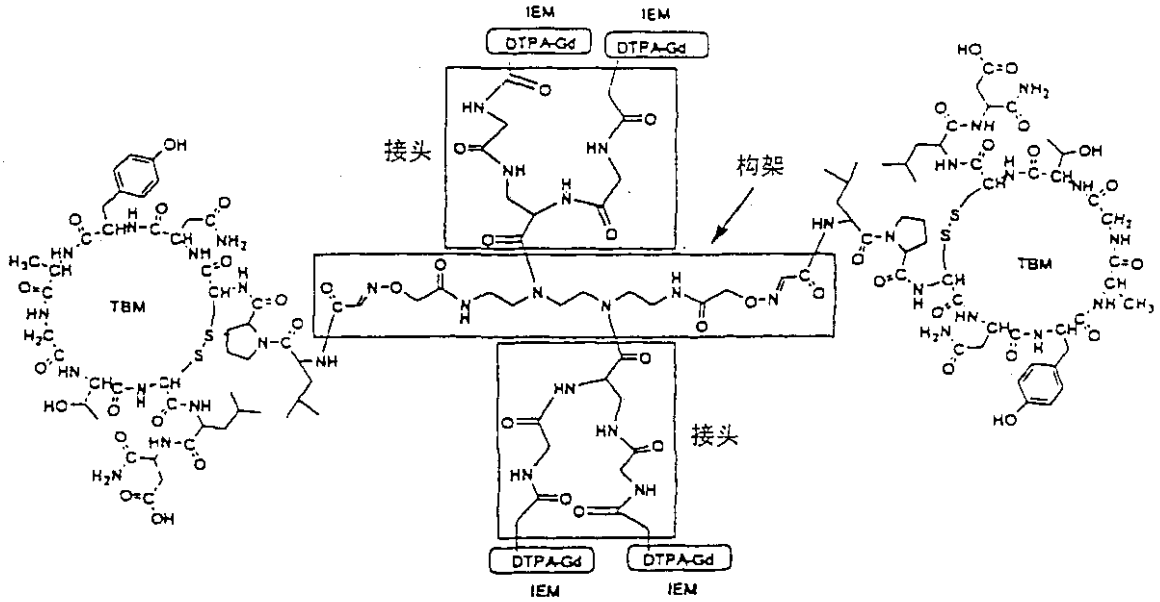
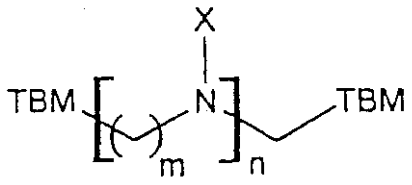


图 6 带有支链连接基的多位点结合多体的例子



式(V)显示了含有重复胺亚结构的线性构架的通式，



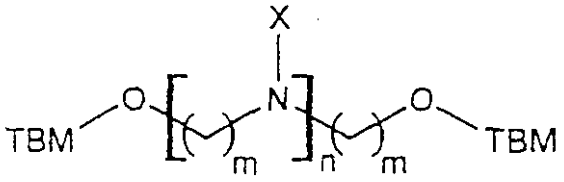
(V)

X＝L-IEM、TBM 或 IEM

其中，m 可以是 1-10 的整数，n 可以为 2-10 的整数。较佳地，m 为 1-2、2-4、4-6、6-8、或 8-10。较佳地，n 为 2-4、4-6、6-8 或 8-10。

式(VI)显示了含有重复胺亚结构和末端氧的可以形成醚-键连的 TBM 的线

状构架的结构通式，



(VI)

X＝L-IEM、TBM 或 IEM

其中 m 可以是 1-10，n 可以为 2-10。较佳地，m 为 1-2、2-4、4-6、6-8、或 8-10。较佳地，n 为 2-4、4-6、6-8、或 8-10。

构架结构并非限制于含有胺的重复亚结构，也不限于含有末端氧或氮的结构。所述的构架可以含有任何可以连接 IEM(任选通过连接基)和 TBM 的重复亚结构。组成该构架的碳原子可以任选地被杂原子(选自氧、氮、硫、磷和卤素)取代或置换。构架还可以含有取代基如短链烃(1-10 个碳原子)。这些烃侧链也可任选地被选自氧、氮、硫、磷和卤素的杂原子取代或置换。因此所述的构架可以含有许多常规的有机基团，例如磷酸二酯、氨基甲酸酯、硫酸酯和磺酰基。同样，连接于这种构架结构上的取代基也可以包括常规的有机基团，如磷酸二酯、氨基甲酸酯、硫酸酯、磺酰基和氨基酸。

图 7 显示了一些线状和支链聚胺构架的说明性例子及含有这些构架的造影剂的相应实例。图 8 提供了低聚体构架的例子和含有这些构架的造影剂的相应例子。在本发明中含有杂原子的构架是优选的例子，因为杂原子使 IEM 和 TBM 易于连接(任选通过连接基)。

图 7 线状和支链聚胺构架的例子

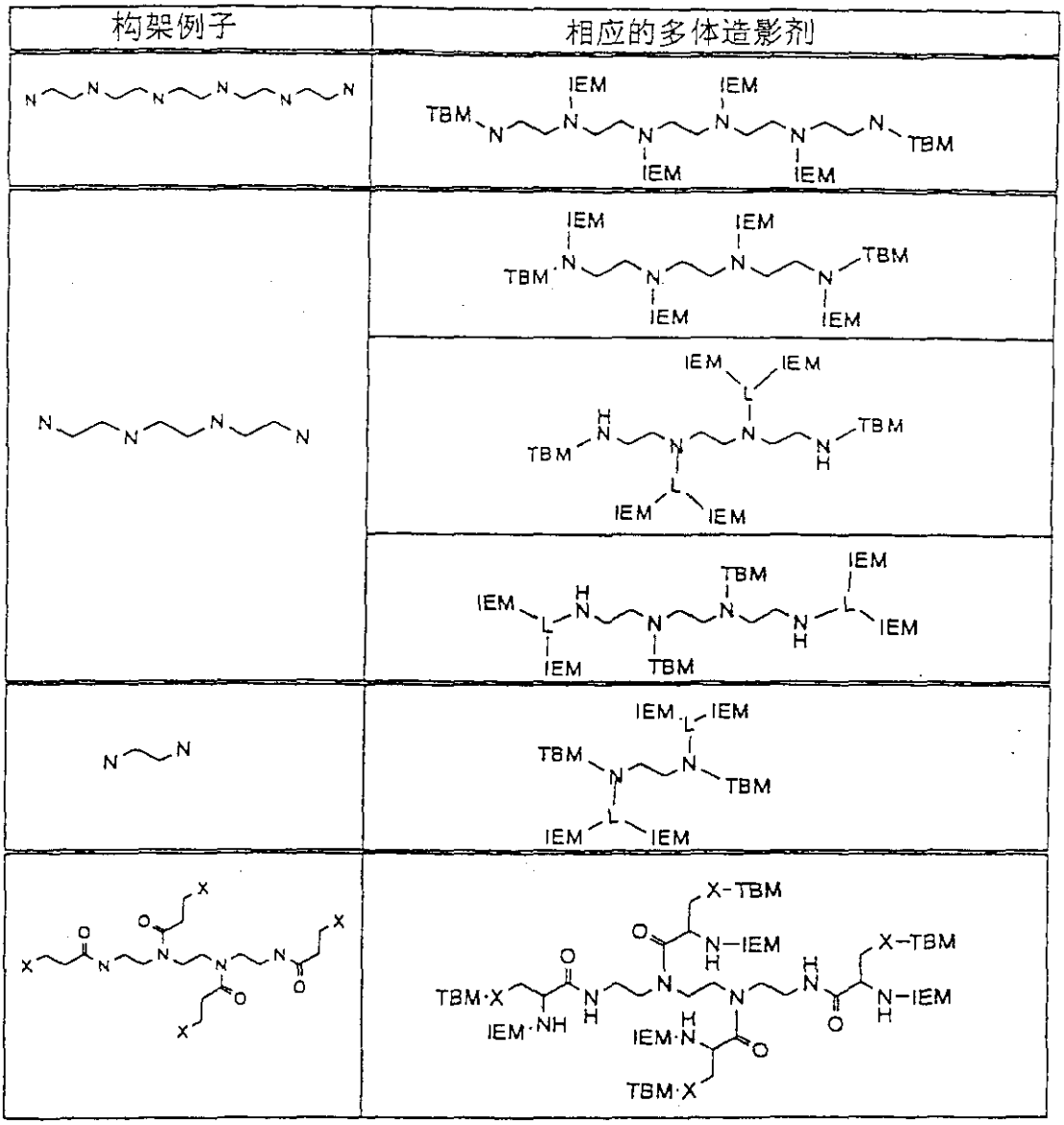
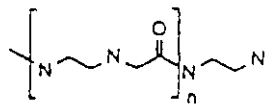
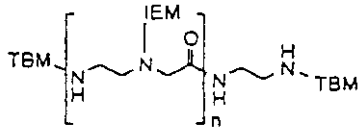
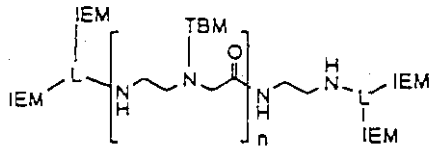
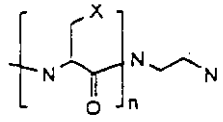
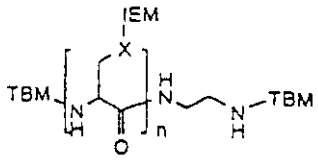
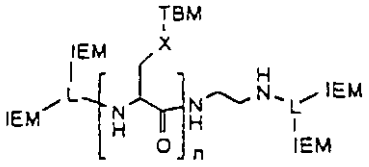
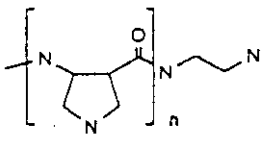
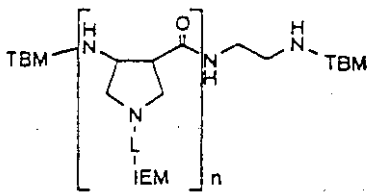
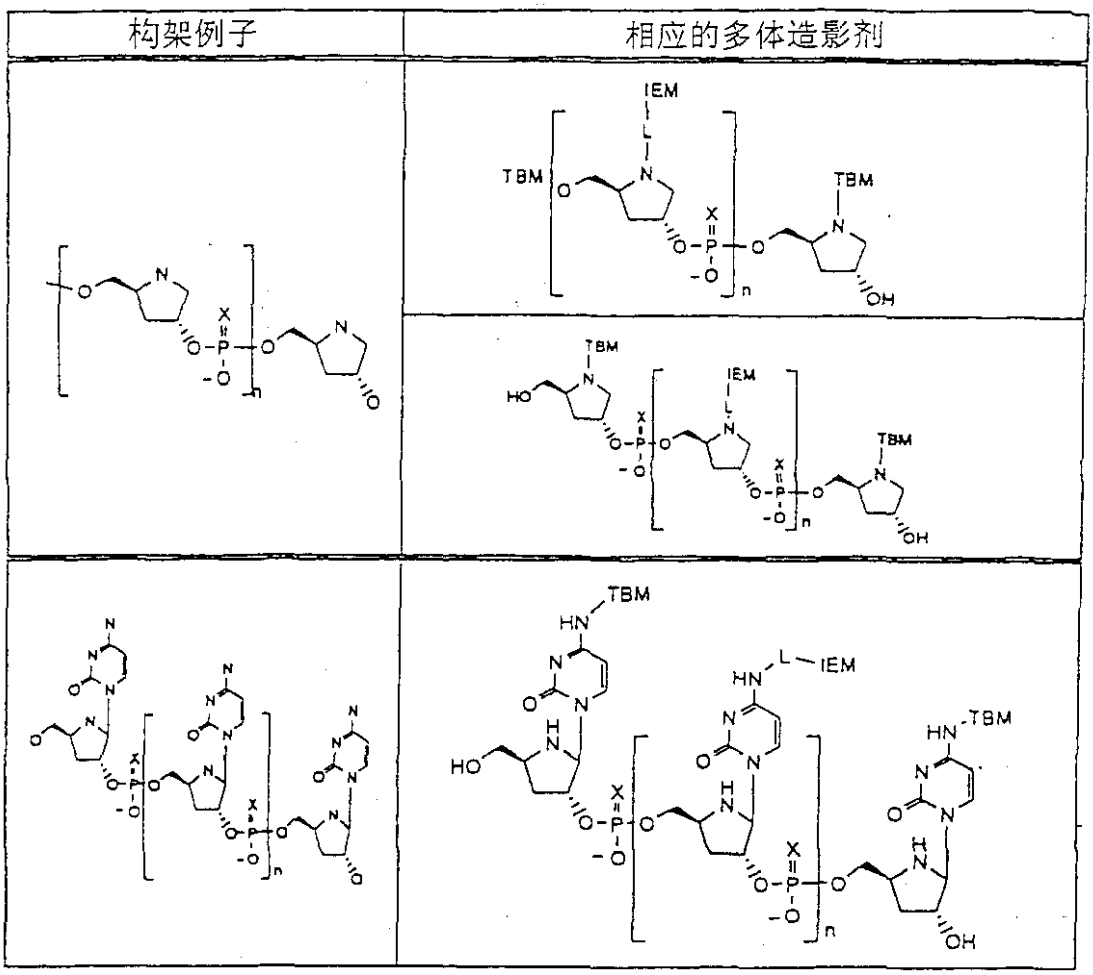


图 8 低聚构架的例子

构架例子	相应的多体造影剂
	
	
	
	
	

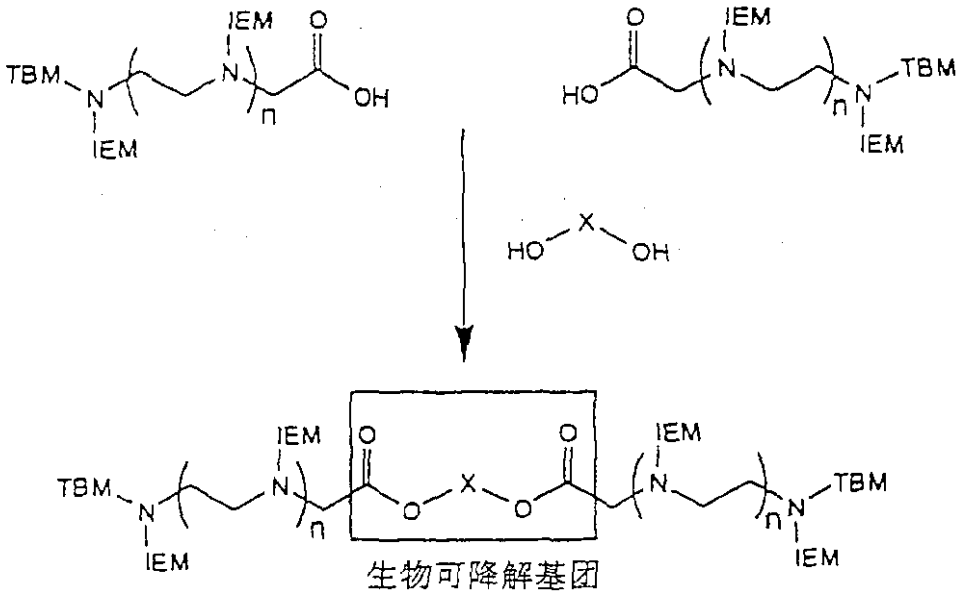
X=N、O、S　　L=连接基



X=N、O、S　　L=连接基

这种构架的其它优选了例子是在体内易降解的构架主链。生物可降解造影剂具有能被如哺乳动物体内存在的酶降解的构架。较佳地这种构架含有 1 个或多个生物可降解基团(可以特异性地被酶降解)。特别优选的生物可降解构架是能被人的酶降解的构架。因为已知存在许多酶促反应，因此生物可降解基团的确切结构多种多样。生物可降解基团特别优选的例子包括(但不限制于)羰基、酯、二酯、磷酸酯、二磷酸酯、磷酸二酯、酸酐、磺酰基、硫酸酯和氨基甲酸酯。这些生物可降解基团可以让多体造影剂迅速代谢并减少中毒的风险。图 9 显示了多体生物可降解构架的一个例子。在该例子中，该构架是在含有 2 个末端醇的分子的存在下由两个羧酸缩合形成的。这种缩合反应的特定反应条件是本领域已知的。

图 9 生物可降解构架的例子

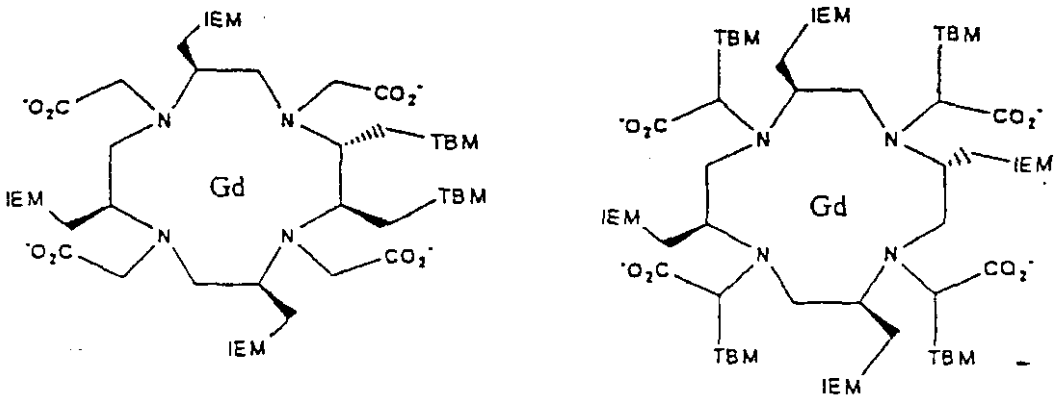


X＝连接部分

另外，当 pH 或温度变化或用超声波或光能时可以切割生物可降解基团。这些基团可参见前体药物文献。(Kratz,F.,Beyer,U.和 Schutte M.T.,Crit.Rev.Ther. Drug Carrier Syst.16:第 245-88 页(1999)；Dougherty,T.J.,Gomer,C.J.Henderson,B. W.,Jori,G.Kessel, D.,Korbelik,M.,Moan,J.,和 Peng,Q.J.Natl.Cancer Inst.90:第 889-905 页(1998)；Wang,W.,Jiang,J.,Ballard,C.E.,和 Wang, B.Curr.Pharm.Des.5:第 265-87 页(1999))。

含有螯合金属离子的环状元件的构架是另一类型本发明的优选实施例。特别优选的是含有 IEM 的构架，它螯合钆或提供构架内的以及连接于构架的分开 IEM 中的钆的螯合。图 10 显示了这种构架。

图 10 用部分钆复合物作为构架的多体的例子

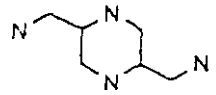
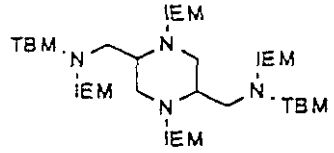
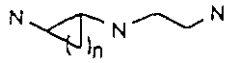
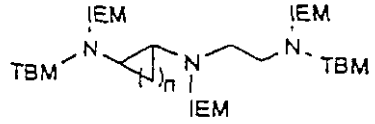
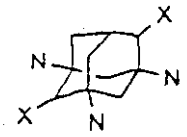
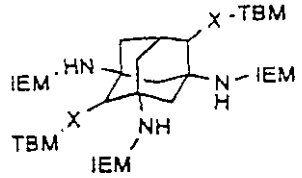
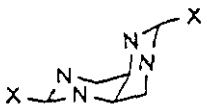
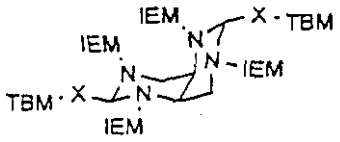
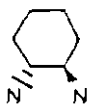
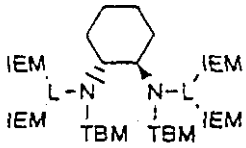
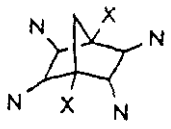
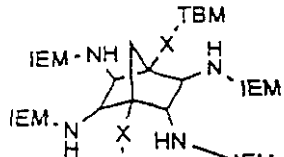
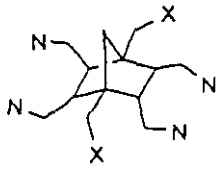
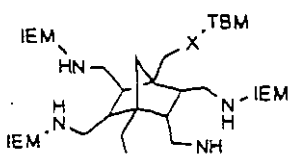


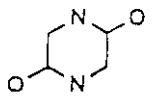
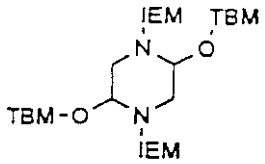
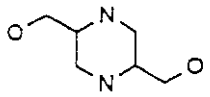
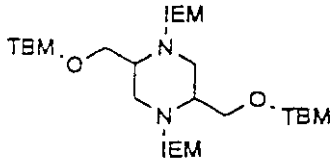
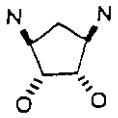
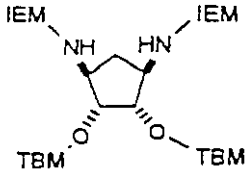
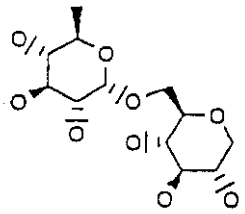
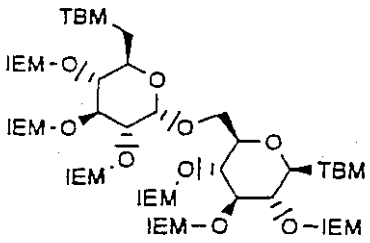
将构架分成开链类和环状类并不表示这些类型间的相互排斥。已在考虑结合有限制运动的环状元件的开链结构。这种构架的环状部分可以是同素环或杂环或多环。因此，这些环可以是高度受限的如环丙基环或可以是构象受限较少的环如环己基环。

构架的开链和环状部分都可以任选地被选自氧、氮、硫、磷和卤素的杂原子取代或置换。这些构架还可含有取代基如短链烃(1-10 个碳原子)。这些烃侧链可以任选地被选自氧、氮、硫、磷和卤素的杂原子取代或置换。所以这些构架在其开链和环状部分可以含有许多常规的有机基团。一些例子包括(但不限制于)羰基、酯、二酯、磷酸酯、磷酸二酯、酸酐、磺酰基、硫酸酯和氨基甲酸酯。

这些环还可包括常规生物环状化合物(如碳水化合物)的衍生物。环状部分的优选例子包括多环系统如萘烷的杂环衍生物。其它例子包括(但不限制于)含有 3-7 个原子的碳环，其中至多 4 个原子被选自 O、S、C(O)、S(O)、$S(O)_2$ 和 NH 的部分任选地取代。这些环任选地被甲基及其衍生物、烷基、链烯基、或炔基(含 2-100 个碳)取代，其中至多 10 个碳原子被选自氧、氮、硫、磷和卤素的杂原子取代或被选自 O、S、CO、S(O)、$S(O)_2$ 和 NH 的部分置换。就如一些构架如以氨基酸为基础的构架可能包括 TBM 一样，构架同样也可包括 IEM。IEM 可以是任何有机部分、金属离子或螯合物。优选的例子是那些带有环状 IEM 的，更优选的是为金属螯合物的环状 IEM。图 11 显示了线性和环状组合构架的优选例子及相应的含有这些构架的造影剂的例子。

图 11 包括环状部分构架的例子

构架-聚胺例子	相应的多体造影剂
	
	
	
	
	
	
	

构架-聚胺醇或醚衍生物例子	相应的多体造影剂
	
	
	
构架-碳水化合物衍生物例子	相应的多体造影剂
	

X=N、O、S、CH2

n=1-8

任选的连接基

本发明一些实施例中造影剂的特征是含有将 IEM 连接于构架的任选连接基。在本文的其它部分描述影像增强部分(IEM)和寻靶部分(TBM)。连接基部分(L)可以是任何小的亚单位，其含有 1-30 个通过单键或多键共价连接的碳原子，其中至多 10 个碳原子可以被 O、N、P、S、F、Cl、Br、H 或 I 取代。连接基具有将 IEM 连接于构架的功能。连接基的例子包括线性或分支的被官能团(如羰基、酯、酰胺、胺、脲、硫醚、芳基、磷酸酯、磺胺等)取代的链烷、链烯或炔。某些实施例中优选的连接基含有 2 个或多个官能团，其中一个连接于构架，其它连接 IEM。

官能团可以是相同的或不同的。所述官能团的例子包括(但不限制于)酮、酯、酰胺、醚、碳酸酯、磺胺、链烷、链烯、炔和氨基甲酸酯。制备连接基的

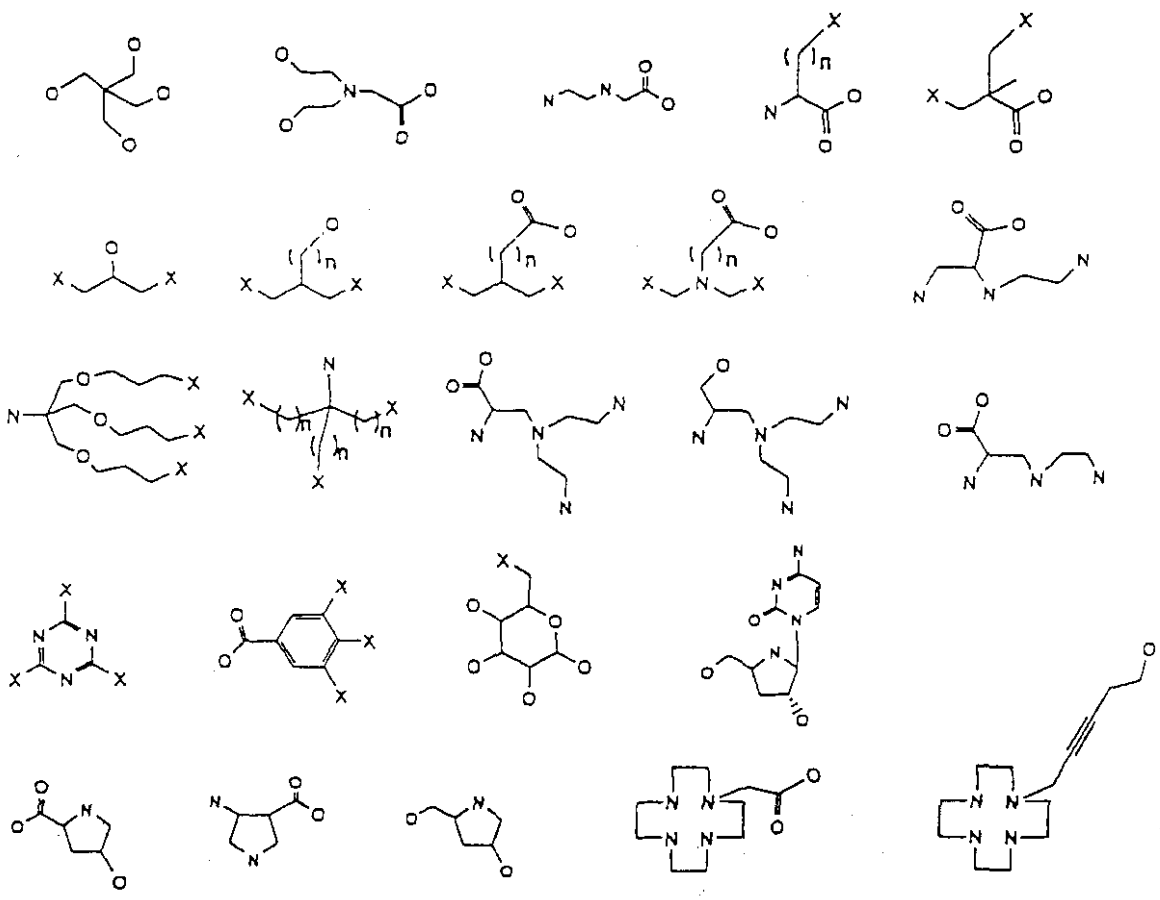
优选试剂的例子是氨基酸，尤其是甘氨酸、丙氨酸、丝氨酸、高丝氨酸、苏氨酸、酪氨酸、半胱氨酸、氨基苯丙氨酸、赖氨酸、鸟氨酸、2,4-二氨基丁酸、二氨基丙酸、羟基脯氨酸、天冬氨酸和谷氨酸；二醇，尤其是乙二醇；二卤化物，尤其是二氯乙烷、2-巯基乙醇、2-氨基乙醇、1,2-二氨基乙醇；二羧酸，特别是乙二酸、丙二酸、羟基丁二酸、丁二酸、反式丁烯二酸、戊二酸和己二酸；和其它双官能团、三官能团和多官能团的小分子。

其它连接基(并非限制)可以是脲、醛缩醇、缩酮、二酯、羰基、硫脲、砜、硫酯、酯、醚、二硫化物、内酯、亚胺、磷酰基或磷酸二酯键；取代的或未取代的饱和或不饱和烷基链；线性、分支或环状一种或不同氨基酸的氨基酸链(如血纤蛋白结合部分的 N-或 C-端的延伸)；丙二酸、丁二酸、戊二酸、己二酸和庚二酸；己酸、简单二胺和二醇。

优选连接基的分子量是明确限定的。其分子量的范围可以为从 100 以下到 1000 以上。较佳地，连接基的分子量小于 200，更佳地是小于 100。另外，理想的是用可以体内生物降解的连接基从而为本发明的造影剂提供有效的排泄途径。取决于它们在连接基上的位置，这些生物可降解的官能团包括酯、二酯、酰胺、磷酸酯、醚、醛缩醇和缩酮官能团。

通常，可以用已知的方法将金属螯合物或其它 IEM 偶联于连接基，并将连接基偶联于 TBM。参见如 WO95/28967、WO98/18496、WO98/18497。本发明考虑在任何位点连接螯合物，只要该金属螯合物保留了其紧密结合金属的能力(以使毒性最小化)。图 12 显示了一些连接基的例子，为简化省去了氢原子。

图 12 具有多个连接点连接基的例子



X=N、O、S

与多位点结合相互作用关联的对比增强

本发明在其不同实施例中改进了所有顺磁金属(如钆)靶结合的中心、多体螯合结构的平均弛豫率。表 1 和表 2 显示的例子表明多体 IEM 结构仅通过单个 TBM 对靶的结合是不足以将平均每个 Gd(III)的结合弛豫率改善到可比较的单个 IEM 结构中所观察到的水平。虽然通过单个 TBM 的结合降低了多体的总转动相关时间，但各金属(如钆)螯合物显然依旧以不受限制的形式转动。这种过量的运动降低了各金属中心的弛豫率。出人意料的是，发现即使多体包括具有两个芳环的 TBM 也同样如此。McMurry 等人的 PCT WO 96/23526 表明，用含有两个或多个芳环(以非平面取向)的血浆蛋白质结合部分(PPBM)可改善含有单个 IEM 造影剂的白蛋白结合弛豫率。如表 1 所示，用这种 TBM(二苯基环己基)能为单个 IEM(MS-325)的情况提供优良的弛豫率增强，而对其多体类似物的每个 IEM，却提供惊人低的白蛋白结合弛豫率。为了进一步改善寻靶多体的弛

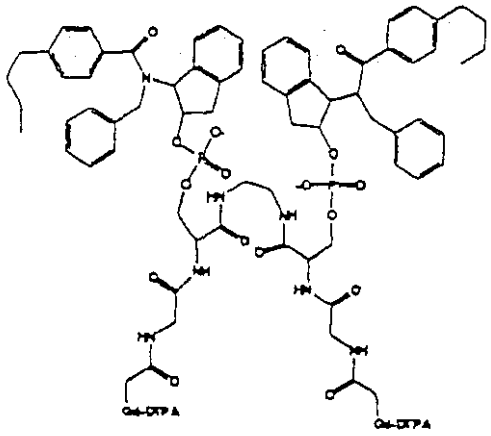
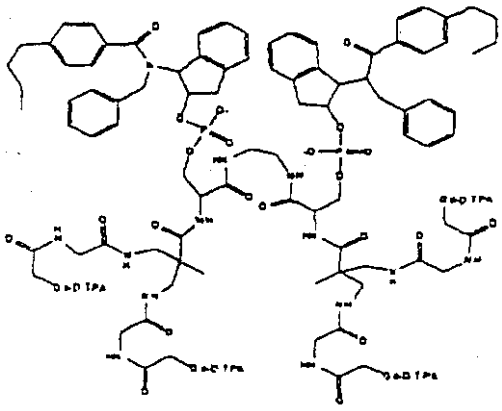
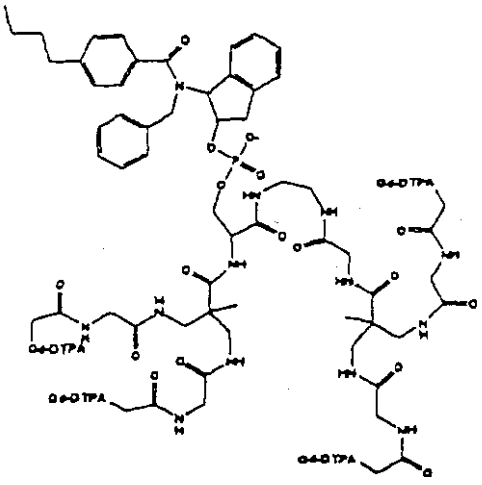
豫率，必须降低螯合螯合基团和螯合离子的局部运动。

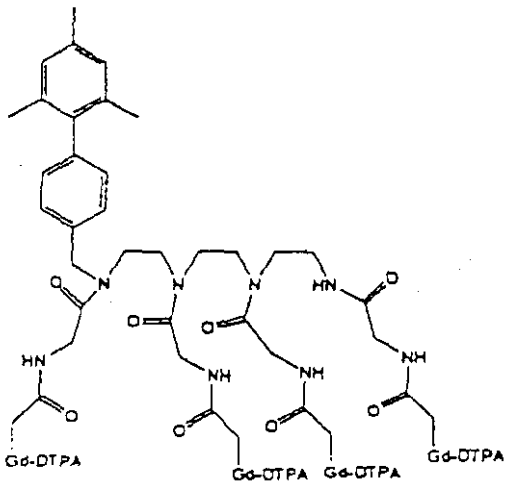
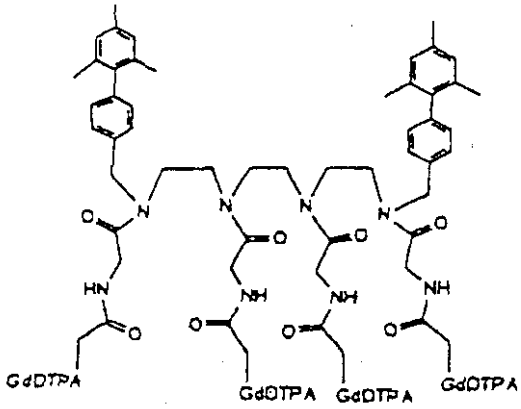
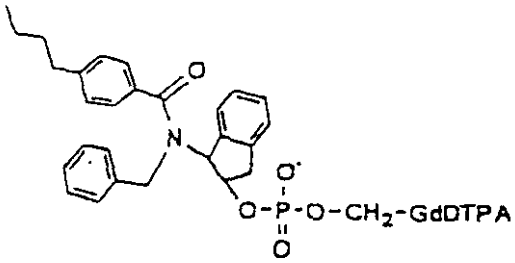
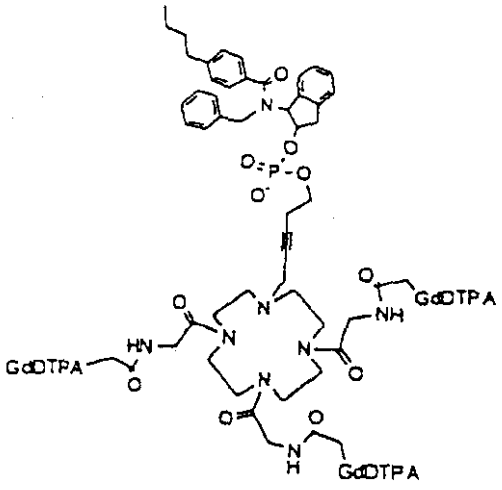
本发明描述了由多位点结合的相互作用及同时柔性的降低而表现出高弛豫率的寻靶多体。将两个或多个 TBM 引入靶(条件是结合时每个 IEM 的弛豫率没有明显减少)，发现显著改善了弛豫率，从而在许多情况中结合状态的造影剂的每 IEM 弛豫率与在单个 IEM 中所观察到的类似(提高了 7-8 倍)。弛豫率(每 IEM)的提高是由于寻靶多体结构(包括连接的 IEM)柔韧性的降低。通常结合时弛豫率增加 1.5 倍或更多。优选的图像清晰度是弛豫率增加至少 2 或 3 倍的结果。更优选的是弛豫率增加 4 倍、5 倍和 6 倍。特别优选的是，弛豫率增加 7-8 倍、9-10 倍或 10 倍以上。在 20MHz、37℃优选的弛豫率为至少 $10mM^{-1}s^{-1}$(每 IEM)，更优选的是至少 20 $mM^{-1}s^{-1}$(每 IEM)，较佳地是至少 25 $mM^{-1}s^{-1}$(每 IEM)，更佳地是至少 $30mM^{-1}s^{-1}$(每 1EM)，佳地是至少 35 $mM^{-1}s^{-1}$(每 IEM)，最佳的是至少 40 $mM^{-1}s^{-1}$(每 IEM)。较佳地，在 20MHz 和 37℃时造影剂总弛豫率大于 60 $mM^{-1}s^{-1}$。

以下数据说明由多位点结合引起的对比度增强。在表 2 所示的 3 个特异性比较中多重 TBM 的益处是显然的。在该表格中，本发明的化合物包括至少 2 个 IEM 和至少 2 个 TBM。将本发明的化合物与仅含有一个 IEM 或一个 TBM 的化合物作比较。此比较表明本发明的化合物的弛豫率(每个 IEM)增加。在该表的测定中，所用的靶标是人血清白蛋白(HSA)。首先，M8-04 和 M8-05 分子的比较表明 IEM 数量的增加而 TBM 保持恒定使总弛豫率相应增加。具体说，该比较表明可以加入 IEM 而每个 Gd(III)离子的弛豫率不会有相当的丢失，只要存在至少两个 TBM 且在该分子中间隔适当的距离。

M8-04 和 M8-05 化合物含有 2 个 TBM，各含有苯并稠合的环戊基、苯基和烷基取代的苯基。这两个 TBM 显然足以限制该分子，从而当 IEM(此时为 DTPA 部分)的数量自 2 个增加到 4 个时，各 IEM 位点上每个 Gd(III)的弛豫率保持相同(在 20MHz 时为 32 与 $32.7mM^{-1}s^{-1}$)。因此，与 IEM 数量的翻倍相同，该分子的总弛豫率也翻倍(2 个 IEM 时为 64 $mM^{-1}s^{-1}$，4 个 IEM 时为 131 $mM^{-1}s^{-1}$)。

表 2 通过多位点结合弛豫率的增加：单 TBM 对双 TBM

化合物编号#	TBM_5 编号 #	IEM_5 编号 #	化学结构	结合 %	$R1_{结合}/Gd^{+3}$ $mM^{-1}S^{-1}$(20MHz)	总 $R1_{mM}$ $mM^{-1}S^{-1}$(20MHz)
M8-04	102	2		99	32	64
M8-05	2	4		97	32.7	131
M8-06	1	4		91.3	27	108

M8-07	1	4		64-1	16	64
M8-08	2	4		93.7	44.1	176.5
M8-09	1	1		99.5	38.7	38.7
M8-10	1	3		66.1	25.7	77.1

其次，M8-06 和 M8-05 分子的比较表明，当保持 IEM 的数量恒定时 TBM 数量的增加将增加每个 IEM 的弛豫率，只要 TBM 的间距足够。在这两种分子中，TBM 具有相同的结构，且 IEM 的结构是相同的钆-螯合的 DTPA 部分。但 M8-06 化合物仅含有一个 TBM 而 M8-05 化合物含有 2 个 TBM。M8-05 化合物中的这两个 TBM 更有效地限制分子，因为每个钆的弛豫率在 20MHz 时自 27.0 $mM^{-1}s^{-1}$ 增加至 32.7 $mM^{-1}s^{-1}$。所以，TBM 数量的增加增加了每个 IEM 的弛豫率，只要 TBM 间距足够。

第三，M8-07 和 M8-08 分子的比较(见表 2)再次表明当保持 IEM 数量恒定时，间距充足的 TBM 数量的增加时每个 IEM 的弛豫率增加。在这两种分子中，TBM 含有相同的烷基取代的联苯基结构，且这两种化合物含有相同数量的 IEM，同时 IEM 的结构是相同的钆-DTPA 部分。M8-07 和 M8-08 分子的核心结构(构架)也是相同的四胺。M8-07 化合物含单个 TBM 和 4 个 IEM，而 M8-08 化合物含有 2 个 TBM 和 4 个 IEM。同样，M8-08 化合物中的 2 个 TBM 更有效地限制分子，因为存在 HSA 时每个钆的结合弛豫率在 20MHz 时自 16.0 $mM^{-1}s^{-1}$ 增加至 44.1 $mM^{-1}s^{-1}$。因此，该计算表明 TBM 数量的翻倍使该分子的总弛豫率的增加到 2 倍以上(2.75 倍，从 1 个 TBM 时的 64.0 $mM^{-1}s^{-1}$ 增加至 2 个 TBM 时的 176.5 $mM^{-1}s^{-1}$)。另一方面，M8-07 和 M8-08 化合物的自由弛豫率相当，在 20MHz 时分别为 9.2 $mM^{-1}s^{-1}$ 和 10.3 $mM^{-1}s^{-1}$。化合物 M8-07 和 M8-08 的 $R1_{结合}$ 与 $R1_{游离}$的比分别为 1.7 和 4.3，这表明 M8-08 化合物可以提供更好的靶和背景之间对比度的增强。由于 M8-08 也增加 HSA 亲和力并因此具有较高的靶特异性(与 M8-07 相比)，由这种多位点结合多体产生的对比度的增强比有一个 TBM 的多体更优越。

最后，该表还包括化合物 M8-09 和 M8-10 的数据。这些数据是用于提供含有单个 TBM 和多个 IEM 的分子的典型弛豫率的。M8-09 和 M8-10 分子的比较也表明，简单地将 IEM 添加到含有一定 TBM 的分子不能成比例地增加总弛豫率，因为 IEM 数量的 3 倍增加仅得到弛豫率的 2 倍增加。

因此，在两个分开的位点限制分子将增加分子总体的和造影剂局部螯合区域的弛豫率(即降低转动相关时间)。但是，TBM 必须分开足够的距离，这就有效减少了分子结合靶标时整个分子的柔韧性。从而使添加至 4 个 IEM 时每个

IEM 的平均 $RI_{结合}$不会显著减少。多位点结合惊人地限制了过整个多体螯合物结构的柔韧性。因此，对包括螯合物的 IEM 而言，随着多位点结合后降低了局部螯合物的运动。结果，与类似的多位点螯合化合物(仅通过一个 TBM 结合，因此对靶向的位点不是“假-环化”或“拉链状”)相比，观察到 MRI 信号惊人地增加。这些多体/多位点结合结构还进一步增加了对比度的强化，因为在未结合状态它们也产生信号，但该信号比具有刚性分子结构的造影剂所产生的弱，而且它们具有改善的与靶标的亲和力。简而言之，本发明提供了化合物、组合物和它们的使用方法，其中由于多位点结合相互作用(多个 TBM)的结果，每个 IEM 的弛豫率不会因 IEM 的添加而减少，从而改善了对比度。

用途

血池成像具有许多潜在的诊断和治疗益处。循环系统的详细成像可以为早期检测提供信息，如动脉瘤、栓塞或血栓和其它凝块、和血流受限的区域(如动脉硬化的冠状动脉中存在的)。可用高分辨率血池成像更精确诊断的其它常规循环疾病是与以下疾病相关的循环缺陷：糖尿病、心脏病、淋巴水肿、周围性血管疾病、雷诺氏现象、静脉炎；和其它对血管内部损伤、心杂音、静脉曲张；和由瓣膜紊乱和脉管炎引起的其它疾病。同样，直接针对特异性靶标的造影剂可以改善对疾病，如由嗜中性白细胞缺陷导致的中性白细胞减少症的诊断。另外，MR、光学成像和其它形式的成像比已有方法(需要手术切割和全身麻醉下插入导管)的侵害性更小。

按本文公开制备的 MRI 和光学造影剂可以与常规 MRI 和光学造影剂相同的方式使用。当对血栓成像时，可能优选 MR 方法和脉冲序列来提高血栓与背景血液和组织的对比度。这些方法包括(但不限制于)辐射血管造影序列(black blood angiography sequence)，即设法使血液变深如快速自旋回声序列(fast spin echo sequence)和血流-破坏梯度回声序列(flow-spoiled gradient echo sequence)。这些方法还包括不依赖血流的方法，其由于对比增强的血栓和血流及组织间 T_1 的差异来提高对比差异，如反向回收制备的或饱和回收制备的序列将增加血栓和背景组织间的对比度。T_2 技术的制备方法证明也是有用的。最后，用于磁化传递方法的制剂可以改善与本发明试剂的对比度。

可以按适用于静脉内给予人或动物模型系统的药物组合物常规流程的方法配制此组合物。通常，用于静脉内施用的组合物是无菌等渗缓冲水溶液配制的溶液。需要时该组合物也可含有增溶剂和局部麻醉剂(如利多卡因)来缓解注射位点的疼痛。通常，将组分分开或以单位剂量形式，例如干的冻干粉末或无水浓缩物混合在一起给予。可将此组合物存储于密封的容器中如安瓿或小袋中，以活性单位指明活性成分的量。当组合物是以输液给予时，可将其分散于含有无菌的药用级别的“注射用水”或盐水的输液瓶中。当以注射给予此组合物时，可提供一安瓿注射用的无菌水或盐水，从而在施用前混合该成分。

本发明的药物组合物包括本发明的化合物及其药学上可接受的盐，和任何药学上可接受的成分、赋形剂、载体、佐剂或运载体。

较佳地，以可注射组合物的形式将此高弛豫率的多体造影剂给予患者。优选给予 MRI 造影剂的方法是肠胃外的，即静脉内、动脉内、鞘内、间质内或腔内。可按与其它诊断剂或治疗剂相类似的方法，将本发明的药物组合物给予哺乳动物(包括人)。施用的剂量和施用的方式取决于各种因素，包括患者的年龄、体重、性别、患者的症状和遗传因素，最终可由临床医生在试验确定各种剂量后进行本文所述的成像来决定。一般而言，诊断敏感性或治疗有效所需的剂量范围约为 0.001 到 50,000μg/kg(主体体重)，较佳地在 0.01 到 25.0μg/kg(主体体重)范围之间。可按本文所公开的经验地确定最佳剂量。

在以下实施例中将进一步说明本发明实例中一些高弛豫率多体组合物的合成和用途。以下实施例中所包括的具体参数仅是为说明本发明的实施，对本发明的范围无任何限制。虽然以上已描述了许多实例和特征，本领域技术人员可以理解在本发明的精神和权利要求范围内还可以对所述的实施例和特征进行改善和变化。虽然以描述了许多实例和特征，但本领域技术人员可以理解在本发明的精神和权利要求范围内还可以对所述的实施例和特征进行改善和变化。

实施例

实施例 1 测定弛豫率的方法

用在 0.47 特斯拉(20MHz H-1 拉莫尔频率)和 37℃操作的 Bruker NMS-120

Minnispec NMR 分光光度计评估本发明化合物的弛豫率。用该装置的软件通过逆转恢复脉冲序列(inversion recovery pulse sequence)确定水质子的 T_1。通过制备 4 份独立样品在靶标存在下(通常为 4.5%HSA)测定弛豫率。第一份在磷酸盐缓冲盐水(PBS)中仅含有 4.5%HSA，其它三份在 PBS 中除含有 4.5%HSA 以外，还分别含有 20、30 和 40μM Gd(III)。将样品在 37℃培养至少 15 分钟以确保进行 T_1 测定前温度的平衡。用感应偶合的等离子体-质谱(ICP-MS)确定样品中 Gd(III)的含量。通过以 s^{-1} 表示的豫率速率(1/T1) 对 Gd(III)浓度(mM)作曲线确定弛豫率(每个 Gd(III)离子)。与该数据拟合的线的斜率即为弛豫率。还以类似的方式(但无靶标)测定了无靶标时化合物的弛豫率。

在另外的实验中如用超滤或平衡渗析确定了这些条件下结合于靶标的品种的浓度。由已知的结合于靶标的品种的浓度、存在靶标时的弛豫率、无靶标时的弛豫率可以计算本文所述的平均结合弛豫率。

实施例 2 测试血纤蛋白结合本发明造影剂的实验模型

在这些实施例中所述对血块(血栓)成像具有高弛豫率和特异性的造影剂能够区分血块和循环中的纤维蛋白原。其可以提供对病症发展的各种状态时血栓的形成进行灵敏有效的探测。可以用其来诊断是否存在早期和晚期血栓。

可以使用的一种动物模型是兔颈静脉血栓模型(颈静脉被钳制)。在两处钳制该静脉，除去这两个钳制处之间的兔血。在两处钳制间加入人血纤蛋白原、兔红血细胞和凝血酶，从而产生含有人血纤蛋白的血栓。通常将这种血栓老化(aged)30 分钟。

在这种兔颈静脉模型中，常规测定仪器是用破坏梯度(spoiled gradiet)(SPGR)MRI 为 36/5/30deg 的 1.5Tesla。另外，3D GRE 44/10/30deg 可以用于 1.5 Tesla 仪器进行成像方法。再者，用于 1.5Tesla 可以进行 IR2000/10/700 法。

实施例 3 实例化合物：制备

本发明的优选实例是具有图 13 所示结构的用于 MRI 成像的造影剂。

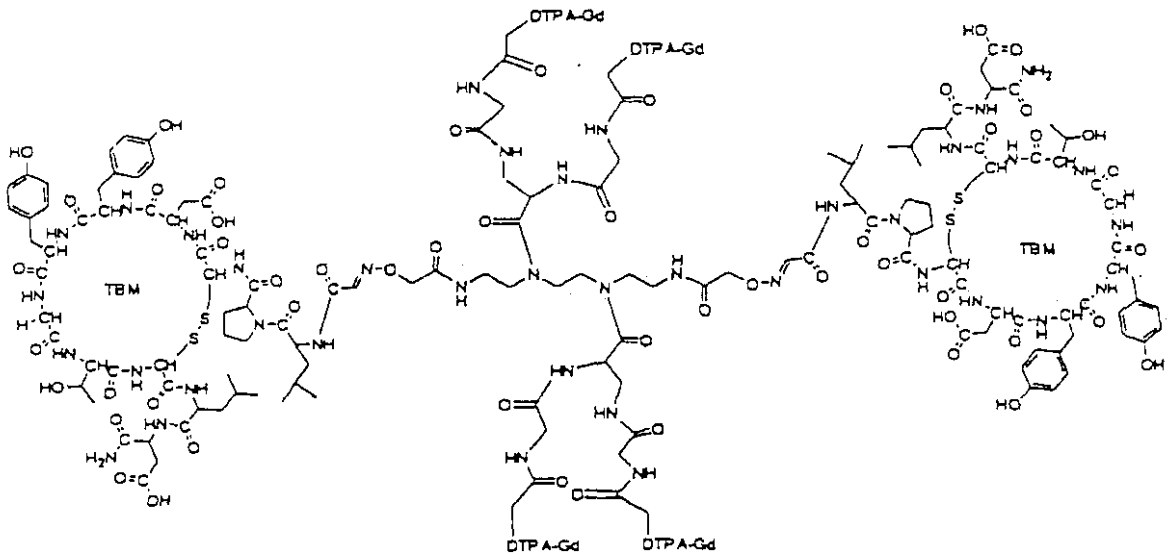
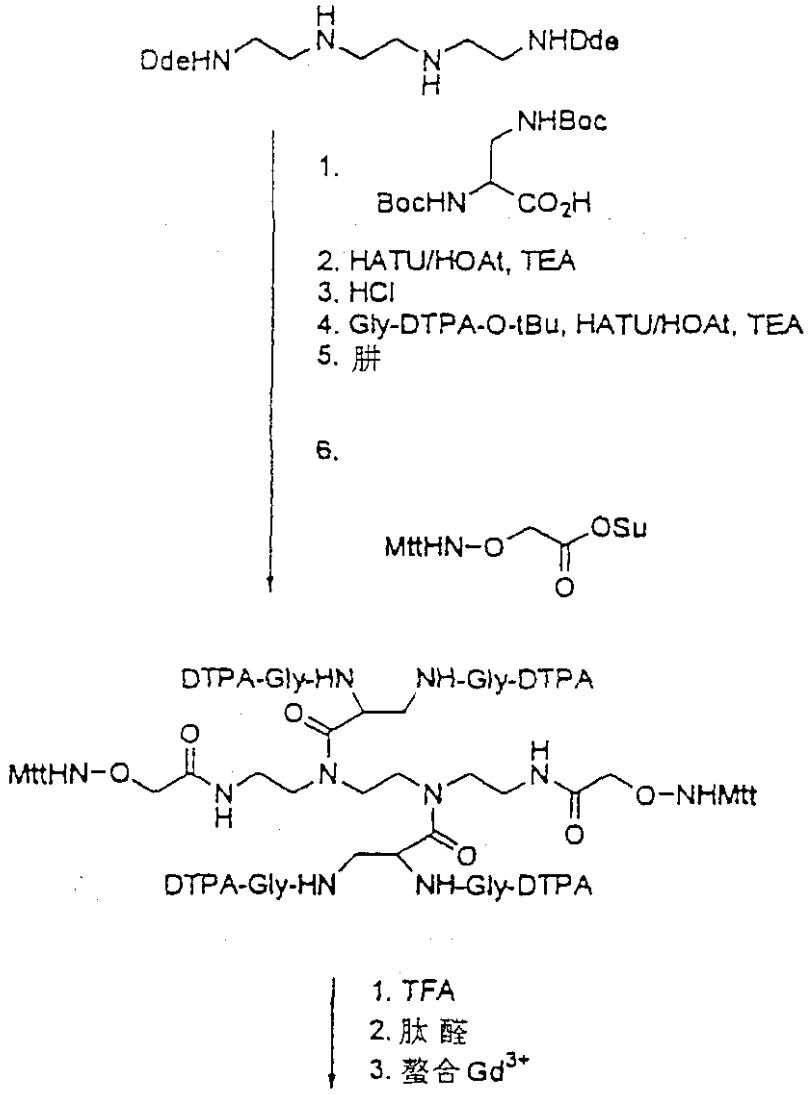


图 13 中的造影剂含有 4 个用作 IEM 的 DTPA-Gd 部分。IEM 通过包括一系列重复酰胺的连接基连接于乙二胺构架。TBM 是两个表现出对血纤蛋白高亲和力的肽。这两个肽可以通过在两个半胱氨酸残基间形成二硫键而环化。TBM 通过肟和酰胺键连接于构架。

在 37℃、10μM 造影剂、2.5mg/ml 血纤蛋白、50mM Tris、150mM NaCl、2mM Ca^{2+}、pH 7.4 时，原型血栓剂与血纤蛋白 DD(E)氨基酸片段的结合为 51%结合。在 20MHz 和 37℃时结合化合物的弛豫率为 101.4$mM^{-1}s^{-1}$，每个 Gd 螯合物为 25.4$mM^{-1}s^{-1}$。游离造影剂的弛豫率为 67.7 $mM^{-1}s^{-1}$，每个 Gd 螯合物为 16.9 $mM^{-1}s^{-1}$。该造影剂在结合时弛豫率大为提高。图 14 显示了作为原型 MR 试剂的血栓肽多体的合成。虽然显示了特定的肽和造影剂，但本领域技术人员可以理解可以用其它肽代替化合物中的，而且化合物中所用的肽不必相同。

图 14 靶向人血纤蛋白的多体造影剂的合成



M8-11

实验部分

二 Dde-四胺的合成

将四胺(Fluka)(1.50ml)与 Dde-OH(NovaBiochem)(4.0g)在 30mlEtOH 中反应。回流此清澈的浅黄色溶液 16 小时。反应完成后，真空浓缩反应混合物得到干燥的残留物。将残留物溶解于 250ml 乙醚和 2N HCl 溶液中。分开浅黄色酸性水层，加入 50%NaOH 直到 pH 达到 12，然后用 EtOAc 提取。用盐水洗涤 EtOAc 层，干燥(Na_2SO_4)并过滤。真空蒸发滤液得到浅黄色块状固体(2.9g)，将其在快速硅胶柱上纯化，用 EtOAc/MeOH(2:3)和 EtOAc/(含有 1%的 MeOH)(2:3)洗脱得到呈浅黄色固体的所需产物(2.3g)。

^{1}H-NMR(300MHz,CDCl3): δ 1.02(s,6H),2.34(s,4H,2),2.56(s,),2.79(s,2H), 2.92 -2.94(t,2H),3.48-3.54(q,2H),198.2。

MS:m/z475.2$(M+H)^+$

二 Dde-四胺二-Dpr$(Boc)_2$的合成

将 Boc-Dpr(Boc)-OH.DCHA(4.85g)悬浮于 60ml EtOAc(含有 12.0ml 2M H_2SO_4)中。振荡该烧瓶，分开 EtOAc 层。用 EtOAc 提取水层。合并 EtOAc 层，用盐水洗涤，无水 $MgSO_4$ 干燥并过滤。真空蒸发滤液，将所得的固体干燥后得到 3.23g 呈白色晶状固体的 Boc-Dpr(Boc)-OH 游离酸。

^{1}H-NMR(300MHz,DMSO-d_6): δ 1.35(s,18H,),3.30(s,2H),3.96-3.98(m,1H), 6.87-6.90(d,1H)

0℃将作为游离酸的 Boc-Dpr(Boc)-OH(1.52g)溶解于 15ml DCM 中。在其中加入 HOAt(0.68g)和 DIEA(0.35ml)，0℃搅拌此清澈的溶液。然后将上述二 Dde-四胺加到此溶液中，然后再加入 HATU(1.90g)和 2mmolTEA。加入无水 DMF(5ml)，搅拌此反应物 36 小时。真空蒸发溶剂，用 150mlEtOAc 吸收残留物，用 1N HCl、饱和 $NaHCO_3$、盐水洗涤，然后无水 Na_2SO_4 干燥，过滤并真空蒸发得到白色固体(1.92g)。在快速硅胶柱上层析纯化该固体，用 EtOAc(5%MeOH)洗脱得到呈白色固体的产物(1.3g)。

^{1}H-NMR(300MHz,$CDCl_3$): δ 1.01(s,6H),1.35(s,18H),2.34(s,4H), 2.58 (s,3H), 3.30(s,2H),3.44-3.67(bm，6H),3.85-4.10(m,1H),5.44-5.52(d,2H),5.74(bs,1H)。

MS:m/z 1047.7$(M+H)^+$

二-Dde-四胺-diDpr.HCl 盐的合成

将二 Dde-四胺-diDpr$(BOC)_2$溶解于 40ml 4N HCl/二噁烷中，搅拌 10 小时。用冷乙醚研磨此悬浮液，真空蒸发得到大量白色固体。用高真空泵干燥这些固体，得到以 HCl 盐形式的所需产物(1.09g)。

MS:m/z647.3$(M+H)^+$

合成二 Dde-二 Dpr-四-GlyDTPA-OtBu

0℃将 Gly-DTPA 五-叔-丁酯(Gly-DTPA-O-tBu)溶解于 10ml DMF 中。加入

HOAt(0.41g)和 DIEA(0.52ml)，在 0℃搅拌此溶液。将上述二-Dde-四胺-diDpr 盐溶解于 3ml DMF 中，并加入 DIEA(0.13ml)。将 HATU(1.14g)和额外的 DIEA(0.09ml)加入该混合物中。搅拌此黄色溶液 36 小时。真空除去溶剂，用 EtOAc 吸收残留物。用 1N HCl、饱和 $NaHCO_3$、盐水洗涤有机层，无水 Na_2SO_4 干燥，过滤并真空干燥得到浅黄色固体(3.15g)。用制备型 RP-HPLC(C-4, ACN/H_2O)纯化。混合含有所需化合物的组分，冷冻干燥得到白色固体(0.25g)。

MS:m/z 1244.4$(M+3H)^{3+}$;933.9$(M+4H)^{4+}$

四胺-四-CN-GlyDTPA-O-tBu 的合成

将二 Dde-四胺-二 Dpr-四-GlyDTPA-O-tBu(0.38g)溶解于 8ml 2%v/v 肼的 DMF 中，室温搅拌 10 分钟。真空浓缩此反应混合物，并用 CH_3CN 吸收残留物，用预备型 RP-HPLC[C-4,CAN/H_2O]纯化。混合含有所需化合物的组分，冷冻干燥得到白色固体(0.25g)。

MS:m/z 1702.1$(M+2H)^{2+}$;1135.1$(M+3H)^{3+}$

二-MeO-三苯甲基-AoA-四-Gly-DTPA-O-tBu 的合成

0℃将四胺-四-Gly-DPTA-O-tBu(98mg)溶解于 3mlDMF 中。加入 TEA(9μl)和甲氧基三苯甲基氨基氧乙酸琥珀酰亚胺酯(MeO-Trt-AoA-Osu)(29mg)，搅拌过夜，加入 MeOH,蒸去溶剂，用 DCM 提取残留物，用 10%柠檬酸水溶液、盐水洗涤，无水 $MgSO_4$ 干燥。用制备型 RP-HPLC(C-4, ACN/H_2O)进一步纯化产物。混合含有所需产物的组分，冷冻干燥得到白色固体(87mg)。

MS:m/z 2047.5$(M+2H)^{2+}$;1365.3$(M+3H)^{3+}$

AoA-四-GlyDTPA-OH 的合成

将二-MeO 三苯甲基-AoA-四-GlyDTPA-O-tBu(85mg)溶解于 10mlCH_3Cl/苯硫基甲烷/DCM/TIS(64/16/16/4)中，在 0℃搅拌 3 小时。TIS 指$(iPr)_3SiH$。用 20ml 水稀释此反应混合物，用乙醚提取。在制备型 RP-HPLC(C-18, ACN/NH_4OAc)进一步纯化水层。分离产物，冷冻干燥得到白色固体(29mg)。

MS:m/z 1214.4$(M+2H)^{2+}$;809.5$(M+3H)^{3+}$

SLPCDYYGTCLD-NH_2 的氧化

将肽 c[SLPCDYYGTCLD-NH_2] (10mM 在 NaPi 缓冲液中，pH＝6.8)与 $NaIO_4$(20mM)反应。用乙二醇淬灭氧化。在 C-18Sep-Pak 柱上纯化反应物。用含有 0.1%TFA 的 80%CH_3CN 洗脱产物。真空离心除去溶剂，将所需的产物α-N-乙醛酰-c[SLPCDYYGTCLD-NH_2]冷冻干燥成白色粉末。

MS:m/z 1315.5(M+H)+

化学选择性连接-最后装配：M8-11 的合成

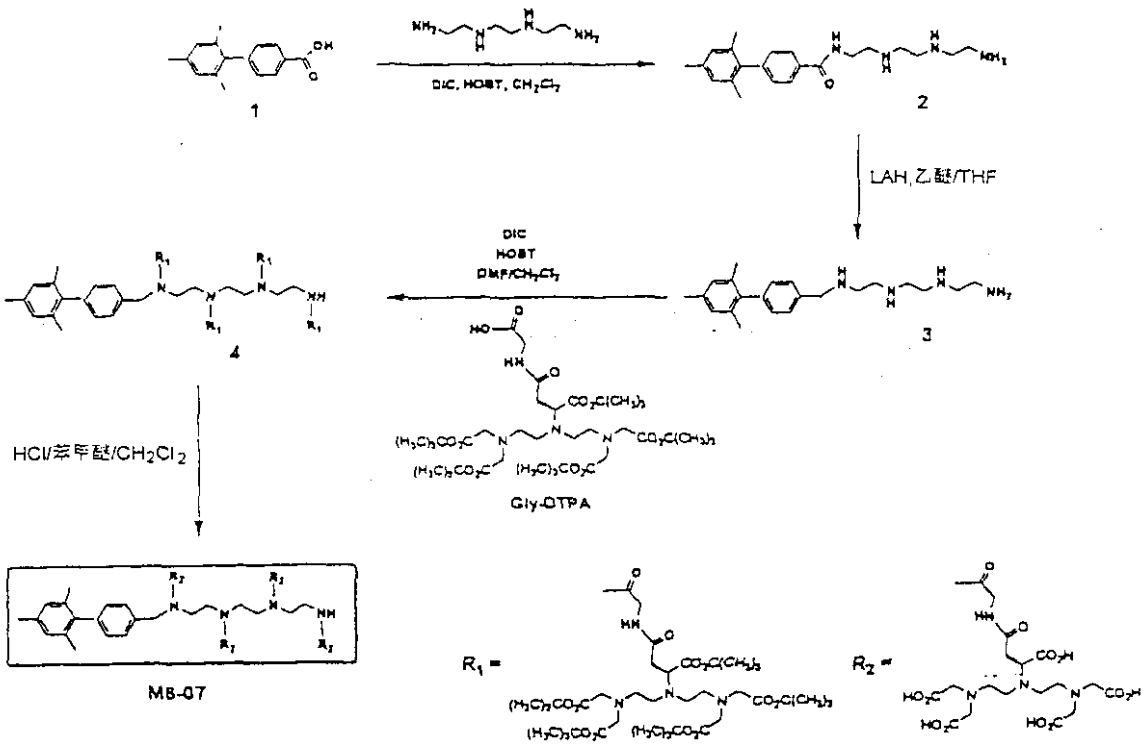
22 ℃，将 α-N-乙醛酰-c[SLPCDYYGTCLD-NH_2](13.2mg)和 AoA-四 DPTA-OH(12.3mg)在 20mM 乙酸钠缓冲液(pH 4.6)中反应。用半制备型 RP-HPLC(C-18,CH_4CH/5mm NH_4OAc)纯化反应物。用标准方法将其转化成钆复合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

MS:m/z 1674.6$(m+3H)^{3+}$;1256.3$(m+4H)^{4+}$

实施例 4：带有三亚乙基四胺构架的造影剂的合成

用 Di-Dde-四胺-diDpr 和上述流程，以及用二氨基-BOC 取代的乙烷连接基和 Gly-DTPA-OtBu IEM 取代，可以将上述各种螯合物结合于三亚乙基四胺构架。螯合物可以是相同的或不同的，产生同质螯合物造影剂或包含异质螯合物的造影剂。

实施例 5M8-07 的合成



在 4-苯基苯甲酸(0.7g)和三亚乙基四胺(4.26g)的 CH_2Cl_2(200ml)溶液中加入 HOBt(0.89g,5.83mmol)和 DIC(0.74g)。室温搅拌此混合物过夜。过滤得到的沉淀物，减压下除去溶剂得到黄色油状物。将反应混合物加样到 C4 柱上进行制备型-HPLC(洗脱液：0.1%TFA/H_2O/CH_3CN)得到呈白色泡沫的 TFA 盐的单酰胺(0.63g)。

LC-MS：(m/e) 369.1(M^+)

室温，在上述单酰胺(0.63g)的乙醚(100ml)和 THF(100ml)溶液中缓慢加入 LAH(1.30g)。回流此混合物 2 小时，然后在室温搅拌过夜。在此混合物中逐滴加入水以淬灭 LAH。过滤除去得到的沉淀，减压下除去溶剂得到无色油状物。将反应物加样到 C4 柱上进行制备型-HPLC(洗脱液：0.1%TFA/H_2O/CH_3CN)得到呈白色泡沫的 TFA 胺盐(180mg)。

LC-MS：(m/e) 354.4(M^+)

在 TFA 胺盐(180mg)和二异丙基乙胺(287mg)的 DMF(50ml)溶液中加入 Gly-DTPA-OtBu(1.88g)的 CH_2Cl_2(50ml)溶液、HOBt(370mg)和 DIC(301mg)。室温搅拌此混合物过夜。减压下除去溶剂得到黄色油状物。将此反应混合物加到 C4 柱上进行制备型-HPLC(洗脱液：0.1%TFA/H_2O/CH_3CN)得到呈白色固体的粗

产物(0.36g)。

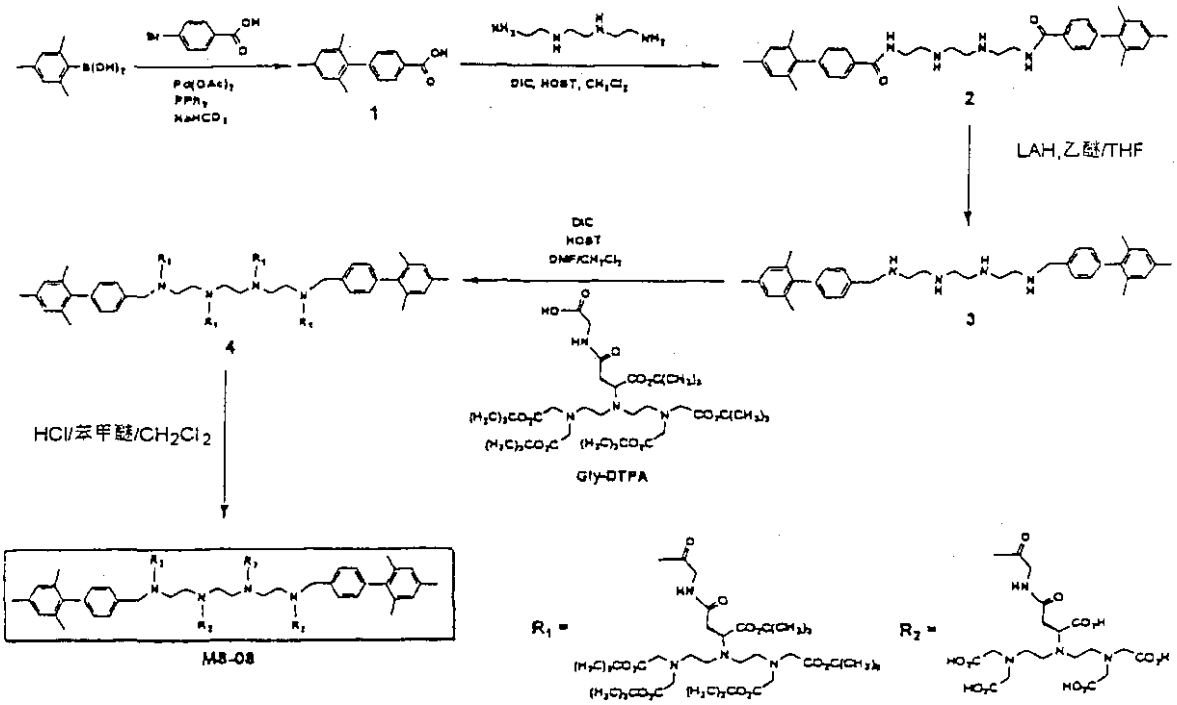
LC-MS: (m/e)1719.4(M^{2+}),1147.2(M^{3+})

在上述白色固体(0.19g)的 CH_2Cl_2(4.5ml)和苯甲醚(4.5ml)溶液中逐滴加入 4.5ml 12N HCl。室温搅拌此混合物 3 小时。在此混合物中加入 40ml 水，用乙醚洗涤 3 次。冷冻干燥水溶液，得到粗产物，然后将其加样到 C18 柱上进行制备型-HPLC(洗脱液：100mM AcONH4/CH3CN)得到白色固体(50mg)。

LC-MS: (m/e)1158.6(M^{3+}),772.8(M^{3+})

按标准方法将此物转化成钆的复合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

实施例 6：M8-08 的合成



4-苯基苯甲酸。在苯基硼酸(10g)和 4-溴苯甲酸(12.9g)的 1-丙醇(150ml)和 DME(200ml)溶液中加入三苯基膦(0.128g)、2M 碳酸钠溶液(37ml)和水(30ml)。氮气气氛下在此混合物中加入乙酸钯(82mg)。加热回流此混合物过夜。移去热源后，加入 100ml 水，搅拌 2.5 小时同时边冷却至室温。用 150ml 乙酸乙酯稀

释颜色变暗的混合物，将两相分开。用饱和碳酸氢钠溶液洗涤有机层若干次，直到 TLC 显示完全除去 4-溴苯甲酸(R_f＝0.55,洗脱液：CH_2Cl_2/CH_3OH＝5)。用 200ml 1N NaOH 溶液提取此溶液 3 次。在合并的水层中加入约 50ml 12N HCl 使 pH 达到 3。过滤得到的沉淀物，用水洗涤，干燥得到呈白色固体的 4-苯基苯甲酸(8.81g)。R_f＝0.75(洗脱液：CH_2Cl_2/CH_3OH＝5)

^{1}H-NMR(300MHz,$CDCl_3$)：δ 1.997(s,6H),2.340(s,3H),6.961(s,2H),7.274(d,J＝8.1Hz,2H),8.177(d,J＝8.1Hz,2H)。

在 4-苯基苯甲酸(1.5g)和三亚乙基四胺(0.43g)的 CH_2Cl_2(60ml)溶液中加入 HOBt(0.96g)和 DIC(0.79g)。室温搅拌此混合物过夜。过滤得到的沉淀物，干燥得到白色固体(1.45g)。

LC-MS：(m/e) 591.3(M^+)

室温，在上述白色固体(0.45g)的乙醚(20ml)和 THF(80ml)溶液中缓慢加入 LAH(0.33g)。回流此混合物 2 小时，然后在室温搅拌过夜。在此混合物中逐滴加入水以淬灭 LAH。过滤除去得到的沉淀，减压下除去溶剂得到浅黄色油状物。将反应物加样到 C4 柱上进行制备型-HPLC(洗脱液：0.1%TFA/H_2O/CH_3CN)得到呈白色固体的 TFA 胺盐(140mg)。

LC-MS：(m/e) 564.6(M^+)

在此 TFA 胺盐(50mg)和二异丙基乙胺(38mg)的 DMF(30ml)溶液中加入 Gly-DTPA-OtBu(193mg)的 CH_2Cl_2(30ml)溶液、HOBt(37.5mg)和 DIC(31mg)。室温搅拌此混合物过夜。减压下除去溶剂得到褐色油状物。将此反应混合物加到 C4 柱上进行制备型-HPLC(洗脱液：0.1%TFA/H_2O/CH_3CN)得到呈浅黄色固体的粗产物。

LC-MS: (m/e)1824.2(M^{2+}),1216.3(M^{3+}),912.5(M^{4+})

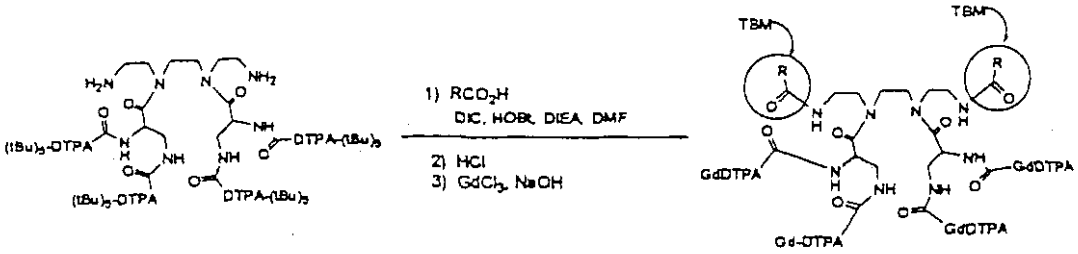
在此浅黄色固体(0.58g)的 CH_2Cl_2(5ml)和苯甲醚(5ml)溶液中逐滴加入 10ml 12N HCl。室温搅拌此混合物 3 小时。在此混合物中加入 40ml 水，用乙醚洗涤

得到的混合物 3 次。冷冻干燥水溶液，得到粗产物，然后将其加样到 C18 柱上进行制备型-HPLC(洗脱液： 100mM AcONH4/CH3CN)得到白色固体(11mg)。

LC-MS: (m/e)1263.2(M^{2+}),842.4(M^{3+}),632.2(M^{4+})。

按标准方法将此物转化成钆的复合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

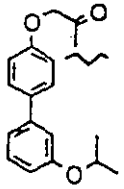
实施例 7：含有两个相同 TBM 的 HSA 结合多体的一般合成

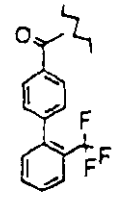
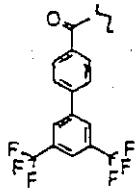
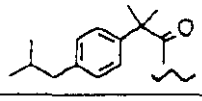
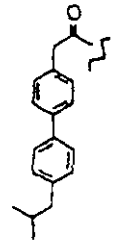
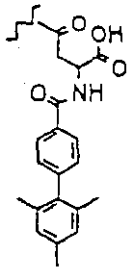
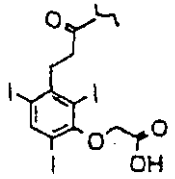
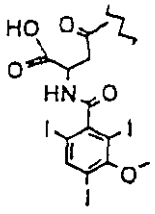


将含有 4 个叔丁酯保护的 DTPA 的二胺溶解于二甲基甲酰胺(0.75ml)中。加入 RCO_3H(3eq)(其表示各种含有 TBM 的羧酸)、DIC(0.052ml,3.3eq)和 HOBt(0.051g,3.3eq)。在逐滴加入 DIEA(0.105ml,6eq)之前将反应混合物冷却至 0℃。室温搅拌此反应混合物共 8.5 小时。用 CH_2Cl_2 稀释此反应混合物，用 0.1N HCl、饱和 $NaHCO_3$ 和盐水洗涤。Na_2SO_4 干燥有机层，真空除去残留的溶剂，得到叔丁酯保护的中间体。用 DCM 吸收产物，在 0℃加入 HCl，搅拌 3 小时。蒸发溶剂得到白色固体，将此固体与 $GdCl_3$(4eq)和 NaOH(12eq)反应得到终产物。

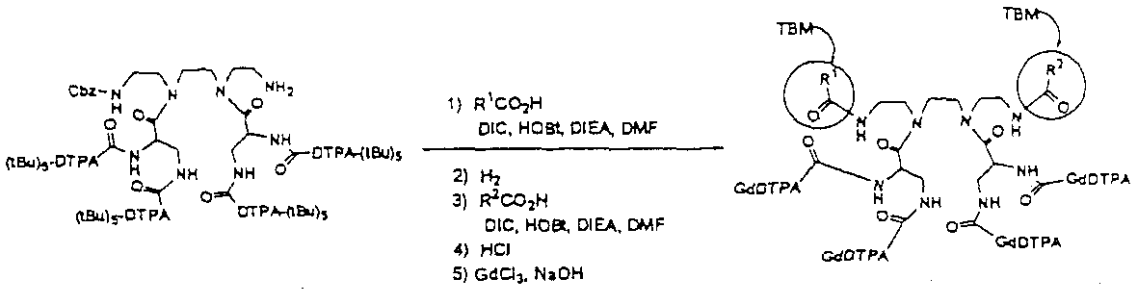
表 3 列出了按此方法合成的含有 2 个相同 TBM 的化合物及 TBM 的结构和质谱数据。

表 3 含有 2 个相同的 TBM 的多体造影剂

化合物 编号#	TBM 的化学结构 在一般合成流程中的“R”	质谱 (m/z, $[M]^{3+}$)
M8-12		1048.5

M8-13		1036.9
M8-14		1080.9
M8-15		1005.0
M8-16		1-36.8
M8-17		1094.1
M8-18		1259.1
M8-19		1288.51

实施例 8：含有两个不同 TBM 的 HSA 结合的多体的一般合成



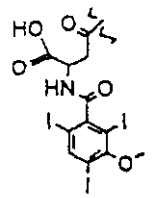
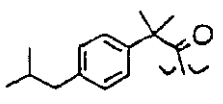
将含有 4 个叔丁酯保护的 DTPA 的 CBz 保护的单胺溶解于二甲基甲酰胺(0.75ml)中。加入 R^1CO_2H(1.5eq)(其表示各种含有 TBM1 的羧酸)、DIC(0.052ml, 1.5eq)和 HOBt(0.051g,1.5eq)。在逐滴加入 DIEA(0.105ml,6eq)之前将反应混合物冷却至 0℃。室温搅拌此反应混合物共 8.5 小时。

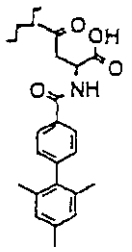
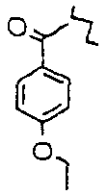
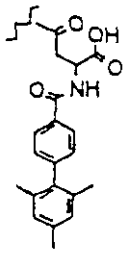
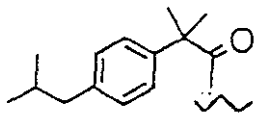
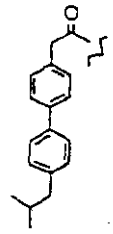
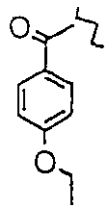
用 CH_2Cl_2 稀释此反应混合物，用 0.1N HCl、饱和 $NaHCO_3$ 和盐水洗涤。Na_2SO_4 干燥有机层，真空除去残留的溶剂。

将此化合物溶解于 EtOAc 中并氢化(5%Pd-C)。滤去催化剂后，将产物与另一羧酸(R^2CO_2H,1.5eq)(代表各种含有 TBM2 的羧酸)、DIC(0.052ml,1.5eq)和 HOBt(0.051g,1.5eq)反应。室温搅拌此反应混合物共 8.5 小时。用 CH_2Cl_2 稀释此反应混合物，用 0.1N HCl、饱和 $NaHCO_3$ 和盐水洗涤。Na_2SO_4 干燥有机层，真空除去残留的溶剂。将产物悬浮于 DCM 中，0℃加入 HCl，搅拌此反应物 3 小时。蒸发掉溶剂后得到白色固体，将此固体与 $GdCl_3$(4eq)和 NaOH(12eq)反应得到终产物。

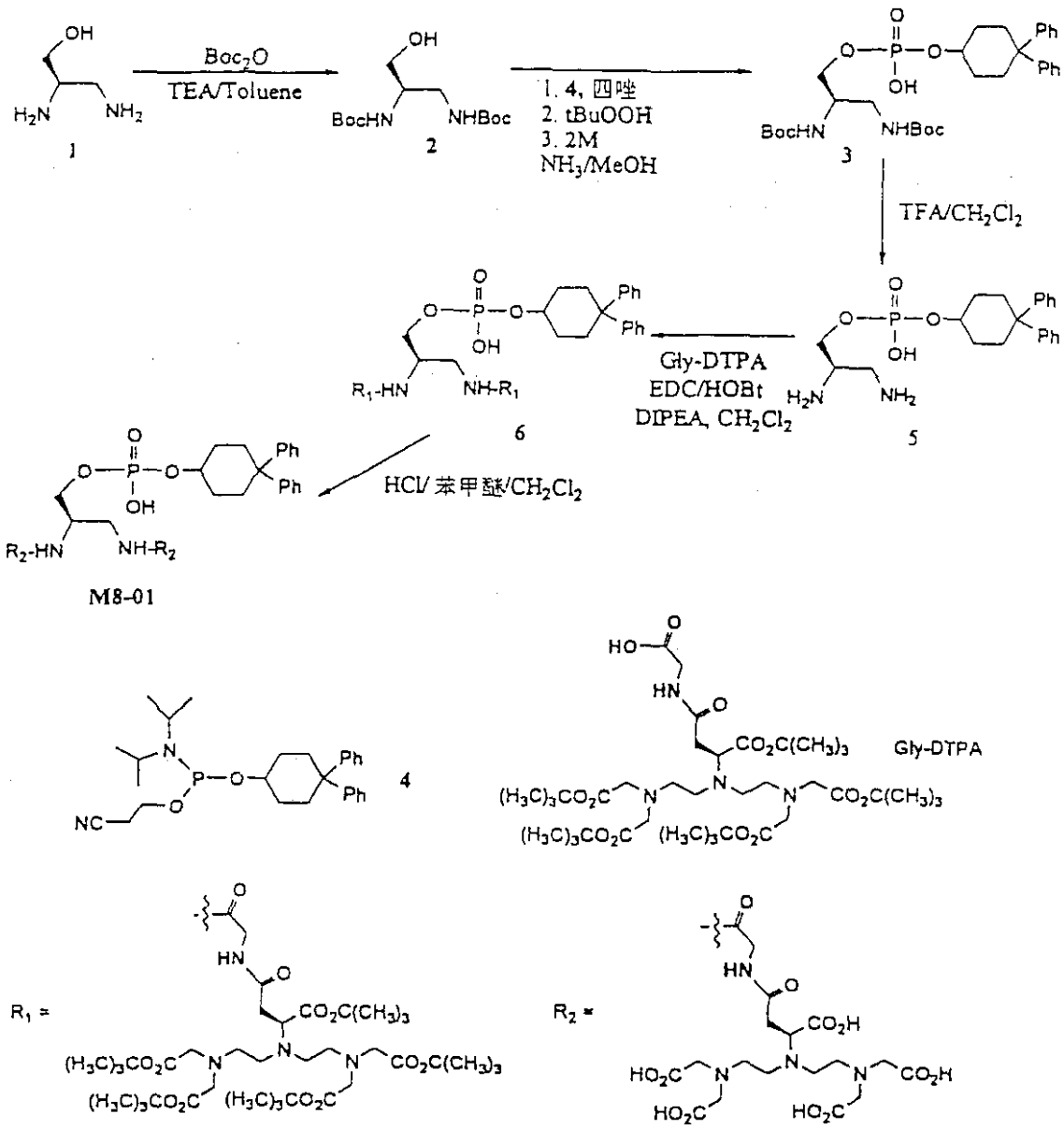
表 4 列出了按此方法合成的化合物及 TBM1 和 TBM2 的结构。需注意在一般合成流程中的“R^1”代表 TBM1，一般合成流程中的“R^2”代表 TBM2。

表 4 含有 2 个不同 TBM 的多体造影剂

化合物编号#	TBM1 的化学结构 一般合成流程中为“R^1”	TBM2 的化学结构 一般合成流程中为“R^2”
M8-20		

M8-21		
M8-22		
M8-23		

实施例 9：M8-01 的合成

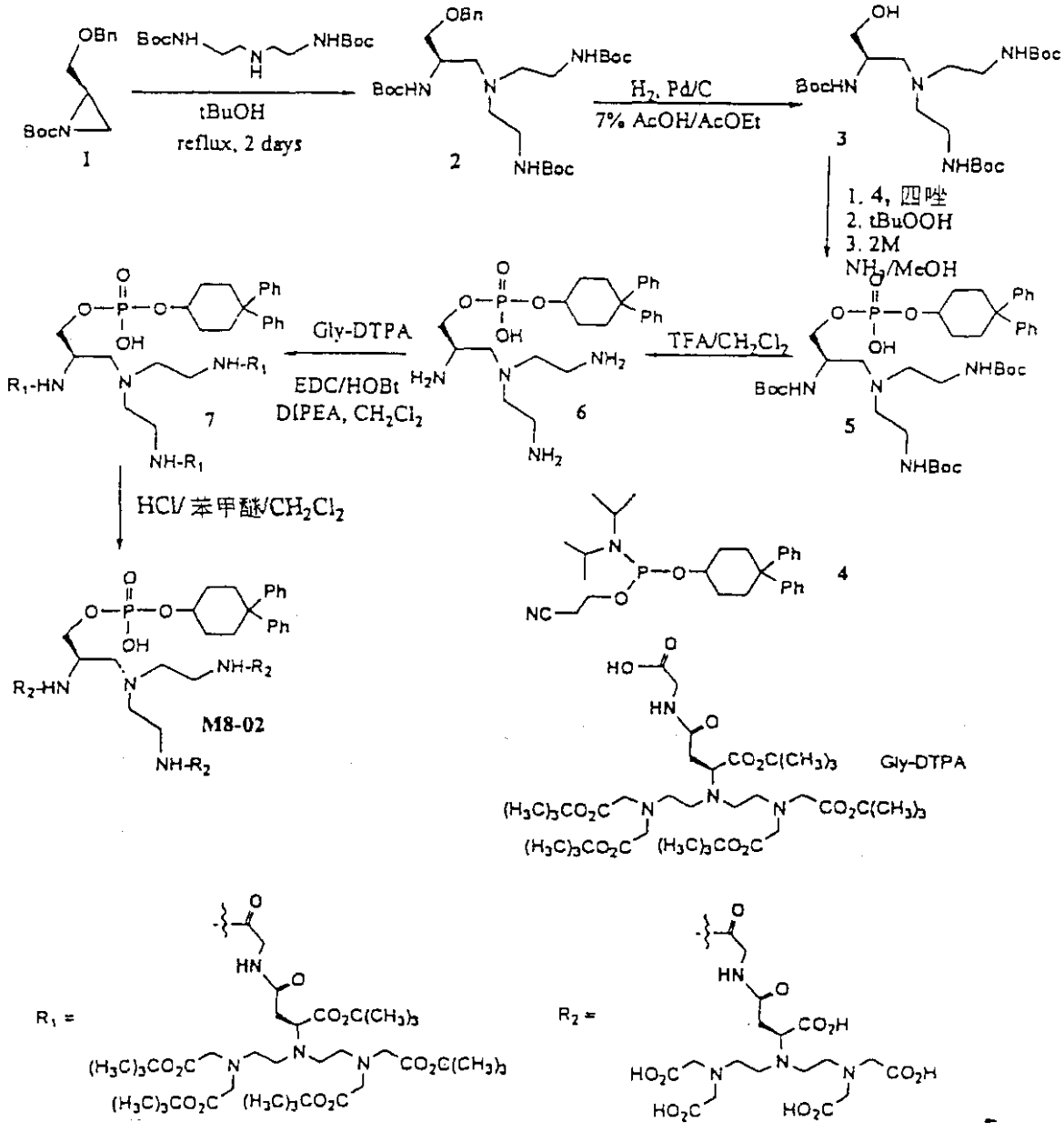


将 1,2-二(Boc-氨基)-3-羟基丙烷(1.0eq)和二苯基环己基亚磷酰胺酸酯(1.1eq)溶解于四氢呋喃(1.5ml/mmol 亚磷酰胺酸酯)中，与分子筛搅拌 30 分钟。室温在此混合物中加入四唑(1.2eq)。搅拌此混合物 45 分钟，^{31}P NMR 显示反应完成。在此混合物中加入叔-丁基氢过氧化物(1.2eq)。搅拌此反应混合物约 1 小时，至 ^{31}P NMR 显示反应完全。过滤除去得到的沉淀和分子筛，然后在滤液中加入二氯甲烷。顺序用硫代硫酸钠溶液、碳酸氢钠溶液和饱和氯化钠溶液洗涤此溶液，硫酸钠干燥，过滤并真空浓缩。在得到的浅黄色油状物中加入 2M 氨的甲醇溶液。搅拌此混合物过夜，然后真空除去溶剂。用硅胶柱层析(乙酸乙酯

/甲醇洗脱)得到磷酸二酯。

在三氟乙酸和二氯甲烷中搅拌使此磷酸二酯去保护，然后将此混合物冷冻干燥得到二胺-二苯基环己基-磷酸二酯。用 4 当量的 Gly-DTPA-O-tBu、EDC 和 HOBt 的二氯甲烷(已加入二异丙基乙胺以增加 pH)搅拌此二胺过夜。用制备型反相 HPLC 纯化此浓缩的反应混合物。在盐酸：苯甲醚：二氯甲烷(4：1：1)中搅拌来切割 2 个 DTPA 单位的叔丁酯，随后按常规方法用 $GdCl_3$ 形成 DTPA-钆螯合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

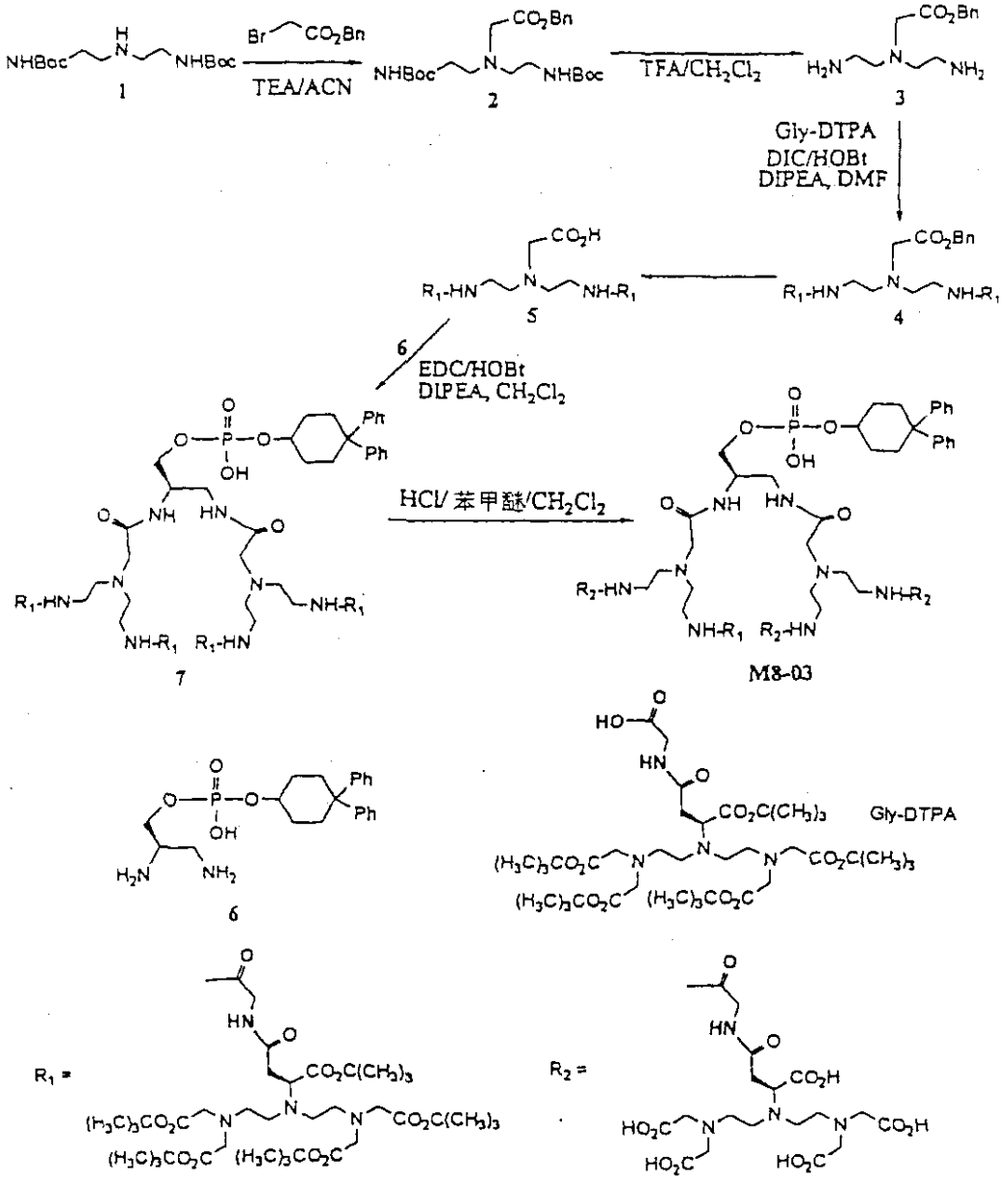
实施例 10：M8-02 的合成



将由 N-Boc-O-苄基-丝氨酸制备的苄氧基亚甲基-N-Boc-氮丙啶(2.82g)和N,N"-二 Boc-二亚乙基三胺(3.9g)在回流的叔丁醇中搅拌 2 天，然后真空除去溶剂，用快速层析纯化残留物(甲醇和二氯甲烷为洗脱液)得到 4.54g 物质。通过 1H NMR 光谱的积分证明 Boc 氢与芳族氢的比例。将这种三 Boc-保护的四胺苄醚(3.8g)溶解于 70ml 7%乙酸的乙酸乙酯(含有 1.0g 10%碳钯)中。将此反应混合物置于 48psi 的氢气中 16 小时。真空下除去反应混合物的溶剂，快速层析纯化(甲醇和二氯甲烷为洗脱液)残留物，得到 3.6g。用上述条件(四唑、叔-丁基氢过氧化物、氨的甲醇溶液)将所得的醇和二苯基环己基亚磷酰胺酸酯反应，得到相应的磷酸二酯。

在 1.0ml TFA 和 1.0ml DCM 中搅拌 3 小时除去此磷酸二酯的三个 Boc 基团，然后冷冻干燥此混合物得到 54ml 三胺。将三胺溶解于 1.0ml 二氯甲烷中，并逐滴加入含有各 6 当量 Gly-DTPA-O-tBu、EDC 和 HOBt 的 2.0ml 二氯甲烷溶液(其中加入 DIEA 将 pH 调节至 9.0)。用制备级 HPLC(C-4 柱，20ml/分钟，30：70 乙腈：水到 100：0 梯度 25 分钟，然后维持 10 分钟)纯化此浓反应物。用质谱验证该化合物的分子量。在盐酸：苯甲醚：二氯甲烷(4：1：1)中搅拌 5 小时来切割 DTPA 亚基的叔丁酯以产生羧酸，然后真空除去溶剂，溶解于水中并冷冻干燥。用常规方法[参见 Lauffer R.B.,等人 Radiology 207:第 529-38 页(1998)]用 $GdCl_3$ 形成 DTPA-钆螯合物。

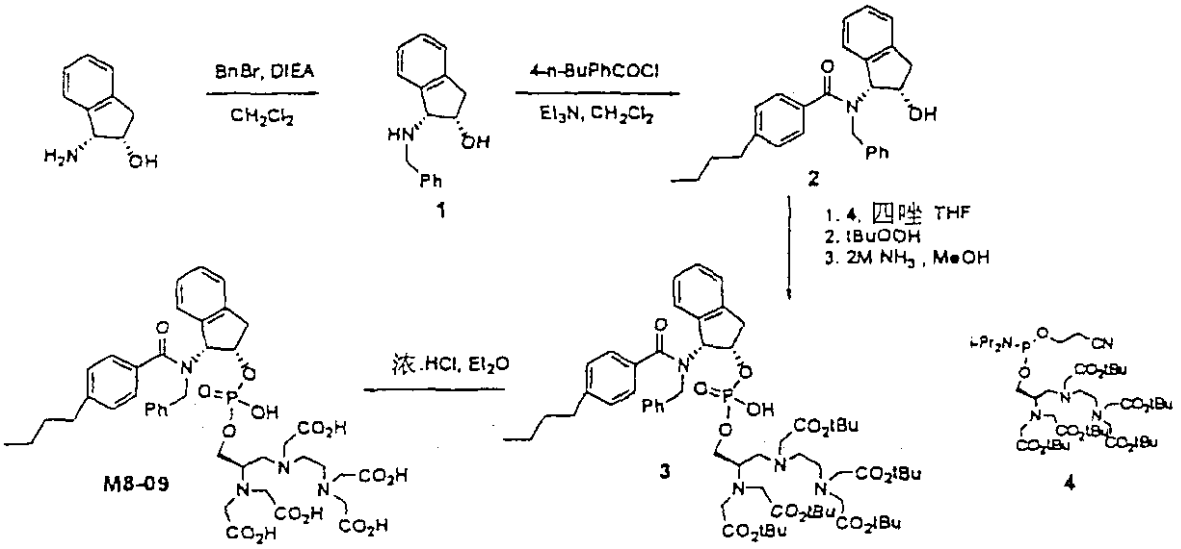
实施例 11：M8-03 的合成



将溴乙酸苄基酯(11ml)的 50ml 乙腈溶液加入 N,N"-二 Boc-二亚乙基三胺(14g)的 50ml 乙腈和 19ml 三乙胺的溶液中，并搅拌 2 小时，然后真空除去溶剂，快速层析(己烷/乙酸乙酯为洗脱液)纯化残留物得到 11g 物质。通过在 1：1 的三氟乙酸和二氯甲烷的混合物中搅拌 3 小时，除去该物质中混合物的 2 个 Boc 保护基团，然后真空除去溶剂，在水和乙醚间分配，冷冻干燥得到 9.2g 物质。在 N,N-二甲基甲酰胺中搅拌 Gly-DTPA-O-tBu、DIC 和 HOBt(各 2.2 当量)45 分钟，然后将此三胺(1.0g)作为二(三氟乙酸铵)溶解于 N,N-二甲基甲酰胺中，并将它们加到反应容器中，加入二异丙基乙胺将 pH 调节至 9.0。

搅拌 12 小时后，用水稀释此溶液，用乙酸乙酯提取，然后顺序用柠檬酸水溶液、饱和碳酸氢钠和饱和氯化钠洗涤。真空浓缩乙酸乙酯，快速层析(己烷/乙酸乙酯)得到 845mg 由质谱证明为四聚物的物质。在氢气气氛中，氢化 800mg 溶解于 10ml 己烷、9ml 甲醇和 1ml 三乙胺(含有 20%钯碳)的此物质，来除去苄基基团，然后经硅藻土过滤并真空浓缩。在含有 EDC 和 HOBt(各 1.2 当量)的二氯甲烷中搅拌此羧酸(750mg)30 分钟，将上述合成 M8-01 中所述的二胺-二苯基环己基-磷酸二酯(80mg)溶解于二氯甲烷中，并加到此羧酸溶液中，加入二异丙基乙胺将 pH 调节至 9.0。几小时后，浓缩此混合物，用制备级 HPCL(C-4 柱，20ml/分钟，30：70 乙腈：水到 100：0 梯度 25 分钟，然后维持 10 分钟)纯化得到 150mg 物质，用质谱验证它的分子量。在盐酸：苯甲醚：二氯甲烷(4：1：1)中搅拌 5 小时，切割 DTPA 亚单位的叔丁酯，以产生羧酸，然后真空除去溶剂，溶解于水中并冷冻干燥(得到 90mg)。按常规方法用 $GdCl_3$ 形成 DTPA-钆螯合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

实施例 12：M8-09 的合成



在(1R-2S)-(+)-顺式-1-氨基-2-茚满醇(15g)的二氯甲烷(90ml)悬浮液中加入二异丙基乙胺(35ml)，然后再加入苄基溴(17.2g)。搅拌此混合物过夜。用水洗涤此溶液，并用 0.1N HCl 溶液提取 2 次，将合并水层的 pH 升至 8，用 CH_2Cl_2 提取此水层 4 次。用饱和氯化钠洗涤合并的有机层，硫酸钠干燥，过滤并浓缩得到 1-苄基氨基-2-茚满醇(15.62g)，用质谱验证其分子量(M^+的 m/e＝239.95)。

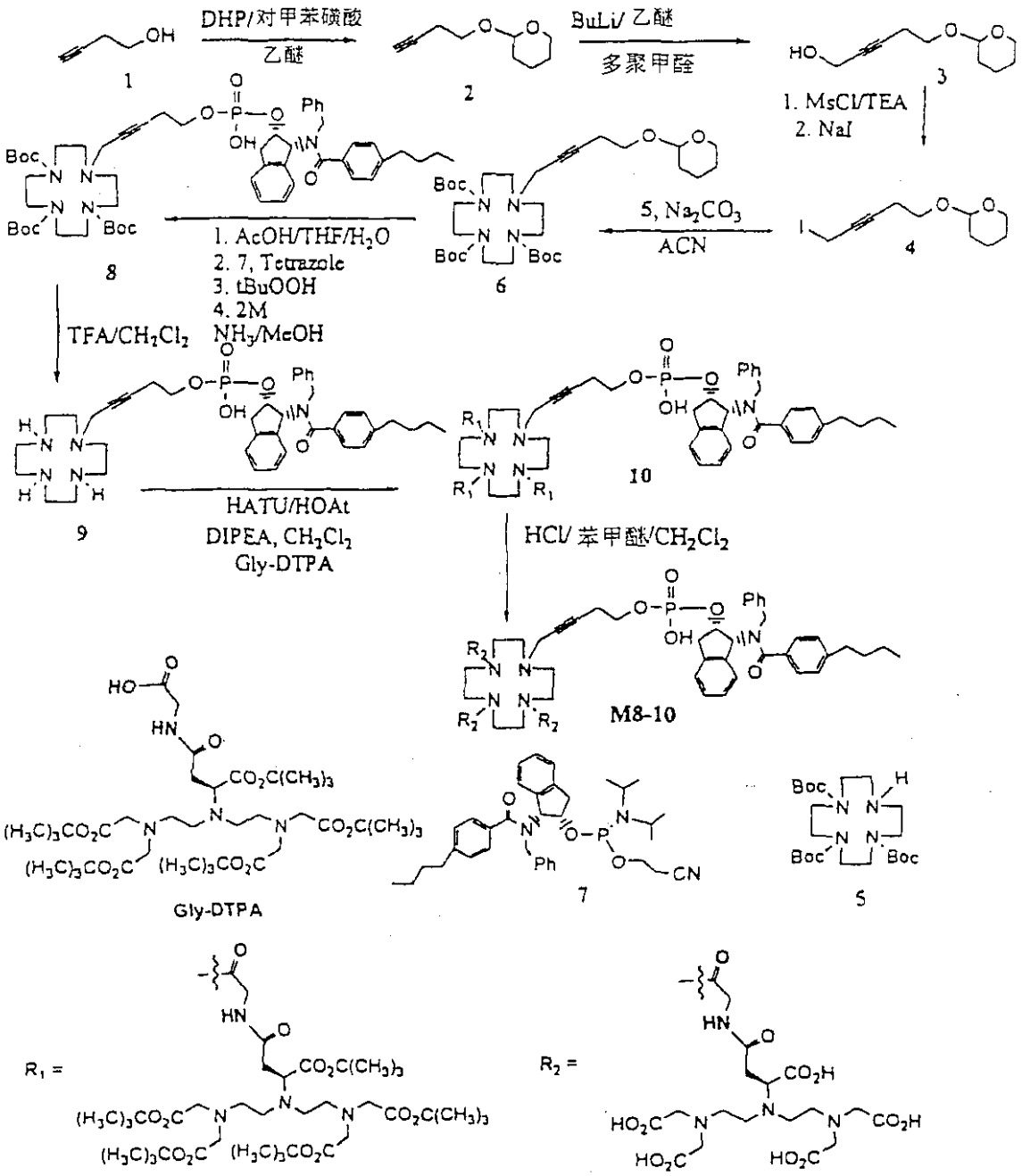
在 1-苄基氨基-2-茚满醇(10.5)和三乙胺(9.2ml)的二氯甲烷(250ml)溶液中逐滴加入 4-丁基苯甲酰氯(9.1ml)。搅拌此混合物 4 小时，然后在真空下浓缩。

将残留物溶解于乙酸乙酯中，并用水洗涤，硫酸钠干燥，过滤，减压下浓缩并用快速层析(己烷/乙酸乙酯为洗脱液)得到 1-对-丁基苯甲酰基-1-苄基氨基-2-茚满醇(12.7g):^{1}H NMR(CDCl3) δ 0.93(t,3H),1.24-1.4(m,2H),1.5-1.65(m,2H),2.6(t,2H),2.8(宽 d,1H),3.07(dd,1H),4.52(m,1H),4.65(m,2H), 5.13(m,1H), 7.09-7.32(m,11H),7.38-7.62(m,2H)。将 1-对-丁基苯甲酰基-1-苄基氨基-2-茚满醇(1.26g)和 DTPA 亚磷酰胺酸酯(2.99g)溶解于四氢呋喃(5ml)中，与分子筛搅拌 30 分钟，然后在其中加入四唑(265mg)，搅拌此混合物 45 分钟。

^{31}P NMR 表明反应完成，在此混合物中加入叔-丁基氢过氧化物(0.433ml)，然后搅拌此反应混合物 1 小时直到 ^{31}P NMR 显示反应完成。过滤除去形成的沉淀和分子筛，然后在滤液中加入二氯甲烷。顺序用硫代硫酸钠溶液、碳酸氢钠溶液和饱和氯化钠溶液洗涤此溶液，硫酸钠干燥，过滤并真空浓缩。在得到的浅黄色油状物中加入 2M 氨的甲醇溶液。搅拌此混合物过夜，然后减压下除去溶剂。快速层析(乙酸乙酯/甲醇为洗脱液)纯化此反应混合物，得到呈白色固体的磷酸二酯：^{31}P NMR(THF-d^8) δ -0.28;LC-MS(m/e)1165.75(M^+)。

通过溶解于二氯甲烷和用 12N 盐酸处理，除去 DTPA 亚单元上的叔丁酯以产生羧酸。几小时后，加入 5N 氢氧化钠水溶液将 pH 调节至 1.5，滤去形成的白色沉淀，用盐酸(pH＝1.5)洗涤 2 次。冷冻干燥沉淀物 48 小时，得到呈白色细粉末的产物：LC-MS(m/e)885.15(M^+)。按常规方法用 $GdCl_3$ 形成 DTPA-钆螯合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

实施例 13：M8-10 的合成



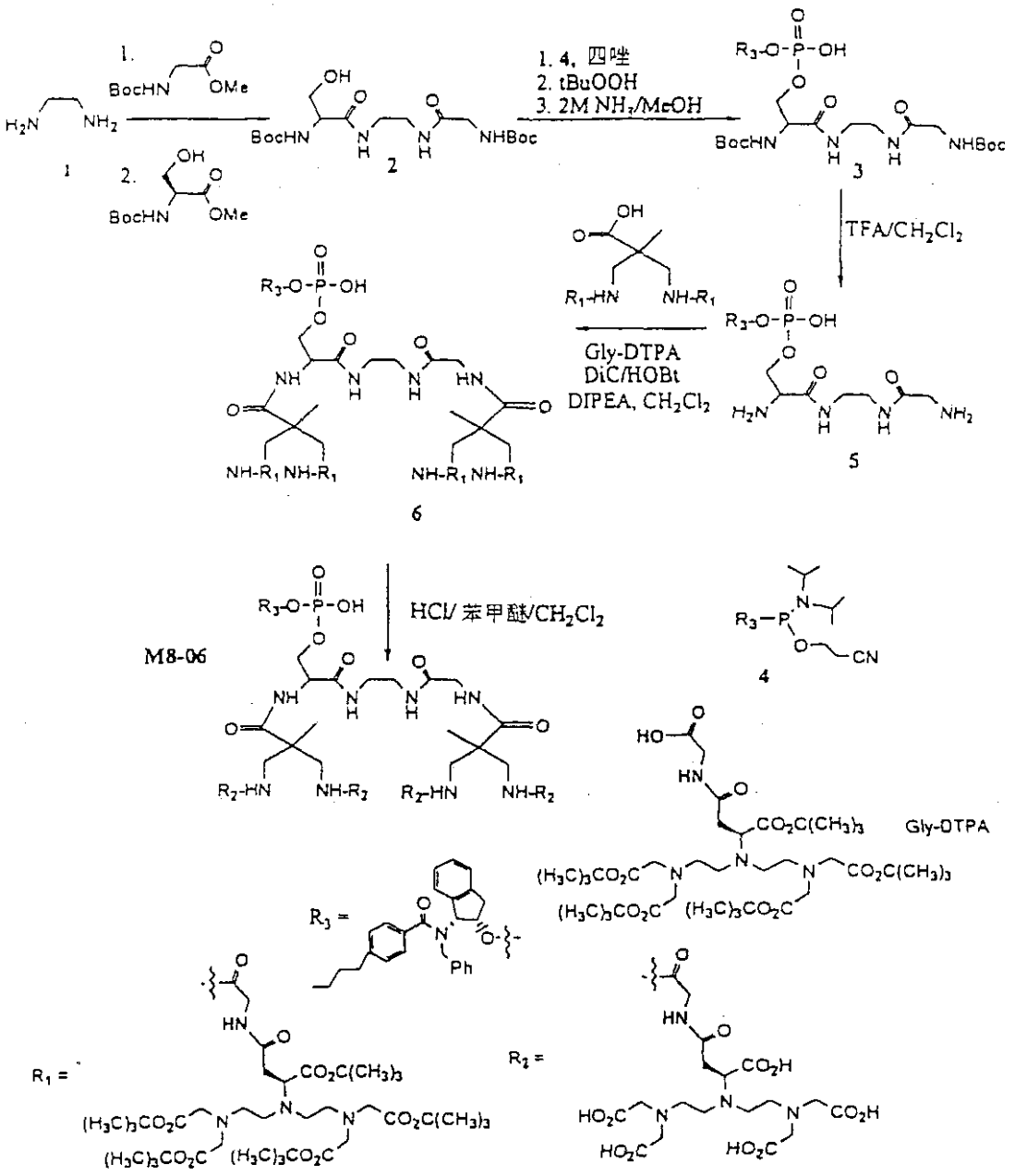
在含有 3,4-二氢吡喃(12g)和对甲苯磺酸(tosic acid)(20mg)的乙醚(250ml)中搅拌 3-丁炔-1-醇(10g)12 小时，然后真空浓缩此溶液，残留物在乙酸乙酯和水之间分配。用饱和氯化钠水溶液洗涤此有机溶液，硫酸钠干燥，并浓缩得到 DHP-保护的醇(18.7g)，其无需进一步鉴定或纯化。将 DHP-醇(18.7g)溶解于乙醚(65ml)中并冷却至-75℃，然后在此温度逐滴加入丁基锂(50.5ml 的 2.0M 溶液)，然后加入多聚甲醛(3.5g)。4 小时后，让此溶液升温至室温，加入水(100ml)。弃去水层，真空浓缩有机层得到油状物，快速层析(乙酸乙酯/己烷为洗脱液)纯化得到

5-THP-2-戊炔-1,5-醇(13g)，由 ^{1}H NMR 证明其结构。

首先用常规方法将炔醇(2.0g)转化成甲磺酸酯(882mg 甲磺酰氯，1.6ml 三乙胺，二氯甲烷)，然后再转化成碘化物(6.2g 碘化钠，无水丙酮)。在三 Boc-1,4,7,10-四氮杂环十二烷(200mg)和碳酸氢钠(200mg)的 8ml 乙腈溶液中搅拌此碘代炔(1.2g)1.5 小时，然后真空浓缩除去溶剂，并快速层析(乙酸乙酯/己烷为洗脱液)纯化，并由 ^{1}H NMR 和 LC-MS 证明此加合物(240mg)。40℃在 4：2：1 的乙酸：四氢呋喃：水(12ml)中搅拌将此加合物(240mg)6 小时来除去 THP 保护，然后用水和乙酸乙酯稀释此反应物，顺序用饱和碳酸氢钠水溶液和氯化钠提取有机层，硫酸钠干燥并浓缩得到醇(250mg)。用上述 M8-01 合成中所述的标准亚磷酰胺酸酯化学(四唑、叔丁基氢过氧化物、氨/甲醇)合成此醇与 1-对-丁基苯甲酰基-1-苄基氨基-2-茚满醇的磷酸二酯。

如上所述通过用酸(三氟乙酸的二氯甲烷溶液)处理除去 1,4,7,10-四氮杂环十二烷上的三个 Boc 保护基团，然后用标准方法将得到的三种胺用 Gly-DTPA-O-tBu 转化成酰胺(HATU/HOAt,二异丙基乙胺，二氯甲烷)。在 4：1：1 的盐酸：苯甲醚：二氯甲烷中搅拌 5 小时除去 DTPA 亚单元的叔丁酯以产生羧酸，然后真空除去溶剂，溶解于水中并冷冻干燥。按常规方法用 $GdCl_3$ 形成 DTPA-钆螯合物(参见 Lauffer R.B.,等人，Radiology 207:第 529-38 页(1998)]。

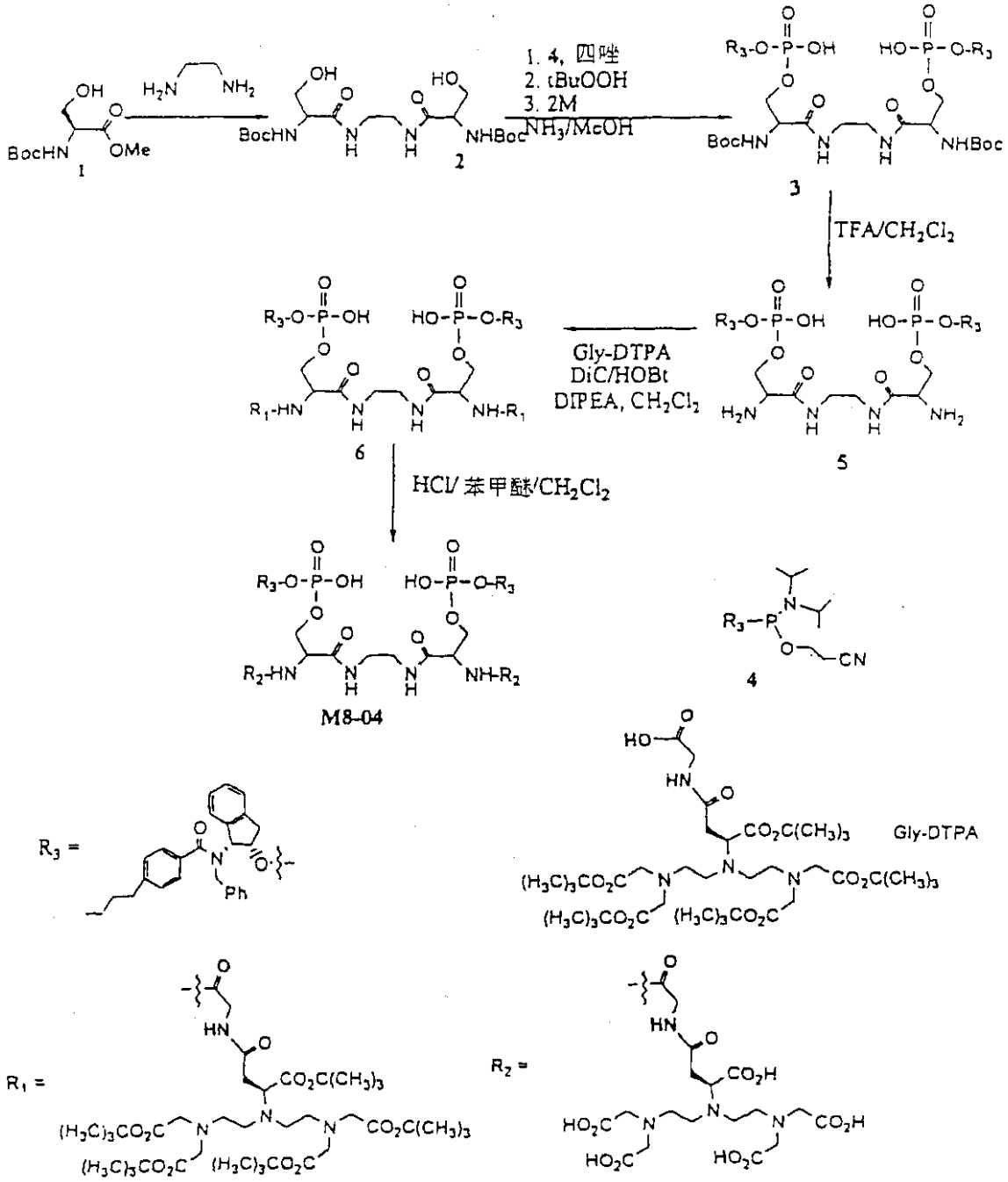
实施例 14：M8-06 的合成



顺序将乙二胺与 N-Boc-甘氨酸甲酯和 N-Boc-丝氨酸甲酯反应形成二酰胺。用上述方法(四唑、叔丁基氢过氧化物、氨/甲醇)将游离的醇和 1-对-丁基苯甲酰基-1-苄基氨基-2-茚满醇转化成磷酸二酯。用上述条件(DIC/HOBt,二异丙基乙胺、二氯甲烷)将苄基-(3-氨基-2-氨基甲基-2-甲基)丙酸酯和 2 当量的 Gly-DTPA-O-tBu 反应形成相应的二酰胺。2 当量此二酰胺与乙二胺衍生物反应以形成四聚体。在 4：1：1 的盐酸：苯甲醚：二氯甲烷中搅拌 5 小时除去 DTPA 亚单元的叔丁酯以产生羧酸，然后真空除去溶剂，溶解于水中并冷冻干燥。按常

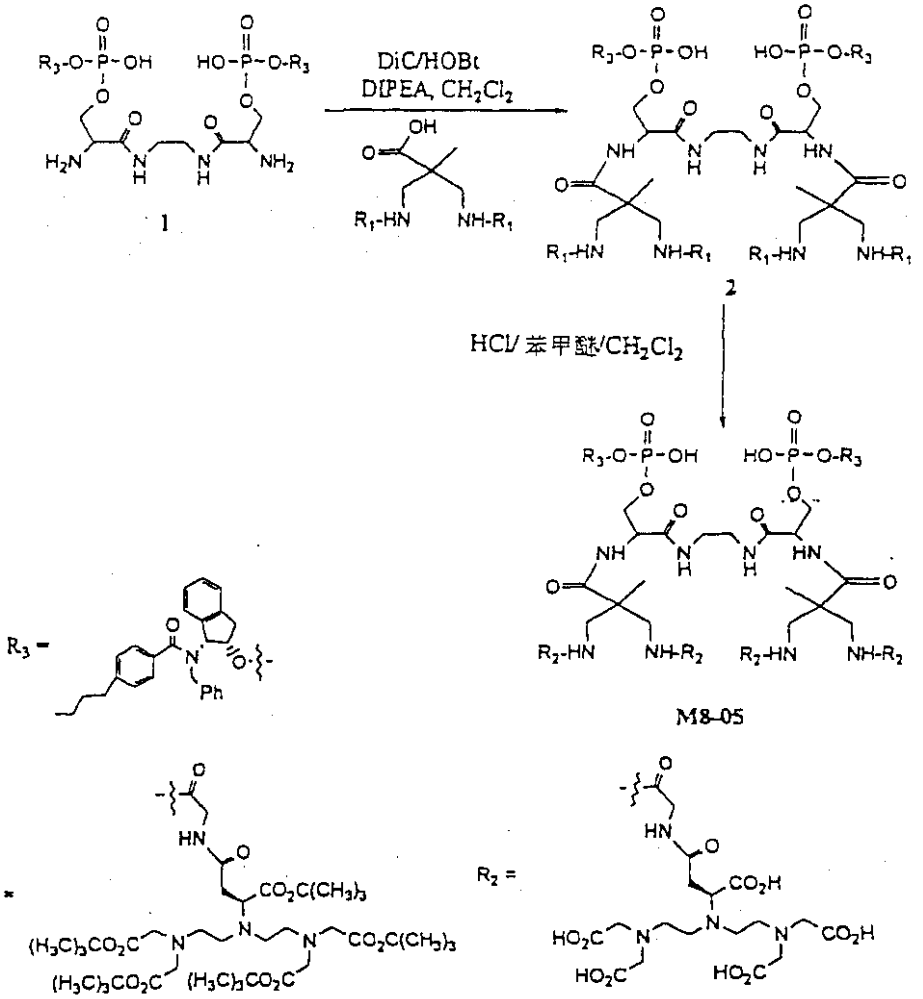
规方法用 $GdCl_3$ 形成 DTPA-钆螯合物(参见 Lauffer R.B.,等人，Radiology 207: 第 529-38 页(1998)]。

实施例 15：M8-04 的合成



将乙二胺与 2 当量 N-Boc-丝氨酸甲酯反应形成二酰胺。将此二酰胺进一步反应形成如上所示的二磷酸酯衍生物。按所示的合成流程并用实施例 14 所述的方法，连接 TBM 并去保护。

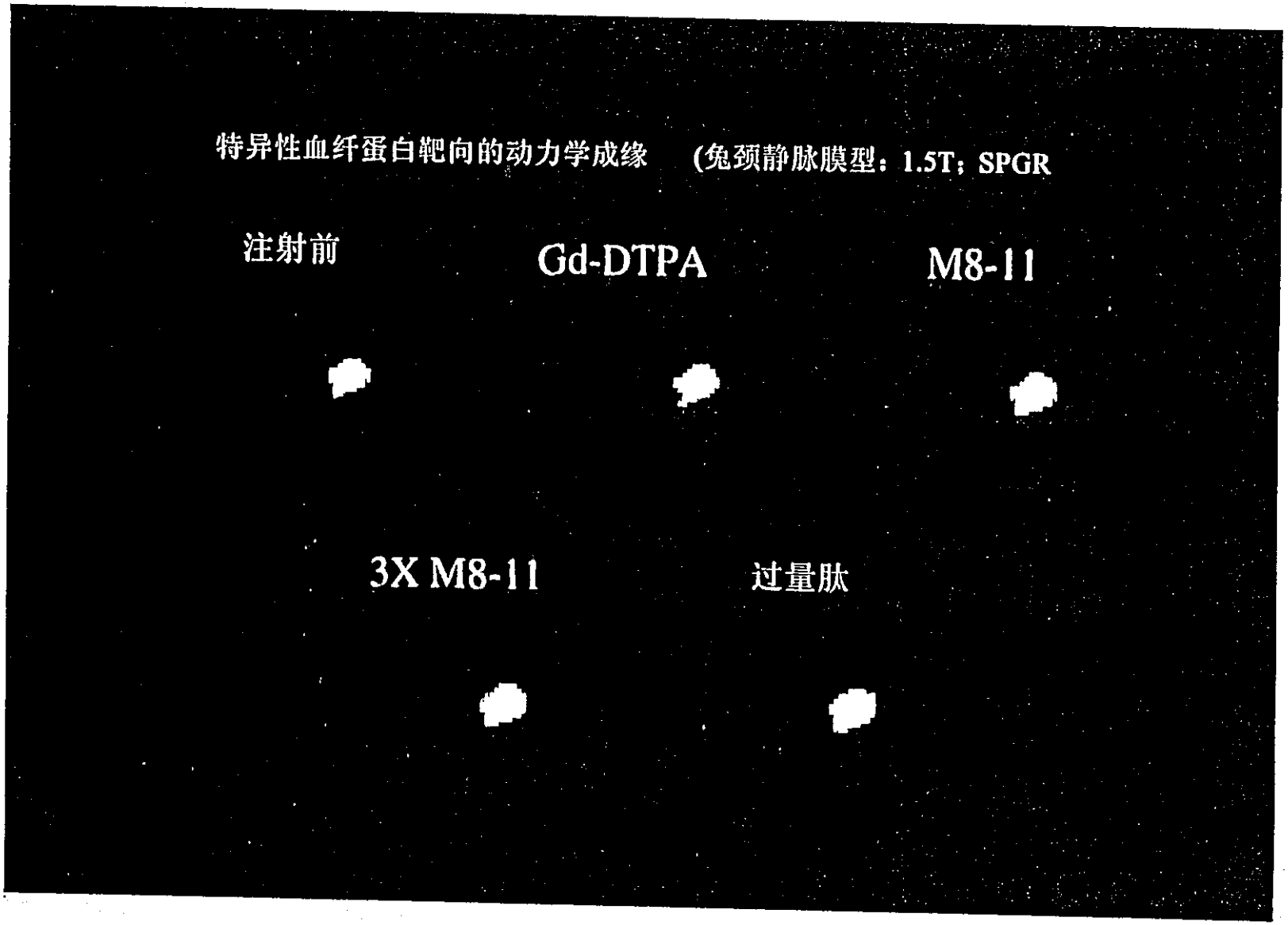
实施例 16：M8-05 的合成



在本合成中用作起始物的二酰胺的二磷酸衍生物与实施例 15 中所示的合成 M8-04 中的中间体相同。按所示的合成流程并用实施例 14 所述的方法，结合 TBM 并去保护。

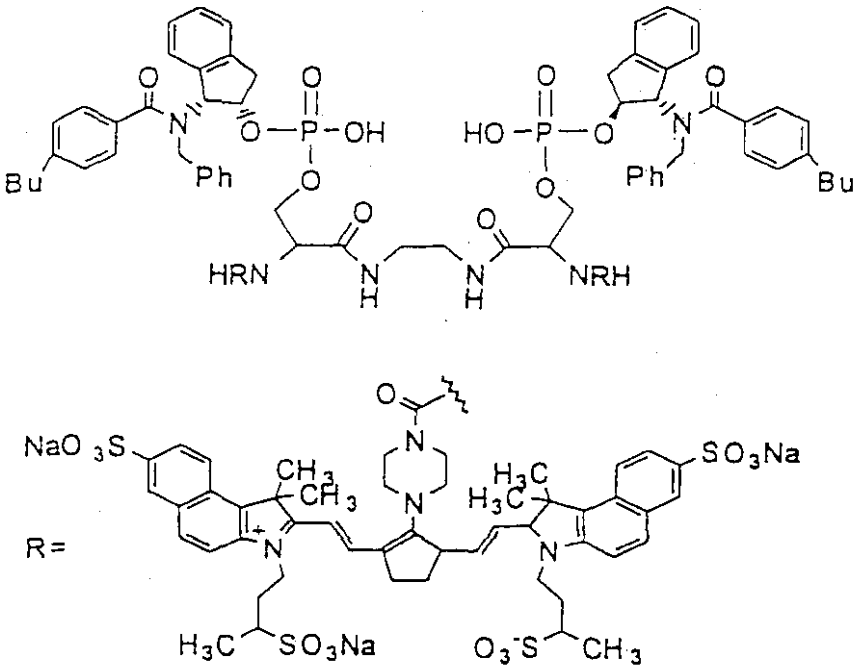
实施例 16-由兔颈静脉模型测定的 M8-11 的结合

图 15 是用实施例 1 所述的试验在一个实验中得到的成像的彩色照片。该实验显示了注射前、用对照非靶向的 Gd-DTPA 化合物注射、用实施例 3 给出的 M8-11 注射、用三倍剂量的 M8-11 注射和用过量序列为 LPCDYYGTCLD 的肽(以单字母氨基酸形式)注射(其与造影剂的 TBM 竞争靶标)的成像。因为无 TBM 的造影剂不结合并因为存在过量肽时结合逆转，由 M8-11 特异性成像凝块。


特异性血纤蛋白靶向的动力学成缘 (兔颈静脉膜型：1.5T；SPGR
注射前
Gd-DTPA
M8-11
3X M8-11
过量肽

实施例 16-近红外光学成像剂

M8-24



近红外荧光成像剂 M8-24 含有适用于光学成像的 IEM(“R”)。由相应荧光染料的羧酸衍生物(如 WO 2000/16810 中所公开的)制备该试剂。按实施例 13 中所示的条件将羧酸衍生物结合于二酰胺的二磷酸衍生物。在实施例 13 中，类似步骤是 Gly-DTPA TBM 结合于二酰胺的二磷酸酯衍生物形成 M8-04。这种白蛋白靶向的红外线造影剂可用于如眼科血管造影和皮肤癌的诊断中。上述光学造影剂可用于 MRI 血池造影剂的任何用途中。另外，技术人员将理解通过构建染料的不同羧酸衍生物，这种光学造影剂的 IEM 可以多种多样。因此这种试剂是适应特定实验标准如特定的激发波长所特制的。