



- (51) International Patent Classification:
A61K 49/06 (2006.01) *A61B 5/055* (2006.01)
A61K 49/18 (2006.01)
- (21) International Application Number:
PCT/AU2012/000415
- (22) International Filing Date:
20 April 2012 (20.04.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
2011901492 20 April 2011 (20.04.2011) AU
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: NUCLEAR MAGNETIC RESONANCE AGENT

(57) Abstract: The invention provides a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.



WO 2012/142670 A1

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NUCLEAR MAGNETIC RESONANCE AGENT

Field of the Invention

- 5 The present invention relates in general to nuclear magnetic resonance (NMR) agents. In particular, the invention relates to NMR agents for use in medical applications such as magnetic resonance imaging (MRI).

Background of the Invention

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NMR is an effect whereby magnetic nuclei in a magnetic field absorb and then re-emit electromagnetic energy. When a given nuclei absorbs electromagnetic energy it is described as being on resonance. Different nuclei within a molecule resonate at different frequencies for a given magnetic field strength, which in turn enables information to be
15 obtained about chemical and structural features of the molecule. The ability to derive such molecular information has seen NMR become an invaluable analytical technique used in a diverse array of applications.

The application of NMR in medical applications such as MRI has promoted substantial
20 advances in medical diagnosis. MRI is a non-invasive technique that provides anatomical information about a subject.

However, techniques for performing MRI are prone to relatively low sensitivity. Such low sensitivity can be offset by increased scan times, but this approach is often not practical.
25 For example, it can be difficult if not impossible to have a subject remain stationary for an extended period of time in order to obtain a suitable image. Furthermore, with significant demand for MRI, coupled with limited MRI facilities, extended image acquisition time frames are simply not desirable.

30 Depending upon the region of a subject to be imaged, conventional MRI can also be limited by its ability to distinguish certain anatomical features.

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Such limitations have led to the development of NMR agents such as contrast agents. Contrast agents can be used in MRI to improve the visibility of anatomical features and can also decrease the timeframe in which a suitable image may be obtained.

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The most common form of contrast agent falls into a class known as a "relaxation" agent. These types of agents function by altering the relaxation times (longitudinal relaxation time T_1 , and/or transverse relaxation time T_2) of nearby water molecules located in tissue, etc. Depending on the type of agent and imaging sequence, such contrast agents can
10 promote a brightening or darkening response in the resulting image. Such agents are typically based on gadolinium complexes or iron oxide particles.

Despite representing a major advance in MRI technology, relaxation contrast agents are not without their limitations. For example, the contrast in anatomical features provided by
15 the agent can be subtle and difficult to interpret. Gadolinium based contrast agents also present toxicity concerns.

Conventional contrast agents are also somewhat limited in the information that can be derived from their use in that they only in effect provide information relating to their
20 general location in a subject.

An opportunity therefore remains to address or ameliorate one or more disadvantages or shortcomings associated with conventional NMR agents and/or their use in medical applications such as MRI, or at least to provide a useful alternative NMR agent and/or
25 method of using such an agent in medical applications such as MRI.

Summary of the Invention

The present invention therefore provides a switchable nuclear magnetic resonance (NMR)
30 agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a

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polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

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It has now been found that an effective and versatile NMR agent can be provided using polymer having covalently coupled thereto one or more specific NMR nuclides selected from D, B, F, Br, P and Si. Those skilled in the art would appreciate that these specific nuclides are NMR visible in their own right and as such the agents in accordance with the invention may be categorised as "direct" signalling or imaging agents. In other words, this class of agent functions differently from the aforementioned "relaxation" agents.

Importantly, the NMR agents in accordance with the invention are switchable in the sense that they (1) present a first form in which the NMR signal of the specified nuclides is diminished, and (2) by undergoing a chemical reaction switch from the first form into a second form in which the NMR signal of the specified nuclides is amplified. In other words, despite being NMR visible and forming part of the agent, the specified nuclides have a low if not absent NMR signal in the first form of the agent, and when the first form of the agent undergoes a chemical reaction it is transformed or switched into a second form of the agent in which the NMR signal of the specified nuclides is increased or appears.

By presenting such unique properties an NMR agent in accordance with the invention can not only provide for a direct NMR signal from the specified nuclides from at least the second form of the agent, but the point in time and location of a chemical reaction can be detected as a result of the agent switching or being transformed from the first form to its second form. In medical applications such as MRI, the agent can therefore not only be used for conventional imaging, but also to image in real time the location of specific biochemical events *in vivo*. The agent may also be designed such that the chemical reaction which promotes switching between its first form and second form also promotes release of a pharmaceutically active agent such as a drug associated with the agent. In that case, the invention enables sophisticated imaging of drug release therapies. Such an

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approach advantageously allows for non-invasive and potentially quantitative analysis of drug release, accumulation, distribution at a site and its efficacy *in vivo*.

These and further applications of the invention are discussed in more detail below.

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In one embodiment, the NMR agent in accordance with the invention is capable of undergoing a chemical reaction in a biological system, said chemical reaction causing the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

10

The biological system may be *in vivo* as in a subject, or it may be *ex vivo* or even *in vitro*.

The present invention also provides a method of detecting a chemical reaction in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing the chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to detect occurrence of the chemical reaction.

The present invention further provides a method of obtaining an image of a region of interest in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing a chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to obtain the image.

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In one embodiment, the medium in which the chemical reaction takes place, or image of a region of interest is obtained, is a biological system.

- 5 The present invention also provides a composition comprising a switchable nuclear magnetic resonance (NMR) agent according to the invention and at least one pharmacologically acceptable carrier.

The present invention further provides a composition for magnetic resonance imaging
10 (MRI) comprising a switchable nuclear magnetic resonance (NMR) agent according to the invention and at least one pharmacologically acceptable carrier.

The present invention also provides use of a switchable nuclear magnetic resonance (NMR) agent according to the invention in a medium.

15

The present invention further provides use of a switchable nuclear magnetic resonance (NMR) agent according to the invention as a magnetic resonance imaging (MRI) agent.

The present invention also provides use of a switchable nuclear magnetic resonance
20 (NMR) agent according to the invention in obtaining an image of a region of interest in a medium.

In another embodiment, the NMR agent further comprises a pharmaceutically active agent.

- 25 In a further embodiment, the NMR agent comprises a plurality of covalently coupled F NMR nuclides.

The specified NMR nuclides are covalently coupled to a polymer chain. The polymer chain will generally be a synthetic polymer chain. In one embodiment, the polymer chain
30 forms part of a microgel or a dendritic polymer. The dendritic polymer may be a dendrimer or a hyperbranched polymer.

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The NMR agent may comprise a plurality of polymer chains.

The NMR agent may comprise a plurality of polymer chains that are associated with each other, for example by way of crosslinking. Such polymer chains may form what is commonly known in the art as a microgel.

The NMR agent in accordance with the invention may conveniently be referred to as a polymeric NMR agent in the sense that the agent that provides for the first and second forms has a polymeric structure. The polymeric structure of the agent may comprise other components such as a pharmaceutically active agent, the likes of which may be covalently bound thereto or physically constrained therein (for example as in encapsulation).

The NMR agent may also comprise one or more radioactive isotopes suitable for use in nuclear medical procedures such as nuclear medical imaging (e.g., positron emission tomography (PET)).

Further aspects and embodiments of the invention are described in more detail below.

20 Brief Description of the Drawings

The invention will herein be described with reference to the following drawings in which:

Figure 1 illustrates ^{19}F images of solutions of polymer wherein the fluorinated segments are in a hydrophobically-trapped environment (acetal protected glycerol methacrylate) (1a) and the resulting image of the same solution following deprotection of the acetal group such that the fluorinated segments are in a hydrophilic environment (1b).

Detailed Description of the Invention

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The present invention provides for a switchable NMR agent. By being "switchable" is

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meant that the agent is capable of being transformed from a first form into a second form as described herein. In accordance with the invention, the transition or switching of the first form into the second form is promoted through the first form of the agent undergoing a chemical reaction. Further detail in relation to the nature of such a chemical reaction is
5 discussed below.

By the NMR agent being "capable" of being transformed from a first form into a second form as described herein is therefore a reference to the agents "switchable" characteristic. Accordingly, the word "capable" is a reference to the agent's latent ability to be
10 transformed from its first form to its second form.

In other words, the agent has latent ability to undergo a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.
15

Or in other words, the agent has potential to undergo a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

20 In yet further words, upon undergoing a chemical reaction the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified.

In still further words, a chemical reaction will cause the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.
25

The NMR agent comprises a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si. By being "covalently coupled" is meant that one or more of said nuclides is covalently coupled, either directly or indirectly, to a polymer chain. Where only one of said nuclides is coupled to a polymer chain, it will be
30 appreciated that the NMR agent in that case must comprise one or more additional polymer chains having at least one or more of said nuclides covalently coupled thereto (i.e. because

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the NMR agent itself must comprise a plurality of said nuclides). The NMR agent may of course comprise a single polymer chain having a plurality of said nuclides covalently coupled thereto.

- 5 Those skilled in the art will appreciate that the NMR nuclides denoted by the atomic symbols D, B, F, Br, P and Si represent the elements deuterium, boron, fluorine, bromine, phosphorus and silicon, respectively.

- Both F and P have a 100% natural abundance and can be more technically represented as
10 ^{19}F and ^{31}P , respectively. The NMR visible nuclide of Si is ^{29}Si which has a 4.7% natural abundance. The nuclide D may also be represented as ^2H and has a natural abundance of only 0.02%. B has two NMR visible nuclides with ^{11}B being the most suitable for use in accordance with the invention and having a natural abundance of 80.4%. Br also has two NMR visible nuclides, with both ^{79}Br and ^{81}Br being suitable for use in accordance with
15 the invention and having a natural abundance of 50.5% and 49.5%, respectively.

NMR nuclides suitable for use in accordance with the invention may therefore also be represented as ^2H , ^{11}B , ^{19}F , ^{79}Br , ^{81}Br , ^{31}P , ^{29}Si .

- 20 For convenience, unless stated otherwise reference hereinafter to "nuclide", "nuclides", "the nuclides" and "said nuclides" or grammatical variances thereof is intended to be a reference to one or more of ^2H , ^{11}B , ^{19}F , ^{79}Br , ^{81}Br , ^{31}P , ^{29}Si .

- Where the NMR agent comprises a nuclide that does not have 100% natural abundance,
25 that nuclide may nevertheless be present in the agent at a relative % abundance higher than its natural abundance due to the agent being prepared using reagents having an enriched abundance of the nuclide.

- Those skilled in the art will appreciate that F, D and Br are monovalent nuclides, whereas
30 B, P and Si are multivalent nuclides. In the case of the multivalent nuclides, in addition to being covalently coupled to a polymer chain the remaining valence sites will of course be

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appropriately occupied by a suitable substituent. For example, the remaining valence sites may be occupied by one of the same or different nuclides.

In one embodiment, the NMR agent comprises a plurality of coupled F NMR nuclides.

5

In a further embodiment, the NMR agent does not comprise an NMR nuclide selected from D, B, Br, P and Si.

Important features of the NMR agents in accordance with the invention are that (1) they
10 have a first form in which the NMR signal of said nuclides is diminished, and (2) the first form of the agent is capable of undergoing a chemical reaction to afford a second form of the agent in which the NMR signal of said nuclides is amplified.

For avoidance of any doubt, reference to the NMR signal of said nuclides being
15 "diminished" and "amplified" is intended to be a relative measureable NMR signal difference between the first and second forms of the agent. In other words, the amplified signal is amplified relative to the signal afforded by the first form of the agent and the diminished signal is diminished relative to the NMR signal afforded by the second form of the agent.

20

The diminished NMR signal of the first form of the agent may be diminished to such an extent that it is undetectable. The diminished NMR signal may also present as a low intensity signal. It will be appreciated that what is meant by an undetectable or low
25 intensity signal is that the signal attained within an acceptable time frame is not adequate for practical application. Conversely, it will be appreciated that what is meant by an amplified signal is that the signal attained within an acceptable time frame is, if not more than, adequate for practical application.

Where the first form of the agent has an undetectable NMR signal, as will be discussed in
30 more detail below, the NMR signal of the nuclides is in effect being suppressed. In that case, reference to an undetectable NMR signal being "amplified" is meant the suppression

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effect is removed such that the nuclides become detectable. In other words, the nuclide NMR signal is "amplified" in the sense that the otherwise NMR visible nuclides transition from having an undetectable signal (i.e. in the first form of the agent) into having a detectable signal (i.e. in the second form of the agent).

5

The NMR agent in accordance with the invention comprises a plurality of the nuclides. Generally, the agent will comprise per polymer chain an amount of nuclides ranging from about 0.3-15 mol% nuclides, for example about 1-10 mol% nuclides, or about 2-6 mol% nuclides.

10

In one embodiment, the NMR signal of said nuclides is diminished by intramolecular mobility confinement of said nuclides, and the NMR signal of said nuclides is amplified by intramolecular mobility liberation of said nuclides.

15 In other words, the invention provides for a switchable polymeric nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished by intramolecular mobility confinement of said
20 nuclides, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified by intramolecular mobility liberation of said nuclides.

Reference herein to the expression "intramolecular mobility" is intended to mean the
25 molecular motion a given atom or substituent group of a molecule may experience relative to another atom or substituent group of that same molecule. For example, an atom or a substituent group that forms part of a polymer chain will have a degree of mobility relative to other atoms or substituent groups that form part of the polymer chain.

30 According to the present invention the NMR signal of the nuclides may be diminished by intramolecular mobility "confinement of said nuclides". In other words, the diminished

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NMR signal of the nuclides is caused by the intramolecular mobility of the nuclides being confined in some way. It will be appreciated that such confinement of mobility is not intended to merely mean that a nuclide is covalently bound to the polymer chain. Confinement of the nuclides must therefore be confinement of their intramolecular mobility above simply being covalently coupled to the polymer chain.

Similarly, the NMR signal of the nuclides is amplified by intramolecular mobility "liberation of said nuclides". In that case, confinement of the nuclides is reduced or removed to thereby liberate and promote the intramolecular mobility of the nuclides.

10 Given that intramolecular mobility of the nuclides is to be retained, it will be appreciated that liberation of this mobility does not embrace a situation where the nuclides are cleaved from the polymer chain.

Conceptually, intramolecular mobility confinement of the nuclides may therefore be

15 considered to be where the molecular motion of the nuclides relative to the remainder of the polymer chain is suppressed. Conversely, intramolecular mobility liberation of said nuclides may be considered to be where suppressed molecular motion of the nuclides relative to the polymer chain is reduced or removed.

20 Those skilled in the art will appreciate that molecular motion of a given nuclide plays an important part in the ability of the nuclides to produce an NMR signal. Without wishing to be limited by theory, restricted intramolecular motion is believed to promote an increase in spin-spin relaxation of a nuclide, the effect of which is to diminish an NMR signal of that nuclide. Conversely, more free intramolecular motion of a nuclide is believed to promote

25 a decrease in spin-spin relaxation of the nuclide which in turn gives rise to an amplified NMR signal of that nuclide.

In one embodiment, the NMR signal of said nuclides may therefore be diminished by intramolecular mobility confinement of said nuclides which causes an increase in spin-spin

30 relaxation of said nuclides, and the NMR signal of said nuclides is amplified by intramolecular mobility liberation of said nuclides which causes a decrease in spin-spin

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relaxation of said nuclides.

Without wishing to be limited by theory, it is believed that by promoting intramolecular mobility confinement and intramolecular mobility liberation of nuclides in accordance
5 with the invention, the nuclides provide for a diminished or amplified NMR signal as a result of an increase or decrease in dipole-dipole or quadrupolar interactions of the nuclides.

In other words, confinement and liberation of the nuclides effects the spin-spin relaxation
10 of said nuclides, with this effect on the spin-spin relaxation being promoted by dipole-dipole or quadrupolar interactions of the nuclides.

In a further aspect, the present invention therefore provides a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides
15 selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished by an increase in dipole-dipole or quadrupolar interactions of said nuclides, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the
20 NMR signal of said nuclides is amplified by a decrease in dipole-dipole or quadrupolar interactions of said nuclides.

For the most part, the intensity of an NMR signal is believed to be dictated by the mobility of a given nuclide. This is particularly true for polymer chains according to the present
25 invention in which the molecular motion of a nuclide is limited through being covalently bound to a relatively high molecular weight species (relative to small molecules). As molecular motion of the nuclide slows down or becomes confined, the NMR signal diminishes until a point is reached whereby the nuclide presents as a "solid-like material" where the NMR signal can become undetectable.

30

Those skilled in the art will appreciate that an important NMR parameter associated with

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mobility of a given nuclide and NMR signal intensity is the so-called spin-spin relaxation time T_2 . The value of T_2 (in units of time) is a measure of the range of local magnetic fields experienced by a nuclide in an NMR analysis. The NMR signal intensity is directly related to the inverse of the T_2 . In general, small molecules presenting nuclides with
5 relatively unconfined mobility exhibit a high T_2 and may be of the order of several seconds in duration. In contrast, nuclides in a rigid solid have confined mobility and as such typically exhibit relatively short T_2 values in the order of only several micro seconds. Depending upon the degree of molecular motion, T_2 values between these two extremes are possible.

10

Those skilled in the art will also appreciate that the NMR parameter T_2 is strongly influenced by a number of intra- and inter-nuclear interactions. Such interactions can be categorised into three classes, namely dipole-dipole couplings, quadrupolar couplings and electron-nuclear couplings.

15

In the context of intramolecular mobility of a given nuclide, dipole-dipole and quadrupolar couplings are believed to play an important role in influencing the signal intensity of the nuclides.

20 In contrast, electron-nuclear couplings are believed to influence NMR signal intensity when a source of free electrons comes sufficiently close to interact with a given nuclide. Increased interaction results in diminishing the NMR signal of the nuclide even if its intramolecular mobility is not confined. The source of free electrons may be provided by metallic species or so-called paramagnetic species which possess un-paired electrons.

25

The diminished and amplified NMR signal that operates in accordance with the invention is believed to result predominantly (i.e. greater than 50%, or 60%, or 80%) through dipole-dipole and/or quadrupolar couplings.

30 Of the nuclides used in accordance with the invention, F, P and Si are believed to be primarily influenced through dipole-dipole couplings, whereas D, B and Br are believed to

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be primarily influenced through quadrupolar couplings.

In the context of the nuclides, the terms "interactions" and "couplings" may be used interchangeably.

5

In one embodiment, the NMR signal provided for and in accordance with the invention is not diminished or amplified through electron-nuclear interactions. In other words, in one embodiment the NMR agent in accordance with the invention does not comprise a source of free electrons such as that which may be found in metallic species or paramagnetic species.

10

The nuclides used in accordance with the invention are covalently coupled to a polymer chain. The nuclides may be covalently coupled directly to the polymer backbone of the polymer chain or they may be indirectly covalently coupled to the polymer chain through one or more atoms or substituent groups.

15

The polymer chain will generally be a synthetic polymer chain. By the polymer chain being "synthetic" is meant that the polymer does not have a structure corresponding to a naturally occurring polymer such as, for example, a peptide. Accordingly, a "synthetic" polymer is not intended to embrace structures of naturally occurring polymers derived from biomolecules such as amino acids, carbohydrates and nucleic acids.

20

A polymer chain used in accordance with the invention will generally have a number average molecular weight (M_n) ranging up to about 10,000 kg/mol, for example from about 2-500 kg/mol, or from about 4-200 kg/mol.

25

As used herein, M_n values are those determined by size exclusion chromatography coupled with a multi-angle laser light scattering (MALLS) detector and a refractive index detector to provide absolute molecular weights and size distributions.

30

It has been found that synthetic polymer chemistry may be used to effectively and

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efficiently control structural features of polymer chains in accordance with the invention to enhance the "switchability" of the agents.

5 Provided that the requirements of the NMR agent in accordance with the invention are met, there is no particular limitation concerning the polymerisation technique that can be used to prepare polymer chains used in accordance with the invention.

10 In one embodiment, the polymer chains are prepared by condensation polymerisation. The condensation polymerisation may be performed using the appropriate combination of poly-carboxylic acids (and ester derivatives thereof), poly-ols, poly-amines, poly-acyl chlorides, lactones and hydroxy-acids.

15 In the context of condensation polymerisation, reference to "poly-" carboxylic acids (and ester derivatives thereof), ols, amines and acyl chlorides is intended to mean that such monomers have two or more carboxylic acid (and ester derivatives thereof), alcohol, amine, acyl chloride groups, respectively.

20 Condensation polymerisation is a form of step-growth polymerisation where monomers react to form polymer and in doing so release a low molecular weight species such as water or methanol. Such reactions are commonly performed using a catalyst. Examples of condensation catalysts include Lewis acids such as antimony trioxide, titanium oxide and dibutyl tin dilaurate.

25 In another embodiment, the polymer chains used in accordance with the invention are derived from ethylenically unsaturated monomers. Polymerisation of the ethylenically unsaturated monomers may be conducted using a living or non-living polymerisation technique.

30 Living polymerisation is generally considered in the art to be a form of chain polymerisation in which irreversible chain termination is substantially absent. An important feature of living polymerisation is that polymer chains will continue to grow

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while monomer and reaction conditions to support polymerisation are provided. Polymer chains prepared by living polymerisation can advantageously exhibit a well defined molecular architecture, a predetermined molecular weight and narrow molecular weight distribution or low polydispersity.

5

Examples of living polymerisation include ionic polymerisation and controlled radical polymerisation (CRP). Examples of CRP include, but are not limited to, iniferter polymerisation, stable free radical mediated polymerisation (SFRP), atom transfer radical polymerisation (ATRP), and reversible addition fragmentation chain transfer (RAFT) polymerisation.

10

In one embodiment of the invention, the polymer chains are prepared using a living polymerisation technique. Equipment, conditions, and reagents for performing living polymerisation are well known to those skilled in the art.

15

Where ethylenically unsaturated monomers are to be polymerised by a living polymerisation technique, it will generally be necessary to make use of a so-called living polymerisation agent. By "living polymerisation agent" is meant a compound that can participate in and control or mediate the living polymerisation of one or more ethylenically unsaturated monomers so as to form a living polymer chain (i.e. a polymer chain that has been formed according to a living polymerisation technique).

20

Living polymerisation agents include, but are not limited to, those which promote a living polymerisation technique selected from ionic polymerisation and CRP.

25

In one embodiment of the invention, the polymer chain is prepared by ionic polymerisation.

30

Living ionic polymerisation is a form of addition polymerisation whereby the kinetic-chain carriers are ions or ion pairs. The polymerisation proceeds via anionic or cationic kinetic-chain carriers. In other words, the propagating species will either carry a negative or

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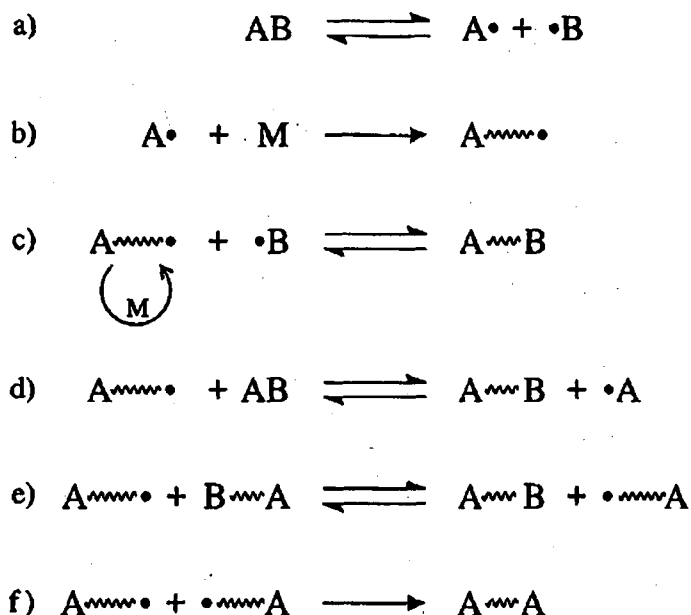
positive charge, and as such there will also be an associated counter cation or counter anion, respectively. For example, in the case of anionic polymerisation, the living polymerisation agent might be represented as $I^{\ominus}M^{\oplus}$, where I represents an organo-anion (e.g. an optionally substituted alkyl anion) and M represents an associated countercation, or in the case of living cationic polymerisation, the living polymerisation agent might be represented as $I^{\oplus}M^{\ominus}$, where I represents an organo-cation (e.g. an optionally substituted alkyl cation) and M represents an associated counteranion. Suitable agents for conducting anionic and cationic living polymerisation are well known to those skilled in the art and include, but are not limited to, aprotic acids (e.g. aluminium trichloride, boron trifluoride), protonic (Bronstead) acids, stable carbenium-ion salts, organometallic compounds (e.g. N-butyl lithium, cumyl potassium) and Ziegler-Natta catalysts (e.g. triethyl aluminium and titanium tetrachloride).

In one embodiment of the invention, the polymer chain is prepared by CRP.

In a further embodiment of the invention, the polymer chain is prepared by iniferter polymerisation.

Iniferter polymerisation is a well known form of CRP, and is generally understood to proceed by a mechanism illustrated below in Scheme 1.

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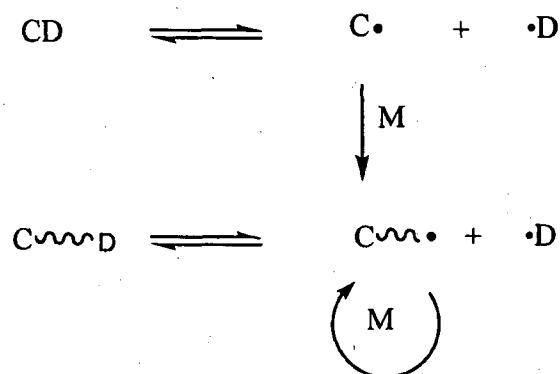
Scheme 1: General mechanism of controlled radical polymerisation with iniferters.

With reference to Scheme 1, the iniferter agent AB dissociates chemically, thermally or photochemically to produce a reactive radical species A and generally a relatively stable radical species B (for symmetrical iniferters the radical species B will be the same as the radical species A) (step a). The radical species A can initiate polymerisation of monomer M (in step b) and may be deactivated by coupling with radical species B (in step c). Transfer to the iniferter (in step d) and/or transfer to dormant polymer (in step e) followed by termination (in step f) characterise iniferter chemistry. Suitable iniferter agents are well known to those skilled in the art, and include, but are not limited to, dithiocarbonate, disulphide, and thiuram disulphide compounds.

In a further embodiment of the invention, the polymer chains are prepared by SFRP.

As suggested by its name, this mode of radical polymerisation involves the generation of a stable radical species as illustrated below in Scheme 2.

- 19 -



Scheme 2: General mechanism of controlled radical polymerisation with stable free radical mediated polymerisation.

5

With reference to Scheme 2, SFRP agent CD dissociates to produce an active radical species C and a stable radical species D. The active radical species C reacts with monomer M, which resulting propagating chain may recombine with the stable radical species D. Unlike iniferter agents, SFRP agents do not provide for a transfer step. Suitable agents for conducting SFRP are well known to those skilled in the art, and include, but are not limited to, moieties capable of generating phenoxy and nitroxy radicals. Where the agent generates a nitroxy radical, the polymerisation technique is more commonly known as nitroxide mediated polymerisation (NMP).

10

15 Examples of SFRP agents capable of generating phenoxy radicals include those comprising a phenoxy group substituted in the 2 and 6 positions by bulky groups such as tert-alkyl (e.g. t-butyl), phenyl or dimethylbenzyl, and optionally substituted at the 4 position by an alkyl, alkyloxy, aryl, or aryloxy group or by a heteroatom containing group (e.g. S, N or O) such dimethylamino or diphenylamino group. Thiophenoxy analogues of such phenoxy containing agents are also contemplated.

20

SFRP agents capable of generating nitroxy radicals include those comprising the substituent $\text{R}^1\text{R}^2\text{N-O}\cdot$, where R^1 and R^2 are tertiary alkyl groups, or where R^1 and R^2 together with the N atom form a cyclic structure, preferably having tertiary branching at the positions α to the N atom. Examples of such nitroxy substituents include 2,2,5,5-

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- 20 -

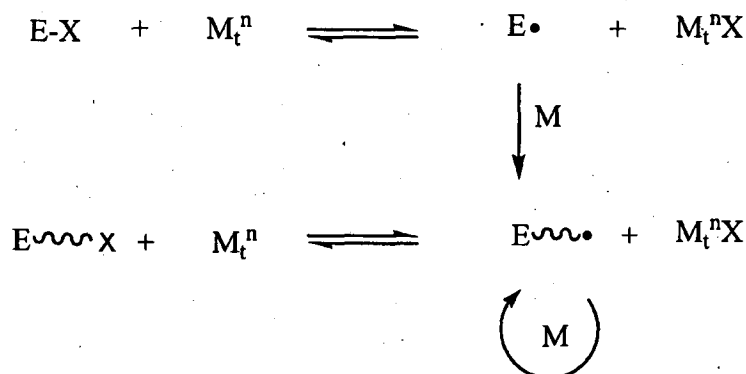
tetraalkylpyrrolidinoxyl, as well as those in which the 5-membered heterocycle ring is fused to an alicyclic or aromatic ring, hindered aliphatic dialkylaminoxyl and iminoxyl substituents. A common nitroso substituent employed in SFRP is 2,2,6,6-tetramethyl-1-piperidinoxyl.

5

In another embodiment of the invention, the polymer chain is prepared by ATRP.

ATRP generally employs a transition metal catalyst to reversibly deactivate a propagating radical by transfer of a transferable atom or group such as a halogen atom to the propagating polymer chain, thereby reducing the oxidation state of the metal catalyst as illustrated below in Scheme 3.

10



Scheme 3: General mechanism of controlled radical polymerisation with atom transfer radical polymerisation.

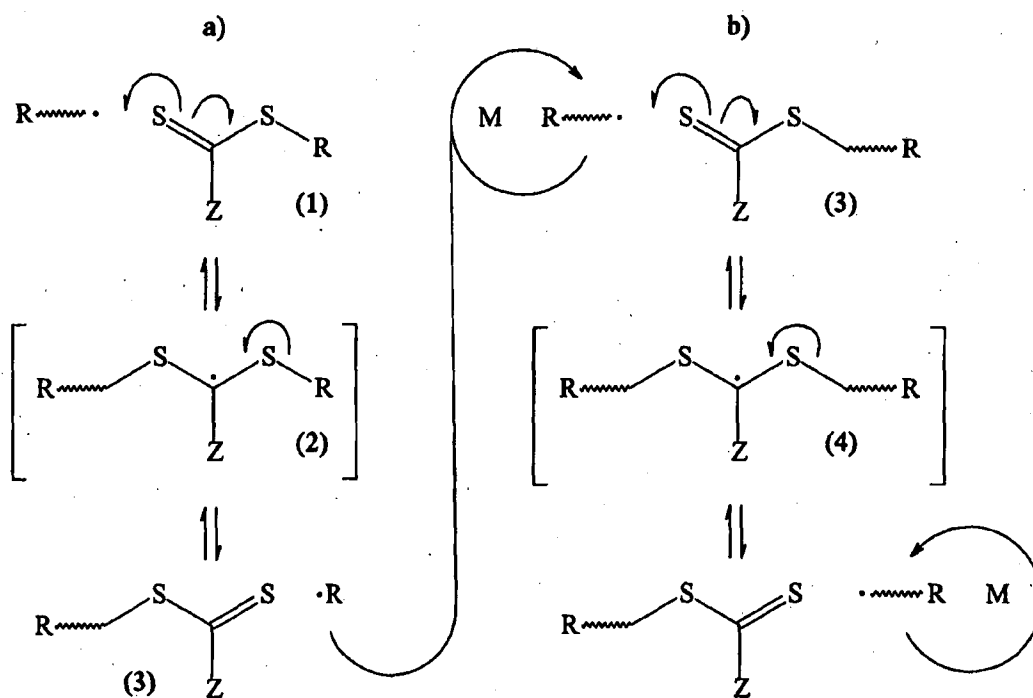
With reference to Scheme 3, a transferable group or atom (X, e.g. halide, hydroxyl, C₁-C₆-alkoxy, cyano, cyanato, thiocyanato or azido) is transferred from the organic compound (E) to a transition metal catalyst (M_t, e.g. copper, iron, gold, silver, mercury, palladium, platinum, cobalt, manganese, ruthenium, molybdenum, niobium, or zinc) having oxidation number (n), upon which a radical species is formed that initiates polymerisation with monomer (M). As part of this process, the metal complex is oxidised (M_tⁿ⁺¹X). A similar reaction sequence is then established between the propagating polymer chain and the dormant X end-capped polymer chains.

25

- 21 -

In a further embodiment of the invention, the polymer chain is prepared by RAFT polymerisation.

- 5 RAFT polymerisation is well known in the art and is believed to operate through the mechanism outlined below in Scheme 4.



- 10 **Scheme 4:** General mechanism of controlled radical polymerisation with reversible addition fragmentation chain transfer polymerisation.

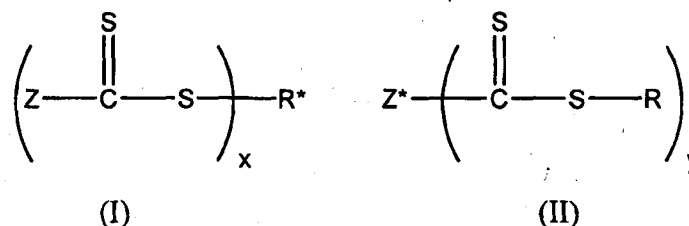
With reference to Scheme 4, RAFT polymerisation is believed to proceed through initial reaction sequence (a) that involves reaction of a RAFT agent (1) with a propagating
 15 radical. The labile intermediate radical species (2) that is formed fragments to form a temporarily deactivated dormant polymer species (3) together a radical (R) derived from the RAFT agent. This radical can then promote polymerisation of monomer (M), thereby

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reinitiating polymerisation. The propagating polymer chain can then react with the dormant polymer species (3) to promote the reaction sequence (b) that is similar to reaction sequence (a). Thus, a labile intermediate radical (4) is formed and subsequently fragments to form again a dormant polymer species together with a radical which is capable of further chain growth.

RAFT agents suitable for use in accordance with the invention comprise a thiocarbonylthio group (which is a divalent moiety represented by: $-\text{C}(\text{S})\text{S}-$). Examples of RAFT agents are described in Moad G.; Rizzardo, E; Thang S, H. Polymer 2008, 49, 1079-1131 (the entire contents of which are incorporated herein by reference) and include xanthate, dithioester, dithiocarbonate, dithiocarbamate and trithiocarbonate compounds.

A RAFT agent suitable for use in accordance with the invention may be represented by general formula (I) or (II):



where Z and R are groups, and R^* and Z^* are x-valent and y-valent groups, respectively, that are independently selected such that the agent can function as a RAFT agent in the polymerisation of one or more ethylenically unsaturated monomers; x is an integer ≥ 1 ; and y is an integer ≥ 2 .

In order to function as a RAFT agent in the polymerisation of one or more ethylenically unsaturated monomers, those skilled in the art will appreciate that R and R^* will typically be an optionally substituted organic group that function as a free radical leaving group under the polymerisation conditions employed and yet, as a free radical leaving group, retain the ability to reinitiate polymerisation. Those skilled in the art will also appreciate that Z and Z^* will typically be an optionally substituted organic group that function to give

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a suitably high reactivity of the C=S moiety in the RAFT agent towards free radical addition without slowing the rate of fragmentation of the RAFT-adduct radical to the extent that polymerisation is unduly retarded.

- 5 In formula (I), R* is a x-valent group, with x being an integer ≥ 1 . Accordingly, R* may be mono-valent, di-valent, tri-valent or of higher valency. For example, R* may be an optionally substituted polymer chain, with the remainder of the RAFT agent depicted in formula (I) presented as multiple groups pendant from the polymer chain. Generally, x will be an integer ranging from 1 to about 20, for example from about 2 to about 10, or
10 from 1 to about 5:

Similarly, in formula (II), Z* is a y-valent group, with y being an integer ≥ 2 . Accordingly, Z* may be di-valent, tri-valent or of higher valency. Generally, y will be an integer ranging from 2 to about 20, for example from about 2 to about 10, or from 2 to
15 about 5.

Examples of R in RAFT agents used in accordance with the invention include optionally substituted, and in the case of R* in RAFT agents used in accordance with the invention include a x-valent form of optionally substituted, alkyl, alkenyl, alkynyl, aryl, acyl,
20 carbocyclyl, heterocyclyl, heteroaryl, alkylthio, alkenylthio, alkynylthio, arylthio, acylthio, carbocyclylthio, heterocyclylthio, heteroarylthio, alkylalkenyl, alkylalkynyl, alkylaryl, alkylacyl, alkylcarbocyclyl, alkylheterocyclyl, alkylheteroaryl, alkyloxyalkyl, alkenyloxyalkyl, alkynyloxyalkyl, aryloxyalkyl, alkylacyloxy, alkylcarbocycloxy, alkylheterocycloxy, alkylheteroaryloxy, alkylthioalkyl, alkenylthioalkyl,
25 alkynylthioalkyl, arylthioalkyl, alkylacylthio, alkylcarbocyclylthio, alkylheterocyclylthio, alkylheteroarylthio, alkylalkenylalkyl, alkylalkynylalkyl, alkylarylalkyl, alkylacylalkyl, arylalkylaryl, arylalkenylaryl, arylalkynylaryl, arylacylaryl, arylacyl, arylcarbocyclyl, arylheterocyclyl, arylheteroaryl, alkenyloxyaryl, alkynyloxyaryl, aryloxyaryl, alkylthioaryl, alkenylthioaryl, alkynylthioaryl, arylthioaryl, arylacylthio,
30 arylcarbocyclylthio, arylheterocyclylthio, arylheteroarylthio, and a polymer chain.

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For avoidance of any doubt reference herein to "optionally substituted", alkyl, alkenyl... etc, is intended to mean each group such as alkyl and alkenyl is optionally substituted.

Examples of R in RAFT agents used in accordance with the invention also include optionally substituted, and in the case of R* in RAFT agents used in accordance with the invention also include an x-valent form of optionally substituted, alkyl; saturated, unsaturated or aromatic carbocyclic or heterocyclic ring; alkylthio; dialkylamino; an organometallic species; and a polymer chain.

More, specific examples of R in RAFT agents used in accordance with the invention include optionally substituted, and in the case of R* in RAFT agents used in accordance with the invention include an x-valent form of optionally substituted, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₆-C₁₈ aryl, C₁-C₁₈ acyl, C₃-C₁₈ carbocyclyl, C₂-C₁₈ heterocyclyl, C₃-C₁₈ heteroaryl, C₁-C₁₈ alkylthio, C₂-C₁₈ alkenylthio, C₂-C₁₈ alkynylthio, C₆-C₁₈ arylthio, C₁-C₁₈ acylthio, C₃-C₁₈ carbocyclylthio, C₂-C₁₈ heterocyclylthio, C₃-C₁₈ heteroarylthio, C₃-C₁₈ alkylalkenyl, C₃-C₁₈ alkylalkynyl, C₇-C₂₄ alkylaryl, C₂-C₁₈ alkylacyl, C₄-C₁₈ alkylcarbocyclyl, C₃-C₁₈ alkylheterocyclyl, C₄-C₁₈ alkylheteroaryl, C₂-C₁₈ alkylalkoxyalkyl, C₃-C₁₈ alkenylalkoxyalkyl, C₃-C₁₈ alkynylalkoxyalkyl, C₇-C₂₄ arylalkoxyalkyl, C₂-C₁₈ alkylalkoxy, C₂-C₁₈ alkylthioalkyl, C₃-C₁₈ alkenylthioalkyl, C₃-C₁₈ alkynylthioalkyl, C₇-C₂₄ arylthioalkyl, C₂-C₁₈ alkylacylthio, C₄-C₁₈ alkylcarbocyclylthio, C₃-C₁₈ alkylheterocyclylthio, C₄-C₁₈ alkylheteroarylthio, C₄-C₁₈ alkylalkenylalkyl, C₄-C₁₈ alkylalkynylalkyl, C₈-C₂₄ alkylarylalkyl, C₃-C₁₈ alkylacylalkyl, C₁₃-C₂₄ arylalkylaryl, C₁₄-C₂₄ arylalkenylaryl, C₁₄-C₂₄ arylalkynylaryl, C₁₃-C₂₄ arylacylaryl, C₇-C₁₈ arylacyl, C₉-C₁₈ arylcarbocyclyl, C₈-C₁₈ arylheterocyclyl, C₉-C₁₈ arylheteroaryl, C₈-C₁₈ alkenylalkoxyaryl, C₈-C₁₈ alkynylalkoxyaryl, C₁₂-C₂₄ arylalkoxyaryl, alkylthioaryl, C₈-C₁₈ alkenylthioaryl, C₈-C₁₈ alkynylthioaryl, C₁₂-C₂₄ arylthioaryl, C₇-C₁₈ arylacylthio, C₉-C₁₈ arylcarbocyclylthio, C₈-C₁₈ arylheterocyclylthio, C₉-C₁₈ arylheteroarylthio, and a polymer chain having a number average molecular weight in the range of about 500 to about 80,000, for example in the range of about 500 to about 30,000

30

Where R in RAFT agents used in accordance with the invention include, and in the case of

- 25 -

R* in RAFT agents used in accordance with the invention include an x-valent form of, an optionally substituted polymer chain, the polymers chain may be formed by any suitable polymerisation process such as radical, ionic, coordination, step-growth or condensation polymerisation. The polymer chains may comprise homopolymer, block polymer, 5 multiblock polymer, gradient copolymer, or random or statistical copolymer chains and may have various architectures such as linear, star, branched, graft, or brush.

Examples of Z in RAFT agents used in accordance with the invention include optionally substituted, and in the case of Z* in RAFT agents used in accordance with the invention 10 include a y-valent form of optionally substituted: F, Cl, Br, I, alkyl, aryl, acyl, amino, carbocyclyl, heterocyclyl, heteroaryl, alkyloxy, aryloxy, acyloxy, acylamino, carbocyclyloxy, heterocyclyloxy, heteroaryloxy, alkylthio, arylthio, acylthio, carbocyclylthio, heterocyclylthio, heteroarylthio, alkylaryl, alkylacyl, alkylcarbocyclyl, alkylheterocyclyl, alkylheteroaryl, alkyloxyalkyl, aryloxyalkyl, alkylacyloxy, 15 alkylcarbocyclyloxy, alkylheterocyclyloxy, alkylheteroaryloxy, alkylthioalkyl, arylthioalkyl, alkylacylthio, alkylcarbocyclylthio, alkylheterocyclylthio, alkylheteroarylthio, alkylarylalkyl, alkylacylalkyl, arylalkylaryl, arylacylaryl, arylacyl, arylcarbocyclyl, arylheterocyclyl, arylheteroaryl, aryloxyaryl, arylacyloxy, arylcarbocyclyloxy, arylheterocyclyloxy, arylheteroaryloxy, alkylthioaryl, arylthioaryl, 20 arylacylthio, arylcarbocyclylthio, arylheterocyclylthio, arylheteroarylthio, dialkyloxy-, diheterocyclyloxy- or diaryloxy- phosphinyl, dialkyl-, diheterocyclyl- or diaryl-phosphinyl, cyano (i.e. -CN), and -S-R, where R is as defined in respect of formula (II).

More specific examples of Z in RAFT agents used in accordance with the invention 25 include optionally substituted, and in the case of Z* in RAFT agents used in accordance with the invention include a y-valent form of optionally substituted: F, Cl, C₁-C₁₈ alkyl, C₆-C₁₈ aryl, C₁-C₁₈ acyl, amino, C₃-C₁₈ carbocyclyl, C₂-C₁₈ heterocyclyl, C₃-C₁₈ heteroaryl, C₁-C₁₈ alkyloxy, C₆-C₁₈ aryloxy, C₁-C₁₈ acyloxy, C₃-C₁₈ carbocyclyloxy, C₂-C₁₈ heterocyclyloxy, C₃-C₁₈ heteroaryloxy, C₁-C₁₈ alkylthio, C₆-C₁₈ arylthio, C₁-C₁₈ acylthio, C₃-C₁₈ carbocyclylthio, C₂-C₁₈ heterocyclylthio, C₃-C₁₈ heteroarylthio, C₇-C₂₄ 30 alkylaryl, C₂-C₁₈ alkylacyl, C₄-C₁₈ alkylcarbocyclyl, C₃-C₁₈ alkylheterocyclyl, C₄-C₁₈

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alkylheteroaryl, C₂-C₁₈ alkyloxyalkyl, C₇-C₂₄ aryloxyalkyl, C₂-C₁₈ alkylacyloxy, C₄-C₁₈ alkylcarbocycloxy, C₃-C₁₈ alkylheterocycloxy, C₄-C₁₈ alkylheteroaryloxy, C₂-C₁₈ alkylthioalkyl, C₇-C₂₄ arylthioalkyl, C₂-C₁₈ alkylacylthio, C₄-C₁₈ alkylcarbocyclylthio, C₃-C₁₈ alkylheterocyclylthio, C₄-C₁₈ alkylheteroarylthio, C₈-C₂₄ alkylarylalkyl, C₃-C₁₈ alkylacylalkyl, C₁₃-C₂₄ arylalkylaryl, C₁₃-C₂₄ arylacylaryl, C₇-C₁₈ arylacyl, C₉-C₁₈ arylcarbocyclyl, C₈-C₁₈ arylheterocyclyl, C₉-C₁₈ arylheteroaryl, C₁₂-C₂₄ aryloxyaryl, C₇-C₁₈ arylacyloxy, C₉-C₁₈ arylcarbocycloxy, C₈-C₁₈ arylheterocycloxy, C₉-C₁₈ arylheteroaryloxy, C₇-C₁₈ alkylthioaryl, C₁₂-C₂₄ arylthioaryl, C₇-C₁₈ arylacylthio, C₉-C₁₈ arylcarbocyclylthio, C₈-C₁₈ arylheterocyclylthio, C₉-C₁₈ arylheteroarylthio, dialkyloxy-,
 10 diheterocycloxy- or diaryloxy- phosphinyl (i.e. -P(=O)OR^k₂), dialkyl-, diheterocyclyl- or diaryl- phosphinyl (i.e. -P(=O)R^k₂), where R^k is selected from optionally substituted C₁-C₁₈ alkyl, optionally substituted C₆-C₁₈ aryl, optionally substituted C₂-C₁₈ heterocyclyl, and optionally substituted C₇-C₂₄ alkylaryl, cyano (i.e. -CN), and -S-R, where R is as defined in respect of formula (II).

15

In one embodiment, the RAFT agent used in accordance with the invention is a trithiocarbonate RAFT agent and Z or Z* is an optionally substituted alkylthio group.

In the lists herein defining groups from which Z, Z*, R and R* may be selected, each alkyl,
 20 alkenyl, alkynyl, aryl, carbocyclyl, heteroaryl, heterocyclyl, and polymer chain moiety may be optionally substituted. For avoidance of any doubt, where a given Z, Z*, R or R* contains two or more of such moieties (e.g. alkylaryl), each of such moieties may be optionally substituted with one, two, three or more optional substituents as herein defined.

25 In the lists herein defining groups from which Z, Z*, R and R* may be selected, where a given Z, Z*, R or R* contains two or more subgroups (e.g. [group A][group B]), the order of the subgroups is not intended to be limited to the order in which they are presented. Thus, a Z, Z*, R or R* with two subgroups defined as [group A][group B] (e.g. alkylaryl) is intended to also be a reference to a Z, Z*, R or R* with two subgroups defined as [group
 30 B][group A] (e.g. arylalkyl).

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The Z, Z*, R or R* may be branched and/or optionally substituted. Where the Z, Z*, R or R* comprises an optionally substituted alkyl moiety, an optional substituent includes where a -CH₂- group in the alkyl chain is replaced by a group selected from -O-, -S-, -NR^a-, -C(O)- (i.e. carbonyl), -C(O)O- (i.e. ester), and -C(O)NR^a- (i.e. amide), where R^a may be
5 selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, carbocyclyl, heteroaryl, heterocyclyl, arylalkyl, and acyl.

Reference herein to a x-valent, y-valent, multi-valent or di-valent "form of...." is intended to mean that the specified group is a x-valent, y-valent, multi-valent or di-valent radical,
10 respectively. For example, where x or y is 2, the specified group is intended to be a divalent radical. In that case, a divalent alkyl group is in effect an alkylene group (e.g. -CH₂-). Similarly, the divalent form of the group alkylaryl may, for example, be represented by -(C₆H₄)-CH₂-, a divalent alkylarylalkyl group may, for example, be represented by -CH₂-(C₆H₄)-CH₂-, a divalent alkyloxy group may, for example, be
15 represented by -CH₂-O-, and a divalent alkyloxyalkyl group may, for example, be represented by -CH₂-O-CH₂-. Where the term "optionally substituted" is used in combination with such a x-valent, y-valent, multi-valent or di-valent groups, that group may or may not be substituted or fused as herein described. Where the x-valent, y-valent, multi-valent, di-valent groups comprise two or more subgroups, for example [group
20 A][group B][group C] (e.g. alkylarylalkyl), if viable one or more of such subgroups may be optionally substituted. Those skilled in the art will appreciate how to apply this rationale in providing for higher valent forms.

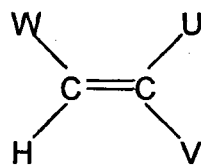
In one embodiment of the invention, a polymer chain used in accordance with the
25 invention is derived from one or more ethylenically unsaturated monomers. A polymer chain so formed may be a homopolymer or copolymer. Factors that determine copolymerisability of ethylenically unsaturated monomers are well documented in the art. For example, see: Greenlee, R. Z., in Polymer Handbook 3rd edition (Brandup, J, and Immergut, E. H. Eds) Wiley: New York, 1989, p II/53.

30

Suitable examples of ethylenically unsaturated monomers that may be used to prepare a

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polymer chain in accordance with the invention include those of formula (III):



(III)

5 where U and W are independently selected from $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{R}^1$, $-\text{COR}^1$, $-\text{CSR}^1$, $-\text{CSOR}^1$, $-\text{COSR}^1$, $-\text{CONH}_2$, $-\text{CONHR}^1$, $-\text{CONR}^1_2$, hydrogen, halogen and optionally substituted C_1 - C_4 alkyl or U and W form together a lactone, anhydride or imide ring that may itself be optionally substituted, where the optional substituents are independently selected from hydroxy, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{R}^1$, $-\text{COR}^1$, $-\text{CSR}^1$, $-\text{CSOR}^1$,
10 $-\text{COSR}^1$, $-\text{CN}$, $-\text{CONH}_2$, $-\text{CONHR}^1$, $-\text{CONR}^1_2$, $-\text{OR}^1$, $-\text{SR}^1$, $-\text{O}_2\text{CR}^1$, $-\text{SCOR}^1$, and $-\text{OCSR}^1$;

V is selected from hydrogen, R^1 , $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{R}^1$, $-\text{COR}^1$, $-\text{CSR}^1$, $-\text{CSOR}^1$, $-\text{COSR}^1$, $-\text{CONH}_2$, $-\text{CONHR}^1$, $-\text{CONR}^1_2$, $-\text{OR}^1$, $-\text{SR}^1$, $-\text{O}_2\text{CR}^1$, $-\text{SCOR}^1$, and $-\text{OCSR}^1$;
15

where the or each R^1 is independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted alkylaryl, optionally substituted alkylheteroaryl, and an optionally substituted polymer chain.
20

The or each R^1 may also be independently selected from optionally substituted C_1 - C_{22} alkyl, optionally substituted C_2 - C_{22} alkenyl, optionally substituted C_2 - C_{22} alkynyl, optionally substituted C_6 - C_{18} aryl, optionally substituted C_3 - C_{18} heteroaryl, optionally substituted C_3 - C_{18} carbocyclyl, optionally substituted C_2 - C_{18} heterocyclyl, optionally substituted C_7 - C_{24} arylalkyl, optionally substituted C_4 - C_{18} heteroarylalkyl, optionally
25

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substituted C₇-C₂₄ alkylaryl, optionally substituted C₄-C₁₈ alkylheteroaryl, and an optionally substituted polymer chain.

In one embodiment, R¹ may be independently selected from optionally substituted C₁-C₆ alkyl.

Examples of optional substituents for R¹ include those selected from alkyleneoxidyl (epoxy), hydroxy, alkoxy, acyl, acyloxy, formyl, alkylcarbonyl, carboxy, sulfonic acid, alkoxy- or aryloxy-carbonyl, isocyanato, cyano, silyl, halo, amino, including salts and derivatives thereof. Examples polymer chains include those selected from polyalkylene oxide, polyarylene ether and polyalkylene ether.

Examples of monomers of formula (III) include maleic anhydride, N-alkylmaleimide, N-arylmaleimide, dialkyl fumarate and cyclopolymerisable monomers, acrylate and methacrylate esters, acrylic and methacrylic acid, styrene, acrylamide, methacrylamide, and methacrylonitrile, mixtures of these monomers, and mixtures of these monomers with other monomers.

Other examples of monomers of formula (III) include: methyl methacrylate, ethyl methacrylate, propyl methacrylate (all isomers), butyl methacrylate (all isomers), 2-ethylhexyl methacrylate, isobornyl methacrylate, methacrylic acid, benzyl methacrylate, phenyl methacrylate, methacrylonitrile, alpha-methylstyrene, methyl acrylate, ethyl acrylate, propyl acrylate (all isomers), butyl acrylate (all isomers), 2-ethylhexyl acrylate, isobornyl acrylate, acrylic acid, benzyl acrylate, phenyl acrylate, acrylonitrile, styrene, functional methacrylates, acrylates and styrenes selected from glycidyl methacrylate, 2-hydroxyethyl methacrylate, hydroxypropyl methacrylate (all isomers), hydroxybutyl methacrylate (all isomers), N,N-dimethylaminoethyl methacrylate, N,N-diethylaminoethyl methacrylate, triethyleneglycol methacrylate, itaconic anhydride, itaconic acid, glycidyl acrylate, 2-hydroxyethyl acrylate, hydroxypropyl acrylate (all isomers), hydroxybutyl acrylate (all isomers), N,N-dimethylaminoethyl acrylate, N,N-diethylaminoethyl acrylate, triethyleneglycol acrylate, methacrylamide, N-methylacrylamide, N,N-

- 30 -

- dimethylacrylamide, N-tert-butylmethacrylamide, N-n-butylmethacrylamide, N-methylolmethacrylamide, N-ethylolmethacrylamide, N-tert-butylacrylamide, N-n-butylacrylamide, N-methylolacrylamide, N-ethylolacrylamide, vinyl benzoic acid (all isomers), diethylamino styrene (all isomers), alpha-methylvinyl benzoic acid (all isomers),
- 5 diethylamino alpha-methylstyrene (all isomers), p-vinylbenzene sulfonic acid, p-vinylbenzene sulfonic sodium salt, trimethoxysilylpropyl methacrylate, triethoxysilylpropyl methacrylate, tributoxysilylpropyl methacrylate, dimethoxymethylsilylpropyl methacrylate, diethoxymethylsilylpropyl methacrylate, dibutoxymethylsilylpropyl methacrylate, diisopropoxymethylsilylpropyl methacrylate,
- 10 dimethoxysilylpropyl methacrylate, diethoxysilylpropyl methacrylate, dibutoxysilylpropyl methacrylate, diisopropoxysilylpropyl methacrylate, trimethoxysilylpropyl acrylate, triethoxysilylpropyl acrylate, tributoxysilylpropylacrylate, dimethoxymethylsilylpropyl acrylate, diethoxymethylsilylpropyl acrylate, dibutoxymethylsilylpropyl acrylate, diisopropoxymethylsilylpropyl acrylate, dimethoxysilylpropyl acrylate,
- 15 diethoxysilylpropyl acrylate, dibutoxysilylpropyl acrylate, diisopropoxysilylpropyl acrylate, vinyl acetate, vinyl butyrate, vinyl benzoate, vinyl chloride, vinyl fluoride, vinyl bromide, maleic anhydride, N-phenylmaleimide, N-butylmaleimide, N-vinylpyrrolidone, N-vinylcarbazole, butadiene, ethylene and chloroprene. This list is not exhaustive.
- 20 Further monomers suitable for use in preparing the polymer chains are discussed below.

When describing certain aspects of the present invention, it may be convenient to refer to certain constituent components of the NMR agent in terms of their polarity (i.e. their polar or apolar character), or alternatively in terms of their hydrophilic or hydrophobic character.

- 25 Terms such as polar, apolar, hydrophilic and hydrophobic are generally used in the art to convey favourable or unfavourable interactions between one component relative to another (e.g. attractive or repulsive interactions) and not to define absolute qualities of a particular component.
- 30 For example, polar or hydrophilic components are more likely to be wetted or solvated by an aqueous medium such as water (attractive interaction), whereas apolar or hydrophobic

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components are less likely to be wetted or solvated by an aqueous medium such as water (repulsive interaction).

For example, it may be convenient to refer to a polymer chain or section or region thereof
5 as having hydrophilic or hydrophobic character. In that case, the polymer chain or section or region thereof will generally be derived from one or more monomers having respective hydrophilic or hydrophobic character.

As a guide only, examples of hydrophobic ethylenically unsaturated monomers include,
10 but are not limited to, styrene, alpha-methyl styrene, butyl acrylate, butyl methacrylate, amyl methacrylate, hexyl methacrylate, lauryl methacrylate, stearyl methacrylate, ethyl hexyl methacrylate, crotyl methacrylate, cinnamyl methacrylate, oleyl methacrylate, ricinoleyl methacrylate, vinyl butyrate, vinyl tert-butyrate, vinyl stearate and vinyl laurate.

15 As a guide only, examples of hydrophilic ethylenically unsaturated monomers include, but are not limited to, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, hydroxypropyl methacrylate, acrylamide and methacrylamide, hydroxyethyl acrylate, N-methylacrylamide, N,N-dimethylacrylamide or dimethylaminoethyl methacrylate, or copolymers thereof.

20

Where a free radical polymerisation technique is to be used in polymerising one or more ethylenically unsaturated monomers so as to form a polymer chain in accordance with the invention, the polymerisation will usually require initiation from a source of free radicals.

25 A source of initiating radicals can be provided by any suitable means of generating free radicals, such as the thermally induced homolytic scission of suitable compound(s) (thermal initiators such as peroxides, peroxyesters, or azo compounds), the spontaneous generation from monomers (e.g. styrene), redox initiating systems, photochemical initiating systems or high energy radiation such as electron beam, X- or gamma-radiation.

30

Thermal initiators are generally chosen to have an appropriate half life at the temperature

of polymerisation. These initiators can include one or more of the following compounds:

2,2'-azobis(isobutyronitrile), 2,2'-azobis(2-cyanobutane), dimethyl 2,2'-
 azobis(isobutyrate), 4,4'-azobis(4-cyanovaleric acid), 1,1'-
 5 azobis(cyclohexanecarbonitrile), 2-(t-butylazo)-2-cyanopropane, 2,2'-azobis{2-
 methyl-N-[1,1-bis(hydroxymethyl)-2-hydroxyethyl]propionamide}, 2,2'-azobis[2-
 methyl-N-(2-hydroxyethyl)propionamide], 2,2'-azobis(N,N'-
 dimethyleneisobutyramidine) dihydrochloride, 2,2'-azobis(2-amidinopropane)
 dihydrochloride, 2,2'-azobis(N,N'-dimethyleneisobutyramidine), 2,2'-azobis{2-
 10 methyl-N-[1,1-bis(hydroxymethyl)-2-hydroxyethyl]propionamide}, 2,2'-azobis{2-
 methyl-N-[1,1-bis(hydroxymethyl)-2-ethyl]propionamide}, 2,2'-azobis[2-methyl-
 N-(2-hydroxyethyl)propionamide], 2,2'-azobis(isobutyramide) dihydrate, 2,2'-
 azobis(2,2,4-trimethylpentane), 2,2'-azobis(2-methylpropane), t-butyl
 peroxyacetate, t-butyl peroxybenzoate, t-butyl peroxyneodecanoate, t-butylperoxy
 15 isobutyrate, t-amyl peroxy-pivalate, t-butyl peroxy-pivalate, diisopropyl
 peroxydicarbonate, dicyclohexyl peroxydicarbonate, dicumyl peroxide, dibenzoyl
 peroxide, dilauroyl peroxide, potassium peroxydisulfate, ammonium
 peroxydisulfate, di-t-butyl hyponitrite, dicumyl hyponitrite. This list is not
 exhaustive.

20

Photochemical initiator systems are generally chosen to have an appropriate quantum yield for radical production under the conditions of the polymerisation. Examples include benzoin derivatives, benzophenone, acyl phosphine oxides, and photo-redox systems.

25 Redox initiator systems are generally chosen to have an appropriate rate of radical production under the conditions of the polymerisation; these initiating systems can include, but are not limited to, combinations of the following oxidants and reductants:

oxidants: potassium, peroxydisulfate, hydrogen peroxide, t-butyl hydroperoxide.

30

reductants: iron (II), titanium (III), potassium thiosulfite, potassium bisulfite.

Other suitable initiating systems are described in commonly available texts. See, for example, Moad and Solomon "The Chemistry of Free Radical Polymerisation", Pergamon, London, 1995, pp 53-95.

5

Initiators that are more readily solvated in hydrophilic media include, but are not limited to, 4,4'-azobis(cyanovaleric acid), 2,2'-azobis{2-methyl-N-[1,1-bis(hydroxymethyl)-2-hydroxyethyl]propionamide}, 2,2'-azobis[2-methyl-N-(2-hydroxyethyl)propionamide], 2,2'-azobis(N,N'-dimethyleneisobutyramidine), 2,2'-azobis(N,N'-

10 dimethyleneisobutyramidine) dihydrochloride, 2,2'-azobis(2-amidinopropane) dihydrochloride, 2,2'-azobis{2-methyl-N-[1,1-bis(hydroxymethyl)-2-ethyl]propionamide}, 2,2'-azobis[2-methyl-N-(2-hydroxyethyl)propionamide], 2,2'-azobis(isobutyramide) dihydrate, and derivatives thereof.

15 Initiators that are more readily solvated in hydrophobic media include azo compounds exemplified by the well known material 2,2'-azobisisobutyronitrile. Other suitable initiator compounds include the acyl peroxide class such as acetyl and benzoyl peroxide as well as alkyl peroxides such as cumyl and t-butyl peroxides. Hydroperoxides such as t-butyl and cumyl hydroperoxides are also widely used.

20

When preparing a polymer chain in accordance with the invention it may be desirable to promote crosslinking between polymer chains.

Those skilled in the art will appreciate that the crosslinking of polymer chains may be
25 achieved in numerous ways. For example, crosslinking may be achieved using multi-ethylenically unsaturated monomers. In that case, crosslinking is typically derived through a free radical reaction mechanism.

Alternatively, crosslinking may be achieved using ethylenically unsaturated monomers which also contain a reactive functional group that is not susceptible to taking part in free
30 radical polymerisation reactions (i.e. "functionalised" unsaturated monomers). In that case, such monomers may be incorporated into the polymer backbone through polymerisation of

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the unsaturated group, and the resulting pendant functional group provides means through which crosslinking may occur. By utilising monomers that provide complementary pairs of reactive functional groups (i.e. groups that will react with each other), the pairs of reactive functional groups can react through non-radical reaction mechanisms to provide
5 crosslinks.

A variation on using complementary pairs of reactive functional groups is where the monomers are provided with non-complementary reactive functional groups. In that case, the functional groups will not react with each other but instead provide sites which can subsequently be reacted with a crosslinking agent to form the crosslinks. It will be
10 appreciated that such crosslinking agents will be used in an amount to react with substantially all of the non-complementary reactive functional groups. Formation of the crosslinks under these circumstances will generally occur after polymerisation of the monomers.

A combination of these crosslinking techniques may be used.

15 In the context of generating a crosslink, the terms "multi-ethylenically unsaturated monomers" and "functionalised unsaturated monomers" mentioned above can conveniently and collectively also be referred to herein as "crosslinking ethylenically unsaturated monomers" or "crosslinking monomers". By the general term "crosslinking ethylenically unsaturated monomers" or "crosslinking monomers" it is meant an ethylenically
20 unsaturated monomer through which a crosslink is or will be derived.

It will be appreciated that not all unsaturated monomers that contain a functional group can be used for the purpose of functioning as a crosslinking monomer. For example, despite being a functionalised unsaturated monomer acrylic acid should not be considered as a crosslinking monomer unless it is used to provide a site through which a crosslink is to be
25 derived.

Examples of suitable multi-ethylenically unsaturated monomers that may be used to promote crosslinking include ethylene glycol di(meth)acrylate, triethylene glycol di(meth)acrylate, tetraethylene glycol di(meth)acrylate, 1,3-butylene glycol

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di(meth)acrylate, trimethylolpropane tri(meth)acrylate, 1,4-butanediol di(meth)acrylate, neopentyl glycol di(meth)acrylate, 1,6-hexanediol di(meth)acrylate, pentaerythritol di(meth)acrylate, pentaerythritol tri(meth)acrylate, pentaerythritol tetra(meth)acrylate, glycerol di(meth)acrylate, glycerol allyloxy di(meth)acrylate, 1,1,1-
 5 tris(hydroxymethyl)ethane di(meth)acrylate, 1,1,1-tris(hydroxymethyl)ethane tri(meth)acrylate, 1,1,1-tris(hydroxymethyl)propane di(meth)acrylate, 1,1,1-tris(hydroxymethyl)propane tri(meth)acrylate, triallyl cyanurate, triallyl isocyanurate, triallyl trimellitate, diallyl phthalate, diallyl terephthalate, divinyl benzene, methylol (meth)acrylamide, triallylamine, oleyl maleate, glyceryl propoxy triacrylate, allyl
 10 methacrylate, methacrylic anhydride and methylenebis (meth) acrylamide.

Examples of suitable ethylenically unsaturated monomers which contain a reactive functional group that is not susceptible to taking part in free radical polymerisation reactions include acetoacetoxyethyl methacrylate, glycidyl methacrylate, *N*-methylolacrylamide, (isobutoxymethyl)acrylamide, hydroxyethyl acrylate, *t*-butyl-
 15 carbodiimidoethyl methacrylate, acrylic acid, γ -methacryloxypropyltriisopropoxysilane, 2-isocynoethyl methacrylate and diacetone acrylamide.

Examples of suitable pairs of monomers mentioned directly above that provide complementary reactive functional groups include *N*-methylolacrylamide and itself, (isobutoxymethyl)acrylamide and itself, γ -methacryloxypropyltriisopropoxysilane and
 20 itself, 2-isocynoethyl methacrylate and hydroxyethyl acrylate, and *t*-butyl-carbodiimidoethyl methacrylate and acrylic acid.

Examples of suitable crosslinking agents that can react with the reactive functional groups of one or more of the functionalised unsaturated monomers mentioned above include hexamethylene diamine, melamine, trimethylolpropane tris(2-methyl-1-aziridine
 25 propionate) and adipic bishydrazide. Examples of pairs of crosslinking agents and functionalised unsaturated monomers that provide complementary reactive groups include hexamethylene diamine and acetoacetoxyethyl methacrylate, hexamethylene diamine and glycidyl methacrylate, melamine and hydroxyethyl acrylate, trimethylolpropane tris(2-

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methyl-1-aziridine propionate) and acrylic acid, adipic bishydrazide and diacetone acrylamide.

5 An important feature of the present invention is that the NMR agent comprises a plurality of the covalently coupled nuclides. One or more of the nuclides is coupled to a polymer chain. Accordingly, the NMR agent may comprise a plurality of polymer chains having at least one nuclide covalently coupled to each polymer chain. Alternatively, the NMR agent may comprise a polymer chain to which a plurality of nuclides is covalently coupled.

10 There are no particular limitations concerning the means by which a nuclide is covalently coupled to a polymer chain. For example, a nuclide may be covalently coupled to a monomer from which the polymer chain is derived. In that case, one or more of said nuclides will be covalently coupled to a monomer residue of a polymer chain.

15 Alternatively, a polymer chain may be prepared using a functionalised monomer. In that context, a "functionalised monomer" is intended to mean a monomer having at least one functional group that can participate in a polymerisation reaction so as to form the polymer chain and at least one other functional group that does not participate in the polymerisation reaction.

20

A polymer chain prepared using such a functionalised monomer will therefore comprise a monomer residue having covalently bound thereto a reactive functional group. Examples of ethylenically unsaturated monomers which contain a reactive functional group that is not susceptible to taking part in free radical polymerisation reactions are described herein.

25 A polymer chain prepared using such a monomer will comprise a reactive functional group that may undergo subsequent chemical reaction so as to covalently couple a nuclide to the polymer chain.

30 Those skilled in the art will appreciate that methods of forming a polymer chain may require the use of an initiator. For example, free radical polymerisation reactions typically require the use of a free radical initiator or a residue thereof may as a part of the

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polymerisation reaction become covalently bound to the polymer chain. The initiator may therefore comprise one or more nuclides and through such a reaction process in effect covalently couple the nuclides to the so formed polymer chain.

- 5 Those skilled in the art will also appreciate that methods of forming a polymer chain may require the use of a chain transfer agent. For example, a free radical polymerisation reaction may utilise a RAFT agent, and a residue of that agent will become covalently bound to the polymer chain. The chain transfer agent may therefore comprise one or more nuclides and through such a reaction process in effect covalently couple the nuclides to the
10 so formed polymer chain.

Where a "living polymerisation agent" functions as a chain transfer agent, reference herein to a "chain transfer agent" is intended to embrace a living polymerisation agent.

- 15 In one embodiment, the one or more nuclides are therefore covalently coupled to a monomer residue that forms part of the polymer chain.

In an alternative embodiment, the one or more nuclides are covalently coupled to an initiator residue that forms part of the polymer chain.

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In a further embodiment, the one or more nuclides are covalently coupled to a chain transfer agent residue that forms part of the polymer chain.

- In yet a further embodiment, the polymer chain is prepared by polymerising one or more
25 monomers having covalently coupled thereto one or more NMR nuclides selected from D, B, F, Br, P and Si.

- In still a further embodiment, the polymer chain is prepared using an initiator and/or chain transfer agent which as part of the polymerisation reaction one or both become covalently
30 bound to the so formed polymer chain, wherein the initiator and/or chain transfer agent have covalently bound thereto one or more NMR nuclides selected from D, B, F, Br, P and

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Si.

In another embodiment, the polymer chain is prepared using one or more functionalised monomers, the resulting polymer chain comprising at least one reactive functional group
 5 derived from the one or more functionalised monomers, wherein the at least one functional group of the polymer chain is reacted with a compound comprising one or more nuclide selected from D, B, F, Br, P and Si so as to covalently couple said one or more nuclides to the polymer chain.

10 In one embodiment, the NMR agent comprises a plurality of covalently coupled F NMR nuclides. In that case, the polymer chain may comprise one or more fluorine substituted groups selected from trifluoromethyl, perfluoroethyl, 2,2,2-trifluoroethyl, perfluoropropyl, perfluoroisopropyl, bis(trifluoromethyl)methyl, tris(trifluoromethyl)methyl, 2,2,3,3,3-
 15 pentafluoropropyl, perfluorobutyl, trifluoromethylphenyl, 1,3-di(trifluoromethyl) phenyl, trifluoromethylbenzyl, 1,3-di(trifluoromethyl)benzyl, tris(trifluoromethyl)-methylphenyl, tris(trifluoromethyl)-methylbenzyl, fluorophenyl, difluorophenyl, pentafluorophenyl and pentafluorobenzyl. In one embodiment the covalently coupled F NMR nuclides are selected from trifluoromethyl, tris(trifluoromethyl) methyl, perfluoroisopropyl, tris(trifluoro-methyl) methylbenzyl and pentafluorobenzyl.

20

In a further embodiment, the polymer chain is prepared using a monomer, initiator and/or chain transfer agent which form a covalently bound residue of the resulting polymer chain, wherein at least one of the monomer, initiator and chain transfer agent comprise a fluorine substituted group selected from trifluoromethyl, perfluoroethyl, 2,2,2-trifluoroethyl,
 25 perfluoropropyl, perfluoroisopropyl, bis(trifluoromethyl)methyl, tris(trifluoromethyl)methyl, 2,2,3,3,3-pentafluoropropyl, perfluorobutyl, trifluoromethylphenyl, 1,3-di(trifluoromethyl) phenyl, trifluoromethylbenzyl, 1,3-di(trifluoromethyl)benzyl, tris(trifluoromethyl)-methylphenyl, tris(trifluoromethyl)-methylbenzyl, fluorophenyl, difluorophenyl, pentafluorophenyl and pentafluorobenzyl. In
 30 one embodiment the covalently coupled F NMR nuclides are selected from trifluoromethyl, tris(trifluoromethyl) methyl, perfluoroisopropyl, tris(trifluoro-methyl)

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methylbenzyl and pentafluorobenzyl.

Specific examples of monomers comprising one or more nuclides that may be used to prepare the polymer chain include trifluoroethyl acrylate (tFEA), 1H,1H,2H,2H-perfluorodecyl(meth)acrylate, 1H,1H,2H,2H-perfluorooctyl(meth)acrylate, heptadecylfluorodecyl(meth)acrylate, pentafluorophenyl(meth)acrylate trifluoroethyl methacrylate (TFEMA), hexafluoroisopropyl acrylate (HFPA) and hexafluoroisopropyl methacrylate (HFPA), perfluoro-t-butyl ether of ethoxyethyl acrylate, perfluoro-t-butyl ether of ethoxyethyl methacrylate, 2,2,3,3-tetrafluoropropyl acrylate, and 2,2,3,3-tetrafluoropropyl methacrylate.

Specific examples of initiators comprising one or more nuclides that may be used in preparing the polymer chain include (per)fluoro alkyl halides, (per)fluoroalkyl isobutyryl bromides, (per)fluoro alkyl azo compounds, and (per)fluoro aryl azo compounds.

15

Specific examples of chain transfer agents comprising one or more nuclides that may be used in preparing the polymer chain include benzyl fluoro dithioformate, s-thiobenzoyl-1H,1H,2H,2H-perfluorodecanethiol, s-thiobenzoyl-1H,1H,2-keto-3-oxa-4H,4H,5H,5H-perfluoroendecanethiol, s-thiobenzoyl-3-keto-4-oxa-5H,5H,6H,6H-perfluorododecane-2-thiol, and s-thiobenzoyl-2-methyl-3-keto-4-oxa-5H,5H,6H,6H-perfluorododecane-2-thiol.

20

Provided that the polymer chain provides for the required features of the NMR agent in accordance with the invention, there is no particular limitation concerning the structure of the polymer chain.

25

In one embodiment, the polymer chain is a linear polymer chain. In that case, the NMR agent may comprise a plurality of linear polymer chains. The plurality of linear polymer chains may be associated so as to form a three dimensional structure such as a microgel. Polymer chains that form a microgel structure may be crosslinked to each other so as to retain the integrity of the three dimensional structure. In that case, the microgel *per se* may not be considered in a strict sense to comprise "linear" polymer chains. Nevertheless, it is

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common within the art to often refer to microgels as an association of crosslinked linear polymer chains due to at least one technique by which they are commonly prepared.

- Microgels are generally prepared by one or two approaches. The first of these is commonly referred to as a "self-assembly" approach. In that case, linear polymer chains are prepared such that they are capable of forming a micelle structure. Polymer chains capable of forming a micelle structure will generally exhibit amphipathic character. A conventional micelle structure presented in an aqueous liquid medium will typically be made up of assembled polymer chains having a section or region that exhibits hydrophilic character and is solvated by the surrounding aqueous liquid medium, with a hydrophobic section or region of the polymer chain forming the centre or core of the micelle structure. This type of micelle formation is commonly referred to as a "normal phase micelle" or an "oil-in water micelle". "Inverse" or "reverse" micelles may also be formed where the liquid medium is hydrophobic in character and the hydrophobic section or region of the assembled polymer chains is solvated by the hydrophobic liquid medium, with the hydrophilic section or region of the polymer chains forming the centre or core of the micelle structure. Inverse or reverse micelles are also commonly referred to as "water-in-oil micelles".
- Micelle structures typically exist in a form of equilibrium with their surrounding liquid medium in that the polymer chains that form the micelle structures are free to move away from the structure into the liquid medium. To stabilise the micelle structures from "falling apart", the polymer chains may be provided with reactive functional groups that can function as a site to form a crosslink with one or more of the other polymer chains. Such stabilised micelle structures are known as microgels.

- Microgels may also be prepared by a so called "*in situ*" approach. In that case, a three dimensional crosslinked network of polymer chains may be prepared in a one step procedure without transition through a micelle structure. In that case, polymer chains are formed and simultaneously crosslinked during formation of the microgel.

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Microgels may be prepared using polymerisation techniques outlined herein well known to those skilled in the art.

In one embodiment, the microgel is of a type that can absorb and can be swollen by a hydrophilic liquid.

In a further embodiment, the microgel is of a type that can absorb and can be swollen by an aqueous liquid.

In one embodiment, the one or more said nuclides are covalently coupled to a polymer chain that forms part of a microgel structure.

The polymer chain may also be or form part of a dendritic polymer. Dendritic polymers are a class of synthetic tree-like macromolecules and include dendrimers and hyperbranched polymers.

Dendrimers are known in the art to have completely branched star-like topologies, whereas hyperbranched polymers are known in the art to have imperfectly branched or irregular structures. Both dendrimer and hyperbranched polymer molecules are composed of repeating units that typically emanate from a central core structure or molecule.

Dendritic polymers may be prepared using polymerisation techniques well known to those skilled in the art, including those outlined herein.

Synthetic techniques for hyperbranched polymers can be divided into two general categories. The first category involves techniques of the so called "single-monomer methodology" (SMM) where the polymers are prepared by polymerisation of so called "AB_x, AB^{*} or a latent AB_x monomer". The second category includes techniques of the so called "double-monomer methodology" (DMM) whereby the direct polymerisation of at least two types of monomers or a pair of monomers generates the hyperbranched polymer.

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The SMM category includes a step-growth polycondensation approach for producing, for example, polyphenylene, polyester, polyamide and polycarbonate hyperbranched polymers, self-condensing vinyl polymerisation (SCVP) for producing, for example, polystyrene, poly(methacrylate) and poly(acrylate) hyperbranched polymers,
5 multibranching ring-opening polymerisation (SCROP) for producing, for example, polyamine, polyether and polyester hyperbranched polymers, and proton-transfer polymerisation (PTP) for producing, for example, polysiloxane and polyester hyperbranched polymers with epoxy or hydroxyl end groups.

10 The DDM category includes so called " $A_2 + B_3$ " methodology for producing, for example, polyamide, polycarbonate and polyurea hyperbranched polymers, and couple-monomer methodology (CMM), which is a combination of SMM and " $A_2 + B_3$ " methodologies, for producing, for example, poly(sulphone amine), poly(ester amine) and poly(urea urethane) hyperbranched polymers.

15

Techniques for preparing hyperbranched polymers are well known to those skilled in the art, for example, as described by Mitsutoshi Jikei, Masa-aki Kakimoto, Prog. Polym. Sci. 26 (2001) 1233-1285, the entire contents of which are incorporated herein by cross reference.

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In one embodiment of the invention, the one or more of said nuclides are covalently bound to a polymer chain that is or forms part of a dendritic polymer. The dendritic polymer may be a dendrimer or a hyperbranched polymer.

25 In the context of forming dendritic polymer structures, it will generally be necessary to use one or more multi functional monomers. Such monomers have more than one reactive sites at which polymerisation may take place and are often referred to as branching monomers. Examples of such branching monomers include multi-ethylenically unsaturated monomers and poly-carboxylic acids (and ester derivatives thereof), poly-ols,
30 poly-amines, and poly-acyl chlorides as described herein.

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Where the polymer chain forms part of a larger polymer structure such a microgel or dendritic polymer, the "polymer chain" may be conveniently referred to as a "microgel" or a "dendritic polymer". In that case, the nuclides may be referred to as being covalently coupled to a "microgel" or a "dendritic polymer".

5

An important feature of NMR agent in accordance with the invention is that it can provide for a first and second form in which the NMR signal of the nuclides is diminished and amplified, respectively. For convenience, the transition of the agent from its first form to its second form may herein be described as the agent "switching" or being "switched" from the first form to the second form.

10

According to the present invention, switching of the NMR agent occurs as a result of the first form of the agent undergoing a chemical reaction to provide for the second form of the agent. As described herein, the first form of the agent may provide for intramolecular mobility confinement of the nuclides, and this form of the agent undergoes a chemical reaction to provide for intramolecular mobility liberation of the nuclides.

15

There is no particular limitation concerning the type of chemical reaction that can promote switching of the NMR agent. For example, the chemical reaction may result in the cleavage of a covalent bond associated with the polymer chain. Alternatively, the chemical reaction may result in decomplexation of a complexed moiety associated with the polymer chain. Such decomplexation may be promoted itself through cleavage of a covalent bond associated with the polymer chain.

20

In one embodiment, the chemical reaction that causes the first form of the agent to switch to the second form promotes cleavage of a covalent bond associated with (i.e. forms part of) the polymer chain, said cleavage of the covalent bond not resulting in the covalently coupled nuclides being cleaved from the polymer chain.

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By said cleavage of the covalent bond not resulting in the covalently coupled nuclides being cleaved from the polymer chain is meant that after cleavage of the covalent bond the

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nuclides must still be covalently coupled to a polymer chain.

In one embodiment, the nuclides of the NMR agent in its first form are in a state of intramolecular mobility confinement. In other words, the polymer chain presents a structure in which the intramolecular mobility of the nuclides is restricted or confined such
5 that their NMR signal is diminished or absent.

In another embodiment, the intramolecular mobility confinement of said nuclides occurs as a result of the nuclides being located within a hydrophobic environment. For example, the
10 nuclides of the NMR agent may be located within a section or region of a microgel or dendritic polymer which is substantially hydrophobic in character whereby the hydrophobic character causes intramolecular mobility confinement of the nuclides. This might be achieved by covalently coupling, complexing or encapsulating a hydrophobic moiety or material so as to promote a hydrophobic environment in the section or region
15 comprising the nuclides. Such a structure presents as the first form of the NMR agent. Decomplexation or cleavage of a covalent bond associated with this form of the agent may promote release of the hydrophobic moiety or material from the agent, thereby reducing or removing the intramolecular mobility confinement of the nuclides so as to provide for intramolecular mobility liberation of the nuclides.

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In one embodiment, the chemical reaction causes release from the agent of a hydrophobic moiety which in turn causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

25 For example, the one or more nuclides may be covalently coupled to a polymer chain that forms part of a microgel or dendritic polymer structure, the polymer chain further comprising a hydrophobic moiety covalently coupled thereto such that the NMR signal of said nuclides is diminished. In that case, the nuclides will be of a type that preferentially associate with covalently coupled or complexed hydrophobic character and within the
30 structure of the polymer consequently have their intramolecular mobility confined. This structure of the agent represents the first form of the agent. The chemical reaction then

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promotes decomplexation or cleavage of the covalently coupled hydrophobic moiety from the polymer chain, which in turn provides intramolecular mobility liberation of the nuclides.

- 5 Alternatively, the one or more nuclides may be covalently coupled to a polymer chain that forms part of a microgel or dendritic polymer having a crosslinked structure. The crosslinked polymer structure having encapsulated therein hydrophobic material such that the NMR signal of said nuclides is diminished. In that case, the nuclides will be of a type that preferentially associate with encapsulated hydrophobic character and within the
- 10 structure of the polymer consequently have their intramolecular mobility confined. This structure of the agent represents the first form of the agent. The chemical reaction then promotes cleavage of the crosslinks which enables the hydrophobic material to be released from the polymer, and in turn provides intramolecular mobility liberation of the nuclides.
- 15 Where a hydrophobic moiety or material is used to promote intramolecular mobility confinement of the nuclides, it may be desirable to have covalently coupled in the proximity of the nuclides a hydrophilic moiety to facilitate intramolecular mobility liberation of the nuclides. For example, when *in vivo* the NMR agent may be present within a hydrophilic liquid environment, such as a predominantly aqueous liquid
- 20 environment. In that case, in its first form intramolecular mobility of the nuclides may be confined by being associated with covalently coupled hydrophobic moieties or encapsulated hydrophobic material in and around the location of the nuclides. Release of the hydrophobic moieties or hydrophobic material from the agent will then enable aqueous liquid to more readily interact with the nuclides, the process of which may be facilitated by
- 25 hydrophilic moieties covalently coupled in the proximity of the nuclides.

More effective interaction of the polymer chain comprising the nuclides with the aqueous liquid can enhance intramolecular mobility liberation of the nuclides. In one example, the covalently coupled hydrophobic moiety may be a protected hydrophilic group, whereby

30 deprotection of the hydrophobic moiety affords a covalently coupled hydrophilic group. This might be achieved by, for example, acetal deprotection.

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In one embodiment, the second form of the agent comprises an aqueous swollen polymer structure which promotes intramolecular mobility liberation of the nuclides.

- 5 In one embodiment, the covalently coupled hydrophobic moiety, the complexed hydrophobic moiety or the encapsulated hydrophobic material may be a pharmaceutically active agent. In that case, the NMR agent in accordance with the invention not only provides for a switch that promotes improved NMR-visibility of the nuclides contained therein, but the chemical reaction that causes the NMR signal of the nuclides to be
- 10 amplified also promotes release of a pharmaceutically active agent from the NMR agent. Such versatility of the NMR agent can allow analysis of drug distribution and accumulation of the target site of a subject while facilitating quantification of delivery.

- In one embodiment, the chemical reaction further causes a pharmaceutically active agent to
- 15 be released from the NMR agent.

- In one embodiment, the polymer chain further comprises a covalently coupled, complexed or encapsulated pharmaceutically active agent. The pharmaceutically active agent may be covalently coupled or complexed to the polymer chain via a monomer, initiator and/or
- 20 chain transfer agent residue that forms part of the polymer chain. The pharmaceutically active agent may be covalently coupled or complexed to the polymer chain during the process of forming the polymer chain, or subsequently covalently coupled or complexed to the polymer chain after it has been formed. Where the pharmaceutically active agent is covalently coupled or complexed to the polymer chain after it has been formed, the
- 25 polymer chain will of course be prepared with one or more suitable functional groups that will enable the pharmaceutically active agent to be covalently coupled or complexed thereto.

- Switching of the NMR agent may also occur through decomplexation of a moiety
- 30 complexed to the polymer chain. For example, the polymer chain may present an ionic charge such as a cationic charge or an anionic charge. Complexation of the polymer chain

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with an oppositely charged moiety can result in a densely packed complexed structure which causes intramolecular mobility confinement of the nuclides. Decomplexation of the complexed structure through a chemical reaction can promote intramolecular mobility liberation of the nuclides.

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For example, the one or more nuclides may be covalently coupled to a cationic polymer chain, the cationic polymer chain forming a neutral complexed structure with an anionic moiety. In that case, the complexed structure promotes intramolecular mobility confinement of the nuclides. This structure of the agent represents the first form of the agent. The chemical reaction then promotes decomplexation of the complexed structure, which in turn provides intramolecular mobility liberation of the nuclides.

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In one embodiment, the one or more of said nuclides are covalently coupled to an ionically charged polymer chain.

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In a further embodiment, the NMR signal of said nuclides is diminished due to the ionically charged polymer chain being complexed with an oppositely ionically charged moiety.

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In a further embodiment, the NMR signal of said nuclides is amplified due to decomplexation of the ionically charged polymer chain and the opposite ionically charged moiety.

In a further embodiment, the polymer chain is cationically charged.

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Where the NMR agent is in a complexed form, there is no particular limitation concerning the type of chemical reaction that can cause this first form of the agent to switch into the second and decomplexed form of the agent in which the NMR signal of the nuclides is amplified. The chemical reaction may be, for example an ion exchange reaction or as a result of a change in pH.

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The complexed form of such an agent may also comprise one or more covalent bonds between the ionically charged polymer and the opposite ionically charged moiety. In that case, the cleavage of such covalent bonds may also promote decomplexation.

- 5 In one embodiment, the opposite ionically charged moiety that may form a complex with the ionically charged polymer chain may be a pharmaceutically active agent such as an oligonucleotide. Accordingly, the complexed form of the NMR agent may also provide for drug release therapy as herein described.
- 10 The chemical reaction that promotes switching of the first form of the agent into its second form may be a pH, redox or enzyme based reaction. Those skilled in the art will appreciate that such reactions, or the conditions to promote such reactions, may occur *in vivo* in connection with a variety of biochemical events. For example, abnormal enzyme activity of certain enzymes is often observed in cancer or cancer related diseases, cardiovascular
- 15 diseases, diseases of the central nervous system, in inflammations and infections. Determination of abnormal enzyme activity and identification of tissue or cell showing abnormal enzyme activity can therefore provide valuable diagnostic information.

- The NMR agents in accordance with the invention can advantageously be tailor designed
- 20 such that the chemical reaction which causes the first form of the agent to switch into the second form of the agent is promoted through a biochemical stimulus such as a change in pH, redox environment or by certain enzyme activity.

- Where the chemical reaction promotes cleavage of a covalent bond associated with the
- 25 polymer, the covalent bond may form part of a redox degradable linkage, for example a disulfide linkage, or an acid hydrolysable linkage, for example an oxime, imine, ester, hydrazone or acetal linkage. Alternatively, the cleaved covalent bond may form part of an enzyme degradable linkage, for example a peptide linkage. In other words, the covalent bond may be enzymatically cleaved.

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Such degradable linkages may be used to covalently couple a pharmaceutically active

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agent and/or hydrophobic moiety to a polymer chain used in accordance with the invention, or alternatively used in the formation of a crosslink that crosslinks polymer chains used in accordance with the invention.

- 5 In one embodiment, the chemical reaction promotes cleavage of a hydrazone, oxime, imine, ester, disulfide and/or peptide group.

As illustrative examples only, pharmaceutically active agents such as maytansine, doxorubicine, and paclitaxel may be covalently coupled to a polymer chain in accordance
10 with the invention through a disulfide group, a hydrazone group and an acetal group, respectively.

The present invention therefore also provides a method of detecting a chemical reaction in a medium, the method comprising introducing to the medium a switchable nuclear
15 magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing the chemical reaction in the medium the first form of the agent switches into a second form in which the
20 NMR signal of said nuclides is amplified, said amplified NMR signal being used to detect occurrence of the chemical reaction.

In one embodiment, the medium in which the chemical reaction takes place is a biological system.
25

Where the biological system is a subject, the method of detecting the chemical reaction will of course comprise administering the switchable NMR agent to the subject.

In one embodiment, the chemical reaction detected is an enzyme reacting with the NMR
30 agent. For example, the NMR agent may be used to detect enzyme activity in the subject. In that case, the method of detecting a chemical reaction may be described as a method of

- 50 -

detecting enzyme activity in a subject, the method comprising administering to the subject a switchable NMR agent in accordance with the invention.

According to such methods of the invention, the amplified NMR signal is used to detect
5 occurrence of the chemical reaction. Accordingly, it will be appreciated that the chemical reaction is required to occur within a suitable NMR detector such as a MRI apparatus or an NMR spectrometer.

As used herein the expression "pharmaceutically active agent" is intended to mean a drug
10 or any other similar chemical or biological material or compound suitable for administration to a subject, that induces a desired biological or pharmacological effect, which may include but is not limited to (1) having a prophylactic effect on the subject and preventing an undesired biological effect such as preventing an infection, (2) alleviating a condition caused by a disease, for example, alleviating pain or inflammation caused as a
15 result of disease, and/or (3) either alleviating, reducing, or completely eliminating the disease from the subject. The effect may be local, such as providing for a local anaesthetic effect, or it may be systemic.

This invention is not intended to be limited to the use of specific pharmaceutically active
20 agents. Rather in the context of pharmaceutically active agents it is concerned with the delivery of agents that exist in the state of the art or that may later be established as active agents and that are suitable for delivery by the present invention.

Such agents include broad classes of compounds normally delivered to a subject. In
25 general this includes but is not limited to: antiinfectives such as antibiotics and antiviral agents; analgesics and analgesic combinations; anorexics; helminthics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiabetic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations; antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics;
30 antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations including potassium and calcium channel blockers, beta-blockers, alpha-

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blockers, and antiarrhythmics; antihypertensives; diuretics and antidiuretics; vasodilators; including general coronary, peripheral and cerebral; central nervous system stimulants; vasoconstrictors; cough and cold preparations including decongestants; hormones such as estradiol and other steroids, including corticosteroids; hypnotics; immunosuppressives; 5 muscle relaxants; parasympatholytics; psychostimulants; sedatives; and tranquilizers; probiotics.

In one embodiment, the pharmaceutically active agent used in accordance with the invention is a cellular toxin.

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Reference to cellular toxin herein should be understood to mean any proteinaceous or non-proteinaceous molecule or group of molecules which will either retard cell growth or induce cell death, for example either by directly killing the cell or else delivering a signal which induces apoptosis. That is, the toxin may be either cytostatic or cytotoxic. It would 15 be appreciated by the person of skill in the art that the present invention can be designed to deliver one cellular toxin or multiple cellular toxins (i.e. a "cocktail" of toxin). The decision in relation to how best to proceed can be made by the person of skill in the art as a matter of routine procedure. For example, depending on the diagnosis of the subject, certain specific toxins or combinations of toxins are regarded as particularly desirable to 20 use. It would be appreciated that the body of knowledge in relation to the characteristics and use of cytotoxic agents is extensive and the person of skill in the art could design an administration protocol to meet the parameters of the present invention as a matter of routine procedure.

25 Reference to "cellular toxin" herein should therefore be understood as a reference to any agent which acts to damage or destroy cells. Without limiting the present invention to any one theory or mode of action, many such agents function via the induction of apoptotic processes. However, this is not the only mechanism by which such agents function and it is conceivable that the subject damage or cell death may be induced by some other 30 mechanism. Examples of cytotoxic agents include, but are not limited to, Actinomycin D, Adriamycin, Arsenic Trioxide, Asparaginase, Bleomycin, Busulfan, Camptothecin, and Irinotecan.

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Carboplatinum, Carmustine, Chlorambucil, Cisplatin, Corticosteroids, Colicheamicin, Cyclophosphamide, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Gemzar, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Lomustine, Maytansine, Melphalan, Mercaptopurine, 5 Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Platinol, Platinex, Procarbazine, Raltitrexel, Rixin, Steroids, Streptozocin, Taxol, Taxotere, Thioguanine, Thiotepa, Tomudex, Topotecan, Treosulfan, Trihydrate, Vinblastine, Vincristine, Vindesine, Vinorelbine, Vinorelbine, duanomycin, dactinomycin, esorubisin, mafosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, Mitomycin C, mithramycin, prednisone, 10 hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, hexamethylmelamine, pentamethylmelamine, amsacrine, chlorambudil, methylcyclohexylnitrosurea, nitrogen mustards, Cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, deoxyco-formycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), colchicine, trimetrexate, teniposide, diethylstilbestrol.

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However, reference to "cellular toxin" should also be understood to extend to any other molecule which is perhaps not traditionally regarded as a cytotoxic agent but nevertheless falls within the scope of the present definition on the basis that it induces cellular damage, for example DNA damage, such as nucleophosmin or agents which induce cellular damage as part of a synergistic process with another agent. Examples include catalytic antibodies, 20 prodrugs, CHK1/2 inhibitor (such as CBP-501 or AZD7762), histone deacetylase inhibitor (such as vorinostat), tumor necrosis factor related apoptosis inducing ligand or BH3 mimetic (such as ABT737), small molecule inhibitors such as the tyrosine kinase inhibitors imatinib mesylate (Glivec®), gefitinib (Iressa®) and erlotinib (Tarceva®), and the 25 monoclonal antibodies (mAb) such as rituximab (Mabthera®) and trastuzumab (Herceptin®).

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Combination treatments may include, for example, gemcitabine together with a CHK1/2 inhibitor or irinotecan together with a CHK1/2 inhibitor.

The cellular toxin may be a molecule which functions as an RNA interference mechanism.

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Without limiting the present invention to any one theory or mode of action "RNA interference" broadly describes a mechanism of gene silencing which is based on degrading or otherwise preventing the translation of mRNA in a sequence specific manner. In terms of the application of this technology to selectively knocking down gene expression, exogenous double stranded RNA (dsRNA) specific to a gene sought to be
5 knocked down can be introduced into the intracellular environment. Once the dsRNA enters the cell, it is cleaved by an RNaseIII-like enzyme, Dicer, into double stranded small interfering RNAs (siRNAs) 21-23 nucleotides in length that contain 2 nucleotide overhangs on the 3' ends. In an ATP dependent step, the siRNAs become integrated into a
10 multi-subunit protein complex known as the RNAi induced silencing complex (RISC), which guides the siRNAs to the target RNA sequence. The siRNA unwinds and the antisense strand remains bound to RISC and directs degradation of the complementary target mRNA sequence by a combination of endo- and exonucleases. However, whereas the RNAi mechanism was originally identified in the context of its role as a microbial
15 defence mechanism in higher eukaryotes, it is also known that RNAi based gene expression knockdown can also function as a mechanism to regulate endogenous gene expression. Specifically, microRNA (miRNA) is a form of endogenous single-stranded RNA which is typically 20-25 nucleotides and is endogenously transcribed from DNA, but not translated into protein. The DNA sequence that codes for an miRNA gene generally
20 includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair form a double stranded RNA hairpin loop, this forming the primary miRNA structure (pri-miRNA). A nuclear enzyme cleaves the base of the hairpin to form pre-miRNA. The pre-miRNA molecule is then actively transported out of the
25 nucleus into the cytoplasm where the Dicer enzyme cuts 20-25 nucleotides from the base of the hairpin to release the mature miRNA.

Although both of the RNA interference mechanisms detailed above effectively achieve the same outcome, being selective gene expression knockdown, RNAi based on the use of
30 exogenously administered dsRNA generally results in mRNA degradation while RNAi based on the actions of miRNAs generally results in translational repression by a

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mechanism which does not involve mRNA degradation. The RNA interference which is contemplated in the context of the present invention should be understood to encompass reference to both of these RNAi gene knockdown mechanisms. The induction of this miRNA based knockdown mechanism could be achieved by administering, in accordance
5 with the method of the invention, exogenous RNA oligonucleotides of the same sequence as an miRNA, pre-miRNA or pri-miRNA molecules. However, it should be understood that these exogenous RNA oligonucleotides may lead to either mRNA degradation (analogous to that observed with the introduction of an exogenous siRNA population) or mRNA translational repression, this being akin to the mechanism by which the endogenous
10 miRNA molecules function. In terms of using a cellular toxin in accordance with the invention, the occurrence of either gene knockdown mechanism is acceptable.

The RNA interference mechanism herein discussed is effected via the use of an RNA oligonucleotide which can induce an RNA interference mechanism. Reference to an
15 "RNA oligonucleotide" should therefore be understood as a reference to an RNA nucleic acid molecule which is double stranded or single stranded and is capable of either effecting the induction of an RNA interference mechanism directed to knocking down the expression of a gene targeted or downregulating or preventing the onset of such a mechanism. In this regard, the subject oligonucleotide may be capable of directly
20 modulating an RNA interference mechanism or it may require further processing, such as is characteristic of hairpin double stranded RNA which requires excision of the hairpin region, longer double stranded RNA molecules which require cleavage by dicer or precursor molecules such as pre-miRNA which similarly require cleavage. The subject oligonucleotide may be double stranded (as is typical in the context of effecting RNA
25 interference) or single stranded (as may be the case if one is seeking only to produce a RNA oligonucleotide suitable for binding to an endogenously expressed gene). Examples of RNA oligonucleotides suitable for use in the context of the present invention include, but are not limited to:

- 30 (i) *long double stranded RNA (dsRNA)* – these are generally produced as a result of the hybridisation of a sense RNA strand and an antisense RNA

strand which are each separately transcribed by their own vector. Such double stranded molecules are not characterised by a hairpin loop. These molecules are required to be cleaved by an enzyme such as Dicer in order to generate short interfering RNA (siRNA) duplexes. This cleavage event preferably occurs in the cell in which the dsRNA is transcribed.

(ii) *hairpin double stranded RNA (hairpin dsRNA)* – these molecules exhibit a stem-loop configuration and are generally the result of the transcription of a construct with inverted repeat sequences which are separated by a nucleotide spacer region, such as an intron. These molecules are generally of longer RNA molecules which require both the hairpin loop to be cleaved off and the resultant linear double stranded molecules to be cleaved by Dicer in order to generate siRNA. This type of molecule has the advantage of being expressible by a single vector.

(iii) *short interfering RNA (siRNA)* – these can be synthetically generated or, recombinantly expressed by the promoter based expression of a vector comprising tandem sense and antisense strands each characterised by its own promoter and a 4-5 thymidine transcription termination site. This enables the generation of 2 separate transcripts which subsequently anneal. These transcripts are generally of the order of 20-25 nucleotides in length. Accordingly, these molecules require no further cleavage to enable their functionality in the RNAi pathway.

(iv) *short hairpin RNA (shRNA)* – these molecules are also known as “small hairpin RNA” and are similar in length to the siRNA molecules but with the exception that they are expressed from a vector comprising inverted repeat sequences of the 20-25 nucleotide RNA molecule, the inverted repeats being separated by a nucleotide spacer. Subsequently to cleavage of the hairpin (loop) region, there is generated a functional siRNA molecule.

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- (v) *micro RNA/small temporal RNA (miRNA/stRNA)* – miRNA and stRNA are generally understood to represent naturally occurring endogenously expressed molecules. Accordingly, although the design and administration of a molecule intended to mimic the activity of a miRNA will take the form of a synthetically generated or recombinantly expressed siRNA molecule, the method of the present invention nevertheless extends to the design and expression of oligonucleotides intended to mimic miRNA, pri-miRNA or pre-miRNA molecules by virtue of exhibiting essentially identical RNA sequences and overall structure. Such recombinantly generated molecules may be referred to as either miRNAs or siRNAs.
- (vi) miRNAs which mediate spatial development (sdRNAs), the stress response (srRNAs) or cell cycle (ccRNAs).
- (vii) RNA oligonucleotides designed to hybridise and prevent the functioning of endogenously expressed miRNA or stRNA or exogenously introduced siRNA. It would be appreciated that these molecules are not designed to invoke the RNA interference mechanism but, rather, prevent the upregulation of this pathway by the miRNA and/or siRNA molecules which are present in the intracellular environment. In terms of their effect on the miRNA to which they hybridise, this is reflective of more classical antisense inhibition.

It will be appreciated that the person of skill in the art can determine the most suitable RNA oligonucleotide for use in any given situation. For example, although it is preferable that the subject oligonucleotide exhibits 100% complementarity to its target nucleic acid molecule, the oligonucleotide may nevertheless exhibit some degree of mismatch to the extent that hybridisation sufficient to induce an RNA interference response in a sequence specific manner is enabled. Accordingly, it is preferred that the oligonucleotide comprises at least 70% sequence complementarity, more preferably at least 90% complementarity and even more preferably, 95%, 96%, 97%, 98% 99% or 100% sequence complementarity.

In another example pertaining to the design of oligonucleotides suitable for use in the present invention, it is within the skill of the person of skill in the art to determine the particular structure and length of the subject oligonucleotide, for example whether it takes
5 the form of dsRNA, hairpin dsRNA, siRNA, shRNA, miRNA, pre-miRNA, pri-miRNA etc. For example, it is generally understood that stem-loop RNA structures, such as hairpin dsRNA and shRNA, are more efficient in terms of achieving gene knockdown than, for example, double stranded DNA which is generated utilising two constructs separately coding the sense and antisense RNA strands. Still further, the nature and length of the
10 intervening spacer region can impact on the functionality of a given stem-loop RNA molecule. In yet still another example, the choice of long dsRNA, which requires cleavage by an enzyme such as Dicer, or short dsRNA (such as siRNA or shRNA) can be relevant if there is a risk that in the context of the particular cellular environment an interferon response could be generated, this being a more significant risk where long dsRNA is used
15 than where short dsRNA molecules are utilised. In still yet another example, whether a single stranded or double stranded nucleic acid molecule is required to be used will also depend on the functional outcome which is sought. For example, to the extent that one is targeting an endogenously expressed miRNA with an antisense molecule, it would generally be appropriate to design a single stranded RNA oligonucleotide suitable for
20 specifically hybridising to the subject miRNA. However, to the extent that it is sought to induce RNA interference, a double stranded siRNA molecule is required. This may be designed as a long dsRNA molecule which undergoes further cleavage or an siRNA.

To facilitate or enhance diagnostic or therapeutic applications of the NMR agent in
25 accordance with the invention, the polymer chain may also comprise one or more ligands to target delivery of the agent within a subject. By a "ligand" in this context is meant a molecule that binds to or interacts with a target molecule or cell within the subject. For example, the ligand can be a small molecule, hormone, growth factor, steroid, protein, antibody, antibody fragment, peptide or polypeptide, or mimetic thereof. Thus the ligand
30 may be a molecule that combined to a receptor expressed on the surface of a target cell or conversely, to a molecule expressed on the surface of a target cell. The specific chemical

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composition of the ligand will be primarily selected based on the diseased state or condition to be diagnosed or treated.

Targets to which ligands can be selected to bind with include a wide variety of molecules including for example, cell signalling molecules, antibodies and antibody fragments, proteins and cell surface receptors.

Generally, such ligands will be covalently bound to the polymer chain.

10 In one embodiment, the polymer chain therefore further comprises a targeting ligand covalently coupled thereto.

In another embodiment, the polymer chain further comprises a targeting ligand covalently coupled thereto that targets tissue expressing an enzyme that promotes the chemical
15 reaction.

The NMR agent according to the invention may also be suitably adapted for use in imaging techniques not reliant upon NMR. The NMR agent may therefore be adapted for use in applications such as ultrasound, X-ray, Computed Tomography (CT), Single Photon
20 Emission Computed Tomography (SPECT), and PET.

In one embodiment, the NMR agent comprises a radioactive isotope suitable for use in nuclear medical procedures such as nuclear imaging (e.g. PET). Examples of suitable radioactive isotopes include ^{99m}Tc , ^{67}Ga , ^{64}Cu , ^{89}Zr and ^{18}F . In one embodiment, the
25 polymer chain further comprises covalently coupled thereto a radioactive isotope. The radioactive isotope may be provided in the form of a complex, with the complex being covalently coupled to the polymer chain. In that case, it will be appreciated that the radioactive isotope *per se* is not directly or indirectly covalently coupled to the polymer chain.

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Accordingly, in another embodiment the polymer further comprises covalently coupled

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thereto a complexed moiety, wherein the complex moiety comprises a radioactive isotope.

Incorporation of a suitable co-imaging agent with the NMR agent in accordance with the invention can greatly enhance utility of the agent in diagnostic imaging.

5

Those skilled in the art will appreciate that NMR provides the underlying principle in the function of MRI. Accordingly, the NMR agents according to the present invention are particularly useful in MRI applications.

- 10 The present invention therefore further provides a method of obtaining an image or region of interest in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in
- 15 which the NMR signal of said nuclides is diminished, wherein upon undergoing a chemical reaction in the medium the first form of the agents switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to obtain the image.
- 20 In one embodiment, the method involves obtaining an image of a region of interest in a biological system.

In a further embodiment, the method involves obtaining an image of a region of interest in a subject. Such an image will generally be acquired using MRI.

25

Where the NMR agent in accordance with the invention is used in medical applications, it will usually be provided in the form of a composition comprising at least one pharmacologically acceptable carrier.

- 30 The present invention therefore provides a composition for magnetic resonance imaging (MRI) comprising a switchable nuclear magnetic resonance (NMR) agent according to the

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invention and at least one pharmacologically acceptable carrier.

The present invention further provides use of an NMR agent in accordance with the invention in the manufacture of a composition for obtaining a diagnostic image.

5

In the context of using the NMR agent or composition thereof in MRI, the agent/composition will generally be administered to a subject. Where the NMR agent or composition comprising the agent is to be administered to a subject, they should of course be suitable for administration to the subject.

10

By the term "subject" is meant either an animal or human subject. By "animal" is meant primates, livestock animals (including cows, horses, sheep, pigs and goats), companion animals (including dogs, cats, rabbits and guinea pigs), and captive wild animals (including those commonly found in a zoo environment). Laboratory animals such as rabbits, mice, rats, guinea pigs and hamsters are also contemplated as they may provide a convenient test system. In some embodiments, the subject is a human subject.

15

By being "suitable" for administration to a subject is meant that administration of the agent or composition to a subject will not result in unacceptable toxicity, including allergenic responses and disease states.

20

By "administration" of the agent or composition to a subject is meant that the agent or composition is presented such that it can be or is transferred to the subject. There is no particular limitation on the mode of administration, but this will generally be by way of oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous, intrathecal, and intraspinal), inhalation (including nebulisation), rectal and vaginal modes.

25

Compositions in accordance with the invention comprise a pharmacologically acceptable carrier. By "pharmacologically acceptable" is meant that the carrier, or other constituent of the composition (e.g. stabiliser) is suitable for administration to a subject in their own right. In other words, administration of such material, to a subject will not result in

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unacceptable toxicity, including allergenic responses and disease states.

As a guide only, a person skilled in the art may consider "pharmacologically acceptable" as an entity approved by a regulatory agency of a federal or state government or listed in the
5 US Pharmacopeia or other generally recognised pharmacopeia for use in animals, and more particularly humans.

Having said this, those skilled in the art will appreciate that the suitability of a composition for administration to a subject and whether or not a given NMR agent or carrier would be
10 considered pharmacologically acceptable, will to some extent depend upon the mode of administration selected. Thus, the mode of administration may need to be considered when evaluating whether a given composition is suitable for administration to a subject or pharmacologically acceptable.

15 The carrier used in compositions of the invention may comprise of one or more different liquids. Suitable pharmacologically acceptable liquid carriers are described in Martin, Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA, (1990), and include, but are not limited to, liquids that may be sterilised such as water and oils, including those of petroleum, animal, vegetable, mineral or synthetic origin, such as peanut
20 oil, soya bean oil, mineral oil, sesame oil, and the like. Other suitable liquids include methylene glycol, propylene glycol, polyethylene glycol, polypropylene glycol, ethanol, isopropyl alcohol, benzyl alcohol. Water or soluble saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions.

25

The compositions of the invention may comprise one or more pharmacologically acceptable additives known to those in the art. For example, one or more additives such as wetting agents, de-foaming agents, surfactants, buffers, electrolytes, preservatives, colourings, flavourings and sweeteners.

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Where the NMR agent comprises a pharmaceutically active agent, the agent or

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composition comprising the agent may comprise a pharmaceutical adjuvant which may improve the efficacy or potency of the pharmaceutically active agent.

5 The particular nature of carrier and any other additive (if present) will in part depend upon the intended application of the NMR agent/composition. Those skilled in the art will be able to select a suitable liquid carrier and additive (if present) for the intended application.

10 The NMR agent described herein (generally as part of a composition) may be administered in, as appropriate, a treatment, inhibitory, or diagnostic effective amount. A treatment, inhibitory, or diagnostic effective amount is intended to include an amount which, when administered according to the desired dosing regimen, achieves a desired therapeutic or diagnostic effect, including one or more of: alleviating the symptoms of, preventing or delaying the onset of, inhibiting or slowing the progression of, diagnosing, or halting or reversing altogether the onset or progression of a particular condition being treated and/or
15 assessed.

Suitable dosage amounts and dosing regimens to achieve this can be determined by an attending physician and may depend on the particular condition being treated or diagnosed, the severity of the condition as well the general age, health and weight of the subject.

20

Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. Suitable dosages of the NMR agent *per se* may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage may be in the range of 1 µg to 1 g per kg of body weight
25 per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage may be in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage may be in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another embodiment, the dosage may be in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per body weight
30 per dosage.

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The NMR agent/compositions in accordance with the invention may be administered in a single dose or a series of doses.

Where the NMR agent/compositions in accordance with the invention are suitable for parenteral administration, they will generally be in the form of an aqueous or non-aqueous isotonic sterile injection solution that may contain one or more of an anti-oxidant, buffer, bactericide or solute which renders the composition isotonic with the blood of the intended subject. Such compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials.

10

Where the NMR agent in accordance with the invention is provided in the form of a liquid composition, the polymer chain will generally need to be maintained in a suspended or dispersed state within the liquid carrier. The polymer chain may have as part of its structure sections or regions that facilitate maintaining the polymer chain within a dispersed state throughout the liquid medium. For example, the polymer chain may comprise stabilising polymeric moieties such as polyethylene glycol (PEG) segment.

15

In one embodiment, the NMR agent is provided in a liquid carrier with the polymer chain being in a dispersed state within the carrier, wherein polymer is maintained in that dispersed state without use of a stabiliser. In other words, the polymer chain is self-stabilising.

20

The polymer chain may also be maintained in a dispersed state within a liquid carrier using a stabiliser. Suitable pharmaceutically acceptable stabilisers include, but are not limited to, various polymers, low molecular weight oligomers, natural products, and surfactant stabilisers including nonionic, anionic, cationic, ionic, and zwitterionic surfactants.

25

Representative examples of stabilisers include hydroxypropyl methylcellulose (now known as hypromellose), hydroxypropylcellulose, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol

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monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (e.g., macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (e.g., the commercially available Tweens® such as e.g., Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (e.g., Carbowaxes 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, citric acid, triethanolamine, polyvinyl alcohol (PVA), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (e.g., Pluronic F68® and F 108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, NJ.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-I 1 0®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-IOG® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2(CON(CH_3)-CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl (-D-glucopyranoside; n-decyl (-D- maltopyranoside; n-dodecyl (-D-glucopyranoside; n-dodecyl (-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-(-D-glucopyranoside; n-heptyl (-D- thioglucoside; n-hexyl (-D-glucopyranoside; nonanoyl-N-methylglucamide; n- noyl (-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl (-D- glucopyranoside; octyl (-D-thioglucopyranoside; PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

Examples of useful cationic stabilisers include, but are not limited to, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilisers, poly-n- methylpyridinium, anthryl pyridinium chloride, cationic

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phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr)₃ hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate. Other useful cationic stabilisers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quaternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C 12- 15dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulfate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide, N-alkyl (C 12- 18)dimethylbenzyl ammonium chloride, N-alkyl (C14- 18)dimethyl-benzyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C 12- 14) dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylanionium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C12-14) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C12, C15, C17 trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyltrimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALQUAT 336), POLYQUAT, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stealkonium chloride compounds (such as stearyltrimonium

chloride and distearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL and ALKAQUAT (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl
5 pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

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Such exemplary cationic stabilisers and other useful cationic stabilisers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry*,
15 (Marcel Dekker, 1990).

A further list of stabilisers include benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quaternary phosphorous compound, a pyridinium compound,
20 an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quaternary ammonium compounds. Examples of quaternary ammonium compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride (Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium
25 POE (10) oleyl ether phosphate, diethanolammonium POE (3) oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium

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chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procaine hydrochloride, cocobetaine, stearyltrimonium bentonite, stearyltrimonium hectorite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these stabilisers are known pharmacologically acceptable excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated herein by reference.

The NMR agents in accordance with the invention are particularly suitable for use in biological systems. By "biological system" is meant a subject, an individual cell, a collection of cells such as a cell culture, an organ, a tissue, and a multi-cell organism such as subjects. Accordingly, the NMR agent may be used or applied *in vivo*, *ex vivo* or *in vitro*. Where the NMR agent is used or applied *ex vivo* or *in vitro*, its use or application will be with biological material. For example, the agent may be used to obtain an image of a region of interest in a biological material, or the agent may be used to detect a chemical reaction in a biological material.

By the expression "biological material" is meant a substance derived or obtained from a living organism. Illustrated examples of biological materials include, but are not limited to, cells, tissues, blood, blood components, proteins (including recombinant proteins, transgenic proteins and proteinaceous materials), peptides, and enzymes.

Where the NMR agent in accordance with the invention is used to obtain an image or a region of interest in a medium, the nuclides of the agent will generally provide for in the second form of the agent in which their NMR signal is amplified a T_2 the value of at least 5ms, for example at least 10 ms or at least 20 ms, or at least 30 ms, or at least 40 ms, or at

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least 70 ms, or at least 100 ms.

Detection of the NMR signal provided by the NMR agent in accordance with the invention may be obtained using NMR detectors well known to those skilled in the art, for example
5 an NMR spectrometer or MRI apparatus.

NMR agents in accordance with the invention are particularly versatile and are not limited by the potential toxic nature of conventional gadolinium and iron oxide particle based agents. The agents also exhibit an ability to (1) absolutely quantify the number of
10 molecules in a volume element, (2) deliver and confirm that delivery through observation of an amplified NMR signal, and (3) to follow successive processes such as the passage through a membrane and then binding to a particular target molecule.

As used herein, the term "alkyl", used either alone or in compound words denotes straight
15 chain, branched or cyclic alkyl, preferably C₁₋₂₀ alkyl, e.g. C₁₋₁₀ or C₁₋₆. Examples of straight chain and branched alkyl include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, *t*-butyl, *n*-pentyl, 1,2-dimethylpropyl, 1,1-dimethyl-propyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-
20 trimethylpropyl, heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethyl-pentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-methyloctyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-,
25 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2-pentylheptyl and the like. Examples of
30 cyclic alkyl include mono- or polycyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and the like.

Where an alkyl group is referred to generally as "propyl", "butyl" etc, it will be understood that this can refer to any of straight, branched and cyclic isomers where appropriate. An alkyl group may be optionally substituted by one or more optional substituents as herein defined.

5

The term "alkenyl" as used herein denotes groups formed from straight chain, branched or cyclic hydrocarbon residues containing at least one carbon to carbon double bond including ethylenically mono-, di- or polyunsaturated alkyl or cycloalkyl groups as previously defined, preferably C₂₋₂₀ alkenyl (e.g. C₂₋₁₀ or C₂₋₆). Examples of alkenyl
10 include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-
15 cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl. An alkenyl group may be optionally substituted by one or more optional substituents as herein defined.

As used herein the term "alkynyl" denotes groups formed from straight chain, branched or cyclic hydrocarbon residues containing at least one carbon-carbon triple bond including
20 ethylenically mono-, di- or polyunsaturated alkyl or cycloalkyl groups as previously defined. Unless the number of carbon atoms is specified the term preferably refers to C₂₋₂₀ alkynyl (e.g. C₂₋₁₀ or C₂₋₆). Examples include ethynyl, 1-propynyl, 2-propynyl, and butynyl isomers, and pentynyl isomers. An alkynyl group may be optionally substituted by one or more optional substituents as herein defined.

25

The term "halogen" ("halo") denotes fluorine, chlorine, bromine or iodine (fluoro, chloro, bromo or iodo).

The term "aryl" (or "carboaryl") denotes any of single, polynuclear, conjugated and fused
30 residues of aromatic hydrocarbon ring systems (e.g. C₆₋₂₄ or C₆₋₁₈). Examples of aryl include phenyl, biphenyl, terphenyl, quaterphenyl, naphthyl, tetrahydronaphthyl,

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anthracenyl, dihydroanthracenyl, benzanthracenyl, dibenzanthracenyl, phenanthrenyl, fluorenyl, pyrenyl, idenyl, azulenyl, chrysenyl. Preferred aryl include phenyl and naphthyl. An aryl group may or may not be optionally substituted by one or more optional substituents as herein defined. The term "arylene" is intended to denote the divalent form
5 of aryl.

The term "carbocyclyl" includes any of non-aromatic monocyclic, polycyclic, fused or conjugated hydrocarbon residues, preferably C_{3-20} (e.g. C_{3-10} or C_{3-8}). The rings may be saturated, e.g. cycloalkyl, or may possess one or more double bonds (cycloalkenyl) and/or
10 one or more triple bonds (cycloalkynyl). Particularly preferred carbocyclyl moieties are 5-6-membered or 9-10 membered ring systems. Suitable examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cyclopentenyl, cyclohexenyl, cyclooctenyl, cyclopentadienyl, cyclohexadienyl, cyclooctatetraenyl, indanyl, decalynyl and indenyl. A carbocyclyl group may be optionally
15 substituted by one or more optional substituents as herein defined. The term "carbocyclylene" is intended to denote the divalent form of carbocyclyl.

The term "heteroatom" or "hetero" as used herein in its broadest sense refers to any atom other than a carbon atom which may be a member of a cyclic organic group. Particular
20 examples of heteroatoms include nitrogen, oxygen, sulfur, phosphorous, boron, silicon, selenium and tellurium, more particularly nitrogen, oxygen and sulfur.

The term "heterocyclyl" when used alone or in compound words includes any of monocyclic, polycyclic, fused or conjugated hydrocarbon residues, preferably C_{3-20} (e.g.
25 C_{3-10} or C_{3-8}) wherein one or more carbon atoms are replaced by a heteroatom so as to provide a non-aromatic residue. Suitable heteroatoms include O, N, S, P and Se, particularly O, N and S. Where two or more carbon atoms are replaced, this may be by two or more of the same heteroatom or by different heteroatoms. The heterocyclyl group may be saturated or partially unsaturated, i.e. possess one or more double bonds.
30 Particularly preferred heterocyclyl are 5-6 and 9-10 membered heterocyclyl. Suitable examples of heterocyclyl groups may include azridinyl, oxiranyl, thiiranyl, azetidiny,

oxetanyl, thietanyl, 2H-pyrrolyl, pyrrolidinyl, pyrrolinyl, piperidyl, piperazinyl, morpholinyl, indolinyl, imidazolidinyl, imidazolynyl, pyrazolidinyl, thiomorpholinyl, dioxanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyrrolyl, tetrahydrothiophenyl, pyrazolinyl, dioxalanyl, thiazolidinyl, isoxazolidinyl, dihydropyranyl, oxazinyl, thiazinyl, 5 thiomorpholinyl, oxathianyl, dithianyl, trioxanyl, thiadiazinyl, dithiazinyl, trithianyl, azepinyl, oxepinyl, thiepinyl, indenyl, indanyl, 3H-indolyl, isoindolynyl, 4H-quinolazynyl, chromenyl, chromanyl, isochromanyl, pyranyl and dihydropyranyl. A heterocyclyl group may be optionally substituted by one or more optional substituents as herein defined. The term "heterocyclylene" is intended to denote the divalent form of heterocyclyl.

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The term "heteroaryl" includes any of monocyclic, polycyclic, fused or conjugated hydrocarbon residues, wherein one or more carbon atoms are replaced by a heteroatom so as to provide an aromatic residue. Preferred heteroaryl have 3-20 ring atoms, e.g. 3-10. Particularly preferred heteroaryl are 5-6 and 9-10 membered bicyclic ring systems. 15 Suitable heteroatoms include, O, N, S, P and Se, particularly O, N and S. Where two or more carbon atoms are replaced, this may be by two or more of the same heteroatom or by different heteroatoms. Suitable examples of heteroaryl groups may include pyridyl, pyrrolyl, thienyl, imidazolyl, furanyl, benzothienyl, isobenzothienyl, benzofuranyl, isobenzofuranyl, indolyl, isoindolyl, pyrazolyl, pyrazinyl, pyrimidinyl, pyridazinyl, 20 indolizinyl, quinolyl, isoquinolyl, phthalazinyl, 1,5-naphthyridinyl, quinoxalinyl, quinoxalinyl, quinolizyl, oxazolyl, thiazolyl, isothiazolyl, isoxazolyl, triazolyl, oxadiazolyl, oxatriazolyl, triazinyl, and furazanyl. A heteroaryl group may be optionally substituted by one or more optional substituents as herein defined. The term "heteroarylene" is intended to denote the divalent form of heteroaryl.

25

The term "acyl" either alone or in compound words denotes a group containing the moiety C=O (and not being a carboxylic acid, ester or amide) Preferred acyl includes C(O)-R^e, wherein R^e is hydrogen or an alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocyclyl, or heterocyclyl residue. Examples of acyl include formyl, straight chain or branched alkanoyl 30 (e.g. C₁₋₂₀) such as acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl,

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- dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl and icosanoyl; cycloalkylcarbonyl such as cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl and cyclohexylcarbonyl; aroyl such as benzoyl, toluoyl and naphthoyl; aralkanoyl such as phenylalkanoyl (e.g. phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutyryl, phenylpentanoyl and phenylhexanoyl) and naphthylalkanoyl (e.g. naphthylacetyl, naphthylpropanoyl and naphthylbutanoyl); aralkenoyl such as phenylalkenoyl (e.g. phenylpropenoyl, phenylbutenoyl, phenylmethacryloyl, phenylpentenoyl and phenylhexenoyl) and naphthylalkenoyl (e.g. naphthylpropenoyl, naphthylbutenoyl and naphthylpentenoyl);
- 5 aryloxyalkanoyl such as phenoxyacetyl and phenoxypropionyl; arylthiocarbamoyl such as phenylthiocarbamoyl; arylglyoxyloyl such as phenylglyoxyloyl and naphthylglyoxyloyl; arylsulfonyl such as phenylsulfonyl and naphthylsulfonyl; heterocycliccarbonyl; heterocyclicalkanoyl such as thienylacetyl, thienylpropanoyl, thienylbutanoyl, thienylpentanoyl, thienylhexanoyl, thiazolylacetyl, thiadiazolylacetyl and tetrazolylacetyl;
- 10 heterocyclicalkenoyl such as heterocyclicpropenoyl, heterocyclicbutenoyl, heterocyclicpentenoyl and heterocyclichexenoyl; and heterocyclicglyoxyloyl such as thiazolylglyoxyloyl and thienylglyoxyloyl. The R^e residue may be optionally substituted as described herein.
- 15
- 20 The term "sulfoxide", either alone or in a compound word, refers to a group $-S(O)R^f$ wherein R^f is selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, carbocyclyl, and aralkyl. Examples of preferred R^f include C₁₋₂₀alkyl, phenyl and benzyl.
- 25 The term "sulfonyl", either alone or in a compound word, refers to a group $S(O)_2R^f$, wherein R^f is selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, carbocyclyl and aralkyl. Examples of preferred R^f include C₁₋₂₀alkyl, phenyl and benzyl.
- 30 The term "sulfonamide", either alone or in a compound word, refers to a group $S(O)NR^fR^f$ wherein each R^f is independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl,

heteroaryl, heterocyclyl, carbocyclyl, and aralkyl. Examples of preferred R^f include C_{1-20} alkyl, phenyl and benzyl. In one embodiment at least one R^f is hydrogen. In another embodiment, both R^f are hydrogen.

- 5 The term, "amino" is used here in its broadest sense as understood in the art and includes groups of the formula NR^aR^b wherein R^a and R^b may be any independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, carbocyclyl, heteroaryl, heterocyclyl, arylalkyl, and acyl. R^a and R^b , together with the nitrogen to which they are attached, may also form a monocyclic, or polycyclic ring system e.g. a 3-10 membered ring, particularly, 5-6 and 9-10 10 membered systems. Examples of "amino" include NH_2 , NH alkyl (e.g. C_{1-20} alkyl), NH aryl (e.g. NH phenyl), NH aralkyl (e.g. NH benzyl), NH acyl (e.g. $NHC(O)C_{1-20}$ alkyl, $NHC(O)$ phenyl), N alkylalkyl (wherein each alkyl, for example C_{1-20} , may be the same or different) and 5 or 6 membered rings, optionally containing one or more same or different heteroatoms (e.g. O, N and S).

15

- The term "amido" is used here in its broadest sense as understood in the art and includes groups having the formula $C(O)NR^aR^b$, wherein R^a and R^b are as defined as above. Examples of amido include $C(O)NH_2$, $C(O)NH$ alkyl (e.g. C_{1-20} alkyl), $C(O)NH$ aryl (e.g. $C(O)NH$ phenyl), $C(O)NH$ aralkyl (e.g. $C(O)NH$ benzyl), $C(O)NH$ acyl (e.g. $C(O)NHC(O)C_{1-20}$ alkyl, $C(O)NHC(O)$ phenyl), $C(O)N$ alkylalkyl (wherein each alkyl, for example C_{1-20} , may be the same or different) and 5 or 6 membered rings, optionally containing one or more same or different heteroatoms (e.g. O, N and S).

- 25 The term "carboxy ester" is used here in its broadest sense as understood in the art and includes groups having the formula CO_2R^g , wherein R^g may be selected from groups including alkyl, alkenyl, alkynyl, aryl, carbocyclyl, heteroaryl, heterocyclyl, aralkyl, and acyl. Examples of carboxy ester include CO_2C_{1-20} alkyl, CO_2 aryl (e.g. CO_2 phenyl), CO_2 aralkyl (e.g. CO_2 benzyl).

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As used herein, the term "aryloxy" refers to an "aryl" group attached through an oxygen bridge. Examples of aryloxy substituents include phenoxy, biphenyloxy, naphthyloxy and the like.

- 5 As used herein, the term "acyloxy" refers to an "acyl" group wherein the "acyl" group is in turn attached through an oxygen atom. Examples of "acyloxy" include hexylcarbonyloxy (heptanoyloxy), cyclopentylcarbonyloxy, benzoyloxy, 4-chlorobenzoyloxy, decylcarbonyloxy (undecanoyloxy), propylcarbonyloxy (butanoyloxy), octylcarbonyloxy (nonanoyloxy), biphenylcarbonyloxy (eg 4-phenylbenzoyloxy), naphthylcarbonyloxy (eg
10 1-naphthoyloxy) and the like.

As used herein, the term "alkyloxycarbonyl" refers to a "alkyloxy" group attached through a carbonyl group. Examples of "alkyloxycarbonyl" groups include butylformate, sec-butylformate, hexylformate, octylformate, decylformate, cyclopentylformate and the like.

- 15 As used herein, the term "arylalkyl" refers to groups formed from straight or branched chain alkanes substituted with an aromatic ring. Examples of arylalkyl include phenylmethyl (benzyl), phenylethyl and phenylpropyl.

- As used herein, the term "alkylaryl" refers to groups formed from aryl groups substituted
20 with a straight chain or branched alkane. Examples of alkylaryl include methylphenyl and isopropylphenyl.

- In this specification "optionally substituted" is taken to mean that a group may or may not be substituted or fused (so as to form a condensed polycyclic group) with one, two, three
25 or more of organic and inorganic groups, including those selected from: alkyl, alkenyl, alkynyl, carbocyclyl, aryl, heterocyclyl, heteroaryl, acyl, aralkyl, alkaryl, alkheterocyclyl, alkheteroaryl, alkcarbocyclyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, halocarbocyclyl, haloheterocyclyl, haloheteroaryl, haloacyl, haloaryalkyl, hydroxy, hydroxyalkyl, hydroxyalkenyl, hydroxyalkynyl, hydroxycarbocyclyl, hydroxyaryl,
30 hydroxyheterocyclyl, hydroxyheteroaryl, hydroxyacyl, hydroxyaralkyl, alkoxyalkyl, alkoxyalkenyl, alkoxyalkynyl, alkoxy carbocyclyl, alkoxyaryl, alkoxyheterocyclyl,

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alkoxyheteroaryl, alkoxyacyl, alkoxyaralkyl, alkoxy, alkenyloxy, alkynyloxy, aryloxy, carbocyclyloxy, aralkyloxy, heteroaryloxy, heterocyclyloxy, acyloxy, haloalkoxy, haloalkenyloxy, haloalkynyloxy, haloaryloxy, halocarbocyclyloxy, haloaralkyloxy, haloheteroaryloxy, haloheterocyclyloxy, haloacyloxy, nitro, nitroalkyl, nitroalkenyl, 5 nitroalkynyl, nitroaryl, nitroheterocyclyl, nitroheteroaryl, nitrocarbocyclyl, nitroacyl, nitroaralkyl, amino (NH₂), alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, aralkylamino, diaralkylamino, acylamino, diacylamino, heterocyclamino, heteroarylamino, carboxy, carboxyester, amido, alkylsulphonyloxy, arylsulphenyloxy, alkylsulphenyl, arylsulphenyl, thio, alkylthio, alkenylthio, alkynylthio, 10 arylthio, aralkylthio, carbocyclylthio, heterocyclylthio, heteroarylthio, acylthio, sulfoxide, sulfonyl, sulfonamide, aminoalkyl, aminoalkenyl, aminoalkynyl, aminocarbocyclyl, aminoaryl, aminoheterocyclyl, aminoheteroaryl, aminoacyl, aminoaralkyl, thioalkyl, thioalkenyl, thioalkynyl, thiocarbocyclyl, thioaryl, thioheterocyclyl, thioheteroaryl, thioacyl, thioaralkyl, carboxyalkyl, carboxyalkenyl, carboxyalkynyl, carboxycarbocyclyl, 15 carboxyaryl, carboxyheterocyclyl, carboxyheteroaryl, carboxyacyl, carboxyaralkyl, carboxyesteralkyl, carboxyesteralkenyl, carboxyesteralkynyl, carboxyestercarbocyclyl, carboxyesteraryl, carboxyesterheterocyclyl, carboxyesterheteroaryl, carboxyesteracyl, carboxyesteraralkyl, amidoalkyl, amidoalkenyl, amidoalkynyl, amidocarbocyclyl, amidoaryl, amidoheterocyclyl, amidoheteroaryl, amidoacyl, amidoaralkyl, formylalkyl, 20 formylalkenyl, formylalkynyl, formylcarbocyclyl, formylaryl, formylheterocyclyl, formylheteroaryl, formylacyl, formylaralkyl, acylalkyl, acylalkenyl, acylalkynyl, acylcarbocyclyl, acylaryl, acylheterocyclyl, acylheteroaryl, acylacyl, acylaralkyl, sulfoxidealkyl, sulfoxidealkenyl, sulfoxidealkynyl, sulfoxidecarbocyclyl, sulfoxidearyl, sulfoxideheterocyclyl, sulfoxideheteroaryl, sulfoxideacyl, sulfoxidearalkyl, sulfonylalkyl, 25 sulfonylalkenyl, sulfonylalkynyl, sulfonylcarbocyclyl, sulfonylaryl, sulfonylheterocyclyl, sulfonylheteroaryl, sulfonylacyl, sulfonylaralkyl, sulfonamidoalkyl, sulfonamidoalkenyl, sulfonamidoalkynyl, sulfonamidocarbocyclyl, sulfonamidoaryl, sulfonamidoheterocyclyl, sulfonamidoheteroaryl, sulfonamidoacyl, sulfonamidoaralkyl, nitroalkyl, nitroalkenyl, nitroalkynyl, nitrocarbocyclyl, nitroaryl, nitroheterocyclyl, nitroheteroaryl, nitroacyl, 30 nitroaralkyl, cyano, sulfate, phosphate, triarylmethyl, triarylamino, oxadiazole, and carbazole groups. Optional substitution may also be taken to refer to where a -CH₂- group

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in a chain or ring is replaced by a group selected from -O-, -S-, -NR^a-, -C(O)- (i.e. carbonyl), -C(O)O- (i.e. ester), and -C(O)NR^a- (i.e. amide), where R^a is as defined herein.

Preferred optional substituents include alkyl, (e.g. C₁₋₆ alkyl such as methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl), hydroxyalkyl (e.g. hydroxymethyl, hydroxyethyl, hydroxypropyl), alkoxyalkyl (e.g. methoxymethyl, methoxyethyl, methoxypropyl, ethoxymethyl, ethoxyethyl, ethoxypropyl etc) alkoxy (e.g. C₁₋₆ alkoxy such as methoxy, ethoxy, propoxy, butoxy, cyclopropoxy, cyclobutoxy), halo, trifluoromethyl, trichloromethyl, tribromomethyl, hydroxy, phenyl (which itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆ alkyl, C₁₋₆ alkoxy, haloC₁₋₆alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), benzyl (wherein benzyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆alkyl, C₁₋₆ alkoxy, haloC₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), phenoxy (wherein phenyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆ alkyl, C₁₋₆ alkoxy, haloC₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), benzyloxy (wherein benzyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆ alkyl, C₁₋₆ alkoxy, haloC₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), amino, alkylamino (e.g. C₁₋₆ alkyl, such as methylamino, ethylamino, propylamino etc), dialkylamino (e.g. C₁₋₆ alkyl, such as dimethylamino, diethylamino, dipropylamino), acylamino (e.g. NHC(O)CH₃), phenylamino (wherein phenyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆ alkyl, C₁₋₆ alkoxy, haloC₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), nitro, formyl, -C(O)-alkyl (e.g. C₁₋₆ alkyl, such as acetyl), O-C(O)-alkyl (e.g. C₁₋₆alkyl, such as acetyloxy), benzoyl (wherein the phenyl group itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyC₁₋₆ alkyl, C₁₋₆ alkoxy, haloC₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆alkyl, and amino), replacement of CH₂ with C=O, CO₂H, CO₂alkyl (e.g. C₁₋₆ alkyl such as methyl ester, ethyl ester, propyl ester, butyl ester), CO₂phenyl (wherein phenyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyl C₁₋₆ alkyl, C₁₋₆ alkoxy, halo C₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), CONH₂, CONHphenyl (wherein phenyl itself may be further substituted e.g., by C₁₋₆ alkyl, halo, hydroxy, hydroxyl C₁₋₆ alkyl, C₁₋₆ alkoxy, halo C₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), CONHbenzyl (wherein benzyl itself may be further substituted e.g., by

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C₁₋₆ alkyl, halo, hydroxy hydroxyl C₁₋₆ alkyl, C₁₋₆ alkoxy, halo C₁₋₆ alkyl, cyano, nitro OC(O)C₁₋₆ alkyl, and amino), CONHalkyl (e.g. C₁₋₆ alkyl such as methyl ester, ethyl ester, propyl ester, butyl amide) CONHdialkyl (e.g. C₁₋₆ alkyl) aminoalkyl (e.g., HN C₁₋₆ alkyl-, C₁₋₆alkylHN-C₁₋₆ alkyl- and (C₁₋₆ alkyl)₂N-C₁₋₆ alkyl-), thioalkyl (e.g., HS C₁₋₆ alkyl-),
 5 carboxyalkyl (e.g., HO₂CC₁₋₆ alkyl-), carboxyesteralkyl (e.g., C₁₋₆ alkylO₂CC₁₋₆ alkyl-), amidoalkyl (e.g., H₂N(O)CC₁₋₆ alkyl-, H(C₁₋₆ alkyl)N(O)CC₁₋₆ alkyl-), formylalkyl (e.g., OHCC₁₋₆alkyl-), acylalkyl (e.g., C₁₋₆ alkyl(O)CC₁₋₆ alkyl-), nitroalkyl (e.g., O₂NC₁₋₆ alkyl-), sulfoxidealkyl (e.g., R(O)SC₁₋₆ alkyl, such as C₁₋₆ alkyl(O)SC₁₋₆ alkyl-), sulfonylalkyl (e.g., R(O)₂SC₁₋₆ alkyl- such as C₁₋₆ alkyl(O)₂SC₁₋₆ alkyl-), sulfonamidoalkyl (e.g.,
 10 ₂HRN(O)SC₁₋₆ alkyl, H(C₁₋₆ alkyl)N(O)SC₁₋₆ alkyl-), triarylmethyl, triaryl amino, oxadiazole, and carbazole.

The invention will now be described with reference to the following non-limiting examples.

15

Examples

Example 1

20 **Production of acetal-protected hyperbranched ¹⁹F imaging agent (P1): hyperbranched solketal methacrylate-co-trifluoroethyl methacrylate.**

The title compound was synthesised as follows:

0.48 mL of ethyleneglycol dimethacrylate (2.54e-3 mol), 7.97 g solketal methacrylate
 25 (SMA, 3.98e-2 mol)), 1.26 mL of 2,2,2-trifluoroethyl acrylate (FEA, 9.96e-3 mol)), 870 mg 2-dodecylsulfanyl thiocarbonyl sulfanyl-2-methylpropionic acid (2.39 e-3 mol) and 35 mg 2,2-azobisisobutyronitrile (AIBN, 2.1e-4 mol) were dissolved up in 50 mL tetrahydrofuran (THF) in a round bottom flask. The solution was then purged with argon for 20 minutes to remove oxygen. The round bottom flask was heated to 65°C, and the
 30 reaction was allowed to proceed for 24 hours. The crude reaction mixture was then precipitated into hexane to remove residual monomer and dried in a vacuum oven.

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^1H NMR (CDCl_3): 0.87-1.06 ppm (br, m, CH backbone), 1.23 ppm (s, $\text{SCH}_2\text{C}_{11}\text{H}_{20}$), 1.37 ppm (d, $(\text{CH}_3)_2\text{CO}_2\text{R}$), 1.83 (br, CH_2 backbone), 3.63 ppm, (s, $\text{SCH}_2\text{C}_{11}\text{H}_{23}$) 3.7-4.3 ppm (br, m, $\text{OCH}_2\text{CHCH}_2$), 4.29 ppm (CH_2CF_3). Relative mol % incorporation of components from NMR: 21% FEA, 79% SMA.

5

Molecular weight analysis:

M_N	M_W	PDI
8900	19900	2.24

Example 2

10

Production of polyethyleneglycol-stabilised P1 (P2): hyperbranched polyethyleneglycolmethacrylate-block-P1 copolymers (P2)

The title compound was synthesised as follows:

15 2 g of P1 (2.25×10^{-4} mol) was dissolved up in 10 mL of THF with 1.10 mL of (polyethylene glycol) methacrylates ($M_w=475$) (2.5×10^{-3} mol) and 3.3 mg AIBN (2.0×10^{-5} mol). The solution was purged with argon for 10 minutes and then heated to 65°C and allowed to react for 24 hours. Crude reaction mixture was precipitated into hexane. The product was then dialysed in water for 4 days in a 3,500 MWCO dialysis snakeskin, and then lyophilised.

20 ^1H NMR (CDCl_3): 0.87-1.06 ppm (br, m, CH backbone), 1.23 ppm (s, $\text{SCH}_2\text{C}_{11}\text{H}_{20}$), 1.37 ppm (d, $(\text{CH}_3)_2\text{CO}_2\text{R}$), 1.58 ppm (br, s, CH_2 backbone PEGMA), 1.83 (br, CH_2 backbone), 3.36 ppm (s, OCH_3) 3.62 ppm ($\text{CH}_2\text{CH}_2\text{O}$), 3.63 ppm, (s, $\text{SCH}_2\text{C}_{11}\text{H}_{23}$) 3.7-4.3 ppm (br, m, $\text{OCH}_2\text{CHCH}_2$), 4.29 ppm (CH_2CF_3).

25 Molecular weight analysis:

M_N	M_W	PDI
10400	20900	2.02

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^{19}F T_2 relaxation time at 7 T: 0.341 ms

Example 3

5 **Production of deprotected P2 (P3): hyperbranched polyethyleneglycolmethacrylate-block-(polyglycerolmethacrylate-co-trifluoroethylacrylate).**

The title compound was synthesised as follows:

10 10 mg of P2 was dissolved in 1 mL of 50:50 10% hydrochloric acid in tetrahydrofuran (THF) solution and allowed to react for 5 hours in an uncapped 20 mL vial, until solution became noticeably clearer. The remaining THF was blown off with nitrogen gas.

^{19}F T_2 relaxation time at 7 T: 28 ms

15 ^{19}F T_2 measurements were recorded on a Bruker 300 MHz wide-bore system (7 T) using a 5 mm birdcage resonator. Typically, the polymer was dissolved in water to a concentration of 20 mg/mL. The samples were analysed using a standard spin echo pulse sequence with a repetition time of 3 seconds. The ^{19}F images were recorded on the same instrument using a spin-echo 3D pulse sequence. Sample phantoms were made up as described for the T_2 measurements. A matrix with dimensions of 128x96x8 was collected with two repetitions giving an overall scan time of 68 minutes. An example of a slice from images collected for the acetal-protected monomer (P2) and the deprotected monomer (P3) are shown in Figure 1.

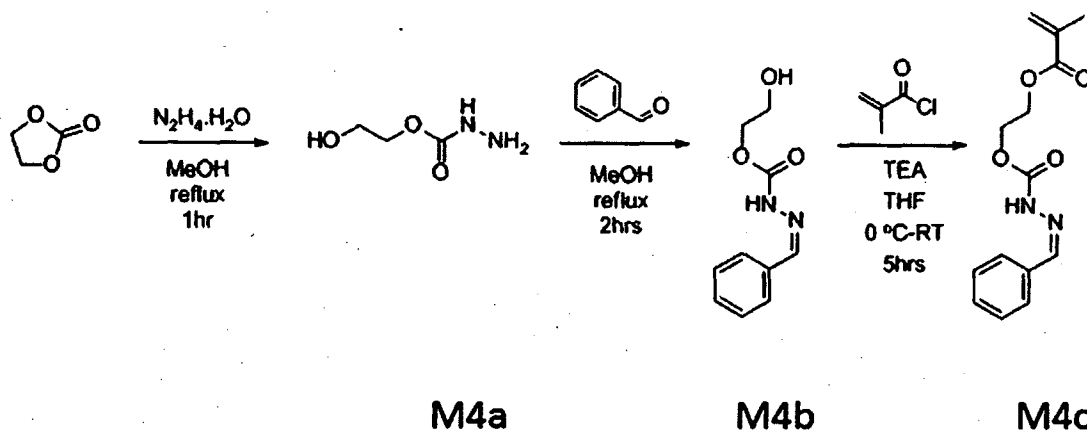
25 **Example 4**

Production of hydrazone-protected hyperbranched ^{19}F imaging agent (P4): hyperbranched 2-(methacryloyloxy)ethyl 2-benzylidene hydrazinecarboxylate-co-trifluoroethyl methacrylate-co-polyethyleneglycol monomethylether methacrylate.

30

The title compound was synthesised as follows:

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Synthesis of 2-hydroxyethyl hydrazinecarboxylate (M4a): Ethylene carbonate (8.2 g, 4.7 M) was added to methanol (20 mL) and stirred until solubilised. Hydrazine hydrate (4.6 mL, 4.7 M) was added in one batch and heat was generated. This solution was then refluxed for 1 hr then cooled. The solution was then reduced in volume, whereby a white precipitate formed. This was then collected by vacuum filtration and washed with a small volume of cold methanol then diethyl ether. The solid was then recrystallised in a minimum volume of boiling ethanol to form white crystals (10.1 g, 90.3 %). ¹H NMR (500 MHz, d₆-DMSO, δ, ppm): 0.88 (s, 3H), 1.40 (s, 3H), 1.44 (s, 3H), 1.95 (s, 3H), 3.61-3.71 (dd, ²J = 38.4 Hz, ³J = 10 Hz, 4H), 4.22 (s, 2H), 5.56 (t, J = 1.6 Hz, 1H), 6.10 (t, J = 1 Hz, 1H)

Synthesis of 2-hydroxyethyl 2-benzylidenehydrazine carboxylate (M4b): 2-hydroxyethyl hydrazinecarboxylate (5.1 g, 2.1 M) was added to methanol (10 mL) and stirred. Benzaldehyde (4.2 mL, 2.1 M) in methanol (10 mL) was added to this solution at room temperature and stirred for 5 min, whereby heat evolved. The solution was then refluxed for 2 hrs and then cooled to room temperature. The solvent was then removed *in vacuo* and the product solidified on cooling to give a waxy white solid (7.9 g, 91.2 %). This product was used without further purification. ¹H NMR (500 MHz, d₆-DMSO, δ, ppm): 0.88 (s, 3H), 1.40 (s, 3H), 1.44 (s, 3H), 1.95 (s, 3H), 3.61-3.71 (dd, ²J = 38.4 Hz, ³J = 10 Hz, 4H), 4.22 (s, 2H), 5.56 (t, J = 1.6 Hz, 1H), 6.10 (t, J = 1 Hz, 1H)

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Synthesis of 2-(methacryloyloxy)ethyl 2-benzylidene hydrazinecarboxylate (M4c): 2-hydroxyethyl 2-benzylidenehydrazine carboxylate (20 g,) was added to tetrahydrofuran (200 mL). To this solution was added triethylamine (14.7 mL) and the whole solution was stirred at 0 °C. Methacryloyl chloride (10.3 mL,) in tetrahydrofuran (50 mL) was added drop-wise over a 30 min period. After addition, the solution was stirred at room temperature for 5 hrs before methanol (5 mL) was added to quench any remaining acid chloride. The solution was then reduced in volume to dryness and redissolved in ethyl acetate (100 mL). This turbid solution was then extracted with saturated NaHSO₄ solution (3 x 50 mL), followed by Milli-Q water (50 mL) and finally brine (50 mL). The organic phase was then dried over MgSO₄, filtered and reduced in volume to dryness. Column chromatography on silica gel (petroleum spirit/ethyl acetate: 3/2) of this crude product afforded the desired monomer as a clear, highly viscous liquid (15.5 g, 58.4 %). ¹H NMR (500 MHz, d₆-DMSO, δ, ppm): 0.88 (s, 3H), 1.40 (s, 3H), 1.44 (s, 3H), 1.95 (s, 3H), 3.61-3.71 (dd, ²J = 38.4 Hz, ³J = 10 Hz, 4H), 4.22 (s, 2H), 5.56 (t, J = 1.6 Hz, 1H), 6.10 (t, J = 1 Hz, 1H).

Synthesis of RAFT agent 2-(((butylthio)carbonothioyl)thio)-2-methylpropanoic acid (BMP): This RAFT agent was synthesised according to the method of McCormick and coworkers.[1] ¹H NMR (500 MHz, CDCl₃, δ, ppm): 0.93 (t, J = 7.5 Hz, 3H), 1.43 (m, J = 7.5 Hz, 2H), 1.66 (m, J = 7.5 Hz, 2H), 1.72 (s, 6H), 3.29 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃, δ, ppm): 13.59, 22.09, 25.20, 29.84, 36.73, 55.53, 178.48, and 220.82.

Production of hyperbranched 2-(methacryloyloxy)ethyl 2-benzylidene hydrazinecarboxylate-co-trifluoroethyl acrylate-co-polyethyleneglycol monomethylether methacrylate (P4): PEGMA (2.49 g, 1.05 M), 4Mc (0.47 g, 0.25 M), EGDMA (0.096 mL, 0.10 M), BMP (0.12 g, 0.09 M), AIBN (7.5 mg, 9.14 x 10⁻³ M), and 1,4-dioxane (5 mL) were added to a 15 mL Schlenk flask equipped with a magnetic stirrer then purged with argon for 20 min. The flask was placed in a temperature controlled oil bath at 65 °C for 48 hrs. The flask was then recharged with PEGMA (3.35 g, 1.41 M), AIBN (6.7 mg, 8.16 x 10⁻³ M), and dioxane (5 mL) and polymerised for another 48 hrs at 65 °C. After this time the reaction was halted by quenching in ice and then exposure to air.

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The polymerisation mixture was then precipitated into petroleum spirit (BP 40-60) at 0 °C and stirred for 10 min. The supernatant was then decanted off the viscous polymer film. This film was then dried for 1 h under high vacuum at 25 °C. The polymer was then dissolved in Milli-Q water and dialysed against 10 mM phosphate buffer (pH ~ 8) for 3 days, after which it was lyophilised and characterised by SEC ($M_n = 59K$, PDI = 2.31). This polymer incorporated 6.8% hydrazone-monomer (by 1H NMR).
 ^{19}F T_2 relaxation time at 11.75T: 32 ms.

Example 5

10

Production of deprotected P4 (P5): hyperbranched 2-(methacryloyloxy)ethyl hydrazinecarboxylate-co-trifluoroethyl acrylate-co-polyethyleneglycol monomethylether methacrylate (P5).

50 mg P4 was dissolved in 1 mL of 9:1 mixture of H_2O/D_2O . 3 drops of concentrated HCL was added to the mixture and shaken for 1 minute.
 ^{19}F T_2 relaxation time at 11.75T: 39 ms.

Example 6

20 **Production of hyperbranched 2-phenyl-1,3-dioxan-5-yl methylmethacrylate-co-trifluoroethyl methacrylate-polyethyleneglycol monomethylether methacrylate (P6).**

The title compound was synthesised via a three-step procedure as follows.

Synthesis of hyperbranched polyethyleneglycol monomethyl ether methacrylate-co-trifluoroethyl acrylate-co-solketal methacrylate (P6a) . PEGMA (12.96 g, 0.73 M), solketal methacrylate (synthesised according to reference [2]) (1.37 g, 0.18 M), EGDMA (0.40 mL, 5.69×10^{-2} M), BMP (0.48 g, 5.69×10^{-2} M), AIBN (34.8 mg, 5.65×10^{-3} M), and tetrahydrofuran (24 mL) were added to a 100 mL Schlenk flask equipped with a magnetic stirrer then purged with argon for 20 min. The flask was placed in a temperature controlled oil bath at 70 °C for 48 hrs. The reaction was halted by quenching in ice and then exposure to air. The polymerization mixture was then precipitated into petroleum

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spirit (BP 40-60) at 0 °C and stirred for 10 min. The supernatant was then decanted off the viscous polymer film. This film was then dried for 1 h under high vacuum at 25 °C. The polymer was then dissolved in Milli-Q water and dialysed against water for 3 days, after which it was lyophilised and characterised by GPC. (M_n = 36K, PDI = 1.35). This
5 polymer contained 20 mol% SMA by ^1H NMR.

Deprotection of solketal methacrylate (P6b). The HBP PEGMA-co-TFEA-co-SMA (P6a) (11.46 g, 3.2×10^{-3} M) was ring opened by stirring in 10% HCl in water (100 mL) at room temperature overnight. The polymer was then pH balanced to neutral using 50 wt%
10 NaOH solution and then dialysed against water for 3 days, after which it was lyophilised and used without further characterisation.

Synthesis of title compound (P6) : HBP-OH (P6b) (2 g), benzaldehyde dimethyl acetal (400 μL), *p*-toluene sulfonic acid (50 mg) in *N,N'*-dimethylformamide (10 mL) at 50 °C
15 under house vacuum for 48 hrs. The solution was then precipitated into 2:1 diethyl ether/petroleum spirit (BP 40-60) at 0 °C and stirred for 10 min. The supernatant was then decanted off the viscous polymer film. This film was then dried for 1 h under high vacuum at 25 °C. The polymer was then dissolved in 10 mM phosphate buffer (pH ~ 7.4) and dialysed against pH 7.4 phosphate buffer for 3 days, after which it was lyophilised.
20 ^{19}F T_2 relaxation time at 11.75 T: 30 ms (20 mg/mL solution in 9:1 $\text{H}_2\text{O}:\text{D}_2\text{O}$)

Example 7

Production of deprotected P6 (P7): hyperbranched polyethyleneglycolmethacrylate-co-trifluoroethyl acrylate-co-glycerol methacrylate.
25

To a solution of P6 (20 mg/mL in H_2O) was added 3 drops of concentrated hydrochloric acid. The solution was shaken vigorously for 2 minutes.

^{19}F T_2 relaxation time at 11.75 T: 72 ms (20 mg/mL solution in 9:1 $\text{H}_2\text{O}:\text{D}_2\text{O}$)
30

The reference in this specification to any prior publication (or information derived from it),

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or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

5

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

CLAIMS:

1. A switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one
5 or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.
2. The switchable nuclear magnetic resonance (NMR) agent according to claim 1,
10 wherein the NMR signal is diminished or amplified through dipole-dipole couplings between said nuclides.
3. The switchable nuclear magnetic resonance (NMR) agent according to claim 1 or 2, wherein one or more F nuclides are covalently coupled to the polymer chain.
4. The switchable nuclear magnetic resonance (NMR) agent according to any one of
15 claims 1 to 3, wherein the polymer chain comprises one or more F substituted groups selected from trifluoromethyl, perfluoroethyl, 2,2,2-trifluoroethyl, perfluoropropyl, perfluoroisopropyl, bis(trifluoromethyl)methyl, tris(trifluoromethyl)methyl, 2,2,3,3,3-pentafluoropropyl, perfluorobutyl, trifluoromethylphenyl, 1,3-di(trifluoromethyl) phenyl, trifluoromethylbenzyl, 1,3-di(trifluoromethyl)benzyl, tris(trifluoromethyl)-methylphenyl,
20 tris(trifluoromethyl)-methylbenzyl, fluorophenyl, difluorophenyl, pentafluorophenyl and pentafluorobenzyl.
5. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 4, wherein the polymer chain further comprises a targeting ligand covalently coupled thereto.
- 25 6. The switchable nuclear magnetic resonance (NMR) agent according to claim 5, wherein the ligand is selected from a hormone, growth factor, steroid, protein, antibody, antibody fragment, peptide, polypeptide, or mimetic thereof.
7. The switchable nuclear magnetic resonance (NMR) agent according to any one of

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claims 1 to 6, wherein the polymer chain further comprises a pharmaceutically active agent covalently coupled or complexed thereto.

8. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 7, wherein said one or more nuclides are covalently coupled to an ionically charged polymer chain.

9. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 8, wherein the polymer chain forms part of a polymer microgel or dendritic polymer.

10. A method of detecting a chemical reaction in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing the chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to detect occurrence of the chemical reaction.

11. The method of detecting a chemical reaction in a medium according to claim 10, wherein the medium in which the chemical reaction takes place is a biological system.

12. The method of detecting a chemical reaction in a medium according to claim 10 or 11, wherein the chemical reaction that causes the first form of the agent to switch to the second form promotes cleavage of a covalent bond that forms part of the polymer chain, said cleavage of the covalent bond not resulting in the covalently coupled nuclide(s) being cleaved from the polymer chain.

13. The method of detecting a chemical reaction in a medium according to any one of claims 10 to 12, wherein the chemical reaction causes release from the agent of a hydrophobic moiety, the release of which causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

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14. The method of detecting a chemical reaction in a medium according to claim 13, wherein the hydrophobic moiety is a pharmaceutically active agent.
15. The method of detecting a chemical reaction in a medium according to any one of claims 10 to 13, wherein the chemical reaction is promoted through a change in pH, a
5 change in redox environment, or through enzyme activity.
16. A method of obtaining an image of a region of interest in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to
10 polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing a chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to obtain the image.
17. The method of obtaining an image of a region of interest in a medium according to
15 claim 15, wherein the medium in which the image is obtained is a biological system.
18. The method of obtaining an image of a region of interest in a medium according to claim 17, wherein the biological system is a subject.
19. The method of obtaining an image of a region of interest in a medium according to claim 18, wherein the polymer chain further comprises a targeting ligand covalently
20 coupled thereto that targets in the subject tissue expressing an enzyme that promotes the chemical reaction.
20. The method of obtaining an image of a region of interest in a medium according to any one of claims 16 to 19, wherein the polymer chain further comprises a pharmaceutically active agent covalently coupled or complexed thereto.
- 25 21. The method of obtaining an image of a region of interest in a medium according to claim 20, wherein the chemical reaction causes release from the agent of the pharmaceutically active agent, the release of which causes the first form of the agent to

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switch into a second form in which the NMR signal of said nuclides is amplified.

22. A composition for magnetic resonance imaging (MRI) comprising a switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 9.

CLAIMS:

1. A switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one
5 or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein the agent is capable of undergoing a chemical reaction that causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.
2. The switchable nuclear magnetic resonance (NMR) agent according to claim 1,
10 wherein the NMR signal is diminished or amplified through dipole-dipole couplings between said nuclides.
3. The switchable nuclear magnetic resonance (NMR) agent according to claim 1 or 2, wherein one or more F nuclides are covalently coupled to the polymer chain.
4. The switchable nuclear magnetic resonance (NMR) agent according to any one of
15 claims 1 to 3, wherein the polymer chain comprises one or more F substituted groups selected from trifluoromethyl, perfluoroethyl, 2,2,2-trifluoroethyl, perfluoropropyl, perfluoroisopropyl, bis(trifluoromethyl)methyl, tris(trifluoromethyl)methyl, 2,2,3,3,3-pentafluoropropyl, perfluorobutyl, trifluoromethylphenyl, 1,3-di(trifluoromethyl) phenyl, trifluoromethylbenzyl, 1,3-di(trifluoromethyl)benzyl, tris(trifluoromethyl)-methylphenyl,
20 tris(trifluoromethyl)-methylbenzyl, fluorophenyl, difluorophenyl, pentafluorophenyl and pentafluorobenzyl.
5. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 4, wherein the polymer chain further comprises a targeting ligand covalently coupled thereto.
- 25 6. The switchable nuclear magnetic resonance (NMR) agent according to claim 5, wherein the ligand is selected from a hormone, growth factor, steroid, protein, antibody, antibody fragment, peptide, polypeptide, or mimetic thereof.
7. The switchable nuclear magnetic resonance (NMR) agent according to any one of

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claims 1 to 6, wherein the polymer chain further comprises a pharmaceutically active agent covalently coupled or complexed thereto.

8. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 7, wherein said one or more nuclides are covalently coupled to an ionically charged polymer chain.

9. The switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 8, wherein the polymer chain forms part of a polymer microgel or dendritic polymer.

10. A method of detecting a chemical reaction in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing the chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to detect occurrence of the chemical reaction.

11. The method of detecting a chemical reaction in a medium according to claim 10, wherein the medium in which the chemical reaction takes place is a biological system.

12. The method of detecting a chemical reaction in a medium according to claim 10 or 11, wherein the chemical reaction that causes the first form of the agent to switch to the second form promotes cleavage of a covalent bond that forms part of the polymer chain, said cleavage of the covalent bond not resulting in the covalently coupled nuclide(s) being cleaved from the polymer chain.

13. The method of detecting a chemical reaction in a medium according to any one of claims 10 to 12, wherein the chemical reaction causes release from the agent of a hydrophobic moiety, the release of which causes the first form of the agent to switch into a second form in which the NMR signal of said nuclides is amplified.

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14. The method of detecting a chemical reaction in a medium according to claim 13, wherein the hydrophobic moiety is a pharmaceutically active agent.
15. The method of detecting a chemical reaction in a medium according to any one of claims 10 to 13, wherein the chemical reaction is promoted through a change in pH, a
5 change in redox environment, or through enzyme activity.
16. A method of obtaining an image of a region of interest in a medium, the method comprising introducing to the medium a switchable nuclear magnetic resonance (NMR) agent comprising a plurality of covalently coupled NMR nuclides selected from one or more of D, B, F, Br, P and Si, one or more of said nuclides being covalently coupled to a
10 polymer chain, said agent having a first form in which the NMR signal of said nuclides is diminished, wherein upon undergoing a chemical reaction in the medium the first form of the agent switches into a second form in which the NMR signal of said nuclides is amplified, said amplified NMR signal being used to obtain the image.
17. The method of obtaining an image of a region of interest in a medium according to
15 claim 15, wherein the medium in which the image is obtained is a biological system.
18. The method of obtaining an image of a region of interest in a medium according to claim 17, wherein the biological system is a subject.
19. The method of obtaining an image of a region of interest in a medium according to claim 18, wherein the polymer chain further comprises a targeting ligand covalently
20 coupled thereto that targets in the subject tissue expressing an enzyme that promotes the chemical reaction.
20. The method of obtaining an image of a region of interest in a medium according to any one of claims 16 to 19, wherein the polymer chain further comprises a pharmaceutically active agent covalently coupled or complexed thereto.
- 25 21. The method of obtaining an image of a region of interest in a medium according to claim 20, wherein the chemical reaction causes release from the agent of the pharmaceutically active agent, the release of which causes the first form of the agent to

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switch into a second form in which the NMR signal of said nuclides is amplified.

22. A composition for magnetic resonance imaging (MRI) comprising a switchable nuclear magnetic resonance (NMR) agent according to any one of claims 1 to 9.

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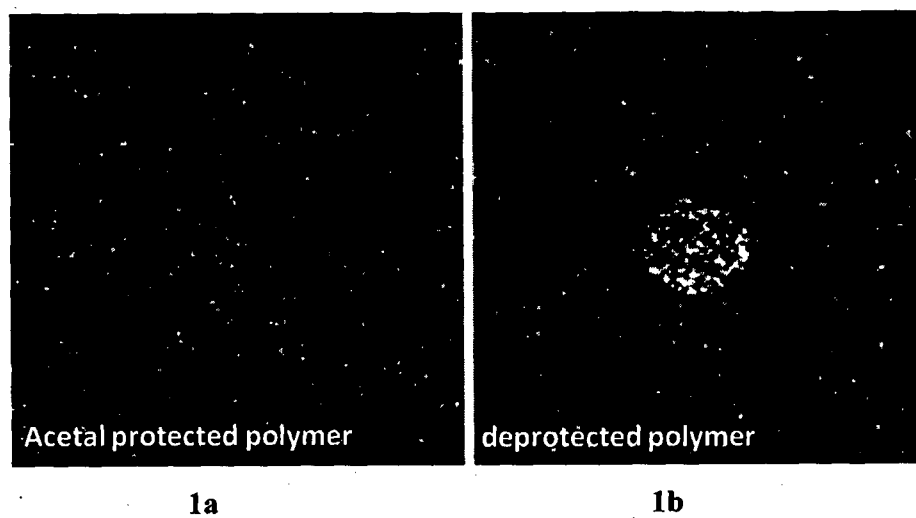


Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2012/000415

A. CLASSIFICATION OF SUBJECT MATTER A61K 49/06 (OCT 2005) A61K 49/18 (OCT 2005) A61B 5/055 (OCT 2005)					
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED					
Minimum documentation searched (classification system followed by classification symbols)					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) HCAPlus, Medline, BIOSIS, WPIDS. Keywords: NMR, MRI, Magnetic resonance, switch, polymer, nuclide, agent, macromolecule, micelle, image. [Google - inventor names only].					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
	Documents are listed in the continuation of Box C				
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex					
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="width: 33%; vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> <td style="width: 33%;"></td> </tr> </table>			* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
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Date of the actual completion of the international search 16 July 2012		Date of mailing of the international search report 30 July 2012			
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au Facsimile No.: +61 2 6283 7999		Authorized officer James-Robert Cram AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0399359645			

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2012/000415
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X Y	THOMAS, R. N., & GUO, C. "Surface-Functionalised, Probe-Containing, Polymeric Nanospheres for Biomedical Imaging", Spectroscopy, 2002, 16, 387-398. Abstract, conclusion, section 4, table 3, section 4.5 Whole document	1, 3-9, 22 2, 10-21
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X Y	WO 1991/012824 A2 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 05 September 1991 Abstract, page 3, 4, 10, 13 Whole document	1, 3-4, 22 2, 5-21
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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2012/000415
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	Abstract, examples	1, 3, 16, 22
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2012/000415	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2012/000415	
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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 2012/0148500 A1	14 Jun 2012	EP 2468305 A1	27 Jun 2012
		US 2012148500 A1	14 Jun 2012
End of Annex			
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