Composition comprising acetic anhydride and a gadolinium complex, and method for the use in hyperpolarisation mri analysis.

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(54) Title: COMPOSITION COMPRISING ACETIC ANHYDRIDE AND A GADOLINIUM COMPLEX, AND METHOD FOR THE USE IN HYPERPOLARISATION MRI ANALYSIS

(57) Abstract: The present invention generally relates to a composition comprising acetic anhydride, a DNP agent and a gadolinium complex and its use for the preparation of hyperpolarised imaging agent for MR diagnostic analysis.

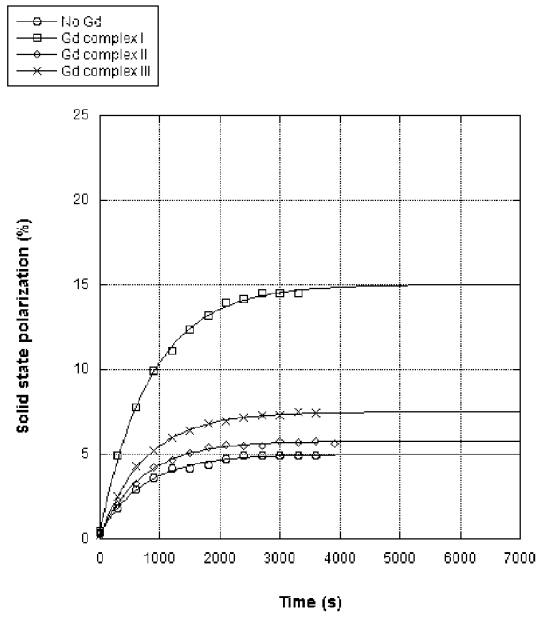

Figure 1.

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COMPOSITION COMPRISING ACETIC ANHYDRIDE AND A GADOLINIUM COMPLEX, AND METHOD FOR THE USE IN HYPERPOLARISATION MRI ANALYSIS.

The present invention generally relates to a composition comprising acetic anhydride, a DNP agent and a gadolinium complex and its use for the preparation of a hyperpolarised imaging agent for MR diagnostic analysis.

BACKGROUND OF THE INVENTION

MRI is a non invasive technique with broad diagnostic value. The technique has gained wide clinical acceptance and is of great importance in diagnostic medicine. However, despite significant technological advancements (increasing field strength and improvement in technology), applications of MRI are limited by an intrinsically low sensitivity.

Some alternatives to enhance its sensitivity have been developed which involve *ex-vivo* nuclear spin polarisation of agents, prior to administration and consequent *in-vivo* MR signal measurement.

EP1544634 discloses some of said alternative techniques, comprising among others, Dynamic Nuclear Polarisation (DNP), Para Hydrogen Induced (PHI) polarisation and Polarisation Transfer (PT) from a hyperpolarised noble gas.

WO9935508 describes a method for obtaining hyperpolarised high T1 agents by dynamic nuclear polarisation (DNP) whereby polarisation of a sample is carried out by a polarising agent or so-called DNP agent, which is a compound comprising unpaired electron.

WO2007064226 generally describes the use of paramagnetic metal ions, optionally added in chelated form, to enhance the hyperpolarisation level of a substrate, when subjected to hyperpolarisation MRI experiments.

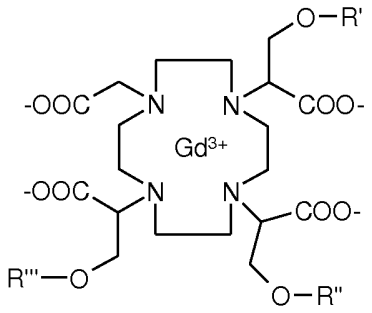
We have now found that when a composition comprising acetic anhydride, a DNP agent and a suitable gadolinium complex of formula (A), is used in DNP experiments, an unexpectedly high degree of polarisation of the acetic anhydride system can be achieved. Even further, when the hyperpolarised acetic anhydride thus obtained is contacted with an aqueous carrier, the anhydride is converted to acetic acid, maintaining the high degree of polarisation and thus employable e.g. as MRI contrast agent.

Hence, according to the invention, the gadolinium complex of formula (A) is particularly advantageous when used as DNP hyperpolarisation enhancer for acetic anhydride based systems. For these and other advantages, which may be better appreciated by the skilled person upon reading the detailed description of the invention, the present invention provides a substantial innovative contribution over the state of the art.

SUMMARY OF THE INVENTION

A first aspect of the invention refers to a composition comprising:

- acetic anhydride,
- a DNP agent, and
- a gadolinium complex of formula (A):



(A)

wherein:

R', R'' and R''' are independently selected from: optionally substituted phenyl, (C_1-C_6)alkylene-phenyl and C_6-C_{10} heterocyclic ring.

It is a further aspect of the invention the use of said composition for the preparation of hyperpolarised acetic anhydride in DNP experiment. In another aspect, the invention refers to the use of the gadolinium complex of general formula (A) as hyperpolarisation enhancer in dynamic nuclear polarisation (DNP) experiments.

The invention also relates to a process for preparing hyperpolarised acetic acid for the use in a method of magnetic resonance investigation, comprising:

a) subjecting the composition of the present invention as previously defined to dynamic nuclear polarisation (DNP) methods to obtain hyperpolarised acetic anhydride; and

b) contacting the hyperpolarised acetic anhydride of step a) with an aqueous carrier to convert said hyperpolarised acetic anhydride to hyperpolarised acetic acid; and optionally

c) removing the gadolinium complex of formula (A) and the DNP agent.

It is herein also provided a method for operating an MRI system comprising the steps of:

a) submitting a subject, which has been positioned in said MRI system and treated with hyperpolarised acetic acid obtained according to the above process, to a radiation frequency selected to excite nuclear spin transitions in a non-zero nuclear spin nuclei of said active substrate; and

b) recording a MR signal from said excited nuclei.

In a further aspect, the present invention relates to a method for operating a MRI system comprising the steps of:

a) submitting a subject pre-treated with hyperpolarised acetic acid obtained according to the above process, which has been positioned in said MRI system, to a radiation frequency selected to excite nuclear spin transitions in a non-zero nuclear spin nuclei of said active substrate; and

b) recording a MR signal from said excited nuclei.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Acetic anhydride solid state polarisation build up curves. The figure shows the degree of hyperpolarisation obtainable by adding three different gadolinium complexes to a mixture of acetic anhydride and Finlandic radical, in DNP experiments.

Figure 2: Butyric anhydride solid state polarisation build up curves. The figure shows the degree of hyperpolarisation obtainable by adding three different gadolinium complexes to a mixture of butyric anhydride and Finlandic radical, in DNP experiments.

Figure 3: Finlandic acid radical formula.

Figure 4: Gadolinium complexes I, II, III formulae.

DETAILED DESCRIPTION OF THE INVENTION

The term (C1-C6)alkylene-phenyl comprises within its meaning a bivalent linear or branched alkenyl group having from 1 to 6 atom carbons, such as:

methylene, ethylene, propylene and the like, linked to an optionally substituted phenyl group.

The term optionally substituted phenyl group comprises within its meaning a phenyl ring optionally substituted with e.g. one or more substituents such as C1-C6 linear or branched alkyl groups like, for instance, methyl, ethyl, propyl and the like.

The term C6-C10 heterocyclic ring comprises within its meaning an aromatic or aliphatic carbon ring having from 6 to 10 atoms, interrupted by one or more heteroatoms selected from: N, O, P and S.

The term "polarising agent" or "DNP agent" or "radical" comprises within its meaning a compound comprising unpaired electron. During the DNP process, energy, generally provided in the form of microwave irradiation, initially excites the DNP agent. Upon decay to the ground state, a transfer of polarisation occurs from the unpaired electron off the DNP agent to the NMR active nuclei of the sample, i.e. the acetic anhydride.

The term "glass form" comprises within its meaning a solid solution or an amorphous (non-crystalline) solid form.

The term "glass-forming agent" or "glassing agent" comprises within its meaning a compound which prevents crystallization and promotes the formation of a glass form.

The expression "aqueous carrier" comprises within its meaning any aqueous solvent, solvent mixtures or solutions that are tolerated by the human or non-human animal body, for use in *in-vivo* diagnostic applications.

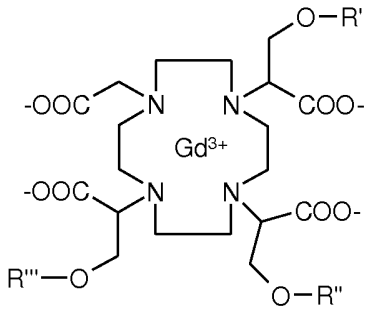
Generally, the carrier is sterile and physiologically tolerable, such as sterile water, purified water such as water for injection (WFI), physiological saline solution, optionally properly buffered.

In this respect, in some cases, the obtained aqueous solution (comprising the hyperpolarised acetic anhydride) may subsequently be admixed with further additives in order to render it physiologically acceptable for *in-vivo* diagnostic applications. Examples of suitable additives are pH regulating agents such as organic or inorganic bases (*e.g.* alkaline metal bases) or organic or inorganic acids or buffers.

In a first aspect, the invention relates to a composition for the use in DNP experiments for the preparation of hyperpolarised acetic anhydride, comprising:

- acetic anhydride, a

- DNP agent, and
- a gadolinium complex of formula (A):



(A)

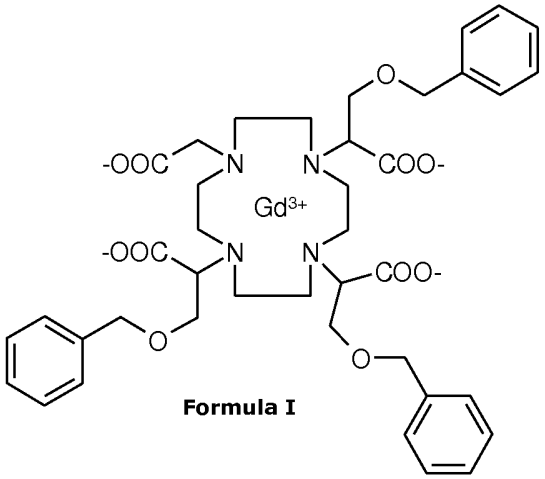
wherein:

R', R'' and R''' are independently selected from: optionally substituted phenyl, (C_1-C_6)alkylene-phenyl and C_6-C_{10} heterocyclic ring.

Preferably R', R'' and R''' are the same.

In a preferred embodiment, the composition is a liquid solution having a concentration of from about 2M to about 12 M with respect to the acetic anhydride, preferably from 5 to 10 M, even more preferably from 8 to 10M.

In a further embodiment, the present composition comprises: acetic anhydride, a DNP agent as herewith set forth and a gadolinium complex of the above formula (A), wherein R is the same a (C1-C6)alkenyl-phenyl, more preferably, the gadolinium complex of formula (A) is [[α1,α4,α7-tris[(phenylmethoxy)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetato(4-)] gadolinate(1-)]hydrogen, of formula:



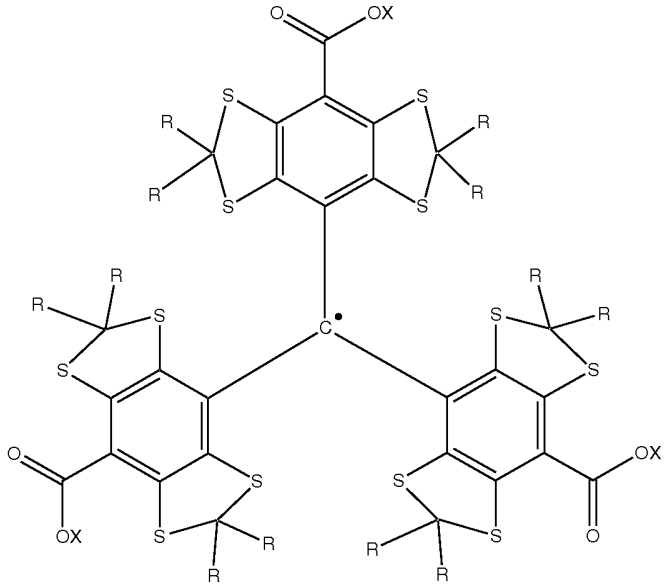
Formula I

In this respect, the gadolinium complex of formula (I) can be prepared e.g. according to Aime S. et al. Inorg. Chem.1992, 31, 1100-1103.

Preferred concentrations of the gadolinium complex are from 0.5 mM to 4 mM , more preferably from 1 mM to 2.5 mM.

As regards the DNP agent, commonly known as "radical", it should be stable in the reaction media of choice, and at least partially soluble in the system in order to obtain a homogenous distribution and an optimal concentration of the electron spin relative to the nuclear spin. Typically, the polarising agent is added in an amount of from 5 mM to 50 mM to the mixture undergoing DNP, more preferably from 8 to 18 mM. Due in particular to their efficient polarisation properties, the use of trityl radicals as polarising agents is preferred, such as, for instance those described in WO-A-99/35508, WO-A-88/10419, WO-A-90/00904, WO-A-91/12024, WO-A-93/02711 or WO-A-96/39367 and herein included by reference.

According to a still preferred embodiment, a radical of the following general formula (B) can advantageously be employed as polarising agent:



Formula (B)

wherein:

R the same or different, represents a straight chain or branched C1-C6-alkyl group optionally substituted by one or more hydroxyl group, methoxy group, or a group of formula $-(CH_2)_n-O-R2$, wherein n is 1, 2 or 3;

R2 is a straight chain or branched C1-C6-alkyl group, optionally substituted by one or more hydroxyl groups or methoxy groups; and

X is independently selected from: hydrogen H, an alkaline metal, *e.g.* Na, K, Cs, an optionally substituted straight or branched C1-C6 alkyl group, optionally interrupted by Sulphur or Oxygen atoms, and an optionally substituted aliphatic or aromatic C3-C8 cyclic group or hetero group.

Preferably, said radical is a compound of the above formula (B) which is soluble in acetic anhydride *e.g.* a compound of formula (B), wherein X is hydrogen, or wherein X is selected from hydrophobic moieties such as methyl, -CD3, ethyl, tert-butyl or phenyl.

The selected DNP agent or radical is preferably the trityl radical, e.g. the compound of the above formula (B) wherein R is CH3 or CD3, generally present in the composition at a concentration from 5 mM to 25 mM, whereas, preferred concentrations are from 10 mM to 15 mM.

The above mentioned radicals of general formula (B) are known and commercially available (e.g. from Sigma-Aldrich).

The present composition comprising the acetic anhydride, a DNP agent, e.g. of formula (B), and a paramagnetic complex of formula (A) can be prepared by mixing together the three components, e.g. by means of methods known in the art, such as stirring, vortexing and/or sonication.

Advantageously, the present composition does not require the presence of any additional glassing agent (such as glycerol or DMSO) since upon freezing the present composition spontaneously forms a glass, suitable for the DNP experiment.

Even further, as supported in the herein below experimental part, the composition of the invention when subjected to DNP experiments, allows the preparation of hyperpolarised acetic anhydride with an unexpectedly high degree of polarisation, with respect to similar mixtures comprising different gadolinium complexes, such as e.g. Gd-DOTAM of formula (II) or a gadolinium complex of formula (III) as indicated in Figure 4. In fact, the use of the complex of formula (I) ([[α1,α4,α7-tris[(phenylmethoxy)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetato(4-)] gadolinate(1-)]hydrogen) in the presence of a DNP agent as formerly described, allows the preparation of acetic anhydride with a degree of polarisation 2 times higher than the degree otherwise obtainable by using Gd-DOTAM (II) or the complex of formula (III), without impairing the polarisation time, as shown in Figure I. The result is even more surprising when considering a similar system wherein the acetic anhydride is replaced by butyric anhydride. The anhydrides are polar aprotic liquids which

are in fact able to form a glass form by themselves (e.g. without the addition of any glassing agent) upon freezing.

However, in the case of butyric anhydride, and contrary to what happen for the similar acetic anhydride system, the degree of polarisation obtained using the complex of formula (I) is basically the same obtainable by using the other gadolinium complexes of formula (II) and (III) (i.e. 1,5-1.7 time higher) as clearly indicated in the figure 2 herein below. This means that the present composition comprising the complex of general formula (A), as above defined, as DNP hyperpolarisation enhancer can be advantageously used in a process for the preparation of hyperpolarised acetic anhydride to be further used as precursor for the preparation of hyperpolarised acetic acid as herein below described. Conveniently in fact, because of the high degree of polarisation achievable, the hyperpolarised acetic anhydride obtained when the present composition is used in DNP experiments, can be contacted with an aqueous carrier to be hydrolyzed to acetic acid, substantially maintaining the high degree of polarisation

Therefore, in a further aspect, the invention refers to a process for the preparation of hyperpolarised acetic acid, said process comprising the steps of:

a) subjecting the composition of the present invention as previously defined to dynamic nuclear polarisation (DNP) methods to obtain hyperpolarised acetic anhydride; and
b) contacting the hyperpolarised acetic anhydride of step a) with an aqueous carrier to convert said hyperpolarised acetic anhydride to hyperpolarised acetic acid; and optionally
c) removing the gadolinium complex of formula (A) and the DNP agent.

In step a), the composition of the present invention, comprising acetic anhydride, a DNP agent and a gadolinium complex of formula (A), preferably of formula (I), is hyperpolarised by Dynamic Nuclear Polarisation (DNP), as described, for instance, in WO 98/58272 and WO0196895. Practically, the composition is cooled and /or frozen, in such a way that a glass form is formed. As afore mentioned, advantageously, and differently from other similar organic substrates, the present composition does not require the addition of any glass forming agent since the acetic anhydride system spontaneously forms a glass upon freezing. Preferably, the frozen composition is irradiated at a frequency

comprised from about 94GHz to about 200mW. The thus hyperpolarised acetic anhydride composition may be monitored using e.g. MR spectroscopy or MRI techniques, as commonly used in the art.

The solid polarised acetic anhydride, is then converted into liquid hyperpolarised acetic acid, according to step b) of the present process. Typically, the solid hyperpolarised composition obtained after step a) is dissolved in an appropriate aqueous carrier, optionally containing additives such as buffers, pH regulating agents, and the like. Preferably, the hyperpolarised acetic anhydride system is dissolved in an aqueous carrier, to result in a physiologically tolerable solution comprising hyperpolarised acetic acid. In this respect, preferred aqueous carriers are selected from: saline, water, aqueous solutions containing a suitable amount of a base, such as NaOH or other organic or inorganic compounds with basic aqueous reaction (e.g. tromethamine or trisodium phosphate), or of an inorganic or organic acid such as phosphoric acid, hydrochloric acid, citric acid or acetic acid, capable of promoting the hydrolysis. Typically, the base is present in an amount of about 1 to 5 mole equivalents to the anhydride, preferably about 2 to 4 mole.

The aqueous carrier is added to the anhydride mixture in order to have a quantitative transformation of the anhydride into the acid, wherein "quantitative transformation" means that the conversion is of at least 20%, preferably 50% or more, even more preferably 75%, 90% or more. After the dissolution step, the active acetic acid is present in the aqueous solution and the resulting pH may be adjusted at physiologically acceptable values generally by adding suitable acid or basic buffers thereto. The precise concentration will of course depend upon a range of factors such as, *inter alia*, toxicity and administration route. In general, optimal concentrations will in most cases lie in the range from 10 mM to 150 mM, particularly from 40 to 80 mM.

According to the last step of the present process, the gadolinium complex of formula (A) and/or the DNP agent, along with any reaction products thereof, are optionally removed from the final composition. If the hyperpolarised acetic acid thus obtained is intended to be used as a MR imaging agent in living human or animal being, both the trityl radical and the paramagnetic metal complex are preferably removed from the liquid composition. In this respect, upon dissolution of the solid hyperpolarised acetic anhydride composition the DNP agent and/or the paramagnetic metal ion might precipitate and thus may be easily separated from the liquid by filtration. If no precipitation occurs or if such

precipitation partially occurs, the DNP agent and the chosen paramagnetic complex may be removed by chromatographic separation techniques according to methods known in the art, such as reversed phase liquid chromatography and the like.

After removal of the paramagnetic complex of formula (A) and/or the DNP agent, or reaction derivatives thereof, the liquid sample may be checked for residual paramagnetic metal ion and/or DNP agent traces, by e.g. UV/visible absorption measurements or electrochemical detection, fluorescent measurements and the like.

In a preferred embodiment, the acetic anhydride is enriched with non-zero nuclear spin nuclei, such as 13C. The term "enriched" means that the concentration of the non-zero spin nuclei in the compound is above the typical value of natural abundance of said nuclei, preferably above at least 10% of natural abundance, more preferably above at least 25%, and even more preferably above at least 75% of its natural abundance and most preferably above at least 90% of its natural abundance. The enrichment will in particular be concentrated on an atom position, for which a chemical transformation of the molecule, or a chemical or magnetic changes of the environment of the molecule, will be measurable as a change of its chemical shift. Said non-zero nuclei confer to the substrate a T1 relaxation time of at least 5 seconds (indicated with s), preferably of at least 10 s, preferably of at least 20 s, preferably of at least 30 s, and even more preferably of at least 40 s, measured in a solution subjected to a magnetic fields of from about 0.5 mT to about 20 T (Tesla). The enrichment may include either selective enrichments of one or more sites within the molecule or uniform enrichment of all sites. To this extent, commercially available enriched acetic anhydride can be suitably employed or, in case, the enrichment can be achieved by chemical synthesis, or biological labeling, according to well known prior art teachings.

As extensively reported, the present invention provides a composition comprising acetic anhydride, a DNP agent and a gadolinium complex of general formula (A), preferably the complex of formula (I), for the preparation of hyperpolarised acetic anhydride with a remarkably high degree of polarisation, and suitable for the use in the preparation of a MRI contrast agent, when converted into the active acetic acid as above explained. In this respect, the dosage of the solution should be kept as low as possible whilst still providing a detectable contrast response. The dosage of the MR imaging substrate obtained

according to the present method will vary depending on, for instance, the nature of the tissue or organ of interest and the measuring apparatus.

In particular, the hyperpolarised hydrolysed substrate (i.e. the active acetic acid) can be administered into the vascular system or directly into an organ or muscle tissue, or by subdermal or subcutaneous route, as the case may be. It has to be noted in this respect that the physical features of the solution to be administered (such as the temperature, density and the like) have to be physiologically tolerable in order to reduce the risks associated with the selected route of administration.

It will be clear that the present method should be carried out within the frame of time in which the hyperpolarised acetic acid remains significantly polarised, shortly after being subjected to the chemical conversion of the solid acetic anhydride mixture thereof. This means that the sample, either human or non-human animal body, should be available close to the area in which the polarisation takes place.

Due to the high polarisation level achievable, the present process and the method of the present invention may find clinical application in a variety of imaging investigations such as, but not limited to, the vascular/angiographic imaging, interventional applications, perfusion mapping or metabolic/molecular imaging

In particular, in another aspect, the present invention relates to a method for operating an MRI system comprising the steps of:

a) submitting a subject, which has been positioned in said MRI system and treated with a hyperpolarised active acetic acid obtained according to the above process, to a radiation frequency selected to excite nuclear spin transitions in a non-zero nuclear spin nuclei of said active substrate; and

b) recording a MR signal from said excited nuclei.

In a further aspect, the present invention relates to a method for operating a MRI system comprising the steps of:

a) submitting a subject pre-treated with a hyperpolarised active acetic acid obtained according to the above process, which has been positioned in said MRI system, to a radiation frequency selected to excite nuclear spin transitions in a non-zero nuclear spin nuclei of said active substrate; and

b) recording a MR signal from said excited nuclei.

According to a further aspect, the present invention relates to the use of a gadolinium complex of formula (A) as above defined, for the preparation of hyperpolarised acetic anhydride in dynamic nuclear polarisation (DNP) experiments. As clearly evident from the experimental part herein below, in fact, the use of a complex of formula (A), e.g. wherein R is a benzyl group, (i.e. the compound of formula (I)) results in an unexpected enhancement in the polarisation level when acetic anhydride is subjected to DNP experiment. Contrary to the similar butyric anhydride system, in fact, the gadolinium complex of formula (I) leads to an enhancement of about 2 times in the case of acetic anhydride vs an enhancement of about 1.5 in the case of butyric anhydride.

The following examples are intended to better define the invention, without posing any limitation thereof.

EXPERIMENTAL PART

Materials

The following materials are employed in the subsequent examples:

Finlandic acidTris(8-carboxy-2,2,6,6-tetramethylbenzo[1,2-d:4,5-d']-bis(1,3)dithiol-4-yl), carboxylic acid form, Figure 3

Gadolinium complex I, [[α1,α4,α7-tris[(phenylmethoxy)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetato(4-)] gadolinate(1-)]hydrogen, Figure 4

Gadolinium complex II [10-[2-(dioctadecylamino)-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)] gadolinium, Figure 4

Gadolinium complex III, [10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)]gadolinium, Figure 4

Example 1 DNP preparation of acetic anhydride in the presence of a trityl radical as DNP agent

Finlandic acid (1.90 mg, 1.83 µmol) was dissolved in unlabeled acetic anhydride (110 µl, 119.7 mg). Several rounds of sonication and whirling were performed to fully dissolve the radical. The concentration of the radical in this solution was 16.7 mM. A fraction of this solution (30 µl, 32.3 mg) was mixed with 1,1'-13C2 acetic anhydride (10 µl, 11.0 mg, 0.10 µmol) yielding a solution with a radical concentration of 12.5 mM . A sample of this solution (43 mg containing 0.2 mmol 13C) was hyperpolarised with an exponential time constant of 670 s and an end polarisation value of 5 %, Figure 1.

Example 2 DNP preparation of acetic anhydride in the presence of Gd-chelate I as paramagnetic metal ion a trityl radical as DNP agent

Finlandic acid (6.80 mg, 6.56 µmol) was dissolved in unlabeled acetic anhydride (393 µl, 427 mg). Several rounds of sonication and whirling were performed to fully dissolve the radical. The concentration of the radical in this solution was 16.7 mM. Mortared gadolinium complex II (0.84 mg, 0.79 µmol) was added to the solution. The mixture was ultra-sonicated for 1 minute and then whirl-mixed. This sonication / whirl-mixing procedure was repeated two times after which the solution was centrifuged at 10000 RPM for 3 minutes. A fraction of this solution (30 µl, 32.5 mg) was mixed with 1,1'-13C2 acetic anhydride (10 µl, 11.0 mg, 0.10 µmol) yielding a solution with a radical concentration of 12.5 mM . A sample of this solution (43.3 mg containing 0.2 mmol 13C) was hyperpolarised with an exponential time constant of 690 s and an end polarisation value of 6 %, Figure 1. In comparison to ex. 1 the addition of gadolinium complex II does not change the polarisation properties of the sample significantly.

Example 3 DNP preparation of acetic anhydride in the presence of complex (I) as paramagnetic metal ion a trityl radical as DNP agent

Finlandic acid (8.94 mg, 8.62 µmol) was dissolved in unlabeled acetic anhydride (516 µl, 566 mg). Several rounds of sonication and whirling were performed to fully dissolve the radical. The concentration of the radical in this solution was 16.7 mM. Mortared gadolinium complex I (0.76 mg, 0.82 µmol) was added to 413 µl of the solution. The mixture was ultra-sonicated for 1 minute and then whirl-mixed. This sonication / whirl-mixing procedure was repeated two times after which the solution was centrifuged at 10000 RPM for 3 minutes. A fraction of this solution (30 µl, 33 mg) was mixed with 1,1'-13C2 acetic anhydride (10 µl, 11.0 mg, 0.10 µmol) yielding a solution with a radical concentration of 12.5 mM . A sample of this solution (43.7 mg containing 0.2 mmol 13C) was hyperpolarised with an exponential time constant of 690 s and an end polarisation value of 15 %, Figure 1. In comparison to ex. 1 the addition of gadolinium complex I increase the end polarisation of the sample by a factor of 2 without increasing the polarisation build-up time.

Example 4 DNP preparation of acetic anhydride in the presence of complex III as paramagnetic metal ion a trityl radical as DNP agent

Finlandic acid (9.62 mg, 9.28 µmol) was dissolved in unlabeled acetic anhydride (556 µl, 608 mg). Several rounds of sonication and whirling were

performed to fully dissolve the radical. The concentration of the radical in this solution was 16.7 mM. Mortared gadolinium complex III (0.35 mg, 0.62 µmol) was added to 313 µl of the solution. The mixture was ultra-sonicated for 1 minute and then whirl-mixed. This sonication / whirl-mixing procedure was repeated two times after which the solution was centrifuged at 10000 RPM for 3 minutes. A fraction of this solution (30 µl, 33 mg) was mixed with 1,1'-13C2 acetic anhydride (10 µl, 11.0 mg, 0.10 µmol) yielding a solution with a radical concentration of 12.5 mM. A sample of this solution (43.5 mg containing 0.2 mmol 13C) was hyperpolarised with an exponential time constant of 750 s and an end polarisation value of 7.5 %, Figure 1. In comparison to ex. 1 the addition of gadolinium complex III results in a small increase of the end polarisation of the sample.

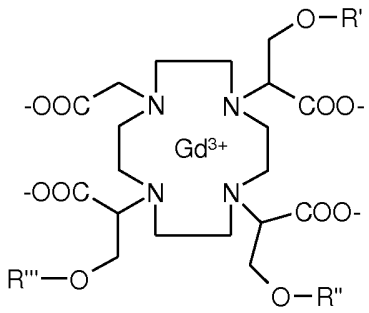
Example 5 DNP preparation, dissolution and hydrolysis of acetic anhydride in the presence of a trityl radical as DNP agent

A stock solution of radical was prepared by dissolving Finlandic acid (2.7 mg, 2.6 µmol) in unlabeled acetic anhydride (22.2 mg) yielding a solution with a concentration of 105 µmol / g . A part (2.1 mg) of this stock solution was mixed with 1,1'-$^{13}C_2$ acetic anhydride (15 µl, 17.2 mg, 0.165 mmol) to make a 12.8 mM solution. From this solution a sample (17.1 mg, 0.15 mmol) was hyperpolarised with an exponential time constant of 525 s and an end polarisation value of 5 %. The sample was dissolved in 5 ml water with added NaOH (12 M, 39 µl) and allowed to hydrolyze for 15 s and then neutralized by mixing with phosphate buffer (300 µl, 1M, pH 7.3) with addition of HCl (14 µl, 12M). The sample was transferred to an NMR spectrometer and the acquisition was started 30 s after the dissolution recording a time series of 1D spectra to follow signal decay. From the NMR spectra it was clear that the hydrolysis was complete at the start of the acquisition. The pH was 6.8 after neutralization and the recorded T_1 of the hydrolysis product was 45 s.

CLAIMS

1. A composition comprising:

- acetic anhydride,
- a DNP agent, and
- a gadolinium complex of formula (A):

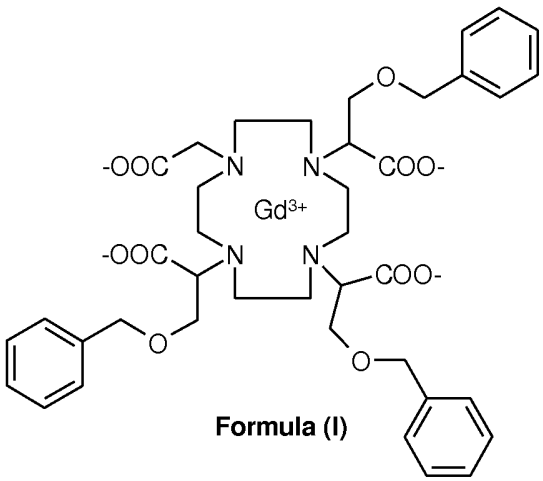


(A)

wherein:

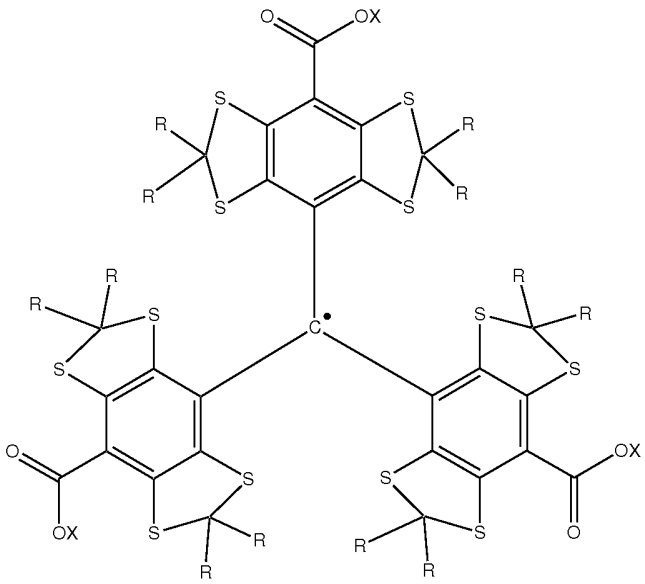
R', R'' and R''' are independently selected from: optionally substituted phenyl, (C_1-C_6)alkylene-phenyl and C_6-C_{10} heterocyclic ring.

2. A composition according to claim 1 wherein the gadolinium complex is [[α1,α4,α7-tris[(phenylmethoxy)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetato(4-)] gadolinate(1-)]hydrogen, of formula (I):



Formula (I)

3. A composition according to claims 1-2 wherein the DNP agent is a compound of formula (B):



Formula (B)

wherein:

R the same or different, represents a straight chain or branched C1-C6-alkyl group optionally deuterated and substituted by one or more hydroxyl group, methoxy group, or a group of formula $-(CH_2)_n-O-R2$, wherein n is 1, 2 or 3;

R2 is a straight or branched C1-C6-alkyl group, optionally substituted by one or more hydroxyl groups or methoxy groups; and

X is independently selected from: H, an alkaline metal, an optionally substituted straight or branched C1-C6 alkyl group, optionally interrupted by Sulphur or Oxygen atoms, and an optionally substituted aliphatic or aromatic C3-C8 cyclic group or hetero group.

4. A composition according to claim 3 wherein in formula (B) R is CH3 or CD3.

5. A composition according to claim 1 wherein the DNP agent is a trityl radical as defined in claim 4 and the gadolinium complex is as defined in claim 2.

6. The use of the composition of claims 1-5 for the preparation of hyperpolarised acetic anhydride by DNP experiments.

7. A process for the preparation of liquid hyperpolarised acetic acid, said process comprising the steps of:

a) subjecting the composition as defined in claims 1-5 to dynamic nuclear polarisation (DNP) to obtain hyperpolarised acetic anhydride; and

b) contacting the hyperpolarised acetic anhydride of step a) with an aqueous carrier to transform said hyperpolarised acetic anhydride in hyperpolarised acetic acid; and optionally

c) removing the gadolinium complex of formula (A) and the DNP agent.

8. A method for operating a MRI system comprising the steps of:

a) submitting a subject, which has been positioned in said MRI system and treated with hyperpolarised acetic acid obtained according to the process of claim 7 or 8, to a radiation frequency selected to excite nuclear spin transitions in a non-zero nuclear spin nuclei of said active substrate; and

b) recording a MR signal from said excited nuclei.

9. Use of a gadolinium complex of formula (A) as defined in claim 1 or 2, for the preparation of hyperpolarised acetic anhydride by DNP experiments.

DRAWINGS

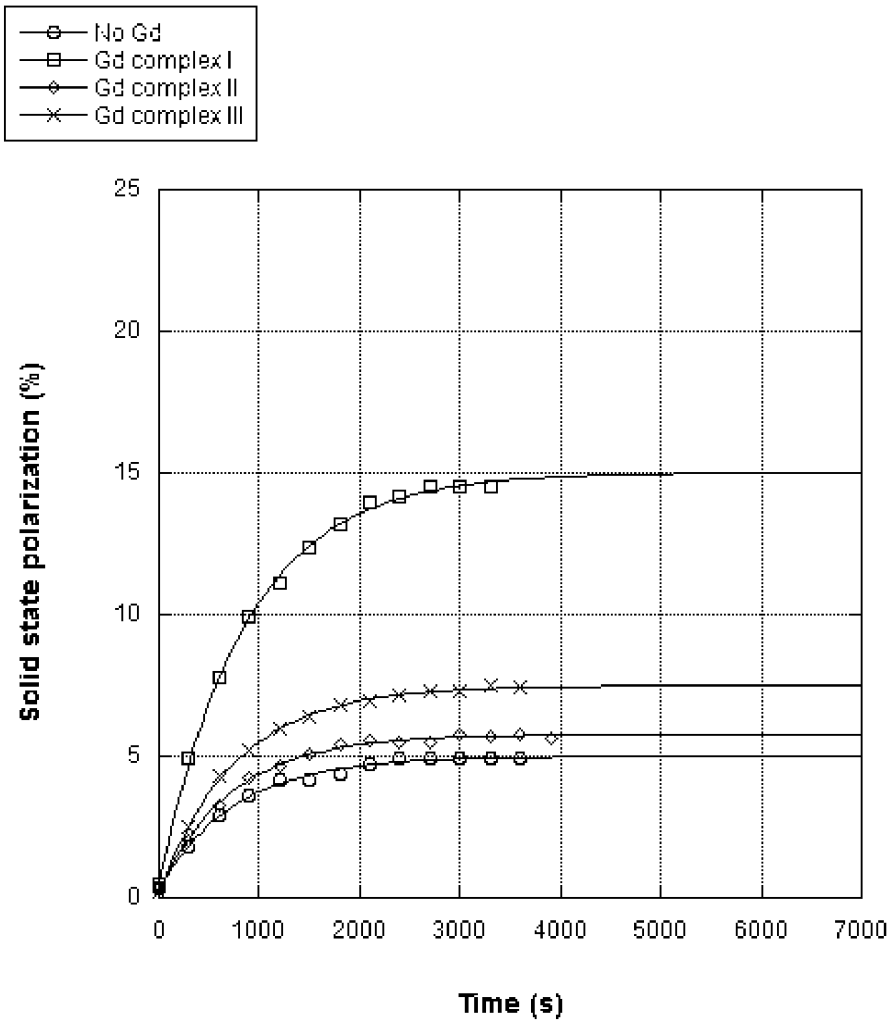


Figure 1.

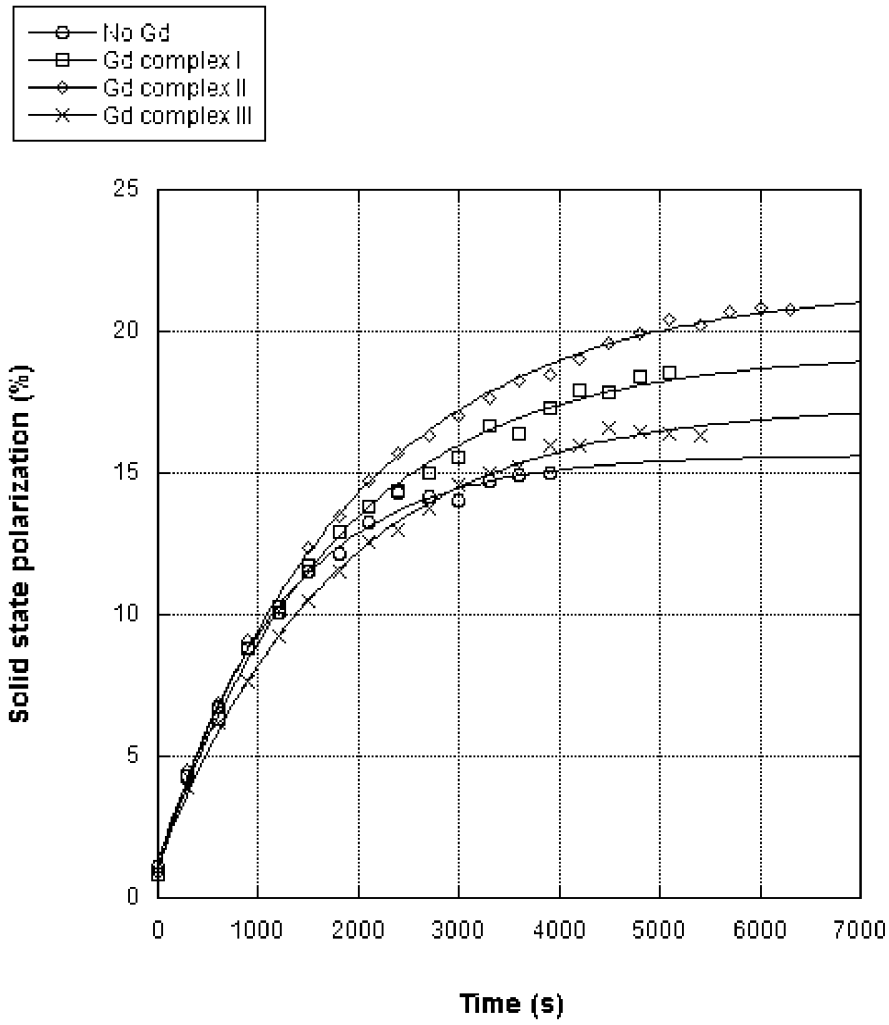

No Gd
Gd complex I
Gd complex II
Gd complex III
Solid state polarization (%)
0
5
10
15
20
25
0
1000
2000
3000
4000
5000
6000
7000
Time (s)

Figure 2.

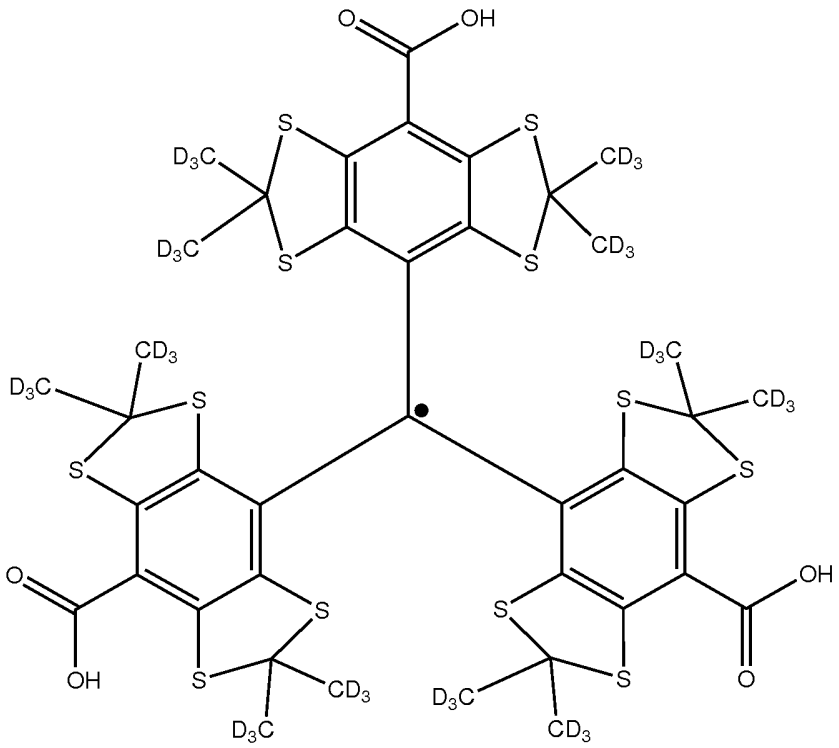
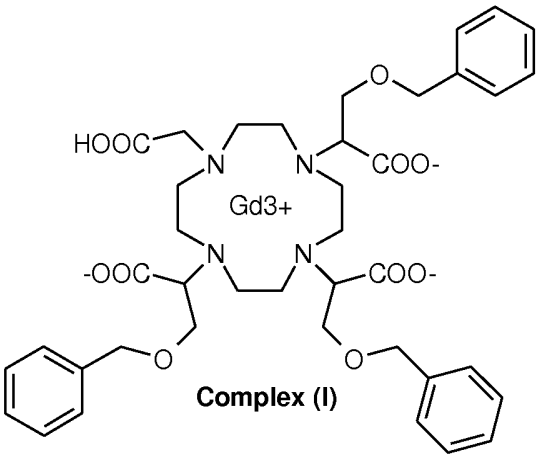
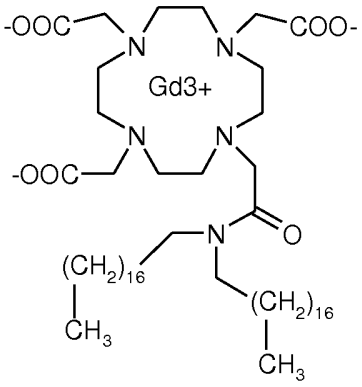

O
OH
S
S
D3C
CD3
D3C
CD3
S
S
CD3
D3C
D3C
S
S
CD3
S
S
O
OH
S
S
CD3
D3C
S
S
D3C
CD3
O
OH

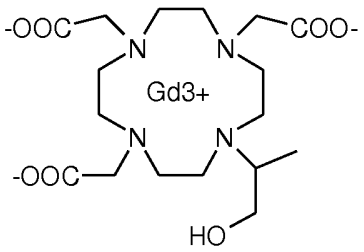
Figure 3.



Complex (I)



Complex (II)



Complex (III)

Figure 4.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/074292

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K49/10
ADD.

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)
A61K

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)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVID M WILSON ET AL: "Generation of hyperpolarized substrates by secondary labeling with [1,1-C-13] acetic anhydride", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC; US, vol. 106, no. 14, 7 April 2009 (2009-04-07), pages 5503-5507, XP002641409, ISSN: 0027-8424, DOI: 10.1073/PNAS.0810190106 [retrieved on 2009-03-10] abstract ----- -/--	1-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2013	30/01/2013

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/074292

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SILVIO AIME ET AL: "Synthesis and NMRD Studies of Gd3+ Complexes of Macrocyclic Polyamino Polycarboxylic Ligands Bearing beta-Benzyloxy-alpha-propionic Residues", INORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY, EASTON, US, vol. 31, no. 6, 18 March 1992 (1992-03-18) , pages 1100-1103, XP002120082, ISSN: 0020-1669, DOI: 10.1021/IC00032A035 pages 1100-1101 -----	1-9
A	HOVLAND R. ET AL.: "PREPARATION AND IN VITRO EVALUATION OF GDDOTA-(BOM)4; A NOVEL ANGIOGRAPHIC MRI CONTRAST AGENT", ORG. BIOMOL CHEM., vol. 1, 10 April 2003 (2003-04-10), pages 1707-1710, XP002672385, pages 1707-1708 -----	1-9

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