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INT CL **A61K, C07B, C07C, C07F, G01R**
Other: **EPODOC, WPI, TXTE, INSEPC, XPESP**

(54) Abstract Title: **Hyperpolarizing nuclei**

(57) The present invention provides a process for producing a compound comprising a hyperpolarized nucleus. Parahydrogen or a hyperpolarized derivative thereof and a compound comprising a hyperpolarizable nucleus is arranged an ordered environment, in particular as ligands of a metal complex. The hyperpolarization is directly transferable from the parahydrogen or derivative to the hyperpolarizable nucleus. The ligand containing the newly hyperpolarized nucleus is then separated from the metal complex.

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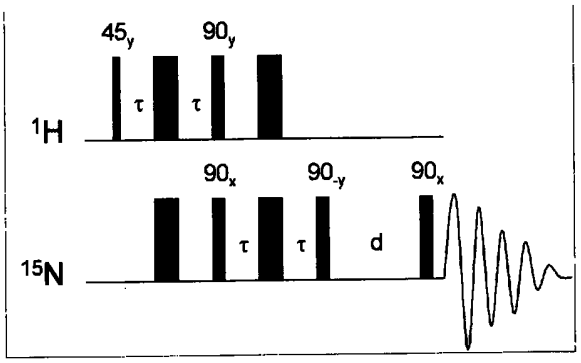
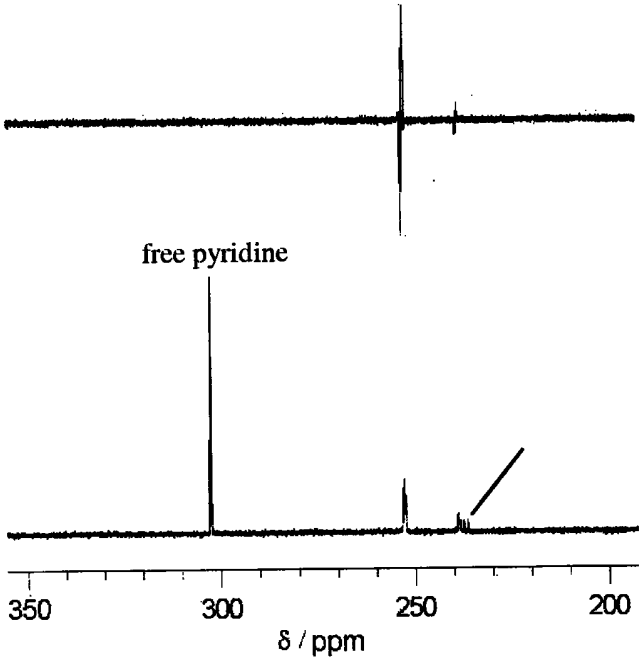

45y
90y
τ
τ
1H
90x
90-y
90x
τ
τ
d
15N

Fig. 1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350
300
250
200
δ / ppm

Fig. 2

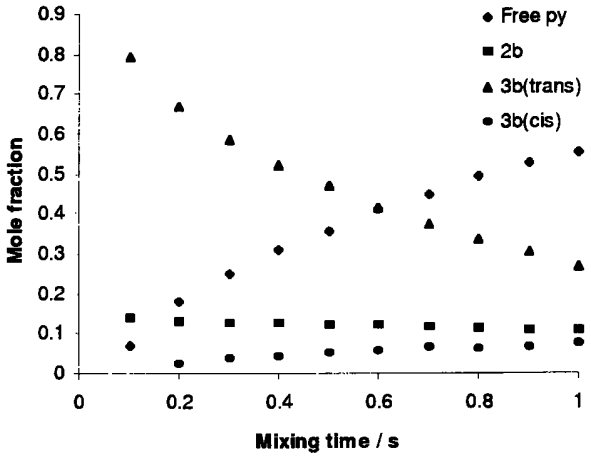
Free py
2b
3b(trans)
3b(cis)
Mole fraction
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
Mixing time / s

Fig. 3

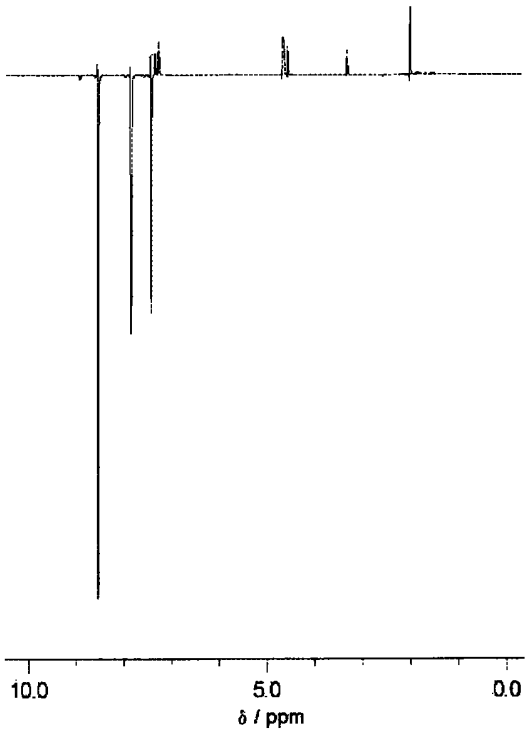
10.0
5.0
0.0
δ / ppm

Fig. 4

PROCESS

Field of the Invention

This invention relates to nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) In particular, the invention relates to processes, devices and compounds for hyperpolarizing nuclei.

Background to the Invention

NMR and MRI involve the detection of the transition of nuclear spins between an excited state and a ground state in an applied magnetic field. Because the energy difference between these states is often small, the usual Boltzmann distribution of chemically identical nuclei is such that at room temperature the populations of nuclear spin states which are in dynamic equilibrium are almost identical Since the strength of the detected signal in magnetic resonance experiments is proportional to the population difference, NMR signals are typically weak

The strength of detectable NMR signals can however be enhanced by hyperpolarizing the magnetic nuclei. Hyperpolarization (also known as pre-polarization) in this context refers to a process in which a significant excess of magnetic nuclei are induced into the same spin state This results in a large increase in available signal due to the much larger inequality of populations across the energy levels. In order for a hyperpolarized state to be useful, it is important that the spin state is sufficiently long lived to provide useful information, i.e. that the relaxation time of the spin state is 'long'. The rules governing the relaxation rates of nuclear spins are complex but known. It suffices to say that certain nuclei and spins systems have relaxation times which may extend to hours, days, months or even years.

There are a number of ways to induce certain nuclei into a hyperpolarized state. The simplest way is to cool the material to very low temperatures in the presence of a magnetic field, which will favour population of the lower energy state in which the spins of the nuclei are aligned with the applied magnetic field. This method is suitable for the production of hyperpolarized monatomic gases such as xenon or helium-3 The polarization levels of these nuclei have also been increased via the use of laser-based technologies

One molecule that can be readily polarized is dihydrogen Dihydrogen exists in various spin states, in which the spins of the individual nuclei are either aligned (ortho, the higher energy state), or opposed (para, the lower energy spin state) Para-hydrogen (p-H_2) is a nuclear spin isomer of dihydrogen with the spin configuration $\alpha\beta$-$\beta\alpha$. Para-hydrogen has no net magnetic moment and is therefore unobservable in this form by magnetic resonance methods The ortho forms however retain magnetic resonance activity. The binuclear spin system of dihydrogen can be hyperpolarized simply by cooling to low temperature in the presence of a suitable catalyst which promotes conversion to the lower energy para-hydrogen state In this process, the role of the catalyst is to perturb the dihydrogen molecule and thereby reduce its symmetry; otherwise a quantum mechanical selection rule prevents interconversion between the two spin states Once separated from the catalyst and returned to room temperature, the para-hydrogen spin state may last for over a year in the absence of external effects.

Nuclei can be hyperpolarized by a process known as para-hydrogen induced polarization (PHIP). PHIP has proved to be highly efficient and has currently achieved greater enhancement of heteronuclei NMR signals than other methods known in the art. PHIP is generally the result of a chemical reaction in which the para-hydrogen nuclei are transferred into another molecule having certain symmetry properties Under the right circumstances, the spin state of the para-hydrogen molecule is preserved in the spins of the two hydrogen atoms which become part of the new molecule. If other NMR-active nuclei are within coupling distance of the hydrogen nuclei, spin polarization of those nuclei can be transferred spontaneously. In this way, the signals of heteronuclei such as ^{13}C, ^{15}N and ^{31}P can be enhanced By way of example, WO 99/24080 describes a PHIP process in which para-hydrogen is added across a symmetrical carbon-carbon double bond containing a ^{13}C centre In one example of such a process, Wilkinson's catalyst is first reduced by addition of para-hydrogen, followed by addition of an ethylene ligand The resulting hydride ligands then undergo a migratory insertion reaction with the ethylene ligand, which subsequently dissociates from the complex to form uncoordinated hyperpolarized ethane. An overview of PHIP is given in Blazina et al, Dalton Trans., 2004, 2601-2609.

Conventional PHIP processes involve the chemical addition of para-hydrogen to hydrogenatable substrates, usually organic substrates containing double and triple bonds. This processes are therefore limited to substrates capable of undergoing

dihydrogenation. Furthermore, hydrogen equivalence is not preserved at all stages, which leads to some loss of hyperpolarization through relaxation.

Summary of the Invention

The present invention is based at least in part on a discovery that by suitably arranging parahydrogen or a derivative thereof and a hyperpolarizable nucleus in an ordered environment, hyperpolarization can be transferred directly from the parahydrogen nuclei to the hyperpolarizable nucleus, i.e without the need to chemically incorporate the parahydrogen into a compound comprising the hyperpolarizable nucleus. It has also been discovered that the hyperpolarized state of the nucleus is substantially retained when the compound is removed from the ordered environment

Accordingly, the present invention provides a process for producing a compound comprising hyperpolarized nucleus, which comprises·

(a) arranging parahydrogen or a hyperpolarized derivative thereof and a compound comprising a hyperpolarizable nucleus in an ordered environment such that hyperpolarization is directly transferable from the parahydrogen or derivative to the hyperpolarizable nucleus;

(b) directly transferring hyperpolarization from the parahydrogen or derivative to the hyperpolarizable nucleus, and

(c) separating the compound comprising the hyperpolarized nucleus from the ordered environment

By way of illustration, a process of the invention may involve the use of a metal complex comprising a pair of hydride ligands whose nuclei are hyperpolarized and a ligand comprising a hyperpolarizable nucleus. When the ligands are suitably arranged about the metal centre, hyperpolarization can be transferred directly from the hydride ligands to the hyperpolarizable nucleus.

Thus, the invention includes, but is not limited to, a process for producing a compound comprising a hyperpolarized nucleus from a hydrogenated metal complex, wherein the complex comprises a pair of hydride ligands whose nuclei are hyperpolarized and a ligand comprising a hyperpolarizable nucleus, and wherein the hydride ligands are arranged such that hyperpolarization is directly transferable from the hydride ligands to the hyperpolarizable nucleus, the process comprising directly transferring

hyperpolarization from the hydride ligands to the hyperpolarizable nucleus and separating the ligand comprising the hyperpolarized nucleus from the complex.

The invention also provides a device for producing a compound comprising a hyperpolarized nucleus, which comprises a reaction chamber comprising:

a) an inlet for a fluid enriched with para-hydrogen; and

b) a metal complex attached to a support, wherein the complex is hydrogenatable or hydrogenated with parahydrogen

The metal complex of said device may comprise a ligand which is a compound comprising a hyperpolarizable nucleus. The device may further comprise an inlet for a solution comprising said ligand in unbound form

Also provided is a hydrogenated metal complex comprising a pair of hydride ligands whose nuclei are hyperpolarized and a ligand comprising a hyperpolarizable nucleus, wherein the hydride ligands are arranged such hyperpolarization is directly transferable to the hyperpolarizable nucleus

Compounds obtained by a process of the invention may be useful as magnetic resonance (MR) imaging agents Accordingly, the use of compounds polarized in this way in diagnosis or therapy also forms part of the invention. In one aspect, the invention provides a composition comprising a compound comprising a nucleus which has been hyperpolarized by a process of the invention and a physiologically acceptable carrier or excipient

Processes of the present invention are advantageous in several respects over conventional hyperpolarization processes. A process of the invention may be used to hyperpolarize nuclei in a wide variety of compounds, without requiring that the compounds be capable of undergoing hydrogenation. For example, many metabolites comprise heteroatoms such as nitrogen, phosphorus or carbon, all of which are capable of coordinating to transition metal centres *via* suitable interactions Thus, the invention is particularly suited for forming metabolic magnetic resonance (MR) contrast agents The ligand can become substantially polarized by cycling of this process. Furthermore, addition of dihydrogen to transition metal complexes is a reversible process or can be promoted by the addition of a suitable sacrificial hydrogen acceptor or light source. Thus, with the right transition metal system, a continuous supply of parahydrogen can be

provided that is in rapid equilibrium with the dihydride metal complex, thereby promoting the generation of a hyperpolarized metal complex.

Brief Description of the Drawings

Fig 1 shows a PH-INEPT+EXSY pulse sequence. Pulses shown are $180_x°$ unless otherwise stated.

Fig 2 shows (top) a PH-INEPT spectrum of a sample using $[Ir(COD)(P\{p\text{-tolyl}\}_3)_2]BF_4$ ad ^{15}N-pyridine showing signals for $[Ir(P\{p\text{-tolyl}\}_3)(H)_2(py)_3]^+$ (major) $[Ir(P\{p\text{-tolyl}\}_3)_2(H)_2(py)_2]^+$ (minor), (bottom) PH-INEPT+EXSY spectrum of the same sample with 500 ms reaction delay

Fig. 3 is a plot of the fraction of overall signal integration for pyridine ligands of $[Ir(P\{p\text{-tolyl}\}_3)(H)_2(py)_3]^+$, $[Ir(P\{p\text{-tolyl}\}_3)_2(H)_2(py)_2]^+$ and polarised free pyridine

Fig 4 is an ^{1}H NMR spectrum of a sample using the PCy_3 supported iridium complex showing emission signals for spontaneously polarised pyridine.

Description of Various Embodiments

According to the present invention, parahydrogen or a hyperpolarized derivative thereof and a compound comprising a hyperpolarizable nucleus are arranged in an ordered environment such that hyperpolarization is directly transferable from the parahydrogen or derivative to the hyperpolarizable nucleus. The hyperpolarizable nucleus is then hyperpolarized by directly transferring hyperpolarization from the parahydrogen or derivative to the hyperpolarizable nucleus. The compound comprising the hyperpolarized nucleus can then be removed from the ordered environment and used as required, e.g as a contrast agent.

A process of the invention utilises parahydrogen or a hyperpolarized derivative thereof. The hyperpolarized derivative may comprise a pair of hydride ligands whose nuclei are hyperpolarized Hyperpolarization is transferred directly from the parahydrogen or derivative to a compound comprising one or more hyperpolarizable nuclei. By way of example, the or each hyperpolarizable nucleus may be selected from ^{1}H, ^{13}C, ^{15}N, ^{29}Si and ^{31}P nuclei An ordered environment is utilised to ensure that the parahydrogen or

derivative and the hyperpolarizable nucleus are suitably arranged so as to facilitate direct transfer of hyperpolarization. Suitable ordered environments will be apparent to those skilled in the art and include complexes, in particular metal complexes, and the like

The compound comprising the hyperpolarizable nucleus may be organic or inorganic in nature Typically, the compound will comprise one or more atoms selected from hydrogen, carbon, nitrogen, oxygen, silicon, sulphur and phosphorus. Where the compound is a ligand, it may be a mono-, bi- or multi-dentate ligand. Included are compounds, especially ligands, comprising one or more heterocyclic groups, in particular one or more heterocyclic groups comprising ^{15}N For example, the compound may comprise one or more groups selected from pyridine and derivatives thereof (e g 3- or 4-methylpyridine), nicotinamide, nicotine, pyridazine, purine, quinoline, quinazoline, quinoxaline and quinine. In one embodiment, the compound does not comprise an aliphatic unsaturated carbon-carbon bond. Of mention are compounds which are metabolites. Thus, the compound comprising the hyperpolarizable nucleus may be selected from amino acids, proteins, carbohydrates, nucleotides, drugs, prodrugs, coenzymes, cofactors and other materials that contain hyperpolarizable nuclei. Where the compound is a ligand, it is preferably labile, such that it can readily dissociate from the metal complex, e g when in equilibrium with unbound ligand in solution

In a preferred embodiment, the present invention involves the use of a metal complex which has been hydrogenated with para-hydrogen and which comprises a ligand which is a compound comprising a hyperpolarizable nucleus The ligand may comprise one or more hyperpolarizable nuclei By way of example, the or each hyperpolarizable nucleus may be selected from ^{1}H, ^{13}C, ^{15}N, ^{29}Si and ^{31}P nuclei. Of mention are ligands comprising one or more ^{13}C or ^{15}N nuclei, in particular one or more ^{15}N nuclei In one embodiment, the ligand is attached directly to the metal *via* an atom comprising the said hyperpolarizable nucleus.

The metal complex will usually be a transition metal complex, for example comprising a metal atom selected from Ru, Rh, Ir, W, Pd and Pt. The complex will usually comprise one or more ligands in addition to the ligand comprising the hyperpolarizable nucleus. These one or more other ligands may comprise organic or inorganic ligands and may be mono-, bi- or multidentate in nature. These one or more remaining ligands may play a role in controlling the activity and stability of the metal centre In one embodiment, the

metal complex comprises one or more phosphine ligands in addition to the ligand to be hyperpolarized The metal complex may be attached to a solid support, for example a polymer support Attachment will usually be made through a ligand which links the metal centre to the support Suitable linkers are known in the art For example, the linker may comprise one or more in-chain atoms selected from C, O, N, S, P and Si The linker comprises a siloxane moiety for attachment to the support and/or a phosphine moiety for attachment to the metal of the complex. In embodiments, the linker is a group of the following formula -O-Si$(OMe)_2$-$(CH_2)_n$-P$(Cy)_2$-, wherein n is 0 upwards (e g 0, 1, 2, 3, 4, 5 or 6) and Cy is cyclohexyl.

In one embodiment, the hydrogenated metal complex is an octahedral complex. In this case, the complex may comprise hydride ligands arranged relatively *cis* and one or more ligands comprising a hyperpolarizable nucleus arranged *trans* thereto One of the remaining ligands may, for example, act as a linker which tethers the complex to a support

The hydrogenated metal complex may be obtained by reacting parahydrogen with a hydrogenatable metal complex comprising the ligand comprising the hyperpolarizable nucleus. Alternatively, the hydrogenated metal complex may be obtained by reacting a ligand comprising the hyperpolarizable nucleus with a metal complex hydrogenated with parahydrogen

Hydrogenation of the complex may be achieved by contacting the complex with a fluid, typically a solution, containing dissolved para-hydrogen, preferably such that the resulting hydride ligands are in equilibrium with the para-hydrogen in solution Fluids enriched with para-hydrogen are particularly suitable in this regard. The term "enriched hydrogen" as used herein includes reference to hydrogen in which there is a higher than equilibrium proportion of para-hydrogen, for example where the proportion of para-hydrogen is more than 25%, e.g. more than 30%, e.g. 45% or more, e g 60% or more, e g. 90% or more, in particular 99% or more. Enriched hydrogen may be obtained catalytically at low temperatures e.g. at 160 K or less, preferably at 80 K or less or more preferably at about 20 K The parahydrogen thus formed may be stored for long periods, preferably at low temperature, e g 18 to 20 K Alternatively the parahydrogen may be stored in pressurized gas form in containers with non-magnetic and non-paramagnetic inner surfaces, e g a gold or deuterated polymer coated container Parahydrogen may also be obtained by electrolysis. The hydrogenation step may be

performed in the liquid or gaseous phase, and preferably in the absence of materials which would promote relaxation.

The ligands are arranged such that the hyperpolarization is directly transferable from the hydride ligands to hyperpolarizable nucleus, i e hyperpolarization is transferable without first chemically incorporating the hydride ligands into the compound comprising the hyperpolarizable nucleus When a metal complex is hydrogenated with para-hydrogen, the resulting hydride ligands are normally formed in a *cis* arrangement In this arrangement, transfer of hyperpolarization from the hydride ligands to the hyperpolarizable nucleus will normally be possible, especially when the ligand comprising the hyperpolarizable nucleus is located *trans* to the hydride ligands.

Hyperpolarization of the hyperpolarizable nucleus by the parahydrogen or derivative ligands may occur spontaneously. In general, spontaneous polarization will occur when the transitions associated with the NMR signals are close in energy and therefore mix. This situation can be readily achieved in a low field, but may also be achieved by the application of a suitable train of radio frequencies.

If spontaneous transfer is not possible, pulse sequences of electromagnetic radiation can be applied to the system which will result in polarization transfer. Examples of suitable sequences can be found in the Figures herein and in Blazina et al, Dalton Trans., 2004, 2601-2609

After hyperpolarization has been transferred, the compound comprising the hyperpolarized nucleus can then be separated from the ordered environment and the parahydrogen or derivative thereof. Separation may be achieved using physical and or chemical means. Where a hydrogenated metal complex forms the ordered environment, the ligand comprising the hyperpolarized nucleus is separated from the complex. In this regard, the ligand is preferably chemically or physically labile Where the ligand is labile, dissociation of the ligand from the complex may be achieved by contacting the complex with a solution comprising the ligand in unbound form Equilibrium may be established between the bound and unbound ligand, facilitating dissociation of the hyperpolarized ligand from the nucleus.

Hyperpolarized compounds of the invention may be suitable for use in high resolution NMR experiments. In this case, the compound should preferably be strongly polarizable

(for example, to a level of greater than 5%, preferably greater than 10%, more preferably greater than 25%) Collection of ^{1}H, ^{13}C, ^{15}N, ^{31}P or ^{29}Si signals should be facilitated.

The invention is particularly suited to the production of MR imaging agents. In this case, the compound should preferably be strongly polarizable (for example, to a level of greater than 5%, preferably greater than 10%, more preferably greater than 25%) and have a non-hydrogen MR imaging nucleus with a long T_1 relaxation time under physiological conditions, e g ^{13}C, ^{15}N or ^{29}Si By a long T_1 relaxation time is meant that T_1 is such that once polarised, the MR imaging agent will remain so for a period sufficiently long to allow the imaging procedure to be carried out in a comfortable time span Significant polarization should therefore be retained for at least 1 s, preferably for at least 60 s, more preferably for at least 100 s and especially for 1000 s or longer. Furthermore, the chemical shift, or even better the coupling constant of the signal from the imaging nucleus should preferably be influenced by physiological parameters (e.g morphology, pH, metabolism, temperature, oxygen tension or calcium concentration). For example, influence by pH can be used as a general disease marker, whilst influence by metabolism may be a cancer marker Alternatively, the MR imaging agent may conveniently be a material which is transformed (e.g. at a rate such that its half-life is no more than 10 xT_1 of the reporter nucleus, preferably no more than 1 xT_1) in the subject under study to a material in which the reporter nucleus has a different coupling constant or chemical shift

The MR imaging agents may be administered to a sample and the sample subsequently exposed to radiation of a frequency selected to excite nuclear spin transitions of one or more hyperpolarized nuclei present in the imaging agent. The magnetic resonance signals of the nuclei can then be detected The detected signals can then be used to generate an image, biological functional data or dynamic flow data.

MR imaging agents may be used to image a subject, for example, selected from a human or animal, a cell culture, a membrane-free culture or a chemical reaction medium Thus, it may be preferable for the MR imaging agents to have negligible toxicity Such agents have both *in vitro* and *in vivo* usage.

The MR imaging agent may be administered parenterally, e.g. by bolus injection, by intravenous or intra-arterial injection or, where the lungs are to be imaged, in spray form, e.g. by aerosol spray. Oral and rectal administration may also be used

MR imaging agents may be conveniently formulated with conventional pharmaceutical or veterinary carriers or excipients. Formulations of the invention may thus comprise one or more components selected from stabilizers, antioxidants, osmolality adjusting agents, solubilizing agents, emulsifiers, viscosity enhancers and buffers. Preferably, these components are not paramagnetic, superparamagnetic, ferromagnetic or ferrimagnetic. The formulation may be in forms suitable for parenteral (e.g. intravenous or intraarterial) or enteral (e g oral or rectal) application, for example for application directly into body cavities having external voidance ducts (such as the lungs, the gastrointestinal tract, the bladder and the uterus), or for injection or infusion into the cardiovascular system. However, solutions, suspensions and dispersions in physiological tolerable carriers (e.g. water) will generally be preferred.

Where the MR imaging agent is to be injected, it may be convenient to inject simultaneously at a series of administration sites such that a greater proportion of the vascular tree may be visualized before the polarisation is lost through relaxation. Intra-arterial injection is useful for preparing angiograms and intravenous injection for imaging larger arteries and the vascular tree.

Parenterally administrable forms should of course be sterile and free from physiologically unacceptable agents and from paramagnetic, superparamagnetic, ferromagnetic or ferrimagnetic contaminants, and should have low osmolality to minimize irritation or other adverse effects upon administration and thus the formulation should preferably be isotonic or slightly hypertonic Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as sodium chloride solution, Ringer's solution, dextrose solution, dextrose and sodium Chloride solution, lactated Ringer's solution and other solutions such as are described in Remington's Pharmaceutical Sciences, 15th ed , Easton: Mack Publishing Co , pp. 1405-1412 and 1461-1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The compositions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the MR imaging agents and which will not interfere with the manufacture, storage or use of the products

For use in *in vivo* imaging, the formulation, which normally will be substantially isotonic, may conveniently be administered at a concentration sufficient to yield a 1 μM to 1M

concentration of the MR imaging agent in the imaging zone. However, the precise concentration and dosage will of course depend upon a range of factors such as toxicity, the organ targeting ability of the MR imaging agent, and the administration route. The optimum concentration for the MR imaging agent represents a balance between various factors. In general, optimum concentrations will typically range from about 0.1 mM to about 10 M, especially from about 0.2 mM to about 1 M, more especially from about 0.5 mM to about 500 mM. Formulations for intravenous or intraarterial administration may, for example, contain the MR imaging agent in concentrations of from about 10 mM to about 10 M, especially from about 50 mM to about 500 mM For bolus injection the concentration may conveniently range from about 0.1 mM to about 10M, especially from about 0.2 mM to about 10 M, in particular from about 0 5 mM to 1 M, more particularly from about 10 mM to about 500 mM, yet still more particularly from about 10 mM to about 300 mM

The dosages of the MR imaging agent used according to the process of the present invention will vary according to the precise nature of the MR imaging agents used, of the tissue or organ of interest and of the measuring apparatus. Typically, the dosage should be kept as low as possible whilst still achieving a detectable contrast effect. By way of example, the dosage may range from 1 to 1000 mg/kg, e.g from 2 to 500 mg/kg, especially from 3 to 300 mg/kg.

Once the MR imaging agent has been administered to the subject, the MR signals may be detected using procedures known in the art. For example, it may be advantageous to use fast single shot imaging sequences e g. EPI, RARE or FSE. MR signals may be conveniently converted into two- or three-dimensional image data or into functional, flow or perfusion data by conventional manipulations. By imaging, it will be appreciated that not just production of two- or three-dimensional morphological images is covered The images produced may be representations of the value or temporal change in value of a physiological parameter such as temperature, pH, oxygen tension and the like. Morphological images however will generally be produced For *in vivo* imaging, the MR imaging agent should of course be physiologically tolerable or be capable of being presented in a physiologically tolerable form.

The following Examples illustrate the invention

Example 1: Preparation of hydride complexes

$Ir(H)_2(PPh_3)_2(pyridine)_2^+$ was generated by bubbling H_2 through a solution of $[Ir(COD)(PPh_3)_2]^+$ (COD = cycloocta-1,5-diene) in dichloromethane in the presence of excess pyridine and isolated in the solid state as has been previously described by Rosales *et al* (*Dalton Trans.*, 2004, 2952.) When the same reaction was carried out using ^{15}N-labelled pyridine under *p*-H_2, the hydride resonances of both $Ir(H)_2(PPh_3)_2(pyridine)_2^+$ and $Ir(H)_2(PPh_3)(pyridine)_3^+$ were substantially enhanced This is attributable to the second order spin system that is generated when the hydride ligands become magnetically inequivalent. The size of the enhanced hydride resonance of $Ir(H)_2(PPh_3)(pyridine)_3^+$ was much larger than that of $Ir(H)_2(PPh_3)_2(pyridine)_2^+$ in contrast to their relative intensities when using ^{14}N-pyridine This suggests that the latter complex exchanges *p*-H_2 at a greater rate.

Analogues of these species were generated for $[Ir(COD)((P\{p\text{-tolyl}\}_3)_2]^+$ and $[Ir(COD)((P\{C_6H_4\text{-}p\text{-}OMe\}_3)_2]^+$. When the hydrogenation was carried out in the presence of ^{15}N-pyridine using equimolar solutions of these complexes, similar hydride signals were observed to those generated using the PPh_3 system When the signal to noise ratio of the hydride ligands of the product dihydride complexes was monitored, it was observed that the signals reduce in intensity the trend PPh_3 > $P\{p\text{-tolyl}\}_3$ > $P\{C_6H_4\text{-}p\text{-}OMe\}$ which suggests that the increased basicity of the phosphine leads to a reduction in the catalytic rate and hence reduction in the recycling of para-hydrogen nuclei into these hydride positions.

Example 2: Polarization transfer to ^{15}N

A series of heteronuclear polarization experiments were utilised in order to transfer the enhanced polarization from hydride resonances to the ^{15}N of pyridine using PHIP adapted INEPT and INADAQUATE experiments (Haake *et al*, *J. Am. Chem. Soc.*, 1996, 118, 8688) These experiments included PH-INEPT, refocused PH-INEPT+, INEPT(+π/4) and PH-INADAQUATE experiments. When recorded under identical conditions and with the signal to noise of the experiments normalised with respect to the relative signal strength of hydride resonances in ^1H spectra recorded immediately before each experiment, it was shown that the most efficient polarization transfer was achieved using the PH-INEPT experiment. The refocused version of this experiment only afforded about a third of the ^{15}N signal intensity, most likely due to transverse relaxation of the ^{15}N

signal during the refocusing echo The INEPT(+π/4) and PH-INADAQUATE experiments were found to be the least effective

p-H_2 derived polarization was transferred from hydride ligands to the ^{15}N of pyridine in the complex $Ir(H)_2$(pyridine)$(PPh_3)_2Cl$. The experiment described above was used as a preparative sequence in an EXSY type experiment. Here, a refocused sequence was necessary to generate observable in-phase ^{15}N signals and hence the PH-INEPT+ sequence was used as a preparative block for a new PH-INEPT+EXSY experiment This preparative sequence was followed by a $90°_{-y}$ pulse to store the ^{15}N polarization as +*z*-magnetisation, a delay for ligand dissociation and then a further 90° read pulse. The signal for free ^{15}N-pyridine was found to be enhanced by such an approach

When tricyclohexylphosphine (PCy_3) ligands were used in place of PPh_3 ligands, the exchange rate of both H_2 and pyridine was substantially enhanced. Under these conditions, the ^1H NMR spectra of the associated samples using either labelled or non-labelled pyridine contained large emission signals in the aromatic region of the spectrum at 7.43, 7.84 and 8 54 ppm due to the free substrate. These signals were most readily observed immediately after shaking the NMR tube in low field and then placing it in the spectrometer This suggests that (i) under low field conditions there is spontaneous transfer of the PHIP effect to the protons of pyridine when the pyridine ligand is temporarily ligated to the iridium hydride complex; and (ii) exchange of ligated and free pyridine leads to the polarization of a portion of the free pyridine without having protons derived from *p*-H_2 chemically incorporated into the molecule. When 4-methylpyridine or purine was used in place of pyridine, related enhancements effects were also observed. Under these conditions, when ^{15}N labelled pyridine was employed, substantial enhancement of the free pyridine ^{15}N signal was also evident.

In a further experiment, a sample of $[Ir(COD)(PCy_3)(py)]BF_4$ (COD = cycloocta-1,5-diene, Cy = cyclohexyl, py = pyridine) was dissolved in methanol-d_4 at 300 K in an NMR tube. Excess pyridine (1-10 μL) was then added and the NMR tube pressurised with *para*-H_2. When the resultant reaction was monitored by ^1H NMR spectroscopy, the appearance of emission signals of substantial intensity in the organic region of the spectrum between δ8 and δ6 was observed. The strongest of these peaks was observed at δ7.84 and 8.54 along with an enhanced signal that is in absorption at δ7.43. These peaks are coincident with those of free pyridine. The peaks were only observable with these enhanced characteristics when the observation process was started immediately

after shaking the NMR tube outside the magnet and introducing it into the NMR spectrometer. Furthermore, the signals decayed over the course of a few successive scans when the magnetisation was read and partially destroyed using a hard +45 ° excitation pulse The resonances of free pyridine were therefore observed to have been hyperpolarized

In a similar manner, the following ligands were also hyperpolarized 4-methylpyridine, 3-methylpyridine, nicotinamide, nicotine, pyridazine, purine, quinoline, quinazoline, quinoxaline and quinine.

This Example demonstrates that the reversible binding of a substrate and *para*-hydrogen can be utilised to hyperpolarize a substrate without the need to chemically incorporate nuclei from parahydrogen with the molecule. Thus no overall chemical reaction is required in contrast with previous studies

Example 3

This Example illustrates further procedures for obtaining and polarising transition metal complexes

Radio frequency-assisted polarisation transfer

1 mg of hydrogenatable complex was dissolved in 500 μL of d_4-methanol in an NMR tube fitted with a Young's tap top Approximately 1 mg of the substrate to be polarised (and 1 to 10 μL of a sacrificial hydrogen acceptor such as 1-phenylprop-1-yne is added if necessary). The sample was then degassed and pressurised by 3 to 3.5 bar of p-H_2. The tube was then shaken to dissolve the gas and transferred into the NMR spectrometer NMR spectra were then recorded using the PH-INEPT+EXSY sequence over a range of reaction delay times that varied between 50 ms and >1s PH-INEPT and PH-INEPT+ spectra were also recorded to give control spectra correlating to reaction time t = 0s

Figs 1 to 3 are spectral traces which confirm that the ^{15}N signal of free pyridine and other suitable ligands were enhanced using this approach.

Spontaneous polarisation transfer

1 mg of a hydrogenatable complex such as $[Ir(COD)(PR_3)_2]BF_4$, $[Ir(COD)(PR_3)(py)]BF_4$, or $[Ir(COD)(py)_2]BF_4/PR_3$ where R is a suitable donor such as Cy, was dissolved in 500 µL of d_4-methanol in an NMR tube fitted with a Young's tap top To this sample was added approximately 1 mg of substrate to be polarised (and 1 to 10 µL of a suitable hydrogen acceptor such as 1-phenylprop-1-yne if necessary) The samples were then degassed and then pressurised to 3 to 3.5 bar with p-H_2 The samples were then shaken to dissolve the gas and immediately transferred into the spectrometer Recording of ^1H NMR (or a single can heteronuclear spectrum, e g ^{13}C, ^{15}N) spectra commenced as soon as the sample arrived in the probe head Under these conditions specific substrate resonances were polarised, as illustrated in Fig. 4.

Procedure for testing effectiveness of phosphine ligands

2 mg of $[Ir(COD)(py)_2]BF_4$ (COD = 1,5-cyclooctadiene, py = pyridine) was dissolved in 500 µL degassed d_4-methanol containing 1 µL of pyridine in an NMR tube fitted with a Young's tap top in a glove box To this sample was added 1 to 2 equivalents of the desired phosphine to generate $[Ir(COD)(PR_3)_x(py)_y]^+$ *in situ*. After locking and shimming the spectrometer on the sample, the sample was then pressurised to 3 to 3.5 bar with p-H_2, shaken to dissolve the gas and immediately transferred into the spectrometer. Recording of spectra commenced as soon as the sample arrived in the probe head. Effectiveness of the polarisation was estimated from comparative integration of polarised and solvent signals in the ^1H NMR spectrum

Based on this procedure, the phosphines PCy_3 and tris-(ortho-tolyl)-phosphine were found to provide particularly enhanced signals

Claims

1 A process for producing a compound comprising a hyperpolarized nucleus, which comprises.

(a) arranging parahydrogen or a hyperpolarized derivative thereof and a compound comprising a hyperpolarizable nucleus in an ordered environment such that hyperpolarization is directly transferable from the parahydrogen or derivative to the hyperpolarizable nucleus,

(b) directly transferring hyperpolarization from the parahydrogen or derivative to the hyperpolarizable nucleus; and

(c) separating the compound comprising the hyperpolarized nucleus from the ordered environment and the parahydrogen or derivative thereof.

2. A process according to claim 1, wherein the parahydrogen is present in parahydrogen-enriched hydrogen

3. A process according to claim 1, wherein the parahydrogen derivative comprises a pair of hydride ligands whose nuclei are hyperpolarized.

4. A process according to claim 3, wherein the ordered environment comprises a hydrogenated metal complex comprising a pair of hydride ligands whose nuclei are hyperpolarized and a ligand comprising a hyperpolarized nucleus, and wherein the process comprises directly transferring hyperpolarization from the hydride ligands to the hyperpolarizable nucleus and separating the ligand comprising the hyperpolarized nucleus from the complex

5 A process according to claim 4, wherein the hydrogenated metal complex is formed by reacting parahydrogen with a hydrogenatable metal complex comprising the ligand comprising the hyperpolarizable nucleus

6. A process according to claim 4, wherein the hydrogenated metal complex is formed by reacting a ligand comprising the hyperpolarizable nucleus with a metal complex hydrogenated with parahydrogen.

7 A process according to claim 5 or claim 6, wherein the reaction is conducted in a fluid comprising para-hydrogen

8 A process according to claim 7, wherein the fluid comprises parahydrogen-enriched hydrogen.

9. A process according to claim 7 or claim 8, wherein the hydride ligands are in equilibrium with the hydrogen in said solution

10 A process according to any of claims 3 to 9, wherein the hydride ligands are in a *cis* arrangement.

11 A process according to any of claims 4 to 10, wherein the ligand is attached to the metal *via* an atom containing said hyperpolarizable nucleus.

12 A process according to any of claims 4 to 11, wherein the metal complex is present in a solution comprising said ligand in unbound form.

13 A process according to claim 12, wherein the bound ligand is labile and in equilibrium with unbound ligand.

14. A process according to any of claims 4 to 13, wherein the complex is bound to a support.

15. A process according to any of claims 4 to 14, wherein the complex is a transition metal complex.

16. A process according to claim 15, wherein the complex comprises a transition metal selected from Ru, Rh, Ir, W, Pd and Pt.

17. A process according to any preceding claim, wherein the compound comprising the hyperpolarizable nucleus is a metabolite, a drug or a prodrug.

18. A process according to any preceding claim, wherein the hyperpolarizable nucleus is a ^{1}H, ^{29}Si, ^{13}C, ^{15}N or ^{31}P nucleus

19. A process according to any preceding claim, wherein hyperpolarization is transferred spontaneously.

20. A process according to any of claims 1 to 16, wherein hyperpolarization is transferred using a pulse sequence

21 A device for producing a compound comprising a hyperpolarized nucleus, which comprises a reaction chamber comprising.

a) an inlet for a fluid enriched with para-hydrogen, and

b) a metal complex attached to a support, wherein the complex is hydrogenatable or hydrogenated with parahydrogen.

22. A device according to claim 21, wherein the metal complex is hydrogenated with parahydrogen.

23. A device according to claim 21, wherein the metal complex is hydrogenatable with parahydrogen

24. A device according to any of claims 21 to 23, wherein the metal complex comprises a ligand which is a compound comprising a hyperpolarizable nucleus.

25. A device according to any of claims 21 to 24, further comprising an inlet for a solution comprising a ligand in unbound form, wherein the ligand is a compound comprising a hyperpolarizable nucleus.

26 A device according to any of claims 21 to 25, further comprising an outlet for one or more fluids.

27 A compound comprising at least one hyperpolarized nucleus, obtainable by a process of any of claims 1 to 20.

28 A compound according to claim 27, which is a metabolite

29. A compound according to claim 27 or claim 28, wherein the hyperpolarized nucleus is a heteroatomic nucleus.

30. A compound of any of claims 27 to 29, for use in diagnosis or therapy

31. Use of a compound of any of claims 27 to 29 as a magnetic resonance (MR) contrast agent.

32 A composition comprising a compound of any of claims 27 to 29 and a physiologically acceptable carrier or excipient.

33. A hydrogenated metal complex comprising a pair of hydride ligands whose nuclei are hyperpolarized and a ligand comprising a hyperpolarizable nucleus, wherein the hydride ligands are arranged such hyperpolarization can be directly transferred to the hyperpolarizable nucleus.

34. A complex according to claim 33, wherein the ligand is a labile ligand

35. A complex according to claim 33 or claim 34, wherein the ligand is a metabolite

36. A complex according to any of claims 33 to 35, wherein the hyperpolarized nucleus is a heteroatomic nucleus.

37. A complex according to any of claims 33 to 36, which is attached to a solid support.

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Application No: GB0711624.7 **Examiner:** Mr Peter Davies

Claims searched: 1 - 20, 27 - 32 **Date of search:** 26 November 2007

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 17, 18, 20, 27 - 32 at least	WO 2007/044867 A2 (HUNTINGTON MEDICAL) - especially pages 19 to 22
X	1, 17, 18, 20, 27 - 32 at least	WO 2004/019995 A2 (AMERSHAM) - pages 11 and 12 especially
X	1, 17 -19, 27 - 32 at least	US 2002/0137965 A1 (AXELSSON ET AL.) - see abstract
X	1, 17 - 19, 27 - 32 at least	US 6278893 B1 (NYCOMED) - columns 17 - 22, figure 3 especially
X	1, 17 - 19, 27 - 32 at least	WO 99/24080 A1 (NYCOMED)
X	1, 17 - 19, 27 - 32 at least	Magnetic Resonance Imaging, 2005, vol. 23, Goldman et al., "Hyperpolarzation of 13C through order transfer from parahydrogen: A new contrast agent for MRI", pp 153-157 ref: XP004843472 , INSPEC abstract 8608223
X	1 and 27 at least	Inorganica Chimica Acta, 2007, vol. 360, Ahlquist et al., "Rhodium(I) hydrogenation in water: Kinetic studies and the detection of an intermediate using 13C{1H} PHIP NMR spectroscopy", pp 1621 - 1627 ref: XP005918401, sections 2.5 and 3 in particular

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art
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Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[X] :

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Worldwide search of patent documents classified in the following areas of the IPC

A61K; C07B; C07C; C07F; G01R

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, TXTE, INSEPC, XPESP

International Classification:

Subclass	Subgroup	Valid From
G01R	0033/28	01/01/2006
A61K	0049/12	01/01/2006
C07F	0013/00	01/01/2006