Title: CHELATING AMPHIPHILES

Abstract: The present invention provides an amphiphile metal chelating agent useful for example as medical contrast agents and for nuclear medicine applications comprising a chelating headgroup that contains at least one donor atom, wherein the head group is capable of forming a complex with a metal ion, and R1 that includes one or more Y groups, where each Y is the same or different and is a hydrophobic saturated or unsaturated substituent, and R2 which is similar to R1 or is hydrogen, and wherein the agent is capable of self-assembling into a lyotropic or thermotropic phase selected from the group consisting of: lamellar, bicontinuous cubic, hexagonal, and sponge phases.
Chelating amphiphiles

Field of the invention

The present invention relates to amphiphilic metal chelating agents capable of self assembly. This invention also relates to the use of amphiphilic metal chelating agents as medical contrast agents and for nuclear medicine applications.

Background of the invention

Metal chelating agents are organic compounds capable of forming a complex with a metal ion. One particular application of metal chelating agents is their medical diagnostic applications as medical contrast agents for use in, for example, magnetic resonance imaging (MRI).

MRI is a technique that exploits the behaviour of protons in solvent molecules in a magnetic field to produce diagnostic images. Paramagnetic ions such as manganese and gadolinium commonly reduce both spin-lattice ($T_1$) and spin-spin relaxation time ($T_2$) of the solvent protons present in tissues to produce a higher contrast image. However, owing to their high toxicity, the "bare" or uncomplexed metals are often unsuitable for use as diagnostic agents in vivo. In these circumstances, it is necessary to sequester the metal by using a chelating agent. The metal-chelate complex is capable of being safely administered to a patient in need of such treatment to enhance diagnostic images without the toxicity effects of the uncomplexed metal.

Metal chelating agents used as MRI contrast agents are generally based upon aminopolyacetic acid compounds, such as diethylenetriaminepentaacetic acid (DTPA) and their cyclic analogues such as $^{1}$,y.$^i$O-tetraazacyclododecane-$^{1}$,y.$i$O-tetraacetic acid (DOTA). However, these contrast agents lack specificity in relation to their distribution into specific organs or tissues. Furthermore, their efficacy as $T_i$ agents, commonly evaluated by its proton relaxivity enhancement ($ri=1/T_i$) (also known as the paramagnetic spin-lattice relaxation rate) is not optimal.
Several approaches have been investigated to improve the properties of MRI contrast agents. One such approach is to covalently attach the chelating agent to high molecular weight molecules such as proteins, polymers or phospholipid type molecules. Another approach has been to non-covalently incorporate metal chelating agents into a supramolecular structure, such as an inert amphiphile self-assembled matrix. In this approach, the amphiphile self-assembled matrix is physiologically inert, acting essentially as a pharmaceutical carrier or excipient. The amphiphile components that comprise the self-assembled matrix cannot form a metal chelate, and accordingly have no capacity to act as diagnostic agents.

In spite of these developments, the contrast agents described above still suffer from drawbacks. For instance, owing to the need to either covalently attach the chelating agent to a large molecule or incorporate the agent into an inert amphiphile matrix, the number of the chelating sites is still relatively small in comparison to the mass of the administered agent. Accordingly, the number of metal ions capable of being incorporated into the diagnostic agent - the so-called 'payload' is undesirably low. Accordingly, there remains a need to develop new diagnostic agents with improved properties.

Further, by way of background, an amphiphile is a molecule that possesses a hydrophilic portion attached to a hydrophobic domain. The self-assembly behaviour of amphiphiles in solvent arises because of the preferential interaction between the solvent and either the hydrophilic or hydrophobic portion of the amphiphilic molecule. When an amphiphile is exposed to a polar solvent, the hydrophilic portion of the amphiphile tends to preferentially interact with the polar solvent, mostly water or a buffer system, resulting in the formation of hydrophilic domains ('solvent domain'). The hydrophobic portion of the amphiphile molecules tend to be excluded from this domain, resulting in the de facto formation of a hydrophobic domain ('amphiphile domain'). It is in this self-assembled form that amphiphiles are capable of acting as an inert carrier or matrix into which biologically active molecules may be incorporated.

Self-assembled structures may exhibit a variety of orientational orders. If long-range orientational order is observed within the self-assembled structure at equilibrium, the
self assembled structure is termed a 'mesophase', a 'lyotropic liquid crystalline phase', a "lyotropic phase" or, as used herein, simply a 'phase'.

There are two principal types of liquid crystalline phases: thermotropic liquid crystals and lyotropic liquid crystals. Thermotropic liquid crystals can be formed by heating a crystalline solid or by cooling an isotropic melt of an appropriate solute. Lyotropic liquid crystals may be formed by addition of a solvent to an appropriate solid or liquid amphiphile. The manipulation of parameters such as amphiphile concentration and chemical structure, solvent composition, temperature and pressure may result in the amphiphile-solvent mixture adopting lyotropic phases with distinctive characteristics.

Lyotropic phases may be classified in terms of the curvature of the interface between the hydrophilic and hydrophobic domains. The curvature between these hydrophilic and hydrophobic domains is dependent upon several factors, including the concentration and molecular structure of the amphiphile. When the interface displays net curvature towards the hydrophobic domain, the phase is termed 'normal'. When the interface displays net curvature towards the hydrophilic domain, the phase is termed 'inverse' or 'reverse'. If the net curvature of the system approaches zero, then the resulting phase may possess a lamellar-type structure that consists of planar amphiphile bilayers separated by solvent domains. Alternatively, the net curvature may approach zero if each point on the surface is as convex in one dimension as it is concave in another dimension; such phases are referred to as "bicontinuous cubic" phases. Examples of particular phases that can be formed by self-assembled structures include but are not limited to: micellar (normal and inverse), hexagonal (normal and inverse), lamellar, cubic (normal, inverse and bicontinuous), normal and inverse micellar cubic, ribbon, mesh, or noncubic 'sponge' bicontinuous phases and other intermediate phases such as rhombohedral, tetragonal and monoclinic phases.

Bulk phases that are thermodynamically stable in excess water may be dispersed to form particles (so-called "colloidosomes") that retain the internal structure of the non-dispersed bulk phases. Phases that are suitable for dispersion into colloidosomes include lamellar, inverse bicontinuous cubic, inverse hexagonal, inverse micellar cubic and L3 ('sponge'). When colloidosomes possess the internal structure of an inverse
bicontinuous cubic phase, the particles are colloquially referred to as cubosomes. Similarly, when the particles possess the internal structure of an inverse hexagonal phase, they are referred to as hexosomes. When the particles possess the internal structure of a lamellar phase, they are referred to as liposomes. Colloidal particles may also be formed from 'sponge' phases.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

**Summary of the invention**

The current invention relates to amphiphile metal chelating agents that are capable of self assembly. The agents, when complexed to an appropriate metal and dispersed into colloidal particles, may be used as medical contrast agents, particularly as MRi contrast agents.

In one aspect, the present invention provides an amphiphile metal chelating agent of the general formula (I):

$$ R_1^-A^-R_2 $$

in which:

- $A$ is a chelating headgroup that contains at least one donor atom, wherein the head group is capable of forming a complex with a metal ion, the metal ion selected from the group consisting of transition metal ions, lanthanide metal ions and alkaline rare earth metals.

- $R_i$ is selected from the group consisting of a substituent according to formula (a), a substituent according to formula (b), a substituent according to formula (c), and a substituent according to formula (d),
wherein

Xi is a difunctional spacer group linked to a hydrophobic group Y on one end and to the chelating head group on the other end,

X2 is a trifunctional spacer group linked to two hydrophobic groups Y on one end and the chelating head group on the other end,

X3 is a tetra functional spacer group linked to three hydrophobic groups Y on one end and the chelating head group on the other end,

wherein the functional groups are selected from carboxylic acids, alcohols, amines, thiols, halides, azides, isocyanates and isothiocyanates,

each Y is the same or different and is a hydrophobic saturated or unsaturated substituent with a linear chain length of between 10 and 30 carbon atoms selected from the group consisting of alkyl, alkenyl, alkynyl, branched alkyl, branched alkenyl and branched alkynyl, substituted alkyl, substituted alkenyl and substituted alkynyl,

R2 is selected from the group consisting of a substituent according to formula (a), a substituent according to formula (b), a substituent according to formula (c), a substituent according to formula (d) and hydrogen,

and wherein the compounds according to formula (I) are capable of self-assembling into a lyotropic or thermotropic phase selected from the group consisting of: lamellar, bicontinuous cubic, hexagonal, and sponge phases.
In one aspect, the compound self-assembles into one or more lyotropic or thermotropic phases under physiological conditions that are lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic.

Preferably, the headgroup forms a stable complex with the metal ion.

In one embodiment, each \( Y \) is identical.

Compounds according to formula (I) are neutral or possess a net negative charge under physiological conditions in one aspect of the invention. By physiological conditions is meant typical human body temperature and body fluid pH, for embodiments of the invention useful for diagnostic imaging compositions. Compounds according to formula (I) also may form either thermotropic liquid crystalline phases or lyotropic liquid crystalline phases. They may also form bulk crystalline phases.

In another aspect, the present invention provides a metal-complexed chelating amphiphile agent of the general formula (II):

\[
M^{n+} \quad R_1^-A-R_2^- \tag{II}
\]

wherein \( M \) is a metal ion;

\( n \) may be 1 to 7; and

\( R_1, A \) and \( R_2 \) are defined as above.

Preferably, \( n \) is 1, 2 or 3.

In another aspect, the present invention provides a metal-complexed amphiphile agent according to formula (Ha):

\[
M^{n+} \quad (/\text{V}-R_1^-)^p \tag{Ha}
\]
wherein $A'$ is a carboxyl group;

$p$ is 1, 2 or 3; and

$R_i, M$ and $n$ are defined as above.

In one aspect, the agent self-assembles into one or more lyotropic or thermotropic phases under physiological conditions that are lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic.

In preferred embodiments, $R_2$ is either hydrogen or formula (a).

Another aspect of this invention provides a self-assembled structure of chelating amphiphiles according to formula (I), (II) or (Ma).

Another aspect of this invention provides colloidal particles derived from the self-assembled structure of chelating amphiphiles according to formula (I), (II) or (Ma). The colloidal particles according to the current invention may be selected from the group consisting of colloidosomes and solid-lipid particles.

Preferably, $M$ is selected from the group consisting of alkaline rare earth metal ions, transition metal ions and lanthanide metal ions with paramagnetic, magnetic, fluorescence and/or radioisotope properties.

Without wishing to be bound by theory, it is believed that the colloidal particles according to the present invention represent desirable contrast agents, and in particular desirable MRI contrast agents. The metal complexed compounds may also find use in nuclear medicine applications or as fluorescence imaging agents. The inherent nature of the self-assembled structures of metal complexed compounds according to the present invention provides a high payload of the relevant metal ion within the self assembled structure. Furthermore the dispersion into colloidal particles may provide an enhanced local concentration of the relevant ion at the target site, providing higher contrast images and also delivering a higher payload of the desired radionuclide to the site of treatment for nuclear medicine applications.
The processes to generate these colloidal particles allows for the manufacture of self assembled particles of tuneable size. Tuneable size is believed to confer several advantages to the use of the self-assembled structures according to the present invention in vivo, such as, for example, passive targeting to particular organs or tissues.

The colloidosomes according to the present invention are particularly suited as MRI contrast agents, as the internal architecture of the self-assembled structure provides an environment for exchanging the bound solvent proton to a paramagnetic ions and the solvent proton in the channels of the mesoporous particles. The internal architecture is believed to significantly increase the water proton relaxation time and subsequently provide higher contrast images.

The self-assembled structures according to the current invention may comprise any one or more of a bulk lyotropic phase selected from the following group: lamellar, inverse bicontinuous cubic, inverse hexagonal, inverse micellar cubic and L₃ ('sponge') phases.

The bulk phase may also be a non-lyotropic crystalline phase. The colloidosomes derived from the bulk phases may be selected from the following group: liposomes, cubosomes, hexosomes and "sponge" particles. Preferably, the colloidosomes are liposomes, inverse hexosomes, inverse cubosomes and inverse micellar cubosomes.

The invention also provides a self-assembled structure of one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT and Gd-DTPA-BT in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase. Typically, only one of these compounds would be in the self-assembled structure. The self-assembled structure is sufficiently stable under physiological conditions for its intended use.

Similarly, the invention also provides a dispersion of submicron- or nano-particles of a self-assembled structure of one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT and Gd-DTPA-
BT, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

In another embodiment, the invention provides a compound selected from the group consisting of Gd-oleate and Gd-phytanate. The invention extends to a self-assembled structure of one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase, and also to a dispersion of submicron- and nano-particles of self-assembled structure of one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

As indicated above, a self-assembled structure according to the invention can optionally be incorporated within a non-chelating amphiphile such as myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide, wherein the structure forms one or more of lamellar, inverse hexagonal, inverse bicontinuous cubic and inverse micellar cubic phases. Also, the invention includes a composition including a dispersion of submicron- and nano-particles of a self-assembled structure of one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT, Gd-DTPA-BT and a non-chelating amphiphile such as myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes. In this way, it is possible, for example, to further enhance the relaxivity of the incorporated chelating amphiphile.

A self-assembled structure of one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate may also be incorporated with a non-chelating amphiphile such as myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide, wherein the structure forms one or more of lamellar, inverse hexagonal, inverse bicontinuous cubic and inverse micellar cubic phases. Similarly, a composition is provided including a dispersion of submicron- and nano-particles of a self-assembled structure of one or more compounds selected from the group consisting...
of Gd-oleate and Gd-phytanate, and a non-chelating amphiphile such as myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

In the above forms of the invention where a self-assembled structure is formed, the self-assembled structure is sufficiently stable under physiological conditions for its intended use.

In these embodiments, a mixture of components is contemplated to obtain optimal functionality of the invention. The invention doesn't, however, require a mixture and typically only one type of chelating amphiphile is used.

Another aspect of the current invention relates to a process for preparing colloidosomes according to the current invention, the process comprising the steps of:

forming a bulk lyotropic or thermotropic phase comprising compounds according to formula (I), (II) or (Ha); and

fragmenting said bulk lyotropic or thermotropic phase.

A preferred aspect of the current invention relates to a process for preparing colloidosomes according to the current invention, the process comprising the steps of:

addition of an aqueous solvent to a chelating amphiphile according to the present invention; and

dispersing the amphiphile-solvent mixture with an aqueous solution.

Another aspect of this invention relates to the use of the colloidal particles derived from the self-assembled structure of formula (II) or (Ma) according to the current invention as medical contrast agents.

The colloidal particles may be suitable for use as MRI contrast agents, CT contrast agents, SPECT contrast agents, PET contrast agents or fluorescence imaging agents.
Another aspect of this invention relates to the use of the colloidal particles derived from the self-assembled structure of formula (II) or (Ma) according to the current invention as nuclear medicine agents.

Another aspect of this invention relates to a diagnostic composition according to the present invention, comprising an effective amount of colloidal particles including compounds of formula (II) or (Ha) according to the current invention.

Another aspect of this invention relates to a composition suitable for use in fluorescence imaging, the composition comprising an effective amount of colloidal particles including compounds of formula (II) or (Ha) according to the current invention.

Another aspect of this invention relates to the diagnosis of a disease state by administration of an effective amount of a composition according to the current invention to a patient requiring diagnosis. A suitable composition is the colloidal particles derived from the self-assembled structure of formula (II) or (Ma). The invention also includes the use of colloidal particles derived from the self-assembled structure according to the current invention for the manufacture of a medicament for the diagnosis of a disease state. Further, the invention provides a compound of formula (II) or (Ha) for use in a diagnostic imaging composition.

**Brief description of the drawings**

Table 1: Size average and *in vitro* relaxivity values of various colloidal particles of EDTA and DTPA conjugates complexed with manganese and gadolinium.

Table 2: Size average and *in vitro* relaxivity values of various colloidal particles prepared from Gd-DTPA-MP incorporated into phytantriol at various molar ratios, as well as Gd-DTPA-MP incorporated into phytanylethanolamide at 5% molar ratio.

Table 3: Relaxivities of colloidal dispersions of Ln-oleates.

Table 4: Relaxivity of colloidal dispersions of Gd-oleate/myverol mixtures.
Table 5: Relaxivity of colloidal dispersions of Ln-phytanate (bracketed results are for Ln-phytanate in phytantriol).

Table 6: Relaxivity values of colloidal particles made from Gd-DTPA amphiphiles after addition of Zn ions (2 h post addition). The values in brackets show the results after 3 days equilibration.

Figure 1: Schematic picture of the different phases that can occur upon hydration of different amphiphiles. Abbreviations for different mesophases are micellar (Li); micellar cubic (h), normal hexagonal (Hi), bicontinuous cubic (V1), lamellar (L), inverse bicontinuous cubic (V2), inverse hexagonal (H2), inverse micellar cubic (I2), and inverse micellar (L2), where subscripts 1 and 2 refer to "normal" and "inverse" phases, respectively.

Figure 2: Water penetration behaviour into EDTA-BO observed under a cross polarised microscope by adding 100 mM sodium acetate solution at (a) 25°C; (b) 37°C; (c) 40°C; and (d) 45°C.

Figure 3: Water penetration behaviour into EDTA-BO observed under a cross polarised microscope by adding 2 M sodium acetate solution at (a) 25°C; (b) 37°C; (c) 60°C; and (d) back to room temperature.

Figure 4: Synchrotron SAXS/VAXS pattern of EDTA-BO in the presence of excess aqueous solution (a) 100 mM sodium acetate; (b) 200 mM sodium acetate; (c) 1 M sodium acetate; and (d) 2 M sodium acetate; conducted at different temperatures.

Figure 5: Water penetration behaviour into EDTA-MP observed under a cross polarised microscope by adding 100 mM sodium acetate solution at room temperature. An anisotropic phase typical of lamellar phase is formed at the boundary with water.

Figure 6: SAXS patterns obtained from hydrated EDTA-MP with excess amount of 70 wt% (a) water; (b) 100 mM Na-acetate; (c) 200 mM Na-acetate; and (d) comparison of diffraction patterns at hydration with water, 100 and 200 mM Na-acetate and at room temperature.
Figure 7: Water penetration behaviour into EDTA-BP observed under cross polarised microscope by adding sodium acetate solution at 100 mM concentration conducted at (a) room temperature; (b) 37°C; (c) 40°C; and (d) 50°C.

Figure 8: SAXS patterns at various temperatures obtained from hydrated EDTA-BP at excess amount of 70 wt% (a) water; (b) 100 mM Na-acetate; (c) 1 M Na-acetate; and (d) comparison between the diffraction patterns at different ionic state and at room temperature. Asterisks (*) show the diffraction peaks related to inverse micellar cubic phase and arrows (>) show those for the inverse hexagonal phase.

Figure 9: Water penetration behaviour into DTPA-BP observed under cross polarised microscope by adding sodium acetate solution at 100 mM concentration at (a) 21°C; (b) 25°C; (c) 37°C; and (d) 50°C.

Figure 10: Synchrotron SAXS patterns of DTPA-BP hydrated with excess amount of 70 wt% (a) 100 mM sodium acetate; (b) 200 mM sodium acetate; and (c) 500 mM sodium acetate conducted at various temperatures.

Figure 11: Synchrotron SAXS patterns of Gd-EDTA-MP suspensions at different ratios of Gd to EDTA-MP.

Figure 12: Synchrotron SAXS patterns of EDTA-BP at (a) different pH of the solution; (b) pH=5 and at different temperatures; and (c) different molar ratios of Mn to EDTA-BP. The non-complexed EDTA-BP sample displayed nanostructured hexagonal particles with a lattice parameter of 6.52±0.05 nm at room temperature and at pH=5.7 (black line). The lattice parameter decreased to 6.38±0.05 nm on addition of Mn at 0.1/1 (red line) and this change was accompanied by an additional broad peak at approximately 0.15 Å⁻¹. The lattice parameter gradually decreased by addition of further Mn ions to the dispersion and at the ratio of 0.6/1 the lattice parameter decreased to 5.82±0.05 nm. Simultaneously the intensity of the broad diffraction peak increased and shifted to slightly higher q values as well as the emergence of an extra peak at 0.30 Å⁻¹.

Figure 13: Synchrotron SAXS patterns of dispersed particles of (a) DTPA-BP at different temperatures; (b) Gd-DTPA-BP at a molar ratio of 1:2 at different temperatures.
Figure 14: Synchrotron SAXS patterns of dispersed nanoparticles of Gd-DTPA-MP/phytantriol (1% molar ratio). The diffraction peaks are in the ratio of $\sqrt{2} : \sqrt{4} : \sqrt{8}$, consistent with the inverse cubic $Im\overline{3}m$ phase. The lattice parameter at $25^\circ C$ was $10.65 \pm 0.05$ nm, and decreased to $10.16 \pm 0.05$ nm at $37^\circ C$.

Figure 15: Cryo TEM micrographs of (a) EDTA-MP; (b) Mn-EDTA-MP; and (c) Gd-EDTA-MP; scale bars are 200 nm.

Figure 16: Cryo TEM micrographs of (a) EDTA-BP; (b,c) Mn-EDTA-BP at a molar ratio of 0.6/1. scale bars are 100 nm.

Figure 17: Cryo TEM micrographs of EDTA-BO; scale bars are 200 nm.

Figure 18: Cryo TEM micrographs of DTPA-BP; scale bars are 200 nm.

Figure 19: Cryo TEM micrographs of (a) Gd-DTPA-MP/phytanylethanolamide (5% molar ratio); scale bar is 100 nm and (b) Gd-DTPA-MP/phytantriol (1% molar ratio); scale bar is 200 nm.

Figure 20: Fluorescent response of Eu-oleate versus concentration.

Figure 21: Plot of proton (a) longitudinal relaxivity $r_1$ and (b) transverse relaxivity $r_2$ at 20 MHz and room temperature with a MINISPEC from Bruker versus the concentration of gadolinium (III) phytanate.

Figure 22: MS of Gd-DTPA-MP (a) before addition of extra metal ions (b) exposed to zinc, (c) magnesium and (d) calcium. No transmetalation occurred and the extra peaks in the range of 900-1200 belong to adducts of Gd-DTPA-MP with zinc, magnesium and calcium.

Figure 23: $T_1$- weighted MR images; Magnevist at 2.2 mM (spot a); sheep blood only (spot b); Gd-EDTA-BO at 1.3 and 2.5 mM (spots c and d); Gd-EDTA-MP at 0.8 and 1.54 mM (spots e and f); Gd-EDTA-BP at 0.532 and 1.024 mM (spots g and h); water only (spot i) and Magnevist at 8.2 mM (spot j).
Figure 24: TVweighted MR images; Magnevist at 2.1 mM and 8.33 mM (spots a and e); Gd-DTPA-BP at 0.71 mM and 1.28 mM (spots b and c); water only (spot d); and blood only (spot f). The other spots are unrelated samples.

**Detailed description of the embodiments**

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

It will be noted that various terms employed in the specification, examples and claims have meanings that will be understood by one of ordinary skill in the art. However, for clarity of meaning intended in this document, certain terms are defined below.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

The term "stable complex" as used throughout the specification means a complex formed between a metal ion and a chelating agent that is thermodynamically and kinetically stable under physiological conditions. The thermodynamic stability refers to the complex stability rate in the presence of endogenous ions and biomolecules, whereas the kinetic stability is related to the metal complex decomposition and decomplexation half life under physiological conditions. It would be preferred that the kinetic stability of the complexes are higher, desirably orders of magnitude higher, than the complex half life in vivo so that the complex will remain intact before being excreted from the body.

The term "head group" as used throughout the specification means the polar portion of the structure of the amphiphile. It may encompass the substituent A, or both the substituent A and the metal ion M when the chelating metal agent according to the present invention forms a stable complex with a metal ion. Similarly, it may encompass substituent A', or both the substituent A' and the metal ion M.
The term "contrast agent" as used throughout the specification is understood to mean an agent that provides assistance in distinguishing between the appearance of two or more tissues during medical imaging analysis. Medical imaging analysis includes, but is not limited to techniques such as MRI, CT, PET and SPECT.

The term "self-assembled structure" as used throughout the specification is understood to mean an aggregate of amphiphiles that possess some degree of internal organisational order. The self-assembled structures may be formed by contacting the amphiphile with solvent. Self-assembled structures include a bulk crystalline phase, a bulk lyotropic phase, a bulk thermotropic phase, a colloidal particle that displays the same internal structure as a bulk lyotropic phase (a so-called "colloidosome"), or a solid lipid particle.

The term "bulk phase" as used throughout the specification is understood to mean a lyotropic or thermotropic phase. It also includes a bulk crystalline phase. Examples of bulk phases contemplated by the present invention include are not limited to phases that display the following morphologies: micellar (Li) micellar cubic (h); normal hexagonal (H₁); bicontinuous cubic (V₁); lamellar (Lₐ); inverse bicontinuous cubic (V₂); inverse hexagonal (H₂); inverse micellar cubic (I₂) and sponge (L₃) phases.

The term "colloidal particle" as used throughout the specification means a "colloidosome" or a solid lipid particle.

The term "colloidosome" as used throughout the specification means a colloidal particle that possesses the same internal nanostructure of a bulk lyotropic phase.

The term "solid lipid particle" as used throughout the specification means a colloidal particle of the amphiphile of the invention, where the colloidal particle comprises a core of the neat amphiphile and usually will be stabilised by a surface layer of surfactant. The neat amphiphile core may be in a crystalline, microcrystalline, liquid crystalline or a non-crystalline form. It will be understood that the term "particle" refers to particles that may be nanoparticles or microparticles based on their average size. Often such particles are referred to as "solid lipid nanoparticles" although they may in fact be in a size range of
microparticles. This form of self-assembled structure does not swell upon contact with excess solvent.

The term "hexagonal phase" as used throughout the specification is understood to mean an amphiphile phase consisting of long, rod-like micelles packed into a hexagonal array. A "normal hexagonal phase" is a hexagonal phase consisting of long, rod-like normal micelles, whilst an "inverse hexagonal phase" is a hexagonal phase consisting of long, rod-like inverse micelles. The normal hexagonal phase may be referred to as the "Hi phase" and the inverse hexagonal phase may be referred to as the "Hn phase". When a colloidosome possesses the internal structure of a bulk hexagonal phase the colloidosome may be referred to as a "hexosome".

The term "lamellar phase" as used throughout the specification is understood to mean a stacked bilayer arrangement, where the opposing monolayers of the hydrophilic portion of the amphiphile molecules may either be in contact by hydrogen bonding or by ionic bonding; or alternatively may be separated by a polar solvent in the case of a lyotropic lamellar phase. The hydrophobic portion of the amphiphile molecule of the back-to-back layers are in intimate contact to form a hydrophobic layer. The planar lamellar phase is referred to as the "U phase".

The term "cubic phase" as used throughout the specification is understood to refer to two main classes of phases: micellar cubic and bicontinuous cubic. "Micellar cubic phase" refers to a phase consisting of micelles arranged in a cubic array. A "normal micellar cubic phase" or "I" phase consists of normal micelles arranged in a cubic array, whilst an "inverse micellar cubic phase" or "In" phase consists of inverse micelles arranged in a cubic array.

"Bicontinuous cubic phase" refers to a family of closely related phases that consist of a single curved lipid bilayer that forms a complex network that separates the polar solvent space into two continuous, but non-intersecting volumes. Bicontinuous cubic phases possess long range order based upon a cubic unit cell. Bicontinuous cubic phases have zero mean curvature; that is, at all points on the surface of the amphiphile bilayer, the surface is as convex as it is concave. Bicontinuous cubic phases may be of the normal
("vι phase") or inverse ("vn phase") type. Several types of long range orientational orders have been observed for bicontinuous cubic phases; the orientational order in these phases correspond to space groups la3d, Pn3m, and Im3m. When a colloidosome possesses the internal structure of a bulk cubic phase the colloidosome may be referred to as a "cubosome".

The term "sponge phase" or "L3 phase" as used throughout the specification is understood to refer to a phase that resembles a bicontinuous cubic phase, in that it possesses an amphiphile bilayer that separates the polar solvent space into two unconnected volumes, but it does not possess long range order. Accordingly, these phases are analogous to a "melted cubic phase".

The term "diagnostic composition" as used throughout the specification is understood to mean a composition comprising a diagnostically effective amount of a chelating amphiphile according to the current invention and at least one pharmaceutically acceptable carrier, excipient, diluent, additive or vehicle selected based upon the intended form of administration, and consistent with conventional pharmaceutical practices.

The terms "biologically active agent", "diagnostically active agent" "active agent" and "active ingredient" as used throughout the specification are understood to mean any substance that is intended for the diagnosis of a state in a biological system.

The term "effective amount" when used in the context of a contrast agent throughout the specification is the amount of a contrast agent according to the present invention required to effect the desired contrast in an image. An effective amount of a contrast agent will vary according to factors such as the age, sex, and weight of a subject, and the ability of the substance to elicit contrast in the tissues or organs in the subject.

As used herein, "diagnostically effective amount" relates to the amount or dose of a compound according to the present invention, or composition thereof, that will lead to one or more desired effects. A diagnostically effective amount of a substance will vary
according to factors such as the disease state, age, sex, and weight of a subject, and the ability of the substance to elicit a desired response in the subject.

The abbreviations throughout this specification are as follows:


EDTA-MP = N-mono-(carboxy-3,7,1 1,15-tetramethylhexadecanyl)-ethylenediamine-N-N'-triacetic acid.

EDTA-BP = N,N'-b/s-(carboxy-3,7,1 1,15-tetramethylhexadecanyl)-ethylenediamine-N-N'-diacetic acid.

DTPA-MP = N-mono-(carboxy-3,7,1 1,15-tetramethylhexadecanyl)-diethylenetriamine-N-N',N''-tetraacetic acid.

DTPA-BP = N,N''-/)/s-(carboxy-3,7,1 1,15-tetramethylhexadecanyl)-diethylenetriamine-N-N',N''-triacetic acid.


DTPA- monotocopherol (DTPA-MT) = N-mono-(carboxy-2,5,7,8-Tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanyl) diethylenetriamine-N-N',N''-tetraacetic acid.

DTPA-Tris-trilaurate = N-mono-(2-amido-Tris(O,O',O"'-tri-dodecanoyl)methyl-1,3-propane) diethylenetriamine-N-N' \_N"-tetraacetic acid.

DTPA-b's(Tris-trilaurate) = N,N"-b/s-(2-amido-Tris(O,O' \_O"'-tri-dodecanoyl)methyl-1,3-propane) diethylenetriamine-N-N',N"-triacetic acid.

Accordingly, in one aspect, the present invention provides compounds according to formula (I), (II) and (Ha) that are capable of self-assembling into lyotropic phases.

Particular embodiments of A according to the present invention include ethylenediaminetetraacetic acid (EDTA), i^\_y.I-O-tetraazacyclododecane-I,4,T,1-O-tetraacetic acid (DOTA), diethylenetriaminopentaacetic acid (DTPA) and Tris(2-aminoethyl)hexaacetic acid (TTAHA).

In particular embodiments, Y has a linear chain length of 10 to 30 carbon atoms. Y is generally hydrophobic. In one embodiment, Y is alpha-tocopherol. In another embodiment, Y is an isoprenoid. In other embodiments, Y is an hydroxylated alkyl or hydroxylated alkenyl group. Preferred embodiments of Y are: alkyl, alkenyl, branched alkyl and alkenyl (isoprenoid), hydroxylated alkyl or hydroxylated alkenyl groups, of 10 to 30 carbon atoms. Preferably, the chain length is 10 to 24 carbon atoms, and more preferably 12 to 20 carbon atoms. Y in specific embodiments has 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms. Optionally, Y is an N-acyl chain of these.

In this specification including the claims, isoprenoid (sometimes referred to in the art as terpenoid) is used to refer to an isoprenoid in the traditional sense as well as in a sense specific to this specification. In the traditional sense, an isoprenoid is understood to be an organic compound consisting of repeating isoprene units, namely 2-methyl-1,3-butadiene, being a branched chain unsaturated hydrocarbon. Each isoprene unit, prior to addition to an isoprenoid chain, has the formula \text{CH}_2\text{C}(\text{CH}_3)\text{CHCH}_2. The resultant isoprenoid chain retains some carbon-carbon double bonds. In this specification, the term isoprenoid also includes a chain (which may be derived from an isoprene as just described) but absent all carbon-carbon double bonds (ie saturated) but otherwise retaining the structural pattern of an isoprenoid. Such a chain may, for instance, be
formed by the addition of partially hydrogenated isoprene units. In other words, as used throughout this specification the term isoprenoid may refer to fully saturated or partially saturated chains derived from isoprene units. Other functional groups may also be present on the isoprenoid chain. The skeleton of the isoprene units can be discerned in repeated occurrence in the isoprenoid. The skeleton of isoprenoids may differ from strict additivity of isoprene units by loss or shift of a fragment, commonly a methyl group.

In particular embodiments, one or more $Y$ groups is selected from the group consisting of phytanyl, oleyl, tocopheryl, lauryl, oleoyl, 3,7-dimethyl octanoyl, myristoyl, linoleoyl, 3,7,11-trimethyl-dodecyl, palmitoyl, 3,7,11,15-tetramethyl-hexadecyl, stearoyl, arachidonoyl, linolenoyl and octanoyl.

In some embodiments according to the current invention, $X_1$, $X_2$ and $X_3$ are linker groups. A "linker" refers to a group that acts as a spacer between the headgroup $A$ and the hydrophobic group(s) $Y$. Linkers are bifunctional in the case of $X_1$, are trifunctional in the case of $X_2$, and are tetrafunctional in the case of $X_3$. $X_1$, $X_2$ and $X_3$ each contain at least one functional group (an "attachment site") to anchor the group $Y$ at one site in the molecule, and another functional group at another attachment site to anchor the group $A$. "Functional" in this context means a group that has reacted to form a bond within the amphiphile. $X_1$, $X_2$ and $X_3$ may contain additional functional groups that are unreacted (such as $-\text{NH}_2$ or $-\text{COOH}$), but which could be activated by a separate reaction. Examples of functional groups are ethers, esters, amides, carbamates, imides, imines, carbonates, thioethers, thioesters, disulfides.

For instance, $X_1$, $X_2$, $X_3$ has at least one functional group attached to a moiety selected from the group consisting of heteroatoms, alkyl, alkenyl, alkyne, where these may be cyclic and/or include further heteroatoms and functional substituents (such as carbonyl, carboxylic, amide, hydroxyl, ether, amine), or a combination of any of these. Particular embodiments of $X_2$ include: diethanolamine, propane-1,2,3-tricarboxylic acid, cysteine, aspartic acid, asparagine, serine, tyrosine, arginine, histidine, threonine, lysine, glutamic acid and glutamine.
Particular embodiments of $X_3$ include: citric acid and tris(hydroxymethyl)aminomethane (Tris). The examples provided for $X_1$, $X_2$ and $X_3$ are not intended to be an exhaustive list and the current invention contemplates other embodiments of $X_1$, $X_2$ and $X_3$.

In another embodiment according to the current invention, $M$ is a metal ion that is selected from the group consisting of paramagnetic lanthanide and paramagnetic transition metal ions. In a preferred embodiment, $M$ is selected from the group consisting of Gd, Eu, Dy, Mn, or Fe. In another embodiment, $M$ is a radionuclide. In another preferred embodiment, $M$ is selected from the group consisting of $^{99}$Tc, $^{111}$In, $^{90}$Y, $^{68}$Ga, $^{67}$Ga, $^{66}$Ga, $^{61}$Cu, $^{68}$Cu, $^{166}$Ho, $^{153}$Sm or $^{212}$Bi. When $M$ is a radionuclide, the radionuclide preferably emits beta radiation, gamma radiation and/or positrons.

It will be understood by the skilled addressee that the value of $n$ is dependent upon the nature of the metal ion.

It will further be understood by the skilled addressee that the choice of $M$ is dependent upon the application of the metal chelating agent. The appropriate metal ion for the relevant application may be selected from any of those that are considered appropriate by the skilled addressee. By way of non-limiting example, if the chelating agent according to the current invention is used as an MRI contrast agent, suitable metal ions include paramagnetic metal ions, such as, for example, gadolinium, manganese and iron. When the chelating agents according to the present invention are used as MRI contrast agents, the skilled addressee will further recognise that the choice of $M$ will also depend upon whether the chelating agent is intended to be used as a $T_1$ or $T_2$ contrast agent. When the chelating agents according to the present invention are intended to be used as $T_1$ contrast agents, the preferred metal is gadolinium and manganese. When the chelating agents according to the present invention are intended to be used as $T_2$ contrast agents, the preferred metal is iron.

Again, by way of non-limiting example, when the metal chelating agent according to the current invention is used as a CT contrast agent, suitable metal ions include, for example, barium. When the chelating agent according to the current invention is used as a PET contrast agent, suitable metal ions include positron emitting radionuclide ions,
such as, for example $^{177}$Lu. When the chelating agent according to the current invention is used as a SPECT contrast agent, suitable metal ions include gamma radiation emitting radionuclide ions, such as, for example $^{111}$In, $^{99}$Tc and $^{67}$Ga. When the metal chelating agent according to the current invention is used in nuclear medicine applications, suitable metal ions include $^{99}$Tc. When the metal chelating agent according to the current invention is used in fluorescence applications, suitable metal ions include Eu.

It will also be understood by the skilled addressee that the selection of Ri and $R_2$ in relation to A for compounds according to formula (I), or alternatively the selection of Ri and $R_2$ in relation to both M and A for compounds according to formula (II) will dictate whether a chelating amphiphile will form a lyotropic phase according to the current invention. Similar considerations are present for compounds according to formula (Ma).

Formation of the desired phases of the current invention requires a balance between the specific hydrophilic and hydrophobic domains. In general, the interplay between the volume of the head group and hydrophobic tail (molecular geometry) of the amphiphile is very important in determining lyotropic phase behaviour. The relationship between the molecular geometry and the phase behaviour can be described by the critical packing parameter (CPP). CPP is defined as $CPP = \frac{v}{a_0 l_c}$, where $v$ is molecular volume, $a_0$ is the cross-sectional area of the surfactant head group, and $l_c$ corresponds to the hydrophobic tail length. Since the development of this formula, CPP has been used widely in speculating the mesophase behaviour based on the curvature of the molecule. For a molecule with a small head group and a bulky hydrophobic tail, the CPP is commonly greater than 1, thereby inducing a mean negative interfacial curvature and potentially form an inverse mesophase.

Substituents Ri and $R_2$ according to the current invention are selected based upon formation of a CPP greater than one when considered in context of the chelating head group A (or M and A) according to the current invention. Figure 1 illustrates this balance. The phases to the left of the lamellar phases have a critical packing density of less than 1 and often they happen at lower concentrations of the amphiphiles. The phases to the right of the lamellar phases have a CPP of more than 1 and usually occur
at higher concentration of the amphiphiles. The CPP is not constant for an amphiphile molecule and changes with external factors such as temperature, pressure, concentration of the amphiphile and pH, as well as some additional solvents and additives. However, this parameter can be used as a simple speculation of the phases that may occur upon hydration of the amphiphiles at room temperature or physiological temperature and at physiological pHs and pressure.

In order to have a lyotropic liquid crystalline phase, it is necessary that the hydrophobic chains remains fluid in the self-assembled state. Accordingly, unsaturated and branched chains are generally used, as these structural features prevent the hydrophobic chains from packing into a crystalline form. Hydrophilic headgroups that have a relatively small effective headgroup area impart a low net charge. As a result, the CPP is designed to be equal to 1 for lamellar phases and greater than one for inverse phases. Accordingly, in one aspect of the invention, Y is as described above and selected so that it has either a CPP equal to 1, or in a other embodiment greater than 1.

In addition to the phases shown in Figure 1, less common phases can also occur upon hydration of amphiphiles such as sponge phase (L3). This phase has a bicontinuous sponge-like structure with a lipid bilayer separating the polar solvent space into two unconnected sections similar to bicontinuous cubic phases. However, unlike bicontinuous cubic phases, sponge phases do not possess long range order and their internal structure can be envisioned as a melted cubic phase.

Particularly preferred embodiments of compounds according to the present invention include

EDTA-MO, formula (III);

EDTA-BO, formula (IV);

EDTA-MP; formula (V);

EDTA-BP, formula (VI);
DTPA-monophytanyl, formula (VII);

DTPA-bisphytanyl, formula (VIII);

DTPA-MO, formula (IX);

DTPA-BO, formula (X);

DTPA-monotocopherol, formula (XI);

DTPA-bistocopherol, formula (XII); and

metal complexed derivatives thereof.

Also, DTPA-Tris-trilaurate, formula (XIII) and DTPA-b/s(Tris-trilaurate), formula (XIV) and metal complexed derivatives thereof are additional embodiment.

These are represented as follows:
The synthesis of the compounds according to formula (III) to (XIV) of the current invention may be carried out according to methods known to those skilled in the art. Reaction conditions for the synthesis of compounds according to the current invention
would be readily determined by one of ordinary skill in the art and are also exemplified in the accompanying examples.

The synthesis of some of the preferred embodiments according to the present invention may be carried out according to Schemes 1 and 2:

$$R_1^-A-R_2 \quad \xrightarrow{M^{n+}} \quad R_1^-A^- - R_2 M^{n+}$$

Scheme 2

The starting materials and reagents used to synthesise the compounds according to the current invention are either available from commercial suppliers such as, for example, the Aldrich Chemical Company (Milwaukee, WI), Bachem (Torrance, CA), Sigma Chemical Company (St. Louis, MO), Lancaster Synthesis (Ward Hill, MA).

Another aspect according to the current invention provides self assembled structures of compounds according to formula (I), (II) or (Ma).

In one embodiment, the self-assembled structures of the current invention comprise at least one solvent system and at least one amphiphile domain, wherein the amphiphile domain comprises at least one of the compounds of according to the general formula O), (II) or (Ia).
The solvent system of the current invention comprises at least one polar solvent. Examples of suitable solvents include solvents conventionally used for amphiphile self-assembly, such as, for example, but not limited to, the following: water, glycerol, propylene glycol, propylene carbonate, glycofurol or mixtures thereof.

The solvent may also comprise other components, including e.g. salts, pH buffering agents, sugars such as glucose and sucrose. In addition to the amphiphilic chelating agents, the composition of the current invention may also comprise at least one other amphiphile that is capable of self-assembly behaviour. Amphiphiles capable of self-assembly behaviour are known to those skilled in the art and are described in various publications, such as, for example, Drummond and Fong (Drummond 1999) Laughlin (Laughlin 1996, 2000) the *Handbook of Lipid Research* (Small 1986). Examples of amphiphiles that are capable of self-assembly include, but are not limited to: surfactants, lipids, and block copolymers.

The self-assembled structures of the current invention may also comprise at least one other component intended to stabilise the self-assembled structure. Examples of stabilising reagents are triblock copolymers of PEG-PPO-PEG of different building blocks and more specifically poloxamer 407, as well as PEG lipid stabilising reagents such as polysorbate (for example, polysorbate 80).

In one embodiment, the self-assembled structure of the current invention comprises at least one bulk phase.

The bulk phase of the current invention comprises at least one phase selected from the following group: lamellar, normal hexagonal, normal micellar cubic, normal bicontinuous cubic, inverse bicontinuous cubic, L₃ ‘sponge’, and inverse hexagonal. Preferably, the bulk phase comprises at least one phase selected from the group consisting of inverse hexagonal, inverse bicontinuous cubic phase, inverse micellar cubic phase, L₃ ‘sponge’ phase and lamellar phases. Most preferably, the bulk phase comprises inverse hexagonal, inverse bicontinuous cubic and lamellar phases.
In a preferred embodiment, the bulk phases according to the current invention may be readily produced at a temperature range of about room temperature to about 50 °C and be stable within this temperature range for at least several months.

Preferred embodiments according to the current invention are bulk lyotropic inverse phases. The thermodynamic stability of the lyotropic phases according to the present invention means that the bulk phase maintains its primary higher ordered structure upon dilution in excess aqueous solvent, although the lattice parameter might be changed due to the swelling of the amphiphile in water. Most preferably, the lyotropic phase according to the current invention is an inverse bicontinuous cubic phase, an inverse micellar cubic phase, or an inverse hexagonal phase.

It will be recognised by one skilled in the art that the observed lyotropic phase is dependent upon temperature. The bulk phases according to the current invention are stable between room temperature and physiological temperature, are preferably stable at temperatures from about 35 °C to about 40 °C and are most preferably stable from about 35 °C to about 37 °C.

Processes for preparing bulk phases according to the current invention are known to those skilled in the art. In one embodiment, bulk phases according to the present invention may be prepared by addition of appropriate buffer to each amphiphile in the appropriate concentration. Examples of appropriate buffers include but are not limited to physiologically acceptable buffers, such as, saline, sodium acetate, sodium carbonate, glucose and sucrose buffers.

In another embodiment, the preferred inverse cubic phases according to the current invention are prepared by mechanically mixing molten lipid between room temperature and about 50 °C until an optically clear and visually homogenous sample are obtained.

Optionally, addition of a co-solvent such as, for example, ethanol in the range of 10 to about 20% by weight may assist the homogenisation process.
A further aspect of the invention relates to self assembled structures of the current invention that are dispersed into one or more colloidal particles that retain the internal structure of the bulk phase. Such particles are referred to as "colloidosomes".

In one embodiment, the colloidosomes according to the current invention are selected from the following group: liposomes, cubosomes, hexosomes and "sponge" type particles. In a preferred embodiment, the colloidosomes are selected from the following group: cubosomes and hexosomes; most preferably, the colloidal particles are cubosomes.

In a particularly preferred embodiment according to the current invention, the colloidosomes are derived from an inverse phase. The thermodynamic stability of the inverse lyotropic phases according to the present invention means that the bulk phases can progressively be diluted in excess aqueous solvent and dispersed into colloidosomes while maintaining the same liquid crystalline structures as that of bulk phases.

The colloidosomes according to the current invention may be prepared according to processes known to those skilled in the art. For example, colloidosomes may be prepared by hydration of a thin lipid film in water or saline solution. In addition, sugars such as glucose, dextrose might be added to the media. Inverse phase colloidosomes, such as inverse cubosomes and hexosomes, may be hydrated in water to form gel like bulk phases that can be consequently dispersed into particles by using shear forces such as sonication and high pressure homogenisation in the presence of stabilising agents.

It will be recognised by one of ordinary skill in the art that in order to prepare stable colloidosomes it is necessary to add a stabilisation agent or fragmentation agent. Suitable fragmentation agents are known to those skilled in the art and include, for example, poloxamer or polysorbate. Poloxamer is the most widely used stabilising agent for inverse phase colloidosomes and is a block copolymer of polyethylene glycol (PEG) and polypropylene oxide (PPO). In a preferred embodiment according to the current invention, the stabilising agent is a triblock copolymer of PEG-PPO-PEG of
different building blocks. In a particularly preferred embodiment according to the current invention, the stabilisation agent is poloxamer 407.

In one embodiment, the colloidosomes are prepared by dispersing a bulk lyotropic phase. The bulk lyotropic phases of the current invention may be dispersed by dropwise addition of an ethanolic solution of the bulk phases into water containing a stabilising reagent. Alternatively, the bulk lyotropic phase may be dispersed by adding water containing at least one stabilising reagent to the bulk phases. The size of these particles can be controlled by means of vortexing, sonication, filtration, extrusion and homogenisation, techniques well known to one skilled in the art.

In a preferred embodiment, colloidosome dispersions according to the current invention are prepared by dissolving an appropriate amount of the neat chelating amphiphile and a surfactant in a water miscible solvent. The water miscible solvent may be one or more solvents selected from the group consisting of ethanol, propanol, and butanol; is preferably a solvent selected from the group consisting of ethanol and propanol and is most preferably ethanol. The amphiphile-surfactant mixture is well mixed under vortex until the solvent-surfactant-amphiphile mixture is homogeneous. Optionally, the mixture may be heated to facilitate dissolution of the amphiphile and surfactant into the water miscible solvent. Typically, the mixture is heated to temperatures around physiological temperature (for example, temperatures less than about 40°C) to facilitate dissolution. The dissolved mixture is then added in a controlled manner to an aqueous solution. Preferably, the aqueous solution is sodium acetate buffer. Preferably, the amphiphile-surfactant mixture is added dropwise to the water or the aqueous buffer. Preferably, the water to which the mixture is being added is agitated; most preferably, the water is being agitated by means of a vortex.

The coarse colloidosomes prepared according to this embodiment may optionally be subject to one or more additional processing steps. Such processing methods are known to those skilled in the art and include, for example, sonication, probe sonication, high pressure homogenisation, and stepwise extrusion through membranes. The membranes employed for stepwise extrusion may possess pore sizes including, for
example, 0.8, 0.4, 0.2, 0.1 and 0.05 µm. In one embodiment, the processing step is a size selection process.

In a preferred embodiment, the coarse colloidosome preparation is further processed by means of passing through a series of polycarbonate (PC) membranes. The size range of the membranes will be selected by a person skilled in the art according to the desired particle size of the final product. The equipment which may be used for this processing step is known to those skilled in the art, but may include, for example, a mini-extruder.

According to another aspect of the invention, the colloidal particle is a solid-lipid particle.

A preferred aspect of the current invention seeks to provide solid-lipid particles comprised of at least one chelating amphiphile according to the present invention. Solid lipid particles according to the current invention may be manufactured by processes known to those skilled in the art. See, for example, Mehnert and Mader. (Mehnert 2001).

The appropriate process used to manufacture solid lipid particles according to the current invention may be selected according to the physicochemical properties of the chelating amphiphile of the current invention.

In one embodiment, the solid lipid particles of the current invention are prepared according to mechanical methods. According to this embodiment, one or more stabilisers are added to the neat amphiphile. Examples of stabilisers include, but are not limited to triblock polymers (for example, poloxamer 407). The amount of stabiliser added to the neat amphiphile may be between about 1 to about 10% (w/w). Depending on the nature of the amphiphile and the stabiliser, the amount of stabiliser added may be between about 5 to about 10% (w/w). Optionally, other additives may be added to the amphiphile. Other additives are known to those skilled in the art and may include, for example ethanol, propanol and butanol to ease the high viscosity of the bulk phases. The amphiphile mix is then melted, and water is added to the melted amphiphile mixture. To prepare the initial bulk phases, usually 20 to about 30% by weight of the amphiphile is added to water, usually at room temperature (about 22 to about 25°C).
The amphiphile-water mixture is then sheared using methods known to those skilled in the art. In a preferred embodiment, the amphiphile-water mixture is sheared using rough homogenization. The mixture may then undergo further processing to produce particles of desirable size and polydispersity. Methods of further processing are known to those skilled in the art and may include, for example, high pressure homogenization, ultrasonication, and filtration through different membranes with known pore sizes.

It will be recognised by the skilled addressee that the size of the colloidal particles of the current invention will depend upon the intended use. For example, for intravenous administration the preferred colloidal particle size range is commonly between about 30 nm and about 10 µm. More preferably, the size range is between about 30 nm and about 1 µm for intravenous application.

For delivery of colloidal particles into specific organs, such as the liver, and passive targeting to tumours, particle sizes of between about 30 nm to about 1000 nm are contemplated. More preferably particle sizes are about 30 nm to less than about 500 nm. Particularly preferred are colloidal particles of sizes between about 30 nm to about 300 nm. Without wishing to be bound by theory, it is believed that particles of the size between 30 - 300 nm are passively targeted to cancer cells, owing to their enhanced permeation and retention time in the leakier and chaotic neovasculature of solid tumours.

It will be further recognised by the skilled addressee that self-assembled structures comprising compounds according to formula (II) or (Ma) may be prepared by at least one of two methods. In one aspect, compounds according to formula (II) or (Ha) are subjected to conditions that facilitate their self assembly to generate self assembled structures of compounds according to formula (II) or (Ua). In another aspect, compounds according to formula (I) or the non-metallated precursors of formula (Ha) are subjected to conditions to facilitate their self assembly. The resultant self assembled structures according to formula (I) or the non-metallated precursors of formula (Ha) are then subjected to conditions that facilitate the transmetalation of the constituent amphiphiles, resulting in the formation of self assembled structures comprising compounds according to formula (II) or (Ha).
Another aspect of this invention relates to the use of colloidal particles derived from the self-assembled structures or compositions thereof according to the present invention as a diagnostic agent or contrast agent.

A further aspect of this invention relates to diagnostic compositions of the current invention. In one embodiment, the diagnostic composition according to the present invention comprises at least one of the compounds according to formula (I), (II) or (Ma). In another embodiment, the diagnostic composition comprises colloidal particles derived from at least one self-assembled structure according to the current invention. In one embodiment, the diagnostic composition according to the current invention may be freeze-dried, spray freeze dried, lyophilised or a spray-dried powder.

Diagnostic compositions according to the present invention may include pharmaceutically acceptable carriers, excipients, diluents, additives and vehicles selected based upon the intended form of administration, and consistent with conventional pharmaceutical practices. Suitable pharmaceutical carriers, excipients, diluents, additives and vehicles are known to those skilled in the art and are described in publications, such as, for example Remington: The Science and Practice of Pharmacy.

The diagnostic compositions according to the present invention may further include adjuvants that include, but are not limited to: preservatives, wetting agents or antimicrobial agents. Other adjuvants include, but are not limited to: cryoprotectants, spray drying adjuvants, buffers, isotonically adjusting agents, and phi adjusting materials.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 5,000 mg of an active ingredient, preferably contain between 20 and 1,000 mg of an active ingredient, and most preferably between 100 and 750 mg of an active ingredient.
It will be recognised that the intended form of administration is the form of colloidal particles.

The dosage regimen of the colloidal particles derived from the self-assembled structure or compositions thereof according to the current invention will vary depending upon known factors such as the pharmacodynamic characteristics of the compounds, self-assembled structures, colloid particles and compositions thereof of the current invention, and their mode and route of administration; the age, sex, health, medical condition, and weight of the patient, any concurrent treatment, the frequency of treatment, the renal, hepatic and cardiovascular and otherwise general health status of the patient in need of such treatment, and can readily be determined by standard clinical techniques.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

The examples that follow are intended to illustrate, but in no way limit, the present invention.

**General experimental and characterisation methodology.**

**Materials:** EDTA dianhydride, phytol and oleyl alcohol, oleic acid and all Lanthanide and transition metal chloride or acetate salts were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). All other reagents and solvents were of analytical grade and obtained from Sigma-Aldrich. Phytanol was synthesised by hydrogenation of phytol according to the procedures reported in Burns et al/ Aus J Chem, 1999, 52,387-395.

**Nuclear magnetic resonance spectroscopy (NMR):** NMR spectra were recorded on a Bruker 200 Spectrometer.

**High Performance Liquid Chromatography (HPLC):** Analytical HPLC was performed on Waters HPLC equipment (Waters Corporation, Milford, MA, USA.)
Electrospray Ionisation Mass Spectrometry (ESI-MS): ESI-MS spectra of the samples was performed on a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) equipped with ESI and APCI interfaces. Samples were infused using a syringe pump or using the LC/MS mode.

Water Penetration: The water penetration behaviour of the bulk amphiphile samples was analysed by using cross polarised light microscopy (POM). Typically 1-2 mg of each sample was placed on a microscope slide and heated until melted. Samples that were degraded before melting were used as the crystalline material. A cover-slip was placed on the melted sample and the sample was cooled to room temperature before addition of water. The microscope slide was placed into a Linkam PE94 hot stage (Linkam Scientific Instruments Ltd; Surry, England) and the temperature was controlled by a central processor PE94. Water was added to the edges of the cover-slips by a syringe, which resulted in instant water penetration into the sample by capillary action between the two glass surfaces, and generated a concentration gradient from 100% water to 100% neat amphiphile. The edges of the coverslips were then sealed with transparent nail-polish to avoid evaporation of the water. The samples were then heated at 2°C/min and phase transition behaviours were examined. Water penetration scans were performed between ambient temperature to temperatures at which the neat amphiphile melted, or otherwise, up to 100°C. The interaction of the water or aqueous buffer and the chelating amphiphiles and their metal complexes was observed with an Olympus GX51 inverted optical microscope (Olympus Australia Pty. Ltd.; Melbourne, Australia) in the presence or absence of crossed polarising lenses. Images were captured with an Olympus c-5060 digital camera (Olympus Australia Pty. Ltd.; Melbourne, Australia).

Cryogenic Transmission Electron Microscopy (Cryo-TEM): A laboratory-built humidity-controlled vitrification system was used to prepare the samples for Cryo-TEM. Humidity was kept close to 80% for all experiments, and ambient temperature was 22°C. A 4 µL aliquot of the sample was pipetted onto a 300-mesh copper grid coated with lacy formvar-carbon film (ProSciTech, Thuringowa, Queensland). After 30 seconds adsorption time the grid was blotted manually using Whatman 541 filter paper,
between 2 and 10 seconds. Blotting time was optimised for each sample. The grid was then plunged into liquid ethane cooled by liquid nitrogen. Frozen grids were stored in liquid nitrogen until required.

The samples were examined using a Gatan 626 cryoholder (Gatan, Pleasanton, CA, USA) and Tecnai 12 Transmission Electron Microscope (FEI, Eindhoven, The Netherlands) at an operating voltage of 120 kV. At all times low dose procedures were followed, using an electron dose of 8-10 electrons/Å² for all imaging. Images were recorded using a Megaview III CCD camera and AnalySIS camera control software (Olympus.)

10 **Small Angle X-ray Scattering:** SAXS analyses were performed on an in-house built system comprising of a Luzzati-Guinier type camera, which was connected to an X-ray generator (XRG 2500, Inel, France) operating at 40 kV and 30 mA with a sealed-tube Cu anode (Long fine focus, Philips). An 8° offcut bent quartz crystal (purchased from Inel, France) and 2 sets of vertical and horizontal slits were used to convert the divergent polychromatic X-ray beam into a focused line-shaped beam of Cu Kα radiation (\( \lambda = 0.154 \text{ nm} \)). The home-built flight tube, including a semitransparent Ni-filter as a beam stop, was evacuated to reduce parasitic scattering. The adjustable sample-detector distance was determined to be 250 mm by using Silver Behenate as a secondary standard.

20 The 2D scattering pattern was recorded on a CCD camera with a 4-port readout (QuadRO: 4320-4/1-75 X-ray camera system) from Princeton Instruments, which is a division of Roper Scientific, Inc. (Trenton, NJ, USA). This detector features a 2084 X 2084 pixel array with 24 μm X 24 μm pixel size, and is operated at -40°C to reduce thermally generated charge. The software Winview 32 (Princeton Instruments) was used to collect the 2D data, and the software SAXSQuant (Anton Paar, Graz) to conduct all primary data handling such as background subtraction and integration of the 2D pattern into the 1D scattering function \( I(q) \), where \( q \) is the length of the scattering vector defined by \( q = (4\pi/\lambda)\sin(\theta/2) \), where \( \lambda \) is the wavelength and \( \theta \) is the scattering angle. The raw data were exported and plotted using Origin software.
Alternatively, the SAXS analyses were performed at the Australian Synchrotron facility in Melbourne.

**Example 1: Chelating EDTA/DTPA groups conjugated to long hydrophobic chains**

1(a) Synthesis

5 EDTA-MO, formula (III) and EDTA-BO, formula (IV)

EDTA dianhydride (2.56 g, 10 mM) was added to a solution of anhydrous DMF/pyridine (40 mL/5 mL). The reaction mixture was heated to 100 °C until the EDTA-dianhydride dissolved completely. The temperature was then reduced to 50°C and oleyl alcohol (4.02 g, 15 mM), dissolved in 5 mL of tetrahydrofuran (THF), was added to the reaction mixture. The reaction mixture was stirred for 3 h. After addition of 10 mL of water, the reaction mixture was stirred for an additional 1 h, followed by evaporation of the solvents under reduced pressure. The oily residue was redissolved in ethanol/sodium acetate solution and applied to a C18 prep HPLC column (50 mm × 200 mm, Waters, Madison, USA). The pure conjugates were separated and eluted by a stepwise gradient method from buffer A: H₂O/ethanol 90/10, to buffer B: ethanol. The mono-oleyl conjugate eluted at 85% buffer B and the bis-oleyl conjugate eluted at 100% buffer B. The pure title compounds were dried under reduced pressure to obtain 0.4 g of compound (III) and 4.2 g of compound (IV), with an overall yield of 70%.

EDTA-MO: MS: 542.36. ¹H NMR in DMSO-d6: 5.33 (m, 2H, =CH-CH₂), 4.01 (t, 2H, CH₂-O), 3.53 (s, 2H, N-CH₂-CO), 3.44 (s, 6H, N-CH₂-CO), 2.75 (t, 4H, N-CH₂), 1.98 (m, 4H, =CH-CH₂), 1.55 (m, 2H, O-CH₂-CH₂), 1.25 (m, 22H, CH₂), 0.85 (t, 3H, CH₃); Elemental analysis: Calculated; C: 61.97, H:9.29, N:5.16, found; C: 63.59, H:9.66, N:4.74, Na<1.

EDTA-BO: MS: 792.63 ¹H NMR in DMSO-d6: 5.33 (t, 4H, =CH-CH₂), 4.01 (t, 4H, CH₂-O), 3.52 (s, 4H, N-CH₂-CO), 3.42 (s, 4H, N-CH₂-CO), 2.73 (t, 4H, N-CH₂), 1.99 (m, 8H, =CH-CH₂), 1.55 (m, 4H, O-CH₂-CH₂), 1.24 (m, 44H, CH₂), 0.85 (t, 6H, CH₃); Elemental analysis: Calculated; C: 69.85, H:10.85, N:3.47, found; C: 69.70, H:10.85, N:3.47, Na<0.7.
EDTA-MP, formula (V) and EDTA-BP, formula (VI)

EDTA dianhydride (2.56 g, 10 mmol) was added to a solution of anhydrous DMF/ pyridine (40 mL/5 mL). The reaction mixture was heated to 100°C until the EDTA-dianhydride dissolved completely. The temperature was then reduced to 50°C and phytanol (5.36 g, 18 mmol), dissolved in 10 mL THF, was added to the reaction mixture. The reaction mixture was stirred overnight at 90°C. Water was added to the reaction mixture and the oily residue was completely dissolved. The reaction mixture was stirred for 3 h, followed by addition of 10 mL of water, and stirring for an additional 1 h. The solvents were then removed under reduced pressure. The residue was redissolved in ethanol/sodium acetate solution and applied to a C18 prep HPLC column (50 mm X 200 mm, Waters, Madison, USA). The pure conjugates were separated and eluted by a stepwise gradient method from buffer A: (H2O/ethanol 90/10), to buffer B: (ethanol). Compound (V) eluted at 90% buffer B and compound (VI) eluted at 100% buffer B. The pure title compounds were dried under reduced pressure to obtain 1.0 g of compound (V) and 3.9 g of compound (VI), with an overall yield of 61%.

EDTA-MP; MS: 572.36, 1H NMR in DMSO-d6: 4.05 (t, 2H, CH2-O), 3.53 (s, 2H, N-CH2-CO), 3.47 (s, 6H, N-CH2-CO), 2.76 (t, 4H, N-CH2), 1.84-0.95 (m, 24H, CH-CH2, O-CH2-CH2, CH-CH2), 0.94-0.72 (m, 15H, CH-CH3); Elemental analysis; Calculated: C: 62.8, H:10.01, N:4.88, found; C: 66.11, H:10.66, N:4.02.

EDTA-BP; MS: 852, 1H NMR in MeOD: 4.2 (t, 4H, CH2-O), 3.9 (s, 4H, N-CH2-CO), 3.7 (s, 4H, N-CH2-CO), 3.2 (t, 4H, N-CH2), 1.84 -0.94 (m, 48H, CH-CH2, O-CH2-CH2, CH-CH2), 0.94-0.72 (m, 3OH, CH3). Elemental analysis; Calculated: C: 70.38, H:11.34, N:3.28, found; C: 69.65, H:10.51, N:3.27, Na<1.

DTPA-MP, formula (VII) and DTPA-BP, formula (VIII)

DTPA-bis-an hydride (1.79 g, 5 mM) was added to a solution of anhydrous DMF/ pyridine (40 mL/5 mL). The reaction mixture was heated to 130 °C until the DTPA- bis-anhydride was completely soluble. The temperature was then reduced to 90 °C and phytanol (2.98 g, 10 mM), dissolved in 5 mL of THF, was added to the reaction mixture. The reaction mixture was stirred overnight at 90 °C. Water was added to the reaction mixture and
which was stirred for an additional 1 h to hydrolyze the unreacted anhydrides, followed by concentration under reduced pressure. The oily residue was redissolved in ethanol/sodium acetate solution and applied to a C18 prep HPLC column (50 mm X 200 mm, Waters, Madison, USA). The pure conjugates were obtained by a stepwise gradient method from buffer A: H$_2$O/ethanol 90/10, to buffer B: ethanol. The mono phytanyl conjugate eluted at 80% buffer B and the bis-phytanyl conjugate eluted at 95% buffer B. The pure title compounds were dried under reduced pressure to obtain 0.89 g of compound the mono-derivative and 0.24 g of the bis-derivative, with an overall yield of 23%. DTPA-monophytanyl (DTPA-MP); MS: 672.93. $^1$H NMR in MeOD, 4.2 (t, 2H, CH$_2$-O), 3.94 (s, 2H, N- CH$_2$ -CO), 3.64 (s, 2H, N- CH$_2$ -CO), 3.58 (s, 6H, N- CH$_2$ -CO), 3.48 (t, 4H, N- CH$_2$), 3.19 (t, 4H, N- CH$_2$ -CO), 1.84-0.95 (m, 24 H, CH-CH$_2$, O-CH$_2$-CH$_2$, CH-CH$_2$), 0.94-0.72 (m, 15 H, CH-CH$_3$). Elemental analysis: Calculated; C: 60.6, H:9.42, N:6.24, found; C: 61.80, H;9.85, N:5.74.

DTPA-BP; MS: 953.93, $^1$H NMR in MeOD: 4.17 (t, 4H, CH$_2$-O), 3.94(s, 2H, N- CH$_2$ -CO), 3.64 (s, 4H, N- CH$_2$ -CO), 3.58 (s, 4H, N- CH$_2$ -CO), 3.48 (t, 4H, N- CH$_2$), 3.19 (t, 4H, N- CH$_2$ -CO), 1.84 -0.94 (m, 48 H, CH-CH$_2$, 0-CH$_2$-CH$_2$, CH-CH$_2$), 0.94-0.72 (m, 30H, CH$_3$) Elemental analysis: Calculated; C:67.97, H:10.88, N:4.4, found: C: 67.96, H;10.81, N:4.38.

**Mn-EDTA-MO, formula (XV)**

2 ml of 2 M sodium acetate solution was added to a solution of EDTA-MO (400 mg, 0.738 mmol) in 10 ml of water. Manganese acetate (181 mg, 0.738 mmol), dissolved in 4 ml of water, was added drop-wise to the reaction mixture. The reaction mixture turned to a milky brown solution. The complexation reaction continued overnight and was then tested by ESI/MS which proved complete disappearance of the peak due to the parent amphiphile and the appearance of the peak at 625. All the solvent was removed under reduced pressure and 40 ml additional water was added to the precipitate, which was then stirred overnight. The precipitate was filtered and dried under vacuum to yield the title compound.
1 ml of 2 M sodium acetate solution was added to a solution of EDTA-BO (793 mg, 1 mmol) in 10 ml of ethanol. The pH of the sample was around 6.72. Manganese acetate (245 mg, 1 mmol), dissolved in 6 ml of water, was added to the reaction mixture and the reaction mixture turned to a milky solution. The complexation reaction continued overnight and was then tested by ESI/MS, which proved complete disappearance of the peak due to the parent amphiphile and the appearance of the peak at 905.27, equivalent to the mass of sodium acetate salt of the complex. Ethanol was removed under reduced pressure and 50 ml additional water was added to the precipitate, which was then stirred overnight. The precipitate was filtered and dried under vacuum to yield 500 mg of the complexed sample.

\(^1\text{H NMR showed broadening and nearly disappearance of the EDTA} \; ^1\text{H peaks due to the proximity to the paramagnetic Mn ions.}

2 ml of 2 M sodium acetate solution was added to a solution of EDTA-MP (572 mg, 1 mmol) in 10 mL of water. Manganese acetate (245 mg, 1 mmol), dissolved in 6 mL of water, was added to the reaction mixture and the reaction mixture turned to a milky brown solution. The complexation reaction continued overnight and was then tested by ESI/MS, which proved complete disappearance of the peak due to the parent amphiphile and the appearance of the peak at 625. All the solvent was removed under reduced pressure and 50 mL additional water was added to the precipitate, which was then stirred overnight. The precipitate was filtered and dried under vacuum to yield the title compound.
Mn-EDTA-BP, formula (XVIII)

1 mL of 2 M sodium acetate solution was added to a solution of EDTA-BP (666 mg, 0.78 mmol) in 20 mL of ethanol. Manganese acetate (191 mg, 0.78 mmol), dissolved in 2 mL of water, was added drop-wise to the reaction mixture and the reaction mixture turned to a milky white solution. The complexation reaction continued overnight. Ethanol was removed under reduced pressure and 50 mL additional water was added to the precipitate, which was then stirred overnight. The precipitate was filtered and dried under vacuum to yield 500 mg of the complexed sample.

$^1$H NMR showed broadening and nearly disappearance of the EDTA. $^1$H peaks due to the proximity to the paramagnetic Mn ions. Elemental analysis; Calculated; C: 66.27, H:10.46, N:3.09, Mn:6.06 found; C: 65.37, H:10.51, N:3.18, Mn:5.8.

Gd-EDTA-MP, formula (XIX)

10 mL of 2 M sodium acetate solution (pH=6.43) and 10 mL of ethanol were added to 800 mg (1.39 mmol) of EDTA-MP. Gd-acetate (467 mg, 1.4 mmol), dissolved in 5 mL of water, was added to the above solution. The reaction mixture was stirred for 60 min at room temperature. MS of the sample showed complete disappearance of the mass peak due to the precursor amphiphile. Ethanol was evaporated and the aqueous solution was extracted with chloroform. The insoluble white precipitate at the boundary of the water/chloroform interface was filtered and washed with water to yield 0.6 g of
pure complex. The chloroform extract was also evaporated to yield 0.55 g of the complex with some impurity. NMR of both samples showed very broad peaks of the amphiphile molecules, typical of the complexed compounds associated with paramagnetic metal ions.

![Diagram](XIX)

**Gd-DTPA-MP, formula (XX)**

4 mL of 1 M sodium acetate solution was added to a solution of DTPA-MP (500 mg, 0.742 mmol) in 5 mL of THF. Gd-acetate (267 mg, 0.8 mmol), dissolved in 4 mL of water, was added drop-wise to the reaction mixture and the reaction mixture turned to a milky white solution. The complexation reaction continued overnight. THF was removed under reduced pressure and the residue was redissolved in ethanol/water and purified on a semi prep C18 column. The pure fractions were collected to yield 220 mg of the title compound with an overall yield of 35%. ICP showed inclusion of 14.6% of Gd in this sample.
Example 1(b): Preparation of bulk lyotropic phases

Self assembled bulk phases of the samples of Example 1 were prepared by adding aqueous solutions of 100 mM, 200 mM, 500 mM, 1 M and 2 M sodium acetate to each neat amphiphile. Various ratios of amphiphile/aqueous solution from 20% up to an excess amount of aqueous solution were made by using 50-100 mg of amphiphile. The samples were kept in sealed tubes. The samples were heated, vortexed and centrifuged to acquire homogenous mixtures. The mixtures were then transferred to 1-mm quartz capillary tubes, centrifuged for 10-30 min at 2000g and flame sealed. Samples were then stored at room temperature for at least 1 hour before being characterised. The following examples look more closely at the phases as they form.

Example 1(c): Characterisation of the lyotropic phase behaviour of EDTA chelating amphiphile

Lyotropic behaviour of the chelating amphiphiles was studied by water penetration scan technique by using polarised optical microscopy (POM). Although only lamellar and hexagonal mesophases exhibit distinct birefringence under a polarised microscope, the other mesophases could also be distinguished by the discontinuation of refractive indices at their boundaries. Using this technique, a gradient of hydration is produced within the sample from fully hydrated amphiphiles at the boundary of the water to the neat amphiphile in the middle. All compounds showed $S_A$ phases in their pure state by exhibiting anisotropic structure. This was also confirmed by XRD of the neat
amphiphiles. The results of the XRD (not shown) demonstrated the lamellar crystalline structure for most of the amphiphiles.

POM of EDTA-MO demonstrated an isotropic phase at excess water near the outer boundary of the amphiphile in contact with 100 mM sodium acetate solution, and a lamellar phase at lower water concentrations next to the neat amphiphile. The isotropic phase demonstrated round circular bubbles, typical of micellar phase. The lamellar phase birefringence continued up to 60°C, at which temperature, the lamellar phase band narrowed down and was substituted by a hexagonal phase.

Phase behaviour was also confirmed by small angle X-ray scattering (SAXS). The results of the SAXS analysis confirmed the phases observed by POM. The hydrated amphiphile with EDTA-MO/sodium acetate solution demonstrated a lamellar crystalline structure with a lattice parameter of 4.40±0.012 nm and a lamellar liquid crystalline with d-spacing of 5.71±0.07 nm. The lamellar crystalline phase has a very comparable lattice parameter with the solid crystalline material measured by XRD (4.34 nm ± 0.02); which indicates that the EDTA-MO at 30% sodium acetate solution (100 mM) is not fully hydrated and is composed of lamellar liquid crystalline and lamellar crystalline phases. This amphiphile at higher concentrations of water (30% amphiphile, 70% sodium acetate solution) demonstrated a lamellar crystalline phase with nearly the same dimension as the solid crystalline material (4.40±0.01) with an unidentified peak at q = 0.56 nm⁻¹, which might be characteristic of micellar domains formed by this chelating amphiphile.

The birefringence pattern of the EDTA-BO amphiphile hydrated with 100 mM Na acetate is shown in Figure 2. At room temperature, this amphiphile showed myelin structure at the boundary with water, Figure 2(a), followed by a swollen crystalline phase with a somewhat different birefringence of the neat amphiphile. By raising the temperature to 35°C and further to 37°C, an isotropic phase appeared between the myelin structure and the neat amphiphile (Figure 2(b)). The isotropic phase had typical characteristics of cubic phases due to the formation of non-circular bubbles and the stiffness of the band. By further raising the temperature to 40°C (Figure 2(c)), the myelin structure disappeared and only one isotropic phase formed at the boundary with water.
Finally, further heating of the sample to 45°C resulted in an additional isotropic phase, a LC lamellar phase (U), and a third isotropic phase between the $L_\alpha$ and the neat amphiphile, shown in Figure 2(d). This trend continued up to 60°C, at which temperature a few distinct birefringence appeared between the isotropic phase and the neat amphiphile. The conical shape of the new band suggested the formation of an inverse hexagonal structure above 60°C and this trend continued up to 100°C.

Addition of 2 M sodium acetate to EDTA-BO resulted in the appearance of a lamellar liquid crystalline phase at excess water and at room temperature, followed by an isotropic phase between the liquid crystalline $L_\alpha$ and the crystalline lamellar neat amphiphile as shown in POM images of Figure 3(a). By raising the temperature to 37°C, the isotropic phase expanded further and an additional non-isotropic phase formed between the isotropic phase and the neat amphiphile (Figure 3(b)). The isotropic phase expanded broadly by raising the temperature, however, a narrow band birefringence appeared at around 37°C, which continued up to 60°C, at which temperature a clear hexagonal birefringence was formed between the two isotropic bands. This trend continued up to 71.8X, at which temperature the anisotropic texture of the neat amphiphile turned to an isotropic phase due to melting of the neat amphiphiles consistent with the melting point of the neat amphiphile obtained by DSC.

The lyotropic phase behaviour of EDTA-BO at excess amount of aqueous buffer swollen with different concentrations of sodium acetate and over a broad temperature range was also assessed by synchrotron SAXS/AXS analyses, shown in Figure 4. The lyotropic phase behaviour with excess sodium acetate solution (100 mM) at 25°C displayed only lamellar crystalline phase, displaying a d-spacing of 3.26±0.05 nm. At 37°C the lamellar crystalline phase transformed to a lyotropic inverse bicontinuous cubic phase with a lattice parameter of 22.8±0.11 nm. This trend continued up to 45°C, at which temperature the inverse bicontinuous cubic phase transformed to an inverse hexagonal phase and this lyotropic phase was retained up to 60°C; the highest temperature that this analysis was conducted.

The same sample hydrated with excess sodium acetate solution (200 mM) showed similar lyotropic phases as above (Figure 4(b)). By raising the ionic state of the head
groups more significantly by swelling of this amphiphile with higher concentration of sodium acetate solution (500 mM, 1 M and 2 M), its lytropic transformed to an inverse cubic phase at around 37°C and retained it's inverse cubic phase up to 70°C (Figure 4(c) and 4(d)). This finding confirmed our observation with the POM and that this amphiphile has a very rich polymorphism, forming lamellar liquid crystalline at room temperature, inverse cubic phases close to physiological condition and hexagonal phases at higher temperatures. Phase behaviour studies of EDTA-MP, observed by POM, showed myelin structures followed by a lamellar liquid crystalline phase (Figure 5). This transition continued up to 50°C, where the myelin structure disappeared and it transformed to only a lamellar liquid crystalline phase. This sample retained the same behaviour up to 70°C at which temperature, the neat amphiphile and the liquid crystalline phase melted away. This observation was also in agreement with the DSC result where a broad melting temperature range with an onset at 48°C was demonstrated. Similar phase behaviour was also supported by the SAXS analysis of EDTA-MP swelling in 100 mM sodium acetate solution shown in Figure 6. EDTA-MP lyotropic liquid crystalline phase behaviour in binary (amphiphile/water) and ternary systems (amphiphile/water/Na-acetate) was examined by synchrotron SAXS and is shown in Figure 6. At room temperature, this amphiphile hydrated with water displayed two phases in equilibrium; a lamellar crystalline and an L_α phase, both showing Bragg peaks in the ratio of 1:2:3. By raising the temperature, the Bragg peaks relating to the lamellar crystalline phase diminished and transformed to a single phase, an L_α liquid crystalline phase, below 4°C. The lattice parameter of the lamellar crystalline phase was consistent with the largest lattice parameter of the polycrystalline neat amphiphile (4.66 ±0.05 nm), demonstrating a non-swelled crystalline phase. The lattice parameter for the L_α phase decreased from 8.52 nm at 25°C to 8.17 nm at 50°C, which is consistent with the behaviour of mesophases in general and is due to the chain splay increase. Similarly, this amphiphile, when hydrated with 100 mM Na-acetate, displayed two phases as shown in Figure 6b. The lattice parameter of the L_α phase hydrated with 100 mM Na-acetate was slightly less than that hydrated with water. This is possibly reflected by the different state of the headgroups ionization, electrostatic interaction between the charges, as well as the effects on the intramolecular hydrogen bonding within the headgroups. It's assumed that at this ionic condition only one of the amine groups is protonated and therefore a partial hydrogen bonding between carboxylates
and protonated amine groups occurs. This will consequently result in a smaller lattice parameter at this ionic condition. By increasing the temperature, the lattice parameter of the \( L_\alpha \) phase decreased slightly, which can be reflected by chain splay increases at higher temperatures.

5 The crystalline lamellar structure disappeared when hydrated with 200 mM Na-acetate and displayed a single \( L_\alpha \) phase. This may indicate the complete hydration of the headgroups at this ionic concentration. The lattice parameter of the lamellar liquid crystalline phase at 200 mM Na-acetate was slightly larger than that hydrated with 100 mM Na-acetate, but comparable to the \( L_\alpha \) phase hydrated with water.

10 Phase behaviour studies of EDTA-BP observed by POM (Figure 7) demonstrated spontaneous formation of birefringence typical of inverse hexagonal phases upon hydration at \( 21^0\text{C} \). This hexagonal band continued to broaden by raising the temperature. The same trend continued up to \( 100^0\text{C} \). In addition the neat amphiphile also showed a thermotropic phase and transformed into a hexagonal phase by raising the temperature up to \( 100^0\text{C} \).

SAXS/AXS analysis of the lyotropic phase behaviour of EDTA-BP, swollen with pure water, sodium acetate at 100 mM, 500 mM and 1 M concentration and at various temperatures are shown in Figure 8(a-d). Like its oleyl counterpart, this amphiphile also demonstrated a very rich polymorphism with the different ionic states of the head groups. While most of the head groups are protonated (swelling with pure water), it displayed only a hexagonal phase from room temperature up to \( 50^0\text{C} \). Addition of Na-acetate (100 mM, 500 mM and 1 M) yielded two phases in equilibrium where, as well as the inverse hexagonal phase, a 3D micellar cubic phase (Fd3m or \( \text{In} \)) was also identified (Figure 8b). Increasing the concentration of sodium acetate from 0 to 100 mM and above increases the shielding effect on the self-assembled structures due to the higher ionic state of the solvent and therefore, the \( \text{pK}_a \) values for the amine and acetate ions are lowered. It is most likely that at this ionic condition, only one of the amine groups is protonated and therefore a partial hydrogen bonding between carboxylates and protonated amine groups occurs. The decrease in the size of the headgroup lowers the critical packing parameter or shape factor \( \text{CPP}=v/(\text{kao}) \). This can move the hexagonal
phase towards the micellar cubic phase with Bragg peaks in the ratio \(V_3, \sqrt{6}, V_{11}, V_{12}, V_{16}, V_{19}, V_{24}, V_{27}\). A small shift in \(q\) value occurs with increasing temperature which translates to smaller lattice parameters. The \(H_n\) and Fd3m phases exist in a prolonged metastable equilibrium state, and were observed for samples with extended equilibration times (7 days). The lattice parameter of the inverse hexagonal phase at 25°C increased from 4.33 nm in water to 5.07, 5.76 and 5.99 nm in aqueous buffer solutions at 100 mM, 500 mM and 1 M, respectively. The relative contribution of electrostatic repulsion and intramolecular hydrogen bonding gauged the shift towards micellar cubic phases or hexagonal phases. The population equilibrium between the two phases favoured the \(H_n\) phase at higher buffer concentration of 500 mM and 1 M concentrations (Figure 8c-d). The preference for hexagonal phases over micellar cubic phases at higher concentrations of sodium acetate is presumably due to the high ionic state of the solution, contributing to deprotonation of the amine groups and breaking the intramolecular hydrogen bonding within the headgroups. Thus the headgroups are more negatively charged which allows for larger headgroups and therefore a smaller shape factor, reversing the phase behaviour towards hexagonal phases (Figure 8d).

Phase behaviour of DTPA-BP observed by POM, demonstrated an isotropic phase at the boundary with water at room temperature (Figure 9). By raising the temperature to 37 °C, the isotropic phase expanded to a broader band and continued to expand by raising the temperature until 50°C where an extra anisotropic phase appeared between the isotropic band and the neat amphiphile and simultaneously the anisotropic texture of the neat amphiphile disappeared to an isotropic phase. The isotropic phase close to the boundary and at excess water is most likely due to an inverse bicontinuous cubic phase.

The majority of the DTPA chelating amphiphiles such as DTPA-MP, DTPA-MO and DTPA-BO displayed only lamellar and micellar phases at bulk phases and at 100% excess water. The only exception to those was DTPA-BP, which displayed higher ordered nanostructures as shown in Figure 10(a-c). This amphiphile displayed a very broad peak and non-well resolved peaks at lower temperatures up to 40°C. This phase is most likely due to an inverse bicontinuous cubic phase. The bicontinuous cubic phase then transformed to a hexagonal phase at higher temperatures, which is consistent with
the phase behaviour of amphiphiles. At a higher concentration of Na-acetate (500 mM), the bicontinuous cubic and lamellar liquid crystalline (U) phases coexisted. At higher temperatures the bicontinuous cubic phase transformed into a hexagonal phase, which co-existed with an L\textsubscript{α} phase.

**Example 1(d): Preparation of colloidal particles**

EDTA amphiphiles were constituted into colloidal particles by injection of their ethanolic solution at 100 mM concentration into a sodium acetate buffer solution. The final concentration of the amphiphiles was 20 mM (pH=5.2-5.7). These were then vortexed vigorously to prepare homogenous hydrated samples. These colloidal particles were also prepared by direct hydration of the neat amphiphiles with sodium acetate solution at a final concentration of 20 mM. The dispersions made by the latter method were subjected to ultrasonication, high pressure homogenization and extrusion through 200 and 100 nm pore membranes. Particle sizes were measured at 37°C with a laser light scattering submicron particle sizer (Malvern instruments, Malvern, Worcestershire, UK). The mean sizes of the colloidal particles complexed with Gd are shown in Table 1.

**Example 1(e): Synchrotron SAXS of colloidal particles**

In order to obtain definitive structural information on the mesophases in dispersed particles, synchrotron SAXS was used to acquire high resolution diffraction patterns of our submicron sized particles. Dispersions of 20 mM amphiphiles in 100 mM Na-acetate solution were used for all the analyses and the subsequent complexation reactions with Mn or Gd. It should be noted that no additional surfactant such as poloxamer, commonly used for the dispersion of the inverse phase, was used in this study.

**EDTA-MP**

EDTA-MP dispersed in 100 mM Na-acetate solution and at an apparent pH of ~5.5 mainly formed liposomal particles with a typical SAXS pattern shown in Figure 11. The complexation reactions of this amphiphile with Mn or Gd were conducted subsequent to the suspension of the chelating amphiphiles in 100 mM Na-acetate solution. The fast
The kinetics of complexation was confirmed by MS analysis through subsequent incremental addition of metal ions to the amphiphile dispersion (data not shown). The complexation reaction for Gd-EDTA-MP at different molar ratios of Gd to EDTA-MP was also monitored using synchrotron SAXS (coloured lines in Figure 11). The diffraction pattern of the neat amphiphile dispersion of EDTA-MP is consistent with liposomal particles. The SAXS pattern of the complexed dispersion displayed sharper diffraction peaks as the electron dense Gd-ions provide greater contrast for scattering. Incorporation of Gd also increases the thickness of the membrane bilayer as evidenced by the shift of the peak at 0.07 Å⁻¹.

**EDTA-BP**

The effect of the pH on the internal nanostructures of the EDTA-BP amphiphile was examined to understand the structural changes with pH and the optimum pH for subsequent complexation reaction. The pH of the EDTA-BP dispersion at 100 mM sodium acetate was measured to be approximately 5.7. Subsequently, the pH of this dispersion was reduced to 5 and 4 by addition of 10 mM HCl solution, or was raised to higher pH by addition of 10 mM sodium hydroxide solution. The scattering pattern of these dispersions at different pHs are shown in Figure 12a. At pH=5.7, the dispersed particles displayed two sets of diffraction peaks consistent with an inverse hexagonal structure in the ratio 1:√3: √4 and a smaller set of diffraction peaks in the ratio √2: V4: V0, consistent with an inverse bicontinuous cubic Im3m phase. The lattice parameter for the two phases was calculated to be 6.52±0.05 nm for the inverse hexosomes and 18.5±0.05 nm for the inverse Im3m cubosomes. At lower pHs of 5 and 4, the diffraction patterns of the dispersions displayed only one set of peaks consistent with an inverse hexagonal structure. The lattice parameter of the hexosomes decreased to 5.87±0.05 nm at pH=5 and further to 5.42±0.05 nm at pH=4. The decrease in the lattice parameter is most likely due to protonation of the amine groups in the head group and as noted before more intramolecular hydrogen bonding. This causes less repulsion forces between the head groups, the formation of smaller water channels, and a higher curvature of the interface. Inverse hexagonal nanostructures were retained for pH<6. At a higher pH, around 6, the diffraction pattern drastically altered to a very broad peak at 0.12 Å⁻¹, which looks like the typical diffraction pattern for liposomes as evidenced by
cryo-TEM discussed in the following sections. We conclude that the additional set of peaks we observed in the sample at pH=5.7 is a bicontinuous cubic phase of symmetry Im3m due to the location in the phase diagram between the flat interface of liposomes and the curved interface in the hexosomes. However, its range of existence seems to be very small (between pH=5.7 and pH=6).

The possibility of phase transition of the dispersed sample at higher temperatures was investigated by conducting synchrotron SAXS of EDTA-BP dispersions at pH=5.0 and at three different temperatures up to 50°C (Figure 12b). The inverse hexagonal diffraction pattern was retained at all three different temperatures. The lattice parameter slightly increased from 5.87±0.05 nm at 25°C to 5.93±0.05 nm at 37°C and further to 5.95±0.05 nm at 50°C.

The complexation of EDTA-BP amphiphile dispersions with Mn was also investigated by Synchrotron SAXS as shown in Figure 12c. Addition of incremental Mn ions to EDTA-BP dispersions resulted in the appearance of two broad peaks at approximately q=0.15 and 0.3 Å⁻¹. Simultaneously, the diffraction peaks consistent with the hexagonal nanostructures in the ratio of 1/1, V3, h4, as well as the diffraction peaks owing to the Im3m cubic nanostructures decreased significantly upon addition of 0.1 molar ratio of Mn to EDTA-BP. The emergence of a broad diffraction peak may reflect the formation of less ordered emulsified particles co-existent with hexagonal nanostructures. This was proved by visualization of the dispersed particle using cryo-TEM which is discussed in the following section. Both sets of diffraction peaks steadily shifted to higher q-values, that is, smaller lattice parameters with addition of Mn. The lattice parameter of the precursor amphiphiles EDTA-BP was 6.52±0.05 nm, and decreased to 5.82±0.05 nm at a molar ratio of Mn/EDTA-BP=0.6. Less charges on the headgroups and thus less intermolecular repulsion between the headgroups in the hexagonal arrays reflects a decreased d-spacing between unit cells. As noted before, further increase of Mn induced precipitation of the samples. This is most likely due to the reduction of charges in the amphiphile headgroups by complexation with Mn and transformation to less charged particles.

DTPA-BP
DTPA-BP dispersed in 100 mM Na-acetate solution mainly formed liposomal particles with a typical SAXS pattern shown in Figure 13a. Complexation of Gd with this amphiphile at a 1:2 ratio of metal to ligands transformed the liposomal particles to nanostructured particles with completely different scattering patterns. The new scattering pattern displayed a set of diffraction peaks in the ratio 1:V3: V4, consistent with the nanostructures of hexosome particles. The lattice parameter of these hexosomes at 25°C was 6.84±0.05 nm and increased slightly to 6.88±0.05 nm at higher temperatures.

**Incorporation of Gd-DTPA-MP into neutral amphiphile phytanolethanolamide and phytantriol**

Gd-DTPA-MP was incorporated into phytanolethanolamide and phytantriol, which are known to form cubosomes at room temperature and above. The complexed amphiphile solution in dichloromethane (DCM) was added to the DCM solution of phytanolethanolamide and phytantriol at different molar ratios and vortexed for a few minutes. DCM was rotary evaporated and further dried under a freeze dryer overnight. The mixed dried samples (50 mg) were dispersed in 2.5 ml of water (containing 5 mg of F127 stabiliser). The mixtures were homogenised by bath sonication and further homogenised under Ultratarrax for 5 min with a speed of 20000 rpm at 40°C. All of the dispersions were almost transparent except the mixture at a ratio of 99/1 of phytantriol/Gd-DTPA-MP (1% Gd-DTPA-MP) which looked milky. The mixture of phytanolethanolamide/ Gd-DTPA-MP was prepared only at 5% molar ratio of Gd-DTPA-MP. The dispersed nanoparticles of this mixture also looked milky. The particles were sized by dynamic light scattering, as previously described. The Synchrotron SAXS pattern of these particles were examined and the majority of phytantriol/ Gd-DTPA-MP mixed dispersions displayed scattering patterns consistent with liposomal particles. Only 1% Gd-DTPA-MP dispersions displayed a set of diffraction peaks in the ratio V2: V4: X6, consistent with the inverse bicontinuous cubic Im3m phase, as shown in Figure 14. The lattice parameter at 25°C was 10.65±0.05 nm, and decreased to 10.16 ±0.05 nm at 37°C.

**Example 1(f): Characterisation of colloidal particles' morphology by Cryo-TEM**
The influence of the metal component on the structural morphology of the colloidal particles was studied by Cryo TEM and the results are shown in Figures 15-19.

**EDTA-MP**

EDTA-MP amphiphile hydrated with 100 mM sodium acetate (Figure 15) exhibited a mixture of long thread-like micellar phase, and some uni-lamellar vesicles, which coexisted in the dispersion (15(a)). This result is in agreement with the SAXS analysis and water penetration scans. Complexation of this amphiphile with Mn or Gd ions (Figure 15(b-c)) resulted in predominant formation of unilamellar liposomes.

**EDTA-BP**

Cryo-TEM of typical particles of EDTA-BP dispersions is shown in Figure 16. A mixture of particles with internal nanostructure and unilamellar vesicles was observed at 10 mM concentration of the sample (16(a)). The particles with internal nanostructures were very sensitive to the electron beams and melted when exposed to higher energy beams for examination at higher magnification, which impeded verification of the detailed internal nanostructure of these particles. Given that water penetration scans and SAXS analysis showed the formation of inverse hexagonal phase within a broad range of temperature, ionic concentration and water content, it can be assumed that particles with internal nanostructures are forming hexosomes.

Cryo TEM micrographs of Mn-EDTA-BP dispersions are shown in Figure 16(b-c).

**EDTA-BO**

CryoTEM micrographs of EDTA-BO dispersions are shown in Figure 17(a-d). This amphiphile exhibited a mixture of cubosomes, unilamellar and multilamellar particles.

**DTPA-MP & DTPA-BP**

CryoTEM micrographs of DTPA-MP and DTPA-BP also demonstrated particles of unilamellar and multilamellar structures. The micrographs of the DTPA-BP are shown in
Figure 18. However, as was revealed by synchrotron SAXS analyses, shown in Figure 13(b), complexation of this amphiphile with Gd induced hexagonal nanostructures.

**Dispersion of Gd-DTPA-MP mixed with neutral amphiphiles in aqueous solution**

Dispersion of the precomplexed amphiphiles such as Gd-EDTA-MP, Gd-DTPA-MP, Mn-EDTA-BP and Mn-EDTA-BO was achieved by the addition of non-chelating amphiphiles such as phytanylethanolamide and phytantriol. A typical Cryo-TEM micrograph of the colloidal particles made by dispersion of a mixture of Gd-DTPA-MP/phytanylethanolamide and Gd-DTPA-MP/phytantriol (5% and 1% molar ratio), respectively are shown in Figure 19(a,b).

The former dispersion displayed hexosomes, whereas the latter showed cubosome structure, consistent with synchrotron SAXS analysis.

**Example 1(g): Assessment of T₁ and T₂ relaxation times**

The proton longitudinal and transverse relaxation time (T₁ and T₂) for the EDTA and DTPA-chelating amphiphilic colloidal particles were measured at 35°C on a MARAN ultra (Oxford instruments, Abingdon, UK) at a field strength of 0.53 T. T₁ was measured with an inversion recovery sequence, while T₂ was measured with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The relaxivity parameters, r₁ and r₂, were then calculated as an inverse of relaxation time per mM.

Relaxivity of Mn and Gd EDTA and DTPA conjugates are listed in Table 1. Most of these chelating amphiphilic colloidal particles demonstrated several fold increase in longitudinal relaxivity over the commercial samples, Magnevist, suggesting that these conjugates have promising relaxivity properties as contrast agents. Relaxivity of Gd-DTPA-MP incorporated within phytantriol and phytanylethanolamide nanostructured particles are also listed in Table 2. The longitudinal relaxivity of all these nanostructured particles were several fold higher than that of Magnevist. Incorporation of 1% of Gd-DTPA-MP within cubsomes of phytantriol had the highest Pt value.

**Example 2: Ln oleates**
This example examines the behaviour of eight lanthanide (III) oleates, their chelation and self-assembly structures, using the same characterisation methods described in the previous examples, except that the SAXS experiments were performed using an X-Ray beamline of the Advanced Photon Source, Argonne, Illinois.

5 Example 2(a): Synthesis

The synthesis of lanthide (such as La, Ce, Nd, Sm, Eu, Gd, Tb and Dy) oleates was based on a two-step precipitation method as shown in the following equations:

\[
C_{17}H_{33} - c OOH + NaOH \rightarrow C_{17}H_{33} - coo^-Na^+ + H_2O
\]

\[
3C_{17}H_{33} - COO^-Na^+ + LnCl_3 \rightarrow (C_{17}H_{33} - COO^-)_3Ln^{3+} + 3NaCl
\]

Oleic acid (1.448 g, 5.1 mmol) was dissolved in 1:1 ethanol/water (20 ml) with stirring at 50°C in a water bath. To the stirring oleic acid solution, an equivalent amount of 0.5 M NaOH was added dropwise to convert the oleic acid to Na oleate. The mixture was stirred for an additional 30 min at 50°C. The solution of Na oleate solution was then added dropwise with stirring to the solution of lanthanide chloride hexahydrate (1.8 mmol), which was dissolved in 1:1 ethanol/water (20 ml), at room temperature. The addition of Na oleate solution resulted in a white precipitate. The mixture was left to stir for an additional 60 min at room temperature after the Na oleate was completely mixed. The precipitate was then filtered and washed with hot water (3 x 50 ml), cold ethanol (3 x 50 ml) and acetone (3 x 50 ml). Under the reaction conditions used here, the lanthanide oleates were typically in monohydrate or hemihydrate form with the exception of Ce oleate which is anhydrous.

Example 2 (b): Dispersion Procedures

The Ln (lanthide) oleates (Ln = Eu, Gd, Tb and Dy) were dispersed in water to form particles. First, the Ln oleates were dissolved slowly in the minimum amount of 2-
methyl-2 propanol at 60°C to form a homogenous solution. This was followed by the addition of 10 w/w% of the stabiliser Pluronic F127. The mixture of Ln oleate and F127 was added to 50 ml Milli-Q water with mixing under ultratarrax for 5 min with a speed of 15,000 rpm at 80°C. The dispersion was immediately passed through a high pressure homogeniser using pressures of 10000 psi for 6 passes at 60°C. The solution appeared milky, indicating the formation of colloidal particles. The particles were sized by dynamic light scattering measurement. The particle size distributions (D<sub>10</sub>, D<sub>50</sub>, D<sub>90</sub>) for these dispersions are provided in Table 3.

**Example 2(c): Characterisation**

The X-ray analysis showed that the neat bulk lanthanide soaps have a lamellar bilayer structure at room temperature. POM scans showed that all the bulk Ln oleates form a lamellar phase in the presence of excess water. SAXS and XRD confirmed that the bulk Ln oleates do not swell in excess water. Select bulk Ln oleates were dispersed in water to form non-swelling lamellar sub-micron colloidal particles, confirmed by DLS and SAXS measurements.

**Example 2(d): Assessment of T<sub>i</sub> and T<sub>2</sub> relaxation times**

For particle dispersions of Ln oleates, T<sub>i</sub> and T<sub>2</sub>, were measured at 20 MHz (0.47 T) at room temperature with a MINISPEC from Bruker. The data show that the Gd oleate dispersion significantly reduces the relaxation time (T<sub>i</sub> and T<sub>2</sub>) relative to pure water (T<sub>i</sub> = T<sub>2</sub> = 2 s), and that the relaxation rate increases linearly with the concentration of Gd oleate. Tb and Dy oleate, whilst significantly reducing the transverse proton relaxation time, have very long longitudinal relaxation times. Although the longitudinal relaxivity of Gd oleate is much higher than that of the other Ln oleates, it is slightly lower than that of Magnevist under the same conditions. The transverse relaxivity of Gd oleate is very similar to that of Tb oleate, Dy oleate and Magnevist. Both the longitudinal and transverse relaxivities for Eu oleate, which is not paramagnetic, are very small. The relatively high relaxivities of Gd, Tb and Dy oleates, comparable to those of the commercially available agent, Magnevist, combined with their ability to form sub-micron
colloidal particles exemplifies the potential of this form of ordered non-swelling, nanostructured self-assembly colloids as MRI contrast agents.

**Example 2 (e): Luminescent Behaviour of Europium (III) Oleate**

Fluorescence excitation and emission spectra for Eu oleate particle dispersions were measured on a Perkin-Elmer Model LS-50B fluorimeter (cut-off filter: 430 nm, slit width: 10 nm). The optical time-resolved properties of Eu oleate particle dispersions were investigated by a time-resolved fluorimeter (PHERAsstar, BMGLABTECH) with excitation at 300 nm and detection of emission at 620 nm. Measurements were performed in 96-well microliter plates. Background counts were 500-600, and were subtracted from sample readings. The time-resolved fluorescence measurements as plotted in Figure 20 demonstrate that colloidal dispersions of Eu(III) oleate exhibit strong luminescence. Thus these rare earth metal soaps exemplify the potential of self-assembled chelating amphiphiles as contrast agents in medical imaging modalities such as magnetic resonance imaging (MRI) as well as in fluorescence imaging.

**Example 2(f): Incorporation of chelating amphiphiles with non-chelating amphiphile colloidal particles**

In this example Gd oleate is added to an inverse bicontinuous cubic phase forming system to investigate the potential of these dual systems to act as magnetic MRI contrast agents.

DSC, SAXS and Cryo-TEM measurements of the Gd oleate/Myverol systems indicate that Gd oleate is at least partially incorporated within the bicontinuous cubic phase of Myverol. However at Gd oleate concentrations greater than 1 weight % partial phase separation of the system may occur with formation of a Gd oleate rich lamellar phase as well as the bicontinuous cubic phase. Bulk Gd oleate/Myverol mixtures can be dispersed into stable colloidal dispersions. SAXS and Cryo-TEM measurements of these dispersions indicate that the presence of Gd oleate in the Myverol system prevents the formation of cubosomes from the bulk cubic phase. Instead the dispersion consists of putative Gd-oleate-rich non-swelling lamellar particles as well as colloidal...
particles lacking ordered internal structure. The proton relaxivities $r_1$ and $r_2$ of Myverol dispersion containing Gd-oleate were determined and are listed in Table 4.

The proton relaxivities, $r_1$ and $r_2$, of phytantriol dispersions containing Gd, Tb, Dy and Eu phytanate at 10 weight % were also determined and are listed in Table 5 (in brackets). Incorporation within a cubosome seems to have had negligible effect on the transverse relaxivity. In contrast a substantial increase in longitudinal relaxivity was observed for all samples and, for 10 wt% Gd phytanate in phytantriol, the longitudinal relaxivity measured is higher than that of the commercial contrast agent. A similar increase in relaxivity was observed for Gd oleate incorporated within Myverol cubosomes, see Example 4. Incorporation within a bicontinuous cubic phase, which has both a high surface area and continuous water networks, may facilitate the co-ordination of more water molecules around the paramagnetic ion, increasing the inner-sphere relaxivity. The bicontinuous cubic phase may also partially impede the rotational movement of the metal ion, adding to the increase in relaxation rate. Note the effect of incorporation within a bicontinuous cubic phase is much more pronounced for the Ln oleates than for the phytanates studied here.
Example 3: Lanthide metal phytanates

Example 3(a): Synthesis

Synthesis of phytanic acid

Oxidation of phytanol was carried out by dissolving 30 g of phytanol in 60 ml. acetic acid and 1200 ml. acetone. A solution of 24 g chromium trioxide dissolved in 30 ml. water was added dropwise to the above solution, over an ice bath. After the addition was complete, the mixture was stirred at room for 90 min and then the reaction was allowed to proceed to completion overnight. Thereafter, 500 ml. water was added, followed by powdered sodium bisulfite to quench the oxidation. The precipitated chromium salts were filtered off using a Buchner funnel and a Whatman #4 filter paper and the solvent evaporated. The predominantly acetone mixture that first evaporated was used to wash the chromium salts. The sticky material obtained after the evaporation was suspended in 400 ml. water and extracted (overnight) into 500 ml. ether, thereby removing most of the chromium salts. The aqueous layer was washed with ether several times and added to the product and the solvent evaporated. After workup and distillation, about 20 g of pure phytanic acid was collected.

Synthesis of lanthanide salts of phytanic acid

The synthesis of the metal soaps was achieved via double decomposition. Briefly, addition of NaOH to obtain the sodium soap of the fatty acid is followed by double decomposition with the lanthanide/transition metal salt (hexahydrated, either a lanthanide chloride or a nitrate). Zn, La, Ce, Nd, Sm, Eu, Gd, Tb and Dy salts were synthesised. Subsequently, the product was washed with water, ethanol and acetone. Thereafter, the dried product was recrystallised from pentanol: water (5:1) before the final freeze drying. The zinc soap formed a white dispersion with very fine particles, which was separated out by filtration having added excess zinc to promote aggregation. The zinc soap was washed with water, ethanol and acetone. It was soluble in acetone and fairly soluble in ethanol. Unlike the other lanthanide soaps, zinc phytanate failed to recrystallise from 1-pentanol and hence the solvent was evaporated to recover the
metal soap before freeze drying. The zinc soap is a viscous/oily liquid at room 
temperature and forms solid white particles at -20°C. This is in contrast to Gd phytanate 
which has a melting point at approximately 32°C and appears glassy (almost 
transparent).

5 Example 3(b): Characterisation

Characterisation of the bulk phases of the above materials was carried as before, 
except for the SAXS experiments which were carried out using the SOL beamline at 
Imperial College London. X-Rays were produced using a Phillips PW2213/20 generator 
operating at 40 kV and 30 mA through an AEG type 50/21 X-ray tube.

Several of the salts form a liquid crystalline hexagonal columnar mesophase at room 
temperature in the presence of water, and Sm phytanate forms this phase even when dry. SAXS experiments following prolonged equilibration in excess water over a period 
of at least one month showed that Sm, Eu and Tb phytanate contain distinct diffraction 
peaks characteristic of a hexagonal columnar phase. Although Nd phytanate and Dy 
phytanate displayed broadened diffraction peaks, these were still in a ratio consistent 
with the formation of a hexagonal phase. The remaining Ln phytanate samples (Ln = 
La, Ce and Gd) displayed one broad peak roughly coincident with the first order 
reflection of the more ordered hexagonal phases. These results suggest that whilst 
some water has been taken up by these samples, they are virtually non-swelling in 
water.

Example 3(c): Dispersion into colloidal particles

The dispersion of these bulk phases into colloidal particles was carried out by mixing 
the material in water to form particles. These were then dissolved in phytantriol (with the 
addition of CHCl₃) at 60°C and later evaporated off CHCl₃ using a rotor vapour. This 
was further dried under a freeze dryer overnight. 500 mg of this solution (50 mg of the 
soap sample) was dissolved in 49.5 mL water (containing 50 mg of F127 stabiliser). The 
mixture was homogenised under ultratarrax for 5 min with a speed of 15 000 - 20 000 
rpm at 80°C and immediately passed through a high pressure homogeniser using
pressures of 10000 psi for 4-5 passes at 60°C. All resulting solutions were milky in appearance indicating the formation of colloidal particles. The particles were sized by dynamic light scattering measurement as previously described. The results are given in Table 5.

5 Example 3(d): Relaxivity

As before, the relaxivity of these colloidal particles were measured at 20 MHz (0.47 T) and room temperature with a MINISPEC from Bruker.

Gd phytanate displays a transverse relaxivity similar to that of Magnevist, although it has a lower longitudinal relaxivity. A plot of proton longitudinal and transverse relaxivity ($r_1$ and $r_2$) at 20 MHz and at room temperature versus the concentration of Gd-phytanate is shown in Figure 21. In contrast, Tb and Dy phytanate display relaxivity values, both longitudinal and transverse, considerably below those of the commercial agent. Eu phytanate, which is not paramagnetic, has very low relaxivity values as expected. The relatively high transverse relaxivity of Gd phytanate, combined with its ability to form dispersed particles of hexasomes, suggests its potential as a MRI contrast agent.

The proton relaxivities, $r_1$ and $r_2$, of Gd, Tb, Dy and Eu phytanate incorporated into phytantriol at 10 weight % were also determined and are listed in Table 5 (in brackets). A substantial increase in longitudinal relaxivity was observed for all samples and for 10 wt% Gd phytanate in phytantriol, the longitudinal relaxivity measured is higher than that of the commercial contrast agent. A similar increase in relaxivity was observed for Gd oleate incorporated within Myverol cubosomes, see Example 2. Incorporation within a bicontinuous cubic phase, which has both a high surface area and continuous water networks, may facilitate the co-ordination of more water molecules around the paramagnetic ion, increasing the inner-sphere relaxivity. The cubic phase may also partially impede the rotational movement of the metal ion, adding to the increase in relaxation rate.

Example 4: Transmetalation reactions, stability
Kinetic and thermodynamic stability of the Gd complexes are very important factors in determining their stability \textit{in vitro} and \textit{in vivo}. Predicting the amount of the free gadolinium which might form by transmetalation of Gd-contrast agent and formation of chelates of endogenous cations such as zinc, calcium, copper and iron, is very important for determining the release of the free Gd ions, which are very toxic to the body. Among the endogenous cations, zinc is the prevalent cation for replacement of Gd due to the high concentration of zinc in the blood. Copper concentration is relatively low in the blood serum and calcium has less affinity to organic ligands. Iron is also relatively protected by proteins and is not readily available for transmetalation. The transmetalation of Gd-contrast agent with the zinc cation can produce zinc chelates which are excreted from urine. However, releasing Gd ions will generate the toxic Gd ions that can be deposited on tissues in the form of salts with phosphate, citrate and other endogenous anions. Among the currents agents available for clinical use, there are significant differences in the stability and liability in releasing toxic Gd ions between the linear contrast agent and macrocyclic contrast agents. Gd-DTPA-MP according to the present invention is an ionic micellar particle and Gd-DTPA-BP is a non-ionic liposomal nanoparticle. The effect of zinc cation \textit{in vitro} on the stability of these Gd-complexed chelating amphiphiles was examined by measuring their relaxivity before and after addition of the zinc cation by using low field NMR. In addition, the complex suspension of each conjugate was tested for their molecular mass by MS/ESI, followed by the exchange of the Gd from chelating head groups with zinc, calcium and magnesium. The molecular mass of the Gd-complex and any changes in molecular mass by addition of zinc, copper or magnesium were further followed up to determine the transmetalation at molecular level. This method has limitations with the complexed molecules which were neutrally charged which demonstrate only very weak or nearly no signal by MS/ESI such as Gd-DTPA-BP.

DTPA-MP and DTPA-BP particles were dispersed in 100 mM sodium acetate solution and were made at 20 mM concentration of the amphiphile. Gd acetate solutions were added to the amphiphile dispersions in stoichiometric ratios of 1/2 and 1/1 for the DTPA-BP and DTPA-MP respectively. Owing to the non-ionic property of Gd-DTPA-BP, and based on the knowledge that DTPA-BP forms mostly liposomal particles, a lesser amount of Gd ions was used to keep the particles charged and prevent precipitation of
the dispersion. Partial complexation of the DTP-BP also invokes an increase in the stability of the complex. In general, complexes with overall negative charges are reported to be more stable than neutral complexes and often an excess amount of chelating agents are applied to improve the stability of the neutral complexes. Therefore, by incorporating less Gd to the DTPA-BP complexes, the existence of free Gd is minimised and on the other hand, due to the complexation of some of the Gd ions to the inner layer of the amphiphile in liposomal particles, the outer layers maintains some of its head groups uncomplexed and charged, which in fact facilitates stability of the particles. The Ti and T2 of these samples were measured before addition of zinc chloride solution, which is shown in Table 6.

It should be noted that all the measurements were performed at 37 °C and the first analysis were examined after 2 hours of addition of zinc ions to the suspension. The relaxivity of each suspension was also measured after 3 days and the results are shown in the brackets. For comparison, the relaxivity of the Magnevist was measured simultaneously as the reference material.

The relaxivity values of Gd-DTPA-MP increased significantly by addition of zinc ions up to 154% in r₁ and 158% in r₂ value. The relaxivity measurement of both Gd and Gd+Zn complexed sample decreased relatively after 3 days, although there was no noticeable precipitation in both dispersions. Despite a small decrease of the relaxivity value after 3 days compared to after 2 hours, still the relaxivity value of Zn added Gd-DTPA-MP conjugate was much higher in comparison to the parent complex measurement (136% of the original value for r₁). In contrast to Gd-DTPA-MP which is a negatively charged complex, Gd-DTPA-BP (a neutrally charged complex) exhibited significant reduction of relaxivity over addition of Zn ions, which is consistent with the results obtained by other groups when using neutral complexes such as Gadodiamide.

The typical MS results, obtained by addition of zinc chloride compared with that of the original Gd complexed of DTPA-MP are shown in Figure 22. Addition of the zinc ions has induced the production of the zinc adducts with the negative ions of MS=963.67. Similarly, addition of magnesium and calcium had a similar effect on the Gd-complexed DTPA-MP inducing of Mg-Gd-DTPA-MP and Ca-Gd-DTPA-MP of MS =923.53 and
939.7. In addition, no apparent peaks related to the transmetalated complex such as Zn-DTPA-MP, Mg-DTPA-MP or Ca-DTPA-MP was detected.

4. *In situ* experiments

The relaxivity of Gd complexed with different amphiphiles was examined in sheep blood by using a Bruker Biospec 4.7 Tesla animal MRI scanner. Ti weight images of different Gd complexes are shown in Figures 23 and 24. Gd-DTPA-BP demonstrated comparable contrast intensity to that of Magnevist, despite the fact that the concentration of this amphiphile was less than Magnevist. The Gd-particles made according to the present invention have a very high payload. Accordingly, the contrast intensity of the colloidal particles in tissues may be much higher compared with the low molecular weight Magnevist and other commercial agents. The Gd complexes of DTPA amphiphiles may hold promise as contrast agents, specifically targeted at diseased tissues such as cancer.
<table>
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<th>Complex conjugate</th>
<th>$r_1$(mM$^{-1}$s$^{-1}$)</th>
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Table 1
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<td>10%</td>
<td>15.3</td>
<td>157</td>
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<td>20%</td>
<td>11.5</td>
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<td>9.7</td>
<td>213</td>
<td>0.230</td>
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<td>40%</td>
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<td>100%</td>
<td>12.0</td>
<td>123</td>
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<tr>
<td>Gd/DTPAM/ PHYTANOLETHANOLAMIDE 5%</td>
<td>10.2</td>
<td>363</td>
<td>0.344</td>
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<tr>
<td><strong>Magnevist</strong></td>
<td>3.4-3.5</td>
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**Table 2**
<table>
<thead>
<tr>
<th>Dispersions</th>
<th>Particle size (nm)</th>
<th>Relaxivity (mM$^{-1}$ s$^{-1}$)</th>
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<tbody>
<tr>
<td></td>
<td>$D_{10}$</td>
<td>$D_{50}$</td>
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<tr>
<td>Eu oleate</td>
<td>160</td>
<td>420</td>
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<tr>
<td>Gd oleate</td>
<td>280</td>
<td>530</td>
</tr>
<tr>
<td>Tb oleate</td>
<td>250</td>
<td>650</td>
</tr>
<tr>
<td>Dy oleate</td>
<td>260</td>
<td>600</td>
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<tr>
<td>Magnevist</td>
<td>-</td>
<td>-</td>
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Table 3
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<tr>
<th>% of Gd Oleate in Myverol</th>
<th>Particle size (nm)</th>
<th>Relaxivity (mM⁻¹s⁻¹)</th>
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<tr>
<td>0</td>
<td>D₁₀ 102  D₅₀ 158  D₉₀ 235</td>
<td>r₁ 15.62  r₂ 22.17</td>
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<td>0.1</td>
<td>161  291  486</td>
<td>15.62  22.17</td>
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<td>0.5</td>
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<td>20.29  26.06</td>
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<td>1</td>
<td>68   226  410</td>
<td>34.24  27.34</td>
</tr>
<tr>
<td>5</td>
<td>82   143  230</td>
<td>19.01  26.11</td>
</tr>
<tr>
<td>10</td>
<td>97   236  470</td>
<td>13.60  20.30</td>
</tr>
<tr>
<td>20</td>
<td>128  275  488</td>
<td>9.31   13.35</td>
</tr>
<tr>
<td>30</td>
<td>173  323  860</td>
<td>4.71   7.64</td>
</tr>
<tr>
<td>40</td>
<td>86   204  854</td>
<td>3.59   6.16</td>
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<td>50</td>
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<td>60</td>
<td>256  599  1473</td>
<td>1.86   4.21</td>
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<td>80</td>
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<td>1.72   3.81</td>
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<td>2.78   6.06</td>
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<td>4.91   6.26</td>
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Table 4
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<tr>
<td></td>
<td>D₁₀</td>
<td>D₅₀</td>
</tr>
<tr>
<td>Eu phytanate</td>
<td>451 (461)</td>
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<tr>
<td>Gd phytanate</td>
<td>462 (98)</td>
<td>645 (240)</td>
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<tr>
<td>Tb phytanate</td>
<td>458 (454)</td>
<td>629 (616)</td>
</tr>
<tr>
<td>Dy phytanate</td>
<td>461 (447)</td>
<td>639 (698)</td>
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<tr>
<td>Magnevist</td>
<td>-</td>
<td>-</td>
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</table>

Table 5
<table>
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<tr>
<th>Complex</th>
<th>( T_1 ) (mS)</th>
<th>( r_1 ) (mM(^{-1})s(^{-1}))</th>
<th>( T_2 ) (mS)</th>
<th>( r_2 ) (mM(^{-1})s(^{-1}))</th>
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<td>Gd-DTPA-MP (20 mM)</td>
<td>3.03</td>
<td>16.5</td>
<td>2.63</td>
<td>18.75</td>
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<tr>
<td></td>
<td>(3.670)</td>
<td>(13.63)</td>
<td>(2.093)</td>
<td>(23.58)</td>
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<tr>
<td>Zn/Gd-DTPA-MP (1/1.8)</td>
<td>2.18</td>
<td>25.42</td>
<td>1.88</td>
<td>29.80</td>
</tr>
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<td></td>
<td>(2.46)</td>
<td>(22.52)</td>
<td>(2.57)</td>
<td>(21.79)</td>
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<tr>
<td>Gd-DTPA-BP (10 mM)</td>
<td>17.83</td>
<td>5.60</td>
<td>14.71</td>
<td>6.8</td>
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<td>(17.64)</td>
<td>(5.66)</td>
<td>(13.05)</td>
<td>(7.6)</td>
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<tr>
<td>Zn/Gd-DTPA-BP (2/1)</td>
<td>38.24</td>
<td>3.15</td>
<td>46.85</td>
<td>2.57</td>
</tr>
<tr>
<td></td>
<td>(144.41)</td>
<td>(0.83)</td>
<td>(84.98)</td>
<td>(1.41)</td>
</tr>
<tr>
<td>Magnevist (500 mM)</td>
<td>0.43</td>
<td>4.7</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
1. A compound according to formula (II):

\[ \text{i} \nu \text{r}^+ \quad R_1= \text{A} \equiv R_2 \quad ("I") \]

wherein

A is a chelating headgroup that contains at least one donor atom, wherein the
head group is capable of forming a complex with a metal ion, the metal ion
selected from the group consisting of transition metal ions, lanthanide metal
ions and alkaline rare earth metals,

Ri is selected from the group consisting of a substituent according to formula (a), a
substituent according to formula (b), a substituent according to formula (c), and
a substituent according to formula (d),

\[ \text{Y} \quad (a) \quad \text{Y} \quad \text{X}_2 \quad (c) \quad \text{Y} \quad \text{X}_3 \quad (d) \]

\[ \text{Y} \quad \text{X}_1 \quad (b) \quad \text{Y} \quad \text{Y} \quad \text{Y} \quad \text{Y} \]

wherein

\( X_1 \) is a difunctional spacer group linked to a hydrophobic group Y on one end
and to the chelating head group on the other end,

\( X_2 \) is a trifunctional spacer group linked to two hydrophobic groups Y on one
end and the chelating head group on the other end,

\( X_3 \) is a tetra functional spacer group linked to three hydrophobic groups Y on
one end and the chelating head group on the other end,
wherein the functional groups are selected from carboxylic acids, alcohols, amines, thiols, halides, azides, isocyanates and isothiocyanates,

each Y is the same or different and is a hydrophobic saturated or unsaturated substituent with a linear chain length of between 10 and 30 carbon atoms selected from the group consisting of alkyl, alkenyl, alkynyl, branched alkyl, branched alkenyl and branched alkynyl, substituted alkyl, substituted alkenyl and substituted alkynyl,

R\textsubscript{2} is selected from the group consisting of a substituent according to formula (a), a substituent according to formula (b), a substituent according to formula (c), a substituent according to formula (d) or hydrogen,

M is a metal ion;

n is 1, 2 or 3;

and wherein the compound self-assembles into one or more lyotropic or thermotropic phases that are lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic.

2. A compound according to formula (Ma):

\[ M^{n+} (/V-R_1^p) \]  

wherein

A’ is a carboxyl group;

p is 1, 2 or 3;

R\textsubscript{i}, M and n are as defined in claim 1,
and wherein the compound self-assembles into one or more lyotropic or thermotropic phases that are lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic.

3. A compound according to formula (I):

\[
R_1-A-R_2 \quad (I)
\]

wherein

- **A** is a chelating headgroup that contains at least one donor atom, wherein the head group is capable of forming a complex with a metal ion, the metal ion selected from the group consisting of transition metal ions, lanthanide metal ions and alkaline rare earth metals.

- **R₁** is selected from the group consisting of a substituent according to formula (a), a substituent according to formula (b), a substituent according to formula (c), and a substituent according to formula (d),

\[
\begin{align*}
Y - (a) & \quad Y - (c) & \quad Y - (d) \\
Y - X_1 - (b) & \quad Y & \quad Y \\
\end{align*}
\]

wherein

- **X₁** is a difunctional spacer group linked to a hydrophobic group Y on one end and to the chelating head group on the other end,

- **X₂** is a trifunctional spacer group linked to two hydrophobic groups Y on one end and the chelating head group on the other end,
$X_3$ is a tetra functional spacer group linked to three hydrophobic groups $Y$ on one end and the chelating head group on the other end,

wherein the functional groups are selected from carboxylic acids, alcohols, amines, thiols, halides, azides, isocyanates and isothiocyanates,

each $Y$ is the same or different and is a hydrophobic saturated or unsaturated substituent with a linear chain length of between 10 and 30 carbon atoms selected from the group consisting of alkyl, alkenyl, alkynyl, branched alkyl, branched alkenyl and branched alkynyl, substituted alkyl, substituted alkenyl and substituted alkynyl,

$R_2$ is selected from the group consisting of a substituent according to formula (a), a substituent according to formula (b), a substituent according to formula (c), a substituent according to formula (d) and hydrogen,

and wherein the compound self-assembles into one or more lyotropic or thermotropic phases that are lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic.


6. A self-assembled structure of one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT and Gd-DTPA-BT, in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase.
7. A dispersion of submicron- or nano-particles of a self-assembled structure of one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT and Gd-DTPA-BT, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

8. A compound selected from the group consisting of Gd-oleate and Gd-phytanate.

9. A diagnostic imaging composition including an effective amount of a compound according to any one of claims 1, 2 or 5 together with at least one pharmaceutically acceptable carrier, excipient, diluent, additive or vehicle.

10. A self-assembled structure of a non-chelating amphiphile and one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT and Gd-DTPA-BT in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase.

11. A composition including a dispersion of submicron- and nano-particles of a self-assembled structure of a non-chelating amphiphile and one or more compounds selected from the group consisting of Mn-EDTA-MO, Mn-EDTA-BO, Mn-EDTA-MP, Mn-EDTA-BP, Gd-EDTA-MP, Gd-DTPA-MP, Gd-DTPA-BP, Gd-DTPA-MO, Gd-DTPA-BO, Gd-DTPA-MT, Gd-DTPA-BT, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

12. A self-assembled structure of one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase.

13. A dispersion of submicron- and nano-particles of self-assembled structure of one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.
14. A self-assembled structure of a non-chelating amphiphile and one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate in the form of a lamellar, inverse hexagonal, inverse bicontinuous cubic or inverse micellar cubic phase.

15. A composition including a dispersion of submicron- and nano-particles of a self-assembled structure of a non-chelating amphiphile and one or more compounds selected from the group consisting of Gd-oleate and Gd-phytanate, the particles being liposomes, inverse hexosomes, inverse cubosomes or inverse micellar cubosomes.

16. A composition according to claim 11 or 15 in which the non-chelating amphiphile is one or more of myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide.

17. A self-assembled structure according to claim 10 or 14 in which the non-chelating amphiphile is one or more of myverol, phytantriol, oleoylethanolamide and phytanoylethanolamide.

18. A diagnostic imaging composition including an effective amount of:

- a self-assembled structure according to any one of claims 6, 10, 12, 14 or 17,
- a dispersion of particles according to any one of claims 7 or 13, or
- a composition according to any one of claims 11, 15 or 16,

together with at least one pharmaceutically acceptable carrier, excipient, diluent, additive or vehicle.
FIGURE 5

Neat amphiphile

Lα

100 mM Na-Acetate
FIGURE 6

(c) Intensity (a.u.)

(d) Intensity (a.u.)

- 50°C
- 45°C
- 40°C
- 37°C
- 30°C
- 25°C

- hydrated with 200mMNa-Acetate
- hydrated with 100mMNa-Acetate
- hydrated with water
- neat amphiphile

q(Å⁻¹)
FIGURE 8

(a)

Intensity (a.u.)

$q (\text{Å}^{-1})$

(b)

Intensity (a.u.)

$q (\text{Å}^{-1})$
FIGURE 8

(c) 50°C  
45°C  
40°C  
37°C  
30°C  
25°C

(d) 1M Na-Acetate  
500mM Na-Acetate  
100mM Na-Acetate

Intensity (a.u.)

q(Å⁻¹)

Intensity (a.u.)

q(Å⁻¹)
FIGURE 13

(a)

Intensity (a.u.)

$q(\text{Å}^{-1})$

(b)

Intensity (a.u.)

$q(\text{Å}^{-1})$
FIGURE 15

(a)

(b)

(c)
FIGURE 21

$r_1 = 2.40 \text{ mM}^{-1}\text{s}^{-1}$

$1/T_1/s$

$r_2 = 5.38 \text{ mM}^{-1}\text{s}^{-1}$

$1/T_2/s^{-1}$

[Service Phytanate] (mM)
# INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/AU20 10/000672

---

### A. CLASSIFICATION OF SUBJECT MATTER

<table>
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<td>A61K 47/22 (2006.01) C07C 53/126 (2006.01)</td>
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<td>A61K 49/10 (2006.01) C07D 311/72 (2006.01)</td>
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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)

Electronic database consulted during the international search (name of data base and, where practicable, search terms used)

Chemical Abstracts registry file - structure search based on the compounds of claims 4-7 and 10-11.

Medline, Chemical Abstracts, BIOSIS, WPI/DS - keywords: chelat?, ((imaging or contrast or diagnostic) (w) agent), contrast media, amphiphil?, amphipathic?, surface active agent, oleate?, oleic?, phytanic?, phytanate?, self-assembl?

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>Takeshita T. et al. &quot;Synthesis of EDTA-Monoalkyl Ester Chelates and Evaluation of the Surface Active Properties&quot; Journal of the American Oil Chemists' Society (1980) 57(12): 430-434 See page 430, left-hand column, last paragraph; Figure 2; Table 1; Title; Abstract</td>
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Chemical Abstracts Plus accession number 1983:615349 & CAS Registry File RN 87926-87-2 See whole abstract 1, 3, 4

Further documents are listed in the continuation of Box C

See patent family annex

---

Date of the actual completion of the international search

23 August 2010

Date of mailing of the international search report

8 SEP 2010

Name and mailing address of the ISA/AU

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Authorized officer

ANDREW BRYCE

AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No: +61 2 6283 3 132

Form PCT/ISA/210 (second sheet) (July 2009)
## INTERNATIONAL SEARCH REPORT

### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>WO 2010/060131 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION), 3 June 2010</td>
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A | See abstract; Figure 2; Page 377, left-hand column, first paragraph |


A | See page 144, left-hand column |


A | See Figure 1 |


A | See abstract |


A | See Examples pages 113-19 |


A | See page 7, lines 21-25; Page 9, lines 21-30; Page 19, lines 15-23 |


A | See abstract; Figures 1, 9 and 10; Page 6643, left-hand column |

Form PCT/ISA/2 10 (continuation of second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- See Supplemental Box

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos:
   1, 3-7, 9-11 and 16-18, as far as they relate to the specific compounds defined in claims 4-7 and 10-11.

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

□ No protest accompanied the payment of additional search fees.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (July 2009)
The International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This International Searching Authority has found that there are different inventions as follows:

1. Claims 1, 3-7, 9-11 and 16-18 (in part). It is considered that compounds of formulas (II) and (I) where moiety A is derived from EDTA, comprise a first distinguishing feature.

2. Claims 1, 3-7, 9-11 and 16-18 (in part). It is considered that compounds of formulas (II) and (I) where moiety A is derived from DTPA, comprise a second distinguishing feature.

3. Claims 2, 8, 9 and 12-18 (in part). It is considered that compounds of formula (Ha) where A' is a carboxyl group and Y is an oleate moiety, comprise a third distinguishing feature.

4. Claims 2, 8, 9 and 12-18 (in part). It is considered that compounds of formula (Ha) where A' is a carboxyl group and Y is a phytanate moiety, comprise a fourth distinguishing feature.

5. Claims 1, 3 and 9 (in part). It is considered that compounds of formulas (II) and (I), where moiety A is a chelating headgroup other than those derived from EDTA or DTPA, comprise a fifth distinguishing feature.

6. Claims 2 and 9 (in part). It is considered that compounds of formula (Ha) where A' is a carboxyl group and Y is a hydrophobic substituent other than an oleate or a phytanate, comprise a sixth distinguishing feature.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The only feature common to all of the claims is that they relate to compounds that are amphiphilic, capable of binding to a metal, and can self-assemble. However this concept is not novel in the light of:

- Mulder W.J.M. et al. "A Liposomal System for Contrast-Enhanced Magnetic Resonance Imaging of Molecular Targets" Bioconjugate Chemistry (2004) volume 15, pages 799-806, see Figure 1 which discloses a liposome comprising Gd-DTP A-bis(stearylamide)


- Mulder W. J. M. et al. "Lipid-based nanoparticles for contrast-enhanced MRI and molecular imaging" NMR in Biomedicine (2006) 19: 142-164, which discloses the self-assembly of amphiphilic molecules into well defined structures, including cubic, lamellar and hexagonal phases (see page 144, left-hand column).

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art. Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention a posteriori.

The claims have been searched for inventions 1 and 2 (claims 1, 3-7, 9-11 and 16-18), as far as they relate to the specific compounds defined in claims 4-7 and 10-11.
This Annex lists the known "A" publication level 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.

<table>
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX