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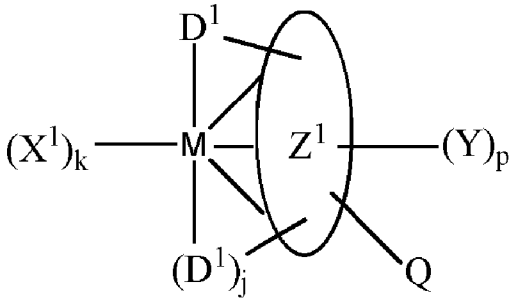


(I)

(57) Abstract: The present invention relates to a method of labelling biological molecules with ^{18}F, via attachment of fluorine to a metal complex, where the metal complex is conjugated to the biological molecule. The invention highlights the incorporation of hydrogen bonding (H- bonding) into the metal complex scaffold, and how this can be utilised to improve the kinetics of fluoride incorporation. Also provided are pharmaceutical compositions, kits and methods of in vivo imaging using compounds of Formula (I).

WO 2014/177690 A1

Metal Complexes and their Fluorination.

Field of the Invention.

The present invention relates to a method of labelling biological molecules with ^{18}F, *via* attachment of fluorine to a metal complex, where the metal complex is conjugated to the biological molecule. The invention highlights the incorporation of hydrogen bonding (H-bonding) into the metal complex scaffold, and how this can be utilised to improve the kinetics of fluoride incorporation. Also provided are pharmaceutical compositions, kits and methods of *in vivo* imaging.

Background to the Invention.

The ^{18}F radiolabelling of biological molecules, to obtain radiotracers suitable for *in vivo* imaging is an area of continued interest [Schirrmacher *et al*. Mini-Rev.Org.Chem., 4(4), 317-329 (2007)]. Whilst there are many methods for direct (single-step) labelling of small molecules with ^{18}F, these methods are generally not suitable for application to peptides (and larger macromolecules). The presence of amino acids such as lysine and arginine make standard strategies of incorporation of fluoride *via* nucleophilic substitution difficult, due to:

(i) hydrogen bonding interactions between the fluoride and these amino acid functionalities, thus reducing the nucleophilicity of the fluoride ion; and/or

(ii) the requirement to use higher temperatures which can cause the degradation or disruption of the peptide/protein structure.

Inorganic chemistry approaches to improved radiofluorination methods have been reviewed by Smith *et al* [Dalton Trans., 40, 6196-6205 (2011)].

WO 2009/079024 (McBride *et al*) discloses an 'inorganic' method of labeling a molecule with ^{18}F comprising:

a) reacting the ^{18}F with a metal to form an ^{18}F metal complex; and

b) attaching the ^{18}F metal complex to a molecule to form one or more ^{18}F labeled molecules to be administered to a subject.

WO 2009/079024 teaches that suitable metals for the metal complex are selected from aluminium, gallium, indium, lutetium and thallium.

Example 3 of WO 2009/079024 provides the ^{18}F-labelling of various metal complexes of the chelate-peptide conjugate IMP 272:

DOTA-Gln-Ala-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2

IMP 272

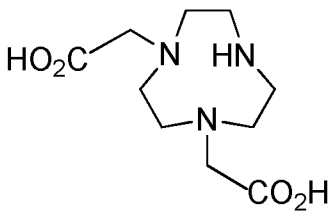
where DOTA = 1,4,7,10-tetraazacyclododecanetetraacetic acid,
HSG = the histamine succinyl glycyl group.

The ^{18}F-radiolabelling results reported were: indium (24%), gallium (36%), zirconium (15%), lutetium (37%) and yttrium (2%).

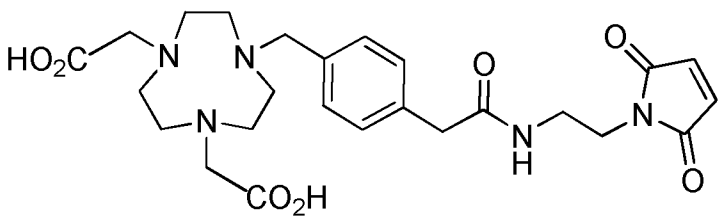
WO 2011/068965 discloses a method of labeling a molecule with ^{18}F or ^{19}F comprising attaching a complex of ^{18}F or ^{19}F and a group IIIA metal to a chelating moiety, wherein the chelating moiety is conjugated to the molecule or the chelating moiety is later attached to the molecule. WO 2011/068965 states that the metals of group IIIA (aluminium, gallium, indium, and thallium) are suitable for F binding, but that aluminium is preferred.

McBride *et al* subsequently reported [J.Nucl.Med., 50(6), 991-998 (2009) at page 994] that Ga, In, Zr, Lu and Y do not bind the IMP 272 peptide as well as the aluminium complex, and that the metal complexes of the alternative metals (Ga, In, Zr, Lu and Y) were unstable in water.

More recent publications have focused on aluminium as the metal of choice since the aluminium-fluoride bond is one of the strongest metal-fluoride bonds, and the AlF_n complex is stable *in vivo* – and optimizing the aluminium chelator used [McBride *et al*, Bioconj.Chem., 21(7), 1331-1340 (2010); Bioconj.Chem., 22, 1793-1803 (2011) and Appl.Rad.Isot., 70, 200-204 (2012)]. Preferred chelators are based on the NODA system, with NODA-MPAEM used to conjugate to biomolecules:



NODA



NODA-MPAEM

The prior art methods of WO 2009/079024, WO 2011/068965 and associated publications do, however have some disadvantages:

(a) the kinetics of formation of the Al-^{18}F bond requires the use of higher temperatures for ^{18}F-radiolabelling, and many biomolecules are temperature-sensitive;

(b) the pH range (pH 3.8 to 4.2) for ^{18}F-radiolabelling these metal complexes is relatively narrow, due to the need to avoid hydrolysis of the aluminium. This will not be compatible with all biomolecules due to acid-sensitive instability or risks of aggregation.

There is therefore still a need for alternative ^{18}F-radiolabelling methods which permit efficient radiofluorination of a range of biological molecules, under mild conditions (of e.g. temperature and pH). Ideally such methods are suitable for aqueous conditions - since ^{18}F is typically available as an aqueous solution and some biomolecules may not tolerate organic solvents. The capability of performing the labelling in aqueous or predominantly aqueous conditions, will eliminate the requirement to dry the [^{18}F] fluoride, which is typically required for traditional ^{18}F chemistries involving "nucleophilic substitution". This has the benefit that it may further simplify the ^{18}F radiofluorination chemistry *via* a reduction of process steps. Reduction of process steps and in particular, the reduction of the radiosynthesis time has benefit in minimising loss of yield due to radioactive decay.

The Present Invention.

The present invention provides a versatile method for radiolabelling biomolecules, and in particular peptides:

(i) at lower temperatures (preferably room temperature);

(ii) in aqueous (or predominantly aqueous) conditions;

(iii) in a pH range which can be adjusted or adapted to match the properties of the biomolecule/peptide;

(iv) where the ^{18}F-labelled agents exhibit high *in vivo* stability.

The present inventors have found that both the choice of metal ion and chelate scaffold are critical in the design of high affinity fluoride binders. The metal ions and the metal complexes of the present invention have several advantages:

(a) the metal ion exhibits a high affinity for fluoride in water and at medium pH;

(b) the metal centre has a preferred coordination number and limited redox ability - which simplifies the speciation and chemistry;

(c) the kinetics of substitution of the ligand being replaced by fluoride ion are fast enough (and sufficiently complete) to take up fluoride in the time available (based upon the half-life of ^{18}F), but the resulting metal fluoride bond is sufficiently strong that metal-bound fluoride is not easily lost in purification or *in vivo*;

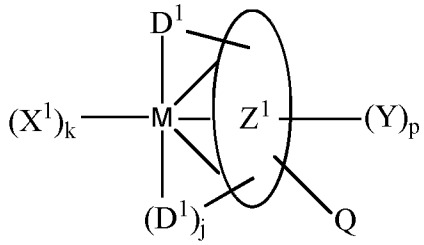
(d) the precursor for ^{18}F labelling is a single, well-defined species which can be readily synthesized and purified.

The above characteristics of the metal complexes of the present invention mean that the metal complex of interest can be conjugated to the biological targeting moiety, and purified as necessary before the ^{18}F-radiofluorination step. That is advantageous over prior art approaches for the reasons described above.

Without wishing to be constrained by theory, the present inventors believe that the pendant Y^1 group of the non-radioactive precursor metal complex of Formula (II) will have increased affinity for fluoride compared to the corresponding unfunctionalised (i.e. lacking a pendant Y^1 group) precursor metal complex. The functional group Y^1 contains a hydrogen bond donor group that is expected to facilitate the approach of fluoride ion to the metal, thus increasing rates of [^{18}F]-fluoride uptake – and ultimately producing faster incorporation of ^{18}F. That in turn provides more efficient ^{18}F-radiolabelling of biomolecules.

Detailed Description of the Invention.

In a first aspect, the present invention provides an imaging agent which comprises an ^{18}F-labelled compound of Formula I:



(I)

where:

X^1 is independently Br, Cl, ^{19}F or ^{18}F,

with the proviso that at least one X^1 is ^{18}F;

M is Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+};

Z^1 is a chelating agent having a donor set of 3 amine donors and one or two D^1 groups, wherein all 3 amine donors and the donor atom(s) of D^1 are bound to M,

wherein Z^1 has at least one Y group, and also a Q group covalently conjugated thereto;

Y is independently $-(A^1)_x-Y^1$ or $-(A^1)_x-Y^1-Q$;

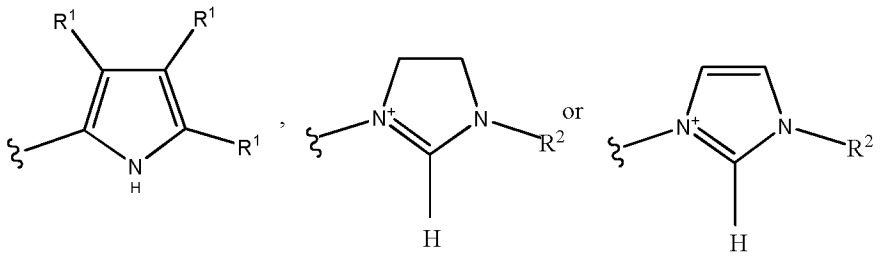
each D^1 is independently a group of formula $-(A^2)_p-D$, where D is a metal coordinating coordinating group chosen from $-CO_2H$, -OH, -SH, $-PO_3H_2$ or C_{2-8} nitrogen-containing heteroaryl;

each A^1 is independently $-CH_2-$ or –O-, and each A^2 is independently an A^1 group or $-C_6H_4-$ provided that neither Y nor D^1 comprises any -O-O- bonds;

Y^1 is $-NHR^a$, $-NH(CH_2)_2NHR^a$, $-NH(CH_2)_3NHR^a$, $-(C=O)NHR^a$,

$-NH(C=O)R^a$, $-NH(C=NH)NHR^a$, $-OR^a$, a Y^2 group or a Y^3 group;

Y^2 is:



Y^3 is Arg, Lys, Asn, Gln, Ser, Thr or Tyr;

wherein R^a is independently H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

and wherein each R^1 is independently C_{1-4} alkyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

and R^2 is independently H, C_{1-4} alkyl or $Si(C_{1-4}$ alkyl$)_3$;

j is 0 or 1;

k is (2-j);

each p is independently 1, 2 or 3;

x is an integer of value 1 to 6;

Q is –L-[BTM];

L is a synthetic linker group of formula $–(A)_m-$ wherein each A is independently – CR_2-,-CR=CR- , -C≡C- , $-CR_2CO_2-$, $-CO_2CR_2-$, -NRCO- , -CONR- , -CR=N-O-, -NR(C=O)NR-, -NR(C=S)NR-, $-SO_2NR-$, $-NRSO_2-$, $-CR_2OCR_2-$, $-CR_2SCR_2-$, $-CR_2NRCR_2-$, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, -Ar-, -NR-Ar-, -O-Ar-, -Ar-(CO)-, an amino acid, a sugar or a monodisperse polyethyleneglycol (PEG) building block,

wherein each R is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

m is an integer of value 1 to 20;

each Ar is independently a C_{5-12} arylene group, or a C_{3-12} heteroarylene group;

BTM is a biological targeting moiety.

The imaging agents of Formula I of the first aspect comprise a metal complex of a trivalent metal ion (M), i.e. where the metal is in the M(III) oxidation state (M^{3+}). By the term "metal complex" is meant a coordination complex of a metal. Suitable such metal complexes comprise the chelating agent, Z^1. The metal complex of Formula I thus comprises the following donor atoms covalently bound to M, either:

(i) a pentadentate chelating agent having the 3 amine donors of the chelating agent Z^1 and 2 D^1 groups (j =1), plus one halogen (X^1) group; or

(ii) a tetradentatate chelating agent having the 3 amine donors of the chelating agent Z^1 and one D^1 group (j = 0), plus two halogen (X^1) groups.

Suitable metals of the invention (M) include aluminium, gallium, indium, scandium, yttrium, holmium, erbium, terbium, ytterbium or lutetium.

By the term "imaging agent" is meant a compound suitable for imaging the mammalian body. Preferably, the mammal is an intact mammalian body *in vivo*, and is more preferably a human subject. Such imaging agents are designed to have minimal pharmacological effect on the mammalian subject to be imaged. Preferably, the imaging agent can be administered to the mammalian body in a minimally invasive manner, i.e. without a substantial health risk to the mammalian subject when carried out under professional medical expertise. Such minimally invasive administration is preferably intravenous administration into a peripheral vein of said subject, without the need for local or general anaesthetic.

The term "*in vivo* imaging" as used herein refers to those techniques that non-invasively produce images of all or part of an internal aspect of a mammalian subject. A preferred imaging technique of the present invention is positron emission tomography (PET).

By the term "C_{2-8} nitrogen-containing heteroaryl" is meant a heterocyclic aryl ring of 2 to 8 carbon atoms, containing at least one nitrogen (N) heteroatom. Suitable such rings include imidazole (e.g. 2-imidazole or 4-imidazole), benzimidazole or triazole. Preferred such rings incorporate at least two heteroatoms. Preferably, all the heteroatoms are *N* heteroatoms, such that more preferred such rings comprise at least 2 *N* heteroatoms, and no other heteraotoms.

The terms "comprising" or "comprises" have their conventional meaning throughout this application and imply that the agent or composition must have the essential features or components listed, but that others may be present in addition. The term 'comprising' includes as a preferred subset "consisting essentially of" which means that the composition has the components listed without other features or components` being present.

By the term "biological targeting moiety" (BTM) is meant a compound which, after administration, is taken up selectively or localises at a particular site of the mammalian body *in vivo*. Such sites may for example be implicated in a particular disease state or be indicative of how an organ or metabolic process is functioning.

In Formula I, the Y, D^1 and/or Q groups are conjugated to either the backbone of the chelating agent (Z^1), or to the N donor atoms of Z^1. When Y is $-(A^1)_x-Y^1-Q$, that means that the BTM and the Y^1 group are attached as part of the same substituent on Z^1.

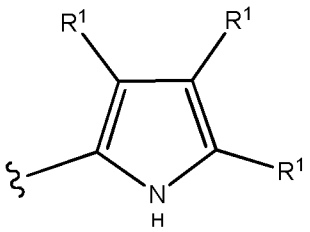
Preferred embodiments.

The D^1 group(s) is preferably located as an *N*-substituent on the amine donor atom(s) of Z^1. D^1 is preferably an anionic metal donor. D^1 is preferably $-(CH_2)_{p-1}-C_6H_4-OH$, $-(CH_2)_{p-1}-C_6H_4-SH$, $-(CH_2)_pCO_2H$ or $-(CH_2)_pPO_3H_2$, more preferably $-(CH_2)_pCO_2H$ and is most preferably $-(CH_2)CO_2H$.

When Y is a Y^3 group, Q is preferably located at Y, i.e. y is 0, and Y is preferably $-(A^1)_x-Y^1-Q$. It is particularly convenient that, when the BTM is a peptide, Y^1 is a Y^3 group so that potentially both Y^1 and BTM may form part of the same peptide conjugated to the chelator Z^1.

When Y is a Y^1 or Y^2 group, Q is preferably located at either the chelator backbone or as an *N*-substituent of an amine donor atom of Z^1.

In Formula I, Y^1 is preferably an amide of formula $-(C=O)NHR^a$ or $-NH(C=O)R^a$, or a Y^2 pyrrole group of formula Y^{2a}:



Y^{2a}

Preferred Y^{2a} pyrrole groups have each $R^1 = C_{1-4}$ alkyl, more preferably methyl or dimethyl-ethyl.

The BTM may be of synthetic or natural origin, but is preferably synthetic. The term "synthetic" has its conventional meaning, i.e. man-made as opposed to being isolated from natural sources eg. from the mammalian body. Such compounds have the advantage that their manufacture and impurity profile can be fully controlled.

Monoclonal antibodies and fragments thereof of natural origin are therefore outside the scope of the term 'synthetic' as used herein. The molecular weight of the BTM is preferably up to 30,000 Daltons. More preferably, the molecular weight is in the range 200 to 20,000 Daltons, most preferably 300 to 18,000 Daltons, with 400 to 16,000 Daltons being especially preferred. When the BTM is a non-peptide, the molecular weight of the BTM is preferably up to 3,000 Daltons, more preferably 200 to 2,500 Daltons, most preferably 300 to 2,000 Daltons, with 400 to 1,500 Daltons being especially preferred.

The biological targeting moiety preferably comprises: a 3-100 mer peptide, peptide analogue, peptoid or peptide mimetic which may be a linear or cyclic peptide or combination thereof; a single amino acid; an enzyme substrate, enzyme antagonist enzyme agonist (including partial agonist) or enzyme inhibitor; receptor-binding compound (including a receptor substrate, antagonist, agonist or substrate); oligonucleotides, or oligo-DNA or oligo-RNA fragments. The enzyme and/or receptor is preferably endogenous to the mammalian subject.

By the term "peptide" is meant a compound comprising two or more amino acids, as defined below, linked by a peptide bond (ie. an amide bond linking the amine of one amino acid to the carboxyl of another). The term "peptide mimetic" or "mimetic" refers to biologically active compounds that mimic the biological activity of a peptide or a protein but are no longer peptidic in chemical nature, that is, they no longer contain any peptide bonds (that is, amide bonds between amino acids). Here, the term peptide mimetic is used in a broader sense to include molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. The term "peptide analogue" refers to peptides comprising one or more amino acid analogues, as described below. See also *Synthesis of Peptides and Peptidomimetics*, M. Goodman *et al*, Houben-Weyl E22c, Thieme.

By the term "amino acid" is meant an *L*- or *D*-amino acid, amino acid analogue (e.g. naphthylalanine) or amino acid mimetic which may be naturally occurring or of purely synthetic origin, and may be optically pure, i.e. a single enantiomer and hence chiral, or a mixture of enantiomers. Conventional 3-letter or single letter abbreviations for amino acids are used herein. Preferably the amino acids of the

present invention are optically pure. By the term "amino acid mimetic" is meant synthetic analogues of naturally occurring amino acids which are isosteres, i.e. have been designed to mimic the steric and electronic structure of the natural compound. Such isosteres are well known to those skilled in the art and include but are not limited to depsipeptides, retro-inverso peptides, thioamides, cycloalkanes or 1,5-disubstituted tetrazoles [see M. Goodman, Biopolymers, 24, 137, (1985)]. Radiolabelled amino acids such as tyrosine, histidine or proline are known to be useful *in vivo* imaging agents.

When the BTM is an enzyme substrate, enzyme antagonist, enzyme agonist, enzyme inhibitor or receptor-binding compound it is preferably a non-peptide, and more preferably is synthetic. By the term "non-peptide" is meant a compound which does not comprise any peptide bonds, ie. an amide bond between two amino acid residues. Suitable enzyme substrates, antagonists, agonists or inhibitors include glucose and glucose analogues; fatty acids, or elastase, Angiotensin II or metalloproteinase inhibitors. The enzyme of the enzyme substrate, antagonist, agonist or inhibitor is preferably endogenous to the mammalian subject. Suitable synthetic receptor-binding compounds include estradiol, estrogen, progestin, progesterone and other steroid hormones; ligands for the dopamine D-1 or D-2 receptor, or dopamine transporter such as tropanes; and ligands for the serotonin receptor. The receptor of the receptor-binding compound is preferably endogenous to the mammalian subject.

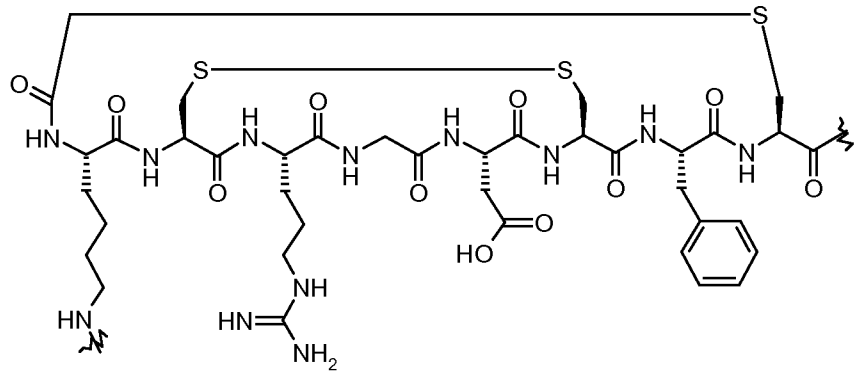
The BTM is most preferably a 3-100 mer peptide or peptide analogue. When the BTM is a peptide, it is preferably a 4-30 mer peptide, and most preferably a 5 to 28-mer peptide.

When the BTM is an enzyme substrate, enzyme antagonist, enzyme agonist or enzyme inhibitor, preferred such biological targeting molecules of the present invention are synthetic, drug-like small molecules i.e. pharmaceutical molecules. Preferred dopamine transporter ligands such as tropanes; fatty acids; dopamine D-2 receptor ligands; benzamides; amphetamines; benzylguanidines, iomazenil, benzofuran (IBF) or hippuric acid. Tropane agents are described by Morgan and Nowotnik [Drug News Perspect., 12(3), 137-145 (1999).

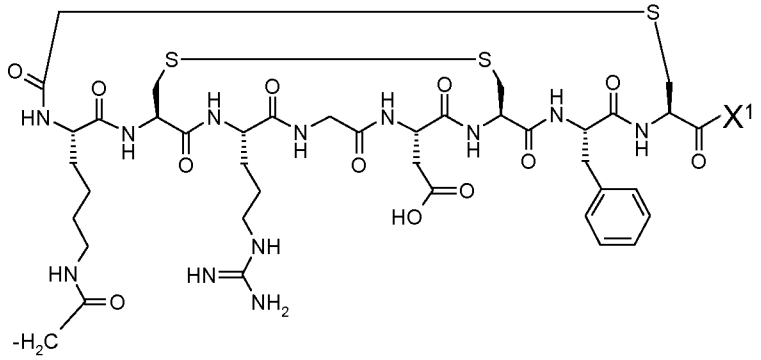
When the BTM is a peptide, preferred such peptides include:

- somatostatin, octreotide and analogues,
- peptides which bind to the ST receptor, where ST refers to the heat-stable toxin produced by *E.coli* and other micro-organisms;
- bombesin;
- vasoactive intestinal peptide;
- neurotensin;
- laminin fragments,
- N-formyl chemotactic peptides for targeting sites of leucocyte accumulation,
- Platelet factor 4 (PF4) and fragments thereof,
- RGD (Arg-Gly-Asp)-containing peptides, which may eg. target angiogenesis [R.Pasqualini *et al.*, Nat Biotechnol. 1997 Jun;15(6):542-6]; [E. Ruoslahti, Kidney Int. 1997 May;51(5):1413-7].
- peptide fragments of α_2-antiplasmin, fibronectin or beta-casein, fibrinogen or thrombospondin. The amino acid sequences of α_2-antiplasmin, fibronectin, beta-casein, fibrinogen and thrombospondin can be found in the following references: α_2-antiplasmin precursor [M.Tone *et al.*, J.Biochem, 102, 1033, (1987)]; beta-casein [L.Hansson *et al,* Gene, 139, 193, (1994)]; fibronectin [A.Gutman *et al*, FEBS Lett., 207, 145, (1996)]; thrombospondin-1 precursor [V.Dixit *et al*, Proc. Natl. Acad. Sci., USA, 83, 5449, (1986)]; R.F.Doolittle, Ann. Rev. Biochem., 53, 195, (1984);
- peptides which are substrates or inhibitors of angiotensin, such as: angiotensin II orAngiotensin I.

Preferred BTM peptides are RGD peptides. A more preferred such RGD peptide comprises the fragment:

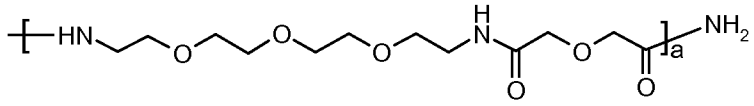


A most preferred such RGD peptide is when the BTM is a peptide of formula (A):



(A)

wherein X^1 is either $-NH_2$ or



wherein a is an integer of from 1 to 10.

In Formula A, a is preferably 1.

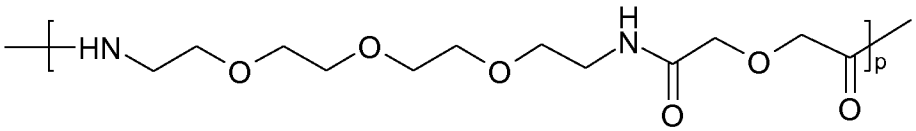
When the BTM is a peptide, one or both termini of the peptide, preferably both, have conjugated thereto a metabolism inhibiting group (M^{IG}). Having both peptide termini protected in this way is important for *in vivo* imaging applications, since otherwise rapid metabolism would be expected with consequent loss of selective binding affinity for the BTM peptide. By the term "metabolism inhibiting group" (M^{IG}) is meant a biocompatible group which inhibits or suppresses enzyme, especially peptidase such as carboxypeptidase, metabolism of the BTM peptide at either the amino terminus or carboxy terminus. Such groups are particularly important for *in vivo* applications, and are well known to those skilled in the art and are suitably chosen from, for the peptide amine terminus:

N-acylated groups $-NH(C=O)R^G$ where the acyl group $-(C=O)R^G$ has R^G chosen from: C_{1-6} alkyl, C_{3-10} aryl groups or comprises a polyethyleneglycol (PEG) building block. Suitable PEG groups are described for the linker group (L^1), below. Preferred

such PEG groups are the biomodifiers of Formulae Bio1 or Bio2 (below). Preferred such amino terminus M^{IG} groups are acetyl, benzyloxycarbonyl or trifluoroacetyl, most preferably acetyl.

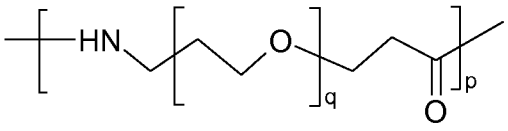
Suitable metabolism inhibiting groups for the peptide carboxyl terminus include: carboxamide, *tert*-butyl ester, benzyl ester, cyclohexyl ester, amino alcohol or a polyethyleneglycol (PEG) building block. A suitable M^{IG} group for the carboxy terminal amino acid residue of the BTM peptide is where the terminal amine of the amino acid residue is N-alkylated with a C_{1-4} alkyl group, preferably a methyl group. Preferred such M^{IG} groups are carboxamide or PEG, most preferred such groups are carboxamide.

When the linker group (L) comprises a peptide chain of 1 to 10 amino acid residues, the amino acid residues are preferably chosen from glycine, lysine, arginine, aspartic acid, glutamic acid or serine. When L comprises a PEG moiety, it preferably comprises units derived from oligomerisation of the monodisperse PEG-like structures of Formulae Bio1 or Bio2:



(Bio1)

17-amino-5-oxo-6-aza-3, 9, 12, 15-tetraoxaheptadecanoic acid of Formula Bio1 wherein p is an integer from 1 to 10. Alternatively, a PEG-like structure based on a propionic acid derivative of Formula Bio2 can be used:



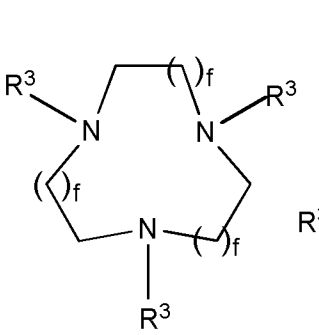
(Bio2)

where p is as defined for Formula Bio1 and q is an integer from 3 to 15. In Formula Bio2, p is preferably 1 or 2, and q is preferably 5 to 12.

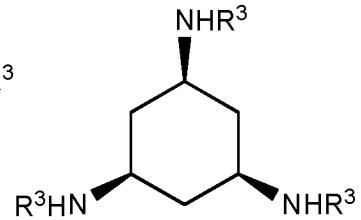
When the linker group does not comprise PEG or a peptide chain, preferred L groups have a backbone chain of linked atoms which make up the $-(A)_m-$ moiety of 2 to 10 atoms, most preferably 2 to 5 atoms, with 2 or 3 atoms being especially preferred.

BTM peptides which are not commercially available can be synthesised by solid phase peptide synthesis as described in P. Lloyd-Williams, F. Albericio and E. Girald; *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, 1997.

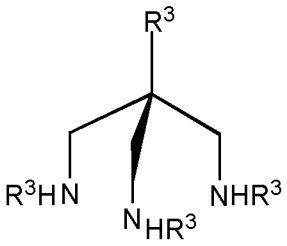
In a preferred embodiment, the chelating agent Z^1 of the first aspect is of Formula Z^a, Z^b or Z^c:



(Z^a)



(Z^b)



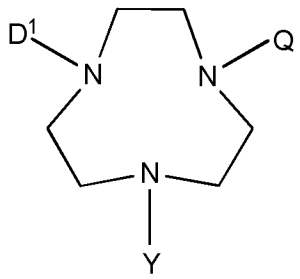
(Z^c)

where each R^3 is independently H, C_{1-4} alkyl, C_{2-4} alkoxyalkyl, C_{1-4} hydroxyalkyl, a D^1 group, a Y group or a Q group;

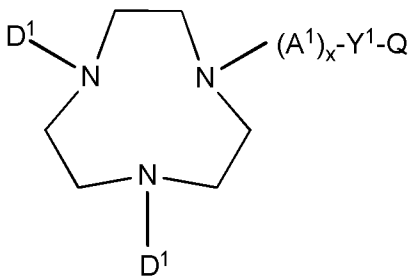
each f is independently 1 or 2.

In Formula Z^a, preferably each f = 1.

More preferred chelators of Formula (Z^a) are of Formula Z^{aa} or Z^{ab}:



(Z^{aa})



(Z^{ab})

where D^1, Q, Y, A^1, Y^1 and x are as defined for Formula I.

In Formulae Z^a, Z^b, Z^c, Z^{aa} and Z^{ab}, x, D^1, Y and Q, and preferred aspects thereof are as defined for Formula I. In particular, D^1 is preferably a Y^{2a} group, where Y^{2a} is as defined above.

In Formula I, each X^1 group is preferably independently Cl, ^{19}F or ^{18}F.

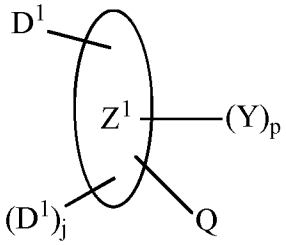
In Formula I, M is preferably Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+} or Y^{3+}; more preferably Ga^{3+}, In^{3+}, Sc^{3+} or Y^{3+}; most preferably Ga^{3+} or In^{3+}; with Ga^{3+} being the ideal.

Preferably, the imaging agent is provided in sterile form, i.e. in a form suitable for mammalian administration as is described in the fifth aspect (below).

The imaging agents of the first aspect can be obtained as described in the second aspect (below).

In a second aspect, the present invention provides a method of preparation of the imaging agent of the first aspect, which method comprises reaction of a precursor with a supply of [^{18}F]-fluoride or [^{18}F]NaF, optionally in the presence of [^{19}F]-fluoride, in a suitable solvent,

wherein said precursor comprises a metal complex of a chelator of Formula II:



(II)

where Z^1, D^1, Y, Q, j and p are as defined in are as defined in the first aspect; and where said metal is chosen from: Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+}.

The metal of the metal complex precursor can be radioactive or non-radioactive. When the metal is radioactive, suitable radiometal isotopes include ^{67}Ga, ^{68}Ga and ^{111}In. Preferably, the metal of the metal complex of the precursor is non-radioactive. Hence, the precursor used in the second aspect is preferably non-radioactive.

The [^{18}F]-fluoride may either be:

(i) delivered directly from a cyclotron and formulated using an ion exchange cartridge and appropriate eluent; or

(ii) in the form of GMP [^{18}F]NaF produced on an automated platform in a GMP facility.

The production of [^{18}F]-fluoride suitable for radiopharmaceutical applications is well-known in the art, and has been reviewed by Hjelstuen *et al* [Eur.J.Pharm.Biopharm., 78(3), 307-313 (2011)], and Jacobson *et al* [Curr.Top.Med.Chem., 10(11), 1048-1059 (2010)]. [^{18}F]NaF can be produced using an "automated synthesizer" as described in the sixth aspect (below).

The "suitable solvent" includes: acetonitrile, a C_{1-4} alkylalcohol, dimethylformamide, tetrahydrofuran, or dimethylsulfoxide, or aqueous mixtures of any thereof, or water. Aqueous buffers can be used in the pH range of 4-8, more preferably 5-7. A preferred solvent is aqueous in nature, and is more preferably a biocompatible carrier solvent as defined in the fourth aspect (below).

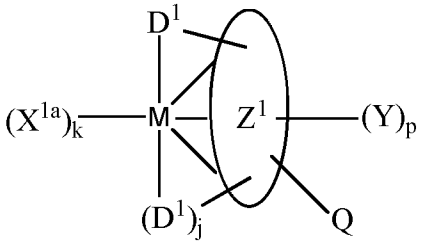
The ^{19}F-carrier (when used) may be in the form of:

(a) alkaline metal salt (eg NaF, KF, CsF etc); or

(b) in the presence of "non-metallic counter ions" (eg $[R_4N]F$ where R = alkyl), $[Ar_4P]F$; $[Ar_3S]F$

(c) metal cryptand counterions eg [K(kryptofix 2.2.2)]F, [Na(kryptofix 2.2.2)]F, [N(18-crown-6)]F etc.

The metal complex precursor of the second aspect is preferably of Formula III:



(III)

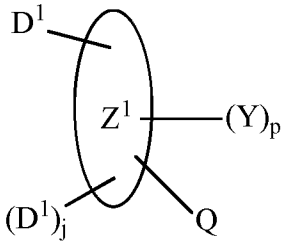
where X^{1a} is independently Br or Cl, and j and k are as defined for Formula (I).

Preferred embodiments of M, Z^1, Y, Q and p in Formula III are as defined in the first aspect (above). For the precursor of Formula III, it is preferred that X^{1a} = Cl. Preferably, the precursor is provided in sterile form, to facilitate the preparation of

imaging agents in pharmaceutical composition form – as is described in the fifth aspect (below).

The precursor used in the second aspect can be obtained as described in the fourth aspect (below).

In a third aspect, the present invention provides a chelating agent of Formula II:



(II)

where Z^1, D^1, Y, Q, j and p are as defined in the first aspect.

Preferred embodiments of Z^1, D^1, Y, Q, j and p in the third aspect are as defined in the first aspect. In particular, the chelator is preferably of Formula Z^a, Z^b or Z^c, Z^{aa} and preferred embodiments thereof as described above. The chelator of Formula II has a single Q group conjugated thereto.

The chelating agent of the third aspect can be obtained as described in the fourth aspect (below).

In a fourth aspect, the present invention provides a metal complex of the chelating agent of Formula II of the third aspect, where said metal is Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+}.

Preferred aspects of the chelating agent in the fourth aspect are as described in the first and third aspects (above).

The metal of the metal complex of the fourth aspect can be radioactive or non-radioactive. When the metal is radioactive, suitable radiometal isotopes include ^{67}Ga,

^{68}Ga and ^{111}In. Preferably, the metal of the metal complex of the fourth aspect is non-radioactive. More preferably, the metal complex is the precursor of Formula III as defined in the second aspect (above). The Q group of the precursor of Formula III preferably comprises a BTM which is chosen from: a 3-100 mer peptide, an enzyme substrate, an enzyme antagonist an enzyme agonist, an enzyme inhibitor or a receptor-binding compound.

Preferred aspects of the precursor of Formula III in are as described in the second aspect of the invention (above). Preferred aspects of the Q group and BTM in the fourth aspect are as described in the first aspect of the invention (above). The precursor is preferably "in a form suitable for mammalian administration" as defined below, most preferably in lyophilized form.

A preferred method of preparation of the precursor *via* metal complex formation with the chelator of Formula II described in the second aspect. The chelator can be prepared by literature methods, and modifications thereof. The precursor can be obtained by the bifunctional chelate approach. The term "bifunctional chelate" has its conventional meaning, and refers to a chelating agent having covalently attached thereto a pendant functional group. The functional group is used as a reactive site to attach the chelator to the BTM. The bifunctional chelate approach and associated syntheses have been described by Bartholoma *et al* [Chem.Rev., 110(5), 2903-2920 (2010)]; Chakraborty *et al* [Curr.Top.Med.Chem., 10(11), 1113-1134 (2010)] and Brechbiel *et al* [Quart.J.Nucl.Med.Mol.Imaging, 52(2), 166-173 (2008)]. The functional group of the present invention is preferably an amine, carboxylic acid or activated ester, more preferably a primary amine or an activated ester. Bifunctional chelators having a pendant amine functional group can be conjugated to the carboxyl group of a BTM. Bifunctional chelators having a carboxyl or activated ester functional group can be conjugated to an amine group of a BTM.

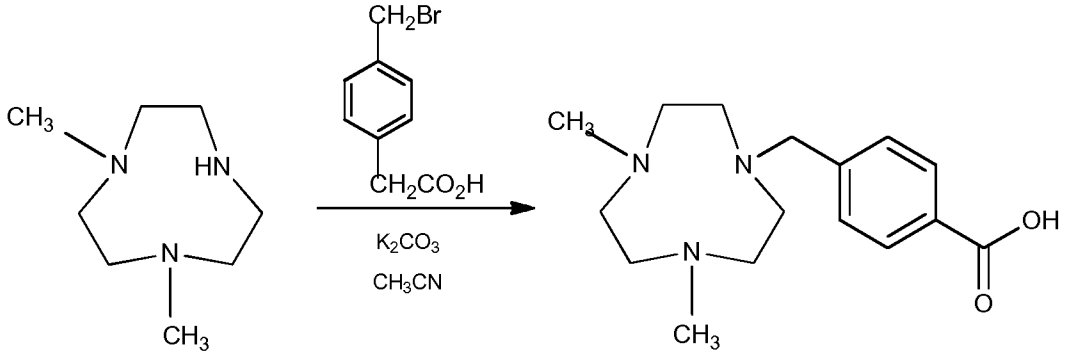
When preparing Ga(III) complexes, the use of anhydrous $GaCl_3$ as the Ga(III) source in the labelling work is challenging due to the high sensitivity of this compound to hydrolysis, which makes weighing out and manipulating the very small quantities necessary for labelling work difficult. A preferred source of Ga(III) is $Ga(NO_3)_3.nH_2O$ (commercially available from Sigma-Aldrich). The composition of

the salt has been determined as the nona-hydrate. $Ga(NO_3)_3.nH_2O$ is soluble and stable in aqueous media (pH ~3). This has the advantage of being able to deliver small quantities of the salt and subsequently diluting it in aqueous or buffered solution to the desired concentration, without risk of degradation. A further advantage is that the nitrate anion can be readily exchanged for fluoride under radiolabelling conditions. Finally, $Ga(NO_3)_3$ in dilute HNO_3 forms the hexa-aqua species $[Ga(H_2O)_6]^{3+}$, which is the reference standard used in ^{71}Ga NMR spectroscopy (δ = 0). This has the additional advantage of permitting tracking the course of reactions using ^{71}Ga NMR.

By the term "activated ester" or "active ester" is meant an ester derivative of the associated carboxylic acid which is designed to be a better leaving group, and hence permit more facile reaction with nucleophile, such as amines. Examples of suitable active esters are: *N*-hydroxysuccinimide (NHS); sulfo-succinimidyl ester; pentafluorophenol; pentafluorothiophenol; *para*-nitrophenol; hydroxybenzotriazole and PyBOP (i.e. benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate). Preferred active esters are *N*-hydroxysuccinimide or pentafluorophenol esters, especially *N*-hydroxysuccinimide esters.

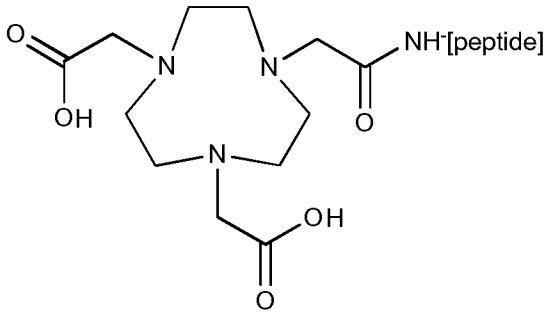
When a bifunctional chelator having a carboxyl functional group is conjugated to an amine group of a BTM, an activating agent is used. By the term "activating agent" is meant a reagent used to facilitate coupling between an amine and a carboxylic acid to generate an amide. Suitable such activating agents are known in the art and include carbodiimides such as EDC [*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide and *N*,*N'*-dialkylcarbodiimides such as dicyclohexylcarbodiimide or diisopropylcarbodiimide; and triazoles such as HBTU [*O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate], HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate], and PyBOP [benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate]. Such activating agents are commercially available. Further details are given in *March's Advanced Organic Chemistry*, 5th Edition, pages 508-510, Wiley Interscience (2001). A preferred such activating agent is EDC.

The chelator-BTM conjugates of the Z^a type can be prepared using analogous chemistry to McBride *et al* [Bioconj.Chem., 21(7), 1331-1340 (2010); Bioconj.Chem., 22, 1793-1803 (2011) and Appl.Rad.Isot., 70, 200-204 (2012)], e.g:



The starting material 1,4-dimethyl-tacn can be obtained by the method of Wieghardt *et al* [Inorg.Synth., 32, 75-81 (1998); Z.Anorg.Allg.Chem., 608, 60-68 (1992)]. Tacn and Me_3-tacn are commercially available. Me_3-tacn can also be obtained by the method of Wieghardt *et al* [Inorg Chem., 21, 3086 (1982)]. *N*-functionalised tacn chelators can be obtained by the method of Martin *et al* [J. Org. Chem., 47, 412 (1982)] or Mahapatra *et al* [J.Am. Chem. Soc., 118, 11555 (1996)]. Backbone-functionalised tacn chelators are described by Kuppers *et al* [Inorg. Chem., 25, 2400 (1986)].

McBride *et al* [Bioconj.Chem., 21(7), 1331-1340 (2010)] include the synthesis of a peptide-conjugated chelator IMP461:



IMP461

Peptide = -*D*-Ala-*D*-Lys(HSG)-*D*-Tyr-*D*-Lys(HSG)-NH_2,

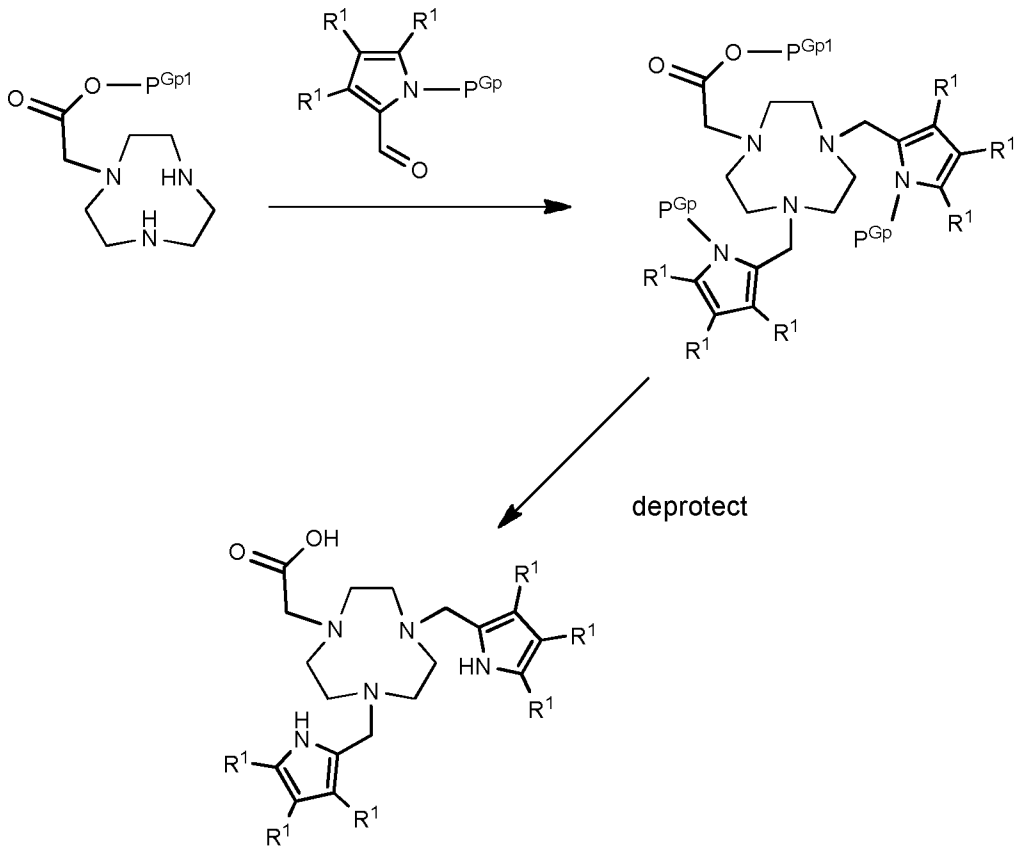
HSG = histamine-succinyl-glycine

By the term "protecting group" is meant a group which inhibits or suppresses undesirable chemical reactions, but which is designed to be sufficiently reactive that it may be cleaved from the functional group in question under mild enough conditions

that do not modify the rest of the molecule. After deprotection the desired product is obtained. Amine protecting groups are well known to those skilled in the art and are suitably chosen from: Boc (where Boc is tert-butyloxycarbonyl), Fmoc (where Fmoc is fluorenylmethoxycarbonyl), trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] or Npys (i.e. 3-nitro-2-pyridine sulfenyl). Suitable thiol protecting groups are Trt (Trityl), Acm (acetamidomethyl), t-Bu (tert-butyl), tert-Butylthio, methoxybenzyl, methylbenzyl or Npys (3-nitro-2-pyridine sulfenyl). The use of further protecting groups are described in *Protective Groups in Organic Synthesis*, 4th Edition, Theorodora W. Greene and Peter G. M. Wuts, [Wiley Blackwell, (2006)]. Preferred pyrrole protecting groups are Boc and Fmoc, most preferably Boc.

Amide functionalized chelators are described in Table 1 and the supporting Examples. Pyrrole-functionalised chelators can be prepared as described in Scheme 1:

Scheme 1.



where: R^1 is independently C_{1-4} alkyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl; P^{Gp} is a protecting group.

The alkylated pyrrole aldehyde 3,5-dimethyl-4-ethyl-pyrrole-2-carboxaldehyde is commercially available (Sigma-Aldrich). 3,4,5-Trimethylpyrrole-2-carboxaldehyde, 3,5-dimethyl-4-ethyl-pyrrole-2-carboxaldehyde and 4,5-dimethyl-3-ethyl-pyrrole-2-carboxaldehyde can be prepared as described by Clezy *et al* [Aust.J.Chem., 42, 775-786 (1989)]. The condensation of the pyrrole aldehyde with tacn can be carried out by first protecting the pyrrole by the method of Davies *et al* for 1-(*tert*-butoxycarbonyl)pyrrole-2-carboxaldehyde [J.Org.Chem., 61, 2305-2313 (1996)], followed by reductive amination [*March's Advanced Organic Chemistry*, 5th Edition, pages 1187-1189, Wiley Interscience (2001)]. The deprotection is carried out by standard methods as described in Greene and Wuts (above). The synthesis of Scheme 1 can be readily adapted to systems wherein alternative *N*-functionalised tacn starting materials are used so that one or two pyrrole substituents are attached.

In a fifth aspect, the present invention provides a radiopharmaceutical composition which comprises the imaging agent of the first aspect, together with a biocompatible carrier, in a form suitable for mammalian administration.

Preferred aspects of the imaging agent in the fifth aspect are as described in the first aspect of the present invention (above).

By the phrase "in a form suitable for mammalian administration" is meant a composition which is sterile, pyrogen-free, lacks compounds which produce toxic or adverse effects, and is formulated at a biocompatible pH (approximately pH 4.0 to 10.5). Such compositions lack particulates which could risk causing emboli *in vivo*, and are formulated so that precipitation does not occur on contact with biological fluids (e.g. blood). Such compositions also contain only biologically compatible excipients, and are preferably isotonic.

The "biocompatible carrier" is a fluid, especially a liquid, in which the imaging agent can be suspended or preferably dissolved, such that the composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which

may advantageously be balanced so that the final product for injection is isotonic); an aqueous buffer solution comprising a biocompatible buffering agent (e.g. phosphate buffer); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). Preferably the biocompatible carrier is pyrogen-free water for injection, isotonic saline or phosphate buffer.

The imaging agents and biocompatible carrier are each supplied in suitable vials or vessels which comprise a sealed container which permits maintenance of sterile integrity and/or radioactive safety, plus optionally an inert headspace gas (eg. nitrogen or argon), whilst permitting addition and withdrawal of solutions by syringe or cannula. A preferred such container is a septum-sealed vial, wherein the gas-tight closure is crimped on with an overseal (typically of aluminium). The closure is suitable for single or multiple puncturing with a hypodermic needle (e.g. a crimped-on septum seal closure) whilst maintaining sterile integrity. Such containers have the additional advantage that the closure can withstand vacuum if desired (eg. to change the headspace gas or degas solutions), and withstand pressure changes such as reductions in pressure without permitting ingress of external atmospheric gases, such as oxygen or water vapour.

Preferred multiple dose containers comprise a single bulk vial which contains multiple patient doses, whereby single patient doses can thus be withdrawn into clinical grade syringes at various time intervals during the viable lifetime of the preparation to suit the clinical situation. Pre-filled syringes are designed to contain a single human dose, or "unit dose" and are therefore preferably a disposable or other syringe suitable for clinical use. The pharmaceutical compositions of the present invention preferably have a dosage suitable for a single patient and are provided in a suitable syringe or container, as described above.

The pharmaceutical composition may contain additional optional excipients such as: an antimicrobial preservative, pH-adjusting agent, filler, radioprotectant, solubiliser or osmolality adjusting agent. By the term "radioprotectant" is meant a compound which

inhibits degradation reactions, such as redox processes, by trapping highly-reactive free radicals, such as oxygen-containing free radicals arising from the radiolysis of water. The radioprotectants of the present invention are suitably chosen from: ascorbic acid, *para*-aminobenzoic acid (i.e. 4-aminobenzoic acid), gentisic acid (i.e. 2,5-dihydroxybenzoic acid) and salts thereof with a biocompatible cation. By the term "biocompatible cation" (B^c) is meant a positively charged counterion which forms a salt with an ionised, negatively charged group, where said positively charged counterion is also non-toxic and hence suitable for administration to the mammalian body, especially the human body. Examples of suitable biocompatible cations include: the alkali metals sodium or potassium; the alkaline earth metals calcium and magnesium; and the ammonium ion. Preferred biocompatible cations are sodium and potassium, most preferably sodium.

By the term "solubiliser" is meant an additive present in the composition which increases the solubility of the imaging agent in the solvent. A preferred such solvent is aqueous media, and hence the solubiliser preferably improves solubility in water. Suitable such solubilisers include: C_{1-4} alcohols; glycerine; polyethylene glycol (PEG); propylene glycol; polyoxyethylene sorbitan monooleate; sorbitan monooloeate; polysorbates; poly(oxyethylene)poly(oxypropylene)poly(oxyethylene) block copolymers (Pluronics^{TM}); cyclodextrins (e.g. alpha, beta or gamma cyclodextrin, hydroxypropyl-β-cyclodextrin or hydroxypropyl-γ-cyclodextrin) and lecithin.

By the term "antimicrobial preservative" is meant an agent which inhibits the growth of potentially harmful micro-organisms such as bacteria, yeasts or moulds. The antimicrobial preservative may also exhibit some bactericidal properties, depending on the dosage employed. The main role of the antimicrobial preservative(s) of the present invention is to inhibit the growth of any such micro-organism in the pharmaceutical composition. The antimicrobial preservative may, however, also optionally be used to inhibit the growth of potentially harmful micro-organisms in one or more components of kits used to prepare said composition prior to administration. Suitable antimicrobial preservative(s) include: the parabens, i.e. methyl, ethyl, propyl or butyl paraben or

mixtures thereof; benzyl alcohol; phenol; cresol; cetrimide and thiomersal. Preferred antimicrobial preservative(s) are the parabens.

The term "pH-adjusting agent" means a compound or mixture of compounds useful to ensure that the pH of the composition is within acceptable limits (approximately pH 4.0 to 10.5) for human or mammalian administration. Suitable such pH-adjusting agents include pharmaceutically acceptable buffers, such as tricine, phosphate or TRIS [i.e. *tris*(hydroxymethyl)aminomethane], and pharmaceutically acceptable bases such as sodium carbonate, sodium bicarbonate or mixtures thereof.

By the term "filler" is meant a pharmaceutically acceptable bulking agent which may facilitate material handling during production and lyophilisation. Suitable fillers include inorganic salts such as sodium chloride, and water soluble sugars or sugar alcohols such as sucrose, maltose, mannitol or trehalose.

The radiopharmaceutical compositions of the fifth aspect may be prepared under aseptic manufacture (i.e. clean room) conditions to give the desired sterile, non-pyrogenic product. It is preferred that the key components, especially the associated reagents plus those parts of the apparatus which come into contact with the imaging agent (e.g. vials) are sterile. The components and reagents can be sterilised by methods known in the art, including: sterile filtration, terminal sterilisation using e.g. gamma-irradiation, autoclaving, dry heat or chemical treatment (e.g. with ethylene oxide). It is preferred to sterilise some components in advance, so that the minimum number of manipulations needs to be carried out. As a precaution, however, it is preferred to include at least a sterile filtration step as the final step in the preparation of the pharmaceutical composition.

The radiopharmaceutical compositions of the present invention may be prepared by various methods:

(i) aseptic manufacture techniques in which the ^{18}F-radiolabelling step is carried out in a clean room environment;

(ii) terminal sterilisation, in which the ^{18}F-radiolabelling is carried out without using aseptic manufacture and then sterilised at the last step [e.g. by

gamma irradiation, autoclaving dry heat or chemical treatment (e.g. with ethylene oxide)];

(iii) aseptic manufacture techniques in which the ^{18}F-radiolabelling step is carried out using an automated synthesizer apparatus.

Method (iii) is preferred, and is described more fully in the sixth aspect (below).

In a sixth aspect, the present invention provides a method of preparation of the radiopharmaceutical composition of the fifth aspect, which comprises carrying out the method of preparation of the second aspect using an automated synthesizer apparatus.

Preferred aspects of the imaging agent, precursor and composition in the sixth aspect are as described in the first, second and fourth, and fifth aspects of the present invention respectively.

By the term "automated synthesizer" is meant an automated module based on the principle of unit operations as described by Satyamurthy *et al* [Clin.Positr.Imag., 2(5), 233-253 (1999)]. The term 'unit operations' means that complex processes are reduced to a series of simple operations or reactions, which can be applied to a range of materials. Such automated synthesizers are preferred for the method of the present invention especially when a radiopharmaceutical composition is desired. They are commercially available from a range of suppliers [Satyamurthy *et al,* above], including: GE Healthcare; CTI Inc; Ion Beam Applications S.A. (Chemin du Cyclotron 3, B-1348 Louvain-La-Neuve, Belgium); Raytest (Germany) and Bioscan (USA).

Commercial automated synthesizers also provide suitable containers for the liquid radioactive waste generated as a result of the radiopharmaceutical preparation. Automated synthesizers are not typically provided with radiation shielding, since they are designed to be employed in a suitably configured radioactive work cell. The radioactive work cell provides suitable radiation shielding to protect the operator from potential radiation dose, as well as ventilation to remove chemical and/or radioactive vapours. The automated synthesizer preferably comprises a cassette.

By the term "cassette" is meant a piece of apparatus designed to fit removably and interchangeably onto an automated synthesizer apparatus (as defined above), in such a way that mechanical movement of moving parts of the synthesizer controls the operation of the cassette from outside the cassette, i.e. externally. Suitable cassettes comprise a linear array of valves, each linked to a port where reagents or vials can be attached, by either needle puncture of an inverted septum-sealed vial, or by gas-tight, marrying joints. Each valve has a male-female joint which interfaces with a corresponding moving arm of the automated synthesizer. External rotation of the arm thus controls the opening or closing of the valve when the cassette is attached to the automated synthesizer. Additional moving parts of the automated synthesizer are designed to clip onto syringe plunger tips, and thus raise or depress syringe barrels.

The cassette is versatile, typically having several positions where reagents can be attached, and several suitable for attachment of syringe vials of reagents or chromatography cartridges (e.g. solid phase extraction or SPE). The cassette always comprises a reaction vessel. Such reaction vessels are preferably 1 to 10 cm^3, most preferably 2 to 5 cm^3 in volume and are configured such that 3 or more ports of the cassette are connected thereto, to permit transfer of reagents or solvents from various ports on the cassette. Preferably the cassette has 15 to 40 valves in a linear array, most preferably 20 to 30, with 25 being especially preferred. The valves of the cassette are preferably each identical, and most preferably are 3-way valves. The cassettes are designed to be suitable for radiopharmaceutical manufacture and are therefore manufactured from materials which are of pharmaceutical grade and ideally also are resistant to radiolysis.

Preferred automated synthesizers of the present invention comprise a disposable or single use cassette which comprises all the reagents, reaction vessels and apparatus necessary to carry out the preparation of a given batch of radiofluorinated radiopharmaceutical. The cassette means that the automated synthesizer has the flexibility to be capable of making a variety of different radiopharmaceuticals with minimal risk of cross-contamination, by simply changing the cassette. The cassette approach also has the advantages of: simplified set-up hence reduced risk of operator error; improved GMP (Good Manufacturing Practice) compliance; multi-tracer capability; rapid change between production runs; pre-run automated diagnostic

checking of the cassette and reagents; automated barcode cross-check of chemical reagents *vs* the synthesis to be carried out; reagent traceability; single-use and hence no risk of cross-contamination, tamper and abuse resistance.

Included in this aspect of the invention, is the use of an automated synthesizer apparatus to prepare the radiopharmaceutical composition of the second aspect.

Included in this aspect of the invention, is the use of a suitable cassette in conjunction with an automated synthesizer apparatus to prepare the radiopharmaceutical composition of the second aspect.

In the sixth aspect, the precursor is preferably provided in sterile, lyophilized form. The lyophilized precursor is preferably provided as a non-radioactive kit in a pharmaceutical grade container, preferably a septum-sealed vial, as is described in the fifth aspect (above).

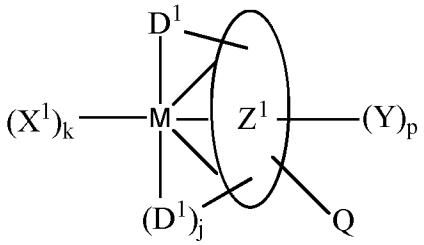
In a seventh aspect, the present invention provides a method of imaging the human or animal body which comprises generating an image of at least a part of said body to which the imaging agent of the first aspect, or the composition of the fifth aspect has distributed using PET, wherein said imaging agent or composition has been previously administered to said body.

Preferred aspects of the imaging agent or composition in the sixth aspect are as described in the first and fifth aspects respectively of the present invention (above).

Also included in the invention is a method of diagnosis of the human or animal body which comprises the imaging method of the sixth aspect.

CLAIMS.

1. An imaging agent which comprises an ^{18}F-labelled compound of Formula I:



(I)

where:

X^1 is independently Br, Cl, ^{19}F or ^{18}F,

with the proviso that at least one X^1 is ^{18}F;

M is Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+};

Z^1 is a chelating agent having a donor set of 3 amine donors and one or two D^1 groups, wherein all 3 amine donors and the donor atom(s) of D^1 are bound to M,

wherein Z^1 has at least one Y group, and also a Q group covalently conjugated thereto;

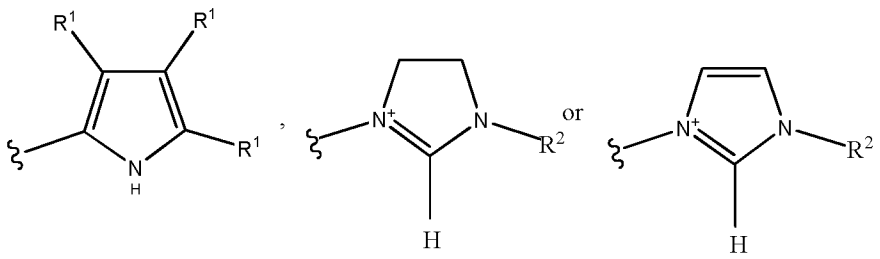
Y is independently $-(A^1)_x-Y^1$ or $-(A^1)_x-Y^1-Q$;

each D^1 is independently a group of formula $-(A^2)_p-D$, where D is a metal coordinating coordinating group chosen from $-CO_2H$, -OH, -SH, $-PO_3H_2$ or C_{2-8} nitrogen-containing heteroaryl;

each A^1 is independently $-CH_2-$ or –O-, and each A^2 is independently an A^1 group or $-C_6H_4-$ provided that neither Y nor D^1 comprises any -O-O- bonds;

Y^1 is $-NHR^a$, $-NH(CH_2)_2NHR^a$, $-NH(CH_2)_3NHR^a$, $-(C=O)NHR^a$, $-NH(C=O)R^a$, $-NH(C=NH)NHR^a$, $-OR^a$, a Y^2 group or a Y^3 group;

Y^2 is:



Y^3 is Arg, Lys, Asn, Gln, Ser, Thr or Tyr;

wherein R^a is independently H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

and wherein each R^1 is independently C_{1-4} alkyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

and R^2 is independently H, C_{1-4} alkyl or $Si(C_{1-4}$ alkyl$)_3$;

j is 0 or 1;

k is (2-j);

each p is independently 1, 2 or 3;

x is an integer of value 1 to 6;

Q is –L-[BTM];

L is a synthetic linker group of formula $–(A)_m-$ wherein each A is independently – CR_2-,-CR=CR- , -C≡C- , $-CR_2CO_2-$, $-CO_2CR_2-$, -NRCO- , -CONR- , -CR=N-O-, -NR(C=O)NR-, -NR(C=S)NR-, $-SO_2NR-$, $-NRSO_2-$, $-CR_2OCR_2-$, $-CR_2SCR_2-$, $-CR_2NRCR_2-$, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, -Ar-, -NR-Ar-, -O-Ar-, -Ar-(CO)-, an amino acid, a sugar or a monodisperse polyethyleneglycol (PEG) building block,

wherein each R is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{2-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

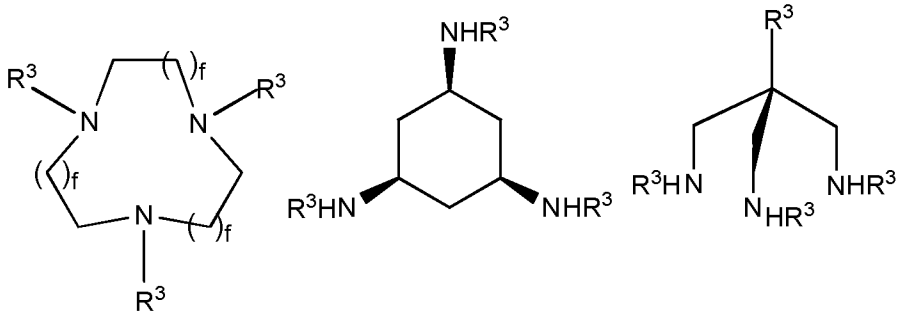
m is an integer of value 1 to 20;

each Ar is independently a C_{5-12} arylene group, or a C_{3-12} heteroarylene group;

BTM is a biological targeting moiety.

2. The imaging agent of claim 1, where each Y group is covalently conjugated to a different amine donor atom of Z^1.

3. The imaging agent of claim 1 or claim 2, where Z^1 is of Formula Z^a, Z^b or Z^c:

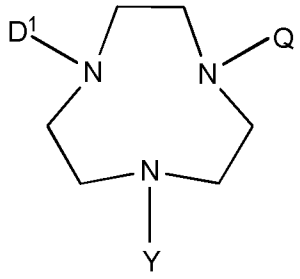


(Z^a) (Z^b) (Z^c)

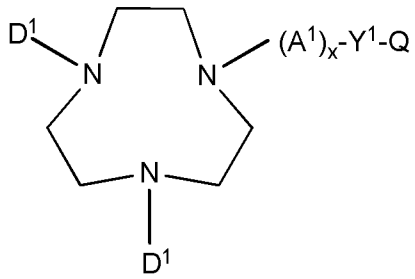
where each R^3 is independently H, C_{1-4} alkyl, C_{2-4} alkoxyalkyl, C_{1-4} hydroxyalkyl, a D^1 group, a Y group or a Q group;

each f is independently 1 or 2.

4. The imaging agent of claim 3, where Z^a is of Formula Z^{aa} or Z^{ab}:



(Z^{aa})



(Z^{ab})

where D^1, Q, Y, A^1, Y^1 and x are as defined for Formula I.

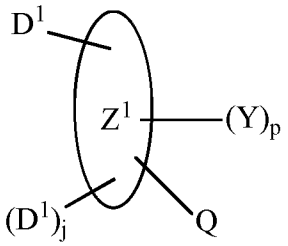
5. The imaging agent of any one of claims 1 to 4, where M is Ga^{3+} or In^{3+}.

6. The imaging agent of any one of claims 1 to 5, where each X^1 is independently Cl, ^{19}F or ^{18}F.

7. The imaging agent of any one of claims 1 to 6, where the BTM is chosen from: a single amino acid, a 3-100 mer peptide, an enzyme substrate, an enzyme antagonist an enzyme agonist, an enzyme inhibitor or a receptor-binding compound.

8. A method of preparation of the imaging agent of any one of claims 1 to 7, which comprises reaction of a precursor with a supply of [^{18}F]-fluoride or [^{18}F]NaF, optionally in the presence of [^{19}F]-fluoride, in a suitable solvent,

wherein said precursor comprises a metal complex of a chelator of Formula II:

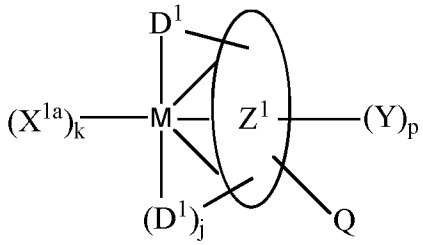


(II)

where Z^1, D^1, Y, Q, j, and p are as defined in any one of claims 1 to 5;

and where said metal is chosen from: Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+}.

9. The method of claim 8, where said precursor is of Formula III:



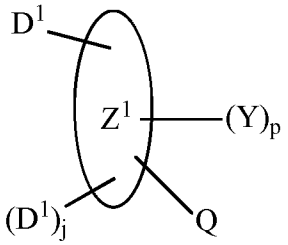
(III)

where X^{1a} is independently Br or Cl.

10. The method of claim 8 or claim 9, where Z^1 is of Formula Z^a, Z^b or Z^c as defined in claim 3, or of Formula Z^{aa} or Formula Z^{ab} as defined in claim 4.

11. The method of claim 9 or claim 10, where X^{1a} = Cl.

12. A chelating agent of Formula II:



(II)

where Z^1, D^1, Y, Q, j and p are as defined in any one of claims 1 to 4.

13. A metal complex of the chelating agent of Formula II as defined in claim 12, where said metal is Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} or Lu^{3+}.

14. The metal complex of claim 13, which is the precursor of Formula III as defined in any one of claims 9 to 11.

15. The metal complex of claim 14, wherein Q in said precursor of Formula III comprises a BTM which is chosen from: a 3-100 mer peptide, an enzyme substrate, an enzyme antagonist an enzyme agonist, an enzyme inhibitor or a receptor-binding compound.

16. A radiopharmaceutical composition which comprises the imaging agent of any one of claims 1 to 7, together with a biocompatible carrier, in a form suitable for mammalian administration.

17. A method of preparation of the radiopharmaceutical composition of claim 16, which comprises carrying out the method of any one of claims 8 to 11 using an automated synthesizer apparatus.

18. The method of claim 17, where the automated synthesizer apparatus comprises a cassette which comprises the non-radioactive reagents necessary to carry out the method of any one of claims 8 to 11.

19. The method of claim 17 or claim 18, where the precursor is provided in sterile, lyophilized form.

20. A method of imaging the human or animal body which comprises generating an image of at least a part of said body to which the imaging agent of any one of claims 1 to 7, or the composition of claim 16 has distributed using PET, wherein said agent or composition has been previously administered to said body.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/058986

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/082618 A2 (IMMUNOMEDICS INC [US]; D SOUZA CHRISTOPHER A [US]; MCBRIDE WILLIAM J [) 21 June 2012 (2012-06-21) paragraph [00155]; claims 1,4,7,18,19,36,40 paragraph [0002] paragraph [00243] paragraph [00268] paragraph [00355] paragraph [00470] -----	1-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 2014	07/07/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Johnson, Claire

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/058986

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012082618 A2	21-06-2012	AU 2011344110 A1	02-05-2013
		CA 2814953 A1	21-06-2012
		CN 103370063 A	23-10-2013
		EP 2651411 A2	23-10-2013
		WO 2012082618 A2	21-06-2012