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(54) Title: RADIO-PHARMACEUTICAL COMPLEXES

(57) Abstract: A tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor. Methods of treatment utilising such complexes and methods of formation of such complexes are provided.

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## Radio-Pharmaceutical Complexes

### FIELD OF THE INVENTION

The present invention relates to complexes of thorium isotopes and particularly with complexes of thorium-227 with certain octadentate ligands conjugated to tissue targeting moieties. The invention also relates to the treatment of disease, particularly neoplastic diseases, involving the administration of such complexes.

### BACKGROUND TO THE INVENTION

Specific cell killing can be essential for the successful treatment of a variety of diseases in mammalian subjects. Typical examples of this are in the treatment of malignant diseases such as sarcomas and carcinomas. However the selective elimination of certain cell types can also play a key role in the treatment of other diseases, especially hyperplastic and neoplastic diseases.

The most common methods of selective treatment are currently surgery, chemotherapy and external beam irradiation. Targeted radionuclide therapy is, however, a promising and developing area with the potential to deliver highly cytotoxic radiation to unwanted cell types. The most common forms of radiopharmaceutical currently authorised for use in humans employ beta-emitting and/or gamma-emitting radionuclides. There has, however, been some interest in the use of alpha-emitting radionuclides in therapy because of their potential for more specific cell killing.

The radiation range of typical alpha emitters in physiological surroundings is generally less than 100 micrometers, the equivalent of only a few cell diameters. This makes these sources well suited for the treatment of tumours, including micrometastases, because they have the range to reach neighbouring cells within a tumour but if they are well targeted then little of the radiated energy will pass beyond the target cells. Thus, not every cell need be targeted but damage to surrounding healthy tissue may be minimised (see Feinendegen et al., *Radiat Res* 148:195-201 (1997)). In contrast, a beta particle has a range of 1 mm or more in water (see Wilbur, *Antibody Immuncon Radiopharm* 4: 85-96 (1991)).

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The energy of alpha-particle radiation is high in comparison with that carried by beta particles, gamma rays and X-rays, typically being 5-8 MeV, or 5 to 10 times that of a beta particle and 20 or more times the energy of a gamma ray. Thus, this deposition of a large amount of energy over a very short distance gives  $\alpha$ -radiation an exceptionally high linear energy transfer (LET), high relative biological efficacy (RBE) and low oxygen enhancement ratio (OER) compared to gamma and beta radiation (see Hall, "Radiobiology for the radiologist", Fifth edition, Lippincott Williams & Wilkins, Philadelphia PA, USA, 2000). This explains the exceptional cytotoxicity of alpha emitting radionuclides and also imposes stringent demands on the biological targeting of such isotopes and upon the level of control and study of alpha emitting radionuclide distribution which is necessary in order to avoid unacceptable side effects.

Table 1 below shows the physical decay properties of the alpha emitters so far broadly proposed in the literature as possibly having therapeutic efficacy.

Table 1

Candidate nuclide	$T_{1/2}^*$	Clinically tested for
$^{225}\text{Ac}$	10.0 days	leukaemia
$^{211}\text{At}$	7.2 hours	glioblastoma
$^{213}\text{Bi}$	46 minutes	leukaemia
$^{223}\text{Ra}$	11.4 days	skeletal metastases
$^{224}\text{Ra}$	3.66 days	ankylosing spondylitis

\* Half life

So far, with regards to the application in radioimmunotherapy the main attention has been focused on  $^{211}\text{At}$ ,  $^{213}\text{Bi}$  and  $^{225}\text{Ac}$  and these three nuclides have been explored in clinical immunotherapy trials.

Several of the radionuclides which have been proposed are short-lived, i.e. have half lives of less than 12 hours. Such a short half-life makes it difficult to produce and distribute radiopharmaceuticals based upon these radionuclides in a commercial manner. Administration of a short-lived nuclide also increases the proportion of the radiation dose which will be emitted in the body before the target site is reached.

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The recoil energy from alpha-emission will in many cases cause the release of daughter nuclides from the position of decay of the parent. This recoil energy is sufficient to break many daughter nuclei out from the chemical environment which may have held the parent, e.g. where the parent was complexed by a ligand such as a chelating agent. This will occur even where the daughter is chemically compatible with, i.e. complexable by, the same ligand. Equally, where the daughter nuclide is a gas, particularly a noble gas such as radon, or is chemically incompatible with the ligand, this release effect will be even greater. When daughter nuclides have half-lives of more than a few seconds, they can diffuse away into the blood system, unrestrained by the complexant which held the parent. These free radioactive daughters can then cause undesired systemic toxicity.

The use of Thorium-227 ( $T_{1/2} = 18.7$  days) under conditions where control of the  $^{223}\text{Ra}$  daughter isotope is maintained was proposed a few years ago (see WO 01/60417 and WO 02/05859). This was in situations where a carrier system is used which allows the daughter nuclides to be retained by a closed environment. In one case, the radionuclide is disposed within a liposome and the substantial size of the liposome (as compared to recoil distance) helps retain daughter nuclides within the liposome. In the second case, bone-seeking complexes of the radionuclide are used which incorporate into the bone matrix and therefore restrict release of the daughter nuclides. These are potentially highly advantageous methods, but the administration of liposomes is not desirable in some circumstances and there are many diseases of soft tissue in which the radionuclides cannot be surrounded by a mineralised matrix so as to retain the daughter isotopes.

More recently, it was established that the toxicity of the  $^{223}\text{Ra}$  daughter nuclei released upon decay of  $^{227}\text{Th}$  could be tolerated in the mammalian body to a much greater extent than would be predicted from prior tests on comparable nuclei. In the absence of the specific means of retaining the radium daughters of thorium-227 discussed above, the publicly available information regarding radium toxicity made it clear that it was not possible to use thorium-227 as a therapeutic agent since the dosages required to achieve a therapeutic effect from thorium-227 decay would result in a highly toxic and possibly lethal dosage of radiation from the decay of the radium daughters, i.e. there is no therapeutic window.

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WO 04/091668 describes the unexpected finding that a therapeutic treatment window does exist in which a therapeutically effective amount of a targeted thorium-227 radionuclide can be administered to a subject (typically a mammal) without generating an amount of radium-223 sufficient to cause unacceptable myelotoxicity. This can therefore be used for treatment and prophylaxis of all types of diseases at both bony and soft-tissue sites.

In view of the above developments, it is now possible to employ alpha-emitting thorium-227 nuclei in endoradionuclide therapy without lethal myelotoxicity resulting from the generated  $^{223}\text{Ra}$ . Nonetheless, the therapeutic window remains relatively narrow and it is in all cases desirable to administer no more alpha-emitting radioisotope to a subject than absolutely necessary. Useful exploitation of this new therapeutic window would therefore be greatly enhanced if the alpha-emitting thorium-227 nuclei could be complexed and targeted with a high degree of reliability.

Because radionuclides are constantly decaying, the time spent handling the material between isolation and administration to the subject is of great importance. It would also be of considerable value if the alpha-emitting thorium nuclei could be complexed, targeted and/or administered in a form which was quick and convenient to prepare, preferably requiring few steps, short incubation periods and/or temperatures not irreversibly affecting the properties of the targeting entity. Furthermore, processes which can be conducted in solvents that do not need removal before administration (essentially in aqueous solution) have the considerable advantage of avoiding a solvent evaporation or dialysis step.

In view of the need for selectivity in the delivery of cytotoxic agents in therapy, there is an evident need for targeting of alpha-radionuclide complexes. However, conjugates of suitable chelators with a small targeting peptide or small protein tend to show poor solubility in aqueous systems because the small biomolecule cannot keep the insoluble chelate in solution. Poor solubility leads to aggregation and precipitation. Aggregates are unacceptable in a drug preparation to be administered to human subjects and evidently precipitation renders a composition entirely unusable.

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Furthermore, also with a larger targeting peptide/protein, such as a monoclonal antibody, the chelator will be exposed on the surface of the conjugate as a hydrophobic 'spot'. This might in some contexts lead to issues with micro aggregation.

In a biological system, such as in a human patient, hydrophobicity in general is associated with undesirable uptake in the liver. Evidently this is much more serious with highly cytotoxic agents such as alpha-emitters than for typical drug compounds. Hydrophobicity of the chelator also increases the risk of an immune response, as hydrophobicity facilitates stronger binding of antibodies produced by the host's immune system. Again this is of particular concern with alpha-emitters due to their exceptional cytotoxicity. There is thus evidently a considerable need of methods for the selective delivery of alpha-emitting thorium radionuclides by conjugates having increased hydrophilicity, particularly of the ligand portion, so as to address one or more of the issues discussed above.

Octadentate chelating agents containing hydroxypyridinone groups have previously been shown to be suitable for coordinating the alpha emitter thorium-277, for subsequent attachment to a targeting moiety (WO2011098611). Octadentate chelators were described, containing four 3,2- hydroxypyridinone groups joined by linker groups to an amine-based scaffold, having a separate reactive group used for conjugation to a targeting molecule. Preferred structures of the previous invention contained 3,2-hydroxypyridinone groups having a methyl substituted nitrogen in position 1 of the heterocyclic ring, and were linked to the amine based scaffold by a amine bond involving an formic acid attached at position 4, as shown in by the compounds ALG-DD-NCS, ALG1005-38, Bb-1-HOPO-1-DEBN. In the experiment where one of these hydroxypyridinone containing molecules was conjugated to a tumour targeting antibody, the molecule was dissolved in the organic solvent DMSO since it could not be dissolved in aqueous buffers.

CD22 is a sugar binding transmembrane receptor expressed in certain mammalian cells, particularly in B cells where it functions as an inhibitory receptor for B cell receptor signalling. It has been considered a possible a target for antibody based therapy.

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The present inventors have now unexpectedly established that the use of a 4+ thorium-227 ion complexed by an octadentate hydroxypyridinone (HOPO)-type ligand comprising four HOPO moieties of which at least one is substituted with a suitable solubilising moiety can provide a dramatic improvement in solubility and corresponding properties of the complex. Furthermore, coupling of such a ligand to a CD22-binding targeting moiety can provide a conjugate having advantageous properties.

#### SUMMARY OF THE INVENTION

Viewed from a first aspect the present invention therefore provides a tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position (1-position) with a hydroxyalkyl solubilising group wherein the tissue targeting moiety has binding affinity for the CD22 receptor. In one embodiment such complexes are soluble in pure water.

In a preferred embodiment, the octadentate ligand comprises at least one 3,2-HOPO moiety, and preferably 2, 3, or 4 3,2-HOPO moieties. In a further preferred embodiment, at least 2, preferably at least 3 and most preferably all 4 HOPO moieties comprise hydroxyalkyl solubilising moieties at the N-position.

Preferred targeting moieties include polyclonal and particularly monoclonal antibodies and fragments thereof. Specific binding fragments such as Fab, Fab', Fab'2 and single-chain specific binding antibodies are typical fragments.

In such complexes (and preferably in all aspects of the current invention) the thorium ion will generally be complexed by the octadentate hydroxypyridinone-containing ligand, which in turn will be attached to the tissue targeting moiety by any suitable means. Such means may include direct covalent attachment or attachment by means of any suitable specific binding pair (e.g. biotin/avidin type binding pairs). Any suitable attachment may be used but direct covalent bonding or use of a covalent or binding-pair linker moiety will be typical methods. Covalent ester or amide bonds are preferred methods.

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Viewed from a further aspect the invention provides the use of a tissue targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor (including any such complex described herein) in the manufacture of a medicament for the treatment of hyperplastic or neoplastic disease including any such disease described herein.

In a corresponding aspect, the invention provides a method of treatment of a human or non-human animal (particularly one in need thereof) comprising administration of at least one tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor (including any such complex described herein). Such a method is preferably for the treatment of hyperplastic or neoplastic disease including any such disease described herein. Such a method is typically carried out on a human or non-human mammalian subject, such as one in need thereof.

In a further corresponding embodiment, the invention provides for a tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor (including all such complexes as disclosed herein) for use in therapy, and in particular for use in the treatment of hyperplastic and/or neoplastic disease including any such diseases and methods described herein.

Viewed from a further aspect the invention provides a pharmaceutical composition comprising a tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising

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group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor (including any such complex described herein) together with at least one pharmaceutical carrier or excipient.

So as to distinguish from thorium complexes of the most abundant naturally occurring thorium isotope, i.e. thorium-232 (half-life  $10^{10}$  years and effectively non-radioactive), it should be understood that the thorium complexes and the compositions thereof claimed herein include the alpha-emitting thorium radioisotope (i.e. at least one isotope of thorium with a half-life of less than  $10^3$  years, e.g. thorium-227) at greater than natural relative abundance, e.g. at least 20% greater. This need not affect the definition of the method of the invention where a therapeutically effective amount of a radioactive thorium, such as thorium-227 is explicitly required, but will preferably be the case in all aspects.

In all aspects of the invention, it is preferable that the alpha-emitting thorium ion is an ion of thorium-227. The 4+ ion of thorium is a preferable ion for use in the complexes of the present invention. Correspondingly, the 4+ ion of thorium-227 is highly preferred.

Viewed from a yet still further aspect the invention also provides a kit for use in a method according to the invention, said kit comprising a tissue targeting moiety, conjugated or conjugatable to an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties, where at least one of the four HOPO moieties is substituted at the N-position (1-position) with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor. All binding moieties and ligands preferably being those described herein. Such a kit will optionally and preferably include an alpha-emitting thorium radionuclide, such as  $^{227}\text{Th}$ .

In a further aspect the invention furthermore provides a method for the formation of a tissue-targeting complex, said method comprising coupling a tissue targeting moiety to an octadentate hydroxypyridinone-containing ligand in aqueous solution, the complex comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue

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targeting moiety has binding affinity for the CD22 receptor. Such a method may be conducted in the substantial absence of any organic solvent.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, "tissue targeting" is used herein to indicate that the substance in question (particularly when in the form of a tissue-targeting complex as described herein), serves to localise itself (and particularly to localise any conjugated thorium complex) preferentially to at least one tissue site at which its presence (e.g. to deliver a radioactive decay) is desired. Thus a tissue targeting group or moiety serves to provide greater than average localisation to at least one desired site in the body of a subject following administration to that subject. The targeting moiety in the present case will be selected to bind specifically to cell-surface receptor CD22. This may be reflected, for example by having 100 or more times greater binding affinity for cells expressing CD22 than for non-CD22 expressing cells. It is believed that CD22 is expressed and/or over-expressed in cells having certain disease states (as indicated herein) and thus the CD22 specific binder may serve to target the complex to such disease-affected cells. Similarly a tissue targeting moiety may bind to cell-surface markers (e.g. CD22 receptors) present on cells in the vicinity of disease affected cells. CD22 cell-surface markers may be more heavily expressed on diseased cell surfaces than on healthy cell surfaces or more heavily expressed on cell surfaces during periods of growth or replication than during dormant phases. In one embodiment, a CD22 specific binding ligand may be used in combination with another binder for a disease-specific cell-surface marker, thus giving a dual-binding complex.

The tissue targeting moiety may also comprise two or more components collectively having the effect of targeting the thorium complex to the desired tissue(s). This may be, for example, where one component is administered first and binds to a particular tissue, tumour or cell-type (a tissue-binding agent) and a second and/or further component (linking agent) is administered simultaneously, or preferably subsequently, which binds *in vivo* to the tissue-binding agent. The linking agent would be conjugated directly or indirectly to the complexed alpha-emitting thorium and thus collectively the tissue-binding and linking agents would form a tissue-targeting moiety. Suitable specific binding pairs suitable for providing the tissue

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binding agent and linking agent with mutual affinity are well known in the art (e.g. biotin with avidin or streptavidin).

The various aspects of the invention as described herein relate to treatment of disease, particularly for the selective targeting of diseased tissue, as well as relating to complexes, conjugates, medicaments, formulation, kits etc useful in such methods. In all aspects, the diseased tissue may reside at a single site in the body (for example in the case of a localised solid tumour) or may reside at a plurality of sites (for example where several joints are affected in arthritis or in the case of a distributed or metastasised cancerous disease).

The diseased tissue to be targeted may be at a soft tissue site, at a calcified tissue site or a plurality of sites which may all be in soft tissue, all in calcified tissue or may include at least one soft tissue site and/or at least one calcified tissue site. In one embodiment, at least one soft tissue site is targeted. The sites of targeting and the sites of origin of the disease may be the same, but alternatively may be different (such as where metastatic sites are specifically targeted). Where more than one site is involved this may include the site of origin or may be a plurality of secondary sites.

The term "soft tissue" is used herein to indicate tissues which do not have a "hard" mineralised matrix. In particular, soft tissues as used herein may be any tissues that are not skeletal tissues. Correspondingly, "soft tissue disease" as used herein indicates a disease occurring in a "soft tissue" as used herein. The invention is particularly suitable for the treatment of cancers and "soft tissue disease" thus encompasses carcinomas, sarcomas, myelomas, leuukemias, lymphomas and mixed type cancers occurring in any "soft" (i.e. non-mineralised) tissue, as well as other non-cancerous diseases of such tissue. Cancerous "soft tissue disease" includes solid tumours occurring in soft tissues as well as metastatic and micro-metastatic tumours. Indeed, the soft tissue disease may comprise a primary solid tumour of soft tissue and at least one metastatic tumour of soft tissue in the same patient. Alternatively, the "soft tissue disease" may consist of only a primary tumour or only metastases with the primary tumour being a skeletal disease. Particularly suitable for treatment and/or targeting in all appropriate aspects of the invention are hematological neoplasms and especially neoplastic diseases of lymphoid cells, such as lymphomas and lymphoid leukemias, including Non-Hodgkin's Lymphoma,

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B-cell neoplasms of B-cell lymphomas. Similarly, any neoplastic diseases of bone marrow, spine (especially spinal cord) lymph nodes and/or blood cells are suitable for treatment and/or targeting in all appropriate aspects of the invention.

Some examples of B-cell neoplasms that are suitable for treatment and/or targeting in appropriate aspects of the present invention include:

Chronic lymphocytic leukemia/Small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma (such as Waldenström macroglobulinemia), Splenic marginal zone lymphoma, Plasma cell neoplasms (e.g. Plasma cell myeloma, Plasmacytoma, Monoclonal immunoglobulin deposition diseases, Heavy chain diseases), Extranodal marginal zone B cell lymphoma (MALT lymphoma), Nodal marginal zone B cell lymphoma (NMZL), Follicular lymphoma, Mantle cell lymphoma, Diffuse large B cell lymphoma, Mediastinal (thymic) large B cell lymphoma, Intravascular large B cell lymphoma, Primary effusion lymphoma and Burkitt lymphoma/leukemia.

It is a key recent finding that certain alpha-radioactive thorium isotopes (e.g.  $^{227}\text{Th}$ ) may be administered in an amount that is both therapeutically effective and does not generate intolerable myelotoxicity. As used herein, the term "acceptably non-myelotoxic" is used to indicate that, most importantly, the amount of radium-223 generated by decay of the administered thorium-227 radioisotope is generally not sufficient to be directly lethal to the subject. It will be clear to the skilled worker, however, that the amount of marrow damage (and the probability of a lethal reaction) which will be an acceptable side-effect of such treatment will vary significantly with the type of disease being treated, the goals of the treatment regimen, and the prognosis for the subject. Although the preferred subjects for the present invention are humans, other mammals, particularly dogs, will benefit from the use of the invention and the level of acceptable marrow damage may also reflect the species of the subject. The level of marrow damage acceptable will generally be greater in the treatment of malignant disease than for non-malignant disease. One well known measure of the level of myelotoxicity is the neutrophil cell count and, in the present invention, an acceptably non-myelotoxic amount of  $^{223}\text{Ra}$  will typically be an amount controlled such that the neutrophil fraction at its lowest point (nadir) is no less than 10% of the count prior to treatment. Preferably, the acceptably non-myelotoxic amount of  $^{223}\text{Ra}$  will be an amount such that the

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neutrophil cell fraction is at least 20% at nadir and more preferably at least 30%. A nadir neutrophil cell fraction of at least 40% is most preferred.

In addition, radioactive thorium (e.g.  $^{227}\text{Th}$ ) containing compounds may be used in high dose regimens where the myelotoxicity of the generated radium (e.g.  $^{223}\text{Ra}$ ) would normally be intolerable when stem cell support or a comparable recovery method is included. In such cases, the neutrophil cell count may be reduced to below 10% at nadir and exceptionally will be reduced to 5% or if necessary below 5%, providing suitable precautions are taken and subsequent stem cell support is given. Such techniques are well known in the art.

A thorium isotope of particular interest in the present invention is thorium-227, and thorium-227 is the preferred isotope for all references to thorium herein where context allows. Thorium-227 is relatively easy to produce and can be prepared indirectly from neutron irradiated  $^{226}\text{Ra}$ , which will contain the mother nuclide of  $^{227}\text{Th}$ , i.e.  $^{227}\text{Ac}$  ( $T_{1/2} = 22$  years). Actinium-227 can quite easily be separated from the  $^{226}\text{Ra}$  target and used as a generator for  $^{227}\text{Th}$ . This process can be scaled to industrial scale if necessary, and hence the supply problem seen with most other alpha-emitters considered candidates for molecular targeted radiotherapy can be avoided.

Thorium-227 decays via radium-223. In this case the primary daughter has a half-life of 11.4 days. From a pure  $^{227}\text{Th}$  source, only moderate amounts of radium are produced during the first few days. However, the potential toxicity of  $^{223}\text{Ra}$  is higher than that of  $^{227}\text{Th}$  since the emission from  $^{223}\text{Ra}$  of an alpha particle is followed within minutes by three further alpha particles from the short-lived daughters (see Table 2 below which sets out the decay series for thorium-227).

Table 2

Nuclide	Decay mode	Mean particle energy (MeV)	Half-life
$^{227}\text{Th}$	$\alpha$	6.02	18.72 days
$^{223}\text{Ra}$	$\alpha$	5.78	11.43 days
$^{219}\text{Rn}$	$\alpha$	6.88	3.96 seconds

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$^{215}\text{Po}$	$\alpha$	7.53	1.78 ms
$^{211}\text{Pb}$	$\beta$	0.45	36.1 minutes
$^{211}\text{Bi}$	$\alpha$	6.67	2.17 minutes
$^{207}\text{TI}$	$\beta$	1.42	4.77 minutes
$^{207}\text{Pb}$			Stable

Partly because it generates potentially harmful decay products, thorium-227 ( $T_{1/2} = 18.7$  days) has not been widely considered for alpha particle therapy.

Thorium-227 may be administered in amounts sufficient to provide desirable therapeutic effects without generating so much radium-223 as to cause intolerable bone marrow suppression. It is desirable to maintain the daughter isotopes in the targeted region so that further therapeutic effects may be derived from their decay. However, it is not necessary to maintain control of the thorium decay products in order to have a useful therapeutic effect without inducing unacceptable myelotoxicity.

Assuming the tumour cell killing effect will be mainly from thorium-227 and not from its daughters, the likely therapeutic dose of this isotope can be established by comparison with other alpha emitters. For example, for astatine-211, therapeutic doses in animals have been typically 2-10 MBq per kg. By correcting for half-life and energy the corresponding dosage for thorium-227 would be at least 36-200 kBq per kg of bodyweight. This would set a lower limit on the amount of  $^{227}\text{Th}$  that could usefully be administered in expectation of a therapeutic effect. This calculation assumes comparable retention of astatine and thorium. Clearly however the 18.7 day half-life of the thorium will most likely result in greater elimination of this isotope before its decay. This calculated dosage should therefore normally be considered to be the minimum effective amount. The therapeutic dose expressed in terms of fully retained  $^{227}\text{Th}$  (i.e.  $^{227}\text{Th}$  which is not eliminated from the body) will typically be at least 18 or 25 kBq/kg, preferably at least 36 kBq/kg and more preferably at least 75 kBq/kg, for example 100 kBq/kg or more. Greater amounts of thorium would be expected to have greater therapeutic effect but cannot be administered if intolerable side effects will result. Equally, if the thorium is administered in a form having a short biological half-life (i.e. the half life before elimination from the body still carrying the thorium), then greater amounts of the

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radioisotope will be required for a therapeutic effect because much of the thorium will be eliminated before it decays. There will, however, be a corresponding decrease in the amount of radium-223 generated. The above amounts of thorium-227 to be administered when the isotope is fully retained may easily be related to equivalent doses with shorter biological half-lives. Such calculations are well known in the art and given in WO 04/091668 (e.g. in the text and in Examples 1 and 2).

If a radiolabelled compound releases daughter nuclides, it is important to know the fate, if applicable, of any radioactive daughter nuclide(s). With  $^{227}\text{Th}$ , the main daughter product is  $^{223}\text{Ra}$ , which is under clinical evaluation because of its bone seeking properties. Radium-223 clears blood very rapidly and is either concentrated in the skeleton or excreted via intestinal and renal routes (see Larsen, *J Nucl Med* 43(5, Supplement): 160P (2002)). Radium-223 released *in vivo* from  $^{227}\text{Th}$  may therefore not affect healthy soft tissue to a great extent. In the study by Müller in *Int. J. Radiat. Biol.* 20:233-243 (1971) on the distribution of  $^{227}\text{Th}$  as the dissolved citrate salt, it was found that  $^{223}\text{Ra}$  generated from  $^{227}\text{Th}$  in soft tissues was readily redistributed to bone or was excreted. The known toxicity of alpha emitting radium, particularly to the bone marrow, is thus an issue with thorium dosages.

It was established for the first time in WO 04/091668 that, in fact, a dose of at least 200 kBq/kg of  $^{223}\text{Ra}$  can be administered and tolerated in human subjects. These data are presented in that publication. Therefore, it can now be seen that, quite unexpectedly, a therapeutic window does exist in which a therapeutically effective amount of  $^{227}\text{Th}$  (such as greater than 36 kBq/kg) can be administered to a mammalian subject without the expectation that such a subject will suffer an unacceptable risk of serious or even lethal myelotoxicity. Nonetheless, it is extremely important that the best use of this therapeutic window be made and therefore it is essential that the radioactive thorium be quickly and efficiently complexed, and held with very high affinity so that the greatest possible proportion of the dose is delivered to the target site.

The amount of  $^{223}\text{Ra}$  generated from a  $^{227}\text{Th}$  pharmaceutical will depend on the biological half-life of the radiolabelled compound. The ideal situation would be to use a complex with a rapid tumour uptake, including internalization into tumour cell, strong tumour retention and a short biological half-life in normal tissues.

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Complexes with less than ideal biological half-life can however be useful as long as the dose of  $^{223}\text{Ra}$  is maintained within the tolerable level. The amount of radium-223 generated *in vivo* will be a factor of the amount of thorium administered and the biological retention time of the thorium complex. The amount of radium-223 generated in any particular case can be easily calculated by one of ordinary skill. The maximum administrable amount of  $^{227}\text{Th}$  will be determined by the amount of radium generated *in vivo* and must be less than the amount that will produce an intolerable level of side effects, particularly myelotoxicity. This amount will generally be less than 300 kBq/kg, particularly less than 200 kBq/kg and more preferably less than 170 kBq/kg (e.g. less than 130 kBq/kg). The minimum effective dose will be determined by the cytotoxicity of the thorium, the susceptibility of the diseased tissue to generated alpha irradiation and the degree to which the thorium is efficiently combined, held and delivered by the targeting complex (being the combination of the ligand and the targeting moiety in this case).

In the method of invention, the thorium complex is desirably administered at a thorium-227 dosage of 18 to 400 kBq/kg bodyweight, preferably 36 to 200 kBq/kg, (such as 50 to 200 kBq/kg) more preferably 75 to 170 kBq/kg, especially 100 to 130 kBq/kg. Correspondingly, a single dosage until may comprise around any of these ranges multiplied by a suitable bodyweight, such as 30 to 150 Kg, preferably 40 to 100 Kg (e.g. a range of 540 kBq to 4000 kBq per dose etc). The thorium dosage, the complexing agent and the administration route will moreover desirably be such that the radium-223 dosage generated *in vivo* is less than 300 kBq/kg, more preferably less than 200 kBq/kg, still more preferably less than 150 kBq/kg, especially less than 100 kBq/kg. Again, this will provide an exposure to  $^{223}\text{Ra}$  indicated by multiplying these ranges by any of the bodyweights indicated. The above dose levels are preferably the fully retained dose of  $^{227}\text{Th}$  but may be the administered dose taking into account that some  $^{227}\text{Th}$  will be cleared from the body before it decays.

Where the biological half-life of the  $^{227}\text{Th}$  complex is short compared to the physical half-life (e.g. less than 7 days, especially less than 3 days) significantly larger administered doses may be needed to provide the equivalent retained dose. Thus, for example, a fully retained dose of 150 kBq/kg is equivalent to a complex with a 5 day half-life administered at a dose of 711 kBq/kg. The equivalent administered

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dose for any appropriate retained doses may be calculated from the biological clearance rate of the complex using methods well known in the art.

Since the decay of one <sup>227</sup>Th nucleus provides one <sup>223</sup>Ra atom, the retention and therapeutic activity of the <sup>227</sup>Th will be directly related to the <sup>223</sup>Ra dose suffered by the patient. The amount of <sup>223</sup>Ra generated in any particular situation can be calculated using well known methods.

In a preferred embodiment, the present invention therefore provides a method for the treatment of disease in a mammalian subject (as described herein), said method comprising administering to said subject a therapeutically effective quantity of a conjugate comprising a tissue targeting moiety, an octadentate ligand (especially any of those described herein) and a radioactive thorium isotope (e.g. thorium-227).

It is obviously desirable to minimise the exposure of a subject to the <sup>223</sup>Ra daughter isotope, unless the properties of this are usefully employed. In particular, the amount of radium-223 generated *in vivo* will typically be greater than 40 kBq/kg, e.g. greater than 60 kBq/Kg. In some cases it will be necessary for the <sup>223</sup>Ra generated *in vivo* to be more than 80 kBq/kg, e.g. greater than 100 or 115 kBq/kg.

Thorium-227 labelled conjugates in appropriate carrier solutions may be administered intravenously, intracavitory (e.g. intraperitoneally), subcutaneously, orally or topically, as a single application or in a fractionated application regimen. Preferably the complexes conjugated to a targeting moiety will be administered as solutions by a parenteral (e.g. transcutaneous) route, especially intravenously or by an intracavitory route. Preferably, the compositions of the present invention will be formulated in sterile solution for parenteral administration.

Thorium-227 in the methods and products of the present invention can be used alone or in combination with other treatment modalities including surgery, external beam radiation therapy, chemotherapy, other radionuclides, or tissue temperature adjustment etc. This forms a further, preferred embodiment of the method of the invention and formulations/medicaments may correspondingly comprise at least one additional therapeutically active agent such as another radioactive agent or a chemotherapeutic agent.

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In one particularly preferred embodiment the subject is also subjected to stem cell treatment and/or other supportive therapy to reduce the effects of radium-223 induced myelotoxicity.

According to this invention  $^{227}\text{Th}$  may be complexed by targeting complexing agents. Typically the targeting moieties will have a molecular weight from 100 g/mol to several million g/mol (particularly 100 g/mol to 1 million g/mol), and will preferably have affinity for a disease-related receptor either directly, and/or will comprise a suitable pre-administered binder (e.g. biotin or avidin) bound to a molecule that has been targeted to the disease in advance of administering  $^{227}\text{Th}$ . Suitable targeting moieties include poly- and oligo-peptides, proteins, DNA and RNA fragments, aptamers etc, preferably a protein, e.g. avidin, streptavidin, a polyclonal or monoclonal antibody (including IgG and IgM type antibodies), or a mixture of proteins or fragments or constructs of protein. Antibodies, antibody constructs, fragments of antibodies (e.g. Fab fragments or any fragment comprising at least one antigen binding region(s)), constructs of fragments (e.g. single chain antibodies) or a mixture thereof are particularly preferred. Suitable fragments particularly include Fab, F(ab')<sub>2</sub>, Fab' and/or scFv. Antibody constructs may be of any antibody or fragment indicated herein.

In one aspect, the specific binder (tissue targeting moiety) may be an peptide with sequence similarity or identity with at least one sequence as set out below:

Light Chain:

Murine DIQLTQSPSLAVSAGENVTMS**C**KSSQSVLYSAHKNYL**A**WYQQKPGQSP  
Humanised -----SA-V-DR-----KA

Murine KLIY**W**ASTRE**S**GVPDRFTGSGSTDFTLTISRVQVEDLAIYY**C**HQYL**S**  
Humanised -----S-S-----F---SL-P--I-T-----

Murine **W**TFGGGTKLEIKR (SeqID1)  
Humanised ----- (SeqID2)

Heavy Chain:

Murine QVQLQESGAELSKPGASVKMSCKASGYTF**T**S**Y**W**L**HWIKQRPGQGLEWIG  
H'ised1 -----Q---VK---S---V-----VR-A-----

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H'ised2	----VQ----VK---S---V-----VR-A-----
Murine	<b>YINPRNDYTEYNQNFKD</b> <u>KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR</u>
H'ised1	-----I---E-TN---E---R---T-F-F---
H'ised2	-----I---E-TN---E---R---T-F-F---
Murine	<b>RDITTFYWGQGTTLVSS</b> (SeqID3)
H'ised1	-----V---- (SeqID4)
H'ised2	-----V---- (SeqID5)

In the above sequences, "-" in the Humanised (H'ised) sequences indicates that the residue is unchanged from the murine sequence.

In the above sequences (SeqID1-5), the bold regions are believed to be the key specific-binding regions (CDRs), the underlined regions are believed to be of secondary importance in binding and the unemphasised regions are believed to represent structural, rather than specific binding regions.

In all aspects of the invention, the tissue targeting moiety may have a sequence having substantial sequence identity or substantial sequence similarity to at least one of any of those sequences set out in SeqID 1 to 5. Substantial sequence identity/similarity may be taken as having a sequence similarity/identity of at least 80% to the complete sequences and/or at least 90% to the specific binding regions (those regions shown in bold in the above sequences and optionally those sections underlined). Preferable sequence similarity or more preferably identity may be at least 92%, 95%, 97%, 98% or 99% for the bold regions and preferably also for the full sequences. Sequence similarity and/or identity may be determined using the "BestFit" program of the Genetics Computer Group Version 10 software package from the University of Wisconsin. The program uses the local had algorithm of Smith and Waterman with default values: Gap creation penalty=8, Gap extension penalty=2, Average match=2.912, average mismatch 2.003.

A tissue targeting moiety may comprise more than one peptide sequence, in which case at least one, and preferably all sequences may (independently) conform to the above-described sequence similarity and preferably sequence identity with any of SeqID1-5.

A tissue targeting moiety will have binding affinity for CD22 and in one embodiment may also have a sequence with up to about 40 variations for the full domains (preferably 0 to 30 variations). Variants may be by insertion, deletion and/or

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substitution and may be contiguous or non-contiguous with respect to seqIDs 1-5. Substitutions or insertions will typically be by means of at least one of the 20 amino acids of the genetic code and substitutions will most generally be conservative substitutions. However, in one embodiment, at least one insertion and/or substitution may be made to an amino acid having a reactive side-chain useful for linking to the ligand moiety. Such a side-chain may comprise, for example, at least one thiol, amine, alcohol, acid, or amide group or any protected equivalent thereof (e.g. ester, thioester etc). Protective groups are well known in organic chemistry and may be selected from standard texts such as "Protective Groups in Organic Chemistry" by Theodora Greene (incorporated herein by reference).

Generally, the octadentate ligand is conjugated directly or indirectly (e.g. via a linker moiety) to the targeting moiety. General constructs of this type; i.e. of active (e.g. therapeutically or diagnostically active) metal - complexing moiety - optional linker moiety - targeting moiety, are well known in the fields of targeted radiopharmaceuticals and targeted imaging agents. However, little or no work is available assessing the suitability of various ligands for specific use with thorium 4+ ions. In this regard reference may be had for example to "Handbook of Targeted Delivery of Imaging Agents", Ed. Torchilin, CRC Press, 1995.

The most relevant previous work on thorium ions with hydroxypyridinone ligands was published as WO2011/098611 and discloses the relative ease of generation of thorium ions complexed with octadentate HOPO-containing ligands.

Previously known chelators for thorium also include the polyaminopolyacid chelators which comprise a linear, cyclic or branched polyazaalkane backbone with acidic (e.g. carboxyalkyl) groups attached at backbone nitrogens. Examples of such chelators include DOTA derivatives such as p-isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bz-DOTA) and DTPA derivatives such as p-isothiocyanatobenzyl-diethylenetriaminepentaacetic acid ( p-SCN- Bz-DTPA), the first being cyclic chelators, the latter linear chelators.

Derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid have been previously exemplified, but standard methods cannot easily be used to chelate thorium with DOTA derivatives. Heating of the DOTA derivative with the metal provides the chelate effectively, but often in low yields. There is a tendency for at

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least a portion of the ligand to irreversibly denature during the procedure. Furthermore, because of its relatively high susceptibility to irreversible denaturation, it is generally necessary to avoid attachment of the targeting moiety until all heating steps are completed. This adds an extra chemical step (with all necessary work-up and separation) which must be carried out during the decay lifetime of the alpha-emitting thorium isotope. Obviously it is preferable not to handle alpha-emitting material in this way or to generate corresponding waste to a greater extent than necessary. Furthermore, all time spent preparing the conjugate wastes a proportion of the thorium which will decay during this preparatory period.

It is preferred that the complexes of alpha-emitting thorium and an octadentate ligand in all aspects of the present invention are formed or formable without heating above 60°C (e.g. without heating above 50°C), preferably without heating above 38°C and most preferably without heating above 25°C.

It is additionally preferred that the conjugate of the targeting moiety and the octadentate ligand be prepared prior to addition of the alpha-emitting thorium isotope (e.g.  $^{227}\text{Th}^{4+}$  ion). The products of the invention are thus preferably formed or formable by complexation of alpha-emitting thorium isotope (e.g.  $^{227}\text{Th}^{4+}$  ion) by a conjugate of an octadentate ligand and a tissue-targeting moiety.

The chelators may be non-phosphonate molecules and in one embodiment of the present invention the  $^{227}\text{Th}$  will not be attached to any phosphonate or other bone-targeting group nor administered with such materials.

The present inventors have now established that complexes comprising octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide are highly amenable to generation at room temperature and/or physiological temperature (e.g. at 20°C or 37°C). Such complexes may be generated rapidly and furthermore since the temperature of generation is comparatively low the complexation of the thorium component may take place after the ligand moiety has been bound or otherwise conjugated to the tissue-targeting moiety, thus reducing the number of steps required after addition of the radioisotope.

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In addition to the above, the more water soluble nature of the octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties wherein at least one HOPO moiety comprises a hydroxyalkyl solubilising group serves to further improve the ease of manufacture of the complete conjugate. Specifically, during manufacturing of the conjugate a hydrophobic chelator, such as a previously known octadentate ligand, has to be dissolved in an organic solvent, such as DMSO or DMA. Removal of all traces of the organic solvent after conjugation is necessary but difficult with such non-volatile polar organic solvents and complete removal is difficult to prove analytically. Time spent in analysis is obviously undesirable where an alpha-emitter has been incorporated because the radionuclide continues to decay and the potency of the conjugate reduces with time.

Due to the requirement for an organic solvent, a hydrophobic chelator is challenging to combine not only with proteinaceous targeting molecules but even more so with alternative targeting molecules that are more hydrophilic, including nanoparticles having PEG or dextrane on the surface.

A PEG or alternative hydrophilic highly water soluble spacer may be desired for biological reasons, such as prolonged halflife or reducing an immune response. The manufacturing of the chelator – PEG unit prior to conjugation to the protein is also challenging due to the difference in solubility properties of the two parts. A PEG, or similar, spacer introduces more hydrophilicity into the molecule, between the chelating moiety and the carrier protein. However, this only moves the chelator further away from the carrier protein, while the hydrophobicity of the chelator is not affected. Therefore a hydrophobic chelator may still be recognized as a hydrophobic spot on the surface of the (PEGylated) targeting molecule and generate undesirable reactions as discussed herein above.

Various types of targeting compounds that may be linked to thorium (e.g. thorium-227) via an octadentate chelator (comprising a coupling moiety as described herein). The targeting moiety may be selected from known targeting groups, which include monoclonal or polyclonal antibodies, growth factors, peptides, hormones and hormone analogues, folate and folate derivatives, botin, avidin and streptavidin or analogues thereof. Other possible targeting groups include RNA, DNA, or fragments thereof (such as aptamer), oligonucleotides, carbohydrates, lipids or

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compounds made by combining such groups with or without proteins etc. PEG moieties may be included as indicated above, such as to increase the biological retention time and/or reduce the immune stimulation.

The tissue targeting moiety may, in one embodiment, exclude bone-seekers, liposomes and folate conjugated antibodies or antibody fragments. Alternatively, such moieties may be included.

The thorium (e.g. thorium-227) labelled molecules of the invention may be used for the treatment of cancerous or non-cancerous diseases by targeting disease-related receptors. Typically, such a medical use of  $^{227}\text{Th}$  will be by radioimmunotherapy based on linking  $^{227}\text{Th}$  by a chelator to an antibody, an antibody fragment, or a construct of antibody or antibody fragments for the treatment of cancerous or non-cancerous diseases. The use of  $^{227}\text{Th}$  in methods and pharmaceuticals according to the present invention is particularly suitable for the treatment of any form of cancer including carcinomas, sarcomas, lymphomas and leukemias, especially cancer of the lung, breast, prostate, bladder, kidney, stomach, pancreas, oesophagus, brain, ovary, uterus, oral cancer, colorectal cancer, melanoma, multiple myeloma and non-Hodgkin's lymphoma.

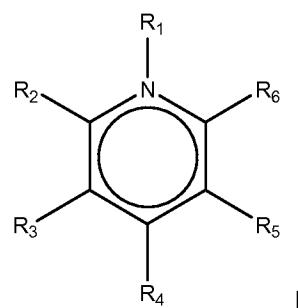
The amount of  $^{223}\text{Ra}$  released could be diminished if the molecule carrying  $^{227}\text{Th}$  has a short biological retention half-time *in vivo* because the radionuclide will mostly be eliminated before a high proportion of the  $^{227}\text{Th}$  has decayed to  $^{223}\text{Ra}$ . The amount of  $^{227}\text{Th}$  would, however, need to be increased in order to remain therapeutically effective, according to the present invention. If the complexing agent is selected so as to deliver the  $^{227}\text{Th}$  into the interior of the targeted cells, this will further increase the specific cytotoxicity and reduce the systemic toxic effect of the radioactive daughters because of at least partial retention of daughter isotopes at the tumour site. Both of these features widen the  $^{227}\text{Th}$  therapeutic window and thus form preferred embodiments of the invention.

In a further embodiment of the invention, patients with both soft tissue and skeletal disease may be treated both by the  $^{227}\text{Th}$  and by the  $^{223}\text{Ra}$  generated *in vivo* by the administered thorium. In this particularly advantageous aspect, an extra therapeutic component to the treatment is derived from the acceptably non-myelotoxic amount of  $^{223}\text{Ra}$  by the targeting of the skeletal disease. In this

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therapeutic method,  $^{227}\text{Th}$  is typically utilised to treat primary and/or metastatic cancer of soft tissue by suitable targeting thereto and the  $^{223}\text{Ra}$  generated from the  $^{227}\text{Th}$  decay is utilised to treat related skeletal disease in the same subject. This skeletal disease may be metastases to the skeleton resulting from a primary soft-tissue cancer, or may be the primary disease where the soft-tissue treatment is to counter a metastatic cancer. Occasionally the soft tissue and skeletal diseases may be unrelated (e.g. the additional treatment of a skeletal disease in a patient with a rheumatological soft-tissue disease).

A key aspect of the present invention in all respects is the use of an octadentate ligand, particularly an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties. Such ligands will typically comprise at least four chelating groups each independently having the following substituted pyridine structure (I):



Wherein  $R_1$  is an optional N-substituent solubilising group which will be present in at least one of the four moieties of formula I and may be present in 2, 3 or all 4 such moieties.  $R_1$  may thus be absent or may be selected from OH and hydroxyalkyl moieties. Suitable hydroxyalkyl moieties will comprise at least one OH group but may optionally comprise more than one, such as two, three or four OH groups. One or two OH groups are most preferred on the hydroxyalkyl moiety.

The nitrogen on the pyridinone ring of HOPO moiety (especially the 3,2-HOPO and 2,3-HOPO) is a suitable point for introducing hydrophilic substituents without grossly affecting the properties of the ring, and importantly, which will face outwards after conjugating the molecule to a carrier protein or other targeting molecule. We have previously shown that a chelator based on a pyrimidone ring having a methyl group in this position is suitable for chelation of thorium ions. The novel chelators have alternative groups introduced, including a hydroxyethyl at the N-position.

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Surprisingly, the minor change from methyl to hydroxylethyl resulted in a chelator which was completely soluble in pure water. This molecule and some related examples are shown below.

As used herein, all hydrocarbyl moieties are independently selected from short hydrocarbyl groups, such as C1 to C8 hydrocarbyl, including C1 to C8 alkyl, alkenyl or alkynyl groups. Correspondingly, alkyl groups will typically be straight or branched chain C1 to C8 alkyl groups such as methyl, ethyl, n- or iso-propyl, n-, iso- or sec-butyl and so forth.

Highly preferred R<sub>1</sub> groups include straight or branched chain alkyl groups (such as those indicated above) having one, two or more hydroxy groups attached to a carbon atom of the alkyl chain. Some highly preferred hydroxyalkyl groups include hydroxymethyl, hydroxyethyl, hydroxy n-propyl, hydroxy iso-propyl, di-hydroxy n-propyl (e.g. 1,2-, 2,3- or 1,3-di-hydroxy propyl), hydroxy n-butyl, di-hydroxy n-butyl and tri-hydroxy n-butyl with hydroxyethyl being most highly preferred. In one embodiment, each of the 4 HOPO moieties of the octadentate ligand will comprise a hydroxylakyl (such as hydroxyethyl) group at position R<sub>1</sub>. In a further embodiment, all four HOPO moieties will comprise the same hydroxyalkyl group (e.g. all 4 HOPO groups will be N-substituted with hydroxyethyl or all 4 will be substituted with di-hydroxy propyl).

In a highly preferred embodiment, all 4 HOPO groups will be the same HOPO group selected from 3,2 HOPO and 2,3, HOPO groups. In a further highly preferred embodiment (which may optionally be combined with the previous), all four HOPO groups will be N-substituted with the same hydroxyalkly group selected from hydroxymethyl, hydroxyethyl, hydroxy propyl, hydroxybutyl, dihydroxypropyl and dihydroxybutyl. Of this list hydroxyethyl, hydroxypropyl and dihydroxypropyl are most preferred.

In Formula I, groups R<sub>2</sub> to R<sub>6</sub> may each independently be selected from H, OH, =O, short hydrocarbyl (as described herein), a linker moiety (as described herein) and/or a coupling moiety (as described herein). Generally, at least one of groups R<sub>2</sub> to R<sub>6</sub> will be OH. Generally, at least one of groups R<sub>2</sub> to R<sub>6</sub> will be =O. Generally, at least one of groups R<sub>2</sub> to R<sub>6</sub> will be a linker moiety (as described herein). Preferably, exactly one of groups R<sub>2</sub> to R<sub>6</sub> will be =O. Preferably exactly

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one of groups  $R_2$  to  $R_6$  will be OH. Preferably exactly one of groups  $R_2$  to  $R_6$  will be a linker moiety (as described herein). The remaining groups  $R_2$  to  $R_6$  may be any of those moieties indicated herein, but are preferably H. Where a linker moiety or any additional linker, template or chelating groups attached to a linker moiety do not comprise a coupling moiety then one of groups  $R_1$  to  $R_6$  is preferably a coupling moiety (as described herein).

In a preferred embodiment, one of groups  $R_2$  to  $R_6$  will be OH and one of  $R_2$  to  $R_6$  will be =O and the OH and =O groups will be on neighbouring atoms of the ring. Thus, in a preferred embodiment, OH and =O may be on atoms 2,3; 3,2; 3,4; or 4,3 respectively (numbering from the nitrogen as would be expected). Octadentate ligands having at least one chelating moiety wherein OH and =O groups are present at positions 3 and 2 respectively are highly preferred. The octadentate ligands may have 2, 3 or 4 such chelating groups, where 2 or 4 such groups are highly preferred. N-substituted 3,2-HOPO moieties are highly preferred as all four complexing moieties of the octadentate ligand.

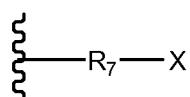
Suitable chelating moieties may be formed by methods known in the art, including the methods described in US 5,624,901 (e.g. examples 1 and 2) and WO2008/063721 (both incorporated herein by reference).

As used herein, the term "linker moiety" ( $R_L$  in formula II) is used to indicate a chemical entity which serves to join at least two chelating groups in the octadentate ligands, which form a key component in various aspects of the invention. Linker moieties may also join the octadentate ligand portion to the tissue targeting moiety. Typically, each chelating group (e.g. those of formula I above and/or formula II below) will be bi-dentate and so four chelating groups, of which at least one is of formula I, will typically be present in the ligand. Such chelating groups are joined to each other by means of their linker moieties. Thus, a linker moiety (e.g. group  $R_L$  below) may be shared between more than one chelating group of formula I and/or II. The linker moieties may also serve as the point of attachment between the complexing part of the octadentate ligand and the targeting moiety. In such a case, at least one linker moiety will join to a coupling moiety ( $R_C$ ). Suitable linker moieties include short hydrocarbyl groups, such as C1 to C12 hydrocarbyl, including C1 to C12 alkyl, alkenyl or alkynyl group, including methyl, ethyl, propyl, butyl, pentyl and/or hexyl groups of all topologies.

Linker moieties may also be or comprise any other suitably robust chemical linkages including esters, ethers, amine and/or amide groups. The total number of atoms joining two chelating moieties (counting by the shortest path if more than one path exists) will generally be limited, so as to constrain the chelating moieties in a suitable arrangement for complex formation. Thus, linker moieties will typically be chosen to provide no more than 15 atoms between chelating moieties, preferably, 1 to 12 atoms, and more preferably 1 to 10 atoms between chelating moieties. Where a linker moiety joins two chelating moieties directly, the linker will typically be 1 to 12 atoms in length, preferably 2 to 10 (such as ethyl, propyl, n-butyl etc). Where the linker moiety joins to a central template (see below) then each linker may be shorter with two separate linkers joining the chelating moieties. A linker length of 1 to 8 atoms, preferably 1 to 6 atoms may be preferred in this case (methyl, ethyl and propyl being suitable, as are groups such as these having an ester, ether or amide linkage at one end or both).

In addition to the linker moiety, which primarily serves to link the various chelating groups of the octadentate ligand to each other and/or to a central template, the octadentate preferably further comprises a “coupling moiety” ( $R_C$ ). The function of the coupling moiety is to link the octadentate ligand to the targeting moiety. This may be achieved by either covalent or non-covalent attachment (e.g. by a specific binding pair such as biotin/avidin (streptavidin). Linker moieties as described above form possible coupling moieties. Preferably coupling moieties will be covalently linked to the chelating groups, either by direct covalent attachment to one of the chelating groups or more typically by attachment to a linker moiety or template. Should two or more coupling moieties be used, each can be attached to any of the available sites such as on any template, linker or chelating group.

In one embodiment, the coupling moiety may have the structure:



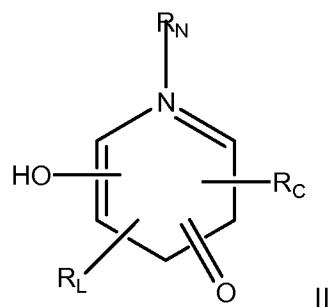
wherein  $R_7$  is a bridging moiety, which is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or

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unsubstituted heteroaryl; and X is a targeting moiety or a reactive functional group. The preferred bridging moieties include all those groups indicated herein as suitable linker moieties. Preferred targeting moieties include all of those described herein and preferred reactive X groups include any group capable of forming a covalent linkage to a targeting moiety, including, for example, COOH, OH, SH, NHR and COH groups, where the R of NHR may be H or any of the short hydrocarbyl groups described herein. Highly preferred groups for attachment onto the targeting moiety include epsilon-amines of lysine residues and thiol groups of cysteine residues. Non-limiting examples of suitable reactive X groups, include N-hydroxysuccimidylesters, imidoesters, acylhalides, N-maleimides, alpha-halo acetyl and isothiocyanates, where the latter three are suitable for reaction with a thiol group.

The coupling moiety is preferably attached, so that the resulting coupled octadentate ligand will be able to undergo formation of stable metal ion complexes. The coupling moiety will thus preferably link to the linker, template or chelating moiety at a site which does not significantly interfere with the complexation. Such a site will preferably be on the linker or template, more preferably at a position distant from the surface binding to the target.

Preferred chelating groups include those of formula II below:



In the above formula II, the =O moiety represents a keto-group attached to any carbon of the pyridine ring, the -OH represents a hydroxy moiety attached to any carbon of the pyridine ring and the -R<sub>L</sub> represents a linker moiety which attaches the hydroxypyridinone moiety to other complexing moieties so as to form the overall octadentate ligand. Any linker moiety described herein is suitable as R<sub>L</sub> including short hydrocarbyl groups, such as C1 to C8 hydrocarbyl, including C1 to C8 alkyl,

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alkenyl or alkynyl group, including methyl, ethyl, propyl, butyl, pentyl and/or hexyl groups of all topologies.  $R_L$  may join the ring of formula II at any carbon of the pyridine ring. The  $R_L$  groups may then in turn bond directly to another chelating moiety, to another linker group and/or to a central atom or group, such as a ring or other template (as described herein). The linkers, chelating groups and optional template moieties are selected so as to form an appropriate octadentate ligand.

In one preferred embodiment the -OH and =O moieties of formula II reside on neighbouring atoms of the pyridine ring, such that 2,3-, 3,2-; 4,3-; and 3,4-hydroxypyridinone derivatives are all highly suitable.

Moiety  $R_L$  resides on the nitrogen of the pyridine ring. Group  $R_N$  may be absent in some groups of formula II where more than one different group of formula II is present in the octadentate ligand. However, at least one  $R_N$  group in each octadentate ligand will be a hydroxylalkyl group as indicated herein.

In one preferred embodiment, at least one 3,2- hydroxypyridinone moiety is present in the octadentate ligand structure. This may evidently be substituted by any of the various substituent moieties indicated herein.

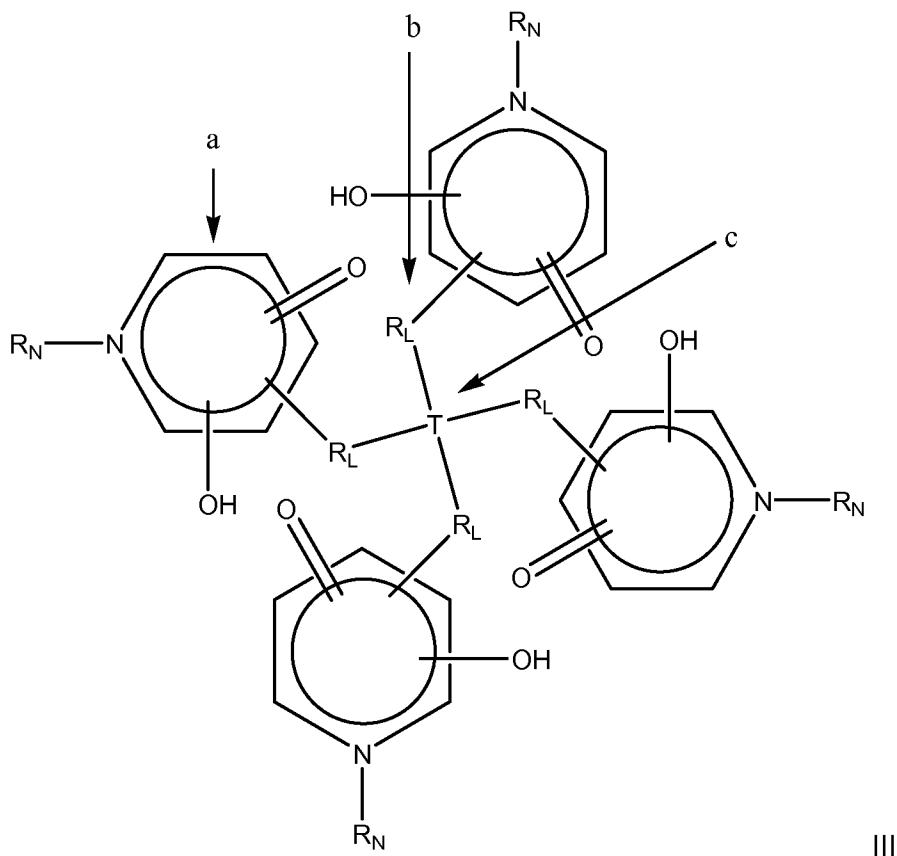
Since each of the moieties of formula II has two potentially complexing oxygens, one embodiment of the present invention provides for an octadentate ligand comprising at least 2, preferably at least 3 and most preferably 4 independently chosen moieties of formula II. Each moiety of formula II may have an independent substitution pattern, but in one preferred embodiment, at least one moiety is a 3,2-hydroxypyridinone moiety. The ligand may include 2, 3 or 4 3,2- hydroxypyridinone moieties (substituted as appropriate, as described herein).

Each moiety of formula I or II in the octadentate ligand may be joined to the remainder of the ligand by any appropriate linker group as discussed herein and in any appropriate topology. For example, four groups of formula I may be joined by their linker groups to a backbone so as to form a linear ligand, or may be bridged by linker groups to form a "oligomer" type structure, which may be linear or cyclic. Alternatively, the ligand moieties of formulae I and/or II may be joined in a "cross" or "star" topography to a central atom or group, each by a linker (e.g. " $R_L$ " moiety). Linker ( $R_L$ ) moieties may join solely through carbon-carbon bonds, or may attach to

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each other, to other chelating groups, to a backbone, template, coupling moiety or other linker by any appropriately robust functionality including an amine, amide, ester, ether, thio-ether or disulphide bond.

A "stellar" arrangement is indicated in formula III below:



Wherein all groups and positions are as indicated above and "T" is additionally a central atom or template group, such as a carbon atom, hydrocarby chain (such as any of those described herein above), aliphatic or aromatic ring (including heterocyclic rings) or fused ring system. The most basic template would be a single carbon, which would then attach to each of the chelating moieties by their linking groups. Longer chains, such as ethyl or propyl are equally viable with two chelating moieties attaching to each end of the template. Evidently, any suitably robust linkage may be used in joining the template and linker moieties including carbon-carbon bonds, ester, ether, amine, amide, thio-ether or disulphide bonds.

Evidently, in the structures of formula II III, IV and IVb, those positions of the pyridine ring(s) which are not otherwise substituted (e.g. by a linker or coupling

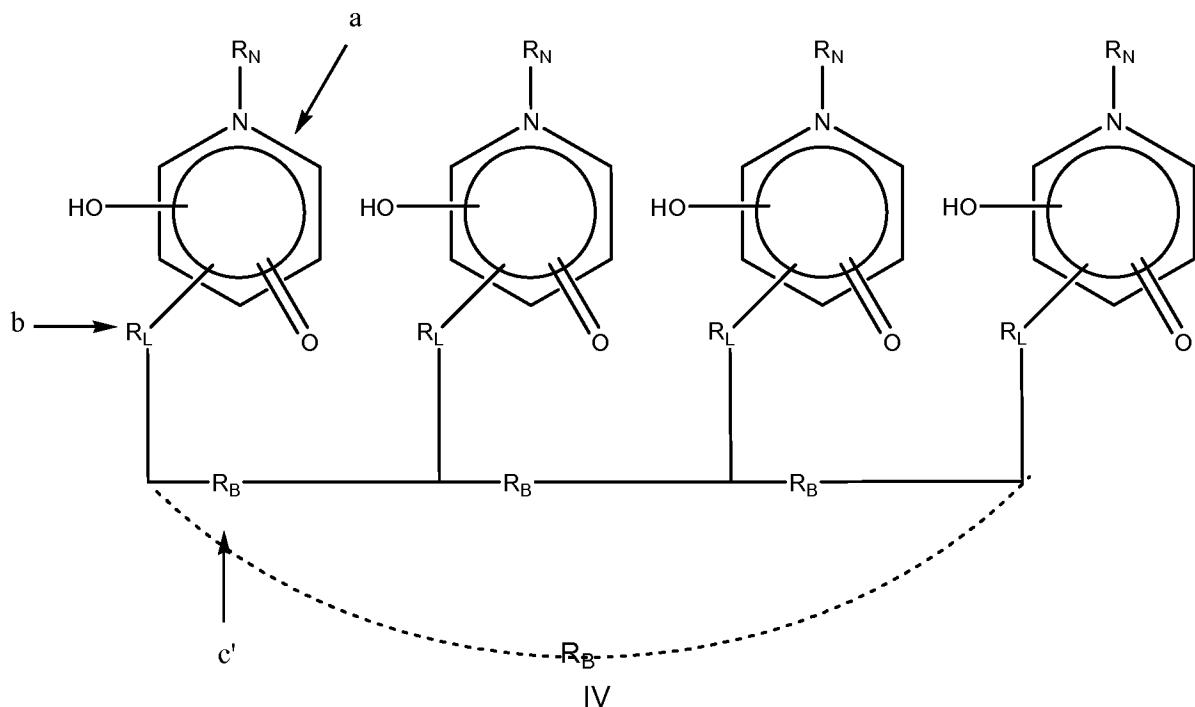
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moiety) may carry substituents described for  $R_1$  to  $R_5$  in Formula I, as appropriate. In particular, small alkyl substituents, such as methyl, ethyl or propyl groups may be present at any position.

The octadentate ligand will generally additionally comprise at least one coupling moiety as described above. This may be any suitable structure including any of those indicated herein and will terminate with the targeting moiety, a specific binder or a functional group capable of linking to such a targeting moiety or specific binder.

The coupling moiety may attach to any suitable point of the linker, template or chelating moiety, such as at points a), b) and/or c) as indicated in formula III. The attachment of the coupling moiety may be by any suitably robust linkage such as carbon-carbon bonds, ester, ether, amine, amide, thio-ether or disulphide bonds. Similarly, groups capable of forming any such linkages to the targeting moiety are suitable for the functional end of the coupling moiety and that moiety will terminate with such groups when attached to the targeting part.

An alternative, "backbone" type structure is indicated below in Formula IV



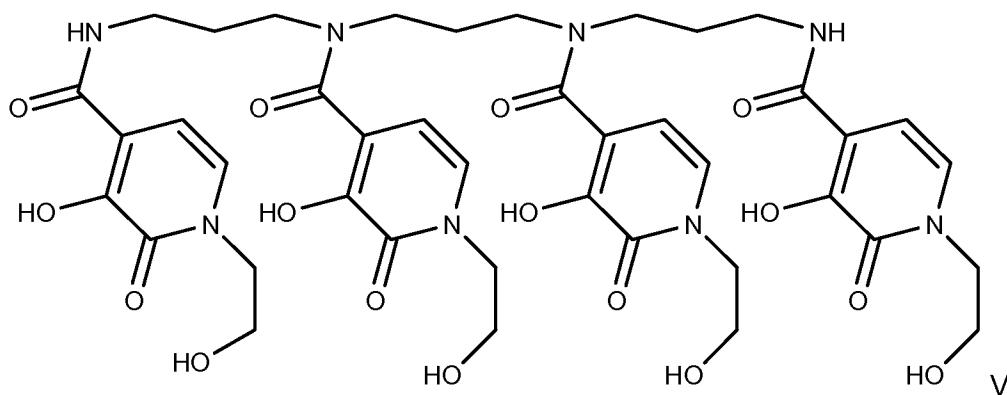
Wherein all groups and positions are as indicated above and " $R_B$ " is additionally a backbone moiety, which will typically be of similar structure and function to any of

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the linker moieties indicated herein, and thus any definition of a linker moiety may be taken to apply to the backbone moiety where context allow. Suitable backbone moieties will form a scaffold upon which the chelating moieties are attached by means of their linker groups. Usually three or four backbone moieties are required. Typically this will be three for a linear backbone or four if the backbone is cyclised. Particularly preferred backbone moieties include short hydrocarbon chains (such as those described herein) optionally having a heteroatom or functional moiety at one or both ends. Amine and amide groups are particularly suitable in this respect.

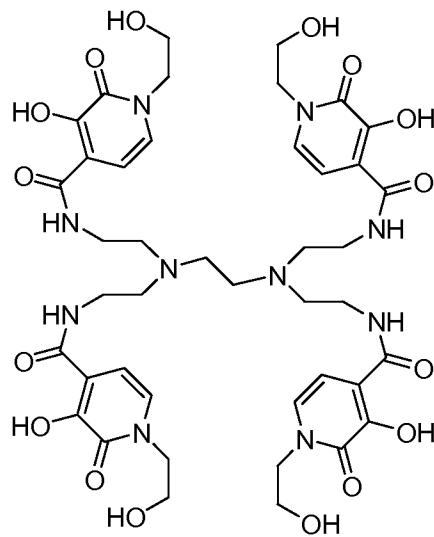
The coupling moiety may attach to any suitable point of the linker, backbone or chelating moiety, such as at points a), b) and/or c') as indicated in formula IV. The attachment of the coupling moiety may be by any suitably robust linkage such as carbon-carbon bonds, ester, ether, amine, amide, thio-ether or disulphide bonds. Similarly, groups capable of forming any such linkages to the targeting moiety are suitable for the functional end of the coupling moiety and that moiety will terminate with such groups when attached to the targeting part.

An example of a "backbone" type octadentate ligand having four 3,2-HOPO chelating moieties (each with a hydroxyethyl solubilising group) attached to a backbone by amide linker groups would be formula V as follows:



Evidently, a linker group  $R_L$  may be added at any suitable point on this molecule, such as at one of the secondary amine groups or as a branching point on any of the backbone alkyl groups. All small alkyl groups such as the backbone propylene or the n-substituting ethylene groups may be substituted with other small alkylanes such as any of those described herein (methylene, ethylene, propylene, and butylene being highly suitable among those).

Exemplary "templated" octadentate ligands, each having four 3,2-HOPO chelating moieties linked by ethyl amide groups to ethyl and propyl diamine respectively would be formula VI as follows:

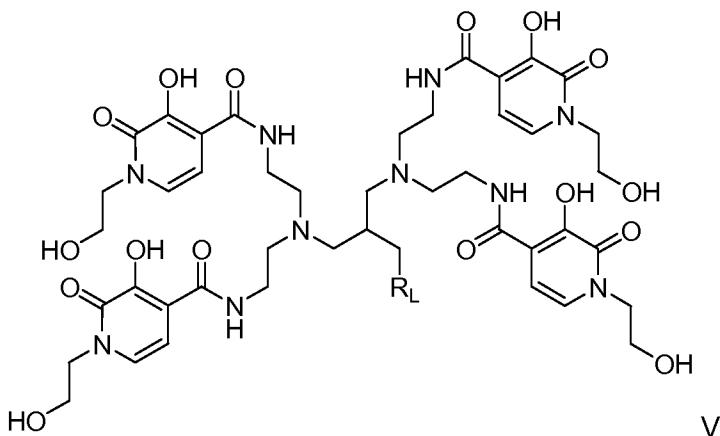


VI

Evidently, any of the alkylene groups, shown in formula VI as ethylene moieties may be independently substituted with other small alkylene groups such as methylene, propylene or n-butylene. It is preferred that some symmetry be retained in the molecule and thus, for example, the central ethylene group might be substituted with a propylene while the other ethylene groups remain, or the two ethylenes linking the HOPO moieties to one or both central tertiary amines may be replaced with methylene or propylene. Similarly, as discussed herein, the N-substituting groups may be replaced with any other hydroxyalkyl group as discussed herein throughout.

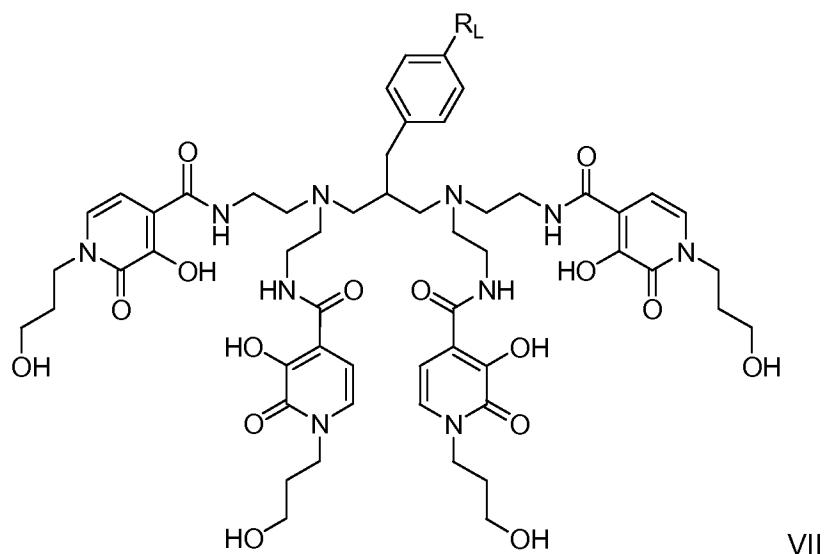
As indicated above, the octadentate ligand will typically include a coupling moiety which may join to the remainder of the ligand at any point. A suitable point for linker attachment is shown below in formula VI:

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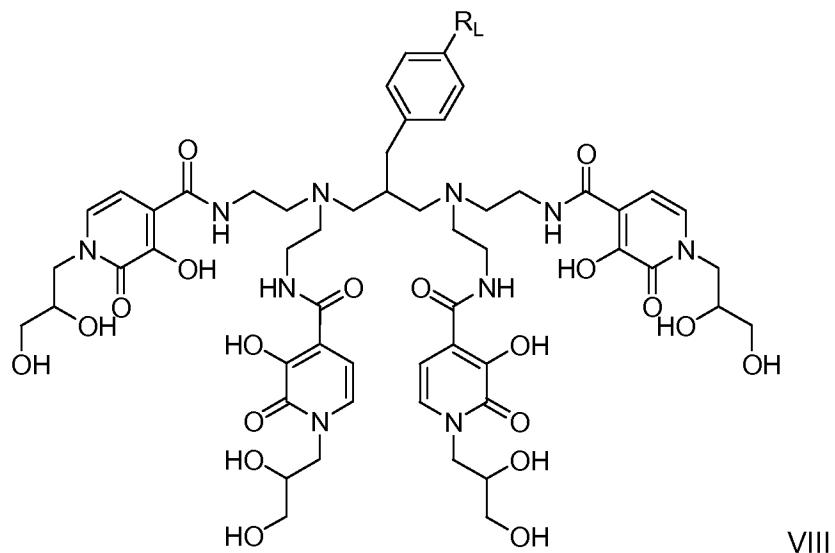


Wherein  $R_L$  is any suitable linking moiety, particularly for attachment to a tissue targeting group. A short hydrocarbyl group such as a C1 to C8 cyclic, branched or straight chain aromatic or aliphatic group terminating in an active group such as an amine is highly suitable as group  $R_L$  in formula VI and herein throughout.

Highly preferred octadentate ligands showing suitable sites for ligand attachment include those of formulae VII and VIII below:

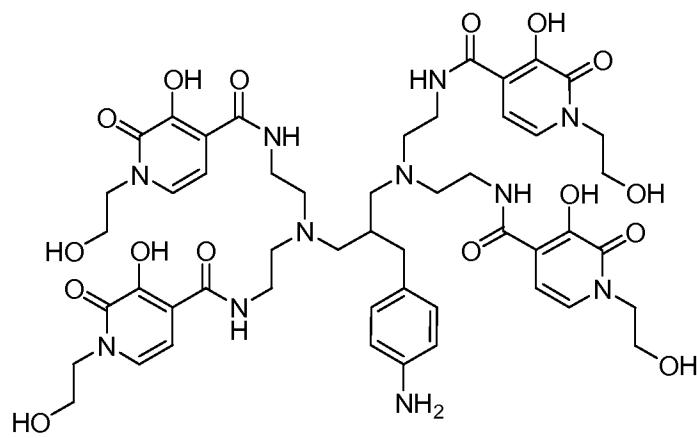


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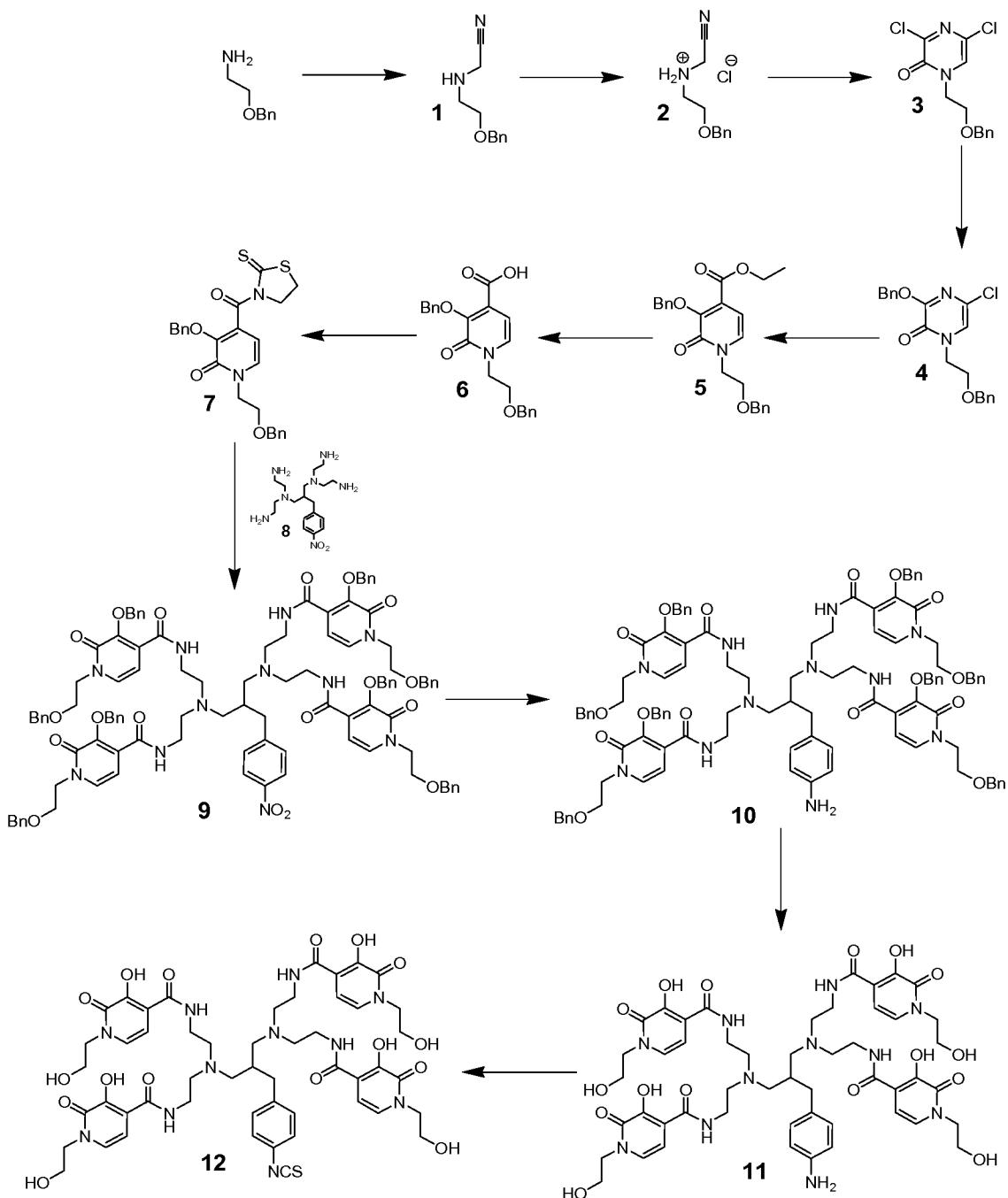
Wherein in formulae VII and VIII  $R_L$  may be any suitable linker group or reactive moiety as described herein.  $R_L$  will typically form the point of attachment of the ligand to the targeting moiety and thus any suitable reactive group can be used for this attachment either directly or using a further linker. Suitable reactive moieties for  $R_L$  in formulae VII and VIII include  $NH_2$  and  $NCS$  groups.

An exemplary compound with a functionalized moiety terminating the coupling moiety, according to this embodiment, is structure IX below (the linker phenylamine group may evidently be substituted with any other  $R_L$  group as indicated herein as appropriate, such as  $NCS$  in compound 12):



The synthesis of compound IX is described herein below and follows the following synthetic route:

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All documents referred to herein are hereby incorporated by reference, including Gordon AEV et al, Rational design of sequestering agents for plutonium and other actinides. Chem. Rev. 2003, 103, 4207-4282, PCT Patent Application WO 2008/063721 A2 and T.N. Lambert et al., Tetrahedron Letters 43 (2002) 7379-7383.

In the methods of formation of the complexes of the present invention, it is preferred that the reaction be carried out in aqueous solution. This has several advantages.

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Firstly, it removes the burden on the manufacturer to remove all solvent to below acceptable levels and certify that removal. Secondly it reduces waste and most importantly it speeds production by avoiding a separation or removal step. In the context of the present radiopharmaceuticals, it is important that synthesis be carried out as rapidly as possible since the radioisotope will be decaying at all times and time spent in preparation wastes valuable material and introduces contaminant daughter isotopes.

In one embodiment, the method comprises forming a first aqueous solution of octadentate hydroxypyridinone-containing ligand (as described herein throughout) and a second aqueous solution of a tissue targeting moiety (as described herein throughout) and contacting said first and said second aqueous solutions.

In a related embodiment, the method of formation of the present invention is carried out in the substantial absence of any organic solvent. In this context, and "organic solvent" takes its natural meaning of a material which is liquid at or around room temperature and which comprises at least one carbon. Such organic solvents typically comprise hydrocarbon, alcohol, ester, amide, ester and/or halogenated moieties and such solvents are preferably present at no more than 1% (e.g. 0.0001 to 1%), preferably no more than 0.5% and most preferably no more than 0.2% by weight in the aqueous solutions referred to herein. For the avoidance of doubt, the targeting moieties and ligands referred to herein are not encompassed by the term "organic solvent". Certain organic materials, such as organic acids, amines and their salts may be present at somewhat higher concentrations so as to act as pH buffers in the aqueous solvent. Where present these will typically be at a concentration of no more than 10% (e.g. 0.001 to 10%), preferably no more than 5%, more preferably no more than 1% by weight.

Suitable coupling moieties are discussed in detail above and all groups and moieties discussed herein as coupling and/or linking groups may appropriately be used for coupling the targeting moiety to the ligand. Some preferred coupling groups include amide, ester, ether and amine coupling groups. Esters and amides may conveniently be formed by means of generation of an activated ester groups from a carboxylic acid. Such a carboxylic acid may be present on the targeting moiety, on the coupling moiety and/or on the ligand moiety and will typically react with an alcohol or amine to form an ester or amide. Such methods are very well

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known in the art and may utilise well known activating reagents including N-hydroxy maleimide, carbodiimide and/or azodicarboxylate activating reagents such as DCC DIC DEAD DIAD etc.

**Brief Summary of the Figures:**

Figure 1: SEC-UV chromatogram of AGC1115 at 280 nm (A) and 335 nm (B). The average chelator-to-antibody ratio (CAR) is approximately 0.9.

Figure 2 Binding of AGC1100 and AGC1115 analysed by flow cytometry on CD22-positive Raji cells. Detection was done using mouse anti-human IgG Fc, PE conjugated secondary antibody and median fluorescence intensity (MFI) was plotted against log concentration in nM of primary antibody. Trastuzumab was used as an isotype control.

Figure 3: Ramos cells incubated with the Th-227 labelled AGC0015 conjugated C22-binding mAb AGC1115 (filled circles), the Th-227 labelled AGC0015 conjugated control mAb trastuzumab (filled squares), or culture medium (filled diamonds). Both mAbs were labelled with Th-227 to the same specific activity (44 kBq/µg), and used at 3 nM (A).

The invention will now be illustrated by the following non-limiting Examples . All compounds exemplified in the examples form preferred embodiments of the invention (including preferred intermediates and precursors) and may be used individually or in any combination in any aspect where context allows. Thus, for example, each and all of compounds 2 to 4 of Example 2, compound 10 of Example 3 and compound 7 of Example 4 form preferred embodiments of their various types.

In the Examples, the following antibodies and antibody conjugates are referred to:

AG01100 - Anti-CD22 antibody as generated in Example 3

AG01115 - AG01100 conjugated to a high-solubility conjugator (12)

**Example 1 –Isolation of pure thorium-227**

Thorium-227 is isolated from an actinium-227 cow. Actinium-227 was produced through thermal neutron irradiation of Radium-226 followed by the decay of Radium-227 ( $t_{1/2}=42.2$  m) to Actinium-227. Thorium-227 was selectively retained

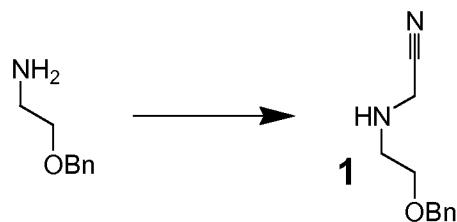
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from an Actinium-227 decay mixture in 8 M  $\text{HNO}_3$  solution by anion exchange chromatography. A column of 2 mm internal diameter, length 30 mm, containing 70 mg of AG®1-X8 resin (200–400 mesh, nitrate form) was used. After Actinium-227, Radium-223 and daughters had eluted from the column, Thorium-227 was extracted from the column with 12 M HCl. The eluate containing

Thorium-227 was evaporated to dryness and the residue resuspended in 0.01 M HCl.

### Example 2 – Synthesis of compound 12

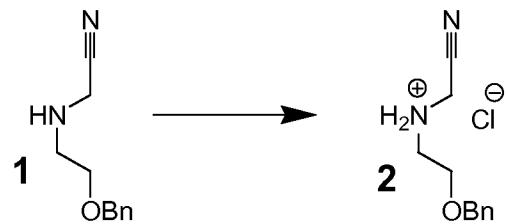
#### Step 1



2-benzyloxyethylamine (31g, 207mmol) and glycolonitrile (16mL, 70% solution in water, 207mmol) was dissolved in 300mL EtOH (abs) and refluxed for 4 h. The volatiles were removed under reduced pressure. The crude product (24.7g, 130mmol) was carried on to the next step without further purification.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz): 2.92(m, 2H), 3.58-3.62(m, 4H), 4.51(s, 2H), 7.25-7.37(m, 5H)

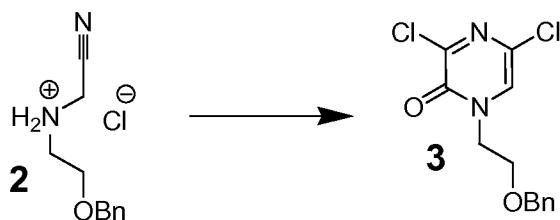
#### Step 2



1 (24.7g, 130mmol) was dissolved in dry ether. HCl (g) was bubbled through the solution for 30 minutes. The precipitate was filtered off and dried under reduced pressure, giving the desired product (27.8g, 122.6mmol). The product was carried on to the next step without further purification or analysis.

#### Step 3

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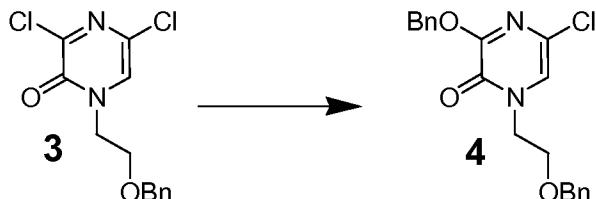


**2** (27.8g, 122.6mmol) was dissolved in 230mL chlorobenzene at room temperature. Oxallyl chloride (45mL, 530mmol) dissolved in 100mL chlorobenzene was added drop wise over 30 minutes at room temperature. The reaction mixture was stirred at room temperature for 45 hours. The reaction was carefully quenched by drop wise addition of 100mL water. The phases were separated, and the aqueous phase was extracted with 3\*100mL DCM. The organic phases were combined and washed with 100mL brine. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and the volatiles were removed under reduced pressure. The crude product was purified by dry flash chromatography on  $\text{SiO}_2$  using a gradient of MeOH (0-2%) in DCM, yielding the desired product (21.2g, 70.8mmol).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400MHz): 3.71-3.76(m, 2H), 4.06-4.12(m, 2H), 4.47(s, 2H), 7.217-7.22(m, 2H), 7.26-7.36(m, 4H)

MS(ESI-pos, m/z): 321.0

#### Step 4



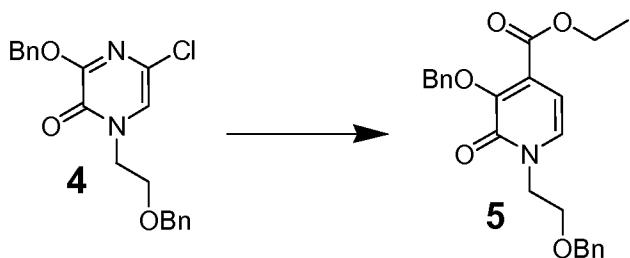
Sodium hydride (60% dispersion, 3.60g, 90mmol) was stirred in 50mL THF at 0 °C and benzyl alcohol (8.3mL, 80mmol) was added drop wise over 10 minutes. The reaction mixture was stirred for 30 minutes at 0 °C before **3** (21.2g, 70.8mmol) dissolved in 100mL THF was added drop wise at 0 °C. The reaction mixture was stirred in the dark over night at room temperature. 50mL HCl in dioxane (4M) was added drop wise before the reaction mixture was reduced *in vacuo*. 500mL DCM was added, followed by 200mL water. The phases were separated and the aqueous phase was extracted with 200mL DCM. The organic phases were combined and washed with 100mL brine. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and the volatiles were removed under reduced pressure. Dry flash chromatography on  $\text{SiO}_2$  using a gradient of MeOH (0-6%) in DCM gave the desired product (25.6g, 69mmol).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 300MHz): 3.69-3.75(m, 2H), 4.01-4.07(m, 2H), 4.46(s, 2H), 5.37(s, 2H), 6.97(s, 1H), 7.19-7.39(m, 8H), 7.44-7.51(m, 2H)

MS(ESI-pos, m/z): 371.1, 763.2

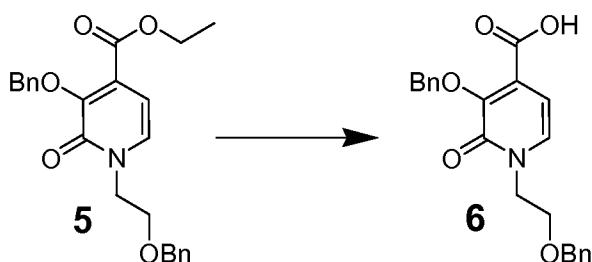
#### Step 5

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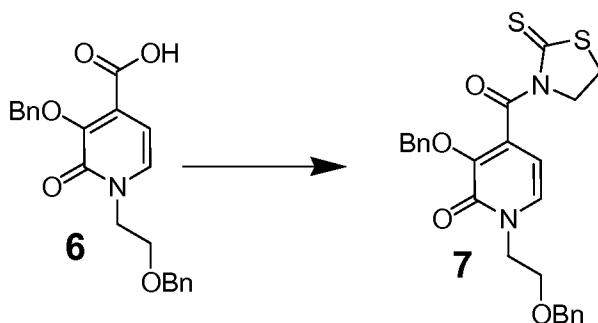
**4** (25.6g, 69mmol) and ethyl propiolate (41mL, 0.4mol) was heated at 140 °C for 5 hours. The reaction mixture was cooled down to room temperature and the reaction mixture was purified by dry flash chromatography on SiO<sub>2</sub>. A gradient of MeOH (0-10%) in DCM gave the desired product as an inseparable mixture of the desired 4-isomer together with the 5-isomer. This mixture (28.6g, ~65mmol) was used directly in the next step without further purification.

### Step 6



**5** (28.6g, ~65mmol), as obtained in the previous step, was dissolved in 300mL THF at 0 °C. 100mL KOH (1M, *aq*) was added, and the reaction mixture was stirred for 40 hours at room temperature. HCl (1M, *aq*) was added until pH~2 (125mL) and the aqueous phase was extracted with 3\*250mL CHCl<sub>3</sub>. The organic phases were combined and washed with 100mL brine, filtered and the volatiles were removed *in vacuo*. The obtained material (25.9g, ~65mmol) was used without in the next step without further purification or analysis.

### Step 7

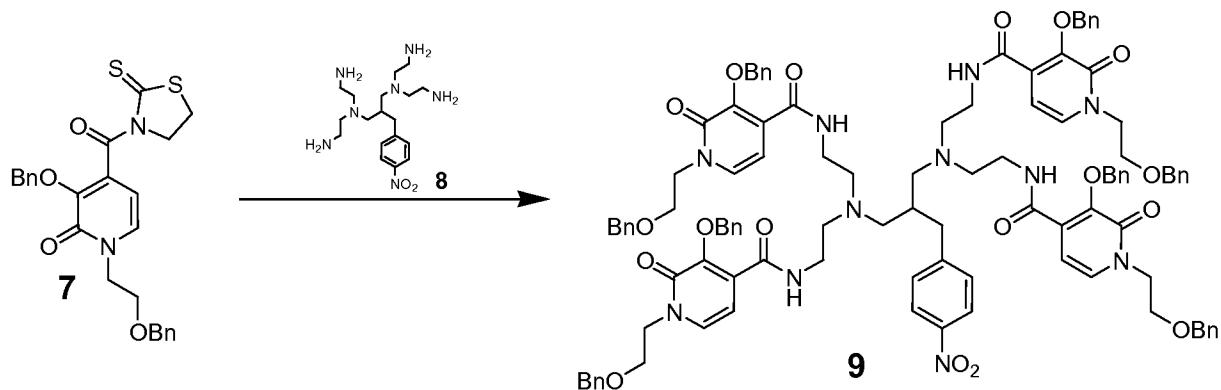


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**6** (25.9g, ~64mmol), as obtained in the previous step, was partially dissolved in 400mL DCM. 2-Thiazoline-2-thiol (8.94g, 75mmol) and DMAP (0.86g, 7mmol) was added, followed by DCC (15.48g, 75mmol). The reaction mixture was stirred at room temperature over night. The reaction mixture was filtered through a Celite-pad and the Celite-pad was washed with 100mL DCM. The volatiles were removed *in vacuo*. The product mixture was purified by dry flash chromatography on  $\text{SiO}_2$  using first a gradient of DCM (50-100%) in heptane, followed by a gradient of THF (0-15%) in DCM. The appropriate fractions were reduced *in vacuo*, giving a mixture of products. This inpure mixture was purified by flash chromatography on  $\text{SiO}_2$  using a gradient of EtOAc (25-75%) in heptane. The appropriate fractions were reduced *in vacuo*, giving a mixture of products. Finally, to get the desired product, the product mixture was purified by dry flash chromatography on RP18-silica using a gradient of MeCN (25-75%) in water. This gave the desired product (8.65g, 18mmol).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 300MHz): 2.90(t,  $J=7.3\text{Hz}$ , 2H), 3.77-3.84(m, 2H), 4.18-4.23(m, 2H), 4.35(t,  $J=7.3\text{Hz}$ , 2H), 4.51(s, 2H), 5.33(s, 2H), 6.11(d,  $7.0\text{Hz}$ , 1H), 7.21-7.48(m, 11H)  
 MS(ESI-pos, m/z): 503.1

### Step 8

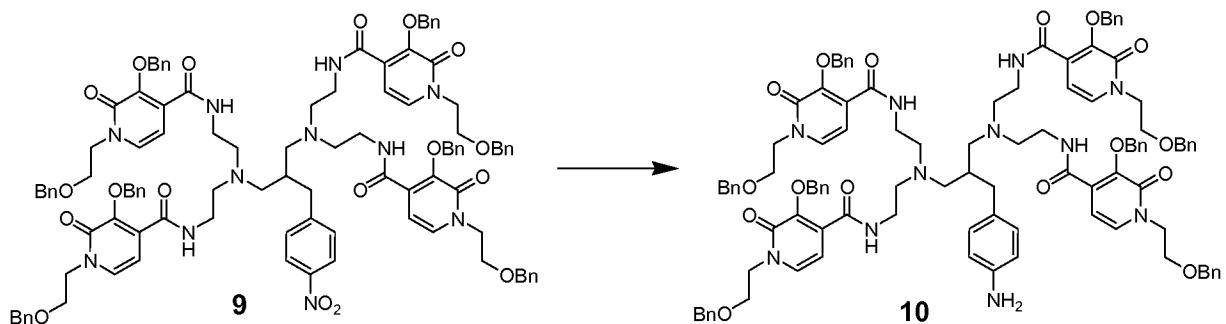


**7** (5.77g, 12mmol) and **8** (1.44g, 2.4mmol) were partially dissolved in 40mL DMPU. DBU (2.7mL, 18mmol) was added drop wise. The reaction was stirred for 4 days at room temperature. Purification by dry flash chromatography on  $\text{SiO}_2$  using a gradient of DCM and MeOH in EtOAc gave the desired product (3.93g, 2.15mmol).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400MHz): 2.20-2.32(m, 10H), 2.44-2.50(m, 2H), 3.05-3.20(m, 10H), 3.23-3.27(m, 1H), 3.69-3.77(m, 8H), 4.06-4.15(m, 8H), 4.43(s, 8H), 5.24(s, 8H), 6.62(d,  $J=7.2\text{Hz}$ , 4H), 7.13(d,  $J=7.2\text{Hz}$ , 4H), 7.16-7.38(m, 42H), 7.82-7.93(m, 6H)

### Step 9

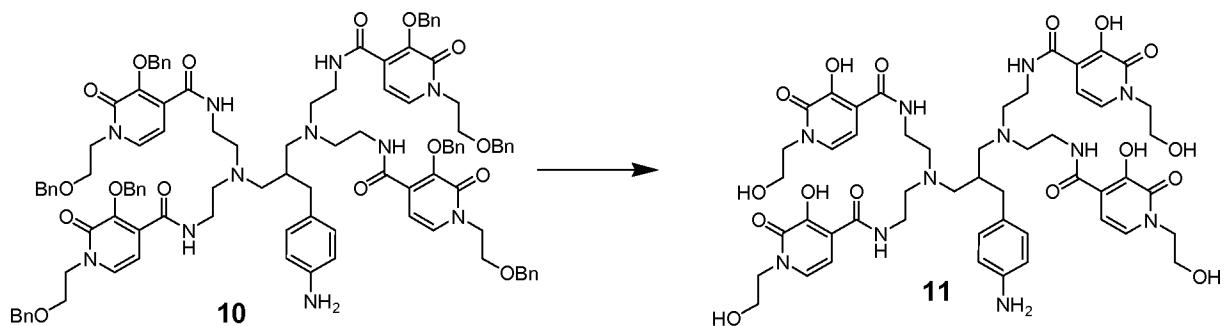
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**9** (3.93g, 2.15mmol) was dissolved in 300mL EtOH at room temperature. 60mL water was added, followed by NH<sub>4</sub>Cl (5.94g, 32.3mmol). The reaction mixture was to 60 °C before iron powder (1.80g, 32.3mmol) was added. The reaction mixture was stirred at 60 °C for 1 hour. The reaction mixture was cooled down to room temperature and 400mL DCM and 100mL water was added. The reaction mixture was filtered, and the organic phase was washed with 100mL water and 100mL brine. The aqueous phases were combined and back extracted with 3\*100mL DCM. The organic phases were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the volatiles were removed under reduced pressure. The product mixture was purified by dry flash chromatography on SiO<sub>2</sub> using a gradient of MeOH (0-7%) in DCM gave the desired product (3.52g, 1.96mmol).

MS(ESI-pos, m/z): 899.2

### Step 10

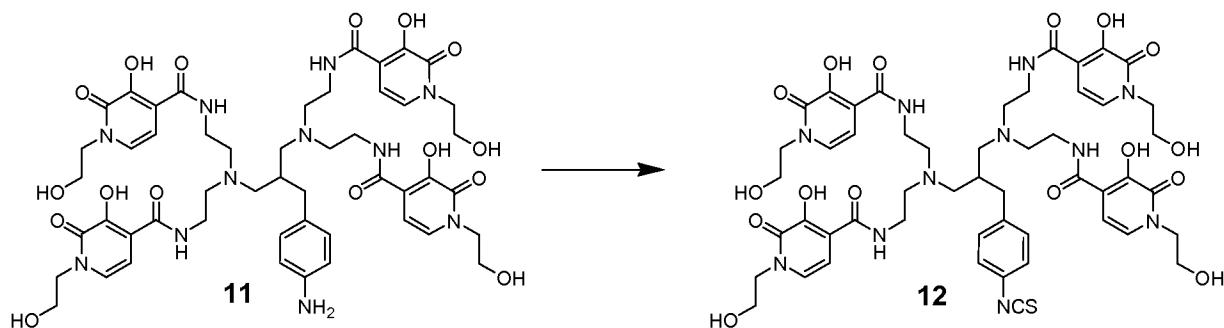


**10** (1.00g, 0.56mmol), Pd(OH)<sub>2</sub>/C (Pearlman's catalyst, 1.00g) and 10mL AcOH was placed in a pressure reactor. The reactor was evacuated by water aspirator and H<sub>2</sub> was introduced (7 bar). The reaction mixture was stirred for 1 hour before the pressure was released and 5mL HCl (6M, *aq*) was added to the reaction mixture. The reactor was evacuated as before and H<sub>2</sub> was once again introduced (7 bar). After stirring for 7 days, HPLC indicated full conversion. The reaction mixture was filtered and the volatiles were removed under reduced pressure. The residue was dissolved in MeOH/MeCN (1:1) and the

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product was precipitated by addition of  $\text{Et}_2\text{O}$ . The solids were collected by centrifugation and decanting the supernatant before the product was dried *in vacuo* (484mg, 0.45mmol).  
 $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 400MHz): 2.70-2.95(m, 2H), 3.00-3.10(m, 2H), 3.15-3.65(m, 19H), 3.75-4.23(m, 16H), 6.25(bs, 4H), 7.04(d,  $J=7.0\text{Hz}$ , 4H), 7.44(d,  $J=8.2\text{Hz}$ , 2H), 7.57(d,  $J=8.2\text{Hz}$ , 2H)  
 $\text{MS}(\text{ESI-pos, m/z})$ : 1076.4

### Step 11



Compound 11 (20mg, 18 $\mu\text{mol}$ ) was dissolved in 3mL MeCN and 3mL water. 20  $\mu\text{L}$  thiophosgene was added. The reaction mixture was stirred rigidly for 1 hour. The volatiles were removed under reduced pressure and the residue was dissolved in 4mL MeCN. The product was precipitated by adding the acetonitrile phase to 40mL  $\text{Et}_2\text{O}$ . The solids were collected by centrifugation and decanting the supernatant before the product was dried *in vacuo* (10mg, 9 $\mu\text{mol}$ ).

$\text{MS}(\text{ESI-pos, m/z})$ : 1118.4

### Example 3: Generation of the anti-CD22 monoclonal antibody (AGC1100).

The sequence of the monoclonal antibody (mAb) hLL2, also called epratuzumab, here denoted AGC1100, was constructed as described in (1). The mAb used in the current examples was produced by Immunomedics Inc, New Jersey, USA.

Production of this mAb could for example be done in Chinese hamster ovarian suspension (CHO-S) cells, transfected with a plasmid encoding the genes encoding the light and the heavy chain. First stable clones would be selected for using standard procedures. Following approximately 14 days in a single-use bioreactor, the monoclonal antibody may be harvested after filtration of the supernatant.

AGC1100 would be further purified by protein A affinity chromatography (MabSelect SuRe, Atoll, Weingarten/Germany), followed by an ion exchange step. A third purification step based on electrostatic and hydrophobicity could be used to remove aggregates and potentially remaining impurities. The identity of AGC1100 would be confirmed by isoelectric focusing, SDS-PAGE analysis, N-terminal sequencing and

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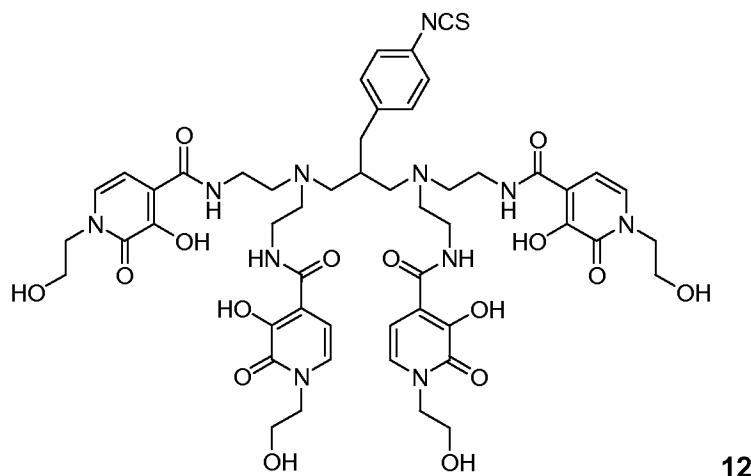
LC/MS analysis. Sample purity would be further analyzed by size-exclusion chromatography (SEC).

References:

(1) Leung, Goldenberg, Dion, Pellegrini, Shevitz, Shih, and Hansen. Molecular Immunology 32: 1413-27, 1995.

**Example 4: Conjugation of AGC1100 with the chelator AGC0015.**

The antibody AGC1100 was conjugated with the water soluble chelator AGC0015.(12) The conjugation reaction was performed in a 1:1 (v/v) mixture of PBS mixed with 70 mM borate buffer pH 8.5. The chelator, AGC0015 is as shown below:



The chelator, AGC0015 (12 above), was dissolved in metal-free water before it was added to the conjugation reaction. A nominal molar chelator to antibody ratio of 1.3:1 was used and the reaction was incubated for 22 hours at 21°C. At the end of reaction time the antibody fraction was separated from free chelator by size exclusion chromatography on an ÄKTA Purifier (GE Healthcare), using a HiLoad Superdex 200 16/600 PG column (GE Healthcare; code.no. 28-9893-35) and 0.9% NaCl 100 mM citrate buffer pH 5.5 as mobile phase. The final chelator-antibody-ratio (CAR) of purified conjugate was determined by HPLC size exclusion chromatography-UV (SEC-UV) analysis. The CAR determination was done on an Agilent 1200 series HPLC system (Agilent Technologies), column TSKgel SuperSW 3000, 4.6 x 300 mm, 4 µm particles (Tosoh Bioscience, part no. 18675) maintained at room temperature and mobile phase 300 mM NaCl 200 mM ammonium acetate pH 6.8 (isocratic elution) with a total run time of 15 minutes. The injection volume was 5 µl and the LC flow rate was 0.35 ml/min. The UV signals were monitored at 280 and 335 nm, corresponding to mAb and chelator absorbance maximum,

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respectively. Representative results of a CAR-determination are presented in Figure 1.

**Example 5: Chelation of Antibody/Chelator conjugate AGC1115 with Th-227**

Thorium-227 ( $^{227}\text{Th}$ ) as a 4+ ion was isolated from an actinium-227 ( $^{227}\text{Ac}$ ) generator system.  $^{227}\text{Th}$  was selectively retained from a  $^{227}\text{Ac}$  decay mixture in 8 M HNO<sub>3</sub> by anion exchange chromatography, where negatively charged nitrate complexes are formed with  $^{227}\text{Th}^{4+}$ .  $^{227}\text{Ac}$  and daughter nuclides were washed off the column and  $^{227}\text{Th}$  was eluted in 12 M HCl. The  $^{227}\text{Th}$ -eluate was evaporated to dryness and the residue dissolved in 0.5 M HCl.

In the chelation reaction the antibody-conjugate AGC1115 was incubated for 15 minutes in 0.9% NaCl 100 mM citrate buffer, pH 5.5 at 21°C/room temperature in the presence of 1 MBq  $^{227}\text{Th}$  per 0.5 mg antibody conjugate. The high molecular fraction containing radio labelled antibody-conjugate was separated from free  $^{227}\text{Th}$  and daughter nuclides by size exclusion chromatography using NAP-5 DNA Grade columns (GE Healthcare). The labelling efficiency was typically 96-98%, including potential loss in the NAP-5 desalting step.

**Example 6: Binding analysis of AGC1115 and AGC1100 to CD22-positive Raji cells by flow cytometry.**

Binding of AGC1115 and AGC1100 (anti-human CD22, Immunomedics; hLL2, #1003164, 10 mg/ml) to CD22-positive Raji cells (ATCC, #CCL-86) was analysed by flow cytometry. The EC<sub>50</sub> value determined from the fitted curve was used for comparison of the antibody versus the antibody conjugate binding potency. This analysis was used to confirm that antibody conjugate binding potency to CD22 was unaffected by the conjugation procedure.

Raji cells were grown in RPMI 1640 (PAA; #E15-840) in the presence of 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. For the flow cytometry analysis 50 ml cell culture was harvested by centrifugation at 4°C for 5 min at 340xg. Cells were resuspended and washed twice in 10 ml PBS, supplemented with 1% FBS, and pelleted by centrifugation at 4°C for 5 min at 340xg. Subsequently, 20 µl of the preparation of resuspended cells was diluted 1:500 in Coulter Isoton II Diluent, and counted using Beckman Coulter Z2 instrumentation (Beckman Coulter; CA, USA). The preparation was adjusted to a cell density of 1x10<sup>6</sup> cells/ml and 100 µL was transferred to each well in a V-shaped bottom 96-well plate (Nunc/Fisher Scientific; NH, USA). Cells were spun down and re-suspended after decantation, which resulted in an approximate volume of 50 µl cell suspension per well.

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AGC1115 and AGC1100 was diluted to 50 µg/ml and titrated in twelve points in 3-fold dilution steps. An isotype control antibody (trastuzumab) was prepared accordingly. 100 µl from each dilution of the antibody was added to the wells containing Raji cells. After incubation for 1.5 h at 4°C, the cells were spun down and washed twice with 200 µl cold PBS, supplemented with 1% FBS. PE conjugated mouse anti-human IgG Fc (BioLegend; #409304) was used as a secondary antibody reagent for detection of human mAb. The secondary antibody reagent was prepared at 1 µg/ml in PBS, supplemented with 1% FBS. 100 µl from the secondary antibody reagent was subsequently added to each well, before incubation for 1 h at 4°C in the dark. The cells were washed twice, as described above, and resuspended in 200 µl PBS, supplemented with 1% FBS. All samples were analysed in a V-shaped bottom 96-well plate. Fluorescent signal was recorded on a Beckman Coulter Cell Lab Quanta SC MPL flow cytometer (Beckman Coulter; CA, USA). Median values (MFI) were exported to an Excel sheet and plotted against the concentration ([nM]).

Data was fitted using the “log(agonist) vs. response – Variable slope (four parameters)” binding model in GraphPadPrism (PrismSoftware; CA, USA) and the EC<sub>50</sub> values was calculated from the fit (Figure 2). Direct staining of the Raji cells with secondary antibody showed low background, MFI values of approximately 1 (0.5-1% of the AGC1115 MFI values).

The calculated EC<sub>50</sub> values of the fitted titration curves of AGC1100 and AGC1115 were 9 nM and 6 nM, respectively, and indicated that the binding potency of the conjugate AGC1115 was comparable to AGC1100.

#### **Example 7: Th-227-induced cell cytotoxicity by AGC1115-Th-227.**

In vitro cell cytotoxicity was investigated in CD22 positive Ramos cells (see Example 6). AGC1115 and the control trastuzumab conjugated with AGC0015 were used to chelate Th-227 to a specific activity of 44 kBq/µg.

Ramos cells were grown at 37°C with 5 % CO<sub>2</sub>, and split 1:5 three times a week. The day before the assay the culture medium (Iscove's Modified Dulbecco's Medium (IMDM) with 20 % FBS and 1 % Penicillin/Streptomycin) was replaced by new medium and the volume adjusted to give 400 000 cells per mL. About, 1 600 000 cell (4 mL) were added to each well in a 6 well plate. The plate was incubated until next day for addition of labelled mAb, or culture medium.

After adding labelled mAb, or culture medium, the plate was incubated for 4 more hours. In the experiment AGC1115 or trastuzumab-AGC0015 was added to each well to a final concentration of 3 nM. Following incubation, the cells were washed

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twice in culture medium, and the ATP in the supernatant and in the pellet was measured. The cells were then split 1:2 and incubated in culture medium at 37°C with 5% CO<sub>2</sub>. The same procedure, but with only one wash, was repeated at days 3, 5 and 7.

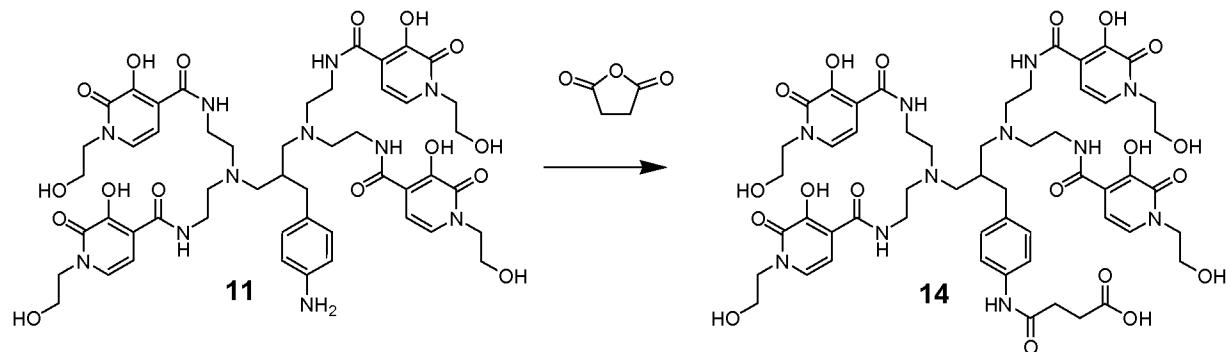
A quantification of ATP was used as a measure of cell viability at different sample times (CellTiter-Glo Luminescent cell viability assay from Promega), resulting in the curves shown in Figure 3. The Ramos cell binding AGC1115-Th-227 resulted in cellular toxicity, in contrast to the Th-227 labelled control construct, not binding to Ramos cells.

### Example 8 – Acid Derivative

Making an acid derivative of the water soluble chelator enabling alternative coupling chemistries .

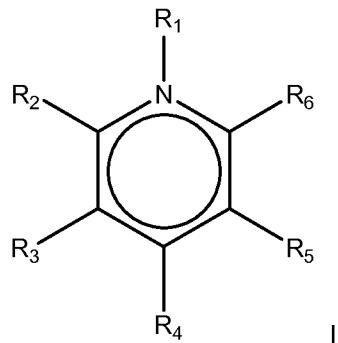
This example shows the successful synthesis of an acid derivative. This derivative of the chelator enables, for example, formation of an amide bond with an epsilon amine of the tumour targeting protein.

The present example shows the synthesis of the soluble chelator and starts out from substance **11** (Example 2). 43 mg (~0.04 mmol) of substance **11** was dissolved in 4 mL DMSO, 4 mL acetonitrile, and 30 $\mu$ L NEt<sub>3</sub>. 6 mg of succinic anhydride was added (0.06 mmol). LC/MS analysis of the reaction mix after 22 hours reaction at room temperature showed that substance **15** had formed. Some contaminant diacylated side product was formed. Adding the anhydride in portions should minimize the ester formation and improve molar yield of product **14**. HPLC analysis of the resulting reaction mixture is shown in Figure 6.



## Claims

1. A tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor.
2. The complex as claimed in claim 1 comprising at least one 3,2-HOPO moiety.
3. The complex as claimed in claim 1 or claim 2 wherein all 4 HOPO moieties comprise hydroxyalkyl solubilising moieties at the N-position.
4. The complex as claimed in claim 1 or claim 2 comprising an octadentate ligand comprising four chelating moieties of formula I



Wherein R<sub>1</sub> is an optional N-substituent solubilising group which will be present in at least one of the four moieties of formula I; groups R<sub>2</sub> to R<sub>6</sub> are each independently selected from H, OH, =O, short hydrocarbyl groups, linker moieties and/or coupling moieties wherein one of R<sub>2</sub> to R<sub>6</sub> is OH and one of R<sub>2</sub> to R<sub>6</sub> is =O.

5. The complex as claimed in claim 4 wherein at least one of groups R<sub>1</sub> to R<sub>6</sub> is a linker moiety.

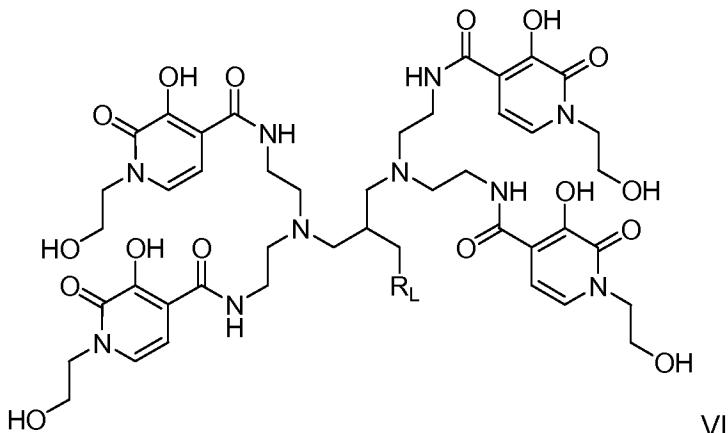
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6. The complex as claimed in any of claims 4 to 5 comprising four 3,2-hydroxypyridinone moieties.

7. The complex as claimed in any preceding claim wherein the N-substituents on each of the four HOPO groups are each independently chosen from HO-, HOCH<sub>2</sub>-, HOCH<sub>2</sub>CH<sub>2</sub>-, HO-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, HO-CH(CH<sub>3</sub>)CH<sub>2</sub>-, HO-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, HO-CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>-, HO-CH(CH<sub>2</sub>CH<sub>3</sub>)CH<sub>2</sub>-, HO-C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>-, HO-CH(CH<sub>3</sub>)CH(CH<sub>3</sub>)- and HOCH<sub>2</sub>CH(CH<sub>2</sub>CH<sub>3</sub>)-.

8. The complex as claimed in any of claims 1 to 7, wherein said ion of an alpha-emitting thorium radionuclide is the 4+ ion of an alpha-emitting thorium radionuclide such as <sup>227</sup>Th.

9. A complex as claimed in any of claims 1 to 8 comprising a ligand moiety of formula VI:



Wherein R<sub>L</sub> is any suitable linker moiety.

10. A complex as claimed in any preceding claim wherein the tissue targeting moiety is a monoclonal or polyclonal antibody, an antibody fragment (such as Fab, F(ab')<sub>2</sub>, Fab' or scFv), or a construct of such antibodies and/or fragments.

11. A complex as claimed in any preceding claim wherein the tissue targeting moiety comprises at least one peptide chain having at least 90% sequence similarity with at least one of the following sequences:

Light Chain:

DIQLTQSPSSLAVSAGENVTMSC**KSSQSVLYSANHKNYLA**WYQQKPGQSPKLLIY**WASTRE**  
**S**GVPDFRTGSGSGTDFLTISRVQVEDLAIYYCHQYLSSWTFGGGTKLEIKR (SeqID1)

DIQLTQSPSSLASAAVEDRTMSC**KSSQSVLYSANHKNYLA**WYQQKPGQAKLLIY**WASTRE**  
**S**GVPSRFSGSGSGTDFFTISSLQPEDIATYYCHQYLSSWTFGGGTKLEIKR (SeqID2)

Heavy Chain:

QVQLQESGAELSKPGASVKMSCKASGYTFT**SYWLH**WIKQRPGQGLEWIG**YINPRNDYTEYN**  
**QNFKD**KATLTADKSSSTAYMQLSSLTSEDAVYYCAR**RDITTFY**WGQGTTTVSS  
(SeqID3)

QVQLQQSGAEVKKPGSSVKVSCKASGYTFT**SYWLH**WVRQAPGQGLEWIG**YINPRNDYTEYN**  
**QNFKD**KATITADESTNTAYMELSSLRSEDTAFYFCAR**RDITTFY**WGQGTTTVSS  
(SeqID4)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFT**SYWLH**WVRQAPGQGLEWIG**YINPRNDYTEYN**  
**QNFKD**KATITADESTNTAYMELSSLRSEDTAFYFCAR**RDITTFY**WGQGTTTVSS  
(SeqID5)

12. Use of a tissue targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxylalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor, in the manufacture of a medicament for the treatment of hyperplastic or neoplastic disease.

13. Use as claimed in claim 12 wherein said tissue-targeting complex is a complex as claimed in any of claims 1 to 11.

14. Use as claimed in claim 12 or claim 13 wherein said disease is a carcinoma, sarcoma, myeloma, leukemia, lymphoma or mixed type cancer including Non-Hodgkin's Lymphoma or B-cell neoplasms.

15. A method of treatment of a human or non-human animal (particularly one in need thereof) comprising administration of at least one tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-

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position with a hydroxyalkyl solubilising group wherein the tissue targeting moiety has binding affinity for the CD22 receptor.

16. The method of claim 15 wherein said tissue-targeting complex is a complex as claimed in any of claims 1 to 11.

17. The method as claimed in claim 15 or claim 16 for the treatment of hyperplastic or neoplastic disease, such as a carcinoma, sarcoma, myeloma, leukemia, lymphoma or mixed type cancer, including Non-Hodgkin's Lymphoma or B-cell neoplasms.

18 A tissue targeting complex as claimed in any of claims 1 to 11 for use in therapy.

19. A tissue targeting complex as claimed in claim 18 for use in the treatment of hyperplastic and/or neoplastic disease such as a carcinoma, sarcoma, myeloma, leukemia, lymphoma or mixed type cancer including Non-Hodgkin's Lymphoma or B-cell neoplasms.

20. A pharmaceutical composition comprising a tissue-targeting complex comprising a tissue targeting moiety, an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group wherein the tissue targeting moiety has binding affinity for the CD22 receptor, together with at least one pharmaceutical carrier or excipient.

21. The pharmaceutical composition as claimed in claim 20 comprising a tissue-targeting complex as a complex as claimed in any of claims 1 to 11.

22. A kit for use in a method according to any of claims 15 to 17, said kit comprising a tissue targeting moiety, conjugated or conjugatable to an octadentate hydroxypyridinone-containing ligand comprising four HOPO moieties, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the

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CD22 receptor, said kit optionally and preferably include an alpha-emitting thorium radionuclide, such as  $^{227}\text{Th}$ .

23. A method of formation of a tissue-targeting complex, said method comprising coupling a tissue targeting moiety to an octadentate hydroxypyridinone-containing ligand in aqueous solution, the complex comprising four HOPO moieties and the ion of an alpha-emitting thorium radionuclide, where at least one of the four HOPO moieties is substituted at the N-position with a hydroxyalkyl solubilising group and wherein the tissue targeting moiety has binding affinity for the CD22 receptor.

24. The method of claim 23 comprising preparing a first aqueous solution of octadentate hydroxypyridinone-containing ligand and a second aqueous solution of said tissue targeting moiety and contacting said first and said second aqueous solutions.

25. The method of claim 24 wherein said contacting is conducted at below 40°C.

26. The method of claim 24 or claim 25 wherein said contacting is conducted in the substantial absence of any organic solvent.

27. The method of any of claims 23 to 26 wherein said coupling yields an amide, ester, ether or amine bond between the chelate and the antibody.

28. The method of claim 27 wherein said ester or amide linkage is formed by means of at least one activated ester group, for example formed by means of at least one N-hydroxy maleimide, carbodiimide or azodicarboxylate coupling reagent.

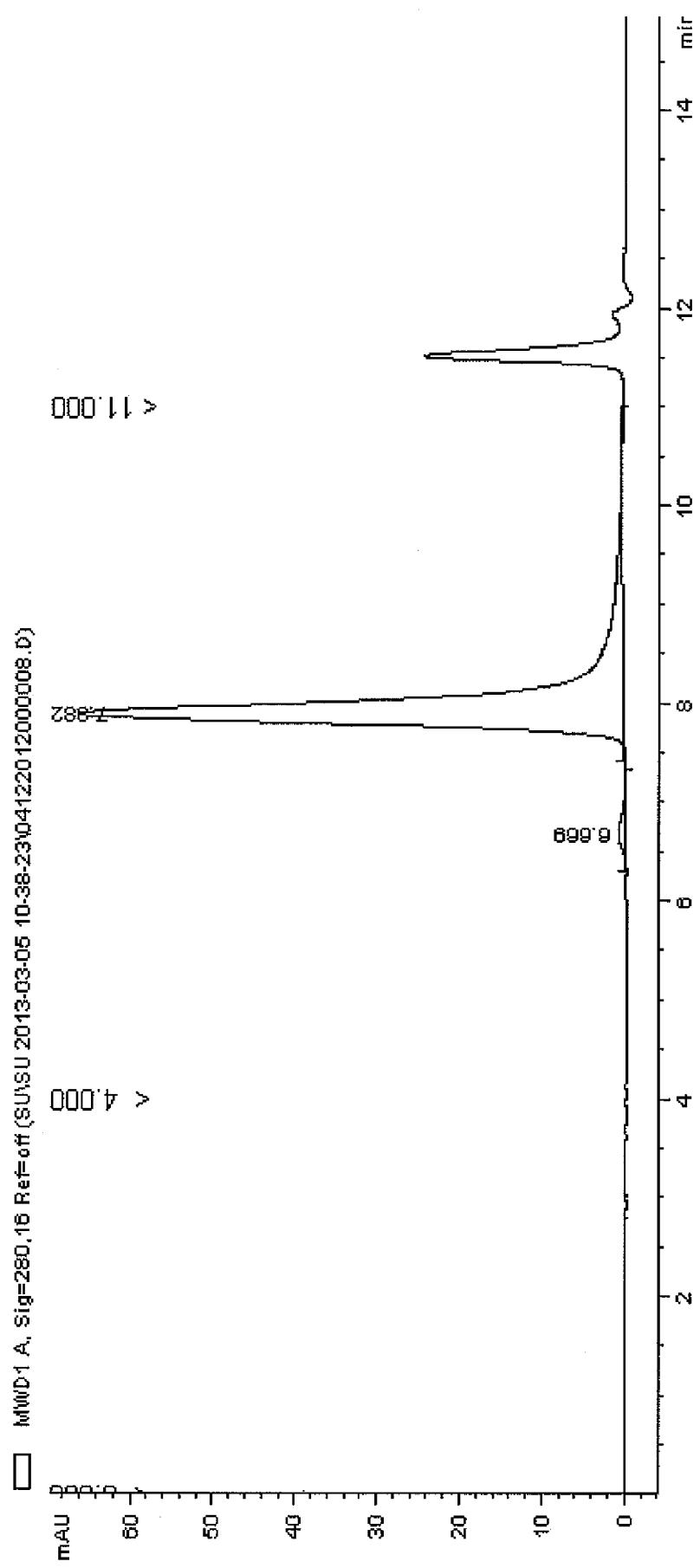


Figure 1a)

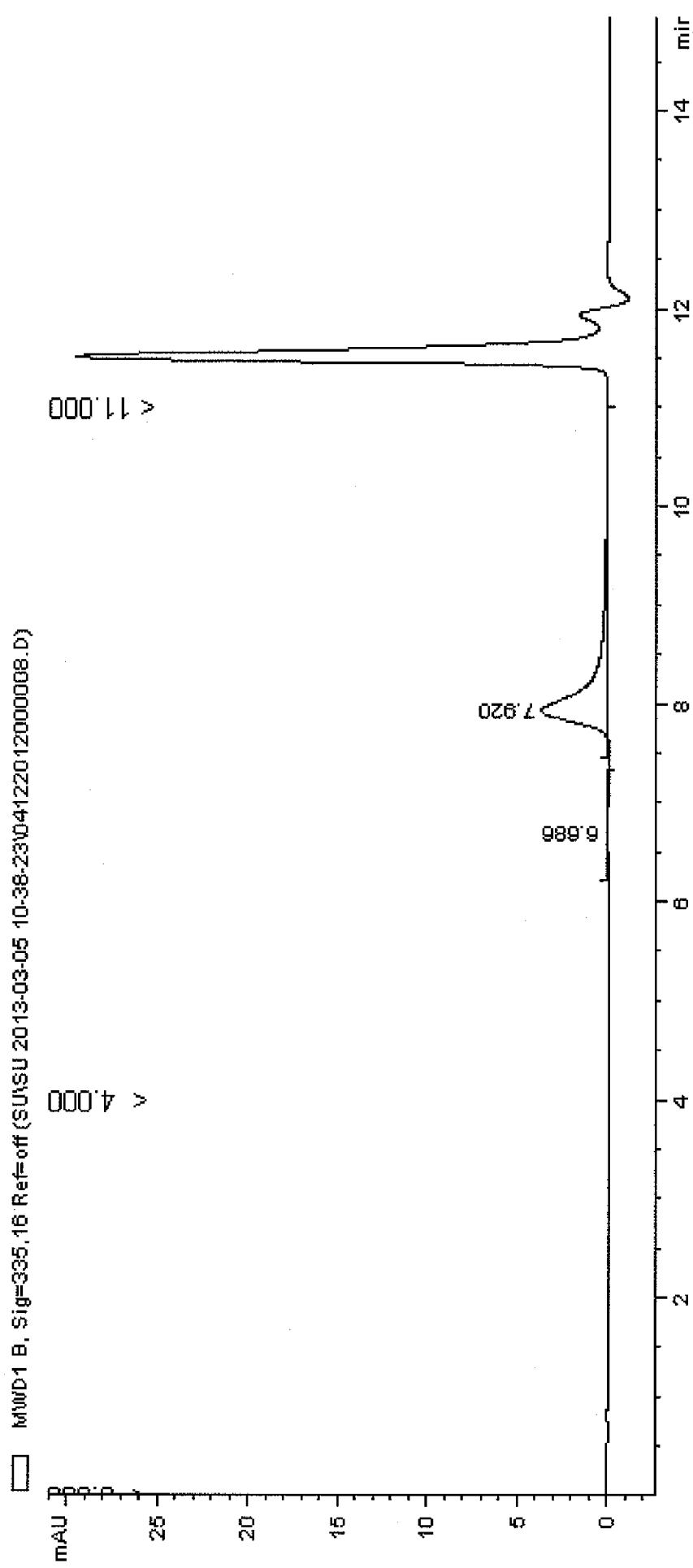


Figure 1b

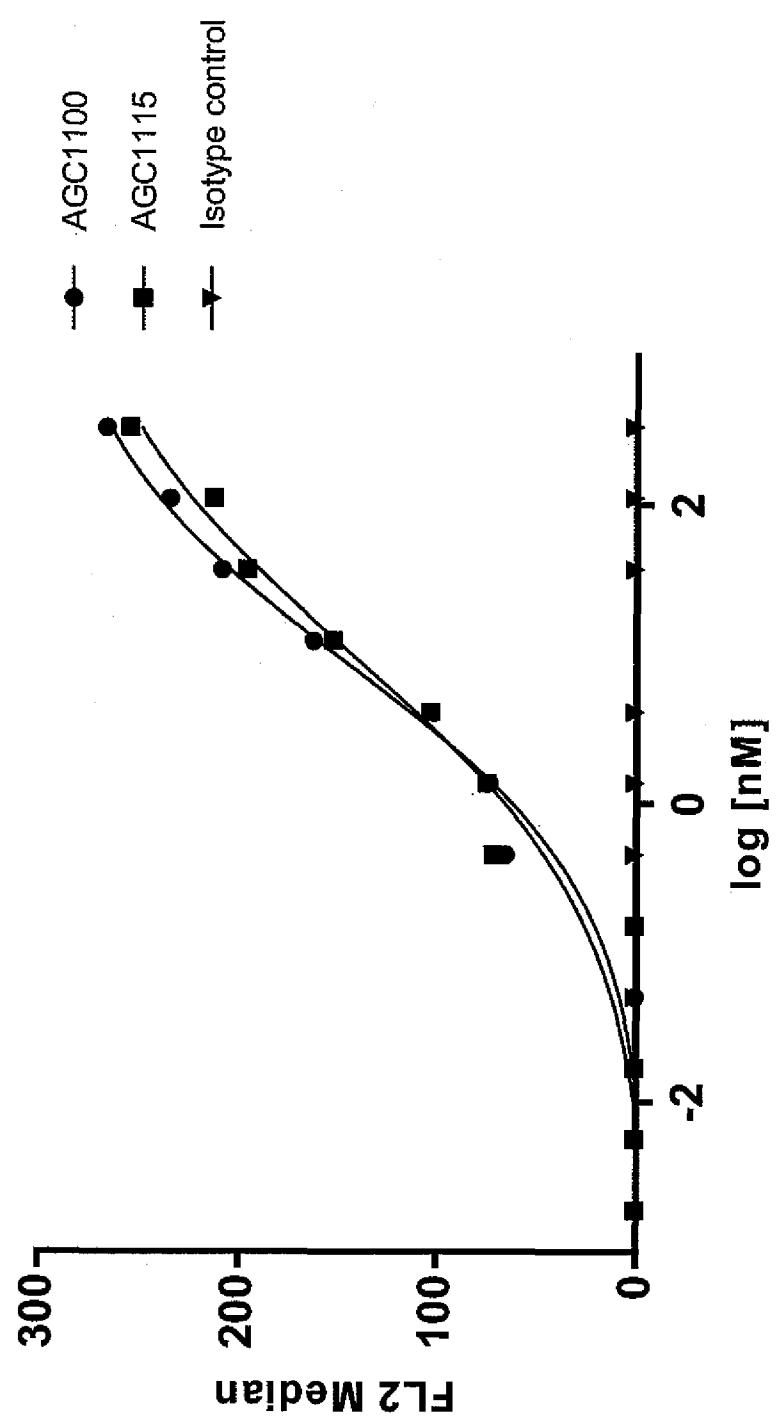


Figure 2

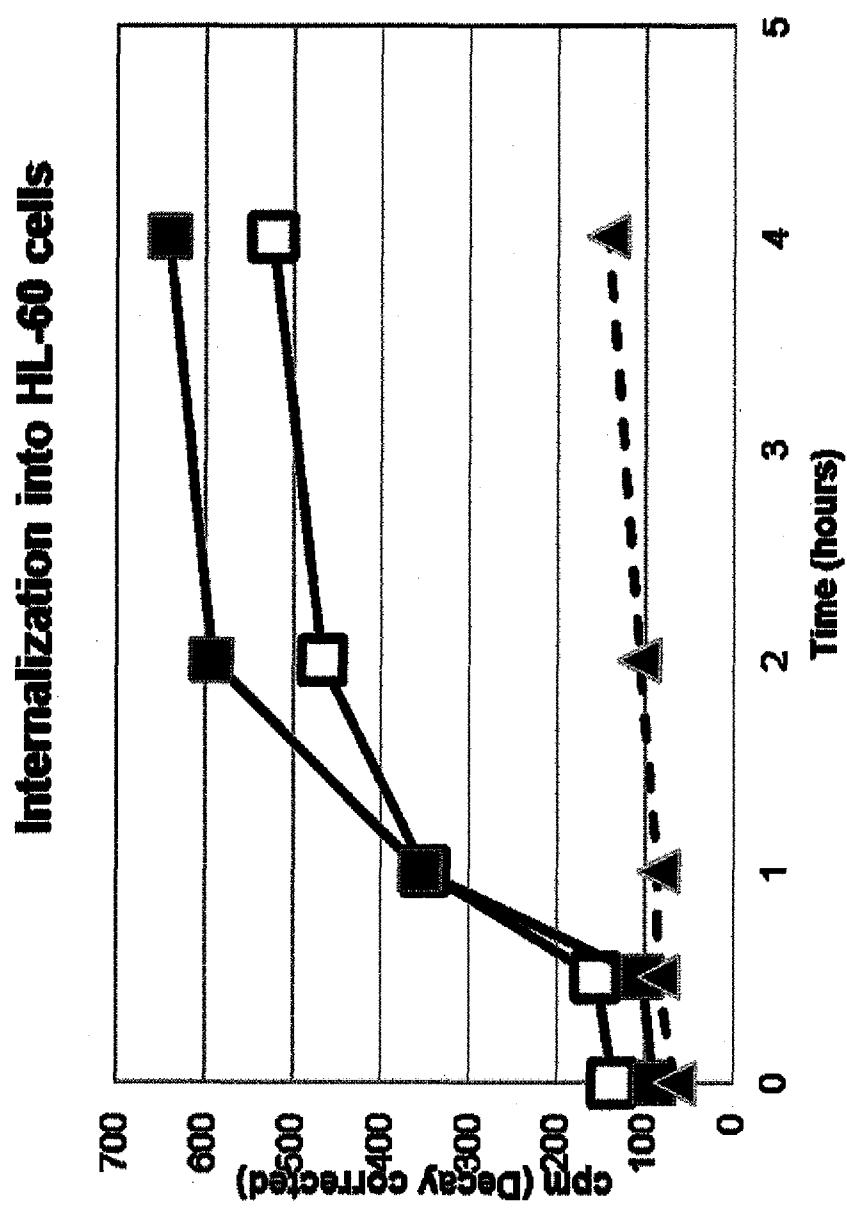


Figure 3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2013/059839

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. A61K51/04 A61K51/10 A61P35/00  
ADD. A61K103/40

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, WPI Data, BIOSIS, EMBASE**

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/098611 A2 (ALGETA AS [NO]; RAMDAHL THOMAS [NO]) 18 August 2011 (2011-08-18) cited in the application page 4, 5th full paragraph to page 5, 1st full paragraph page 15, 3rd full paragraph to page 16, line 1 page 16, 3rd full paragraph to page 17, 3rd full paragraph page 25, line 4 to page 26, line 3 and formulae V, VI and VII examples 2, 8 ----- Y WO 2008/085064 A2 (GE HEALTHCARE AS [NO]; WADSWORTH HARRY JOHN [GB]; NEWINGTON IAN MARTIN) 17 July 2008 (2008-07-17) page 3, line 15 - page 4, line 3 examples 4-10,12,13,17 -----	1-28 1-28 -/-
Y		

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  
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"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

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Date of the actual completion of the international search  <b>7 August 2013</b>	Date of mailing of the international search report  <b>16/08/2013</b>
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <b>Birikaki, Lemonia</b>

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2013/059839

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2006/003123 A2 (EUROPEAN COMMUNITY [BE]; MORGENSTERN ALFRED [DE]; APOSTOLIDIS CHRISTOS) 12 January 2006 (2006-01-12) page 7, lines 24-27 page 10, lines 20-28 page 11, line 20 - page 12, line 12 -----	1-28
1		

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No

PCT/EP2013/059839

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