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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.

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 Immunocon Radiopharm 4: 85-96 (1991)).

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 α-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}Ac	10.0 days	leukaemia
^{211}At	7.2 hours	glioblastoma
^{213}Bi	46 minutes	leukaemia
^{223}Ra	11.4 days	skeletal metastases
^{224}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}At, ^{213}Bi and ^{225}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.

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}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}Ra daughter nuclei released upon decay of ^{227}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.

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}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.

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 an 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 hydroxypyiridinone 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.

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.

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

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. thorioum-227) at greater than natural relative abundance, eg 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}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

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 call-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

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,

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}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}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}Ra will be an amount such that the

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}Th) containing compounds may be used in high dose regimens where the myelotoxicity of the generated radium (e.g. ^{223}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}Ra, which will contain the mother nuclide of ^{227}Th, i.e. ^{227}Ac ($T_{1/2}$ = 22 years). Actinium-227 can quite easily be separated from the ^{226}Ra target and used as a generator for ^{227}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}Th source, only moderate amounts of radium are produced during the first few days. However, the potential toxicity of ^{223}Ra is higher than that of ^{227}Th since the emission from ^{223}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}Th	α	6.02	18.72 days
^{223}Ra	α	5.78	11.43 days
^{219}Rn	α	6.88	3.96 seconds

^{215}Po	α	7.53	1.78 ms
^{211}Pb	β	0.45	36.1 minutes
^{211}Bi	α	6.67	2.17 minutes
^{207}Tl	β	1.42	4.77 minutes
^{207}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}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}Th (i.e. ^{227}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

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 an 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}Th, the main daughter product is ^{223}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}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}Th as the dissolved citrate salt, it was found that ^{223}Ra generated from ^{227}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}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}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}Ra generated from a ^{227}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.

Complexes with less than ideal biological half-life can however be useful as long as the dose of ^{223}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}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 300kBq/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}Ra indicated by multiplying these ranges by any of the bodyweights indicated. The above dose levels are preferably the fully retained dose of ^{227}Th but may be the administered dose taking into account that some ^{227}Th will be cleared from the body before it decays.

Where the biological half-life of the ^{227}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

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 ^{227}Th nucleus provides one ^{223}Ra atom, the retention and therapeutic activity of the ^{227}Th will be directly related to the ^{223}Ra dose suffered by the patient. The amount of ^{223}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 ^{223}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 ^{223}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, intracavitary (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 intracavitary 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.

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}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}Th. Suitable targeting moieties include poly- and oligo-peptides, proteins, DNA and RNA fragments, aptamers etc, preferably a protein, e.g. avidin, strepatavidin, 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 particuarly include Fab, F(ab')$_2$, 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    DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLAWYQQKPGQSP
Humanised ------------SA-V-DR----------------------------KA

Murine    KLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSS
Humanised ---------------S--S---------F---SL-P--I-T---------

Murine    WTFGGGTKLEIKR (SeqID1)
Humanised ------------- (SeqID2)
```

Heavy Chain:

```
Murine    QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGLEWIG
H'ised1   -----Q----VK---S---V----------------VR-A---------
```

H'ised2 ----VQ----VK---S---V----------VR-A---------

Murine **YINPRNDYTEYNQNFKD**KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR
H'ised1 ----------I---E-TN----E----R---T-F-F---
H'ised2 ----------I---E-TN----E----R---T-F-F---

Murine **RDITTFY**WGQGTTLTVSS (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

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 thoruim 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

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 spend 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. $^{227}Th^{4+}$ ion). The products of the invention are thus preferably formed or formable by complexation of alpha-emitting thorium isotope (e.g. $^{227}Th^{4+}$ 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}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.

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 octodentate 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

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.

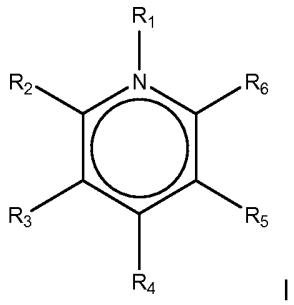
The thorium (e.g. thorium-227) labelled molecules of the invention may be used for the treatment of cancerous or non-cancerous diseases by targeting disease-related receptors. Typically, such a medical use of ^{227}Th will be by radioimmunotherapy based on linking ^{227}Th by a chelator to an antibody, an antibody fragment, or a construct of antibody or antibody fragments for the treatment of cancerous or non-cancerous diseases. The use of ^{227}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}Ra released could be diminished if the molecule carrying ^{227}Th has a short biological retention half-time in vivo because the radionuclide will mostly be eliminated before a high proportion of the ^{227}Th has decayed to ^{223}Ra. The amount of ^{227}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}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}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}Th and by the ^{223}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}Ra by the targeting of the skeletal disease. In this

therapeutic method, ^{227}Th is typically utilised to treat primary and/or metastatic cancer of soft tissue by suitable targeting thereto and the ^{223}Ra generated from the ^{227}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):



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.

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-propy, n-, iso- or sec-butyl and so forth.

Highly preferred R_1 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_1. 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_2 to R_6 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_2 to R_6 will be OH. Generally, at least one of groups R_2 to R_6 will be =O. Generally, at least one of groups R_2 to R_6 will be a linker moiety (as described herein). Preferably, exactly one of groups R_2 to R_6 will be =O. Preferably exactly

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.

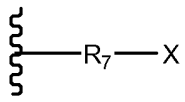
Suitable chelating moieties may be formed by methods known in the art, including the methods described in US 5,624,901 (e.g. examples 1 and 2) and WO2008/063721 (both incorporated herein by reference).

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:

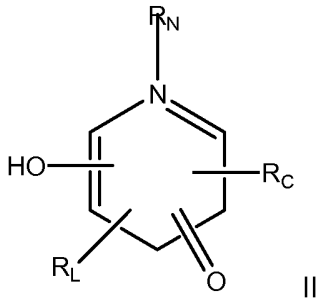


wherein R_7 is a bridging moiety, which is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or

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:



II

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_L 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_L including short hydrocarbyl groups, such as C1 to C8 hydrocarbyl, including C1 to C8 alkyl,

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 hydroxyalkyl group as indicated herein.

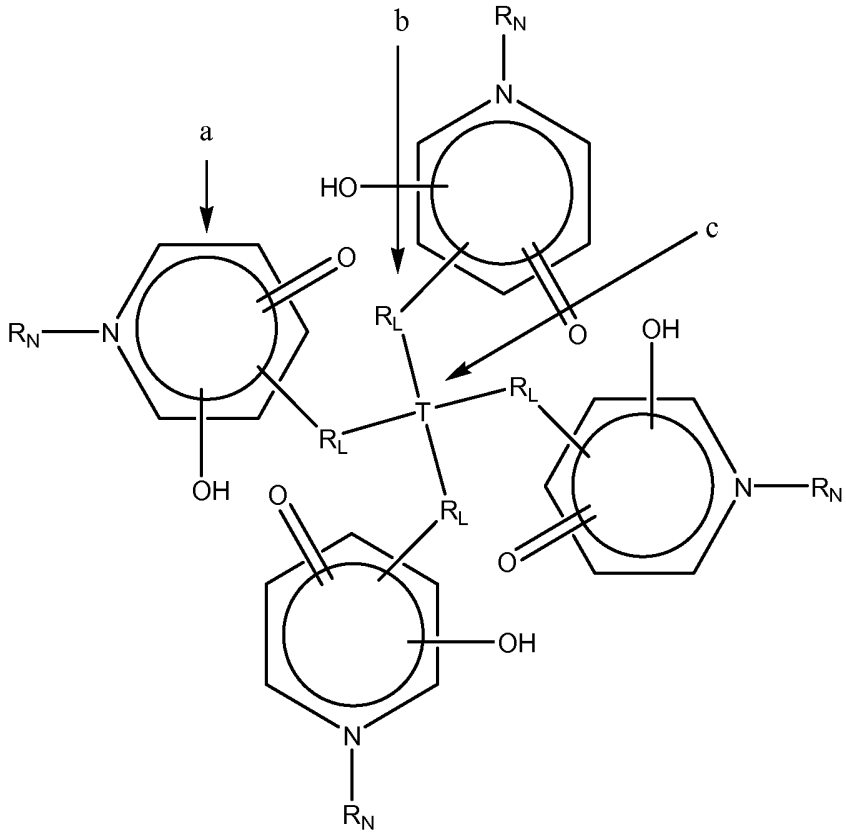
In one preferred embodiment, at least one 3,2- hydroxypyridinone moiety is present in the octadentate ligand structure. This may evidently be substituted by any of the various substituent moieties indicated herein.

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

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:



III

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.

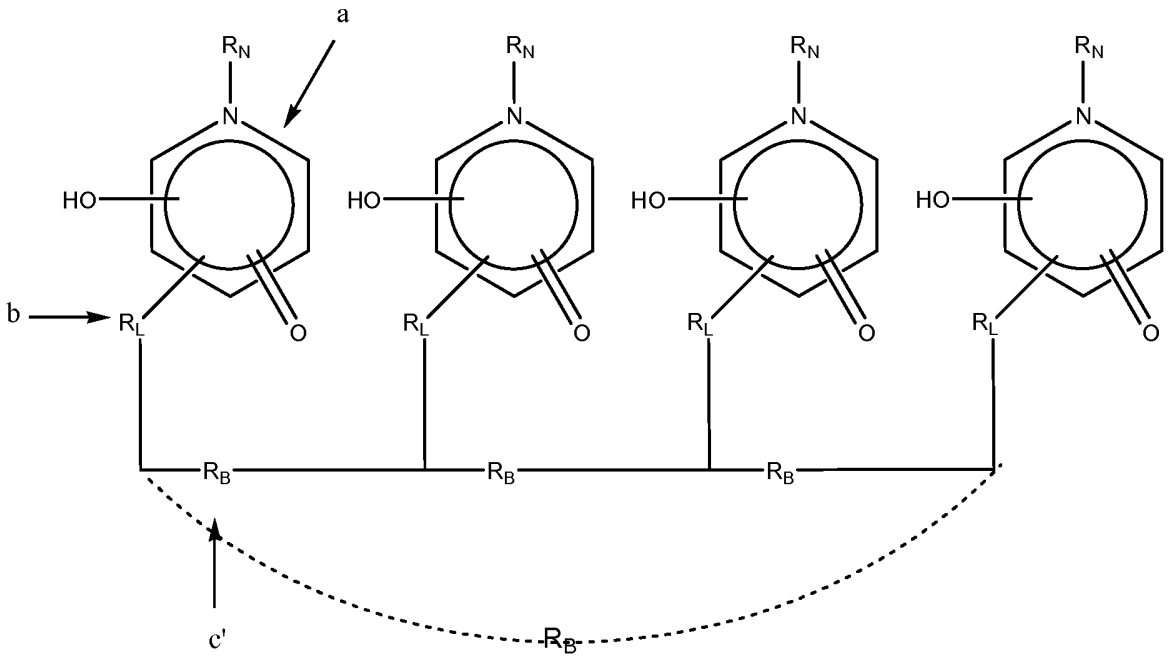
Evidently, in the structures of formula II III, IV and IVb, those positions of the pyridine ring(s) which are not otherwise substituted (e.g by a linker or coupling

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



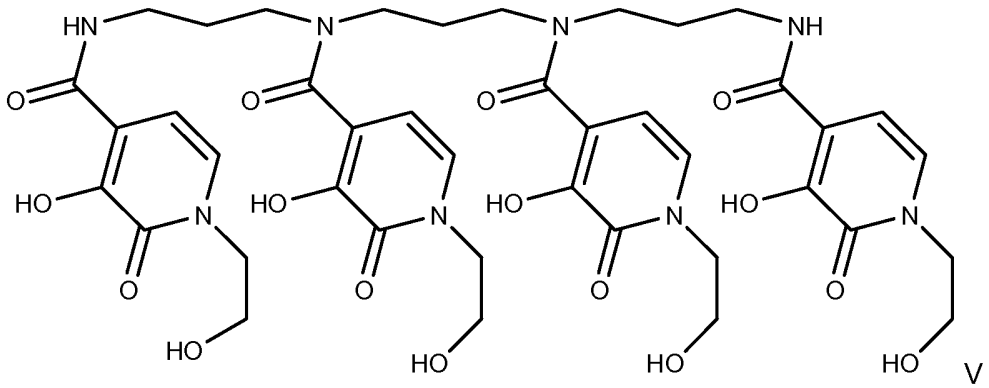
IV

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

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.

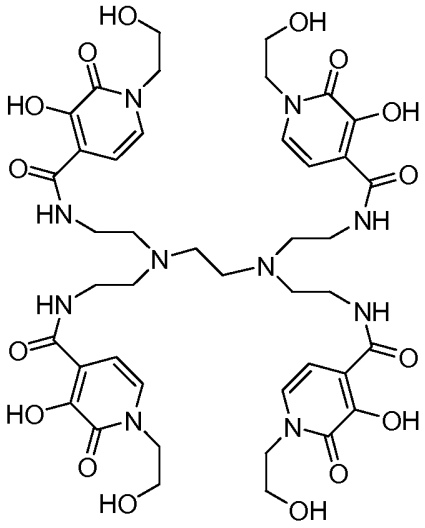
An example of a "backbone" type octadentate ligand having four 3,2-HOPO chelating moieties (each with a hydroxyethyl solubilising group) attached to a backbone by amide linker groups would be formula V as follows:



V

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 alkylenes such as any of those described herein (methylene, ethylene, propylene, and butylene being highly suitable among those).

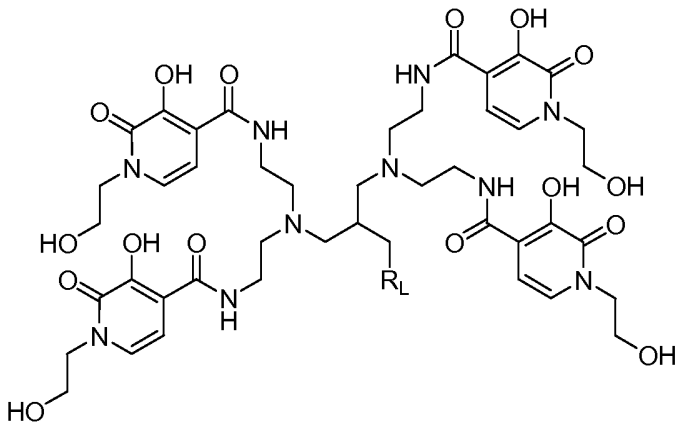
Exemplary "templated" octadentate ligands, each having four 3,2-HOPO chelating moieties linked by ethyl amide groups to ethyl and propyl diamine respectively would be formula VI as follows:



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.

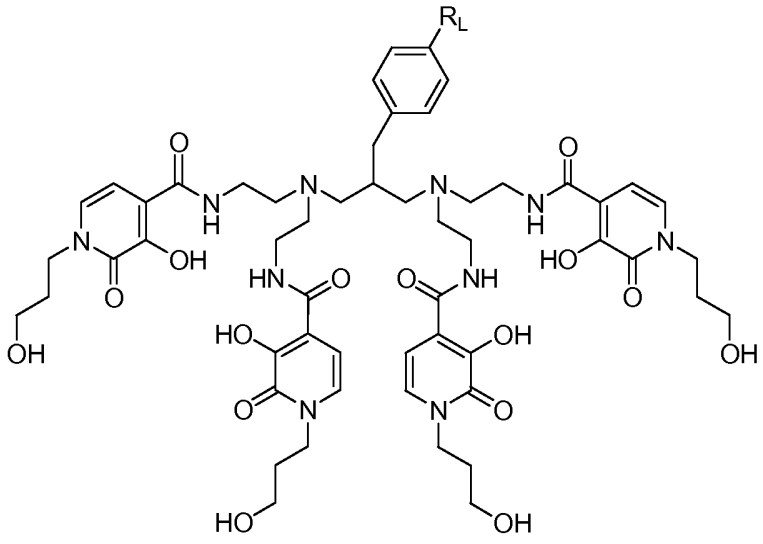
As indicated above, the octadentate ligand will typically include a coupling moiety which may join to the remainder of the ligand at any point. A suitable point for linker attachment is shown below in formula VI:



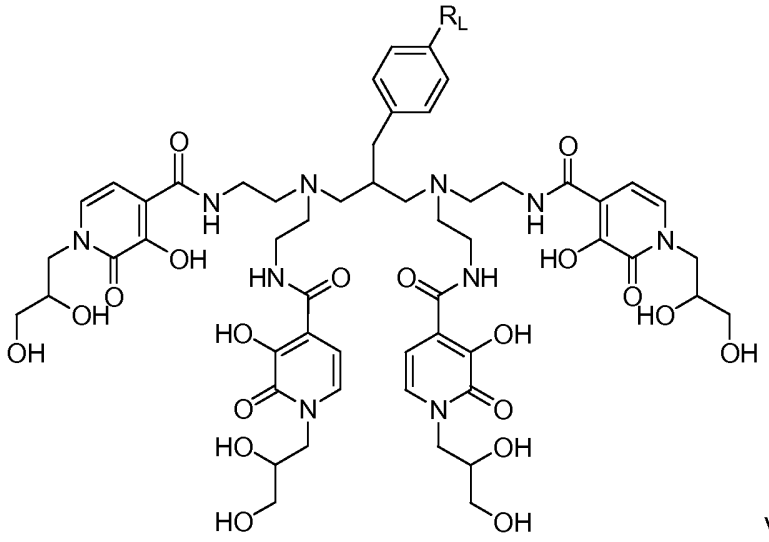
VI

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:



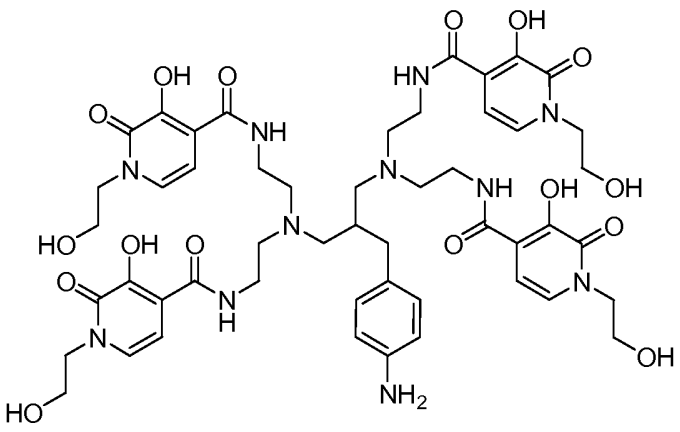
VII



VIII

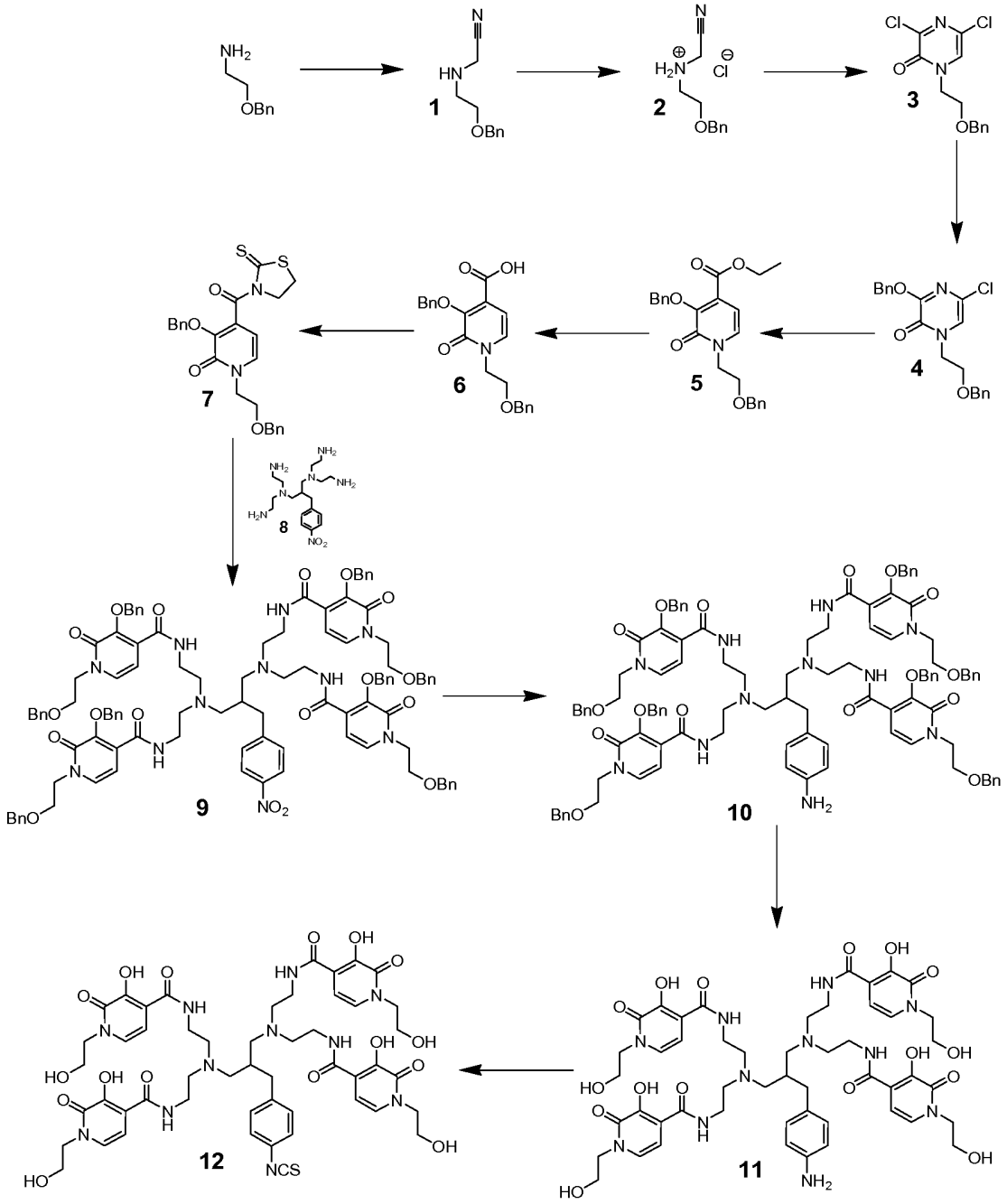
Wherein in formulae VII and VIII R_L may be any suitable linker group or reactive moiety as described herein. R_L will typically form the point of attachment of the ligand to the targeting moiety and thus any suitable reactive group can be used for this attachment either directly or using a further linker.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



IX

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



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.

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

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

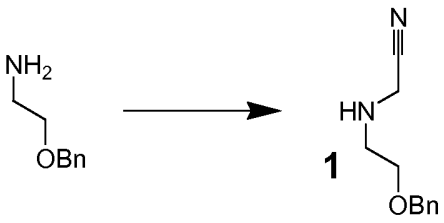
Thorium-227 is isolated from an actinium-227 cow. Actinium-227 was produced through thermal neutron irradiation of Radium-226 followed by the decay of Radium-227 (t1/2=42.2 m) to Actinium-227. Thorium-227 was selectively retained

from an Actinium-227 decay mixture in 8 M 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

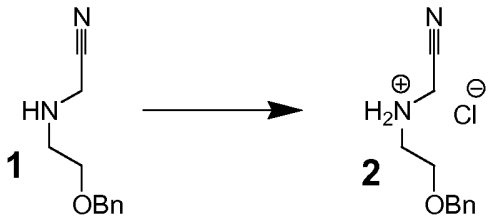
Step 1



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.

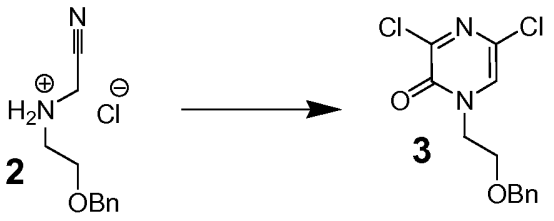
^{1}H-NMR ($CDCl_3$, 400MHz): 2.92(m, 2H), 3.58-3.62(m, 4H), 4.51(s, 2H), 7.25-7.37(m, 5H)

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

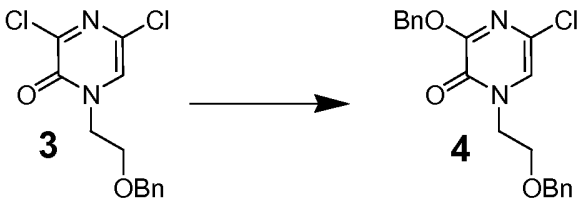


2 (27.8g, 122.6mmol) was dissolved in 230mL chlorobenzene at room temperature. Oxallyl chloride (45mL, 530mmol) dissolved in 100mL chlorobenzene was added drop wise over 30 minutes at room temperature. The reaction mixture was stirred at room temperature for 45 hours. The reaction was carefully quenched 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 Na_2SO_4, filtered and the volatiles were removed under reduced pressure. The crude product was purified by dry flash chromatography on SiO_2 using a gradient of MeOH (0-2%) in DCM, yielding the desired product (21.2g, 70.8mmol).

^{1}H-NMR ($CDCl_3$, 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

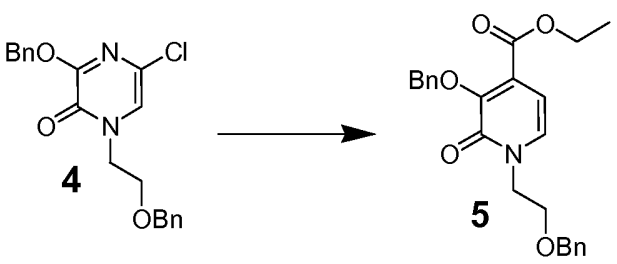


Sodium hydride (60% dispersion, 3.60g, 90mmol) was stirred in 50mL THF at 0 °C and benzyl alcohol (8.3mL, 80mmol) was added drop wise over 10 minutes. The reaction mixture was stirred for 30 minutes at 0 °C before **3** (21.2g, 70.8mmol) dissolved in 100mL THF was added drop wise at 0 °C. The reaction 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 Na_2SO_4, filtered and the volatiles were removed under reduced pressure. Dry flash chromatography on SiO_2 using a gradient of MeOH (0-6%) in DCM gave the desired product (25.6g, 69mmol).

^{1}H-NMR ($CDCl_3$, 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)

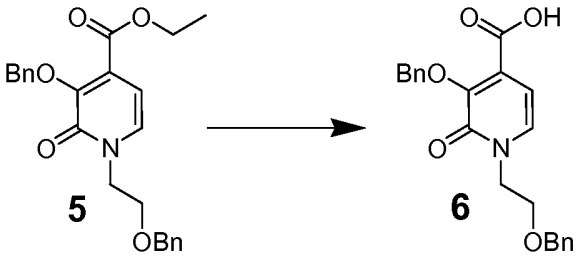
MS(ESI-pos, m/z): 371.1, 763.2

Step 5



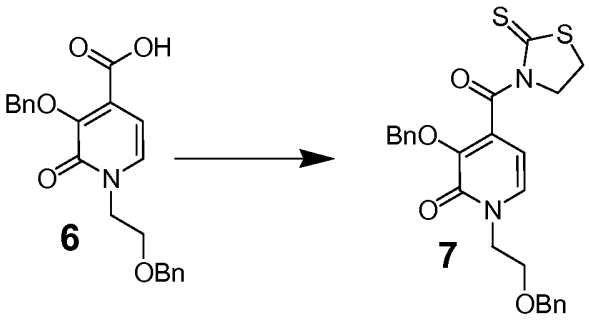
4 (25.6g, 69mmol) and ethyl propiolate (41mL, 0.4mol) was heated at 140 °C for 5 hours. The reaction mixture was cooled down to room temperature and the reaction mixture was purified by dry flash chromatography on SiO_2. A gradient of MeOH (0-10%) in DCM gave the desired product as an inseparable mixture 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 $CHCl_3$. 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

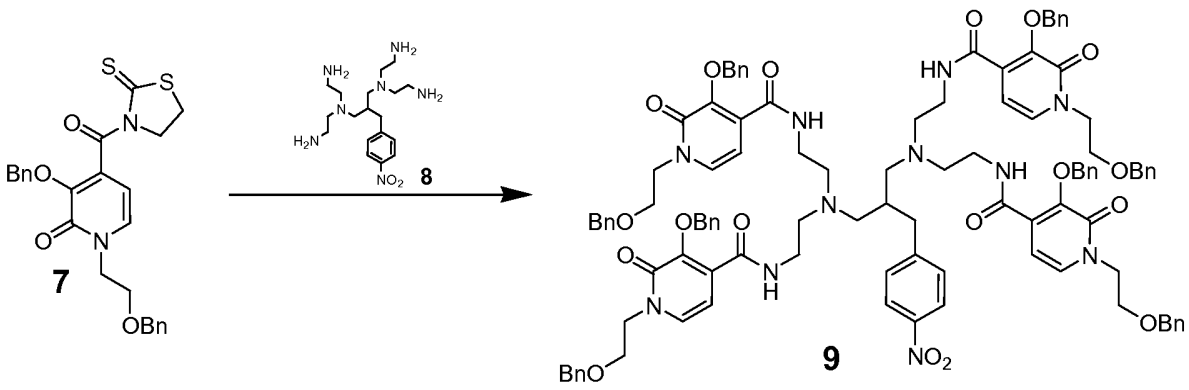


6 (25.9g, ~64mmol), as obtained in the previous step, was partially dissolved in 400mL DCM. 2-Thiazoline-2-thiol (8.94g, 75mmol) and DMAP (0.86g, 7mmol) was added, followed by DCC (15.48g, 75mmol). The reaction mixture was stirred at room temperature over night. The reaction mixture was filtered through 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 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 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}H-NMR ($CDCl_3$, 300MHz): 2.90(t, *J*=7.3Hz, 2H), 3.77-3.84(m, 2H), 4.18-4.23(m, 2H), 4.35(t, *J*=7.3Hz, 2H), 4.51(s, 2H), 5.33(s, 2H), 6.11(d, 7.0Hz, 1H), 7.21-7.48(m, 11H)

MS(ESI-pos, m/z): 503.1

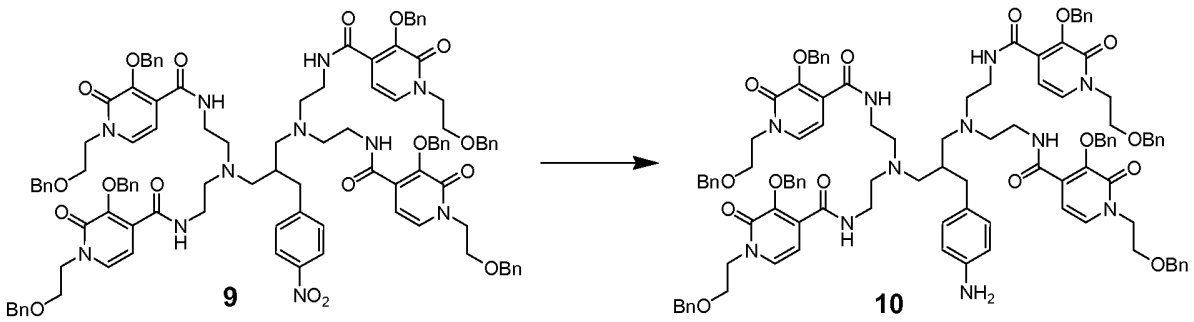
Step 8



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 SiO_2using a gradient of DCM and MeOH in EtOAc gave the desired product (3.93g, 2.15mmol).

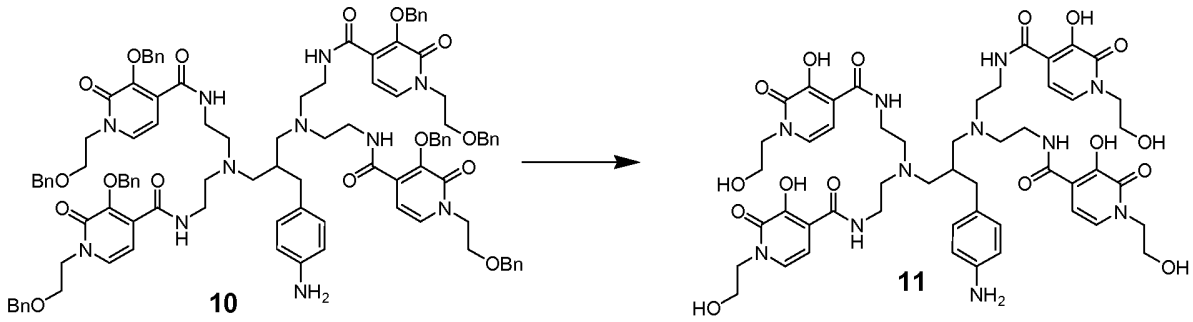
^{1}H-NMR ($CDCl_3$, 400MHz): 2.20-2.32(m, 10H), 2.44-2.50(m, 2H), 3.05-3.20(m, 10H), 3.23-3.27(m, 1H), 3.69-3.77(m, 8H), 4.06-4.15(m, 8H), 4.43(s, 8H), 5.24(s, 8H), 6.62(d, *J*=7.2Hz, 4H), 7.13(d, *J*=7.2Hz, 4H), 7.16-7.38(m, 42H), 7.82-7.93(m, 6H)

Step 9



9 (3.93g, 2.15mmol) was dissolved in 300mL EtOH at room temperature. 60mL water was added, followed by NH_4Cl (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_2SO_4, filtered and the volatiles were removed under reduced pressure. The product mixture was purified by dry flash chromatography on SiO2 using a gradient of MeOH (0-7%) in DCM gave the desired product (3.52g, 1.96mmol).
MS(ESI-pos, m/z): 899.2

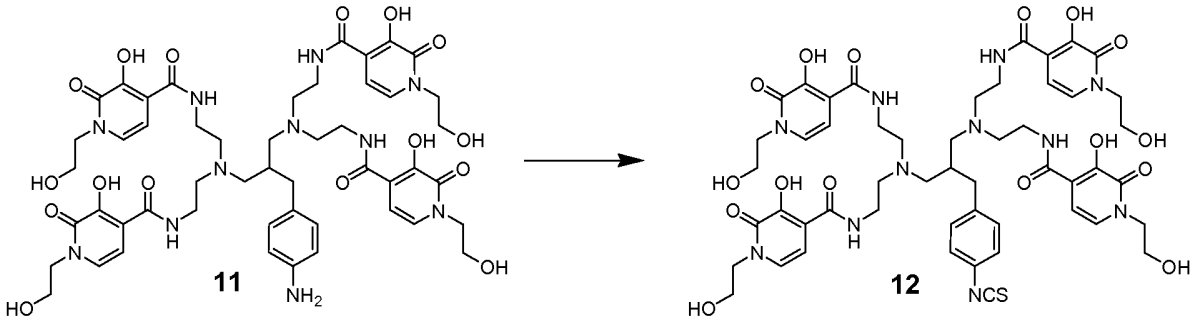
Step 10



10 (1.00g, 0.56mmol), $Pd(OH)_2/C$ (Pearlman's catalyst, 1.00g) and 10mL AcOH was placed in a pressure reactor. The reactor was evactuated by water aspirator and H_2 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_2 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

product was precipitated by addition of Et_2O. The solids were collected by centrifugation and decanting the supernatant before the product was dried *in vacuo* (484mg, 0.45mmol).
^{1}H-NMR (D_2O, 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=7.0Hz, 4H), 7.44(d, *J*=8.2Hz, 2H), 7.57(d, *J*=8.2Hz, 2H)
MS(ESI-pos, m/z): 1076.4

Step 11



Compound 11 (20mg, 18µmol) was dissolved in 3mL MeCN and 3mL water. 20 µL 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 acetontrile phase to 40mL Et_2O. The solids were collected by centrifugation and decanting the supernatant before the product was dried *in vacuo* (10mg, 9µmol).
MS(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

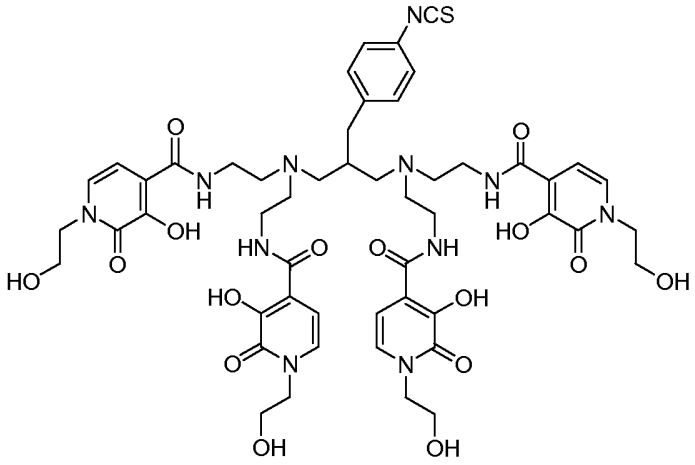
LC/MS analysis. Sample purity would be further analyzed by size-exclusion chromatography (SEC).

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:



12

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,

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}Th) as a 4+ ion was isolated from an actinium-227 (^{227}Ac) generator system. ^{227}Th was selectively retained from a ^{227}Ac decay mixture in 8 M HNO_3 by anion exchange chromatography, where negatively charged nitrate complexes are formed with $^{227}Th^{4+}$. ^{227}Ac and daughter nuclides were washed off the column and ^{227}Th was eluted in 12 M HCl. The ^{227}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}Th per 0.5 mg antibody conjugate. The high molecular fraction containing radio labelled antibody-conjugate was separated from free ^{227}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_{50} 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 $1x10^6$ 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.

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_{50} 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_{50} 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_2, 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

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_2. The same procedure, but with only one wash, was repeated at days 3, 5 and 7.

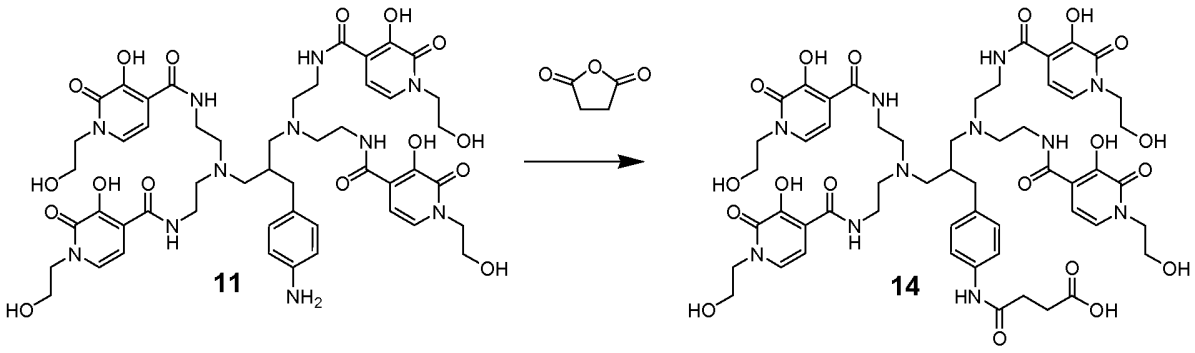
A quantification of ATP was used as a measure of cell viability at different sample times (CellTiter-Glo Luminescent cell viability assay from Promega), resulting in the curves shown in Figure 3. The Ramos cell binding AGC1115-Th-227 resulted in cellular toxicity, in contrast to the Th-227 labelled control 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 30µL NEt_3. 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.



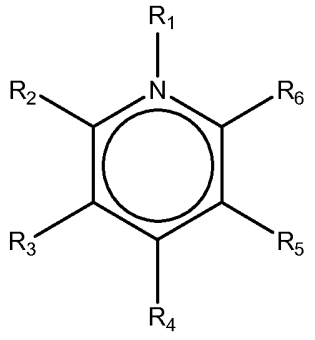
Claims

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



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; groups R_2 to R_6 are each independently selected from H, OH, =O, short hydrocarbyl groups, linker moieties and/or coupling moieties wherein one of R_2 to R_6 is OH and one of R_2 to R_6 is =O.

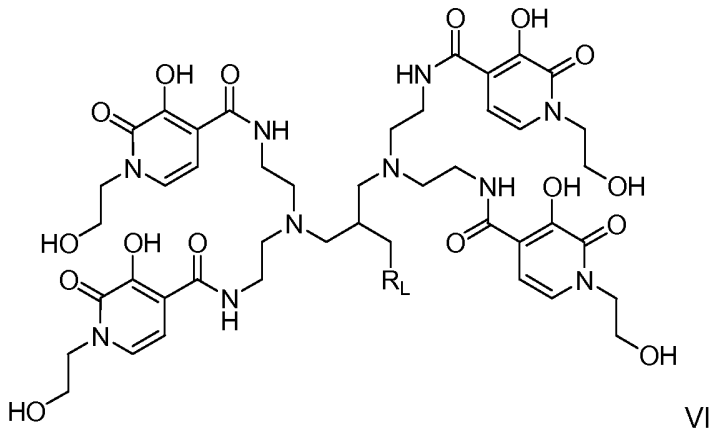
5. The complex as claimed in claim 4 wherein at least one of groups R_1 to R_6 is a linker moiety.

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-, $HOCH_2$-, $HOCH_2CH_2$-, HO-$CH_2CH_2CH_2$-, HO-$CH(CH_3)CH_2$-, HO-$CH_2CH_2CH_2CH_2$-, HO-$CH(CH_3)CH_2CH_2$-, HO-$CH(CH_2CH_3)CH_2$-, HO-$C(CH_3)_2CH_2$-, HO-$CH(CH_3)CH(CH_3)$- and $HOCH_2CH(CH_2CH_3)$-.

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 ^{227}Th.

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



VI

Wherein R_L 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, $F(ab')_2$, 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**
SGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYC**HQYLSSWT**FGGGTKLEIKR (SeqID1)

DIQLTQSPSSLASAAVEDRTMSC**KSSQSVLYSANHKNYLA**WYQQKPGQKAKLLIY**WASTRE**
SGVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**HQYLSSWT**FGGGTKLEIKR (SeqID2)

Heavy Chain:

QVQLQESGAELSKPGASVKMSCKASGYTFT**SYWLH**WIKQRPGQGLEWIG**YINPRNDYTEYN**
QNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCAR**RDITTFY**WGQGTTLTVSS
(SeqID3)

QVQLQQSGAEVKKPGSSVKVSCKASGYTFT**SYWLH**WVRQAPGQGLEWIG**YINPRNDYTEYN**
QNFKDKATITADESTNTAYMELSSLRSEDTAFYFCAR**RDITTFY**WGQGTTVTVSS
(SeqID4)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFT**SYWLH**WVRQAPGQGLEWIG**YINPRNDYTEYN**
QNFKDKATITADESTNTAYMELSSLRSEDTAFYFCAR**RDITTFY**WGQGTTVTVSS
(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 hydroxyalkyl 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-

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 is 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

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

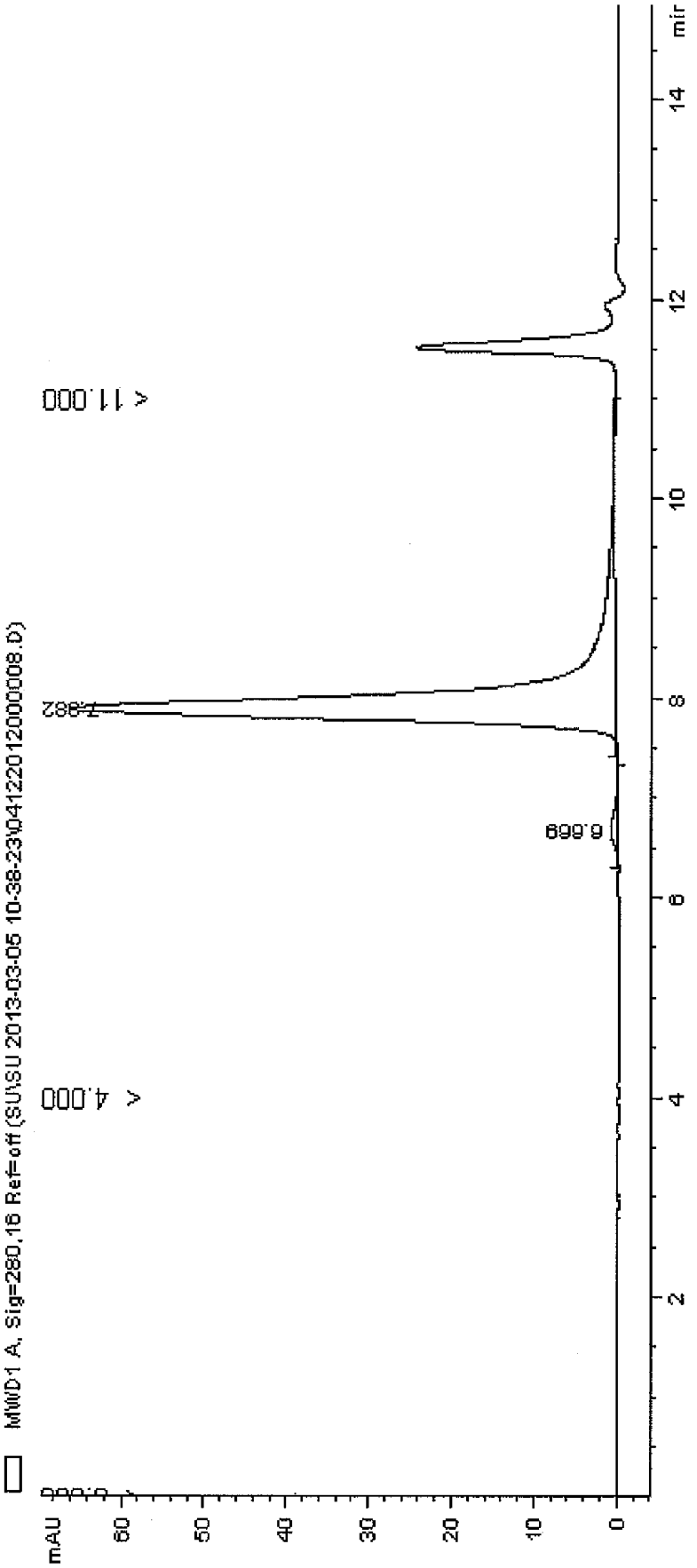
24. The method of claim 23 comprising preparing a first aqueous solution of octadentate hydroxypyridinone-containing ligand and a second aqueous solution of said tissue targeting moiety and contacting said first and said second aqueous solutions.

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.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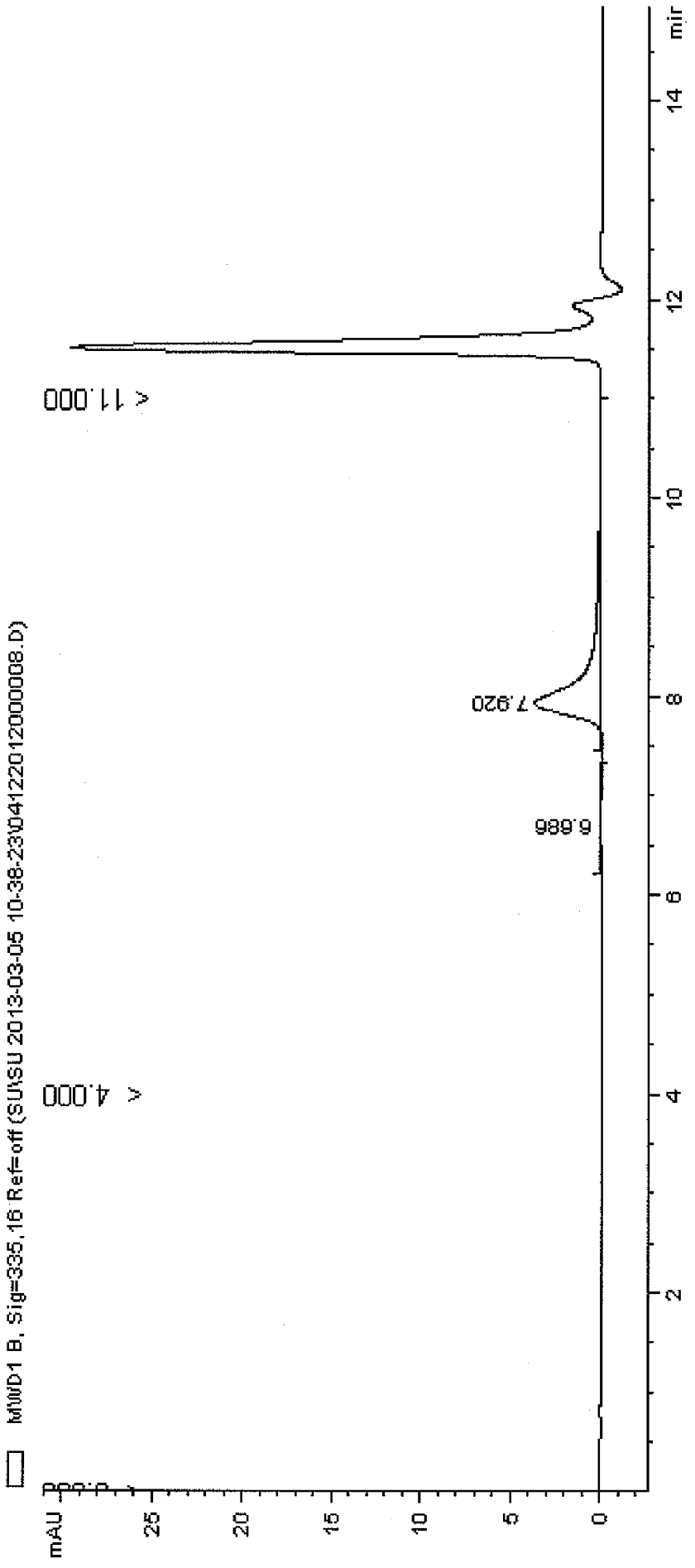
mAU
60
50
40
30
20
10
0
2
4
6
8
10
12
14
min
7.882
6.669
A 4.000
A 11.000

Figure 1a)


mAU
25
20
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10
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0
2
4
6
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10
12
14
min
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7.920
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Figure 1b

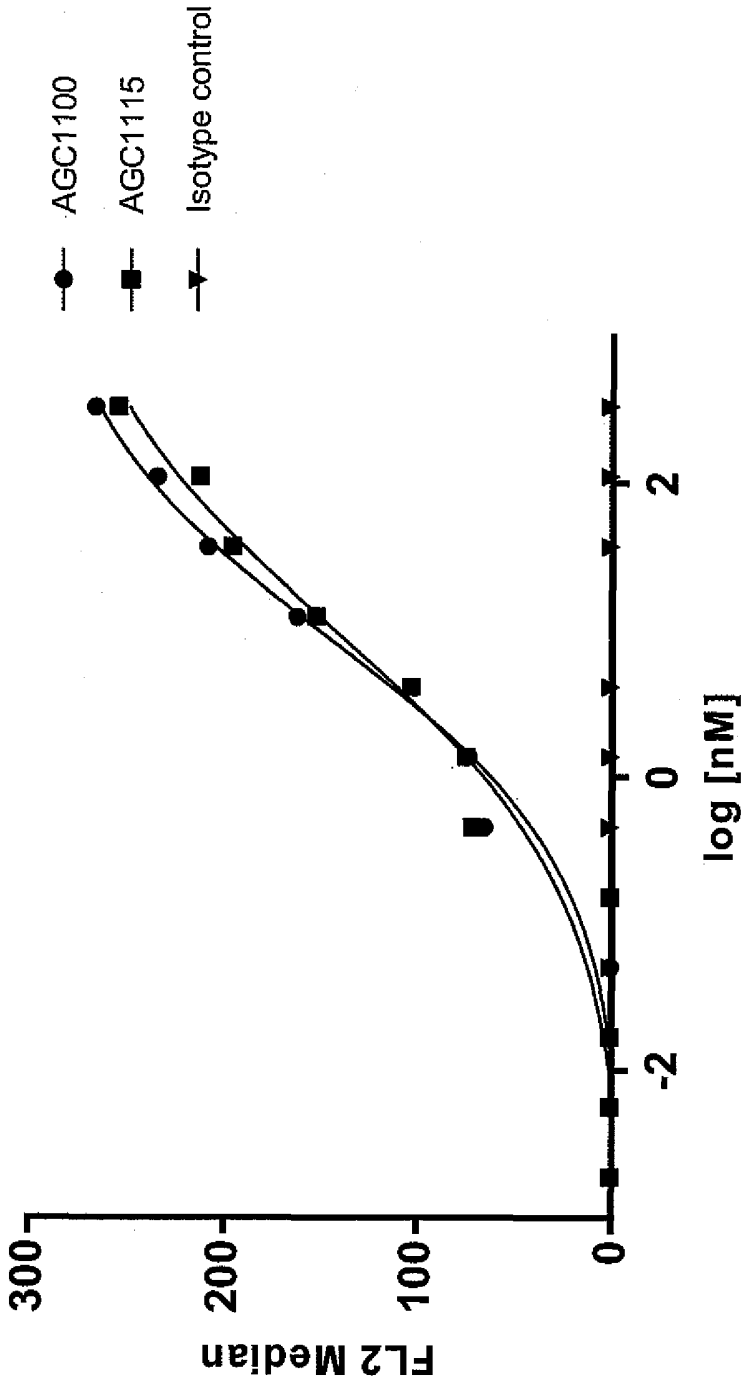
AGC1100
AGC1115
Isotype control
FL2 Median
300
200
100
0
-2
0
2
log [nM]

Figure 2

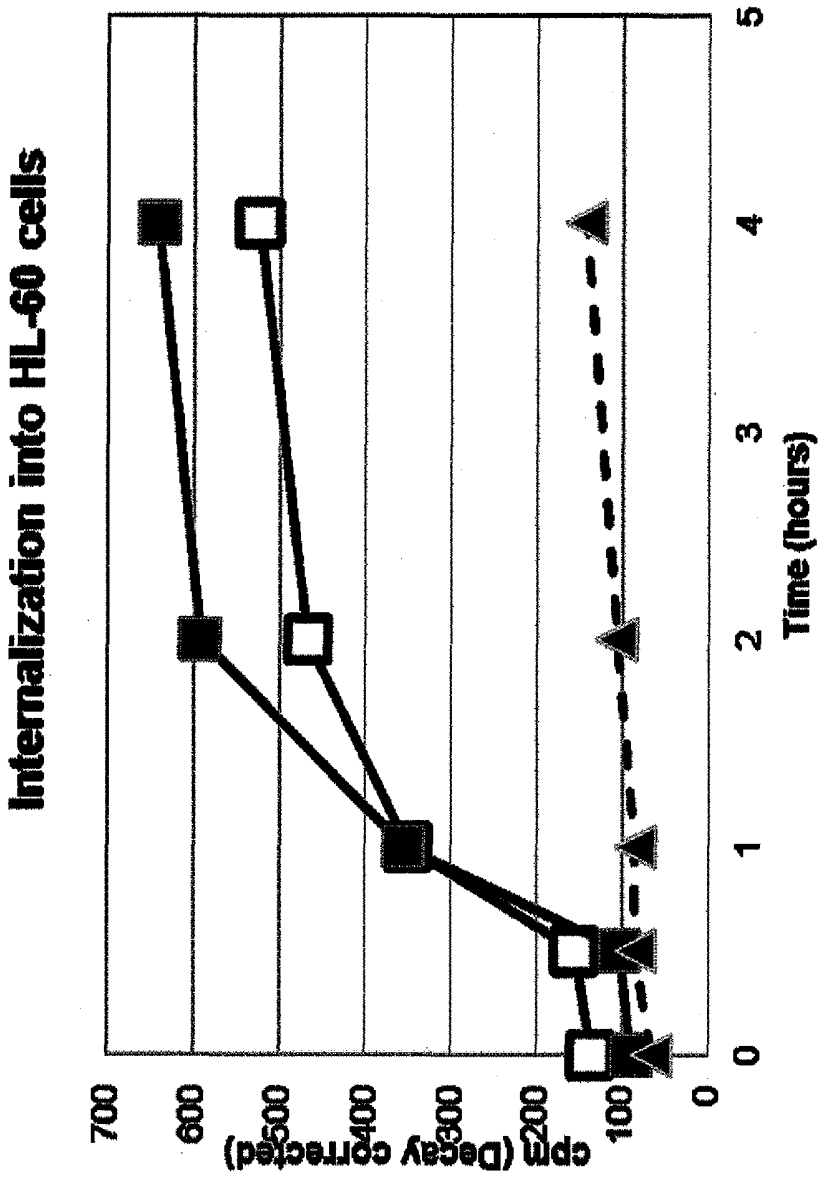
Internalization into HL-60 cells
cpm (Decay corrected)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Time (hours)

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	1-28
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

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2013	16/08/2013

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

1

Form PCT/ISA/210 (second sheet) (April 2005)

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

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011098611 A2	18-08-2011	AU 2011214281 A1	30-08-2012
		CA 2789433 A1	18-08-2011
		EP 2533817 A2	19-12-2012
		JP 2013519658 A	30-05-2013
		KR 20120130769 A	03-12-2012
		SG 183279 A1	27-09-2012
		US 2013183235 A1	18-07-2013
		WO 2011098611 A2	18-08-2011
WO 2008085064 A2	17-07-2008	EP 2104662 A2	30-09-2009
		US 2010056787 A1	04-03-2010
		WO 2008085064 A2	17-07-2008
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		CA 2570191 A1	12-01-2006
		EP 1763372 A2	21-03-2007
		EP 2497501 A2	12-09-2012
		WO 2006003123 A2	12-01-2006

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(87)PCT国际申请的公布数据
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(74)专利代理机构 北京派特恩知识产权代理有限公司 11270
代理人 浦彩华 武晨燕
(51)Int.Cl.
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A61K 51/10(2006.01)
A61P 35/00(2006.01)
A61K 103/40(2006.01)

权利要求书3页 说明书29页
序列表3页 附图4页

(54)发明名称

放射性药物络合物

(57)摘要

本文公开了一种组织靶向络合物，其包含组织靶向部分、包含四个HOPO部分的含羟基吡啶酮的八齿配体以及α发射钍放射性核素的离子，其中所述四个HOPO部分中的至少一个在N位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对CD22受体的结合亲和力。本文提供了利用此类络合物的治疗方法和此类络合物的形成方法。

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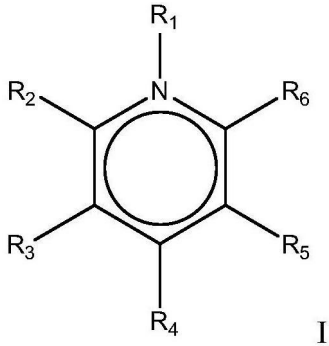
1. 一种组织靶向络合物，其包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中所述四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。

2. 如权利要求 1 所述的络合物，其包含至少一个 3,2-HOPO 部分。

3. 如权利要求 1 或权利要求 2 所述的络合物，其中全部 4 个 HOPO 部分包含在所述 N 位的羟基烷基增溶部分。

4. 如权利要求 1 或权利要求 2 所述的络合物，其包含含有四个式 I 的螯合部分的八齿配体



I

其中 R_1 为任选的 N 取代基增溶基团，其将存在于所述四个式 I 部分中的至少一个中；基团 R_2 至 R_6 各自独立地选自 H、OH、＝ O、短烃基、连接子部分和 / 或偶联部分，其中 R_2 至 R_6 中的一个为 OH 并且 R_2 至 R_6 中的一个为＝ O。

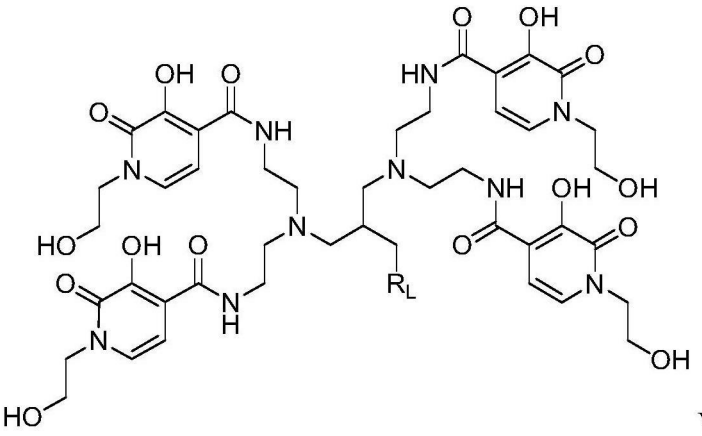
5. 如权利要求 4 所述的络合物，其中基团 R_1 至 R_6 中的至少一个是连接子部分。

6. 如权利要求 4 至 5 中任一项所述的络合物，其包含四个 3,2- 羟基吡啶酮部分。

7. 如前述权利要求中任一项所述的络合物，其中在所述四个 HOPO 基团中的每一个上的 N 取代基各自独立地选自 HO-、$HOCH_2$-、$HOCH_2CH_2$-、HO-$CH_2CH_2CH_2$-、HO-$CH(CH_3)CH_2$-、HO-$CH_2CH_2CH_2CH_2$-、HO-$CH(CH_3)CH_2CH_2$-、HO-$CH(CH_2CH_3)CH_2$-、HO-$C(CH_3)_2CH_2$-、HO-$CH(CH_3)CH(CH_3)$- 和 $HOCH_2CH(CH_2CH_3)$-。

8. 如权利要求 1 至 7 中任一项所述的络合物，其中 α 发射钍放射性核素的所述离子是 α 发射钍放射性核素诸如 ^{227}Th 的 4+ 离子。

9. 一种如权利要求 1 至 8 中任一项所述的络合物，其包含式 VI 的配体部分：



VI

其中 R_L 是任何合适的连接子部分。

10. 一种如前述权利要求中任一项所述的络合物，其中所述组织靶向部分是单克隆或

多克隆抗体、抗体片段（诸如 Fab、$F(ab')_2$、Fab' 或 scFv）、或此类抗体和 / 或片段的构建体。

11. 一种如前述权利要求中任一项所述的络合物，其中所述组织靶向部分包含与至少一个下列序列具有至少 90％序列相似性的至少一条肽链：

轻链：

DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSSWTFGGGTKLEIKR(SeqID1)

DIQLTQSPSSLASAAVEDRTMSCKSSQSVLYSANHKNYLAWYQQKPGQKAKLLIYWALTRESGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCHQYLSSWTFGGGTKLEIKR(SeqID2)

重链：

QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGLEWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGTTLTVSS(SeqID3)

QVQLQQSGAEVKKPGSSVKVSCKASGYTFTSYWLHWVRQAPGQGLEWIGYINPRNDYTEYN

QNFKDKATITADESTNTAYMELSSLRSEDTAFYFCARRDITTFYWGQGTTVTVSS(SeqID4)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYWLHWVRQAPGQGLEWIGYINPRNDYTEYNQNFKDKATITADESTNTAYMELSSLRSEDTAFYFCARRDITTFYWGQGTTVTVSS(SeqID5)

12. 一种组织靶向络合物用于制造供治疗增生性或肿瘤性疾病的药物的用途，所述组织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及所述 α 发射钍放射性核素的离子，其中所述四个 HOPO 部分中的至少一个在所述 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。

13. 如权利要求 12 所述的用途，其中所述组织靶向络合物是如权利要求 1 至 11 中任一项所述的络合物。

14. 如权利要求 12 或权利要求 13 所述的用途，其中所述疾病是癌瘤、肉瘤、骨髓瘤、白血病、淋巴瘤或混合型癌症，包括非霍奇金淋巴瘤或 B 细胞肿瘤。

15. 一种治疗人或非人动物（特别是有需要者）的方法，其包括施用至少一种组织靶向络合物，所述组织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及所述 α 发射钍放射性核素的离子，其中所述四个 HOPO 部分中的至少一个在所述 N 位被羟基烷基增溶基团取代，其中所述组织靶向部分具有对所述 CD22 受体的结合亲和力。

16. 如权利要求 15 所述的方法，其中所述组织靶向络合物是如权利要求 1 至 11 中任一项所述的络合物。

17. 如权利要求 15 或权利要求 16 所述的方法，其用于治疗增生性和 / 或肿瘤性疾病，诸如癌瘤、肉瘤、骨髓瘤、白血病、淋巴瘤或混合型癌症，包括非霍奇金淋巴瘤或 B 细胞肿瘤。

18. 一种如权利要求 1 至 11 中任一项所述的组织靶向络合物，其用于疗法中。

19. 一种如权利要求 18 所述的组织靶向络合物，其用于治疗增生性和 / 或肿瘤性疾病，例如癌瘤、肉瘤、骨髓瘤、白血病、淋巴瘤或混合型癌症，包括非霍奇金淋巴瘤或 B 细胞肿瘤。

20. 一种药物组合物，其包含组织靶向络合物以及至少一种药物载体或赋形剂，所述组

织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及所述 α 发射钍放射性核素的离子，其中所述四个 HOPO 部分中的至少一个在所述 N 位被羟基烷基增溶基团取代，其中所述组织靶向部分具有对所述 CD22 受体的结合亲和力。

21. 如权利要求 20 所述的药物组合物，其所包含的组织靶向络合物是如权利要求 1 至 11 中任一项所述的络合物。

22. 一种用于根据权利要求 15 至 17 中任一项所述的方法中的试剂盒，所述试剂盒包含与或可与包含四个 HOPO 部分的含羟基吡啶酮的八齿配体缀合的组织靶向部分，其中所述四个 HOPO 部分中的至少一个在所述 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对所述 CD22 受体的结合亲和力，所述试剂盒任选地且优选地包括 α 发射钍放射性核素诸如 ^{227}Th。

23. 一种形成组织靶向络合物的方法，所述方法包括使组织靶向部分与含羟基吡啶酮的八齿配体在水溶液中偶联，所述络合物包含四个 HOPO 部分和所述 α 发射钍放射性核素的离子，其中所述四个 HOPO 部分中的至少一个在所述 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对所述 CD22 受体的结合亲和力。

24. 如权利要求 23 所述的方法，其包括制备含羟基吡啶酮的八齿配体的第一水溶液和所述组织靶向部分的第二水溶液并且使所述第一水溶液与所述第二水溶液接触。

25. 如权利要求 24 所述的方法，其中所述接触在低于 40℃下进行。

26. 如权利要求 24 或权利要求 25 所述的方法，其中所述接触在基本上不存在任何有机溶剂的情况下进行。

27. 如权利要求 23 至 26 中任一项所述的方法，其中所述偶联在所述螯合物与所述抗体之间产生酰胺、酯、醚或胺键。

28. 如权利要求 27 所述的方法，其中所述酯或酰胺键借助于至少一个活化酯基形成，例如借助于至少一个 N- 羟基马来酰亚胺、碳化二亚胺或偶氮二羧酸酯偶联试剂形成。

放射性药物络合物

技术领域

[0001] 本发明涉及钍同位素的络合物并且特别涉及钍 -227 与某些缀合至组织靶向部分的八齿配体的络合物。本发明还涉及疾病，特别是肿瘤性疾病的治疗，其包括施用此类络合物。

背景技术

[0002] 特异性细胞杀伤对于哺乳动物受试者中多种疾病的成功治疗可为必不可少的。这种情形的典型实例是恶性疾病如肉瘤和癌瘤的治疗。然而，选择性消除某些细胞类型在其它疾病，尤其是增生性和肿瘤性疾病的治疗中也可起到关键作用。

[0003] 目前选择性治疗的最常见方法是手术、化疗和外照射。然而，靶向放射性核素疗法是一种有潜力向有害的细胞类型递送高细胞毒性放射的有希望和正在发展中的领域。目前批准用于人的最常见形式的放射性药物使用 β 发射和 / 或 γ 发射放射性核素。然而，由于 α 发射放射性核素用于更特异性细胞杀伤的潜力，其在疗法中的使用已受到一些关注。

[0004] 典型的 α 发射体在生理环境中的放射范围一般小于 100 微米，仅相当于几个细胞直径。这使得这些源非常适合于肿瘤（包括微转移）的治疗，因为它们具有到达肿瘤内的邻近细胞的范围，但如果它们被准确靶向，则几乎没有放射能量将越过靶细胞。因此，并非每个细胞都需要被靶向，而对周围健康组织的损伤可降到最小（参见 Feinendegen 等，Radiat Res148:195-201(1997))。相比之下，β 粒子在水中具有 1mm 或更大的范围（参见 Wilbur,Antibody Immunocon Radiopharm 4:85-96(1991))。

[0005] 相比于由 β 粒子、γ 射线和 X 射线所带的能量，α 粒子放射的能量较高，通常为 5-8MeV，或为 β 粒子的 5 至 10 倍以及 γ 射线的能量的 20 倍或更大。因此，大量能量在很短距离上的这种沉积使得 α 放射相比于 γ 和 β 放射具有异常高的线性能量转移 (LET)、高的相对生物功效 (RBE) 和低的氧增强比 (OER)（参见 Hall,"Radiobiology for the radiologist", 第五版，Lippincott Williams&Wilkins,Philadelphia PA,USA,2000)。这解释了 α 发射放射性核素的异常细胞毒性并且还对此类同位素的生物靶向以及 α 发射放射性核素分布的控制水平和研究施加了严格要求，为避免不可接受的副作用，这是必要的。

[0006] 下表 1 示出了迄今为止在文献中尽可能宽泛提出的具有治疗功效的 α 发射体的物理衰变特性。

[0007] 表 1

[0008]

候选核素	$T_{1/2}$*	临床测试用于
^{225}Ac	10.0 天	白血病
^{211}At	7.2 小时	胶质母细胞瘤

^{213}Bi	46 分钟	白血病
^{223}Ra	11.4 天	骨转移
^{224}Ra	3.66 天	强直性脊柱炎

[0009] * 半衰期

[0010] 迄今为止，关于在放射免疫疗法中的应用，主要注意力集中在 ^{211}At、^{213}Bi 和 ^{225}Ac，并且这三种核素已经被探索用于临床免疫疗法试验中。

[0011] 已经提出的若干种放射性核素是短寿命的，即具有小于 12 小时的半衰期。如此短的半衰期使得难以以商业方式生产和分配基于这些放射性核素的放射性药物。短寿命的核素的施用也增加了放射剂量的比例，所述放射剂量将在到达靶位点之前在体内发射。

[0012] 来自 α 发射的反冲能在许多情况下将造成子体核素从母体衰变位置释放。这种反冲能足以从例如其中母体与配体如螯合剂络合的可保持母体的化学环境中分裂出许多子体核素。即使其中子体与相同配体化学相容、即可与相同配体络合，这也将发生。同样，在子体核素是气体，特别是惰性气体如氡气，或者与配体化学不相容时，这种释放作用将甚至会更大。当子体核素具有超过几秒钟的半衰期时，它们可以扩散进入不受保持母体的配位剂约束的血液系统中。这些游离的放射性子体然后可造成不希望的全身毒性。

[0013] 几年前提出在其中维持 ^{223}Ra 子同位素的控制的条件下使用钍 -227($T_{1/2}$= 18.7 天)(参见 WO 01/60417 和 WO 02/05859)。这是其中使用允许由封闭环境滞留子体核素的载体系统的情形。在一种情况下，放射性核素被配置在脂质体内并且大尺寸的脂质体（与反冲距离相比）有助于将子体核素滞留在脂质体内。在第二种情况下，使用放射性核素的趋骨性络合物，其并入骨基质中并因此限制了子体核素的释放。这些是潜在非常有利的方法，但脂质体的施用在一些情况下是不希望的，并且存在许多其中放射性核素不能被矿化基质包围以滞留子同位素的软组织疾病。

[0014] 最近，已确定在 ^{227}Th 衰变后释放的 ^{223}Ra 子核的毒性可相比于根据关于类似核的先前测试所预测在更大的程度上在哺乳动物体内耐受。在不存在上文讨论的滞留钍 -227 的镭子体的具体方式的情况下，关于镭毒性的可公开获得的信息使得清楚认识到，不可能使用钍 -227 作为治疗剂，这是因为从钍 -227 衰变实现治疗作用所需的剂量将产生来自镭子体衰变的高毒性且可能致命剂量的放射，即不存在治疗窗。

[0015] WO 04/091668 描述一种意想不到的发现，即存在治疗性治疗窗，其中可向受试者（通常是哺乳动物）施用治疗有效量的靶向钍 -227 放射性核素而不产生足以造成不可接受的骨髓毒性的量的镭 -223。这可因此用于治疗和预防在骨和软组织部位处所有类型的疾病。

[0016] 鉴于上述发展，现在有可能在内放射性核素疗法中使用 α 发射钍 -227 核而不具有由所生成的 ^{223}Ra 引起的致命骨髓毒性。然而，治疗窗保持相对较窄，并且在所有情况下希望向受试者施用不超过绝对必要量的 α 发射放射性同位素。如果 α 发射钍 -227 核可被络合并以高可靠性程度靶向，则这种新治疗窗的有效利用因此将会极大增强。

[0017] 因为放射性核素不断衰变，所以在分离与向受试者施用之间处理材料所花费的时间非常重要。如果 α 发射钍核可以快速且便于制备、优选需要很少步骤、短温育期和 / 或

不会不可逆影响靶向实体的性质的温度的形式络合、靶向和/或施用，则其也将具有相当大的价值。此外，可在施用前无需去除的溶剂中（基本上在水溶液中）进行的工艺具有避免溶剂蒸发或渗析步骤的显著优点。

[0018] 鉴于在治疗中对于细胞毒性剂递送的选择性的需要，存在对于 α 放射性核素络合物的靶向的明显需要。然而，合适的螯合剂与小的靶向肽或小的蛋白质的缀合物倾向于在水性系统中显示不良溶解性，这是因为小的生物分子不能将不溶性螯合物保持在溶液中。不良溶解性导致聚集和沉淀。在待施用至人受试者的药物制剂中，聚集体是不可接受的，并且明显沉淀使得组合物完全不可用。

[0019] 此外，还对于较大的靶向肽/蛋白质，如单克隆抗体，螯合剂将作为疏水性‘点’暴露在缀合物的表面上。这在某些情况下可能会导致微聚集问题。

[0020] 在生物系统中，例如在人患者中，疏水性一般与肝脏中不希望的吸收有关。显然这对于高细胞毒性剂如 α 发射体比对于典型的药物化合物更为严重。螯合剂的疏水性也增加了免疫反应的风险，因为疏水性促进由宿主的免疫系统所产生抗体的更强结合。此外，由于 α 发射体的异常细胞毒性，其特别受到关注。因此显然非常需要通过具有增强的亲水性（特别是配体部分）的缀合物来选择性递送 α 发射钍放射性核素的方法，以解决一种或多种上文所讨论的问题。

[0021] 含有羟基吡啶酮基团的八齿螯合剂先前已显示适于配位 α 发射体钍 -277，用于随后连接至靶向部分 (WO2011098611)。描述了八齿螯合剂，其含有通过连接子基团接合至胺类骨架的四个 3,2- 羟基吡啶酮基团，所述胺类骨架具有用于缀合至靶向分子的单独反应性基团。先前发明的优选结构含有在杂环的 1 位中具有甲基取代氮的 3,2- 羟基吡啶酮基团，并且通过包括在 4 位连接的甲酸的胺键连接至胺类骨架，如由化合物 ALG-DD-NCS、ALG1005-38、Bb-1-HOPO-1-DEBN 所示。在其中这些含羟基吡啶酮的分子之一缀合至靶向肿瘤的抗体的实验中，将该分子溶解在有机溶剂 DMSO 中，因为它不能溶解在水性缓冲液中。

[0022] CD22 是在某些哺乳动物细胞、特别是 B 细胞中表达的糖结合跨膜受体，在所述细胞中它充当 B 细胞受体信号传导的抑制受体。它已被认为是基于抗体的疗法的可能靶标。

[0023] 本发明人现在已出乎意料地确定，使用由包含四个 HOPO 部分（其中至少一个被合适的增溶部分取代）的羟基吡啶酮 (HOPO) 型八齿配体络合的 4+ 钍 -227 离子可提供络合物的溶解性和相应特性的显著改善。此外，这种配体与 CD22 结合靶向部分的偶联可提供具有有利特性的缀合物。

发明内容

[0024] 从第一方面来看，本发明因此提供一种组织靶向络合物，其包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位 (1 位）被羟基烷基增溶基团取代，其中所述组织靶向部分具有对 CD22 受体的结合亲和力。在一个实施方案中，此类络合物可溶于纯水中。

[0025] 在一个优选的实施方案中，八齿配体包含至少一个 3,2-HOPO 部分，并且优选 2、3 或 4 个 3,2-HOPO 部分。在另一个优选的实施方案中，至少 2 个、优选至少 3 个并且最优选全部 4 个 HOPO 部分包含在 N 位的羟基烷基增溶部分。

[0026] 优选的靶向部分包括多克隆和特别地单克隆抗体和其片段。特异性结合片段诸如

Fab、Fab'、Fab'2 和单链特异性结合抗体是典型的片段。

[0027] 在这些络合物（以及优选在本发明的所有方面）中，钍离子一般将由含羟基吡啶酮的八齿配体络合，所述八齿配体又将通过任何合适的方式连接至组织靶向部分。这些方式可包括直接共价连接或借助于任何合适的特异性结合对（例如生物素 / 抗生物素蛋白型结合对）连接。可使用任何合适的连接，但直接共价键合或使用共价或结合对连接子部分将是典型的方法。共价酯或酰胺键是优选的方法。

[0028] 从另一个方面来看，本发明提供组织靶向络合物（包括本文所述的任何此类络合物）用于制造供治疗增生性或肿瘤性疾病（包括本文所述的任何此类疾病）的药物的用途，所述组织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。

[0029] 在一个相应的方面，本发明提供一种治疗人或非人动物（特别是有需要者）的方法，其包括施用至少一种组织靶向络合物（包括本文所述的任何此类络合物），所述组织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。这种方法优选用于治疗增生性或肿瘤性疾病，包括本文所述的任何此类疾病。这种方法通常对人或非人哺乳动物受试者，诸如有需要者进行。

[0030] 在另一个相应的实施方案中，本发明提供一种组织靶向络合物（包括如本文所公开的所有此类络合物），其包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力，所述组织靶向络合物是用于疗法中，并且特别是用于治疗增生性和 / 或肿瘤性疾病（包括任何此类疾病）和本文所述的方法。

[0031] 从另一个方面来看，本发明提供一种药物组合物，其包含组织靶向络合物（包括本文所述的所有此类络合物）以及至少一种药物载体或赋形剂，所述组织靶向络合物包含组织靶向部分、包含四个 HOPO 部分的含羟基吡啶酮的八齿配体以及 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。

[0032] 为与最丰富的天然存在的钍同位素，即钍 -232（半衰期 10^{10} 年并且有效非放射性）的钍络合物相区别，应理解，本文所要求保护的钍络合物和其组合物包括比天然相对丰度更大，例如大至少 20％的 α 发射钍放射性同位素（即半衰期小于 10^{3} 年的至少一种钍的同位素，例如钍 -227）。这无需影响其中明确要求治疗有效量的放射性钍诸如钍 -227 的本发明方法的定义，但在所有方面优选将是这种情况。

[0033] 在本发明的所有方面，α 发射钍离子优选是钍 -227 的离子。钍的 4+ 离子是优选用于本发明络合物中的离子。相应地，非常优选钍 -227 的 4+ 离子。

[0034] 从另一个方面来看，本发明还提供一种用于根据本发明的方法中的试剂盒，所述试剂盒包含缀合或可缀合至包含四个 HOPO 部分的含羟基吡啶酮的八齿配体的组织靶向部分，其中四个 HOPO 部分的至少一个在 N 位（1 位）被羟基烷基增溶基团取代并且其中所述

组织靶向部分具有对 CD22 受体的结合亲和力。所有结合部分和配体优选是本文所述的那些。这种试剂盒将任选地且优选地包括 α 发射钍放射性核素，诸如 ^{227}Th。

[0035] 在另一个方面，本发明此外提供一种用于形成组织靶向络合物的方法，所述方法包括使组织靶向部分与含羟基吡啶酮的八齿配体在水溶液中偶联，所述络合物包含四个 HOPO 部分和 α 发射钍放射性核素的离子，其中四个 HOPO 部分中的至少一个在 N 位被羟基烷基增溶基团取代并且其中所述组织靶向部分具有对 CD22 受体的结合亲和力。这种方法可在基本上不存在任何有机溶剂的情况下进行。

具体实施方式

[0036] 在本发明的情形下，“组织靶向”在本文用于指示所讨论的物质（特别是当呈如本文所述的组织靶向络合物形式时）用于优先自身定位（并且特别是定位任何缀合的钍络合物）至需要其存在（例如以递送放射性衰变）的至少一个组织部位。因此，在施用至受试者后，组织靶向基团或部分用于向所述受试者体内的至少一个所需部位提供大于平均值的定位。在本发明情况下靶向部分应经过选择以特异性结合至细胞表面受体 CD22。这可例如通过对于表达 CD22 的细胞的结合亲和力是对于非表达 CD22 的细胞的结合亲和力的 100 倍或更多来反映。认为 CD22 在具有某些疾病状态（如本文所示）的细胞中表达和 / 或过度表达并且因此 CD22 特异性结合物可用于将络合物靶向此类受疾病影响的细胞。类似地，组织靶向部分可结合至受疾病影响细胞附近的细胞上存在的细胞表面标志物（例如 CD22 受体）。CD22 细胞表面标志物可在患病细胞表面上比健康细胞表面上更多表达或者在生长或复制期间比在休眠期间在细胞表面上更多表达。在一个实施方案中，CD22 特异性结合配体可与疾病特异性细胞表面标志物的另一种结合物组合使用，因此得到双重结合络合物。

[0037] 组织靶向部分还可包含共同具有将钍络合物靶向所需组织的作用的两种或更多种成分。这可能是例如其中首先施用一种成分并结合至特定组织、肿瘤或细胞类型（组织结合剂），并且同时或优选随后施用第二种和 / 或其它成分（连接剂），其在体内结合至所述组织结合剂。连接剂将直接或间接缀合至络合的 α 发射钍并且因此组织结合剂和连接剂将共同形成组织靶向部分。适于向组织结合剂和连接剂提供相互亲和力的合适的特异性结合对是本领域中众所周知的（例如生物素与抗生物素蛋白或链霉亲和素）。

[0038] 如本文所述的本发明的多个方面涉及疾病的治疗，特别是用于选择性靶向患病组织，以及涉及可用于所述方法的络合物、缀合物、药物、制剂、试剂盒等。在所有方面，患病组织可能位于体内的单一位点（例如在局部化实体肿瘤的情况下）或者可位于多个位点（例如其中在关节炎中或者在分布式或转移式癌性疾病中有若干个关节受影响）。

[0039] 待靶向的患病组织可处于软组织位点、钙化组织位点或多个位点，其可全部在软组织中，全部在钙化组织中或者可包括至少一个软组织位点和 / 或至少一个钙化组织位点。在一个实施方案中，靶向至少一个软组织位点。靶向位点和疾病起源位点可能相同，但或者可能不同（例如其中特异性靶向转移位点）。在涉及一个以上位点的情况中，这可包括起源位点或者可为多个二级位点。

[0040] 术语“软组织”在本文用于指示不具有“硬质”矿化基质的组织。特别地，如本文所用的软组织可为并非骨骼组织的任何组织。相应地，如本文所用的“软组织疾病”指示在如本文所用的“软组织”中存在的疾病。本发明特别适于治疗癌症并且“软组织疾病”因此

涵盖在任何“软质”(即非矿化)组织中存在的癌瘤、肉瘤、骨髓瘤、白血病、淋巴瘤和混合型癌症,以及所述组织的其它非癌性疾病。癌性“软组织疾病”包括软组织中存在的实体肿瘤以及转移性和微转移性肿瘤。实际上,软组织疾病可包括软组织的原发性实体肿瘤和在同一患者中的软组织的至少一种转移性肿瘤。或者,“软组织疾病”可仅由原发性肿瘤或仅由转移瘤组成,其中所述原发性肿瘤是骨骼疾病。在本发明的所有适当方面特别适于治疗和/或靶向的是血液肿瘤以及尤其是淋巴细胞的肿瘤性疾病,例如淋巴瘤和淋巴样白血病,包括非霍奇金氏淋巴瘤、B细胞淋巴瘤的B细胞肿瘤。类似地,在本发明的所有适当方面,骨髓、脊柱(尤其是脊髓)、淋巴结和/或血液细胞的任何肿瘤性疾病也适于治疗和/或靶向。

[0041] 在本发明的适当方面适于治疗和/或靶向的B细胞肿瘤的一些实例包括:

[0042] 慢性淋巴细胞性白血病/小淋巴细胞淋巴瘤、B细胞幼淋巴细胞性白血病、淋巴浆细胞性淋巴瘤(例如瓦尔登斯特伦巨球蛋白血症(Waldenström macroglobulinemia))、脾边缘区淋巴瘤、浆细胞肿瘤(例如浆细胞骨髓瘤、浆细胞瘤、单克隆免疫球蛋白沉积病、重链疾病)、结外边缘区B细胞淋巴瘤(MALT淋巴瘤)、淋巴结边缘区B细胞淋巴瘤(NMZL)、滤泡性淋巴瘤、套细胞淋巴瘤、弥漫性大B细胞淋巴瘤、纵膈(胸腺)大B细胞淋巴瘤、血管内大B细胞淋巴瘤、原发性渗出性淋巴瘤和伯基特淋巴瘤/白血病。

[0043] 最近的一个关键发现是,某些α放射性钍同位素(例如^{227}Th)可以在治疗上有效并且不会产生不可耐受的骨髓毒性的量施用。如本文所用,术语“可接受的非骨髓毒性”用于指示,最重要的是,通过所施用的钍-227放射性同位素的衰变所产生的镭-223的量一般不足以对受试者直接致命。然而,本领域技术人员将清楚知道,将为所述治疗的可接受副作用的骨髓损伤的量(以及致命反应的可能性)将随着所治疗疾病的类型、治疗方案的目标以及对于受试者的预后而显著改变。尽管本发明的优选受试者是人,但其它哺乳动物,特别是狗将从本发明的使用中获益,并且可接受的骨髓损伤水平也可反映受试者的物种。在恶性疾病的治疗中,相比于非恶性疾病,可接受的骨髓损伤水平一般将更大。骨髓毒性水平的一个众所周知的量度是嗜中性粒细胞计数,并且在本发明中,可接受的非骨髓毒性量的^{223}Ra通常将是经过控制以使得在最低点(最低限度)时嗜中性粒细胞分率不少于在治疗之前的计数的10%的量。可接受的非骨髓毒性量的^{223}Ra优选将是使得嗜中性粒细胞分率为最低限度时的至少20%并且更优选至少30%的量。至少40%的最低限度嗜中性粒细胞分率是最优选的。

[0044] 另外,含放射性钍(例如^{227}Th)的化合物可以高剂量方案使用,其中当包括干细胞支持或类似的恢复方法时,所产生的镭(例如^{223}Ra)的骨髓毒性通常将是不可耐受的。在这些情况下,嗜中性粒细胞计数可降至低于最低限度时的10%并且例外情况下将降至5%或必要时低于5%,从而采取合适的预防措施并且给予后续干细胞支持。这些技术是本领域中众所周知的。

[0045] 本发明中特别关注的钍同位素是钍-227,并且在情形允许时对于本文中钍的所有提及,钍-227是优选的同位素。钍-227相对容易产生并且可以从中子辐照的^{226}Ra间接制备,其将含有^{227}Th的母体核素,即^{227}Ac($T_{1/2}$= 22年)。锕-227可以相当容易地与^{226}Ra靶标分离并用作^{227}Th的发生器。必要时可将这种工艺扩展到工业规模,并且因此可以避免关于被视为分子靶向放射疗法的候选物的大多数其它α发射体可见的供应问题。

[0046] 钍-227通过镭-223衰变。在这种情况下,原发性子体具有11.4天的半衰期。从

纯 ^{227}Th 源，在前几天内仅产生中等量的镭。然而，^{223}Ra 的潜在毒性高于 ^{227}Th，这是因为 α 粒子从 ^{223}Ra 中发射之后，接着数分钟内从短寿命子体产生三个其它 α 粒子（参见下表 2，其阐述钍 -227 的衰变系列）。

[0047] 表 2

[0048]

核素	衰变模式	平均粒子能量 (MeV)	半衰期
^{227}Th	α	6.02	18.72 天
^{223}Ra	α	5.78	11.43 天
^{219}Rn	α	6.88	3.96 秒
^{215}Po	α	7.53	1.78 毫秒
^{211}Pb	β	0.45	36.1 分钟
^{211}Bi	α	6.67	2.17 分钟
^{207}TI	β	1.42	4.77 分钟
^{207}Pb			稳定

[0049] 部分原因是因为其产生潜在有害的衰变产物，钍 -227($T_{1/2}$= 18.7 天）还没有被广泛考虑用于 α 粒子疗法。

[0050] 钍 -227 可以足以提供所需治疗作用而不产生那么多的镭 -223 以致于造成不可耐受的骨髓抑制的量施用。理想的是将子同位素保持在靶向区域中以使得可从其衰变得到其它治疗作用。然而，为了获得有用的治疗作用而不诱导不可接受的骨髓毒性，不必维持钍衰变产物的控制。

[0051] 假设肿瘤细胞杀伤作用将主要来自钍 -227 而非来自其子体，可通过与其它 α 发射体进行比较来确定这种同位素的可能治疗剂量。例如，对于砹 -211，在动物中的治疗剂量通常是 2-10MBq/kg。通过校正半衰期和能量，钍 -227 的相应剂量将是至少 36-200kBq/kg 体重。这将设定预期治疗作用时可有效施用的 ^{227}Th 量下限。这种计算假定砹和钍的类似滞留。然而，显然钍的 18.7 天半衰期将很可能导致这种同位素在其衰变前的更大消除。这种计算剂量因此应通常被认为是最低有效量。根据完全滞留的 ^{227}Th（即未从体内消除的 ^{227}Th）表达的治疗剂量通常将为至少 18 或 25kBq/kg，优选至少 36kBq/kg 并且最优选至少 75kBq/kg，例如 100kBq/kg 或更多。更大量的钍将预期具有更大的治疗作用，但如果将产生不可耐受的副作用，则不能施用。同样地，如果钍以具有短生物半衰期（即在从仍带有钍的体内消除之前的半衰期）的形式施用，则为获得治疗作用将需要更大量的放射性同位素，因为大量的钍将在其衰变之前被消除。然而，所生成的镭 -223 的量将存在相应降低。当同位素被完全滞留时待施用的钍 -227 的上述量可容易地与具有较短生物半衰期的等效剂量相关。这些计算是本领域中众所周知的并且在 WO 04/091668 中（例如正文中以及实施例

1 和 2 中）给出。

[0052] 如果放射性标记的化合物释放子体核素，则重要的是知道任何放射性子体核素的命运（如果适用的话）。对于 ^{227}Th，主要子体产物是 ^{223}Ra，由于其趋骨特性，其正处于临床评价中。镭 -223 非常快速地清除血液并且集中在骨骼中或通过肠道和肾脏途径排泄（参见 Larsen, J Nucl Med 43(5, 增刊):160P(2002)）。在体内从 ^{227}Th 释放的镭 -223 因此可能不会在较大程度上影响健康软组织。在 Müller 于 Int. J. Radiat. Biol. 20:233-243(1971) 中关于呈溶解的柠檬酸盐形式的 ^{227}Th 分布的研究中，发现在软组织中从 ^{227}Th 产生的 ^{223}Ra 容易再分配至骨骼或排泄。因此 α 发射镭的已知毒性，特别是对于骨髓的已知毒性是伴随钍剂量的问题。

[0053] 在 WO 04/091668 中首次确定，实际上，剂量为至少 200kBq/kg 的 ^{223}Ra 可施用并在人受试者中耐受。这些数据呈现在所述公开案中。因此，现在可以看出，相当意外地，存在治疗窗，其中可向哺乳动物受试者施用治疗有效量的 ^{227}Th（例如大于 36kBq/kg）而预期这种受试者不会遭受不可接受的严重或甚至致命的骨髓毒性的风险。然而，极其重要的是，进行这种治疗窗的最佳使用，并且因此必要的的是放射性钍快速并有效地络合，并且以非常高的亲和力保持以使得最大可能比例的剂量被递送至靶位点。

[0054] 从 ^{227}Th 药物产生的 ^{223}Ra 的量将取决于放射性标记的化合物的生物半衰期。理想情况将是使用具有快速肿瘤吸收的络合物，所述肿瘤吸收包括内化至肿瘤细胞中、强烈肿瘤滞留以及在正常组织中的短生物半衰期。然而只要 ^{223}Ra 的剂量保持在可耐受的水平内，具有小于理想生物半衰期的络合物就可以是有用的。在体内产生的镭 -223 的量将是所施用的钍的量和钍络合物的生物滞留时间的因素。在任何特定情况下，本领域普通技术人员可容易地计算所产生的镭 -223 的量。^{227}Th 的最大可施用量将由体内产生的镭的量确定并且必须小于将产生不可耐受水平的副作用，特别是骨髓毒性的量。这个量一般将小于 300kBq/kg，特别是小于 200kBq/kg 并且更优选小于 170kBq/kg（例如小于 130kBq/kg）。最小有效剂量将由钍的细胞毒性、患病组织对所产生的 α 照射的易感性以及钍被靶向络合物（在这种情况下为配体与靶向部分的组合）有效组合、保持和递送的程度确定。

[0055] 在本发明的方法中，希望钍络合物以 18 至 400kBq/kg 体重、优选 36 至 200kBq/kg（例如 50 至 200kBq/kg）、更优选 75 至 170kBq/kg、尤其 100 至 130kBq/kg 的钍 -227 剂量施用。相应地，单一剂量可包含大约任何这些范围乘以合适的体重，例如 30 至 150Kg、优选 40 至 100Kg（例如每剂 540kBq 至 4000KBq 的范围等）。此外将需要钍剂量、络合剂和施用途径以使得体内产生的镭 -223 剂量小于 300kBq/kg、更优选小于 200kBq/kg、仍更优选小于 150kBq/kg、尤其小于 100kBq/kg。此外，这将提供由这些范围乘以任何所示体重所指示的 ^{223}Ra 暴露。上述剂量水平优选是完全滞留的 ^{227}Th 剂量，但考虑到一些 ^{227}Th 将在其衰变前从体内清除，其可为所施用剂量。

[0056] 在 ^{227}Th 络合物的生物半衰期相比于物理半衰期（例如小于 7 天，尤其是小于 3 天）较短时，可能需要显著较大的所施用剂量来提供等效滞留剂量。因此，例如，150kBq/kg 的完全滞留剂量等效于以 711kBq/kg 的剂量施用的具有 5 天半衰期的络合物。可使用本领域中众所周知的方法由络合物的生物清除率来计算任何适当滞留剂量的等效施用剂量。

[0057] 由于一个 ^{227}Th 核的衰变提供一个 ^{223}Ra 原子，^{227}Th 的滞留和治疗活性将与患者所遭受的 ^{223}Ra 剂量直接相关。可使用众所周知的方法来计算在任何特定情形下所产生的 ^{223}Ra

的量。

[0058] 在一个优选的实施方案中，本发明因此提供一种用于治疗哺乳动物受试者（如本文所述）中的疾病的方法，所述方法包括向所述受试者施用治疗有效量的缀合物，所述缀合物包含组织靶向部分、八齿配体（尤其是任何本文所述的那些）和放射性钍同位素（例如钍 -227）。

[0059] 除非其特性被有效使用，否则显然需要将受试者对于 ^{223}Ra 子同位素的暴露降至最小。特别是，体内产生的镭 -223 的量通常将大于 40kBq/kg，例如大于 60kBq/Kg。在一些情况下，体内产生的 ^{223}Ra 将必需大于 80kBq/kg，例如大于 100 或 115kBq/kg。

[0060] 在适当的载体溶液中的钍 -227 标记的缀合物可经静脉内、腔内（例如腹膜内）、皮下、经口或经局部、以单次应用或以分次应用方案施用。缀合至靶向部分的络合物优选将以溶液形式通过肠胃外（例如经皮）途径、尤其是静脉内或通过腔内途径施用。优选地，本发明的组合物将配制成无菌溶液以用于肠胃外施用。

[0061] 在本发明的方法和产物中的钍 -227 可单独使用或与其它治疗形式组合使用，所述治疗形式包括手术、外照射疗法、化疗、其它放射性核素、或组织温度调节等。这构成了本发明方法的另一个优选的实施方案，并且制剂 / 药物可相应地包含至少一种其它治疗活性剂如另一种放射性剂或化疗剂。

[0062] 在一个特定优选的实施方案中，受试者还经历干细胞治疗和 / 或其它支持疗法以降低镭 -223 诱导的骨髓毒性的作用。

[0063] 根据本发明，^{227}Th 可由靶向络合剂络合。靶向部分通常将具有 100g/mol 至数百万 g/mol（特别是 100g/mol 至 1 百万 g/mol）的分子量，并且优选将直接具有对于疾病相关受体的亲和力，和 / 或将包含结合至在施用 ^{227}Th 之前已靶向疾病的分子的合适的预施用结合物（例如生物素或抗生物素蛋白）。合适的靶向部分包括多肽和寡肽、蛋白质、DNA 和 RNA 片段、适体等，优选为蛋白质，例如抗生物素蛋白、链霉亲和素、多克隆或单克隆抗体（包括 IgG 和 IgM 型抗体）、或蛋白质或蛋白质片段或构建体的混合物。抗体、抗体构建体、抗体片段（例如 Fab 片段或包含至少一个抗原结合区域的任何片段）、片段构建体（例如单链抗体）或其混合物是特别优选的。合适的片段特别包括 Fab、F(ab')$_2$、Fab' 和 / 或 scFv。抗体构建体可为本文所示的任何抗体或片段。

[0064] 在一个方面，特异性结合物（组织靶向部分）可为与如下所示的至少一个序列具有序列相似性或同一性的肽：

[0065] 轻链：

[0066]

```
鼠      DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLAWYQQKPGQSP
人源化  ------------SA-V-DR----------------------------KA

鼠      KLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSS
人源化  ---------------S--S---------F---SL-P--I-T---------

鼠      WTFGGGTKLEIKR (SeqID1)
人源化  ------------- (SeqID2)
```

[0067] 重链：

[0068]

鼠	QVQLQESGAELSKPGASVKMSCKASGYTFT**SYWLH**WIKQRPGQGLEWIG
H'ised1	-----Q----VK---S---V----------------VR-A---------
H'ised2	----VQ----VK---S---V----------------VR-A---------
鼠	**YINPRNDYTEYNQNFKD**KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR
H'ised1	--------------------I---E-TN----E----R---T-F-F---
H'ised2	--------------------I---E-TN----E----R---T-F-F---
鼠	**RDITTFY**WGQGTTLTVSS (SeqID3)
H'ised1	-------------V---- (SeqID4)
H'ised2	-------------V---- (SeqID5)

[0069] 在上述序列中,人源化 (H'ised) 序列中的“-”指示残基相比于鼠序列未改变。

[0070] 在上述序列 (SeqID1-5) 中,粗体区域被认为是关键的特异性结合区域 (CDR),加下划线的区域被认为在结合中具有次要重要性并且未强调的区域被认为表示结构性而非特异性结合区域。

[0071] 在本发明的所有方面,组织靶向部分可具有与任何 SeqID 1 至 5 中所示那些序列中的至少一种具有实质序列同一性或实质序列相似性的序列。实质序列同一性 / 相似性可视为与完整序列具有至少 80％的序列相似性 / 同一性和 / 或与特异性结合区域 (在上述序列中以粗体显示的那些区域和任选地加下划线的那些部分) 具有至少 90％的序列相似性 / 同一性。对于粗体区域以及优选也对于全序列,优选的序列相似性或更优选同一性可为至少 92％、95％、97％、98％或 99％。可使用来自威斯康星大学 (University of Wisconsin) 的 Genetics Computer Group 第 10 版软件包的“BestFit”程序测定序列相似性和 / 或同一性。所述程序使用 Smith 和 Waterman 的局部算法 (local had algorithm),其中默认值为 :空位产生罚分＝ 8,空位延伸罚分＝ 2,平均匹配＝ 2.912,平均错配 2.003。

[0072] 组织靶向部分可包含一个以上肽序列,在这种情况下至少一个以及优选所有序列可 (独立地) 符合与任何 SeqID1-5 的上述序列相似性以及优选地序列同一性。

[0073] 组织靶向部分将具有对 CD22 的结合亲和力并且在一个实施方案中还可具有对于完全结构域具有多至约 40 种变化 (优选 0 至 30 种变化) 的序列。变体可通过插入、缺失和 / 或取代得到并且可关于 seqID 1-5 为连续或非连续的。取代或插入通常将借助于遗传密码的 20 种氨基酸中的至少一种并且取代最通常将为保守性取代。然而,在一个实施方案中,可对具有可用于连接至配体部分的反应性侧链的氨基酸进行至少一个插入和 / 或取代。这种侧链可包含例如至少一个硫醇、胺、醇、酸或酰胺基或其任何保护等效物 (例如酯、硫酯等)。保护基是有机化学中众所周知的并且可选自诸如 Theodora Greene 的“Protective Groups in Organic Chemistry”等标准教科书 (以引用的方式并入本文中)。

[0074] 一般来说,八齿配体直接或间接 (例如通过连接子部分) 缀合至靶向部分。这种类型的一般构建体 ;即活性 (例如治疗或诊断活性) 金属 - 络合部分 - 任选的连接子部分 - 靶向部分,是靶向放射性药物和靶向造影剂领域中众所周知的。然而,很少或没有研究评估各种配体对于钍 4+ 离子特异性使用的适宜性。就此而言,例如可参考 ″Handbook of Targeted Delivery of Imaging Agents″,Torchilin 编 ,CRC Press,1995。

[0075] 对于具有羟基吡啶酮配体的钍离子的最相关先前研究以 WO2011/098611 公布并

且公开了产生与含 HOPO 的八齿配体络合的钍离子的相对容易性。

[0076] 先前已知的钍螯合剂也包括聚氨基聚酸螯合剂，其包含在主链氮上连接有酸性基团（例如羧基烷基）的线性、环状或分支聚氮杂烷烃主链。这些螯合剂的实例包括 DOTA 衍生物如对异硫氰基苄基 -1,4,7,10- 四氮杂环十二烷 -1,4,7,10- 四乙酸 (p-SCN-Bz-DOTA) 以及 DTPA 衍生物如对异硫氰基苄基 - 二亚乙基三胺五乙酸 (p-SCN-Bz-DTPA)，前者为环状螯合剂，后者为线性螯合剂。

[0077] 先前已例示 1,4,7,10- 四氮杂环十二烷 -1,4,7,10- 四乙酸的衍生物，但标准方法不能容易地用于螯合钍与 DOTA 衍生物。将 DOTA 衍生物与金属一起加热有效提供螯合剂，但通常产率较低。存在配体的至少一部分在程序期间不可逆变性的趋势。此外，由于其对于不可逆变性的相对高的易感性，一般有必要避免靶向部分的连接直至完成所有的加热步骤。这增加了一个额外的化学步骤（具有所有必要的处理和分离），其必须在 α 发射钍同位素的衰变寿命期间进行。显然优选不以这种方式处理 α 发射材料或者在比必要更大的程度上产生相应的废物。此外，制备缀合物所花费的所有时间浪费了一部分钍，其将在这个制备期内衰变。

[0078] 在本发明的所有方面，α 发射钍和八齿配体的络合物优选是在或可在不具有高于 60℃加热（例如不具有高于 50℃加热）、优选不具有高于 38℃加热并且最优选不具有高于 25℃加热的情况下形成。

[0079] 另外优选的是在添加 α 发射钍同位素（例如 $^{227}Th^{4+}$ 离子）之前制备靶向部分与八齿配体的缀合物。本发明的产物因此优选是或可通过八齿配体与组织靶向部分的缀合物对 α 发射钍同位素（例如 $^{227}Th^{4+}$ 离子）的络合而形成。

[0080] 螯合剂可为非膦酸酯分子，并且在本发明的一个实施方案中，^{227}Th 将不会连接至任何膦酸酯或其它靶向骨骼的基团，并且不与所述材料一起施用。

[0081] 本发明人现已确定，包含含有四个 HOPO 部分的含羟基吡啶酮的八齿配体与 α 发射钍放射性核素的离子的络合物非常适于在室温和 / 或生理温度下（例如 20℃或 37℃下）产生。这种络合物可迅速产生并且此外由于产生温度相对较低，在配体部分已经结合或以其它方式缀合至组织靶向部分之后可发生钍成分的络合，因此降低在加入放射性同位素之后所需的步骤的数目。

[0082] 除上述之外，包含四个 HOPO 部分（其中至少一个 HOPO 部分包含羟基烷基增溶基团）的含羟基吡啶酮的八齿配体的更易溶于水的特性用于进一步提高完整缀合物的制造容易性。具体地，在缀合物制造期间，必须将疏水性螯合剂如先前已知的八齿配体溶解在有机溶剂如 DMSO 或 DMA 中。在缀合后有必要除去所有微量的有机溶剂，但对于这些非挥发性极性有机溶剂是困难的并且难以通过分析证明完全去除。分析所花费的时间显然是不希望的，其中已并入 α 发射体，因为放射性核素持续衰变并且缀合物的效能随时间降低。

[0083] 由于需要有机溶剂，疏水性螯合剂不仅对于与蛋白质类靶向分子组合具挑战性，而且对于与更具亲水性的替代靶向分子组合更具挑战性，所述替代靶向分子包括在表面上具有 PEG 或右旋糖苷的纳米粒子。

[0084] 出于生物学原因如延长的半衰期或降低免疫反应，可能需要 PEG 或替代的亲水性高水溶性间隔基。由于两个部分的溶解度性质的差异，螯合剂 -PEG 单元在缀合至蛋白质之前的制造也具挑战性。PEG 或类似的间隔基向分子中的螯合部分与载体蛋白之间引入更大

亲水性。然而，这仅使螯合剂移动以进一步远离载体蛋白，而螯合剂的疏水性不受影响。因此，疏水性螯合剂仍可被识别为（聚乙二醇化）靶向分子的表面上的疏水性点并产生如上文所讨论的不希望的反应。

[0085] 多种类型的靶向化合物可通过八齿螯合剂（包含如本文所述的偶联部分）连接至钍（例如钍 -227）。靶向部分可选自已知的靶向基团，其包括单克隆或多克隆抗体、生长因子、肽、激素和激素类似物、叶酸和叶酸衍生物、生物素、抗生物素蛋白和链霉亲和素或其类似物。其它可能的靶向基团包括 RNA、DNA 或其片段（例如适体）、寡核苷酸、碳水化合物、脂质或通过在存在或不存在蛋白质等的情况下组合这些基团而制备的化合物。可如上文所示包括 PEG 部分，例如以增加生物滞留时间和 / 或降低免疫刺激。

[0086] 在一个实施方案中，组织靶向部分可排除趋骨物、脂质体和叶酸缀合抗体或抗体片段。或者，可包括这些部分。

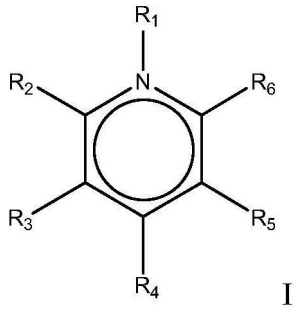
[0087] 本发明的钍（例如钍 -227）标记的分子可通过靶向疾病相关受体而用于治疗癌性或非癌性疾病。通常，基于通过螯合剂将 ^{227}Th 连接至抗体、抗体片段、或抗体或抗体片段的构建体，^{227}Th 的这种医学用途将通过放射免疫疗法用于治疗癌性或非癌性疾病。^{227}Th 在根据本发明的方法和药物中的用途特别适于治疗任何形式的癌症，包括癌瘤、肉瘤、淋巴瘤和白血病、特别是肺癌、乳腺癌、前列腺癌、膀胱癌、肾癌、胃癌、胰腺癌、食道癌、脑癌、卵巢癌、子宫癌、口腔癌、结肠直肠癌、黑素瘤、多发性骨髓瘤和非霍奇金氏淋巴瘤。

[0088] 如果携带 ^{227}Th 的分子在体内具有短的生物滞留半衰期，则所释放的 ^{223}Ra 的量可降至最小，因为在高比例的 ^{227}Th 衰变为 ^{223}Ra 之前，这种放射性核素将大部分被消除。然而，根据本发明，^{227}Th 的量将需要增加以保持治疗有效性。如果选择络合剂以将 ^{227}Th 递送至靶向细胞的内部，则由于子同位素至少部分滞留在肿瘤位点处，这将进一步增加特异性细胞毒性并降低放射性子体的全身毒性作用。这些特征都加宽了 ^{227}Th 治疗窗并因此构成本发明的优选实施方案。

[0089] 在本发明的另一个实施方案中，具有软组织和骨骼疾病的患者可由 ^{227}Th 和由所施用的钍在体内产生的 ^{223}Ra 来治疗。在这个特别有利的方面，通过靶向骨骼疾病，对于治疗的额外治疗成分源自可接受的非骨髓毒性量的 ^{223}Ra。在这种治疗方法中，^{227}Th 通常通过适当靶向原发性和 / 或转移性软组织癌而用于治疗原发性和 / 或转移性软组织癌并且由 ^{227}Th 衰变产生的 ^{223}Ra 被用于治疗同一受试者中的相关骨骼疾病。这种骨骼疾病可能是由原发性软组织癌产生的骨骼转移，或者可为其中软组织治疗是抵抗转移性癌症的原发性疾病。偶尔，软组织和骨骼疾病可能无关（例如在患有风湿性软组织疾病的患者中额外治疗骨骼疾病）。

[0090] 在所有方面，本发明的一个关键方面是使用八齿配体，特别是包含四个 HOPO 部分的含羟基吡啶酮的八齿配体。这些配体通常将包含至少四个螯合基团，其各自独立地具有下列被取代的吡啶结构 (I)：

[0091]



I

[0092] 其中 R_1为任选的 N 取代基增溶基团，其将存在于四个式 I 部分中的至少一个中并且可存在于 2、3 或全部 4 个这些部分中。R_1因此可不存在或者可选自 OH 和羟基烷基部分。合适的羟基烷基部分将包含至少一个 OH 基团，但可任选包含一个以上、例如两个、三个或四个 OH 基团。在羟基烷基部分上，一个或两个 OH 基团是优选的。

[0093] HOPO 部分（特别是 3,2-HOPO 和 2,3-HOPO）的吡啶酮环上的氮是适于引入亲水性取代基而不会非常影响环的性质的点，并且重要的是，其将在分子与载体蛋白或其它靶向分子缀合后面向外部。先前已显示，基于在这个位置上具有甲基的嘧啶酮环的螯合剂适于螯合钍离子。新型的螯合剂具有在 N 位引入的替代基团，包括羟基乙基。令人惊讶的是，从甲基到羟基乙基的微小变化产生完全可溶于纯水中的螯合剂。下文显示这种分子和一些相关的实例。

[0094] 如本文所用，所有烃基部分独立地选自短烃基，例如 C1 至 C8 烃基，包括 C1 至 C8 烷基、烯基或炔基。相应地，烷基通常将为直链或支链 C1 至 C8 烷基如甲基、乙基、正丙基或异丙基、正丁基、异丁基或仲丁基等。

[0095] 非常优选的 R_1基团包括具有连接至烷基链的碳原子的一个、两个或更多个羟基的直链或支链烷基（例如上文所示那些）。一些非常优选的羟基烷基包括羟基甲基、羟基乙基、羟基正丙基、羟基异丙基、二羟基正丙基（例如 1,2- 二羟基丙基、2,3- 二羟基丙基或 1,3- 二羟基丙基）、羟基正丁基、二羟基正丁基和三羟基正丁基，其中羟基乙基是最优选的。在一个实施方案中，八齿配体的 4 个 HOPO 部分各自将包含在位置 R_1处的羟基烷基（例如羟基乙基）。在另一个实施方案中，所有四个 HOPO 部分将包含相同的羟基烷基（例如所有 4 个 HOPO 基团将在 N 位被羟基乙基取代或者所有 4 个 HOPO 基团将被二羟基丙基取代）。

[0096] 在一个非常优选的实施方案中，所有 4 个 HOPO 基团将为选自 3,2HOPO 和 2,3HOPO 基团的相同 HOPO 基团。在另一个非常优选的实施方案（其可任选地与前述组合）中，所有 4 个 HOPO 基团将在 N 位被选自羟基甲基、羟基乙基、羟基丙基、羟基丁基、二羟基丙基和二羟基丁基的相同羟基烷基取代。在这个列表中，羟基乙基、羟基丙基和二羟基丙基是最优选的。

[0097] 在式 I 中，基团 R_2至 R_6可各自独立地选自 H、OH、＝ O、短烃基（如本文所述）、连接子部分（如本文所述）和 / 或偶联部分（如本文所述）。一般来说，基团 R_2至 R_6中的至少一个将为 OH。一般来说，基团 R_2至 R_6中的至少一个将为＝ O。一般来说，基团 R_2至 R_6中的至少一个将为连接子部分（如本文所述）。基团 R_2至 R_6中优选只有一个将为＝ O。基团 R_2至 R_6中优选只有一个将为 OH。基团 R_2至 R_6中优选只有一个将为连接子部分（如本文所述）。其余基团 R_2至 R_6可为任何本文所示的那些部分，但优选为 H。在连接子部分或连接至连接子部分的任何其它连接子、模板或螯合基团不包含偶联部分时，基团 R_1至 R_6中的一

个优选为偶联部分（如本文所述）。

[0098] 在一个优选的实施方案中，基团R_2至R_6中的一个将为OH并且R_2至R_6中的一个将为＝O，并且OH和＝O基团将在环的相邻原子上。因此，在一个优选的实施方案中，OH和＝O可分别在原子2,3；3,2；3,4；或4,3上（如将预期从氮开始编号）。具有至少一个螯合部分的八齿配体是非常优选的，其中OH和＝O基团分别存在于位置3和位置2。八齿配体可具有2、3或4个这种螯合基团，其中2或4个这种基团是非常优选的。N上取代的3,2-HOPO部分非常优选作为八齿配体的所有四个络合部分。

[0099] 合适的螯合部分可通过本领域中已知的方法形成，包括US 5,624,901（例如实施例1和2）和WO2008/063721（都以引用的方式并入本文中）中所述的方法。

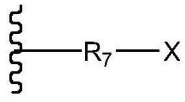
[0100] 如本文所用，术语“连接子部分”（式II中的R_L）用于指示用于接合八齿配体中的至少两个螯合基团的化学实体，其在本发明的多个方面形成关键成分。连接子部分还可将八齿配体部分接合至组织靶向部分。通常，每个螯合基团（例如上文式I和/或下文式II的那些）将为双齿的并且因此四个螯合基团（其中至少一个具有式I）通常将存在于配体中。这些螯合基团借助于其连接子部分而彼此接合。因此，在一个以上式I和/或II的螯合基团之间可共用连接子部分（例如下文基团R_L）。连接子部分还可充当八齿配体与靶向部分的络合部分之间的连接点。在这种情况下，至少一个连接子部分将接合至偶联部分（R_C）。合适的连接子部分包括短烃基，例如C1至C12烃基，包括C1至C12烷基、烯基或炔基，包括所有拓扑结构的甲基、乙基、丙基、丁基、戊基和/或己基。

[0101] 连接子部分还可为或包含任何其它适当稳固的化学键，包括酯、醚、胺和/或酰胺基团。接合两个螯合部分的原子总数（如果存在一个以上路径，则通过最短的路径计数）一般将是有限的，以便以适于络合物形成的布置来限制螯合部分。因此，连接子部分通常将经过选择以在螯合部分之间提供不超过15个原子，优选地在螯合部分之间提供1至12个原子并且更优选1至10个原子。在连接子部分直接接合两个螯合部分时，连接子长度通常将为1至12个原子，优选2至10个原子（例如乙基、丙基、正丁基等）。在连接子部分接合至中心模板（参见下文）时，则每一连接子可能较短，其中两个独立的连接子接合螯合部分。在这种情况下，1至8个原子，优选1至6个原子的连接子长度可为优选的（甲基、乙基和丙基是合适的，诸如在一端或两端具有酯、醚或酰胺键的这些基团也是如此）。

[0102] 除了主要用于将八齿配体的各种螯合基团彼此连接和/或连接至中心模板的连接子部分之外，八齿配体优选进一步包含“偶联部分”（R_C）。偶联部分的功能是将八齿配体连接至靶向部分。这可通过共价或非共价连接（例如通过特异性结合对如生物素/抗生物素蛋白（链霉亲和素））来实现。如上文所述的连接子部分形成可能的偶联部分。偶联部分优选将通过直接共价连接至一个螯合基团或者更通常通过连接至连接子部分或模板而共价连接至螯合基团。应使用两个或更多个偶联部分，各自可连接至例如任何模板、连接子或螯合基团上的任何可用位点。

[0103] 在一个实施方案中，偶联部分可具有以下结构：

[0104]



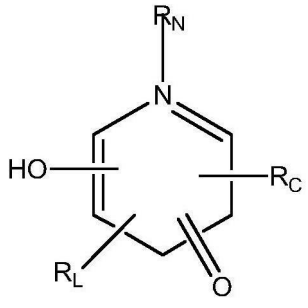
[0105] 其中R_7为桥联部分，其为选自被取代或未被取代的烷基、被取代或未被取代的杂

烷基、被取代或未被取代的杂环烷基、被取代或未被取代的芳基以及被取代或未被取代的杂芳基的成员；并且 X 是靶向部分或反应性官能团。优选的桥联部分包括本文指示为合适的连接子部分的所有这些基团。优选的靶向部分包括本文所述的所有那些靶向部分并且优选的反应性 X 基团包括能够与靶向部分形成共价键的任何基团，包括例如 COOH、OH、SH、NHR 和 COH 基团，其中 NHR 的 R 可为 H 或本文所述的任何短烃基。用于连接至靶向部分上的非常优选的基团包括赖氨酸残基的 ε－胺和半胱氨酸残基的硫醇基团。合适的反应性 X 基团的非限制性实例包括 N- 羟基琥珀酰亚胺酯、酰亚胺酯、酰基卤、N- 马来酰亚胺、α－卤基乙酰基和异硫氰酸酯，其中后三种适于与硫醇基团反应。

[0106] 优选连接偶联部分，以使得所得偶联的八齿配体将能够经历稳定的金属离子络合物的形成。偶联部分因此将优选在不显著干扰络合的位点处连接至连接子、模板或螯合部分。这种位点优选将在连接子或模板上，更优选在远离结合至靶标的表面的位置处。

[0107] 优选的螯合基团包括以下式 II 的那些：

[0108]



II

[0109] 在上式 II 中，＝O 部分表示连接至吡啶环的任何碳的酮基，-OH 表示连接至吡啶环的任何碳的羟基部分，并且 $-R_L$ 表示将羟基吡啶酮部分连接至其它络合部分以形成整个八齿配体的连接子部分。本文所述的任何连接子部分可适用作包括短烃基的 R_L，所述短烃基例如 C1 至 C8 烃基，包括 C1 至 C8 烷基、烯基或炔基，包括所有拓扑结构的甲基、乙基、丙基、丁基、戊基和 / 或己基。R_L 可在吡啶环的任何碳处接合式 II 的环。R_L 基团然后又可直接键合至另一个螯合部分、另一个连接子基团和 / 或中心原子或基团，例如环或其它模板（如本文所述）。连接子、螯合基团和任选的模板部分经过选择以形成适当的八齿配体。

[0110] 在一个优选的实施方案中，式 II 的 -OH 和＝O 部分处在吡啶环的相邻原子上，以使得 2,3-、3,2-、4,3- 和 3,4- 羟基吡啶酮衍生物都非常合适。

[0111] 部分 R_L 处在吡啶环的氮上。在一些式 II 基团中可能不存在基团 R_N，其中在八齿配体中存在一个以上不同的式 II 基团。然而，每一八齿配体中的至少一个 R_N 基团将为如本文所示的羟基烷基。

[0112] 在一个优选的实施方案中，至少一个 3,2- 羟基吡啶酮部分存在于八齿配体结构中。这显然可被任何本文所示的各种取代基部分取代。

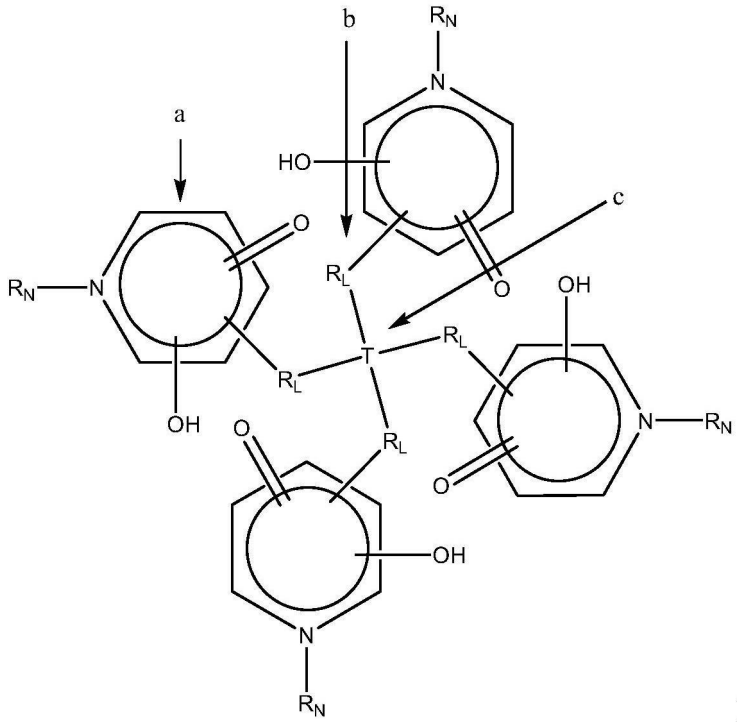
[0113] 由于每一式 II 的部分具有两个可能络合的氧，因此本发明的一个实施方案提供包含至少 2 个、优选至少 3 个并且最优选 4 个独立选择的式 II 部分的八齿配体。每一式 II 的部分可具有独立的取代模式，但在一个优选的实施方案中，至少一个部分是 3,2- 羟基吡啶酮部分。配体可包括 2、3 或 4 个 3,2- 羟基吡啶酮部分（适当时被取代，如本文所述）。

[0114] 八齿配体中的每一式 I 或 II 的部分可通过如本文所讨论并且呈任何适当拓扑结

构的任何适当连接子基团接合至配体的其余部分。例如，式 I 的四个基团可通过其连接子基团接合至主链以形成线性配体，或者可通过连接子基团桥联以形成“寡聚物”型结构，其可为线性的或环状的。或者，式 I 和 / 或 II 的配体部分可各自通过连接子（例如“R_L”部分）以“十字”或“星形”外形接合至中心原子或基团。连接子（R_L）部分可仅通过碳碳键接合，或者可通过任何适当稳固的官能团（包括胺、酰胺、酯、醚、硫醚或二硫键）彼此连接，连接至其它螯合基团、主链、模板、偶联部分或其它连接子。

[0115] “星形”布置示于下式 III 中：

[0116]



[0117] 其中所有基团和位置如上文所指示并且“T”另外为中心原子或模板基团，例如碳原子、烃链（例如任何上文所述的那些）、脂族或芳族环（包括杂环）或稠环系统。最基本的模板将是单一碳，其然后将通过其连接基团连接至各螯合部分。较长链如乙基或丙基同样可行，其中两个螯合部分连接至模板的每一端。显然，任何适当稳固的键可用于接合模板与连接子部分，包括碳碳键、酯、醚、胺、酰胺、硫醚或二硫键。

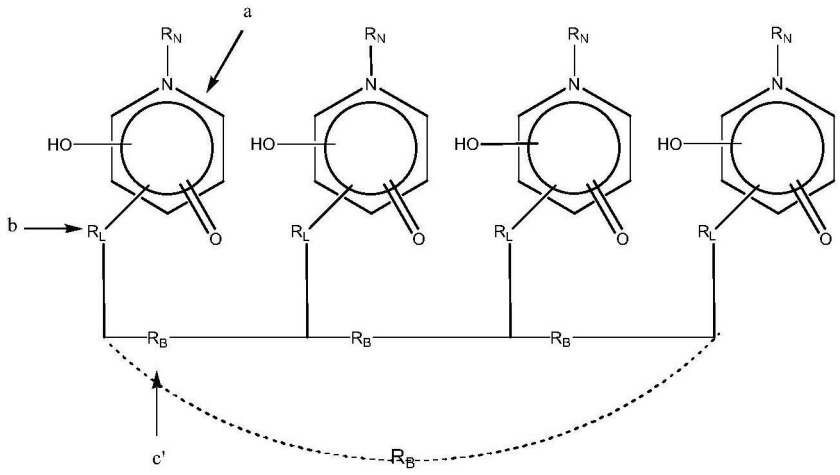
[0118] 显然，在式 II、III、IV 和 IVb 的结构中，适当时，并未以其它方式被取代（例如被连接子或偶联部分取代）的吡啶环的那些位置可带有在式 I 中关于 R_1 至 R_5 所述的取代基。特别是，小的烷基取代基如甲基、乙基或丙基可存在于任何位置。

[0119] 八齿配体一般将另外包含至少一个如上文所述的偶联部分。这可为任何合适的结构，包括本文所示的那些中的任何一种，并且将以靶向部分、特异性结合物或能够连接至这种靶向部分或特异性结合物的官能团封端。

[0120] 偶联部分可连接至连接子、模板或螯合部分的任何合适点，例如在如式 III 中所示的点 a)、b) 和 / 或 c) 处。偶联部分的连接可通过任何适当稳固的键，例如碳碳键、酯、醚、胺、酰胺、硫醚或二硫键。类似地，能够与靶向部分形成任何这些键的基团适于偶联部分的官能末端并且所述部分当连接至靶向部分时将以这些基团封端。

[0121] 作为替代，“主链”型结构示于以下式 IV 中

[0122]



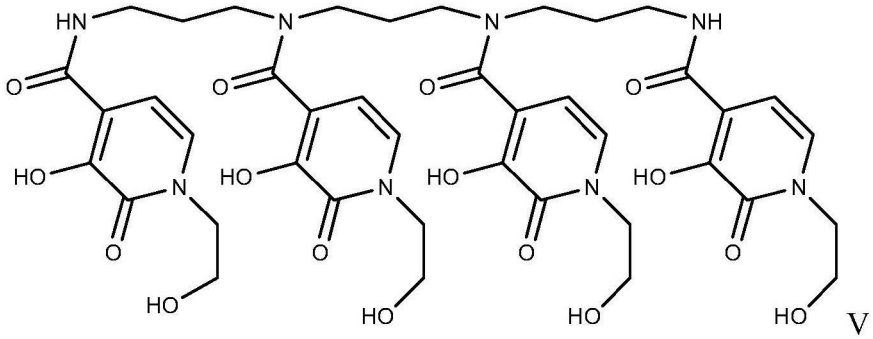
IV

[0123] 其中所有基团和位置都如上文所示并且“R_B”另外为主链部分，其通常将具有类似结构并用于任何本文所示的连接子部分，并且因此在情形允许时，连接子部分的任何定义可适用于主链部分。合适的主链部分将形成骨架，其后借助于其连接子基团连接螯合部分。通常需要三个或四个主链部分。通常对于线性主链将是三个，或者如果主链被环化，则将是四个。特别优选的主链部分包括任选在一端或两端具有杂原子或官能部分的短烃链（例如本文所述的那些）。在这方面，胺和酰胺基团是特别合适的。

[0124] 偶联部分可连接至连接子、主链或螯合部分的任何合适点，例如在如式 IV 中所示的点 a)、b) 和 / 或 c’）处。偶联部分的连接可能是通过任何适当稳固的键，例如碳碳键、酯、醚、胺、酰胺、硫醚或二硫键。类似地，能够与靶向部分形成任何这些键的基团适于偶联部分的官能末端并且所述部分当连接至靶向部分时将以这些基团封端。

[0125] 通过酰胺连接子基团连接至主链的具有四个 3,2-HOPO 螯合部分（各自具有一个羟基乙基增溶基团）的“主链”型八齿配体的实例将是如以下式 V：

[0126]

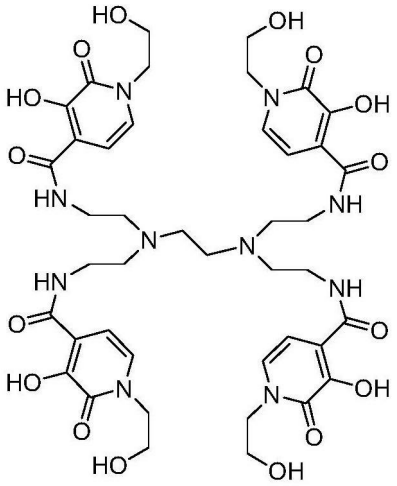


V

[0127] 显然，连接子基团 R_L 可在这个分子上的任何合适点添加，例如在一个仲胺基处或以任何主链烷基上的分支点形式添加。所有小的烷基如主链亚丙基或 n 取代亚乙基可用其它小的亚烷基如任何本文所述的那些（其中亚甲基、亚乙基、亚丙基和亚丁基是非常合适的）取代。

[0128] 各自具有分别通过乙基酰胺基连接至乙基和丙基二胺的四个 3,2-HOPO 螯合部分的示例性“模板化”八齿配体将是如以下式 VI：

[0129]

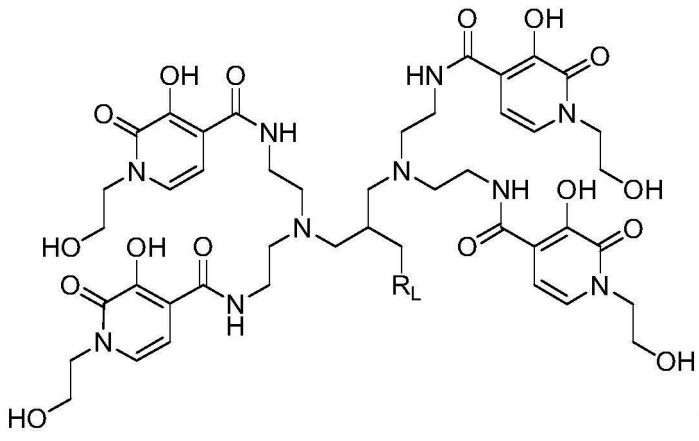


VI

[0130] 显然，在式 VI 中显示为亚乙基部分的任何亚烷基可用其它小的亚烷基如亚甲基、亚丙基或亚正丁基独立地取代。优选在分子中保留一些对称性并且因此例如中心亚乙基可被亚丙基取代，而其它亚乙基保留，或者将 HOPO 部分连接至一个或两个中心叔胺的两个亚乙基可被亚甲基或亚丙基置换。类似地，如本文所讨论，N 取代基团可被如整个本文中所讨论的任何其它羟基烷基置换。

[0131] 如上文所示，八齿配体通常将包括可在任何点接合至配体的其余部分的偶联部分。适用于连接子连接的点示于以下式 VI 中：

[0132]

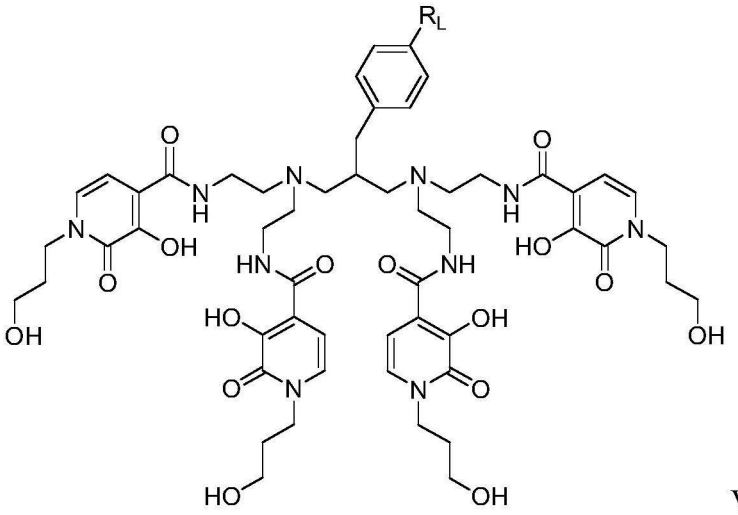


VI

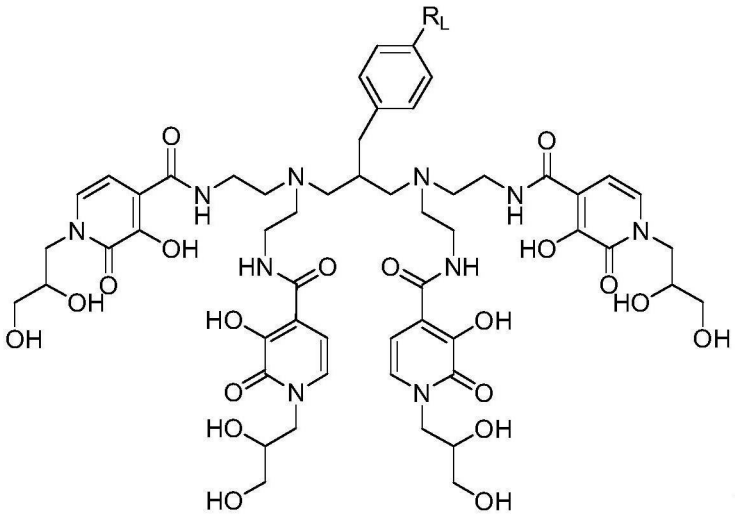
[0133] 其中 R_L 是任何合适的连接部分，特别用于连接至组织靶向基团。在活性基团如胺中封端的短烃基如 C1 至 C8 环状、支链或直链芳族或脂族基团非常适合作为式 VI 和整个本文中的基团 R_L。

[0134] 显示适用于配体连接的位点的非常优选的八齿配体包括以下式 VII 和 VIII 的那些：

[0135]



VII

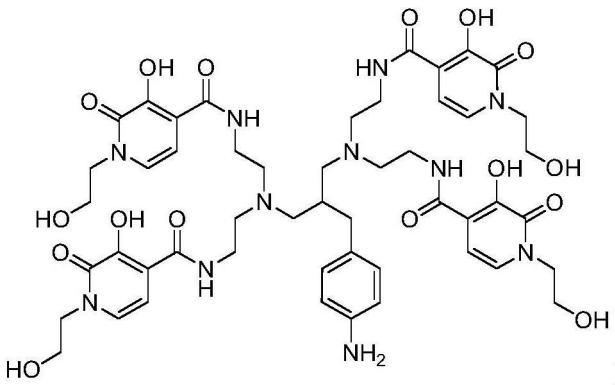


VIII

[0136] 其中在式 VII 和 VIII 中，R_L可为如本文所述的任何合适的连接子基团或反应性部分。R_L通常将形成配体与靶向部分的连接点并且因此任何合适的反应性基团可以直接地或使用另一个连接子而用于这种连接。在式 VII 和 VIII 中适用于 R_L的反应性部分包括 NH_2 和 NCS 基团。

[0137] 根据这个实施方案，以官能化部分封端偶联部分的示例性化合物是以下的结构 IX（连接子苯胺基团适当时显然可被如本文所示的任何其它 R_L基团取代，例如化合物 12 中的 NCS）：

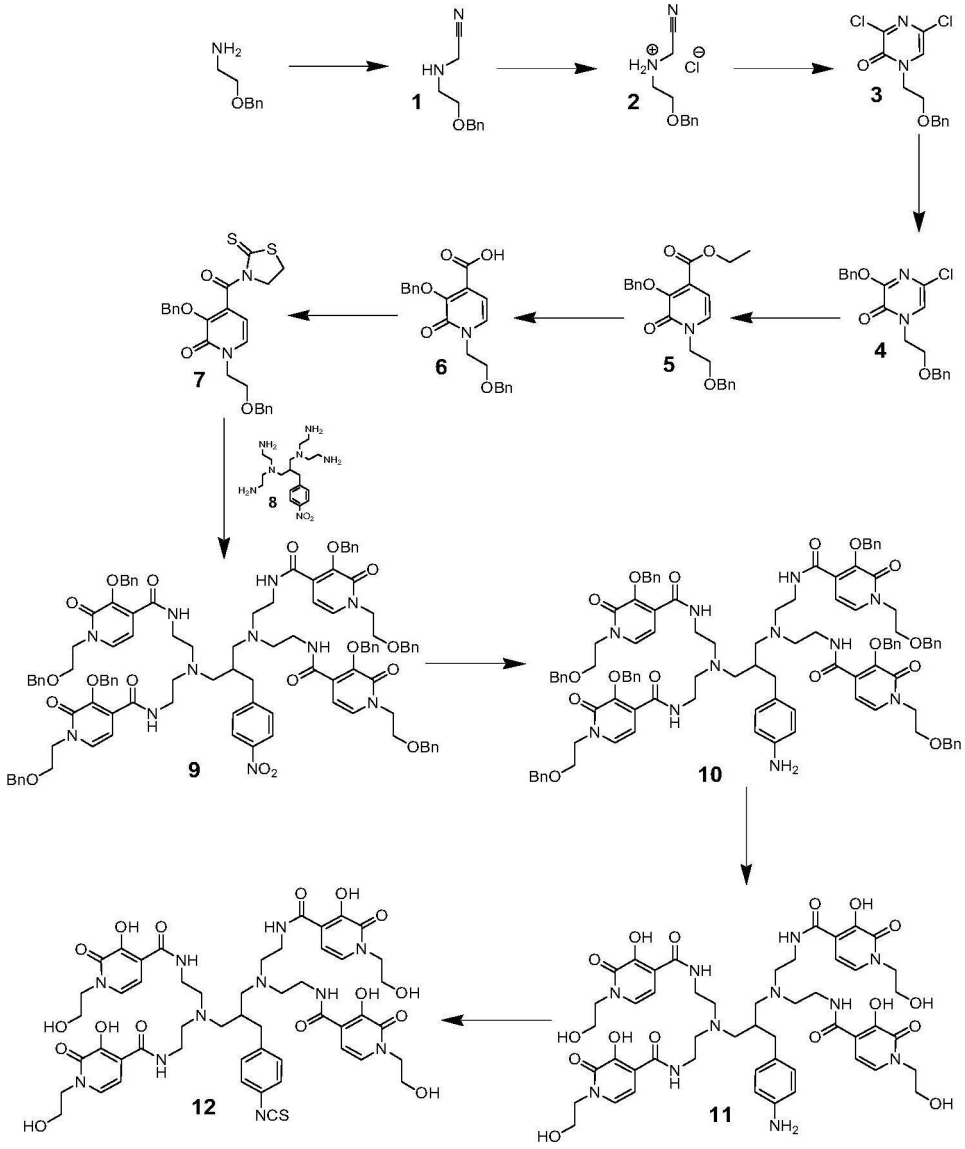
[0138]



IX

[0139] 化合物 IX 的合成描述于以下并且遵循下列合成路线：

[0140]



[0141] 本文提及的所有文献特此以引用的方式并入，包括 Gordon AEV 等，Rational design of sequestering agents for plutonium and other actinides. Chem. Rev. 2003, 103, 4207-4282；PCT 专 利 申 请 WO 2008/063721A2 和 T. N. Lambert 等，Tetrahedron Letters 43(2002)7379-7383。

[0142] 在本发明的络合物的形成方法中，优选在水溶液中进行反应。这具有若干优点。首先，其消除了制造商去除所有溶剂至可接受水平以下并验证所述去除的负担。其次，其减少废物并且最重要的是其通过避免分离或去除步骤而加速生产。在本发明放射性药物的情形下，重要的是尽可能快速地进行合成，因为放射性同位素将在任何时候衰变并且制备所花费的时间浪费了有价值的物质并引入了污染物子同位素。

[0143] 在一个实施方案中，所述方法包括形成含羟基吡啶酮的八齿配体（如整个本文中所述）的第一水溶液和组织靶向部分（如整个本文中所述）的第二水溶液并且使所述第一水溶液与所述第二水溶液接触。

[0144] 在一个相关实施方案中，在基本上不存在任何有机溶剂的情况下进行本发明的形成方法。在这种情形下，并且“有机溶剂”采用其在室温或大约室温下为液体并且其包含至

少一个碳的材料的天然含义。这些有机溶剂通常包含烃、醇、酯、酰胺、酯和/或卤代部分并且这些溶剂优选在本文提及的水溶液中以不超过1重量%(例如0.0001至1重量%)、优选不超过0.5重量%并且最优选不超过0.2重量%的量存在。为了避免疑问,术语"有机溶剂"不涵盖本文提及的靶向部分和配体。某些有机物质如有机酸、胺和其盐可以稍微较高的浓度存在以充当水性溶剂中的pH缓冲剂。在存在时,这些通常将为不超过10重量%(例如0.001至10重量%)、优选不超过5重量%、更优选不超过1重量%的浓度。

[0145] 合适的偶联部分在上文详细讨论并且本文作为偶联和/或连接基团讨论的所有基团和部分可适当用于将靶向部分偶联至配体。一些优选的偶联基团包括酰胺、酯、醚和胺偶联基团。宜借助于从羧酸生成活化酯基来形成酯和酰胺。这种羧酸可存在于靶向部分上、偶联部分上和/或配体部分上并且通常将与醇或胺反应以形成酯或酰胺。这些方法是本领域中众所周知的并且可利用众所周知的活化试剂,包括N-羟基马来酰亚胺、碳化二亚胺和/或偶氮二羧酸酯活化试剂如DCC、DIC、DEAD、DIAD等。

附图说明

[0146] 图1:AGC1115在280nm(A)和335nm(B)下的SEC-UV色谱图。平均螯合剂/抗体比率(CAR)为约0.9。

[0147] 图2通过流式细胞术在CD22阳性Raji细胞上分析的AGC1100和AGC1115的结合。使用小鼠抗人IgG Fc、PE缀合的二抗进行检测,并且绘制中值荧光强度(MFI)对一抗的对数浓度(nM)的曲线。将曲妥珠单抗用作同种型对照物。

[0148] 图3:与Th-227标记的AGC0015缀合的C22结合mAb AGC1115(实心圆形)、Th-227标记的AGC0015缀合的对照mAb曲妥珠单抗(实心正方形)或培养基(实心菱形)温育的Ramos细胞。这两种mAb都用Th-227标记至相同特异性活性(44kBq/μg),并且以3nM(A)使用。

[0149] 现将通过以下非限制性实施例来说明本发明。在实施例中示例的所有化合物构成本发明的优选实施方案(包括优选的中间体和前体)并且在情形允许时在任何方面可单独地使用或以任何组合使用。因此,例如,每个以及所有的实施例2的化合物2至4、实施例3的化合物10和实施例4的化合物7构成其各种类型的优选实施方案。

[0150] 在实施例中,提及下列抗体和抗体缀合物:

[0151] AG01100-如在实施例3中所产生的抗CD22抗体

[0152] AG01115-缀合至高溶解性配体(12)的AG01100

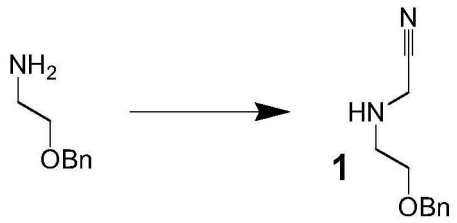
[0153] 实施例1-纯钍-227的分离

[0154] 从锕-227源分离出钍-227。锕-227是通过以下方式产生:镭-226的热中子辐照,接着镭-227衰变(t1/2=42.2m)为锕-227。通过阴离子交换色谱法在8M HNO_3溶液中从锕-227衰变混合物选择性滞留钍-227。使用含有70mg的AG® 1-X8树脂(200-400目,硝酸盐形式)的2mm内径且长度为30mm的柱。在锕-227、镭-223和子体已从柱中洗脱后,用12M HCl从柱中提取钍-227。将含有钍-227的洗脱液蒸发至干燥并且将残余物再次悬浮在0.01M HCl中。

[0155] 实施例2-化合物12的合成

[0156] 步骤1

[0157]

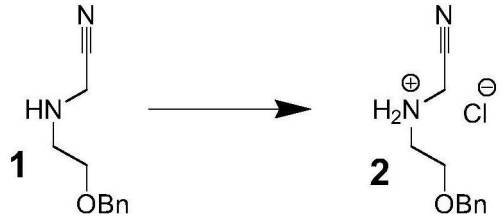


[0158] 将 2- 苄氧基乙胺 (31g,207mmol) 和乙醇腈 (16mL,70％水溶液,207mmol) 溶解在 300mL EtOH(绝对) 中并且回流 4 小时。减压除去挥发物。粗产物 (24.7g,130mmol) 不经进一步纯化即继续进行下一步骤。

[0159] ^{1}H-NMR($CDCl_3$,400MHz):2.92(m,2H),3.58-3.62(m,4H),4.51(s,2H),7.25-7.37(m,5H)

[0160] 步骤 2

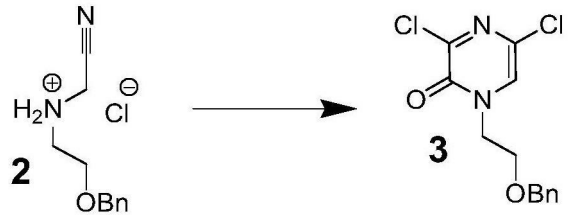
[0161]



[0162] 将 1(24.7g,130mmol) 溶解在无水乙醚中。使 HCl(气) 鼓泡通过溶液持续 30 分钟。过滤沉淀物并减压干燥,得到所需产物 (27.8g,122.6mmol。所述产物不经进一步纯化或分析即继续进行下一步骤。

[0163] 步骤 3

[0164]



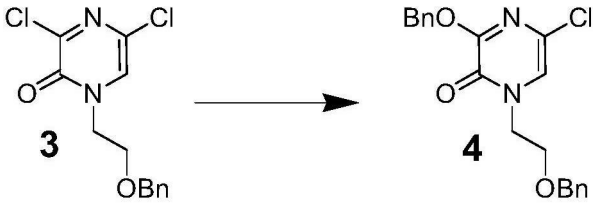
[0165] 在室温下将 2(27.8g,122.6mmol) 溶解在 230mL 氯苯中。在室温下经 30 分钟滴加溶解在 100mL 氯苯中的草酰氯 (45mL,530mmol)。在室温下搅拌反应混合物 45 小时。通过滴加 100mL 水小心地中止反应。分离各相,并且用 3*100mL DCM 萃取水相。将有机相合并并用 100mL 盐水洗涤。有机相经 Na_2SO_4 干燥,过滤并且减压除去挥发物。通过干式快速色谱法在 SiO_2 上使用含 MeOH(0-2％) 的 DCM 梯度纯化粗产物,得到所需产物 (21.2g,70.8mmol)。

[0166] ^{1}H-NMR($CDCl_3$,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)

[0167] MS(ESI 正离子模式 ,m/z,):321.0

[0168] 步骤 4

[0169]



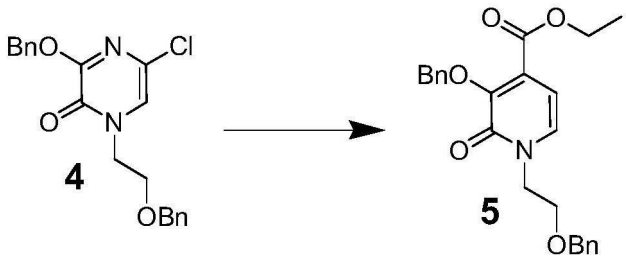
[0170] 在0℃下将氢化钠(60％分散液,3.60g,90mmol)在50mL THF中搅拌并且经10分钟滴加苄醇(8.3mL,80mmol)。在0℃下搅拌反应混合物30分钟,随后在0℃下滴加溶解在100mL THF中的3(21.2g,70.8mmol)。在室温下将反应混合物在黑暗中搅拌过夜。滴加含50mL HCl的二噁烷(4M),然后在真空中缩减反应混合物。加入500mL DCM,接着加入200mL水。分离各相并且用200mL DCM萃取水相。将有机相合并并用100mL盐水洗涤。有机相经Na_2SO_4干燥,过滤并且减压除去挥发物。在SiO_2上使用含MeOH(0-6％)的DCM梯度进行干式快速色谱法,得到所需产物(25.6g,69mmol)。

[0171] ^{1}H-NMR($CDCl_3$,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)

[0172] MS(ESI正离子模式,m/z):371.1,763.2

[0173] 步骤5

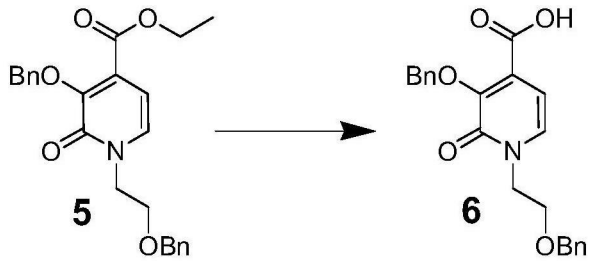
[0174]



[0175] 在140℃下加热4(25.6g,69mmol)和丙炔酸乙酯(41mL,0.4mol)持续5小时。将反应混合物冷却至室温并且通过在SiO_2上进行干式快速色谱法来纯化反应混合物。利用含MeOH(0-10％)的DCM梯度,得到呈所需4-异构体与5-异构体的不可分离的混合物形式的所需产物。这种混合物(28.6g,约65mmol)不经进一步纯化即直接用于下一步骤中。

[0176] 步骤6

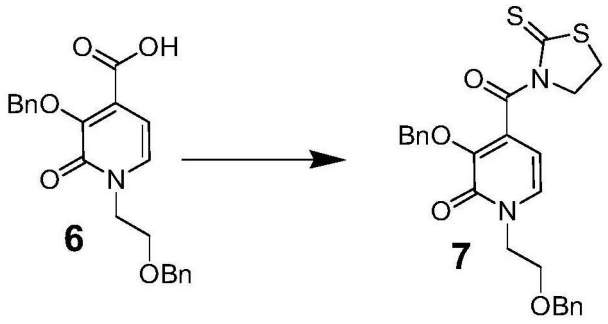
[0177]



[0178] 在0℃下将如先前步骤中所获得的5(28.6g,约65mmol)溶解在300mL THF中。加入100mL KOH(1M,水溶液),并且在室温下搅拌反应混合物40小时。加入HCL(1M,水溶液)直至pH值为约2(125mL)并且用3*250mL $CHCl_3$萃取水相。将有机相合并并用100mL盐水洗涤,过滤并且在真空中除去挥发物。所得物质(25.9g,约65mmol)不经进一步纯化或分析即直接用于下一步骤中。

[0179] 步骤 7

[0180]



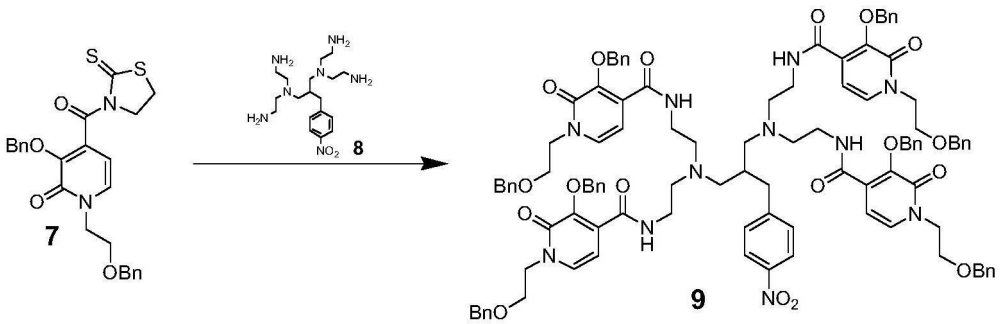
[0181] 将如先前步骤中所获得的 6(25.9g,约 64mmol) 部分溶解在 400mL DCM 中。加入 2- 噻唑啉 -2- 硫醇 (8.94g,75mmol) 和 DMAP(0.86g,7mmol),接着加入 DCC(15.48g,75mmol)。在室温下搅拌反应混合物过夜。通过 Celite 垫过滤反应混合物并且用 100mL DCM 洗涤 Celite 垫。在真空中除去挥发物。通过干式快速色谱法在 SiO_2 上依次使用含 DCM(50-100%) 的庚烷梯度和含 THF(0-15%) 的 DCM 梯度纯化产物混合物。将适当馏分在真空中缩减,得到产物的混合物。通过快速色谱法在 SiO_2 上使用含 EtOAc(25-75%) 的庚烷梯度纯化这种不纯的混合物。将适当馏分在真空中缩减,得到产物的混合物。最后,为得到所需产物,在 RP18 二氧化硅上使用含 MeCN(25-75%) 的水的梯度通过干式快速色谱法纯化产物混合物。这得到所需产物 (8.65g,18mmol)。

[0182] ^{1}H-NMR($CDCl_3$, 300MHz):2.90(t, J = 7.3Hz, 2H), 3.77-3.84(m, 2H), 4.18-4.23(m, 2H), 4.35(t, J = 7.3Hz, 2H), 4.51(s, 2H), 5.33(s, 2H), 6.11(d, 7.0Hz, 1H), 7.21-7.48(m, 11H)

[0183] MS(ESI 正离子模式 ,m/z):503.1

[0184] 步骤 8

[0185]

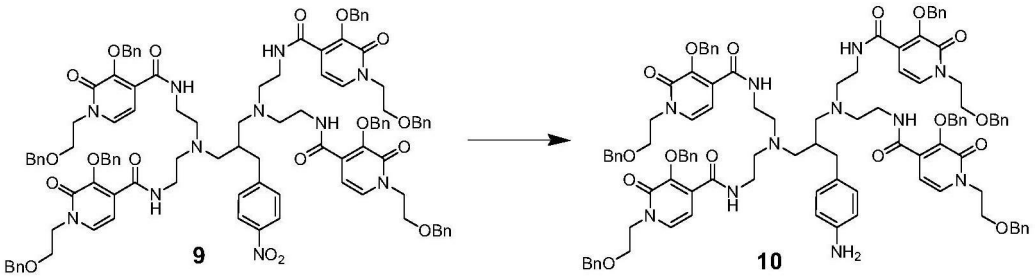


[0186] 将 7(5.77g,12mmol) 和 8(1.44g,2.4mmol) 部分溶解在 40mL DMPU 中。滴加 DBU(2.7mL,18mmol)。在室温下搅拌反应物 4 天。通过干式快速色谱法在 SiO_2 上使用含 DCM 和 MeOH 的 EtOAc 梯度纯化,得到所需产物 (3.93g,2.15mmol)。

[0187] ^{1}H-NMR($CDCl_3$, 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 = 7.2Hz, 4H), 7.13(d, J = 7.2Hz, 4H), 7.16-7.38(m, 42H), 7.82-7.93(m, 6H)

[0188] 步骤 9

[0189]

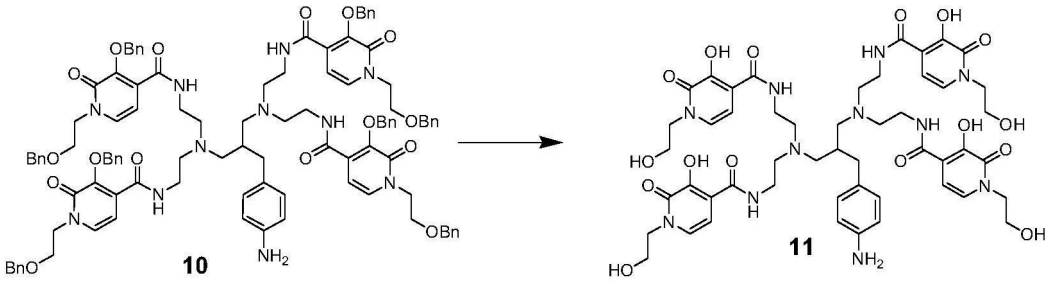


[0190] 在室温下将 9(3.93g,2.15mmol) 溶解在 300mL EtOH 中。加入 60mL 水,接着加入 NH_4Cl(5.94g,32.3mmol)。将反应混合物加热至 60℃,然后加入铁粉 (1.80g,32.3mmol)。在 60℃下搅拌反应混合物 1 小时。将反应混合物冷却至室温并且加入 400mL DCM 和 100mL 水。过滤反应混合物,并且用 100mL 水和 100mL 盐水洗涤有机相。将水相合并并用 3*100mL DCM 反萃取。将有机相合并,经 Na_2SO_4 干燥,过滤并且减压除去挥发物。通过干式快速色谱法在 SiO2 上使用含 MeOH(0-7%) 的 DCM 梯度纯化产物混合物,得到所需产物 (3.52g,1.96mmol)。

[0191] MS(ESI 正离子模式 ,m/z):899.2

[0192] 步骤 10

[0193]



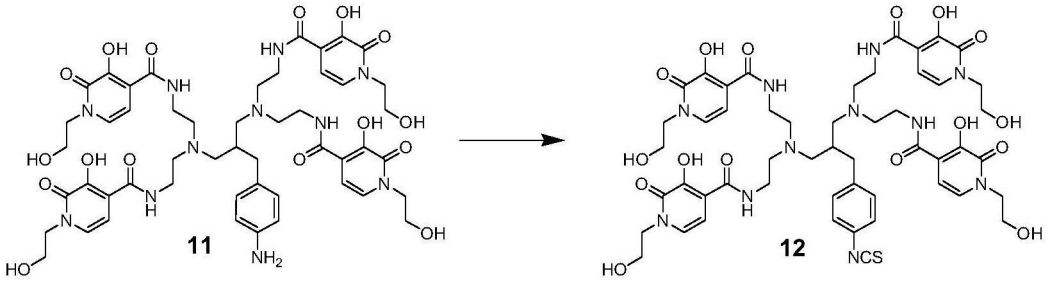
[0194] 将 10(1.00g,0.56mmol)、$Pd(OH)_2/C$(Pearlman 催化剂,1.00g) 和 10mL AcOH 置于压力反应器中。通过吸水器排空反应器并且引入 H_2(7 巴)。将反应混合物搅拌 1 小时,然后释放压力并且将 5mL HCl(6M,水溶液) 加入反应混合物中。如先前所述排空反应器并且再次引入 H_2(7 巴)。在搅拌 7 天后,HPLC 指示完全转化。过滤反应混合物并且减压除去挥发物。将残余物溶解在 MeOH/MeCN(1:1) 中并且通过加入 Et_2O 析出产物。通过离心并且倾析出上清液收集固体,然后在真空中干燥产物 (484mg,0.45mmol)。

[0195] ^{1}H-NMR(D_2O,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 = 7.0Hz,4H),7.44(d,J = 8.2Hz,2H),7.57(d,J = 8.2Hz,2H)

[0196] MS(ESI 正离子模式 ,m/z):1076.4

[0197] 步骤 11

[0198]



[0199] 将化合物 11(20mg,18μmol) 溶解在 3mL MeCN 和 3mL 水中。加入 20μL 硫光气。将反应混合物快速搅拌 1 小时。减压除去挥发物并且将残余物溶解在 4mL MeCN 中。通过向 40mL Et_2O 中加入乙腈相来析出产物。通过离心并且倾析出上清液收集固体,然后在真空中干燥产物 (10mg,9μmol)。

[0200] MS(ESI 正离子模式,m/z):1118.4

[0201] 实施例 3:抗 CD22 单克隆抗体 (AGC1100) 的生成。

[0202] 如 (1) 中所述构建单克隆抗体 (mAb)hLL2(也称为依帕珠单抗,本文表示为 AGC1100) 的序列。本实施例中使用的 mAb 是由 Immunomedics Inc,New Jersey,USA 制备。可例如在用对编码轻链和重链的基因进行编码的质粒转染的中国仓鼠卵巢悬浮 (CHO-S) 细胞中进行这种 mAb 的产生。首先将使用标准程序选择稳定的克隆。在单次使用的生物反应器中约 14 天后,可在上清液过滤后收集单克隆抗体。将通过蛋白质 A 亲和色谱法 (MabSelect SuRe,Atoll,Weingarten/Germany) 以及接着离子交换步骤进一步纯化 AGC1100。可使用基于静电和疏水性的第三纯化步骤除去聚集体和潜在的残余杂质。将通过等电聚焦、SDS-PAGE 分析、N 端测序和 LC/MS 分析来确认 AGC1100 的身份。将通过尺寸排阻色谱法 (SEC) 进一步分析样品纯度。

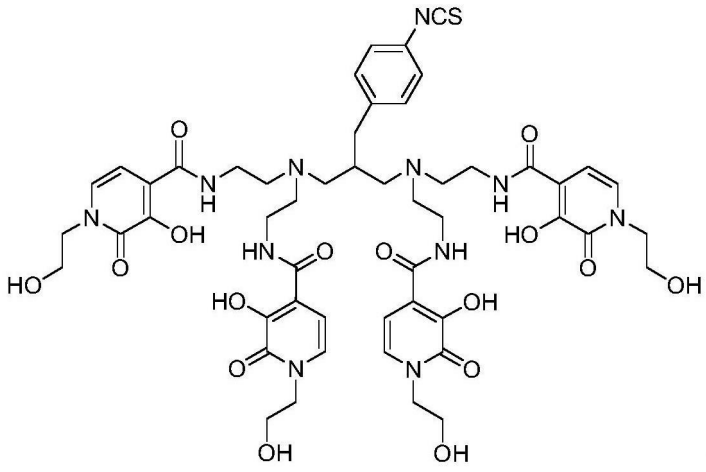
[0203] 参考文献:

[0204] (1)Leung,Goldenberg,Dion,Pellegrini,Shevitz,Shih,and Hansen.Molecular Immunology32:1413-27,1995。

[0205] 实施例 4:AGC1100 与螯合剂 AGC0015 的缀合。

[0206] 将抗体 AGC1100 与水溶性螯合剂 AGC0015 缀合。(12) 在 PBS 与 70mM 硼酸盐缓冲液 (pH 8.5) 混合的 1:1(v/v) 混合物中进行缀合反应。螯合剂 AGC0015 如以下所示:

[0207]



12

[0208] 将螯合剂 AGC0015(以上 12) 溶解在不含金属的水中,然后将其加入缀合反应中。

使用标称摩尔比率为1.3:1的螯合剂/抗体并且在21℃下将反应温育22小时。在反应时间结束时,通过尺寸排阻色谱法在ÄKTA纯化器(GE Healthcare)上使用HiLoad Superdex 20016/600PG柱(GE Healthcare;代码号28-9893-35)并使用0.9% NaCl、100mM柠檬酸盐缓冲液(pH 5.5)作为流动相,从游离螯合剂中分离出抗体馏分。通过HPLC尺寸排阻色谱法-UV(SEC-UV)分析测定纯化缀合物的最终螯合剂/抗体比率(CAR)。在Agilent 1200系列HPLC系统(AgilentTechnologies)上进行CAR测定,其中柱TSKgel SuperSW 3000,4.6×300mm,4μm粒子(TosohBioscience,部件号18675)维持在室温下并且流动相为300mM NaCl、200mM乙酸铵pH 6.8(等度洗脱),其中总操作时间为15分钟。注射体积为5μl并且LC流速为0.35ml/min。在280和335nm下监测UV信号,分别对应于mAb和螯合剂最大吸光度。CAR测定的代表性结果呈现于图1中。

[0209] 实施例5:抗体/螯合剂缀合物AGC1115与Th-227的螯合

[0210] 从锕-227(^{227}Ac)发生器系统中分离出呈4+离子形式的钍-227(^{227}Th)。通过阴离子交换色谱法在8M HNO_3中从^{227}Ac衰变混合物中选择性滞留^{227}Th,其中用$^{227}Th^{4+}$形成带负电的硝酸盐络合物。从柱中洗出^{227}Ac和子体核素并且在12M HCl中洗脱出^{227}Th。将^{227}Th-洗脱物蒸发至干燥并且将残余物溶解在0.5M HCl中。

[0211] 在螯合反应中,在每0.5mg抗体缀合物1MBq ^{227}Th的存在下在21℃/室温下将抗体-缀合物AGC1115在0.9% NaCl 100mM柠檬酸盐缓冲液(pH 5.5)中温育15分钟。通过尺寸排阻色谱法使用NAP-5DNA Grade柱(GE Healthcare)从游离的^{227}Th和子体核素中分离出含有放射性标记的抗体-缀合物的高分子馏分。标记效率通常为96-98%,包括在NAP-5脱盐步骤中的潜在损失。

[0212] 实施例6:通过流式细胞术进行AGC1115和AGC1100与CD22阳性Raji细胞的结合分析。

[0213] 通过流式细胞术分析AGC1115和AGC1100(抗人CD22,Immunomedics;hLL2,#1003164,10mg/ml)与CD22阳性Raji细胞(ATCC,#CCL-86)的结合。将从拟合曲线测定的EC_{50}值用于比较抗体相对于抗体缀合物结合效能。这种分析用于证实,抗体缀合物与CD22的结合效能不受缀合程序影响。

[0214] 在10%胎牛血清(FBS)和1%青霉素/链霉素存在下使Raji细胞在RPMI 1640(PAA;#E15-840)中生长。对于流式细胞术分析,通过在4℃下在340×g下离心5分钟来收集50ml细胞培养物。将细胞再次悬浮并在补充有1% FBS的10ml PBS中洗涤两次,并通过在4℃下在340×g下离心5分钟来形成沉淀。随后,将20μl再次悬浮的细胞的制剂以1:500稀释于Coulter Isoton II稀释剂中,并使用Beckman Coulter Z2仪器(Beckman Coulter;CA,USA)计数。将制剂调节至1×10^6个细胞/毫升的细胞密度并且将100μL转移至V形底96孔板(Nunc/Fisher Scientific;NH,USA)的每个孔中。将细胞短暂离心并在倾析后再次悬浮,将得到每个孔50μl细胞悬浮液的近似体积。

[0215] 将AGC1115和AGC1100稀释至50μg/ml并以3倍稀释步骤在12个点滴定。相应地制备同种型对照抗体(曲妥珠单抗)。将来自每一抗体稀释液的100μl加入含有Raji细胞的孔中。在4℃下温育1.5小时后,将细胞短暂离心并以补充有1% FBS的200μl冷PBS洗涤两次。将PE缀合的小鼠抗人IgG Fc(BioLegend;#409304)用作用于检测人mAb的二抗试剂。所述二抗试剂是在补充有1% FBS的PBS中以1μg/ml制备。随后将来自二

抗试剂的 100μl 加入每个孔，然后在 4℃下在黑暗中温育 1 小时。如上文所述将细胞洗涤两次，并且再次悬浮在补充有 1% FBS 的 200μl PBS 中。在 V 形底 96 孔板中分析所有样品。将荧光信号记录在 Beckham Coulter Cell Lab Quanta SC MPL 流式细胞仪 (Beckman Coulter ;CA,USA) 上。将中值 (MFI) 导出到 Excel 图表中并针对浓度 ([nM]) 绘制曲线。

[0216] 在 GraphPadPrism(PrismSoftware ;CA,USA) 中使用“log(激动剂) 对反应 - 可变斜率 (四个参数)”结合模型拟合数据并且根据拟合计算 EC_{50} 值 (图 2)。用二抗使 Raji 细胞直接染色显示低本底，MFI 值为约 1(AGC1115MFI 值的 0.5-1%)。

[0217] AGC1100 和 AGC1115 的拟合滴定曲线的所计算 EC_{50} 值分别为 9nM 和 6nM，并且指示缀合物 AGC1115 的结合效能与 AGC1100 类似。

[0218] 实施例 7 :AGC1115-Th-227 的 Th-227 诱导的细胞毒性。

[0219] 在 CD22 阳性 Ramos 细胞中研究体外细胞毒性 (参见实施例 6)。将 AGC1115 和与 AGC0015 缀合的对照曲妥珠单抗用于螯合 Th-227 至特异性活性为 44kBq/μg。

[0220] 使 Ramos 细胞在 37℃与 5% CO_2 下生长，并且每周三次分成 1:5。在测定前一天，用新培养基更换培养基 (具有 20% FBS 和 1% 青霉素 / 链霉素的伊斯科夫改良达尔伯克培养基 (IMDM)) 并且调节体积以得到 400000 个细胞 / 毫升。将约 1600000 个细胞 (4mL) 加入 6 孔板的每个孔。将板温育直至第二天，以加入标记的 mAb 或培养基。

[0221] 在加入标记的 mAb 或培养基之后，将板再温育 4 小时。在实验中，将 AGC1115 或曲妥珠单抗 -AGC0015 加入每个孔至最终浓度为 3nM。在温育后，将细胞在培养基中洗涤两次，并且测量上清液和沉淀中的 ATP。然后将细胞分成 1:2 并在 37℃与 5% CO_2 下在培养基中温育。在第 3 天、第 5 天和第 7 天重复相同程序，但仅洗涤一次。

[0222] 将 ATP 的定量用作不同样品时间下的细胞活力的量度 (来自 Promega 的 CellTiter-Glo 发光细胞活力测定)，产生图 3 中所示的曲线。与 Th-227 标记的对照构建体 (未结合至 Ramos 细胞) 相反，结合 AGC1115-Th-227 的 Ramos 细胞产生细胞毒性，

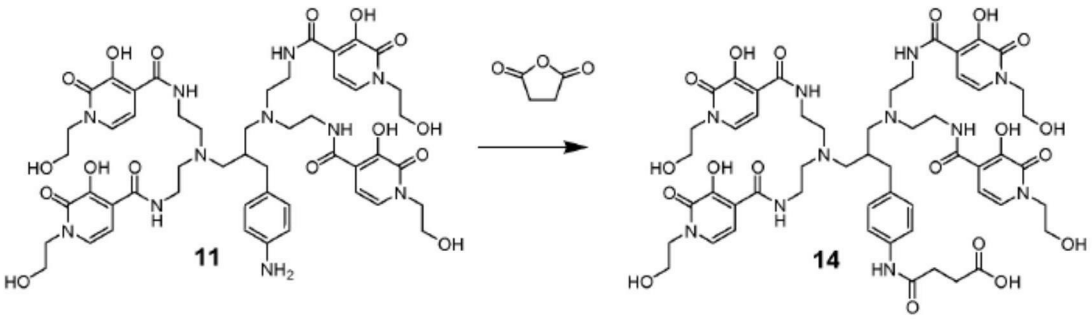
[0223] 实施例 8—酸衍生物

[0224] 制备水溶性螯合剂的酸衍生物能够得到替代偶联化学。

[0225] 这个实施例显示酸衍生物的成功合成。这种螯合剂的衍生物能够例如与肿瘤靶向蛋白质的 ε 胺形成酰胺键。

[0226] 本实施例显示可溶性螯合剂的合成并从物质 11(实施例 2) 起始。将 43mg(约 0.04mmol) 物质 11 溶解在 4mL DMSO、4mL 乙腈和 30μL NEt_3 中。加入 6mg 琥珀酸酐 (0.06mmol)。在室温下反应 22 小时后反应混合物的 LC/MS 分析显示已形成物质 15。形成一些污染物二酰化副产物。以多份加入酸酐会使酯形成降至最少并提高产物 14 的摩尔产率。所得反应混合物的 HPLC 分析示于图 6 中。

[0227]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35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

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35 40 45

[0002]

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50 55 60

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65 70 75 80

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20 25 30

Trp Leu His Trp Ile Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Arg Asn Asp Tyr Thr Glu Tyr Asn Gln Asn Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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Ala Arg Arg Asp Ile Thr Thr Phe Tyr Trp Gly Gln Gly Thr Thr Leu
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Thr Val Ser Ser
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35 40 45

Gly Tyr Ile Asn Pro Arg Asn Asp Tyr Thr Glu Tyr Asn Gln Asn Phe
50 55 60

Lys Asp Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Asn Thr Ala Tyr
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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Phe Tyr Phe Cys
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Thr Val Ser Ser
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35 40 45

Gly Tyr Ile Asn Pro Arg Asn Asp Tyr Thr Glu Tyr Asn Gln Asn Phe
50 55 60

Lys Asp Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Phe Tyr Phe Cys
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Thr Val Ser Ser
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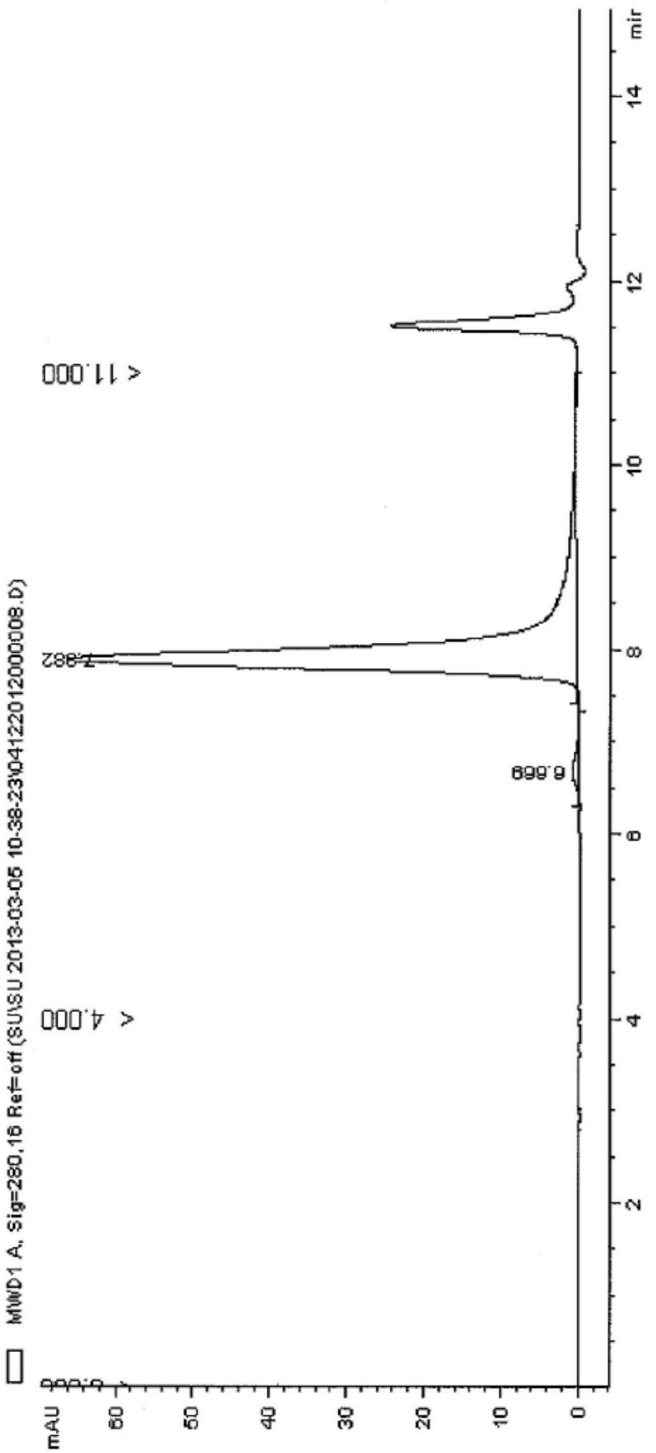
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图 1a)

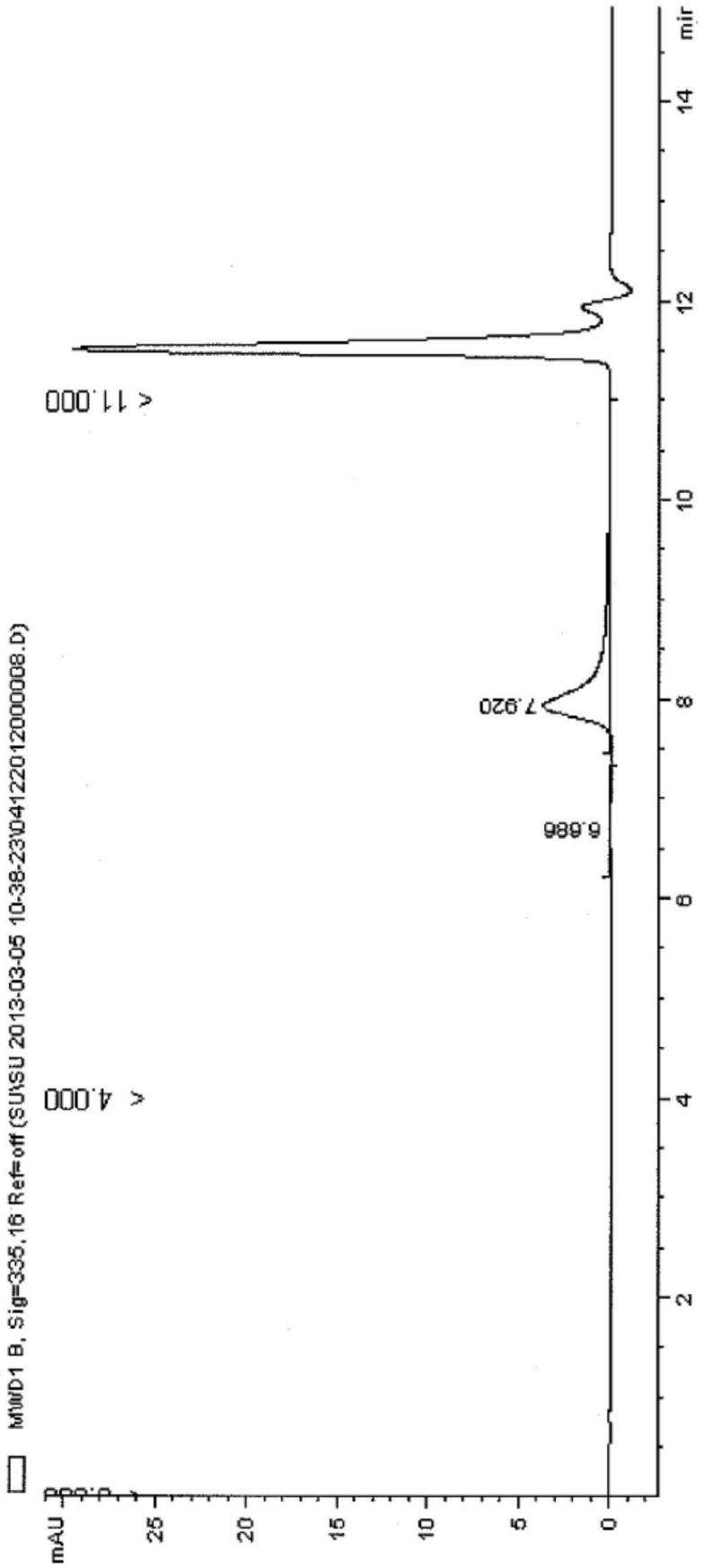
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图 1b

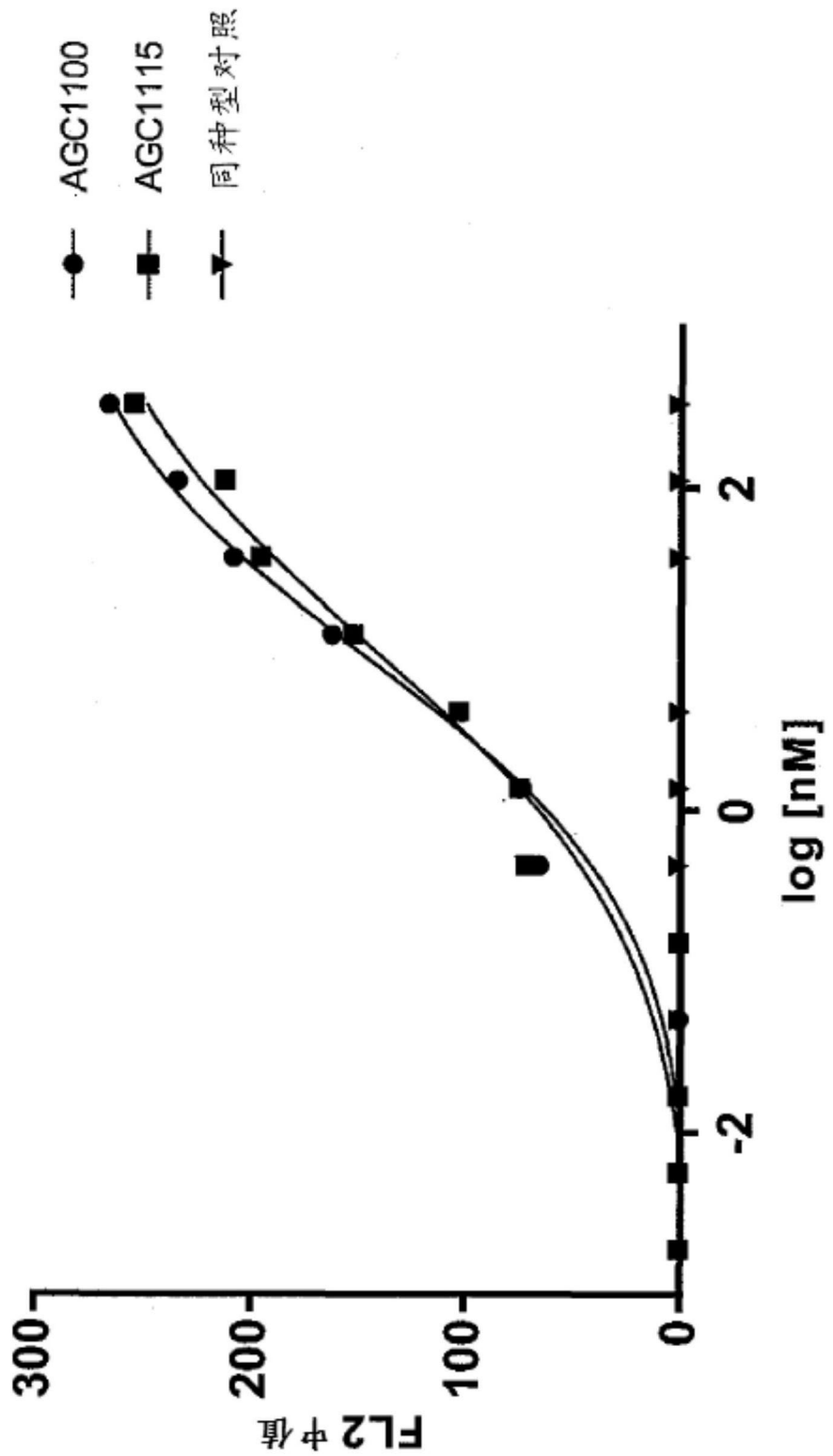

图 2

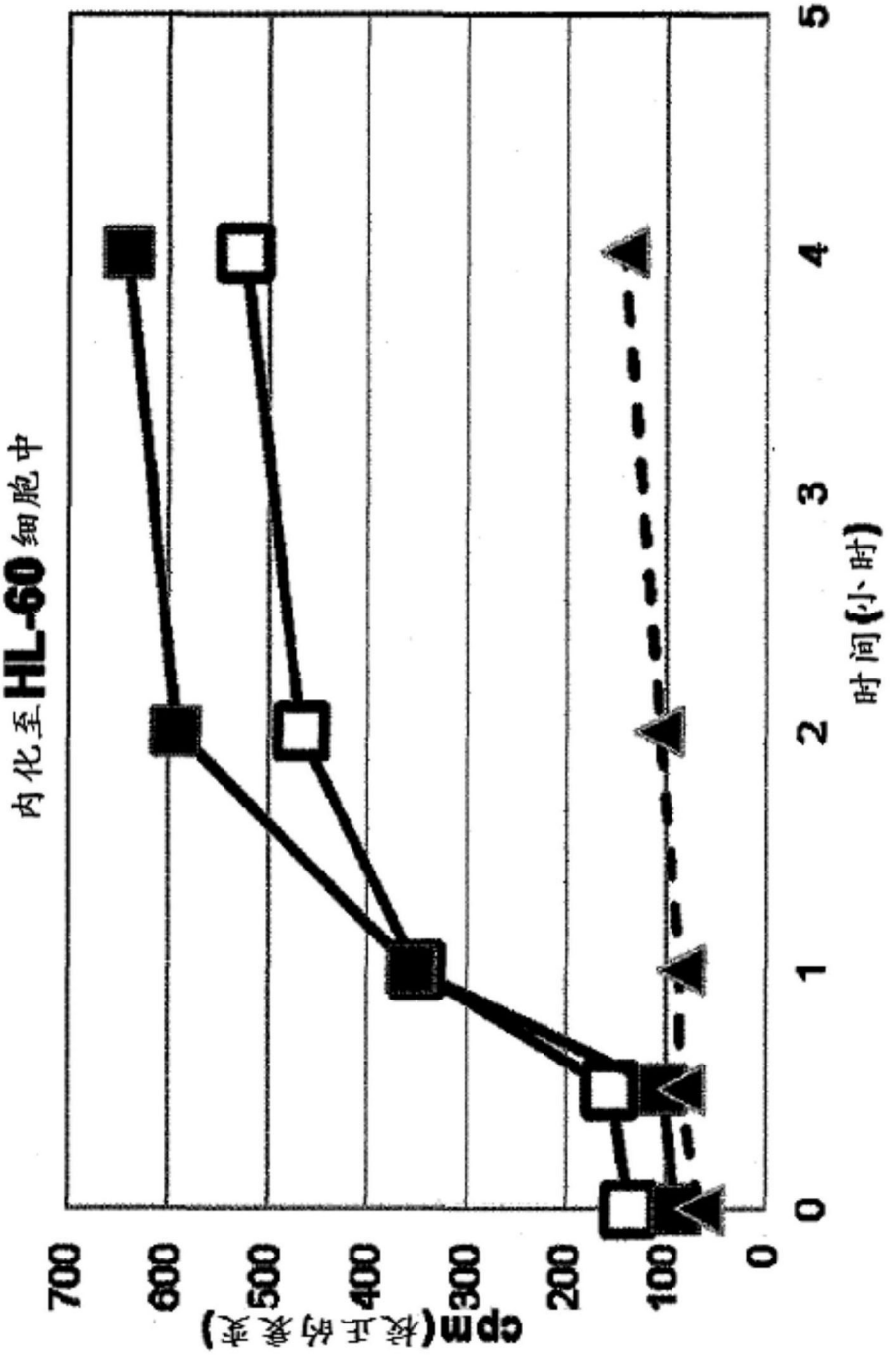
内化至HL-60细胞中
cpm(校正的衰变)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
时间(小时)

图 3