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(73) Patenthaver: **Universität Zürich, Prorektorat MNW , Rämistrasse 71, 8006 Zürich, Schweiz**
Universitätsspital Basel, Rechtsdienst , Hebelstrasse 32, 4031 Basel, Schweiz

(72) Opfinder: **MINDT, Thomas, L., Pfeffingerstrasse 88, CH-4053 Basel, Schweiz**
BAUMAN, Andreas, Nikolaus-Singeisen-Weg 1c, 79650 Schopfheim, Tyskland
Patra, Malay, 66 Homer Ave, apartment 310, Cambridge, MA 02138, USA
GASSER, Gilles, 6, Square de Port Royal, F-75013 Paris, Frankrig

(74) Fuldmægtig i Danmark: **Novagraaf Brevets, Bâtiment O2, 2 rue Sarah Bernhardt CS90017, F-92665 Asnières-sur-Seine cedex, Frankrig**

(54) Benævnelse: **MULTIDENTATE, BIFUNKTIONELLE CHELATERINGSMIDLER TIL RADIONUKLIDKOMPLEKSDANNELSE I DIAGNOSTIK OG TERAPI**

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WO-A1-2014/164988
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MALAY PATRA ET AL: "An octadentate bifunctional chelating agent for the development of stable zirconium-89 based molecular imaging probes", CHEMICAL COMMUNICATIONS > CHEMICAL COMMUNICATIONS - CHEMCOM, vol. 50, no. 78, 1 January 2014 (2014-01-01), pages 11523-11525, XP055145739, GB ISSN: 1359-7345, DOI: 10.1039/C4CC05558F
AMRUTA R. POREDDY ET AL: "Hydroxamate-Based Iron Chelators: Combinatorial Syntheses of Desferrioxamine B Analogues and Evaluation of Binding Affinities", JOURNAL OF COMBINATORIAL CHEMISTRY, vol. 6, no. 2, 1 March 2004 (2004-03-01), pages 239-254, XP055195053, ISSN: 1520-4766, DOI: 10.1021/cc030039a

DESCRIPTION

[0001] The present invention relates to multidentate ligand systems and metal complexes thereof, and to their use in diagnostics and therapy.

Background of the invention

[0002] ^{89}Zr is a metallic radionuclide with promising characteristics for application in medical diagnosis. For in vivo diagnostics or therapeutic applications where radio-labelled compositions are employed, it is important that the radionuclide specifically localizes to target tissues. To provide specific binding to, or absorption by, the particular cells or tissue(s) of interest, radionuclides are generally coupled to targeting agents. Typically, metallic radionuclides are bound to a chelating agent, and the chelating agent is coupled to a targeting moiety, which provides the radiolabelled composition with the ability to bind selectively to a specific population of target cells or tissue(s).

[0003] ^{89}Zr radiolabelled compounds may be employed in a variety of techniques, including PET (positron emission tomography) diagnostics, in particular in the field of immuno-PET, where antibody-based radiotracers are used to image tumours based on the expression of tumour-associated antigens on tumour cells. Radionuclides with suitable half-lives are often employed in nuclear medicine. The half-live of ^{89}Zr, which is 78.4 h, matches the biological half-lives of antibodies. This makes ^{89}Zr a favourable radiometal for application in immuno-PET, which often requires imaging at late time points due to slow accumulation (e.g. several days) of the radioconjugate in the target tissue.

[0004] Besides PET radionuclides such as ^{89}Zr, ^{44}Sc, ^{64}Cu, and ^{68}Ga, gamma ray emitting metallic radionuclides such as ^{67}Ga, ^{111}In, or ^{99m}Tc may also be employed for imaging studies in immunodiagnostics, particularly in single-photon emission computed tomography (SPECT). In addition, particle emitting radionuclides such as ^{177}Lu, ^{90}Y or ^{213}Bi may be employed in therapeutic applications.

[0005] Chelation describes a process in which a metal ion or a pre-complexed form thereof, reacts with a chelating agent to form a coordinated chelate complex, in which the metal is coordinatively bound to the chelating agent at two or more sites. The sequestration properties of chelating agents are not only exploited in nuclear medicine in form of radiopharmaceuticals, but also in detoxification techniques. It may also be applied to the development of MRI contrast agent based on gadolinium (Gd) or iron (Fe).

[0006] Known tetrahydroxamate chelators have linkers of 7 atoms or less for complexation with metal ions (Poreddy et al. 2003, J. Combo Chem. 2004, 6, 239- 254; WO0004868;

WO2014164988).

[0007] Desferrioxamine (DFO, CAS No. 70-51-9) is a hexadentate chelator, which is clinically approved for the treatment of iron poisoning. ^{89}Zr has been conjugated to a functionalized derivative of DFO (Fischer et al. Molecules 2013, 18, 6469-6490). DFO coordinates only six of the eight available coordination sites of the metal atom, while water molecules occupy the remaining positions. The inability of DFO to bind all coordination sites of Zr^{4+} results in an unfavourable stability of the complex in vivo. Dissociation of ^{89}Zr from the conjugate in vivo results in its accumulation in radiation sensitive tissue (red bone marrow, specifically). Such unspecific deposition of radioactivity in vivo poses serious limitations to ^{89}Zr-based radiopharmaceuticals for clinical applications due to the radiation burden to the patients.

[0008] The objective of the present invention is to provide improved means for delivering radionuclides, particularly ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{177}Lu, and ^{90}Y, for diagnostics and/or therapeutic use. This objective is attained by the subject-matter of the independent claims.

Terms and definitions

[0009] Amino acid sequences are given from amino to carboxyl terminus. Capital letters for sequence positions refer to L-amino acids in the one-letter code (Stryer, Biochemistry, 3rd ed. p. 21).

[0010] A C_1-C_4 alkyl in the context of the present specification signifies a saturated linear or branched hydrocarbon having 1, 2, 3 or 4 carbon atoms, wherein one carbon-carbon bond may be unsaturated and one CH_2 moiety may be exchanged for oxygen, sulphur or nitrogen (an ether, thioether, sulfoxide, sulfone or amine bridge). Non-limiting examples for a C_1-C_4 alkyl are methyl, ethyl, propyl, prop-2-enyl, n-butyl, 2-methylpropyl, *tert*-butyl, but-3-enyl, prop-2-inyl and but-3-inyl.

[0011] A C_1-C_5 alkyl in the context of the present specification signifies a saturated linear or branched hydrocarbon having 1, 2, 3, 4 or 5 carbon atoms, wherein one carbon-carbon bond may be unsaturated and one CH_2 moiety may be exchanged for oxygen, sulphur or nitrogen (an ether, thioether, sulfoxide, sulfone or amine bridge). Non-limiting examples for a C_1-C_5 alkyl include the examples given for C_1-C_4 alkyl above, and additionally 3-methylbut-2-enyl, 2-methylbut-3-enyl, 3-methylbut-3-enyl, n-pentyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1,2-dimethylpropyl and pent-4-inyl.

[0012] A C_1-C_8 alkyl in the context of the present specification signifies a saturated linear or branched hydrocarbon having from one to 8 carbon atoms and includes the definitions for C_1-C_4 alkyl and C_1-C_5 alkyl given above.

[0013] A saturated alkyl is an alkyl having only saturated carbon-carbon bonds. A partially unsaturated alkyl is an alkyl comprising isolated or conjugated carbon-carbon double bonds, but not aromatic double bonds.

[0014] The term arene (or: "aryl") in the context of the present specification signifies a cyclic aromatic C_5-C_{10} hydrocarbon. Examples of arene include, without being restricted to, phenyl, naphtyl and heteroarene. A heteroarene ("heteroaryl") in the context of the present invention is an arene that comprises one or several nitrogen, oxygen, and/or sulphur atoms as part of the aromatic system. Examples for heteroarene include, without being restricted to, pyrrole, thiophene, furan, imidazole, pyrazole, thiazole, oxazole, pyridine, thiazine, quinolone, benzofuran and indole. An arene or a heteroarene in the context of the invention additionally may be substituted by one or more alkyl groups. In certain preferred embodiments, the heteroarene is unsubstituted.

[0015] The term "substituted" refers to the addition of a substituent group to a parent compound.

[0016] "Substituent groups" can be protected or unprotected and can be added to one available site or to many available sites in a parent compound.

[0017] "Protected" substituent groups are reactive substituent groups such as a hydroxyl group, an amine or sulfhydryl group, a carboxylic acid group or an carboxylic acid amide group within an organic molecule, which are derivatized to the effect of decreasing their reactivity during a conversion step of the organic molecule, while retaining the ability to restore the substituent group readily by cleavage of the protecting group. Protecting groups and their use are reviewed in Wuts, Greene's protective groups in organic synthesis, Wiley 5th edition 2014 (ISBN 978-1118057483).

[0018] Substituent groups may also be further substituted with other substituent groups and may be attached directly or by a linking group such as an alkyl or hydrocarbyl group to a parent compound. "Substituent groups" amenable herein include, without limitation, halogen, oxygen, nitrogen, sulphur, hydroxyl, alkyl, alkenyl, alkynyl, acyl (-$C(O)R^a$), carboxyl (-$C(O)OR^a$), aliphatic groups, alicyclic groups, alkoxy, substituted oxy (-OR^a), aryl, aralkyl, heterocyclic radical, heteroaryl, heteroarylalkyl, amino (-$N(R^b)(R^c)$), imino (=NR^b), amido (-$C(O)N(R^b)(R^c)$ or -$N(R^b)C(O)R^a$), hydrazine derivates (-$C(NH)NR^aR^b$), tetrazole (CN_4H_2), azido ($-N_3$), nitro ($-NO_2$), cyano (-CN), isocyano (-NC), cyanato (-OCN), isocyanato (-NCO), thiocyanato (-SCN); isothiocyanato (-NCS); carbamido (-$OC(O)N(R^b)(R^c)$ or -$N(R^b)C(O)OR^a$), thiol (-SR^b), sulfinyl (-$S(O)R^b$), sulfonyl (-$S(O)_2R^b$), sulfonamidyl (-$S(O)_2N(R^b)(R^c)$or -$N(R^b)S(O)_2R^b$) and fluorinated compounds $-CF_3$, $-OCF_3$, $-SCF_3$, $-SOCF_3$ or $-SO_2CF_3$, wherein each R^a, R^b and R^c is, independently, H or a further substituent group with a preferred list including without limitation, H, alkyl, alkenyl, alkynyl, aliphatic, alkoxy, acyl, aryl, heteroaryl,

alicyclic, heterocyclic and heteroarylalkyl.

[0019] An unsubstituted linker chain is a chain constituted only of a linear chain of linker atoms N (nitrogen), C (carbon) and O (oxygen), bound to hydrogen atoms where appropriate. Non-limiting examples are methylene (-CH_2-), ethyl (-CH_2CH_2-) or ethoxyethyl (-CH_2CH_2-O-CH_2CH_2-) linker.

[0020] A substituted linker chain is a chain constituted of a linear chain of linker atoms N (nitrogen), C (carbon) and O (oxygen), wherein some or all linker atoms are bound to substitute moieties as defined above.

[0021] Identity in the context of the present specification is a single quantitative parameter representing the result of a sequence comparison position by position. Methods of sequence comparison are known in the art; the BLAST algorithm available publicly is an example.

Summary of the invention

[0022] The present invention relates to a ligand able to coordinate Zirconium ions and other related metal ions by four hydroxamate groups, wherein the hydroxamate moieties are spaced by between 10 and 12 bonds, particularly 10 bonds, in other words the hydroxamates are separated by linkers having between 9 and 11, particularly 9 carbon or hetero atoms between them. The ligand is attached, or attachable, covalently to a moiety conferring targeting specificity onto the ligand. The moiety conferring targeting specificity may be an antibody or a ligand for a tissue-specific receptor.

[0023] The invention further relates to a complex between the ligand of the invention, and a metal atom coordinated by the ligand.

[0024] The invention further relates to the use of such complex in diagnosis or therapy of disease.

Detailed description of the invention

[0025] According to a first aspect of the invention, a ligand characterized by a general formula I:

$$R^1 - D - X - D - X - D - X - D- E - R^2 \qquad (I)$$

is provided, wherein

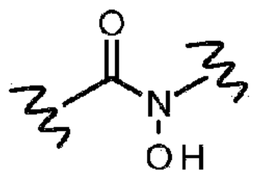
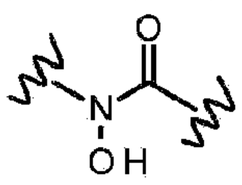
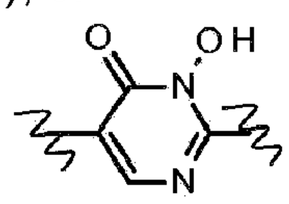
each D is a hydroxamate moiety, each X is a spacer separating two hydroxamate moieties, R^1

is a non-reactive terminal moiety, R^2 is a moiety conferring tissue specificity or the ability to link the molecule to a tissue specific moiety, and E is an optional spacer separating the ligand and R^2.

[0026] Said ligand is characterized by a general formula I:

$$R^{1}\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}E\text{-}R^{2} \qquad (I),$$

wherein

- D is C(O)N(OH) (

) or N(OH)C(O) (

), or

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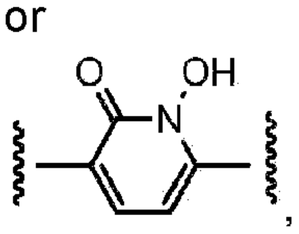
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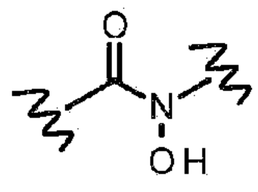
in particular D is

,
- each X independently of any other X is a saturated or partially unsaturated, substituted or unsubstituted linker comprising a chain of 9, 10 or 11, in certain embodiments particularly 9, atoms selected from any of N, C and O,
- R^1 is or comprises H, a C_1-C_5 alkyl, a C_3-C_6 cycloalkyl, an arene, and/or a heteroarene;
- E is a saturated or partially unsaturated, substituted or unsubstituted linker comprising a chain of 1 - 50 atoms selected from C, O, N and S and
- R^2 is
    1. a) an OH, NH_2, SH, COOH, CHO, N_3, SCN, CH_2X (X = Cl, Br, I), activated ester (e.g., NHS, tetra-or pentafluoro phenol derivatives), an ene-one-system (alpha, beta unsaturated carbonyl, a Michael acceptor system (e.g., maleimide)) a diene/dienophile (Diels Alder), an alkene, or an alkyne,
    2. b) a first click moiety capable of selectively forming a covalent bond with a second click moiety R^3 under reaction conditions not leading to a covalent reaction of R^2

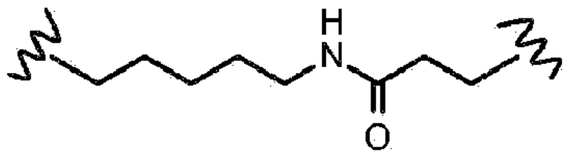
or R^3 with natural occurring polypeptides, in particular with proteins, or

3. c) an antibody, an oligopeptide, a polypeptide, a polynucleotide, a liposome, a polymerosome, a phospholipid, a vitamin, a monosaccharide, an oligosaccharide, a nanoparticle, or a drug-like molecule having a molecular weight less than (<) 3000 U, or a moiety that specifically binds to a target site on cells and/or tissues with an association constant of lower than (<) 10^{-6} mol/l, $<10^{-7}$ mol/l, $<10^{-8}$ mol/l or $<10^{-9}$ mol/l, or
4. d) a solid support.

[0027] In certain embodiments, D is a substituted or unsubstituted pyrimidinone or pyridinone moiety.

[0028] In certain embodiments, X is a saturated or partially unsaturated, substituted or unsubstituted linker comprising 9, 10, or 11 atoms, particularly 9 atoms within the chain (the counted atoms constituting the chain backbone; i.e. an ethyl group $\underline{C}H_2$-$\underline{C}H_2$ counts as two atoms; the chains -$\underline{C}$(O)$\underline{N}$H$\underline{C}H_2\underline{C}H_2\underline{C}H_2$- and -$\underline{C}(O)\underline{N}H\underline{C}$H($CH_3$)$\underline{C}H_2\underline{C}H_2$- both count as 5 atoms; counted atoms are underlined) selected from CH_2, NR, CO and O, with R being H or C_1-C_4 alkyl. In certain embodiments, R is H.

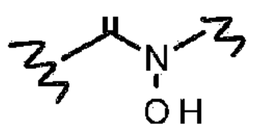
[0029] In certain embodiments, X is unsubstituted. In certain embodiments, X is an unsubstituted linear chain consisting of C_1-C_5 alkyl moieties bridged (linked) by COO (ester) or CONH (amide) moieties. In certain embodiments, X is



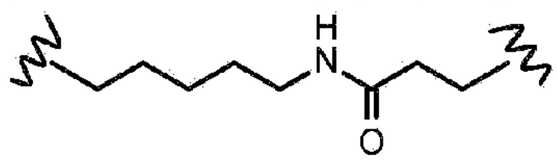
[0030] In certain embodiments, R^1 is CH_3, CH_2CH_3, or a saturated or partially unsaturated C_3-C_6 cyclic alkyl moiety, or a monoanular or bianular aryl or heteroaryl moiety, particularly a saturated or partially unsaturated C_3-C_6 cyclic alkyl moiety, or a monoanular or bianular aryl or heteroaryl moiety consisting of between three and fifteen carbon or hetero (N, C, O, S) atoms (and hydrogen as appropriate).

[0031] In certain embodiments, E is a saturated or partially unsaturated, substituted or unsubstituted linker comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 5 to 25, 10 to 30, 20 to 50 or 30 to 50 atoms selected from C, O, N and S (the counted atoms constituting the chain backbone and are counted as exemplified for X above). In certain embodiments, E is integral with R^2.

[0032] In certain embodiments, D is



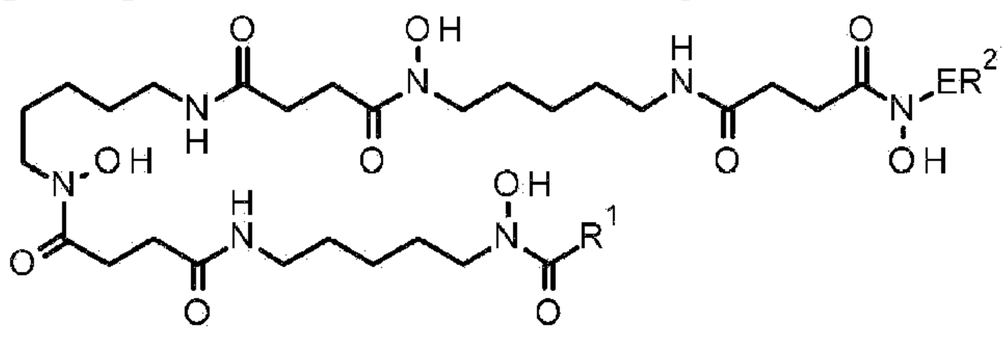
(in text form: C(O)N(OH)), each X is (all X are)



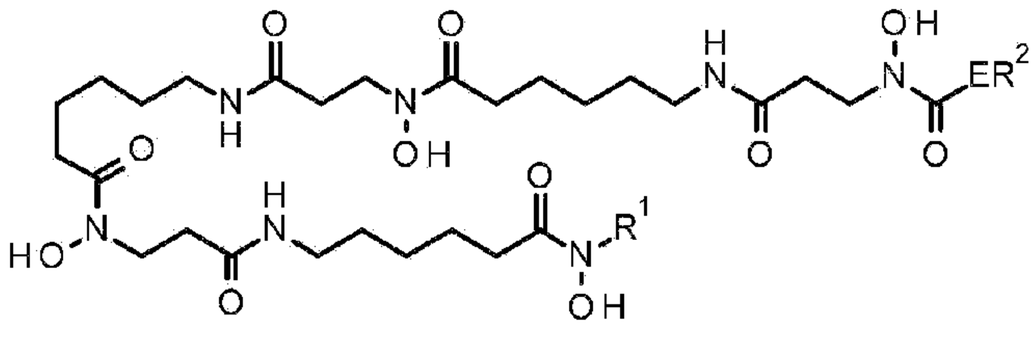
R^1 is methyl, ethyl or propyl, and

E is $(CH_2)_5$ NH-.

[0033] In one embodiment, the ligand is characterized by the following formula:



[0034] In another embodiment, the ligand is characterized by the following formula:



[0035] In certain embodiments, R^2 comprises, or is, an antibody, an oligopeptide, a polypeptide or protein, a polynucleotide, a liposome, a polymerosome, a phospholipid, a vitamin, a monosaccharide, an oligosaccharide, a nanoparticle, or a drug-like molecule having a molecular mass less than (<) 3000 U, any of which is selective for a disease specific ligand, a cell specific ligand or a tissue specific ligand.

[0036] In certain embodiments, R^2 is a vitamin selected from folic acid, biotin, retinol, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, ascorbic acid, cyanocobalamin, cholecalciferol, tocopherol, and phylloquinone.

[0037] In certain embodiments, R^2 comprises, or is, a biotin molecule. Biotin may be bound to the ligand by conjugation to an amine, sulfhydryl and/or OH via its valeric acid side chain. Noncovalent binding of biotin to avidin or streptavidin, which in turn is covalently linked to a high-specificity ligand such as an antibody, can be employed to enhance the versatility of the ocatadentate ligand of the invention, for example for use in radionuclide-based diagnosis and therapy. In certain embodiments, R^2 comprises an avidin or streptavidin molecule conjugated to the ligand allowing for binding non-covalently to biotin-labelled molecules.

[0038] In certain embodiments, R^2 is an OH, SH, COOH, SCN, activated ester, or an ene-one-

system (an alpha, beta unsaturated carbonyl, also referred to as a Michael acceptor system).

[0039] In certain embodiments, R^2 comprises, or is, one partner of two partners forming a so-called click reaction couple. In such embodiments, R^2 is a first click moiety capable of forming a covalent bond selectively with a second click moiety R^3 under reaction conditions not leading to a covalent reaction of R^2 or R^3 with natural occurring polypeptides, in particular with proteins. The click reactive groups are meant to conjugate the ligand to molecules of interest and at the same time provide the possibility of novel pre-targeting approaches. In certain embodiments, R^2 is selected from an azide, an alkyne, a tetrazine, a cyclooctine and a trans-cyclooctene, Suitable click reaction partners are well known in the art.

[0040] In certain embodiments, R^2 comprises, or is, a drug like moiety having a molecular mass of more then 160 u but less than 1000 u, less than 700 u, or less than 500 u, comprises up to five hydrogen bond donators (e.g., oxygen and or nitrogen atoms with one H attached), up to ten hydrogen bond acceptors (e.g., oxygen or nitrogen atoms) and an octanol-water partition coefficient logP of below 5.6 (any of these characteristics applied to the isolated R^2 moiety, without regard to the rest of compound I). These are the so-called "Lipinski" rules of 5 (originally, referring to molecules between 160 and 500 u) for drug-like compounds.

[0041] In certain embodiments, R^2 comprises, or is, an antibody, an antibody fragment, an antibody-like molecule, an oligopeptide or a nucleic acid aptamer molecule of 10 to 75 nucleotides in length, capable of binding to a cell specific or tissue specific target, particularly a cancer specific target.

[0042] An antibody fragment may be a Fab domain or an Fv domain of an antibody, or a single-chain antibody fragment, which is a fusion protein consisting of the variable regions of light and heavy chains of an antibody connected by a peptide linker. The R^2 moiety may also be a single domain antibody, consisting of an isolated variable domain from a heavy or light chain. Additionally, an antibody may also be a heavy-chain antibody consisting of only heavy chains such as antibodies found in camelids. An antibody-like molecule may be a repeat protein, such as a designed ankyrin repeat protein (Molecular Partners, Zurich).

[0043] Methods for generating antibodies against cell specific or tissue specific targets are known in the art. They include, for example, immunization of mice with human isolated targets, or immunization of mice with expression vectors encoding the target (DNA immunization).

[0044] Suitable R^2 moieties according to the above aspect of the invention may also be developed by evolutive methods such as phage display, ribosome display or SELEX, wherein polypeptides or oligonucleotides are selected due to their binding affinity to a target of interest. Additionally, the binding affinity of an identified R^2 moiety may be improved by cycles of evolution of the amino acid sequence or nucleotide sequence and selection of the evolved R^2

moiety may be effected based on the required affinity.

[0045] In embodiments where R^2 comprises an oligopeptide, R^2 may be a peptide that can bind to a recognition site of a receptor situated on the surface of a tissue-specific cell.

[0046] Alternatively, R^2 may comprise a soluble polypeptide comprising a contiguous amino acid sequence of at least 8 amino acid residues taken from the protein sequence of a soluble polypeptide ligand of a cell- or tissue specific receptor, wherein said soluble polypeptide binds to said receptor with a dissociation constant of 10^{-6} mol/l or lower, particularly with a dissociation constant of $\leq 10^{-8}$ mol/l.

[0047] In certain embodiments, R^2 comprises, or is, a monosaccharide consisting of three to seven carbon atoms. Examples are glyceraldehyde (C3), erythrose or threose (C4), arabinose, ribose or xylose (C5) glucose, mannose, galactose or fructose (C6) or sedoheptulose (C7). The sugar alcohols and amino sugars of C3 to C7 monosaccharides are included in the group of monosaccharides according to the definition used herein.

[0048] An oligosaccharide is a molecule consisting of two to ten of the same or different monosaccharides according to the above definition. A polysaccharide comprises more than ten monosaccharides.

[0049] In certain embodiments, R^2 comprises, or is, a vesicle-like molecule enclosing a solution comprising nutrients and/or drugs, which is used for administration of said nutrients and/or drugs (see Mishra et al., Journal of Biomedical Materials Research, Part A (2013), 101A(12), 3646-3660).

[0050] In certain embodiments, R^2 comprises, or is, a lipid structure with amphipathic character used as vesicular carrier for enhanced delivery of nutrients and/or drugs, thereby improving bioavailability and reducing toxicity.

[0051] In certain embodiments, R^2 is an alkyne or azide moiety, an alkyne, a tetrazine, a cyclooctine, a trans-cyclooctene, a carboxy group, an amino group, iodine, bromine, chlorine, succinimide, thiol group, a cyclooctyne moiety, biotin, avidin, or streptavidin.

[0052] In certain embodiments, R^2 is a nanomaterial, more particularly a nanoparticle. Nanoparticles may be selected from gold, silica, lipids, polymeric, metal or metal oxide compositions, wherein said metals are selected from iron, manganese, or titanium. The sizes of nanoparticles cover a range between 1 and 500 nanometers. They have unique physicochemical properties due to their large surface area to mass ratio and high activity, which differs from bulk materials of the same composition. For diagnostic and therapeutic applications, nanoparticles may be covalently linked to amino acids, antibodies, aptamers, avidin, streptavidin, peptides, polypeptides, polynucleotides, and/or nucleotides, which

specifically bind to their biological targets. Drug-coated or liposomal nanoparticles may be used as carrier particles for targeted drug delivery. Metal oxide nanoparticles conjugated to radioisotopes enable monitoring of their biodistribution in vivo.

[0053] "Nucleotides" in the context of the present specification are nucleic acid or nucleic acid analogue building blocks, oligomers of which are capable of forming selective hybrids with DNA or RNA oligomers or artificial nucleic acid oligomers on the basis of base pairing. The term nucleotides in this context includes the classic ribonucleotide building blocks adenosine, guanosine, uridine (and ribosylthymin), cytidine, the classic deoxyribonucleotides deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine and deoxycytidine. It further includes analogues of nucleic acids such as phosphotioates, 2'O-methylphosphothioates, peptide nucleic acids (PNA; N-(2-aminoethyl)-glycine units linked by peptide linkage, with the nucleobase attached to the alpha-carbon of the glycine) or locked nucleic acids (LNA; 2'O, 4'C methylene bridged RNA building blocks). The hybridizing sequence may be composed of any of the above nucleotides, or mixtures thereof.

Targeting moieties:

[0054] In certain embodiments, R^2 is bombesin (CAS No. 31362-50-2). In certain embodiments, R^2 comprises a functional homologue of bombesin, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 1 (bombesin)
SEQ ID NO 1: QQRLGNQWAVGHLM

[0055] In certain embodiments, R^2 is somatostatin (CAS No. 38916-34-6). In certain embodiments, R^2 comprises a functional homologue of somatostatin, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 2 (somatostatin).
SEQ ID NO 2:
MLSCRLQCALAALSIVLALGCVTGAPSDPRLRQFLQKSLAAAAGKQELAKYFLAELLSEPNQ
TENDALEPEDLSQAAEQDEMRLELQRSANSNPAMAPRERKAGCKNFFWKTFTSC

[0056] In certain embodiments, R^2 is gastrin (UniProt ID: P01350). In certain embodiments, R^2 comprises a functional homologue of gastrin, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 3 (gastrin).
SEQ ID NO 3:
MQRLCVYVLIFALALAAFSEASWKPRSQQPDAPLGTGANRDLELPWLEQQGPASHHRRQL
GPQGPPHLVADPSKKQGPWLEEEEEAYGWMDFGRRSAEDEN

[0057] In certain embodiments, R^2 comprises a peptide sequence of trans-activator of transcription protein (tat) (Pfam ID: PF00539). In certain embodiments, R^2 comprises the

amino acid sequence of the protein transduction domain identical to SEQ ID NO 4 (tat peptide transduction domain).
SEQ ID NO 4: YGRKKRRQRRR

[0058] In certain embodiments, R^2 is prostate-specific antigen (PSA) (UniProt ID: P07288). In certain embodiments, R^2 comprises a functional homologue of PSA, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 5 (PSA).
SEQ ID NO 5:
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWV
LTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLM
LLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVC
AQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLY
TKVVHYRKWIKDTIVANP

[0059] In certain embodiments, R^2 is neuropeptide Y (NPY) (UniProt ID: P01303). In certain embodiments, R^2 comprises a functional homologue of NPY, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 6 (NPY).
SEQ ID NO 6:
MLGNKRLGLSGLTLALSLLVCLGALAEAYPSKPDNPGEDAPAEDMARYYSALRHYINLITRQ
RYGKRSSPETLISDLLMRESTENVPRTRLEDPAMW

[0060] In certain embodiments, R^2 is octreotide (CAS No. 83150-76-9), also known as "Sandostatin" (Novartis Pharmaceuticals), identical to SEQ NO 7. Octreotide is an octapeptide that pharmacologically mimics natural somatostatin, though it is a more potent inhibitor of growth hormone, glucagon, and insulin than somatostatin.
SEQ ID NO 7: FCFWKYCT-ol (Disulfide bridge: 2-7)

[0061] In certain embodiments, R^2 is gastric inhibitory polypeptide (GIP) (UniProt ID: P09681). In certain embodiments, R^2 comprises a functional homologue of GIP, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 8 (GIP).
SEQ ID NO 8:
MVATKTFALLLLSLFLAVGLGEKKEGHFSALPSLPVGSHAKVSSPQPRGPRYAEGTFISDYSI
AMDKIHQQDFVNWLLAQKGKKNDWKHNITQREARALELASQANRKEEEAVEPQSSPAKNP
SDEDLLRDLLIQELLACLLDQTNLCRLRSR

[0062] In certain embodiments, R^2 is neurokinin A (NKA) (UniProt ID: P20366). In certain embodiments, R^2 comprises a functional homologue of NKA, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 9 (NKA).
SEQ ID NO 9:
MKILVALAVFFLVSTQLFAEEIGANDDLNYWSDWYDSDQIKEELPEPFEHLLQRIARRPKPQ

QFFGLMGKRDADSSIEKQVALLKALYGHGQISHKRHKTDSFVGLMGKRALNSVAYERSAM
QNYERRR

[0063] In certain embodiments, R^2 is neurotensin (UniPro ID: P30990). In certain embodiments, R^2 comprises a functional homologue of neurotensin, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 10 (NKA).
SEQ ID NO 10:
MMAGMKIQLVCMLLLAFSSWSLCSDSEEEMKALEADFLTNMHTSKISKAHVPSWKMTLLNV
CSLVNNLNSPAEETGEVHEEELVARRKLPTALDGFSLEAMLTIYQLHKICHSRAFQHWELIQ
EDILDTGNDKNGKEEVIKRKIPYILKRQLYENKPRRPYILKRDSYYY

[0064] In certain embodiments, R_2 is exendin-3 (CAS No. 130357-25-4). In certain embodiments, R_2 comprises a functional homologue of exendin-3, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 11 (exendin-3).
SEQ ID NO 11: HGGGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2

[0065] In certain embodiments, R^2 is exendin-4 (CAS No. 141758-74-9). In certain embodiments, R^2 comprises a functional homologue of exendin-4, having an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, 99% identity to SEQ ID NO 12 (exendin-4).SEQ ID NO 12: HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2

[0066] In certain embodiments, R^2 is substance P (SP) comprising the amino acid sequence identical to SEQ ID NO 13 (substance P).
SEQ ID NO 13: RPKPQQFFGLM

[0067] According to a second aspect of the invention, a compound formed by complex formation between the ligand according to the first aspect of the invention and a metal atom is provided. The metal atom is coordinatively bound to the D moieties of the ligand. In certain embodiments, the metal atom is octa-coordinated, i.e. eight atoms of the ligand collaborate in complex formation with the atom (the coordination number is 8), particularly where the metal is Zr or Y, more particularly where the metal is ^{89}Zr or ^{90}Y.

[0068] In certain embodiments, the metal atom is hexa-coordinated, i.e. six atoms of the ligand collaborate in complex formation with the atom, particularly where the metal is Ga or In, more particularly where the metal is ^{111}In, ^{67}Ga or ^{68}Ga. In certain embodiments, the metal atom is tetra-coordinated. In certain embodiments, the metal atom is penta-coordinated.

[0069] In certain embodiments, the metal has the oxidation number +1, +2, +3, +4, + 5, +6, or +7. In certain embodiments, the metal has the oxidation number +3, +4, +5 or +6. In certain embodiments, the metal has a coordination number from 4 to 8.

[0070] In certain embodiments, the metal is comprised in any one of the groups 3, 4, 6, 7, 9, 10, 11, 13, 15, the lanthanide group of elements, or the actinide group of elements of the periodic table of the elements. Groups are assigned in accordance to current IUPAC practice, older designations refer to the "scandium group" (IIIA) for group 3, "titanium group" (IVA) for group 4, "actinides group" for group 6, "manganese group" for group 7, "lanthanides group" comprising group, 9, 10, and 11, "boron group" for group 13, and "nitrogen group" for group 15.

[0071] In certain embodiments, the metal is selected from zirconium (Zr), yttrium (Y), gallium (Ga), technetium (Tc), indium (In), copper (Cu), terbium (Tb), scandium (Sc), lutetium (Lu), rhenium (Re), bismuth (Bi), europium (Eu), gold (Au), iron (Fe), magnesium (Mg) or gadolinium (Gd).

[0072] In certain embodiments, the metal is a metallic radionuclide. A radionuclide, or a radioactive nuclide, is an atom with an unstable nucleus, which undergoes radioactive decay, resulting in the emission of gamma ray(s) or subatomic particles such as positrons, alpha or beta particles, or Auger electrons. These emissions constitute ionizing radiation. Radionuclides occur naturally, or can be produced artificially. Radionuclides are often referred to as radioactive isotopes or radioisotopes.

[0073] In certain embodiments, the metal is selected from ^{44}Sc, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{99m}Tc, ^{111}In, ^{nat}Gd, ^{nat}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, or ^{213}Bi. In certain embodiments, the metal is ^{67}Ga, ^{68}Ga, ^{89}Zr, or ^{90}Y.

[0074] In certain embodiments, the complex is an octadentate complex and the metal is selected from ^{67}Ga, ^{68}Ga, ^{89}Zr, or ^{90}Y.

[0075] The invention is not limited to octadentate coordinated complexes. In certain embodiments, the complex may comprise Ga or In and may be 6-coordinate. In certain embodiments, the complex may comprise Zr or Y and may be 8-coordinate. In certain embodiments, the complex may comprise Tc and may be 4-coordinate. Even if a metal does not require all coordination sites provided by the ligand system, the ligand will increase the stability of the complex simply by providing higher "concentration" of coordinating groups in the near vicinity of the metal which will protect it from trans-chelation.

[0076] According to a third aspect of the invention, a method for the synthesis of a complex according to the second aspect of the invention is provided, wherein a ligand according to the first aspect of the invention is reacted with a metal, particularly a metal of group 3, 4, 6, 7, 9, 10, 11, 13, 15, the lanthanide group of elements, or the actinide group of elements of the periodic table is reacted with the ligand.

[0077] In certain embodiments, the complex is formed by providing a ligand according to the

invention, and adding a metal. In certain embodiments, the metal is selected from Ga, Tc, In, Cu, Tb, Sc, Lu, Re, Bi, Eu, Gd, Zr, or Y.

[0078] In certain embodiments, the metal is selected from the isotopes ^{67}Ga, ^{68}Ga, ^{89}Zr, or ^{90}Y.

[0079] In certain embodiments, the complex is isolated after synthesis by high-performance liquid chromatography (HPLC).

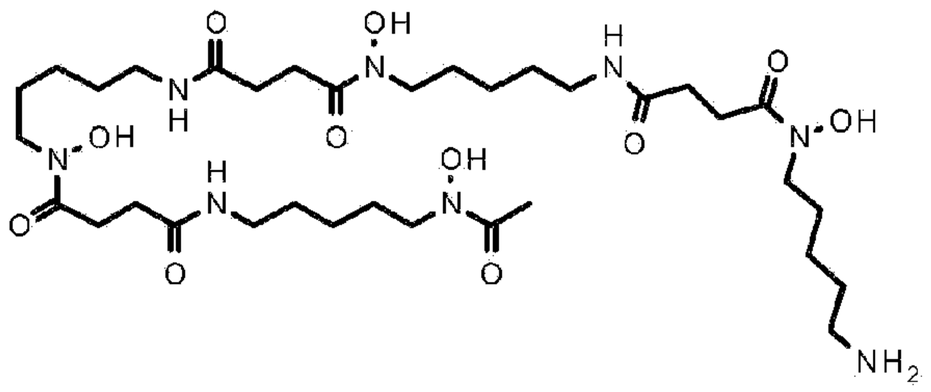
[0080] In certain embodiments, an octa co-ordinated complex I (b) is formed by reaction of a ligand I (a) with a metal comprised in any one of the groups 3, 4, 6, 7, 9, 10, 11, 13, or 15 of the periodic table of the elements. In certain embodiments the metal is zirconium. In certain embodiments, said complex I is formed by reaction of said ligand (a) with zirconium chloride ($ZrCl_4$) (CAS No. 10026-11-6). Figure 2 shows the calculated structure of the octa co-ordinated complex I.

[0081] In another embodiment, an octa co-ordinated complex II (d) is formed by reaction of a ligand II (c) with a metal comprised in any one of the groups 3, 4, 6, 7, 9, 10, 11, 13, or 15 of the periodic table of the elements. In certain embodiments the metal is zirconium. In certain embodiments, said complex II is formed by reaction of said ligand II with zirconium (IV) acetylacetonate (CAS No. 10026-11-6).

[0082] In yet another embodiment, ligand III (e) is synthesised from a peptide precursor compound. In certain embodiments, said peptide precursor compound is a modified amino acid sequence of bombesin.
SEQ ID NO 14: $(\beta\text{-A})_3$-QWAVGHL-14Nle-$CONH_2$

[0083] In certain embodiments, complex III is formed by reaction of said ligand III (e) with a metal comprised in any one of groups 3, 4, 6, 7, 9, 10, 11, 13, or 15 of the periodic table of the elements. In certain embodiments the metal is zirconium. In certain embodiments, ligand III is radiolabelled using commercial ^{89}zirconium oxalate. Figure 1 shows stability of complex III in comparison to a hexadentate ^{89}zirconium-complex.

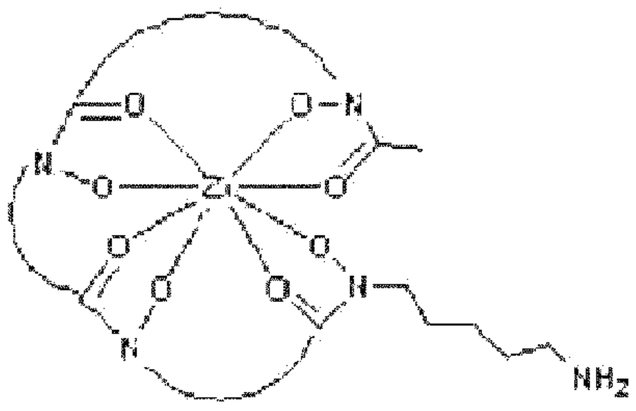
[0084] Particular embodiments of this aspect of the invention are:



ligand I

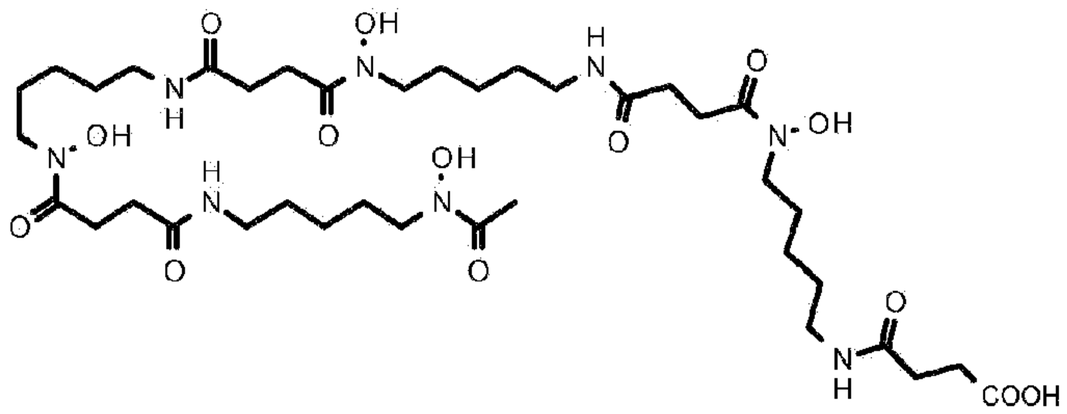
1. a. N-[5-[[4-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxo-butanoyl]-

hydroxyamino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentyl]-N'-(5-aminopentyl)-N'-hydroxy-butanediamide (ligand I) and its Zr complex I:



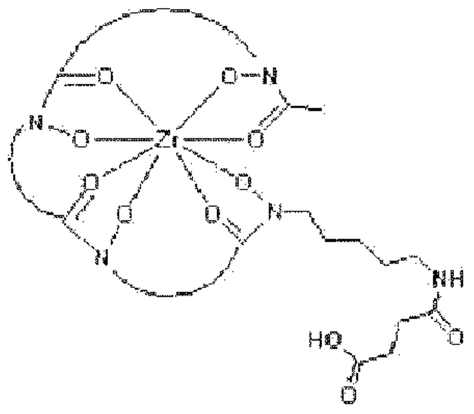
complex I

2. b. Octa co-ordinated complex I



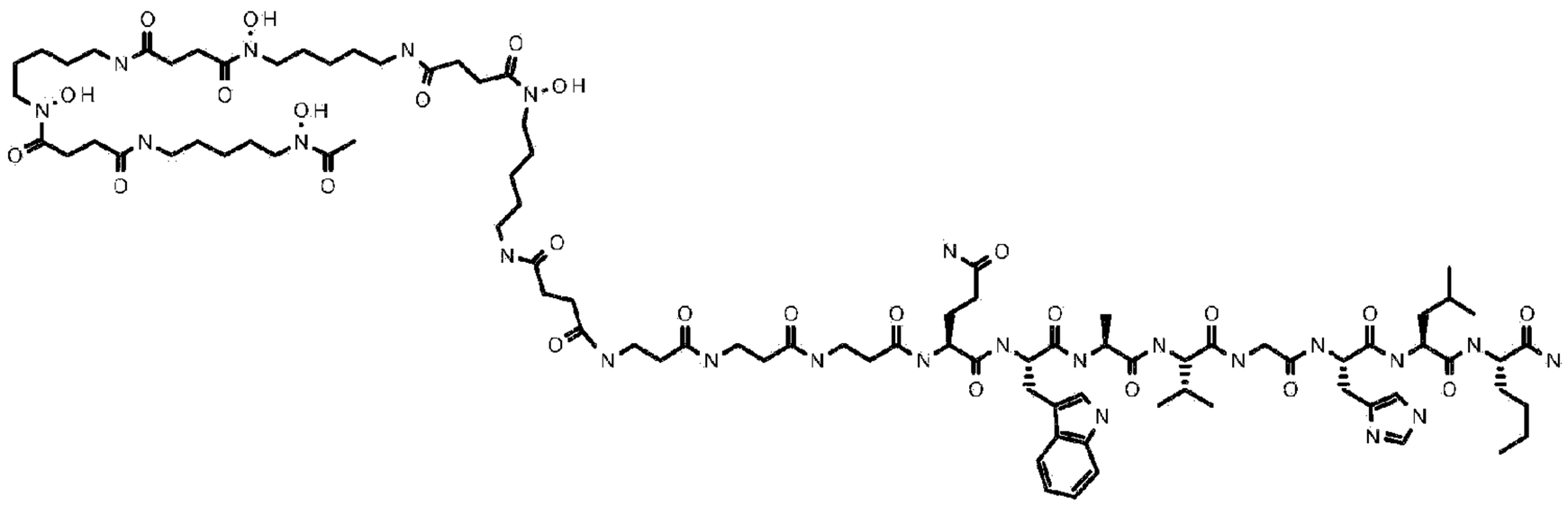
ligand II

3. c. 4-[5-[[4-[5-[[4-[5-[[4-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxo-butanoyl]-hydroxyamino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoic acid (ligand II) and its Zr complex II:



complex II

4. d. Octa co-ordinated complex II



(Ligand III) (amide hydrogens not shown)

5. e. (2S)-2-[3-[3-[3-[[4-[5-[[4-[5-[[4-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]amino]propanoylamino]propanoylamino]propanoylamino]-N-[(1S)-2-[[(1S)-2-[[1-[[2-[[(1S)-2-[[(1S)-1-[[(1S)-1-carbamoylpentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-imidazol-4-ylmethyl)-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-2-

methylpropyl]amino]-1-methyl-2-oxo-ethyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxoethyl]pentanediamide. (Ligand III)

6. f. Octa co-ordinated complex III (see structure under Example h).

[0085] Other particular embodiments relate to molecules comprising ligand I, II or III complexed to ^{89}Zr, and covalently linked to any of the above named targeting moieties.

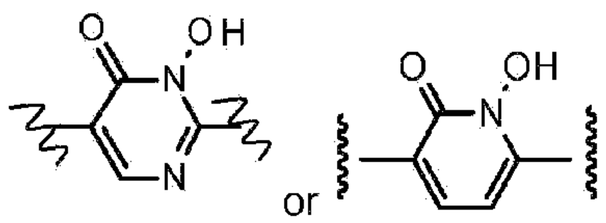
[0086] According to an alternative aspect, the invention provides a ligand characterized by the features as outlined in the following items:

Item 1: a ligand characterized by a general formula I:

$$R^1\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}E\text{-}R^2 \qquad (I),$$

wherein

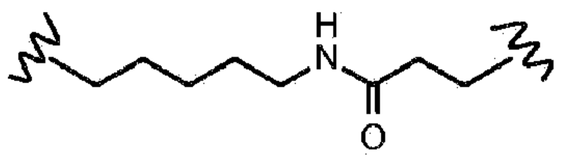
- D is



- each X independently of any other X is a saturated or partially unsaturated, substituted or unsubstituted linker comprising a chain of 9, 10 or 11, in some embodiments particularly 9 atoms selected from any of N, C, O, in particular CH_2, NH, CO, O, with R being H or C_1-C_4 alkyl, or each X independently of any other X is a saturated or unsaturated, substituted or unsubstituted heterocyclic moiety,
- R^1 is or comprises a C_1-C_5 alkyl, a C_3-C_6 cycloalkyl, an arene, and/or a heteroarene,
- E is a saturated or partially unsaturated, substituted or unsubstituted linker comprising a chain comprising 1 - 50 atoms and
- R^2 is
    1. a) an OH, SH, COOH, SCN, activated ester, or an ene-one-system ;
    2. b) a first click moiety capable of forming a covalent bond selectively with a second click moiety R^3 under reaction conditions not leading to a covalent reaction of R^2 or R^3 with natural occurring polypeptides, in particular with proteins, or
    3. c) a targeting moiety that specifically binds to a target site on cells and/or tissues with an association constant of lower than (<) 10^{-6} mol/l, $<10^{-7}$ mol/l, $<10^{-8}$ mol/l or $<10^{-9}$ mol/l, particularly an antibody, an oligopeptide, a polypeptide, a polynucleotide, a liposome, a polymerosome, a phospholipid, a vitamin, a monosaccharide, an oligosaccharide, a nanoparticle, or a drug-like molecule having a molecular weight less than (<) 3000 U, or
    4. d) a solid support.

Item 2: The ligand according to item 1, wherein

- R^2 is
    1. i. an alkyne or azide moiety, an alkyne, a tetrazine, a cyclooctine, a trans-cyclooctene, a carboxy group, an amino group, iodine, bromine, chlorine, succinimide, thiol group, a cyclooctyne moiety, biotin, avidin, or streptavidin,
    2. ii. an antibody, a polypeptide, a polypeptide, a polynucleotide, a liposome, a polymerosome, a phospholipid, a vitamin, a monosaccharide, an oligosaccharide, a nanoparticle, or a drug-like molecule having a molecular weight less than (<) 3000 U;
- each X is



,

- R^1 is methyl, ethyl or propyl and
- E is $(CH_2)_5$ - NH-.

Item 3: The ligand according to any one of the above items,

wherein R^2 comprises a polypeptide being a functional homologue of bombesin, somatostatin, gastrin, trans-activator of transcription peptide, prostate-specific antigen, neuropeptide Y, octreotide, gastric inhibitory polypeptide, neurokinin A, neurotensin, exendin-3, exendin-4, or substance P, said peptide comprising an amino acid sequence of at least 80%, 85%, 90%, 95%, 98%, or 99% identity to SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, or SEQ ID NO 13, respectively.

Item 4: A complex comprising a ligand according to any one of the above items, coordinatively bound to a metal atom, particularly a metal comprised in group 3, 4, 6, 7, 9, 10, 11, 13, 15, the lanthanide group of elements, or the actinide group of elements of the periodic table.

Item 5: The complex according to item 4, wherein the metal is selected from Ga, Tc, In, Cu, Tb, Sc, Lu, Re, Bi, Eu, Gd, Zr, or Y.

Item 6: The complex according to any one of items 4 or 5, wherein the metal is one of ^{44}Sc, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{99m}Tc, ^{111}In, ^{nat}Eu, ^{nat}Gd, ^{177}Lu, ^{161}Tb, ^{186}Re, ^{188}Re, or ^{213}Bi.

Item 7: The complex according to any one of items 4 to 6, wherein the metal

- has the oxidation number +1, +2, +3, +4, +5, +6 or +7, and/or
- forms an 8-coordinate complex with the ligand.

Item 8: A complex according to any one of items 4 to 7 for use as a pharmaceutical, particularly for use in a method of treatment of neoplastic disease (cancer).

Item 9: A complex according to any one of items 4 to 7, for use in a method of medical diagnosis or radio-immunotherapy, particularly for use in positron emission tomography and/or

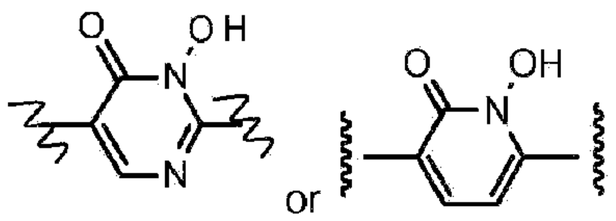
single-photon emission computed tomography.

Item 10: A complex according to any one of item 4 to 7 for use in diagnosis of neoplastic disease.

Item 11: A solid support comprising a ligand covalently attached to said solid support, said ligand being characterized by a general formula I

$$R^1\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}E\text{-}R^2 \qquad (I)$$

- wherein D is



- each X independently of any other X is a saturated or partially unsaturated, substituted or unsubstituted linker comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11, in some embodiments particularly 9 atoms selected from any of N, C, O, in particular CH_2, NH, CO, O, with R being H or C_1-C_4 alkyl, or each X independently of any other X is a saturated or unsaturated, substituted or unsubstituted heterocyclic moiety,
- R^1 comprises a C_1-C_5 alkyl, a C_3-C_6 cycloalkyl, an arene, and/or a heteroarene, and
- E is a saturated or partially unsaturated, substituted or unsubstituted chain comprising 1 - 50 atoms and R^2 is the attachment site to said solid support.

Item 12: The solid support according to item 11, wherein said solid support is a nanoparticle.

[0087] In certain embodiments, the complex is purified via liquid chromatography, high-performance liquid chromatography (HPLC), size exclusion chromatography (gel permeation chromatography), affinity chromatography, or ion exchange chromatography.

[0088] According another aspect of the invention, the complex according to the invention is used as a diagnostic, particularly for cancer diagnostics.

[0089] In certain embodiments, the complex is used in radioimmunotherapy.

[0090] In certain embodiments, the complex comprising ^{44}Sc, ^{67}Ga, ^{68}Ga, ^{89}Zr ^{nat}Eu, and ^{nat}Gd, ^{nat}Fe is provided for use in diagnostic imaging, particularly for cancer diagnostics.

[0091] In certain embodiments, the complex is used in positron emission tomography or in single-photon emission computed tomography (SPECT or SPET).

[0092] In radioimmunotherapy, an antibody or an antibody fragment, which is labelled with a

radionuclide, is used to deliver cytotoxic radiation to a target cell or to image a cell for targeting by an external radiation source. In its specific application to cancer therapy, an antibody, which is specific for a tumour-associated antigen, is used to deliver a lethal dose of radiation to the tumour cells or to specifically image radiation dosimetry at the tumour cell. Radioimmunotherapy requires a tumour cell to express an antigen that is unique to the neoplasm or that is not accessible in normal cells.

[0093] According another aspect of the invention, the complex according to the invention is used as a pharmaceutical.

[0094] In certain embodiments, the complex is used in cancer therapy.

[0095] In certain embodiments, the complex is conjugated to receptor-specific molecules comprising an antibody, an oligopeptide, a polypeptide, or a small molecule compound for targeting cancer-type specific receptors and/or receptors overexpressed in certain cancer types.

[0096] Breast cancer-specific receptors comprise estrogen / progesterone receptor and human epidermal growth factor 2 receptor (HER2). In certain embodiments, the complex is conjugated to estrogen receptor agonists comprising "indene-based molecules". These molecules have a common "indene" building block as part of their chemical structure. In certain embodiments, the complex is conjugated to molecules selected from 17-beta-estradiol, estrone, raloxifene, estriol, genistein, the monoclonal antibody trastuzumab known as "Herceptin" (Roche), or selective estrogen receptor modulators (SERMs).

[0097] Pancreatic cancer and lung cancer are characterised by overexpression of various specific receptors and polypeptides. In certain embodiments, the complex is conjugated to an epidermal growth factor receptor (EGFR)-specific polypeptide or polypeptides comprising epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), the EGFR antagonist potato carboxypeptidase inhibitor (PCI), amphiregulin, insulin-like growth factor 1 (IGF1), or insulin-like growth factor 2 (IGF2). In certain embodiments, the complex is conjugated to compounds comprising gefitinib (CAS No. 184475-35-2), erlotinib (CAS No. 183321-74-6), erlotinib-hydrochloride (CAS No. 183319-69-9), cetuximab (CAS No. 205923-56-4), rituximab or compounds capable of specifically binding to insulin-like growth factor receptors (IGFRs).

[0098] Gastric cancer-specific receptors comprise HER2 and CXC chemokine receptors (CXCR1 to CXCR7). In certain embodiments, the complex is conjugated to HER2-specific monoclonal antibodies comprising trastuzumab (CAS No. 180288-69-1) or pertuzumab (CAS No. 380610-27-5). In certain embodiments, the complex is conjugated to CXC chemokine receptor-specific agonists or antagonists.

[0099] Prostate cancer development and progression has been shown to correlate with overexpression of androgen receptor (AR) and/or epidermal growth factor receptor (EGFR). In

certain embodiments, the complex is conjugated to prostate-specific antigen (PSA) or to an antibody capable of specifically binding to extracellular signal-regulated protein kinases (ERKs). It has also been shown that some prostate cancer types develop and progress independently of androgen receptor signalling. In another embodiment, the complex is conjugated to an antibody capable of specifically binding to B-cell lymphoma 2 (BCL-2) (UniProt ID: P10415) for targeting androgen-independent prostate cancer cells in patients. In certain embodiments, the complex is conjugated to polypeptides or compounds capable of specifically binding to vitronectin receptors (Alpha-v beta-3) (UniProt ID: P04004), selected from angiogenesis inhibitors comprising vitaxin (CAS No. 892553-42-3), or bevacizumab (CAS No. 216974-75-3). In certain embodiments, the complex is conjugated to glycosylated RGD-containing peptides (RGD-peptides) (Haubner et al., J. Nucl. Med. 2001, 42(2), 326-336; Zhao et al., Plos ONE 8(4), 2013, e61043; Zhang et al., Cancer Res. 2007, 67(4), 1555-1562).

[0100] Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. These enzymes can break down connective tissue and overexpression of MMPs has been observed in pathological conditions comprising inflammation, cancer invasion, metastasis and angiogenesis. In certain embodiments, the complex is conjugated to a matrix metalloproteinase inhibiting compound, in particular an MMP inhibitor selected from the group comprising marimastat (CAS No. 154039-60-8), doxycycline (CAS No. 24390-14-5), or cipemastat (CAS No. 190648-49-8). In certain embodiments, the complex is conjugated to specific tissue inhibitors of metalloproteinases (TIMPs). TIMPs are polypeptides comprising TIMP1, TIMP2, TIMP3, and TIMP4.

[0101] Positron emission tomography (PET) is a functional imaging technique applied in nuclear medicine, whereby a three-dimensional image (e.g. of functional processes) in the body is produced. The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide, which is introduced into the body in form of a pharmaceutical compound.

[0102] Similar to PET, single-photon emission computed tomography (SPECT) is also applied as a three-dimensional diagnostic imaging technique using gamma rays emitted by radioisotopes. In contrast with PET, the tracer used in SPECT emits gamma radiation that is measured directly, whereas a PET tracer emits positrons that annihilate with near-by electrons, which are a few milimeters away, causing two gamma photons to be emitted in opposite directions. Therefore, PET scanners detect emissions that occur coincidently in time, thereby providing more radiation event localisation information and thus resulting in higher resolution images compared to SPECT.

[0103] Similarly, a dosage form comprising a complex according to the present invention is provided. In certain embodiments, the dosage form is for intravenous administration or subcutaneous administration.

[0104] Wherever alternatives for single separable features such as, for example, a metal or radionuclide, ligand element or medical indication are laid out herein as "embodiments", it is to be understood that such alternatives may be combined freely to form discrete embodiments of

the invention disclosed herein. Thus, any of the alternative embodiments for a D or X may be combined with any of the alternative embodiments of metal or radionuclide, and these combinations may be combined with any instance of R^3 mentioned herein.

[0105] The invention is further illustrated by the following examples and figures, from which further embodiments and advantages can be drawn. These examples are meant to illustrate the invention but not to limit its scope.

Short description of the figures

[0106]

Fig. 1

shows a stability profile of the octa co-ordinated complex III (ligand-conjugated complex; top two lines) in comparison to the hexadentate complex known in the art (DFO-conjugated complex; bottom two curves). The graph shows results of competition experiments using 300-fold excess of free DFO in a 24 hour time (Challenging against 0.1 mM DFO-mesylate in 0.5 M HEPES (pH 7.3)). X axis: time; Y axis: percentage of intact complex.

Fig. 2

shows the calculated structure of the complex formed by reaction of ligand I with zirconium chloride. The atoms are depicted in different colours comprising zirconium (center), oxygen (gray), nitrogen (white with bold line), and carbon (white).

Fig. 3

shows the synthesis of complex III

General Methods

[0107] Materials: All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over molecular sieves. All preparations were carried out using standard Schlenk techniques. Instrumentation and methods: **Instrumentation and Methods.** *^1H and ^{13}C NMR spectra* were recorded in deuterated solvents on 400 (^{1}H: 400 MHz, ^{13}C: 100.6 MHz) or 500 (^{1}H: 500 MHz, ^{13}C: 126 MHz) MHz spectrometers at room temperature. The chemical shifts, δ, are reported in ppm (parts per million). The residual solvent peaks have been used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). The ^{1}H and ^{13}C signals were assigned with the help of 2D NMR techniques. *Elemental microanalyses* were performed on a LecoCHNS-932 elemental analyser. *ESI-MS* were performed using a

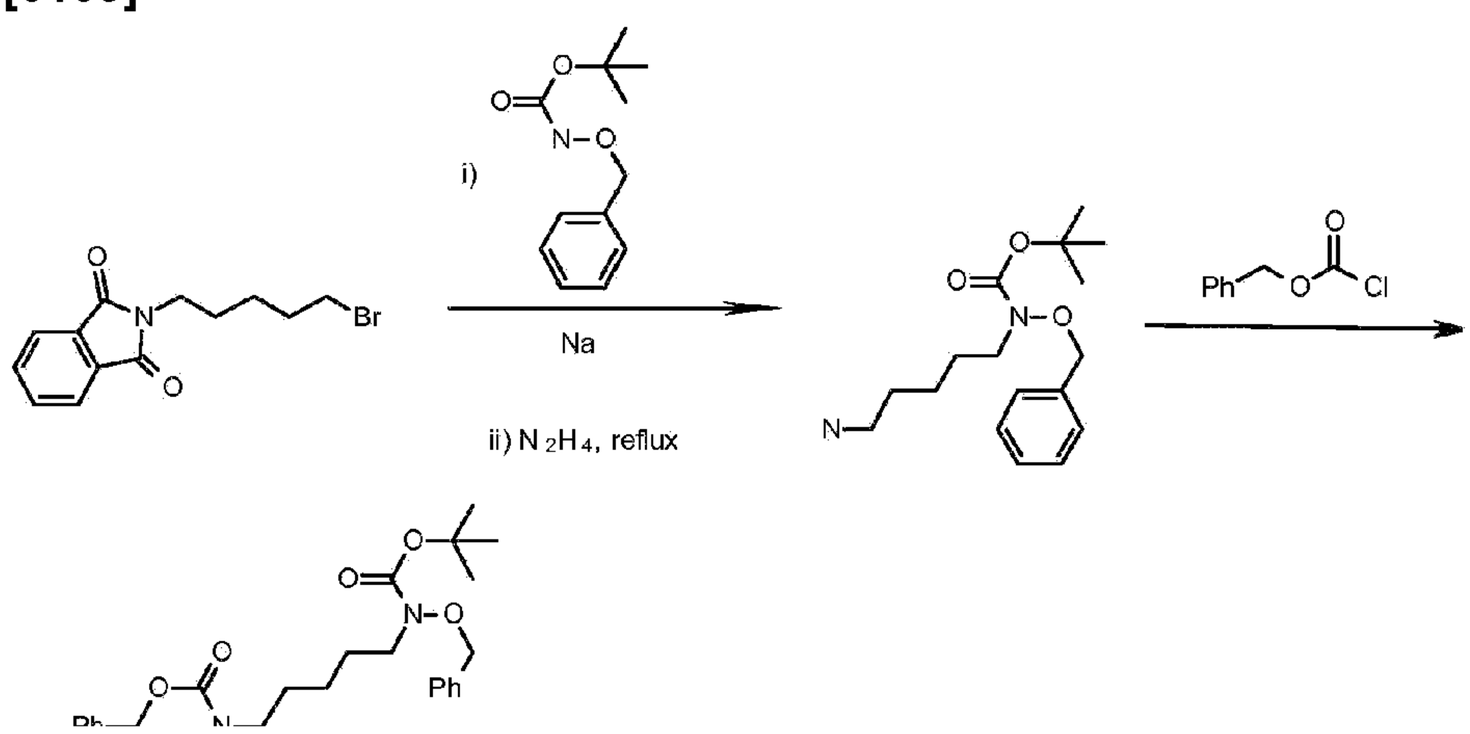
Bruker Daltonics HCT 6000 mass spectrometer. *LC-MS spectra* were recorded on a HPLC apparatus (*Acquity Ultra Performance LC, Waters*) that was connected to a mass spectrometer (Bruker Esquire 6000) operated in ESI mode. A Nucleosil 100-5 C18 (250 x 3 mm) reverse phase column was used with a flow rate of 0.5 mL min^{-1} and UV-absorption was measured at 254 nm. The runs were performed with a linear gradient of A (acetonitrile (Sigma-Aldrich HPLC-grade) and B (distilled water containing 0.02% TFA and 0.05% HCOOH): t = 0-3 min, 5% A; t = 17 min, 100% A; t = 20 min, 100% A; t = 21 min, 5% A; t = 25 min, 5% A.

[0108] Synthesis of the peptide precursor compound characterising a modified amino acid sequence of bombesin (SEQ ID NO. 14) was performed on an Applied Biosystem automatic peptide synthesizer using Rink Amide resin and standard Fmoc-protocoll. Analysis of peptide precursor, ligand I and ligand II were performed on a HPLC system using a Phenomenex Jupiter column, 4µ Proteo 90A, 250 x 4.6 mm, with a flow rate of 2 mL / min. Analysis of peptide precursor compound and ligand I were performed with a linear gradient of HPLC-grade solvent A (acetonitrile) from Sigma-Aldrich and B (distilled water containing 0.1% TFA). Peptide precursor: t = 0 min, 5% A; t = 12.5 min, 50% A; t = 13.5 min, 95% A; t = 14.5 min, 95% A; t = 15 min, 5% A; t = 17 min, 5% A. Ligand I: t = 0 min, 10% A; t = 12.5 min, 40% A; t = 13.5 min, 95% A; t = 14.5 min, 95% A; t = 15 min, 10% A; t = 17 min, 10% A. Ligand II: t = 0 min, 15% A; t = 12.5 min, 40% A; t = 13.5 min, 95% A; t = 14.5 min, 95% A; t = 15 min, 15% A; t = 17 min, 15% A. MS acquisitions were performed in the full scan mode in the mass range from m/z 100 to 2000 at 20'000 resolution and 1 scan per second. Masses were calibrated with a 2 mmol/l solution of sodium formate over m/z 158 to 1450 mass range with accuracy below 2 ppm.

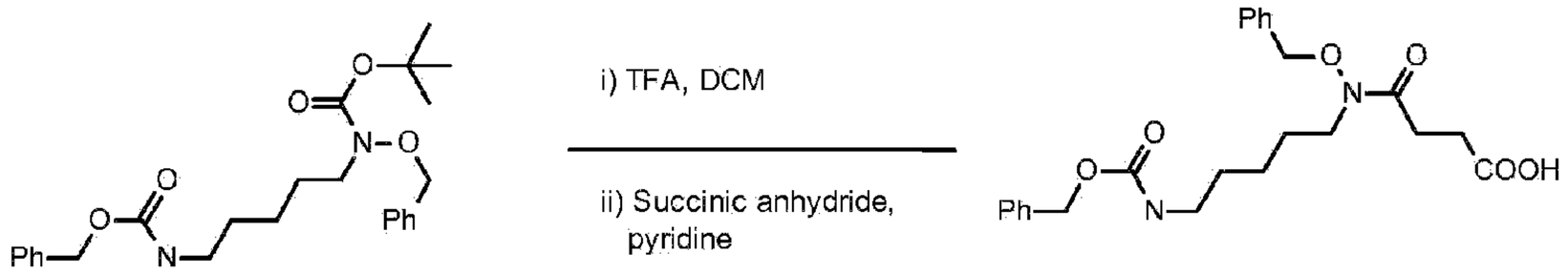
Examples

a. Synthesis of compound a (4-[benzyloxy-[5-(benzyloxycarbonylamino)pentyl]amino]-4-oxo-butanoic acid) was synthesised following the procedures described in *Na. Chem. Biol.*, 2007, 3, 652-656, and *Na. Chem. Biol.*, 2007, 3, 652-656.

[0109]



t-butyl N-benzyloxy-N-[5-(benzyloxycarbonylamino)pentyl]carbamate

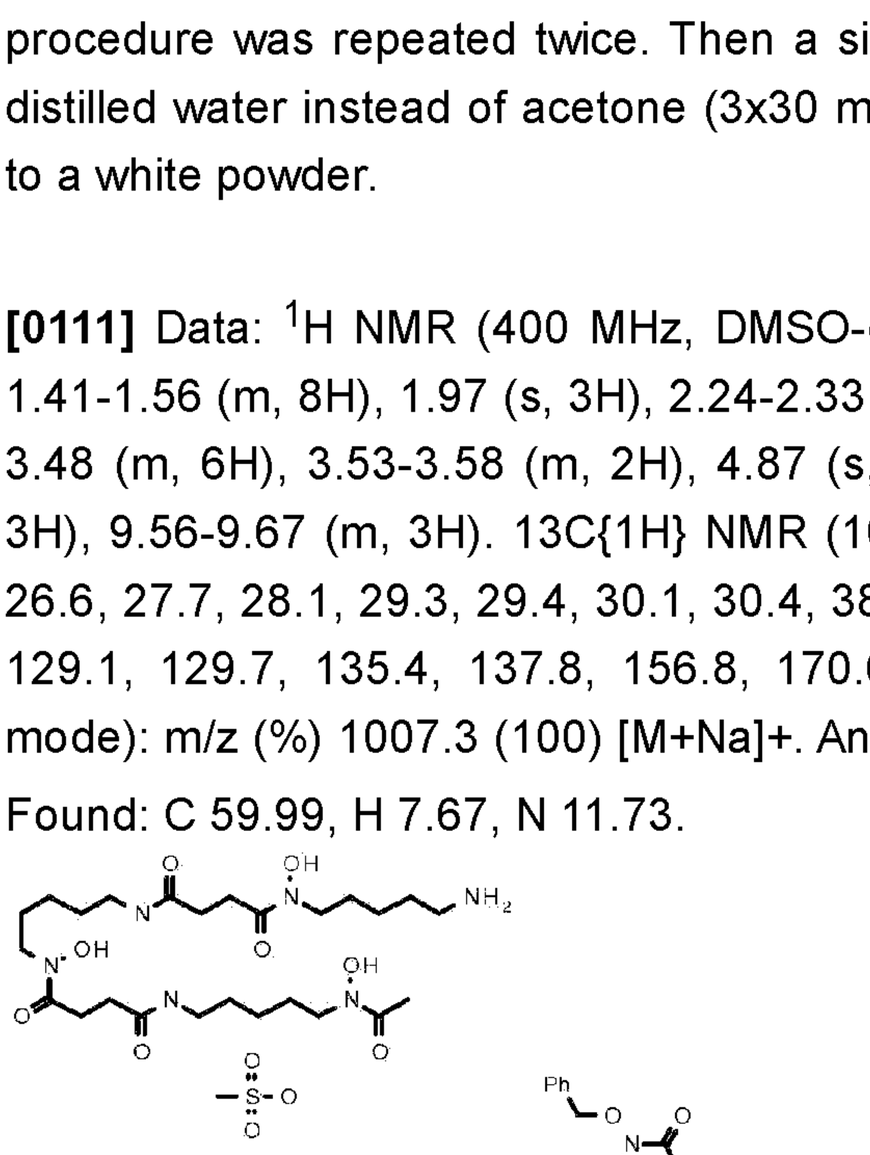


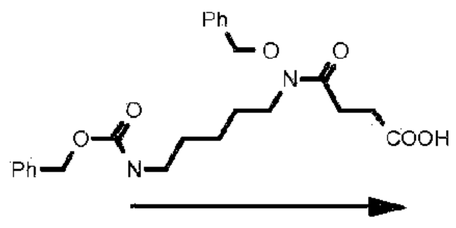
Compound a

b. Synthesis of compound b [(benzyl N-[5-[[4-[5-[[4-[5-[[4-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-hydroxy-amino]pentylamino]-4-oxo-butanoyl]-benzyloxyamino]pentyl]carbamate)]:

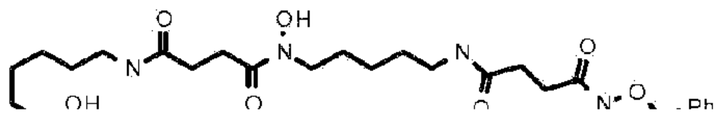
[0110] To a stirred solution of carboxylic acid (1.13 g, 2.6 mmol), addition of HATU (1.46 g, 3.8 mmol) in DMF (10 mL), and DIPEA (0.66 g, 5.1 mmol) was carried out under N_2 atmosphere. After stirring the mixture for 40 min at room temperature, deferoxamine mesylate salt (1.68 g, 2.6 mmol) followed by DIPEA (0.66 g, 5.1 mmol) and 4-methyl morpholine (2 mL) were added. The mixture was then stirred for 48 h at room temperature. The solvent was removed using a high vacuum pump. Addition of 50 mL ice cold acetone to the resulting paste resulted in a white solid, which was isolated by centrifugation, followed by decantation of the acetone. This procedure was repeated twice. Then a similar washing procedure was followed using double distilled water instead of acetone (3x30 mL). The wet white solid was lyophilized to give result to a white powder.

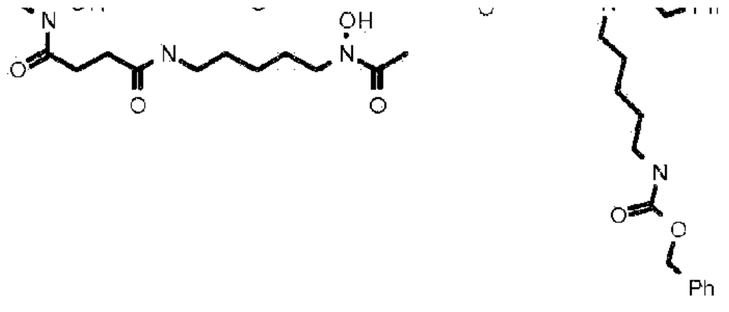
[0111] Data: ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) 1.16-1.27 (m, 8H), 1.32-1.43 (m, 8H), 1.41-1.56 (m, 8H), 1.97 (s, 3H), 2.24-2.33 (m, 6H), 2.54-2.65 (m, 6H), 2.94-3.03 (m, 8H), 3.43-3.48 (m, 6H), 3.53-3.58 (m, 2H), 4.87 (s, 2H), 4.99 (s, 2H), 7.17-7.47 (m, 11H), 7.76 (s, br, 3H), 9.56-9.67 (m, 3H). 13C{1H} NMR (100 MHz, DMSO-d6): δ (ppm) 20.8, 23.8, 23.9, 26.5, 26.6, 27.7, 28.1, 29.3, 29.4, 30.1, 30.4, 38.9, 45.1, 47.3, 47.6, 65.5, 75.9, 128.2, 128.7, 128.9, 129.1, 129.7, 135.4, 137.8, 156.8, 170.6, 171.4, 171.7, 172.4. ESI-MS (positive detection mode): m/z (%) 1007.3 (100) [M+Na]+. Anal. Calcd for $C_{49}H_{76}N_8O_{13}$: C 59.74, H 7.78, N 11.37. Found: C 59.99, H 7.67, N 11.73.





HATU, DIPEA



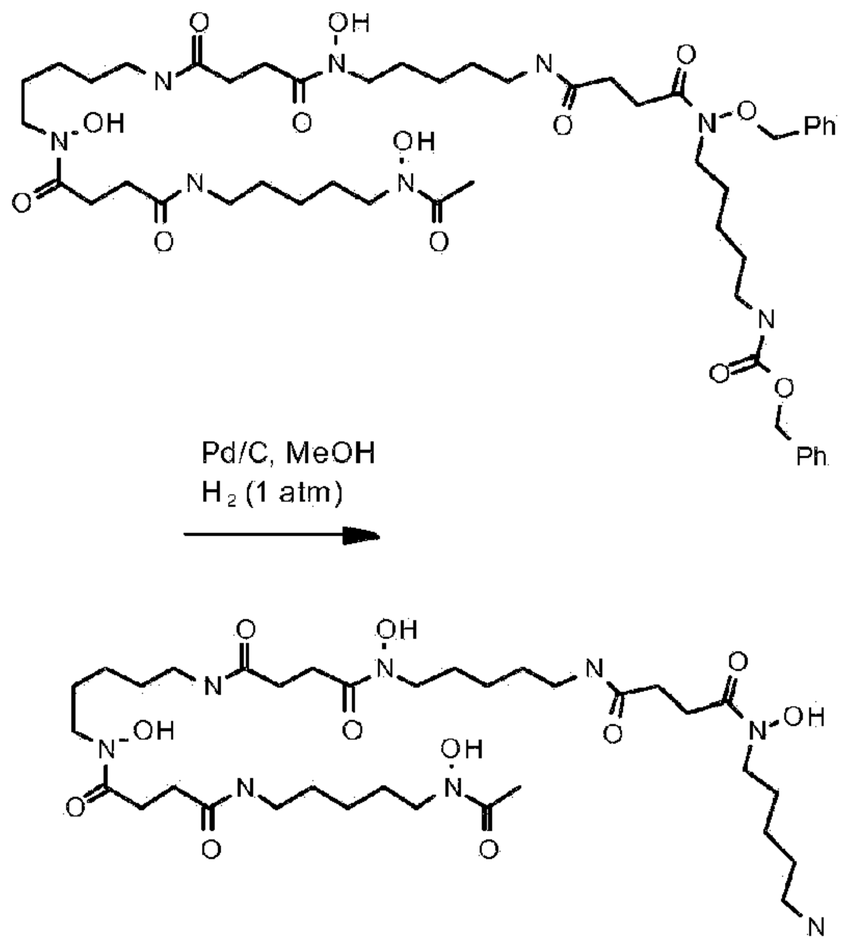


Compound b

c. Synthesis of ligand I:

[0112] A mixture of compound b (125 mg, 0.13 mmol) and 100 mL of MeOH was sonicated for 10 min in an ultrasonic bath. To the resulting suspension, 10% Pd/C (38 mg) was added and hydrogenation was carried out for 6 h under H_2 (1 bar) atmosphere. The reaction mixture was then filtered by cotton plug followed by a filter paper. The solution was evaporated to give a white solid that was washed with 10 mL of acetonitrile and 20 mL of Et_2O and dried.

[0113] Data: 1H NMR (500 MHz, DMSO-d6): δ (ppm) 1.19-1.28 (m, 8H), 1.33-1.39 (m, 7H), 1.46-1.53 (m, 9H), 1.97 (s, 3H), 2.24-2.33 (m, 8H), 2.55-2.60 (m, 6H), 2.73-2,76 (m, 2H), 2.97-3.03 (m, 6H), 3.41-3.49 (m, 8H), 7.77 (s, br, 4H), 9.60 (s, br, 3H). 13C{1H} NMR (125 MHz, DMSO-d6): δ (ppm) 20.8, 23.3, 23.9, 26.2, 26.5, 27.1, 27.9, 28.1, 29.2, 30.2, 30.3, 38.9, 39.2, 47.2, 47.5, 170.6, 171.7, 172.4, 172.5. ESI-MS (positive detection mode): m/z (%) 761.5 (100) [M+H]+. Anal. Calcd for $C_{34}H_{64}N_8O_{11}$: C 53.67, H 8.48, N 14.73. Found: C 53.60, H 8.25, N 14.66.



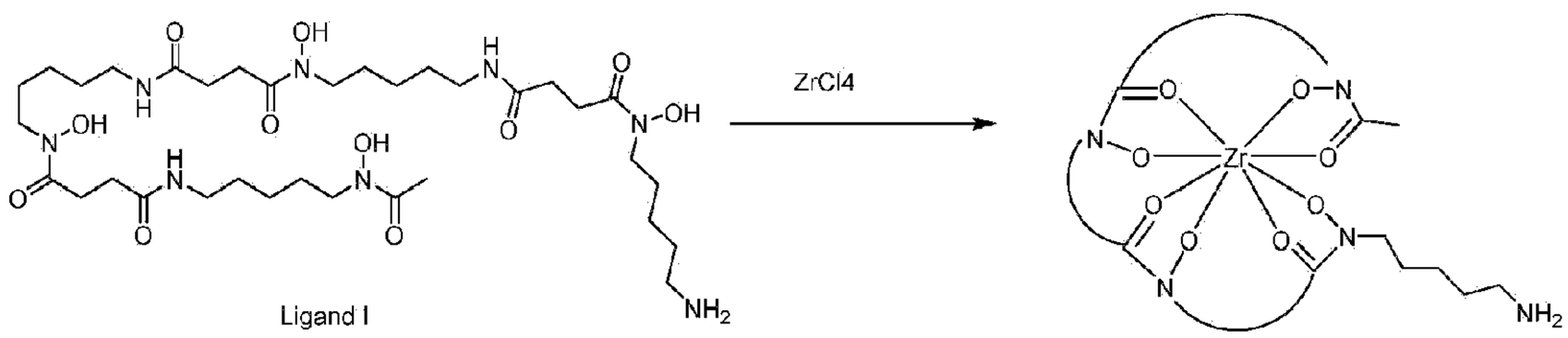
Ligand I

d. Synthesis of complex I:

[0114] 600 uL (0.0072 mM) of a solution of the ligand I in 0.1 M HCl was added to 200 uL of the solution of $ZrCl_4$ in 0.1 M HCl. The pH of the mixture was then slowly adjusted to ca. 8 by slow addition of 0.1 M K_2CO_3 solution and stirred for addition 40 min. Then the mixture was lyophilized to give white solid. Formation of the desired product was confirmed by the single peak observed in the LC-MS analysis that has the expected mass.

[0115] For scaling up, to a suspension of the ligand (21.8 mg) in 667 uL of 0.1mM HCl, a solution (667 uL, 10 mg/mL) of $ZrCl_4$ in 0.1M HCl was added and stirred for 10 min. The suspension slowly disappears during this time. The pH of the mixture was then slowly adjusted to ca. 8 by slow addition of 0.1 M K_2CO_3 solution, stirred for additional 1.5 h and then lyophilized to give a white solid powder.

[0116] Data: t_R (LC-MS) 9.3 min. Mass 847.3 [M+H]+.

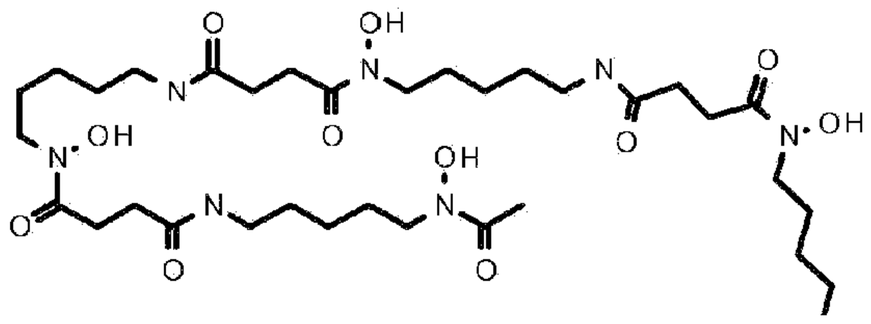


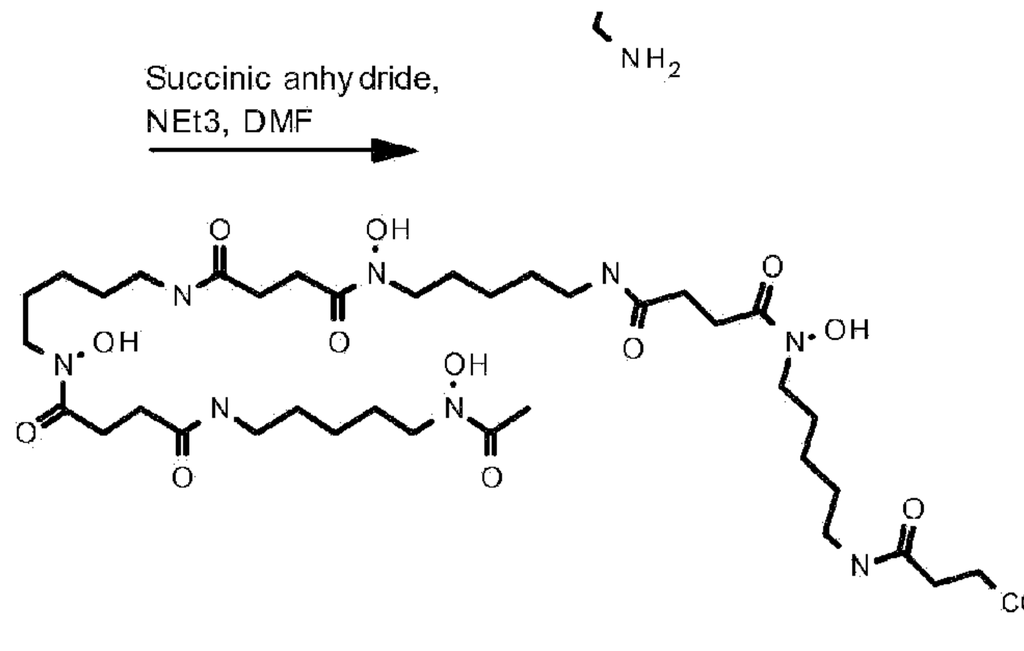
e. Synthesis of ligand II

[0117] To a stirred mixture of ligand I (20 mg, 0.03 mmol) and succinic anhydride (3.9 mg, 0.04 mmol) in 2 mL DMF, NEt_3 (7.6 mg, 0.07 mmol) was added under N_2 atmosphere. After 48 h, DMF was removed in vacuum and the white solid obtained was washed with small portions acetone and Et_2O.

[0118] Data: 1H NMR (500 MHz, DMSO-d6): δ (ppm) 1.18-1.26 (m, 8H), 1.34-1.42 (m, 8H), 1.46-1.54 (m, 8H), 1.97 (s, 3H), 2.24-2.33 (m, 8H), 2.39-2.43 (m, 2H), 2.56-2.61 (m, 6H), 2.97-3.03 (m, 8H), 3.41-3.49 (m, 8H), 7.77 (s, br, 4H), 9.64 (s, br, 3H), 12.04 (s, br, 1H). 13C{1H} NMR (125 MHz, DMSO-d6): δ (ppm) 20.8, 23.9, 26.4, 28.1, 29.2, 29.6, 30.3, 30.4, 38.9, 47.2, 47.4, 170.6, 171.2, 171.7, 172.4, 174.3. ESI-MS (positive detection mode): m/z (%) 883.4 (100) [M+Na]+. Anal. Calcd for $C_{38}H_{68}N_8O_{14}$: C 53.01, H 7.96, N 13.01. Found: C 52.89, H 7.73, N 12.88.

1H NMR (500 MHz, DMSO-d6)



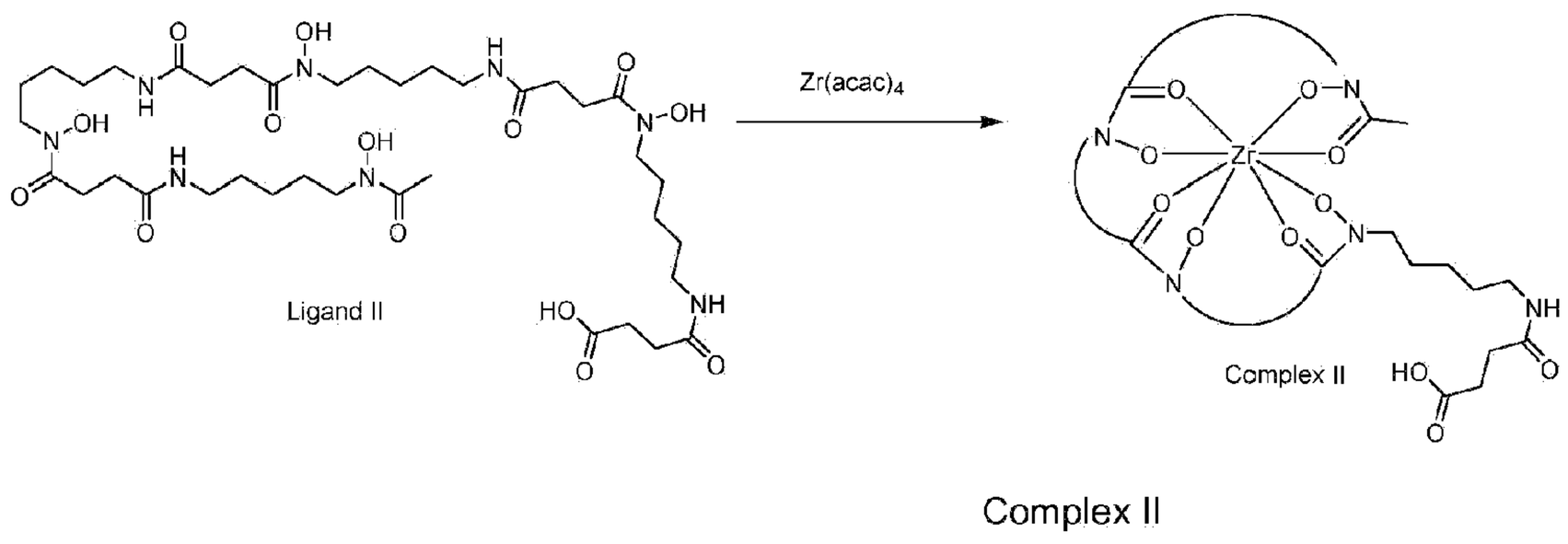


Ligand II

f. Synthesis of complex II

[0119] Ligand II is not soluble either in 0.1 M HCl or in H_2O. The complexation was carried out in MeOH using zirconium (IV) acetylacetonate [$Zr(acac)_4$]. A mixture of ligand II (50 mg, 0.058 mmol) and $Zr(acac)_4$ (28.3 mg, 0.058 mmol) in 15 mL MeOH was heated at reflux for 15 h with continuous stirring under N_2 atmosphere. The solvent was then removed and the residue was then washed with Et_2O (20 mL) and acetone (10 mL). The compound was obtained as off white solid (48 mg, 87 %). The compound is it not soluble in common organic solvents including DMSO. When trying to dissolve in 0.5 % NaOD in D_2O, decomposition of the complex was observed. Therefore due to the limited solubility NMR spectra of the compound could not be obtained. However, the formation of the complex can be confirmed using LC-MS. Elemental analysis calculated for $C_{38}H_{64}N_8O_{14}Zr•6H_2O•(CH_3)_2CO$, C 44.19, H 7.42, N 10.06. Found C 44.11, H 7.04, N 10.16.

[0120] Data: t_R (LC-MS) 8.7 and 8.8 min. Mass 947.3 [M+H]+

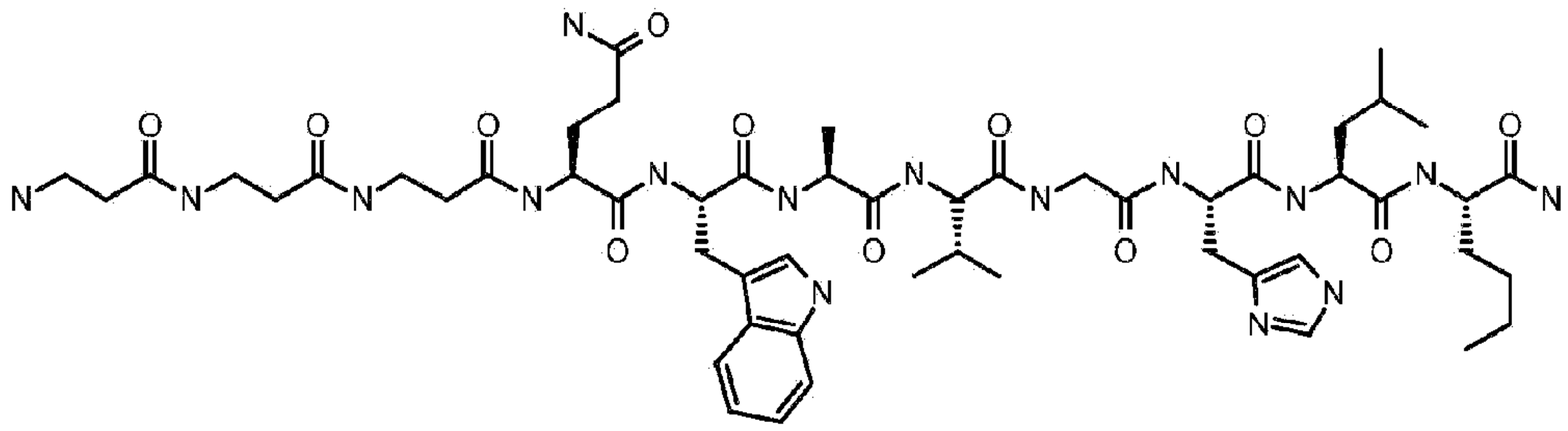


Ligand II

Complex II

g. Synthesis of a modified amino acid sequence of bombesin BBN_{Ago}(beta-Ala)$_3$, identical to SEQ ID NO 14: (β-Ala)$_3$-Gln-Trp-Ala-Val-Gly-Hi-Leu-14Nle-$CONH_2$

[0121] Synthesis was performed on a solid phase synthesis using an Applied Biosystem automatic peptide synthesizer with low-loaded Rink Amid resin (on a Phenomenex Jupiter 250 x 4.6 mm column) and standard Fmoc-protocoll, followed by HPLC analysis to test for chemical purity. Molecular Weight: 1135.32 g/mol; Chemical Formula: $C_{53}H_{82}N_{16}O_{12}$



h. Synthesis of ligand III

[0122] Coupling of ligand II to $BBN_{Ago}(beta\text{-}Ala)_3$ was done by manual synthesis. 50-60 mg (9-12 µmol) of peptide resin was Fmoc-deprotected with 20% Piperidine/DMF (5x 2 mL, 3-5 min each). MP-Zr-19 (2 eq.) was dissolved in 1 mL DMF (peptide grade) and shortly warmed up in a heating block at 60-70 °C, then vortexed and sonicated. The solution was transfered to an eppendorff tube containing HATU (2 eq), and DIPEA (4 eq.) was added. The mixture was transfered to a syringe reactor containing the swollen peptide resin. After 3 h the reaction was stopped and the coupling procedure repeated over night. The peptide conjugate was cleaved from the resin with ~ 0.6 mL TFA/phenol/TIS/water (87.5/5/2.5/5) for 3 h and afterwards precipitated in ice cold Et_2O. The pellet was broken and washed three times with Et_2O. The remaining pellet was purified by preparative HPLC. Combined HPLC fractions were concentrated and lyophilized. ESI-MS spectra were recorded at positive electrospray ionization mode on a Bruker Esquire 3000 plus (Bruker Daltonics GmbH, Bremen, Germany) at the University of Basel. MALDI-MS analysis was performed on an Applied Biosystem 4800 TOF-TOF.

[0123] Characterization HPLC: t_R= 9.6 min (85_60, analyt.); t_R= 8.9 min (75_68, analyt.), t_R= 14.8 min (75_65, prep.)

[0124] Characterization ESI-MS: m/z= 990.1 $[M+2H]^{2+}$, 1979.8 [M+H]+

[0125] Characterization MALDI-MS: m/z= 1978.1 $[M+H]^+$, additional signals due to degradation of chelator during measurement (e.g. 1333.7, 1533.8 break of Hydroxamate-bonds).

[0126] Molecular Weight: 1978.29 g/mol; Chemical Formula: $C_{91}H_{148}N_{24}O_{25}$ Formula of ligand III and reaction: see Fig. 3

Radiolabelling and Quality Control of ^{89}Zr-ABP-27 and ^{89}Zr-ABP-28

i) <u>Synthesis of complex III</u>

[0127] Zr-89 was obtained in 1 M oxalic acid from Perkin Elmer. ABP-27 (M_w= 1978,29 g/mol) was prepared in lyophilized aliquots of 50 µg/50 µL and dissolved with deionized water upon starting the labeling. 10-30 µL (11.2-28.1 MBq) was taken from the Zr-89 stock solution and filled up to 200 µL with 1.0 M oxalic acid. 90 µL of 2.0 M Na_2CO_3 was added and incubated for 3 min at RT. 300 µL 0.5 M HEPES (pH 7.3), 710 µL of peptide solution (10 µg/10 µL (5 nmol) + 700 µL H_2O) and 300 µL 0.5 M HEPES (pH 7.3) were added. The pH was checked with pH-strips and ranged from pH 7.0-7.3. The reaction solution was incubated for 240 min at ambient temperature. Quality control of radiolabelling reactions were performed by means of HPLC and ITLC. Reversed phase HPLC was done on a Bischof system equipped with HPLC pumps 2250, a λ-1010 UV-detector, a Berthold LB509 radioflow detector and a Jupiter, 4µ Proteo 90A, 250 x 4.6 mm column from Phenomenex. The column was eluted with mixtures of acetonitrile (solvent A) and water with 0.1 % trifluoroacetic acid (TFA) (solvent B) at a flow rate of 2 mL/min and a linear gradient: 0 min 80% B, 12.5 min 60% B, 13.5 min 5% B, 14.5 min 5% B, 15 min 80% B, 17 min 80% B. 20 µL of labeling solution were diluted in 50 µL 0.1 mM Desferrioxamine in 0.5 M HEPES solution (pH 7.3) and 10 µL were injected into HPLC. Radiochemical purity was determined by manual integration and determined to be between 94-97 %. ITLC was done using Biodex green ITLC-Strips. 2 µL of labelling solution were spotted directly on the strip and developed with citric acid solution (20 mM, pH 4.8) as eluent. The strip was read out with a Cyclone Plus Phosphorimager and a MultiSensitive storage phosphor screen from Perkin Elmer. Radiochemical yield was determined by manual integration and determined to be >95 %

j) <u>Synthesis and Characterisation of DFO*-NCS:</u>

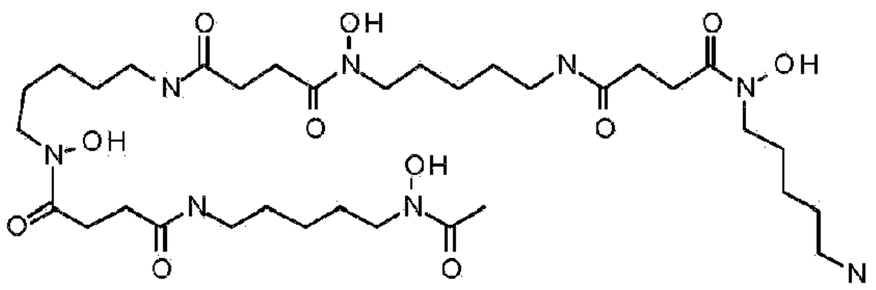
<u>Method:</u>

[0128] DFO* (50 mg, 0.066 mmol) was dissolved in a mixture isopropanol/water (4:1, 8 mL) by means of sonication and heating. The solution was then added at room temperature drop wise to a solution of phenylenediisothiocyanate (126 mg, 0.66 mmol) in chloroform (8 mL) containing triethylamine (0.079 mmol, 0.011 mL). The reaction was stirred at room temperature. After 24h UPLC-MS indicated complete conversion of DFO*. The solvents were concentrated under reduced pressure and the resulting white solid was washed with diethylether (4 x 10 mL). The product was further purified by preparative HPLC (Nucleodur Preparative column (ISIS-C18 16 x 250 mm 5 µm)) at a flow rate of 8 mL min^{-1} with a linear gradient of A (acetonitrile (Sigma-Aldrich HPLC-grade) and B (distilled water containing 0.1% TFA): t=0 min A 36% B 64%, t=15 min A 44% B 56%, t=17 min A 36% B 64%. After

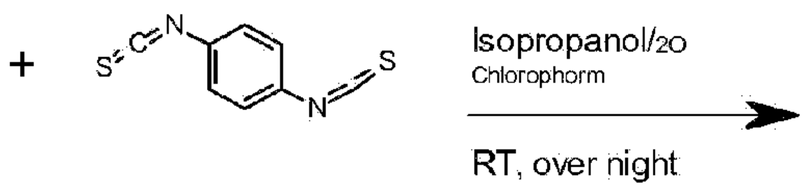
lyophilisation, the product was obtained as a white powder (6 mg, 10 %).

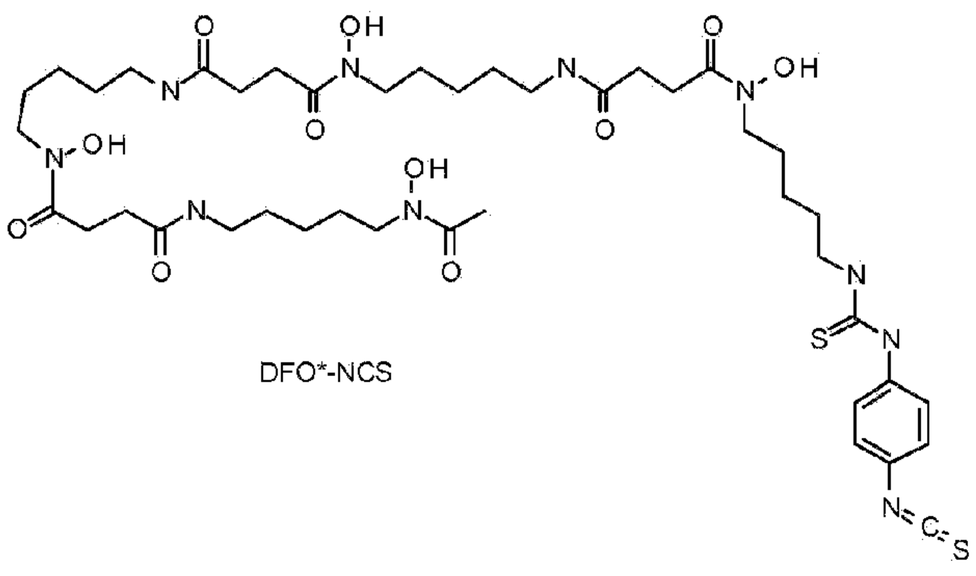
^{1}H NMR (600 MHz, DMSO-d_6): δ(ppm) 9.57-9.71 (m, br, 5H), 7.90 (s, br, 1H), 7.77 (s, br, 3H), 7.52-7.56 (d, 2H), 7.34-7.38 (d, 2H), 3.41-3.49 (m, 10H), 2.97-3.03 (m, 6H), 2.55-2.60 (m, 6H), 2.23-2.3 (m, 6H), 1.97 (s, 3H), 1.48-1.51 (m, 10H), 1.36-1.39 (m, 6H), 1.19-1.21 (m, 8H). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d6): δ(ppm) 180.1, 172.0, 171.3, 170.2, 139.3, 132.6, 126.2, 124.6, 123.1, 47.1, 46.8, 43.7, 38.4, 29.9, 28.8, 28.0, 27.6, 27.1, 26.0, 23.6, 23.5, 20.4. Some carbon signals were not observed due to overlapping signals. HR-ESI-MS: calcd for $C_{42}H_{68}N_{10}O_{11}S_2$/z $[M+H^+]^+$ 953.45887, found 953.45832, calcd for C42H68N10O11NaS2/z $[M+Na^+]^+$ 975.44027, found 975.44027.

[0129] ^{1}H and ^{13}C NMR measurements were carried out on Bruker AV-600 (CP-TCI (CryoPorbe)) spectrometer and referenced to residual solvent peaks. High resolution ESI-MS spectra were recorded using a Bruker ESQUIRE-LC quadrupole ion trap instrument.



DFO*



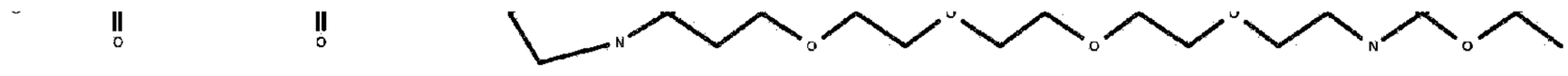


DFO*-NCS

Reaction scheme

k) Synthesis of PEG complex:

[0130]



[0131] R1= Methyl

D= Hydroxymate

X= $[CH_2]_5$-N-[C=O]-$[CH_2]_2$

SEQUENCE LISTING

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<110> Universitaet Zuerich

<120> Multidentate Bifunctional Chelating Agents for Radionuclide Complexation in Diagnostics and Therapy

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<170> PatentIn version 3.5

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Ala Lys Tyr Phe Leu Ala Glu Leu Leu Ser Glu Pro Asn Gln Thr Glu
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Phe Thr Ser Cys
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Gln Gln Gly Pro Ala Ser His His Arg Arg Gln Leu Gly Pro Gln Gly
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His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu
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Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg
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His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
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Phe Gly Leu Met Gly Lys Arg Asp Ala Asp Ser Ser Ile Glu Lys Gln
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Val Ala Leu Leu Lys Ala Leu Tyr Gly His Gly Gln Ile Ser His Lys
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Arg His Lys Thr Asp Ser Phe Val Gly Leu Met Gly Lys Arg Ala Leu
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Arg

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Ala His Val Pro Ser Trp Lys Met Thr Leu Leu Asn Val Cys Ser Leu
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Val Asn Asn Leu Asn Ser Pro Ala Glu Glu Thr Gly Glu Val His Glu
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Glu Glu Leu Val Ala Arg Arg Lys Leu Pro Thr Ala Leu Asp Gly Phe
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Ser Leu Glu Ala Met Leu Thr Ile Tyr Gln Leu His Lys Ile Cys His
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Ser Arg Ala Phe Gln His Trp Glu Leu Ile Gln Glu Asp Ile Leu Asp
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Thr Gly Asn Asp Lys Asn Gly Lys Glu Glu Val Ile Lys Arg Lys Ile
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO0004868A [0006]
- WO2014164988A [0006]

Non-patent literature cited in the description

- **POREDDY et al.**J. Combo Chem., 2003, vol. 6, 239-254 [0006]
- CHEMICAL ABSTRACTS, 70-51-9 [0007]
- **FISCHER et al.**Molecules, 2013, vol. 18, 6469-6490 [0007]
- **STRYER**Biochemistry21- [0009]
- **WUTS**Greene's protective groups in organic synthesisWiley20140000 [0017]
- **MISHRA et al.**Journal of Biomedical Materials Research, 2013, vol. 101A, 123646-3660 [0049]
- CHEMICAL ABSTRACTS, 31362-50-2 [0054]
- CHEMICAL ABSTRACTS, 38916-34-6 [0055]
- CHEMICAL ABSTRACTS, 83150-76-9 [0060]
- CHEMICAL ABSTRACTS, 130357-25-4 [0064]
- CHEMICAL ABSTRACTS, 141758-74-9 [0065]
- CHEMICAL ABSTRACTS, 10026-11-6 [0080] [0081]
- CHEMICAL ABSTRACTS, 184475-35-2 [0097]
- CHEMICAL ABSTRACTS, 183321-74-6 [0097]
- CHEMICAL ABSTRACTS, 183319-69-9 [0097]
- CHEMICAL ABSTRACTS, 205923-56-4 [0097]
- CHEMICAL ABSTRACTS, 180288-69-1 [0098]
- CHEMICAL ABSTRACTS, 380610-27-5 [0098]
- CHEMICAL ABSTRACTS, 892553-42-3 [0099]
- CHEMICAL ABSTRACTS, 216974-75-3 [0099]
- **HAUBNER et al.**J. Nucl. Med., 2001, vol. 42, 2326-336 [0099]

- **ZHAO et al.**Plos ONE, 2013, vol. 8, 4e61043- [0099]
- **ZHANG et al.**Cancer Res., 2007, vol. 67, 41555-1562 [0099]
- CHEMICAL ABSTRACTS, 154039-60-8 [0100]
- CHEMICAL ABSTRACTS, 24390-14-5 [0100]
- CHEMICAL ABSTRACTS, 190648-49-8 [0100]

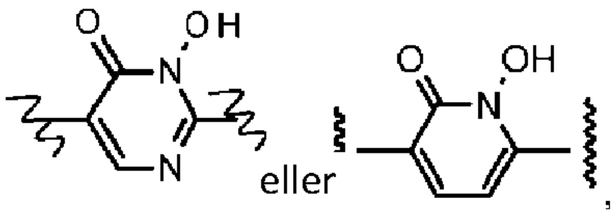
Patentkrav

1. Ligand, der er **kendetegnet ved** en almen formel I:

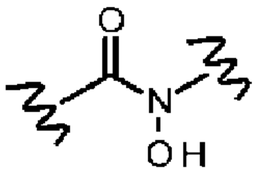
$$R^1\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}E\text{-}R^2 \qquad (I),$$

hvor

- D er C(O)N(OH) eller N(OH)C(O), eller



D navnlig er



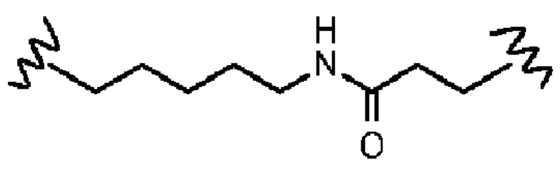
(udskrevet: C(O)N(OH)),

- hvert X uafhængigt af et hvilket som helst andet X er en mættet eller delvist umættet, substitueret eller ikke-substitueret linker, der omfatter en kæde af 9 til 11 atomer udvalgt fra en hvilken som helst af N, C, O
- R^1 er en H, C_1-C_5-alkyl, en C_3-C_6-cycloalkyl, en aren og/eller en heteroaren,
- E er en linker, der omfatter 1 - 50 atomer udvalgt fra C, O, N og S, og
- R^2 er

a) OH, NH_2, SH, COOH, CHO, N_3, SCN, CH_2X, aktiveret ester, et en-on-system, en dien/dienophil, en alken eller en alkyn, hvor X er Cl, Br eller I, eller

b) en første klikdel, der kan danne en kovalent binding selektivt med en anden klikdel R^3 under reaktionsbetingelser, der ikke fører til en kovalent reaktion af R^2 eller R^3 med naturligt forekommende polypeptider, hvor klikdelen R^2 navnlig er udvalgt fra et azid, en alkyn, et tetrazin, et cyclooctin og en trans-cycloocten.

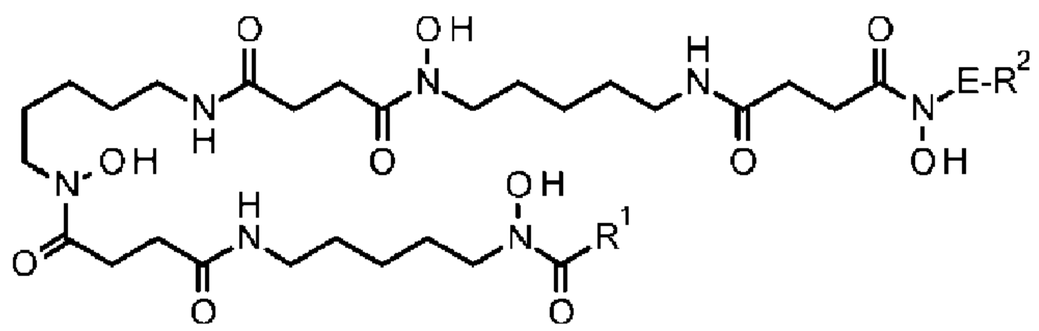
2. Ligand ifølge krav 1, hvor X er en ikke-substitueret lineær kæde, der består af C_1-C_5-alkyldele sammenkoblet (forbundet) af COO- (ester) dele eller CONH- (amid) dele, hvor X navnlig er



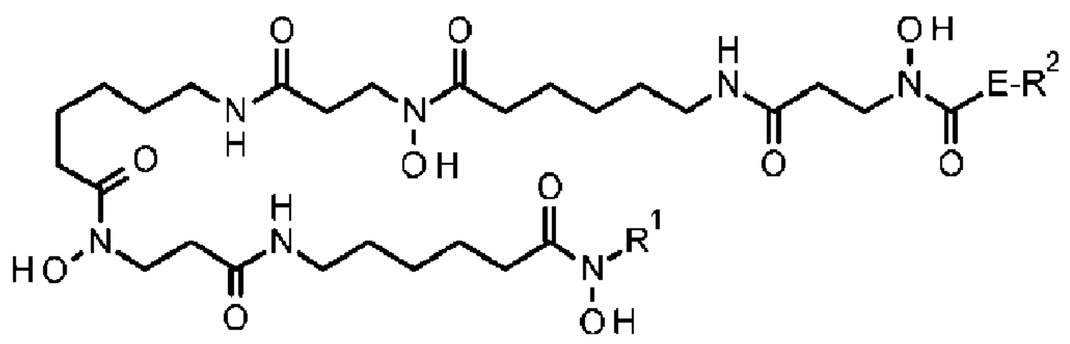
3. Ligand ifølge et hvilket som helst af de foregående krav, hvor E omfatter 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 5 til 25, 10 til 30, 20 til 50 eller 30 til 50 atomer udvalgt fra C, O, N og S.

4. Ligand ifølge et hvilket som helst af de foregående krav, hvor R^2 er en alkyn- eller aziddel, en alkyn, et tetrazin, et cyclooctin, en trans-cycloocten, en carboxygruppe, en aminogruppe, iod, brom, chlor, succinimid, en thiolgruppe, en cyclooctyndel, biotin, avidin eller streptavidin.

5. Ligand ifølge et hvilket som helst af de foregående krav, beskrevet ved formlerne



eller



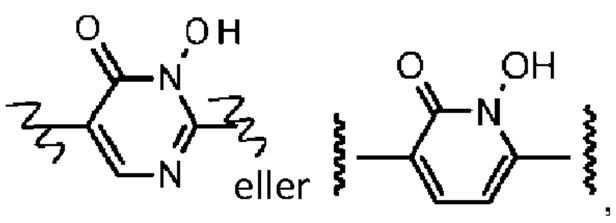
hvor E, R^1 og R^2 har den samme betydning som anført i et hvilket som helst af de foregående krav.

6. Konjugeret ligand **kendetegnet ved** en almen formel I:

$$R^1\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}X\text{-}D\text{-}E\text{-}R^2 \qquad (I),$$

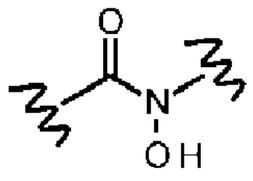
hvor

- D er C(O)N(OH) eller N(OH)C(O), eller



eller

D navnlig er



(udskrevet: C(O)N(OH)),

- hvert X uafhængigt af et hvilket som helst andet X er en mættet eller delvist umættet, substitueret eller ikke-substitueret linker, der omfatter en kæde af 9 til 11 atomer udvalgt fra en hvilken som helst af N, C, O
- R^1 er en C_1-C_5-alkyl, en C_3-C_6-cycloalkyl, en aren og/eller en heteroaren,
- E er en linker, der omfatter 1 - 50 atomer udvalgt fra C, O, N og S, og
- R^2 er en målrettet del, der specifikt binder til et målsted på celler og/eller væv med en associeringskonstant, der er lavere end (<) 10^{-6} mol/l, navnlig et antistof, et oligopeptid, et polypeptid, et polynukleotid, et liposom, en polymerosom, et phospholipid, et vitamin, et monosaccharid, et oligosaccharid, en nanopartikel, eller ert lægemiddellignende molekyle med en molekylvægt på mindre end (<) 3000 U.

7. Konjugeret ligand ifølge krav 6, hvor R^2 omfatter et polypeptid, der er en funktionel homolog af bombesin, somatostatin, gastrin, transaktivator af transskriptionspeptid, prostataspecifikt antigen, neuropeptid Y, octreotid, gastrisk hæmmende polypeptid, neurokinin A, neurotensin, exendin-3, exendin-4 eller stof P, hvilket peptid omfatter en aminosyresekvens med mindst 80 %, 85 %, 90 %, 95 %, 98 %, eller 99 % identitet med henholdsvis SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12 eller SEQ ID NO 13.

8. Kompleks, der omfatter en ligand ifølge et hvilket som helst af de foregående krav, koordineret bundet til et metalatom, navnlig et metal, der indgår i gruppe 3, 4, 6, 7, 9, 10, 11, 13, 15, lanthanidgruppen af elementer, eller actinidgruppen af elementer fra det periodiske system, mere navnlig et metal udvalgt fra Ga, Tc, In, Cu, Tb, Sc, Lu, Re, Bi, Eu, Gd, Zr eller Y, endnu mere navnlig et metal udvalgt fra ^{44}Sc, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{99m}Tc, ^{111}In, ^{nat}Eu, ^{nat}Gd, ^{177}Lu, ^{161}Tb, ^{186}Re, ^{188}Re

eller ^{213}Bi.

9. Kompleks ifølge krav 8, hvor metallet

- har oxidationsnummeret +1, +2, +3, +4, +5, +6 eller +7, og/eller
- danner et 8-koordineret kompleks med liganden.

10. Fremgangsmåde til syntase af et kompleks ifølge krav 8 til 9, hvilken fremgangsmåde omfatter følgende trin:

(a) tilvejebringelse af en ligand ifølge et hvilket som helst af kravene 1 til 7;

(b) tilsætning af et metal udvalgt fra et element af gruppen 3, 4, 6, 7, 9, 10, 11, 13 eller 15, lanthanidgruppen af elementer, eller actinidgruppen af elementer fra det periodiske system, navnlig et metal udvalgt fra Ga, Zr, Lu eller Y, mere navnlig en isotop udvalgt fra ^{67}Ga, ^{68}Ga,^{89}Zr, ^{177}Lu eller ^{90}Y.

(c) og eventuelt isolering af komplekset.

11. Kompleks ifølge et hvilket som helst af kravene 8 til 9, eller et kompleks opnået ved en fremgangsmåde ifølge krav 10, til anvendelse som et farmaceutisk middel, navnlig til anvendelse i en fremgangsmåde til behandling af neoplastisk sygdom (cancer).

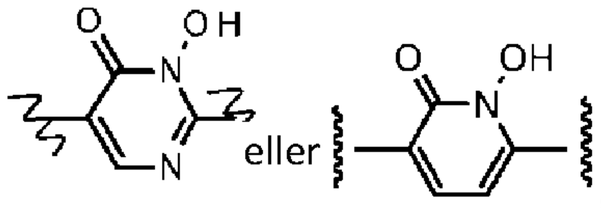
12. Kompleks ifølge et hvilket som helst af kravene 8 til 9, eller et kompleks opnået ved en fremgangsmåde ifølge krav 10, til anvendelse i en fremgangsmåde til medicinsk diagnosticering eller radioimmunterapi, navnlig til anvendelse i positronemissionstomografi og/eller enkeltfoton-emissionscomputertomografi.

13. Kompleks ifølge et hvilket som helst af kravene 8 til 9, eller et kompleks opnået ved en fremgangsmåde ifølge krav 10, til anvendelse ved diagnosticering af neoplastisk sygdom.

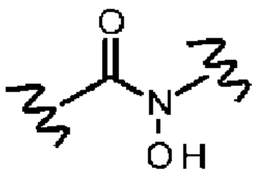
14. Fast bærer omfattende en ligand kovalent bundet til den faste bærer, hvilken ligand er **kendetegnet ved** en almen formel I

$$R^1\text{-D-X-D-X-D-X-D-E-}R^2 \qquad \text{(I)}$$

- hvor D er C(O)N(OH) eller N(OH)C(O), eller



D navnlig er



(udskrevet: C(O)N(OH)),

- hvert X uafhængigt af et hvilket som helst andet X er en mættet eller delvist umættet, substitueret eller ikke-substitueret linker, der omfatter en kæde af 9 til 11 atomer udvalgt fra en hvilken som helst af N, C, O;
- R^1 er en C_1-C_5-alkyl, eller en C_3-C_6-cycloalkyl, en aren og/eller en heteroaren,
- E er en lineær linker, der omfatter 1 - 50 atomer udvalgt fra C, O, N og S og
- R^2 er bindingsstedet til den faste bærer.

15. Fast bærer ifølge krav 14, hvor den faste bærer er en nanopartikel, navnlig en nanopartikel udvalgt fra guld, siliciumdioxid, lipider, polymer-, metal- eller metaloxidsammensætninger, der omfatter metallerne jern, mangan eller titan.

DRAWINGS

Fig. 1

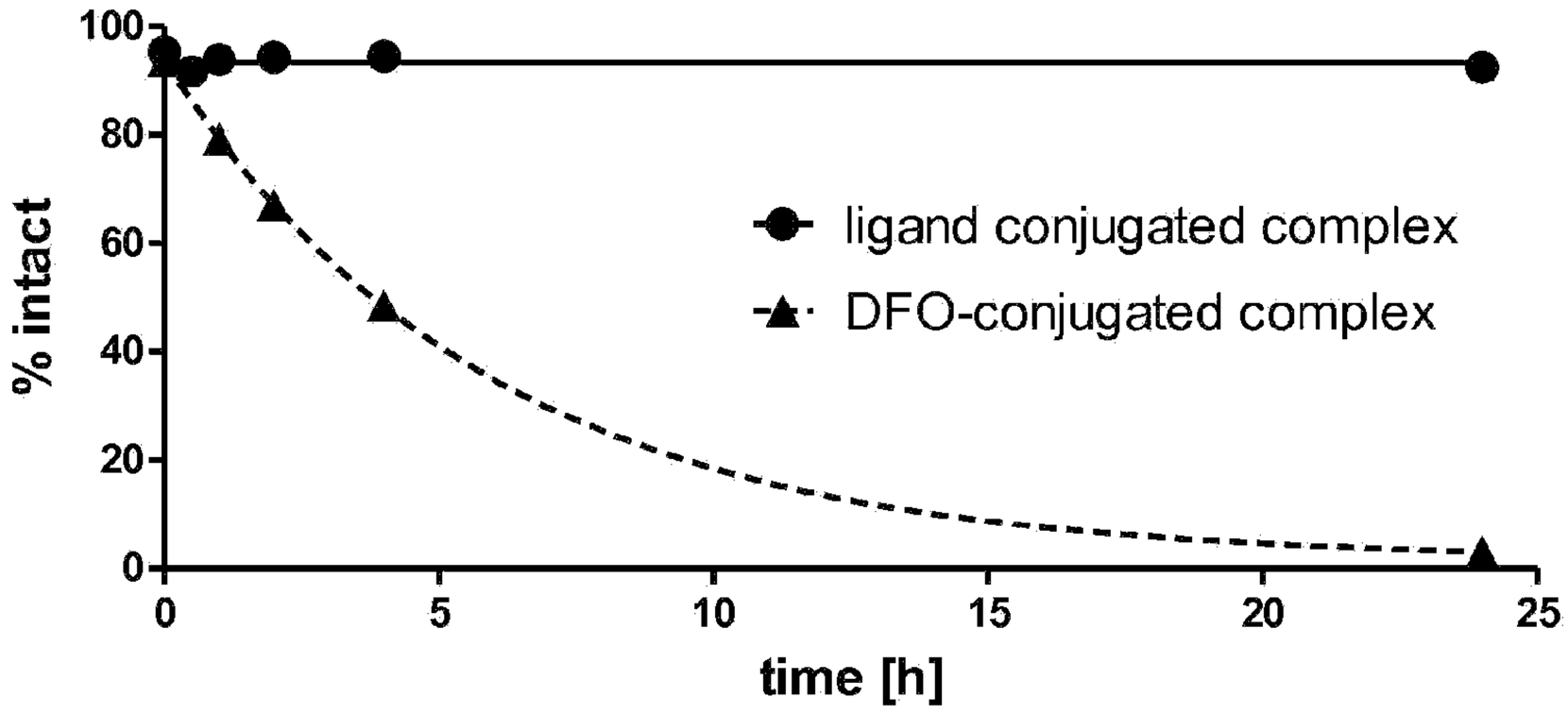


Fig. 2

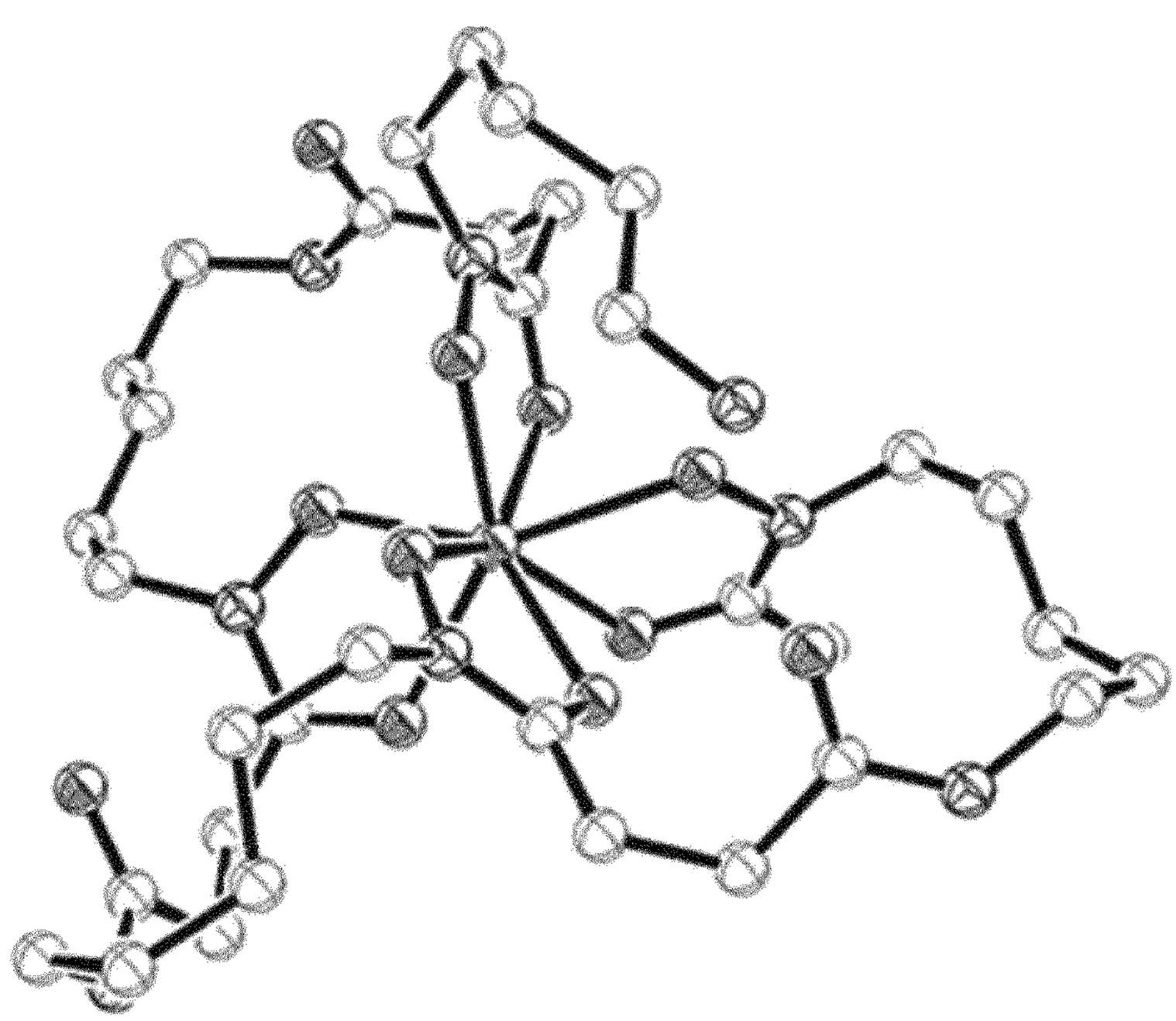
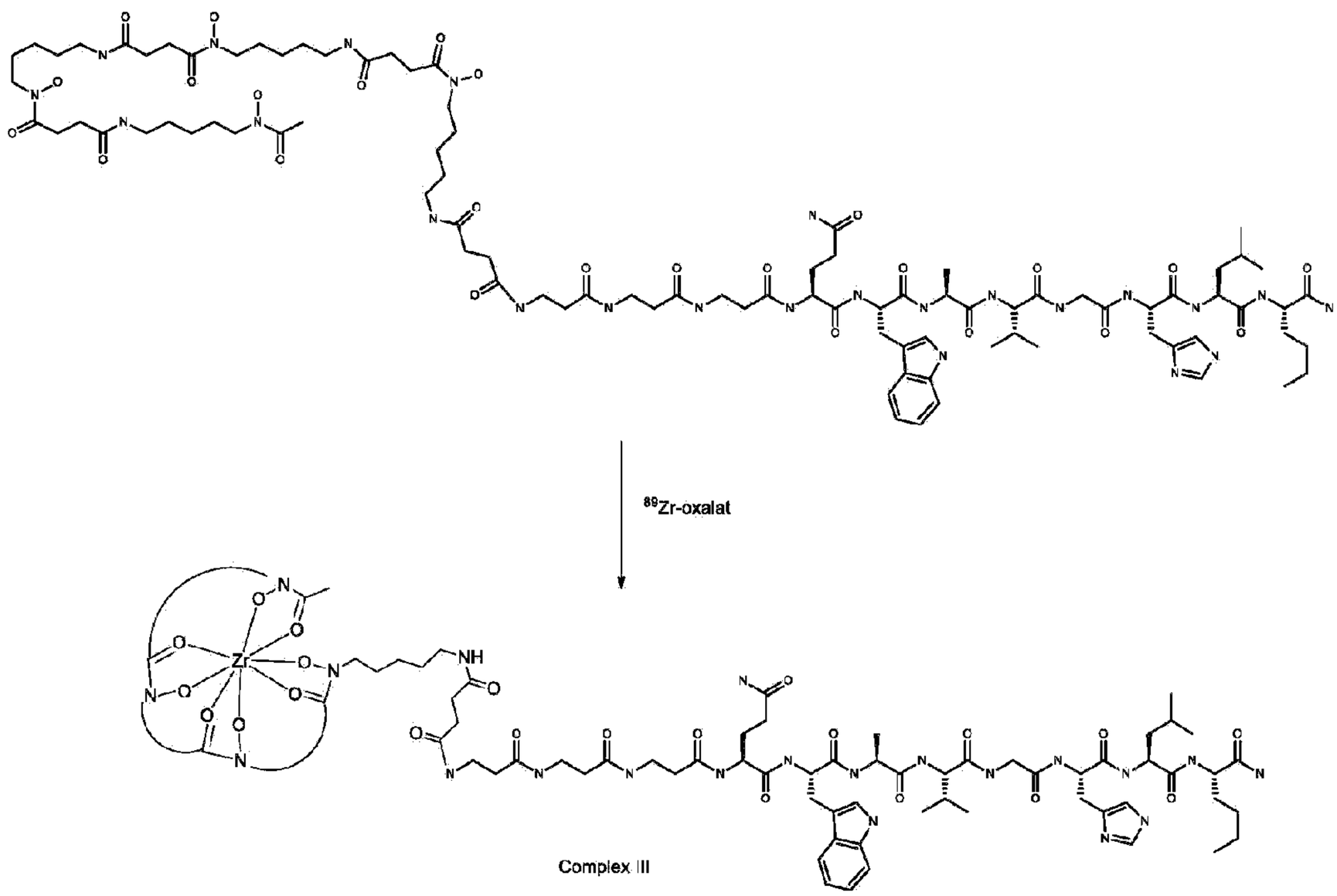


Fig. 3



Complex III