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CHEMICAL ABSTRACTS, vol. 86, no. 19, 9
May 1977, page 524, abstract no. 139 627d,
Columbus, Ohio, US; **SOCIEDAD ANON,**
DABEER, "Phenol derivatives of hydroxylal-
kylenediaminoacetic acids and their Salts";
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Description

The present invention concerns chelants possessing ortho ligating functionality, complexes and conjugates thereof, processes for their preparation and formulations for their use in cancer diagnostics and/or therapy.

Functionalized chelants, or bifunctional coordinators, are known to be capable of being covalently attached to an antibody having specificity for cancer or tumor cell epitopes or antigens. Radionuclide complexes of such antibody/chelant conjugates are useful in diagnostic and/or therapeutic applications as a means of conveying the radionuclide to a cancer or tumor cell. See, for example, Meares et al., *Anal. Biochem.* **142**, 68-78, (1984); and Krejcarek et al., *Biochem. and Biophys. Res. Comm.* **77**, 581-585 (1977).

Aminocarboxylic acid chelating agents have been known and studied in the literature for several years. Typical of the aminocarboxylic acids are nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), diethylenetriaminepentaacetic acid (DTPA) and trans-1,2-diaminocyclohexanetetraacetic acid (CDTA). Numerous bifunctional chelating agents based on aminocarboxylic acids have been proposed and prepared. For example the cyclic dianhydride of DTPA (Hnatowich et al. *Science*, **220**, 613-615, 1983; U.S. Patent 4,479,930) and mixed carboxycarbonic anhydrides of DTPA (Gansow, U.S. Patents 4,454,106 and 4,472,509; Krejcarek et al., *Biochem. and Biophys. Res. Comm.* **77**, 581-585, 1977) have been reported in the literature. When the anhydrides are coupled to proteins the coupling proceeds via formation of an amide bond thus leaving four of the original five carboxymethyl groups on the diethylenetriamine (DETA) backbone (Hnatowich et al., *Int. J. Appl. Isot.* **33**, 327-332, 1982). In addition, U.S. Patents 4,432,907 and 4,352,751 disclose bifunctional chelating agents useful for binding metal ions to "organic species such as organic target molecules or antibodies." As in the above, coupling is obtained via an amide group through the utilization of diaminotetraacetic acid dianhydrides. Examples of anhydrides include dianhydrides of EDTA, CDTA, propylenediaminetetraacetic acid and phenylene 1,2-diaminetetraacetic acid. A recent U.S. Patent 4,647,447 discloses several complex salts formed from the anion of a complexing acid for use in various diagnostic techniques. Conjugation via a carboxyl group of the complexing acid is taught which gives a linkage through an amide bond.

Another class of bifunctional chelating agent based on aminocarboxylic acid functionality is also well documented in the literature. Thus, Sundberg et al. in the *J. of Med. Chem.* **17**(12), 1304 (1974) discloses bifunctional analogs of EDTA. Representative of these compounds are 1-(p-nitrophenyl)-ethylenediaminetetraacetic acid 1-(p-aminophenyl)ethylenediaminetetraacetic acid and 1-(p-benzenediazonium)ethylenediaminetetraacetic acid. Coupling to proteins through the para-substituent and the binding of radio-active metal ions to the chelating group is discussed. The compounds are also disclosed in *Biochem. Biophys. Res. Comm.* **75**(1), 149 (1977) and in U.S. Patents 3,994,966 and 4,043,998. It is important to note that attachment of the aromatic group to the EDTA structure is through a carbon of the ethylenediamine backbone. Optically active bifunctional chelating agents based on EDTA, HEDTA and DTPA are disclosed in U.S. Patent 4,622,420. Also in this reference the attachment of the aminocarboxylic acid functionality to the rest of the bifunctional chelating molecule is through a carbon of the ethyleneamine backbone. In these compounds an alkylene group links the aromatic group (which contains the functionality needed for attachment to the protein) to the carbon of the polyamine which contains the chelating functionality. Other references to such compounds include Brechbiel et al. *Inorg. Chem.* **25**, 2772-2781 (1986), U.S. Patent 4,647,447 and a published PCT application having International Publication Number WO 86/06384. More recently, certain macrocyclic bifunctional chelating agents and the use of their copper chelate conjugates for diagnostic or therapeutic applications have been disclosed in U.S. Patent 4,678,667. Attachment of the aminocarboxylic acid functionality to the rest of the bifunctional chelating molecule is through a ring carbon of the cyclic polyamine backbone. Thus, a linker, attached at one end to a ring carbon of the cyclic polyamine, is also attached at its other end to a functional group capable of reacting with the protein.

Another class of bifunctional chelating agent, also worthy of note, consists of compounds wherein the chelating moiety, i.e. the aminocarboxylic acid, of the molecule is attached through a nitrogen to the functional group of the molecule containing the moiety capable of reacting with the protein. As an example Mikola et al. in a published PCT application (International Publication Number WO 84/03698, published 9/27/1984) disclose a bifunctional chelating agent prepared by reacting p-nitrobenzylbromide with DETA followed by reaction with bromoacetic acid to make the aminocarboxylic acid. The nitro group is reduced to the corresponding amine group and is then converted to the isothiocyanate group by reaction with thiophosgene. These compounds are bifunctional chelating agents which can be conjugated to bio-organic molecules for use as diagnostic agents capable of chelating lanthanides. Since attachment of the linker portion of the molecule is through one of the nitrogens of the aminocarboxylic acid, then one potential

aminocarboxyl group is lost for chelation. Thus, a DETA-based bifunctional chelant containing four (not five) acid groups is prepared. In this respect this class of bifunctional chelant is similar to those where attachment to the protein is through an amide group with subsequent loss of a carboxyl chelating group.

In the *J. Radioanalytical Chem.* 57(12), 553-564 (1980), Paik et al. disclose the use of p-nitrobenzyl-bromide in a reaction with a "blocked" diethylenetriamine, i.e. bis-(2-phthalimidoethyl)amine followed by deblocking procedures and carboxymethylation using chloroacetic acid, to give N'-p-nitrobenzyl-diethylenetriamine N,N,N'',N''-tetraacetic acid. Again, since the attachment is through a nitrogen, a tetraacetic acid derivative is obtained. Conjugation of the bifunctional chelating agent and chelating with indium is discussed. Substitution on the nitrogen atom is also taught by Eckelman et al. in the *J. Pharm. Sci.* 64(4), (1975) by reacting amines such as "ethylenediamine or diethylenetriamine with the appropriate alkyl bromide before carboxymethylation." The compounds are proposed as potential radiopharmaceutical imaging agents.

Recently Carney, Rogers, and Johnson disclosed (3rd. International Conference on Monoclonal Antibodies; San Diego, California - February 4-6, 1988) abstracts entitled "Absence of Intrinsically Higher Tissue Uptake from Indium-111 Labeled Antibodies: Co-administration of Indium-111 and Iodine-125 Labeled B72.3 in a Nude Mouse Model" and "Influence of Chelator Denticity on the Biodistribution of Indium-111 Labeled B72.3 Immunoconjugates in Nude Mice". The biodistribution of indium-111 complexed with an EDTA and DTPA bifunctional chelating agent is disclosed. Attachment of the aromatic ring to the EDTA/DTPA moieties is through an acetate radical. Previously Hunt et al. in U.S. Patents 4,088,747 and 4,091,088 (1978) disclosed ethylenediaminediacetic acid (EDDA) based chelating agents wherein attachment of an aromatic ring to the EDDA moiety is through the alkylene or acetate radical. The compounds are taught to be useful as chelates for studying hepatobiliary function. The preferred metal is technetium-99m. Indium-111 and indium 113 are also taught as useful radionuclides for imaging.

Martell et al. in the *Inorganica Chimica Acta* 138, 215-230 (1987) disclose an iron chelating agent for treating Cooley's anemia. The ligands used were analogs of EDTA with amino and carboxylate donor groups, or having additional donor groups present like phenolic or phenolic groups substituted on pyridine rings: aminophosphonic acid or ester groups with additional phenolate and amino donors; macrocyclic polyamines having carboxylate and/or phenolate donor groups; trishydroxamic acids; triscatechols; and multidentate ligands with coordinating amide groups.

The development of bone metastases is a common and often catastrophic event for a cancer patient. The pain, pathological fractures, frequent neurological deficits and forced immobility caused by these metastatic lesions significantly decrease the quality of life for the cancer patient. The number of patients that contract metastatic disease is large since nearly 50% of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastases. Bone metastases are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix and other tumors, and collectively, these represent less than 20% of patients who develop bone metastases. Metastatic bone cancer is rarely life threatening and occasionally patients live for years following the discovery of the bone lesions. Initially, treatment goals center on relieving pain, reducing requirements for narcotic medication and increasing ambulation. Clearly, it is hoped that some of the cancers can be cured.

The use of radionuclides for treatment of cancer metastatic to the bone dates back to the early 1950's. It has been proposed to inject a radioactive particle-emitting nuclide in a suitable form for the treatment of calcific lesions. It is desirable that such nuclides be concentrated in the area of the bone lesion with minimal amounts reaching the soft tissue and normal bone. Radioactive phosphorus (P-32 and P-33) compounds have been proposed, but the nuclear and biolocalization properties limit the utility of these compounds. [Kaplan, E., et al., *J. Nuc. Med.* 1(1), 1, (1960); (U.S. Patent 3,965,254)].

Another attempt to treat bone cancer has been made using phosphorus compounds containing a boron residue. The compounds were injected into the body (intravenously) and accumulated in the skeletal system. The treatment area was then irradiated with neutrons in order to activate the boron and give a therapeutic radiation dose. (U.S. Patent 4,399,817).

In the above mentioned procedures, it is not possible to give therapeutic doses to the tumor without substantial damage to normal tissues. In many cases, especially for metastatic bone lesions, the tumor has spread throughout the skeletal system and amputation or irradiation is not practical. (*Seminars in Nuclear Medicine* IX(2), April, 1979).

The use of Re-186 complexed with a diphosphonate has also been proposed. [Mathieu, L. et al., *Int. J. App. Rad. & Isot.* 30, 725-727 (1979); Weinenger, J., Ketring, A. R., et al., *J. Nuc. Med.* 24(5), 125, (1983)]. However, the preparation and purification needed for this complex limits its utility and wide application.

Strontium-89 has also been proposed for patients with metastatic bone lesions. However, the long half-life (50.4 days), high blood levels and low lesion to normal bone ratios can be disadvantageous. [Firusian, N.,

Mellin, P., Schmidt, C. G., *The Journal of Urology*, 116, 764, (1976); Schmidt, C. G., Firusian, N., *Int. J. Clin. Pharmacol.*, 93, 199-205, (1974)].

A palliative treatment of bone metastases has been reported which employed I-131 labelled α -amino-(3-iodo-4-hydroxybenzylidene)diphosphonate [Eisenhut, M., *J. Nuc. Med.* 25(12), 1356-1361, (1984)]. The use of radioiodine as a therapeutic radionuclide is less than desirable due to the well known tendency of iodine to localize in the thyroid. Eisenhut lists iodide as one of the possible metabolites of this compound. In addition, any I-131 left over from the iodination reaction and not separated in the washing procedure also constitutes a threat to the thyroid.

Aminocarboxylic acids are known to chelate metal ions. Particularly stable chelates are formed with metals from the alkaline earth and transition metal series.

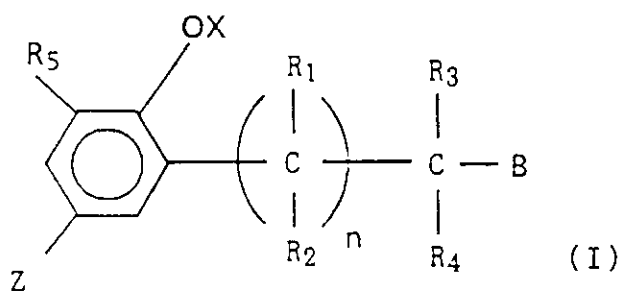
O'Mara et al. (*J. Nuc. Med.* 10, 49-51, 1969) have prepared rare earth complexes of aminocarboxylic acids at chelant to metal ratios of 10:1. They find good skeletal properties and propose their use as diagnostic skeletal agents. In addition to high bone uptake, high amounts of radiation were observed in muscle and/or liver. Of the rare earth nuclides evaluated Sm-153 and Er-171 were indicated as having the most suitable characteristics for imaging in humans. The utility of these agents for therapy, however, is not suggested.

Rosoff, B. et al., *Int. J. App. Rad. and Isot.* 14, 129-135 (1963), disclose complexes of EDTA and NTA with certain radionuclides, namely Sc-46, Y-91, La-140 and Sm-153. The relationship of the stability constant of these complexes to urinary excretion is shown. Chelant to metal molar ratios of 5:1 were employed and high concentrations of radioactivity were observed in the liver, spleen, kidney, lung and bone.

The present invention is directed to novel chelants possessing ortho ligating functionality, which chelant forms complexes with metals, especially "radioactive" metals having rare earth-type chemistry. Preferred radioactive metals include samarium-153 (^{153}Sm), holmium-166 (^{166}Ho), yttrium-90 (^{90}Y), promethium-149 (^{149}Pm), gadolinium-159 (^{159}Gd), lanthanum-140 (^{140}La), lutetium-177 (^{177}Lu), ytterbium-175 (^{175}Yb), scandium-47 (^{47}Sc) and praseodymium-142 (^{142}Pr). The complexes so formed can be used by themselves or can be attached to an antibody or fragment thereof and used for therapeutic and/or diagnostic purposes. The complexes and/or conjugates can be formulated for *in vivo* or *in vitro* uses. Preferred uses of the formulated conjugates is the treatment of cancer in animals, especially humans.

In addition certain of the chelant-radionuclide complexes can be effectively employed in compositions useful as therapeutic and/or diagnostic agents for calcific tumors and/or in compositions useful as therapeutic agents for the relief of bone pain.

More specifically, the present invention is directed to a bifunctional chelant possessing ortho ligating functionality having the formula



wherein:

Z is a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof selected from amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyl, bromoacetamido or maleimido;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄CO₂H;

R₁, R₂, R₃ and R₄ each are independently hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group with the proviso that when n = 0 and R₅ = H, then one of R₃ or R₄ must be CO₂H;

R₅ is hydrogen or (CR₁R₂)_nCR₃R₄B;

B represents a linear or branched amine or polyalkylene amine where at least one of the amine hydrogens has been substituted with a CR₃R₄CO₂H group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

It is preferred that the carboxyl group (when present) be attached to the first or second carbon from the nitrogen of the B group, i.e. the carbon α or β to the nitrogen in the chelant moiety. Preferred compounds of formula I are those where n is 0; or R₁, R₂, R₃ and R₄ are each hydrogen; or n is 0 and one of R₃ or R₄ is hydrogen and the other is COOH; or X is hydrogen.

The present invention is also directed to radioactive metal ion complexes, especially radioactive rare-earth type metal ion complexes, and to conjugates formed with the aforementioned complexes and antibody or antibody fragments. In addition, the present invention also includes formulations having the chelant-radionuclide complexes and/or the conjugates of the invention and a pharmaceutically acceptable carrier. Typically the pharmaceutically acceptable carrier in these formulations is in liquid form. The invention also includes a method for the diagnosis or treatment of a disease state, especially cancer, in a mammal by the administration to the mammal an effective amount of the formulation.

As used herein, the following indicated terms have these meanings: "synthetic linkers" include any synthetic organic or inorganic linkers which are capable of being covalently attached to an antibody or antibody fragment preferred synthetic linkers are biodegradable synthetic linkers which are stable in the serum of a patient but which have a potential for enzymatic cleavage within an organ of clearance for the radioisotope, for example biodegradable peptides or peptide containing groups. It is desirable that the nature and/or position of Z be such that it does not appreciably interfere with the chelation reaction.

The term "C₁-C₃" alkyl includes methyl, ethyl, n-propyl and isopropyl.

The terms "linear or branched amine or polyalkylene amine" mean straight or branched chain alkyl moieties that contain at least one, and usually more than one, nitrogen atom.

As used herein, the term "mammal" means animals that nourish their young with milk secreted by mammary glands, preferably warm blooded mammals, more preferably humans.

"Antibody" refers to any polyclonal, monoclonal, chimeric antibody or heteroantibody, preferably a monoclonal antibody; "antibody fragment" includes Fab fragments and F(ab')₂ fragments, and any portion of an antibody having specificity toward a desired epitope or epitopes. When using the term "radioactive metal chelate/antibody conjugate" or "conjugate", the "antibody" is meant to include whole antibodies and/or antibody fragments, including semisynthetic or genetically engineered variants thereof. Preferred antibodies are CC-49 and antibody fragments such as Fab and F(ab')₂. Other possible antibodies are CC-83 and B72.3. The hybridoma cell line B72.3 is deposited American Type Culture Collection (ATCC), having the accession number ATCC HB 8108. The other murine monoclonal antibodies bind to epitopes of TAG-72, a tumor associated antigen.

As used herein, "radioactive metal complex" or "complex" refers to a complex of the compound of the invention, e.g. formula I, complexed with a rare-earth type metal ion, especially a radioactive rare-earth type metal ion, where at least one metal atom is chelated or sequestered; "radioactive metal ion chelate/antibody conjugate" or "radioactive metal ion conjugate" refers to a radioactive metal ion conjugate that is covalently attached to an antibody or antibody fragment; "radioactive" when used in conjunction with the word "metal ion" refers to one or more isotopes of the rare-earth type elements that emit particles and/or photons, such as ¹⁵³Sm, ¹⁶⁶Ho, ⁹⁰Y, ¹⁴⁹Pm, ¹⁵⁹Gd, ¹⁴⁰La, ¹⁷⁷Lu, ¹⁷⁵Yb, ⁴⁷Sc and ¹⁴²Pr. The terms "bifunctional coordinator", "bifunctional chelating agent" and "functionalized chelant" are used interchangeably and refer to compounds that have a chelant moiety capable of chelating a metal ion and a linker/spacer moiety covalently bonded to the chelant moiety that is capable of serving as a means to covalently attach to an antibody or antibody fragment.

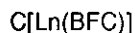
As used herein, "pharmaceutically acceptable salt" means any salt of a compound of formula (I) which is sufficiently non-toxic to be useful in therapy or diagnosis of mammals. Thus, the salts are useful in accordance with this invention. Representative of those salts formed by standard reactions from both organic and inorganic sources include, for example, sulfuric, hydrochloric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, d-camphoric, glutaric, glycolic, phthalic, tartaric, formic, lauric, steric, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic acids and other suitable acids. Also included are salts formed by standard reactions from both organic and inorganic sources such as ammonium, alkali metal ions, alkaline earth metal ions, and other similar ions. Particularly preferred are the salts of the compounds of formula (I) where the salt is potassium, sodium, ammonium, or mixtures thereof.

The bifunctional chelating agents described herein (represented by formula I) can be used to chelate or sequester the rare-earth type metal ions, particularly radioactive rare-earth type metal ions, so as to form metal ion chelates (also referred to herein as "complexes"). The complexes, because of the presence of the functionalizing moiety (represented by "Z" in formula I), can be attached to functionalized supports, such as functionalized polymeric supports, or preferably covalently attached to antibodies or antibody fragments. Thus the complexes described herein may be covalently attached to an antibody or antibody fragment and

are referred to herein as "conjugates".

The antibodies or antibody fragments which may be used in the conjugates described herein can be prepared by techniques well known in the art. Highly specific monoclonal antibodies can be produced by hybridization techniques well known in the art, see for example, Kohler and Milstein [*Nature* 256, 495-497 (1975); and *Eur. J. Immunol.* 6, 511-519 (1976)]. Such antibodies normally have a highly specific reactivity. In the antibody targeted radioactive metal ion conjugates, antibodies directed against any desired antigen or hapten may be used. Preferably the antibodies which are used in the radioactive metal ion conjugates are monoclonal antibodies, or fragments thereof having high specificity for a desired epitope(s). Antibodies used in the present invention may be directed against, for example, tumors, bacteria, fungi, viruses, parasites, mycoplasma, differentiation and other cell membrane antigens, pathogen surface antigens, toxins, enzymes, allergens, drugs and any biologically active molecules. Some examples of antibodies or antibody fragments are CC-11, CC-15, CC-30, CC-46, CC-49 F(ab')₂, CC-49, CC-83, CC-83 F(ab')₂, CC-92 and B72.3. [See D. Colcher et al., *Cancer Res.* 48, 4597-4603 (Aug. 15, 1988) for CC-49, CC-83 and B72.3 antibodies.] The following CC antibodies have been deposited in ATCC as follows: CC-11 as HB 9455; CC-15 as HB 9460; CC-30 as HB 9457; CC-46 as HB 9458; CC-49 as HB 9459; CC-83 as HB 9453; and CC-92 as HB 9454. B72.3 has been deposited in ATCC as HB 8108. A more complete list of antigens can be found in U.S. Patent 4,193,983. The radioactive metal ion chelate/antibody conjugates of the present invention are particularly preferred for the diagnosis and treatment of various cancers.

The preferred rare-earth type (lanthanide or pseudo-lanthanide) complexes of the present invention are represented by the formula.



wherein: Ln is a rare-earth metal (lanthanide) ion, such as Ce³⁺, Pr³⁺, Nd³⁺, Pm³⁺, Sm³⁺, Eu³⁺, Gd³⁺, Tb³⁺, Dy³⁺, Ho³⁺, Er³⁺, Tm³⁺, Yb³⁺ and Lu³⁺, or pseudo-lanthanide metal ion, such as Sc³⁺, Y³⁺ and La³⁺; BFC represents a bifunctional chelant; and C represents a pharmaceutically acceptable ion or group of ions of sufficient charge to render the entire complex neutral. If the BFC contains four or more negatively charged moieties, then C is a cation or group of cations such as H⁺, Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Ra²⁺, NH₄⁺, N(CH₃)₄⁺, N(C₂H₅)₄⁺, N(C₃H₇)₄⁺, N(C₄H₉)₄⁺, As(C₆H₅)₄⁺, [(C₆H₅)₃P=]₂N⁺ and other protonated amines. If the BFC contains three negatively charged moieties, then C is not required. If the BFC contains two negatively charged moieties, then C is an anion such as F⁻, Cl⁻, Br⁻, I⁻, ClO₄⁻, BF₄⁻, H₂PO₄⁻, HCO₃⁻, HCO₂⁻, CH₃SO₃⁻, H₃C-C₆H₄-SO₃⁻, PF₆⁻, CH₃CO₂⁻ and B(C₆H₅)₄⁻.

This invention is used with a physiologically acceptable carrier, excipient or vehicle therefor. The methods for preparing such formulations are well known. The formulations may be in the form of a suspension, injectable solution or other suitable formulations. Physiologically acceptable suspending media, with or without adjuvants, may be used.

An "effective amount" of the formulation is used for therapy. The dose will vary depending on the disease being treated. Although *in vitro* diagnostics can be performed with the formulations of this invention, *in vivo* diagnostics are also contemplated using formulations of this invention. The conjugates and formulations of this invention can also be used in radioimmuno guided surgery (RIGS); however, other metals which could be used for this purpose also include ^{99m}Tc, ¹¹¹In, ^{113m}In, ⁶⁷Ga and ⁶⁸Ga.

When the chelant-radionuclide complexes of this invention are to be used for the treatment of bone cancer certain criteria must be met. While the properties of the radionuclide are important, the overall properties of the composition containing the radionuclide-chelant complex is the determining factor. The disadvantages of any one property may be overcome by the superiority of one or more of the properties of either ligand or radionuclide and their combination, as employed in the composition must be considered in toto.

The following is a discussion of those criteria which must be considered in choosing any particular combination (i.e., complex) of radionuclide and ligand employed in the compositions of the invention. Radionuclide-chelant complexes, when used in the absence of an appropriate excess of the ligands employed in the invention may not be therapeutically useful or effective.

There is a need, therefore, for compositions possessing the following criteria by which it is possible to deliver therapeutic radiation doses to calcific tumors with minimal doses to soft tissue.

The radionuclide must be delivered preferentially to the bone rather than to soft tissue. Most particularly, uptake in either liver or blood is undesirable.

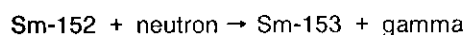
The radionuclide should be cleared rapidly from non-osseous tissue to avoid unnecessary damage to such tissues, e.g., it should clear rapidly from the blood.

The proposed use for some of the compositions of this invention is the therapeutic treatment of calcific tumors in animals. As used herein, the term "calcific tumors" includes primary tumors, where the skeletal system is the first site of involvement, and metastatic bone cancer where the neoplasm spreads from other primary sites, such as prostate and breast, into the skeletal system. This invention provides a means of alleviating pain and/or reducing the size of, and/or inhibiting the growth and/or spread of, or causing regression of and/or destroying the calcific tumors by delivering a therapeutic radiation dose.

The composition may be administered as a single dose or as multiple doses over a longer period of time. Delivery of the radionuclide to the tumor must be in sufficient amounts to provide the benefits referred to above.

Other uses of some of the chelants of the present invention may include the removal of undesirable metals (i.e. iron) from the body, magnetic resonance imaging, attachment to polymeric supports for various purposes, e.g. as diagnostic agents, and removal of lanthanide metal or pseudo-lanthanide metal ion by selective extraction. In addition the metal-ligand complexes used to deliver radionuclides to calcific sites may have utility for the ablation of bone marrow (i.e. for bone marrow transplants).

Radionuclides can be produced in several ways. In a nuclear reactor a nuclide is bombarded with neutrons to obtain a radionuclide, e.g.



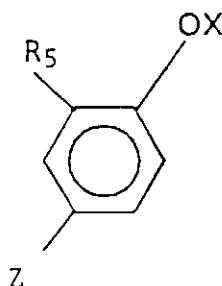
Another method of obtaining radionuclides is to bombard nuclides with particles produced by a linear accelerator or a cyclotron. Yet another way is to isolate the radionuclide from a mixture of fission products. The method of obtaining the nuclides employed in the present invention is not critical thereto.

The chelating agents disclosed herein can be prepared in ways well known to the art. Thus, for example, see *Chelating Agents and Metal Chelates*, Dwyer & Mellor, Academic Press (1964), Chapter 7. See also methods for making amino acids in *Synthetic Production and Utilization of Amino Acids*, (edited by Kameko, et al.) John Wiley & Sons (1974).

When Z (in the formula) is chosen to be an electrophilic moiety it can be prepared by methods known to the art. Such methods may be found in *Acc. Chem. Res.* 17, 202-209 (1984).

Examples of some of the methods which may be used to prepare the chelants of formula I are:

A) reacting a compound of the formula



wherein: Z has the meaning given for formula I

X is hydrogen;

R₅ is hydrogen or (CR₁R₂)_nCR₃R₄T, where R₁, R₂, R₃ and R₄ each are independently hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group, n is 0 or 1, and T represents a linear or branched amine or polyalkylene amine where at least one of the amine hydrogens has been substituted with a CR₃R₄CO₂H group; or

a pharmaceutically acceptable salt thereof;

with a compound B and an aldehyde or aldehyde precursor equivalent, where B represents a linear or branched amine or polyalkylene amine where there is at least one amine hydrogen;

in the presence of caustic and a suitable solvent, at a temperature of 20 °C or less, followed by heating and separating the desired product of formula I;

B) reacting the product obtained from Step (A) with a halo-(CR₁R₂)_nCR₃R₄ acid, at a pH of 9 or higher, in the presence of caustic, at a temperature of 20 °C or less, to provide the compound of formula I where at least one of R₁, R₂, R₃ and R₄ is CO₂H;

C) hydrolyzing the product of Step (B) where Z is NHC(O)CH_3 with NaOH in H_2O , to provide the product of formula I where Z is NH_2 .

D) reacting the product obtained from Step (A) with glycolonitrile, in caustic, at a pH of 9 or higher, at a temperature of 20°C or less, followed by hydrolysis of the cyano group with HCl in H_2O , to provide the

E) hydrolyzing the product of Step (A) where Z is NHC(O)CH_3 with DCl in D_2O , with heating, to provide the product of formula I where Z is NH_2 ; and

F) reacting the product obtained from any one of Steps (A) through (E), where Z is NH_2 , with thiophosgene to provide the product of formula I where Z is isothiocyanato.

The reaction conditions and reagents for the various steps above are as follows. When the temperature is " 20°C or less" this is usually accomplished by use of an ice/water bath. "Heating" is done either at reflux or above room temperature. The preferred "caustic" is sodium hydroxide, but any suitable base the is able to maintain the desired pH without adverse effect on the product formed from the reaction is acceptable. A "suitable solvent" is inert and provides solubility for the reactants, examples of such solvents are water, and alcohols such as methanol. The desired product may be separated by any conventional methods, for example precipitation from a solvent such as acetone.

The complexes of formula I are prepared by conventional methods, for example by reacting the chelant with the metal under conditions such that the metal is sequestered by the chelant. Frequently, the chelant is in excess to that of the metal.

The conjugates of formula I are prepared by conventional methods, for example by covalently attaching the complex to an antibody or antibody fragment.

The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the use of the invention. Structures of compounds with reference to the generic formula I are shown in Table I.

Preparation of Starting Materials

Example A. PREPARATION OF UNSYMMETRICAL ETHYLENEDIAMINEDIACETIC ACID.

Deionized water (60.6 g), 98% N-acetylenediamine (20.4 g, 0.2 mole), and bromoacetic acid (55.7 g, 0.40 mole) were added to a reaction vessel and cooled in an ice-water bath. The pH of the mix was adjusted, while stirring, to approximately 8.1 with 25% sodium hydroxide solution. The temperature of the mix was maintained at less than 20°C during the caustic addition. The ice-water bath was removed and the pH maintained between 7 and 8 by the addition of 25% sodium hydroxide solution. The temperature was controlled at less than 37°C by periodically cooling with an ice-water bath. The reaction mixture was stirred and maintained as above for approximately 31 hours and then transferred to a round-bottom reaction flask equipped with a water-cooled reflux condenser, magnetic stirrer bar, thermometer, addition funnel and a heating mantle. Sodium hydroxide solution (40.1 g of 50% solution) was added and the mix heated, with stirring, at reflux for approximately 15 hours and then cooled and filtered using a medium glass frit funnel and vacuum. The filtrate was transferred quantitatively (using deionized water) to a beaker and cooled in an ice-bath to less than 25°C . Deionized water (100 ml) was added with stirring and the pH adjusted to approximately 4 with concentrated hydrochloric acid while maintaining the temperature at less than 25°C . The mix was filtered using a medium glass frit funnel and vacuum. Approximately 1200 ml of ethanol were added to a large beaker and stirred with a magnetic stirrer bar. The filtrate from above was added to the ethanol with thorough mixing. An oily material forms which gradually turns into a white solid. Agitation was continued for two hours at which time the solids were collected by filtering using a medium glass frit funnel and vacuum. The solids were allowed to dry by exposure to air for about 1.5 hours and then placed in a vacuum oven and dried at $55\text{--}60^\circ\text{C}$ for several hours. Approximately 42.9 g of white solids containing inorganic salt were collected and identified as unsymmetrical ethylenediaminediacetic acid by proton and carbon NMR.

Example B. PREPARATION OF 2-OXO-1-PIPERAZINEACETIC ACID; LACTAM OF ETHYLENEDIAMINEDIACETIC ACID.

Deionized water (150 g), 25.0 g (0.14 mole) of symmetrical ethylenediaminediacetic acid, and 28 g of concentrated hydrochloric acid were added to a round-bottom reaction flask equipped with a thermometer, temperature controller, water-cooled reflux condenser, and heating mantle. The mixture was stirred with a magnetic stirrer bar and heated at reflux for four hours and cooled. The contents were filtered using a

medium glass frit funnel and vacuum. The pH of the filtrate was adjusted to approximately 1.5 with 50% sodium hydroxide solution and filtered with a medium glass frit funnel using vacuum. The pH of the filtrate was adjusted to about 5 with 50% sodium hydroxide solution and the volatiles removed (*in vacuo*) at a temperature of 60-70 °C. The solids were dried in a vacuum oven at 55-60 °C for several hours. The lactam of symmetrical ethylenediaminediacetic acid was confirmed by proton and carbon NMR.

Example C. PREPARATION OF 2-OXO-1,4-PIPERAZINEDIACETIC ACID; LACTAM OF ETHYLENEDIAMINETRIACETIC ACID.

Approximately 40.8 g of 2-oxo-1-piperazineacetic acid, prepared by the procedure of Example B, and 70 g of deionized water were added to a beaker and stirred for several hours with a magnetic stirrer bar. The contents were filtered using a medium glass frit funnel and vacuum. The filtrate and 20.0 g of bromoacetic acid were added to a beaker and stirred till all the bromoacetic acid had dissolved. The pH was adjusted to approximately 7 with 25% sodium hydroxide solution. The temperature was maintained at less than 25 °C during the caustic addition by cooling in an ice-water bath. The ice-water bath was removed and the mix allowed to stir for approximately 4-5 hours at approximately 35 °C while maintaining the pH at about 7 by the periodic addition of 25% sodium hydroxide solution. The reaction mix was allowed to stand for several hours and then concentrated (*in vacuo*) to a weight of approximately 90-100 g and filtered using a medium glass frit funnel and vacuum. Volatiles were removed (*in vacuo*) from the filtrate at a temperature of 55-60 °C and the material dried in a vacuum oven at 55-60 °C for several hours. The lactam of ethylenediaminetriacetic acid was confirmed by proton and carbon NMR.

Example D. PREPARATION OF TRISODIUM ETHYLENEDIAMINETRIACETIC ACID.

Approximately 44.5 g of the crude 2-oxo-1,4-piperazinediacetic acid, prepared by the procedure of Example C, and 280 g of deionized water were added to a beaker and agitated till the lactam had dissolved. Caustic solution (110 g, 50%) was added with agitation. The temperature was maintained at less than 25 °C by cooling in an ice-bath. Hydrolysis was then achieved by immersing tubes containing the solution into a water bath controlled at 87 °C. After 15 minutes, the solutions were removed and cooled in an ice-water bath. Analysis by proton and carbon NMR confirmed the presence of trisodium ethylenediaminetriacetic acid in the alkaline hydrolysis medium.

Example E. PREPARATION OF 4-DIETHYLENETRIAMINEACETIC ACID.

To a flask equipped with a water-cooled reflux condenser, magnetic stirrer and thermometer were added 75.0 g of phthalic anhydride, 350.5 g of acetic acid and 26.0 g of diethylenetriamine. The mix was stirred and heated at approximately 116 °C for 1.5 hours and then cooled. Volatiles were removed under vacuum at 65-70 °C until a weight of 218 g was obtained. The mixture was poured into 600 g of ethanol with stirring. After two hours the solids were filtered using a medium glass frit funnel. The solids were washed twice with 500 ml of ethanol and then dried in a vacuum oven at 60-65 °C. Approximately 66 g of material of the diphthaloyl compound were collected.

The ethyl ester of the diphthaloyl compound was prepared by adding 65.6 g of the above prepared diphthaloyl compound, 17.7 g of sodium carbonate and 800 ml of ethanol to a flask equipped with a water-cooled reflux condenser, addition funnel, mechanical stirrer and a thermometer with a temperature controller. Ethyl bromoacetate (51.0 g) was added over a 15 minute period to the stirred mixture and then heated at reflux for 16 hours. Ethanol was removed (200 ml) by distillation using a Dean-Stark distillation trap and the remaining reaction mixture cooled to less than 5 °C by the addition of crushed ice. The mixture was cooled for an additional 5 hours in an ice bath and filtered using a medium glass frit funnel. The solids were washed twice with ethanol and dried in a vacuum oven at 65-70 °C. Approximately 81 g of ethyl 1,7-diphthaloyl-4-diethylenetriamineacetate were obtained. In 30.32 g of water and 76.4 g of concentrated hydrochloric acid was dissolved 20.1 g (0.045 moles) of ethyl 1,7-diphthaloyl-4-diethylenetriamineacetate with heating to 93 °C and the mixture held at 93 °C for 6.5 hours. The resulting white precipitate was filtered and washed with water. The combined filtrate was concentrated at 60 °C under vacuum to give a white solid. NMR analysis indicated that the phthaloyl groups were not completely hydrolyzed. The two solids were then combined and added to concentrated hydrochloric acid with a small amount of water. The slurry was then heated to reflux for 6 hours, cooled to room temperature and filtered to give 12.3 g of phthalic acid. The filtrate was then evaporated under vacuum to give 13.9 g of product as a yellow solid. The product was dissolved in water by the addition of 6 g of 50% sodium hydroxide and treated with activated

charcoal at 100 °C followed by filtration and evaporation under vacuum to give 15.2 g 4-diethylenetriamineacetic acid.

Preparation of Final Products

5

Example 1a) PREPARATION OF 2-[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)AMINO]-2-(5-ACETAMIDO-2-HYDROXYPHENYL)-ETHANOIC ACID.

Deionized water (10.3 g), 98% 4-acetamidophenol (15.1 g 0.1 mole), 50% aqueous glyoxylic acid (14.8 g, 0.1 mole), and methanol (50.5 g) were added to a beaker and mixed using a magnetic stirrer bar. Unsymmetrical ethylenediaminediacetic acid (19.5 g), prepared by the procedure of Example A, was added and the mix cooled in an ice-water bath. The pH of the mix was adjusted, while stirring, to approximately 8.0 with 50% sodium hydroxide solution. The temperature of the mix was maintained at less than 20 °C during the caustic addition. The ice-water bath was removed and the mix adjusted to pH 8.7 and stirred at 25-32 °C for approximately 2 hours. The mix was transferred to a round-bottom reaction flask equipped with a water-cooled reflux condenser, magnetic stirrer bar, thermometer, and a heating mantle. The mix was heated, with stirring, at 70 °C for 8 hours and then cooled and filtered using a medium glass frit funnel and vacuum. The solids were allowed to dry by exposure to air for 7 hours and then placed in a vacuum oven and dried at 55-60 °C for several hours. Approximately 29.6 g of solids were collected. The material was then agitated with approximately 300 g of acetone and filtered using a medium glass frit funnel and house vacuum. The solids were washed once again with an additional 300 g of acetone, air dried and then placed in a vacuum oven for one hour at 60 °C. Approximately 26.7 g of 2-[(2-{[bis(carboxymethyl)]amino}ethyl)-amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid, sodium salt were collected. These solids and 180 g of deionized water were placed in a beaker and stirred with a magnetic stirrer bar. The pH was adjusted to 2.2 with concentrated hydrochloric acid at which point the acid form of the product began to precipitate from solution. The product was collected by filtration and washed with approximately 150 g of deionized water. The product, 2-[(2-{[bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)-ethanoic acid was dried in a vacuum oven at 55-60 °C for several hours. Approximately 14.2 g of product were obtained. Proton NMR verified the structure of the product. (See Table I.)

30

b) PREPARATION OF 2-[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)AMINO]-2-[5-ACETAMIDO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID.

Deionized water (4.5 g), bromoacetic acid (2.0 g) and 2-[(2-{[bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid (2.5 g), prepared by the procedure of part (a) were added to a small reaction vessel and cooled in an ice-water bath. The pH of the mix was adjusted, while stirring, to approximately 9.3 with 25% sodium hydroxide solution. The temperature of the mix was maintained at less than 20 °C during the caustic addition. The ice-water bath was removed and the mix allowed to stir for 48 hours at a temperature of 35-40 °C while maintaining the pH between 10.5 and 11.5 by the periodic addition of 25% sodium hydroxide solution. A portion of the reaction mixture (10.2 g) was added to a beaker and stirred with a magnetic stirrer bar. Acetone (125 g) was added to the solution over a 15-minute period resulting in the precipitation of an oil. The acetone portion was removed by decanting and an additional 50 g of acetone added to the precipitate, mixed, and the acetone layer removed. The oil was air dried and then dried in a vacuum oven at 60-65 °C for about two hours to give a crispy yellow solid. The product was purified by anion exchange chromatography on Q-Sepharose™ from Pharmacia Inc. on a 15 mm X 500 mm column eluting with a gradient of 0-30% formic acid over two hours at a rate of 3 ml/min and collecting fractions. The fractions were monitored by UV absorption and the appropriate fractions combined and lyophilized to give the desired product. (See Table I.)

c) PREPARATION OF 2-[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID, PENTA SODIUM SALT.

Approximately 40 mg of 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid, prepared by the procedure of part (b) was dissolved in 700 µl of D₂O and adjusted with NaOD/D₂O to pH 13. Hydrolysis of the N-acetyl group to the corresponding aniline functionality proceeded at ambient temperature and was followed by proton NMR which confirmed the structure. (See Table I.)

Example 2 a) PREPARATION OF 2-[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CYANOMETHYL)-AMINO]-2-(5-ACETAMIDO-2-HYDROXYPHENYL)ETHANOIC ACID.

Deionized water (3.1 g) and 2.5 g of 2-[(2-{[bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid, prepared by the procedure of Example 1 a) were added to a small glass vessel and cooled in an ice-water bath. The pH was adjusted to 9.8-9.9 with 25% sodium hydroxide solution. The temperature of the mix was maintained at less than 20 °C during the caustic addition. The ice-bath was removed and 1.0 g of an aqueous 40% glycolonitrile solution was added with mixing and the pH adjusted to 9.9-10.0 with 25% sodium hydroxide solution. The mix was transferred to a small reaction flask equipped with a thermometer containing a temperature controller, water cooled reflux condenser, and heating mantle. The reaction mixture was stirred with a magnetic stirrer bar and heated at 49-50 °C for eight hours, cooled and allowed to stand at ambient temperature for 72 hours. A portion of the reaction mixture (8.5 g) was added to a beaker and stirred with a magnetic stirrer bar. Acetone (146 g) was added to the solution over a 10-minute period, resulting in the precipitation of a solid material. The acetone portion was removed by decanting and an additional 50 g of acetone added to the precipitate, mixed, and the acetone layer removed. The material was dried in a vacuum oven at 60-65 °C for about four hours. Approximately 2.9 g of product was collected. Proton NMR supported the desired aminoacetonitrile derivative. (See Table I.)

b) PREPARATION OF 2-[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)AMINO]-2-(5-AMINO-2-HYDROXYPHENYL)ETHANOIC ACID.

Approximately 1.0 g of 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(cyanomethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid, prepared above by the procedure of part (a) was hydrolyzed under acidic conditions to convert the aminoacetonitrile functionality to the corresponding acetate group and the N-acetyl group to the aniline group. The aminoacetonitrile compound, 2.2 g of D₂O, and 7.8 g of 20% DCl were added to a glass tube. The tube was placed in a temperature-controlled water bath at 88-89 °C for a total of 33 minutes and then removed and cooled. The hydrolysis was followed by proton NMR. The solution was then freeze-dried and lyophilized to give 1.3 g of solids. The product was purified by anion exchange (Q-Sepharose™) on a 15 mm X 500 mm column eluting with a gradient of 0-1 M acetic acid over one hour at a rate of 3 ml/min and collecting 6 ml fractions. The fractions were monitored by UV absorption and the appropriate fractions were combined and lyophilized to give the desired product. (See Table I.)

Example 3 a) PREPARATION OF 2-[BIS(2-{[(BIS(CARBOXYMETHYL)]AMINO}ETHYL)AMINO)-2-[5-ACETAMIDO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID AND 2-{[2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)AMINO]ETHYL} (CARBOXYMETHYL)AMINO]-2-[5-ACETAMIDO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID.

Deionized water (24.8 g), 15.1 g (0.1 mole) of 98% 4-acetamidophenol, and 14.8 g of 50% aqueous glyoxylic acid (0.1 mole) were added to a beaker and cooled in an ice-water bath. The pH of the mix was adjusted to 3.3 with 25% sodium hydroxide solution while maintaining the temperature at less than 20 °C. DETA (9.8 g) was then added. Once again the temperature was kept below 20 °C by cooling in an ice-water bath. The pH after addition of the DETA was approximately 10.2. The mix was transferred to a reaction flask equipped with a thermometer, a temperature controller, water-cooled reflux condenser, and heating mantle. The reaction mixture was stirred with a magnetic stirrer bar and heated at 75 °C for approximately seven hours and cooled. Acetone (1400 g) was added to a large beaker and stirred with a magnetic stirrer bar. Approximately 40 g of the reaction solution prepared above was added over a 10 minute period resulting in the precipitation of a solid material. The acetone portion was removed by decanting and an additional 1460 g of acetone added and the solid titrated and mixed thoroughly under acetone. The solids were recovered by filtering using a medium glass frit funnel and vacuum. The solids were washed with a copious amount of acetone and then dried in a vacuum oven at a temperature of 60-65 °C for several hours. Approximately 7.8 g of solids were recovered with proton NMR showing a mixture of the desired isomers of the DETA compound.

Deionized water (5.3 g) and 4 g of the above isolated solids were added to a beaker and stirred with a magnetic stirrer bar for about three hours at which time the solids had completely dissolved. Bromoacetic acid (10.1 g) was added with stirring and the mix cooled in an ice-water bath. The pH of the mix was adjusted to approximately 11 with 25% sodium hydroxide solution. The temperature was maintained at less than 20 °C during the caustic addition. The ice-water bath was removed and the mix allowed to stir for 50

hours at a temperature of 35-40 °C while maintaining the pH between approximately 10.5-11.5 by the periodic addition of 25% sodium hydroxide solution. Acetone (240 g) was added to a beaker and stirred with a magnetic stirrer bar. Approximately 5 g of the reaction solution was added to the acetone resulting in the precipitation of a solid. The acetone portion was decanted and an additional 245 g of acetone added, mixed and the acetone layer removed. The solids were collected by filtering using a medium glass frit funnel and vacuum. The solids were washed with acetone and then dried in a vacuum oven at 55-60 °C for several hours. Approximately 2.6 g of solids were collected. (See Table I.)

b) PREPARATION OF 2-[BIS(2-{[(BIS(CARBOXYMETHYL)]AMINO}ETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID AND 2-[{2-[(2-{[(BIS(CARBOXYMETHYL)]AMINO}ETHYL)-(CARBOXYMETHYL)AMINO]ETHYL} (CARBOXYMETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID.

Approximately 376 mg of 2-[bis(2-{[(bis(carboxymethyl)]amino)ethyl]amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid and 2-[{2-[(2-{[(bis(carboxymethyl)]amino)ethyl)(carboxymethyl)amino]-ethyl} (carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid, prepared by the procedure of part (a) was dissolved in 1.0 g of D₂O and treated with 5 drops of 37% DCl. The acidic solution was then heated at 80 °C for 2 hours after which time the proton NMR spectrum indicated that virtually all of the acetanilide groups had been converted to aniline groups and acetic acid. The solution was then frozen in a dry-ice acetone bath and lyophilized overnight to yield the desired product as a light brown solid. (See Table I.)

Example 4 PREPARATION OF 2-[{2-[(2-{[(BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)-AMINO]ETHYL}(CARBOXYMETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID.

In 40 ml of water was dissolved 8.0 g of 4-diethylenetriamineacetic acid, prepared by the procedure of Example E, and then the mixture was cooled in an ice bath. To this cooled solution was added 6.08 g (0.04 moles) of 4-acetamidophenol and a chilled solution of 5.95 g (0.04 moles) of a 50 weight % solution of glyoxylic acid in water. While keeping the slurry at less than 20 °C with the ice bath, a 2.5 ml portion of 50 weight % sodium hydroxide was added. The resulting slurry at pH 8.75 was heated slowly to 80 °C, held at this temperature for 4.5 hours with stirring, then allowed to cool overnight. The solution was then evaporated under vacuum to about 25 ml volume and added to 300 ml of acetone. The acetone was decanted from the resulting solid. The solid was washed several times with acetone and dried to give 26.1 g of product as a dark sticky solid. A 26.05 g portion of this solid was dissolved in 50 ml of water. Into this solution was dissolved 26.7 g (0.192 moles) of bromoacetic acid. The resulting solution was cooled in an ice bath, the pH adjusted to 10.5 with 50 weight % sodium hydroxide, allowed to warm to room temperature, and then heated to 46 °C. The temperature was kept at 46 °C and the pH kept at 10.5 by addition of 50 weight % sodium hydroxide for about 23 hours. The volume was then reduced to 50 ml under vacuum. The concentrated solution was added to 500 ml of acetone with vigorous stirring and the resulting precipitate allowed to settle. The acetone was decanted and an additional 400 ml of acetone was added, vigorously stirred and then decanted. A final wash in the same manner using 100 ml of acetone was done. The solid was dried under vacuum to give 52.55 g of crispy brown solid. A 2.00 g sample of this brown solid was dissolved in 20 ml of water and treated with 1.48 g of concentrated hydrochloric acid. This solution was heated at 80 °C until analysis by proton NMR indicated complete hydrolysis of the N-acetyl moiety. The solution was then freeze dried to give 2.13 g of brown solid containing the title product. (See Table I.)

Example 5 a) PREPARATION OF 2,6-BIS{[(2-{[(BIS(CARBOXYMETHYL)]AMINO}ETHYL)-(CARBOXYMETHYL)]AMINOMETHYL}-4-(ACETAMIDO)PHENOL.

The alkaline trisodium ethylenediaminetriacetic acid solution, prepared by the procedure of Example D, was cooled in an ice-bath and hydrochloric acid added with stirring to obtain a pH of about 13.8. The temperature was maintained at less than 35 °C during the acid addition. Volatiles were removed (*in vacuo*) at ambient temperature to a weight of 210 g. The solids were removed by filtering on a medium glass frit funnel using vacuum. The filtrate was transferred to a 250 ml round-bottom flask equipped with a water-cooled reflux condenser, magnetic stirrer bar, thermometer, temperature controller, heating mantle, and addition funnel. The pH was adjusted to about 11 with hydrochloric acid. The temperature was maintained at less than 30 °C during the acid addition. The mix was heated to approximately 40 °C and 11.6 g of 37%

aqueous formaldehyde solution added dropwise from the addition funnel over a 35 minute period. The reaction mixture was stirred and heated for an additional 30 minutes and then cooled. The solution was adjusted with 25% sodium hydroxide solution to a pH of about 9.8 and transferred to an addition funnel. To a beaker was added 10.3 g of 98% 4-acetamidophenol, 25.2 g of deionized water, and 9.5 g of 25% sodium hydroxide solution. The mix was stirred till complete dissolution was obtained. The solution was transferred to a round-bottom reaction flask equipped as described above and heating and stirring initiated. The mix was heated to approximately 65 °C at which point the formaldehyde adduct solution prepared above was added dropwise over approximately a one hour period. The reaction was stirred and heated at 65 °C for an additional 12 hours and then cooled. Acetone (150 g) was added to a beaker and stirred with a magnetic stirrer bar. Approximately 10 g of the crude reaction mixture was added to the acetone resulting in the precipitation of an oily material. The acetone portion was decanted and an additional 150 g of acetone added to the precipitate, mixed, and the acetone layer removed. The material was dried in a vacuum oven at 55-60 °C for several hours. Approximately 3.1 g of solids were collected. Approximately 165 mg of the solids were dissolved in a minimum of water and loaded onto a Q-Sepharose™ (from Pharmacia Inc.) column [1.5 cm X 50 cm., acetate form] and eluted using a gradient of 0 to 1 M ammonium acetate over two hours at 2 ml/min. The absorbance at 300 nm was observed. The product was contained in the third major peak. This was isolated and freeze-dried to yield 36.4 mg of solids which was characterized by proton and carbon NMR and fast atom bombardment mass spectrometry as 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)]aminomethyl}-4-(acetamido)phenol. (See Table I.)

b) PREPARATION OF 2,6-BIS{[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)]AMINOMETHYL}-4-(AMINO)PHENOL.

Approximately 264 mg. of 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)]aminomethyl}-4-(acetamido)phenol, prepared by the procedure of part (a) was placed in a 5 mm NMR tube and dissolved in a mixture of D₂O (0.5 ml) and DCL (0.5 ml, 20%). The NMR tube was placed in a hot water bath (85 °C) for short periods of time and the reaction progress monitored by NMR (disappearance of the acetamide methyl protons and appearance of acetic acid). After 35 minutes the reaction was complete. The reaction mixture was freeze-dried to yield the crude amine hydrochloride as a dark solid material. The crude product was dissolved in a minimum amount of water and loaded onto a Q-Sepharose™ column (1.5 cm X 50 cm, acetate form) and eluted using a gradient of 0 to 1 M ammonium acetate over three hours at 2 ml/min. The absorbance at 300 nm was observed. The product was contained in the third major peak. This was isolated and freeze-dried to leave a pale amber solid (122 mg) which was a mixture of the desired amine product and ammonium chloride. The product mixture was characterized by proton and carbon NMR and elemental analysis. The salt-containing product (250 mg from combined batches) was further purified on Q-Sepharose™ (1.5 cm X 50 cm, formate form) using a gradient of 0 to 10% formic acid over four hours. The absorbance at 300 nm was observed. The first major peak contained the desired product. This was isolated and freeze-dried to yield 8.3 mg of a white crystalline solid. The structure was confirmed by proton and carbon NMR and fast atom bombardment mass spectrometry. (See Table I.)

Example 6 PREPARATION OF 2,6-BIS{[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)(CARBOXYMETHYL)]AMINOMETHYL}-4-(ISOTHIOCYANATO)PHENOL.

Product containing 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)]aminomethyl}-4-(amino)phenol and inorganic salt (208 mg, 15% in NH₄Cl), prepared by the procedure of Example 5 b) was dissolved in a minimum amount of water and passed through a Sephadex™ G-10 (Pharmacia, Inc.) desalting column (1 cm X 35 cm). The salt-free amine was eluted with water and freeze-dried (11.5 mg). The amine was dissolved in water (10 ml) and placed in a round-bottom reaction flask. Thiophosgene (0.015 ml, 10 eq) dissolved in methylene chloride (1 ml) was added. The reaction mixture was stirred at room temperature for one hour. The mixture was then washed with several portions of methylene chloride to remove excess thiophosgene and the aqueous layer freeze-dried to give the crude isothiocyanato product which was characterized by fast atom bombardment mass spectrometry. (See Table I.)

Example U. PREPARATION OF N,N'-DI(2-HYDROXY, 5-ACETAMIDOBENZYL)ETHYLENEDIAMINE-N,N'-DIACETIC ACID. (Comparative)

Ethylenediamine-N,N'-diacetic acid (10 g, 0.056 mole), 25 g of deionized water, 7.0 g of 50% sodium hydroxide solution, and 5.0 g of methanol were added to a round-bottom reaction flask equipped with a

water-cooled reflux condenser, mechanical stirrer, thermometer with a temperature controller, and an addition funnel. The reaction mixture was heated to 55 °C. Aqueous 37% formaldehyde solution (9.2 g, 0.11 mole) was weighed into the addition funnel and added over a twenty minute period. The reaction mixture was heated at 55 °C for one hour and then cooled and transferred to another addition funnel. To a reaction flask, equipped as above, were added 17.2 g of 4-acetamidophenol (0.11 mole), 36 g of deionized water, 2.0 g of 50% sodium hydroxide solution, and 36 g of methanol. The mix was heated to 65 °C and the aqueous formaldehyde/ethylenediamine-N,N'-diacetic acid adduct solution added over a one hour and fifteen minute period. The reaction mixture was heated an additional 12 hours at 64-65 °C and then cooled. A portion of the reaction product was concentrated and the methanol removed under vacuum. The solution was adjusted to pH 1.5-2.0 with hydrochloric acid resulting in the precipitation of the acetyl product. The material was filtered, washed with deionized water, and dried in a vacuum oven at 55-60 °C for several hours. The structure was confirmed by proton NMR.

EXAMPLE V. PREPARATION OF N,N'-DI(2-HYDROXY-5-AMINOBENZYL)ETHYLENEDIAMINE-N,N'-DIACETIC ACID, HYDROCHLORIDE. (Comparative)

To approximately 0.9 g of the product isolated in Example U were added 12.5 g of deionized water and 8 g of concentrated hydrochloric acid. The solution was heated at reflux and stirred for one hour in a round-bottom reaction flask. The volatiles were removed (*in vacuo*), and the amine hydrochloride product dried in a vacuum oven at 50-60 °C for several hours. The structure was confirmed by proton NMR.

Example W. PREPARATION OF ETHYLENEDIAMINEDI[(2-HYDROXY-5-ACETAMIDOPHENYL)ACETIC ACID]. (Comparative)

Aqueous (50%) glyoxylic acid (30.0 g, 0.20 mole), 98% 4-acetamidophenol (30.9 g, 0.20 mole) and deionized water (22 g) were added to a round-bottom reaction flask equipped with a water-cooled reflux condenser, mechanical stirrer and a thermometer with a temperature controller. The flask was cooled with an ice-water bath and 19.0 g of 50% sodium hydroxide solution added slowly with stirring while maintaining the temperature below 30 °C. Ethylenediamine (6.1 g, 0.10 mole) was added at a temperature less than 30 °C. The ice bath was removed and the reaction mixture heated and stirred for five hours at 85-86 °C. Approximately 20 g of the aqueous reaction product was treated with 10 g of ethyl ether. The ether layer was removed and the procedure repeated again. The aqueous portion was then adjusted to a pH of approximately 4.2 with hydrochloric acid and agitated with 35 g of acetone. The acetone layer was removed and discarded. To the remaining material was added 65 g of methanol with stirring. The resulting solids were filtered and dried in a vacuum oven at 55-60 °C for several hours.

EXAMPLE X. PREPARATION OF ETHYLENEDIAMINEDI[(2-HYDROXY-5-AMINOPHENYL)ACETIC ACID] (Comparative)

To approximately 4.5 g of the above solids were added 6 g of deionized water and 21 g of concentrated hydrochloric acid. The mix was filtered and 6 g of water added. The solution was placed in a round-bottom reaction flask equipped with a water-cooled reflux condenser, mechanical stirrer, and a thermometer. The solution was heated for one hour at 100-103 °C and then cooled. The volatiles were removed *in vacuo* and the product, the hydrochloride of ethylenediaminedi(2-hydroxy-5-aminophenyl)acetic acid, was dried in a vacuum oven at 60 °C for several hours. Hydrolysis of the acetyl functionality was followed by proton NMR.

COMPLEX PREPARATION AND PERCENT COMPLEX DETERMINATION.

In the following examples the following terms were used: conc. means concentrated; OAc means the acetate moiety, OCOCH_3 ; TLC means thin layer chromatography; ambient temperature means room temperature or about 20 to 25 °C; overnight means from about 9 to 18 hours; SP-Sephadex™ C-25 resin is a cation exchange resin having sulfonic acid functionality, sold by Pharmacia, Inc.

The yttrium and/or samarium complexes of several of the compounds were prepared and percent complexation determined as follows:

Yttrium Complex Preparation:

Complexes were made by preparing a 0.0003M yttrium solution in water. ($\text{YCl}_3 \cdot 6\text{H}_2\text{O}$, 303.26 g/mole; $\text{Y}(\text{OAc})_3$, 12.1% H_2O). Radioactive YCl_3 (Oakridge National Laboratories) was added to give the desired number of counts. Ten μl of ligand solution (at 0.03M) were added to 990 μl of the Y solution, giving a ligand-to-metal ratio of 1:1. (Ten times the amount of ligand solution was used for a 10:1 ligand to metal ratio.) The pH was then adjusted to 7.4 using microliter quantities of hydrochloric acid or sodium hydroxide. The solution was then tested for the amount of complexed yttrium using the cation exchange method given below.

Percent Complex Determination:

A disposable 10 ml plastic (Biorad) column was filled with 1 to 2 ml of water-swelled Sephadex™ C-25 cation exchange resin. The water was pressure eluted to the top of the resin. Fifteen μl of the complex (or more if counts were low) were added to the top of the resin. This was followed by 2 ml of 4:1 (V:V) isotonic saline:conc. ammonium hydroxide solution as an eluent which was allowed to drip into a counting tube. This was also pressure eluted to the top of the resin. An additional 2 ml of the eluent were added and the column pressure eluted to remove all liquid. The dried resin was then placed in a third counting tube and the three tubes counted on a NaI well counter using a Canberra multichannel analyzer linked to a computer. The percent complex was determined by dividing the number of counts in the two elutions by the total counts in the elutions plus the column, all times 100. By this method, uncomplexed yttrium was retained on the column.

Samarium Complex Preparation/% Complex Determination:

Samarium complexes were formed as described previously for yttrium complexes except that 0.0003M samarium was prepared by dissolution of Sm_2O_3 (348.7 g/mole) in 0.1M hydrochloric acid. Radioactive Sm-153 was obtained as a 0.0003M solution in 0.1M hydrochloric acid from the University of Missouri Research Reactor, Columbia, Missouri. Percent complex determination was made in the same manner as for the yttrium complex. Results are summarized in Table II.

Table II

Complexation Data			
Complex Example No.	Compound of Ex. No.	% Complex (10:1)	
		Y	Sm
16	1 c	>99	
17	2 b	99	
18	3 b		96**
19	4		>99**
20	5 b	99	
21	5 b		98
22	5 b	98*	
23	5 b		98*

*Ligand/metal ratio was about 1:1;

**Ligand/metal ratio was about 50:1.

Examples I - VI and Comparative Examples A-F *IN VIVO* SCREENING OF BIFUNCTIONAL CHELATES.

The stability of certain rare earth chelates has been correlated with *in-vivo* testing in animals. For example, Rosoff, et al. in the *International Journal of Applied Radiation and Isotopes*, 14, 129-135 (1963), report on the distribution of radioactive rare earth chelates in mice for certain aminocarboxylic acids. The correlation found was that *in vivo* "the competition between the chelating agent and body constituents (inorganic and organic) for the rare-earth ion, determines its deposition and excretion." The strong rare-earth chelates are believed to dissociate very little and be excreted, while the weak and intermediate strength chelates dissociate more readily and thus are deposited in organs such as the liver. However, concentration of radionuclide in the liver is not always due to weak complex formation, but in some cases is due to the affinity that the metal chelate has for the liver (see Comparative Examples A & B in Table III). Compounds have, in fact, been prepared and utilized for the evaluation of liver function (Fritzberg, Alan R., *Radiopharmaceuticals: Progress and Clinical Perspectives* 1, (1986); U.S. Patents 4,088,747 and 4,091,088 (Hunt et al.))

The biodistribution of several of the samarium and/or yttrium chelates disclosed herein was determined and the percent dose in the liver used as an *in vivo* screening procedure to qualitatively estimate the stability of the chelates. Chelates of NTA and EDTA are included for comparison. Also samarium was injected as samarium chloride in unchelated form.

Sprague-Dawley rats weighing from 150 to 200 g were purchased from Charles River Laboratories. These animals were placed in cages and fed water and food *ad libitum*. The animals were acclimated for at least five days before use. Prior to injection of complex, the animals were placed under a heat lamp (15 to 30 minutes) to dilate the tail vein. Then, the animal was placed in a restraining cage, the tail cleaned with an alcohol swipe, and the animal injected (50 to 200 μ l) via the tail vein. After injection, the animal was placed in another cage for two hours after which time the animal was sacrificed by cervical dislocation. The animal was then dissected, the parts rinsed with deionized H₂O, patted dry, and weighed into a tared counting vial. Whatever size of injection was made, at least three standards of the same material were prepared and counted with the animal parts. Percent of dose is the number of counts in the organ divided by the number of counts in the standard times 100 (see Table III).

Table III

Biodistribution Data			
Biology Example No.	Compound of Ex. No.*	Metal	% Injected Dose in Liver
I	1 c	Y	0.22
II	2 b	Y	0.38
III	5 b	Y	0.28
IV	5 b	Y	0.18
V	5 b	Sm	0.35
VI	5 b	Sm	0.26
(A)	U(comp)	Sm	12
(B)	X(comp)	Sm	24
(C)	EDTA	Sm	8.4
(D)	EDTA	Sm	4.4
(E)	NTA	Sm	8.6
(F)	SmCl ₃	Sm	39

* Complexes were prepared at ligand/metal ratios of 10:1 for Example I-III and V; at 1:1 for Examples IV & VI; at 5:1 for Example C; and at about 300:1 for Examples D and E.

Example VII

The 1:1 complex of yttrium with 1-(p-aminobenzyl)diethylenetriaminepentaacetic acid (ABDTPA), a well known bifunctional chelant used in the literature, and with the ligand of Example 5 b) (now Ex. VII) were prepared using the techniques described earlier. Several 100 microliter aliquots were then withdrawn into separate centrifuge tubes. Excess metal was added such that the total volume change is minimized and the time noted. One-half hour after metal addition, the percent complex was determined by the Sephadex™ C-25 method and this was compared to the original amount of complex. The percent complex versus added metal gives an indication as to the lability of the ligand-metal complex. Results are given below and are compared to the EDTA-yttrium complex.

TABLE IV

Complex Study			
Metal/Ligand Molar Ratio	% Complex		
	Ex. VII	ABDTPA	EDTA
1	95	97	98
10	93	95	86
100	90	92	78
250	90	87	48
500	79	70	16

Example VIII.

A 0.18M/L solution of 1-(p-aminobenzyl)diethylenetriaminepentaacetic acid (ABDTPA) and an identical 0.18M/L solution of the ligand of Example 5 b) were prepared in 0.5M sodium acetate buffer at pH 6.5. The solutions were then treated with 1.5 equivalents of yttrium-90 as 0.03M/L yttrium chloride. The pH of the resulting complex was 5-6. Excess Y-90 was removed by passing the complex through a one ml bed volume of Chelex™ resin (Bio-Rad Laboratories). The concentration of the complex in this purified form was 0.0013M. An appropriate amount of the solution was added to 1.7×10^{-9} moles of aldehyde containing CC-46 monoclonal antibody to give a 40:1 ratio of complex to antibody. After one hour exposure a 236 molar excess (over antibody) of NaCNBH₃ was added and the solutions allowed to set for approximately one hour. After this time the antibody (and any covalently attached complex) was separated from unbound complex by Sephadex™ G-25 gel filtration. This procedure gave an average of 5.0 complexes per antibody for 1-(p-aminobenzyl)diethylenetriaminepentaacetic acid and an average of 5.4 complexes per antibody for the ligand of Example 12.

Example IX.

In order to demonstrate the inertness of the antibody-complex conjugates of Example VIII, the conjugates were challenged with an excess of diethylenetriaminepentaacetic acid (DTPA) in the following manner. The purified antibody-complexes were added to HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) at pH 7.4 and treated with an appropriate amount of 0.1 M DTPA solution (pH 7.4) to ensure a 1000 fold molar excess of DTPA over complex attached to antibody. After one hour an aliquot was removed and the antibody-complex conjugates were separated from low molecular weight substances using gel-filtration. The results indicate that the ABDTPA system lost over 98% of the yttrium while the system using the ligand of Example 12 lost approximately 39% of the yttrium.

EXAMPLE X. PREPARATION OF 2,6-BIS{[(2-{[BIS(CARBOXYMETHYL)]AMINO}ETHYL)-(CARBOXYMETHYL)]AMINOMETHYL}-4-(AMINO)PHENOL, SAMARIUM COMPLEX

A samarium solution was prepared by combining in a 1 ml vial radioactive ¹⁵³Sm (200 μl of a 3×10^{-4} M solution in 0.1M hydrochloric acid, 6×10^{-5} mmole) and "cold" SmCl₃·6H₂O (4.8 mg, 1.31×10^{-2}

mmole). This solution was added to 2,6-bis{[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)]-aminomethyl}-4-(acetamido)phenol (3.2 mg, 5.31×10^{-3} mmole), prepared by the procedure of Example 5 a). The pH was then adjusted to 7 by the addition of sodium hydroxide (40 μ l of a 1.0M solution). The percent complex was determined to be 68% using the SephadexTM C-25 method.

The above complex was purified by anion exchange chromatography (Q-SepharoseTM, 1.5 cm X 21 cm, 0 to 1M NaCl over 30 min, 2 ml/min, detection at 285 nm). Complex-containing fractions (1 ml each, 6 ml total) were combined and the percent complex was determined to be 95%.

EXAMPLE XI. CONJUGATION OF 2,6-BIS{[(2-[[BIS(CARBOXYMETHYL)]AMINO}ETHYL)-(CARBOXYMETHYL)]AMINOMETHYL}-4-(AMINO)PHENOL, SAMARIUM COMPLEX TO CC-46 MONOCLONAL ANTIBODY

Sodium bicarbonate (60 mg, 7.14×10^{-1} mmole) was placed in a one dram glass vial and the complex solution from Example X was added (1 ml, about 8.8×10^{-4} mmole). Thiophosgene (10 μ l, 1.31×10^{-1} mmole) in chloroform (1 ml) was added and the vial was sealed. The mixture was shaken for 15 minutes, after which the aqueous layer was washed twice with chloroform (1 ml portions). Percent complex was checked and found to be 96%.

The above isothiocyanate Sm complex (100 μ l, about 8.8×10^{-5} mmole) was combined with CC-46 monoclonal antibody (100 μ l of an 8 mg/ml solution, about 5.3×10^{-6} mmole) and allowed to stand for 24 hours. The amount of complex conjugated to antibody was determined to be 46% by size exclusion chromatography.

EXAMPLE XII. PREPARATION OF 2-[BIS(2-[[[BIS(CARBOXYMETHYL)]AMINO}ETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID AND 2-[[2-[(2-[[BIS(CARBOXYMETHYL)]-AMINO}ETHYL)(CARBOXYMETHYL)AMINO]ETHYL] (CARBOXYMETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)PHENYL]ETHANOIC ACID, SAMARIUM COMPLEX

A solution of the ligands from Example 3 b) was prepared by dissolving 266 mg of the lyophilized solid in 1 ml of water. A 33.85 μ l aliquot of this solution was treated with 1 ml of 3×10^{-4} M SmCl₃ in 0.1N hydrochloric acid containing a tracer amount of radioactive ¹⁵³Sm. The pH of the complex solution was adjusted to about 13 using 50 weight % sodium hydroxide and then adjusted to about pH 7.5 using 1.0N hydrochloric acid. The percent Sm that was complexed was determined as described in Examples 16 through 23 and was found to be 100%.

The inertness of the complex was demonstrated by placing two 500 μ l aliquots of the complex solution in separate vials. One portion was treated with 1-2 μ l portions of 0.1N hydrochloric acid until the pH was lowered and the other portion was treated with 0.1N sodium hydroxide to bring the pH up. The complexes were allowed to set for 5-10 minutes at each pH change, then they were sampled to determine the percent complexation at that pH by the method described for Examples 16 through 23. The results are shown in the following table.

Table V

pH	% Complexed
1	98
2	100
3	100
4	100
5	100
7	100
9	100
11	100
13	100

EXAMPLE XIII. PREPARATION OF 2-[(2-[(2-[[BIS(CARBOXYMETHYL)AMINO]ETHYL)-(CARBOXYMETHYL)AMINO]ETHYL) (CARBOXYMETHYL)AMINO]-2-[5-AMINO-2-(CARBOXYMETHYLOXY)-PHENYL]ETHANOIC ACID, SAMARIUM COMPLEX

A solution of the ligand from Example 4 was prepared by dissolving 13.9 mg of the brownish solid in 772 μ l of water. A complex was prepared by dissolving 500 μ l of this ligand solution in 1 ml of 3×10^{-4} M SmCl_3 (containing 0.1N hydrochloric acid) that had been spiked with radioactive ^{153}Sm . The pH of the complex solution was adjusted to about 7 by the addition of 1.0N sodium hydroxide. The percent complexation was determined by the method described for Examples 16 through 23 and found to be 96%.

The inertness of the complex was demonstrated by placing two 500 μ l aliquots of the complex solution in separate vials. One portion was treated with 1-2 μ l portions of 1.0N hydrochloric acid until the pH was lowered and the other portion was treated with 0.1N, 1.0N and 50 weight % sodium hydroxide to bring the pH up. The complexes were allowed to set for about 5 minutes at each pH change, then they were sampled to determine the percent complexation at that pH by the method described for Examples 16 through 23.

The results are shown in the following table.

Table VI

pH	% Complexed
1	91
2	88
3	92
5	96
7	96
9	99
12	98
13	99

TABLE 1: continued

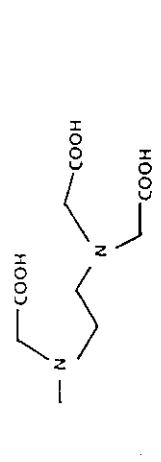
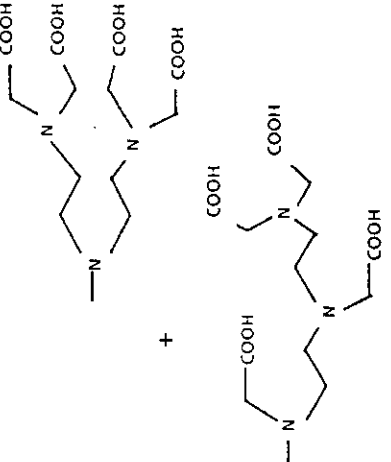
Example No.	Z	X	R ₅	n	R ₁	R ₂	R ₃	R ₄	B
2 b	-NH ₂	-H	H	0	-	-	-H	-COOH	
3 a	NHCOCH ₃	CH ₂ CO ₂ H	H	0	-	-	-H	-COOH	

TABLE 1: continued

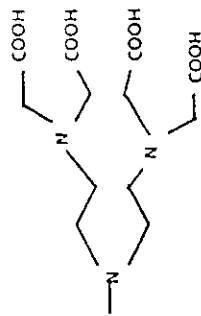
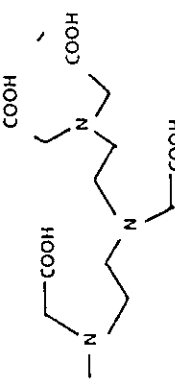
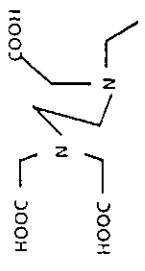
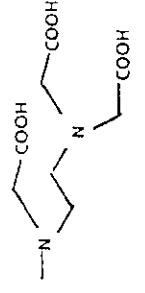
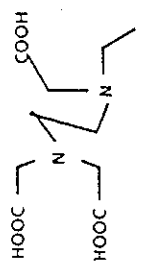
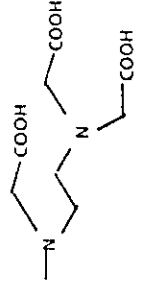
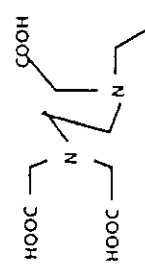
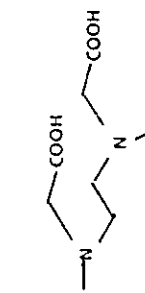
Example No.	Z	X	R ₅	n	R ₁	R ₂	R ₃	R ₄	B
3 b)	-NH ₂	CH ₂ COOH	H	0	-	-	-H	-COOH	
4	-NH ₂	CH ₂ COOH	H	0	-	-	-H	-COOH	

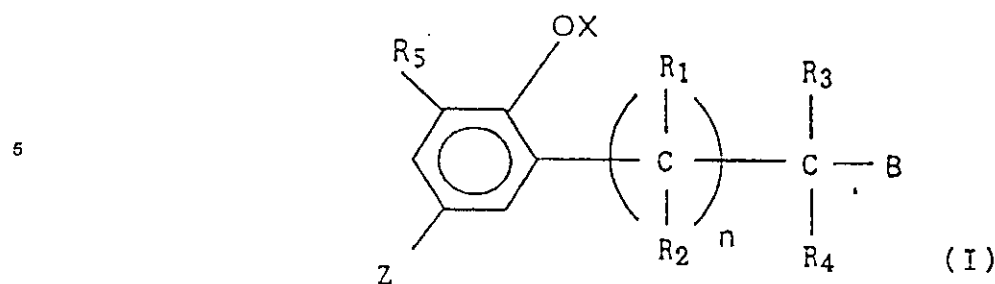
TABLE 1: continued

Example No.	Z	X	R ₅	n	R ₁	R ₂	R ₃	R ₄	B
5 a	NHCOCH ₃	-H		0	-	-	-H	-H	
5 b	-NH ₂	-H		0	-	-	-H	-H	
6	-NCS	-H		0	-	-	-H	-H	

Claims

Claims for the following Contracting States : DE, GB, FR, IT, NL, SE, CH, BE, LI, AT, LU

1. A bifunctional chelant possessing ortho ligating functionality having the formula



wherein:

15 Z is a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof selected from amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyl, bromoacetamido or maleimido;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄CO₂H;

R₁, R₂, R₃ and R₄ each are independently hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group with the proviso that when n = 0 and R₅ = H, then one of R₃ or R₄ must be CO₂H;

20 R₅ is hydrogen or (CR₁R₂)_nCR₃R₄B;

B represents a linear or branched amine or polyalkylene amine where at least one of the amine hydrogens has been substituted with a CR₃R₄CO₂H group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

25

2. The bifunctional chelant of Claim 1 wherein n is 0.
3. The bifunctional chelant of Claim 1 or 2 wherein R₄ is CO₂H.
- 30 4. The bifunctional chelant of Claim 1, 2 or 3 wherein X is hydrogen.
5. The bifunctional chelant of any one of Claims 1 through 4 wherein R₁, R₂ and R₃ are each hydrogen.
6. The bifunctional chelant of Claim 1 which is 2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)-amino] -2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid.
- 35 7. The bifunctional chelant of Claim 1 which is 2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)-amino] -2-(5-amino-2-hydroxyphenyl)ethanoic acid.
- 40 8. The bifunctional chelant of Claim 1 which is 2,6-bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(amino)phenol.
9. The bifunctional chelant of Claim 1 which is 2,6-bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(isothiocyanato)phenol.
- 45 10. A complex which comprises a chelant as claimed in any one of Claims 1 through 9, or a pharmaceutically acceptable salt thereof; complexed with a metal ion selected from La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc.
- 50 11. A complex of Claim 10 wherein the metal ion is ¹⁵³Sm, ¹⁶⁶Ho, ⁹⁰Y, ¹⁴⁹Pm, ¹⁵³Gd, ¹⁴⁰La, ¹⁷⁷Lu, ¹⁷⁵Yb, ⁴⁷Sc or ¹⁴²Pr.
12. A conjugate comprising a complex as claimed in Claim 10 or 11 covalently attached to an antibody or antibody fragment.
- 55 13. A conjugate of Claim 12 wherein the antibody or fragment thereof is a monoclonal antibody or fragment thereof.

14. A conjugate of Claim 13 wherein the antibody or antibody fragment is CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ or B72.3.

15. A pharmaceutical formulation comprising a complex as claimed in Claim 10 or 11 and a physiologically acceptable carrier.

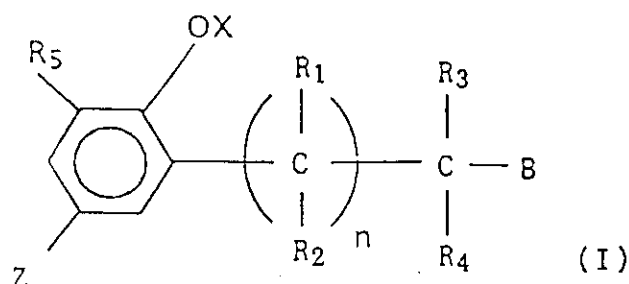
16. A pharmaceutical formulation comprising a conjugate as claimed in Claim 12 and a physiologically acceptable carrier.

17. A pharmaceutical formulation according to claim 15 or 16 for the treatment of cancer.

18. A pharmaceutical formulation according to claim 15 or 16 for the treatment of bone pain.

Claims for the following Contracting States : ES, GR

1. A process for preparing a bifunctional chelant possessing ortho ligating functionality having the formula



wherein:

Z is a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof selected from amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyl, bromoacetamido or maleimido;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄COOH;

R₁, R₂, R₃ and R₄ each are independently hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group with the proviso that when n=0 and R₅=H, then one of R₃ or R₄ must be CO₂H;

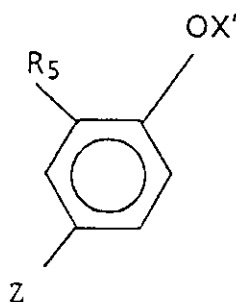
R₅ is hydrogen or (CR₁R₂)_nCR₃R₄B;

B represents a linear or branched amine or polyalkylene amine where at least one of the amine hydrogens has been substituted with a CR₃R₄COOH group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof, which comprises:

A) reacting a compound of the formula



wherein:

Z is defined as before;

X' is hydrogen;

R₅ is hydrogen or (CR₁R₂)_nCR₃R₄T, where R₁, R₂, R₃ and R₄ each are independently

hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group, n is 0 or 1, and T represents a linear or branched amine or polyalkylene amine where at least one of the amine hydrogens has been substituted with a CR₃R₄CO₂H group; or

a pharmaceutically acceptable salt thereof;

with a compound B and an aldehyde or aldehyde precursor equivalent, where B represents a linear or branched amine or polyalkylene amine where there is at least one amine hydrogen;

in the presence of caustic and a suitable solvent, at a temperature of 20 °C or less, followed by heating and separating the desired product of formula I;

B) reacting the product obtained from Step (A) with a halo-(CR₁R₂)_nCR₃R₄ acid, at a pH of 9 or higher, in the presence of caustic, at a temperature of 20 °C or less, to provide the compounds of formula I, where at least one of R₁, R₂, R₃ and R₄ is CO₂H;

C) hydrolyzing the product of Step (B) where Z is NHC(O)CH₃ with NaOH in H₂O, to provide the products of formula I, where Z is NH₂;

D) reacting the product obtained from Step (A) with glycolnitrile, in caustic, at a pH of 9 or higher, at a temperature of 20 °C or less, followed by hydrolysis of the cyano group with HCl in H₂O, to provide the products of formula I, where at least one of R₁, R₂, R₃ and R₄ is CO₂H;

E) hydrolyzing the product of Step (A) where Z is NHC(O)CH₃ with DCl in D₂O, with heating, to provide the products of formula I, where Z is NH₂; or

F) reacting the product obtained from any one of Steps (A) through (E), where Z is NH₂, with thiophosgene to provide the products of formula I, where Z is isothiocyanato.

2. A process of Claim 1, wherein n is 0.

3. A process of Claim 1, wherein R₄ is CO₂H.

4. A process of Claim 1, wherein X is hydrogen.

5. A process of Claim 1, wherein R₁, R₂ and R₃ are each hydrogen.

6. A process of Claim 1, wherein the caustic is sodium hydroxide.

7. The process of Claim 1, step (C), for preparing 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)-amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid which comprises reacting 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]-ethanoic acid with NaOH in H₂O.

8. The process of Claim 1, step (D), for preparing 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)-amino]-2-(5-amino-2-hydroxyphenyl)ethanoic acid which comprises reacting 2-[(2-{[bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid with glycolnitrile and NaOH, to form 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(cyanomethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)-ethanoic acid, followed by hydrolysis with HCl in H₂O.

9. The process of Claim 1, step (E), for preparing 2-[(2-{[bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]ethyl-(carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid which comprises reacting 2-[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)amino]ethyl-(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid with DCl in D₂O.

10. The process of Claim 1, step (A), for preparing 2-[(2-{[bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]ethyl-(carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid which comprises reacting diethylenetriamineacetic acid and 4-acetamidophenol with glyoxylic acid.

11. The process of Claim 1, step (E), for preparing 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)aminomethyl]-4-(amino)phenol} which comprises reacting 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)aminomethyl]-4-(acetamido)phenol} with DCl in D₂O.

12. The process of Claim 1, step (F), for preparing 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)aminomethyl]-4-(isothiocyanato)phenol} which comprises reacting 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)aminomethyl]-4-(amino)phenol} with thiophosgene.

13. A process for preparing a complex of a compound as claimed in any one of Claims 1 through 12, or a pharmaceutically acceptable salt thereof, complexed with a metal ion selected from La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc, which comprises reacting the compound with the metal ion.

14. A process for preparing a complex of Claim 13, wherein the metal ion is ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc or ^{142}Pr .

15. A process for preparing a conjugate comprising a complex as claimed in Claim 13 or 14 covalently attached to an antibody or antibody fragment, which comprises reacting the complex with the antibody or antibody fragment.

16. A process for preparing a conjugate of Claim 15, wherein the antibody or fragment is a monoclonal antibody or fragment thereof.

17. A process for preparing a conjugate of Claim 16, wherein the antibody or antibody fragment is CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ or B72.3.

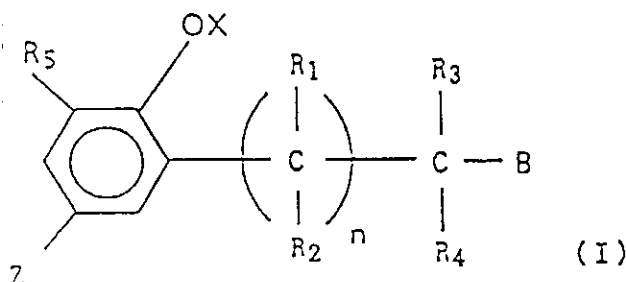
18. A process for preparing a pharmaceutical formulation comprising a complex as claimed in Claim 13 or 14, which comprises reacting the complex with a physiologically acceptable carrier.

19. A process for preparing a pharmaceutical formulation comprising a conjugate as claimed in any one of Claims 15 through 17, which comprises reacting the conjugate with a physiologically acceptable carrier.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : DE, GB, FR, IT, NL, SE, CH, BE, LI, AT, LU

1. Bifunktioneller Chelatbildner, der eine Ortho-Ligationsfunktionalität besitzt, mit der Formel



wobei:

Z ein synthetischer Linker ist, der die Bildung einer Komplexbildung mit einem Radionuklid nicht beeinträchtigt und der mit einem Antikörper oder einem Fragment davon verbunden werden kann, ausgewählt aus Amino, Isothiocyanato, Semicarbazid, Thiosemicarbazid, Carboxyl, Bromacetamido oder Maleimido,

X Wasserstoff, C₁-C₃ Alkyl oder CR₃R₄CO₂H ist,

R₁, R₂, R₃ und R₄ unabhängig jeweils Wasserstoff, Hydroxy, CO₂H oder eine C₁-C₃-Alkylgruppe sind unter der Bedingung, daß, wenn n = 0 und R₅ = H entweder R₃ oder R₄ CO₂H sein muß,

R₅ Wasserstoff oder (CR₁R₂)_nCR₃R₄B ist,

B ein lineares oder verzweigtes Amin oder Polyalkylenamin darstellt, wobei mindestens einer der Aminwasserstoffe mit einer CR₃R₄CO₂H-Gruppe substituiert worden ist,

n 0 oder 1 ist oder

ein pharmazeutisch verträgliches Salz davon.

2. Bifunktioneller Chelatbildner nach Anspruch 1, wobei n 0 ist.

3. Bifunktioneller Chelatbildner nach Anspruch 1 oder 2, wobei R₄ CO₂H ist.

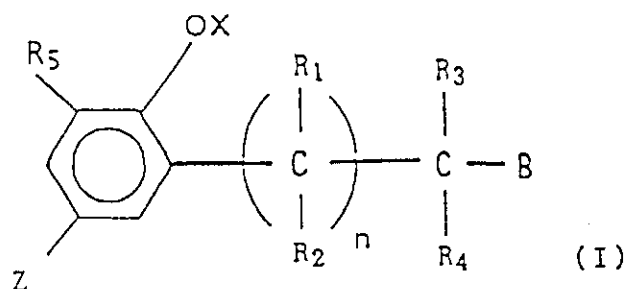
4. Bifunktioneller Chelatbildner nach Anspruch 1, 2 oder 3, wobei X Wasserstoff ist.
5. Bifunktioneller Chelatbildner nach einem der Ansprüche 1 bis 4, wobei R₁, R₂ und R₃ jeweils Wasserstoff sind.
- 5 6. Bifunktioneller Chelatbildner nach Anspruch 1, der 2-[(2-[[Bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethansäure ist.
7. Bifunktioneller Chelatbildner nach Anspruch 1, der 2-[(2-[[Bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)amino]-2-(5-amino-2-hydroxyphenyl)ethansäure ist.
- 10 8. Bifunktioneller Chelatbildner nach Anspruch 1, der 2,6-Bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(amino)phenol ist.
- 15 9. Bifunktioneller Chelatbildner nach Anspruch 1, der 2,6-Bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(isothiocyanato)phenol ist.
10. Komplex, der einen Chelatbildner nach einem der Ansprüche 1 bis 9 oder ein pharmazeutisch verträgliches Salz davon umfaßt,
- 20 komplexiert mit einem Metallion ausgewählt aus La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y oder Sc.
11. Komplex nach Anspruch 10, wobei das Metallion ¹⁵³Sm, ¹⁶⁶Ho, ⁹⁰Y, ¹⁴⁹Pm, ¹⁵⁹Gd, ¹⁴⁰La, ¹⁷⁷Lu, ¹⁷⁵Yb, ⁴⁷Sc oder ¹⁴²Pr ist.
- 25 12. Konjugat, umfassend einen Komplex nach Anspruch 10 oder 11, welcher kovalent an einen Antikörper oder ein Antikörperfragment gebunden ist.
13. Konjugat nach Anspruch 12, wobei der Antikörper oder das Fragment davon ein monoklonaler Antikörper oder ein Fragment davon ist.
- 30 14. Konjugat nach Anspruch 13, wobei der Antikörper oder das Antikörperfragment CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ oder B72.3 ist.
- 35 15. Pharmazeutische Formulierung, umfassend einen Komplex nach Anspruch 10 oder 11 und einen physiologisch verträglichen Träger.
16. Pharmazeutische Formulierung, umfassend ein Konjugat nach Anspruch 12 und einen physiologisch verträglichen Träger.
- 40 17. Pharmazeutische Formulierung nach Anspruch 15 oder 16 zur Behandlung von Krebs.
18. Pharmazeutische Formulierung nach Anspruch 15 oder 16 zur Behandlung von Knochenschmerzen.

45 **Patentansprüche für folgende Vertragsstaaten : ES, GR**

1. Verfahren zum Herstellen eines bifunktionellen Chelatbildners, der eine Ortho-Ligationsfunktionalität besitzt, mit der Formel

50

55



wobei:

Z ein synthetischer Linker ist, der die Bildung einer Komplexbildung mit einem Radionuklid nicht beeinträchtigt und der mit einem Antikörper oder einem Fragment davon verbunden werden kann, ausgewählt aus Amino, Isothiocyanato, Semicarbazid, Thiosemicarbazid, Carboxyl, Bromacetamido oder Maleimido,

X Wasserstoff, C₁-C₃ Alkyl oder CR₃R₄CO₂H ist,

R₁, R₂, R₃ und R₄ jeweils unabhängig Wasserstoff, Hydroxy, CO₂H oder eine C₁-C₃-Alkylgruppe sind unter der Bedingung, daß, wenn n = 0 und R₅ = H entweder R₃ oder R₄ CO₂H sein muß,

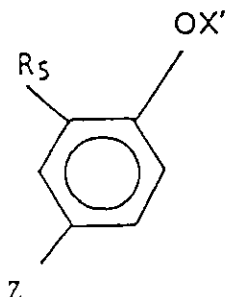
R₅ Wasserstoff oder (CR₁R₂)_nCR₃R₄B ist,

B ein lineares oder verzweigtes Amin oder Polyalkylenamin darstellt, wobei mindestens einer der Aminwasserstoffe mit einer CR₃R₄CO₂H-Gruppe substituiert worden ist,

n 0 oder 1 ist oder

ein pharmazeutisch verträgliches Salz davon, welches umfaßt:

A) Umsetzen einer Verbindung der Formel



wobei:

Z wie vorstehend definiert ist,

X' Wasserstoff ist;

R₅ Wasserstoff oder (CR₁R₂)_nCR₃R₄T ist, wobei R₁, R₂, R₃ und R₄ jeweils unabhängig Wasserstoff, Hydroxy, CO₂H oder eine C₁-C₃-Alkylgruppe sind, n 0 oder 1 ist und T ein lineares oder verzweigtes Amin oder Polyalkylenamin darstellt, bei dem mindestens einer der Aminwasserstoffe mit einer CR₃R₄CO₂H-Gruppe substituiert worden ist oder

ein pharmazeutisch verträgliches Salz davon;

mit einer Verbindung B und einem Aldehyd oder einem Aldehyd entsprechenden Vorläufer, wobei B ein lineares oder verzweigtes Amin oder Polyalkylenamin darstellt, bei dem mindestens ein Aminwasserstoff vorhanden ist;

in der Gegenwart von Alkali und einem geeigneten Lösungsmittel, bei einer Temperatur von 20 °C oder weniger, gefolgt von Erhitzen und Abtrennen des gewünschten Produkts nach Formel I;

B) Umsetzen des aus Schritt (A) erhaltenen Produkts mit einer Halogen-(CR₁R₂)_nCR₃R₄-Säure bei einem pH von 9 oder höher in der Gegenwart von Alkali bei einer Temperatur von 20 °C oder weniger, um die Verbindungen nach Formel I bereitzustellen, bei denen mindestens einer von R₁, R₂, R₃ und R₄ CO₂H ist;

C) Hydrolysieren des Produkts aus Schritt (B), bei dem Z NHC(O)CH₃ ist, mit NaOH in H₂O, um die Produkte nach Formel I bereitzustellen, bei denen Z NH₂ ist;

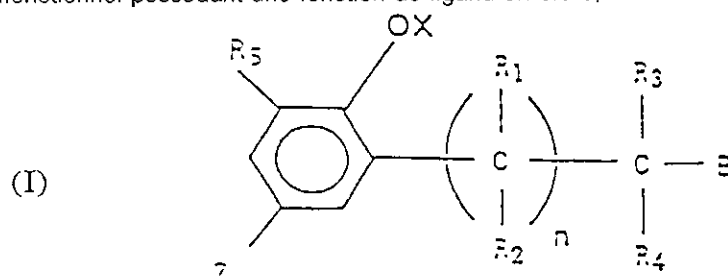
- D) Umsetzen des in Schritt (A) erhaltenen Produkts mit Glycolnitril in Alkali, bei einem pH von 9 oder höher, bei einer Temperatur von 20°C oder weniger, gefolgt von Hydrolyse der Cyangruppe mit HCl in H₂O, um die Produkte nach Formel I bereitzustellen, bei denen mindestens einer aus R₁, R₂, R₃ und R₄ CO₂H ist;
- 5 E) Hydrolysieren des Produkts aus Schritt (A), bei dem Z NHC(O)CH₃ ist, mit DCl in D₂O unter Erhitzen, um die Produkte nach Formel I bereitzustellen, bei denen Z NH₂ ist oder
- F) Umsetzen des aus einem der Schritte (A) bis (E) erhaltenen Produkts, bei dem Z NH₂ ist mit Thiophosgen, um die Produkte nach Formel I bereitzustellen, bei denen Z Isothiocyanato ist.
- 10 2. Verfahren nach Anspruch 1, wobei n 0 ist.
3. Verfahren nach Anspruch 1, wobei R₄ CO₂H ist.
4. Verfahren nach Anspruch 1, wobei X Wasserstoff ist.
- 15 5. Verfahren nach Anspruch 1, wobei R₁, R₂ und R₃ jeweils Wasserstoff sind.
6. Verfahren nach Anspruch 1, wobei das Alkali Natriumhydroxid ist.
- 20 7. Verfahren nach Anspruch 1, Schritt (C) zum Herstellen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethansäure, welches umfaßt das Umsetzen von 2-[(2-{[Bis-(carboxymethyl)]amino}ethyl)(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethansäure mit NaOH in H₂O.
- 25 8. Verfahren nach Anspruch 1, Schritt (D) zum Herstellen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]-2-(5-amino-2-hydroxyphenyl)ethansäure, welches umfaßt das Umsetzen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethansäure mit Glycolnitril und NaOH um 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)(cyanomethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethansäure zu bilden, gefolgt von Hydrolyse mit HCl in H₂O.
- 30 9. Verfahren nach Anspruch 1, Schritt (E) zum Herstellen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]ethyl{(carboxymethyl)amino}-2-[5-amino-2-(carboxymethyloxy)phenyl]-ethansäure, welches umfaßt das Umsetzen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]ethyl{(carboxymethyl)amino}-2-[5-acetamido-2-(carboxymethyloxy)phenyl]-ethansäure mit DCl in D₂O.
- 35 10. Verfahren nach Anspruch 1, Schritt (A) zum Herstellen von 2-[(2-{[Bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)amino]ethyl{(carboxymethyl)amino}-2-[5-amino-2-(carboxymethyloxy)phenyl]-ethansäure, welches umfaßt das Umsetzen von Diethylentriaminessigsäure und 4-Azetamidophenol mit Glyoxalsäure.
- 40 11. Verfahren nach Anspruch 1, Schritt (E) zum Herstellen von 2,6-Bis{[(2-{[bis(carboxymethyl)]-amino}ethyl)(carboxymethyl)aminomethyl]-4-(amino)phenol, welches umfaßt das Umsetzen von 2,6-bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)aminomethyl]-4-(acetamido)phenol mit DCl in D₂O.
- 45 12. Verfahren nach Anspruch 1, Schritt (F) zum Herstellen von 2,6-Bis{[(2-{[bis(carboxymethyl)]-amino}ethyl)(carboxymethyl)aminomethyl]-4-(isothiocyanato)phenol, welches umfaßt das Umsetzen von 2,6-Bis{[(2-{[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)aminomethyl]-4-(amino)phenol mit Thiophosgen.
- 50 13. Verfahren zum Herstellen eines Komplexes einer Verbindung nach einem der Ansprüche 1 bis 12 oder eines pharmazeutisch verträglichen Salzes davon, komplexiert mit einem Metallion, ausgewählt aus La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y oder Sc, welches umfaßt das Umsetzen der Verbindung mit dem Metallion.
- 55 14. Verfahren zum Herstellen eines Komplex nach Anspruch 13, wobei das Metallion ¹⁵³Sm, ¹⁶⁶Ho, ⁹⁰Y, ¹⁴⁹Pm, ¹⁵⁹Gd, ¹⁴⁰La, ¹⁷⁷Lu, ¹⁷⁵Yb, ⁴⁷Sc oder ¹⁴²Pr ist.

15. Verfahren zum Herstellen eines Konjugats, umfassend einen Komplex nach einem der Ansprüche 13 oder 14, welcher kovalent an einen Antikörper oder ein Antikörperfragment gebunden ist, welches das Umsetzen des Komplexes mit dem Antikörper oder dem Antikörperfragment umfaßt.
- 5 16. Verfahren zum Herstellen eines Konjugats nach Anspruch 15, wobei der Antikörper oder das Fragment ein monoklonaler Antikörper oder ein Fragment davon ist.
17. Verfahren zum Herstellen eines Konjugats nach Anspruch 16, wobei der Antikörper oder das Antikörperfragment CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ oder B72.3 ist.
- 10 18. Verfahren zum Herstellen einer pharmazeutischen Formulierung, umfassend einen Komplex nach Anspruch 13 oder 14, welches das Umsetzen des Komplexes mit einem physiologisch verträglichen Träger umfaßt.
- 15 19. Verfahren zum Herstellen einer pharmazeutischen Formulierung, umfassend ein Konjugat nach einem der Ansprüche 15 bis 17, welches das Umsetzen des Konjugats mit einem physiologisch verträglichen Träger umfaßt.

Revendications

20 Revendications pour les Etats contractants suivants : DE, GB, FR, IT, NL, SE, CH, BE, LI, AT, LU

1. Agent chélatant bifonctionnel possédant une fonction de ligand en ortho, de formule



dans laquelle

Z

représente un groupe de liaison synthétique qui n'interfère pas avec la complexation d'un radionucléide et qui peut être lié à un anticorps ou un fragment de celui-ci, choisi parmi les groupes amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyle, bromoacétamido ou maléimido,

X

R₁, R₂, R₃ et R₄

représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle en C₁₋₃ ou CR₃R₄CO₂H, hydroxy, CO₂H ou un groupe alkyle en C₁₋₃, avec la réserve que, lorsque n = 0 et R₅ = H, un des substituants R₃ ou R₄ doit être un groupe CO₂H,

R₅

B

représente un atome d'hydrogène ou un groupe (CR₁R₂)_nCR₃R₄B, représente une amine linéaire ou ramifiée ou une poly(alkylène amine) dans laquelle au moins un des atomes d'hydrogène de l'amine a été substitué par un groupe CR₃R₄COOH,

n

est égal à 0 ou 1, ou

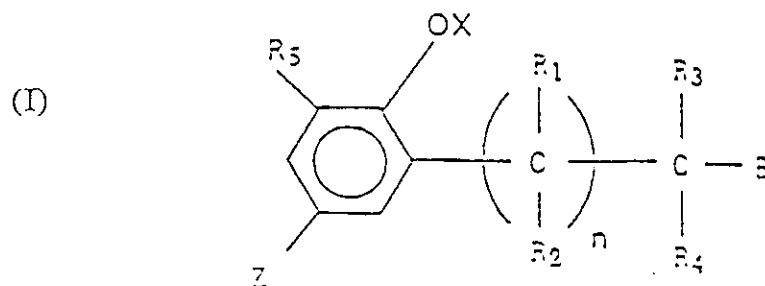
un sel acceptable d'un point de vue pharmaceutique d'un tel composé.

- 50 2. Agent chélatant bifonctionnel conforme à la revendication 1 dans lequel n est égal à 0.
3. Agent chélatant bifonctionnel conforme à la revendication 1 ou 2 dans lequel R₄ représente un groupe CO₂H.
- 55 4. Agent chélatant bifonctionnel conforme à la revendication 1, 2 ou 3 dans lequel X représente un atome d'hydrogène.

5. Agent chélatant bifonctionnel conforme à une quelconque des revendications 1 à 4 dans lequel R_1 , R_2 et R_3 sont chacun un atome d'hydrogène.
6. Agent chélatant bifonctionnel conforme à la revendication 1 qui est l'acide 2-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)amino]-2-[5-amino-2-(carboxyméthoxy)phényl]éthanoïque.
7. Agent chélatant bifonctionnel conforme à la revendication 1 qui est l'acide 2-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)amino]-2-(5-amino-2-hydroxyphényl)éthanoïque.
8. Agent chélatant bifonctionnel conforme à la revendication 1 qui est le 2,6-bis-{[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)aminométhyl]-4-(amino)phénol}.
9. Agent chélatant bifonctionnel conforme à la revendication 1 qui est le 2,6-bis-{[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)aminométhyl]-4-(isothiocyanato)phénol}.
10. Complexe constitué d'un agent chélatant conforme à une quelconque des revendications 1 à 9 ou un sel acceptable d'un point de vue pharmaceutique d'un tel composé, formant un complexe avec un ion métallique choisi parmi La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y ou Sc.
11. Complexe conforme à la revendication 10 dans lequel l'ion métallique est le ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc ou ^{142}Pr .
12. Conjugué contenant un complexe conforme à la revendication 10 ou 11 lié covalentement à un anticorps ou un fragment d'anticorps.
13. Conjugué conforme à la revendication 12 dans lequel l'anticorps ou le fragment de celui-ci est un anticorps monoclonal ou un fragment de celui-ci.
14. Conjugué conforme à la revendication 13 dans lequel l'anticorps ou le fragment d'anticorps sont CC-46, CC-49, CC49 F(ab')₂, CC-83, CC-83 F(ab')₂ ou B72.3.
15. Formulation pharmaceutique contenant un complexe conforme à la revendication 10 ou 11 et un véhicule physiologiquement acceptable.
16. Formulation pharmaceutique contenant un conjugué conforme à la revendication 12 et un véhicule physiologiquement acceptable.
17. Formulation pharmaceutique conforme à la revendication 15 ou 16 pour le traitement du cancer.
18. Formulation pharmaceutique conforme à la revendication 15 ou 16 pour le traitement de douleurs osseuses.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé pour préparer un agent chélatant bifonctionnel possédant une fonction de ligand en ortho, de formule



dans laquelle

Z représente un groupe de liaison synthétique qui n'interfère pas avec la complexation d'un radionucléide et qui peut être lié à un anticorps ou un fragment de celui-ci, choisi parmi les groupes amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyle, bromoacétamido ou maléimido,

X représente un atome d'hydrogène, un groupe alkyle en C_{1-3} ou $CR_3R_4CO_2H$,
 R_1, R_2, R_3 et R_4 représentant chacun indépendamment un atome d'hydrogène, un groupe hydroxy, CO_2H ou un groupe alkyle en C_{1-3} avec la réserve que, lorsque $n = 0$ et $R_5 = H$, un des substituants R_3 ou R_4 doit être un groupe CO_2H ,

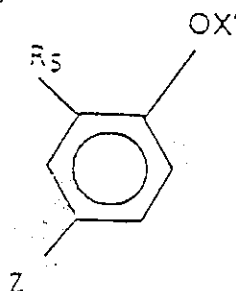
R_5 représente un atome d'hydrogène ou un groupe $(CR_1R_2)_nCR_3R_4B$,

B représente une amine linéaire ou ramifiée ou une poly(alkylène amine) dans laquelle au moins un des atomes d'hydrogène de l'amine a été substitué par un groupe CR_3R_4COOH ,

n est égal à 0 ou 1, ou

un sel acceptable d'un point de vue pharmaceutique d'un tel composé, lequel procédé consiste

A) à faire réagir un composé de formule



dans laquelle

Z est défini comme ci-dessus,

X' représente un atome d'hydrogène,

R_5 représente un atome d'hydrogène ou un groupe $(CR_1R_2)_nCR_3R_4T$, où R_1, R_2, R_3 et R_4 représentent indépendamment un atome d'hydrogène, un groupe hydroxy, un groupe CO_2H ou un groupe alkyle en C_{1-3} , n est égal à 0 ou 1 et T représente une amine linéaire ou ramifiée ou une poly(alkylène amine) dans laquelle ou moins une des atomes d'hydrogène de l'amine a été remplacé par un groupe $CR_3R_4CO_2H$, ou un sel acceptable d'un point de vue pharmaceutique d'un tel composé, avec un composé B et un équivalent d'un aldéhyde ou d'un précurseur d'aldéhyde, où B représente une amine linéaire ou ramifiée ou une poly(alkylène amine) contenant au moins un atome d'hydrogène sur la fonction amine, en présence d'une solution caustique et d'un solvant approprié et à une température inférieure ou égale à $20^\circ C$, puis à chauffer et à séparer le produit recherché de formule I,

B) à faire réagir le produit obtenu dans l'étape (A) avec un halogénure d'un $(CR_1R_2)_nCR_3R_4$ -acide à un pH supérieur ou égal à 9 en présence d'une solution caustique à une température inférieure ou égale à $20^\circ C$, pour obtenir le composé de formule I où ou moins un des substituants R_1, R_2, R_3 et R_4 est un groupe CO_2H ,

C) à hydrolyser le produit obtenu dans l'étape (B) où Z représente un groupe $NHC(O)CH_3$ avec du NaOH dans de l'eau, pour obtenir les produits de formule I où Z est un groupe NH_2 ,

D) à faire réagir le produit obtenu dans l'étape (A) avec du glycolnitrile dans une solution caustique à un pH supérieur ou égal à 9 et à une température inférieure ou égale à $20^\circ C$, puis à hydrolyser le groupe cyano avec de l'acide chlorhydrique en solution aqueuse pour obtenir un produit de formule I, où au moins un des substituants R_1, R_2, R_3 et R_4 est un groupe CO_2H ,

E) à hydrolyser le produit de l'étape (A) où Z représente un groupe $NHC(O)CH_3$ avec du DCl en solution dans D_2O sous chauffage pour obtenir un produit de formule I, où Z représente un groupe NH_2 , ou

F) à faire réagir le produit obtenu dans une quelconque des étapes (A) à (E) où Z représente un groupe NH_2 , avec du thiophosgène pour obtenir les produits de formule I où Z représente un groupe isothiocyanato.

2. Procédé conforme à la revendication 1 dans lequel n est égal à 0.

3. Procédé conforme à la revendication 1 dans lequel R_4 représente un groupe CO_2H .
4. Procédé conforme à la revendication 1 dans lequel X représente un atome d'hydrogène.
5. Procédé conforme à la revendication 1 dans lequel R_1 , R_2 et R_3 sont chacun un atome d'hydrogène.
6. Procédé conforme à la revendication 1 dans lequel la substance caustique est l'hydroxyde de sodium.
7. Procédé conforme à la revendication 1, étape (C), pour préparer l'acide 2-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)amino]-2-[5-amino-2-(carboxyméthoxy)phényl]éthanoïque qui consiste à faire réagir l'acide 2-[(2{[bis(carboxyméthyl)]amino}éthyl)(carboxyméthyl)amino]-2-[5-acétamido-2-(carboxyméthoxy)phényl]éthanoïque avec du NaOH dans de l'eau.
8. Procédé conforme à la revendication 1, étape (D), pour préparer l'acide 2-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)amino]-2-(5-amino-2-hydroxyphényl)éthanoïque qui consiste à faire réagir l'acide 2-[(2{[bis(carboxyméthyl)]amino}éthyl)amino]-2-(5-acétamido-2-hydroxyphényl)éthanoïque avec du glycolnitrile et du NaOH, pour former l'acide 2-[(2{[bis(carboxyméthyl)]amino}éthyl)(cyanométhyl)amino]-2-(5-acétamido-hydroxyphényl)éthanoïque, puis à l'hydrolyser par de l'HCl dans H_2O .
9. Procédé conforme à la revendication 1, étape (E), pour préparer l'acide 2-[(2-[(2{[bis(carboxyméthyl)]-amino}éthyl)-(carboxyméthyl)amino]éthyl)(carboxyméthyl)amino]-2-[5-amino-2-(carboxyméthoxy)phényl]éthanoïque qui consiste à faire réagir l'acide 2-[(2-[(2{[bis(carboxyméthyl)]amino}éthyl)-(carboxyméthyl)amino]éthyl)(carboxyméthyl)amino]-2-[5-acétamido-2-(carboxyméthoxy)phényl]éthanoïque avec du DCl dans du D_2O .
10. Procédé conforme à la revendication 1, étape (A), pour préparer l'acide 2-[(2-[(2{[bis(carboxyméthyl)]-amino}éthyl)-(carboxyméthyl)amino]éthyl)(carboxyméthyl)amino]-2-[5-amino-2-(carboxyméthoxy)phényl]éthanoïque qui consiste à faire réagir l'acide diéthylènetriamineacétique et l'acétamidophénol avec l'acide glyoxylique.
11. Procédé conforme à la revendication 1, étape (E), pour préparer le 2,6-bis-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)aminométhyl]-4-(amino)phénol qui consiste à faire réagir le 2,6-bis-[(2-[(2{[bis(carboxyméthyl)]amino}éthyl)(carboxyméthyl)aminométhyl]-4-(acétamido)phénol avec DCl dans D_2O .
12. Procédé conforme à la revendication 1, étape (F) pour préparer le 2,6-bis-[(2{[bis(carboxyméthyl)]-amino}éthyl)(carboxyméthyl)aminométhyl]-4-(isothiocyanato)phénol qui consiste à faire réagir le 2,6-bis-[(2{[bis(carboxyméthyl)]amino}éthyl)(carboxyméthyl)aminométhyl]-4-(amino)phénol avec du thiophosgène.
13. Procédé pour préparer un complexe d'un composé conforme à une quelconque des revendications 1 à 12 ou un sel acceptable d'un point de vue pharmaceutique d'un tel composé, formant un complexe avec un ion métallique choisi parmi La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y ou Sc, lequel procédé consiste à faire réagir le composé avec l'ion métallique.
14. Procédé pour préparer un complexe conforme à la revendication 13 dans lequel l'ion métallique est le ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc ou ^{142}Pr .
15. Procédé pour préparer un conjugué contenant un complexe conforme à la revendication 13 ou 14 lié covalentement à un anticorps ou un fragment d'anticorps, lequel procédé consiste à faire réagir le complexe avec l'anticorps ou le fragment d'anticorps.
16. Procédé pour préparer un conjugué conforme à la revendication 15 dans lequel l'anticorps ou le fragment de celui-ci est un anticorps monoclonal ou un fragment de celui-ci.
17. Procédé pour préparer un conjugué conforme à la revendication 16 dans lequel l'anticorps ou le fragment d'anticorps sont CC-46, CC-49, CC49 F(ab')₂, CC-83, CC-83 F(ab')₂ ou B72.3.

18. Procédé pour préparer une formulation pharmaceutique contenant un complexe conforme à la revendication 13 ou 14 qui consiste à faire réagir le complexe avec un support physiologiquement acceptable.

5 **19.** Procédé pour préparer une formulation pharmaceutique contenant un conjugué conforme à une quelconque des revendications 15 à 17 qui consiste à faire réagir le conjugué avec un support physiologiquement acceptable.

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