639899

SPRUSON & FERGUSON

**Australia**

**Patents Act 1990**

**NOTICE OF ENTITLEMENT**

I, Richard G. Waterman, of 2030 Dow Center, Abbott Road, Midland, Michigan 48640, United States of America, being authorised by the Applicant/Nominated Person in respect of Application No 48282/90 state the following:-

The Applicant/Nominated Person has entitlement from the actual inventors as follows:-

The Applicant/Nominated Person is the assignee of the actual inventors.

The Applicant/Nominated Person is entitled to rely on the application listed in the Declaration under Article 8 of the PCT as follows:

The Applicant/Nominated Person is the assignee of the basic applicants.

The basic application listed on the Declaration under Article 8 of the PCT is the application first made in a Convention country in respect of the invention.

DATED this 18 day of May 1993
at Midland, Michigan 48640, U.S.A.

The Dow Chemical Company

SIGNATURE

Richard G. Waterman
General Patent Counsel

IRN: 138028

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48282/92
17-08.93

Dow Case No. 32,813D IW

**AUSTRALIA** **AUSTRALIA**
**Patent Declaration**

*Patents Act*

# DECLARATION FOR A PATENT APPLICATION

INSTRUCTIONS
(a) Insert "Convention" if applicable
(b) Insert FULL name(s) of applicant(s)

In support of the (a) CONVENTION application made by (b)
THE DOW CHEMICAL COMPANY
2030 Dow Center, Abbott Road
Midland, Michigan 48640, U.S.A.
(hereinafter called "applicant(s)") for a patent (c) for an invention entitled (d)

(c) Insert "of addition" if applicable
(d) Insert TITLE of invention

MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE

(e) Insert FULL name(s) AND address(es) of declarant(s) (See headnote*)

I/~~We~~ (e) Richard G. Waterman, General Patent Counsel
THE DOW CHEMICAL COMPANY
2030 Dow Center, Abbott Road
Midland, Michigan 48640, U.S.A.
do solemnly and sincerely declare as follows:

~~1. I am/We are the applicant(s).~~
(or, *in the case of an application by a body corporate*)
1. I am/~~We are~~ authorized to make this declaration on behalf of the applicant(~~s~~).
~~2. I am/We are the actual inventor(s) of the invention.~~
(or, *where the applicant(s) is/are not the actual inventor(s)*)
2. (f)

(f) Insert FULL name(s) AND address(es) of actual inventor(s)

Jaime Simon, Rte. 1 Box 120-G, Angleton, Texas 77515 USA
David A. Wilson, 229 San Saba, Richwood, Texas 77531 USA
Joseph R. Garlich,301 Southern Oaks,Lake Jackson,Texas 77566 USA
David E. Troutner,402 Edgewood Avenue,Columbia,Missouri 65201 USA

All of the above are citizens of the United States of America
All of the above are chemists by profession

(g) Recite how applicant(s) derive(s) title from actual inventor(s) (See headnote**)

~~is~~/are the actual inventor(s) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows:
(g) The applicant Company is the assignee of the said invention from the said actual inventor(s).

(h) Insert country, filing date, and basic applicant(s) for the/or EACH basic application

(**Note:** *Paragraphs 3 and 4 apply* only *to Convention applications*)
3. The basic application(~~s~~) for patent or similar protection on which the application is based is/~~are~~ identified by **country, filing date, and basic applicant(s)** as follows:
(h)

United States of America filed on December 15, 1989 in the names of Jaime Simon, Joseph R. Garlich, David A. Wilson and David E. Troutner

4. The basic application(~~s~~) referred to in paragraph 3 hereof was/~~were~~ the first application(~~s~~) made in a Convention country in respect of the invention the subject of the application.

(k) Insert PLACE of signing

(l) Insert DATE of signing

(m) Signature(s) of declarant(s)

CORP.
SEAL

Declared at (k) Midland, Michigan 48640, U.S.A.
Dated (l) 25 July 19 90
(m) THE DOW CHEMICAL COMPANY
SIGNATURE
By: R R Waterman
RICHARD G. WATERMAN
General Patent Counsel

To: The Commissioner of Patents

Agent: Spruson & Ferguson

AU9048282

(12) PATENT ABRIDGMENT (11) Document No. AU-B-48282/90
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 639899

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**MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE**

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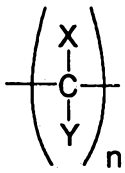
(56) Prior Art Documents
**AU 47009/89 A61K 43/00 31/675**
**AU 45440/89 A61K 33/24 31/66 43/00**

(57) Claim

1. A method for the therapeutic treatment or prophylaxis of calcific tumors in an animal having one or more calcific tumors, which method comprises administering to said animal a therapeutically effective amount of a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene radical of the formula



(I)

wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when $n > 1$, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

10. A method for the therapeutic treatment or prophylaxis of calcific tumors in an animal having one or more calcific tumors, which method comprises administering to said

.../2

animal a therapeutically effective amount of a composition which comprises a complex of (1) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid, or a physiologically acceptable salt thereof, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

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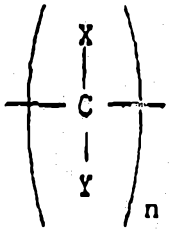
(54) Title: MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE




(I)

(57) Abstract

Particle emitting radionuclides, e.g. Samarium-153, have been complexed with certain macrocyclic aminophosphonic acids wherein the nitrogen and phosphorus are interconnected by an alkylene group or substituted alkylene group. A composition is now disclosed which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene radical of formula (I), wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when n > 1, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175.

# MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE

The present invention concerns macrocyclic aminophosphonic acid complexes for the treatment of cancer, especially the treatment of calcific tumors and for the relief of bone pain, the method of treatment of calcific tumors, and compositions and formulations having as their active ingredient a radionuclide complexed with a macrocyclic aminophosphonic acid, and the process for preparing the macrocyclic aminophosphonic acid complexes.

The development of bone metastasis 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 percent of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastasis. Bone metastasis are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix and other tumors, but collectively, these represent less than 20 percent of patients who develop bone metastasis. 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, thus 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 use of these compounds. [See for example, Kaplan, E., et al., *Journal of Nuclear Medicine* 1(1), 1 (1960) and 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. (See U.S. Patent 4,399,817).

The use of radionuclides for calcific tumor therapy is discussed in published European patent application 176,288 where the use of Sm-153, Gd-159, Ho-166, Lu-177 or Yb-175 complexed with certain ligands selected from ethylenediaminetetraacetic acid

(EDTA) or hydroxyethylethylenediaminetriacetic acid (HEEDTA) is disclosed.

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 external beam irradiation is not practical. (See Seminars in Nuclear Medicine, Vol. IX, No. 2, April, 1979).

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

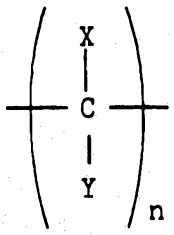
Strontium-89 has also been proposed for patients with metastatic bone lesions. However, the long half-life (50.4 days), high blood levels and low lesion to normal bone ratios limit the utility. [See Firusian, N., Mellin, P., Schmidt, C. G., *The Journal of Urology* 116, 764 (1976); Schmidt, C. G., Firusian, N., *Int. J. Clin. Pharmacol.* 93, 199-205, (1974).]

A palliative treatment of bone metastasis has been reported which employed I-131 labeled α-amino-(3-iodo-4-hydroxybenzylidene)diphosphonate [Eisenhut, M., *Journal of Nuclear Medicine* 25(12), 1356-1361 (1984)]. The use of radioactive iodine 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.

Surprisingly, the present invention overcomes many of the above noted problems. The present invention concerns at least one composition having a radionuclide complexed with a macrocyclic aminophosphonic acid, such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or its physiologically acceptable salt, which composition causes minimal damage to normal tissue when administered in the method of the invention. Surprisingly, the present complex is more effective at a lower ligand to metal molar ratio than has been known previously in the art.

Particularly, this invention concerns a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula

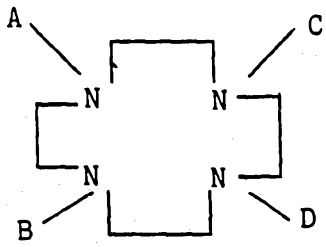


(I)

wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable

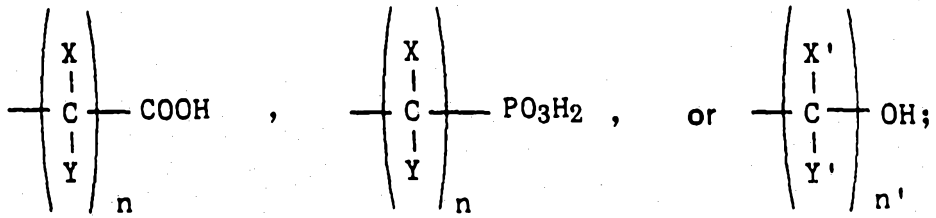
salts of the acid radicals; and n is 1-3, with the proviso that when n>1, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, and

wherein the resulting composition is therapeutically effective. Particularly preferred are macrocyclic moieties of Formula (I) where X and Y are hydrogen and n is 1. Especially preferred are a certain macrocyclic aminophosphonic acid of the structure




(II)

wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula




and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined before; X' and Y' are independently hydrogen, methyl or ethyl

radicals; n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group. The preferred macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP). The composition can be administered as a formulation with suitable pharmaceutically acceptable carriers. The present invention includes the use of the complex, composition or formulation described herein in combination with one or more other agents, drugs, treatments and/or radiation sources which assist in therapy of calcific tumors or relief of bone pain .

Certain compositions containing these complexes have been found useful for therapy of calcific tumors in animals. The administration of the therapeutic compositions can be palliative to the animal, for example by alleviating pain and/or inhibiting tumor growth and/or causing regression of tumors and/or destroying the tumors. As will be more fully discussed later, the properties of the radionuclide, of the macrocyclic aminophosphonic acid and of the complex formed therefrom are important considerations in determining the effectiveness of any particular composition employed for such treatment.

In addition, the present invention also includes formulations having at least one of the radionuclide(s) complexed with at least one of the macrocyclic aminophosphonic acids as defined above, especially those macrocyclic aminophosphonic acids of Formula (II), and a pharmaceutically acceptable carrier, excipient or vehicle therefor. The methods for preparing such formulations are well known. The formulations are sterile and may be in the form of a

suspension, injectable solution or other suitable pharmaceutically acceptable formulations. Pharmaceutically acceptable suspending media, with or without adjuvants, may be used. The sterile compositions are suitable for administration to an animal wherein the composition is defined as before and has the radionuclide in dosage form present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal, preferably at least 0.2 mCi per kilogram of body weight of said animal..

Particle-emitting radionuclides employed in the compositions of the invention are capable of delivering a high enough localized ionization density to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors, and/or destroy the tumor and are capable of forming complexes with the macrocyclic aminophosphonic acid ligands described herein. The radionuclides found to be useful in the practice of the invention are Samarium-153 (Sm-153), Holmium-166 (Ho-166), Ytterbium-175 (Yb-175), Lutetium-177 (Lu-177), Yttrium-90 (Y-90) and Gadolinium-159 (Gd-159).

For the purpose of convenience, the compositions having a radionuclide-macrocyclic aminophosphonic acid complex of the present invention will frequently be referred to herein as "radionuclide compositions" or "compositions" and the macrocyclic aminophosphonic acid derivative referred to as the "ligand" or "chelant".

As used herein, the term "animals" means warm blooded mammals, including humans, and is meant to

encompass animals in need of treatment for calcific tumors or in need of relief of bone pain.

The term "calcific tumors" includes primary tumors, where the skeletal system is the first site of involvement, invasive tumors where the primary tumor invades the skeletal system or other tissue tumors which calcify, and metastatic bone cancer where the neoplasm spreads from other primary sites, e.g. prostate and breast, into the skeletal system.

For the purpose of the present invention, the complexes described herein and physiologically acceptable salts thereof are considered equivalent in the therapeutically effective compositions. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand or ligands employed and which will not cause a significant adverse physiological effect when administered to an animal at dosages consistent with good pharmacological practice; some examples of such practice are described herein. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary amines and the like. Physiologically acceptable salts may be prepared by treating the macrocyclic aminophosphonic acid as defined above, especially those of Formula (II), with an appropriate base.

The formulations of the present invention are in the solid or liquid form containing the active

radionuclide complexed with the ligand. These formulations may be in kit form such that the two components are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier. Additionally, for stability and other factors, if the formulations are complexed with the radionuclide prior to shipment to the ultimate user, the formulation having the complex and a buffer present are frozen in a kit form, and which frozen formulation is later thawed prior to use.

Injectable compositions of the present invention may be either in suspension or solution form. In the preparation of suitable formulations it will be recognized that, in general, the water solubility of the salt is greater than the free acid. In solution form the complex (or when desired the separate components) is dissolved in a pharmaceutically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, water, aqueous alcohols, glycols, and phosphonate or carbonate esters. Such aqueous solutions contain no more than 50 percent of the organic solvent by volume.

Injectable suspensions as compositions of the present invention require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinyl-pyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethlycellulose. Suitable physiologically acceptable adjuvants, if necessary to keep the complex in suspension, may be chosen from among thickners such as carboxymethyl-

cellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters. Many substances which effect the hydrophibicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silcone antifoams, sorbitol, and sugars are all useful suspending agents.

Complexes employed in the compositions or formulations of the present invention must fit certain criteria insofar as possible as discussed below.

One criteria concerns the selection of the radionuclide. While the properties of the radionuclide are important, the overall properties of the composition containing the radionuclide-macrocyclic aminophosphonic acid 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.

There is a need 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. For example, the radionuclide must be delivered preferentially to the bone rather than to soft tissue. Most particularly, uptake of the radionuclide in either liver or blood is undesirable. Additionally, 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 the compositions and formulations 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, or other tissue tumors which calcify, or 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 or formulation 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.

The "effective amount" or "therapeutically effective amount" of radionuclide composition to be administered to treat calcific tumors will vary according to factors such as the age, weight and health of the patient, the calcific tumor being treated, the treatment regimen selected as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The compositions of this

invention may also be employed at doses which are useful but not therapeutic.

A suitable dose of the composition or formulation of this invention for use in this invention is at least about 0.02 mCi per Kg of body weight. A "therapeutically effective dose" of the composition or formulation of this invention for use in this invention is at least about 0.2 mCi per Kg of body weight.

The effective amount used to treat calcific tumors will typically be administered, generally by administration into the bloodstream, in a single dose or multipule doses. The amounts to be administered to achieve such treatment are readily determined by one skilled in the art employing standard procedures.

The radionuclide and ligand may be combined under any conditions which allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is all that is required. The complex formed is by a chemical bond and results in a relatively stable radionuclide composition, e.g. stable to the disassociation of the radionuclide from the ligand.

The macrocyclic aminophosphonic acid complexes when administered at a ligand to metal molar ratio of at least about 1:1, preferably from 1:1 to 3:1, more preferably from 1:1 to 1.5:1, give biodistributions that are consistent with excellent skeletal agents. By contrast, certain other aminophosphonic acid complexes result in some localization in soft tissue (e.g. liver) if excess amounts of ligand are not used. A large excess of ligand is undesirable since uncomplexed ligand

may be toxic to the patient or may result in cardiac arrest or hypocalcemic convulsions. In addition, the macrocyclic aminophosphonic acid ligands are useful when large amounts of metal are required (i.e. for metals that have a low specific activity). In this case, the macrocyclic aminophosphonic acid ligands have the ability to deposit larger amounts of activity in the bone than is possible when using non-cyclic aminophosphonic acid ligands.

A preferred embodiment of the present invention is a therapeutically effective composition or formulation containing complexes of at least one radionuclide of Gd-159, Ho-166, Lu-177, Sm-153, Y-90 and Yb-175 with DOTMP or a physiologically acceptable salt(s) thereof.

Combinations of the various above noted radionuclides can be administered for the therapeutic treatment of calcific tumors. The combinations can be complexed as herein described by complexing them simultaneously, mixing two separately complexed radionuclides, or administering two different complexed radionuclides sequentially. It may be possible to achieve the same beneficial results of high delivery of the radionuclide to the area of the tumor, but with little soft tissue damage, by administering the ligand and the radionuclide in a manner which allows formation of the radionuclide-chelant complex *in situ* such as by simultaneous or near simultaneous administration of the radionuclide and an appropriate amount of ligand or by the administration of ligand and a radionuclide complexed with a weaker ligand, i.e., one which undergoes ligand exchange with the ligands of this invention, such that the desired

radionuclide-chelant complex is formed via ligand exchange *in situ*. The composition or formulation may be administered as a single dose or as multiple doses over a longer period of time.

Aminophosphonic acids can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive amine hydrogen with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof. The amine precursor (1,4,7,10-tetraazacyclododecane) employed in making the macrocyclic aminophosphonic acids is a commercially available material.

Methods for carboxyalkylating to give amine derivatives containing a carboxyalkyl group are well known (U.S. 3,726,912) as are the methods which give alkyl phosphonic and hydroxyalkyl (U.S. 3,398,198) substituents on the amine nitrogens.

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

$$\text{Sm-152} + \text{neutron} \longrightarrow \text{Sm-153} + \text{gamma}.$$

Another process for obtaining radionuclides is by bombarding nuclides with linear accelerator or cyclotron-produced particles. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The process for obtaining

the radionuclide is not critical to the present invention.

For example, to irradiate $Sm_2O_3$ for production of Sm-153, the desired amount of target was first weighed into a quartz vial, the vial was flame sealed under vacuum and welded into an aluminum can. The can was irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial was removed and transferred to a glove box, crushed into a glass vial which was then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1 to 4M HCl was then added to the vial via syringe to dissolve the $Sm_2O_3$. Once dissolved, the solution was diluted to the appropriate volume by addition of water. The solution was removed from the original dissolution vial which contains chards of the crushed quartz vial and transferred via syringe to a clean glass serum vial. This solution was then used for complex preparation. Similar procedures can be used to prepare Lu-177, Yb-175, Gd-159, Y-90 and Ho-166.

The invention described herein provides a means of delivering a therapeutic amount of radioactivity to calcific tumors. However, it may also be desirable to administer a "sub-therapeutic" amount (i.e. "useful amount") to determine the fate of the radionuclide using a scintillation camera prior to administering a therapeutic dose. Therapeutic doses will be administered in sufficient amounts to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors and/or kill the tumor. Amounts of radionuclide needed to provide the desired therapeutic dose will be determined experimentally and

optimized for each particular composition. The amount of radioactivity required to deliver a therapeutic dose will vary with the individual composition employed. For example, less activity will be needed for radionuclides with longer half-lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The composition to be administered may be given in a single treatment or fractionated into several portions and administered at different times. Administering the composition in fractionated doses may make it possible to minimize damage to non-target tissue. Such multiple dose administration may be more effective.

The compositions of the present invention may be used in conjunction with other active agents and/or ingredients that enhance the therapeutic effectiveness of the compositions and/or facilitate easier administration of the compositions.

Studies to determine the qualitative biodistribution of the various radionuclides were conducted by injecting the compositions into rats and obtaining the gamma ray images of the entire animal at various times up to two hours after injection.

Quantitative biodistributions were obtained by injecting 50-100 microliters of the composition into the tail vein of unanesthetized male Sprague Dawley rats. The rats were then placed in cages lined with absorbent paper in order to collect all urine excreted prior to sacrifice. After a given period of time, the rats were sacrificed by cervical dislocation and the various tissues dissected. The samples were then rinsed with saline, blotted dry on absorbent paper and

weighed. The radioactivity in the samples was measured with a NaI scintillation counter.

The following examples are included to aid in the understanding of the invention but are not to be construed as limiting the invention.

Preparation of Starting Materials

Example A: Preparation of DOTMP

In a 100-mL three necked round-bottomed flask equipped with a thermometer, reflux condenser, and heating mantle was added 3.48 g (20.2 mmole) of 1,4,7,10-tetraazacyclododecane and 14 ml of water. This solution was treated with 17.2 mL of concentrated HCl and 7.2 g of $H_3PO_3$ (87.8 mmole) and heated to 105°C. The refluxing suspension was stirrred vigorously and treated dropwise with 13 g (160.2 mmole) of formaldehyde (37 wt percent in water) over a one hour period. At the end of this time the reaction was heated at reflux an additional 2 hours after which the heat was removed and the reaction solution allowed to cool and set at room temperature for 62.5 hours. The reaction solution was then concentrated *in vacuo* at 40°C to a viscous reddish brown semisolid. A 30 mL portion of water was added to the semisolid which started to dissolve but then began to solidify. The whole suspension was then poured into 400 mL of acetone with vigorously stirring. The resulting off-white precipitate was vacuum filtered and dried overnight to give 10.69 g (97 percent yield) of crude DOTMP. A 2.0 g (3.65 mmole) sample of the crude DOTMP was dissolved in 2 mL of water by the addition of 700 µL of concentrated ammonium hydroxide (10.0 mmole) in 100 µL portions to give a solution at pH of 2-3.

This solution was then added all at once to 4.5 mL of 3N HCl (13.5 mmole), mixed well, and allowed to set. Within one hour small squarish crystals had begun to form on the sides of the glass below the surface of the liquid. The crystal growth was allowed to continue undisturbed for an additional 111 hours after which time the crystals were gently bumped off of the vessel walls, filtered, washed with 3 mL portions of water, four times, and air dried to constant weight to give 1.19 g (60 percent yield) of white crystalline solid DOTMP.

Example B: Preparation of DOTMP

A 250 mL three-necked, round-bottomed flask was loaded with 6.96 g (0.04 moles) of 1,4,7,10-tetraazacyclododecane. To this flask was added 14.5 g (0.177 moles) of phosphorous acid, 30 mL of deionized water and 28 mL of concentrated hydrochloric acid (0.336 moles).

The flask was attached to a reflux condenser and fitted with a stir bar, and a thermometer adapted with a thermowatch controller. A separate solution of 26.0 g (0.32 moles) of aqueous 37 percent formaldehyde solution was added to a 100 mL addition funnel and attached to the flask. The flask was brought to reflux temperature (about 105°C) with vigorous stirring. The formaldehyde solution was added dropwise over a 30-40 minute interval. The solution was heated and stirred for an additional three hours then cooled slowly to ambient temperature.

The reaction solution was transferred to a 500 mL round-bottomed flask and attached to a rotary evaporation apparatus. The solution was taken down to a

viscous, amber semi-solid (note - temperature never exceeded 40°C). This semi-solid was treated with approximately 300 mL of HPLC grade acetone producing a light brown, sticky viscous oil. This oil was dissolved in 22 mL of water and added slowly with vigorous stirring to 1L of acetone. The acetone was decanted and the light colored oil dried under vacuum to give 16.6 g (76 percent yield) of crude DOTMP. To 13.1 g of this crude DOTMP was added 39.3 g of deionized water along with a seed crystal and the solution allowed to stand overnight. The resulting precipitate was vacuum filtered, washed with cold water, and dried under vacuum to give 4.75 g of DOTMP (36 percent yield).

A further purification was performed by dissolving 3.0 g (5.47 mmole) of DOTMP from above in 3 mL of water by the addition of 2.2 mL (31.5 mmole) of concentrated ammonium hydroxide. This solution was made acidic by the addition of 2.4 mL (28.8 mmole) of concentrated HCl at which time a white solid precipitated. This precipitate was vacuum filtered and dried to give 2.42 g (81 percent yield) of purified DOTMP characterized by a singlet at 11.5 ppm (relative to 85 percent $H_3PO_4$) in the $^{31}P$ decoupled NMR spectrum.

Example C: Preparation of Sm-153

Sm-153 can be produced in a reactor such as the University of Missouri Research Reactor. Sm-153 is produced by irradiating 99.06 percent enriched $^{152}Sm_2O_3$ in the first row reflector at a neutron flux of $8 \times 10^{13}$ neutron/$cm^2$·sec. Irradiations were generally carried out for 50 to 60 hours, yielding a Sm-153 specific activity of 1000-1300 Ci/g.

To irradiate $Sm_2O_3$ for production of Sm-153, the desired amount of target is first weighed into a quartz vial, the vial flame sealed under vacuum and welded into an aluminum can. The can is irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial is removed and transferred to a glove box, opened into a glass vial which is then sealed. An appropriate amount of a solution of hydrochloric acid is then added to the vial via syringe in order to dissolve the $Sm_2O_3$. Once the $Sm_2O_3$ is dissolved, the Samarium solution is diluted to the appropriate volume by addition of water. The solution is removed from the original dissolution vial which contains the chards of the quartz irradiation vial, and transferred via syringe to a clean glass serum vial.

Example D: Preparation of Ho-166

Holmium-166 is prepared by weighing 0.5-1.0 mg of $Ho_2O_3$ into a quartz vial. The vial is sealed and placed in an aluminum can which is welded shut. The sample is irradiated (usually for about 24-72 hours) in the reactor (first row reflector, neutron flux of $8 \times 10^{13}$ neutron/$cm^2$·sec). After irradiation, the vial is opened and the oxide is dissolved using 4N HCl. Heating may be necessary. Water is then used to dilute the sample to an appropriate volume.

Example E: Preparation of Gd-159

Gadolinium-159 is prepared by sealing gadolinium oxide (1.1 mg) in a quartz vial. The vial is welded inside an aluminum can and irradiated for 30 hours in a reactor at a neutron flux of $8 \times 10^{13}$

neutron/$cm^2$·sec. The contents of the quartz vial is dissolved using HCl. Water is added to obtain a solution of Gd-159 in 0.1N HCl.

Example F: Preparation of Y-90

A non-radioactive Ytterium (Y) solution was prepared by dissolving 15.1 mg of $YCl_3 \cdot 6H_2O$ in 11.24 mL of water. A quantity of 1500 µL of this solution was added to a vial containing 0.5 mL of Y-90 solution (prepared by neutron irradiation of 1 mg of $Y_2O_3$ followed by dissolution in 1N HCl to give a final volume of 0.5 mL).

Example G: Preparation of Yb-175 and Lu-177

When the procedure of Examples C, D, E or F are repeated using the appropriate oxide, the radioisotopes of Ytterbium-175 (Yb-175) and Lutetium-177 (Lu-177) are prepared.

Preparation of Final Products

Example 1: Preparation and Biodistribution of Sm-DOTMP and Sm-153-DOTMP

The ligand of Example A (22 mg) was dissolved in 878 µl of distilled water and 15 µl of 50 percent NaOH. A volume of 15 µl of this solution was transferred to a vial containing 1.5 mL of Sm solution (0.3 mM Sm in 0.1N HCl spiked with 2 µl of Sm-153 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Sm found as a complex was >99 percent as determined by ion exchange chromatography. This yielded a solution containing Sm at 0.3 mM with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table I. The numbers represent the average of 3 rats per data point.

TABLE I
% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP[1]

| Tissue | % Dose |
|---|---|
| Bone | 58.1 |
| Liver | 0.06 |
| Kidney | 0.27 |
| Spleen | 0.004 |
| Muscle | 0.15 |
| Blood | 0.004 |

[1] Ligand to Sm Molar Ratio of approximately 1.5

Example 2: Preparation and Biodistribution of Ho-DOTMP and Ho-166-DOTMP

The ligand of Example A (22 mg) was dissolved in 878 µL of distilled water and 15 µL of 50 percent NaOH. A volume of 30 µL of this solution was transferred to a vial containing 1.5 mL of Ho solution (0.6 mM Ho in 0.1N HCl spiked with 2 µL of Ho-166 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Ho found as a complex was greater than 99 percent as determined by ion exchange chromatography. This yielded a solution containing 0.6 mM Ho with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Ho solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table II. The numbers represent the average of 3 rats per data point.

TABLE II
% INJECTED DOSE IN SEVERAL TISSUES FOR Ho-DOTMP[1]

| Tissue | % Dose |
|---|---|
| Bone | 57 |
| Liver | 0.07 |
| Kidney | 0.4 |
| Spleen | 0.006 |
| Muscle | 0.3 |
| Blood | 0.07 |

[1] Ligand to Ho Molar Ratio of approximately 1.5

Example 3: Preparation and Biodistribution of Sm-DOTMP, Sm-153-DOTMP, Ho-DOTMP and Ho-166-DOTMP

A quantity of 14.5 mg of the ligand of Example B was placed in a vial and dissolved in 760 µL of water and 5 µL of 50 percent NaOH. A volume of 1100 µL of Sm solution (0.3 mM Sm in 0.1N HCl) which was spiked with Sm-153, was placed in a separate vial and 10 µL of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 3 plastic columns containing 1.5 mL of cation exchange resin (Sephadex™ C-25 from Pharmacia). The amount of Sm as a complex was determined to be 99 percent by cation exchange chromatography.

A volume of 1100 µL of Ho solution (0.6 mM Ho in 0.1N HCl) which was spiked with Ho-166, was placed in a separate vial and 20 µL of the above ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 2 plastic columns containing 1.5 mL of cation exchange resin (Sephadex C-25 from Pharmacia). The amount of Ho as a

complex was determined to be 99 percent by cation exchange chromatography.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table III. The numbers represent the average of 3 rats per data point.

TABLE III
% INJECTED DOSE IN SEVERAL TISSUES FOR DOTMP METAL COMPLEXES

| Tissue | Sm | Ho |
|---|---|---|
| Bone | 50 | 64 |
| Liver | 0.37 | 0.19 |
| Kidney | 0.29 | 0.32 |
| Spleen | 0.04 | 0.05 |
| Muscle | 0.49 | 0.22 |
| Blood | 0.12 | 0.17 |

Example 4: Preparation and Biodistribution of Gd-DOTMP and Gd-159-DOTMP

The ligand of Example B (14.5 mg) was placed in a vial and dissolved in 760 µL of water and 5 µL of 50 percent NaOH. A volume of 1000 µL of Gd solution (0.3 mM Gd in 0.1N HCl) which contained tracer quantities of Gd-159, was placed in a separate vial and 15 µL of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the amount of Gd as a complex was determined to be >99 percent by cation exchange chromatography.

A Sprague Dawley rat was allowed to acclimate for five days then injected with 175 µL of the solution described above via a tail vein. The rat weighed 155 g at the time of injection. After 2 hours the rat was killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 175 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IV.

TABLE IV
% INJECTED DOSE IN SEVERAL TISSUES
FOR Gd-DOTMP[1]

| Tissue | % Dose |
|---|---|
| Bone | 50 |
| Liver | 0.08 |
| Kidney | 0.25 |
| Spleen | None Detected* |
| Muscle | 0.08 |
| Blood | 0.06 |

[1] Ligand to Gd molar ratio of approximately 1.5
*counts in the spleen were below background background

Example 5: Preparation and Biodistribution of Lu-DOTMP and Lu-177-DOTMP

The ligand of Example B (15.8 mg) was dissolved in 963 µL of distilled water and 8 µL of 50 percent NaOH. A volume of 15 µL of this solution was transferred to a vial containing 1.5 mL of Lu solution (0.3 mM Lu in 0.1N HCl spiked with 2 µL of Lu-177 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Lu found as a complex was >99 percent by ion exchange chromatography. This yielded a solution containing 0.3 mM Lu with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Lu solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI

scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table V. The numbers represent the average of 3 rats per data point.

TABLE V
% INJECTED DOSE IN SEVERAL TISSUES FOR Lu-DOTMP[1]

| Tissue | % Dose |
|---|---|
| Bone | 54 |
| Liver | 0.08 |
| Kidney | 0.3 |
| Spleen | 0.006 |
| Muscle | 0.04 |
| Blood | 0.09 |

[1] Ligand to Lu molar ratio of approximately 1.5

Example 6: Preparation and Biodistribution of Y-DOTMP and Y-90-DOTMP

To the solution of Y and Y-90 prepared in Example F was added 200 µl (0.0266 moles) of DOTMP from Example B in water and the pH of the solution adjusted to 7.5 using 50 percent NaOH and 1N NaOH. The percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 1.7.

Sprague Dawley rats were allowed to acclimate for eight days then injected with 150 µL of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VI. The numbers represent the average of 5 rats per data point.

TABLE VI
% INJECTED DOSE IN SEVERAL TISSUES
FOR Y-DOTMP[1]

| Tissue | % Dose |
|---|---|
| Bone | 33 |
| Liver | 0.06 |
| Kidney | 0.35 |
| Spleen | 0.01 |
| Muscle | 0.31 |
| Blood | 0.12 |

[1] Ligand to Y molar ratio of approximately 1.7

Example W (Comparative)

To a vial containing 0.5 mL of Y-90 solution (prepared by the irradiation of 1 mg of $Y_2O_3$ followed by dissolution in 1.1N HCl to give a final volume of 0.5 mL) was added 1.5 mL of water to give a $8.86 \times 10^{-3}$ molar solution of Y containing tracer Y-90. To 2 mL ($1.772 \times 10^{-5}$ mole) of this solution was added 133 µL ($1.676 \times 10^{-4}$ mole) of 1.26M ethylenediaminetetramethylenephosphonic acid (EDTMP) solution where upon the solution became turbid. The solution cleared up upon addition of 50 µL of 50 percent NaOH. To this solution was added 40 µL ($5.04 \times 10^{-5}$ mole) more of 1.26M EDTMP solution. The pH of the resulting solution was 7.5 and the percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 123.

Sprague Dawley rats were allowed to acclimate for eight days then injected with 150 µL of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table W. The numbers represent the average of 5 rats per data point.

TABLE W
% INJECTED DOSE IN SEVERAL TISSUES
FOR Y-EDTMP[1]

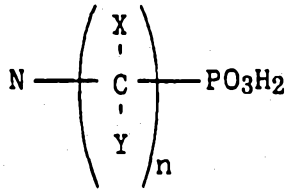
| Tissue | % Dose |
|---|---|
| Bone | 30 |
| Liver | 0.09 |
| Kidney | 0.30 |
| Spleen | 0.01 |
| Muscle | 0.58 |
| Blood | 0.15 |

[1] Ligand to Y molar ratio of approximately 123

(There are no Examples X and Y.)

Example Z (Comparative)

In a method similar to that previously used, compositions were prepared containing complexes of Sm-153 with several commercially available phosphonic acids which do not contain the alkylene linkage between the nitrogen and the phosphorus atoms (which linkage is required in the present ligand).



The two hour biolocalization of Sm-153 in rats for these compositions was determined as previously described. The results are given in Table X. The ligands used include methylendiphosphonic acid (MDP) and hydroxyethylidinediphosphonic acid (HEDP) which contain a $P-CH_2-PO_3H_2$ and a $P-C(CH_3)(OH)-PO_3H_2$ linkage, respectively; pyrophosphate (PYP) which contains a $P-O-PO_3H_2$ linkage; and imidodiphosphate (IDP) which contains a $N-PO_3H_2$ linkage. Metal complexes of these ligands are known skeletal agents. For example, Tc complexes of MDP, HEDP, and PYP have been used commercially as diagnostic bone agents. However, these ligands were inadequate for selectively delivering Sm-153 to the skeletal system as exemplified by the large fraction of the radioactivity found in the liver and/or blood.

Table Z shows the biolocalization of Sm-153 in rats two hours after injection and the results represent the percent of injected dose in tissue.

TABLE Z

| % Dose In | Sm-153 MDP | Sm-153 HEDP | Sm-153 PYP | Sm-153 IDP |
|---|---|---|---|---|
| Bone | 2 | 21 | 2 | 0.6 |
| Liver | 85 | 3.5 | 73 | 36 |
| Blood | 0.23 | 13 | 0.23 | 0.04 |

The numbers given in Table Z for Sm-153-MDP, Sm-153-HEDP, Sm-153-PYP and Sm-153-IDP represent the average of the results of five, five, three and three rats, respectively.

Example 7: Preparation of Sm-DOTMP or Ho-DOTMP Kit Using HEPES Buffer

A 0.1M solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma™ Chemical Co., St. Louis, MO) at a pH of 7.43 was prepared. A 0.0066M solution of DOTMP was prepared by dissolving 68.2 mg (1.084 x 10-4 µmole) of DOTMP in 16.4285 mL of 1N NaOH. Into each of seven 10 mL serum vials was placed 0.600 mL (3.96 mole) of DOTMP solution and 3.00 mL of 0.1M HEPES buffer solution. Each serum vial was then placed in a dry ice/acetone bath until the liquid was frozen and then placed in a Virtis Freeze Dryer Apparatus overnight which gave the aqueous components as a dry white powder in the bottom of the serum vials. The serum vials were then stoppered and sealed by crimping. These kits were formulated to receive 6 mL of either $SmCl_3$ ($3 \times 10^{-4}$ mole) or $HoCl_3$ ($6 \times 10^{-4}$ mole) in 0.1N HCl.

Example 8: Reconstitution of Sm-DOTMP or Ho-DOTMP Kit Containing HEPES Buffer

A 6.0 mL addition of $SmCl_3$ ($3 \times 10^{-4}$M spiked with Sm-153 in 0.1N HCl) was made to one of the kits described in Example 7. The pH of the resulting reconstituted kit was 7.5 and the percent of Sm that was complexed was determined using cation exchange chromatography to be >99 percent.

Similarly, a 6.0 mL addition of $HoCl_3$ ($6 \times 10^{-4}$M spiked with Ho-166) in 0.1N HCl was made to

one of the kits described in Example 7. The pH of the resulting solution was 7.5 and the percent of Ho that was complexed was determined using cation exchange chromatography to be >97 percent.

Example 9: Reconstitution and Biodistribution of Sm-HEPES-DOTMP Kits

A kit from Example 8 was treated with 6.0 mL of $SmCl_3$ ($3 \times 10^{-4}$M spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was 7.5 and the percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VII. The numbers represent the average of 3 rats per data point.

TABLE VII
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP/HEPES BUFFER

| Tissue | % Dose |
|---|---|
| Bone | 58 |
| Liver | 0.06 |
| Kidney | 0.29 |
| Spleen | 0.01 |
| Muscle | 0.18 |
| Blood | 0.06 |

Example 10: Preparation of Sm-DOTMP Kits Using Bicarbonate Buffer

A 0.009M solution of DOTMP at pH 6.66 was prepared by adding 141.5 mg (2.25 x 10-4 mole) of DOTMP to 9 mL of 1N NaOH and diluting to 25 mL final volume. A 0.4M solution of sodium bicarbonate ($NaHCO_3$) was prepared by dissolving 8.4 g of $NaHCO_3$ in 250 mL of water. Kits were prepared by adding 3.0 mL of $NaHCO_3$ solution and 0.300 mL of DOTMP solution to each of seven 10 mL serum vials and treating them as described in Example 7 to give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of $SmCl_3$ ($3 \times 10^{-4}$M) in 0.1N HCl which would give a ligand to metal ratio of 1.5:1.

Example 11: Reconstitution and Biodistribution of Sm-DOTMP Kits Using Bicarbonate Buffer

A kit from Example 10 was treated with 6.0 mL of $SmCl_3$ ($3 \times 10^{-4}$M spiked with Sm-153) in 0.1N HCl.

The pH of the resulting solution was 6.55 and was adjusted to 7.27 by the addition of 60 µL of 1N NaOH. The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VIII. The numbers represent the average of 3 rats per data point.

TABLE VIII
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP[1]/BICARBONATE

| Tissue | % Dose |
|---|---|
| Bone | 65 |
| Liver | 0.07 |
| Kidney | 0.34 |
| Spleen | 0.01 |
| Muscle | 0.30 |
| Blood | 0.04 |

[1]Ligand to Sm molar ratio of approximately 1.5

Example 12: Preparation of DOTMP Kit Using Excess Base

A 0.009M solution of DOTMP was prepared as described in Example 10 except more NaOH was added such that the final solution was pH 10.66. Kits were prepared by adding 0.300 mL of DOTMP solution and 0.700 mL of 1.0N NaOH solution to each of five 10 mL serum vials and treating them as described in Example 7 to give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of $SmCl_3$ ($3 \times 10^{-4}$M) in 0.1N HCl which would give a ligand to metal ratio of 1.5:1.

Example 13: Reconstitution and Biodistribution of DOTMP Kits Using Excess Base and Phosphate Buffer

A kit from Example 12 was treated with 5.4 mL of $SmCl_3$ ($3 \times 10^{-4}$M spiked with Sm-153) in 0.1N HCl and 0.6 mL of $SmCl_3$ ($3 \times 10^{-4}$M spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was between 10 and 11. The pH was adjusted to 7.79 by the addition of 0.200 mL of 1.05M phosphate buffer (pH 7.49). The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a

multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IX. The numbers represent the average of 5 rats per data point.

TABLE IX
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP[1]/PHOSPHATE

| Tissue | % Dose |
|---|---|
| Bone | 59 |
| Liver | 0.85 |
| Kidney | 0.41 |
| Spleen | 0.03 |
| Muscle | 0.35 |
| Blood | 0.11 |

[1]Ligand to Sm molar ratio of approximately 1.5

Example 14: Preparation of 18 mL Ho-DOTMP Kits

A 0.009M solution of DOTMP at pH 6.66 was prepared as described in Example 10 except more NaOH was added such that the final solution was at pH 10.19. Kits were prepared by adding 1.800 mL of DOTMP solution and 2.100 mL of 1N NaOH solution to each of twelve 20 mL serum vials. These vials were then treated as described in Example 7 to give the final kits containing a white, dry solid. These kits were formulated to receive 18.0 mL of $HoCl_3$ ($6 \times 10^{-4}$M) which would give a ligand to metal ratio of 1.5:1.

Example 15: Reconstitution and Biodistribution of 18 mL Ho-DOTMP Kits

A kit from Example 14 was treated with 18.0 mL of $HoCl_3$ ($6 \times 10^{-4}$M spiked with Ho-166) in 0.1N HCl. The solution was then treated with 0.6 mL of 1.05M phosphate buffer (pH 7.49) which brought the pH down to 7.53. The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table X. The numbers represent the average of 5 rats per data point.

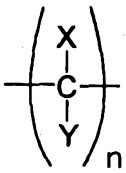
TABLE X
% INJECTED DOSE IN SEVERAL TISSUES
FOR Ho-DOTMP[1]/PHOSPHATE

| Tissue | % Dose |
|---|---|
| Bone | 60 |
| Liver | 0.12 |
| Kidney | 0.35 |
| Spleen | 0.08 |
| Muscle | 0.21 |
| Blood | 0.04 |

[1]Ligand to Ho molar ratio of approximately 1.5

The claims defining the invention are as follows:-

1. A method for the therapeutic treatment or prophylaxis of calcific tumors in an animal having one or more calcific tumors, which method comprises administering to said animal a therapeutically effective amount of a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene radical of the formula

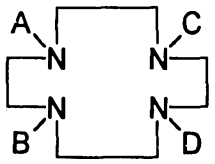


(I)

wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when $n > 1$, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

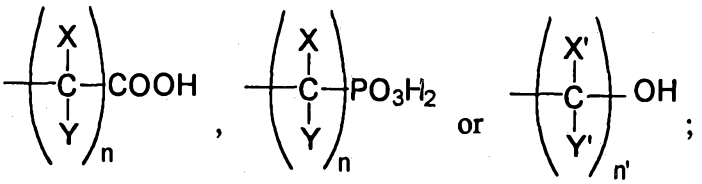
2. The method of claim 1 wherein X and Y are hydrogen and n is 1.

3. The method of claim 1 wherein the macrocyclic aminophosphic acid has the structure



(II)

wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula



and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined in claim 1; X' and Y' are independently hydrogen, methyl or ethyl radicals; n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group.

4. The method of any one of claims 1-3 wherein the radionuclide is Gd-159.

5. The method of any one of claims 1-3 wherein the radionuclide is Sm-153.

6. The method of any one of cliams 1-3 wherein the radionuclide is Lu-177.

7. The method of any one of claims 1-3 wherein the radionuclide is Yb-175.

8. The method of any one of claims 1-3 wherein the radionuclide is Ho-166.

9. The method of any one of claims 1-3 wherein the radionuclide is Y-90.

10. A method for the therapeutic treatment or prophylaxis of calcific tumors in an animal having one or more calcific tumors, which method comprises administering to said animal a therapeutically effective amount of a composition which comprises a complex of (1) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid, or a physiologically acceptable salt thereof, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-165, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

11. The method of claim 10 wherein the radionuclide is Sm-153.

12. The method of claim 10 wherein the radionuclide is Lu-177.

13. The method of claim 10 wherein the radionuclide is Ho-166.

14. The method of claim 10 wherein the radionuclide is Y-90.

15.. A method according to any one of the preceding claims wherein the radionuclide in dosage form is present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal.

16. The method of claim 15 wherein the radionuclide in dosage form is present in an amount containing at least 0.2mCi per kilogram of body weight of said animal.

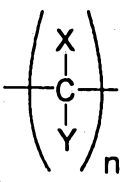
17. The method of any one of the preceding claims wherein the ligand to metal molar ratio is at least about 1:1.

18. The method of claim 17 wherein the ligand to metal molar ratio is from 1:1 to 3:1.

19. The method of claim 17 wherein the ligand to metal molar ratio is from 1:1 to 1.5:1.

20. The method of any one of the preceding claims, wherein the animal is a human.

21. A method for the therapeutic treatment of an animal having bone pain, which method comprises administering to said animal a therapeutically effective amount of a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene radical of the formula



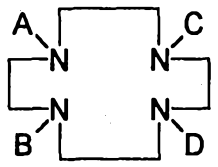
(I)

wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts

of the acid radicals; and n is 1-3, with the proviso that when n>1, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

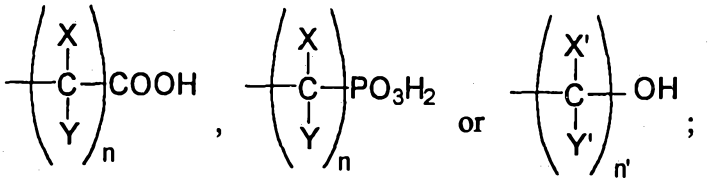
22. The method of claim 21 wherein X and Y are hydrogen and n is 1.

23. The method of claim 21 wherein the macrocyclic aminophosphic acid has the structure




(II)

wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula




and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined in claim 1; X' and Y' are independently hydrogen, methyl or ethyl radicals; n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group.

24. The method of any one of claims 21-23 wherein the radionuclide is Gd-159.

25. The method of any one of claims 21-23 wherein the radionuclide is Sm-153.

26. The method of any one of cliams 21-23 wherein the radionuclide is Lu-177.

27. The method of any one of claims 21-23 wherein the radionuclide is Yb-175.

28. The method of any one of claims 21-23 wherein the radionuclide is Ho-166.

29. The method of any one of claims 21-23 wherein the radionuclide is Y-90.

30. A method for the therapeutic treatment of an animal having bone pain, which method comprises administering to said animal a therapeutically effective amount of a composition which comprises a complex of (1) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid, or a physiologically acceptable salt thereof, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, together with at least one pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.

31. The method of claim 30 wherein the radionuclide is Sm-153.

32. The method of claim 30 wherein the radionuclide is Lu-177.

33. The method of claim 30 wherein the radionuclide is Ho-166.

34. The method of claim 30 wherein the radionuclide is Y-90.

35. A method according to any one of claims 21-34, wherein the radionuclide in dosage form is present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal.

36. The method of claim 35 wherein the radionuclide in dosage form is present in an amount containing at least 0.2mCi per kilogram of body weight of said animal.

37. The method of any one of claims 21-36, wherein the ligand to metal molar ratio is at least about 1:1.

38. The method of claim 37 wherein the ligand to metal molar ratio is from 1:1 to 3:1.

39. The method of claim 37 wherein the ligand to metal molar ratio is from 1:1 to 1.5:1.

40. The method of any one of claims 21-39, wherein the animal is a human.

41. A method for the therapeutic treatment or prophylaxis of calcific tumors in an animal having one or more calcific tumors, which method comprises administering to said animal a therapeutically effective amount of a composition as defined in claim 1, which composition is substantially as herein described with reference to any one of Examples 1 to 15.

42. A method for the therapeutic treatment of an animal having bone pain, which method comprises administering to said animal a therapeutically effective amount of a composition as defined in claim 1, which composition is substantially as herein described with reference to any one of Examples 1 to 15.

43. The method of claim 41 or claim 42, wherein the animal is a human.

**Dated 9 June, 1993**
**The Dow Chemical Company**

**Patent Attorneys for the Applicant/Nominated Person**
**SPRUSON & FERGUSON**

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/05782

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A 61 K 43/00, 49/02

**II. FIELDS SEARCHED**

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| U.S. | 424/1.1., 424/9, 534/10 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

STN MESSENGER Structure Search

**III. DOCUMENTS CONSIDERED TO BE RELEVANT** [9]

| Category * | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| A | EP, A, 287,465 (GUERBET S.A.) 19 OCTOBER 1988. See the entire document. | 1-10 |
| A | EP, A, 0,164,843 (THE DOW CHEMICAL COMPANY) 18 DECEMBER 1985, see the entire document. | 18-20 |
| Y P | US, A, 4,885,363 (TWEEDLE et al.) 05 DECEMBER 1989. See the entire document. | 1-27 |
| P | US, A, 4,882,142 (SIMON et al.) 21 NOVEMBER 1989. See the abstract. | 1-17,22-27 |
| A,P | US, a, 4,853,209 (KAPLAN et al.) 01 AUGUST 1989. See the abstract. | |
| Y | US, A, 3,965,254 (TOFE et al.) 22 JUNE 1976 See the abstract | 18-20 |
| Y | US, A, 4,017,595 (SUBRAMANIAN et al.) 12 APRIL 1977 See column 1, line 5 bridging column 2, line10. | 18-20 |
| Y | US, A, 4,187,284 (ROLLESTON et al.) 05 FEBRUARY 1980 See column 1, lines 23-54. | 18-20 |

* Special categories of cited documents: [10]

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

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

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

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

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

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

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

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

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 15 FEBRUARY 1990 | 22 MAR 1990 |
| International Searching Authority | Signature of Authorized Officer |
| ISA/US | Veronica Treese for JOHN M. COVERT |

Form PCT/ISA/210 (second sheet) (Rev.11-87)

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

| | | |
|---|---|---|
| Y | Int. J. Applied Radiation and Isotopes, Volume 14, issued 1963 (Northern Ireland) ROSOFF et al., "Distribution and Excretion of Radioactive Rare-Earth Compounds in Mice", see p. 132, second column bridging column 3, first column; see page 134, bottom half, first column. | 18-20 |
| Y | Chemical Abstracts, Volume 87, issued 1977 (Columbus,Ohio,USA) G. Subramanian et al., "Indium-113m labeled polyfunctional phosphonates as bone imaging agents", abstract No. 179938h, Nucl.-Med. (Stuttgart) Suppl., 1977, 14, 671-8 (Eng). | 18-20 |

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☒ Claim numbers 18-20, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

**VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [4]**

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.