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(54) **Title:** TRICYCLOPOLYAZAMACROCYCLOPHOSPHONIC ACIDS, COMPLEXES AND DERIVATIVES THEREOF, FOR USE AS CONTRAST AGENTS

(57) **Abstract**

Tri- and tetra-cyclopolyazamacrocyclophosphonic acid compounds and their derivatives are disclosed which may form inert complexes with Gd, Mn or Fe ions. The overall charge of the complex can be varied to alter the *in vivo* biolocalization. The complexes are useful as contrast agents for diagnostic purposes.

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TRICYCLOPOLYAZAMACROCYCLOPHOSPHONIC ACIDS, COMPLEXES AND DERIVATIVES THEREOF, FOR USE AS CONTRAST AGENTS

This invention concerns ligands that are tricyclopolyazamacrocyclophosphonic acids, complexes and derivatives thereof, for use as contrast agents in magnetic resonance imaging (MRI). To better understand this invention, a brief background on MRI is provided in the following section.

MRI is a non-invasive diagnostic technique which produces well resolved cross-sectional images of soft tissue within an animal body, preferably a human body. This technique is based upon the property of certain atomic nuclei (e.g. water protons) which possess a magnetic moment [as defined by mathematical equations; see G M. Barrow, Physical Chemistry, 3rd Ed., McGraw-Hill, NY (1973)] to align in an applied magnetic field. Once aligned, this equilibrium state can be perturbed by applying an external radio frequency (RF) pulse which causes the protons to be tilted out of alignment with the magnetic field. When the RF pulse is terminated, the nuclei return to their equilibrium state and the time required for this to occur is known as the relaxation time. The relaxation time consists of two parameters known as spin-lattice (T1) and spin-spin (T2) relaxation and it is these relaxation measurements which give information on the degree of molecular organization and interaction of protons with the surrounding environment.

Since the water content of living tissue is substantial and variations in content and environment exist among tissue types, diagnostic images of biological organisms are obtained which reflect proton density and relaxation times. The greater the differences in relaxation times (T1 and T2) of protons present in tissue being examined, the greater will be the contrast in the obtained image [e.g., *J. Magnetic Resonance* 33, 83-106 (1979)].

It is known that paramagnetic chelates possessing a symmetric electronic ground state can dramatically affect the T1 and T2 relaxation rates of juxtaposed water protons and that the effectiveness of the chelate in this regard is related, in part, to the number of unpaired electrons producing the magnetic moment [e.g., *Magnetic Resonance Annual*, 231-266, Raven Press, NY (1985)]. It has also been shown that when a paramagnetic chelate of this type is administered to a living animal, its effect on the T1 and T2 of various tissues can be directly observed in the magnetic resonance (MR) images with increased contrast being observed in the areas of chelate localization. It has therefore been proposed that stable, non-toxic paramagnetic chelates be administered to animals in order to increase the diagnostic information obtained by MRI [e.g., *Frontiers of Biol. Energetics* I, 752-759 (1978); *J. Nucl. Med.* 25, 506-513 (1984); Proc. of NMR Imaging Symp. (Oct. 26-27, 1980); F. A. Cotton *et al.*, *Adv. Inorg. Chem.* 634-639 (1966)]. Paramagnetic metal chelates used in this manner are referred to as contrast enhancement agents or contrast agents.

There are a number of paramagnetic metal ions which can be considered when undertaking the design of an MRI contrast agent. In practice, however, the most useful paramagnetic metal ions are gadolinium (Gd^{+3}), iron (Fe^{+3}), manganese (Mn^{+2}) and (Mn^{+3}), and chromium (Cr^{+3}), because these ions exert the greatest effect on water protons by virtue of their large magnetic moments. In a non-complexed form (e.g. $GdCl_3$), these metal ions are toxic to an animal, thereby precluding their use in the simple salt form. Therefore, a fundamental role of the organic chelating agent (also referred to as a ligand) is to render the paramagnetic metal non-toxic to the animal while preserving its desirable influence on T1 and T2 relaxation rates of the surrounding water protons.

Art in the MRI field is quite extensive, such that the following summary, not intended to be exhaustive, is provided only as a review of this area and other compounds that are possibly similar in structure. U.S. Patent 4,899,755 discloses a method of alternating the proton NMR relaxation times in the liver or bile duct of an animal using Fe^{+3}-ethylene-bis(2-hydroxyphenylglycine) complexes and its derivatives, and suggests among various other compounds the possible use of a pyridine macrocyclomethylenecarboxylic acid. U.S. Patent 4,880,008 (a CIP of U.S. Patent 4,899,755) discloses additional imaging data for liver tissue of rats, but without any additional complexes being shown. U S Patent 4,980,148 discloses gadolinium complexes for MRI which are non-cyclic compounds. C. J. Broan *et al.* [*J. Chem. Soc., Chem. Commun.*, 1739-1741 (1990)] describe some bifunctional macrocyclic phosphinic acid compounds. C. J. Broan *et al.* [*J. Chem. Soc., Chem. Commun.*, 1738-1739 (1990)] describe compounds that are triazabicyclo compounds. I. K. Adzamli *et al.* [*J. Med. Chem.* 32, 139-144 (1989)] describes acyclic phosphonate derivatives of gadolinium complexes for NMR imaging.

At the present time, the only commercial contrast agents available in the U.S. are the complex of gadolinium with diethylenetriaminepentaacetic acid (DTPA-Gd^{+3} - Magnevist™ by Schering) and a DO3A derivative [1,4,7-tris(carboxymethyl)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecanato]gadolinium (Prohance™ by Squibb). Magnevist™ and Prohance™ are considered as non-specific/perfusion agents since they freely distribute in extracellular fluid followed by efficient elimination through the renal system. Magnevist™ has proven to be extremely valuable in the diagnosis of brain lesions since the accompanying breakdown of the blood/brain barrier allows perfusion of the contrast agent into the affected regions. In addition to Magnevist™, Guerbet is commercially marketing a macrocyclic perfusion agent [1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclo-dodecanato]gadolinium (Dotarem™) which presently is only available in Europe. Prohance™ is shown to have fewer side effects than Magnevist™. A number of other potential contrast agents are in various stages of development.

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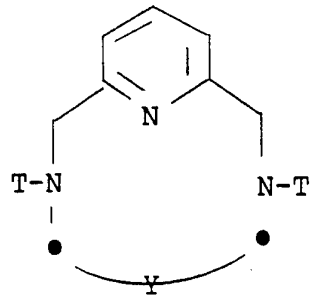
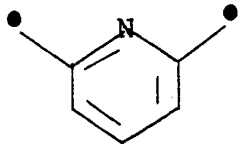
The present invention is directed to novel ligands that are tri- and tetra-cyclopolyazamacrocyclic compounds, and derivatives thereof, of the formula:



(I)

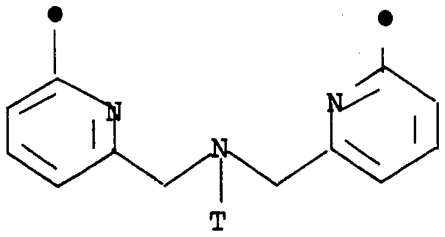
wherein:

Y is either



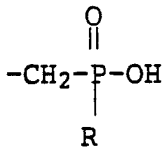
(A)

or



(B)

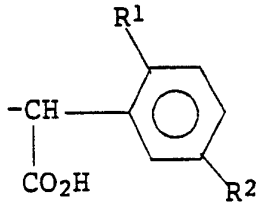
T is $-CH_2-COOH$, or



;

where: R is OH, C_1-C_5 alkyl or -O-(C_1-C_5 alkyl);

with the proviso that both T moieties are the same or one T is



;

R^1 is OH or OCH_3;

R^2 is NO_2, NH_2, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl; or

pharmaceutically-acceptable salts thereof.

When the above ligands of Formula (I) have:

all T equal $-CH_2-P(O)ROH$, where R is OH, or the pharmaceutically-acceptable salts thereof, the ligands are referred to herein as BP2P for (A) and TP3P for (B);

all T equal $-CH_2-COOH$, or the pharmaceutically-acceptable salts thereof, the ligands are referred to herein as BP2A (A) and TP3A for (B);

all T equal $-CH_2-P(O)ROH$, where R is $-O-(C_1-C_5$ alkyl), or the pharmaceutically-acceptable salts thereof, the ligands are referred to herein as BP2(O-Alk)P for (A) and TP3(O-Alk)P for (B); and

all T equal $-CH_2-P(O)ROH$, where R is C_1-C_5 alkyl, or the pharmaceutically-acceptable salts thereof, the ligands are referred to herein as BP2(Alk)P for (A) and TP3(Alk)P for (B).

The complexes of this invention can be designed to provide a specific overall charge which advantageously influences the *in vivo* biolocalization and image contrast. For example, when the metal ion is + 3 the following can be obtained:

an overall charge of -3 - when Formula (I) is TP3P, which are useful as calcific tissue contrast agents;

an overall charge of -1 - when Formula (I) is BP2P, which are useful as calcific tissue contrast agents;

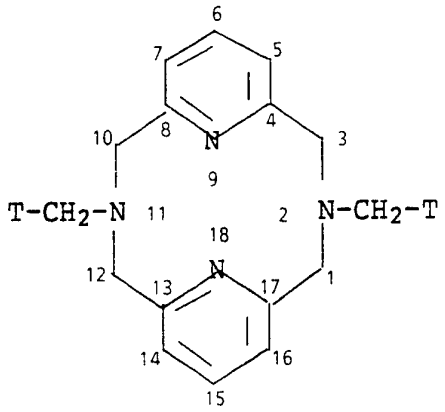
an overall charge of 0 - when Formula (I) is TP3A, TP3(O-Alk)P, or TP3(Alk)P, which are useful as general perfusion contrast agents; and

an overall charge of + 1 - when Formula (I) is BP2A, BP2(O-Alk)P, or BP2(Alk)P, which are useful as calcific tissue contrast agents.

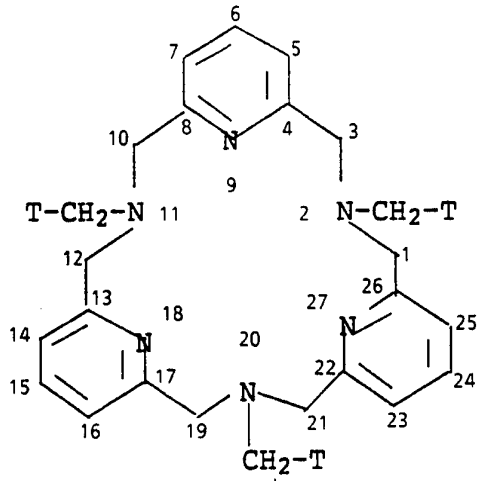
The complexes may be formulated to be in a pharmaceutically acceptable form for administration to an animal.

When one T term is other than $-CH_2-COOH$ or $-CH_2-PO_2HR$, then the compound is a bifunctional ligand/complex and may be linked through R^2 to a biologically active molecule.

The compounds of Formula (I) are numbered for nomenclature purposes as follows:



(IA)



(IB)

One aspect of the present invention concerns development of contrast agents having synthetic modifications to the paramagnetic chelate enabling site specific delivery of the contrast agent to a desired tissue. The advantage being increased contrast in the areas of interest based upon tissue affinity as opposed to contrast arising from non-specific perfusion which may or may not be apparent with an extracellular agent. The specificity of the ligand of Formula (I) may be controlled by adjusting the total charge and lipophilic character of the complex. The overall range of the charge of the complex is from -3 to + 1 as indicated above. For example, for a complex having 2 or 3 PO_3H_2 groups (BP2P and TP3P), the overall charge is highly negative and bone uptake is expected; whereas when the overall charge of the complex is 0 (thus neutral, TP3A, TP3(O-Alk)P, and TP3(Alk)P), the complex may have the ability to cross the blood brain barrier and normal brain uptake may be possible. Unexpectedly, for a complex

having an overall charge of + 1 [BP2A, BP2(O-Alk)P, and BP2(Alk)P], the complex displays bone uptake.

While not wishing to be bound by theory, it is believed that when a charged complex of the invention is made (e.g. possibly -3 for bone, -1 for liver, or + 1 for heart), the variations in that chelate ionic charge can influence biolocalization.

Tissue specificity may also be realized by ionic or covalent attachment of the chelate to a naturally occurring or synthetic molecule (e.g. through R^2) having specificity for a desired target tissue. One possible application of this approach is through the use of chelate conjugated monoclonal antibodies which would transport the paramagnetic chelate to diseased tissue enabling visualization by MRI. In addition, attachment of a paramagnetic chelate to a macromolecule can further increase the contrast agent efficiency resulting in improved contrast relative to the unbound chelate. Recent work by Lauffer (U.S. Patents 4,880,008 and 4,899,755) has demonstrated that variations in lipophilicity can result in tissue-specific agents and that increased lipophilic character favors non-covalent interactions with blood proteins resulting in enhancement of relaxivity.

The terms used in Formula (I) and for this invention are further defined as follows:

"C_1-C_5 alkyl" includes both straight and branched chain alkyl groups.

"Animal" includes a warmblooded mammal, preferably a human being.

"Complex" refers to a complex of the compound of the invention, e.g. Formula (I), complexed with a metal ion, where at least one metal atom is chelated or sequestered.

"Biologically active material" refers to a dextran, peptide, or molecules that have specific affinity for a receptor, or preferably antibodies or antibody fragments.

"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. Possible antibodies are 1116-NS-19-9 (anti-colorectal carcinoma), 1116-NS-3d (anti-CEA), 703D4 (anti-human lung cancer), 704A1 (anti-human lung cancer), CC49, CC83 and B72.3. The hybridoma cell lines 1116-NS-19-9, 1116-NS-3d, 703D4, 704A1, CC49, CC83 and B72.3 are deposited with the American Type Culture Collection, having the accession numbers ATCC HB 8059, ATCC CRL 8019, ATCC HB 8301, ATCC HB 8302, ATCC HB 9459, ATCC HB 9453 and ATCC HB 8108, respectively.

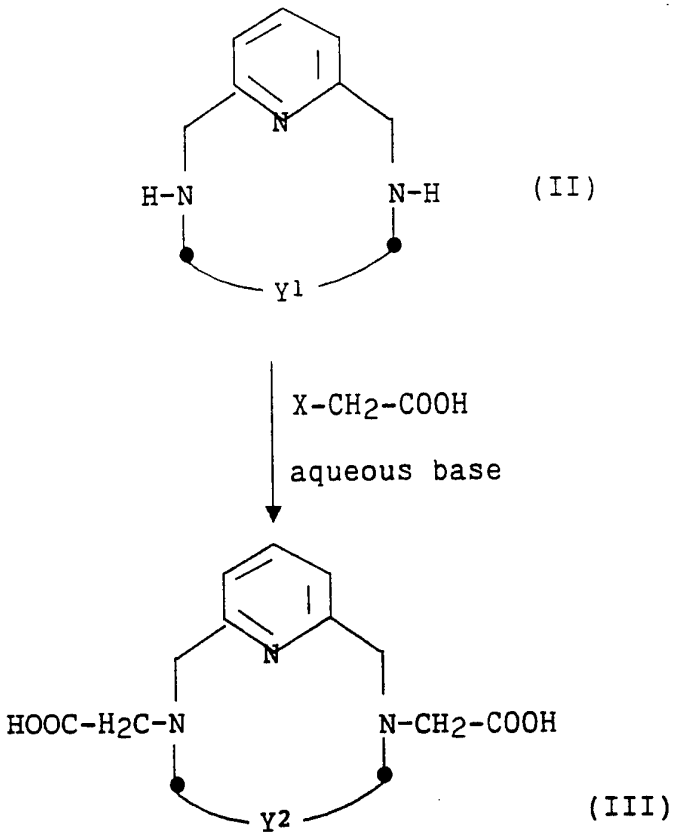
The bifunctional chelating agents described herein [represented by Formula (I)] can be used to chelate or sequester the metal ions so as to form metal ion chelates (also referred to herein as "complexes"). The complexes, because of the presence of the functionalizing moiety [represented by R^2 in Formula (I)], can be covalently attached to biologically active materials, such as dextran, molecules that have specific affinity for a receptor, or preferably covalently attached to antibodies or antibody fragments. Thus the complexes described herein may be covalently attached to an antibody or antibody fragment or have specific affinity for a receptor and are referred to herein as "conjugates"

As used herein, "pharmaceutically-acceptable salts" means any salt or mixtures of salts of a compound of Formula (I) which is sufficiently non-toxic to be useful in diagnosis of animals, preferably 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, palmoic, mucic, glutamic, gluconic acid, 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 or 1-deoxy-1-(methylamino)-D-glucitol, 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, or ammonium. Also included are mixtures of the above salts.

The compounds of Formula (I) are prepared by various processes. Typical general synthetic approaches to such processes are provided by the reaction schemes given below.

In Scheme 1, the compounds of Formula (I) are prepared wherein Y is (A) or (B), and all T moieties are $-CH_2-COOH$.

Scheme 1



(II)

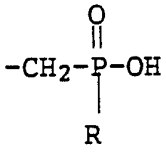
(III)

wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

X is chloro or bromo atom; and Y^2 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with $-CH_2-COOH$.

The aqueous base is alkali metal hydroxide, such as sodium hydroxide or potassium hydroxide. The pH of the reaction is maintained about 8-10. Temperature is between about 60-90°C, and pressure is not critical so that ambient pressure is used.

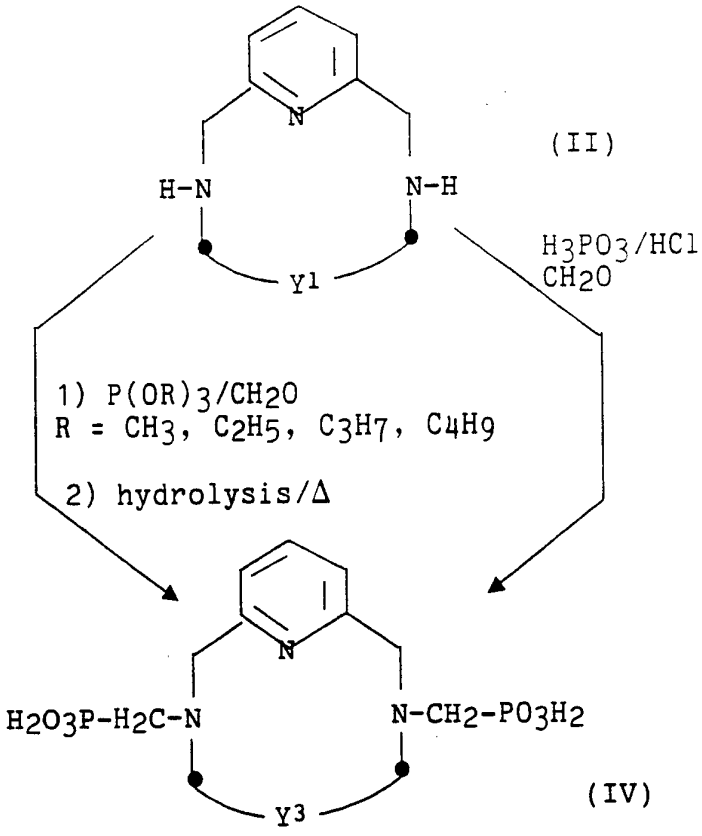
In Scheme 2, the compounds of Formula (I) are prepared wherein Y is (A) or (B), and all T moieties are



:

where: R is OH.

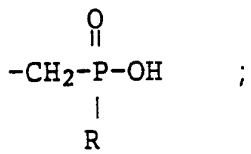
Scheme 2



wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; Y^3 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with $-CH_2-PO_3H_2$.

The hydrolysis in Scheme 2 is done by known aqueous acid conditions, such as using 3 to 12M hydrochloric acid. The pH of the reaction is maintained below 3. Temperature is at reflux. Pressure is not critical so that ambient pressure is used. Alternatively, when the reaction is run in one step, phosphorous acid, hydrochloric acid and excess formaldehyde are used. The pH of the reaction is below 2. Temperature is at reflux. Pressure is not critical so that ambient pressure is used.

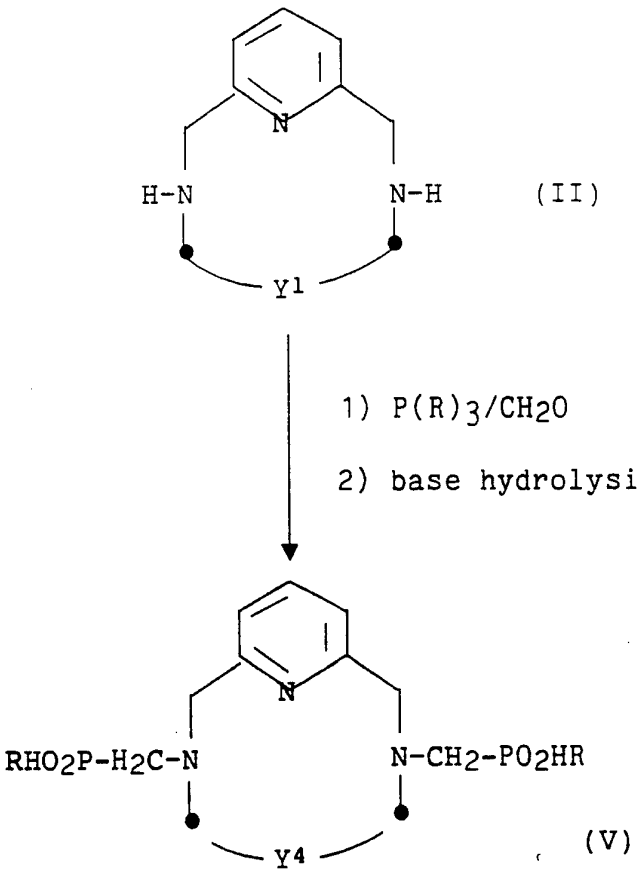
In Scheme 3, the compounds of Formula (I) are prepared wherein Y is (A) or (B), all T moieties are



;

where: R is -O-(C_1-C_5 alkyl).

Scheme 3

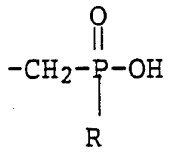


wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; Y^4 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with -CH_2-PO_2HR; and

R is -O-(C_1-C_5 alkyl).

The hydrolysis in Scheme 3 is done under known aqueous basic conditions, such as using excess alkali metal hydroxide, such as sodium or potassium hydroxide. The pH of the reaction is above 9. Temperature is at reflux. Pressure is not critical so that ambient pressure is used.

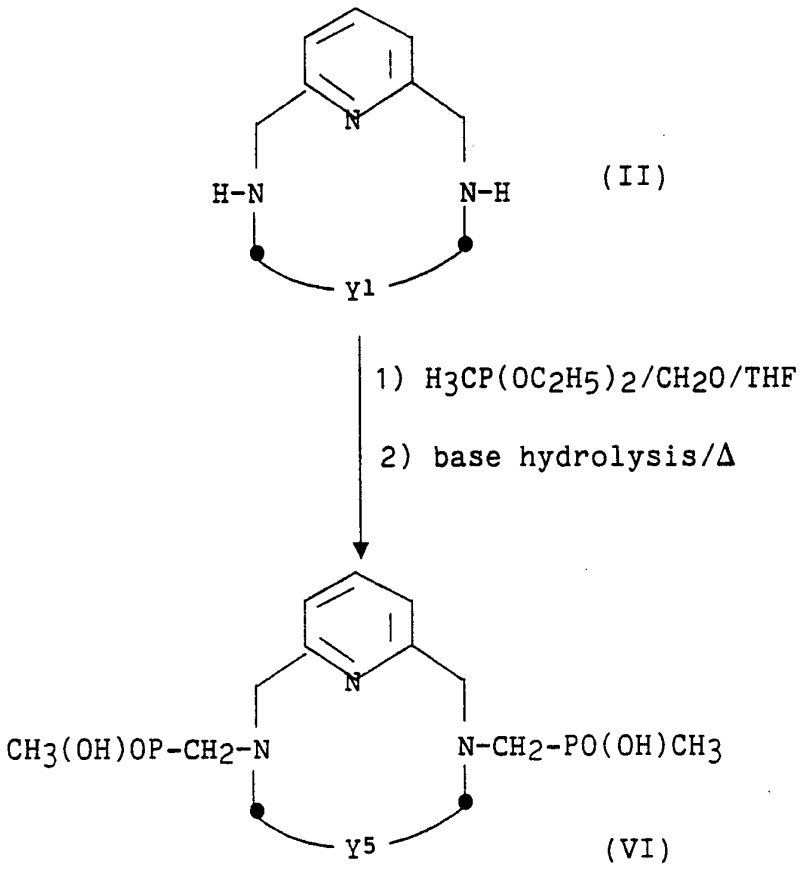
In Scheme 4, the compounds of Formula (I) are prepared wherein Y is (A) or (B), all T moieties are



where: R is C_1-C_5 alkyl.

Scheme 4 shows the preparation of the compounds of Formula (I) when R is methyl.

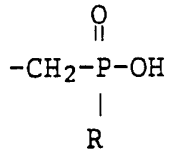
Scheme 4



wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; Y^5 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with $-CH_2-PO(OH)CH_3$.

The hydrolysis in Scheme 4 is done under known aqueous basic conditions, such as using excess alkali metal hydroxide, such as sodium or potassium hydroxide. The pH of the reaction is above 9. Temperature is at reflux. Pressure is not critical so that ambient pressure is used

In Scheme 5, the compounds of Formula (I) are prepared wherein Y is (A) or (B), all T moieties are

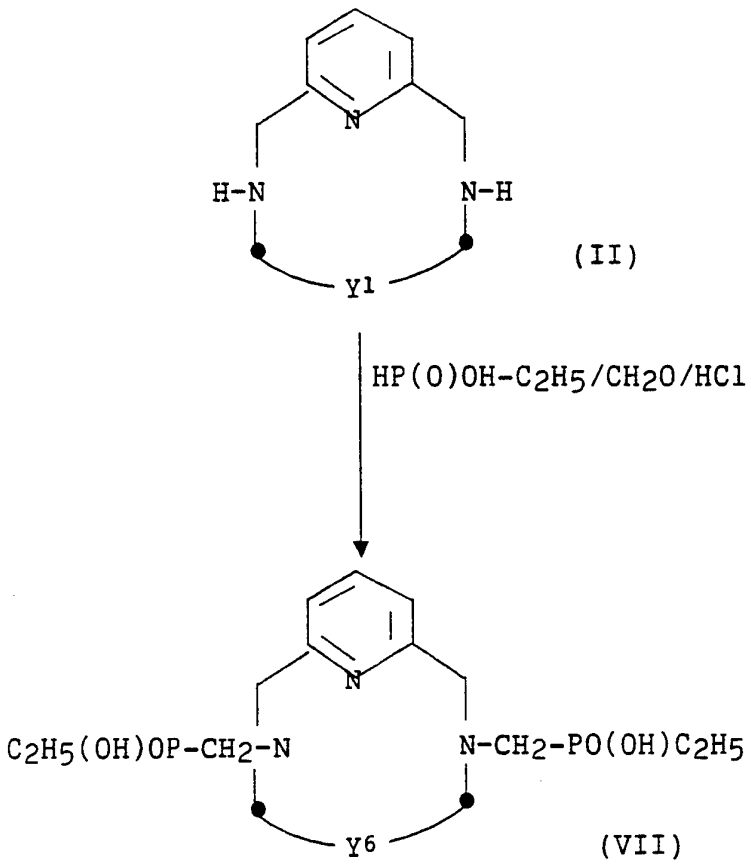


;

where: R is C_1-C_5 alkyl.

Scheme 5 shows the preparation of the compounds of Formula (I) when R is ethyl.

Scheme 5

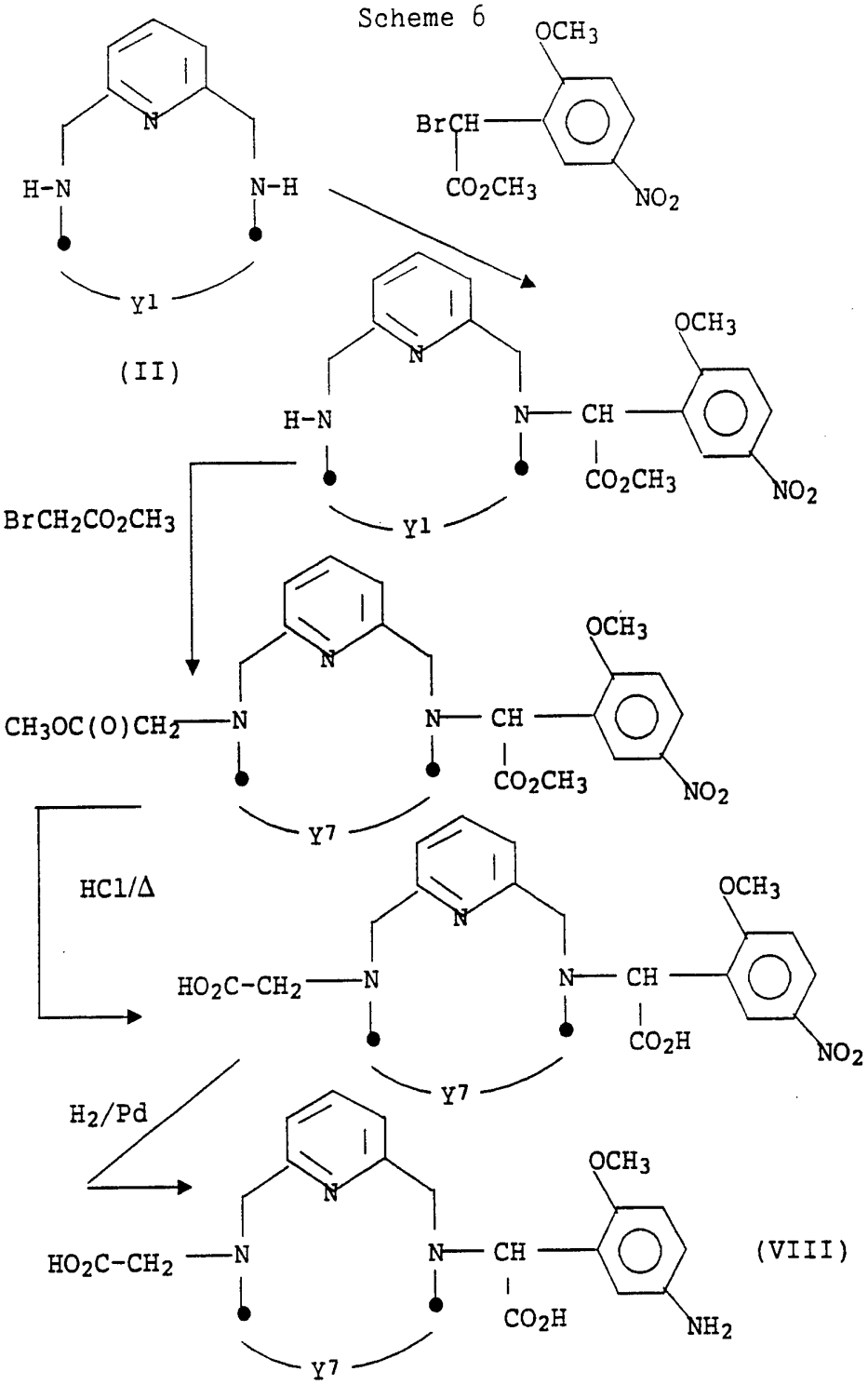


wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; Y^6 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with $-CH_2-PO(OH)C_2H_5$.

The reaction in Scheme 5 is under acidic conditions with a hydrochloric acid. The pH of the reaction is below 3. Temperature is at reflux. Pressure is not critical so that ambient pressure is used.

Scheme 6 shows the preparation of the compounds of Formula (I) when one T is a bifunctional moiety; and the other T is defined above for Formula (I).

Scheme 6



wherein: Y^1 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; Y^7 is defined as before for Y when (A) and when (B) the nitrogen atom is substituted with $-CH_2-CO_2H$.

In the above Schemes, the general process description illustrates specific steps that may be used to accomplish a desired reaction step. The general description of these process steps follows.

The general synthetic methodology for preparing the 2:2 and 3:3 macrocyclic precursors outlined in Schemes 1-6 is described by Pappalerdo *et al., J. Org. Chem.* 53, 3521-3529 (1988). The carboxylate derivatives described in Scheme 1 are prepared by conventional alkylation procedures utilizing chloro- or bromo-acetic acid under basic aqueous conditions and utilizing the appropriate secondary amine macrocyclic starting material.

The phosphonic acid derivatives outlined in Scheme 2 can be prepared by initial alkylation of the amine with a trialkyl phosphite and paraformaldehyde, resulting in an organic soluble perester. This ester is then hydrolyzed under refluxing acid conditions to give the desired aminophosphonic acids. Alternatively, the phosphonic acid can be prepared under acidic conditions by employing phosphorous acid in combination with formaldehyde and hydrochloric acid.

Phosphonate half esters are prepared as shown in Scheme 3 by initial formation of the dialkyl phosphonate ester, followed by hydrolysis under basic conditions. Base hydrolysis gives exclusive conversion to the half ester.

Scheme 4 illustrates the methodology for synthesizing the methyl phosphinate derivatives using diethoxymethylphosphine as the nucleophile and paraformaldehyde. Condensation can be conducted in solvents such as tetrahydrofuran (THF), dimethylformamide (DMF), dioxane, acetonitrile, or alcoholic media. The resulting phosphinate ester is then hydrolyzed under acidic conditions (e.g. 6N HCl, 80-100°C) or basic conditions (excess base, 40-100°C) to give the corresponding methylphosphonic acid. Alternatively, as outlined in Scheme 5, the method devised by A. D. Sherry *et al.* (*Inorg. Chem.*, submitted 1991) using ethylphosphonic acid generated *in situ* can be used to obtain phosphinate derivatives having increased lipophilic character.

Scheme 6 illustrates the preparation of bifunctional compounds of Formula (I) which may then be attached to a biologically active material.

The metal ions used to form the complexes of this invention are Gd^{+3}, Mn^{+2}, Fe^{+3} and available commercially, e.g. from Aldrich Chemical Company. The anion present is halide, preferably chloride, or salt free (metal oxide).

A "paramagnetic nuclide" of this invention means a metal ion which displays spin angular momentum and/or orbital angular momentum. The two types of momentum combine to give the observed paramagnetic moment in a manner that depends largely on the atoms bearing the unpaired electron and, to a lesser extent, upon the environment of such atoms. The paramagnetic nuclides found to be useful in the practice of the invention are gadolinium (Gd^{+3}), iron (Fe^{+3}) and manganese (Mn^{+2}), with Gd^{+3} being preferred.

The complexes are prepared by methods well known in the art. Thus, for example, see Chelating Agents and Metal Chelates, Dwyer & Meilor, 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). An example of the preparation of a complex involves reacting a bicyclopolyazamacrocyclophosphonic acid with the metal ion under aqueous conditions at a pH from 5 to 7. The complex formed is by a chemical bond and results in a stable paramagnetic nuclide composition, e.g. stable to the disassociation of the paramagnetic nuclide from the ligand

The complexes of the present invention are 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. A large excess of ligand is undesirable since uncomplexed ligand may be toxic to the animal or may result in cardiac arrest or hypocalcemic convulsions.

This invention is used with a physiologically acceptable carrier, excipient or vehicle therefore. 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 diagnosis. The dose will vary depending on the disease and physical parameters of the animal, such as weight. *In vivo* diagnostics are also contemplated using formulations of this invention.

Other uses of some of the chelants of the present invention may include the removal of undesirable metals (i.e. iron) from the body, attachment to polymeric supports for various purposes, e.g. as diagnostic agents, and removal of metal ions by selective extraction. The ligands of Formula (I) having in at least two R terms T equal to $P(O)R^1OH$ may be used for metal ion control as scale inhibitors. Some of these ligands can be used in less than stoichiometric amounts. Similar uses are known for compounds described in U.S. Patents 2,609,390; 3,331,773; 3,336,221; and 3,434,969.

The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention.

Some terms used in the following examples are defined as follows:

LC = liquid chromatography, purifications were carried out at low pressure using Dionex 2010i system fitted with a hand-packed Q-Sepharose™ anion exchange column (23 x 2 cm).

DMF = dimethylformamide.
AcOH = acetic acid.
g = gram(s).
mg = milligrams.
kg = kilogram(s).
mL = milliliter(s).
µL = microliter(s).

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pH Stability General Procedure

A stock $^{159}GdCl_3$ (or $^{153}SmCl_3$) solution was prepared by adding 2 µL of $3x10^{-4}M$ $^{159}GdCl_3$ in 0.1N HCl to 2 mL of a $3x10^{-4}M$ $GdCl_3$ carrier solution. Appropriate ligand solutions were then prepared in deionized water. The 1:1 ligand/metal complexes were then prepared by combining the ligands (dissolved in 100-500 µL of deionized water) with 2 mL of the stock $^{159}GdCl_3$ solution, followed by through mixing to give an acidic solution (pH = 2). The pH of the solution was then raised to 7.0 using 0.1N NaOH. The percent metal as a complex was then determined by passing a sample of the complex solution through a Sephadex™ G-50 column eluting with 4:1 saline (85% NaCl/NH_4OH) and collecting 2 x 3 mL fractions. The amount of radioactivity in the combined elutions was then compared with that left on the resin (non-complexed metal is retained on the resin). The pH stability profile was generated by adjusting the pH of an aliquot of the complex solution using 1M NaOH or 1M HCl and determining the percent of the metal existing as a complex using the ion exchange method described above. The Sm results are known by experimental comparison to be identical for complexation and biodistribution of the ligands of this invention.

SYNTHESIS OF LIGANDS.

General Materials and Methods.

All reagents were obtained from commercial suppliers and used as received without further purification. NMR spectra were recorded on a Bruker AC-250 MHz spectrometer equipped with a multi-nuclear quad probe (^1H, ^{13}C, ^{31}P, and ^{19}F) at 297°K unless otherwise indicated. ^1H spectra in D_2O were recorded by employing solvent suppression pulse sequence ("PRESAT", homo-nuclear presaturation). ^1H spectra are referenced to residual chloroform (in $CDCl_3$) at δ7.26 or external dioxane (in D_2O) at δ3.55. ^{13}C and ^{31}P spectra reported are proton decoupled (broad band). Assignments of $^{13}C\{^1H\}$ chemical shifts were aided by DEPT (Distortionless Enhancement by Polarization Transfer) experiments. $^{13}C\{^1H\}$ spectra are referenced to center peak of $CDCl_3$ at δ77.00 (in $CDCl_3$) and external dioxane at δ66.66 (in D_2O). $^{31}P\{^1H\}$ spectra were referenced to external 85% H_3PO_4 at δ0.00. Melting points were determined by capillary melt methods and were uncorrected. Semipreparative ion-exchange chromatographic separations were performed at low pressure (<600 psi) using a standard glass column fitted with hand-packed Q-Sepharose™ (anion exchange) or SP--Sepharose™ (cation exchange) glass column, and with on-line UV detector at 263 nm for eluent monitoring. GC/MS spectra were performed on a Hewlett Packard 5890A Gas Chromatograph/ 5970 Mass Selective Detector.

STARTING MATERIALS

Example A

Preparation of N,N'-ditosyl-2,11-diaza[3.3](2,6)pydinophane.

To a stirred solution of tosylamide, sodium salt (TsNHNa), (13.52 g, 70 mmol) in anhydrous DMF (1.3 L) at 80°C was added dropwise (1.5 hr) under a N_2 atmosphere a solution of

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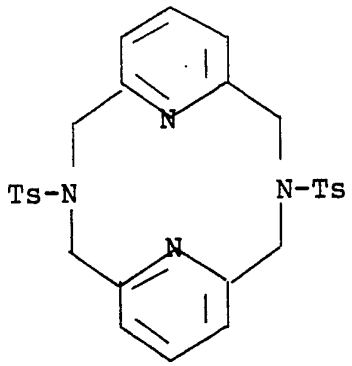
2,6-bis(chloromethyl)pyridine (12.32 g, 70 mmol) in DMF (200 mL). After l hr, solid TsNHNa (13.52 g, 70 mmol) was added all at once, and the mixture was stirred at 80°C for additional 16 hr. The reaction mixture was then cooled to room temperature and decanted. The solvent was removed *in vacuo* and the resulting residue was combined with acetone and filtered to give a waxy solid which was continuously extracted (Soxlet) with acetone (300 mL) for 48 hr. The product was isolated as the precipitate at the bottom of the heating flask. Upon drying, the product was isolated as white powder (5.24 g, 27%): mp = 246 - 248°C and further characterized by:

^{1}H NMR (DMSO-d6)

δ 2.42 (s, 6H), 4.38 (s, 8H), 6.99 (d, 4H), 7.40-7.49 (m, 6H), 7.88 (d, 4H); and

^{13}C {^{1}H} NMR (DMSO-d6)

δ 20.91, 55.55, 122.12, 126.92, 129.88, 135.99, 137.03, 143.30, 154.71; and is illustrated by the formula



Example B

Preparation of 2,11-diaza[3.3](2,6)pydinophane.

N,N'-ditosyl-2,11-diaza[3.3](2,6)pydinophane (5.24 g, 9.5 mmol), prepared in Example A, was dissolved in 90% H_2SO_4 (48 mL) and heated at 110°C with stirring for 2 hr. After the solution was cooled to room temperature, it was slowly diluted with deionized water (50 mL) while chilling in an ice bath. The resulting solution was then poured into a 25% NaOH solution (200 mL) which was cooled in an ice bath. The resulting white solid was extracted with $CHCl_3$ (3 X 100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to dryness to give the titled product as a waxy white solid (1.69 g, 74%) and further characterized by:

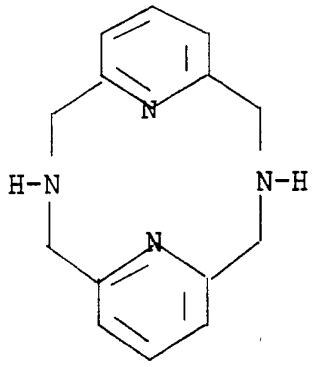
^{1}H NMR ($CDCl_3$)

δ 3.91 (s, 8 H), 6.47 (d, 4H), 7.06 (t, 2H); and

^{13}C {^{1}H} NMR ($CDCl_3$)

δ 55.89, 119.73, 135.71, 159.36;

CG/mass spectra *m/z* M + 240; and is illustrated by the formula



Example C

Preparation of N,N',N"-tritosyl-2,11,20-triaza[3.3.3](2,6)pydinophane.

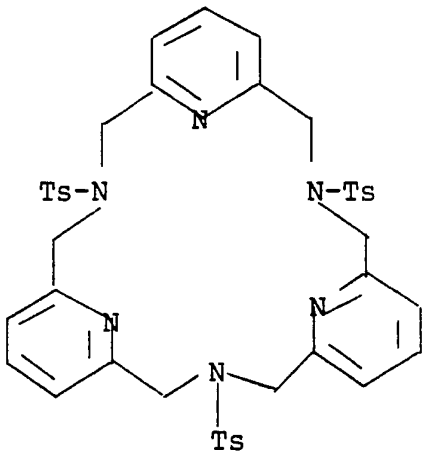
Isolated as a by-product from the reaction used for preparation of N,N'-ditosyl-2,11-diaza[3.3](2,6)pydinophane, prepared in Example A, (remaining part on the extractor thimble after Soxlet extraction) mp 260 - 262°C; and further characterized by:

^{1}H NMR (DMSO-d6)

δ 2.31 (s, 9H), 4.08 (s, 12H), 7.01 (d, 4H), 7.30 (d, 6H), 7.52 (t, 3H), 7.67 (d, 6H); and

^{13}C {^{1}H} NMR (DMSO-d6)

δ 20.92, 54.03, 120.55, 127.13, 129.83, 135.12, 136.83, 143.45, 155.47; and is illustrated by the formula:



Example D

Preparation of 2,11,20-triaza[3.3.3](2,6)pydinophane.

N,N',N"-tritosyl-2,11,20-triaza[3.3.3](2,6)pydinophane (0.5 g, 0.61 mmol), prepared in Example C was dissolved in 90% H_2SO_4 (6 mL) and heated at 110°C with stirring for 2 hr. The solution was then cooled to room temperature and slowly diluted with deionized water (6 mL) while chilling in a ice bath. The resulting solution was then poured into a 25% NaOH solution (22 ml) which was cold in an ice bath. The resulting white solid was extracted

with $CHCl_3$ (2 X 30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to dryness to give the titled product as a waxy white solid (0.167 g, 76%); and further characterized by:

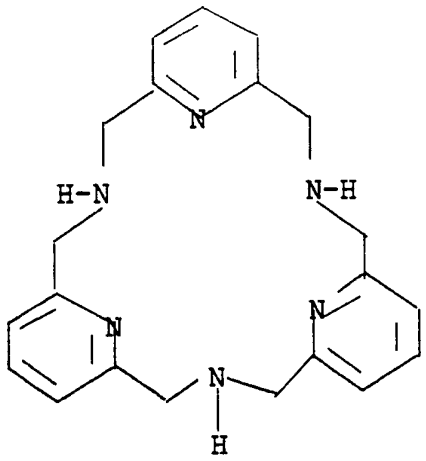
^1H NMR ($CDCl_3$)

δ 3.03 (s, 3H), 3.93 (s, 12H), 7.08 (d, 6H), 7.54 (t, 3H); and

^{13}C {^1H} NMR ($CDCl_3$)

δ 54.58, 120.72, 136.50, 158.64;

GC/mass spectra *m/z* M+ 360; and is illustrated by the formula:



Example E

Preparation of N,N'-bis(methylenedimethylphosphonate)-2,11-diaza[3.3](2,6)pydinophane.

The product prepared in Example B, 2,11-diaza[3.3](2,6)pydinophane (276.4 mg, 1.15 mmol) was combined with paraformaldehyde (138 mg, 4.60 mmol, excess) and trimethylphosphite (0.814 mL, 855 mg, 4.60 mmol, excess). After the mixture was gently stirred for 10 min to obtain a well mixed slurry, it was heated to 85°C and maintained for 1 hr. After the excess reagents and byproducts were removed *in vacuo* (1 hr at 110°C/0.01 mmHg), the dark brown residue was dissolved in $CHCl_3$ (20 mL) and washed with deionized water (5 X 15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed *in vacuo* to give the product as a yellow waxy solid (363 mg, 65%); and further characterized by:

^1H NMR ($CDCl_3$)

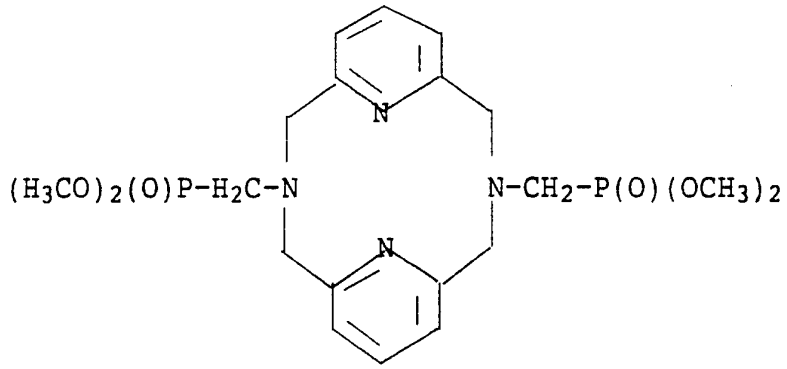
δ 3.39 (d, 4H), 3.88 (d, 12H), 4.08 (s, 8H), 6.84 (d, 4H), 7.13 (t, 2H); and

^{13}C {^1H} NMR ($CDCl_3$)

δ 52.75 (d), 54.88 (d), 65.21 (d), 122.71, 135.69, 157.14; and

^{31}P NMR ($CDCl_3$)

δ 27.22; and is illustrated by the formula



Example F

Preparation of N,N'-bis(methylenediethylphosphonate)-2,11-diaza[3.3](2,6)pydinophane.

The product prepared in Example B, 2,11-diaza[3.3](2,6)pydinophane (0.47 g, 1.96 mmol) was combined with paraformaldehyde (235 mg, 7.84 mmol, excess) and triethylphosphite (1.34 mL, 1.30 g, 7.84 mmol, excess). After the mixture was gently stirred for 10 min to obtain a well mixed slurry, it was heated to 90°C for 1 hr. After the excess reagents and byproducts were removed *in vacuo* (1 hr at 125°C/0.01mmHg), the resulting dark brown residue was dissolved in $CHCl_3$ (20 mL) and washed with deionized water (5 X 15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed *in vacuo* to give the product as a yellow waxy solid (957 mg, 91%); and further characterized by:

^1H NMR ($CDCl_3$)

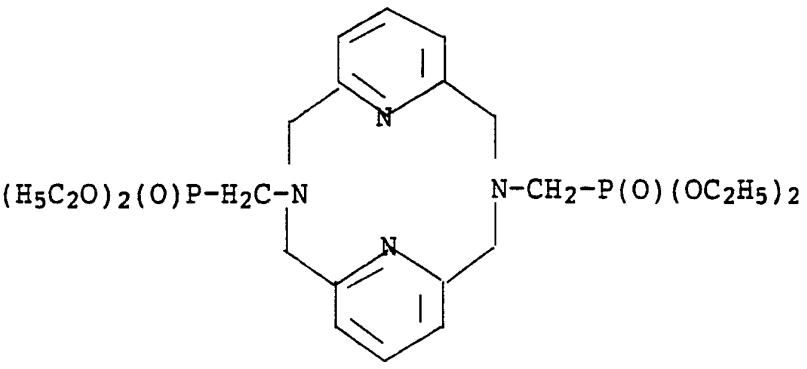
δ 1.24 (t, 12H), 3.20 (d, 4H), 3.94 (s, 8H), 4.07 (q, 8H), 6.71 (d, 4H), 6.98 (t, 2H); and

$^{13}C\{^1H\}$ NMR ($CDCl_3$)

δ 16.48, 55.36 (d), 61.75 (d), 65.14 (d), 122.52, 135.41, 157.04; and

$^{31}P\{^1H\}$ NMR ($CDCl_3$)

δ 24.60; and is illustrated by the formula



FINAL PRODUCTS

Ligands: Preparation of tri- and dimethylenecarboxylic acids as shown in Scheme 1.

Example 1

Preparation of N,N'-diacetic acid-2,11-diaza[3.3](2,6)pydinophane (BP2A).

To a stirred aqueous solution (1 mL) of 2,11-diaza[3.3](2,6)pydinophane, prepared in Example B, (82.4 mg, 0.58 mmol) was added bromoacetic acid (275 mg, 1.98 mmol, excess) and the pH of the reaction mixture was maintained above 10 by adding small portions of conc. NaOH until no more caustic was needed to keep pH > 11 (~30 min). The reaction mixture was then heated (60°C) for 2 hr, then the reaction mixture was cooled to room temperature, the pH of the reaction mixture adjusted to 7. The solution was then chromatographed on cation exchange (SP-Sepharose™) column (1.5 X 50 cm) eluting first with deionized water then with 1M HCl. The acidic fraction containing product was evaporated to dryness, followed by co-evaporation with fresh deionized water (3 X 2 mL) to eliminate excess HCl. The final product was isolated as a white solid upon freeze drying of the concentrated aqueous solution from above; and characterized by:

^{1}H NMR (D_2O)

δ 4.17 (s, 4H), 4.43 (s, 8H), 7.15 (d, 4H), 7.66 (t, 2H); and

^{13}C {^{1}H} NMR (D_2O)

δ 61.44, 61.70, 127.46, 146.84, 154.37, 175.62.

Example 2

Preparation of N,N',N"-triacetic acid-2,11,20-diaza[3.3.3](2,6)pydinophane (TP3A).

To a stirred aqueous solution (1 mL) of 2,11,20-triaza[3.3.3](2,6)pydinophane, prepared in Example D, (54.5 mg, 0.15 mmol) was added bromoacetic acid (100 mg, 0.72 mmol, excess) and the pH of the reaction mixture was maintained above 10 by adding small portions of conc. NaOH until no more caustic was needed to keep the final pH > 11 (~30 min). The reaction mixture was then heated (60°C) for 2 hr, cooled to room temperature, and pH adjusted to 7. The aqueous solution was then chromatographed on a cation exchange (SP-Sepharose™) column (1.5 X 50 cm) eluting first with deionized water then with 1M HCl. The acidic fraction containing product was evaporated to dryness, followed by coevaporation with fresh deionized water (3 X 2 mL) to eliminate excess HCl. The final product was isolated as white solid upon freeze drying of the concentrated aqueous solution from above; and characterized by:

^{1}H NMR (D_2O)

δ 4.12 (s, 6H), 4.68 (s, 12H), 7.41 (d, 6H), 7.81 (t, 3H); and

^{13}C {^{1}H} NMR (D_2O)

δ 57.32, 60.53, 128.57, 143.42, 152.42, 171.17.

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Ligands: Preparation of dimethylenephosphonic acids as shown in Scheme 2.

Example 3

Preparation of N,N'-bis(methylenephosphonic acid)-2,11-diaza[3.3](2,6)pydinophane (BP2P).

A conc. HCl solution (37%,4 mL) of N,N'-bis(methylenedimethylphosphonate)-2,11-diaza[3.3](2,6)pydinophane, prepared in Example E, (255 mg, 0.53 mmol) was heated at reflux for 2.5 hr. After cooling, the solution was evaporated to dryness, followed by co-evaporation with fresh deionized water (3 X 2 mL) to eliminate excess HCl. The final product was isolated as a hydroscopic brown solid upon freeze-drying of the concentrated aqueous solution; and characterized by:

^{1}H NMR (D_2O)

δ 3.55 (d, 4H), 4.46 (br s, 8H), 6.90 (d, 4H), 7.37 (t, 2H); and

^{13}C {^{1}H} NMR (D_2O)

δ 57.80 (d), 63.74 (d), 127.02, 144.18, 152.96; and

^{31}P {^{1}H} NMR (D_2O)

δ 11.71.Ligands: Preparation of dimethylenephosphonate half esters as shown in Scheme 3.

Example 4

Preparation of N,N'-bis(methylenephosphonic acid ethyl ester)-2,11-diaza[3.3](2,6)pydinophane (BP2EP).

The product prepared in Example F, N,N'-bis(methylenediethylphosphonate)-2,11-diaza[3.3](2,6)pydinophane, (957 mg, 1.77 mmol) was combined with 0.1M KOH (7.2 mL) and heated at 60°C for 16 hr. The solution was then cooled and freeze-dried to give a residue which was dissolved into $CHCl_3/C_2H_5OH$ (95/5), and filtered. Upon evaporation of solvent and concentration *in vacuo*, the product was isolated as a pale yellow powder (657 mg, 66%); and characterized by:

^{1}H NMR (D_2O)

δ 1.10 (t, 6H), 2.97 (d, 4H), 3.81 (q, 4H), 3.84 (s, 8H), 6.73 (d, 4H), 7.09 (t, 2H); and

^{13}C {^{1}H} NMR (D_2O)

δ 18.98, 58.76 (d), 63.69 (d), 66.53 (d), 126.35, 140.09, 159.37; and

^{31}P {^{1}H} NMR (D_2O)

δ 20.65.

Complexes: Preparation of metal/ligand complexes for biodistribution studies.

General Procedure

Metal ligand complexes were prepared by various methods. The methods included mixing of the metal and ligand in aqueous solution and adjusting the pH to the desired value. Complexation was done in solutions containing salts and/or buffers as well as water. Sometimes heated solutions were found to give higher complex yields than when the complexation was done at ambient temperatures.

For example, a solution of the ligand is prepared by dissolving the ligand in deionized water (about pH = 2). A ligand/metal complex was then prepared by combining the ligand solution with aqueous $SmCl_3 \cdot H_2O$ ($3x10^{-4}M$ in 0.01N HCl) containing tracer $^{153}SmCl_3$. After thorough mixing, the percent metal as a complex was determined by passing a sample of the complex solution through a Sephadex™ column, eluting with 4:1 saline (0.85% $NaCl/NH_4OH$), and collecting 2 x 3 mL fractions. The amount of radioactivity in the combined elutions was then compared with that left on the resin. Under these conditions, complex was removed with the eluent and non-complexed metal is retained on the resin. By this method complexation was determined to usually be about 95% or greater.

Using the above procedure, the complexes of samarium with

N,N'-diacetic acid-2,11-diaza[3.3](2,6)pydinophane (BP2A), ligand:metal ratio of 4:1, 99%;

N,N',N"-triacetic acid-2,11,20-diaza[3.3.3](2,6)pydinophane (TP3A), ligand:metal ratio of 3:1, 98.6%;

N,N'-bis(methylenephosphonic acid)-2,11-diaza[3.3](2,6)pydinophane (BP2P), ligand:metal ratio of 3:1, 95%; and

N,N'-bis(methylenephosphonic acid ethyl ester)-2,11-diaza[3.3](2,6)pydinophane (BP2EP), ligand:metal ratio of 4:1, 98%;

were made. However, in an analogous manner the corresponding gadolinium complexes may be prepared.

BIODISTRIBUTION

General Procedure

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the complex solution via a tail vein. The rats weighed between 150 and 250 g at the time of injection. After 30 min. 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 dose in blood was estimated assuming blood to be 6.5% of the total body weight. The percent dose in bone was estimated by multiplying the percent dose in the femur by 25. The percent dose in muscle was estimated assuming muscle to be 43% of the total body weight.

In addition to organ biodistribution, chelates of the compounds of Formula (I) were evaluated for efficiency of bone localization since phosphonates are known for their ability to bind to hydroxyapitite. The results of these tests are given below.

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EXAMPLE I

When the complex ^{153}Sm[N,N'-diacetic acid-2,11-diaza[3.3](2,6)pydinophane] (^{153}Sm-BP2A) was evaluated, the results are given in Table I below. The numbers represent the average of a minimum of 2 rats per data point at 2 hours post injection.

TABLE I
BIODISTRIBUTION OF ^{153}Sm-BP2A
% INJECTED DOSE

ORGAN	AVERAGE
Bone	27.98
Liver	0.68
Kidney	0.95
Spleen	0.01
Muscle	0.80
Blood	0.43
Heart	0.02
Lung	0.06
Brain	0.01
Stomach	0.05
Small Intestine	0.80
Large Intestine	0.04

TABLE I
RATIOS FOR ^{153}Sm-BP2A
AVERAGE % INJECTED DOSE
FOR ^{159}Gd-BP2EP

Bone/Blood	Bone/Muscle	Bone/Liver	Bone/Spleen
65	35	41	2,798

EXAMPLE II

When the complex ^{153}Sm[N,N',N"-triacetic acid-2,11,20-diaza[3.3.3](2,6)pydinophane] (^{153}Sm-TP3A) was evaluated, the results are given in Table II below. The numbers represent 1 rat per data point at 2 hours post injection.

TABLE II
BIODISTRIBUTION OF ^{153}Sm-TP3A
% INJECTED DOSE

ORGAN	% INJECTED DOSE
Bone	1.495
Liver	0.570
Kidney	0.639
Spleen	0.068
Muscle	0.833
Blood	0.437
Heart	0.016
Lung	0.047
Stomach	0.036
Small Intestine	1.426
Large Intestine	0.156

TABLE II
RATIOS FOR ^{153}Sm-TP3A
AVERAGE % INJECTED DOSE

Bone/Blood	Bone/Muscle	Bone/Liver	Bone/Spleen
3	2	3	22

EXAMPLE III

When the complex ^{153}Sm[N,N'-bis(methylenephosphonic acid)-2,11-diaza[3.3](2,6)pydinophane] (^{153}Sm-BP2P) was evaluated, the results are given in Table III below. The numbers represent 3 rats per data point at 2 hours post injection.

TABLE III
BIODISTRIBUTION OF ^{153}Sm-BP2P
% INJECTED DOSE

ORGAN	AVERAGE
Bone	60.08
Liver	3.71
Kidney	1.21
Spleen	0.05
Muscle	1.53
Blood	0.87
Heart	0.07
Lung	0.17
Brain	0.01
Stomach	0.20
Small Intestine	0.39
Large Intestine	0.13

TABLE III
RATIOS FOR ^{153}Sm-BP2P
AVERAGE % INJECTED DOSE

Bone/Blood	Bone/Muscle	Bone/Liver	Bone/Spleen
69	39	16	1,201

EXAMPLE IV

When the complex ^{153}Sm[N,N'-bis(methylenephosphonic acid ethyl ester)-2,11-diaza[3.3](2,6)pydinophane] (^{153}Sm-BP2EP) was evaluated, the results are given in Table IV below. The numbers represent 3 rats per data point at 2 hours post injection.

TABLE IV
BIODISTRIBUTION OF ^{153}Sm-BP2EP
% INJECTED DOSE

ORGAN	AVERAGE
Bone	39.82
Liver	5.97
Kidney	0.74
Spleen	0.32
Muscle	1.21
Blood	0.45
Heart	0.04
Lung	0.19
Brain	0.01
Stomach	0.36
Small Intestine	0.33
Large Intestine	0.25

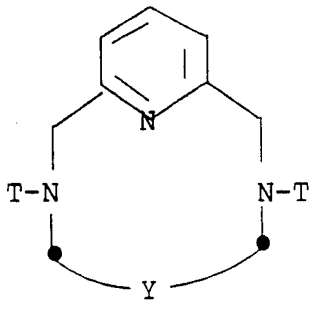
TABLE IV
RATIOS FOR ^{153}Sm-BP2E
AVERAGE % INJECTED DOSE

Bone/Blood	Bone/Muscle	Bone/Liver	Bone/Spleen
88	33	7	124

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

WHAT IS CLAIMED IS:

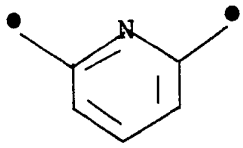
1. Tri- and tetra-cyclopolyazamacrocyclic compounds of the formula



(I)

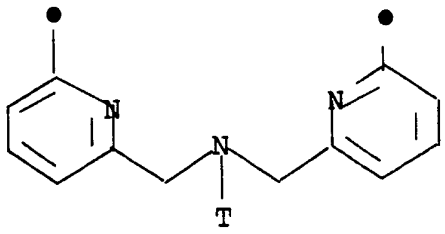
wherein:

Y is either



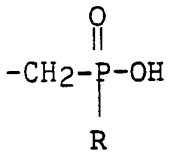
(A)

or



(B)

T is $-CH_2-COOH$ or

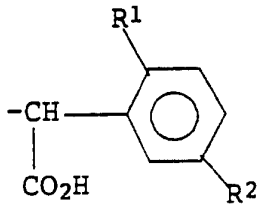


;

where: R is OH, C_1-C_5 alkyl or -O-(C_1-C_5 alkyl);

with the proviso that both T moieties are the same or

one T is



;

R^1 is OH or OCH_3;

R^2 is NO_2, NH_2, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl; or

pharmaceutically-acceptable salts thereof.

2. A compound of Claim 1 wherein all T equal to $-CH_2-P(O)ROH$, where R is OH.

3. A compound of Claim 2 wherein the compound is of Formula (IA) has two T equal to $-CH_2-P(O)ROH$, where R is OH, or pharmaceutically-acceptable salts thereof, and is named as N,N'-bis(methylenephosphonic acid)-2,11-diaza[3.3](2,6)pydinophane.

4. A compound of Claim 1 wherein all T equal $-CH_2-COOH$.

5. A compound of Claim 4 wherein the compound is of Formula (IA) has two T equal to $-CH_2-COOH$, or pharmaceutically-acceptable salts thereof, and is named as N,N'-diacetic acid-2,11-diaza[3.3](2,6)pydinophane.

6. A compound of Claim 4 wherein the compound is of Formula (IB) has three T equal to $-CH_2-COOH$, or pharmaceutically-acceptable salts thereof, and is named as N,N',N"-triacetic acid-2,11,20-diaza[3.3.3](2,6)pydinophane.

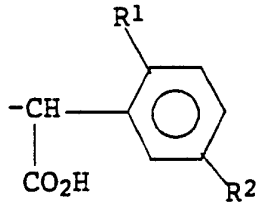
7. A compound of Claim 1 wherein all T equal $-CH_2-P(O)ROH$, where R is $-O-(C_1-C_5$ alkyl).

8. A compound of Claim 7 wherein the compound is of Formula (IA) has two T equal to $-CH_2-P(O)ROH$, where R is $-O-(C_1-C_5$ alkyl), or pharmaceutically-acceptable salts thereof.

9. A compound of Claim 8 wherein R is ethoxy, or pharmaceutically-acceptable salts thereof, and is named as N,N'-bis(methylenephosphonic acid ethyl ester)-2,11-diaza[3.3](2,6)pydinophane.

10. A compound of Claim 1 wherein all T equal $-CH_2-P(O)ROH$, where R is C_1-C_5 alkyl.

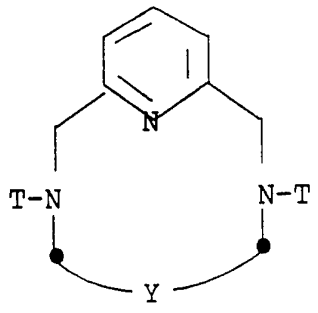
11. A compound of Claim 1 where one T is



;

where R^1 and R^2 are defined as in Claim 1; and all the other T terms are $-CH_2-COOH$.

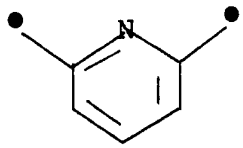
12. A complex which comprises a tri- and tetra-cyclopolyazamacrocyclic compound of the formula



(I)

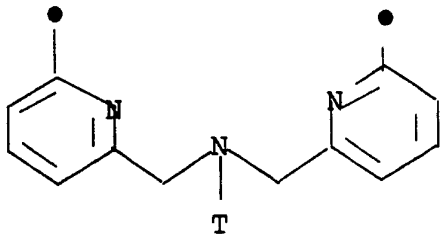
wherein:

Y is either



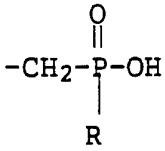
(A)

or



(B)

T is $-CH_2-COOH$ or

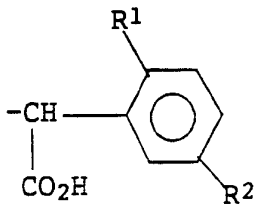


;

where: R is OH, C_1-C_5 alkyl or -O-(C_1-C_5 alkyl);

with the proviso that both T moieties are the same; or

one T is



;

R^1 is OH or OCH_3;

R^2 is NO_2, NH_2, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl; or

pharmaceutically-acceptable salts thereof;

complexed with a metal ion selected from Gd^{+3}, Mn^{+2} or Fe^{+3}.

13. A complex of Claim 12 wherein the metal is Gd^{+3}.

14. A complex of Claim 12 wherein all T equal to $-CH_2-P(O)ROH$, where R is OH, and the metal is Gd^{+3}.

15. A complex of Claim 14 wherein the compound is of Formula (IA) has two T equal to $-CH_2-P(O)ROH$, where R is OH, or pharmaceutically-acceptable salts thereof, and is named as N,N'-bis(methylenephosphonic acid)-2,11-diaza[3.3](2,6)pydinophane.

16. A complex of Claim 12 wherein all T equal $-CH_2-COOH$, and the metal is Gd^{+3}.

17. A complex of Claim 16 wherein the compound is of Formula (IA) has two T equal to $-CH_2-COOH$, or pharmaceutically-acceptable salts thereof, and is named as N,N'-diacetic acid-2,11-diaza[3.3](2,6)pydinophane.

18. A complex of Claim 16 wherein the compound is of Formula (IB) has three T equal to $-CH_2-COOH$, or pharmaceutically-acceptable salts thereof, and is named as N,N',N"-triacetic acid-2,11,20-diaza[3.3.3](2,6)pydinophane.

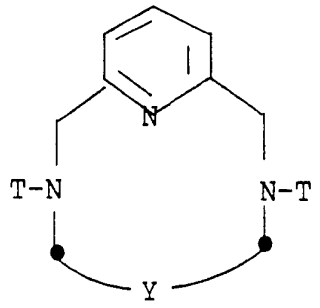
19. A complex of Claim 12 wherein all T equal $-CH_2-P(O)ROH$, where R is $-O-(C_1-C_5$ alkyl), and the metal is Gd^{+3}.

20. A complex of Claim 19 wherein the compound is of Formula (IA) has two T equal to $-CH_2-P(O)ROH$, where R is $-O-(C_1-C_5$ alkyl), or pharmaceutically-acceptable salts thereof.

21. A complex of Claim 20 wherein R is ethoxy, or pharmaceutically-acceptable salts thereof, and is named as N,N'-bis(methylenephosphonic acid ethyl ester)-2,11-diaza[3.3](2,6)pydinophane.

22. A complex of Claim 12 wherein all T equal $-CH_2-P(O)ROH$, where R is C_1-C_5 alkyl, and the metal is Gd^{+3}.

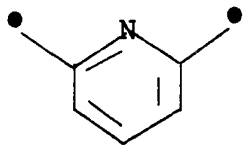
23. A conjugate which comprises a compound of the formula



(I)

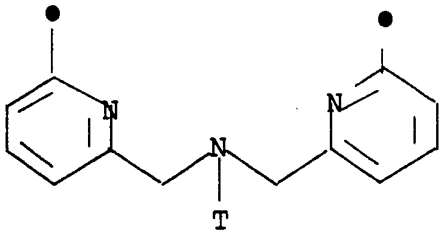
wherein:

Y is either



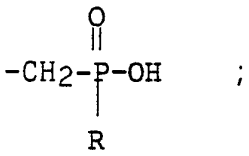
(A)

or



(B)

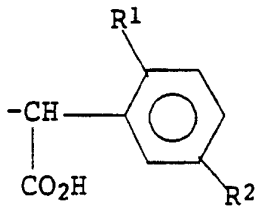
one T is $-CH_2-COOH$ or



;

where: R is OH, C_1-C_5 alkyl or -O-(C_1-C_5 alkyl);

and the other T is



;

R^1 is OH or OCH_3;

R^2 is NO_2, NH_2, isothiocyanato, semicarbazido, thiosemicarbazido, maleimido, bromoacetamido or carboxyl; or

pharmaceutically-acceptable salts thereof;

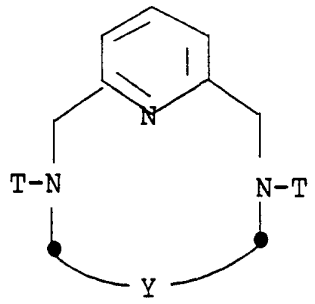
complexed with a metal ion selected from Gd^{+3}, Mn^{+2} or Fe^{+3}; and

covalently attached to a biologically active material.

24. A pharmaceutical formulation comprising a complex of of any one of Claims 12-22 with a pharmaceutically-acceptable carrier.

25. A method for the diagnosis of a disease state in an animal which comprises administering to said animal an effective amount of the formulation of Claim 23.

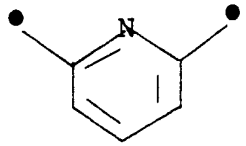
26. A process for preparing tri- or tetra-cyclopolyazamacrocyclic compounds of the formula



(I)

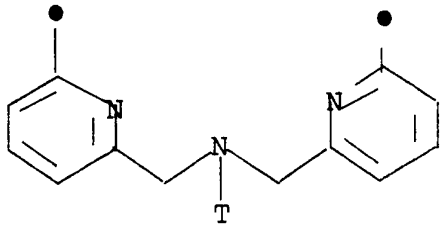
wherein:

Y is either



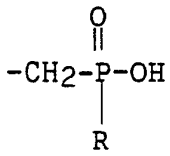
(A)

or



(B)

T is $-CH_2-COOH$ or



;

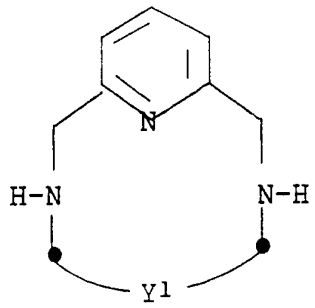
where: R is OH, C_1-C_5 alkyl or -O-(C_1-C_5 alkyl);

with the proviso that both T moieties are the same; or

pharmaceutically-acceptable salts thereof;

which comprises any one of the following:

(A) reacting a compound of the formula



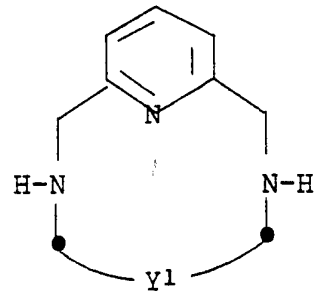
(II)

wherein: Y^1 is defined as in Formula (I) for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

with X-CH_2-COOH, where X is cholro or bromo atom, in the presence of an aqueous alkali metal hydroxide, at a pH between about 8-10, at a temperature between about 60-90°C;

to provide a compound of Formula (I) above where (A) has two T equal -CH_2-COOH and where (B) has three T equal -CH_2-COOH; or

(B) reacting a compound of the formula



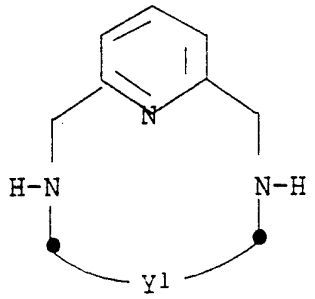
(II)

wherein: Y^1 is defined as in Formula (I) for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

with phosphorous acid in hydrochloric acid and excess formaldehyde, at a pH below about 2, at reflux temperature;

to provide a compound of Formula (I) above where (A) has two T equal -CH_2-PO_3H_2 and where (B) has three T equal -CH_2-PO_3H_2; or

(C) reacting a compound of the formula



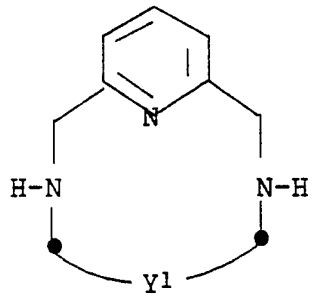
(II)

wherein: Y^1 is defined as in Formula (I) for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

with $P(OR)_3$ where R is C_1-C_4 alkyl in formaldehyde, followed by hydrolysis using 3 to 12M hydrochloric acid, at a pH below about 3, at reflux temperature;

to provide a compound of Formula (I) above where (A) has two T equal $-CH_2-PO_3H_2$ and where (B) has three T equal $-CH_2-PO_3H_2$; or

(D) reacting a compound of the formula



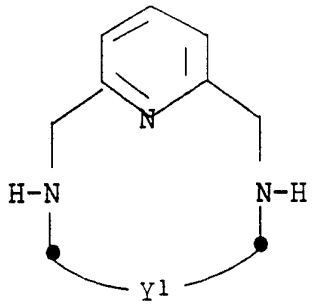
(II)

wherein: Y^1 is defined as in Formula (I) for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

with $P(OR)_3$ where R is C_1-C_4 alkyl in formaldehyde, followed by hydrolysis using excess alkali metal hydroxide, at a pH above about 12, at reflux temperature;

to provide a compound of Formula (I) above where (A) has two T equal $-CH_2-PO_2HR$ where R is -O-(C_1-C_5 alkyl) and where (B) has three T equal $-CH_2-PO_2HR$ where R is -O-(C_1-C_5 alkyl); or

(E) reacting a compound of the formula



(II)

wherein: Y^1 is defined as in Formula (I) for Y when (A) and when (B) the nitrogen atom is substituted with hydrogen; and

with H_3C-$P(OEt)_2$ in formaldehyde and tetrahydrofuran, followed by hydrolysis using excess alkali metal hydroxide, at a pH above about 12, at reflux temperature or with $HP(O)OH$-C_2H_5 in formaldehyde and hydrochloric acid, at a pH below about 3, at reflux temperature;

to provide a compound of Formula (I) above where (A) has two T equal $-CH_2$-$PO(OH)R$ where R is C_1-C_5 alkyl and where (B) has three T equal $-CH_2$-$PO(OH)R$ where R is C_1-C_5 alkyl.

INTERNATIONAL SEARCH REPORT

Int. ional application No.
PCT/US94/05071

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :540/465, 472; 530/386, 387.1; 424/9; 514/80, 286, 293
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/465, 472; 530/386, 387.1; 424/9; 514/80, 286, 293

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS STN STRUCTURE SEARCH (also in parent file)
APS TEXT: "pydinophane" or "pyridinophane" (in parent file)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF ORGANIC CHEMISTRY, Volume 53, No. 15, issued 1988, Bottino et al, "Reaction of Tosylamide Monosodium Salt with Bis(halomethyl) Compounds: An Easy Entry to Symmetrical N-Tosyl Aza Macrocycles," pages 3521-3529, see pgs. 3525-3526.	1-26
X	INORGANIC CHEMISTRY, Volume 19, No. 5, issued 1980, J.F. Desreux, "Nuclear Magnetic Resonance Spectroscopy of Lanthanide Complexes with a Tetraacetic Tetraza Macrocycle. Unusual Conformation Properties," pages 1319-1324, see page 1320, Experimental Section.	26
X	US, A, 3,434,969 (RALSTON) 25 MARCH 1969, see column 3, lines 13-75.	26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 17 JULY 1994	Date of mailing of the international search report 01 AUG 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Philip I. Datlow Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/US94/05071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SYNTHESIS, Volume 12, issued 1987, I.A. Natchev, "Phosphono and Phosphino Analogues and Derivatives of the Natural Aminocarboxylic Acids and Peptides 1. Synthesis and Enzyme-Substrate Interactions of N-Phosphono-, and N-Phosphinomethylated Cyclic Amides", pages 1079-1084, see page 1082, left column, conversion of 4 to 5-8.	26
X,P	US, A, 5,247,077 (PARKER ET AL) 21 SEPTEMBER 1993, see columns 10-11, example 1.	26
X	INORGANIC CHEMISTRY, Volume 30, No. 26, issued 1991, I. Lazar et al., "Synthesis and Complexation Properties of a New Macrocyclic Polyaza Polyphosphinate Ligand, DOTEP (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methyleneet hylphosphinate))", pages 5016-5019, see page 5016.	26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05071

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
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, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

(telephone practice)
Please See Extra Sheet.

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

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

4. ☐ 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 claims Nos.:

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US94/05071

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 31/44, 31/675; C07D 471/18, 471/22; C07F 9/30, 9/38, 9/40; C07K 13/00; G01N 24/08

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-22, 24, 26 (in part), drawn to macrocyclic compounds of formula I, complexes thereof, composition thereof, and process of reacting formula II with a haloalkyl acid, classified in class 540, subclasses 465, 472
II. Claims 23 and 25, drawn to conjugates of compounds of formula I with biological materials, classified in class 530, various subclasses
III. Claim 26 (in part), drawn to reacting formula II with a phosphorus acid (process B), classified in class 540, subclass 472
IV. Claim 26 (in part), drawn to reacting formula II with a phophorus acid ester group (processes C-E), classified in class 540, subclass 472
This application lack unity of invention since the it contains claims to more than one of the combinations of categories set forth in 37 CFR 1.475(b). See 37 CFR 1.475(c). Also, it is noted that Group I is the main invention, and all other products and processes are separate inventions under 37 CFR 1.475(d).