

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

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

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

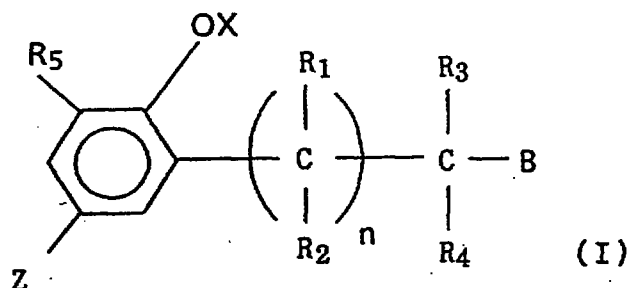
Rosoff, B. et al., *Int. j. App. Rad. and Isot.* 14, 129-135 (1963), disclose complexes of EDTA and NTA with certain radionuclides, namely Sc-46, Y-91, La-140 and Sm-153. The relationship of the stability constant of these complexes of urinary excretion is shown. Chelant to metal molar ratios of 5:1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

The present invention is directed to novel chelants possessing ortho ligating functionality, which chelant forms complexes with metals, especially "radioactive" metals having rare earth-type chemistry. Preferred radioactive metals include samarium-153 (^{153}Sm), holmium-166 (^{166}Ho), yttrium-90 (^{90}Y), promethium-149 (^{149}Pm), gadolinium-159 (^{159}Gd), lanthanum-140 (^{140}La), lutetium-177 (^{177}Lu), ytterbium-175

(¹⁷⁵Yb), scandium-47 (⁴⁷Sc) and praseodymium-142 (¹⁴²Pr). The complexes so formed can be used by themselves or can be attached to any antibody or fragment thereof and used for therapeutic and/or diagnostic purposes. The complexes and/or conjugates can be formulated for *in vivo* or *in vitro* uses. Preferred uses of the formulated conjugates is the treatment of cancer in animals, especially humans.

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

More specifically, the present invention provides a bifunctional chelant possessing ortho ligating functionality having the formula



wherein:

Z is an electrophilic or nucleophilic moiety which allows for covalent attachment to an antibody or fragment thereof or a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof;

X is hydrogen, C_1-C_3 alkyl or $CR_3R_4CO_2H$;

R_1 , R_2 , R_3 and R_4 each are independently hydrogen, hydroxy, CO_2H or a C_1-C_3 alkyl group;

R_5 is hydrogen or $(CR_1R_2)_nCR_3R_4B'$;

B. represents a linear or branched polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

B' represents a linear or branched amine or polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

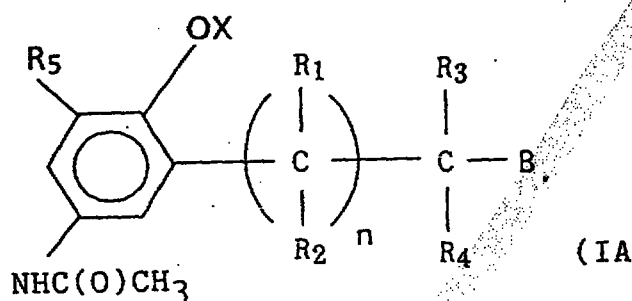
n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

It is preferred that the carboxyl group (when present) be attached to the first or second carbon from the nitrogen of the B group, i.e. the carbon α or β to the nitrogen in the chelant moiety. Preferred compounds of formula I are those where n is 0; or R_1 , R_2 , R_3 and R_4

are each hydrogen; or n is 0 and one of R_3 or R_4 is hydrogen and the other is COOH ; or X is hydrogen. When the chelants are to be used as bifunctional chelating agents, then Z is preferably amino, isothiocyanato, semicarbazide, thiosemicarbazide, carboxyl, bromoacetamido or maleimido.

The present invention further provides a bifunctional chelant possessing ortho ligating functionality having the formula



wherein:

X is hydrogen, $\text{C}_1\text{-C}_3$ alkyl or $\text{CR}_3\text{R}_4\text{CO}_2\text{H}$;

R_1 , R_2 , R_3 and R_4 each are independently hydrogen, hydroxy, CO_2H or a $\text{C}_1\text{-C}_3$ alkyl group;

R_5 is hydrogen or $(\text{CR}_1\text{R}_2)_n\text{CR}_3\text{R}_4\text{B}'$;

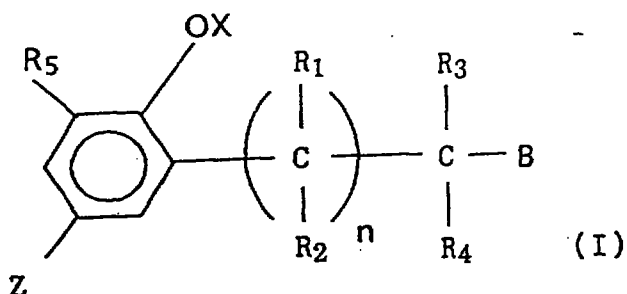
B represents a linear or branched polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $\text{CR}_3\text{R}_4\text{CO}_2\text{H}$ group;

B' represents a linear or branched amine or polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $\text{CR}_3\text{R}_4\text{CO}_2\text{H}$ group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

The present invention still further provides a complex which comprises a chelant having the formula



wherein:

Z is an electrophilic or nucleophilic moiety which allows for covalent attachment to an antibody or fragment thereof or a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof;

X is hydrogen, C_1-C_3 alkyl or $CR_3R_4CO_2H$;

R_1 , R_2 , R_3 and R_4 each are independently hydrogen, hydroxy, CO_2H or a C_1-C_3 alkyl group;

R_5 is hydrogen or $(CR_1R_2)_nCR_3R_4B'$;

B represents a linear or branched polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

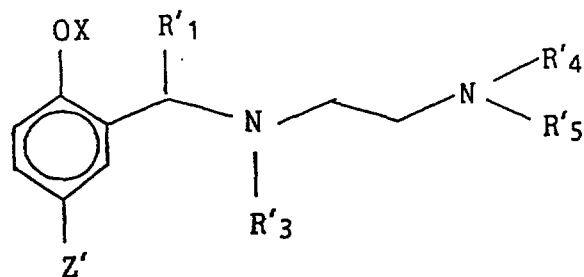
B' represents a linear or branched amine or polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof;

complexed with a metal ion selected from La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc.

The present invention still further provides a chelant possessing ortho ligating functionality having the formula



II

wherein Z' is hydrogen, NH_2 , NO_2 , $NHC(O)CH_3$ or $N(R')_2$, where R' is hydrogen or C_1-C_3 alkyl;

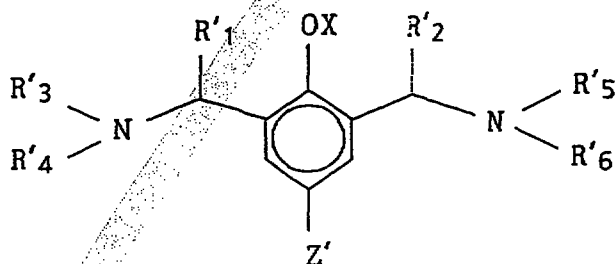
X is hydrogen, C_1-C_3 alkyl or CR_3R_4COOH ;

R'_1 is hydrogen or $COOH$;

R'_3 , R'_4 and R'_5 are independently hydrogen or CR_3R_4COOH , with the proviso that at least one of R'_1 , R'_3 , R'_4 and R'_5 is hydrogen; or

a pharmaceutically acceptable salt thereof.

The present invention still further provides, a chelant possessing ortho ligating functionality having the formula



III

wherein Z' is hydrogen, NH_2 , NO_2 , $NHC(O)CH_3$ or $N(R')_2$, where R' is hydrogen or C_1-C_3 alkyl;

X is hydrogen or C_1-C_3 alkyl or CR_3R_4COOH ;

R'_1 and R'_2 each are hydrogen or $COOH$, with the proviso that at least one is $COOH$;

R'_3 , R'_4 , R'_5 and R'_6 are independently

hydrogen or $\text{CR}_3\text{R}_4\text{COOH}$, with the proviso that at least three are $\text{CR}_3\text{R}_4\text{COOH}$; or

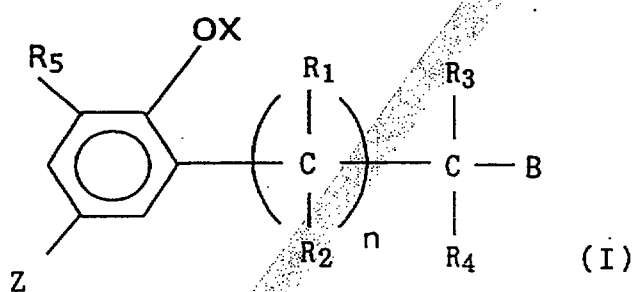
a pharmaceutically acceptable salt thereof.

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

As used herein, the following indicated terms have these meanings: with respect to the definition of Z; "electrophilic" moieties include, but are not limited to, isothiocyanate, bromoacetamide, maleimide, imidoester, thiophthalimide, N-hydroxysuccinimyl ester, pyridyl disulfide and phenyl azide; suitable "nucleophilic" moieties include, but are not limited to, carboxyl, amino, acyl hydrazide, semicarbazide, and thiosemicarbazide; "synthetic linkers" include any synthetic organic or inorganic linkers which are capable of being covalently attached to an antibody or antibody fragment, preferred synthetic linkers are biodegradable synthetic linkers which are stable in the serum of a patient but which have a potential for enzymatic cleavage within an organ of clearance for the radioisotope, for example biodegradable peptides or peptide

CLAIMS

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



wherein:

Z is an electrophilic or nucleophilic moiety which allows for covalent attachment to an antibody or fragment thereof or a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄CO₂H;

R_1 , R_2 , R_3 and R_4 each are independently hydrogen, hydroxy, CO_2H or a $\text{C}_1\text{-C}_3$ alkyl group;

R_5 is hydrogen or $(\text{CR}_1\text{R}_2)_n\text{CR}_3\text{R}_4\text{B}'$;

B represents a linear or branched polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $\text{CR}_3\text{R}_4\text{CO}_2\text{H}$ group;

B' represents a linear or branched amine or polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $\text{CR}_3\text{R}_4\text{CO}_2\text{H}$ group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

2. The bifunctional chelant of Claim 1 wherein n is 0.

3. The bifunctional chelant of Claim 1 or 2 wherein R_4 is CO_2H .

4. The bifunctional chelant of Claim 1, 2 or 3 wherein X is hydrogen.

5. The bifunctional chelant of any one of Claims 1 through 4 wherein R_1 , R_2 and R_3 are each hydrogen.

6. The bifunctional chelant of any one of Claims 1 through 5 wherein Z is amino, isothiocyanato, semicarbazido, thiosemicarbazido, carboxyl, bromoacetamido or maleimido.

7. The bifunctional chelant of Claim 1 which is 2-[(2-[[bis(carboxymethyl)amino]ethyl)(carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid.

8. The bifunctional chelant of Claim 1 which is 2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)amino]-2-(5-amino-2-hydroxyphenyl)ethanoic acid.

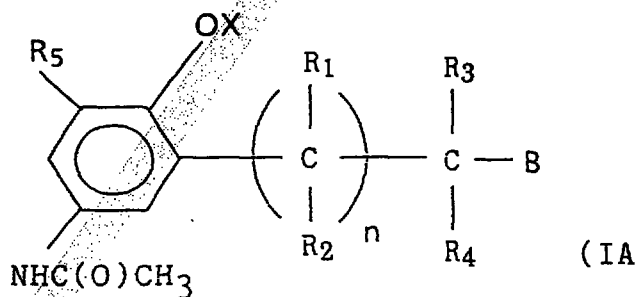
9. The bifunctional chelant of Claim 1 which is 2-[bis(2-[[bis(carboxymethyl)]amino]ethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid.

10. The bifunctional chelant of Claim 1 which is 2-[[2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)amino]ethyl](carboxymethyl)amino]-2-[5-amino-2-(carboxymethyloxy)phenyl]ethanoic acid.

11. The bifunctional chelant of Claim 1 which is 2,6-bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(amino)phenol.

12. The bifunctional chelant of Claim 1 which is 2,6-bis{[(2-[[bis(carboxymethyl)]amino]ethyl)-(carboxymethyl)]aminomethyl}-4-(isothiocyanato)phenol.

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



wherein:

X is hydrogen, C₁-C₃ alkyl or CR₃R₄CO₂H;

R_1 , R_2 , R_3 and R_4 each are independently hydrogen, hydroxy, CO_2H or a C_1 - C_3 alkyl group;

R_5 is hydrogen or $(CR_1R_2)_nCR_3R_4B'$;

B represents a linear or branched polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

B' represents a linear or branched amine or polyalkylene polyamine where at least one of the amine hydrogens has been substituted with a $CR_3R_4CO_2H$ group;

n is 0 or 1; or

a pharmaceutically acceptable salt thereof.

14. The bifunctional chelant of Claim 13 which is 2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid.

15. The bifunctional chelant of Claim 13 which is 2-[bis(2-[[bis(carboxymethyl)]amino]ethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid.

16. The bifunctional chelant of Claim 13 which is 2-[[2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)amino]ethyl](carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid.

17. The bifunctional chelant of Claim 13 which is 2-[(2-[(2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)amino]ethyl)(carboxymethyl)amino]ethyl)(carboxymethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid.

18. The bifunctional chelant of Claim 13 which is 2-[(2-[(2-[[bis(carboxymethyl)]amino]ethyl)(carboxymethyl)

amino}ethyl)(2-{{[bis(carboxymethyl)]amino}ethyl)amino]-2-[5-acetamido-2-(carboxymethyloxy)phenyl]ethanoic acid.

19. The bifunctional chelant of Claim 13 which is 2,6-bis{[(2-{{[bis(carboxymethyl)]amino}ethyl)-(carboxymethyl)]aminomethyl}-4-(acetamido)phenol.

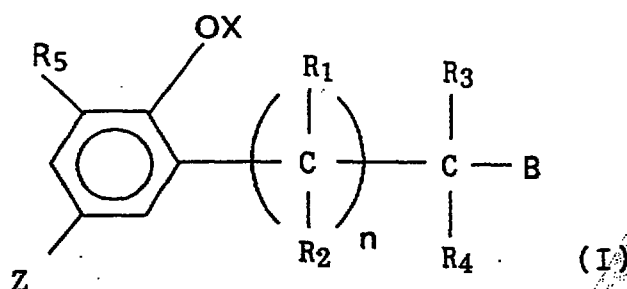
20. The bifunctional chelant of Claim 13 which is 2-({[bis(carboxymethyl)]amino}methyl)-6-({[bis(carboxymethyl)]amino}ethyl)(carboxymethyl)aminomethyl}-4-(acetamido)phenol.

21. The bifunctional chelant of Claim 13 which is 2,6-bis{(bis(carboxymethyl)amino)(carboxymethyl)-4-(acetamido)phenol.

22. The bifunctional chelant of Claim 13 which is 2-[(2-{{[bis(carboxymethyl)]amino}ethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid.

23. The bifunctional chelant of Claim 13 which is 2-[(2-{{[bis(carboxymethyl)]amino}ethyl)(cyanomethyl)amino]-2-(5-acetamido-2-hydroxyphenyl)ethanoic acid.

24. A complex which comprises a chelant having the formula



wherein:

Z is an electrophilic or nucleophilic moiety which allows for covalent attachment to an antibody or fragment thereof or a synthetic linker which does not interfere with the formation of complexation with a radionuclide and which can be attached to an antibody or fragment thereof;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄CO₂H;

R₁, R₂, R₃ and R₄ each are independently hydrogen, hydroxy, CO₂H or a C₁-C₃ alkyl group;

R₅ is hydrogen or (CR₁R₂)_nCR₃R₄B';

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

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

n is 0 or 1; or

a pharmaceutically acceptable salt thereof;

complexed with a metal ion selected from La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc.

25. A complex of Claim 24 wherein the metal ion is ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc or ^{142}Pr .

26. A complex of Claim 22 wherein the chelant is as claimed in any one of Claims 2 through 12.

27. A conjugate which comprises a complex as claimed in any one of Claims 24 through 26; covalently attached to an antibody or antibody fragment.

28. A conjugate of Claim 27 wherein the antibody or fragment thereof is a monoclonal antibody or fragment thereof.

29. A conjugate of Claim 28 wherein the antibody or antibody fragment is CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ or B72.3.

30. A pharmaceutical formulation comprising a complex as claimed in any one of Claims 24 through 26, and a physiologically acceptable carrier.

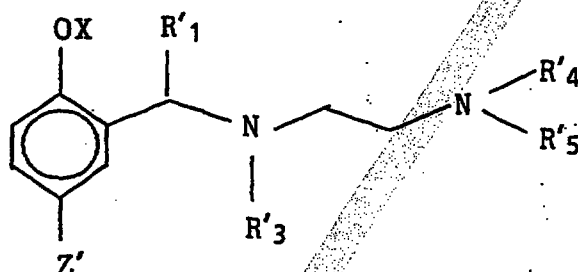
31. A pharmaceutical formulation comprising a conjugate as claimed in any one of Claims 27 through 29, and a physiologically acceptable carrier.

32. A method for the diagnosis and/or treatment of a disease state in a mammal which comprises administering to said mammal an effective amount of the formulation of Claim 30 or 31.

33. A method of Claim 32 wherein the disease state is cancer.

34. A method for the therapeutic treatment of an animal having bone pain which comprises administering to said animal a therapeutically effective amount of the formulation of Claim 30 or 31.

35. A chelant possessing ortho ligating functionality having the formula



II

wherein Z' is hydrogen, NH₂, NO₂, NHC(O)CH₃ or N(R')₂, where R' is hydrogen or C₁-C₃ alkyl;

X is hydrogen, C₁-C₃ alkyl or CR₃R₄COOH;

R'₁ is hydrogen or COOH;

R'₃, R'₄ and R'₅ are independently hydrogen or CR₃R₄COOH, with the proviso that at least one of R'₁, R'₃, R'₄ and R'₅ is hydrogen; or

a pharmaceutically acceptable salt thereof.

36. The chelant of Claim 35 wherein R'₁ is COOH, R'₃ is hydrogen, and R'₄ and R'₅ are CH₂COOH.

37. The chelant of Claim 36 wherein Z' is NHC(O)CH₃, and X is hydrogen.

38. A complex which comprises a chelant as claimed in any one of Claims 35 through 37;

complexed with a metal ion selected from La, Ce,

Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc.

39. A complex of Claim 38 wherein the metal ion is ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc or ^{142}Pr .

40. A conjugate which comprises a complex as claimed in Claim 38 or 39; covalently attached to an antibody or antibody fragment.

41. A conjugate of Claim 40 wherein the antibody or fragment thereof is a monoclonal antibody or fragment thereof.

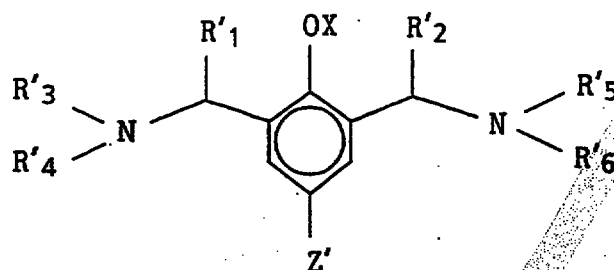
42. A conjugate of Claim 41 wherein the antibody or antibody fragment is CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ or B72.3.

43. A pharmaceutical formulation comprising a complex as claimed in Claim 38 or 39, and a physiologically acceptable carrier.

44. A pharmaceutical formulation comprising a conjugate as claimed in any one of Claims 40 through 41, and a physiologically acceptable carrier.

45. A method for the diagnosis and/or treatment of a disease state in a mammal which comprises administering to said mammal an effective amount of the formulation of Claim 43 or 44.

46. A chelant possessing ortho ligating functionality having the formula



III

wherein Z' is hydrogen, NH_2 , NO_2 , $NHC(O)CH_3$ or $N(R')_2$, where R' is hydrogen or C_1-C_3 alkyl;

X is hydrogen or C_1-C_3 alkyl or CR_3R_4COOH ;

R'_1 and R'_2 each are hydrogen or $COOH$, with the proviso that at least one is $COOH$;

R'_3 , R'_4 , R'_5 and R'_6 are independently hydrogen or CR_3R_4COOH , with the proviso that at least three are CR_3R_4COOH ; or

a pharmaceutically acceptable salt thereof.

47. The chelant of Claim 46 wherein R'_1 and R'_2 are $COOH$, R'_3 , R'_4 , R'_5 and R'_6 are CH_2COOH .

48. The chelant of Claim 47 wherein Z' is $NHC(O)CH_3$ and X is hydrogen.

49. A complex which comprises a chelant as claimed in any one of Claims 46 through 48;

complexed with a metal ion selected from La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y or Sc.

50. A complex of Claim 49 wherein the metal ion is ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{159}Gd , ^{140}La , ^{177}Lu , ^{175}Yb , ^{47}Sc or ^{142}Pr .

51. A conjugate which comprises a complex as claimed in Claim 49 or 50;
covalently attached to an antibody or antibody fragment.

52. A conjugate of Claim 51 wherein the antibody or fragment thereof is a monoclonal antibody or fragment thereof.

53. A conjugate of Claim 53 wherein the antibody or antibody fragment is CC-46, CC-49, CC-49 F(ab')₂, CC-83, CC-83 F(ab')₂ or B72.3.

54. A pharmaceutical formulation comprising a complex as claimed in Claim 49 or 50, and a physiologically acceptable carrier.

55. A pharmaceutical formulation comprising a conjugate as claimed in any one of Claims 51 through 53, and a physiologically acceptable carrier.

56. A method for the diagnosis and/or treatment of a disease state in a mammal which comprises administering to said mammal an effective amount of the formulation of Claim 54 or 55.

CHELANTS POSSESSING ORTHO LIGATING FUNCTIONALITY AND COMPLEXES THEREOF

ABSTRACT

A group of functionalized amine chelants having ortho ligating functionality that form complexes with rare-earth type metal ions are disclosed. The complexes can be covalently attached to an antibody or antibody fragment and used for therapeutic and/or diagnostic purposes. In addition certain of the chelant-radionuclide complexes can be effectively employed in compositions useful as therapeutic and/or diagnostic agents for calcific tumors and/or relief of bone pain.