

Physiologically tolerable metal complex salts for use
in NMR diagnosis

The invention relates to physiologically tolerable metal complex salts and the use thereof in NMR diagnosis.

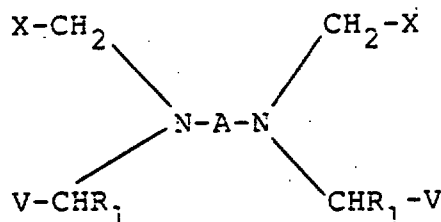
5 Complex compounds and their salts have been used for a long time in medicine, for example as auxiliaries for the administration of sparingly soluble ions (for example iron) and as antidotes (calcium or zinc complexes being preferred in this case) for detoxication in the
10 case of inadvertent incorporation of heavy metals or their radioactive isotopes.

The present application and copending application No. 85.29903 (publication No. 2 169 599) are divisional applications from application No. 84.01486 (publication
15 No. 2 137 612).

We have now found that certain physiologically tolerable complex salts containing one or more central elements having the atomic numbers of from 21 to 29, 42, 44 and from 58 to 70 can be used for the manufacture of
20 preparations that are surprisingly outstandingly suitable for use in NMR diagnostics.

The present invention provides a diagnostic preparation which comprises

(i) a physiologically tolerable, non-radioactive complex
25 salt of the general formula I



(I)

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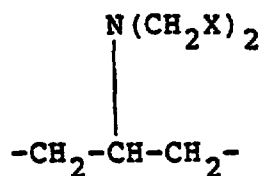
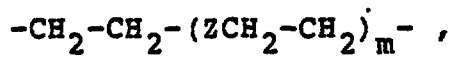
in which

X represents the radicals $-\text{COOY}$, $-\text{PO}_3\text{HY}$ or $-\text{CONHOY}$ wherein each Y represents a hydrogen atom, a metal equivalent, the metal being selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58 to 70, or a physiologically tolerable cation of an inorganic or organic base or amino acid,

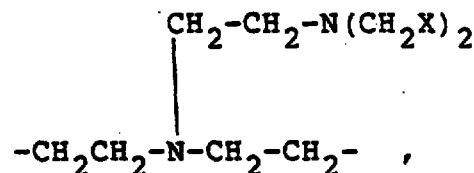
R_1 represents in each case a hydrogen atom or a methyl group

and in which either

a) A represents the group $-\text{CHR}_2-\text{CHR}_3-$,



or



15 in which

X has the meanings given above,

R_2 and R_3 together represent a trimethylene group or a tetramethylene group, or each represent a hydrogen atom, an alkyl radical containing up to 6 carbon atoms, a phenyl radical or a benzyl radical,

$\underline{m}$ represents the integer 1, 2 or 3,

and

20

Z represents an oxygen atom or a sulphur atom or the group



in which

5 X has the meanings given above
and

R₄ represents an alkyl radical
containing up to 6 carbon atoms,

and

10 each V has the same meaning as X or represents the
group -CH₂OH or -COB,

in which

B represents a protein or lipid radical or an
-NH(CH₂)_nX radical,

15 in which

X has the meanings given above
and

n represents an integer from 1 to 12,

with the proviso that at least one of the symbols V

20 represents a -COB group, as defined above, and at least
two of the substituents Y are metal equivalents
selected from elements having atomic numbers of from 21
to 29, 42, 44 and from 58 to 70,

or

25 b) A represents the group -CHR₂-CHR₃-

in which

R_2 represents a hydrogen atom

and

R_3 represents the group $-(CH_2)_p-C_6H_4-W$ -protein

5 in which

p represents 0 or 1,

W represents $-NN-$, $-NHCOCH_2-$

or $-NHCS-$

and

10 $-protein$ represents a protein radical,

and

each V has the same meaning as X or represents the group $-CH_2OH$,

with the proviso that at least two of the substituents

15 Y are metal equivalents selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58 to 70,

and

(ii) a physiologically tolerable carrier.

20 Thus, the physiologically tolerable complex salt of the general formula I may be regarded as containing

(a) a central element selected from elements having atomic numbers of from 21 to 29 inclusive, 42, 44 and from 58 to 70 inclusive, and (b) a radical of a

25 physiologically tolerable complex-forming acid and, if desired, (c) a radical selected from radicals of inorganic and organic bases and amino acids.

The physiologically tolerable complex salt of the general formula I may contain more than one such central element (a) and more than one such radical (c).

For the intended use of the diagnostic agent
5 according to the invention, the element or elements having an atomic number mentioned above, which forms the central element or elements of the physiologically tolerable complex salt, must not, of course, be radioactive.

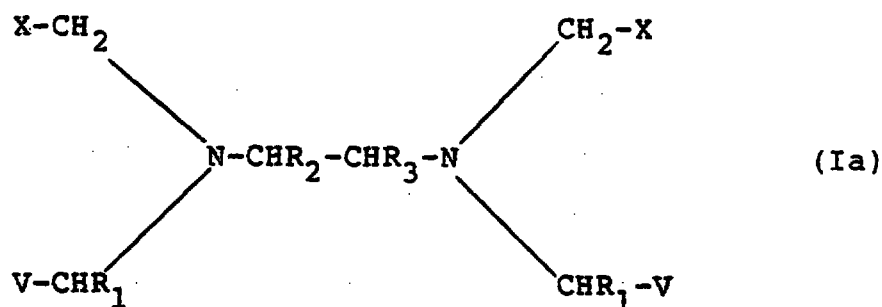
10 Since a preparation of the invention is to be used in NMR diagnostics (see European Patent Application 71 564), the central element of the complex salt must be paramagnetic. Such elements are especially the divalent and trivalent elements having
15 an atomic number of from 21 to 29, 42, 44 and from 58 to 70. Suitable elements are, for example, chromium(III), manganese(II), iron(III), iron(II), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III) and ytterbium(III).
20 Especially preferred, owing to their strong magnetic moment, are gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III).

The complex-forming acids from which the physiologically tolerable complex salts of the general
25 formula I may be derived are, especially, those containing carboxymethylene groups.

The complex-forming acids from which the

physiologically tolerable complex salts of the general formula I are derived can, as conjugates, be bonded to biomolecules that are known to become especially concentrated in the organ or organ part under examination. Such biomolecules are, for example, proteins or lipids. There come into consideration conjugates with protein hormones, such as insulin, and more especially conjugates with albumens, such as human serum albumen, antibodies, such as, for example, monoclonal antibodies specific to tumour-associated antigens, or antimyosin. The diagnostic preparations formed therefrom are suitable, for example, for use in tumour and infarct diagnosis. For examinations of the liver there are suitable, for example, conjugates or inclusion compounds with liposomes, which are used, for example, as unilamellar or multilamellar phosphatidylcholinecholesterol vesicles. The conjugates are formed either by way of a carboxy group of the complex-forming acid or, in the case of proteins, also by way of a $-(CH_2)_p-C_6H_4-W-$ group as defined above under R_3 . In the conjugate formation of some complex-forming acids with proteins or lipids, several acid radicals may be bonded to the macromolecular biomolecule. In that case, each complex-forming acid radical may carry one central element.

Suitable complex salts of the general formula I above are, for example, those of the general formula Ia

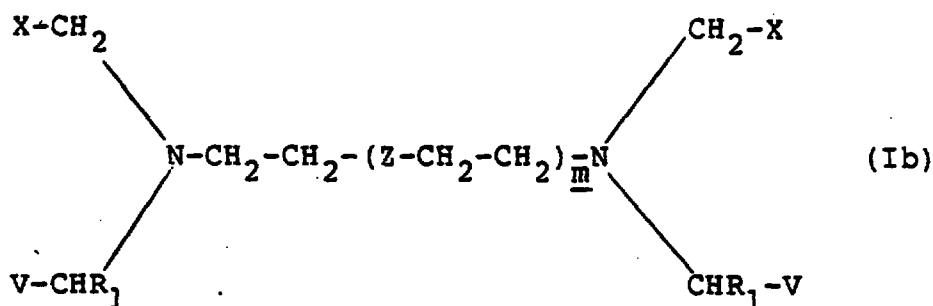


in which X, V, R₁, R₂ and R₃ have the meanings given above.

The following complex-forming acids, inter alia,
 5 are suitable for the manufacture of the complex salts
 of the general formula Ia:-

ethylenediaminetetraacetic acid, ethylenediaminetetra-
 acetohydroxamic acid, trans-1,2-cyclohexylenediamine-
 tetraacetic acid, DL-2,3-butylenediaminetetraacetic
 10 acid, DL-1,2-butylenediaminetetraacetic acid, DL-1,2-
 propylenediaminetetraacetic acid, 1,2-diphenylethylene-
 diaminetetraacetic acid, ethylenedinitrolotetrakis-
 (methane-phosphonic acid) and N-(2-hydroxyethyl)-
 ethylenediaminetriacetic acid.

15 Other suitable complex salts of the general
 formula I are, for example, those of the general
 formula Ib



in which X, V, Z, R₁ and m have the meanings given above. If Z represents an oxygen atom or a sulphur atom, complex salts in which m represents 1 or 2 are preferred.

The following complex-forming acids, inter alia, are suitable for the manufacture of the complex salts of the general formula Ib:

diethylenetriaminepentaacetic acid, triethylenetetraminehexaacetic acid, tetraethylenepentamineheptaacetic acid, 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid, 3,9-bis-(1-carboxyethyl)-6-carboxymethyl-3,6,9-triazaundecanoic diacid, diethylenetriaminepentakis-(methylenephosphonic acid), 1,10-diaza-4,7-dioxadecane-1,1,10,10-tetraacetic acid and 1,10-diaza-4,7-dithiadecane-1,1,10,10-tetraacetic acid.

Other complex-forming acids that are suitable for the manufacture of the complex salts of the general formula I are, for example:

1,2,3-tris-[bis-(carboxymethyl)-amino]-propane and nitrolotris-(ethylenenitrolo)-hexaacetic acid.

If not all of the acid hydrogen atoms of the complex-forming acid are substituted by the central element or elements, it is advantageous, for the purpose of increasing the solubility of the complex salt, to substitute the remaining hydrogen atoms by physiologically tolerable cations of inorganic and/or organic bases or amino acids. Suitable inorganic cations are for example, lithium, potassium or, especially, sodium. Suitable cations of organic bases are, inter alia, those of primary, secondary or tertiary amines, such as, for example, ethanolamine, diethanolamine, morpholine, glucamine, N,N-dimethyl-glucamine or, especially, N-methylglucamine. Suitable cations of amino acids are, for example, those of lysine, arginine or ornithine.

The complex-forming acids required for the formation of the complex salts of the general formula I are known or can be manufactured in a manner known per se.

For example, 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21-24-pentaazapentatriacontanoic diacid is manufactured in the following manner, which is an improvement to the method proposed by R. A. Bulman et al. in Naturwissenschaften 68, (1981) 483:

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17.85 g (= 50 mmol) of 1,5-bis-(2,6-dioxomorpholino)-3-azapentane-3-acetic acid are suspended in 400 ml of dry dimethylformamide and, after the addition of 20.13 g (= 100 mmol) of 11-aminoundecanoic acid, the whole is heated at 70°C for 6 hours. The clear solution is concentrated in vacuo. The yellow oily residue is stirred with 500 ml of water at room temperature. In so doing, an almost white, voluminous solid precipitates which is suction-filtered and washed several times with water. For further purification, the resulting product is introduced into 200 ml of acetone and the whole is stirred for 30 minutes at room temperature. After suction-filtering and drying in vacuo at 50°C, 36.9 g (= 97 % of the theoretical yield) of a white powder of melting point 134-138°C are obtained.

Conjugation of the complex-forming acids with biomolecules is likewise effected according to methods known per se, for example by reacting the nucleophilic groups of the biomolecule, such as, for example, amino, hydroxy, thio or imidazole groups, with an activated derivative of the complex-forming acid.

Activated derivatives of the complex-forming acid which come into consideration are, for example, acid chlorides, acid anhydrides, activated esters, nitrenes or isothiocyanates. Conversely, it is also possible to react an activated biomolecule with the complex-forming

acid.

For conjugation with proteins, substituents of the structure $-C_6H_4N_2^+$ or $-C_6H_4NHCOCH_2$ -halogen may also be considered.

5 The manufacture of the complex salts of the general formula I can likewise be carried out in a manner known per se by dissolving or suspending the metal oxide or metal salt (for example the nitrate, chloride or sulphate) of the element having an atomic
10 number of from 21 to 29, 42, 44 or from 58 to 70 in water and/or a lower alcohol (such as methanol, ethanol or isopropanol) and adding a solution or suspension of the equivalent amount of the complex-forming acid in water and/or a lower alcohol, and stirring, if
15 necessary while warming or heating to boiling point, until the reaction is complete. If the complex salt formed is insoluble in the solvent used, it is isolated by filtration. If it is soluble, it can be isolated by concentrating the solution to dryness by evaporation,
20 for example by means of spray-drying.

 If acid groups are still present in the resulting complex salt, it is often advantageous to convert the acid complex salt into a neutral complex salt or salts by means of inorganic and/or organic bases or amino
25 acids that form physiologically tolerable cations and to isolate the neutral salt. In many cases, this is indeed unavoidable since the dissociation of the

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complex salt is so suppressed by the shift in the pH value to neutral that only in that manner can uniform products be at all isolated or at least purified.

5 The manufacture is advantageously carried out with the aid of organic bases or basic amino acids. It can, however, also be advantageous if the neutralisation is carried out by means of inorganic bases (hydroxides, carbonates or bicarbonates) of sodium, potassium or lithium.

10 For the manufacture of the neutral salts there may, for example, be added to the acid complex salts in aqueous solution or suspension as much of the desired base as is necessary to obtain the neutral point. The resulting solution can subsequently be
15 concentrated to dryness in vacuo. It is frequently of advantage to precipitate the resulting neutral salts by adding water-miscible solvents, such as, for example, lower alcohols (methanol, ethanol, isopropanol, etc.), lower ketones (acetone, etc.), and polar ethers (tetra-
20 hydrofuran, dioxane, 1,2-dimethoxyethane, etc.), and thus obtain crystallisates that are easily isolated and readily purified. It has been found especially advantageous to add the desired base to the reaction mixture during the complex formation and thereby
25 dispense with one process step.

If the acid complex salts contain several free acid groups, it is often advantageous to produce

neutral mixed salts that contain both inorganic and organic physiologically tolerable cations as ions of opposite charge. This can be effected, for example, by reacting the complex-forming acid in aqueous suspension or solution with the oxide or salt of the element supplying the central element and with half the amount of organic base required for neutralisation, isolating the complex salt formed, if desired purifying it, and then adding to it the amount of inorganic base required for complete neutralisation. The order in which the bases are added can also be reversed.

The manufacture of the diagnostic preparations according to the invention is likewise effected in a manner known per se by suspending or dissolving the complex salts in an aqueous medium, for example water or physiological salt solution, optionally with the addition of the additives customary in galenical pharmacy, and subsequently sterilising the solution or suspension. Suitable additives are, for example, physiologically tolerable buffers (such as, for example, tromethamine hydrochloride), small additions of complex formers (such as, for example, diethylenetriaminepentaacetic acid) or, if necessary, electrolytes (such as, for example, sodium chloride).

In principle, it is also possible to manufacture the diagnostic preparations of the invention even without isolating the complex salts. In each case,

particular care must be taken to effect the chelate formation in such a manner that the salts and salt solutions according to the invention are virtually free of non-complexed toxically acting metal ions. This can
 5 be ensured, for example, with the aid of colour indicators, such as xylenol orange, by test titrations during the manufacturing process. The invention also therefore provides processes for the manufacture of the complex salts and of the aforesaid preparations
 10 containing them. As a final safeguard, there is always purification of the isolated complex salt.

If suspensions of the complex salts in water or physiological salt solution are desired for oral administration or other purposes, a sparingly soluble
 15 complex salt is mixed with one or more auxiliaries customary in galenical pharmacy and/or surfactants and/or aromatic substances for taste correction.

The diagnostic preparations of the invention contain preferably from 1 μ mol to 1 mol per litre of
 20 the complex salt and are, as a rule, administered in doses of from 0.001 to 5 mmol/kg. They are intended for oral, and especially parenteral, administration. If desired, they may be in a dosage form suitable for administration orally, neurally or intravasally. When
 25 the preparations are in a form suitable for injections, they may be contained in ampoules.

The diagnostic preparations of the invention meet

the many requirements for suitability as contrast agents for nuclear spin tomography. For example, after oral or parenteral administration, they are outstandingly suitable for improving the information that can be provided by the image obtained with the aid of nuclear spin tomography, as a result of increasing the signal intensity. They also exhibit the high activity necessary to keep to a minimum the amount of foreign substances introduced into the body and the good tolerability necessary to maintain the non-invasive character of the examination (the compounds mentioned in J. Comput. Tomography 5,6: 543-46 (1981), in Radiology 144, 343 (1982) and in Brevet Special de Medicament No. 484 M (1960) are, for example, too toxic). The ready water-solubility of the complex salts used in the preparations of the invention enables the preparation of highly concentrated solutions, so that the volume introduced into the circulation can be kept within reasonable limits and the dilution by body fluid can be compensated, that is to say the NMR diagnostic preparations must be 100 to 1000 times more water-soluble than is necessary for NMR spectroscopy. Furthermore, the diagnostic preparations of the invention are not only highly stable in vitro but also exhibit a surprisingly high stability in vivo, so that the per se toxic ions that are not covalently bonded in the complexes are released or exchanged only

extremely slowly. The conjugates with proteins and antibodies which are used, for example, for the diagnosis of tumours bring about a surprisingly high intensification of the signal at such a low dosage that it is possible to use in this case solutions of correspondingly low concentration.

Especially low doses (under 1 mg/kg) and therewith solutions of lower concentrations (1 $\mu\text{mol/l}$ to 5 mmol/l) than are specified in EP 71 564 are required for organ-specific NMR diagnostics, for example for detecting tumours and coronary infarcts.

The invention also provides physiologically tolerable, non-radioactive complex salts of the general formula I, as defined above. In those complex salts of the general formula I containing more than two of the radicals represented by X, it may be advantageous for at least one of the substituents Y to be a physiologically tolerable cation of an organic base or amino acid.

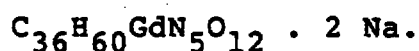
The present invention further provides the use of a physiologically tolerable, non-radioactive complex salt of the general formula I, as defined above, for the manufacture of a preparation for a method of NMR diagnosis of the human or animal body.

The following Examples illustrate the invention:-

Example 1

Preparation of the disodium salt of the gadolinium(III) complex of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-

5 12,15,18,21,24-pentaazapentatriacontanoic diacid,



15.2 g (= 20 mmol) of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid are suspended in 400 ml of water and the
10 suspension is heated to 95°C. 7.43 g (= 20 mmol) of gadolinium(III) chloride hexahydrate, dissolved in 60 ml of water, are slowly added dropwise. The whole is maintained at this temperature for 2 hours and then, in order to neutralise the hydrochloric acid formed, 60 ml
15 of 1N sodium hydroxide solution are added.

When the reaction is complete (testing with xylenol orange) the precipitate obtained is filtered and washed free of sodium chloride with water. 17.60 g (96 % of the theoretical yield) of a water-insoluble,
20 white powder of melting point 290-292°C are obtained.

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Gadolinium(III) complex of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid.

Analysis:

5	(calculated)	C 47.30	H 6.84	N 7.66	Gd 17.20
	(found)	C 47.13	H 6.83	N 7.60	Gd 17.06

14.6 g (= 16 mmol) of the gadolinium(III) complex so obtained are suspended in 200 ml of water, and 31.4 l of 1N sodium hydroxide solution are added dropwise thereto. After 1 hour a clear solution is obtained which is filtered and then concentrated in vacuo. After drying in vacuo at 80°C 13.2 g (87 % of the theoretical yield) of a readily water-soluble, white powder of melting point 279-285°C are obtained.

15 Analysis:

	(calculated)	C 45.13	H 6.31	N 7.31	Gd 16.41	Na 4.80
	(found)	C 45.20	H 6.12	N 7.28	Gd 16.26	Na 4.75

In analogous manner there is obtained, using N-methylglucamine in place of sodium hydroxide solution,
the di-N-methylglucamine salt of the gadolinium(III)

complex of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,
15,18,21,24-pentaazapentatriacontanoic diacid,
 $C_{50}H_{96}GdN_7O_{22}$.

Example 2

- 5 Preparation of a solution of the disodium salt of the
gadolinium(III) complex of 13,23-dioxo-15,18,21-tris-
(carboxymethyl)-12,15,18,21,24-pentaazapentatria-
contanoic diacid
-

- 392.0 g (= 400 mmol) of the salt described in
10 Example 1 are made into a paste in 500 ml of water
p.i. (pro injectione) and dissolved by making up
the volume to 1000 ml with water p.i. while heating
slightly. The solution is placed in bottles and
heat-sterilised.

15 Example 3

Preparation of a solution of the di-N-methylglucamine
salt of the gadolinium(III) complex of 13,23-dioxo-15,
18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapenta-
triacontanoic diacid

- 20 130.4 g (= 100 mmol) of the di-N-methylglucamine
complex salt listed in Example 1 are made into a paste

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in 250 ml of water p.i. and dissolved while heating. The solution is made up to 500 ml with water p.i., introduced into ampoules and heat-sterilised.

Example 4

- 5 Preparation of a solution of the gadolinium(III) complex of the conjugate of diethylenetriaminepentaacetic acid with human serum albumen
-

- 10 10 mg of 1,5-bis-(2,6-dioxomorpholino)-3-azapentane-3-acetic acid (that is the anhydride of diethylenetriaminepentaacetic acid) are added to 20 ml of a solution of 3 mg of the protein in a 0.05 molar sodium bicarbonate buffer (pH 7-8). The whole is stirred for 30 minutes at room temperature and is then dialysed against a 0.3 molar sodium phosphate buffer.
- 15 50 mg of gadolinium(III) acetate are then added and purification is effected by gel chromatography over a Sephadex G25 column; Sephadex is a Registered Trade Mark. The fraction obtained is sterile-filtered and placed in multi-dose phials. Freeze-drying produces a
- 20 dry preparation that can be stored.

In an analogous manner, there is obtained with immunoglobulin a solution of the corresponding complex conjugate.

Example 5

Preparation of a solution of the gadolinium(III) complex of the conjugate of diethylenetriaminepentaacetic acid (DTPA) with a monoclonal antibody

5 1 mg of a mixed DTPA anhydride (obtained, for example, from DTPA and isobutyl chloroformate) is added to 20 μ l of a solution of 0.3 mg of a monoclonal antibody in a 0.05 molar sodium bicarbonate buffer (pH 7-8) and the whole is stirred for 30 minutes at room
10 temperature. Dialysis is carried out against a 0.3 molar sodium phosphate buffer, and 2 mg of the gadolinium(III) complex of ethylenediaminetetraacetic acid (EDTA) are added to the antibody fraction
15 obtained. After purification by gel chromatography over Sephadex G25, the sterile-filtered solution is placed in multi-dose phials and freeze-dried.

 Using the mixed anhydride of trans-1,2-diaminocyclohexanetetraacetic acid (CDTA) there is obtained, in analogous manner, a solution of the corresponding
20 gadolinium(III) complex of the CDTA antibody.

 Using the manganese(II) complex of ethylenediaminetetraacetic acid there is obtained in an analogous manner the manganese(II) complexes of the antibodies coupled with DTPA or CDTA.

Example 6

Preparation of a solution of the gadolinium(III) complex of the conjugate of 1-phenyl-ethylenediamine-tetraacetic acid with immunoglobulin

5 According to the procedure described in J. Med. Chem. 1974, vol. 17, p. 1307, a 2 % solution of the protein in a 0.12 molar sodium bicarbonate solution containing 0.01 mol of ethylenediaminetetraacetic acid is cooled to +4°C and there is added dropwise the
10 proportion, equivalent to the protein, of a freshly prepared ice-cold diazonium salt solution of 1-(p-aminophenyl)-ethylenediaminetetraacetic acid. The whole is stirred overnight (pH 8.1) at +4°C and is then dialysed against a 0.1 molar sodium citrate
15 solution. When dialysis is complete, an excess of gadolinium(III) chloride is added to the solution of the conjugate and ultra-filtration is carried out to remove ions. Finally, the sterile-filtered solution is placed in multi-dose phials and freeze-dried.

Example 7

Preparation of a colloidal dispersion of a Mn(II)-CDTA-lipid conjugate

0.1 mmol of distearoylphosphatidylethanolamine
5 and 0.1 mmol of the bisanhydride of trans-1,2-diamino-
cyclohexanetetraacetic acid in 50 ml of water are
stirred at room temperature for 24 hours. 0.1 mmol of
manganese(II) carbonate is added and stirring is again
carried out at room temperature for 6 hours. After
10 purification over a Sephadex G50 column, the sterile-
filtered solution is placed in multi-dose phials and
freeze-dried.

A colloidal dispersion of the gadolinium-DTPA-
lipid conjugate can be obtained analogously with
15 gadolinium(III) oxide.

Example 8

Preparation of liposomes charged with gadolinium(III)-
DTPA

According to the procedure described in Proc.
20 Natl. Acad. Sci. U.S.A. 75, 4194, a lipid mixture
comprising 75 mol % of egg phosphatidylcholine and
25 mol % of cholesterol is prepared as a dry substance.

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500 mg thereof are dissolved in 30 ml of diethyl ether and, in an ultrasonic bath, 3 ml of a 0.1 molar solution of the di-N-methylglucamine salt of the gadolinium(III) complex of diethylenetriaminepenta-
5 acetic acid in water p.i. are added dropwise thereto. When the addition of the solution is complete, the exposure to ultrasonic waves is continued for 10 minutes and then concentration is carried out in a rotary evaporator. The gel-like residue is suspended
10 in a 0.125 molar sodium chloride solution and, at 0°C, repeatedly freed of non-encapsulated contrast agent portions by centrifugation (20000 g/29 minutes). Finally, the liposomes so obtained are freeze-dried in multi-dose phials. The preparation is administered as
15 a colloidal dispersion in a 0.9 % by weight sodium chloride solution.

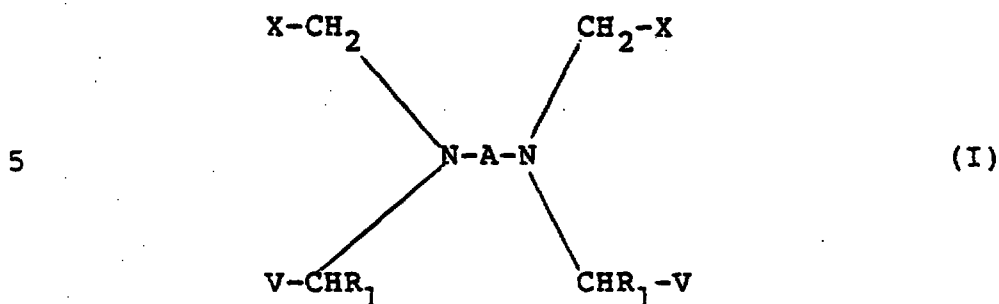


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Claims

1. A diagnostic preparation which comprises
(i) a physiologically tolerable, non-radioactive
complex salt of the general formula I



in which

10 X represents the radicals -COOY , $\text{-PO}_3\text{HY}$ or -CONHOY
wherein each Y represents a hydrogen atom, a metal
equivalent, the metal being selected from elements
having atomic numbers of from 21 to 29, 42, 44 and
from 58 to 70, or a physiologically tolerable
cation of an inorganic or organic base or amino
acid,

15 R_1 represents in each case a hydrogen atom or a
methyl group

and in which either

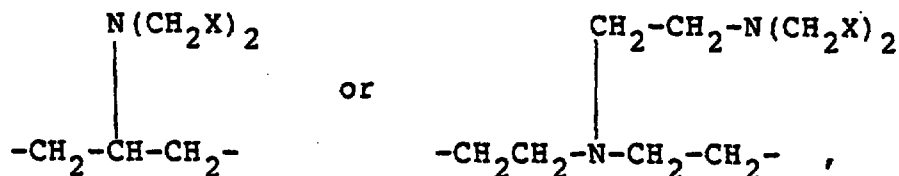


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a) A represents the group $-\text{CHR}_2-\text{CHR}_3-$,

$-\text{CH}_2-\text{CH}_2-(\text{ZCH}_2-\text{CH}_2)_{\underline{m}}-$,



in which

5

X has the meanings given above,

R_2 and R_3 together represent a trimethylene group
or a tetramethylene group,

or each represent a hydrogen atom,
an alkyl radical containing up to 6
carbon atoms, a phenyl radical
or a benzyl radical,

10

$\underline{m}$ represents the integer 1, 2 or 3,

and

Z represents an oxygen atom or a sulphur
atom or the group

15



in which

X has the meanings given above
and

20

R_4 represents an alkyl radical
containing up to 6 carbon atoms,

and



45

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each V has the same meaning as X or represents the group $-\text{CH}_2\text{OH}$ or $-\text{COB}$,

in which

B represents a protein or lipid radical or an
5 $-\text{NH}(\text{CH}_2)_n\text{X}$ radical,

in which

X has the meanings given above
and

n represents an integer from 1 to 12,
10 with the proviso that at least one of the symbols V represents a $-\text{COB}$ group, as defined above, and at least two of the substituents Y are metal equivalents selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58
15 to 70,

or

b) A represents the group $-\text{CHR}_2-\text{CHR}_3-$

in which

R_2 represents a hydrogen atom
20 and

R_3 represents the group $-(\text{CH}_2)_p-\text{C}_6\text{H}_4-\text{W}-\text{protein}$

in which

p represents 0 or 1,

W represents $-\text{NN}-$, $-\text{NHCOCH}_2-$

25 or $-\text{NHCS}-$

and

$-\text{protein}$ represents a protein radical,



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and

each V has the same meaning as X or represents the group $-\text{CH}_2\text{OH}$,

with the proviso that at least two of the substituents

5 Y are metal equivalents selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58 to 70,

and

(ii) a physiologically tolerable carrier.

10 2. A diagnostic preparation as claimed in claim 1, wherein the carrier (ii) is an aqueous carrier.

3. A diagnostic preparation as claimed in claim 2, wherein the carrier (ii) is water or physiological salt solution, and the complex salt (i) is dissolved or
15 suspended in it.

4. A diagnostic preparation as claimed in claim 2 or claim 3, wherein the complex salt (i) is present in a concentration of from 1 μmol to 1 mol per litre.

5. A diagnostic preparation as claimed in any one of
20 claims 1 to 4, wherein the complex salt (i) is derived from diethylenetriaminepentaacetic acid.

6. A diagnostic preparation as claimed in any one of



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claims 1 to 4, wherein the complex salt (i) is derived from ethylenediaminetetraacetic acid.

7. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is derived
5 from trans-1,2-cyclohexylenediaminetetraacetic acid or 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid.

8. A diagnostic preparation as claimed in any one of claims 1 to 7, wherein the complex salt (i) is derived
10 from a complex-forming acid linked as a conjugate with a biomolecule.

9. A diagnostic preparation as claimed in claim 8, wherein the biomolecule is a lipid.

10. A diagnostic preparation as claimed in claim 8,
15 wherein the biomolecule is a protein.

11. A diagnostic preparation as claimed in claim 10, wherein the protein is insulin.

12. A diagnostic preparation as claimed in claim 10, wherein the protein is an albumen.

20 13. A diagnostic preparation as claimed in claim 10,



wherein the protein is a monoclonal antibody.

14. A diagnostic preparation as claimed in claim 13, wherein the monoclonal antibody is specific to tumour-associated antigens.
- 5 15. A diagnostic preparation as claimed in any one of claims 1 to 7, wherein the complex salt (i) is derived from a complex-forming acid forming a conjugate or inclusion compound with a liposome.
- 10 16. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the di-N-methylglucamine salt of the gadolinium(III) complex of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid.
- 15 17. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the gadolinium(III) complex of the conjugate of diethylenetriaminepentaacetic acid with immunoglobulin.
- 20 18. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the gadolinium(III) complex of the conjugate of diethylenetriaminepentaacetic acid with human serum albumen.



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19. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the gadolinium(III) complex of the conjugate of diethylenetriaminepentaacetic acid with a monoclonal antibody.
20. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the manganese(II) complex of the conjugate of trans-1,2-cyclohexylenediaminetetraacetic acid with a monoclonal antibody.
21. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the manganese(II) complex of a lipid conjugate of trans-1,2-cyclohexylenediaminetetraacetic acid.
22. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the gadolinium(III) complex of an inclusion compound of diethylenetriaminepentaacetic acid with a liposome.
23. A diagnostic preparation as claimed in any one of claims 1 to 4, wherein the complex salt (i) is the disodium salt of the gadolinium(III) complex of 13,23-dioxo-15,18,21-tris-(carboxymethyl)-12,15,18,21,24-pentaazapentatriacontanoic diacid.



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24. A diagnostic preparation as claimed in claim 1, substantially as described in any one of Examples 2 to 8 herein.

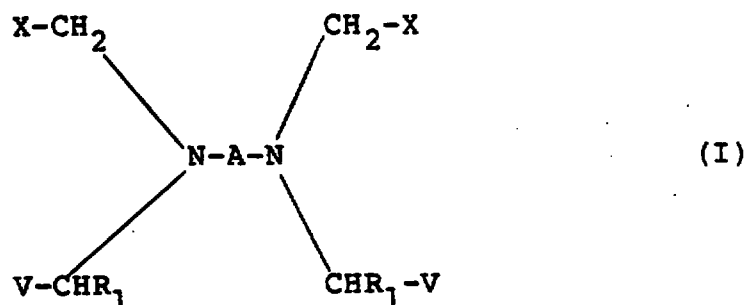
25. A diagnostic preparation as claimed in any one of
5 claims 1 to 23, which is in a dosage form suitable for administration orally, neurally or intravasally.

26. An ampoule containing a diagnostic preparation as claimed in any one of claims 1 to 23, in a form suitable for injection.

10 27. A process for the manufacture of a diagnostic preparation as claimed in any one of claims 1 to 23, wherein the complex salt (i) is dissolved or suspended in water or physiological salt solution, and is made up, if desired with the incorporation of one or more
15 physiologically tolerable adjuncts, in a form that is suitable for intravasal or oral administration.

28. A process for the manufacture of a diagnostic preparation as claimed in claim 1, conducted substantially as described in any one of Examples 2 to
20 8 herein.

29. A physiologically tolerable, non-radioactive complex salt of the general formula I



in which

X represents the radicals -COOY , $\text{-PO}_3\text{HY}$ or -CONHOY wherein each Y represents a hydrogen atom, a metal equivalent, the metal being selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58 to 70, or a physiologically tolerable cation of an inorganic or organic base or amino acid,

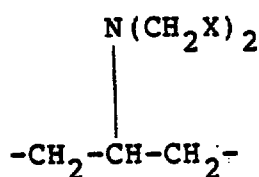
R_1 represents in each case a hydrogen atom or a methyl group

and in which either

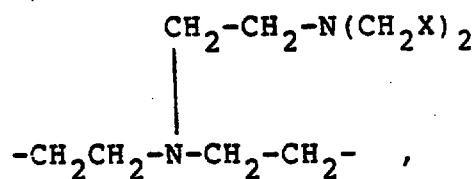
a) A represents the group $\text{-CHR}_2\text{-CHR}_3\text{-}$,



15



or





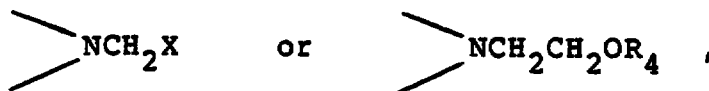
in which

X has the meanings given above,
R₂ and R₃ together represent a trimethylene group
or a tetramethylene group,
5 or each represent a hydrogen atom,
an alkyl radical containing up to 6
carbon atoms, a phenyl radical
or a benzyl radical,

m represents the integer 1, 2 or 3,

10 and

Z represents an oxygen atom or a sulphur
atom or the group



in which

15 X has the meanings given above
and

R₄ represents an alkyl radical
containing up to 6 carbon atoms,

and

20 each V has the same meaning as X or represents the
group -CH₂OH or -COB,

in which

B represents a protein or lipid radical or an
-NH(CH₂)_nX radical,



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in which

X has the meanings given above

and

n represents an integer from 1 to 12,

5 with the proviso that at least one of the symbols V represents a -COB group, as defined above, and at least two of the substituents Y are metal equivalents selected from elements having atomic numbers of from 21 to 29, 42, 44 and from 58 to 70,

10 or

b) A represents the group -CHR₂-CHR₃-

in which

R₂ represents a hydrogen atom

and

15 R₃ represents the group -(CH₂)_p-C₆H₄-W-protein

in which

p represents 0 or 1,

W represents -NN-, -NHCOCH₂-

or -NHCS-

20 and

-protein represents a protein radical,

and

each V has the same meaning as X or represents the group -CH₂OH,

25 with the proviso that at least two of the substituents Y are metal equivalents selected from elements having



atomic numbers of from 21 to 29, 42, 44 and from 58 to 70.

30. A physiologically tolerable complex salt as claimed in claim 29, which contains more than two of the radicals represented by X and in which at least one of the substituents Y is a physiologically tolerable cation of an organic base or amino acid.
31. Any one of the physiologically tolerable complex salts as claimed in claim 29, specified in any one of the Examples herein.
32. A process for the manufacture of a physiologically tolerable complex salt as claimed in any one of claims 29 to 31, substantially as described herein.
33. A physiologically tolerable complex salt as claimed in claim 29, for use in a method of NMR diagnosis of the human or animal body.
34. A physiologically tolerable complex salt as claimed in claim 30, for use in a method of NMR diagnosis of the human or animal body.
35. A physiologically tolerable complex salt as claimed in claim 31, for use in a method of NMR



55.

diagnosis of the human or animal body.

36. The use of a physiologically tolerable complex salt as claimed in claim 29, for the manufacture of a preparation for a method of NMR diagnosis of the human
5 or animal body.

37. The use of a physiologically tolerable complex salt as claimed in claim 30, for the manufacture of a preparation for a method of NMR diagnosis of the human or animal body.

10 38. The use of a physiologically tolerable complex salt as claimed in claim 31, for the manufacture of a preparation for a method of NMR diagnosis of the human or animal body.
