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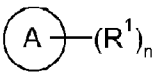
(54) Titre : COMPOSES DE CHELATES DE GADOLINIUM POUR UTILISATION DANS L'IMAGERIE PAR RESONANCE MAGNETIQUE

(54) Title: GADOLINIUM CHELATE COMPOUNDS FOR USE IN MAGNETIC RESONANCE IMAGING



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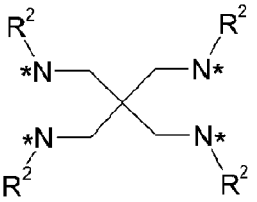

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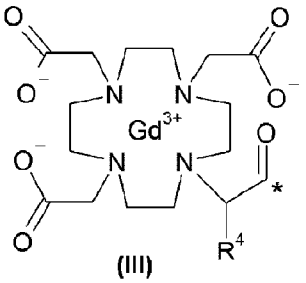

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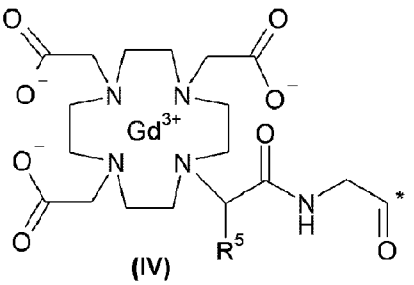

(IV)

(57) Abrégé/Abstract:
The present invention relates to high relaxivity extracellular gadolinium chelate complexes of general formula (I), (see formula I), in which:

A represents (see formula II) where * indicates the point of attachment of with R^1; R^1 represents R^3; n represents an integer of

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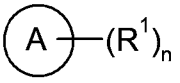
(57) **Abrégé(suite)/Abstract(continued)**:
4; R^2 represents a hydrogen atom; R^3 represents a group selected from:
(see formula III), and (see formula IV), where * indicates the point of attachment with the rest of the molecule; R^4 represents a hydrogen atom; R^5 represents a hydrogen atom or a methyl group; or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same. The present invention also relates to methods of preparing said compounds, and to the use of said compounds as MRI contrast agents.

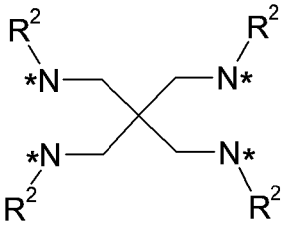
ABSTRACT

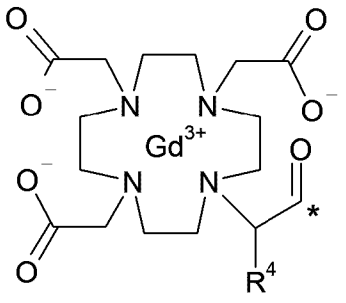
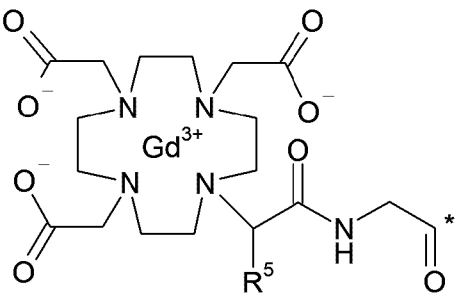
The present invention relates to high relaxivity extracellular gadolinium chelate complexes of general formula (**I**),



(**I**) ,

in which:

A represents  where * indicates the point of attachment of with R^1; R^1 represents R^3; n represents an integer of 4; R^2 represents a hydrogen atom; R^3 represents a group selected from:

 , and  ,

where * indicates the point of attachment with the rest of the molecule; R^4 represents a hydrogen atom; R^5 represents a hydrogen atom or a methyl group; or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same. The present invention also relates to methods of preparing said compounds, and to the use of said compounds as MRI contrast agents.

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GADOLINIUM CHELATE COMPOUNDS FOR USE IN MAGNETIC RESONANCE IMAGING

FIELD OF THE INVENTION

The present invention relates to the items characterized in the patent claims, namely to new high relaxivity extracellular gadolinium chelates based on low molecular weight core polyamines, to methods of preparing said compounds, to the use of said compounds as MRI contrast agents and to their use in a mammalian body.

BACKGROUND

1. Introduction

Nine gadolinium-based contrast agents (GBCAs) have been approved for clinical use: gadopentetate dimeglumine (Magnevist®), gadoterate meglumine (Dotarem®), gadoteridol (ProHance®), gadodiamide (Omniscan®), gadobutrol (Gadovist®), gadoversetamide (OptiMARK®), gadoxetic acid (Primovist®), gadobenate dimeglumine (MultiHance®) and gadofosveset trisodium (Vasovist®/Ablavar®). With the exception of gadoxetic acid, gadobenate dimeglumine and gadofosveset trisodium, the GBCAs exhibit a strictly extracellular passive distribution in the body and are excreted exclusively via the kidney.

Gadoxetic acid and gadobenate dimeglumine exhibit a different pharmacokinetic profile than the other agents. In addition to the extracellular distribution, they are taken up and are also excreted partially via the liver. This allows, besides the classical imaging possibilities (e.g. central nervous system, angiography, extremities, heart, head/face/neck, abdomen and breast imaging), also liver imaging due to the enhancement of liver parenchyma caused by the GBCAs uptake in hepatocytes.

In contrast to the other GBCAs gadofosveset trisodium shows no passive diffusion in the body and remains in the vascular space. The prolonged period in the blood vessels caused by the reversible binding to HSA (human serum albumin) allows high resolution MR angiographies.

The various GBCAs differ in their efficacy which is given by their longitudinal (r1) and transversal (r2) relaxivity and is dependent on magnetic field strengths, temperature and different intrinsic factors of the metal chelates. The intrinsic relaxivity influencing parameters are mainly the number of water molecules directly bound to the gadolinium (so-called inner-sphere water, q), the mean residence time of the inner sphere water molecules (τm), the number and residence times of water molecules in the second hydration sphere (so-called second sphere water) and the rotational diffusion (τr) (Helm L. et. al., Future Med Chem.

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2010; 2: 385-396). In terms of their relaxivity all the commercially available GBCAs are very similar to each other and derived from a range of 4 to 7 L $mmol^{-1}s^{-1}$.

Strategies for increasing the sensitivity of GBCAs are frequently described in the literature (Caravan P. et. al. Chem. Soc. Rev., 2006, 35, 512-523, Helm et.al. Future Med Chem. 2010; 2:385-396, Jacques V. Invest Radiol. 2010;45:613-624). One of the strategies is the increase of the inner sphere water molecules (q) that are water molecules which are directly coordinated to the gadolinium ion in the chelate. As the examples of AAZTA and HOPO-based ligands show, the increase of the inner sphere water molecules from one to two leads to a significant increase in relaxivity. Another strategy to increase the relaxivity is the slowing of the rotational diffusion of the molecule. The so-called tumbling rate (τr, see introduction) describes the tumbling of the molecule in solution and is mainly affected by the molecular size and protein binding of the GBCA (Merbach A.S. et. al., The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging, 2013, ISBN: 978-1-119-99176-2).

A further important characteristic of the GBCAs is their complex stability. The potential of the GBCAs to release free toxic Gd^{3+} ions is a major safety issue and of utmost importance in particular for patients with end-stage renal disease. Nephrogenic systemic fibrosis (NSF) is a rare and serious syndrome that is associated with the exposure to GBCAs in patients with severe kidney failure. NSF involves fibrotic changes in the skin and many organs. In 2010, the Food and Drug Administration (FDA) published revised labeling recommendations for four GBCAs which have been principally implicated in NSF, including gadodiamide (Omniscan®), gadobenate dimeglumine (MultiHance®), gadopentetate dimeglumine (Magnevist®) and gadoversetamide (OptiMARK®) (Yang L et. al. Radiology. 2012;265:248-253). At first glance the stability of all GBCAs is very high, but significant differences exist between the linear and macrocyclic agents and between the ionic and nonionic representatives of the linear agents. The macrocyclic GBCAs possess the highest complex stabilities (Frenzel T. et. al. Invest Radiol. 2008; 43:817-828). Due to the better awareness of risk patients, the use of lower doses and more widespread use of the macrocyclic GBCAs the incidence of NSF has decreased in the last years (Wang Y. et.al. Radiology. 2011;260:105-111 and Becker S. et.al. *Nephron Clin Pract.* 2012; 121:c91-c94).

The crucial issue for clinical applications is in vivo stability. The kinetic inertness combined with the thermodynamic stability is particularly with regard to the risk of nephrogenic systemic fibrosis (NSF) the best predictor of the in vivo toxicity of q=2 chelates (Merbach A.S. et. al., The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging, 2013, ISBN: 978-1-119-99176-2, page 157-208). The complexes with q=2 show two-fold enhancement of relaxivity but, unfortunately, they have a lower stability than q=1 compounds (Hermann P. et.al. Dalton Trans., 2008, 3027-3047).

2. Description of the Prior Art, Problem to be solved and its Solution

Several macrocyclic compounds are described in the prior art.

EP1931673 B1 and EP2457914 B1 relate to pyDO3A (q=2), DO3A and DOTA compounds comprising short aminoalcohol chains and metal complexes for medical imaging.

Macrocyclic lanthanide DO3A- and DOTA-like GBCAs with high relaxivities are described in the prior art.

Ranganathan R.S. et.al.(Investigative Radiology 1998;33:779-797) investigated the effect of multimerization on the relaxivity of macrocyclic gadolinium chelates. WO199531444 relates to monomeric and multimeric compounds having enhanced relaxivities.

US 5679810 relates to linear oligomer polychelant compounds and chelates formed therewith, having alternating chelant and linker moieties bound together by amide or ester moieties, and to their use in diagnostic imaging.

US 5650133 relates to dichelants, in particular compounds having two macrocyclic chelant groups linked by a bridge containing an ester or amide bond, and to metal chelates thereof, and to their use in diagnostic imaging.

WO 97/32862 A1 describes gadolinium polychelants as magnetic resonance imaging agents which are linking at least two units of chelant to the amino groups of a target carrier structure (like e.g. a protein, aminoacid or peptide).

US 2007/202047 relates to gadolinium chelate compounds for use in magnetic resonance imaging, which are derived from a chelating molecule selected from 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (DOTA) and diethylentriaminepentaacetic acid (DTPA), wherein at least one of the carboxylic groups of the chelating molecule is reacted with an amine.

GBCAs with higher relaxivity offer on the one hand the opportunity of a significant dose reduction and on the other an increased sensitivity in the MRI examination of many diseases using the standard dose (Giesel FL. et.al. Eur Radiol 2010, 20: 2461–2474).

However, there is an unmet medical need to provide GBCAs for general use in magnetic resonance imaging, which:

- exhibit high relaxivity,
- show a favorable pharmacokinetic profile,
- are completely excreted,
- are chemically stable,
- exhibit high water solubility,
- offer the potential for a significant dose reduction,
- are suitable for imaging of different body regions, and
- are very well-tolerated.

The state of the art described above does not describe the specific high relaxivity extracellular gadolinium chelate compounds of general formula (I) of the present invention as defined herein, or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same, as described and defined herein, and as hereinafter referred to as "compounds of the present invention".

It has now been found, and this constitutes the basis of the present invention, that said compounds of the present invention have surprisingly and advantageous properties.

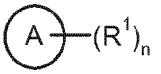
In particular, said compounds of the present invention have been found to exhibit a balanced profile of a high relaxivity, a favorable pharmacokinetic profile, a complete excretion, a high stability, a high solubility, the potential for a significant dose reduction and the potential for whole body imaging, and they may therefore be used as contrast agents for magnetic resonance imaging (MRI).

SUMMARY

The present invention describes a new class of high relaxivity extracellular gadolinium chelate complexes, methods for their preparation and their use as MRI contrast agents.

DESCRIPTION of the INVENTION

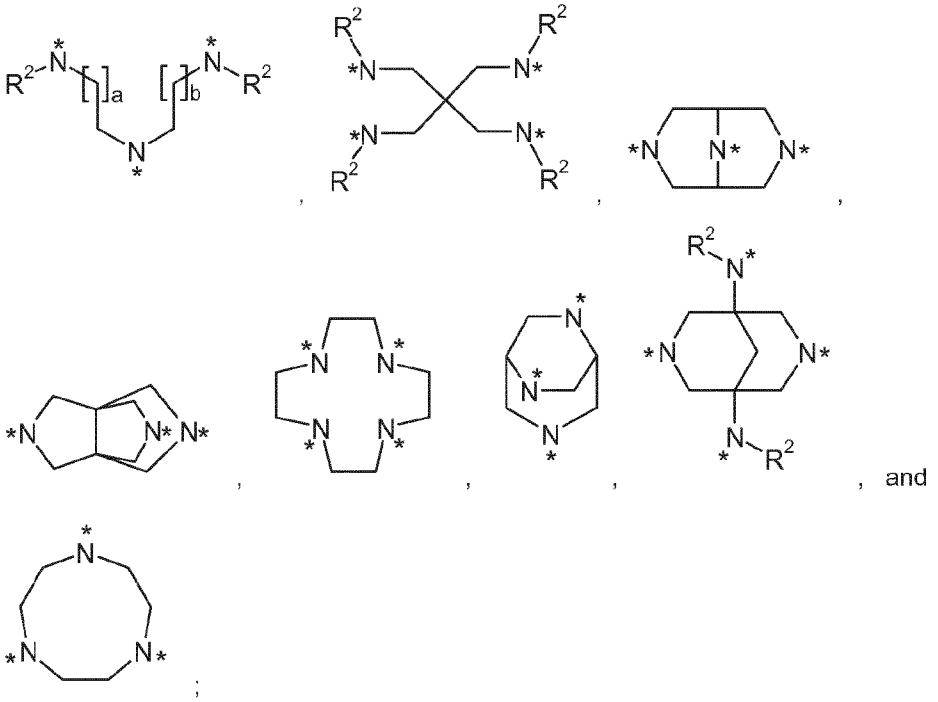
In accordance with a first aspect, the present invention covers compounds of general formula (I), comprising 4, 5, 6, 7 or 8 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups,



(I),

in which :

A represents a group selected from:



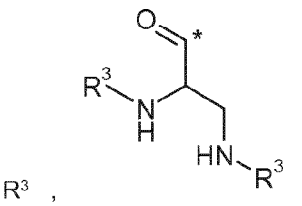
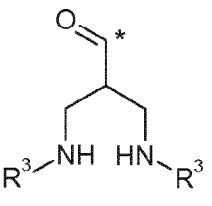
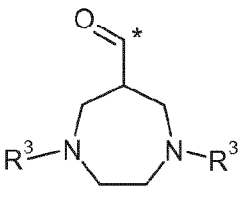
;

in which groups a and b represent, independently from each other, an integer of 1 or 2 ;

and,

in which groups * indicates the point of attachment of said group with R^1 ;

R^1 represents, independently from each other, a hydrogen atom or a group selected from :

R^3 , , , and ,

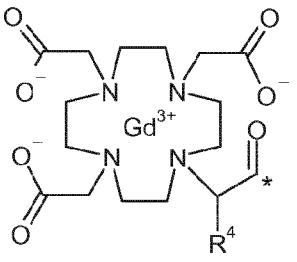
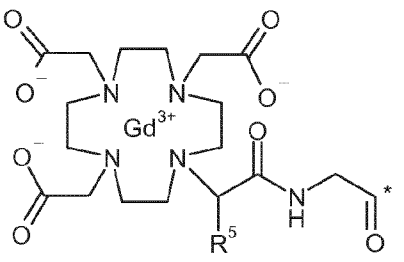
in which groups * indicates the point of attachment of said group with A ,

with the proviso that only one of the substituents R^1 may represent a hydrogen atom ;

n represents an integer of 3 or 4 ;

R^2 represents, independently from each other, a hydrogen atom or a methyl group ;

R^3 represents a group selected from :

, and ;

in which groups * indicates the point of attachment of said group with the rest of the molecule ;

R^4 represents, independently from each other, a hydrogen atom or a methyl group ;

R^5 represents, independently from each other, a hydrogen atom or a methyl group ;

or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

The compounds of this invention may contain one or more asymmetric centre, depending upon the location and nature of the various substituents desired. Asymmetric carbon atoms may be present in the (R) or (S) configuration, which can result in racemic mixtures in the case of a single asymmetric centre, and in diastereomeric mixtures in the case of multiple

asymmetric centres. In certain instances, asymmetry may also be present due to restricted rotation about a given bond, for example, the central bond adjoining two substituted aromatic rings of the specified compounds.

Preferred compounds are those which produce the more desirable biological activity. Separated, pure or partially purified isomers and stereoisomers or racemic or diastereomeric mixtures of the compounds of this invention are also included within the scope of the present invention. The purification and the separation of such materials can be accomplished by standard techniques known in the art.

The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example, by the formation of diastereoisomeric salts using an optically active acid or base or formation of covalent diastereomers. Examples of appropriate acids are tartaric, diacetyltartaric, ditoluoyltartaric and camphorsulfonic acid. Mixtures of diastereoisomers can be separated into their individual diastereomers on the basis of their physical and/or chemical differences by methods known in the art, for example, by chromatography or fractional crystallisation. The optically active bases or acids are then liberated from the separated diastereomeric salts. A different process for separation of optical isomers involves the use of chiral chromatography (*e.g.*, chiral HPLC columns), with or without conventional derivatisation, optimally chosen to maximise the separation of the enantiomers. Suitable chiral HPLC columns are manufactured by Daicel, *e.g.*, Chiracel OD and Chiracel OJ among many others, all routinely selectable. Enzymatic separations, with or without derivatisation, are also useful. The optically active compounds of this invention can likewise be obtained by chiral syntheses utilizing optically active starting materials.

In order to limit different types of isomers from each other reference is made to IUPAC Rules Section E (Pure Appl Chem 45, 11-30, 1976).

The present invention includes all possible stereoisomers of the compounds of the present invention as single stereoisomers, or as any mixture of said stereoisomers, *e.g.* R- or S-isomers, or E- or Z-isomers, in any ratio. Isolation of a single stereoisomer, *e.g.* a single enantiomer or a single diastereomer, of a compound of the present invention may be achieved by any suitable state of the art method, such as chromatography, especially chiral chromatography, for example.

Further, the compounds of the present invention can exist as N-oxides, which are defined in that at least one nitrogen of the compounds of the present invention is oxidised. The present invention includes all such possible N-oxides.

The present invention also relates to useful forms of the compounds as disclosed herein, such as metabolites, hydrates, solvates, salts, in particular pharmaceutically acceptable salts, and co-precipitates.

The compounds of the present invention can exist as a hydrate, or as a solvate, wherein the compounds of the present invention contain polar solvents, in particular water, methanol or ethanol for example as structural element of the crystal lattice of the compounds. The amount of polar solvents, in particular water, may exist in a stoichiometric or non-stoichiometric ratio. In the case of stoichiometric solvates, *e.g.* a hydrate, hemi-, (semi-), mono-, sesqui-, di-, tri-, tetra-, penta- *etc.* solvates or hydrates, respectively, are possible. The present invention includes all such hydrates or solvates.

Further, the compounds of the present invention can exist in the form of a salt. Said salt may be either an inorganic or organic addition salt, particularly any pharmaceutically acceptable inorganic or organic addition salt, customarily used in pharmacy.

The term "pharmaceutically acceptable salt" refers to a relatively non-toxic, inorganic or organic acid addition salt of a compound of the present invention. For example, see S. M. Berge, *et al.* "Pharmaceutical Salts," J. Pharm. Sci. **1977**, 66, 1-19. The production of especially neutral salts is described in US 5,560,903.

Pharmaceutically acceptable salts of the compounds according to the invention include salts of mineral acids and carboxylic acids, for example, without being limited thereto, salts of hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid, aspartic acid and glutamic acid.

Those skilled in the art will further recognise that acid addition salts of the claimed compounds may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods.

The present invention includes all possible salts of the compounds of the present invention as single salts, or as any mixture of said salts, in any ratio.

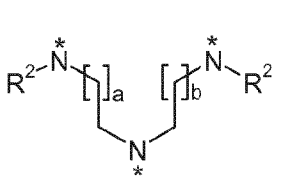
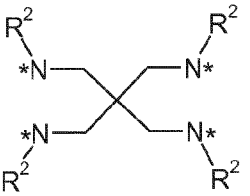
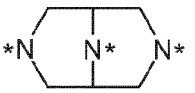
In the present text, in particular in the Experimental Section, for the synthesis of intermediates and of examples of the present invention, when a compound is mentioned as a salt form with the corresponding base or acid, the exact stoichiometric composition of said

salt form, as obtained by the respective preparation and/or purification process, is, in most cases, unknown.

This applies analogously to cases in which synthesis intermediates or example compounds or salts thereof have been obtained, by the preparation and/or purification processes described, as solvates, such as hydrates with (if defined) unknown stoichiometric composition.

In accordance with a second embodiment of the first aspect, the present invention covers compounds of general formula (I), *supra*, comprising 4, 5 or 6, gadolinium [4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups, wherein :

(A) represents a group selected from:


, 
, 
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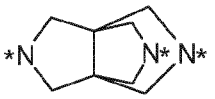
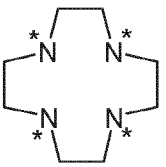
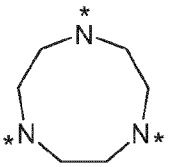

, 
, and 
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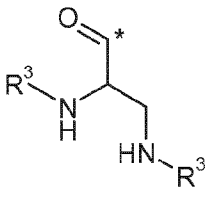
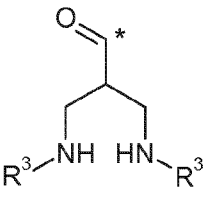
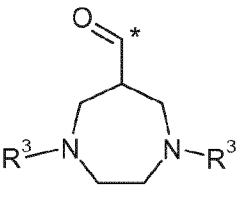
in which groups a and b represent, independently from each other, an integer of 1 or 2 ;

and ,

in which groups * indicates the point of attachment of said group with R^1 ;

R^1 represents, independently from each other, a hydrogen atom or a group selected from :

R3 ,  ,  , and  ,

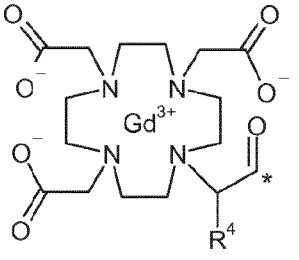
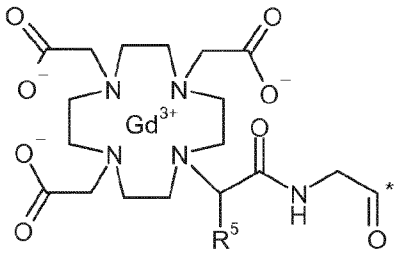
in which groups * indicates the point of attachment of said group with A ,

with the proviso that only one of the substituents R^1 may represent a hydrogen atom ;

n represents an integer of 3 or 4 ;

R^2 represents, independently from each other, a hydrogen atom or a methyl group ;

R^3 represents a group selected from :

 , and  ;

in which groups * indicates the point of attachment of said group with the rest of the molecule ;

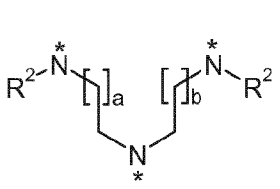
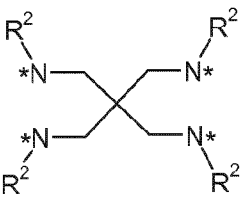
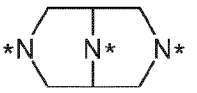
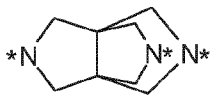
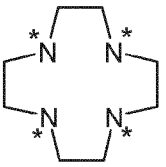
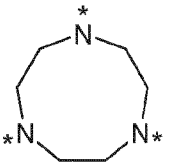
R^4 represents, independently from each other, a hydrogen atom or a methyl group ;

R^5 represents, independently from each other, a hydrogen atom or a methyl group ;

or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

In accordance with a third embodiment of the first aspect, the present invention covers compounds of general formula (I), *supra*, comprising 4, 5 or 6, gadolinium [4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups, wherein :

(A) represents a group selected from:

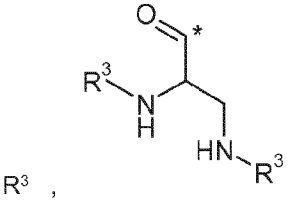
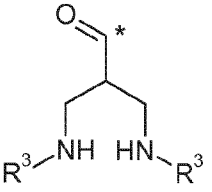
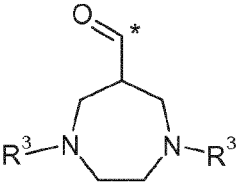







in which groups a and b represent an integer of 1 ;

and ,

in which groups * indicates the point of attachment of said group with R^1 ;

R^1 represents, independently from each other, a hydrogen atom or a group selected from :

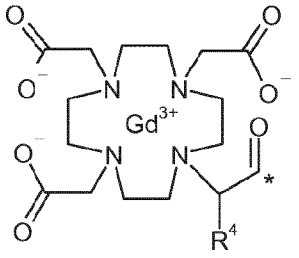
in which groups * indicates the point of attachment of said group with A ,

with the proviso that only one of the substituents R^1 may represent a hydrogen atom ;

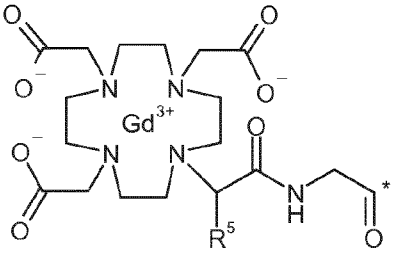
n represents an integer of 3 or 4 ;

R^2 represents a hydrogen atom ;

R^3 represents a group selected from :



, and



;

in which groups * indicates the point of attachment of said group with the rest of the molecule ;

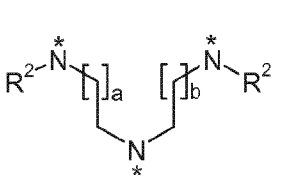
R^4 represents a hydrogen atom ;

R^5 represents a hydrogen atom or a methyl group ;

or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

In accordance with a fourth embodiment of the first aspect, the present invention covers compounds of general formula (I), *supra*, comprising 4, 5 or 6, gadolinium [4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups, wherein :

(A) represents a group selected from:



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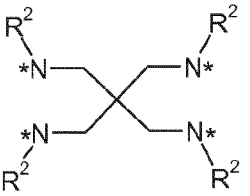

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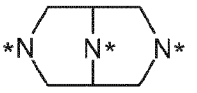

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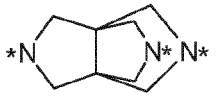

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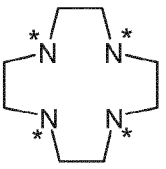
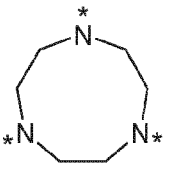


, and



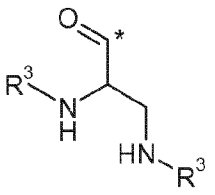
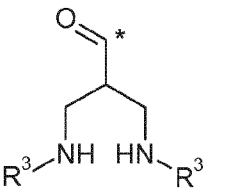
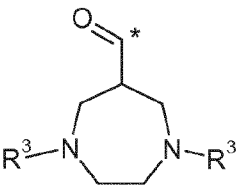
;

in which groups a and b represent an integer of 1 ;

and ,

in which groups * indicates the point of attachment of said group with R^1 ;

R^1 represents, independently from each other, a hydrogen atom or a group selected from :


, 
, and 
,

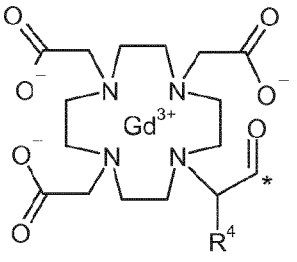
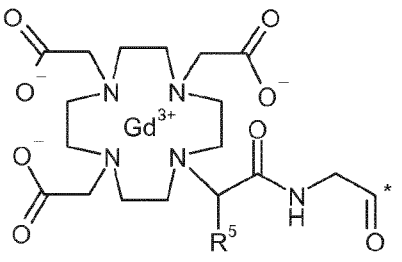
in which groups * indicates the point of attachment of said group with A ,

with the proviso that only one of the substituents R^1 may represent a hydrogen atom ;

n represents an integer of 3 or 4 ;

R^2 represents a hydrogen atom ;

R^3 represents a group selected from :


, and 
;

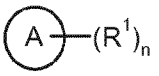
in which groups * indicates the point of attachment of said group with the rest of the molecule ;

R^4 represents a hydrogen atom ;

R^5 represents a methyl group ;

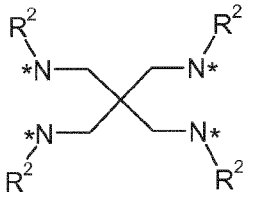
or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

In accordance with another aspect, the present invention covers compounds of general formula (I),



(I),

in which :

represents a  group,

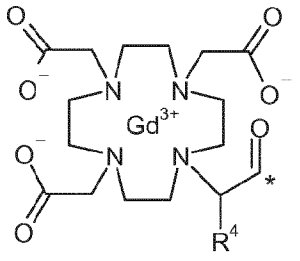
in which group * indicates the point of attachment of said group with R^1 ;

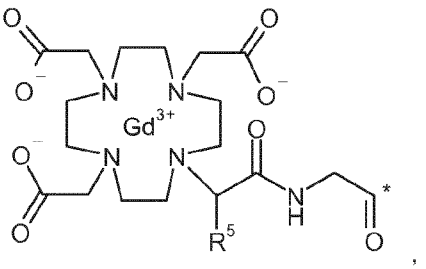
R^1 represents a group R^3 ;

n represents an integer of 4 ;

R^2 represents a hydrogen atom ;

R^3 represents a group selected from :



, and  ,

in which groups * indicates the point of attachment of said group with the rest of the molecule ;

R^4 represents a hydrogen atom ;

R^5 represents a hydrogen atom or a methyl group ;

or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 4, 5, 6, 7 or 8 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 4, 5 or 6 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 4 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 5 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl] groups.

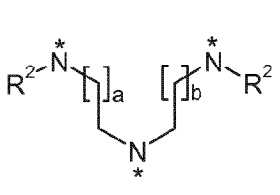
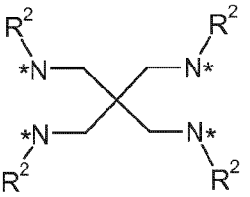
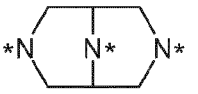
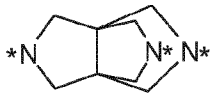
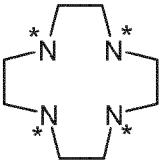
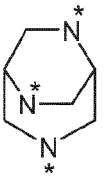
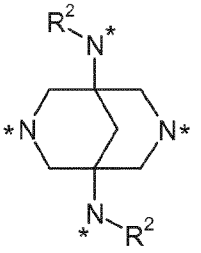
In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 6 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 7 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), comprising 8 gadolinium [4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl] groups.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

represents a group selected from: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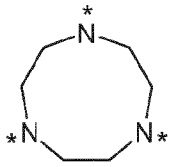

;

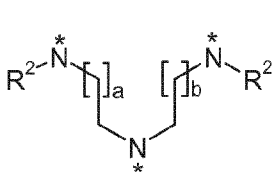
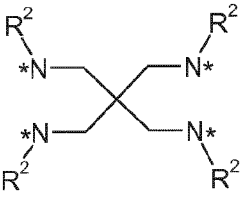
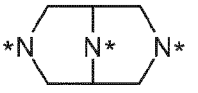
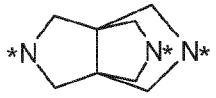
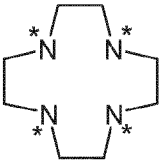
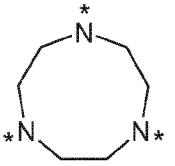
in which groups a and b represent, independently from each other, an integer of 1 or 2 ;

and,

in which groups * indicates the point of attachment of said group with R^1 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

(A) represents a group selected from:






and

;

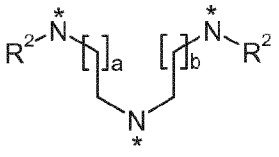
in which groups a and b represent, independently from each other, an integer of 1 or 2 ;

and ,

in which groups * indicates the point of attachment of said group with R^1 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

(A) represents a group selected from: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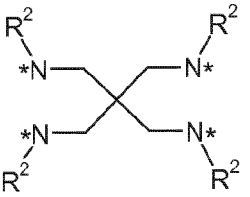
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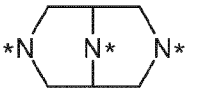
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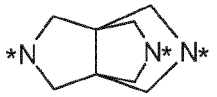
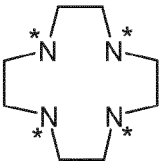
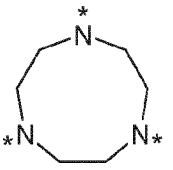
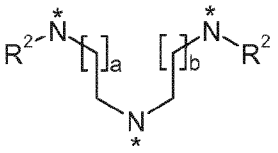

,

, and

;

in which groups a and b represent an integer of 1 ;

and ,

in which groups * indicates the point of attachment of said group with R^1 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

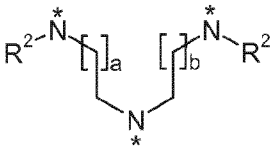
(A) represents a  group ,

in which groups a and b represent, independently from each other, an integer of 1 or 2 ;

and ,

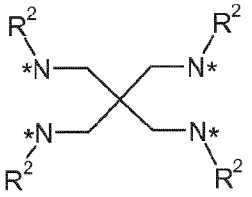
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

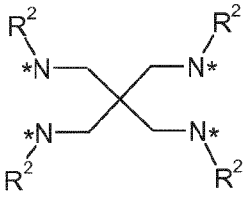
in which groups a and b represent an integer of 1 ;
and ,
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

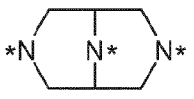
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

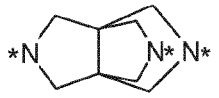
in which group * indicates the point of attachment of said group with R^1,
and
R^2 represents a hydrogen atom.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

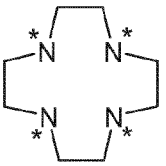
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

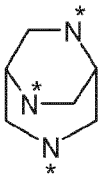
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

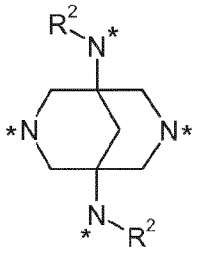
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

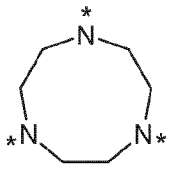
in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

in which group * indicates the point of attachment of said group with R^1.

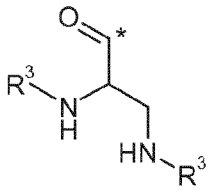
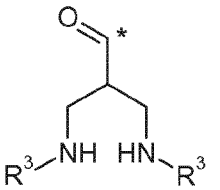
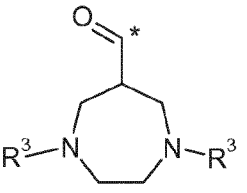
In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

A represents a  group ,

in which group * indicates the point of attachment of said group with R^1.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

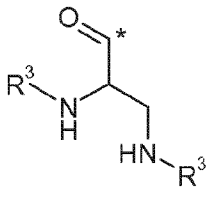
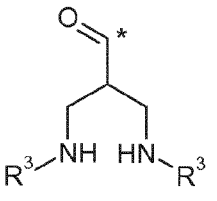
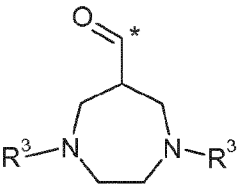
R^1 represents, independently from each other, a hydrogen atom or a group selected from :

R^3 ,  ,  , and  ,

in which groups * indicates the point of attachment of said group with A ,
with the proviso that only one of the substituents R^1 may represent a hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

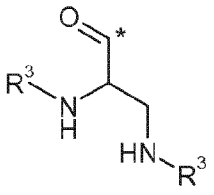
R^1 represents, independently from each other a group selected from :


, 
, and 
,

in which groups * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

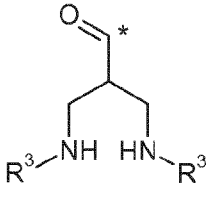
R^1 represents, independently from each other a group selected from :


, and
,

in which groups * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

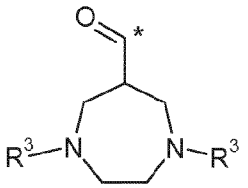
R^1 represents, independently from each other a group selected from :


, and
,

in which group * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^1 represents, independently from each other a group selected from :



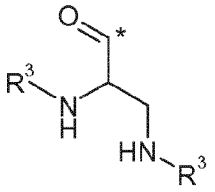
R^3 , and ,

in which group * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

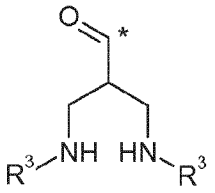
R^1 represents a group R^3 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^1 represents a  group ,

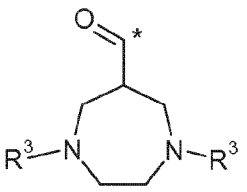
in which group * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^1 represents a  group ,

in which group * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

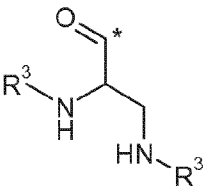
R^1 represents a  group ,

in which group * indicates the point of attachment of said group with A .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

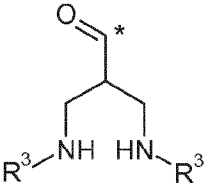
R^1 represents, independently from each other, a hydrogen atom or a R^3 group ,
with the proviso that only one of the substituents R^1 may represent a hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :



R^1 represents, independently from each other, a hydrogen atom or a group
in which group * indicates the point of attachment of said group with A ,
with the proviso that only one of the substituents R^1 may represent a hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

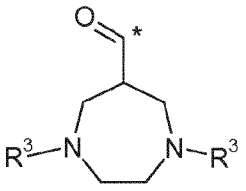


R^1 represents, independently from each other, a hydrogen atom or a group,

in which group * indicates the point of attachment of said group with A ,
with the proviso that only one of the substituents R^1 may represent a hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^1 represents, independently from each other, a hydrogen atom or a



group ,

in which group * indicates the point of attachment of said group with A ,
with the proviso that only one of the substituents R^1 may represent a hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

n represents an integer of 3 or 4 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

n represents an integer of 3 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

n represents an integer of 4 .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^2 represents, independently from each other, a hydrogen atom or a methyl group .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

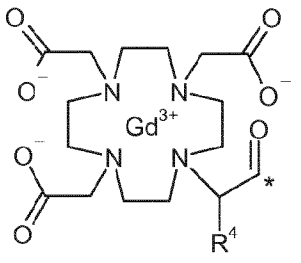
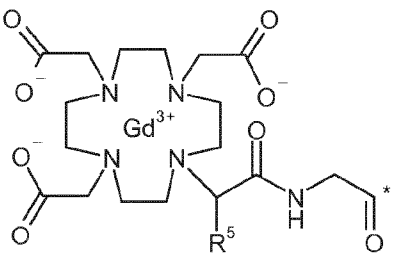
R^2 represents a hydrogen atom.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^2 represents a methyl group.

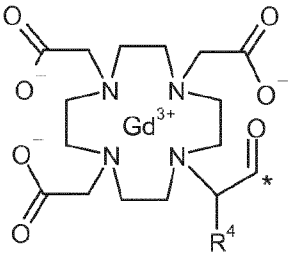
In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a group selected from :


, and

;

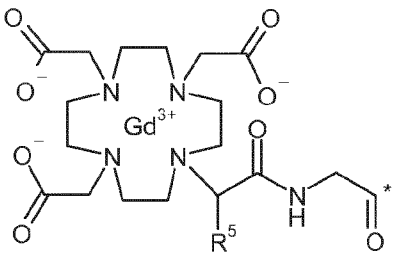
in which groups * indicates the point of attachment of said group with the rest of the molecule.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a  group ,

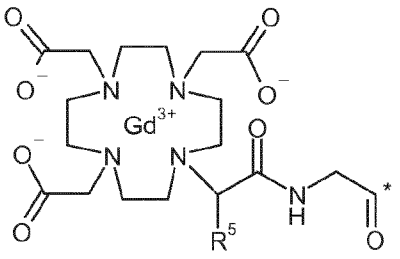
in which group * indicates the point of attachment of said group with the rest of the molecule.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a  group ;

in which group * indicates the point of attachment of said group with the rest of the molecule.

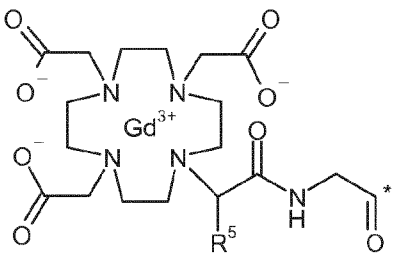
In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a  group ;

in which group * indicates the point of attachment of said group with the rest of the molecule ; and

R^5 represents a hydrogen atom or a methyl group.

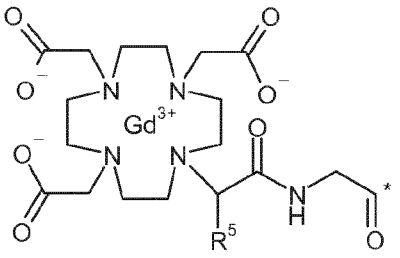
In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a  group ;

in which group * indicates the point of attachment of said group with the rest of the molecule ; and

R^5 represents a hydrogen atom.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^3 represents a  group ;

in which group * indicates the point of attachment of said group with the rest of the molecule ; and

R^5 represents a methyl group.

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^4 represents, independently from each other, a hydrogen atom or a methyl group .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^4 represents hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^4 represents a methyl group .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^5 represents, independently from each other, a hydrogen atom or a methyl group .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^5 represents hydrogen atom .

In a further embodiment of the above-mentioned aspect, the invention relates to compounds of formula (I), wherein :

R^5 represents a methyl group .

It is to be understood that the present invention relates also to any combination of the embodiments described above.

Another embodiment of the first aspect are compounds of formula (I) selected from the group consisting of:

Pentagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,18,22,25-hexaoxo-26-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-14-[({2-[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-9,19-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14,17,21,24-heptaazaheptacosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]-acetate ,

Hexagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,15,19,22-hexaoxo-23-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,16-bis({[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-11-(2-{[3-{[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoyl}amino)acetyl]amino}-2-({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraaza-cyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)propanoyl]amino}ethyl)-4,7,11,14,18,21-hexaazatetracosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate ,

Tetragadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate ,

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate ,

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate ,

Pentagadolinium [4-(1-{[2-(bis{2-[({1,4-bis[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl]propanoyl}amino)acetyl]-1,4-diazepan-6-yl}carbonyl)amino]ethyl}-amino)-2-oxoethyl]amino}-1-oxopropan-2-yl)-7,10-bis(carboxylatomethyl)-1,4,7,10-tetra-azacyclododecan-1-yl]acetate ,

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2"""",2""""',2"""""',2"""""",2""""""',2"""""""',2""""""""',2"""""""",2""""""""', 2"""""""",2""""""""',2"""""""""'-{ethane-1,2-diylcarbamoyl-1,4-diazepane-6,1,4-triyltris[(2-oxo-ethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetra-yl]}octadecaacetate ,

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2"""",2""""',2"""""',2"""""",2""""""',2"""""""',2""""""""',2"""""""",2""""""""', 2"""""""",2""""""""',2"""""""""'-(1,4,7-triazonane-1,4,7-triyltris{carbonyl-1,4-diazepane-6,1,4-triyl-bis[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})octadecaacetate ,

Tetragadolinium 2,2',2",2"',2"",2""',2"""',2"""",2""""',2"""""',2"""""",2""""""',2"""""""',2""""""""'-{1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetrayltetrakis[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}dodecaacetate ,

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2"""",2""""',2"""""',2"""""",2""""""',2"""""""',2""""""""',2"""""""",2""""""""', 2"""""""",2""""""""',2"""""""""'-{3,7,10-triazatricyclo[3.3.3.$0^{1,5}$]undecane-3,7,10-triyltris[carbonyl-(3,6,11,14-tetraoxo-4,7,10,13-tetraazahexadecane-8,2,15-triyl)di-1,4,7,10-tetraazacyclodo-decane-10,1,4,7-tetrayl]}octadecaacetate ,

Tetragadolinium 2,2',2",2"',2"",2""',2"""',2"""",2""""',2"""""',2"""""",2""""""',2"""""""',2""""""""'-(3,7,9-triazabicyclo-[3.3.1]nonane-3,7-diylbis{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})dodecaacetate ,

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate , and

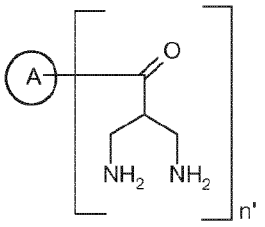
Tetragadolinium [4,10-bis(carboxylatomethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate ,

or a stereoisomer, a tautomer, an N-oxide, a hydrate, a solvate, or a salt thereof, or a mixture of same.

In accordance with another aspect, the present invention covers methods of preparing compounds of the present invention, said methods comprising the steps as described in the Experimental Section herein.

In accordance with a further aspect, the present invention covers intermediate compounds which are useful for the preparation of the compounds of general formula (I), *supra*.

Particularly, the inventions covers compounds of general formula (II-a) :

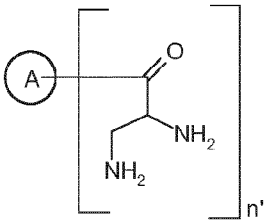


(II-a)

in which (A) is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof;

and

compounds of general formula (II-b) :

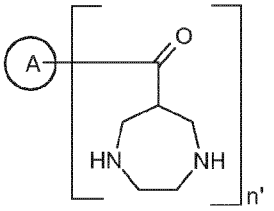


(II-b)

in which A is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof;

and

compounds of general formula (II-c) :

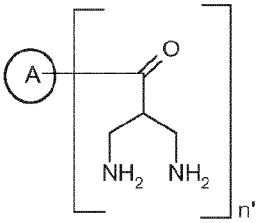


(II-c)

in which A is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof.

More particularly still, the present invention covers the intermediate compounds which are disclosed in the example section of this text, *infra*.

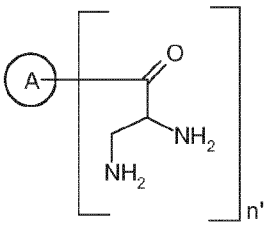
In accordance with a further aspect, the present invention covers the use of the compounds of general formula (II-a) :



(II-a)

in which  is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof, for the preparation of a compound of general formula (I) as defined *supra*.

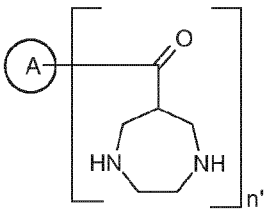
In accordance with a further aspect, the present invention covers the use of the compounds of general formula (II-b) :



(II-b)

in which  is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof, for the preparation of a compound of general formula (I) as defined *supra*.

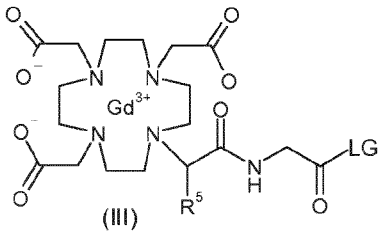
In accordance with a further aspect, the present invention covers the use of the compounds of general formula (II-c) :



(II-c)

in which  is as defined for the compounds of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, and salts thereof, for the preparation of a compound of general formula (I) as defined *supra*.

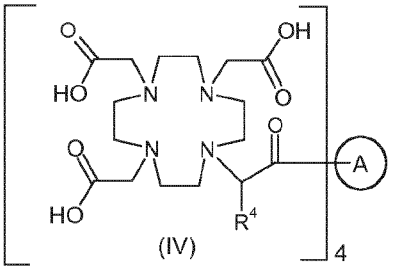
In accordance with a further aspect, the present invention covers the use of the compounds of general formula (III) :



(III)

in which R^5 is as defined for the compounds of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *infra*, for the preparation of a compound of general formula (I) as defined *supra*.

In accordance with a further aspect, the present invention covers the use of the compounds of general formula (IV) :



(IV)

in which R^4 is as defined for the compounds of general formula (I), *supra*, and (A) represents a tetraamine as defined for the compounds of general formula (I), *supra*, for the preparation of a compound of general formula (I) as defined *supra*.

Another aspect of the invention is the use of a compound of general formula (**I**) for diagnostic imaging.
Preferably, the use of a compound of the invention in the diagnosis is performed using magnetic resonance imaging (MRI).

Another aspect of the invention are compounds of general formula (**I**) for use in diagnostic imaging.

Another aspect of the invention are compounds of general formula (**I**) for use in magnetic resonance imaging (MRI).

The invention also contains compounds of general formula (**I**) for the manufacture of diagnostic agents.

Another aspect of the invention is the use of the compounds of general formula (I) or mixtures thereof for the manufacture of diagnostic agents.

Another aspect of the invention is the use of the compounds of general formula (I) or mixtures thereof for the manufacture of diagnostic agents for magnetic resonance imaging (MRI).

Another aspect of the invention is a method of imaging body tissue in a patient, comprising the steps of administering to the patient an effective amount of one or more compounds of general formula (I) in a pharmaceutically acceptable carrier, and subjecting the patient to NMR tomography. Such a method is described in US 5,560,903.

For the manufacture of diagnostic agents, for example the administration to human or animal subjects, the compounds of general formula (I) or mixtures will conveniently be formulated together with pharmaceutical carriers or excipient. The contrast media of the invention may conveniently contain pharmaceutical formulation aids, for example stabilizers, antioxidants, pH adjusting agents, flavors, and the like. Production of the diagnostic media according to the invention is also performed in a way known in the art, see US 5,560,903. They may be formulated for parenteral or enteral administration or for direct administration into body cavities. For example, parenteral formulations contain a sterile solution or suspension in a dose of 0.0001-5 mmol gadolinium/kg body weight, especially 0.005-0.5 mmol gadolinium/kg body weight of the compound of formula (I) according to this invention. Thus the media of the invention may be in conventional pharmaceutical formulations such as solutions, suspensions, dispersions, syrups, etc. in physiologically acceptable carrier media, preferably in water for injections. When the contrast medium is formulated for parenteral administration, it will be preferably isotonic or hypertonic and close to pH 7.4.

In a further aspect, the invention is directed to a method of diagnosing and health monitoring of patients. This method comprises a) administering to a human in need of such diagnosis a compound of the invention for detecting the compound in the human as described above and herein, and b) measuring the signal arising from the administration of the compound to the human, preferably by magnetic resonance imaging (MRI).

GENERAL SYNTHESIS

The compounds according to the invention can be prepared according to the following schemes 1 through 12.

The schemes and procedures described below illustrate synthetic routes to the compounds of general formula (I) of the invention and are not intended to be limiting. It is obvious to the person skilled in the art that the order of transformations as exemplified in the schemes can be modified in various ways. The order of transformations exemplified in the schemes is therefore not intended to be limiting. Appropriate protecting groups and their introduction and cleavage are well-known to the person skilled in the art (see for example T.W. Greene and P.G.M. Wuts in *Protective Groups in Organic Synthesis*, 3rd edition, Wiley 1999). Specific examples are described in the subsequent paragraphs.

The term "amine-protecting group" as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, which is chosen from but not limited to a class of protecting groups namely carbamates, amides, imides, N-alkyl amines, N-aryl amines, imines, enamines, boranes, N-P protecting groups, N-sulfenyl, N-sulfonyl and N-silyl, and which is chosen from but not limited to those described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, third edition, page 494-653. The "amine-protecting group" is preferably carbobenzyloxy (Cbz), *p*-methoxybenzyl carbonyl (Moz or MeOZ), *tert*-butyloxycarbonyl (BOC), 9-fluorenylmethyloxycarbonyl (FMOC), benzyl (Bn), *p*-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), *p*-methoxyphenyl (PMP), triphenylmethyl (Trityl), methoxyphenyl diphenylmethyl (MMT) or the protected amino group is a 1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl (phthalimido) or an azido group.

The term "carboxyl-protecting group" as employed herein by itself or as part of another group is known or obvious to someone skilled in the art, which is chosen from but not limited to a class of protecting groups namely esters, amides and hydrazides, and which is chosen from but not limited to those described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, third edition, page 369-453. The "carboxyl-protecting group" is preferably methyl, ethyl, propyl, butyl, *tert*-butyl, allyl, benzyl, 4-methoxybenzyl or 4-methoxyphenyl.

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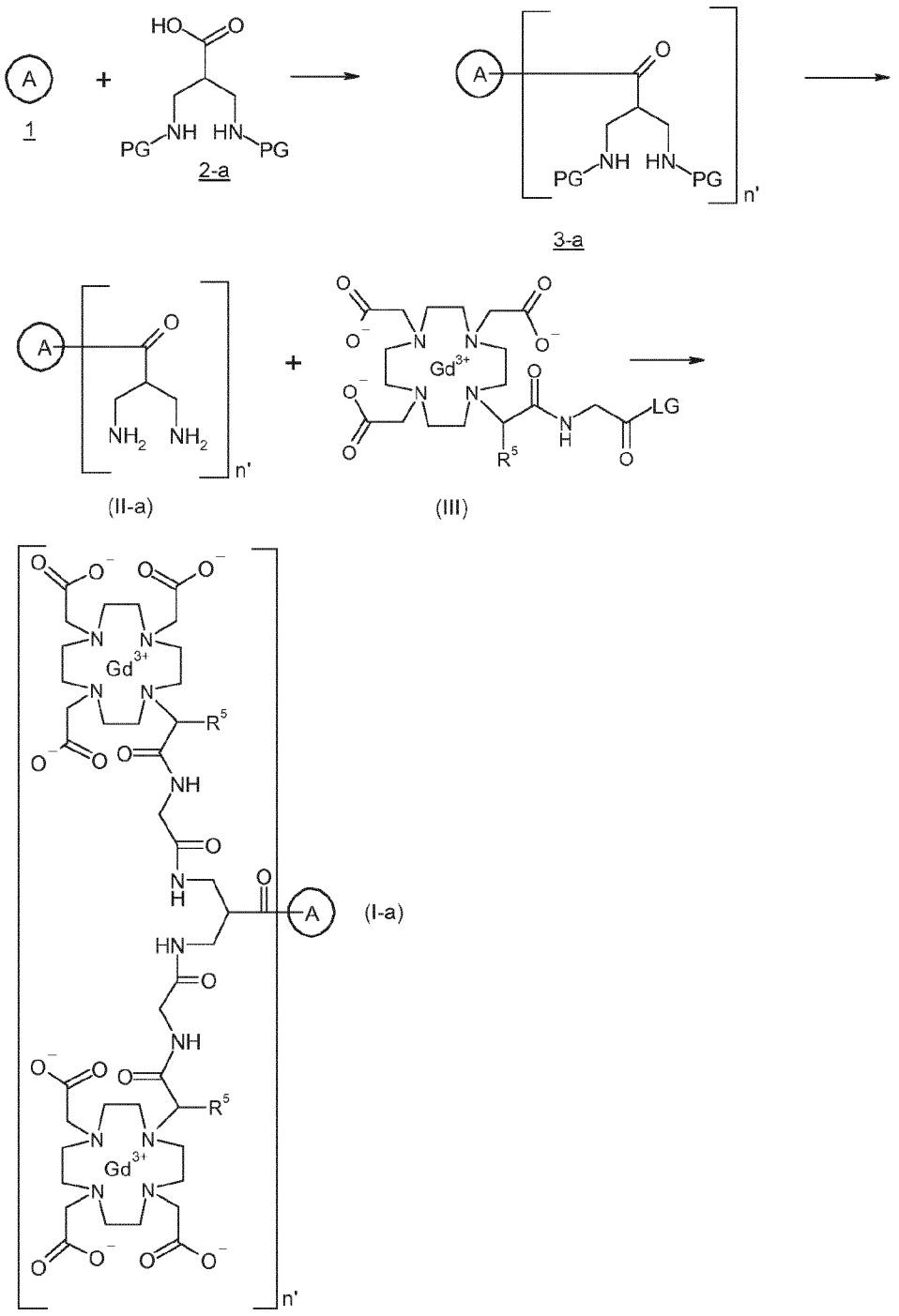
A route for the preparation of compounds of general formula (I-a) is described in Scheme 1.

Scheme 1



Scheme 1: Route for the preparation of compounds of general formula (I-a), wherein

(A) and R^5 have the meaning as given for general formula (I), *supra*, n' represents an integer of 2, 3 and 4, and PG represents an amine-protecting group, such as for example a *tert*-butyloxycarbonyl group (BOC) or a group as defined below.

The starting materials 1 are either commercially available polyamines or salts thereof [for example CAS 111-40-0, CAS 28634-67-5, CAS 4730-54-5, CAS 4742-00-1, CAS 294-90-6] or polyamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature or in the experimental part, *infra* [for example CAS 41077-50-3].

A triamine or tetraamine 1 or a salt thereof is reacted with a protected 3-amino-2-(aminomethyl)propionic acid 2-a, [for example CAS 496974-25-5] or a salt thereof, leading to an intermediate 3-a. Suitable amine-protecting groups for 3-amino-2-(aminomethyl)propionic acid are for example carbobenzyloxy (Cbz), *p*-methoxybenzyl carbonyl (Moz or MeOZ), *tert*-butyloxycarbonyl (BOC), 9-fluorenylmethyloxycarbonyl (FMOC), benzyl (Bn), *p*-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), *p*-methoxyphenyl (PMP), triphenylmethyl (Trityl), methoxyphenyl diphenylmethyl (MMT) or the protected amino group is a 1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl (phthalimido) or an azido group. The coupling reaction of polyamines 1 with propionic acid derivatives 2-a is carried out employing standard peptide coupling conditions, such as for example coupling in the presence of HATU and *N,N*-diisopropylethylamine, in a suitable solvent such as for example *N,N*-dimethylformamide, in a temperature range from room temperature up to 80°C, to furnish the intermediates of general formula 3-a.

Deprotection of intermediates of general formula 3-a leading to intermediates of general formula (II-a) or salts thereof is performed in analogy to methods described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, second edition, page 309-405. The amine-protecting group *tert*-butyloxycarbonyl (BOC) is removed by dissolving a BOC-protected intermediate of general formula 3-a in a suitable solvent, such as for example an alcohol, tetrahydrofuran, dioxane or *N,N*-dimethylformamide, or a mixture thereof, by adding suitable acids, such as for example aqueous hydrochloric or hydrobromic acid or trifluoroacetic acid in organic solvents like dichloromethane. The deprotection reaction is carried out at temperatures ranging from room temperature to the boiling point of the respective solvent or solvent mixture, preferably the reaction is carried out at temperatures ranging from room temperature to 80°C.

Intermediates of general formula (II-a) or salts thereof are reacted with Gd-complexes of the general formula (III), which are activated by a leaving group (LG), such as for example

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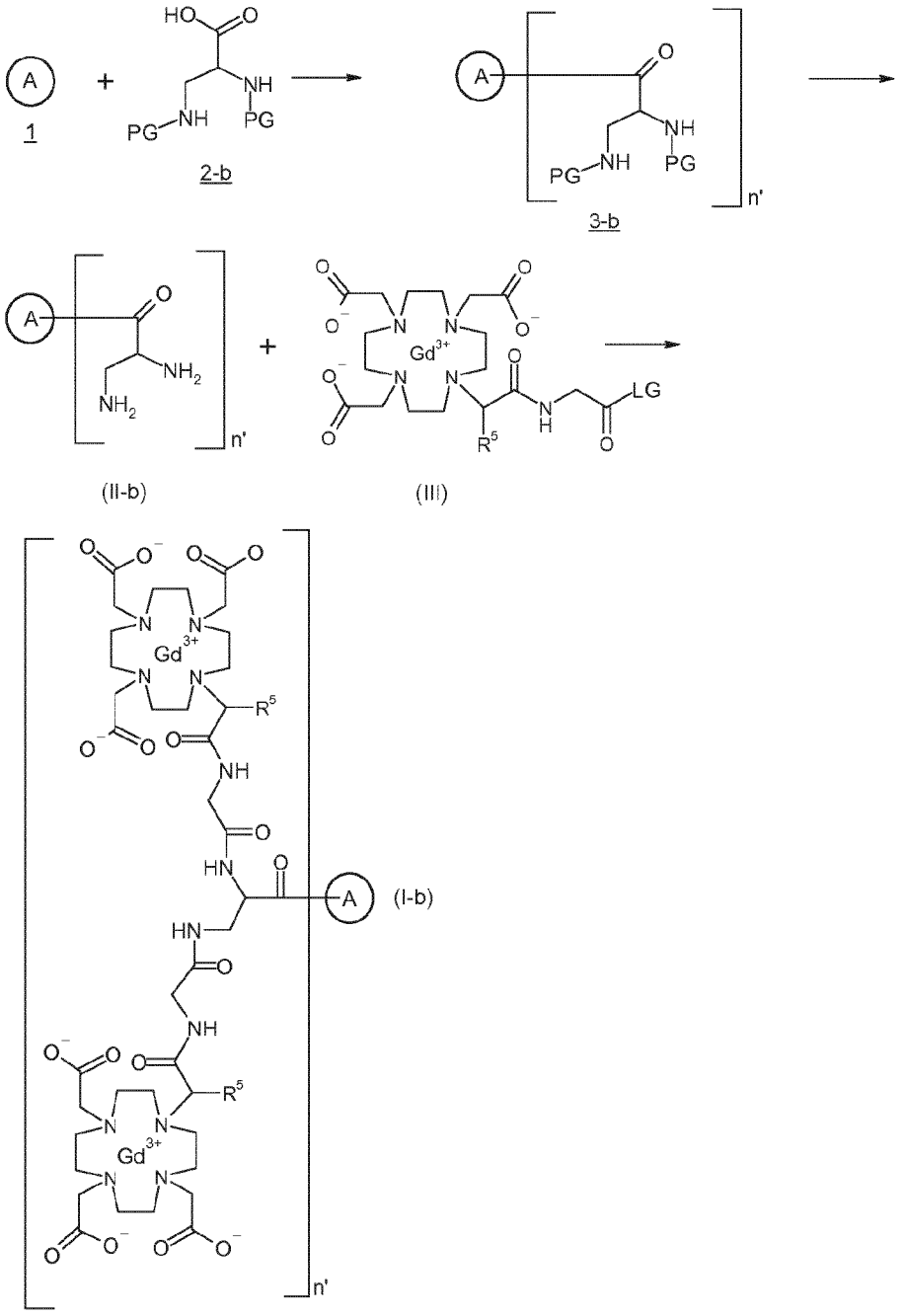
pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione, hydroxybenzotriazole or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to compounds of the general formula (I-a). The preparation of activated esters is well known to the person skilled in the art and is described in detail for example by C.A. Montalbetti and V. Falque in Tetrahedron **61** (2005), page 10827-10852. For example, the preparation of gadolinium 2,2',2"-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate is described in detail in WO 2001051095 A2. The reaction of intermediates of general formula (II-a) with the activated Gd-complexes of general formula (III) is carried out in a suitable solvent, such as for example dimethyl sulfoxide, *N,N*-dimethylformamide, pyridine or a mixture thereof, optionally the reaction is carried out in the presence of a base. Suitable bases are for example trialkylamines, such as for example triethylamine or *N,N*-diisopropylethylamine. The reaction is carried out at temperatures ranging from room temperature to 100℃, preferably the reaction is carried out at temperatures ranging from 50℃ to 70℃.

A route for the preparation of compounds of general formula (I-b) is described in Scheme 2.

Scheme 2



Scheme 2: Route for the preparation of compounds of general formula (I-b), wherein

Ⓐ and R^5 have the meaning as given for general formula (I), *supra*, n' represents an integer of 2, 3 and 4, and PG represents an amine-protecting group, such as for example a *tert*-butyloxycarbonyl group (BOC) or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*.

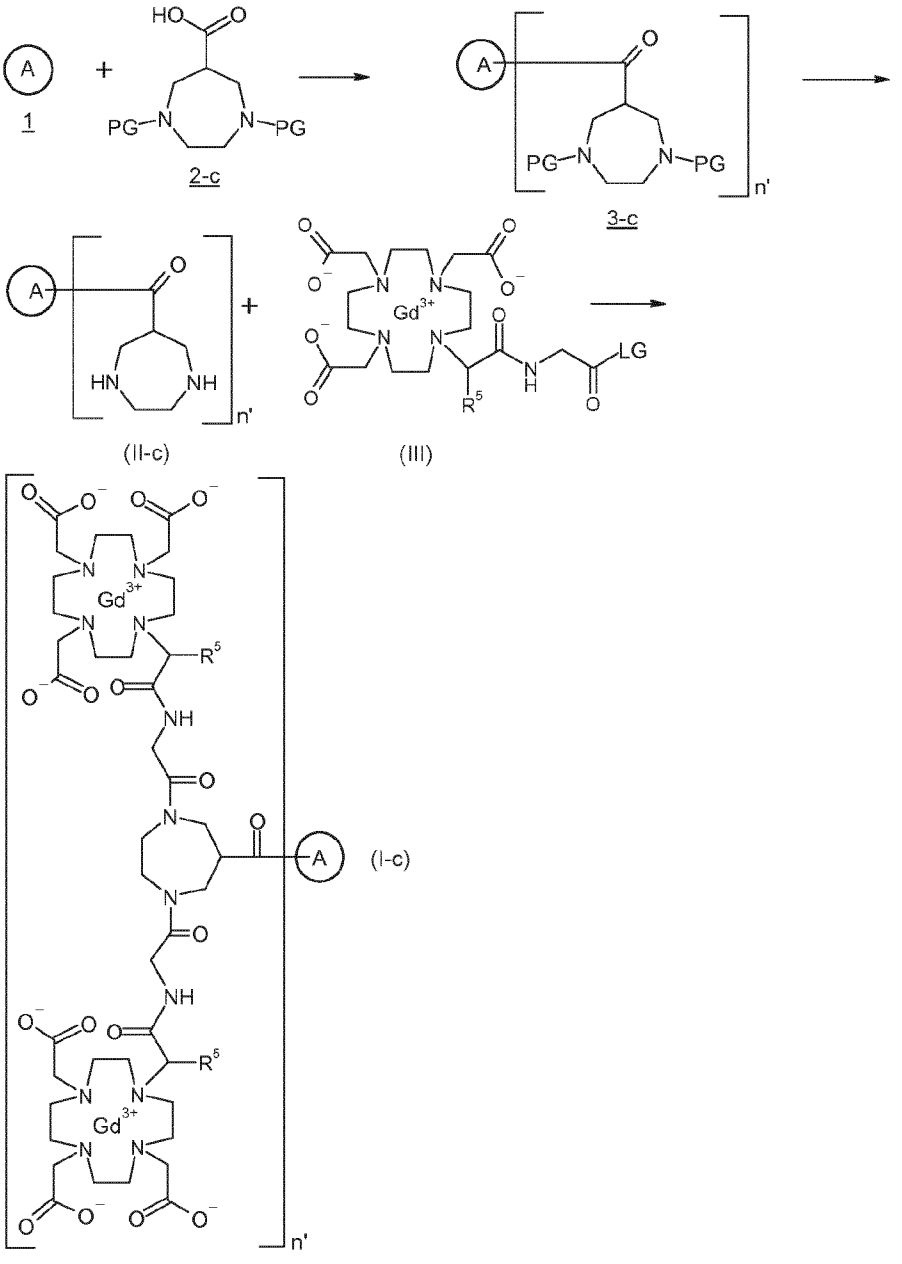
The compounds of general formula (I-b) are synthesized in analogy to the compounds of general formula (I-a), as described above.

The starting materials 1 are either commercially available polyamines or salts thereof [for example CAS 111-40-0, CAS 28634-67-5, CAS 4730-54-5, CAS 4742-00-1, CAS 294-90-6] or polyamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature or in the experimental part, *infra* [for example CAS 41077-50-3].

A triamine or tetraamine 1 or a salt thereof is reacted with a protected 2,3-diaminopropionic acid 2-b [for example CAS 201472-68-6] or a salt thereof, to furnish an intermediate of general formula 3-b, which after deprotection furnishes an intermediate of general formula (II-b) or a salt thereof. In the final step an intermediate of general formula (II-b) or a salt thereof is reacted with a Gd-complex of the general formula (III), leading to a compound of the general formula (I-b).

A route for the preparation of compounds of general formula (I-c) is described in Scheme 3.

Scheme 3



Scheme 3: Route for the preparation of compounds of general formula (I-c), wherein

(A) and R^5 have the meaning as given for general formula (I), *supra*, n' represents an integer of 2, 3 and 4, and PG represents an amine-protecting group, such as for example a

tert-butyloxycarbonyl group (BOC) or a group as definened for the synthesis of the compounds of the general formula (I-a) *supra*.

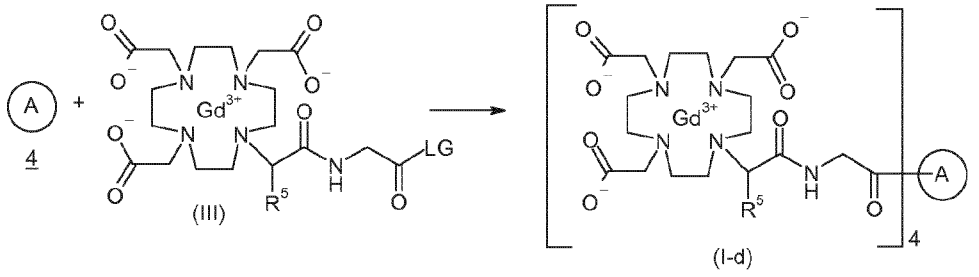
The compounds of general formula (I-c) are synthesized in analogy to the compounds of general formula (I-a), as described above.

The starting materials 1 are either commercially available polyamines or salts thereof [for example CAS 111-40-0, CAS 28634-67-5, CAS 4730-54-5, CAS 4742-00-1, CAS 294-90-6] or polyamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature or in the experimental part, *infra* [for example CAS 41077-50-3].

A triamine or tetraamine 1 or a salt thereof is reacted with a protected 1,4-diazepane-6-carboxylic acid 2-c, which can be synthesized as described in the experimental part *infra*, starting from methyl 1,4-dibenzyl-1,4-diazepane-6-carboxylate [see US 5,866,562], to furnish an intermediate of general formula 3-c, which after deprotection furnishes an intermediate of general formula (II-c) or a salt thereof. In the final step an intermediate of general formula (II-c) or a salt thereof is reacted with a Gd-complex of the general formula (III), leading to a compound of the general formula (I-c).

A route for the preparation of compounds of general formula (I-d) is described in Scheme 4.

Scheme 4



Scheme 4: Route for the preparation of compounds of general formula (I-d), wherein

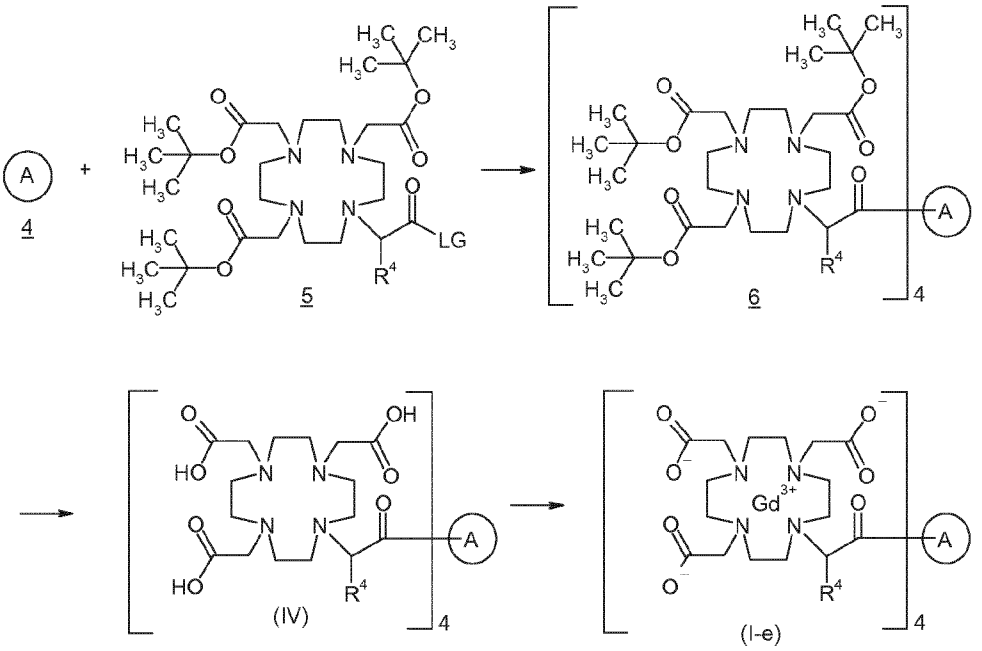
R^5 has the meaning as given for general formula (I), *supra*, (A) represents a tetraamine as given for general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*.

The starting materials 4 are either commercially available tetraamines or salts thereof [for example CAS 4742-00-1, CAS 294-90-6] or tetraamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature.

A tetraamine 4 or a salt thereof is reacted with a Gd-complex of the general formula (III), which is activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione, hydroxybenzotriazole or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to a compound of the general formula (I-d). The preparation of activated esters is well known to the person skilled in the art and is described in detail for example by C.A. Montalbetti and V. Falque in Tetrahedron **61** (2005), page 10827-10852. For example, the preparation of gadolinium 2,2',2"-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate is described in detail in WO 2001051095 A2. The reaction of polyamine 4 or a salt thereof with the activated Gd-complexes of general formula (III) is carried out in a suitable solvent, such as for example dimethyl sulfoxide, *N,N*-dimethylformamide, pyridine or a mixture thereof, optionally the reaction is carried out in the presence of a base. Suitable bases are for example trialkylamines, such as for example triethylamine or *N,N*-diisopropylethylamine. The reaction is carried out at temperatures ranging from room temperature to 100°C, preferably the reaction is carried out at temperatures ranging from 50°C to 70°C.

A route for the preparation of compounds of general formula (I-e) is described in Scheme 5.

Scheme 5



Scheme 5: Route for the preparation of compounds of general formula (I-e), wherein

R^4 has the meaning as given for general formula (I), *supra*, (A) represents a tetraamine as given for general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*.

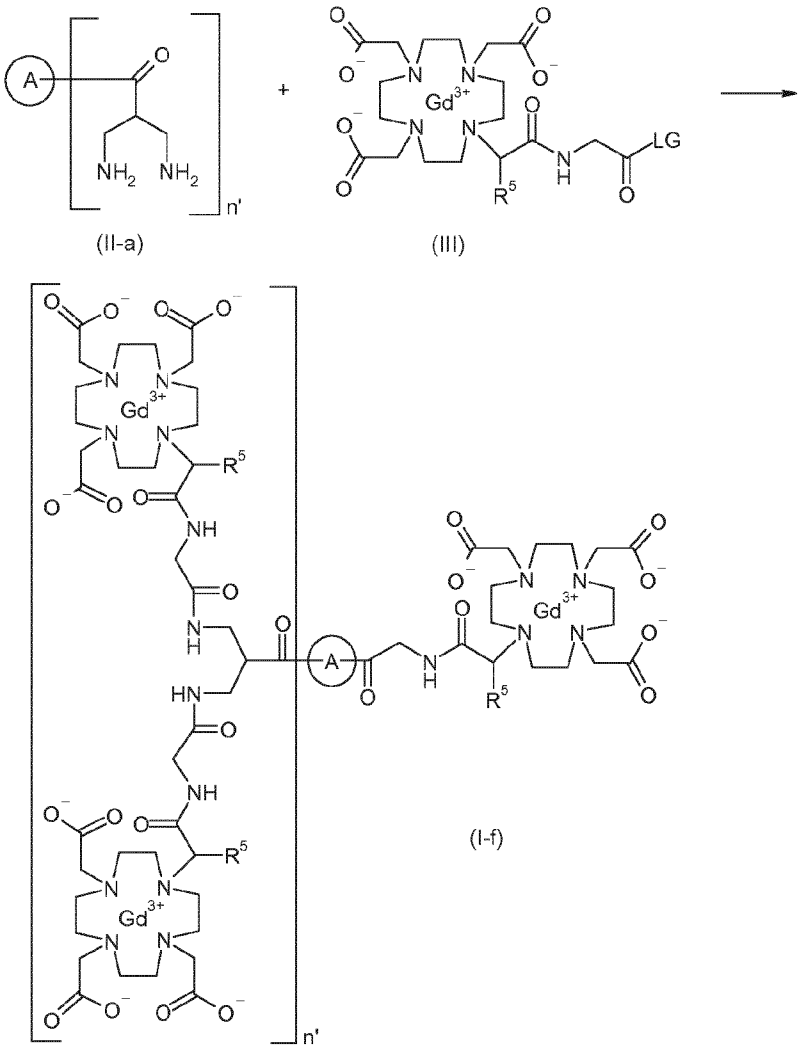
The starting materials 4 are either commercially available tetraamines or salts thereof [for example CAS 4742-00-1, CAS 294-90-6] or tetraamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature.

A tetraamine 4 or a salt thereof is reacted with a [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivative 5, which is activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione [for example, the synthesis of tri-tert-butyl 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate is described in detail by Cong Li *et al.*, *J. Am.Chem.Soc.* **2006**, *128*, p.15072-15073; S3-5 and Galibert *et al.*, Biorg. and Med. Chem. Letters 20 (2010), 5422 - 5425] or hydroxybenzotriozole, leading to an intermediate 6. The preparation of activated esters is well known to the person skilled in the

art and is described in detail for example by C.A. Montalbetti and V. Falque in Tetrahedron **61** (2005), page 10827-10852. The coupling reaction of polyamines 4 with [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivatives 5 is carried out in a suitable solvent, such as for example *N,N*-dimethylformamide or dimethyl sulfoxide, or a mixture thereof, in a temperature range from room temperature up to 80℃, to furnish the intermediates 6. Cleavage of the carboxyl-protecting groups of intermediates 6 to yield the intermediates of general formula (IV) can be achieved as described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, second edition, page 245-247. The deprotection is, for example, performed by dissolving and stirring of intermediates 6 in trifluoroacetic acid at room temperature for several hours. The complexation of intermediates of general formula (IV) with suitable gadolinium (III) compounds or salts, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, is well known to a person skilled in the art. The intermediates of general formula (IV) are dissolved in water and after adding of suitable gadolinium (III) compounds the resulting mixtures are stirred in a temperature range from room temperature up to 100℃ at pH = 1-7 for several hours, to furnish the compounds of general formula (I-e). Intermediates of general formula (IV) are, for example, dissolved in water, gadolinium triacetate tetrahydrate is added, the pH is adjusted to 3.5 - 5.5 by addition of a suitable base, such as for example aqueous sodium hydroxide solution. The reaction is carried out at temperatures ranging from 50℃ to 80 ℃, leading to compounds of general formula (I-e).

A route for the preparation of compounds of general formula (I-f) is described in Scheme 6.

Scheme 6



Scheme 6: Route for the preparation of compounds of general formula (I-f), wherein

n' represents an integer of 2, if (A) represents a triamine as defined *supra*, or

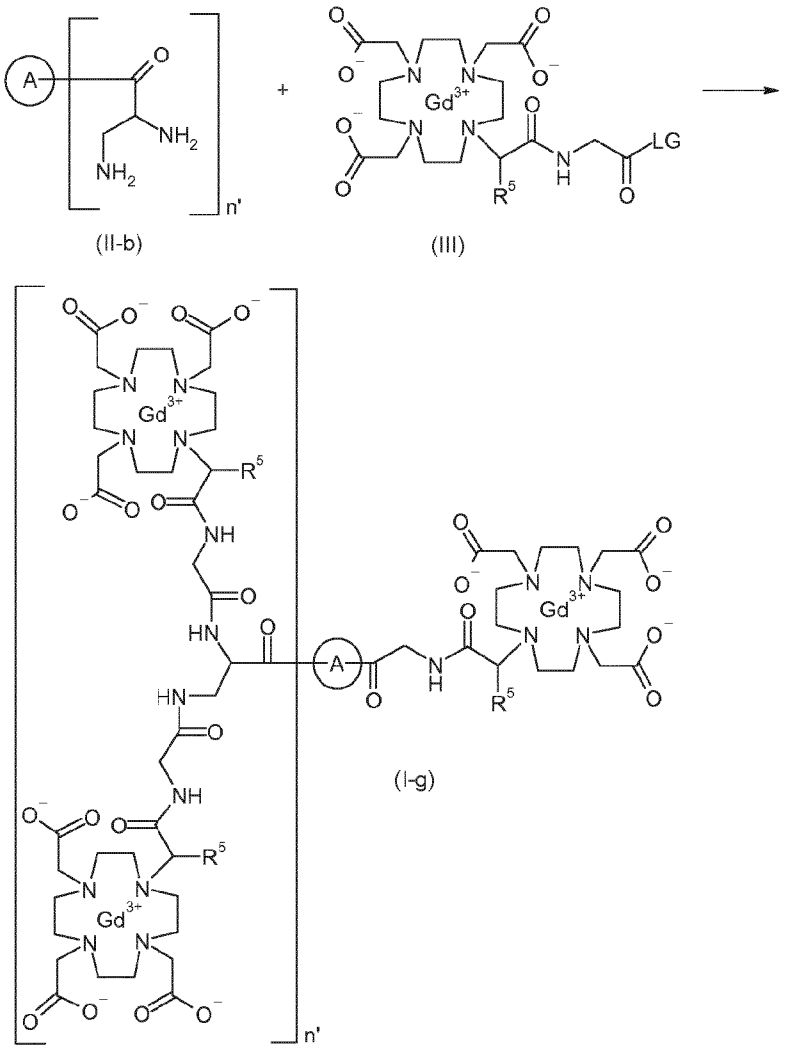
n' represents an integer of 3, if (A) represents a tetraamine as defined *supra*,

and R^5 has the meaning as given for general formula (I), *supra*, and LG represents activating leaving groups, such as for example 4-nitrophenol or a group as defined below.

Intermediates of general formula (II-a) or salts thereof, as described in Scheme 1 and in the experimental part *infra*, wherein n' represents an integer of 2 and  represents a triamine core as defined *supra*, or intermediates of general formula (II-a) or salts thereof, wherein n' represents an integer of 3 and  represents a tetraamine core as defined *supra*, are reacted with Gd-complexes of the general formula (III), which are activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione, hydroxybenzotriozole or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to compounds of the general formula (I-f). The preparation of activated esters is well known to the person skilled in the art and is described in detail for example by C.A. Montalbetti and V. Falque in Tetrahedron **61** (2005), page 10827-10852. For example, the preparation of gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate is described in detail in WO 2001051095 A2. The reaction of intermediates of general formula (II-a) or salts thereof with the activated Gd-complexes of general formula (III) is carried out in a suitable solvent, such as for example dimethyl sulfoxide, *N,N*-dimethylformamide, pyridine or a mixture thereof, optionally the reaction is carried out in the presence of a base. Suitable bases are for example trialkylamines, such as for example triethylamine or *N,N*-diisopropylethylamine. The reaction is carried out at temperatures ranging from room temperature to 100℃, preferably the reaction is carried out at temperatures ranging from 50℃ to 70℃.

A route for the preparation of compounds of general formula (I-g) is described in Scheme 7.

Scheme 7



Scheme 7: Route for the preparation of compounds of general formula (I-g), wherein

n' represents an integer of 2, if (A) represents a triamine as defined *supra*, or

n' represents an integer of 3 if (A) represents a tetraamine as defined *supra*,

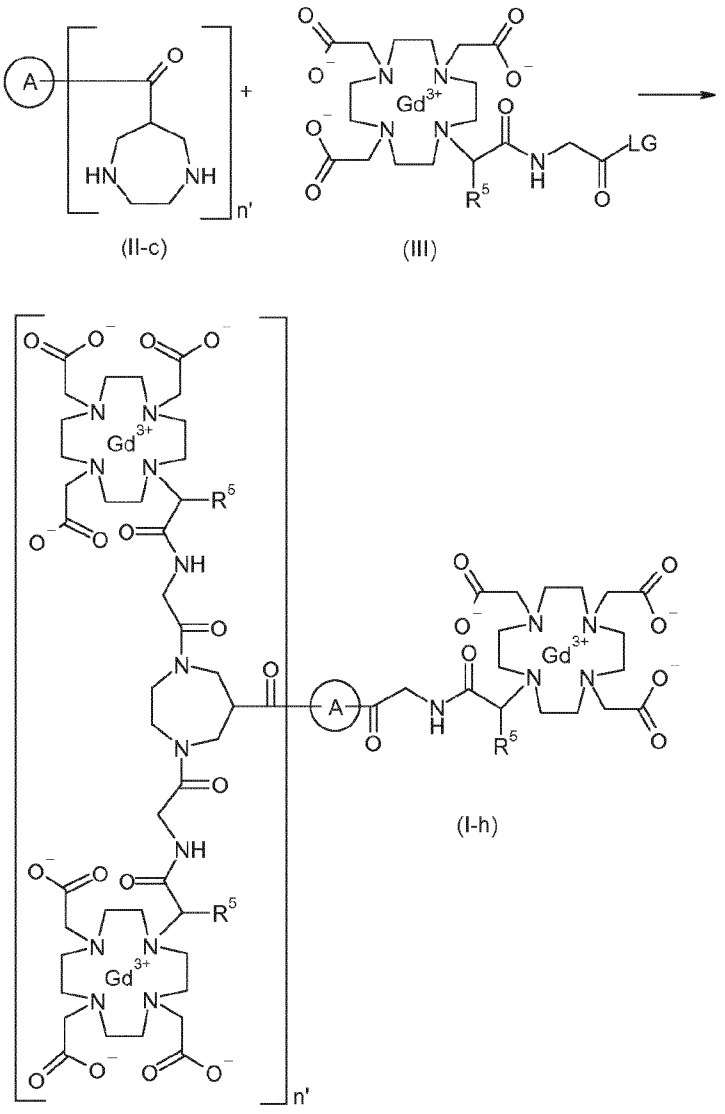
and R^5 has the meaning as given for general formula (I), *supra*, and LG represents activating leaving groups, such as for example 4-nitrophenol or a group as defined below.

The compounds of general formula (I-g) are synthesized in analogy to the compounds of general formula (I-f), as described above.

Intermediates of general formula (II-b) or salts thereof, as described in Scheme 2, wherein n' represents an integer of 2 and  represents a triamine core as defined *supra*, or intermediates of general formula (II-b) or salts thereof, wherein n' represents an integer of 3 and  represents a tetraamine core as defined *supra*, are reacted with Gd-complexes of the general formula (III), which are activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione, hydroxybenzotriazole or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to compounds of the general formula (I-g).

A route for the preparation of compounds of general formula (I-h) is described in Scheme 8.

Scheme 8



Scheme 8: Route for the preparation of compounds of general formula (I-h), wherein

n' represents an integer of 2, if (A) represents a triamine as defined *supra*, or

n' represents an integer of 3, if (A) represents a tetraamine as defined *supra*,

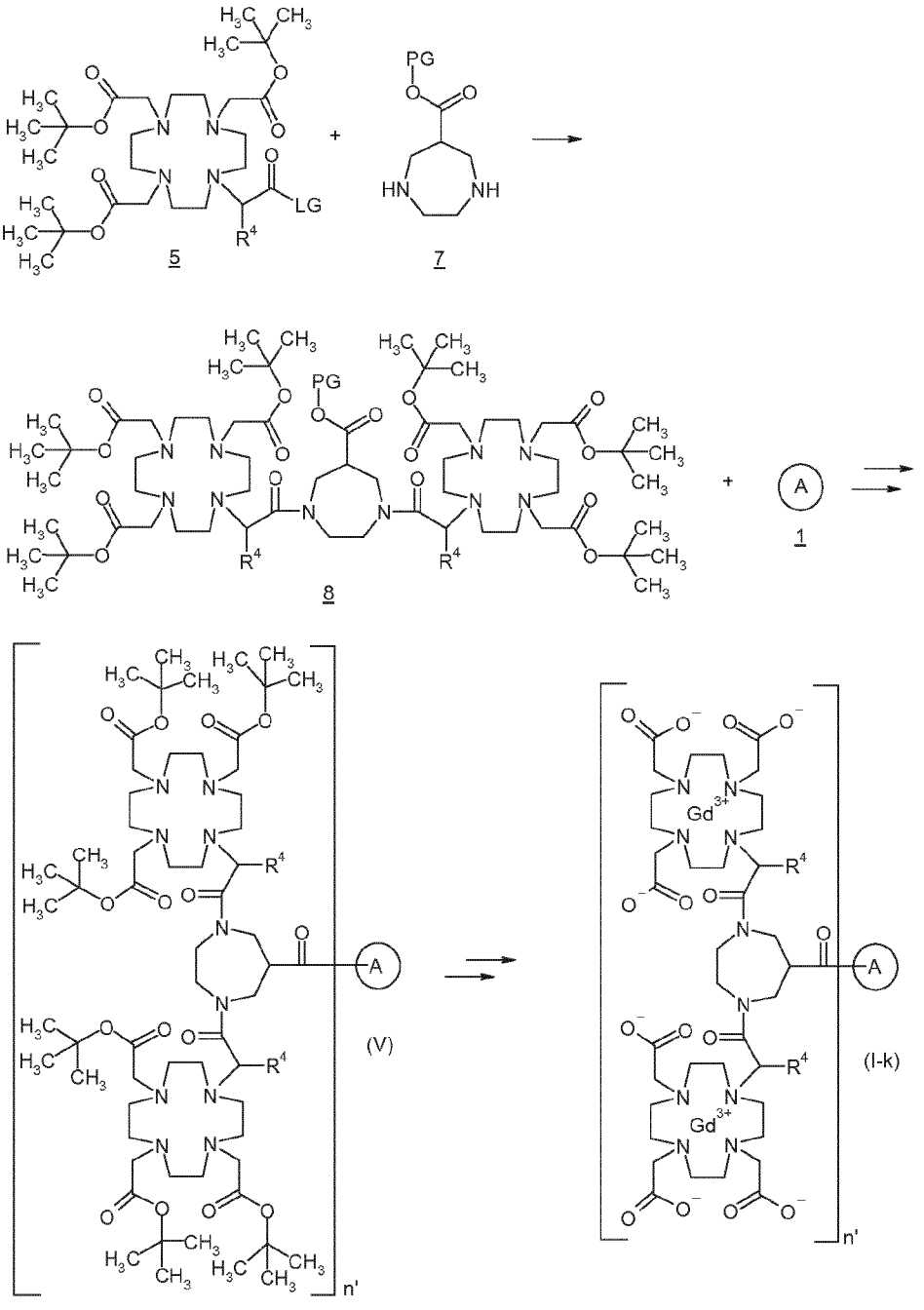
and R^5 has the meaning as given for general formula (I), *supra*, and LG represents activating leaving groups, such as for example 4-nitrophenol or a group as defined below.

The compounds of general formula (I-h) are synthesized in analogy to the compounds of general formula (I-f), as described above.

Intermediates of general formula (II-c) or salts thereof, as described in Scheme 3, wherein n' represents an integer of 2 and  represents a triamine core as defined *supra*, or intermediates of general formula (II-c) or salts thereof, wherein n' represents an integer of 3 and  represents a tetraamine core as defined *supra*, are reacted with Gd-complexes of the general formula (III), which are activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione, hydroxybenzotriazole or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to compounds of the general formula (I-h).

A route for the preparation of compounds of general formula (I-k) is described in Scheme 9.

Scheme 9



Scheme 9: Route for the preparation of compounds of general formula (I-k), wherein

Ⓐ and R^4 have the meaning as given for general formula (I), *supra*, n' represents an integer of 2, 3 and 4, LG represents activating leaving groups, such as for example 1-hydroxypyrrolidine-2,5-dione, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*, and PG represents a carboxyl-protecting group, such as for example a methyl or ethyl group.

The starting materials 1 are either commercially available polyamines or salts thereof [for example CAS 111-40-0, CAS 28634-67-5, CAS 4730-54-5, CAS 4742-00-1, CAS 294-90-6] or polyamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature or in the experimental part, *infra* [for example CAS 41077-50-3].

Diamines 7 or salts thereof are commercially available [for example CAS 1417898-94-2] or can be synthesized by methods which are well known to a person skilled in the art. Diamines 7 or salts thereof can be reacted with a [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivative 5, which is activated by a leaving group (LG), such as for example pentafluorophenol, 4-nitrophenol, 1-hydroxypyrrolidine-2,5-dione [for example, the synthesis of tri-tert-butyl 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate is described in detail by Cong Li *et al.*, *J. Am.Chem.Soc.* **2006**, *128*, p.15072-15073; S3-5 and. Galibert *et al.*, *Biorg. and Med. Chem. Letters* **2010**, *20* , p.5422-5425] or hydroxybenzotriazole, leading to intermediates 8. The preparation of activated esters is well known to the person skilled in the art and is described in detail for example by C.A. Montalbetti and V. Falque in *Tetrahedron* **2005**, *61* page 10827-10852. The protection group PG of intermediates 8 can be cleaved under basic conditions, such as for example by treatment with alkali metal hydroxides, such as for example lithium hydroxide, in water or a mixture of water and tetrahydrofurane, to yield the corresponding salt of the carboxylic acid. This salt can be coupled with polyamines 1 employing standard peptide coupling conditions, such as for example coupling in the presence of HATU and 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-ol in the presence of *N*,*N*-diisopropylethylamine, in a suitable solvent, such as for example dichloromethane, at room temperature, to furnish the intermediates of general formula (V). Cleavage of the carboxyl-protecting groups of intermediates of general formula (V) can be achieved employing standard conditions, such as for example, by dissolving and stirring of intermediates (V) in aqueous hydrochloric acid at room temperature. The subsequent complexation with suitable gadolinium (III) compounds or salts, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, is well known to a person skilled in the art, and can, for example, be achieved by the reaction with suitable gadolinium (III) compounds in a temperature range from room temperature up

to 100℃ at pH = 1-7 for several hours, to furnish the compounds of general formula (I-k). The raw carboxylic acids derived from the compounds of general formula (V) are, for example, reacted with gadolinium trioxide at 80℃, leading to compounds of general formula (I-k).

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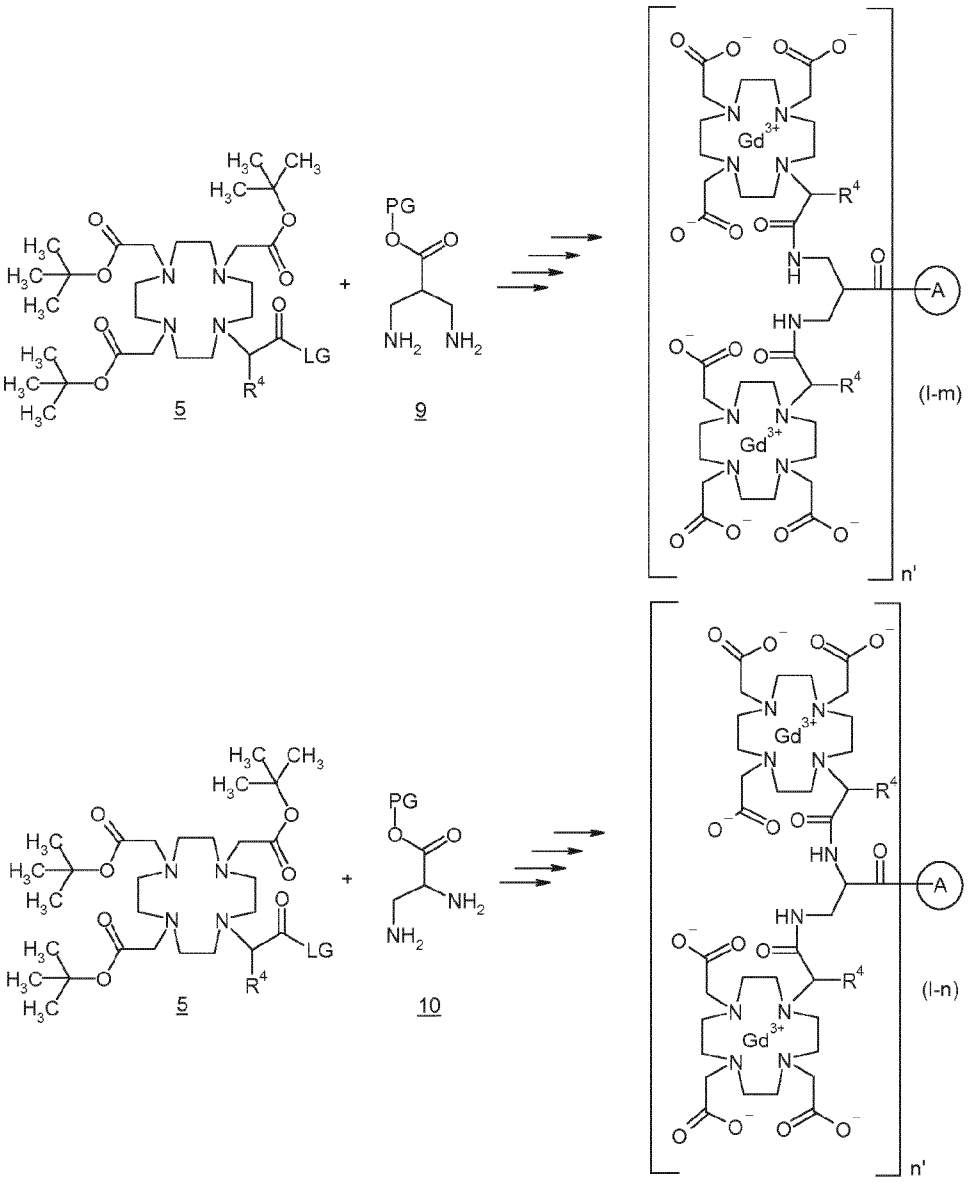
A route for the preparation of compounds of general formulae (I-m) and (I-n) is described in Scheme 10.

Scheme 10



Scheme 10: Route for the preparation of compounds of general formulae (I-m) and (I-n), wherein

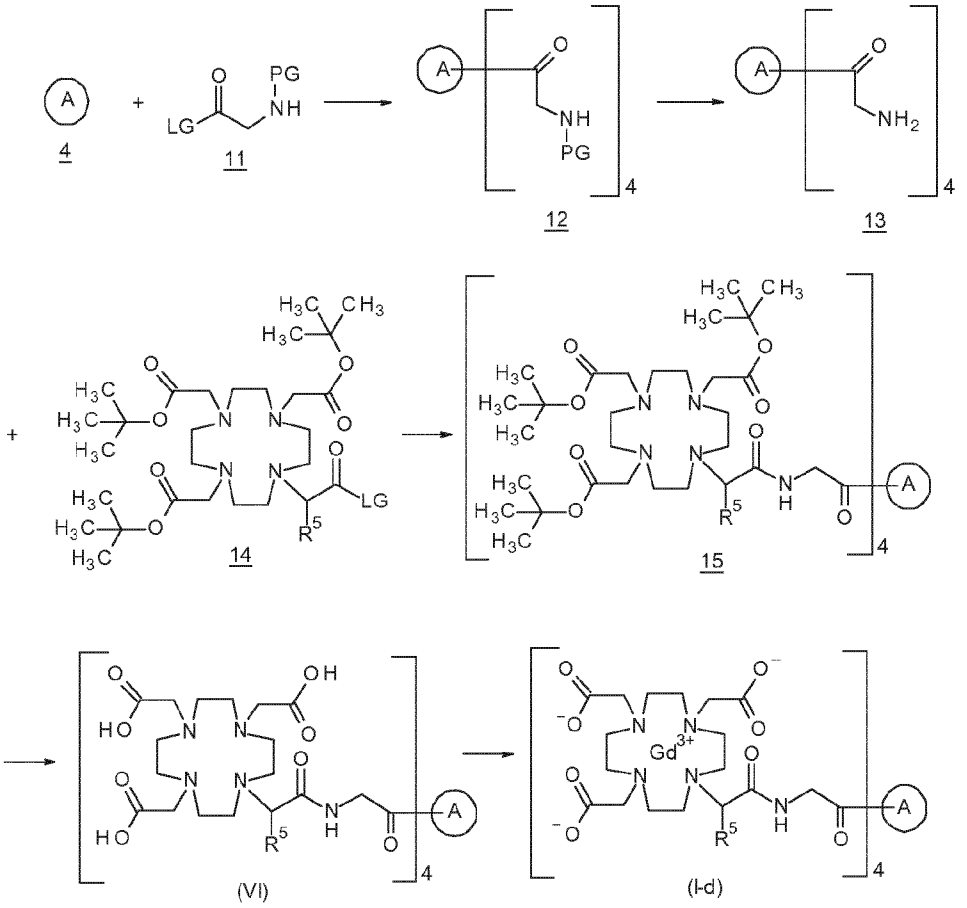
(A) and R^4 have the meaning as given for general formula (I), *supra*, n' represents an integer of 2, 3 and 4, LG represents activating leaving groups, such as for example 1-hydroxypyrrolidine-2,5-dione, or a group as defined for the synthesis of the compounds of the

general formula (I-a) *supra*, and PG represents a carboxyl-protecting group, such as for example a methyl or ethyl group.

When instead of the diamines of formula 7, as described in Scheme 9, diamines of formulae 9 and 10 or salts thereof are used in the analoguous synthesis as described in Scheme 9, the compounds of general formulae (I-m) and (I-n) can be obtained.
Diamines 9 or salts thereof are commercially available [for example CAS 159029-33-1, CAS 440644-06-4] or can be synthesized by methods which are well known to a person skilled in the art.
Diamines 10 or salts thereof are commercially available [for example CAS 20610-20-2, CAS 6059-44-5] or can be synthesized by methods which are well known to a person skilled in the art.

An alternative route to the one described in Scheme 4 for the preparation of compounds of general formula (I-d) is described in Scheme 11.

Scheme 11



Scheme 11: Alternative route for the preparation of compounds of general formula (I-d), wherein

R^5 has the meaning as given for general formula (I), *supra*, (A) represents a tetraamine as given for general formula (I), *supra*, and LG represents an activating leaving group, such as for example 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*.

The starting materials 4 are either commercially available tetraamines or salts thereof [for example CAS 4742-00-1, CAS 294-90-6] or tetraamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described

in the literature. The starting materials 14 are either commercially available or known from the literature or can be synthesized in analogy to compounds which are described in the literature, e.g. by step-wise alkylation of the cyclen core.

A tetraamine 4 or a salt thereof is reacted with an amino acid derivative 11, which is activated by a leaving group (LG), such as for example 1-hydroxypyrrolidine-2,5-dione, pentafluorophenol, 4-nitrophenol or 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol, leading to an intermediate 12. The preparation of activated esters is well known to the person skilled in the art and is described in detail for example by C.A. Montalbetti and V. Falque in Tetrahedron **61** (2005), page 10827-10852. The coupling reactions of polyamines 4 with amino acid derivatives 11 are carried out in a suitable solvent, such as for example dichloromethane or *N,N*-dimethylformamide, in a temperature range from room temperature up to 50°C, to furnish the intermediates 12. Cleavage of the amino protecting groups (PG) of intermediates 12 to yield the intermediates 13 can be achieved as described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, second edition. In case of *tert*-butoxycarbonyl protecting groups the deprotection is, for example, performed by reacting intermediates 12 with HCl in CPME in a suitable solvent, such as for example CPME or 1,4-dioxane or a mixture thereof in a temperature range from 0°C to room temperature for several hours.

A tetraamine 13 or a salt thereof is reacted with a [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivative 14, which is activated by a leaving group (LG), such as for example 3*H*-[1,2,3]triazolo[4,5-b]pyridin-3-ol, 4-nitrophenol or 1-hydroxypyrrolidine-2,5-dione leading to an intermediate 15. The coupling reaction of tetraamines 13 with [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivatives 14 is carried out in a suitable solvent, such as for example *N,N*-dimethylacetamide or dimethyl sulfoxide, or a mixture thereof, in a temperature range from room temperature to 80°C, to furnish the intermedia tes 15.

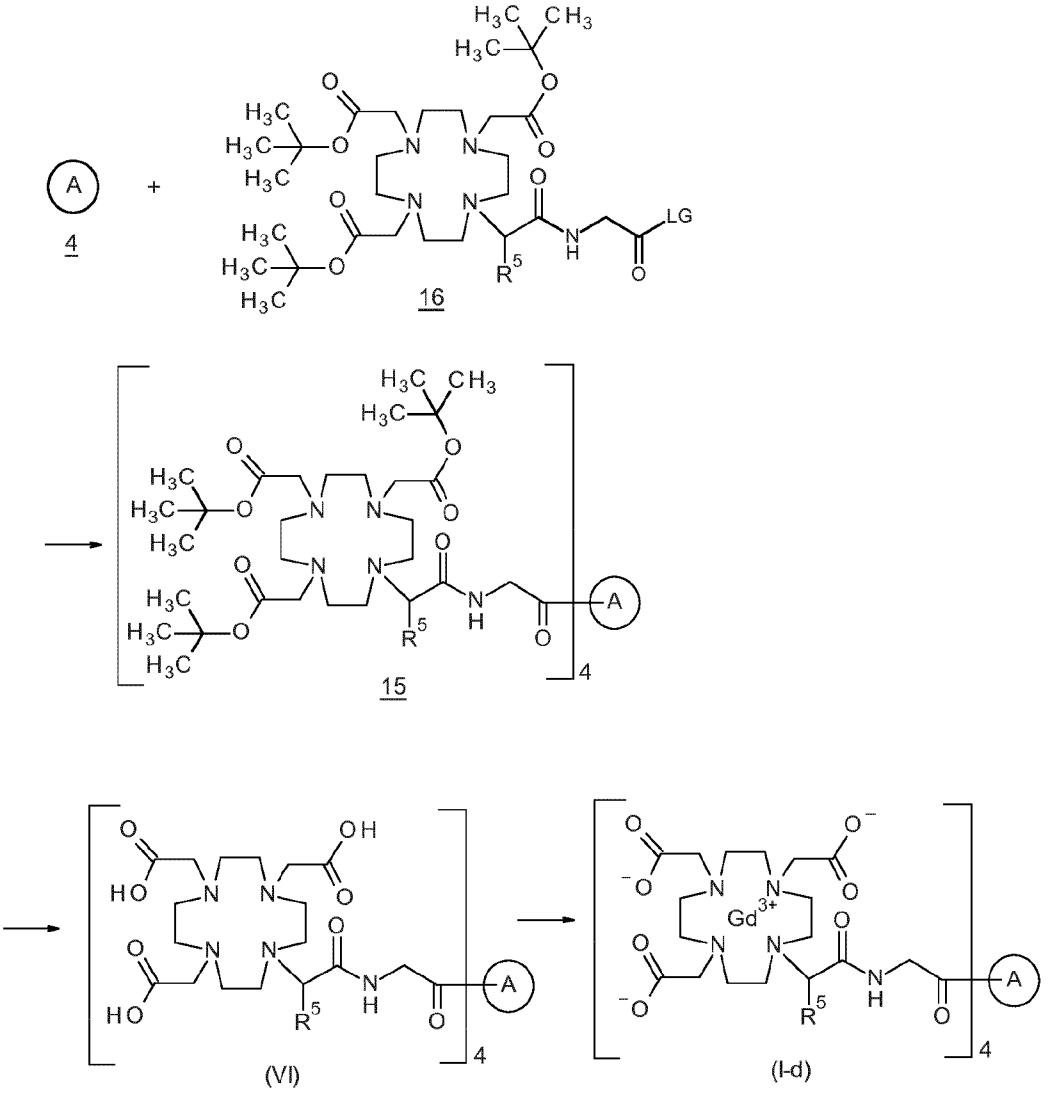
Cleavage of the carboxyl-protecting groups of intermediates 15 to yield the intermediates of general formula (VI) can be achieved as described in the textbook Greene and Wuts, Protecting groups in Organic Synthesis, second edition, page 245-247. The deprotection is, for example, performed by dissolving and stirring of intermediates 15 in trifluoroacetic acid at room temperature for several hours.

The complexation of intermediates of general formula (VI) with suitable gadolinium (III) compounds or salts, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, is well known to a person skilled in the art. The intermediates of general formula (VI) are dissolved in water and after adding of suitable gadolinium (III) compounds the resulting mixtures are stirred in a temperature range from room temperature up to 100°C at pH = 1-7 for several hours, to furnish the compounds of general formula (I-d). Intermediates of general formula (VI) are, for

example, dissolved in water, gadolinium triacetate tetrahydrate is added and the pH is adjusted to 3.5 - 5.5 by addition of a suitable base, such as for example aqueous sodium hydroxide solution. The reaction is carried out at temperatures ranging from 50℃ to 80℃, leading to compounds of general formula (I-d).

An alternative route to the one described in Scheme 4 for the preparation of compounds of general formula (I-d) is described in Scheme 12.

Scheme 12



Scheme 12: Alternative route for the preparation of compounds of general formula (I-d), wherein

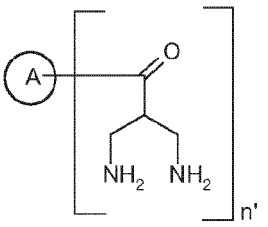
R^5 has the meaning as given for general formula (I), *supra*,  represents a tetraamine as given for general formula (I), *supra*, and LG represents an activating leaving group, such as for example 3H-[1,2,3]triazolo[4,5-b]pyridine-3-ol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*.

The starting materials 4 are either commercially available tetraamines or salts thereof [for example CAS 4742-00-1, CAS 294-90-6] or tetraamines or salts thereof which are known from the literature, or which can be prepared in analogy to compounds which are described in the literature. The starting materials 16 are either known from the literature or can be synthesized in analogy to compounds which are described in the literature, e.g. by step-wise alkylation of the cyclen core.

A tetraamine 4 or a salt thereof is reacted with a [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivative 16, which is activated by a leaving group (LG), such as for example 3*H*-[1,2,3]triazolo[4,5-b]pyridin-3-ol, 4-nitrophenol or 1-hydroxypyrrolidine-2,5-dione leading to an intermediate 15. The coupling reaction of tetraamines 4 with [4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid derivatives 16 is carried out in a suitable solvent, such as for example *N,N*-dimethylformamide, to furnish the intermediates 16.

The complexation of intermediates of general formula (VI) with suitable gadolinium (III) compounds or salts, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, is well known to a person skilled in the art. The intermediates of general formula (VI) are dissolved in water and after adding of suitable gadolinium (III) compounds the resulting mixtures are stirred in a temperature range from room temperature up to 100℃ at pH = 1-7 for several hours, to furnish the compounds of general formula (I-d). Intermediates of general formula (VI) are, for example, dissolved in water, gadolinium triacetate tetrahydrate is added and the pH is adjusted to 3.5 - 5.5 by addition of a suitable base, such as for example aqueous sodium hydroxide solution. The reaction is carried out at temperatures ranging from 50℃ to 80℃, leading to compounds of general formula (I-d).

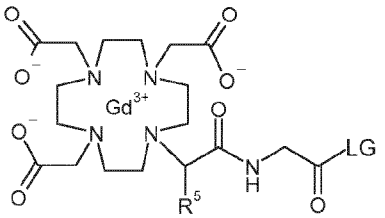
In accordance with an embodiment, the present invention also relates to a method of preparing a compound of general formula (I-a) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (II-a) :



(II-a)

in which  is as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, or a salt thereof,

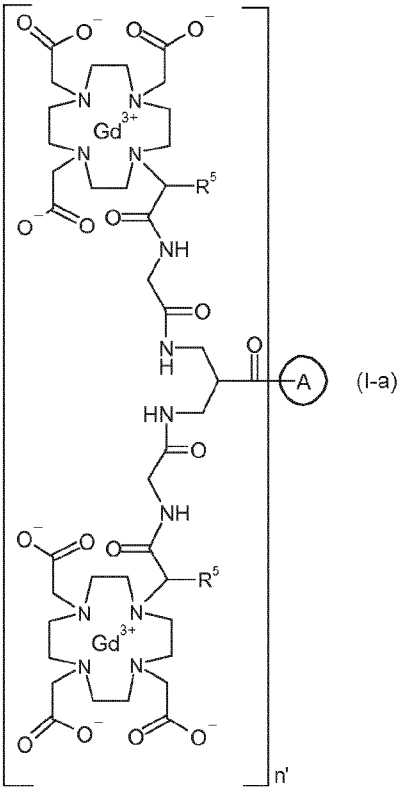
to react with a compound of general formula (III) :



(III)

in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

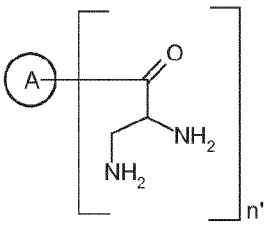
thereby giving a compound of general formula (I-a) :



(I-a)

in which (A) and R^5 are as defined for the compound of general formula (I) *supra*, and n' represents an integer of 2, 3 and 4.

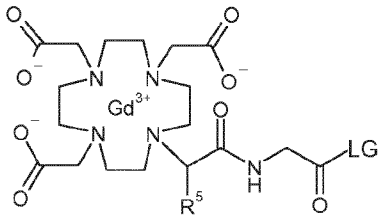
In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-b) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (II-b) :



(II-b)

in which  is as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, or a salt thereof,

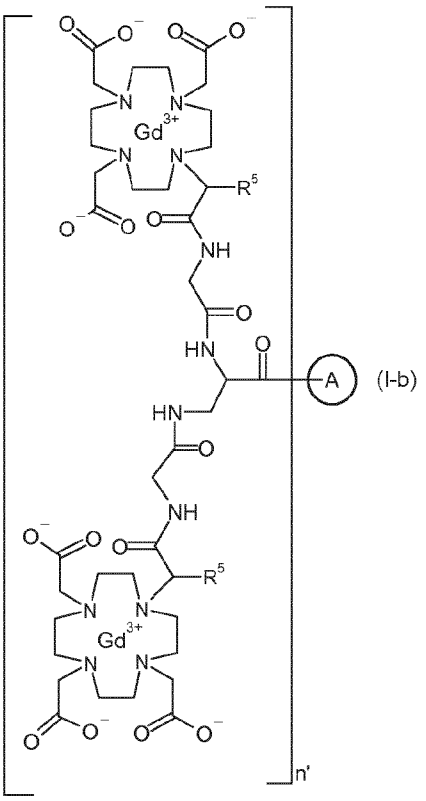
to react with a compound of general formula (III) :



(III)

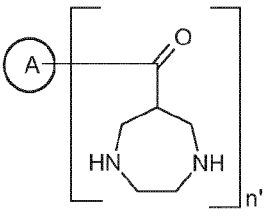
in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

thereby giving a compound of general formula (I-b) :



in which (A) and R^5 are as defined for the compound of general formula (I) *supra*, and n' represents an integer of 2, 3 and 4.

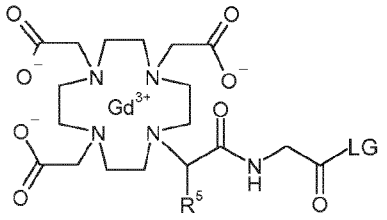
In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-c) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (II-c) :



(II-c)

in which (A) is as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4, or a salt thereof,

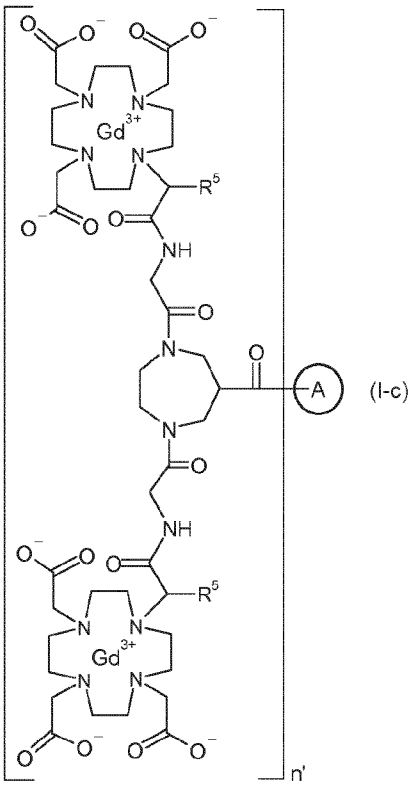
to react with a compound of general formula (III) :



(III)

in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

thereby giving a compound of general formula (I-c) :



(I-c)

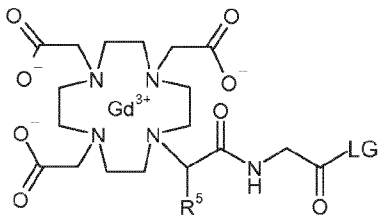
in which (A) and R^5 are as defined for the compound of general formula (I) *supra*, and n' represents an integer of 2, 3 and 4.

In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-d) as defined *supra*, said method comprising the step of allowing a compound of formula 4,

4

in which  is a tetraamine as defined for the compound of general formula (I), *supra*, or a salt thereof,

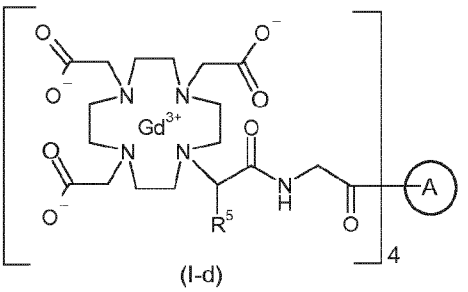
to react with a compound of general formula (III) :



(III)

in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

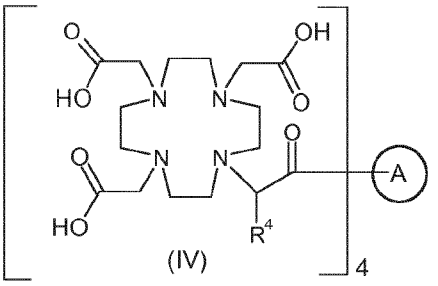
thereby giving a compound of general formula (I-d) :



(I-d)

in which R^5 is as defined for the compound of general formula (I) *supra*, and  is a tetraamine as defined for the compound of general formula (I), *supra*.

In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-e) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (IV) :

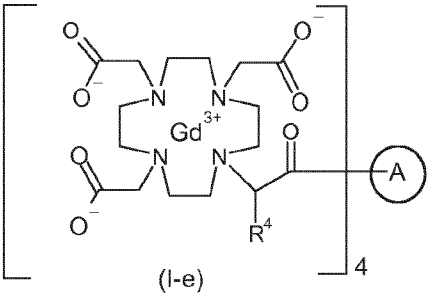


(IV)

in which R^4 is as defined for the compound of general formula (I), *supra*, and A is a tetraamine as defined for the compound of general formula (I), *supra*,

to react with a gadolinium (III) compound, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, or with a salt thereof,

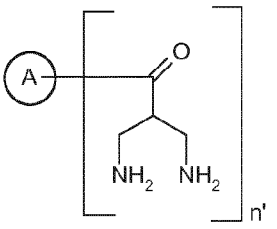
thereby giving a compound of general formula (I-e) :



(I-e)

in which R^4 is as defined for the compound of general formula (I), *supra*, and A is a tetraamine as defined for the compound of general formula (I), *supra*.

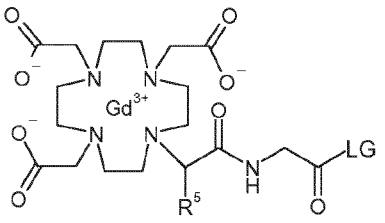
In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-f) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (II-a) :



(II-a)

in which (A) is a triamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, or a salt thereof, or in which (A) is a tetraamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 3, or a salt thereof,

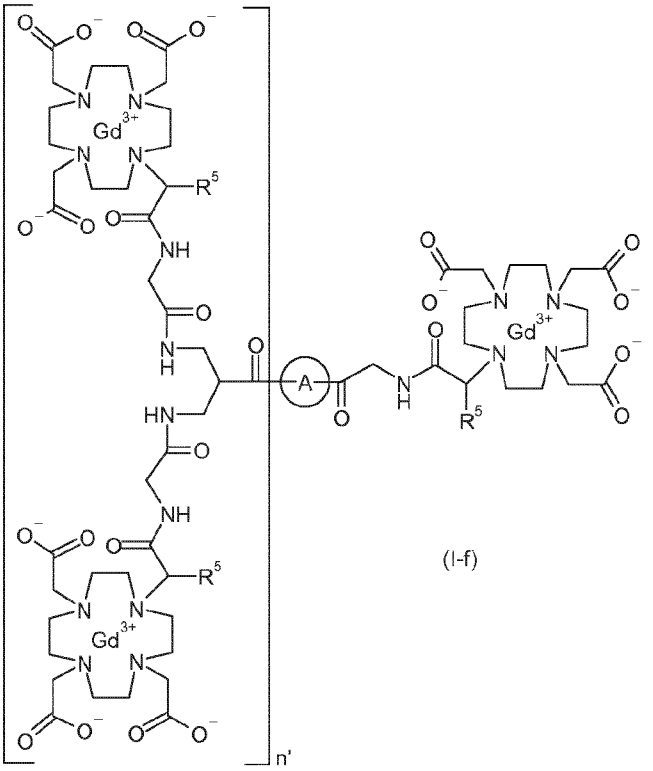
to react with a compound of general formula (III) :



(III)

in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

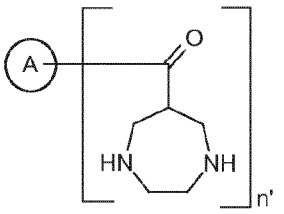
thereby giving a compound of general formula (I-f) :



(I-f)

in which R^5 is as defined for the compound of general formula (I), *supra*, and in which (A) is a triamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, or in which (A) is a tetraamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 3.

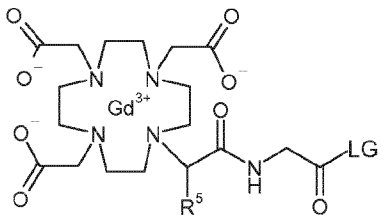
In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-h) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (II-c) :



(II-c)

in which A is a triamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, or a salt thereof, or in which A is a tetraamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 3, or a salt thereof,

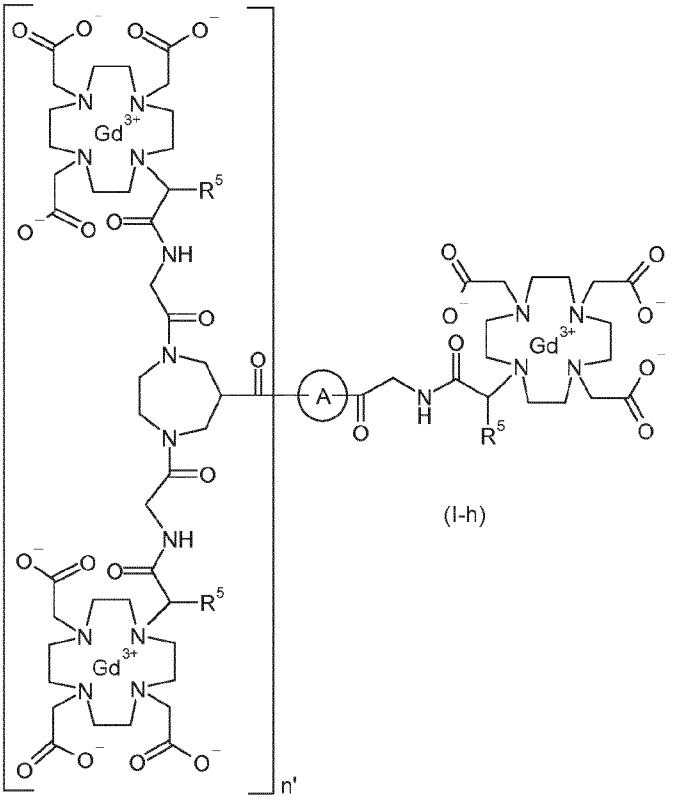
to react with a compound of general formula (III) :



(III)

in which R^5 is as defined for the compound of general formula (I), *supra*, and LG represents an activating leaving group, such as for example 4-nitrophenol, or a group as defined for the synthesis of the compounds of the general formula (I-a) *supra*,

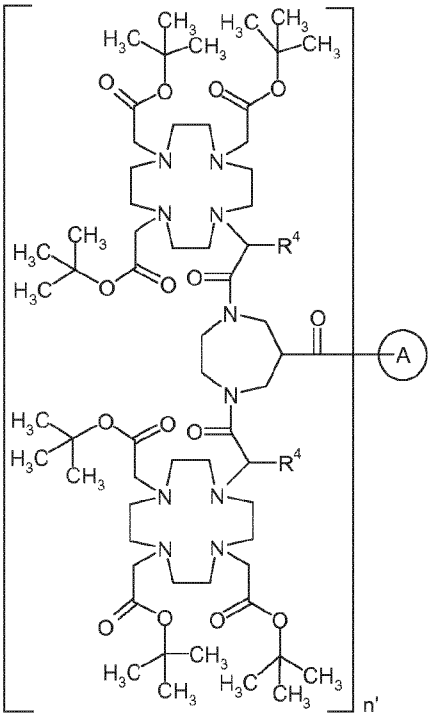
thereby giving a compound of general formula (I-h) :



(I-h)

in which R^5 is as defined for the compound of general formula (I), *supra*, and in which (A) is a triamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, or in which (A) is a tetraamine as defined for the compound of general formula (I), *supra*, and n' represents an integer of 3.

In accordance with another embodiment, the present invention also relates to a method of preparing a compound of general formula (I-k) as defined *supra*, said method comprising the step of allowing an intermediate compound of general formula (V) :



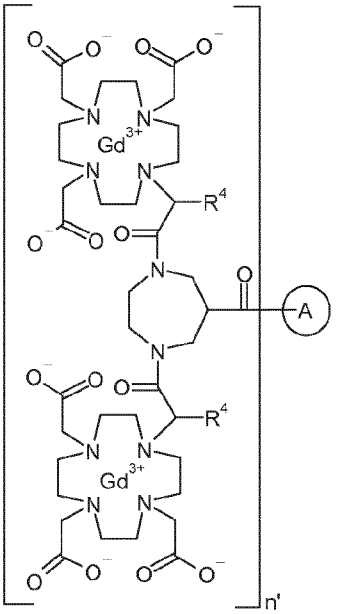
(V)

in which (A) and R^4 are as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4,

in a first step to react with an acid, such as for example aqueous hydrochloric acid, and

in a second step to react with a gadolinium (III) compound, such as for example gadolinium trioxide, gadolinium triacetate or hydrates of gadolinium triacetate, gadolinium trichloride or gadolinium trinitrate, or with a salt thereof,

thereby giving a compound of general formula (I-k) :



(I-k)

in which (A) and R^4 are as defined for the compound of general formula (I), *supra*, and n' represents an integer of 2, 3 and 4.

DESCRIPTION OF THE FIGURES

Figure 1 shows the blood plasma kinetic of Example 3 versus Gadovist® in rats. The pharmacokinetic profile of Example 3 is comparable to that of Gadovist®.

Figure 2 shows the evolution of the relative water proton paramagnetic longitudinal relaxation rate $R_1^P(t)/R_1^P(0)$ versus time of Example 3, Reference compound 1 (Gadovist®), Reference compound 2 (Magnevist®) and Reference compound 3 (Primovist®). The stability of Example 3 is comparable to the high stability macrocyclic Reference compound 1 (Gadovist®).

Figure 3 shows the magnetic resonance angiography data in male New Zealand white rabbits: (A) 30 µmol Gd/kg bw Reference compound 1 (Gadovist®); (B) 30 µmol Gd/kg bw Example 3 and (C) 100 µmol Gd/kg bw Reference compound 1. The contrast enhancement of the low dose protocol with Example 3 (B) is comparable to that of the standard dose of Reference compound 1 (C). Furthermore, the image quality of the low dose protocol of Example 3 (B) is significantly better than the low dose protocol of Reference compound 1 (A). The angiography study demonstrates the potential for Example 3 for a significant dose reduction.

Figure 4 MR images before and after administration of contrast agent. Representative images of the head and neck region before and 1.4 min after administration of Example 3 (A) and reference compound 1 (B). The strong signal enhancement is visible for example in the heart, the tongue and the neck muscle.

Figure 5 MR images before and after administration of contrast agent. Representative images of the abdominal region before and 0.5 min after administration of Example 3 (A) and reference compound 1 (B). The strong signal enhancement is visible for example in the aorta, kidney, liver and spleen.

Figure 6 MR images before and after administration of contrast agent. Representative images of the pelvis region before and 2.9 min after administration of Example 3 (A) and reference compound 1 (B). The strong signal enhancement is visible for example in the vascular system (vessels) and the extremity muscles.

Figure 7 MRI signal enhancements for different body regions.
Signal enhancement over time after administration of Example 3 and Reference compound 1 (Gadovist®) for tongue, chops muscle, liver, spleen, aorta and extremity muscle. No differences in the time course of signal changes were observed between Example 3 and reference compound 1. This demonstrates identical pharmacokinetic properties and indicates the potential of Example 3 for the imaging of different body regions. As expected from the approximately 2-fold higher relaxivity (see example A) the observed contrast enhancements of Example 3 were higher compared to that of reference compound 1 (Gadovist®). The vertical bars represent the standard deviation.

Figure 8 Correlation of tissue gadolinium concentration and MRI signal enhancement.
The gadolinium concentration was measured in tissue samples of the brain, tongue, liver, spleen, blood and extremity muscle (muscle) and respective MRI signal changes determined in-vivo, after administration of Example 3 and reference compound 1. The vertical and horizontal error bars represent the standard deviation. The dotted lines represent the linear regression between gadolinium concentration and MRI signal change.

Figure 9 Diffusion of different contrast agents through semipermeable membranes (20 kDa).
Dynamic CT measurements were performed to show the ability of different contrast agents to diffuse through a semipermeable membrane. (A) CT images of Example 1, 2, 3, 4, 5 and 6 in comparison to that of Reference compound 1 (Gadovist®) and 4 (Gadomer). A representative measurement region for the signal evaluation over time is indicated in the image A1.

Figure 10 Signal analysis of dynamic CT diffusion phantom study over time. Signal in Hounsfield units (HU) over time of the dialysis cassette in fetal bovine solution for Example 1-6 and reference compounds 1 and 4 demonstrate that contrary to Reference compound 4 (Gadomer) all of the investigated compound are able to pass the semipermeable membrane (20 kDa).

Figure 11 Contrast-enhanced magnetic resonance images of GS9L brain tumors in rats (marked with white arrows). (A) Intraindividual comparison of Reference compound 1 (Gadovist®) and Example 3 at the same dose of 0.1 mmol Gd/kg body weight (bw). Example 3 showed higher lesion-to-brain contrast and an excellent demarcation of the tumor rim. (B) Comparison of the Reference compound 1 (Gadovist®) at 0.3 mmol Gd/kg bw and Example 3 at 0.1 mmol Gd/kw bw. Example 3 showed similar lesion-to-brain contrast at one third of the dose of Reference compound 1.

EXPERIMENTAL SECTION

Abbreviations

ACN	acetonitrile
AUC	area under the curve
br	broad signal (in NMR data)
bw	body weight
CPME	cyclopentyl methyl ether
CPMG	Carr-Purcell-Meiboom-Gill (MRI sequence)
C_{Gd}	concentration of the compound normalized to the Gadolinium
CI	chemical ionisation
Cl_{tot}	total clearance
d	day(s)
DAD	diode array detector
DCM	dichloromethane
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	deuterated dimethylsulfoxide
ECCM	extracellular contrast media
EI	electron ionisation
ELSD	evaporative light scattering detector
ESI	electrospray ionisation
FBS	fetal bovine serum
h	hour
HATU	*N*-[(dimethylamino)(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)-methylidene]-*N*-methylmethanaminium hexafluorophosphate
HCOOH	formic acid
HPLC	high performance liquid chromatography
HU	Hounsfield units
IR	inversion recovery
kDa	kilo Dalton
LCMS	liquid chromatography-mass spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
MRI	magnetic resonance imaging
MRT	mean residence time
MS	mass spectrometry

m	multiplet
min	minute(s)
NMR	nuclear magnetic resonance spectroscopy : chemical shifts (δ) are given in ppm.
r_i	(where i=1, 2) relaxivities in L $mmol^{-1}$ s^{-1}
Rt.	retention time
s	singlet
RC	reference compound
R_i	(where i=1, 2) relaxation rates ($1/T_{1,2}$)
$R_{i(0)}$	relaxation rate of the respective solvent
$T_{1,2}$	relaxation time
T	Tesla
t	triplet
t½ α	plasma half-life, compartment V1
t½ β	plasma half-life, compartment V2
t½ γ	plasma half-life, compartment V3
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TI	inversion time
UPLC	ultra performance liquid chromatography
V1 + V2	volume, compartments V1+V2
V_c (V1)	volume, central compartment V1
$V_{d,ss}$	volume of distribution at steady state

Materials and Instrumentation

The chemicals used for the synthetic work were of reagent grade quality and were used as obtained.

All reagents, for which the synthesis is not described in the experimental section, are either commercially available, or are known compounds or may be formed from known compounds by known methods by a person skilled in the art.

^{1}H-NMR spectra were measured in $CDCl_3$, D_2O or DMSO-d_6, respectively (room temperature, Bruker Avance 400 spectrometer, resonance frequency: 400.20 MHz for ^{1}H or Bruker Avance 300 spectrometer, resonance frequency: 300.13 MHz for ^{1}H. Chemical shifts

are given in ppm relative to sodium (trimethylsilyl)propionate-d_4 (D_2O) or tetramethylsilane (DMSO-d_6) as external standards (δ = 0 ppm).

The compounds and intermediates produced according to the methods of the invention may require purification. Purification of organic compounds is well known to the person skilled in the art and there may be several ways of purifying the same compound. In some cases, no purification may be necessary. In some cases, the compounds may be purified by crystallization. In some cases, impurities may be stirred out using a suitable solvent. In some cases, the compounds may be purified by chromatography, particularly flash column chromatography, using for example prepacked silica gel cartridges, e.g. Biotage SNAP cartidges KP-Sil® or KP-NH® in combination with a Biotage autopurifier system (SP4® or Isolera Four®) and eluents such as gradients of hexane/ethyl acetate or DCM/methanol. In some cases, the compounds may be purified by preparative HPLC using for example a Waters autopurifier equipped with a diode array detector and/or on-line electrospray ionization mass spectrometer in combination with a suitable prepacked reverse phase column and eluents such as gradients of water and acetonitrile which may contain additives such as trifluoroacetic acid, formic acid or aqueous ammonia.

Examples were analyzed and characterized by the following HPLC based analytical methods to determine characteristic retention time and mass spectrum:

Method 1: UPLC (ACN-HCOOH):
Instrument: Waters Acquity™ UPLC-MS SQD 3001; column: Acquity UPLC BEH C18 1.7 µm, 50x2.1mm; eluent A: water + 0.1% formic acid, eluent B: acetonitril; gradient: 0-1.6 min 1-99% B, 1.6-2.0 min 99% B; flow 0.8 mL/min; temperature: 60 °C; injection: 2 µl; DAD scan: 210-400 nm; ELSD.

Method 2: UPLC (ACN-HCOOH polar):
Instrument: Waters Acquity UPLC-MS SQD 3001; column: Acquity UPLC BEH C18 1.7 µm, 50x2.1mm; eluent A: water + 0.1% formic acid, eluent B: acetonitril; gradient: 0-1.7 min 1-45% B, 1.7-2.0 min 45-99% B; flow 0.8 mL/min; temperature: 60 °C; injection: 2 µl; DAD scan: 210-400 nm; ELSD.

Method 3: UPLC (ACN-HCOOH long run):
Instrument: Waters Acquity UPLC-MS SQD 3001; column: Acquity UPLC BEH C18 1.7 µm, 50x2.1mm; eluent A: water + 0.1% formic acid, eluent B: acetonitril; gradient: 0-4.5 min 0-10% B; flow 0.8 mL/min; temperature: 60 °C; injection: 2 µl; DAD scan: 210-400 nm; ELSD.

Date Reçue/Date Received 2020-12-07

Method 4: UPLC ($ACN-NH_3$):

Instrument: Waters Acquity UPLC-MS ZQ2000; column: Acquity UPLC BEH C18 1.7 µm, 50x2.1 mm; Eluent A: water + 0.2% ammonia , eluent B: acetonitrile; gradient: 0-1.6 min 1-99% B, 1.6-2.0 min 99% B; flow rate 0.8 mL/min; temperature: 60 °C; injection: 1 µL; DAD scan: 210-400 nm; ELSD.

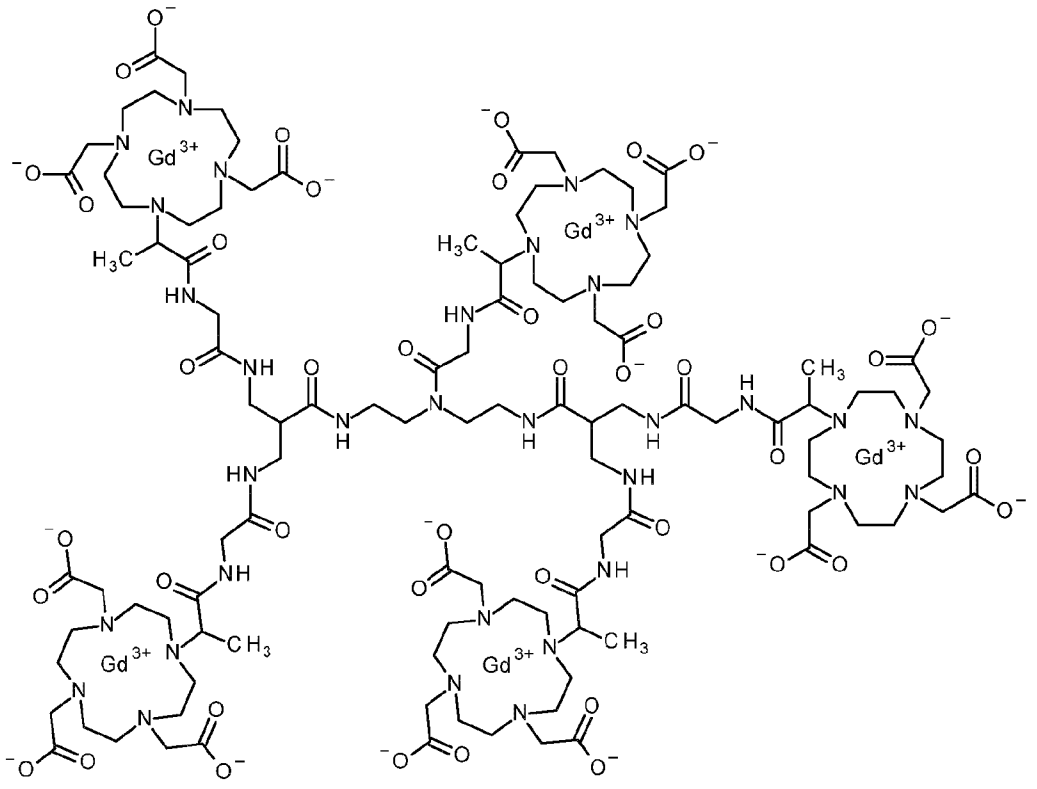
Method 5: LC-MS:

Instrument: Agilent 1290 UHPLCMS Tof; column: BEH C 18 (Waters) 1.7 µm, 50x2.1 mm; eluent A: water + 0.05 vol-% formic acid (99%), eluent B: acetonitrile + 0.05% formic acid; gradient: 0-1.7 min 98-10% A, 1.7-2.0 min 10% A, 2.0-2.5 min 10-98% A, flow 1.2 mL/min; temperature: 60 °C; DAD scan: 210-400 nm.

Example Compounds

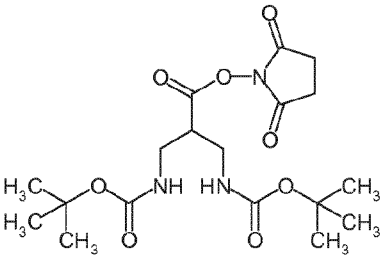
Example 1

Pentagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,18,22,25-hexaoxo-26-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-14-[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-9,19-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoyl}amino)acetyl]amino}methyl)-4,7,11,14,17,21,24-heptaazaheptacosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate



Example 1a

Di-*tert*-butyl (2-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}propane-1,3-diyl)biscarbamate



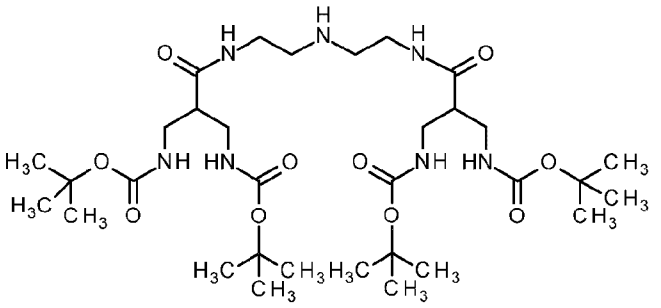
3.60 g (11.3 mmol, 1 eq.) 3-[(*tert*-butoxycarbonyl)amino]-2-{[(*tert*-butoxycarbonyl)amino]-methyl}propanoic acid (see WO 2006/136460 A2) and 1.43 g (12.4 mmol, 1.1 eq.) 1-hydroxypyrrolidine-2,5-dione were dissolved in 120 mL THF. To the reaction mixture was added dropwise a solution of 2.57 g (12.4 mmol, 1.1 eq.) *N,N'*-dicyclohexylcarbodiimide in 60 mL THF. After stirring for 3 hours at room temperature, the resulting suspension was cooled to 0℃ and the precipitated urea was filtere d off. The clear solution was evaporated to dryness yielding 5.50 g (13.24 mmol, 117 %) of the title compound.

UPLC (ACN-HCOOH): Rt. = 1.15 min.

MS (ES^+): m/z = 416.3 $(M + H)^+$.

Example 1b

***Tert*-butyl (7,17-bis{[(*tert*-butoxycarbonyl)amino]methyl}-2,2-dimethyl-4,8,16-trioxo-3-oxa-5,9,12,15-tetraazaoctadecan-18-yl)carbamate**



4.70 g (11.3 mmol, 2.22 eq.) Di-*tert*-butyl (2-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}propane-1,3-diyl)biscarbamate (example 1a) were dissolved in 120 mL THF. To the reaction mixture

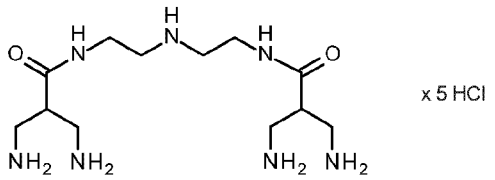
was added dropwise a solution of 0.53 g (5.10 mmol, 1 eq.) *N*-(2-aminoethyl)ethane-1,2-diamine and 1.14 g (11.3 mmol, 2.22 eq.) triethylamine in 40 mL THF. After stirring for 3 hours at room temperature, the resulting suspension was diluted with dichloromethane. The organic solution was washed with aqueous sodium hydroxide (0.1 M), with water and was dried over sodium sulfate. The crude product was isolated by evaporation under reduced pressure and was purified by silica gel chromatography yielding 2.81 g (3.99 mmol, 78%) of the title compound.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.36 (s, 36 H), 2.39 - 2.47 (m, 3 H), 2.52 - 2.58 (m, 4 H), 2.95 - 3.20 (m, 12 H), 6.64 (t, 4 H), 7.72 (t, 2 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.06 min.
MS (ES^+): m/z = 704.6 (M^+ + H).

Example 1c

***N,N'*-(Iminodiethane-2,1-diyl)bis[3-amino-2-(aminomethyl)propanamide] pentahydrochloride**



600 mg (0.85 mmol) *Tert*-butyl (7,17-bis{[(*tert*-butoxycarbonyl)amino]methyl}-2,2-dimethyl-4,8,16-trioxo-3-oxa-5,9,12,15-tetraazaoctadecan-18-yl)carbamate (example 1b) were dissolved in 9.6 mL methanol and 2.85 mL aqueous hydrochloric acid (37%). The reaction mixture was heated under stirring for 2 hours at 50℃. For isolation the suspension was evaporated to dryness yielding 423 mg (0.87 mmol, 102%) of the title compound.

^{1}H-NMR (400 MHz, D_2O): δ = 3.04 - 3.15 (m, 2 H), 3.17 - 3.27 (m, 8 H), 3.29 - 3.38 (m, 4 H), 3.55 (t, 4 H) ppm.

UPLC (ACN-HCOOH): Rt. = 0.19 min.
MS (ES^+): m/z = 304.2 $(M + H)^+$, free base.

Example 1

Pentagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,18,22,25-hexaoxo-26-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-14-[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-9,19-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoyl}amino)acetyl]amino}methyl)-4,7,11,14,17,21,24-heptaazaheptacosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate

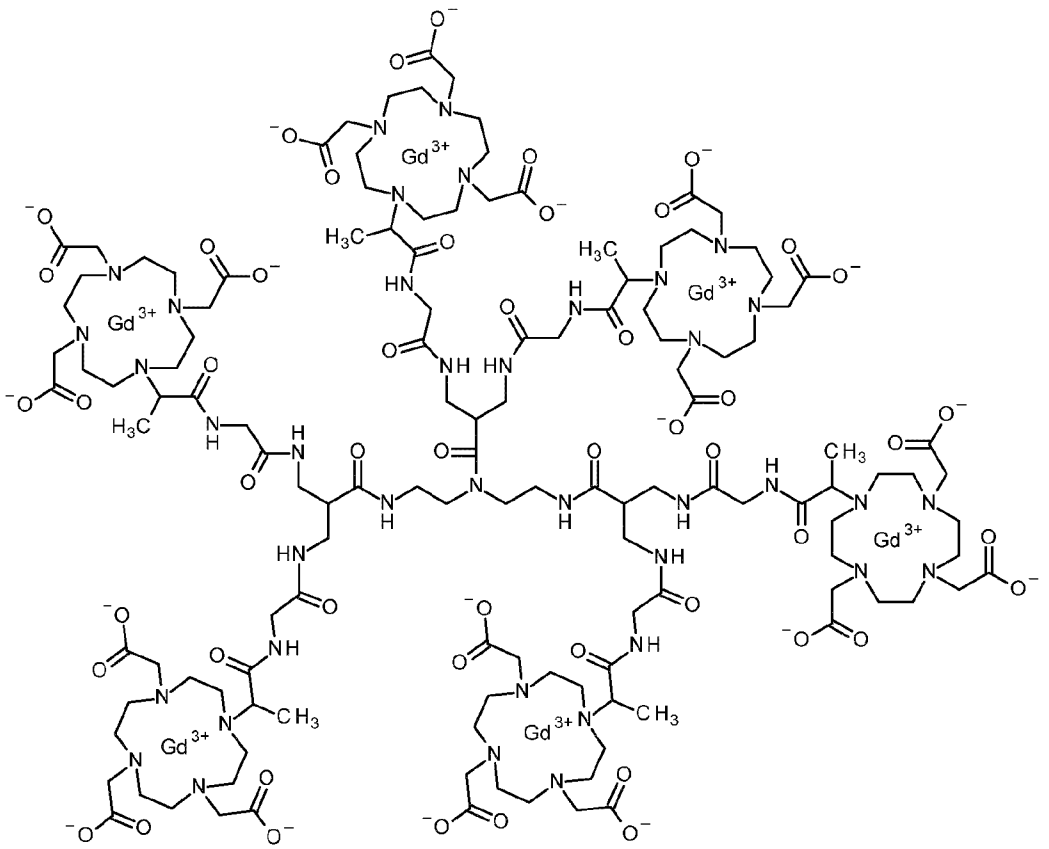
150 mg (309 µmol, 1 eq.) *N,N'*-(Iminodiethane-2,1-diyl)bis[3-amino-2-(aminomethyl)-propanamide] pentahydrochloride (example 1c) were dissolved in 60 mL DMSO. After adding of 499 mg (3.86 mmol, 12.5 eq.) *N,N*-diisopropylethylamine and 4.06 g (5.40 mmol, 17.5 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2) the resulting reaction mixture was stirred and heated for 8 hours at 50°C. The cooled solution was concentrated under reduced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 668 mg (64%, 199 µmol) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.46 min.

MS (ES^-): m/z (z = 2) = 1680.5 $(M - 2H)^{2-}$; (ES^+): m/z (z =3) = 1121.3 $(M + H)^{3+}$, m/z (z = 4) = 841.4 $[(M + H)^{4+}$.

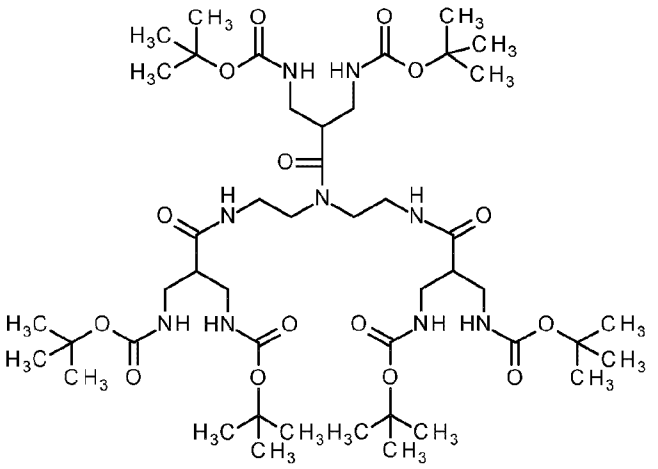
Example 2

Hexagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,15,19,22-hexaoxo-23-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,16-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-11-{2-{[3-{[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclo-dodecan-1-yl]propanoyl}amino)acetyl]amino}-2-({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)propanoyl]-amino}ethyl)-4,7,11,14,18,21-hexaazatetracosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate



Example 2a

***Tert*-butyl (12-{2-[(3-[(*tert*-butoxycarbonyl)amino]-2-{[(*tert*-butoxycarbonyl)amino]-methyl}propanoyl)amino]ethyl}-7,14-bis{[(*tert*-butoxycarbonyl)amino]methyl}-2,2-dimethyl-4,8,13-trioxo-3-oxa-5,9,12-triazapentadecan-15-yl)carbamate**



890 mg (2.80 mmol, 3 eq.) 3-[(*Tert*-butoxycarbonyl)amino]-2-{[(*tert*-butoxycarbonyl)amino]-methyl}propanoic acid (see WO 2006/136460 A2) were dissolved in 22 mL DMF. To the solution were added 434 mg (3.36 mmol, 3.6 eq.) *N*,*N*-diisopropylethylamine and 1.28 g (3.36 mmol, 3.6 eq.) HATU. The resulting reaction mixture was stirred for 2 hours at room temperature. After dropwise adding of a solution of 96.1 mg (0.93 mmol, 1 eq.) *N*-(2-amino-ethyl)ethane-1,2-diamine and of 434 mg (3.36 mmol, 3.6 eq.) *N*,*N*-diisopropylethylamine in 9 mL DMF, the resulting reaction mixture was heated under stirring for 3 hours at 70℃. After cooling and diluting with dichloromethane, the solution was washed with aqueous sodium hydroxide (0.1 M), aqueous citric acid (1%) and water and was dried over sodium sulfate. The crude product was isolated by evaporation under reduced pressure and was purified by silica gel chromatography yielding 451 mg (0.45 mmol, 48%) of the title compound.

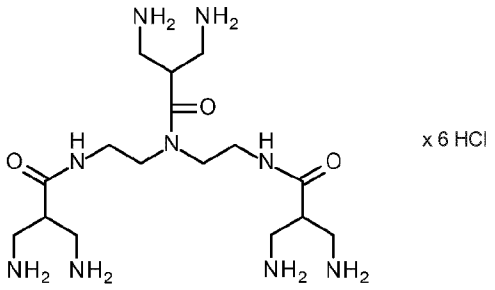
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.37 (s, 54 H), 2.36 - 2.49 (m, 3 H), 2.81 - 3.30 (m, 17 H), 3.36 - 3.70 (m, 3 H), 6.16 - 6.92 (m, 6 H), 7.77 - 8.35 (m, 2 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.49 min.

MS (ES^+): m/z = 1004.6 $(M + H)^+$.

Example 2b

3-Amino-*N*,*N*-bis(2-{[3-amino-2-(aminomethyl)propanoyl]amino}ethyl)-2-(aminomethyl)-propanamide hexahydrochloride



581 mg (0.58 mmol) *Tert*-butyl (12-{2-[(3-[(tert-butoxycarbonyl)amino]-2-{[(*tert*-butoxycarbonyl)amino]methyl}propanoyl)amino]ethyl}-7,14-bis{[(*tert*-butoxycarbonyl)amino]methyl}-2,2-dimethyl-4,8,13-trioxo-3-oxa-5,9,12-triazapentadecan-15-yl)carbamate (example 2a) were dissolved in 9.3 mL methanol and 2.9 mL aqueous hydrochloric acid (37%). The reaction mixture was heated under stirring for 2 hours at 50°C. For isolation the suspension was evaporated to dryness yielding 376 mg (0.60 mmol, 103%) of the title compound.

^{1}H-NMR (400 MHz, D_2O): δ = 3.13 - 3.27 (m, 2 H), 3.28 - 3.85 (m, 21 H) ppm.

UPLC (ACN-HCOOH): Rt. = 0.19 min.
MS (ES^+): m/z = 404.3 $(M + H)^+$, free base.

Example 2

Hexagadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,10,15,19,22-hexaoxo-23-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,16-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-11-(2-{[3-{[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclo-dodecan-1-yl]propanoyl}amino)acetyl]amino}-2-({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)propanoyl]-amino}ethyl)-4,7,11,14,18,21-hexaazatetracosan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate

150 mg (241 µmol, 1 eq.) 3-Amino-*N*,*N*-bis(2-{[3-amino-2-(aminomethyl)propanoyl]amino}-ethyl)-2-(aminomethyl)propanamide hexahydrochloride (example 2b) were dissolved in 60

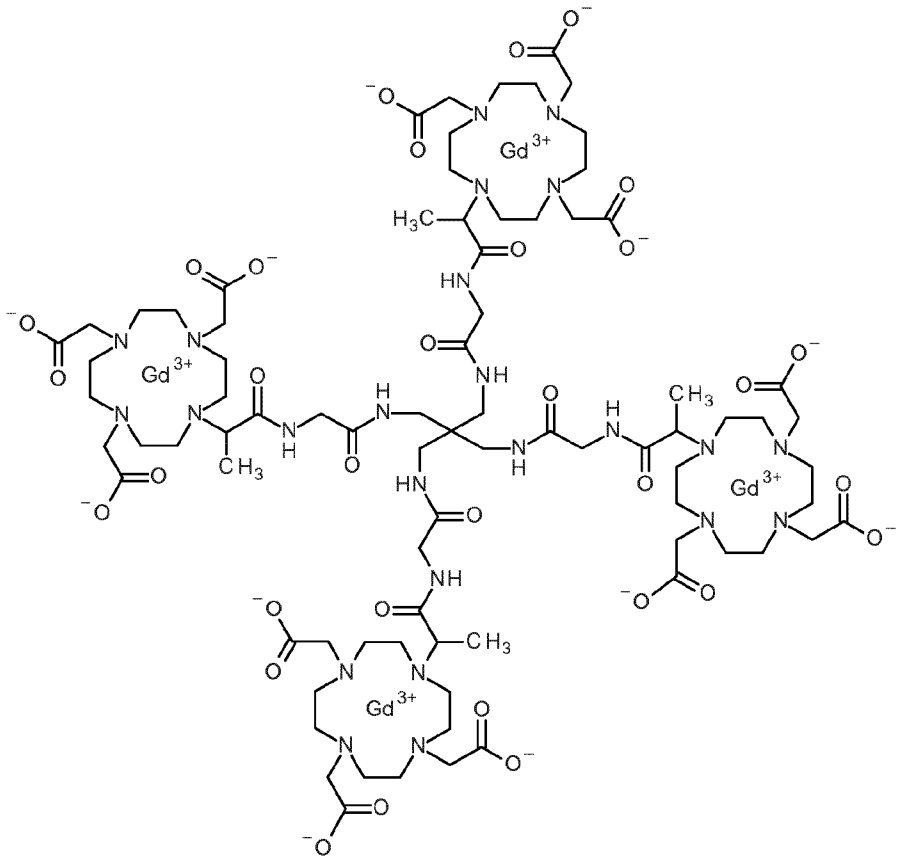
mL DMSO. After adding of 467 mg (3.62 mmol, 15 eq.) *N,N*-diisopropylethylamine and 3.80 g (5.06 mmol, 21 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2) the resulting reaction mixture was stirred and heated for 8 hours at 50℃. The cooled solution was concentrated under reduced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 677 mg (166 µmol, 69%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.44 min.

MS (ES^+): m/z (z = 3) = 1357.4 $(M + 3H)^{3+}$, m/z (z = 4) = 1018.8 $(M + 4H)^{4+}$], m/z (z = 5) = 815.7 $(M + 5H)^{5+}$.

Example 3

Tetragadolinium [4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate



225 mg (1.65 mmol, 1 eq.) 2,2-Bis(aminomethyl)propane-1,3-diamine (see W. Hayes et al., Tetrahedron 59 (2003), 7983 - 7996) were dissolved in 240 mL DMSO. After addition of 1.71 g (13.2 mmol, 8 eq.) *N,N*-diisopropylethylamine and 14.9 g (19.85 mmol, 12 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2) the resulting reaction mixture was stirred and heated for 8 hours at 50°C. The cooled solution was concentrated under reduced pressure to a final volume of 40 - 50 mL. The concentrate was poured under stirring in 600 mL ethyl acetate, the formed precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water

using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 3.42 g (80%, 1.33 mmol) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.42 min.

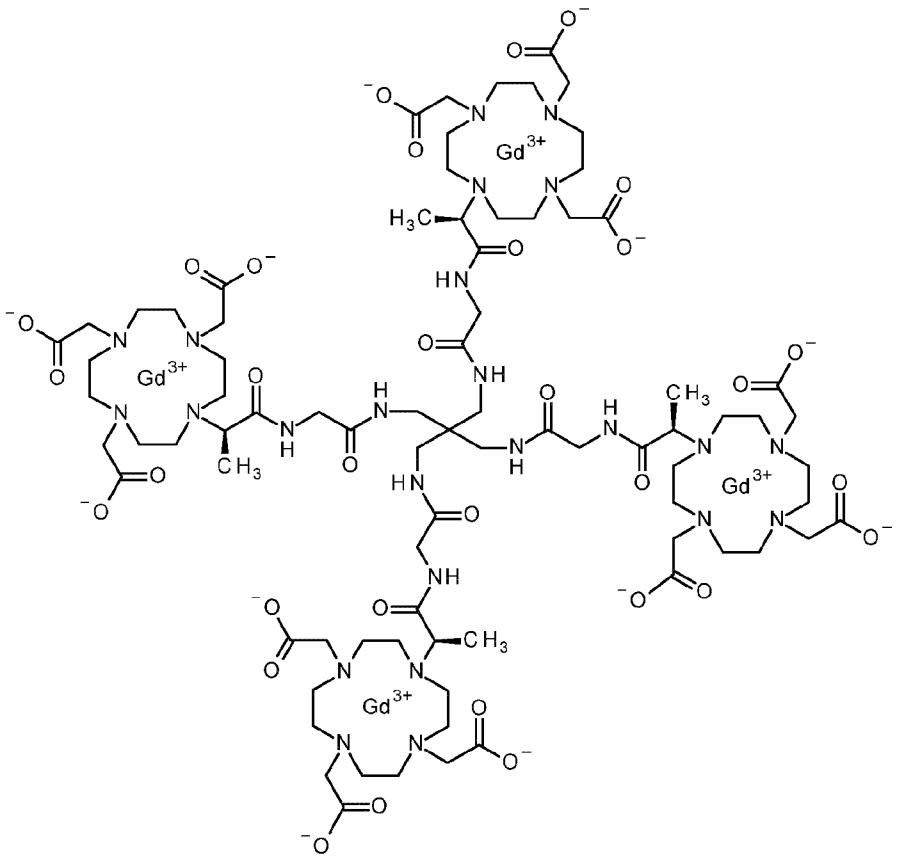
MS (ES^+): m/z (z = 2) = 1290.4 $(M + H)^{2+}$, m/z (z = 3) = 860.7 $(M + H)^{3+}$.

Example 3 comprises a mixture of stereoisomers, which exhibit the following absolute configurations:

all-R, all-S, RRRS, SSSR, RRSS.

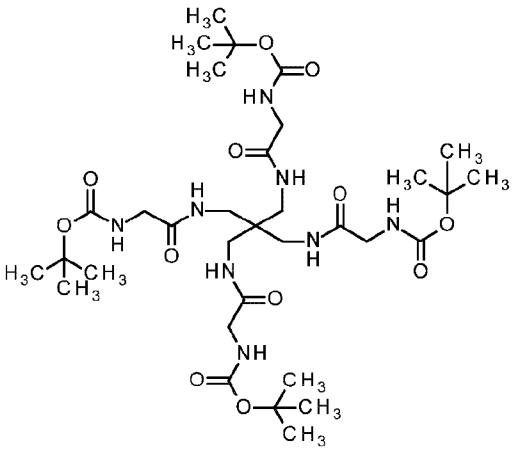
Example 3-1

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)-acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate



Example 3-1a

***Tert*-butyl {10,10-bis[({[(*tert*-butoxycarbonyl)amino]acetyl}amino)methyl]-2,2-dimethyl-4,7,13-trioxo-3-oxa-5,8,12-triazatetradecan-14-yl}carbamate**



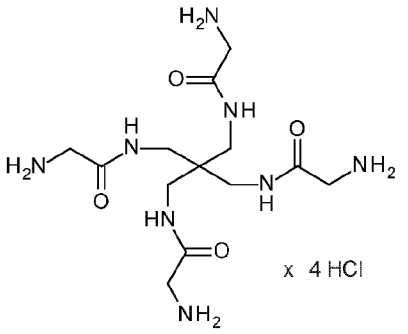
A mixture of 2,2-bis(aminomethyl)propane-1,3-diamine tetrahydrochloride (851 mg, 3.06 mmol, 1 eq.; see W. Hayes et al., Tetrahedron 59 (2003), 7983 - 7996) in dichloromethane (50 mL) was treated with *N,N*-diisopropylethylamine (6.00 eq., 3.20 mL, 18.4 mmol) and 2,5-dioxopyrrolidin-1-yl *N*-(*tert*-butoxycarbonyl)glycinate (CAS No. [3392-07-2]; 6.00 eq., 5.00 g, 18.4 mmol) and stirred at room temperature for 2.5 days. The reaction mixture was diluted with water, the formed precipitate filtered off and washed with water and dichloromethane. The precipitated material was subjected to silica gel chromatography (dichloromethane / methanol) to give the title compound (800 mg, 34%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.36 (s, br, 36H), 2.74 - 2.76 (m, 8H), 3.48 - 3.50 (m, 8H), 6.96 (s, br, 0.4H*), 7.40 - 7.42 (m, 3.6H*), 7.91 - 8.00 (m, 4H) ppm.

LC-MS (ES^+): m/z = 761.4 $(M + H)^+$; Rt. = 1.16 min.

Example 3-1b

2-Amino-*N*-(3-[(aminoacetyl)amino]-2,2-bis{[(aminoacetyl)amino]methyl}propyl)acetamide tetrahydrochloride



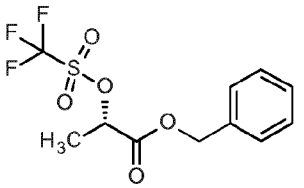
A suspension of *tert*-butyl {10,10-bis[({[(*tert*-butoxycarbonyl)amino]acetyl}amino)methyl]-2,2-dimethyl-4,7,13-trioxo-3-oxa-5,8,12-triazatetradecan-14-yl}carbamate (1.00 eq., 800 mg, 1.05 mmol) from example 11a in CPME (10 mL) was cooled to 0 °C and treated dropwise with HCl in CPME (10 eq., 3.5 mL of a 3 M solution, 10.5 mmol). The reaction mixture was stirred at 0 °C for 1 h and at rt overnight upon wh ich dioxane (4 mL) and another amount of HCl in CPME (30 eq., 11 mL of a 3 M solution, 32 mmol) were added and stirring at rt continued for 2 days. The resulting suspension was concentrated in vacuo to give the title compound (575 mg, quant.) which was not further purified.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.17 - 3.18 (m, 8H), 3.59 - 3.61 (m, 8H), 8.21 (s, br, 12H), 8.55 (t, 4H) ppm.

LC-MS (ES^+): m/z = 361.2 $(M - 3HCl - Cl)^+$; Rt. = 0.10 min.

Example 3-1c

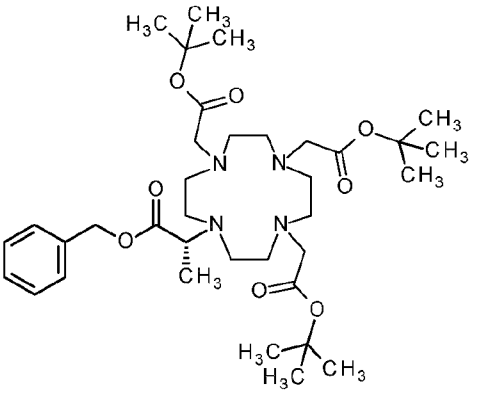
Benzyl (2S)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate



Prepared according to H.C.J. Ottenheim et al., Tetrahedron 44 (1988), 5583 – 5595: A solution of (S)-(-)-lactic acid benzyl ester (CAS No. [56777-24-3]; 1.00 eq., 5.00 g, 27.7 mmol) in dry dichloromethane (95 mL) was cooled to 0 °C and treated with trifluoromethanesulfonic anhydride (CAS No. [358-23-6]; 1.1 eq., 5.2 mL, 8.6 g, 31 mmol). After stirring for 5 min 2,6-dimethylpyridine (1.15 eq., 3.72 mL, 3.42 g) was added and stirring continued for another 5 min. The obtained reaction mixture was directly used in the next step.

Example 3-1d

Benzyl (2R)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoate



A solution of tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (CAS No. [122555-91-3]; 1.00 eq., 9.52 g, 18.5 mmol) in dry dichoromethane (75 mL) was cooled to 0 °C and treated with the reaction mixture of benzyl (2S)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate in dichloromethane prepared in example 3-1c; and *N,N*-diisopropyl-

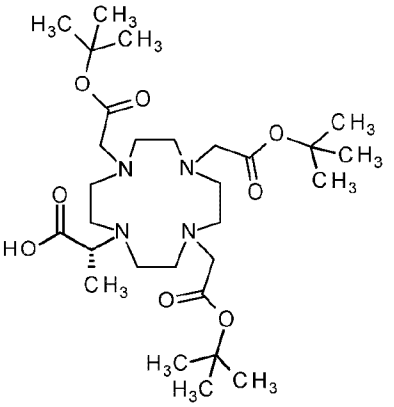
ethylamine (3.0 eq, 9.7 mL, 55 mmol). The resulting solution was stirred at rt for 6 days upon which it was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The obtained material was purified by amino phase silica gel chromatography (KP-NH®, hexane/ethyl acetate to dichloromethane/methanol) to give the title compound (1.92 g, 14%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.20 (d, 3H), 1.37 - 1.45 (m, 27H), 1.98 - 2.01 (m, 3H), 2.08 - 2.24 (m, 5H), 2.57 - 2.84 (m, 7H), 2.94 - 3.11 (m, 4H), 3.38 - 3.48 (m, 3H), 3.75 (q, 1H), 5.07 - 5.17 (m, 2H), 7.32 - 7.40 (m, 5H) ppm.

LC-MS (ES^+): m/z = 677.5 $(M + H)^+$, m/z (z =2) = 339.2 $(M + H)^{2+}$; Rt. = 1.06 min.

Example 3-1e

(2R)-2-[4,7,10-Tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoic acid



A solution of benzyl (2R)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoate (example 3-1d; 1.92 g, 2.84 mmol) in methanol (17.5 mL) was treated with Pd/C (10wt%; 0.050 eq., 151 mg, 0.14 mmol) and stirred under a hydrogen atmosphere at room temperature for 20 hours. The reaction mixture was filtrated over Celite®, washed with methanol and the filtrate concentrated in vacuo to give the title compound (1.51 g, 88%) which was not further purified.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.11 (s, br, 3H), 1.42 - 1.43 (m, 27H), 1.97 - 2.13 (m, 5H), 2.56 - 2.82 (m, 7H), 2.97 - 3.07 (m, 4H), 3.34 - 3.53 (m, 7H), 12.8 (s, br, 1H) ppm.

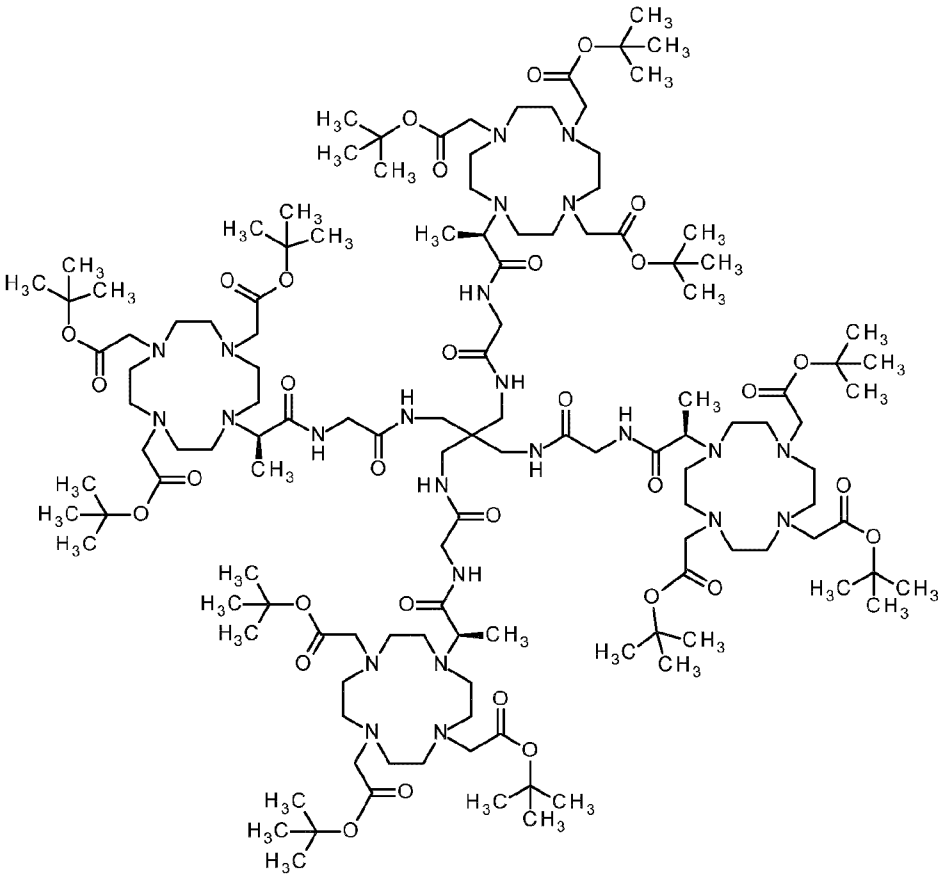
UPLC (ACN-NH_3): Rt. = 1.31 min.

MS (ES^+): m/z = 587 $(M + H)^+$.

LC-MS (ES^+): m/z = 587 $(M + H)^+$, m/z (z =2) = 294.2 $(M + H)^{2+}$; Rt. = 0.79 min.

Example 3-1f

***Tert*-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}-amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclo-dodecan-1-yl}acetate**



A mixture of (2R)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoic acid (example 3-1e; 12.0 eq., 1.50 g, 2.56 mmol) in *N,N*-dimethylacetamide (15 mL) was treated with HATU (14.4 eq., 1.17 g, 3.07 mmol) and *N,N*-diisopropylethylamine (14.4 eq, 534 µL, 3.07 mmol) and stirred at rt for 20 minutes. A suspension of 2-amino-*N*-(3-[(aminoacetyl)amino]-2,2-bis{[(aminoacetyl)amino]methyl}propyl)acetamide tetrahydrochloride (example 3-1b; 1.00 eq., 108 mg, 213 µmol) in *N,N*-dimethylacetamide (6 mL) was added and the resulting mixture stirred at 50 °C overnight. The reaction mixture was concentrated under reduced pressure and the obtained residue subjected to amino phase silica gel chromatography (KP-NH®, ethyl acetate to ethyl acetate/methanol) to give the title compound (260 mg, 42%).

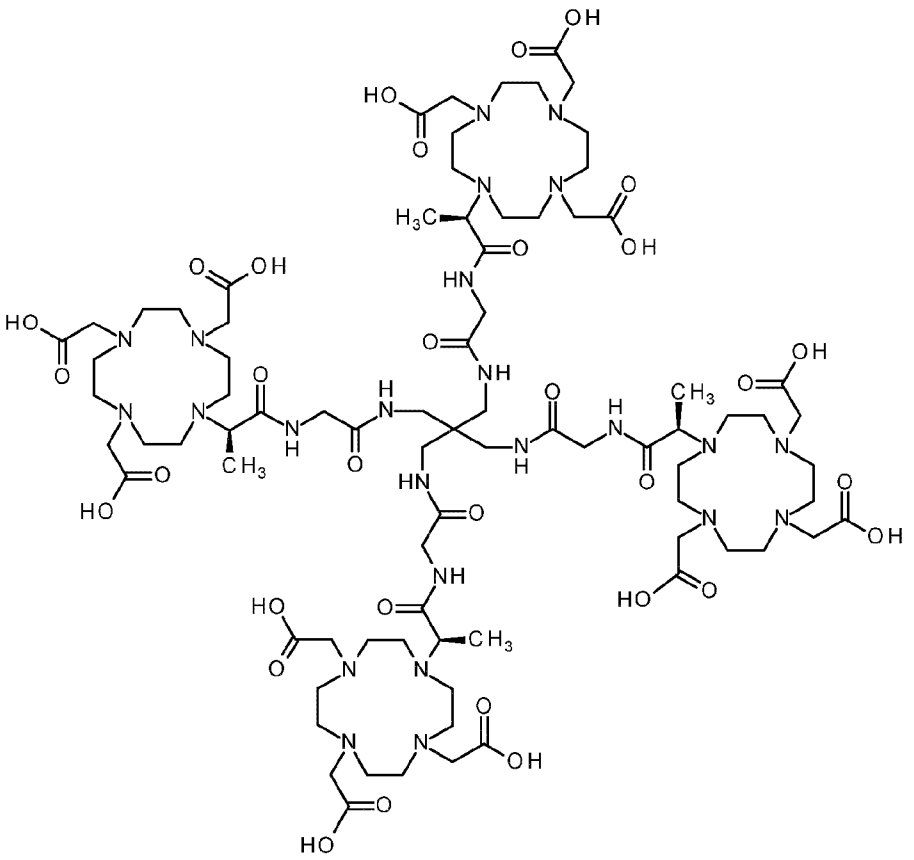
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.03 (s, br, 5H), 1.28 (s, br, 7H), 1.36 - 1.43 (m, 108H), 1.87 - 2.24 (m, 23H), 2.42 (s, br, 4H), 2.53 - 2.84 (m, 41H), 2.97 - 3.18 (m, 17H), 3.28 (s, br, 5H), 3.39 - 3.46 (m, 6H), 3.58 (s, br, 7H), 3.76 (s, br, 2H), 4.01 (s, br, 3H), 7.81 (s, br, 5H), 8.33 (s, br, 2H), 9.27 (s, br, 1H) ppm.

UPLC (ACN-NH_3): Rt. = 1.23 min.
MS (ES^+): m/z (z =4) = 660 $(M + H)^{4+}$.

LC-MS (ES^+): m/z (z =2) = 1318 $(M + H)^{2+}$, m/z (z =3) = 879 $(M + H)^{3+}$, m/z (z =4) = 660 $(M + H)^{4+}$; Rt. = 0.94 min.

Example 3-1g

{4,10-Bis(carboxymethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid



Tert-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}-acetate (example 3-1f; 260 mg, 0.099 mmol) was treated with TFA (25 mL) under stirring at room temperature overnight. The reaction mixture was concentrated under reduced pressure, the obtained residue taken up with water (20 mL) and lyophilized. The crude product was used without further characterization in the next chemical step.

Example 3-1

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)-acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclo-dodecan-1-yl}acetate

The crude material {4,10-bis(carboxymethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid from example 3-1g was dissolved in water (20 mL). Tris(acetato-kappaO)gadolinium tetrahydrate (298 mg, 0.734 mmol) was added and the reaction mixture stirred at 70 °C for 2 h. The pH value of the resulting solution was adjusted to 4.5 by addition of aqueous sodium hydroxide solution (2 N) and stirring at 70 °C continued for 2 days. The resulting solution was ultrafiltrated with water (7x100 mL) using an 1 kDa membrane and the final retentate was lyophilized yielding the title compound (70 mg, 27% over two steps).

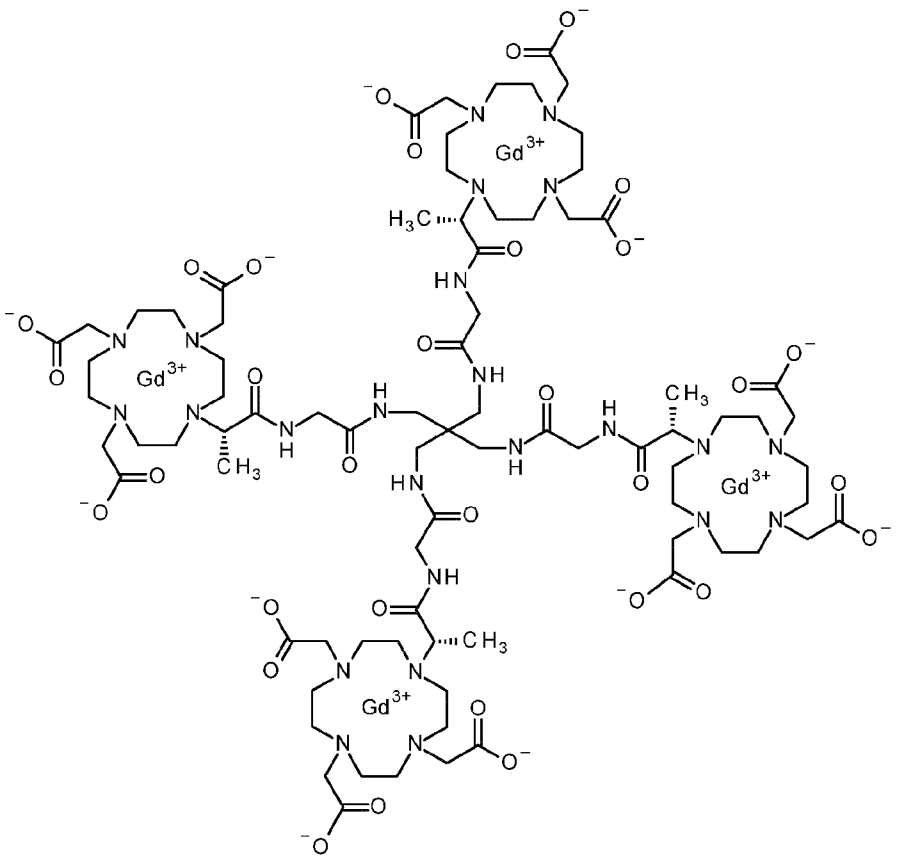
UPLC (ACN-HCOOH): Rt. = 0.39 min.

MS (ES^+): m/z (z = 2) = 1290.1 $(M + H)^{2+}$, m/z (z = 3) = 860.3 $(M + H)^{3+}$.

LC-MS (ES^+): m/z (z = 2) = 1290.3 $(M + H)^{2+}$, m/z (z = 3) = 860.9 $(M + H)^{3+}$, m/z (z = 4) = 645.6 $(M + H)^{4+}$; Rt. = 0.25 min.

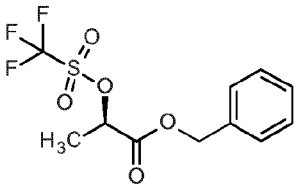
Example 3-2

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}-acetate



Example 3-2a

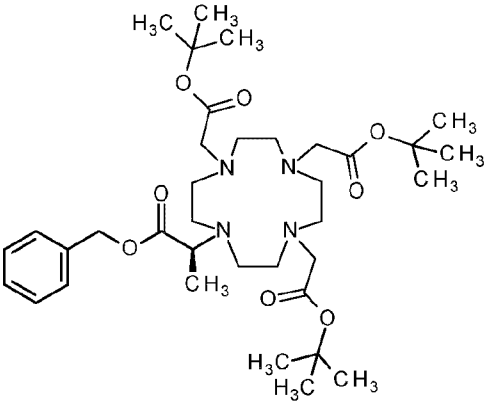
Benzyl (2R)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate



Prepared in analogy to the corresponding S-isomer (example 3-1c) from (R)-(+)-lactic acid benzyl ester (CAS No. [74094-05-6]; 8.00 g, 44.4 mmol) in dichloromethane. The obtained reaction mixture was directly used in the next step.

Example 3-2b

Benzyl (2S)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoate

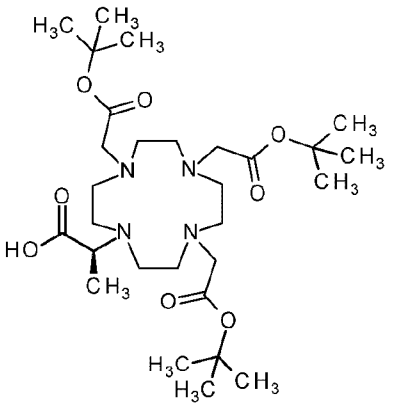


Prepared in analogy to the corresponding R-isomer (example 3-1d) from tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (CAS No. [122555-91-3]; 1.00 eq., 15.2 g, 29.6 mmol) and the reaction mixture of benzyl (2R)-2-{[(trifluoromethyl)sulfonyl]oxy}propanoate in dichloromethane prepared in example 3-2a.

LC-MS (ES^+): m/z = 677.4 $(M + H)^+$, m/z (z =2) = 339.2 $(M + H)^{2+}$; Rt. = 0.94 min.

Example 3-2c

(2S)-2-[4,7,10-Tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-propanoic acid



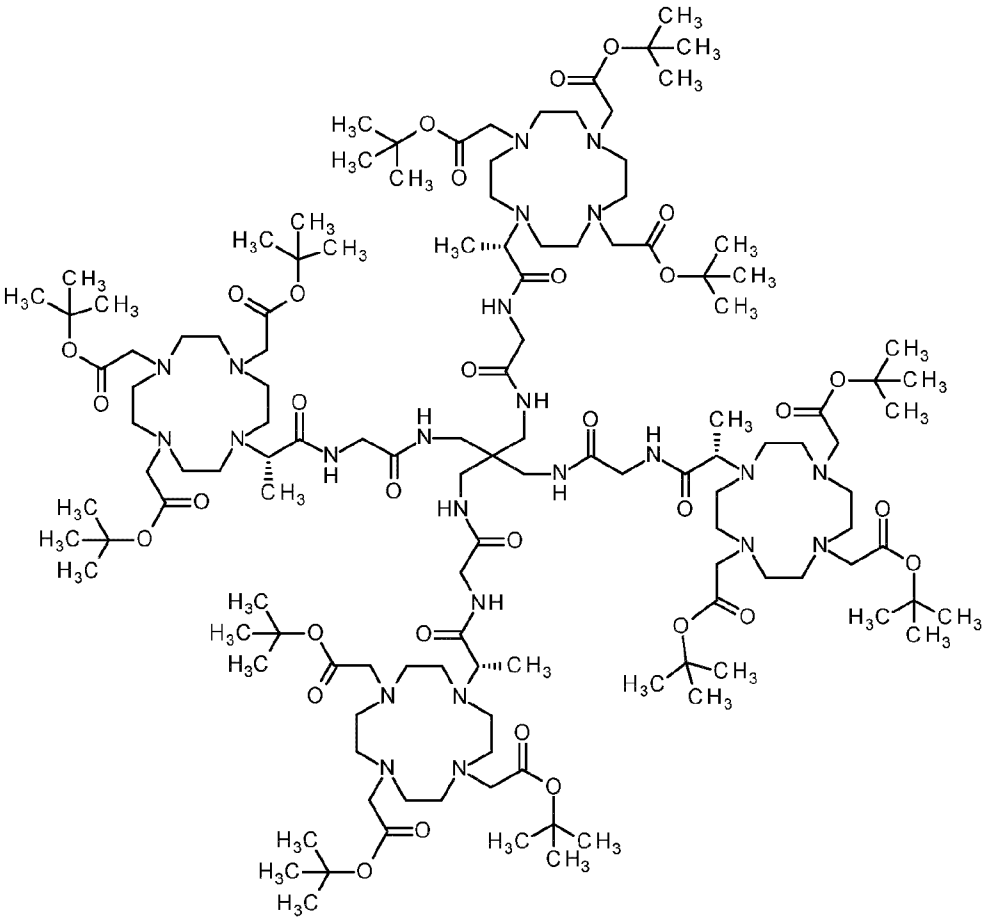
Prepared in analogy to the corresponding R-isomer (example 3-1e) from benzyl (2S)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoate (example 3-2b).

UPLC (ACN-NH_3): Rt. = 1.31 min.
MS (ES^+): m/z = 587 $(M + H)^+$.

LC-MS (ES^+): m/z = 587.4 $(M + H)^+$, m/z (z =2) = 294.2 $(M + H)^{2+}$; Rt. = 0.82 min.

Example 3-2d

***Tert*-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}-amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraaza-cyclododecan-1-yl}acetate**

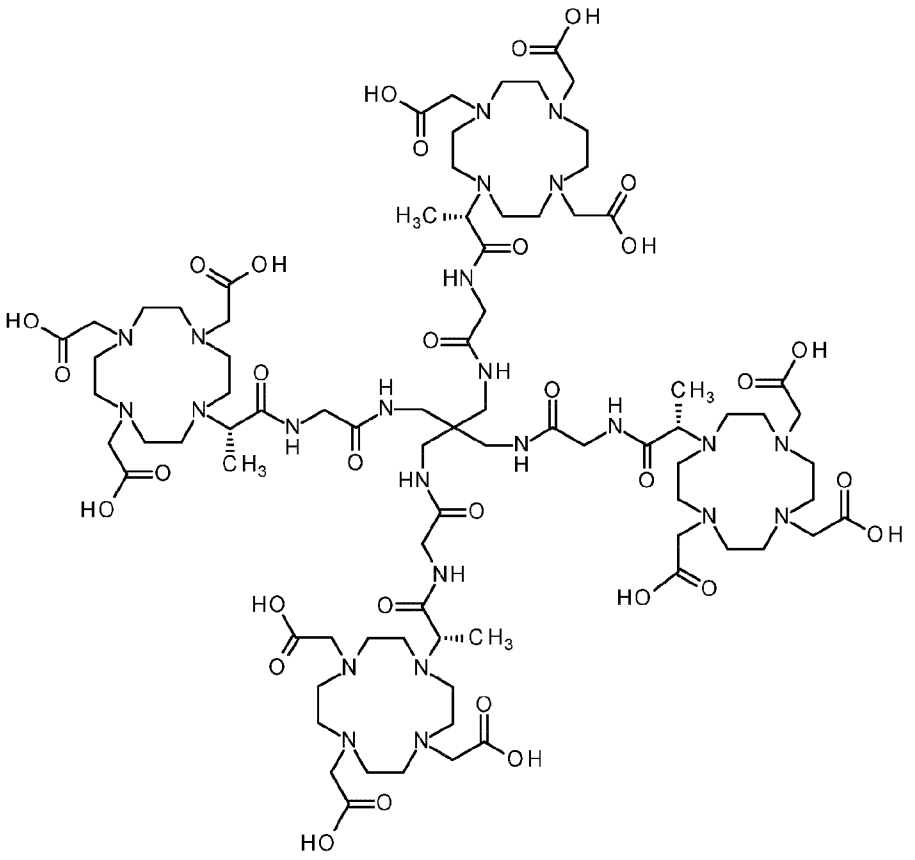


Prepared in analogy to the corresponding R-isomer (example 3-1f) from (2S)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoic acid (example 3-2c) and 2-amino-*N*-(3-[(aminoacetyl)amino]-2,2-bis{[(aminoacetyl)amino]methyl}propyl)acet-amide tetrahydrochloride (example 3-1b).

LC-MS (ES^+): m/z (z =2) = 1318 $(M + H)^{2+}$, m/z (z =3) = 879 $(M + H)^{3+}$, m/z (z =4) = 660 $(M + H)^{4+}$; Rt. = 0.95 min.

Example 3-2e

{4,10-Bis(carboxymethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid



Prepared in analogy to the corresponding R-isomer (example 3-1g) from *tert*-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate (example 3-2d). The crude product was used without further characterization in the next chemical step.

Example 3-2

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}-acetate

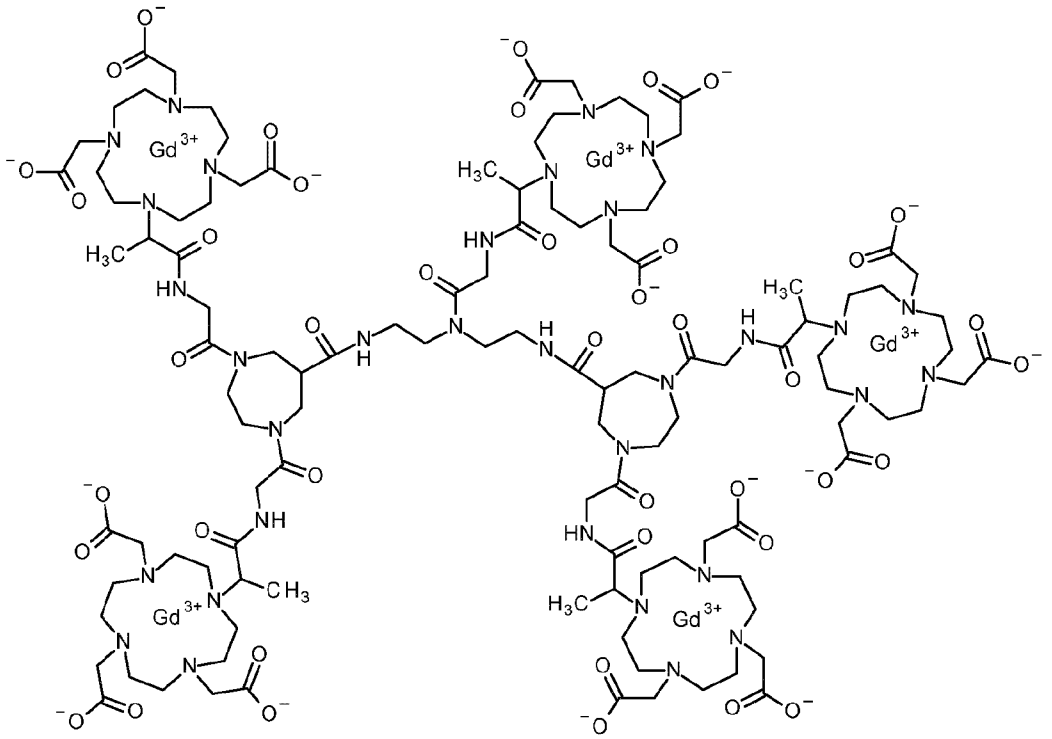
Prepared in analogy to the corresponding R-isomer (example 3-1) from {4,10-bis(carboxymethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-cyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid (example 3-2e) and tris(acetato-kappaO)gadolinium tetrahydrate at pH 4.5. The resulting reaction solution was ultrafiltrated with water (8x100 mL) using an 1 kDa membrane and the final retentate lyophilized and purified by preparative HPLC.

UPLC (ACN-HCOOH): Rt. = 0.41 min.
MS (ES^+): m/z (z = 2) = 1290 $(M + H)^{2+}$, m/z (z = 3) = 861 $(M + H)^{3+}$.

LC-MS (ES^+): m/z (z = 2) = 1290 $(M + H)^{2+}$, m/z (z = 3) = 860 $(M + H)^{3+}$, m/z (z = 4) = 645.6 $(M + H)^{4+}$; Rt. = 0.23 min.

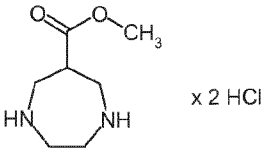
Example 4

Pentagadolinium [4-(1-{[2-(bis{2-[({1,4-bis[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-1,4-diazepan-6-yl}carbonyl)-amino]ethyl}amino)-2-oxoethyl]amino}-1-oxopropan-2-yl)-7,10-bis(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetate



Example 4a

6-(Methoxycarbonyl)-1,4-diazepanediium dichloride



x 2 HCl

6.00 g (17.7 mmol) Methyl 1,4-dibenzyl-1,4-diazepane-6-carboxylate [see US 5,866,562] were dissolved in 30 mL methanol. After adding of 6 mL aqueous hydrochloric acid (37%), 6 mL water and 600 mg palladium on charcoal (10%), the reaction mixture was hydrogenated (1 atm) for 17 hours at 40°C. The cata lyst was filtered off and the solution was evaporated under reduced pressure yielding 4.1 g (17.7 mmol, 100%) of the title compound.

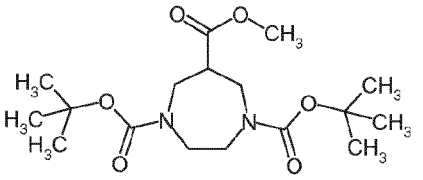
^{1}H-NMR (400 MHz, D_2O): δ = 3.62 - 3.84 (m, 9 H), 3.87 (s, 3 H) ppm.

UPLC (ACN-HCOOH): Rt. = 0.20 min.

MS (ES^+): m/z = 159.1 $(M + H)^+$, free base.

Example 4b

1,4-Di-*tert*-butyl 6-methyl 1,4-diazepane-1,4,6-tricarboxylate



4.00 g (17.3 mmol, 1 eq.) 6-(Methoxycarbonyl)-1,4-diazepanediium dichloride (example 4a) were dissolved in 80 mL DMF. After addition of 7.71 g (76.2 mmol, 4.4 eq.) trimethyl amine and 8.31 g (38.1 mmol, 2.2 eq.) di-*tert*-butyl dicarbonate, the resulting reaction mixture was stirred overnight at room temperature. The suspension was filtered, the filtrate evaporated under reduced pressure and diluted with ethyl acetate. The resulting solution was washed with aqueous citric acid (pH = 3 - 4), half saturated aqueous sodium bicarbonate, was dried over sodium sulfate and evaporated under reduced pressure yielding 4.92 g (13.7 mmol, 79%) of the title compound.

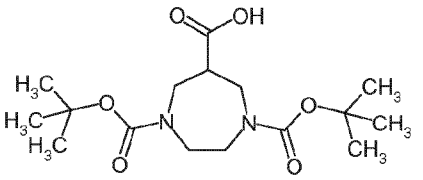
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.36 (s, 18 H), 2.69 - 3.27 (m, 4 H), 3.35 - 4.00 (m, 5 H), 3.62 (s, 3 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.32 min.

MS (ES^+): m/z = 359.2 $(M + H)^+$.

Example 4c

1,4-Bis(*tert*-butoxycarbonyl)-1,4-diazepane-6-carboxylic acid



4.86 g (13.66 mmol) 1,4-Di-*tert*-butyl 6-methyl 1,4-diazepane-1,4,6-tricarboxylate (example 4b) were dissolved in 82 mL THF. After adding of 27 mL aqueous sodium hydroxide (2 M) the resulting reaction mixture was stirred for 20 hours at room temperature, was diluted with water and was acidified (pH = 3 - 4) by addition of citric acid. The crude product was extracted with dichloromethane, the organic layer was washed with brine, dried over sodium sulfate and was evaporated to dryness yielding 4.67 g (12.4 mmol, 91%) of the title compound.

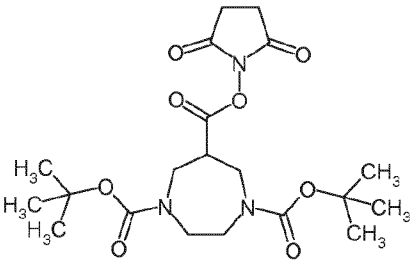
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.38 (s, 18 H), 2.58 - 2.86 (m, 1 H), 2.94 - 4.00 (m, 8 H), 12.50 (s, br, 1 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.12 min.

MS (ES^+): m/z = 345.2 $(M + H)^+$.

Example 4d

Di-*tert*-butyl 6-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}-1,4-diazepane-1,4-dicarboxylate



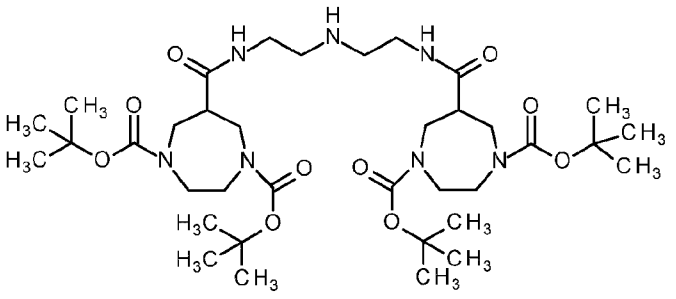
1.76 g (5.11 mmol, 1 eq.) 1,4-Bis(*tert*-butoxycarbonyl)-1,4-diazepane-6-carboxylic acid (example 4c) and 0.65 g (5.62 mmol, 1.1 eq) 1-hydroxypyrrolidine-2,5-dione were dissolved in 50 mL THF. A solution of 1.16 g (5.62 mmol, 1.1 eq.) *N,N'*-dicyclohexylcarbodiimide in 30 mL THF was added and the resulting reaction mixture was refluxed for 5 hours. The suspension was cooled to 0℃ and the precipitated u rea was filtered off. The final solution of the activated ester was directly used for the next chemical step.

UPLC (ACN-HCOOH): Rt. = 1.24 min.

MS (ES^+): m/z = 442.3 $(M + H)^+$.

Example 4e

Tetra-*tert*-butyl 6,6'-[iminobis(ethane-2,1-diylcarbamoyl)]bis(1,4-diazepane-1,4-dicarboxylate)



To the solution of the activated ester (5.11 mmol, 2.2 eq.) di-*tert*-butyl 6-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}-1,4-diazepane-1,4-dicarboxylate from example 4d were added

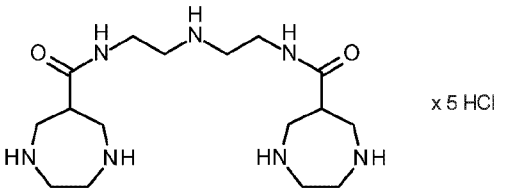
517 mg (5.11 mmol, 2.2 eq.) triethylamine and 240 mg (2.32 mmol, 1 eq.) *N*-(2-aminoethyl)ethane-1,2-diamine. The resulting reaction mixture was stirred for 20 hours at room temperature and was diluted with dichloromethane. The solution was washed with aqueous sodium hydroxide (0.1 M), with water and was dried over sodium sulfate. The crude product was isolated by evaporation and was purified by silica gel chromatography yielding 1.20 g (1.59 mmol, 68%) of the title compound.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.37 (s, 36 H), 2.51 - 2.70 (m, 7 H), 2.85 - 3.28 (m, 12 H), 3.45 - 4.10 (m, 8 H), 7.69 - 8.27 (m, 2 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.20 min.
MS (ES^+): m/z = 756.7 $(M + H)^+$.

Example 4f

***N,N'*-(Iminodiethane-2,1-diyl)bis(1,4-diazepane-6-carboxamide) pentahydrochloride**



385 mg (0.51 mmol) Tetra-*tert*-butyl 6,6'-[iminobis(ethane-2,1-diylcarbamoyl)]bis(1,4-diazepane-1,4-dicarboxylate) (example 4e) were dissolved in 5.7 mL methanol and 1.7 mL aqueous hydrochloric acid (37%). The reaction mixture was heated under stirring for 2 hours at 50℃. For isolation the suspension was evaporated to dryness yielding 277 mg (0.51 mmol, 100%) of the title compound.

^{1}H-NMR (400 MHz, D_2O): δ = 3.18 (t, 4 H), 3.32 - 3.40 (m, 2 H), 3.51 (t, 4 H), 3.57 - 3.69 (m, 16 H) ppm.

UPLC (ACN-HCOOH): Rt. = 0.24 min.
MS (ES^+): m/z = 356.3 $(M + H)^+$, free base.

Example 4

Pentagadolinium [4-(1-{[2-(bis{2-[({1,4-bis[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]-1,4-diazepan-6-yl}carbonyl)-amino]ethyl}amino)-2-oxoethyl]amino}-1-oxopropan-2-yl)-7,10-bis(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetate

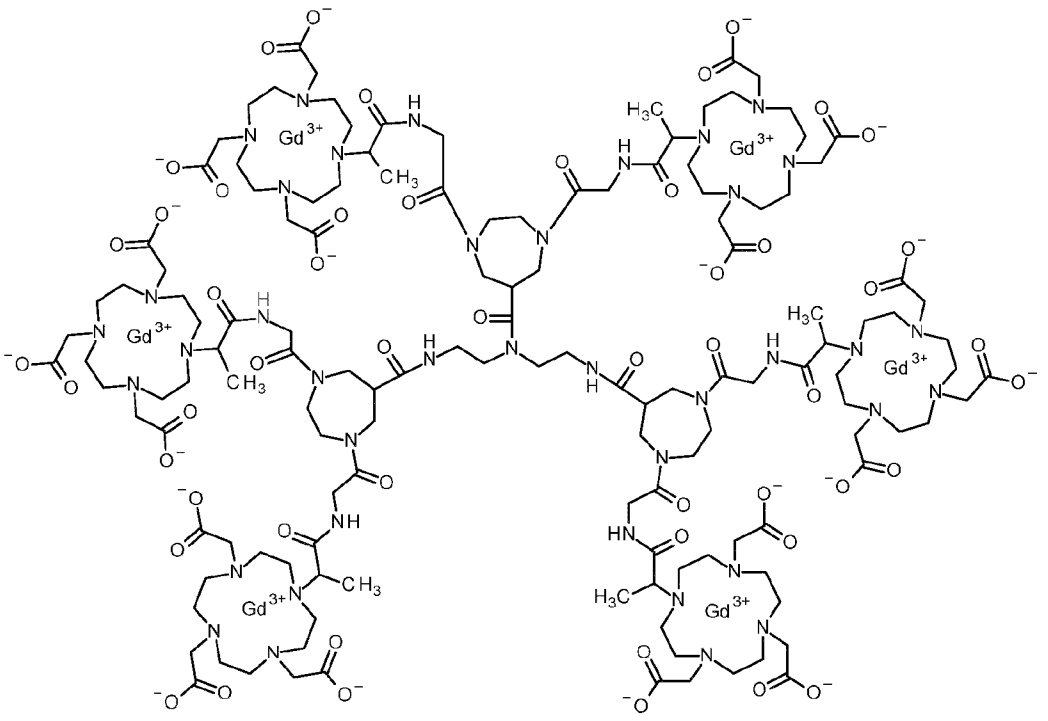
150 mg (279 µmol, 1 eq.) *N,N'*-(Iminodiethane-2,1-diyl)bis(1,4-diazepane-6-carboxamide) pentahydrochloride (example 4f) were dissolved in 60 mL DMSO. After addition of 451 mg (3.49 mmol, 12.5 eq.) *N,N*-diisopropylethylamine and 3.67 g (4.88 mmol, 17.5 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2), the resulting reaction mixture was stirred and heated for 8 hours at 50°C. The cooled solution was concentrated under reduced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 672 mg (197 µmol, 70%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.43 min.

MS (ES^-): m/z (z = 2) = 1706.3 $(M - 2H)^{2-}$ m; (ES^+): m/z (z = 4) = 854.5 $(M + 4H)^{4+}$.

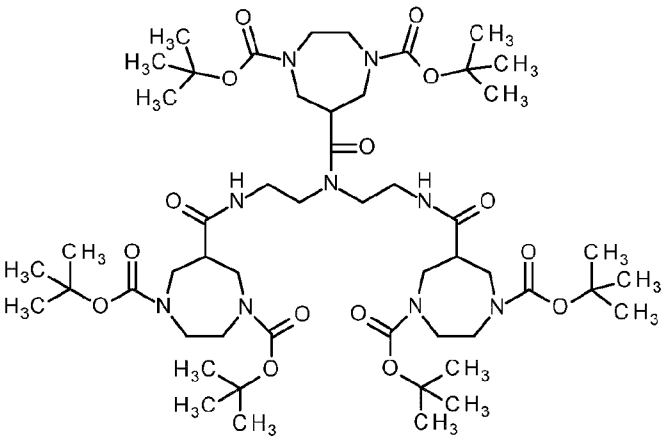
Example 5

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2""""',2"""""',2""""""',2"""""""',2""""""""',2"""""""""',2""""""""""',2"""""""""",2""""""""""",2"""""""""""",2""""""""""""",2"""""""""""""-{ethane-1,2-diylcarbamoyl-1,4-diazepane-6,1,4-triyltris[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}octadecaacetate



Example 5a

Hexa-*tert*-butyl 6,6',6''-(ethane-1,2-diylcarbamoyl)tris(1,4-diazepane-1,4-dicarboxylate)



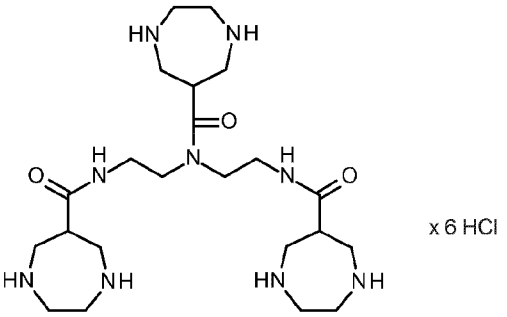
1.20 g (3.48 mmol, 3 eq.) 1,4-Bis(*tert*-butoxycarbonyl)-1,4-diazepane-6-carboxylic acid (example 4c), 540 mg (4.18 mmol, 3.6 eq.) diisopropylethylamine and 1.59 g (4.18 mmol, 3.6 eq.) HATU were dissolved in 30 mL DMF and stirred for 2 hours at room temperature. After drop wise addition of a solution of 120 mg (1.16 mmol, 1 eq.) *N*-(2-aminoethyl)ethane-1,2-diamine and of 540 mg (4.18 mmol, 3.6 eq.) *N,N*-diisopropylethylamine in 8 mL DMF, the resulting reaction mixture was heated under stirring for 3 hours at 70°C. After cooling and diluting with dichloromethane, the solution was washed with aqueous sodium hydroxide (0.1 M), with aqueous citric acid (1%), with water and was dried over sodium sulfate. The crude product was isolated by evaporation under reduced pressure and was purified by silica gel chromatography yielding 660 mg (0.61 mmol, 52%) of the title compound.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.38 (s, 54 H), 2.55 - 4.06 (m, 35 H), 7.90 - 8.52 (m, 2 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.64 min.
MS (ES^+): m/z = 1082.7 $(M + H)^+$.

Example 5b

***N,N*-Bis{2-[(1,4-diazepan-6-ylcarbonyl)amino]ethyl}-1,4-diazepane-6-carboxamide hexahydrochloride**



654 mg (0.60 mmol) Hexa-*tert*-butyl 6,6',6''-(ethane-1,2-diylcarbamoyl)tris(1,4-diazepane-1,4-dicarboxylate) (example 5a) were dissolved in 6.8 mL methanol and 3 mL aqueous hydrochloric acid (37%). The reaction mixture was heated under stirring for 2.5 hours at 50℃. For isolation the suspension was evaporated to dryness yielding 441 mg (0.63 mmol, 105%) of the title compound.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.20 - 3.71 (m, 35 H), 8.50 - 8.80 ppm (m, 2 H), 9.76 (s, br, 12 H).

UPLC (ACN-HCOOH): Rt. = 0.19 min.
MS (ES^+): m/z = 482.3 $(M + H)^+$, free base.

Example 5

Hexagadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''',2''''''''''',2'''''''''''',2''''''''''''',2'''''''''''''',2''''''''''''''-{ethane-1,2-diylcarbamoyl-1,4-diazepane-6,1,4-triyltris[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}octadecaacetate

150 mg (214 µmol, 1 eq.) *N,N*-Bis{2-[(1,4-diazepan-6-ylcarbonyl)amino]ethyl}-1,4-diazepane-6-carboxamide hexahydrochloride (example 5b) were dissolved in 60 mL DMSO. After adding of 0.42 g (3.21 mmol, 15 eq.) *N,N*-diisopropylethylamine and 3.38 g (4.50 mmol, 21 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2), the resulting

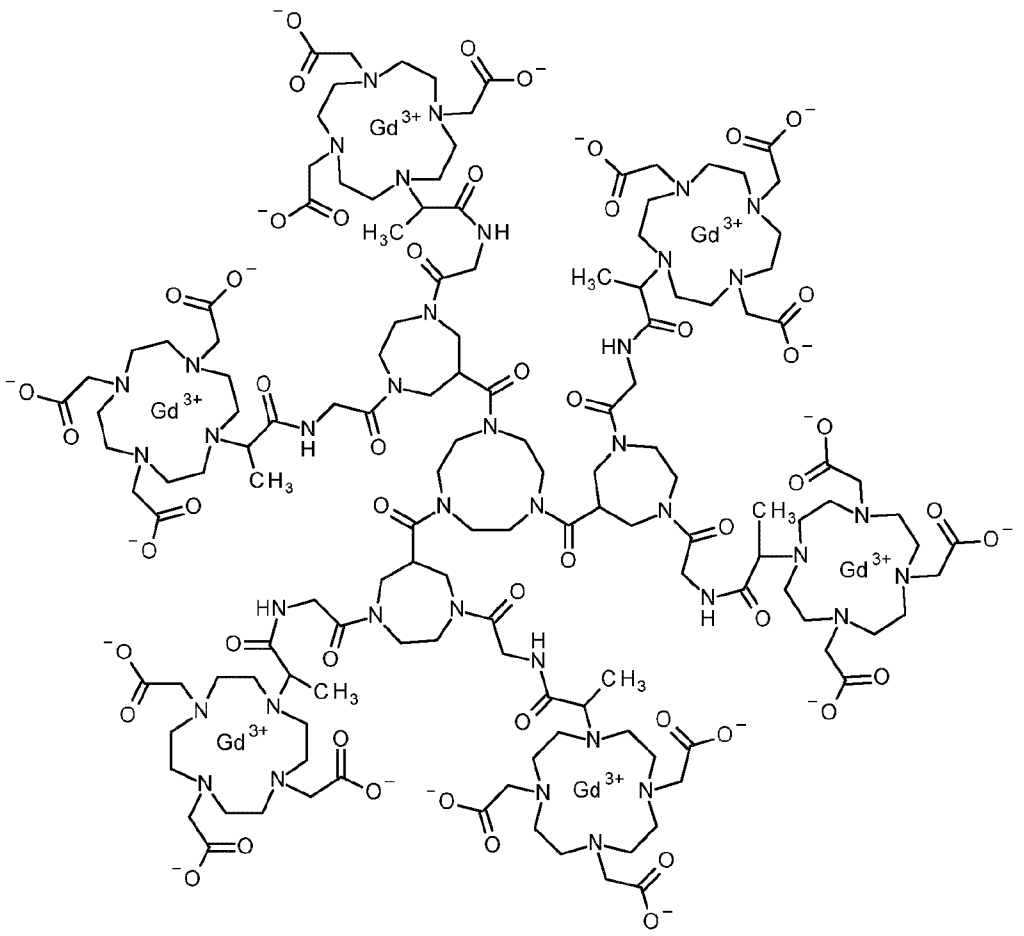
reaction mixture was stirred and heated for 8 hours at 50℃. The cooled solution was concentrated under reduced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using a 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 595 mg (143 µmol, 67%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.41 min.

MS (ES^+): m/z (z = 3) = 1384.6 $(M + H)^{3+}$, m/z (z = 4) = 1039.5 $(M + H)^{4+}$, m/z (z = 5) = 831.6 $(M + H)^{5+}$.

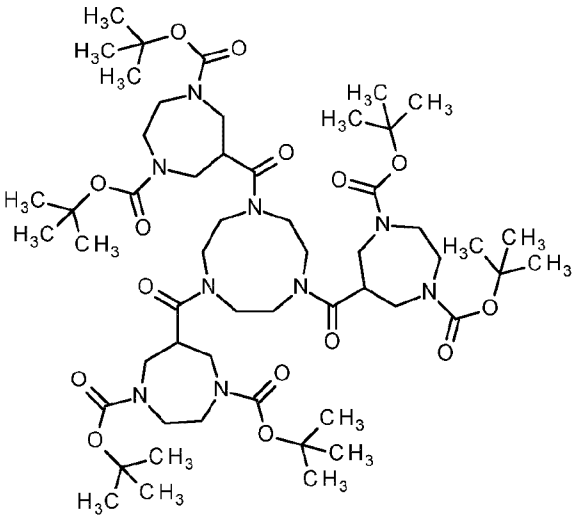
Example 6

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2""""',2"""""',2""""""',2"""""""',2""""""""',2"""""""""',2""""""""""',2"""""""""",2""""""""""',2"""""""""""',2""""""""""""'-(1,4,7-triazonane-1,4,7-triyltris{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})octadecaacetate



Example 6a

Hexa-*tert*-butyl 6,6',6"-(1,4,7-triazonane-1,4,7-triyltricarbonyl)tris(1,4-diazepane-1,4-dicarboxylate)



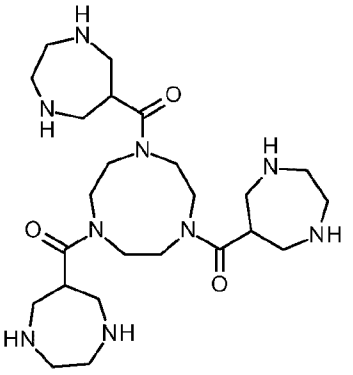
800 mg (2.32 mmol, 3 eq.) 1,4-Bis(*tert*-butoxycarbonyl)-1,4-diazepane-6-carboxylic acid (example 4c), 360 mg (2.79 mmol, 3.6 eq.) diisopropylethylamine and 1.06 g (2.79 mmol, 3.6 eq.) HATU were dissolved in 20 mL DMF and stirred for 2 hours at room temperature. After dropwise adding of a solution of 100 mg (774 µmol, 1 eq.) 1,4,7-triazonane trihydrochloride and of 360 mg (2.79 mmol, 3.6 eq.) *N,N*-diisopropylethylamine in 5 mL DMF, the resulting reaction mixture was heated under stirring for 3 hours at 70℃. After cooling and diluting with dichloromethane, the solution was washed with aqueous sodium hydroxide (0.1 M), with aqueous citric acid (1%), with water and was dried over sodium sulfate. The crude product was isolated by evaporation under reduced pressure and was purified by silica gel chromatography yielding 545 mg (492 µmol, 63%) of the title compound.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.47 (s, 54 H), 2.85 - 4.45 (m, 39 H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.73 min.
MS (ES^+): m/z = 1108.8 $(M + H)^+$.

Example 6b

1,4,7-Triazonane-1,4,7-triyltris(1,4-diazepan-6-ylmethanone) hexahydrochloride



x 6 HCl

380 mg (343 µmol) Hexa-*tert*-butyl 6,6',6''-(1,4,7-triazonane-1,4,7-triyltricarbonyl)tris(1,4-diazepane-1,4-dicarboxylate) (example 6a) were dissolved in 3.90 mL methanol and 1.72 mL aqueous hydrochloric acid (37%). The reaction mixture was heated under stirring for 2.5 hours at 50°C. For isolation the suspension was evaporated to dryness yielding 257 mg (354 µmol, 103%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.19 min.
MS (ES^+): m/z = 508.4 $(M + H)^+$, free base.

Example 6

Hexagadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''',2''''''''''',2'''''''''''',2''''''''''''',2'''''''''''''',2''''''''''''''-(1,4,7-triazonane-1,4,7-triyltris{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})octadecaacetate

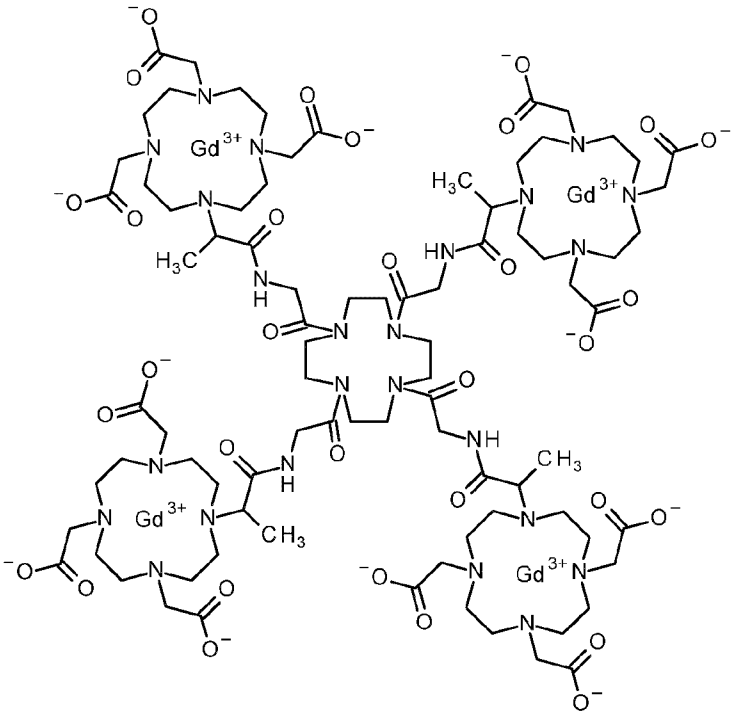
175 mg (241 µmol, 1 eq.) 1,4,7-Triazonane-1,4,7-triyltris(1,4-diazepan-6-ylmethanone) hexahydrochloride (example 6b) were dissolved in 60 mL DMSO. After adding of 467 mg (3.61 mmol, 15 eq.) *N,N*-diisopropylethylamine and 3.80 g (5.06 mmol, 21 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2), the resulting reaction mixture was stirred and heated for 8 hours at 50°C. The cooled solution was concentrated under reduced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed precipitate was filtered off and was dried in

vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 590 mg (141 µmol, 58%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.43 min.
MS (ES^+): m/z (z = 3) = 1393.1 $(M + 3H)^{3+}$, m/z (z =4) = 1045.5 $(M + 4H)^{4+}$, m/z (z = 5) = 837.0 $[(M + 5H)^{5+}$.

Example 7

Tetragadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''',2''''''''''-{1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayltetrakis[(2-oxoethane-2,1-diyl)imino(1-oxopropane-1,2-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}dodecaacetate



35 mg (203 µmol, 1 eq.) 1,4,7,10-Tetraazacyclododecane were dissolved in 60 mL DMSO. After adding of 2.14 g (2.84 mmol, 14 eq.) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (see WO 2001051095 A2), the resulting reaction mixture was stirred and heated for 8 hours at 50℃. The cooled solution was concentrated under re duced pressure to a final volume of 15 - 20 mL. The concentrate was poured under stirring in 400 mL ethyl acetate, the formed

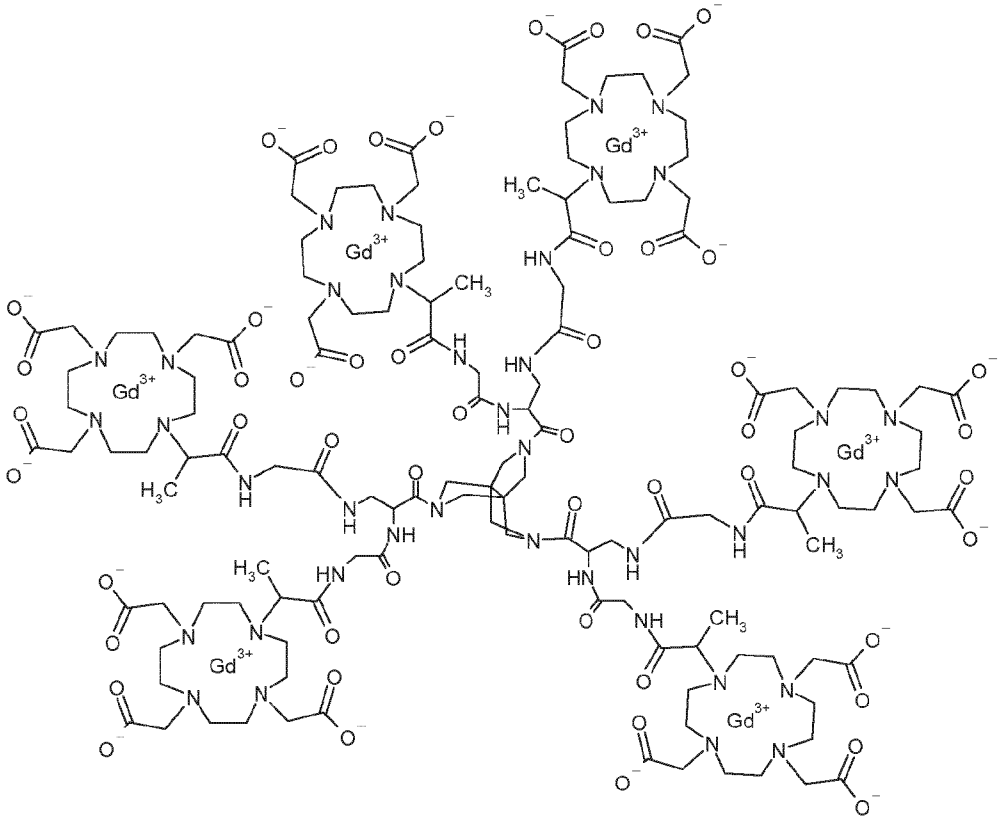
precipitate was filtered off and was dried in vacuo. The solid was dissolved in water, the resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 28 mg (10.6 µmol, 5%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.41 min.

MS (ES^+): m/z (z = 2) = 1311.7 $(M + 2H)^{2+}$, m/z (z = 3) = 873.1 $(M + 3H)^{3+}$.

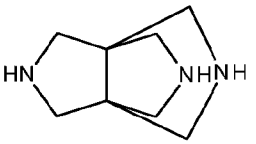
Example 8

Hexagadolinium 2,2',2",2"',2"",2""',2"""',2""""',2"""""',2""""""',2"""""""',2""""""""',2"""""""""',2""""""""",2"""""""""',2"""""""""",2""""""""""',2"""""""""""-{3,7,10-triazatricyclo[3.3.3.0^{1,5}]undecane-3,7,10-triyltris[carbonyl(3,6,11,14-tetraoxo-4,7,10,13-tetraazahexadecane-8,2,15-triyl)di-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}octadecaacetate



Example 8a

Tetrahydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole

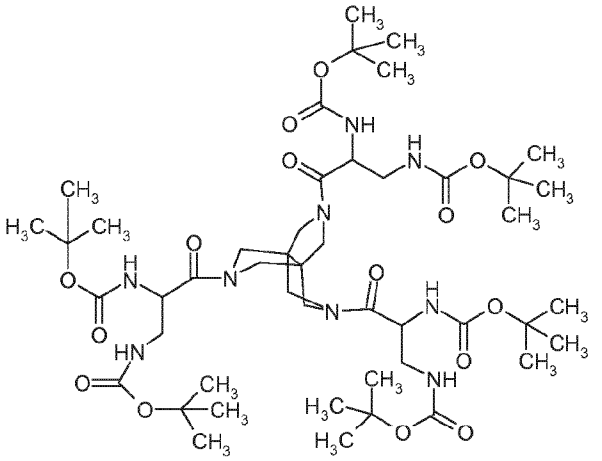


4.0 g (6.5 mmol) 2,5,8-Tris((4-methylphenyl)sulfonyl)tetrahydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole (prepared via the procedures outlined in *J. Org. Chem.* **1996**, *61*, 8897-8903) was refluxed in 44 mL aqueous hydrobromic acid (47%) and 24 mL acetic acid for 18 hours. The solvent was removed in vacuo, the residue dissolved in water and the aqueous phase was washed two times with dichloromethane. The aqueous phase was lyophilized and taken up in a small amount of water and passed through an anionic exchange column (DOWEX 1X8) by elution with water. The basic fraction was collected and concentrated to yield 0.89 g of tetrahydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole as free base.

^{1}H-NMR (400 MHz, D_2O): δ = 2.74 (s, 12 H) ppm.

Example 8b

***Tert-butyl*-{1-[5,8-bis{2,3-bis[(*tert*-butoxycarbonyl)amino]propanoyl}dihydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrol-2(3*H*)-yl]-3-[(*tert*-butoxycarbonyl)amino]-1-oxopropan-2-yl}carbamate**

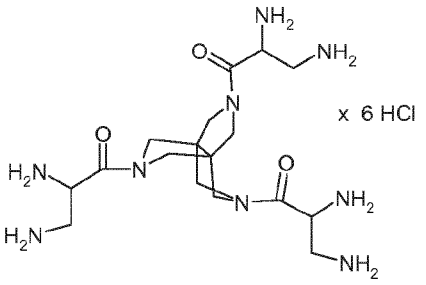


A solution prepared from 431.5 mg (0.89 mmol, CAS [201472-68-6]) *N*-(*tert*-butoxycarbonyl)-3-[(*tert*-butoxycarbonyl)amino]alanine *N*,*N*-dicyclohexylammonium salt, 0.44 mL (2.54 mmol) *N*,*N*-diisopropylethylamine and 386 mg (1.0 mmol) HATU in 4.3 mL DMF was added to 38.9 mg (254 µmol) of tetrahydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole in 2 mL DMF. After stirring the combined mixture for 20 min at room temperature the solvent was removed in vacuo and the residue purified by chromatography on amino phase silica gel (ethyl acetate in hexane, 0 to 100%) followed by preparative HPLC (C18-Chromatorex 10 µm, acetonitrile in water + 0.1% formic acid, 65% to 100%) to yield 68.6 mg of *tert*-butyl-{1-[5,8-bis{2,3-bis[(*tert*-butoxycarbonyl)amino]propanoyl}dihydro-1*H*,4*H*-3*a*,6*a*-(methanoimino-methano)pyrrolo[3,4-*c*]pyrrol-2(3*H*)-yl]-3-[(*tert*-butoxycarbonyl)amino]-1-oxopropan-2-yl}-carbamate.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.43 s, br, 54H), 3.34 - 3.97 (m, 18H), 4.48 (s, br, 3H), 5.01-5.67(m, 6H) ppm.

UPLC (ACN-HCOOH): Rt. = 1.48 min.
MS (ES^+): m/z = 1012.6 $(M + H)^+$.

Example 8c
3,3',3''-[1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole-2,5,8(3*H*,6*H*)-triyl]-tris(3-oxopropane-1,2-diaminium) hexachloride



65 mg (60 µmol) *Tert*-butyl-{1-[5,8-bis{2,3-bis[(*tert*-butoxycarbonyl)amino]propanoyl} dihydro-1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrol-2(3*H*)-yl]-3-[(*tert*-butoxy-carbonyl)amino]-1-oxopropan-2-yl}carbamate (example 8b) were dissolved in 2.0 mL DMF and 0.48 mL hydrochloric acid in dioxane (4 M, 0.19 mmol) were added. The reaction mixture was heated under microwave radiation for 10 min at 80℃ while stirring. The solvent was removed in vacuo, the residue taken up in a small amount of water and lyophilized to yield

38.9 mg of 3,3',3''-[1*H*,4*H*-3*a*,6*a*-(methanoiminomethano)pyrrolo[3,4-*c*]pyrrole-2,5,8(3*H*,6*H*)-triyl]tris(3-oxopropane-1,2-diaminium) hexachloride.

^{1}H-NMR (600 MHz, D_2O): δ = 3.40 - 3.50 (m, 3H), 3.52 - 3.56 (m, 3H), 3.79 - 4.19 (m, 12H), 4.51 – 4.54 (m, 3H) ppm.

UPLC (ACN-HCOOH): Rt. = 0.20 min.
MS (ES^+): m/z = 412.3($[M + H)^+$, free base.

Example 8

Hexagadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''',2''''''''''',2'''''''''''',2''''''''''''',2'''''''''''''',2''''''''''''''-{3,7,10-triazatricyclo[3.3.3.0^{1,5}]undecane-3,7,10-triyltris[carbonyl(3,6,11,14-tetraoxo-4,7,10,13-tetraazahexadecane-8,2,15-triyl)di-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]}octadecaacetate

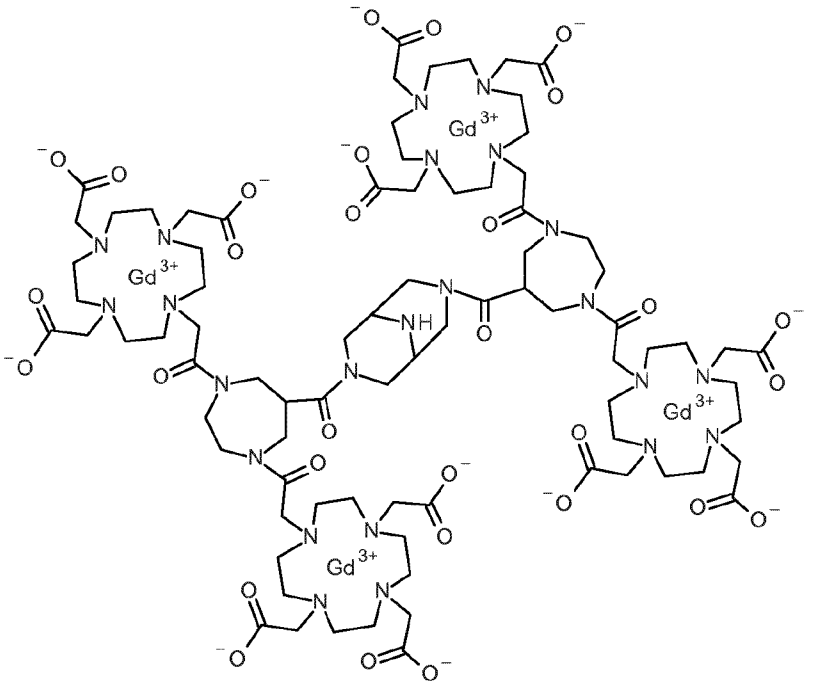
30 mg (48 µmol) 3,3',3''-[1*H*,4*H*-3*a*,6*a*-(Methanoiminomethano)pyrrolo[3,4-*c*]pyrrole-2,5,8(3*H*,6*H*)-triyl]tris(3-oxopropane-1,2-diaminium) hexachloride (example 8c) were dissolved in a mixture of 1.8 mL DMSO, 1.8 mL DMF, and 116 µL pyridine. At 60℃ 281 mg (0.38 mmol, WO 2001051095 A2) of gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate were added followed by 44 µL trimethylamine and the resulting reaction mixture was stirred for 15 hours at 60℃ and at room temperature for two days. Another amount of gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate (56 mg, 75 µmol) and trimethylamine (5.4 µL) was added at 60℃ and stirring at 60℃ was continued for 15 hours. The solvent was removed in vacuo, the residue taken up in 200 mL of water and the resulting solution was ultrafiltrated using a 1 kDa membrane. After diluting the retentate two times with additional 200 mL of deionized water and continuing the ultrafiltration the final retentate was lyophilized. The residue was dissolved in a mixture of 1.6 mL DMSO, 1.6 mL DMF, and 105 µL pyridine and addition of 261 mg (0.35 mmol) gadolinium 2,2',2''-[10-(1-{[2-(4-nitrophenoxy)-2-oxoethyl]amino}-1-oxopropan-2-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]triacetate and 48 µL triethylamine at 60℃ was repeated a third time. After stirring for 18 hours at 60℃ the ultrafiltration procedure using a 1 kDa membrane was repeated and the retentate after three 200 mL filtrations was lyophilized. The crude product was purified by preparative HPLC (XBrigde C18, 5µm, acetonitrile in water + 0.1% formic acid, 0% to 7%) to yield 51 mg of the title compound.

UPLC (ACN-HCOOH long run): Rt. = 2.95 min.

MS (ES^+): m/z (z =3) = 1360.4 $(M + 3H)^{3+}$, m/z (z =4) = 1021.3 $(M + 4H)^{4+}$, m/z (z = 5) = 817.5 $(M + 5H)^{5+}$.

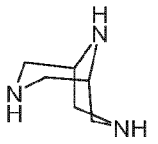
Example 9

Tetragadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''''-(3,7,9-triaza-bicyclo[3.3.1]nonane-3,7-diylbis{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})dodecaacetate



Example 9a

3,7,9-Triazabicyclo[3.3.1]nonane

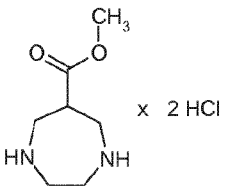


220 mg (0.49 mmol) 3,9-Dibenzyl-7-(phenylsulfonyl)-3,7,9-triazabicyclo[3.3.1]nonane (prepared via the procedures outlined in *Tetrahedron Letters,* **2005**, *46,* 5577-5580) was refluxed in 3.4 mL aqueous hydrobromic acid (47%) and 1.8 mL acetic acid for 17 hours. The solvent was removed in vacuo, the residue dissolved in water and the aqueous phase was washed two times with dichloromethane. The aqueous phase was lyophilized and taken up in a small amount of water and passed through an anionic exchange column (DOWEX 1X8) by elution with water. The basic fraction was collected and concentrated to yield 29.6 mg of 3,7,9-triazabicyclo[3.3.1]nonane as free base.

^{1}H-NMR (400 MHz, D_2O): δ = 2.88 (t, 2H), 3.15 (d, 8H) ppm.

Example 9b

6-(Methoxycarbonyl)-1,4-diazepanediium dichloride

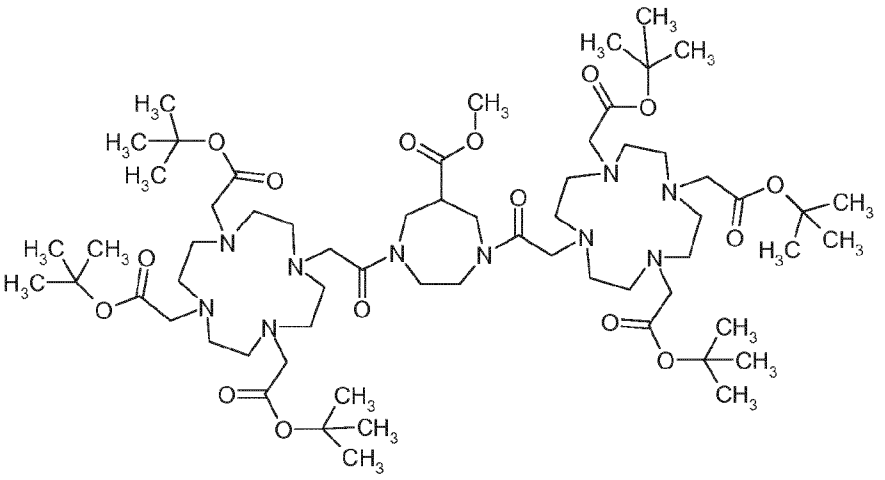


To 8.3 g (24.5 mmol) methyl 1,4-dibenzyl-1,4-diazepane-6-carboxylate (prepared in analogy to US005866562A, p.9) in 42 mL methanol were added 8.3 mL concentrated hydrochloric acid, 2 mL of water and 830 mg palladium on charcoal (10%). The suspension was stirred under a hydrogen atmosphere for 5 hours at 40℃ and 17 hours at room temperature. The mixture was filtrated trough a path of celite and the filtrate concentrated in vacuo upon which toluene was added two times and removed in vacuo. The residue was dissolved in water and lyophilized to yield 5.65 g of 6-(methoxycarbonyl)-1,4-diazepanediium dichloride.

^{1}H-NMR (400 MHz, D_2O): δ = 3.49 - 3.68 (m, 9H), 3.70 - 3.73 (m, 4H), 3.75 (s, 3H) ppm.

Example 9c

Methyl 1,4-bis{[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}-1,4-diazepane-6-carboxylate



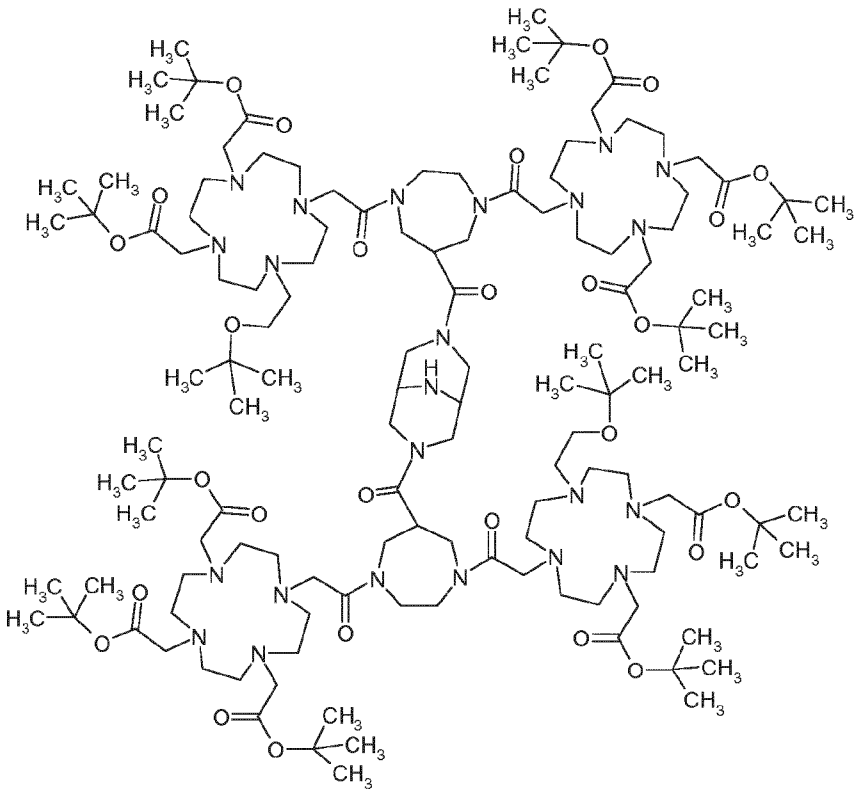
To 200 mg (0.78 mmol) of 6-(methoxycarbonyl)-1,4-diazepanediium dichloride in 10 mL dichloromethane were added 10 mL (6.2 mmol) *N,N*-diisopropylethylamine and the mixture stirred for 5 min at room temperature. 1.04 g (1.56 mmol) tri-*tert*-butyl 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (prepared in analogy to Cong Li *et al.*, *J. Am.Chem.Soc.* **2006**, *128*, p.15072-15073; S3-5 and Galibert *et al.*, Bioorg. Med. Chem. Letters 2010 (20), 5422-5425) was added and the mixture was stirred for 18 hours at room temperature. The solvent was removed under reduced pressure and the residue was purified by chromatography on amino phase silica gel (ethyl acetate in hexane, 20 to 100%, then ethanol in ethyl acetate 0 to 100%) to yield 210 mg of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.94 min.

MS (ES^+): m/z = 1267.6 $(M + 1H)^+$

Example 9d

Dodeca-*tert*-butyl 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''',2''''''''''-(3,7,9-triaza-bicyclo[3.3.1]nonane-3,7-diylbis{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})dodecaacetate



305 mg (0.24 mmol) Methyl 1,4-bis{[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetra azacyclododecan-1-yl]acetyl}-1,4-diazepane-6-carboxylate (example 9c) were dissolved in 3.9 mL THF and a solution of 6.6 mg lithium hydroxide in 0.87 mL water was added. After stirring for 15 min the solvent was removed under reduced pressure and the raw lithium 1,4-bis{[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}-1,4-diazepane-6-carboxylate (300 mg) was dissolved in 2.0 mL dichloromethane. 120 µL (0.71 mmol) *N,N*-Diisopropylethylamine, 112 mg (0.30 mmol) HATU and 40 mg (0.30 mmol) 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-ol were added and after stirring for 15 min a solution of 15 mg (0.12 mmol) of 3,7,9-triazabicyclo[3.3.1]nonane in 1 mL dichloromethane was added and the mixture was stirred for 3 days. To additional 170 mg of raw lithium 1,4-bis{[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}-1,4-diazepane-6-carboxylate in 1 mL dichloromethane were added 67 mg (0.18 mmol) HATU, 24 mg (0.18 mmol) 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-ol over 15 min and 50 µL *N,N*-

diisopropylethylamine. After stirring for 15 minutes the freshly prepared HATU solution was added to the reaction mixture. After one day the solvent was removed under reduced pressure upon which toluene was added six times and removed in vacuo. The residue was purified by chromatography on amino phase silica gel (ethyl acetate in hexane, 0 to 100%, then ethanol in ethyl acetate 0 to 40%) to yield 181 mg of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.78-0.84 min.
MS (ES^-): m/z (z = 2) = 1298.7 $(M - 2H)^{2-}$

Example 9

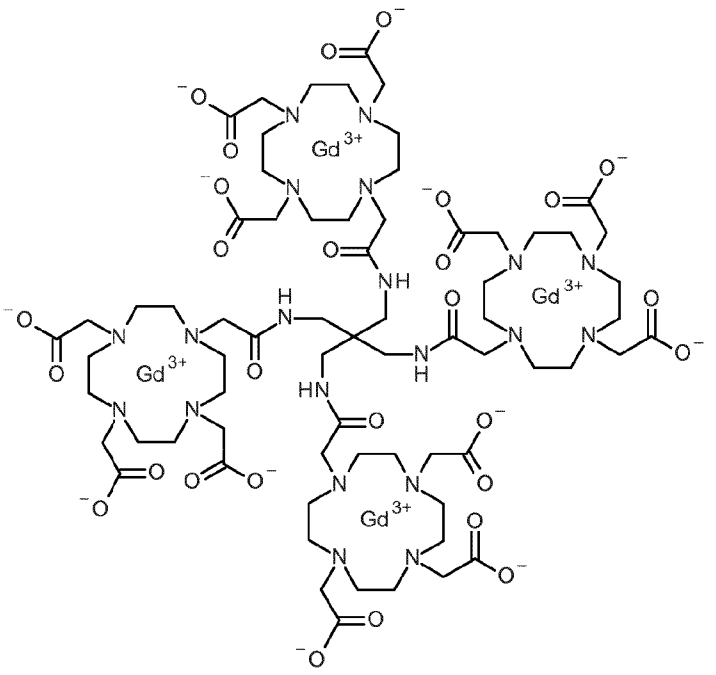
Tetragadolinium 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''-(3,7,9-triaza-bicyclo[3.3.1]nonane-3,7-diylbis{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})dodecaacetate

390 mg (mmol) Dodeca-*tert*-butyl 2,2',2'',2''',2'''',2''''',2'''''',2''''''',2'''''''',2''''''''',2'''''''''-(3,7,9-triazabicyclo [3.3.1]nonane-3,7-diylbis{carbonyl-1,4-diazepane-6,1,4-triylbis[(2-oxoethane-2,1-diyl)-1,4,7,10-tetraazacyclododecane-10,1,4,7-tetrayl]})dodecaacetate (example 9d) were dissolved in 10.8 mL water and the solution was adjusted to pH 2.5 by addition of aqueous hydrochloric acid (2M). 440 mg (1.25 mmol) Gadolinium(III)oxide were added and the mixture was stirred at 80℃ for 17 hours, while the pH of the suspension changed to pH 5. The mixture was diluted with water, sonicated and filtrated. The filtrate was ultrafiltrated using a 1 kDa membrane. After diluting the retentate two times with additional 100 mL of deionized water and continuing the ultrafiltration the final retentate was lyophilized. The crude product was purified by preparative HPLC (C18 YMC-ODS AQ, 10 µm, acetonitrile in water + 0.1% formic acid, 1% to 10%) to yield 14.5 mg of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.34 min.
MS (ES^+): m/z (z = 2) = 1272.9 $(M + 2H)^{2+}$

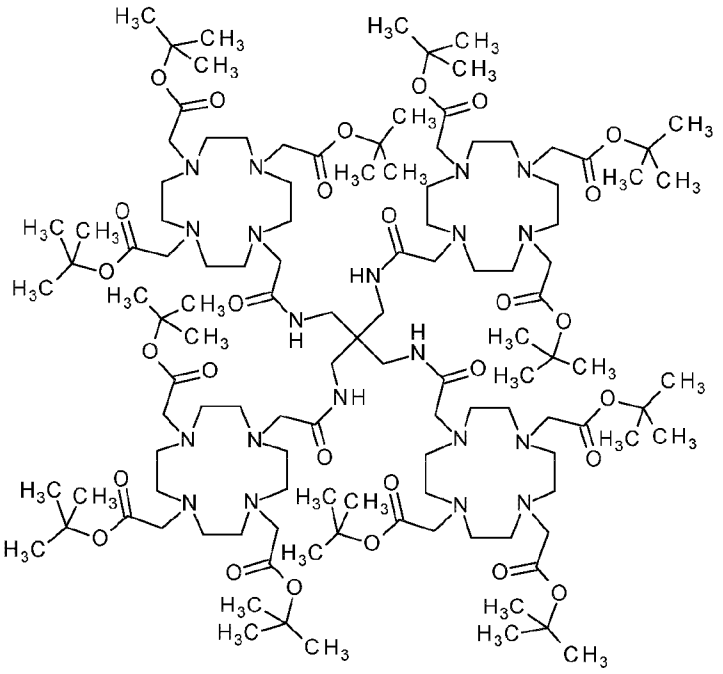
Example 10

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate



Example 10a

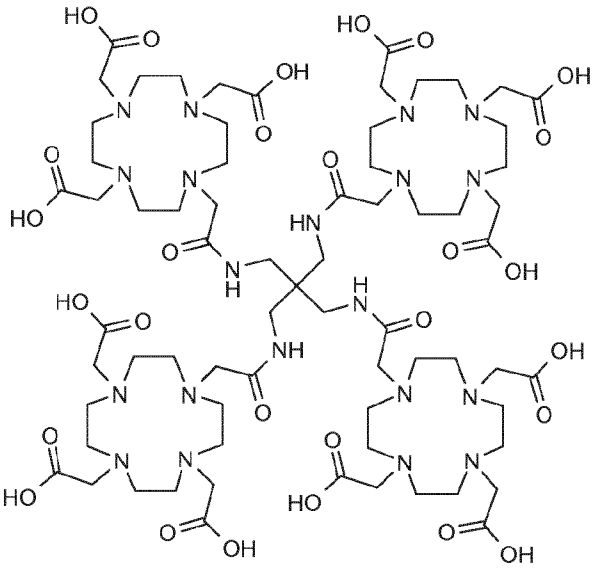
***Tert*-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]-propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate**



6.6 mg (49.8 µmol, 1 eq.) 2,2-Bis(aminomethyl)propane-1,3-diamine (see W. Hayes et al., Tetrahedron 59 (2003), 7983 - 7996) were dissolved in 7 mL DMSO. After adding of 77 mg (0.6 mmol, 12 eq.) *N,N*-diisopropylethylamine and 400 mg (0.6 mmol, 12 eq.) tri-*tert*-butyl 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (see M. Galibert et al., Bioorg. Med. Chem. Letters 2010 (20), 5422-5425 and *J. Am.Chem.Soc.* **2006**, *128*, p.15072-15073; S3-5) the resulting reaction mixture was stirred and heated over night at 50℃. The cooled s olution was concentrated under reduced pressure. The crude product was used without further characterization for the next chemical step.

Example 10b

{4,10-bis(carboxymethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-cyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetra-azacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclo-dodecan-1-yl}acetic acid



The crude *tert*-butyl {4,10-bis(2-*tert*-butoxy-2-oxoethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}-amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate from example 10a was dissolved in 40 mL TFA. The resulting solution was stirred overnight at room temperature and was concentrated under reduced pressure. The crude product was used without further characterization for the next chemical step.

Example 10

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}-amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate

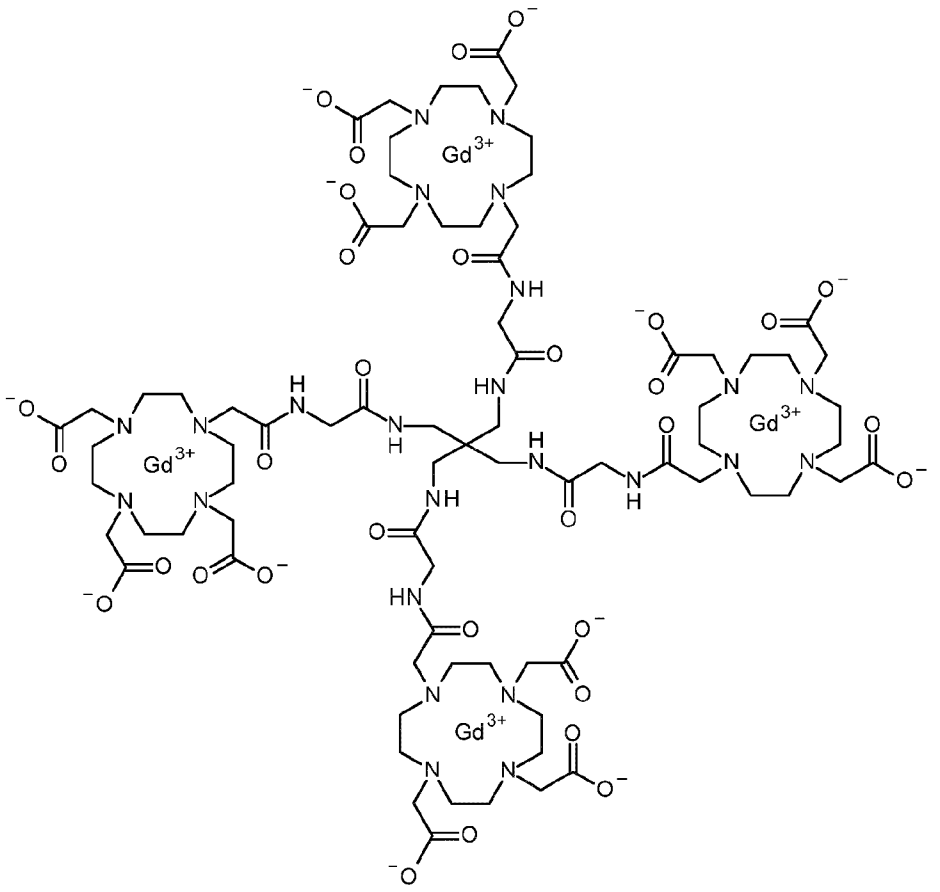
The crude {4,10-bis(carboxymethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetra-

azacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid from example 10b was dissolved in 10 mL water. After addition of 326 mg of tris(acetato-kappaO)gadolinium tetrahydrate the pH value of the resulting solution was adjusted to 3.5 - 4.5 by addition of aqueous sodium hydroxide solution. The reaction mixture was heated under stirring overnight at 70℃. The resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 65 mg (28 µmol, 46%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.40 min.
MS (ES^+): m/z (z = 2) = 1149.7 $(M + 2H)^{2+}$, m/z (z =3) = 766.0 $(M + 3H)^{3+}$.

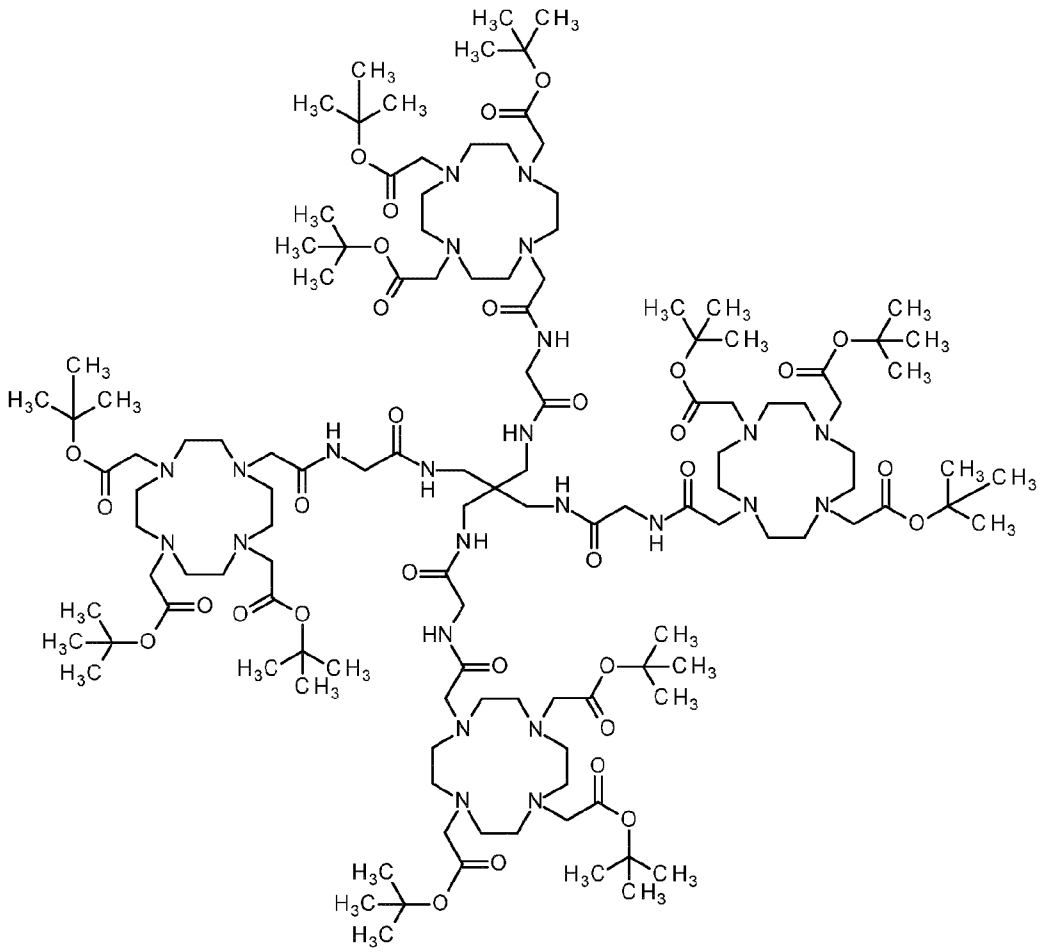
Example 11

Tetragadolinium [4,10-bis(carboxylatomethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}-methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate



Example 11a

***Tert*-butyl [4,10-bis(2-tert-butoxy-2-oxoethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}-methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate**

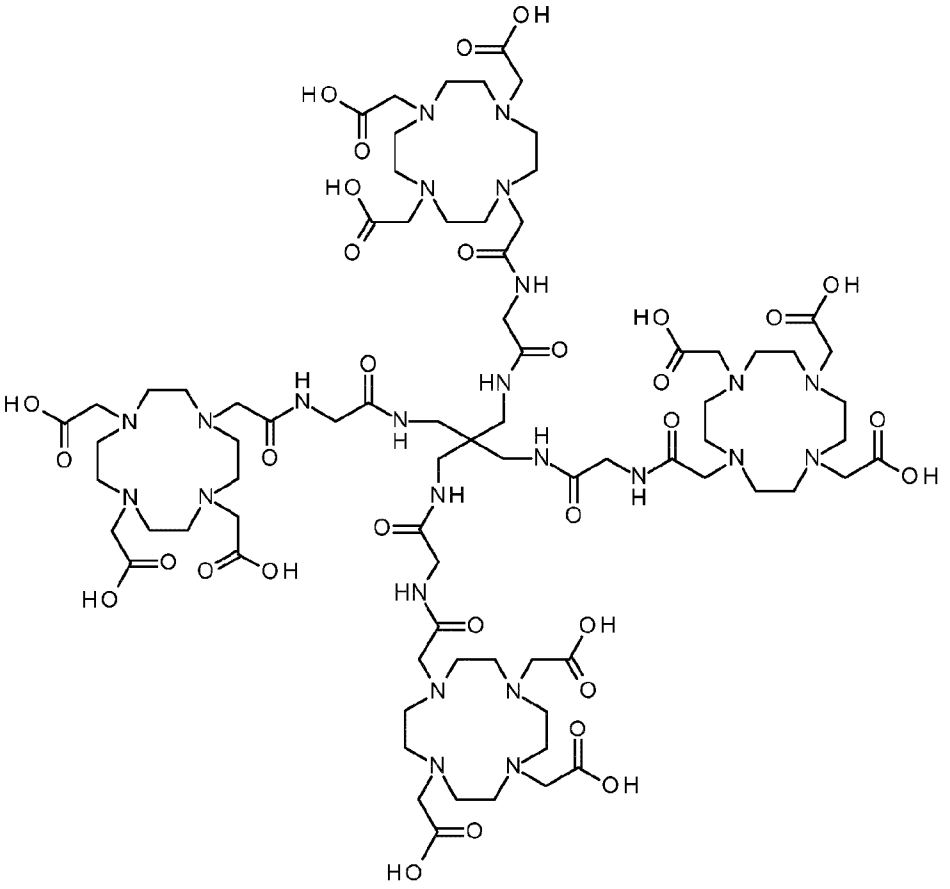


2.99 g (4.75 mmol, 12 eq.) N-{[4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}glycine (see M. Suchy et al., Org. Biomol. Chem. 2010, 8, 2560 - 2566) and 732 mg (5.70 mmol, 14.4 eq.) ethyldiisopropylamine were dissolved in 40 mL N,N-dimethylformamide. After addition of 2.17 g 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU; 5.70 mmol, 14.4 eq.) the reaction mixture was stirred for 15 minutes at room temperature. 100.1 mg (396 µmol, 1 eq.) 2,2-bis(ammoniomethyl)propane-1,3-diaminium tetrachloride (see W. Hayes et al., Tetrahedron 59 (2003), 7983 - 7996) and 982.7 mg (7.60 mmol, 19.2 eq.) ethyldiisopropyl-

amine were added and the resulting reaction mixture was stirred over night at 50℃. The cooled solution was concentrated under reduced pressure. The crude product was used without further characterization for the next chemical step.

Example 11b

[4,10-bis(carboxymethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-cyclododecan-1-yl]acetyl}amino)acetyl]amino}methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid



The crude *tert*-butyl [4,10-bis(2-tert-butoxy-2-oxoethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(2-tert-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}-methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate from

example 11a was dissolved in 125 mL TFA. The resulting solution was stirred for 2 hours at 70℃, overnight at room temperature and was concentrated under reduced pressure. The oily product was dissolved in 200 mL water, was isolated by lyophilisation and was used without further characterization for the next chemical step.

Example 11

Tetragadolinium [4,10-bis(carboxylatomethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}-methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate

The crude [4,10-bis(carboxymethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-cyclododecan-1-yl]acetyl}amino)acetyl]amino}methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid from example 11b was dissolved in 100 mL water. After addition of 2.89 g of tris(acetato-kappaO)gadolinium tetrahydrate the pH value of the resulting solution was adjusted to 3.0 - 3.5 by addition of aqueous sodium hydroxide solution. The reaction mixture was heated under stirring for 24 hours at 70℃. The resulting solution was ultrafiltrated with water using an 1 kDa membrane and the final retentate was lyophilized. The crude product was purified by RP-chromatography yielding 296 mg (120 µmol, 30%) of the title compound.

UPLC (ACN-HCOOH): Rt. = 0.41 min.

MS (ES^+): m/z (z = 2) = 1262.8 $(M + 2H)^{2+}$, m/z (z =3) = 841.5 $(M + 3H)^{3+}$.

Reference compound 1

Gadovist® (gadobutrol, Bayer AG, Leverkusen, Germany)

Reference compound 2

Magnevist® (gadopentetate dimeglumine, Bayer AG, Leverkusen, Germany)

Reference compound 3

Primovist® (gadoxetate disodium, Bayer AG, Leverkusen, Germany)

Reference compound 4

Gadomer-17 was synthesized as described in EP0836485B1, Example 1k.

In vitro and in vivo characterisation of Example compounds

Examples were tested in selected assays one or more times. When tested more than once, data are reported as either average values or as median values, wherein

- the average value, also referred to as the arithmetic mean value, represents the sum of the values obtained divided by the number of times tested, and
- the median value represents the middle number of the group of values when ranked in ascending or descending order. If the number of values in the data set is odd, the median is the middle value. If the number of values in the data set is even, the median is the arithmetic mean of the two middle values.

Examples were synthesized one or more times. When synthesized more than once, data from assays represent average values or median values calculated utilizing data sets obtained from testing of one or more synthetic batch.

Example A

Relaxivity measurements at 1.4 T

Relaxivity measurements at 1.41 T were performed using a MiniSpec mq60 spectrometer (Bruker Analytik, Karlsruhe, Germany) operating at a resonance frequency of 60 MHz and a temperature of 37°C. The T_1 relaxation times were determined using the standard inversion recovery (IR) method with a fixed relaxation delay of at least 5 x T_1. The variable inversion time (TI) was calculated automatically by the standard software of the MiniSpec mq60 (8 steps). The T_2 measurements were done by using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, applying a relaxation delay of at least 5 x T_1.

Each relaxivity measurement was performed using three different Gd concentrations (3 concentrations between 0.05 and 2 mM). The T_1 and T_2 relaxation times of the example compounds 1-10 were measured in different media for example in water, fetal bovine serum (FBS, Sigma, F7524) and human plasma.

Human plasma preparation: For each experiment fresh blood was taken from a volunteer using 10 mL citrate-tubes (Sarstedt S-Monovette 02.1067.001, 10 mL, Citrate). The 10 mL citrate- tubes were carefully inverted 10 times to mix blood and anticoagulant and centrifuged for 15 minutes at 1811 g at room temperature (Eppendorf, Centrifuge 5810R).

The relaxivities r_i (where i=1, 2) were calculated on the basis of the measured relaxation rates R_i in water and plasma:

$$R_i = R_{i(0)} + r_i [C_{Gd}],$$

where $R_{i(0)}$ represent the relaxation rate of the respective solvent and C_{Gd} the concentration of the compound normalized to the Gadolinium. The Gadolinium concentrations of the investigated solutions were verified by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS Agilent 7500a, Waldbronn, Germany).

The determined relaxivity values are summarized in Table 1.

Table 1: Relaxivities of investigated compounds in water, fetal bovine serum (FBS) and human plasma at 1.41 T and relaxivities of Reference compounds 1-4 (RC1-RC4) at 1.5 T in water and bovine plasma. All values were measured at 37°C, are normalized to Gd and given in L $mmol^{-1}$ s^{-1}.

Example No	r_1 water*	r_2 water*	r_1 FBS*	r_2 FBS*	r_1 human plasma*	r_2 human plasma*
1	11.1	12.9	13.2	16.3	13.0	19.5
2	12.1	14.2	13.4	16.4	13.9	17.6
3	10.1	11.7	11.5	13.7	11.8	14.7
3-1	9.5	11.1	n.d.	n.d.	10.4	13.1
3-2	9.4	10.8	n.d.	n.d.	11.4	14.2
4	11.5	13.5	13.3	16.0	13.2	16.5
5	13.0	15.2	14.6	18.1	14.3	17.7
6	13.4	15.7	14.2	17.5	14.6	18.6
7	10.8	12.6	11.7	14.4	12.1	14.9
8	12.5	14.5	14.5	17.9	14.6	18.1
9	7.4	8.5	8.8	10.4	n.d.	n.d.
10	7.3	8.3	9.2	10.7	9.7	11.3
RC1^	3.3	3.9	5.2	6.1	n.d.	n.d.
RC2^	3.3	3.9	4.1	4.6	n.d.	n.d.
RC3^	4.7	5.1	6.9	8.7	n.d.	n.d.
RC4^	17.3	22	16	19	n.d.	n.d.

* values are depicted in L $mmol^{-1}$ s^{-1}

^ Relaxivities from reference compounds from Rohrer et. al. (Invest. Radiol. 2005; 40, 11: 715-724), bovine plasma (Kreaber GmbH, Pharmaceutical Raw Material, Ellerbek, Germany)

Relaxivity measurements at 3.0 T

Relaxivity measurements at 3.0 T were performed with a whole body 3.0 T MRI Scanner (Philips Intera, Philips Healthcare, Hamburg, Deutschland) using a knee-coil (SENSE-Knee-8, Philips Healthcare, Hamburg, Deutschland). The sample tubes (CryoTubetm Vials, Thermo Scientific 1.8 mL, Roskilde, Denmark) were positioned in 3 rows of 4 and 5 tubes in a plastic holder in a box filled with water. The temperature was adjusted to 37℃. For the MRI sequence the shortest possible echo-time (TE) with 7.46 milliseconds was used. The inversion times were chosen to optimize the sequence to measure T_1 values corresponding to the estimated T_1 range of all relaxation times of contrast media containing solutions. The following inversion times (TIs) were applied: 50, 100, 150, 200, 300, 500, 700, 1000, 1400, 2100, 3200, and 4500 milliseconds. The sequence was run with a constant relaxation delay of 3.4 seconds after the registration of the last echo (variable TR in the range from 3450 to 7900 milliseconds). For details of the fit procedure, see Rohrer et.al. (Invest. Radiol. 2005; 40, 11: 715-724). The experimental matrix of the phantom measurement was 320 x 320.

The relaxivities were evaluated using three different concentrations of each compound (3 concentrations between 0.05 and 2 mM).

The T_1 relaxation times of the Example compounds 1-6 were measured in water and human plasma. Human plasma preparation: For each experiment fresh blood was taken from a volunteer using 10 mL citrate-tubes (Sarstedt S-Monovette 02.1067.001, 10 mL, Citrate). The 10 mL citrate- tubes were carefully inverted 10 times to mix blood and anticoagulant and centrifuged for 15 minutes at 1811 g at room temperature (Eppendorf, Centrifuge 5810R).

The relaxivities r_i (where i=1, 2) were calculated on the basis of the measured relaxation rates R_i in water and plasma:

$$R_i = R_{i(0)} + r_i [C_{Gd}],$$

where $R_{i(0)}$ represent the relaxation rate of the respective solvent and C_{Gd} the concentration of the compound normalized to the Gadolium (Table 2).

Table 2: Relaxivities (normalized to Gd) in water and human plasma at 3.0 T and 37℃ [L $mmol^{-1}$ s^{-1}]

Example No	r_1 water*	r_1 human plasma*
1	9.5 ± 0.2	10.8 ± 0.1
2	9.2 ± 0.3	11.4 ± 0.1
3	9.2 ± 0.3	10.2 ± 0.2
3-1	8.9 ± 0.2	10.1 ± 0.1
3-2	9.0 ± 0.4	11.4 ± 0.2
4	10.1 ± 0.2	11.8 ± 0.3
5	10.8 ± 0.3	12.4 ± 0.2
6	11.3 ± 0.4	12.8 ± 0.3
RC1^	3.2 ± 0.3	5.0 ± 0.3
RC2^	3.1 ± 0.3	3.7 ± 0.2
RC3^	4.3 ± 0.3	6.2 ± 0.3
RC4^	13.0 ± 0.7	13 ± 1

* Average ± standard deviation, values are depicted in L $mmol^{-1}$ s^{-1}

Example B

Pharmocokinetic parameters

Pharmacokinetic parameters of the compound of Example 3 were determined in male rats (Han-Wistar, 220-230 g, n=3). The compound was administered as a sterile aqueous solution (52.5 mmol Gd/L) as a bolus in the tail vein of the animals. The dose was 0.1 mmol Gd/kg. Blood was sampled 1, 3, 5, 10, 15, 30, 60, 90, 120, 240, 360, 480 and 1440 min post injection and the Gd concentration was determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS Agilent 7500a, Waldbronn, Germany). The blood level was converted to plasma concentrations by division by 0.625 (plasma fraction of rat blood, assuming strictly extracellular distribution). As a control, 3 animals were treated in the same way with Gadovist®, a low molecular weight contrast agent. The time courses of the blood plasma levels are shown in Figure 1.

The fit of the obtained data to a three compartment model (Phoenix – WinNonlin) yielded the pharmacokinetic parameters which are shown in Table 3.

Table 3: Time courses of blood plasma levels

Parameter		unit	Gadovist®		Example 3	
			mean	SD	mean	SD
t½ α	Half-life, compartment V1	[min]	1.6	0.4	1.7	0.3
t½ β	Half-life, compartment V2	[min]	20.5	1.9	18.2	3.4
t½ γ	Half-life, compartment V3	[min]	232	126	133	22.0
MRT	Mean residence time	[min]	30.1	3.8	24.1	4.4
AUC_∞	Area under the curve (to infinity)	[µmol/l*min]	11500	1180	9040	1220
V_c (V1)	Volume, central compartment V1	[l/kg]	0.14	0.01	0.11	0.01
V2	Volume, compartment V2	[l/kg]	0.12	0.01	0.15	0.01
V1 + V2	Volume, compartments V1+V2	[l/kg]	0.25	0.02	0.26	0.01
$V_{d,ss}$	Volume of distribution at steady state	[l/kg]	0.28	0.02	0.28	0.01
Cl_{tot}	Total Clearance	[ml/min*kg]	9.30	0.9	11.8	1.7

Example C

Excretion and residual organ gadolinium concentration after 5 days

The excretion and organ distribution of Example 3 were determined in male rats (Han-Wistar, 100-110 g, n=3). The compound was administered as a sterile aqueous solution (54 mmol Gd/L) as a bolus in the tail vein of the animals. The dose was 0.1 mmol Gd/kg. Urine was collected in the following time periods 0-1 h, 1-3 h, 3-6 h, 6-24 h, 1-2 d and 2-5 d post injection and feces 0-1 d, 1-2 d and 2-5 d post injection. As a control, 3 animals were treated in the same way with Gadovist®, a low molecular weight contrast agent. On day 7 the animals were sacrificed and the following organs were excised: blood, liver, kidney, spleen, heart, lung, brain, mesenteric lymph nodes, muscle, skin, stomach, gut, bone and bone marrow. The remaining carcass was freeze dried and ground to a fine powder. The Gd concentration in the organs and the carcass was determined by ICP-MS (ICP-MS Agilent 7500a, Waldbronn, Germany). The results of the organ distribution of Example 3 and Reference compound 1 (Gadovist®) are summarized in Table 4. The Example 3 is excreted quickly via the kidneys. After 3 h 95.8% ± 3.4% of the injected dose was found in urine and 96.9% ± 3.7% after 5 days. About 1.4% ± 0.6% was excreted via the feces. Less than 0.5% of the administered dose was present in the body 7 days after the injection. The individual organs contained less than 0.03% of the injected dose, except the kidney which is the excretion organ.

Table 4: Excretion and organ distribution of Gadovist® and Example 3 in rats

	Gadovist® [% Dose]	**Example 3** [% Dose]
Time period post injection	**Urine**	**Urine**
0-1 h	91.28 ± 2.69 %	90.36 ± 4.4 %
1-3 h	7.38 ± 1.50 %	5.43 ± 1.04 %
3-6 h	0.22 ± 0.08 %	0.46 ± 0.38 %
6-24 h	0.28 ± 0.03 %	0.17 ± 0.02 %
1-2 d	0.20 ± 0.02 %	0.14 ± 0.01 %
2-5 d	0.64 ± 0.18 %	0.34 ± 0.03 %
Time period post injection	**Feces**	**Feces**
0-1 d	1.47 ± 1.38 %	1.13 ± 0.62 %
1-2 d	0.13 ± 0.08 %	0.10 ± 0.02 %
2-5 d	0.13 ± 0.02 %	0.13 ± 0.01 %
Time point post injection	**Σ organs and carcass**	**Σ organs and carcass**
7 d	0.50 ± 0.07 %	0.49 ± 0.01 %
Total recovery	**101.9 ± 0.4 %**	**98.8 ± 3.1 %**

Example D

Chemical stability

Examples 1, 2, 3 and 6 were separately dissolved in 10 mM Tris-HCl buffer, pH 7.4 at a final concentration of 5 mmol Gd/L. An aliquot was removed and the rest of the clear and colorless solution was autoclaved at 121℃ for 20 m in. After autoclaving, the solution was still clear and colorless. The aliquot removed before and after autoclaving was analyzed by HPLC-ICP-MS to determine the integrity of the compound.

HPLC: Column: Hypercarb 2.5 mm x 15 cm. Solvent A: 0.1% formic acid in water. Solvent B: acetonitrile. Gradient from 100% A to 5% A + 95% B in 10 min. Flow 1 ml/min. Detection by ICP-MS, tuned to ^{158}Gd. The chromatograms, displaying the intensity of the detected Gd, were visually compared. No changes in the chromatograms before and after autoclaving were detected. The compounds were stable during the autoclaving procedure.

Example E

Gadolinium release after the addition of zinc and phosphate

The proton relaxometric protocol for the transmetallation assessment for the stability determination of MRI contrast media is described in Laurent S. et al. (Invest. Radiol. 2001; 36, 2: 115-122). The technique is based on measurement of the evolution of the water proton paramagnetic longitudinal relaxation rate in phosphate buffer (pH 7.00, 26 mmol/L, KH_2PO_4 Merck, Hessen, Germany) containing 2.5 mmol/L gadolinium complex and 2.5 mmol/L $ZnCl_2$ Sigma-Aldrich, Munich, Germany). Hundret microliters of a 250 mmol/L solution of $ZnCl_2$ were added to 10 mL of a buffered solution of paramagnetic complex (Reference compounds 1-4 and Example 3). The mixture was vigorously stirred, and 300 µL were taken out for the relaxometric study at 0 min, 60 min, 120 min, 3 h, 4 h, 5 h, 24 h, 48 h and 72 h. The measurements were performed on a MiniSpec mq60 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 60 MHz and 37℃. The results of Example 3 in comparison to Reference compound 1 (Gadovist®), Reference compound 2 (Magnevist®) and Reference compound 3 (Primovist®) are shown in Figure 2. If Gadolinium transmetallation is triggered by the Zn^{2+} ions in a phosphate-buffered solution, then free released Gd^{3+} would react with the free PO_4^{3-} ions to form $GdPO_4$. Due to the low solubility of $GdPO_4$ a part of the Gadolinium precipitates as solid and has no further influence on the longitudinal relaxation rate of water. A decrease of the proton relaxation rate would be observed for Gadolinium chelates with a low stability [see linear contrast media in Figure 2: Reference compounds 2 (Magnevist®) and 3 (Primovist®)]. The stability of Example 3 is comparable to the high stability of Reference compound 1 (Gadovist®).

Example F

Gd-complex stabilities in human plasma at 37℃, 15 d

Examples 3 and 10 were separately dissolved in human plasma at 1 mmol Gd/L. As a reference for released Gd^{3+} 0.1 mmol/L Gadolinium chloride ($GdCl_3$) was dissolved in human plasma. The plasma samples were incubated for 15 days at 37℃ under 5% CO_2 atmosphere to maintain the pH at 7.4. Aliquots were taken at the start and end of the incubation. The amount of Gd^{3+} released from the complexes was determined by HPLC-ICP-MS. Column: Chelating Sepharose (HiTrap, 1mL). Solvent A: 10 mM BisTris-HCl pH 6.0. Solvent B: 15 mM HNO3. Gradient: 3 min at 100% A, from 3 to 10 min at 100% B. Flow 1 mL/min. Detection by ICP-MS, tuned to ^{158}Gd. The chromatograms, displaying the intensity of the detected Gd, were evaluated by peak area analysis. The size of the peak of Gd^{3+}, eluting after the change from solvent A to B, was recorded. For both compounds the increase of this peak and thus the release of Gd^{3+} was below the limit of quantification (< 0.1% of the injected total amount of Gadolinium). Both Gd-complexes are stable under physiological conditions.

Example G

Water solubility

The water solubility of the compounds was determined at room temperature (20℃) in 0.5 mL buffer solution (10 mM Tris-HCl) in the microcentrifuge tubes (Eppendorf, 2.0 mL safe-lock caps). The solid compound was added stepwise to the buffer solution. The suspension was mixed using a shaker (Heidolph Reax 2000) and treated 5 min in an ultrasound bath (Bandelin, Sonorex Super RK255H) The suspension was stored at room temperature (20℃) over night and final Gadolinium concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS). The results are summarized in Table 5.

Table 5: Solubilities of compounds in water at 20℃.

Example No	Solubility [mmol Gd/L]
1	>1200
2	>1200
3	>1400
4	>1200
5	>1100
6	>1100
7	>1400
8	>1000
9	>800
10	>800

Example H

Contrast-enhanced magnetic resonance angiography (CE-MRA)

The potential of a significant dose reduction was shown by an intraindividual comparison of 100 µmol Gadolinium per kilogram body weight [100 µmol Gd/ kg bw], which is comparable to the human standard dose, and a low dose protocol using 30 µmol Gadolinium per kilogram body weight. Reference compound 1 (Gadovist®), as an approved representative of the Gadolinium-based MRI contrast agents, was used in both dose protocols (100 µmol Gd/kg bw and 30 µmol Gd/kg bw) and compared to Example 3 (30 µmol Gd/ kg bw).

The contrast-enhanced magnetic resonance angiography study was performed at a clinical 1.5 T Scanner (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany). For optimal signal exploitation, a standard spine coil was used for the data acquisition. The study was done using male New Zealand white rabbits (weight 2.5-2.9 kg, n=6, Charles River Kisslegg). All animals were initially anesthetized using a body weight-adjusted intramuscular injection of a mixture (1+2) of xylazin hydrochlorid (20 mg/mL, Rompun 2%, Bayer Vital GmbH,

Leverkusen) and ketamine hydrochlorid (100 mg/mL, Ketavet, Pfizer, Pharmacia GmbH, Berlin) using 1 mL/kg body weight. The continuous anesthesia of the intubated animals (endotracheal tube, Rueschelit Super Safe Clear, cuff 3.0 mm, Willy Ruesch AG, Kernen, Germany) was achieved by the intravenous injection of 0.9 mg propofol per kilogram per hour (10 mg/mL, Propofol-Lipuro 1%, B. Braun Melsungen AG, Melsungen, Germany). The continuous intravenous injection was performed using a MR infusion system (Continuum MR Infusion System, Medrad Europe B. V., AE Beek, Deutschland). The tracheal respiration (SV 900C, Maquet, Rastatt, Germany) was performed with 55% oxygen, forty breaths per minute and a breathing volume of 7 mL per kilogram body weight per minute.

Based on a localizer sequence oriented in coronal, axial and sagittal directions the anatomic course of the aorta was acquired. The time to peak was determined using a small intravenous test bolus (0.25 mL/2.5-2.7 kg or 0.3 mL/2.8-2.9 kg bw, Reference compound 1) and a 3D FLASH sequence (test bolus sequence: repetition time: 36.4 millisecond, echo time 1.45 millisecond, flip angle: 30 degree, spatial resolution: 1.0x0.8x17 mm). The angiography 3D FLASH sequence was characterized by a repetition time of 3.24 milliseconds, an echo time of 1.17 milliseconds, a flip angle of 25 degree and a slice thickness of 0.94 mm. The field of view of 141x300 mm was combined with a matrix of 150x320 resulting in a spatial resolution of 0.9x0.9x0.9 mm and a whole acquisition time of 13 seconds per 3D block. The 3D FLASH sequence was performed once before and immediately after injection of the contrast agent. The time interval for the intraindividual comparison between the different contrast agent applications was twenty to thirty minutes (n=3 animals).

The resulting magnetic resonance angiographs of the intraindividual comparison in rabbits are depicted in Figure 3: (A) 30 µmol Gd/kg bw Reference compound 1 (Gadovist®); (B) 30 µmol Gd/kg bw Example 3 and (C) 100 µmol Gd/kg bw Reference compound 1. The contrast enhancement of the low dose protocol with Example 3 (B) is comparable to that of the standard dose of Reference compound 1 (C). Furthermore, the image quality of the low dose protocol of Example 3 (B) is significantly better than the low dose protocol of Reference compound 1 (A). The angiography study demonstrates the potential of Example 3 for a significant dose reduction.

Example J

Whole body imaging

Classical extracellular Gadolinium-based contrast agents exhibit a rapid extracellular passive distribution in the whole body and are excreted exclusively via the kidney. The fast extracellular distribution in the whole body enables the classical imaging possibilities as for example angiography and the imaging of the central nervous system, extremities, heart,

head/face/neck, abdomen and breast. The comparability of the pharmacokinetic and diagnostic behavior of Reference compound 1 (Gadovist®) and other ECCM has been shown and forms the basis for bridging the efficacy to all body parts usually imaged in the diagnostic workup of a variety of diseases (Tombach B et.al., Eur Radiol 2002;12(6):1550-1556). The described contrast-enhanced magnetic resonance study compares the pharmacokinetic distribution and the diagnostic performance of Example 3 to Reference compound 1 (Gadovist®), as an approved representative of the Gadolinium-based MRI contrast agents.

To demonstrate that Example 3 has the same mode of action, MRI signal intensity over time and Gd concentrations were determined in various tissues. The study was performed at a clinical whole body MRI equipped with body spine coil, abdomen flex coil, neck coil (1.5 T Magnetom Avanto, Siemens Healthcare, Erlangen, Germany). The study was done using male New Zealand white rabbits (weight 2.3-3.0 kg, n=8, Charles River Kisslegg). All animals were initially anesthetized using a body weight-adjusted intramuscular injection of a mixture (1+2) of xylazin hydrochlorid (20 mg/mL, Rompun 2%, Bayer Vital GmbH, Leverkusen) and ketamine hydrochlorid (100 mg/mL, Ketavet, Pfizer, Pharmacia GmbH, Berlin) using 1 mL/kg body weight. The continuous anesthesia of the intubated animals (endotracheal tube, Rueschelit Super Safe Clear, cuff 3.0 mm, Willy Ruesch AG, Kernen, Germany) was achieved by the intravenous injection of 0.9 mg propofol per kilogram per hour (10 mg/mL, Propofol-Lipuro 1%, B. Braun Melsungen AG, Melsungen, Germany). The continuous intravenous injection was performed using a MR infusion system (Continuum MR Infusion System, Medrad Europe B. V., AE Beek, Deutschland). The tracheal respiration (SV 900C, Maquet, Rastatt, Germany) was performed with 55% oxygen, forty breaths per minute and a breathing volume of 7 mL per kilogram body weight per minute.

Dynamic MRI measurements up to 22 min post injection with subsequent quantitative signal analysis (Siemens Mean Curve software (SYNGO Task Card, Siemens Healthcare, Erlangen, Germany), were performed for three different regions head and neck (brain, tongue, chops muscle, neck muscle), abdomen (spleen, liver, blood) and pelvis (extremity muscle). For the three different slice groups a 3D T1-weighted Vibe sequence was used (TR=4.74ms, TE=2.38, flip=10°, 1:29 min). The dynamic measurements of the three slice groups (Head/Neck: 1:29 min, Abdomen: 0:49 min, Pelvis: 1:16 min) were done up to 22 min post injection: 1. Head/Neck: baseline, 1.4, 5.2, 8.9, 12.8, 16.5, 20.4 min, 2. Abdomen: baseline, 0.5, 4.3, 8.1, 11.9, 15.7, 19.5 min and 3. Pelvis: baseline, 2.9, 6.7, 10.5, 14.4, 18.1, 22.0 min. At 30 min post injection the animals were sacrificed and the Gd concentrations were measured using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS Agilent 7500a, Waldbronn, Germany) in the following tissue samples: blood, brain, tongue, liver and extremity muscle. A quantitative image evaluation was performed for the 30 min time point

p.i. due to the combination of the quantitative ICP-MS Gadolinium concentrations and the MRI region-of-interest analysis.

The administration of the contrast agent leads to a signal increase in the vascular system and in the extravascular, extracellular space of the body. The signal enhancement is based on the pharmacokinetic and physicochemical properties of the contrast agents. Figure 4 shows representative images of the head and neck region before and 1.4 min after administration of Example 3 and Reference compound 1. Figure 5 shows representative abdominal images before and 0.5 min after administration of Example 3 and Reference compound 1. Figure 6 shows representative images of the pelvis region before and 0.5 min after administration of Example 3 and Reference compound 1. All images show a clear signal enhancement for example in the heart, tongue, aorta, kidney, liver, spleen, the whole vascular system and muscles.

The signal-time curves show the signal change over time after contrast agent administration and represent the contrast agent pharmacokinetics in the respective tissue (Figure 7). In all investigated tissues a rapid increase of signal intensity was observed after contrast agent injection which was followed by a continuous signal decrease. The degree of these contrast enhancements is tissue specific. However, no differences in the time course of contrast enhancements were observed between Example 3 and Reference compound 1. This demonstrates identical pharmacokinetic properties and shows that Example 3 is suitable for different body regions (Figures 7). The amplitude of contrast enhancement depends on tissue characteristics, especially on tissue perfusion and the physicochemical properties, especially on relaxivity. As expected from the approximately 2-fold higher relaxivity (see Example A) the contrast enhancement using Example 3 is higher compared to that of Reference compound 1.

The relation between Gadolinium concentration and MRI signal change were investigated by comparing the amount of Gadolinium in tissue 30 min p.i. with the signal change at the MRI measurement performed at 19.5 min p.i. (abdomen), 20.4 min p.i. (head and neck) and 22.0 min p.i. (pelvis). The respective data for Example 3 and Reference compound 1 are shown in Figure 8. A linear correlation between the Gadolinium concentrations in various tissues and the respective MRI signal changes were observed. This demonstrates that the efficacy of Example 3 and Reference compound 1 are independent of the body region or tissue investigated. A slight deviation from this correlation was observed for the spleen, which shows a higher MRI signal enhancement than it would be expected from the Gadolinium tissue concentration. This was observed for both contrast agents and relates to the significantly higher blood volume of the spleen in comparison to other organs and tissues. Consequently the spleen loses much of its Gadolinium concentration by the exsanguination which in turn results in a mismatch between in-vivo imaging and ex-vivo Gadolinium

determination. The correlation between signal change and tissue Gadolinium concentration of all other tissues and organs, which represents the respective relaxivity, depends on the efficacy of the contrast agent used. A larger slope was determined for Example 3 (1.9) than for Reference compound 1 (1.0), which is in good agreement with the known higher relaxivity of Example 3 (Figure 8; see also relaxivity data described in Example A).

Example K

Dynamic CT diffusion phantom study

As indicated in Example A the Reference compound 4 has a relaxivity which is in a similar range as the compounds of the present invention. Following intravenous injection, all clinically approved small monomer GBCAs (gadopentetate dimeglumine, gadoterate meglumine, gadoteridol, gadodiamide, gadobutrol and gadoversetamide) distribute in the blood and extravascular/extracellular space by passive distribution (Aime S et. al., J Magn Reson Imaging. 2009; 30, 1259-1267). Contrast agents with a high protein binding, for example gadofosveset trisodium with a prolonged period in the blood vessels caused by the reversible binding to HSA, or large hydrodynamic sizes as for example Reference compound 4 are hindered to pass the vessel wall. For good imaging results a fast diffusion through the vessel walls is required due to the fast renal excretion of GBCAs.

The described dynamic CT diffusion study compares the ability of Examples 1, 2, 3, 4, 5, 6 and Reference compounds 1 and 4 to pass a semipermeable membrane (20 kDa). A 128-row clinical CT device (SOMATOM Definition, 128; Siemens Healthcare, Forchheim, Germany) was used to monitor the diffusion through a semipermeable membrane at 100 kV and 104 mA. Single measurements were performed at 0 min, 1 min, 2 min, 3 min, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 60 min, 2 h, 3 h, 5 h, 7 h, 22 h, 24 h, 30 h, 46 h and 48 h after placing the dialysis cassette (Slide-A-Lyser, 20,000 MWCO, 0.1-0.5 mL Capacity, Thermo Scientific, Roskilde, Denmark) filled with contrast agent in fetal bovine serum solution (FBS, Sigma, F7524). The images were reconstructed with a slice thickness of 2.4 mm and a B30 convolution kernel. The used concentration in the dialysis cassettes of the investigated Examples 1, 2, 3, 4, 5, 6 and Reference compounds 1 and 4 was 20 mmol Gd/L. The imaging results for all investigated Examples and the Reference compounds 1 and 4 for the time points 0 min and 48 h after placing the cassettes in the FBS solution are depicted in Figure 9. For image analysis, regions of interest were manually drawn on 1 centrally located slice for each time point (a representative measurement region is indicated in Figure 9: Image 1A). The results of the Hounsfield units (HU) of the analyzed regions over time are shown in Figure 10. The calculated diffusion half-lifes of the investigated Examples and Reference compounds are summarized in Table 6.

Table 6: Diffusion half-live through a semipermeable membrane (20 kDa)

Example No	Diffusion half-live (20kDa) [h]
1	39
2	39
3	11
4	21
5	24
6	36
RC 1	2
RC 4	~90000

The Figure 10 and the calculated half-life data show, similar to the Reference compound 1 (Gadovist®) and in contrast to the Reference compound 4, that the Examples 1-6 are able to pass the semipermeable membrane. Furthermore the data of the investigated compounds show contrary to other high relaxivity agents, which have a high protein binding or very slow tumbling rates (e.g. Reference compound 4), that the compounds of the present invention have hydrodynamic dimensions which can overcome barriers in a timely manner. These findings indicate the ability of the compounds of the invention to overcome barriers as for example endothelial walls in the vascular system, which is a requirement for whole body imaging.

Example L

Evaluation of potential side effects

None of the investigated example compounds showed undesired negative side effects in animals after application. Additionally the off target activity of the Example 3 was screened in commercial radioligand binding and enzyme assays (LeadProfilingScreen®, Eurofins Panlabs, Taipei, Taiwan) and revealed no critical finding.

Example M

Contrast-enhanced MRI of brain tumors in rats

The potential of a significant dose reduction was shown by an intraindividual comparison of 0.3 mmol Gadolinium per kilogram body weight (300 µmol Gd/ kg bw) and a low dose protocol using 0.1 mmol Gadolinium per kilogram body weight (100 µmol Gd/kg bw). Reference compound 1 (Gadovist®), as an approved representative of the Gadolinium-

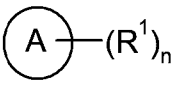
based MRI contrast agents, was used in both dose protocols (0.3 mmol Gd/kg bw and 0.1 mmol Gd/kg bw) and compared to Example 3 (0.1 mmol Gd/ kg bw).

GS9L cell line (European Collection of Cell Cultures, Cancer Res 1990;50:138-141; J Neurosurg 1971;34:335) were grown in Dulbecco's Modified Eagle Medium (DMEM, GlutaMAX™, Ref: 31966-021, Gibco) supplement with 10% fetal bovine serum (FBS, Sigma F75249) and 1 % Penicillin-Streptomycin (10.000 units/mL, Gibco). The study was done using male Fisher rats (F344, weight 170-240 g, n=4, Charles River Kisslegg). Inoculation was performed under ketamine/xylazine anesthesia using a body weight-adjusted intramuscular injection of a mixture (1+2) of xylazin hydrochlorid (20 mg/mL, Rompun 2%, Bayer Vital GmbH, Leverkusen) and ketamine hydrochlorid (100 mg/mL, Ketavet, Pfizer, Pharmacia GmbH, Berlin) using 1 mL/kg body weight.. For orthotopically intracerebral implantation anesthetized animals were fixed in a stereotactic apparatus and 1.0E+06 GS9L cells suspended in a volume of 5 µl medium were injected slowly into the brain using a Hamilton syringe.

The contrast-enhanced MRI study was performed at a clinical 1.5 T Scanner (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany). A rat head coil (coil and animal holder for rats, RAPID Biomedical GmbH) was used for the data acquisition. The rats were anesthetized using a mixture of isoflurane (2.25 %), oxygen gas (ca. 0.5 L/min) and nitrous oxide (flow ca. 1 L/min). MR Imaging was done using a 3D turbo-spin echo sequence (12 1 mm slices in a 3 D block, field of view: 80 mm (33% oversampling), repetition time: 500 millisecond, echo time 19 millisecond, spatial resolution: 0.3x0.3x1.0 mm). The animals were imaged at two consecutive days. The first day the Reference compound 1 (Gadovist®) and the Example 3 were intraindividually compared at the same dose of 0.1 mmol Gd/kg bw, which is comparable to the human standard dose. The second day the Reference compound 1 (Gadovist®) at 0.3 mmol Gd/kg bw, which is comparable to the triple human dose (clinically approved in certain CNS indications), was compared to the standard dose of Example 3 (0.1 mmol Gd/kg bw). The resulting MR images of the GS9L rat brain tumors are depicted in Figure 11: (A) *Intraindividual comparison of Reference compound 1 (Gadovist®) and Example 3 at the same dose of 0.1 mmol Gd/kg body weight (bw).* Example 3 showed at the same dose higher lesion-to-brain contrast and an excellent demarcation of the tumor rim. (B) Comparison of the Reference compound 1 (Gadovist®) at 0.3 mmol Gd/kg bw (triple dose) and Example 3 at 0.1 mmol Gd/kw bw (standard dose). Example 3 showed similar lesion-to-brain contrast at one third of the dose of Reference compound 1.

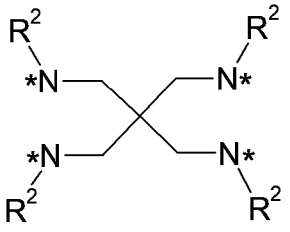
CLAIMS:

1. A compound of general formula (**I**),



(**I**) ,

in which:

A represents a  group,

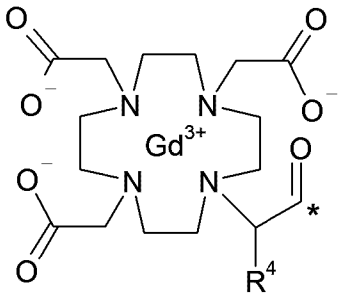
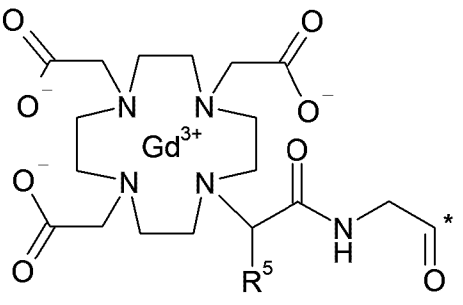
in which group * indicates the point of attachment of said group with R^1;

R^1 represents a group R^3;

n represents an integer of 4;

R^2 represents a hydrogen atom;

R^3 represents a group selected from:

 , and  ,

in which groups * indicates the point of attachment of said group with the rest of the molecule;

Date Reçue/Date Received 2020-12-07

R^4 represents a hydrogen atom;

R^5 represents a hydrogen atom or a methyl group;

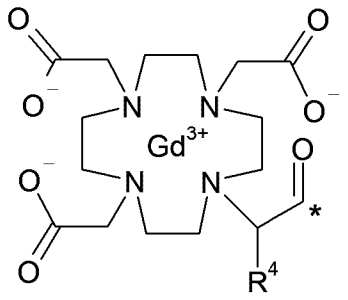
or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same.

2. The compound according to claim 1, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, in which:

R^5 represents a methyl group.

3. The compound according to claim 1, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, in which:

R^3 represents a

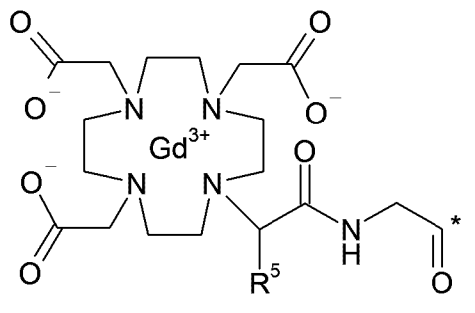


group.

4. The compound according to claim 1 or 2, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, in which:

R^3 represents a

Date Reçue/Date Received 2020-12-07



group.

5. The compound:

Tetragadolinium[4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate,

Tetragadolinium{4,10-bis(carboxylatomethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate,

Tetragadolinium{4,10-bis(carboxylatomethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate,

Tetragadolinium {4,10-bis(carboxylatomethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetate, or

Tetragadolinium[4,10-bis(carboxylatomethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(carboxylato-methyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate,

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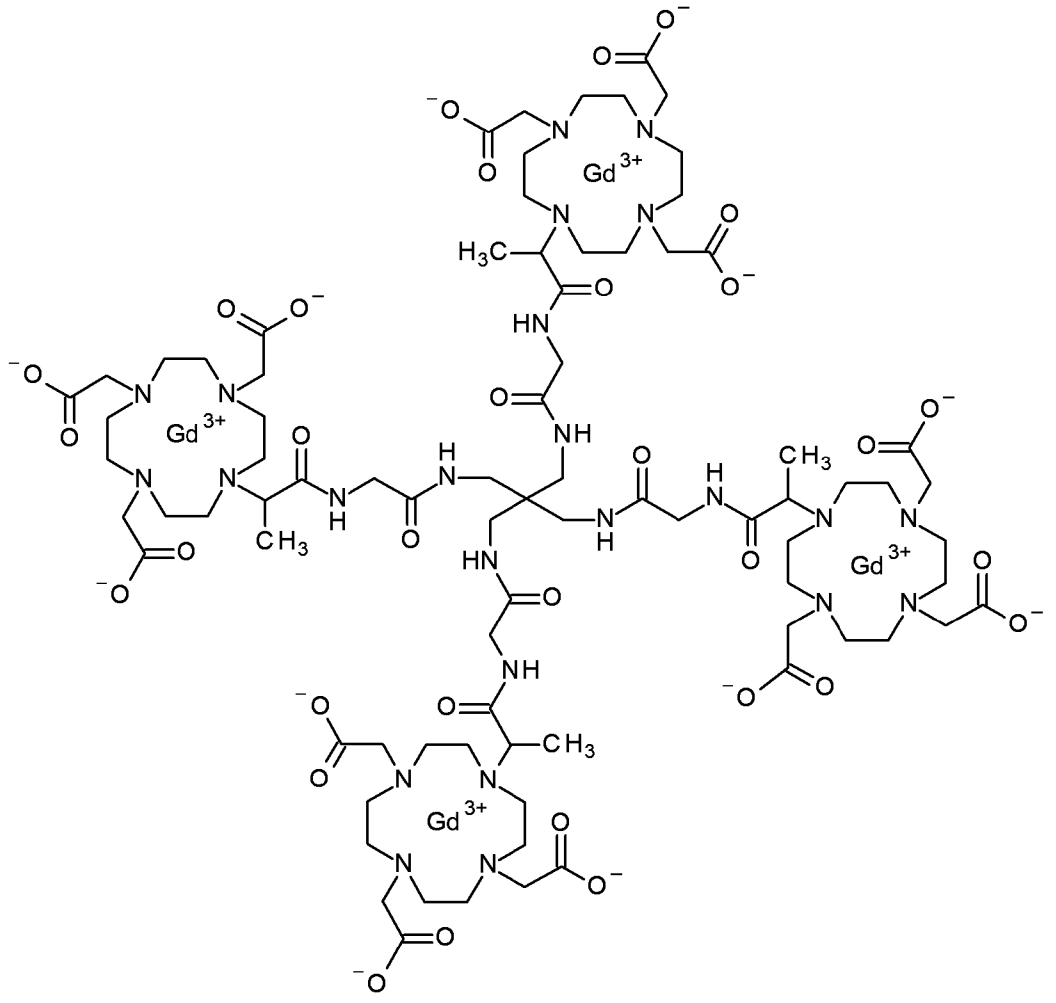
or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same.

6. The compound:

Tetragadolinium[4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris-(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}-methyl)-4,7,11,14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetate,



,

or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same.

7. A method of preparing a compound of general formula (I-d)

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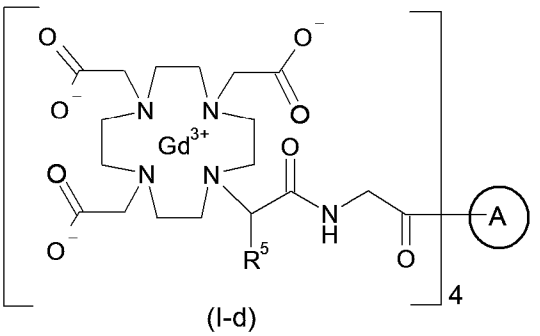
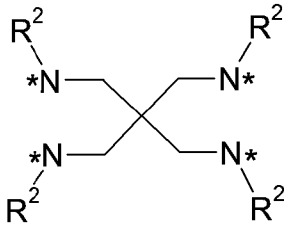


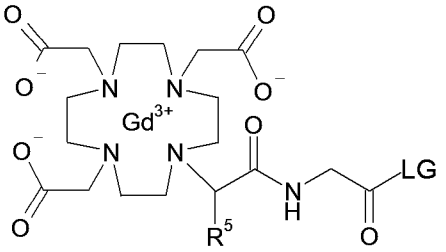
(I-d)

in which R^5 represents a hydrogen atom or a methyl group, and  represents a

 group as defined in claim 1,

said method comprising the step of allowing a tetraamine which is 2,2-bis(aminomethyl)propane-1,3-diamine, or a salt thereof,

to react with a compound of general formula (III):



(III)

in which R^5 represents a hydrogen atom or a methyl group, and LG represents an activating leaving group,

thereby giving a compound of general formula (I-d).

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8. The method according to claim 7, wherein LG represents 4-nitrophenol.

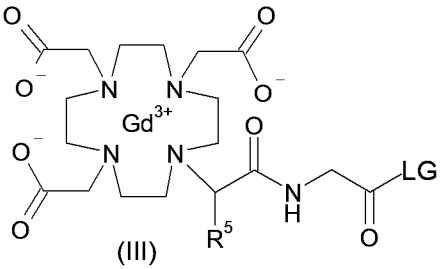
9. Use of a compound of any one of claims 1 to 6, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, for magnetic resonance imaging (MRI).

10. Compound according to any one of claims 1 to 6, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, for use in magnetic resonance imaging (MRI).

11. Use of a compound according to any one of claims 1 to 6, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, for the manufacture of a contrast agent for magnetic resonance imaging.

12. A diagnostic agent for use in magnetic resonance imaging body tissue in a patient, comprising an effective amount of one or more compounds according to any one of claims 1 to 6, or a stereoisomer, a tautomer, a hydrate, or a solvate thereof, or a mixture of same, in a pharmaceutically acceptable carrier.

13. Use of a compound of general formula (III):



(III)

in which R^5 represents a hydrogen atom or a methyl group, and LG represents an activating leaving group,
for the preparation of a compound of general formula (I) according to claim 1, 2, 4, 5 or 6.

14. Use according to claim 13, wherein LG is 4-nitrophenol.

15. A compound of general formula (**I'**),

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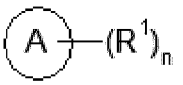
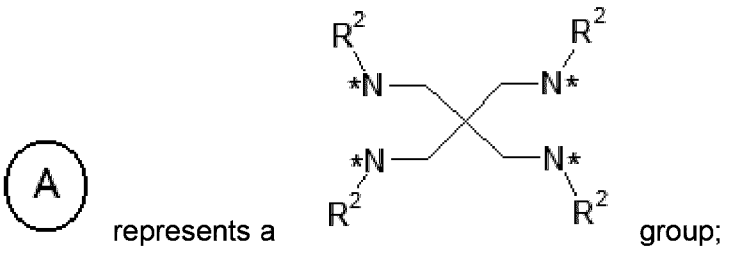


(**I'**),

in which:



represents a group;

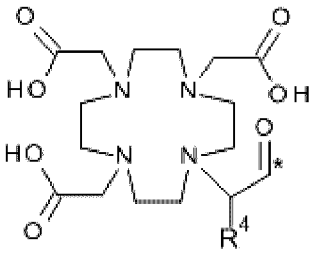
in which group * indicates the point of attachment of said group with R^1;

R^1 represents a group R^3;

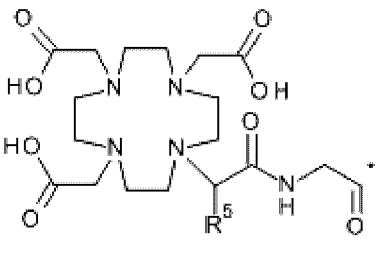
n represents an integer of 4;

R^2 represents a hydrogen atom;

R^3 represents a group selected from:



, and



,

in which groups * indicates the point of attachment of said group with the rest of the molecule;

R^4 represents a hydrogen atom;

R^5 represents a hydrogen atom or a methyl group;

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or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, or a mixture of same.

16. The compound:

[4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid,

{4,10-Bis(carboxymethyl)-7-[(2R,16R)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2R)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid,

{4,10-Bis(carboxymethyl)-7-[(2S,16S)-3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({(2S)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,14-tetraazaheptadecan-2-yl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid,

{4,10-bis(carboxymethyl)-7-[2-oxo-2-({3-({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)-2,2-bis[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)methyl]propyl}amino)ethyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic acid, or

[4,10-bis(carboxymethyl)-7-{2,5,11,14-tetraoxo-15-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-8,8-bis({[({[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}amino)acetyl]amino}methyl)-3,6,10,13-tetraazapentadec-1-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid,
or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, or a mixture of same.

17. The compound:

[4,10-bis(carboxylatomethyl)-7-{3,6,12,15-tetraoxo-16-[4,7,10-tris(carboxylatomethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-9,9-bis({[({2-[4,7,10-tris(carboxylatomethyl)-1,4,7,

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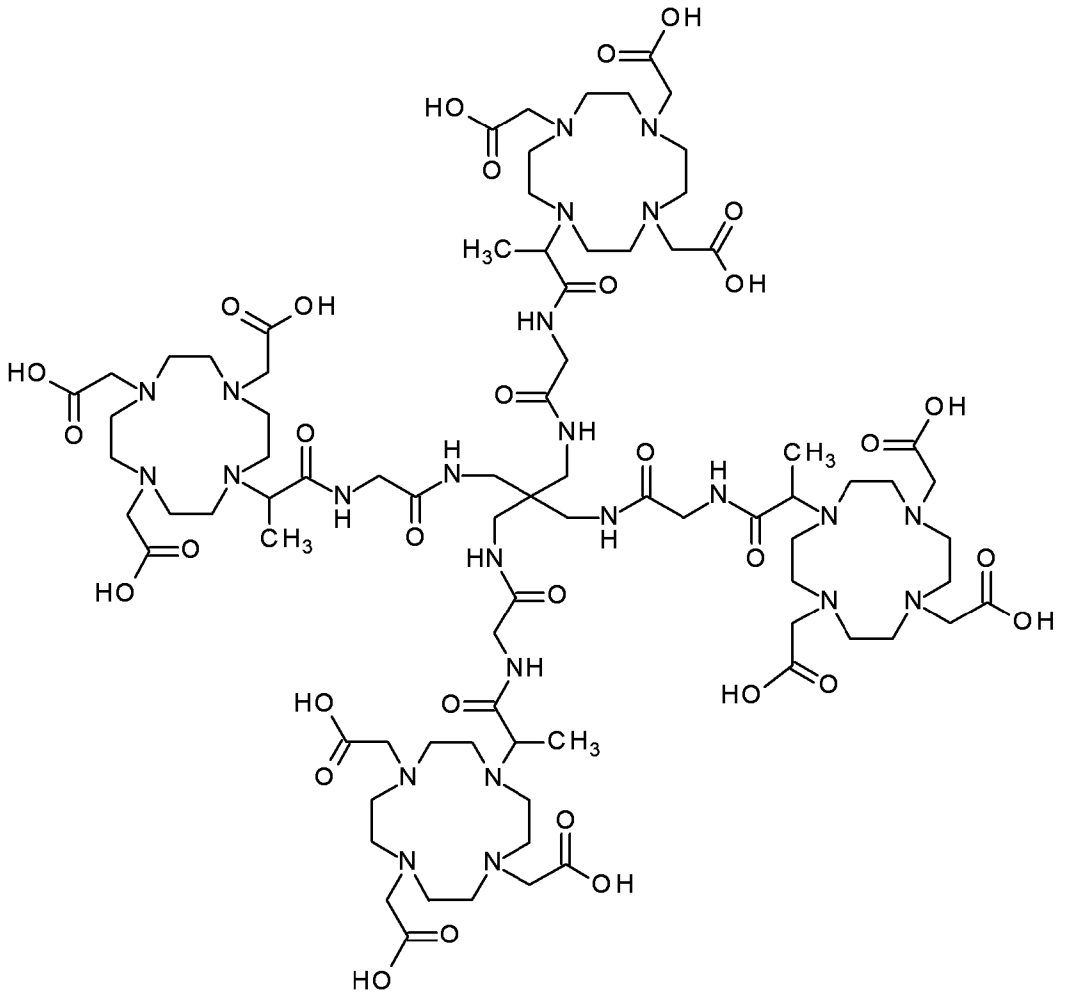
10-tetraazacyclododecan-1-yl]propanoyl}amino)acetyl]amino}methyl)-4,7,11,
14-tetraazaheptadecan-2-yl}-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid,



or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, or a mixture of same.

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FIGURES

Figure 1

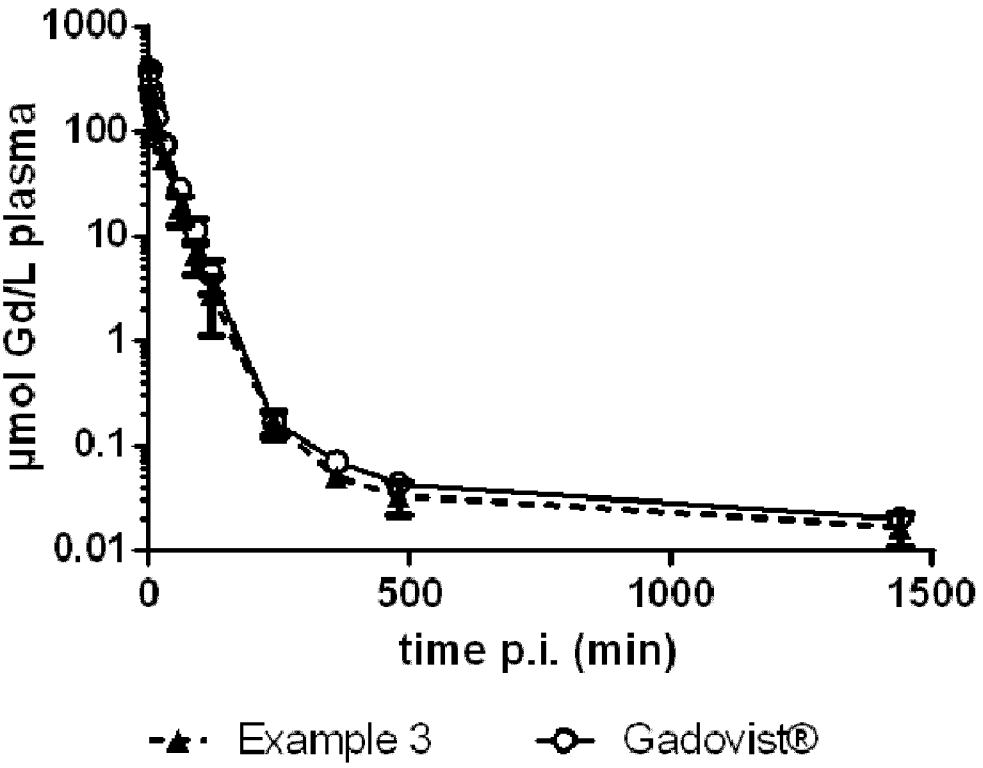


Figure 2

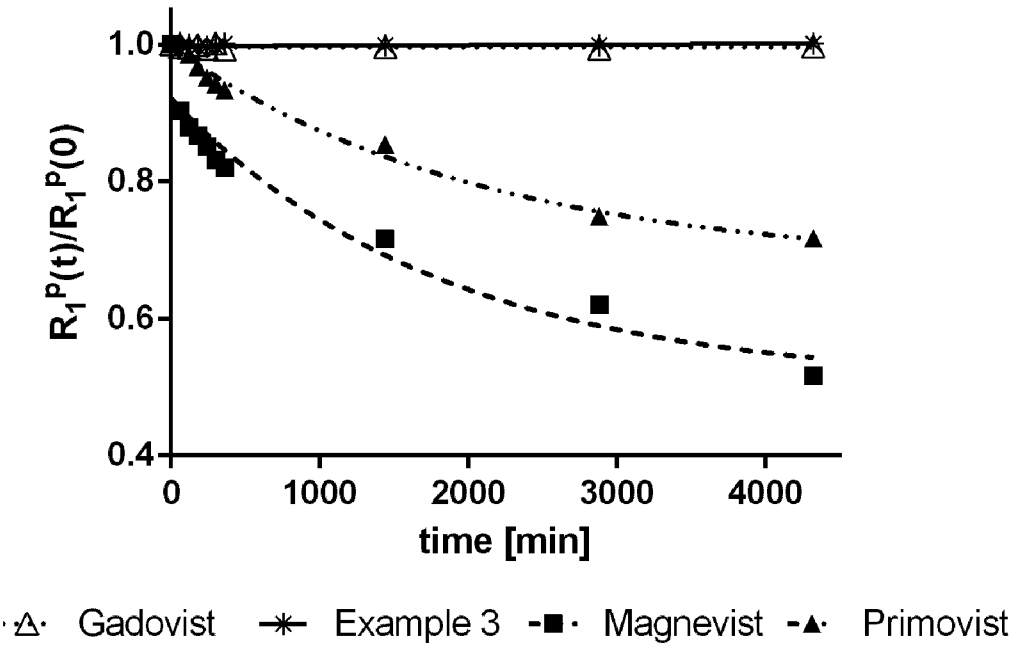

1.0
0.8
0.6
0.4
$R_1^P(t)/R_1^P(0)$
0
1000
2000
3000
4000
time [min]
Gadovist
Example 3
Magnevist
Primovist

Figure 3

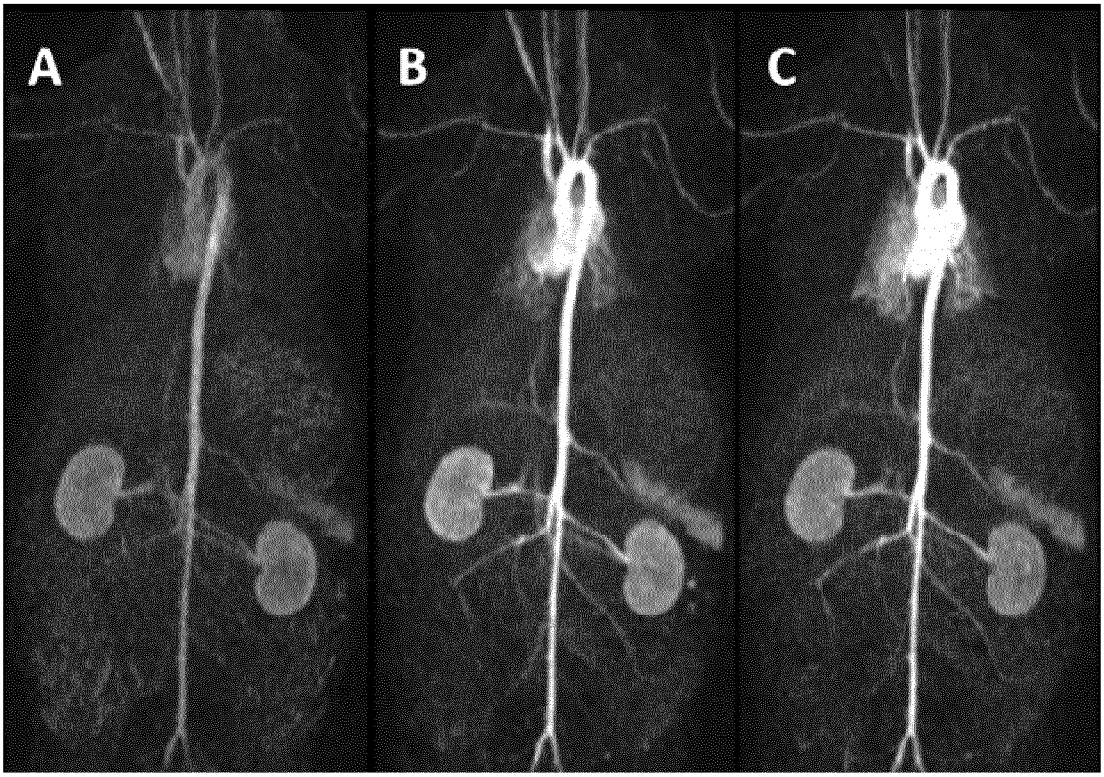
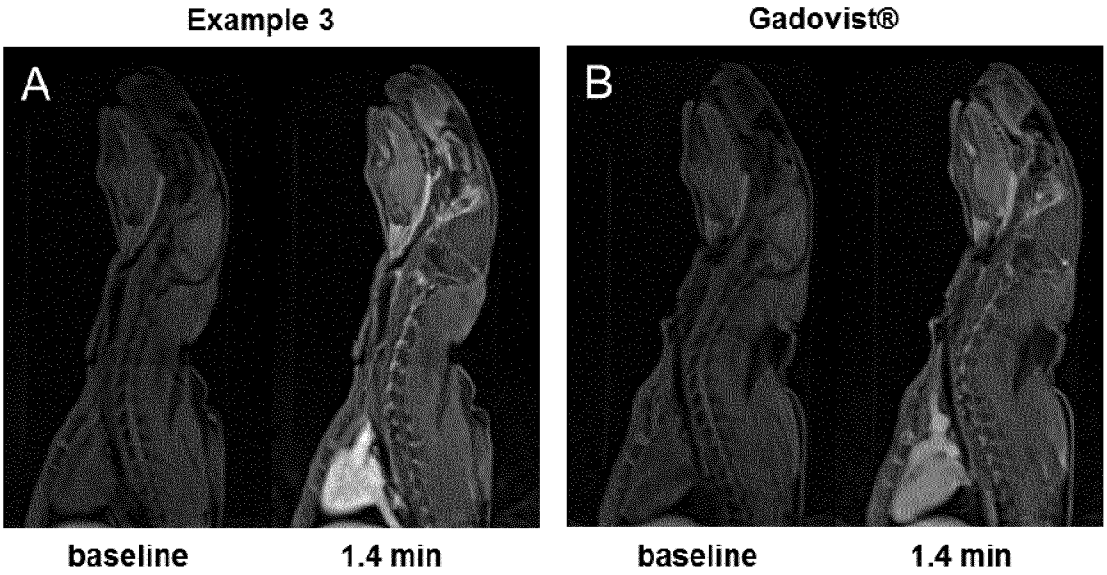


Figure 4



5

Figure 5

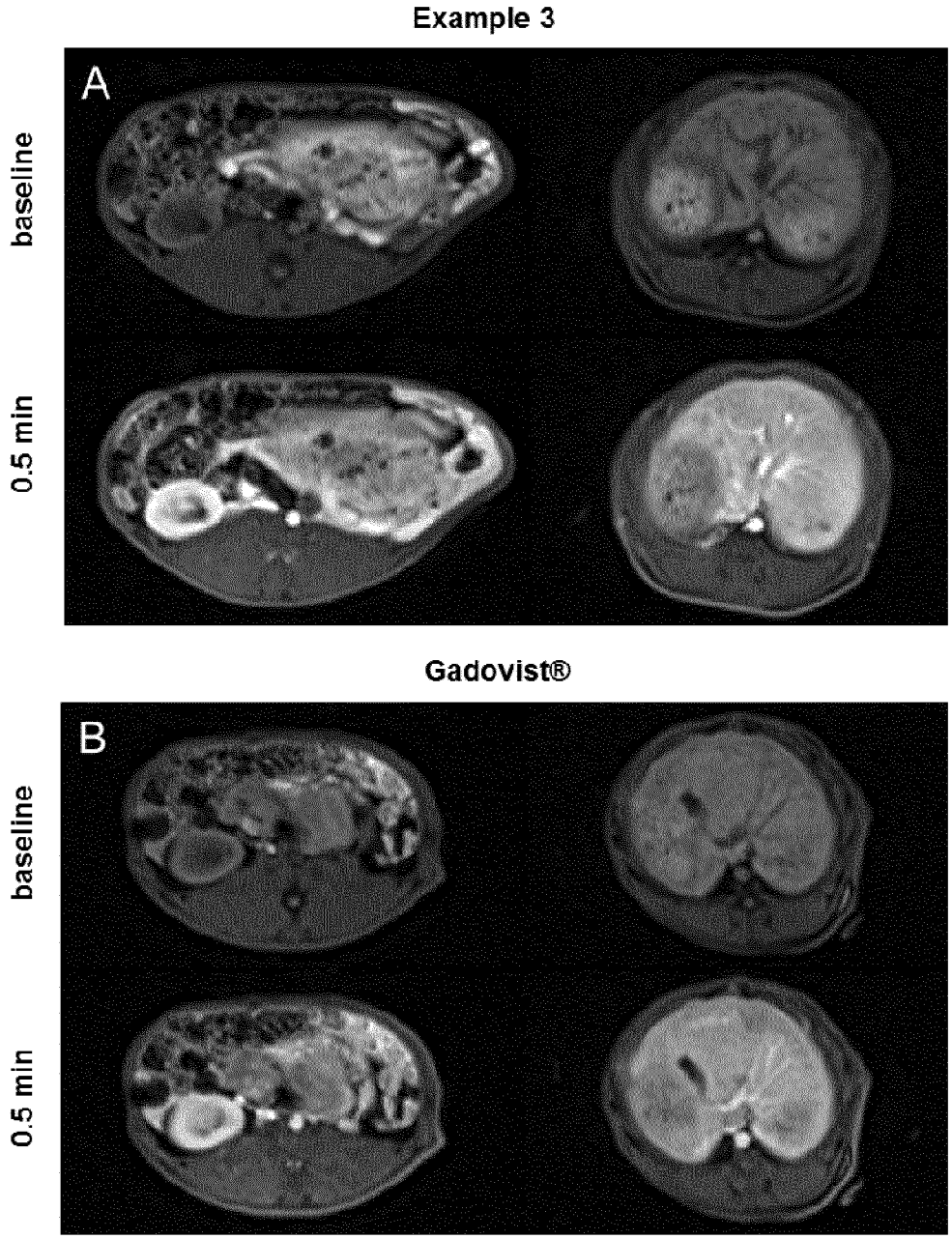


Figure 6

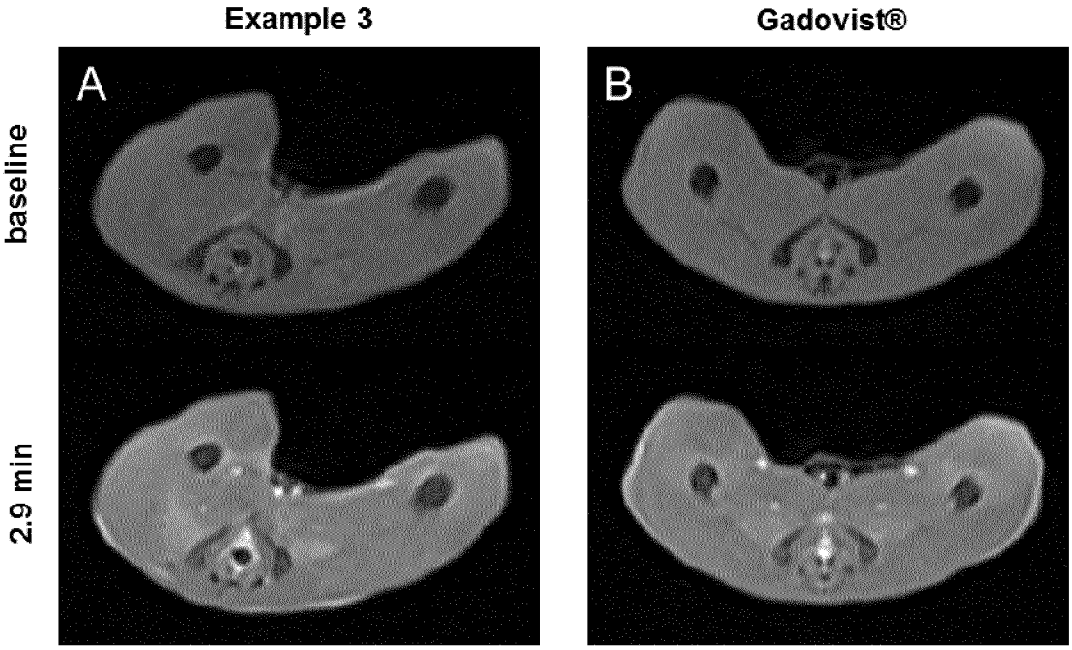


Figure 7

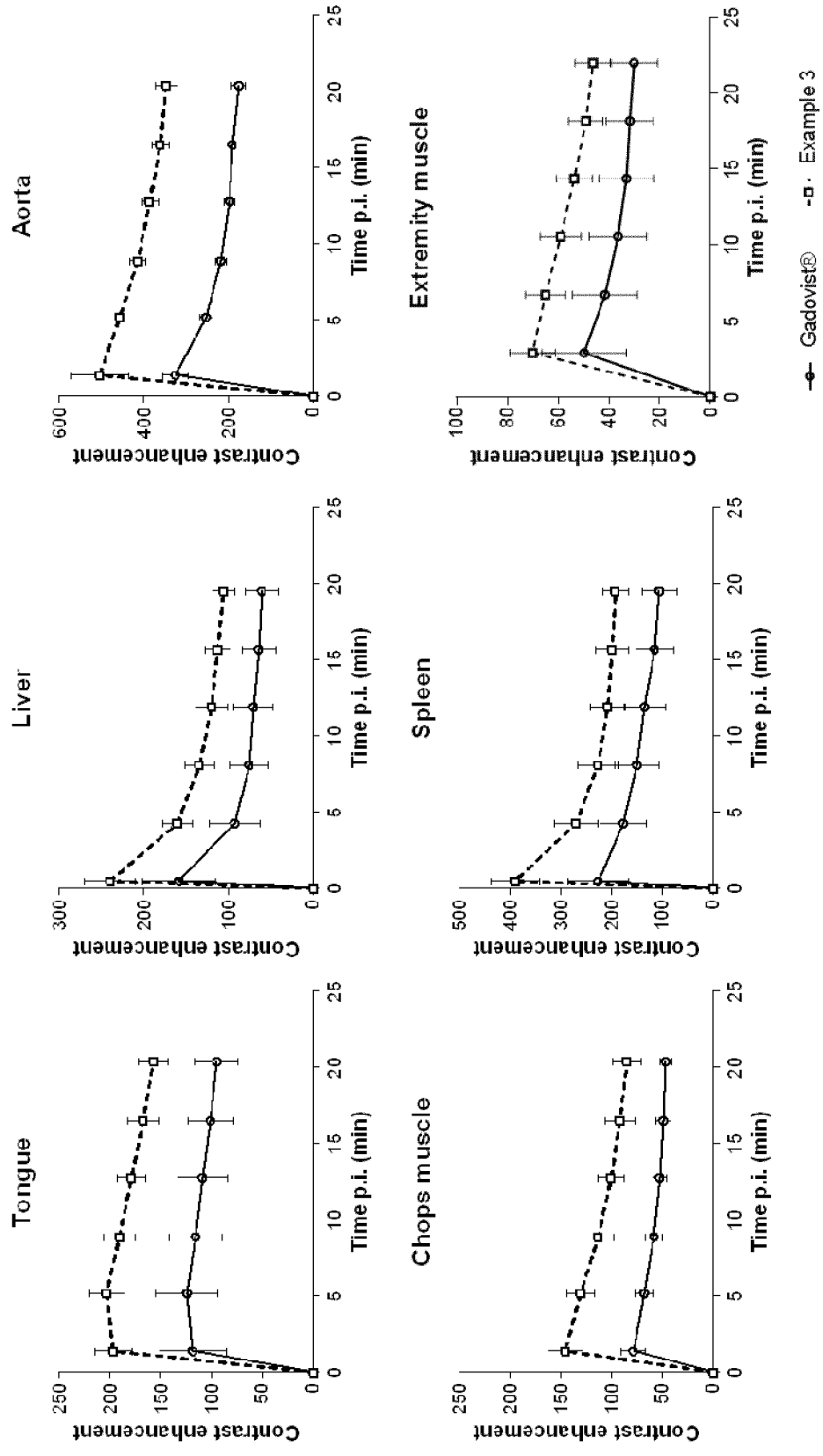

Aorta
Liver
Tongue
Extremity muscle
Spleen
Chops muscle
Contrast enhancement
Time p.i. (min)
Gadovist®
Example 3

Figure 8

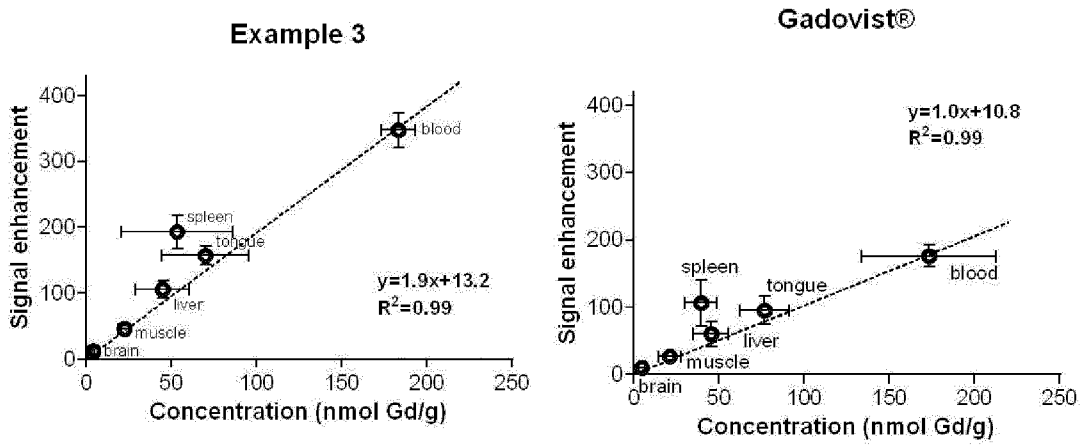

Example 3
Signal enhancement
Concentration (nmol Gd/g)
y=1.9x+13.2
R2=0.99
blood
spleen
tongue
liver
muscle
brain
Gadovist®
y=1.0x+10.8
R2=0.99
blood
spleen
tongue
liver
muscle
brain

Figure 9

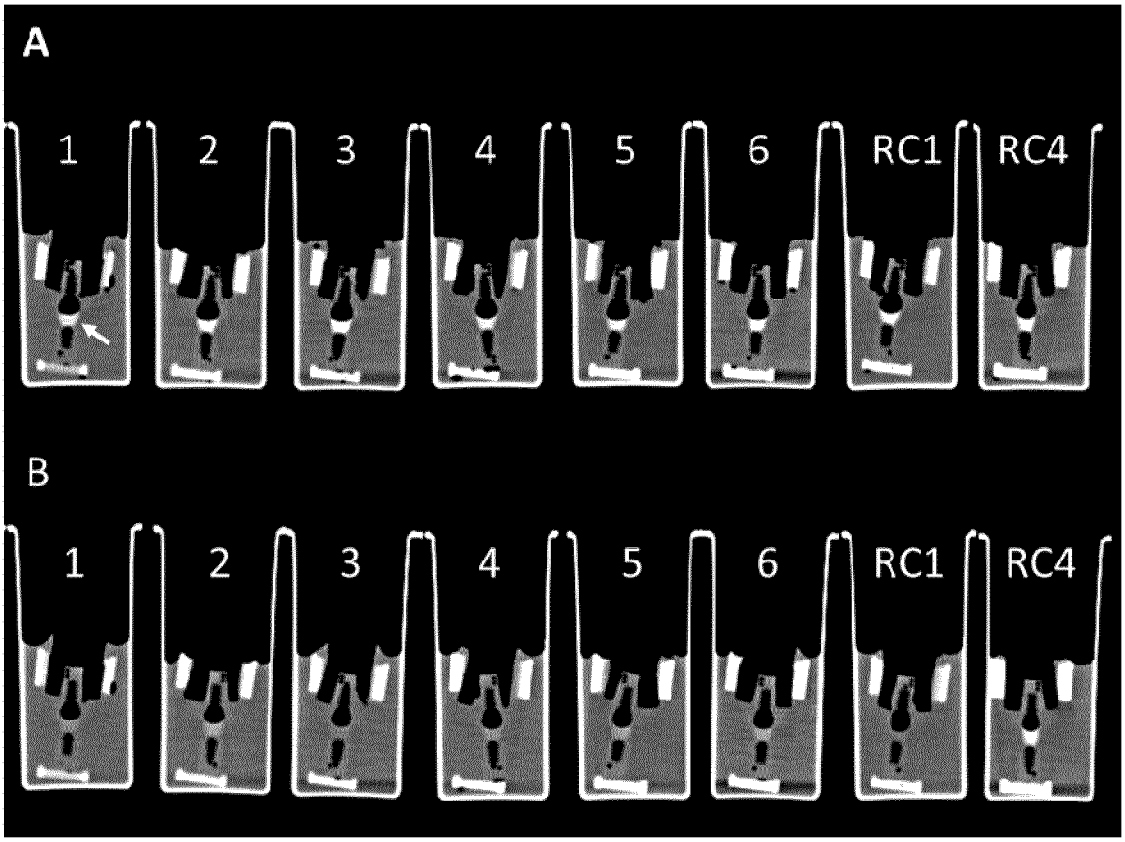


Figure 10

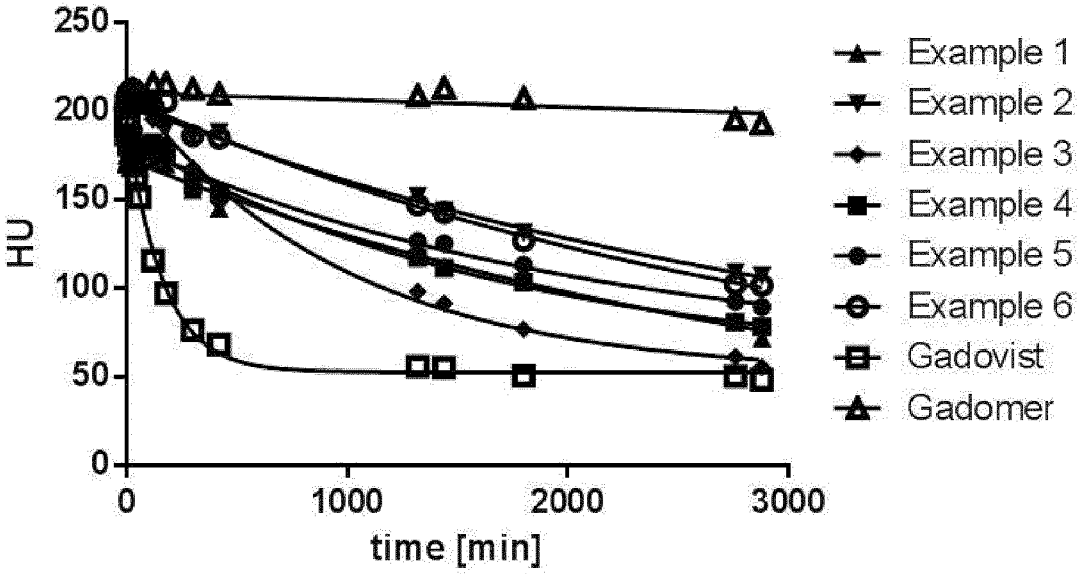
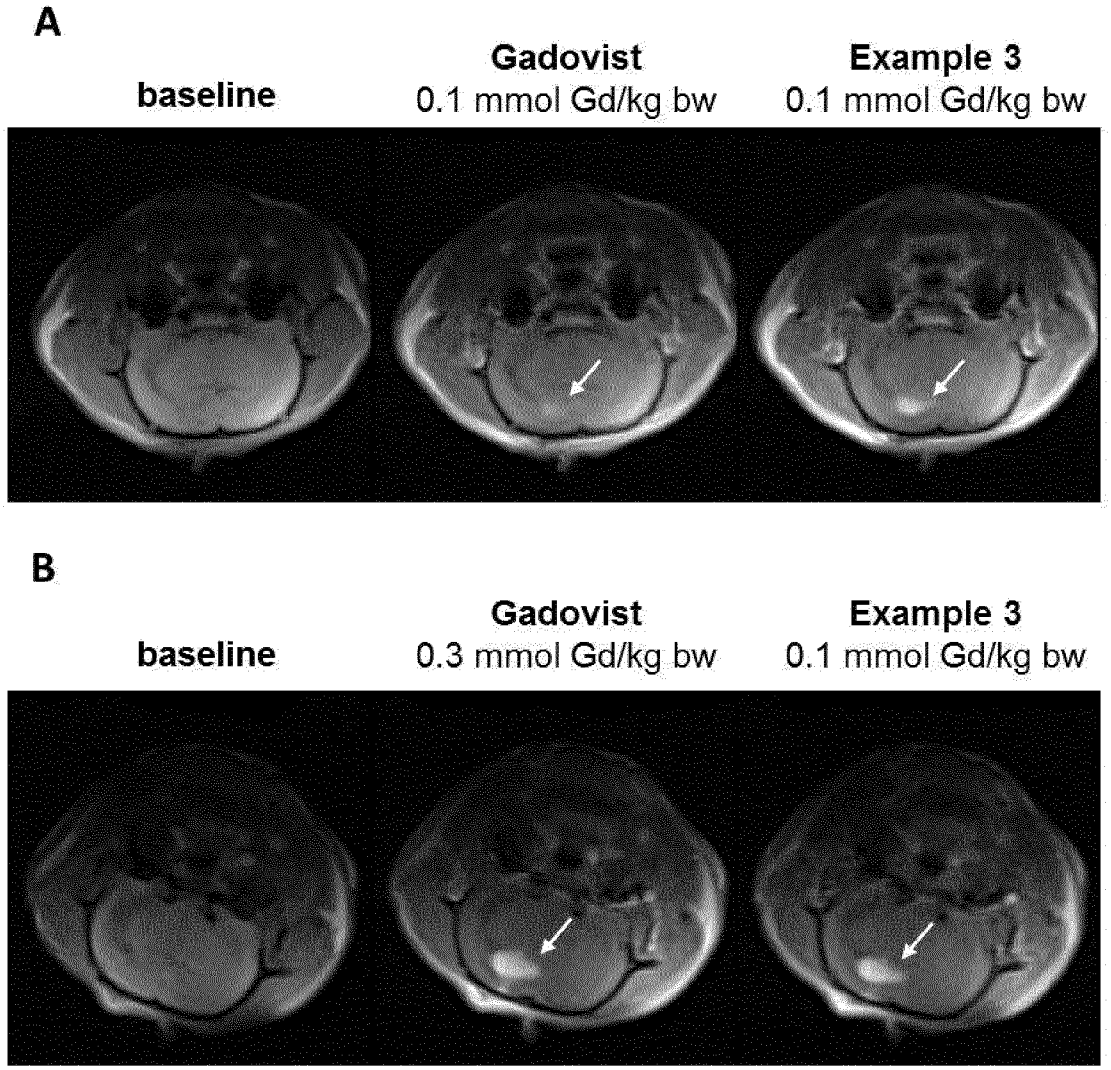
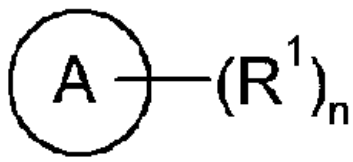
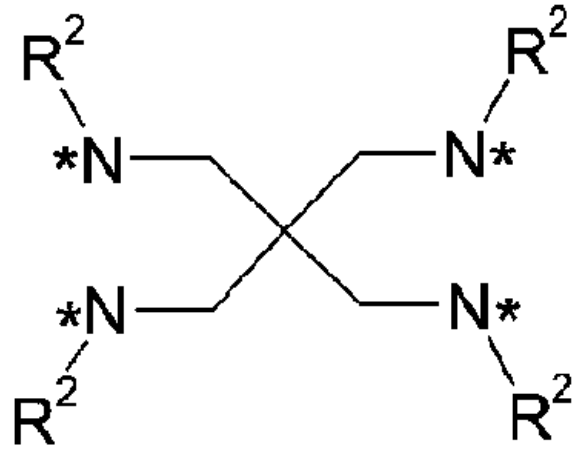

250
200
150
100
50
0
HU
0
1000
2000
3000
time [min]
Example 1
Example 2
Example 3
Example 4
Example 5
Example 6
Gadovist
Gadomer

Figure 11

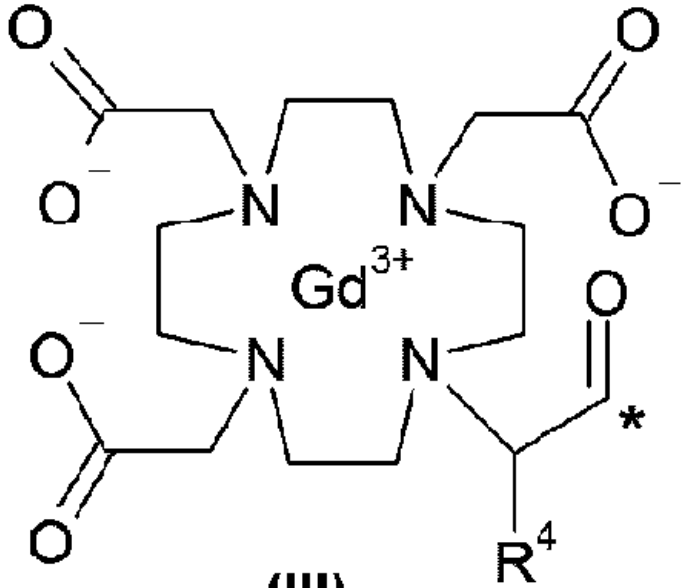

A
baseline
Gadovist
0.1 mmol Gd/kg bw
Example 3
0.1 mmol Gd/kg bw
B
baseline
Gadovist
0.3 mmol Gd/kg bw
Example 3
0.1 mmol Gd/kg bw



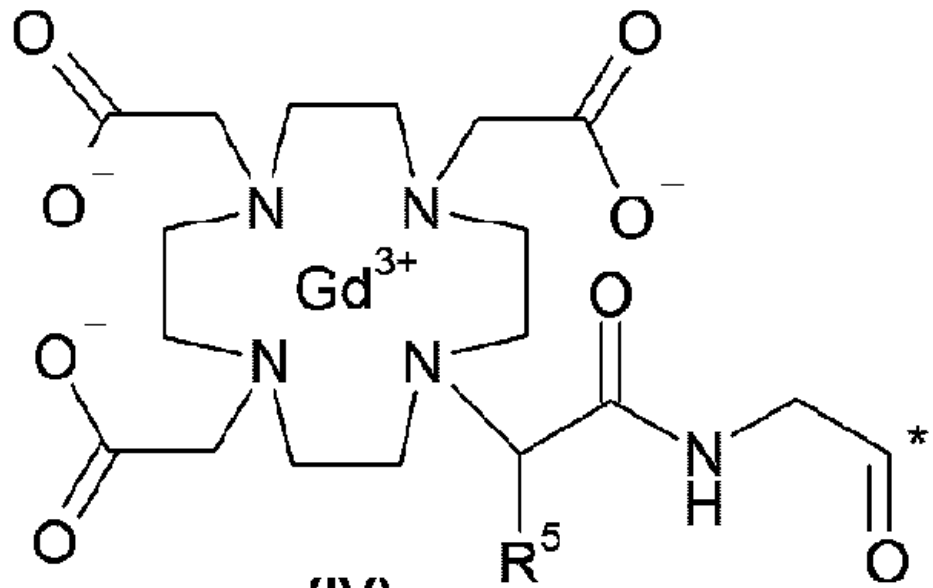
(I)



(II)



(III)



(IV)