Title: STABILIZATION OF PEPTIDIC RADIOPHARMACEUTICALS USING URIC ACID OR DERIVATIVES THEREOF

Abstract: The present invention relates to novel stabilizing agents (stabilizers) of radiopharmaceutical compositions used for diagnosis and therapy. In particular, the invention relates to use of an uric acid derivative to increase the shelf-life of diagnostic and therapeutic peptidic radiopharmaceuticals by reducing the side products originated from radiolysis.
Stabilization of peptidic radiopharmaceuticals using uric acid or derivatives thereof

The present invention relates to novel stabilizing agents (stabilizers) of radiopharmaceutical compositions used for diagnosis and therapy. In particular the invention relates to the use of an uric acid derivative to increase the shelf-life of diagnostic and therapeutic peptidic radiopharmaceuticals by reducing the side products originated from radiolysis.

Introduction

Peptidic diagnostic and therapeutic radiopharmaceuticals are often not stable, particularly at high activity levels. At high radioactivity concentrations e.g. during radiolabeling the peptidic moieties are often very susceptible to decomposition or modifications by radiolytic processes. Since the target specificity of the radioisotope labeled pharmaceutical peptidic pharmaceutical is largely dependent on the integrity of the peptide motif, radiolytic decomposition may lead to decreased diagnostic and therapeutic efficacy and unwanted radiation toxicity (Liu, S. and D.S. Edwards, Bioconjugate Chemistry, 2001. 12(4): p. 554-558). The mechanism for radiolytic decomposition is thought to be caused by free radicals such as hydroxyl and superoxide radicals formed in the presence of a large amount of ionizing radiation arising from the radioisotopes. Known radical scavengers such as human serum albumin (Kishore R, Early JF, Krohn KA, et al., Int J of Radial Appl Instrum Part B, Nucl Med and Biol 1986;4:457-459), gentisic acid, and ascorbic acid have been applied successfully as stabilizers for radiolabeled antibodies, but if they fail the strong demand of alternative stabilizers becomes evident.

Background

Galtium-68 is a metallic, generator-produced radionuclide that has become widely available through commercial generators from a number of suppliers for radiopharmaceutical equipment (e.g. Eckert & Ziegier Radiopharm AG, Berlin, Germany; Veenstra Instruments, Netherlands; Scintomics GmbH, Furstenfeldbruck, Germany). Its production is independent of an on-site cyclotron and the availability of this positron emitter has given rise to a continuously increasing number of clinical studies (Al-Nahhas, A., et al., Anticancer Research, 2007. 27(6B): p. 4087-4094). With well established procedures (Ocak, M., et al., Applied Radiation and Isotopes, 2010.

Beside $^{68}$Ga a number of radionuclides are routinely employed in diagnostic use, such as Tc-99m, In-111, F-18, and TI-201. Other radionuclides are in therapeutic use, such as Y-90, 1131, P-32, Sr-89, Sm-153.


Chemical decomposition may limit a radiopharmaceutical's shelf life by decreasing the radiochemical purity of the agent over time. For example, the peptide or even the radionuclide itself might be susceptible to oxidation as a consequence of the exposure to ionizing radiation. In addition, the radiation emitted from a radionuclide can break chemical bonds of a peptide or other components of the composition, thus causing autoradiofysis (Cyr J., US patent 6881396).

Thus, many radiopharmaceuticals require stabilizing agent(s) to maximize shelf life. Such stabilizing agent(s) must be non-toxic and must be able to maintain the product's radiochemical purity for an acceptable shelf-life as well as during use. In addition, an
acceptable radiopharmaceutical stabilizing agent should not interfere with the radiolabeling process and, if required, the stabilizing agent should be easily removable before application of the radiopharmaceutical.

5 Problem to be solved by the invention and its solution

Radiolysis is a phenomenon in which the formation of ionized molecules leads to side products and degradation products. This is known from a number of different applications, e.g. from $^{18}$F-labelled radiopharmaceuticals (Jacobson, M.S., H.R. Dankwart, and D.W. Mahoney, Applied Radiation and Isotopes, 2009. 67(6): p. 990-995) or the field of metalioradiopharmaceuticals. There, where for therapeutics a high radioactivity concentration is produced, radiolysis is a known problem and different approaches have been investigated in order to avoid radiolytic side products. The use of gentisic acid and ascorbic acid as scavenger is common (Liu, S. and D.S. Edwards, Bioconjugate Chemistry, 2001. 12(4): p. 554-558) but was found to be insufficient in the studied cases. Another influential factor described in the literature is the presence of oxygen in the reactants. Liu et al. (Liu, S., C.E. Eilars, and D.S. Edwards, Bioconjugate Chemistry, 2003. 14(5): p. 1052-1056) present a radiosynthesis with exclusion of oxygen to avoid radiolytic degradation. In the studied cases due to the setting of the radiosynthesis which comprises the use of kits and cassettes, the presence of oxygen cannot be excluded.

Labeling the DOTA-RM2 peptide with $^{68}$Ga at high specific activities commonly used stabilizers e.g. gentisic acid and ascorbic acid failed and a high amount of side products was observed.

Thus, there is a need to provide a stabilizing agent avoiding production of side products due to radiolysis or oxidation while fulfilling the requirements for GMP compliant patient application in a clinical setting.

Summary of the invention

It has now been surprisingly found that the radiolabelling quality and shelf life of peptidic radiopharmaceutical compositions may be significantly increased by an addition of a stabilizing amount of uric acid or a derivative thereof.

In one embodiment, the invention provides a composition comprising a radiopharmaceutical precursor and a stabilizing amount of uric acid or a derivative thereof.
In another embodiment, the invention provides a method of stabilizing a radiopharmaceutical comprising the steps of:
combining a precursor of said pharmaceutical with a stabilizing amount of uric acid or a derivative thereof in a container; and
adding a radionuclide to the container.

In a further embodiment, the invention provides a method for stabilizing the peptide radiopharmaceutical by adding the stabilizing amount of an uric acid derivative during the radiolabelling.

In a further embodiment, the invention provides a method for stabilizing the peptide radiopharmaceutical by adding the stabilizing amount of an uric acid derivative right after the radiolabelling.

In a further embodiment, the invention provides a kit comprising a sealed vial containing a predetermined quantity of a radiopharmaceutical precursor and a stabilizing amount of uric acid or a derivative thereof.

In a further embodiment, the present invention relates to the use of uric acid or derivatives thereof for stabilizing a peptidic radiopharmaceutical.

In a further embodiment, the present invention relates to the use of uric acid or derivatives thereof in a stabilizing amount thereof, being in the range of 1 μg/mL to 1,000 μg/mL for stabilizing a peptidic radiopharmaceutical.

In a further embodiment, the present invention relates to the use of a kit comprising a sealed vial containing a predetermined quantity of a radiopharmaceutical precursor and a stabilizing amount of uric acid or a derivative thereof for stabilizing a peptidic radiopharmaceutical.

**Description**

In a first aspect, the invention is directed to a method for stabilizing a peptidic radiopharmaceutical,
characterized in that uric acid derivatives are used as stabilizing agent.

Sub-embodiments:
Peptidic radiopharmaceutical

in a sub-embodiment, the peptidic radiopharmaceutical is a natural or synthetic peptide labeled with a radioisotope or a complex comprising a radioisotope and a chelator wherein the natural or synthetic peptide is suitable for being labeled with a radioisotope.

In a sub-embodiment, the natural or synthetic peptide is a peptide comprising of 4 to 700 amino acids wherein the amino acids may be selected from natural and synthetic amino acids and also may comprise modified natural and non-natural amino acids.

Preferably, the peptide is of 4 to 50 amino acids.

More preferably, the peptide is of 4 to 15 amino acids.

Even more preferably, the peptide is, but is not limited to, somatostatin and derivatives thereof and related peptides, somatostatin receptor specific peptides, neuropeptide Y and derivatives thereof and related peptides, neuropeptide Y1 and the analogs thereof, bombesin and derivatives thereof and related peptides, gastrin, gastrin releasing peptide and the derivatives thereof and related peptides, epidermal growth factor (EGF of various origin), insulin growth factor (IGF) and IGF-1, integrins (αβ1, αβ2, αβ5, αβ6), LHRH agonists and antagonists, transforming growth factors, particularly TGF-α; angiotensin; cholecystokinin receptor peptides, cholecystokinin (CCK) and the analogs thereof; neurotensin and the analogs thereof, thyrotropin releasing hormone, pituitary adenylate cyclase activating peptide (PACAP) and the related peptides thereof, chemokines, substrates and inhibitors for cell surface matrix metalloproteinase, prolactin and the analogs thereof, tumor necrosis factor, interleukins (IL-1, IL-2, IL-4 or IL-6), interferons, vasoactive intestinal peptide (VIP) and the related peptides thereof.

Even more preferably, the peptide is selected from the group comprising bombesin and bombesin analogs, preferably those having the sequences listed herein below, somatostatin and somatostatin analogs, preferably those having the sequences listed herein below, neuropeptide Y1 and the analogs thereof, preferably those having the sequences listed herein below, vasoactive intestinal peptide (VIP) and the analogs thereof.

Even more preferably, the peptide is bombesin and the analogs thereof.

In a sub-embodiment, the peptide is bombesin and the analogs thereof.

Bombesin is a fourteen amino acid peptide that is an analog of human Gastrin releasing peptide (GRP) that binds with high specificity to human GRP receptors present in prostate tumor, breast tumor and metastasis.
The invention is related to peptides as listed above but also to their analog peptides wherein analog peptides have mutated amino acids compared to original peptide and retain biological activity of the original peptide.

Peptides are preferably selected from but not limited to

Seq ID 1: D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$ (peptide RM2);
Seq ID 2: D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ(CH$_2$OH-CH$_2$)-(CH$_2$)$_2$CH$_3$;
Seq ID 3: D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ(CH$_2$NH)-Phe-NH$_2$;
Seq ID 4: D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ(CH$_2$NH)-Cys-NH$_2$;
Seq ID 5: Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$;
Seq ID 6: Gln-Trp-Ala-Val-Gly-His-Leuψ(CH$_2$OH-CH$_2$)-(CH$_2$)$_2$CH$_3$;
Seq ID 7: Gln-Trp-Ala-Val-Gly-Hts-Leuψ(CH$_2$NH)-Phe-NH$_2$;
Seq ID 8: Gln-Trp-Ala-Val-Gly-His-Leuψ(CH$_2$NH)-Cys-NH$_2$;
Seq ID 9: Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH$_2$;
Seq ID 10: Gln-Trp-Ala-Val-NMeGly-His-Leuψ(CH$_2$OH-CH$_2$)-(CH$_2$)$_2$CH$_3$;
Seq ID 11: Gln-Trp-Ala-Val-NMeGly-His-Leuψ(CH$_2$NH)-Phe-NH$_2$;
Seq ID 12: Gln-Trp-Ala-Val-NMeGly-His-Leuψ(CH$_2$NH)-Cys-NH$_2$;
Seq ID 13: D-Phe-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH$_2$;
Seq ID 14: D-Phe-Gln-Trp-Ala-Val-NMeGly-His-Leuψ(CH$_2$OH-CH$_2$)-(CH$_2$)$_2$CH$_3$;
Seq ID 15: D-Phe-Gln-Trp-Ala-Val-NMeGly-Hts-Leuψ(CH$_2$NH)-Phe-NH$_2$;
Seq ID 16: D-Phe-Gln-Trp-Ala-Val-NMeGly-His-Leuψ(CH$_2$NH)-Cys-NH$_2$;
Seq ID 17: D-PHE-CYS TYR D TRP LYS THR CYS THR OH, (DISULFIDE BOND) (peptide TATE);
Seq ID 18: D-Phe-cyclo[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr(o) (peptide TOC).

Peptidic radiopharmaceutical are preferably selected from but not limited to

[$^{68}$Ga]-DOTA-Gly-aminobenzoyl-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$;
[$^{68}$Ga]-DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$;
[$^{68}$Ga]-DOTA-4-amino-1-piperidine-4-carboxylicacid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$;
[$^{68}$Ga]-DOTA-5-amino-4,7,10,13-tetraoxapentadecanoic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$;
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-(15-amino-4,7,10,13-tetraoxapentadecanoic acid)-(4-amino-1-carboxymethyl-piperidine)-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH\_\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-diaminobutyric acid-D-Phe-Gin-Trp-Ala-Val-Gly-His-Sta-Leu-NH\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-4-(2-aminoethyl)-1-carboxymethyl-piperazine-D-Phe-Gln-Trp-Ata-Val-Gly-Hts-Sta-Leu-NH\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-LeuDOTA-(5-amino-3-oxa-pent)-succinamic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-(15-amino-4,7,10,13-tetraoxapentadecanoic acid)-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-15-amino-4,7,10,13-tetraoxapentadecanoic acid-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly4His-Le\_\_\_\_\_\_\_^{(\text{CHOH-CH\_\_\_\_\_\_\_2-HCH\_\_\_\_\_\_\_2)_2}-CH\_\_\_\_\_\_\_3;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le\_\_\_\_\_\_\_^{(\text{CHOH-CH\_\_\_\_\_\_2})-(CH\_\_\_\_\_\_\_2)-CH\_\_\_\_\_\_\_3;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly4His-Le\_\_\_\_\_\_\_^{(\text{CH\_\_\_\_\_\_2NH})}-Phe-NH\_\_\_\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le\_\_\_\_\_\_\_^{(\text{CH\_\_\_\_\_\_2NH})}-Cys-NH\_\_\_\_\_\_\_2;  \\
\textsuperscript{[\textsuperscript{65}Ga]}-DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH\_\_\_\_2, \textsuperscript{[\textsuperscript{65}Ga]}-DOTA-RM2  \\
3-cyano-4-[\textsuperscript{18}F]fluorobenzylo-Ala(SO\_\_\_\_3H)-Ala(SO\_\_\_\_3H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH\_\_\_\_2
3-cyano-4-[1^{18}F]fluorobenzoyl-Ala(S0_3H)-Ava-Gln-Trp-Ala-Val-NMMeGly-His-Sta-Leu-NH_2

5 $^{68}$Ga-DOTATOC

$^{68}$Ga-DOTATATE
Preferably:

\[^{88}\text{Ga}]-\text{DOTA} \sim \text{4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Afa-Val-Gly-His-Sta-Leu-NH}_2, \quad {^{88}\text{Ga}}-\text{DOTA-RM2}

3-cyano-4-[^{18}\text{F}]\text{fluorobenzoyl-Ala(S0\text{}_{3\text{H}})-Ala(S0\text{}_{3\text{H}})-Ava-Gli-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH}_2

3-cyano-4-[^{18}\text{F}]\text{fluorobenzoyl-Ala(S0\text{}_{3\text{H}})-Ava-G[Trp-Ala-Val-NMeGly-His-Sta-Leu-NH}_2

\[^{68}\text{Ga}]-\text{DOTATOC}\
Radioisotope or complex comprising a metal radioisotope and a chelator.

In a sub-embodiment, the radioisotope is selected from the group comprising carbon-11 (¹¹C), nitrogen-13 (¹³N), oxygen-15 (¹⁵O), bromine-75 (⁷⁵Br), bromine-76 (⁷⁶Br), iodine-124 (¹²⁴I) and fluorine-18 (¹⁸F). Preferably, the radioisotope is selected from the group comprising bromine-75 (⁷⁵Br), bromine-76 (⁷⁶Br), iodine-124 (¹²⁴I) and fluorine-18 (¹⁸F). More preferably, the radioisotope is fluorine-18 (¹⁸F).

In a sub-embodiment, the metal radioisotope complexed to a chelator is selected from the group comprising ¹⁷⁷Lu, ⁹⁰Y, ¹³¹I, ⁹⁹ᵐTc, ⁶⁷Ga, ⁵⁸Fe, ⁸⁸Ga, ⁷³As, ¹¹¹In, ⁹⁷Ru, ²⁰³Pb, ⁶²Cu, ⁶⁴Cu, ⁵¹Cr, ⁵²ᵐMn, and ¹⁵⁷Gd. Preferably, the metal radioisotope is selected from...
the group comprising $^{65}$Tc, $^{67}$Ga, $^{68}$Ga, and $^{111}$In. More preferably, the metal radioisotope is $^{68}$Ga.

In a sub-embodiment, the chelator complexing the metal radioisotope is selected from the group comprising:

- DOTA-, NODASA-, NODAGA-, NOTA-, DTPA-, EDTA-, TETA-, and TRITA-based chelators,
- CE-DTS, DADT derivative, triamidethiol derivative, DADS derivative, hydrazinonicotinic acid, and bis(hydroxoamamide) derivative

wherein DOTA stands for 1,4,7,10-tetraazacyclododecane-N,N',N" tetraacetic acid,

DTPA stands for diethylenetriaminepentaacetic acid,

EDTA stands for ethylenediamine-N,N'-tetraacetic acid,

TETA stands for 1,4,8,1 1-tetraazacyclododecane-1, 4,8,11-tetraacetic acid,

NOTA stands for 1,4,7-triazacyclononanetriacetic acid,

CE-DTS stands for 3-{4-[(5E,7E)-3,10-dithioxo-2,4,5,8,9,1 1-hexaazadodeca-5,7-dien-6-yl]phenyl}propanotc acid,

DADT derivative stands for 4-methyl-3,4-bis[(2-methyl-2-sulfanylpropyl)amino]pentanoic acid,

triamidethiol derivative stands for N-(sulfanylacetyl)glycylglycylglycine,

DADS stands for N-(sulfanylacetyl)-3-[(sulfanylacetyl)amino]alanine, and bis(hydroxoamamide) derivative stands for 4-amino-N'-hydroxy-N-(3-[[Z]-{hydroxyimino}methyl]amino)propyl]benzenecarboximidamide.

Preferably, the chelator is selected from the group comprising:

- DOTA-, NOTA-, DTPA-, and TETA-based chelators.
Preferably, $^{99m}\text{Tc}$ or $^{186/188}\text{Re}$ chelators are selected from the group comprising:
Uric acid derivatives

Uric acid derivatives mean uric acid as such, derivatives thereof and/or mixture thereof.
In a sub-embodiment, the uric acid derivatives are compounds of formula I

\[
\begin{align*}
\text{I}
\end{align*}
\]

wherein

- \(R_1\) is Hydrogen, substituted or unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group, \(\text{CH}_3-(\text{CH}_2)_m^o-(\text{CH}_2-\text{CH}_2)_n\text{-CH}_2\text{-CH}_2\), \(n = 0, 1, 2\) or 3 and \(m = 0\) or 1 or substituted or unsubstituted acyi group,
- \(R_2\) is Hydrogen, substituted or unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group, \(\text{CH}_3-(\text{CH}_2)_m^o-(\text{CH}_2-\text{CH}_2)_n\text{-CH}_2\text{-CH}_2\), \(n = 0, 1, 2\) or 3 and \(m = 0\) or 1 or substituted or unsubstituted acyi group,
- \(R_3\) is Hydrogen, substituted or unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group, \(\text{CH}_3-(\text{CH}_2)_m^o-(\text{CH}_2-\text{CH}_2)_n\text{-CH}_2\text{-CH}_2\), \(n = 0, 1, 2\) or 3 and \(m = 0\) or 1 or substituted or unsubstituted acyi group, and
- \(R_4\) is Hydrogen, substituted or unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group, \(\text{CH}_3-(\text{CH}_2)_m^o-(\text{CH}_2-\text{CH}_2)_n\text{-CH}_2\text{-CH}_2\), \(n = 0, 1, 2\) or 3 and \(m = 0\) or 1 or substituted or unsubstituted acyi group.

In a sub-embodiment, \(R_1, R_2, R_3\) and \(R_4\) are independently from each other Hydrogen, or substituted or unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group.

In a sub-embodiment, \(R_1, R_2, R_3\) and \(R_4\) are independently from each other Hydrogen, \(\text{CH}_3-(\text{CH}_2)_m^o-(\text{CH}_2-\text{CH}_2)_n\text{-CH}_2\text{-CH}_2\), \(n = 1, 2\) or 3 and \(m = 0\) or 1.

In a sub-embodiment, \(R_1, R_2, R_3\) and \(R_4\) are independently from each other Hydrogen, or substituted or unsubstituted acyi group.

Preferably, unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group is methyl or ethyl. More preferably, unsubstituted \(\text{C}_1-\text{C}_3\) alkyl group is methyl.

Preferably, substituted alkyl group is substituted with one or two substituents defined as hydroxyl, hydroxymethyl, methyl, ethyl, methoxy, methoxymethyl, ethoxy, or 2-
methoxyethoxy. More preferably, substituted C$_1$-C$_3$ alkyl group is 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxypropyl, 2,3-dihydroxypropyl, 2-methoxyethyl, or 2-(2-methoxyethoxy)ethyl.

Preferably, CH$_3$(CH$_2$)$_m$O-(CH$_2$O)$_n$-CH$_2$-CH$_2$ is defined such as $n = 0$ and $m = 0$ or $n = 1$ and $m = 1$.

Preferably, unsubstituted acyl group is acetyl. Preferably, substituted acyl group is substituted with one substituent defined as hydroxyl, methyl, ethyl, methoxy, ethoxy, or 2-methoxyethoxy. More preferably, substituted acyl group is hydroxyacetyl, or methoxyacetyl.

In a sub-embodiment, R$_1$, R$_2$, R$_3$ and R$_4$ are independently from each other Hydrogen methyl or ethyl.

In a sub-embodiment, R$_1$, R$_2$, R$_3$ and R$_4$ are independently from each other Hydrogen or methyl optionally substituted with hydroxymethyl or methoxymethyl.

In a sub-embodiment, R$_1$, R$_2$, R$_3$ and R$_4$ are Hydrogen, i.e. uric acid.

Preferably, compound of formula 1 is selected from but not limited to uric acid:

1-methyluric acid:
3-methyluric acid:

7-methyluric acid:

9-methyluric acid:

1,3-Dimethyluric acid:

1,7-Dimethyluric acid:
1,9-Dimethyluric acid:

3,7-Dimethyluric acid:

Tetramethyluric acid:

In a sub-embodiment, the concentrations of the uric acid derivatives are in the range of 1 µg/mL to 1,000 µg/mL preferably of 10 pg/mL to 100 pg/mL uric acid derivatives and more preferably of 20 pg/mL to 60 pg/mL uric acid derivatives. Even more preferably, the concentration of the uric acid derivative is 40 pg/mL.

Method of stabilizing
in a sub-embodiment, the invention is directed to a method for stabilizing a peptidic radiopharmaceutical
characterized in that uric acid derivatives are used as stabilizing agent
wherein the uric acid derivatives are compound of formula I

\[
\begin{align*}
\text{I} & \\
R_1 & \text{is Hydrogen, substituted or unsubstituted } C_1-C_3 \text{ alkyl group, } CH_3-(CH_2)_m-0-(CH_2-C_2-0)_n-CH_2CH_2, n = 0, 1, 2 \text{ or } 3 \text{ and } m = 0 \text{ or } 1 \text{ or substituted or unsubstituted acyl group,} \\
R_2 & \text{is Hydrogen, substituted or unsubstituted } C_1-C_3 \text{ alkyl group, } CH_3-(CH_2)_m-0-(CH_2-C_2-0)_n-CH_2CH_2, n = 0, 1, 2 \text{ or } 3 \text{ and } m = 0 \text{ or } 1 \text{ or substituted or unsubstituted acyl group,} \\
R_3 & \text{is Hydrogen, substituted or unsubstituted } C_1-C_3 \text{ alkyl group, } CH_3-(CH_2)_m-0-(CH_2-C_2-0)_n-CH_2CH_2, n = 0, 1, 2 \text{ or } 3 \text{ and } m = 0 \text{ or } 1 \text{ or substituted or unsubstituted acyl group,} \quad \text{and} \\
R_4 & \text{is Hydrogen, substituted or unsubstituted } C_1-C_3 \text{ alkyl group, } CH_3-(CH_2)_m-0-(CH_2-C_2-0)_n-CH_2CH_2, n = 0, 1, 2 \text{ or } 3 \text{ and } m = 0 \text{ or } 1 \text{ or substituted or unsubstituted acyl group.}
\end{align*}
\]

Preferably, the method is defined as referring to peptidic radiopharmaceuticals selected from

\begin{align*}
\text{[}^{68}\text{Ga}3\text{-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2, \quad \text{[}^{68}\text{Ga}3\text{-DOTA-RM2}; \\
3\text{-cyano-4-[}^{18}\text{F}]\text{fluorobenzoyl-Ala(SO}_3\text{H)-Ala(SO}_3\text{H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH}_2; \\
3\text{-cyano-4-[}^{18}\text{F}]\text{fluorobenzoyl-Ala(SO}_3\text{H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH}_2; \\
\text{[}^{68}\text{Ga}-\text{DOTATOC}; \text{ or} \\
\text{[}^{68}\text{Ga}-\text{DOTATATE} \text{ and}
\end{align*}
the uric acid derivative is selected from
uric acid, 1-methyluric acid, 3-methyluric acid, 7-methyluric acid, 9-methyluric acid, 1,3-
Dimethyluric acid, 1,7-Dimethyluric acid, 1,9-Dimethyluric acid, 3,7-Dimethyluric acid,
or Tetramethyluric acid.

More preferably, the method is defined as referring to the peptidic radiopharmaceutical
[^68Ga]-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-
Leu-NH₂, ^68Ga-DOTA-RM2.

In a sub-embodiment, the invention is directed to a method for stabilizing a peptidic
radiopharmaceutical,
characterized in that uric acid derivative(s) is/are used as stabilizing agent
comprising the steps,
- adding uric acid derivative(s) to a peptidic radiopharmaceutical
  precursor,
- adding uric acid derivative(s) into the reaction mixture of a radiolabeling
  reaction or
- adding uric acid derivative(s) to the freshly prepared peptidic
  radiopharmaceutical.

In a further sub-embodiment, the method for stabilizing a peptidic radiopharmaceutical,
characterized in that uric acid derivative(s) are used as stabilizing agent,
comprising the steps
- adding uric acid derivative(s) to a peptidic radiopharmaceutical
  precursor,
- adding uric acid derivative(s) into the reaction mixture of a radiolabeling
  reaction or
- adding uric acid derivative(s) to the freshly prepared peptidic
  radiopharmaceutical,

wherein the uric acid derivative(s) are compounds of formula I
wherein

R₁ is Hydrogen, substituted or unsubstituted C₁-C₃ alkyl group, CH₃-(CH₂)m-O-(CH₂-C₂H₂)n-CH₂CH₂, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group,

R₂ is Hydrogen, substituted or unsubstituted C₁-C₃ alkyl group, CH₃-(CH₂)m-O-(CH₂-C₂H₂)n-CH₂CH₂, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group,

R₃ is Hydrogen, substituted or unsubstituted C₁-C₃ alkyl group, CH₃-(CH₂)m-O-(CH₂-C₂H₂)n-CH₂CH₂, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group, and

R₄ is Hydrogen, substituted or unsubstituted C₁-C₃ alkyl group, CH₃-(CH₂)m-O-(CH₂-C₂H₂)n-CH₂CH₂, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group.

Preferably, the method is defined as referring to peptidic radiopharmaceuticals selected from

[^⁶⁸Ga]-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-Hfs-Sta-Leu-NH₂, ⁶⁸Ga-DOTA-RM2;

3-cyano-4-[¹⁷¹F]fluorobenzooyl-Ala(S₀₃H)-Aia(S₀₃H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH₂;

3-cyano-4·¹⁸F]fluorobenzoyl-Ala(S₀₃H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH₂;

[^⁶⁸Ga]-DOTATOC; or

[^⁶⁸Ga]-DOTATATE, and

the uric acid derivative is selected from
uric acid, 1-methyluric acid, 3-methyluric acid, 7-methyluric acid, 9-methyluric acid, 1,3-Dimethy luric acid, 1,7-Dimethyluric acid, 1,9-Dimethyluric acid, 3,7-Dimethyluric acid, or Tetramethyluric acid.

More preferably, the method is defined as referring to the peptidic radiopharmaceutical \[^{68}\text{Ga}]\text{DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2, \[^{68}\text{Ga}]\text{DOTA-RM2}.\]

In a further sub-embodiment, the method is directed to uric acid derivatives that are added to peptidic radiopharmaceutical precursors such peptidic radiopharmaceutical precursors are well known in the art.

In a sub-embodiment, the method is directed to uric acid derivatives that are added into the reaction mixture of a radiolabeling reaction.

In a sub-embodiment, the method is directed to uric acid derivatives that is added to the freshly prepared peptidic radiopharmaceutical, wherein freshly prepared means that the radiolabeling synthesis is substantially completed.

Preferably, the peptidic radiopharmaceutical precursor is selected from but not limited to

\[
\text{DOTA-Gly-aminobenzoyl-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]
\[
\text{DOTA-4-amino-1-carboxymethyl-piperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]
\[
\text{DOTA-4-amino-1-piperidtne-4-carboxylicacid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]
\[
\text{DOTA-15-amino-4,7,10,13-tetraoxapentadecanoic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]
\[
\text{DOTA-(15-amino-4,7,10,13-tetraoxapentadecanoic acid)-(4-amino-1-carboxymethyl-piperidine)-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]
\[
\text{DOTA-diaminobutyricacid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2; \]

DOTA-4-(2-aminoethyl)-1-carboxymethyl-piperazine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH₂;

LeuDOTA-(5-amino-3-oxa-pentyl)-succinamic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH₂;

DOTA-4-amino-1-carboxymethyl-pipendine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le^{(CHOH-CH₂)(CH₂)₂-CH₃};

DOTA-(15-amino-4,7,10,13-tetraoxapentadecanoic acid-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le^{(CHOH-CH₂HCH₂)₂-CH₃};

DOTA-15-amino-4,7,10,13-tetraoxapentadecanoic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le^{(CHOH-CH₂)₂-CH₃};

DOTA-4-amino-1-carboxymethyl-pipendine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le^{CH₂NH}-Phe-NH₂;

DOTA-15-amino-4,7,10,13-tetraoxapentadecanoic acid-D-Phe-Gln-Trp-Ala-Val-Gly-His-Le^{CH₂NH}-Cys-NH₂;

DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH₂, DOTA-RM2

3-cyano-4-trimethylammoniobenzoyl-Ala(S0,S0)-Ala(S0,S0)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH₂ trifluoroacetate

3-cyano-4-trimethylammoniobenzoyl-Ala(S0,S0)-Ala(S0,S0)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH₂ trifluoroacetate
Preferably:

DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-\textsubscript{NH\textsubscript{2}}, DOTA-RM2
3-cyano-4-trimethylammoniobenzoyl-Ala(SO$_3$H)-Ala(SO$_3$H)-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH$_2$ trifluoroacetate

5 3-cyano-4-trimethylammoniobenzoyl-Ala(SO$_3$H)-Ava-Gin-Trp-Aia-Val-NMeGly-His-Sta-Leu-NH$_2$ trifluoroacetate
More preferably, DOTA-4-amino-1-carboxymeihylpiperidine-D-Phe-Gln-Trp-Ala-Val-Giy-His-Sta-Leu-NH₂, DOTA-RM2

The invention is directed to a method defined as above and by the scope of all or some of the preferred features and sub-embodiments as combined to each other.
In a **second aspect**, the invention is directed to compositions comprising

- a peptidic radiopharmaceutical and
- uric acid derivatives used as stabilizing agent.

5 The peptidic radiopharmaceutical and uric acid derivatives are as disclosed above.

In a **third aspect**, the invention is directed to compositions comprising

- a peptidic radiopharmaceutical precursor and
- uric acid derivatives used as stabilizing agent.

10 The peptidic radiopharmaceutical precursor and uric acid derivatives are as disclosed above.

In a **fourth aspect**, the invention is directed to a kit comprising

- a peptidic radiopharmaceutical precursor and
- uric acid derivatives used as stabilizing agent.

15 The peptidic radiopharmaceutical precursor and uric acid derivatives are as disclosed above.

The preferred features and sub-embodiments disclosed in the first aspect are herein incorporated in the second, third and fourth aspects.

In a **fifth aspect**, the invention is directed to the use of uric acid or derivatives thereof for stabilizing a peptidic radiopharmaceutical comprising

- the use of uric acid or derivatives thereof in a stabilizing amount, being in the range of 1 µg/mL to 1.000 µg/mL for stabilizing a peptidic radiopharmaceutical
- the use of a kit comprising a sealed vial containing a predetermined quantity of a radiopharmaceutical precursor and a stabilizing amount of uric acid or a derivative thereof for stabilizing a peptidic radiopharmaceutical.
The peptidic radiopharmaceutical precursor and uric acid derivatives are as disclosed above.

The preferred features and sub-embodiments disclosed in the first aspect are herein incorporated in the fifth aspect.

Definitions

The term "stabilizing agent" or "stabilizer" refers to a chemical which inhibits radiolysis reactions.

The term "radiopharmaceutical" refers to a radioactive compound used in radiotherapy or diagnosis in the field of nuclear medicine wherein the radioactive compound is used as tracer in the radiotherapy or diagnosis of diseases.

The term "peptidic radiopharmaceutical" refers to a radiopharmaceutical as defined above comprising a natural or synthetic peptide containing two or more natural or synthetic amino acids linked by the carboxylic acid of one amino acid to the amino group of another including modifications of these peptide bonds such as methylation or reduction. The natural or synthetic peptide of the radiopharmaceutical is interfering with mammal cell structures such as receptors or enzymes.

As used hereinafter in the description of the invention and in the claims, the term "amino acid" means any molecule comprising at least one amino group and at least one carboxylic group, but which has no peptide bond within the molecule. In other words, an amino acid is a molecule that has a carboxylic acid functionality and an amine nitrogen having at least one free hydrogen, preferably in alpha position thereto, but no amide bond in the molecule structure. Thus, a dipeptide having a free amino group at the N-terminus and a free carboxylic group at the C-terminus is not to be considered as a single "amino acid" in the above definition. The amide bond between two adjacent amino acid residues which is obtained from such a condensation is defined as "peptide bond". Optionally, the nitrogen atoms of the polyamide backbone (indicated as NH above) may be independently alkylated, e.g., with d-Ce-alkyl, preferably CH₃.

An amide bond as used herein means any covalent bond having the structure
wherein the carbonyl group is provided by one molecule and the NH-group is provided by the other molecule to be joined. The amide bonds between two adjacent amino acid residues which are obtained from such a polycondensation are defined as "peptide bonds". Optionally, the nitrogen atoms of the polyamide backbone (indicated as NH above) may be independently alkylated, e.g., with -CrCe-alkyl, preferably -CH₃.

As used hereinafter in the description of the invention and in the claims, an amino acid residue is derived from the corresponding amino acid by forming a peptide bond with another amino acid.

As used hereinafter in the description of the invention and in the claims, an amino acid sequence may comprise naturally occurring and/or synthetic / artificial amino acid residues, proteinogenic and/or non-proteinogenic amino acid residues. The non-proteinogenic amino acid residues may be further classified as (a) homo analogues of proteinogenic amino acids, (b) β-homo analogues of proteinogenic amino acid residues and (c) further non-proteinogenic amino acid residues.

Accordingly, the amino acid residues may be derived from the corresponding amino acids, e.g., from

- proteinogenic amino acids, namely Ala, Arg, Asn, Asp, Cys, Gin, Glu, Gly, His, lle, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val; or
- non-proteinogenic amino acids, such as
  - homo analogues of proteinogenic amino acids wherein the sidechain has been extended by a methylene group, e.g., homoaalanine (Hal), homoarginine (Har), homocysteine (Hey), homoglutamine (Hgl), homohistidine (Hhi), homoisoleucine (Hil), homoleucine (Hie), homolysine (Hly), homomethionine (Hme), homophenylalanine (Hph), homoproline (Hpr), homoserine (Hse), homothreonine (Hth), homotryptophane (Htr), homotyrosine (Hty) and homovaline (Hva);
  - β-homo analogues of proteinogenic amino acids wherein a methylene group has been inserted between the α-carbon and the carboxyl group yielding β-amino acids, e.g., β-homoalanine (β-Hal), β-homoarginine (β-Har), β-homoasparagine
(β-HAs), β-homocysteine (pHcy), β-homoglutamine (pHgl), β-homohistidine (βHHI), β-homotsoleucine (βHll), β-homoleucine (βHII), β-homoiysine (βHY), β-homomethionine (βHHβ), β-homophenylalanine (βHpη), β-homoproline (βHPr), β-homoserine (βHse), β-homothreonine (βHθn), β-homotryptophane (βHII), β-homotyrosine (βHθ) and β-homovaline (βHtva);

- Further non-proteinogenic amino acids, e.g., α-aminoadipic acid (Aad), β-aminoacidic acid (pAad), α-aminoisobutyric acid (Abu), a-aminoisobutyric acid (Aib), β-alanine (βAα), 4-aminoobutyric acid (4-Abu), 5-aminovaleric acid (5-Ava), 6-aminohexanoic acid (6-Ahx), 8-aminoocotanoic acid (8-Aoc), 9-amimononanoic acid (9-Anc), 10-aminodecanoic acid (10-Adc), 12-aminododecanoic acid (12-Ado), a-aminosuberic acid (Asu), azetidine-2-carboxylic acid (Aze), β-cyclohexylalanine (Cha), altrulline (Cit), dehydroalanine (Dha), γ-carboxyglutamic acid (Gla), a-cyclohexylglycine (Chg), propargylglycine (Pra), pyroglutamic acid (Gip), a-tert-butylglycine (Tie), 4-benzoylphenylalanine (Bpa), δ-hydroxylysine (Hyl), 4-hydroxyproline (Hyp), allo-isoleucine (aile), lanthionine (Lan), (1-naphthyl)alanine (1-Nal), (2-naphthyl)alanine (2-Nal), norleucine (Nle), norvaline (Nva), ornithine (Orn), phenylglycin (Phe), pipecolic acid (Pip), sarcosine (Sar), selenocysteine (Sec), statine (Sta), β-thienylalanine (Thi), 1,2,3,4-tetrahydroisochinoline-3-carboxylic acid (Tic), allo-threonine (aThr), thtazolidine-4-carboxylic acid (Thz), γ-amino-butryric acid (GABA), iso-cysteine (iso-Cys), diaminopropionic acid (Dpr), 2,4-diaminobutyric acid (Dab), 3,4-diaminobutyric acid (Dab), biphenylyalanine (Bip), phenylalanine substituted in para-position with -C₄-C₆ alkyl, -halide, -NH₂, -CO₂H or Phe(4-R) (wherein R = -C₄-C₆ alkyl, -halide, -NH₂, or -CO₂H); peptide nucleic acids (PNA, cf., P.E. Nielsen, Ace. Chem. Res., 32, 624-30);

- or their N-alkylated analogues, such as their N-methylated analogues.

Cyclic amino acids may be proteinogenic or non-proteinogenic, such as Pro, Aze, Gip, Hyp, Pip, Tic and Thz.

For further examples and details reference can be made to, e.g., J.H. Jones, J. Peptide ScL, 2003, 9.

As used herein, the term "alkyl" refers to a C₄-C₆ straight chain or branched chain alkyl group such as, for example methyl, ethyl, propyl, isopropyl, butyl, isobutyi, tert-butyl,
penty!, isopenty!, neopentyl, heptyi, hexyl, decyl. Preferably, alkyl is \( C_1-C_8 \) straight chain or branched chain alkyl or \( C_7-C_{10} \) straight chain or branched chain alkyl.

Whenever the term "substituted" is used, it is meant to indicate that one or more hydrogens on the atom indicated in the expression using "substituted" is / are replaced by one or multiple moieties from the group comprising halogen, hydroxyl, nitro, \( C_1-C_8 \)-alkylcarbonyl, cyano, thfluoramethyl, d-Ce-alkylsulfanyl, \( C_1-C_8 \)-alkyl, \( C_1-C_8 \)-alkoxy and (VCe-alkylsulfanyl), provided that the regular valency of the respective atom is not exceeded, and that the substitution results in a chemically stable compound, / e. a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into a pharmaceutical composition.

As used herein, \( C_n-C_m \) indicates the range of number of carbon atoms the respective moiety may feature, illustrated by but not limited to e.g. \( C_1-C_2 \)-alkyl or \( C_1-C_6 \) alkoxy, which may feature 1, 2, 3, 4, 5, or 6 carbon atoms not covering optional additional substitution.

As used herein, the term "carrier" refers to microcrystalline cellulose, lactose, mannitol.

As used herein, the term "solvents" refers to liquid polyethylene glycols, ethanol, corn oil, cottonseed oil, glycerol, isopropanol, mineral oil, oleic acid, peanut oil, purified water, water for injection, sterile water for injection and sterile water for irrigation.

Metal radioisotopes such as \(^{99m}\text{Tc}\) or \(^{186/188}\text{Re}\) can be bound to peptides using two methods. The direct coordination of these metals is applicable in particular to proteins (high molecular weight peptides). The other approach involves the linkage of a bifunctional chelating agent (Arano Y., Annals of Nuclear Medicine Vol. 16, No. 2, 79-93, 2002) such as exemplarily depicted above.

The expression "stabilizing amount" with the context of the present invention denotes concentrations of the uric acid or derivatives thereof in the range of 1 pg/mL to 1.000 pg/mL, preferably of 10 \( \mu \text{g/mL} \) to 100 \( \mu \text{g/mL} \) uric acid derivatives and more preferably
of 20 µg/mL to 60 µg/mL uric acid derivatives. Even more preferably, the concentration of the uric acid derivative is 40 µg/mL.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOS</td>
<td>End of synthesis</td>
</tr>
<tr>
<td>ESI-TOF</td>
<td>Electrospray-lonization Time-of-Flight</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>LA</td>
<td>Low Activity</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography / Mass Spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>RCP</td>
<td>Radiochemical purity</td>
</tr>
<tr>
<td>RP</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>RCY</td>
<td>Radiochemical yield</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion current</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>VWD</td>
<td>Variable wavelength detector</td>
</tr>
</tbody>
</table>
Experimental Section

Table 1: List of chemicals and further consumables

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Purity</th>
<th>Provider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>Glacial; ≥99.99%</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Acetonitrile (MeCN)</td>
<td>Reag. Ph. Eur</td>
<td>Merck</td>
</tr>
<tr>
<td>Acetone</td>
<td>for analysis EMSURE® ACS, ISO, Reag. Ph Eur</td>
<td>Merck</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Ph. Eur.</td>
<td>Fluka</td>
</tr>
<tr>
<td>DOTA-RM1</td>
<td>70%</td>
<td>BSP (NeoMPS)</td>
</tr>
<tr>
<td>DOTATATE</td>
<td>&gt;99%</td>
<td>Bachem</td>
</tr>
<tr>
<td>DOTATOC</td>
<td>96.6%</td>
<td>Poly Peptide</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Ph. Eur</td>
<td>KAZ</td>
</tr>
<tr>
<td>Formic acid 98–100% (HCOOH)</td>
<td>ACS, Reag. Ph. Eur</td>
<td>Merck</td>
</tr>
<tr>
<td>Ga(NO₃)₃ hydrate</td>
<td>99.9998 % (trace metal basis)</td>
<td>Acros Organics</td>
</tr>
<tr>
<td>Gentic acid</td>
<td>≥98%</td>
<td>Fluka</td>
</tr>
<tr>
<td>HEPES (N-2-Hydroxyethylpiperazin- N'-2-ethansulfonsäure)</td>
<td>≥99%</td>
<td>Merck</td>
</tr>
<tr>
<td>Sodium phosphate dibasic dihydrate (NaH₂PO₄·2H₂O)</td>
<td>Ph. Eur</td>
<td>Fluka</td>
</tr>
<tr>
<td>Sulfuric acid (H₂SO₄)</td>
<td>95-97%, puriss p.a</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Hydrochloric acid 30% (HCl)</td>
<td>Ultrapur</td>
<td>Merck</td>
</tr>
<tr>
<td>Sodium acetate anhydrous</td>
<td>Trace Select; ≥99.999%</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Sodium chloride solution 0.9%</td>
<td>Ph. Eur</td>
<td>B. Braun</td>
</tr>
<tr>
<td>Chemical</td>
<td>Purity</td>
<td>Provider</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>TFA</td>
<td>For spectroscopy</td>
<td>Merck</td>
</tr>
<tr>
<td>Uric acid</td>
<td>99%</td>
<td>ABCR</td>
</tr>
<tr>
<td>Water</td>
<td>Trace Select</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Water for Injection</td>
<td>Ph. Eur</td>
<td>Fresenius Kabi</td>
</tr>
</tbody>
</table>
1 Radiolabeling of Peptides

1.1 General Synthesis of $^{68}$Ga-DOTA-RM2 (compound 2)

Most of the $^{68}$Ga labeling syntheses described below were performed starting with compound 1 which is a DOTA-conjugated Bombesin derivative (Ga-DOTA-RM2) for radiolabeling. Precursor 1 is stored at -20°C in fractions of 28 µg dissolved in 40 ml water. The stability of 1 was determined by HPLC over a period of 5 months. No degradation was observed.

RM2 is a modified sequence of peptide Bombesin. The peptide sequence of RM2 contains 9 amino acid as described below

D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$

Radiochemical yields are corrected for decay unless stated otherwise.

Figure 1 shows the radiolabeling of DOTA-RM2 (compound 1) with 68Ga to give $^{68}$Ga-DOTA-RM2 (compound 2).

1.2 Gallium-68 generators

Two $^{68}$Ge/$^{68}$Ga generators (IGG 100, Eckert & Ziegier Eurotope GmbH) of different activities (generator A = 740 MBq; generator B = 1850 MBq) were used for the labeling experiments. $^{68}$Gallium was obtained by elution with 0.1 M HCl (7 ml_). The syntheses were performed using an automated synthesis module (Modular-Lab PharmTracer, Eckert & Ziegier Eurotope GmbH) and disposable synthesis cassettes (C4-Ga68-PP or C4-Ga68-FR; Eckert & Ziegier Eurotope GmbH).

Programs for the fractionation method and the pre-purification method were provided by Eckert & Ziegler Eurotope GmbH. The impact of both methods on the impurity profile was tested.

1.3 No-Carrier-Added $^{68}$Ga Syntheses

1.3.1 Syntheses of $^{68}$Ga-DOTA-RM2 Using the Pre-purification Method

The $^{68}$Ga eluate was pre-purified using a cation exchange cartridge (Strata-X-C, Phenomenex). A solution of 98% acetone / 0.02 M HCl (0.4 mL) was used to elute $^{68}$Ga from the cartridge. Sodium acetate buffer (0.2 M; 2 mL; pH 4) and peptide 1 (28 µg dissolved in 40 µL water) was pre-filled into the reactor vessel. The reaction mixture was heated to 95 °C for 400 s.

After radiolabeling, the reaction mixture was cooled down to approx. 50 °C and diluted with saline (3 mL). The mixture was passed through a pre-conditioned SepPak C18 cartridge (pre-conditioned with 6 mL ethanol (50%), 6 mL saline) in order to immobilize the drug substance on the cartridge. Educts and impurities were washed away with saline (3 mL). The product was eluted from the cartridge with 1 mL ethanol (50%) and passed through a 0.22 µm sterile filter. The final product (2) was collected in a vial and formulated with saline (9 mL) to yield a final batch volume of 10 mL.

1.3.2 Syntheses of $^{68}$Ga-DOTA-RM2 Using the Fractionation Method

The $^{68}$Ga eluate was collected in three fractions (fraction 1 with 2.4 mL and fraction 3 with 2 mL were discarded). Only fraction 2 (1.7 mL) containing the main radioactivity (approx. 80% of the total radioactivity) was transferred into the reactor vessel. The reactor was prefilled with HEPES buffer (2.5 M; 350 µL), water (450 µL) and peptide 1 (28 µg) dissolved in water (40 µL). The reaction mixture was heated to 95 °C for 400 s.

The purification steps of the crude product were the same as described in the pre-purification method.

After radiolabeling, the reaction mixture was cooled down to approx. 50 °C and diluted with saline (3 mL). The mixture was passed through a pre-conditioned SepPak C18 cartridge (pre-conditioned with 6 mL ethanol (50%), 6 mL saline) in order to immobilize the drug substance on the cartridge. Educts and impurities were washed away with...
saline (3 mL). The product was eluted from the cartridge with 1 mL ethanol (50%) and passed through a 0.22 pm sterile filter. The final product was collected in a vial and formulated with saline (9 mL) to yield a final batch volume of 10 mL.

1.3.3 Syntheses of DOTATATE and DOTATOC Using the Pre-purification Method

The $^{68}$Ga eluate was pre-purified using a cation exchange cartridge (Strata-X-C, Phenomenex). A solution of 98% acetone / 0.02 M HCl (0.4 mL) was used to elute $^{68}$Ga from the cartridge. Sodium acetate buffer (0.2 M; 2 mL; pH 4) and peptide DOTATATE and DOTATOC, respectively, (50 µg peptide dissolved in 50 pL water) was pre-filled into the reactor vessel. The reaction mixture was heated to 95 °C for 400 s.

After radiolabeling, the reaction mixture was cooled down to approx. 50 °C and diluted with saline (3 mL). The mixture was passed through a pre-conditioned SepPak C18 cartridge (pre-conditioned with 6 mL ethanol (50%), 6 mL saline) in order to immobilize the drug substance on the cartridge. Educts and impurities were washed away with saline (3 mL). The product was eluted from the cartridge with 1 mL ethanol (50%) and passed through a 0.22 µm sterile filter. The final product was collected in a vial and formulated with saline (9 mL) to yield a final batch volume of 10 mL.

1.4 Carrier Added Syntheses of $^{68}$Ga-DOTA-RM2 Using the Fractionation Method

The carrier added radiolabeling experiment was performed with the fractionation method. The fraction that was used for the labeling contained a starting activity of 930 MBq. The reactor vial was pre-filled with peptide 1 (3 mg) dissolved in water (490 µL) and HEPES buffer (2.5 M; 350 µL). After 100 s of heating the solution at 95 °C, Ga(N0$_3$)$_3$·n H$_2$O (1.04 mg) dissolved in HCl (0.1 M; 10 pL) was added to the reaction mixture and kept stirring for another 300 s at 95 °C. The purification steps were the same as mentioned above.

1.4.1 LC-MS Measurements

The products of the carrier added syntheses were allowed to decay and were analyzed within 1 week after synthesis by LC-MS (ESI-TOF, LCT Premier, Waters; column:
ACE 3 C18; 3 µm 100Å, 50 mm × 4.6 mm; gradient method from 17% B, to 25% B within 20 min; eluent A: water with 0.1% HCOOH; eluent B: MeCN with 0.1% HCOOH). The LC-MS measurements allowed the structure assignment of the unknown radiochemical impurities. 

Figure 2 shows a LC-MS TIC of the reaction mixture obtained from a carrier added radiosynthesis without addition of a stabilizer.

After decay of the carrier added Ga-DOTA-RM2 sample, LC-MS analysis was performed (cf. Figure 2). The main peak at a retention time $R_t = 6.53$ min represents $^{69m}$Ga-DOTA-RM2 ($M = 1706.6$ g/mol) (detected as $[M+2H]^{2+/2}$; $m/z = 853.3$). The $m/z$ ratios of the more polar side products in the range of 4.2 to 5.6 min were determined to be $m/z = 861.4$ and $m/z = 869.4$, respectively. The mass difference of $m/z = +8$ is interpreted as the detection of $[M+2H+0]^{2+/2}$, whereas $m/z = +16$ indicates the detection of the molecule after insertion of two oxygen atoms as $[M+2H+20]^{2+/2}$. The structure assignment was realized by using the isotope pattern calculator of the MassLynx 4.1 software. The calculated isotope patterns of the detected compounds mentioned above are in perfect agreement with the experimental data (cf. Figure 3.1, 3.2, 3.3 and 3.4).

Figure 3.1, 3.2, 3.3 and 3.4 shows calculated isotope patterns of Ga-DOTA-RM2 derivatives with two ($m/z = 869$) and one ($m/z = 861$) additional oxygen atoms introduced by radiolytic processes, and unchanged Ga-DOTA-RM2 ($m/z = 853$) (first three spectra 3.1 - 3.3,) as well as the corresponding experimental isotope patterns of all three Ga-DOTA-RM2 species (3.4).

The data support the hypothesis of the formation of radiolytically oxidized products of $^{68}$Ga-DOTA-RM2 with one and two additional oxygen atoms, respectively.

1.5 Quality Control! - Analytical HPLC

1.5.1 Determination of Chemical and Radiochemical Purity

Analytical HPLC was performed with a gradient RP-HPLC system, equipped with an UV-detector and a NaI(Tl) well-type scintillation detector for radioactivity detection. Data acquisition and interpretation were carried out with associated software. An analytical column ACE 3 C18 (3 µm; 100Å, 50 mm × 4.6 mm) was used with a flow rate of 2 mL/min, a wavelength of 230 nm for method 1 and 2 and a wavelength of 225 nm
for methods 3-5, respectively. The eluents used for method 1 and 2 were acidified with TFA, methods 3-5 with $H_2SO_4$.

**Method 1**

Mobile phase A: water with 0.027% $H_2SO_4$; B: MeCN with 0.027% $H_2SO_4$

0 min, 0% B, 10 min 40% B, 10.1 min 90% B, 12.1 min 90% B, 12.3 min 0% B, 16 min 0% B

**Method 2**

Mobile phase A: water with 0.027% $H_2SO_4$; B: MeCN with 0.027% $H_2SO_4$

0 min 15% B, 40 min 25% B

**Method 3**

Mobile phase A: water with 0.027% $H_2SO_4$; B: MeCN with 0.027% $H_2SO_4$

0 min, 17% B; 12 min, 25% B; 14 min, 75% B; 15 min, 17% B, 17 min, 17% B.

1.5.2 **Determination of Uric Acid**

The quantification of uric acid in the final product was performed using an analytical HPLC with a gradient RP-HPLC system (Agilent 1100 Series) equipped with an UV-detector (VWD). Data acquisition and interpretation were carried out with associated software (ChemStation Software). An analytical column Phenomenex Luna 5 µm C18 (5 µm, 100 A, 250 mm * 4.6 mm) was used with a flow rate of 1 mUmin. The detector wavelength was set to 288 nm for 0 - 12 min and to 225 nm for 12.01 - 18 min.

**Method 4**

Mobile phase A: 10 mM NaH$_2$PO$_4$, mobile phase B: acetonitrile

0 min, 0% B, 12 min 0% B, 13 min 50% B, 15 min 50% B, 17 min 0% B, 18 min 0% B
1.6 Influence of the Starting Radioactivities of $^{68}$Ga on the RCP of $^{68}$Ga-DOTA-RM2

1.6.1 Experiments with Low Starting Radioactivity (up to 240 MBq $^{68}$Ga)

Low radioactivity batches were conducted by two different methods (LA1 and LA2, respectively). For method LA1, the generator was eluted as usual, and then the program was stopped after drawing up all the activity in the syringe. The $^{68}$Ga was allowed to decay to the desired starting radioactivity. For method LA2, the generator was eluted short time after the last elution. Due to this short regeneration period, low starting radioactivities of $^{68}$Ga were obtained.

The low activity batches were carried out with starting activities ranging from 170 to 240 MBq obtained from generator A.

In Figure 4, the HPLC chromatograms of a low activity batch with a starting activity of 230 MBq and a product activity of 84 MBq (RCY 46%) are depicted. Radiochemical purity varies depending on the HPLC method used: 95% (method 1) and 93% (method 2), respectively. Method 2 with a shallower gradient is able to separate the unknown radiochemical impurities from the main product.

Figure 4 shows HPLC radiochromatograms of $^{68}$Ga-DOTA-RM2 (low activity batch). The RCP was determined to be of 95% (HPLC method 1, top) and 93% with the optimized HPLC method 2 (bottom).

Nine low radioactivity syntheses were conducted with radiochemical yields of (69 ± 9)% using the fractionation as well as pre-purification method. The radiochemical purities were determined to be (94 ± 2)% using HPLC method 1.

1.6.2 Experiments with High Starting Radioactivity (> 720 MBq $^{68}$Ga)

High radioactivity batches were performed with starting radioactivities ranging from 720 to 1300 MBq obtained from generator B.

The high radiochemical purities obtained with the generator A could not be reproduced with generator B. Three batches showed a radiochemical purity of 89% (method 1).

As an example, Figure 5 shows the HPLC chromatogram of a batch produced with a starting radioactivity of 900 MBq. The radioactivity of the product at EOS was 445 MBq.
A radiochemical purity of 83% (method 2) and 89% (method 1), respectively, were determined for this batch. Seven syntheses with high starting radioactivities were performed with radiochemical yields of (64 ± 9)% using the fractionation as well as the pre-purification method. The radiochemical purities were determined to be (82 ± 13)% using HPLC method 1 or method 3.

Figure 5 shows HPLC radiochromatograms from a 68Ga-DOTA-RM2 high radioactivity batch. With HPLC method 1 (top), the RCP was determined to be of 89% in contrast to method 2 with a better resolution and separation of impurities (83% RCP, bottom).

Based on HPLC method 2, the method 3 was developed with a shorter total run time, but with a similar shallow gradient (cf. Figure 6).

Figure 6 shows the HPLC radiochromatogram of 68Ga-DOTA-RM2 obtained with a shallow gradient but shorter run time (method 3). The radiochemical impurities are completely separated from the product peak. The data clearly show a correlation between starting radioactivity and radiochemical purity: the lower the starting radioactivity, the better the radiochemical purity. These results were independent of the use of either method LA1 or LA2 and independent of the used buffer (HEPES or sodium acetate).

1.7 68Ga-Labeling in Presence of Putative Stabilizers

1.7.1 Ascorbic Acid, Gentisic Acid and Sodium Thiosulfate
The use of ascorbic acid completely inhibited the formation of oxygenated 68Ga-DOTA-RM2 side products. However, a new radiochemical impurity was detected as an unresolved peak after the 68Ga-DOTA-RM2 peak (cf. Figure 7). Hence, the radiochemical purity decreased to (78 ± 1)%,$n = 3$. Furthermore, the RCY decreased remarkably to (34 ± 1)%,$n = 3$. However, based on these results obtained from experiments with three different concentrations of ascorbic acid (0.1 mg, 1 mg, and 10 mg), no dependence of the RCP and RCY, respectively, on the concentration was observed.
Figure 7 shows HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 after addition of 0.1 mg ascorbic acid as stabilizer to the reaction mixture. Besides the successful suppression of the formation of oxygenated side products, an additional unknown radiochemical impurity at a retention time of 7.6 min was detected.

Similar results with the detection of a well pronounced radiochemical impurity were obtained using gentisic acid (52% RCP, 20% RCY) and sodium thiosulfate (61% RCP, 35% RCY), respectively (cf. Figure 8).

Figure 8 shows HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 stabilized with 1 mg gentisic acid as stabilizer (top) and 20 uL 10% sodium thiosulfate solution (bottom), respectively.

### 1.7.2 Caffein and Xanthine

$^{68}$Ga-DOTA-RM2 syntheses were performed using 0.1 mg caffeine and xanthine, respectively.

By the addition of caffeine to the reaction mixture, $^{68}$Ga-DOTA-RM2 was obtained in low RCY (14%) and with a low RCP (33%). Analytical HPLC of the drug product revealed the formation of radiolysis products as well as the unknown radiochemical impurity at $R_t = 7$ min. This impurity was already observed during the experiments with ascorbic acid (cf. Figure ).

Figure 9 shows the HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 using caffeine as a putative stabilizer.

Using xanthine as potential stabilizer, the obtained RCY (2.3%) was extremely low. Therefore, the RCP could not be determined. 19% of the starting activity was found in the reactor, 48% of starting activity was lost in the waste.
1.7.3 Uric Acid

1.7.3.1 Stabilizing Effect in the $^{68}$Ga-DOTA-RM2 Synthesis

By addition of 0.1 mg uric acid, the radiolysis reactions of $^{68}$Ga-DOTA-RM2 could be inhibited without negative influence on the radiochemical yield ($62 \pm 8\%$, $n = 2$). The radiochemical purity increased significantly to ($96 \pm 1\%$) (cf. Figure 10).

Figure 10 shows typical HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 obtained with high radiochemical purity using 0.1 mg uric acid as stabilizer.

1.7.3.2 Stabilizing Effect in the $^{68}$Ga-DOTATOC Syntheses

By addition of 0.1 mg uric acid to the reaction mixture, $^{68}$Ga-DOTATOC was obtained in high RCY (77%) and with excellent RCP (99%). Manufacturing of $^{68}$Ga-DOTATOC without a stabilizer resulted in a RCP of only ($84 \pm 10\%$) and RCY of ($73 \pm 4\%$, $n = 2$) (cf. Figure 11).

Figure 11 shows the HPLC radiochromatograms (method 3) of $^{68}$Ga-DOTATOC manufactured without stabilizer (top) and with high radiochemical purity (99%) using uric acid as stabilizer (bottom).

1.7.3.3 Stabilizing Effect in the $^{68}$Ga-DOTATATE Syntheses

$^{68}$Ga-DOTATATE was obtained in high RCY (79%) and with excellent RCP (>99%). Without stabilizer, the RCP of $^{68}$Ga-DOTATATE (77% RCY) was 86%, $n = 1$ (cf. Figure 12).

Figure 12 shows HPLC radiochromatograms (method 3) of $^{68}$Ga-DOTATOC manufactured without stabilizer (86% RCP, top) and with high radiochemical purity (>99%) using uric acid as stabilizer (bottom).

1.7.3.4 Quantification of Uric Acid in the Drug Product

The concentrations of uric acid in the final product were determined using the validated analytical method 4. After cleaning step on the solid phase cartridge the concentrations of uric acid in three decayed $^{68}$Ga-DOTA-RM2 batches were below the LOD (< 0.1 pg/mL).
1.7.4 **1-Methyluric Acid**
Performing a $^{68}$Ga-DOTA-RM2 synthesis, with a starting radioactivity of 1248 MBq the obtained RCY (40%, 406MBq) and RCP (93.2%) are lower than using uric acid. The RCP of 93.3% was much higher than using ascorbic acid, gentisic acid or sodium thiosulfate but lower than using uric acid (cf. Figure 13).

Figure 13 shows the HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a RCP of 93.3% achieved by adding 1-Methyluric acid to the reaction mixture.

1.7.5 **1,3-Dimethyluric Acid**
Using 1,3-Dimethyluric acid as stabilizer in a $^{68}$Ga-DOTA-RM2 synthesis, the obtained RCY (19.9%, 191.6MBq) with a starting radioactivity of 1182 MBq was significantly lower than using uric acid. The RCP of 88.5% was much higher than using ascorbic acid, gentisic acid or sodium thiosulfate but lower than using uric acid (cf. Figure ).

Figure 14 shows the HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a radiochemical purity of 88.5% achieved by adding 1,3-Dimethyluric acid to the reaction mixture.

1.7.6 **Tetramethyluric Acid**
Performing a $^{68}$Ga-DOTA-RM2 synthesis, the RCY (57%, 466 MBq) with Tetramethyluric acid as stabilizer using a starting radioactivity of 1005 MBq was higher compared to the yields obtained with 1-methyluric acid and 1,3-dimethyluric acid. However, the RCP (91.7 %) is lower than using uric acid (cf. Figure 10 and Figure 15).

Figure 15 shows the HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a RCP of 91.7% achieved by adding Tetramethyluric acid to the reaction mixture.
2 Description of the Figures

Figure 1: Radioabelaiirig of DOTA-RM2 (compound 1) with $^{68}$Ga to give $^{68}$Ga-DOTA-RM2 (compound 2)

Figure 2: LC-MS TIC of the reaction mixture obtained from a carrier added radiosynthesis without addition of a stabilizer

Figure 3.1, 3.2, 3.3 and 3.4: Calculated isotope patterns of Ga-DOTA-RM2 derivatives with two (m/z = 869) and one (m/z = 861) additional oxygen atoms introduced by radtolytic processes, and unchanged Ga-DOTA-RM2 (m/z = 853) (first three spectra, 3.1, 3.2 and 3.3) as well as the corresponding experimental isotope patterns of all three Ga-DOTA-RM2 species (3.4).

Figure 4: HPLC radiochromatograms of $^{68}$Ga-DOTA-RM2 (low radioactivity batch). The RCP was determined to be of 95% (HPLC method 1, top) and 93% with the optimized HPLC method 2 (bottom).

Figure 5: HPLC radiochromatograms from a $^{68}$Ga-DOTA-RM2 high radioactivity batch. With HPLC method 1 (top), the RCP was determined to be of 89% in contrast to method 2 with a better resolution and separation of impurities (83% RCP, bottom).

Figure 6: HPLC radiochromatogram of $^{68}$Ga-DOTA-RM2 obtained with a shallow gradient but shorter run time (method 3). The radiochemical impurities are completely separated from the product peak.

Figure 7: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 after addition of 0.1 mg ascorbic acid as stabilizer to the reaction mixture. Besides the successful suppression of the formation of oxygenated side products, an additional unknown radiochemical impurity at a retention time of 7.6 min was detected

Figure 8: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 stabilized with 1 mg gentisic acid as stabilizer (top) and 20 uL 10% sodium thiosulfate solution (bottom), respectively.
Figure 9: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 using caffeine as a putative stabilizer

Figure 10: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 obtained with high radiochemical purity using 0.1 mg uric acid as stabilizer

Figure 11: HPLC radiochromatograms (method 3) of $^{68}$Ga-DOTA-TOC manufactured without stabilizer (top) and with high radiochemical purity (99%) using uric acid as stabilizer (bottom)

Figure 12: HPLC radiochromatograms (method 3) of $^{68}$Ga-DOTA-TOC manufactured without stabilizer (86% RCP, top) and with high radiochemical purity (>99%) using uric acid as stabilizer (bottom)

Figure 13: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a RCP of 93.3% achieved by adding 1-Methyluric acid to the reaction mixture

Figure 14: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a radiochemical purity of 88.5% achieved by adding 1,3-Dimethyluric acid to the reaction mixture

Figure 15: HPLC radiochromatogram (method 3) of $^{68}$Ga-DOTA-RM2 with a RCP of 91.7% achieved by adding Tetramethyluric acid to the reaction mixture
Claims

1. A method for stabilizing a peptidic radiopharmaceutical characterized in that uric acid derivatives are used as stabilizing agent.

2. The method according to claim 1 wherein the uric acid derivatives are compounds of formula I

[Diagram]

wherein

R1 is Hydrogen, substituted or unsubstituted C1-C3 alkyl group, CH3(CH2)m-0-(CH2)2 CH2-0)n CH2-CH2, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group,

R2 is Hydrogen, substituted or unsubstituted C1-C3 alkyl group, CH3(CH2)m-0-(CH2)2 CH2-0)n CH2-CH2, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group,

R3 is Hydrogen, substituted or unsubstituted C1-C3 alkyl group, CH3(CH2)m-0-(CH2)2 CH2-0)n CH2-CH2, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group, and

R4 is Hydrogen, substituted or unsubstituted C1-C3 alkyl group, CH3(CH2)m-0-(CH2)2 CH2-0)n CH2-CH2, n = 0, 1, 2 or 3 and m = 0 or 1 or substituted or unsubstituted acyl group.

3. The method according to claim 1 or 2 wherein uric acid derivative is selected from uric acid, 1-methyluric acid, 3-methyluric acid, 7-methyluric acid, 9-methyluric acid, 1,3-Dimethyluric acid, 1,7-Dimethyluric acid, 1,9-Dimethyluric acid, 3,7-Dimethyluric acid, or Tetramethyluric acid.
4. The method according to claims 1, 2 or 3 wherein the peptidic radiopharmaceutical is selected from

\[ ^{68}\text{Ga}]\text{-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH}_2, \quad \text{or} \quad \text{DOTA-RM2}; \]

5. The method according to claim 4 wherein the peptidic radiopharmaceutical is

\[ ^{68}\text{Ga}]\text{-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Vai-Gly-His-Sta-Leu-}
\text{NH}_2, \quad \text{or} \quad \text{DOTA-RM2}. \]

6. The method according to claims 1 to 5 comprising the steps

- adding uric acid derivative(s) to a peptidic radiopharmaceutical precursor,
- adding uric acid derivative(s) into the reaction mixture of a radiofabeling reaction or
- adding uric acid derivative(s) to the freshly prepared peptidic radiopharmaceutical.

7. The method according to claim 6 wherein the peptidic radiopharmaceutical precursor is

\text{DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Aia-Val-Giy-His-Sta-Leu-}
\text{NH}_2, \quad \text{DOTA-RM2}.

8. A composition comprising

- a peptidic radiopharmaceutical and
9. A composition comprising
   -a peptidic radiopharmaceutical precursor and
   -uric acid derivatives used as stabilizing agent.

10. A kit comprising
    -a peptidic radiopharmaceutical precursor and
    -uric acid derivatives used as stabilizing agent.

11. Use of a kit according to claim 10 for stabilizing a peptidic radiopharmaceutical.

12. Use of uric acid or derivatives thereof for stabilizing a peptidic radiopharmaceutical.

13. Use according to claim 12, characterized by using a stabilizing amount of uric acid or derivatives thereof in the range of 1 Mg/mL to 1.000 μg/mL.
Fig 1:

1 (DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$, DOTA-RM2) → $^{68}$Ga[GaCl$_2$], pH 4
400s, 95°C

5 ($^{68}$Ga-DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$, $^{68}$Ga-DOTA-RM2)
Fig 2:
Fig 3.1, 3.2, 3.3, and 3.4:
Fig 4 top:
Fig 4 bottom:
Fig 5 top:
Fig 5 bottom:
Fig 6:

[Graph showing a peak at 6.99 min]
Fig 7:
Fig 8:
Fig 9:
Fig 10:
Fig 11:
Fig 12:
Fig 13:
Fig 14:
Fig 15:
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K51/08

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols): A61K

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

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

EPO-Internal, WPI Data, BEILSTEIN Data, BIOSIS, CHEMABS Data, EMBASE.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 02/067859 A2 (BRISTOL MYERS SQUIBB PHARMA CO [US]) 6 September 2002 (2002-09-06) page 1, line 1 - page 13, line 33</td>
<td>1-13</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:
  A: document defining the general state of the art which is not considered to be of particular relevance.
  E: earlier application or patent but published on or after the international filing date.
  L: document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
  O: document referring to an oral disclosure, use, exhibition or other means.
  P: document published prior to the international filing date but later than the priority date claimed.

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

X: document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.

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

A: document member of the same patent family.

Date of the actual completion of the international search: 15 May 2013

Date of mailing of the international search report: 24/05/2013

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer
Kukol ka, Flori an

Form PCT/ISA/210 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR 0207315 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2438204 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1503680 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CZ 20032597 A3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1365813 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HU 0304083 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2004529884 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX PA03007595 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2002122769 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 02067859 A2</td>
</tr>
<tr>
<td>US 5393512 A</td>
<td>28-02-1995</td>
<td>NONE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2413538 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2002122768 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0204030 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2003161753 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2004091388 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2004009143 A1</td>
</tr>
</tbody>
</table>