

^{18}F -labelled folates**5 Field of Invention**

The present invention is directed towards new ^{18}F -folate radiopharmaceuticals, wherein fluorine-18 is covalently linked through a triazole- or tetrazole-linker to a folate or derivative thereof, a method of their preparation, as well as
10 their use in diagnosis and monitoring of cancer and inflammatory and autoimmune diseases and therapy thereof.

Background

Cell-specific targeting for delivery of effector moieties such
15 as diagnostic or therapeutic agents is a widely researched field and has led to the development of non-invasive diagnostic and/or therapeutic medical applications. In particular in the field of nuclear medicine procedures and treatments, which employ radioactive materials emitting electromagnetic radiations as
20 γ -rays or photons or particle emitting radiation, selective localization of these radioactive materials in targeted cells or tissues is required to achieve either high signal intensity for visualization of specific tissues, assessing a disease and/or monitoring effects of therapeutic treatments, or high radiation
25 dose, for delivering adequate doses of ionizing radiation to a specified diseased site, without the risk of radiation injury in other e.g. healthy tissues. It is thus of crucial interest to determine and assess cell-specific structures and in particular structures that are present in case of tumors (i.e. cancer) or
30 inflammatory and autoimmune diseases, such as receptors,

antigens, haptens and the like which can be specifically targeted by the respective biological vehicles.

The folate receptor (FR) has been identified as one of these structures. The FR is a high-affinity ($K_D < 10^{-9}$ M) membrane-associated protein. In normal tissues and organs FR-expression is highly restricted to only a few organs (e.g. kidney, lungs, choroids plexus, and placenta), where it largely occurs at the luminal surface of epithelial cells and is therefore not supplied with folate in the circulation. The FR-alpha is frequently overexpressed on a wide variety of specific cell types, such as epithelial tumours (e.g. ovarian, cervical, endometrial, breast, colorectal, kidney, lung, nasopharyngeal), whereas the FR-beta is frequently overexpressed in leukaemia cells (approx. 70 % of acute myelogenous leukaemia (AML) are FR-beta positive). Both may therefore be used as a valuable tumour marker for selective tumour-targeting (Elnakat and Ratnam, Adv. Drug Deliv. Rev. 2004; 56:1067-84). In addition it has recently been discovered that activated (but not resting) synovial macrophages in patients diagnosed with rheumatoid arthritis possess a functionally active FR-beta (Nakashima-Matsushita et al, Arthritis & Rheumatism, 1999, 42(8): 1609-16). Therefore activated macrophages can be selectively targeted with folate conjugates in arthritic joints, a capability that opens possibilities for the diagnosis and treatment of rheumatoid arthritis (Paulos et al, Adv. Drug Deliv. Rev. 2004; 56:1205-17).

Folic acid, which is based on a pteridine skeleton which is conjugated through a benzoylamino moiety to a glutamate, and its derivatives have thus been intensively studied over the past 15 years as targeting agents for the delivery of therapeutic and/or diagnostic agents to cell populations bearing folate receptors in order to achieve a selective concentration of therapeutic and/or diagnostic agents in such cells relative to normal cells.

Various folic acid derivatives and conjugates are known and have been (pre)clinically evaluated, including folate radiopharmaceuticals (Leamon and Low, Drug Discov. Today 2001; 6:44-51; US 4,276,280), fluorinated folate chemotherapeutics (US 4,628,090), folate-conjugates with chemotherapeutic agents (Leamon and Reddy, Adv. Drug Deliv. Rev. 2004; 56:1127-41; Leamon et al, Bioconjugate Chem. 2005; 16:803-11), with proteins and protein toxins (Ward et al, J. Drug Target. 2000; 8:119-23; Leamon et al, J. Biol. Chem. 1993; 268:24847-54; Leamon and Low, J. Drug Target. 1994; 2:101-12), with antisense oligonucleotides (Li et al, Pharm. Res. 1998; 15:1540-45; Zhao and Lee, Adv. Drug Deliv. Rev. 2004; 56:1193-204), with liposomes (Lee and Low, Biochim. Biophys. Acta-Biomembr. 1995; 1233:134-44; Gabizon et al, Adv. Drug Deliv. Rev. 2004; 56:1177-92), with hapten molecules (Paulos et al, Adv. Drug Deliv. Rev. 2004; 56:1205-17), with MRI contrast agents (Konda et al, Magn. Reson. Mat. Phys. Biol. Med. 2001; 12:104-13) etc. Typically all of these derivatives and conjugates have been modified at the glutamate portion of folic acid which lends itself to known carboxylic acid coupling methodology.

Folate radiopharmaceuticals can be in particular very useful for an improved diagnosis and evaluation of the effectiveness of cancer and inflammatory and autoimmune disease therapy. This may include assessment and/or prediction of a treatment response and consequently improvement of radiation dosimetry. Typical visualization techniques suitable for radioimaging are known in the art and include positron emission tomography (PET), planar or single photon emission computerized tomography (SPECT) imaging, gamma cameras, scintillation, and the like.

Both PET and SPECT use radiotracers to image, map and measure activities of target sites of choice. Yet while PET uses positron emitting nuclides which require a nearby cyclotron, SPECT uses single photon emitting nuclides which are available

by generator systems, which may make its use more convenient. However SPECT provides less sensitivity than PET and beside a few approaches quantification methods are lacking. In case of PET, the positron annihilation results in two gamma rays of 511 keV which provide the basis for well developed quantification methods. Thus PET is one of the most sophisticated functional imaging technologies to assess regional uptake and affinity of ligands or metabolic substrates in brain and other organs and thus provides measures of imaging based on metabolic activity. This is for example achieved by administering a positron emitting isotope to a subject, and as it undergoes radioactive decay the gamma rays resulting from the positron/electron annihilation are detected by the PET scanner.

Factors that need to be considered in the selection of a suitable isotope useful for PET include sufficient half-life of the positron-emitting isotope to permit preparation of a diagnostic composition optionally in a pharmaceutically acceptable carrier prior to administration to the patient, and sufficient remaining half-life to yield sufficient activity to permit extra-corporeal measurement by a PET scan. Furthermore, a suitable isotope should have a sufficiently short half-life to limit patient exposure to unnecessary radiation. Typically, a suitable radiopharmaceutical for PET may be based on a metal isotope, such as gallium or copper. These two require however a chelator for entrapment of the metal, which may have an effect on steric and chemical properties. Alternatively a radiopharmaceutical may be based on a covalently linked isotope which provides minimal structural alteration. Radionuclides used for covalent attachment and suitable for PET scanning are typically isotopes with short half lives such as ^{11}C (ca. 20 min), ^{13}N (ca. 10 min), ^{15}O (ca. 2 min), ^{18}F (ca. 110 min).

To date, a number of chelate-based folate radiopharmaceuticals have been synthesized and successfully evaluated as diagnostic

agents for imaging folate receptor-positive tumors. The most widely studied derivatives were labeled either with ^{111}In and $^{99\text{m}}\text{Tc}$ (Siegel et al., J. Nucl. Med. 2003, 44:700; Müller et al., J. Organomet. Chem. 2004, 689:4712) for SPECT or with ^{68}Ga for PET (Mathias et al., Nucl. Med. Biol. 2003, 30(7):725). However, all of the above need a suitable chelating agent, which is typically linked to folic acid through its glutamate portion.

Thus a folate radiopharmaceutical having a covalently linked isotope would be of great interest. In particular a ^{18}F -labeled folate radiopharmaceutical would be most suitable for PET Imaging because of its excellent imaging characteristics which would fulfil all of the above considerations. Compared with other suitable radionuclides (^{11}C , ^{13}N , ^{15}O), ^{18}F is very useful because of its long half-life of approximately 110 minutes and because it decays by emitting positrons having the lowest positron energy, which allows for the sharpest images with a high-resolution PET. Furthermore, the longer half-life of ^{18}F also allows for syntheses that are more complex and satellite distribution to PET centers with no radiochemistry facilities.

Yet, the structure of folic acid does not lend itself to direct radiolabeling with ^{18}F . Thus to date, there have been only very few ^{18}F -labeled folic acid derivatives reported in the literature (Bettio et al., J. Nucl. Med., 2006, 47(7), 1153; WO 2006/071754). Moreover, the currently reported radiosyntheses are time-consuming and give only low radiochemical yields of less than 5% (Bettio et al., J. Nucl. Med., 2006, 47(7), 1153). Thus currently known ^{18}F -labeled folates or derivatives thereof are not able to fill the need for specific radiopharmaceuticals suitable for metabolic imaging of tumors to improve diagnosis and treatment of cancer and inflammatory and autoimmune diseases.

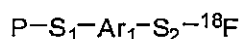
Applicants have now found efficient and versatile methods for

production of new ^{18}F -labeled folate radiopharmaceuticals wherein fluorine-18 is linked through a triazole or tetrazole-linker to a folate or derivative thereof, such as e.g. to the glutamate functionality of folic acid. Preliminary *in-vitro* studies suggested their suitability as powerful diagnostic agents for FR-positive tumours.

Summary of the Invention

The present invention is in a first aspect directed to new ^{18}F -folate radiopharmaceuticals (hereinafter also called compounds of the invention), wherein fluorine-18 is linked through a triazole or tetrazole linker to a folate or derivative thereof, such as to the glutamate functionality of folate or derivative.

In one specific embodiment, the new folate radiopharmaceuticals are compounds of formula I,



I

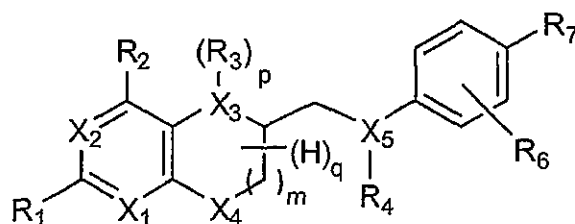
wherein

P is a folate or derivative thereof,
 S_1 , S_2 are independently of each other a single bond or a spacer, and
 Ar_1 is a triazole or tetrazole.

Preferably S_1 and S_2 are independently of each other a single bond or a spacer unit, such as straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one -CN, -Hal, -OH, - NH_2 , -SH, - SO_3H or - NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -O-CO-, - NR' -, -N=, - NR' -CO-, -CO- NR' -, - NR' -

CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'- or a five- or six-membered aromatic ring, which is unsubstituted or substituted with -CN, -Hal, -NO₂, -COR' or -COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof.

More specifically the present invention is directed towards compounds having formula II



II

wherein

X₁ to X₅ are independently of each other C or N

R₁ and R₂ are independently of each other H, Hal, -OR', -NHR', C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and (C₁-C₁₂ alkylamino)carbonyl, wherein R' is H or C₁-C₆ alkyl,

R₃, R₄ are independently of each other H, formyl, iminomethyl, nitroso, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, halosubstituted C₁-C₁₂ alkanoyl,

R₅ is H, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and (C₁-C₁₂ alkylamino)carbonyl,

R₆, R₇ are independently of each other straight-chain or

branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, or a group of the formula III



III

5 wherein S₁ and S₂ are independently of each other a single bond or a spacer unit, such as straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of the non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -O-
 10 CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'- or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof, and

15 Ar₁ is a triazole or a tetrazole,

with the proviso that one of R₆ and R₇ is a group of formula III,

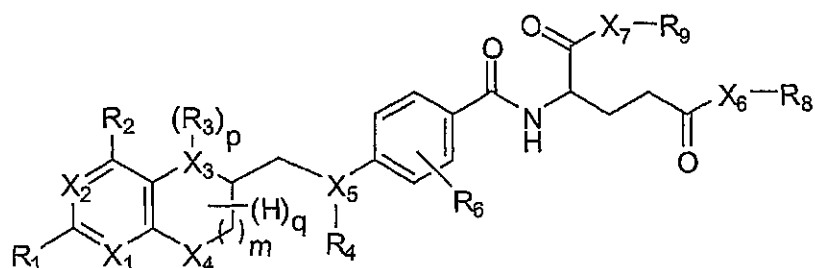
m is 0 or 1,

p is 0, 1 or 2, and

q has a value of 1 to 7.

20

In a further specific embodiment the present invention contemplates a compound of formula IV

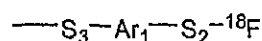


IV

wherein

X_6 , X_7 are independently of each other C, N or O,

- 5 R_8 , R_9 are independently of each other H or straight-chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-, or a group of
- 10 the formula V



V

- wherein S_2 , S_3 are independently of each other a single bond or a spacer unit, such as straight chain or branched C_1 - C_{12} alkyl,
- 15 which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -O-CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, - SO_3R' -, -PR'-, or a five- or six-membered
- 20 aromatic ring, which is unsubstituted or substituted with CN,

Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, with the proviso that no two heteroatoms are adjacent to each other,

Ar₁ is a triazole or a tetrazole, and

- 5 X₁ to X₅, R₁ to R₆, m, p and q, are as defined hereinabove, with the proviso that either R₆ is a group of formula III or one of R₈ and R₉ is a group of formula V.

10 In a further aspect the present invention is directed to a method of their preparation. More specifically, the compounds of the invention may be obtained using a 1,3-dipolar cycloaddition of azides with alkynes or alkyne substitutes, such as nitriles in high yields.

- 15 In another aspect the present invention is directed to pharmaceutical compositions of the compounds of the invention.

In yet another aspect the present invention is directed to the use in diagnosis and monitoring of therapy of cancer and inflammatory and autoimmune diseases in vitro or in vivo.

- 20 In one embodiment, the present invention is directed towards uses of the compounds of the invention for diagnostic imaging of a cell or population of cells expressing a folate-receptor.

More specifically the present invention includes methods for diagnostic imaging of a cell or population of cells expressing a
25 folate-receptor, which includes for example methods for in vitro detection of a cell expressing the folate receptor, for example a tumor cell or an activated macrophage, in a tissue sample. Such methods may also be performed in vivo.

Thus, in a further embodiment the present invention is directed

towards uses of the compounds of the invention for convenient and effective administration to a subject in need for diagnostic imaging and/or monitoring of therapy of cancer and inflammatory and autoimmune diseases. The subject of the methods of the present invention is preferably a mammal, such as an animal or a human, preferably a human.

Such methods of the invention may be performed in combination with any other methods of diagnosis or therapy of cancer and inflammatory and autoimmune diseases including methods using other already developed diagnostic and/or therapeutic agents and utilizing x-ray computed tomography (CT), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), single photon emission computed tomography (SPECT), optical imaging, and ultrasound.

Other features and advantages of the invention will be apparent from the following detailed description thereof and from the claims.

Brief Description of Figures

Figure 1. Synthesis of ^{18}F -Click folate (γ -(4-(4-(4-[^{18}F]fluorobutyl)-1,2,3-triazol-1-yl)butyl) folic acid amide.

Figure 2. Synthesis of γ -(4-azido-butyonyl)-folic acid amide.

Figure 3. Specific uptake in folate receptor-positive tissues as shown by ex vivo biodistribution studies using ^{18}F -click folate.

Figure 4. PET images using ^{18}F -click folate in comparison to PET images using [^{18}F]FDG.

Detailed Description of the Invention

The present invention is in a first aspect directed to new ^{18}F -
5 folate radiopharmaceuticals (hereinafter also called compounds
of the invention), wherein fluorine-18 is linked through a
triazole or a tetrazole linker to a folate or derivative
thereof, such as to the glutamate functionality of folic acid.

The term "folate" as used herein, comprises compounds based on a
10 condensed pyrimidine heterocycle, which is linked to an
aminobenzoyl moiety carrying in para-position a group S_1 as
defined hereinafter. As used herein a "condensed pyrimidine
heterocycle" includes a pyrimidine fused with a further 5- or 6-
membered heterocycle, such as a pteridine or a pyrrolopyrimidine
15 bicycle. Preferred representatives of folates as used herein are
based on a folate skeleton, i.e. pteroyl-glutamic acid or N-
[4(pteridin-6-ylmethylamino)benzoyl]-glutamic acid), and
derivatives thereof and includes optionally substituted folic
acid, folinic acid, pteropolyglutamic acid, and folate receptor-
20 binding pteridines such as tetrahydropterins, dihydrofolates,
tetrahydrofolates, and their deaza and dideaza analogs. Folic
acid is the preferred basic structure used for the compounds of
this invention. The terms "deaza" and "dideaza" analogs refers
to the art recognized analogs having a carbon atom substituted
25 for one or two nitrogen atoms in the naturally occurring folic
acid structure. For example, the deaza analogs include the 1-
deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs. The
dideaza analogs include, for example, 1,5-dideaza, 5,10-dideaza,
8,10-dideaza, and 5,8-dideaza analogs. Preferred deaza analogs
30 compounds include N-[4-[2-[(6R)-2-amino-1,4,5,6,7,8-hexahydro-4-
oxopyrido[2,3-d]pyrimidin-6-yl]ethyl]benzoyl]-L-glutamic acid
(Lometrexol) and N-[4-[1-[(2,4-diamino-6-

pteridinyl)methyl]propyl]benzoyl]-L-glutamic acid (Edatrexate).

More specifically, the new folate radiopharmaceuticals are compounds of formula I,



I

wherein

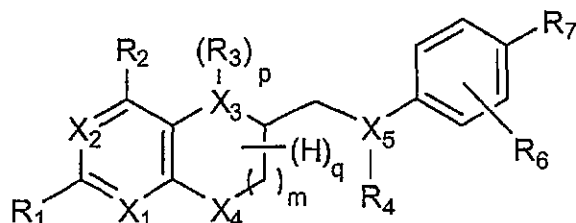
P is a folate or derivative thereof,

S₁, S₂ are independently of each other a single bond or a
 10 spacer, and

Ar₁ is a triazole or tetrazole.

Preferably, S₁ and S₂ are independently of each other a single
 bond or a spacer unit, such as straight-chain or branched C1-C12
 15 alkyl, which is unsubstituted or substituted by at least one CN,
 Hal, or NO₂, and wherein one or more of the non-adjacent CH₂
 groups may independently be replaced by -O-, -CO-, -CO-O-, -O-
 CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -
 NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'-, or a five- or
 20 six-membered aromatic ring, which is unsubstituted or
 substituted with CN, Hal, NO₂, COR' or COOR', wherein R'
 represents H or C1-C6 alkyl, or a combination thereof.

In another specific embodiment, the new folate
 25 radiopharmaceuticals are compounds of formula II



II

wherein

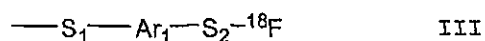
X_1 to X_5 are independently of each other C or N,

5 R_1 and R_2 are independently of each other H, Hal, $-OR'$, $-NHR'$, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, and (C1-C12 alkylamino)carbonyl, wherein R' is H or C1-C6 alkyl,

10 R_3 , R_4 are independently of each other H, formyl, iminomethyl, nitroso, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, halosubstituted C1-C12 alkanoyl,

R_5 is H, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, and (C1-C12 alkylamino)carbonyl,

15 R_6 , R_7 are independently of each other straight-chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , or a group of the formula III



20 wherein S_1 and S_2 are independently of each other a single bond or a spacer unit, such as straight-chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-O-$

CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'-, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof, and

Ar₁ is a triazole or a tetrazole,

with the proviso that one of R₆ and R₇ is a group of formula III,

m is 0 or 1,

p is 0, 1 or 2, and

q has a value of 1 to 7.

In a preferred embodiment R₆ is CN, Hal, NO₂, or a group R₅ and R₇ is a group of formula III.

In another preferred embodiment R₇ is CN, Hal, NO₂, or a group R₅ and R₆ is a group of formula III.

It is understood, that the abbreviations "N" and "C" are representative for all possible degrees of saturation, i.e. N includes -NH- and -N= linkages and C includes -CH₂- and -CH= linkages.

It is further understood, that (H)_q represents all H substituents on the indicated ring (i.e. on X₃, C₆, C₇ and X₄). For example q = 5 for a fully saturated unsubstituted analog (X₃ = X₄ = N, p = 0) or q = 7 for a fully saturated unsubstituted 5,8-dideaza analog (X₃ = X₄ = C, p = 0) and q = 1 for a fully unsaturated

analog with $X_3 = X_4 = N$, $p = 0$.

S_1 is preferably a single bond or a spacer unit, such as straight-chain or branched C_1 - C_{18} alkyl, which is unsubstituted
 5 or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -NR'-, -NR'-CO-, -CO-NR'-, -CH=CH-, -C≡C-, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR', or COOR', wherein R'
 10 represents H or C_1 - C_6 alkyl, or a combination thereof, more preferably a single bond or a spacer unit, such as straight-chain or branched C_1 - C_{18} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of non-adjacent CH_2 groups may independently be replaced
 15 by -O-, -CO-, -CO-O-, -NR'-, -NR'-CO-, -CO-NR'-, -CH=CH-, -C≡C-, or an unsubstituted five- or six-membered aromatic ring.

In a preferred embodiment, S_1 is a single bond or a straight-chain or branched C_1 - C_{12} alkyl, wherein one or more of
 20 non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -NR'-, -NR'-CO-, -CO-NR'-, -CH=CH-, -C≡C-.

In another preferred embodiment S_1 may represent an amino acid, i.e. a compounds with both an amino group (e.g., NH_2 or NH_3^+) and a carboxylic acid group (e.g., COOH or COO^-). In a specific
 25 embodiment, the amino acid may be an α -amino acid, a β -amino acid, a D-amino acid or an L-amino acid. The amino acid may be a naturally occurring amino acid (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine,

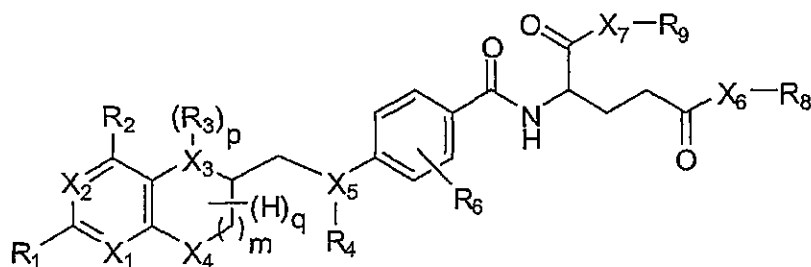
glutamine, aspartic acid, glutamic acid, lysine, arginine, or histidine, etc.) or it may be a derivative thereof. Examples of derivatives include optionally substituted amino acids, e.g. having one or more substituents selected from CN, Hal, and/or NO₂. The amino acid may also include any other non-naturally occurring amino acids, such as e.g. norleucine, norvaline, L- or D- naphthalanine, ornithine, homoarginine and others well known in the peptide art (see for for example in M. Bodanzsky, "Principles of Peptide Synthesis," 1st and 2nd revised ed., Springer- Verlag, New York, NY, 1984 and 1993, and Stewart and Young, "Solid Phase Peptide Synthesis," 2nd ed., Pierce Chemical Co., Rockford, IL, 1984, both of which are incorporated herein by reference). Amino acids and amino acid analogs/derivatives can be purchased commercially (Sigma Chemical Co.; Advanced Chemtech) or synthesized using methods known in the art. In another specific embodiment, the amino acid may also be part of a polyamino acid (also termed polypeptide), wherein a plurality of same or different amino acids as defined hereinabove are covalently linked, i.e. linked through conventional peptide or other bonds. Preferred amino acids include for example glutamic acid, aspartic acid, glutamine, aspartine, lysine, arginine, cystein, and derivatives thereof and preferred polyamino acids include homopolymers the respective homopolymers thereof (i.e. polyglutamic acid, polyaspartic acid, etc). Most preferred are optionally substituted aspartic and glutamic acid.

S₂ is preferably a single bond or a straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof, more preferably a single bond or a straight-chain or

branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂.

Most preferably, S₂ is a single bond or a straight-chain or
5 branched C₁-C₁₂ alkyl.

As indicated hereinabove, preferred representatives of folates as used herein are based on a folate skeleton wherein S₁ is a glutamic acid residue, and derivatives thereof. Thus in another
10 specific embodiment, the new folate radiopharmaceuticals are compounds of formula IV

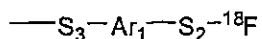


IV

wherein

15 X₆, X₇ are independently of each other C, N or O,

R₈, R₉ are independently of each other H or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of embedded, non-adjacent CH₂ groups may independently be replaced
20 by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-, or a group of the formula V



V

wherein S_2 , S_3 are independently of each other a single bond or a spacer unit, such as straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by $-\text{O}-$, $-\text{CO}-$, $-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-$, $-\text{NR}'-$, $-\text{N}=\text{}$, $-\text{NR}'-\text{CO}-$, $-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{NR}'-$, $-\text{CH}=\text{CH}-$, $-\text{C}\equiv\text{C}-$, $-\text{S}-$, $-\text{SO}_3\text{R}'-$, $-\text{PR}'-$, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or COOR' , wherein R' represents H or C_1 - C_6 alkyl, or a combination thereof,

Ar_1 is a triazole or a tetrazole,

and

X_1 to X_5 , R_1 to R_6 , m , p and q , are as defined according to compound of formula II and preferred embodiments as defined hereinafter,

with the proviso that either R_6 is a group of formula III or one of R_8 and R_9 is a group of formula V.

20

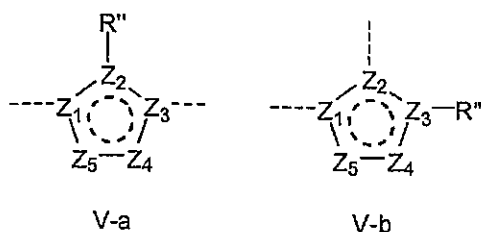
S_2 and S_3 are preferably independently of each other a single bond or a straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or COOR' , wherein R' represents H or C_1 - C_6 alkyl, or a combination thereof, more preferably straight chain or branched C_1 - C_{12} alkyl, which is

unsubstituted or substituted by at least one CN, Hal, or NO₂.

Most preferably S₂ and S₃ are independently of each other a single bond or a straight chain or branched C₁-C₁₂ alkyl.

5

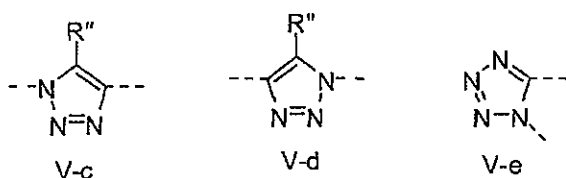
Ar₁ is a triazole or tetrazole of formula V-a and V-b



wherein the dotted lines represent linking sites to the spacer groups, and

10 Z₁ to Z₅ are independently of each other C or N, such that sum of all N is 3 or 4,

and is preferably selected from formulae V-c, V-d, and V-e



15 wherein the dotted lines represent linking sites to the spacer groups and R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR',

20 wherein R' represents H or C₁-C₆ alkyl.

In a specific embodiment R_6 is CN, Hal, NO_2 , or a group R_5 and one of R_8 and R_9 is a group of formula V.

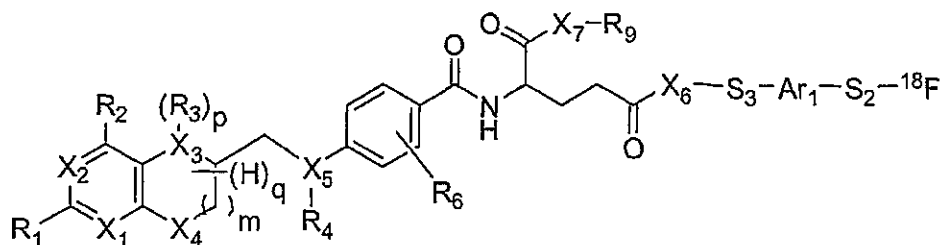
- 5 In another specific embodiment R_6 is CN, Hal, NO_2 , or a group R_5 , H or is straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and R_9 is a group of formula V.
- 10 In a further specific embodiment R_6 is CN, Hal, NO_2 , or a group R_5 , R_9 is H or straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -
- 15 $CH=CH$ -, $-C\equiv C$ -, and R_8 is a group of formula V.

In a further specific embodiment R_8 and R_9 are independently of each other H or straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -

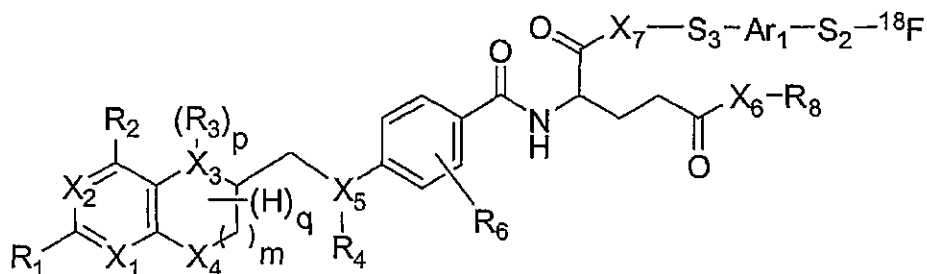
20 $CH=CH$ -, $-C\equiv C$ -, and R_6 is a group of formula V.

Thus, in another specific embodiment, the new folate

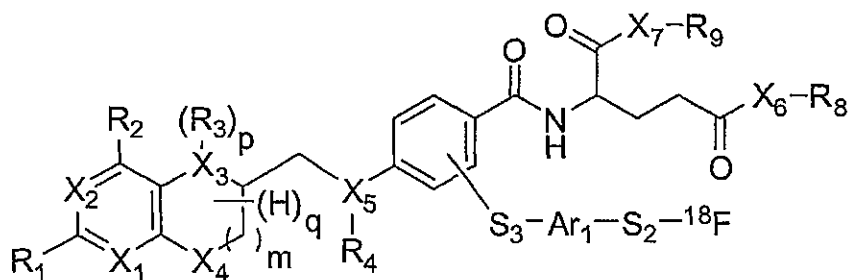
25 radiopharmaceuticals are compounds of formulae VI, VIa or VIb,



VI



VIa



VIb

wherein

X_1 to X_5 are independently of each other C or N,

X_6 , X_7 are independently of each other C, N or O,

10 R_1 and R_2 are independently of each other H, Hal, $-OR'$, $-NHR'$, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, and (C1-C12 alkylamino)carbonyl, wherein R' is H or C1-C6 alkyl,

15 R_3 , R_4 are independently of each other H, formyl, iminomethyl, nitroso, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, halosubstituted C1-C12 alkanoyl,

R_6 is H, CN, Hal, NO_2 , $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_1\text{-C}_{12}$ alkoxy, $\text{C}_1\text{-C}_{12}$ alkanoyl, $\text{C}_2\text{-C}_{12}$ alkenyl, $\text{C}_2\text{-C}_{12}$ alkynyl, ($\text{C}_1\text{-C}_{12}$ alkoxy)carbonyl, or ($\text{C}_1\text{-C}_{12}$ alkylamino)carbonyl,

R_8, R_9 are independently of each other H or straight chain or
5 branched $\text{C}_1\text{-C}_{12}$ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by $-\text{O}-$, $-\text{CO}-$, $-\text{CO}-\text{O}-$, $-\text{CO}-\text{NR}'-$, $-\text{CH}=\text{CH}-$, $-\text{C}\equiv\text{C}-$,,

S_2, S_3 are independently of each other a single bond or a spacer
10 unit, such as straight-chain or branched $\text{C}_1\text{-C}_{12}$ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by $-\text{O}-$, $-\text{CO}-$, $-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-$, $-\text{NR}'-$, $-\text{N}=\text{N}-$, $-\text{NR}'-\text{CO}-$, $-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{NR}'-$,
15 $-\text{CH}=\text{CH}-$, $-\text{C}\equiv\text{C}-$, $-\text{S}-$, $-\text{SO}_3\text{R}'-$, $-\text{PR}'-$, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or COOR' , wherein R' represents H or $\text{C}_1\text{-C}_6$ alkyl, or a combination thereof,

Ar_1 is a triazole or a tetrazole,

20 m is 0 or 1,

p is 0, 1 or 2, and

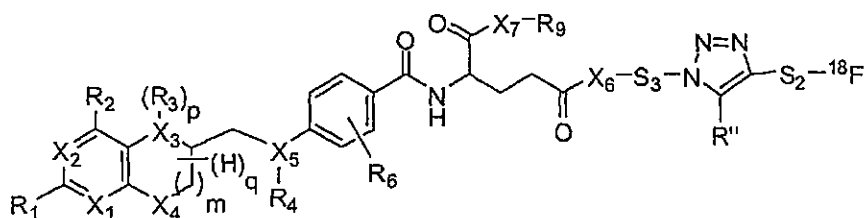
q has a value of 1 to 7.

S_2 and S_3 are preferably independently of each other a single
25 bond or a straight-chain or branched $\text{C}_1\text{-C}_{12}$ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or COOR' , wherein R'

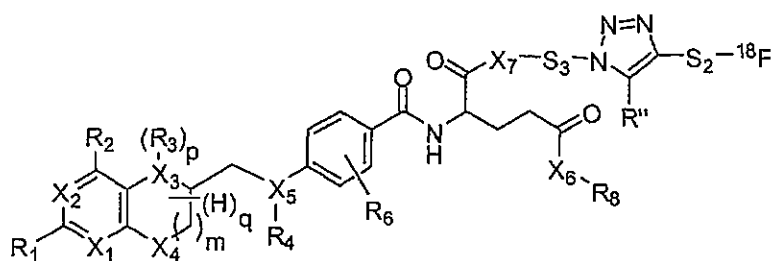
represents H or C₁-C₆ alkyl, or a combination thereof, more preferably straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂.

- 5 Most preferably, S₂ and S₃ are independently of each other a single bond or a straight chain or branched C₁-C₁₂ alkyl.

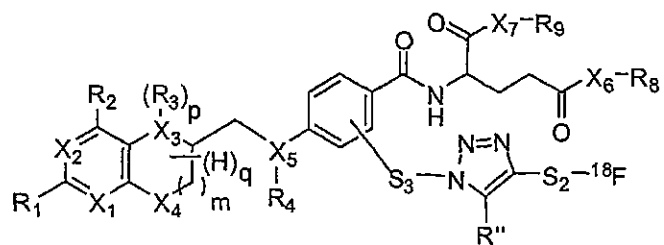
In a further specific embodiment, the new folate radiopharmaceuticals are compounds of formulae VIIa to VIIf or
10 VIIIfa to VIIIf,



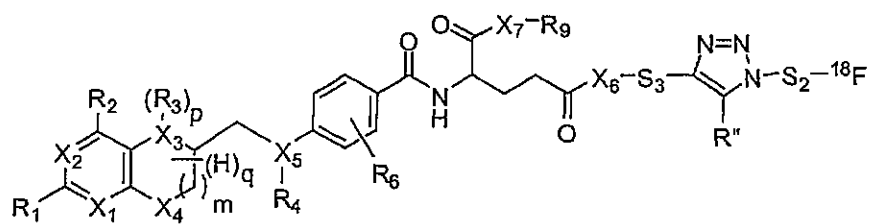
VIIa



VIIb

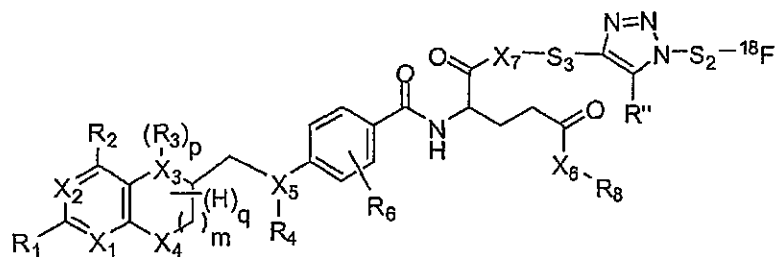


VIIc

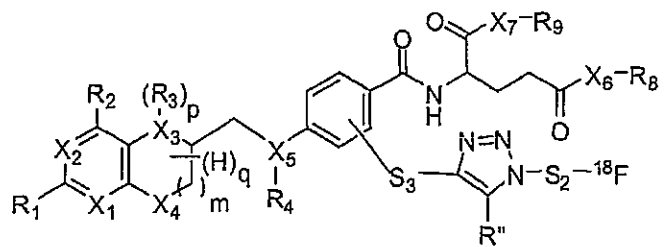


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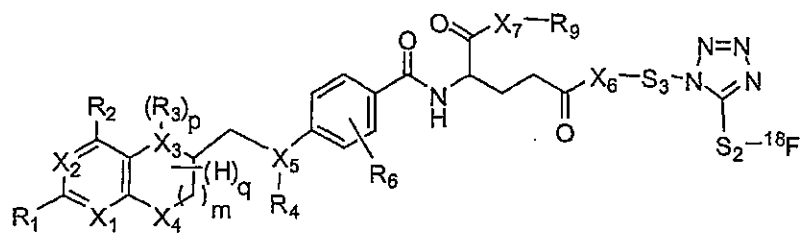
VIId



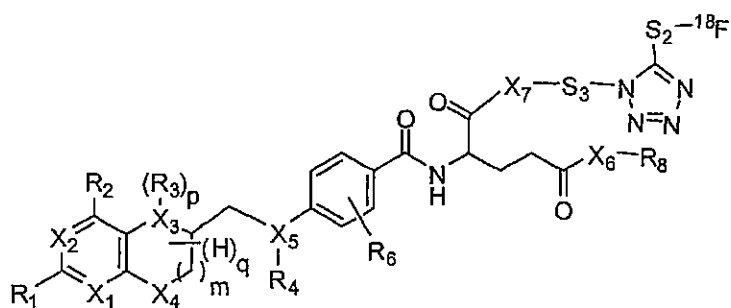
VIIe



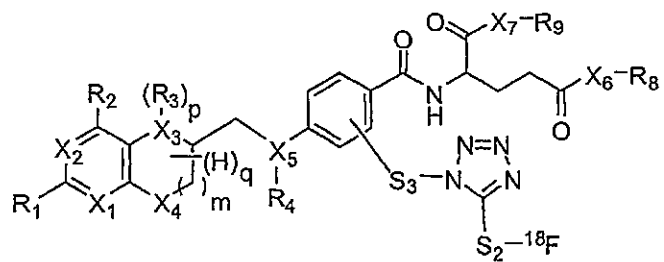
VIIIf



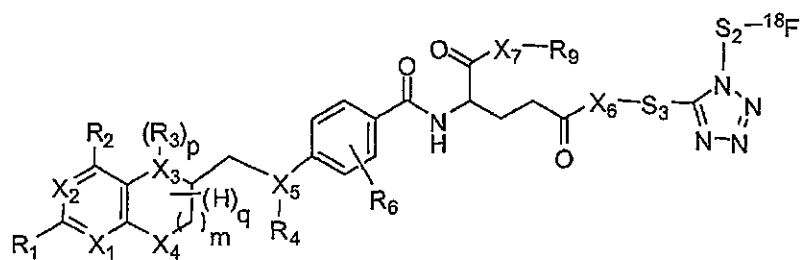
VIIIa



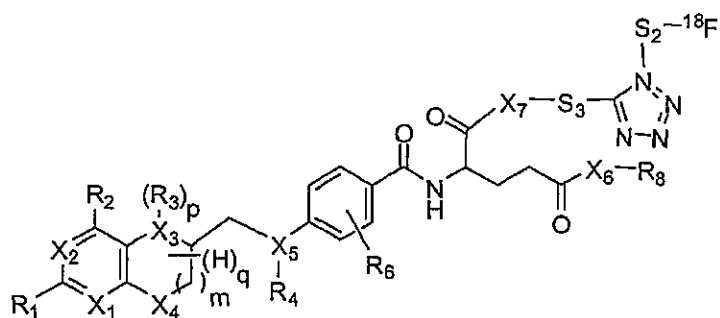
VIIIb



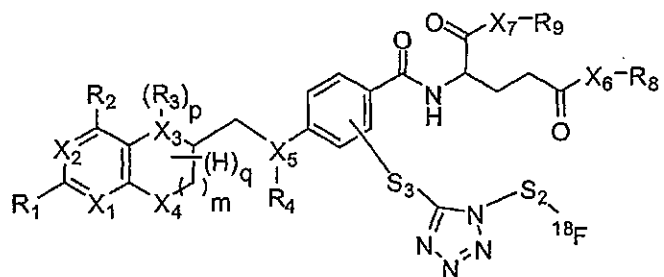
VIIIc



VIIIId



VIIIIf



VIIIId

wherein

X_1 to X_5 are independently of each other C or N,

X_6, X_7 are independently of each other C, N or O,

R_1 and R_2 are independently of each other H, Hal, $-OR'$, $-NHR'$, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and (C₁-C₁₂ alkylamino)carbonyl, wherein R' is H or C₁-C₆ alkyl,

R_3, R_4 are independently of each other H, formyl, iminomethyl, nitroso, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, halosubstituted C₁-C₁₂ alkanoyl,

R_6 is H, CN, Hal, NO₂, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, or (C₁-C₁₂ alkylamino)carbonyl,

R_8, R_9 are independently of each other H or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of embedded, non-adjacent CH₂ groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$,

S_2, S_3 are independently of each other a single bond or a spacer unit, such as straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of the non-adjacent CH₂ groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-O-CO-$, $-NR'-$, $-N=$, $-NR'-CO-$, $-CO-NR'-$, $-NR'-CO-O-$, $-O-CO-NR'-$, $-NR'-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$, $-S-$, $-SO_3R'-$, $-PR'-$, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof,

R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', or straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂, or a

five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl,

m is 0 or 1,

5 p is 0, 1 or 2, and

q has a value of 1 to 7.

Further preferred embodiments as defined hereinabove apply also to compounds of formulae VI to VIII.

10

Preferably, R₁ and R₂ may independently of each other H, alkyl, -OR₅, -NHR', more preferably -OR', -NHR', wherein R' represents H or C₁-C₆ alkyl.

15 Preferably, R₃ is H, formyl, C₁-C₁₂ alkyl or C₁-C₁₂ alkanoyl.

Preferably, R₄ is H, nitroso, C₁-C₁₂ alkoxy, or C₁-C₁₂ alkanoyl.

Preferably, R₅ is H, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, (C₁-C₁₂ alkoxy)carbonyl, or (C₁-C₁₂ alkylamino)carbonyl, more preferably H or C₁-C₄ alkyl.

20

Preferably, R₆ is H, CN, Hal, NO₂, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, or (C₁-C₁₂ alkoxy)carbonyl, more preferably H, CN, Hal, NO₂, or C₁-C₈ alkyl.

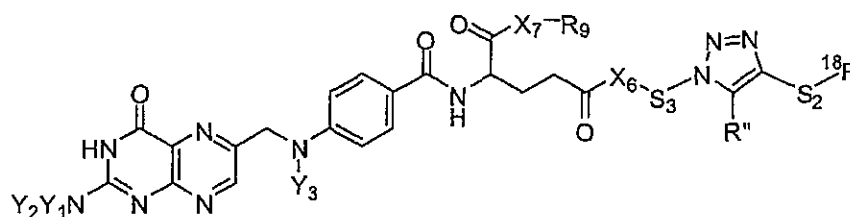
25

One specific embodiment of the compounds of the invention includes for example compounds wherein

(a) X_1 to X_5 are N, R_1 is NY_1Y_2 , R_2 is O, R_4 is Y_3 , m is 1, p is 0 or 1 and q is 1 or 3, or

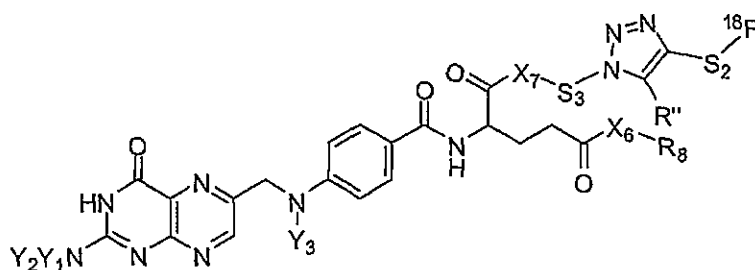
5 (b) X_1 to X_5 are N, R_1 is NY_1Y_2 , R_2 is NH_2 , R_4 is Y_3 , m is 1, p is 0 and q is 1.

Thus, in a further specific embodiment the present invention is for example directed to compounds of formulae IX, IXa, X or Xa,

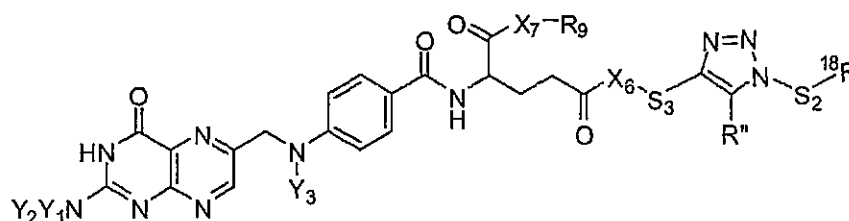


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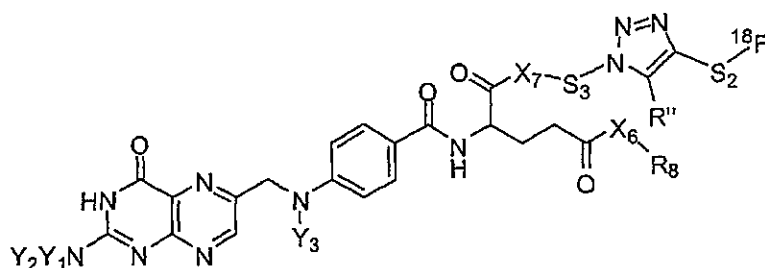
IX



IXa



X



Xa

wherein,

5 X₆, X₇ are independently of each other C, N or O,

Y₁, Y₂ are independently of each other selected from H, formyl, straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of embedded, non-adjacent CH₂ groups may
 10 independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-,
 , -C≡C-,

Y₃ is selected from H, formyl, nitroso, straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of
 15 embedded, non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-,

R₈, R₉ are independently of each other H or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of
 20 embedded, non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-,

R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', or straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H,

or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, and

S₂, S₃ are independently of each other a single bond or a spacer unit, such as straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of the non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -O-CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'- or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof.

Preferably R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', or straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂.

The term "alkyl", when used singly or in combination, refers preferably to straight chain or branched alkyl groups containing 1 to 12 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. More preferred alkyl groups contain 1 to 8, more preferably 1 to 4 carbon atoms.

As used herein, the term "alkenyl", singly or in combination with other groups, refers to straight chain or branched alkyl

groups containing 2 to 12 carbon atoms, such as methylene, ethylene, propylene, isopropylene, butylene, t-butylene, sec-butylene, isobutylene, amylene, isoamylene, pentylene, isopentylene, hexylene and the like. The preferred alkenyl
5 groups contain 2 to 6 carbon atoms.

The term "alkynyl" as used herein refers to a linear or branched chain of carbon atoms with one or more carbon-carbon triple bonds. The preferred alkynyl groups contain 2 to 12, more
10 preferably 2 to 6 carbon atoms.

The term "alkoxy" as used herein refers to alkyl, as defined above, substituted with oxygen, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, tert-butoxy and the like.
15

The term "alkanoyl" as used herein refers to formyl, or alkyl, as defined above, terminally-substituted with a carbonyl such as acetyl, propanoyl, butanoyl, pentanoyl and the like.

20 The term "alkylamino" as used herein refers to alkyl, as defined above, substituted with nitrogen, including both monoalkylamino such as methylamino, ethylamino, propylamino, tert-butylamino, and the like, and dialkylamino such as dimethylamino, diethylamino, methylpropylamino, and the like.

25 The term "halo" as used herein refers to any Group 17 element and includes fluoro, chloro, bromo, iodo, and astatine(o).

In a further aspect the present invention provides a method of
30 synthesizing a compound of the invention. Applicants have found

that the compounds of the invention may be obtained in an effective manner by a process which comprises a cycloaddition of an azide with an alkyne or alkyne substitute under thermal conditions or in the presence of a catalyst. These reactions are
5 known as Huisgen 1,3-dipolar cycloaddition (thermal conditions) and Click-Reaction (catalytic conditions) and have been described in the art (Kolb and Sharpless, *Drug Discovery Today* 2003, 8, 1128; Kolb et al. *Angew. Chem. Int. Ed.* 2001, 40, 2004; Rostovtsev, V. V. et al. *Angew. Chem. Int. Ed.* 2002, 41, 2596; US
10 2005/02222427; WO 06/116629). More specifically compounds of formula I wherein Ar^1 is a triazole are obtained by cycloaddition of an azide R_a-N_3 with an alkyne $R_b-C\equiv C-R_c$ and compounds of formula I wherein Ar^1 is a tetrazole are obtained by cycloaddition of an azide R_a-N_3 with a cyanide $R_b-C\equiv N$. All
15 possible combinations are contemplated herein, i.e. R_a being the folate derivative and R_b being the ^{18}F -labelled group as well as R_b being the folate derivative and R_a being the ^{18}F -labelled group. Thus the modular and versatile nature of the reaction allows employing a wide variety of linkers to couple the
20 radioisotope to folic acid.

In one specific embodiment the cycloaddition is performed under thermal conditions, i.e. at temperatures ranging from 10 to 200 °C, preferably from 10 to 100 °C.

25 In another embodiment the cycloaddition is performed in the presence of a catalyst, such as a transition metal complex, such as Ru and Cu(I). Preferred catalysts are Cu(I) salts, such as Cu(I) chloride, bromide, iodide. Alternatively Cu(I) can be obtained by in situ reduction of a Cu(II) salt. This reaction
30 can be performed in a variety of protic or aprotic solvents, such as for example methanol, ethanol, 2-propanol, tertiary-butanol, n-butanol and/or water or buffered solutions thereof, at a wide range of temperatures (such as between 10 and 100 °C,

preferably room temperature) and varying pH (such as from 4 to 12), under oxidative or reducing conditions and in the presence of other functional groups with no need for protecting groups.

It will be obvious for a skilled person to select appropriate conditions (see also US 2005/0222427 which is incorporated herein by reference as well as references cited therein).

The ^{18}F -labelled starting material, i.e. the ^{18}F -labelled alkyne, alkyne substitute or azide, for the cycloaddition reaction is obtained in a displacement reaction with the respective alkyne, alkyne substitute or azide having a suitable leaving group. The leaving group on the alkyne may be any common leaving group known in the art and includes for example halogen, nitro, diazonium salts, sulfonate esters, including mesylate, tosylate, pentafluorobenzoate, and the like. Typically, the displacement reaction is performed in a polar aprotic solvent selected from acetonitrile, acetone, 1,4-dioxane, tetrahydrofuran (THF), N-methylpyrrolidinone (NMP), dimethoxyethane (DME), dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO) and hexamethylphosphoramide (HMPA) and mixtures thereof.

Thus in one exemplary reaction, an alkyne or alkyne substitute of choice, provided with a suitable leaving group, was labelled with a ^{18}F fluoride activated by phase transfer catalysts such as tetrabutylammonium carbonate or aminopolyethers (e.g. Kryptofix[®] 2.2.2) in combination with potassium carbonate or oxalate in a displacement reaction under standard conditions (for example acetonitrile, 100 °C, 10-15 min). The obtained ^{18}F -labelled alkyne was co-distilled with a suitable solvent to a catalyst of choice (e.g. Cu(I)I). To this mixture the azidofolic acid or derivative of choice was added under standard conditions (for example Na ascorbate in DMF, H₂O and DIPEA at 80°C) to give the final labelled product with a RCY of 25 -

35 %.

Alternatively, an azide of choice, provided with a suitable leaving group, was labelled with a [^{18}F]fluoride activated by phase transfer catalysts such as tetrabutylammonium carbonate or aminopolyethers (e.g. Kryptofix[®] 2.2.2) in combination with potassium carbonate or oxalate to give the [^{18}F]-labelled azide, which was subsequently coupled to the alkyne substituted folic acid or derivative of choice in a catalyzed 1,3-dipolar cycloaddition under standard conditions.

10

In a further aspect the present invention provides uses of folate radiopharmaceuticals of the invention for convenient and effective administration to a subject in need for diagnostic imaging.

15 Thus the present invention provides a method for diagnostic imaging of a cell or population of cells expressing a folate-receptor, said method comprising the steps of administering at least one folate radiopharmaceutical of the invention in a diagnostic imaging amount, and obtaining a diagnostic image of
20 said cell or population of cells.

Such imaging may be performed on a cell or population of cells expressing a folate-receptor in vitro or in vivo.

Thus, the present invention provides a method for in vitro detection of a cell expressing the folate receptor in a tissue
25 sample which includes contacting said tissue sample with at least one folate radiopharmaceutical of the invention in effective amounts and for sufficient time and conditions to allow binding to occur and detecting such binding by PET imaging.

30 In a further aspect the present invention provides uses of folate radiopharmaceuticals of the present invention for

convenient and effective administration to a subject in need for diagnostic imaging or monitoring of therapy of cancer and inflammatory and autoimmune diseases.

5 In another aspect the present invention provides a method for simultaneous diagnosis and therapy, comprising the steps of administering to a subject in need thereof at least one folate radiopharmaceutical of the present invention in a diagnostically effective amount in combination with a therapeutically active, and obtaining a diagnostic image of said tissues to follow the
10 course of treatment.

The subject of the methods of the present invention is preferably a mammal, such as an animal or a human, preferably a human.

15 The dosage depends on the nature of the effect desired, such as the form of diagnosis or therapy, on the kind and frequency of treatment, on the diagnostic instrumentation, on the form of application of the preparation, and on the age, weight, nutrition and condition of the recipient, kind of concurrent treatment, if any.

20 However, the most preferred dosage can be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. This typically involves adjustment of a standard dose, e.g., reduction of the dose if the patient has a low body weight.

25 Treatment can commence with a smaller amount, below the optimum amount, which can be increased in order to achieve the optimum effect.

The imaging procedure in the PET scanner takes place from within minutes to 2-4 hours after administration of the radiotracer.

30 The schedule depends on the imaging target and kinetics of the radiotracer as well as the desired information.

The preferred route of administration of the folate radiopharmaceuticals of the present invention is by intravenous injection.

5 The suitable forms for injection include sterile aqueous solutions or dispersions of the above mentioned folate radiopharmaceuticals of the present invention. Typically the radiopharmaceutical will be formulated in physiological buffer solutions.

10 The folate radiopharmaceuticals can undergo sterilization by any art recognized technique, including but not limited to, addition of antibacterial or antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Preferably they undergo a sterile filtration before administration eliminating the need of additional sterilisation
15 agents.

For a solution to be injected a preferred unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging of the organ or tumor in vivo can take place, if desired, from within minutes to 2-4 hours after the radiolabeled
20 reagent has been administered to a subject to allow a sufficient amount of the administered dose to accumulate in the targeted area of choice.

25 The folate radiopharmaceuticals of the invention may also be used for in vitro detection of a cell expressing the folate receptor in a tissue biopsy taken from a subject. Thus in a further embodiment the present invention provides a method for in vitro detection of a cell expressing the folate receptor, e.g. a tumor cell, in a tissue sample which includes contacting
30 said tissue sample with a folate radiopharmaceutical of the present invention in effective amounts and for sufficient time and conditions to allow binding to occur and detecting such

binding by imaging techniques.

Samples can be collected by procedures known to the skilled person, e.g., by collecting a tissue biopsy or a body fluid, by aspirating for tracheal or pulmonary samples and the like.

5 Tissue samples to be tested include any tissue suspected to contain a cell expressing a folate receptor, such as tumour cells, epithelial cells, kidneys, gastrointestinal or the hepatobiliary system, and others. Samples can be sectioned, e.g., with a microtome, to facilitate microscopic examination
10 and observation. Samples can also be fixed with an appropriate fixative either before or after incubation with one of the folate radiopharmaceuticals of the present invention to improve the histological quality of sample tissues.

Time and conditions sufficient for binding of a folate
15 radiopharmaceutical of the present invention to a folate receptor on the cell include standard tissue culture conditions, i.e. samples can be cultured in vitro and incubated with one of the complexes or compositions of the present invention in physiological media. Such conditions are well known to the
20 skilled person. Alternatively, samples can be fixed and then incubated with a folate radiopharmaceutical of the present invention in an isotonic or physiological buffer.

For all applications it is convenient to prepare the compounds
25 of the present invention at, or near, the site where they are to be used.

All of the compounds and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light
30 of the present disclosure. It will be apparent to those of skill in the art that variations may be applied to the present

invention without departing from the scope of the invention. The Examples provided herein are intended to be illustrative and are not exhaustive; therefore the illustrated Examples should not be viewed as limiting the invention in any way.

5

Examples

Materials and Methods

Infrared spectra were recorded on a Jasco FT/IR-6200 ATR-IR. Nuclear magnetic resonance spectra were recorded with a Bruker
10 400 MHz or 500 MHz spectrometer with the corresponding solvent signals as an internal standard. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm). Values of the coupling constant, J , are given in Hertz (Hz); the following abbreviations are used in the experimental
15 section for the description of ^1H -NMR spectra: singlet (s), doublet (d), triplet (t), multiplet (m), doublet of doublets (dd). The chemical shifts of complex multiplets are given as the range of their occurrence. Low resolution mass spectra (LR-MS) were recorded with a Micromass Quattro microTM API LC-ESI.

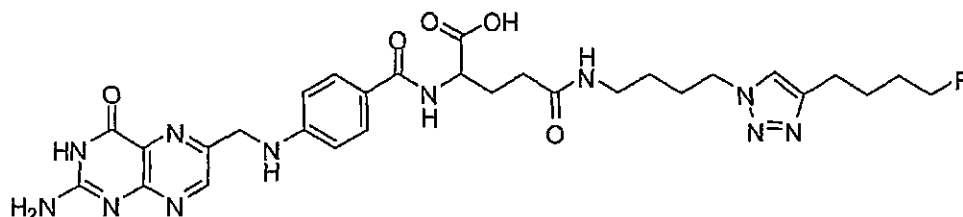
20 Water sensitive reactions were run under argon in flame-dried glass ware. Reactions were monitored by thin layer chromatography (TLC, performed on EM Science 0.25 mm thick, precoated silica gel 60 F-254 glass supported plates) or HPLC. HPLC was performed on a Merck-Hitachi L-7000 system equipped
25 with an L-7400 tunable absorption detector. Analytical HPLC was performed with an XBridge[®] column (C18, 5 μm , 4.6 x 150 mm, Waters) using the following solvent system (1 mL/min): 0.1 % TFA_{aq} (solvent A), acetonitril (solvent B), 1 mL/min; 0-1 min, 95 % A; 1-15 min, 95 $\rightarrow$ 5 % A; 15-20 min, 5 % A; 20 $\rightarrow$ 22 min, 5 $\rightarrow$ 95 % A;
30 22 $\rightarrow$ 25 min, 95 % A. Semi-prep HPLC was performed with XBridge[®] semiprep column (C18, 5 μm , 10 x 150 mm, Waters), 3 mL/min, isochratic NH_4HCO_3 (10 mM, 88 %) / CH_3CN (12 %). All chemicals

were used as supplied unlike stated otherwise.

Production of n.c.a. [^{18}F]fluoride N.c.a. [^{18}F]fluoride was produced via the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction at a Cyclone 18/9 cyclotron (IBA, Belgium). Isotopically 97% enriched [^{18}O]water was irradiated by a 16 MeV proton beam using a 2.1 ml liquid target. The [^{18}F]fluoride/[^{18}O]water solution was transferred from the target to a manipulator equipped syntheses hotcell using a helium stream.

[^{18}F]fluoride (~20 - 30 GBq) was trapped on an anion exchange cartridge (Sep-Pak[®] Light Accell Plus QMA, Waters AG), preconditioned with 5 ml 0.5M potassium carbonate solution and 5 ml water, while the [^{18}O]water was recovered for recycling. The [^{18}F]fluoride was directly eluted into a 10 ml sealed reaction vessel using a solution of potassium carbonate (2 mg) and Kryptofix[®] 2.2.2 (10 mg) in 1.5 ml acetonitrile/water (4:1). At 85 - 90 °C the solvents were removed by vacuum and a stream of nitrogen. Subsequently, 1 ml of dry acetonitrile was added three times and evaporated to dryness.

20 Example 1: Synthesis of ^{19}F -click folate standard



(a) Synthesis of 6- ^{19}F -fluoro-1-hexyne

5-hexyn-1-yl p-tosylate (330 mg, 1.3 mmol) (prepared by tosylation of 5-hexyne-1-ol according to the procedure described by van Hest et al. J. Am. Chem. Soc. 2000, 122, 1282), Kryptofix[®] 2.2.2 (620 mg, 1.6 mmol) and KF (97 mg, 1.6 mmol) in dry THF (12 mL) were stirred at reflux for 20 hours. Fractionated distillation of the volatile components at ambient pressure gave

6- ^{19}F]fluoro-1-hexyne as a 42% solution in THF (200mg, 62 %):
 Bp: 65-70 °C / 1 atm.; ^1H -NMR (CDCl_3) δ 4.51 (t, 1H, J = 6.0 Hz),
 4.40 (t, 1H, J = 6.0 Hz), 2.23 (dt, 2H, J = 7.6 and 2.8 Hz),
 1.95 (t, 1H, J = 2.8 Hz), 1.80-1.73 (m, 2H), 1.65-1.61 (m, 2H)
 5 ppm.

(b) Synthesis of γ -(4-azido-butyonyl)-folic acid amide

References numbers see Figure 2.

N-BOC-amino-butane-azide (2) was prepared from mono-BOC-
 10 diaminobutane (1) according to the procedure described by Link
 et al. (*J. Am. Chem. Soc.* 2004, 126, 10598). Deprotection of the
 intermediate 2 in CH_2Cl_2 /TFA (20%) at room temperature over night
 yielded, after drying *in-vacuo*, the TFA salt of 4-azido-butane-
 amine (3) as a colorless oil (0.44 g, 94 %): ^1H -NMR (CDCl_3) δ
 15 4.54 (bs, 1H), 3.27 (t, 2H, J = 6.5 Hz), 3.25-3.10 (m, 2H),
 1.63-1.50 (m, 4H), 1.14 (s, 9H) ppm; LR-MS: $[\text{M}+\text{H}]^+ = 215.21$
 (calc. for $\text{C}_9\text{H}_{18}\text{N}_4\text{O}_2$: 214.26).

In a flamed-dried flask under argon was dissolved BocGluOMe (4,
 261 mg, 1.0 mmol) in dry DMF (5 mL, over molecular sieves 4 Å)
 20 and Et_3N (210 μL , 1.5 equiv) was added. HBTU (380 mg, 1.0 mmol)
 was added at 0 °C and the mixture was stirred for half an hour.
 The solution of the activated acid was transferred via cannula
 to a solution of amine TFA salt 3 (228 mg, 1.0 mmol) in dry DMF
 (5 mL) containing Et_3N (210 μL , 1.5 equiv) at 0 °C. After 2 hrs,
 25 the mixture was warmed to rt and stirred over night. Removal of
 volatile components under reduced pressure and purification of
 the residue by flash chromatography on silicagel with CH_2Cl_2 /MeOH
 (60:1 to 30:1) provided product 5 as a colorless oil (330 mg, 92
 %). Intermediate 5 was deprotected in CH_2Cl_2 /TFA (20%) at room
 30 temperature over night yielding, after drying *in-vacuo*, the TFA
 salt of Glu(4-azido-butylamide)OMe (6) as a pale yellow oil (740
 mg, quantitative): ^1H -NMR (CDCl_3) δ 10.15-8.60 (bs, 3H), 6.74 (t,

1H, $J = 5.6$ Hz), 4.14 (dd, 1H, $J = 7.7$ and 3.7 Hz), 3.80 (s, 3H), 3.31-3.26 (m, 2H), 3.25-3.18 (m, 2H), 2.59-2.45 (m, 2H), 2.38-2.27 (m, 1H), 2.24-2.13 (m, 1H), 1.63-1.50 (m, 4H) ppm; LR-MS: $[M+H]^+ = 258.23$ (calc. for $C_{10}H_{19}N_5O_3$: 257.29).

5 In a flamed-dried flask under argon was suspended N2-N,N-dimethylaminomethylene-10-formyl-pteronic acid **7** (198 mg, 0.5 mmol) in dry DMF (10 mL, over molecular sieves 4 Å) and Et_3N (104 μ L, 0.75 mmol) was added. HBTU (380 mg, 0.5 mmol) was added at 0 °C and the mixture was stirred for one hour. To the resulting
10 orange solution was added at 0 °C a solution of amine TFA salt **6** (186 mg, 0.5 mmol) in dry DMF (9 mL) containing Et_3N (210 μ L, 1.5 mmol). The resulting clear yellow solution was stirred at 0 °C for one hour and then allowed to warm to rt. Removal of volatile components under reduced pressure and purification of the
15 residue by flash chromatography on silicagel with $CH_2Cl_2/MeOH$ (17:1 to 10:1) provided product **8** as a yellow solid (290 mg, 92 %). Azido folate **8** (63 mg, 0.1 mmol) was dissolved in 1 M NaOH (3 mL) and stirred at rt over night. The resulting turbid solution was cleared by filtration through Celite™. The pH of
20 the yellow solution was adjusted to pH ~2 by addition of HCl (first 37 % HCl, then 1 M HCl) which resulted in precipitation of the product. The suspension was centrifuged (10 min at 3500 rpm), the pale yellow supernatant decanted and the solid product dried under reduced pressure to give the penta-hydrochloride
25 salt of γ -(4-azido-butyonyl)-folic acid amide **9** as a yellow powder (75 mg, quantitative): mp > 200 °C; 1H -NMR (DMSO- d_6) δ 12.21-11.95 (bs, 1H), 8.64 (s, 1H), 8.18 (d, 1H, $J = 7.2$ Hz), 7.85 (t, 1H, $J = 5.7$ Hz), 7.65 (d, 2H, $J = 9.0$ Hz), 7.00-6.82 (bs, 2H), 6.93 (t, 1H, $J = 6.2$ Hz), 6.64 (d, 2H, $J = 9.0$ Hz),
30 4.49 (d, 2H, $J = 5.9$ Hz), 4.32-4.22 (m, 1H), 3.29 (t, 2H, $J = 6.8$ Hz), 3.03 (q, 2H, $J = 6.5$ Hz), 3.09-2.96 (m, 2H), 2.12-1.83 (m, 2H), 1.55-1.45 (m, 2H), 1.45-1.35 (m, 2H) (one NH not observed) ppm; ^{13}C -NMR (DMSO- d_6) δ 173.8, 171.5, 166.3, 160.8,

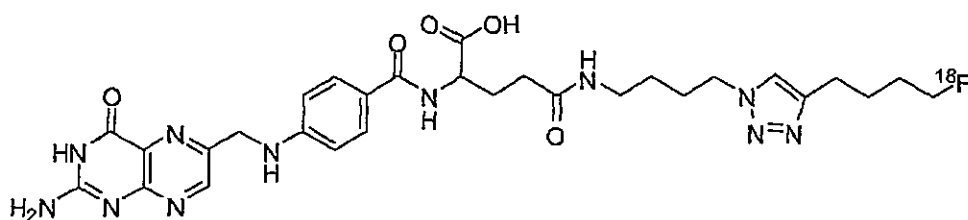
156.6, 153.8, 150.8, 148.7, 148.5, 128.9, 127.9, 121.3, 111.2, 52.2, 50.3, 45.9, 37.9, 32.0, 26.6, 26.3, 25.7 ppm; HR-MS: $[M]^+ = 537.2127$ (calc. for $C_{23}H_{27}N_{11}O_5$: 537.2197); elemental analysis (calculated %-values for $C_{23}H_{27}N_{11}O_5(HCl)_5$ in parenthesis) C 39.16
 5 (38.38), H 4.09 (4.48), N 21.43 (21.40), O (11.11), Cl (24.63).

(c) Synthesis of ^{19}F -click folate standard

γ -(4-Azidobutyl)-folic acid amide (penta-hydrochloride salt, 14 mg, 0.02 mmol) was suspended in t BuOH/ H_2O (1:1, 2 mL) and 6-
 10 $[^{19}F]$ fluoro-1-hexyne (48 mg, 0.2 mmol), $Cu(OAc)_2$ (0.8 mg, 20 mol%) and sodium ascorbate (1.5 mg, 40 mol%) were added. The mixture was stirred at 75 °C for 30 min after which HPLC indicated completed conversion of the azide starting material. The mixture was cooled to room temperature and the resulting
 15 suspension was dissolved by addition of a few drops NaOH 1M. Addition of HCl (1 M) to a final pH of 2 resulted in precipitation of the product. The mixture was centrifuged (10 min at 3500 rpm) and the pale yellow supernatant decanted. Drying of solid provided a brown powder (20 mg). The crude
 20 product was dissolved in NaOH 0.1 M (0.4 mL) and purified by semi-prep HPLC. The collected fractions containing the product were concentrated under reduced pressure and the residue dissolved in NaOH (1 M, 1 mL). Addition of HCl (1 M) to a final pH of 2 resulted in precipitation of the product. Centrifugation
 25 (10 min at 3500 rpm), decantation of the pale yellow supernatant and drying of the solid provided the penta-hydrochloride salt of the final product as a yellow powder (9 mg, 53 %): 1H -NMR (DMSO- d_6) δ 12.02-11.85 (bs, 1H), 8.66 (s, 1H), 8.19 (d, 1H, J = 7.8 Hz), 7.86-7.80 (m, 2H), 7.65 (d, 2H, J = 8.7 Hz), 7.35-6.95
 30 (broad m, 2H), 6.65 (d, 2H, J = 8.7 Hz), 4.53-4.47 (m, 3H), 4.40 (t, 1H, J = 5.8 Hz), 4.32-4.23 (m, 3H), 3.54-3.32 (bs, 1H), 3.08-2.97 (m, 2H), 2.62 (t, 2H, J = 6.6 Hz), 2.22-2.13 (m, 2H), 2.09-2.00 (m, 1H), 1.98-1.88 (m, 1H), 1.79-1.60 (m, 6H), 1.36-

1.26 (m, 2H) ppm; ^{13}C -NMR (DMSO- d_6) δ 173.8, 171.5, 166.3, 160.7, 155.0, 153.5, 150.7, 149.1, 148.5, 146.5, 129.0, 128.0, 121.7, 121.4, 111.2, 84.2, 83.0, 52.2, 48.8, 45.9, 37.7, 32.0, 29.3, 27.2, 26.5, 26.1, 24.8, 24.5 ppm; LR-MS: $[\text{M}+\text{H}]^+ = 638.24$
 5 (calc. for $\text{C}_{29}\text{H}_{36}\text{FN}_{11}\text{O}_5$: 637.67); elemental analysis (calculated %-values for $\text{C}_{29}\text{H}_{36}\text{N}_{11}\text{O}_5(\text{HCl})_5$ in parenthesis) C 42.72 (42.48), H 5.18 (5.04), N 18.17 (18.79), O (9.76), F (2.32), Cl (21.62).

Example 2: Synthesis of ^{18}F -click folate



10

To the obtained dry ^{18}F fluoride-cryptate complex 5-hexyn-1-yl p-tosylate (25 μl) in 1.2 ml acetonitrile was added. The reaction vessel was connected via a PTFE tubing with a second reaction vessel containing Copper(I)iodide (2.5 mg) and it was
 15 placed in a dry-ice/iso-propanol cooling bath. The reaction mixture in reaction vessel one was heated to 95 - 100 $^{\circ}\text{C}$ while the ^{18}F -labelled product 6- ^{18}F fluoro-1-hexyne was co-distilled with acetonitrile to the second reaction vessel (bp (6- ^{18}F fluoro-1-hexyne) = 106 $^{\circ}\text{C}$). 6- ^{18}F fluoro-1-hexyne was given
 20 in a radiochemical yield of 70 - 85 % and a radiochemical purity of 90 - 98 %. After 12 min the second reaction vessel containing 6- ^{18}F fluoro-1-hexyne in ca. 0.8 - 1.0 ml acetonitrile was disconnected and allowed to warm to room temperature. To this mixture 0.3 ml water, 25 μl DIEA, 25 mg sodium ascorbate in
 25 0.15 ml water and γ -(4-azido-butyonyl)-folic acid amide (see Example 1(b)) in 0.2 ml DMF are added successively. The mixture was heated to 80 $^{\circ}\text{C}$ and was allowed to cool down to 60 $^{\circ}\text{C}$ within 20 min. The ^{18}F -Click-folate content of the crude mixture was 55 - 65 %.

For semi-preparative HPLC purification the mixture was diluted with 2 ml HPLC solvent. The separation was carried out on a RP 18 column (Phenomenex[®] Gemini 5 μ C18, 250 x 10 mm) using a gradient as follows. Solvent A = 10 mM ammonium bicarbonate solution, B = methanol, 0 - 30 min: A: 90% $\rightarrow$ 40%, 30 - 40 min: A: 40%, , 40 - 45 min: A: 40% $\rightarrow$ 90%.

The HPLC solvent of the isolated ¹⁸F-click folate was removed under reduced pressure and a stream of nitrogen at 100 °C. The product was formulated in physiological phosphate buffer solution.

Example 3: In vitro, in vivo and ex vivo studies using ¹⁸F-click folate

The ¹⁸F-click folate was employed in in vitro plasma stability studies and in metabolite studies using liver microsomes.

¹⁸F-click folate was incubated with both human and murine plasma at 37 °C in a shaking water bath. Aliquots were taken at different time points and analysed by HPLC. The tracer showed a high stability. No defluorination or other radioactive degradation products were detected over a period of 0 - 120 min.

Metabolites studies using human and murine liver microsomes were carried out at 37 °C over a time span of 0 - 30 min. Aliquots were taken at 0, 5, 15 and 30 min. The studies showed no radioactive metabolites or degradation of the ¹⁸F-click folate over 30 min.

¹⁸F-click folate was applied in ex vivo biodistribution studies using eight nude mice bearing KB xenografts tumors. ~2 MBq of the radiotracer were injected into each animal. In a blockade group (4 animals) 200 μ g natural folic acid was injected 10 min

prior to the radiotracer. The animals were scarified 45 min post injection. The folate receptor-positive KB tumors show a high specific uptake of the radiotracer with a ratio of 94 % specific blockade. Furthermore a specific uptake with 78 % specific
5 blockade was also found in the kidneys, which are known to express the folate receptor. High unspecific uptake was found in gallbladder, intestine and faeces which points to a strong hepatobiliary elimination of the radiotracer.

Figure 3 shows the high specific uptake of the ^{18}F -click folate
10 in folate receptor-positive tissues.

In vivo PET imaging using the ^{18}F -click folate was performed in nude mice bearing KB xenografts tumors. ~11 MBq of the radiotracer were injected into each animal. In the blockade group 200 μg natural folic acid was injected 10 min prior to the
15 radiotracer. The PET scans were acquired from 15 min to 45 min post injection.

The KB tumors could be imaged by PET using ^{18}F -click folate. Due to a low tumor-to-background ratio, the KB tumors are only moderately visualized. A high accumulation of radioactivity in
20 the abdominal region (gallbladder, intestines) points to a strong hepatobiliary elimination of the radiotracer.

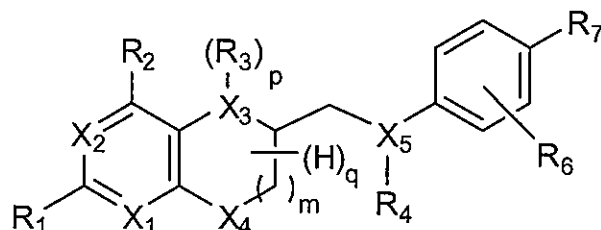
PET imaging using [^{18}F]FDG was performed with the same animals four days after the ^{18}F -click folate studies. ~9 MBq of [^{18}F]FDG were injected into each animal. The PET scans were acquired from
25 30 min to 60 min post injection. The [^{18}F]FDG scans were not able to image the folate receptor-positive KB tumors. Although [^{18}F]FDG is the most commonly used radiotracer in PET tumor imaging, it is known that [^{18}F]FDG is not suitable for the imaging of KB xenografts tumors (Bettio et al, J. Nucl. Med.
30 1998; 47: 1153-60).

Figure 4 show the PET images using ^{18}F -click folate (left hand side) in comparison to PET images using [^{18}F]FDG (right hand

side), the arrows indicate the position of the KB xenografts tumors.

Claims

1. A compound having formula II



II

5 wherein

X₁ to X₅ are independently of each other C or N,

R₁ and R₂ are independently of each other H, Hal, -OR', -NHR',
C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂
alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and
10 (C₁-C₁₂ alkylamino)carbonyl, wherein R' is H or C₁-C₆
alkyl,

R₃, R₄ are independently of each other H, formyl, iminomethyl,
nitroso, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl,
halosubstituted C₁-C₁₂ alkanoyl,

15 R₅ is H, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂
alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and (C₁-
C₁₂ alkylamino)carbonyl,

R₆, R₇ are independently of each other straight-chain or
branched C₁-C₁₂ alkyl, which is unsubstituted or
20 substituted by at least one CN, Hal, or NO₂, or a group
of the formula III



III

wherein S_1 and S_2 are independently of each other a single bond or a spacer unit, such as straight-chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by $-\text{O}-$, $-\text{CO}-$, $-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-$, $-\text{NR}'-$, $-\text{N}=\text{}$, $-\text{NR}'-\text{CO}-$, $-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{O}-$, $-\text{O}-\text{CO}-\text{NR}'-$, $-\text{NR}'-\text{CO}-\text{NR}'-$, $-\text{CH}=\text{CH}-$, $-\text{C}\equiv\text{C}-$, $-\text{S}-$, $-\text{SO}_3\text{R}'-$, $-\text{PR}'-$, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or COOR' , wherein R' represents H or C_1 - C_6 alkyl, or a combination thereof, and

Ar_1 is a triazole or a tetrazole,

with the proviso that one of R_6 and R_7 is a group of formula III,

m is 0 or 1,

p is 0, 1 or 2, and

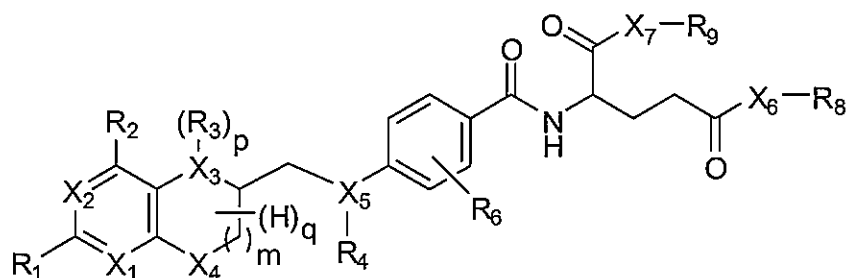
q has a value of 1 to 7.

2. A compound according to claim 1, wherein R_6 is straight-chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and R_7 is a group of formula III.

3. A compound according to claims 1 or 2, wherein R_7 is

straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and R₆ is a group of formula III.

- 5 4. A compound according to any preceding claim wherein S₁ is
a single bond or a spacer unit, such as straight-chain or
branched C₁-C₁₈ alkyl, which is unsubstituted or
substituted by at least one CN, Hal, or NO₂, and wherein
one or more of non-adjacent CH₂ groups may independently
10 be replaced
by -O-, -CO-, -CO-O-, -NR'-, -NR'-CO-, -CO-NR'-, -CH=CH-,
-C≡C-, or a five- or six-membered aromatic ring, which
is unsubstituted or substituted with CN, Hal, NO₂, COR',
or COOR', wherein R' represents H or C₁-C₆ alkyl, or a
15 combination thereof.
5. A compound according to any preceding claim wherein S₂ is
a single bond or a straight-chain or branched C₁-C₁₂
alkyl, which is unsubstituted or substituted by at least
20 one CN, Hal, or NO₂, or a five- or six-membered aromatic
ring, which is unsubstituted or substituted with CN, Hal,
NO₂, COR', or COOR', wherein R' represents H or C₁-C₆
alkyl, or a combination thereof, more preferably a single
bond or a straight-chain or branched C₁-C₁₂ alkyl, which
25 is unsubstituted or substituted by at least one CN, Hal,
or NO₂.
6. A compound according to claim 1 having formula IV

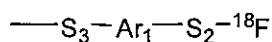


IV

wherein

X_6 , X_7 are independently of each other C, N or O

- 5 R_8 , R_9 are independently of each other H or straight-chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-, or a group of the formula V
- 10



V

- wherein S_2 , S_3 are independently of each other a single bond or a spacer unit, such as straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by -O-, -CO-, -CO-O-, -O-CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -PR'-, or a five- or six-membered aromatic
- 15
- 20

ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof,

Ar₁ is a triazole or a tetrazole,

5 and

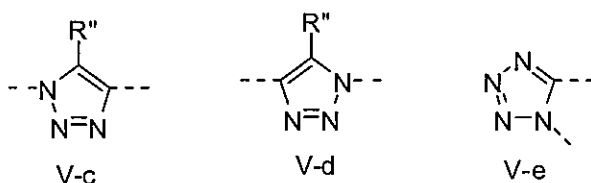
X₁ to X₅, R₁ to R₆, p and q, are as defined in claim 3,

with the proviso that either R₆ is a group of formula III or at least one of R₈ and R₉ is a group of formula V.

- 10 7. A compound according to claim 6, wherein S₂ and S₃ are independently of each other a single bond or a straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or
 15 substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof, more preferably straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂.

20

8. A compound according to any preceding claim wherein Ar₁ is selected from formulae V-c, V-d and V-e



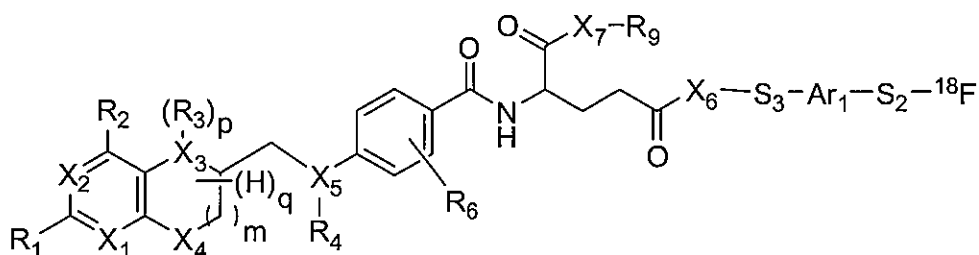
wherein the dotted lines represent linking sites to the

spacer groups and R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', wherein R' represents H or C₁-C₆ alkyl, or straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof.

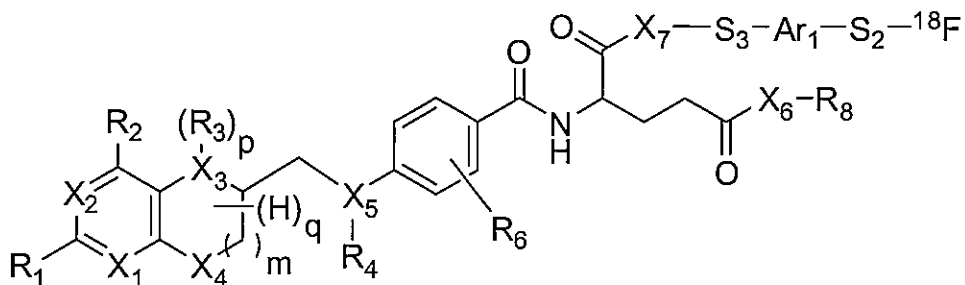
9. A compound according to claim 6, wherein R₆ is CN, Hal, NO₂, or a group R₅ and at least one of R₈ and R₉ is a group of formula V.
10. A compound according to claim 6, wherein R₆ is CN, Hal, NO₂, or a group R₅, R₈ is H or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal or NO₂, wherein one or more of embedded, non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-, and R₉ is a group of formula V.
11. A compound according to claim 6, wherein R₆ is CN, Hal, NO₂, or a group R₅, R₉ is H or straight chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal or NO₂, wherein one or more of embedded, non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-, -CH=CH-, -C≡C-, and R₈ is a group of formula V.

12. A compound according to claim 6, wherein R_8 and R_9 are independently of each other H or straight chain or branched C_1 - C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal or NO_2 , wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$, and R_6 is a group of formula V.

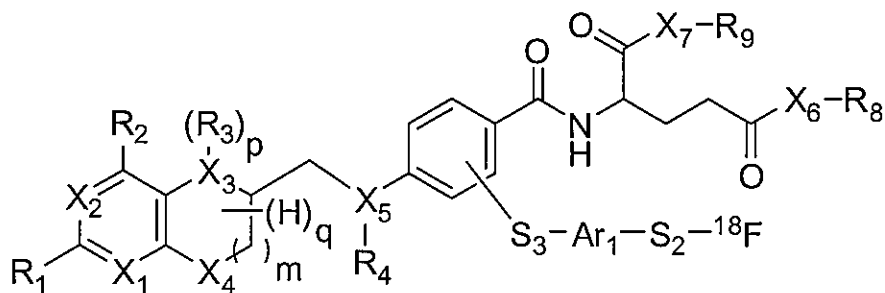
13. A compound according to claim 1 having formulae VI, VIa or VIb,



VI



VIa



VIb

wherein

X₁ to X₅ are independently of each other C or N,

X₆, X₇ are independently of each other C, N or O,

R₁ and R₂ are independently of each other H, Hal, -OR', -NHR',
 5 C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₂-C₁₂
 alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and
 (C₁-C₁₂ alkylamino)carbonyl, wherein R' is H or C₁-C₆
 alkyl,

10 R₃, R₄ are independently of each other H, formyl, iminomethyl,
 nitroso, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl,
 halosubstituted C₁-C₁₂ alkanoyl,

R₆ is H, CN, Hal, NO₂, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂
 alkanoyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, (C₁-C₁₂
 alkoxy)carbonyl, or (C₁-C₁₂ alkylamino)carbonyl,

15 R₈, R₉ are independently of each other H or straight chain or
 branched C₁-C₁₂ alkyl, which is unsubstituted or
 substituted by at least one CN, Hal, or NO₂, and wherein
 one or more of embedded, non-adjacent CH₂ groups may
 independently be replaced by -O-, -CO-, -CO-O-, -CO-NR'-,
 20 -CH=CH-, -C≡C-, ,

S₂, S₃ are independently of each other a single bond or a spacer
 unit, such as straight-chain or branched C₁-C₁₂ alkyl,
 which is unsubstituted or substituted by at least one CN,
 Hal, or NO₂, and wherein one or more of the non-adjacent
 25 CH₂ groups may independently be replaced by -O-, -CO-, -
 CO-O-, -O-CO-, -NR'-, -N=, -NR'-CO-, -CO-NR'-, -NR'-CO-O-,
 -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, -S-, -SO₃R'-, -
 PR'-, or a five- or six-membered aromatic ring, which is
 unsubstituted or substituted with CN, Hal, NO₂, COR', or

COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof,

Ar₁ is a triazole or a tetrazole,

m is 0 or 1,

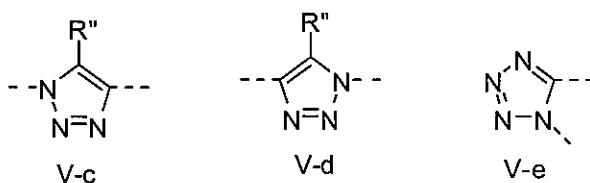
5 p is 0, 1 or 2, and

q has a value of 1 to 7.

14. A compound according to claim 13 wherein S₂ and S₃ are independently of each other a single bond or a
10 straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl.

15

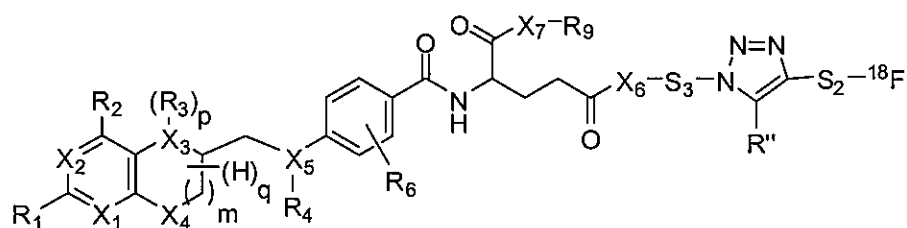
15. A compound according to claims 13 or 14, wherein Ar₁ is selected from formulae V-c, V-d and V-e



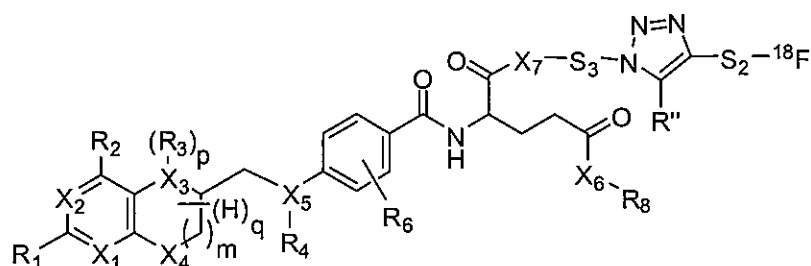
20 wherein the dotted lines represent linking sites to the spacer groups and R'' is H, Hal, NH-COR', NH-SO₂R', CO₂R', COR', wherein R' represents H or C₁-C₆ alkyl, or straight-chain or branched C₁-C₁₂ alkyl, which is unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂, or a five- or six-membered
25 aromatic ring, which is unsubstituted or substituted with

CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, or a combination thereof.

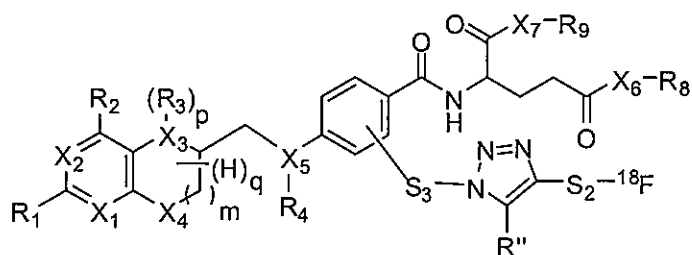
16. A compound according to any preceding claim of formulae
 5 VIIa to VIIf or VIIIa to VIIIf,



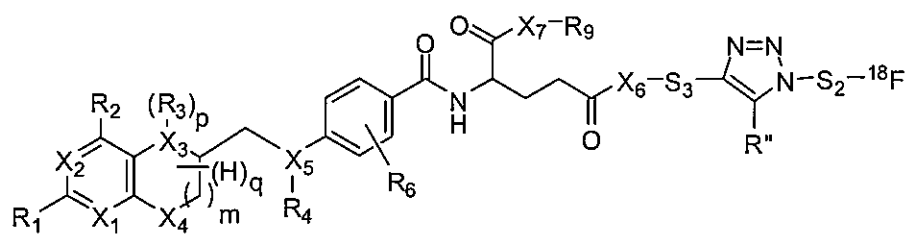
VIIa



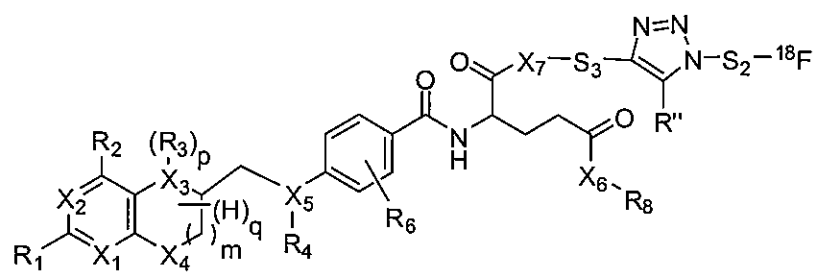
VIIb



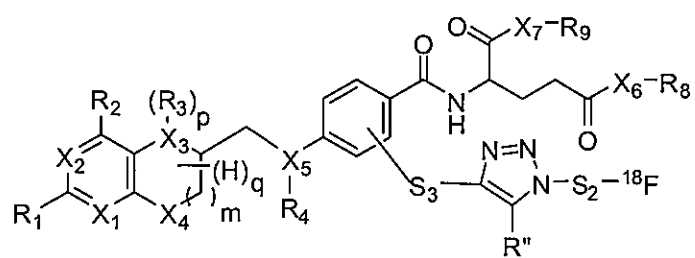
VIIc



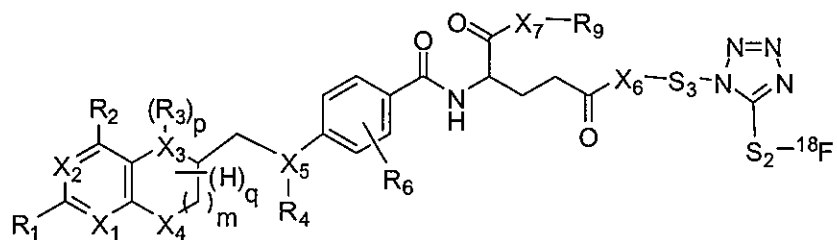
VIIId



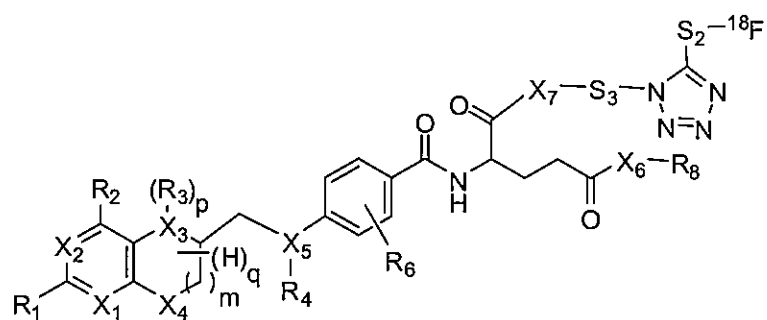
VIIIf



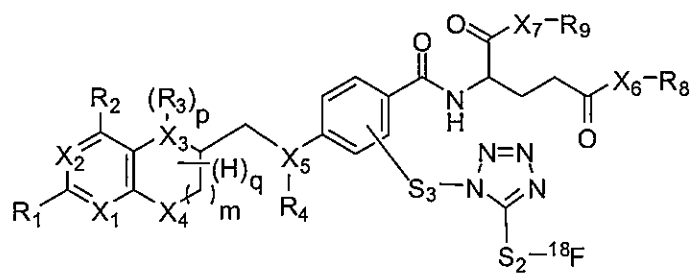
VIIId



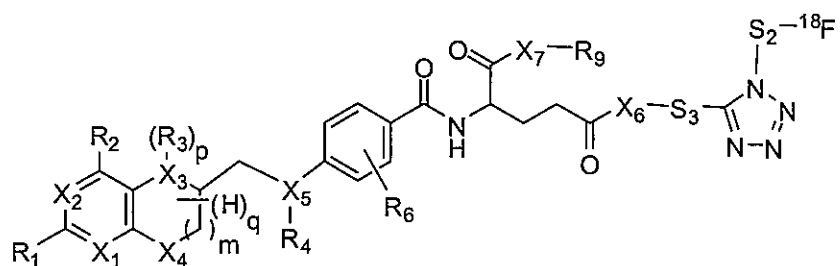
VIIIa



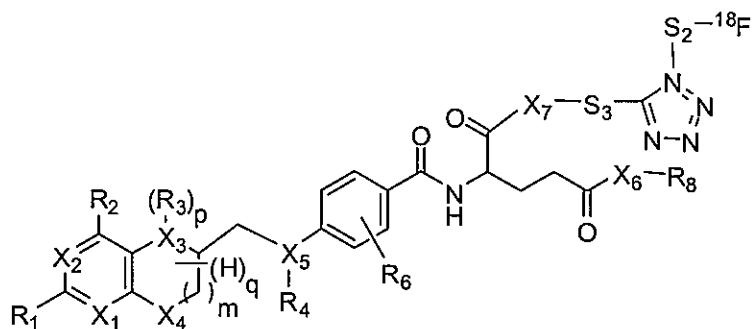
VIIIb



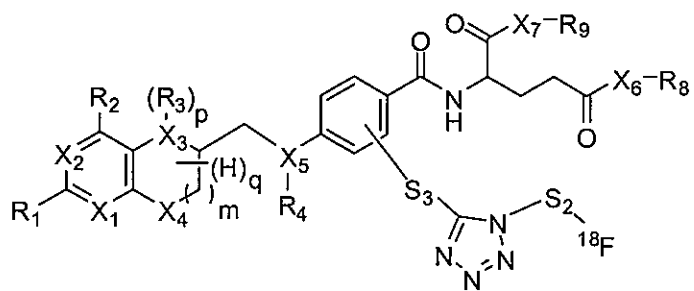
VIIIc



VIIIId



VIIIIf



VIIIId

wherein

X_1 to X_5 are independently of each other C or N,

X_6 , X_7 are independently of each other C, N or O,

R_1, R_2 are independently of each other H, Hal, $-OR'$, $-NHR'$, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, and (C1-C12 alkylamino)carbonyl, wherein R' is H or C1-C6 alkyl,

R_3, R_4 are independently of each other H, formyl, iminomethyl, nitroso, C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, halosubstituted C1-C12 alkanoyl,

R_6 is H, CN, Hal, NO_2 , C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, or (C1-C12 alkylamino)carbonyl,

R_8, R_9 are independently of each other H or straight chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$,,

S_2, S_3 are independently of each other a single bond or a spacer unit, such as straight-chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of the non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-O-CO-$, $-NR'-$, $-N=$, $-NR'-CO-$, $-CO-NR'-$, $-NR'-CO-O-$, $-O-CO-NR'-$, $-NR'-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$, $-S-$, $-SO_3R'-$, $-PR'-$, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or $COOR'$, wherein R' represents H or C1-C6 alkyl, or a combination thereof,

R'' is H, Hal, $NH-COR'$, $NH-SO_2R'$, CO_2R' , COR' , or straight-chain or branched C1-C12 alkyl, which is

unsubstituted or substituted by at least one CN, Hal, OH, NH₂, SO₃H, SH, CO₂H, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C₁-C₆ alkyl, and

m is 0 or 1,

p is 0, 1 or 2, and

q has a value of 1 to 7.

10 17. A compound according to any preceding claim, wherein R₁ and R₂ are independently of each other H, alkyl, -OR', -NHR', wherein R' represents H or C₁-C₆ alkyl.

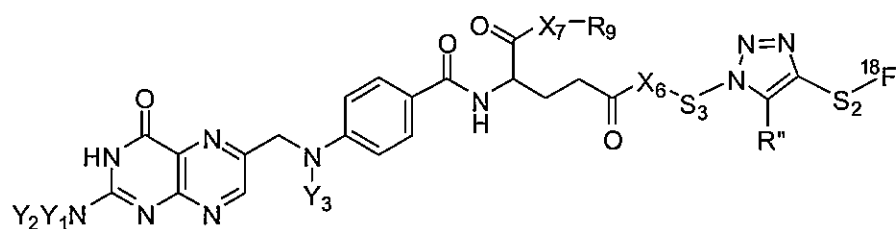
15 18. A compound according to any preceding claim, wherein R₃ is H, formyl, C₁-C₁₂ alkyl or C₁-C₁₂ alkanoyl.

19. A compound according to any preceding claim, wherein R₄ is H, nitroso, C₁-C₁₂ alkoxy, or C₁-C₁₂ alkanoyl.

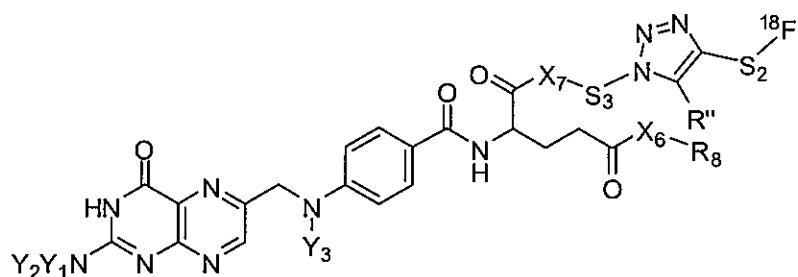
20 20. A compound according to any preceding claim, wherein R₅ is H, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, (C₁-C₁₂ alkoxy)carbonyl, or (C₁-C₁₂ alkylamino)carbonyl.

25 21. A compound according to any preceding claim, wherein R₆ is H, CN, Hal, NO₂, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, or (C₁-C₁₂ alkoxy)carbonyl.

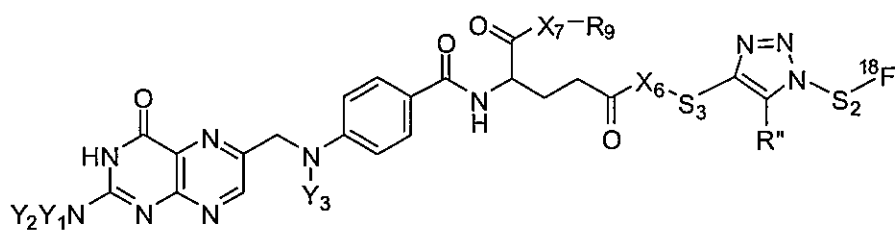
22. A compound according to any preceding claim having formulae IX, IXa, X or Xa,



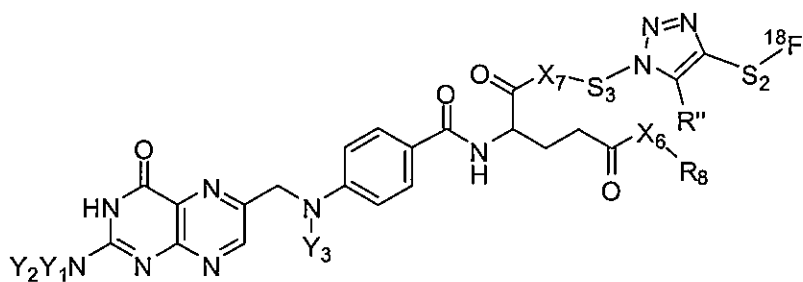
IX



IXa



X



Xa

wherein,

X_6, X_7 are independently of each other C, N or O,

Y_1, Y_2 are independently of each other selected from H, formyl,
 5 straight chain or branched C_1-C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$,

10 Y_3 is selected from H, formyl, nitroso, straight chain or branched C_1-C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$,
 15 $-CH=CH-$, $-C\equiv C-$,

R_8, R_9 are independently of each other H or straight chain or branched C_1-C_{12} alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2 , and wherein one or more of embedded, non-adjacent CH_2 groups may
 20 independently be replaced by $-O-$, $-CO-$, $-CO-O-$, $-CO-NR'-$, $-CH=CH-$, $-C\equiv C-$,

R'' is H, Hal, $NH-COR'$, $NH-SO_2R'$, CO_2R' , COR' , wherein R' represents H or C_1-C_6 alkyl, or straight-chain or branched C_1-C_{12} alkyl, which is unsubstituted or
 25 substituted by at least one CN, Hal, OH, NH_2 , SO_3H , SH, CO_2H , or NO_2 , or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2 , COR' , or $COOR'$, wherein R' represents H or C_1-C_6 alkyl, or a combination thereof and

S₂, S₃ are independently of each other a single bond or a spacer unit, such as straight-chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, and wherein one or more of the non-adjacent CH₂ groups may independently be replaced by -O-, -CO-, -CO-O-, -O-CO-, -NR'-, -NR'-CO-, -CO-NR'-, -NR'-CO-O-, -O-CO-NR'-, -NR'-CO-NR'-, -CH=CH-, -C≡C-, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C1-C6 alkyl, or a combination thereof.

23. A compound according to claim 22, wherein S₂ and S₃ are independently of each other straight-chain or branched C1-C8 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO₂, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO₂, COR', or COOR', wherein R' represents H or C1-C6 alkyl.

24. A method for preparing a compound according to claim 1 comprising the steps of (a) reacting an azido-derivatized folic acid with an ¹⁸F-labelled alkyne or alkyne substitute in a 1,3-cycloaddition and (b) isolating the compound.

25. Method according to claim 24, wherein the ¹⁸F-labelled alkyne or alkyne substitute is prepared in a displacement reaction by reacting an alkyne or alkyne substitute provided with a leaving group with [¹⁸F]fluoride to give

the ^{18}F -labelled alkyne or alkyne substitute.

26. A method for preparing a compound according to claim 1 comprising the steps of (a) reacting a folic acid derivatized with an alkyne or alkyne substitute with an ^{18}F -labelled azide in a 1,3-cycloaddition and (b) isolating the compound.
27. Method according to claim 26, wherein the ^{18}F -labelled azide is prepared in a displacement reaction by reacting an azide provided with a leaving group with ^{18}F fluoride to give the ^{18}F -labelled azide.
28. Method according to claims 24 to 27, wherein the cycloaddition is performed either under thermal conditions or in the presence of a catalyst.
29. Method according to claim 24, comprising the steps of (a) reacting a (C2-C12)alk-1-yne or substitute thereof, provided with a suitable leaving group, with ^{18}F fluoride, and (b) coupling the obtained product with an azido-folic acid or derivative in a Cu(I)-catalyzed 1,3-dipolar cycloaddition.
30. Method according to claim 26, comprising the steps of (a) reacting a (C1-C12)azide, provided with a suitable leaving group, with ^{18}F fluoride, and (b) coupling the obtained product with an folic acid derivatized with an alkynyl or substitute thereof in a Cu(I)-catalyzed 1,3-dipolar cycloaddition.

31. Compound according to claims 1 to 23 for use in diagnostic imaging of a cell or population of cells expressing a folate-receptor in vitro or in vivo.

5

32. Compound according to claims 1 to 23 for use in convenient and effective administration to a subject in need for diagnostic imaging.

10 33. Compound according to claims 1 to 23 for use in a method for diagnostic imaging of a cell or population of cells expressing a folate-receptor, said method comprising the steps of administering at least one compound according to claims 1 to 23 in a diagnostic imaging amount, and
15 obtaining a diagnostic image of said cell or population of cells.

34. Compound according to claim 33, wherein the diagnostic imaging is performed of a cell or population of cells
20 expressing a folate-receptor in vitro or in vivo.

35. Method for in vitro detection of a cell expressing the folate receptor in a tissue sample which includes contacting said tissue sample with a compound according to claims 1 to 23 in effective amounts and for sufficient
25 time and conditions to allow binding to occur and detecting such binding by PET imaging.

36. Compound according to claims 1 to 23 for use in a method
30 of diagnostic imaging or monitoring a subject comprising

the steps of (i) administering at least one compound according to claims 1 to 23 in a diagnostic imaging amount, and (ii) performing diagnostic imaging using PET by detecting a signal from said at least one compound.

5

37. Compound according to claims 1 to 23 for use in a method of monitoring therapy of cancer and inflammatory and autoimmune diseases in a subject comprising the steps of (i) administering to a subject in need thereof at least one compound according to claims 1 to 23 in a diagnostic imaging amount in combination with a therapeutically active, and (ii) performing diagnostic imaging using PET by detecting a signal from said at least one compound to follow the course of therapy of cancer and inflammatory and autoimmune diseases.

10

15

38. Compound of claims 36 or 37 used in combination with any other methods of diagnosis or therapy of cancer and inflammatory and autoimmune diseases.