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(54) A METHOD FOR THE PREPARATION OF 18F-LABELLED FOLATES

VERFAHREN ZUR HERSTELLUNG VON 18F-MARKIERTEN FOLATEN

PROCÉDÉ POUR LA PREPARATION DE FOLATES MARQUÉS AU 18F

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- **BETRIO A ET AL: "Synthesis and Preclinical Evaluation of a Folic Acid Derivative Labeled with 18F for PET Imaging of Folate Receptor-Positive Tumors" JOURNAL OF NUCLEAR MEDICINE,, vol. 47, 1 January 2005 (2005-01-01), pages 1153-1160, XP002488687 cited in the application**
- **MARIK ET AL: "Click for PET: rapid preparation of [<18>F]fluoropeptides using Cu<I> catalyzed 1,3-dipolar cycloaddition" TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, vol. 47, no. 37, 11 September 2006 (2006-09-11), pages 6681-6684, XP005707475 ISSN: 0040-4039**

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Description

Field of Invention

[0001] The present invention is directed towards a new method of synthesis of ^{18}F-labeled pteroate or folate radiopharmaceuticals, wherein fluorine-18 is attached to a pteroate (or folate) or derivative thereof, through direct radiolabeling with ^{18}[F]fluoride.

Background

[0002] Cell-specific targeting for delivery of effector moieties such 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 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 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 inflammatory and autoimmune diseases, such as receptors, anti-gens, haptens and the like which can be specifically targeted by the respective biological vehicles.

[0003] The folate receptor (FR) has been identified as one of these structures. In normal tissues and organs FR-expression is highly restricted to only a few organs (e.g. kidney, lungs, choroids plexus, and placenta). Yet, 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), and 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).

[0004] Various folic acid derivatives and conjugates are known and have been (pre)clinically evaluated. In particular, folate radiopharmaceuticals have increasingly gained importance in the field of nuclear medicine and can be very useful for an improved diagnosis and evaluation of the effectiveness of therapy of cancer and inflammatory and autoimmune diseases, such as assessment and/or prediction of a treatment response and consequently improvement of radiation dosimetry. A typical visualization technique which is suitable for radioimaging is PET. PET uses isotopes with short half lives, which are either covalently linked to its carrier or via a chelating moiety. Suitable isotopes include for example ^{11}C (ca. 20 min), ^{13}N (ca. 10 min), ^{15}O (ca. 2 min), and ^{18}F (ca. 110 min) as covalently bound nuclides and for example ^{68}Ga (ca. 68 min) which is usually linked by a chelating system.

[0005] Clearly 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 be 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.

[0006] Yet, the structure of folic acid does not lend itself to direct radiolabeling with ^{18}F. Thus, to date, mainly 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 ^{99m}Tc for SPECT (Siegel et al., J. Nucl. Med. 2003, 44:700; Müller et al., J. Organomet. Chem. 2004, 689:4712) or with ^{68}Ga for PET (Mathias et al., Nucl. Med. Biol. 2003, 30(7):725). In contrast, only very few folic acid derivatives have been reported in the literature which have been labelled with ^{18}F (Bettio et al., J. Nucl. Med., 2006, 47(7), 1153; WO 2006/071754). Typically, an intermediate of choice was radiofluorinated to obtain an ^{18}F-labelling intermediate, which is subsequently activated and purified in order to be subjected to coupling to a functional group within folic acid, such as the carboxylic acid group within the glutamate part of folic acid. Clearly, such a multi-step radiosynthesis is time-consuming and in fact gave typically only low radiochemical yields of less than 5% (Bettio et al., J. Nucl. Med., 2006, 47(7), 1153).

[0007] Thus, there is still a great need for an efficient and versatile approach for preparing directly radiolabeled ^{18}F-folates or derivatives thereof, which addresses one or more of the above discussed drawbacks.

[0008] Applicants have now found an efficient and versatile method of synthesis of new ^{18}F-labeled folate radiopharmaceuticals overcoming the drawbacks of conventional labelling methods, wherein fluorine-18 is attached to a folic acid or derivative thereof through direct radiolabeling with ^{18}[F]fluoride.

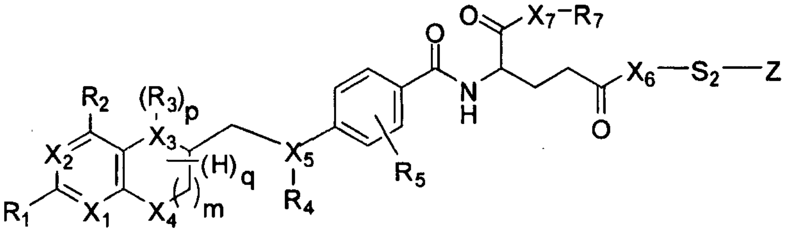
[0009] Thus the present method is a time-saving and convenient direct ^{18}F-labelling method, wherein no prosthetic groups are necessary and suitable precursors which carry only amide bounded activated groups as moieties for direct ^{18}F-labelling are easy accessible.

[0010] In addition the present method allows regioselective preparation and labelling of the α- or γ- isomer with no need for separation, which is known to be difficult and time-consuming.

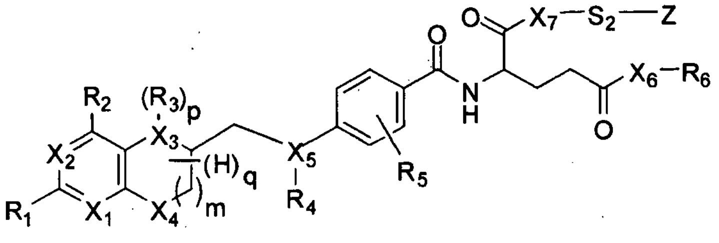
Summary of the Invention

[0011] Thus, the present invention is directed to a new method of synthesis of ^{18}F-labeled pteroate or folate radiopharmaceuticals (hereinafter also called method of the invention), wherein fluorine-18 is attached to a folate or derivative thereof through direct radiolabeling with ^{18}[F]fluoride.

[0012] More specifically the present invention is directed towards a method of direct radiolabeling of the glutamate moiety of a folate compound with ^{18}F, comprising the step of reacting a compound of formula VII or VIIa with ^{18}F,



VII



VIIa

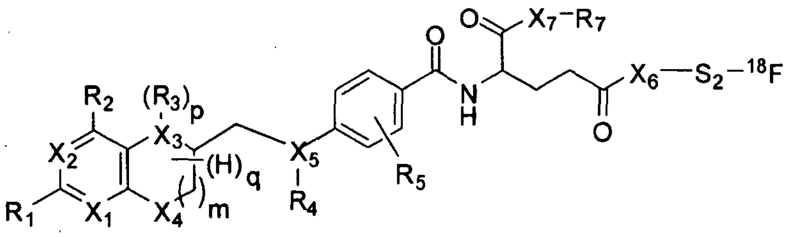
wherein

X_1 to X_5	are N,
X_6, X_7	are independently of each other O or N,
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, or (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_5	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, (C1-C12 alkylamino)carbonyl,
S_2	is a 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-, or a five-or six-membered aromatic ring having 0, 1 or 2 heteroa-toms, which is unsubstituted or substituted with CN, Hal, NO_2, COR', or COOR', wherein R' represents H or C1-C6 al-kyl, or a combination thereof,

m	1,
p	is 0, 1 or 2,
q	has a value of 1 to 7,
Z	is a leaving group, and wherein 18[F]fluoride is acti-vated by phase transfer catalysts such as tetrabutylammonium carbonate or aminopolyethers (e.g. Kryptofix© 2.2.2) in combination with potassium carbonate or oxalate,

to obtain a ^{18}F-labeled compound of general formula VI or VIa



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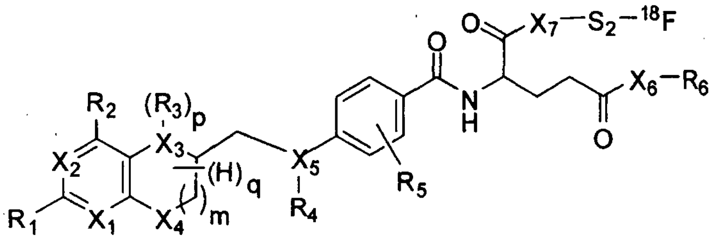
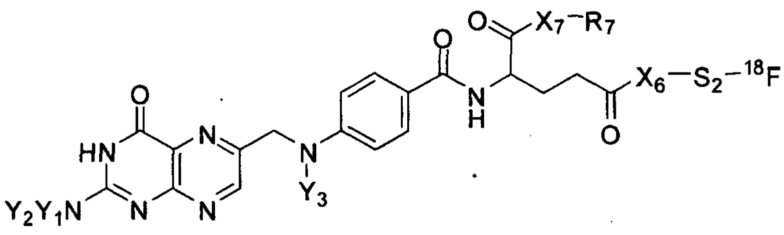


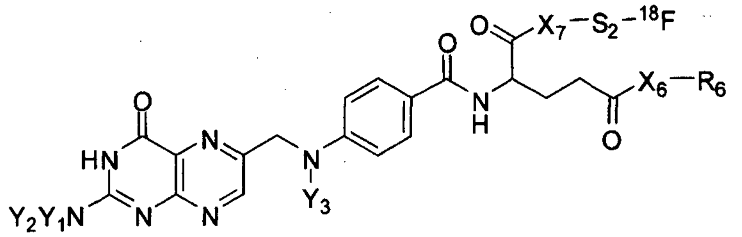
VIa.

[0013] Preferably, Z is a leaving group such as Hal, NO_2, diazonium salts, sulfonate esters, including mesylate CH_3SO_2O-, tosylate $CH_3C_6H_4SO_2O$-, pentafluorobenzoate, triflateCF_3SO_2O-, iodonium salts -I+R'''$_2$, dialkyl/-aryl silanes -SiOHR'''$_2$, and silanols - SiHR'''$_2$, wherein R''' is independently a straight-chain or branched $C_{(1-24)}$ alkyl group or an optionally substituted carbocyclic and heterocyclic group comprising five-, six- or ten-membered ring systems and the like.

[0014] In a further specific embodiment the present invention contemplates a method of direct radiolabeling wherein the obtained compound has the formula VIII or VIIIa,



VIII



VIIIa

wherein

X_6, X_7	are independently of each other N or O,
Y_1, Y_2	are independently of each other selected from H, straight chain or branched C_1-C_4 alkyl,
Y_3	is selected from H, formyl, nitroso, straight chain or branched C1-C12 alkyl,
R_6, R_7	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
S2	is straight-chain or branched C1-C12 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or N02, and wherein one or more of the non-adjacent CH2 groups may independently be replaced by -O-, or a five-or six-membered aromatic ring having 0, 1 or 2 heteroatoms, which is unsubstituted or substituted with CN, Hal, N02, COR', or COOR', wherein R' represents H or C1-C6 alkyl, or a combination thereof.

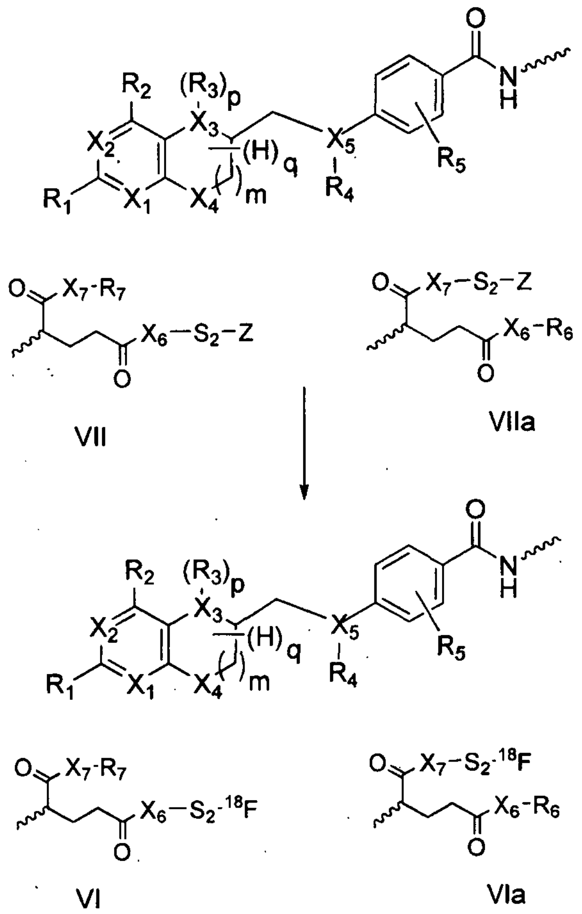
[0015] Other features and advantages of the invention will be apparent from the following detailed description thereof and from the claims.

Detailed Description of the Invention

[0016] The present invention is directed to a new method of synthesis of ^{18}F-labeled pteroate or folate radiopharmaceuticals (hereinafter also called method of the invention), wherein fluorine-18 is attached through direct radiolabeling with ^{18}[F]fluoride (hereinafter also abbreviated by "^{18}F").

[0017] The term "folate" as used herein, includes compounds based on a folate skeleton (i.e. based on pteroyl-glutamic acid or N-[4(pteridin-6-ylmethylamino)benzoyl]-glutamic acid), and derivatives thereof. These include optionally substituted folic acid, folinic acid, pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates.

[0018] More specifically the present invention is directed towards a method of synthesis depicted in Scheme 1, wherein a precursor VII or VIIa, having a leaving group Z is directly radiolabeled with ^{18}F to obtain a compound of general formula I having formula VI or VIa.



Scheme 1. Synthesis scheme

[0019] It was found that a folic acid moiety of general formulae VII or VIIa, respectively, may be ^{18}F-radiolabeled easily and efficiently in a direct manner and thus with no need for synthesis and purification of an intermediate ^{18}F-labelling agent.

[0020] Preferably, the direct radiolabeling of precursor VII or VIIa is performed with ^{18}F activated by phase transfer catalysts such as tetrabutylamonium carbonate or aminopolyethers (e.g. Kryptofix© 2.2.2) in combination with potassium carbonate or oxalate. In a specific embiodiment, ^{18}F is activated with Kryptofix, in a polar aprotic solvent selected from acetonitrile, acetone, 1 ,4-dioxane, tetrahydrofuran (THF), N-methylpyrrolidinone (NMP), di-methoxyethane (DME), dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethytsulfoxide (DMSO) and hexa-methylphosphoramide (HMPA) and mixtures thereof.

[0021] The leaving group Z may be any common leaving group known in the art and includes for example Hal, NO_2, diazonium salts, sulfonate esters, including mesylate CH_3SO_2O-, tosylate $CH_3C_6H_4SO_2O$-, pentafluorobenzoate, triflateCF_3SO_2O-, iodonium salts $-I^+R'''_2$, dialkyl/-aryl silanes $-SiOHR'''_2$, and silanols -SiHR"+'2, wherein R''' is independently a straight-chain or branched $C_{(1-24)}$ alkyl group or an optionally substituted carbocyclic and heterocyclic group comprising five-, six- or ten-membered ring systems and the like.

[0022] Groups X_1 to X_5, R_1 to R_5, p and q are defining the nature of the folate group in more detailed manner. A person skilled in the art would though know the range of these groups within the folic acid skeleton.

[0023] In a preferred embodiment, R_1 and R_2 are independently of each other represent H, alkyl, -OR', -NHR', more preferably -OR', - NHR.

[0024] In a preferred embodiment, R_3 is H, C1-C12 alkyl or C1-C12 alkanoyl.

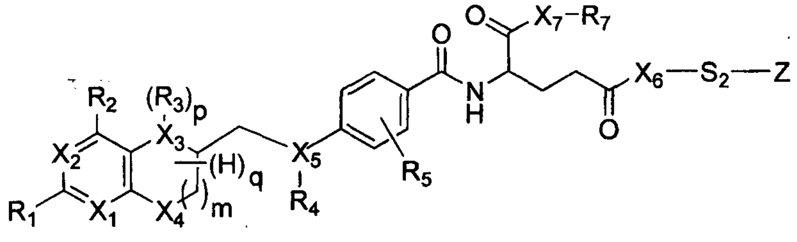
[0025] In another preferred embodiment, R_4 is H, nitroso, C1-C12 alkoxy, or C1-C12 alkanoyl.

[0026] It is understood, that (H)q represents all H substituents on the indicated ring (i.e. on X3, C6, C7 and X4), thus q may have a value of 1 to 7. For example q = 7 stands for a fully saturated 5,8-dideaza analog (X3 = X4 = C) and q = 1 for a fully unsaturated analog with X3 = X4 = N.

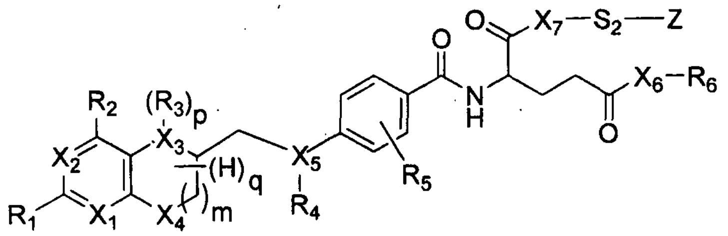
[0027] It is also understood, that p depends on the nature of X and the aromaticity of the ring and thus may be 0, 1 or 2.

[0028] It is further understood, that the abbreviation "N" is representative for all possible degrees of saturation and includes -NH-and -N= linkages.

[0029] In a specific embodiment, the present invention is directed towards a method of direct radiolabeling in accordance with scheme 1, of the glutamate moiety of a folic acid or derivative thereof with ^{18}F, comprising the step of reacting a compound of formulae VII or VIIa with ^{18}F,



VII



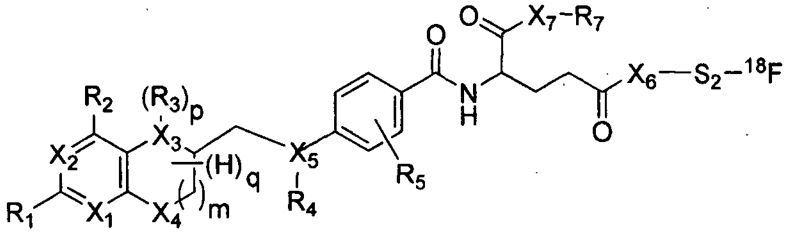
VIIa

wherein

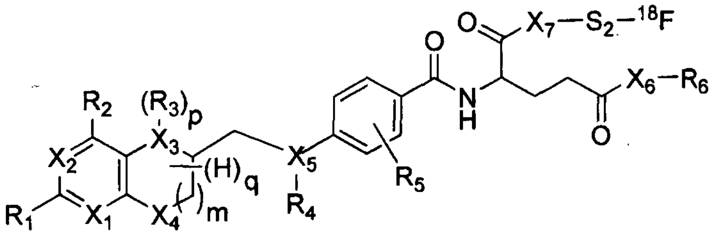
X_1 to X_5	are N,
X_6, X_7	are independently of each other O or N,
R_1, R_2	are independently of each other H, Hal, -OR', -NHR', C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, or (C1-C12 alkyl-amino)carbonyl, wherein R' is H or C1-C6 alkyl,
R_3, 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_5	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, and (C1-C12 alkylamino)carbonyl,
R_6, R_7	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,
S_2	is 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-, or a five-or six-membered aromatic ring having 0, 1 or 2 heteroatoms, 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,
m	is 1,
p	is 0, 1 or 2,
q	has a value of 1 to 7,

Z is a leaving group, and

[0030] wherein ^{18}F is activated by phase transfer catalysts such as tetrabutylamonium carbonate or aminopolyethers (e.g. Kryptofix© 2.2.2) in combination with potassium carbonate or oxalate, to obtain a ^{18}F-labeled compound of general formulae VI or VIa,



VI

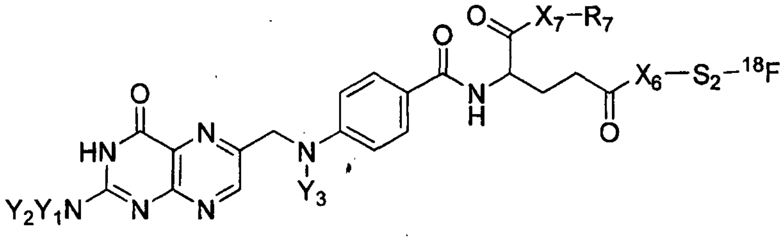


VIa

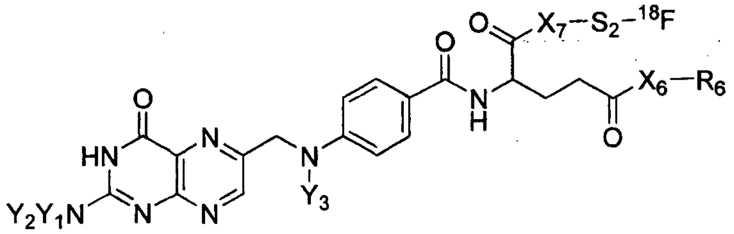
[0031] S_2 is preferably straight chain or branched C1-C8 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2, or a five- or six-membered aromatic ring, which is unsubstituted or substituted with CN, Hal, NO_2, COR', or COOR', wherein R' represents H or C1-C6 alkyl, or a combination thereof, more preferably straight-chain or branched C1-C6 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2.

[0032] A further specific embodiment of the compounds of the invention includes for example compounds wherein X_1 to X_5 are N, R_1 is NY_1Y_2, R_2 is O, R_4 is Y_3, p is 0 and q is 1.

[0033] Thus, in a further specific embodiment the obtained compound is a compound of formulae VIII or VIIIa,



VIII



VIIIa

X_6, X_7	are independently of each other N or O,
Y_1, Y_2	are independently of each other selected from H, straight chain or branched C_1-C_4 alkyl,
Y_3	is selected from H, formyl, nitroso, straight chain or branched C1-C12 alkyl,
R_6, R_7	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
S_2	is 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-, or a five-or six-membered aromatic ring having 0, 1 or 2 heteroatoms, 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.

[0034] The term "alkyl", when used singly or in combination, refers 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 and the like. The preferred alkyl groups contain 1 to 8, more preferably 1 to 4 carbon atoms.

[0035] 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 groups contain 2 to 6 carbon atoms.

[0036] 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 preferably 2 to 6 carbon atoms.

[0037] 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.

[0038] 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. 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.

[0039] The term "halo" as used herein refers to any Group 7 element and includes fluoro, chloro, bromo, iodo, and astatine(o).

[0040] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light 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.

Examples

Materials and Methods

[0041] Infrared spectra were recorded on a Jasco FT/IR-6200 ATR-IR. Nuclear magnetic resonance spectra were recorded with a Bruker 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 section for the description of ^{1}H-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 micro™ API LC-ESI.

[0042] 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 with an L-7400 tunable absorption detector. Analytical HPLC was performed with an XBridge® column (C18, 5μm, 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→5 % A; 15-20 min, 5 % A; 20→22 min, 5→95 % A; 22→25 min, 95 % A. Semi-prep HPLC was performed with XBridge® semiprep column (C18, 5μm, 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.

Example 1: Synthesis of γ-2-fluoroethyl-folic acid

(a) Synthesis of γ-Glu(fluoroethyl) methyl ester

[0043] In a flame dried flask was added BOC-Glu-OMe (556mg, 2.13mmol), dry DMF (10ml) and Et3N (0.9ml, 1.9 e.q.). The reaction mixture was cooled to 0 °C, and HBTU (808mg, 1 e.q.) was added and the reaction mixture was stirred for' 10mins. A solution of 2-fluoroethylamine (212ml, 1 e.q.) in dry DMF. (10ml) and Et3N (0.9ml, 1.9 e.q.) was added dropwise to the reaction mixture at 0 °C. The reaction was stirred for 2h, allowed to warm to r.t. and stirred overnight. Water (10ml) was added to the reaction mixture, and it was extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated in vacuo to give a pale yellow oil. ^1H-NMR (300 MHz, DMSO-d_6) [ppm]: 7.78 (bs, NH); 6.60 (bs, NH) ; 4.77 (bs, OH) ; 4.62 (t, CH) ; 3.47 (m, CH_3) ; 3. 37 (t, CH_2) ; 3.11 (t, CH_2) ; 2.89 (t, CH_2) ; 2.73 (t, CH_2); 1.39 (s, CH_3)

(b) Synthesis of γ-2-fluoroethyl-folic acid

[0044] γ-Glu(fluoroethyl) methyl ester (450mg, 1.48mmol) was dissolved in excess TFA/CH_2Cl_2 (1:1) until complete BOC deprotection occurred as monitored by TLC/HPLC. Excess TFA/CH_2Cl_2 was removed under vacuo to give the TFA salt of Glu(hydroxyethyl) methyl ester as pale yellow oil, which was directly used in the coupling reaction with N2-N,N-dimethylaminomethylene-10-formyl-pteroic acid according to the procedures outlined in EP A 07 105 987 and EP A 07 105 984.

Example 2: Synthesis of γ-(2-(p-toluenesulfonyl)ethyl) folic acid amide

(a) Synthesis of BOC-Glu(hydroxyethyl) methyl ester

[0045] In a flame dried flask was added BOC-Glu-OMe (556mg, 2.13mmol), dry DMF (10ml) and Et3N (0.9ml, 1.9 e.q.). The reaction mixture was cooled to 0 °C, and HBTU (808mg, 1 e.q.) was added and the reaction mixture was stirred for 10mins. A solution of amino alcohol (0.13ml, 1 e.q.) in dry DMF (10ml) and Et3N (0.9ml, 1.9 e.q.) was added dropwise to the reaction mixture at 0 °C. The reaction was stirred for 2h, allowed to warm to r.t. and stirred overnight. Water (10ml) was added to the reaction mixture, and it was extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated in vacuo to give a pale yellow oil. ^1H-NMR (300 MHz, DMSO-d_6) [ppm] : 9.40 (bs, NH); 7.72 (bs, NH); 4.52 (t, CH); 3.54 (m, CH_3); 3.44 (t, CH_2) ; 3.14 (t, CH_2); 2.56 (t, CH_2); 2.20 (t, CH_2) ; 1.24 (s, CH_3)

[0046] (b) Synthesis of γ-(2-(p-toluenesulfonyl)ethyl) folic acid amide BOC-Glu(hydroxyethyl) methyl ester (450mg, 1.48mmol) was dissolved in excess TFA/CH_2Cl_2 (1:1) until complete BOC deprotection occurred as monitored by TLC/HPLC. Excess TFA/CH_2Cl_2 was removed under vacuo to give the TFA salt of Glu(hydroxyethyl) methyl ester as pale yellow oil, which was directly used in the coupling reaction with N2-N,N-dimethylaminomethylene-10-formyl-pteroic acid according to the procedures outlined in EP A 07 105 987 and EP A 07 105 984. γ-(2-hydroxyethyl) folic acid amide (26 μmol) was dissolved in dichloromethane (10 ml) and cooled to 0 °C. Et_3N (10 μl, 1.5 eq.) and TsCl (7 mg, 1. 4 eq.) were added and the reaction mixture was stirred for 2h at 0 °C. Then the mixture was allowed to warm to room temperature and stirred overnight.

The reaction mixture was poured into water (15 ml), layers were separated. The aqueous phase was extracted with dichloromethane and combined organic phases were dried over $MgSO_4$. The solvents were removed under vacuo. The product was purified by column chromatography using silica gel and an eluent system of pentane and ethyl acetate to give a yellow oil.

[0047] This compound serves then as precursor for a direct aliphatic nucleophilic ^{18}F-labelling according to literature procedures (Coenen, H.H. PET Chemistry - The Driving Force in Molecular Imaging, Schubiger, P.A.; Lehmann, L.;

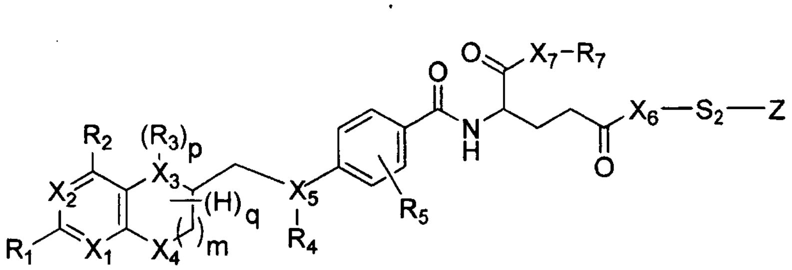
Friebe, M., Eds.; Springer: Berlin, 2007, pp. 15-50).

Summary

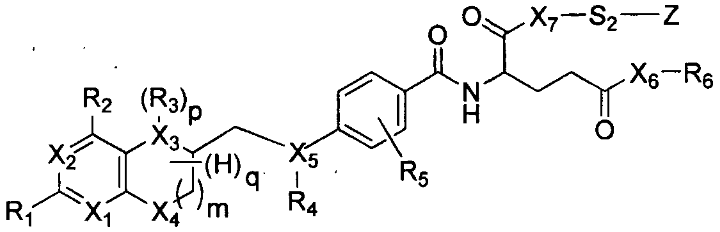
[0048] The present invention is directed towards a new method of synthesis of ^{18}F-folate radiopharmaceuticals, wherein fluorine-18 is attached to a folate or derivative thereof, through direct radiolabeling with ^{18}[F]fluoride.

Claims

1. A method of direct radiolabeling of the glutamate moiety of a folate compound with ^{18}F comprising the step of reacting a compound of formulae VII or VIIa with ^{18}F,



VII



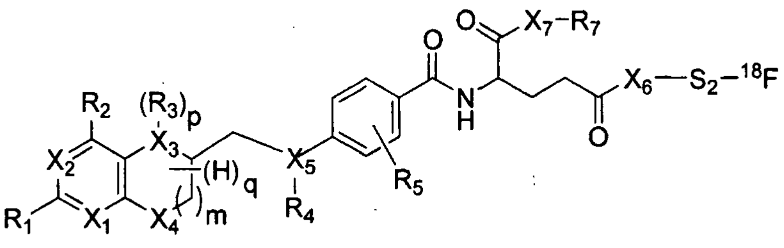
VIIa

wherein

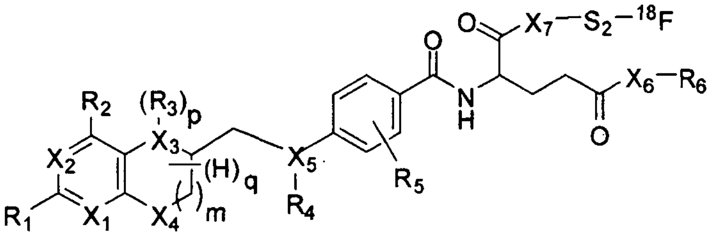
X_1 to X_5 are N,
X_6, X_7 are independently of each other O or N,
R_1, R_2 are independently of each other H, Hal, -OR', -NHR', C1-C12 alkyl, C1-C12 alkoxy, C1-C12 alkanoyl, C2-C12 alkenyl, C2-C12 alkynyl, (C1-C12 alkoxy)carbonyl, or (C1-C12 alkylamino)carbonyl, wherein R' is H or C1-C6 alkyl,
R_3, 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_5 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, and (C1-C12 alkylamino)carbonyl,
R_6, R_7 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,
S_2 is straight-chain or branched C1-C12 alkyl, which is un-substituted 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-; or a five-or six-membered aromatic ring having 0, 1 or 2 heteroatoms, 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,
m is 1,
p is 0, 1 or 2,
q has a value of 1 to 7,
Z is a leaving group, and

wherein ^{18}F is activated by phase transfer catalysts such as tetrabutylamonium carbonate or aminopolyethers (e.g. Kryptofix© 2.2.2) in combination with potassium carbonate or oxalate, to obtain a ^{18}F-labeled compound of general formulae VI or VIa,



VI



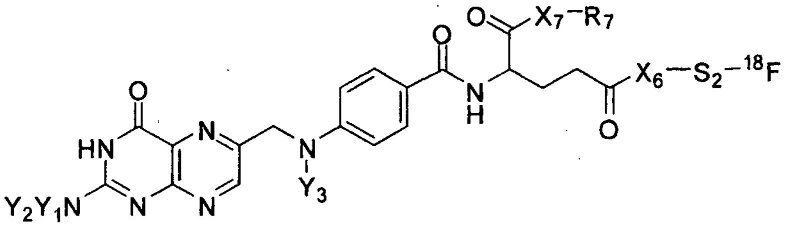
VIa.

2. A method according to claim 1, wherein ^{18}F is activated with Kryptofix© 2.2.2 in a polar solvent selected from acetonitrile, acetone, 1,4-dioxane, tetrahydrofuran (THF), N-methylpyrrolidinone (NMP), di-methoxyethane (DME), dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethytsulfoxide (DMS), hexa-methylphosphoramide (HMPA) and mixtures thereof.

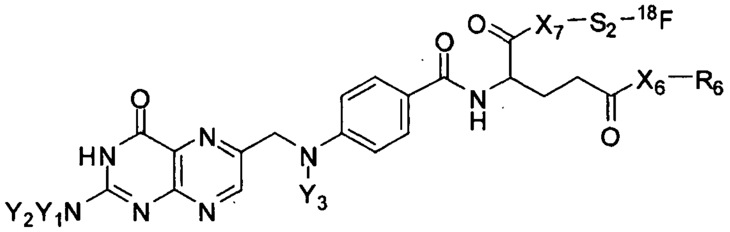
3. A method according to claims 1 or 2, wherein Z is selected from Hal, NO_2, diazonium salts, sulfonate esters, including mesylate, tosylate, pentafluorobenzoate, triflate, iodonium salts, dialkyl/-aryl silanes, and silanols.

4. A method according to any preceding claim, wherein S_2 is straight chain or branched C1-C6 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2

5. A method according to any preceding claim, wherein the obtained compound has the formulae VIII or VIIIa,



VIII



VIIIa

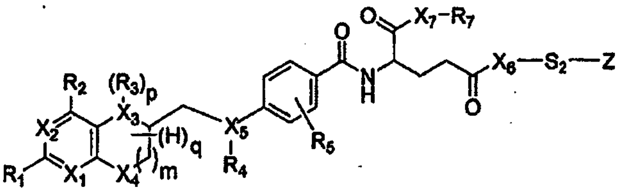
wherein,

X_6, X_7 are independently of each other N or O,
Y_1, Y_2 are independently of each other selected from H, straight chain or branched C_1-C_4 alkyl,
Y_3 is selected from H, formyl, nitroso, straight chain or branched C1-C12 alkyl,
R_6, R_7 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
S_2 is 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-; or a five-or six-membered aromatic ring having 0, 1 or 2 heteroatoms, 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.

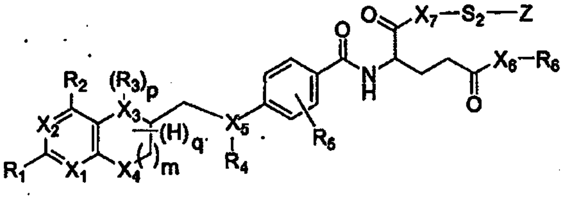
6. A method according to claim 5, wherein S_2 is straight-chain or branched C1-C6 alkyl, which is unsubstituted or substituted by at least one CN, Hal, or NO_2.

Patentansprüche

1. Verfahren zur direkten radioaktiven Markierung des Glutamat-Anteils einer Folatverbindung mit ^{18}F, der den Schritt der Reaktion einer Verbindung der Formel VII oder VIIa mit ^{18}F umfasst,



VII

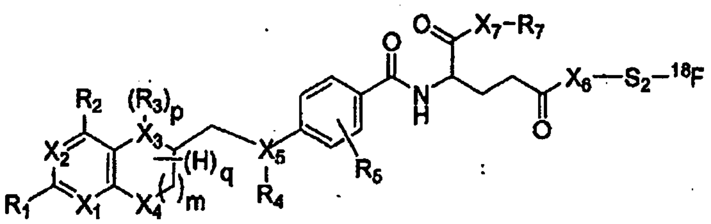


VIIa

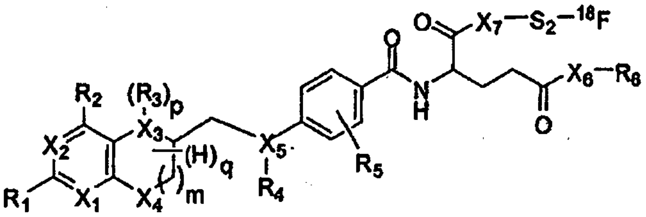
wobei

X_1 bis X_5 N sind,
X_6, X_7 unabhängig voneinander O oder N sind,
R_1, R_2 unabhängig voneinander H, Hal, -OR', NHR', C1-C12-Akyl, C1-C12-Alkoxy, C1-C12-Alkanoyl, C2-C12-Alkenyl, C2-C12 Alkynyl (C1-C12-Alkoxy)-Carbonyl oder (C1-C12-Alkylamino)-Carbonyl sind, wobei R' H oder C1-C6-Akyl ist,
R_3, R_4 sind unabhängig voneinander H, Formyl, Iminomethyl, Nitroso, C1-C12-Alkyl, C1-C12-Alkoxy, C1-C12-Alkanoyl, Halo-substituiertes C1-C12-Alkanoyl sind,
R_5 H, CN, Hal, NO_2, C1-C12-Alkyl, C1-C12-Alkoxy, C1-C12-Alkanoyl, C2-C12-Alkenyl, C2-C12-Alkynyl, (C1-C12-Alkoxy)-Carbonyl und (C1-C12 Alkylamino)-Carbonyl ist,
R_6, R_7 unabhängig voneinander H oder geradkettiges oder verzweigtkettiges C1-C12-Alkyl sind, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist,
S_2 geradkettiges oder verzweigtkettiges C1-C12-Alkyl ist, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist, und wobei eine oder mehrere der nicht-benachbarten CH_2-Gruppen unabhängig durch -O- oder einen fünf- oder sechsgliedrigen aromatischen Ring mit 0, 1 oder 2 Heteroatomen ersetzt werden kann, der unsubstituiert oder durch CN, Hal, NO_2, COR' oder COOR' substituiert ist, wobei R' H oder C1-C6-Alkyl oder eine Kombination davon darstellt,
m 1 ist,
p 0, 1 oder 2 ist,
q einen Wert von 1 bis 7 hat,
z eine Abgangsgruppe ist, und

wobei ^{18}F durch Phasen-Transfer-Katalysatoren wie Tetrabutylammoniumkarbonat oder Aminopolyether (z. B. Kryptofix© 2.2.2) in Kombination mit Kaliumkarbonat oder Oxalat aktiviert ist,
um eine ^{18}F-markierte Verbindung der allgemeinen Formel VI oder VIa zu erhalten,



VI



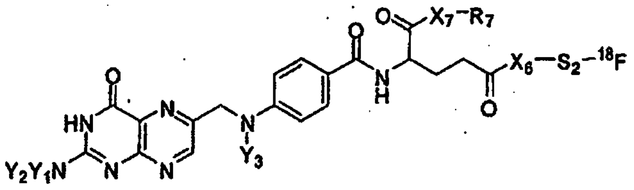
VIa.

2. Verfahren nach Anspruch 1, wobei ^{18}F durch Kryptofix© 2.2.2 in einem polaren Lösungsmittel ausgewählt aus Acetonitril, Aceton, 1,4-Dioxan, Tetrahydrofuran (THF), N-Methylpyrrolidinon (NMP), Di-Methoxyethan (DME), N, N-Dimethylformamid (DMF), Dimethylsulfoxid (DMS), Hexa-Methylphosphoramid (HMPA) und Mischungen davon aktiviert ist.

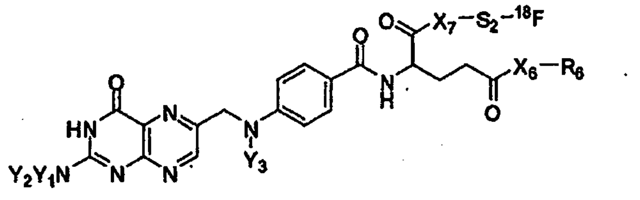
3. Verfahren nach Anspruch 1 oder 2, wobei Z aus Hal, No_2, Diazoniumsalzen, Sulfonsäure-Estern einschließlich Mesylat, Tosylat, Pentafluorobenzoat, Triflat, Iodonium-Salze, Dialkyl/Diaryl-Silane und Silanol ausgewählt ist.

4. Verfahren nach einem der vorhergehenden Ansprüche, wobei S_2 ein geradkettiges oder verzweigtkettiges C1-C6-Alkyl ist, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist,

5. Verfahren nach einem der vorhergehenden Ansprüche, wobei die erhaltene Verbindung die Formel, VIII oder VIIIa hat,



VIII



VIIIa

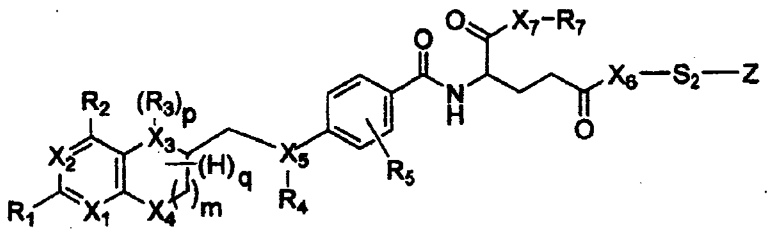
wobei

X_6, X_7 unabhängig voneinander N oder O sind,
Y_1, Y_2 unabhängig voneinander aus H, geradkettigem oder verzweigtkettigem C1-C4-Alkyl gewählt sind,
Y_3 aus H, Formyl, Nitroso, geradkettigem oder verzweigtkettigem C1-C12-Alkyl ausgewählt ist,
R_6, R_7 unabhängig voneinander H oder geradkettiges oder verzweigtkettiges C1-C12-Alkyl sind, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist, und
S_2 geradkettiges oder verzweigtkettiges C1-C12-Alkyl ist, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist, und wobei eine oder mehrere der nicht-benachbarten CH_2-Gruppen unabhängig durch -O- oder einen fünf- oder sechsgliedrigen aromatischen Ring mit 0, 1 oder 2 Heteroatomen ersetzt werden kann, der unsubstituiert oder durch CN, Hal, NO_2, COR' oder COOR' substituiert ist, wobei R' H oder C1-C6-Alkyl oder eine Kombination davon darstellt.

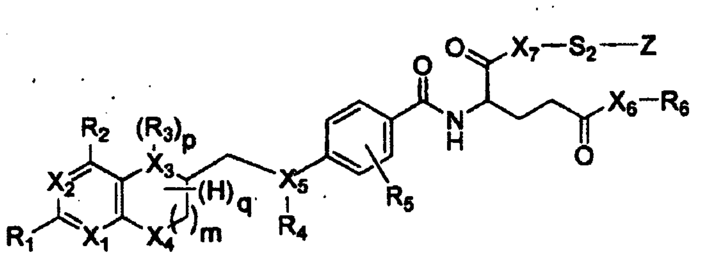
6. Verfahren nach Anspruch 5, wobei S_2 ein geradkettiges oder verzweigtkettiges C1-C6-Alkyl ist, das unsubstituiert oder mindestens von einem CN, Hal oder NO_2 substituiert ist.

Revendications

1. Procédé de radiomarquage direct du motif glutamate d'un composé folate avec du ^{18}F comprenant l'étape de réaction d'un composé de formule VII ou VIIa avec le ^{18}F,



VII

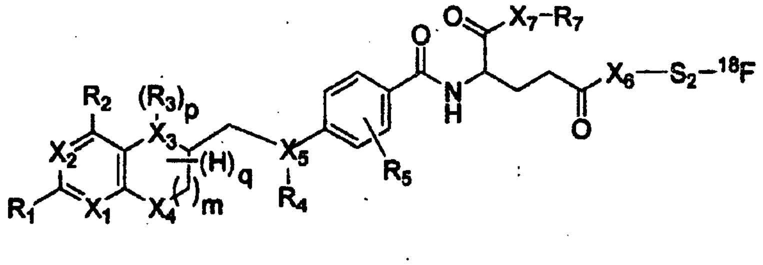


VIIa

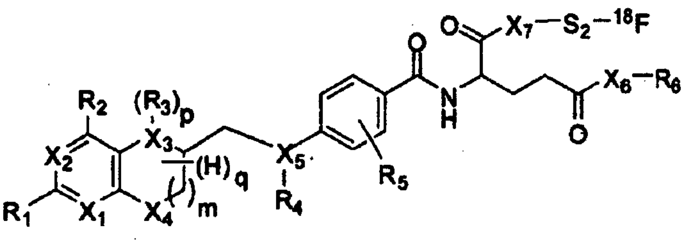
dans lesquelles,

X_1 à X_5 sont N,
X_6, X_7 sont, indépendamment l'un de l'autre, O ou N,
R_1, R_2 sont, indépendamment l'un de l'autre, H, Hal, -OR', -NHR', un groupe alkyle en C_1-C_{12}, un groupe alcoxy en C_1-C_{12}, un groupe alcanoyle en C_1-C_{12}, un groupe alcényle en C_2-C_{12}, un groupe alcynyle en C_2-C_{12}, un groupe (alcoxy en C_1-C_{12})carbonyle, ou un groupe (alkylamino en C_1-C_{12})carbonyle, R' étant H ou un groupe alkyle en C_1-C_6,
R_3, R_4 sont, indépendamment l'un de l'autre, H, un groupe formyle, iminométhyle, nitroso, alkyle en C_1-C_{12}, alcoxy en C_1-C_{12}, alcanoyle en C_1-C_{12}, alcanoyle en C_1-C_{12} halogéno-substitué,
R_5 est H, CN, Hal, NO_2, un groupe alkyle en C_1-C_{12}, alcoxy en C_1-C_{12}, alcanoyle en C_1-C_{12}, alcényle en C_2-C_{12}, alcynyle en C_2-C_{12}, (alcoxy en C_1-C_{12})carbonyle et (alkylamino en C_1-C_{12})carbonyle,
R_6, R_7 sont, indépendamment l'un de l'autre, H ou un groupe alkyle en C1-C12 linéaire ou ramifié, qui est non substitué ou substitué par au moins un CN, Hal ou NO_2,
S_2 est un groupe alkyle en C_1-C_{12} linéaire ou ramifié, qui est non substitué ou substitué par au moins un CN, Hal ou NO_2, et dans lequel un ou plusieurs des groupes CH_2 non adjacents peuvent être remplacés indépendamment par -O- ; ou un noyau aromatique à 5 ou 6 chaînons ayant 0, 1 ou 2 hétéroatomes, qui est non substitué ou substitué par CN, Hal, NO_2, COR' ou COOR', dans lequel R' représente H ou un groupe alkyle en C_1-C_6 ou une combinaison de ceux-ci,
m vaut 1,
p vaut 0, 1 ou 2,
q a une valeur de 1 à 7,
Z est un groupe labile, et

dans lequel ^{18}F est activé par catalyseurs de transfert de phase tels que le carbonate de tétrabutylammonium ou des aminopolyéthers (par exemple, Kryptofix© 2.2.2) combinés à du carbonate ou de l'oxalate de potassium, afin d'obtenir un composé marqué au ^{18}F de formule générale VI ou VIa,



VI



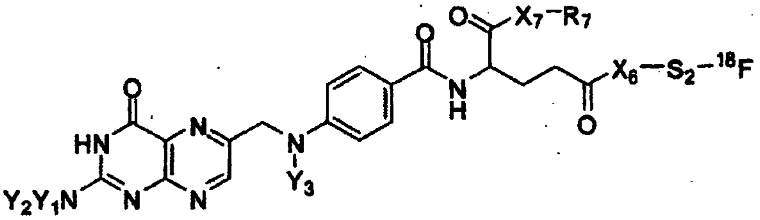
VIa.

2. Procédé selon la revendication 1, dans lequel le ^{18}F est activé avec du Kryptofix© 2.2.2 dans un solvant polaire choisi parmi l'acétonitrile, l'acétone, le 1,4-dioxane, le tétrahydrofurane (THF), la N-méthylpyrrolidinone (NMP), le diméthoxyéthane (DME), le diméthylacétamide (DMA), le N,N-diméthylformamide (DMF), le diméthylsulfoxyde (DMS), l'hexaméthylphosphoramide (HMPA) et des mélanges de ceux-ci.

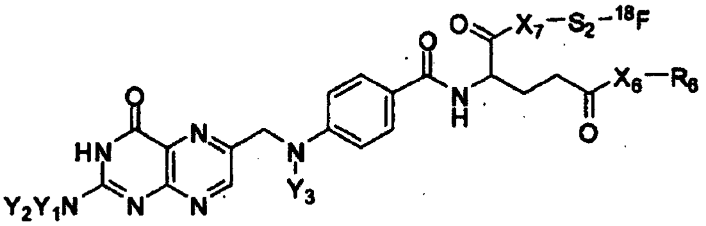
3. Procédé selon les revendications 1 ou 2, dans lequel Z est choisi parmi Hal, NO_2, les sels de diazonium, les esters sulfonates, comprenant le mésylate, le tosylate, le pentafluorobenzoate, le triflate, les sels d'iodinium, les dialkyl/-aryl silanes et les silanols.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel S_2 est un groupe alkyle en C1-C6 linéaire ou ramifié qui est non substitué ou substitué par au moins un CN, Hal ou NO_2.

5. Procédé selon l'une quelconque des revendications précédentes, dans lequel le composé obtenu est de formule VIII ou VIIIa,



VIII



VIIIa

dans lesquelles,

X_6, X_7 sont, indépendamment l'un de l'autre, N ou O,
Y_1, Y_2 sont choisis indépendamment l'un de l'autre parmi H, un groupe alkyle en C_1-C_4 linéaire ou ramifié,
Y_3 est choisi parmi H, les groupes formyle, nitroso, alkyle en C1-C12 linéaire ou ramifié,
R_6, R_7 sont, indépendamment l'un de l'autre, H ou un groupe alkyle en C1-C12 linéaire ou ramifié, qui est non substitué ou substitué par au moins un CN, Hal ou NO_2, et
S_2 est un groupe alkyle en C1-C12 linéaire ou ramifié, qui est non substitué ou substitué par au moins un CN, Hal ou NO_2, et dans lequel un ou plusieurs des groupes CH_2 non adjacents peuvent être remplacés indépendamment par -O- ; ou un noyau aromatique à 5 ou 6 chaînons ayant 0, 1 ou 2 hétéroatomes, qui est non substitué ou substitué par CN, Hal, NO_2, COR' ou COOR', R' représentant H ou un groupe alkyle en C_1-C_6 ou des combinaisons de ceux-ci.

6. Procédé selon la revendication 5, dans lequel S_2 est un groupe alkyle en C_1-C_6 linéaire ou ramifié, qui est non substitué ou substitué par au moins un CN, Hal ou NO_2.

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- WO 2006071754 A **[0006]**
- EP 07105987 A **[0044] [0046]**
- EP 07105984 A **[0044] [0046]**

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- **NAKASHIMA-MATSUSHITA et al.** *Arthritis & Rheumatism,* 1999, vol. 42 (8), 1609-16 **[0003]**
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专利名称(译)	一种制备18f标记的叶酸盐的方法		
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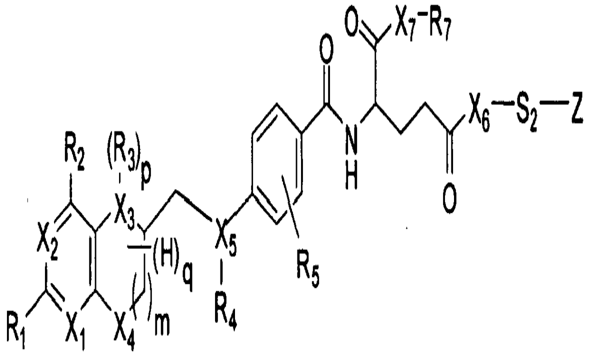
摘要(译)

本发明涉及一种合成18F-叶酸放射性药物的新方法，其中通过用18 [F]氟化物和18F-叶酸直接放射性标记将氟-18连接到翼状酸酯（或叶酸）或其衍生物上。通过这种合成方法获得的放射性药物及其在癌症治疗和炎症和自身免疫疾病治疗的诊断和监测中的用途。



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