

DIAGNOSTIC COMPOUNDS

The present invention relates to new peptide-based compounds and their use
5 for diagnostic imaging using positron emission tomography (PET). More
specifically the invention relates to the use of such peptide-based compounds
as targeting vectors that bind to receptors associated with angiogenesis, in
particular integrin receptors, for example, the $\alpha v \beta 3$ integrin receptor. Such
compounds may thus be used for diagnosis or therapy of, for example,
10 malignant diseases, heart diseases, endometriosis, inflammation-related
diseases, rheumatoid arthritis and Kaposi's sarcoma. It further relates to
methods and reagents for production of such peptide-based compounds.

The application of radiolabelled bioactive peptides for diagnostic imaging is
15 gaining importance in nuclear medicine. Biologically active molecules which
selectively interact with specific cell types are useful for the delivery of
radioactivity to target tissues. For example, radiolabelled peptides have
significant potential for the delivery of radionuclides to tumours, infarcts, and
infected tissues for diagnostic imaging and radiotherapy. ^{18}F , with its half-life
20 of approximately 110 minutes, is the positron-emitting nuclide of choice for
many receptor imaging studies. Therefore, ^{18}F -labelled bioactive peptides
have great clinical potential because of their utility in PET to quantitatively
detect and characterise a wide variety of diseases.

25 New blood vessels can be formed by two different mechanisms:
vasculogenesis or angiogenesis. Angiogenesis is the formation of new blood
vessels by branching from existing vessels. The primary stimulus for this
process may be inadequate supply of nutrients and oxygen (hypoxia) to cells
in a tissue. The cells may respond by secreting angiogenic factors, of which
30 there are many; one example, which is frequently referred to, is vascular
endothelial growth factor (VEGF). These factors initiate the secretion of
proteolytic enzymes that break down the proteins of the basement membrane,
as well as inhibitors that limit the action of these potentially harmful enzymes.

The other prominent effect of angiogenic factors is to cause endothelial cells to migrate and divide. Endothelial cells that are attached to the basement membrane, which forms a continuous sheet around blood vessels on the contraluminal side, do not undergo mitosis. The combined effect of loss of
5 attachment and signals from the receptors for angiogenic factors is to cause the endothelial cells to move, multiply, and rearrange themselves, and finally to synthesise a basement membrane around the new vessels.

Angiogenesis is prominent in the growth and remodelling of tissues, including
10 wound healing and inflammatory processes. Tumours must initiate angiogenesis when they reach millimetre size in order to keep up their rate of growth. Angiogenesis is accompanied by characteristic changes in endothelial cells and their environment. The surface of these cells is remodelled in preparation for migration, and cryptic structures are exposed where the
15 basement membrane is degraded, in addition to the variety of proteins which are involved in effecting and controlling proteolysis. In the case of tumours, the resulting network of blood vessels is usually disorganised, with the formation of sharp kinks and also arteriovenous shunts. Inhibition of angiogenesis is also considered to be a promising strategy for antitumour
20 therapy. The transformations accompanying angiogenesis are also very promising for diagnosis, one example being malignant disease, but the concept also shows great promise in inflammation and a variety of inflammation-related diseases, including atherosclerosis, the macrophages of early atherosclerotic lesions being potential sources of angiogenic factors.

25

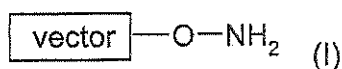
Many ligands involved in cell adhesion contain the tripeptide sequence arginine-glycine-aspartic acid (RGD). The RGD sequence appears to act as a primary recognition site between the ligands presenting this sequence and receptors on the surface of cells. It is generally believed that secondary
30 interactions between the ligand and receptor enhance the specificity of the interaction. These secondary interactions might take place between moieties

of the ligand and receptor that are immediately adjacent to the RGD sequence or at sites that are distant from the RGD sequence.

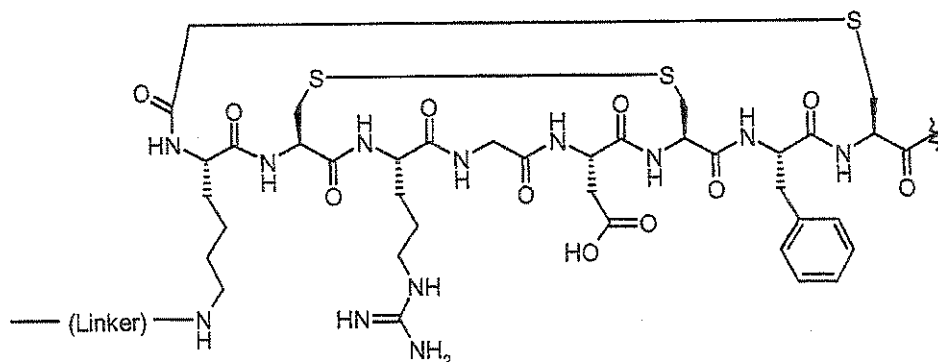
The efficient targeting and imaging of integrin receptors associated with angiogenesis *in vivo* demands therefore a selective, high affinity RGD based vector that is chemically robust and stable. Furthermore, the route of excretion is an important factor when designing imaging agents in order to reduce problems with background.

WO 03/006491 describes peptide-based compounds which target integrin receptors associated with angiogenesis. However, there exists a need for further such peptide-based compounds having utility for diagnostic imaging techniques such as PET. Co-pending International application PCT/GB2004/001052 describes methods suitable for labelling biologically active vectors with ^{18}F . But there is still a need for peptide-based compounds which may be prepared rapidly and efficiently and yet still have the desirable biological activity.

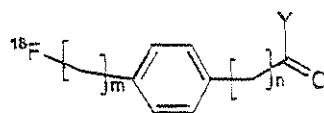
In a first aspect, the present invention provides a method for radiofluorination comprising reaction of a compound of formula (I):



wherein the vector comprises the fragment:



with a compound of formula (II):



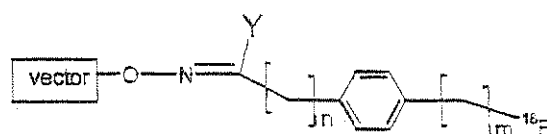
(II)

wherein:

n is an integer of 0 to 20;

m is an integer of 0 to 10;

- 5 Y is hydrogen, C₁₋₆alkyl (such as methyl), or phenyl
to give a compound of formula (III):

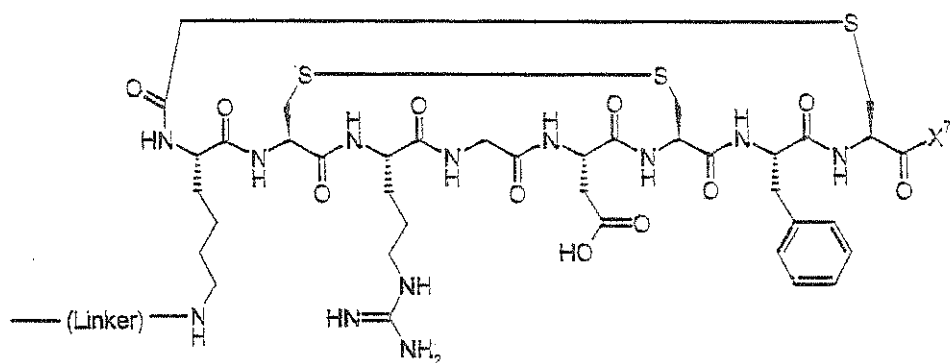


(III)

- 10 wherein m, n, and Y are defined as for the compound of formula (II) and the
vector is as defined for the compound of formula (I).

This reaction may be effected in a suitable solvent, for example, in an
aqueous buffer in the pH range 1 to 11, suitably 2 to 11, more suitably 2 to 6,
15 and at a non-extreme temperature of from 5 to 100 °C, suitably 20 to 70°C,
preferably at ambient temperature.

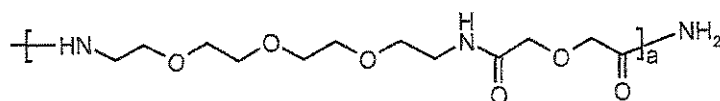
In one particular embodiment, the vector in formula (I) or (III) is of formula (A):



(A)

20

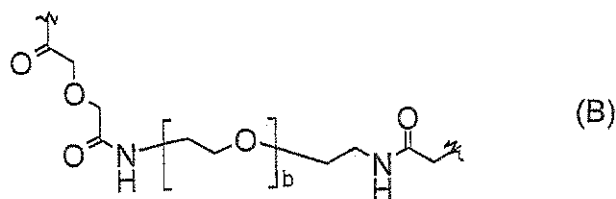
wherein X^7 is either $-NH_2$ or



5 wherein a is an integer of from 1 to 10, preferably a is 1.

The Linker forming part of the vector in the compound of formula (I) is chosen to provide good *in vivo* pharmacokinetics, such as favourable excretion characteristics in the resultant conjugate of formula (III). The use of linker groups with different lipophilicities and or charge can significantly change the *in vivo* pharmacokinetics of the peptide to suit the diagnostic need. For example, where it is desirable for a conjugate of formula (III) to be cleared from the body by renal excretion, a hydrophilic linker is used, and where it is desirable for clearance to be by hepatobiliary excretion a hydrophobic linker is used. Linkers including a polyethylene glycol moiety have been found to slow blood clearance which is desirable in some circumstances.

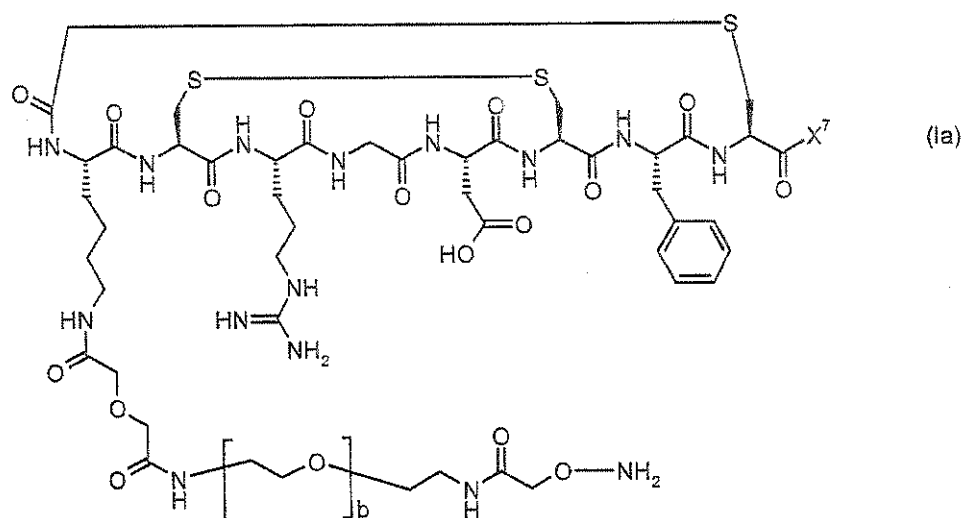
The Linker forming part of the vector in the compound of formula (I) is a C_{1-60} hydrocarbyl group, suitably a C_{1-30} hydrocarbyl group, optionally including 1 to 30 heteroatoms, suitably 1 to 10 heteroatoms such as oxygen or nitrogen. Suitable Linker groups include alkyl, alkenyl, alkynyl chains, aromatic, polyaromatic, and heteroaromatic rings, and polymers comprising ethyleneglycol, amino acid, or carbohydrate subunits. Preferably, the Linker forming part of the vector in the compound of formula (I) comprises a polyethylene glycol subunit, most preferably the Linker is of formula B:



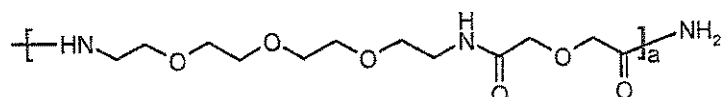
wherein b is an integer of from 2 to 20, and is preferably 3 to 10, most preferably 5.

The term "hydrocarbonyl group" means an organic substituent consisting of carbon and hydrogen, such groups may include saturated, unsaturated, or aromatic portions.

Accordingly, preferred compounds of formula (I) are those of formula (Ia):



wherein X^7 is either $-\text{NH}_2$ or



wherein a is an integer of from 1 to 10, preferably a is 1 and b is an integer of from 2 to 20 and is preferably 3 to 10, most preferably 5.

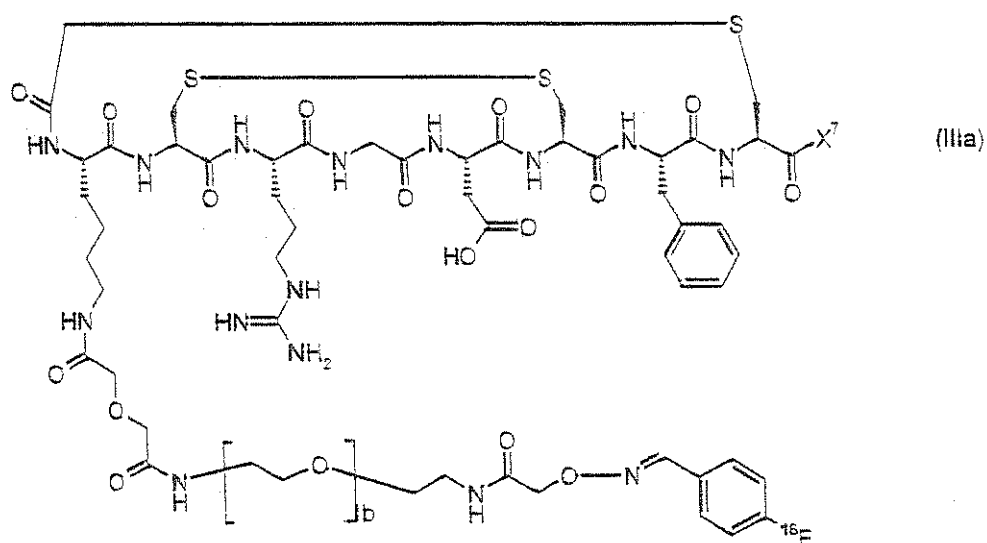
Preferred compounds of formula (II) are those where m is 0, n is 0, and Y is hydrogen.

Compounds of formula (I) and (III) may be prepared by standard methods of peptide synthesis, for example, solid-phase peptide synthesis, for example, as described in Atherton, E. and Sheppard, R.C.; "Solid Phase Synthesis"; IRL Press: Oxford, 1989. Incorporation of the aminoxy group in a compound of formula (I) may be achieved by formation of a stable amide bond formed by

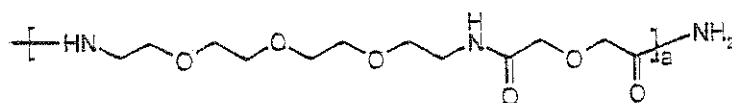
reaction of a peptide amine function with an activated acid and introduced either during or following the peptide synthesis.

In another embodiment, the present disclosure provides compounds of formula (I) and (Ia) as defined above having use as reagents useful for the production of radiolabelled peptide-based compounds.

In a further embodiment the present disclosure provides radiolabelled conjugates of formula (III) or a salt thereof, as defined above. Preferred compounds of formula (III) are those of formula (IIIa):

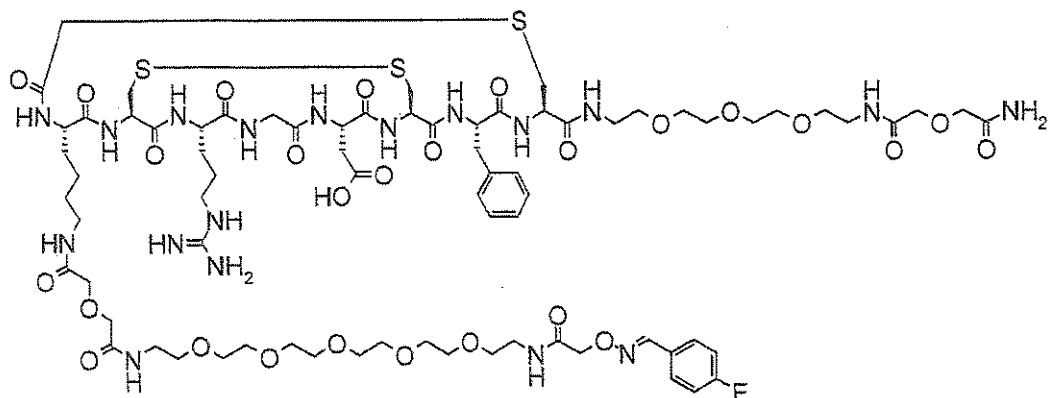


or a salt thereof, wherein X^7 is either $-NH_2$ or



wherein a is an integer of from 1 to 10, preferably a is 1 and b is an integer of from 2 to 20 and is preferably 3 to 10, most preferably 5.

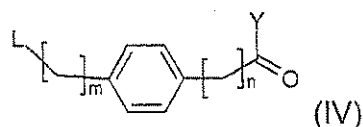
One particularly preferred compound of formula (III) is:



Suitable salts of the compounds of formula (III) and (IIIa) include
 5 pharmaceutically acceptable acid additions salts such as those formed from
 hydrochloric, hydrobromic, sulphuric, citric, tartaric, phosphoric, lactic, pyruvic,
 acetic, trifluoroacetic, succinic, oxalic, fumaric, maleic, oxalacetic,
 methanesulphonic, ethanesulphonic, p-toluenesulphonic, benzenesulphonic,
 and isoethionic acids.

10

Compounds of formula (II) may be prepared from the corresponding
 precursors of formula (IV):



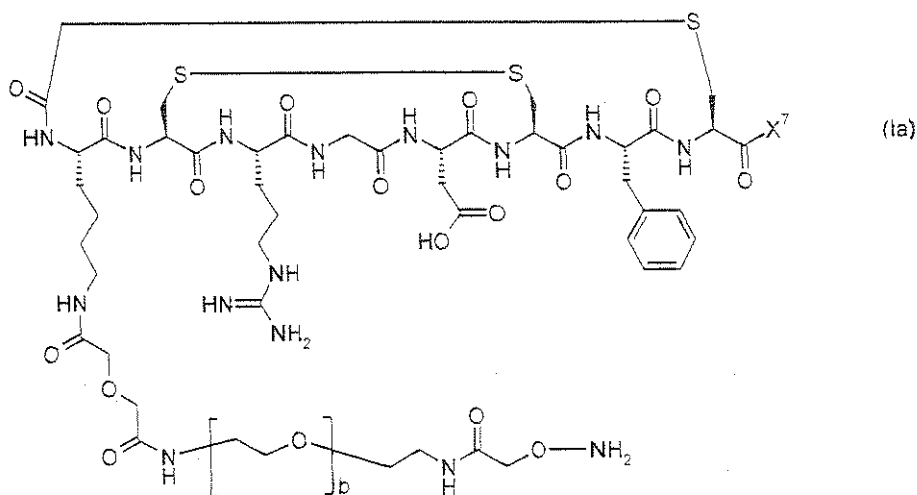
15

or a protected derivative thereof, wherein L is a leaving group preferably when
 $m \geq 1$, L is p-toluenesulphonate, trifluoromethanesulphonate, or
 methanesulphonate or a halide and when m is 0 L is p-trialkyl ammonium salt
 20 or p-nitro, and Y, m, and n are as described for the compound of formula (II);
 by reaction with cyclotron produced aqueous [^{18}F]-fluoride, suitably pre-
 activated by evaporation from a base (for example, from tetrabutylammonium
 or K_2CO_3 /Kryptofix-222), in a suitable solvent such as acetonitrile, N,N-
 dimethylformamide, or dimethyl sulphoxide, typically at ambient or at elevated

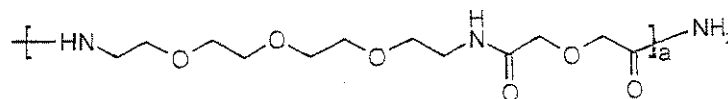
temperature, for example up to 140°C. The aldehyde or ketone function of compounds of formula (II) can also be rapidly generated from their protected precursors such as acetals or ketals by simple acid treatment following fluorination.

As shown in the *in vitro* competition binding assay below, the compounds of formula (I) and (Ia) bind to receptors associated with angiogenesis. These compounds may thus be useful for treatment, *in vivo* diagnosis and imaging of diseases and conditions associated with angiogenesis.

In a first aspect, there is provided a method for radiofluorination comprising reaction of a compound of formula (Ia):

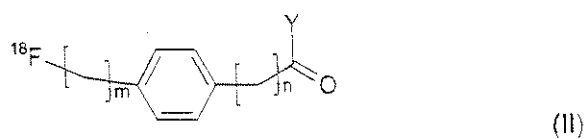


wherein X⁷ is either -NH₂ or



wherein a is an integer of from 1 to 10, and b is an integer of from 2 to 20,

with a compound of formula (II):



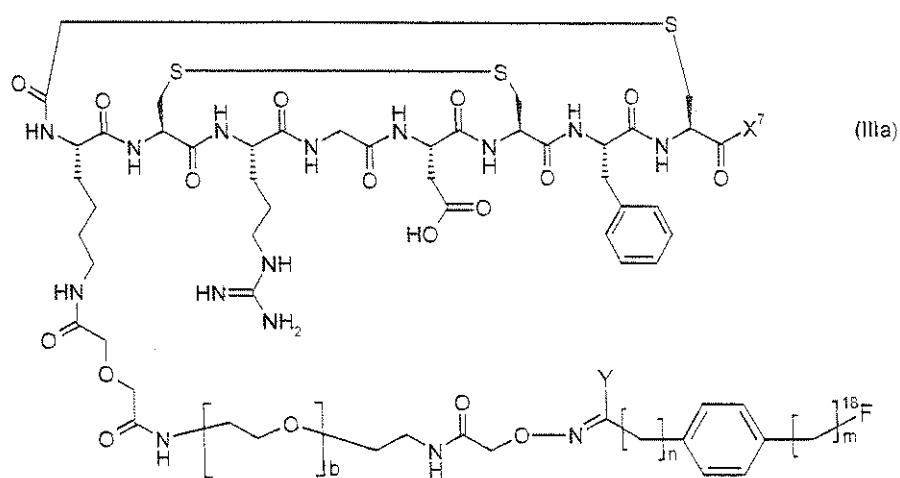
wherein:

n is an integer of 0 to 20;

m is an integer of 0 to 10;

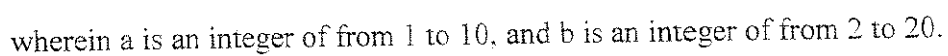
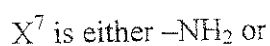
Y is hydrogen, C₁₋₆alkyl (such as methyl), or phenyl

to give a compound of formula (IIIa):



wherein m, n, and Y are defined as for the compound of formula (II) and the vector is as defined for the compound of formula (Ia).

In a third aspect, there is provided a compound of formula (IIIa).



In a fifth aspect, there is provided a compound of formula (IIIa) or a salt thereof, as defined above, for use in medicine.

In a sixth aspect, there is provided a compound or salt as defined above for use in the *in vivo* diagnosis or imaging, for example by PET, of a disease or condition associated with angiogenesis.

In a seventh aspect, there is provided a compound or salt as defined above wherein the *in vivo* diagnosis or imaging comprises administering a radiopharmaceutical to a body, for example into the vascular system and generating an image of at least a part of said body to which said radiopharmaceutical has distributed using PET, wherein said radiopharmaceutical comprises a compound of formula (IIIa) or a salt thereof as defined above.

In an eighth aspect, there is provided a compound or salt as defined above for use in a method of monitoring the effect of treatment of a human or animal body with a drug to combat a condition associated with cancer, preferably angiogenesis, said method comprising administering to said body a compound of formula (IIIa) or a salt thereof as defined above and detecting the uptake of said compound by cell receptors, said administration and detection optionally but preferably being effected repeatedly.

In a ninth aspect, there is provided use of a radiolabelled conjugate of formula (IIIa) or a salt thereof as defined above for the manufacture of a radiopharmaceutical for use in a method of *in vivo* imaging, suitably PET, and preferably for imaging of a disease or condition associated with angiogenesis; involving administration of said radiopharmaceutical to a human or animal body and generation of an image of at least part of said body.

The term "diseases and conditions associated with angiogenesis" includes those diseases and conditions referred to below. Reference is also made in this regard to WO 98/47541.

Diseases and conditions associated with angiogenesis include different forms of cancer and metastasis, for example, breast, skin, colorectal, pancreatic, prostate, lung or ovarian cancer.

Other diseases and conditions associated with angiogenesis are inflammation (for example, chronic inflammation), atherosclerosis, rheumatoid arthritis and gingivitis.

Further diseases and conditions associated with angiogenesis are arteriovenous malformations, astrocytomas, choriocarcinomas, glioblastomas, gliomas, hemangiomas (childhood, capillary), hepatomas, hyperplastic endometrium, ischemic myocardium, endometriosis, Kaposi sarcoma, macular degeneration, melanoma, neuroblastomas, occluding peripheral artery disease, osteoarthritis, psoriasis, retinopathy (diabetic, proliferative), scleroderma, seminomas and ulcerative colitis.

The present invention also provides a radiopharmaceutical composition comprising an effective amount (e.g. an amount effective for use in *in vivo* PET imaging) of a compound of general formula (III) or (IIIa) or a salt thereof,

as defined above; together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

5 A preferred embodiment of the invention relates to a compound of general formula (III) or (IIIa) or a salt thereof, as defined above, for use in medicine, particularly in the *in vivo* diagnosis or imaging, for example by PET, of a disease or condition associated with angiogenesis.

10 The radiolabelled conjugates of formula (III) or (IIIa) may be administered to patients for PET imaging in amounts sufficient to yield the desired signal, typical radionuclide dosages of 0.01 to 100 mCi, preferably 0.1 to 50 mCi, most preferably 1 to 20 mCi, will normally be sufficient per 70kg bodyweight.

15 The radiolabelled conjugates of formula (III) or (IIIa) may therefore be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized.

20

Viewed from a further aspect the invention provides the use of a radiolabelled conjugate of formula (III) or (IIIa) or a salt thereof as defined above for the manufacture of a radiopharmaceutical for use in a method of *in vivo* imaging, suitably PET, and preferably for imaging of a disease or condition associated
25 with angiogenesis; involving administration of said radiopharmaceutical to a human or animal body and generation of an image of at least part of said body.

Viewed from a still further aspect the invention provides a method for *in vivo*
30 diagnosis or imaging of a disease or condition associated with angiogenesis involving administering a radiopharmaceutical to said body, e.g. into the vascular system and generating an image of at least a part of said body to

which said radiopharmaceutical has distributed using PET, wherein said radiopharmaceutical comprises a radiolabelled conjugate of formula (III) or (IIIa) or a salt thereof.

5 Viewed from a further aspect the invention provides a method of monitoring the effect of treatment of a human or animal body with a drug to combat a condition associated with cancer, preferably angiogenesis, e.g. a cytotoxic agent, said method comprising administering to said body a radiolabelled conjugate of formula (III) or (IIIa) or a salt thereof and detecting the uptake of
10 said conjugate by cell receptors, preferably endothelial cell receptors and in particular $\alpha v \beta 3$ receptors, said administration and detection optionally but preferably being effected repeatedly, e.g. before, during and after treatment with said drug.

15 In yet another embodiment of the instant invention, there is provided a kit for the preparation of a radiofluorinated tracer comprising a prosthetic group of formula (II) and a compound of formula (I).

In use of the kits, the compound of formula (II) would be added to the
20 compound of formula (I) respectively which may suitably be dissolved in aqueous buffer (pH 1-11). After reaction at a non-extreme temperature for 1 to 70 minutes, the labelled peptide may be purified, for example, by solid-phase extraction (SPE) or high performance liquid chromatography (HPLC) and collected.

25

EXAMPLES

The invention is illustrated by way of examples in which the following abbreviations are used:

30

HPLC: high performance liquid chromatography

NMR: nuclear magnetic resonance

TFA: trifluoroacetic acid.

hr(s) : hour(s)

min(s) : minute(s)

DMAP : 4-(dimethylamino)pyridine

THF : tetrahydrofuran

5 DCM : dichloromethane

DMF : N,N-dimethylformamide

TBAF: tetrabutylammonium fluoride

MeOH: methanol

TLC: thin layer chromatography

10 TIS: triisopropylsilane

DMSO: dimethylsulphoxide

PBS: phosphate buffered saline

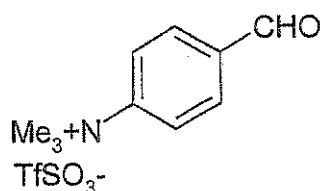
PyAOP : [7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium-hexafluorophosphate]

15 Boc: t-butoxycarbonyl

RT: room temperature

Example 1 Preparation of 4-trimethylammonium benzaldehyde triflate (compound 1)

20



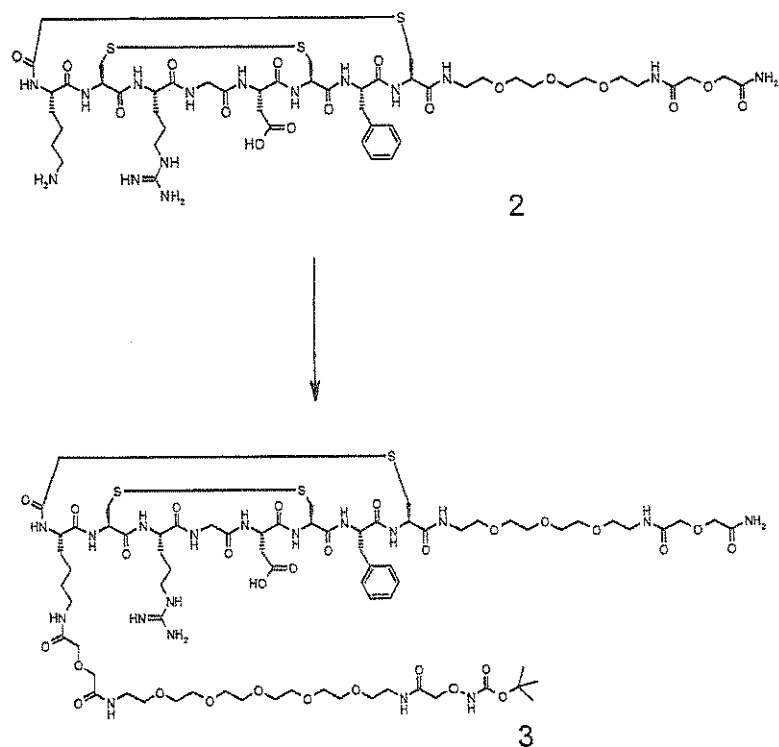
1

This compound was synthesised according to the procedure described by Haka et al (J. Labelled Cpds. & Radiopharms 1989 27(7) 823).

25

Example 2 - Preparation of Peptide Precursor (Compound 3)

The peptide, Compound 2 was synthesised using standard peptide synthesis.



(a) 1,17-Diazido-3,6,9,12,15-pentaoxaheptadecane

- 5 A solution of dry hexaethylene glycol (25 g, 88 mmol) and methanesulphonyl chloride (22.3 g, 195 mmol) in dry THF (125 mL) was kept under argon and cooled to 0 °C in an ice/water bath. A solution of triethylamine (19.7 g, 195 mmol) in dry THF (25 mL) was added dropwise over 45 min. After 1 hr the cooling bath was removed and the reaction was stirred for another for 4 hrs.
- 10 Water (55 mL) was then added to the mixture, followed by sodium hydrogencarbonate (5.3 g, to pH 8) and sodium azide (12.7 g, 195 mmol). THF was removed by distillation and the aqueous solution was refluxed for 24 h (two layers were formed). The mixture was cooled, ether (100 mL) was added and the aqueous phase was saturated with sodium chloride. The
- 15 phases were separated and the aqueous phase was extracted with ether (4 x 50 mL). The combined organic phases were washed with brine (2 x 50 mL) and dried (MgSO₄). Filtration and evaporation of the solvent gave 26 g (89 %) of a yellow oil. The product was used in the next step without further purification.

(b) 17-Azido-3,6,9,12,15-pentaoxaheptadecanamine

To a vigorously stirred suspension of 1,17-diazido-3,6,9,12,15-pentaoxaheptadecane (25 g, 75 mmol) in 5 % HCl (200 mL) was added a solution of triphenylphosphine (19.2 g, 73 mmol) in ether (150 mL) over 3 hrs
5 at room temperature. The reaction mixture was stirred for additional 24 hrs. The phases were separated and the aqueous phase was extracted with dichloromethane (3 x 40 mL). The aqueous phase was cooled in an ice/water bath and the pH was adjusted to 12 by addition of solid potassium hydroxide. The aqueous phase was concentrated and the product was taken up in
10 dichloromethane (150 mL). The organic phase was dried (Na_2SO_4) and concentrated giving of 22 g (95 %) of a yellow oil. The product was identified by electrospray mass spectrometry (ESI-MS) (MH^+ calculated: 307.19; found 307.4). The crude oil was used in the next step without further purification.

(c) 23-Azido-5-oxo-6-aza-3,9,12,15,18,21-hexaoxatricosanoic acid

To a solution of 17-azido-3,6,9,12,15-pentaoxaheptadecanamine (15 g, 50 mmol) in dichloromethane (100 mL) was added diglycolic anhydride (Acros, 6.4 g, 55 mmol). The reaction mixture was stirred overnight. The reaction was
20 monitored by ESI-MS analysis, and more reagents were added to drive the reaction to completion. The solution was concentrated to give a yellow residue which was dissolved in water (250 mL). The product was isolated from the aqueous phase by continuous extraction with dichloromethane over night. Drying and evaporation of the solvent gave a yield of 18 g (85 %). The product was characterized by ESI-MS analysis (MH^+ calculated: 423.20;
25 found 423.4). The product was used in the next step without further purification.

(d) 23-Amino-5-oxo-6-aza-3,9,12,15,18,21-hexaoxatricosanoic acid

23-Azido-5-oxo-6-aza-3,9,12,15,18,21-hexaoxatricosanoic acid (9.0 g, 21
30 mmol) was dissolved in water (50 mL) and reduced using $\text{H}_2(\text{g})$ -Pd/C (10 %). The reaction was run until ESI-MS analysis showed complete conversion to

the desired product (MH^+ calculated: 397.2; found 397.6). The crude product was used in the next step without further purification.

(e) (Boc-aminooxy)acetyl-PEG(6)-diglycolic acid

- 5 A solution of dicyclohexycarbodiimide (515 mg, 2.50 mmol) in dioxan (2.5 mL) was added dropwise to a solution of (Boc-aminooxy)acetic acid (477 mg, 2.50 mmol) and N-hydroxysuccinimide (287 mg, 2.50 mmol) in dioxan (2.5 mL). The reaction was stirred at RT for 1h and filtered. The filtrate was transferred to a reaction vessel containing a solution of 23-amino-5-oxo-6-aza-
10 3,9,12,15,18,21-hexaoxatricosanoic acid (1.0 g, 2.5 mmol) and N-methylmorpholine (278 μ l, 2.50 mmol) in water (5 mL). The mixture was stirred at RT for 30 min. ESI-MS analysis showed complete conversion to the desired product (MH^+ calculated: 570.28; found 570.6).

- The crude product was purified by preparative HPLC (column: Phenomenex
15 Luna 5 μ C18 (2) 250 x 21.20 mm, detection: 214 nm, gradient: 0-50 % B over 60 min where A = H₂O/0.1 % TFA and B = acetonitrile/0.1 % TFA, flow rate: 10 mL/min) affording 500 mg (38 %) of pure product.

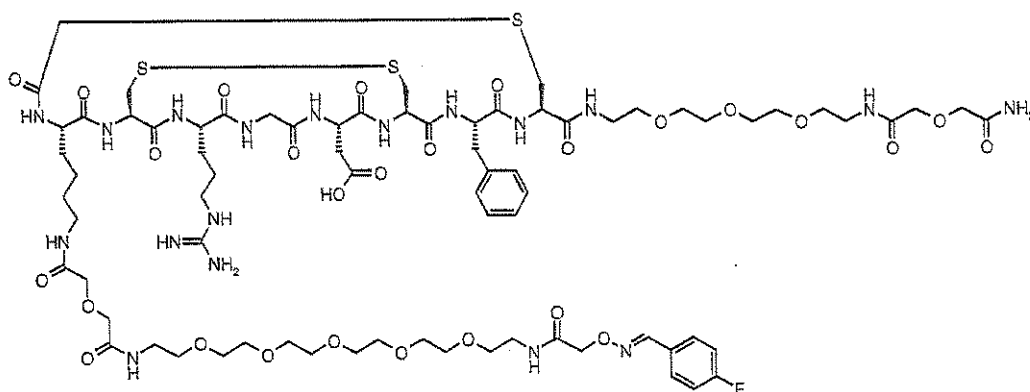
- The product was analyzed by HPLC (column: Phenomenex Luna 3 μ C18 (2),
50 x 2.00 mm, detection: 214 nm, gradient: 0-50 % B over 10 min where A =
20 H₂O/0.1 % TFA and B = acetonitrile/0.1 % TFA, flow rate: 0.75 mL/min, R_t = 5.52 min). Further confirmation was carried out by NMR analysis.

(f) Conjugation of (Boc-aminooxy)acetyl-PEG(6)-diglycolic acid to Compound 2

- 25 (Boc-aminooxy)acetyl-PEG(6)-diglycolic acid (0.15 mmol, 85 mg) and PyAOP (0.13 mmol, 68 mg) were dissolved in DMF (2 mL). N-methylmorpholine (0.20 mmol, 20 μ L) was added and the mixture was stirred for 10 min. A solution of Compound 2 (0.100 mmol, 126 mg) and N-methylmorpholine (0.20 mmol, 20 μ L) in DMF (4 mL) was added and the reaction mixture was stirred for 25 min.
30 Additional N-methylmorpholine (0.20 mmol, 20 μ L) was added and the mixture was stirred for another 15 min. DMF was evaporated *in vacuo* and the product was taken up in 10 % acetonitrile-water and purified by preparative HPLC

(column: Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm, detection: UV 214 nm, gradient: 5-50 % B over 40 min where A = H₂O/0.1 % TFA and B = acetonitrile/0.1 % TFA, flow rate: 10 mL/min,) affording 100 mg semi-pure product. A second purification step where TFA was replaced by HCOOH (gradient: 0-30 % B, otherwise same conditions as above) afforded 89 mg (50 %). The product was analysed by HPLC (column: Phenomenex Luna 3 μ C18 (2) 50 x 2 mm, detection: UV 214 nm, gradient: 0-30 % B over 10 min where A = H₂O/0.1 % HCOOH and B = acetonitrile/0.1 % HCOOH, flow rate: 0.3 mL/min, Rt: 10.21 min). Further product characterisation was carried out using ESI-MS (MH₂²⁺ calculated: 905.4, found: 906.0).

Example 3 - Chemoselective ligation of ¹⁸F-fluorobenzaldehyde to Compound 3 to give Compound 4



15

4

Deprotection of peptide 3 was carried out by addition of TFA containing 5% water to 10mg of peptide. The Boc-deprotected peptide (5.9 mg, 0.0044 mmol) in 1 ml water was added to 4-fluoro benzaldehyde (Compound 1) (1.1 mg, 0.94 μ l, 0.0089 mmol) in 1 ml acetonitrile. pH of the mixture was 3.5. After 45 minutes at 70 degrees the mixture was purified by reverse phase preparative chromatography twice (Phenomenex Luna C18 column, 00G-4253-N0; solvents: A= water + 0.1% TFA / B= CH₃CN + 0.1% TFA, gradient: 10-40 % B over 30 min ; flow 5.0 ml /minute; detected at 214 nm), affording 2.0 mg (32 %) of pure compound (analytical HPLC: Phenomenex Luna C18 column, 00G-4252-E0; solvents: A= water + 0.1% TFA / B= CH₃CN + 0.1%

25

TFA, gradient: 10-50 % B over 20 min ; flow 1.0 ml /minute; retention time 16.3 minutes, detected at 214 and 254 nm). Further characterisation was carried out using mass spectrometry, giving m/z value 1437.2. [M-H⁺].

5 Example 4: Radiosynthesis of ¹⁸F-compound 4

Method 1

¹⁸F-fluoride (up to 370MBq) was azeotropically dried in the presence of Kryptofix 222 (5mg in 0.5ml acetonitrile) and potassium carbonate (50µl 0.1M solution in water) by heating under N₂ to 110°C for 20mins. During this time
10 3x0.5ml acetonitrile were added and evaporated. After cooling to <40°C, a solution of trimethylammonium benzaldehyde triflate (1mg in 0.4ml DMSO) was added. The reaction vessel was sealed and heated to 90°C for 15 mins to effect labelling. Meanwhile, Compound 3 (6mg) was treated with 5% water in TFA (200µl) for 5 mins at RT. The solvents were then removed in vacuo. The
15 deprotected peptide was redissolved in 0.1M NH₄OAc buffer, pH4 (0.4ml) and combined with 4-¹⁸F-fluorobenzaldehyde in the reaction vessel. The reaction vessel was sealed and heated to 70°C for 15mins to effect conjugation. After cooling to room temperature, the product was obtained by preparative radio HPLC (column Phenomenex Luna C18(2) 5 µm 10 x 100 mm, solvents: A =
20 water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 15-25% B over 5 min; 25%B for 12 mins; 25-50%B over 10 mins; flow 4.0 ml/min, UV detection at 210 and 254 nm). The product fraction was diluted with water (10ml) and loaded onto a SepPak C18-plus cartridge (conditioned with 10ml EtOH and 20ml H₂O). Compound 4 was eluted in ethanol (1ml). The ethanol was
25 removed in vacuo and compound 4 was formulated in PBS.

Method 2

a) Radiosynthesis of ¹⁸F-fluorobenzaldehyde

¹⁸F-Fluoride (up to 370MBq) is azeotropically dried in the presence of
30 Kryptofix 222 (5mg in 0.5ml acetonitrile) and potassium carbonate (50µl 0.1M solution in water) by heating under N₂ to 110°C for 20mins. During this time 3 x 0.5ml acetonitrile are added and evaporated. After cooling to <40°C, a

solution of trimethylammonium benzaldehyde triflate (1mg in 0.4ml DMSO) is added. The reaction vessel is sealed and heated to 90°C for 15mins to effect labelling. The crude reaction mixture is cooled to room temperature and diluted by addition of water. The mixture will be passed sequentially through ion exchange cartridges (preconditioned with ethanol (or acetonitrile) and water) and eluted in an acetonitrile/water mixture. The eluate will be concentrated using a C18 Seppak, and the fluorobenzaldehyde will be eluted in acetonitrile.

10 b) Conjugation of Compound 3 and 4-¹⁸F-fluorobenzaldehyde

Compound 3 is treated with 5% water in TFA for 5mins at room temperature. The solvents are then removed by evaporation under vacuum. The peptide is redissolved in 0.1M NH₄OAc buffer, pH4 (0.5ml) and combined with 4-¹⁸F-fluorobenzaldehyde in the reaction vessel. The reaction vessel is sealed and heated to 70°C for 15mins to effect conjugation. After cooling to room temperature, the product is obtained by preparative radio HPLC (as described for method 1) or by SPE.

20 Biological Data

Binding Studies

Using cell membrane preparations known to express the $\alpha v \beta 3$ integrin receptor, competitive binding studies were carried out using ¹²⁵I-echistatin and the F-19 labelled peptides as competing ligand. Binding curves were obtained and K_i's calculated using Prism™ software.

Compound 4, had a K_i value of 10 nM.

30 Biodistribution in Lewis lung tumours

Mice (male C57BL/6, ca. 25 g) were injected sub-cutaneously into the inner right thigh with Lewis lung carcinoma (LLC) cells (0.1 mL, 1x10⁷ cells/mL in medium). Animals were monitored for tumour growth for up to 15 days, with

this time selected during model development as it showed the highest level of angiogenesis.

To determine the biodistribution of ^{18}F -compounds, tumour-bearing animals were injected with test article (0.1 mL, 5-10 MBq/mL) as an intravenous bolus via the tail vein. At various times post injection animals were euthanased. Muscle, kidneys, urine, lung, liver, stomach, small intestine, large intestine, thyroid, tumour were dissected and a blood sample taken. Dissected tissues and blood samples were weighed and counted (Wallac automatic gamma counter system). At least three animals per time point were studied. Results are expressed as %id and %id per gram of tissue.

Table 1 shows biodistribution of Compound 4 in the mouse Lewis Lung tumour model. Summarised data over time. Average data (n>3) of 5 independent experiments, presented as Mean (SD).

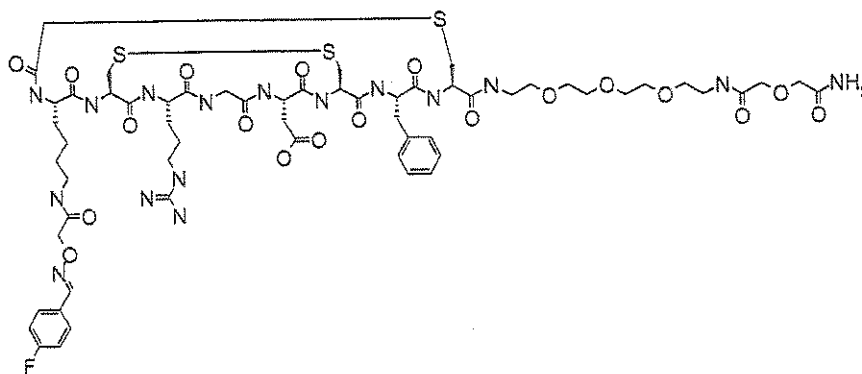
Table 1

TIME (MINS P.I.)	BLOOD %ID/G	MUSCLE %ID/G	LUNG %ID/G	LIVER %ID/G	TUMOUR %ID/G
5	6.35 (2.34)	1.78 (0.57)	6.54 (1.71)	6.01 (1.03)	2.69 (0.53)
60	0.84 (0.39)	0.56 (0.23)	2.12 (0.90)	1.48 (0.65)	1.84 (0.45)
120	0.45 (0.13)	0.27 (0.07)	1.17 (0.28)	0.89 (0.29)	1.49 (0.32)

TIME (MINS P.I.)	TUMOUR: BLOOD	TUMOUR: MUSCLE	TUMOUR: LUNG	TUMOUR: LIVER
5	0.48	1.62	0.45	0.46
60	2.27	3.60	0.95	1.35

120	3.31	5.80	1.27	1.75
-----	------	------	------	------

As comparison, biodistribution of Compound 5 in the mouse Lewis Lung tumour model is shown in Table 2. Summarised data over time. Average data (n>3) of 5 independent experiments, presented as Mean (SD).



5

5

Table 2

TIME (MINS P.I.)	BLOOD %ID/G	MUSCLE %ID/G	LUNG %ID/G	LIVER %ID/G	TUMOUR %ID/G
5	7.30 (1.3)	2.27 (0.6)	8.67 (1.4)	7.6 (0.9)	4.10 (0.9)
60	0.90 (0.2)	0.87 (0.3)	3.37 (0.5)	3.70 (0.9)	2.07 (0.3)
120	0.71 (0.2)	0.44 (0.1)	2.03 (0.4)	3.28 (0.9)	1.12 (0.3)

10

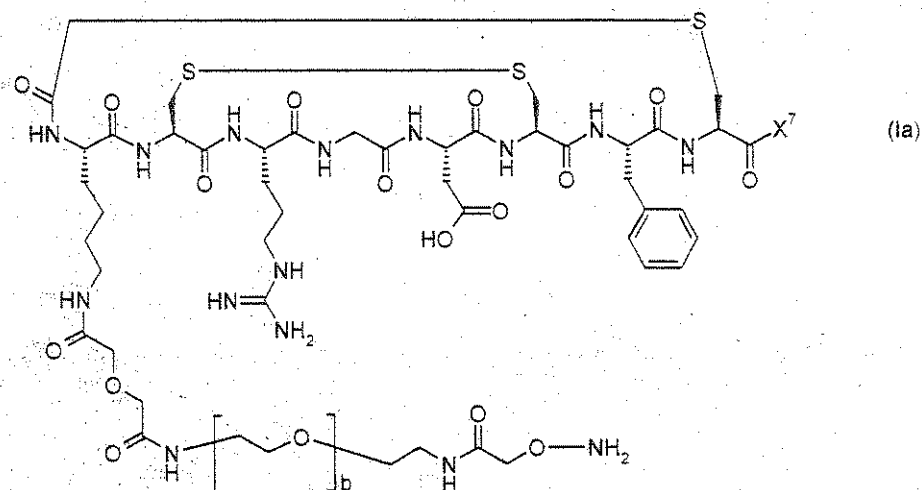
TIME (MINS P.I.)	TUMOUR: BLOOD	TUMOUR: MUSCLE	TUMOUR: LUNG	TUMOUR: LIVER
5	0.6	1.8	0.5	0.5

60	2.3	2.6	0.6	0.6
120	1.6	2.6	0.6	0.3

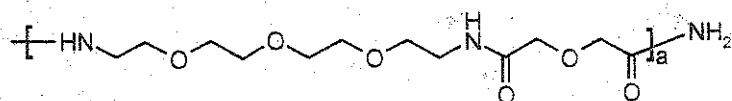
The additional PEG moiety in compound 4 imparts significantly more favourable *in vivo* characteristics. Specifically the remaining activity present in background tissues such as blood, muscle, lung and liver for compound 4
5 after 120 minutes is substantially less than for compound 5. Subsequently tumour:background ratios are significantly better thus enabling imaging.

CLAIMS

1: A method for radiofluorination comprising reaction of a compound of formula (Ia):

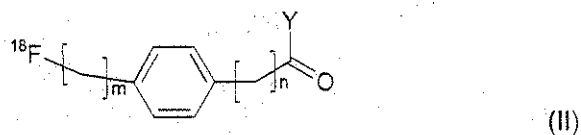


wherein X^7 is either $-NH_2$ or



wherein a is an integer of from 1 to 10, and b is an integer of from 2 to 20,

with a compound of formula (II):



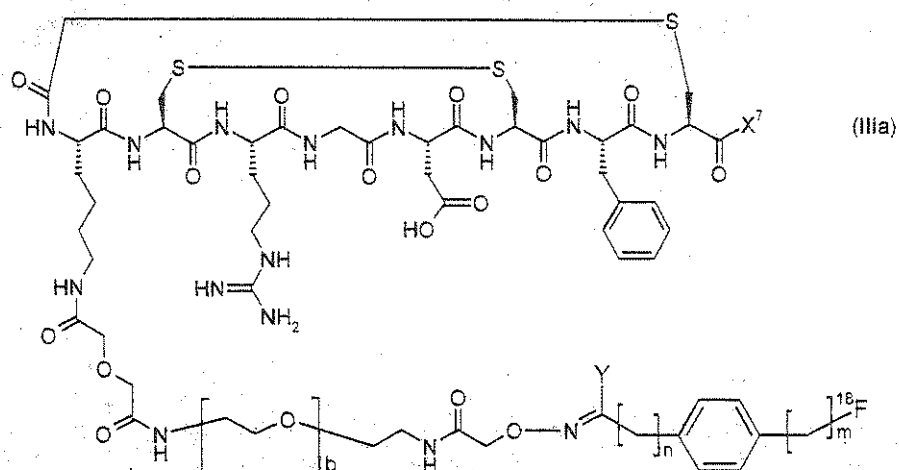
wherein:

n is an integer of 0 to 20;

m is an integer of 0 to 10;

Y is hydrogen, C₁₋₆alkyl (such as methyl), or phenyl

to give a compound of formula (IIIa):



wherein m, n, and Y are defined as for the compound of formula (II) and the vector is as defined for the compound of formula (Ia).

10

15

20

25

30

2. A method according to claim 1 wherein a is 1.

3. A method according to claim 1 or 2 wherein b is 3 to 10, and is preferably 5.

5

10

15

20

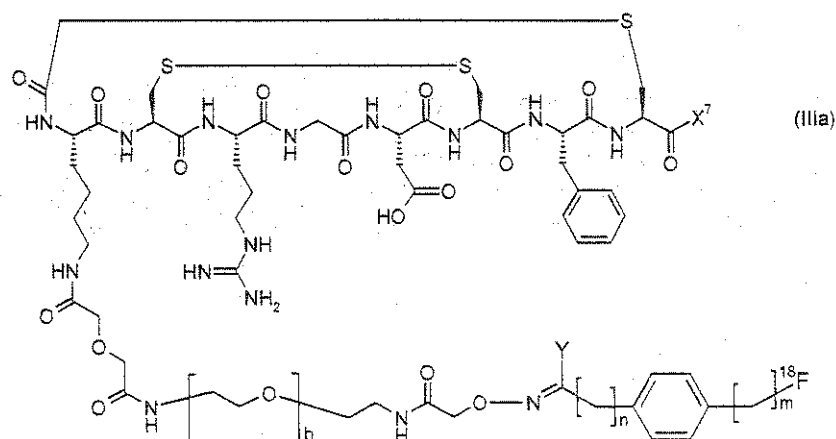
25

30

35

40

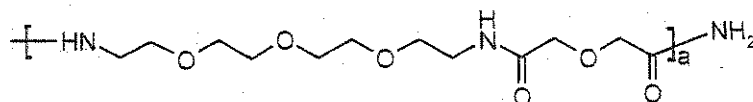
4. A compound of formula (Ia) as defined in any of claims 1 to 3.
5. A compound of formula (IIIa).



or a salt thereof wherein n is an integer of 0 to 20, m is an integer of 0 to 10,

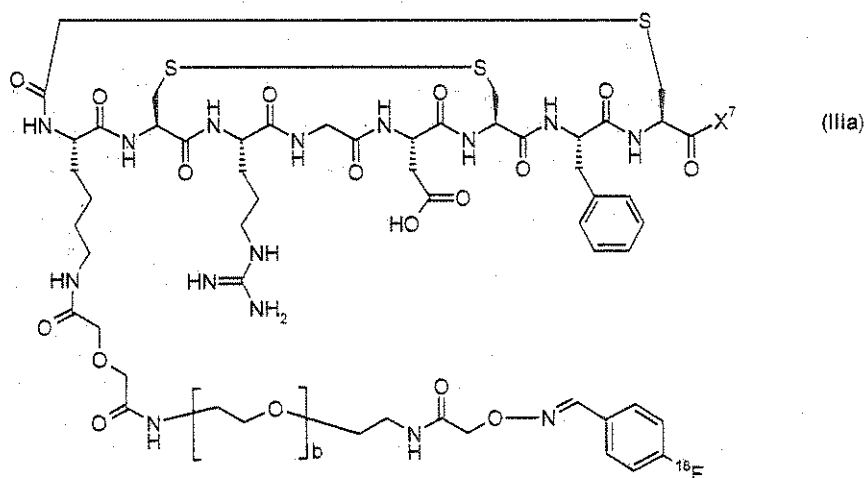
- 5 Y is hydrogen, C₁₋₆alkyl (such as methyl), or phenyl;

X⁷ is either -NH₂ or



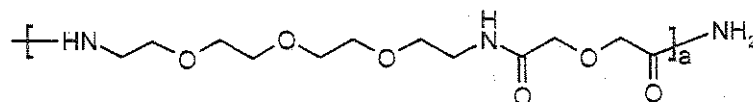
wherein a is an integer of from 1 to 10, and b is an integer of from 2 to 20.

6. A compound of formula (IIIa) according to claim 5 of formula :



10

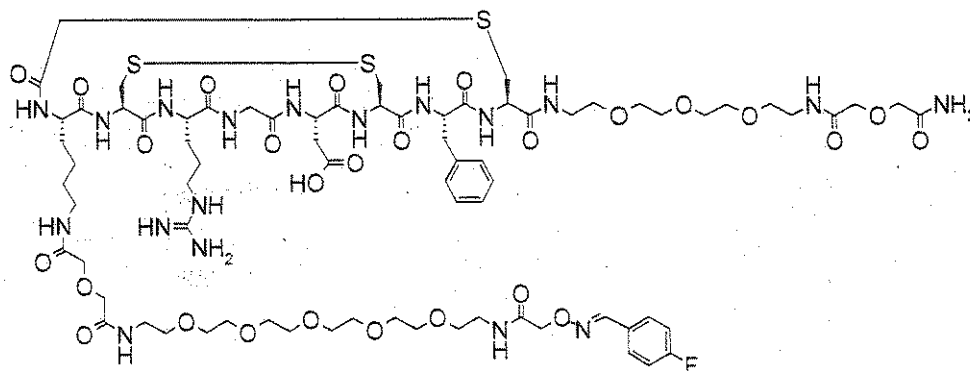
or a salt thereof, wherein X⁷ is either -NH₂ or



wherein a is an integer of from 1 to 10, preferably a is 1 and b is an integer of from 2 to 20 and is preferably 3 to 10, most preferably 5.

15

7. A compound of formula (IIIa) which is:



8. A radiopharmaceutical composition comprising an effective amount of a compound of formula (IIIa) or a salt thereof, as defined in any of claims 5 to 7; together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

9. A compound of formula (IIIa) or a salt thereof, as defined in any of claims 5 to 7, for use in medicine.

10. A compound or salt according to claim 9 for use in the *in vivo* diagnosis or imaging, for example by PET, of a disease or condition associated with angiogenesis.

11. A compound or salt according to claim 10 wherein the *in vivo* diagnosis or imaging comprises administering a radiopharmaceutical to a body, for example into the vascular system and generating an image of at least a part of said body to which said radiopharmaceutical has distributed using PET, wherein said radiopharmaceutical comprises a compound of formula (IIIa) or a salt thereof as defined in any of claims 5 to 7.

12. A compound or salt according to claim 9 for use in a method of monitoring the effect of treatment of a human or animal body with a drug to combat a condition associated with cancer, preferably angiogenesis, said method comprising administering to said body a compound of formula (IIIa) or a salt thereof as defined in any of claims 5 to 7 and detecting the uptake of said compound by cell receptors, said administration and detection optionally but preferably being effected repeatedly.

13. Use of a radiolabelled conjugate of formula (IIIa) or a salt thereof as defined in any of claims 5 to 7 for the manufacture of a radiopharmaceutical for use in a method of *in vivo* imaging, suitably PET, and preferably for imaging of a disease or condition associated with angiogenesis; involving administration of said radiopharmaceutical to a human or animal body and generation of an image of at least part of said body.