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(54) Title: RADIOIODINATION METHOD

(57) Abstract: The present invention provides a method for the synthesis of radioiodinated compounds which is advantageous over prior art methods. Using a hydrazine or an aminoxy in place of a primary amine for indirect radioiodination facilitates a much quicker reaction thus reducing reaction time and increasing the yield. In addition, where there are primary amines in the molecule to be radioiodinated, such as the N-terminus of a peptide or lysine residues, reaction at the hydrazine or aminoxy is greatly favoured.

## RADIOIODINATION METHOD

### Technical Field of the Invention

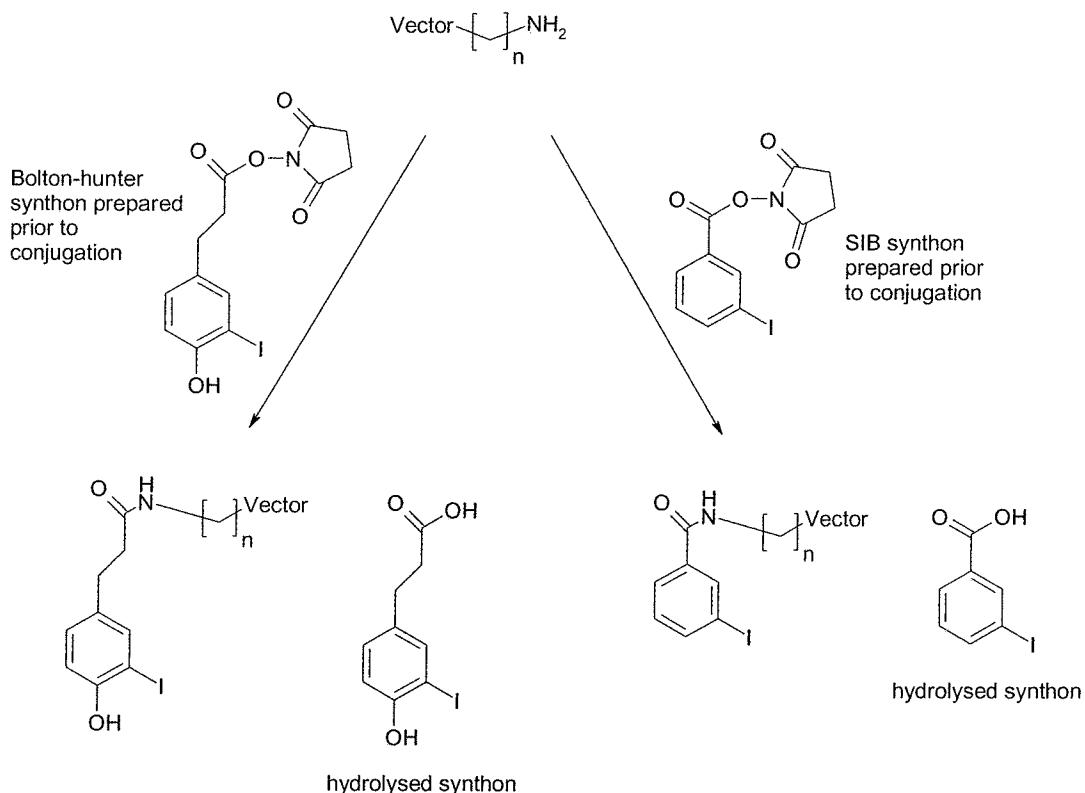
The present invention relates to the synthesis of radioiodinated compounds, and in particular to indirect radioiodination methods. The method of the 5 present invention provides advantages over presently-known indirect radioiodination methods.

### Description of Related Art

Radioiodination is most easily carried out *via* direct radioiodination by reacting radioiodine with a suitable precursor compound. For example, the precursor 10 compound may comprise: an aryl iodide or bromide (to permit radioiodine exchange); an activated precursor compound aryl ring (e.g. a phenol group); an organometallic precursor compound (e.g. trialkyltin, trialkylsilyl or organoboron compound); or an organic precursor compound such as triazenes or a good leaving group for nucleophilic substitution such as an iodonium salt.

15 Although simple to perform, direct radioiodination has disadvantages, especially when applied to the radioiodination of biomolecules such as proteins. For example, radioiodination of proteins by direct electrophilic substitution of hydroxyl groups on tyrosine residues cannot be applied to proteins lacking tyrosine residues. Furthermore, proteins radioiodinated by electrophilic 20 methods often exhibit reduced biological function resulting from direct exposure to oxidising conditions. Another problem with direct radioiodination is that is that proteins labelled in this way often undergo *in vivo* deiodination due to the structural similarity of iodotyrosine residues with thyroid hormones. To address these problems, indirect radioiodination methods have been developed. The 25 known indirect radioiodination methods comprise formation of a radioiodinated synthon, which is then conjugated to a protein under mild conditions through modification of lysine  $\epsilon$ -amino groups (for a review see Wilbur 1992 Bioconj Chem; 3: 433-70). Currently the most commonly used indirect radioiodination method involves conjugation of an iodo Bolton-Hunter reagent, or more

typically *N*-succinimidyl-3-iodo-benzoate (SIB), to a primary amine present in the vector, as illustrated in scheme 1 below:



**Scheme 1: Known indirect radioiodination routes**

5 A typical such reactions takes 30 minutes at 37°C, pH 7.5-8, 50mM, in sodium phosphate buffer. The reaction generally proceeds more quickly at higher pH. However, as the pH is increased so the hydrolysis of the NHS ester increases and the subsequent yields can be compromised for that step. The yields also vary depending on the vector, its solubility, position of the amine functionality  
 10 and the system chosen for the conjugation.

The formation of hydrazones by the action of hydrazine on ketones or aldehydes is known (March's Advanced Organic Chemistry 5<sup>th</sup> Edition, 2001, by Smith and March; Chapter 16 pp 1192-4), and can be used for the chemoselective coupling of a carbonyl functionality to a hydrazine-functionalised compound. In the art of radiochemistry, the hydrazine-functionalised compound 6-hydrazinonicotinic acid (HYNIC) is a well-known  
 15

bifunctional ligand for  $^{99m}\text{Tc}$  labelling of biomolecules (see for example Abrams *et al* 1990 *J Nuc Med*; 31: 2022-8). HYNIC has also more recently been reported as an attractive route to label biomolecules with  $^{18}\text{F}$ . Chang *et al* (2005 *Bioconj Chem*; 16: 1329-33) describe the preparation of  $^{18}\text{F}$  labelled 5 human serum albumin (HSA) by conjugation of [ $^{18}\text{F}$ ]fluorobenzaldehyde ( $[^{18}\text{F}]$ FBA) with HYNIC-HSA *via* hydrazone formation. The same group also reported application of this method in the preparation of an  $^{18}\text{F}$  labelled RGD peptide (Lee *et al* 2006 *Nuc Med Biol*; 33: 677-83). Bruus-Jensen *et al* (2006 *Nuc Med Biol*; 33: 173-83) investigated the method in the formation of various 10 other  $^{18}\text{F}$  labelled peptides and reported that it was a fast and straightforward radiolabeling method leading to high yields under mild acidic conditions. In these reports, it was found that the optimum temperature for hydrazone formation ranged between 50-70°C. This temperature range is not the most ideal for reactions comprising biomolecules, which are ideally carried out at 15 temperatures closer to physiological temperature.

There is scope to provide improved methods for the radioiodination of biomolecules.

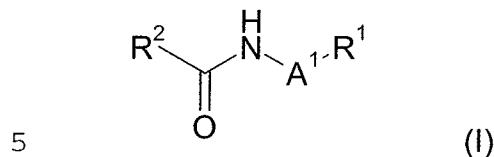
### **Summary of the Invention**

The present invention provides a method for the synthesis of radioiodinated 20 biomolecules which is advantageous over presently-known methods. Using reaction between a hydrazine or an aminoxy derivative and an active ester facilitates a much quicker reaction thus reducing reaction time and increasing the yield as compared with the known reaction between an amine and an active ester. In addition, where there are primary amines in the molecule to be 25 radioiodinated, such as the N-terminus of a peptide or lysine residues, reaction at the hydrazine or aminoxy group is greatly favoured. Furthermore, the method of the invention provides good yields at temperatures that mimic those of the native state of the biomolecule. This provides the advantage of optimal preservation of the biomolecule's structure and function.

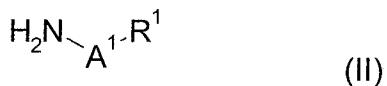
## Detailed Description of the Invention

### Method for Synthesis of a Radioiodinated Compound

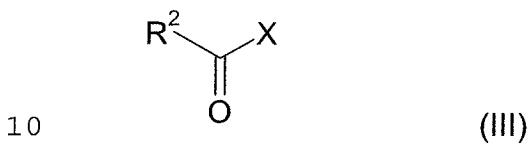
In one aspect, the present invention relates to a method for the synthesis of a radioiodinated compound of Formula I:



or a salt or solvate thereof, said method comprising reaction of a compound of Formula II:



with a compound of Formula III:



wherein:

$\text{A}^1$  is either NH or O;

one of  $\text{R}^1$  and  $\text{R}^2$  is the group  $-\text{L}^1-\text{Ar}^1$  wherein:

$\text{L}^1$  is a bond or is a bivalent linker comprising 1-3  $\text{L}^*$  linker units wherein  $\text{L}^*$  is selected from  $-\text{CO}-$ ,  $-\text{CR}'_2-$ ,  $-\text{CR}'=\text{CR}'-$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{CR}'_2\text{CO}_2-$ ,  $-\text{CO}_2\text{CR}'_2-$ ,  $-\text{NR}'-$ ,  $-\text{NR}'\text{CO}-$ ,  $-\text{CONR}'-$ ,  $-\text{NR}'-$ ,  $-(\text{C}=\text{O})\text{NR}'-$ ,  $-\text{NR}'(\text{C}=\text{S})\text{NR}'-$ ,  $-\text{SO}_2\text{NR}'-$ ,  $-\text{NR}'\text{SO}_2-$ ,  $-\text{CR}'_2\text{OCR}'_2-$ ,  $-\text{CR}'_2\text{SCR}'_2-$ ,  $-\text{CR}'_2\text{NR}'\text{CR}'_2-$ , a  $\text{C}_{5-12}$  arylene group, and a  $\text{C}_{3-12}$  heteroarylene group, wherein  $\text{R}'$  is hydrogen or  $\text{C}_{1-3}$  alkyl; and,

$\text{Ar}^1$  is a 6-membered  $\text{C}_{3-6}$  aryl group, substituted with radioiodine, and with 0-3 other substituents selected from  $\text{C}_{1-3}$  alkyl, halo, amino, carboxyl,

hydroxyl, or protected versions thereof, and wherein said aryl group has 0-3 heteroatoms selected from N, S and O;

and the other of R<sup>1</sup> and R<sup>2</sup> is the group -L<sup>2</sup>-R<sup>\*</sup>, wherein:

5 L<sup>2</sup> is a bond or is a bivalent linker comprising 1-6 L<sup>\*</sup> linker units wherein L<sup>\*</sup> is as defined for L<sup>1</sup>; and,

R<sup>\*</sup> is a biomolecule;

wherein R<sup>1</sup> and R<sup>2</sup> optionally comprise suitable protecting groups;

and wherein X represents an active ester group.

Definitions for Method of the Invention

10 The term “radioiodinated compound” means a compound comprising radioiodine, i.e. at least one radioiodine atom, and specifically in the context of the present invention, a compound comprising a radioiodoaryl group. A “radioiodoaryl group” is an aryl group, as defined herein, comprising a radioiodine atom. A “radioiodine atom” may be any radioactive isotope of iodine, preferably <sup>123</sup>I, <sup>124</sup>I, <sup>125</sup>I, or <sup>131</sup>I, and most preferably <sup>123</sup>I, <sup>124</sup>I, or <sup>131</sup>I.

15

Suitable salts according to the phrase “salt or solvate thereof” used in the present invention include (i) physiologically acceptable acid addition salts such as those derived from mineral acids, for example hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and those derived from 20 organic acids, for example tartaric, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, glycollic, gluconic, succinic, methanesulphonic, and para-toluenesulphonic acids; and (ii) physiologically acceptable base salts such as ammonium salts, alkali metal salts (for example those of sodium and potassium), alkaline earth metal salts (for example those of calcium and magnesium), salts with organic bases such as triethanolamine, N-methyl-D-glucamine, piperidine, pyridine, piperazine, and morpholine, and salts with amino acids such as arginine and lysine.

In its broadest sense, the term "reaction of" refers to mixing a solution of the compound of Formula II with a solution of the compound of Formula III. The solutions may be aqueous or organic. An "aqueous" solution is a solution wherein the solvent comprises water. The term "organic" when referring to a

5 solution refers to a solution wherein the solvent is a carbon-containing solvent, including such solvents as tetrahydrofuran (THF), dichloromethane (DCM) and dimethylformamide (DMF). Typically, mixing a solution of the compound of Formula II with a solution of the compound of Formula III is carried out at a defined temperature over a defined period of time. It is preferred that the

10 method of the invention is carried out at a temperature less than 50°C, but not significantly less than room temperature. A preferred temperature for carrying out the method of the invention is therefore in the range 15-45°C, most preferably in the range 20-40°C and especially preferably 35-40°C. An advantage of the present invention over the prior art methods is that it can

15 successfully be carried out at 37°C.

An "active ester" is an ester made with an alcohol whose structure allows it to be easily displaced by nucleophiles such as amines, forming a stable linkage. In a preferred embodiment, the active ester is selected from N-hydroxysuccinimidyl (NHS) ester, pentafluorophenyl ester, and

20 hydroxybenzotriazole (HOBT) ester. A most preferred active ester is NHS ester.

The term "alkyl" used either alone or as part of another group is defined herein as any straight, or branched saturated or unsaturated  $C_nH_{2n+1}$  group, wherein unless otherwise specified n is an integer between 1 and 10. Alkyl groups

25 include for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, 1-methylpropyl, pentyl, isopentyl, sec-pentyl, hexyl, heptyl, and octyl.

The term "cycloalkyl" refers to an alkyl group as defined herein which is cyclic.

The term "aryl" is defined herein as any mono-, bi- or tri-cyclic  $C_{5-14}$  molecular fragment or group comprising at least one aromatic ring, and preferably having

30 5 to 6 ring members in each ring. The term aryl embraces purely aromatic

radicals such as phenyl, naphthyl, tetrahydronaphthyl, indane, and biphenyl, as well as radicals comprising at least one aromatic ring fused with one or more cycloalkyl or heterocycloalkyl rings.

The term "amino" means an  $-\text{NH}_2$  group.

5 The term "hydroxyl" means an  $-\text{OH}$  group.

The term "nitro" means an  $-\text{NO}_2$  group.

The term "halo" relates to a halogen atom selected from iodine, fluorine, bromine and chlorine.

The term "carboxyl" means a  $-\text{COOH}$  group.

10 The term "heteroatom" refers to any atom in a hydrocarbon moiety that is not carbon or hydrogen. In the context of the present invention, heteroatoms are selected from nitrogen, oxygen and sulfur.

15 The term "biomolecule" as used herein means a component or product of a cell such as a peptide, protein, antibody, carbohydrate, lipid, or nucleic acid, or synthetic versions thereof. Because they are subject to denaturation, e.g. at high temperatures or due to strongly acidic or basic conditions, biomolecules are advantageously radioiodinated indirectly and at temperatures as close to physiological temperature as possible.

20 The term "physiological temperature" is taken herein to refer most specifically to temperatures in the range 20-40°C and preferably 35-40°C.

The term "alkylene", used either alone or in combination, refers to a straight or branched chain or cyclic bivalent aliphatic radical having a specified number of carbon atoms. Examples of alkylanes as used herein include, but are not limited to, methylene, ethylene, propylene, butylene and the like.

25 The term "cycloalkylene" refers to a cyclic bivalent aliphatic radical.

The term "arylene", used either alone or in combination, refers to bivalent

unsaturated aromatic carboxylic radicals having a single ring, such as phenylene, or multiple condensed rings, such as naphthylene or anthrylene. Examples of arlenes as used herein include, but are not limited to, benzene-1,2-diyl, benzene-1,3-diyl, benzene-1,4-diyl, naphthalene-1,8-diyl, and the like.

5 The term "heteroarylene" refers to an arylene group comprising one or more heteroatoms selected from N, S or O.

A "suitable protecting group" is a group which inhibits or suppresses undesirable chemical reactions, but which is designed to be sufficiently reactive that it may be cleaved from the functional group in question under mild enough 10 conditions that do not modify the rest of the molecule. After deprotection the desired product is obtained. Protecting groups are well known to those skilled in the art and are suitably chosen from, for amine groups: Boc (where Boc is *tert*-butyloxycarbonyl), Fmoc (where Fmoc is fluorenylmethoxycarbonyl), trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] or Npys (i.e. 3-nitro-2-pyridine sulfenyl); and for 15 carboxyl groups: methyl ester, *tert*-butyl ester or benzyl ester. For hydroxyl groups, suitable protecting groups are: methyl, ethyl or *tert*-butyl; alkoxyethyl or alkoxyethyl; benzyl; acetyl; benzoyl; trityl (Trt) or trialkylsilyl such as tetrabutylmethoxysilyl. The use of further protecting groups are described in 20 'Protective Groups in Organic Synthesis', Theodorora W. Greene and Peter G. M. Wuts, (Fourth Edition, John Wiley & Sons Inc., 2007).

#### How to Obtain Compounds of Formulas II and III

The compounds of Formulas II and III may be obtained by methods known to the person skilled in the art.

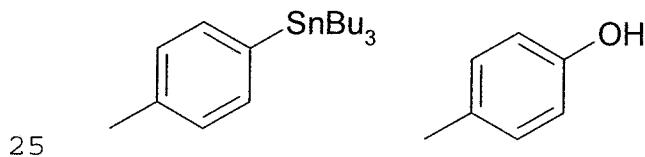
25 To obtain a compound of either Formula II or Formula III comprising the group -L<sup>1</sup>-Ar<sup>1</sup>, i.e. comprising a radioiodoaryl group, a starting compound can be used that comprises a derivative which either undergoes electrophilic or nucleophilic radioiodination or undergoes condensation with a labelled aldehyde or ketone. Examples of the first category are:

(a) organometallic derivatives such as a trialkylstannane (e.g. trimethylstannyl or tributylstannyl), or a trialkylsilane (e.g. trimethylsilyl) or an organoboron compound (e.g. boronate esters or organotrifluoroborates);

5 (b) aromatic rings activated towards electrophilic iodination (e.g. phenols) and aromatic rings activated towards nucleophilic iodination (e.g. aryl iodonium salt aryl diazonium, aryl trialkylammonium salts or nitroaryl derivatives).

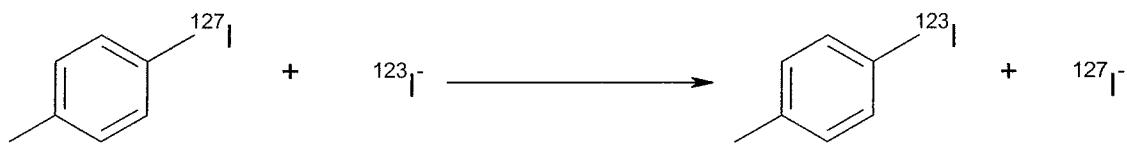
The starting compound preferably comprises: an aryl iodide or bromide (to 10 permit radioiodine exchange); an activated precursor compound aryl ring (e.g. a phenol group); an organometallic precursor compound (e.g. trialkyltin, trialkylsilyl or organoboron compound); or an organic precursor compound such as triazenes or a good leaving group for nucleophilic substitution such as an iodonium salt. Precursor compounds and methods of introducing radioiodine 15 into organic molecules are described by Bolton (J. Lab Comp Radiopharm 2002; 45: 485-528). Precursor compounds and methods of introducing radioiodine into proteins are described by Wilbur (Bioconj Chem 1992; 3(6): 433-470). Suitable boronate ester organoboron compounds and their preparation are described by Kabalaka *et al* (Nucl Med Biol, 2002; 29: 841-843 20 and 2003; 30: 369-373). Suitable organotrifluoroborates and their preparation are described by Kabalaka *et al* (Nucl Med Biol, 2004; 31: 935-938). Preferred starting compounds for radioiodination comprise an organometallic precursor compound, most preferably a trialkyltin.

Examples of aryl groups to which radioiodine can be attached are given below:

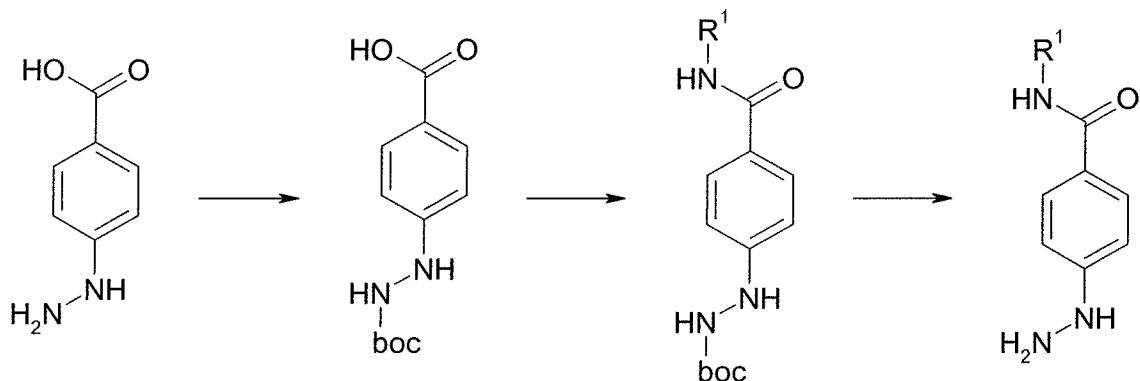


Both contain substituents which permit facile radioiodine substitution onto the aromatic ring to form the group Ar<sup>1</sup>. Ar<sup>1</sup> substituents containing radioactive

iodine can be synthesised by direct iodination *via* radiohalogen exchange, e.g.



In one embodiment,  $\text{A}^1$  of Formula II is NH, such that the compound of Formula II is a hydrazine. A wide variety of hydrazine compounds are readily available commercially that can be converted to compounds of Formula II wherein  $\text{A}^1$  is NH using standard methods of organic chemistry. For example, iodophenylhydrazine is commercially available, which can be converted by radioiodine exchange to a compound of Formula II wherein  $\text{A}^1$  is NH, and  $\text{R}^1$  is radioiodophenyl. Also, commercially-available hydrazino-benzoic acid may be readily converted into hydrazine compounds of Formula II, e.g.:



wherein boc is a tert-butoxycarbonyl protecting group, and  $\text{R}^1$  is as defined herein.

Where  $\text{R}^1$  is  $-\text{L}^2\text{-R}^*$ , the route for obtaining the compound of Formula II has to be sufficiently mild in order to be suitable for functionalisation without loss of function of the biomolecule. A route similar to that depicted above wherein the starting hydrazine-containing compound is HYNIC is well-known in the art of radiochemistry for the functionalisation of biomolecules with hydrazine. An advantage of the HYNIC moiety for introduction of a hydrazine functional group is that it can be conjugated to a biomolecule relatively easily and under mild conditions. For example, *N*-hydroxysuccinimidyl-HYNIC can be used to treat

lysine residues in a peptide or protein (Rennen *et al* 2000 Nuc Med Biol; 27: 599-604), or HYNIC-maleamide can be used by reacting the maleamide group with cysteine residues (Banerjee *et al* 2005 Dalton Trans; 24: 3886-97).

An early report using a HYNIC moiety for  $^{99m}$ Tc labelling of biomolecules 5 (Abrams *et al* 1990 J Nuc Med; 31: 12) describes how to incorporate a hydrazine group into IgG by conjugating HYNIC to IgG. The resultant nicotinyl hydrazine-modified IgG can be conjugated with  $^{99m}$ Tc-glucoheptonate, such that the end nitrogen of the hydrazine acts to complete co-ordination of  $^{99m}$ Tc. Others have applied similar methods for  $^{99m}$ Tc labelling of other proteins, and of 10 peptides, demonstrating the versatility of this approach. Blankenberg *et al* (1998 PNAS; 95(11) 6349-54) describe  $^{99m}$ Tc labelling of annexin V, Rennen *et al* (2004 Chest; 126(6): 1954-61) describe  $^{99m}$ Tc labelling of interleukin-8, Oyen *et al* (2000 Eur J Nuc Med; 27: 392-9) describe  $^{99m}$ Tc labelling of the glycoprotein IIa/IIIb receptor antagonist DMP444, and Faintuch *et al* (2005 15 Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry; 35(1): 43-51) describe  $^{99m}$ Tc labelling of Bombesin<sub>7-14</sub> NH<sub>2</sub>.

HYNIC groups can be site-specifically conjugated to peptides by immobilising the protected peptide on a solid phase, coupling HYNIC, deprotecting the peptide and then cleaving the deprotected peptide from the solid support 20 (Surfraz *et al* 2007 J Med Chem; 50: 1418-22).

HYNIC has also been reported in a route for  $^{18}$ F labelling of biomolecules, see e.g. Lee *et al* (2006 Nuc Med Biol; 33: 677-83); and, Bruus-Jensen *et al* (2006 Nuc Med Biol; 33: 173-83). These publications disclose functionalisation of a biomolecule with HYNIC. The hydrazine group of the HYNIC-functionalised 25 biomolecule reacts with the aldehyde of  $^{18}$ F-fluorobenzaldehyde to form a stable hydrazone bond, thereby providing an  $^{18}$ F-labelled biomolecule.

In a preferred embodiment of the method of the invention, A<sup>1</sup> in the compound of Formula II is NH, and R<sup>1</sup> is the group -L<sup>2</sup>-R<sup>\*</sup>. A preferred biomolecule in the context of the present invention is a peptide, a protein, or an antibody. The 30 terms “peptide”, “protein” and “antibody” have their common meaning in the art,

as now briefly described. A peptide is a short polymer formed from the linking, in a defined order, of amino acids, wherein one amino acid residue is linked to the next by an amide bond. Conventionally, peptides are regarded as comprising up to 50 amino acid residues. Proteins are polypeptide molecules 5 or consist of multiple polypeptide subunits. Antibodies, also known as immunoglobulins, are gamma globulin proteins that are used by the immune systems of vertebrates.

In another embodiment, A<sup>1</sup> of Formula II is O, such that the compound of Formula II is an aminoxy compound. Like hydrazines, aminoxy compounds are 10 hypernucleophiles and provide the advantage over amino compounds of being more reactive. Some aminoxy compounds are available commercially that can act as starting points for obtaining a variety of compounds of Formula II wherein A<sup>1</sup> is O, e.g. L-alpha-Aminoxy-beta-phenylpropionic acid hydrobromide (Apollo Scientific Ltd.), aminoxyisobutyric acid hydrochloride (Fine and 15 Performance Chemicals Ltd.).

The prior art describes how to obtain aminoxy analogues of peptide compounds by replacement of an amino group with aminoxy (see for example Briggs and Morely 1979 JCS Perkin I: 2138-43; Yang *et al* 2001 J Org Chem; 66(22): 7303-12; Yang *et al* 1999 J Am Chem Soc; 121 (3): 589-590). 20 Aminoxy-functionalised lipids are reported by Perouzel *et al* (2003 Bioconj Chem; 14: 884-98).

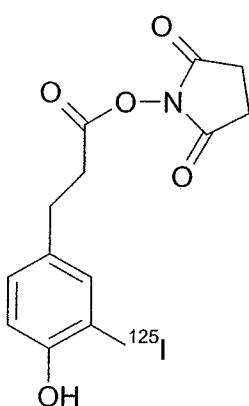
The compound of Formula III is an active ester. Where R<sup>2</sup> is -L<sup>1</sup>-Ar<sup>1</sup> the compound of Formula III is a radioiodoaryl functionalised with an active ester. Where R<sup>2</sup> is -L<sup>2</sup>-R<sup>\*</sup> the compound of Formula III is a biomolecule functionalised 25 with an active ester.

Many active esters are readily available commercially. Conversion of a carboxylic acid to an active ester may be carried out by reaction with carbodiimide/phenol or transesterification reagent, e.g. pentafluorophenyl trifluoroacetate or *p*-nitrophenyl trifluoroacetate. It is well-known in the art to 30 use active esters in the synthesis of peptide compounds (see for example

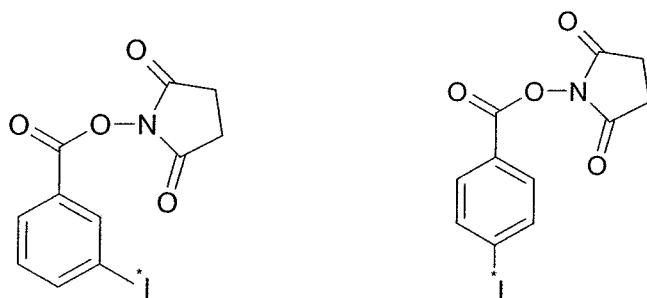
Grant "Synthetic Peptides" 2002; Oxford University Press: 137-139; Anderson *et al* 1963 J Am Chem Soc; 85: 3039). A preferred active ester of the present invention is *N*-Hydroxysuccinimide (NHS) ester.

In a preferred embodiment, the compound of Formula III comprises -L<sup>1</sup>-Ar<sup>1</sup>,  
 5 and such compounds are known in the art of indirect radioiodination. Preferably, L<sup>1</sup> is a bond or is a C<sub>1-3</sub> alkylene linker. Ar<sup>1</sup> is preferably radioiodophenyl or radioiodophenol, i.e. phenyl substituted with radioiodine and optionally substituted with hydroxyl.

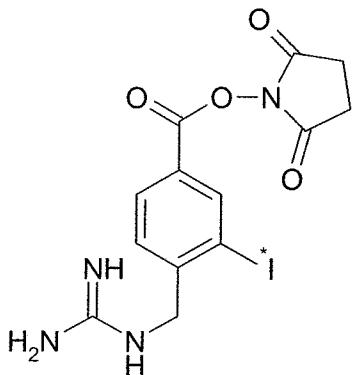
Indirect radioiodination methods typically involve conjugation of an iodine  
 10 moiety functionalised with an NHS ester with a primary amine. For example, Bolton and Hunter (1973 Biochem J;133: 529-39) describe labelling of proteins with <sup>125</sup>I by reaction of <sup>125</sup>I iodinated 3-(4-hydroxyphenyl)propionic acid NHS ester (illustrated below) with free amino groups in the protein molecule.



15 Koziorowski *et al* (1998 Appl Radiat Isot; 49(8): 955-9) teach how to obtain radioiodinated NHS 3- and 4-iodobenzoate (illustrated below, wherein <sup>\*</sup>I is radioiodine), which are commonly-used indirect radioiodination agents.



Another protocol involves reacting the free amino groups of a protein with *N*-succinimidyl 4-guanidinomethyl-3-[<sup>125</sup>I]iodobenzoate (illustrated below; Vaidyanathan & Zalutsky 2007 Nature Protocols; 2: 282-6).

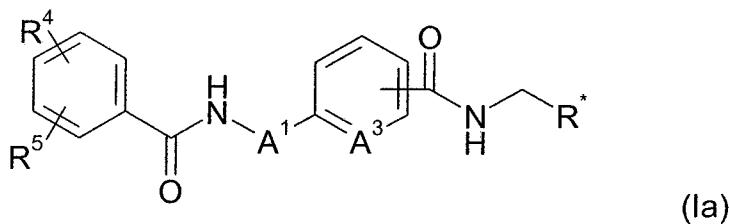


5 The above-described radioiodinated NHS ester compounds are preferred for use in the method of the present invention as compounds of Formula III wherein R<sup>2</sup> is -L-Ar<sup>1</sup>.

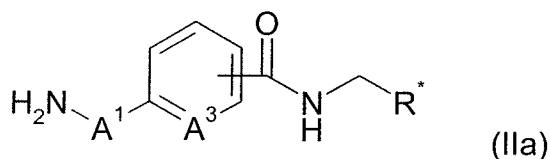
In a preferred embodiment, L<sup>1</sup> and L<sup>2</sup> are independently either a bond or a C<sub>1-3</sub> alkylene linker.

10 Preferred Compounds

In a preferred embodiment, said compound of Formula I is a compound of Formula Ia:

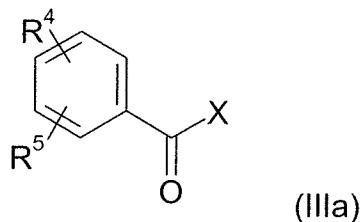


said compound of Formula II is a compound of Formula IIa:



15

said compound of Formula III is a compound of Formula IIIa:



wherein A<sup>3</sup> is N or CH, R<sup>\*</sup> is as defined above, one of R<sup>4</sup> and R<sup>5</sup> is radioiodine and the other of R<sup>4</sup> and R<sup>5</sup> is hydrogen or hydroxyl, and X is an active ester as defined above.

Preferably, A<sup>3</sup> is CH, which provides the advantage that the reaction can proceed more specifically at the A<sup>1</sup>-NH<sub>2</sub> due to the absence of a heteroatom on the aryl ring. This is because the nitrogen heteroatom withdraws electrons from the hydrazine NH<sub>2</sub> making this less reactive towards the active ester. When A<sup>3</sup> is CH, i.e. the ring is a phenyl ring, the electron withdrawing effect will not be exerted.

In a preferred embodiment, the method of the invention is used to obtain a radiopharmaceutical composition (described as a separate aspect of the invention below). In this case, the method of the invention further comprises one or more of the following steps:

- 15 (i) removal of any protecting groups from the radioiodinated compound of Formula I; and/or,
- (ii) admixing the radioiodinated compound of Formula I with a biocompatible carrier to form a radiopharmaceutical composition; and/or,
- 20 (iii) sterilisation of the radiopharmaceutical composition of step (ii); and/or
- (iv) depyrogenation of the radiopharmaceutical composition of step (ii).

Removal of protecting groups may be carried out by methods well-known to the

person skilled in the art (Greene and Wuts, *supra*).

Methods for sterilisation and depyrogenation are also well-known to the skilled person.

“Sterilisation” refers to any process that effectively kills or eliminates 5 transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) from a surface, equipment, article of food or medication, or biological culture medium. Sterilisation can be achieved through application of heat, chemicals, irradiation, high pressure or filtration.

“Depyrogenation” refers to the removal of pyrogens from solution, most 10 commonly from injectable pharmaceuticals. A “pyrogen” is defined as any substance that can cause a fever, e.g. the bacterial substance lipopolysaccharide (LPS), present in the cell wall of some bacteria, is a pyrogen. Depyrogenation may be achieved through filtration, distillation, chromatography, or inactivation.

15 Automated Synthesis

In a preferred aspect, the method of the invention, as suitably and preferably defined herein, is automated. Automated processes are particularly useful in the synthesis of radioactive compounds as radiation exposure to the operator is reduced. PET radiotracers in particular are now often conveniently prepared 20 on an automated radiosynthesis apparatus. There are several commercially-available examples of such apparatus, including TRACERlab<sup>TM</sup> and FASTlab<sup>TM</sup> (both from GE Healthcare). Such apparatus commonly comprises a “cassette”, often disposable, in which the radiochemistry is performed, which is fitted to the apparatus in order to perform a radiosynthesis. The cassette normally includes 25 fluid pathways, a reaction vessel, and ports for receiving reagent vials as well as any solid-phase extraction cartridges used in post-radiosynthetic clean up steps.

### Radioiodinated Compounds

The radioiodinated compound of Formula I, as suitably and preferably defined herein for the method of the invention, is an *in vivo* imaging agent wherein the radioiodine substituent of Ar<sup>1</sup> comprises <sup>123</sup>I, <sup>124</sup>I or <sup>131</sup>I. By the term "*in vivo* imaging agent" is meant a compound designed to target a particular physiology or pathophysiology in a mammal, and which can be detected following its administration to the mammalian body *in vivo*. When the radioiodine-containing compound obtained by the method of the present invention is an *in vivo* imaging agent, the iodine atom in the radioiodoaryl moiety is selected from <sup>123</sup>I, <sup>124</sup>I, and <sup>131</sup>I. The isotopes <sup>123</sup>I and <sup>131</sup>I emit gamma rays, which can be detected using single-photon emission tomography (SPECT). The isotope <sup>124</sup>I emits positrons, which can be detected using positron emission tomography (PET). Preferred isotopes of iodine for the *in vivo* imaging agent of the invention are <sup>123</sup>I and <sup>124</sup>I, most preferably <sup>123</sup>I.

15    Radiopharmaceutical Composition

The radioiodinated compound obtained by the method of the invention can be used for the preparation of a radiopharmaceutical composition, comprising the radioiodinated compound of Formula I as defined herein together with a biocompatible carrier suitable for mammalian administration.

20    A "radiopharmaceutical composition" is defined in the present invention as a formulation comprising the radioiodinated compound of the present invention together with a biocompatible carrier in a form suitable for mammalian administration, preferably administration to humans.

25    The "biocompatible carrier" is a fluid, especially a liquid, in which the radioiodine-containing compound as defined herein is suspended or dissolved, such that the radiopharmaceutical composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier medium is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution

such as saline (which may advantageously be balanced so that the final product for injection is either isotonic or not hypotonic); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). The biocompatible carrier medium may also comprise biocompatible organic solvents such as ethanol. Such organic solvents are useful to solubilise more lipophilic compounds or formulations. Preferably the biocompatible carrier medium is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier medium for intravenous injection is suitably in the range 4.0 to 10.5.

The radiopharmaceutical composition may be administered parenterally, i.e. by injection, and is most preferably an aqueous solution. Such a composition may optionally contain further ingredients such as buffers; pharmaceutically acceptable solubilisers (e.g. cyclodextrins or surfactants such as Pluronic, Tween or phospholipids); pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or *para*-aminobenzoic acid).

#### Kit and Cassette

20 The method of the invention may conveniently be carried out by means of a kit. Therefore, in another aspect, the present invention provides a kit for carrying out the method of the invention, as suitably and preferably defined herein, wherein said kit comprises:

25 (i) a first vessel comprising either the compound of Formula II as defined herein, or the compound of Formula IIa as defined herein; and,

(ii) a second vessel comprising either the compound of Formula III as defined herein, or the compound of Formula IIIa as defined herein.

When the method of the invention is automated, the first and second vessels of the kit may be housed in a disposable or removable cassette designed for use

with an automated synthesis apparatus. Therefore, in another aspect, the present invention further provides a cassette for an automated synthesis apparatus comprising the components as defined above for the kit of the invention.

- 5 It is particularly convenient to use a kit or a cassette as described above for the preparation of the radiopharmaceutical composition of the invention. Therefore in a preferred embodiment, either the kit or the cassette of the invention is used in the preparation of the radiopharmaceutical composition of the invention as described herein.
- 10 In a further aspect, the present invention provides for the use of the kit or of the cassette of the invention to carry out the method of the invention as described herein.

For the kit and the use thereof, the cassette and the use thereof, Formulas I-III and Formulas Ia-IIia, and preferred embodiments thereof, are as described  
15 above for the method of the invention.

#### **Brief Description of the Examples**

Unless otherwise specified, all reagents described in the Examples below were obtained from Sigma-Aldrich or BDH.

50 mM pH7.4 phosphate buffer was prepared using 40.5ml 0.2M Na<sub>2</sub>HPO<sub>4</sub> +  
20 9.5 ml 0.2M NaH<sub>2</sub>PO<sub>4</sub> made up to 200ml with water.

0.2M pH4 ammonium acetate buffer was prepared using 18ml 0.2M NH<sub>4</sub>OAc +82ml 0.2M HOAc made up to 1 litre with water.

Example 1 is a comparative example describing a route for indirect iodination based on prior art teachings relating to <sup>18</sup>F labelling *via* conjugation of a  
25 HYNIC-functionalised biomolecule with <sup>18</sup>F-fluorobenzaldehyde.  
Radiochemical purity (RCP) was 15% with the radioiodination step carried out at 70°C.

Example 2 describes an indirect radioiodination method of the invention comprising reaction of a HYNIC type hydrazine, 2-hydrazinopyridine with *N*-succinimidyl-3-iodo benzoic acid. RCP was 21% at 37°C. The method of this example has the advantage over the prior art method described in Example 1  
5 that it is carried out at physiological temperature, a temperature which is optimal for the preservation of biomolecular structure and function. In addition, the method of Example 2 demonstrated an improved yield over the prior art method of Example 1.

Example 3 describes the synthesis of 3-trimethylstannyl-benzoic acid 2,5-dioxo-  
10 pyrrolidin-1-yl ester, an intermediate used in the method of Example 2.

Example 4 demonstrates that NHS ester reacts more readily with hydrazine groups than with amino groups.

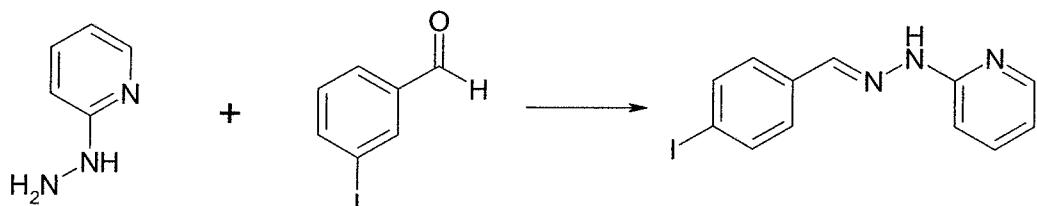
#### **Abbreviations used in the Examples**

Ac	acetyl
15 DCC	dicyclohexylcarbodiimide
DMF	dimethylformamide
h	hour(s)
HPLC	high performance liquid chromatography
LCMS	liquid chromatography mass spectrometry
20 Lys	lysine
MeOH	methanol
min	minute(s)
MS	mass spectrometry
m/z	mass-to-charge ratio

NHS	<i>N</i> -Hydroxysuccinimide
NMR	nuclear magnetic resonance
RCP	radiochemical purity
TFA	trifluoroacetic acid
5	THF tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
<i>t</i> <sub>R</sub>	retention time
UV	ultraviolet

10 **Examples**

**Comparative Example 1: Synthesis of *N*-[1-(4-*Iodo-phenyl*)-meth-(*E*)-*ylidene*]-*N*'-pyridin-2-yl-hydrazine**



**1(i) Reaction carried out with <sup>127</sup>I**

15 <sup>127</sup>I conjugation of 4-iodobenzaldehyde and 2-hydrazinopyridine was performed in order to purify the product and analyse by mass spectrometry to confirm its identity.

2-hydrazinopyridine and 4-iodobenzaldehyde were dissolved in ethanol to give 9.2mM and 4.3mM solutions.

20 To a 10ml silanised sealed glass vial the following were added in the given

order:-

10 $\mu$ l 2-hydrazinopyridine ( $9.2 \times 10^{-8}$  moles)

170 $\mu$ l 50 mM pH7.4 phosphate buffer

21 $\mu$ l 4-iodobenzaldehyde ( $9.1 \times 10^{-8}$  moles)

5 The preparation was heated at 37°C for 20 minutes before analysing by HPLC:

Column - Phenomenex Luna C18(2) 5 $\mu$  4.6×150mm

Flow – 1ml/min

Detection – UV 254nm and Bioscan radio detector

Solvent A – 0.1%TFA in water

10 Solvent B – 0.1%TFA in acetonitrile

Gradient –	0min 0% B
	20min 70% B
	25min 90% B
	27min 90% B
15	28min 0% B
	35min 0% B

The peak for N-[1-(4-iodo-phenyl)-meth-(E)-ylidene]-N'-pyridin-2-yl-hydrazine was HPLC purified. Mass spectrometry showed a peak at 324 which is the correct mass for the expected product.

20 1(ii) Reaction carried out with  $^{123}I$

2-hydrazinopyridine was dissolved in ethanol to give a 9.2mM solution. 5 $\mu$ l of peracetic acid was diluted in 5ml water to give a 5mM solution.

#### Synthon Preparation

25 37 $\mu$ l 2.8mM 4-tri-n-butyltinbenzaldehyde (prepared according to the method of Sessler *et al* J Am Chem Soc 1995; 117(2): 704-14) in ethanol ( $1.0 \times 10^{-7}$  moles) was added to a silanised sealed 1.5ml 'V' shaped vial. 200 $\mu$ l 0.2M pH4 ammonium acetate buffer and 10 $\mu$ l 1mM Na $^{127}I$  in 0.05M NaOH ( $1.0 \times 10^{-7}$

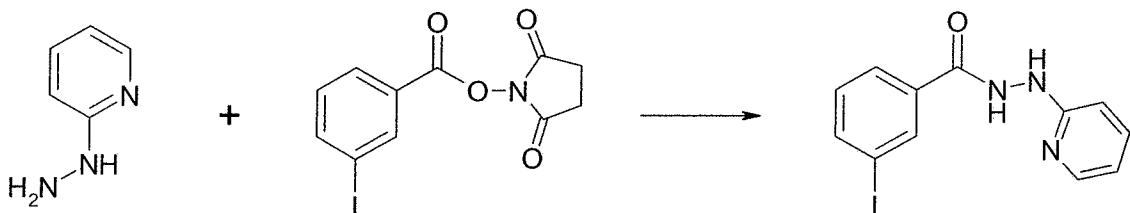
$^8$ moles) were added to a  $^{123}\text{I}$  vial. 10 $\mu\text{l}$  5mM peracetic acid was added to the  $^{123}\text{I}$  vial. The contents of the  $^{123}\text{I}$  vial were transferred to the silanised 1.5ml 'V' shaped vial.

Conjugation

5 4.4 $\mu\text{l}$  2-hydrazinopyridine ( $4.0 \times 10^{-8}$  moles), 100 $\mu\text{l}$  crude 4-iodobenzaldehyde and 100 $\mu\text{l}$  50mM pH7.4 phosphate buffer were added to a silanised sealed 10ml glass vial and the prep heated at 70°C for 10 minutes.

HPLC, carried out as per Example 1, showed 15% conjugate at  $t_{\text{R}}$  14.1min. There was 38% iodide and 47% synthon remaining.

10 **Example 2: Synthesis of 3-iodo-benzoic acid N'-pyridin-2-yl-hydrazide**



2(i) Reaction carried out with  $^{127}\text{I}$

15 3-iodo-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester was prepared by reaction of 3-iodo benzoic acid (1g, 4mmol), N-hydroxysuccinimide (464mg, 4mmol) and dicyclohexylcarbodiimide (DCC) (4mls of a 1M solution in dichloromethane, 4mmol). The reaction mixture was stirred at room temperature for 6 hours in 10ml DMF. The resulting white precipitate was filtered and discarded and the resulting filtrate reduced *in vacuo* and purified by column chromatography giving a 29% yield.

20  $^{127}\text{I}$  conjugation of 3-iodo-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester and 2-hydrazinopyridine was performed in order to purify the product and analyse by mass spectrometry to confirm its identity.

2-hydrazinopyridine was dissolved in ethanol to give a 9.2mM solution.

To a 10ml sealed silanised glass vial the following were added in the given order:-

20 $\mu$ l 2-hydrazinopyridine ( $1.8 \times 10^{-7}$  moles)

180 $\mu$ l 50mM pH7.4 phosphate buffer

5 63 $\mu$ l 2.9mM 3-iodo-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester ( $1.8 \times 10^{-7}$  moles)

The preparation was heated at 37°C for 30 minutes before analysing by HPLC as described above for Example 1. The peak for 3-iodo-benzoic acid N'-pyridin-2-yl-hydrazide was HPLC purified and analysed by mass spectrometry. Mass spectrometry showed a peak at 340 which is the correct mass for the

10 expected product.

#### 2(ii) Reaction carried out with $^{123}\text{I}$

##### 2(ii)(a) Synthon preparation

2-hydrazinopyridine was dissolved in ethanol to give a 17mM solution. 10 $\mu$ l of peracetic acid was diluted in 5ml water then 100 $\mu$ l of this solution diluted to 1ml with water to give a 1mM solution. 3-trimethylstannyl-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (synthesis described in Example 3 below) was dissolved in 1% acetic acid in methanol to give a 0.26mM solution. A C18 SepPak was conditioned with 5ml acetonitrile followed by 10mls water.

20 38 $\mu$ l 3-trimethylstannyl-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester solution

( $1.0 \times 10^{-8}$  moles) was added to a silanised sealed 1.5ml 'V' shaped vial.

200 $\mu$ l 0.2M pH4 ammonium acetate buffer and 10 $\mu$ l 1mM  $\text{Na}^{127}\text{I}$  in 0.05M

NaOH ( $1.0 \times 10^{-8}$  moles) were added to a  $^{123}\text{I}$  vial.

The contents of the  $^{123}\text{I}$  vial were transferred to the silanised 1.5ml 'V' shaped vial.

25 The crude synthon was loaded on the pre prepared C18 Sep-Pak. Iodide was eluted with 5ml water. The synthon was eluted with 2.5ml acetonitrile into a

silanised vial. The acetonitrile was removed under high vacuum.

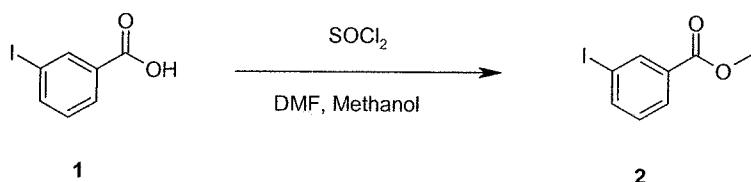
2(ii)(c) Conjugation

6 $\mu$ l 2-hydrazinopyridine ( $1.0 \times 10^{-7}$  moles) and 194 $\mu$ l 50 mM pH7.4 phosphate buffers were added to the dried synthon and the prep heated at 37°C for 30 minutes.

HPLC showed 21% product at  $t_R$  11.3min. There was 8% synthon left in the crude reaction.

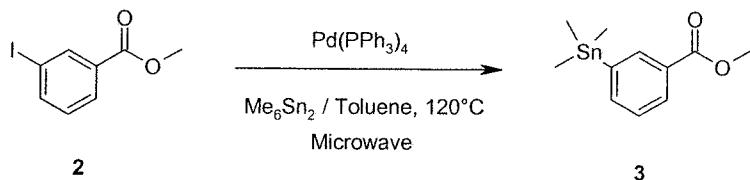
**Example 3: Synthesis of 3-trimethylstannyli-benzoic acid 2,5-dioxo-pyridin-1-yl ester**

10 3(i) Methyl 3-iodobenzoate (2)



One drop of dimethylformamide was added to a stirring suspension of 3-iodobenzoic acid (1.0g, 4.03 mmol), in thionyl chloride (20 ml). The mixture was then heated at 80°C for 18 hours. After cooling to ambient temperature, the solvent was completely evaporated, then methanol (20 ml) was slowly added and the mixture stirred at ambient temperature for 30 minutes. Evaporation of methanol afforded the crude product as oil which solidified on standing at ambient temperature. The crude product was purified by flash chromatography using ethyl acetate/ hexane (1:1) and the pure product was obtained as slightly yellow crystals (824 mg, 78.4%).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$  3.95 (s, 3H,  $\text{CH}_3$ ), 7.20 (t, 1H), 7.9 (d, 1H), 8.0 (d, 1H), 8.20 (s, 1H)

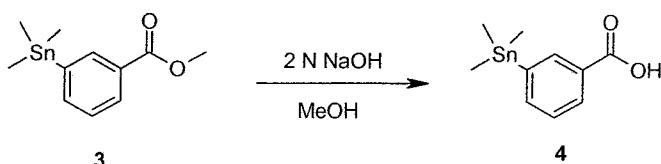
3(ii) 3-Trimethylstannyl-benzoic acid methyl ester (3)

To a mixture of methyl 3-iodobenzoate 200 mg, 0.76 mmol), and hexamethylditin (643  $\mu$ l, 3.1 mmol) in toluene (8 ml) was added tetrakis (triphenylphosphine) palladium (0) (180 mg, 0.155 mmol (10 mol %)). The reaction tube was flushed with argon, capped and heated in a microwave at 120°C for 15 minutes. After cooling, TLC (ethyl acetate/hexane, 1:1) showed complete conversion to product. Then the black suspension was filtered (filter paper) and the resulting dark coloured solution was evaporated to dryness. The 10 residue was dissolved in ethyl acetate (15 ml) and extracted with water (6x10 ml).

Then the organic phase was dried ( $\text{MgSO}_4$ ) filtered and evaporated under reduced pressure to afford the crude product as a dark oil (463 mg) which was purified by flash chromatography (Ethyl acetate: hexane, 1:1) to afford a slightly 15 yellow oil. Yield: 344 mg (74%).

**NB:** Microwave irradiation was carried out in a Personal Chemistry Emrys synthesizer.

<sup>1</sup>H NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$  0.32 (s, 9H, Sn(CH<sub>3</sub>)<sub>3</sub>), 3.95(s, 3H, CH<sub>3</sub>), 7.40 (t, 1H), 7.69 (d, 1H), 8.0 (d, 1H), 8.20 (s, 1H)

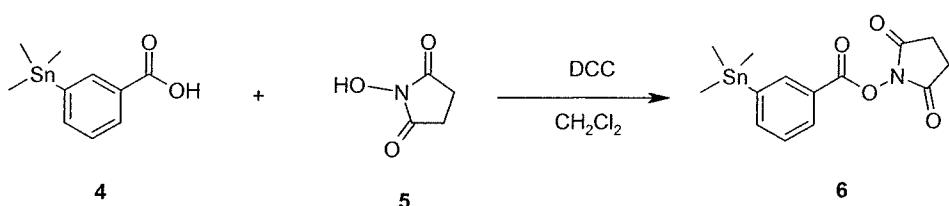
20 3(iii) 3-Trimethylstannyl-benzoic acid (4)

3-Trimethylstannyl-benzoic acid methyl ester (148 mg, 0.50 mmol) was

dissolved in methanol (3 ml) and while stirring at ambient temperature, aqueous 2N sodium hydroxide (1ml, 2 mmol) was slowly added after which the mixture was stirred at this temperature for 3 hours when monitoring by HPLC indicated that the hydrolysis was completed. Then the mixture was evaporated to dryness and the residue re-dissolved in water (4 ml) and acidification by slow addition of 1M hydrochloric acid resulted in precipitation of the product. The mixture extracted with dichloromethane (3x10ml) and the combined dichloromethane phases was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated to afford the crude product as a white solid material. It was used in the next step without further purification. Yield: 107 mg (76%).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$  0.39 (s, 9H,  $\text{Sn}(\text{CH}_3)_3$ ), 7.42 (t, 1H), 7.72 (d, 1H), 8.02 (d, 1H), 8.15 (s, 1H)

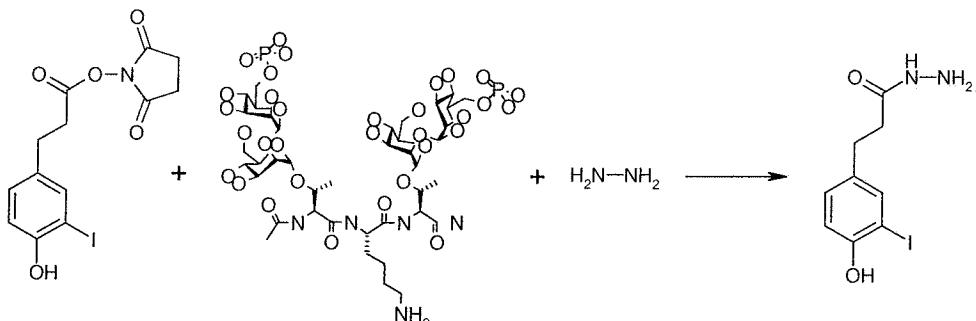
3(iv) 3-Trimethylstannyl-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (6)



To a solution of 3-trimethylstannyl-benzoic acid (101 mg, 0.35 mmol) in dichloromethane (3 ml) was added *N,N*'-dicyclohexylcarbodiimide, DCC (72 mg, 0.35 mmol) followed by *N*-hydroxy-succinimide (40.28 mg, 0.35 mmol). The clear and colourless mixture was left to stir at ambient temperature and a white precipitate started to appear after ca. 15 minutes. The reaction as stopped after 1 hour when TLC (ethyl acetate/hexane, 1:1) showed complete disappearance of the starting material. The mixture was filtered and the residue that was recovered after evaporation of the solvent was purified by flash chromatography using ethyl acetate/ hexane, 1:1. The pure product was obtained as colourless oil. Yield: 90 mg (67%).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$  0.25 (s, 9H,  $\text{Sn}(\text{CH}_3)_3$ ), 2.88 (broad s, 4H,  $(\text{CH}_2)_2$ ), 7.42 (t, 1H), 7.74 (d, 1H), 8.03 (d, 1H), 8.20 (s, 1H)

**Example 4: Synthesis of 3-(4-Hydroxy-3-iodo-phenyl)-propionic acid hydrazide**



5mg of Ac-Thr(Mannose-6-Phosphate-Mannose)-Lys-Thr(Mannose-6-Phosphate-Mannose)-NH<sub>2</sub> ("glycopeptide"; synthesized according to methods disclosed by Christensen *et al* J Chem Soc Perkin Trans 1994; 10: 1299-1310) was reacted with 1.7mg of iodinated Bolton-Hunter NHS ester ("BH"; Wako pure chemical industries 199-09341) and sym-Collidine (1.1μL; Fluka 27690) were mixed in 1ml DMF (Rathburn PTS6020) and the mixture (cloudy solution) was heated at 35°C for 2h. All BH was consumed and a new peak corresponding to BH-hydrazine conjugate was observed as sole product. More BH (0.5mg) and sym-Collidine (1.1μL) were added and stirring at 35°C was continued for 2h. Most BH was consumed and the peak corresponding to BH-hydrazine had increased. Acetone (100μL) was added in order to react with remaining residual hydrazine and the mixture stirred over night at room temperature. More BH (2.0 mg) was then added and stirring continued at 35°C for 90min. No consumption of BH was observed indicating that all hydrazine was quenched. More sym-Collidine (2.2 μL) was added and stirring continued at 35°C for 2 hrs. No product had been formed. Water (500 μL) was added to better dissolve the starting material and stirring continued at 35°C overnight. Small amount of product was formed. More water (2 mL) and BH (1.8 mg) were added, then THF (2 mL) was added to better dissolve BH, and stirring continued over night. More of the product was formed together with hydrolysed BH. The solvents were partly removed *in vacuo* and the residue diluted with water/0.1 % TFA (8 mL) and subjected to HPLC purification.

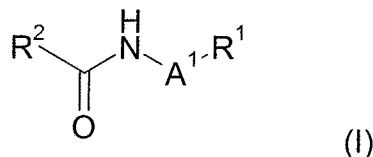
Monitoring of the above-described reaction was carried out by LCMS using a Phenomenex Luna 3 $\mu$  C18 (2) 20 x 2 mm column, with detection at 214 nm. Solvent A: H<sub>2</sub>O/0.1 % TFA; solvent B: CH<sub>3</sub>CN/0.1 % TFA; flow: 0.6 mL/min; gradient: 0-30 % B over 5 min, t<sub>R</sub>: 3.11 min (product); 3.40 min (BH-hydrazine),  
5 found m/z: 1471.9 (product); 306.9 (BH-hydrazine), expected MH<sup>+</sup>: 1472.3 (P); 307 (BH-hydrazine).

Purification was carried out by HPLC using a Phenomenex Luna 5 $\mu$  C18 (2) 250 x 21.20 mm column, with detection at 214 nm. Solvent A: H<sub>2</sub>O/0.1 % TFA; solvent B: CH<sub>3</sub>CN/0.1 % TFA; flow: 10 mL/min; gradient: 0-30 % B over 40 min;  
10 t<sub>R</sub>: 36-40 min.

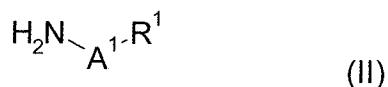
Analysis of purified product was carried out by LCMS using a Phenomenex Luna 3 $\mu$  C18 (2) 20 x 2 mm column with detection at 214 nm. Solvent A: H<sub>2</sub>O/0.1 % TFA; solvent B: CH<sub>3</sub>CN/0.1 % TFA; flow: 0.6 mL/min; gradient: 0-30 % B over 5 min; t<sub>R</sub>: 3.13 min (P). Found m/z: 1471.9, expected MH<sup>+</sup>: 1472.3.  
15 Yield 0.9 mg (15 %).

## Claims

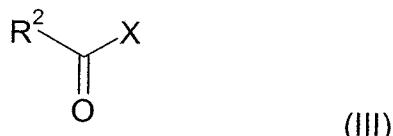
1) A method for the synthesis of a radioiodinated compound of Formula I:



or a salt or solvate thereof, said method comprising reaction of a compound of  
5 Formula II:



with a compound of Formula III:



wherein:

10  $\text{A}^1$  is either NH or O;

one of  $\text{R}^1$  and  $\text{R}^2$  is the group  $-\text{L}^1-\text{Ar}^1$  wherein:

15  $\text{L}^1$  is a bond or is a bivalent linker comprising 1-3  $\text{L}'$  linker units wherein  $\text{L}'$  is selected from  $-\text{CO}-$ ,  $-\text{CR}'_2-$ ,  $-\text{CR}'=\text{CR}'-$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{CR}'_2\text{CO}_2-$ ,  $-\text{CO}_2\text{CR}'_2-$ ,  $-\text{NR}'-$ ,  $-\text{NR}'\text{CO}-$ ,  $-\text{CONR}'-$ ,  $-\text{NR}'-$ ,  $-(\text{C}=\text{O})\text{NR}'-$ ,  $-\text{NR}'(\text{C}=\text{S})\text{NR}'-$ ,  $-\text{SO}_2\text{NR}'-$ ,  $-\text{NR}'\text{SO}_2-$ ,  $-\text{CR}'_2\text{OCR}'_2-$ ,  $-\text{CR}'_2\text{SCR}'_2-$ ,  $-\text{CR}'_2\text{NR}'\text{CR}'_2-$ , a  $\text{C}_{5-12}$  arylene group, and a  $\text{C}_{3-12}$  heteroarylene group, wherein  $\text{R}'$  is hydrogen or  $\text{C}_{1-3}$  alkyl; and,

20  $\text{Ar}^1$  is a 6-membered  $\text{C}_{3-6}$  aryl group, substituted with radioiodine, and with 0-3 other substituents selected from  $\text{C}_{1-3}$  alkyl, halo, amino, carboxyl, hydroxyl, or protected versions thereof, and wherein said aryl group has 0-3 heteroatoms selected from N, S and O;

and the other of R<sup>1</sup> and R<sup>2</sup> is the group -L<sup>2</sup>-R<sup>\*</sup>, wherein:

L<sup>2</sup> is a bond or is a bivalent linker comprising 1-6 L<sup>\*</sup> linker units wherein L<sup>\*</sup> is as defined for L<sup>1</sup>; and,

R<sup>\*</sup> is a biomolecule;

5 wherein R<sup>1</sup> and R<sup>2</sup> optionally comprise suitable protecting groups;

and wherein X represents an active ester group.

2) The method as defined in Claim 1 wherein A<sup>1</sup> is NH.

3) The method as defined in Claim 1 wherein A<sup>1</sup> is O.

4) The method as defined in any one of Claims 1-3 wherein L<sup>1</sup> is a bond or a C<sub>1-3</sub> alkylene linker.

5) The method as defined in any one of Claims 1-4 wherein L<sup>2</sup> is a bond or a C<sub>1-3</sub> alkylene linker.

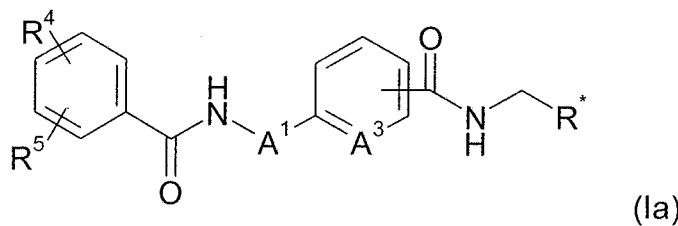
6) The method as defined in any one of Claims 1-5 wherein R<sup>1</sup> is the group -L<sup>2</sup>-R<sup>\*</sup> and R<sup>2</sup> is the group -L<sup>1</sup>-Ar<sup>1</sup>.

15 7) The method as defined in any one of Claims 1-6 wherein Ar<sup>1</sup> is phenyl substituted with radioiodine, and optionally substituted with hydroxyl or guanidine.

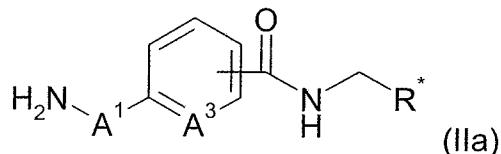
8) The method as defined in any one of Claims 1-7 wherein R<sup>\*</sup> is a biomolecule that is an antibody, a peptide or a protein.

20 9) The method as defined in Claim 1 wherein:

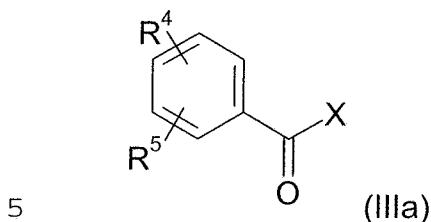
said compound of Formula I is a compound of Formula Ia:



said compound of Formula II is a compound of Formula IIa:



said compound of Formula III is a compound of Formula IIIa:



wherein  $A^3$  is N or CH,  $R^*$  is as defined in any one of Claims 1 or 8, one of  $R^4$  and  $R^5$  is radioiodine and the other of  $R^4$  and  $R^5$  is hydrogen or hydroxyl, and X is as defined in Claim 1.

10) The method as defined in Claim 9 wherein  $A^1$  is NH.

10 11) The method as defined in Claim 9 wherein  $A^1$  is O.

12) The method as defined in any one of Claims 9-11 wherein  $A^3$  is CH.

13) The method as defined in any one of Claims 9-12 wherein one of  $R^4$  and  $R^5$  is radioiodine and the other is hydrogen.

14) The method as defined in any one of Claims 1-13 wherein said radioiodinated compound is an *in vivo* imaging agent and the radioiodine substituent of  $Ar^1$  comprises  $^{123}I$ ,  $^{124}I$  or  $^{131}I$ .

15) The method as defined in any one of Claims 1-14 further comprising one or

more of the following steps in any order:

(i) removal of any protecting groups from the radioiodinated compound of Formula I; and/or,

5 (ii) admixing the radioiodinated compound of Formula I with a biocompatible carrier to form a radiopharmaceutical composition; and/or,

(iii) sterilisation of the radiopharmaceutical composition of step (ii); and/or

(iv) depyrogenation of the radiopharmaceutical composition of step (ii).

16) The method as defined in any one of Claims 1-15 which is automated.

17) The method as defined in Claim 16 which is carried out on an automated  
10 synthesis apparatus.

18) A radiopharmaceutical composition comprising the radioiodinated compound of Formula I as defined in the method of any one of Claims 1-14 together with a biocompatible carrier in a form suitable for mammalian administration.

19) A kit for carrying out the method as defined in any one of Claims 1-17  
15 comprising:

(i) a first vessel comprising either the compound of Formula II as defined in the method of any one of Claims 1-8, or the compound of Formula IIa as defined in the method of any one of Claims 9-12; and,

20 (ii) a second vessel comprising either the compound of Formula III as defined in the method of any one of Claims 1 and 4-8, or the compound of Formula IIIa as defined in the method of either Claim 9 or Claim 13.

20) A cassette for carrying out the method as defined in Claim 17, said cassette comprising first and second vessels as defined for the kit of Claim 19.

25 21) Use of the kit as defined in Claim 19 to carry out the method as defined in any

one of Claims 1-17.

22) Use of the cassette as defined in Claim 20 to carry out the method as defined in Claim 17.

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/051053

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C07C243/38 A61K51/04  
ADD.

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
C07C A61K

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

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

EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 279 887 A (BALDWIN R M ET AL.) 21 July 1981 (1981-07-21) abstract; claims 1-7; example 13 Table; column 2 ----- -/-	1-7,14, 15,18-22 8-13,16, 17
Y		

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2010	27/05/2010
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Jenn, Thierry

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/051053

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RANDERATH ET AL.: "3-([3- <sup>125</sup> I]iodo-4-hydroxyphenyl)propionyl carbohydrazide, a new radioiodination reagent for ultrasensitive detection and determination of periodate-oxidized nucleoside derivatives and other carbonyl compounds" ANALYTICAL BIOCHEMISTRY, vol. 115, no. 2, August 1981 (1981-08), pages 391-397, XP024828669 ACADEMIC PRESS INC, NEW YORK ISSN: 0003-2697 DOI: 10.1016/0003-2697(81)90023-3 [retrieved on 1981-08-01] abstract; figures 1,4	1,2,4-7, 14,15, 18-22
Y	-----	8-13,16, 17
X	LIU W ET AL.: "Chemical modification of ribonucleosides with N-acetyl-3,5-di[ <sup>125</sup> I]iodotyrosyl hydrazide" BIOORGANIC CHEMISTRY, vol. 14, no. 1, 1 March 1986 (1986-03-01), pages 1-7, XP024022462 ACADEMIC PRESS INC., NEW YORK, NY, U ISSN: 0045-2068 DOI: 10.1016/0045-2068(86)90012-X [retrieved on 1986-03-01] abstract	1,2,4-7, 14,15, 18-22
Y	page 5, line 42 - page 6, line 2 page 7, line 1 - line 15	8-13,16, 17
X	SOMAWARDHANA C W ET AL.: "Synthesis of 2-[ <sup>123</sup> I] and <sup>124</sup> I]-iodoisonicotinic acid hydrazide - Potential radiotracers for tuberculosis" APPLIED RADIATION AND ISOTOPES, vol. 42, no. 3, 1991, pages 215-220, XP24707195 GB ISSN: 0883-2889 DOI: 10.1016/0883-2889(91)90079-G abstract; figure 2	1,2,4-7, 14,15, 18-22
Y	page 218	8-13,16, 17
X	WO 2006/010165 A2 (ONCOLOGIC INC [US] ET AL.) 26 January 2006 (2006-01-26) figure 46; example 13; compounds 5344,5345	18
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/051053

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALDWIN R M ET AL.: "Structure-activity relationships of iodine-123-labeled o-iodobenzamide derivatives" JOURNAL OF RADIOANALYTICAL CHEMISTRY, vol. 65, no. 1-2, 1981, pages 269-277, XP009131750 ISSN: 0022-4081 abstract; figure 1; tables 2,3; compounds 12,13 page 270, paragraph 3 – page 272, paragraph 1 -----	18

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No  
**PCT/EP2010/051053**

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WO 2006010165	A2 26-01-2006	AU 2005265425 A1		26-01-2006
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