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(54) Titre : PROCEDE DE CRISTALLISATION DE DERIVES D'INDOLE TRICYCLIQUES
(54) Title: CRYSTALLIZATION PROCESS OF TRICYCLIC INDOLE DERIVATIVES

(57) Abrégé/Abstract:
The present invention relates to a composition comprising a tricyclic indole compound. The composition has a higher purity and better impurity profile than known compositions comprising said tricyclic indole compound and as a consequence has superior properties, particularly when said compound is destined for use in vivo as a therapeutic or diagnostic agent. Also provided by the present invention is a method to make the composition of the invention, a pharmaceutical composition comprising the composition of the invention, and use of the composition of the invention in a medical method.

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(54) **Title**: CRYSTALLIZATION PROCESS OF TRICYCLIC INDOLE DERIVATIVES

(57) **Abstract**: The present invention relates to a composition comprising a tricyclic indole compound. The composition has a higher purity and better impurity profile than known compositions comprising said tricyclic indole compound and as a consequence has superior properties, particularly when said compound is destined for use in vivo as a therapeutic or diagnostic agent. Also provided by the present invention is a method to make the composition of the invention, a pharmaceutical composition comprising the composition of the invention, and use of the composition of the invention in a medical method.

CRYSTALLIZATION PROCESS OF TRICYCLIC INDOLE DERIVATIVES

Technical Field of the Invention

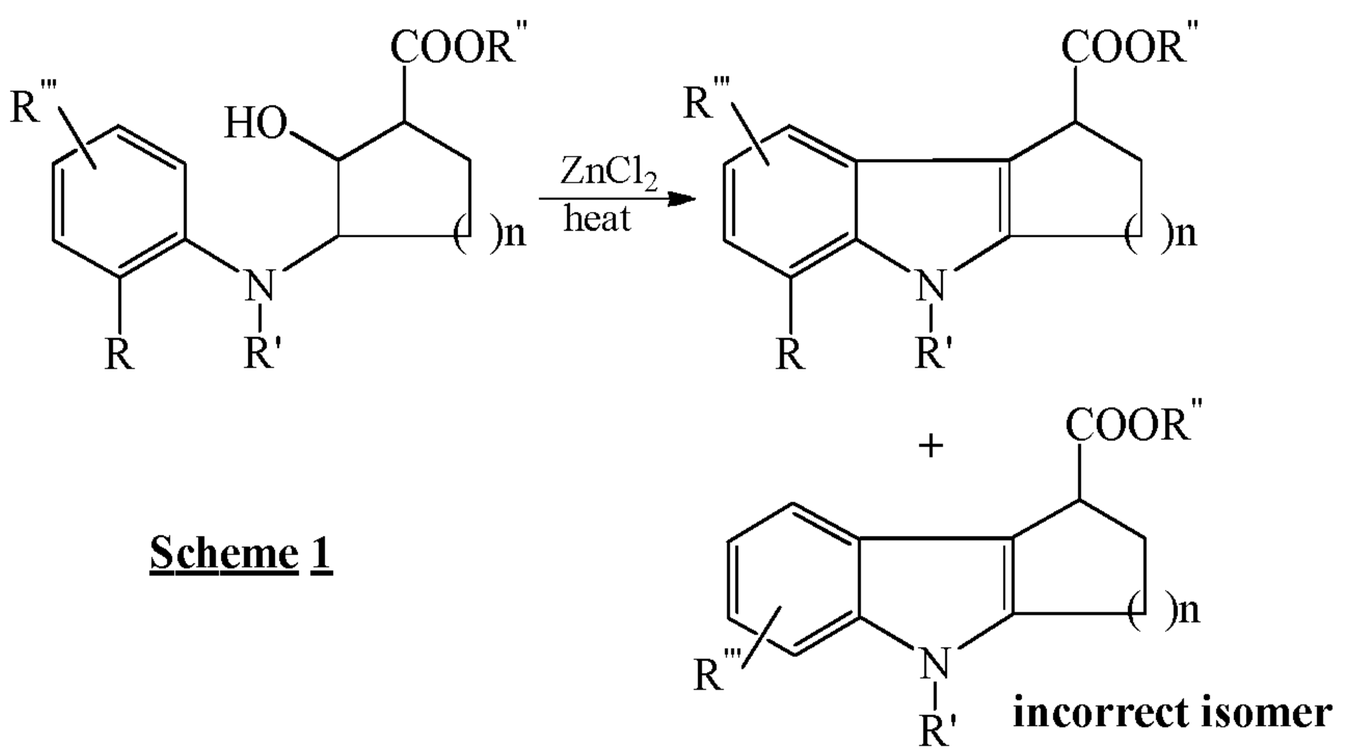
The present invention relates to a composition comprising a tricyclic indole compound. More specifically the present invention relates to wherein said composition has a more favourable impurity profile as compared with known compositions comprising said compound.

Description of Related Art

Tricyclic indole compounds are known in the art and have been reported to have application variously as melatonin antagonists (Davies 1998 J Med Chem; 41: 451-467), secretory phospholipase A_2 inhibitors (Anderson *et al* EP 0952149 A1), treatment for Alzheimer's disease (Wantanabe WO 99/25340), treatment of inflammatory diseases such as septic shock (Kinnick *et al* WO 03/014082 and WO 03/016277) and binders of high affinity to translocator protein (TSPO, formerly known as peripheral benzodiazepine receptor; Wadsworth *et al* (WO 2010/109007).

The synthesis of these tricyclic indole compounds comprises a condensation reaction between an analine and a bromo oxocycloalkanecarboxylate, followed by cyclization in the presence of a zinc halide. One problem with this cyclization reaction is that more than one cyclized isomer can result, as illustrated in Scheme 1 below:

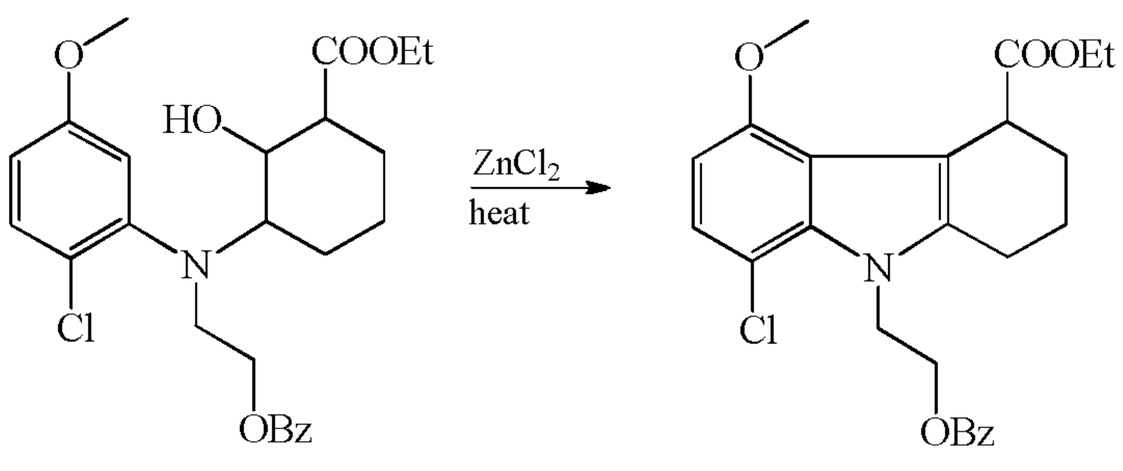
Scheme 1



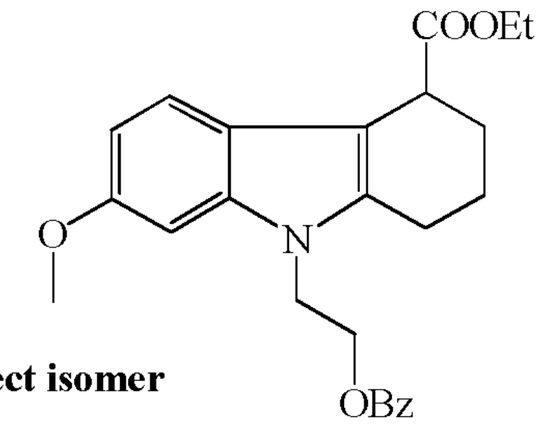
The incorrect isomer is formed when the R group reacts with the –OH. This incorrect

isomer has similar reactivity to the correct isomer and as a consequence when any further steps are taken to modify the correct isomer, a respective incorrect isomer is generated in the reaction mixture. This is particularly problematic if the resultant compound is intended for *in vivo* use, as the incorrect isomer will likely compete with the correct isomer for binding to the intended biological target.

In the method described by Kinnick *et al* (WO 03/014082), a chloro group was introduced at the R position illustrated in Scheme 1 with the aim of forcing the cyclization reaction to take place in just one way and result in only the correct cyclized isomer. This strategy was applied by Wadsworth *et al* (WO 2010/109007) in the cyclization reaction illustrated in Scheme 2 below (where Et = ethyl and Bz = benzyl):



Scheme 2



incorrect isomer

Work up and chromatographic purification of the resultant reaction mixture was followed by removal of the chloro group and conversion of the ethyl to diethyl amine to obtain a key intermediate, which in turn was purified using crystallization from diethyl ether. Purity of the key intermediate was still only 71%. When investigating this particular reaction, the present inventors have found that the purified reaction mixture still contains an amount of the incorrect isomer, which is evidently difficult to remove.

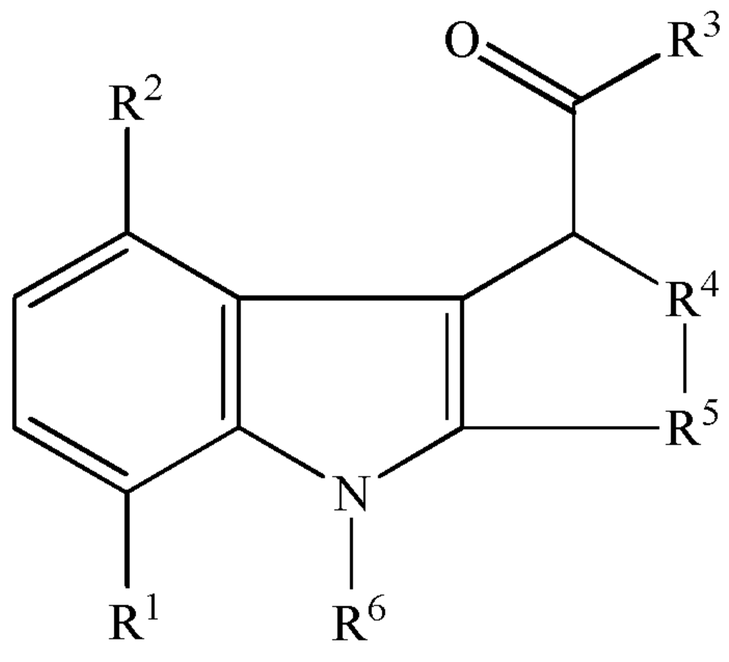
There is therefore a need for a method to obtain these and similar tricyclic indole compounds where the amount of incorrect isomer is reduced or preferably eliminated.

Summary of the Invention

The present invention relates to a composition comprising a tricyclic indole compound wherein the quantity of an incorrect isomer in said composition is reduced. The composition therefore has a higher purity and better impurity profile than known compositions comprising said tricyclic indole compound and as a consequence has superior properties, particularly when said compound is destined for use *in vivo* as a therapeutic or diagnostic agent. Also provided by the present invention is a method to make the composition of the invention, a pharmaceutical composition comprising the composition of the invention, and use of the composition of the invention in a medical method.

Detailed Description of the Preferred Embodiments

In one aspect, the present invention provides a composition comprising a compound of Formula I:



(I)

wherein:

R^1 is hydrogen, C_{1-3} alkyl, C_{1-3} alkoxy, or halo;

R^2 is hydroxyl, halo, cyano, C_{1-3} alkyl, C_{1-3} alkoxy, C_{1-3} fluoroalkyl, or C_{1-3} fluoroalkoxy;

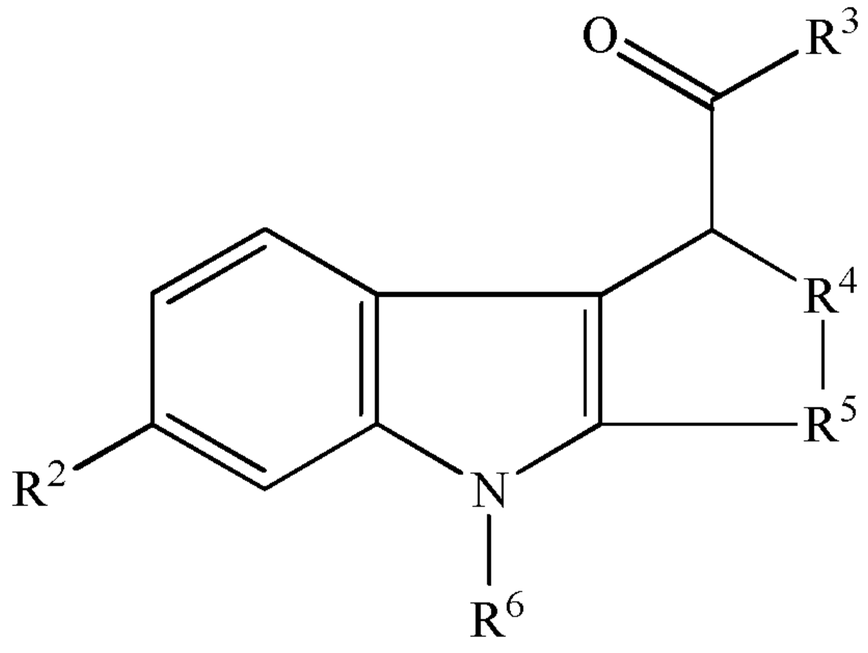
R^3 is $-N-R^7R^8$ wherein R^7 and R^8 are hydrogen, C_{1-6} alkyl, C_{7-10} arylalkyl or, together with R^7, forms a nitrogen-containing C_{4-6} aliphatic ring;

R^4 is O, S, SO, SO_2 or CH_2;

R^5 is CH_2, CH_2-CH_2, $CH(CH_3)$-CH_2 or CH_2-CH_2-CH_2;

R^6 is $–A^1$-R^9 wherein A^1 is a bond or C_{1-10} alkylene, and R^9 is hydrogen, fluoro or a leaving group, or R^9 is the group $–O$-R^{10} wherein R^{10} is hydrogen, C_{1-3} alkyl, C_{3-6} aryl, C_{7-10} arylalkyl, or a hydroxyl protecting group;

wherein said composition comprises no more than 1% of a compound of Formula II:



(II)

wherein R^2 to R^6 are as defined for Formula I.

The term "alkyl" used either alone or as part of another group is defined as any straight $–C_nH_{2n+1}$ group, branched $–C_nH_{2n+1}$ group wherein n is >3, or cyclic $–C_nH_{2n-1}$ group where n is >2. Non-limiting examples of alkyl groups include methyl, ethyl, propyl, isobutyl, cyclopropyl and cyclobutyl.

The term "alkoxy" refers to an alkyl group as defined above comprising an ether linkage, and the term "ether linkage" refers to the group -C-O-C-. Non-limiting examples of alkoxy groups include, methoxy, ethoxy, and propoxy.

The term "halo" or "halogen" is taken to mean any one of chloro, fluoro, bromo or iodo.

The term "hydroxyl" refers to the group –OH.

The term "cyano" refers to the group –CN.

The terms "fluoroalkyl" and "fluoroalkoxy"refer respectively to an alkyl group and an alkoxy group as defined above wherein a hydrogen is replaced with a fluoro.

The term "arylalkyl" refers to an aryl-substituted alkylene group wherein "aryl" refers to any molecular fragment or group which is derived from a monocyclic or polycyclic aromatic hydrocarbon, or a monocyclic or polycyclic heteroaromatic hydrocarbon and "alkylene" refers to a divalent linear $-C_nH_{2n}-$ group.

A "nitrogen-containing C_{4-6} aliphatic ring" is a saturated C_{4-6} alkyl ring comprising a nitrogen heteroatom. Examples include pyrolidinyl, piperidinyl and morpholinyl rings.

The term "leaving group" refers to a molecular fragment that departs with a pair of electrons in heterolytic bond cleavage. Non-limiting examples of suitable leaving groups include halo groups selected from chloro, iodo, or bromo, aryl or alkyl sulfonates such as tosylate, triflate, nosylate or mesylate.

The term "protecting group" is meant 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 to obtain the desired product under mild enough conditions that do not modify the rest of the molecule. Protecting groups are well-known in the art and are discussed in detail in 'Protective Groups in Organic Synthesis', by Greene and Wuts (Fourth Edition, John Wiley & Sons, 2007). Non-limiting examples of suitable protecting groups for hydroxyl include acetyl ($-COCH_3$), benzoyl ($-COC_6H_5$), benzyl ($-CH_2C_6H_5$), β-methoxyethoxymethyl ether (MEM), dimethoxytrityl (DMT) and methoxymethyl ether (MOM).

In a first preferred embodiment R^1 is halo and in a second preferred embodiment R^1 is hydrogen. When R^1 is halo it is most preferably chloro or bromo, and especially preferably chloro.

In a preferred embodiment R^2 is halo, C_{1-3} alkoxy or C_{1-3} fluoroalkoxy, most preferably hydrogen, halo or C_{1-3} alkoxy, especially preferably hydrogen, fluoro or methoxy, and most especially preferably methoxy.

In a preferred embodiment, R^3 is $-N\text{-}R^7R^8$ wherein R^7 and R^8 are C_{1-6} alkyl or C_{7-10} arylalkyl, most preferably wherein R^7 and R^8 are C_{1-3} alkyl, especially preferably wherein R^7 and R^8 are both ethyl.

In a preferred embodiment R^4 is CH_2.

In a preferred embodiment, R^5 is CH_2-CH_2.

In a first preferred embodiment, R^6 is–A^1-R^9 wherein A^1 is C_{1-10} alkylene, most preferably C_{1-3} alkylene and especially preferably ethylene, and R^9 is the group –O-R^{10} wherein R^{10} is C_{7-10} arylalkyl or a hydroxyl protecting group, most preferably wherein R^{10} is a hydroxyl protecting group.

In a second preferred embodiment R^6 is –A^1-R^9 wherein A^1 is C_{1-10} alkylene, most preferably C_{1-3} alkylene and especially preferably ethylene, and R^9 is hydrogen, fluoro or a leaving group. Where R^9 is fluoro it is preferably [^{18}F]fluoro, such that the composition of the invention is an "*in vivo* imaging composition". Where R^9 is a leaving group the composition of the invention is a "precursor composition" that can be reacted with [^{18}F]fluoride to obtain the *in vivo* imaging composition. The leaving group is preferably halo, or an aryl or alkyl sulfonate, most preferably an aryl or alkyl sulfonate, and especially preferably tosylate, triflate, nosylate or mesylate.

The term "no more than" should be understood to mean any amount less than the quoted percent quantity. Therefore no more than 1% means any amount between 0-1%. In an ideal embodiment of the composition of the present invention there is 0% of said compound of Formula II in the composition of the invention. However, in reality, it may be that at least a trace amount of the compound of Formula II remains in the composition, i.e. no more than 1% could e.g. refer to 0.1-1%.

In a first preferred composition of the present invention:

R^1 is halo, preferably chloro or bromo, and most preferably chloro;

R^2 is halo, C_{1-3} alkoxy or C_{1-3} fluoroalkoxy, preferably hydrogen, halo or C_{1-3} alkoxy, most preferably hydrogen, fluoro or methoxy, and especially preferably methoxy;

R^3 is –N-R^7R^8 wherein R^7 and R^8 are C_{1-6} alkyl or C_{7-10} arylalkyl, preferably wherein R^7 and R^8 are C_{1-3} alkyl, most preferably wherein R^7 and R^8 are both ethyl;

R^4 is CH_2;

R^5 is CH_2-CH_2; and,

R^6 is–A^1-R^9 wherein A^1 is C_{1-10} alkylene, most preferably C_{1-3} alkylene and especially preferably ethylene, and R^9 is the group –O-R^{10} wherein R^{10} is C_{7-10} arylalkyl or a hydroxyl protecting group, preferably wherein R^{10} is a hydroxyl protecting group.

In a second preferred composition of the present invention:

R^1 is hydrogen;

R^2 is halo, C_{1-3} alkoxy or C_{1-3} fluoroalkoxy, preferably hydrogen, halo or C_{1-3} alkoxy, most preferably hydrogen, fluoro or methoxy, and especially preferably methoxy;

R^3 is –N-R^7R^8 wherein R^7 and R^8 are C_{1-6} alkyl or C_{7-10} arylalkyl, preferably wherein R^7 and R^8 are C_{1-3} alkyl, most preferably wherein R^7 and R^8 are both ethyl;

R^4 is CH_2;

R^5 is CH_2-CH_2; and,

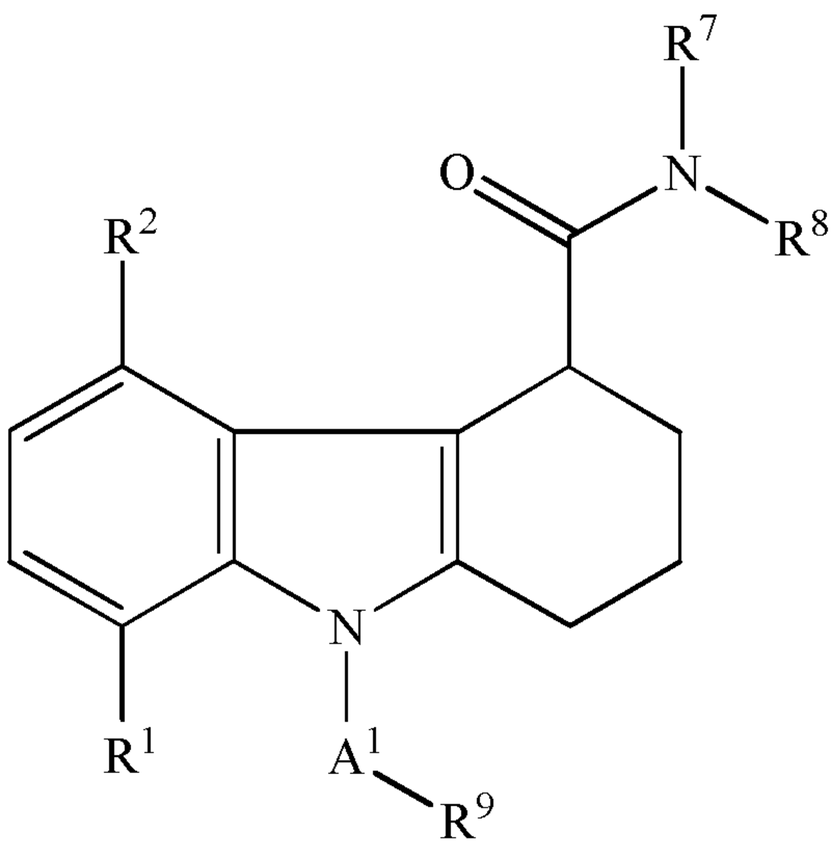
R^6 is –A^1-R^9 wherein A^1 is C_{1-10} alkylene, most preferably C_{1-3} alkylene and especially preferably ethylene, and R^9 is hydrogen, fluoro, or a leaving group, preferably wherein R^9 is fluoro or a leaving group, wherein said fluoro is [^{18}F]fluoro and wherein said leaving group is preferably halo, or an aryl or alkyl sulfonate, most preferably an aryl or alkyl sulfonate, and especially preferably tosylate, triflate, nosylate or mesylate. This second preferred composition can therefore either be an *in vivo* imaging composition or a precursor composition of the invention.

The compound of Formula I and the compound of Formula II of the composition of the present invention as defined above may each comprise a chiral centre. All forms of such isomer, including enantiomers and diastereoisomers, are encompassed by the present invention. The compound of Formula I and the compound of Formula II may be present in the composition of the present invention as racemic mixture or as an enantiomerically-enriched mixture, or the racemic mixture may be separated using well-known techniques

and an individual enantiomer maybe used alone. Preferably, the composition of the invention comprises the S-enantiomer of said compound of Formula I and said compound of Formula II.

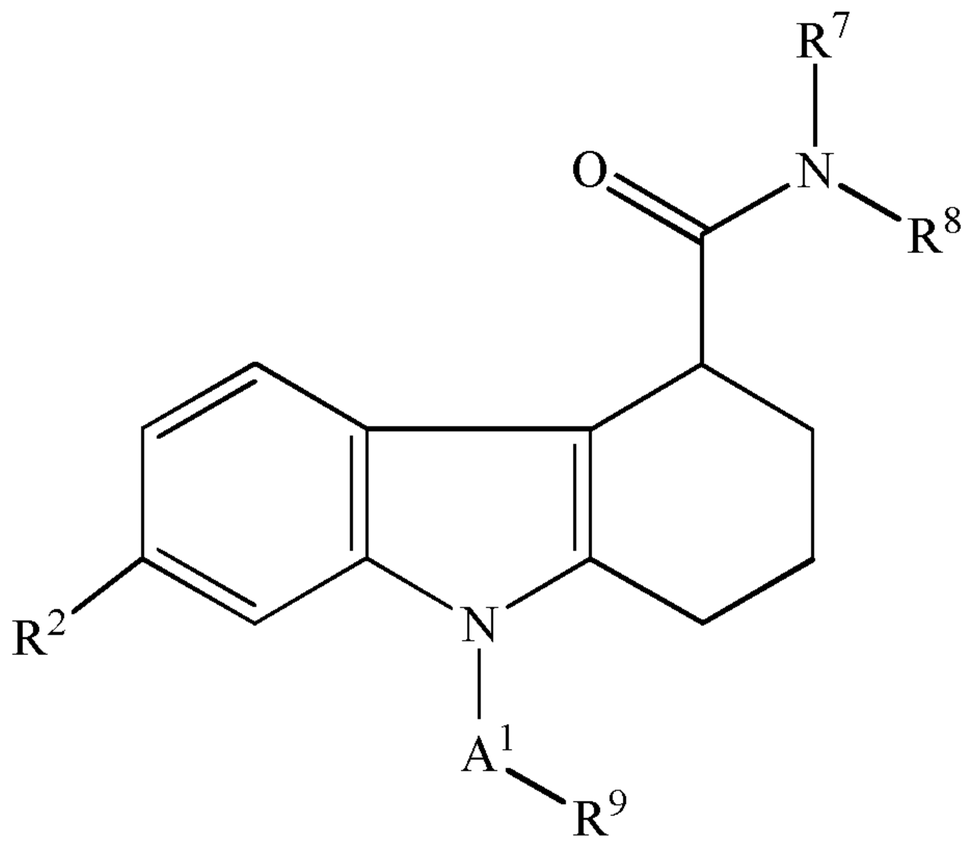
In a preferred embodiment, the composition of the present invention comprises no more than 0.5% of said compound of Formula II, most preferably no more than 0.3%, especially preferably no more than 0.2%, and most especially preferably no more than 0.1%.

In a particularly preferred composition of the present invention, said compound of Formula I is a compound of Formula Ia:



(Ia)

wherein each of R^1, R^2, R^7, R^8, R^9 and A^1 are as variously defined hereinabove and said compound of Formula II is a compound of Formula IIa:



(IIa)

wherein each of R^2, R^7, R^8, R^9 and A^1 are as variously defined hereinabove.

Preferably for this particularly preferred composition of the invention:

R^1 is hydrogen;

R^2 is fluoro or methoxy;

R^7 and R^8 are C_{1-6} alkyl;

R^9 is hydrogen, fluoro or a leaving group; and,

A^1 is C_{1-10} alkylene.

Most preferably for this particularly preferred composition of the invention:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are C_{1-3} alkyl;

R^9 is [^{18}F]fluoro or an aryl or alkyl sulfonate; and,

A^1 is C_{1-3} alkylene.

Especially preferably for this particularly preferred composition of the invention:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are methyl or ethyl;

R^9 is [^{18}F]fluoro, tosylate, triflate, nosylate or mesylate; and,

A^1 is C_{1-3} alkylene.

Most especially preferably for this particularly preferred composition of the invention:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are both ethyl;

R^9 is [^{18}F]fluoro or mesylate; and,

A^1 is ethylene.

Where an above-defined particularly preferred composition of the invention comprises ^{18}F it is an *in vivo* imaging composition, and where it comprises a leaving group, it is a precursor composition.

In another aspect, the present invention comprises a method to obtain the composition as defined hereinabove wherein said method comprises crystallization of a reaction mixture comprising said compound of Formula I as defined hereinabove, and said compound of Formula II as defined hereinabove, wherein said crystallization is carried out in a suitable organic solvent in the presence of a catalytic amount of a weak organic base in order to obtain said composition.

The term "catalytic amount" means an amount of a substance used in a chemical reaction as a catalyst and is generally much smaller than the stoichiometric amounts of either reactants or products.

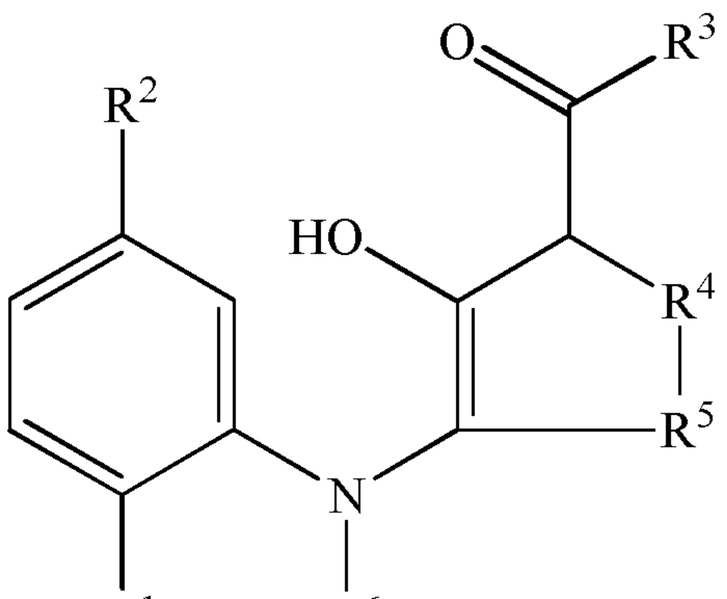
The term "suitable organic solvent" encompasses non-polar solvents and polar aprotic

solvents, suitably having a dielectric constant of between 3.5-8. Examples of suitable organic solvents for use in the method of the present invention include diethyl ether, ethyl acetate, tetrahydrofuran (THF) and diisopropylether. Diethyl ether is preferred.

The term "weak organic base" refers to an organic compound which acts as a base. Organic bases are generally proton acceptors and usually contain nitrogen atoms, which can easily be protonated. Amines and nitrogen-containing heterocyclic compounds are organic bases. Non-limiting examples include pyridine, alkyl amines, morpholine, imidazole, benzimidazole, histidine, phosphazene bases and hydroxides of some organic cations. In the context of the present invention alkyl amines are preferred, e.g. N,N-diisopropyl amine, triethyl amine or diethyl amine.

The present inventors have found that when using the method of the invention a very good quality product is obtained having optimum yield. Please refer to Example 1 wherein a method to obtain the composition of the present invention is described. It can be seen that by applying the method of the invention to the purification of a key intermediate in the synthesis, the amount of incorrect isomer remaining is significantly less than when the prior art method for purification of this intermediate is used.

Preferably, the reaction mixture for use in the method of the invention is obtained using a method comprising cyclization of a compound of Formula III:



(III)

wherein:

R^1 is as suitably and preferably defined hereinabove;

R^2 is as suitably and preferably defined hereinabove;

R^3 is as suitably and preferably defined hereinabove;

R^4 is as suitably and preferably defined hereinabove;

R^5 is as suitably and preferably defined hereinabove; and,

R^6 is as suitably and preferably defined hereinabove;

wherein said cyclization is carried out by reaction of said compound of Formula III with a zinc halide.

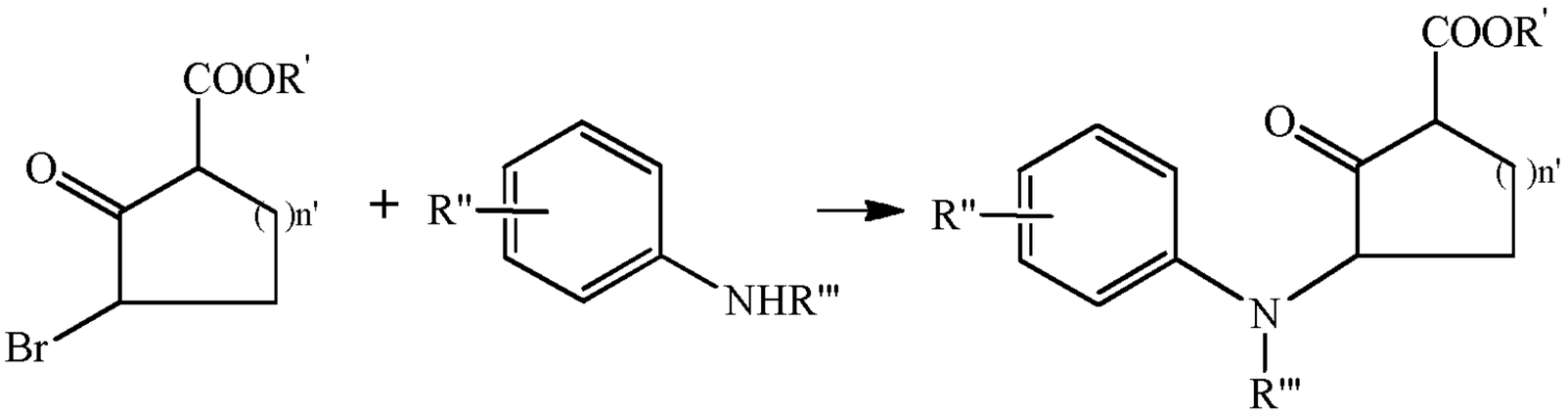
It is preferred that said zinc halide is zinc chloride or zinc bromide, most preferably zinc chloride.

In a particularly preferred embodiment said zinc chloride is added lot-wise. The term "lot-wise" means introduction of a reagent to a reaction using more than one addition. In the context of the present invention said more than one addition comprises a first addition and a second addition wherein said second addition is carried out at least 6 hours after said first addition. Said lot-wise addition preferably further comprises a third addition wherein said third addition is carried out said second addition.

Cyclization of said compound of Formula III is preferably carried out wherein R^1 is halogen, preferably chloro, and wherein R^6 comprises a protecting group. This is to ensure that the cyclization reaction results in as much of the correct isomer as possible. The R^1 and R^6 group can be converted subsequently using methods well-known to the person skilled in the art to obtain other R^1 and R^6 groups as defined above.

Compounds of Formula III can be obtained from commercial starting materials using or adapting methods described in the prior art. Reference is made in this regard to the teachings of Julia & Lenzi (Bulletin de la Société de France 1962: 2262-2263), Davies *et al* (J Med Chem 1998; 41: 451-467), Kinnick *et al* (WO 2003/014082 and WO 2003/016277), Anderson *et al* (EP0952149 B1) and Wadsworth *et al* (WO 2010/109007). In each of these publications compounds of Formula III are obtained by

condensation reaction between an analine and a bromo oxocycloalkanecarboxylate as illustrated in Scheme 3 below:



Scheme 3

In the above scheme R' is an R^3 group as defined herein, R" is an R^1 and/or an R^2 group as defined herein, R''' is an R^6 group as defined herein and n' is an integer of 1-3.

In another aspect, the present invention provides a pharmaceutical composition comprising the composition of the invention together with a biocompatible carrier suitable for mammalian administration.

The "biocompatible carrier" is a fluid, especially a liquid, in which the composition of the invention is suspended or dissolved, such that the pharmaceutical composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier 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 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 is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier for intravenous injection is suitably in the range 4.0 to 10.5.

In a yet further aspect, the present invention provides for use of the pharmaceutical

composition of the invention in a medical method, wherein said medical method is preferably either a method for treatment or a method for diagnosis of a pathological condition. In particular, the pharmaceutical composition of the present invention is useful in the treatment or diagnosis of a pathological condition comprising inflammation.

Where the composition of the invention is an *in vivo* imaging composition as referred to above, i.e. wherein R^6 comprises [^{18}F]fluoro, the medical method is preferably a method of *in vivo* imaging comprising:

(i) administering said pharmaceutical composition to a subject;

(ii) detecting signals emitted by the [^{18}F]fluoro comprised in said pharmaceutical composition;

(iii) generating an image representative of the location and/or amount of said signals.

The "subject" of the invention can be any human or animal subject. Preferably the subject of the invention is a mammal. Most preferably, said subject is an intact mammalian body *in vivo*. In an especially preferred embodiment, the subject of the invention is a human.

"Administering" the in this *in vivo* imaging method is preferably carried out parenterally, and most preferably intravenously.

The "detecting" step of the method of the invention involves detection of signals emitted by the [^{18}F]fluoro by means of a detector sensitive to said signals, i.e. a positron-emission tomography (PET) detector.

The "generating" step of the method of the invention is carried out by a computer which applies a reconstruction algorithm to the acquired signal data to yield a dataset. This dataset is then manipulated to generate images showing the location and/or amount of signals emitted by said [^{18}F]fluoro.

The *in vivo* imaging composition of the invention is readily obtained by reaction with

[^{18}F]fluoride of a precursor composition as defined above, i.e. a composition of the invention wherein R^6 comprises a leaving group as defined hereinabove. [^{18}F]-fluoride ion ($^{18}F^-$) is normally obtained as an aqueous solution from the nuclear reaction $^{18}O(p,n)^{18}F$ and is made reactive by the addition of a cationic counterion and the subsequent removal of water. Removal of water is commonly carried out by application of heat and use of a solvent such as acetonitrile to provide a lower boiling azeotrope. A "cationic counterion" is a positively-charged counterion examples of which include large but soft metal ions such as rubidium or caesium, potassium complexed with a cryptand, or tetraalkylammonium salts. A preferred cationic counterion is a metal complex of a cryptand, most preferably wherein said metal is potassium and wherein said cryptand is Kryptofix 222.

In another aspect the present invention provides the pharmaceutical composition of the invention for use in any of the above-defined medical methods.

In a yet further aspect, the present invention provides for use of the composition of the invention in the manufacture of the pharmaceutical composition of the invention for use in any of the above-defined medical methods.

In a further aspect the present invention provides a kit suitable for making the *in vivo* imaging composition of the invention, wherein said kit comprises said precursor composition. A specialised kit, or "cassette", may be used to prepare the *in vivo* imaging composition of the present invention on an automated radiosynthesis apparatus. By the term "cassette" is meant a piece of apparatus designed to fit removably and interchangeably onto an automated radiosynthesis apparatus, in such a way that mechanical movement of moving parts of the synthesizer controls the operation of the cassette from outside the cassette, i.e. externally. [^{18}F]-radiotracers are now often conveniently prepared on automated radiosynthesis apparatuses. By the term "automated radiosynthesis apparatus" is meant an automated module based on the principle of unit operations as described by Satyamurthy *et al* (1999 Clin Positr Imag; 2(5): 233-253). The term "unit operations" means that complex processes are reduced to a series of simple operations or reactions, which can be applied to a range of materials. Such automated radiosynthesis apparatuses are commercially available from a

range of suppliers (Satyamurthy *et al,* above), including: GE Healthcare; CTI Inc; Ion Beam Applications S.A. (Chemin du Cyclotron 3, B-1348 Louvain-La-Neuve, Belgium); Raytest (Germany) and Bioscan (USA).

A commercial automated radiosynthesis apparatus also provides suitable containers for the liquid radioactive waste generated as a result of the radiosynthesis. Automated radiosynthesis apparatuses are not typically provided with radiation shielding, since they are designed to be employed in a suitably configured radioactive work cell. The radioactive work cell provides suitable radiation shielding to protect the operator from potential radiation dose, as well as ventilation to remove chemical and/or radioactive vapours. Suitable cassettes comprise a linear array of valves, each linked to a port where reagents or vials can be attached, by either needle puncture of an inverted septum-sealed vial, or by gas-tight, marrying joints. Each valve has a male-female joint which interfaces with a corresponding moving arm of the automated radiosynthesis apparatus. External rotation of the arm thus controls the opening or closing of the valve when the cassette is attached to the automated radiosynthesis apparatus. Additional moving parts of the automated radiosynthesis apparatus are designed to clip onto syringe plunger tips, and thus raise or depress syringe barrels.

The cassette is versatile, typically having several positions where reagents can be attached, and several suitable for attachment of syringe vials of reagents or chromatography cartridges (e.g. for SPE). The cassette always comprises a reaction vessel. Such reaction vessels are preferably 0.5 to 10 mL, more preferably 0.5 to 5 mL and most preferably 0.5 to 4 mL in volume and are configured such that 3 or more ports of the cassette are connected thereto, to permit transfer of reagents or solvents from various ports on the cassette. Preferably the cassette has 15 to 40 valves in a linear array, most preferably 20 to 30, with 25 being especially preferred. The valves of the cassette are preferably each identical, and most preferably are 3-way valves. The cassettes are designed to be suitable for radiopharmaceutical manufacture and are therefore manufactured from materials which are of pharmaceutical grade and ideally also are resistant to radiolysis.

Preferred automated radiosynthesis apparatuses of the present invention comprise a disposable or single use cassette which comprises all the reagents, reaction vessels and

apparatus necessary to carry out the preparation of a given batch of the *in vivo* imaging composition of the invention.

The following non-limiting examples serve to illustrate the invention in more detail.

Brief Description of the Examples

Example 1 describes a method to obtain a composition comprising a compound of Formula I as defined herein and a compound of Formula II as defined herein, wherein a prior art method is compared with the method of the present invention.

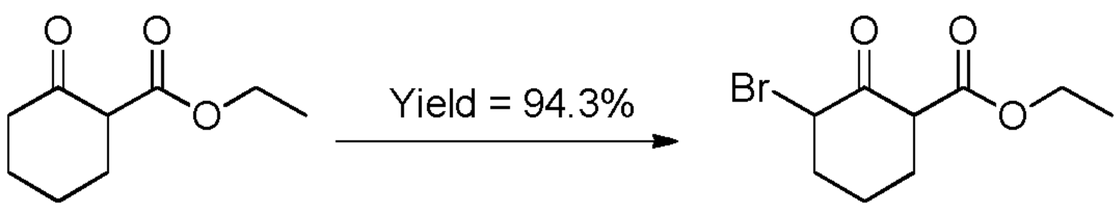
List of Abbreviations used in the Examples

OMs: mesylate

Example 1: Synthesis of N,N-diethyl-9-(2-[^{18}F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide

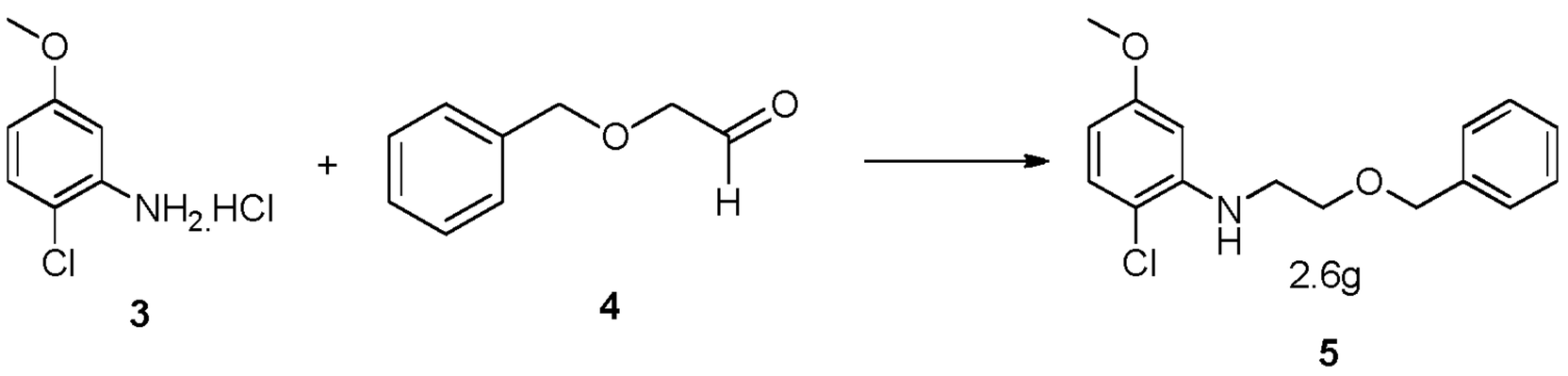
The compound N,N-diethyl-9-(2-[^{18}F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide was synthesised using the following steps:

Step 1: Synthesis of ethyl 3-bromo-2-oxocyclohexanecarboxylate



1 **2**

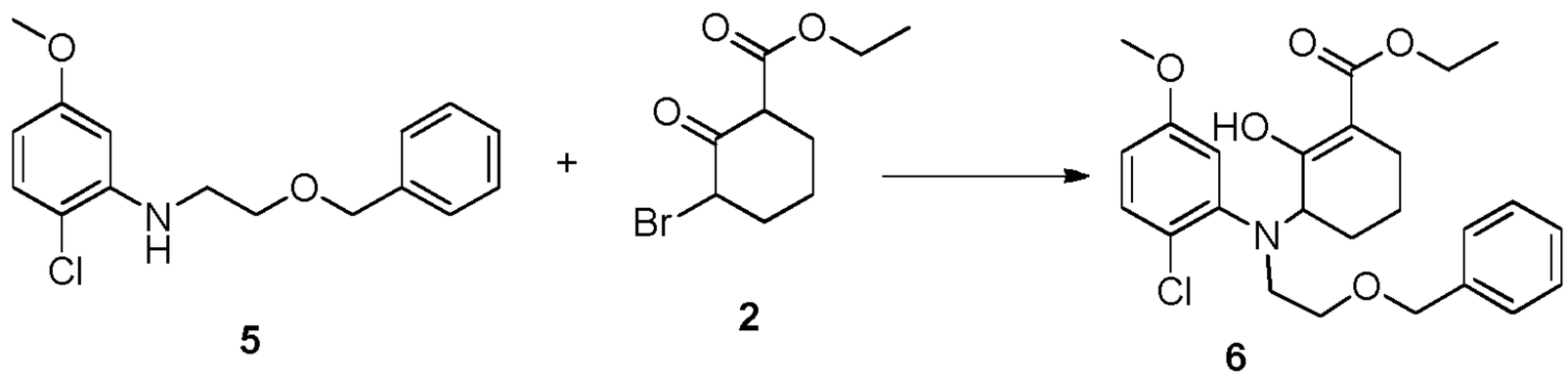
Step 2: Synthesis of N-(2-(benzyloxy)ethyl)-2-chloro-5-methoxyaniline



3 **4** **5**

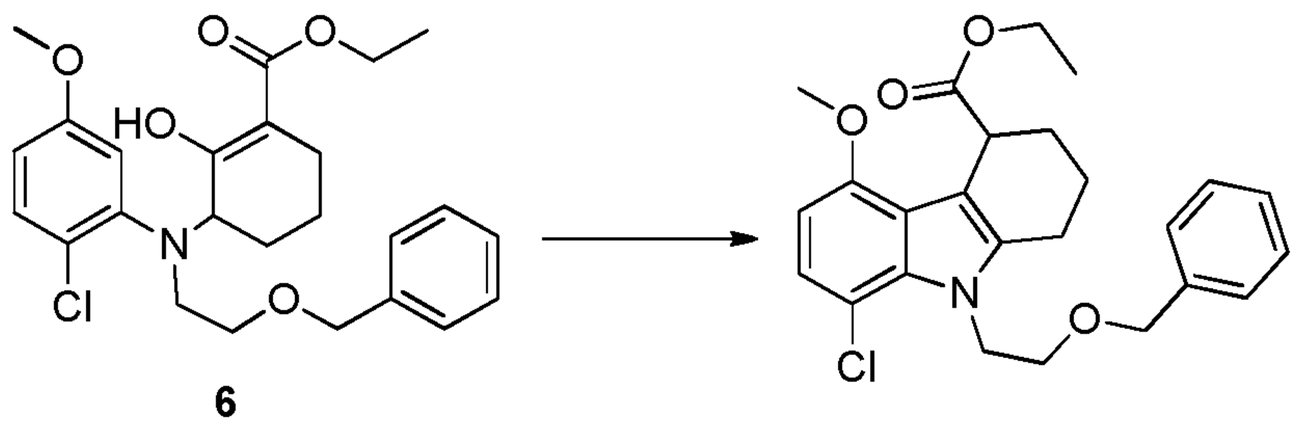
Step 3: Synthesis of ethyl 3-((2-(benzyloxy)ethyl)(2-chloro-5-methoxyphenyl)amino)-2-

hydroxycyclohex-1-enecarboxylate



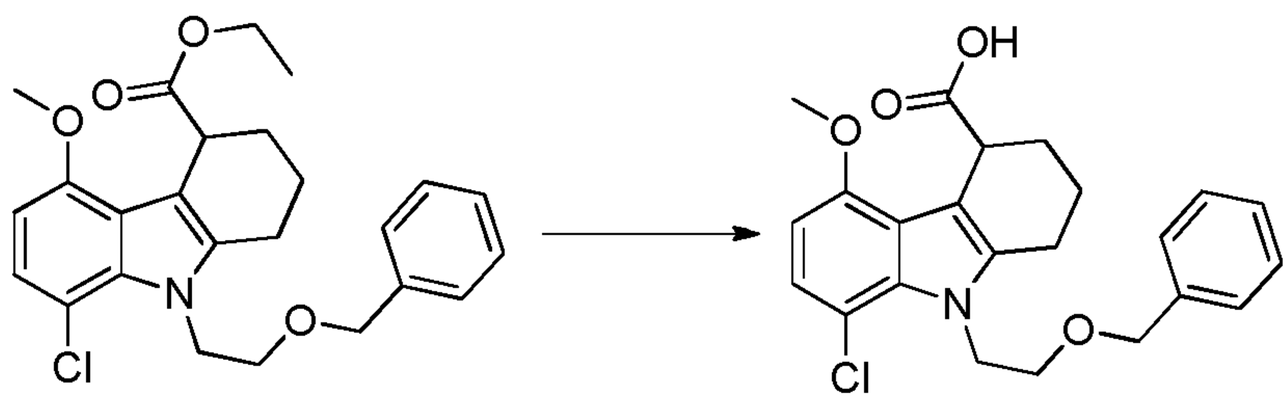
5 6

Step 4: Synthesis of ethyl 9-(2-(benzyloxy)ethyl)-8-chloro-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxylate



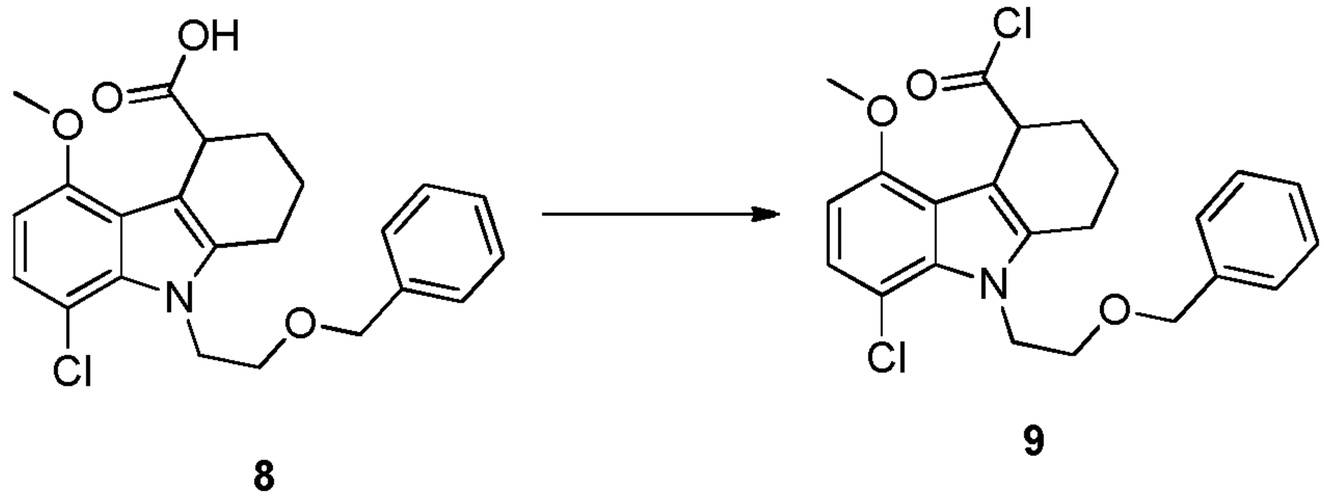
6 7

Step 5: Synthesis of 9-(2-(benzyloxy)ethyl)-8-chloro-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxylic acid



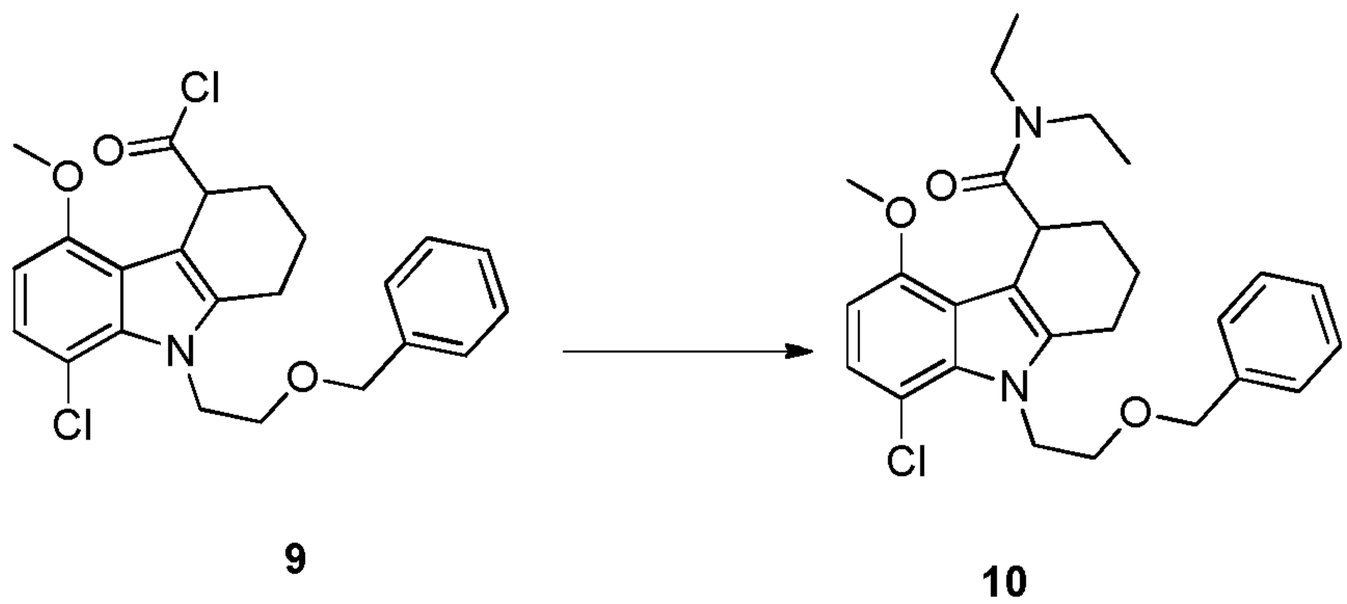
7 8

Step 6: Synthesis of 9-(2-(benzyloxy)ethyl)-8-chloro-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carbonyl chloride



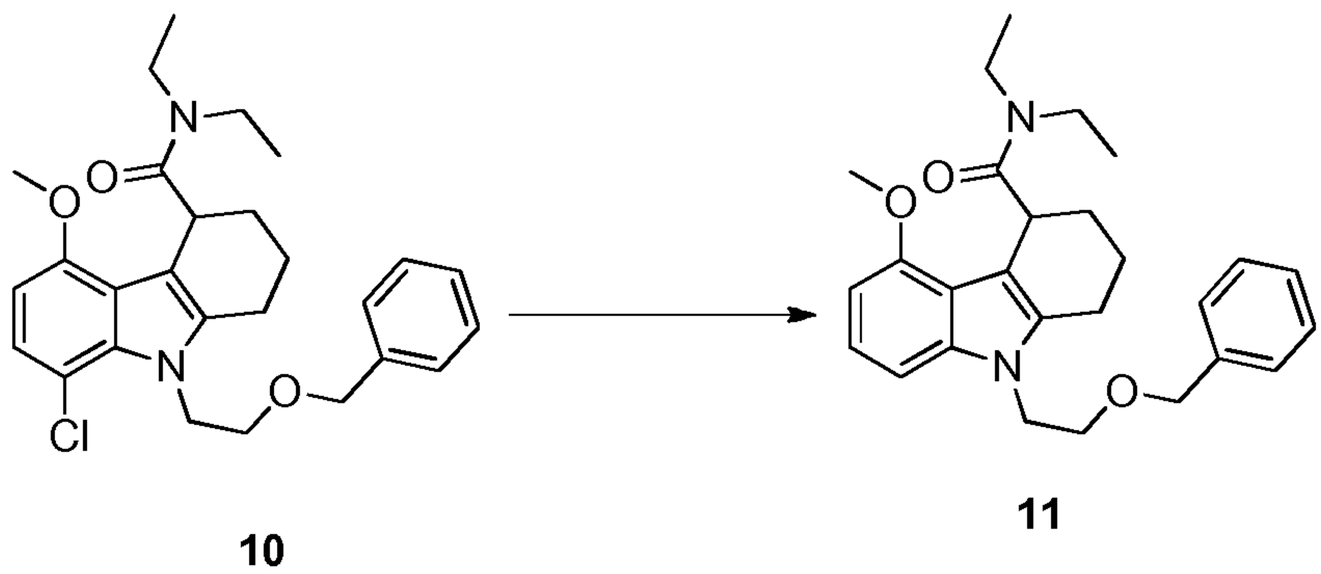
8 9

Step 7: Synthesis of 9-(2-(benzyloxy)ethyl)-8-chloro-N,N-diethyl-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide



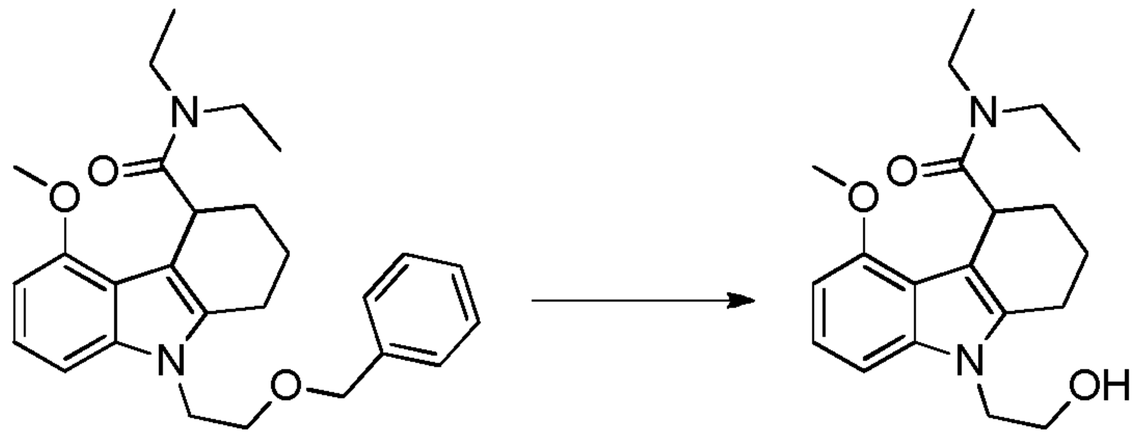
9 10

Step 8: Synthesis of 9-(2-(benzyloxy)ethyl)-N,N-diethyl-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide



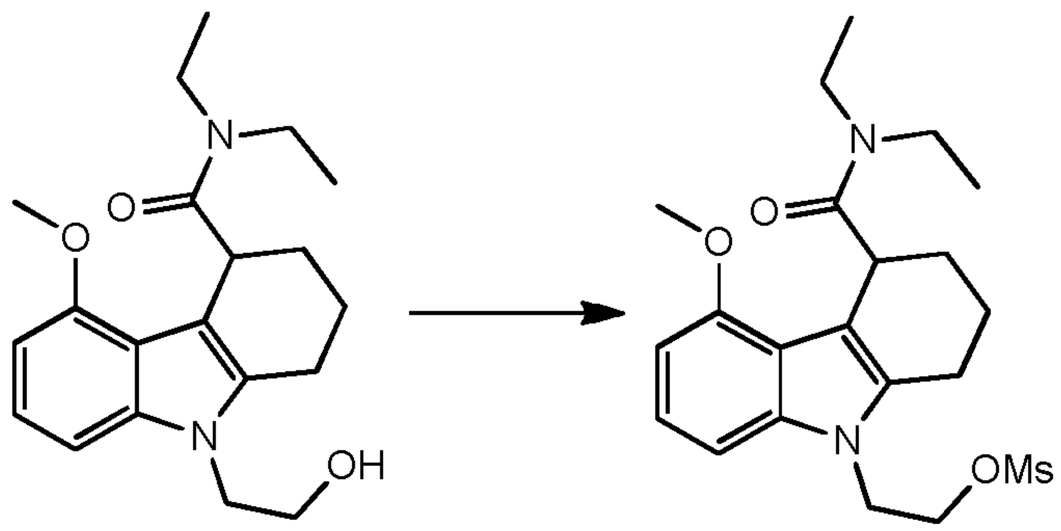
10 11

Step 9: Synthesis of N,N-diethyl-9-(2-hydroxyethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide



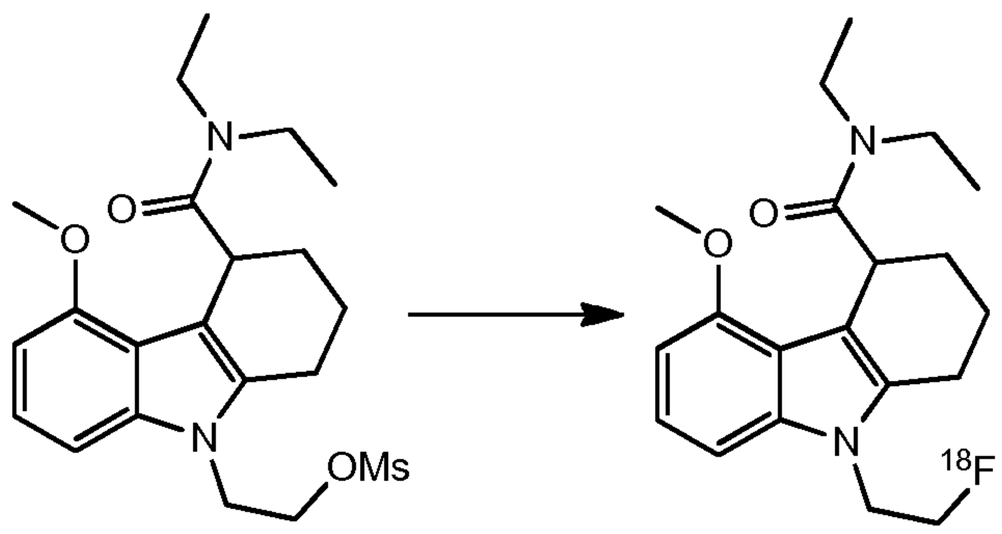
11 12

Step 10: Synthesis of 2-(4-(diethylcarbamoyl)-5-methoxy-3,4-dihydro-1H-carbazol-9(2H)-yl)ethyl methanesulfonate



12 13

Step 11: Synthesis of N,N-diethyl-9-(2-[^{18}F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide



13 14

In the prior art method (Wadsworth *et al* WO 2010/109007 Example 1), intermediate 10 above was purified by crystallization from diethyl ether (Wadsworth *et al* WO 2010/109007 Example 1(i)). The method of the present invention was carried out as generally described by Wadsworth *et al* (WO 2010/109007). However, in the method of

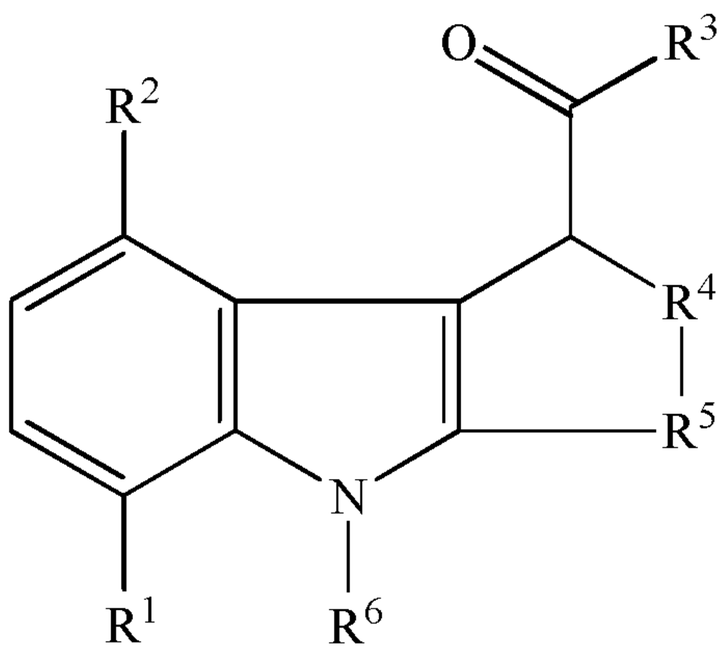
the present invention, intermediate 10 was purified by crystallization from diethyl ether in the presence of diethyl amine.

Table 1: shows the per cent yield of the desired product along with the amount of incorrect isomer impurity (where measured) in brackets thereafter. With the method of the invention it can be seen that the amount of the incorrect isomer in intermediate 10, which was purified using the method of the invention was only 0.2%.

Intermediate	**6**	**7**	**8**	**10**	**12**	**13**
Prior Art	95.10	78.20 (6.4)	90.40 (7.6)	92.18 (7.0)	93.15 (4.9)	91.75 (5.1)
Prior Art	91.28	77.97 (6.9)	82 (9.0)	95.40 (3.2)	91.10 (3.3)	92.87 (2.3)
Invention	95.00 (6.5)	84.00 (6.5)	87.27 (5.8)	97.92 (0.2)	-	-

Claims

(1) A composition comprising a compound of Formula I:



(I)

wherein:

R^1 is hydrogen, C_{1-3} alkyl, C_{1-3} alkoxy, or halo;

R^2 is hydroxyl, halo, cyano, C_{1-3} alkyl, C_{1-3} alkoxy, C_{1-3} fluoroalkyl, or C_{1-3} fluoroalkoxy;

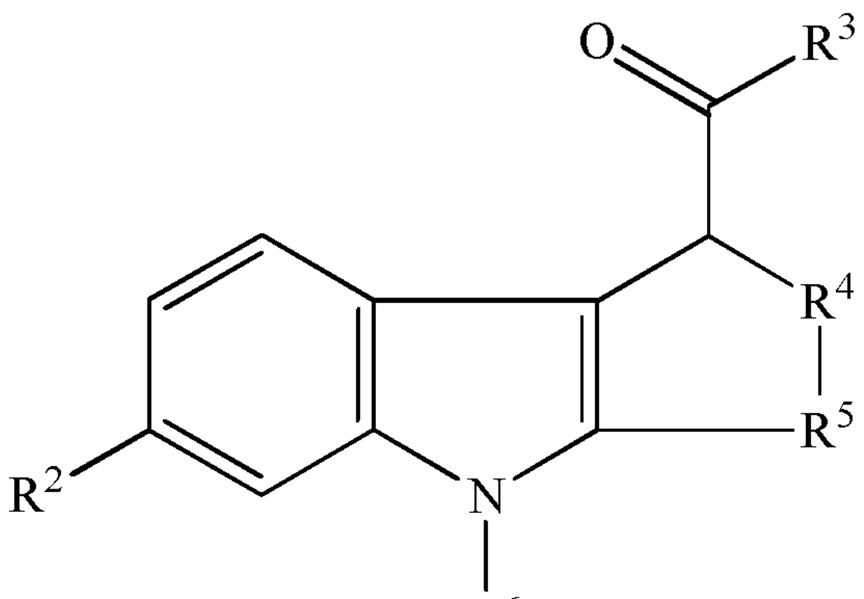
R^3 is $-N-R^7R^8$ wherein R^7 and R^8 are hydrogen, C_{1-6} alkyl, C_{7-10} arylalkyl or, together with R^7, forms a nitrogen-containing C_{4-6} aliphatic ring;

R^4 is O, S, SO, SO_2 or CH_2;

R^5 is CH_2, CH_2-CH_2, $CH(CH_3)$-CH_2 or CH_2-CH_2-CH_2;

R^6 is $-A^1-R^9$ wherein A^1 is a bond or C_{1-10} alkylene, and R^9 is hydrogen, fluoro or a leaving group, or R^9 is the group $-O-R^{10}$ wherein R^{10} is hydrogen, C_{1-3} alkyl, C_{3-6} aryl, C_{7-10} arylalkyl, or a hydroxyl protecting group;

wherein said composition comprises no more than 1% of a compound of Formula II:



(II)

wherein R^2 to R^6 are as defined for Formula I.

(2) The composition as defined in Claim 1 wherein R^1 is halo.

(3) The composition as defined in either Claim 1 or Claim 2 wherein R^1 is chloro or bromo.

(4) The composition as defined in any one of Claims 1-3 wherein R^1 is chloro.

(5) The composition as defined in any one of Claims 1-4 wherein R^6 is–A^1-R^9 wherein A^1 is C_{1-10} alkylene and R^9 is the group –O-R^{10} wherein R^{10} is C_{7-10} arylalkyl or a hydroxyl protecting group.

(6) The composition as defined in any one of Claims 1-5 R^6 is–A^1-R^9 wherein A^1 is C_{1-10} alkylene and R^9 is the group –O-R^{10} and wherein R^{10} is a hydroxyl protecting group.

(7) The composition as defined in Claim 1 wherein R^1 is hydrogen.

(8) The composition as defined in either Claim 1 or Claim 7 wherein R^6 is –A^1-R^9 wherein A^1 is C_{1-10} alkylene and R^9 is hydrogen, fluoro or a leaving group.

(9) The composition as defined in any one of Claims 1, 7 or 8 wherein R^6 is –A^1-R^9 wherein A^1 is C_{1-10} alkylene and R^9 is hydrogen.

(10) The composition as defined in any one of Claims 1, 7 or 8 wherein R^6 is –A^1-R^9 wherein A^1 is C_{1-10} alkylene and R^9 is a leaving group.

(11) The composition as defined in any one of Claims 1, 7 or 8 wherein R^6 is –A^1-R^9

wherein A^1 is C_{1-10} alkylene and R^9 is fluoro.

(12) The composition as defined in any one of Claims 1, 7, 8 or 11 wherein R^6 is $-A^1-R^9$ wherein A^1 is C_{1-10} alkylene and R^9 is [^{18}F]fluoro.

(13) The composition as defined in any one of Claims 1-12 wherein said composition comprises the S-enantiomer of said compound of Formula I and said compound of Formula II.

(14) The composition as defined in any one of Claims 1-13 wherein R^6 is $-A^1-R^9$ wherein A^1 is C_{1-3} alkylene.

(15) The composition as defined in any one of Claims 1-14 wherein R^6 is $-A^1-R^9$ wherein A^1 is ethylene.

(16) The composition as defined in any one of Claims 1-15 wherein R^2 is halo, C_{1-3} alkoxy or C_{1-3} fluoroalkoxy.

(17) The composition as defined in any one of Claims 1-16 wherein R^2 is halo or C_{1-3} alkoxy.

(18) The composition as defined in any one of Claims 1-17 wherein R^2 is fluoro or methoxy.

(19) The composition as defined in any one of Claims 1-18 wherein R^2 is methoxy.

(20) The composition as defined in any one of Claims 1-19 wherein R^3 is $-N-R^7R^8$ wherein R^7 and R^8 are C_{1-6} alkyl or C_{7-10} arylalkyl.

(21) The composition as defined in any one of Claims 1-20 wherein R^3 is $-N-R^7R^8$ wherein R^7 and R^8 are C_{1-3} alkyl.

(22) The composition as defined in any one of Claims 1-21 wherein R^3 is $-N-R^7R^8$ wherein R^7 and R^8 are both ethyl.

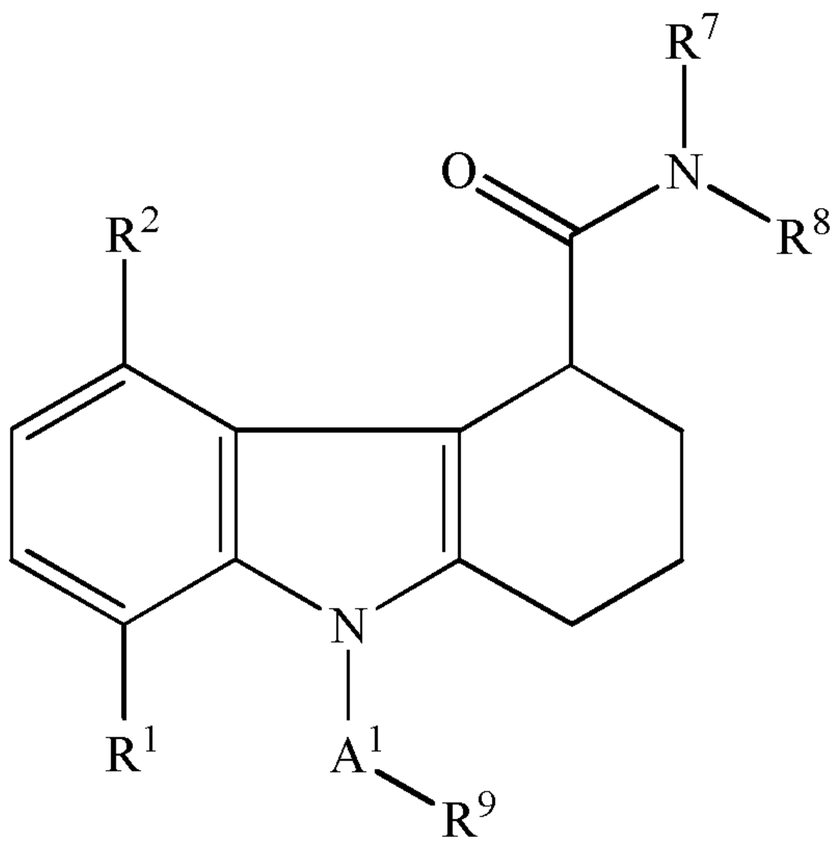
(23) The composition as defined in any one of Claims 1-22 wherein R^4 is CH_2.

(24) The composition as defined in any one of Claims 1-23 wherein R^5 is CH_2-CH_2.

(25) The composition as defined in any one of Claims 1-24 which comprises no more than 0.5% of said compound of Formula II.

(26) The composition as defined in Claim 25 which comprises no more than 0.3% of said compound of Formula II.

(27) The composition as defined in any one of Claims 1-26 wherein said compound of Formula I is a compound of Formula Ia:



(Ia)

wherein:

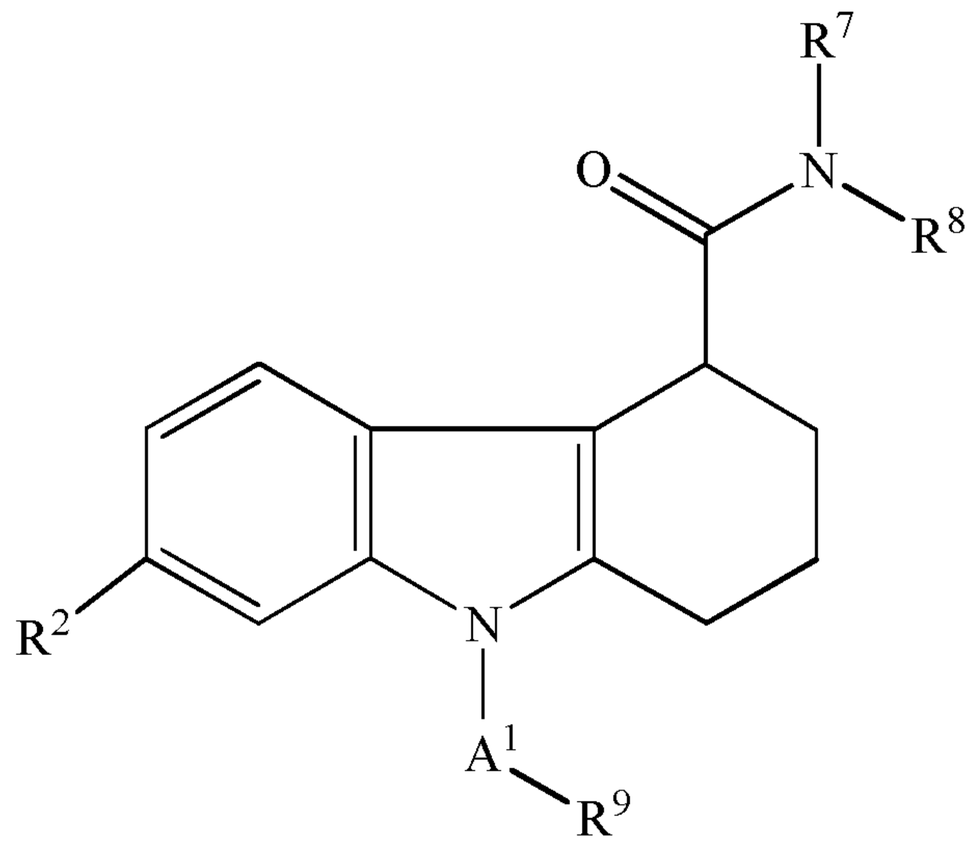
R^1 is as defined in any one of Claims 1-3 and 7;

R^2 is as defined in any one of Claims 1 and 16-19;

R^7 and R^8 are as defined in any one of Claims 1 and 20-22;

R^9 and A^1 are as defined in any one of Claims 1, 5, 6, 8-12, 14 and 15;

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



(IIa)

wherein each of R^2, R^7, R^8, R^9 and A^1 are as defined for Formula Ia.

(28) The composition as defined in Claim 27 wherein:

R^1 is hydrogen;

R^2 is fluoro or methoxy;

R^7 and R^8 are C_{1-6} alkyl;

R^9 is hydrogen, fluoro or a leaving group; and,

A^1 is C_{1-10} alkylene.

(29) The composition as defined in either Claim 27 or Claim 28 wherein:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are C_{1-3} alkyl;

R^9 is [^{18}F]fluoro or an aryl or alkyl sulfonate; and,

A^1 is C_{1-3} alkylene.

(30) The composition as defined in any one of Claims 27-29 wherein:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are methyl or ethyl;

R^9 is [^{18}F]fluoro, tosylate, triflate, nosylate or mesylate; and,

A^1 is C_{1-3} alkylene.

(31) The composition as defined in any one of Claims 27-30 wherein:

R^1 is hydrogen;

R^2 is methoxy;

R^7 and R^8 are both ethyl;

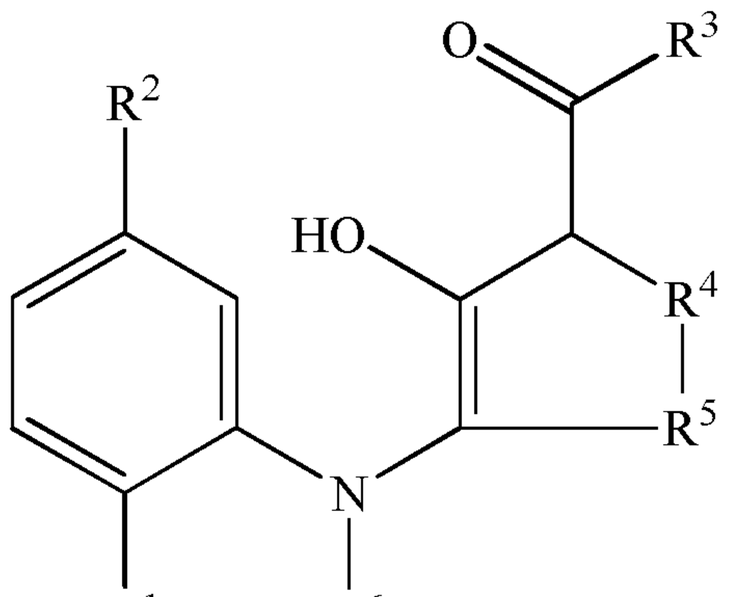
R^9 is [^{18}F]fluoro or mesylate; and,

A^1 is ethylene.

(32) A method to obtain the composition as defined in any one of Claims 1-31 wherein said method comprises crystallization of a reaction mixture comprising said compound of Formula I as defined in any one of Claims 1-24 and 27-31, and said compound of Formula II as defined in any one of Claims 1, 5, 6, 8-24 and 27-31, wherein said crystallization is carried out in a suitable organic solvent in the presence of a catalytic amount of a weak organic base in order to obtain said composition.

(33) The method as defined in Claim 32 wherein said weak organic base is selected from N,N-diisopropyl ethylamine or diethyl amine.

(34) The method as defined in either Claims 32 or Claim 33 wherein said reaction mixture is obtained using a method comprising cyclization of a compound of Formula III:



(III)

wherein:

R^1 is as defined in any one of Claims 1-3 and 7;

R^2 is as defined in any one of Claims 1 and 16-19;

R^3 is as defined in any one of Claims 1 and 20-22;

R^4 is as defined in either Claim 1 or Claim 23;

R^5 is as defined in either Claim 1 or Claim 24; and,

R^6 is as defined in any one of Claims 1, 5, 6, 8-12, 14 and 15;

wherein said cyclization is carried out by reaction of said compound of Formula III with a zinc halide.

(35) The method as defined in Claim 34 wherein said zinc halide is zinc chloride or zinc bromide.

(36) The method as defined in either Claim 34 or Claim 35 wherein said zinc halide is zinc chloride.

(37) A pharmaceutical composition comprising the composition as defined in any one of Claims 1-31 together with a biocompatible carrier suitable for mammalian administration.

(38) Use of the pharmaceutical composition as defined in Claim 37 in a medical method.

(39) The use as defined in Claim 38 wherein said medical method is treatment of a pathological condition.

(40) The use as defined in Claim 39 wherein said medical method is diagnosis of a pathological condition.

(41) A method of *in vivo* imaging comprising:

(i) administering to a subject the pharmaceutical composition as defined in Claim 37 wherein R^9 is [^{18}F]fluoro;

(ii) detecting signals emitted by the [^{18}F]fluoro comprised in said pharmaceutical composition;

(iii) generating an image representative of the location and/or amount of said signals.

(42) A kit comprising the composition as defined in Claim 1 any one of Claims 1-4, 7, 8, 10 and 13-27 wherein R^6 is $–A^1$-R^9 wherein A^1 is a bond or C_{1-10} alkylene, and R^9 is a leaving group.

(43) The kit as defined in Claim 42 which is a cassette for use with an automated radiosynthesis apparatus.