



- (51) International Patent Classification:
C07H 19/167 (2006.01)
- (21) International Application Number:
PCT/US2012/035263
- (22) International Filing Date:
26 April 2012 (26.04.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/479,658 27 April 2011 (27.04.2011) US
- (71) Applicant (for all designated States except US): **RELI-
ABLE BIOPHARMACEUTICAL CORPORATION**
[US/US]; 1945 Walton Road, St. Louis, MO 63114 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WOOLDRIDGE,
Luzviminda, T.** [US/US]; 9326 Crawford Avenue, Brent-
wood, MO 63144 (US). **MA, Chun** [US/US]; 1007 Re-
gency Manor Drive, Ballwin, MO 63011 (US). **NADJI,
Sourena** [US/US]; 3 Bon Aire Drive, Olivette, MO 63132
(US).
- (74) Agent: **LESSLER, Jay P.**; Blank Rome LLP, The
Chrysler Building, 405 Lexington Ave., New York, NY
10174-0208 (US).

- (81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))



WO 2012/149196 A1

(54) Title: IMPROVED PROCESSES FOR THE PREPARATION OF REGADENOSON AND A NEW CRYSTALLINE FORM THEREOF

(57) Abstract: This disclosure relates to an improved process for the preparation of regadenoson, pharmaceutically acceptable salts thereof, and hydrates thereof, and for the preparation of intermediates useful in the synthesis of regadenoson. The disclosure also relates to a new crystalline form of regadenoson. Processes for the preparation of the crystalline form, compositions containing the crystalline form, and methods of use thereof are also described.

IMPROVED PROCESSES FOR THE PREPARATION OF REGADENOSON AND A NEW CRYSTALLINE FORM THEREOF

This application claims the benefit of U.S. Provisional Application No. 61/479,658, filed April 27, 2011, the entire contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to an improved process for the preparation of regadenoson, pharmaceutically acceptable salts thereof, and hydrates thereof, and for the preparation of intermediates useful in the synthesis of regadenoson. The present invention also relates to a new crystalline form of regadenoson. Processes for the preparation of the crystalline form, compositions containing the crystalline form, and methods of use thereof are also described.

BACKGROUND OF THE INVENTION

Regadenoson [(1-{9-[(4S, 2R, 3R, 5R)-3,4-dihydroxy-5-(hydroxymethyl)oxalan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamine] is a selective A_{2A}-adenosine receptor agonist that is a coronary vasodilator. It is currently marketed in the form of a monohydrate as a pharmacologic stress agent indicated for radionuclide myocardial perfusion imaging (MPI) in patients unable to undergo adequate exercise stress.

U.S. Patent No. 8,106,183 describes amorphous regadenoson, and three forms of regadenoson, referred to as Form A (a monohydrate), Form B and Form C.

The synthesis of regadenoson is described, for example, in U.S. Patent Nos. 6,403,567 and 7,183,264. The syntheses disclosed are multi-step processes that proceed via 2-hydrazinoadenosine, which is prepared from the corresponding iodo-derivative (2-iodoadenosine). Although these methods are suited to small scale syntheses, they are not well suited for large scale syntheses. Moreover, the yields are low and the staining nature of iodine makes the purification of the 2-iodoadenosine intermediate problematic. It is desirable, therefore,

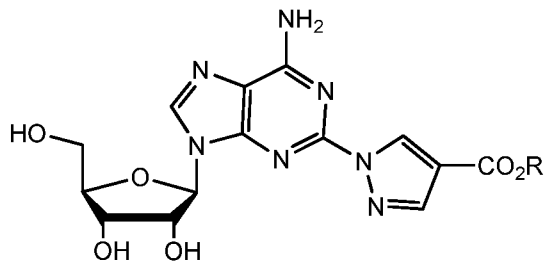
to find new methods of synthesis that provide a convenient method for making large quantities of the material in good yield and high purity.

SUMMARY OF THE INVENTION

The present inventors have found that regadenoson, and hydrates thereof (such as regadenoson monohydrate) may be prepared in high purity and high yield in one step using a 2-haloadenosine (e.g., 2-fluoroadenosine) as a starting material. The present inventors surprisingly found that 2-hydrazinoadenosine may be prepared from 2-fluoroadenosine in higher yield and at higher conversion rate than from the corresponding 2-iodo or 2-chloroadenosine derivatives.

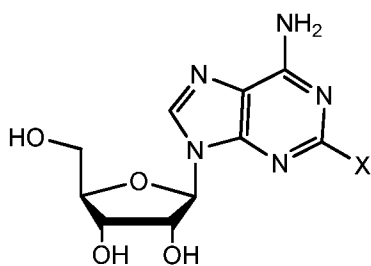
In one aspect, the present invention relates to a process for the preparation of regadenoson, derivatives thereof, pharmaceutically acceptable salts thereof, and hydrates thereof, which comprises contacting a 2-haloadenosine with a 4-N-alkylcarboxamide pyrazole (e.g., 4-N-methylcarboxamide pyrazole) or a 4-carboxylate pyrazole in the presence of a metal catalyst and a base.

Thus, in one aspect, the present invention relates to a process for the preparation of a compound of Formula (I), and pharmaceutically acceptable salts thereof, and hydrates thereof:

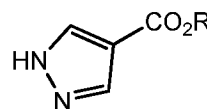


(I)

comprising contacting a compound of Formula (II) with a compound of Formula (III) in the presence of a metal catalyst and a base



(II)



(III)

wherein R is C₁₋₆ alkyl (e.g., C₁₋₄ alkyl such as methyl, ethyl, isopropyl); and

X is a halogen, such as F, Cl, Br or I.

The compound of Formula (I) may be converted to regadenoson by aminating the carboxylate group of the compound (e.g., by reaction with methylamine)

In a preferred aspect, the present invention relates to a process for the preparation of regadenoson, and hydrates thereof, which comprises contacting 2-fluoroadenosine with 4-N-methylcarboxamide pyrazole in the presence of a metal catalyst and a base.

In another aspect, the present invention relates to a process for the preparation of 2-hydrazinoadenosine comprising contacting 2-fluoroadenosine or 2-bromoadenosine with hydrazine. In a preferred embodiment, 2-fluoroadenosine is reacted with hydrazine. The 2-hydrazinoadenosine may further be converted to regadenoson, or a hydrate thereof.

In yet another aspect, the invention relates to a process for the purification of regadenoson (for example, regadenoson prepared by a method described herein) by subjecting the regadenoson to reverse phase chromatography. The column can be eluted with a water / alcohol (preferably water / methanol).

In yet another aspect, the invention relates to regadenoson having at least 98% purity (e.g., at least 99% or at least 99.5% purity) and comprising 2-fluoroadenosine in an amount up to about 0.2%, based upon the total weight of the regadenoson. The regadenoson may be incorporated into a pharmaceutical composition, such as a parenteral solution.

In yet another aspect, the invention relates to a composition comprising (a) regadenoson, and (b) 2-fluoroadenosine in an amount up to about 0.2%, based upon the total weight of the

composition. The composition can be a pharmaceutical composition, such as a parenteral solution.

In yet another aspect, the invention relates to a pharmaceutical composition comprising regadenoson made by a process of the present invention.

The present inventors have also discovered a new crystalline form of regadenoson, referred to herein as Form D. Thus, in a further aspect, the present invention relates to a novel crystalline form of regadenoson. In another aspect, the present invention relates to a process for the preparation of the new crystalline form. In certain embodiments, the new crystalline form may be prepared by a process that does not involve isolating any intermediate regadenoson compound. In additional aspects, the present invention relates to compositions containing the crystalline form, and to methods of use thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a ^1H nuclear magnetic resonance (NMR) spectrum of regadenoson monohydrate.

Figure 2 is a High-Performance Liquid Chromatography (HPLC) trace for regadenoson monohydrate.

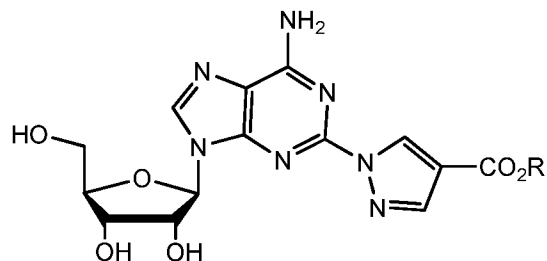
Figure 3 is an HPLC trace for the formation of 2-hydrazinoadenosine (A) by reaction of hydrazine with a 1:1:1 mixture of 2-fluoroadenosine (B), 2-chloroadenosine (C) and 2-iodoadenosine (D).

Figure 4 is an X-ray powder diffraction pattern of Form D of regadenoson.

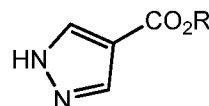
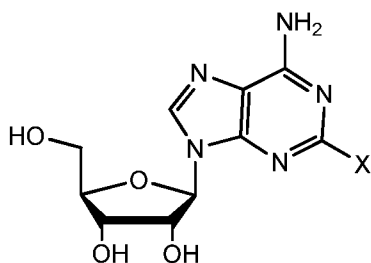
DETAILED DESCRIPTION OF THE INVENTION

Improved Processes for the Preparation Of Regadenoson and Derivatives Thereof and Hydrates Thereof

In one aspect, the present invention relates to a process for the preparation of a compound of Formula (I), and pharmaceutically acceptable salts thereof, and hydrates thereof:



comprising contacting a compound of Formula (II) with a compound of Formula (III) in the presence of a metal catalyst and a base

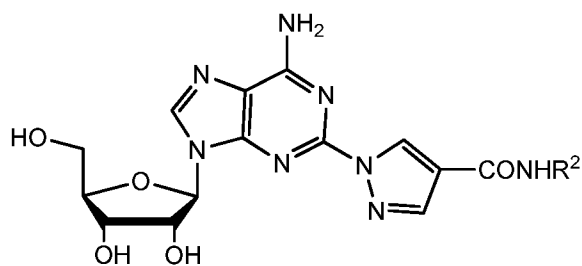


wherein R is C₁₋₆ alkyl (e.g., C₁₋₄ alkyl such as methyl, ethyl, isopropyl); and

X is a halogen, such as F, Cl, Br or I.

In a preferred embodiment, X is F. In another embodiment, R is methyl.

In one embodiment, the process further comprises converting the compound of formula (I) to a compound of Formula (IV)

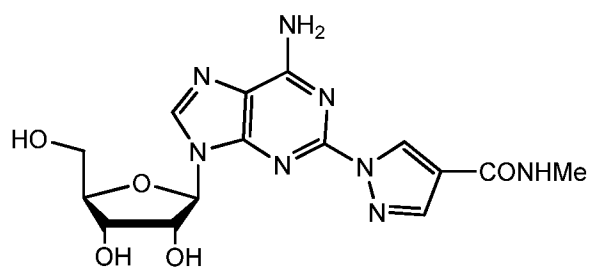


(IV)

wherein R^2 is C_{1-6} alkyl (e.g., C_{1-4} alkyl such as methyl, ethyl, isopropyl).

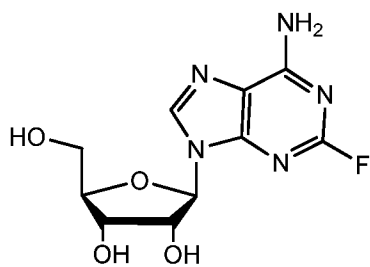
For example, the compound of Formula (I) may be converted to a compound of Formula (IV) by reaction with a primary amine, such as a C_{1-6} alkylamine (e.g., methyl amine).

In another aspect, the present invention relates to a process for the preparation of regadenoson of Formula (IA), pharmaceutically acceptable salts thereof, and hydrates thereof:

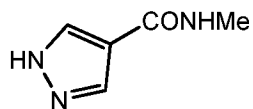


(IA)

which comprises contacting 2-fluoroadenosine of Formula (IIA) with 4-N-methylcarboxamide pyrazole of Formula (IIIA) in the presence of a metal catalyst and a base.



(IIA)



(IIIA)

In certain embodiments, the reaction is conducted in a polar solvent. For example, the solvent may be an organic solvent that is miscible with DMSO, such as acetonitrile, dimethylsulfoxide, dimethyl formamide, dichloroethane, dichloromethane, methanol, ethanol, and mixtures thereof. In certain embodiments, the reaction is conducted in a mixture (for example a 1:2 mixture) of dimethylsulfoxide and acetonitrile. In some embodiments, the solvent is dimethylsulfoxide containing from about 10 % to about 50% acetonitrile, for example, dimethylsulfoxide containing about 25% acetonitrile.

In additional embodiments, the base has a pK of at least 8 (e.g., a pK of from about 9 to about 12). For example, the base may be selected from sodium hydroxide, potassium hydroxide, triethylamine, cyclic amidine bases (such as, e.g., 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,4-diazabicyclo[2.2.2]octane (DABCO)) and combinations thereof. In one embodiment, the base is DBU. In another embodiment, the base is DABCO. In certain embodiments, the amount of base is about 3 equivalents, based on the amount of compound of formula (II). In further embodiments, the metal catalyst is a copper catalyst. For example, the metal catalyst is a copper (II) catalyst. In one embodiment, the copper (II) catalyst is chelated to a resin, for example, a resin attached to iminodiacetic acid. In certain embodiments, the amount of catalyst is about 0.1 equivalent, based on the amount of compound of formula (II).

In further embodiments, the reaction is conducted at a temperature of between about 60 °C and about 90 °C, for example between about 70 °C and about 85 °C, such as at about 80 °C.

In additional embodiments, the reaction proceeds for between about 12 and about 24 hours, such as between about 12 and about 18 hours.

In certain embodiments, the regadenoson is in the form of a hydrate, for example a monohydrate.

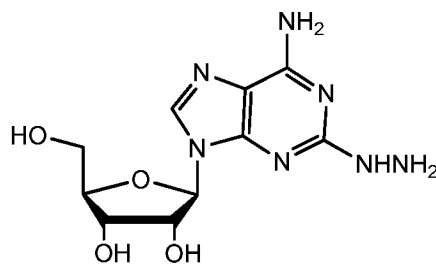
Figures 1 and 2, respectively, depict the ¹H NMR spectrum (DMSO-d₆) and HPLC trace of regadenoson monohydrate, prepared according to a process of the present invention.

In further embodiments, the regadenoson, pharmaceutically acceptable salt thereof, or hydrate thereof, is greater than about 75% pure, for example, greater than about 80% pure, greater than about 85% pure, greater than about 90% pure, greater than about 95% pure, greater

than about 97.5% pure, greater than about 99% pure, greater than about 99.5% pure or greater than about 99.9% pure.

In additional embodiments, the process further comprises converting regadenoson, or a hydrate thereof, to a pharmaceutically acceptable salt thereof. Acid addition salts may be prepared in a standard manner in a suitable solvent from the parent compound and an excess of acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic, methanesulfonic, and the like. Cationic salts may be prepared by reaction with an excess of an alkaline reagent, such as hydroxide, carbonate or alkoxide, containing the appropriate cation. Cations such as Na^+ , K^+ and Ca^{+2} are examples of cations present in pharmaceutically acceptable salts.

In another aspect, the present invention relates to a process for the preparation of 2-hydrazinoadenosine of Formula (V):



(V)

comprising contacting 2-fluoroadenosine or 2-bromoadenosine with hydrazine.

In a preferred embodiment, 2-fluoroadenosine is reacted with hydrazine. In certain embodiments, a molar excess of 2-fluoroadenosine is used. In some embodiments, the molar excess of hydrazine to 2-fluoroadenosine is from about 1.5 to about 20, such as from about 2 to about 12, from about 3 to about 6. In one embodiment, the molar excess of hydrazine to 2-fluoroadenosine is about a 3 molar excess.

In certain embodiments, the reaction is conducted in a polar solvent. For example, the solvent may be an organic solvent that is miscible with DMSO, such as acetonitrile, dimethylsulfoxide, dimethyl formamide, dichloroethane, dichloromethane, methanol, ethanol,

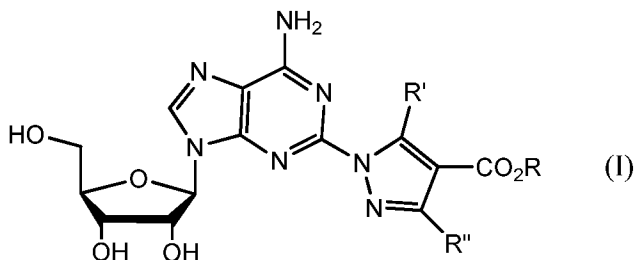
and mixtures thereof. For example, in one embodiment, the solvent is dimethylsulfoxide containing from about 10 % to about 50% acetonitrile.

In further embodiments, the reaction is conducted at a temperature of between about 60 °C and about 90 °C, for example between about 70 °C and about 85 °C, such as at about 75 °C.

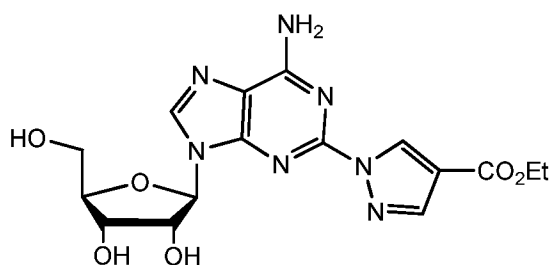
Figure 3 shows a HPLC vs. time trace for reaction of a 1:1:1 mixture of 2-fluoro, 2-chloro- and 2-iodoadenosine with 18 equivalents of hydrazine in dimethylsulfoxide at 75 °C. The inventors surprisingly found that the 2-fluoroadenosine was completely converted to 2-hydrazineadeosine within 5 hours at 75 °C. In contrast, the corresponding 2-iodoadenosine derivative achieved only 50% conversion to 2-hydrazineadeosine, and the 2-chlorodensoinse achieved only 20% conversion to 2-hydrazineadeosine under the same reaction conditions.

In certain embodiments, the process further comprises converting 2-hydrazinoadenosine of Formula (V) to regadenoson, or a hydrate thereof.

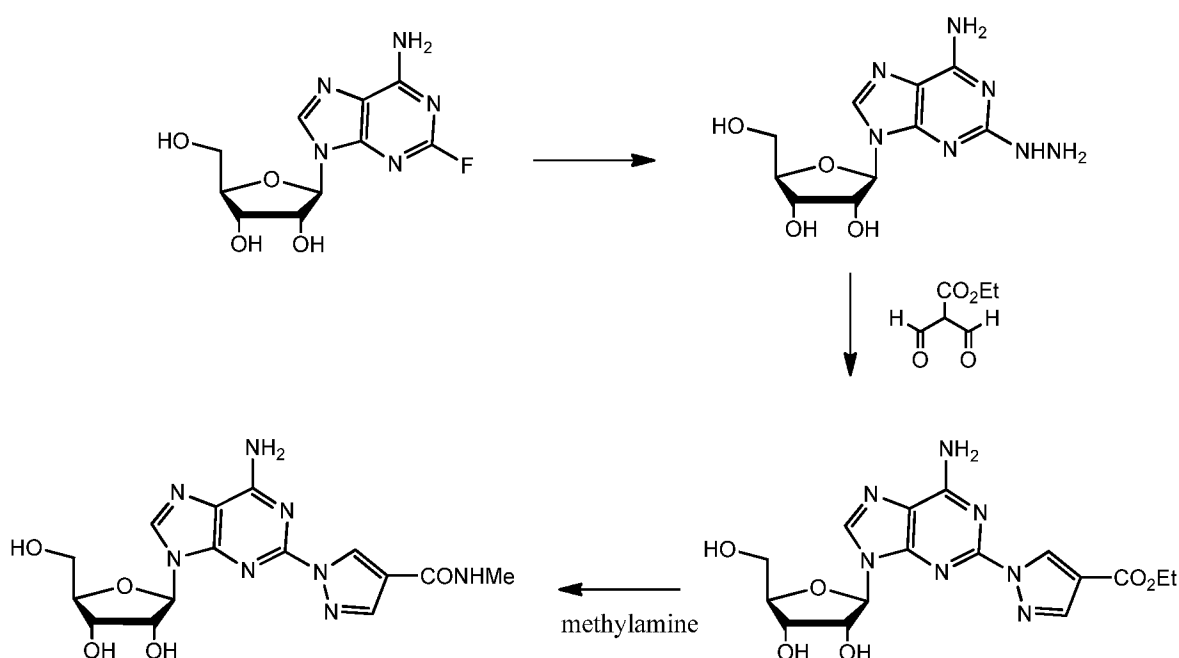
For example, the 2-hydrazinoadenosine of Formula (V) may be contacted with a compound of formula $[R'C(O)CH(CO_2R)C(O)R'']$ to afford a compound of Formula (I):



in which R is C₁₋₆ alkyl and R' and R'' are, independently, hydrogen, C₁₋₆ alkyl or aryl, with the proviso that at least one of R' and R'' is hydrogen. In one embodiment, R' and R'' are both hydrogen. The compound of Formula (I) may be further converted to regadenoson. In an exemplary embodiment, the 2-hydrazinoadenosine of Formula (V) may be contacted with ethyl 2-formyl-3-oxopropionate [HC(O)CH(CO₂Et)C(O)H] to afford



which may subsequently be converted to regadenoson, or a hydrate thereof, by contacting it with methylamine, according to the procedures outlined, for example, in U.S. Patent Nos. 6,403,567, 7,183,264 and 7,732,595, as shown below:



Regadenoson can be purified by any method known in the art. Applicants have discovered that one particularly effective method of purification is by subjecting the regadenoson to reverse phase chromatography. The column can be eluted with water / alcohol (preferably water / methanol). For example, the regadenoson can be dissolved in 1:1 mixture of DMSO and methanol, loaded to a reverse phase column, and eluted with water/methanol (gradient with a 5% increase of MeOH). The fractions with the desired HPLC purity, for example, with 99.9% or greater purity, can be combined, filtered and dried (e.g., in a vacuum oven). The reverse phase chromatography can be repeated on fractions have a purity of 99.8% or lower (for example, a purity of between 50% and 99.8%).

Prior to (or alternatively to) performing the reverse phase chromatography, the regadenoson may undergo one or more purification steps, such as (a) filtering, (b) co-evaporation with xylene, and (c) precipitation (for example, from acetonitrile) followed by filtration.

New Crystalline Form of Regadenoson (Form D)

In another aspect, the present invention provides a novel crystalline form of regadenoson which can be identified by one or more analytical methods. Form D is stable under inert conditions. The X-ray powder diffraction (XRPD) pattern of the crystalline form (Form D) is provided in Figure 4.

In a further embodiment, the crystalline form of regadenoson (Form D) is characterized by a X-ray powder diffraction pattern substantially as shown in Figure 4. With respect to the term "substantially," one skilled in the art would understand that the relative intensities of the peaks can vary, depending upon the sample preparation technique, the sample mounting procedure and the particular instrument employed.

In another embodiment, the crystalline form of regadenoson (Form D) contains between about 0.8 and about 1.7 % by weight of water of hydration.

Form D of regadenoson can be in solid form (such as a powder) which is useful for preparing solutions containing regadenoson, such as a parenteral formulation of regadenoson.

In one embodiment, the invention provides a composition comprising Form D of regadenoson. In another embodiment, the invention provides a composition comprising Form D of regadenoson and one or more other solid state forms of regadenoson, such as Form A, B or C of regadenoson.

The crystalline form of regadenoson (Form D) of the invention can be administered alone or as an active ingredient of a formulation. Thus, the present invention also includes pharmaceutical compositions of the crystalline form of regadenoson (Form D) containing, for example, one or more pharmaceutically acceptable carriers.

Compositions

Parenteral solutions may be prepared, for example, by dissolving regadenoson (such as Form D of regadenoson, or regadenoson made by a process of the present invention) in an aqueous solution (such as saline) and optionally adding one or more pharmaceutically acceptable excipients. Thus, in another aspect, the present invention relates to a method of preparing a parenteral solution of regadenoson comprising (i) dissolving (a) Form D of regadenoson, or (b) regadenoson made by a process of the present invention, in an aqueous solution and (ii) optionally adding one or more pharmaceutically acceptable excipients.

Numerous standard references are available that describe procedures for preparing various formulations suitable for administering regadenoson, including Form D of regadenoson and regadenoson made by a process of the present invention. Examples of potential formulations and preparations are contained, for example, in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association (fifth edition); Pharmaceutical Dosage Forms: Tablets (Lieberman, Lachman and Schwartz, editors) third edition, published by Marcel Dekker, Inc.; as well as Remington's Pharmaceutical Sciences (Arthur Osol, editor), (seventeenth edition).

Administration of the regadenoson (such as Form D of regadenoson) may be accomplished according to patient needs, for example, orally, nasally, parenterally (subcutaneously, intravenously, intramuscularly, intrasternally and by infusion), by inhalation, rectally, vaginally, topically and by ocular administration.

Various solid oral dosage forms can be used for administering the regadenoson (such as Form D of regadenoson) including such solid forms as tablets, gelcaps, capsules, caplets, granules, lozenges and bulk powders. The regadenoson can be administered alone or combined with various pharmaceutically acceptable carriers, diluents (such as sucrose, mannitol, lactose, and starches) and excipients known in the art, including, but not limited, to suspending agents, solubilizers, buffering agents, binders, disintegrants, preservatives, colorants, flavorants, lubricants and the like. Time release capsules, tablets and gels are also advantageous in administering the compounds of the present invention.

Suppositories for rectal administration of the regadenoson (such as Form D of regadenoson) can be prepared by mixing the compound with a suitable excipient such as cocoa

butter, salicylates and polyethylene glycols. Formulations for vaginal administration can be in the form of a pessary, tampon, cream, gel, past foam, or spray formula containing, in addition to the active ingredient, such suitable carriers as are known in the art.

For topical administration, the pharmaceutical composition can be in the form of creams, ointments, liniments, lotions, emulsions, suspensions, gels, solutions, pastes, powders, sprays, and drops suitable for administration to the skin, eye, ear or nose. Topical administration may also involve transdermal administration via means such as transdermal patches.

Aerosol formulations suitable for administering via inhalation also can be made. For example, for treatment of disorders of the respiratory tract, the compounds according to the invention can be administered by inhalation in the form of a powder (e.g., micronized) or in the form of atomized solutions or suspensions. The aerosol formulation can be placed into a pressurized acceptable propellant.

Methods of Treatment

The present invention further provides methods for treating a condition which requires modulation of an adenosine receptor, e.g., an A_{2A} receptor. The methods involve administering a therapeutically effective amount of (i) regadenoson of the present invention (e.g., Form D of regadenoson, or regadenoson made by a process of the present invention) or (ii) a regadenoson formulation (such as a parenteral solution) prepared from regadenoson of the present invention, to a patient in need thereof. One embodiment is a method of stimulating coronary vasodilation, producing coronary vasodilation.

In additional embodiments, the present invention provides methods of myocardial perfusion imaging that involve administering (i) regadenoson of the present invention or (ii) a regadenoson formulation (such as a parenteral solution) prepared from regadenoson of the present invention, and a radionuclide.

In some embodiments, the compound or composition of the present invention is administered as a mono-therapy. In other embodiments, the compound or composition of the present invention is administered as part of a combination therapy. For example, a compound or

composition of the present invention may be used in combination with other drugs or therapies that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which compounds and compositions of the present invention are useful.

In certain embodiments, regadenoson of the present invention is administered (or used to prepare a formulation such as a parenteral solution) in an amount of about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 1 mg, about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 5.5 mg, about 6 mg, about 6.5 mg, about 7 mg, about 7.5 mg, about 8 mg, about 8.5 mg, about 9 mg, about 9.5 mg or about 10 mg. For example, the active ingredient is administered in an amount of about 0.4 mg, e.g., as an injection containing 0.4 mg regadenoson of the present invention (such as Form D) per 5 mL solution (such as saline) (0.08 mg/mL). In additional embodiments, the amount administered is sufficient to stress the heart and induce a coronary steal situation. In additional embodiments, the amount administered is sufficient for imaging heart or coronary activity in the patient.

Definitions

An “effective amount” refers to the amount of a compound of the present invention that, when administered to a patient (e.g., a mammal) for treating a disorder, is sufficient to effect such treatment for the disorder, or an amount of a compound that is sufficient for modulating a adenosine receptor (e.g., A_{2A} receptor) to achieve the objectives of the invention. The “effective amount” will vary depending on the compound, the disease and its severity and the age, weight, etc., of the patient to be treated.

A subject or patient in whom administration of the therapeutic compound is an effective therapeutic regimen for a disease or disorder is preferably a human, but can be any animal, including a laboratory animal in the context of a clinical trial or screening or activity experiment. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods, compounds and compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, humans, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine,

caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, and avian species, such as chickens, turkeys, and songbirds, i.e., for veterinary medical use.

The following examples are merely illustrative of the present invention and should not be construed as limiting the scope of the invention in any way as many variations and equivalents that are encompassed by the present invention will become apparent to those skilled in the art upon reading the present disclosure.

EXAMPLES

2-Fluoroadenosine and ethyl pyrazole-4-carboxylate are available from Sigma-Aldrich (St. Louis, MO). Chelex 100 resin is available from Bio-Rad Laboratories (Hercules, CA). The HPLC method for the preparation of N-methyl-4-carboxamide and regadenoson was carried out on an Altima C18 column, 5 μ , 4.6 mm x 250 mm, using a gradient of 2 – 70 % over 15 min of Buffer B (Buffer A = 0.1% triethylamine / 0.1% Acetic Acid / 3% acetonitrile by volume in DI water, pH ~4.5; Buffer B = 10% Buffer A in acetonitrile). The purification of crude regadenoson was carried out on a reverse phase resin using a gradient of 0 - 70% methanol / water.

X-Ray Powder Diffractions analysis was performed using a Rigaku Ultima IV X-ray diffractometer. The x-ray generator employed a Cu tube operated at 40kV. The experimental conditions were as follows: Bragg-Brentano focusing method geometry; Incident and receiving Soller slits = 5 degrees; divergent and scattered slits = 2/3 degree; divergent height limiting slit = 10mm; receiving slit = 0.3mm; scan speed = 0.2 degrees/minute; sampling width = 0.02 degree; scintillation detector with monochromator.

EXAMPLE 1

Synthesis of N-Methyl-4-carboxamide

20 g (143 mmol, 1 equiv) of ethyl pyrazole-4-carboxylate and 200 mL (2310 mmol, 16.2 equiv) of a 40 % aqueous solution of methylamine were added to a three-necked flask equipped

with a condenser and a heating mantle. The mixture was stirred to aid dissolution, and heated to 65 °C for 2 hours. The reaction was monitored using HPLC at 220 nm with a C18 column. The reaction mixture was then concentrated *in vacuo* to obtain a syrup / solid. The crude product was co-evaporated with acetonitrile (3 x 200 mL). 100 mL of acetonitrile was then added to the solids and the mixture was stirred for several hours until the solids were well suspended. The solids were then isolated by filtration, washed with 100 mL acetonitrile, and dried in an oven at 40°C to afford 14.4 g (80 % yield) of N-methyl-4-carboxamide with a purity of 93.5% by HPLC.

EXAMPLE 2

Synthesis of IDAAR-Cu⁺²

This preparation has reported in the literature. See, e.g., *Chinese Chemical Letters*, (21(1), 51-54, 2010).

An Erlenmeyer flask was charged with 350 mL of water and 75 g of Chelex 100 resin. With stirring, an aqueous solution of copper sulfate pentahydrate (59 g in 350 mL of water) was slowly added over a period of 15 minutes. The resulting slurry was stirred for 2 hours, then filtered. The resulting solids were washed with 100 - 200 mL of water and dried in a vacuum oven at 50 °C for 16 hours to afford 18 g of IDAAR-Cu⁺². The copper content of the product was determined to be 11 wt % using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

EXAMPLE 3

Synthesis of Regadenoson Monohydrate

5 g (17.5 mmol, 1 equiv) of 2-fluoroadenosine, 3.07g (24.5 mmol, 1.4 equiv) of N-methylpyrazole-4-carboxamide, and 32 mL of dimethylsulfoxide were added under a nitrogen atmosphere to a dry 3-necked reaction flask equipped with a condenser and a heating mantle.. The mixture was stirred to afford a solution. 100 mL of acetonitrile was then added followed by the addition of 2.2 g of IDAAR-Cu²⁺ and 5.34 g (5.24 mL, 35.1 mmol, 2 equiv) of diazabicycloundecene (DBU). The reaction mixture was heated to 70 – 80 °C overnight and monitored by HPLC at 260 nm with a C18 column until the reaction was complete. Then, the reaction mixture was evaporated *in vacuo* to remove most of the acetonitrile. The remaining

dimethylsulfoxide solution was purified by reverse phase chromatography using methanol and water. The product was dried *in vacuo* at a temperature that did not exceed 40° C to afford 3 g (44% yield) of regadenoson monohydrate.

EXAMPLE 4

Synthesis of 2-Hydrazineadenosine

2-fluoroadenosine (4g, 14 mmol) was dissolved in 100 mL ethanol in a 300 mL three-necked flask. Hydrazine hydrate (4.1 mL, 6 equivalents, 84 mmol) was added and the mixture was heated to reflux for 1 hour. The reaction mixture was allowed to cool to room temperature and stirred overnight (16 hours). The resulting white precipitate was isolated by filtration and dried in oven at 40°C overnight to afford 2-hydrazinoadenosine (yield: 94%, 3.5g, 96% purity).

EXAMPLE 5

Synthesis of Regadenoson Form D

2-Fluoroadenosine (45 g, 0.158 mol., 1 eq.), 4-(N-methylcarboxamido)pyrazole (27.64 g, 0.221 mol., 1.4 eq.), dimethylsulfoxide (DMSO) (320 mL) and acetonitrile (960 mL) were added to a dry 3000 ml 3-neck reaction flask equipped with a condenser and heating mantle. After stirring for 10 minutes, IDAAR-Cu (20.07 g, 0.032 mol., 0.2 eq.) and DBU (48.0 g, 0.316 mol., 2 eq.) were added. The resulting mixture was then heated to 65°C overnight (18 hours).

The reaction mixture was then filtered and the filtrate was evaporated followed by 2 x 500 mL co-evaporation with xylene. The residue was diluted with 5 L acetonitrile, transferred to a 10 L flask and kept in a cold room (4°C) overnight. The resulting white precipitate was isolated by filtration and stirred in 1.8 L of water. The mixture was heated to 80° C for 2 hours, then allowed to cool in a cold room (4°C) overnight.

The white precipitate was isolated by filtration, then dissolved in 200 ml of 1:1 mixture of DMSO and methanol. The clear and slightly yellow solution was loaded to a reverse phase column (10 L) and eluted with water/methanol (gradient with a 5% increase of MeOH every 10 L).

The fractions with HPLC purity of more than 99.9% were combined and concentrated to

a paste. The supernatant liquid was decanted and the flask heated in an oil-bath at 150° C under reduced pressure of 20mmHg for 6 hours to afford 6.2 g of Regadenoson Form D as white solid (99.94% HPLC, KF analysis 0.8%).

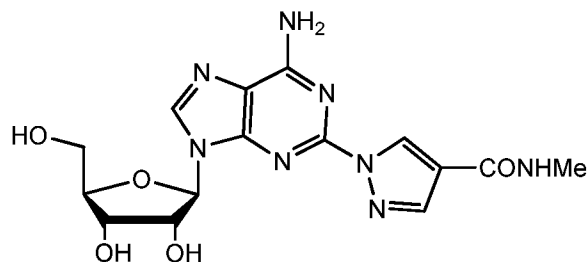
The fractions with HPLC purity between 50 and 99.8% (~ 23g of product as indicated by HPLC) were combined and subjected to a second purification stage.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

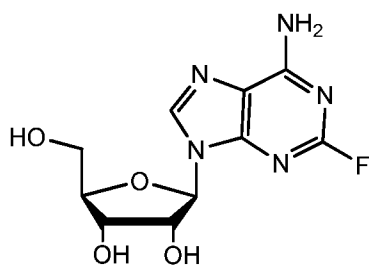
WHAT IS CLAIMED IS:

1. A process for the preparation of regadenoson of Formula (IA), a pharmaceutically acceptable salt thereof, or a hydrate thereof:

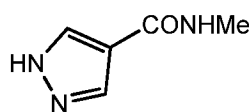


(IA)

comprising contacting 2-fluoroadenosine of Formula (IIA) with 4-N-methylcarboxamide pyrazole of Formula (IIIA):



(IIA)

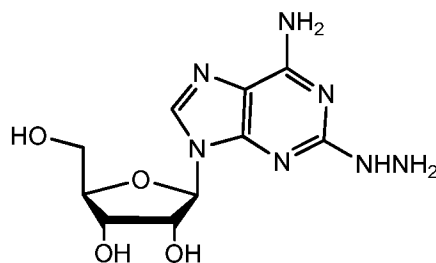


(IIIA)

in the presence of a metal catalyst and a base.

2. The process of claim 1, wherein the reaction is conducted in a solvent selected from acetonitrile, dimethylsulfoxide, and mixtures thereof.
3. The process of claim 1 or 2, wherein the base is selected from sodium hydroxide, potassium hydroxide, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,4-diazabicyclo[2.2.2]octane (DABCO), and combinations thereof.
4. The process of claim 3, wherein the base is DBU.
5. The process of any one of claims 1-4, wherein the metal catalyst is a copper (II) catalyst.

6. The process of claim 5, wherein the copper (II) catalyst is chelated to a resin.
7. The process of any one of claims 1-6, wherein the reaction is conducted at a temperature of between about 60 °C and about 90 °C.
8. The process of any one of claims 1-7, wherein the regadenoson of Formula (IA) is a monohydrate.
9. The process of any one of claims 1-8, further comprising converting the regadenoson, or hydrate thereof, to a pharmaceutically acceptable salt thereof.
10. A process for the preparation of 2-hydrazinoadenosine of Formula (V):

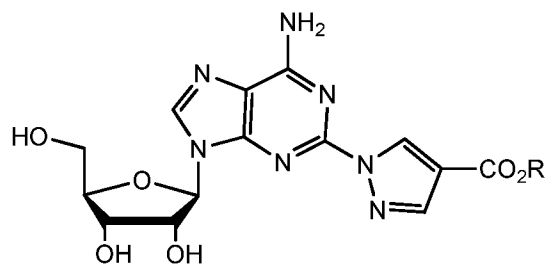


(V)

comprising contacting 2-fluoroadenosine with hydrazine.

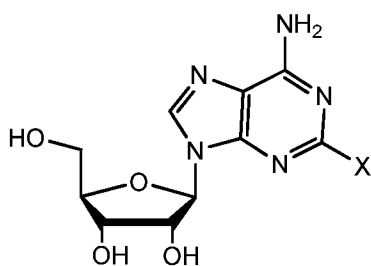
11. The process of claim 11, wherein the molar ratio of hydrazine to 2-fluoroadenosine is from about 3 to about 6.
12. The process of claim 10 or 11, wherein the reaction is conducted in an organic solvent selected from acetonitrile, dimethylsulfoxide, and mixtures thereof.
13. The process of any one of claims 10-12, wherein the reaction is conducted at a temperature of between about 60 °C and about 90 °C.
14. The process of any one of claims 10-13, further comprising converting the 2-hydrazinoadenosine of Formula (IV) to regadenoson, a pharmaceutically acceptable salt thereof, or a hydrate thereof.

15. A process for the preparation of a compound of Formula (I), or a pharmaceutically acceptable salt thereof or a hydrate thereof:

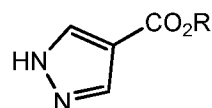


(I)

comprising contacting a compound of Formula (II) with a compound of Formula (III) in the presence of a metal catalyst and a base



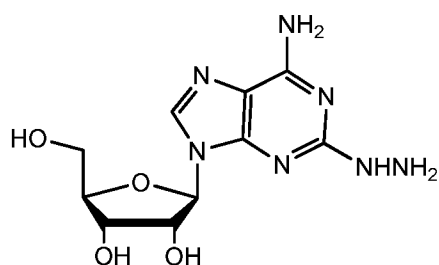
(II)



(III)

wherein R is C₁₋₆ alkyl; and X is a halogen.

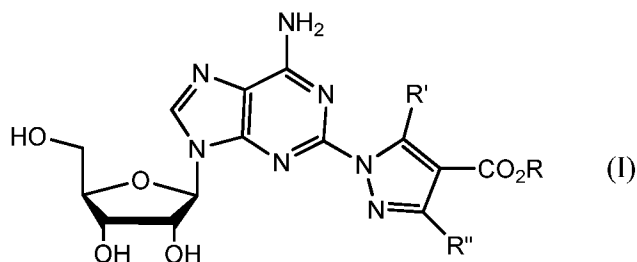
16. A process for the preparation of 2-hydrazinoadenosine of Formula (V):



(V)

comprising contacting 2-bromoadenosine with hydrazine.

17. The process of claim 10 or 16, further comprising contacting the compound of Formula (V) with $[R'C(O)CH(CO_2R)C(O)R'']$ to afford a compound of Formula (I):



wherein R is C₁₋₆ alkyl and R' and R'' are, independently, hydrogen, C₁₋₆ alkyl or aryl, with the proviso that at least one of R' and R'' is hydrogen.

18. The process of claim 17, wherein R' and R'' are hydrogen.

19. The process of claim 18, further comprising converting the compound of Formula (I) to regadenoson, or a pharmaceutically acceptable salt thereof, or a hydrate thereof.

20. The process of claim 19, wherein the converting step comprises reacting the compound of Formula (I) with methylamine.

21. The process of any one of claims 1-9, 14, 19, and 20, further comprising the step of subjecting the regadenoson to reverse phase chromatography.

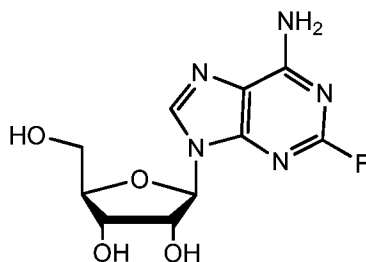
22. The process of claim 21, wherein the column is eluted with water and methanol.

23. A process for purifying regadenoson comprising the step of subjecting the regadenoson to reverse phase chromatography.

24. The process of claim 23, wherein the column is eluted with water and methanol.

25. Regadenoson made by a process of any of claims 1-9, 14, and 19-24.

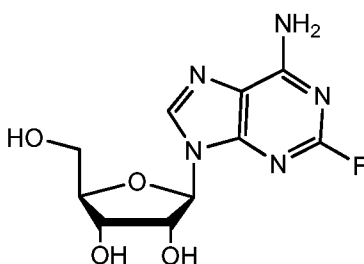
26. Regadenoson having at least 98% purity and comprising 2-fluoroadenosine of Formula (IIA)



(IIA)

in an amount up to about 0.2%, based upon the total weight of the regadenoson.

27. A composition comprising:
- regadenoson, and
 - 2-fluoroadenosine of Formula (IIA)



(IIA)

in an amount up to about 0.2%, based upon the total weight of the composition.

28. A parenteral solution of the regadenoson of claim 26.
29. A crystalline form of regadenoson (Form D) having an X-Ray powder diffraction pattern substantially as shown in Figure 4.
30. The crystalline form of regadenoson (Form D) of claim 29, wherein the crystalline form contains between about 0.8 and about 1.7 % by weight of water of hydration.
31. A pharmaceutical composition comprising a crystalline form of claim 29 or 30 and a pharmaceutically acceptable carrier.

32. A method of preparing a parenteral solution of regadenoson comprising dissolving Form D of regadenoson in an aqueous solution and optionally adding one or more pharmaceutically acceptable excipients.
33. A parenteral solution of regadenoson, prepared by the method of claim 32.

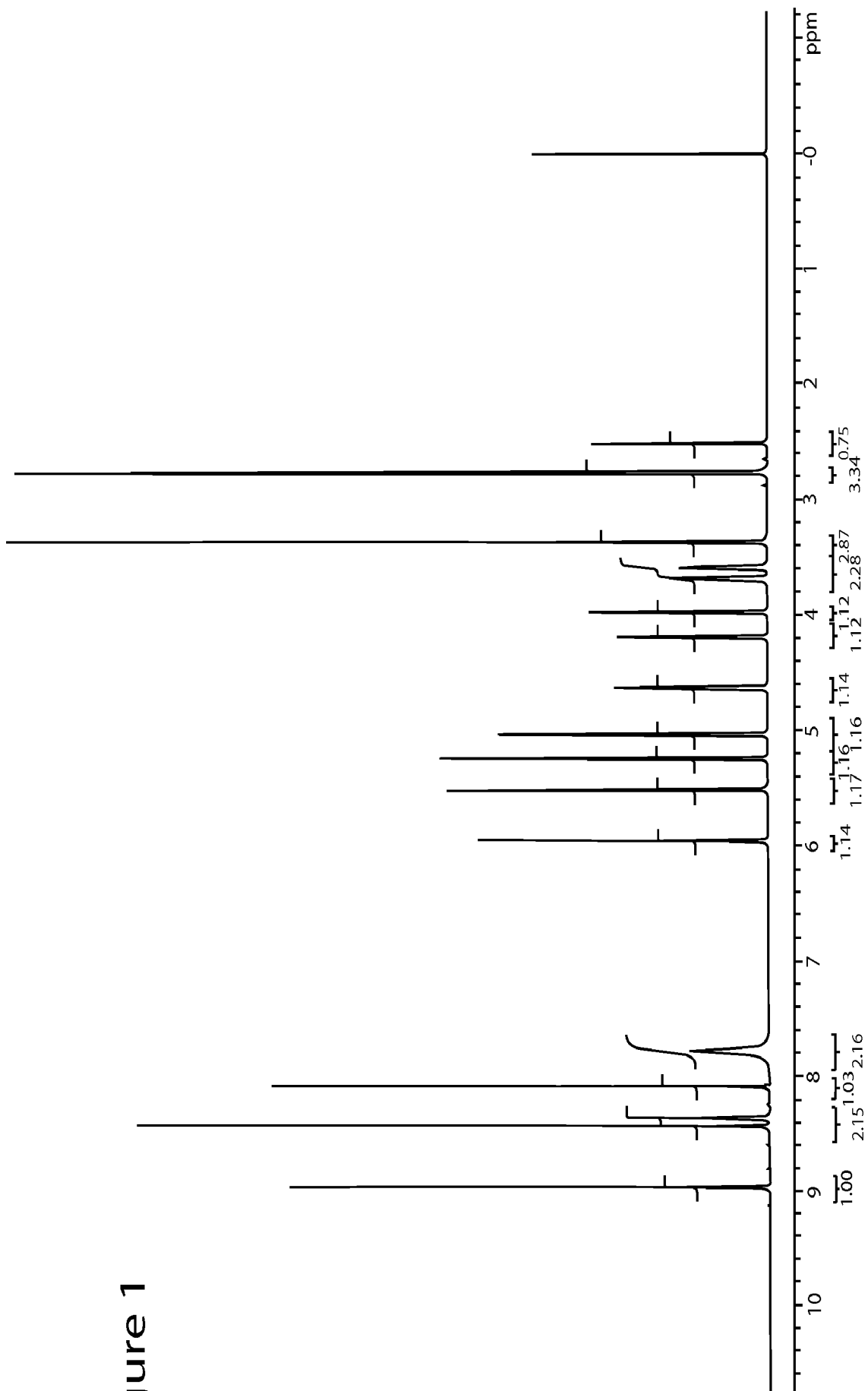


Figure 1

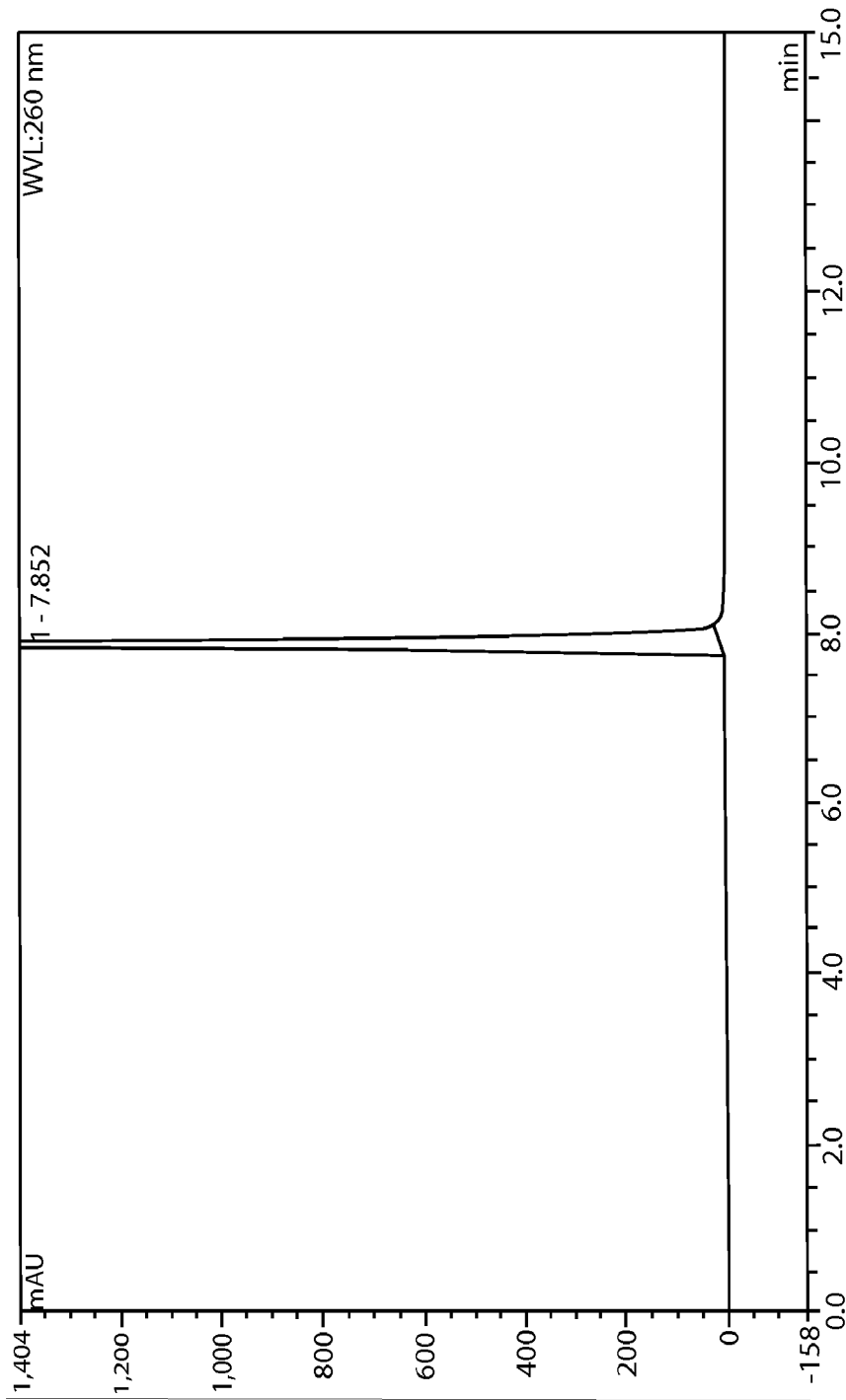


Figure 2

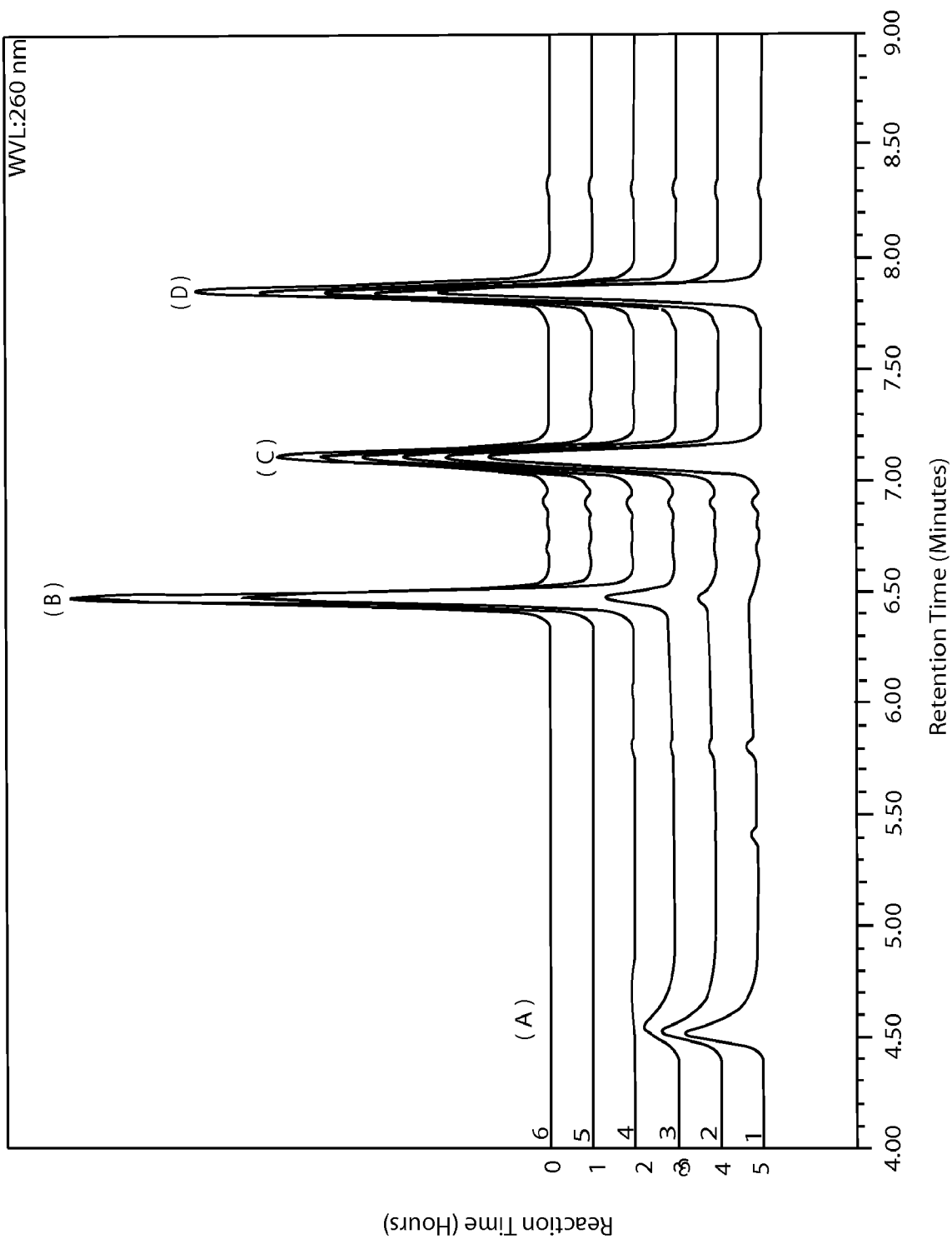


Figure 3

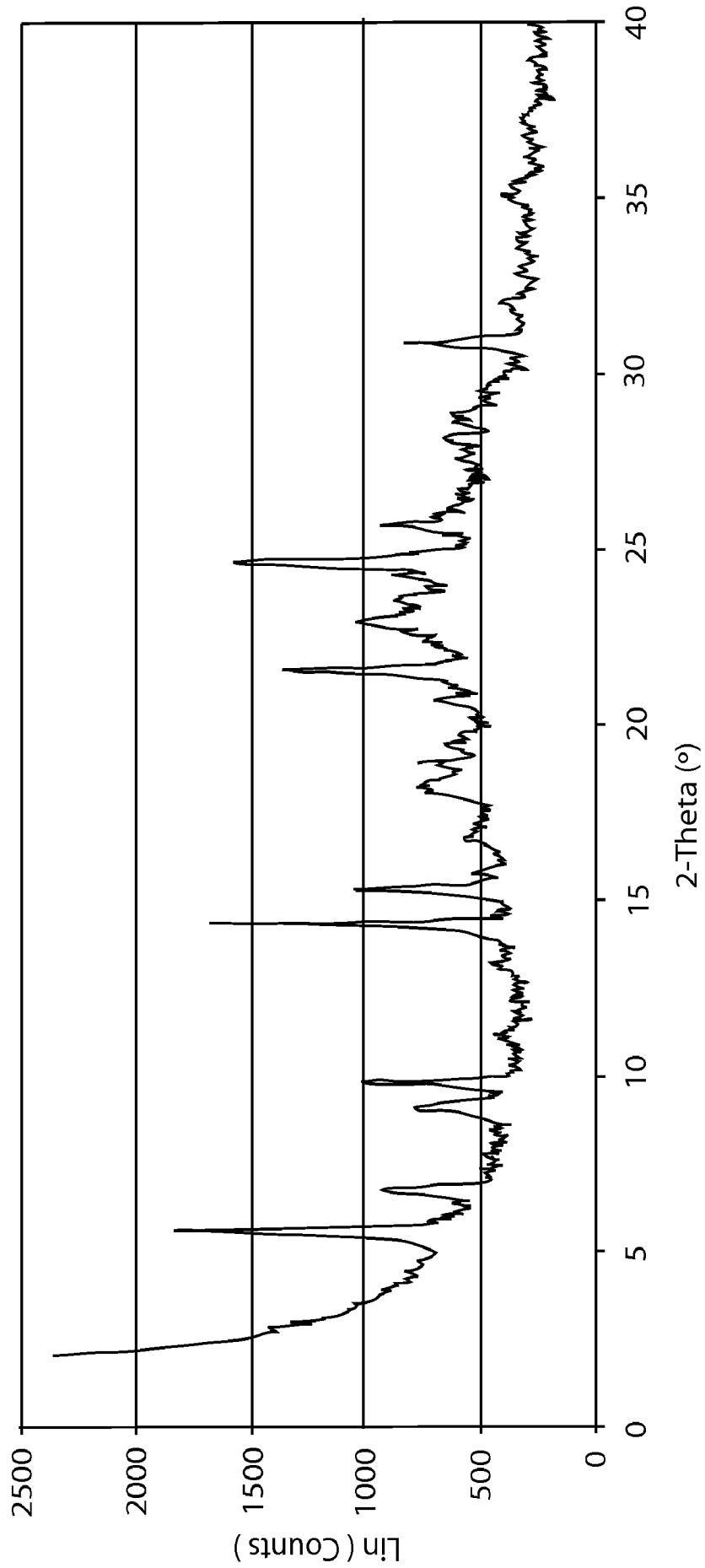


Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/035263

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07H19/167
 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)
 C07H

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 practicable, search terms used)
 EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 6 403 567 B1 (ZABLOCKI JEFF A [US] ET AL) 11 June 2002 (2002-06-11) cited in the application column 9 - column 13 example 5	25 1-9,21, 22
X A	US 7 732 595 B2 (ZABLOCKI JEFF [US] ET AL) 8 June 2010 (2010-06-08) cited in the application reaction scheme 1 examples 4,6	25 1-9,21, 22

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 application or patent 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 14 June 2012	Date of mailing of the international search report 25/09/2012
---	--

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 Nikolai, Joachim
--	--

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/035263

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9(completely); 21, 22, 25(partially)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9(completely); 21, 22, 25(partially)

Process for the preparation of regadenoson. Regadenoson made by a process of claim 1.

2. claims: 10-14, 16-20(completely); 21, 22, 25(partially)

Process for the preparation of 2-hydrazinoadenosine. Regadenoson made by a process of claims 14 and 19-22.

3. claim: 15

Process for preparing a compound of formula (I)

4. claims: 23, 24(completely); 25(partially)

Process for purifying regadenoson. Regadenoson made by a process of claims 23-24.

5. claims: 26-28

Composition comprising regadenoson and 2-fluoroadenosine

6. claims: 29-33

Crystalline form of regadenoson (Form D) and method of preparing a parental solution of regadenoson comprising dissolving Form D of regadenoson.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/035263

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6403567	B1	11-06-2002	AR 024441 A1 02-10-2002
			AT 256141 T 15-12-2003
			AU 760806 B2 22-05-2003
			BR 0011856 A 10-02-2004
			CA 2377746 A1 28-12-2000
			CN 1358191 A 10-07-2002
			DE 60007127 D1 22-01-2004
			DE 60007127 T2 16-09-2004
			DE 122011000010 I1 16-06-2011
			DK 1189916 T3 22-03-2004
			EP 1189916 A2 27-03-2002
			ES 2209974 T3 01-07-2004
			HK 1044952 A1 25-02-2005
			IL 147198 A 10-04-2006
			JP 4727880 B2 20-07-2011
			JP 2003506461 A 18-02-2003
			LU 91785 I2 31-03-2011
			MX PA01013325 A 02-07-2002
			NO 2011002 I1 07-03-2011
			NO 20016350 A 18-02-2002
			NZ 516334 A 28-05-2004
			PT 1189916 E 31-03-2004
			TR 200200588 T2 23-09-2002
			TW I230161 B 01-04-2005
			US 6403567 B1 11-06-2002
			US 6642210 B1 04-11-2003
			US 2004038928 A1 26-02-2004
			US 2006052332 A1 09-03-2006
			US 2007203090 A1 30-08-2007
			US 2010160620 A1 24-06-2010
			US 2011144320 A1 16-06-2011
			WO 0078779 A2 28-12-2000
			ZA 200200301 A 26-11-2003
US 7732595	B2	08-06-2010	AU 2007212542 A1 16-08-2007
			CA 2640089 A1 16-08-2007
			CN 102260311 A 30-11-2011
			EP 1989214 A1 12-11-2008
			JP 2009525347 A 09-07-2009
			KR 20080090491 A 08-10-2008
			NZ 570239 A 30-09-2011
			US 2007225247 A1 27-09-2007
			US 2007265445 A1 15-11-2007
			US 2010179313 A1 15-07-2010
			US 2010267953 A1 21-10-2010
			US 2011257387 A1 20-10-2011
			US 2012165350 A1 28-06-2012
			WO 2007092372 A1 16-08-2007