

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
10 November 2005 (10.11.2005)

PCT

(10) International Publication Number
WO 2005/105708 A1

(51) International Patent Classification⁷: **C07B 59/00**

(21) International Application Number:
PCT/JP2005/006547

(22) International Filing Date: 28 March 2005 (28.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/566921 3 May 2004 (03.05.2004) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL ¹⁵O-LABELED MONOSACCHARIDE AND PRODUCING METHOD THEREOF

(57) Abstract: This invention relates to novel ¹⁵O-labeled monosaccharide useful for positron emission tomography (PET) and producing method thereof.



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1.

DESCRIPTION

Novel ^{15}O labeled monosaccharide and producing method thereof



5 Technical Field

This invention relates to novel ^{15}O -labeled monosaccharide useful for positron emission tomography (PET) and producing method thereof.

10 Background Art

Positron emission tomography (PET) is a noninvasive bio-imaging technology which can be used to diagnose functions or disorders of a variety of organs such as brain and heart. In PET, a radioactive tracer is administrated to a subject to
15 determine the distribution of the tracer in the body of the subject. To date, [^{18}F]2-fluoro-2-deoxyglucose (FDG) have been used as the most useful PET tracer. FDG can be used to determine sugar metabolism quantitatively and have lead to progressive improvement for brain study or cancer diagnosis. However, the
20 number of times of PET measurement per one day is limited due to long half-life of ^{18}F (110 min). Moreover, since ^{18}F -labeled compound is non-natural, the behavior of ^{18}F -labeled compound in a body is different from that of the corresponding natural compound. Further, although synthesis of glucose labeled with
25 ^{15}O at C1 position has been tried, such ^{15}O -labeled glucose has been failed to be synthesized. Moreover, OH group at the C1

position of glucose molecule is unstable and easily subjected to exchange reaction with H₂O in blood. Therefore, such labeled glucose should not be suitable for PET measurement.

5 Summary of the invention

The present inventors have established novel ¹⁵O-labeled monosaccharide and producing method thereof to overcome such shortcomings of conventional labeled compounds for PET. The method for producing ¹⁵O-labeled monosaccharide of the present
10 invention is partially based on the alcohol production method described in WO98/34983.

In one aspect, the present invention provides ¹⁵O-labeled monosaccharide which is labeled with ¹⁵O at hydroxymethyl group in the monosaccharide molecule.

15 In another aspect, the present invention provides a method for producing ¹⁵O-labeled monosaccharide, comprising reacting a monosaccharide, which is substituted with a halogen at hydroxyl of hydroxymethyl group in the monosaccharide molecule, with ¹⁵O oxygen in the presence of an organotin compound and a reducing
20 agent, wherein said reacting occurs either in the absence of a radical initiator or in the presence of not more than 0.3 equivalent, based on the halogenated monosaccharide, of a radical initiator to provide ¹⁵O-labeled monosaccharide which is labeled with ¹⁵O at the hydroxymethyl group in the monosaccharide molecule.

25 In another aspect, the present invention provides a method for diagnosing a whole body, organs or tissues of a subject,

comprising administering the above ^{15}O -labeled monosaccharide to the subject to measure metabolism of the ^{15}O -labeled monosaccharide in the subject.

In another aspect, the present invention provides a kit
5 for producing ^{15}O -labeled monosaccharide, comprising a monosaccharide which is substituted with a halogen at hydroxyl of hydroxymethyl group in the monosaccharide molecule, an organotin compound and a reducing agent, wherein the kit is used to react the halogenated monosaccharide with ^{15}O oxygen in the
10 presence of the organotin compound and the reducing agent to produce a ^{15}O -labeled monosaccharide which is labeled with ^{15}O at the hydroxymethyl group in the monosaccharide molecule.

The method of the invention can be used to produce ^{15}O -labeled monosaccharide rapidly with high yield. Since ^{15}O has short
15 half-life (2 min), the ^{15}O -labeled monosaccharide of the present invention can be used to practice more than once PET measurement per one day for a subject and with only little radioactive dose exposed to the subject. Moreover, the behavior of the ^{15}O -labeled monosaccharide of the present invention in a body is more similar
20 to that of natural compound than that of ^{18}F -labeled compound. Further, since the ^{15}O -labeled monosaccharide of the present invention is labeled with ^{15}O at the hydroxymethyl group of the monosaccharide molecule (e.g., C6 position of hexose or C5 position of pentose), it is more stable in a body than
25 monosaccharide labeled with ^{15}O at C1 position. Therefore, the

^{15}O -labeled monosaccharide of the present invention can be used in PET measurement to accomplish more successful imaging.

Brief description of the drawings

5 Fig. 1 shows the glass filter reactor used for radical hydroxylation of halogenated glucose in the Example. Numbers in this figure are length in mm.

 Fig. 2 shows the purification system used for purification of ^{15}O 2-deoxy-D-glucose.

10 Fig. 3 shows the cyclotron system used for generation of ^{15}O oxygen and introduction of it into the reaction system.

 Fig. 4 shows the results of PET scans for rat performed by using several of labeled compounds. Fig. 4A and 4B show integrated PET image from 15 min to 30 min after administration of ^{15}O 2-deoxy-D-glucose and ^{18}F FDG, respectively. Fig. 4C and 4D show PET image of initial blood flow from 0 sec to 90 sec and from 15 min to 30 min after administration of ^{15}O H_2O , respectively.

 Fig. 5 shows the results of PET scans for mouse performed by using several of labeled compounds. Fig. 5A and 5B show integrated PET image from 15 min to 30 min after administration of ^{15}O 2-deoxy-D-glucose and ^{18}F FDG, respectively. Fig. 5C and 5D show PET image of initial blood flow from 0 sec to 90 sec and from 15 min to 30 min after administration of ^{15}O H_2O , respectively.

25

Detailed description of the present invention

The present invention is now described in detail.

The "monosaccharide" useful for the present invention includes any monosaccharide and derivative thereof known in the art. Preferably, the "monosaccharide" useful for the present invention is hexose or pentose. Hexose useful for the present invention includes D-glucose, D-galactose, D-mannose, D-fructose and derivative thereof. Pentose useful for the present invention includes D-xylose, D-ribose, D-deoxyribose, D-arabinose and derivative thereof. Preferably, the "monosaccharide" useful for the present invention is D-glucose or derivative thereof, and most preferably, D-glucose or 2-deoxy-D-glucose.

The term "labeled with ^{15}O at hydroxymethyl group in the monosaccharide molecule" means that hydroxymethyl ($-\text{CH}_2\text{OH}$) group in the monosaccharide molecule is labeled with ^{15}O to form $-\text{CH}_2^{15}\text{[O]H}$ group. More specifically, the term means, for example, that if the monosaccharide is a hexose, the free hydroxyl on C6 carbon of the hexose is labeled with ^{15}O , and that if the monosaccharide is a pentose, the free hydroxyl on C5 carbon of the pentose is labeled with ^{15}O .

The "a monosaccharide which is substituted with a halogen" or "a halogenated monosaccharide" in the present invention is used as a precursor in the method for producing ^{15}O -labeled monosaccharide of the present invention. Each of the terms is referred to a monosaccharide having a halogenated methyl group

(-CH₂X, X is a halogen atom) formed by substituting a halogen for hydroxyl of hydroxymethyl group in the monosaccharide molecule.

The "halogen" in the halogenated monosaccharide to be used
5 in the present invention includes chloro, iodo, bromo and so on, iodo is the most preferred halogen for purposes of the invention.

The "organotin compound" useful for the present invention includes, but is not limited to, organotin hydrides (e.g., trialkyltin hydrides and triaryltin hydrides) and organotin
10 halides (e.g., tributyltin chloride, dibutyl(t-butyl)tin chloride and triaryltin halides (e.g. triphenyltin chloride)). Preferably, organotin compound includes trialkyltin hydrides. Most preferable organotin compound is tributyltin hydrides.

The amount of the organotin compound to be used in the present
15 invention may be a catalyst amount, is preferably 1.0-6.0 equivalents, and more preferably 2.0-5.0 equivalents, based on the substrate halogenated monosaccharide.

The "reducing agent" useful for the present invention includes, but is not limited to, reducing agents known in the
20 art such as phosphine, sulfide, selenide, telluride, arsine, stibane, bismuthane or derivative thereof, borohydrides. Preferred reducing agent for use in the present invention is triphenylphosphine.

The amount of the reducing agent to be used in the present
25 invention is sufficient for reducing peroxide produced in the

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reaction, and preferably at least 1 equivalent based on the substrate halogenated monosaccharide.

The reaction according to the present invention can be carried out using various solvents which do not interfere with the reaction. Such solvent includes fluorine-solvent (e.g., benzofluoride, benzotrifluoride, perfluorodecalin, etc.), alcohol (e.g. ethanol, isopropyl alcohol, butanol, t-butanol, etc.), BTX, and ethers (e.g. tetrahydrofuran etc.), inclusive of mixtures thereof, among others. Those skilled in the art can select suitable solvent for the reaction based on, for example, solubility of the halogenated monosaccharide or ^{15}O oxygen in the reaction system. Preferred solvent is a mixture of fluorine-solvent and alcohol.

The reaction temperature for use in the present invention is not particularly restricted provided that the radical reaction is enabled to proceed, although the reaction is preferably conducted at not less than 50°C , and more preferably $75-85^{\circ}\text{C}$.

^{15}O oxygen used in the present invention can be produced, for example, by generating the nuclear reaction $^{15}\text{N}(\text{p}, \text{n})^{15}\text{O}$ by proton bombardment from a cyclotron on target gas containing $^{16}\text{O}_2$ and $^{15}\text{N}_2$, or by generating the nuclear reaction $^{15}\text{N}(\text{d}, \text{n})^{15}\text{O}$ by deuteron bombardment from a cyclotron on target gas containing $^{16}\text{O}_2$ and $^{15}\text{N}_2$. Then the target gas containing ^{15}O oxygen produced is mixed with cold carrier gas and introduced into the reaction system (Fig. 3).

Preferably, prior to introduction of ^{15}O oxygen in the reaction system, carrier gas containing oxygen ($^{16}\text{O}_2$) is introduced into the reaction system to initiate radical reaction. Preferably, the initiation of the radical reaction is monitored, and the introduction of ^{15}O oxygen into the reaction system is started simultaneously with the initiation of the radical reaction. The initiation of the radical reaction can be easily monitored by those skilled in the art using an analytical technique (e.g., TLC) known in the art

Concentration of $^{16}\text{O}_2$ in the cold carrier gas introduced into the reaction system to initiate radical reaction is preferably at least 1.5% and more preferably 1.5%-5.0%, although the concentration is suitably adjusted by those skilled in the art based on, for example, reaction conditions such as induction time of the radical reaction. Concentration of $^{16}\text{O}_2$ in the target gas is preferably equivalent to the concentration of $^{16}\text{O}_2$ in the cold carrier gas, although it is suitably adjusted by those skilled in the art based on, for example, yield of ^{15}O -labeled monosaccharide produced.

In the method for producing ^{15}O -labeled monosaccharide of the present invention, the radical reaction can be caused without substantially using "radical initiator". However, "radical initiator" may be introduced into the reaction system for promoting the reaction or for suppressing production of reductants as byproduct. If "radical initiator" is used in the method of the present invention, the amount of the "radical initiator" is

preferably no more than 0.3 equivalents and more preferably no more than 0.03 equivalents, based on the halogenated monosaccharide.

The "radical initiator" for use in the present invention includes, but is not limited, to AIBN and dibenzoyl peroxide.

The method for producing ^{15}O -labeled monosaccharide of the present invention can be carried out by using any reaction vessel known in the art or a reaction vessel which those skilled in the art can easily make using any experimental appliance known in the art (e.g., glass filter). For example, a preferred reaction vessel useful for the method of the present invention is a reaction vessel comprising a glass filter at the bottom as shown in the following examples, wherein ^{15}O oxygen in the mixed gas can bubble very finely in the reaction vessel. Such reaction vessel can be made by those skilled in the art referring the teaching of the specification. Preferably, the reaction vessel has longitudinally long form for dissolving sufficient ^{15}O oxygen in the reaction system.

The kit for producing ^{15}O -labeled monosaccharide of the present invention comprises a halogenated monosaccharide, an organotin compound and a reducing agent, wherein the kit is used to react the halogenated monosaccharide with ^{15}O oxygen in the presence of the organotin compound and the reducing agent to produce ^{15}O -labeled monosaccharide of the present invention. The ^{15}O oxygen can be supplied from, for example, a cyclotron equipped in an institution (e.g., a hospital) wherein a diagnosis of a

subject is practiced. Preferably, the kit of the present invention further comprise no more than 0.3 equivalents, based on the halogenated monosaccharide, of radical initiator. In one embodiment, the kit of the present invention further comprises
5 a reaction vessel in which the reaction can be practiced to produce ^{15}O -labeled monosaccharide. Preferably, the reaction vessel may be a vessel in a disposable cassette format known in the art to be able to produce ^{15}O -labeled monosaccharide easily and rapidly.

The ^{15}O -labeled monosaccharide of the present invention
10 can be used to diagnose a whole body, organs or tissues of a subject. The diagnosing method comprises administering the ^{15}O -labeled monosaccharide of the present invention to the subject to measure metabolism of the ^{15}O -labeled monosaccharide in the subject. Such measurement can be carried out by using any medical imaging method
15 known in the art such as positron emission tomography. The organ or tissue diagnosed by using the ^{15}O -labeled monosaccharide of the present invention includes, but is not to be limited, brain, heart or tumor tissue which metabolizes glucose actively. Further, due to much shorter half-life of ^{15}O than that of ^{18}F ,
20 the ^{15}O -labeled monosaccharide of the present invention can be used to measure diurnal variation of sugar metabolism in one subject over time.

If the ^{15}O -labeled monosaccharide of the present invention is used as a diagnostic agent, the reaction product is preferably
25 purified to remove the organotin compound and the reducing agent in the reaction mixture. Such purification method includes, but

is not to be limited, solid-phase extraction using silica gel column (e.g., Sep-Pak C18) and HPLC.

The reaction conditions of the present invention have been described above. In conducting a specific reaction, it is necessary to optimize the reaction conditions according to the structure of the halogenated monosaccharide and the species and amount of the organotin compound, among other variables, but such optimization is a matter which can be made easily by any one skilled in the art. Further, the method for producing ¹⁵O-labeled monosaccharide of the present invention is applicable to disaccharide (e.g., maltose, sucrose, lactose) and oligosaccharide provided that the saccharides have a free hydroxyl group on C6 carbon of hexose residue or C5 carbon of pentose residue in the molecule.

The following examples illustrate the present invention in further detail.

Example

Materials

Unless otherwise noted, chemicals were used as they are.

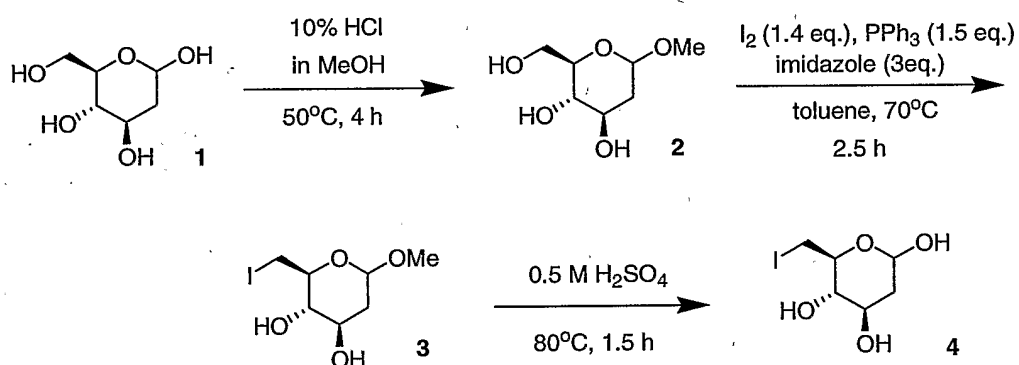
2-Butanol (Kanto Chemical) was distilled from CaH₂ prior to use. Benzotrifluoride, perfluorodecalin and AIBN were purchased from Acros, Fluorochem Ltd. and Wako Pure Chemicals, respectively. n-Bu₃SnH was obtained from Aldrich and used without further purification. N₂/O₂ gas (98.5/1.5) was obtained from Nippon Sanso Co., Ltd.

12.

Equipments

Hydroxylation of 2,6-dideoxy-6-iodo-D-glucose and 6-iodo-6-deoxy-D-glucose was performed in the glass filter reactor as shown in Fig. 1. Sep-Pak cartridges were purchased from Waters. The cyclotron is OSCAR-12 (NKK/Oxford). The target gas consisted of 1.5% O₂ in ¹⁵N₂. PET scans were obtained on Planar Positron Imaging System (Hamamatsu Photonics K.K.).

10 Example 1: Synthesis of 2,6-dideoxy-6-iodo-D-glucose



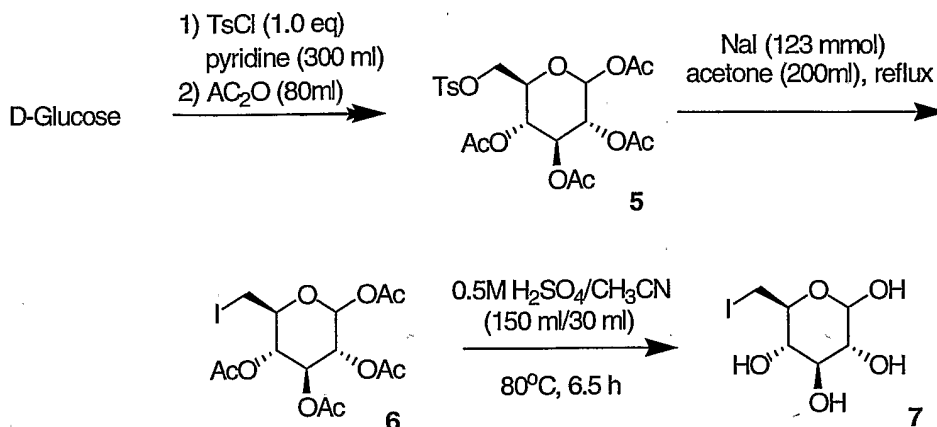
2-deoxy-D-glucose (1) (Aldrich) (5.0 g, 30 mmol) was placed in a flask, and HCl solution in methanol (10%, 60 ml) was added. The resulting mixture was heated at 50°C overnight. After cooling to room temperature, silver carbonate was added, and the mixture was vigorously stirred by when generation of CO₂ gas have finished. The reaction mixture was filtrated through Celite, and the resultant solution was evaporated. Toluene (20 ml) was added to the solution, and the resultant mixture was evaporated to remove the residual methanol. Toluene (250 ml) was added and dissolved in the concentrated residue, and imidazole (6.12 g, 90.0 mmol),

triphenylphosphine (11.8 g, 45.0 mmol) and iodine (11.5g, 42.0 mmol) were added. Again, toluene (200 ml) was added, and the resultant mixture was vigorously stirred under N₂ flow at 70°C for 2.5 h. After the reaction completed, the supernatant in the reaction vessel was transferred to another vessel and evaporated. On the other hand, chloroform was added to the gummy solid component in the reaction vessel to give a suspension, and the suspension was filtrated through Celite. The filtrate was evaporated and combined to the concentrated supernatant solution obtained previously. The combined solution was subjected to column chromatography (chloroform:methanol = 20:1, twice) to afford 5.9 g of compound 3. The yield is 68% based on compound 1.

Compound 3 (5.9 g) was placed in a flask, and sulfuric acid (0.5 M aqueous solution, 200 ml) was added. The solution was stirred at 80°C for 1.5 h. After cooling to room temperature, sodium hydrogencarbonate was carefully added portionwise to the acidic solution to neutralize it. The resultant mixture was then directly evaporated (bath temp. no more than 40°C). The evaporation was stopped when the volume of the solution reduced to about 10-20 ml. Methanol (100 ml) was added to the resultant concentrated solution, which led to growth white solid of sodium sulfate. The solution was filtrate through Celite, and the filtrate was evaporated at no more than 30°C. The concentrated residue was subjected to column chromatography (chloroform:methanol = 8:1) repeatedly to afford 4.2 g of 2,6-dideoxy-6-iodo-D-glucose (compound 4). The yield is 51% based

on compound 1.

Example 2: Synthesis of 6-iodo-6-dideoxy-D-glucose



5 D-Glucose (20.0 g, 0.111 mol) was placed in a 1000-mL round-bottomed flask, and pyridine (300 ml) was added. Some of glucose remained undissolved. *p*-Toluenesulfonyl chloride (22.0g, 0.115 mol) was added at ambient temperature. After stirring for 11 h, acetic anhydride (80 ml, 0.83 mol) was added in one portion.

10 Gentle exothermic reaction took place. After being stirred for 1.5 h, the mixture was evaporated. Ethanol (200 ml) was added to a residual oil. The oil was dissolved and soon a white crystal appeared. After the mixture stood undisturbed at -10°C for 27 h, the crystal was collected on a glass filter, washed with cold

15 ethanol (25 ml, twice), and dried under reduced pressure. 6-*O*-(*p*-Toluenesulfonyl)-1,2,3,4-tetra-*O*-acetyl-β-D-glucose (compound 5) was obtained in 33% yield (18.5g, 36.8 mmol).

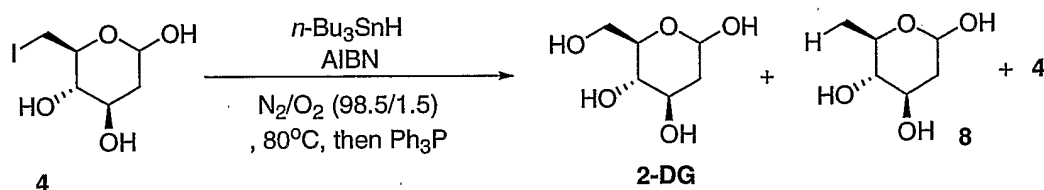
6-*O*-(*p*-Toluenesulfonyl)-1,2,3,4-tetra-*O*-acetyl-β-D-glucose (5, 18.5 g, 36.8 mmol) was placed in a 500-mL round-bottomed

20 flask. Acetone (200 ml) and sodium iodide (18.5g, 123 mmol) were

added. The resulting mixture was heated at reflux for 20 h. The reaction proceeded gradually. The mixture was poured into 1000 ml of water, and the resulting solid was filtered with a glass filter. Recrystallization from ethanol afforded 6-deoxy-6-iodo-1,2,3,4-tetra-O-acetyl- β -D-glucose (compound 6) in 65% yield (11.0 g, 24.0 mmol).

6-Deoxy-6-iodo-1,2,3,4-tetra-O-acetyl- β -D-glucose (6, 6.87 g, 15.0 mmol) was placed in a 500-mL round-bottomed flask. Sulfuric acid (0.5 M aqueous solution, 150 ml) and acetonitrile (30 ml) were added to 6, and the mixture was heated at 80°C for 6.5 h. After cooling to room temperature, sodium hydrogencarbonate was carefully added portionwise to the acidic solution. Neutralization was checked with indicator paper, and the mixture was then directly evaporated (bath temp. 40°C). Before complete removal of solvent (ca. 10-20 ml), methanol (100 ml) was added to the flask, which led to growth of white precipitate. Filtration through Celite, concentration of the filtrate (bath temp. 30°C), and silica gel column purification (chloroform:methanol = 3:1) afforded 6-deoxy-6-iodo-D-glucose (compound 7) (2.16 g, 7.44 mmol, 50%).

Example 3: Radical hydroxylation of 2,6-dideoxy-6-iodo-D-glucose in the glass filter reactor (1)



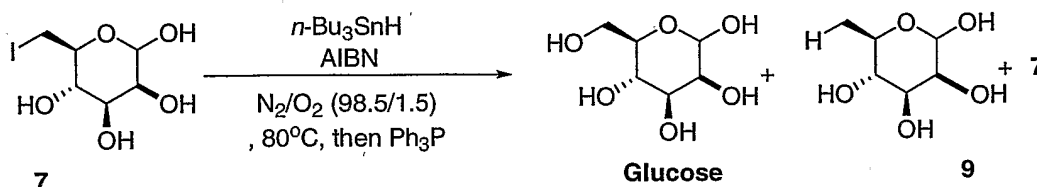
2,6-dideoxy-6-iodo-D-glucose (4) (162 mg, 0.600 mmol) as obtained in Example 1 and AIBN (2.4 mg, 0.015 mmol) were placed in a 20-mL vial. 2-Butanol (1.8 ml) was added, and 4 was dissolved. Benzotrifluoride (12.0 ml) and perfluorodecalin (2.7 ml) were then added. Tributyltin hydride (485 μ l, 1.80 mmol) was introduced by a microsyringe. Note that addition by a normal syringe may cause initiation of the reaction before bubbling, giving only compound 8. The homogeneous solution was transferred with a Pasteur pipette into the glass filter reactor. The reactor was then immersed in an oil bath (80°C) with bubbling of mixed gas ($N_2/O_2 = 98.5/1.5$, 200 ml/min). The glass filter made very fine bubbles. TLC analyses were done every 30 sec, which indicated that the reaction actually started at 1.5 min and completed at 4.0 min after the heating started. After 7.0 min, the reaction mixture was transferred to a solution of triphenylphosphine (157 mg, 0.600 mmol) in toluene (1.5 ml). The reactor was rinsed with 10 ml of methanol once. Concentration under reduced pressure afforded a mixture of oil and viscous residue. Toluene (10 ml) was added, and the resulting supernatant was passed through a Sep-Pak Cartridge silica (long body, conditioned with 10 ml of toluene prior to use). Here the gummy residue, which consisted of glycosides, was not soluble in toluene. The eluent containing tin and phosphine compounds was thrown away. Again, toluene was added to the gummy residue, and the toluene layer was passed through the same cartridge. The cartridge was then washed with methanol (5 ml + 5 ml) by using the same syringe that was employed to take

up the toluene solutions. The methanolic eluent was added the gummy glycosides. Evaporation and ^1H NMR measurement (1,1,2,2-tetrachloromethane as an internal standard) revealed that the mixture consisted of 0.182 mmol (31% based on **4**, 54% based on O_2 that passed through the solution during the radical chain) of 2-deoxy-D-glucose (2-DG) and 0.402 mmol (67%) of **8**, in addition to a trace of tin and phosphine impurities.

Example 4: Radical hydroxylation of 2,6-dideoxy-6-iodo-D-glucose in the glass filter reactor (2)

The method used in this example was substantially same as the method shown in the above Example 3. A mixture of **4** (270 mg, 1.0 mmol), tributyltin hydride (3.0 mmol) and AIBN (0.025 mmol) in α,α,α -trifluorotoluene (20.0 ml)/perfluorodecalin (4.5 ml)/2-butanol (3.0 ml) was heated at 80°C with concomitant bubbling of N_2/O_2 (98.5/1.5, 180 ml/min for 0 to 2 min 40 sec, 200 ml/min for 2 min 40 sec to 6 min 20 sec). TLC analyses were done every 30 sec, which indicated that the reaction actually started at 3 min 20 sec and completed at 6.5 min after the heating started. After 7.0 min, workup with triphenylphosphine (1.0 mmol) reduced hydroperoxide into 2-DG. ^1H NMR measurement revealed that the mixture consisted of 0.280 mmol (28% based on **4**, 70% based on O_2 that passed through the solution during the radical chain) of 2-DG and 0.72 mmol (72%) of **8**, in addition to a trace of tin and phosphine impurities.

Example 5: Radical hydroxylation of 6-iodo-6-dideoxy-D-glucose in the glass filter reactor



6-iodo-6-dideoxy-D-glucose (7) (261 mg, 0.900 mmol) as
 5 obtained in Example 2 and AIBN (3.6 mg, 0.015 mmol) were placed
 in a 20-mL vial. 2-Butanol (3.5 ml) was added, and 7 was dissolved.
 Benzotrifluoride (15.0 ml) and perfluorodecalin (2.0 ml) were
 then added. Tributyltin hydride (484 μ l, 1.80 mmol) was
 introduced by a microsyringe. The homogeneous solution was
 10 transferred with a Pasteur pipette into the glass filter reactor.
 The reactor was then immersed in an oil bath (80°C) with bubbling
 of mixed gas (N₂/O₂ = 98.5/1.5, 200 ml/min). TLC analyses were
 done every 30 sec, which indicated that the reaction actually
 started at 2.0 min and completed at 5.0 min after the heating
 15 started. After 7.0 min, the reaction mixture was transferred to
 a solution of triphenylphosphine (236 mg, 0.900 mmol) in toluene
 (2.0 ml). The reactor was rinsed with 10 ml of methanol twice.
 Workup, purification, and NMR analysis as shown in Example 3
 revealed that the mixture consisted of 0.198 mmol (22% based on
 20 7, 50% based on O₂ that passed through the solution during the
 radical chain) of glucose, 0.569 mmol (63%) of 9 and 0.137 mmol
 (15%) of 7 in addition to a trace of tin and phosphine impurities.

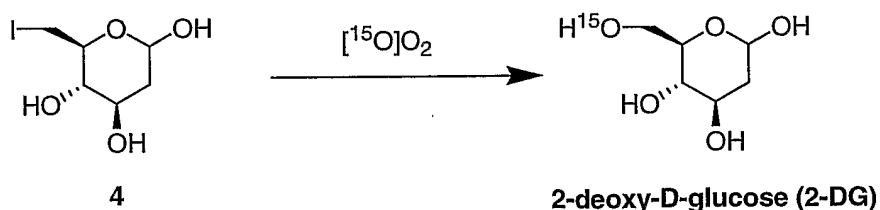
Example 6: Hydroxylation of 2,6-dideoxy-6-iodo-D-glucose in the

glass filter reactor followed by rapid purification

For administration to an animal, the produced 2-DG is necessary to be separated from tin-compound and phosphine-compound. Therefore, the present inventors constructed a purification system as shown in Fig. 2 and purified the produced 2-DG by using the system.

After the reaction was performed for 4 min as shown in Example 3, triphenylphosphine (157 mg, 0.600 mmol in 2 mL of PhCF₃) was added to the reaction mixture, and bubbling continued for 10 sec. Water (3 ml) was added, and bubbling continued for 20 sec. The whole mixture was transferred to a reservoir and then stood undisturbed for 20 sec to float an aqueous layer (ca. 2.3 ml) on a fluorous organic layer. The lower layer was eluted away through a Sep-Pak C18 cartridge (conditioned with 10 ml of PhCF₃) by using a reduced pressure. The elution stopped at the boundary of the two layers. The aqueous layer on the top of the cartridge was then passed through another Sep-Pak C18 cartridge (conditioned with 10 ml of methanol and then with 10 ml of water). The eluate was a tin- and phosphine-free aqueous solution of 2-DG (17%, 0.103 mmol), 4 (trace) and 8 (47%, 0.280 mmol).

Example 7: PET imaging using ¹⁵[O] 2-deoxy-D-glucose



A solution of 2,6-dideoxy-6-iodo-D-glucose (274 mg, 1.0

mmol), tributyltin hydride (807 μ l, 3.0 mmol) and AIBN (4.0 mg, 0.025 mmol) in PhCF₃ (20.0 ml)/C₁₀F₁₈ (4.5 ml)/2-BuOH (3.0 ml) was placed in the reactor, which was on the automated synthetic apparatus (Fig. 3). Proton bombardment (beam current 50 μ A) on target gas started (N₂/O₂ = 98.5/1.5, target gas pressure = 6 kg/cm²). The beginning of the bombardment was defined as 0 sec for convenience. Cold gas (N₂/O₂ = 1773/3, 180 ml/min) was provided and heating with temperature-controlled air (80°C) started simultaneously at 2 min 40 sec. At 4.0 min, the target gas was evacuated from the targeting area in the cyclotron system (20 ml/min). The target gas was mixed with cold N₂/O₂ gas (target gas/N₂/O₂ = 20/177/3, 200 ml/min), and the resulting gas was then supplied to the reaction vessel. At 4 min 40 sec, the RI counter at the detector indicated arrival of hot gas. At 5 min 20 sec, the bombardment stopped. However, evacuation of the target gas continued. Triphenylphosphine (262 mg, 1.0 mmol in 1.5 ml of PhCF₃) was added at 6.0 min. At 6 min 10 sec, all the mixture was transferred from the reactor to a Sep-Pak Vac Silica cartridge (conditioned with 10 ml of PhCF₃) (A in Fig. 3) and the eluent was removed. Saline (3.0 ml) was consequently passed through the Sep-Pak Vac Silica cartridge. The eluted aqueous solution was passed through Sep-Pak C18 cartridge (conditioned with 10 ml of acetonitrile and then with 10 ml of saline) (B in Fig. 3) and afforded to a tin- and phosphine-free aqueous solution of ¹⁵[O]2-deoxy-D-glucose ([¹⁵O]2-DG) (ca. 3 ml, 15-20 mCi).

For rat imaging, 1 mL of the [¹⁵O]2-DG solution was injected

from his tail vein. [^{15}O] 2-DG-PET scans could be performed over 30 min after the administration. Integrated PET image from 15 min to 30 min appeared accumulation of [^{15}O] 2-DG in heart, kidney and bladder (Fig. 4A). For comparison with [^{15}O] 2-DG, [^{18}F] FDG-PET scans were also performed, and integrated PET image for same period appeared accumulation of [^{18}F] FDG-PET like that of [^{15}O] 2-DG (Fig. 4B). These images were clearly different from the image of initial blood flow from 0 sec to 90 sec (Fig. 4C) and from 15 min to 30 min (Fig. 4D) of [^{15}O] H_2O . Similar results were also obtained for mouse imaging (Fig. 5). These results proves that [^{15}O] 2-DG can be used as a PET tracer like [^{18}F] FDG.

CLAIMS

1. A ^{15}O -labeled monosaccharide which is labeled with ^{15}O at hydroxymethyl group in the monosaccharide molecule.

5 2. The ^{15}O -labeled monosaccharide of claim 1, wherein the monosaccharide is hexose or pentose.

3. The ^{15}O -labeled monosaccharide of claim 1, wherein the monosaccharide is D-glucose or 2-deoxy-D-glucose.

10

4. A method for producing ^{15}O -labeled monosaccharide, comprising reacting a monosaccharide, which is substituted with a halogen at hydroxyl of hydroxymethyl group in the monosaccharide molecule, with ^{15}O oxygen in the presence of an organotin compound and a reducing agent, wherein said reacting occurs either in the absence of a radical initiator or in the presence of not more than 0.3 equivalent, based on the halogenated monosaccharide, of a radical initiator to provide ^{15}O -labeled monosaccharide which is labeled with ^{15}O at the hydroxymethyl group in the monosaccharide molecule.

15 20

5. The method of claim 4, wherein the monosaccharide is hexose or pentose.

25 6. The method of claim 5, wherein the monosaccharide is D-glucose or 2-deoxy-D-glucose.

7. The method of claim 4, wherein the halogen is iodo.

8. The method of claim 4, wherein the amount of the organotin
5 compound is 2.0-5.0 equivalents based on the halogenated
monosaccharide.

9. The method of claim 4, wherein the organotin compound
is an organotin hydride.

10

10. The method of claim 9, wherein the organotin compound
is trialkyltin hydrides.

15

11. The method of claim 4, wherein the reducing agent is
a phosphine.

12. The method of claim 11, wherein the reducing agent is
triphenylphosphine.

20

13. The method of claim 4, wherein in the reacting step,
gas containing $^{16}\text{O}_2$ is introduced into the reaction to initiate
radical reaction prior to introduction of the ^{15}O oxygen.

25

14. The method of claim 13, wherein the amount of $^{16}\text{O}_2$ in
the gas is at least 1.5%.

15. A method for diagnosing a whole body, organs or tissues of a subject, comprising administering the ^{15}O -labeled monosaccharide of any one of claims 1-3 to the subject to measure metabolism of the ^{15}O -labeled monosaccharide in the subject.

5

16. The method of claim 15, wherein the measurement is carried out by using positron emission tomography.

17. The method of claim 15, wherein the organ is brain or
10 heart.

18. The method of claim 15, wherein the tissue is tumor tissue.

19. The method of claim 15, wherein a diurnal variation of the metabolism of the ^{15}O -labeled monosaccharide is measured in the subject over time.

20. A kit for producing ^{15}O -labeled monosaccharide,
20 comprising a monosaccharide which is substituted with a halogen at hydroxyl of hydroxymethyl group in the monosaccharide molecule, an organotin compound and a reducing agent, wherein the kit is used to react the halogenated monosaccharide with ^{15}O oxygen in the presence of the organotin compound and the reducing agent
25 to produce a ^{15}O -labeled monosaccharide which is labeled with ^{15}O at the hydroxymethyl group in the monosaccharide molecule.

21. The kit of claim 20, wherein the monosaccharide is hexose or pentose.

5 22. The kit of claim 21, wherein the monosaccharide is D-glucose or 2-deoxy-D-glucose.

23. The kit of claim 20, wherein the halogen is iodo.

10 24. The kit of claim 20, wherein the amount of the organotin compound is 2.0-5.0 equivalents based on the halogenated monosaccharide.

15 25. The kit of claim 20, wherein the organotin compound is an organotin hydride.

26. The kit of claim 25, wherein the organotin compound is trialkyltin hydrides.

20 27. The kit of claim 20, wherein the reducing agent is a phosphine.

28. The kit of claim 27, wherein the reducing agent is triphenylphosphine.

25

29. The kit of claim 20, wherein the kit further comprises

a radical initiator not more than 0.3 equivalent based on the halogenated monosaccharide.

30. The kit of claim 20, wherein the kit further comprises
5 a reaction vessel, wherein the reaction can be practiced to produce
¹⁵O-labeled saccharide.

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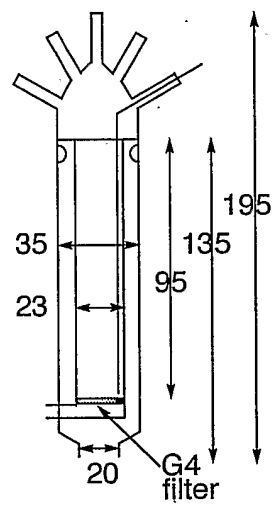


Figure 1

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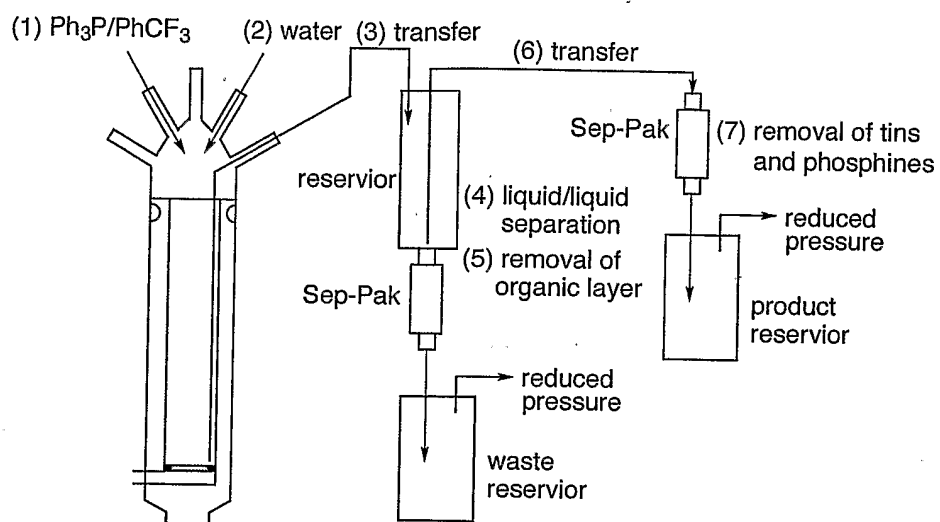


Figure 2

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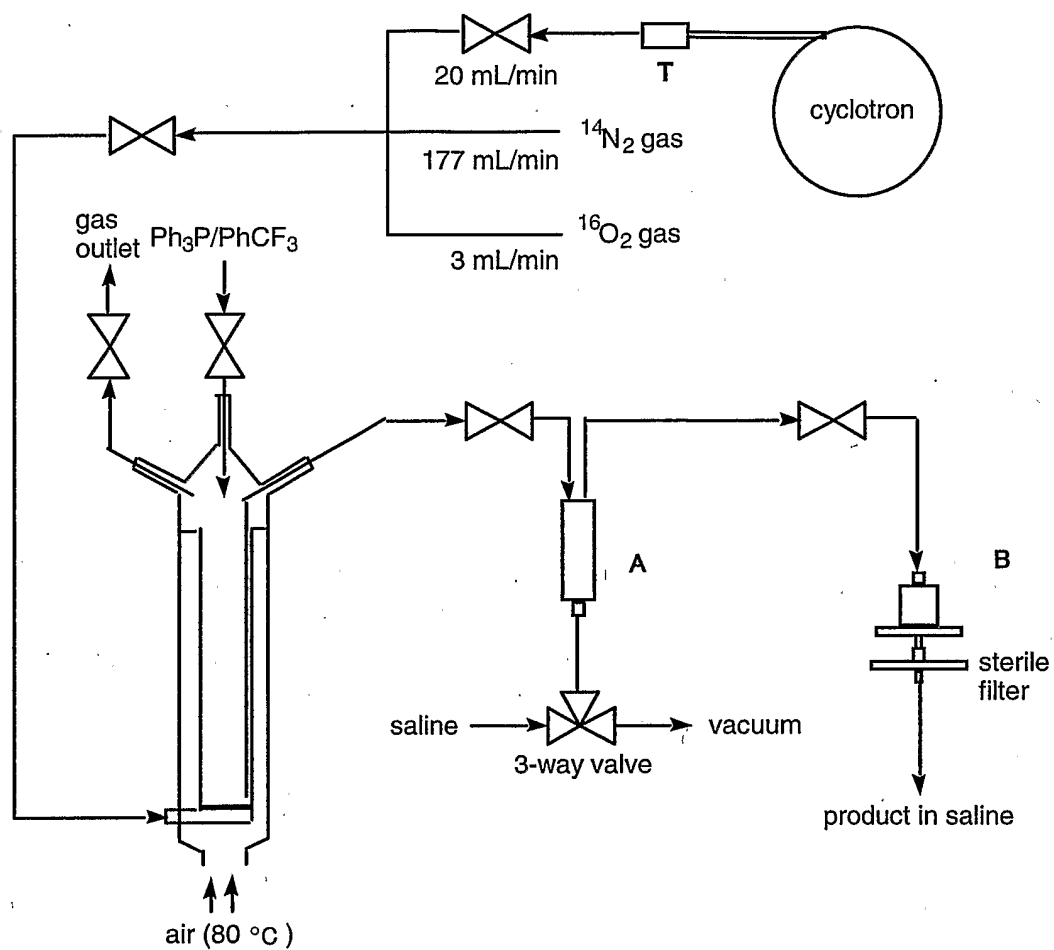


Figure 3.

4/5



Figure 4A

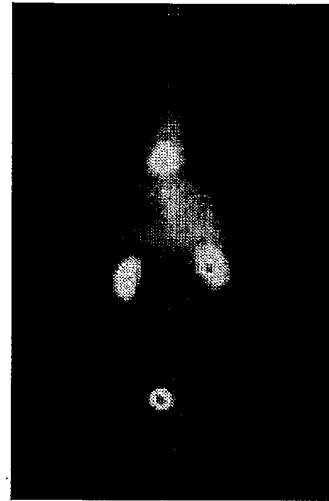


Figure 4B

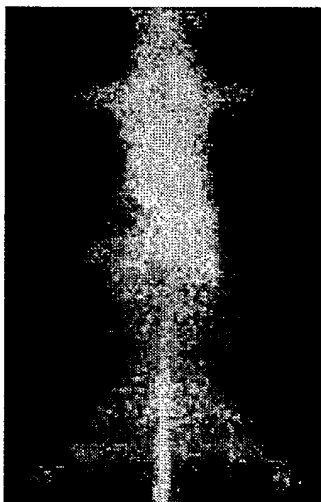


Figure 4C



Figure 4D

Figure 4

5/5

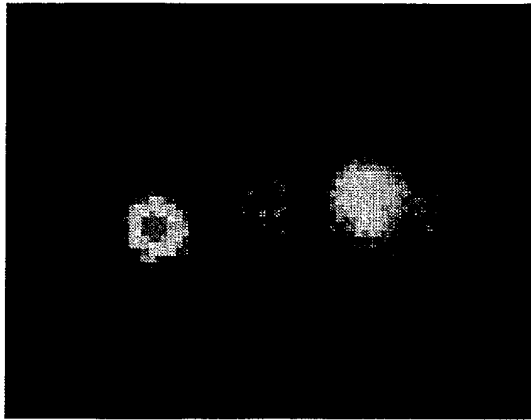


Figure 5A

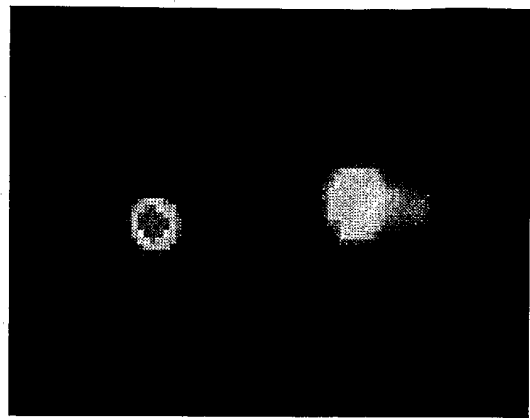


Figure 5B

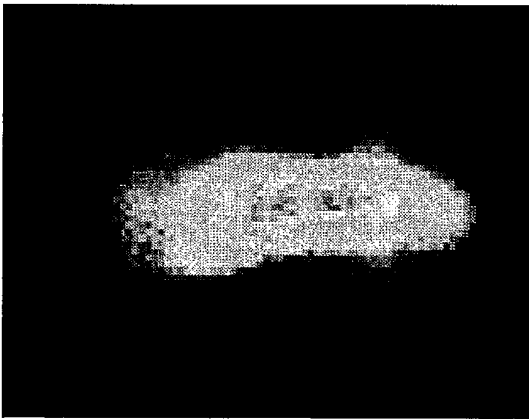


Figure 5C

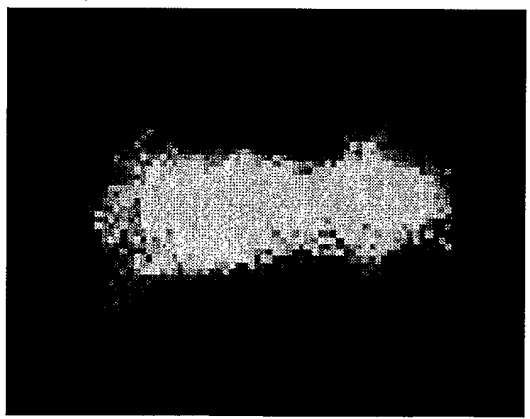


Figure 5D

Figure 5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP2005/006547

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07B59/00

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)

IPC 7 C07B

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

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

EPO-Internal, CHEM ABS Data, BEILSTEIN 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.
A	EP 0 588 480 A (GENERAL ELECTRIC COMPANY) 23 March 1994 (1994-03-23) the whole document -----	1,4,15, 20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

6 July 2005

Date of mailing of the international search report

21/07/2005

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2005/006547

Box 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:

Although claims 15-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
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 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:

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 fee, this Authority did not invite payment of any additional fee.
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.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP2005/006547

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0588480	A	23-03-1994	US 5264570 A	23-11-1993
			CA 2101623 A1	15-04-1995
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			JP 6157572 A	03-06-1994