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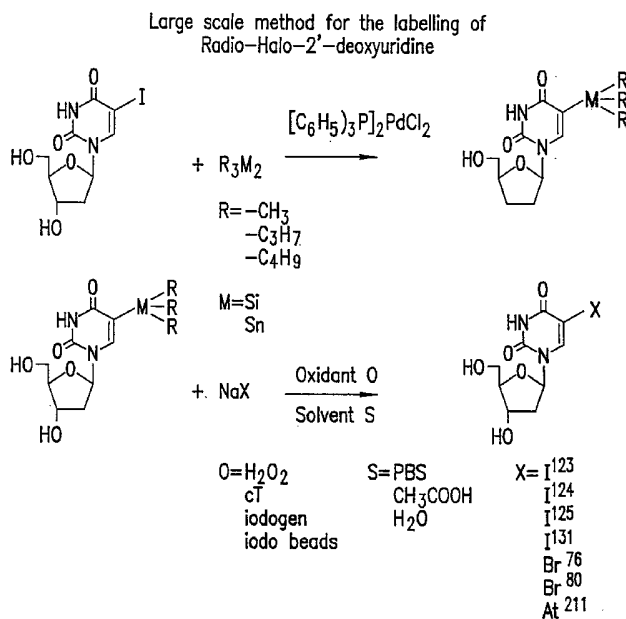
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(54) Title: SYSTEM AND METHOD FOR THE LARGE SCALE LABELING OF COMPOUNDS WITH RADIOHALOGENS



(57) Abstract: The invention relates to a new method for producing radio-halogenated compounds having very high specific radioactivity. The method improves the stability of the radio-labeled compound such that its biological and/or chemical activity is retained. The main aspect of the invention is that vitamin C and/or ethanol is added to the radiolabeled compound at either or both of just following attachment of the radio-halogen and during purification of the radio-halogenated compound from the labeling mixture, or during packaging for storage.



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.

SYSTEM AND METHOD FOR THE LARGE SCALE LABELING OF COMPOUNDS WITH RADIOHALOGENS

RELATED APPLICATIONS

5 The present application claims priority under 35 U.S.C § 119 of U.S. Provisional Application 60/299,762, filed June 22, 2001.

Field of the Invention

 The present invention is generally related to a large scale method for making radio-halogenated compounds, preferably radio-labeled nucleotides and nucleosides (“radionucleotides”,
10 “radionucleosides”), steroids, or proteins such as antibodies. The invention resides in part in the finding that addition of an antioxidant, preferably ethanol or vitamin C (ascorbic acid), or a mixture of both, to the radiolabeled compound during its preparation and/or storage, allows retention of biological or chemical activity of the radiolabeled compound. One embodiment of the invention is a novel technique for large-scale production of deoxyuridines labeled with radioisotopes of
15 halogens (“radiohalogens”).

Background of the Invention

 Many diagnostic medical procedures require radiation sources that are introduced into a tissue or ingested by the patient. Such radiation sources preferably have a lifetime of a few hours - enough time to complete the diagnosis, but short enough that damage to tissue from the radiation is
20 avoided and the radioisotope does not decay. Such radiation sources are preferably not chemically poisonous. 5'-radio-halogenated-2'-deoxyuridine labeled with radioactive isotopes is such a radiation source. Specifically, iododeoxyuridine labeled with radioisotopes of iodine (“radioiodine”) is such a radiation source.

 The lifetime of radioactive isotopes of halogens varies from minutes to days.
25 Radiohalogens are not chemically poisonous in tracer quantities. Radioactive isotopes of halogens have therefore found many uses in making medical and radiopharmaceutical products. One such product is iododeoxyuridine labeled with radioisotopes of iodine. One potential important use is the treatment of neoplastic meningitis with methotrexate and 5-[¹²⁵I]iodo-2'-deoxyuridine (¹²⁵IudR). Iododeoxyuridine labeled with radioisotopes of iodine may also prove to be significant
30 therapy for cancer. See, e.g., U.S. Patent 5,077,034, hereby incorporated by reference. Additional uses may include treatment of colorectal tumors, lung tumors and arteriolosclerosis.

 Radiohalogenated steroids and proteins can be used to image binding sites for these molecules in a subject or in in vitro assays of binding of such ligands to their receptors.

Several methods are used to label compounds with radiohalogens. These methods are limited by the amount of product they produce and the chemical purity and stability of the compound produced. An additional limitation is the time required to produce the compound must be compatible with the short life of some radioisotopes.

5 Technical and economic considerations are critical factors in choosing a method for the production of radio-halogenated compounds. Accordingly, a better, more efficient and less costly method of producing radio-halogenated compounds, such as steroids, proteins or nucleotides and nucleosides, for example, iododeoxyuridine labeled with radioisotopes of iodine is needed.

10 The present invention for large-scale production is simplified, rapid, produces higher yields, can be scaled up and provides an increase in stability of the radio-labeled compound. Typical procedures produce quantities of the scale 50 to 500 micro Ci. This novel method can produce batches of 100 milli Ci to 1 Ci in less time than the methods of the prior art. Specific activities of 1000 to 3000 Ci/mmol can be achieved in the product for labeled nucleoside, nucleotide or derivatives thereof or for labeled steroid products. Specific activities of from 10 to 40
15 mCi/nanogram can be achieved for labeled protein or labeled peptide products.

Brief Description of the Drawing

Other aspects and advantages of the present invention will become apparent upon reading the detailed description and accompanying drawing given herein below, which is given by way of illustration only, and which is not limitative of the present invention, wherein:

20 Figure 1 shows the synthesis of labeled iododeoxyuridine.

Detailed Description of the Invention

The invention presents a method for preparing radiohalogen labeled compounds. The radiohalogen is can be a radioisotope of any halogen, such as a radioactive isotope of fluorine or bromine, but is preferably a radioiodine selected from ^{125}I , ^{123}I and ^{131}I . The compounds to be
25 prepared can be labeled nucleosides, nucleotides, or derivatives thereof, or can be steroids, or proteins or peptides. Preferred steroids are estradiol, progesterone and 17 (OH) progesterone. Preferred proteins are antibodies, such anti-TSH antibody.

In a preferred embodiment of the invention, radiolabeled nucleotides or nucleosides, such as deoxypyrimidine, particularly deoxyuridine, are labeled with radioisotopes of halogens. The
30 method of the invention accomplishes the synthesis of the radiolabeled compound using less time and in larger quantities. Due to less radiologic damage, the resulting product is more stable with respect to biological and/or chemical activity than the products obtained in the prior art.

The present invention constitutes an improvement over prior art methods for producing radiolabeled steroids, antibodies, and nucleosides, nucleotides and derivatives thereof. Pyrimidine nucleoside or nucleotides, e.g. uridine, thymine or cytosine, and derivatives thereof, are preferred nucleotides and nucleosides to be used in the invention. The invention can be applied to the synthesis of any labeled pyrimidine nucleosides or derivatives such as ribonucleosides, deoxyribonucleosides and 2', 3' dideoxynucleosides and 5' sugar-derivatized nucleosides such as 5'-galactosyl derivatives and similarly to corresponding mono-, di- and triphosphonucleotides.

The invention can also be applied to the production of radiohalogen labeled steroids and proteins. Being commonly used in imaging or therapeutic protocols, antibodies are a preferred embodiment of a protein to be used in the present invention.

Briefly, the invention resides in the discovery that addition of an antioxidant to the labeling reaction solution after the labeling reaction has proceeded, with maintenance of the presence of the antioxidant during separation and packaging, results in production of a labeled product having higher biological activity of the pyrimidine nucleoside or nucleotide or derivative thereof.

In a preferred embodiment a pyrimidine nucleoside, for example, iododeoxyuridine, is labeled with a radioisotope of iodine.

The synthesis of halogenated pyrimidine nucleosides is known in the art. Exemplary syntheses are described by Kassis et al. in U.S. Patents 5,720,935, 4,851,520 and PCT publication WO 01/05439, each of which is hereby incorporated by reference in its entirety and for all purposes. Another synthesis is described by Baranowska-Kortylewicz et al., U.S. Patent 5,468,853, hereby incorporated by reference in its entirety and for all purposes. Baranowska-Kortylewicz et al. also describe the synthesis of 5' sugar derivatized pyrimidine nucleosides and their use in treatment of cancers; see WO 01/03745, hereby incorporated by reference in its entirety and for all purposes.

Similarly, methods for radioactive labeling of steroid compounds and proteins with a radiohalogen, including radioiodine, are also known in the art. *See, e.g.* Melo e Silva et al., *Appl. Radiat. Isot.* 54:227-239 (2000) and U.S. Patent 5,302,700, respectively.

The present invention is an improvement upon these synthetic methods. For labeling of nucleotides or nucleosides, generally the methods of Kassis et al. or Baranowska-Kortylewicz are followed, but an antioxidant is included after the radiohalogenation reaction so that the labeled product is protected from degradation during separation and storage steps. The resulting process allows the preparation of product having very high specific activity.

In a preferred embodiment of the process of the invention, related to labeling of a

nucleoside or derivative thereof, a halogenated pyrimidine nucleoside, or derivative as noted above, is dissolved in solvent, preferably 1,4 dioxane, at a temperature of 40 to 60 °C, preferably about 50 °C. The solution is cooled to 20 to 30 °C, preferably to room temperature and an alkylmetallic catalyst (preferably, but not limited to, bis(tributyltin)), and triphenylphosphine palladium are added. The solution is heated to 100 to 110 °C, preferably to about 105 °C and maintained for 5 to 7 hours, preferably about 6 hours, under an inert atmosphere, preferably nitrogen or argon. The solvent is removed by evaporation, preferably under vacuum, and then the metallated (stannylated) product is obtained by flash chromatography on silica gel using a mobile phase comprising chloroform and methanol. The methanol is added as a linear gradient, to a final ratio of chloroform:methanol 4:1. Fractions are collected and the metallated (stannylated) pyrimidine is detected by thin layer chromatography. The product shows an R_f of 0.3 on thin layer chromatography on silica gel using a mobile phase of hexane:ether 1:1. The metallated pyrimidine is then reacted with Na¹²⁵I or other radiohalogen in a solution containing hydrogen peroxide or iodogen or other oxidant and a buffer, preferably a phosphate buffer. Iodogen tends to produce a good yield of high specific activity product. However it is not water soluble and so may contaminate the sample, thus rendering it unsuitable for medical use. Therefore, if iodogen is used in preparing a medical sample, care should be taken to as completely purify the product from the iodogen as possible. Hydrogen peroxide as an oxidant is water soluble and so can be easily removed during the downstream purification, but a lower yield of product is typically obtained. If hydrogen peroxide is used as the oxidant, it is typically used at a concentration of 0.1 to 0.5 mg/ml.

This oxidation step is generally described in U.S. Patent 5,720,935. The pH should be acidic to neutral. That is, the pH should be about 7, preferably from 5 to 8, more preferably from 6 to 8 or 7 to 8 and is typically 7.4. Potassium salts of the halogen can also be used. The mixture is stirred for 1 to 5 minutes, preferably two to four minutes, more preferably about two minutes. Then an antioxidant, preferably vitamin C, is added to a concentration of 0.1 to 1 mg/ml, preferably from 0.3 to 1 mg/ml, more preferably about 0.5 mg/ml. The solution is then chromatographed over C₁₈ reverse phase matrix (e.g. Sep-Pak C18 cartridge) which has been previously washed with ethanol and then equilibrated with water. The column is then washed with water to remove unwanted material and the radiolabeled pyrimidine nucleoside (or other derivative thereof) is eluted with ethanol. Antioxidant, preferably vitamin C, can then be added to 0.1 to 1 mg/ml, preferably to 0.3 to 1 mg/ml, more preferably about 0.5 mg/ml, to the solution for final packaging and storage.

The product is packaged in any manner typical in the art, for example in a glass syringe bottle or screw-cap tube.

The amount of the antioxidant that is added is independent of the amount of labeled compound. However, if the solution of labeled compound contains more than 100 mCi, the

antioxidant should be added at both the separation step and to the final product for packaging. On the other hand, if the solution of labeled compound contains less than 100 mCi, it is sufficient to add the antioxidant only during the separation step and then to package the final product in ethanol.

5 The final product is preferably packaged at a radioactive concentration of 100 to 200 mCi/ml.

Ethanol at a concentration of 95 to 100%, preferably at 100% can be used as the antioxidant in place of, or in combination with vitamin C to the extent that the radio-labeled compound is soluble in ethanol. Thus, when radio-labeled steroids are the intended product, ethanol can suitably be used at both the column purification and packaging steps. On the other
10 hand, because antibodies and other proteins tend to denature upon contact with ethanol, vitamin C is the preferred antioxidant to be used when radio-labeled proteins are produced according to the invention.

Example 1

Production of radio-labeled IUdR

15 Seven mmol of unlabeled iododeoxuridine (IudR, 2.5 g) was dissolved in 100 ml anhydrous dioxane at 50 °C. The solution was then cooled to room temperature. 0.125 g of bis(triphenyl phosphine) palladium dichloride and 6 g (15 mmol) of hexabutyliditin were added and the mixture was refluxed for 6 hours at 110 °C under argon. The solvent was removed under vacuum and the product was redissolved in chloroform:methanol 4:1 and purified by chromatography over a silica
20 gel column, eluting with a linear gradient of 0 to 20% methanol in chloroform. The product elutes in the range of 10 to 15% methanol. (The gradient of 0 to 20% methanol is run over 50 collection tubes; the product elutes in tubes 25 to 30.) The product is identified by ultraviolet fluorescence or iodine staining of a silica TLC plate developed with hexane:ether 1:1. The desired Bu₃SnUdR product was obtained as a pale yellow oil that exhibits a R_f of 0.3.

25 Na¹²⁵I (200 mCi, produced by MDS Nordion) was added to a solution of 100 µg of Bu₃SnUdR in 250 ml of phosphate buffered saline, pH 7.4 containing 0.1 mg iodogen and the reaction was allowed to proceed for two minutes. Ascorbic acid was added to a concentration of 0.5 mg/ml and the solution was loaded onto a Sep-Pak™ C18 cartridge that was previously washed with 10 ml of ethanol, then washed three times, 10 mls each, with water. The cartridge was washed
30 once with 1 ml water to remove free iodine and ¹²⁵IUdR was eluted with 1 ml of ethanol, collecting the fractions containing radioactivity. 0.5 mg Ascorbic acid was added as a solution at 1 mg/ml in ethanol to the eluate. 180 mCi of ¹²⁵IUdR at a specific activity of about 2000 Ci/mmol was obtained.

Example 2**Labeling Proteins**

132 microlitres of 0.1 molecular potassium phosphate buffer at pH 8.2 was added to a vial containing 100 micrograms of polyclonal anti-TSH antibody and stirred for 10 seconds. 7 mCi of Na¹²⁵I was added to the solution. 40 microlitres of chloramine T (CT) buffer solution at 1 mg/ml was added and stirred for 1 minute. 40 microlitres of ascorbic acid buffer solution at 5 mg/ml was further added and stirred for 10 seconds. The iodinated polyclonal anti-TSH solution was then loaded into a G75 column (10 ml) and eluted with 0.01 M phosphate buffered saline at pH 7.4. 15 fractions in 13 X 100 MM test tubes were collected at the rate of 0.5 ml/tube at 2 minutes per tube (the product elutes in tubes 6-10). 1 ml of ascorbic acid buffer was added to a concentration of 1 mg/ml of 5% BSA phosphate saline buffer at pH 7.4. 4 mCi of ¹²⁵I anti-TSH was obtained.

Example 3**Labeling Steroids**

Fifty microlitres of DMF and 100 microlitres of 0.2 M phosphate buffer at pH 7.4 was added to a vial containing 4 micrograms of Estradiol-6-CMO-Histamine Aliquot. 10 mCi of sodium I-¹²⁵ was added to the solution and stirred for ~10 seconds. 50 microlitres of chloramine T (CT) solution at 1 mg/ml was further added to the mixture and stirred for 3 minutes. 50 microlitres of ascorbic acid buffer was then added at 5 mg/ml and injected into an HPLC, using a methanol/water (MeOH/H₂O) gradient. 0.5 ml fractions containing radioactivity were collected after 45 minutes. 10 ml of ethanol were added. 2.1 mCi of ¹²⁵I Estradiol at a specific activity of about 2000 Ci/mmol was obtained.

What is claimed is:

1. A method for synthesis of a radioiodinated deoxypyrimidine comprising:
 - i) dissolving a 5-iododeoxypyrimidine in 1,4 dioxane at a temperature of 40 to 60 °C;
 - ii) cooling the solution to 20 to 30 °C and adding bis(tributyltin) and
5 triphenylphosphine palladium;
 - iii) heating the solution to 100 to 110 °C for about 6 hours under an argon atmosphere;
 - iv) removing the solvent by rotary evaporation;
 - v) chromatographing the mixture by flash chromatography over a silica gel matrix using a mobile phase consisting of chloroform/methanol 4:1;
 - 10 vi) reacting the product with Na¹²⁵I in phosphate buffer containing hydrogen peroxide, for about 2 minutes;
 - vii) adding a solution of ascorbic acid to the reaction;
 - viii) binding the resulting 5-¹²⁵I-deoxypyrimidine from the reaction mixture to a C18 reverse phase chromatography matrix;
 - 15 ix) washing the matrix with water;
 - x) eluting the 5-¹²⁵I-deoxypyrimidine with ethanol;
 - xi) adding ascorbic acid to the eluate.
2. The method of claim 1, in which the amount of ascorbic acid added at step vii) is from 0.1 to 1 mg/ml.
- 20 3. The method of claim 1, in which the amount of ascorbic acid added at step xi) is from 0.1 to 1 mg/ml.
4. The method of claim 1, wherein the deoxypyrimidine is deoxyuridine.
5. A method for producing a radiohalogen labeled pyrimidine nucleoside, comprising:
 - i) applying the radiohalogen labeled pyrimidine nucleoside to a chromatographic
25 stationary phase in a solution comprising an antioxidant;
 - ii) washing the stationary phase having the ¹²⁵I-labeled pyrimidine nucleoside bound

thereto; and

iii) eluting the radiohalogen labeled pyrimidine nucleoside from the stationary phase to obtain an eluate.

6. The method of claim 5, further comprising:

5 iv) adding an antioxidant to the eluate.

7. The method of claim 5, wherein the antioxidant is ascorbic acid.

8. The method of claim 6, wherein the antioxidant in step iv) is ascorbic acid.

9. The method of claim 6, wherein the antioxidant in both of steps i) and iv) is ascorbic acid.

10. The method of claim 5, wherein the concentration of antioxidant is from 0.1 to 1 mg/ml.

10 11. The method of claim 9, wherein the concentration of ascorbic acid is from 0.1 to 1 mg/ml.

12. The method of claim 5, wherein the stationary phase is a C18 reverse phase matrix, the column is washed with water in step ii) and the radiohalogen labeled pyrimidine nucleoside is eluted with ethanol in step iii).

13. The method of claim 7, wherein the stationary phase is a C18 reverse phase matrix, the
15 column is washed with water in step ii) and the radiohalogen labeled pyrimidine nucleoside is eluted with ethanol in step iii).

14. The method of claim 9, wherein the stationary phase is a C18 reverse phase matrix, the column is washed with water in step ii) and the radiohalogen labeled pyrimidine nucleoside is eluted with ethanol in step iii).

20 15. The method of claim 5, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.

16. The method of claim 7, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.

25 17. The method of claim 9, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.

18. The method of claim 12, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.

19. The method of claim 13, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.
20. The method of claim 14, in which the radiohalogen labeled pyrimidine nucleoside is 125I-labeled deoxyuridine.
- 5 21. A composition comprising an 125I-labeled deoxypyrimidine, ethanol and ascorbic acid.
22. A method for synthesis of a radiohalogenated steroid compound comprising:
- i) reacting a 6-CMO-histamine derivative of the steroid with sodium 125I in the presence of chloramine T to obtain an 125I-labeled steroid;
 - ii) adding ascorbic acid to a concentration of from 1 to 5 mg/ml;
 - 10 iii) purifying the 125I-labeled steroid by chromatography over a reverse-phase solid phase and eluting with a methanol/water (MeOH/H₂O) gradient into a volume of methanol/water;
 - iv) adding ethanol to the purified ¹²⁵I-labeled steroid in an amount of from 5 to 20 volumes based upon the volume of methanol/water.
23. A composition comprising an 125I-labeled steroid compound and a stabilizing solution
15 selected from the group consisting of ethanol, an ascorbic acid solution and a solution of ethanol and ascorbic acid.
24. The method of claim 23, wherein the steroid is estradiol, progesterone or 17-hydroxy progesterone.
25. The composition of claim 24, wherein the steroid is estradiol, progesterone or 17-hydroxy
20 progesterone.
26. The composition of claim 24, wherein the radiohalogen-labeled steroid has a specific activity of from 1000 to 2000 Ci/mmol.
27. A method for preparing an ¹²⁵I-labeled protein comprising:
- i) reacting the protein Na¹²⁵I in the presence of chloramine T (CT) in a buffer
25 comprising ascorbic acid buffer at a concentration of from 1 to 5 mg/ml to obtain a labeled protein;
 - ii) purifying the labeled protein from the chloramine T by gel filtration chromatography in a buffer that optionally contains ascorbic acid at a concentration of from 1 to 5 mg/ml; and

- 10 -

iii) collecting the labeled protein in a buffer comprising ascorbic acid at a concentration of from 1 to 5 mg/ml.

28. A composition comprising a radiohalogen-labeled protein in a buffer comprising ascorbic acid, wherein the radiohalogen-labeled protein has a specific activity of from 10 to 40
5 mCi/nanogram of protein.

29. The composition of claim 28, wherein the protein is an antibody.

1/1

Large scale method for the labelling of
Radio-Halo-2'-deoxyuridine

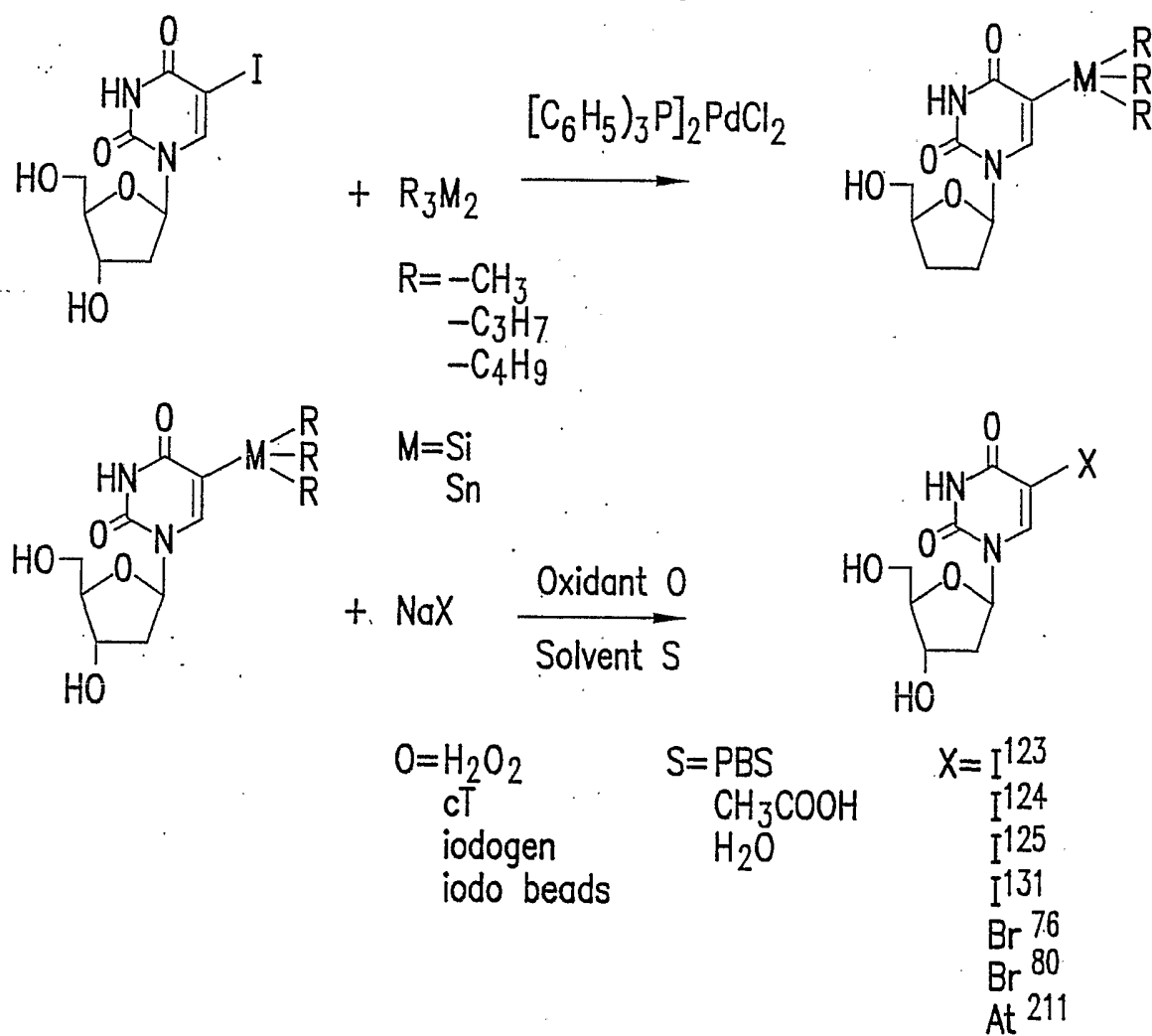


FIG.1