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With amended claims.

(54) Title: METHOD OF MAKING RADIOIODINATED PYRIMIDINE

(57) Abstract

The method of making radioiodinated pyrimidine nucleoside or nucleotide which comprises contacting a water-insoluble halomercuri pyrimidine nucleoside or nucleotide with an aqueous medium containing a dissolved radioactive iodide ion and an oxidizing agent, the molar amounts of said nucleoside or nucleotide and said oxidizing agent being in excess of the molar amount of said iodide, whereby water-soluble radioactive iodinated pyrimidine nucleoside or nucleotide is formed in solution, and separating residual water-insoluble halomercuri pyrimidine nucleoside or nucleotide from said solution.

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METHOD OF MAKING RADIOIODINATED PYRIMIDINE NUCLEOSIDE OR NUCLEOTIDE

This invention relates to a method of making radioiodinated pyrimidine nucleosides and nucleotides and pertains more specifically to a heterogeneous phase reaction for making the desired compounds in a form which is readily purifiable, for example by a simple HPLC procedure.

Radioiodinated pyrimidine nucleosides such as 5-iodo-2'-deoxyuridine have been used for many years to label the DNA of proliferating cells in studies of tumor transplantation and of DNA structure among other uses. Radioiodination methods for making such compounds have included electrophilic substitution of hydrogen in the pyrimidine ring by reacting water-soluble pyrimidine nucleosides in aqueous solution with sodium iodide and nitric acid as described by Keough et al., J.Labelled Compd. Radiopharm., Vol. 14, pp. 83-90 (1978) and with sodium iodide and chloramine-T as described by Bakker et al., Int. J. Appl. Radiat. Isot., Vol. 32, pp. 176-178 (1981). Although the radiochemical yields in these processes are high, and satisfactory separation of the product from residual sodium iodide can usually be achieved by chromatography, complete removal of unreacted pyrimidine nucleoside is very difficult because of the more than 20,000-fold excess of this starting material used in the synthesis and requires complicated and lengthy procedures.

There has now been discovered a facile procedure for the preparation of radioiodinated pyrimidine nucleosides and nucleotides which comprises contacting a water-insoluble halomercuri-pyrimidine nucleoside or nucleotide with an aqueous medium containing a dissolved radioactive iodide ion and an oxidizing agent, the molar amount of said nucleoside or nucleotide being in excess of the molar amount of said iodide, whereby water-soluble radioactive iodinated

pyrimidine nucleoside or nucleotide is formed in solution, and separating residual water-insoluble halomercuri-pyrimidine nucleoside or nucleotide from said solution. The separation can readily be carried out by conventional filtration procedures. The method is suitable for no-carrier-added syntheses and, following the separation step, requires only a minimal purification process, for example, by conventional HPLC procedures, to produce a product of extremely high purity.

The process of the present invention can be applied to any water-insoluble halomercuri-pyrimidine nucleoside such as 5-chloromercuri cytidine, 5-chloromercuri-2'-deoxycytidine, 5-chloromercuri uridine, 5-chloromercuri-2'-deoxyuridine, or water-insoluble halomercuri-pyrimidine nucleotide such as 5-chloromercuri-cytidine-5'-mono-, di, or tri-phosphate, 5-chloromercuri-2'-deoxycytidine-5'-mono-, di-, or tri-phosphate, 5-chloromercuri-uridine-5'-mono-, di-, or tri-phosphate, or 5-chloromercuri-2'-deoxyuridine-5'-mono-, di-, or tri-phosphate; the corresponding 5-fluoromercuri compounds, and the like may also be used. The water-insoluble nucleosides and nucleotides can be made by well-known procedures as described, for example, in Bergstrom et al., J. Carbohyr., Nucleosides, Nucleotides, Vol. 4, pp. 257-269 (1977).

The radioactive iodide ion may be employed in the form of any water-soluble salt, e.g. an alkali metal salt such as the sodium salt of ^{123}I, ^{125}I, ^{131}I, preferably in carrier-free form.

The oxidizing agent used may be either water-soluble as in the case of Chloramine-T or nitric acid; or it may be water-insoluble as in the case of Iodogen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril).

Both the halomercuri-pyrimidine nucleoside or nucleotide and the oxidizing agent must be employed in molar excess with respect to the iodide ion. Water-insoluble oxidizing agents are preferred because the excess agent is removed from the aqueous medium after reaction completion in the same filtration step by which the excess halomercuri-pyrimidine nucleoside or nucleotide is removed, thus simplifying still more the subsequent high performance liquid chromatography separation used to remove the small amounts of water-soluble by-products present. The amount of excess of these reagents is not critical, but it is usually convenient and desirable to employ at least 1800 molar excess of each, with respect to iodide ion. Larger excesses are limited only by economics and convenience.

The concentration of iodide ion in the aqueous reaction medium is not critical and it may range from 0.1 mCi/mL to 100 mCi/mL, preferably from 0.1 mCi/mL to 10 mCi/mL.

The reaction may be carried out at temperatures up to 100°C but optimum results are obtained at 20°-30°C for 1 to 3 hours. Longer or shorter times may be employed at different temperatures and/or iodide concentrations if desired.

After filtration of the reaction mixture to remove excess halomercuri-pyrimidine nucleoside or nucleotide and excess oxidizing agent (when a water-insoluble oxidizing agent is used), the reaction mixture is subjected to high performance liquid chromatography preferably on a reverse phase column (Bonda Pak C_{18}) using methyl alcohol/distilled water 20/80) as eluant. Eluates can be assayed by ultraviolet absorption and by gamma ray detection.

The following specific examples are intended to illustrate more fully the nature of the invention without acting as a limitation upon its scope.

Example 1

Preparation of 5-chloromercuri-2'-deoxyuridine

2'-Deoxyuridine (0.50 g, 2.20 mmol) was dissolved in 2 mL water and the solution was heated to 50°C. To this solution mercuric acetate (0.74 g, 2.32 mmol) in 3 mL water was added. The mixture was brought to 50°C and an additional 1 mL water was added. The reaction was allowed to proceed for 2.5 h at 50°C resulting in a thick white suspension. The heating bath was removed and the mixture cooled to about 40°C. Sodium chloride (0.32 g, 5.45 mmol) in 1 mL water was added to the reaction mixture, and it was stirred for 1 hour. The suspension was filtered, and the white precipitate was washed successively with 0.5 mL 0.1 M NaCl, 0.5 mL water, 0.5 mL 95% ethanol and 0.5 mL diethyl ether. The precipitate was dried in vacuo at about 60°C for 24 hours giving 0.59 g (57.7%) 5-chloromercuri-2'-deoxyuridine: mp. 210-211°C with decomposition (lit. mp. 210.5-211°C; Bergstrom, 1977); ^{1}Hnmr (1 M NaCN/D_2O) 2.35 ppm. (2H, m, C2'H_2), 3.85 ppm. (2H, m, C5'H_2), 4.01 ppm. (1H, m, C3'H), 4.46 ppm. (1H, m, C4'H), 6.33 ppm. (1H, t, C1'H), 7.72 ppm. (1H, s, C6H).

Example 2

Preparation of 5-fluoromercuri-2'-deoxyuridine

2'-Deoxyuridine (0.50 g, 2.20 mmol) was reacted with mercuric acetate (0.74 g, 2.32 mmol) as described in Example 1 above. Sodium fluoride (0.23 g, 5.45 mmol) in 1 mL water was added after the reaction mixture had cooled to about 40°C. The suspension was stirred for 1 hour and filtered. The precipitate was washed successively with 1 mL 0.1 M sodium fluoride, 1 mL

water, 0.5 mL 95% ethanol and 0.5 mL diethyl ether. Drying in vacuo at 60°C gave 0.48 g (49.1%) 5-fluoromercuri-2'-deoxyuridine: mp. 250-257°C with decomposition; ^{1}Hnmr (1 M NaCN/D_2O) 2.38 ppm. (2H, t, C2'H_2), 3.84 ppm. (2H, m, C5'H_2), 4.00 ppm. (1H, m, C3'H), 4.47 ppm. (1H, m, C4'H), 6.35 ppm. (1H, t, C1'H), 7.51 ppm (1H, s, C6H).

Example 3

Preparation of 5-iodo-2'-deoxyuridine (IUdR)

Iodogen (10 mg, 23.1 µmol) and freshly prepared 5-chloromercuri-2'-deoxyuridine (10 mg, 21.6 µmol) were suspended in 0.4 mL water. To this mixture a solution of sodium iodide (3 mg, 20 µmol) in 0.4 mL water was added. The mixture was allowed to react at room temperature for 24 hours during which time it was vigorously stirred. Filtration of the mixture through a 0.22 m Millex filter gave 0.78 mL of clear solution. This was chromatographed by HPLC giving 2.9 mg (41%) IUdR with a retention time (R_T) of 7.1 min (identical to an authentic sample). The HPLC revealed the presence of two other components (R_T = 2.2 min, 3.31%; R_T = 16.9 min, 9.95%) (Fig. 1). The first peak was also observed when a solution of pure NaI was injected; the last peak was identified as some oxidized form of iodine generated from NaI in the presence of Iodogen, presumably ICl). This latter peak was not observed during the no-carrier-added radioiodination. The synthesis was repeated several times varying the reaction time and the ratio of the reagents. The crude filtrate (prior to HPLC) from one such experiment was analyzed for mercury and 0.2 g/mL was found.

The retention time of Iodogen was established as follows: a saturated solution of Iodogen in methanol was prepared and chromatographed (15 minutes isocratic

H_2O/CH_3OH, 80/20 by volume, followed by a linear gradient to 100% methanol in 10 min); R_T of Iodogen = 29 minutes. Sodium iodide was added to the saturated solution of Iodogen, and the mixture was allowed to react for 3 hours after which it was quenched with $Na_2S_2O_5$. The filtrate was chromatographed as described for Iodogen; R_T of the by-product = 3.2 minutes.

Example 4

Preparation of 5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]IUdR)

To the dry mixture of freshly prepared 5-chloromercuri-2'-deoxyuridine (4 mg, 8.6 µmol) and Iodogen (4 mg, 9.3 µmol) was added 1.4 mCi sodium [^{125}I] iodide in 0.3 mL water. The mixture was stirred in a closed 2-mL reaction vial at room temperature for 2 hours. The suspension was withdrawn from the reaction vessel into a 5-mL syringe and filtered through a 0.22 µm Millex filter. The reaction vial and filter were washed 4 times with 0.2 mL water. The combined filtrates were injected into the HPLC, and the radioactive content of each fraction determined. Fractions from the peak with an R_T = 7.1 minutes, corresponding to that of an authentic sample, afforded 5-[^{125}I]iodo-2'-deoxyuridine in 57.1% radiochemical yield (0.8 mCi). The radiochemical purity of the crude [^{125}I]IUdR in the filtrate was 99.1%, sodium [^{125}I]iodide accounted for the remaining 0.9% of the radioactivity (R_T = 2.2 minutes).

There is shown in Table 1 below a summary of yield and purity of [^{125}I]IUdR obtained by varying the conditions of the foregoing example.

TABLE 1

Summary of Yields and Purity of [^{125}I]IUdR Obtained Under Different Reaction Conditions

Solvent	Reaction time (h)	ClHgUdR:Iodogen[a]	Percent purity	Percent yield
water	1	3:3	77.0	22.4
water	3	10:2.5	97.8	36.7
water	1	10:10	84.0	44.0
water	2[c]	10:10	99.1±0.4	54.8±1.6

a. mg/mL:mg/mL

c. four runs

Example 5

Preparation of 5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]IUdR)

The radioiodination of 5-fluoro-2'-deoxyuridine was conducted by substituting 8.6 µmol of it for the 5-chloromercuri-2'-deoxyuridine of Example 4. Approximately 100% of the radioactivity was recovered in the filtrate; the yield of [^{125}I]IUdR was about 50% and the remainder of the radioactivity was recovered in the sodium iodide fraction.

Example 6

Preparation of 5-[^{123}I]iodo-2'-deoxyuridine ([^{123}I]IUdR)

To a dry mixture of 5-chloromecuri-2'-deoxyuridine (4 mg, 8.6 mol) and Iodogen (4 mg, 9.3 mol) was added 2.1 mCi sodium [^{123}I]iodide in 0.3 mL water. The pH of the sodium iodide solution was adjusted to about 7 using 0.1 M acetic acid. The mixture was stirred in a closed 2-mL reaction vial at room temperature for 3 hours. The suspension was withdrawn from the reaction vessel into a 5-mL syringe and filtered through a 0.22 m Millex filter. The reaction vial and the filter were washed 4 times with 0.2 mL water. The combined filtrates were injected into the HPLC, and the fraction were collected and counted in a dose calibrator. The radioactive fractions which corresponded to an authentic sample (R_T = 7.1 minutes) afforded 5-[^{123}I]iodo-2'-deoxyuridine in 65.7% radiochemical yield (1.37 mCi). The radiochemical purity of the crude [^{123}I]IUdR in the filtrate was 98%. Sodium [^{123}I]iodide accounted for 1.2% of the radioactivity (R_T = 2.2 minutes), and the remaining 0.8% was associated with unidentified species with R_T = 4.1 minutes.

Similar results can be obtained when other water-insoluble halomercuri pyrimidine nucleosides or nucleotides are employed.

What is claimed is:

1. The method of making radioiodinated pyrimidine nucleoside or nucleotide which comprises contacting a water-insoluble halomercuri pyrimidine nucleoside or nucleotide with an aqueous medium containing a dissolved radioactive iodide ion and an oxidizing agent, the molar amounts of said nucleoside or nucleotide and said oxidizing agent being in excess of the molar amount of said iodide, whereby water-soluble radioactive iodinated pyrimidine nucleoside or nucleotide is formed in solution, and

separating residual water-insoluble material including halomercuri pyrimidine nucleoside or nucleotide from said solution.

2. The method as claimed in claim 1 in which said insoluble nucleoside or nucleotide is 5-chloromercuri pyrimidine nucleoside or nucleotide.

3. The method as claimed in claim 2 in which said insoluble nucleoside is 5-chloromercuri-2'-deoxyuridine.

4. The method as claimed in claim 1 in which said insoluble nucleoside or nucleotide is fluoromercuri pyrimidine nucleoside or nucleotide.

5. The method as claimed in claim 4 in which said insoluble nucleoside is 5-fluoromercuri-2'-deoxyuridine.

6. The method as claimed in claim 1 in which said oxidizing agent is water-insoluble.

7. The method as claimed in claim 1 in which said oxidizing agent is Iodogen.

8. The method as claimed in claim 2 in which said oxidizing agent is water-insoluble.

9. The method as claimed in claim 2 in which said oxidizing agent is Iodogen.

10. The method as claimed in claim 3 in which said oxidizing agent is water-insoluble.

11. The method as claimed in claim 3 in which said oxidizing agent is Iodogen.

12. The method as claimed in claim 4 in which said oxidizing agent is water-insoluble.

13. The method as claimed in claim 4 in which said oxidizing agent is Iodogen.

14. The method as claimed in claim 5 in which said oxidizing agent is water-insoluble.

15. The method as claimed in claim 5 in which said oxidizing agent is Iodogen.

AMENDED CLAIMS
[received by the International Bureau on 27 June 1989 (27.06.89)
original claims 1-7 replaced by new claims 1-4 (1 page)]

1. The method of making radioiodinated pyrimidine nucleoside or nucleotide which comprises contacting a water-insoluble halomercuri pyrimidine nucleoside or nucleotide with an aqueous medium containing a dissolved radioactive iodide ion and iodogen, the molar amounts of said nucleoside or nucleotide and said iodogen being in excess of the molar amount of said iodide, whereby water-soluble radioactive iodinated pyrimidine nucleoside or nucleotide is formed in solution, and

separating residual water-insoluble material including halomercuri pyrimidine nucleoside or nucleotide from said solution.

2. The method as claimed in claim 1 in which said insoluble nucleoside or nucleotide is 5-chloromercuri pyrimidine nucleoside or nucleotide.

3. The method as claimed in claim 2 in which said insoluble nucleoside is 5-chloromercuri-2'-deoxyuridine.

4. The method as claimed in claim 1 in which said insoluble nucleoside or nucleotide is fluoromercuri pyrimidine nucleoside or nucleotide.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00358

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C07H 1/00

U.S. CL.: 536/23, 29; 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/23, 29; 424/1.1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DATABASES SEARCHED: APS, CAS online

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Chemical Abstracts, Volume 102, No. 13, Issued 01 April 1985 (Columbus, Ohio, USA), Ute Linz, "Chemical and Biological Consequences of the Radioactive Decay of Iodine -125 in Plasmid DNA," see page 327, column 2, the abstract no. 108959h, Radiation Res. 1985, 101(2), 262-78 (Eng).	1-5
Y	Chemical Abstracts, Volume 102, No. 19, issued 13 May 1985 (Columbus, Ohio, USA), Yip Lee, "The Synthesis of [^{36}Cl]-, [^{82}Br]-, and [^{123}I]- Label 1-(3'-Chloro-(Bromo and Iodo)-3'-Deoxy-Beta-D-Arabinofuranosyl) Uracil," see page 652, column 2, the abstract No. 167078t, Int. J. Appl. Radiat. Isot. 1984, 35(11), 1053-6 (Eng).	1-5

* Special categories of cited documents: [10]

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"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

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 May 1989	12 JUN 1989
International Searching Authority	Signature of Authorized Officer
ISA/US	JAMES O. WILSON

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 4,649,039 (GARLICK) 10 March 1987 See column 2, lines 9 to 36.	1-15
A	US, A, 3,135,787 (RESTIVO) 02 June 1964, see column 3, example 5.	1-3
A	UA, A, 2,949,451 (HOFFER) 16 August 1960, see column 5, example 3a.	4-5

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . , 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 [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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 of the international application.

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

3. ☐ 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 claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.