Synthesis of 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodo[2-^{14}C] uracil

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SUMMARY

The synthesis of the title compound (7) is described. [2-^{14}C] cytosine (1) is treated with an aqueous mixture of sodium bisulfite and sodium sulfate at 80°C for 30 min. The resulting solid is then treated with aqueous sodium hydroxide and passed through a cation exchange column, producing [2-^{14}C] uracil (2). Iodionation with iodic acid and iodine in acetic acid yielded 5-iodo- [2-^{14}C]uracil (3) Treatment of (3) with hexamethyldisilazane formed the trimethylsilated pyrimidine. Condensation with 3-0-acetyl-5-0-benzoyl-2-deoxy-2-fluoro-D-arabinosyl bromide produced a mixture of α, β isomes. Separation by column chromatography and saponification with aqueous potassium carbonate yielded the title compound.

Key Words:

1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodo[2-^{14}C] uracil,antiviral, anticancer.

INTRODUCTION

1-β-D-Arabinofuranosyluracil is devoid of antiviral and anticancer activity. The 5-halogeno analogues show antiviral activity in cell culture[1] and in vivo[2]. They also have shown antiherpes virus activity in culture and were also active against experimental herpes keratitis in rabbits[3].

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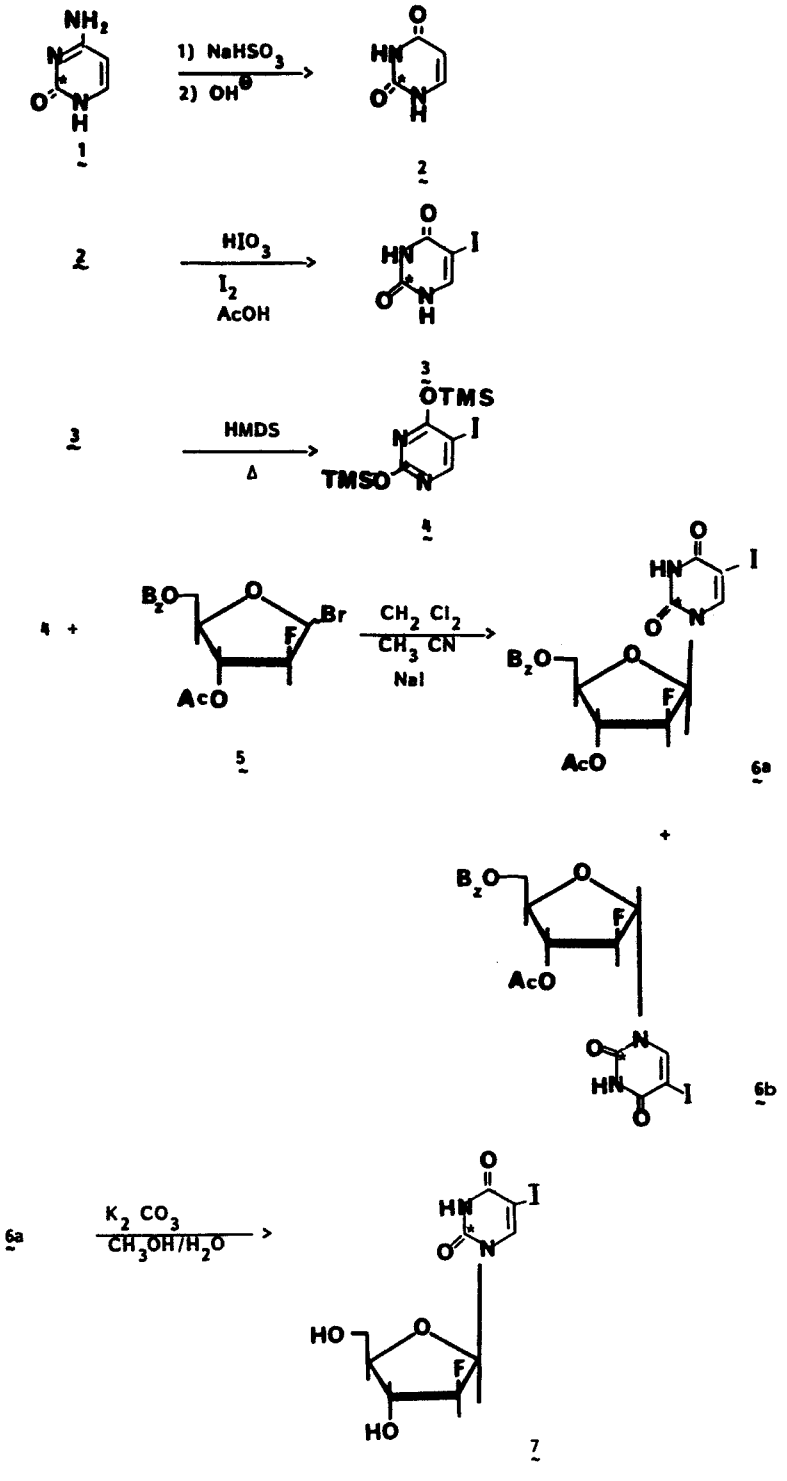
Synthetic Pathway



*Position of Radiolabel

It is apparent from the above-mentioned data that the nature of the substituent at C-5 of the pyrimidine nucleoside is an important factor in determination of biological activity.

This report describes the synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofluranosyl)-5-iodo-[2-^{14}C] uracil.

MATERIALS

The [2-^{14}C] Cytosine was purchased from Amersham Corporation. All chemicals used in the synthesis were purchased commercially and used without any purification. All other solvents were either distilled or analytical reagent quality. Thin layer chromatography plates used were Analtech silca gel GF, scored 10 x 20 cm, 250 microns and high pressure liquid chromatography was carried out on Waters Associates instrumentation. Radioactivity was measured by a Beckman LS 9000 liquid scintillation counter. Nuclear magnetic resonance was measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and Mettler Microanalytical M5AS balance.

EXPERIMENTAL

[2-^{14}C] Uracil

Into a 50 ml erlenmeyer flask was added, H_2O (20 ml), [2-^{14}C] cytosine (271 mg., 100 mCi 41 mCi/mmol), non-radioactive cytosine (618 mg), sodium bisulfite (4.99 g) and sodium sulfate (1.51 g). This was immersed into a preheated oil bath at 80°C and stirred at this temperature for 35 min. The reaction was then stirred at room temperature for 30 min and then at 0°C for 30 min. The resulting solid was removed by filtration and dried under high vacuum for 16 hrs, yielding (1.75 g). This solid was suspended in H_2O (20 ml) and pH adjusted to pH 12 with 30% sodium hydroxide. The resulting solution was applied to a cation exchange column (Dowex 50W-X8, 100 ml) and eluted with water. Concentration of eluent (H_2O) under reduced pressure produced a solid (787 mg) yield = 88%.

Thin Layer Chromatography:

Eluent-methanol(10); ammonium hydroxide (0.1), Plates - Analtech silica gel, Visualization Iodine vapors, Compound Rf = 0.76.

High Pressure Liquid Chromatography:

This was carried out on Waters Associates instrumentation with the following parameters: Eluent - water (100%), Flow Rate - 2 ml/min., Detector - ultraviolet at 280 nm., Temperature - 22.5°C, Column - Waters Associates C-18, Retention Time - 2.95 min.

5-Iodo-[2-^{14}C] uracil (3)

Into a 250 ml round-bottom flask was placed (2) (787 mg), iodic acid (347 mg), iodine (765 mg), water (29 ml) acetic acid (42 ml) and carbon tetrachloride (22 ml). This mixture was immersed into a preheated oil bath at 80°C and stirred at this temperature for 16 hrs. The reaction was cooled to room temperature and then concentrated, under reduced pressure, to a solid which was dried under high vacuum for 4 hrs. The solid was then suspended in H_2O (10 ml), stirred at room temperature for 15 mins and then removed by filtration and dried. This produced desired product (1.20 g) yield = 72%.

Thin Layer Chromatography:

Eluent - Chloroform (10); Methanol (1) Plates - Analtech silica gel, Visualization - iodine vapors, Compound - Rf = 0.38.

High Pressure Liquid Chromatography:

This was carried out on Waters Associates instrumentation having the following parameters: Eluent - Water (100%), Flow Rate - 2 ml/min, Detector -ultraviolet at 280 nm, Temperature - 22.5°C, Column - Waters Associates C-18 Retention Time - 9.74 min.

2,4-bisl (trimethylsilyl) oxy]-5-iodo- [2-^{14}C]pyrimidine (4)

Into a 100 ml round-bottom flask was placed (3) (1.20 g), hexamethyldisilazane (60 ml) and ammonium sulfate (18 mg). This was heated under reflux for 1 hr and then

stirred at room temperature for 16 hrs. The reaction was then concentrated under reduced pressure to a yellow viscous oil (1.9 g) yield = 99%. This material was used directly.

1-(3-O-Acetyl-5-O-benzoyl-2-deoxy-2-fluoro- β-D-arabinofuranosyl) -5-iodo [2-^{14}C]uracil (6a)

To a solution of (5) [4] (1.45 g) in dry methylene chloride (25 ml) and dry acetonitrile (5 ml) was added (4) (1.9 g) followed by sodium iodide (602 mg) and the reaction stirred at room temperature for 7 days. The reaction was concentrated to dryness yielding a solid. This mixture was purified by two column chromatographic systems. The first column chromatography system used was chloroform (10) - methanol (1) and the second system was ether. This produced desired product (270 mg) yield = 13%.

Thin Layer Chromatography:

System One - eluent - chloroform (10); methanol (1), Plates - Analtech silica gel, Visualization - ultraviolet light at 254 nm. Compound - Rf = 0.76 (mixture of α and β isomers).

System Two - eluent - ether, Plates - Analtech silica gel, Visualization -ultraviolet light at 254 nm.

Compound - Rf = 0.54 (β isomer)

Rf = 0.42 (α isomer)

High Pressure Liquid Chromatography:

This was carried out on Waters Associates instrumentation having the following parameters: Eluent - gradient from 100% A (6% acetonitrile, water) to 50% A and 50% B (100% acetonitrile) Flow Rate - 2 ml/min. Detector - ultraviolet at 280 nm. Temperature - 22.5°C Column - Waters Associates C-18, Retention Time - 26 min.

1-(2-Deoxy-2-fluoro- β-D-arabinofuranosyl)-5-iodo- [2-^{14}C] uracil (7)

To a solution of (6a) (270 mg) in methanol (5 ml) was added IN potassium carbonate

solution (0.53 ml), and the mixture stirred at room temperature for 16 hrs. To the clear solution was added isopropyl alcohol (30 ml) and the reaction concentrated under reduced pressure to a solid. This was purified by column chromatography yielding desired product (140 mg), with a radiochemical purity of 99% and a specific activity of 24.6 μCi/mg, yield = 71%.

Thin Layer Chromatography:

Eluent - chloroform (10) - methanol (1), Plates - Analtech silica gel, Visualization - ultraviolet light at 254 nm. Compound - Rf = 0.18.

High Pressure Liquid Chromatography:

This was Waters Associates instrumentation having the following parameters: Eluent - 6% acetonitrile, water Flow Rate - 2 ml/min. Detector - ultraviolet at 280 nm. Temperature - 22.5°C Column - Waters Associates C-18 Retention Time - 8.5 min.

RESULTS AND DISCUSSION

[2-^{14}C]Cytosine was converted to [2-^{14}C]uracil by the method of H. Hayatsu et al[5]. [2-^{14}C]cytosine was chosen for the starting material rather than [2-^{14}C] uracil due to economic considerations. Treatment of the generated [2-^{14}C]uracil with iodic acid, iodine, acetic acid, water and carbon tetrachloride at 80°C for 16 hrs produced a 72% yield of 5-iodo-[2-^{14}C] uracil after ion exchange chromatography. Heating in neat hexamethyldisilazane produced the trimethylsilated pyrimidine. Condensation of 3-0-acetyl-5-0-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide[4] with 2,4-bis[(trimethysilyl)oxy]-5-iodopyrimidine in the presence of sodium iodide for 7 days produced a mixture of α and β isomers in a 1 to 3 ratio. Two column chromatography systems were used to separate the isomers. The first chromatography system separated the mixture of isomers away from unreacted materials and impurities, and the second chromatography system separated the isomers. Deprotecting was achieved by using 1N potassium carbonate in methanol for 16 hrs. Purification by column

chromatography yielded 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodo-[2-^{14}C]uracil (140 mg) having a radiochemical purity of 99% and a specific activity of 24.6 μCi/mg. All experimental conditions were optimized using non-radiolabelled materials.

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