SYNTHESIS OF (S)-1-[3-HYDROXY-2-(PHOSPHONYLMETHOXY) PROPYL]-[2-^{14}C]CYTOSINE ((S)-[^{14}C] HPMPC)

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SUMMARY

The synthesis of (S)-[^{14}C]HPMPC (4) is described. Heating [2-^{14}C]cytosine (1) in N,N-dimethylformamide with (R)-3-O-benzyl-2-O-[(diethylphosphonyl)methyl]-1-O-(methylsulfonyl) glycerol in the presence of cesium carbonate gave (S)-1-[3-benzyloxy-2-(diethylphosphonylmethoxy) propyl]-[2-^{14}C]cytosine (2). Reduction with palladium hydroxide on carbon in cyclohexene yielded (S)-1-[3-hydroxy-2-(diethylphosphonylmethoxy)propyl]-[2-^{14}C] cytosine (3). Deprotection of the diethylphosphonate ester with bromotrimethylsilane and treatment with water produced 190 mg of [^{14}C] HPMPC (4) having a specific activity of 21.2 μCi/mg and a radiochemical purity 98.5% in an overall yield of 5.6%.

KEY WORDS

Antiviral, [^{14}C] HPMPC, (S)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl]-[2-^{14}C]cytosine.

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INTRODUCTION

(S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC) is a potent and selective inhibitor of herpes viruses, including herpes simplex types 1 and 2 and human cytomegalovirus[1,2]. During the development of HPMPC, pharmacokinetic and drug disposition studies were essential to understand its absorption, tissue distribution, metabolism and elimination in various animal models used in the investigation of safety and efficacy.

This report describes the synthesis of [^{14}C] HPMPC.

EXPERIMENTAL

Materials

[2-^{14}C]Cytosine was purchased from Amersham Corporation. All other reagents were ACS grade or the highest quality commercially obtainable. NMR spectra were obtained on a Bruker Spectrospin 360 MHz instrument, using tetramethylsilane as an internal standard. Radioactivity was measured by a Beckman LS9000 liquid scintillator. TLC Plates: Silica gel, 250μ GF (Analtech). Method: Mobile phase, as indicated; visualization, UV 254 nm. All the high pressure liquid chromatography was carried out on Jones and Whatman Instrumentation.

(S)-1-[3-Benzyloxy-2-(diethylphosphonylmethoxy)propyl]-[2-^{14}C]cytosine (2)

A solution of (R)-3-O-benzyl-2-O-[(diethylphosphonyl)methyl]-1-O-(methylsulfonyl) glycerol (5.0 g, 0.012 mol) in N,N-dimethylformamide (25 ml) was vigorously stirred and heated at 90°C in a 250 ml, three-necked, round-bottomed flask equipped with a magnetical stirrer. Cytosine (1.38 g, 0.012 mol) and [2-^{14}C]cytosine (198 mg, 0.0017 mol, 100 mCi, 56 mCi/mmol) were added in one portion followed by addition of cesium carbonate (7.95 g, 0.024 mol). The reaction mixture was stirred at 90°C for 2.5 h and then was allowed to cool to room temperature. The insoluble material was removed by filtration. The filtrate was concentrated to give a yellow oil, which was purified by flash-column chromatography on silica gel (120 g) eluting with a gradient of 5%-->10%-->15% methanol/methylene chloride. This produced 800 mg of desired N-alkylated product (2) (TLC: 10% MeOH/CH_2Cl_2, Rf = 0.5). Yield = 13.4%.

(S)-1-[3-Hydroxy-2-(diethylphosphonylmethoxy)propyl]-[2-^{14}C]cytosine (3)

A mixture of (S)-1-[3-benzyloxy-2-(diethylphosphonylmethoxy)propyl]-[2-^{14}C] cytosine (2) (800 mg, 1.9 mmol) and palladium hydroxide on carbon

(800 mg, 20%) in 1:1 ethanol/cyclohexene (15 ml) was heated at reflux. Thin layer chromatography showed no further consumption of starting material after 7 h. The reaction mixture was filtered through a celite pad and washed with hot ethanol. The filtrate was concentrated in vacuo and then redissolved in 1:1 ethanol/cyclohexene (15 ml). Palladium hydroxide on carbon (800 mg, 20%) was added and the reaction mixture was heated at reflux for 8 h. Thin layer chromatography indicated 99% of product. The mixture was filtered while hot through a celite pad, rinsing the collected solid with hot ethanol. The filtrate was concentrated in vacuo to give a pale yellow oil. Purification by flash-column chromatography on silica gel (60 g) eluting with a gradient of 7.5%-->15% methanol/methylene chloride afforded 350 mg of product (3) as a white foam. (TLC:30% MeOH/CH_2Cl_2, Rf = 0.3). Yield = 65%.

(S)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]-[2-^{14}C]cytosine (4)

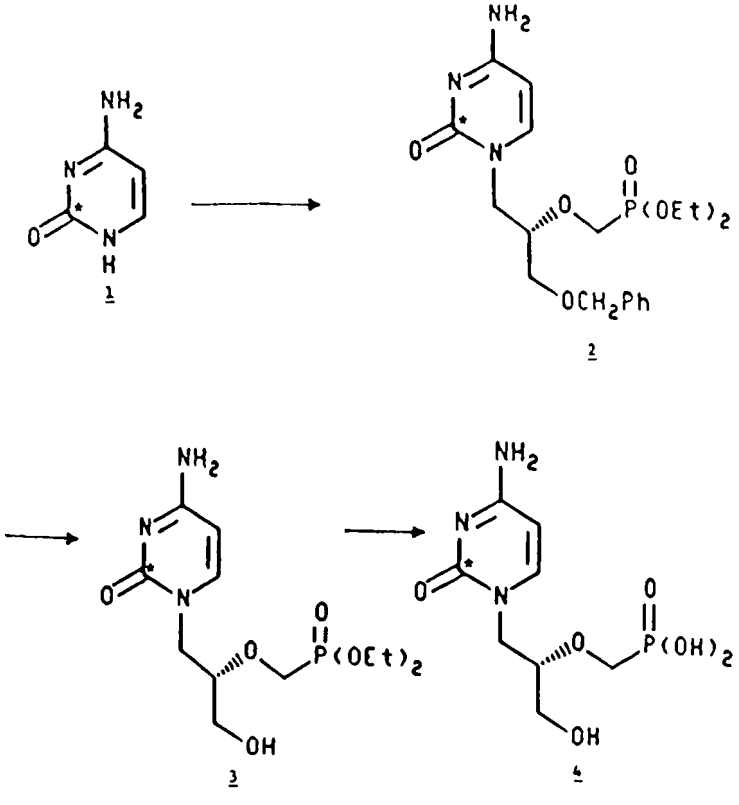
A solution of (S)-1-[3-hydroxy-2-(diethylphosphonyl)methoxy]propyl-[2-^{14}C]cytosine (3) (350 mg, 1.04 mmol) in acetonitrile (3.5 ml) was treated with bromotrimethylsilane (1 ml, 10 mmol) dropwise via syringe over 5 min. The resulting yellow solution was stirred at room-temperature for 4 h and the reaction mixture was concentrated in vacuo. The residual oil was placed under high-vacuum for 15 h, then dissolved in water (0.25 ml) and ethanol (1.5 ml) was added. The solution was stirred at room temperature for 1 h after which time a solid precipitated. The white solid was removed by filtration, washed with ethanol (0.25 ml) and dried in vacuo. This produced 190 mg of product (4) having a radiochemical purity of 98.5% and a specific activity of 21.2 μCi/mg. Yield = 65%. Radiochemical purity was determinded by high pressure liquid chromatography. This was carried out on Jones instrumentation with the following parameters: Eluent - 75% tetrabutylammonium phosphate (0.005 M) plus dibasic sodium phosphate (0.003M) and 25% acetonitrile. Flow-Rate - 2 ml/min. Detector - Ultraviolet at 280 nm. Temperature - 22.5%. Column - Jones Apex C-18. Retention - Time - 9.0 min.

RESULTS AND DISCUSSION

Coupling of [2-^{14}C]cytosine with (R)-3-O-benzyl-2-O-[(diethylphosphonyl)methyl]-1-O-(methylsulfonyl) glycerol[1] in the presence of cesium carbonate in DMF at 90°C afforded the N-alkylated product (2). Completion of the [^{14}C] HPMPC synthesis required unmasking of the primary hydroxyl and the phosphonic acid groups. Removal of the benzyl protecting group was achieved by transfer hydrogenation with 20% $Pd(OH)_2$ on carbon in

cyclohexene/ethanol. This provided, after flash-column chromatography, the diethylester of [^{14}C]HPMPC (3). Final deprotection was accomplished by treatment with bromotrimethylsilane in CH_3CN. Concentration of the reaction mixture, followed by treatment of the residue with water and ethanol afforded [^{14}C]HPMPC (4) as a crystalline solid having a radiochemical purity of 98.5% and a specific activity of 21.2 μCi/mg in an overall yield of 5.6%. All experimental conditions were optimized using nonradiolabelled materials.

SYNTHETIC PATHWAY



* = position of radiolabel

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