SYNTHESIS OF [2-^{14}C] 3′-DEOXYTHYMIDIN-2′-ENE (d4T) AND [5-^{125}I] 3′-AZIDO-2′, 3′-DIDEOXY-5-IODOURIDINE: POTENT INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV-1)

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SUMMARY

The syntheses of [2-^{14}C] 3′-deoxythymidin-2′-ene (d4T) and [5-^{125}I] 3′-azido-2′, 3′-dideoxy-5-iodouridine in a one-pot operation are described.

Key words: 3′-Azido- and 2′, 3′-unsaturated deoxyribonucleosides, [2-^{14}C] 3′-deoxythymidin-2′-ene (d4T), [5-^{125}I] 3′-azido- 2′, 3′-dideoxy-5-iodouridine, HIV-1, HPLC.

INTRODUCTION

Various 3′-azido- and 2′, 3′-unsaturated deoxyribonucleosides are potent inhibitors of retroviruses including the human immunodeficiency virus (HIV-1), which is responsible for the acquired immunodeficiency syndrome (AIDS)[2-10]. Furman et al.[11] investigated the metabolism of 3′-azido-3′-deoxythymidine (AZT) and found it to be sequentially phosphorylated to the corresponding 5′-mono-, di-, and triphosphate analogues by cellular enzymes. The AZT-triphosphate inhibits the utilization of dTTP by reverse transcriptase and may be incorporated in the terminal position of DNA, thereby preventing elongation. In order to study the metabolism and mechanism of action of 3′-deoxythymidin-2′-ene (3′-deoxy-2′, 3′-didehydrothymidine, d4T) and 3′-azido-2′, 3′-dideoxy-5-iodouridine (3′-N_3-IUdR), two potent inhibitors of HIV-1[4-9], we have synthesized the radioactive [2-^{14}C] 3′-deoxythymidin-2′-ene and [5-^{125}I] 3′-azido-2′, 3′-dideoxy-5-iodouridine. The details of the syntheses are described in this report.

CHEMISTRY

[2-^{14}C] Labeled 3′-deoxythymidin-2′-ene (4) was synthesized by the methodology of Horwitz et al.[12] with modification. The synthesis was performed in a one-pot operation.

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Starting with [2-^{14}C] thymidine (1), the desired final product was isolated at the end of the operation by reverse phase HPLC, using a μBondapak C18 semipreparative column.

[5-^{125}I] Labeled 3′-azido-2′, 3′-dideoxy-5-iodouridine (6) was synthesized by treating 3′-azido-2′, 3′-dideoxyuridine[13] (5, 25 mg, 0.1 mmol) with silver trifluoroacetate (44 mg, 0.2 mmol) and iodine (76 mg of iodine plus 10 mCi of $^{125}I_2$ in 0.1 mL of water, pH 9-10) in dry dioxane[14]. The final product was isolated by a preparative silica gel TLC plate (2 mm, Merck), using ethyl acetate as eluting solvent.

EXPERIMENTAL

[2-^{14}C] 3′-Deoxythymidin-2′-ene (4).

An aqueous solution of [2-^{14}C] thymidine (1, 10.9 mg, 0.045 mmol, 2.5 mCi) in 5 mL of water was transferred to a 10 mL-round bottom flask containing nonradioactive thymidine (25 mg, 0.104 mmol). The solution was evaporated to dryness. The residue was co-evaporated with absolute ethanol (3 x 1 mL) and pyridine (3 x 1 mL) in vacuo at room temperature. To the residue 1 mL of pyridine was added. The solution was stirred at 0°C (ice-water bath), and methanesulfonyl chloride (59.2 mg, 0.51 mmol) was added slowly in small fractions to the solution. The reaction mixture was kept in the refrigerator overnight and then treated with 0.1 mL of water with stirring for 1h. The solvents were evaporated under reduced pressure at >30°C, and the residue was co-evaporated with absolute ethanol (3 x 1 mL) to dryness in vacuo to yield [2-^{14}C] 3′, 5′-di-O-methanesulfonylthymidine (2), which was used immediately for the next step without further purification.

A solution of crude compound 2 in 1 mL of water containing 1.2 mL of 1 N NaOH was refluxed for 2h. The solution was cooled to room temperature and neutralized with glacial acetic acid to pH 6-7. The solution was evaporated to dryness in vacuo. The resultant residue was co-evaporated with absolute ethanol (3 x 1 mL) and then dried under vacuum for 5h to afford [2-^{14}C] 1-(2-deoxy-3, 5-epoxy-β-D-threopentosyl)thymine (3), which was again used for the next preparation without further purification.

Potassium t-butoxide (44 mg, 0.39 mmol) was added to a solution of compound 3 in 1 mL of dry DMSO. The reaction mixture was stirred at room temperature overnight (15h), and then neutralized with dilute aqueous acetic acid to pH 6.5-7. The solution was evaporated to dryness below 40°C in vacuo to produce the crude final product 4.

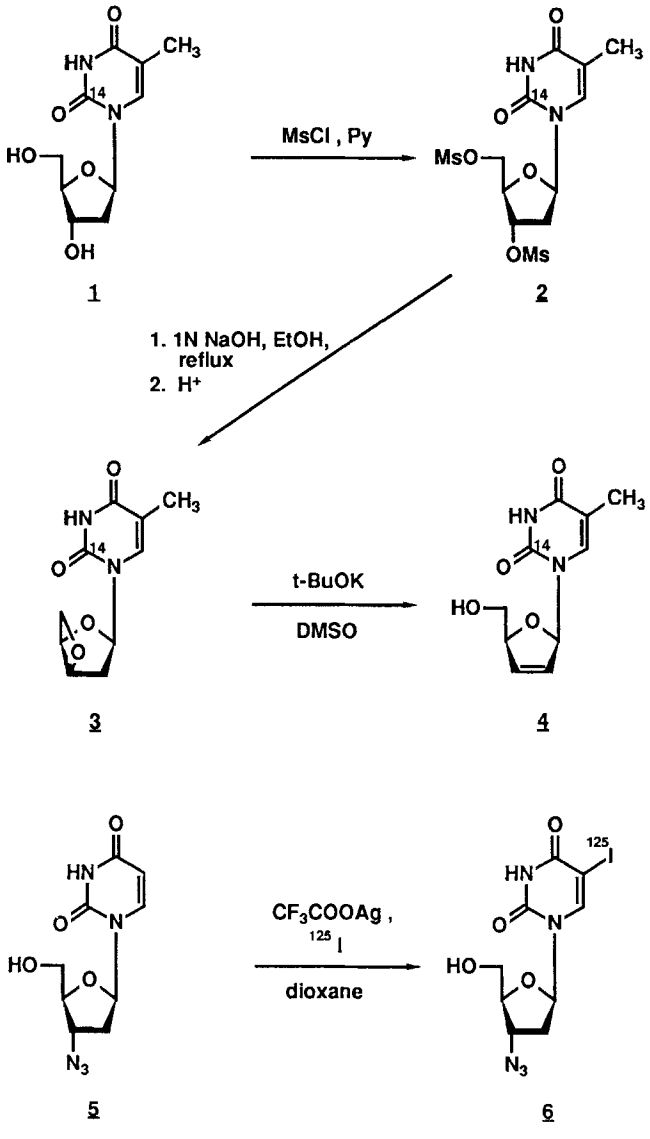
The progress of the reactions described above was monitored by TLC (silica gel), using the unlabeled authentic samples previously synthesized in our laboratory as references. The respective R_f values in CH_2Cl_2-EtOH (8:1) are as follows: (1) For compound 2, R_f 0.7, (2) For compound 3, R_f 0.55, (3) For compound 4, R_f 0.46.

After preliminary clean-up over a small silica gel column, the crude product was dried in vacuo and redissolved in 2.0 mL 0.1 M ammonium acetate (pH 5.5) for HPLC purification. Reverse phase chromatography was performed using a μBondapak C18 semipreparative column (19 x 150 cm, Waters) at a flow rate of 4.0 mL/min. with a 0-20% gradient of CH_3CN into 0.1 M ammonium acetate (pH 5.5) in 40 min. Under these conditions, d4T eluted with a retention time of 39.5 minutes. The product-containing fractions from successive injections were pooled and evaporated repeatedly in vacuo to remove the volatile salts. The yield was 15.4 mmoles (10.4%) by UV spectroscopy, and the specific activity was 16.5 Ci/mol. Finally, the radiochemical purity was determined by coinjecting a small sample of the [^{14}C]-d4T with nonradioactive d4T on a Nova-Pak C18 cartridge column (Waters) at a flow rate of 3.0 mL/min. The chromatogram was run using a 0-20% gradient of CH_3CN into 0.1 M ammonium acetate (pH 5.5) over 40 min. and the product eluted with a retention time of 14.3 min. Radiochemical purity was determined to be 93.5%. For biochemical experiments, the compound was repurified immediately prior to use with the semi-preparative HPLC system previously described, yielding a product of 99% radiochemical purity with a recovery of 95%.

[5-^{125}I] 3′-Azido-2′, 3′-dideoxy-5-iodouridine (6).

A suspension of 3′-azido-2′, 3′-dideoxyuridine[13] (25 mg, 0.1 mmol) and silver trifluoroacetate (44.2 mg, 0.2 mmol) in 5 mL of dry dioxane was stirred at O°C. A solution of iodine (76 mg of nonradioactive I_2 plus 10 mCi of $^{125}I_2$ in 0.1 mL of water, pH 8-10; $^{125}I_2$ is from New England Nuclear) in 5 mL of dioxane was added dropwise to this suspension at 0°C. The resulting mixture was stirred for 5h at room temperature, after which saturated aqueous sodium bicarbonate solution (3 mL) was then added. The mixture was filtered through a celite pad, and the solvents were evaporated to dryness. The remaining residue was dissolved in 4 mL of methanol and passed through a short silica gel (0.5 g) column. The eluate was evaporated under reduced pressure to dryness to yield the crude product.

Scheme 1



The crude product was dissolved in 0.5 mL methanol and spotted on a preparative silica gel TLC plate (2 mm, Merck). The plate was developed in ethyl acetate, and visualized under UV light. The product ran with $R_f = 0.66$, while the starting material ran with $R_f = 0.23$. The panel containing the product was carefully scraped from the plate and packed into a scintered glass funnel. The product was eluted with MeOH - CH_2Cl_2 (1:1), and the solvent was removed in vacuo to give the final product (11.3% yield).

Radiochemical purity was determined by analytical reverse phase HPLC on a μBondapak C18 10μ cartridge column (Waters). The chromatography system employed a 0 to 50% gradient of CH_3CN into 0.1 M ammonium acetate (pH 5.5) over 50 min. at a flow rate of 3.0 mL/min. The product eluted with a retention time of 20.0 minutes, and the radiochemical purity was 74.7%. Prior to biochemical experiments, the product was repurified over a semi-preparative RPC18 Column (Waters) employing the same gradient at a flow rate of 4 mL/min. yielding a product of 98% purity with recovery of 92%.

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