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Short Research Article

Synthesis of tritium-labeled 2′,3′-dideoxy-2′,3′-didehydrothymidine†

G. V. SIDOROV[1,*], N. F. MYASOEDOV[1], M. V. JASKO[2] and YU. S. SKOBLOV[2]

[1] Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq. 2, Moscow 123182, Russia
[2] Institute of Molecular Biology, Russian Academy of Sciences, Vavilov St. 32, Moscow 119991, Russia

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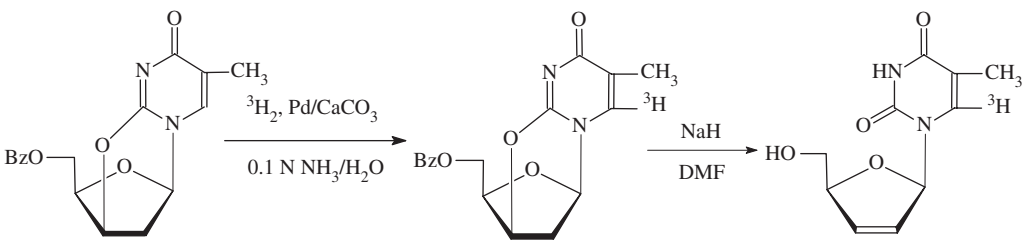
Introduction

The mechanisms of action, metabolic pathways, and cellular uptake of 2′,3′-dideoxy-2′,3′-didehydrothymidine have been intensively studied in recent years.[1] These studies require tritium-labeled compound at high specific radioactivity (A_{mol} > 10 Ci/mmol). A new method of synthesis of tritium-labeled 2′,3′-dideoxy-2′,3′-didehydrothymidine was developed. The first stage was the preparation of tritium-labeled 5′-*O*-benzoyl-2,3′-anhydrothymidine by catalytic isotope exchange with gaseous tritium in solution. The synthesized [6-^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine was transformed into [6-^{3}H]2′,3′-dideoxy-2′,3′-didehydrothymidine in one stage, in an elimination reaction catalysed by sodium hydride in DMF.

Results and discussion

Synthesis was carried out according to Scheme 1:

In the first step we synthesized [6-^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine. Previous NMR studies had shown that tritium replaces position 6 of pyrimidine.[2] The second step was an elimination reaction resulting in the formation of [6-^{3}H]2′,3′-dideoxy-2′,3′-didehydrothymidine. During this step the *O*-benzoyl residue was removed quantitatively. Most likely excess of NaH causes degradation the DMF with the formation of dimethylamine, which removes *O*-benzoyl group. A 'cold' synthesis of 2′,3′-dideoxy-2′,3′-didehydrothymidine was also carried out and the NMR spectrum (Figure 1) confirmed the formation of 2′,3′-dideoxy-2′,3′-didehydrothymidine.



(**I**) (**II**) (**III**)

Scheme 1

Synthesis of tritium-labeled (6-^{3}H)5′-*O*-benzoyl-2,3′-anhydrothymidine (II)

5′-*O*-Benzoyl-2,3′-anhydrothymidine (**I**) (6.5 mg, 19.8 μmol) was placed in a reaction glass reaction vial, then 500 μl of 0.1 N ammonia in 50% aqueous ethanol and 90 mg of 5% Pd/$CaCO_3$ (Fluka) as catalyst were added. The vial was then frozen in liquid nitrogen, evacuated,

*Correspondence to: G. V. Sidorov, Institute of Molecular Genetics, Russian Academy of Science, Kurchatov Sq. 2, Moscow 123182, Russia. E-mail: sidgv@img.ras.ru
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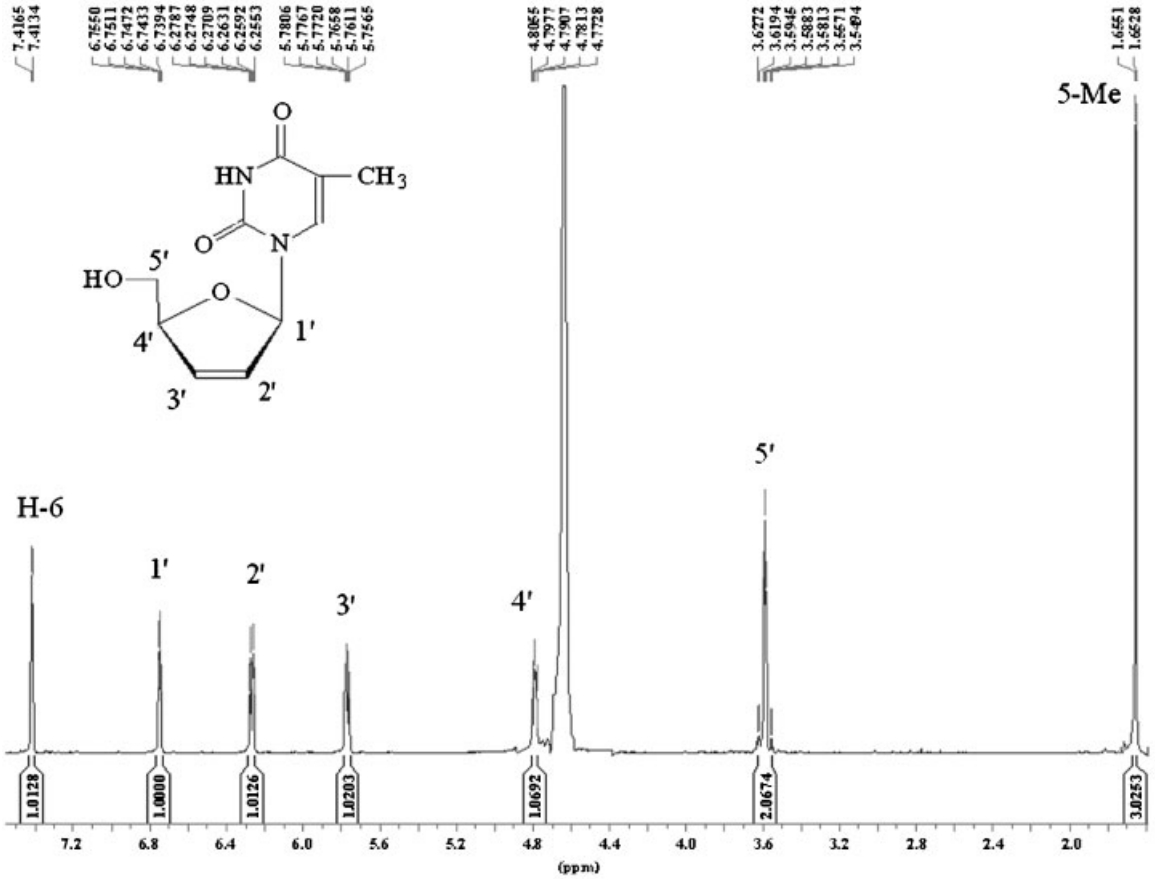

Figure 1 NMR spectrum of 2′,3′-dideoxy-2′,3′-didehydrothymidine ('cold' synthesis) in D_2O. AMX III-400 (Bruker) spectrometer with the 400 MHz operating frequency for ^1H.

and filled with gaseous tritium to a pressure of 400 mmHg. Upon defrosting, the reaction mixture was stirred at room temperature for 20 h. After the reaction was completed, tritium was removed from the vial and the catalyst separated by centrifugation. Labile tritium was removed by evaporation with 10 ml of 50% ethanol. Isolation of the product was achieved by HPLC. As a result, 170 mCi of product was obtained with a specific radioactivity of 21.6 Ci/mmol, in 38% yield.

Synthesis of tritium-labeled 2′,3′-dideoxy-2′,3′-didehydrothymidine (III)

[6-^{3}H]5′-*O*-benzoyl-2,3′-anhydrothymidine (**II**) (1.3 mg, 85 mCi) was dissolved in 0.1 ml of absolute *N*,*N*-dimethylformamide, then about 1 mg of NaH (80% in oil) was added and the solution was stirred at room temperature for 18 h. Then 10 ml of 10% acetic acid was added. The solution was evaporated in vacuum, co-evaporated with water (4 × 0.2 ml) and then with ethanol (2 × 0.4 ml). Isolation of the product was achieved by HPLC. As a result, 17 mCi of product was obtained with a specific radioactivity of 18.5 Ci/mmol, in 20% yield. The radiochemical purity of the product as estimated by thin layer chromatography was above 97%. [6-^{3}H]2′,3′-dideoxy-2′,3′-didehydrothymidine was stored at −20°C as a solution of 1 mCi/ml in 1:1 (v/v) water–ethanol.

HPLC was conducted using a 13-μm (10 × 250 mm) Nucleosil C18 (Macherey–Nagel) column, elution rate 1.5 ml/min, detection UV 260 nm. The mobile phase:

- 5′-*O*-Benzoyl-2,3′-anhydrothymidine. 60% Methanol in water, retention time 13.1 min.
- 2′,3′-Dideoxy-2′,3′-didehydrothymidine. Solvents: A – water; B – methanol. Program: 0–40% B/A in 40 min, retention time 33.9 min.

TLC was carried out on Silufol plates (Czech Republic).

- 5′-*O*-Benzoyl-2,3′-anhydrothymidine in chloroform–ethanol (95:5 v/v) solvent system, $R_f = 0.35$.
- 2′,3′-Dideoxy-2′,3′-didehydrothymidine chloroform–ethanol (9:1 v/v) solvent system, $R_f = 0.28$.

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