AN IMPROVED SYNTHESIS OF [5'-^{3}H]-3'-AZIDO-3'-DEOXYTHYMIDINE, TRITIATED ZIDOVUDINE

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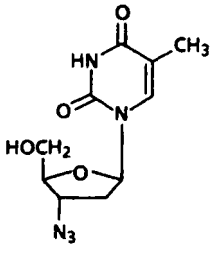
SUMMARY

[5'-^{3}H]-3'-Azido-3'-deoxythymidine (3) was prepared at a specific activity of 14.0 Ci/mmol by a two-step synthetic sequence involving controlled oxidation of the unlabelled compound (1) to the 5'-aldehyde (2) followed by reduction with [^{3}H]-$NaBH_4$. Purification was performed by preparative TLC. The radiochemical purity was 99.2%.

Key Words: [^{3}H]-zidovudine, BW A509U, azidothymidine, AZT, RETROVIR®, radioimmunoassay.

INTRODUCTION

The antiviral agent zidovudine (1, 3'-azido-3'-deoxythymidine, azidothymidine, AZT, BW A509U, RETROVIR®) blocks infection of susceptible host cells *in vitro* by the AIDS virus (Human Immunodeficiency Virus) (1,2).



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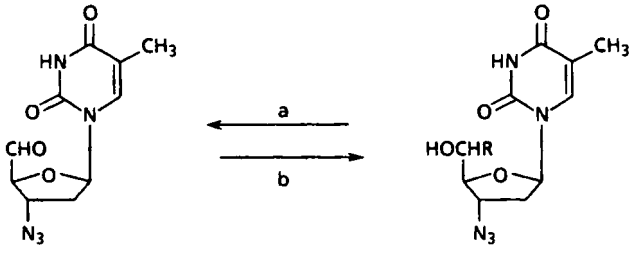
Zidovudine received FDA approval on March 19, 1987 for the management of certain adult patients with symptomatic HIV infection (AIDS and advanced AIDS Related Complex-ARC) who have a history of Pneumocystis carinii pneumonia or a CD4 (T4) lymphocyte cell count of less than 200.

Zidovudine is currently undergoing clinical studies in AIDS patients. Development of a radio-immunoassay for analysis of clinical trial samples requires a high specific activity tritium labelled form of zidovudine. The studies demand that the radiolabelled product should be chemically stable, should have a high chemical and radiochemical purity, and a minimum acceptable specific activity of 10-12 Ci/mmol. This paper describes the preparation of tritium labelled zidovudine with a specific activity of 14.0 Ci/mmol by a short two-step oxidation-reduction sequence in 30.0% overall yield from unlabelled zidovudine.

RESULTS AND DISCUSSION

A lower-yielding synthesis of [^{3}H]-zidovudine at a lower specific activity for use in some antibacterial studies has been reported (3). Zidovudine (1) was oxidized with pyridinium dichromate in dry methylene chloride (4), then purified by chromatography on silica gel to give the 5'-aldehyde (2) in 16% yield. Subsequent reduction with [^{3}H]-$NaBH_4$ gave a 56% yield (9.0% overall yield) of [^{3}H]-zidovudine (3) with a specific activity of 2.4 Ci/mmol. However, a higher specific activity was required for anti-viral radioimmunoassays, and a more efficient higher-yielding synthesis was desirable.

Attempts were made to improve the yield of the 5'-aldehyde (2). Further oxidations with pyridinium dichromate, and also with pyridinium chlorochromate (5) were performed, but no dramatic increase in the yield of the 5'-aldehyde was observed.



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1 (R = H)
3 (R = [^{3}H])

Reagents: a) Pyridinium trifluoroacetate, 1,3-dicyclohexylcarbodiimide, DMSO.

b) [^{3}H]$NaBH_4$, 2-propanol/H_2O

However, oxidation of zidovudine (1) with dry DMSO and 1,3-dicyclohexylcarbodiimide in the presence of pyridinium trifluoroacetate (6), followed by chromatography on silica gel in two different

solvents, gave a 65% yield of the 5'-aldehyde (2) as single-spot material by TLC. The 5'-aldehyde proton appeared in the NMR spectrum at 9.6 ppm relative to tetramethylsilane.

Reduction with [^{3}H]-$NaBH_4$ in 2-propanol/H_2O followed by preparative TLC on silica gel afforded the tritiated product (3) in 46% yield. The chemical and radiochemical purity of (3) was >99% by TLC and plate scanning. The specific activity of (3) was found to be 14.0 Ci/mmol.

Development of a radioimmunoassay system is in progress.

EXPERIMENTAL

Sodium boro[^{3}H]hydride was obtained from Amersham International plc at a batch specific activity of ~50 Ci/mmol. 3'-Azido-3'-deoxythymidine was obtained from Burroughs Wellcome Co., Chemical Development Laboratories. 1,3-Dicyclohexylcarbodiimide and pyridinium trifluoroacetate were purchased from Aldrich Chemical Company. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources. Thin layer chromatography was performed on 5 X 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (Merck). Preparative TLC was performed on a 20 X 20 cm glass plate pre-coated with 2 mm silica gel 60 (Merck). Column chromatography was performed on silica gel 60 (230-400 mesh) as supplied by Merck. The specific activity was determined by counting an aliquot of a solution whose concentration had been determined by UV spectroscopy, using the extinction coefficient of the unlabelled zidovudine. Radiochemical purity was determined on the TLC plate with a Bioscan System 200 Imaging Scanner.

3'-Azido-3',5'-dideoxy-5'-oxothymidine (2)

To a solution of 3'-azido-3'-deoxythymidine (1) (2.00 g, 7.48 mmol) and pyridinium trifluoroacetate (0.722 g, 3.74 mmol, 0.5 eq.) in dry DMSO (30 mL, dried twice with Type 4A Molecular Sieves) was added 1,3-dicyclohexylcarbodiimide (DCC) (4.63 g, 22.4 mmol, 3 eq.). The oxidation was followed by TLC on silica gel, using $CHCl_3/CH_3OH$ (9/1, v/v). For a 15 cm elution, R_f of 1 = 0.33, and R_f of 2 = 0.36. After stirring at room temperature for 2.5 hours, methanol (1.0 mL) was added, and the reaction mixture was filtered to remove 1,3-dicyclohexylurea. The filtrate was evaporated under high vacuum at 40°C to near dryness, and the residue was dissolved in ethyl acetate and pre-loaded onto 20 g of silica gel. The material was chromatographed on a silica gel column (50 mm diameter X 200 mm length) using ethyl acetate. The resulting product was rechromatographed on silica gel using $CHCl_3/CH_3OH$ (19/1, v/v). Removal of the solvents under high vacuum gave the desired aldehyde (2) (1.30 g) as a foam in 65% yield, and shown to be single-spot material by TLC. ^{1}H NMR (80 MHz, DMSO-d_6) δ (TMS) 9.6 (s). Due to

its instability, the compound was not fully characterized. The aldehyde was used immediately in the following reaction.

[5'-^{3}H]-3'-Azido-3'-deoxythymidine (3)

Sodium boro[^{3}H]hydride (2.47 mg, 3.0 Ci at ~50 Ci/mmol) was added in 2-propanol/H_2O (3/1; 2.4 mL) to a stirred solution of the 5'-aldehyde (2) (32.8 mg) in 2-propanol (0.6 mL) at 25°C under argon. After 60 minutes, TLC (silica gel, $CHCl_3/CH_3OH$ (9/1, v/v)) showed zero residual aldehyde. Acetone (0.8 mL) was added and, after 5 minutes, 1*N* HCl was added to pH ~6. After evaporation to dryness, the residue was dissolved in methanol (1.0 mL) and applied to a silica gel preparative plate, and the plate was developed in $CHCl_3/CH_3OH$ (9/1, v/v) for 1.75 hours. Removal of the band at R_f 0.30 corresponding to authentic zidovudine, followed by extraction with acetone (50 mL), filtration, evaporation to dryness, dissolution in EtOAc (10 mL), filtration and evaporation to dryness under high vacuum afforded 15.1 mg of [5'-^{3}H]-3'-azido-3'-deoxythymidine (3) as a colorless glass in 46% yield with specific activity 14.0 Ci/mmol. TLC on silica gel in $CHCl_3/CH_3OH$ (9/1, v/v) showed single-spot material with R_f value (0.35) identical to that of authentic zidovudine. The radiochemical purity was found by plate scanning to be 99.2%.

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