SYNTHESES OF [2-^{14}C] 2,5'-ANHYDRO-3'-AZIDO-3'-DEOXYTHYMIDINE AND [2-^{14}C] 2,5'-ANHYDRO-3'-AZIDO-2',3'-DIDEOXY-5-IODOURIDINE: INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV-1)

Tai-Shun Lin*, Mao-Chin Liu, E. Michael August,[1] Evelyn M. Birks, and William H. Prusoff
Department of Pharmacology and Comprehensive Cancer Center, Yale University
School of Medicine, New Haven, Connecticut 06510

SUMMARY

The syntheses of [2-^{14}C] 2,5'-anhydro-3'-azido-3'-deoxythymidine and [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine in a one pot operation under mild conditions are described.

Key words: AZT, [2-^{14}C] 2,5'-anhydro-3'-azido-3'-deoxythymidine, [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine, HPLC, mild reaction condition.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is the result of an infection by the human immunodeficiency virus (HIV).[2,3] Although at the present time there is no cure for AIDS, 3'-azido-3'-deoxythymidine (AZT) has been extensively used in the treatment of patients with AIDS in order to prolong their lives.[4,5] Recently, we have reported[6] the syntheses and antiviral activities of several 2,5'-anhydro analogues of 3'-azido-3'-deoxythymidine (AZT), 3'-azido-2',3'-dideoxyuridine (AZDU), 3'-azido-2',3'-dideoxy-5-halouridines, and 3'-deoxythymidine against human immunodeficiency virus (HIV-1) and Rauscher-Murine leukemia virus (R-MuLV). 2,5'-Anhydro-3'-azido-3'-deoxythymidine (anhydro-AZT) was the most active compound among these anhydro derivatives. As anhydro-AZT is quite stable in a neutral environment,[6] it was necessary to determine whether its biological activity was due to the compound itself or to a conversion to AZT, which could be produced from the enzymatic hydrolysis of anhydro-AZT in cells by some unknown mechanism. Further evidence to suggest that the anhydro nucleosides have their unique biological activity was provided by Simpson et al.[7] who investigated the effect of these compounds on mitochondrial DNA synthesis. At 25 μM, AZT inhibited the uptake of [^{3}H] dATP into mitochondrial DNA by 51%, whereas anhydro-AZT inhibited DNA synthesis by only 5%.

0362–4803/91/121315–07$05.00

Received 15 July, 1991
Revised 19 August, 1991

Conversely, at a concentration of 25 μM, 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine (anhydro-5-I-AZDU) inhibited mitochondrial DNA synthesis more strongly than did the parent compound, 3'-azido-2',3'-dideoxy-5-iodouridine, with a ratio of 100% versus 12%. Anhydro-5-I-AZDU would not be expected to show greater biological activity if such activity was solely dependent upon conversion of an inactive prodrug to a active (parent) species.

In order to study the molecular basis of the antiviral activity as well as the inhibitory activity to mitochondrial DNA synthesis concerning these anhydro nucleoside derivatives, we have synthesized [2-^{14}C] 2,5'-anhydro-3'-azido-3'-deoxythymidine ([2-^{14}C] anhydro-AZT, 3) and [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine ([2-^{14}C] anhydo-5-I-AZDU, 7). The details of the syntheses are described in this report.

CHEMISTRY

[2-^{14}C] 2,5'-Anhydro-3'-azido-3'-deoxythymidine ([2-^{14}C] anhydro-AZT, 3) and [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine ([2-^{14}C] anhydro-5-I-AZDU, 7) were synthesized by the methodology developed in our laboratory[6] with some modifications. The syntheses were performed in a one pot operation and are outlined in Scheme I. Tosylation of [2-^{14}C] 3'-azido-3'-deoxythymidine (1) with p-toluenesulfonyl chloride in anhydrous pyridine at room temperature yielded the tosylate 2, which was then reacted with 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) in anhydrous acetonitrile to give [2-^{14}C] anhydro-AZT (3). Because compound 3 is not stable in the presence of DBU under reflux, it was desirable to adopt a milder reaction condition. This reaction could be carried out smoothly at room temperature (~25 °C) for 2 days. Iodination[8,9] of [2-^{14}C] 3'-azido-2',3'-dideoxyuridine (4) with silver trifluoroacetate and iodine in dry dioxane produced the 5-iodo analogue 5, which was consecutively treated with p-toluenesulfonyl chloride in anhydrous pyridine and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in anhydrous acetonitrile to furnish the final product 7. The [2-^{14}C] label was preferable over the [5-^{125}I] label in compound 7 since ^{14}C has a much longer half-life ($t_{1/2}$) than ^{125}I; the starting material, [2-^{14}C]-3'-azido-2',3'-dideoxyuridine, is commercially available; and $^{125}I_2$ is much more hazardous to use in the synthesis.

For most of the reactions, the crude products produced in each step were semi-purified by passing through a short silica gel (2-3 g) column, eluted with appropriate solvents. This simple procedure was essential to remove some impurities, which would otherwise interfere with the next preparation or affect the stability of the products.

EXPERIMENTAL

[2-^{14}C] 2,5'-Anhydro-3'-azido-3'-deoxythymidine (3).

A methanolic solution of [2-^{14}C] 3'-azido-3'-deoxythymidine (1, 0.5 mCi, in 3 mL of methanol, purchased from Moravek Biochemicals Inc.) was transferred to a 10 mL round-bottom flask. The solution was evaporated in vacuo at room temperature to dryness and the residue was coevaporated with dry dioxane (3 x 1 mL) in vacuo at room temperature. To the residue 15 mg (0.056 mmol) of nonradioactive 3'-azido-3'-deoxythymidine (AZT) and 0.2 mL of dry pyridine were added. The solution was stirred at 0 °C (ice-water bath), and p-toluenesulfonyl chloride (15 mg, 0.079 mmol) was added slowly to the solution in small fractions. The resulting mixture was stirred at 0-5 °C for 30 min and then at room temperature for 2 days. The reaction mixture was cooled to 0 °C (ice-water bath) and water (0.1 mL) was added with stirring for 1 h. The solvents were evaporated under reduced pressure at <30 °C and the residue was coevaporated with anhydrous acetonitrile (3 x 0.5 mL) in vacuo to dryness to yield [2-^{14}C] 3'-azido-5'-O-(p-tolylsulfonyl)-3'-deoxythymidine (2), which was used immediately for the next step without further purification.

To a cooled solution (ice-water bath) of the crude compound 2 in anhydrous acetonitrile was added 51 mg (0.34 mmol) of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in small fractions. The reaction mixture was stirred at room temperature for 2 days and then passed through a short silica gel (2 g) column, eluted with CH_3CN/CH_3OH (10:1, v/v). The fractions containing the target compound (monitored by TLC, CH_3CN/CH_3OH, 3:1, v/v, R_f 0.47) were combined and evaporated under reduced pressure to afford the crude product.

The progress of the reactions described above was monitored by TLC (EM precoated silica gel sheets containing a fluoroscent indicator) using the unlabeled authentic samples previously synthesized in our laboratory as references. The respective R_f values in CH_2Cl_2/CH_3OH (16:1, v/v) are as follows: (1) compound 1, R_f 0.40, (2) compound 2, R_f 0.69, (3) compound 3, R_f 0.36.

HPLC purification of the crude product was carried out on a Partisil 10/25 M9 column (Whatman) at a flow rate of 3 mL/min using isocratic elution in 5% methanol/95% acetonitrile (v/v). Under these conditions, AZT eluted with a retention time of 5 min and anhydro-AZT eluted with a retention time of 19 min. The product-containing fractions were pooled and evaporated in vacuo. Based on the UV spectrum, a yield of 8.9 μmole (2.2 mg, ~10%) [2-^{14}C] anhydro-AZT

was obtained. Unreacted [2-^{14}C] AZT was likewise recovered from the HPLC run and stored for further use. The radiochemical purity of the product was determined by rechromatography on an 8 mm x 10 cm μBondapak C-18 analytical cartridge column (Waters) employing isocratic elution of 5% acetonitrile in 0.1 M ammonium acetate (pH 5.5) at 3 mL/min. Under these conditions, [2-^{14}C] anhydro-AZT and [2-^{14}C] AZT had retention times of 9 and 16.5 min, respectively. The product was found to be 98.8% radiochemically pure with less than 0.1% [2-^{14}C] AZT present as a contaminant. The product was stored at -20 °C in a methanolic solution where breakdown to the extent of 2% (generation of AZT) was noted upon 15 months of storage.

[2-^{14}C] 2,5'-Anhydro-3'-azido-2',3'-dideoxy-5-iodouridine (7).

A solution of [2-^{14}C] 3'-azido-2',3'-dideoxyuridine (4, 1 mCi, in 2 mL of 50% aqueous ethanol, purchased from Sigma) was transferred to a 10 mL round-bottom flask containing nonradioactive 3'-azido-2',3'-dideoxyuridine (AZDU, 4, 50 mg, 0.2 mmol). The solution was evaporated in vacuo to dryness and the residue was coevaporated with anhydrous dioxane (4 x 2 mL) at room temperature in vacuo. To this residue silver trifluoroacetate (55 mg, 0.25 mmol) and dioxane (2.5 mL) were added. The reaction mixture was stirred at 5 °C (ice-water bath) and a solution of iodine (65 mg, 0.26 mmol) in dioxane (2 mL) was added in small portions to this suspension at 5-10 °C. The resulting mixture was stirred at room temperature for 3 h, after which saturated aqueous sodium bicarbonate solution (2 mL) was added. The mixture was filtered through a celite pad, washing with dioxane (2 mL). The combined filtrate and washings were evaporated in vacuo to dryness. The remaining residue was dissolved in 2 mL of ethyl acetate and passed through a short silica gel (3 g) column eluted with CH_2Cl_2/AcOEt (1:1, v/v). The fractions (~15 mL, R_f 0.62) were combined and evaporated in vacuo to yield [2-^{14}C] 3'-azido-2',3'-dideoxy-5-iodouridine (5), which was used immediately for the next step without further purification.

Crude compound 5 was coevaporated with anhydrous pyridine (2 x 1 mL) in vacuo and the residue was dissolved again in 0.4 mL of anhydrous pyridine. To the cooled solution (0 °C, ice-water bath) with stirring was added p-toluenesulfonic chloride (80 mg, 0.42 mmol). The reaction mixture was stirred at room temperature for 2 days and then cooled to 0 °C and water (0.1 mL) was added with stirring for 1 h. The solvents were evaporated at room temperature in vacuo. The residue was coevaporated with CH_2Cl_2 (3 x 1 mL) in vacuo and dissolved in CH_2Cl_2 (2 mL). The solution was passed through a short silica gel (2 g) column eluted with CH_2Cl_2/

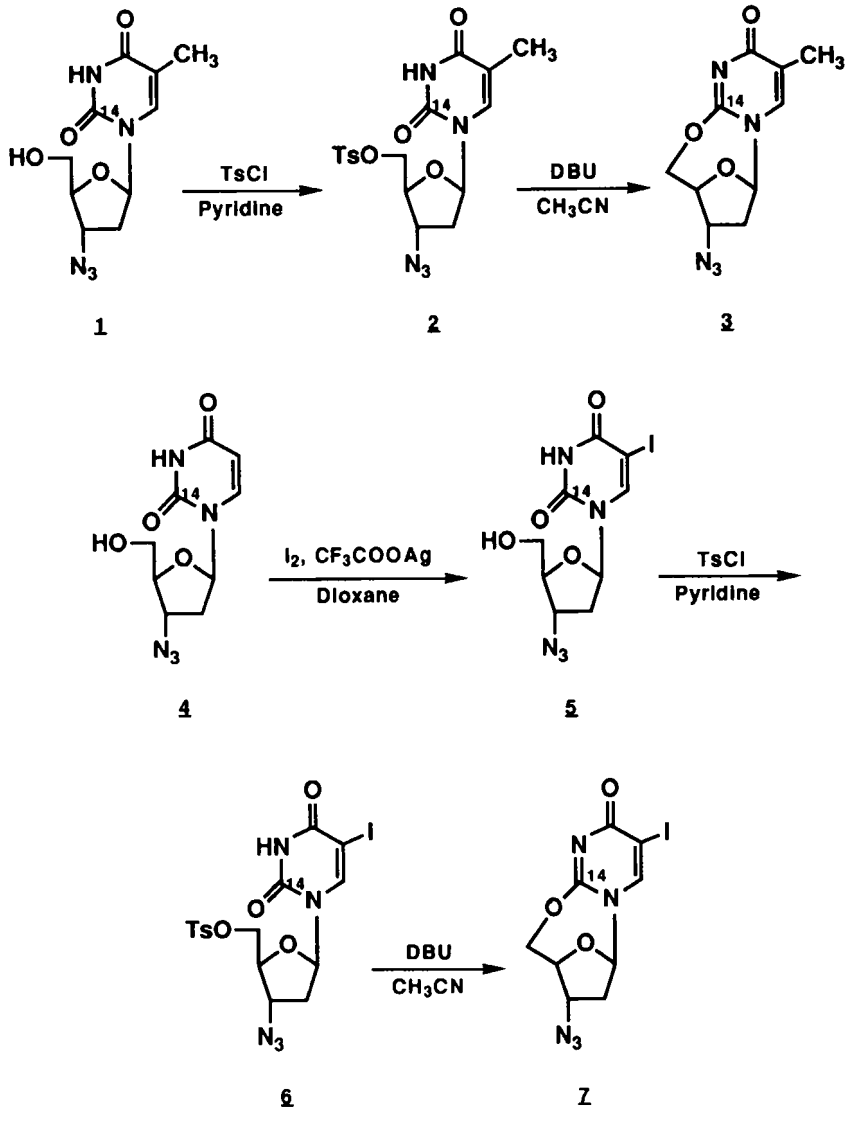
AcOEt (1:1, v/v). The fractions containing the desired compound (monitored by TLC, R_f 0.82) were combined and evaporated in vacuo to yield [2-^{14}C] 3'-azido-5'-O-(p-tolylsulfonyl)-2',3'-dideoxy-5-iodouridine (6), which was again used for the next preparation without further purification.

1,8-Diazabicyclo[5,4,0]undec-7-ene (DBU, 80 mg, 0.53 mmol) was added to a cooled solution (ice-water bath) of compound 6 in 0.4 mL of anhydrous acetonitrile. The reaction mixture was stirred at room temperature for 2 days and then filtered through a celite pad, washing with 4 mL of anhydrous acetonitrile. The filtrate and washings were combined and evaporated in vacuo to dryness. The residue was dissolved in 2 mL of actonitrile and passed through a short silica gel (2 g) column, eluted first with CH_2Cl_2/AcOEt (1:1, v/v, 10 mL) to remove the unreactive starting material and then with CH_2Cl_2/AcOEt (1:3, v/v). The fractions containing the final product (monitored by TLC, CH_2Cl_2/CH_3OH, 15:1, v/v, R_f 0.32) were combined and evaporated in vacuo to give [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine (7).

The progress of the reactions was monitored by TLC as previously described. The respective R_f values in CH_2Cl_2/AcOEt (1:1, v/v) are as follows: (1) compound 4, R_f 0.16, (2) compound 5, R_f 0.60, (3) compound 6, R_f 0.74, (4) compound 7, R_f 0.14.

HPLC purification of the crude product was carried out on a Partisil 10/25 M9 column (Whatman) at a flow rate of 1 mL/min using isocratic elution in 0.5% methanol/99.5% acetonitrile (v/v). Under these conditions, the retention times were as follows: [2-^{14}C] 3'-azido-5'-O-(p-tolylsulfonyl)-2',3'-dideoxy-5-iodouridine (6), 13 min; [2-^{14}C] 3'-azido-2',3'-dideoxy-5-iodouridine (5), 14.4 min; [2-^{14}C] 3'-azido-2',3'-dideoxyuridine (4), 22 min; and [2-^{14}C] 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-iodouridine (7), 29 min. The product-containing fractions were pooled and evaporated in vacuo. Based on the UV spectrum, a yield of 8.9 μmole (2.2 mg, overall yield ~3.1%) [2-^{14}C] anhydro-5-I-AZDU (7) was obtained. The radiochemical purity of the product was determined by HPLC chromatography on an 8 mm x 10 cm μBondapak C-18 analytical cartridge column (Waters) employing isocratic elution of 5% acetonitrile in 0.1 M ammonium acetate (pH 5.5) at 3 mL/min for 20 min, followed by a 10 min gradient to 50% acetonitrile and then maintained at 50% acetonitrile. Under these conditions, the retention times were: [2-^{14}C] AZDU (4), 8 min; [2-^{14}C] anhydro-5-I-AZDU (7), 12 min; [2-^{14}C] 5-I-AZDU (5), 24.5 min; and [2-^{14}C] 5'-O-Ts-5-I-AZDU (6), 30 min. The product was found to be 98.02% radiochemically pure with contaminants of 1.44% of AZDU, 0.43% of 5-IAZDU, and 0.11% of 5'-O-Ts-5-I-AZDU. The product was stored at -20 °C as an acetonitrile solution.

Scheme 1


HO
HN
O
CH3
14
N
O
N3
TsCl
Pyridine
TsO
DBU
CH3CN
1
2
3
I2, CF3COOAg
Dioxane
4
5
6
7

ACKNOWLEDGMENT

This research was supported by PHS grants AI-29430 (to T.S.L.) and CA-05262 (to W.H.P.) awarded by the National Institutes of Health, DHHS.

REFERENCES AND NOTES

1) Present address: Laboratory of Biological Chemistry, National Cancer Institute, National Institutes of Health, Bldg. 37, Room 5E10, Bethesda, MD 20892.

2) Barre-Sinoussi, F.; Chermann, J. C.; Montagnier, L. Science (Washington, D. C.), 220: 868 (1983).

3) Border, S.; Gallo, R. C. Annu. Rev. Immunol., 3: 321 (1985).

4) Mitsuya, H.; Weinhold, K. F.;Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Border, S. Proc. Natl. Acad. Sci. U.S.A., 82: 7096 (1985).

5) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R. T.; Jackson, G. G.; Durack, D. T.; King, D. N. Engl. J. Med., 317: 185 (1987).

6) Lin, T. S.; Shen, Z. Y.; August, E. M.; Brankovan, V.; Yang, H.; Ghazzouli, I. and Prusoff, W. H. J. Med. Chem., 32: 1891 (1989).

7) Simpson, M. V.; Chin, C. D.; Keilbaugh, S. A.; Lin, T. S.; Prusoff, W. H. Biochem Pharmacol., 38: 1033 (1989).

8) Lin, T. S.; Gao, Y. S.; August, E. M.; Qian, H. Y. and Prusoff, W. H. J. Labelled Compounds and Radiopharmaceuticals., 27: 669 (1989).

9) Kobayashi, R.; Yamamoto, K.; Asai, T.; Nakano, N.; Kumadaka, H. J. Chem. Soc. Perkin 1: 2755 (1980).