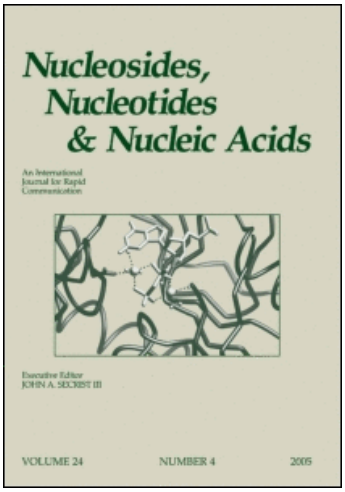

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AN IMPROVED SYNTHESIS OF AZIDOTHYMIDINE

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Abstract: A convenient and high yielding procedure is described for a direct conversion of thymidine (**1**) into 2,3′-anhydrothymidine (**2**) using the Mitsunobu reaction. Isolation and characterization of two new compounds, **3** and **4**, are discussed. AZT has been synthesized from **1** in two steps , in 62% overall yield, by heating **2** with NaN_3 in DMF.

Azidothymidine (**5**, AZT)[1] is the first clinically approved drug for treating acquired immunodeficiency syndrome (AIDS).[2] However, serious side effects such as short plasma half-life[3] and bone marrow suppression[4] were reported on clinical administration. In order to increase its therapeutic efficacy, several prodrugs of AZT were synthesized by conjugating AZT with various compounds such as amino acid,[5,6] heterocyclic acids,[5] retinoic acid,[5] sugars,[7] and lipids.[8] Various steroidal carboxylic ester[9] and phosphotriester[10] conjugates of AZT were synthesized in our laboratory. The demand for large amounts of AZT, to synthesize its steroidal prodrugs, prompted us to establish an efficient and cost-cutting procedure for the synthesis of AZT.

AZT can be synthesized in two steps from thymidine (**1**) by converting **1** into 2,3′-anhydrothymidine (**2**) followed by azidation at the 3′-position of **2** effecting the opening of the anhydride ring (Fig.1). Kowollik *et al* [11] converted **1** into **2** by heating with two equivalents of 2-chloro-1-diethylamino-1,1,2-trifluoroethane at 70°C to give 75% yield. However, this procedure has been reported[12] to give only 40% yield in large scale preparation. Reese *et al* [13] reported a different method to obtain **2** in 65% yield by heating **1** with diphenyl sulfite at 156°C. Although the latter method is preferable to the former, maintenance of the reaction temperature at 156+/-1°C and the difficult work-up limit its application for scaling up the reaction. The use of the Mitsunobu reaction[14] for the convenient intramolecular cyclization[15] of 5′-O-protected thymidines[16-20] to give corresponding 2,3′-anhydro derivatives has led us to study the same reaction on

unprotected thymidine to avoid protection and deprotection of the 5′-OH group of **1**. A similar method on unprotected thymidine was reported by Shibuya *et al* [21] using Ph_3P or Bu_3P and diethyl azodicarboxylate or diphenyl azodicarboxylate to give a 76% yield of **2**. We report here a mild and improved procedure, of the Mitsunobu reaction, for a high yielding conversion of **1** into **2** with a facile separation of **2** from the reaction mixture. Isolation and characterization of two new products, **3** and **4**, which gave more insight into the above Mitsunobu reaction, are also discussed. Compound **2** was converted into AZT by azidation at its 3′-position.

RESULTS AND DISCUSSION

A mixture of **1** (Fig.1) and PPh_3 in acetonitrile was treated with diisopropyl azodicarboxylate (DIAD) at room temperature. After 10 min. the reaction mixture was cooled; the major product was precipitated in chilled ethyl acetate as white, virtually pure, granular solid. The product obtained in 81% yield was identified as **2** by comparing its physical and spectral properties.[22,23] The filtrate was concentrated and separated on silica gel using column chromatography to give an additional 10% of **2** and a mixture of two closely migrating compounds. Further separation of this mixture on silica gel using radial chromatography gave two pure compounds. These two new compounds were characterized as 3′-deoxy-3′β-(N,N′-diisopropyloxycarbonylhydrazino)thymidine (**3**) and 2,3′-anhydro-4′,5′-dehydro-5′-deoxythymidine (**4**) (Scheme 1) based on their spectral data (experimental section).

The proton assignments of **3** and **4** were further confirmed by selective decoupling experiments. The proton assignment of 2′-α and -βH is based on the NMR of 2′-deoxy nucleosides.[24] Irradiation of 1′-H of **4** resulted in the collapse of the *ddd* of 2′-αH to a *dd*. There was no noticeable change in the broad doublet of 2′-βH. This indicated that while the coupling between 1′-αH and 2′-αH is substantial, the coupling between 1′-αH and 2′-βH is much smaller. The same observation also became evident upon irradiating 3′-H. It further demonstrated a poor coupling between 3′-αH and 2′-βH. The identity of 1′-, 2′-, and 3′-protons of **3** was established by their similar decoupling patterns to those of **4**. These results indicate a β-substitution at the 3′-position of **3** in agreement with an S_N2 mechanism of the Mitsunobu reaction. Similar alkylhydrazine-N,N′-dicarboxylates were reported in the literature.[14]

The characterization of the two new products, **3** and **4**, gave more insight of the course of the reaction. The formation of **2**, **3**, and **4** can be explained by the plausible mechanism depicted in Scheme 1. The zwitter ion **A** on reacting with **1** gives the bisoxyphosphonium salt **BC**. Cleavage of oxyphosphonium group at the 3′-position of **B**

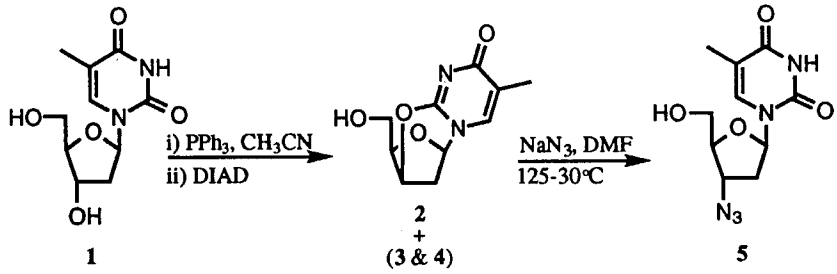
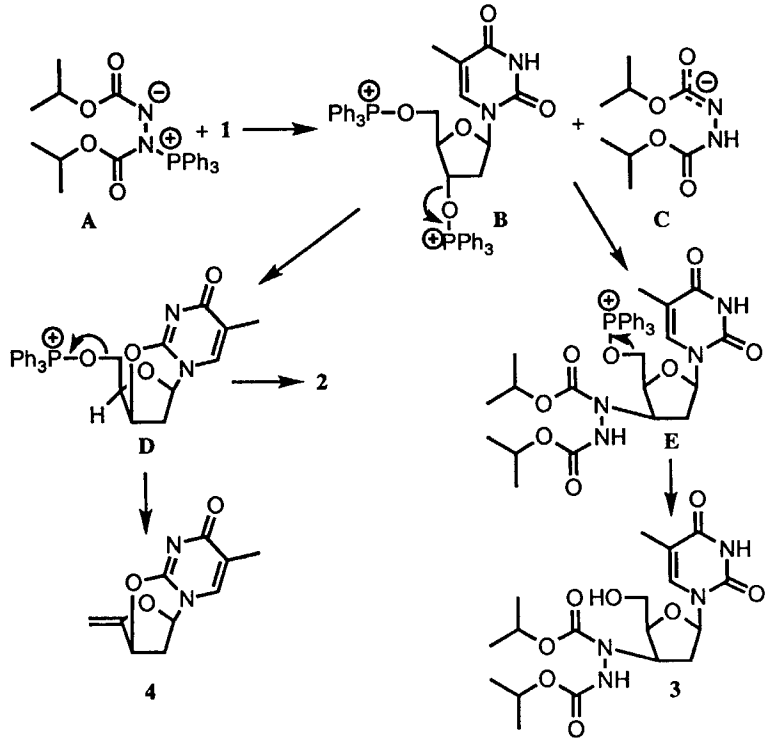


FIGURE 1



SCHEME 1

results in either intramolecular nucleophilic attack by the C2-oxygen to form **D** or an interionic attack by **C** to form **E**. Olefin **4** is formed by an 1,2-elimination[14] from **D**. Nucleophilic substitution at the 5'-position of **D** and **E** by water during work-up gives **2** and **3** respectively.

A careful study of this reaction in different solvents such as CH_2Cl_2, tetrahydrofuran (THF) and N,N'-dimethyl formamide (DMF) did not give better results. Hence, in an effort to improve the yield of **2**, the reaction was studied at reduced temperature. When the reaction was conducted at 0°C **2** was obtained in 88% yield. No detectable amount of the byproduct, **3** or **4**, was obtained from the filtrate. Thus **2** was obtained in the highest yield of all the previous methods reported for the direct conversion of **1** into **2**.[11-13,21]

This procedure was also found to be suitable for a large scale preparation from the following experiment. A 25-fold increase of the amount of reactants did not considerably lower the yield of **2**. On reacting 0.1 mole of **1** under these conditions, **2** was obtained in 85% yield.

Compound **2** was converted into AZT by heating with NaN_3 in DMF. Removal of the solvent followed by purification on silica gel using column chromatography gave AZT. The physical and spectral characteristics of AZT thus obtained are in full agreement with the reported data.[25,26]

In summary, the Mitsunobu reaction on unprotected thymidine and its mechanism were studied by carefully isolating two new products, **3** and **4**. This study has resulted in a high yielding procedure for the direct conversion of **1** into **2**. Using this procedure, AZT has been synthesized in two steps from **1**, in an overall yield of 62%. This procedure is short, simple and offers a superior yield of AZT than the previously reported two step synthesis.[13]

EXPERIMENTAL SECTION

Acetonitrile, DMF of anhydrous grade (Sure/Seal™), DIAD, triphenylphosphine, and thymidine were purchased from Aldrich Chemical Co. THF was freshly distilled from Na/Benzophenone under N_2 atmosphere. Thin layer chromatography (TLC) was performed on precoated silica gel plastic sheets $60F_{254}$ (0.2 mm) EM in a 1:9 mixture of methanol and chloroform. Reagents and compounds were detected under short wavelength UV light and also by heating, after spraying 3% sulfuric acid in ethanol (v/v). Silica gel 60 (70-230 mesh ASTM) EM Science was used for column chromatography. Products were purified on a chromatotron, Harrison Research Ltd., using rotars coated with silica gel

$60GF_{254}$ EM Reagents (1 or 2 mm). Duration of the reactions was monitored by TLC in chloroform-methanol (9:1). All reactions were carried out under N_2 atmosphere.

Melting points (uncorrected) were recorded using a Mel-Temp apparatus. ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker/IBM-SY200 spectrometer at 270 MHz using tetramethylsilane (TMS) as an internal standard. The chemical shifts of NMR spectra were expressed in parts per million (ppm). FAB MS spectra were obtained on a Finnigan MAT95Q using a 3:1 mixture of dithiothreitol and dithioerythritol as matrix with 5% trifluoroacetic acid added.

General Procedure

A mixture of **1** (1g, 4.1 mmol) and two molar equivalents of PPh_3 was dried together by co-evaporating anhydrous THF and suspended in CH_3CN. To this reaction mixture, two molar equivalents of neat DIAD was added slowly with vigorous stirring. The reaction was complete in 10 min. at room temperature and 5h at O°C.The reaction mixture was cooled to -20°C and poured into a chilled (-20°C) solvent, diethyl ether or ethyl acetate, with vigorous stirring. The major product was separated as a precipitate which was filtered, washed with solvent, and dried. The filtrate was concentrated under reduced pressure. More product, obtained by a similar repeated precipitation, was pooled together and purified by crystallization. The final concentrate of the filtrate was initially eluted with CH_2Cl_2 on silica gel using column chromatography to remove unreacted PPh_3, DIAD, triphenylphosphine oxide, and N,N'-diisopropyloxycarbonylhydrazine. Further elution with a 2:98 mixture of methanol and CH_2Cl_2 gave a mixture which showed two closely running compounds on TLC, R_f 0.42 and 0.46. This mixture was separated into two pure components by careful elution with a 2:98 mixture of methanol and chloroform by repeated radial chromatography on chromatotron.

2,3'-Anhydrothymidine **2**. A dried mixture of **1** (1g, 4.1 mmol) and PPh_3 (2.16g, 8.2 mmol) was suspended in 50 mL of acetonitrile and cooled to -15°C in ice-methanol bath. DIAD (1.62 mL, 8.2 mmol) was slowly added during 15 min. with vigorous stirring maintaining the reaction temperature below -5°C. The stirring was continued for 5h at 0°C. The reaction mixture was cooled to -20°C and poured with stirring into ethyl acetate at the same temperature. The precipitate was washed thoroughly with cold ethyl acetate and dried under vacuum on P_2O_5 overnight to give 815 mg of **2**. It melted at 230°C (aq. CH_3OH) (lit.[22] m.p. 230°C).

Similarly, a mixture of **1** (25g, 103 mmol) and finely ground PPh_3 (54g, 206 mmol) was dried with anhydrous THF, suspended in 800 mL of CH_3CN and cooled to -15°C in ice-methanol bath. Ice-cold neat DIAD (41 mL, 206 mmol) was slowly added

with vigorous stirring during 2h maintaining the reaction temperature below -10°C and continued the stirring for another 5h at -10°C. A similar work-up gave 20g of **2**.

3'-Deoxy-3'β-(N,N'-diisopropyloxycarbonylhydrazino)thymidine **3**. It was obtained as pale yellow fluffy semi-solid in 7 and 5% yields when the reaction was conducted at room temperature in DMF and acetonitrile respectively. ^{1}H NMR δ 1.22 (*d*, 6H, *J* = 6.4 Hz, -CH(C<u>H</u>$_3$)$_2$), 1.26 (*d*, 6H, *J* = 6.4 Hz, -CH(C<u>H</u>$_3$)$_2$), 1.94 (*s*, 3H, 5-CH$_3$), 2.48 (br.*d*, 1H, J_{gem} = 11.2 Hz, 2'β-H), 2.68 (*ddd*, 1H, *Jgem* = 12.7 Hz, J_{vic} = 2.9 and 3.4 Hz, 2'α-H), 3.78 (*m*, 2H, 5'-H), 4.52 (*m*, 1H, 4'-H), 4.92 (*h*, 2H, J_{vic} = 6.4 Hz, 2 x C<u>H</u>Me$_2$), 5.29 (*m*, 1H, 3'-H), 5.52 (*d*, 1H, *Jvic* = 3.9 Hz, 1'-H), 6.80 (br. *s*, 1H, N-N'-H), and 6.98 (*s*, 1H, 6-H). FAB MS m/e 411.1847 ($C_{18}H_{27}N_4O_7$ requires 411.1725) (MH^+-H_2O, 100).

2,3'-Anhydro-4',5'-dehydro-5'-deoxythymidine **4**. It was obtained in 5 and 4% yields when the reaction was conducted at room temperature in DMF and acetonitrile respectively. It gave colorless needles from hexane-chloroform, m.p. 180°C. ^{1}H NMR δ 1.90 (*d*, 3H, $J_{6,Me}$ = 1.0 Hz, 5-CH$_3$), 2.45 (*ddd*, 1H, *Jgem* = 12.7 Hz and J_{vic} = 2.9 Hz, 2'α-H), 2.80 (*d*, 1H, *Jgem* = 12.70 Hz, 2'β-H), 4.68 (*d*, 2H, *Jgem* = 2.9 Hz, 2 x 5'-H), 5.28 (*m*, 1H, 3'-H), 5.77 (*d*, 1H, J_{vic} = 3.9 Hz, 1'-H), and 7.04 (*m*, 1H, 6-H). FAB MS m/e 207.0714 ($C_{10}H_{11}N_2O_3$ requires 207.0769) (MH^+, 100).

3'-Azido-3'-deoxythymidine **5**. Compound **2** (3g, 11.2 mmol) was heated with NaN_3 (1.5g, 22.4 mmol) in DMF for 3h. The solvent was removed under reduced pressure. The resulting pale brown residue was chromatographed on silica gel eluting with 1:3 mixture of ethyl acetate and hexane to give **5** (2.53g, 71%) as a white solid, which was crystallized to colorless needles from water, m.p. 120°C (lit.[25] m.p. 119-121°C).

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