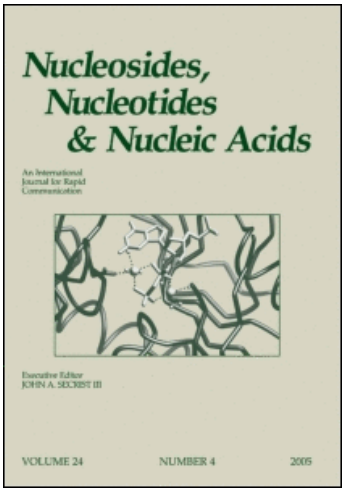

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3-(3-Azido-2,3-Dideoxy-β-D-*erythro* pentofuranosyl)-thymine from 3'-Azido-3'-deoxythymidine (AZT). An Intriguing Rearrangement

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3-(3-AZIDO-2,3-DIDEOXY-ß-D-*erythro*PENTOFURANOSYL)-THYMINE FROM 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT). AN INTRIGUING REARRANGEMENT[1]

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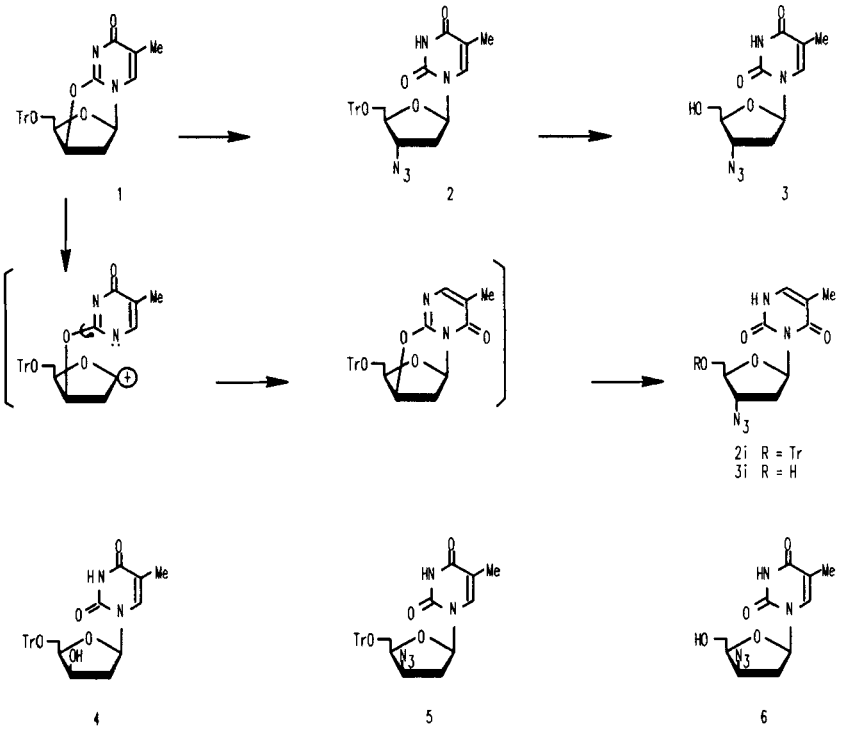
Abstract: During the course of preparation of 3'-azido-3'-deoxythymidine (AZT), we observed consistent formation of an isomer of AZT (2-4%) which was isolated and the structure established as 3-(3-azido-2,3-dideoxy-ß-D-*erythro*pentofuranosyl)thymine. In a more detailed study, this rearrangement was found to occur during the treatment of 2,3'-anhydro-5'-O-tritylthymidine (**1**) with LiN_3 in aqueous DMF.

3'-Azido-3'-deoxythymidine (**3**, AZT) is the only clinical drug currently available for the treatment of acquired immunodeficiency syndrome (AIDS).[2] This nucleoside was originally synthesized in 1964 by Horwitz *et al.*[3], and later by others.[4,5] We had also obtained this compound by direct treatment of 3'-O-mesyl-5'-O-tritylthymidine with NaN_3 in DMF.[6] Subsequently, we followed the Horwitz procedure, since the isolation of the desired product is easier and higher yield is obtained.

5-O-Trityl-2,3'-anhydrothymidine (**1**)[7] was treated with LiN_3 in DMF, and the 3'-azido-3'-deoxy product **2** was purified on a silica gel column. Chromatographically homogeneous **2** was de-O-tritylated in two ways: in *n*-butanol/trifluoroacetic acid (*n*-BuOH:TFA 3:1 v/v) at room temperature, or in aq. 80% HOAc at 55 °C. In the TFA/BuOH detritylation, no glucosyl bond cleavage was apparent. Traces of thymine were detected on TLC after HOAc treatment of **2**. Compound **3** was isolated by column chromatography ($CHCl_3$/EtOH 95:5) followed by crystallization from isopropanol. The mother liquors of

crystallization were found to contain a mixture of two compounds. After fractional crystallization, additional crops of **3** were isolated. The mother liquor which contained two components in approximately equal amounts was subjected to HPLC (C_{18} column with 50% aq. MeOH). The second component was isolated in 2.7% yield overall from **1**. The ^1H NMR, MS, UV, and elemental analyses established this second component to be an isomerr of AZT (**3i**). (See Experimental Section).

Experiments were repeated in order to establish in which step this rearrangement occurred. Compound **1** was purified by



Scheme 1

recrystallization from methanol. No isomeric impurity was found in the mother liquor. Treattment of **1** with LiN_3 in DMF containing a small amount of H_2O gave a mixture from which the 3'-azido products **2** and **2i** (72% yield) and **1-(5-O-trityl-2-deoxy-ß-D-threo-pentofuranosyl)thymine (4)** (5.7% yield from **1**) were obtained.

The 3'-azido products fraction was applied on preparative TLC plates (Et_2O) and **2** and its isomer **2i** separated from **1** in 55% and 4.4% yield, respectively.

Both **2** and **2i** were de-O-tritylated separately, using n-BuOH/TFA or 80% aq. HOAc, to give the corresponding nucleosides **3** and **3i**. No evidence of isomerization was obtained. We conclude, therefore, that isomerization occurred during the LiN_3 treatment of **1**, and the isomer is not an O-gycosylated derivative which is sensitive to acid and should be hydrolyzed during detritylation. The ^1H NMR spectrum of **3i** is totally different from that of the alpha-AZT.[8-10]

The structure of **3i** was established as 3-(3-azido-2,3-dideoxy-ß-D-erythropentofuranosyl)thymine by UV, IR, ^1H NMR and mass spectrometric analyses. The large basochromic shift (Δ 29.5 nm) in base (λmax at 269.0 in acid and neutral to λmax at 298.5 in base) clearly indicates that the glycosyl moiety in **3i** attaches at N3 rather than N1 of the thymine ring.[11] The presence of the N_3 function is demonstrated by the strong IR absorption band at 2100 cm^{-1}. The ^1H NMR spectrum of **3i** shows that the presence of on primary OH group (exchangeable triplet at δ 4.84) establishing the furanosyl structure of the glycosyl moiety. Thus, **3i** has one of the four possible isomeric structures: anomers of 3-(3-azido-2,3-dideoxy-D-erythropentofuranosyl)thymine and 3-(3-azido-2,3-dideoxy-D-threo-pentofuranosyl)thymine. The threo structure can be ruled out by ^1H NMR analyses: Except for the shape of the anomeric signal (quartet at 6.53) and the chemical shift of H2' (δ 2.81), overall ^1H NMR pattern is very similar to that of AZT and quite different than that of 1-(3-azido-2,3-dideoxy-ß-D-threopentofuranosyl)thymine,[6] the "up" azido isomer of AZT. (See Table 1). In the beta-erythro structure **3i**, the chemical shift of H2' should show large paramagnetic shift due to close proximity to the carbonyl group at C2 of thymine, whereas in the alpha isomer, H2" should shift to lower field. In the beta structure, H2' should couple strongly with H3', and H2" should couple strongly with H1'. On the other hand, in the alpha anomer H2' should couple strong with both H1' and H3', but H2" should only weakly couple with both H1' and H3'. The presence of at least one large coupling for both H2' ($J_{1',2'}$ = 4.67 and $J_{2',3'}$ = 8.51 Hz) and H2"

($J_{1',2''}$ = 8.78 and $J_{2'',3'}$ = 6.59 Hz) (Table 1), thus, established the beta-erythro structure for **3i**.

A plausible mechanism for the formation of **3i** would be cleavage of the glycosyl linkage in **1** followed by glycosylation at N3 prior to nucleophilic attack of azide ion at C3′ from the alpha side (Scheme 1). Although N1 to N3 glycosyl rearrangement in anhydronucleosides has been reported to occur in very acidic conditions,[12,13] our finding that similar rearrangement takes place under weakly basic conditions should be intriguing. It should also be noted that Cook et al.[14] reported the formation of 3-(2,3,5-trideoxy-3,5-diiodo-ß-D-erythropentofuranosyl)-5-fluorouracil via 2,3′-anhydro-3-(2,5-dideoxy-5-iodo-ß-D-threopentofuranosyl)-5-fluorouracil upon treatment of 2′-deoxy-5-fluorouridine with excess methyltriphenoxyphosphonium iodide.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. ^{1}H NMR spectra were recorded on a JEOL 90QX using Me_4Si as the internal standard and Me_2SO-d_6 as solvent. Preparative separation was effected on 20 x 20 cm, 1 mm silica gel GF plates purchased from Analtech, Newark, NJ. IR spectra were measured on a Perkin-Elmer Infracord Spectrometer. Analytical separations by HPLC were carried out on a u-Bondepack™ C_{18} column (flow rate 1 mL/min, 30% aq. MeOH). Preparative HPLC separations were achieved on Dynamax Macro HPLC C_{18} column (flow rate 5 mL/min, 30% aq. MeOH). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Reaction of 2,3'-Anhydrothymidine (1) with LiN_3.

A mixture of **1**[5] (1.864 g, 4 mmol), LiN_3 (706 mg, 14.4 mmol) in DMF (18 mL) and H_2O (2.5 mL) was stirred until an homogeneous solution was obtained. The solution was gently heated under reflux for 11 h when only traces of **1** were detected by TLC. The solution was concentrated in vacuo to a small volume, and then poured into H_2O (1 L). The precipitates were collected, air-dried, redissolved in a small volume of $CHCl_3$ and chromatographed on a silica gel column,

Table 1. 90-MHz ^1H NMR Spectral Data in Me_2SO-d_6

	Chemical Shifts of the Protons in ppm (δ) from Me_4Si[a]										
Compd	NH	Trityl	H6	H1′	OH	H3′	H4′	H5′,5″	H2′	H2″	Me	Coupling const. (Hz)
2	11.37 (s,1)	7.53-7.26 (m,16)		6.13 (t,1)		4.59 (m,1)	3.87 (m,1)	3.24 (m,2)	2.52-2.32 (m,2)		1.56 (s,3)	$J_{1',2'}$ = 6.31 $J_{1',2''}$ = 6.31
2i	10.91 (s,1)	7.45-7.23 (m,16)		6.59 (dd,1)		4.45 (m,1)	3.88 (m,1)	3.22 (m,2)	2.84 (m,1)	2.25 (m,1)	1.74 (s,3)	$J_{1',2'}$ = 3.57 $J_{1',2''}$ = 9.19
4	11.27 (s,1)	8.31-7.25 (m,16)		6.11 (dd,1)	5.20 (d,1)	4.13 (m,2)		3.84-3.22 (m,2)	2.65-2.46 (m,2)		1.65 (s,3)	$J_{1',2'}$ = 0.05 $J_{1',2''}$ = 6.03
5	11.34 (s,1)	7.45-7.27 (m,16)		6.07 (dd,1)		4.58 (m,1)	4.28 (m,1)	3.32-3.25 (m,2)	2.88-1.90 (m,2)		1.66 (s,3)	$J_{1',2'}$ = 3.46 $J_{1',2''}$ = 7.87
3	11.30 (s,1)		7.67 (s,1)	6.09 (dd,1)	5.21 (t,1)	4.40 (m,1)	3.89-3.75 (m,1)	3.67-3.42 (m,2)	2.31 (m,2)		1.78 (s,3)	$J_{1',2'}$ = 6.6 $J_{1',2''}$ = 6.3
3i	10.90 (s,1)		7.30 (s,1)	6.53 (dd,1)	4.84 (t,1)	4.39 (m,1)	3.80-3.69 (m,1)	3.60-3.42 (m,2)	2.81 (ddd,1)	2.15 (ddd,1)	1.75 (s,3)	$J_{1',2'}$ = 4.67 $J_{2',2''}$ = 13.37 $J_{1',2''}$ = 8.78 $J_{2',3'}$ = 8.51 $J_{2'',3'}$ = 6.59
6			7.48 (s,1)	6.04 (dd,1)		4.47 (m,1)	4.01 (m,1)	3.73-3.66 (m,2)	2.88-1.93 (m,2)		1.79 (s,1)	$J_{1',2'}$ = 3.72 $J_{1',2''}$ = 7.85

[a] Signals are designated as: s, singlet; d, doublet; t, triplet; dd, double doublet; ddd, double double doublet; m, multiplet. Following the observed multiplicities are the numbers of protons as estimated by integration.

which was washed successively with $CHCl_3$, $CHCl_3$-EtOH (100 µL v/v), and $CHCl_3$-EtOH (50:1 v/v). A mixture of **2** and its isomer **2i** (total 1.460 g, 72%) was eluted first followed by **1-(2-deoxy-5-O-trityl-ß-D-threopentofuranosyl)thymine (4)** (0.110g, 5.7%) which was crystallized from MeOH, mp 239-240 °C (lit.[15] mp 240-241 °C). MS (CI) m/z 483 (M - H). ^{1}H NMR δ 11.27 (s, 1H, NH exchangeable), 8.31-7.25 (m, 16H, Tr, H6), 6.11 (dd, 1H, H1', $J_{1',2'}$ = 6.03, $J_{1',2''}$ = 0.05 Hz), 5.20 (d, 1H, 3'-OH, exchangeable), 4.13 (m, 2H, H3',4'), 3.84-3.22 (m, 2H, H5',5"), 2.65-2.46 (m, 2H, H2',2"), 1.65 (s, 3H, 5-Me).

The mixture of **2** and **2i** (130 mg) was dissolved in a small amount of $CHCl_3$ and applied on 3 preparative silica gel plates, which were developed twice with Et_2O. Compound **2** (100 mg) was obtained from the upper UV abosorbing band after extraction with $CHCl_3$-EtOH (19:1 v/v), and **2i** (8.0 mg) from the lower band. A larger amount of the mixture (775 mg) was dissolved in Et_2O and applied on a silica gel column (25 x 2 cm), which was washed with Et_2O. Pure AZT **2** (400 mg) was eluted first, followed by an isomeric mixture (239 mg). The **2i**-rich fraction (134 mg) was applied on silica gel plates, and the anomers were separated: pure **2** (87 mg) and **2i** (45 mg) were obtained.

Compound **2**: MS (CI) m/z 508 (M - H), IR (KBr) 2,100 cm^{-1} (N_3), ^{1}H NMR δ 11.37 (s, 1H, NH, exchangeable), 7.53-7.26 (m, 16H, Tr, H6), 6.13 (t, 1H, H1', $J_{1',2'} = J_{1',2''}$ = 6.31 Hz), 4.59 (m, 1H, H3'), 3.87 (m, 1H, H4'), 3.24 (m, 2H, H5',5"), 2.52-2.32 (m, 2H, H2',2"), 1.56 (s, 3H, 5-Me).

Anal Calcd. for $C_{29}H_{27}N_5O_4$.1/4 EtOH: C, 67.99, H, 5.51, N, 13.44. Found: C, 68.43, H, 5.74, N, 13.59. The content of EtOH in the analytical sample was also determined by ^{1}H NMR.

Compound **2i**: MS (CI) m/z 508 (M - H), IR (KBr) 2,100 cm^{-1} (N_3), ^{1}H NMR δ 10.91 (s, 1H, NH, exchangeable), 7.45-7.23 (m, 16H, Tr, H6), 6.59 (dd, 1H, H1', $J_{1',2'}$ = 9.15, $J_{1',2''}$ = 3.57 Hz), 4.45 (m, 1H, H3'), 3.88 (m, 1H, H4'), 3.22 (m, 2H, H5',5"), 2.84 (m, 1H, H2'), 2.25 (m, 1H, H2"), 1.74 (s, 3H, 5-Me).

Anal Calcd. for $C_{29}H_{27}N_5O_4$.1/4 EtOH: C, 67.99, H, 5.51, N, 13.44. Found: C, 67.72, H, 5.34, N, 11.87. This compound resisted crystallization, and was used directly without further purification.

De-O-tritylation of 2 with nBuOH/TFA

Compound **2** (93 mg, 0.18 mmol) was dissolved in a mixture of nBuOH and TFA (3:1 v/v, 40 mL). After 1 h at room temperature, the solution was diluted with nBuOH (80 mL), and then concentrated in vacuo. The residue was coevaporated with toluene (3 x 50 mL), and partitioned between Et_2O (30 mL) and H_2O (30 mL). The aqueous layer was washed with Et_2O (3 x 10 mL). After concentration of the aqueous layer, the residue (no traces of **3i** were detected by HPLC) was purified by chromatography on a silica gel column ($CHCl_3$-EtOH, 49:1 v/v) to give 42 mg of **3** (88%), mp 121-122 °C (from iPrOH) (lit.[9] mp 122-123 °C). MS (CI) m/z 266 (M - H), IR (KBr) 2,100 cm^{-1} (N_3), UV $\lambda max(H_2O-MeOH)$ 263 nm. ^1H NMR δ 11.30 (s, 1H, NH, exchangeable), 7.67 (s, 1H, H6), 6.09 (dd, 1H, H1', $J_{1',2'}$ = 6.6, $J_{1',2''}$ = 6.3 Hz), 5.21 (t, 1H, 5'-OH, exchangeable), 4.40 (m, 1H, H3'), 3.89-3.75 (m, 1H, H4'), 3.67-3.42 (m, 2H, H5',5"), 2.31 (m, 2H, H2',2"), 1.78 (s, 3H, 5-Me). ^{13}C NMR 12.20 (Me), 36.20 (C2'), 60.14 (C3'), 60.79 (C5'), 83.39 (C1' or C4'), 83.98 (C4' or C1'), 109.50 (C5), 135.99 (C6), 150.40 (C2 or C4), 163.67 (C4 or C2).

Anal Calcd for $C_{10}H_{13}N_5O_4$: C, 44.94, H, 4.90, N, 26.21. Found: C, 44.89, H, 4.97, N, 26.12.

In a similar manner, **2i** gave **3i**, mp 104-106 °C (from 2-butanone), MS (CI) m/z 266 (M - H), IR (KBr) 2,100 cm^{-1} (N_3), UV λmax(1N HCl) 269.0nm (ϵ 7,840), $\lambda max(H_2O-MeOH)$ 268.5 (7,800), λmax(1N NaOH) 298.5 (8,130). ^1H NMR δ 10.90 (s, 1H, NH, exchangeable), 7.30 (s, 1H, H6), 6.53 (dd, 1H, H1', $J_{1',2'}$ = 4.67, $J_{1',2''}$ = 8.78 Hz), 4.84 (t, 1H, 5'-OH, exchangeable), 4.39 (m, 1H, H3'), 3.76-3.42 (m, 3H, H4',5',5''), 2.81 (ddd, 1H, H2', $J_{2',2''}$ = 13.37, $J_{1',2'}$ = 4.67, $J_{2',3'}$ = 8.51 Hz), 2.15 (ddd, 1H, H2'', $J_{2',2''}$ = 13.37, $J_{1',2''}$ = 8.78, $J_{2'',3'}$ = 6.59 Hz), 1.75 (s, 3H, 5-Me). ^{13}C NMR δ 12.25 (Me), 34.20 (C2'), 61.77 (C3'), 61.93 (C5'), 80.51 (C1'), 84.36 (C4'), 107.55 (C5), 136.91 (C6), 150.67 (C2 or C4), 163.62 (C4 or C2).

Anal Calcd. for $C_{10}H_{13}N_5O_4.1/8\ C_4H_8O$ (2-butanone): C, 45.65, H, 5.11. N, 25.35. Found: C, 45.80, H, 5.27, N, 25.63. The presence of a small amount of 2-butanone in the analytical sample was detected by ^1H NMR.

De-O-tritylation of 2 with 80% Aq. HOAc.

A mixture of **2** (146 mg, 0.28 mmol) in 80% aq. HOAc (3 mL) was stirred at 55 °C for 1 h (traces of thymine were detected on TLC), and then concentrated in vacuo. The residue was partitioned between Et_2O and H_2O. From the aqueous layer (no traces of **3i** were detected), **3** (63 mg, 86%) was obtained after chromatographic purification and crystallization from iPrOH, mp 121-122 °C.

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