SYNTHESIS OF 2',3'-DIDEHYDRO-3'-DEOXYTHYMIDINE AND ITS ACTIVITY AGAINST HIV

G. A. Tolstikov, A. G. Mustafin,
R. R. Gataullin, I. B. Abdrakhmanov,
A. G. Plyasunova, and A. G. Pokrovskii

UDC 547.963.32

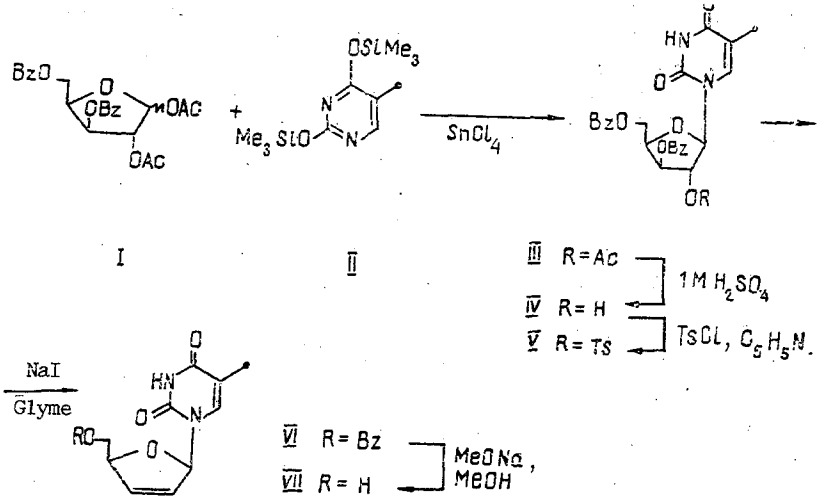
2,3-Didehydro-3'-deoxythymidine has been obtained with an overall yield of 10% by the condensation of 1,2-di-O-acetyl-3,5-di-O-benzoyl-D-xylofuranose with bis-(dimethylsilyl)thymine and a series of subsequent transformations. The anti-HIV activity of the compound obtained was studied on the model of MT-4 lymphoid cells primarily infected with HIV-1. It was found that the substance effectively inhibits the reproduction of HIV-1 in a cell culture.

Some 2',3'-dideoxynucleosides, and also their 2',3'-unsaturated analogues, are promising anti-HIV drugs and are undergoing the stage of clinical trials at the present time [1, 2].

Among the substances of this group, particular interest is presented by D4T (2',3'-didehydro-3'-deoxythymidine), the metabolism of which in the cell differs substantially from the metabolism of azidothymidine (AZT). AZT readily passes into the monophosphate form but inhibits thymidylate kinase, which is necessary for subsequent phosphorylation [3]. In contrast to this, D4T is phosphorylated to the monophosphate considerably less well but, since it does not inhibit thymidylate kinase, the subsequent phosphorylation stages take place considerably more effectively than for AZT [3].

It is assumed that the absence of an accumulation of D4T in the form of the monophosphate makes this substance preferable to AZT [4].

In the present communication we describe the synthesis and anti-HIV activity of 2,3'-didehydro-3'-deoxythymidine (VII). The condensation of 1,2-di-O-acetyl-3,5-di-O-benzoyl-D-xylofuranose (I) [5] with bis(trimethylsilyl)thymine (II) in acetonitrile solution in the presence of $SnCl_4$ gave 2'-O-acetyl-3',5'-di-O-benzoyl-β-D-xylofuranosylthymine (III) with a yield of 73%. The treatment of compound (III) with a 1 M solution of H_2SO_4 led to nucleoside (IV), the tosylation of which gave substance (V). Boiling the tosylate (V) with NaI led to the unsaturated 2'-enofuranoside (VI) with a yield of 30%, and the treatment of this with MeONa in methanol gave compound (VII) with a yield of 85%.



Institute of Organic Chemistry, Ural Branch, Russian Academy of Sciences, Ufa. Vector Scientific-Production Association, Kil'tsovo, Novosibirsk Province. Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 133-136, January-February, 1993. Original article submitted February 20, 1992.

0009-3130/93/2901-0113 $12.50

TABLE 1. Influence of D4T on the Reproduction of HIV-I in a Cell Culture

Name of the substance	Concentration of the substance, μg/ml	Number of live cells, %	Inhibition, %, of	
			activity of the viral reverse transcriptase	accumulation of viral antigen
Thymidine	0.05	81.72±12.11	98.40±0.20	82.33±2.86
D4T	10.00	69.35± 2.80	97.04±0.66	62.00±2.52
"	5.0	83.78± 8.27	98.16±1.17	79.20±2.78
"	1.0	88.64±15.28	97.79±2.26	69.10±5.35
"	0.1	77.63±15.05	66.01±7.38	-
"	0.05	65.71± 2.10	37.25±1.12	-
Control (uninfected MT-4 cells)	-	90.83± 6.33	-	-
Control (MT-4 cells infected with HIV)	-	34.26± 2.45	0	0

The antiviral activity of 2',3'-didehydro-3'-deoxythymidine (VII) was studied on the model of MT-4 lymphoid cells primarily infected with HIV-1, and it was established that D4T is characterized by high values of the selectivity index and is less toxic for the cells than AZT. The results that we obtained (Table 1) agree well with those of E. De Clercq [3].

Thus, the facts presented show the possibility of synthesizing the promising anti-HIV drug D4T from the readily accessible 1-β-D-xylofuranosylthymine.

EXPERIMENTAL

UV absorption spectra were taken on a Shimadzu UV 365 instrument. ^1H and ^{13}C NMR spectra were taken on a Bruker AM-300 instrument with working frequencies of 300 and 75 MHz. Values of δ (ppm) relative to tetramethylsilane and spin-spin coupling constants (Hz) are given. Specific angles of rotation were determined with the aid of a Perkin-Elmer 241 MC polarimeter. Column chromatography was conducted on silica gel LS 40/100 (Czechoslovakia).

1-(2-O-Acetyl-3,5-di-O-benzoyl-β-D-xylofuranosyl)thymine (III). A solution of 8.48 g (31.4 mmole) of bis(trimethylsilyl)thymine (II) and 13.2 g (29.8 mmole) of 1,2-di-O-acetyl-3,5-do-O-benzoyl-D-xylofuranose in 150 ml of acetonitrile was treated with 4.5 ml of $SnCl_4$, and the reaction mixture was left at room temperature for 4 h. Then it was treated with 40 g of dry $NaHCO_3$, and 15 ml of water was added dropwise with vigorous stirring. Stirring was continued for another 30 min, and the precipitate was filtered off and washed with chloroform (2 × 100 ml). The combined filtrates were washed with water, with saturated $NaHCO_3$ solution and with water again and were filtered through a thin layer of silica gel. The silica gel was washed with chloroform (2 × 20 ml) and the combined filtrates were evaporated under reduced pressure, giving the analytically pure substance (III) in the form of a foam, which was used in the subsequent reaction without further purification. Yield 10.98 g (73%).

1-(3,5-Di-O-benzoyl-β-D-xylofuranosyl)thymine (IV). A mixture of 5.08 g (10 mmole) of compound (III), 35 ml of acetonitrile, and 5.5 ml of 1 M aqueous H_2SO_4 was heated at 75°C for 18 h. After cooling to room temperature, the reaction mixture was neutralized with solid $NaHCO_3$ and was evaporated under reduced pressure (40°C) to a syrupy state. The residue was treated with water and was extracted with ethyl acetate. The organic layer was washed with 5% $NaHCO_3$ solution and with water. After drying over Na_2SO_4, the solvent was evaporated off under reduced pressure (40°C) to dryness. The residue was dissolved in 15 ml of chloroform and a few drops of water were added. The product crystallized in the form of an amorphous powder containing 1 molecule of $CHCl_3$ and one molecule of H_2O, which were readily eliminated on heating (35°C) in vacuum. Yield 3.26 g (70%).

1-(2-O-Tosyl-3,5-di-O-benzoyl-β-D-xylofuranosyl)thymine (V). p-Toluenesulfonyl chloride (8 g; mmole) was added in three portions to a solution of 4.66 g (10 mmole) of compound (IV) in 50 ml of dry pyridine. After 24 h, the reaction mixture was diluted at 0°C with a 50% aqueous solution of pyridine and was extracted with methylene chloride (3 × 50 ml); the combined extracts were washed with $NaHCO_3$ solution and with water, and they were dried

with Na_2SO_4 and evaporated under reduced pressure (32°C). The residue was chromatographed (chloroform) giving 4.72 g (76.2%) of the tosylate (V) in the form of amorphous crystals, $[\alpha]_D^{20}$ + 24° (c 2.5; $CHCl_3$). ^1H NMR spectrum ($CDCl_3$): 170 (3H, s, CH_3), 2.35 (3H, s,CH_3), 4.61 (1H, dd, J_{gem} = 12.35, $J_{5'a,4'}$ = 5.36, H-5'a), 4.67 (1H, dd, $J_{5'b,4'}$ = 4.07, H-5'b), 6.12 (1H, d, $J_{1',2'}$ = 3.95, H-1'), 5.31 (1H, dd, $J_{2',3'}$ = 3.70, H-2'), 5.85 (1H, dd, $J_{3',4'}$= 5.00, H-3'), 4.82 (1H, q, H-4'), 7.10 = 8.00 (14H, m, PhH), 7.44 (1H, s, H-6), 9.17 (1H, q, NH). ^{13}C NMR spectrum ($CDCl_3$): 87.16 (C-1'), 82.83 (C-2'), 74.60 (C-3'), 77.93 (C-4') 61.80 (C-5'), 149.94 (C-2), 163.26 (C-4), 111.58 (C-5), 134.70 (C-6), 12.19 (C-5''), 164.75 (C=O), 165.94 (C=O), 128.13; 130.09, 132.69; 133.50; 134.09; 149.89 (aromatic C), 21.65 (Ph−CH_3). Found %: C 61.00, H 4.44, N 4.07, S 5.12. $C_{31}H_{28}N_2O_{10}S$. Calculated %: C 60.00, H 4.52, N 4.51, S 5.16.

5'-O Benzoyl-2',3'-didehydro-3'-deoxythymidine (VI). A mixture of 2.48 g (4 mmole) of the tosylate (V), 3.1 g (20 mmole) of NaI, and 30 ml of 1,2-dimethoxyethane was boiled for 38 h. Then it was evaporated, the residue was washed with water and CH_2Cl_2 (3 × 60 ml), and the combined extracts were washed with water, with 5% $Na_2S_2O_3$ solution, and with water again, and were dried with Na_2SO_4. The solvent was evaporated off under reduced pressure and the residue was chromatographed (chloroform). This gave a 0.4 g (30%) of product (VI), mp 156-158°C, $[\alpha]_D^{26}$ − 98° (c 1; $CHCl_3$). ^1H NMR spectrum ($CDCl_3$): 1.52 (3H, d, J = 1.15, CH_3), 7.00 (1H, ddd, $J_{1',2}$ = 3.80, $J_{1',3'}$ = 1.60, H-1'), 5.95 (1H, de, $J_{2'3'}$ = 6.00, $J_{2'4}$, = 2.20, H-2'), 6.41 (1H, dt $J_{3'4'}$ = 1.75, H-3'), 5.16 (1H, m,H-4'), 4.59 (1H, dd, $J_{5'a,4'}$ = 3.06, J_{gem} = 12.40, H-5a', 4.62 (1H, dd, $J_{5'b,4'}$ = 3.75, H-5'b), 7.08 (1H, q, J = 1.15, H-6), 7.40-8.05 (5H, m, PhH), 8.90 (1H, s, NH). ^{13}C NMR spectrum ($CDCl_3$): 89.96 (C-1'), 127.46 (C-2'), 133.33 (C-3'), 84.55 (C-4'), 65.11 (C-5'), 150.81 (C-2), 163.70 (C-4), 111.36 (C-5), 135.15 (C-6), 12.03 (C-5''), 166.29 (C=O), 128.72; 129.70; 135.68 (Ph). Found %: C 62.33, H 4.48, N 8.03. $C_{17}H_{16}N_2O_5$. Calculated %: C 62.19, H 4.88, N 8.54.

2',3'-Didehydro-3'-deoxythymidine (VII). A mixture of 0.3 g (0.91 mole) of compound (VI), 2.39 mmole of sodium methanolate, and 9 ml of methanol was stirred for 1 h and was then neutralized with KU-2 cation-exchange resin which was then filtered off and washed with CH_3OH. The combined filtrate was evaporated to dryness and the residue was chromatographed with the eluent $CH_2Cl_2(CH_3OH)-NH_4OH$ (conc.) (90(10):1). This gave 0.174 g (85%) of compound (VII), mp 164-166°C (lit. 165-166°C) [2]. The purity of the substance was not less than 99%.

In the study of its anti-HIV activity, the substance D4T in Hanks' solution (100 μg/ml-1 μg/ml was added to a suspension of lymphoid cells infected with HIV-1 to a final concentration of the substance of 0.01-10.0 mg/ml. The inhibition of the reproduction of the HIV-1 in the cells was judged from the fall in the activity of reverse transcriptase and the fall in the accumulation of the viral antigen (by the method of enzyme-mediated immunoassay) in the culture liquid on the fourth day of cultivation as compared with a control (without the addition of the substance).

For comparison we used one of the most effective drugs for the treatment of AIDS — azidothymidine (AZT). Table 1 gives the results of the experiments.

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