# Synthesis of Novel Pyrimidine Nucleoside Analogues



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Two new tetrazolopyrimidinedeoxynucleosides were synthesized and their physico-chemical data are described. Results of preliminary analyses of their biological properties are also reported.

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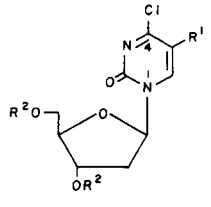
In recent years considerable efforts have been directed toward the synthesis of analogues of nucleosides, not naturally occurring, in view of their potential antimetobolite activity, as possible therapeutic agents [1-3]. In fact, among others, two analogues of deoxycytidine, arabinosylcytidine and 5-azadeoxycytidine are now used in the treatment of leukemic patients [4].

Nucleoside analogues exhibit their action either by interfering with enzymes of nucleotide metabolism or, being incorporated into DNA; therefore they have been of relevent interest in the study of the mechanism of enzyme action and cellular differentiation [5].

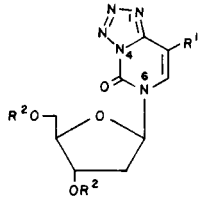
In a previous paper [6], the authors had described the synthesis of a chlorine derivative **1** of thymidine which was shown to be a good starting product for different substitutions in position 4 of the pyrimidine ring.

The present paper reports expoitation of the versatility of **1** in gaining the 6-(2-deoxy-β-D-ribosyl)-8-methyltetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one (**3**) in consideration of the well-known biological activities of tetrazole derivatives [7].

Furthermore, we also synthesized the deoxyuridine analogue of **1** [4], from which it was possible to obtain good yields of compound **5** and **6**. Compound **1** readily obtained in good yield from 3',5'-diacetylthymidine [6], was treated with excess sodium azide in anhydrous dimethylformamide, thus producing, as expected, through an azidoazomethine-tetrazole isomerism, compound **2** $[\alpha]_D$ − 12.1, mp = 114-115°.



1, 4



2, 3, 5, 6

| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | $CH_3$ | $COCH_3$ |
| 2 | $CH_3$ | $COCH_3$ |
| 3 | $CH_3$ | H |
| 4 | H | $COCH_3$ |
| 5 | H | $COCH_3$ |
| 6 | H | H |

Structure **2** was confirmed by ms and ir (experimental), $^1H$ and $^{13}C$ nmr (Tables 1 and 2) spectra and uv absorptions [λ max (chloroform) = 252 nm (ε = 8500), 274 nm (s)] which matched those of 8-methyltetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one [8].

Hydrolysis of the acetyl groups of **2** with diluted hydrochloric acid afforded derivative **3** as white crystals, $[\alpha]_D$ = 31.4, mp = 143-144°.

Synthesis of 1-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribosyl)-4-chloropyrimidin-2(1*H*)-one (**4**), $[\alpha]_D$ = 52.2, was carried out starting from 3',5'-diacetyl-2'-deoxyuridine by reaction with thionyl chloride and dimethylformamide in anhydrous chloroform under reflux for 1.5 hours; $^1H$ and $^{13}C$ nmr (see Tables 1, 2); uv spectra and elemental analyses

Table 1

$^1H$ NMR (250 MHz) Chemical Shifts of Compounds **2**, **3**, **4**, **5**, **6** [a]

| | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|
| H-5 | | | 6.41 d | | |
| H-6 | | | 7.99 d | | |
| H-7 | 7.45 bs | 8.11 bs | | 7.77 d | 8.33 d |
| H-8 | | | | 6.90 d | 7.01 d |
| $CH_3$ | 2.42 bs | 2.41 bs | | | |
| H-1' | 6.50 dd | 6.53 dd | 6.11 dd | 6.42 dd | 6.53 dd |
| | a 2.68 m | | a 2.86 m | a 2.69 m | |
| $H_2$-2' | | 2.45 m | | | 2.45 m |
| | b 2.33 m | | b 2.01 m | b 2.30 m | |
| H-3' | 5.26 m | 4.50 m | 5.18 m | 5.22 m | 4.50 m |
| H-4' | 4.35 m | 4.04 m | 4.34 m | 4.35 m | 4.06 m |
| $H_2$-5' | 4.41 m | 3.87 m | 4.41 m | 4.42 m | 3.85 m |
| | 2.14 s | | 2.08 s | 2.09 s | |
| 2 $CH_3CO$ | 2.14 s | | 2.03 s | 2.05 s | |

[a] All chemical shift values are given in δ (ppm) relative to TMS, s = singlet, d = doublet, dd = double doublet, m = multiplet, b = broad. The spectra were carried out in deuteriochloroform for compounds **2**, **4**, **5** and perdeuteriomethanol for compounds **3** and **6**.

were consistent with structure **4**, which was further confirmed by its conversion into 2'-deoxycytidine by reaction with methanolic ammonia.

Synthesis of 6-(2-deoxy-3,5-di-*O*-acetyl-β-D-ribosyl)tetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one (**5**), $[\alpha]_D$ = 28.4, mp = 125-126° and of its deacetylated analogue **6**, $[\alpha]_D$ = 60.6, mp 154-156°, were performed in the same way as those of **2** and **3**, respectively.

Structures **5** and **6** were assigned on the basis of elemental analyses and their spectral data by comparison with those of the analogue thymidine derivatives.

Preliminary analysis of the biological activity of compounds **2** and **3** was made by determining their cytotoxic effect of murine L-1210 leukemic cells and on human HL-60 myeloid leukemic cells in culture. These compounds, at a concentration of 10 μg/ml, showed a weak growth inhibitory effect (~ 10%) on both types of cells.

Table 2

$^{13}C$ NMR (62.9 MHz) Chemical Shifts of Compounds **2**, **3**, **4**, **5**, **6** [a]

| | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|
| C-2 | | | 166.8 s | | |
| C-4 | | | 152.9 s | | |
| C-5 | 151.4 s | 153.1 s | 105.1 d | 150.7 s | 152.7 s |
| C-6 | | | 142.8 d | | |
| C-7 | 129.6 d | 133.1 d | | 133.5 d | 137.0 d |
| C-8 | 104.5 s | 105.5 s | | 94.2 d | 94.1 d |
| C-9 | 142.2 s | 144.3 s | | 142.4 s | 144.5 s |
| $CH_3$ | 13.0 q | 12.8 q | | | |
| C-1' | 86.7 d | 88.2 d | 88.0 d | 87.0 d | 89.8 d |
| C-2' | 38.1 t | 40.2 t | 39.0 t | 38.3 t | 42.0 t |
| C-3' | 83.1 d | 87.9 d | 83.4 d | 83.2 d | 88.6 d |
| C-4' | 73.8 d | 71.1 d | 73.9 d | 73.8 d | 72.0 d |
| C-5' | 63.4 t | 62.0 t | 63.5 t | 63.5 t | 62.6 t |
| | 20.6 q | | 20.7 q | 20.7 q | |
| 2 $CH_3CO$ | 20.6 q | | 20.7 q | 20.7 q | |
| 2 $CH_3CO$ | 170.3 s | | 170.1 s | 170.1 s | |
| | 170.1 s | | 170.1 s | 170.1 s | |

[a] All chemical shift values are given in δ (ppm) relative to TMS, s = singlet, d = doublet, t = triplet, q = quartet in the off resonance spectra. The spectra were carried out in deuteriochlorofom for compounds **2**, **4**,**5**, in deuterium/oxide for compound **3** and in perdeuteriomethanol for compound **6**.

## EXPERIMENTAL

All the reagents for the synthesis are commercially available (Merck). The $^1H$ and $^{13}C$ nmr Fourier-transform spectra were recorded with a Bruker WM-250. The ir spectra were recorded with a Perkin-Elmer 390 spectrometer. The uv spectra were taken on a Perkin-Elmer 550 S spectrophotometer. The ms were taken on a Kratos MS 50 instrument.

6-(2-Deoxy-3,5-di-*O*-acetyl-β-D-ribosyl)-8-methyltetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one (**2**).

Compound **1** (300 mg) was treated in anhydrous dimethylformamide with excess sodium azide at room temperature for 1 hour. The mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness *in vacuo* and the residue was chromatographed on tlc (silica gel, eluent chloroform/methanol 95:5); the band Rf 0.43 (uv light), eluted with chloroform/methanol, 9:1, afforded 275 mg of **2** (90% yield). Recrystallization from methanol gave an analytically pure sample, mp = 114-115°; $[\alpha]_D$ = 12.1 (c = 1 in chloroform); λ max = 252 nm (ε = 8500), 274 nm (s); ms gave significant ions at m/z 352 ($MH^+$), 232 ($MH^+$-2 $CH_3COOH$), 201 (diacetylated sugar moiety), 152 (base moiety + 2H); ν max chloroform = 1740 $cm^{-1}$.

*Anal.* Calcd. for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.84; N, 19.94. Found: C, 47.98; H, 4.90; N, 20.11.

6-(2-Deoxy-β-D-ribosyl)-8-methyltetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one (**3**).

Compound **2** (200 mg) was treated with hydrochloric acid 0.09 *M* (20 ml) in chloroform/methanol, 9:1 under reflux for 1.5 hours. The resulting mixture was carefully neutralized with concentrated ammonia and evaporated to dryness *in vacuo* and the residue was chromatographed on plc (silica gel, eluent chloroform/methanol, 85:15) and the band Rf 0.30 (uv light) eluted with chloroform/methanol, 1:1 afforded 90 mg of **3** (60% yield). Recrystallization from methanol gave an analytically pure sample, mp = 143-144°, $[\alpha]_D$ = 31.4 (c, = 1 in methanol); λ max methanol = 252 nm (ε = 8000), 274 nm (s).

*Anal.* Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.87; N, 26.22. Found: C, 44.89; H, 4.96; N, 26.23.

1-(2-Deoxy-3,5-di-*O*-acetyl-β-D-ribosyl)-4-chlorpyrimidin-2(1*H*)-one (**4**).

Dimethylformamide (0.1 ml) and 1.0 ml of freshly distilled thionyl chloride were added to a solution of 3',5'-diacetyl-2'-deoxyuridine (500 mg) in anhydrous chloroform (15 ml) and the mixture was refluxed for 1.5 hours. After cooling, the solution was washed with aqueous sodium bicarbonate and water (5 × 10 ml). The organic layer was dried with sodium sulphate, evaporated to dryness *in vacuo* and the residue was chromatographed on a silicagel column using increasing amounts of methanol in chloroform. The fractions eluted with chloroform/methanol, 98:2 yielded 350 mg (66% yield) of pure **4**; $[\alpha]_D$ = 52.2 (c = 1 in chloroform); λ max chloroform = 308 nm (ε = 4500); ms: significant ions at m/z 330 ($M^+$), 210 ($M^+$-2 $CH_3COOH$), 201 (diacetylated sugar moiety), 129 (base moiety); ν max chloroform = 1730 $cm^{-1}$.

*Anal.* Calcd. for $C_{13}H_{15}ClN_2O_6$: C, 47.27; H, 4.55; Cl, 10.68; N, 8.48. Found: C, 47.38; H, 4.60; Cl, 10.67; N, 8.35.

Compound **4**, by treatment with concentrated methanolic ammonia, at 50° for 8 hours was converted into 2'-deoxycytidine.

6-(2-Deoxy-3,5-di-*O*-acetyl-β-D-ribosyl)tetrazolo[1,5-*c*]pyrmidine-5(6*H*)-one (**5**).

Compound **4** (300 mg) was treated in anhydrous dimethylformamide with excess sodium azide at room temperature for 1 hour. The mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness *in vacuo* and the residue was chromatographed on plc (silicagel, eluent chloroform/methanol, 95:5); the band Rf 0.39 (uv light), eluted with chloroform/methanol, 9:1, afforded 260 mg of **5** (85% yield). Recrystallization from methanol gave an analytically pure sample, mp = 125-126°; $[\alpha]_D$ = 28.4 (c = 1 in chloroform); λ max chloroform = 253 nm (ε = 10800), 273 nm (s); ms: significant ions at m/z 338 ($MH^+$), 218 ($MH^+$-2 $CH_3COOH$), 201 (diacetylated sugar moiety), 138 (base moiety + H); ν max chloroform = 1740 $cm^{-1}$.

*Anal.* Calcd. for $C_{13}H_{15}N_5O_6$: C, 46.29; H, 4.45; N, 20.77. Found: C, 46.40; H, 4.48; N, 20.65.

6-(2-Deoxy-β-D-ribosyl)tetrazolo[1,5-*c*]pyrimidin-5(6*H*)-one (**6**).

Compound **5** (200 mg) was treated with hydrochloric acid 0.09 *M* (20 ml) in water/methanol, 9:1, under reflux. The resulting mixture was carefully neutralized with concentrated ammonia, evaporated to dryness *in vacuo* and the residue chromatographed on plc (silicagel, eluent chloroform/methanol 85:15); the band Rf 0.30 (uv light) was eluted with chloroform/methanol, 1:1 affording 100 mg of **6** (67% yield). Recrystallization from methanol gave an analytically pure sample, mp = 154-156°; $[\alpha]_D$ = 60.6 (c = 1 in methanol); λ max methanol = 253 nm (ε = 9000), 272 nm

(s).

*Anal.* Calcd. for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.35; N, 27.67. Found: C, 42.74; H, 4.45; N, 27.69.

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