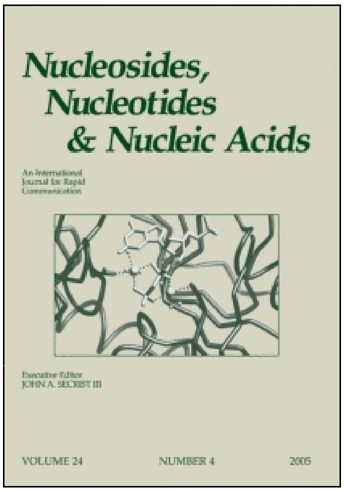

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SYNTHESIS OF 4-SUBSTITUTED PYRIMIDINE 2',3'-DIDEOXYNUCLEOSIDES

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ABSTRACT

Reaction of 5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-deoxythymidine with triphenylphosphine/carbon tetrachloride, followed by deprotection of the 5'-hydroxyl group, afforded the 4-chloro derivative 3 from which some 4-substituted pyrimidin-2(1H)one-2',3'-dideoxyribosides were obtained by nucleophilic substitution under very mild conditions.

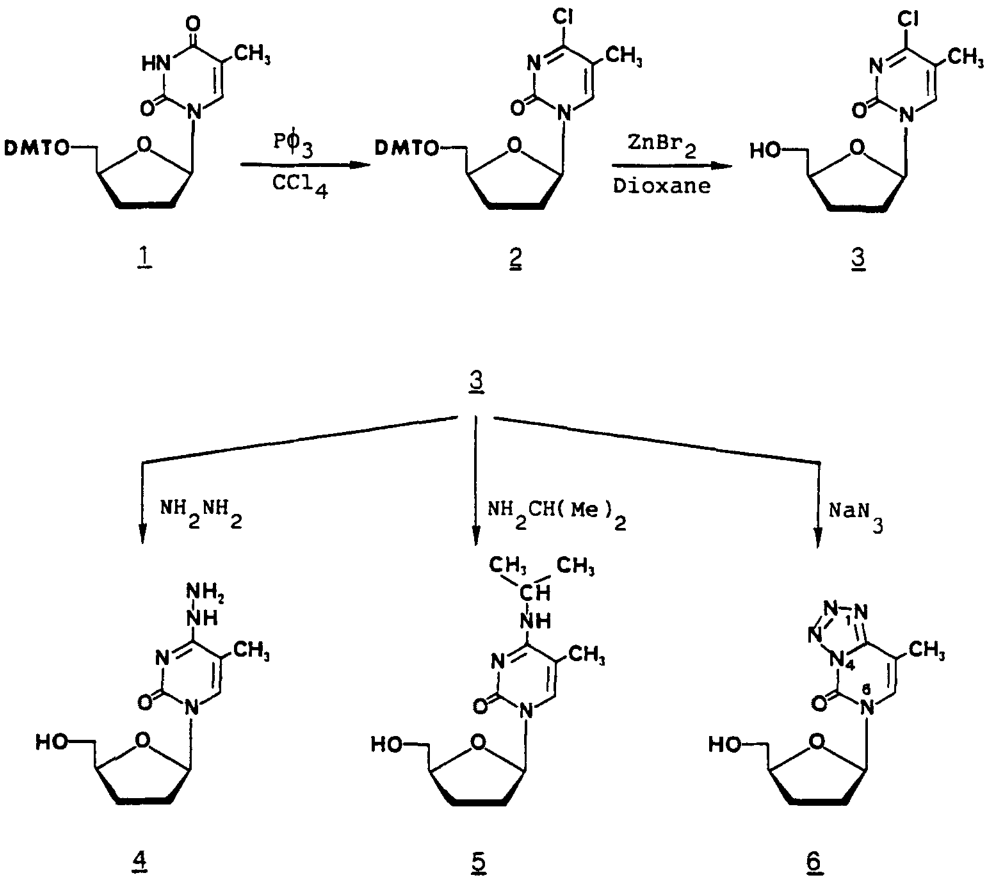
2',3'-Dideoxynucleosides, such as 3'-deoxy-3'-azidothymidine (AZT)[1] and 2',3'-dideoxycytidine (ddC)[2] are potent and selective inhibitors of the replication of the human immunodeficiency virus (HIV). The 5'-triphosphate of AZT competes with TTP and serves as chain-terminating inhibitor of HIV reverse transcriptase[3].

However, the severe toxicological problems associated with the common 2',3'-dideoxynucleosides, such as bone marrow suppression and other side effects[4], have directed our interest towards the synthesis of modified 2',3'-dideoxynucleosides with the aim of obtaining chemotherapeutically viable derivatives.

In contrast to the large number of pyrimidine 2',3'-dideoxynucleosides that have been synthesized, relatively few syntheses of such base modified compounds have been described so far[5].

A general route leading to various 2',3'-dideoxynucleosides with a different substituent at a fixed position of the base requires a 2',3'-dideoxynucleoside with a leaving group on this position.

We describe here the synthesis of the compound 3 having the chlorine atom prone to nucleophilic substitution under very mild conditions[6]. The synthesis and the characterization of the base-modified nucleosides (4-6) are also reported.



DMT = dimethoxytriphenylmethyl

As starting product to obtain 3 we used 5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-deoxythymidine (1), synthesized from 3'-deoxythymidine according to a reported procedure[7].

In order to prevent the cleavage[8] of the N-glycosidic bond, which is very acid labile in 2',3'-dideoxynucleosides[10], we chose for the chlorination the adduct of triphenylphosphine and CCl_4 which leads, in essentially-neutral conditions, to the formation of the 4-chloro derivative 2 in high yields[11].

The 4,4'-dimethoxytriphenylmethyl group was removed by treatment of 2 with $ZnBr_2$ in anhydrous dioxane [14] for 30 min at 40 °C, thus obtaining 3.
^1H and ^{13}C NMR, UV spectra and elemental analyses were consistent with structures 2 and 3. A further confirmation consisted in converting 3 into 5-methyl-2',3'-dideoxycytidine by reaction with methanolic ammonia at room temp. for 30 min.

Compound 3, by treatment with anhydrous hydrazine or isopropylamine for 30 min at room temp., afforded 4 and 5 respectively, in good yields. Analogously by treatment of 3 with NaN_3 in DMF the tetrazolo[1,5-c]pyrimidin-5(6H)-one derivative 6 was obtained. Structures 4-6 were corroborated by spectroscopic data.

The above results confirm that 3 reacts rapidly with nucleophiles under very mild conditions and the process can be considered as an useful alternative to the known procedures for the preparation of 4-substituted pyrimidine nucleoside derivatives [15-17].

Analyses of the biological activity of compounds 3-6 are in progress.

Table 1. ^1H NMR (270 MHz) Chemical shifts (J in Hz) of compounds 1-6.

Position	1	2	3	4	5	6
H-6	7.63 bs	8.25 bs	8.77 bs	7.86 bs	7.90 bs	
H-7						8.27 bs
CH_3	1.50 bs	1.59 bs	2.11 bs	1.94 bs	1.96 bs	2.41 bs
H-1'	6.11 dd (6.4;3.8)	6.02 dd (6.8;2.1)	5.95 dd (6.4;2.1)	6.08 dd (6.7;3.0)	6.06 dd (6.4;4.7)	6.32 dd (6.4;2.8)
H_a-2'	2.40 m	2.57 m	2.50 m	2.43 m	2.42 m	2.57 m
H_b-2' H_2-3'	2.20-1.85	2.15-1.88	2.23-1.87	2.08-1.91	2.10-1.90	2.04-2.27
H-4'	4.23 m	4.31 m	4.27 m	4.17 m	4.15 m	4.28 m
H_2-5'	3.37 * (10.7;3.0) (4.0)	3.44 * (10.7;2.6) (3.3)	3.92 ** (12.0;2.6) (3.8)	3.84 * (12.2;3.7) (3.7)	3.81 * (12.4;3.0) (3.8)	3.90 * (12.4;2.8) (3.7)
Phenyl	7.47-6.79	7.44-6.79				
2 OCH_3	3.79 s	3.80 s				
HO-C-5'			4.05 triplet (5.1)			
$C\underline{H}(CH_3)_2$					4.44 septet (6.4)	
$CH(C\underline{H}_3)_2$					1.25 d (6.4)	

Chemical shifts are given in ppm relative to TMS. s = Singlet, d = doublet dd = double doublet, m = multiplet, bs = broad singlet. * : AB part of an ABX system; ** : AB part of an ABX system. The AB part is further coupled with $\underline{H}$O-C-5' (J = 5.1 Hz). The spectra were carried out in $CDCl_3$ for compounds 1-2, in $(CD_3)_2CO$ for 3 and in CD_3OD for 4-6.

Table 2. ^{13}C NMR (67.9 MHz) Chemical shifts of compounds 1-6

Position	1	2	3	4	5	6
C-2	163.8 s	167.7 s	167.3 s	169.7 s	164.3 s	
C-4	150.3 s	153.3 s	153.6 s	157.8 s	159.0 s	
C-5	110.3 s	112.1 s	111.7 s	103.9 s	104.6 s	153.8 s
C-6	144.4 d	142.2 d	144.8 d	138.5 d	140.0 d	
C-7						133.9 d
C-8						104.0 s
C-9						144.5 s
CH_3	12.0 q	15.1 q	15.6 q	13.1 q	13.6 q	13.9 q
C-1'	86.1 d	90.5 d	89.0 d	88.5 d	88.4 d	89.6 d
C-2'	32.7 t	33.5 t	34.0 t	34.2 t	34.3 t	34.4 t
C-3'	25.8 t	26.4 t	24.2 t	26.0 t	26.1 t	25.6 t
C-4'	80.3 d	82.1 d	84.4 d	83.6 d	83.4 d	84.7 d
C-5'	64.5 t	67.9 t	62.5 t	64.0 t	64.1 t	63.4 t
$\underline{C}H(CH_3)_2$						43.9 d
$CH(\underline{C}H_3)_2$						22.7 q

Chemical shifts are given in ppm relative to TMS. s = Singlet, d = doublet, t = triplet, q = quartet in the off resonance spectra. The spectra were carried out in CD_3OD for compounds 4-6, in $CDCl_3$ for 1-2 and in $(CD_3)_2CO$ for 3.

EXPERIMENTAL

General procedure

The ^1H and ^{13}C NMR spectra were recorded with a Bruker WM 270 instrument. UV spectra were taken on a Perkin-Elmer lambda 7 spectrophotometer. All chemical shifts were expressed in ppm on the respect of the residual solvent signal. FAB mass spectra were determined with a double-focusing mass spectrometer (ZAB 2SE) on samples dissolved in glycerol-thioglycerol on a Cu probe tip and inserted into a source at 10^{-4} Torr pressure of Xe. The sample was bombarded with Xe atoms of 9.5 kV energy. Analytical and preparative TLC were carried out on silica gel plates (Merck, Kieselgel 60 F254, 20x20 cm). The products were visualized by UV light at 254 nm. Column chromatographies were performed on silica gel (Merck, Kieselgel 60, 0.063 - 0.200 mm), and on neutral alumina B III (Merck, 0.063 - 0.200 mm). Optical rotations were measured with a Perkin-Elmer 141 polarimeter at 25°C.

5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-deoxythymidine (1)

To a solution of 3'-deoxythymidine (1.0 mmol,226 mg) and 4-dimethylaminopyridine (0.05 mmol, 6 mg) in anhydrous pyridine (5 ml),4,4'-dimethoxytriphenylmethyl chloride (1.3 mmol, 440 mg) and triethylamine (1.4 mmol, 194 μl) were added and the resulting solution was kept for 1 h at 70°C. The reaction was stopped by addition of water (2 ml) and the resulting solution was evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (60 cm column length, 2.0 cm I.D.) using increasing amounts of CH_3OH in $CHCl_3$-pyridine (99.8:0.2, v/v, flow rate 4.0 ml/min). The fractions which were eluted with $CHCl_3$-pyridine/CH_3OH (98:2, v/v) yielded 203 mg of pure 1 (90% yield), which was crystallized from a CH_3OH/H_2O mixture; m.p. 94-96°C; $[\alpha]_D$ = -2.0° (c = 1.2 in $CHCl_3$). Anal. Calcd. for $C_{31}H_{32}N_2O_6$: C, 70.24; H, 6.10; N, 5.30. Found: C, 70.30; H, 6.17; N, 5.12. UV (CH_3OH) λmax 270 nm (ε= 9200), 233 nm (ε= 18600). FAB MS gave the quasi-molecular ion $(MH)^+$ at m/z 529.

1-[2,3-Dideoxy-ß-D-glycero-pentofuranosyl-5-O-(4,4'-dimethoxytriphenylmethyl)]-4-chloro-5-methylpyrimidin-2(1H)-one (2)

A mixture of 1 (0.5 mmol, 264 mg) and triphenylphosphine (0.6 mmol, 157 mg) was dissolved in 2 ml of anhydrous CCl_4 and kept at gently reflux. After 1 h, TLC analysis (silica gel, 0.25 mm eluent $CHCl_3$/ethyl acetate, 8:2, v/v) showed the almost complete conversion of 1 in a new product (Rf 0.55). The solution was filtered and the filtrate was applied to a column of neutral alumina B III (40 cm column length, 1.5 cm I.D.) packed in $CHCl_3$/hexane (4:6, v/v). The column was developed using increasing amounts of $CHCl_3$ in hexane. The fractions which were eluted with $CHCl_3$/hexane (7:3, v/v, flow rate 3.0 ml/min) afforded 220 mg of pure 2 (80 % yield)[18] as a white foam. $[\alpha]_D$= + 38.0°(c = 1 in $CHCl_3$). UV (CH_3OH) λmax 315 nm (ε= 5700), 275 nm (ε= 3800), 267 nm (ε = 3400), 235 nm (shoulder) (ε = 15000). FAB MS gave the quasi-molecular ion $[(MH)^+, {}^{35}Cl]$ at m/z 547.

1-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-4-chloro-5-methylpyrimidin-2(1H)-one (3).

To the compound 2 (220 mg, 0.4 mmol) was added a saturated solution (8 ml) of $ZnBr_2$ in dioxane and the mixture kept at 40°C. After 30 min the mixture was concentrated *in vacuo*, and then purified by preparative TLC (silica gel 0.5 mm, 10 plates, eluent $CHCl_3/CH_3OH$ 9:1,v/v). The band Rf 0.30 was scratched from the plates and eluted from the silica with $CHCl_3/CH_3OH$ (7:3,v/v) to afford 88 mg of 3 (90 % yield) which was crystallized from benzene; m.p. 83-85 °C; $[\alpha]_D$ = + 105.5°(c = 0.9 in acetone). Anal.Calcd. for $C_{10}H_{13}ClN_2O_3$: C, 49.06; H, 5.36; N, 11.45. Found: C, 49.16; H, 5.41; N, 11.60. UV (CH_3OH) λmax 315 nm (ε = 5500). FAB MS gave significant ions at m/z: 245 $[(MH)^+, {}^{35}Cl]$ and 209 $(MH - HCl)^+$.

1-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-4-hydrazino-5-methylpyrimidin-2(1H)-one (4)

Compound 3 (100 mg, 0.41 mmol), dissolved in anhydrous dimethylformamide (1 ml) was treated with an excess (2 ml) of anhydrous hydrazine at room temperature for 30 min. The resulting solution was evaporated in vacuo. The residue was purified by preparative TLC (silica gel, 4 plates, 0.5 mm, eluent $CHCl_3/CH_3OH$, 65:35,v/v). The band Rf 0.25 was scratched from the plates and eluted from the silica with CH_3OH to afford 88 mg of 4 (90% yield)[18]. $[\alpha]_D$ = +19.4° (c = 0.9 in CH_3OH); UV (CH_3OH) λmax 278 nm (ε=7000). FAB MS gave significant ions at m/z: 241 $(MH)^+$ and 141 (base residue +2 H$)^+$.

1-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-4-N-isopropylamino-5-methylpyrimidin-2(1H)-one (5)

Compound 3 (100 mg, 0.41 mmol) was dissolved in anhydrous dimethylformamide (1 ml) and treated with an excess (2 ml) of isopropylamine at room temperature for 30 min. The resulting solution was evaporated in vacuo and the residue was purified by preparative TLC (silica gel, 4 plates, 0.5 mm, eluent $CHCl_3/CH_3OH$ 9:1,v/v). The band Rf 0.3 was scratched from the plates and eluted from the silica with $CHCl_3/CH_3OH$ (7:3,v/v) to afford 99 mg of 5 (90 % yield)[18]. $[\alpha]_D$ = + 49.2° (c = 1 in CH_3OH); UV (CH_3OH) λmax 278 nm (ε = 8000). FAB MS gave significant ions at m/z: 268 $(MH)^+$ and 168 (base residue + 2H$)^+$.

6-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-8-methyltetrazolo[1,5-c]pyrimidin-5(6H)-one (6)

Compound 3 (150 mg, 0.61 mmol) was treated in anhydrous dimethylformamide (1.5 ml) with an excess of sodium azide at room temperature for 1 h under stirring. The mixture was diluted with water (5 ml) and extracted with $CHCl_3$ (4 x 5ml). The organic phases were evaporated to dryness *in vacuo* and the residue was purified by preparative TLC (silica gel, 6 plates, 0.5 mm, eluent $CHCl_3/CH_3OH$ 9:1,v/v); the band Rf 0.35 was scratched from the plates and eluted from the silica with $CHCl_3/CH_3OH$ (7:3,v/v) to afford 130 mg of 6 (85 % yield), which was crystallized from CH_3OH; m.p. 147-148°C; $[\alpha]_D = + 47.8°$ (c = 1.1 in CH_3OH). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.78; H, 5.22; N, 27.89. Found: C, 47.86; H, 5.19; N, 27.80. UV (CH_3OH) λmax 256 nm (ε = 8100); 273 nm (shoulder). FAB MS gave significant ions at m/z: 252 $(MH)^+$ and 152 (base residue + $2H)^+$.

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