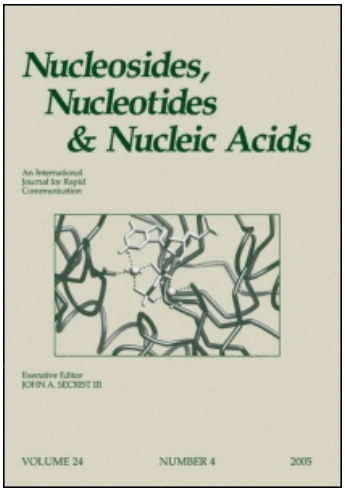

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Synthetic Nucleosides and Nucleotides. XX[1]. Synthesis of Various 1-β-D-Xylofuranosyl-5-Alkyluracils and Related Nucleosides

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SYNTHETIC NUCLEOSIDES AND NUCLEOTIDES. XX.[1] SYNTHESIS OF VARIOUS 1-β-D-XYLOFURANOSYL-5-ALKYLURACILS AND RELATED NUCLEOSIDES.

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Abstract

Treatment of D-xylose (1) with 0.5% methanolic hydrogen chloride under controlled conditions followed by benzoylation and acetolysis afforded crystalline 1-O-acetyl-2,3,5-tri-O-benzoyl-α-D-xylofuranose (4) in good yield. Coupling of 4 with 2,4-bis-trimethylsilyl derivatives of 5-alkyluracils (methyl, ethyl, propyl and butyl)(5a-5d), 5-fluorouracil (5e) and uracil (5f) in acetonitrile in the presence of stannic chloride gave 1-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-nucleosides (6a-6f). Saponification of 6 with sodium methoxide afforded 1-β-D-xylofuranosyl-5-substituted uracils (7a-7f). Condensation of 4 with free adenine in similar fashion and deblocking gave carcinostatic 9-β-D-xylofuranosyladenine (7g).

Spurred largely through the usefulness of 5-substituted 2'-deoxyuridines and 1-β-D-arabinofuranosyl-5-alkyluracils in chemotherapy against Herpes simplex viruses (HSV),[2-5] considerable efforts have been devoted to the synthesis of 5-substituted uracil nucleosides.

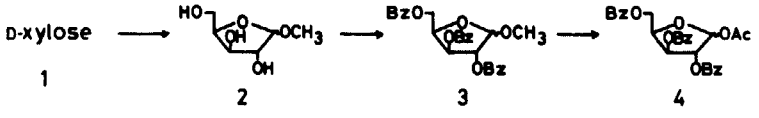
In our previous papers, we reported the synthesis, antitumor and antiviral activities of various 5-fluoropyrimidine nucleosides,[6] 5-alkyluridines and 1-β-D-arabinofuranosyl-5-alkyluracils.[7-9] We also reported that the triphosphates of 1-β-D-arabinofuranosyl-5-alkyluracils[10] and 1-β-D-arabinofuranosyl-5-halogenouracils[11] showed strong inhibitory effects on DNA polymerases from murine cells and oncorna virus. On the other hand, 9-β-D-xylofuranosyladenine possesses antitumor activity.[12] This information prompted us to synthesize the nucleosides related to 5-substituted uracil bearing β-D-xylofuranosyl moieties.

Suitably protected 1-O-acetyl-D-xylofuranose, the key intermediate for present study, is not easily available. For example, fully acylated xylofuranoses including 1-O-acetyl-2,3,5-tri-O-benzoyl-α-D-xylofuranose (4)[13] were prepared from D-xylose (1) via many steps only in low yields. Thus, we decided to develop a facile synthesis of 4 starting from D-xylose. In our study, the best yield was obtained by stirring 1 at 20°C for 5 hr in methanol in the presence of 0.5% hydrogen chloride (w/w). After neutralization and evaporation of excess methanol, the residue was treated

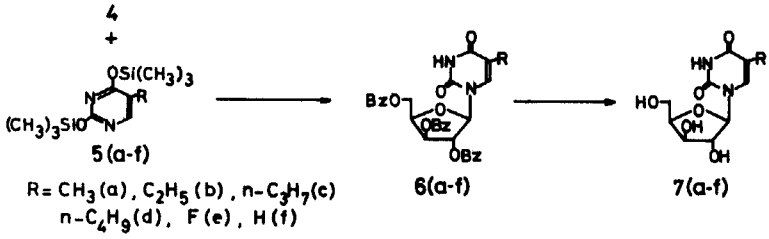
with benzoyl chloride in dry pyridine to afford a mixture of methyl 2,3,5-tri-O-benzoyl-α(and β)-D-xylofuranoside (3). Acetolysis of this product in acetic anhydride-acetic acid mixture in the presence of concentrated sulfuric acid gave anomeric 1-O-acetyl-2,3,5-tri-O-benzoyl-D-xylofuranose (4) in good yield. The α-anomer was easily crystallized from methanol. The overall yield based on the amount of D-xylose was 45-55%.

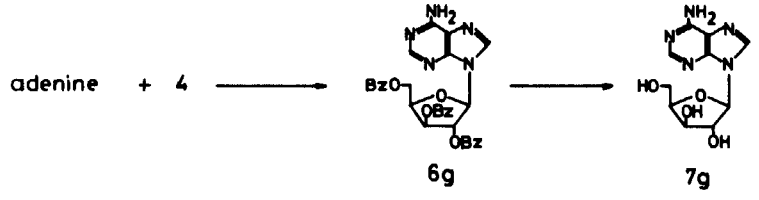
The coupling reaction of compound 4 with 2,4-bis-trimethylsilyloxy-5-substituted uracils (5a-5e) was carried out in acetonitrile in the presence of stannic chloride at room temperature for 3-5 hr to afford crystalline tribenzoates 6a-6e in 80-90% yields. 2,4-Bis-trimethylsilyloxypyrimidine (5f) was also coupled with 4 under similar conditions to give the tribenzoate of 1-β-D-xylofuranosylurasyl (6f) in 80% yield together with a small amount of 3-xylofuranosyl isomer (5%).

In the purine series, condensation of 4 with free adenine in the presence of stannic chloride[14] gave 9-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-adenine (6g) in good yield. These protected xylofuranosyl nucleosides were saponified by sodium methoxide in methanol to give the title compounds (7a-7g) in good yields.
The structures of these compounds were confirmed by elemental analysis, mass spectra, UV and NMR spectrophotometric measurements. The assignment of the β-configuration to 7a-7f was made by comparing the sign of the CD band associated with B_{2u} electronic transition with that of the appropriate nucleoside of known configuration.



Scheme 1. Preparation of 4 from D-xylose.





Scheme 2. Synthesis of β-D-xylofuranosyl nucleosides.

The observed positive sign and amplitude of the CD band of 7a-7f were in accord with those found for 1-β-D-pentofuranosylpyrimidines.[7] The relative intensities of CD bands of compound 7a-7f were found to be intermediate to those of their 1-β-D-ribofuranosyl- and 1-β-D-arabinofuranosyl counterparts.[7]

Preliminary screening results showed that 7a-7e inhibit neither Herpes simplex type 1 in cultured human embryonic lung fibroblast cells nor cultured mouse leukemic L5178Y cells at 100 μg/ml. For comparison, known compounds, 1-β-D-xylofuranosyluracil (7f) inhibits L5178Y cells at 100 μg/ml and 9-β-D-xylofuranosyladenine (7g) inhibits L5178Y at 4.5 μg/ml.

These results showed that the 5-substituent on uracil nucleus strongly affect enzymatic phosphorylation of nucleosides with cellular uridine-cytidine kinase. In contrast, 7g could be phosphorylated by the cellular adenosine kinase system and finally activated form, 9-β-D-xylofuranosyladenine 5'-triphosphate may inhibit DNA-dependent RNA polymerases in vivo.

On the other hand, chemically synthesized 5'-triphosphates of compound 7a-7f showed strong inhibitory effects on DNA-dependent RNA polymerases I and II purified from cherry salmon *(Oncorhynchus masou)* liver nuclei and the results are reported in a separate paper.[15]

Experimental

Melting points were determined on a Yanaco Model MP-3 apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-300 recording spectrophotometer. CD spectra were recorded on a JASCO Model 20 automatic recording spectropolarimeter. NMR spectra were obtained on a R20B Hitachi high resolution NMR spectrophotometer with tetramethylsilane as an internal standard.

1-O-Acetyl-2,3,5-tri-O-benzoyl-α-D-xylofuranose (4)

Well dried D-xylose (1)(30 g) was suspended in freshly distilled methanol (500 ml) containing 0.5% hydrogen chloride (w/w). The mixture was stirred at 20°C for 5 hr. The resulting clear solution of anomeric methyl D-xylofuranosides (2) was neutralized with anhydrous potassium carbonate. The mixture was filtered and the filter cake washed with small amount of dry methanol. The filtrate and washings were combined and the solvent was removed in vacuo. The residue was co-evaporated first with benzene (50 ml x 2) and then with anhydrous pyridine (50 ml x 2) to remove any remaining methanol. To a solution of crude 2 in anhydrous pyridine (200 ml) was added benzoyl chloride (90 ml, 3.9 eq.) dropwise under stirring and cooling in an ice-bath. The resulting solution was further stirred at room temperature overnight. The reaction mixture was evaporated under diminished pressure and the residue treated with distilled water (200 ml) and chloroform (200 ml). After vigorous shaking, the organic layer was separated and the aqueous phase was was extracted again with chloroform (100 ml). The combined chloroform extracts were washed first with saturated sodium bicarbonate (100 ml x 3) and then water (100 ml x 3).

After the chloroform solution was dried over magnesium sulfate, the solvent was removed under reduced pressure to afford crude methyl 2,3,5-tri-O-benzoyl-D-xylofuranoside (3)(95.2 g). Compound 3 (95.2 g) was dissolved in a mixture of acetic acid (160 ml) and acetic anhydride (40 ml). To this solution was dropwise added concentrated sulfuric acid (25 ml) with stirring under cooling in an ice-bath. After being stirred at room temperature overnight, the reaction mixture was vigorously agitated and scratched to give 1-O-acetyl-2,3,5-tri-O-benzoyl- D-xylofuranose (4) as crystals. The crystals were collected by suction filtration and washed with a small amount of acetic acid. The filtrate and washings were combined, treated with sodium acetate (40 g), and evaporated to dryness. The residue was dissolved in chloroform (200 ml) and washed with distilled water (200 ml). The organic layer was washed with saturated aqueous sodium bicarbonate and distilled water (200 ml each). The solvent was removed in vacuo and the residue were crystallized from methanol to give crystalline α-anomer (4). The combined crystals were recrystallized from methanol to afford analytically pure 4, 45.2 g (44.5% based on the amount of 1). mp 124-126° (lit.[13] 127-128.5°). $[\alpha]_D^{14}$ = +154° (c=1.2, $CHCl_3$) lit.[13] $[\alpha]_D^{31}$ = +147° (c=1.0, $CHCl_3$). NMR: (ppm), 7.5-8.2 (m, 15H, benzoyl), 6.8 (d, 1H, 1'-H, $J_{1'-2'}$ = 5Hz), 2.1 (s, 3H, acetyl). Anal. Calcd for $C_{28}H_{24}O_9$: C, 66.67; H, 4.76. Found: C, 66.42; H, 4.84.

Similar experiments were repeated in different scales to give compound 4 in 45-55% yields based on the amount of 1. Uncrystallized β-anomer may be also used for the xylosidation reaction.

General Procedure for the Synthesis of 1-(2,3,5-Tri-O-benzoyl-β-D-xylofuranosyl)-5-substituted uracils (6a-6f)

Condensation of compound 4 and 2,4-bis-trimethylsilyloxy-5-substituted pyrimidines[6-7] (5a-5e) was carried out using 5-10 mmoles of starting material. A typical procedure on a 10 mmole scale is as follows: The compound 4 and a silylated pyrimidine (10 mmole of each) were dissolved in 20 ml of anhydrous acetonitrile, and to this solution was added dropwise stannic chloride (2.6 g) in anhydrous acetonitrile (10 ml) at an ice-bath temperature. After being stirred at room temperature overnight, the reaction mixture was quenched with distilled water (15 ml) with the pH adjusted to 8 by addition of sodium bicarbonate. The mixture was filtered through Celite and the filter cake was washed well with hot chloroform (100 ml x 5). The filtrate and washings were combined and washed with distilled water (300 ml x 4). The chloroform layer was dried over magnesium sulfate and evaporated to dryness. The residual gum (ca. 5.4 g) was chromatographed on a column of silica gel (150 g). Elution was performed stepwise with chloroform-ethyl acetate (9:1, 4:1 and 2:1, v/v). The fractions which were eluted with the 2:1 mixture were collected and concentrated to dryness to give a thin-layer chromatographycally homogeneous (silica gel, chloroform-ethyl acetate, 4:1, v/v) gum which was crystallized from methanol or ethanol to afford 1-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-5-substituted uracils (6a-6f). Yields of the compounds, elemental analytical results and melting points are summarized in Table 1.

Table 1. Analytical Data of 1-(2,3,5-Tri-O-benzoyl-β-D-xylofuranosyl)-5-substituted uracils.

Compounds	Yields (%)	mp (°C)	Formula	Anal. Calcd. (%) (Found, %) C,	H,	N,
6a	88	198-199	$C_{31}H_{26}O_9N_2$	65.26 (65.30)	4.59 (4.59)	4.91 (5.02)
6b	85	163-165	$C_{32}H_{28}O_9N_2$	65.75 (65.50)	4.83 (4.75)	4.79 (4.68)
6c	78	168-169	$C_{33}H_{30}O_9N_2$	66.21 (66.21)	5.05 (5.01)	4.68 (4.60)
6d	90	159-161	$C_{34}H_{32}O_9N_2$	66.66 (66.52)	5.27 (5.31)	4.57 (4.44)
6e	94	178-179	$C_{30}H_{23}O_9N_2F$	62.72 (62.66)	4.04 (3.95)	4.88 (4.76)

Table 2. Analytical results of 1-β-D-Xylofuranosyl-5-substituted uracils and 9-β-D-Xylofuranosyladenine (7a-7g)

Compounds	Yields (%)*	mp (°C)	Formula	Anal. Calcd (%) (Found, %) C,	H,	N,
7a	93	164-165	$C_{10}H_{14}O_6N_2$	46.51 (46.32)	5.47 (5.47)	10.85 (10.93)
7b	81	200-201	$C_{11}H_{16}O_6N_2$	48.52 (48.50)	5.92 (5.96)	10.29) (10.17)
7c	49	155-159	$C_{12}H_{18}O_6N_2$	50.34 (50.44)	6.34 (6.44)	9.79 (9.77)
7d	83	157-159	$C_{13}H_{20}O_6N_2$	51.99 (51.93)	6.71 (6.76)	9.33 (9.23)
7e	68	192-193	$C_9H_{11}O_6N_2F$	41.22 (41.04)	4.23 (4.67)	10.68 (10.73)
7f	62*	156-157	$C_9H_{12}O_6N_2$	44.26 (44.18)	4.95 (5.10)	11.47 (11.44)
7g	63*	188-190	$C_{10}H_{13}N_5O_4$	44.94 (44.83)	4.90 (4.96)	26.21 (26.20)

*Yields from compound 4.

Table 3. Spectrophotometric Properties of 1-β-D-Xylofuranosyl-5-substituted uracils. (7a - 7f)

Compound	UV, λmax (nm) (ε) in H_2O in 0.01 N NaOH	CD in H_2O	NMR (d_6-DMSO) δ (ppm)
7a,	265 (9600)	$[\theta]_{271}$ = +10000	7.65, 1H, s, H_6
	265 (7300)		5.67, 1H, s, 1'H
			2.50, 3H, s, 5-CH_3
7b,	267 (9800)	$[\theta]_{270}$= +11000	7.66, 1H, s, H-6
	265 (7500)		5.69, 1H, s, 1'-H
			2.18, 2H, q, 5- -CH_2-
			1.02, 3H, t, 5-CH_3
7c,	267 (10500)	$[\theta]_{271}$= +12600	7.65, 1H, s, H_6
			5.68, 1H, s, 1'H
			2.14, 2H, dt, -CH_2-
			1.42, 2H, t, -CH_2-
			0.86, 3H, t, 5-CH_3
7d,	267 (11300)	$[\theta]_{271}$= +11300	7.65, 1H, s, H-6
	265 (7400)		5.69, 1H, s, 1'H
			2.17, 2H, t, -CH_2-
			1.26-1.39, 4H, m, -CH_2-CH_2-
			0.87, 3H, t, 5-CH_3
7e,	271 (8700)	$[\theta]_{270}$=+13900	8.00, 1H, d, H-6
	270 (6700)		5.65, 1H, s, 1'H
7f,	263 (10100)	$[\theta]_{264}$=+14100	7.76, 1H, d, H-6
			5.65, 1H, s, 1'H
			5.63, 1H, d, 5-H

1-β-D-Xylofuranosyl-5-substituted uracils (7a-7f)

Compound (6a-6f) (10 mmole) was dissolved in 0.05 M methanolic sodium methoxide (40 ml) and stirred at room temperature overnight. Distilled water (10 ml) was added to the reaction mixture and then mixed with Dowex 50 (H^+-form) for removal of sodium ion.

The resin was removed by filtration and washed with 80% aqueous methanol. The filtrate and washings were combined and evaporated under reduced pressure. The residue was dissolved in 20 ml of water and the solution was extracted with chloroform (5 ml x 4) for removal of methyl benzoate. The aqueous phase was evaporated and the residue crystallized from the appropriate solvent (methanol or ethanol) to give crystalline 7a-7f. Yields, melting points and elemental analytical results of these compounds are summarized in Table 2.

9-β-D-Xylofuranosyladenine (7g)

Adenine (1.35 g, 10 mmole) was suspended in a solution of compound 4 (5.04 g, 10 mmole) in 40 ml of anhydrous acetonitrile. Stannic chloride (1.72 ml, 1.5 eq.l in 20 ml of dry acetonitrile was added and the mixture was stirred at room temperature. After 30 min, the reaction mixture became clear. Thin-layer chromatographic analysis of this solution (silica gel, chloroform-methanol, 9:1, v/v) showed three spots with Rf values of 0.1, 0.42 and 0.80, corresponding to adenine benzoylated nucleoside and 4, respectively. After stirring the reaction mixture overnight, the TLC spots corresponding to adenine and 4 almost disappeared. The reaction mixture was concentrated to a small volume (ca. 5 ml), and sodium bicarbonate (6 g) and distilled water (20 ml) were added. When the vigorous evolution of carbon dioxide had ceased, the mixture was filtered through Celite and the filter cake was washed with hot chloroform (50 ml x 4). The chloroform layer was separated and washed with water, and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a glassy gum, which was dissolved in chloroform and applied to a column of silica gel (100 g). Elution was performed with chloroform-ethyl acetate (9:1, v/v) (300 ml) and chloroform-methanol (9:1, v/v)(350 ml). A small amount of sugar was eluted first. The fractions containing the nucleoside were combined and evaporated under reduced pressure and the residue was crystallized from ethyl acetate to give 9-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-adenine (6g)(3.94 g)(68%), mp 110-114°. The compound 6g was dissolved in 0.05 M, methanolic sodium methoxide (50 ml) and the solution stirred at room temperature overnight. After the usual workup, the crude product was purified by a Dowex 1 (OH^- form) column using 30% aqueous methanol as eluent. The fractions containing nucleoside were combined and evaporated. The residue was crystallized from methanol to give fine needles, (1.7 g, 93%), mp 188-190°. Elemental analytical results are summarized in Table 2.

Assay of Antiviral and Cytostatic Activities

Antiviral activities of these compounds were assayed using herpes simplex virus type 1 in cultured human embryonic lung fibroblast under the conditions which are described in our previous paper.[7] Cytostatic activities were assayed by a similar method using mouse leukemic L5178Y cells in culture described previously.[16]

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