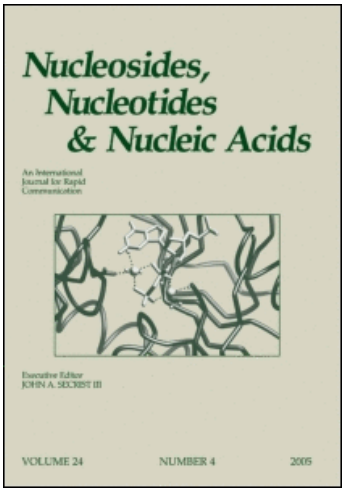

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Synthesis of 5-Fluoro-1-(3-*O*-benzoyl-4, 6-dideoxy- β-L-*GLYCERO*-hex-3-enopyranos-2-ulosyl)uracil

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SYNTHESIS OF 5-FLUORO-1-(3-O-BENZOYL-4, 6-DIDEOXY- β -L-GLYCERO-HEX-3-ENOPYRANOS-2-ULOSYL)URACIL

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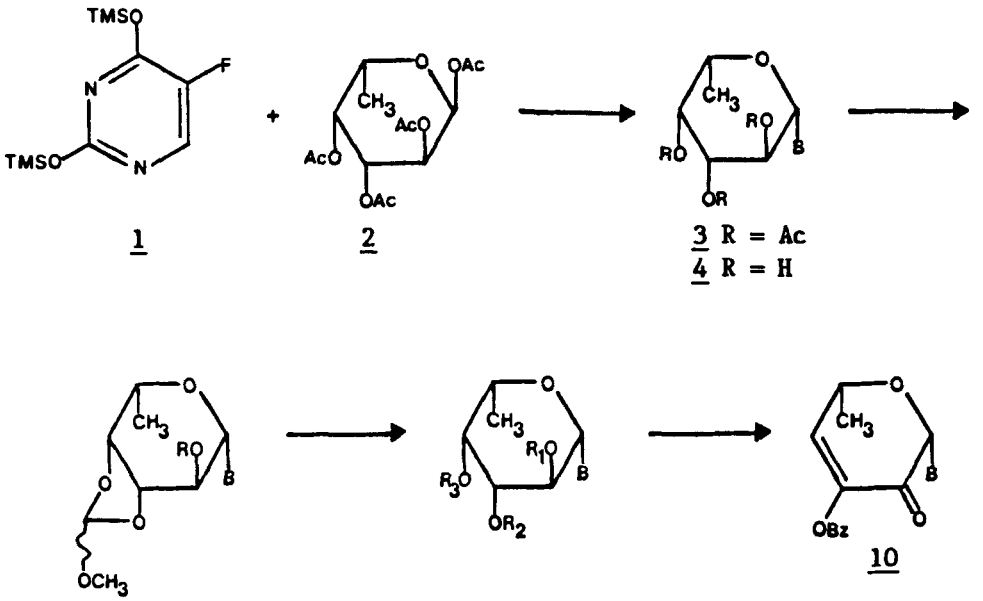
Abstract: Synthesis of the title compound, an unsaturated ketohexopyranosyl nucleoside of 5-fluorouracil is reported. It was prepared by oxidation of the corresponding dibenzoylhexopyranosyl nucleoside with pyridinium dichromate/molecular sieves system.

Several α,β -unsaturated ketohexopyranosyl purine nucleosides with L-rhamnose and L-fucose have been reported to possess significant in vitro and in vivo antiproliferative activity against various cancer cells[1]. The α,β -unsaturated keto system has been found extremely necessary for the activity[1]. These observations aroused our interest in the synthesis and activity evaluation of various corresponding pyrimidine nucleosides. Earlier we found that 5-fluoro-1-(3-O-benzoyl-2, 6-dideoxy- α -L-glycero-hex-2-enopyranos-4-ulosyl)uracil has anticancer activity in both in vitro and in vivo test systems[2]. The synthesis of various corresponding 5-substituted nucleosides for activity correlation studies is in progress in our laboratory. Here, we report the synthesis (Scheme 1) of the title compound, a 2'-ketonucleoside prepared with a view to investigating the effect of positional variation of unsaturation and the keto group on activity.

The triacetyl nucleoside 3 was synthesized according to the procedure of Vorbrüggen[3]. The $J_{1',2'}$ = 8.0 Hz indicates β configuration in $^1C_4$ conformation. The synthesis of 3', 4'-di-O-benzoate 9 was achieved through sequential protection-deprotection reactions (Scheme 1). The signal for H-1' was found as a broad doublet or doublet of doublets in the ^1H NMR spectra of most of the compounds. The complex nature of



1 2

3 R = Ac
4 R = H

5 R = H
6 R = TBDMS

7 R_1 = TBDMS, R_2 = R_3 = H
8a R_1 = TBDMS, R_2 = H, R_3 = Bz
8b R_1 = TBDMS, R_2 = R_3 = Bz
9 R_1 = H, R_2 = R_3 = Bz

10

B = -1-(5-fluorouracil)
TMS = trimethylsilyl
TBDMS = t-butyldimethylsilyl

the signal is attributed to coupling to fluorine at C-5, since its substitution by chlorine results in collapse to a sharp doublet[4].

As expected, a marked difference between the reactivity of the 3' and 4'-hydroxyl groups was found during benzoylation of 7 with benzoic anhydride/4-dimethylaminopyridine. One of them underwent benzoylation within 15 min to yield mono-O-benzoyl nucleoside 8a, while the other took 16 h to yield di-O-benzoyl nucleoside 8b. The ^{1}H NMR spectrum [$(CD_3)_2CO$] of 8a showed a downfield shift for one proton (compared to 7) at δ 5.50, a partially resolved doublet of 2.0 Hz. Likewise, the ^{1}H NMR spectrum of 8b showed downfield shifts for two protons at δ 5.55, a partially resolved broad doublet of 8.0 Hz and at δ 5.65, a partially resolved doublet of 2.0 Hz. In the 3', 4'-di-O-benzoyl nucleoside 9, the signals

became a sharp doublet of doublets at δ 5.65, J = 8.0 and 2.0 Hz and a doublet at δ 5.75, J = 2.0 Hz. On the basis of a $^1C_4$ conformation ($J_{1',2'}$ = 8.0 Hz) these were tentatively assigned to H-3' (dd) and H-4' (d). Thus, it is speculated that 4'-hydroxyl was benzoylated much faster than 3'-hydroxyl and the bulky 2'-O-TBDMS group was responsible for very slow rate of benzoylation of the latter, despite its being equatorial.

Oxidation of 9 with pyridinium dichromate/molecular sieves system[5] followed by in situ elimination of 4'-O-benzoyloxy group as benzoic acid furnished the title compound 10. The ^1H NMR spectrum ($CDCl_3$) of 10 showed a doublet for H-1' at δ 6.95 ($J_{H,F}$ = 1.5 Hz), a broad singlet for H-4' at δ 6.45, a multiplet for H-5' at δ 5.00 and a doublet for H-6' at δ 1.55 ($J_{5',6'}$ = 7.0 Hz) which confirms presence of the 3', 4'-unsaturated-2'-keto system. Anticancer activity of 10 is under investigation.

EXPERIMENTAL SECTION

Melting points (uncorrected) were recorded using a Mel-Temp apparatus. Elemental analyses were performed by Galbraith Laboratories, Inc. ^1H NMR spectra were recorded on a Bruker/IBM-SY200 spectrometer at 270 MHz using $(CH_3)_4Si$ as internal standard. Optical rotations were recorded on a quick polarimeter, model 52 of Rudolph Instruments. Thin layer chromatography (TLC) was performed on a precoated silica gel plastic sheets $60F_{254}$ (0.2 mm) EM Reagents and compounds were detected under short wavelength UV light and by spraying with 3% sulfuric acid in ethanol (w/v). Silica gel 60 (70-230 mesh ASTM) was used for column chromatography. Completion of the reactions was monitored in appropriate solvent systems. Solvent systems: (A) ethyl acetate-hexane (1:8), (B) ethyl acetate-hexane (1:2), (C) ethyl acetate-hexane (1:1), (D) ethyl acetate-hexane (2:1), (E) chloroform-ethyl acetate (1:1) and (F) chloroform-methanol (4:1). Solvents were evaporated under vaccum at about 40 °C.

5-Fluoro-1-(2,3,4-tri-O-acetyl-6-deoxy-β-L-galactopyranosyl)uracil (3).

A mixture of 5-fluorouracil (26 g, 0.20 mol), hexamethyldisilazane (105.2 mL, 0.50 mol) and saccharin[6] (457.50 mg, 2.50 mmol) in dry dichloroethane (200 mL) was stirred and heated under reflux vigorously with the exclusion of moisture until dissolution of all the 5-fluorouracil and for a further 0.5 h. The solution was cooled in an ice-bath

and a solution of tetra-O-acetyl- α -L-fucose (66.4 g., 0.20 mol) in dry dichloroethane (100 mL) was added. Subsequently a solution of stannic chloride (29 mL, 0.25 mol) in dry dichloroethane (25 mL) was added in small portions with vigorous shaking of the reaction mixture. After stirring for 12 h, the reaction mixture was diluted with ethyl acetate (200 mL) and water (150 mL) and neutralized with saturated sodium bicarbonate solution. The precipitate was filtered over hyflo super cel and washed with ethyl acetate to dissolve all the nucleoside. The combined organic layer was washed with water (2x200 mL), separated, dried (Na_2SO_4), filtered and concentrated to afford 3 (56.28 g, 70%): mp 158 °C (ethanol); R_f 0.4 (solvent system C); $[\alpha]_D^{20}$-40.0° (c 0.25, methanol); ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3 H, J = 7.0 Hz, H-6'), 2.00 and 2.25 (2 s, 6 H and 3 H respectively, 3 x $-COCH_3$), 4.05 (q, 1 H, J = 7.0 Hz, H-5'), 5.20 (m, 2 H, H-2' and 3'), 5.35 (d, 1 H, J = 2.0 Hz, H-4'), 5.80 (broad d, 1 H, J = 8.0 Hz, H-1'), 7.45 (d, 1 H, J = 8.0 Hz, H-6). Anal. calcd. for $C_{16}H_{19}FN_2O_9$: C, 47.76; H, 4.72; N, 6.96. Found: C, 48.15; H, 5.02; N, 6.46.

5-Fluoro-1-(6-deoxy- β -L-galactopyranosyl)uracil (4).

To stirred dry ice-cold methanol (2 L) saturated with ammonia gas was added 3 (20.10 g, 0.05 mol). After 24 h, the solution was evaporated and the residue was crystallized from ethanol to afford 4 (12.4 g, 90%): mp 288-290 °C; R_f 0.5 (solvent system F); $[\alpha]_D^{20}$ - 64.0° (c 0.25, methanol); ^{1}H NMR (CD_3OD) δ 1.00 (d, 3 H, J = 7.0 Hz, H-6'), 3.30 to 3.50 (m, 3 H, H-2', 3' and 4'), 3.60 (q, 1 H, J = 7.0 Hz, H-5'), 5.20 (dd, 1 H, J = 9.0 and 1.8 Hz, H-1'), 7.90 (d, 1 H, J = 8.0 Hz, H-6). Anal. calcd. for $C_{10}H_{13}FN_2O_6$: C, 43.48; H, 4.71; N, 10.14. Found: C, 43.27; H, 4.90; N, 10.00.

5-Fluoro-1-(6-deoxy-3, 4-O-methoxymethylene- β -L-galactopyranosyl)-uracil (5).

A solution of 4 (27.6 g, 0.1 mol), trimethyl orthoformate (54.63 mL, 0.5 mol) and p-toluenesulfonic acid (380 mg, 2.0 mmol) in dry dimethylformamide (50 mL) was stirred for 6 h. The solution was then neutralized with saturated sodium bicarbonate solution and evaporated. The residue was chromatographed over a silica gel column eluting with solvent system D to afford 5 (25.44 g, 80%) which was found to be a mixture of two diastereomers in the ratio of 1:5 (^{1}H NMR): R_f 0.45 (solvent system D). Small amount of pure major isomer was separated from the mixture by

repeated crystallization from ethyl acetate-hexane. Rest of the mixture could not be resolved due to cocrystallization of the both isomers. Major isomer: mp 172-174 °C; $[\alpha]_D^{20}$ -120.0° (c 0.10, methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 1.25 (d, 3 H, J = 7.0 Hz, H-6'), 3.30 (s, 3 H, $-OCH_3$), 3.85 (dd, 1 H, J = 7.2 and 6.3 Hz, H-3'), 4.15 to 4.25 (m, 2 H, H-4' and 5'), 4.50 (t, 1 H, J = 7.2 Hz, H-2'), 5.40 (dd, 1 H, J = 7.2 and 1.5 Hz, H-1'), 5.90 (s, 1 H, $\underline{H}COCH_3$), 7.70 (d, 1 H, J = 8.0 Hz, H-6). Anal. calcd. for $C_{12}H_{15}FN_2O_7$: C, 45,28; H, 4.71; N, 8.80. Found: C, 44.93; H, 4.79, N, 8.51. Minor isomer: ^{1}H NMR [$(CD_3)_2CO$][7] δ 3.40 (s, 3 H, $-OCH_3$), 4.10 (dd, 1 H, J = 7.2 and 6.3 Hz, H-3'), 4.30 (t, 1 H, J = 7.2 Hz, H-2'), 5.80 (s, 1 H, $\underline{H}COCH_3$).

5-Fluoro-1-(2-O-t-butyldimethylsilyl-6-deoxy-3,4-O-methoxymethylene-β-L-galactopyranosyl)uracil (6).

To a stirred mixture of t-butyldimethylsilyl chloride (9.5 g, 0.063 mol), imidazole (9.5 g, 0.139 mol) and 4-dimethylaminopyridine (500 mg, 4.09 mmol) in dry dimethylformamide (15 mL) was added 5 (15.9 g, 0.05 mol). After 15 h, solvent was evaporated, and the residue was dissolved in ethyl acetate (500 mL) and washed with water (2x200 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The oily residue was chromatographed over a silica gel column eluting with solvent system C to afford 6 (17.28 g, 80%) which was found to be a mixture of two diastereomers in the ratio of 1:10 (^{1}H NMR): R_f 0.7 (solvent system C). Small amount of pure major isomer was separated from the mixture by crystallization from chloroform. Rest of the mixture was used for subsequent reaction without further separation of the isomers. Major isomer: mp 170-174 °C; $[\alpha]_D^{20}$ -52.0° (c 0.25, methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 0.05 and 0.20 (2 s, 6 H, 2 x $-SiCH_3$), 0.90 (s, 9 H, -t-butyl), 1.30 (d, 3 H, J = 7.0 Hz, H-6'), 3.40 (s, 3 H, $-OCH_3$), 3.80 (dd, 1 H, J = 7.2 and 6.3 Hz, H-3'), 4.10 to 4.25 (m, 2 H, H-4' and 5'), 4.35 (t, 1 H, J = 7.2 Hz, H-2'), 5.55 (dd, 1 H, J = 7.2 and 1.8 Hz, H-1'), 5.95 (s, 1 H, $\underline{H}COCH_3$), 7.50 (d, 1 H, J = 8.0 Hz, H-6). Anal. calcd. for $C_{18}H_{29}FN_2O_7Si$: C, 50.00; H, 6.71; N, 6.48. Found: C, 49.68; H, 6.76; N, 6.00. Minor isomer: ^{1}H NMR [$(CD_3)_2CO$][7] δ 3.45 (s, 1 H, $-OCH_3$), 5.85 (s, 1 H, $\underline{H}COCH_3$).

5-Fluoro-1-(2-O-t-butyldimethylsilyl-6-deoxy-β-L-galactopyranosyl)-uracil (7).

To a stirred solution of 6 (21.6 g, 0.05 mol) in dry methanol (50 mL) was added p-toluenesulfonic acid till it became acidic [pH2]. After

0.5 h, the solution was neutralized with IR-45 resin and filtered. The filtrate was evaporated to afford 7 (15.6 g, 80%): mp 220-222 °C (ethyl acetate-hexane); R_f 0.25 (solvent system C); $[\alpha]_D^{20}$ -20.0° (c 0.5, methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 0.05 and 0.20 (2 s, 6 H, 2 x -$SiCH_3$), 0.80 (s, 9 H, -t-butyl), 1.30 (d, 3 H, J = 7.0 Hz, H-6'), 3.60 to 3.70 and 3.85 to 4.00 (2 m, 1 H and 3 H respectively, H-2', 3', 4' and 5'), 5.55 (broad d, 1 H, J = 9.0 Hz, H-1'), 7.95 (d, 1 H, J = 8.0 Hz, H-6). Anal. calcd. for $C_{16}H_{27}FN_2O_6Si$: C, 49.23; H, 6.92; N, 7.17. Found: C, 48.84; H, 7.16; N, 7.46.

5-Fluoro-1-(2-O-t-butyldimethylsilyl-4-O-benzoyl-6-deoxy-β-L-galactopyranosyl)uracil (8a).

A mixture of 7 (19.5 g, 0.05 mol), benzoic anhydride (22.6 g, 0.10 mol) and 4-dimethylaminopyridine (500 mg, 4.09 mmol) in dry pyridine (20 mL) was stirred for 15 min. The pyridine was evaporated and traces of it was removed by coevaporation with toluene (2x50 mL). The residue was chromatographed over a silica gel column eluting first with solvent system A to remove benzoic anhydride and then with solvent system B to afford a mixture of 8a and benzoic acid. The mixture was dissolved in ethyl acetate (200 mL), washed with a saturated sodium carbonate solution (2x50 mL) and then with water (2x50 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated to afford 8a (21 g, 85%): mp 242-244 °C (ethyl acetate-hexane); R_f 0.6 (solvent system C); $[\alpha]_D^{20}$-48° (c 0.25, methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 0.05 and 0.20 (2 s, 6 H, 2 x -$SiCH_3$), 0.80 (s, 9 H, -t-butyl), 1.20 (d, 3 H, J = 7.0 Hz, H-6'), 4.10 to 4.35 (m, 3 H, H-2', 3' and 5'), 5.50 (partially resolved d, 1 H, J = 2.0 Hz, H-4'), 5.70 (broad s, 1 H, H-1'), 7.55 (t, 2 H, J = 8.0 Hz, Ar-H, m to CO), 7.65 (t, 1 H, J = 8.0 Hz, Ar-H, p to CO), 7.90 (d, 1 H, J = 8.0 Hz, H-6), 8.20 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO).

5-Fluoro-1-(2-O-t-butyldimethylsilyl-3,4-di-O-benzoyl-6-deoxy-β-L-galactopyranosyl)uracil (8b).

A mixture of 7 (19.5 g, 0.05 mol), benzoic anhydride (90.4 g, 0.40 mol) and 4-dimethylaminopyridine (500 mg, 4.09 mmol) in dry pyridine (15 mL) was stirred. After 15 min, TLC (solvent system C) of the reaction mixture indicated complete convertion of 7 into 8a which was completely converted into 8b after 16 h. Work up and purification were done similar to that described for 8a, affording 8b (20.93 g, 70%): mp 254-225 °C (benzene-hexane); R_f 0.8 (solvent system C); $[\alpha]_D^{20}$-40.0° (c 0.25,

methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 0.05 and 0.20 (2 s, 6 H, 2 x $-SiCH_3$), 0.80 (s, 9 H, -t-butyl), 1.30 (d, 3 H, J = 7.0 Hz, H-6'), 4.50 to 4.70 (m, 2 H, H-2' and 5'), 5.55 (partially resolved broad d, 1 H, J = 8.0 Hz, H-3'), 5.65 (partially resolved d, 1 H, J = 2.0 Hz, H-4'), 5.95 (broad s, 1 H, H-1'), 7.40 (t, 2 H, J = 8.0 Hz, Ar-H, m to CO), 7.50 to 7.65 (m, 3 H, Ar-H), 7.70 (t, 1 H, J = 8.0 Hz, Ar-H, p to CO), 7.85 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO), 8.00 (d, 1 H, J = 8.0 Hz, H-6), 8.15 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO). Anal. calcd. for $C_{30}H_{35}FN_2O_8Si$: C, 60.20; H, 5.85; N, 4.68. Found: C, 60.66; H, 5.95; N, 4.92.

5-Fluoro-1-(3,4-di-O-benzoyl-6-deoxy-β-L-galactopyranosyl)uracil (9).

A solution of 8b (14.95 g, 25 mmol) in trifluoroacetic acid (45 mL) and dry methenol (5 mL) was stirred and heated at 75 °C for 6 h and then evaporated. The residue was suspended in a saturated solution of sodium carbonate (20 mL), extracted with ethyl acetate (2x100 mL) and washed with water (2x20 mL). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated. The oily residue was dried in a desiccator for 25 h to afford 9 (8.47, 70%) which was used as such for subsequent oxidation; R_f 0.4 (solvent system C); $[\alpha]_D^{20}$ -40.0° (c 0.25, methanol); ^{1}H NMR [$(CD_3)_2CO$] δ 1.30 (d, 3 H, J = 7.0 Hz, H-6'), 4.50 to 4.70 (m, 2 H, H-2' and 5'), 5.15 (broad s, 1 H, -OH), 5.65 (dd, 1 H, J = 8.0 and 2.0 Hz, H-3'), 5.75 (d, 1 H, J = 2.0 Hz, H-4'), 5.90 (d, 1 H, J = 8.0 Hz, H-1'), 7.40 (t, 2 H, J = 8.0 Hz, Ar-H, m to CO), 7.50 to 7.65 (m, 3 H, Ar-H), 7.70 (t, 1 H, J = 8.0 Hz, Ar-H, p to CO), 7.85 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO), 8.00 (d, 1 H, J = 8.0 Hz, H-6), 8.15 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO), 10.65 (broad s, 1 H, NH).

5-Fluoro-1-(3-O-benzoyl-4,6-dideoxy-β-L-glycero-hex-3-enopyranos-2-ulosyl)uracil (10).

A mixture of 9 (4.84 g, 10 mmol), pyridinium dichromate (6 g, 15.95 mmol), and 3 Å molecular sieves (5 g, freshly powdered and dried over phosphorus pentoxide in vacuo at 360 °C for 10 min) was stirred in dry dichloromethane (100 mL) under anhydrous condition for 6 h and then diluted with ethyl acetate (250 mL). After stirring for 0.5 h, the reaction mixture was filtered through a 2.0 cm thick layer of fine (~40 μm) silica gel and the silica gel was washed with ethyl acetate (200 mL). The filtrate was evaporated and the residue was chromatographed over a silica gel column eluting with solvent system D to afford

10 (2.16 g, 60%): mp 194-195 °C (benzene); R_f 0.55 (solvent system E); $[\alpha]_D^{20}$ + 40.0° (c 0.25, methanol); ^{1}H NMR ($CDCl_3$) δ 1.55 (d, 3 H, J = 7.0 Hz, H-6'), 5.00 (m, 1 H, H-5'), 6.45 (broad s, 1 H, H-4'), 6.95 (d, 1 H, J = 1.5 Hz, H-1'), 7.25 (d, 1 H, J = 8.0 Hz, H-6), 7.50 (t, 2 H, J = 8.0 Hz, Ar-H, m to CO), 7.65 (d, 1 H, J = 8.0 Hz, Ar-H, p to CO), 8.15 (d, 2 H, J = 8.0 Hz, Ar-H, o to CO). Anal. calcd. for $C_{17}H_{13}FN_2O_6$: C, 56.66; H, 3.61; N, 7.77. Found: C, 56.81; H, 3.62; N, 7.70.

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