

Dedicated to the memory of Professor Sorin Roșca (1938–2021)

HIGH YIELD SYNTHESIS OF α -D-GLUCOPYRANOSYL 5-FLUOROURACIL WITHOUT COLUMN CHROMATOGRAPHY PURIFICATION

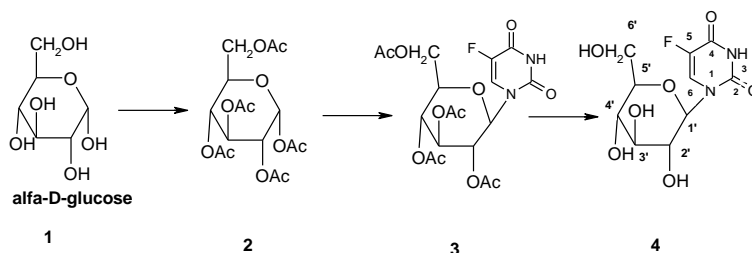
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Glucopyranosyl-5-fluorouracil was synthesized by a sequence of three high yield reactions: acetylation of α -D-glucose, substitution of pentaacetyl-glucopyranose **2** with 2,4-bis-trimethylsilyl-5-fluorouracil and hydrolysis of the tetracetylated nucleoside **3** to β -D-glucopyranosyl-5-fluorouracil **4**. In all sequence, the compounds were pure isolated by simple crystallization.



INTRODUCTION

Glucopyranosyl-5-fluorouracil was studied for its anticancer activity,¹ inhibitor for glycogen phosphorylase,² for its action as an immunoregulator (it has an effect of increasing the inhibition of immunoglobulin production),³ etc.

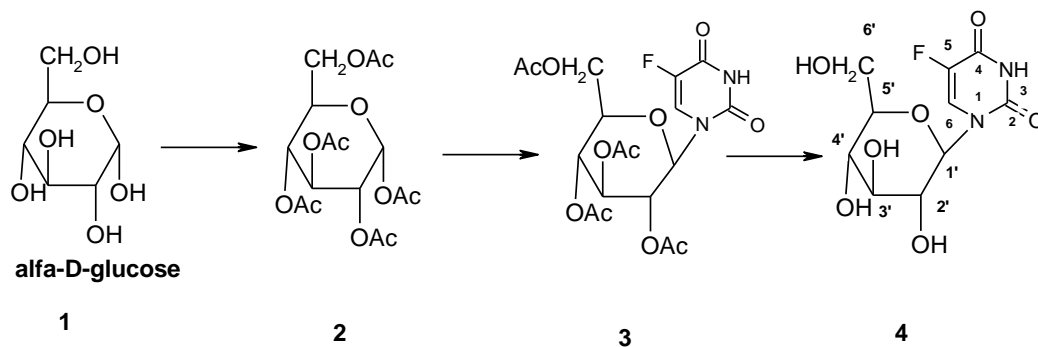
We had to synthesize quickly glucopyranosyl-5-fluorouracil in around ten grams quantity to be used by a partner in an anticancer study. Browsing the literature data, we found a few synthesis variants, which use mainly as starting glucose intermediates pentaacetylglucose,³⁻⁶ tetraacetyl-1-bromide,⁷ or 1-chloro,⁸ 1-acetyl-tetrabenzoate glucose. In these syntheses, the purification of the compounds is performed by column chromatography, which needs time and solvents. We chose to start the synthesis of glucopyranosyl-5-fluorouracil from pentaacetylglucose, easily obtained from α -D-glucose, by

Vorbrueggen reaction (a Hilbert-Johnson type reaction) with bis-O-trimethylsilylated 5-fluorouracil, followed by deprotection of the acetyl groups, as shown in Scheme 1. To our surprise, we found that all three steps of the synthesis could be performed without column chromatography purification, the isolation of all compounds was done by crystallization; the compounds remained in the mother liquors was not isolated.

RESULTS AND DISCUSSION

The proposed synthesis of glucopyranosyl-5-fluorouracil, presented in Scheme 1, was realized in three steps: 1) acetylation of α -D-glucose **1**, 2) N-glycosylation of O²,O⁴-bis-trimethylsilylated 5-fluorouracil and 3) hydrolysis of 2',3',4',6' acetyl groups.

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Scheme 1 – Synthesis of glucopyranosyl-5-fluorouracil from α -D-glucose.

The pentaacetyl-glucose **2** was obtained by acetylation of α -D-glucose **1** with acetic anhydride and pyridine^{6,9} or sodium acetate¹⁰ as base and the product was isolated by crystallization^{9b} or by flash chromatography purification.¹⁰ A review article presents the synthetic methods to obtain α -D-glucose pentaacetate, even enzymatic methods.¹¹ We acetylated α -D-glucose **1** by a published procedure^{9b} (but using a longer time for acetylation, 72 h, instead of 24 h) and isolated pentaacetyl-glucose by simple crystallization in 82.8% yield. The mother liquors and the crude product obtained by extraction of aqueous phase was reused in another reaction with the same quantities of α -D-glucose, acetic anhydride and pyridine and pentaacetyl-glucose was isolated by crystallization in 96.9% yield, in a combined yield of 89.8% for both reactions.

The next well known Vorbrueggen reaction (a silyl-Hilbert Johnson reaction) for synthesis of nucleosides involve the reaction of pentaacetylglucose with 2,4-bis-trimethylsilyl-5-fluorouracil in the presence of a stoichiometric amount (required to secure high-yielding N-glycosylation) of a strong Lewis acid (known also as a Friedel-Crafts catalysts) promoter (*e.g.*, SnCl_4 ,⁴ TMSOTf ^{8b} or $\text{BF}_3 \cdot \text{OEt}_2$). The literature mentioned a few catalysts, instead of the Lewis acid promoters, like 2-methyl-5-phenylbenzoxazolium perchlorates,^{6,11} solid-supported acidic oxazolium perchlorates,^{5, 12} which gave high-yield glycosylation (91-98%), but these methods were not taken into consideration. 2,4-Bis-trimethylsilyl-pyrimidines are generally obtained by trimethylsilylation of pyrimidines with hexamethyldisilazane in the presence of $(\text{NH}_4)_2\text{SO}_4$,^{8a} saccharin,² trimethylsilyl chloride,⁴ pyridine,⁶ with or without a solvent (like 1,2-dichloroethane) at rt or by heating at elevated temperature, sometimes by using MW.¹¹ In final, hexamethyldisilazane in excess is removed by distillation.

We silylated 5-fluorouracil with hexamethyldisilazane and trimethylchlorosilane at rt in acetonitrile, in the presence of pentaacetylglucose **2**, and then 2 equivalents of SnCl_4 in acetonitrile was added during 15 min. under stirring. Stirring was continued at 45-55°C over the weekend (~72h) and, after work-up, the crude product was crystallized from dichloromethane-hexane, resulting directly the pure compound **3** in 85.0% yield. The compound remained in the mother liquor was not isolated. Usually, the excess hexamethyldisilazane is removed before the substitution,^{6,8} but we added SnCl_4 directly to the trimethylsilylation reaction mixture, as we observed in the literature.⁴ The excess of SnCl_4 (2 equivalents instead of 1.37) and a longer reaction time (72 h, instead of 24 h) increased not only the yield of compound **3**, from 65% (purified by flash chromatography) to greater than 85.0%, by also made easier the isolation. The compound **3** was obtained pure by simple crystallization.

As the final step, the acetyl groups of the compound **3** were removed by hydrolysis with 5M NH_3 in MeOH for 72 h (over weekend). The crude compound was crystallized from ethanol-dichloromethane, resulting the pure nucleoside compound **4** in 93.5% yield.

In this sequence, the intermediate compounds, **2** and **3**, and the final nucleoside **4** were obtained in very good yields only through crystallization and this route makes it one of the most attractive options to quickly synthesize 5-fluorouracil-glucoside. No flash chromatography was used to recover the intermediates or the final compound, which remained in the mother liquors.

EXPERIMENTAL

The progress of the reactions was monitored by TLC on Merck silica gel 60 or 60F₂₅₄ plates eluted with the solvent

systems: I, ethyl acetate-hexane-acetic acid, 5:4:0.1; II, dichloromethane-methanol, 9:1, III, ethyl acetate-hexane-acetic acid, 5:1:0.1; IV, chloroform-methanol, 95:5. Spots were developed in UV and with 15% H₂SO₄ in MeOH (heating at 110°C, 10 min.). IR spectra were recorded on FT-IR Bruker Vertex 70 spectrometer by ATR and frequencies were expressed in cm⁻¹, with the following abbreviations: w = weak, m = medium, s = strong, v = very, br = broad. ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker 300 and 500 MHz spectrometer, chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra 2D-NMR were made for the correct assignment of NMR signals. The numbering of the atoms in the compounds is presented in Scheme 1.

1. Synthesis of (2R,3R,4S,5R,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetraol tetraacetate

The compound was synthesized based on the literature data^{9b}: to α -D-glucose (22.1 g, 0.1224 mol) in pyridine (154 mL, 1.92 mol), cooled on an ice-water bath, acetic anhydride (110 mL, 1.089 mol) was added in four portions. The reaction mixture warmed to about 45°C and α -D-glucose dissolved in ~ 30 min. The reaction mixture was stirred over weekend (72 h) at rt (α -D-glucose was dissolved in ~ 30 min), monitoring the reaction mixture by TLC (dichloromethane-methanol, 95:5, R_f 2 = 0.76). The reaction mixture was poured onto crashed ice (400g) and water (400 mL) and stirred ~ 1.5 h. The crystallized compound **2** was filtered off, washed with water (3x100 mL) and dried at rt, resulting 39.58 g (82.8% yield) of pure pentaacetylglucose [(2R,3R,4S,5R,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetraol tetraacetate], **2**, m.p. = 107.1-109.3°C (Catalog Sigma: m.p. = 109-111°C), [α]_D 98.9° (1% in CDCl₃) (Catalog Sigma: [α]_D >= 98° (1% in ethanol)), IR: 1736vs, 1363m, 1233s, 1234vs, 1105m, 1032s, 1018s, 938m, ¹H-NMR-300 MHz (CDCl₃, δ ppm, *J* Hz): 6.26 (d, 1H, H-1, 3.9), 5.40 (t, 1H, H-3, 9.9), 5.07 (t, 1H, H-2, 10.2), 5.03 (dd, 1H, H-4, 4.6, 10.2), 4.20 (dd, 1H, H-6, 4.1, 12.4), 4.08-4.00 (m, 2H, H-5, H-6), 2.11 (s, 3H, CH₃CO), 2.03 (s, 3H, CH₃CO), 1.97 (s, 3H, CH₃CO), 1.96 (s, 3H, CH₃CO), 1.95 (s, 3H, CH₃CO), ¹³C NMR-75 MHz (CDCl₃, δ ppm): 170.81, 170.39, 169.82, 169.56, 168.93 (COCH₃), 89.15 (C-1), 76.74 (C, C-3), 72.27 (C-5), 69.27 (C-2), 67.94 (C-4), 61.54 (C-6), 21.02, 20.83, 20.80, 20.70, 20.58 (5s, CH₃CO); NMR is closely to the literature data.^{7b,10}

The filtrate was extracted with EtOAc (3x150 mL), the organic phases were washed with saturated soln. NaHCO₃ (150 mL), brine (2x100 mL), dried (Na₂SO₄) and concentrated in vacuo. The resulting residue was recycled in another reaction with the same quantities of reagents. In the second reaction, 46.3 g (96.9 % yield) pure pentaacetyl-glucose, **2**, were obtained (total amount for both reactions 85.88 g, 89.8 % yield).

2. Synthesis of (2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate, **3**

Pentaacetyl-glucose **2** (40 mmol, 15.6 g) and 5-FU (46 mmol, 6g) were dissolved in 400 mL acetonitrile, hexamethyldisilazane (48 mmol, 10 mL) and then trimethylsilyl chloride (50 mmol, 6.4 mL) were added under argon and the mixture was stirred for 50 min. A solution of 9.9 mL (22.04 g, 84.6 mmol) SnCl₄ in 40 mL acetonitrile was added in 15 min. (the solution became clear), then the solution was stirred at 45-55°C for

72 h (over weekend), monitoring the evolution of the reaction by TLC (dichloromethane-methanol, 95:5, R_f 2 = 0.76, R_f 3 = 0.48). The bulk of the acetonitrile was distilled under reduced pressure, 200 mL ethyl acetate was added, the solution was cooled on an ice-bath, 250 mL water were added under stirring, the clear phases were separated, the organic phase was washed with sat. soln. NaHCO₃ (100 mL), brine (100 mL), dried (Na₂SO₄), filtered and concentrated under reduced pressure (The aqueous phases were extracted with 150 mL ethyl acetate and unified with the organic phase). The product was crystallized from dichloromethane-hexane, resulting 15.67 g (85.0%) pure compound **3**, (2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate, m.p. = 155.3-157.6°C [lit⁹, mp 100-102°C], ¹H-NMR-300 MHz (CDCl₃, δ ppm, *J* Hz): 11.56 (s, 1H, NH), 7.56 (d, 1H, H-6, 6.0), 5.82 (dd, 1H, H-1', 1.6, 9.3), 5.34 (t, 1H, H-3', 9.3), 5.18 (t, 1H, H-2', 9.3), 5.12 (t, 1H, H-4', 9.3), 4.18 (dd, 1H, H-6', 5.2, 12.6), 4.05 (dd, 1H, H-6', 2.5, 12.6), 3.90 (m, 1H, H-5'), 2.02 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO), 1.95 (s, 3H, CH₃CO), 1.93 (s, 3H, CH₃CO), ¹³C NMR-75 MHz (CDCl₃, δ ppm): 170.16, 169.38 (2C), 169.14 (COCH₃), 156.7 (C-4, *J* = 26.6 Hz), 156.09 (C-2), 140.57 (d, C-5, *J* = 237.9), 123.29 (d, C-6, *J* = 34.3), 80.06 (C-1'), 74.29 (C-3'), 72.27 (C-5'), 69.04 (C-2'), 67.45 (C-4'), 61.47 (C-6'), 20.42, 20.20, 20.08 (3s for 4 CH₃CO groups). For NMR see ref.^{4,6}

3. 5-fluoro-1-((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)pyrimidine-2,4(1H,3H)-dione, **4**

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate, **3**, (15.67 g, 34 mmol) were dissolved in 5M NH₃ in MeOH (250 mL) and stirred over weekend, monitoring the end of the reaction by TLC (dichloromethane-methanol, 4:1, R_f 3 = 0.96, R_f 4 = 0.55). The volatiles were removed under reduced pressure, co-evaporated with anhydrous ethanol and crystallized from ethanol-dichloromethane, resulting 9.3 g (93.5%) (in two fractions) of pure crystallized compound **4**, m.p. 235.8-238.6°C, [α]_D = +51.5° (1% in water), IR: 3605m (v_{OH}), 3328vs (v_{NH} disassociated), 3035m (v_{=CH}), 2919m (v_{CH2} asym), 2871s-2818sm (v_{CH2} sym), 1715vs (v_{C=O}), 1673vs (v_{C=O}), 1601s (v_{C=C}), 1475s (δ CH₂), 1370s (v_{CH2}), 1088s (v_{C-F}), 1016s (v_{CO}), 883s (δ -CH), ¹H-NMR-500 MHz (DMSO-*d*₆, δ ppm, *J* Hz): 11.86 (s, 1H, NH), 8.11 (d, 1H, H-6, 7.0), 5.35 (s, OH), 5.31 (d, 1H, 1H, H-1', 9.2), 5.20 (brs, 1H, OH), 5.07 (brs, 1H, OH), 4.54 (brt, 1H, OH-6'), 3.69 (d, 1H, H-6', 11.7), 3.43 (brt, 2H, H-6', H-2', 6.9), 3.29 (t, 2H, H-3', H-5', 8.2), 3.17 (t, 1H, H-4', 9.1), ¹H-NMR-500 MHz (DMSO-*d*₆ + TFA, δ ppm, *J* Hz): 11.82 (s, 1H, NH), 8.05 (d, 1H, H-6, 6.9), 5.32 (d, 1H, H-1', 9.1), 3.69 (d, 1H, H-6', 11.7), 3.45 (dd, 1H, H-6', 5.7, 11.7), 3.42 (brt, 1H, H-2', 9.2), 3.31 (t, 2H, H-3', H-5', 8.7), 3.19 (t, 1H, H-4', 9.1), ¹³C NMR-125 MHz (DMSO-*d*₆, δ ppm, *J* Hz): 157.09 (C-4, *J* = 25 Hz), 149.56 (C-2), 140.09 (d, C-5, *J* = 228.7 Hz), 125.92 (d, C-6, *J* = 25 Hz), 82.87 (C-1'), 79.86 (C-3'), 76.60 (C-5'), 70.75 (C-2'), 69.35 (C-4'), 60.95 (C-6').

¹H-NMR presents all protons linked to carbon atoms and to oxygen atoms of the hydroxyl groups. The hydroxyl protons were moved (shifted) with TFA to higher field, and the protons 6' and 2' appears as a dd and a brt. The ¹³C-NMR presents clearly the signals for the carbon atoms of the molecule, and HETCOR presents the correlation between the carbon atoms and the hydrogen atoms (Figure 1). These spectra confirm the structure of the molecule.

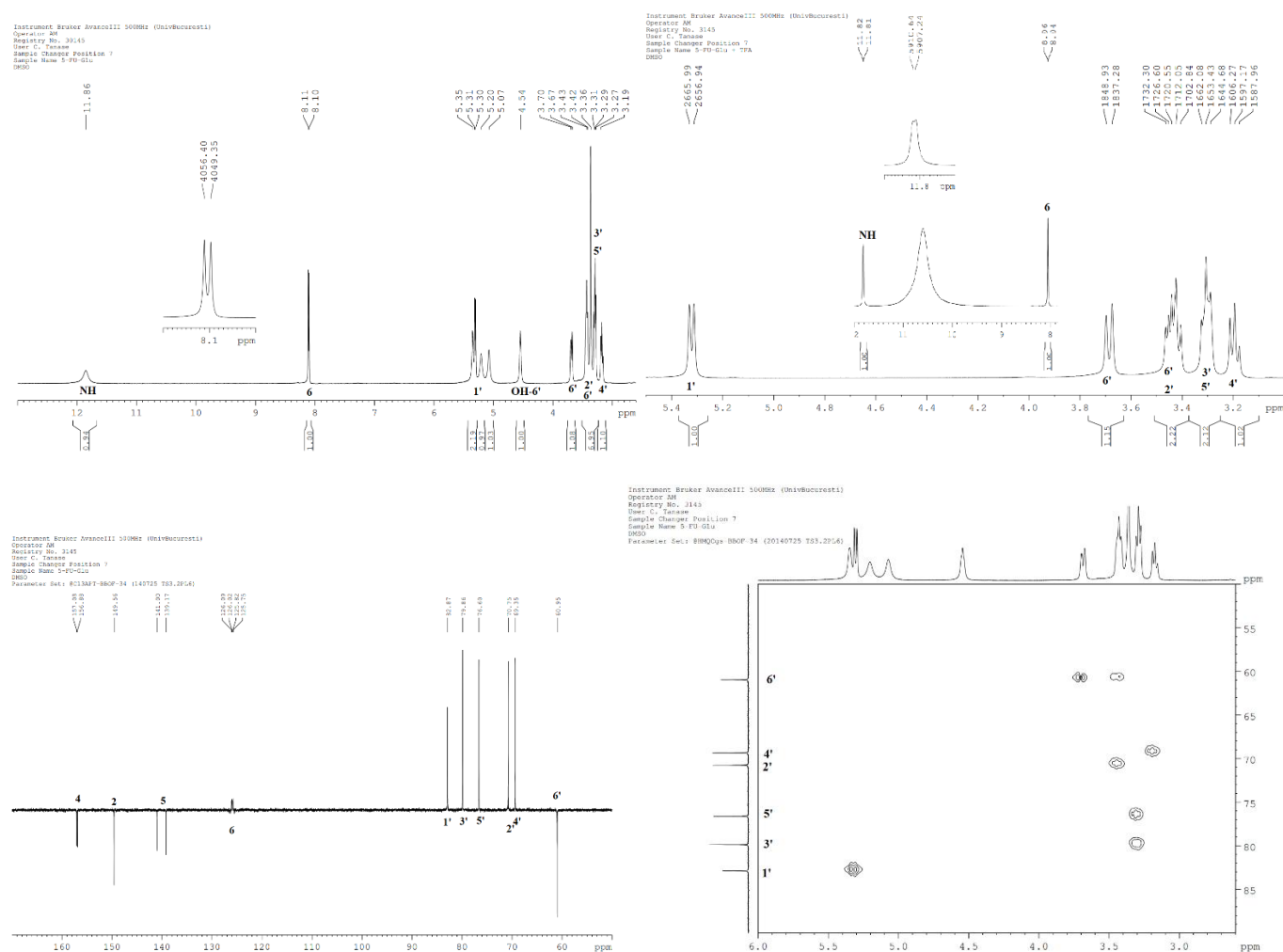


Fig. 1 – ^1H , ^1H (+TFA)- ^{13}C -, and 2D-NMR in $\text{DMSO}-d_6$ spectra of the nucleoside compound **4**.

CONCLUSIONS

Glucopyranosyl-5-fluorouracil was quickly and efficiently synthesized from α -D-glucose in three high yield reactions. In the first step, the acetylation of α -D-glucose was simple realized with acetic anhydride in pyridine, and the crystallized compound was isolated by filtration. In the second step, 5-fluorouracil was trimethylsilylated with hexamethyldisilazane and trimethylsilyl chloride, in the presence of pentaacetylglucose, and the next Vorbrueggen reaction of 2,4-bis-trimethylsilyl-5-fluorouracil with **2** was performed with an excess of SnCl_4 (2 equivalent /pentaacetylglucose **2**) for 72 hours; the tetraacetylated glucopyranosyl 5-fluorouracil was pure isolated by simple crystallization from dichloromethane-hexane in very good yield (85.0%). The final step, the hydrolysis of acetyl groups, was realized with 5M NH_3 in methanol at rt, and the pure product **4**

was crystallized from ethanol-dichloromethane in very good yield (93.5%). All steps are realized in very good yields and the isolation of pure compounds was achieved only by crystallization. The compound remained in the mother liquors was not isolated. The sequence is easy and productive.

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