Carbohydrate Research, 62 (1978) 185–190

Note

An alternative, convenient synthesis of 1-(2-amino-2-deoxy-β-D-glucopyranosyl)uracil*

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(Received June 20th, 1977; accepted for publication, August 8th, 1977)

Following the first reports on the interference of tunicamycin with the biosynthesis of glycoproteins in mammalian[1] and microbial cells[2], this inhibition has been extensively investigated[3–8]. Apparently, the mechanism of the suppression involves the blocking of the formation of the lipid intermediate, P^1-(2-acetamido-2-deoxy-α-D-glucopyranosyl) P^2-poly(isoprenyl) diphosphate[7]. So far, the exact structure of tunicamycin is not known, except that it probably contains (per molecule), at least one uracil residue, one 2-acetamido-2-deoxy-D-glucopyranose residue, and one 2-amino-2-deoxy-D-glucose residue acylated with an unsaturated fatty acid[9]. In order to test the biological activity of similar compounds, especially the role of the fatty acid residue, it was necessary to develop a convenient synthesis of the known[10] starting compound, 1-(2-amino-2-deoxy-β-D-glucopyranosyl)uracil (**11**). The use of a mercury derivative of uracil for direct glycosylation at N-1, as successfully developed for the synthesis of 9-(2-amino-2-deoxy-D-glucopyranosyl)adenine[11], was avoided because of the difficulty in preparing the pure mercury salt of uracil[12]. The alternative method that is based on the reaction of a trimethylsilylated purine derivative with a glycosyl halide in the presence of mercuric cyanide, to give a purine nucleoside in high yield[13], appeared more convenient for the synthesis of **11**. As readily removable, and conveniently introduced, N-protecting groups for 2-amino-2-deoxy-D-glucose, the trifluoroacetyl[14] and 2,4-dinitrophenyl[15,16] groups were selected.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl (**4**) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[16,17] (**5**) was each condensed with 2,4-bis(trimethylsilyl)uracil[18,19] (**6**) in the presence of

*Amino Sugars CXIII. This is publication No. 745 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School and Massachusetts General Hospital. This investigation was supported by Grant AM-03564 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health.

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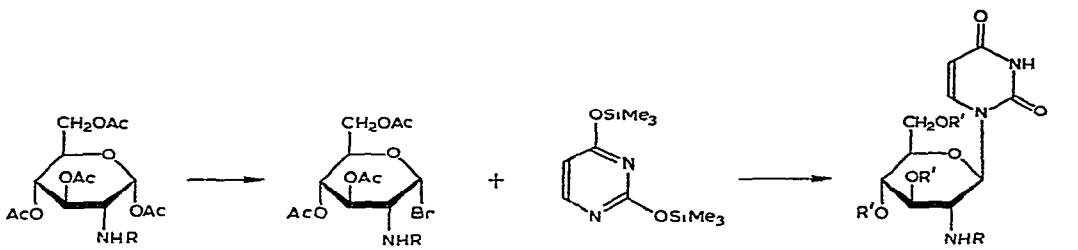
mercuric cyanide, to give 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]uracil (**7**) and 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]uracil (**8**), respectively. The bromide **4** was prepared by treatment of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride[20] (**1**) with trifluoroacetic anhydride in the presence of pyridine, to give 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**2**), which was treated with hydrogen bromide in acetic acid to afford the bromide **4** (not isolated).

In the condensation of **6** with the bromides **4** and **5**, mercuric cyanide appeared to prompt the decomposition of the bromides, and the yield of **7** and **8** was not sufficiently high for further investigation.



1 R = H • HCl
2 R = CF_3CO
3 R = DNP

4 R = CF_3CO
5 R = DNP

6

7 R = CF_3CO, R' = Ac
8 R = DNP, R' = Ac
9 R = CF_3CO, R' = H
10 R = DNP, R' = H
11 R = R' = H

DNP = 2,4-$(NO_2)_2C_6H_3$

In a second approach, the trimethylsilyl derivative **6** was directly coupled with 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranose[21] (**3**) in the presence of stannic chloride, according to the method of Niedballa and Vorbrüggen[22], to give **8** rapidly. Under similar conditions, **6** reacted with **4** to give **7** in high yield.

Treatment of **7** and **8**, respectively, with methanolic triethylamine at room temperature gave the *O*-deacetylated nucleoside derivatives **9** and **10**, respectively. Prolonged treatment of **7** with methanolic ammonia afforded the completely deacylated derivative 1-(2-amino-2-deoxy-β-D-glucopyranosyl)uracil[10] (**11**). On treatment of the protected nucleoside derivative **8** with barium hydroxide solution, **11** was also obtained, but the yield was inferior.

EXPERIMENTAL

General methods. — Evaporations were performed *in vacuo*. Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting points". Rotations were determined for solutions in a 1-dm semimicro tube with a Perkin–Elmer Model 141 polarimeter. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model 237. The

homogeneity of compounds was ascertained by ascending t.l.c. on precoated plates of Silica Gel (Merck), with solvents (v/v): *A*, 19:1 chloroform–ethanol; *B*, 4:1 benzene–acetone; *C*, 1:1 benzene–ethyl acetate; *D*, 19:1 chloroform–methanol; *E*, 1:1 methanol–ethyl acetate; and *F*, 7:3 ethyl acetate–ethanol. Spots were detected by spraying the plates with 20% sulfuric acid and heating for a few min at 200°. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**2**). — To a suspension of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[20] (**1**, 6.5 g) in dichloromethane (65 ml) and pyridine (10 ml) was added, with cooling and stirring, trifluoroacetic anhydride (10 ml). After being stirred overnight, the solution was washed with ice–water, dried (sodium sulfate), and evaporated. The resulting syrup crystallized from ethanol–ether–hexane, to yield long needles (5.2 g, 69%), m.p. 119–120°, $[\alpha]_D^{21}$ +66° (*c* 1.5, chloroform); ν_{max}^{KBr} 3350 (NH), 1750 (OAc), 1730, 1710, 1550, 1375, 1225, and 1175 cm^{-1} (ester); t.l.c. (solvent *A*): R_F 0.3.

Anal. Calc. for $C_{16}H_{20}F_3NO_{10}$: C, 43.35; H, 4.55; F, 12.86; N, 3.16. Found: C, 43.39; H, 4.45; F, 12.70; N, 3.14.

2,4-Bis(trimethylsilyl)uracil[18,19] (**6**). — This compound was prepared by a slight modification of the method of Wittenburg[18]. To a suspension of uracil (1.12 g) in hexamethyldisilazane (6 ml) were added a few drops of chlorotrimethylsilane. The mixture was boiled under reflux with exclusion of moisture for 2 h, or until the suspension had clarified. The hexamethyldisilazane was evaporated, and anhydrous toluene (20 ml) was repeatedly added and evaporated. The residue (2.5 g) was used without further purification.

1-[3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]uracil (**7**). — *A*. To a solution of **6** (2.5 g) in toluene (30 ml) were added **4** (2.3 g; obtained by treatment of **2** with hydrogen bromide in acetic acid, and used without isolation) and mercuric cyanide (1.25 g). The mixture was boiled under reflux for 3 h, and then stirred overnight at room temperature. The solvents were evaporated *in vacuo*, and the residue was extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated, to give a syrup (600 mg) that showed several spots (R_F 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0) in t.l.c. (solvent *D*), and was resolved on chromatoplates (20 × 20 cm, thickness 2 mm). The zone having R_F 0.4 (solvent *A*) and 0.3 (solvent *D*) was removed, and extracted with chloroform. Evaporation of the extract gave **7** as a syrup that crystallized from ethanol–ether–hexane (250 mg, 10%), m.p. 149–150°, $[\alpha]_D^{21}$ +22.7° (*c* 1.2, chloroform); ν_{max}^{KBr} 1750 (OAc), 1725 (CO of uracil), 1575, 1635 (uracil C=C), and 1375 cm^{-1} (CF_3); λ_{max}^{EtOH} 257 nm (ε_{mM} 12.15); t.l.c. (solvent *A*): R_F 0.4.

Anal. Calc. for $C_{18}H_{20}F_3N_3O_{10}$: C, 43.64; H, 4.07; F, 11.51; N, 8.48. Found: C, 43.55; H, 4.10; F, 11.44; N, 8.40.

B. To a solution of **6** (2.5 g) in acetonitrile (15 ml) were added **2** (2 g) and a solution of stannic chloride (0.2 ml) in dry 1,2-dichloroethane (2 ml), and the mixture was boiled under reflux for 4 h. Stannic chloride (0.4 ml) in dry 1,2-dichloroethane

(2 ml) was then added, and the mixture was boiled under reflux for 4 h, cooled, and evaporated. A solution of the residue in ethyl acetate (150 ml) was treated with a cold, saturated solution of sodium hydrogencarbonate until the solution was neutral, the suspended, inorganic material was filtered off, and the ethyl acetate layer was separated from the filtrate, washed with water, dried (sodium sulfate), and evaporated. The residual syrup was dissolved in the minimal volume of ethanol, and the solution diluted with ether and hexane, to afford **7** (1.7 g, 80%), m.p. 149–150° (with shrinking at 128–130°); the i.r. spectrum and t.l.c. mobility were identical with those of the compound described under *A*.

1-[3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]uracil (**8**). — *A*. To a solution of **6** (2.5 g) in toluene (30 ml) were added 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[17] (**5**, 4 g) and mercuric cyanide (2.4 g). The mixture was stirred and boiled under reflux for 3 h, and then kept for 24 h at room temperature. The dark-brown mixture was evaporated, the residue was extracted with chloroform (250 ml), and the undissolved material was filtered off. The filtrate was washed repeatedly with 30% aqueous potassium iodide to remove the mercury cyanide, dried (sodium sulfate), and evaporated, to give an amorphous powder (4 g); t.l.c. (solvent *B*) showed one main, yellow component (R_F 0.2) and several other spots (R_F 0.38, 0.4, 0.7, and 1). The crude product (1 g) was resolved on five preparative chromatoplates (20 × 20 cm, thickness 2 mm), and the zone having R_F 0.2 was removed, and extracted with acetone. Evaporation of the extract gave **8** as a glass that crystallized from ethanol (yield 200 mg, 19%), m.p. 258–260° (shrinking at 156–158°), $[\alpha]_D^{21}$ +71° (*c* 0.4, chloroform); ν_{max}^{KBr} 1740 (OAc), 1620, 1590 (aryl C=C), 1525, 1330 (NO_2), and 550 cm^{-1} (substituted benzene); λ_{max}^{EtOH} 257 (ε_{mM} 11.95) and 340 nm (ε_{mM} 10.30); t.l.c. (solvent C): R_F 0.3.

Anal. Calc. for $C_{22}H_{23}N_5O_{13}$: C, 46.73; H, 4.10; N, 12.38; O, 36.78. Found: C, 46.70; H, 4.08; N, 11.97; O, 36.66.

B. To a solution of **6** (2.5 g) in acetonitrile (15 ml) were added **3** (2.1 g) and a solution of stannic chloride (0.2 ml) in dry 1,2-dichloroethane (2 ml). The mixture was boiled under reflux for 4 h, and re-treated with stannic chloride (0.4 ml) in dry 1,2-dichloroethane (2 ml) under reflux for 4 h. The dark-brown mixture was evaporated, the residue was dissolved in ethyl acetate, and the solution treated with a cold, saturated solution of sodium hydrogencarbonate until it was neutral. The suspension was filtered, and the ethyl acetate layer in the filtrate was separated, washed with water, dried (sodium sulfate), and evaporated, to give a glass (1.8 g) that, in t.l.c. (solvent *C*), showed one major, yellow component (R_F 0.3) and several other yellow spots. The crude product (600 mg) was resolved on chromatoplates, as described earlier, to give **8** after crystallization from methanol (yield 350 mg, 45%), m.p. 258–260° (shrinking at 156–158°); the i.r. spectra and t.l.c. mobility were identical with those of the compound described under *A*.

1-[2-Deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]uracil (**9**). — A solution of **7** (100 mg) in methanol (9 ml) was treated with triethylamine (1 ml) for 24 h at room temperature, evaporated (hot-water bath) to dryness under a stream of nitrogen,

and the residue crystallized from 2-propanol–ether, to give **9** (65 mg, 90%), m.p. 186–187° (with shrinking at 142°), $[\alpha]_D^{21}$ +35° (*c* 1.3, ethanol); ν_{max}^{KBr} 3400–3250 (OH, NH), and 1700, 1560 cm^{-1} (uracil C=C); λ_{max}^{EtOH} 257 nm (ε_{mM} 13.00); t.l.c. (solvent *F*): R_F 0.75.

Anal. Calc. for $C_{12}H_{14}F_3N_3O_7$: C, 39.03; H, 3.82; N, 11.38. Found: C, 38.97; H, 3.92; N, 11.24.

1-[2-Deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]uracil (**10**). — Triethylamine (0.5 ml) was added to a solution of **8** (50 mg) in methanol (4.5 ml). After 24 h at room temperature, the solution was evaporated. Attempts to crystallize the amorphous residue (35 mg, 70%) were unsuccessful. An analytical sample was purified by dissolution in ethanol and precipitation with ether; m.p. 180–210° (dec.), $[\alpha]_D^{21}$ −83° (*c* 0.9, methanol); ν_{max}^{KBr} 3450–3300 (OH, NH), 1690, 1610, 1590 (aryl C=C), 1525, 1330 (NO_2), and 550 cm^{-1} (substituted benzene); λ_{max}^{EtOH} 257 (ε_{mM} 13.40) and 345 nm (ε_{mM} 12.80); t.l.c. (solvent *F*): R_F 0.27.

Anal. Calc. for $C_{16}H_{17}N_5O_{10} \cdot H_2O$: C, 44.16; H, 4.36; N, 15.15; O, 36.33. Found: C, 43.85; H, 4.44; N, 14.57; O, 35.94.

1-(2-Amino-2-deoxy-β-D-glucopyranosyl)uracil (**11**). — *A.* Triethylamine (1 ml) was added to a solution of **7** in methanol (9 ml). After 4 days at room temperature, the solution was evaporated, and the residue was dissolved in methanol (20 ml). Dry ammonia was passed through the solution for 1 h at 0°, and, after 4 days at room temperature the solution was evaporated. To the residue was twice added ethanol (20 ml), followed by evaporation, and the amorphous powder then crystallized from ethanol–water (yield 35 mg, 63%), m.p. 245–246° (dec.), $[\alpha]_D^{21}$ +11.7° (*c* 0.5, water); {lit.[10] m.p. 241–242° (dec.), $[\alpha]_D^{25}$ +15.66° (water)}; λ_{max}^{KBr} 3500, 3350 (OH), 3290 and 3250 (NH, NH_2), 1725, 1675 (uracil CO), and 1600–1475 cm^{-1} (uracil C=C); $\lambda_{max}^{H_2O}$ 257 nm (ε_{mM} 12.25); t.l.c. (solvent *E*): R_F 0.3; descending paper chromatography with Whatman No. 4 paper (6:4:3 butanol–pyridine–water): R_F 0.39.

Anal. Calc. for $C_{10}H_{15}N_3O_{16}$: C, 43.96; H, 5.53; N, 15.38; O, 35.13. Found: C, 43.82; H, 5.60; N, 15.33; O, 34.97.

B. A mixture of **8** (200 mg) and barium hydroxide (1.0 g) in water (30 ml) was boiled under reflux for 2 h. The mixture was cooled, and treated with M sulfuric acid (to pH 3.0), the barium sulfate was filtered off, and the orange filtrate was washed with ethyl acetate (3 × 10 ml). The aqueous layer was stirred with barium carbonate (to give pH 8.0), and filtered through Celite. The filtrate was evaporated to dryness, and the residue crystallized from ethanol–water, to yield **11** (50 mg, 52%), m.p. 244–245° (dec.) (softening at 235°); the i.r. spectra and t.l.c. mobility were identical with those of the compound described under *A*.

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