Synthesis of 1-(4-Amino-4-deoxy- β -D-glucopyranosyl)uracil

By Tadao KONDO and Toshio GOTO

*Department of Agricultural Chemistry, Faculty of Agriculture
Nagoya University, Chikusa, Nagoya*

Received September 10, 1970

Discovery of the antibiotics such as gougerotin and blasticidin S has drawn a growing attention to the synthesis of the nucleosides containing 4-amino-4-deoxy sugar moiety. Recently Fox *et al.*¹⁾ have succeeded in synthesizing a cytosine nucleoside, 1-(4-amino-4-deoxy- β -D-glucopyranosyl)cytosine, in the course of a total synthesis of C-substance, a degradation product of gougerotin. In this report a synthesis of 1-(4-amino-4-deoxy- β -D-glucopyranosyl)uracil (I) is described.

Fox *et al.*¹⁾ used as a starting material for the synthesis of the cytosine nucleoside methyl 2,3,6-tri-O-benzoyl-4-O-mesyl- α -D-galactoside, 4-position of which was already differentiated from 2, 3, and 6 positions by introduction of a mesyl group, which was finally converted to an amino group. Our route consists in the synthesis of an uracil nucleoside from acetobromogalactose followed by differentiation of the 4-position of the sugar moiety of the nucleoside by selective benzylation.

Condensation of acetobromogalactose with 2,4-(dimethylsiloxy)pyrimidine²⁾ afforded a mixture of nucleosides. Since this condensation was accompanied with partial deacetylation, the product was acetylated with acetic anhydride and pyridine to give a mixture of α - and β -anomers of 1-(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl)uracil in a ratio of *ca.* 1:4 (calculated from its NMR integration

curve). Crystallization from isopropanol gave the β -anomer (II) in nicely crystalline form. The α -anomer (III) was obtained only in an amorphous solid from the mother liquor after repeated tlc separations. The UV absorption spectra of II (257 nm) and III (258 nm) indicate an N₁-glycosyl linkage of uracil. The spacing of anomeric doublet in NMR spectrum of II (δ 5.90; J =9.4 Hz) and the positive Cotton effect³⁾ show the glycosyl linkage to be in β -configuration of pyranose type, while α -configuration can be assigned to the glycosyl linkage of III from the coupling constant of the anomeric doublet (δ 6.25; J =2.5 Hz) and the negative Cotton effect, although the possibility of furanose type structure of the latter cannot be eliminated rigorously from the examination of the NMR spectrum. Deacetylation of the β -anomer II with sodium methoxide afforded crystalline 1- β -D-galactopyranosyluracil (IV) in 75% yield. This compound was first synthesized by Hilbert⁴⁾ from acetobromogalactose and 2,4-diethoxypyrimidine followed by hydrolysis with methanolic hydrogen chloride in a low yield.

The next step is the selective benzylation of IV to the 2,3,6-tribenzoate V having free hydroxyl group at the 4-position. Since the difference in reactivity between axial and equatorial hydroxyl group in methyl α -D-

1) K. A. Watanabe, M. P. Kotick and J. J. Fox, *Chem. Pharm. Bull.*, **17**, 416 (1969); *J. Org. Chem.*, **35**, 231 (1970).

2) T. Nishimura, B. Shimizu and I. Iwai, *Chem. Pharm. Bull.*, **12**, 1471 (1964).

3) T. L. V. Ulbricht, J. P. Jennings, P. M. Scope and N. Klyne, *Tetrahedron Letters*, **1964**, 695; A. F. Cook and W. G. Overend, *J. Chem. Soc. (C)*, **1966**, 1549.

4) G. E. Hilbert, *J. Am. Chem. Soc.*, **59**, 330 (1937).

galactoside had been used by Reist *et al.*⁵⁾ for the selective benzylation of the glycoside to methyl 2,3,6-tri-O-benzoyl- α -D-galactoside and since the anomeric proton coupling constant in the NMR spectrum of IV (8.1 Hz) showed that the sugar part of the nucleoside IV is to be a pyranose ring of chair conformation indicating that the C₄-hydroxyl group has an axial orientation, a direct benzylation of the nucleoside IV was carried out; the more restricted condition than that used for the preparation of the methyl tribenzoyl-galactoside was actually necessary. Thus, treatment with 3.0 eq. of benzoyl chloride in pyridine at -12°C afforded the desired tribenzoate (V) in 68% yield, but a mixture of the tri- and tetra-benzoates was obtained by treatment with 3.5 eq. of benzoyl chloride at 0°C and a mixture of di- and tri-benzoates with 3.0 eq. of benzoyl chloride at -15° to -18°C .

Mesylation of the tribenzoate V gave in an 81% yield crystalline 4-O-mesylate VI, which on treatment with sodium azide in hexamethylphosphoric triamide afforded an amorphous material (VII), which was isolated by means of tlc. The structure of VII was assigned as 1-(4-azido-4-deoxy-2,3,6-tri-O-benzoyl- β -D-glucopyranosyl)uracil by analysis of its NMR spectrum using proton spin decoupling technic; the coupling constants, $J_{1',2'}=9.5$ Hz, $J_{2',3'}=9.5$ Hz, and $J_{3',4'}=ca. 9$ Hz, indicate *trans* arrangements of the substituents at 1', 2', 3' and 4' positions (glucopyranose type), and that the chemical shift of 4'-H was higher than that of 2'-H and 3'-H by 1.5 ppm indicates the azido group at 4'-position.

Hydrolysis of the azido-tribenzoate VII with sodium methoxide gave 4-azido-4-deoxy nucleoside VIII which was then hydrogenated over 10% palladium on charcoal to give the 4-amino-4-deoxy nucleoside I, NMR spectrum

of which (anomeric proton at δ 5.63, $J=8.8$ Hz) indicates a β -pyranosyl type structure.

FIG. 1.

EXPERIMENTAL

Mps were determined on a micro hot stage and uncorrected. The following spectrometers were used for spectral measurements: (IR) JASCO IR-G; (NMR) JEOL JNM-4H-100 (100 MHz); (UV) Hitachi EPS-3T; (ORD) Yanagimoto SPR-185. Unless otherwise indicated chemical shifts (δ) were expressed in ppm from internal TMS and coupling constants (J) in Hz. Silica gel G was used for tlc.

1-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)uracil (II). Acetobromogalactose (20 g) was intimately mixed with 2,4-bis(trimethylsiloxy)pyrimidine (20 ml) in a flask which was then evacuated and closed by a stop-cock. The mixture was fused at 80°C and then heated at 140°C for 45 min. After being cooled, the reaction mixture was treated with methanol (20 ml) and evaporated to dryness. The residue was added to a mixture of pyridine (40 ml) and acetic anhydride (30 ml) and the mixture was allowed to stand at room temp. for 20 hr. It was then poured into a cold saturated aqueous sodium bicarbonate solution (300 ml) and the aqueous mixture was extracted with dichloromethane. The organic layer was dried over calcium chloride and evaporated to dryness to give a syrup (23 g). Crystallization from isopropanol yielded needles, mp $106\sim 108^{\circ}\text{C}$ (7.3 g, 34%). *Anal.* Found: C, 49.99; H, 5.88; N, 5.26. Calcd. for $\text{C}_{18}\text{H}_{22}\text{O}_{11}\text{N}_2\cdot(\text{CH}_3)_2\text{CHOH}$: C, 50.01; H,

5) E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker and L. Goodman, *J. Org. Chem.*, **30**, 2312 (1965).

6.01; N, 5.55%. $\lambda_{\text{max}}^{\text{MeOH}}$ 257 nm (ϵ 8900); $[\alpha]_{\text{D}} + 30^\circ$ (MeOH, $c=0.58$), $[\phi]_{293}^{\text{peak}} + 6960^\circ$; $\delta(\text{CDCl}_3)$ 5.51 (1H, d*, $J=1.5$, 4'-H), 5.84 (1H, br. d, $J=8.1$, 5-H), 5.90 (1H, d*, $J=9.4^{**}$, 1'-H), 7.39 (1H, d, $J=8.1$, 6-H), 9.50 (1H, br. s, OH).

The α -anomer (III) was isolated as amorphous powder from the mother liquor by means of tlc on silica gel. Characterization was made only by analysis of IR, UV, and NMR spectra as well as optical dispersion measurements. $\lambda_{\text{max}}^{\text{MeOH}}$ 258 nm (ϵ 7600); $[\alpha]_{\text{D}} + 17^\circ$ (MeOH, $c=0.36$), $[\phi]_{277}^{\text{trough}} - 8870^\circ$; $\delta(\text{CDCl}_3)$ 2.07, 2.08, 2.16, 2.18 (s, $4 \times \text{CH}_3\text{CO}$), 5.70 (1H, d, $J=8.1$, 5-H), 6.25 (1H, d, $J=2.5$, 1'-H), 7.44 (1H, d, $J=8.1$, 6-H), 9.50 (1H, br. s, OH).

1- β -D-Galactopyranosyluracil (IV). A suspension of the tetraacetate of the β -anomer (II) (7.31 g) in methanolic 1N sodium methoxide (73 ml) was refluxed for 1.5 hr. After being cooled at room temp., it was neutralized to pH 7 by addition of Dowex 50 (H^+ type) resin. The resin was removed by filtration and the filtrate was evaporated to a small volume and treated with ethanol to give the nucleoside IV as crystalline mass (3.40 g, 76%). Recrystallization from water-ethanol gave needles, mp 234°C (lit.⁴¹ mp $250 \sim 251^\circ\text{C}$). *Anal.* Found: C, 43.41; H, 5.13; N, 9.88. Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_7\text{N}_2$: C, 43.80; H, 5.15. N, 10.22%. $\lambda_{\text{max}}^{\text{MeOH}}$ 260 nm (ϵ 9700); δ (DMSO- d_6) 5.33 (1H, d, $J=8.3$, 1'-H), 5.70 (1H, dd, $J=8.1$, 1.5, 5-H), 7.64 (1H, d, $J=8.1$, 6-H), 11.24 (1H, d, $J=1.5$, OH).

1-(2,3,6-Tri-O-benzoyl- β -D-galactopyranosyl)uracil (V). To a solution of the nucleoside IV (2.39 g) in anhydrous pyridine (45 ml) was added benzoyl chloride (3.68 g) dropwise with vigorous stirring at -12°C to -15°C in a period of 45 min. After being allowed to stand below 0°C for 10 hr, the reaction mixture was set aside at 10°C for 20 hr. The precipitate resulted was filtered and treated with hot chloroform to give the tribenzoate V as needles (3.25 g, 68%). Recrystallization from chloroform afforded an analytical sample, mp $217 \sim 219^\circ\text{C}$. *Anal.* Found: C, 63.06; H, 4.38; N, 4.75. Calcd. for $\text{C}_{31}\text{H}_{26}\text{O}_{10}\text{N}_2$: C, 63.48; H, 4.47; N, 4.78%. $\lambda_{\text{max}}^{\text{MeOH}}$ 232 nm (ϵ 39200), 260_{sh} (ϵ 9500); $\delta(\text{DMSO}-d_6)$ 6.26 (1H, d, $J=6.3$, 1'-H).

1-(2,3,6-Tri-O-benzoyl-4-O-methanesulfonyl- β -D-galactopyranosyl)uracil (VI). To a solution of the tribenzoate V (1.00 g) in anhydrous pyridine (10 ml) was added dropwise methanesulfonyl chloride (0.75 ml) with stirring at 0°C , and the mixture was allowed to stand at room temp. for 5 hr. It was then poured dropwise into a cold saturated sodium bicarbonate solution (300 ml) with stirring. The precipitate resulted was filtered, dried in air and dissolved in dichloromethane. The solution was treated with Norit A and evaporated to dryness to give crystalline residue. Recrystallization from ethanol gave needles, mp $207 \sim 208^\circ\text{C}$ (0.91 g, 81%). *Anal.* Found: C, 57.95; H, 4.22; N, 4.00. Calcd. for $\text{C}_{32}\text{H}_{28}\text{O}_{12}\text{N}_2\text{S}$: C, 57.82; H, 4.24; N, 4.21%. $\lambda_{\text{max}}^{\text{MeOH}}$ 232 nm (ϵ 39200), 260 nm (ϵ 9500); $\delta(\text{CDCl}_3)$ 3.10 (3H, s, CH_3), 5.54 (1H, d*, $J=2.5$, 4'-H), 6.24 (1H, d*, $J=7.5$, 1'-H).

1-(4-Azido-4-deoxy- β -D-glucopyranosyl)uracil (VIII). A suspension of the mesylate VI (1.69 g) and powdered sodium azide (0.93 g) in hexamethylphosphoric triamide (6 ml) was heated at 80°C with stirring for 5 hr. The reaction mixture was cooled, and poured into water (300 ml). The aqueous solution was extracted three times with chloroform (200 ml each) and the chloroform layer was washed twice with water, dried over calcium chloride, and evaporated to dryness to give the crude azide as a syrup (1.71 g). From the syrup was isolated amorphous 1-(4-azido-4-deoxy-2,3,6-tribenzoyl- β -D-glucopyranosyl)uracil (VII) (1.31 g, 84%) by means of tlc on silica gel G using benzene-ethyl acetate (2:1) as the solvent. ν_{KBr} 2100 cm^{-1} (N_3); $\lambda_{\text{max}}^{\text{MeOH}}$ 232 nm (ϵ 38800), 260_{sh} nm (ϵ 11300); $\delta(\text{CDCl}_3)$ 3.8~4.3 (2H, m, 4', 5'-H), 4.5~4.9 (2H, 6', 6'-H), 5.52 (1H, t, $J=9.5$, 2'-H), 5.76 (1H, br. d, $J=8.0$, 5-H), 5.91 (1H, dd, $J=9.5$ and ca. 9, 3'-H), 6.19 (1H, d, $J=9.5$, 1'-H); $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ were confirmed by proton decoupling technic.

To the azide-tribenzoate VII (0.751 g) was added methanolic 1N sodium methoxide (10 ml) at 0°C and the reaction mixture was allowed to stand at room temp. for 1.5 hr and then neutralized by addition of Dowex 50 (H^+ type). After removal of the resin by filtration the filtrate was evaporated to dryness and the residue was washed thrice with ether (15 ml each) and then dissolved in water. The aqueous solution was evaporated to dryness to give crystalline residue (0.264 g, 79%). Recrystallization from aceto-

* With small virtual couplings.

** J was obtained from the NMR spectrum taken in C_6D_6 .

* With small virtual couplings.

nitrile gave analytical sample of the azide VIII, mp 226°C (dec.). *Anal.* Found: C, 40.17; H, 4.30; N, 22.89. Calcd. for $C_{10}H_{13}O_6N_5$: C, 40.14; H, 4.38; N, 23.41%. ν_{KBr} 2100 cm^{-1} (N_3); λ_{max}^{MeOH} 260 nm (ϵ 8100); $\delta(D_2O)$; acetone was used as an internal standard and taken as 2.21 ppm) 5.58 (1H, d*, $J=8.8$, 1'-H), 5.94 (1H, d, $J=8.1$, 5-H), 7.78 (1H, d, $J=8.1$, 6-H).

1-(4-Amino-4-deoxy- β -D-glucopyranosyl)uracil (I). To a solution of the azide VIII (0.120 g) in ethanol-water (1:1) (4.5 ml) was added 10% palladium on charcoal (40 mg) and the mixture was hydrogenated at room

temp. under H_2 (1 atm) for 3 hr. The catalyst was removed by filtration and the filtrate was evaporated to a small volume, from which the crude amine I was separated as crystals (0.100 g, 86%). Recrystallization from aqueous ethanol gave analytical sample (dried over P_2O_5 at 90°C *in vacuo*), mp 245~247°C (dec.). *Anal.* Found: C, 41.55; H, 5.37; N, 14.42. Calcd. for $C_{10}H_{15}O_6N_3 \cdot H_2O$: C, 41.23; H, 5.88; N, 14.43%. λ_{max}^{MeOH} 260 nm (ϵ 11700); $\delta(D_2O)$; acetone was used as an internal standard and taken as 2.21 ppm) 5.63 (1H, d, $J=8.8$, 1'-H), 5.96 (1H, br. d, $J=8.1$, 5-H), 7.87 (1H, d, $J=8.1$, 6-H).