# Synthesis of 5-Substituted Pyrimidines. II[1,2]

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2,4-Dibenzyloxy-5-lithiopyrimidine has been condensed with 5-O-acetyl-2,3-O-isopropylidine-D-ribonolactone, 2,3,5,6-di-O-isopropylidine-γ-D-mannolactone, and 2,3,5,6-di-O-isopropylidine-γ-D-gulonolactone. The condensation products were reduced with sodium borohydride and then hydrolyzed in dilute acid to remove the remaining protective groups. ψ-Uridine was obtained in a 10% yield. 5-α-D-Mannitoluracil and 5-β-D-gulitoluracil were obtained in yields of 13 and 15%, respectively.

The synthesis of 5-substituted pyrimidines by the reaction of 2,4-dibenzyloxy-5-lithiopyrimidine and diisopropylidine aldehydopentoses was described in an earlier paper.[1] The method has been extended to include the reaction of the lithiopyrimidines with protected sugar lactones. Whereas the preparation of derivatives of aldehydo sugars often requires several steps, the lactones are readily available in good yield.

Pseudouridine was prepared by condensing 5-O-acetyl-2,3-O-isopropylidine-D-ribonolactone (II) with 2,4-dibenzyloxy-5-lithiopyrimidine (I). The product (III) from the condensation was isolated by chromatography of the reaction mixture on alumina. The infrared spectrum showed no lactone peak but exhibited a peak at 5.75 μ characteristic of an ester function. Reduction with sodium borohydride reduced the anomeric carbon to a carbinol and simultaneously removed the acetyl group. The predominant product was the β isomer which, when treated with 0.2 *N* sulfuric acid to cleave the protecting isopropylidine groups, underwent ring closure to ψ-uridine (V). The yield was 10.1%. The series of reactions are shown in Figure 1.

2,3,5,6-Di-O-isopropylidene-γ-D-mannolactone (VI) and 2,3,5,6-di-O-isopropylidene-γ-D-gulonolactone (X) were also condensed with 2,4-dibenzyloxy-5-lithiopyrimidine and the protecting groups removed in the same manner as was used for the ψ-uridine. Crystalline 5-α-D-mannitoluracil (IX) was obtained in a yield of 13% and 5-β-D-gulitoluracil was obtained as a crystalline product in a yield of 15%. Evidence that the compounds were in the open-chain form was the formation of a yellow spot following chromatography and spraying with benzidene–periodate.[1] The compound forming the yellow derivative had the same $R_f$ value as 5-formyluracil. Previously, 5-formyluracil had been identified as the periodate oxidation product of 5-α-D-arabinotoluracil.

We found in our earlier work[1] that certain β-substituted compounds formed cyclic derivatives more readily than the corresponding α compounds. Since ψ-uridine gives at least three compounds on treatment with strong mineral acids and since we had previously had difficulty preparing a pure compound by the cyclization of 5-α-D-arabinotoluracil, we did not investigate the use of strong acids for cyclizing 5-α-D-mannitoluracil and 5-β-D-gulitoluracil. It is likely that conditions could have been found for preparing the cyclic derivatives. However, closure of the ring by acetylation and deacetylation has been found to be a convenient method for accomplishing this reaction and, at least, in our hands gives one major product.

ORD has been developed recently as a useful tool for the elucidation of the stereochemistry of the anomeric carbon of pyrimidine nucleosides.[3] We had presented evidence that the α anomers of 5-substituted pyrimidines exhibit a positive Cotton effect, whereas the β anomers present a negative Cotton effect.[1] 5-D-Mannitoluracil (IX) showed a positive dispersion curve ($[\Phi]_{257}$ +1465) indicating an α configuration. 5-D-Gulitoluracil exhibited a negative curve ($[\phi]_{257}$ −14,690); this would indicate a β configuration. In order to obtain additional proof for the assignment of the β configuration, 5-D-gulitoluracil (XIII) was treated with acetic anhydride and pyridine to give 5-(2,3,4,6-tetra-O-acetyl-D-gulopyranosyluracil. The acetyl groups were removed and periodate studies[4] were carried out to determine the size of the sugar ring. Two moles of periodate were consumed and one mole of formic acid was liberated; no formaldehyde was present. Consequently XXVI has the sugar moiety in the pyranose form. The nmr spectra of XIV showed the $C_1'$ hydrogen at τ 5.82 (doublet, $J$ = 6 cps); this large coupling constant is indicative of a transdiaxial relationship between hydrogens at $C_1'$ and $C_2'$ and corresponds to the β configuration.[5] Assuming these assignments are correct, the large entering group is adding *trans* to the substituted hydroxyl group on $C_2'$ in each of the three lactones studied. Although data from ORD on pyrimidines substituted in the 5 position is too limited to make conclusive assignments, such data along with nmr and the expected orientation of substitution constitutes good evidence for the assignment of configuration of the compounds V, IX, and XIII.

## Experimental Section

Melting points are corrected and were taken on a Mel-Temp unit. Infrared spectra were determined on a Perkin-Elmer Infracord Spectrophotometer. Ultraviolet absorption spectra were obtained on a Perkin-Elmer recording spectrometer. Rotations were in 0.1 *N* aqueous sodium hydroxide solution, unless otherwise stated, and taken with a Rudolph polarimeter. Nuclear magnetic resonance spectra were determined at 60 Mc on a Varian Associates A-60 recording spectrometer.[6] Optical rotatory dispersion was determined at the University of Wiscon-

(1) Part I in the series: W. Asbun and S. B. Binkley, *J. Org. Chem.*, **31**, 2215 (1966).

(2) (a) Supported by U. S. Public Health Service Grant CY3231 from the U. S. Public Health Service and Training Grant No. 471. (b) Presented in part at the Great Lakes Regional Meeting of the American Chemical Society, Chicago, Ill., June 1966.

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(6) We thank Mr. C. Hershberger for the nmr determinations.

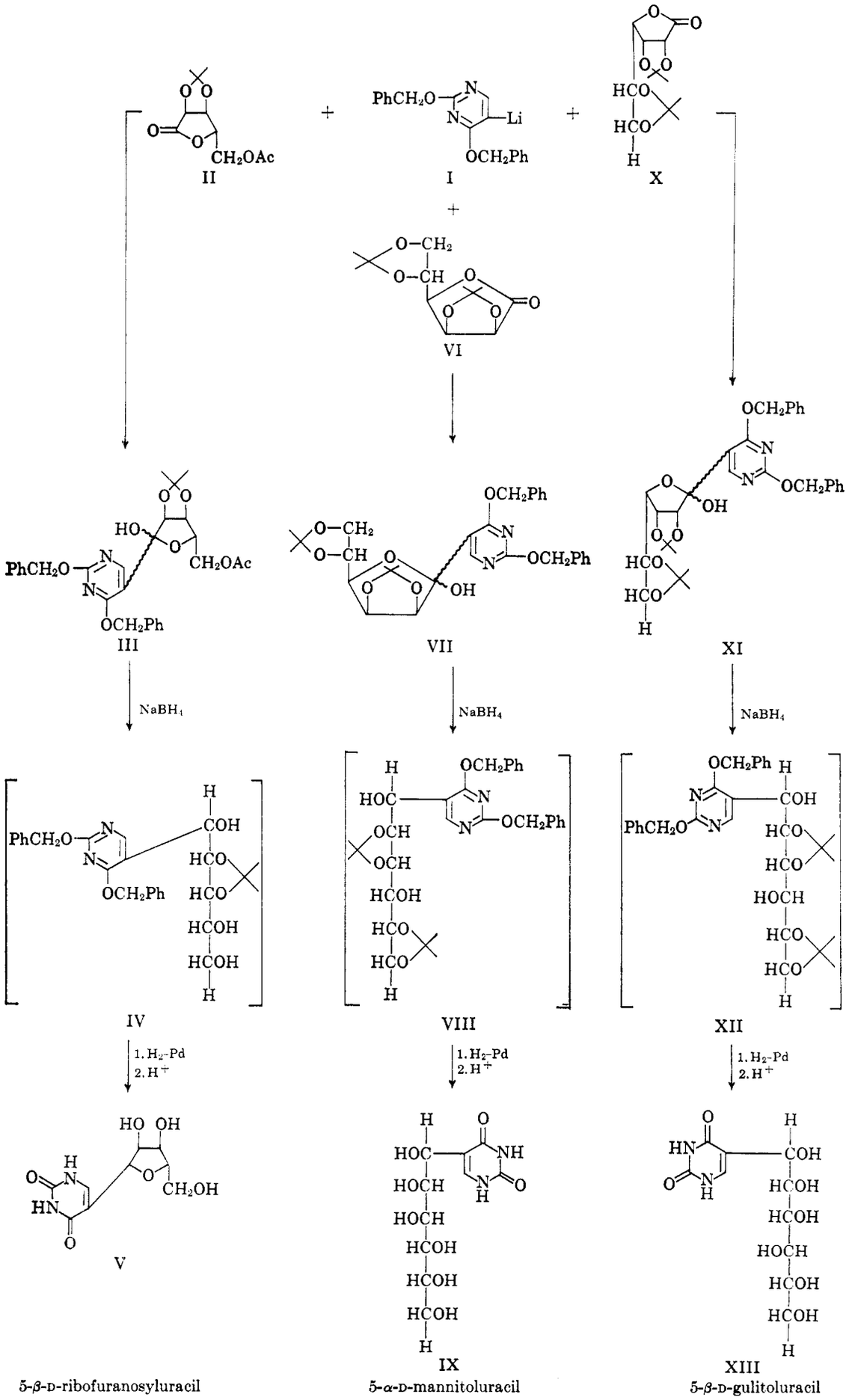
II
I
X
VI
III
VII
XI
NaBH4
IV
VIII
XII
1. H2-Pd
2. H+
V
IX
XIII
5-β-D-ribofuranosyluracil
5-α-D-mannitoluracil
5-β-D-gulitoluracil

Figure 1

sin, at 25° (*c* 0.006, water). Paper chromatography was conducted by the descending technique on Whatman No. 1 paper in methanol–ammonium hydroxide solution (99:1, v/v) with indication by ultraviolet light and the sodium metaperiodate test. Thin layer chromatography was carried out on 0.25-mm thick layers of Whatman silica gel SG41.

**2,3-Isopropylidene-5-O-acetylribonolactone (II).**—D-Ribonolactone (5 g, Sigman Chemical Co.) was treated with acetone (100 ml) and sulfuric acid (2 ml), stirred at room temperature for 3 hr, and then neutralized with dry ammonia. After removing the inorganic salts by filtration, the acetone solution was concentrated to a small volume and the solution treated with petroleum ether (30–60°); upon cooling at 5° the product crystallized (4.8 g, 70.5% yield). Isopropylidene ribonolactone[7] (4.8 g) was acetylated with acetic anhydride (5 ml) in the presence of pyridine (5 ml). The reaction mixture was stirred for 20 hr at room temperature, crushed ice was added, and the solution was extracted with chloroform. The chloroform layer was washed with water and evaporated to dryness. The remaining pyridine was eliminated under high vacuum to give a pale yellow gummy material (5.34 g).

**5-β-D-Ribofuranosyluracil (V).**—The protected sugar lactone (II), (3.45 g, 15 mmoles) was condensed with the pyrimidine-lithio derivative I (20 mmoles) using the same procedure as previously described.[1] The reaction mixture (10.20 g) was chromatographed on alumina (200 g, Merck acid washed). The column was eluted with chloroform (300 ml) and chloroform–1% methanol (400 ml); 100-ml portions were collected. Fractions 4, 5, 6, and 7, (3.7 g) were identical on tlc using chloroform–2% methanol as the solvent. The infrared spectra showed no lactone peak, but an ester peak was observed at 5.75 μ. The product, obtained after removal of the solvent, was dissolved in methanol, sodium borohydride was added, and the mixture was stirred overnight. The methanolic solution was neutralized with 5 *N* hydrochloric acid solution, and the organic solvent removed under vacuum. The residue was suspended in water (100 ml) and the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried, and evaporated to dryness. The contents of the flask were redissolved in ethanol and hydrogenated in the presence of palladium-on-carbon (10%, 1 g) for 3 hr. The catalyst was removed and the isopropylidene groups were cleaved by stirring with 0.2 *N* sulfuric acid for 16 hr. The product crystallized from ethanol to give a compound (0.164 g, 10%) which was identified as 5-β-D-ribofuranosyluracil by comparison with an authentic sample. Paper chromatographic behavior and ultraviolet and infrared spectra were the same. There was no depression of the melting point on mixture with ψ-uridine obtained from natural sources. Chromatography of the mother liquor revealed the presence of two minor components presenting higher $R_f$ values than ψ-uridine.

**5-α-D-Mannitoluracil (IX).**—Diisopropylidene mannolactone[8] (VI) (5.31 g, 20 mmoles) was condensed with I (20 mmoles) using the same procedure indicated above. The product (12.74 g) was chromatographed on alumina (250 g, Merck, acid washed). The column was eluted with chloroform (400 ml) and chloroform–1% methanol (400 ml) and 100-ml fractions were collected. Fractions 6, 7, and 8 (3.22 g) which were identical on tlc were combined. The condensed product was reduced with sodium borohydride as indicated for the preparation of 5-β-D-ribofuranosyluracil. After cleaving the remaining protective groups in the manner described above, the product crystallized from ethanol (0.76 g, 13%), mp 172.5–174.5. It was chromatographically homogenous and gave a yellow spot with benzidene–periodate test indicating the sugar moiety was in the open-chain form. It was recrystallized three times from water to obtain an analytical sample: mp 220–222°; $[\alpha]^{25}D$ −41 (*c* 0.66, 0.1 *N* NaOH); $\lambda_{max}$ ($H_2O$) 266 mμ (ε 10,000, pH 7), 290 mμ (ε 8200, pH 14).

*Anal.* Calcd for $C_{10}H_{16}O_8N_2$: C, 41.10; H, 5.48; N, 9.60. Found: C, 40.84; H, 5.62; N, 9.44.

**5-β-D-Gulitoluracil (XIII).**—Diisopropylidene-D-gulonolactone (XI) (4.65 g, 17.5 mmoles) and I (16 mmoles) were condensed. The product (10.67 g) was chromatographed on alumina (200 g, Merck acid washed). The column was eluted with chloroform (300 ml), chloroform–1% methanol (200 ml), and chloroform–1.5% methanol (400 ml). The fraction eluted with chloroform–1.5% methanol contained the desired material (4.96 g). Reduction with sodium borohydride and removal of the protective groups was achieved in the manner described for the preparation of 5-β-D-ribofuranosyluracil. Upon concentration of the aqueous ethanolic solution and cooling in the refrigerator, a crystalline material was obtained (0.510 g). The crystals gave a single spot on paper chromatography, presenting a yellow color with the benzidene–periodate test. The mother liquor gave a second crop (0.240 g) of the same compound (total yield 14.7%). It was recrystallized three times from water for analysis. When the compound was heated in a capillary tube, it darkened at about 190° and melted with decomposition at 202–204°: $[\alpha]^{25}D$ −203 (*c* 0.59, 0.1 *N* NaOH); $\lambda_{max}$ ($H_2O$) 263 mμ (ε 6400, pH 7), 287 mμ (ε 5300, pH 14).

*Anal.* Calcd for $C_{10}H_{16}O_8N_2$: C, 41.10; H, 5.48; N, 9.60. Found: C, 40.90; H, 5.55; N, 9.64.

**5-(2,3,4,5-Tetra-O-acetyl)-β-D-gulopyranosyluracil (XIV).**—5-β-D-Gulitoluracil (XIII) (0.400 g) was suspended in pyridine (5 ml) and acetic anhydride (5 ml). After stirring at room temperature for 4 days, ice was added and the reaction mixture was extracted with two 20-ml portions of ethyl acetate and with chloroform (20 ml). The organic layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. Pyridine was removed under high vacuum. The white solid residue was dissolved in hot ethyl acetate; crystallization started upon addition of 2 drops of petroleum ether (0.422 g, 69.5% yield), mp 183–185°; the analytical sample was obtained by recrystallizing the compound three times from the same solvent, mp 194–196°; $[\alpha]^{25}D$ −76.5 (*c* 1.11, chloroform).

*Anal.* Calcd for: $C_{18}H_{22}O_{11}N_2$: C, 48.86; H, 4.97; N, 6.33. Found: C, 48.89; H, 5.20; N, 6.19.

**Hydrolysis and Periodate Oxidation of XIV.**—Compound XIV (0.340 g, 1.16 mmoles) was dissolved in methanol (100 ml) and treated with a solution of freshly prepared sodium methoxide (7.40 mmoles). The mixture was stirred for 16 hr and then neutralized with Dowex 50, $H^+$ form. Evaporation of the methanolic solution gave a crystalline solid (XV, 0.192 g) which was chromatographically homogenous.

Periodate oxidation[4] was carried out at 4° in the dark; XV (0.03945 g, 0.144 mmole) was dissolved in water (5 ml) and treated with sodium metaperiodate solution (0.5 *M*, 3 ml); the volume was made up to 10 ml by addition of water. The periodate uptake was determined by the addition of a known amount of sodium arsenite solution; the excess of sodium arsenite was measured with a standardized iodine solution in the presence of soluble starch as indicator. A blank was run simultaneously. After 30 min the solution consumed 0.300 mmole; at the end of 60 min the periodate uptake remained unchanged (calculated 0.288 mmole). In order to measure the formic acid released, 1-ml aliquots of the reaction mixture were treated with 1 ml of ethylene glycol and titrated with 0.012 *N* sodium hydroxide yielding 0.154 mmole of formic acid (theory 0.144 mmole).

**Registry No.**—IX, 15040-85-4; XIII, 15040-86-5; XIV, 15040-87-6.

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