

Note

The anomeric configuration of 3-D-glycosyl-6-methyluracils and the synthesis of related compounds

NAOTAKA YAMAOKA*, EMIKO TAKAHASHI, AND KATURA TUZIMURA

Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori Amamiyama-machi, Sendai (Japan)

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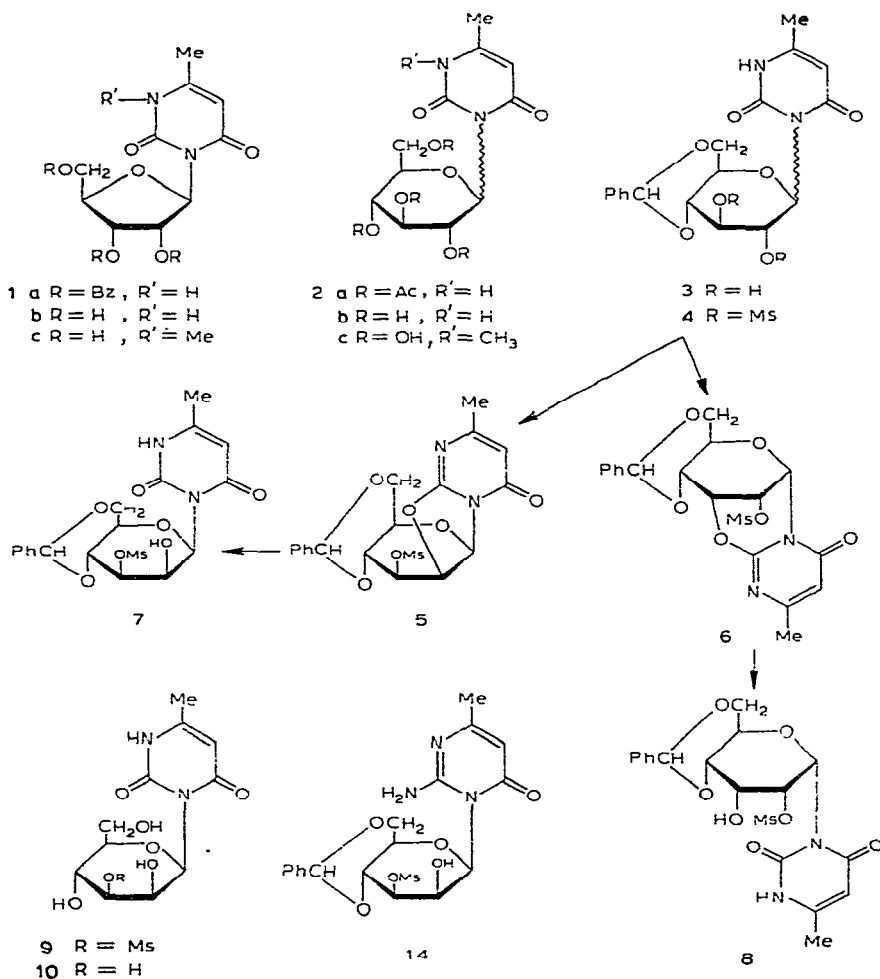
The nitromethane-mercuric cyanide method¹ of nucleoside synthesis usually gives the 1-glycosyl derivative when uracil and 5-substituted uracils are condensed with halogeno sugars², but we have found that the condensation of 6-methyluracil with halogeno sugars gives anomeric mixtures of the 3-glycosyl derivatives.

6-Methyluracil nucleosides could not be synthesised by the mercuric salt method³ and the Hilbert-Johnson method⁴. The latter method gives the "O-glycoside"⁵. Utilization⁶ of the trimethylsilyl derivative of 6-methyluracil gave a poor yield of 6-methyl-3-D-ribofuranosyluracil (**1b**).

Compound **1a** was easily prepared (84%) from 6-methyluracil and tri-*O*-benzoyl-D-ribofuranosyl chloride by the nitromethane-mercuric cyanide method, as was 6-methyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)uracil (**2a**, 94%) from tetra-*O*-acetyl- α -D-glucopyranosyl bromide. The structures of compounds **1a** and **2a** were established by their stability towards methanolic hydrogen chloride⁷, by the formation of 1,6-dimethyluracil by acid hydrolysis of the *N*-methylated derivatives⁸ **1c** and **2c**, and by their u.v. spectra⁹. Compounds **1c** and **2c** were prepared by the methylation of **1b** and **2b** with diazomethane in methanol.

Compounds **1a** and **1b** appeared to be β -D anomers⁶, since the p.m.r. spectrum of **1a** showed a coupling constant, $J_{1',2'}$ of <1 Hz with a half-height width of 3 Hz for H-1'. Compound **2b** was an anomeric mixture, since the p.m.r. spectrum in D₂O showed six peaks near δ 5.7 with $J_{1',2'}$ 9.33 Hz (δ 5.76) for H-1' of one anomer and $J_{1',2'}$ 9.67 Hz (δ 5.65) for H-1' of the other anomer¹⁰, and $J_{5,6}$ 0.7 Hz (δ 5.62) for H-5.

One of the two asymmetric centers of the dialdehyde formed on periodate oxidation of glycosides retains the configuration of the glycosidic carbon atom¹¹. In order to compare **1b** and **2b**, the latter compound was converted into 3- β -D-mannopyranosyl-6-methyluracil. Condensation of **2b** with benzaldehyde-zinc chloride gave 3-(4,6-*O*-benzylidene- α β -D-glucopyranosyl)-6-methyluracil (**3**), which afforded the dimethanesulphonate **4** in good yield. Treatment of **4** with one equivalent of sodium hydroxide in methanol gave a mixture of the 2,2'-anhydro compound (**5**)



and the 2,3'-anhydro compound¹² (6) which could not be separated by chromatography. However, with two equivalents of sodium hydroxide, 4 gave 3-(4,6-*O*-benzylidene-3-*O*-mesyl- β -D-mannopyranosyl)-6-methyluracil (7). Similar treatment of a mixture of the 2,2'- and 2,3'-anhydro compounds (5 and 6) also gave 7. The β -D-anomeric configuration of 7 was established by the coupling constant $J_{1',2'} < 1$ Hz for H-1', and by hydrolysis with aqueous acetic acid to give 3-(3-*O*-mesyl- β -D-mannopyranosyl)-6-methyluracil¹³ (9). Since 9 did not consume periodate, it was identified as a 3'-*O*-mesyl compound. Treatment of 9 with two equivalents of sodium hydroxide gave 3- β -D-mannopyranosyl-6-methyluracil (10), which consumed periodate and had $J_{1',2'} = 0.6$ Hz (δ 6.08) for H-1'. Simple o.r.d. curves were obtained for the nucleosides 1b, 2b, and 10 and for the dialdehyde 11 derived by periodate oxidation. The results (Table I) showed that 1b and 2b were similar anomeric mixtures. This type of o.r.d. data offers a reliable method of determining anomeric purity.

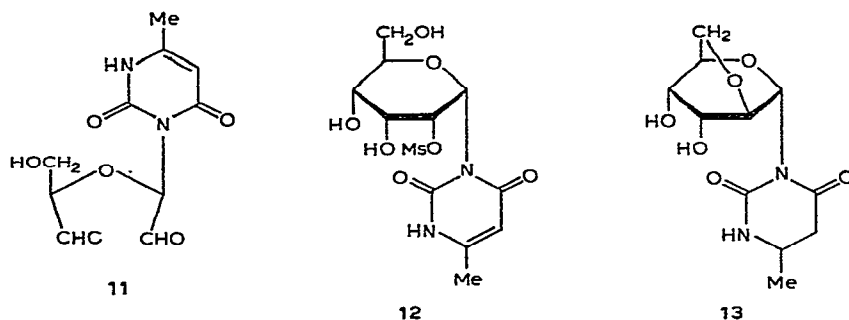


TABLE I

O.R.D. DATA FOR DIALDEHYDES DERIVED BY OXIDATION OF NUCLEOSIDES WITH SODIUM METAPERIODATE (0.04M)

Parent nucleoside	$[M]_{389}^{22\text{ }^a}$	$[M]_{350}^{22\text{ }^a}$
2b	-80°	-410°
10	-25°	-230°
1b	-85°	-400°

^ac 0.01, water.

The structures of the 2,2'-anhydro (5) and 2,3'-anhydro compounds (6) were established by comparison of the u.v. spectra of 3-(4,6-*O*-benzylidene-3-*O*-mesyl- β -D-mannopyranosyl)-6-methylisocytosine¹⁴ (14) and 3,6-dimethylisocytosine¹⁵. Compound 14 was obtained by treatment of the anhydro compounds (5,6) with liquid ammonia in a sealed tube.

Attempted separation of 3-(4,6-*O*-benzylidene-2-*O*-mesyl- α -D-allopyranosyl)-6-methyluracil (8), following treatment of the mixture of 5 and 6 with alkali, was unsuccessful. Treatment of the mother liquor of compound 7, which contained 8, with aqueous acetic acid gave 6-methyluracil and methanesulphonic acid, possibly *via* 12 and 13 since methyl 2,6-anhydro- α -D-altropyranoside is acid-labile¹⁶.

EXPERIMENTAL

P.m.r. spectra were measured, with appropriate internal or external standards of tetramethylsilane, with a JEOL 60 MHz spectrometer. O.r.d. data were measured with a JASCO ORD/UV-5 instrument.

6-Methyl-3-D-riboseyluracil (1b). — A mixture of 6-methyluracil (1.9 g) and mercuric cyanide (5.1 g) was added to nitromethane (500 ml) and dried by azeotropic distillation. The ribofuranosyl chloride (from 10.5 g of tri-*O*-benzoyl-D-ribofuranosyl acetate and ethereal hydrogen chloride) was added dropwise to the mixture during 30 min with continuous, gradual distillation of nitromethane. The mixture was then boiled for 4 h with continuous, gradual distillation of nitromethane and thereafter

evaporated *in vacuo*. The syrupy residue was extracted with chloroform, and the extract was washed with 30% aqueous potassium iodate (2×50 ml) and water (50 ml), dried (Na_2SO_4), and concentrated. The residue was eluted from silica gel with benzene-ether (1:1) to give **1a** as a colourless, amorphous solid (7 g, 82%). N.m.r. data (methyl sulphoxide- d_6): δ 6.55 (s, half-height width 3 Hz, $J_{1',2'} < 1$ Hz, H-1').

A solution of **1a** (7 g) in ethanol (50 ml) and M sodium hydroxide (50 ml) was stored for 1 h and then treated with Dowex-50 (H^+) resin (24 g). The filtrate was evaporated *in vacuo*, and the syrupy residue was treated with a mixture of chloroform and water to remove benzoic acid. The aqueous layer was concentrated and the residue dried to give **1b** (3.1 g) as an amorphous powder, $[\alpha]_D^{22} -18^\circ$ (c 0.08, water), $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 266, $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 234 nm (Found: C, 46.56; H, 5.59; N, 11.08. $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_6$ calc.: C, 46.51; H, 5.49; N, 10.85%). N.m.r. data (methyl sulphoxide- d_6): δ 6.10 (1-proton doublet, $J_{1',2'}$ 3.7 Hz, H-1')

1,6-Dimethyl-3-D-ribofuranosyluracil (1c). — An excess of diazomethane was added to a solution of **1b** (0.2 g) in dichloromethane (50 ml). After 1 day, the mixture was evaporated *in vacuo* to give **1c** (0.1 g), m.p. 159–161° (from ethanol), $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 273, $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 238 nm (Found: C, 48.33; H, 5.93; N, 10.07. $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_6$ calc.: C, 48.52; H, 5.92; N, 10.29%).

6-Methyl-3-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)uracil (2a). — The condensation of 6-methyluracil with tetra-O-acetyl- α -D-glucopyranosyl bromide was carried out as described for **1a** to give **2a** (94%), m.p. 155–157° (from ethanol) (Found: C, 49.87; H, 5.34; N, 6.09. $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_{11}$ calc.: C, 50.00; H, 5.30; N, 6.14%).

3-(α -D-Glucopyranosyl)-6-methyluracil (2b). — A solution of **2a** (1 g) in 50% aqueous ethanol containing 25 ml of M sodium hydroxide was stored for 30 min at room temperature. The mixture was neutralised with Dowex-50 (H^+) resin and evaporated *in vacuo*. The residue was crystallized twice from ethanol to give **2b** (0.6 g, 60%), m.p. 196–200°, $[\alpha]_D^{22}$ ca. 0° (c 0.09, water), $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 266 (ϵ 9,370), $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 234 nm (ϵ 2,036) (Found: C, 45.48; H, 5.64; N, 9.52. $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_7$ calc.: C, 45.83; H, 5.59; N, 9.72%). N.m.r. data (D_2O): δ 5.65 (doublet, $J_{1',2'}$ 9.67 Hz, H-1'), 5.76 (doublet, $J_{1',2'}$ 9.33 Hz, H-1').

3-(4,6-O-Benzylidene- α -D-glucopyranosyl)-6-methyluracil (3). — Compound **2b** (5.2 g) was added to freshly distilled benzaldehyde (80 ml) containing zinc chloride (6 g), and the mixture was stirred for 40 h at room temperature. The mixture was poured into ether (200 ml) with vigorous stirring, and the product was collected, washed with ether and methanol, and recrystallized from hot water to give **3** (6 g, 91%), m.p. 286–288° (Found: C, 57.46; H, 5.34; N, 7.39. $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_7$ calc.: C, 57.44; H, 5.36; N, 7.44%).

3-(4,6-O-Benzylidene-2,3-di-O-mesyl- α -D-glucopyranosyl)-6-methyluracil (4). — A solution of **3** (1.7 g) in dry pyridine (100 ml) at 0° was treated with mesyl chloride (1.4 g), in the usual manner, to give **4** (2.2 g, 90%), m.p. 154–156° (from ethanol). Recrystallization from methanol gave material having m.p. 165–168° (decomp.), $\lambda_{\text{max}}^{\text{EtOH}}$ 264, $\lambda_{\text{min}}^{\text{EtOH}}$ 231 nm (Found: C, 44.89; H, 4.67; N, 5.26; S, 12.10. $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_{11}\text{S}_2$ calc.: C, 45.10; H, 4.54; N, 5.26; S, 12.04%).

3-(4,6-O-Benzylidene-3-O-mesyl- β -D-mannopyranosyl)-6-methyluracil (7). — Compound **4** (6.4 g) dissolved in methanol (700 ml) was treated dropwise with M sodium hydroxide (12 ml). The mixture was stored for 2 h at room temperature, and then evaporated *in vacuo* to small volume and poured into cold water (100 ml). The precipitate (4.4 g, 85%) was collected, washed with cold water, and recrystallized from ethanol to give the mixture **5-6** (2.8 g), m.p. 230–232°, $\lambda_{\max}^{\text{EtOH}}$ 274, $\lambda_{\min}^{\text{EtOH}}$ 236 nm (Found: C, 52.32; H, 4.66; N, 6.45; S, 7.25. $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_8\text{S}$ calc.: C, 52.28; H, 4.62; N, 6.42; S, 7.32%).

The mixture **5-6** (0.3 g) was suspended in 50% aqueous ethanol (20 ml), and M sodium hydroxide (1.4 ml) was added. The solution was stored for 2 h at 40° and then neutralised with Dowex-50 (H^+) resin. The filtrate was evaporated, and the residue was crystallized from aqueous ethanol to give **7** (0.3 g), m.p. 225–228°. Recrystallisation from hot ethanol gave material (0.2 g) having m.p. 238–239° (Found: C, 50.31; H, 4.81; N, 6.17; S, 7.09. $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_9\text{S}$ calc.: C, 50.21; H, 4.88; N, 6.16; S, 7.06%).

3-(3-O-Mesyl- β -D-mannopyranosyl)-6-methyluracil (9). — A suspension of compound **7** (1.9 g) in 80% aqueous acetic acid (50 ml) was refluxed for 1 h and then evaporated *in vacuo*. The syrupy residue was partitioned between chloroform and water. The aqueous layer was evaporated to dryness *in vacuo*, and the residue was crystallized from aqueous ethanol to give **9** (1.2 g), m.p. 173–175°. Recrystallization from aqueous ethanol gave material having m.p. 176–179° (Found: C, 39.19; H, 5.10; N, 7.55; S, 8.65. $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_9\text{S}$ calc.: C, 39.34; H, 4.95; N, 7.65; S, 8.75%).

3- β -D-Mannopyranosyl-6-methyluracil (10). — A suspension of compound **9** (0.6 g) in ethanol (25 ml) containing M sodium hydroxide (3.32 ml) was stored overnight and then neutralized with Dowex-50 (H^+) resin. The filtrate was evaporated *in vacuo*, and the residue was recrystallized from ethanol to give **10** (0.3 g), m.p. 237–238°. $[\alpha]_{\text{D}}^{22} - 32.8^\circ$ (c 0.09, water), $\lambda_{\max}^{\text{H}_2\text{O}}$ 267, $\lambda_{\min}^{\text{H}_2\text{O}}$ 235 nm (Found: C, 45.83; H, 5.59; N, 9.79. $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_7$ calc.: C, 45.88; H, 5.59; N, 9.72%). N.m.r. data (D_2O): δ 6.08 (1-proton doublet, $J_{1',2'} 0.6$ Hz, H-1').

3-(4,6-O-Benzylidene-3-O-mesyl- β -D-mannopyranosyl)-6-methylisocytosine (14). — The mixture **5-6** (0.5 g) was added to liquid ammonia (20 ml) and stored for 4 days at room temperature in a sealed tube. The mixture was then evaporated, and the residue [0.4 g, m.p. 170–175° (decomp.)] was recrystallized from methanol to give **14**, m.p. 173–175° (decomp.); u.v. data (90% aqueous ethanol): $\lambda_{\max}^{0.1\text{M NaOH}}$ 301, 232; $\lambda_{\min}^{0.1\text{M NaOH}}$ 258, $\lambda_{\max}^{\text{neutral}}$ 293, 229; $\lambda_{\min}^{\text{neutral}}$ 251; $\lambda_{\max}^{0.1\text{M HCl}}$ 263; $\lambda_{\min}^{0.1\text{M HCl}}$ 242 nm; n.m.r. data (methyl sulphoxide- d_6): δ 6.38 (1-proton doublet, $J_{1',2'} < 1$ Hz, H-1') (Found: C, 50.14; H, 5.02; N, 9.15; S, 6.92. $\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_8\text{S}$ calc.: C, 50.32; H, 5.11; N, 9.27; S, 7.07%).

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